# Nucleoside Analogs. 6. A Synthesis of Carbocyclic Puromycin Analogs[1)]

Tetsuo SUAMI,* Kin-ichi TADANO, Mitsukuni AYABE, and the late Yasufumi EMORI
*Department of Applied Chemistry, Faculty of Engineering, Keio University, Hiyoshi, Yokohama 223*
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Eight carbocyclic puromycin analogs, in which the furanosyl ring of puromycin is replaced with a cyclopentyl system, were synthesized. Their minimum degeneration concentration (MDC) was determined against *HeLa* cells in a tissue culture. The two analogs were found to be remarkably active.

A nucleoside antibiotic puromycin (**1**) was isolated from a culture broth of *Streptomyces alboniger* by Porter *et al.*,[2)] its synthesis being accomplished by Baker *et al.*[3)] Various analogs have been prepared for clarification of the relationship between structure and biological activity.[4–7)]

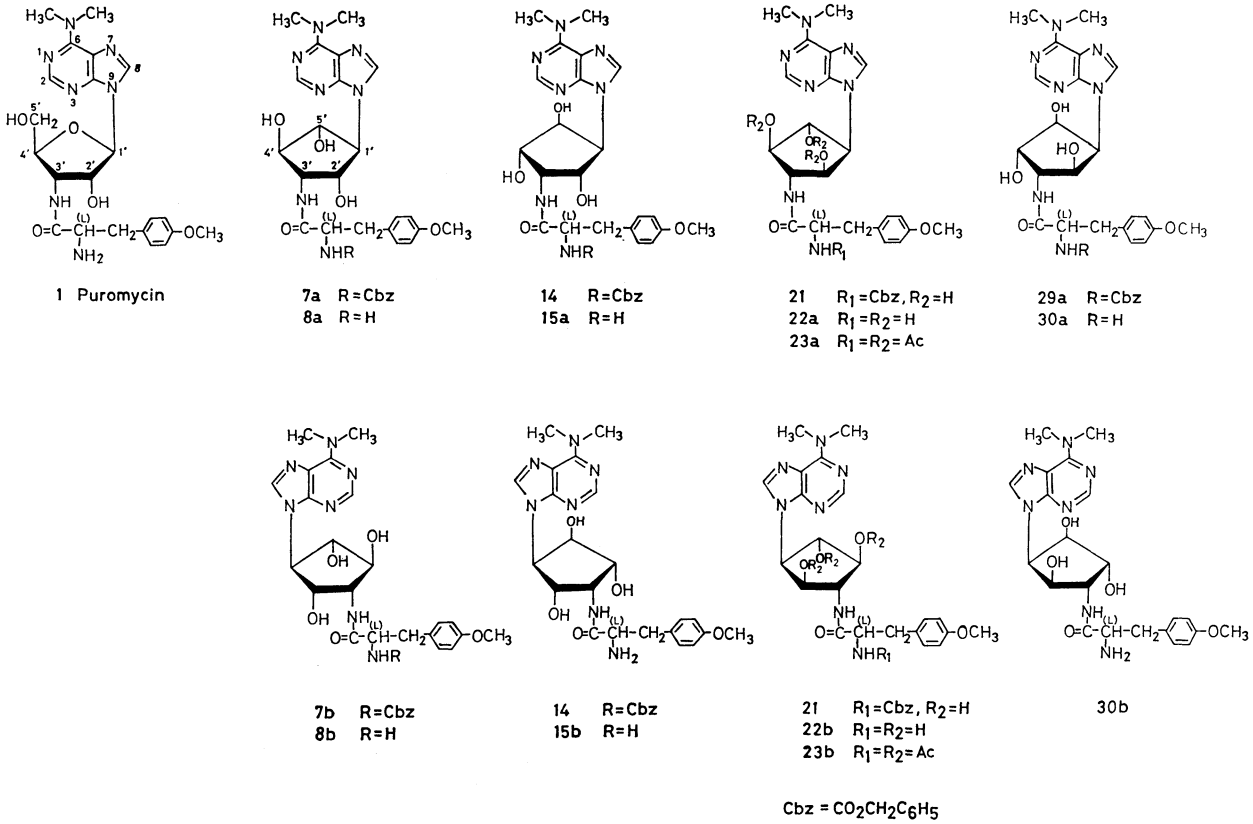
In particular, Daluge and Vince[7)] demonstrated the essential structural requirement for biological activity in puromycin analogs by the synthesis of a carbocyclic analog: 6-dimethylamino-9-[(1*R*,2*R*,3*R*)-2-hydroxy-3-(*p*-methoxyphenyl-L-alanylamino)cyclopentyl]purine. They showed that the removal of the hydroxymethyl moiety from the puromycin molecule is not detrimental to activity and the furanosyl ring can be replaced with a cyclopentane ring with no loss in activity. Their assessment of the structural requirement in the puromycin molecule stimulated us to prepare a carbocyclic puromycin analog in which the 3-amino-3-deoxy-β-D-ribofuranosyl moiety of puromycin is replaced by 3-amino-2,4,5-trihydroxycyclopentyl group.

The structure of puromycin resembles that of the aminoacyl–adenyl terminus of aminoacyl-tRNA, this being strongly connected with its biological activity. Since a cyclopentane ring is conformationally similar to a furanosyl ring, 3-amino-2,4,5-trihydroxycyclopentyl group might replace the furanosyl moiety of puromycin, favorably resulting in biological activity.

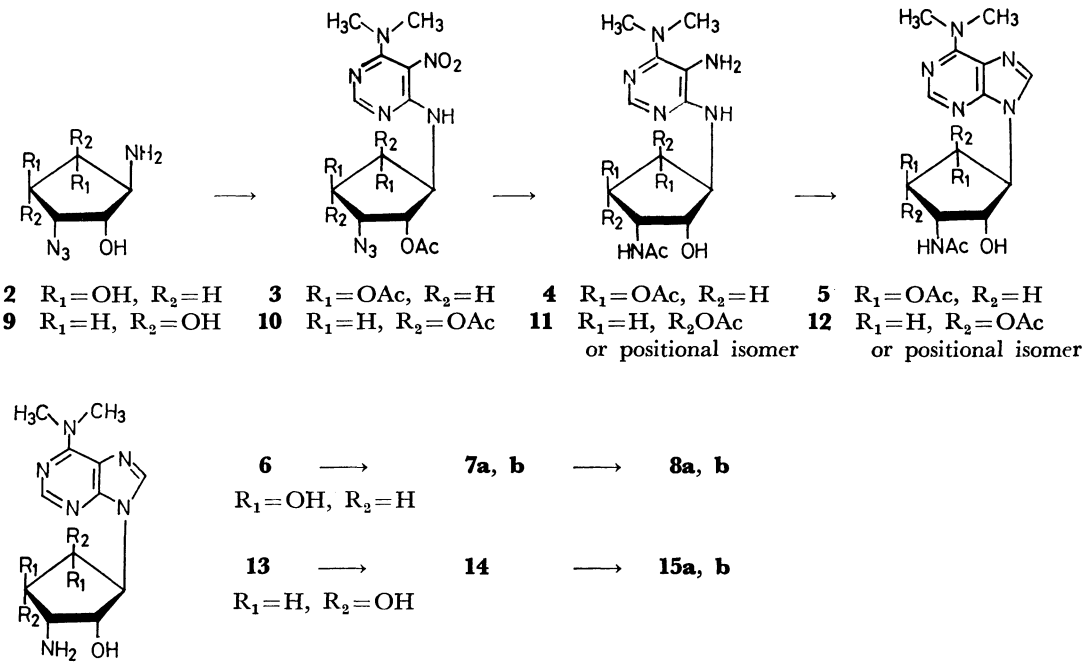
Carbocyclic analogs of adenosine and pyrimidine nucleosides, in which the ribofuranosyl moiety was replaced by the polyhydroxycyclopentyl or polyhydroxycyclohexyl group, have been prepared.[8–12)] In connection with the preceding studies and to extend the Daluge and Vince evaluation of the structural requirement of the puromycin molecule, we have synthesized eight diastereoisomeric 6-dimethylamino-9-[2,4,5-trihydroxy-3-(*p*-methoxyphenyl-L-alanylamino)cyclopentyl]purines (**8a,b, 15a,b, 22a,b,** and **30a,b**), and their biological activities were dtermined against *HeLa* cells.

## Results and Discussion

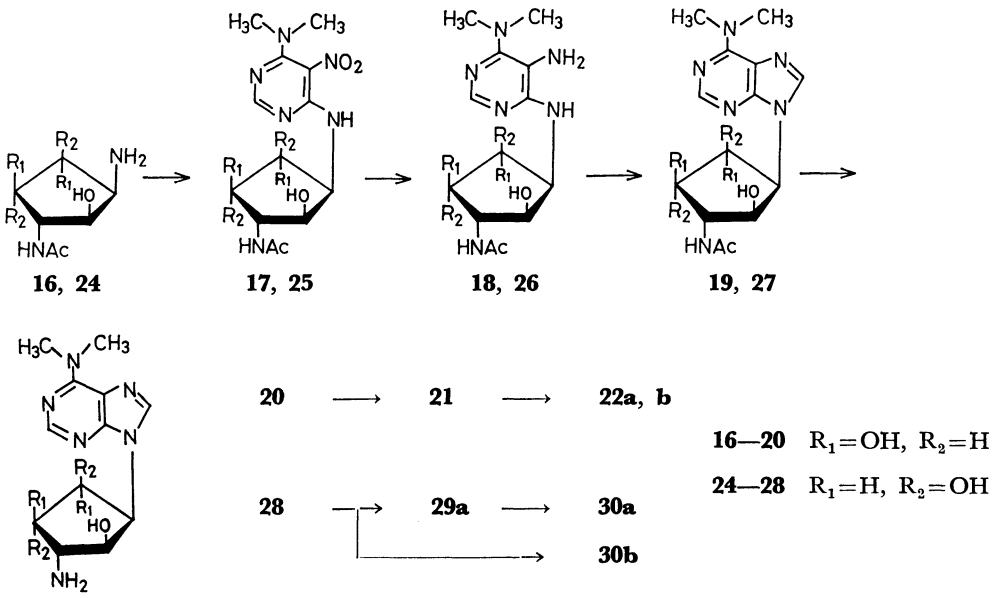
The carbocyclic puromycin analogs were prepared by the reaction processes shown in Schemes 2 and




Scheme 1.




Scheme 2.[19]




Scheme 3.[19]

3. The processes are divided into two categories, 1-amino-3-azido-2,4,5-cyclopentanetriols (**2** and **9**) being used as the starting material in the former two (Scheme 2) and 3-acetamino-1-amino-2,4,5-cyclopentanetriols (**16** and **24**) in the latter two.

When 1-amino-3-azido-2,4,5-cyclopentanetriol[11] (**2**) was heated with 6-chloro-4-dimethylamino-5-nitropyrimidine[13] in 2-methoxyethanol in the presence of triethylamine, 6-[(3-azido-2,4,5-triacetoxycyclopentyl)-amino]-4-dimethylamino-5-nitropyrimidine (**3**) was obtained, after acetylation. The structure of **3** was confirmed by the $^1$H NMR spectrum in which NH revealed a doublet at $\delta$ 8.38 ($J$=7 Hz).

Catalytic hydrogenation of **3** with Raney nickel afforded 6-[(3-acetamido-4,5-diacetoxy-2-hydroxycyclopentyl)amino]-5-amino-4-dimethylaminopyrimidine (**4**). During the course of the reaction, acetyl group migration took place from O to N in the *cis*-arrangement.

Ring closure of **4** was performed with triethyl orthoformate in the presence of *p*-toluenesulfonic acid to give the purine derivative (**5**) in 87% yield. Hydrolysis of **5** in aqueous barium hydroxide, followed by purification on a column of Amberlite CG-120(H$^+$) resin gave 9-(3-amino-2,4,5-trihydroxycyclopentyl)-6-dimethylaminopurine (**6**). Compound **6** was converted into the crystalline hydrochloride which was proved to be the 9-substituted adenine derivative by a characteristic UV absorption.[16]

Table 1. Minimum degeneration concentration (MDC) with *HeLa S3* cells in a tissue culture by the two-fold serial liquid medium dilution method[18]

| Compound | MDC (μg/ml) |
|---|---|
| **8a** | 15.6 |
| **8b** | 250 |
| **15a** | 31.2 |
| **15b** | 125 |
| **22a** | 1000 |
| **22b** | 1000 |
| **30a** | 250 |
| **30b** | 500 |

When **6** was treated with *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[14] and *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide, a mixture of two diastereoisomers (**7a** and **7b**) was obtained, since **6** is of DL form. Compounds **7a** and **7b** were successfully separated by fractional recrystallization. Hydrogenolysis of **7a** and **7b** in glacial acetic acid with palladium black gave **8a** and **8b**, respectively. The overall yields of **8a** and **8b** were 11 and 19%, respectively.

Compounds **15a** and **15b** were obtained by analogous reactions (Scheme 2) in 7 and 8% overall yields, respectively.

On the other hand, when 3-acetamido-1-amino-2,4,5-cyclopentanetriol (**16**) was subjected to react with 6-chloro-4-dimethylamino-5-nitropyrimidine,[13] 6-[(3-acetamido-2,4,5-trihydroxycyclopentyl)amino]-4-dimethylamino-5-nitropyrimidine (**17**) was obtained in 77% yield. The successive reactions leading to **22a** and **22b** were carried out by following the reactions used in the preparation of **8a** and **8b** (Scheme 3). The overall yields of **22a** and **22b** were 8 and 14%, respectively.

By an analogous reaction (Scheme 3), **30a** and **30b** were obtained each in 8% overall yield.

A biological activity of the carbocyclic puromycin analogs thus obtained was determined by the usual two-fold serial liquid medium dilution method with *HeLa S3* cells in a tissue culture. The minimum degeneration concentration (MDC) was appraised, the result being given in Table 1. We see that **8a** and **15a** are remarkably active, in contrast with other diastereoisomers.

The stereochemistry of **8a** and **15a** is tentatively assigned as that depicted in Scheme 1, on the basis of their biological activity and the absolute configuration of puromycin with respect to that on C-1′, 2′, and 3′.

The stereochemistry of **22a** and **30a** is assigned tentatively on the observation that, in the cases of **8a** and **15a**, their specific rotations are more levorotatory than those of the respective diastereoisomers: **8b** and **15b**. Therefore, the absolute configurations of **22a** and **30a** depicted in Scheme 1 are assigned to the strongly levorotatory diastereoisomers.

## Experimental

Melting points were determined in capillary tubes in a liquid bath and are uncorrected. Solutions were evaporated under reduced pressure. Optical rotations were measured with a Japan Spectroscopic DIL-SL polarimeter. $^1$H NMR spectra were recorded at 60 MHz with a Varian A-60D spectrometer for solutions in chloroform-*d*, unless otherwise stated, with tetramethylsilane as an internal standard, the peak positions being given in $\delta$ values.[17] IR spectra were recorded on potassium bromide disks with a Hitachi Perkin-Elmer 225 spectrometer. UV spectra were determined with a Hitachi Perkin-Elmer UV-VIS 139 spectrometer. Acetylation was performed with acetic anhydride and pyridine. Catalytic hydrogenation of a nitro group to an amino group was carried out in the presence of Raney nickel in a hydrogen atmosphere (3.4 kg/cm$^2$) with a Parr apparatus. TLC was performed on Wakogel B-10 (Wako Pure Chemical Co. Ltd.) plates. Silica gel (Wakogel C-300) was used for column chromatography.

DL-*(1,4/2,3,5)-1-Amino-3-azido-2,4,5-cyclopentanetriol (**2**).* The compound was prepared by the method of Tadano *et al.*[11]

*6-[(DL-(1,4/2,3,5)-3-Azido-2,4,5-triacetoxycyclopentyl)amino]-4-dimethylamino-5-nitropyrimidine (**3**).* A mixture of **2** (1.0 g) and 6-chloro-4-dimethylamino-5-nitropyrimidine[13] (2.33 g) in 2-methoxyethanol (50 ml) containing triethylamine (1 ml) was heated at 100 °C for 5 h with mechanical agitation. The mixture was evaporated and the residue was dissolved in warm water (50 ml). The aqueous solution was washed with benzene repeatedly and the aqueous layer was evaporated. The residue was acetylated and the resulting solution was poured into ice cold water. The solution was extracted with chloroform and the chloroform solution was evaporated to give a pale yellow crystalline residue. The residue was washed with warm ethyl acetate to give 1.92 g (72%) of **3**, mp 190—191 °C. $^1$H NMR: $\delta$ 2.06 (s, 3, OAc), 2.14 (s, 3, OAc), 2.15 (s, 3, OAc), 3.08 (s, 6, 2×NCH$_3$), 8.04 (s, 1, pyrimidine H-2), 8.38 (d, 1, $J$=7 Hz, NH).

Found: C, 43.98; H, 4.79; N, 24.26%. Calcd for $C_{17}H_{22}N_8O_8$: C, 43.78; H, 4.76; N, 24.03%.

*6-[(DL-(1,4/2,3,5)-3-Acetamido-4,5-diacetoxy-2-hydroxycyclopentyl)amino]-5-amino-4-dimethylaminopyrimidine (**4**).* Compound **3** (300 mg) was catalytically hydrogenated in ethyl acetate (50 ml) for 3 h. The catalyst was filtered off and the filtrate was evaporated to give 253 mg (96%) of **4** as crystals, mp 206—207 °C (dec). $^1$H NMR (DMSO-$d_6$): $\delta$ 1.94 (s, 3, NAc), 2.05 (s, 3, OAc), 2.06 (s, 3, OAc), 2.81 (s, 6, 2×NCH$_3$), 5.73 (d, 1, $J$=4 Hz, OH), 6.75 (d, 1, $J$=7 Hz, pyrimidine H-6), 8.08 (s, 1, pyrimidine H-2), 8.13 (d, 1, $J$=8 Hz, NH).

Found: C, 50.05; H, 6.50; N, 20.20%. Calcd for $C_{17}H_{26}N_6O_6$: C, 49.75; H, 6.39; N, 20.48%.

*9-[DL-(1,4/2,3,5)-3-Acetamido-4,5-diacetoxy-2-hydroxycyclopentyl]-6-dimethylaminopurine (**5**).* A mixture of **4** (204 mg) and triethyl orthoformate (0.11 ml) in *N*,*N*-dimethylformamide (10 ml) was stirred for 6 h in the presence of *p*-toluenesulfonic acid (9 mg). After being neutralized with Amberlite IRA-400 (OH$^-$) resin, the solution was evaporated. The residue was recrystallized from ethyl acetate to give 182 mg (87%) of **5**, mp 200—201 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 1.95 (s, 3, NAc), 1.97 (s, 3, OAc), 2.12 (s, 3, OAc), 3.59 (s, 6, 2×NCH$_3$), 5.95 (d, 1, $J$=5 Hz, OH), 8.31 (d, 1, $J$=8 Hz, NH), 8.56 (s, 1, purine H-2 or 8), 8.60 (s, 1, purine H-8 or 2).

Found: C, 51.64; H, 5.75; N, 19.74%. Calcd for $C_{18}H_{24}N_6O_6$: C, 51.42; H, 5.75; N, 19.99%.

*9-[DL-(1,4/2,3,5)-3-Amino-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine (6).* A mixture of **5** (1.65 g) and barium hydroxide octahydrate (8.0 g) in water (96 ml) was heated at 100 °C for 3 h with mechanical agitation. Carbon dioxide was bubbled into the solution and barium carbonate was filtered off. The filtrate was concentrated and the residue was purified on a column of Amberlite CG-120($H^+$) resin. After being washed with water, the column was eluted with dil $NH_4OH$ and the effluent was concentrated to give 1.14 g of **6** as a crude product. A part of the product was converted into crystalline hydrochloride, mp 250 °C (dec). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon=1.7\times10^4$), $\lambda_{max}^{H_2O}$ 277 nm ($\varepsilon=1.7\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 277 nm ($\varepsilon=1.7\times10^4$).

Found: C, 43.35; H, 5.74; N, 25.19; Cl, 10.88%. Calcd for $C_{12}H_{19}N_6O_3Cl$: C, 43.57; H, 5.79; N, 25.41; Cl, 10.72%.

*6-Dimethylamino-9-[(1S,2R,4R,5R)-2,4,5-trihydroxy-3-(N-benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (7a)* and *6-Dimethylamino-9-[(1R,2S,4S,5S)-2,4,5-trihydroxy-3-(N-benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)cyclopentyl]-purine (7b).* Dicyclohexylcarbodiimide (DCC, 838 mg) was added under ice-cooling with agitation to a solution of **6** (1.14 g) and *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[14] (1.34 g) in *N,N*-dimethylformamide (DMF, 57 ml) containing *N*-hydroxysuccinimide (468 mg). After 30 min, the solution was left to settle at ambient temperature for 18 h. The mixture was filtered in order to remove *N,N'*-dicyclohexylurea and the filtrate was concentrated. The residue was recrystallized from ethanol to give 670 mg (29%) of **7a** as needles, mp 179—180 °C, $[\alpha]_D^{20}$ −24.4° (*c* 1.07, DMF). $^1H$ NMR (DMSO-$d_6$): 3.62 (s, 6, $2\times NCH_3$), 3.90 (s, 3, $OCH_3$), 5.18 (s, 2, benzyl $CH_2$), 7.34 (q, 4, $C_6H_4$), 7.58 (s, 5, $C_6H_5$), 8.28 (d, 1, $J=8$ Hz, NH on C-3'), 8.54 (s, 1, purine H-2 or 8), 8.57 (s, 1, purine H-8 or 2).

Found: C, 59.62; H, 5.86; N, 16.20%. Calcd for $C_{30}H_{35}N_7O_7$: C, 59.49; H, 5.83; N, 16.19%.

The ethanolic mother liquor of **7a** was stored in a refrigerator to give 1.12 g of a product. Recrystallization from methanol gave 931 mg (40%) of **7b** as needles, mp 210—211 °C, $[\alpha]_D^{20}$ +17.5° (*c* 1.03, DMF). $^1H$ NMR (DMSO-$d_6$): 3.64 (s, 6, $2\times NCH_3$), 3.90 (s, 3, $OCH_3$), 5.21 (s, 2, benzyl $CH_2$), 7.36 (q, 4, $C_6H_4$), 7.62 (s, 5, $C_6H_5$), 8.27 (d, 1, $J=6$ Hz, NH on C-3'), 8.57 (s, 1, purine H-2 or 8), 8.61 (s, 1, purine H-8 or 2).

Found: C, 59.23; H, 5.83; N, 16.33%. Calcd for $C_{30}H_{35}N_7O_7$: C, 59.49; H, 5.83; N, 16.19%.

*6-Dimethylamino-9-[(1S,2R,4R,5R)-2,4,5-trihydroxy-3-(p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (8a).* A solution of **7a** (300 mg) in glacial acetic acid (20 ml) was hydrogenated in the presence of palladium black (200 mg) in a hydrogen atmosphere (1.4 kg/cm²) for 3 h. The catalyst was filtered off, and the filtrate was concentrated. A methanolic solution of the residue was passed through a column of Amberlite IRA-400($OH^-$) resin and the effluent was concentrated. The residue was recrystallized from methanol to give 146 mg (63%) of **8a**, mp 190—191 °C (dec), $[\alpha]_D^{19}$ −55.0° (*c* 0.98, DMF). UV: $\lambda_{max}^{0.1M\ HCl}$ 268 nm ($\varepsilon=2.7\times10^4$), $\lambda_{max}^{H_2O}$ 276 nm ($\varepsilon=1.9\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 275 nm ($\varepsilon=2.1\times10^4$). $^1H$ NMR (DMSO-$d_6$): 3.62 (s, 6, $2\times NCH_3$), 3.89 (s, 3, $OCH_3$), 7.31 (q, 4, $C_6H_4$), 8.54 (s, 1, purine H-2 or 8), 8.57 (s, 1, purine H-8 or 2).

Found: C, 55.92; H, 6.28; N, 20.54%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

*6-Dimethylamino-9-[(1R,2S,4S,5S)-2,4,5-trihydroxy-3-(p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (8b).* Catalytic hydrogenation of **7b** (300 mg) was carried out as in the preparation of **8a**. The product was recrystallized from methanol to give 182 mg (78%) of **8b**, mp 196—197 °C, $[\alpha]_D^{19}$ +0.39° (*c* 0.98, DMF). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon=1.8\times10^4$), $\lambda_{max}^{H_2O}$ 276 nm ($\varepsilon=2.0\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon=2.3\times10^4$). $^1H$ NMR (DMSO-$d_6$): 3.61 (s, 6, $2\times NCH_3$), 3.89 (s, 3, $OCH_3$), 7.35 (q, 4, $C_6H_4$), 8.56 (s, 1, purine H-2 or 8), 8.58 (s, 1, purine H-8 or 2).

Found: C, 55.95; H, 6.18; N, 20.52%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

*DL-(1,5/2,3,4)-1-Amino-3-azido-2,4,5-cyclopentanetriol (9).* Hydrolysis of DL-(1,5/2,3,4)-1-acetamido-3-azido-2,4,5-triacetoxycyclopentane[15] (1.68 g) in 3 M HCl, followed by deionization with Amberlite IRA-400 ($OH^-$) resin gave 837 mg (98%) of **9** as a crude syrup. The product was used for successive reaction without purification.

*6-[(DL-(1,5/2,3,4)-3-Azido-2,4,5-triacetoxycyclopentyl)amino]-4-dimethylamino-5-nitropyrimidine (10).* A mixture of **9** (837 mg) and 6-chloro-4-dimethylamino-5-nitropyrimidine[13] (1.46 g) in 2-methoxyethanol (42 ml) containing triethylamine (1 ml) was heated at 90 °C for 3.5 h. The mixture was treated as in the preparation of **3**. The product was recrystallized from ethanol to give 1.15 g (51%) of **10**, mp 146—147 °C. $^1H$ NMR: 2.03 (s, 3, OAc), 2.11 (s, 3, OAc), 2.15 (s, 3, OAc), 3.05 (s, 6, $2\times NCH_3$), 7.97 (s, 1, pyrimidine H-2), 8.22 (d, 1, $J=6$ Hz, NH).

Found: C, 44.00; H, 4.87; N, 23.70%. Calcd for $C_{17}H_{22}N_8O_8$: C, 43.78; H, 4.76; N, 24.03%.

*6-[(DL-(1,5/2,3,4)-3-Acetamido-2,5-(or 4,5)-diacetoxy-4(or 2)-hydroxycyclopentyl)amino]-5-amino-4-dimethylaminopyrimidine (11).* Compound **10** (1.42 g) was catalytically hydrogenated in ethyl acetate (60 ml). The product was recrystallized from ethanol to give 1.08 g (86%) of **11**, mp 155—156 °C (dec).

Found: C, 49.73; H, 6.53; N, 20.14%. Calcd for $C_{17}H_{26}N_6O_6$: C, 49.75; H, 6.39; N, 20.48%.

*9-[DL-(1,5/2,3,4)-3-Acetamido-2,5-(or 4,5)-diacetoxy-4(or 2)-hydroxycyclopentyl]-6-dimethylaminopurine (12).* A mixture of **11** (902 mg) and triethyl orthoformate (8.6 ml) in DMF (28 ml) containing *p*-toluenesulfonic acid (30 mg) was stirred for 4 h. The reaction mixture was worked up as in the preparation of **5**. The product was washed with warm ethyl acetate to give 770 mg (83%) of **12** as amorphous powder, mp 218—219 °C (dec). $^1H$ NMR (DMSO-$d_6$): 1.89 (s, 3, NAc), 2.03 (s, 6, $2\times OAc$), 3.50 (s, 6, $2\times NCH_3$), 6.14 (d, 1, $J=9$ Hz, NH), 8.50 (s, 1, purine H-2 or 8), 8.62 (s, 1, purine H-8 or 2).

Found: C, 51.26; H, 5.80; N, 20.06%. Calcd for $C_{18}H_{24}N_6O_6$: C, 51.42; H, 5.75; N, 19.99%.

*9-[DL-(1,5/2,3,4)-3-Amino-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine (13).* A mixture of **12** (769 mg) and barium hydroxide octahydrate (4.0 g) in water (51 ml) was heated at 100 °C for 3.5 h. The mixture was worked up as in the preparation of **6** to give 502 mg (93%) of **13** as a crude product. The product was converted into the crystalline hydrochloride in 76% yield, mp 259—260 °C (dec). UV: $\lambda_{max}^{0.1M\ HCl}$ 268 nm ($\varepsilon=2.11\times10^4$), $\lambda_{max}^{H_2O}$ 276 nm ($\varepsilon=2.12\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon=2.10\times10^4$).

Found: C, 39.30; H, 5.43; N, 22.61; Cl, 18.99%. Calcd for $C_{12}H_{20}N_6O_3Cl_2$: C, 39.25; H, 5.49; N, 22.88; Cl, 19.31%.

*A Mixture of 6-Dimethylamino-9-[(1S,2R,4S,5S)-2,4,5-trihydroxy-3-(N-benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)cyclopentyl]purine and 6-Dimethylamino-9-[(1R,2S,4R,5R)-2,4,5-trihydroxy-3-(N-benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (14).* DCC (184 mg) was added under ice-cooling to a solution of **13** (249 mg) and *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[14] (293 mg) in DMF (9 ml) containing *N*-hydroxysuccinimide (103 mg). The mixture was worked up as in the preparation of **7a** and **7b** to give 362 mg (71%) of **14**, mp 125—132 °C. $^1H$ NMR (DMSO-

$d_6$): $\delta$ 3.63 (s, 6, 2×$NCH_3$), 3.90 (s, 3, $OCH_3$), 5.21 (s, 2, benzyl $CH_2$), 8.59 (s, 2, purine H-2 and 8).

Found: C, 59.25; H, 5.81; N, 16.10%. Calcd for $C_{30}H_{35}N_7O_7$: C, 59.49; H, 5.83; N, 16.19%.

*6-Dimethylamino-9-[(1S,2R,4S,5S)-2,4,5-trihydroxy-3-(*p*-methoxyphenyl-*L*-alanylamino)cyclopentyl]purine* (**15a**) *and 6-Dimethylamino-9-[(1R,2S,4R,5R)-2,4,5-trihydroxy-3-(*p*-methoxyphenyl-*L*-alanylamino)cyclopentyl]purine* (**15b**). A solution of **14** (543 mg) in glacial acetic acid (40 ml) was hydrogenated in the presence of palladium black in a hydrogen atmosphere (3.0 kg/cm²) for 3 h. The catalyst was filtered off and the filtrate was evaporated. The methanolic solution of the residue was passed through a column of Amberlite IRA-400($OH^-$) resin. The effluent was evaporated and the residue was purified by column chromatography using 6:1 (v/v) chloroform–methanol. Fractions showing a single spot at $R_f$ 0.25 on TLC in the same solvent system were combined and evaporated to give 137 mg (32%) of **15a**, mp 190—191 °C, $[\alpha]_D^{21}$ −96.4° (*c* 1.00, DMF). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon$=2.39×10⁴), $\lambda_{max}^{H_2O}$ 275 nm ($\varepsilon$=2.51×10⁴), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon$=2.53 × 10⁴). $^1$H NMR (DMSO-$d_6$): $\delta$ 3.64 (s, 6, 2×$NCH_3$), 3.91 (s, 3, $OCH_3$), 8.59 (s, 2, purine H-2 and 8).

Found: C, 55.84; H, 6.09; N, 20.58%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

Fractions showing a single spot at $R_f$ 0.20 on TLC in the same solvent were combined and evaporated to give 145 mg (34%) of **15b**, mp 121—122 °C, $[\alpha]_D^{21}$ +9.0° (*c* 1.00, DMF). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon$=2.11×10⁴), $\lambda_{max}^{H_2O}$ 276 nm ($\varepsilon$=2.17×10⁴), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon$=2.18×10⁴). $^1$H NMR (DMSO-$d_6$): $\delta$ 3.63 (s, 6, 2×$NCH_3$), 3.91 (s, 3, $OCH_3$), 8.59 (s, 2, purine H-2 and 8).

Found: C, 55.82; H, 6.31; N, 20.95%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

DL-*(1,2,4/3,5)-3-Acetamido-1-amino-2,4,5-cyclopentanetriol* (**16**). The compound was prepared by the method of Tadano *et al.*[11]

*6-[*DL-*(1,2,4/3,5)-3-Acetamido-2,4,5-trihydroxycyclopentyl]amino-4-dimethylamino-5-nitropyrimidine* (**17**). A mixture of **16** (990 mg) and 6-chloro-4-dimethylamino-5-nitropyrimidine[13] (1.27 g) in 2-methoxyethanol (51 ml) containing triethylamine (2 ml) was heated at 98 °C for 2 h with mechanical agitation. The solution was evaporated and the residue was recrystallized from ethanol to give 1.43 g (77%) of **17**, mp 216—218 °C (dec).

Found: C, 43.61; H, 5.60; N, 23.42%. Calcd for $C_{13}H_{20}N_6O_6$: C, 43.82; H, 5.66; N, 23.58%.

*6-[(*DL-*(1,2,4/3,5)-3-Acetamido-2,4,5-trihydroxycyclopentyl)-amino]-5-amino-4-dimethylaminopyrimidine* (**18**). Compound **17** (1.43 g) was catalytically hydrogenated in 50% aqueous ethanol (25 ml) for 15 h. The product was recrystallized from ethanol to give 1.19 g (91%) of **18**, mp 187—189 °C (dec).

Found: C, 47.52; H, 7.00; N, 25.31%. Calcd for $C_{13}H_{22}N_6O_4$: C, 47.84; H, 6.80; N, 25.75%.

*9-[*DL-*(1,2,4/3,5)-3-Acetamido-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine* (**19**). A mixture of **18** (552 mg) and formamide (16 ml) was heated at 180 °C for 1.5 h. The solution was evaporated and the residue was dissolved in boiling water. After being decolorized with active charcoal, the solution was settled in a refrigerator to give 427 mg (75%) of **19** as needles, mp 295—297 °C (dec). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon$=2.04×10⁴), $\lambda_{max}^{H_2O}$ 277 nm ($\varepsilon$=2.04×10⁴), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon$=2.33×10⁴).

Found: C, 50.24; H, 6.00; N, 25.24%. Calcd for $C_{14}H_{20}N_6O_4$: C, 49.99; H, 5.99; N, 24.99%.

*9-[*DL-*(1,2,4/3,5)-3-Amino-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine* (**20**). A mixture of **19** (1.48 g) and barium hydroxide octahydrate (19 g) in water (300 ml) was heated under reflux for 4 h. The mixture was worked up as in the preparation of **6** to give 1.16 g (90%) of **20**, mp 204—205 °C (dec). An analytically pure sample was obtained by recrystallization from aqueous ethanol.

Found: C, 48.72; H, 6.20; N, 28.71%. Calcd for $C_{12}H_{18}N_6O_3$: C, 48.97; H, 6.16; N, 28.55%.

*A Mixture of 6-Dimethylamino-9-[(1S,2S,4R,5R)-2,4,5-trihydroxy-3-(*N*-benzyloxycarbonyl-*p*-methoxyphenyl-*L*-alanylamino)cyclopentyl]purine and 6-Dimethylamino-9-[(1R,2R,4S,5S)-2,4,5-trihydroxy-3-(*N*-benzyloxycarbonyl-*p*-methoxyphenyl-*L*-alanylamino)-cyclopentyl]purine* (**21**). To a solution of **20** (218 mg) and *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[14] (256 mg) in DMF (10 ml) containing *N*-hydroxysuccinimide (90 mg), DCC (161 mg) was added under ice-cooling. The mixture was worked up as in the preparation of **7a** and **7b** to give 334 mg (74%) of **21**, mp 214—216 °C.

Found: C, 59.52; H, 5.84: N, 16.37%. Calcd for $C_{30}H_{35}N_7O_7$: C, 59.49; H, 5.83; N, 16.19%.

*6-Dimethylamino-9-[(1S,2S,4R,5R)-2,4,5-trihydroxy-3-(*p*-methoxyphenyl-*L*-alanylamino)cyclopentyl]purine* (**22a**) and *6-Dimethylamino-9-[(1R,2R,4S,5S)-2,4,5-trihydroxy-3-(*p*-methoxyphenyl-*L*-alanylamino)cyclopentyl]purine* (**22b**). A solution of **21** (500 mg) in glacial acetic acid (15 ml) was hydrogenated in the presence of 10% palladium on charcoal in a hydrogen atmosphere (3.0 kg/cm²) for 2 h. The solution was worked up as in the preparation of **8a**, and the product was purified by column chromatography using 3 : 1 (v/v) chloroform–methanol. Fractions showing a single spot at $R_f$ 0.45 on TLC in the same solvent system were combined and concentrated to give 90 mg (23%) of **22a**, mp 259—261 °C (dec), $[\alpha]_D^{20}$ −31.9° (*c*, 0.99, DMF).

Found: C, 55.85; H, 6.24; N, 20.47%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04, H, 6.20; N, 20.79%.

Fractions showing a single spot at $R_f$ 0.31 on TLC in the same solvent were combined and concentrated to give 104 mg (27%) of **22b**, mp 258—260 °C (dec), $[\alpha]_D^{20}$ −3.6° (*c* 1.01, DMF).

Found: C, 56.04; H, 6.19; N, 20.60%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

N,O-*Tetraacetyl Derivative of* **22a** (**23a**). Compound **22a** (30 mg) was acetylated and the product was purified by a column chromatography using 5 : 1 (v/v) benzene–ethanol. The product was washed with ether to give 37 mg (91%) of **23a**, mp 188—189 °C. $^1$H NMR: $\delta$ 1.80 (s, 3, NAc), 1.91 (s, 3, OAc), 1.97 (s, 3, OAc), 2.06 (s, 3, OAc), 3.01 (d, 2, *J*= 7 Hz, $C\underline{H}_2C_6H_4$), 3.46 (s, 6, 2×$NCH_3$), 3.69 (s, 3, $OCH_3$), 6.58 (d, 1, *J*=8 Hz, NH), 6.89 (q, 4, $C_6H_4$), 7.67 (d, 1, *J*= 6 Hz, NH), 7.72 (s, 1, purine H-2 or 8), 8.18 (s, 1, purine H-8 or 2).

Found: C, 56.28; H, 5.90; N, 15.04%. Calcd for $C_{30}H_{37}N_7O_9$: C, 56.33; H, 5.83; N, 15.33%.

N,O-*Tetraacetyl Derivative of* **22b** (**23b**). Compound **22b** (30 mg) was acetylated and the product was purified as in the preparation of **23a** to give 28 mg (69%) of **23b** as a glass. $^1$H NMR: $\delta$ 1.83 (s, 3, NAc), 1.90 (s, 3, OAc), 1.98 (s, 3, OAc), 2.05 (s, 3, OAc), 3.00 (d, 2, *J*=6 Hz, $C\underline{H}_2C_6H_4$), 3.47 (s, 6, 2×$NCH_3$), 3.69 (s, $OCH_3$), 6.45 (d, 1, *J*=8 Hz, NH), 6.87 (q, 4, $CH_2C_6\underline{H}_4$), 7.56 (d, 1, *J*=7 Hz, NH), 7.72 (s, 1, purine H-2 or 8), 8.18 (s, 1, purine H-8 or 2).

Found: C, 56.29; H, 6.09; N, 14.95%. Calcd for $C_{30}H_{37}N_7O_9$: C, 56.33; H, 5.83; N, 15.33%.

DL-*(1,2,5/3,4)-3-Acetamido-1-amino-2,4,5-cyclopentanetriol* (**24**) The compound was prepared by the method of Tadano *et al.*[11]

*6-[(*DL-*(1,2,5/3,4)-3-Acetamido-2,4,5-trihydroxycyclopentyl)-amino]-4-dimethylamino-5-nitropyrimidine* (**25**). A mixture of **24** (402 mg) and 6-chloro-4-dimethylamino-5-nitropyrim-

idine[13] (514 mg) in 2-methoxyethanol (21 ml) containing triethylamine (1 ml) was heated at 80 °C for 2.5 h. The solution was evaporated and the residue was washed with warm ethanol to give 564 mg (75%) of **25**, mp 215—216 °C (dec).

Found: C, 43.96; H, 5.64; N, 23.72%. Calcd for $C_{13}H_{20}N_6O_6$: C, 43.82; H, 5.66; N, 23.58%.

*6-[(DL-(1,2,5/3,4)-3-Acetamido-2,4,5-trihydroxycyclopentyl)-amino]-5-amino-4-dimethylaminopyrimidine (26).* Compound **25** (796 mg) was catalytically hydrogenated in water (50 ml) for 5.5 h. The catalyst was filtered off and the filtrate was evaporated to give 635 mg (87%) of **26**, mp 229—230 °C (dec).

Found: C, 48.13; H, 6.75; N, 26.10%. Calcd for $C_{13}H_{22}N_6O_4$: C, 47.84; H, 6.80; N, 25.75%.

*9-[DL-(1,2,5/3,4)-3-Acetamido-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine (27).* A mixture of **26** (319 mg) and triethyl orthoformate (1.6 ml) in DMF (53 ml) was heated at 67 °C for 4 h in the presence of *p*-toluenesulfonic acid (10 mg). After being neutralized with Amberlite IRA-400 ($OH^-$) resin, the solution was evaporated. The residue was dissolved in 0.1 M HCl (33 ml). After 2 h, the solution was evaporated. The residue was dissolved in water and neutralized with 0.1 M NaOH. The solution was allowed to settle in a refrigerator to give 291 mg (89%) of **27** as crystals, mp 233—234 °C (dec).

Found: C, 49.71; H, 5.95; N, 24.65%. Calcd for $C_{14}H_{20}N_6O_4$: C, 49.99; H, 5.99; N, 24.99%.

*9-[DL-(1,2,5/3,4)-3-Amino-2,4,5-trihydroxycyclopentyl]-6-dimethylaminopurine (28).* A mixture of **27** (340 mg) and barium hydroxide octahydrate (2.7 g) in water (34 ml) was heated at 100 °C for 4 h. The solution was worked up as in the preparation of **6** to give 278 mg (93%) of **28**. UV: $\lambda_{max}^{0.1M\ HCl}$ 268 nm ($\varepsilon=1.48\times10^4$), $\lambda_{max}^{H_2O}$ 275 nm ($\varepsilon=1.64\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 275 nm ($\varepsilon=1.64\times10^4$). $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.63 (s, 6, $2\times NCH_3$), 8.47 s, (1, purine H-2 or 8), 8.58 (s, 1, purine H-8 or 2).

Hydrochloride of **28** was obtained as crystals in 46% yield by recrystallization from methanol, mp 163—164 °C.

Found: C, 41.67; H, 5.97; N, 23.86; Cl, 10.01%. Calcd for $C_{12}H_{19}N_6O_3Cl\cdot H_2O$: C, 41.32; H, 6.06; N, 24.09; Cl, 10.16%.

*6-Dimethylamino-9-[(1S,2S,4S,5S,)-2,4,5-trihydroxy-3-(N-benzyloxycarbonyl-p-methoxyphenyl-L-alanylamino)cyclopentyl] Purine (29a).* DCC (202 mg) was added under ice-cooling with agitation to a solution of **28** (274 mg) and *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[14] (323 mg) in DMF (11 ml) containing *N*-hydroxysuccinimide (113 mg). The mixture was worked up as in the preparation of **7a** to give 165 mg (29%) of **29a**, mp 222—223 °C (dec) (from methanol).

Found: C, 58.52; H, 5.80; N, 15.58%. Calcd for $C_{30}H_{35}N_7O_7$–$CH_3OH$: C, 58.39; H, 6.16; N, 15.38%.

The product was acetylated to give tri-*O*-acetyl derivative of **29a** in a quantitative yield as a syrup. $^1H$ NMR (DMSO-$d_6$): $\delta$ 1.85 (s, 3, OAc), 1.97 (s, 3, OAc), 2.00 (s, 3, OAc), 2.98 (d, 2, $J=7$ Hz, $C\underline{H}_2C_6H_4$), 3.46 (s, 6, $2\times NCH_3$), 3.70 (s, 3, $OCH_3$), 5.02 (s, 2, benzyl $CH_2$), 7.79 (s, 1, purine H-2 or 8), 8.23 (s, 1, purine H-8 or 2).

Found: C, 59.06; H, 5.76; N, 13.14%. Calcd for $C_{36}H_{41}N_7O_{10}$: C, 59.09; H, 5.65; N, 13.40%.

*6-Dimethylamino-6-[(1S,2S,4S,5S)-2,4,5-trihydroxy-3-(p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (30a).* A solution of **29a** (32 mg) in glacial acetic acid (8 ml) was hydrogenated in the presence of palladium black in a hydrogen atmosphere (3.0 kg/cm²) for 4 h. The solution was worked up as in the preparation of **8a** to give 12 mg (48%) of **30a**, mp 139—140 °C (from methanol), $[\alpha]^{19}$ −49.4° (*c* 1.13, methanol). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon=2.11\times10^4$), $\lambda_{max}^{H_2O}$ 275 nm ($\varepsilon=2.15\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 275 nm ($\varepsilon=2.00\times10^4$). $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.64 (s, 6, $2\times NCH_3$), 3.90 (s, 3, $OCH_3$), 8.55 (s, 1, purine H-2 or 8), 8.61 (s, 1, purine H-8 or 2).

Found: C, 55.78; H, 6.35; N, 20.41%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

*6-Dimethylamino-9-[(1R,2R,4R,5R)-2,4,5-trihydroxy-3-(p-methoxyphenyl-L-alanylamino)cyclopentyl]purine (30b).* The methanolic mother liquor of **29a** was concentrated to give 741 mg of a residual syrup. A solution of the residue in glacial acetic acid (14 ml) was hydrogenated in the presence of palladium black in a hydrogen atmosphere (3.0 kg/cm²) for 3 h. The solution was worked up as in the preparation of **8a**. The product was purified by column chromatography using 5 : 1 (v/v) chloroform–methanol. Fractions showing a single spot at $R_f$ 0.27 on TLC in the same solvent were combined and evaporated to give 51 mg of **30a**. Fractions showing a single spot at $R_f$ 0.21 on TLC in the same solvent were combined and evaporated to give 113 mg (26% from **28**) of **30b**, mp 201—202 °C (dec), $[\alpha]_D^{19}$ +42.0° (*c* 1.13, methanol). UV: $\lambda_{max}^{0.1M\ HCl}$ 269 nm ($\varepsilon=1.96\times10^4$), $\lambda_{max}^{H_2O}$ 275 nm ($\varepsilon=2.05\times10^4$), $\lambda_{max}^{0.1M\ NaOH}$ 276 nm ($\varepsilon=2.19\times10^4$). $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.63 (s, 6, $2\times NCH_3$), 3.89 (s, 3, $OCH_3$), 8.53 (s, 1, purine H-2 or 8), 8.59 (s, 1, purine H-8 or 2).

Found: C, 55.78; H, 6.29; N, 20.64%. Calcd for $C_{22}H_{29}N_7O_5$: C, 56.04; H, 6.20; N, 20.79%.

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## References

1) This article is dedicated to the memory of Yasufumi Emori, an exceptionally able coworker who died tragically in a mountain climbing accident in 1976. The numbering of aminocyclitols in this article does not follow the IUPAC and IUB tentative rules for cyclitol nomenclature [*J. Biol. Chem.*, **243**, 5809 (1968)]. Aminocyclitols are numbered in accordance with the numbering of 3-amino-3-deoxy-D-ribofuranose, since it will avoid change in the numbering before and after the condensation of the aminocyclitols with pyrimidine and purine derivatives.

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17) In NMR descriptions s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet.
18) The end point dilution in μg/ml represents the lowest dilution at which degeneration of cells was observed by microscopic observation after incubation at 37 °C for 48 h.
19) In Schemes 2 and 3, the structures depict only one enantiomer of the racemic form actually obtained.