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Synthetic Nucleosides and Nucleotides. XVIII.[1] Synthesis and Cytostatic Activity of 5-Fluoropyrimidine Nucleosides of 3-Amino-3-deoxy-β-D-ribofuranose and Related Compounds

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Treatment of 1,2:5,6-di-O-isopropylidene-3-amino-3-deoxy-α-D-allofuranose (**1**) with trifluoroacetic anhydride afforded crystalline 3-trifluoroacetamido derivative (**2**) in good yield. Selective removal of the 5,6-O-isopropylidene group of **2** by treatment with 70% acetic acid followed by oxidation with periodate and subsequent reduction with sodium borohydride gave crystalline 1,2-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-ribofuranose (**3**) in good yield. *p*-Nitrobenzoylation of **3** followed by acetolysis afforded crystalline 1,2-di-O-acetyl-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-β-D-ribofuranose (**5**). Coupling of the resulting 1-O-acetyl sugar with bis-trimethylsilylated derivatives of N_4-acyl-5-fluorocytosines, N_4-acylcytosines, 5-fluorouracil and N_4-acetyl-5-methylcytosine using $SnCl_4$ afforded the corresponding fully acylated nucleosides (**7**). Saponification of **7** gave 3-amino-3-deoxy-β-D-ribonucleosides (**8a**—**8d**). Alternatively, 2,4-dimethoxy-5-methylpyrimidine was also coupled with **5** followed by treatment with ammonia to give **8d**.

The nucleosides (**8a**—**8d**) thus obtained were examined for cytostatic effect on mouse leukemic L5178Y cells. The compounds tested were active against this system and their ED_{50} values were 0.7 μg/ml, 7 μg/ml, 16 μg/ml and 60 μg/ml, respectively.

Keywords——5-fluoropyrimidine nucleosides; 3′-amino-3′-deoxy-5-fluorocytidine; 3′-amino-3′-deoxy-5-fluorouridine; 3′-amino-3′-deoxycytidine; synthesis; stannic chloride; cytostatic activity; L5178Y cells

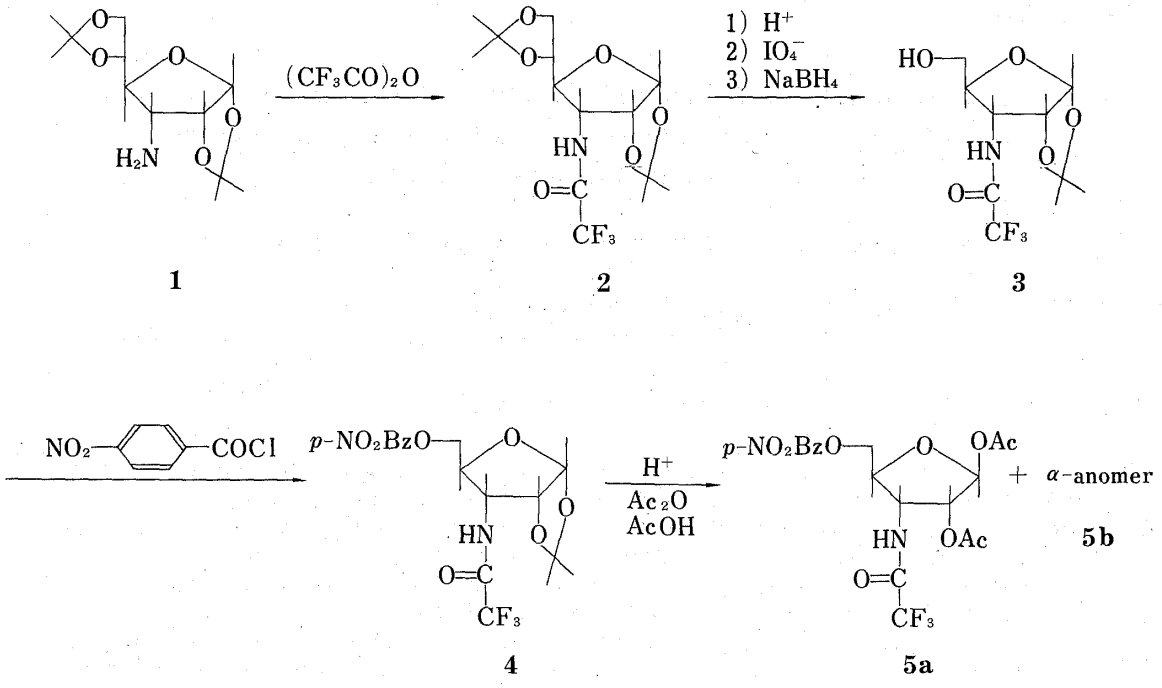
3′-Amino-3′-deoxyadenosine and some of the 3′-aminonucleosides related to the antibiotic puromycin possess various biological activities.[3] On the other hand, 5-fluorouridine and 5-fluorocytidine show remarkable antitumor activities against experimental tumor systems.[4–6] As a part of our program on the design and synthesis of nucleoside analogs having antitumor activities, the synthesis of 5-fluoropyrimidine nucleosides bearing a 3-amino-3-deoxy-β-D-ribofuranose moiety was undertaken, since they correspond to hybrid structures of these active nucleoside groups.

In this paper, we describe the synthesis of various pyrimidine nucleosides having a 3-amino-3-deoxy-β-D-ribofuranose moiety including 3′-amino-3′-deoxy-5-fluorocytidine (**8a**) and 3′-amino-3′-deoxy-5-fluorouridine (**8c**). We also report the growth-inhibitory effects of these compounds on mouse leukemic L5178Y cells in culture.

Synthesis

Even though various procedure have been reported for the synthesis of 3′-amino-3′-deoxyadenosine,[7–11] synthesis of appropriately protected 3-amino-3-deoxy-D-ribose derivatives required many steps and reported yields are extremely low. We have developed a convenient synthesis of the sugar-derivative from 1,2:5,6-di-O-isopropylidene-3-amino-3-deoxy-α-D-allofuranose (**1**)[12] followed by condensation with trimethylsilylated pyrimidine to afford fully protected amino sugar nucleosides.

For the present study, compound **1**[12] was selected as the starting material because **1** can easily be obtained from D-glucose in four steps. Thus, compound **1** was treated with trifluoro-



Ac=acetyl, Bz=benzoyl

Chart 1

acetic anhydride in dichloromethane in the presence of anhydrous pyridine to give crystalline 1,2: 5,6-di-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-allofuranose (**2**), mp 154—155°C, in 80% yield.

For selective removal of the 5,6-isopropylidene group, **2** was treated with 70% aqueous acetic acid at 50°C for 30 min to give 1,2-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-allofuranose as a white powder in almost quantitative yield. Treatment of this material with sodium periodate followed by reduction with sodium borohydride in aqueous methanol at room temperature afforded crystalline 1,2-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-ribofuranose (**3**), mp 153—154°C, in 60% isolated yield based on the amount of **2**. The structure of this compound was identified by comparison of its infrared (IR) and nuclear magnetic resonance (NMR) spectra with those of an authentic specimen from D-xylose[13] and by mixed melting point determination. Compound **3** was treated with *p*-nitrobenzoyl chloride in pyridine to afford crystalline 1,2-O-isopropylidene-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-α-D-ribofuranose (**4**), mp 127—128°C, in 79% yield. By treating compound **4** with acetic acid–acetic anhydride mixture (50: 3, v/v) in the presence of sulfuric acid, 1,2-di-O-acetyl-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-D-ribofuranose (**5**) in nearly quantitative yield. The β-anomer (**5b**) of this sugar (**5a**), mp 171—172.5 °C, could be crystallized from ethyl acetate and *n*-hexane mixture. The ratio of β-anomer (**5a**) to α-amomer (**5b**) in the product was 5: 1 based on its NMR spectra. The configuration of **5a** was assigned from its NMR spectrum, which showed a sharp singlet at δ 6.18 ppm, assignable to the C_1 proton and the coupling constant $J_{1'-2'}$ which was nearly 0.

Nucleosides were synthesized by coupling of **5** with trimethylsilyl derivatives of N_4-acetyl-5-fluorocyctosine (**6a**), N_4-butyryl-5-fluorocytosine (**6b**), N_4-acetylcytosine (**6c**) and N_4-butyrylcytosine (**6d**) in the presence of stannic chloride in anhydrous acetonitrile.[14–17] After usual workup, several protected nucleosides, 2′-O-acetyl-3′-deoxy-3′-trifluoroacetamido-5′-O-*p*-nitrobenzoyl-N_4-acetyl-5-fluorocytidine (**7a**), mp 234—236°C (85%), 2′-O-acetyl-3′-deoxy-3′-trifluoroacetamido-5′-O-*p*-nitrobenzoyl-N_4-acetylcytidine (**7b**), mp 226—227°C (74.5%) and 2′-O-acetyl-3′-deoxy-3′-trifluoroacetamido-5′-O-*p*-nitrobenzoyl-N_4-butyrylcytidine (**7c**), mp

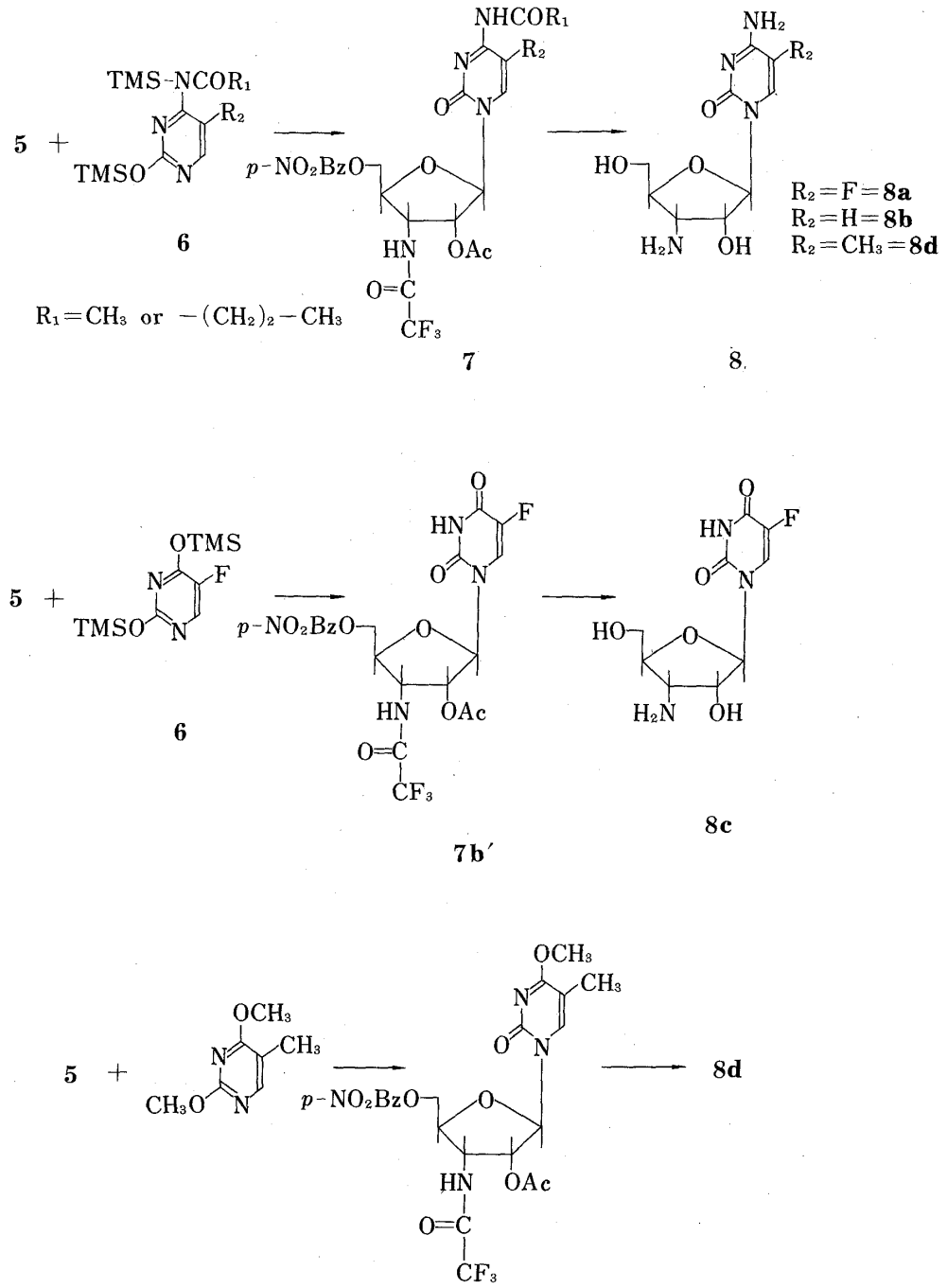


Chart 2

239—240.5°C (56.9%), were obtained as crystals. Other nucleosides did not crystallize from several solvent systems, though they were thin layer chromatographycally homogenous (silica gel, chloroform–ethyl acetate, 9: 1, v/v). To remove the protecting groups on the base and sugar moieties, compounds **7** were treated with methanolic ammonia or sodium methoxide in methanol to give free nucleosides (**8**). The product were finally purified by column chromatography on Dowex 1 (OH^- form). 1-(3′-Amino-3′-deoxy)-β-D-ribofuranosyl-5-fluorouracil (**8c**) was synthesized by coupling of 2,4-bis-trimethylsilyloxy-5-fluoropyrimidine with **5** followed by deblocking of acylated nucleoside (**7b′**).

For the synthesis of 5-methylcytosine nucleoside (**8d**), two methods were employed. In the first, 2,4-bis-trimethylsilyl-N_4-acetyl-5-methylcytosine was coupled with **5** in a manner similar to that described above, followed by deblocking with sodium methoxide to afford **8d**. The second method involved coupling of 2,4-dimethoxy-5-methylpyrimidine with **5**, followed by ammonolysis to give **8d**. The structures of these compounds were confirmed by elemental analysis, ultraviolet (UV) and NMR spectra and ninhydrin reaction. The β-configuration was assigned to **8a**—**d** 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 **8a**—**d** at 270 nm to 280 nm were in accord with those found for 1-β-D-pentofuranosyl pyrimidine derivatives. It should be noted that, throughout the coupling reaction of **5** and **6**, only the β-anomer was isolated from the reaction mixture.

Growth-Inhibitory Effect of Nucleoside Analogs on Mouse Leukemic L5178Y Cells in Culture

The results of cytostatic activity testing of newly synthesized compounds are shown in Table I. The growth-inhibitory activity (ED_{50}) is expressed as the concentration causing 50% inhibition of the cell growth.

Table I. Growth-inhibitory Effect of the Compounds on L5178Y Cells

Compound	Dose (μg/ml) T/C (%)[a]										ED_{50}[b] (μg/ml)
	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.19	
8a				31	50	66	93				7
8b						23	30	46	71	94	0.7
8c			38	59	84	106					16
8d	34	62	86	98							60

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

As can be seen in Table I, 3′-aminonucleosides having various aglycones showed strong growth-inhibitory effects. Among the compounds tested, cytosine and 5-fluorocytosine nucleosides (**8a** and **8b**) were most potent. The 5-fluorouracil counterparts (**8c**) showed less activity and the 5-methylcytosine nucleoside (**8d**) showed only moderate activity. It is especially noteworthy that compound **8b** was active against L5178Y cells. Even though this compound was first synthesized more than twenty years ago,[18] biological activities of this compound have not been reported so far. Compound **8b** as well as **8a**, shows potent antileukemic activity *in vitro*, and *in vivo* experiments are in progress.

Experimental

Melting points were determined on a Yanaco model 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. IR spectra were recorded on a 215 Hitachi grating infrared spectrometer. NMR spectra were obtained on a Hitachi R20-B high resolution NMR spectrometer with tetramethylsilane as an internal standard. Thin-layer chromatography was performed with precoated silica gel 60 F_{254} plates (Merck) and column chromatography was performed with Wako-gel C-200.

1,2: 5,6-Di-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-allofuranose (**2**)——1,2: 5,6-Di-O-isopropylidene-3-amino-3-deoxy-α-D-allofuranose (**1**) (4.84 g) was dissolved in a mixture of dichloromethane (50 ml) and anhydrous pyridine (50 ml). To this solution, trifluoroacetic anhydride (5.26 ml) was added with vigorous stirring and cooling in an ice-bath. The mixture was stirred at room temperature until the starting material (**1**) had disappeared, *i.e.*, the ninhydrin reaction became negative. After 3.5 h, the reaction was nearly completed and the solvent was removed under reduced pressure. The residue was dissolved in 50 ml of dichloromethane and the solution was washed with water (30 ml), followed by saturated sodium bicarbonate

(30 ml×2) and finally with water (30 ml). The dichloromethane layer was dried over magnesium sulfate and evaporated to dryness. The product was homogeneous on a thin-layer chromatogram (Rf=0.50, ethyl acetate, silica gel). The solid was crystallized from di-isopropyl ether to give 4.5 g (61%) of crystals. mp 154—155°C. *Anal.* Calcd for $C_{14}H_{20}O_6F_3N$: C, 47.32; H, 5.63; N, 3.94. Found: C, 47.22; H, 5.43; N, 4.01.

1,2-O-Isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-ribofuranose (3)——Compound **2** (3.55 g, 10 mmol) was dissolved in 70% acetic acid (50 ml) and the solution was heated at 50°C for 30 min. Thin-layer chromatographic analysis of the reaction mixture showed only one spot as visualized by periodate-benzidine reagent. The solvent was removed under reduced pressure at a bath temperature below 30°C. The residue was treated with 50% ethanol (20 ml) and evaporated to dryness. This process was repeated three times to give 3.1 g of crude 1,2-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-allofuranose as a colorless solid. Sodium periodate (2.14 g) was added to a solution of the above compound in 60 ml of distilled water and the pH was adjusted to 7. After the mixture had been stirred at room temperature for 1 h, additional sodium periodate was added and the solution was stirred for 2 h. At this stage, the starting material had nearly disappeared as judged by TLC (silica gel, ethyl acetate). To quench excess periodate, ethylene glycol (1.5 ml) was added to the solution. The solvent was then removed under reduced pressure and the residual solid was extracted with hot chloroform (50 ml×3). The chloroform was evaporated off *in vacuo* to afford the 5-aldehyde derivative as a light yellow syrup. This compound was dissolved in 50% aqueous methanol (50 ml). Sodium borohydride (760 mg) in 10 ml of water was added to the solution and the pH was maintained at 8 by continuous addition of acetic acid. After 3 h, the solvent was evaporated off under reduced pressure. The residue contained a small amount of N-deacylated side product, so 0.5 ml of trifluoroacetic anhydride and 10 ml of anhydrous pyridine was added for re-acylation of free amino groups. After 2 h, the solvent was evaporated off and 100 ml of methanol was added to the residue. The solution was refluxed for 1 h, then the methanol was evaporated off. The residue was crystallized from isopropanol and *n*-hexane mixture to give 1.6 g (57%) of colorless needles. mp 153—154°C. This compound was identified as 1,2-O-isopropylidene-3-deoxy-3-trifluoroacetamido-α-D-ribofuranose (3) by mixed melting point determination and comparison of its IR spectra with that of an authentic specimen,[13] which was prepared from D-xylose. The same experiment was repeated several times and the average yield was 55 to 60% based on the amount of **2**.

1,2-O-Isopropylidene-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-α-D-ribofuranose (4)——*p*-Nitrobenzoyl chloride (3.01 g, 16.2 mmol) in anhydrous benzene (40 ml) was added to compound 3 (3.86 g, 13.5 mmol) in 80 ml of anhydrous pyridine at a temperature below 5°C under exclusion of atmospheric moisture. After being stirred for 2 h at room temperature, the mixture was poured into ice-water mixture (300 ml). The aqueous phase was extracted with benzene (200 ml) and chloroform (200 ml×2). The extracts were combined, washed with water and dried. The solvent was evaporated off and the gummy residue was crystallized from a mixture of ethyl acetate and *n*-hexane to afford 3.45 g of **4** as colorless needles. The mother liquor was evaporated to dryness and the residue was applied to a column of silica gel (20 g). Elution with benzene–chloroform (1: 1, v/v) afforded an additional 1.16 g of **4**. Total yield of **4** was 4.61 g (78.6%), mp 127.5—129°C.This sample was identified by mixed melting point determination and comparison of its IR spectra with that of authentic material.[13]

1,2-Di-O-acetyl-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-β-D-ribofuranose (**5a**)——Compound **4** (4.57 g, 10.2 mmol) was dissolved in a mixture of 100 ml of acetic acid and 6 ml of acetic anhydride. Concentrated sulfuric acid (2.5 ml) in 5 ml of acetic acid was added dropwise to the solution with stirring and cooling in an ice-bath. The mixture was stirred for 15 h at room temperature and then poured into ice-water mixture (400 ml) containing 50 g of sodium acetate. The mixture was extracted with chloroform (300 ml×3) and the extracts were combined and washed successively with water, 5% sodium bicarbonate and then water (300 ml×3 each). The chloroform layer was dried over magnesium sulfate and evaporated to dryness to give a colorless solid, which was crystallized from ethyl acetate and *n*-hexane to give 2.17 g (81.6%) of **5a**, mp 171—172.5°C. The mother liquor contained the α-anomer (**5b**) but it could not be crystallized. *Anal.* Calcd for $C_{18}H_{17}F_3N_2O_{10}$: C, 45.19; H, 3.58; N, 5.86. Found: C, 45.10; H, 3.71; N, 5.91. IR ν_{max}^{KBr} (cm^{-1}): 3320 (NH–CO–CF_3), 3100 (–CH–), 1760—1730 (amide –CO–), 1710 (ester –CO–), 1200 and 715 (–CF_3). $[\alpha]_D^{23}$= +18.1 (c=1, $CHCl_3$). NMR ($CDCl_3$) δ: 9.60 (m, NH, 1H), 8.34 (m, benzene ring, 4H), 6.18 (s, 1-H, 1H), 5.37 (d, 2H, 1H), 4.67 (m, 3, 4, 5, H, 4H), 2.16 and 1.99 (s, acetyl-CH_3 6H), J_{1-2}=0.

1-(2′-O-Acetyl-3′-deoxy-3′-trifluoroacetamido-5′-O-*p*-nitrobenzoyl-β-D-ribofuranosyl-N_4-acetyl-5-fluorocytosine (**7a**)——A solution of **5a** (1.59 g, 3.32 mmol) and trimethylsilylates N_4-acetyl-5-fluorocytosine [derived from N_4-acetyl-5-fluorocytosine (4 mmol)] in dry acetonitrile (40 ml) was treated with $SnCl_4$ (0.86 g, 3.32 mmol) in 6.9 ml of dry acetonitrile under stirring and cooling below 10°C. After the solution had been stirred at room temperature for 5 h, 0.1 equivalent of $SnCl_4$ was added and stirring was continued for an additional 4 h. Sodium bicarbonate (2 g) and distilled water (20 ml) were then added to the solution. After vigorous evolution of carbon dioxide had ceased, the solvent was removed under reduced pressure. The residue was treated with dry benzene (5 ml) and the mixture was evaporated to dryness again. This process was repeated twice. The glassy residue was extracted with boiling acetone (100 ml×4). The combined acetone extracts were evaporated to dryness to give a light yellow gum, which was redissolved in 5 ml of chloroform and applied to a column of silica gel (80 g). The column was first washed with chloroform (300 ml) which eluted a small amount of sugar. Next, elution with chloroform–methanol (9: 1, v/v) gave a fraction

containing the fully acylated nucleoside. The solvent was evaporated off *in vacuo* and the residue was crystallized from boiling ethanol to give 1.2 g (72%) of **7a** as fine needles, mp 234—236°C. *Anal.* Calcd for $C_{22}H_{19}F_4N_5O_{10}$: C, 44.82; H, 3.28; N, 11.88. Found: C, 44.76; H, 3.34; N, 11.67. UV λ_{max}^{EtOH} (nm); 251 and 303. IR ν_{max}^{KBr} (cm^{-1}): 3100 (NH) 1760 (–$COCF_3$), 1730 (benzoyl –CO–) 1710 (acetyl –CO–), 1670 (–$NHCOCH_3$), 1190 and 716 (–CF_3). NMR (DMSO, d_6) δ: 10.8 (m, N_4–NH, 1H), 9.75 (d, NH, 1H), 8.35 (s, aromatic, H6), 5.96 (d, H-1′, 1H, $J_{1'-2'}=3$ Hz), 5.59 (dd, H-2′, 1H), 4.68 (s, H-3′, 4′, 5′, 5″, 4H), 2.1 and 2.28 (s, acetyl, 6H). Similar experiments using N_4-butyryl-5-fluorocytosine gave the corresponding fully acylated nucleoside as gum which was homogeneous on thin-layer chromatography (silica gel, chloroform–ethanol, 9: 1, v/v).

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-5-fluorocytosine (3′-Amino-3′-deoxy-5-fluorocytidine) (8a)——To a solution of **7a** (1.85 g) in 30 ml of absolute methanol was added 1 M sodium methoxide in methanol (20 ml). The mixture was stirred at room temperature for 14.5 h, then the solvent was removed under reduced pressure and the residue was treated with a 1: 1 mixture of ethyl acetate and distilled water (40 ml). The aqueous phase was separated and neutralized with Dowex 50 (H^+-form). The solvent was removed and the residue was dissolved in 10 ml of 30% aqueous methanol. The solution was applied to a column of Dowex 1 (OH^- form, 2.5 cm × 18 cm) with aqueous methanol as the eluent. The fractions containing amino-nucleoside were combined and evaporated to dryness *in vacuo*. The residual solid was crystallized from ethanol to give 340 mg of crystals. mp 176.5—178°C. *Anal.* Calcd for $C_9H_{13}FN_4O_4$: C, 41.54; H, 5.04; N, 21.53. Found: C, 41.66; H, 5.22; N, 21.31. MS *m/e*: 130 (B+1) 129 (B), 113, 101, 87. NMR (DMSO, d_6) δ: 8.40 (d, H-6, 1H, $J_{5,6}=7.5$ Hz), 7.61 (b, N_4–NH_2, 2H), 5.58 (s, 1′-H, 1H). CD (nm): $[\theta]_{280}=+20600$. A similar experiment using N_4-butyryl-5-fluorocytosine as the starting base gave the amino-nucleoside **8a** in 60% yield.

1-(2-O-Acetyl-3-deoxy-3-tri-floroacetamido-5-O-*p*-nitrobenzoyl-α-D-ribofuranosyl)-N_4-acetylcytosine (7b) and 1-(2-O-Acetyl-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-β-D-ribofuranosyl)-N_4-butyrylcytosine (7c)——A solution of trimethylsilyl-N_4-acetylcytosine (1 mmol) and **5a** in anhydrous acetonitrile (20 ml) was treated with $SnCl_4$ (115 μl, 1 mmol) in acetonitrile (5 ml) under stirring. The mixture was stirred for 6 h then evaporated to dryness. After workup as described in the above section, the fully acylated nucleoside was obtained as a colorless glass. This product was crystallized from boiling ethanol to give fine needles, 311 mg (74.5%) of **7b**, mp 226—227°C (lit.[9b] 224—227°C). *Anal.* Calcd for $C_{22}H_{20}F_3N_5O_{10}$: C, 46.24; H, 3.53; N, 12.86. Found: C, 46.34; H, 3.70; N, 12.68. UV λ_{max}^{EtOH} (nm): 295. IR ν_{max}^{KBr} (cm^{-1}): 3200 (NH), 1740 (–O–$COCH_3$) 1720 (benzoyl, –CO–), 1710 (–NH–CO–CF_3). NMR (DMSO, d_6) δ: 11.0 (s, N_4–NH, 1H), 9.75 (d, 3′-NH, 1H), 8.38 (s, aromatic and H-6, 5H), 7.24 (d, H-5, 1H), 5.95 (d, H-1′, 1H, $J_{1'-2'}=3$ Hz), 5.60 (m, H-2′, 1H), 4.68 (s, H-3′, 4′, 5′, 5″, 4H), 6.11 (s, acetyl, 6H).

In a similar experiment employing trimethylsilyl-N_4-butyrylcytosine, **7c** was obtained in a yield of 56.9%, mp 239—240.5°C. *Anal.* Calcd for $C_{24}H_{24}N_5O_{10}$: C, 48.08; H, 4.01; N, 9.51. Found: C, 47.96; H, 4.11; N, 9.99. UV λ_{max}^{EtOH} (nm): 297. NMR (DMSO, d_6) δ: 10.93 (s, N_4–NH, 1H), 9.70 (d, 3′-NH, 1H), 8.34 (s, aromatic, 4H), 8.20 (d, H-6, 1H, $J_{5,6}=7.5$ Hz), 7.26 (d, H-5, 1H), 5.93 (d, 1′-H, 1H, $J_{1'-2'}=2.2$ Hz), 5.60 (dd, 2′-H, 1H), 4.65 (m, H-3′, 4′, 5′, 4H), 2.35 (s, –NH–CO–CH_2–, 2H), 2.10 (s, acetyl CH_3, 3H), 1.52 (m, base –CH_2–CH_3, 2H), 0.90 (t, base –CH_2–CH_2–CH_3, 3H).

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-cytosine (3′-Amino-3′-deoxycytidine) (8b)——Absolute methanol saturated with ammonia (25 ml) was added to a solution of **7c** (500 mg, 0.83 mmol) in absolute methanol (15 ml) in a sealed vessel. The solution was heated at 100°C for 21 h, then the solvent was removed under reduced pressure and the residue was redissolved in 20 ml of distilled water. The solution was extracted with ethyl acetate (20 ml × 2). Thin-layer chromatogrophic analysis (Avicel SF cellulose plate, *n*-butanol–acetic acid–water, 8: 1: 2, v/v/v) of the solution revealed only one spot ($Rf=0.26$) which was positive to ninhydrin spray. The aqueous layer was concentrated to a small volume and applied to a column of Dowex 1 (OH^- form, 1.1 cm × 22 cm). Elution with 10% methanol provided nucleoside (**8b**), between 240 ml and 330 ml. The combined fractions were evaporated to dryness under reduced pressure to give **8b** (145 mg) (72%) as a colorless solid. This product was crystallized from boiling ethanol to give fine needles, mp 225—227°C (lit.[18] 221—223°C, lit.[9b] 220—221°C). *Anal.* Calcd for $C_9H_{14}N_4O_4$: C, 44.62; H, 5.83; N, 23.13. Found: C, 44.40; H, 5.99; N, 22.86. UV $\lambda_{max}^{H_2O}$ (nm): 270. CD (nm) $[\theta]_{271}$: +19300. A similar experiment starting from **7b** afforded the same nucleoside **8b** in 68% yield.

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-5-fluorouracil (3′-Amino-3′-deoxy-5-fluorouridine) (8c)——A solution of **5a** (3.2 mmol) and 2,4-bis-trimethylsilyloxy-5-fluoropyrimidine (967 mg, 3.5 mmol) [derived from 520 mg of 5-fluorouracil] in 40 ml of anhydrous acetonitrile was treated with $SnCl_4$ (0.92 g, 3.5 mmol) in 7.4 ml of acetonitrile under stirring below 10°C. The mixture was stirred at room temperature for 7 h, then sodium bicarbonate (2 g) and distilled water (3 ml) were added and the solvent was removed. The residue was extracted with hot chloroform (100 ml × 4). After removal of the solvent, a slightly yellow foam (1.86 g) was obtained. This material was purified on a column of silica gel (50 g) with chloroform and chloroform–methanol (9: 1, v/v) as eluents to give protected **8c** as a TLC-homogeneous gum (1.43 g, 89.1%). This compound was treated with absolute methanol saturated with ammonia (50 ml) in a stainless steel sealed vessel at 100°C for 20.5 h. The solvent was removed *in vacuo* and the residue was dissolved in distilled water (25 ml). After being extracted with ethyl acetate (25 ml × 2), the aqueous phase was evaporated to dryness under reduced pressure to afford a colorless syrup, which was dissolved in 30% methanol (10 ml) and applied

to a column of Dowex 1 (OH^- form) (3.2 cm × 11 cm). The column was washed with 30% methanol and the desired product was eluted with 0.25 M triethylammonium bicarbonate (pH 8.0) in 30% methanol. The fractions containing the desired product were combined and evaporated to dryness. Aqueous 30% ethanol (20 ml) was added to the residue and the solution was evaporated down again to remove residual triethylammonium bicarbonate and triethylamine. The residue was treated with active carbon and evaporated to dryness to give **8c** as a colorless foam (342 mg) (51.3% yield based on the amount of 5-fluorouracil). The foam was crystallized from ethanol to give colorless needles, mp 119—120°C. *Anal.* Calcd for $C_9H_{12}FN_3O_5$: C, 41.37; H, 4.60; N, 16.09. Found: C, 41.51; H, 4.48; N, 15.98. UV $\lambda_{max}^{H_2O}$ (nm): 268. CD (nm) $[\theta]_{270}$: +14200.

1-(3-Amino-3-deoxy-β-D-ribofuranosyl)-5-methylcytosine (3′-Amino-3′-deoxy-5-methylcytidine) (8d)——Method A: A solution of 2,4-dimethoxy-5-methylpyrimidine (320 mg, 2 mmol) and **5a** (826 mg, 1.7 mmol) in 40 ml of anhydrous acetonitrile was treated with $SnCl_4$ (660 mg, 2.5 mmol) in 10 ml of acetonitrile. The mixture was stirred at room temperature for 18 h, then sodium bicarbonate (1.5 g) and distilled water (3 ml) were added and the mixture was evaporated to dryness. The glassy residue was extracted with boiling acetone (100 ml × 3) and the extract was concentrated. The residue was purified on a column of silica gel (40 g) with chloroform–ethyl acetate (9: 1, v/v) (300 ml) and chloroform–methanol (9: 1, v/v) as eluents to give the desired blocked nucleoside which was tentatively assigned as 1-(2-O-acetyl-3-deoxy-3-trifluoroacetamido-5-O-*p*-nitrobenzoyl-β-D-ribofuranosyl)-4-methoxy-5-methyl-1,2-dihydropyrimidin-2-one. A solution of this syrup (600 mg) in absolute methanol saturated with ammonia (30 ml) was heated at 100°C for 20 h in a stainless steel vessel, then solvent was removed. The residue was dissolved in 10 ml of water and extracted with ethyl acetate (20 ml × 2). The aqueous layer was concentrated to a small volume and applied to a column of Dowex 1 (OH^- form) (1.1 cm × 23 cm). Elution with 30% ethanol gave 137 mg of **8d** as a colorless solid. The product thus obtained was crystallized from ethanol to give tiny needles, mp 138—140°C. *Anal.* Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.87. Found: C, 46.65; H, 6.35; N, 21.69. UV $\lambda_{max}^{H_2O}$ (nm): 275. CD (nm) $[\theta]_{280}$: +19800.

Method B: As similar experiment using bis-N_4,2-trimethylsilyl-N_4-acetyl-5-methylcytosine (1 mmol) and **5a** (1 mmol) gave 118 mg of **8d** which was identical with the specimen prepared by Method A.

Assay Method for Growth-inhibitory Effect of the Compounds 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 remaining cells was counted with a cell counter (Toa micro cell counter, model 1002).

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References and Notes

1) Part XVII of this series: M. Saneyoshi, J. Tohyama, C. Nakayama, S. Takiya, and M. Iwabuchi, *Nucleic Acids Res.*, **9**, 3129 (1981). Part of this work was reported at the 97th Annual Meeting of the Pharmaceutical Society of Japan at Tokyo, 1977 and at the 100th Annual Meeting of the Pharmaceutical Society of Japan at Tokyo, 1980.
2) Present address: *Virology Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo, 105, Japan.*
3) R.J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, pp. 76—91 (1970).
4) C. Heidelberger, *Cancer Res.*, **30**, 1549 (1970) and papers cited therein.
5) J.H. Burchenal, V.E. Currie, M.D. Dowling, J.J. Fox, and I.H. Krakoff, *Ann. N. Y. Acad. Sci.*, **255**, 202 (1975) and papers cited therein.
6) M. Saneyoshi, M. Inomata, and F. Fukuoka, *Chem. Pharm. Bull.*, **26**, 2990 (1978).
7) B.R. Baker, R.E. Schaub, and H.M. Kissman, *J. Am. Chem. Soc.*, **77**, 5911 (1955).
8) E.J. Reist and B.R. Baker, *J. Org. Chem.*, **23**, 1083 (1958).
9) *a*) F.W. Lichtenthaler, P. Voss, and A. Heerd, *Tetrahedron Lett.*, **1974**, 2141; *b*) F.W. Lichtenthaler, E. Cuny, T. Morino, and I. Rychlik, *Chem. Ber.*, **112**, 2588 (1979).
10) R. Mengel and H. Wiedner, *Chem. Ber.*, **109**, 433 (1976).
11) A.V. Azhayev and J. Smrt, *Coll. Czech. Chem. Commn.*, **43**, 1520 (1978).
12) K. Onodera, S. Hirano, and N. Kashimura, *Carbohydr. Res.*, **6**, 276 (1968).
13) A.N. Fujiwara, E.M. Acton, and L. Goodman, *J. Heterocycl. Chem.*, **7**, 891 (1970).
14) U. Niedballa and H. Vorbrüggen, *Angew. Chem.*, **9**, 461 (1970).
15) U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, **39**, 3625 (1974).
16) C. Nakayama, H. Machida, and M. Saneyoshi, *J. Carbohydr. Nucleosides and Nucleotides*, **6**, 295 (1979).
17) M. Saneyoshi and E. Sato, *Chem. Pharm. Bull.*, **27**, 2518 (1979).
18) H.M. Kissman and M.T. Weiss, *J. Am. Chem. Soc.*, **80**, 2575 (1958).