[Chem. Pharm. Bull. 26(8)2340—2345(1978)]

UDC 547.853.7.04 : 547.963.32.04

Synthesis of 6-Cyanocytidine and Its Derivatives (Nucleosides and Nucleotides. XX[1])

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(Received January 13, 1978)

Treatment of 5-bromocytidine or its 2′,3′,5′-tri-*O*-acetate, and 2′,3′-*O*-isopropylidene derivative with sodium cyanide gave the respective 6-cyanocytidine. Methoxide treatment of the 6-cyano compound gave methyl cytidine-6-carboximidate which was converted to cytidine-6-carboxylic acid. Treatment of 2′,3′-*O*-isopropylidene-N^4-acetyl-5-bromocytidine with sodium cyanide afforded the O^6,5′-cyclo-N^4-acetylcytidine through the 6-cyanocytidine as the intermediate.

Keywords——pyrimidine nucleosides; cyclonucleosides; cytidine; nucleophilic substitution; sodium cyanide (cyano compound); NMR; UV

Orotidine, uridine 6-carboxylic acid, is a unique naturally occurring 6-substituted pyrimidine nucleoside[3] and its 5′-phosphate acts as the key intermediate in the biosynthesis of pyrimidine nucleotides.[4] We have recently established a method of introduction of a cyano group into the position 6 of uridine derivatives.[5,6] In addition, 6-mercaptouridine derivatives including uridine 6-sulfonic acid were prepared from 5-bromouridine *via* 6-benzylthiouridines.[7] The present paper deals with the reaction of 5-bromocytidines with sodium cyanide to give the 6-cyanocytidine derivatives and the reactions involving the cyano group. A preliminary results of this work has appeared.[6]

5-Bromocytidine (**1**), the starting material of the present work, was prepared by the action of bromine on cytidine in pyridine–acetic acid without the irradiation.[8] Treatment of **1** with acetic anhydride in the presence of a trace amount of pyridine afforded 2′,3′,5′-tri-*O*-acetyl-N^4-acetyl-5-bromocytidine (**2**). Heating of **2** in methanol with a catalytic amount of acetic acid resulted in a selective removal of the N^4-acetyl group to furnish 2′,3′,5′-tri-*O*-acetyl-5-bromocytidine (**3**). On the other hand, acetylation of **1** with acetic anhydride alone gave N^4-acetyl-5-bromocytidine (**4**), exclusively.

Treatment of **3** with 1.2 equivalent amount of sodium cyanide in dimethylformamide (DMF) at room temperature for 2 hours afforded the 6-cyanocytidine (**5**). The structure of **5** was confirmed by the detection of a nitrile group by infrared (IR) (2250 cm^{-1}) and by the nuclear magnetic resonance (NMR) measurements. Treatment of **5** with sodium methoxide in methanol afforded methyl cytidine-6-carboximidate (**6**) in 80% yield. The structure of **6** was determined by the disappearance of the cyano function by IR and the presence of a methoxy and an imino protons (3.75 and 9.10 ppm, respectively) by the NMR measurements.

In order to find out the best condition of the conversion of the imino-ether group of **6** to the carboxyl group the behaviours of **6** in basic or acidic conditions were checked by UV

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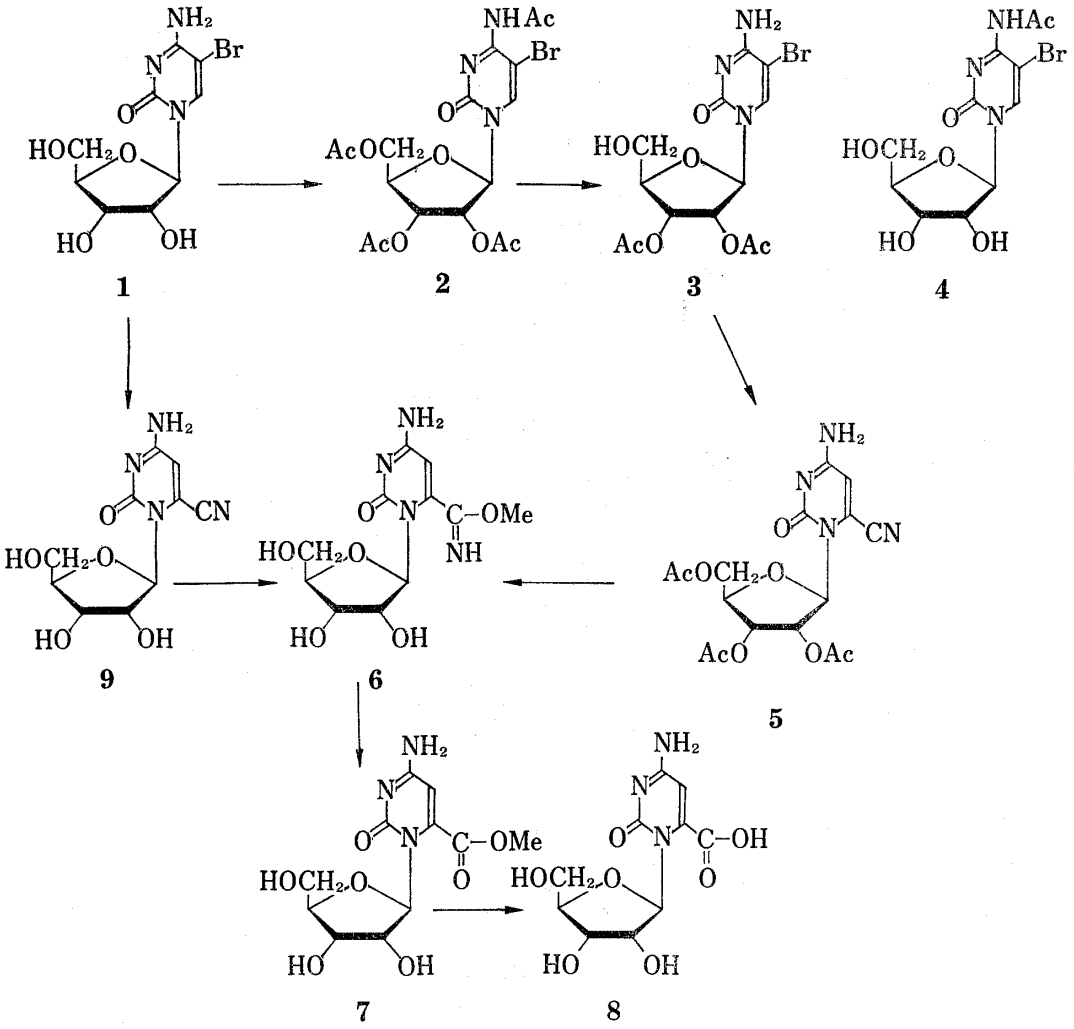

Chart 1

spectral change. The UV absorption maximum of **6** in water (272 nm) shifted rapidly on the addition of sodium hydroxide solution to 285 nm, then slowly shifted back to 274 nm. This spectral change was correlated to the elimination of methanol from **6** to give the 6-cyano compound in the initial step which was slowly hydrolyzed to give the 6-carboxamide. The absorption maximum of **6** in acidic solution (281 nm) shifted slowly to 287 nm which, on addition of alkali, rapidly shifted to 274 nm. This change was correlated to the slow hydrolysis of **6** to the 6-methoxycarbonyl derivative (**7**) which was readily hydrolyzed in an alkaline medium to give the 6-carboxylate. In fact, treatment of **6** with 0.2 N HCl at low temperature for 50 hours followed by the treatment with an alkali, and purification through a DEAE-cellulose column gave cytidine 6-carboxylic acid (**8**) in 76% yield. Compound **8** was deaminated by nitrous acid to give orotidine which confirmed the structure of **8**.

In contrast to the general high stabilities of the glycosylic bonds of pyrimidine nucleosides towards acidic conditions **6** and **8** are rather unstable. Treatment of **6** in 0.1 N HCl at room temperature afforded **7** which was contaminated with cytosine-6-carboxylic acid. Fox and co-workers also observed a partial cleavage of the glycosylic bond of orotidine in 0.5 N H_2SO_4 at 100°.[9] Recently, **8** was prepared by the other route involving the action of carbon dioxide with a 6-lithiocytidine derivative.[10]

9) J.J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, **23**, 295 (1957).
10) L. Pichat and J.-P. Guilbert, *C.R. Acad. Sci. Paris(C)*, **1973**, 1157.

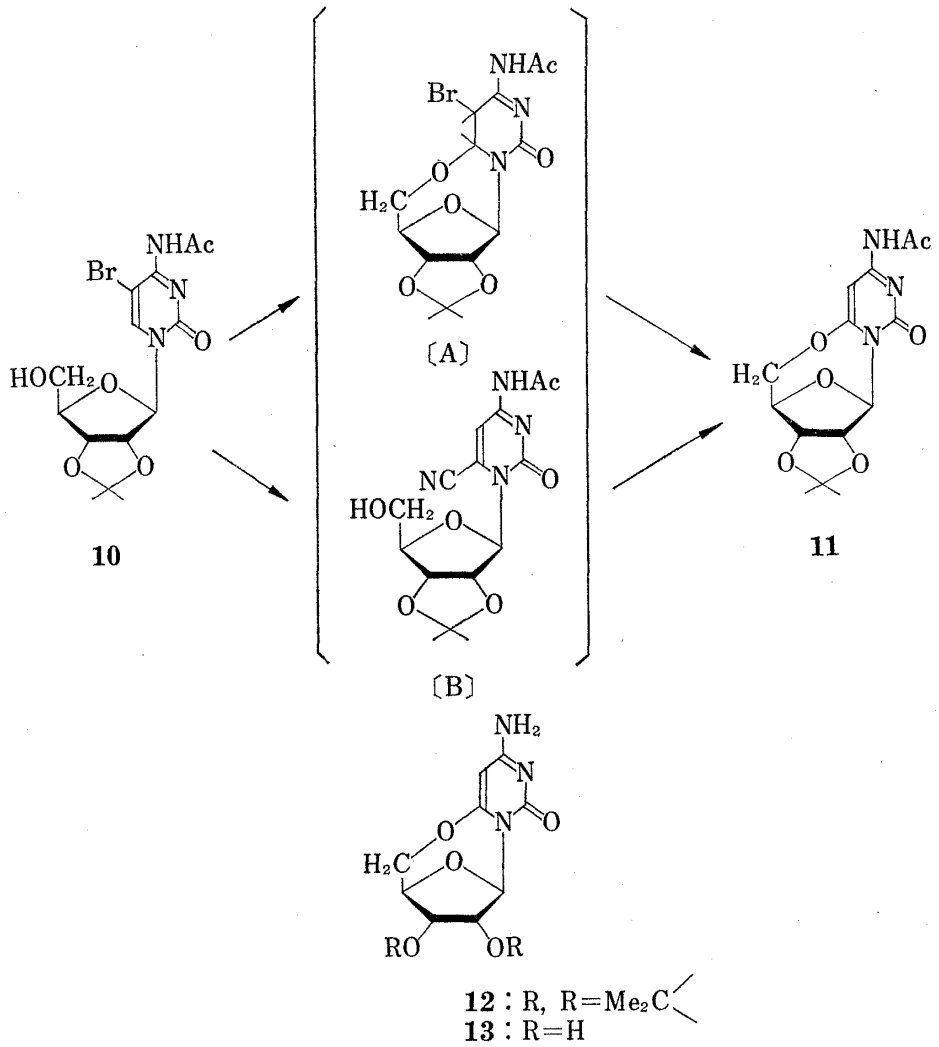

Chart 2

Free 6-cyanocytidine (**9**) can be obtained by the action of sodium cyanide with **1** in dimethylformamide. Treatment of **9** with methoxide in methanol afforded **6**, as expected. The 2′,3′-*O*-isopropylidene derivative of **1** also gave the 6-cyano derivative. On the other hand treatment of 2′,3′-*O*-isopropylidene-N^4-acetyl-5-bromocytidine (**10**, prepared from **4**) with sodium cyanide resulted in a formation of 2′,3′-*O*-isopropylidene-N^4-acetyl-*O*^{6},5′-cyclocytidine (**11**). Since it is well known that the *O*^{6},5′-cyclo-uridine and -cytidine are prepared by the action of the respective 5-halogenopyrimidine nucleosides with the strong base[11] through the 5-halo-*O*^{6},5′-5,6-dihydro intermediate, the formation of **11** from **10** would proceed *via* the similar intermediate (A), or by the different route in which the 6-cyano derivative (B) acts as an intermediate. In order to clarify this point the reaction was carried out under lower temperature (−10°), and the time course of the reaction was checked by the thin layer chromatography (Fig. 1). A rapid consumption of **10** was coincided with the formation of **11** and a 6-cyanocytidine (B), the latter reached the maximum after 10 minutes and then decreased with the increase of **11**, It is clear that the intermediate in the conversion of **10** to **11** was the 6-cyanocytidine (B), although the intermediacy of (A) was not fully excluded. In fact, treatment of 2′,3′-*O*-isopropylidene-6-cyanocytidine with potassium *t*-butoxide gave 2′,3′-*O*-isopropylidene-*O*^{6},5′-cyclocytidine (**12**) in high yield. *O*^{6},5′-cyclocytidine (**13**) was

11) *a*) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **34**, 1390 (1969); *b*) D. Lipkin, C.C. Cori, and M. Sano, *Tetrahedron Lett.*, **1968**, 5593; G.E. Lovett and D. Lipkin, *J. Am. Chem. Soc.*, **95**, 2312 (1973).

prepared similarly *via* the 6-cyano intermediate, which should be an alternative route of the synthesis of **13**.[11b]

This finding suggests that the formation of O^6,5′-cyclouridine derivative by the treatment of 2′,3′-*O*-isopropylidene-5-bromouridine with sodium cyanide in dimethylformamide to give the 6 (and 5)-cyanouridines[6] should also have been explained by the intermediacy of the 6-cyanouridine. These reactions also imply that the 6-cyanocytidine and 6-cyanouridine may serve as the substrates for the preparation of various 6-substituted pyrimidine nucleosides by the intermolecular nucleophilic substitutions. Experiments along these lines are currently undertaken and will be reported separately.

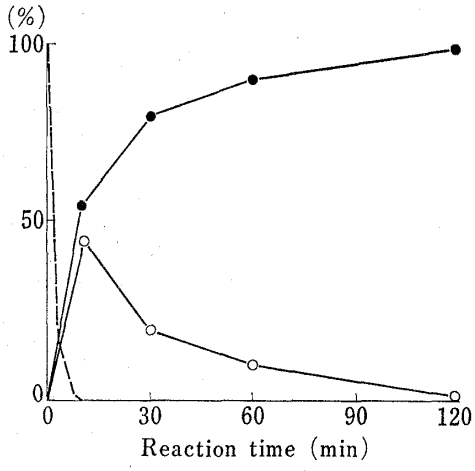

Fig. 1. Time Course of the Reaction of 2′,3′-*O*-Isopropylidene-N^4-acetyl-5-bromocytidine (**10**) with NaCN at −10°

The amount of the intermediate **B** was determined by UV measurements of the eluate from the plate assuming its identity with that of the 5′-*O*-acetate of **B** (see experimental).
——— **10**,
●—● **11**,
○—○ intermediate **B**.

Experimental

The NMR spectra were recorded on a Hitachi R-20B spectrometer using tetramethylsilane as an internal standard. UV spectra were measured on a Shimadzu D-40 and UV 300 spectrophotometer.

5-Bromocytidine (**1**)——Cytidine (20.03 g) was suspended in a mixture of pyridine (200 ml) and AcOH (200 ml) to which was added 4.4 ml of bromine and stirred overnight at room temperature. The solution was concentrated to leave yellow sirup. This was taken in aqueous EtOH and evaporated *in vacuo*. The evaporation was repeated several times and the final residue was dissolved in 200 ml of H_2O and treated with Amberlite IR 410 (OH^- form) resin until the solution became neutral. After the removal of the resin the filtrate was concentrated to dryness and the residue was taken in hot aqueous EtOH. On cooling the colorless needles of **1** (22.90 g, 86.5%) was obtained, mp 180—181.5°; *Anal.* Calcd. for $C_9H_{12}BrN_3O_5$: C, 33.57; H, 3.76; Br, 24.82; N, 13.05. Found: C, 33.43; H, 3.90; Br, 24.91; N, 12.82. UV $\lambda_{max}^{pH\ 7.0}$, 287 nm (ε, 7200), $\lambda_{max}^{1N\ HCl}$, 299 (ε, 11050). NMR (DMSO-d_6), δ: 8.43 (s, 1, H-6), 7.82 and 7.04 (bs, 1+1, 4-NH_2), 5.78 (d, 1, H-1′, $J_{1',2'}$= 3 Hz), 3.4—4.5 (m, 3, H-2′, 3′, 4′), 3.70 (bs, 2, H-5′), 4.7—5.6 (m, 3, HO-2′, 3′, 5′).

2′,3′,5′-Tri-*O*-acetyl-5-bromocytidine (**3**)——A suspension of **1** (19.07 g) in 300 ml of Ac_2O containing 5 drops of pyridine was heated at 70° for 100 min. After cooling the solvent was removed *in vacuo* and EtOH was added, and evaporated repeatedly to leave a mass (**2**). This was dissolved in 500 ml of MeOH and 2 drops of AcOH was added and heated under reflux for 8—10 hr until a single spot on TLC (silica gel, $CHCl_3$–EtOH, 15: 1) at *Rf* 0.33 was attained. On cooling, the colorless crystals separated which were collected and dried to give 24.21 g (92%) of **3**. Recrystallization from EtOH–H_2O gave needles of **3** as a hemi-hydrate, mp 158—159.5°. *Anal.* Calcd. for $C_{15}H_{18}BrN_3O_8 \cdot 1/2H_2O$: C, 39.40; H, 4.19; Br, 17.48; N, 9.19. Found: C, 39.29; H, 4.06; Br, 17.44; N, 9.11. NMR ($CDCl_3$), δ: 7.82 (s, 1, H-6), 8.83 and 5.90 (bs, 1+1, 4-NH_2), 6.03 (d, 1, H-1′, $J_{1',2'}$=3 Hz), 5.43—5.18 (m, 2, H-2′, 3′), 4.38 (bs, 3, H-4′, 5′), 2.21, 2.12, 2.08 (s, 9, AcO).

2′,3′,5′-Tri-*O*-acetyl-6-cyanocytidine (**5**)——Compound **2** (5 g) and 1.2 eq. of NaCN were dissolved in 20 ml of DMF and kept for 2 hr at room temperature. The reaction mixture was diluted with 100 ml of EtOAc and transferred to a separatory funnel, added 300 ml of EtOAc and 50 ml of H_2O, and the aqueous layer was separated. The washing with H_2O was repeated three times and the organic layer was dried over Na_2SO_4 and evaporated to leave a residue. This was crystallized from EtOH–H_2O to give 3.51 g (79.8%) of **5** as a hydrate, mp 70—71.5°, IR (Nujol), 2250 cm^{-1}. *Anal.* Calcd. for $C_{16}H_{18}N_4O_8 \cdot H_2O$: C, 46.60; H, 4.89; N, 13.59. Found: C, 46.22; H, 4.72; N, 13.38. NMR ($CDCl_3$), δ: 8.93 and 7.88 (bs, 1+1, 4-NH_2), 6.81 (s, 1, H-5), 5.81 (s, 1, H-1′), 5.65—5.4 (m, 2, H-2′, 3′), 4.7—4.0 (m, 3, H-4′, 5′), 2.11, 2.07 (s, 9, AcO).

Methyl Cytidine-6-carboximidate (**6**)——Compound **5** (3.51 g) was dissolved in 0.05 N NaOMe in MeOH (40 ml) and kept for 8 hr at room temperature. After neutralization of the solution with Dowex 50W-2(H^+) the filtrate was concentrated and the residue was taken in MeOH–acetone–H_2O (trace) from which colorless crystals of **6**, as a hemi-hydrate, separated, 2.15 g (80.5%), mp 155—157°; UV $\lambda_{max}^{H_2O}$, 272 nm (ε, 7900). *Anal.* Calcd. for $C_{11}H_{16}N_4O_6 \cdot 1/2H_2O$: C, 42.72; H, 5.55; N, 18.11. Found: C, 42.71; H, 5.36; N, 17.93. NMR (DMSO-d_6), δ: 9.10 (bs, 1, imino-H), 7.44 (bs, 2, 4-NH_2), 5.71 (s, 1, H-5), 5.00 (d, 1, H-1′, $J_{1',2'}$=4.5 Hz), 4.62 (m, 1, H-2′), 4.12 (m, 1, H-3′), 3.75 (m, 1, H-4′), 3.60 (m, 2, H-5′), 3.74 (s, 3, MeO), 5.0—4.35 (m, 3, HO-2′, 3′, 5′).

Cytidine 6-Carboxylic Acid (8)——Compound **6** (1.17 g) was dissolved in 0.2 N HCl (30 ml) and kept at 0° for 50 hr. This was made alkaline by adding 6.1 ml of 1 N KOH, kept for 1 hr at 0°, and neutralized and diluted to 1000 ml. The solution was applied to a column of DEAE–cellulose (3.6×37 cm, HCO_3^- form) and washed with H_2O. The elution was performed by a linear gradient of H_2O and 0.05 M triethylammonium bicarbonate. The fractions containing **8** were collected and evaporated to leave a residue. This was dissolved in 4 ml of 1 N HCl and excess of EtOH was added to effect slight turbidity, and kept in a refrigerator. The separated crystals were collected to give 842 mg (75.8%) of **8** as a hemi-hydrate, mp 260° (dec.); UV $\lambda_{max}^{pH\ 7}$, 274 nm (ε, 6940), $\lambda_{max}^{pH\ 2}$, 283 nm (ε, 10300). *Anal.* Calcd. for $C_{10}H_{13}N_3O_7 \cdot 1/2H_2O$: C, 40.55; H, 4.77; N, 14.19. Found: C, 40.62; H, 4.71, N; 14.22. NMR (DMSO-d_6), δ: 8.90 (bs, 1, 4-NH_2), 5.92 (s, 1, H-5), 5.62 (d, 1, H-1′, $J_{1',2'}$=3 Hz), 4.54 (q, 1, H-2′, $J_{2',3'}$=7.5 Hz), 4.11 (t, 1, H-3′, $J_{3',4'}$=7.5 Hz), 3.57 (m, 3, H-4′, 5′).

Acid Treatment of 6——Compound **6** (303 mg) was dissolved in 10 ml of 0.1 N HCl and kept for 6 hr at room temperature, and neutralized with Amberlite IR-410 (OH^- form). The filtrate was concentrated and MeOH was added to the residue to effect precipitation of **7** (170 mg), IR (Nujol); 1760 cm^{-1} (COOMe). This material was contaminated with methyl cytosine-6-carboxylate. A crude **7** (579 mg) was dissolved in 40 ml of H_2O and 2.5 ml of 1 N NaOH and kept for 2 hr at room temperature. After neutralization of the hydrolyzate the solution was applied to a column of DEAE-cellulose (2.8×36 cm, HCO_3^- form) and eluted with a linear gradient of H_2O (3000 ml) and 0.05 M triethylammonium bicarbonate (3000 ml) into two fractions. From the faster eluting fraction **8** was obtained in 52% yield. From the second fraction cytosine-6-carboxylic acid[12] was obtained, 85 mg, mp 294—295° (dec.); UV $\lambda_{max}^{pH\ 7}$, nm (ε): 285 (6500), $\lambda_{max}^{3N\ HCl}$, 298.5 (9000), $\lambda_{max}^{1N\ KOH}$, 298 (4900).

6-Cyanocytidine (9)——Compound **1** (10 g) was dissolved in 20 ml of DMF to which was added 2.0 g of NaCN, and stirred for 2.5 hr at room temperature. Water (20 ml) was added and the solution was quickly neutralized with 10 ml of 1 N HCl while N_2 was bubbling. The solution was concentrated under 40° to leave a residue which was crystallized from H_2O to give 6.1 g (73%) of **9**, mp 160° (dec.); UV $\lambda_{max}^{H_2O}$ 290 nm (ε, 8800), $\lambda_{max}^{1N\ HCl}$ 300 nm (ε, 9900); IR (Nujol), 2250 cm^{-1} (–CN). *Anal.* Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.57; H, 4.52; N, 20.95. NMR (DMSO-d_6), δ: 7.93 and 7.79 (bs, 1+1, 4-NH_2), 6.50 (s, 1, H-5), 5.77 (d, 1, H-1′, $J_{1',2'}$=5.5 Hz), 4.5 (m, 1, H-2′), 4.04 (m, 1, H-3′), 3.63 (m, 2, H-4′, 5′), 5.29, 5.05 and 4.77 (m, 3, HO-2′, 3′, 5′).

To a suspension of 1 g of **9** in 40 ml of MeOH 0.4 ml of 2 N NaOMe in MeOH was added, and stirred for 4 hr at room temperature. The resulting solution was neutralized with Dowex 50W×8(H^+). The resin was filtered, washed with MeOH, and the filtrate and washings were combined and evaporated to dryness. Crystallization of the residue from MeOH–acetone afforded 972 mg (84.6%) of **6**.

2′,3′-*O*-Isopropylidene-6-cyanocytidine——To a suspension of **1** (10 g) in 700 ml of acetone 10 ml of 70% $HClO_4$ was added and stirred for 4 hr at room temperature. After neutralization of the resulting solution with 43 g of K_2CO_3 the mixture was filtered, washed with acetone, and the combined filtrates were evaporated to leave a residue. This was taken in a small volume of MeOH, the insoluble material was removed, and the filtrate was evaporated to give a foam (11.93 g) of 2′,3′-*O*-isopropylidene-5-bromocytidine. A mixture of this compound (8 g) and NaCN (1.14 g) in 16 ml of DMF was kept for 1.5 hr at room temperature. A whole solution was applied to a column of silica gel (Wako C-200, 3.5×40 cm) and washed with 600 ml of $CHCl_3$ to elute DMF. The column was then eluted with EtOH–$CHCl_3$ (8 to 15% EtOH) and the eluates were evaporated to leave a sirup. This was crystallized from acetone–H_2O to give 5.14 g (75.5%) of 2′,3′-*O*-isopropylidene-6-cyanocytidine, mp 140—143°; *Anal.* Calcd. for $C_{13}H_{16}N_4O_5$: C, 50.64; H, 5.23; N, 18.18. Found: C, 50.63; H, 5.18; N, 18.08. IR (Nujol), 2250 cm^{-1} (–CN). NMR (DMSO-d_6), δ: 7.95 and 7.82 (bs, 1+1, 4-NH_2), 6.47 (s, 1, H-5), 5.84 (d, 1, H-1′, $J_{1',2'}$=1.5 Hz), 5.22 (dd, 1, H-2′, $J_{2',3'}$=6 Hz), 4.77 (dd, 1, H-3′, $J_{3',4'}$=4.5 Hz), 4.02 (m, 1, H-4′, $J_{4',5'}$=6.5 Hz), 3.57 (m, 2, H-5′), 1.50 and 1.30 (s, 3+3, Me_2C).

N^4-Acetyl-5-bromocytidine (4)——A suspension of **1** (10.02 g) in 200 ml of acetic anhydride was heated at 70° for 45 min under vigorous stirring. After standing at room temperature the separated white solids were collected, washed with acetone, and dried to give 9.13 g of **4**; UV $\lambda_{max}^{H_2O}$, 313, 221 nm. NMR (DMSO-d_6), δ: 9.58 (bs, 1, 4-NH), 8.82 (s, 1, H-6), 5.71 (d, 1,H-1′, $J_{1',2'}$=1.5 Hz), 3.98 (bs, 3, H-2′, 3′, 4′), 3.70 (bs, 2, H-5′), 2.28 (s, 3, Ac), 5.65—4.9 (3, HO-2′, 3′, 5′). On crystallization of **4** from EtOH–H_2O to the colorless needles resulted in a partial loss of the N^4-acetyl group. The crude **4**, therefore, was used for the further reactions.

2′,3′-*O*-Isopropylidene-N^4-acetyl-5-bromocytidine (10)——Compound **4** (9.0 g) was suspended in 500 ml of acetone and 9 ml of $HClO_4$ (70%) was added, and stirred for 4 hr at room temperature. After neutralization of the solution with 35 g of K_2CO_3 the filtrate and washings were combined and evaporated to leave a solid. This was taken in a small amount of EtOH, the insoluble material removed, evaporated, and the residue was crystallized from hot acetone to give 7.22 g of **10**, mp 175.5—177°; UV $\lambda_{max}^{H_2O}$ 313 nm (ε, 6500), 221 nm (ε, 18400). *Anal.* Calcd. for $C_{14}H_{18}BrN_3O_6$: C, 41.58; H, 4.46; Br, 19.77; N, 10.40. Found: C, 41.37; H, 4.42; Br, 19.58; N, 10.39.

2′,3′-*O*-Isopropylidene-N^4-acetyl-O^6,5′-cyclocytidine (11)——Compound **10** (517 mg) and 70 mg of NaCN were dissolved in 5 ml of DMF and kept for 1 hr at room temperature. To the solution was added 35 ml

12) G.D. Davis, F. Biocchi, R.K. Robins, and C.C. Cheng, *J. Org. Chem.*, **26**, 2755 (1961).

of EtOAc and the precipitate was removed and transferred to a separatory funnel. This was washed with 10 ml of H_2O and the organic layer was evaporated to leave the residue. The crystallization from acetone–MeOH afforded 200 mg of **11**, mp 226.5—227.5°. *Anal.* Calcd. for $C_{14}H_{17}N_3O_6$: C, 52.01; H, 5.30; N, 13.00; Found: C, 52.05; H, 5.33; N, 12.81. NMR (DMSO-d_6), δ: 6.83 (s, 1, H-1′), 6.48 (s, 1, H-5), 4.98 (d, 1, H-2′, $J_{2',3'}$=5.5 Hz), 4.89 (d, 1, H-3′), 4.63 (bs, 1, H-4′), 4.38 (dq, 2, H-5a′, b′, $J_{a,b}$=12 Hz, $J_{a,4'}$=2 Hz, $J_{b,4'}$=1.5 Hz), 1.44, 1.29 (s, 3+3, Me_2C).

2′,3′-*O*-Isopropylidene-*O*^{6},5′-cyclocytidine (12)——2′,3′-*O*-Isopropylidene-6-cyanocytidine (2.5 g) was dissolved in 20 ml of DMF and 100 ml of *t*-BuOH followed by the addition of 1 g of KO*t*-Bu. After stirring for 1.5 hr at room temperature the solution was neutralized by 1 N HCl and concentrated to effect precipitation. The precipitate (**12**, 2.13 g, 97%) was crystallized from MeOH–H_2O, mp>300°. *Anal.* Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38; N, 14.94. Found: C, 51.22; H, 5.36; N, 14.81. UV $\lambda_{max}^{H_2O}$ 270 nm, $\lambda_{max}^{1N\ HCl}$ 276 nm. NMR (DMSO-d_6), δ: 7.27 (bs, 2, 4-NH_2), 6.43 (s, 1, H-1′), 5.38 (s, 1, H-5), 4.92 (d, 1, H-2′, $J_{2',3'}$=5.5 Hz), 4.79 (d, 1, H-3′), 4.53 (s, 1, H-4′), 4.23 (m, 2, H-5a′, b′, $J_{a,b}$=12 Hz, $J_{a,4'}$=1.5 Hz), 1.42, 1.28 (s, 3+3, Me_2C).

***O*^{6},5′-Cyclocytidine (13)**——Compound **1** (3.32 g) was suspended in 34 ml of methyl orthoformate, added 3.02 g of *p*-TsOH·H_2O, and stirred for 1 hr at room temperature. After neutralization of the solution with 1 N NaOMe and removal of the resulting precipitates (*p*-TsONa) the filtrate was concentrated and the residue was applied to a column of silica gel (Mallinckrodt, 100 mesh, 40 g, 2.8×15 cm) with $CHCl_3$. After washing the column with 300 ml of $CHCl_3$ it was kept at room temperature for 3 days. Elution of the column with 5—10% EtOH–$CHCl_3$ afforded 3.17 g of 2′,3′-*O*-methoxymethylidene-5-bromocytidine as a foam. This was treated with 430 mg of NaCN in 5 ml of DMF at room temperature for 1.5 hr under stirring followed by the addition of 970 mg of KO*t*-Bu in 50 ml of *t*-BuOH. The solution was neutralized after 6 hr with 1 N HCl and concentrated to give a crystalline mass (2′,3′-*O*-methoxymethylidene-*O*^{6},5′-cyclocytidine, 1.85 g). One half of the product was dissolved in 200 ml of 20% AcOH and kept for 2 days at room temperature. The mixture was concentrated and the residue was crystallized from H_2O to give prisms of **13** (651 mg, 81.5%), mp>300°; UV $\lambda_{max}^{H_2O}$ 270 nm (ε, 11150), $\lambda_{max}^{1N\ HCl}$ 276 nm (ε, 17200). *Anal.* Calcd. for $C_9H_{11}N_3O_5$: C, 44.81; H, 4.61; N, 17.42. Found: C, 44.94; H, 4.58; N, 17.52. NMR (DMSO-d_6), δ: 7.21 (bs, 2, 4-NH_2), 6.34 (s, 1, H-1′), 6.50 (s, 1, H-5), 4.38—4.1 (m, 3, H-2′, 3′, 4′), 4.18 (q, 2, H-5a′, b′, $J_{a,b}$=13 Hz, $J_{a,4'}$=1.5 Hz).

5′-*O*-Acetyl-2′,3′-*O*-isopropylidene-*N*^{4}-acetyl-6-cyanocytidine——2′,3′-*O*-Isopropylidene-6-cyanocytidine (1.16 g) was taken in 10 ml of pyridine and 6 ml of acetic anhydride was added and kept overnight at room temperature. The mixture was evaporated and the residue was crystallized from hot MeOH to give 1.06 g (72%) of the title compound, mp 169—172°; UV λ_{max}^{EtOH} 276 nm, 310 (sh) nm. IR (Nujol), 2240 cm^{-1}. *Anal.* Calcd. for $C_{17}H_{20}N_4O_7$: C, 52.04; H, 5.14; N, 14.28. Found: C, 52.11; H, 5.04; N, 14.03. NMR ($CDCl_3$), δ: 10.90 (bs, 1, 4-NH), 7.98 (s, 1, H-5), 6.10 (d, 1, H-1′, $J_{1',2'}$=1 Hz), 5.22 (bd, 1, H-2′, $J_{2',3'}$=7 Hz), 4.92 (bd, 1, H-3′), 4.32 (bs, 3, H-4′, 5′), 2.43 (s, 3, NAc), 2.06 (s, 3, OAc), 1.56, 1.35 (s, 3+3, Me_2C).

Acknowledgement This work was supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.