

Synthesis and Antiviral Activity of 6-Chloropurine Arabinoside and Its 2'-Deoxy-2'-fluoro Derivative

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6-Chloropurine arabinoside (**3a**) was obtained by treatment of the 2'-*O*-acetylated congener (**2**) with ammonia in methanol. The 3',5'-di-*O*-tritylated riboside (**6**) was allowed to react with diethylaminosulfur trifluoride (DAST) in the presence of pyridine to give the 2'-deoxy-2'-fluoroarabinoside (**7**), from which 6-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)purine (**3b**) was obtained. The antiviral effects of **3a** and **3b** were assayed against several DNA and RNA viruses. Only **3a** displayed potent activity against varicella-zoster virus (VZV). This antiviral activity was dependent on phosphorylation by the VZV-induced thymidine kinase (TK). Compound **3a** showed moderate activity against other DNA viruses, herpes simplex type 1 (HSV-1) and type 2 (HSV-2), and vaccinia virus. They were equally active against TK⁻ and TK⁺ strains of HSV-1, which suggests that the HSV-1-encoded TK does not play a role in the anti-HSV-1 activity. No activity was noted with any of the compounds against various RNA viruses, including human immunodeficiency virus, at subtoxic concentrations.

Key words 6-chloropurine; arabinoside; diethylaminosulfur trifluoride; antiviral activity; thymidine kinase

Several antiviral agents have been developed for the treatment of DNA virus infections.¹⁾ One of these, 9-(β-D-arabinofuranosyl)adenine (ara-A), a naturally occurring antibiotic, was shown to have activity against DNA viruses.²⁾ In contrast with acyclovir, however, ara-A is phosphorylated in mammalian cells to ara-A 5'-monophosphate by adenosine kinase and deoxycytidine kinase. Therefore, the utility of ara-A for the treatment of diseases caused by DNA viruses has been limited by the toxicity and lack of selectivity.^{2c,3)} Recently, Averett *et al.* reported that 6-methoxypurine arabinoside (ara-M) shows marked activity against varicella-zoster virus (VZV), but no appreciable activity against other herpes viruses (herpes simplex virus (HSV) types 1 and 2).⁴⁾ This selective anti-VZV activity correlates with substrate utilization by the VZV-encoded thymidine kinase (TK).^{4a)} The antiviral activity of 6-chloropurine riboside against some DNA

viruses has also been reported by us.⁵⁾ Therefore, we planned to explore the antiviral activity of 6-chloropurine nucleosides. In this paper, methods for the synthesis of 6-chloropurine arabinoside (**3a**) and its 2'-fluoro derivative (**3b**) are described. The antiviral activity of these compounds against DNA and RNA viruses is also presented.

Synthesis of 6-Substituted Purine Arabinosides 6-Chloropurine arabinoside (**3a**) has been prepared from 9-(β-D-xylofuranosyl)adenine.⁶⁾ Recently, we reported the synthesis of 6-chloro-9-(2-*O*-acetyl-β-D-arabinofuranosyl)purine (**2**) from 6-chloropurine riboside (**1**) in 4 steps in 22% overall yield as follows.^{7a)} Thus, treatment of **1** with 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane afforded the corresponding 3',5'-*O*-(tetraisopropylidisiloxan-1,3-diyl) derivative, which was allowed to react with trifluoromethanesulfonyl chloride in the presence of 4-dimethylaminopyridine (DMAP) to give the 2'-*O*-

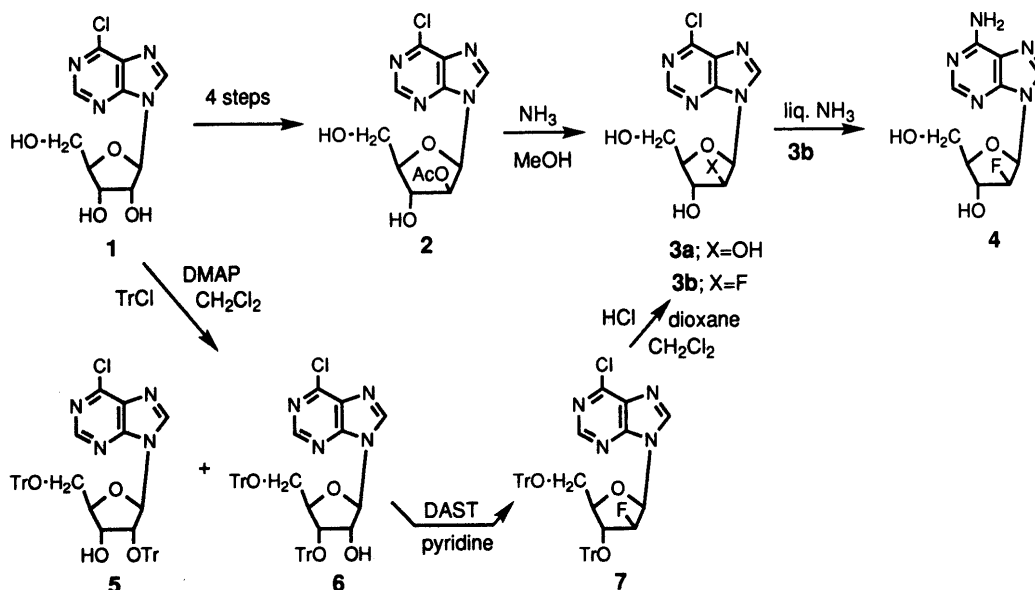
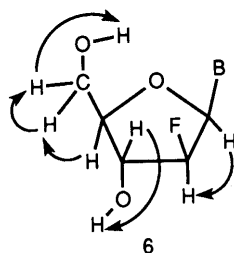


Chart 1

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Fig. 1. NOESY Spectrum of Compound **3b**

triflate. An S_N2 displacement with acetate anion of the 2'-*O*-triflate gave the 2'(*S*)(*ara*)-*O*-acetate (**2**). 6-Chloropurine arabinoside (**3a**) was readily obtained by treatment of **2** with ammonia in methanol in good yield. Another target is an arabinoside bearing fluorine at the 2'-carbon, because 5-alkyluracil 2'-deoxy-2'-fluoroarabinosides are potent anti-herpes agents.⁸⁾ Watanabe *et al.* reported a method to prepare the purine 2'-deoxy-2'-fluoroarabinosides from 2'-triflate using $(Me_2N)_3S(SiMe_3)F_2$.⁹⁾ Since direct fluorination of 6-chloropurine nucleosides with diethylaminosulfur trifluoride (DAST) has been developed,⁷⁾ we applied this method to the synthesis of 6-chloropurine fluoroarabinoside (**7**). Introduction of a triphenylmethyl (trityl) group in 6-chloropurine riboside (**1**) gave the 2',5'-di-*O*-tritylated product (**5**) and 3',5'-di-*O*-tritylated product (**6**) in 22% and 23% yields, respectively. Compound **6** was allowed to react with DAST in the presence of pyridine to give the 2'-deoxy-2'-fluoroarabinoside (**7**) in 87% yield. Attack of N3 of the aglycon on the activated 2'-carbon to form isonucleoside was reported in a similar reaction of N^6,O^3',O^5' -tritrityl-adenosine.¹⁰⁾ It is supposed that the electron-withdrawing 6-chloro group of **6** prohibited nucleophilic attack of N3 of the aglycon on the 2'-carbon of the alkoxy-(diethylamino)sulfur difluoride intermediate. Hydrolysis of **7** with HCl in a mixture of CH_2Cl_2 and dioxane gave 6-chloro-9-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-purine (**3b**). The 1H -NMR spectrum of **3b** indicated that the 2'-fluorine caused a downfield shift of the 2'-proton signal and a large $H2'-C-F$ geminal coupling (52.5 Hz). The nuclear Overhauser effect (NOE) was observed between $H1'$ and $H2'$ as well as $H4'$ and $H5'$ in the two-dimensional NOE (NOESY) spectrum of **3b** and the configuration was identified as the 2'(*S*) arabinoside structure (Fig. 1). Reaction of **3b** with liquid ammonia gave the adenine congener (**4**), for which the measured data were identical with the published values.¹⁰⁾ Therefore, the structure of **3b** was unequivocally determined (Chart 1).

Antiviral Activity The antiviral effects of 6-chloropurine arabinosides (**3a**, **3b**) were assayed according to previously established procedures¹¹⁾ and the results are presented in Tables 1, 2 and 3. Compound **3a** displayed marked activity against VZV (OKA strain, YS strain; IC_{50} : 0.1 $\mu g/ml$) (Table 1). This 50%-inhibitory concentration was at least 500-fold lower than the cytostatic concentration. To elucidate the role of the VZV-encoded TK in the anti-VZV activity of **3a**, the compound was also evaluated against TK-deficient (TK^-) strains of VZV. Compound **3a** proved 100-fold less active against TK^-

Table 1. Antiviral Activity of Compounds **3a** and **3b** against VZV in Human Embryonic Lung (HEL) Cells

Compound	Antiviral activity ^{a)} IC_{50} ($\mu g/ml$)				Cytotoxicity ^{b)} CC_{50} ($\mu g/ml$)
	TK ⁺ VZV OKA strain	TK ⁺ VZV YS strain	TK ⁻ VZV 07/1 strain	TK ⁻ VZV YS/R strain	
3a	0.1	0.1	8	10	> 50
3b	36	39	> 50	43	47
Acyclovir ^{c)}	0.3	0.9	8	22	> 200
Brivudin ^{d)}	0.001	0.003	—	—	> 200

a) 50%-inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. b) 50%-cytotoxic concentration, or concentration required to reduce cell growth by 50%. c) 9-(2-Hydroxyethoxymethyl)guanine. d) *E*-5-(2-Bromovinyl)-2'-deoxyuridine.

Table 2. Antiviral Activity of Compounds **3a** and **3b** against HSV and Vaccinia Virus in HEL Cells

Compound	Minimum inhibitory concentration ^{a)} ($\mu g/ml$)			
	TK ⁺ HSV Type 1 (KOS)	TK ⁺ HSV Type 2 (G)	Vaccinia virus	TK ⁻ HSV Type 1 (B2006)
3a	15	20	2	10
3b	85	100	70	40
Brivudin	0.02	100	2	20
Ribavirin ^{b)}	150	250	40	40
Ganciclovir ^{c)}	0.0007	0.007	100	10
Acyclovir	0.07	0.07	300	10

a) Required to reduce virus-induced cytopathogenicity by 50%. b) 1-(β -D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide. c) 9-(1,3-Dihydroxy-2-propoxy-methyl)guanine.

Table 3. Antiviral Activity of Compounds **3a** and **3b** against Cytomegalovirus in HEL Cells

Compound	Antiviral activity ^{a)} IC_{50} ($\mu g/ml$)		Cytotoxicity ^{b)} CC_{50} ($\mu g/ml$)
	AD-169 strain	Davis strain	
3a	50	35	> 50
3b	> 50	40	47
Acyclovir	0.4	0.4	> 200
Brivudin	0.06	0.16	> 50

a, b) See footnotes to Table 1.

VZV than against TK^+ VZV. This means that the activity of **3a** must at least partially depend on phosphorylation by the virus-induced TK. De Miranda *et al.* demonstrated that the 5'-phosphate of ara-M (ara-MMP) underwent demethoxylation to 9-(β -D-arabinofuranosyl)hypoxanthine 5'-monophosphate (ara-IMP) by the cellular AMP deaminase.^{4c)} Then, ara-IMP was converted to ara-ATP, which acts as a DNA polymerase inhibitor in the VZV-infected cells.^{4b)} Since 6-methoxypurine arabinoside differs from **3a** only by the substitution of a chlorine for a methoxy group at the 6-position of the purine, the 6-chloro congener **3a** may be convertible to ara-ATP by a similar pathway. Compound **3a** showed moderate activity against other DNA viruses, HSV-1 and HSV-2, and vaccinia virus (Table 2). It was equally active against TK^- and TK^+

strains of HSV-1, which suggests that the HSV-1-encoded TK does not play a role in the anti-HSV-1 activity of compound **3a**. Compounds **3a,b** did not show appreciable activity against cytomegalovirus (Table 3). The activity of the test compounds was also examined against RNA viruses, such as influenza A and B, Sindbis, Coxsackie B4, Semliki forest, polio-1, parainfluenza-3, respiratory syncytial, vesicular stomatitis, reovirus-1, Punta Toro, and human immunodeficiency virus types 1 and 2. No activity against these viruses was noted with any of the compounds at subtoxic concentrations.

Experimental

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) without correction. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low-resolution mass spectra (MS) were obtained on a JEOL JMS-AX500 mass spectrometer in the direct-inlet mode. ¹H-NMR spectra were recorded on a Varian Unity 200 (200 MHz) or Unity 600 (600 MHz) in CDCl₃ (or dimethyl sulfoxide (DMSO)-*d*₆) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with Silica gel 60 containing the fluorescent indicator F₂₅₄ were used for thin-layer chromatography and Silica gel 60 (Merck 7734, 60–200 mesh) was employed for column chromatography.

6-Chloro-9-(β-D-arabinofuranosyl)purine (3a) 6-Chloro-9-(2-*O*-acetyl-β-D-arabinofuranosyl)purine (**2**) was prepared from 6-chloropurine riboside (**1**) according to the method reported previously.⁷⁾ Then, **2** (1.45 g, 4.41 mmol) was treated with methanol (50 ml) saturated with ammonia and kept at 4°C for 2 h. Concentration of the solution gave a solid, which was recrystallized from water to give **3a** as white crystals (1.05 g, 83%). mp 167–168°C. MS *m/z*: 286, 288 (M⁺). UV λ_{max} (MeOH) nm: 263. Anal. Calcd for C₁₀H₁₁ClN₄O₄: C, 41.88; H, 3.84; N, 19.55. Found: C, 42.22; H, 3.90; N, 19.23.

Tritylation of 6-Chloropurine Riboside (1) Trityl chloride (16.7 g, 60 mmol) was added to a solution of 6-chloropurine riboside (**1**) (2.86 g, 10 mmol) and DMAP (260 mg, 2.13 mmol) in dry CH₂Cl₂ (200 ml) and triethylamine (8.4 ml) and the solution was heated under reflux for 16 h, then cooled. It was diluted with additional CH₂Cl₂ (500 ml) and then washed with water. The organic layer was dried over MgSO₄ and concentrated to a small volume. This residue was chromatographed over a column of Silica gel G (20 × 30 cm) with 0–50% AcOEt in hexane (2 l). Evaporation of the first fraction gave a solid, which was recrystallized from MeOH to give 6-chloro-9-(2,5-di-*O*-trityl-β-D-ribofuranosyl)purine (**5**) as white crystals (1.67 g, 22%). mp 232–233°C. MS *m/z*: 527, 529 (M⁺ – Tr). UV λ_{max} (MeOH) nm: 265. ¹H-NMR (CDCl₃) δ: 8.58 (1H, s, H-8), 8.28 (1H, s, H-2), 7.02–7.38 (ca. 30H, m, (C₆H₅)₃C × 2), 6.40 (1H, d, *J* = 7.7 Hz, H-1'), 5.19 (1H, dd, *J* = 7.7, 4.5 Hz, H-2'), 4.12 (1H, m, H-4'), 3.26 (1H, dd, *J* = 11.4, 3.2 Hz, H-5'a), 3.07 (1H, m, H-5'b), 3.04 (1H, m, H-3'), 2.30 (1H, d, *J* = 1.1 Hz, 3'-OH). Anal. Calcd for C₄₈H₃₉ClN₄O₄ · 0.4H₂O: C, 74.05; H, 5.15; N, 7.20. Found: C, 73.65; H, 5.10; N, 6.95. The second fraction was evaporated and the residue was crystallized from hexane to afford 6-chloro-9-(3,5-di-*O*-(trityl-β-D-ribofuranosyl)purine (**6**) as pale yellow crystals (1.79 g, 23%). mp 193–195°C. MS *m/z*: 527, 529 (M⁺ – Tr). UV λ_{max} (MeOH) nm: 264. ¹H-NMR (CDCl₃) δ: 8.65 (1H, s, H-8), 8.29 (1H, s, H-2), 7.04–7.45 (ca. 30H, m, (C₆H₅)₃C × 2), 6.14 (1H, d, *J* = 4.9 Hz, H-1'), 4.46 (1H, dd, *J* = 5.5, 2.8 Hz, H-3'), 4.30 (1H, q, *J* = 5.1 Hz, H-2'), 3.71 (1H, m, H-4'), 3.60 (1H, d, *J* = 5.3 Hz, 2'-OH), 3.32 (1H, dd, *J* = 10.9, 1.9 Hz, H-5'a), 2.82 (1H, dd, *J* = 10.9, 4.3 Hz, H-5'b). Anal. Calcd for C₄₈H₃₉ClN₄O₄ · 0.3H₂O: C, 74.23; H, 5.14; N, 7.21. Found: C, 74.50; H, 5.25; N, 6.64.

6-Chloro-9-(2-deoxy-2-fluoro-3,5-di-*O*-trityl-β-D-arabinofuranosyl)purine (7) DAST (1.79 ml, 4.2 eq) was added to an ice-cooled solution of **6** (2.5 g, 3.2 mmol) in dry CH₂Cl₂ (27 ml) and pyridine (3.32 ml), and the solution was refluxed for 6 h under an N₂ atmosphere, then cooled. The solution was poured into 5% NaHCO₃ (240 ml) and the organic layer was washed with water three times (100 ml), dried over MgSO₄ and evaporated to give a syrup, which was evaporated azeotropically with toluene twice (30 ml), then dissolved in a small amount of CH₂Cl₂. The solution was chromatographed over a column of Silica gel G (4.0 × 40 cm) with 0–2% EtOH in CH₂Cl₂ (3 l) to give a pale brownish caramel, which was crystallized from benzene to afford **7** as white crystals

(2.18 g, 87%). mp 247–249°C. MS *m/z*: 529, 531 (M⁺ – Tr). UV λ_{max} (MeOH) nm: 263. ¹H-NMR (CDCl₃) δ: 8.73 (1H, s, H-2), 8.17 (1H, d, *J*_{H_{NC}CF} = 3.5 Hz, H-8), 7.15–7.55 (ca. 30H, m, (C₆H₅)₃C × 2), 6.51 (1H, dd, *J* = 23.0, 1.5 Hz, H-1'), 4.59 (1H, m, H-4'), 4.28 (1H, dd, *J* = 13.5, 1.5 Hz, H-3'), 3.71 (1H, dd, *J* = 50.0, 1.5 Hz, H-2'), 3.15–3.40 (2H, m, H-5'a, H-5'b). Anal. Calcd for C₄₈H₃₈ClFN₄O₃: C, 74.55; H, 4.95; N, 7.24. Found: C, 74.45; H, 5.00; N, 7.14.

6-Chloro-9-(2-deoxy-2-fluoro-*O*-D-arabinofuranosyl)purine (3b) A solution of **7** (5.00 g, 6.47 mmol) in CH₂Cl₂ (70 ml), dioxane (50 ml) and concentrated HCl (0.7 ml) was stirred at room temperature overnight. The solution was neutralized with triethylamine, then CH₂Cl₂ (500 ml) and water (500 ml) were added. The organic layer was extracted with water (500 ml) and the combined aqueous layer was washed with benzene, then evaporated to dryness. The residue was dissolved in a small amount of MeOH and chromatographed over a column of Silica gel G (2.6 × 30 cm) with 0–15% EtOH in CHCl₃ (1.5 l) to give a caramel, which was crystallized from EtOH to afford **3b** as white crystals (1.11 g, 60%). mp 178–180°C. MS *m/z*: 288, 290 (M⁺), 289, 291 (M⁺ + 1). UV λ_{max} (0.05 N HCl) nm: 263. λ_{max} (MeOH) nm: 263.5. ¹H-NMR (DMSO-*d*₆) δ: 8.86 (1H, s, H-8), 8.84 (1H, s, H-2), 6.58 (1H, dd, *J* = 12.7, 4.6 Hz, H-1'), 6.02 (1H, d, *J* = 5.1 Hz, 3'-OH), 5.32 (1H, dt, *J* = 52.5, 4.5 Hz, H-2'), 5.15 (1H, t, *J* = 5.7 Hz, 5'-OH), 4.48 (1H, m, H-3'), 3.92 (1H, q, *J* = 5.4 Hz, H-4'), 3.72 (1H, m, H-5'a), 3.67 (1H, m, H-5'b). Anal. Calcd for C₁₀H₁₀ClFN₄O₃: C, 41.61; H, 3.49; N, 19.41. Found: C, 41.38; H, 3.54; N, 19.16.

9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (4) Compound **3b** (100 mg, 0.35 mmol) was reacted with liquid ammonia (2 ml) in a 10 ml steel bomb at 60°C overnight. After cooling to –60°C, the solution was evaporated carefully and the residue was crystallized from EtOH to afford **4** as white crystals (76 mg, 82%). mp 229–230°C (lit. 232–234°C¹⁰). MS *m/z*: 269 (M⁺). UV λ_{max} (MeOH) nm: 264. ¹H-NMR (DMSO-*d*₆) δ: 8.28 (1H, s, H-8), 8.20 (1H, s, H-2), 7.37 (2H, br s, –NH₂), 6.44 (1H, dd, *J* = 15.0, 4.0 Hz, H-1'), 5.99 (1H, d, *J* = 5.0 Hz, 3'-OH), 5.39 (1H, m, H-2'), 5.15 (1H, d, *J* = 5.7 Hz, 5'-OH), 4.50 (1H, m, H-3'), 3.89 (1H, m, H-4'), 3.63–3.77 (1H, m, H-5'a, H-5'b). Anal. Calcd for C₁₀H₁₂FN₅O₃ · 0.3H₂O: C, 43.73; H, 4.62; N, 25.50. Found: C, 43.89; H, 4.62; N, 25.08.

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