Synthetic Nucleosides and Nucleotides. XXXV.[1] Synthesis and Biological Evaluations of 5-Fluoropyrimidine Nucleosides and Nucleotides of 3-Deoxy-β-D-ribofuranose and Related Compounds

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1-*O*-Acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (1), derived from the antibiotic cordycepin was coupled with trimethylsilylated derivatives (2a—c) of N^4-propionylcytosine, N^4-*p*-toluoyl-5-fluorocytosine and 5-fluorouracil in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) to give fully acylated nucleosides (3a—b and 3d, respectively). Selective removal of the N^4-propionyl group of 3a by treatment with hydrazine hydrate gave 2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (4). Deamination of 4 with sodium nitrite in trifluoroacetic acid afforded 2′,5′-di-*O*-*p*-chlorobenzoyluridine (3c) in good yield. Compounds 3a—d were saponified to give free 3′-deoxycytidine (5a), 5-fluoro-3′-deoxycytidine (5b), 3′-deoxyuridine (5c), and 5-fluoro-3′-deoxyuridine (5d), respectively. These 3′-deoxyribonucleosides (5a—d) were then converted to corresponding 5′-monophosphate and further phosphorylated to the 5′-triphosphates by the phosphoroimidazolidate method.

The nucleosides (5a—d) were examined for growth-inhibitory effects on mouse leukemic L5178Y cells, and their IC_{50} values (μg/ml) were 1.8, 33, 6.5, and 18, respectively. On the other hand, the antiviral activities of these compounds on a rhabdovirus, infectious hematopoietic necrosis virus (IHNV), were moderate (IC_{50} = 100—500 μg/ml in CHSE-214 cells). The 5′-triphosphates showed remarkable inhibitory effects on DNA polymerase β and DNA polymerase α-primase purified from testes of the cherry salmon, *Oncorhynchus masou*, but not on common DNA polymerase α from same source.

Key words 5-fluoropyrimidine 3′-deoxyriboside; L5178Y cell; DNA primase; DNA polymerase α; DNA polymerase β; IHNV

3′-Amino-3′-deoxyadenosine and some 3′-amino-3′-deoxyribonucleosides related to the antibiotic puromycin possess various biological activities.[2] 5-Fluoropyrimidine nucleosides bearing 3-amino-3-deoxyribofuranose were synthesized in our laboratory and found to show strong growth-inhibitory effects against mouse leukemic L5178Y cells in culture.[3] We have also reported the synthesis and antitumor activity of various 5-fluoropyrimidine nucleosides having 5-fluorouracil and 5-fluorocytosine as the base moiety.[4] Various 3′-deoxyribofuranosyl nucleotides synthesized starting from the antibiotic cordycepin[5,6] shows remarkable inhibitory effects on eukaryotic DNA-dependent RNA polymerases I and II.[7,8]

As part of our program on the design and synthesis of nucleoside analogues having biological activities, including antitumor and antiviral activities as well as activity at the 5′-triphosphate level on DNA polymerases and RNA polymerases, we examined the synthesis of 5-fluoropyrimidine nucleosides and nucleotides bearing the 3-deoxy-β-D-ribofuranose moiety. We also describe here the growth-inhibitory effects of newly synthesized nucleosides on mouse leukemic L5178Y cells and the CPE spot reduction activity against a rhabdovirus, infectious hematopoietic necrosis virus (IHNV) in chinook salmon embryo cells (CHSE-214 cells) as well as inhibitory effects of the 5′-triphosphates on DNA polymerases α, and β and DNA primase purified from testes of the developing cherry salmon, *Oncorhynchus masou*.

Synthesis

Several procedures have been reported for the synthesis of 3′-deoxyribonucleosides,[9-13] but the synthesis of appropriately protected 3-deoxy-D-ribose required many steps.[2] We have developed a convenient preparation of the 1-*O*-acetyl derivative of the sugar obtained from the antibiotic cordycepin.[6] For the present work, anomeric 1-*O*-acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (**1**) was selected as a starting material because **1** can easily be obtained in only two steps from cordycepin.

First, bis-trimethylsilyl-N^4-propionylcytosine (**2a**) was coupled with **1** in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) in anhydrous acetonitrile.[14] After usual work up, fully protected 2′,5′-di-*O*-*p*-chlorobenzoyl-N^4-propionyl-3′-deoxycytidine (**3a**) was obtained in 78% yield in crystalline form. For the selective removal of N^4-propionyl group, **3a** was treated with hydrazine hydrate in a 4:1 mixture of pyridine and acetic acid at room temperature to afford crystalline 2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (**4**) in 91% yield. Treatment of **4** with sodium nitrite in trifluoroacetic acid at 0—4 °C for 6 h gave 2′,5′-di-*O*-*p*-chlorobenzoyl-3′-deoxyuridine (**3c**) in 96% yield. The bis-trimethylsilylated derivatives of N^4-*p*-toluoyl-5-fluorocytosine (**2b**) and 5-fluorouracil were coupled with **1** in a similar manner described above to give 2′,5′-di-*O*-*p*-chlorobenzoyl-N^4-*p*-toluoyl-5-fluoro-3′-deoxycytidine (**3b**) (78%) and 2′,5′-di-*O*-*p*-chlorobenzoyl-5-fluoro-3′-deoxyuridine (**3d**) (91%), respectively. Saponification of these protected nucleosides

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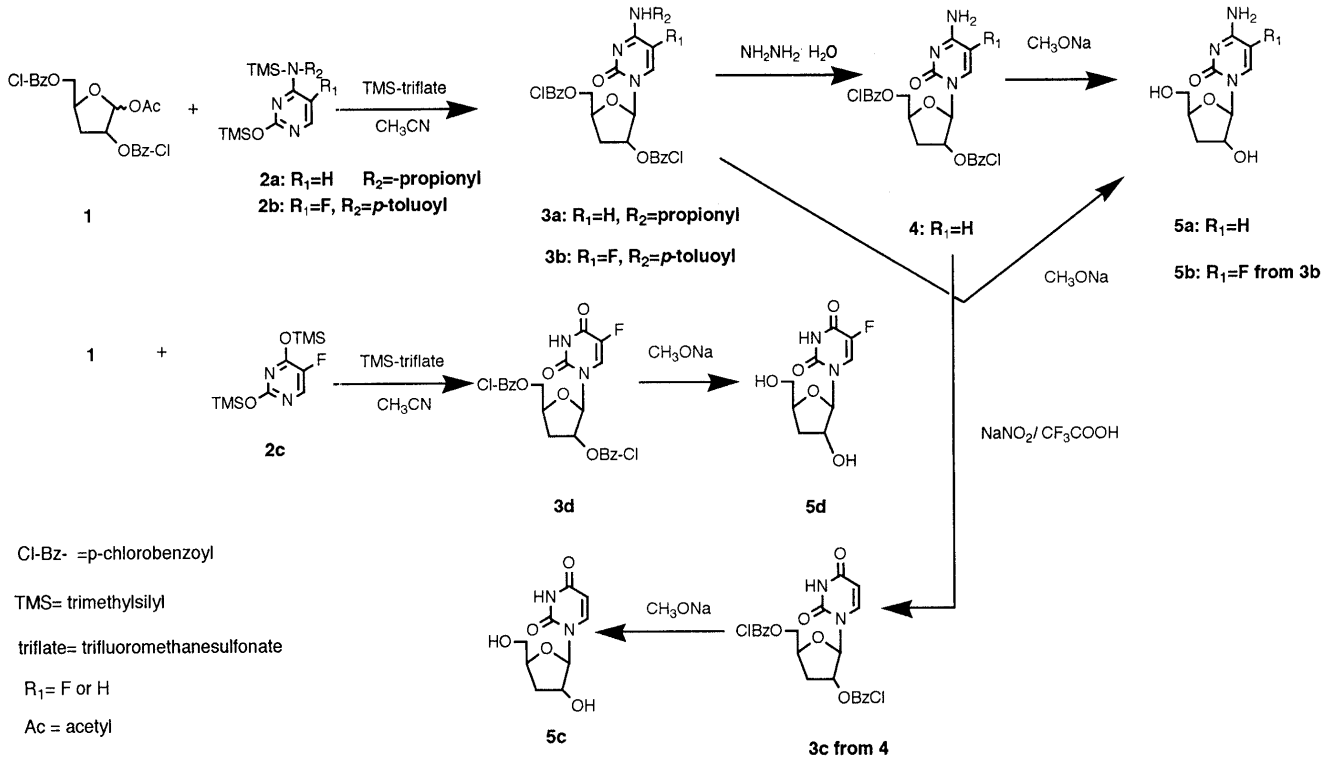

Chart 1

(**3a—d**) with 0.05 M methanolic sodium methoxide at 50 °C for 3—5 h afforded free 3′-deoxycytidine (**5a**), 5-fluoro-3′-deoxycytidine (**5b**), 3′-deoxyuridine (**5c**) and 5-fluoro-3′-deoxyuridine (**5d**) in satisfactory yields. The structures of these compounds were confirmed by elemental analysis, and MS, UV and NMR spectra. The β-configuration of **5a—d** was assigned by comparing the sign of the circular dichroism (CD) band associated with the B_{2u} electronic transition with those of appropriate nucleosides of known configuration. The observed positive sign and amplitude of the CD band of **5a—d** at 270 to 280 nm were in accord with those found for 1-β-D-pentofuranosylpyrimidine derivatives and not 1-α-D-pentofuranosylpyrimidine derivatives.[14] It should be noted that, throughout the coupling reaction using TMS-triflate, only the β-anomer was isolated from the reaction mixture.

For evaluation of the inhibitory effects of these compounds on DNA polymerases α, α-primase and β purified from developing cherry salmon, *Oncorhynchus masou*, testes,[15-19] 5′-triphosphates of **5a—d** and 3′-deoxyguanosine (**5e**) (Yamasa) were synthesized. Phosphorylation was done by the use of phosphorus oxychloride in triethyl phosphate to afford the corresponding 5′-monophosphates of **5a—d** and 3′-deoxyguanosine (**5e**) and further phosphorylation to the 5′-triphosphates was done by the phosphoroimidazolidate method. The resulting 5′-triphosphates (**6a—e**) were purified by DEAE-cellulose column chromatography, paper electrophoresis and preparative paper chromatographies (Table 3).

Growth-Inhibitory Effect of the Nucleoside Analogues on Mouse Leukemic L5178Y Cells The results of growth-inhibitory effect testing of the newly synthesized compounds are shown in Table 1. The cytostatic activity (IC_{50}) is expressed as the concentration causing 50% inhibition of cell growth.

Table 1. Growth-Inhibitory Effect of **5a—d** on L5178Y Cells

Compound	Dose (μg/ml) T/C (%)[a]					IC_{50}[b] (μg/ml)
	100	32	10	3.2	1	
5a	16	17	26	36	64	1.8
5b	18	51	62	86	100	33
5c	7	18	29	82	95	6.5
5d	27	40	61	80	97	18
Cordycepin	10	32	50	92	98	10

a) Expressed as the percentage of L5178Y cells in the treated culture relative to that in controls. *b*) Median inhibitory concentration (μg/ml) for growth-inhibitory effect on L5178Y cells calculated from a log-probit graph.

As can be seen in Table 1, 3′-deoxyribonucleosides with natural bases, cytosine (**5a**) and uracil (**5c**) showed strong effects. However, 5-fluoropyrimidine nucleoside of 3-deoxy-D-ribose, **5b** and **5d**, showed little activity. This may suggest that the fluorine substitution at the 5-position of the pyrimidine nucleus affected the phosphorylation of the nucleoside by uridine–cytidine kinase in L5178Y cells. These results are consistent with our previous study on the cytostatic activity of 3′-amino-3′-deoxyribonucleosides derived from 5-fluorouracil and 5-fluorocytosine.[3] In contrast, 5-fluoro-2′-deoxyuridine and 5-fluoro-2′-deoxycytidine are good substrates for cellular thymidine kinase and deoxycytidine kinase,[2] respectively.

Antiviral Activity of the Compounds against Infectious Hematopoietic Necrosis Virus in CHSE-214 Cells Antiviral activity of **5a—d** and 3′-deoxyadenosine (cordycepin) was determined using the CPE-spot reduction method[20,21] on a rhabdovirus, infectious hematopoietic necrosis virus (IHNV), in cultured CHSE-214 cells. Among

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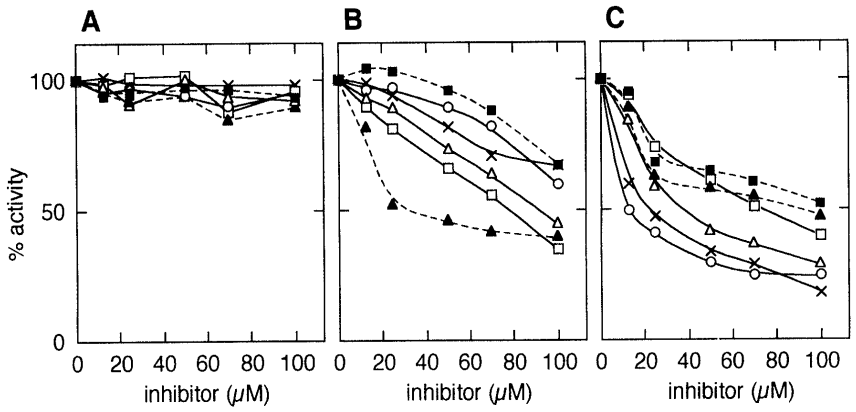

Fig. 1. Inhibitory Effects of Various 3′-Deoxyribonucleotides on DNA Polymerases α (A), β (B) and α-Primase (C)
□—□, 3′-dCTP (**6a**); ■---■, 5F-3′-dCTP (**6b**); △—△, 3′-dUTP (**6c**); ▲---▲, 5F-3′-dUTP (**6d**); ×—×, 3′-dGTP (**6e**); ○—○, 3′-dATP.

Table 2. Kinetic Parameters of Various 3′-Deoxyribonucleotides on DNA Polymerase α-Primase

Compounds	DNA polymerase α-primase	
	K_i (μM)	K_i/K_m
CTP	200 (K_m)	
3′-dCTP (**6a**)	85	0.43
5F-3′-dCTP (**6b**)	77	0.39
UTP	250 (K_m)	
3′-dUTP (**6c**)	84	0.34
5F-3′-dUTP (**6d**)	77	0.32
GTP	143 (K_m)	
3′-dGTP (**6e**)	43	0.30
ATP	800 (K_m)	
3′-dATP	70	0.094

the compounds tested, cordycepin was the most effective (IC_{50}: 8 μg/ml). The other compounds were less active; the IC_{50} values were: **5a** (100 μg/ml), **5b** (500 μg/ml), **5c** (250 μg/ml), **5d** (250 μg/ml).

Inhibitory Effects of the Triphosphates (6a—e and 3′-dATP) on DNA Polymerases α, α-Primase and β The inhibitory effects of various 3′-deoxyribonucleoside 5′-triphosphates (**6a—e**) and 3′-dATP on DNA polymerase α, α-primase and β purified from testes of developing cherry salmon, *Oncorhynchus masou*, testes were examined with activated salmon sperm DNA (for α and β), and M13 single-stranded DNA (for α-primase) as template-primer and template, respectively. The activity without analogues was taken as 100% and the remaining activity (%) at various concentrations of analogues was measured. The resulting inhibition curves are shown in Fig. 1A (α), 1B (β), and 1C (α-primase). As can be seen in Fig. 1A, the compounds did not show strong affinity for DNA polymerase α activity. On the other hand, DNA polymerase β was inhibited by these "ribo type" nucleotide analogues to a remarkable extent. In the selected system of primer RNA synthesis-dependent DNA synthesis (using M13 single-stranded DNA as the template) with DNA polymerase α-primase, these analogues were strongly inhibitory (Fig. 1C). The mode of inhibition of DNA primase by the 3′-deoxyribonucleotides was analyzed by using Lineweaver–Burk plots. All the 3′-deoxyribonucleotides were essentially competitive with the natural substrates which have the same base moieties. Based on these results, the kinetic parameter (K_i value) of each 3′-deoxyribonucleotide was estimated by replotting of apparent K_m *versus* the concentration of inhibitor (Table 2). Interestingly, fluorine-substituted pyrimidine 3′-deoxyribonucleotides still have strong affinity to DNA primase, comparable to 3′-dCTP and 3′-dUTP. This result clearly indicates that the low ability of 5-fluoro nucleosides to inhibit the growth of L5178Y cells is due to the incomplete phosphorylation by uridine-cytidine kinase in the cells. This is the first report concerning "ribo type" nucleotide inhibitors for eukaryotic DNA primase and DNA polymerase β.

Experimental

Melting points were determined on a Yanaco MP-3 apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-300 recording spectrophotometer. MS on a Hitachi RMU-6E mass spectrometer, NMR spectra on a Hitachi R20B high-resolution NMR spectrometer, CD measured on a JASCO model 20 automatic recording spectropolarimeter.

2′,5′-Di-*O*-*p*-chlorobenzoyl-N^4-propionyl-3′-deoxycytidine (3a) TMS-triflate (1 ml, 5.17 mmol) was added to N^4,2-bis-trimethylsilyl-N^4-propionylcytosine (**2a**) (2 g, 6 mmol) derived from 1 g of N^4-propionylcytosine and 1-*O*-acetyl-2,5-di-*O*-*p*-chlorobenzoyl-3-deoxy-D-ribofuranose (**1**)[5] (2.5 g, 5.5 mmol) in 25 ml of anhydrous acetonitrile, in an ice-bath. The solution was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue was treated with saturated aqueous sodium bicarbonate (50 ml) and chloroform (50 ml). After vigorous shaking, the chloroform layer was separated and washed with saturated sodium bicarbonate (50 ml) and distilled water (50 ml). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness and the residue was crystallized from boiling ethanol. Fine needles. 2.4 g (78%), mp 208—210 °C. *Anal.* Calcd for $C_{26}H_{23}Cl_2N_3O_7$: C, 55.73; H, 4.14; Cl, 12.65; N, 7.50. Found: C, 55.87; H, 4.05; Cl, 12.58; N, 7.53. ^{1}H-NMR ($CDCl_3$) δ: 8.93 (s, 1H, H-N_4), 7.3—8.1 (m, 10H, aromatic, H-5 and H-6), 6.05 (d, 1H, H-1′, $J_{1',2'}$ = 0.978), 5.70 (t, 1H, H-2′), 4.4—5.0 (m, 3H, H-4′ and H-5′), 2.50 (q, 2H, –CO–C$\underline{H}_2$–CH_3), 2.0—2.4 (m, 2H, H-3′), 1.18 (t, 3H, –CO–CH_2–C$\underline{H}_3$).

2′,5′-Di-*O*-*p*-chlorobenzoyl-3′-deoxycytidine (4) Hydrazine hydrate (0.3 ml) was added to a solution of **3a** (850 mg, 1.7 mmol) in acetic acid–pyridine (1 : 4, v/v) mixture (10 ml), and the mixture was stirred at room temperature.

After confirmation of the disappearance of **3a** by thin-layer chromatography (TLC) (chloroform–ethanol, 19 : 1, v/v, silica gel), the mixture was evaporated under reduced pressure and the residue was treated with the mixture of ethyl acetate and distilled water (1 : 1) (100 ml). The organic layer was washed well with water (20 ml × 3) and evaporated to dryness. The residue was dissolved in 3 ml of chloroform and applied to a column of silica gel (2.5 cm × 15 cm). Elution was performed with

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chloroform–ethyl acetate (9 : 1). The fractions containing the desired compound were combined and evaporated to dryness under reduced pressure. The residual gum was crystallized from boiling ethanol. Colorless needles. 780 mg (91.2%). mp 124—126 °C. *Anal.* Calcd for $C_{23}H_{19}C_{12}N_3O_6$: C, 54.78; H, 3.80; Cl, 14.06; N, 8.33. Found: C, 55.02; H, 3.77; Cl, 14.01; N, 8.52. ^{1}H-NMR ($CDCl_3$) δ: 7.15 (s, 2H, N_4-H), 7.3—8.3 (m, 10H, aromatic, H-5, H-6), 6.03 (d, 1H, H-1′, $J_{1',2'}$ = 0.977 Hz), 5.73 (t, 1H, H-2′), 4.4—5.0 (m, 3H, H-4′ and H-5′), 2.0—2.4 (m, 2H, H-3′).

2′,5′-Di-*O*-*p*-chlorobenzoyl-3′-deoxyuridine (3c) Sodium nitrite (100 mg) was added to a solution of **4** (1.00 g, 1.99 mmol) in 1 ml of trifluoroacetic acid in ice-bath. The solution was stirred for 2 h and additional sodium nitrite (50 mg × 3) was added. Stirring was continued for 3 h in the ice-bath, then the solvent was removed under reduced pressure. The residue was partitioned between distilled water (10 ml) and chloroform (10 ml). The organic layer was washed with saturated aqueous sodium bicarbonate and distilled water. The chloroform extract was evaporated to dryness, and the residue was crystallized from ethanol. Colorless needles, 0.96 g (96%), mp 203—205 °C. *Anal.* Calcd for $C_{23}H_{18}Cl_2N_2O_7$: C, 54.67; H, 3.59; Cl, 1403; N, 5.54. Found: C, 54.57; H, 3.56; Cl, 13.97; N, 5.64. ^{1}H-NMR ($CDCl_3$) δ: 11.4 (b, 1H, N_3-H), 7.3—8.5 (m, 9H, aromatic and 6-H), 5.92 (t, 1H, 1′-H), 5.8—5.7 (m, 1H, 5-H), 5.58 (t, 1H, 2′-H), 4.68 (s, 3H, 4′-H. 5′-H) 2.25—2.5 (m, 2H, H-3′).

3′-Deoxyuridine (5c) A solution of **3c** (0.96 g) in 35 ml of 0.05 N methanolic sodium methoxide was stirred at 50 °C for 3 h, then diluted with distilled water (15 ml) and neutralized with Dowex 50 (H^+ form). The mixture was filtered and resin was washed well with 50% aqueous methanol (50 ml). The combined filtrate and washings were evaporated to dryness and the residue was partitioned between chloroform and water (50 ml each). The aqueous layer was concentrated to afford a thick gum which was crystallized from boiling methanol. Colorless needles. 0.30 g (65.8%), mp 180—181 °C. (lit.[19] mp 178—179 °C, lit.[10] mp 178 °C, lit.[13] 177—179 °C.) *Anal.* Calcd for $C_9H_{12}N_2O_5$: C, 47.36; H, 5.30; N, 12.27. Found: C, 47.29; H, 5.41; N, 12.30. UV $\lambda_{max}^{H_2O}$ nm (ε): 260 (9800). CD $[\theta]_{260}$ (nm): +18700. MS *m/z*: 228 (M^+), 220 ($M^+ - H_2O$), 117 (sugar), 113 (base). ^{1}H-NMR (DMSO-d_6) δ: 11.29 (d, 1H, N_3-H), 7.97 (d, 1H, 6-H, $J_{5,6}$ = 8.05 Hz), 5.62 (t, 1H, 2′-OH), 5.5—5.7 (m, 2H, 5-H, 1′-H), 5.1 (t, 1H, 5′-OH), 4.1—4.4 (m, 2H, 2′-H, 4′-H), 3.5—3.7 (m, 2H, 5′-H), 1.6—2.0 (m, 2H, 3′-H).

2′,5′-Di-*O*-*p*-chlorobenzoyl-N^4-*p*-toluoyl-5-fluoro-3′-deoxycytidine (3b) N^4,2-Bis-trimethylsilyl-N^4-*p*-toluoyl-5-fluorocytosine (**2b**) (derived from 1.8 g of N^4-*p*-toluoyl-5-fluorocytosine, 6 mmol) and **1** (2.5 g, 5.5 mmol) in 25 ml of anhydrous acetonitrile was treated with TMS-triflate (1 ml) in an ice-bath. The solution was stirred at room temperature and the solvent was removed under reduced pressure after 1 h stirring. The residual foam was partitioned between the saturated aqueous sodium bicarbonate (50 ml) and chloroform (50 ml). After vigorous shaking, the chloroform layer was separated and washed with distilled water (30 ml × 2), then concentrated to dryness. The residue was crystallized from boiling acetone to give colorless prisms. 2.44 g (76%). mp 231—232 °C. *Anal.* Calcd for $C_{31}H_{24}Cl_2FN_3O_7$: C, 58.14; H, 3.77; N, 6.56. Found: C, 58.18; H, 3.78, N, 6.53. ^{1}H-NMR ($CDCl_3$) δ: 13.0 (d, 1H, N^4-H), 7.2—8.4 (m, 13H, Aromatic and H-6), 5.90 (s, 1H, 1′-H), 5.63 (s, 1H, 2′-H), 4.73 (s, 3H, 4′-H, 5′-H), 2.42 [m, CH_3 (*p*-toluoyl), 3′-H].

2′,5′-Di-*O*-*p*-chlorobenzoyl-5-fluoro-3′-deoxyuridine (3d) A solution of **1** (2.5 g) and 2,4-bis-trimethylsilyloxy-5-fluoropyrimidine (**2c**) (derived from 870 mg of 5-fluorouracil) in 40 ml of anhydrous acetonitrile was treated with TMS-triflate (1 ml) under stirring at below 10 °C. After 30 min at room temperature, additional TMS-triflate (1 ml) was added. After 2 h, the coupling reaction was completed as judged from the TLC (chloroform–ethanol, 9 : 1, v/v, silica gel) profile. The solvent was removed *in vacuo* and the residue was partitioned aqueous sodium bicarbonate (50 ml) and chloroform (50 ml).

The organic layer was evaporated and the residue was crystallized from boiling ethanol to give needles. 3.8 g (92%), mp 205—207 °C. *Anal.* Calcd. for $C_{23}H_{17}Cl_2FN_2O_7$: C, 52.77; H, 3.25; N, 5.35. Found: C, 52.90; H, 3.17; N, 5.28. ^{1}H-NMR ($CDCl_3$) δ: 7.5—8.5 (m, 9H, aromatic and H-6), 5.92 (s, 1H, 1′-H), 5.58 (t, 1H, 2′-H), 4.68 (s, 3H, 4′-H, 5′-H), 2.25—2.5 (m, 2H, 3′-H).

3′-Deoxycytidine (5a) A solution of **3a** (3.5 g, 6.25 mmol) in 140 ml of 0.05 N methanolic sodium methoxide was heated at 50 °C for 3 h with stirring, then neutralized with Dowex 50 (H^+-form) and filtered. The resin was washed with a mixture of distilled water–pyridine–ethanol (6 : 1 : 1). The filtrate and washings were combined and evaporated, and the residue was partitioned between chloroform and distilled water. The aqueous layer was mixed with a small amount of *n*-butanol and concentrated to dryness. The residue was dissolved in 3 ml of 30% ethanol and applied to a column of Dowex 1 (OH^- form, 2.5 cm × 30 cm). Elution was performed with 30% ethanol and fractions were monitored by measuring the UV absorbance at 270 nm. The fractions containing the desired nucleoside were combined and evaporated to give a colorless product, which was crystallized from 70% aqueous ethanol. 0.9 g (50%). mp 233—235 °C (lit.[9] 224—230 °C, lit.[13] 223—225 °C). *Anal.* Calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49. Found: C, 47.39; H, 5.76; N, 18.47. UV $\lambda_{max}^{H_2O}$ nm (ε): 272 (9100), $\lambda_{max}^{0.01\,N\,HCl}$ nm (ε): 285 (12800). CD $[\theta]_{271}$ (nm): +18700. MS *m/z*: 227 (M^+), 209 ($M^+ - H_2O$), 112 (base). ^{1}H-NMR (DMSO-d_6) δ: 7.94 (d, 1H, 6-H, $J_{5,6}$ = 7.3 Hz), 7.08 (s, 2H, N^4-H), 5.70 (d, 2H, 5-H, 1′-H), 5.5 (d, 1H, 2′-OH), 5.02 (t, 1H, 5′-OH), 4.25 (m, 1H, 4′-H), 4.09 (m, 1H, 2′-H), 3.6 (m, 2H, 5′-H). 1.8 (m, 2H, 3′-H).

5-Fluoro-3′-deoxycytidine (5b) A solution of 2.0 g of **3b** (3 mmol) in 75 ml of methanolic sodium methoxide (0.05 N) was stirred at 50 °C for 5 h, then concentrated to a small volume, diluted with distilled water (50 ml), neutralized with Dowex 50 (H^+-form) and filtered. The resin was washed with 50% methanol (20 ml × 2).

The filtrate and washings were evaporated to dryness and the residue was crystallized from ethanol. Colorless crystals. 480 mg (64.8%). mp 194—195 °C. *Anal.* Calcd for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; N, 17.13. Found: C, 44.09; H, 5.02; N, 16.93. UV $\lambda_{max}^{H_2O}$ nm (ε): 238 (7600), 289 (7700). CD $[\theta]_{280}$ (nm): +21000. MS *m/z*: 245 (M^+), 227 ($M^+ - H_2O$), 130 (base$^+$). ^{1}H-NMR (DMSO-d_6) δ: 8.35 (d, 1H, 6-H, $J_{5F,6}$ = 7.35 Hz), 7.48—7.64 (d, 2H, N^4-H), 5.57 (s, 1H, 1′-H), 5.52 (d, 1H, 2′-OH), 5.24 (t, 1H, 5′-OH), 4.0—4.4 (m, 2H, 2′-H, 4′-H), 3.5—4.0 (d, 2H, 5′-H), 1.5—2.0 (m, 2H, 3′-H).

5-Fluoro-3′-deoxyuridine (5d) Compound **3d** (1.3 g, 0.5 mmol) in 70 ml of methanolic sodium methoxide (0.05 N) was stirred at 50 °C for 5 h, then concentrated to a small volume. The residue was taken up in 30% methanol then neutralizedwith Dowex 50(H^+-form) . The resin was filtered off, the filtrate was evaporated *in vacuo*, the resulting glassy residue was crystallized from ethanol. 525 mg (81%). mp 169—171 °C. *Anal.* Calcd for $C_9H_{11}FN_2O_6$: C, 43.91; H, 4.50; N, 11.38. Found: C, 43.85; H, 4.40; N, 11.34. UV $\lambda_{max}^{H_2O}$ nm (ε): 270 (7200). CD $[\theta]_{270}$ (nm): +14400. MS *m/z*: 246 (M^+), 228 ($M^+ - H_2O$), 117 (base$^+$). ^{1}H-NMR (DMSO-d_6) δ: 11.7 (d, 1H, N^3-H), 8.45 (d, 1H, 6-H), 5.6 (d, 1H, 2′-OH), 5.5 (s, 1H, 1′-H), 5.29 (t, 1H, 5′-OH), 4.2—4.4 (m, 2H, 2′ and 4′-H), 3.52 (dd, 1H, 5′-H_a), 3.82 (dd, 1H, 5′-H_b, $J_{5'ab}$ = 12.36 Hz), 1.8—2.2 (m, 1H, 3′-H_a), 1.65 (dd, 1H, 3′-H_b, $J_{3'ab}$ = 13.2 Hz).

General Method for Phosphorylation of Nucleosides Phosphoryl chloride (0.75 mmol) was added to 0.25 mmol of synthetic nucleoside [**5a**—**d** and 3′-deoxyguanosine (**5e**)] in 1 ml of triethyl phosphate under cooling below 0 °C. The mixture was stirred for 4 h (**5a**), 5 h (**5b**), 12 h (**5c**), 24 h (**5d**) or 10 h (**5e**) at 0 °C, then poured into 30 ml of saturated aqueous sodium bicarbonate and extracted with chloroform (30 ml × 3). The combined organic layers were re-extracted with distilled water (15 ml). The aqueous layers were combined and diluted with water to give a final volume of 300 ml, and applied to a column of DEAE-cellulose (2.5 × 25 cm, bicarbonate form).

The column was washed with 500 ml of distilled water, and eluted with a linear gradient from distilled water (500 ml) to 0.2 M triethylammonium bicarbonate (500 ml, pH 7.8). Fractions containing desired nucleotide were combined and evaporated to dryness and co-evaporation with 50% aqueous ethanol was carried out to remove residual triethylamine and triethylammonium bicarbonate. The yields from 5′-monophosphate were, 85, 80, 78, 80, and 83.5%, respectively. The obtained 5′-monophosphate was converted to the 5′-triphosphate using phosphoroimidazolidate method as follows. The 5′-monophosphate (0.2 mmol) was dissolved in 1 ml of anhydrous *N*,*N*-dimethylformamide (DMF) and evaporated to dryness. This process was repeated twice, then 1 mmol of *N*,*N*′-carbonyldiimidazole was added to the residue in 2 ml of DMF, and the mixture was stirred for 3.5 h at room temperature. After confirmation of the complete conversion to the 5′-phosphorimidazolidate by paper electrophoresis (in 50 mM triethylammonium bicarbonate, pH 7.8, 700 V, 30 min), 0.8 ml of methanol was added to decompose excess reagent, and the mixture was stirred for 30 min at room temperature. The 2 ml (2 mmol) of 1 M tri-*n*-butylammonium pyrophosphate in DMF was added and the mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure at below 37 °C and the residue was dissolved in distilled

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Table 3. Properties of Synthetic Nucleotide Analogues (**6a—e**)

Compound	UV $\lambda_{max}^{H_2O}$ nm (ε)	ε(P) Found	Paper chromatography[a] (*Rf*)	Paper electrophoresis[b] (*R*-dNTP)[c]
3′-dCTP (**6a**)	272 (9100)	3040	0.78	0.95
5F-3′-dCTP (**6b**)	238 (7600), 289 (7700)	2600	0.83	0.98
3′-dUTP (**6c**)	260 (9800)	3150	0.37	1.06
5F-3′-dUTP (**6d**)	270 (7200)	2380	0.41	0.98
3′-dGTP (**6e**)	252.5 (13700)	4600	0.25	1.05

a) Solvent system was ethanol–0.5 M sodium acetate (pH 7.5, 1 : 1, v/v). *b*) In 0.5 M citrate buffer (pH 3.3, 700 V, 30 min). *c*) Relative value with respect to the corresponding dNTP (2′-deoxy counterparts).

water (10 ml) and the nucleotide was adsorbed on 1 g of activated charcoal. The mixture was filtered through celite. After washing with 100 ml of distilled water, elution from charcoal was carried out with 200 ml of 50% ethanol–3% NH_4OH. The eluate was evaporated and the residue was dissolved into 50 ml of distilled water and applied to a column of DEAE-cellulose (2.9 × 25 cm, bicarbonate form). The column was washed with 250 ml of distilled water, and then eluted with a linear gradient from 0.05 M triethylammonium bicarbonate (500 ml, pH 7.8) to 0.5 M triethylammonium bicarbonate (500 ml, pH 7.8). The combined fractions containing the 5′-triphosphate esters were evaporated and co-evaporated with 50% ethanol to dryness. Analytical results were summarized in Table 3.

Assay Method for Growth-Inhibitory Effect of the Compound on Mouse Leukemic L5178Y Cells in Culture Mouse leukemic L5178Y cells were grown in RPMI-1629 medium (Nissui Seiyaku Co., Ltd.) supplemented with 10% calf serum (Flow Laboratory, Md, U.S.A.) at 37 °C. One volume of the test compound diluted with the same medium was added to 9 volumes of the culture containing between 1.2×10^5 and 1.7×10^5 L5178Y cells/ml. After incubation for 48 h at 37 °C in 5% carbon dioxide, the number of cells remaining was counted with a cell counter (Toa micro cell counter, model 1002).

Assay Method for Antiviral Activity against Infectious Hematopoietic Necrosis Virus in CHSE-214 Cells in Culture Antiviral activity of the compounds (**6a—d**) and cordycepin on a rhabdovirus, IHNV, was determined by the cytopathic effect (CPE) spot reduction method[20,21] as follows. Nearly confluent CHSE-214 cells in wells of a microtiter plate were infected at a concentration forming about 100 CPE spots per well. After the addition of culture medium containing various levels of the test compound, the cultures were incubated for 3 d at 15 °C. After fixing and staining, the CPE spots formed on cell monolayers were counted microscopically.

DNA polymerases α, α-primase and β-DNA polymerases α, α-primase and β[15-19] were purified from developing testes of cherry salmon, *Oncorhynchus masou*, as described previously.

Assay of DNA Polymerase Activities The standard assay mixture (25 μl) for DNA polymerase α contained 100 μg/ml activated salmon sperm DNA, 50 mM Tris–HCl (pH 8.0), 4 mM $MgCl_2$, 15% glycerol, 1 mM dithiothreitol, 400 μg/ml bovine serum albumin, 100 μM each of dATP, dGTP and dCTP, 25 μM [^{3}H]dTTP (5 cpm/pmol), 0—100 μM synthetic nucleotide analogue (**6a—d**) and 0.5 unit of enzyme. In the DNA polymerase β assay system, the same mixture was used except for 50 mM glycine–KOH buffer (pH 9.5) instead of Tris–HCl, and addition of 80 mM KCl was used. DNA polymerase α-primase activity was determined with a mixture (25 μl) containing 100 μg/ml M13 single-stranded DNA, 50 mM Tris–HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol, 400 μg/ml bovine serum albumin, 400 μM each of three ribonucleoside triphosphates (rNTP), 100 μM rNTP (corresponding to the analogue), 100 μM each of dATP, dGTP and dCTP, 25 μM [^{3}H]dTTP (50 cpm/pmol), 3 units of DNA polymerase α-primase, and 0—100 μM analogue. Incubation was performed at 30 °C for 30 min. In any case, after incubation, the reaction mixture was chilled in ice-water and transferred to a DEAE-cellulose paper disc (Whatman DE 81). The discs were washed with 5% Na_2HPO_4 (6 times), water (twice), ethanol (twice) and ether, and dried. The remaining radioactivity was measured with toluene scintillator.

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