Synthesis, Antiviral, Antibacterial and Antitumor Cell Activities of 2′-Deoxy-2′-fluoropuromycin

Tokumi MARUYAMA,*,[a] Kunihiko UTSUMI,[a] Hiroshi TOMIOKA,[a] Masumi KASAMOTO,[a] Yoshiko SATO,[a] Tozef ANNE,[b] and Erik DE CLERCQ[b]

Department of Pharmaceutical Sciences, Tokushima Bunri University,[a] Yamashiro-cho, Tokushima 770, Japan and Rega Institute for Medical Research, Katholieke Universiteit Leuven,[b] Minderbroedersstraat 10, B-3000 Leuven, Belgium. Received November 30, 1994; accepted January 24, 1995

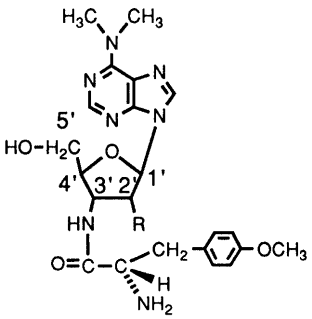
A procedure for the synthesis of 2′-deoxy-2′-fluoropuromycin (1b) was developed. Ring opening of the lyxo-epoxide (4) or nucleophilic displacement of the 3′-*O*-mesylate (5) by an azide ion afforded two azido nucleosides, 6a and 7a. The major product (7a) was reacted with diethylaminosulfur trifluoride (DAST) to give the 2′-fluoronucleoside (8), which was converted to the 3′-aminonucleoside (9) by hydrogenation. Compound 9 was condensed with an amino acid by the conventional method and subsequently deprotected by acid to give 1b. Compounds 1b, 6b and 7b exhibited no selective antiviral activity against several DNA and RNA viruses. Compound 1b had weak antibacterial activity (minimum inhibitory concentration approximately 25—50 μg/ml) and was cytotoxic to several tumor cell lines (L1210, Molt 4, CEM) at a concentration of about 5 μM. This antitumor cell activity may be attributed to inhibition of protein biosynthesis.

Key words puromycin; fluorinated sugar; dimethylaminosulfur trifluoride; antiviral activity; antibacterial activity; antitumor cell activity

Puromycin (**1a**), a nucleoside antibiotic isolated from *S. alboniger*,[1] inhibits protein biosynthesis *in vitro* and *in vivo*[2] and exhibits antitumor activity.[3] Puromycin analogs modified at the base, sugar and amino acid moieties have been prepared to investigate the structure–activity relationship.[4] In particular, Daluge and Vince proved that the 5′-hydroxymethyl group and oxygen of the furanose ring are not necessary for inhibition of protein synthesis.[5] However, the role of the 2′-hydroxyl group of puromycin is not clear, because no 2′-modified analogues, except for 2′-deoxypuromycin, have been prepared.[6] Wohlrab *et al.* reported that 2′-deoxy-2′-fluorocytidine has antiviral activity against herpes simplex virus type 1 (HSV-1).[7] This prompted us to prepare 2′-deoxy-2′-fluoropuromycin (**1b**). In this paper, the synthesis of **1b** *via* the lyxo-epoxide is described, and the antiviral activity, antibacterial activity and antitumor activity against several tumor cell lines are presented.

Chemical Synthesis

The synthesis of the 3′-azido-2′-fluoro-2′,3′-dideoxy analogs of pyrimidine nucleosides has been accomplished, using diethylaminosulfur trifluoride (DAST), from the 3′-azido-3′-deoxyarabinosides.[8] The purine arabinosides were also converted to the corresponding 2′-deoxy-2′-fluororibosides using a similar approach.[9] We adopted this reagent for the synthesis of **8**. Thus, compound **2** was converted to 9-(2,3-anhydro-5-*O*-trityl-β-D-lyxofuranosyl)-6-chloropurine (**3**) as mentioned in a previous report,[9] then the product **3** was treated with 50% aqueous dimethylamine in *N*,*N*-dimethylformamide (DMF) to afford **4**. The lyxo-epoxide **4** was subjected to nucleophilic reaction with NaN_3 to give **6a** and **7a** in 17% and 58% yields, respectively. The ^{1}H-NMR spectrum of the major product **7a** revealed that the signals of H2′ and H3′ appeared at about 4.5—4.6 ppm, and the acetylation of **7a** caused a downfield shift of H2′ (*ca.* 0.8 ppm), indicating that the acetyl group was introduced at 2′-OH. This 3′-preference[10] could be explained in terms of the stability of the intermediary anion as follows: attack of the azido ion at the 3′-carbon resulted in the formation of a 2′-alkoxide ion, which is thought to be stabilized by the electron-withdrawing base moiety. In the case of the 3′-alkoxide ion, the base exerts little stabilizing effect. Since yields were not satisfactory, an alternative route to **7a** was explored. Compound **2** was treated with 6 eq of 50% aqueous dimethylamine in DMF at 4 °C overnight to afford the 3′-*O*-mesylate **5**. This product was treated with NaN_3 in a similar manner to that described for **4** to give **6a** and **7a** in 21% and 78% yields, respectively. The mechanism could be explained in terms of a two-step reaction. Initial attack of the azido ion at 2′-OH resulted in ring closure to form the intermediary lyxo-epoxide **4**, which was then attacked by the nucleophile. The azido-nucleosides (**6b**, **7b**) were obtained by the hydrolysis of the corresponding 5′-*O*-tritylated compounds, **6a** and **7a**. Synthesis of the 2′-fluoride **8** was carried out by the reaction of **7a** with DAST in the presence of pyridine in 72% yield.[9] The ^{1}H-NMR spectrum of **8** indicated that the 2′-fluorine caused a downfield shift and a large H2′-



puromycin (**1a**) : R=OH
2′-deoxy-2′-fluoropuromycin
(**1b**) : R=F

Fig. 1

* To whom correspondence should be addressed.

C-F geminal coupling (52.3 Hz) of the 2′-proton. The configuration of **8** was identified as the riboside structure from the nuclear Overhauser effect (NOE) observed between H2′ and H3′ on the two-dimensional NOE (NOESY) spectrum. Castillon *et al.* reported that neighboring group participation by the azido group occurred in the DAST reaction on the pyranose ring.[11] In contrast, no participation by the 3′-azido group was observed in the nucleophilic displacement of **7**. The 2′-fluoride **8** was hydrogenated under H_2 gas using 5% Pd–C as a catalyst to give the 3′-aminonucleoside **9**. Condensation of **9** with *N*-*tert*-butoxycarbonyl-*p*-methoxyphenyl-L-alanine[12] was performed in the presence of *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in dry DMF to give the protected puromycin analog **10**. Finally, compound **10** was hydrolyzed with 80% CF_3COOH to afford the trifluoroacetate of 2′-deoxy-2′-fluoropuromycin (**1b**), from which free **1b** was obtained. The structures of both the trifluoroacetate and free **1b** were identified from the spectroscopic data in combination with elemental analyses.

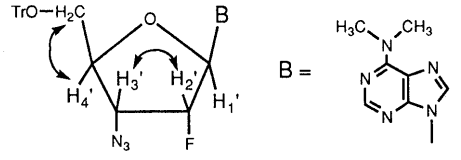


Fig. 2. NOESY Experiment on **8**

Biological Activity

Puromycin is a broad-spectrum antibiotic with antitumor activity.[3] That fact prompted us to explore the antitumor activity of its 2′-deoxy-2′-fluoro analog **1b** towards murine leukemia cells (L1210/0) and human T-lymphocyte cells (Molt/4, CEM/0). For the biological evaluation of **1b**, the trifluoroacetate was chosen because of its solubility in water. Although the azidonucleosides (**6b** and **7b**) displayed no antitumor activity, the puromycin analog **1b** demonstrated appreciable antitumor activity against tumor cell lines (L1210, Molt 4, CEM) and this was also evident from its toxicity for Vero cells (minimum

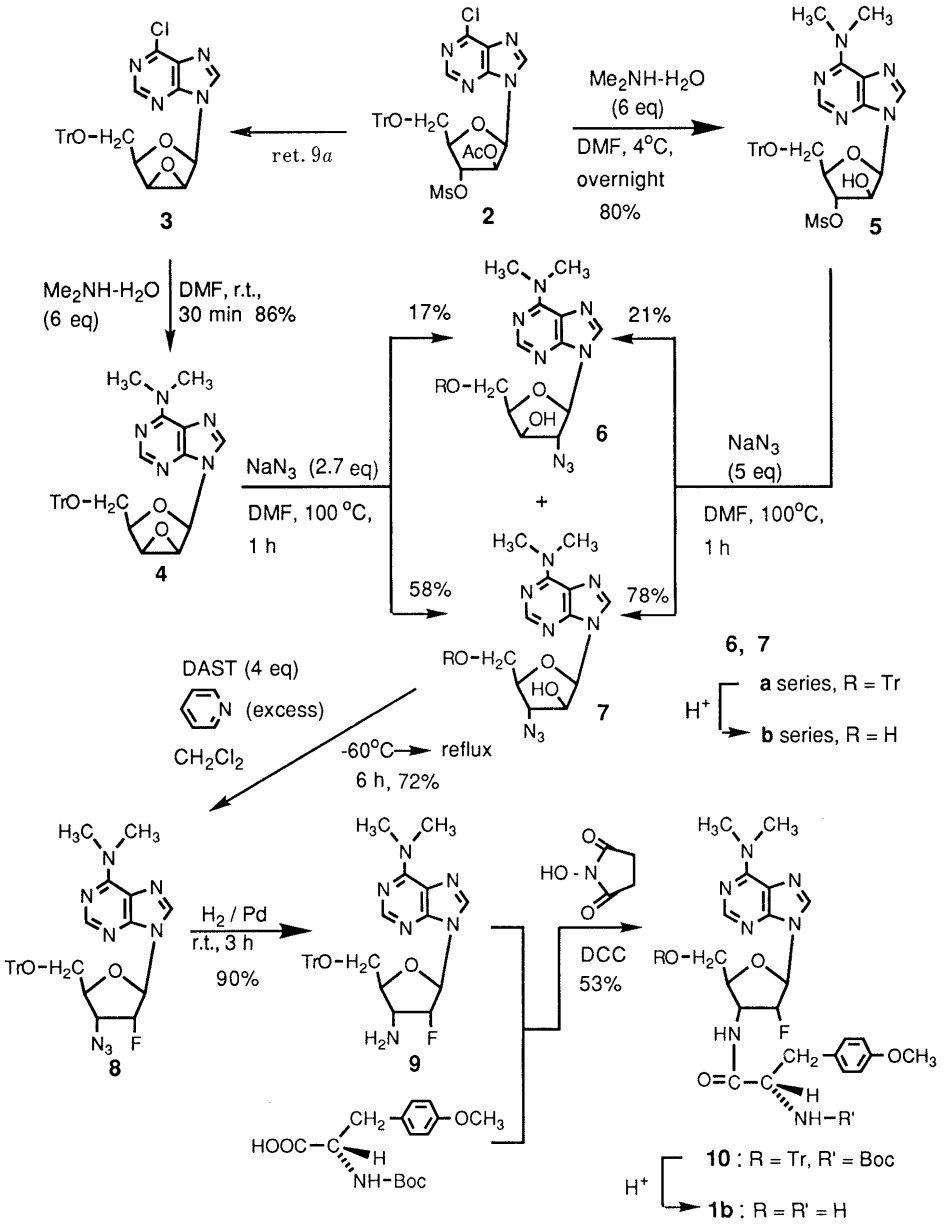


Chart 1

Table 1. Antibacterial Activity of Compound **1b**

Minimum inhibitory concentration			
>50 μg/ml	50 μg/ml	25 μg/ml	12.5 μg/ml
Streptococcus faecalis *Pseudomonas aeruginosa*	*Candida albicans* *Candida tropicalis* *Bacillus cereus* *Bacillus subtilis* *Escherichia coli*	*Micrococcus flavus* *Micrococcus lysodeicticus* *Sarcina lutea* *Serratia marcescens*	*Staphylococcus aureus*

Table 2. Inhibitory Effect of Compounds **1b**, **6b** and **7b** on the Proliferation of Murine Leukemia Cells (L1210/0) and Human T-Lymphocyte Cells (Molt4/C8, CEM/0)

Compound	IC_{50} (μM)[a]		
	L1210/0	Molt4/C8	CEM/0
1b	5.00±3.40	7.55±0.68	5.63±1.40
6b	340±139	>500	238±55
7b	>500	>500	>500

a) 50% Inhibitory concentration, or concentration required to inhibit cell growth by 50%.

cytotoxic concentration, 40 μg/ml). Compound **1b** also showed weak antibacterial activity and its minimum inhibitory concentration (MIC) was 12.5 μg/ml for *Staphylococcus aureus* and 25—50 μg/ml for most other bacteria examined. This activity is almost equal to that of puromycin (MIC for *S. aureus*, 25 μg/ml).[6] This is the first example of a 2′-modified puromycin showing marked biological activity. It is likely that the cytotoxicity and antibacterial activity of **1b** are caused by the inhibition of protein biosynthesis. Daluge and Vince reported that the 5′-hydroxymethyl group and oxygen of the furanose ring are not necessary for the inhibition of protein synthesis,[5] but the 2′-hydroxyl group seems more important, since it is located close to the amino acid group. Koizumi *et al.* demonstrated that the 2′-deoxy analog of puromycin has severely reduced activity.[6] The difference between **1b** and 2′-deoxypuromycin could be explained as follows: it is known that the electronegativity of substituents influences the conformation of the carbohydrate moiety of 2′-substituted nucleosides.[13] For example, introduction of fluorine at the 2′-carbon position favors the 3′-*endo* conformation, which resembles a ribonucleoside.[14] In the ^{1}H-NMR spectrum of **1b**, H1′ appears as a doublet with $J_{1',F}=19.4$ Hz and $J_{1',2'}\approx 0$ Hz in contrast to $J_{1',2'}=6.1$ Hz for 2′-deoxypuromycin.[6] This tendency is in good agreement with that in the adenosine series,[13] in which $J_{1',2'}$ values are 3.1 Hz for 2′-deoxy-2′-fluoroadenosine and 7.7 Hz for 2′-deoxyadenosine. As a consequence, **1b** may be assumed to occur in the 3′-*endo* form, and this conformation would be necessary for the binding to the ribosome. In contrast, 2′-deoxypuromycin should assume a different conformation that is unfavorable for binding to the ribosome. However, it is not excluded that dipole–dipole interaction of 2′-substituents may affect the ribosome-binding activity.

The antiviral effects of **1b** and azidonucleosides **6b**, **7b** were assayed according to previously established procedures.[15] These compounds displayed no activity against several DNA viruses, such as herpes simplex virus type 1 and type 2, and vaccinia virus. No antiviral activity was observed against several RNA viruses, such as vesicular stomatitis, Coxsackie, polio, parainfluenza-3, reo-1, Sindbis and Semliki forest virus. In an antiviral experiment compound **1b** showed some cytotoxicity towards cell cultures (minimum cytotoxic concentrations for Vero cells, HeLa cells and E_6SM cells were 40 μg/ml).

Experimental

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low-resolution mass spectra were obtained on a JEOL JMS-AX500 mass spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on a Varian UNITY 200 (200 MHz) or UNITY 600 (600 MHz) in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with Silica gel 60 containing fluorescent indicator F_{254} were used for thin-layer chromatography and Silica gel 60 (Merck 7734, 60—200 mesh) was employed for column chromatography.

9-(2,3-Anhydro-5-*O*-trityl-β-D-lyxofuranosyl)-N^6,N^6-dimethyladenine (4) The arabinoside **2** was converted to 9-(2,3-anhydro-5-*O*-trityl-β-D-lyxofuranosyl)-6-chloropurine **3** as mentioned in the previous report.[9a] Then, **3** (240 mg, 0.47 mmol) was dissolved in a mixture of DMF (7 ml) and 50% aqueous dimethylamine (0.47 ml, 6 eq) and the solution was stirred for 30 min at room temperature. After evaporation of the solution, the residue was dissolved in a small amount of AcOEt and chromatographed over a column of Silica gel G (2.2×19 cm) using 0—50% AcOEt in benzene (1.2 l) to give a caramel (208 mg, 86%). UV λ_{max}^{MeOH} nm: 272; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 273. ^{1}H-NMR ($CDCl_3$) δ: 8.33, 8.01 (each 1H, s, H2, H8), 7.20—7.47 (15H, m, 5′-OC(C_6H_5)$_3$), 6.34 (1H, s, H1′), 4.24 (1H, t, J=6.4 Hz, H4′), 4.03 (2H, s, H2′ and H3′), 3.53 (6H, s, N(CH_3)$_2$), 3.44 (2H, m, H5′). MS *m/z*: 519 (M^+).

9-(3-*O*-Methanesulfonyl-5-*O*-trityl-β-D-arabinofuranosyl)-N^6,N^6-dimethyladenine (5) The arabinoside **2** (7.67 g, 11.8 mmol) was dissolved in DMF (225 ml) and 50% aqueous dimethylamine (15 ml) was added to the solution. The mixture was kept at 4 °C overnight, then evaporated. The residue was dissolved in $CHCl_3$ (250 ml) and the organic layer was washed with water twice (500 ml), dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed over a column of Silica gel G (3.0×35 cm) using benzene–AcOEt (2 l) and AcOEt (2 l) to give a caramel (5.80 mg, 80%). UV λ_{max}^{MeOH} nm: 271; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 272. ^{1}H-NMR ($CDCl_3$) δ: 8.27 (1H, s, H2), 8.00 (1H, s, H8), 7.2—7.4 (15H, m, 5′-OC(C_6H_5)$_3$), 6.18 (1H, d, J=4.8 Hz, H1′), 4.53 (1H, t, J=4.1 Hz, H2′), 4.73 (1H, m, H3′), 4.23 (1H, q, J=4.5 Hz, H4′), 3.5—3.7 (7H, m, N(CH_3)$_2$ and H5′a), 3.40 (1H, dd, J=4.2, 10.6 Hz, H5′b), 3.10 (3H, s, SO_2CH_3).

Preparation of the 3′-Azidonucleoside (7a) Method A: Sodium azide (362 mg, 2.7 eq) was suspended in a solution of **4** (1.05 g, 2.0 mmol) in DMF (50 ml) and the mixture was stirred at 100 °C for 1 h. After cooling, benzene (25 ml), ether (25 ml) and water (50 ml) were added to it and the organic layer was washed with water (50 ml) three times, dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed over a column of Silica gel G (2.8×30 cm) using 0—4.8% EtOH in $CHCl_3$ (800 ml). Evaporation of the first fraction gave 9-(2-

azido-2-deoxy-5-*O*-trityl-β-D-xylofuranosyl)-N^6,N^6-dimethyladenine (**6a**) (190 mg, 17%). UV λ_{max}^{MeOH} nm: 273.5; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 268; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 273. ^{1}H-NMR ($CDCl_3$) δ: 8.18, 7.80 (each 1H, s, H2, H8), 7.15—7.47 (15H, m, 5′-OC(C_6H_5)$_3$), 5.59 (1H, d, J=1.95 Hz, H1′), 4.53 (1H, d, J=1.95 Hz, H2′), 4.10—4.28 (2H, m, H3′ and H4′), 3.45—3.64 (8H, m, N(CH_3)$_2$ and H5′), 3.11 (3H, s, SO_2CH_3). MS *m/z*: 562 (M^+).

The second fraction was evaporated to afford 9-(3-azido-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)-N^6,N^6-dimethyladenine (**7a**) (666 mg, 58%) as a caramel. UV λ_{max}^{MeOH} nm: 273; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267.5; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 274. ^{1}H-NMR ($CDCl_3$) δ: 8.29 (1H, s, H2), 7.98 (1H, s, H8), 7.22—7.36 (15H, m, 5′-OC(C_6H_5)$_3$), 6.08 (1H, d, J=5.1 Hz, H1′), 4.49—4.62 (2H, m, H2′ and H3′), 3.87 (1H, m, H4′), 3.53 (6H, br, N(CH_3)$_2$), 3.43 (2H, m, H5′). MS *m/z*: 562 (M^+).

Method B: A solution of **5** (632 mg, 1.03 mmol) and NaN_3 (325 mg, 5 eq) in DMF (30 ml) was stirred at 100 °C for 1 h, then cooled. Usual work-up of the solution and Silica gel G chromatography similar to that as mentioned above gave the 2′-azidonucleoside **6a** (122 mg, 21%) and 3′-azidonucleoside **7a** (456 mg, 78%), which were identical with the samples obtained as described above.

2′-*O*-Acetate of 7a Acetic anhydride (0.5 ml) was added to a solution of **7a** (25 mg, 0.045 mmol) in pyridine (1 ml) and the mixture was kept for 1 h at room temperature. A usual work-up of the mixture gave 9-(2-*O*-acetyl-3-azido-3-deoxy-5-*O*-trityl-β-D-arabinofuranosyl)-N^6,N^6-dimethyladenine as a caramel (30 mg, quantitative). UV λ_{max}^{MeOH} nm: 273; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 274. ^{1}H-NMR ($CDCl_3$) δ: 8.28, 7.95 (each 1H, s, H2, H8), 7.20—7.49 (15H, m, 5′-OC(C_6H_5)$_3$), 6.47 (1H, d, J=3.9 Hz, H1′), 5.35 (1H, t, J=3.9 Hz, H2′), 4.54 (1H, dd, J=5.2, 3.9 Hz, H3′), 3.98 (1H, m, H4′), 3.41—3.57 (8H, m, N(CH_3)$_2$ and H5′), 1.82 (3H, s, 2′-OCOC$\underline{H}_3$). MS *m/z*: 604 (M^+).

9-(2-Azido-2-deoxy-β-D-xylofuranosyl)-N^6,N^6-dimethyladenine (6b) A solution of **6a** (200 mg, 0.36 mmol) in 80% aqueous CF_3COOH (3 ml) was stirred at room temperature for 10 min and evaporated *in vacuo* to give a residue, which was evaporated azeotropically with water (3 ml) twice. The solid thus obtained was dissolved in a small amount of $CHCl_3$ and chromatographed over a column of Silica gel G (2.0×20 cm) using 0—7.7% EtOH in $CHCl_3$ (520 ml) to afford a caramel (81.2 mg, 71%). UV λ_{max}^{MeOH} nm: 274; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 268; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 274. ^{1}H-NMR ($CDCl_3$) δ: 8.27, 7.73 (each 1H, s, H2, H8), 6.05 (1H, br, 3′-OH), 5.50 (1H, d, J=6.2 Hz, H1′), 5.45 (1H, br, 5′-OH), 4.81 (1H, t, J=6.2 Hz, H2′), 4.54 (1H, m, H3′), 4.32 (1H, dt, J=6.4, 2.7 Hz, H4′), 3.9—4.1 (2H, m, H5′), 3.55 (6H, br, N(CH_3)$_2$). MS *m/z*: 320 (M^+), 192 (dimethyladenine+CHO)$^+$, 164 (dimethyladenine)$^+$. *Anal.* Calcd for $C_{12}H_{16}N_8O_3\cdot 0.5H_2O$: C, 43.77; H, 5.20; N, 34.03. Found: C, 43.88; H, 5.01; N, 33.91.

9-(3-Azido-3-deoxy-β-D-arabinofuranosyl)-N^6,N^6-dimethyladenine (7b) Compound **7a** (200 mg, 0.36 mmol) was subjected to a similar reaction to obtain **7b** as white crystallines (78.2 mg, 69%), mp 199—202 °C. UV λ_{max}^{MeOH} nm: 274; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 269; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 274. ^{1}H-NMR ($CDCl_3$) δ: 8.26, 8.18 (each 1H, s, H2, H8), 6.23 (1H, d, J=6.4 Hz, H1′), 6.02 (1H, d, J=6.0 Hz, 2′-OH), 5.25 (1H, br, 5′-OH), 4.51 (1H, q, J=6.5 Hz, H2′), 4.30 (1H, t, J=7.7 Hz, H3′), 3.74 (1H, m, H4′), 3.62 (2H, m, H5′), 3.4 (6H, br, N(CH_3)$_2$). MS *m/z*: 320 (M^+), 278 (M^+-N_3), 192 (dimethyladenine+CHO)$^+$, 164 (dimethyladenine)$^+$. *Anal.* Calcd for $C_{12}H_{16}N_8O_3$: C, 45.00; H, 5.04; N, 34.98. Found: C, 44.81; H, 5.05; N, 35.06.

9-(3-Azido-2-fluoro-2,3-dideoxy-5-*O*-trityl-β-D-ribofuranosyl)-N^6,N^6-dimethyladenine (8) A solution of **7a** (3.327 g, 5.92 mmol) in CH_2Cl_2 (165 ml) and pyridine (6.3 ml) was cooled to −60 °C under an N_2 atmosphere and DAST (3.3 ml, 4 eq) was added dropwise to the mixture. The solution was stirred for 6 h at 50 °C and poured into saturated $NaHCO_3$ (350 ml) with stirring, then CH_2Cl_2 (350 ml) was added. The organic layer was washed with water (200 ml) twice, dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed over a column of Silica gel G (2.8×33 cm) with 0—20% AcOEt in benzene (2 l) to give **8** (2.42 g, 72%) as a caramel. UV λ_{max}^{MeOH} nm: 272; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267.5; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 272.5. ^{1}H-NMR ($CDCl_3$) δ: 8.30 (1H, s, H2), 7.92 (1H, s, H8), 7.09—7.40 (15H, m, 5′-OC(C_6H_5)$_3$), 6.14 (1H, dd, J=20.0, 1.7 Hz, H1′), 5.60 (1H, ddd, J=52.3, 4.1, 1.7 Hz, H2′), 4.78 (1H, ddd, J=22.7, 8.6, 4.1 Hz, H3′), 4.27 (1H, m, H4′), 3.53 (6H, br s, N(CH_3)$_2$), 3.46 (2H, m, H5′). MS *m/z*: 564 (M^+).

9-(3-Amino-2-fluoro-2,3-dideoxy-5-*O*-trityl-β-D-ribofuranosyl)-N^6,N^6-dimethyladenine (9) A solution of **8** (500 mg, 0.89 mmol) and 5% Pd–C (150 mg) in EtOH (100 ml) containing AcOH (5 ml) was stirred vigorously under an H_2 atmosphere for 3 h at room temperature. After removal of the catalyst, the solution was chromatographed over a column of Silica gel G (2.8×33 cm) with 0—5% EtOH in $CHCl_3$ (2 l) to give **9** as a caramel (428 mg, 90%). UV λ_{max}^{MeOH} nm: 272; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267; $\lambda_{max}^{0.05\,N\,NaOH}$ nm: 273. ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, s, H2), 7.97 (1H, s, H8), 7.2—7.5 (*ca.* 15H, m, –C(C_6H_5)$_3$), 6.21 (1H, d, J=19.3 Hz, H1′), 5.37 (1H, dd, J=4.2, 53.1 Hz, H2′), 3.9—4.1 (2H, m, H3′, H4′), 3.58 (1H, dd, J=11.3, 2.8 Hz, H5′a), 3.52 (6H, s, N(CH_3)$_2$), (8H, m, 3.40 (1H, dd, J=11.3, 3.8 Hz, H5′b). MS *m/z*: 538 (M^+), 295 (M^+-Tr).

9-[3-(*N*-*tert*-Butoxycarbonyl-*p*-methoxyphenyl-L-alanyl)amino-2-fluoro-2,3-dideoxy-5-*O*-trityl-β-D-ribofuranosyl]-N^6,N^6-dimethyladenine (10) To a mixture of **9** (495 mg, 0.92 mmol), *N*-*tert*-butoxycarbonyl-*p*-methoxyphenyl-L-alanine[12] (260 mg, 0.84 mmol) and *N*-hydroxysuccinimide (120 mg, 1.04 mmol) in dry DMF (30 ml) was added DCC (215 mg, 1.04 mmol) and the whole was stirred at 30 °C overnight. Insoluble material was filtered off, the filtrate was evaporated and the residue was dissolved in benzene (50 ml). The organic layer was extracted with water (30 ml×2), dried over $MgSO_4$ and concentrated to a small volume. This solution was chromatographed over a column of Silica gel G (3.2×40 cm) with 0—2.4% EtOH in $CHCl_3$ (3 l). Evaporation of the first fraction gave the protected puromycin analog **10** as a caramel (437 mg, 58%). UV λ_{max}^{MeOH} nm: 273. ^{1}H-NMR ($CDCl_3$) δ: 8.30 (1H, s, H2), 7.94 (1H, s, H8), 7.2—7.5 (*ca.* 15H, m, –C(C_6H_5)$_3$), 6.89 (2H, d, J=8.1 Hz, two of $C_6\underline{H}_4$OMe), 6.73 (2H, d, J=8.1 Hz, two of $C_6\underline{H}_4$OMe), 6.20 (1H, d, J=18.6 Hz, H1′), 5.88 (1H, br, NHBoc), 5.38 (1H, dd, J=52.8, 4.3 Hz, H2′), 4.95—5.2 (2H, m, H3′, CONH–), 4.21 (1H, m, H4′), 3.86 (1H, m, –C$\underline{H}$(−)CH$_2$–), 3.75 (3H, s, $C_6H_4OC\underline{H}_3$), 3.5 (6H, s, N(CH_3)$_2$), 3.3—3.55 (2H, m, H5′), 2.96 (1H, dd, J=13.3 Hz, one of –CH(−)C$\underline{H}_2$–), 2.73 (1H, dd, J=8.6, 13.3 Hz, one of –CH(−)CH$_2$–), 1.40 (9H, s, –NHCOO(C$\underline{H}_3$)$_3$).

The second fraction was evaporated to recover the starting material (91 mg, 18%).

9-[3-(*p*-Methoxyphenyl-L-alanyl)amino-2-fluoro-2,3-dideoxy-β-D-ribofuranosyl]-N^6,N^6-dimethyladenine (2′-Deoxy-2′-fluoropuromycin, 1b) A solution of **10** (200 mg, 0.25 mmol) in 80% aqueous CF_3COOH (3 ml) was stirred at room temperature for 20 min, then evaporated to dryness under reduced pressure. The residue was evaporated azeotropically with water (4 ml) twice and the residue was taken up in EtOH (2 ml). The solution was chromatographed over a column of Silica gel G (2.3×24 cm) with 0—17% EtOH in $CHCl_3$ (1.2 l) to give the trifluoroacetate of **1b** as white crystals (121 mg, 67%), mp 120 °C. UV λ_{max}^{MeOH} nm: 274, 217; $\lambda_{max}^{0.05\,N\,HCl}$ nm: 267. ^{1}H-NMR (DMSO-d_6) δ: 8.82 (1H, d, J=7.5 Hz, –CONH–), 8.40 (1H, s, H8), 8.23 (3H, s, H2 and NH_2), 7.16 (2H, d, J=8.0 Hz, two of $C_6\underline{H}_4$OMe), 6.89 (2H, d, J=8.0 Hz, two of $C_6\underline{H}_4$OMe), 6.33 (1H, d, J=19.4 Hz, H1′), 5.48 (1H, dd, J=52.1, 4.5 Hz, H2′), 5.17 (1H, br, 5′-OH), 4.85 (1H, ddd, J=26.2, 9.5, 4.6 Hz, H3′), 3.9—4.1 (2H, m, H4′ and –C$\underline{H}$(−)CH$_2$–), 3.74 (3H, s, $C_6H_4OC\underline{H}_3$), 3.62 (1H, d, J=11.4 Hz, H5′a), 3.3—3.5 (H5′b, N(CH_3)$_2$, H_2O), 2.98 (2H, m, –CH(−)C$\underline{H}_2$–). MS *m/z*: 473 (M^+). *Anal.* Calcd for $C_{22}H_{28}FN_7O_4\cdot 2CF_3COOH\cdot H_2O$: C, 43.40; H, 4.48; N, 13.62. Found: C, 43.07; H, 4.46; N, 13.89.

Triethylamine (0.1 ml) was added to a solution of the trifluoroacetate of **1b** (78 mg, 0.067 mmol) in EtOH (3 ml) and the solution was evaporated to dryness. Recrystallization of the residue from a small amount of water gave white crystals (48.4 mg, 95%), mp 128—130 °C. *Anal.* Calcd for $C_{22}H_{28}FN_7O_4\cdot H_2O$: C, 53.76; H, 6.15; N, 19.94. Found: C, 53.91; H, 5.93; N, 19.51.

Acknowledgments This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (No. 04226218 and 05210211) from the Ministry of Education, Science and Culture, Japan.

References and Notes

1) Porter J. N., Hewitt R. I., Hesseltine C. W., Krupka G., Lowery J. A., Wallace W. S., Bohonos N., Williams J. H., *Antibiot. Chemother.*, **2**, 409 (1952).
2) Suhadolnik R. J., "Nucleosides as Biological Probes," John Wiley & Sons, Inc., New York, 1979, pp. 96—102.
3) *a*) Nathans D., "Antibiotics," Vol. 1, ed. by Gottlieb D., Shaw P. D., Springer-Verlag, Berlin, 1967, p. 259; *b*) Suhadolnik R. J., "Nucleoside Antibiotics," John Wiley & Sons, Inc., New York, 1970.
4) Suami T., Tadano K., Ayabe M., Emori Y., *Bull. Chem. Soc. Jpn.*, **51**, 855 (1978) and the references cited therein.
5) Daluge S., Vince R., *J. Med. Chem.*, **15**, 171 (1972).

6) Koizumi F., Oritani T., Yamasita K., *Agric. Biol. Chem.*, **54**, 3093 (1990).
7) Wohlrab F., Jamieson A. T., Hay J., Mengel R., Guschlbauer W., *Biochim. Biophys. Acta*, **824**, 233 (1985).
8) Huang J.-T., Chen L.-C., Wang L., Kim M.-H., Warshaw J. A., Armstrong D., Zhu Q.-Y., Chou T.-C., Watanabe K. A., Matulic-Adamic J., Su T.-L., Fox J. J., Polsky B., Baron P. A., Gold F. W. M., Hardy W. D., Zuckerman E., *J. Med. Chem.*, **34**, 1640 (1991).
9) *a*) Maruyama T., Utzumi K., Sato Y., Richman D. D., *Nucleosides & Nucleotides*, **13**, 527 (1994); *b*) *Idem, ibid.*, **13**, 1219 (1994).
10) *a*) Lee W. W., Benitez A., Goodman L., Baker B. R., *J. Am. Chem. Soc.*, **82**, 2648 (1960); *b*) Reist E. J., Calkins D. F., Goodman L., *J. Org. Chem.*, **32**, 2538 (1967).
11) Castillon S., Dessinges A., Faghih R., Lukacs G., Olesker A., Thang T. T., *J. Org. Chem.*, **50**, 4913 (1985).
12) *N-tert*-Butoxycarbonyl-*p*-methoxyphenyl-L-alanine was prepared by treatment of Boc-L-tyrosine with methyl iodide followed by alkaline hydrolysis.
13) Ikehara M., *Heterocycles*, **21**, 75 (1984).
14) Uesugi S., Miki H., Ikehara M., Iwahashi H., Kyogoku M., *Tetrahedron Lett.*, **1979**, 4073.
15) *a*) De Clercq E., Descamps J., Verhelst G., Walker R. T., Jones A. S., Torrence P. F., Shugar D., *J. Infect. Dis.*, **141**, 563 (1980); *b*) De Clercq E., Holy A., Rosenberg I., Sakuma T., Balzarini J., Maudgal P. C., *Nature* (London), **323**, 464 (1986); *c*) Schols D., De Clercq E., Balzarini J., Baba M., Witvrouw M., Hosoya M., Andrei G., Snoeck R., Neyts J., Pauwels R., Nagy M., Gyorgyi-Edelenyi J., Machovich R., Horvath I., Low M., Gorog S., *Antiviral Chem. Chemother.*, **1**, 233 (1990).