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Synthetic Nucleosides and Nucleotides. XXI.[1] On the Synthesis and Biological Evaluations of 2′-Deoxy-α-D-ribofuranosyl Nucleosides and Nucleotides

TOYOFUMI YAMAGUCHI and MINEO SANEYOSHI*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Sapporo 060, Japan

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N_4-Benzoyl-2′-deoxycytidine (**1**) was converted to its 3′,5′-di-*O*-acetate (**2**). Compound **2** was smoothly anomerized to its α-counterpart (**3**) by reaction with trimethylsilyl trifluoromethanesulfonate (TMS-triflate). Saponification of **3** afforded crystalline α-2′-deoxycytidine (**4**). Similarly, 3′,5′-di-*O*-*p*-toluoyl-2′-deoxythymidine (**5**) was anomerized to the α-anomer (**6**), which was then deblocked to give α-2′-deoxythymidine (**7**).

α-2′-Deoxyadenosine (**8**) and 9-(2-deoxy-α-D-ribofuranosyl)-6-methylthiopurine (**9a**) were prepared by TMS-triflate-catalyzed trans-2-deoxyribosylation from compound **2** to N_6-benzoyladenine and 6-methylthiopurine, respectively. α-5-Fluoro-2′-deoxycytidine (**11a**) and its β-anomer (**11b**) were synthesized by the reaction of the trimethylsilyl derivative of N_4-*p*-toluoyl-5-fluorocytosine with 1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose followed by deblocking.

Among the compounds related to α-2′-deoxyribonucleosides, compound **4** and **11a** showed weak growth-inhibitory activity on mouse leukemic L5178Y cells in culture. Of the nucleoside 5′-triphosphates, α-deoxy ATP had some affinity with DNA polymerase α when activated DNA was used as a template-primer. α-Deoxythymidine 5′-triphosphate (TTP) showed a remarkable inhibitory effect on DNA polymerase β when poly [rA]-oligo dT was used as template-primer.

Keywords——α-2′-deoxyribonucleoside; synthesis; self-anomerization; trans-2-deoxyribosylation; trimethylsilyl trifluoromethanesulfonate (TMS-triflate); cytostatic effect; DNA polymerase α; DNA polymerase β; cherry salmon testes

Recent reports on the biological activities of nucleoside analogues having an α-glycosyl linkage between the base and the sugar have attracted great attention to this category of nucleosides. In particular, the antitumor activity of α-2′-deoxy-6-thioguanosine,[2] the bacteriostatic activity of α-4′-thio-5-fluorouridine,[3] the cytostatic activity of 9-α-D-arabinofuranosyladenine (α-Ara A),[4] 1-α-D-arabinofuranosylcytosine (α-Ara C),[5] and so on have prompted much work on the synthesis and biological evaluations of α-2′-deoxyribonucleosides. In enzyme studies, 9-α-D-arabinofuranosyladenine 5′-triphosphate (α-Ara ATP) showed selective inhibitory action on mammalian DNA polymerase α.[4] These results prompted us to synthesize a series of α-2′-deoxyribonucleosides and nucleotides which might exhibit biological activity in eukaryotic cells and towards DNA polymerases.

Here we report the synthesis of various α-2′-deoxyribonucleosides using a novel self-anomerization reaction of naturally occurring β-2′-deoxyribofuranosylpyrimidines, catalyzed by trimethylsilyl trifluoromethanesulfonate (TMS-triflate).

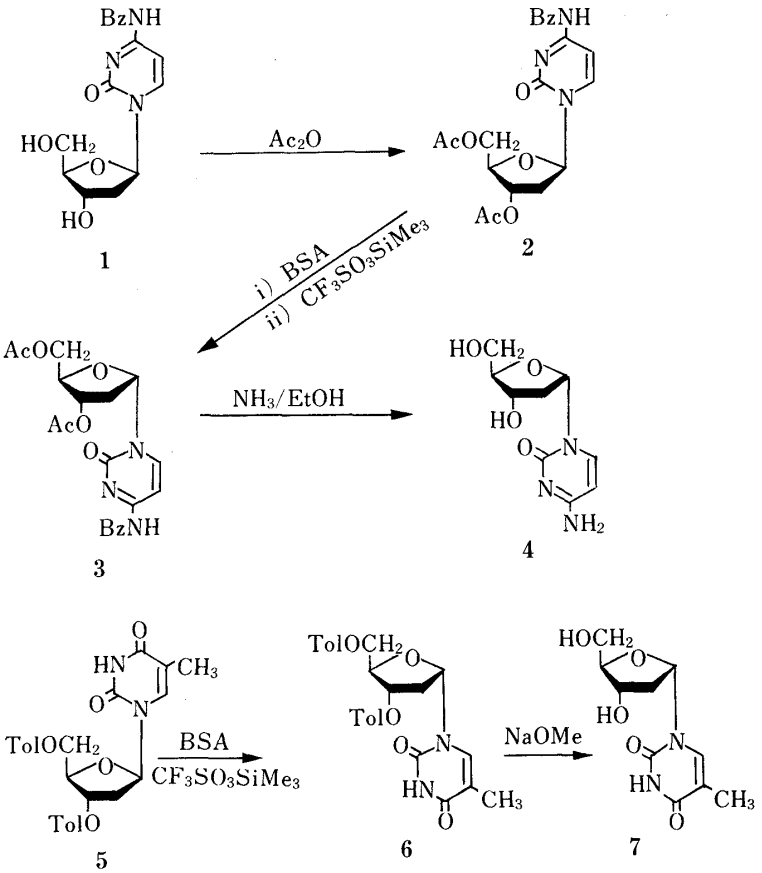
Transdeoxyribosylation from 2′-deoxycytidine derivatives to certain purine bases was also employed for the synthesis of purine-α-2′-deoxyribonucleosides.

The results of biological evaluations are also reported, including examination of the growth-inhibitory effects of the synthesized α-2′-deoxyribonucleosides on mouse leukemic L5178Y cells in culture and the effects of the corresponding 5′-triphosphates on DNA polymerases α and β purified from developing cherry salmon (*Oncorhynchus masou*) testes.

Synthesis

In this paper, we describe first a simple and convenient synthesis of α-2′-dioxyribofuranosylpyrimidines and -purines. The synthetic methods consists of either trimethylsilyl trifluoromethanesulfonate (TMS-triflate)[6]-catalyzed self-anomerization of 2′-deoxycytidine and 2′-deoxythymidine in their fully acylated forms of trans-2′-deoxyribosylation of fully acylated 2′-deoxycytidine (**3**) to certain purine bases such as N_6-benzoyladenine and 6-methylthiopurine.

For the synthesis of α-2′-deoxycytidine (**4**), N_4-benzoyl-2′-deoxycytidine (**1**)[7] was first acetylated to its 3′,5′-di-*O*-acetate (**2**) by treatment with acetic anhydride in anhydrous pyridine, followed by reaction with TMS-triflate and bis(trimethylsilyl)-acetamide (BSA) in dry acetonitrile at 70 °C for 2—3 h. Anomerization of compound **2** occurred, and the content of α-anomer in the reaction mixture reached a maximum within this period. This reaction is thought to occur in two steps as an inter-molecular reaction. First, the trimethylsilylated base is released from the nucleoside and then the liberated active sugar carbonium cation at C_1-position is attacked again by the nucleophilic center (N_1-position of the pyrimidine base). During the reaction, a new spot appeared on a thin-layer chromatogram (TLC) (silica gel, chloroform–ethanol, 9 : 1, v/v) which showed slightly lower movement than the starting β-nucleoside. After usual work-up, the product was separated from residual β-anomer by silica gel column chromatography. The isolated yield of α-anomer (**3**) was around 50% from **2**. Treatment of compound **3** with ethanolic ammonia afforded crude α-2′-deoxycytidine (**4**) and this was further purified on an ion-exchange column (Dowex 1, OH^- form) to give pure **4** in 83% yield. α-Configuration of this material was confirmed by nuclear magnetic resonance



BSA = bis(trimethylsilyl)acetamide
Tol = COC_6H_4Me-*p*

Chart 1

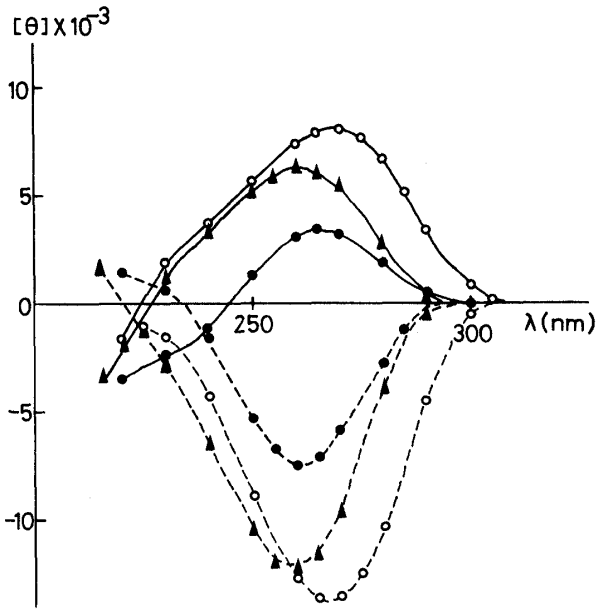


Fig. 1. CD Spectra of Various α-2′-Deoxyribofuranosylpyrimidines and Their β-Anomers

●—●, TdR; ●---●, α-TdR; ○—○, FCdR; ○---○, α-FCdR; ▲—▲, CdR; ▲---▲, α-CdR.

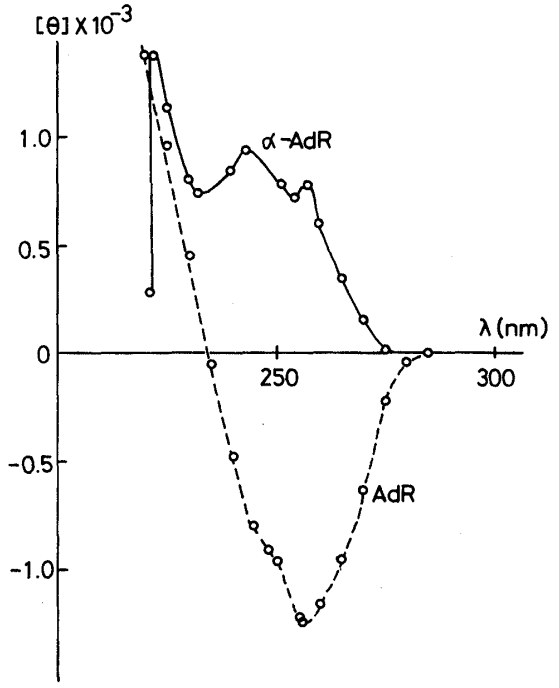


Fig. 2. CD Spectra of α-2′-Deoxyribofuranosyladenine and Its β-Anomer

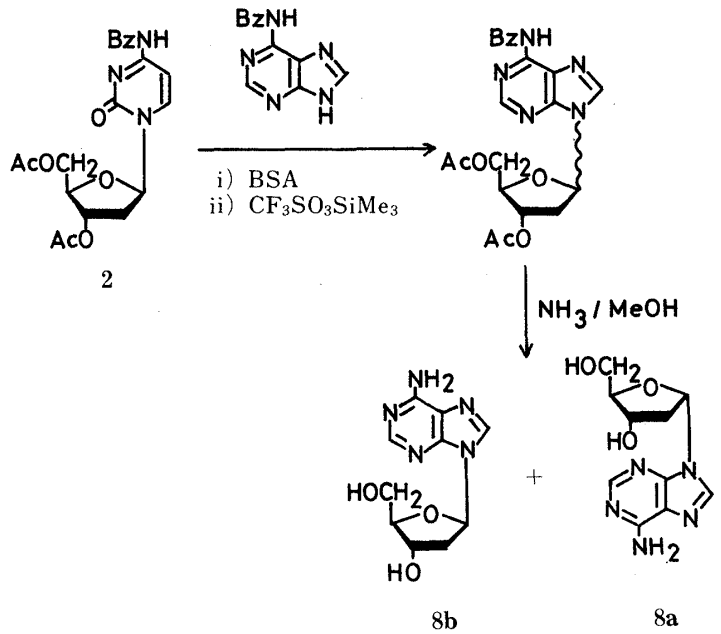


Chart 2

(NMR) spectra, $[\alpha]_D$ and the sign and amplitude of the circular dichroism (CD) spectra.

In the thymine series, 3′,5′-di-*O*-*p*-toluoyl-2′-deoxythymidine (**5**) was treated with BSA and TMS-triflate in a manner similar to that described above. After being heated at 70 °C for 4 h, the reaction mixture showed two spots on TLC (silica gel, chloroform–ethyl acetate, 1 : 1, v/v) with *Rf* values of 0.34 (β-anomer) and 0.25 (α-anomer). The desired α-anomer was separated from residual β-anomer by column chromatography on silica gel. The isolated yield of α-anomer (**6**) was around 30—40%. Compound **6** was saponified with methanolic sodium methoxide to afford crystalline α-2′-deoxythymidine (**7**) in 93% yield. The structure was

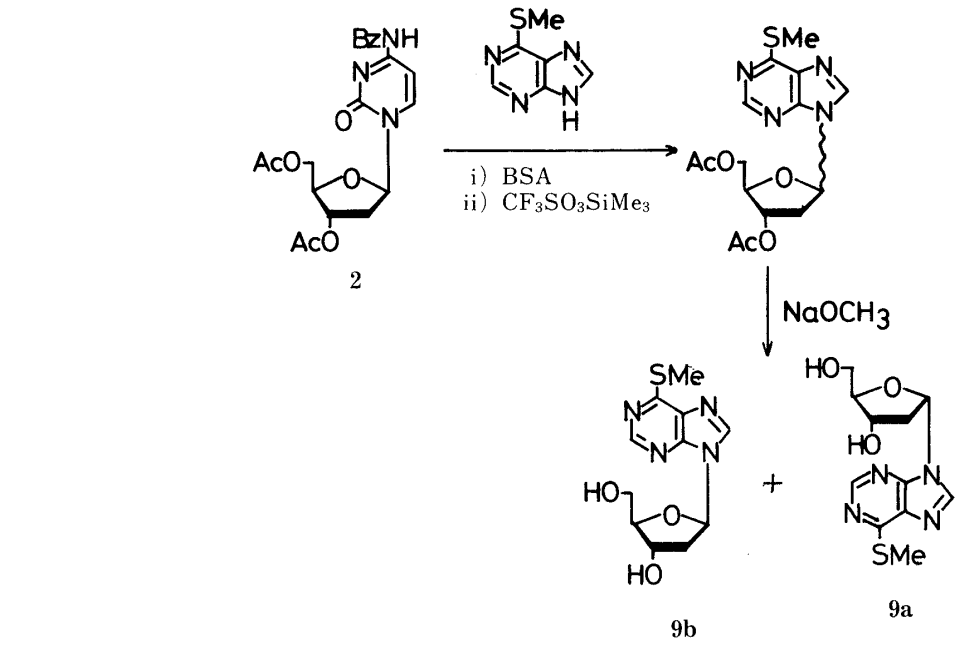


Chart 3

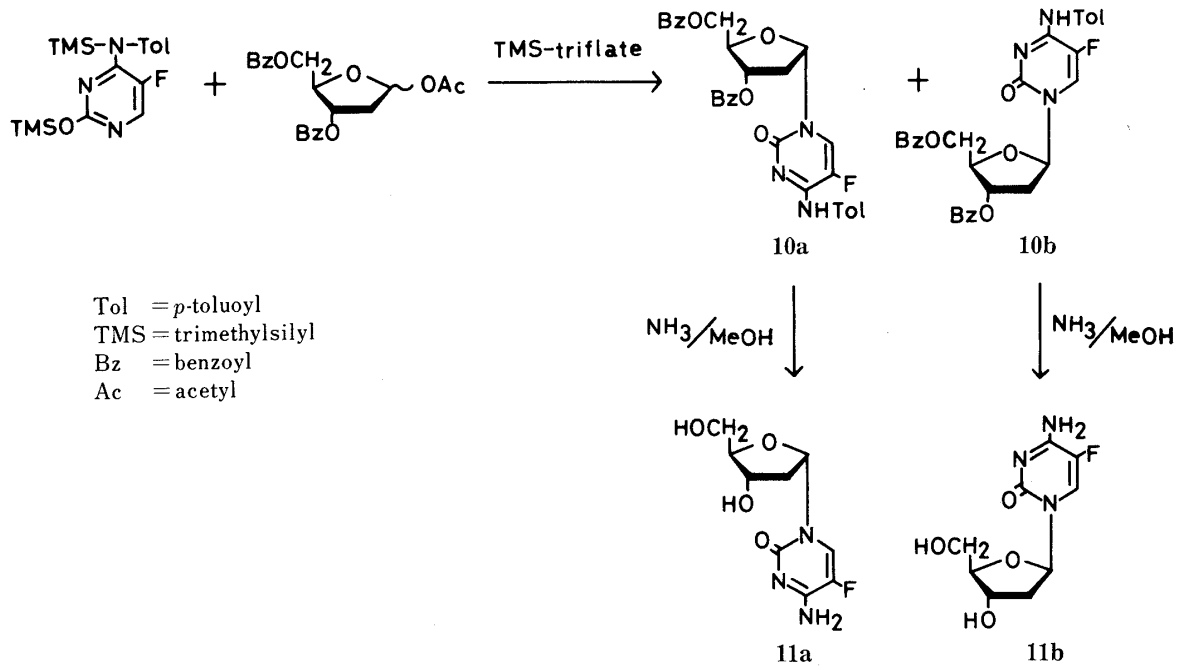


Chart 4

confirmed by elemental analysis, $[\alpha]_D$, ultraviolet (UV), NMR and CD spectroscopy.

Imazawa and Eckstein reported the transglycosylation reaction from acylated 2′,3′-dideoxy-3′-azidothymidine to acylated adenine and guanine.[7] We applied this reaction for the present synthesis. We used compound **2** as a glycosyl donor for this reaction, because of its lability compared with thymidine derivatives. Two kind of purine bases, N_6-benzoyladenine and 6-methylthiopurine were used as glycosyl acceptors. The reaction conditions were quite similar to those described above for the self-anomerization reaction, and the resulting anomeric mixture was separated as follows. In the case of the adenine series, the fully protected anomeric mixture was separated from remaining glycosyl donor **2** by column chromatography on silica gel. Both α- and β-nucleosides were then saponified to free nucleosides. The resulting α-2′-deoxyadenosine (**8a**) and α-2′-deoxyadenosine (**8b**) was

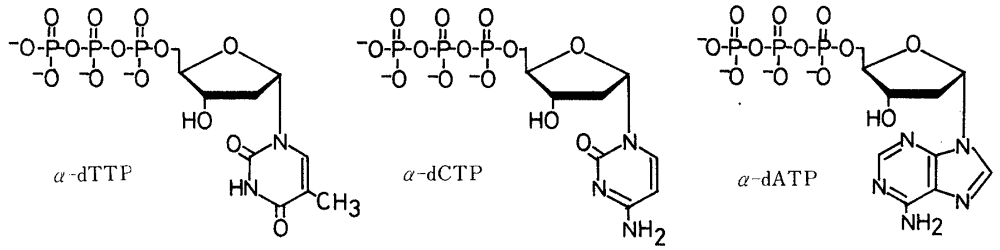


Fig. 3. The Structure of α-2′-Deoxyribonucleotides Tested

separated further by Dowex 1 (OH^- form) column chromatography with distilled water as the eluent. Overall yield of α-2′-deoxyadenosine (**8a**) was 28% based on the amount of **2**. The structure of this compound was confirmed by elemental analysis, as well as UV, NMR and CD spectroscopy. On the other hand, in the case of 6-methylthiopurine, saponified anomeric mixture was separated by repeated silica gel column chromatography. 9-(2-Deoxy-α-D-ribofuranosyl)-6-methylthiopurine (**9a**) was obtained in 36% yield based on the amount of **2**. The yield of β-anomer (**9b**) was 27%. Both purine-α-2′-deoxyribonucleosides showed a positive CD band characteristic of the α-anomer.[9]

Finally, pyrimidine-α-2′-deoxyribonucleoside having a modified base moiety was synthesized by direct deoxyribosylation of trimethylsilylated N_4-*p*-toluoyl-5-fluorocytosine[10] using anomeric 1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose in the presence of TMS-triflate. The latter sugar derivative was prepared from N_6,N_6-3′,5′-tetrabenzoyl-2′-deoxyadenosine by acetolysis. Condensation proceeded very smoothly in only 2 h at room temperature. Isolated yields of the α-anomer (**10a**) and β-anomer (**10b**) were 18% and 31%, respectively. After saponification, free nucleoside was obtained in 58% (α) and 67% (β) yields, respectively.

In previous work, α-2′-deoxyribonucleosides were obtained only as a by-product of the condensation reaction of halo-sugar with mercuri-base or 2,4-dialkoxypyrimidines (Hilbert–Johnson base), and this was not a general synthetic method for α-2′-deoxyribonucleosides. Furthermore, previous methods contained several steps for the preparation of suitably protected 2′-deoxyribosyl derivatives. In contrast, our present synthetic procedure starts from easily obtainable naturally occurring 2′-deoxyribonucleosides, such as 2′-deoxycytidine, 2′-deoxythymidine or 2′-deoxyadenosine, and it seems to be simpler and better than previous methods.

For the evaluation of the inhibitory effects of these α-anomer on DNA-dependent DNA polymerases α and β from cherry salmon (*Oncorhynchus masou*),[11] 5′-triphosphates of α-2′-deoxycytidine (**4**), α-2′-deoxythymidine (**7**) and α-2′-deoxyadenosine (**8a**) were synthesized. These were phosphorylated the use of phosphorus oxychloride in triethylphosphate[12] followed by further phosphorylation to the 5′-triphosphate by the phosphoroimidazolidate method[13] in satisfactory yields.

Biological Activities

Cytostatic activity——Growth-inhibitory effects of α-2′-deoxyribonucleosides on cultured mouse leukemic L5178Y cells were examined. Among the compounds tested, compounds **4** and **10a** were found to be slightly active ($IC_{50} = 60\ \mu g/ml$) against these cells, and the other compounds including **9a** and **9b** were essentially inactive. This finding showed that the affinity of a series of α-2′-deoxyribonucleosides to cellular deoxyribonucleoside kinases is weaker than that of their β-counterparts.

Effect of α-2′-Deoxyribonucleoside 5′-Triphosphates on DNA-Dependent DNA Polymerases α and β from Cherry Salmon (*Oncorhynchus masou*) Testes

Three newly synthesized α-2′-deoxyribonucleoside 5′-triphosphates, α-2′-deoxycytidine

Table I. Growth-Inhibitory Effect of the Test Compounds on L5178Y Cells in Culture

Compound	Dose (μg/ml) T/C (%)[a]								ED_{50}[b] (μg/ml)
	300	150	100	50	25	0.1	0.006	0.003	
4			10	41	99				50
7	104								300
8	81	103							300
9a	113								300
9b	114								300
11a			10	69					60
11b						8	21	94	0.005

a) Percentage of mouse leukemic L5178Y cells in the treated culture relative to that in controls.
b) Median effective dose (μg/ml) for growth-inhibitory effect on L5178Y cells.

Table II. Inhibitory Effect of α-dNTP on Cherry Salmon DNA Polymerases α and β

α-dNTP	DNA polymerase	Template-primer	Labelled NTP	Concentration of inhibitor (μM) Activity remained (%)				
				2.5	5	10	20	40
α-dTTP	α	Activated DNA	^{3}H-dTTP	110	98	100	102	85
	β	Activated DNA	^{3}H-dTTP	115	104	100	90	80
	β	Poly[rA]-oligo dT	^{3}H-dTTP	110	90	80	50	35
α-dATP	α	Activated DNA	^{3}H-dTTP	110	100	85	75	58
	β	Activated DNA	^{3}H-dTTP	100	90	80	75	70
α-dCTP	α	Activated DNA	^{3}H-dTTP	100	100	100	95	90
	α	Activated DNA	^{3}H-dCTP	110	100	105	95	90
	β	Activated DNA	^{3}H-dTTP	115	120	110	100	90
	β	Activated DNA	^{3}H-dCTP	110	100	105	100	95

5′-triphosphate (α-dCTP), α-2′-deoxythymidine 5′-triphosphate (α-dTTP), and α-2′-deoxyadenosine 5′-triphosphate (α-dATP) were examined as inhibitors of DNA synthesis with DNA polymerases α and β *in vitro*. In this system, activated salmon sperm DNA, poly [rA]-oligo dT and poly [dA]-oligo dT as a template-primers.[11] Among them, α-dATP showed a weak inhibitory effect on DNA polymerase α in the presence of activated DNA as a template-primer. On the other hand, α-TTP showed a marked inhibitory effect on DNA polymerase β in the presence of poly [rA]-oligo dT or poly [dA]-oligo dT as a template-primer. α-dCTP was essentially inactive towards both DNA polymerase α and β and this was the case even combination with activated DNA.

In contrast to α-Ara ATP,[4] α-dATP had only a weak inhibitory activity in the DNA polymerase system. This finding may be due to the differences in sugar puckering in these nucleotides and to the affinity of α-dATP, which is weaker than that of α-Ara ATP.

Experimental

General Methods——Melting points were derermined with Yanaco MP-3 apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-300 recording spectrophotometer. CD spectra were obtained on a JASCO model 20 automatic recording spectropolarimeter. Optical rotations were determined with a JASCO polarimeter. NMR spectra were obtained on a JEOL FX-100 NMR spectrometer with tetramethylsilane as an internal standard.

Thin-layer chromatography was performed with pre-coated silica gel 60 F_{254} plates (Merck) and silica gel column chromatography was performed with Wako-gel C-200. Radioactivity was determined with a Beckman LS-230 liquid scintillation counter with toluene scintillator.

3′,5′-Di-*O*-acetyl-N_4-benzoyl-2′-deoxycytidine (2)——Acetic anhydride (6 ml) was added to a solution of N_4-benzoyl-2′-deoxycytidine (**1**) (5.0 g, 15.1 mmol)[7] in 100 ml of anhydrous pyridine. The solution was stirred at 65 °C for 25 h, then the solvent was removed under reduced pressure and the residue was crystallized from ethanol to give colorless needles. 5.3 g (85%) mp 200—202 °C. UV λ_{max}^{EtOH} (nm): 272, 305. ^{1}H-NMR ($CDCl_3$): δ 8.70 (s, 1H, NH), 7.44—8.10 (m, 7H, H-5, H-6 and benzoyl-H), 6.28 (dd, 1H, H-1′, $J_{1'-2'}$ = 5.7 and 7.8 Hz), 5.23 (dd, 1H, H-3′), 4.39 (s, 3H, H-4′, 5′), 1.92—2.96 (m, 8H, H-2′ and acetyl-H). *Anal.* Calcd for $C_{20}H_{21}N_3O_7$: C, 57.82; H, 5.11; N, 10.21. Found: C, 57.70; H, 5.11; N, 10.20.

3′,5′-Di-*O*-acetyl-N_4-benzoyl-α-2′-deoxycytidine (3)——Compound **2** (1 g, 2.4 mmol) was suspended in dry acetonitrile (15 ml) and mixed with bis-trimethylsilylacetamide (BSA) (0.6 ml). The mixture was stirred under heating at 70 °C for 10 min, then TMS-triflate (0.52 ml, 3.1 mmol) was added to the resulting clear solution. The mixture was stirred at 70 °C for 3 h, then the solvent was removed under reduced pressure. The residue was treated with cold saturated aqueous sodium bicarbonate (100 ml). The organic layer was washed with distilled water, dried over anhydrous magnesium sulfate, and evaporated to dryness. The yellow viscous residue was dissolved in a small amount of chloroform and applied to a column of silica gel (20 g). Elution was performed with chloroform containing ethanol (20 : 1, v/v). After evaporation of the combined fractions, the product still showed two spots on TLC (silica gel, chloroform–ethyl acetate, 1 : 1, v/v), Rf = 0.72 (β-anomer) and Rf = 0.67 (α-anomer). The α-anomer was crystallized from ethanol to afford colorless needles. 509 mg (51%). mp 173—175 °C. UV λ_{max}^{EtOH} (nm): 271 and 305. ^{1}H-NMR ($CDCl_3$): δ 8.70 (s, 1H, NH), 7.32—8.40 (m, 7H, H-5, H-6 and benzoyl-H), 6.23 (dd, 1H, H-1′, $J_{1'-2'}$ = 2.0 and 7.0 Hz), 5.15 (d, 1H, H-3′), 4.68 (t, 1H, H-4), 4.13 (d, 2H, H-5′), 2.16—3.02 (m, 2H, H-2′), 2.13 (s, 3H, acetyl), 1.97 (s, 3H, acetyl). *Anal.* Calcd for $C_{20}H_{21}N_3O_7$: C, 57.82; H, 5.11; N, 10.12. Found: C, 58.01; H, 5.10, N, 9.95.

α-2′-Deoxycytidine (4)——Compound **3** (509 mg, 1.2 mmol) was dissolved in ethanol saturated with ammonia (60 ml) at 0 °C in a stainless steel container and the sealed vessel was heated at 100 °C for 13 h. After evaporation of the solvent, the residue was dissolved in distilled water and the solution was washed well with chloroform. The aqueous phase was concentrated to a small volume and applied to a column of Dowex 1 (OH^- form) (1.8 cm × 16 cm). Elution was performed with 30% aqueous ethanol. The fractions showing UV absorption at 270 nm were combined and evaporated to give α-2′-deoxycytidine (**4**). Crystallization from ethanol afforded colorless prisms. 230 mg (82%). mp 203—204 °C (lit.[14] 193—194 °C). UV λ_{max}^{EtOH} (nm) (ε): 272 (7500) $\lambda_{max}^{0.01\ N\ HCl}$ (nm): 281. $[\alpha]_D^{18} = -54°$ (c = 1.2, H_2O). CD, $[\theta]_{259\ nm} = -12200$. ^{1}H-NMR ($D_2O$): 6.4 and 6.16 (dd, 1H, H-1′, $J_{1'-2'}$ = 2.4 and 7.3 Hz). *Anal.* Calcd for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49. Found: C, 47.72; H, 5.80; N, 18.58.

3′,5′-Di-*O*-*p*-toluoyl-α-2′-deoxythymidine (6)——BSA (1.8 ml) was added to a solution of 3′,5′-di-*O*-*p*-toluoyl-2′-deoxythymidine (**5**)[15] (6 g, 12.5 mmol) in dry acetonitrile (108 ml), and the mixture refluxed for 5 min. The resulting clear solution was treated with TMS-triflate (2.7 ml, 16 mmol) and the mixture was stirred at 70 °C for 4 h. The solvent was evaporated off and the residue was mixed with cold saturated aqueous sodium bicarbonate (200 ml). The precipitate thus obtained was collected by filtration and washed well with cold water, then dissolved in chloroform (50 ml). The solution was dried over anhydrous magnesium sulfate, then the solvent was removed under reduced pressure to afford a slightly colored foam (4.4 g). Thin-layer chromatographic analysis of this product (silica gel, chloroform–ethyl acetate, 4 : 1, v/v) showed three spots with Rf values of 0.1 (thymine), 0.25 (α-anomer) and 0.31 (β-anomer). The foam was dissolved in a small amount of chloroform and applied to a column of silica gel (80 g). Elution was performed with chloroform–ethyl acetate (8 : 1, v/v) and fractions containing nucleoside with an Rf value of 0.25 on TLC (chloroform–ethyl acetate, 4 : 1, v/v) were combined and evaporated to give a colorless foam. This material was crystallized from ethanol to afford colorless needles. 1.6 g (27%). mp 145—146 °C (lit.[15] 139 °C). ^{1}H-NMR ($CDCl_3$): δ 8.47 (s, 1H, NH), 7.19—7.98 (m, 9H, H-6 and toluoyl-H), 6.36 (dd, 1H, H-1′, $J_{1'-2'}$ = 2.0 and 6.8 Hz), 5.59 (d, 1H, H-3′), 4.89 (t, 1H, H-4′), 4.50 (d, 2H, H-5′), 2.13—3.05 (m, 8H, H-2′ and toluoyl-methyl), 1.88 (s, 3H, methyl-5). *Anal.* Calcd for $C_{26}H_{26}N_2O_7$: C, 65.26; H, 5.48; N, 5.85. Found: C, 65.01; H, 5.52; N, 6.05.

α-2′-Deoxythymidine (7)——Compound **6** (58 mg, 0.12 mmol) was dissolved in 0.1 M methanolic sodium methoxide (4 ml) and the solution was stirred for 50 min at 40 °C. The reaction mixture was treated with distilled water (2 ml). The solution was neutralized with Dowex 50 (H^+ form) and filtered. The filter cake was washed with 50% aqueous ethanol. The combined filtrate and washings were evaporated under reduced pressure, and the residue was dissolved in 5 ml of water. The solution was washed with chloroform (3 ml × 3), then the aqueous layer was evaporated to dryness and the residual colorless solid was crystallized from ethanol. 27 mg (93%). mp 188—189 °C (lit.[15] 187 °C). UV $\lambda_{max}^{H_2O}$ (nm) (ε): 268 (9880). $[\alpha]_D^{18} = +4.4°$ (c = 1, H_2O). CD, $[\theta]_{260\ nm} = -7500$. ^{1}H-NMR ($D_2O$): 7.79 (s, 1H, H-6), 6.18—6.24 (dd, 1H, H-1′, $J_{1'-2'}$ = 2.9 and 7.3 Hz) 3.63—3.79 (m, 2H, H-5′), 2.15—2.83 (m, 2H, H-2′), 1.93 (s, 3H, methyl-H-5). *Anal.* Calcd for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.83; N, 11.56. Found, C, 49.69; H, 5.77; N, 11.44.

α-2′-Deoxyadenosine (8a)——BSA (3.7 ml, 15 mmol) was added to a solution of compound **2** (1.5 g, 3.6 mmol) and N_6-benzoyladenine (2.3 g, 9.6 mmol) in anhydrous acetonitrile (22 ml). The mixture was heated at 70 °C for 15 min under mechanical stirring to afford a clear solution, to which TMS-triflate (0.8 ml, 4.7 mmol) was added. The

mixture stirred at 70 °C for 4 h. The solvent was removed under reduced pressure and the residue was treated with cold aqueous sodium bicarbonate (100 ml) and chloroform (100 ml). After vigorous shaking, the mixture was filtered by suction and the organic layer was separated and dried with anhydrous magnesium sulfate. The solvent was removed *in vacuo*. The residual yellow sticky gum (1.9 g) was dissolved in a small amount of chloroform and chromatographed on a column of silica gel (50 g) with chloroform containing ethanol (15 : 1, v/v). The fractions containing nucleosides ($Rf = 0.50$ for β, and 0.45 for α on silica gel, chloroform–ethanol, 15 : 1, v/v) were combined and the solvent was evaporated off. This anomeric mixture of 3′,5′-di-*O*-acetyl-N_6-benzoyl-2′-deoxyadenosine[16] (1.3 g) was treated with methanol saturated with ammonia at 0 °C (50 ml) in a sealed stainless steel vessel at 40 °C for 12 h. After evaporation of the solvent, the residue was checked by TLC (silica gel, chloroform–ethanol, 4 : 1, v/v). This residue contained two components with *Rf* values of 0.31 and 0.25 which corresponded to the β- and α-anomer, respectively. The residue was dissolved in distilled water (30 ml) and applied to a column of Dowex 1 (OH^- form) (2.5 cm × 31 cm) with distilled water as an eluent. The fractions containing a material with *Rf* 0.25 were combined and evaporated to give pure **8a**. This compound was crystallized from hot ethanol to afford colorless crystals. 260 mg (28% from **2**). mp 210—211 °C. UV $\lambda_{max}^{H_2O}$ (nm) (ε): 262 (14100). $[\alpha]_D^{18} = +67.0°$ ($c = 1$, H_2O). CD, $[\theta]_{260\,nm} = +1000$. *Anal.* Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.88. Found: C, 47.93; H, 5.26; N, 27.87.

9-(α-2-Deoxy-D-ribofuranosyl)-6-methylthiopurine (9a) and 9-(β-2-Deoxy-D-ribofuranosyl)-6-methylthiopurine (9a)——BSA (7.7 ml, 31 mmol) was added to a solution of compound **2** (3.0 g, 7.2 mmol) and 6-methylthiopurine (2.5 g, 15 mmol) in anhydrous acetonitrile, and the mixture was heated at 70 °C for 10 min. Then TMS-triflate (1.5 ml, 8.7 mmol) was added and the whole was heated at 70 °C for 5 h under continuous stirring. The reaction mixture was extracted with 250 ml of chloroform. The organic layer was dried over magnesium sulfate then concentrated to dryness. The slightly yellow gum (3 g) was dissolved in a small amount of chloroform and applied to a column of silica gel (60 g). Elution was performed with chloroform–ethanol (20 : 1, v/v). The UV-absorbing fractions were collected and evaporated to give a colorless gum which contained the α- and β-anomers (2.7 g). This material was treated with 0.05 M methanolic sodium methoxide (20 ml) at room temperature for 75 min under stirring. After addition of 20 ml of 50% aqueous methanol, the solution was treated with Dowex 50 (H^+ form) to remove sodium ions. The solvent was evaporated off and the residue ($Rf = 0.21$ and $Rf = 0.17$, silica gel, chloroform–ethanol 15 : 1, v/v) was dissolved in a small amount of chloroform. This solution was applied to a column of silica gel (120 g). Elution was carried out with chloroform–ethanol (15 : 1, v/v). Fractions containing material with an *Rf* value of 0.21 were combined and evaporated to give a white solid. This was crystallized from ethanol to afford fine needles. 541 mg (27%). This product was identified as the β-anomer by UV, NMR and direct comparison of its IR spectrum with that of an authentic sample,[17] mp 159—159.5 °C (lit.[17] 158—160 °C). UV λ_{max}^{EtOH} (nm): 290 and 243. ^{1}H-NMR (d_6-DMSO): δ 8.73 (s, 1H, H-2), 8.66 (s, 1H, H-8), 6.43 (t, 1H, H-1′), 5.35 (d, 1H, 5′-OH), 4.99 (t, 1H, 3′-OH), 4.30—4.58 (m, 1H, H-3′), 3.74—3.95 (m, 1H, H-4′), 3.40 (m, 2H, H-5′), 2.18—2.96 (m, 5H, H-2′ and $-SCH_3$). *Anal.* Calcd for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; S, 11.36. Found: C, 46.78; H, 4.98; N, 19.91; S, 11.36.

Fractions containing material which showed a TLC spot at $Rf = 0.17$ were combined and evaporated to give a colorless foam. This material was crystallized from ethyl acetate and ethanol to afford cubic crystals. 739 mg (36%). This product was identified as the α-anomer by comparison with the above β-anomer. mp 94—96 °C. UV λ_{max}^{EtOH} (nm): 290 and 242. ^{1}H-NMR (d_6-DMSO): δ 8.74 (s, 1H, H-2), 8.65 (s, 1H, H-8), 6.47 (dd, 1H, H-1′), 5.49 (d, 1H, 5′-OH), 4.87 (t, 1H, 3′-OH), 4.17—4.34 (m, 2H, H-3′ and H-4′), 3.33—3.57 (m, 2H, H-5′), 2.16—2.96 (m, 5H, H-2′ and SCH_3), 1.07 (t, 1.5H, 1/2 EtOH). *Anal.* Calcd for $C_{11}H_{14}N_4O_3S \cdot 1/2C_2H_5OH$: C, 47.20; H, 5.61; N, 18.35; S, 10.50. Found: C, 47.23; H, 5.49; N, 18.61; S, 10.37.

1-(3,5-Di-*O*-benzoyl-2-deoxy-α-D-ribofuranosyl-5-fluoro-N_4-*p*-toluoylcytosine (10a) and Its β-Anomer (10b)——Anomeric 1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose[14] (4.7 g, 12 mmol) was treated with N_4,2-bis-(trimethylsilyl)-5-fluoro-N_4-*p*-toluoylcytosine (10 mmol) in 100 ml of anhydrous acetonitrile, then TMS-triflate (1.7 ml, 13 mmol) was added and the mixture stirred at room temperature for 1.3 h. Thin-layer chromatographic analysis of the reaction mixture (silica gel, chloroform–ethyl acetate, 4 : 1, v/v), showed four spots with *Rf* values of 0.1 (base), 0.34 (α-anomer), 0.54 (β-anomer) and 0.84 (sugar). The reaction mixture was concentrated to a small volume and mixed with 50 ml of saturated aqueous sodium bicarbonate. The mixture was extracted with chloroform (200 ml) and the chloroform layer was evaporated after being dried over magnesium sulfate. The residue was chromatographed on a column of silica gel (120 g). The faster moving β-anomer was crystallized from ethanol, 1.76 g (31%), mp 196—197 °C. The slower moving α-anomer was crystallized from ethanol, 1.02 g (18%), mp 196—197 °C. These compounds were directly used for the next saponification reaction without further characterization.

1-(2-Deoxy-α-ribofuranosyl)-5-fluorocytosine (α-FCdR) (11a)——Compounds **10a** and **10b** were individually saponified as follows. Compound **10a** (0.8 g, 1.4 mmol) was added to methanolic ammonia saturated at 0 °C (25 ml) in a sealed vessel and then the solution was heated at 40 °C for 15 h. The reaction mixture was evaporated to dryness and the residue was washed well with hot chloroform. The solid material was crystallized from ethanol. 150 mg (58%), mp 195—197 °C (lit.[10] 196—197 °C). $[\alpha]_D^{20} = -89°$ ($c = 1$, H_2O). *Anal.* Calcd for $C_9H_{12}FN_3O_4$: C, 44.93; H, 4.93; N, 17.14. Found: C, 44.47; H, 5.01; N, 17.33.

1-(2-Deoxy-β-D-ribofuranosyl)-5-fluorocytosine (FCdR) (11b)——Compound **10b** (0.8 g) was saponified in a similar fashion to give the β-anomer (**11b**) 230 mg (67%), mp 205—206 °C (lit.[10] 200—205 °C). $[\alpha]_D^{20} = +75°$ ($c = 1$,

TABLE III. Physical Data for α-dNTPs

Compound[a]	UV $\lambda_{max}^{H_2O}$ (nm) (ε)	Calcd ε(P)	Found ε(P)	Yield from nucleoside (%)
α-dCTP	272 (7500)	2500	2460	24
α-dTTP	268 (9880)	3300	3450	38
α-dATP	262 (14100)	4700	4670	42

a) These nucleotides appeared to be homogeneous on paper electrophoresis in 0.05 M sodium citrate (pH 3.3) and paper chromatography in ethanol–1 M sodium acetate (1 : 1, v/v).

H_2O). *Anal.* Calcd for $C_9H_{12}FN_3O_4$: C, 44.08; H, 4.93; N, 17.14. Found: C, 43.94; H, 4.59; N, 17.29.

General Procedure for Phosphorylation of α-2′-Deoxyribonucleosides——α-2′-Deoxyribonucleoside (0.25—0.35 mmol) in triethyl phosphate (1.5 ml) was phosphorylated with phosphorus oxychloride (50 μl, 0.55 mmol) under cooling at −10 °C. Thereaction mixture was stirred for 2.5 h at room temperature and then mixed with saturated aqueous sodium bicarbonate (2 ml) and chloroform (2 ml). The chloroform layer was washed with water (1 ml), and the combined aqueous layer was diluted with distilled water to a final volume of 200 ml. The solution was applied to a column of DEAE-cellulose (2.9 cm × 26.5 cm, bicarbonate form), which was eluted with a linear gradient from water (700 ml) to 0.4 M triethylammonium bicarbonate (pH 8.0, 700 ml). The desired 5′-monophosphate was eluted when the concentration of eluent was 0.16 to 0.20 M. The combined fractions were evaporated to dryness, followed by co-evaporation with 50% aqueous ethanol to remove residual triethylammonium bicarbonate and triethylamine. After checking of the homogeneity by paper chromatography and paper electrophoresis, this 5′-monophosphate was converted to the 5′-triphosphate in the following manner. The 5′-monophosphate (20—30 μmol) (as the triethylammonium salt) in anhydrous dimethylformamide (1 ml) was added to *N,N′*-carbonyldiimidazol (20 mg, 0.12 mmol) and the mixture was stirred at room temperature for 3 h. Methanol (4 μl) was added to the reaction mixture to decompose the excess reagent. The solution was stirred for 30 min at room temperature, then added to a solution of tri-*n*-butylammonium pyrophosphate (0.06 mmol) in dry dimethylformamide. After 4 h, the same amount of pyrophosphate was further added and the whole was stirred for 12 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in 50 ml of distilled water and the pH was adjusted with acetic acid to 4.5. Activated carbon (1 g) was added to the solution and the mixture was stirred for 1 h. The mixture was then filtered and the active carbon was washed well with water. The residue was eluted with 50% aqueous ethanol containing 1% ammonium hydroxide (50 ml). The eluate was evaporated to dryness and residual nucleotide was further purified by DEAE-cellulose column chromatography. The nucleotide mixture in 5 ml of water was applied to a column of DEAE-cellulose (2 cm × 13 cm, bicarbonate form). Elution was performed with a linear gradient from water (250 ml) to 0.5 M triethylammonium bicarbonate (pH 8.0, 250 ml). The fractions containing 5′-triphosphate were combined and evaporated to dryness under reduced pressure. The residue was purified by paper electrophoresis and paper chromatography. Yields, phosphorus analysis data and *Rf* values of the products are summarized in Table III.

Assay Method for Growth-Inhibitory Effect of the Compounds on Mouse Leukemic L5178Y Cells in Culture——Mouse leukemic L5178Y cells[18] were grown in RPMI-1629 medium (Nissui Co., Ltd.) supplemented with 10% calf serum (Flow Laboratory, Md, U.S.A.) at 37 °C. One volume of the compound diluted with the same medium was added to 9 volumes of the culture containing between 1.2×10^5 to 1.7×10^5 L5178Y cells/ml. After incubation for 48 h at 37 °C in 5% carbon dioxide, the number of remaining cells was counted with a cell counter (Toa micro cell counter, model 1002).

Assay of DNA Polymerase α Activity——DNA polymerase α from cherry salmon testes 0.05 unit; specific activity, 78000 units/mg protein[11] was added to a reaction mixture (25 μl) containing 50 mM Tris-HCl (pH 8.0), 4 mM $MgCl_2$, 1 mM dithiothreitol (DTT), dGTP, dCTP, dATP (0.1 mM each), dTTP (25 μM) and ^{3}H-dTTP (8 μCi/ml), 0.4 mg/ml bovine serum albumin, activated salmon sperm DNA (0.1 mg) and 14% glycerol, then 0—100 μM of the α-2′-deoxyribonucleoside 5′-triphosphate was mixed with the solution and the whole was incubated at 20 °C for 20 min. The mixture was transferred to a Whatman DE-81 paper disc, which was washed with 5% sodium hydrogen phosphate, water, ethanol and ether. The remaining radioactivity was counted with a Beckman LS-230 liquid scintillation counter.

Assay of DNA Polymerase β Activity——Method A: A reaction mixture (25 μl) of 50 mM glycine–KOH (pH 9.5), 4 mM $MgCl_2$, 80 mM KCl, 1 mM DTT, 0.1 mM dATP, dGTP, and dCTP, 25 μM dTTP, 8 μCi/ml of ^{3}H-dTTP, bovine serum alubumine (0.4 mg/ml), 14% glycerol and 0.033 unit of cherry salmon DNA polymerase β (specific activity, 190000 units/mg protein)[11] was incubated with 0—100 μM inhibitor at 25 °C for 20 min. In the case of α-dCTP as an inhibitor, dATP, dGTP, dTTP (0.1 mM each), 25 μM dCTP and 8 μCi/ml of ^{3}H-dCTP was replaced with ^{3}H-dTTP in the above reaction mixture. Work-up was similar to that in the case of DNA polymerase α.

Method B: In the case of synthetic template-primer for DNA polymerase β, the assay mixture contained 50 mM

glycine–KOH (pH 9.5), 0.5 M $MnCl_2$, 0.08 M KCl, 0.1 mM DTT, 25 μM dTTP, 8 μCi/ml ^{3}H-dTTP, poly rA (0.8 mg/ml), oligo dT (0.04 mg/ml). Other components, concentrations and incubation conditions were the same as in Method A.

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