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Synthesis of 2,6-Diamino-9-β-D-arabinofuranosylpurine *via* Cyclonucleoside

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2,6-Diacetamido-8-hydroxy-9-(3,5-*O*-diacetyl-2-*O*-tosyl-β-D-ribofuranosyl)purine (III) was cyclized with ammonia in methanol to give a mixture of 2,6-diamino-8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine (V) and 2-acetamido-6-amino-8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine (IV), which was deacetylated to the cyclonucleoside (V) with 40% methylamine. After acetylation, the cyclonucleoside (V) was converted to 2,6-diamino-9-β-D-arabinofuranosylpurine (IX) by cleavage of the anhydro linkage with H_2S in pyridine followed by Raney Ni dethiolation. Alternatively, acetylated arabinofuranosylguanine (X) derived from 8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylguanine was chlorinated with phosphoryl chloride and aminated with ammonia in methanol to afford the arabinoside (IX).

Keywords——2,6-diamino-8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine; 2,6-diamino-9-β-D-arabinofuranosylpurine; cyclization; UV; ^{1}H-NMR; CD

2,6-Diaminopurines, nucleosides and nucleotides possess interesting biological as well as physicochemical properties.[1–6] Elion *et al.*[7] reported that 2,6-diamino-9-β-D-arabinofuranosylpurine (IX) showed high antiviral activity against herpes simplex type 2 and vaccinia viruses containing deoxyribonucleic acid (DNA).

For the synthesis of IX, several procedures have been reported: modification of a preformed arabinoside,[7] condensation of a protected base and arabinose[7] or reduction of a 3′, 5′-*O*-di- protected 2′-ketonucleoside.[8] Chemical and enzymatic methods have been used to prepare the arabinoside (IX) by transglycosylation from cyclocytidine.[9]

Ikehara *et al.* have reported a versatile method for synthesizing 9-β-D-arabinofuranosyladenine (ara-A)[10,11] and 9-β-D-arabinofuranosylguanine (ara-G)[12] *via* corresponding 8, 2′-*O*-cyclonucleosides,[10,11,13] which could be important intermediates for the preparation of biologically interesting arabinofuranosyl derivatives.

We now have developed a method for synthesizing 2,6-diamino-8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine (2,6-diaminopurine-8,2′-*O*-cyclonucleoside) (V), which was then led to IX. We first attempted to synthesize the compound V *via* 2,6-diamino-8-bromo-9-(2-*O*-tosyl-β-D-ribofuranosyl)purine (II)[14] derived from 2,6-diamino-9-β-D-ribofuranosylpurine (I).[14]

In order to convert the 8-bromo to a 8-hydroxy function, II was refluxed with excess sodium acetate in a mixture of acetic anhydride and glacial acetic acid. 2,6-Diacetamido-8-hydroxy-9-(3,5-*O*-diacetyl-2-*O*-tosyl-β-D-ribofuranosyl)purine (III) was obtained in a yield of 95%. The ultraviolet absorption spectrum (UV spectrum) of this compound showed maxima at 302—310 nm, supporting the 8-hydroxy structure (III).

In the course of the cyclization of III in methanolic ammonia at 60 °C for 8 h, we found that two compounds were formed, and they were separated by paper chromatography at pH 10 in yields of 66% and 13%. Their structures were determined to be 2-acetamido-6-amino-8, 2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine (IV) and V, respectively, on the basis

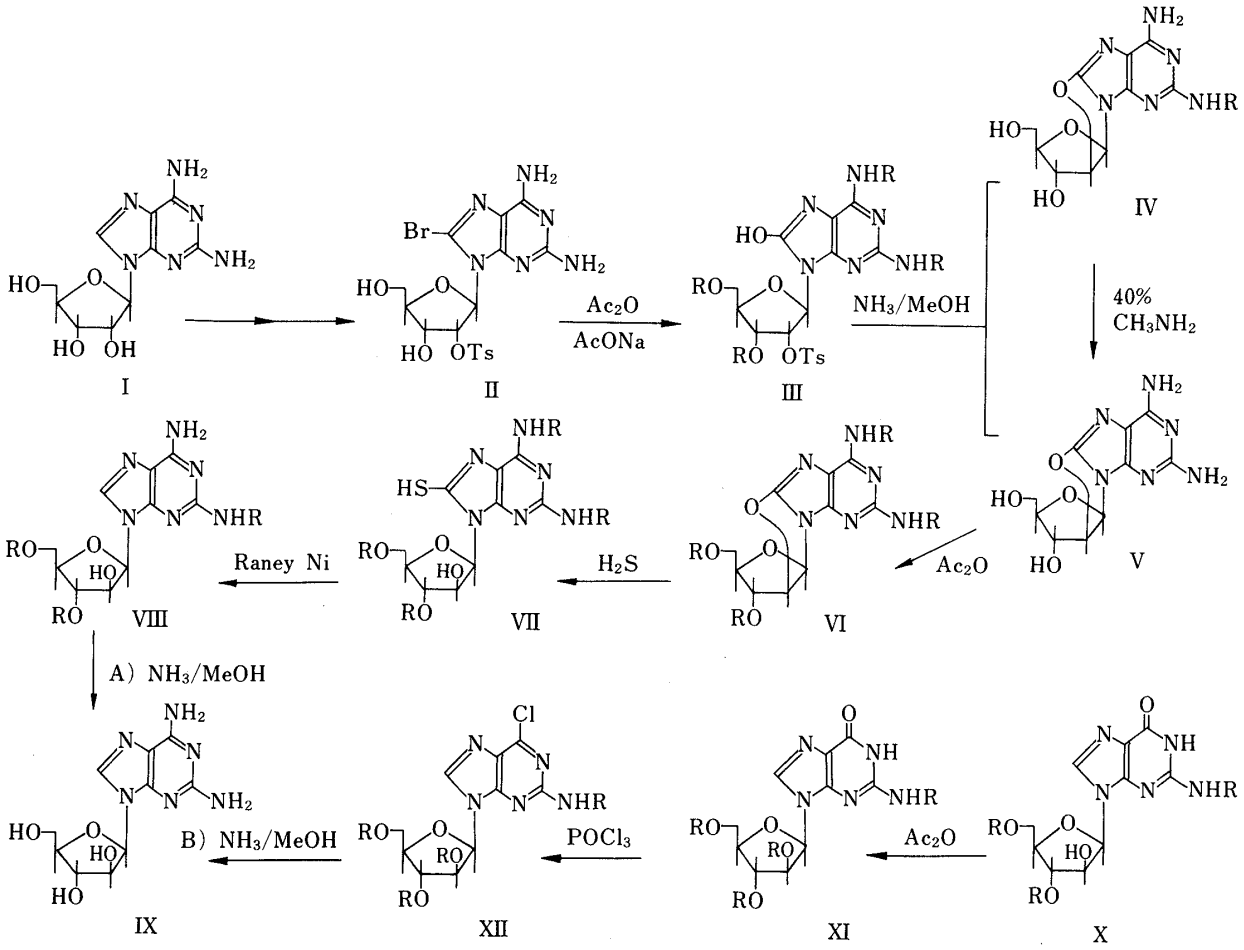

Chart 1

of the UV absorption properties and nuclear magnetic resonance (NMR) spectra. The NMR spectrum of IV showed signals due to the 6-amino group at 6.71 ppm and a methyl and an NH group of 2-acetamide at 2.13 and 9.85 ppm, respectively, showing that this 2-amino group must be acetylated. Compound V showed signals due to the 2 and 6-amino groups at 5.63 and 6.27 ppm. The signals of H-1′ of these compounds (IV and V) appeared as doublets at 6.43 and 6.30 ppm having a coupling constant $J_{1',2'}=6.0$ Hz, which suggested the 8,2′-cyclonucleoside structure.[15,16] The cyclonucleoside (IV) was deacetylated to the desired cyclonucleoside (V) by treatment with 40% methylamine at 0—5 °C for 2 h.

Before cleavage of the anhydro linkage, V was acetylated as usual to give the N^2,N^6,3′,5′-*O*-tetraacetyl-8,2′-*O*-cycloderivative (VI) in a yield of 70%. Then the acetylated cyclo compound (VI) was dissolved in pyridine; the solution was saturated with hydrogen sulfide at −50 °C, and heated at 90 °C for 15 h. 2,6-Diacetamide-8-mercapto-9-(3,5-*O*-diacetyl-β-D-arabinofuranosyl)purine (VII) was obtained in a yield of 62%. The UV absorption maxima at 219, 236, 250 (sh), 287 and 334 nm suggested the 8-mercaptopurine structure.

The mercapto derivative (VII) was then dethiolated by refluxing with Raney nickel in ethanol–water. The UV absorption maximum changed to 268 nm after 30 min. Then the mixture was finally treated with methanolic ammonia at 100 °C for 10 h. Compound IX was obtained in a yield of 67%. The structure of IX was determined on the basis of the UV absorption, mass and ^{1}H-NMR spectra. The latter showed signals of H-1′ as a doublet at 6.59 ppm having a coupling constant of $J_{1',2'}=4.1$ Hz. This value is consistent with the arabino-β-nucleoside structure. Further signals of H-8, 2 and 6-amino groups at 7.76, 6.59

TABLE I. CD Spectroscopic Data for 2,6-Diaminopurine Nucleosides

Compound	λ_{max} (nm)	$[\theta]$	λ_{min} (nm)	$[\theta]$
Riboside (I)	270	$-510^{a,14)}$	250	$-4000^{b,14)}$
			280	$-820^{c,14)}$
Deoxyriboside	265	$-1500^{d,14)}$	250	$-3200^{e,14)}$
			279	$-1700^{f,14)}$
Arabinoside (IX)	267	−760	247	−2500
			275	−900

a) -1.62×10^{-4}. *b*) -4.71×10^{-4}. *c*) -1.69×10^{-4}. *d*) -2.85×10^{-4}.
e) -5.32×10^{-4}. *f*) -3.55×10^{-4}. These values in ref. 14 were erroneous, and were recalculated.

and 5.72 ppm were consistent with the structure (IX). The circular dichroism (CD) spectrum of IX was measured in 0.01 M phosphate buffer solution at pH7.0; a negative Cotton band was seen at around the UV absorption maximum. We compared the CD spectra of 2,6-diamino-9-β-D-2′-deoxyribofuranosylpurine,[14] I[14,17] and IX, which have deoxy, ribo and arabino configurations in the sugar moieties. All the corresponding nucleosides had similar CD profiles. This might be interpreted by assuming that the ribo, deoxy and arabino structures have almost the same torsion angles.[18]

In order to confirm the structure of IX, an alternate synthetic route was investigated. N^2,3′,5′-*O*-Triacetyl-9-β-D-arabinofuranosyl guanosine (X)[19] derived from 8,2′-*O*-cyclo-guanosine was acetylated as usual to give the N^2,2′,3′,5′-*O*-tetraacetyl derivative (XI) in a yield of 87%. This product (XI) was then chlorinated with phosphoryl chloride and *N*,*N*-diethylaniline. The chloro compound (XII) was obtained almost quantitatively. The UV absorption spectrum showed maxima at 227.5, 257.5 and 286 nm. The crude chloro compound (XII) was aminated by treatment with methanolic ammonia at 100 °C for 8 h to give IX in a yield of 78%. This product (IX) proved to be identical with that obtained by cleavage of the anhydro linkage of the 2,6-diaminopurine cyclonucleoside (V) as described above.

Experimental

General Procedures——UV absorption spectra were taken with a Hitachi 340 spectrophotometer. NMR spectra were taken with a Hitachi R-22 spectrometer operated at 90 MHz. DMSO-d_6 was used as the solvent and tetramethylsilane as the internal reference. CD spectra were taken with a JASCO J-500A spectropolarimeter in a 10 mm path-length cell using 0.01 M phosphate buffer solution (pH 7.0) with 1.2 optical density (OD) of nucleoside. Mass spectra (MS) were taken with a JMS-D-100 spectrometer. Thin-layer chromatography (TLC) was performed on Kieselgel HF 254, developed with $CHCl_3$–EtOH mixture. Paper chromatography (PPC) was performed on Toyo Roshi filter paper No. 51-A using the following solvent systems: A, H_2O adjusted to pH 10 with NH_4OH; B, *n*-BuOH–H_2O (86:14, v/v); C, iso-PrOH–conc. NH_4OH–H_2O (7:1:2, v/v).

2,6-Diacetamido-8-hydroxy-9-(3,5-*O*-diacetyl-2-*O*-tosyl-β-D-ribofuranosyl)purine(III)——Compound II[14] (1.13 g, 2.2 mmol) was dissolved in a mixture of AcOH (36 ml) and Ac_2O (36 ml) containing anhyd. NaOAc (3.2 g). The mixture was heated under reflux for 30 min. The solvent was evaporated off *in vacuo* and traces of Ac_2O were decomposed and removed by evaporation several times with EtOH. The residue was suspended in $CHCl_3$ (150 ml) and H_2O (100 ml). The $CHCl_3$ layer was washed twice with saturated $NaHCO_3$, then with H_2O, and finally dried with anhyd. Na_2SO_4. The solvent was evaporated off *in vacuo* to give a crude sample of III as a glass (1.3 g, 95%). UV $\lambda_{max}^{50\%\ EtOH}$ nm: 229, 262, 302; $\lambda_{max}^{50\%\ EtOH(H^+)}$ 229, 262, 302; $\lambda_{max}^{50\%\ EtOH(OH^-)}$ 310. TLC ($CHCl_3$–EtOH, 20:1) *Rf* 0.62. PPC: *Rf* (A) 0.83, *Rf* (B) 0.91.

2,6-Diamino-8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine (V)——The 8-hydroxy compound (III) (936 mg 1.5 mmol) was dissolved in absolute MeOH (100 ml), and ammonia gas was bubbled through the solution for 30 min under cooling at −20 °C. The mixture was sealed in a steel tube and heated at 60 °C for 8 h. After removal of the solvent by evaporation *in vacuo*, the residue showed two spots having *Rf* 0.64 and *Rf* 0.30 on paper chromatography (pH 10). These products were assigned as IV and V, respectively, on the basis of the ^{1}H-NMR

spectra. The mixture was applied to Whatman 3 MM paper and developed with H_2O at pH 10. The two bands of *Rf* 0.64 and *Rf* 0.30 were extracted with H_2O and each extract evaporated to dryness. The residue of the band of *Rf* 0.64 (322 mg 66%) was recrystallzied from EtOH to give the acetylated cyclonucleoside (VI) as colorless needles mp >300 °C. *Anal.* Calcd for $C_{12}H_{14}N_6O_5 \cdot 1/2H_2O$: C, 43.54; H, 4.56; N, 25.38. Found: C, 43.56; H, 4.54; N, 24.83. UV $\lambda_{max}^{H_2O(pH\,1)}$ nm (ε): 268 (18500), 296.5 (9700), $\lambda_{max}^{H_2O(pH\,7)}$ 218 (24200), 286 (15600), $\lambda_{max}^{H_2O(pH\,13)}$ 272 (14100). ^{1}H-NMR δ: 2.13 (3H, s, CH_3), 4.05 (1H, m, H-4′), 4.42 (1H, m, H-3′), 4.92 (1H, br, 5′-OH), 5.90 (1H, br, 3′-OH), 5.63 (1H, d, H-2′), 6.43 (1H, d, H-1′, $J_{1',2'}$=6.0 Hz), 6.71 (2H, s, 6-NH_2), 9.85 (1H, s, 2-AcNH). MS *m/z*: 322 (M^+). PPC: *Rf* (A) 0.64, *Rf* (B) 0.09, *Rf* (C) 0.55.

From the other band of *Rf* 0.30, V was obtained in a yield of 58.7 mg (13%) and recrystallized from H_2O. mp >300 °C. *Anal.* Calcd for $C_{10}H_{12}N_6O_4 \cdot 1/2\,H_2O$: C, 41.52; H, 4.53; N, 29.06. Found: C, 41.41; H, 4.43; N, 28.57. UV $\lambda_{max}^{H_2O(pH\,1)}$ nm (ε): 250 (11900), 301 (10600). $\lambda_{max}^{H_2O(pH\,7)}$ 249 (10700), 284 (8500). $\lambda_{max}^{H_2O(pH\,13)}$ 251 (10100), 285 (8500). ^{1}H-NMR δ: 4.00 (1H, m, H-4′), 4.38 (1H, m, H-3′), 4.93 (1H, br, 5′-OH), 5.53 (1H, d, H-2′), 5.63 (2H, s, 2-NH_2), 6.27 (2H, s, 6-NH_2), 6.30 (1H, d, H-1′, $J_{1',2'}$=6.0 Hz). PPC: *Rf* (A) 0.30, *Rf* (B) 0.03, *Rf* (C) 0.30.

For the deacetylation of IV, 40% methylamine (6 ml) was added to the acetylated cyclonucleoside (VI) (74 mg 0.27 mM). The mixture was stirred at 0—5 °C for 2 h. The solvent was evaporated *in vacuo* and the residue was coevaporated with MeOH. The 8,2′-*O*-cyclonucleoside (V) was obtained in almost quantitative yield and recrystallized from H_2O.

2,6-Diacetamido-8,2′-anhydro-8-hydroxy-9-(3,5-*O*-diacetyl-β-D-arabinofuranosyl)purine (VI)——Compound V (120 mg, 0.43 mmol) was dissolved in pyridine (6.3 ml), and Ac_2O (1.5 ml) was added. The mixture was stirred at room temperature for 20 h. The solvent was evaporated off *in vacuo*, and traces of Ac_2O were removed by coevaporation with EtOH. The residue was suspended in $CHCl_3$ and H_2O. The organic layer was washed twice with saturated $NaHCO_3$ and with H_2O. The solvent was removed by evaporation *in vacuo* to give crude (IV), 135 mg (70%). UV $\lambda_{max}^{50\%\,EtOH}$ nm: 231, 261, 292, $\lambda_{max}^{50\%\,EtOH(H^+)}$ 226, 271, 310, $\lambda_{max}^{50\%\,EtOH(OH^-)}$ 291. TLC ($CHCl_3$–EtOH, 9 : 1) *Rf* 0.61. PPC: *Rf* (A) 0.78, *Rf* (B) 0.83, *Rf* (C) 0.85.

2,6-Diacetamido-8-mercapto-9-(3,5-*O*-diacetyl-β-D-arabinofuranosyl)purine (VII)——Acetylated 8,2′-*O*-cyclonucleoside (VI) (1.76 g, 3.9 mmol) was dissolved in pyridine (50 ml) and cooled at −50 °C. The solution was saturated with dry H_2S at this temperature and the mixture was heated at 90 °C in a steel tube for 15 h. The tube was cooled again and H_2S was removed by flushing with N_2 gas. The solvent was removed *in vacuo* and the residue was coevaporated with toluene then subjected to PLC on silica gel, developed with $CHCl_3$–EtOH, (9 : 1). The *Rf* 0.68 band was extracted with MeOH. The solvent was removed *in vacuo* to give the 8-mercapto compound (VII) in a yield of 1.16 g (62%). An analytical sample was recrystallized from MeOH as colorless needles mp 191—195 °C. *Anal.* Calcd for $C_{18}H_{21}N_6O_8S \cdot 1/2MeOH$: C, 44.58; H, 4.85; N, 16.86. Found: C, 44.60; H, 4.57; N, 16.70. UV $\lambda_{max}^{50\%\,EtOH}$ nm: 219, 236, 350 (sh), 287, 334, $\lambda_{max}^{50\%\,EtOH(H^+)}$ 218, 231, 250, 285, 334, $\lambda_{max}^{50\%\,EtOH(OH^-)}$ 250 (sh), 324. PPC: *Rf* (A) 0.98, *Rf* (B) 0.80, *Rf* (C) 0.91.

2-Acetamido-6-amino-9-(3,5-*O*-diacetyl-β-D-arabinofuranosyl)purine (VIII)——The 8-mercapto derivative (VII) (353 mg, 0.73 mmol) was dissolved in EtOH (30 ml) and H_2O (6 ml). Raney Ni (1 ml wet) was added and the mixture was refluxed for 30 min. The Raney Ni was filtered off and the filtrate was evaorated *in vacuo*. Crude VIII was obtained in a yield of 225 mg (75%). UV $\lambda_{max}^{50\%\,EtOH}$ nm: 223, 268, $\lambda_{max}^{50\%\,EtOH(H^+)}$ 266, $\lambda_{max}^{50\%\,EtOH(OH^-)}$ 269. PPC: *Rf* (A) 0.61, *Rf* (B) 0.57.

2,6-Diamino-9-β-D-arabinofuranosylpurine (IX)——(A): Well-dried acetylated arabino nucleoside (VIII) was dissolved in absolute EtOH (30 ml) and saturated with ammonia gas in a steel tube at −10 °C. The solution was heated at 100 °C for 10 h and the solvent was evaporated off *in vacuo* . The residue was applied to Whatman 3 MM paper and developed with H_2O at pH 10. The band of *Rf* 0.30 was extracted with H_2O and the extract was evaporated *in vacuo*. The residue was recrystallized from H_2O to give IX as colorless plates. mp 257—258 °C (lit [8,9] mp 260—261 °C, mp >250 °C). 104 mg, (67%). *Anal.* Calcd for $C_{10}H_{14}N_6O_4$: C. 42.55; H, 5.00; N, 29.78. Found: C, 42.29; H, 4.96; N, 29.98. UV $\lambda_{max}^{H_2O}$ (pH 7.0) nm (ε): 255 (9700), 279 (10700), $\lambda_{max}^{H_2O\,(pH\,1)}$ 251.8 (11600), 290 (10300), $\lambda_{max}^{H_2O\,(pH\,13)}$ 255 (9700), 279 (10600). NMR δ: 3.67 (3H, m, H-4′ and H-5′), 4.06 (2H, m, H-2′ and H-3′), 5.03 (1H, m, 5′-OH), 5.42 (1H, d, 2′-OH), 5.56 (1H, d, 3′-OH), 5.72 (1H, s, 2-NH_2), 6.06 (1H, d, H-1′, $J_{1',2'}$=4.1 Hz), 6.59 (1H, s, 6-NH_2), 7.76 (1H, s, H-8). PPC: *Rf* (A) 0.30, *Rf* (B) 0.13, *Rf* (C) 0.36. MS *m/z*: 282 (M^+).

(B): The crude 6-chloro derivative (XII) (56 mg 0.12 mmol) was dissolved in absolute MeOH (30 ml) and the solution was saturated with ammonia gas for 30 min under cooling at −15 °C. The mixture was sealed in a steel tube and heated at 100 °C for 8 h. The residue from evaporation of the solution *in vacuo* was applied to Whatman 3 MM paper and developed with solvent A. The band at *Rf* 0.30 was extracted with H_2O, the extract was evaporated and the residue was recrystallized from H_2O. The arabinoside (IX) was obtained as colorless plates in a yield of 26 mg (78%). *Anal.* Calcd for $C_{10}H_{14}N_6O_4$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.26; H, 4.81; N, 29.23. The properties of this product were identical with those of the compound obtained in (A).

***N*^{2},2′,3′,5′-*O*-Tetraacetyl-9-β-D-arabinofuranosylguanine (XI)**——X [19] (126 mg, 0.31 mmol) was dissolved in pyridine (2 ml). Evaporation and addition of pyridine were repeated twice. Then pyridine (2 ml) and Ac_2O (0.5 ml) were added to the residue and the mixture was kept at room temperature for 40 h with stirring. After completion of the reaction had been confirmed by TLC ($CHCl_3$–EtOH, 9 : 1), the solvent was evaporated off *in vacuo* and traces of

AcOH were removed by coevaporation with added toluene. The residue was dissolved in $CHCl_3$ and the solution was washed twice with H_2O. Drying over Na_2SO_4 and evaporation of the solvent gave an amorphous powder (122 mg, 87%). An analytical sample was obtained by recrystallization twice from MeOH. mp 237—239 °C. *Anal.* Calcd for $C_{18}H_{21}N_5O_9$: C, 47.89; H, 4.69; N, 15.51. Found: C, 47.92; H, 4.66; N, 15.28. UV $\lambda_{max}^{50\%\ EtOH}$ nm: 256, 280 (sh), $\lambda_{max}^{50\%\ EtOH\ (H^+)}$ 255, 280 (sh), $\lambda_{max}^{50\%\ EtOH\ (OH^-)}$ 260. TLC ($CHCl_3$–EtOH, 9:1) *Rf* 0.54.

2-Acetamido-6-chloro-9-(2,3,5-*O*-triacetyl-β-D-arabinofuranosyl)purine (XII)——Phosphoryl chloride (0.5 ml) and *N,N*-diethylaniline (0.025 ml) were added to tetraacetylarabinoguanosine (XI) (74 mg, 0.16 mmol), and the mixture was refluxed for 5 min. Excess phosphoryl chloride was evaporated off *in vacuo*, then ice-water was added to the residue. The mixture was stirred for 10 min, then extracted with CH_2Cl_2 (20 ml × 5). The combined extracts were washed with cold H_2O until aqueous layer was neutral and dried over Na_2SO_4. Vacuum evaporation of the solvent gave a yellow caramel in almost quantitative yield. UV $\lambda_{max}^{50\%\ EtOH}$ nm: 227.5, 257.5, 286, $\lambda_{max}^{50\%\ EtOH\ (H^+)}$ 227.5, 257.5, 286, $\lambda_{max}^{50\%\ EtOH\ (OH^-)}$ 285. TLC ($CHCl_3$–EtOH, 9:1) *Rf* 0.72.

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