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The Absolute Stereochemistry of Nebularine–Methanol Photoadduct. A Potential Transition-State Analog of Adenosine Deaminase

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A stereoselective photoaddition of methanol to nebularine and 2′,3′,5′-tri-*O*-acetylnebularine was investigated. The absolute stereochemistry of the nebularine–methanol photoadduct (**2a**), a potential transition-state analog of adenosine deaminase, was determined to be 6(*S*) by X-ray analysis. The addition site of methanol on the purine ring was also chemically demonstrated to be at C(6).

Keywords——photoaddition; nebularine; triacetylnebularine; transition-state analog; X-ray analysis; adenosine deaminase; enzyme inhibitor; coformycin; isocoformycin

In hydrolytic enzyme reactions catalyzed by adenosine deaminase[2a] (adenosine→inosine), guanine deaminase[2b] (guanine→xanthine), and cytidine deaminase[3a] (cytidine→uridine), the displacement of ammonia by water is considered to proceed through an addition–elimination mechanism[4] (Chart 1). The transition-state analog concept developed by Wolfenden was based on the finding that one of the nebularine–methanol photoadducts more strongly inhibits adenosine deaminase than the other. This observation was rationalized by postulating that the enzymatic reaction involves stereospecific displacement from one side of the purine ring. Several known inhibitors of these enzymes are regarded as analogs[5,6] of the tetrahedral intermediate **1**, most notably coformycin[7] (**3a**), 2′-deoxycoformycin[8] (**3b**), and isocoformycin[9] (**4**) for adenosine deaminase, and tetrahydrouridine[3] (**5**) and 5-hydroxy-1,3-diazepin-2-one nucleosides[10] (**6**) for cytidine deaminase. One of the most important factors in the transition-state analog concept is the stereochemistry of the tetrahedral substrate intermediates formed in the course of the enzymatic deamination reaction. However, the absolute structure of the original photoadduct (**2a**) has remained to be determined for the past decade.[5,11] We report here a stereoselective synthesis of the photoadduct **2a** which enabled us to assign the absolute stereochemistry of **2a** by X-ray crystallography.

Previously, we reported a synthesis of coformycin[12] and isocoformycin[9] starting from nebularine. The seven-membered aglycones were formed by a solvolytic ring expansion of the mesylate of the photoadduct, and it became apparent that coformycin could not be produced stereospecifically but was obtained as a mixture of epimers at C (8).[9] The products formed at each step were very sensitive to reaction conditions and more careful investigation was required. In particular, the photoaddition step[11] and the stereochemistry of the photoadducts were carefully reinvestigated.

It was found that the photoaddition proceeded in a highly stereoselective manner. The photoreaction of nebularine with methanol using a quartz filter afforded the same products as reported by Evans and Wolfenden,[11] and we isolated two diastereomers in a ratio of 10 to 1 by column chromatography on silica gel (Wakogel C200, Wako Pure Chemical Co.) with chloroform–methanol (7 : 3) in 82% yield, accompanied by a side product, 6-hydroxymethyl-

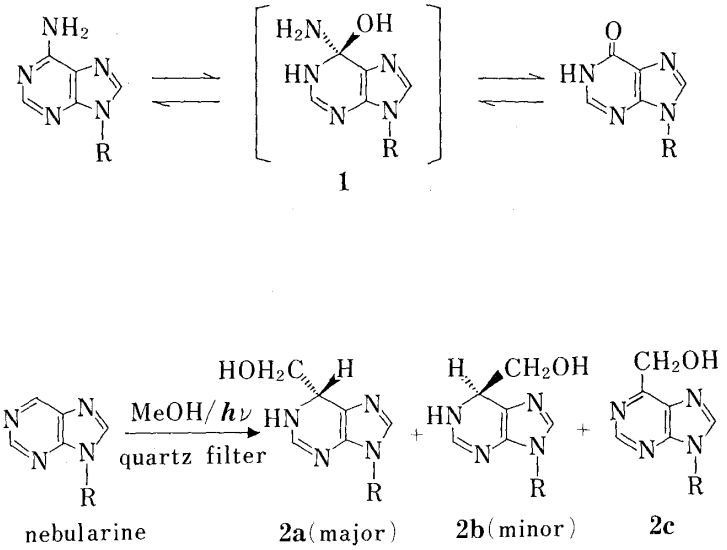


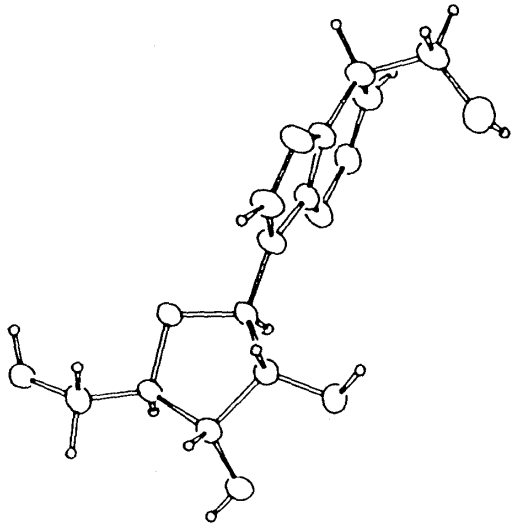
Chart 1

9-β-D-ribofuranosylpurine,[11] **2c** (3.3% yield): $[\alpha]_D^{20}$ −9.7° (c=3.0, MeOH); UV λ_{max}^{MeOH} 263 nm (ε 8600). The major photoadduct, **2a**: $[\alpha]_D^{23}$ −70.0° (c=1.0, MeOH); UV λ_{max}^{pH7} 297 nm (ε 4770), was found to be identical with Wolfenden's sample II,[11] the more active inhibitor of adenosine deaminase, by comparison of the mobility on a thin layer chromatogram (Merck Silica gel G F_{254}, Rf=0.14 in $CHCl_3$: MeOH = 7 : 3) and inhibition of deaminase. The K_i value of **2a** for adenosine deaminase from calf intestinal mucosa was 4.8×10^{-7} M. Evans and Wolfenden reported K_i values of 7.6×10^{-7} M for compound II and 125×10^{-7} M for the diastereoisomer of II (II′), using the enzyme from calf duodenum.[11] The mass spectrometry of **2a** showed m/e 284 (M^+) and 267 (M^+-OH) but there was no peak at m/e 267 in the spectrum of the minor product, **2b**: $[\alpha]_D^{25}$ −44.0° (c=1.5, MeOH); UV λ_{max}^{pH7} 293 nm (ε 4180); Rf=0.08. In the proton nuclear magnetic resonance (^{1}H NMR) spectrum of **2b** in deuterium oxide, a geminal AB-type quartet coupling (J_{AB}=14 Hz) of the methylene protons in the 6-hydroxymethyl group was observed, and this suggested the formation of a rigid intramolecular hydrogen bond,[13] which is consistent with the absence of the dehydrated fragment at m/e 267 in the mass spectrum.

The absolute stereochemistry of the adduct **2a** was determined by X-ray diffraction analysis to be 6(S). The adduct **2a** (200 mg) was dissolved in water (2 ml) in a test tube which was immersed in an acetone bath in a sealed system over one month at room temperature, affording needle crystals (dec. 165—175 °C) suitable for X-ray analysis. A total of 1410 reflections were measured as above the 2δ (I) level out of 1510 independent reflections within the 2θ range of 6° through 150°.

Crystal Data: Photoadduct **2a**, $C_{11}H_{16}N_4O_5$ MW=284.3, monoclinic space group $P2_1$, Z=2, D_{cal}=1.496 g cm^{-3}, a=11.141 (6), b=5.191 (3), c=11.021 (6) Å, β=97.92 (5)°, U=631.3 Å. The structure was solved by the direct method using MULTAN[14] and the locations of all the hydrogen atoms were found by successive use of least-squares calculations and difference Fourier syntheses. The refinement was carried out by block-diagonal least-squares calculations. The molecular structure is illustrated in Fig. 1. The bond lengths, bond angles, torsional angles and molecular conformation were next compared with those of coformycin.[15]

The absolute configuration was deduced from the known configuration of the D-



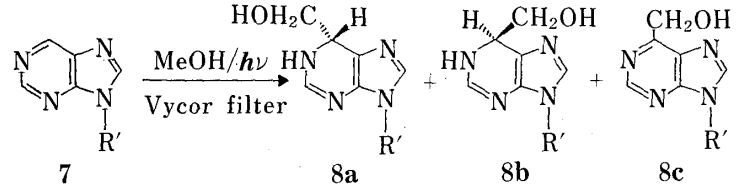
3a : $R^1=R^3=OH$, $R^2=H$
3b : $R^1=OH$, $R^2=R^3=H$
3c : $R^1=H$, $R^2=R^3=OH$

4

5

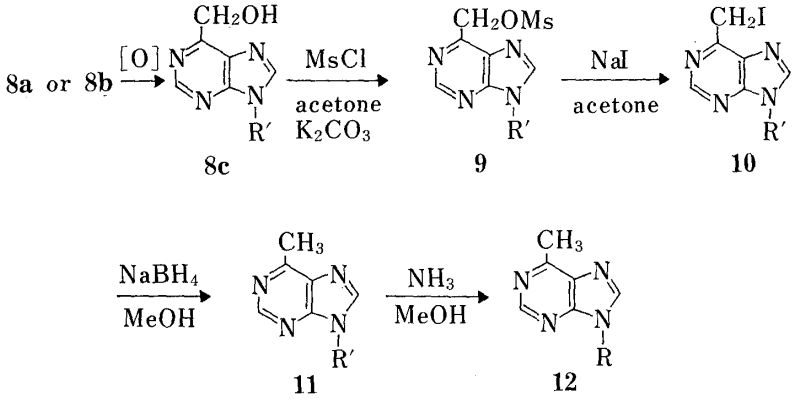
6a : $R^1=OH$, $R^2=H$
6b : $R^1=H$, $R^2=OH$

Fig. 1. Molecular Structure of Photoadduct **2a**



R′ = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl

Chart 2



R′ = 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl
R = β-D-ribofuranosyl

Chart 3

ribofuranoside moiety of nebularine, which had been chemically determined.[16] The attachment of the hydroxymethyl group at C(6) is of the *S*-configuration. The bond lengths and angles in the ribofuranoside moiety are very similar to those found in coformycin, although the puckering of the furanose ring is slightly different. The bond lengths in the base moiety are close to the corresponding ones in coformycin if one disregards the bonds C(7)–C(8) and

C(8)–O(8) in coformycin. The bond angles, on the other hand, are markedly different except for those involved in the imidazole ring. Thus, all the endocyclic angles in the six-membered ring are significantly smaller than the corresponding angles in coformycin, since in the latter compound, the six-membered ring is replaced by the seven-membered diazepine ring. The difference is especially marked at N(3) and C(4), where the endocyclic angles, C(2)–N(3)–C(4) and N(3)–C(4)–C(5), are reduced and the exocyclic angles, C(6)–C(5)–N(7) and N(3)–C(4)–N(9), are increased by several degrees. The glycosyl torsional angle χ[C(8)–N(9)–C(1′)], 68.9°; lies in the range of usual values for *anti* configuration. The torsional angles of the furanose ring are $\tau_0 = 44.6°$, $\tau_1 = -38.1°$, $\tau_2 = -17.2°$, $\tau_3 = -8.4°$, and $\tau_4 = 32.9°$, and the conformation of the ring is C(1′)-*exo*-half-chair, whereas that of coformycin is C(1′)-*exo*-C(2′)-*endo*. The conformation about the C(4′)–C(5′) bond is *gauche-trans*, whereas that of coformycin is *gauche-gauche*.

The present analysis establishes the structure of the photoadduct **2a** to be (6*S*)-1,6-dihydro-6-hydroxymethyl-9-β-D-ribofuranosylpurine. Furthermore, stereoselective photoaddition was also found in the photoreaction of 2′,3′,5′-tri-*O*-acetylnebularine, especially using a Vycor filter (Chart 2). Compared with the case of nebularine, it took much longer to accomplish the photoaddition reaction; after a 10 h irradiation at 254 nm, a small amount of the starting triacetylnebularine **7** was detected by thin layer chromatography, whereas nebularine was fully converted to the photoadducts after about 3 h irradiation. Two diastereomers were isolated by silica gel (Wakogel C200) column chromatography with benzene–acetone (2 : 1 → 1 : 2), in a ratio of 9 : 1 using the Vycor filter. This stereoselectivity was somewhat decreased (to 2 : 1) when the quartz filter was used. The major product, **8a**: $[\alpha]_D^{25}$ −30.0° ($c = 3.0$, MeOH); UV λ_{max}^{MeOH} 299 nm (ε 4750), was identified as the 2′,3′,5′-tri-*O*-acetate of **2a** and the minor one, **8b**: $[\alpha]_D^{25}$ −29.7° ($c = 3.0$, MeOH); UV λ_{max}^{MeOH} 297.5 nm (ε 4150), was the triacetate of **2b**. In addition to the diastereomers (75—85% yield), a by-product, 6-hydroxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (**8c**): $[\alpha]_D^{20}$ −9.7° ($c = 3.0$, MeOH); UV λ_{max}^{MeOH} 263 nm (ε 8600), was obtained in 10—20% yield. Compound **8c** might be derived by oxidative aromatization of the diastreomers or by disproportionation during the photochemical reaction because **8c** was always by-produced under an argon atmosphere. Evidence in support of the assigned structure was obtained from NMR spectra. The signal at 8.11 ppm in the ^{1}H NMR spectrum of **8a** was assigned to the proton H_8 in the imidazole ring from a nuclear Overhauser effect[17] (NOE, 25%) observed between the signal of H_8 and that of the anomeric proton $H_{1'}$ at 6.24 ppm. The assignment of the ribose carbon signals in the ^{13}C NMR spectra was made by reference to the data of Jones *et al.*[18a]

Stereoselective photoaddition might result from the steric effect of the ribose moiety. Favorable rotational conformations about the glycosidic C(1′)–N(9) bond may depend upon the character of the ultraviolet radiation and the solvent. The ^{1}H NMR spectra of the triacetylnebularine **7** in deuterated methanol and chloroform showed nuclear Overhauser effects of 18 and 11%, respectively, between $H_{1'}$ and $H_{8'}$ and this indicates that a *syn* conformation is favored in solution.[17] Examination of a Dreiding molecular model of the compound shows that the *syn* conformation brings the 5′-hydroxymethyl group, irrespective of the protective group on the hydroxyl function, closer to one side of the purine ring, so that addition of methanol to the ring from the other side will be favored. Thus, addition of methanol could take place stereoselectively.

The addition site of methanol on the purine ring was also chemically demonstrated to be at C(6) by transformation of the photoadduct **8a** or **8b** to the known 6-methylpurine riboside[20] (Chart 3). The diastereomer **8a** or **8b** was oxidized with phenyltrimethylammonium perbromide (PTAB)[19] to **8c**, which was then transformed to 6-mesyloxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (**9**) by treatment with mesyl chloride in dry acetone in the presence of an excess of anhydrous potassium carbonate. The mesylate **9** was converted to 6-

iodomethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (**10**) by the action of sodium iodide in acetone at room temperature. The iodide **10** was reduced with sodium borohydride in methanol to 6-methyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (**11**), which was finally deacetylated with methanolic ammonia at room temperature, affording 6-methylpurine riboside (**12**). This was confirmed to be identical with an authentic sample prepared by the method of Gordon *et al.*[20a)]

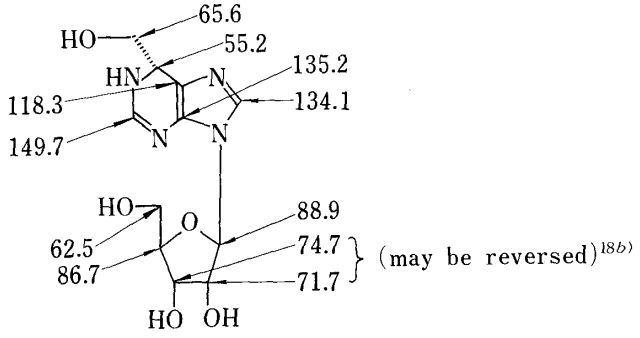
Noteworthy features of the present results include the following: (1) establishment of the absolute configuration at the tetrahedral carbon of the photoadduct **2a** may be useful for an effective approach to the synthesis of potent and selective enzyme inhibitors based on the transition state analog concept,[10,21)] (2) stereochemical considerations and inhibition constants observed for **2a**, **3a**, and **3c**[22)] suggest that stereospecific hydration of adenosine by the enzyme will take place at C(6) of the base from the *re*-face to afford the tetrahedral intermediate **1**, (3) photoaddition of methanol to nebularine or triacetylnebularine is stereoselective, showing that the steric effect of the D-ribose moiety is unexpectedly high.[23)]

Experimental

Materials and Methods——Nebularine was purchased from Sigma Chemical Co., Ltd. Commercial methanol (guaranteed grade) was used without any treatment. Photochemical experiments were carried out with a Riko low-pressure mercury lamp (30 W, 254 nm) in a quartz immersion well using a quartz or Vycor filter. Melting points were taken on a Yamato MP-21 capillary melting apparatus and are uncorrected. Proton NMR spectra were recorded on a Varian HA-100D or A-600 instrument. Chemical shifts are given as δ values with reference to Me_4Si. ^{13}C NMR spectra were obtained on a Varian XL-100 FT spectrometer. The spectra were run at room temperature with all values referenced to ^{13}C of added dioxane, which was adjusted to 67.4 ppm relative to Me_4Si. Infrared (IR) spectra were taken on a Hitachi EPI-32 spectrophotometer in a KBr tablet. Ultraviolet (UV) spectra were observed on a Hitachi 124 spectrophotometer in a 1-cm quartz cell. Specific rotations were measured in a 5-dm cell with a Carl Zeiss LEP-A2 polarimeter. Low- and high-resolution mass spectra (MS) were obtained on a Hitachi RMU-6M or RMU-7M. Thin layer chromatography (TLC) was run on Merck Silica gel G F_{254}. Column chromatography was carried out using Wakogel C200 (Wako Pure Chemical Co., Ltd.).

Photoaddition of Methanol to Nebularine——A solution of nebularine (300 mg, 1.2 mmol) in MeOH (100 ml) was irradiated at 254 nm under a constant stream of argon gas at 0—5 °C for 3 h, until TLC showed no starting nebularine. The ratio of optical density at 298 and 263 nm was 1.83. The solution was then concentrated to dryness under reduced pressure and the residual solid was chromatographed on Wakogel (C200, 10 g) using $CHCl_3$–MeOH (7:3, v/v) to afford 6(*S*)-hydroxymethyl-9-β-D-ribofuranosyl-1,6-dihydropurine (**2a**, 249 mg, 74%), 6(*R*)-hydroxymethyl-9-β-D-ribofuranosyl-1,6-dihydropurine (**2b**, 25 mg, 7.5%) and 6-hydroxymethyl-9-β-D-ribofuranosylpurine (**2c**, 11 mg, 3.3%).

2a: mp 165—175 °C (dec.); $[\alpha]_D^{23}$ −70.0° (c=1.0, MeOH); UV λ_{max}^{pH7} 297 nm (ε 4770); high-resolution mass (MS) *m/e*: 284.1151 (Theor. 284.1119 for $C_{11}H_{16}N_4O_5$), 267.1077 (M^+-OH, Theor. 267.1092 for $C_{11}H_{15}N_4O_4$); IR (KBr): 3350, 1620, 1580, 1500, 1445, 1355, 1205, 1120, 1080, 1040 cm^{-1}; ^1H NMR (100 MHz, D_2O) δ: 4.18 (2H, m, 6-CH_2OH), 4.21 (2H, m, $H_{5'}$), 4.67 (1H, m, $H_{4'}$), 4.74 (1H, m, $H_{3'}$), 5.07 (1H, m, $H_{2'}$), 5.46 (1H, t, 6-CH), 6.18 (1H, d, $H_{1'}$), 7.64 (1H, s, H_2), 8.08 (1H, s, H_8, NOE 11% with $H_{1'}$). The ^{13}C NMR spectrum is summarized in the following structure.



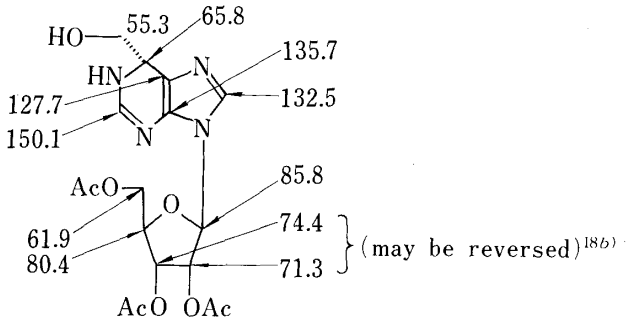
2b: mp 175—185 °C (dec); $[\alpha]_D^{25}$ −44.0° (c=1.5, MeOH); UV λ_{max}^{pH7} 293 nm (ε 4180); MS *m/e*: 284, 283, 193, 163, 151, 135, 121; IR (KBr): 3350, 1615, 1590, 1500, 1445, 1355, 1215, 1125, 1080, 1050 cm^{-1}; ^1H NMR (100 MHz, D_2O) δ: 4.14 (2H, q, AB-type, $|J|$=14 Hz, 6-CH_2OH), 4.26 (2H, m, $H_{5'}$), 4.70 (1H, m, $H_{4'}$), 4.80 (1H, m, $H_{3'}$) 5.08 (1H, m,

$H_{2'}$), 6.21 (1H, d, $H_{1'}$), 7.77 (1H, s, H_2), 8.11 (1H, s, H_8).

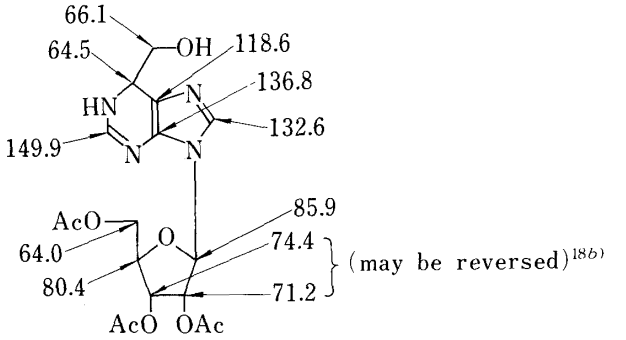
9-2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosylpurine (7)——A solution of 2′,3′,5′-tri-*O*-acetylthioinosine[24] (15 g) in EtOH (1 l) was refluxed with 40 ml of Brown's nickel[25] suspended in EtOH for 4 h until no starting material was detected on TLC. The reaction mixture was then filtered through Hyflo Super-Cel and the filter cake was washed with three 20 ml portions of hot EtOH. The combined filtrate and washings were evaporated under reduced pressure to leave a yellow foam, which was chromatographed on silica gel with benzene–acetone (2 : 1, v/v). This purification afforded **7** (10.4 g, 75%) as a colorless foam: $[\alpha]_D^{20}$ −11.4° (c=1.4, MeOH); UV λ_{max}^{MeOH} 263 nm (ε 8300); MS *m/e*: 379 [(M+1)$^+$], 378 (M$^+$), 335 (M$^+$−Ac), 319 (−OAc), 259 (tri-*O*-acetyl-D-ribose), 149 (base+40); IR (KBr): 1750 (OAc), 1240 (OAc), 1240 (OAc) cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 M Hz) δ; 2.11, 2.13, 2.18, (9H, 3s, Ac), 4.50 (2H, $H_{5'}$), 4.52 (1H, $H_{4'}$), 5.67 (1H, t, $H_{3'}$), 6.09 (1H, t, $H_{2'}$), 6.37 (1H, d, $H_{1'}$), 8.40 (1H, s, H_8, NOE 11% with $H_{1'}$), 9.08 (1H, s, H_2), 9.25 (1H, s, H_6). This product showed a single spot on silica gel TLC with *Rf* 0.40 in benzene–acetone (1 : 2, v/v) and *Rf* 0.50 in EtOAc–MeOH (5 : 1, v/v), and it was so hygroscopic that the elementary analysis gave no reasonable result.

Photoaddition of Methanol to Compound 7——Method A (with a quartz filter): A solution of **7** (1.0 g, 2.65 mmol) in MeOH (200 ml) was irradiated at 254 nm (30 W low-pressure mercury lamp) while argon gas was bubbled into the stirred solution. The temperature was kept at 0—5 °C by immersing the reaction vessel in an ice-water bath. After 10 h the starting material was almost wholly converted into three major products which showed *Rf* values of 0.10 (6(*R*)-hydroxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl-1,6-dihydropurine (**8b**)), 0.20 (6-(*S*)-hydroxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl-1,6-dihydropurine (**8a**)) and 0.43 (6-hydroxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (**8c**) in EtOAc–MeOH (5 : 1, v/v), and 0.03 (**8b**), 0.10 (**8a**) and 0.29 (**8c**) in benzene–acetone (1 : 2, v/v), respectively, on silica gel TLC. Removal of the solvent under reduced pressure left a yellow form (1.08 g), which was chromatographed on silica gel with benzene–acetone (2 : 1→1 : 2, v/v). Some starting material was recovered first (10%), then an aromatized by-product **8c** was eluted (200 mg, 18%). Pure diastereomers **8a** (543 mg, 50%) and **8b** (272 mg, 25%) were isolated in the third and fourth fractions, respectively.

Isomer **8a** was a colorless amorphous solid: mp 58—61 °C; $[\alpha]_D^{23}$ −30° (c=3.0, MeOH); UV λ_{max}^{MeOH} 299 nm (ε 4750); MS *m/e*: 452 [(M+Ac)$^+$], 410 (M$^+$), 392 (M$^+$−H_2O), 379 (−CH_2OH), 337, 295, 259; IR (KBr): 3400, 1750, 1610, 1580, 1495, 1445, 1380, 1235, 1100, 1045 cm^{-1}; ^{1}H NMR (D_2O, 100 MHz) δ: 2.60—2.65 (9H, 3s, Ac), 4.18 (2H, m, 6-C$\underline{H}_2$OH), 4.84 (2H, m, $H_{5'}$), 4.97 (1H, m, $H_{4'}$), 5.45 (1H, t, H_6), 5.98 (1H, t, $H_{3'}$), 6.14 (1H, t, $H_{2'}$), 6.42 (1H, d, J=5 Hz, $H_{1'}$), 7.64 (1H, s, H_2), 8.11 (1H, s, H_8, NOE 25% with $H_{1'}$). The ^{13}C NMR spectrum is summarized in the following structure.



Isomer **8b** was also a colorless amorphous solid: mp 69—72 °C; $[\alpha]_D^{23}$ −29.7 °C (c=3.0, MeOH); UV λ_{max}^{MeOH} 297.5 nm (ε 4150); MS *m/e*: 452 (M+Ac)$^+$, 410 (M$^+$), 368 (−Ac), 193, 151 (base); IR (KBr) 3375, 1745, 1610, 1580, 1500, 1440, 1380, 1235, 1100, 1050 cm^{-1}; ^{1}H NMR (D_2O, 100 MHz) δ: 2.60—2.65 (9H, 3s, Ac), 4.15 (2H, q, $|J|$=12 Hz, 6-C$\underline{H}_2$OH), 4.85 (2H, m, $H_{5'}$), 5.95 (1H, t, $H_{3'}$), 6.16 (1H, t, $H_{2'}$), 6.47 (1H, d, J=5 Hz, $H_{1'}$), 7.27 (1H, s, H_2), 8.11 (1H, s, H_8). The natural ^{13}C NMR spectrum is summarized in the following structure.



Anal. Calcd for $C_{17}H_{22}N_4O_8$: C, 49.75; H, 5.40; N, 13.65; O, 31.19. Found for **8a**: C, 50.01; H, 5.53; N, 13.72; O, 31.51. Found for **8b**: C, 49.85; H, 5.58; N, 12.05; O, 31.62.

Method B (with a Vycor Filter): A solution of **7** (1.08 g) in MeOH (200 ml) was irradiated under an argon atmosphere at 254 nm and the reaction mixture was treated according to Method A. The diastereomers **8a** and **8b** were obtained, **8a** (815 mg, 75%) and **8b** (87 mg, 8%).

Method C (with $Ni(OAc)_2$): A solution of **7** (1.08 g) in MeOH (200 ml) was mixed with $(OAc)_2$ $4H_2O$ (21 mg, 3 mol%) and irradiated at 254 nm under a constant stream of argon gas. After treatment according to Method A, two diastereomers were separated, **8a** (760 mg, 70%) and **8b** (95 mg, 9%).

In the MS of **8a** and **8b**, a peak at *m/e* 452 was always observed; it was accounted for by the recombination of an acetyl fragment with the parent one[26] during the spectrometric measurement.

6-Hydroxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (8c)——Phenyltrimethylammonium perbromide (200 mg, 0.55 mmol) was added to a solution of **8a** (205 mg, 0.5 mmol) in MeOH (20 ml), and after 10 min TLC showed complete conversion of **9a** to **9c**. The reaction mixture was extracted with chloroform and the extract was washed with water, then dried over Na_2SO_4. Removal of the solvent gave crude **8c** as a yellow syrup, which was chromatographed on silica gel with benzene–acetone (2 : 1, v/v) to afford pure **8c** (180 mg, 88%): $[\alpha]_D^{23}$ −9.7° (*c*=3.0, MeOH); UV λ_{max}^{MeOH} 263 nm (ε 8600); MS *m/e*: 451 [(M+1+Ac)$^+$], 409 [(M+1)$^+$], 391 (−H_2O), 365, 349, 259, 193 (base+44). 151 (base+2); IR (KBr): 3450, 2950, 1750, 1600, 1500, 1375, 1340, 1230, 1080, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$, 100 MHz) δ: 4.45 (3H, $H_{5'}$, $H_{4'}$), 5.22 (2H, s, C$\underline{H}_2$OH), 5.71 (1H, m, $H_{3'}$), 6.02 (1H, t, $H_{2'}$), 6.29 (1H, d, *J*= 6.0 Hz, $H_{1'}$), 8.30 (1H, s, H_2), 8.98 (1H, s, H_8).

6-Mesyloxymethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (9)——Anhydrous K_2CO_3 (4 g, 29 mmol) was added to a solution of **8c** (175 mg, 0.43 mmol) in dry acetone (20 ml), an mesyl chloride (0.29 ml, 3.74 mmol) was added all at once with stirring at room temperature. The reaction mixture was then stirred until no starting **8c** could be detected on TLC (*ca.* 24 h). Inorganic solids were filtered off and washed with fresh acetone. The combined filtrate and washings were concentrated under reduced pressure to leave a yellow syrup (182 mg, 88%) which was of satisfactory purity for use in the next step. An analytical sample was obtained in 50% yield by silica gel column chromatography (benzene–acetone=2 : 1, v/v): $[\alpha]_D^{20}$ −18.6° (*c*=1.5, MeOH); UV λ_{max}^{MeOH} 267 nm (ε 8050); MS *m/e*: 487 [(M+1)$^+$], 427, 393, 367, 349, 333, 259, 198, 156, 139, 114; IR (KBr): 3450, 1750, 1600, 1500, 1360, 1340, 1230, 1075, 1045 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ: 3.24 (3H, s, SC$\underline{H}_3$), 5.75 (2H, s, C$\underline{H}_2$OMs), 6.29 (1H, d, $H_{1'}$). This compound gave *Rf* 0.57 in benzene–acetone (1 : 2, v/v).

6-Iodomethyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (10)——The mesylate **9** (180 mg, 0.37 mmol) was dissolved in dry acetone (10 ml), and dry NaI (1 g, 6.7 mmol) was added. The reaction mixture was stirred at room temperature for 4 h until TLC showed no starting compound, then diluted with water (20 ml). The product was extracted with chloroform (20 ml × 2) and the extract was dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a yellow paste (175 mg). Purification by silica gel column chromatography with benzene–acetone (4 : 1) afforded the iodide **10** as a pale yellow syrup (109 mg, 57%); $[\alpha]_D^{24}$ −16.5° (*c*=2.0, MeOH); UV λ_{max}^{MeOH} 278 nm (ε 9200); MS *m/e*: 393 (M$^+$−I), 349, 333, 307, 263, 259, 217, 177, 163, 139, 135; IR (KBr) 3450, 2950, 1745, 1590, 1500, 1375, 1335, 1200—1240 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ: 4.86 (2H, s, C$\underline{H}_2$I), 6.26 (1H, d, $H_{1'}$), 8.30 (1H, s), 8.97 (1H, s). This compound gave *Rf* 0.63 in benzene–acetone (1 : 2).

6-Methyl-9-2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylpurine (11)——$NaBH_4$ (11.4 mg, 0.3 mmol) was added all at once to a solution of **10** (104 mg, 0.2 mmol) in MeOH (5 ml) with stirring at room temperature. After 15 min no starting **10** was observed on TLC, and the reaction mixture was neutralized with 1 N acetic acid, then diluted with water (10 ml), and extracted with CH_2Cl_2. The organic extract was dried over Na_2SO_4 and the solvent was evaporated off to leave a yellow syrup (87 mg). Purification by silica gel column chromatography with benzene–acetone (4 : 1)

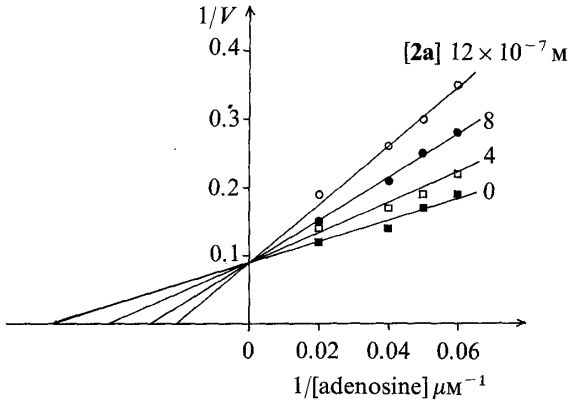


Fig. 2. Inhibition of Adenosine Deaminase by Photoadduct **2a**

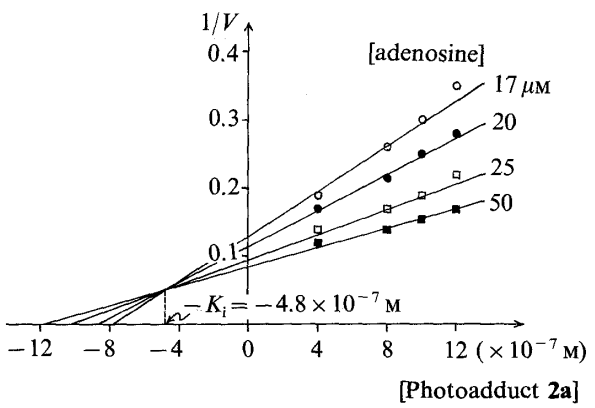


Fig. 3. Determination of K_i for Photoadduct **2a** (Dixon Plots)

gave **11** as a pale yellow syrup (69 mg, 88%): $[\alpha]_D^{23}$ −14.8° (c=4.0, MeOH); UV λ_{max}^{MeOH} 261 nm (ε 7600); MS m/e: 393 [(M+1)$^+$], 392 (M$^+$), 349, 333, 259, 163, 135; IR (KBr): 3450, 2950, 1750, 1600, 1500, 1435, 1380, 1340, 1230, 1095, 1050 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ: 2.87 (3H, s, CH_3), 6.29 (1H, d, $H_{1'}$). This product gave Rf 0.41 in benzene–acetone (1 : 2).

6-Methyl-9-β-D-ribofuranosylpurine (12)——A solution of **11** (66 mg) in MeOH (10 ml) was saturated with ammonia at room temperature and the solution was kept for 2 h, then, after removal of the solvent and excess ammonia, the residual syrup was chromatographed on a silica gel column with benzene–acetone (1 : 2) to give **12** as a colorless solid (41 mg, 75%). An analytical sample was obtained by recrystallization from EtOH: mp 209—210 °C (lit. 209—210 °C); $[\alpha]_D^{23}$ −52.0° (c=1.0, MeOH); UV $\lambda_{max}^{pH5.4}$ 261 nm (ε 7870); UV $\lambda_{max}^{pH1.0}$ 264.5 nm (ε 7300); MS m/e: 267 [(M+1)$^+$], 266 (M$^+$), 236, 235, 177, 163, 149, 135, 121, 73; IR (KBr): 3400, 2930, 1605, 1585, 1500, 1420, 1335, 1320, 1210, 1120, 1095, 1085, 910 cm^{-1}; ^{1}H NMR (60 MHz, D_2O) δ: 2.63 (3H, s, CH_3), 3.93 (2H, m, $H_{5'}$), 6.10 (1H, d, J= 5.5 Hz, $H_{1'}$), 8.58 (1H, s), 8.62 (1H, s). This compound gave Rf 0.41 on silica gel TLC in EtOAc–EtOH–H_2O (3 : 1 : 1, v/v) and Rf 0.06 in benzene–acetone (1 : 2).

Determination of K_i Value of 2a for Adenosine Deaminase——Materials: Adenosine was obtained from a commercial source and purified by recrystallization from water. Adenosine deaminase (Type I, 230 units/mg protein) from calf intestinal mucosa was purchased from Sigma Chemical Co.

Kinetic Studies: Various amounts of adenosine as the substrate were dissolved in a total volume of 1.99 ml containing 50 mM potassium phosphate buffer, pH 7.5, and various concentrations of the photoadduct **2a** as the inhibitor in a quartz cuvette of 1-cm path length.

The enzyme reaction was started by the addition of 100 μl of adenosine deaminase (40 units/l). The reaction was followed by measuring the decrease in absorbancy at 265 nm at room temperature.

Figure 2 presents a Lineweaver–Burk plot constructed from the data obtained. The K_i value of **2a** was obtained as 4.8×10^{-7} M from a Dixon plot (Fig. 3) constructed from the Lineweaver–Bruk plot.

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22) An epimeric mixture of coformycin prepared by ring expansion[9a] of the mesylate of **8a** showed an inhibition constant (K_i) of 3×10^{-10} M compared with the value of 1.5×10^{-10} M of coformycin for formycin A as the substrate. This indicates that the epimer **3c** of coformycin is a weaker inhibitor of adenosine deaminase than **3a**. In the synthetic study on 2′-deoxycoformycin,[8b] the epimer of **3b** was considered to be less active than **3b** as an inhibitor of adenosine deaminase. Isocoformycin **4** and tetrahydrouridine **5** were obtained as an epimeric mixture and may be an equilibrium mixture in solution. However, it may be reasonable to assume that **6a** (seven-membered nucleoside) is a stronger inhibitor than **6b** by analogy with the case of coformycin.
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