and the reaction continued for one more day at 37 °C. TLC showed no further significant change in the ratio of products to starting 5-hydroxy-2′-deoxyuridine. The reaction solution was washed twice with an equal volume of $CHCl_3$, and the aqueous layer was evaporated in vacuo at <40 °C to a deep-red glass. The residue was applied to a silica gel column (2 × 30 cm), and the column was eluted with $CHCl_3$–MeOH (7:1, v/v). Fractions (5 mL) were collected and checked for homogeneity by silica gel TLC ($CHCl_3$–MeOH, 4:1). The fractions of the major product (**2**, R_f ~0.4 in the above system) were collected and applied to a Sephadex LH-20 column (1.5 × 30 cm), which was eluted with MeOH. The yield of chromatographically pure product was 325 mg (23%). Recrystallization from MeOH gave colorless needles of mp 141–142 °C dec. Anal. ($C_{11}H_{13}N_3O_6$) C, H, N. Compound **2** showed a positive Dische test and a negative $FeCl_3$ test. The infrared spectrum of **2** did not reveal the presence of a typical CN absorption at about 2230 cm^{-1}: ^1H NMR (Me_2SO-d_6) δ 11.60 (br s, 1, NH), 7.90 (s, 1, 6 H), 6.14 (t, 1, 1′ H, $J_{1',2'}$ = 6.7 Hz), 5.2 (m, 2, 3′ and 5′ OH), 4.90 (s, 2, OCH_2CN), 4.3 (m, 1, 3′ H), 3.8 (m, 1, 4′ H), 3.6 (m, 2, 5′ H's), 2.10 (dd, 2, 2′ H's); UV λ_{max} 273 nm (ε 8700) at pH 1, 271 (ε 6600) at pH 12. Chemical ionization mass spectrometry gave *m/e* 301 (M + 18) and 284 (M + 1).

As minor products of this reaction, 3-(cyanomethyl)-5-hydroxy-2′-deoxyuridine (**5**, R_f 0.3, ≤1%) and 3-(cyanomethyl)-5-[(cyanomethylene)oxy]-2′-deoxyuridine (**3**, R_f 0.5, 81 mg, 5%) were obtained. The characterization of these compounds is presented below.

3-(Cyanomethyl)-5-[(cyanomethylene)oxy]-2′-deoxyuridine ([[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-(cyanomethyl)-2,4-dioxypyrimidin-5-yl]oxy]acetonitrile; 3) and 3-(Cyanomethyl)-5-hydroxy-2′-deoxyuridine (1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-(cyanomethyl)-5-hydroxypyrimidine-2,4-dione; 5). 5-Hydroxy-2′-deoxyuridine (1.221 g, 5 mmol) was dissolved in KOH solution (5 mL, 5 mmol), and the water was removed by evaporation in vacuo at <40 °C. The residue was further dried in vacuo for 2 h. DMF (100 mL) and iodoacetonitrile (10 mmol, 763 μL) were added, and the resulting solution was stirred vigorously at 37 °C. After 3 h, the reaction became homogeneous. The mixture was maintained at 37 °C for 17 h, after which the DMF solvent was removed in vacuo at ≤40 °C. H_2O (20 mL) was added to the viscous deep-red residue, and the solution was extracted with $CHCl_3$ (2 × 20 mL). The water layer was evaporated at ≤40 °C to a deep-red viscous glass, which was applied to a silica gel column (2 × 30 cm). Elution was with $CHCl_3$–MeOH (7:1). Fractions of 5 mL were checked for homogeneity by TLC ($CHCl_3$–MeOH, 4:1). Fractions containing the major product (R_f ~0.5) were collected and applied to a Sephadex LH 20 column (1.5 × 30 cm), which was eluted with MeOH. Evaporation of the MeOH gave, after drying, 368 mg of **3** (23%) as a glass. Although **3** was pure by TLC and ^1H NMR, repeated attempts to produce crystalline material failed. Compound **3** gave a negative $FeCl_3$ test and a positive Dische test: UV λ_{max} 276 nm at pH 1, 278 at pH 12; ^1H NMR (Me_2SO-d_6) δ 8.12 (s, 1, 6 H), 6.20 (t, 1, 1′ H, *J* = 6.5 Hz), 5.2 (m, 2, 3′ and 5′ OH), 4.93 (s, 1, OCH_2), 4.81 (s, 1, NCH_2), 4.3 (m, 1, 3′ H), 3.8 (m, 1, 4′ H), 3.7 (m, 2, 5′ H's), 2.20 (dd, 2, 2′ H's). The NMR spectrum failed to reveal the presence of any signal corresponding to the pyrimidine N-3 H. Chemical ionization mass spectrometry gave a parent peak at 340 (M + 18).

For further confirmation of the structure of **3**, hydrolysis of **3** was effected by boiling in water (2 h). Sixty-five percent of glycoside bond cleavage was obtained by this procedure. The free base (**4**) was separated from unhydrolyzed **3** by preparative silica gel TLC ($CHCl_3$–MeOH, 4:1). Crystals were obtained by recrystallization from MeOH to give **4**: mp 159–160 °C; IR (KBr) 2264 (CN, very weak) cm^{-1}; UV λ_{max} 272 nm at pH 1; 297 at pH 12; mass spectrum (20 eV, 90 °C), *m/e* 206 (58.5, parent peak), 166 (89.3), 151 (24.2), 83 (100), 28 (27.6); ^1H NMR (D_2O) δ 7.69 (s, 1, 5 H), 4.95 (s, 2, OCH_2), 4.91 (s, 2, NCH_2). Anal. ($C_8H_6N_4O_3$) C, H, N.

Fractions with an R_f ~0.3 ($CHCl_3$–MeOH, 4:1) from the above silica gel chromatography were collected and further purified in the same manner as described for the preparation of **3** above. After the separation, the residue was dissolved in H_2O (5 mL), the resulting solution was passed through Dowex 50 (H^+) resin (0.5 × 3 cm), and the elute was lyophilyzed. The yield of **5** was 66.5 mg (4.7%). The product **5** was amorphous and, like compound **3**, resisted all attempts at crystallization. Compound **5** gave both a positive $FeCl_3$ test and a positive Dische test: UV λ_{max} 281 nm at pH 1; 243 and 312 at pH 12; ^1H NMR (Me_2SO-d_6) δ 8.98 (br, s, 1, 5 OH), 7.51 (s, 1, 6 H), 6.22 (t, 1, 2′ H, *J* = 6.8 Hz), 5.2 (m, 2, 3′ and 5′ OH), 4.82 (s, 2, CH_2CN), 4.3 (m, 1, 3′ H), 3.8 (m, 1, 4′ H), 3.6 (m, 2, 5′ H's), 2.10 (dd, 2, 2′ H's). The ^1H NMR spectrum did not reveal any signal attributable to the pyrimidine N-3 H. The chemical ionization mass spectrum of **5** gave a parent peak at 301 (M + 18).

As a minor product of this reaction in DMF, a trace (<1%) of 5-[(cyanomethylene)oxy]-2′-deoxyuridine (**2**) was obtained.

Synthesis and Biological Evaluation of Certain 2′-Deoxy-β-D-ribo- and -β-D-arabinofuranosyl Nucleosides of Purine-6-carboxamide and 4,8-Diaminopyrimido[5,4-*d*]pyrimidine

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The key intermediate 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile (**7**) was synthesized in four steps from 9-β-D-arabinofuranosylpurine-6-thione (**3**) via 6-(methylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (**6**). Reaction of compound **7** with methanolic ammonia provided the rearranged compound 4-amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**8**). Treatment of **7** with ammonium hydroxide and hydrogen peroxide provided 9-β-D-arabinofuranosylpurine-6-carboxamide (**9**). Compound **7** was also treated with sodium hydrosulfide to yield 9-β-D-arabinofuranosylpurine-6-thiocarboxamide (**10**). Similarly, 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine-6-carbonitrile (**17**) was prepared from 6-chloro-9-(2-deoxy-β-D-*erythro*-pentofluranosyl)purine (**11**) via 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-thione. Compound **17** was converted into 4-amino-8-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]pyrimido[5,4-*d*]pyrimidine (**18**) and 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-carboxamide (**20**), respectively. Compound **2** showed immunosuppressive activity and also inhibited the growth of L-1210 leukemia in mice. Arabinonucleoside analogues **8–10** were inactive when tested against RNA and DNA viruses in cell culture.

The chemical modification of various nucleosides for structure specificity and biological activity relationships has been a major research effort of our group. The introduction of a carboxamide function at the 6 position of

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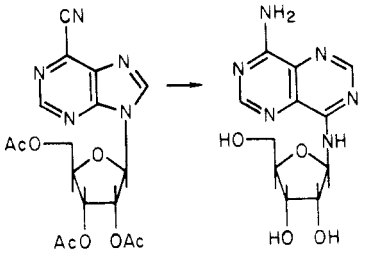
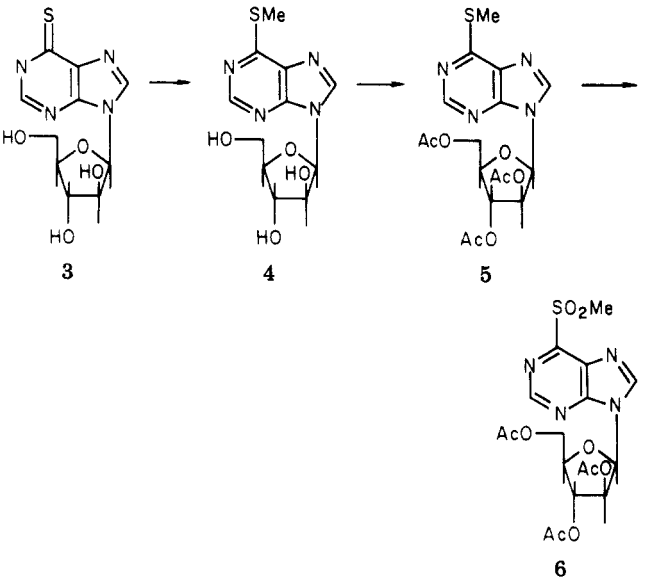
certain naturally ocurring purine nucleosides was recently considered. The nucleoside 6-amino-9-β-D-arabinofuranosylpurine (*ara*-A) is a clinically effective drug against herpes simplex virus infections.[1] A major disadvantage of this nucleoside is its relative insolubility in water which makes its intravenous administration difficult.[2] The 5′-phosphate of *ara*-A tends to increase the solubility of the nucleoside without hampering the biological activity, although 5′-phosphates are known not to be easily transported through the cell membrane.[3,4] The introduction of a carboxamide group due to its polar properties should make the nucleoside readily soluble in water. It could also facilitate the binding of the nucleoside with proteins and nucleic acids which contain such a functionality (–CONH–) as an inherent part of the molecule. Indeed, the carboxamide group is an integral part of several naturally occurring nucleoside antibiotics,[5] such as pyrazomycin, bredinin, and sangivamycin, and is present in various biologically active synthetic nucleosides, such as ribavirin,[6] 5-halo-1-β-D-ribofuranosylimidazole-4-carboxamide,[7] 2-β-D-ribofuranosylthiazole-4-carboxamide,[8] and 9-β-D-ribofuranosylpurine-6-carboxamide,[9] recently reported from our Laboratory.

Chemistry. Traditionally the synthesis of a carboxamide has been readily accomplished via the treatment of a carbonitrile with hydrogen peroxide in an alkaline medium. It has also been observed that the treatment of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine-6-carbonitrile (**1**) with ammonia provided an ammonia adduct,[10] later



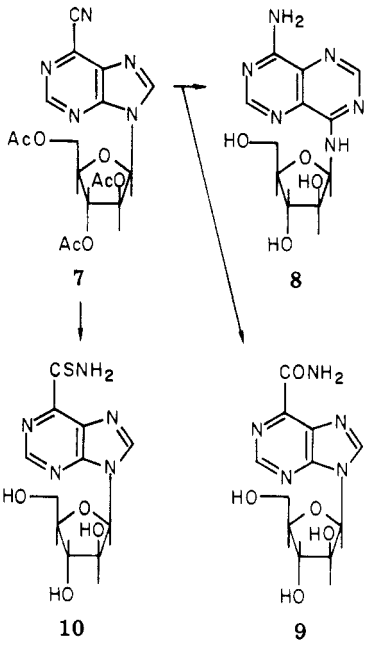
1 **2**

Scheme I



3 **4** **5** **6**

Scheme II



7 **8** **10** **9**

characterized by us as 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**2**) by X-ray crystallography.[11] Compound **2** has exhibited significant antileukemic[12] and immunosuppressive activity (see biological section) and, therefore, it was decided to synthesize the arabino and 2′-deoxynucleoside derivatives of **2** for biological evaluation.

We utilized 9-β-D-arabinofuranosylpurine-6-thione (**3**) as a precursor, which was synthesized following the procedure of Reist et al.[13] These workers have also described

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the methylation of 3 with methyl iodide and potassium carbonate in dimethyl sulfoxide to provide 6-(methylthio)-9-β-D-arabinofuranosylpurine (4). Using a modification of the literature procedure, we methylated compound 3 to provide 4 in almost quantitative yield. The methylthio nucleoside 4 synthesized by us exhibited a melting point of 183–184.5 °C as compared to 99–101 °C as reported in literature.[13] We also prepared compound 4 following the literature procedure[13] and found that both samples when crystallized from methanol had identical UV, ^{1}H NMR, mp (183–184.5 °C), and TLC mobilities. The acetylation of 4 in acetic anhydride using a catalytic amount of 4-(*N*,*N*'-dimethylamino)pyridine provided 6-(methylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-purine (5) as a chromatographically pure syrup in 90% yield. The potassium permanganate oxidation of 5 in acetic acid provided 6-(methylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (6) as a syrup in 80% yield. In the ^{1}H NMR ($CDCl_3$) of 6, all the protons, except acetyls and $C_{4'}$ H and $C_{5'}$ H_2, were shifted downfield as compared to those of 5. The SCH_3 protons had a shift of 0.75 ppm, whereas C_2 H, C_8 H, and $H_{1'}$ were shifted by 0.42, 0.47, and 0.13 ppm, respectively. The downfield shift would be expected due to the sulfonyl group in 6 (Scheme I). The product was of sufficient purity to be used for further reaction without purification. The reaction of sodium cyanide with 6 in dimethylformamide provided the key intermediate 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile (7) in 50% yield after chromatographic purification. Our attempts to crystallize this compound were unsuccessful and it was analyzed as a syrup. Compound 7 did not exhibit appreciable absorption in the IR for the nitrile band but was completely characterized by UV, ^{1}H NMR, and elemental analysis. Once isolated in pure form, compound 7 was found stable at room temperature in contrast to the corresponding ribofuranosyl derivative which has recently been reported[14] as unstable. The availability of nitrile intermediate 7 has led us to synthesize various 6-substituted derivatives (Scheme II).

Treatment of 7 with methanolic ammonia provided the rearranged product 4-amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-*d*]pyrimidine (8), which was characterized as follows. In the ^{1}H NMR of 8 (in Me_2SO-d_6, Me_4Si standard, on a Fourier transform NMR spectrometer, JEOL-FX-900) the 8-NH proton which was coupled with the anomeric proton appeared as a doublet (J = 9.3 Hz) at δ 7.8. The anomeric proton which was further coupled with $C_{2'}$ H appeared as a pair of doublets ($J_{H_{1'}-H_{2'}}$ = 4.0 Hz, $J_{H_{1'}-NH}$ = 9.3 Hz) centered at δ 6.0. The exchange of active hydrogens by D_2O or spin decoupling of the signal centered at δ 7.8 caused collapse of the pair of doublets at δ 6.0 to a doublet ($J_{H_{1'}-H_{2'}}$ = 4.0 Hz) at δ 6.0. The UV spectrum of compound 8 showed a characteristic absorption at wavelengths λ_{max} (H_2O) 292, 303, 310, and 334 nm. The ^{1}H NMR and UV data obtained for compound 8 was comparable to that recorded[11] for ribosyl analogue 2. Reaction of 7 with ammonium hydroxide in the presence of hydrogen peroxide under more carefully controlled conditions provided the desired compound 9-β-D-arabinofuranosylpurine-6-carboxamide (9) as water-soluble needles in 50% yield. The structure of 9 was determined on the basis of ^{1}H NMR which showed, in addition to other protons at appropriate positions, the absorption at δ 8.05 and 8.38 as broad singlets for $CONH_2$ protons which were exchangeable by deuterium oxide. Further structural proof came from elemental analysis and UV absorption, which showed a λ_{max} (pH 1) of 276 nm and λ_{max} (pH 7) of 282 nm for this compound. Further modification of the nitrile derivative 7 has included the substitution of the 6 position by a thiocarboxamide function. The 9-β-D-arabinofuranosylpurine-6-thiocarboxamide (10) was readily obtained in 60% yield when the nitrile intermediate 7 was treated with sodium hydrosulfide.

For the synthesis of similar 2'-deoxynucleoside analogues, 6-chloro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine (11) was required as a precursor, which was synthesized from 2'-deoxyinosine following the procedure of Robins and Basom.[15,16] Compound 11 was treated with sodium hydrosulfide using the literature procedure[15,17] to provide 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-thione (12) in 90% yield. The direct displacement of the chloro group from 11 by methyl mercaptide has been described[15] to provide 6-(methylthio)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (13). It was more conveniently synthesized in our laboratory by methylation of 12 with methyl iodide in 75% yield. Treatment of 13 with pyridine and acetic anhydride produced pure 6-(methylthio)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine (14) as fine needles in 92% yield.

Our attempts to oxidize 14 using a potassium permanganate–acetic acid procedure analogous to that described for the oxidation of 5 were unsuccessful due to the acid lability of the 2'-deoxynucleosides. The oxidation using *m*-chloroperoxybenzoic acid in anhydrous ethyl ether or chloroform provided the crude oxidation product in the crystalline form. In ethyl ether the substrate and the reagent were in solution and the product was insoluble, whereas in chloroform a complete solution was obtained. The pure 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-6-(methylsulfonyl)purine (15) was separated from the crude product by silica gel chromatography and characterized on the basis of UV spectra and ^{1}H NMR in which the signal at δ 3.47 for methyl protons was shifted downfield by 0.77 ppm as compared to the same signal at δ 2.70 for compound 14. This data was similar to that observed in the case of the corresponding arabinonucleoside. During the chromatographic separation of 15, a minor product, presumably 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl-6-(methylsulfinyl)purine (16), was also obtained. Compound 16 had UV absorption at wavelengths identical with that of 15 and in the ^{1}H NMR of 16 the signal at δ 3.18 for *S*-methyl protons was shifted downfield by 0.48 ppm as compared to the same signal for 14. The data suggested structure 16 for the minor product which was not further characterized. The methylsulfonyl group from 15 was conveniently replaced by the cyanide group when 15 was treated with sodium cyanide in dimethylformamide to provide the desired 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine-6-carbonitrile (17). Compound 17 was also formed when the crude oxidation product was treated with sodium cyanide in dimethylformamide. Treatment of 17 with methanolic ammonia provided the rearranged product 4-amino-8-[(2-deoxy-β-D-*erythro*-pentofuranosyl)amino]pyrimido-[5,4-*d*]pyrimidine (18). Compound 17 when reacted with

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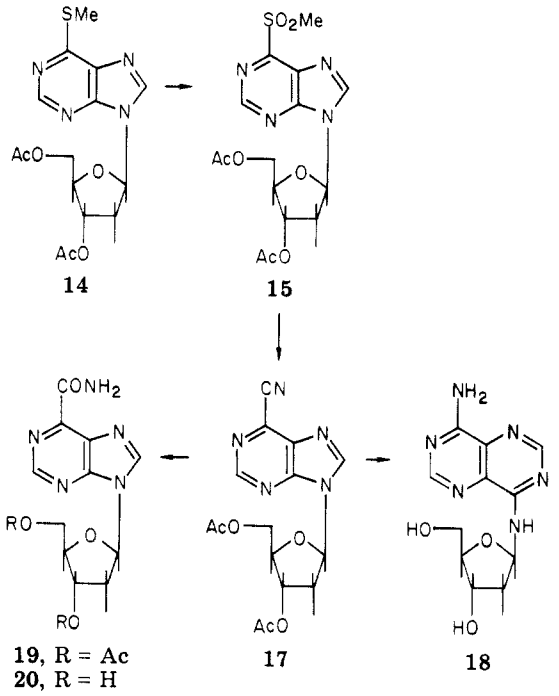
Scheme III



hydrogen peroxide in the presence of ammonium hydroxide provided the desired 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine-6-carboxamide (**19**). Compound **19** on deacetylation provided the desired 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-carboxamide (**20**) (Scheme III).

Biological Evaluation. Compounds **2** and **8** have been evaluated with respect to their ability to suppress cellular and humoral immune responses in mice. As a model for humoral immune responsiveness, mice were immunized by intraperitoneal injections of suspensions of sheep red blood cells (SRBC). Ability to respond to this antigenic stimulus was monitored 4–5 days after immunization by quantitating the amount of circulating SRBC agglutinating antibody in the sera of immunized mice, using a standard microhemagglutination technique.[18] Experimental and control compounds were administered intraperitoneally in pH 7.4 phosphate buffer for 3 consecutive days, beginning either on the day of antigen administration (day 0) or 2 days prior to antigen administration (day −2). Results of these experiments (Table I) are expressed as mean $\log_2$ of hemagglutinin titers for groups of five to seven mice. Hemagglutinin titer is defined as the reciprocal of the highest serum dilution showing positive hemagglutinating settling patterns. As noted in Table I, the riboside **2** was a potent suppressor of circulating antibody (hemagglutinin) formation at 25 (mg/kg)/day when dosing was begun on day 0 and continued for 2 days thereafter. The same number of treatments had no detectable effect when begun on day -2. These data may indicate that certain of the later steps in induction of the immune response are abrogated by the compound, but further investigation will be needed to clarify this point. In contrast to the above, the arabinoside analogue **8** showed no activity when used according to either dosing schedule at doses as high as 100 (mg/kg)/day. The same pattern of suppression with ribosides and inactivity with arabinosides was seen with 6-mercaptopurine riboside (6MPR) and 6-mercaptopurine arabinoside (*ara*-6MP). The data indicate that 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**2**) had about the same suppressive activity on a milligram per kilogram per day basis as cyclophosphamide and cytosine arabinoside (*ara*-C) but was more potent than either 6MP or 6MPR.

Table I. Effect of 4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]-pyrimidine (**2**) and 4-Amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-*d*]pyrimidine (**8**) on Induction of Humoral Immunity[a]

compd	dose, (mg/kg)/day	mean hemagglutination titers: treatment days −2, −1, and 0		mean hemagglutination titers: treatment days 0, +1, and +2	
		drug treated	control	drug treated	control
2	25	8.5	9.5	0	7.0
8	100	8.7	9.5	11.0	10.0
6-mercaptopurine	25			7.5	8.9
	50	7.5	8.1	7.2	8.9
	100	3.4	8.1	4.1	8.9
6-mercaptopurine arabinoside	50	11.3	10.3	7.6	6.5
	100	8.3	10.3	7.2	6.5
	180			8.7	9.1
cytosine arabinoside	25			3.4	7.0
cyclophosphamide	25			1.5	7.1
6-mercaptopurine riboside	50	8.5	8.1	9.1	9.0
	100	8.4	8.1	7.4	9.0
	180			6.4	9.0

[a] Other compounds listed here were used as standards.

Table II. Effect of 4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]-pyrimidine (**2**) and 4-Amino-8-(β-arabinofuranosylamino)-pyrimido[5,4-*d*]pyrimidine (**8**) on Induction of Cellular Immunity

no.	dose, (mg/kg)/day	mean survival time ± SE: drug treated	mean survival time ± SE: control
2	15	21 ± 2.6	12 ± 0.2
8	50	14 ± 1.47	12 ± 0.21
	75	13 ± 0.84	12 ± 0.21
	100	16 ± 1.40	12 ± 0.21

The effect of the compounds on induction of cellular immunity was studied by attempting prolongation of the life (survival time) of full-thickness skin homografts transferred between AKR/J donor and AKD_2F_1/J recipient mice as described by Billingham.[19] Daily intraperitoneal injection of drugs in pH 7.4 phosphate buffer was begun on the day of the graft transfer and continued until rejection. Rejection was defined as that day when each graft had less than 10% viability, as determined by close visual and physical examination. Results are expressed as mean survival times (days) plus or minus standard errors (SE) for groups of four to six mice and are given in Table II. Data from Table II show that **2** extended mean survival times from 12 to 21 days when used at 15 (mg/kg)/day. The arabinoside analogue **8** also showed extension, although this activity was not as profound as with the riboside **2**.

The data indicate that the subject compounds **2** and **8** interfere with induction of cellular and/or humoral immune responses. While the riboside analogue **2** seems to have generalized activity, the arabinoside **8** is specific to the cellular response.

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The ribonucleoside analogue **2** also exhibited significant activity (T/C = 131) against L-1210 leukemia in mice[12] when treated at the doses of 9.4 (mg/kg)/day for 5 days. The arabinonucleoside analogues **8–10** were devoid of any significant antiviral activity when tested against RNA and DNA viruses in cell culture at doses as high as 1000 μg/mL. This inactivity was surprising, especially in view of the potent antiviral activity exhibited by 9-β-D-ribofuranosylpurine-6-carboxamide.[9]

Experimental Section

The physical properties were determined with the following instruments: melting point, Thomas-Hoover apparatus (uncorrected); IR, Beckman Acculab 2 (KBr); UV spectra, Cary 15 UV spectrophotometer (pH 1, 7, and 11); and ^{1}H NMR, Varian EM-390 (Me_4Si). The presence of exchangeable protons was confirmed by ^{1}H NMR spectroscopy in absolute Me_2SO-d_6 by exchange with D_2O followed by reintegration. Elemental analyses were performed by Galbraith laboratories, Inc., Knoxville, Tenn. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical values. Baker analyzed silica gel powder (60–200 mesh) was used for column chromatography and E. Merck silica gel 60 F-254, precoated TLC sheets (0.20 mm) were used to check the purity of the compounds.

6-(Methylthio)-9-β-D-arabinofuranosylpurine (4). A stirred solution of 9-β-D-arabinofuranosylpurine-6-thione[13] (**3**; 56.8 g, 200.0 mmol) in 1.0 N NaOH (200 mL) was cooled in ice (0–5 °C). To this was added a solution of methyl iodide (42.3 g, 300 mmol) and methanol (200 mL) over a period of 15 min. Immediately after complete addition, the crystalline product started to separate, which formed a cake. The cake was broken by adding methanol (200 mL), and the reaction mixture was allowed to stand at room temperature for 15 min. The white crystalline 6-(methylthio)-9-β-D-arabinofuranosylpurine was collected by filtration and recrystallized from methanol: yield 50 g (83%); mp 183–184.5 °C (lit.[13] mp 99–101 °C). The product had identical mp (183–184 °C), UV, ^{1}H NMR, and TLC mobility when compared with those of an authentic sample synthesized by literature[13] procedure and crystallized from methanol: UV λ_{max} (pH 1) 293 nm (ε 17 700); UV λ_{max} (pH 7 or 11) 288 nm (ε 19 300); ^{1}H NMR (Me_2SO-d_6) δ 8.52 (s, 1, C_2 H), 8.72 (s, 1, C_8 H), 2.7 (s, 3, SCH_3), 6.38 (d, 1, J = 4.5 Hz, $C_{1'}$ H), and other sugar protons.

6-(Methylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (5). A suspension of 6-(methylthio)-9-β-D-arabinofuranosylpurine (**4**; 45.0 g, 151.0 mmol) and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (1.1 g) in acetic acid anhydride (225 mL) was stirred at room temperature for 1.25 h. A clear solution was obtained, which was evaporated in vacuo. The residue was dissolved in $CHCl_3$ (500 mL) and washed successively with water (200 mL), aqueous sodium bicarbonate (saturated solution, 200 mL), and water (200 mL). The $CHCl_3$ portion was dried ($MgSO_4$) and evaporated in vacuo to provide chromatographically pure **5** as a colorless syrup: yield 57.0 g (88.9%). A small portion (200 mg) of this syrup was applied to a preparative silica gel plate. It was developed in $CHCl_3$–CH_3COCH_3 (9:1). The main band was cut and eluted chromatographically with ethyl acetate to provide the analytical sample (syrup): UV λ_{max} (pH 1) 293 nm (ε 17 800); UV λ_{max} (pH 7) 288 nm (ε 19 100); UV λ_{max} (pH 11) 288 nm (ε 13 000); ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1, C_2 H), 8.75 (s, 1, C_8 H), 2.83 (s, 3, SCH_3), 6.67 (d, 1, J = 4.5 Hz, $C_{1'}$ H), and other sugar protons. Anal. ($C_{17}H_{20}N_4SO_7$) C, H, N, S.

6-(Methylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (6). To a stirred and cold (0–5 °C) solution of 6-(methylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (**5**; 52.0 g, 122.5 mmol) in acetic acid (650.0 mL) and water (325.0 mL) was added a suspension of $KMnO_4$ (50.0 g, 316.4 mmol) in water (325 mL) over a period of 30 min. The reaction mixture was stirred at 0–5 °C (ice bath) for an additional 1 h and then 30% hydrogen peroxide (ca. 30.7 mL) was added until the solution became colorless. The organic layer was dried ($MgSO_4$) and evaporated to provide pure **6** as a syrup: yield 45.0 g (80.5%). A sample of analytical purity was obtained by preparative thin-layer chromatography in a similar way as described for **5**: UV λ_{max} (pH 1) 275 nm (ε 5500); UV λ_{max} (pH 7) 276 nm (ε 6700); ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1, C_8 H), 8.62 (s, 1, C_2 H), 3.58 (s, 3, SO_2CH_3), 6.80 (d, 1, J = 4.5 Hz, $C_{1'}$H), and other sugar protons. Anal. ($C_{17}H_{20}N_4SO_9$) C, H, N, S.

9-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile (7). To a solution of 6-(methylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine (**6**; 34.0 g, 74.5 mmol) in anhydrous DMF (250 mL) was added granular NaCN (6.36 g, 129.8 mmol). The reaction mixture was stirred at room temperature for 2.5 h to provide a dark-colored solution. The solvent was evaporated in vacuo to leave a residue, which was partitioned with $CHCl_3$ (800 mL) and water (500 mL). The $CHCl_3$ portion was vigorously washed with water (5 × 400 mL), aqueous $NaHCO_3$ (10% solution, 2 × 300 mL), and water (2 × 500 mL), respectively. The chloroform portion was dried ($MgSO_4$) and evaporated in vacuo. The pure product was separated from colored impurities by passing the crude compound through a column packed with silica gel slurry (5 × 55 cm) in $CHCl_3$. The compound was eluted with 5% acetone in $CHCl_3$. The pure fractions were collected and evaporated in vacuo to provide the desired product as a foam: yield 15.0 g (50%); UV λ_{max} (pH 1) 286 nm (ε 11 000); UV λ_{max} (pH 7) 286 nm (ε 9900); UV λ_{max} (pH 11) 266 nm (ε 6700), 324 (5000); ^{1}H NMR ($CDCl_3$) δ 9.1 (s, 1, C_8 H), 8.54 (s, 1, C_2 H), 6.76 (d, 1, J = 4.5 Hz, $C_{1'}$ H), and other sugar protons. Anal. ($C_{17}H_{17}N_5O_7 \cdot 0.5H_2O$) C, H, N.

4-Amino-8-(β-D-arabinofuranosylamino)pyrimido[5,4-*d*]pyrimidine (8). A solution of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile hemihydrate (**7**; 1.0 g, 2.42 mmol) in MeOH–NH_3 (saturated at 0 °C, 50 mL) was stirred in a pressure bottle at room temperature for 2 days. The solvent was evaporated in vacuo and the residue was crystallized from methanol to provide **8**: yield 450 mg (60%); mp 192–193 °C; UV λ_{max} (pH 1) 288 nm (ε 9400), 323 (7300), 338 (5500); UV λ_{max} (pH 7 or 11) 290 nm (ε 13 000), 301 (11 400), 318 (10 600), 333 (7800); ^{1}H NMR (Me_2SO-d_6) δ 8.54 and 8.4 (2 s, 1 and 1, C_2 H and C_6 H), 8.0 (br s, 2, NH_2). Anal. ($C_{11}H_{14}O_4N_6 \cdot H_2O$) C, H, N.

9-β-D-Arabinofuranosylpurine-6-carboxamide (9). To a cold solution of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-purine-6-carbonitrile hemihydrate (**7**; 7.0 g, 16.99 mmol) in methanol (100 mL) and water (100 mL) was added hydrogen peroxide (30% solution, 5.0 mL), and the solution was adjusted to pH 8.5–9 with concentrated NH_4OH. The solution was stirred at room temperature for 5 h and then allowed to stand overnight at 0–5 °C. The solvent was evaporated in vacuo and the residue passed through a column (4 × 45 cm) packed with silica gel in ethyl acetate. The column was eluted with ethyl acetate–1-propanol–water (4:1:2, top layer). The fractions containing the pure product gave, after evaporation, a residue which was recrystallized from methanol–ethyl acetate to provide **9** as fine needles: yield 2.5 g (50%); mp 203–204 °C; UV λ_{max} (pH 1) 276 nm (ε 7800); UV λ_{max} (pH 7) 282 nm (ε 7800); UV λ_{max} (pH 11) 282 nm (ε 7500); IR (KBr) 1685 cm^{-1} ($CONH_2$); ^{1}H NMR (Me_2SO-d_6) δ 9.04 (s, 1, C_8 H), 8.83 (s, 1, C_2 H), 8.38 and 8.05 (s, pair, 2, $CONH_2$), 6.5 (d, 1, J = 4.5 Hz, $C_{1'}$ H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_5$) C, H, N.

9-β-D-Arabinofuranosylpurine-6-thiocarboxamide (10). A solution of 9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine-6-carbonitrile hemihydrate (**7**; 1.0 g, 2.42 mmol) in methanol (10 mL) was added to a solution methoxide (250 mg) in methanol (10 mL) saturated with hydrogen sulfide at 0 °C. The resulting solution was further saturated with hydrogen sulfide at 0 °C and stirred at room temperature in a pressure bottle for 18 h. The solvent was evaporated in vacuo, and the residue was dried and extracted with boiling methanol (30 mL). The methanol portion was evaporated, and the residual product was crystallized from methanol to provide **10** (300 mg, 40%): mp 194–195 °C dec; UV λ_{max} (pH 1) 271 nm (ε 8600); UV λ_{max} (pH 7) 280 nm (ε 10 000); UV λ_{max} (pH 11) 271 nm (ε 7800); ^{1}H NMR (Me_2SO-d_6) δ 10.5 and 10.1 (br s, pair, 2, $CSNH_2$), 9.0 (s, 1, C_8 H), 8.8 (s, 1, C_2 H), 6.5 (d, 1, J = 4.5 Hz, $C_{1'}$ H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_4S$) C, H, N, S.

6-(Methylthio)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine (13). A solution of methyl iodide (8.6 mL) in methanol (43.0 mL) was added dropwise (15 min) to a cold (ice bath) solution of 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine-6-thione (**12**; 5.8 g, 21.6 mmol) in sodium hydroxide (0.25 N, 86.6 mL). The solution

was stirred at 0–5 °C for 2 h, and the solvent was evaporated in vacuo. The residue (syrup) on addition of methanol provided the crystalline compound **13**, which was recrystallized from methanol–ethyl acetate: yield 4.5 g (75%); mp 156–157 °C (lit.[15] 157–158 °C).

6-(Methylthio)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine (14). To an ice-cold solution of anhydrous pyridine (15 mL) and acetic anhydride (10 mL) was added 6-(methylthio)-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**13**; 1.0 g, 3.54 mmol). The suspension became clear within 15 min of stirring. The clear solution was stirred at 0 °C for additional 1.5 h. The solvent was concentrated under vacuum to a mobile syrup. Ice-cold water (5 mL × 4) was added and evaporated under vacuum. A white crystalline product was obtained, which was triturated with cold water (5 mL), filtered, and dried, mp 80–81 °C. After recrystallization from ethyl ether and petroleum ether, the acetyl compound **14** was obtained as shining white needles in **92%** yield (1.2 g): mp 80–81 °C; UV λ_{max} (pH 1) 292 nm (ε 15 700); UV λ_{max} (pH 7) 288 nm (ε 19 500); UV λ_{max} (pH 11) 288 nm (ε 18 200); ^{1}H NMR ($CDCl_3$) δ 8.7 and 8.15 (2 s, 1 and 1, C_8 H and C_2 H), 6.48 (t, 1, $J_{H_{1'}-H_{2'}H_{2''}}$ = 6.8 Hz, $C_{1'}$ H), 2.70 (s, 3, SCH_3). Anal. ($C_{15}H_{18}N_4O_5S$) C, H, N, S.

9-(2-Deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-6-(methylsulfonyl)purine (15). To a cold solution of 6-(methylthio)-9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)purine (**14**; 1.01 g, 3.0 mmol) in ethyl ether (75 mL) was added *m*-chloroperoxybenzoic acid[20] (80–90%, 1.5 g), and the mixture was stirred with exclusion of moisture at 0 °C (ice bath) for 5 h. A crystalline product was obtained, which was collected by filtration and dried (1.1 g), mp 99–102 °C. The crystalline product was found to be the mixture of **15** (major product) and a minor product, probably the corresponding methyl sulfoxide **16**. The mixture, although quite suitable for further reaction for the synthesis of nitrile derivative **17** as described below, was separated by column chromatography. A 400-mg sample of the mixture was passed through a column packed with silica gel in chloroform. It was eluted with chloroform–acetone (9:1). The fractions containing the fast-moving product were collected and concentrated in vacuo. Addition of ethyl ether to the concentrate provided **15** in the crystalline form: yield 300 mg; mp 129–130 °C; UV λ_{max} (MeOH) 275 nm (ε 9500); ^{1}H NMR ($CDCl_3$) δ 9.14 and 8.59 (2 s, 1 and 1, C_8 H and C_2 H), 6.5 (t, 1, $J_{H_{1'}-H_{2'}H_{2''}}$ = 7 Hz, $C_{1'}$ H), 3.47 (s, 3, SO_2CH_3), 2.08 and 2.0 (2 s, 3 and 3, $C_{3'}$ $COCH_3$ and $C_{5'}$ $COCH_3$), and other sugar protons. Anal. ($C_{15}H_{18}N_4O_7S$) C, H, N, S.

The fractions containing the slow-moving product were collected and concentrated in vacuo. Addition of ethyl ether to the concentrate provided 10 mg of a product, probably 9-(2-deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-6-(methylsulfinyl)purine (**16**): UV absorption at identical wavelengths as reported for **15**; ^{1}H NMR ($CDCl_3$) δ 9.18 and 8.48 (2 s, 1 and 1, C_8 H and C_2 H), 3.18 (s, 3, $SOCH_3$), and other protons at positions similar to those for compound **15**.

9-(2-Deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-purine-6-carbonitrile (17). Method A. To a solution of **15** (797 mg, 2.0 mmol) in anhydrous dimethylformamide (6.0 mL) was added sodium cyanide (165 mg, 3.36 mmol), and the mixture was stirred at room temperature for 2.5 h with exclusion of moisture. The dark brown solution was evaporated in vacuo, and the residue was taken in chloroform (50 mL). The chloroform portion was washed vigorously with water (4 × 25 mL) and dried ($MgSO_4$). The chloroform portion was evaporated to provide crude **17**, which was purified by silica gel column chromatography (chloroform–acetone, 12:1) to yield 310 mg (45%) of pure **17** as a syrup. Anal. ($C_{15}H_{15}N_5O_5$) C, H, N.

Method B. The crystalline product (mixture of **15** and **16**) obtained during the synthesis of **15** was treated with sodium cyanide, and the product was isolated exactly as described in method A. The nitrile compound isolated in this experiment was found identical with **17** synthesized by method A.

4-Amino-8-[(2-deoxy-β-D-*erythro*-pentofuranosyl)-amino]pyrimido[5,4-*d*]pyrimidine (18). A solution of **17** (345 mg, 1.0 mmol) in methanolic ammonia (saturated at 0 °C, 15 mL) was stirred in a pressure bottle at room temperature for 24 h. The solvent was evaporated in vacuo, and the residue was crystallized from methanol to provide 166 mg (60%) of **18**: mp 165–166 °C; UV λ_{max} (pH 1) 288 nm (ε 11 400), 337 (8600); UV λ_{max} (pH 7) 290 nm (ε 16 800), 302 (14 700), 318 (13 600), 334 (10 100); UV λ_{max} (pH 11) 290 nm (ε 16 300), 302 (14 300), 318 (13 200), 334 (10 000); ^{1}H NMR (Me_2SO-d_6) δ 8.50 and 8.41 (2 s, 1 and 1, C_2 H and C_6 H), 6.4 (m, 1, $C_{1'}$ H), and other protons. Anal. ($C_{11}H_{14}N_6O_3 \cdot 0.5H_2O$) C, H, N.

9-(2-Deoxy-3,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)-purine-6-carboxamide (19). To a cold solution of nitrile compound **17** (105 mg, 0.3 mmol) in methanol (10 mL) was added a 30% solution of hydrogen peroxide (0.4 mL), and the solution was adjusted to pH 8.5–9 by adding dilute ammonium hydroxide. The solution was kept at 0–5 °C for 16 h and then for 5 h at room temperature. The activated palladium on powdered charcoal (10% catalyst, 200 mg) was added carefully, and the reaction mixture was stirred for 1 h. The catalyst was removed by filtration and the filtrate was evaporated in vacuo. The residue was passed through a column of silica gel and the column was eluted with ethyl acetate–1-propanol–water (4:1:2, top layer) to provide **19**, which was crystallized from methanol and ethyl acetate: yield 50 mg (46%); mp 174–175 °C; UV λ_{max} (pH 1) 240 nm (sh) (ε 5400), 276 (8800); UV λ_{max} (pH 7) 280 nm (ε 8400); UV λ_{max} (pH 11) 282 (ε 9500); ^{1}H NMR (Me_2SO-d_6) δ 9.06 and 8.92 (2 s, 1 and 1, C_2 H and C_8 H), 8.4 and 7.9 (2 br s, 2, $CONH_2$), 6.67 (t, 1, $J_{H_{1'}-H_{2'}H_{2''}}$ = 7.1 Hz, $C_{1'}$ H), 2.13 and 2.03 (2, s, 3 and 3, $C_{3'}$ $COCH_3$ and $C_{5'}$ $COCH_3$), and other sugar protons. Anal. ($C_{15}H_{17}N_5O_6 \cdot 0.5H_2O$) C, H, N.

9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)purine-6-carboxamide (20). A solution of **19** (75 mg, 0.2 mmol) in methanolic ammonia (5 mL, saturated at 0 °C) was stored at room temperature for 5 h. The solvent was evaporated, and the residue was crystallized from methanol and acetone to provide **20**: yield 45 mg (80%); mp 184–185 °C; UV λ_{max} (pH 1) 240 nm (sh) (ε 5000), 277 (8500); UV λ_{max} (pH 7) 280 nm (ε 8100); UV λ_{max} (pH 11) 281 nm (ε 8600); ^{1}H NMR (Me_2SO-d_6) δ 9.08 and 8.98 (2 s, 1 and 1, C_2 H and C_8 H), 8.49 and 8.11 (2 br s, 2, $CONH_2$), 6.56 (t, 1, $J_{H_{1'}-H_{2'}H_{2''}}$ = 7.0 Hz, $C_{1'}$ H), and other sugar protons. Anal. ($C_{11}H_{13}N_5O_4 \cdot 0.5H_2O$) C, H, N.

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