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Synthesis of N^6- or 8-Substituted 9-(β-D-Arabinofuranosyl)-adenines and Their Antiviral Activities against Herpes Simplex and Vaccinia Viruses

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9-(β-D-Arabinofuranosyl)adenine (Ara-A) was synthesized from adenosine 5′-monophosphate in 30% yield *via* 8,2′-O-cycloadenosine as an intermediate. Various 8-substituted-amino Ara-A derivatives were obtained by aminolysis of 8,2′-O-cycloadenosine and N^6-substituted Ara-A derivatives were also obtained by reaction of 6-chloro-9-(β-D-arabinofuranosyl)purine with amines. *In vitro* antiviral activities of the N^6- or 8-substituted Ara-A were determined by the degree of cytopathic effect inhibition.

Keywords——Ara-A; 8-substituted Ara-A; N^6-substituted Ara-A; herpes simplex virus; vaccinia virus

Synthesis of 9-(β-D-arabinofuranosyl)adenine was reported by Lee, *et al.*[2] in 1960 and the biological activities of it have become of interest to a number of chemists and biochemists. As a result, various kinds of 9-(β-D-arabinofuranosyl)adenine (Ara-A) derivatives substituted in the carbohydrate moiety[3] and the heterocyclic base moiety[4] have been synthesized and their biological activities was investigated. However, the derivatives substituted in the N^6 or 8 position of Ara-A have been rarely reported hitherto. The present investigation was undertaken to afford a new synthetic route for Ara-A starting from adenosine 5′-monophosphate and to examine the antiviral activities of N^6- or 8-substituted Ara-A.

It has been previously reported by Ikehara[5] that 8,2′-O-cycloadenosine is a novel and important intermediate for the synthesis of Ara-A. In order to improve the yield of Ara-A, we intended to synthesize 8,2′-O-cycloadenosine from adenosine 5′-monophosphate (I) as shown in Chart 1. The selective *p*-toluenesulfonylation of I was performed by a slightly modified procedure reported by Ikehara[6] and 2′-O-*p*-toluenesulfonyladenosine 5′-monophos-

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phate (II) was obtained in high yield. Although, the product of this reaction contained a small amount of 2′,3′-di-O-*p*-toluenesulfonyladenosine-5′-monophosphate, dephosphorylation reaction was carried out without further purification.

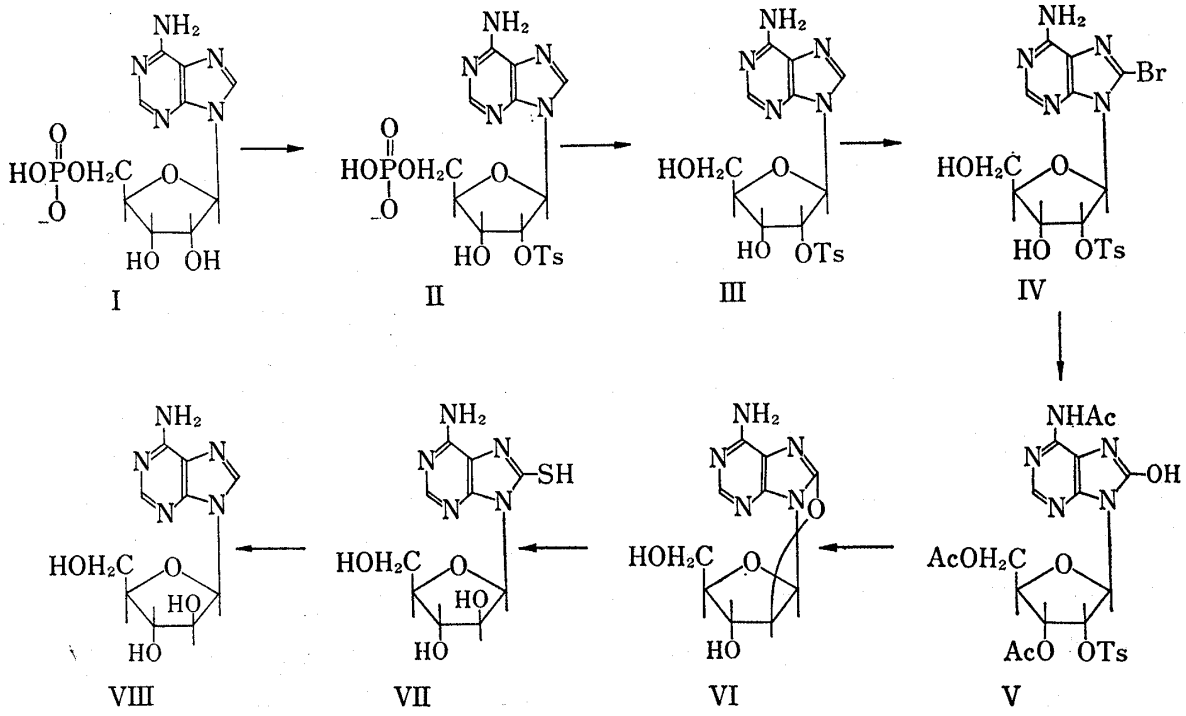

Chart 1

Dephosphorylation by refluxing II with formamide containing 50% phosphate buffer at pH 4.0 for 8 hr gave 2′-O-*p*-toluenesulfonyladenosine (III) with a melting point of 226—228° in 62.8% yield. The structure of this compound was determined by its elemental analysis and by the fact that Ikehara and Uesugi did not find[6] 8,3′-S-cycloadenosine 5′-monophosphate in the products of the cyclization reaction of *p*-toluenesulfonylated 8-bromoadenosine 5′-monophosphate by hydrogen sulfide in pyridine. Bromination of III with bromine at pH 4.0 gave 8-bromo-2′-O-*p*-toluenesulfonyladenosine (IV) in quantitative yield whose properties were in fair agreement with those reported.[7] 8,2′-O-cycloadenosine was obtained in 36% yield from I by a slight modification of the method reported in our previous paper.[8]

9-(β-D-Arabinofuranosyl)adenine was obtained in 83% yield from VI by reaction with hydrogen sulfide in methanol and subsequent desulfurization with Raney Ni. The method described above made it possible to provide 9-(β-D-arabinofuranosyl)adenine in 30% yield from commercially available adenosine 5′-monophosphate.

When 8,2′-O-cycloadenosine was heated with various amines, almost nothing but single products was found on thin-layer chromatography. The reaction conditions, yields, mp, ultraviolet (UV) spectra and peaks of C-2-H on nuclear magnetic resonance (NMR) spectrum are listed in Table I.

The structures of the products were assigned as 8-amino-Ara-A derivatives by their elemental analysis and from the fact that their pattern of NMR spectra was almost the same as that of Ara-A on the carbohydrate moiety. The proposed structures of the compounds were also supported by the reasonable resemblance of their UV spectra with 8-substituted amino-adenosine.[9]

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Table I. Reaction Conditions and Physical Properties of 8-Substituted Amino Ara-A Derivatives

Amine	Solvent	React. cond. (hr)	(°C)	Compound No.	Yield (%)	mp[a] (°C)	UV λ_{max} nm (ε) H^{+b}	H_2O	OH^{-c}	NMR (δ)[d] C–2–H
NH_3	MeOH	10	150	IXa	22	140—146 (m and s) 240 (dec.)	269 (11900)	272 (15000)	275 (15000)	7.93
CH_3NH_2	MeOH	15	100	IXb	67	234—236 (dec.)	276 (15000)	279 (18500)	280 (19000)	7.93
$(CH_3)_2NH$	MeOH	14	110	IXc	44	200—215 (dec.)	288 (12700)	274 (17200)	274 (16800)	8.01
NH_2OH	MeOH	12	100	IXd	67	140—142 (m)	270 (13300)	274 (16500)	276 (16700)	7.91
NH_2NH_2	MeOH	3	Reflux	IXe	49	200—205 (dec.)	266 (14200)	273 (15100)	259 (12500)	8.19
Benzylamine	MeOH	14	Reflux	IXf	42	205—206 (m)	279 (16700)	278 (18800)	282 (18400)	7.95
2,3-Dimethylbenzylamine	PrOH	13	Reflux	IXg	39	130—145	277 (16000)	279 (18100)	281 (18100)	7.94
2,4-Dimethylbenzylamine	PrOH	14	Reflux	IXh	36	126—130	277 (15800)	278 (19300)	281 (19100)	7.97
2,5-Dimethylbenzylamine	PrOH	16	Reflux	IXi	46	217—219	277 (15700)	278 (18800)	280 (18700)	7.96
2,6-Dimethylbenzylamine	PrOH	17	Reflux	IXj	54	172—176	276 (17100)	278 (20400)	281 (20100)	7.98
α-Naphthylmethylamine	PrOH	14	Reflux	IXk	70.6	139—145	281 (19700)	272 (21600)	281 (21200)	8.00
β-Naphthylmethylamine	PrOH	13	Reflux	IXl	75	255—256 (dec.)	276 (24000)	277 (26800)	277 (26200)	7.97

a) m: melt, s: solidify, dec: decomposition.
b) 0.1N HCl.
c) 0.1N NaOH.
d) Taken in DMSO-d_6.

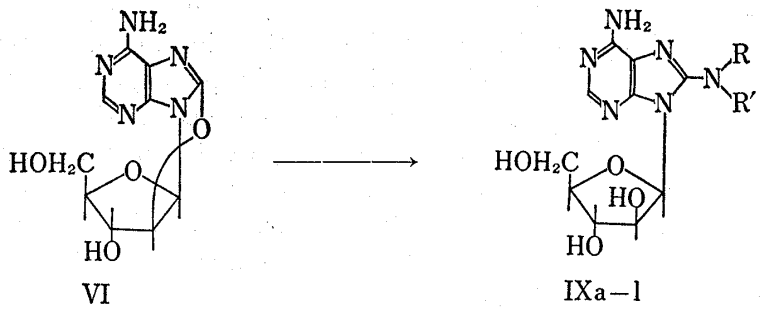

Chart 2

The report[5] that 8-amino-Ara-A and 8,5′-anhydro-8-oxy-9-(β-D-arabinofuranosyl)adenine were obtained by the reaction of 8,2′-O-cycloadenosine with liquid ammonia also supports the proposed structures. In the experimental conditions we employed, a small amount of 8,5′-anhydro-8-oxy-9-(β-D-arabinofuranosyl)adenine was found, but the formation of 8-hydroxyadenine derivatives was almost negligible. These results suggest that the nucleophilic attacks by amines occur on the 8 position of VI selectively. This selectivity is in contrast to the fact that alkyl bond fission occurrs in the case of 8,5′-anhydro-8-oxyadenosine by reaction with various nucleophiles.[10]

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In order to synthesize N^6-substituted Ara-A derivatives, we attempted to obtain the 6-chloro derivative of 8,2′-O-cyclonucleoside from 8,2′-O-cycloinosine by reacting with phosphorous oxychloride or thionyl chloride-N,N-dimethyl formamide (DMF). However cleavage reaction of cyclo-bond always occurred in preference to chlorination of the 6 position. Next, we tried to synthesize N^6-substituted Ara-A derivatives *via* 6-chloro-9-(β-D-arabinofuranosyl)-purine as shown in Chart 3.

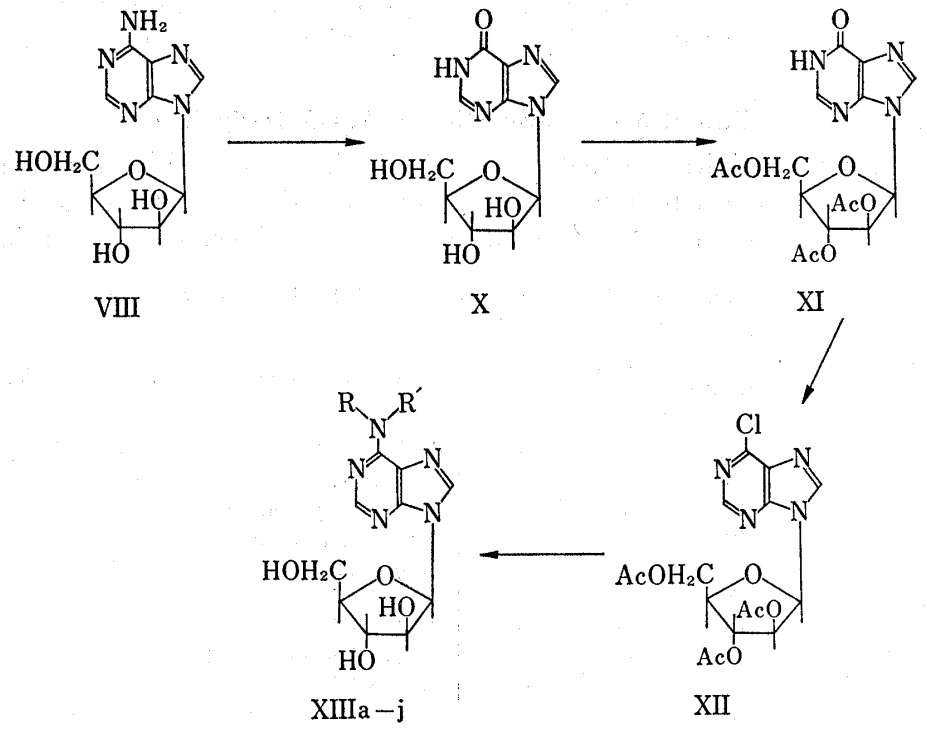

Chart 3

TABLE II. Properties of N^6-Substituted Ara-A

Compound No.	N^6-Substituent	mp (°C)	UV λ_{max} (nm) (ε)			NMR (δ) (cps)			
			$H^{+a)}$	H_2O	$OH^{-b)}$	H-2 or H-8		H-1′ ($J_{1'-2'}$)	Methyl
XIIIa	*o*-Methylbenzyl	231—232	265 (22100)	267 (21800)	267 (22400)	8.25	8.23	6.35 (4.0)	2.36
XIIIb	*m*-Methylbenzyl	221—222	265 (20100)	266 (20100)	266 (20700)	8.23	8.23	6.35 (4.5)	2.37
XIIIc	*p*-Methylbenzyl	172—173	265 (21500)	268 (21600)	268 (22100)	8.21	8.21	6.34 (4.5)	2.35
XIIId	2,3-Dimethylbenzyl	223—226	265 (20500)	267 (20400)	267 (20600)	8.23	8.23	6.34 (4.5)	2.25, 2.25
XIIIe	2,4-Dimethylbenzyl	186—189	266 (23800)	269 (23000)	270 (22500)	8.25	8.25	6.36 (4.5)	2.33, 2.24
XIIIf	2,5-Dimethylbenzyl	231—232	266 (22900)	269 (22800)	269 (23000)	8.24	8.24	6.34 (4.5)	2.33, 2.22
XIIIg	2,6-Dimethylbenzyl	131—133	265 (24300)	269 (23400)	269 (24300)	8.20	8.28	6.34 (4.5)	2.38, 2.38
XIIIh	3,4-Dimethylbenzyl	179—180	267 (23600)	269 (23000)	270 (23500)	8.23	8.23	6.35 (4.5)	2.18, 2.18
XIIIi	α-Naphthylmethyl	242—243	271 (21700)	271 (22100)	271 (22800)	8.25	8.25	6.38 (4.5)	
XIIIj	β-Naphthylmethyl	186—188	267 (27000)	269 (27400)	269 (27800)	8.28	8.26	6.37 (4.0)	

a) 0.1N HCl. *b*) 0.1N NaOH.

Ara-A was deaminated by barium nitrate in 2N acetic acid at room temperature to give 9-(β-D-arabinofuranosyl)hypoxanthine (X) in 73% yield. 2′,3′,5′-Tri-O-acetyl-6-chloro-9-(β-D-arabinofuranosyl)purine (XII)[2] was obtained as a hard foam by acetylation of X and subsequent chlorination with phosphorous oxychloride and diethylaniline hydrochloride in ethyl acetate[11] or DMF-thionyl chloride.[12] Various amines were treated with XII and subsequently deacetylated with methanolic ammonia giving N^6-substituted Ara-A derivatives. The structures of these compounds were decided as XIIIa—j by their elemental analysis, UV spectra, and NMR spectra, respectively. The physical properties of these compounds are shown in Table II.

In Vitro Antiviral Activities of N^6- or 8-Substituted Ara-A Derivatives

In vitro antiviral activities of the compounds obtained above against vaccinia virus and herpes simplex virus were determined by the inhibition of the viral cytopathic effect (CPE) using chick embryo cells. The results of the antiviral experiments are shown in Table III.

Table III. Antiviral Activities of Ara-A Derivatives (*In Vitro*)

8-Substituent	Conc. (μg/ml)	V.V.[a]	H.S.V.[b]	6-Substituent	Conc. (μg/ml)	V.V.[a]	H.S.V.[b]
Br	33	−	−	NH_2(Ara-A)	33	+	+
SH	33	−	−	OH(Ara-I)	100	+	−
NH_2	100	+	−	N^6-*o*-Methylbenzyl	33	−	−
NHOH	100	−	±	N^6-*m*-Methylbenzyl	33	−	−
$NHNH_2$	33	±	−	N^6-*p*-Methylbenzyl	10	−	−
$N(CH_3)_2$	33	−	−	N^6-2,3-Di-Me-benzyl	10	−	−
Benzylamino	100	−	−	N^6-2,4-Di-Me-benzyl	33	±	−
2,3-Di-Me-benzylamino	33	−	−	N^6-2,5-Di-Me-benzyl	33	±	−
2,4-Di-Me-benzylamino	33	−	−	N^6-2,6-Di-Me-benzyl	33	−	−
2,5-Di-Me-benzylamino	33	−	−	N^6-3,4-Di-Me-benzyl	33	−	−
2,6-Di-Me-benzylamino	33	−	−	N^6-α-Naphthylmethyl	33	−	±
α-Naphthylmethylamino	100	±	−	N^6-β-Naphthylmethyl	10	±	+
β-Naphthylmethylamino	10	−	−				

a) Vaccinia virus, strain IHD (cytopathic effect).
b) Herpes simplex virus type II, strain UW (cytopathic effect)
+: complete inhibition of CPE, ±: week but apparent inhibition of CPE, −: no effect.

The data in Table III would seem to indicate that substitution in the 8- position of Ara-A causes to lose its original activities against vaccinia and herpes simplex viruses. It has been reported that Ara-A and its hydrochloride have *anti* conformation in their crystalline state,[13] on the other hand, 8-bromo-Ara-A has *syn* conformation[14] as indicated by the data from X-ray analysis. These facts suggest that the *anti* conformation of the Ara-A plays some role in its antiviral activity. The substitution at the N^6 position of Ara-A also causes to lose its original activities except for the case of N^6-(β-naphthylmethyl)-Ara-A. It is very incomprehensible that N^6-methylbenzyladenosines have potent activities against vaccinia virus,[15] while the arabino-counterparts do not have such activities. This may be due to differences in the mechanism of antiviral activity against vaccinia virus for N^6-methylbenzyladenosine and Ara-A.

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12) M. Ikehara, H. Uno, and F. Ishikawa, *Chem. Pharm. Bull.* (Tokyo), **12**, 276 (1964).
13) T. Hata, S. Sato, M. Kaneko, B. Shimizu, and C. Tamura, *Bull. Chem. Soc. Japan*, **47**, 2758 (1974); A.K. Chwang and M. Sundaralingam, *Acta Cryst.*, **B30**, 2273 (1974); G. Bunick and D. Voet, *Acta Cryst.*, **B30**, 1651 (1974).
14) T. Hata, M. Kaneko, K. Aiba, S. Sato, B. Shimizu, and C. Tamura, Third symposium on Nucleic Acids Chemistry in Japan (1975).
15) M. Kaneko, M. Kimura, T. Nishimura, and B. Shimizu, unpubulished data.

Experimental

Melting points were determined using a Yanagimoto melting points apparatus and were uncorrected. NMR spectra were obtained in DMSO-d_6 on a Varian T-60 spectrometer using TMS as an external standard. UV spectra were obtained using a Hitachi 124 spectrophotometer.

2′-O-*p*-Toluenesulfonyladenosine 5′-monophosphate (II)——To a solution of 150 ml of dioxane and 350 ml of 1 N NaOH solution containing 34.7 g (100 mmol) of adenosine 5′-monophosphate (5′-AMP) was added 22.8 g (120 mmol) of finely crushed *p*-toluenesulfonyl chloride, and the solution was stirred for 15 hr at 0°. The solvent was removed by evaporation *in vacuo* at 50—60° after addition of 100 ml of 1 N HCl aqueous solution. The resulting crystalline mass was suspended to 55 ml of H_2O and the pH of the solution was adjusted to 4.0 with 1 N HCl aqueous solution. H_2O was added until the total volume of the solution became 250 ml. The solution contained a suspension of fine needles, which, though they were yellow in color, could be used in the next dephosphorylation reaction.

2′-O-*p*-Toluenesulfonyladenosine (III)——To the aqueous solution obtained above was added 250 ml of formamide and the solution was heated at refluxing temperature for 8 hr. The reaction solution was allowed to stand for 10 hr at room temperature and the resulting crystals were collected by filtration and washed with 200 ml of H_2O and 50 ml of acetone to give 26.4 g of crude (III). Analytical samples were obtained by recrystallization from 50% aqueous ethanol. mp 226—228°; UV $\lambda_{max}^{0.1N\ HCl\text{-}EtOH}$ nm (ε): 259 (12600); $\lambda_{max}^{H_2O\text{-}EtOH}$ nm (ε): 261.5 (12300); $\lambda_{max}^{0.1N\ NaOH\text{-}EtOH}$ nm (ε): 261.5 (12600). NMR: (DMSO-d_6) δ: 8.07 (1H, s, C_2–H), 8.22 (1H, s, C_8–H), 6.17 (1H, d, $J_{1'-2'}$ 7.0 cps, $C_{1'}$–H). *Anal.* Calcd. for $C_{17}H_{19}N_5O_6S$: C, 48.45; H, 4.55; N, 16.62; S, 7.61. Found: C, 48.64; H, 4.52; N, 16.24; S, 7.72.

8-Bromo-2′-O-*p*-toluenesulfonyladenosine (IV)——To a solution of 2 M acetate buffer of pH 4.0 (190 ml) containing 12.6 ml of bromine, was added 26.4 g of crude compound (III) under stirring at room temperature. After 18 hr stirring, the resulting red suspension was cooled to 0—5° and 19.5 g of $NaHSO_3$ was added to it. The suspension was stirred for 10 min and 126 ml of 5 N NaOH was added dropwise within half an hour, followed by 600 ml of 1 N NaOH. After one hr of stirring, a white crystalline product was collected by filtration, giving 28.36 g of compound (IV). Analytical samples were obtained by recrystallization from 50% aqueous EtOH. mp 210—224° (dec.); UV $\lambda_{max}^{0.1N\ HCl\text{-}EtOH}$ nm (ε): 265 (13400); $\lambda_{max}^{H_2O\text{-}EtOH}$ nm (ε): 265.5 (13300); $\lambda_{max}^{0.1N\ NaOH\text{-}EtOH}$ nm (ε): 265.5 (13300). NMR (DMSO-d_6) δ: 8.07 (1H, s, C_2–H), 6.08 (1H, d, $J_{1'-2'}$ 7.5 cps, $C_{1'}$–H). *Anal.* Calcd. for $C_{17}H_{18}BrN_5O_6S$: C, 40.81; H, 3.63; Br, 15.98; N, 14.00; S, 6.41. Found: C, 40.91; H, 3.65; Br, 15.67; N, 13.50; S, 6.57.

8-Hydroxy-N^6,3′,5′-O-tri-acetyl-2′-O-*p*-toluenesulfonyladenosine (V)——28.35 g (57.0 mmol) of dried 8-bromo-2′-*p*-toluenesulfonyladenosine (IV) was added to AcOH (57 ml), Ac_2O (57 ml) and 17.0 g of anhydrous AcONa. The reaction mixture was refluxed for 3 hr under stirring and exclusion of moisture. After the completion of the reaction, the solvent was removed under reduced pressure and 30 ml of EtOH was added to the residue, then removed by evaporation *in vacuo*. After two or three repetitions of this operation, the residual semi-crystalline product was extracted with two 100 ml portions of $CHCl_3$. The extracts were washed with H_2O and dried over 30 g of $CaCl_2$. Evaporating the solvent under reduced pressure, 35 g of the crude desired product (V) was obtained as a light brown caramel. Although this caramel could be used for the subsequent cyclization reaction, an analytical sample was obtained by crystallization from MeOH. mp 197—199°; UV $\lambda_{max}^{0.1N\ HCl\text{-}EtOH}$ nm (ε): 289.5 (13200); $\lambda_{max}^{H_2O\text{-}EtOH}$ nm (ε): 289 (13300); $\lambda_{max}^{0.1N\ NaOH\text{-}EtCH}$ nm (ε): 270 (9000), 306 (13900). *Anal.* Calcd. for $C_{23}H_{25}N_5O_{10}S$: C, 49.01; H, 4.47; N, 12.43; S, 5.66. Found: C, 49.11; H, 4.48; N, 12.41; S, 5.78.

8,2′-O-Cycloadenosine (VI)——The crude caramel (V) obtained above was dissolved into 140 ml of MeOH and poured into a steel tube. Dry NH_3 gas was passed for about 40 min with ice-salt cooling. The tube was then sealed and heated for about 18 hr at 65—70° with stirring. The tube was allowed to stand at room temperature then stocked overnight in a refrigerator. Precipitated crude 8,2′-O-cycloadenosine was filtered and washed with a small amount of cold MeOH to give 10.53 g of product. This crude 8,2′-O-cycloadenosine was suspended to 150 ml of H_2O and gradually heated to complete dissolution. During the course of the dissolution, the solution was carefully neutralized with 1 N HCl and then allowed to stand in a refrigerator. Thus, 9.63 g of 8,2′-O-cycloadenosine was obtained as white crystals. This sample colored at 190° and decomposed gradually at temperatures above 190°. UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 262 (14600), 280 (sh) (8500); $\lambda_{max}^{H_2O}$ nm (ε): 257 (15000); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 257 (14800). *Anal.* Calcd. for $C_{10}H_{11}N_5O_4 \cdot 1/3H_2O$: C, 44.29; H, 4.46; N, 25.83. Found: C, 44.65; H, 4.75; N, 25.66.

8-Mercapto-9-β-D-arabinofuranosyladenine (VII)——9.63 g (36 mmol) of 8,2′-O-cycloadenosine (VI) was suspended in 430 ml of MeOH in a steel tube and N_2 gas was passed through the suspension for two min. The solution was then saturated with dry H_2S gas under ice-salt cooling. The tube was sealed and heated to about 110° with stirring. After 16 hr heating, the reaction mixture was cooled and N_2 gas was passed through it for 30 min. The solution was concentrated to dryness under reduced pressure to give a crystalline residue in quantitative yield. The residue was dissolved in H_2O and insoluble material was filtered off. This solution could be used without further purification in the subsequent desulfurization reaction. An analytical sample was obtained by crystallization of the residual product from MeOH. mp 154° (dec.); UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 223, 242, 298 (sh), 308 (21900); $\lambda_{max}^{H_2O}$ nm (ε): 238, 298, 305.5 (24800); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 228, 296.5

(21100), 303. NMR (DMSO-d_6) δ: 8.07 (1H, s, C_2–H), 6.70 (1H, d, $C_{1'}$–H, $J_{1'-2'}$ 6.0 cps). *Anal.* Calcd. for $C_{10}H_{13}N_5O_4S \cdot CH_3OH$: C, 39.62; H, 5.51; N, 21.03; S, 9.63. Found: C, 39.85; H, 4.98; N, 20.78; S, 9.71.

9-β-D-Arabinofuranosyladenine (VIII)——To the aqueous solution of 8-mercapto-9-β-D-arabinofuranosyladenine (VII) was added 13 ml of Raney Nickel (Kawaken Fine Chemical NDT-65) and this mixture was refluxed for 3 hr. The insoluble material was filtered off and the filtrate was allowed to stand overnight in a refrigerator. 9-β-D-Arabinofuranosyladenine (VIII, 8.01 g) was obtained as white fine needles. mp 260° (dec.); UV $\lambda_{max}^{0.1N\ HCl}$ nm (ε): 258 (15100); $\lambda_{max}^{H_2O}$ nm (ε): 260 (15400); $\lambda_{max}^{0.1N\ NaOH}$ nm (ε): 260 (15000). NMR (DMSO-d_6) δ: 8.12 (1H, s, C_8–H), 8.18 (1H, s, C_2–H), 7.18 (2H, s, C–NH_2), 6.27 (1H, d, $C_{1'}$–H, $J_{1'-2'}$ 4.8 cps). *Anal.* Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.93; H, 4.91; N, 26.20. Found: C, 44.76; H, 4.92; N, 26.01.

General Procedure for Preparing 8-Substituted Amino Ara-A Derivatives (IXa—e)——8-α-Naphthylmethylamino-9-(β-D-arabinofuranosyl)adenine (IXk): To a solution of 1.06 g of VI (4 mmol) and 2.32 g (12 mmol) of α-naphthylmethylamine hydrochloride in *n*-PrOH was added 1.73 ml of triethylamine and the solution was heated at refluxing temperature for 14 hr. The solvent was removed by evaporation under reduced pressure. EtOH (30 ml) was added to the residue and the resultant crystalline $Et_3N \cdot HCl$ was removed by filtration. The filtrate was condensed to dryness *in vacuo*. The residue was dissolved in MeOH and H_2O was added to the solution. After the solution was allowed to stand in a refrigerator for several hr, the resulting crude crystalline product was collected by filtration and recrystallized from MeOH containing 20% water to give 1.19 g of the desired IXk.

Other 8-substituted amino Ara-A derivatives were obtained by procedures similar to that of IXk. Their physical properties are given in Table I and the data from their elemental analysis are given in Table IV.

Table IV. Analytical Data of 8-Substituted Amino Ara-A Derivatives

Compound No.	Formula	Calcd.			Found		
		C	H	N	C	H	N
IXa	$C_{10}H_{14}N_6O_4 \cdot 2H_2O$	37.73	5.70	26.40	37.81	5.56	26.12
IXb	$C_{11}H_{16}N_6O_4$	44.59	5.44	28.37	44.40	5.49	28.37
IXc	$C_{12}H_{18}N_6O_4$	46.44	5.85	27.07	46.23	5.84	26.78
IXd	$C_{10}H_{14}N_6O_5 \cdot H_2O$	37.98	5.10	26.57	38.23	5.35	26.45
IXe	$C_{10}H_{15}N_7O_4 \cdot H_2O$	38.09	5.43	31.09	38.25	5.33	31.33
IXf	$C_{17}H_{20}N_6O_4$	54.83	5.41	22.57	54.56	5.40	22.52
IXg	$C_{19}H_{24}N_6O_4 \cdot 1/2\ CH_3OH$	56.24	6.29	20.18	56.57	6.40	20.26
IXh	$C_{19}H_{24}N_6O_4 \cdot 3/2\ H_2O$	53.39	6.37	19.67	53.52	6.46	19.92
IXi	$C_{19}H_{24}N_6O_4 \cdot 3/2\ H_2O$	53.39	6.37	19.67	53.46	5.92	19.56
IXj	$C_{19}H_{24}N_6O_4 \cdot H_2O$	54.53	6.26	20.08	54.48	6.18	20.01
IXk	$C_{21}H_{22}N_6O_4 \cdot 2H_2O$	55.01	5.71	18.33	54.93	5.44	18.00
IXl	$C_{21}H_{22}N_6O_4$	59.71	5.25	19.90	60.05	5.32	19.91

9-(β-D-Arabinofuranosyl)hypoxanthine (X)——Ara-A (10.7 g) was dissolved in 800 ml of 2 N AcOH on heating and to the solution was added dropwisely 22.7 g of $Ba(NO_2)_2$ in 200 ml of H_2O with water cooling. At this point, crystalline material appeared. The suspension was stirred for 5 hr at room temperature after which time the crystalline material disappeared and the solution became clear. After the solution was stirred magnetically for about 16 hr at room temperature, the solvent was removed by evaporation *in vacuo*. Addition and evaporation of EtOH were repeated several times and the resultant residue was crystallized from 450 ml of H_2O. After the solution was allowed to stand in a refrigerator for 16 hr, the resulting crystals were collected by filtration to give 7.8 g of the desired (X). The physical properties of this compound (X) were exactly as reported.[4a] mp 246°. *Anal.* Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.77; H, 4.51; N,20.88. Found: C, 44.49; H, 4.55; N, 20.55.

2′,3′,5′-Tri-O-acetyl-9-(β-D-arabinofuranosyl)hypoxanthine (XI)——To a suspension of 7.8 g of the compound (X) in 292 ml of pyridine was added 29.2 ml of Ac_2O and the suspension was heated at 60—70° on an oil bath for 50 min. After 50 min, all insoluble material had disappeared and the solution became clear. The solvent was then removed *in vacuo*, and then addition and evaporation of EtOH was repeated several times. The residual crystalline material was collected and washed with EtOH to give 11.28 g of XI. Analytical samples were obtained by recrystallization from MeOH as prisms, mp 226—227°. *Anal.* Calcd. for $C_{16}H_{18}N_4O_8$: C, 48.73; H, 4.60; N, 14.21. Found: C, 48.70; H, 4.63; N, 14.31. Other physical properties were completely in agreement with those reported.[4a]

6-Chloro-2′,3′,5′-tri-O-acetyl-9-(β-D-arabinofuranosyl)purine (XII)——a) Chlorination with Phosphorous Oxychloride: After drying at 140° and 1 mm Hg for 10 min, diethylaniline hydrochloride (4.73 g) was dissolved in 18 ml of $POCl_3$ and 18 ml of AcOEt on heating. To the solution was added 4.73 g of compound (XI) and the solution was refluxed for 15 min. The solvent was removed by evaporation *in vacuo* and the

residual gummy product was dissolved in 100 ml of dry $CHCl_3$. Dry gaseous NH_3 was passed through the $CHCl_3$ solution under anhydrous conditions and cooled with ice water. After passing NH_3 gas through the solution for 30 min, the resulting insoluble inorganic salt was removed by filtration under anhydrous conditions. The solvent was removed under reduced presure and the resultant gummy product was used in the next reaction.

b) Chlorination with Thionyl Chloride and DMF: To a solution of 2.64 ml of thionyl chloride in 72 ml of dry $CHCl_3$ (distilled in the presence of P_2O_5) was added 2.76 ml of freshly distilled DMF and the solution was stirred for 30 min at room temperature. To the solution was added 4.73 g of compound (XI) and the solution was refluxed for 1.5 hr. $CHCl_3$ was added to the solution and dry gaseous NH_3 was passed for 30 min with ice-salt cooling. The resulting insoluble inorganic salt was removed by filtration and the filtrate was condensed to dryness *in vacuo*. The resulting gummy product was used for the next reaction without further purification.

General Procedure for Preparing N^6-Substituted Ara-A Derivatives (XIIIa—j)——The crude triacetyl-6-chloro-arabinofuranosylpurine (XII) (from 6 mmol of XI) obtained above was dissolved in 50 ml of *n*-PrOH and to the solution were added 2.06 g of 2,3-dimethylbenzylamine hydrochloride and 1.73 ml of triethylamine. The solution was heated at refluxing temperature for 3 hr and the solvent was removed by evaporation under reduced pressure. The residual product was dissolved in $CHCl_3$ and washed with H_2O. After drying and evaporating the solvent, the resulting gummy product was dissolved in MeOH and the solution was saturated with dry NH_3 at 0—5°. The solution was allowed to stand for 16 hr at room temperature. The solvent was removed by evaporation under reduced pressure and the resulting crystalline residue was recrystallized from MeOH to give 1.66 g of the desired XIIIi. Analytical samples were obtained by recrystallization from MeOH.

Other N^6-substituted Ara-A derivatives were obtained by procedures similar to that of XIIIi and their physical properties are given in Table II and the data of their elemental analysis are given in Table V.

TABLE V. Analytical Data of N^6-Substituted Ara-A Derivatives

Compound No.	Formula	Calcd.			Found		
		C	H	N	C	H	N
XIIIa	$C_{18}H_{21}N_5O_4$	58.21	5.70	18.86	58.16	5.83	19.03
XIIIb	$C_{18}H_{21}N_5O_4 \cdot 1/2\ CH_3OH$	57.37	5.98	18.08	57.37	5.98	18.05
XIIIc	$C_{18}H_{21}N_5O_4 \cdot 1/2\ CH_3OH$	57.37	5.98	18.08	57.51	5.85	18.22
XIIId	$C_{19}H_{23}N_5O_4 \cdot 1/2\ H_2O$	57.86	6.13	17.76	58.02	5.72	17.56
XIIIe	$C_{19}H_{23}N_5O_4$	59.21	6.02	18.17	59.45	6.21	18.31
XIIIf	$C_{19}H_{23}N_5O_4$	59.21	6.02	18.17	59.18	6.06	18.25
XIIIg	$C_{19}H_{23}N_5O_4 \cdot 1/2\ H_2O$	57.86	6.13	17.76	57.97	5.87	17.80
XIIIh	$C_{19}H_{23}N_5O_4 \cdot 1/2\ H_2O$	57.86	6.13	17.76	57.48	5.90	17.65
XIIIi	$C_{21}H_{21}N_5O_4 \cdot 1/2\ CH_3OH$	60.91	5.47	16.54	60.76	5.17	16.46
XIIIj	$C_{21}H_{21}N_5O_4 \cdot 1/2\ CH_3OH$	60.91	5.47	16.54	60.99	5.48	16.54

***In Vitro* Antiviral Experiment**——*In vitro* antiviral studies were ran on disposable plastic microplates (Disposo-Trays Model 96 CV-TC Linbro Co.). Ten-day old chick embryo cells were seeded on the plastic panels. The cell monolayers were incubated for 3 days with eagles' minimum essential medium (supplemented with 5% calf serum and 0.293 mg per liter of glutamine) containing test compounds in concentrations, ranging from 100 to 1 μg/per ml. The cytotoxic effect of the compound was determined by microscopic observation of the cells stained by methylene blue. Twenty-four hr monolayers of chick embryo cells were inoculated with 30 plaque forming units of virus per well and the maximum tolerant concentration of the compound was added within 15 min after the virus. Antiviral activity was determined by the inhibition of the viral CPE after 3 days of incubation at 37°.