Synthesis and Antitumor and Antiviral Activities of 1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-iminopyrimidine and Its Derivatives[1]

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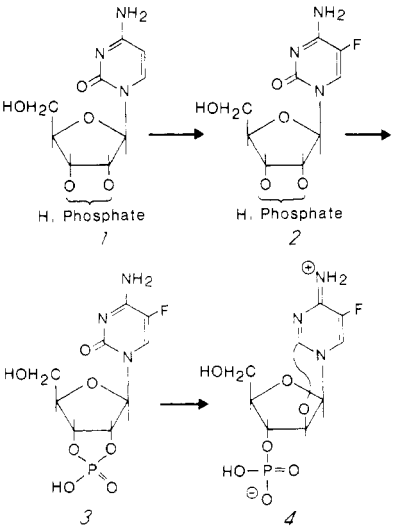
1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-imino-5-fluoropyrimidine (**10**), 1-β-D-arabinofuranosyl-2-amino-1,4(2*H*)-imino-5-fluoropyrimidine 3′-phosphate (**9**), and 1-β-D-arabinofuranosyl-2-amino-1,4(2*H*)-imino-5-chloropyrimidine (**11**) have been synthesized from 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine (**5**), 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine 3′-phosphate (**4**), and 2,2′-anhydro-1-β-D-arabinofuranosyl-5-chlorocytosine (**6**), respectively. 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine 3′-phosphate (**7**), 1-β-D-arabinofuranosyl-2-amino-1,4-(2*H*)-iminopyrimidine (**13**), 1-β-D-arabinofuranosyl-2-amino-1,3(2*H*)-iminopyrimidine 3′-phosphate (**12**), and compounds **4**, **5**, and **9** showed significant in vitro activity against a number of DNA viruses. Compounds **7** and **12** were also effective in vivo against type 1 herpes simplex virus. Compounds **7**, **12**, and **13** were extremely effective in the treatment of mice bearing leukemia L1210.

The usefulness of 1-β-D-arabinofuranosylcytosine (*ara*-C) as antitumor and antiviral agent has been well documented.[3-6] The biological activity of *ara*-C is due to its metabolism to 5′-triphosphate (*ara*-CTP), which is a powerful inhibitor of DNA polymerase.[7,8] *ara*-CTP is also a substrate for DNA polymerase, and some of the cytotoxic effects of *ara*-C can best be explained due to its incorporation into DNA.[7,9,10] However, the clinical application of this drug has been limited because of the difficulty in maintaining the effective serum levels due to its facile deamination,[11-13] resulting in production of biologically inactive 1-β-D-arabinofuranosyluracil (*ara*-U). Since these findings, various attempts have been made to increase the efficacy of the drug either by making it relatively resistant to cytosine nucleoside deaminase or converting it to compounds which slowly decompose to release *ara*-C in the biological systems.

Substitution of *ara*-C with acetyl, methyl, and hydroxyl groups at the 4-amino position provided derivatives with relative resistance toward the human liver or mouse kidney deaminases; however, these compounds showed reduced effectiveness.[14-16] The synthesis of a number of 2′,3′- and 5′-esters of *ara*-C and its 3′,5′-cyclic nucleotides have been reported.[17-22] These derivatives exhibit some improvement over the parent drug. The biological activity of these analogues is due to their transformation to *ara*-C. The most promising derivative, 2,2′-anhydro-1-β-D-arabinofuranosylcytosine (anhydro-*ara*-C) has been shown to be a deaminase-resistant depot form of *ara*-C.[23-25] In comparative studies with *ara*-C, anhydro-*ara*-C has been shown to be more effective against a variety of animal tumor systems,[24] and its mode of action is reported to be due to its slow chemical conversion to *ara*-C.[25] It has also been suggested that anhydro-*ara*-C itself is a substrate for cellular kinases.[26] Synthesis of a number of biologically active 5-halogenated *ara*-C and anhydro-*ara*-C derivatives has also been described.[28-30] These derivatives have shown better therapeutic effects than *ara*-C and anhydro-*ara*-C against the leukemia L1210 system. Anhydro-*ara*-C and 5-F-anhydro-*ara*-C are presently under clinical trial.[31,32] The use of anhydro-*ara*-C is limited due to its toxic side effects.[32-35]

Synthesis of 1-β-D-arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidine hydrochloride (AraAIPy) has been described by Doerr and Fox.[27] These authors showed that AraAIPy was chemically very unstable and readily converted to anhydro-*ara*-C. In this publication, we describe the synthesis and biological activity of AraAIPy derivatives. In view of the chemical instability of AraAIPy (Doerr and Fox[27]), 3′-phosphate derivatives of AraAIPy and 5-F-AraAIPy were prepared. It was assumed that these modifications could provide more stable compounds due to their existence as "zwitterions". Preliminary reports of this work have been presented.[44,45]

Scheme I



Chemistry. Electrophilic fluorination of cytidine 2′-(3′)-monophosphate (**1**) with fluoroxytrifluoromethane[36] (CF_3OF) produced 5-fluorocytidine 2′(3′)-monophosphate (**2**) in 45% yield. Treatment of **2** with ethyl chloroformate at ambient temperature according to the procedure described by Michelson[37] gave 5-fluorocytidine cyclic 2′,3′-phosphate (**3**). Product **3** when heated with chlorotrimethylsilane in the presence of an acid acceptor[38] (pyridine) yielded 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine 3′-phosphate (**4**) (Scheme I). Similarly, 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine[46] (**5**) was prepared by the fluorination of cytidine, followed by treatment with partially hydrolyzed phosphorus oxychloride as described by Kanai et al.[39] 2,2′-Anhydro-1-β-D-arabinofuranosyl-5-chlorocytosine (**6**) was also synthesized from the corresponding cytidine derivative by this procedure in low yield.

All of these compounds were characterized by the usual analytical and spectroscopic means. The UV absorption maxima of products **4**–**8** exhibited no shift from neutral to acidic conditions (pH 1), whereas the corresponding starting cytidine derivatives were protonated at N_3 and therefore showed bathochromic shifts under these conditions. The absorption maxima of compounds **4** and **5** were identical [λ_{max} (MeOH or pH 1) 268 nm] and are in agreement with the literature[29] values. Similarly, the UV spectrum of compound **7** was identical to anhydro-*ara*-C.[39]

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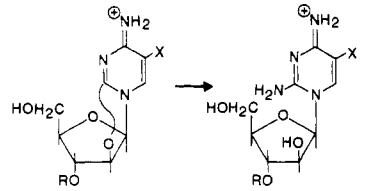
Scheme II



4. X=F, R=phosphate
5. X=F, R=H
6. X=Cl, R=H
7. X=H, R=phosphate
8. R=X=H

9. X=F, R=phosphate
10. X=F, R=H
11. X=Cl, R=H
12. X=H, R=phosphate
13. R=X=H

Table I. Comparative in Vitro Antiviral Activity

compd	virus rating HSV/1	VV	RV/13	MV	PRV
ara-C	1.0	0 9		1.2	0.8
ara-C-3′-P	1.1	0.7	0.8	1.6	1.3
AraAIPy (13)	1.3	0.6		1.3	0.6
AraAIPy-3′-P (12)	1.2	1.1	0.1	1.2	1.1
5-F-anhydro-*ara*-C-3′-P (4)	1 3				
5-F-AraAIPy-3′-P (9)	1.2	1.0	0.3	0.7	1.0
anhydro-*ara*-C-3′-P (7)	1.3	1.2	0.7	1.0	1.0

The ^{1}H NMR spectra of 4–8 gave a doublet for the anomeric proton, indicating the *arabinose* configuration for these derivatives.

Treatment of the anhydro derivatives 4–8 with anhydrous methanolic ammonia at ambient temperature produced the corresponding 1-β-D-arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidines 9–13 (Scheme II). The course of the reaction was followed by thin-layer chromatography (TLC) in systems A, B, or C, in which the products moved slower (less polar) than the starting material. The last traces of ammonia were removed carefully by repeated evaporation from ethanol, and the products were stored in the freezer without any sign of decomposition (TLC) for over a period of 2 years. The UV absorption maxima of AraAIPy derivatives 9–13 typically showed a bathochromic shift of 7–10 nm when compared with the starting anhydro derivatives. As with compounds 4–8, the AraAIPy derivatives 9–13 showed no shift from neutral to cationic species (pH 1), indicating that both of these classes of compounds could not be protonated at the ring nitrogen under these conditions. The protonation of the exocyclic imino group of AraAIPy, anhydro-*ara*-C and other related compounds[40,41] is reported to give a resonant cation. The strong basic strength of the imino group in these derivatives imparts the large degree of resonance stabilization of such a cation. However, AraAIPy derivatives were decomposed rapidly in alkaline conditions, as indicated by the loss in UV absorption. This was not surprising in view of the favorable stereochemistry of the 2′-hydroxyl anion which facilitated the intramolecular nucleophilic attack either at C_2 with the elimination of ammonia or at C_6 (in 5-fluoro derivatives) to form the 6,2′-anhydronucleoside, which subsequently undergoes further cleavage to give the open-chain ureido derivative as reported by Doerr and Fox.[27]

The ^{1}H NMR spectra of anhydro-*ara*-C and AraAIPy derivatives (see Experimental Section), in general, showed a similar pattern: a doublet for the anomeric proton characteristic for the *arabinose* configuration, a set of two doublets for the C_6 and C_5 protons for unsubstituted derivatives, and one doublet for the C_6 proton for 5-fluoro-substituted derivatives.

Table II. Antiherpes Activity of AraAIPy-3′-P and Anhydro-*ara*-C-3′-P in the Herpes Target Organ System

compd	dose, mM/kg	% survivor no. increase
AraAIPy-3′-P (12)	0.14	50
	0.033	0
anhydro-*ara*-C-3′-P (7)	0.14	40
	0.033	0
ara-C	0.14	40
	0.033	0

Table III. Showing Antileukemia Activity of AraAIPy and AraAIPy-3′-P in BDF_1 Female Mice

treatment (ip)	treated/controls, days	wt, day 5	survivor, 60 day	T/C	*P*
anhydro-*ara*-C (8), 600 mg/kg	24.0/8.53	−1.50	6/8	2.81	0.0005
anhydro-*ara*-C-3′-P (7), 600 mg/kg	17.5/8.40	−1.10	0/6	2.07	0.0005
AraAIPy (13), 400 mg/kg	60.0/8.53	−1.12	8/8		
AraAIPy-3′-P (12), 600 mg/kg	22.5/9.2	−0.30	2/6	2.44	0.001

Biological Evaluation. The compounds were screened for their antiviral as well as antitumor properties. The results of in vitro antiviral activity are documented in Table I. All of the compounds tested showed good activity against herpes type 1 and slight activity against rhinovirus type 13 at doses which were not toxic to KB cells. Compounds 7 and 12 exhibited as good or slightly better virus rating against all five viruses as compared to *ara*-C or *ara*-C 3′-phosphate (*ara*-C-3′-P). The drugs were also studied in vivo in a target organ system for their effects against type 1 herpes simplex virus (Table II).[43] Compounds 7 and 12 exhibited appreciable therapeutic activity against intracerebral inoculation of the virus (~10 LD_{50} dose) in swiss albino mice. The results were comparable to or better than *ara*-C or anhydro-*ara*-C-3′-P used as control drugs, as shown by an increase in the number of survivors of the virus-infected mice.

The antitumor effects of the drugs[46,47] were evaluated in the leukemia L1210 system in BDF_1 female mice. Both AraAIPy (13) and its 3′-phosphate 12 showed curative effects (Table III). AraAIPy at 400 mg/kg (five ip daily injections) cured all the leukemic animals. AraAIPy-3′-P at 600 mg/kg gave an ILS (increase in life span) value of 144%, and 33% (2/6) of the animals were cured. Studies on the development of aminoiminopyrimidines as anticancer/antiviral drugs are in progress.

Experimental Section

(A) Chemistry. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained at 90 MHz on a Varian EM-390 spectrophotometer. Ultraviolet spectra were recorded on a Cary 118c spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Thin-layer chromatography (TLC) was performed with Eastman Chromograms coated with either silica gel or cellulose (both with fluorescent indicators), and the components were detected by ultraviolet light. For TLC, the following solvent systems were used: system A, acetonitrile–0.2 M aqueous ammonium chloride (7:3); system B, methanol–benzene (1:3); system C, 60 g of ammonium sulfate and 2 mL of propanol were added to 100 mL of 0.1 M sodium phosphate buffer, pH 6.8 (all systems v/v). Electrophoresis was performed on a high-voltage Savant instrument using 0.2 M acetate buffer, pH 3.6, and 0.1 M phosphate buffer, pH 7.0 (2000 V for 2-h run).

Tributylammonium 5-Fluorocytidine 2′(3′)-Phosphate (2). 2′(3′)-Cytidylic acid, mixed isomers (1, 0.646 g, 2 mmol), were suspended in H_2O (50 mL) and mixed with tri-*n*-butylamine (2 mL). The mixture was stirred at room temperature until all solid dissolved, and the solvent was then removed in vacuo. The residue was evaporated from EtOH (50 mL). The resulting solid was dissolved in MeOH (150 mL) and cooled in a dry ice–acetone bath. Freon II (50 mL) was cooled to −76 °C in dry ice–acetone and trifluoromethyl hypofluorite (CF_3OF, 1 g) was added by a gas-dispersion tube. The resulting Freon solution was added with caution to the MeOH solution. After stirring the solution for 1 h at −76 °C, nitrogen was used to disperse the remaining CF_3OF in the solution. Following removal of the solvent in vacuo, a 10% triethylamine solution in 50% MeOH–water (50 mL) was added, and the resulting solution was maintained at room temperature for 16 h. The solution was reduced to an oil in vacuo. The oil was dissolved in H_2O (25 mL) and applied to a column (27 × 3 cm diameter) of Dowex 50W 8 (H^+) resin. Following development with H_2O, the fractions containing the product were reduced to dryness by freeze-drying to yield 0.307 g (45%) of **2**, which was directly used for the next reaction.

Tributylammonium 5-Fluorocytidine Cyclic 2′,3′-Phosphate (3). Tributylammonium 5-fluorocytidine 2′(3′)-phosphate (**2**, 0.307 g, 0.9 mmol) was dissolved in H_2O (3 mL). Tri-*n*-butylamine (0.72 mL) and ethyl chloroformate (0.19 mL) were added separately, and the resulting solution was stirred at room temperature for 1 h. The solvent was removed in vacuo. Ethanol (25 mL) was added and removed in vacuo, and the process was repeated twice. Dry pyridine (25 mL) was added and removed in vacuo, and the resulting foam of product **3** was used as such for the next reaction.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine 3′-Phosphate (4). Tributylammonium 5-fluorocytidine cyclic 2′,3′-phosphate (**3**, 0.457 g, 0.9 mmol) and tri-*n*-butylamine (0.19 mL) were added to pyridine (5 mL). Chlorotrimethylsilane (1.5 mL) was added, and the resulting solution was heated at 90 °C for 1 h with stirring, in the presence of moisture. The solution was reduced to a gum in vacuo, and ice (50 mL) and $CHCl_3$ (50 mL) were added. The resulting layers were separated, and the water layer was extracted twice more with $CHCl_3$ (50 mL). The water solution was applied to a column (20 × 3 cm diameter) of Dowex 1 × 8 (formate). The column was washed with H_2O (10 mL) and the eluate was applied to another column (25 × 3 cm diameter) of Dowex 50W × 8 (H^+) which was developed with water. The fractions containing product **4** were reduced to a small volume and filtered, and the resulting solution was freeze-dried to obtain a pale powder: yield 0.182 g (63.0%); UV λ_{max} (pH 1) (H_2O) 268 nm, λ_{max} (pH 11) 274; ^1H NMR (Me_2SO-d_6–D_2O) 8.75 (d, 1, $J_{F\text{-}H_6}$ = 5 Hz C_6H), 6.66 (d, 1, $J_{1',2'}$ = 6 Hz $C_{1'}$-H), 5.58 (d, 1, $C_{2'}$-H), 4.55 (br s, 1, $C_{3'}$-H), 4.27 (m, 1, $C_{4'}$-H), $C_{4'}$-H), 3.42 ppm (m, 1, $C_{5'}$-H_6). Anal. ($C_9H_{11}N_3O_7PF\cdot0.5H_2O$) C, H, N.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine Hydrochloride (5). 5-Fluorocytidine hydrochloride (3.692 g, 12.4 mmol) and phosphorous oxychloride (28.4 mL) were added to ethyl acetate (618 mL). Water (5.45 mL) was added dropwise (with caution) to this stirred suspension. Following the addition of the water, the mixture was heated at reflux temperature for 2 h. The mixture was concentrated to a volume of approximately 100 mL and ice (100 mL) was added. The solution was reduced to an oil in vacuo. The oil was dissolved in H_2O (25 mL) and applied to a column (28 × 3 cm diameter) of Dowex 1 × 8 (Cl^-). Following elution of the solution with H_2O (300 mL), the solvent was removed in vacuo and the product **5** recrystallized from EtOH–H_2O: yield 0.781 g, 22.6%; mp 258–262 °C dec; UV λ_{max} (pH 1) 268 nm (ε 11 580), 228 (9200); λ_{max} (H_2O) 268 (11 580), 228 (9200); λ_{max} (pH 11) 274 nm (ε 8240), 240 (9900); ^1H NMR (Me_2SO-d_6) 8.48 (d, 1, $J_{F\text{-}H_6}$ = 5 Hz, C_6H), 6.54 (d, 1, $J_{1,2}$ = 6 Hz, $C_{1'}$-H), 5.43 ppm (d, 1, $C_{2'}$-H). Anal. ($C_9H_{10}N_3O_4F\cdot HCl$) C, H, N.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-chlorocytosine Hydrochloride (6). 5-Chlorocytidine (5.54 g, 20 mmol) and phosphorous oxychloride (45.6 mL) were added to ethyl acetate (600 mL). Water (8.6 mL) was added with caution to this stirred suspension. The mixture was heated at reflux temperature for 2 h and the solution concentrated to an oil in vacuo. Ice–water (100 mL) was added to the oil and the flask shaken to obtain a clear solution. The dark brown solution was applied to a column (10 × 3 cm diameter) of Dowex 50W × 8 (H^+ form). The column was eluted, in succession, with H_2O (500 mL), MeOH (200 mL), and H_2O (150 mL). The product **6** was eluted with 1 N HCl, with the eluent being monitored at 254 nm with an ultraviolet recorder. The solvent was removed in vacuo, and EtOH (100 mL) was added and removed in vacuo. The product was dissolved in a minimum amount of boiling EtOH, and the solution was filtered and concentrated. The solution was cooled at 5 °C for 16 h, and the crystals were collected by filtration and dried in vacuo, yield 1.36 g, 20%. An analytical sample of **6** was prepared by two more recrystallizations from EtOH and dried in vacuo at 110 °C: mp 205–208 °C dec; UV λ_{max} (pH 1) 275 nm (ε 9230), 230 (shoulder) (8100); λ_{max} (pH 7) 275 nm (ε 9230), 230 (shoulder) (8100); λ_{max} (pH 11) 286 nm (ε 6710), 231 (8100); ^1H NMR (D_2O) 8.60 (s, 1, C_6H), 6.72 (d, 1, $J_{1',2'}$ = 5.5 Hz, $C_{1'}$-H), 5.65 ppm (d, 1, $C_{2'}$-H). Anal. ($C_9H_{10}N_3ClO_4\cdot HCl$) C, H, N.

1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-4-iminopyrimidine 3′-Phosphate (12). 2,2′-Anhydrocytidine 3′-phosphate (**7**, 305 mg, 1 mmol) was suspended in MeOH (10 mL) and the suspension treated with methanolic ammonia (20 mL). The reaction was maintained at room temperature (anhydrous conditions) for 3 days. At this stage, TLC (cellulose, systems A and C) showed the reaction was complete. The reaction mixture was evaporated to dryness under reduced pressure and the residue evaporated from MeOH to remove the excess of ammonia. Compound **12** was obtained as a white powder (diammonium salt, yield 300 mg, 85%).

For the analytical sample, a portion of the product **12** (100 mg) was absorbed on a Dowex 1 × 2 (100–200 mesh) formate column (5 × 50 cm), which was eluted with a gradient of water and 0.25 M formic acid. The product-containing fractions were evaported to dryness, and the residue was evaporated from dioxane to remove the last traces of formic acid. The residue was dissolved in H_2O and filtered, and the filtrate was freeze-dried to obtain the free acid of **12** as a white powder: UV λ_{max} (pH 7) 269 nm (ε 8080); λ_{max} (pH 1) 269 nm (ε 8080); λ_{max} (pH 14) 295 nm (ε 2800); ^1H NMR (Me_2SO-d_6–D_2O) 8.40 (br s, 1, NH, exchanges with D_2O), 8.05 (br s, 2, NH_2, overlapping C_6H and exchanges with D_2O), 8.05 (d, 1, $J_{5,6}$ = 6 Hz, C_6H), 5.95 (d, 1, $J_{1',2'}$ = 4.5 Hz), 4.51 (t, 1, peak width = 15 Hz, $C_{2'}$-H), 4.15 (m, 1, $C_{4'}$-H), 4.00 (m, 1, $C_{3'}$-H), 3.75 ppm (m, 2, $C_{5'}$-H_2). Anal. ($C_9H_{15}N_4O_7P\cdot1.75H_2O$) C, H, N.

1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-4-imino-5-fluoropyrimidine 3′-Phosphate (9). 2,2′-Anhydro-5-fluorocytidine 3′-phosphate (**4**, 65 mg, 0.2 mmol) was suspended in MeOH (5.0 mL), and this suspension was treated with dry methanolic ammonia (10 mL). The clear solution was kept at room temperature for 3 days; TLC (system A, cellulose) at this time showed that the reaction was complete. The reaction mixture was evaporated to dryness, and the residue was complete. The reaction mixture was evaporated to dryness, and the residue was evaporated three times from EtOH to obtain **9** as a white product, which was crystallized from aqueous EtOH and stored in a freezer over Drierite: UV λ_{max} (MeOH) 277 nm (ε 7980); λ_{max} (pH 1) 277 nm (ε 7980); λ_{max} (pH 14) 308 nm (sh, decomposes); ^1H NMR (D_2O) 8.30 (d, 1, $J_{F\text{-}H_6}$ = 5.5 Hz, C_6H), 6.05 (d, 1, $J_{1',2'}$ = 4.5 Hz, $C_{1'}$-H), 5.38 (t, 1, peak width = 15 Hz, $C_{2'}$-H), 4.35 (m, 1, $C_{4'}$-H), 4.15 (m, 1, $C_{3'}$H), 3.82 ppm (m, 2, $C_{5'}$-H_2). Anal. ($C_9H_{17}FN_5O_7P\cdot C_2H_5OH$) C, H, N.

1-β-D-Arabinofuranosyl-2-amino-1,4(2*H*)-4-imino-5-fluoropyrimidine Hydrogen Sulfate (10). 2,2′-Anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine hydrochloride (**5**, 56 mg, 0.2 mmol) was dissolved in dry MeOH (5 mL), and cold methanolic ammonia (10 mL) was added. The reaction flask was left at room temperature for 36 h in the absence of moisture. Chromatography (TLC) showed (cellulose plates, systems A and B; silica plates, system B) that the reaction was complete and there was only one UV-absorbing product. The reaction solution was evaporated to dryness. The residue was dissolved in MeOH (25 mL) and the solution absorbed on a Dowex-50 (sulfate form) column; the column was eluted with MeOH (30 mL). The UV-absorbing fractions were combined and evaporated to dryness to provide **10** as a pale solid: yield, 52 mg, 72%; UV λ_{max} (MeOH) 278 nm (ε 6900); λ_{max} (pH 1) 278 nm (ε 7050); λ_{max} (pH 14) 305 nm (sh, decomposes). Anal. ($C_9H_{13}N_4O_4F\cdot H_2SO_4$) C, H, N.

1-β-D-Arabinofuranosyl-2-amino-1,4-imino-5-chloropyrimidine Hydrochloride (11). 2,2′-Anhydro-1-β-D-arabi-

nofuranosyl-5-chlorocytosine hydrochloride (6, 76.5 mg, 0.25 mmol) was suspended in MeOH (5 mL), and cold methanolic ammonia (25 mL) was added. The reaction vessel was sealed and kept at room temperature for 36 h. Thin-layer chromatography (silica, system B) showed that the reaction was complete. The reaction mixture was evaporated to dryness under reduced pressure. The residue was evaporated from MeOH and EtOH to remove the last traces of ammonia. The solid residue was dissolved in MeOH (5 mL) and precipitated with an excess of ether. The product was removed by centrifugation, washed with ether, and dried in vacuo at room temperature: yield 61 mg, 80%; UV λ_{max} (pH 7) 282 nm (ϵ 7300); λ_{max} (pH 1) 282 nm (ϵ 7150); λ_{max} (pH 14) 296 nm (ϵ 3440).

(B) Biological Screening. The antiviral activity in vitro was determined by observing the inhibition of virus-induced cytopathic effects (CPE) in cultures of human carcinoma of nasopharynx (KB) cells grown in disposable plastic microplates. A monolayer (18–24 h) of cells were exposed to approximately 320 cell-culture, 50% effective doses of virus, and an appropriate concentration of each compound was added within 15 min. The cells were observed microscopically for CPE development after 72-h incubation at 37 °C. Degree of CPE inhibition and drug cytotoxicity were scored numerically and used in calculating a virus rating (VR) as described.[42,43] Significance of activity in terms of VR's has been assigned as follows: 0.5, slight or no activity; 0.5–0.9, moderate activity; 1.0, marked activity. The virus rating was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE values (0–4) assigned to T for each drug level was substracted from that of C, and the difference (C – T) was totaled. If partial toxicity was evident at any drug level, the C – T of that level was divided by 2. The sum of C – T values was then divided by ten times the number of test compounds used per drug level. Viruses used in this study were type 1 herpes simplex (HSV/1), vaccinia (VV), rhinovirus type 13 (RV/13), myxoma (MV), and pseudorabis viruses (PRV). Data (Table I) are expressed as the average of many tests and with positive controls of *ara*-C and *ara*-C 3′-phosphate.

The antiviral activity in vivo was determined in a target organ system. Swiss albino mice (six animals per group) were infected intracerebrally (ic) with type 1 herpes simplex virus. Then, 6 h later the animals were given single ic drug injections with the compounds dissolved in Hank's balanced salt solution. The effectiveness of the treatment was computed from the increase in number of survivors as compared to the controls which received only the same amount of Hank's balanced salt solution.

The antileukemia effects of the drugs were evaluated in the leukemia L1210 system. Groups of six to eight BDF_1 female mice (obtained from Simonsen Laboratories, Gilroy, Calif.) were given intraperitoneal (ip) inoculations of 1×10^5 viable L1210 leukemia cells each. Treatments (qd, 1–5) were started 24 h after the tumor implant. All drug solutions were freshly prepared in isotonic sterile saline. The animals in control groups were only given 0.2 mL of sterile saline injection during the treatment period. The results are expressed in terms of number of survivors (cures) after drug treatments and by the value of T/C. In the calculation of T/C values, survivors are not included. Statistical significance of the results (value of *P*) was calculated using a Wilcoxen method for nonparametric distributions.

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References and Notes

(1) Work was done at ICN Nucleic Acid Research Institute, Irvine, Calif. The work was completed at Univerisity of Southern California under Research Grant CA-14089 from the National Cancer Institute, USPHS.
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(46) In animal model studies we have shown that 5-fluoro-anhydro-*ara*-C (**5**) caused an 80% increase in the life span of DBA/2 mice bearing intraperioneal transplants (1×10^5 cells) of leukemia L1210 (200 mg/kg, qd 1–5). During the progress of this work, Fox et al.[27] independently described the antileukemic activity of compound **5**.
(47) Further details of the antileukemic activity of these compounds will be published elsewhere.

Improved Antitumor Effects in 3′-Branched Homologues of 2′-Deoxythioguanosine. Synthesis and Evaluation of Thioguanine Nucleosides of 2,3-Dideoxy-3-(hydroxymethyl)-D-*erythro*-pentofuranose

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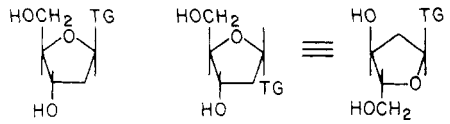
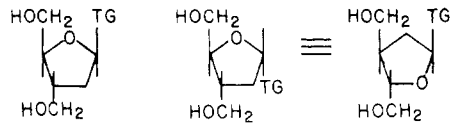
The 3-(hydroxymethyl) branched homologue of 2-deoxyribofuranose was synthesized from the corresponding branched ribofuranose 2-*O*-(*S*-methyl dithiocarbonate) with tributyltin hydride in the first direct, one-step deoxygenation at C-2 of a ribofuranose. Nucleoside coupling afforded the corresponding 3′-branched 2′-deoxyribonucleosides of thioguanine. The α- and β-nucleosides were equally inhibitory to growth of WI-L2 human lymphoblastoid cells, were phosphorylated and incorporated into the DNA of Mecca lymphosarcoma in mice to the same degree, and were more effective in these tests than the parent analogue α-2′-deoxythioguanosine. These results indicate that the hydroxy functions at the 3′ and 5′ positions of 2′-deoxynucleosides are interchangeable on the tumor enzymes, that the furanose ring oxygen and 2′-methylene are correspondingly interchangeable, and that acceptance by the enzymes is improved if primary hydroxyls are provided at both the 3′ and 5′ positions.

A number of thioguanine nucleosides have been synthesized[2-6] and screened for antitumor properties. Almost all of them are active.[6-9] The most important of them are the anomeric pair β- (β-TGdR, 1) and α-2′-



1 (β-TGdR) 2 (α-TGdR) 2a



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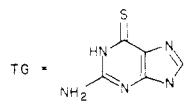

deoxythioguanosine (α-TGdR, **2**),[4,10,11] which are currently undergoing clinical trial.[12] The β anomer is a potent and useful agent, but like most of the thioguanine (TG) nucleosides it suffers rapid enzymatic cleavage to TG, which frustates the potential advantage of the nucleoside over TG.[10] In studies on the biological properties of nucleosides in general, it has been found that the β anomeric configuration is almost invariably required for activity. α-2′-Deoxythioguanosine (**2**) is unique as an α anomer with sufficient activity against experimental tumors to be selected for clinical trial. It is also unique among nucleosides of TG in its resistance to cleavage, as indicated by the absence of thioxanthine and thiouric acid in urine of patients receiving α-TGdR and by the fact that much of the α-TGdR is excreted unchanged.[13] As a result, α-TGdR is especially notable for its low toxicity. It is also less potent that the β anomer, but the low toxicity means that patients can be treated with high doses of α-TGdR without side effects.

The low toxicity may be also, in part, the result of an unusual selectivity of α-TGdR for tumor tissue that has been observed.[10,11] Both α- and β-TGdR are phosphorylated and incorporated into DNA of mouse and human tumor tissues; the extent of incorporation varies with the tissue or enzyme source and appears to be proportional to the resultant carcinostatic effects. Extracts of various normal tissues were tested as well, and β-TGdR was found to be phosphorylated by most of them. However, α-TGdR was not phosphorylated to a significant level in bone marrow cells, an evident explanation for low host toxicity.

Further studies showed that in tumors α-TGdR is incorporated into DNA at the terminal nucleoside position of short fragments, terminating chain growth, while β-TGdR is incorporated into the internal nucleoside positions of DNA.[14] Nevertheless, it seems clear that the α-nucleoside is accepted by many of the enzymes that are responsible for processing β-TGdR and presumably the natural substrate, of β configuration, 2′-deoxyguanosine. It is highly unlikely that there is a separate set of kinases for processing the α anomer, and this suggests that the common assumption of gross structural dissimilarity of anomers, illustrated by structures **1** and **2**, be reexamined in the case of α- and β-TGdR. For either anomer, the purine moiety must occupy the same site on the enzyme. This suggested that **1** be compared with structure **2a** for the α anomer, and it required that the 5′-CH_2OH of **1** be replaced on the enzyme by the 3′-OH of **2a** and vice versa. This also required that the furanose oxygens and 2′-deoxy carbons exchange when one anomer replaces the other on the enzyme.[15] It appeared from this comparison that the α anomer as structure **2a** can simulate the β-anomer **1** reasonably well in terms of shape and placement of key

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