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On the Benzylation of Nucleosides. II.[1] A Novel Synthesis of 2'-O-Benzyluridine

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2'-O-Benzyl-4-methylthiouridine (III) and 2'-O-benzylcytidine (VI) were obtained by respective treatment of 4-methylthiouridine (II) and cytidine (V) with benzyl bromide in the presence of sodium hydride. By this reaction, highly specific benzylation of 2'-hydroxyl group of the ribonucleosides was achieved. The both compounds (III) and (VI) could easily be converted to 2'-O-benzyluridine (IV) which is an important intermediate in the synthesis of oligonucleotide.

The present authors reported that uridine was specifically benzylated at N_3-position of the uracil- and 2'-hydroxyl group of the sugar-moiety to give N_3-benzyluridine and N_3,2'-O-dibenzyluridine[1] by direct treatment of it with benzyl bromide in the presence of sodium hydride. The benzyl residue introduced into the 2'-hydroxyl group was easily removed by hydrogenation with palladium on charcoal but that attacked at N_3-group was highly resistant to the reduction. Therefore, these benzylated products were of little use as intermediates in the synthesis of oligonucleotides.

In order to prevent the benzylation at N_3-position of pyrimidine nucleoside in this reaction, we applied the reaction to (1-β-D-ribofuranosyl)-4-methylthio-2-pyrimidinone, 4-methylthiouridine, (II) and cytidine (V) and found that 4-methylthiouridine gave a product, 2'-O-benzyl-4-methylthiouridine (III), and cytidine gave three products, 2'-O-benzylcytidine (VI), N_3-benzylcytidine (VII) and a dibenzylcytidine (VIII).

2'-O-Benzyl-4-methylthiouridine and 2'-O-benzylcytidine were easily conveted to 2'-O-benzyluridine (IV) by acid hydrolysis and deamination with nitric acid, respectively.

4-Thiouridine (I)[2] was treated with methyl iodide in alkali according to the procedure of Ikehara, *et al.*,[3] who methylated 2',3'-O-isopropylidene-4-thiouridine, to give 4-methylthiouridine (II) and the product was purified by passing through a cellulose column. The pure II which was obtained in a yield of 67.3% coincided in every respect with 4-methylthiouridine reported by Scheit,[4] who obtained the compound by methylation of I with diazomethane.

Since 4-methylthiouridine (II) has no proton at N_3-position, which could be substituted with benzyl group, this compound should be a desirable starting material for the selective benzylation at 2'-hydroxyl group by the method previously reported.[1] The compound (II) was dissolved in a mixture of sodium hydride and dimethylformamide and after the completion of evolution of hydrogen, benzyl bromide was added to the solution. The reaction was allowed to proceed four or five hours at room temperature under stirring. The chloroform-soluble products were taken and separated into a main product (III) and a side product (III') by column chromatography using silica gel. If this reaction was performed at above the room temperature, several additional side products were produced and detected in thin-layer chromatography. The main product (III), which was obtained in a yield of 16.4%, revealed elemental analysis well coincided with that for a monobenzyl-4-methylthiouridine. The ultra-

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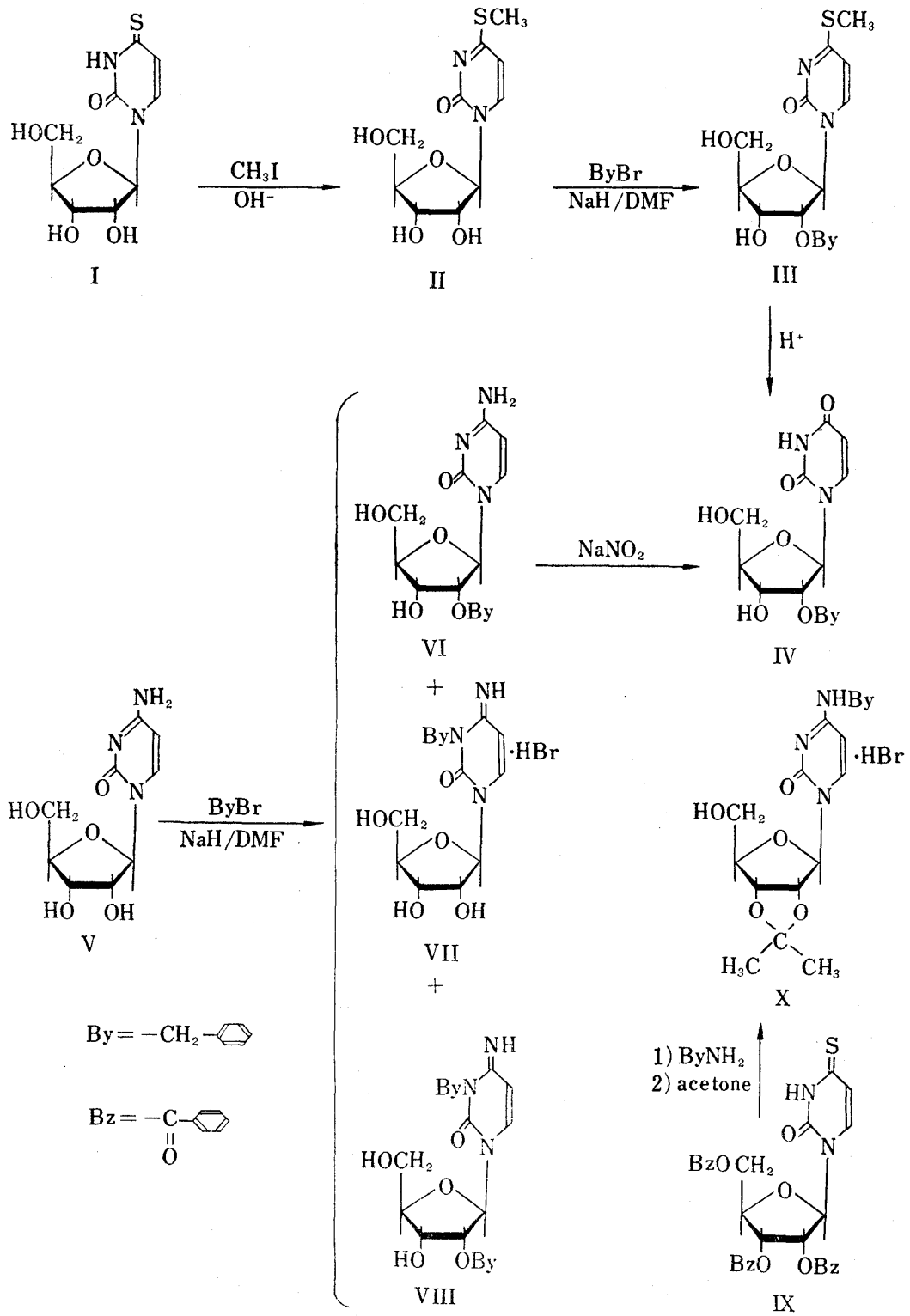

violet absorption spectrum as well as periodate test of III indicated that the benzyl group should be introduced into either 2′- or 3′-hydroxyl group of the sugar moiety. From the behaviors of III′ in thin-layer chromatography and ultraviolet absorption spectra, this side product was assumed to be a 4-methylthiouridine derivative which had two benzyl groups substituted on the sugar moiety.

The compound (III) was treated with aqueous hydrogen chloride to give a product (IV) which did not contain sulfur. This product (IV) was identified by admixture with an au-

thentic specimen of 2′–O–benzyluridine which was generously supplied by Dr. Reese.[5] The infrared absorption spectra and behaviors in paper chromatography of IV were also in good accordance with those of authentic 2′–O–benzyluridine. Thus the product (IV) was determined to be 2′–O–benzyluridine. As the result, starting from 4–thiouridine, we could selectively introduce a benzyl residue into 2′–hydroxyl group of the sugar moiety of uridine.

Cytidine was also benzylated under the similar condition, as was described in the case of 4–methylthiouridine. Paper chromatography of the reaction mixture revealed spots of three products (VI, VII and VIII), the yields of which depended on the reaction conditions. The spots of the VI and VII were found on the chromatogram when 1.5 equimolar sodium hydride and 1.7 equimolar benzyl bromide were used. The third product (VIII) was produced only when 2.5 equimolar sodium hydride and 3 equimolar benzyl bromide were used. The yields of VI and VIII were dependent on the reaction temperature. Thus the product (VI) was mainly obtained when the reaction was performed at room temperature while the compound (VII) was the main product when the reaction was performed at 60°. The yields estimated after the isolation of these products (VI, VII and VIII) obtained by optimal reaction conditions were 12.8, 10.8 and 5.8%, respectively. These yields could be increased by possible improvement of the isolation procedures, because, as is indicated in the "Experimental," the mixture contained considerable amount of the products.

The elemental analysis of the compound (VI) was in good accord with those of a monobenzylcytidine and the behavior of this compound in electrophoresis in borate buffer and its negative reaction to the periodate reagent revealed that the benzyl group of this compound was attached to 2′–O– or 3′–O–position. When the compound (VI) was deaminated with sodium nitrite, it was converted to a product identical with 2′–O–benzyluridine (IV) described above. These results clearly demonstrated that the compound (VI) was 2′–O–benzylcytidine.

The elemental analysis, positive reaction against periodate oxidation and behavior on paper electrophoresis in borate buffer of the compound (VII) showed that VII was a monobenzylcytidine hydrobromide whose benzyl group was substituted on neither 2′– nor 3′–hydroxyl group of the sugar moiety. Furthermore, the compound revealed positive Beilstein reaction and a pK_a value of 8.10 which is characteristic to the N_3–alkylated iminopyrimidines[1,6–9] and is much higher than pK_a value of around 4.0 characteristic to an N_4–alkylated aminopyrimidines.[6,8] From these observations the compound (VII) seemed to be N_3–benzylcytidine hydrobromide.

In order to compare the pK_a value of VII, with that of a N_4–benzylated cytidine derivative, the synthesis of 2′,3′–O–isopropylidene–N_4–benzylcytidine hydrobromide (X) was attempted. 2′,3′,5′–Tri–O–benzoyl–4–thiouridine (IX)[2] was treated with benzylamine according to Fox's method[2] and the resulting N_4–benzylcytidine was converted to a syrupy hydrogen bromide. On paper chromatographic detection, this hydrogen bromide revealed different *Rf* values to that of VII. The syrup was dissolved in acetone and evaporated to furnish a crystalline 2′,3′–O–isopropylidene–N_4–benzylcytidine hydrobromide (X), whose pK_a value was 3.50. Thus, this result further supported the assumption that the compound (VII) is N_3–benzylcytidine hydrobromide.

The compound (VIII) gave elemental analysis data corresponding to those of a dibenzyl cytidine, the ultraviolet absorption spectra and the pK_a value of VIII were quite similar to those of N_3–benzylcytidine hydrobromide (VII) and this compound did not consume periodate. Thus, the product (VIII) was most probably represented by N_3, 2′–O–dibenzylcytidine.

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As the results of this investigation, 2′-O-benzyluridine (IV) and 2′-O-benzylcytidine (VI) were prepared by reaction of benzyl bromide with 4-methylthiouridine (III) and cytidine (V) in the presence of sodium hydride, respectively. It is noticeable that 2′-hydroxyl group of pyrimidine nucleosides are very susceptible to benzylation in the presence of sodium hydride, and this properties revealed a procedure for specific benzylation of the 2′-hydroxyl group of ribonucleosides. In these benzylation, DMF was used as the reaction solvent, because of the advantage to isolate the products, though DMSO has also been found available and some times to stimulate this type of benzylation reaction.

When these 2′-O-benzylated nucleosides were used in the synthesis of oligonucleotides, it is necessary to remove the benzyl group at the final stage of the synthesis. Therefore the hydrogenolysis of these 2′-O-benzylpyrimidine nucleosides was studied. On hydrogenolysis of 2′-O-benzylcytidine with palladium or Raney Ni catalyst, the reduction of the cytosine nucleus more rapidly occurred than the debenzylation, while the benzyl group of 2′-O-benzyluridine could easily be removed to give uridine. These results indicated that of these two 2′-O-benzylpyrimidine nucleosides 2′-O-benzyluridine is more valuable as a material in the synthesis of oligonucleotides and it could easily be obtained from 2′-O-monobenzyl-4-methylthiouridine or 2′-O-monobenzylcytidine.

Experimental

Methods——Paper chromatography was performed on Toyo Roshi No. 53 paper using solvent systems, (1) BuOH–H_2O (84:16), (2) *n*-PrOH–conc. NH_4OH–H_2O (55:10:35), (3) *iso*-PrOH–conc. NH_4OH–H_2O (7:1:2), (4) BuOH–AcOH–H_2O (4:1:2), (5) BuOH–AcOH–H_2O (5:1:4), (6) *iso*-PrOH–H_2O (7:3). Thin-layer chromatography was carried out on Silica Rider (Daiichi Pure Chemicals Co., Ltd.) using solvent systems, (7) AcOEt, (8) AcOEt–$CHCl_3$ (3:1), (9) AcOEt–EtOH (3:1). The *Rf* value of the spot obtained for individual solvent was represented by the symbol (*Rf*) with suffix corresponding to the number of the solvents.

Paper electrophoresis was performed on Toyo Roshi No. 53 paper using the following conditions, (A) in 0.02 M borate at pH 9.3 and 600 V/20 cm, (B) in 0.05 M glycine–NaOH at pH 10.0 and 300 V/20 cm, (C) in BuOH–pyridine–AcOH–H_2O (20:10:2:960) at pH 5.7 and 300 V/20 cm.

Cellulose powder (200—300 mesh) (Toyo Roshi Kaisha, Ltd.) and Kiesel gel (0.05—0.20 mm) (E. Merck Ag. Darmstadt) were used for column chromatographies.

Unless otherwise mentioned the melting points were not corrected.

4-Methylthiouridine (II)——To a solution of 900 mg of 4-thiouridine (I)[2] in 10 ml of 0.4 N NaOH was slowly added 0.293 ml of CH_3I during 10 min under stirring. To the mixture were successively and slowly added 1.6 ml of 0.4 N NaOH and 0.293 ml of CH_3I under stirring. The stirring was continued for additional 3 hr at room temperature and the reaction mixture was evaporated to dryness *in vacuo*. The residual gum was purified through a cellulose column (3×43 cm) using solvent system of BuOH–H_2O (84:16). From the fractions which contained the compound (II), the solvent was removed *in vacuo*, and the residue was recrystallized from EtOH to give white granules, mp 157—157.5° (decomp.), yield 67.3%.[10] UV $\lambda_{max}^{H_2O}$ mμ (ε): 302 (14400), shoulder 280 (9200); $\lambda_{min}^{H_2O}$ 240 (1810); $\lambda_{max}^{1N\,HCl}$ 332 (21800), 270, $\lambda_{min}^{1N\,HCl}$ 286, 240. Rf_1 0.55, Rf_7 0.09. $[\alpha]_D^{30}$ +103° (c=0.4 in H_2O). This compound consumed periodate. *Anal.* Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.79; H, 5.14; N, 10.21. Found: C, 43.97; H, 5.45; N, 10.36.

2′-O-Benzyl-4-methylthiouridine (III) and an Unidentified Product (III′)——In 12 ml of dimethylformamide (DMF) previously dried over molecular shieves 4A was dissolved 95 mg (2.3 mmoles) of NaH (purity, 57—58%) which was washed several times with dry petroleum ether. To the mixture cooled to 0° was added 548 mg (2 mmoles) of 4-methylthiouridine (II) dissolved in 8 ml of DMF, and the solution was stirred at room temperature for 1.5 hr. After completion of the evolution of hydrogen, 230 mg of benzyl bromide was added to the solution and the mixture was stirred at 0° for 15 min. After the mixture was allowed to stand at room temperature for additional 15 min, under ice-cooling, 200 mg of benzyl bromide was added (total amounts of benzyl bromide added was 2.5 mmoles), and the mixture was kept at room temperature for 4—5 hr under stirring until the solution became almost clear. The reaction hitherto described was performed under entire anhydrous conditions. The mixture was poured into 60 ml of ice-water and extracted five times with 20 ml of $CHCl_3$. The starting material (II) unreacted, *ca.* 30—40%, was

10) This compound (II) was obtained by Scheit[4] treating I with diazomethane. mp 158—159°. UV $\lambda_{max}^{pH\,7}$ mμ (ε): 303 (14100), shoulder 282 (9350), $\lambda_{min}^{pH\,7}$ 240.

remained in the aqeuous layer, which was determined by paper chromatography using solvent (1). Detection by thin-layer chromatography of the chloroform layer showed spots of a major and a minor products having *Rf* values of 0.4 and 0.7, respectively (solvent (5)). The chloroform layer was put on a silica gel column (1.5×20 cm) and the products were separated using solvent system of AcOEt–$CHCl_3$ (3:1). The major product (III) was crystallized from acetone to give 119 mg (yield, 16.4%) of rods which were recrystallized from the same solvent to colorless rods, mp 195—195.5°. UV λ_{max}^{EtOH} mμ (ε): 302 (11600), shoulder 280 (9100), λ_{min}^{EtOH} 239 (1720). Rf_1 0.88, Rf_7 0.40, Rf_8 0.25. This compound did not consume HIO_4. *Anal.* Calcd. for $C_{17}H_{20}O_5N_2S$: C, 56.03; H, 5.53; N, 7.69. Found: C, 55.99; H, 5.28; N, 8.03.

Although the minor product (III′) was not identified, from the *Rf* value on thin-layer chromatogram and ultraviolet absorption spectra this compound was assumed to be 4-methylthiouridine substituted with two benzyl groups on the sugar moiety.

2′-O-Benzylcytidine (VI) and 2′-O-Benzylcytidine Hydrochloride (VI′)——To 288 mg (6 mmoles) of NaH previously washed with dry petroleum ether, was added 972 mg (4 mmoles) of dry cytidine (V) dissolved in 40 ml of DMF. The mixture was kept at room temperature for 3 hr under stirring in an anhydrous condition. The dark grey solution turned grey with simultaneous evolution of hydrogen. To this solution was added 1.163 g (6.8 mmoles) of benzyl bromide and the mixture was stirred at room temperature for 2 hr. The paper chromatographical analysis of the light yellow reaction mixture indicated that it contained cytidine (V) (Rf_1 0.12), N_3-benzylcytidine (VII) (Rf_1 0.32), 2′-O-benzylcytidine (VI) (Rf_1 0.57) and dibenzylcytidine (VIII) (Rf_1 0.78) in relative amount of 39.2, 2.2, 46.8 and 5.1%. The reaction mixture was added with *ca.* two fold of water and extracted with $CHCl_3$. The aqueous layer was neutralized with 2 N HCl and evaporated *in vacuo* to obtain a residual gum. The residue was purified by cellulose column (4×58 cm) chromatography using a solvent system of *iso*-PrOH–H_2O (7:3). The fractions which contained the product having Rf_1 0.57 were combined and evaporated to dryness and the residue was crystallized from iso-PrOH–AcOEt–H_2O to give 180 mg of needles, mp 106—107.8° (yield, 12.8%). Recrystallization from EtOH gave colorless needles (VI), mp 183.4° (corr.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 270 (7200); $\lambda_{min}^{H_2O}$ 251 (5700); $\lambda_{max}^{0.1\,N\,HCl}$ 280 (10300); $\lambda_{min}^{0.1\,N\,HCl}$ 241 (1900). Rf_1 0.57, Rf_3 0.86, Rf_5 0.77, Rf_6 0.78. $[\alpha]_D^{28}$ +66° (c=0.4 in H_2O).

On paper electrophoresis this product moved 3.2 cm towards anode in borate buffer, whereas cytidine moved 5.4 cm to the similar direction. This compound did not consume HIO_4. *Anal.* Calcd. for $C_{16}H_{19}O_5N_3 \cdot H_2O$: C, 54.84; H, 6.05; N, 11.51. Found: C, 54.74; H, 5.96; N, 11.81.

In the case of an experimental run, in which a little excess of HCl was used for the neutralization described above, 2′-O-benzylcytidine hydrochloride (VI′) was isolated after the similar treatment for the purification. mp 207.1—208.1° (decomp.) (corr.). UV $\lambda_{max}^{H^+}$ mμ: 281; $\lambda_{max}^{H_2O}$ 275, $\lambda_{max}^{OH^-}$ 270. Rf_1 0.57, Rf_3 0.84, Rf_5 0.76. VI′ did not consume HIO_4 and gave positive to Beilstein test. *Anal.* Calcd. for $C_{16}H_{19}O_5N_3 \cdot HCl \cdot 1/2\,H_2O$: C, 50.72; H, 5.60; N, 11.09. Found: C, 50.93; H, 5.25; N, 10.81.

N_3-Benzyl Cytidine Hydrobromide (VII)——The mixture containing 1.152 g (24 mmoles) of NaH, 3.888 g (16 mmoles) of cytidine and 160 ml of DMF was stirrred at room temperature for 3 hr. To the solution 4.651 g (27 mmoles) of benzyl bromide was added and the mixture was kept at 60° for 2 hr under stirring. The warming at 60° in this reaction step favoured to give larger amount of VII. The solvent was removed *in vacuo* below 50—60°, and the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer which contained 7% of initial total absorbancy at 280 mμ was analyzed by paper chromatography. It contained cytidine (V) (Rf_1 0.12), N_3-benzylcytidine (VII) (Rf_1 0.32), and an unknown product (Rf_1 0.65) in the relative amount of 40.8, 16.5 and 12.2%, respectively, calculated from absorbancy at 280 mμ of cytidine used. The aqueous layer was neutralized with 2 N HCl and evaporated to dryness. The residue was applied on to a cellulose column (4×58 cm) and eluted with a solvent, iso-PrOH–H_2O (7:3). The fractions which contained the product having Rf_1 of 0.32 were combined and the solvent was removed *in vacuo* to dryness to give a gummy residue. Crystallization of the residue from iso-PrOH furnished 718 mg of crystals (VII) (yield, 10.8%) which were recrystallized from iso-PrOH to give fine needles, mp 192.7—195.1° (decomp.) (corr). UV $\lambda_{max}^{0.1\,N\,HCl}$ mμ (ε) :281.5 (12100); $\lambda_{min}^{0.1\,N\,HCl}$ 241.5 (2000); $\lambda_{max}^{H_2O}$ 281.5 (12100); $\lambda_{min}^{H_2O}$ 242 (2200); $\lambda_{max}^{0.1\,N\,NaOH}$ 268 (8400); $\lambda_{min}^{0.1\,N\,NaOH}$ 244 (4600). Rf_1 0.32, Rf_3 0.89, Rf_5 0.65, Rf_6 0.63. $[\alpha]_D^{28}$ +37.0° (c=0.4 in H_2O). This product showed positive Beilstein test and consumed periodate reagent. The pK_a value determined spectrophotometrically was 8.10. Paper electrophoretic mobilities were 6.0 cm (at pH 5.7) and 2.5 cm (at pH 10.0) to the cathode, whereas cytidine moved 2.2 cm (at pH 5.7) and 1.8 cm (at pH 10.0). *Anal.* Calcd. for $C_{16}H_{19}O_5N_3 \cdot HBr$:C, 46.39; H, 4.87; N, 10.15. Found: C, 46.45; H, 4.90; N, 9.90.

On removal of HBr, this product gave a single spot of N_3-benzyl cytidine with Rf_1 0.29, Rf_3 0.85, and Rf_4 0.57.

Dibenzylcytidine (VIII)——To 2.5 g (55 mmoles) of NaH treated as above, was added a mixture of 4.86 g (20 mmoles) of cytidine (V) and 150 ml of DMF. The mixture was stirred at room temperature for 2—3 hr and then treated with 10.23 g (60 mmoles) of benzyl bromide for 30 min below 40° under stirring. The reaction mixture was analyzed by paper chromatography. The amount of the products found in the mixture were as follows, cytidine (V), Rf_1 0.12 (1.28%), 2′-O-monobenzylcytidine (VI), Rf_1 0.57 (43.3%) and dibenzyl cytidine (VIII), Rf_1 0.78 (48.0%). The mixture was poured into H_2O and extracted with ether and subsequently with $CHCl_3$. The ether and chloroform layers were combined, solvents evaporated and the residual gum was dissoved in AcOEt–$CHCl_3$ (1:1) and kept at room temperature overnight. Colorless needles appeared

weighed 205 mg (yield, 5.8%), mp 202.9—204° (corr.). UV $\lambda_{max}^{0.1\,N\,HCl\,(25\%\,EtOH)}$ mμ (ε): 282 (10900); $\lambda_{min}^{0.1\,N\,HCl\,(25\%\,EtOH)}$ 244.5 (2400); $\lambda_{max}^{0.1\,NNaOH\,(25\%\,EtOH)}$ 268 (7380); $\lambda_{min}^{0.1\,N\,NaOH\,(25\%\,EtOH)}$ 244 (4300). Rf_1 0.78. $[\alpha]_D^{28}$ +36.6° (c=0.3 in $CHCl_3$).

The pK_a value determined spectrophotometrically was 8.1. This compound did not consume HIO_4. *Anal.* Calcd. for $C_{23}H_{25}O_5N_3$: C, 65.23; H, 5.95; N, 9.92. Found: C, 65.16; H, 6.14; N, 9.78.

2′-O-Benzyluridine (IV)——A) From 2′-O-Benzyl-4-methylthiouridine (III): 2′-O-Benzyl-4-methylthiouridine (III) (119 mg) was dissolved in 10 ml of EtOH, and to the solution 10 ml of 0.2 N HCl was added. The mixture was kept at 50° for 4 hr. Chloride ion was removed from the mixture with Dowex 1 (HCO_3^-). After removal of the resin from the solution, solvent was evaporated *in vacuo*. Recrystallization of the residue from EtOH gave 44 mg (yield, 44%) of colorless needles, mp 180—181° (corr.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 262 (8400); $\lambda_{min}^{H_2O}$ 233 (2190). Rf_1 0.64, Rf_7 0.27. This compound showed negative reaction with periodate reagent. *Anal.* Calcd. for $C_{16}H_{18}O_6N_2$: C, 57.48; H, 5.40; N, 8.38. Found: C, 57.51; H, 5.42; N, 8.59.

B) From 2′-O-monobenzylcytidine (VI): 2′-O-Benzylcytidine (VI) (240 mg) and $NaNO_2$ (1.64 g) were dissolved in 6 ml of H_2O and to the solution 2.4 ml of AcOH was added. The reaction mixture was kept at room temperature for 6 hr, and then in a cold room overnight. The solvent was removed from the reaction mixture *in vacuo* to obtain a residue. Crystallization of the residue from H_2O afforded 150 mg of colorless needles (yield, 65.8%). Recrystallization from H_2O gave 98 mg of pure material, mp 181.7—182° (corr.). UV $\lambda_{max}^{H_2O}$ mμ (ε): 261.5 (8080); $\lambda_{min}^{H_2O}$ 232 (2300), $\lambda_{max}^{H^+}$ 261; $\lambda_{max}^{OH^-}$ 260. Rf_1 0.64. $[\alpha]_D^{29}$ −35.0° (c=0.25 in H_2O).

This product did not consume HIO_4. *Anal.* Calcd. for $C_{16}H_{18}O_6N_2$: C, 57.48; H, 5.40; N, 8.38. Found: C, 57.70; H, 5.47; N, 8.51.

The both products obtained by method A) and method B) did not show depression in mixed fusion with authentic 2′-O-benzyluridine which was kindly given by Dr. Reese[5] and melted at 181—182°. Their infrared absorption spectra and behaviors on paper chromatogram were also coincided with those of the authentic 2′-O-benzyluridine.

2′,3′-O-Isopropylidene-N_4-benzylcytidine Hydrobromide (X)——A sealed tube which contained 2.22 g of tri-O-benzoyl-4-thiouridine,[2] 28 ml of absolute EtOH and 12 ml of benzylamine was heated at 100° for 24 hr. The tube was cooled, the solvent was removed *in vacuo* and the residue was shaken with H_2O and $CHCl_3$. The aqueous layer was evaporated *in vacuo*, the residue obtained was dissolved in 10 ml of EtOH and the solution was made to pH 1.0 by adding hydrobromic acid. On evaporation of the solvent, the product was obtained as a glass. This glass gave a single spot with Rf_1, 0.50, which consumed periodate reagent. Thus this product must be N_4-benzylcytidine hydrobromide. On removal of HBr, this product gave a single spot having Rf_1 0.64, Rf_2 0.92, Rf_3 0.82 and Rf_4 0.71. When the glass was dissolved in acetone and the solution was evaporated to dryness, a crystalline residue was obtained. Recrystallization of the residue from EtOH yielded pure sample, mp 198—200° (decomp.). UV $\lambda_{max}^{pH\,7}$ mμ (ε): 270 (13900), 239 (10800); $\lambda_{min}^{pH\,7}$ 247 (10500), 228 (9800). Rf_1 0.90, Rf_9 0.50. $[\alpha]_D^{30}$ −29.2° (c=0.3 in EtOH). This compound did not consume HIO_4.

The pK_a value determined spectrophotometrically was 3.50. *Anal.* Calcd. for $C_{19}H_{23}O_5N_3 \cdot HBr$: C, 50.23; H, 5.32; N, 9.25. Found: C, 50.16; H, 5.55; N, 9.47.

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