Synthesis and Antiviral Activity of 5-[(Cyanomethylene)oxy]-2'-deoxyuridine

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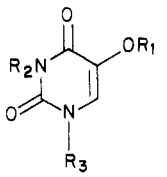
To study the influence of substitution of CN for C≡CH in the anti-herpes virus nucleoside 5-(propynyloxy)-2'-deoxyuridine (1), 5-[(cyanomethylene)oxy]-2'-deoxyuridine (2) was prepared. When the potassium salt of 5-hydroxy-2'-deoxyuridine was reacted with iodoacetonitrile in dry DMF, the bisalkylated product 3-(cyanomethyl)-5-[(cyanomethylene)oxy]-2'-deoxyuridine (3) was the major product with a lesser amount of 3-(cyanomethyl)-5-hydroxy-2'-deoxyuridine (5) and only a trace amount of the desired product (2). In contrast, when 5-hydroxy-2'-deoxyuridine was alkylated in water in the presence of 1 equiv of KOH, compound 2 was the major product. In cultures of primary rabbit kidney (PRK) cells, compound 2 showed an anti-herpes virus activity that was comparable to that of 1 and *ara*-A. Compound 2 did not inhibit incorporation of [*Me*-^{3}H]dThd or [1',2'-^{3}H]dUrd into DNA of PRK cells; however, its anti-herpes virus activity was completely prevented upon the addition of either dThd or dUrd.

Over the past several years, quite a variety of 5-substituted 2'-deoxyuridines have been found to possess potent and selective antiviral activity.[1-3] Many of these thymidine analogues are selective anti-herpes virus agents, and they may owe their selectivity to the differential inhibition of virus-coded, as opposed to host-coded, enzymes of DNA synthesis, such as thymidine kinase and DNA polymerases.[4,5] One of these recently described analogues is 5-(propynyloxy)-2'-deoxyuridine (1),[6] which is a selective anti-herpes virus agent that shows relatively little toxicity to the host cell. Recently, a sulfur analogue of 1 was described which possessed substantial antiviral activity.[3b] To further ascertain the role of steric, as opposed to electronic, factors in the antiviral activity and selectivity of thymidine analogues, we examined the effect of substitution of the cyano group for the acetylene moiety to give 5-[(cyanomethylene)oxy]-2'-deoxyuridine (2).



1, R_1 = $CH_2C{\equiv}CH$; R_2 = H; R_3 = 2-deoxy-β-D-*erythro*-pentofuranosyl
2, R_1 = $CH_2C{\equiv}N$; R_2 = H; R_3 = 2-deoxy-β-D-*erythro*-pentofuranosyl
3, R_1 = $CH_2C{\equiv}N$; R_2 = $CH_2C{\equiv}N$; R_3 = 2-deoxy-β-D-*erythro*-pentofuranosyl
4, R_1 = $CH_2C{\equiv}N$; R_2 = $CH_2C{\equiv}N$; R_3 = H
5, R_1 = H; R_2 = $CH_2C{\equiv}N$; R_3 = 2-deoxy-β-D-*erythro*-pentofuranosyl

Chemistry. The preparation of 5-[(cyanomethylene)-oxy]-2'-deoxyuridine (2) was based on the same methodology previously employed to synthesize 5-(propynyloxy)-2'-deoxyuridine (1), namely, base-catalyzed alkylation of 5-hydroxy-2'-deoxyuridine.[6] This procedure was previously developed by Otter et al.[8,9] for preparation of derivatives of 5-hydroxyuridine. Thus, when 5-hydroxy-2'-deoxyuridine was treated with 1 equiv of KOH and 2 equiv of iodoacetonitrile in DMF/H_2O (1:3), 2 was obtained as the major product (23%) with minor quantities of the dialkylated product, 3-(cyanomethyl)-5-[(cyanomethylene)oxy]-2'-deoxyuridine (3, 5%), and the N-3 alkylated product, 3-(cyanomethyl)-5-hydroxy-2'-deoxyuridine (5, <1%). Under the identical reaction conditions, chloroacetonitrile as the alkylating agent led to no reaction. Also, in the absence of base the reaction failed to proceed.

The structures assigned to compounds 2, 3, and 5 were verified by the following specific characteristics of the nucleosides: (a) In contrast to 5, compounds 2 and 3 failed to develop a yellow color with $FeCl_3$ spray, indicating that a phenolic hydroxyl was not present in the latter two compounds. (b) Compound 5, but not 2 or 3, displayed a base-induced blue shift of ~30 nm when the pH was changed from 1 to 12, characteristic of 1-substituted 5-hydroxyuracils. (c) In the ^{1}H NMR, compound 2 displayed a broad singlet at 11.6 ppm corresponding to the pyrimidine N-3 proton, but 2 had no signal corresponding to the pyrimidine 5-hydroxyl proton. Conversely, compound

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Table I. Antiviral and Antimetabolic Activities of 5-[(Cyanomethylene)oxy]-2'-deoxyuridine (2)

	IC_{50},[a] μg/mL					
compd	HSV-1	HSV-2	TK⁻ HSV-1 (B 2006)	vaccinia	[*Me*-³H]dThd incorporation	[1',2'-³H]dUrd incorporation
2	2 (2–4)	10–100 (10–150)	>400	150	400	>400
1	1 (1–1.5)	19 (7–40)	>200	20	200	100
5-I-2'-dUrd	0.1	0.2 (0.2–0.4)	200	0.4	1	0.3
5-CN-2'-dUrd					>400	8
ara-A	7 (4–10)	3 (2–4)	10	0.4	20	10

[a] Concentration required to reduce virus-induced cytopathogenicity by 50% or [*Me*-³H]dThd (or [1',2'-³H]dUrd) incorporation into host cell DNA by 50%. For herpes simplex virus, the data show the mean values obtained with three different strains (KOS, McIntyre, and F) of HSV-1 and three different strains of HSV-2 (Lyons, G, 196); the range of experimentally obtained values is shown in parentheses. The data for incorporation of dThd and dUrd are representative values obtained from at least two separate experiments.

5 showed a broad singlet at 8.98 ppm corresponding to the pyrimidine 5-hydroxyl proton, but **5** gave no evidence of any signal ascribable to an N-3 proton. Finally, compound **3**'s H^1 NMR was devoid of any signals assignable to the pyrimidine 5-hydroxyl proton or the N-3 proton. (d) Neither compound **3** nor **5** could be obtained crystalline, and satisfactory elemental analyses could not be obtained; however, in addition to other spectral data, both **3** and **5** had molecular weights consistent with the assigned structure according to chemical ionization mass spectra. Moreover, compound **3** was hydrolyzed by boiling water to the free base, 3-(cyanomethyl)-5-[(cyanomethylene)-oxy]uracil (**4**), the structure of which was established by standard means.

The infrared spectra of these analogues are notable, since the usual absorption of the cyano group at ~2230 cm^{-1} is either absent or very weak. This behavior seems to be typical for substances in which the cyano group is borne on the same carbon as some electron-withdrawing atoms.[10]

A completely different result was obtained when the alkylation was carried out with the potassium salt of 5-hydroxy-2'-deoxyuridine in dry DMF. Under these conditions, with 2 equiv of iodoacetonitrile, the dialkylated product (**3**) was obtained as the major product with smaller amounts of the N-3 alkylated product (**5**, ~5%) and only a trace of the 5-O-alkylated compound (**2**). Of the four possible alkylation sites in the pyrimidine base of 5-hydroxy-2'-deoxyuridine, reaction at N-3 as opposed to exocyclic oxygen at C-4 is to be expected, since neutral alkylating agents preferentially form covalent bonds with the more polarizable atom of ambident systems.[11] The drastic change in 5-O as opposed to N-3 alkylation when solvent is changed from DMF–water to DMF might be explained by the relative solvation of the two anion sites. The pK_a of 5-hydroxyuridine has been determined as 7.8.[12] The pK_a of 5-hydroxypyrimidine itself is 6.8,[13] and the pK_a of 4-hydroxy-5-methoxypyrimidine is 8.6.[13] To the extent that these substances may be used as models, then both sites (at N-3 and 5-O) may be partially ionized. Finally, the hypothesis was recently advanced that reaction on the exocyclic (nonring N) site of nucleosides is favored by changes in reaction solvent which increase the S_N1 character of the reaction.[14] The increased anionic solvation in the DMF–H_2O mixtures would present such conditions.

Antiviral and Antimetabolic Activities. Compound **2** was evaluated for antiviral activity in primary rabbit kidney cells infected with various laboratory strains of herpes simplex virus or vaccinia virus. The relative potency of **2** compared to 5-(propynyloxy)-2'-deoxyuridine and 5-iodo-2'-deoxyuridines and *ara*-A was determined by reduction of virus-induced cytopathogenicity. The data of Table I clearly show that 5-[(cyanomethylene)oxy]-2'-deoxyuridine (**2**) possessed the same spectrum and potency of antiviral action as did the 5-(propynyloxy) analogue (**1**). Compound **2** was an order of magnitude less potent than 5-iodo-2'-deoxyuridine. As had been noted before for compound **1**,[15] compound **2** was significantly less effective against the herpes simplex-2 strains. Compound **2**, like **1** and 5-iodo-2'-deoxyuridine, was virtually without activity against a strain of herpes simplex virus that does not induce thymidine kinase. This finding suggests that phosphorylation of **2** by the virus-induced thymidine kinase, or any other enzymatic function that may be deficient in the thymidine kinase deficient virus strain, may be necessary for expression of antiviral activity. The relative lack of activity of **2** toward vaccinia virus holds also for the propynyloxy analogue (**1**) and for a number of other 2'-deoxyuridine analogues, such as 5-propyl-2'-deoxyuridine.[15]

To gain some information on the relative toxicity of **2** toward uninfected cells and to obtain some idea where the site of antiviral action might be, we studied the ability of **2** and related analogues to inhibit [*Me*-³H]dThd and [1',2'-³H]dUrd incorporation into DNA of primary rabbit kidney (PRK) cells. The data in Table I demonstrate that **2** was inactive as an inhibitor of DNA synthesis in PRK cells by both criteria ([Me-³H]dThd and [1',2'-³H]dUrd incorporation). The propynyloxy analogue, **1**, was only slightly inhibitory, as has been noted previously.[16] 5-Iodo-2'-deoxyuridine blocked both [*Me*-³H]dThd and [1',2'-³H]dUrd incorporation, and 5-cyano-2'-deoxyuridine inhibited [1',2'-³H]dUrd but not [*Me*-³H]dThd incorporation. This behavior of the 5-cyano-2'-deoxyuridine has been described previously[16] and was suggested[16] and es-

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Table II. Prevention of Anti-herpes Activity of 5-[(Cyanomethylene)oxy]-2'-deoxyuridine (2) and Related Compounds upon Addition of dThd or dUrd

compd	IC_{50},[a] μg/mL	upon addition of[b] dThd	dUrd
2	1.5	>400	>400
1	0.7	400	40
5-F-2'-dUrd	1	>400	10
ara-A	4	1	1

[a] Concentration required to reduce cytopathogenicity of HSV-1 (KOS) by 50% (in primary rabbit kidney cells). [b] dThd or dUrd added at a final concentration of 100 μg/mL.

tablished as due to inhibition of thymidylate synthetase by the 5'-monophosphate of 5-cyano-2'-deoxyuridine.[17] The inhibitory behavior of 5-iodo-2'-deoxyuridine is probably due to a combination of factors, including its incorporation into host cell and antiviral DNA and inhibition of DNA polymerase.[18a]

Antagonism of the anti-herpes virus activity of 2 and several other related nucleosides with dThd and dUrd was also examined (Table II). The antiviral activity of 2 was completely prevented by either dThd or dUrd when these nucleosides were applied at a concentration of 100 μg/mL. The activity of the propynyloxy derivative (1) was also antagonized by both dThd and dUrd, albeit to a greater extent by dThd than by dUrd. *Ara*-A, which is not known to interfere with thymidine metabolism,[1] showed no loss of its anti-herpes virus activity upon addition of dThd or dUrd. 5-Fluoro-2'-deoxyuridine, a potent inhibitor of thymidylate synthetase, showed complete loss of activity upon addition of dThd, whereas dUrd was less efficient in this regard. These data suggest that 5-[(cyanomethylene)oxy]-2-deoxyuridine (2), unlike 5-fluoro-2-deoxyuridine, does not act as an inhibitor of thymidylate synthetase. Its mechanism of anti-herpes virus action may be at some other locus of thymidine metabolism, possibly the DNA polymerase.

The findings that compound 2 did not inhibit [*Me*-^{3}H]dThd or [1',2'-^{3}H]dUrd incorporation, or vaccinia virus replication, unless concentrations were employed that were significantly higher than those required to inhibit herpes simplex virus replication, point to the selectivity of 2 as an anti-herpes agent. This selectivity was further attested by the fact that compound 2, like 1 (ref 18b), did not inhibit mouse leukemia L1210 cell growth (or [*Me*-^{3}H]-dThd incorporation into L1210 cell DNA), even if applied at a concentration as high as 1 mg/mL.

Discussion

Replacement of the C≡CH group of the propynyloxy analogue, 1 by the C≡N group could influence the following parameters of the side chain.

(a) Steric Bulk. The sizes of the CN group and the C≡CH group are roughly comparable. For instance, the conformational free energy values (*A* values) for these groups in monosubstituted cyclohexanes are 0.17 for CN and 0.18 for C≡CH.[19]

(b) Electronic Properties. The cyano group is a powerful electron-withdrawing group with a σ_p substituent constant approaching that of NO_2.[20] Because of its distance from N-3 H, it is unlikely that the cyano group could cause a significant change of N-3 H pK_a as compared to 1.

(c) Hydrophobicity. The increased hydrogen-bonding ability and increased dipole moment of the CN group contribute to its enhanced hydrophilic nature compared to C≡CH. The hydrophobic (π) constant for the CN moiety is −0.57, while for C≡CH it is +0.4.[21] It appears, therefore, that a dramatic variation in the lipophilicity of R of the OCH_2R side chain at the 5 position of the pyrimidine ring of 2'-deoxyuridine has little, if any, effect on the anti-herpes activity of such nucleosides.

Experimental Section

5-Hydroxy-2'-deoxyuridine was prepared as described elsewhere.[6] 5-Iodo-2'-deoxyuridine was from Serva Feinbiochimica (Heidelberg, West Germany), 5-fluoro-2'-deoxyuridine was from Aldrich Chemical Co. (Milwaukee, WI), and adenosine arabinoside (*ara*-A) was from Parke Davis and Co., (Ann Arbor, MI). 5-Cyano-2'-deoxyuridine and 5-(propynyloxy)-2'-deoxyuridine (1) were prepared as previously described.[6,22] Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were obtained on a Hitachi EPS-3T spectrometer, IR spectra were determined with a Jasco DF-701G instrument, and chemical ionization mass spectra were recorded on a Finnigan 1015D gas chromatograph/mass spectrometer using NH_3. Me_4Si was used as the standard for ^{1}H NMR spectra run in Me_2SO-d_6. DSS was used as the standard for spectra determined in D_2O. ^{1}H NMR spectra were determined with a Hitachi R-20A (60-MHz) instrument, chemical shifts are reported in parts per million (δ), and signals are described as a s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).

Procedures for the determination of antiviral activity and antimetabolic activity have been described elsewhere.[17] Primary rabbit kidney cells were used throughout this study.

For the antiviral experiments, confluent primary rabbit kidney cell cultures in (Falcon) microtrays were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ being the virus dose infective for 50% of the cell cultures) and, immediately after virus adsorption, exposed to varying concentrations of the test compound. Where the antiviral activity of the compounds was measured in the presence of dThd or dUrd, the latter nucleosides were added together with the test compounds. Both dThd and dUrd were used at a final concentration of 100 μg/mL, that is, at the maximum concentration at which dThd and dUrd were themselves not inhibitory to virus replication. Viral cytopathogenicity was recorded as soon as it reached 100% cell destruction in the control (virus-infected, untreated) cell cultures.

5-[(Cyanomethylene)oxy]-2'-deoxyuridine ([[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2,4-dioxopyrimidin-5-yl]oxy]-acetonitrile; 2). 5-Hydroxy-2'-deoxyuridine (1.221 g, 5 mmol) was dissolved in 7 mL of 0.7 N KOH solution (5 mmol, 1 equiv). DMF (2.5 mL) was added, and then iodoacetonitrile (Aldrich Chemical Co., 95% purity, d = 2.31 g/mL, 762 μL or 10 mmol) was added dropwise with stirring. After a few minutes, an homogeneous solution developed. After reaction at 37 °C for 1 day, the progress of the alkylation was checked by silica gel TLC ($CHCl_3$–MeOH, 4:1, v/v). Since the reaction was not complete, an additional quantity (5 mmol) of iodoacetonitrile was added

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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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