Synthesis and Antiviral Activity of Some 7-[(2-Hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine Analogues of Sangivamycin and Toyocamycin

Naveen K. Saxena, Lisa A. Coleman, John C. Drach, and Leroy B. Townsend*
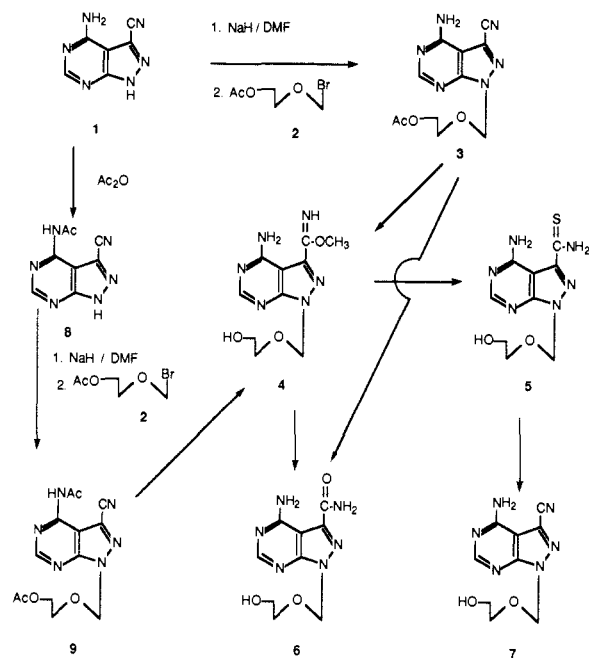
Department of Medicinal Chemistry, College of Pharmacy, Department of Chemistry, College of Literature, Sciences and Arts, and the Department of Biologic and Materials Sciences, School of Dentistry, The University of Michigan, Ann Arbor, Michigan 48109-1065. Received February 23, 1989

The sodium salt of 4-amino-3-cyanopyrazolo[3,4-d]pyrimidine (1) was condensed with (2-acetoxyethoxy)methyl bromide (2) to provide the corresponding protected acyclic nucleoside, 4-amino-3-cyano-1-[(2-acetoxyethoxy)methyl]-pyrazolo[3,4-d]pyrimidine (3). Treatment of 3 with sodium methoxide in methanol provided a good yield of methyl 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-formimidate (4). Treatment of the imidate (4) with sodium hydrogen sulfide gave the thiocarboxamide derivative 5. Aqueous base transformed 4 into 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-carboxamide (6) in good yield. Treatment of 5 with mercuric chloride furnished the toyocamycin analogue 7. Evaluation of compounds 1, 3–7 revealed that only the heterocycle (1) and the thiocarboxamide acyclic nucleoside (5) were active. Compound 5 was the more potent with activity against human cytomegalovirus and herpes simplex virus type 1.

Acyclonucleosides are an important class of compounds having potent and selective antiviral activity.[1] Among the acyclic nucleosides, acyclovir[2] and ganciclovir[3-5] (DHPG) have been key compounds in the search for better drugs. The specificity of these compounds against herpes simplex virus types 1 and 2 (HSV-1, HSV-2) is a consequence of differential substrate specificity between cellular and viral kinases and of biochemical selectivity at the level of DNA polymerases.[6] Both of these compounds also are active against human cytomegalovirus (HCMV) and have been used clinically.[7,8] Each compound, however, suffers from a different problem regarding clinical utility against HCMV. Acyclovir, although not toxic, is only a weak inhibitor of the virus[7] whereas ganciclovir is a more potent inhibitor but is not as free from toxicity.[8]

As a part of our research on antiviral drugs, we have been exploring pyrrolo[2,3-d]pyrimidine nucleosides as potential selective inhibitors of HCMV.[9-13] We have found

Scheme I

that arabinosyl and deoxyribosyl analogues of sangivamycin and toyocamycin[9] as well as acyclic analogues of the 5-halo tubercidins[11] possessed desirable characteristics as inhibitors of HCMV. Because of the potent and partially selective inhibition of HCMV replication by certain of these compounds, we have extended our work to include a different heterocycle. We would now like to report on the synthesis and antiviral activity of some acyclic 3,4-disubstituted pyrazolo[3,4-d]pyrimidine analogues of toyocamycin and sangivamycin.

Results and Discussion

Chemistry. Successful alkylations and glycosylations of pyrrolo[2,3-d]pyrimidines have been reported using various conditions, e.g., DMF/NaH,[14] phase-transfer conditions,[15] aqueous sodium hydroxide,[16] trimethyl-
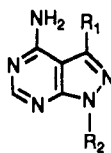
(1) MacCoss, M.; Tolman, R. L.; Ashton, W. T.; Wagner, A. F.; Hannah, J.; Field, A. K.; Karkas, J. D.; Germershausen, J. I. *Chem. Scr.* **1986**, *26*, 113.
(2) Schaeffer, H. J.; Beauchamp, L.; deMiranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature* **1978**, *272*, 583.
(3) Smith, K. O.; Galloway, K. S.; Kennell, W. L.; Oglivie, K. K.; Radatus, B. K. *Antimicrob. Agents Chemother.* **1982**, *22*, 55.
(4) Schaffer, H. J. In *Nucleosides, Nucleotides, and Their Biological Applications*; Rideout, J. L., Henry, D. W., Beacham, L. M., Eds.; Academic Press: New York, 1983; p 1.
(5) Field, A. K.; Davies, M. E.; Dewitt, C.; Berry, H. C.; Lions, R.; Germershausen, J. I.; Karkas, J. D.; Ashton, W. T.; Johnson, D. B. R.; Tolman, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4139.
(6) (a) Drach, J. C. In *Targets for the Design of Antiviral Agents*; DeClercq, E., Walker, R. T., Eds.; Plenum Press: New York, 1983; p 231. (b) Mathews, T.; Boehme, R. *Rev. Infect. Dis.* **1988**, *10*, S490.
(7) Meyers, J. D.; Reed, E. C.; Shepp, D. H.; Thornquist, M.; Dandliker, P. S.; Vicary, C. A.; Florunoy, N.; Kirk, L. E.; Kersey, J. H.; Thomas, E. D.; Balfour, H. H., Jr. *N. Engl. J. Med.* **1988**, *318*, 70.
(8) Collaborative DHPG Treatment Study Group, *N. Engl. J. Med.* **1986**, *314*, 801.
(9) Turk, S. R.; Shipman, C., Jr.; Nassiri, R.; Genzlinger, G.; Krawczyk, S. H.; Townsend, L. B.; Drach, J. C. *Antimicrob. Agents Chemother.* **1987**, *31*, 544.
(10) Saxena, N. K.; Hagenow, B. M.; Genzlinger, G.; Turk, S. R.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1988**, *31*, 1501.
(11) Pudlo, J.; Saxena, N. K.; Nassiri, M. R.; Turk, S. R.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1988**, *31*, 2086.
(12) Gupta, P. K.; Daunert, S.; Nassiri, M. R.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1989**, *32*, 402.
(13) Gupta, P. K.; Nassiri, M. R.; Coleman, L. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1989**, *32*, 1420.
(14) (a) Schram, K. H.; Townsend, L. B. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1253. (b) Hammer, R. H. *J. Pharm. Sci.* **1977**, *55*, 1096.
(15) Seela, F.; Winkeler, M. D. *Carbohydr. Res.* **1983**, *118*, 29 and references cited therein.

Table I. Antiviral Activity and Cytotoxicity of Acyclic Pyrazolo[3,4-d]pyrimidine Nucleosides

compound			50% inhibitory concentration, μM				
	substituent		plaque reduction assay		cytotoxicity		
no.	R_1	R_2	HCMV	HSV-1	HFF^a	BSC^a	KB^b
1	CN	H	68	16	>100	>100	
3	CN	AEM^c	>100^d	>100	>100	>100	54
4	HN=COCH$_3$	HEM	>100	>100	>100	>100	
5	CSNH$_2$	HEM	2.5^e	9	55^e	100	24^f
6	CONH$_2$	HEM	>100^e	>100	>100^e	>100	>100
7	CN	HEM	>100	>100	>100	>100	
acyclovir			63^e	3.9^g	>100^e	>100	>100
ganciclovir (DHPG)			8.7^h	2.3^e	>100^h	>100	1000

^{a} Visual cytotoxicity scored on uninfected HFF or BSC-1 cells at time of HCMV or HSV–1 plaque enumeration. ^{b} Average percent inhibition of DNA, RNA, and protein synthesis determined in KB cells as described in the text. ^{c} Abbreviations used: AEM, 7-(2-acetoxyethoxy)methyl; HEM, 7-(2-hydroxyethoxy)methyl. ^{d} Indicates IC$_{50}$ concentration not reached at noted (highest) concentration. ^{e} Average of two to four experiments. ^{f} Effect on RNA and protein synthesis only. The IC$_{50}$ for effect of [^{3}H]dThd incorporation was 4 μM. ^{g} Average of 11 experiments. ^{h} Average of 54 experiments.

silylation,[16] acid catalyzed fusion procedures.[17] We elected to use the procedure recently reported for the stereospecific synthesis of various 2′-deoxynucleosides of some pyrrolo-[2,3-d]pyrimidines and pyrazolo[3,4-d]pyrimidines.[18,19]

The sodium salt of 4-amino-3-cyanopyrazolo[3,4-d]pyrimidine (1) was generated by the treatment of 1 with sodium hydride in DMF (Scheme I). The sodium salt of 1 was then condensed with (2-acetoxyethoxy)methyl bromide[20] (2) in DMF to furnish the blocked acyclic nucleoside 4-amino-3-cyano-1-[(2-acetoxyethoxy)methyl]-pyrazolo[3,4-d]pyrimidine (3) in about 40% yield, after purification on a silica gel column. The site of alkylation for compound 3 was established to be at N-1 by a direct comparison of the UV spectrum of 3 (λ_{max} (MeOH) at 236.3 and 285.0 nm) with the UV spectrum of the corresponding N-1 ribonucleoside.[19] The N-2 ribonucleoside isomer was reported[18] to occur during ribosylation of the same heterocycle and produced a bathochromic shift of more than 20 nm in the UV spectrum. The presumed N-2 alkylated isomer of 3 was detected but was not isolated. Treatment of compound 3 with NaOMe/MeOH at room temperature and subsequent neutralization with Dowex-50 (H$^+$, washed with methanol) gave methyl 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-formimidate (4) in good yield. A singlet at δ 3.89 (three protons) in the ^{1}H NMR spectrum of 4 and the absence of a peak in the 2220 cm^{-1} region of the IR spectrum confirmed that a conversion of the nitrile group to the methyl formimidate ester group had occurred. An alternate synthesis of 4 involved the condensation of 2 with a pyrazolo[3,4-d]pyrimidine that was more electron deficient in the pyrimidine moiety. Acetylation of the heterocycle 1 with acetic anhydride yielded the acetamido derivative 8. The sodium salt of 8 was then treated with (2-acetoxyethoxy)methyl bromide (2), using the same reaction conditions as those described

for the synthesis of 3, to afford compound 9 in good yield. Because of the electron-withdrawing nature of the acetamido group, the acyclic moiety is much more likely to reside at N-1 or N-2 rather than at N-5 or N-7. Moreover, the treatment of 9 with NaOMe/MeOH gave a compound that was found to be essentially identical (TLC, UV, ^{1}H NMR) to the compound (4) obtained directly from 3. This provided additional support for the structural assignment of 4. For the synthesis of the azasangivamycin analogue, a catalytic amount of sodium hydroxide in water effected a smooth conversion of the imidate 4 into 4-amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-carboxamide (6). An alternate synthesis of 6 involved the treatment of 3 with hydrogen peroxide under basic conditions, which was then followed by a change to acidic conditions to provide 4-amino-1-[(2-hydroxyethoxy)-methyl]pyrazolo[3,4-d]pyrimidine-3-carboxamide (6). In both of the above methods, the yield of 6 was between 38 and 40%. Since the imidate (4) can be considered an activated form of 4-amino-3-cyano-1-[(2-hydroxyethoxy)-methyl]pyrazolo[3,4-d]pyrimidine (7) (vide infra), this prompted us to use 4 as an intermediate for the synthesis of the other desired 4-amino-3-substituted-pyrazolo[3,4-d]pyrimidine acyclic compounds. Sodium hydrogen sulfide in methanol reacted with 4 very rapidly at room temperature. The reaction was totally complete (no trace of starting material as monitored by TLC) in ca. 20 min. The thiocarboxamide analogue (5) was obtained as a light yellow crystalline compound after purification by silica gel column chromatography, followed by crystallization from ethanol. Treatment of 5 with mercuric chloride and triethylamine in dimethylformamide furnished 4-amino-3-cyano-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine (7) ("6-azatoyocamycin analogue").

Biological Evaluation. Compounds were evaluated for activity against HCMV and HSV-1 by means of plaque-reduction assays. Cytotoxicity of each compound was examined in normal human diploid cells (human foreskin fibroblasts, HFF cells), monkey kidney cells (BSC-1 cells), and in a few cases a human neoplastic cell line (KB cells). Table I illustrates that the aglycon (compound 1) possessed weak activity against HCMV and HSV-1. Compound 1 may be active per se or active as a consequence of low-level conversion to the ribosyl nucleotide by cellular salvage enzymes. In a separate experiment we found the ribosyl

(16) Tolman, R. L.; Tolman, G. L.; Robins, R. K.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 799.

(17) Tolman, R. L.; Robins, R. K.; Townsend, L. B. *J. Am. Chem. Soc.* **1968**, *90*, 524.

(18) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379 and references cited therein.

(19) Earl, R. A.; Townsend, L. B. *J. Heterocycl. Chem.* **1974**, *11*, 1033.

(20) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.

nucleoside was active against HCMV and somewhat toxic to HFF cells (IC_{50} = 2.6 and 10 μM, respectively).

All the target nucleosides were inactive except for the thiocarboxamide (compound **5**). This compound was more active than acyclovir and ganciclovir against HCMV in plaque-reduction assays; it also was active against HSV-1. In more detailed studies using yield reduction assays, compound **5** produced 10^4 to 10^5 reductions in HCMV titer at 100 μM; the IC_{90} was 28 μM. This is more potent than the activity of acyclovir against HCMV (10^2 reduction at 100 μM) but less potent than ganciclovir (10^5 reduction at 32 μM). Unlike ganciclovir, however, the activity against HCMV was not completely separated from cytotoxicity. Visual effects on stationary cells (HFF) were noted at 55 μM and effects on growing cells were observed at concentrations as low as 4 μM (Table I and footnote *f*).

The inactivity and low cytotoxicity of the sangivamycin and toyocamycin analogues (compounds **6** and **7**, respectively) is surprising in view of the activity against HCMV of the arabinosyl and deoxyribosyl analogues of sangivamycin and toyocamycin[9] and the cytotoxicity of the parent ribosyl nucleosides.[9,12,21] The inactivity of sangivamycin and toyocamycin analogues as well as the activity of thiocarboxamide analogues also was seen in the acyclic pyrrolo[2,3-*d*]pyrimidine series.[12,13] With either 7-[(2-hydroxyethoxy)methyl]-[13] or 7-[(1,3-dihydroxypropoxy)methyl]pyrrolopyrimidines,[12] the sangivamycin and toyocamycin analogues were weakly active or inactive whereas the thiocarboxamides were active.

Experimental Section

General Procedure. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (¹H NMR) spectra were determined at 270 MHz with an IBM WP 270 SY. The chemical shift values are expressed in δ values (parts per million) relative to the standard chemical shift of the solvent (DMSO-d_6). Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer and the infrared spectra were measured on a Perkin-Elmer 281 spectrophotometer. Elemental analysis were performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (Merck reagents). E. Merck silica gel (230–400 mesh) was used for flash column chromatography. Detection of components on TLC was made by UV light (254 nm). Rotary evaporations were carried out under reduced pressure with the bath temperature below 35 °C unless specified otherwise.

4-Amino-3-cyano-1-[(2-acetoxyethoxy)methyl]pyrazolo-[3,4-*d*]pyrimidine (3). Sodium hydride (0.45 g, 60% in mineral oil) was added to a solution of 4-amino-3-cyanopyrazolo[3,4-*d*]-pyrimidine (1, 3.0 g) in dry DMF (110 mL) at 78 °C in small portions under a nitrogen atmosphere. When all hydrogen evolution had ceased, (2-acetoxyethoxy)methyl bromide (2, 4.8 g) was added dropwise to the reaction mixture, with stirring at 78 °C over a period of 4 h. The reaction mixture was allowed to cool and the solvent was then concentrated in vacuo to about 15 mL. Water (300 mL) was then added and the mixture was immediately extracted with ethyl acetate (2 × 100 mL). The organic layer was separated, washed successively with a saturated solution of $NaHCO_3$ (2 × 100 mL) and water (3 × 50 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a semisolid mass, which was then rotary evaporated onto 1 g of silica gel and applied to the top of a column (1.8 × 25 cm) wet packed with 30 g of silica gel with chloroform as an eluent. Elution of the column with methanol in chloroform (5:95, v:v) yielded a colorless crystalline compound. This solid was recrystallized from ethanol to afford 2.1 g (41%) (colorless needles) of **3**: mp 184–185 °C; ¹H NMR (DMSO-d_6) δ 8.36 (s, 1, C6-H), 7.99 (br s, 2, exchangeable with D_2O, NH_2), 5.75 (s, 2, N1-CH_2), 4.06 (m, 2, CH_2), 3.75 (m, 2, CH_2), 1.93 (s, 3, OAc); IR (KBr) 3420,

(21) Ritch, P. D. *Biochem. Pharmacol.* **1982**, *31*, 2686.

2220 (CN), 1730 (OAc) cm⁻¹; UV $λ_{max}$ nm (ε × 10⁻⁴) pH 7, 233 (0.27) 285 (0.3), pH 1, 232 (0.4) 270 (0.34); pH 11, 235 (0.5) 284 (0.35). Anal. ($C_{11}H_{12}N_6O_3$) C, H, N.

Methyl 4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo-[3,4-*d*]pyrimidine-3-formimidate (4). 4-Amino-3-cyano-1-[(2-acetoxyethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine (3, 1.0 g) was added to a solution of sodium methoxide in dry methanol [prepared from NaOMe (0.35 g) in dry methanol (50 mL)]. The reaction mixture was stirred at room temperature for 1 h. A clear solution occurred after 40 min and the reaction was essentially complete as determined by TLC. The reaction mixture was adjusted to pH 7 by the addition of a small portion of Dowex-50 (H^+ form, prewashed with anhydrous methanol). The solution was then quickly filtered to remove the ion-exchange resin. This was followed by a concentration in vacuo to give a semisolid mass, which was rotary evaporated onto 1.0 g of silica gel and applied to the top of a column (2 × 10 cm) wet packed with 25 g of silica gel with chloroform as an eluent. Elution of the column with 7% methanol in chloroform furnished a colorless syrup, which was crystallized from ethyl acetate to afford 0.35 g (37%) of **4** as colorless needles: mp 145–146 °C; ¹H NMR (DMSO-d_6) δ 8.93 (s, 1 exchangeable with D_2O, C=NH), 8.22 (s, 1, C6-H), 8.01 (s, 2 exchangeable with D_2O, NH_2), 5.71 (s, 2, N1-CH_2), 4.65 (t, 1, *J* = 5.46 and 5.24 Hz, exchangeable with D_2O, OH), 3.83 (s, 3, OMe), 3.53 (m, 2, CH_2), 3.49 (m, 2, CH_2); UV $λ_{max}$ nm (ε × 10⁻⁴) pH 7, 238 (1.5) 283 (1.4), pH 1, 225 (1.7), 232 (1.6) 265 (1.3), pH 11, 238 (1.3) 282 (1.4). Anal. ($C_{10}H_{14}N_6O_3$) C, H, N.

4-Acetamido-3-cyano-1-[(2-acetoxyethoxy)methyl]-pyrazolo[3,4-*d*]pyrimidine (9). A suspension of dry 4-amino-3-cyanopyrazolo[3,4-*d*]pyrimidine (1, 2 g) in a mixture of acetic anhydride (10 mL) and dry pyridine (10 mL) was brought to reflux temperature and vigorously stirred for a total period of 35 min. The solution was then cooled to room temperature and concentrated in vacuo to give a dark solid. Methanol (50 mL) was added and the solution allowed to stand for 0.5 h and this was followed by another concentration in vacuo. The solid was triturated with a mixture of ice and water for 15 min, collected by filtration, and washed with cold water. The moist solid was then dissolved in 200 mL of boiling water, treated with Norit, and filtered while hot. The filtrate was cooled to 0 °C and the tan solid collected by filtration to afford 0.8 g of 4-acetamido-3-cyanopyrazolo[3,4-*d*]pyrimidine (8, 40%), mp >360 °C dec (lit.[19] mp >360 °C).

Sodium hydride (60% emulsion in mineral oil, 0.11 g) was added to a solution of **8** (0.9 g) in dry DMF (20 mL). When all hydrogen evolution had ceased, (2-acetoxyethoxy)methyl bromide (2, 0.87 g) was added at room temperature under a nitrogen atmosphere. The reaction mixture was heated at 75 °C for 2 h and the solvent was then concentrated in vacuo to about 10 mL. Water (100 mL) was added and the product was extracted with ethyl acetate (2 × 50 mL). The organic layer was separated, washed with a saturated solution of $NaHCO_3$ (2 × 50 mL), followed by water (2 × 50 mL), and then dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give a thick syrup, which was purified on a SiO_2 column packed with wet SiO_2 (20 g) in chloroform. Elution of the column (1.5 × 10 cm) with 3% methanol in chloroform yielded a colorless syrup, which was crystallized from ethanol to afford 0.2 g of **9**: mp 116–117 °C; ¹H NMR (DMSO-d_6) δ 11.5 (br s, 1, exchangeable with D_2O, NH), 8.9 (s, 1, C6-H), 5.85 (s, 2, N1-CH_2), 4.1 (m, 2, CH_2), 3.8 (m, 2, CH_2), 2.2 (s, 3, NHAc), 1.91 (s, 3, OAc). Anal. ($C_{13}H_{14}N_6O_4$) C, H, N.

Methyl 4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo-[3,4-*d*]pyrimidine-3-formimidate (4). Compound **9** (0.1 g) was added to a solution of sodium methoxide in methanol [prepared by dissolving NaOMe (0.066 g) in methanol (20 mL)]. The reaction mixture was stirred at room temperature for 1.5 h. The solution was adjusted to pH 7 by the addition of Dowex-50 (H^+, prewashed with methanol) and filtered immediately. The solvent was removed (water aspirator) at 50 °C and the solid was crystallized from ethanol to afford 0.05 g of **4**. TLC, co-TLC, and spectroscopic (¹H NMR, UV) data were in close agreement with the data for the same compound obtained from **3**.

4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-*d*]pyrimidine-3-carboxamide (6). Method 1. Hydrogen peroxide (30%, w/v, 2.22 mL) was added to a stirred solution of compound **3** (0.31 g) in *p*-dioxane (10 mL) containing water (5.3 mL) and $NaHCO_3$ (0.27 g) at 0 °C. The reaction mixture was then brought

to room temperature and stirred for 1 h. Glacial acetic acid was added dropwise to adjust the pH to 2 and the reaction mixture was allowed to stir for an additional 10 min. The reaction was monitored by TLC to ascertain when the starting material was converted into a more polar (lower R_f) compound. The solvent was removed in vacuo to give a solid mass, which was then rotary evaporated onto 1 g of silica gel and applied to the top of a column wet packed with 25 g of silica gel. Elution of the column with 7% methanol in chloroform yielded 0.11 g (39%) of 6 as needles: mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 8.88 (br s, 2, exchangeable with D_2O, $CONH_2$), 8.27 (s, 1, C6-H), 7.98 (br s, 2, exchangeable with D_2O, NH_2), 5.69 (s, 2, N1-CH_2), 4.67 (t, 1, J = 7.64, 5.37 Hz, exchangeable with D_2O, OH), 3.57 (m, 2, CH_2), 3.48 (m, 2, CH_2); IR (KBr) 3340, 1675 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-4}$) pH 7, 235 (0.067) 284 (0.65), pH 1, 224 (1.3) 267 (0.82), pH 11, 237 (0.64) 238 (0.6). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

Method 2. Compound 4 (0.17 g) was added to water (10 mL) containing a 1.2 N solution of NaOH (0.085 mL). The clear solution was stirred at room temperature for 18 h and then adjusted to pH 7 by the addition of glacial acetic acid. The solvent was removed in vacuo to give a solid mass, which was purified on a column packed with wet silica gel (30 g, mesh size 240–400) in ethyl acetate. Elution of the column with 1% methanol in ethyl acetate under pressure applied at the top of the column furnished a colorless crystalline solid, which was recrystallized from ethyl alcohol to afford 6, 0.06 g (38%), mp 221–222 °C.

4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-thiocarboxamide (5). Dry hydrogen sulfide gas was passed through a solution of sodium ethoxide in dry methanol [prepared from NaOMe (0.16 g) in dry methanol (40 mL)] for 5 min. The nucleoside 4 (0.75 g) was added to this solution in one batch and the reaction mixture was then stirred at room temperature for 20 min whereupon some yellow compound precipitated out. The reaction mixture was stirred for an additional 2 h and then allowed to stand at 5 °C for 18 h. The precipitate was collected by filtration and the filtrate was concentrated in vacuo to about 5 mL. Silica gel (1 g) was added and the solvent was removed in vacuo. The silica gel powder was placed on the top of the column prepared with wet silica gel (25 g) in chloroform. Elution of the column with 5% methanol in chloroform furnished a yellow colored compound. This compound was recrystallized from ethanol to afford 0.3 g (23%) of 5: mp 215–216 °C; ^{1}H NMR (DMSO-d_6) δ 10.27 (br s, 2, exchangeable with D_2O, C(S)NH_2), 8.23 (s, 1, C6-H), 9.1 and 8.1 (br s, 2, exchangeable with D_2O, NH_2), 5.73 (s, 2, N1-CH_2), 4.64 (t, 1, J = 5.4 and 5.5 Hz, exchangeable with D_2O, OH), 3.6 (m, 2, CH_2), 3.4 (m, 2, CH_2); UV λ_{max} nm ($\epsilon \times 10^{-4}$), pH 7, 235 (1.9) 258 (1.5), 297 (1.7), pH 1, 224 (1.7) 270 (0.6), pH 11, 237 (2.0) 280 (1.9). Anal. ($C_9H_{12}N_6O_2S$) C, H, N.

4-Amino-3-cyano-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine (7).   4-Amino-1-[(2-hydroxyethoxy)methyl]pyrazolo[3,4-d]pyrimidine-3-thiocarboxamide (5, 1 g) was dissolved in dry DMF (40 mL) and mercuric(II) chloride (0.56 g) was added to this solution followed by the addition of triethylamine (0.52 mL). The reaction mixture was stirred at room temperature for 3 h. The black precipitate was collected by filtration through Celite and the filtrate was removed in vacuo at 40 °C to give a semisolid mass. This solid mass was purified on a column packed with wet silica gel in ethyl acetate. Elution of the column (2 × 20 cm) with 2% methanol in chloroform yielded a colorless crystalline compound after evaporation of all the appropriate UV-absorbing fractions. This compound was recrystallized from methanol to afford 6 as colorless needles: mp 199–200 °C; 0.11 g (48%); ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1, C6-H), 7.97 (br s, 2, exchangeable with D_2O, NH_2), 5.73 (s, 2, N1-CH_2), 4.66 (t, 1, J = 4.5 Hz, exchangeable with D_2O, OH), 3.8–3.5 (m, 4, CH_2); IR (KBr) 3420, 2220 (CN) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-4}$),

pH 7, 234 (0.95) 285 (1.0), pH 1, 232 (1.1), 269 (0.94), pH 11, 235 (0.95), 284 (1.0). Anal. ($C_9H_{10}N_6O_2$) C, H, N.

Antiviral Evaluation. (a) Cells and Viruses. KB cells, an established human cell line derived from an epidermoid oral carcinoma, were routinely grown in minimal essential medium (MEM) with Hank salts [MEM(H)] supplemented with 5% fetal bovine serum. African green monkey kidney (BSC-1) cells and diploid human foreskin fibroblasts (HFF cells) were grown in MEM with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[9] A plaque-purified isolate, P_0, of the Towne strain of HCMV was used in all experiments and was a gift of Dr. Mark Stinski, University of Iowa. The S-148 strain of HSV-1 was provided by Dr. T. W. Schafer of Schering Corp. Stock preparations of HCMV and HSV-1 were prepared and titered as described elsewhere.[9]

(b) Assays for Antiviral Activity. HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that referenced above for titration of HCMV, with the exceptions that the virus inoculum (0.2 mL) contained approximately 50 PFU of HCMV and the compounds to be assayed were dissolved in the overlay medium. Protocols for HCMV titer reduction experiment have been described previously.[9] HSV-1 plaque reduction experiments were performed using monolayer cultures of BSC-1 cells. The assay was performed exactly as referenced above for HSV-1 titration assays except that the 0.2 mL of virus suspension contained approximately 100 PFU of HSV-1 and the compounds to be tested were dissolved in the overlay medium.

(c) Cytotoxicity Assays. Two basic tests for cellular cytotoxicity were routinely employed for compounds examined in antiviral assays. Cytotoxicity produced in HFF and BSC-1 cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 35- and 60-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration. Cytotoxicity in KB cells was determined by measuring the effects of compounds on the incorporation of radioactive precursors into DNA, RNA, and protein as detailed elsewhere.[9]

(d) Data Analysis. Dose–response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against log drug concentration. Fifty percent inhibitory (IC$_{50}$) concentrations were calculated from the regression lines. The three IC$_{50}$'s for inhibition of DNA, RNA, and protein synthesis were averaged to give the values reported in the table for KB cell cytotoxicity. Samples containing positive controls (acyclovir or ganciclovir) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.