Synthesis and Biological Evaluation of Carbocyclic Analogues of Lyxofuranosides of 2-Amino-6-substituted-purines and 2-Amino-6-substituted-8-azapurines[1]

Mark L. Peterson and Robert Vince*

Department of Medicinal Chemistry, University of Minnesota, College of Pharmacy, Health Sciences Unit F, Minneapolis, Minnesota 55455. *Received July 11, 1989*

Carbocyclic analogues of lyxofuranosides of 2-amino-6-substituted-purines and 2-amino-6-substituted-8-azapurines were synthesized from (±)-(1α,2α,3α,5α)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**2**) and 2-amino-4,6-dichloropyrimidine (**3**). The 2-amino-6-chloropurine (**8** and **11**), the 2,6-diaminopurine (**10** and **13**), as well as the guanine (**9**) and 8-azaguanine (**12**) derivatives were all constructed from the key intermediate (±)-(1α,2α,3α,5α)-3-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (**7**) by using established methodology. Compounds **8-13** were evaluated for both antitumor and antiviral activity. None of these materials exhibited appreciable activity against P-388 mouse leukemia cells in vitro. All of these analogues were investigated for activity versus herpes simplex virus type 1 (HSV-1) and influenza virus (IV-A), as well as the human immunodeficiency virus (HIV). Against HSV-1, only compound **9**, the carbocyclic analogue of the lyxofuranoside of guanine, exhibited significant activity, yielding a virus rating (VR) of 2.1. The corresponding 2,6-diamino compound (**10**) demonstrated marginal activity, VR = 0.6, against that virus. The test compounds failed to exhibit inhibition of either IV-A or HIV. Additionally, **9** was tested against human cytomegalovirus (HCMV) and was found to display definite activity at concentrations as low as 32 μM.

In the search for more potent and selective chemotherapeutic agents, nucleosides and their derivatives are among the most widely explored types of compounds. Carbocyclic nucleosides, wherein the furanose oxygen is replaced by a methylene group, are an interesting class of such analogues, many of which possess significant biological properties, including antiviral activity.[2] The potent antitumor agents aristeromycin and neplanocin A are naturally occurring examples of this type of compound. The conformational and structural similarity between the tetrahydrofuran and cyclopentyl rings allows these carbocyclic compounds to display a diverse array of biological effects, which are often absent in the parent furanoside. These structures prove to be more resistant to the action of certain enzymes, such as hydrolases and phosphorylases, that can deactivate the corresponding nucleoside.[3] However, no direct correlation between the activity of a natural nucleoside and its carbocyclic counterpart has been found to exist. Indeed, even within the carbocyclic analogues themselves, it has been found necessary to test a number of different derivatives of a given pseudosugar moiety in order to accurately ascertain their full range of activity.

Nucleosides bearing cyclopentyl groups which have configurations corresponding to those of all of the furanose sugars have been studied to some degree. It has been observed that for a given carbocyclic sugar structure, purine bases other than adenine must be explored. In particular, the 2-amino-6-substituted-purine analogues of the various carbocyclic nucleosides have displayed significant antiviral and/or antitumor activity. For those possessing a ribose-like configuration, the 2,6-diaminopurine derivative was highly active against vaccinia virus and herpes simplex virus type 1 (HSV-1). Several other derivatives of these carbocyclic ribofuranosyl nucleosides possessed diminished activity versus HSV-1.[4,5] More interestingly, it was found that in a series of 2,6-disubstituted purine analogues containing a 2′-deoxy ribo-configurated carbocyclic moiety, all the synthesized structures displayed activity against both HSV-1 and herpes simplex virus type 2 (HSV-2) in vitro.[6] For two of these compounds, the carbocyclic analogue of 2′-deoxyguanosine and the 2,6-diaminopurine derivative thereof, these results have been duplicated in vivo as well. Of these materials, the carbocyclic 2′-deoxyribofuranoside of 2-amino-6-chloro-8-azapurine was found to display the most substantial cytotoxicity toward a human epidermoid carcinoma cell line. Further, the antiviral action exhibited by the 3′-deoxyribofuranoside of guanine and of 2-amino-6-chloropurine for HSV-1 and, to a lesser extent, for HSV-2, was especially significant in that these are the only derivatives containing this particular pseudosugar moiety not devoid of biological activity.[7] In the case of the arabino-configurated analogues, only the 2,6-diaminopurine compound inhibited the growth of HSV-1; however, two of these nucleosides, the carbocyclic arabinofuranosyl guanine and 8-azaguanine, did display appreciable cytotoxicity against P-388 mouse leukemia cells in culture.[8] Likewise, the carbocyclic analogue of the xylofuranoside of guanine and its 8-aza derivative exhibited significant potency in the P-388 assay. More significantly, the former proved to be very active not only against HSV-1 and HSV-2, but also against human cytomegalovirus (HCMV) and varicella-zoster virus (VZV).[9]

In addition, fluorine-substituted carbocyclic guanine compounds have been synthesized and proven to be potent inhibitors of both HSV-1 and HSV-2.[10-12] Most significantly, the carbocyclic analogue of 2′,3′-dideoxy-2′,3′-didehydroguanosine (carbovir), as well as the corresponding 2,6-diaminopurine derivative, recently has been found to possess substantial activity against human immunodeficiency virus (HIV), the etiological agent for acquired im-

(1) A preliminary account of portions of this work was presented at the 196th National Meeting of the American Chemical Society, Los Angeles, CA, Sept 25–30, 1988, Abstract MEDI 9.
(2) Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* **1986**, *6*, 1.
(3) Bennett, L. L., Jr.; Shannon, W. M.; Allan, P. W.; Arnett, G. *Ann. N. Y. Acad. Sci.* **1975**, *255*, 342.
(4) Shealy, Y. F.; Clayton, J. D. *J. Pharm. Sci.* **1973**, *62*, 1432.
(5) Shealy, Y. F.; Clayton, J. D.; Arnett, G.; Shannon, W. M. *J. Med. Chem.* **1984**, *27*, 670.
(6) Shealy, Y. F.; O'Dell, C. A.; Shannon, W. M.; Arnett, G. *J. Med. Chem.* **1984**, *27*, 1416.
(7) Shealy, Y. F.; O'Dell, C. A.; Arnett, G. *J. Med. Chem.* **1987**, *30*, 1090.
(8) Lee, H.; Vince, R. *J. Pharm. Sci.* **1980**, *69*, 1019.
(9) Vince, R.; Turakhia, R. H.; Shannon, W. M.; Arnett, G. *J. Med. Chem.* **1987**, *30*, 2026.
(10) biggadike, K.; Borthwick, A. D.; Exall, A. M.; Kirk, B. E.; Roberts, S. M.; Youds, P. *J. Chem. Soc., Chem. Commun.* **1987**, 1083.
(11) Borthwick, A. D.; Evans, D. N.; Kirk, B. E.; Biggadike, K.; Stephenson, L. Eur. Pat. Appl. EP 212956, March 4, 1987; *Chem. Abstr.* **1987**, *107* 154170v.
(12) Borthwick, A. D.; Butt, S.; Biggadike, K.; Exall, A. M.; Roberts, S. M.; Youds, P. M.; Kirk, B. E.; Booth, B. R.; Cameron, J. M.; Cox, S. W.; Marr, C. L. P.; Shill, M. D. *J. Chem. Soc. Chem. Commun.* **1988**, 656.

0022-2623/90/1833-1214$02.50/0

mune deficiency syndrome (AIDS).[13,14]

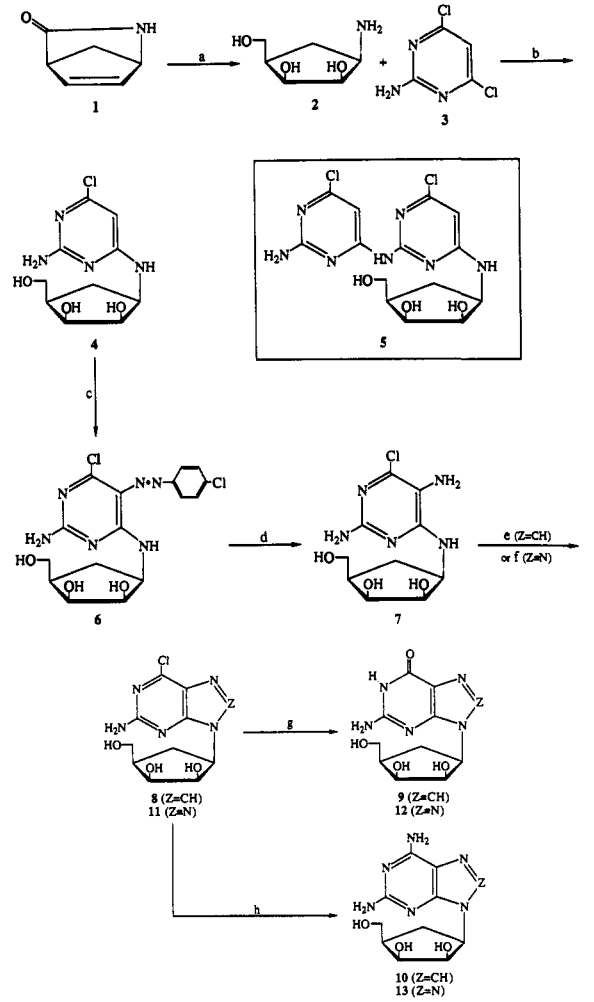
On the basis of this intense interest, it was odd that the cyclopentyl moiety with a configuration that mimics the lyxo orientation of a furanose sugar had not yet been investigated with these purine base substituents. A thorough study of both the α- and β-anomers of the lyxofuranosides of all the natural nucleic acid bases has been reported, but it revealed only one active structure, 9-α-D-lyxofuranosyladenine, which inhibited HSV-1 and HSV-2 both in vitro and in vivo.[15,16] Studies on the carbocyclic lyxoadenine compounds found no appreciable antiviral potency and only the 8-azaadenine derivative exhibited activity against P-388 cells in tissue culture.[17] The 6-methyl-7-deazaadenine lyxofuranosyl analogue was absent of noticeable antitumor or antiviral activity.[18] Surprisingly, however, the 7-deazaguanine derivative has been synthesized and found to exhibit selective inhibition against both HSV-1 and HSV-2 in vitro.[19] In order to satisfy our curiosity about the potential biological activity of 2-amino-6-substituted-purine analogues and their 8-aza derivatives containing the lyxo orientation on the cyclopentyl group, the synthesis of several of these compounds and the evaluation of their biological activity has been completed.

Chemistry

The synthesis of the desired 2-amino-6-substituted-purine derivatives followed a route standard in the field of carbocyclic nucleosides (Scheme I).[4-9] This involved construction first of a cyclopentyl primary amine containing the correct stereochemical configuration and then elaboration of the amine into the heterocyclic base portion of the molecule. The required lyxo-oriented cyclopentyl moiety had been synthesized previously in these laboratories.[17] The same method was employed here. However, it should be noted that a stereoselective approach to the desired stereoisomer, based on glycolization of a related compound with potassium permanganate, which avoided the ion-exchange separation required in this procedure, is known.[20] In addition, a convergent approach to carbocyclic ribo- and lyxoadenine derivatives has recently been described.[21]

The desired, stereochemically correct amino alcohol (±)-(1α,2α,3α,5α)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**2**) was available from the versatile carbocyclic nucleoside precursor 2-azabicyclo[2.2.1]hept-5-en-3-one (**1**) by a straightforward series of reactions.[17] Compound **1** is itself conveniently synthesized on molar scales from the reaction of cyclopentadiene with tosyl cyanide.[22] With amine **2** in hand, the next step was to construct the nucleic acid base. This was accomplished through application of the classical Traube synthetic procedure.[23] The amine

Scheme I[a]



[a] Reagents: (a) refs 17, 22; (b) Et_3N, *n*-BuOH, reflux, 48 h; (c) *p*-$ClC_6H_4N_2^+Cl^-$, NaOAc–HOAc–H_2O, 0 °C → room temperature; (d) Zn–HOAc, EtOH–H_2O, reflux; (e) $HC(OEt)_3$, concentrated HCl (cat.), DMF, room temperature; (f) $NaNO_2$–HOAc–H_2O, 0 °C; (g) 1 N HCl, reflux; (h) NH_3, 75 °C (bomb).

and 2-amino-4,6-dichloropyrimidine (**3**) were refluxed in butanol in the presence of triethylamine to provide the coupled product **4**.[24] It should be noted that, as has been observed by other researchers in the synthesis of these guanine derivatives,[6,7,9] it is necessary to use very pure starting pyrimidine for this reaction. Technical grade material resulted in the formation of a troublesome impurity, which was assigned the structure **5**. This was based on the spectral and analytical data and by analogy with the previously reported work. The requisite 5-amino moiety was introduced onto the pyrimidine ring via a two-step protocol involving first the coupling of **4** with the diazonium salt of *p*-chloroaniline, followed by reduction of the resulting diazo compound **6** with zinc and acetic acid. This gave the slightly unstable triaminopyrimidine **7**.

This key intermediate was then used to synthesize both the purine and 8-azapurine products. Cyclization with triethyl orthoformate in the presence of a catalytic amount of concentrated hydrochloric acid provided the 2-amino-

(13) Vince, R.; Hua, M.; Brownell, J.; Daluge, S.; Lee, F.; Shannon, W. M.; Lavelle, G. C.; Qualls, J.; Weislow, O. S.; Kiser, R.; Canonico, P. G.; Schultz, R. H.; Narayanan, V. L.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1046.

(14) Vince, R.; Hua, M. *J. Med. Chem.* **1989**, *33*, 17.

(15) Gosselin, G.; Bergogne, M.-C.; DeRudder, J.; DeClercq, E.; Imbach, J.-L. *J. Med. Chem.* **1987**, *30*, 982.

(16) Goselin, G.; Bergogne, M.-C.; Imbach, J.-L.; DeRudder, J.; DeClercq, E. *Nucleosides Nucleotides* **1987**, *6*, 65.

(17) Vince, R.; Daluge, S. *J. Org. Chem.* **1980**, *45*, 531.

(18) Legraverend, M.; Lhoste, J. M.; Bechet, J. M.; Bisagni, E. *Eur. J. Med. Chem.-Clin. Ther.* **1983**, *18*, 269.

(19) Legraverend, M.; Ngongo-Tekam, R.-M. N.; Bisagni, E.; Zerial, A. *J. Med. Chem.* **1985**, *28*, 1477.

(20) Kam, B. L.; Oppenheimer, N. J. *J. Org. Chem.* **1981**, *46*, 3268.

(21) Trost, B.; Kuo, G.-H.; Benneche, T. *J. Am. Chem. Soc.* **1988**, *110*, 621.

(22) Daluge, S.; Vince, R. *J. Org. Chem.* **1978**, *43*, 2311.

(23) Lister, J. H. *Fused Pyrimidines*; D. J. Brown, Ed.; Wiley-Interscience: New York, 1971; Part II, pp 31–72.

(24) All new compounds (**4**–**13**) gave spectral data and elemental analyses consistent with the assigned structures. All compounds are racemic mixtures; only one isomer is depicted for clarity.

Table I. Cytotoxicity of Carbocyclic Lyxofuranoside Purine Analogues against P-388 Mouse Leukemia Cells in Vitro[a]

	% inhibition				
compd	100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL	6.25 μg/mL
8	2.8	0	0	0	0
9	71.6	58.4	42.1	9.1	0
10	42.3	11.3	0	0	0
11	99.1	40.1	14.2	0	0
12	63.9	27.4	5.0	0	0
13	58.4	31.5	12.2	0	0
C-Ado[b]	100	100	100	100	100

[a] Reported as percent growth inhibition as determined by the protocol of the National Cancer Institute, ref 25. [b] Positive control: carbocyclic adenosine.

6-chloropurine analogue (8). Liberation of the derivatized amino and hydroxyl groups formed by their reaction with the triethyl orthoformate was necessary, but it was easily accomplished by treatment of the crude product with dilute acid. Alternatively, reaction of **7** with nitrous acid, formed in situ from sodium nitrite and acetic acid, effected ring closure to provide the corresponding 8-azapurine analogue 11. The 6-chloro moiety in 8 and 11 was hydrolyzed by refluxing in dilute acid to provide the guanine (9) and 8-azaguanine (12) derivatives, respectively. The final target structures were accessed by displacement of the halide in 8 and 11 with ammonia to yield the 2,6-diaminopurine compounds 10 and 13, respectively.

Biological Results

The six purine derivatives (8–13) were evaluated as both potential antitumor and antiviral chemotherapeutic agents. The results for a growth inhibition assay[25] using P-388 mouse leukemia cells are given in Table I. These structures did not prove to be significantly cytotoxic to the tumor tissue cultures at concentrations of 6.25–50 μg/mL. One compound, the carbocyclic analogue of lyxofuranosylguanine (9) showed noticeable activity over a short range of concentrations, and another, 2-amino-6-chloro-8-azapurine (11), exhibited almost complete growth inhibition, but only at the highest concentration, 100 μg/mL, employed.

Better results were obtained in screening for antiviral activity. The lyxofuranoside analogues were tested against both a DNA virus, herpes simplex virus type 1, strain E-377 (HSV-1), and an RNA virus, influenza virua type A_0/PR/8/34 (IV-A). A standard method for determining the inhibition of virus-induced cytopathogenic effects (CPE) was used.[26,27] The results are displayed in Table II. The antiviral activity of the compounds is expressed as a virus rating (VR). This was determined by a modification of the method of Ehrlich et al.[28] and is a weighted measurement of the selective antiviral potency of a compound. It takes into account the degree of inhibition of CPE and the degree of toxicity of a given material. None of the compounds exhibited activity against IV-A, but two compounds were appreciably active against HSV-1. The carbocyclic analogue of the lyxofuranoside of guanosine (C-lyxo-G, 9) had a VR of 2.1, indicating significant antiviral activity, while its 2,6-diaminopurine derivative (10) had a VR of 0.6, indicating marginal antiviral activity. The result that the diamino compound displayed slightly diminished activity when compared with its analogous active guanine analogue was consistent with observations in related carbocyclic nucleoside systems.[9,13]

Table II. Evaluation of Carbocyclic Lyxofuranoside Purine Analogues for in Vitro Antiviral Activity

	herpes simplex virus type 1 (E-377 strain) in Vero[a] host cell cultures				influenza virus (type A_0/PR/8/34) in MDCK[b] host cell cultures			
compd	VR[c]	ID_{50}[d]	MTC[e]	TI[f]	VR[c]	ID_{50}[d]	MTC[e]	TI[f]
8	0	–	299.7	–	0	–	299.7	–
9	2.1	83.4	>299.3	>3.6	0	–	>299.3	–
10	0.6	195.2	280.3	1.4	0	–	280.3	–
11	0	–	300.7	–	0.3	–	>256.3	–
12	0	–	300.3	–	0.2	–	95.0	–
13	0	–	>294.8	–	0	–	>294.8	–
			Positive Controls[g]					
Ara-A	2.4	4.8	26.8	5.6				
ACV	6.2	0.9	>255.2	>240.3				
Rib.					3.4	3.1	32	10.3

[a] African green monkey kidney. [b] Madin–Darby canine kidney. [c] VR = virus rating: A VR ≥ 1.0 indicates definite antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate antiviral activity, and a VI < 0.5 usually indicates no significant antiviral activity. Determined by a modification of the method of ref 28. [d] ID_{50} = the minimum drug concentration (μg/mL) that inhibited the CPE by 50%, calculated by using a regression analysis program for semilog curve fitting. [e] MTC = the minimum concentration (μg/mL) at which any cytoxicity was observed. [f] TI = therapeutic index, calculated by dividing the MTC by the ID_{50}. [g] Ara-A: 9-β-D-Arabinofuranosyladenine; ACV; Acyclovir; Rib.: Ribavirin.

Table III. Effect of Carbocyclic Lyxofuranosylguanine (9) Treatment on Yields of Human Cytomegalovirus (HCMV) in MRC5[a] Cell Monolayer Cultures

	compd + HCMV		cytotoxicity	
concn, (μM)	HCMV yield (log_{10} PFU[b]/mL)	HCMV yield reduction (log_{10} PFU[b]/mL)	gross morphology	MTT[c] assay (percent of control)
		Compound 9		
1000	<0.6[d]	>4.8	sl toxic	88
320	2.5	2.9	sl toxic	93
100	3.7	1.7	0	97
32	4.2	1.2	0	103
10	4.8	0.6	–	–
3.2	4.9	0.5	–	–
0[e]	5.4			
		DHPG[f]		
32	2.4	3.0		

[a] MRC5 = human diploid embryonic lung cells. [b] PFU = plaque-forming units. [c] MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. [d] Below detection limit. [e] Virus control. [f] Positive control: 9-[(1,3-Dihydroxy-2-propoxy)methyl]-guanine.

The most active of these compounds, C-lyxo-G (9), was tested for activity against human cytomegalovirus (HCMV) in human diploid embryonic cell (MRC5) cultures by utilizing a virus yield reduction assay procedure.[9] The results are presented in Table III. The material demonstrated a significant effect, reducing the titer of progeny virus by greater than 10 PFU (plaque-forming units) at concentrations as low as 32 μM. At higher concentrations, this effect was even more pronounced. At 1000 μM, virus yields were reduced by greater than $10^{4.8}$ PFU/mL (below the level of detection) when compared with those of untreated cell cultures, and at 320 μM, a yield reduction of $10^{2.9}$ PFU/mL was obtained.

In addition, these compounds were submitted for screening against the human immunodeficiency virus

(25) Geran, R. I.; Greenberg, N. H.; McDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3* (2), 1.
(26) Shannon, W. M.; Arnett, G.; Westbrook, L.; Shealy, Y. F.; O'Dell, C. A.; Brockman, R. W. *Antimicrob. Agents Chemother.* **1981**, *20*, 769.
(27) Shannon, W. M.; Shortnacy, A.; Arnett, G.; Montgomery, J. A. *J. Med. Chem.* **1974**, *17*, 361.
(28) Erlich, J.; Sloan, B. J.; Miller, F. A.; Machamer, H. E. *Ann. N. Y. Acad. Sci.* **1965**, *130*, 5.

(HIV). A microculture tetrazoplium assay procedure to determine the reduction of viral CPE was employed for this testing.[13] Though the purine derivatives did not reveal any cytotoxicity towards the cell lines used in these studies, they, unfortunately, also failed to display any inhibition of HIV at concentrations as high as 125 μg/mL. Further testng on C-lyxo-G is underway to more completely ascertain the spectrum of its antiviral activity.

Experimental Section[29]

Melting points were determined on a Mel-Temp apparatus and are uncorrected. ^{1}H nuclear magnetic resonance spectra were obtained on a Nicolet NT-300 operating at 300.0745 MHz in the solvent listed and are given as parts per million (δ) relative to internal tetramethylsilane (TMS) as reference. Resonances are reported as follows: [apparent multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, others are described), coupling constant (*J*) if appropriate, relative integral, assignment]. In cases where exact assignments of chemically similar resonances are given (with nucleoside numbering), ^{1}H–^{1}H correlation spectra were employed to accurately ascertain them. ^{13}C NMR spectra were determined on the Nicolet NT-300 at 75.461 MHz with peak positions given as δ relative to internal TMS or with respect to the listed solvent as noted for the individual compounds. Fourier transform infrared spectra were taken with a Nicolet 5-DXC instrument and are reported as wavenumbers (cm^{-1}). Major or distinct resolved bands are given and designated as broad (br), very strong (vs), or strong (s) where appropriate to signify their intensity. Ultraviolet spectra were obtained on either a Beckman DU-8 or a Beckman DU-70 spectrometer. Reported wavelengths, in nanometers, are maxima or are designated as a shoulder (sh) or a slight shoulder (slsh). Electron impact (EI) mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer. Chemical ionization (CI) mass spectral data was obtained on a Finnigan 4000 mass spectrometer using the reagent gas indicated for the individual compounds. Fast-atom bombardment (FAB) mass spectral data was recorded with a VG7070E-HF mass spectrometer using the indicated matrix. For CI and FAB spectra, both positive and negative detection modes were employed, thus key ions are reported with a plus (+) or minus (–) to distinguish between the two. The peaks listed are those from the molecular ion, designated M$^+$, and fragments that can be assigned as either plus or minus relative to the molecular ion or the complete purine or pyrimidine portion of the molecule, designated P, as well as other prominent, distinct, or significant peaks. The intensities of the fragments are given parenthetically relative to the base peak, listed as 100. Evaporation in vacuo refers to the use of a rotary evaporator using aspirator pressure unless noted otherwise. Thin-layer chromatography (TLC) was done with 0.25-mm layers of Merck silica gel 60F-254 on glass-backed plates. Plates were visualized by viewing under ultraviolet light and by exposure to iodine vapor. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). The solvent systems used were solvent A, MeOH–$CHCl_3$ (1:5); solvent B, MeOH–$CHCl_3$ (1:3); solvent C, *n*-BuOH–HOAc–H_2O (5:2:3). Chromatography on silica involved dissolving the crude material in a suitable solvent, treatment of this solution with silica gel, followed by evaporation of the solvent, and then drying of the silica under high vacuum. The dried, compound-containing silica was the loaded onto a slurry-packed column of silica gel and eluted with the solvent listed. Pure product-containing fractions as indicated by TLC were combined and evaporated in vacuo. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. 2-Amino-4,6-dichloropyrimidine (**3**) and anhydrous dimethylformamide were obtained from Aldrich Chemical Co., Milwaukee, WI.

(±)-(1α,2α,3α,5α)-3-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (4). To 8.90 g (60.5 mmol) of (±)-(1α,2α,3α,5α)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (**2**)[17] in 250 mL of *n*-BuOH was added 15.0 g (91.2 mmol) of 2-amino-4,6-dichloropyrimidine (**3**) and 51.0 mL of Et_3N. The resulting suspension was heated to reflux under a nitrogen atmosphere for 48 h. The solvent was evaporated in vacuo and the dark residue was partitioned between 250 mL each of CH_2Cl_2 and H_2O. The aqueous layer was extracted twice with 200-mL portions of CH_2Cl_2. TLC (solvent A) indicated that the unreacted pyrimidine was the only component of the organic extracts. The aqueous solution was then stirred with 250 mL of Dowex SBR (OH^-) ion-exchange resin. The resin was removed by filtration and the basic filtrate was evaporated in vacuo. The residue was purified by chromatography on silica with solvent A to yield 12.4 g (74.6%) of the coupled product (R_f = 0.41, solvent B). An analytical sample was prepared by recrystallization from H_2O. Mp: (shrivels 165 °C) 192–194 °C partial dec. UV: λ_{max} 303, 276, 236, 213 nm in 0.1 N HCl; 286, 237, 211 nm in H_2O; 287, 238, 217 nm in 0.1 N NaOH. EIMS (260 °C, 30 eV): *m/z* 276 (M + 2, ^{37}Cl isotope, 14.5), 274 (M$^+$, 42.7), 257 (M – OH, 17.2), 256 (M – H_2O, 9.9), 243 (M – CH_2OH, 16.6), 227 (13.5), 225 (25.6), 215 (16.2), 183 (20.8), 182 (21.6), 173 (45.2), 172 (26.0), 171 (100), 170 (46.1), 169 (58.3), 157 (18.2), 147 (33.5), 146 (26.3), 145 (P + 2 H, 92.1), 144 (P + H, 59.6), 109 (P + H – Cl, 19.3), 81 (15.0), 67 (13.4). IR (KBr): 3550–3000 (br) with maximum bands at 3411, 3304, 3171 and 3099, 2978, 2944, 2884, 1646, 1586 (s), 1487, 1444, 1427, 1357, 1240, 1161, 1100, 1029, 976, 891, 808, 781, 543. ^{1}H NMR (DMSO-d_6): δ 6.78 (br s, 1, N*H*), 6.32 (s, 2, N*H*$_2$), 5.86 (s, 1, C*H* pyrimidine), 4.79 (br s, 1, CHO*H*), 4.57 (br s, 1, CHO*H*), 4.40 (br t, 1, CH_2O*H*), 4.22 (br s, 1, C*H*N), 3.97 (m, 1, C*H*OH), 3.80 (unsymmetrical q, 1, C*H*OH), 3.40–3.58 (pair of m centered at 3.46 and 3.53, total 2, C*H*$_a$*H*$_b$OH), 1.99 (m, 2, C*H*C*H*$_a$H$_b$CHN), 1.37 (poorly resolved m, 1, CHCH$_a$*H*$_b$CHN); the signals at 6.78, 6.32, 4.79, 4.57, and 4.40 disappear upon exchange with D_2O, while the signals at 3.97 and 3.80 become unsymmetrical triplets and those at 3.53 and 3.46 simplify. ^{13}C NMR (DMSO-d_6, TMS ref): δ 163.5, 162.6, 156.9, 93.0, 72.0 (CHOH's not resolved), 60.8, 50.1, 40.9, 32.1. Anal. ($C_{10}H_{15}N_4O_3Cl$): C, H, N.

(±)-(1α,2α,3α,5α)-3-[[2-[(2-Amino-6-chloro-4-pyrimidinyl)amino]-6-chloro-4-pyrimidinyl]amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (5). In an experiment conducted as described above for **4**, starting with 2.06 g (14.0 mmol) of **2** in 100 mL of *n*-BuOH and 12.0 mL of Et_3N, but which utilized 3.44 g of technical grade **3**, 0.96 g (17.1%) of an insoluble, gray material separated out from the reaction residue upon treatment with H_2O and CH_2Cl_2. It was removed by filtration and subsequently recrystallized from EtOH–H_2O to give the analytical sample as a gray powder. Mp: (shrivels 220–230 °C) 250–253 °C. TLC: R_f = 0.52 (solvent B, trails). UV: λ_{max} 323, 265 (slsh), 244 nm in 0.1 N HCl; 305, 262 (sh), 245 (sh), 229 nm in H_2O; 329 (sh), 305, 262 (slsh), 236 nm in 0.1 N NaOH. EIMS (330 °C, 30 eV): *m/z* 403 (M + 2, ^{37}Cl isotope, 19.4), 401 (M$^+$, 28.3), 373 (15.0), 372 (20.4), 370 (M – CH_2OH, 15.9), 344 (14.3), 342 (26.1), 326 (13.7), 302 (28.0), 301 (11.5), 300 (71.7), 299 (26.1), 298 (100), 296 (13.1), 274 (18.5), 273 (23.6), 272 [P (both) + 2 H, 40.8], 271 (26.8), 236 [P (both) + H – Cl, 13.1], 170 (25.5), 81 (18.2), 67 (13.7). IR (KBr): 3600–3000 (br) with maxima at 3480, 3409, 3318 (s) and 3163, 2931, 1645 (s), 1616 (s), 1587 (s), 1554 (s), 1525 (s), 1440 (s), 1363 (s), 1340 (s), 1249, 1089, 1058, 984, 921, 815, 797, 780, 694, 677, 658. ^{1}H NMR (DMSO-d_6): δ 9.68 (s, 1, ArN*H*Ar), 7.59 (s, 1, C*H* pyrimidine), 7.48 (d, *J* = 7.3 Hz, 1, N*H* cyclopentyl), 6.69 (br s, 2, N*H*$_2$), 6.29 (s, 1, C*H* pyrimidine), 4.89 (d, *J* = 5.7 Hz, 1, CHO*H*), 4.62 (d, *J* = 4.7 Hz, 1, CHO*H*), 4.43 (t, *J* = 4.7 Hz, 1, CH_2O*H*), 4.18 (poorly resolved m, 1, C*H*N), 4.06 [poorly resolved m (approximates q), 1, C*H*OH], 3.94 (unsymmetrical q, 1, C*H*OH), 3.38–3.60 (pair of m centered at 3.46 and 3.52, total 2, C*H*$_a$*H*$_b$OH), 2.09 (m, 2, C*H*C*H*$_a$H$_b$CHN), 1.48 (m, 1, CHCH$_a$*H*$_b$CHN); the signals at 9.68, 7.48, 6.69, 4.89, 4.62, and 4.43 disappear upon exchange with D_2O, while the signals at 4.18, 4.06, 3.94, 3.52, and 3.46 simplify. ^{13}C NMR (DMSO-d_6, solvent ref at δ 39.5): δ 163.1, 162.5, 160.4, 159.9, 157.9, 156.6, 97.3, 96.6, 72.1, 72.0, 60.9, 51.5, 41.1, 31.8. Anal. ($C_{14}H_{17}N_7O_3Cl_2 \cdot 0.5H_2O$): C, H, N, Cl.

(±)-(1α,2α,3α,5α)-3-[[2-Amino-6-chloro-5-[(4-chlorophenyl)azo]-4-pyrimidinyl]amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (6). To 1.01 g (7.92 mmol) of *p*-chloroaniline dissolved in 5.6 mL of concentrated hydrochloric acid and 8 mL of H_2O cooled in an ice bath under nitrogen was added a solution of 0.59 g (8.55 mmol) of sodium nitrite in 10 mL of H_2O. A light

(29) In accordance with *Chemical Abstracts* nomenclature, compounds are named as 1,2-cyclopentanediols. Because compounds **9** and **12** contain oxygen substituents on the heterocyclic ring, they are named as a cyclopentylpurine and a 1,2,3-triazolo[4,5-*d*]pyrimidine, respectively.

yellow solution resulted. After stirring at 0 °C for 0.5 h, this cooled solution of *p*-chlorobenzenediazonium chloride was added dropwise to a mixture containing 1.76 g (6.40 mmol) of **4** buffered with 8.89 g of sodium acetate and 31.5 mL of glacial acetic acid in 50 mL of H_2O. A bright orange color formed immediately and an orange precipitate began to fall out of solution shortly thereafter. The mixture was allowed to stir at ambient temperature for 42 h, then it was refrigerated overnight. The precipitated orange solid was collected, washed thoroughly with H_2O, and then dried under vacuum to yield 2.09 g (78.9%) of **6**. This material was sufficiently pure for use in the next reaction. An analytical sample was prepared by recrystallization from MeOH. mp: (discolors starting at 170 °C) 260–261 °C dec. TLC: R_f = 0.65 (solvent B). UV: λ_{max} 428 (slsh), 396, 379 (slsh), 280 nm in THF; 425 (slsh), 394, 379 (slsh), 282 nm in THF–0.1 N HCl; 426 (slsh), 394, 379 (slsh), 282 nm in THF–H_2O; 421 (slsh), 394, 379 (slsh), 282 nm in THF–0.1 N NaOH; 422 (slsh), 388, 376 (slsh), 280 nm in MeOH. EIMS (330 °C, 30 eV): *m/z* 414 (M + 2, ^{37}Cl isotope, 2.0), 412 (M^+, 3.9), 309 (2.0), 288 (5.9), 286 (15.0), 270 (10.5), 268 [M – NH (cyclopentyl), 20.9], 184 (7.8), 183 (7.2), 182 (14.4), 170 (7.2), 127 [P – (N=NAr), 20.3], 111 (C_6H_4Cl, 20.3), 57 (32.7), 55 (34.6), 43 (30.7), 41 (47.1), 28 (100); CIMS (NH_3): *m/z* 413 [$(M + H)^+$, 21.7], 292 (31.6), 290 (94.3), 130 (40.9), 128 [$[P - (N{=}NAr) + H]^+$, 100]; 414 [$(M + 2)^-$, 66.0], 412 (M^-, 100), 289 (21.0). FABMS (DTT·DTE·HCl): *m/z* 413 [$(M + H)^+$]. IR (KBr): 3400–3200 (br) with maxima at 3353 and 3318, 3149 (br), 1653, 1559 (vs), 1484, 1438, 1375, 1302, 1199, 1119, 1086, 836, 784. ^{1}H NMR (DMSO-d_6): δ 10.79 (d, *J* = 7.7 Hz, 1, NH), 7.77 and 7.55 [m(AB), total 4, C*H* phenyl], 7.41 (br s, 2, NH_2), 5.07 (d, *J* = 5.9 Hz, 1, C_2-HO*H*), 4.74 (d, *J* = 4.3 Hz, 1, C_3-HO*H*), 4.47 (partially overlapped quintet, *J* = 7.0 Hz, 1, C*H*N), 4.41 (t, *J* = 5.2 Hz, 1, CH_2O*H*), 4.02 (q, *J* = 4.3 Hz, 1, C_3*H*OH), 3.91 (m, 1, C_2*H*OH), 3.40–3.62 (pair of m centered at 3.43 and 3.57, total 2, C$H_a$$H_b$OH), 2.24 (m, 1, CHC$H_a$$H_b$CHN), 1.99 (m, 1, C*H*C$H_a$$H_b$CHN), 1.34 (m, 1, CHC$H_a$$H_b$CHN); the signals at 10.79, 7.41, 5.07, 4.74, and 4.41 disappear upon exchange with D_2O, while the signals at 4.47, 4.02, 3.91, 3.57, and 3.43 sim plify. Anal. ($C_{16}H_{18}N_6O_3Cl_2$): C, H, N.

(±)-(1α,2α,3α,5α)-3-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]-5-(hydroxymethyl)-1,2-cyclopentanediol (7). A solution of 2.00 g (4.83 mmol) of **6**, 3.22 g (49.3 mmol) of zinc dust, 2.0 mL of glacial acetic acid, 50 mL of H_2O, and 50 mL of EtOH were refluxed under nitrogen for 2.5 h, at which time TLC (solvent B) indicated complete disappearance of starting material. The excess zinc was filtered from the still-warm solution and washed with EtOH, and then the brown filtrate plus washings were evaporated in vacuo. The brown residue was subjected to chromatography on silica using solvent A to give 0.958 g (68.4%) of **7**. Chromatographic purification as rapidly as possible was required to prevent loss of material. It apparently was very sensitive to oxidative decomposition in the crude state as evidenced by the appearance of new, more polar materials on TLC when, in a separate trial, the product was left overnight before chromatography. An analytical sample was prepared by recrystallization from H_2O. Mp: (shrivels and discolors 205–207 °C) 219–221 °C dec. TLC: R_f = 0.38 (solvent B); UV: λ_{max} 297, 237, 210 nm in 0.1 N HCl, 301, 240 (sh), 225 (slsh), 205 nm in H_2O; 302, 241 (sh), 228–235 nm in 0.1 N NaOH. EIMS (250 °C, 30 eV): *m/z* 291 (M + 2, ^{37}Cl isotope, 28.1), 289 (M^+, 88.9), 240 (18.9), 212 (15.5), 186 (39.0), 172 (24.1), 170 (44.3), 161 (33.5), 160 (P + 2 H, 31.4), 159 (P + H, 100). IR (KBr): 3550–3000 (br) with maxima at 3375 and 3262, 2952, 2917, 2882, 1637, 1607 (s), 1578 (s), 1516, 1447 (s), 1359, 1344, 1256, 1124, 1064, 1043, 1016, 983, 946, 864, 775. ^{1}H NMR (DMSO-d_6): δ 6.15 (d, *J* = 8.2 Hz, 1, N*H*), 5.63 (s, 2, NH_2), 4.79 (d, *J* = 6.6 Hz, 1, CHO*H*), 4.65 (d, *J* = 4.6 Hz, 1, CHO*H*), 4.39 (t, *J* = 4.9 Hz, 1, CH_2O*H*), 4.26 (m, 1, C*H*N), 3.98 (m, 1, C*H*OH), 3.83 (substantially obscured m, C*H*OH), 3.81 [br s, 3 (includes resonance at 3.83), NH_2], 3.35–3.60 (pair of m centered at 3.42 and 3.55, total 2, C$H_a$$H_b$OH), 2.01 (m, 2, C*H*C$H_a$$H_b$CHN), 1.39 (m, 1, CHC$H_a$$H_b$CHN); the signals at 6.15, 5.63, 4.79, 4.65, 4.39, and 3.81 disappear upon exchange with D_2O, while the signals at 4.26, 3.98, 3.83, 3.55, and 3.42 simplify. Anal. ($C_{10}H_{16}N_5O_3Cl$): C, H, N.

(±)-(1α,2α,3α,5α)-3-(2-Amino-6-chloro-9*H*-purin-9-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (8). A solution of 0.520 g (1.79 mmol) of **7** in 16 mL of anhydrous DMF and 27.0 mL of triethyl orthoformate was cooled to 0 °C under nitrogen. To this was added 0.53 mL of concentrated HCl. The mixture was stirred under nitrogen at ambient temperature overnight. The volatile materials were removed in vacuo (oil pump, 1–2 Torr) to leave an orange syrup. This was treated with 25 mL of 0.5 N HCl and stirred at room temperature for 2.5 h to hydrolyze the formyl derivatives that had formed. The mixture was then adjusted to pH 9 by addition of 1 N NaOH. The solvent was evaporated in vacuo, and then portions of EtOH were evaporated from the residue. The residue was chromatographed on silica using solvent A to yield 0.374 g (69.6%) of **8** [TLC: R_f = 0.38 (solvent B); 0.55 (solvent C)]. An analytical sample was prepared by recrystallization from absolute EtOH. Mp: (discolors slowly before melting) 229–231 °C dec. UV: λ_{max} 313, 243 (sh), 219 nm in 0.1 N HCl; 306, 244 (sh), 223 nm in H_2O, 306, 246 (sh), 227 nm in 0.1 N NaOH. EIMS (330 °C, 30 eV): *m/z* 301 (M + 2, ^{37}Cl isotope, 10.4), 299 (M^+, 29.0), 268 (M – CH_2OH, 10.9), 225 (15.9), 224 (16.8), 198 (17.9), 196 (49.0), 172 (32.7), 171 (27.0), 170 (P + 2 H, 100), 169 (P + H, 67.8), 160 (17.2), 134 [(P + 2 H) – HCl, 63.3]. IR (KBr): 3557, 3500–3000 (br) with maxima at 3387 (s), 3314 (s), 3206 (s) and 3103 (s), 2955, 2925, 2894, 2874, 1635 (s), 1610 (s), 1568 (s), 1519, 1473, 1417, 1410, 1394, 1378, 1301, 1255, 1228, 1195, 1026, 921, 788. ^{1}H NMR (DMSO-d_6): δ 8.17 (s, 1, C_8*H* purine), 6.81 (br s, 2, NH_2), 5.02 (d, *J* = 5.5 Hz, 1, CHO*H*), 4.94 (d, *J* = 5.0 Hz, 1, CHO*H*), 4.81 (unsymmetrical q, 1, C*H*N), 4.48 (t, *J* = 5.1 Hz, 1, CH_2O*H*), 4.06 (m, 2, C*H*OH's), 3.40–3.70 (pair of m centered at 3.47 and 3.65, total 2, C$H_a$$H_b$OH), 2.25 (m, 1, CHC$H_a$$H_b$CHN), 2.06 (m, 1, C*H*C$H_a$$H_b$CHN), 1.80 (m, 1, CHC$H_a$$H_b$CHN); the signals at 6.81, 5.02, 4.94, and 4.48 disappear upon exchange with D_2O, while the signals at 4.06, 3.65, and 3.47 simplify. Anal. ($C_{11}H_{14}N_5O_3Cl$): C, H, N.

(±)-2-Amino-1,9-dihydro-9-[(1α,2α,3α,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-6*H*-purin-6-one (9). It was found to be most efficient to proceed directly from pyrimidine **7**, without isolation of the intermediate purine **8**. To a solution of 0.353 g (1.23 mmol) of **7** in 10.8 mL of anhydrous DMF and 18.0 mL of triethyl orthoformate cooled in an ice bath under nitrogen was added 0.38 mL of concentrated HCl. The solution was allowed to gradually warm to room temperature and then was stirred overnight. The orange-brown solution was evaporated in vacuo (oil pump, 1–2 Torr) to leave a syrup. This residue was dissolved in 30 mL of 1 N HCl and heated to reflux under nitrogen for 6 h. The reaction mixture was cooled, then the solvent was removed in vacuo to leave an orange solid. This was dissolved in a small amount of H_2O and the pH of the solution was adjusted to 7 by the dropwise addition of 1 N NaOH. A gray solid separated from the neutral solution. It was not isolated, but rather redissolved in the aqueous solution by heating, filtered to remove some dark colored impurities, then cooled. An off-white solid, 0.120 g (34.7%), resulted from this recrystallization. Mp: (shrivels with partial dec at 216–219 °C) 287–290 °C dec. TLC: R_f = 0.35 (solvent C). UV: λ_{max} 277 (sh), 254, 207 nm in 0.1 N HCl; 273 (sh), 252, 206 (sh) nm in H_2O; 268, 257 (sh), 217 nm in 0.1 N NaOH. EIMS (350 °C, 70 eV): *m/z* 281 (M^+, 8.0), 264 (M – OH, 2.7), 178 (10.1), 164 (9.0), 152 (P + 2 H, 30.9), 151 (P + H, 52.7), 150 (P, 27.7), 135 (10.1), 134 (13.3), 110 (9.6), 109 (13.3), 108 (11.2), 67 (17.6), 66 (30.9), 65 (22.3), 44 (80.3), 28 (100). IR (KBr): 3600–3000 (br) with maxima at 3416 (s), 3311 (s) and 3206 (s), 2903, 2861, 2727, 1726 (s), 1685, 1636 (s), 1607 (s), 1573, 1537, 1463, 1414, 1395, 1323, 1180, 1137, 1121, 1099, 1054, 1014, 776, 683, 641. ^{1}H NMR (DMSO-d_6): δ 10.50 (br s, 1, *H*NC=O purine), 7.74 (s, 1, C_8*H* purine), 6.36 (br s, 2, NH_2), 4.93 (d, *J* = 5.9 Hz, 1, CHO*H*), 4.87 (d, *J* = 4.6 Hz, 1, CHO*H*), 4.66 (unsymmetrical q, 1, C*H*N), 4.49 (t, *J* = 5.2 Hz, 1, CH_2O*H*), 4.01 (m, 2, C*H*OH's), 3.42–3.68 (pair of m centered at 3.48 and 3.63, total 2, C$H_a$$H_b$OH), 2.17 (m, 1, CHC$H_a$$H_b$CHN), 2.07 (m, 1, C*H*C$H_a$$H_b$CHN), 1.81 (unsymmetrical q, 1, CHC$H_a$$H_b$CHN); the signals at 10.50, 6.36, 4.93, 4.87, and 4.49 disappear upon exchange with D_2O, while the signals at 4.01, 3.63, and 3.48 simplify. Anal. ($C_{11}H_{15}N_5O_4·H_2O$): C, H, N.

(±)-(1α,2α,3α,5α)-3-(2,6-Diamino-9*H*-purin-9-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (10). Chloropurine **8** (0.228 g, 0.76 mmol) was added to a stainless steel bomb and covered with 2 mL of MeOH. To the resulting solution was added excess liquid ammonia. The sealed bomb was heated to 75 °C for 57 h. Evaporation of the ammonia left a solid, brown residue which was dissolved in hot H_2O, a small quantity of insoluble material

was remofed by filtration, and then the filtrate was adjusted to a volume of approximately 15 mL. Light brown crystals of **10** resulted (0.110 g, 51.7%). Mp: 272–273 °C dec. TLC: R_f = 0.39 (solvent C). UV: λ_{max} 290, 252, 218 nm in 0.1 N HCl; 280, 254, 215 nm in H_2O; 280, 255, 220 nm in 0.1 N NaOH. EIMS (330 °C, 30 eV): m/z 280 (M^+, 56.3), 263 (M – OH, 16.1), 249 (M – CH_2OH, 18.4), 221 (9.5), 207 (8.7), 206 (12.5), 205 (16.4), 193 (15.0), 177 (51.5), 164 (7.7), 163 (9.9), 151 (P + 2 H, 39.4), 150 (P + H, 100), 108 (13.5). IR (KBr): 3508, 3500–3000 (br) with maxima at 3402 (s), 3318 (s) and 3213 (s), 2959, 2917, 1681, 1635 (sh), 1604 (s), 1490, 1475, 1456, 1448, 1416 (s), 1354, 1344, 1313, 1229, 1131, 1066, 1028, 1016, 1006, 992, 789, 633. ^{1}H NMR (DMSO-d_6): δ 7.76 (s, 1, C_8H purine), 6.60 (br s, 2, NH_2), 5.68 (br s, 2, NH_2), 5.02 (partially resolved d, J = 4.0 Hz, 1, CHOH), 4.91 (d, J = 5.6 Hz, 1, CHOH), 4.70 (unsymmetrical q, 1, CHN), 4.47 (t, J = 5.2 Hz, 1, CH_2OH), 4.02 (m, 2, CHOH's), 3.44–3.68 (pair of m centered at 3.49 and 3.64, total 2, CH_aH_bOH), 2.02–2.21 (m, 2, $CHCH_aH_bCHN$), 1.86 (unsymmetrical q, 1, $CHCH_aH_bCHN$); the signals at 6.60, 5.68, 5.02, 4.91, and 4.47 disappear upon exchange with D_2O, while the signals at 4.02, 3.64, and 3.49 simplify. Anal. ($C_{11}H_{16}N_6O_3 \cdot 0.5H_2O$): C, H, N.

(±)-(1α,2α,3α,5α)-3-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (11). Triamino derivative **7** (0.545 g, 1.88 mmol) was dissolved in 15 mL of H_2O and 3.0 mL of glacial acetic acid and then cooled to 0 °C under nitrogen. To this was added a solution of 0.17 g (2.46 mmol) of sodium nitrite in 10 mL of H_2O dropwise over 10 min. The mixture was stirred at 0 °C for 2 h during which time a yellow solid began to precipitate from the solution. It proved most efficient not to isolate this solid separately. After stirring at room temperature for an additional 1 h, the solvent was removed in vacuo (oil pump, 1–2 Torr, T < 35 °C) and the residue was chromatographed on silica with solvent A to give 0.450 g (79.5%) of **11** [TLC (fluoresces under UV light): R_f = 0.46 (solvent B); 0.62 (solvent C)]. Analytically pure material as a yellow-green solid was obtained by recrystallization from absolute EtOH. Mp: 181–185 °C dec, bubbles and turns brown, residue turns black at 220–223 °C. UV: λ_{max} 316, 250 (slsh), 225 nm in 0.1 N HCl; 315, 252 (slsh) nm in H_2O, 315 248 (slsh), 223 nm in 0.1 N NaOH. FABMS (thioglycerol·HCl): m/z 301 [(M + H)$^+$]; 299 [(M – H)$^-$], 335 [(M + Cl)$^-$]. IR (KBr): 3400–3050 (br) with maxima at 3388 (s), 3290 (s) and 3206 (s), 2952, 2924, 1632 (s), 1610 (s), 1571 (s), 1509, 1427, 1409, 1395, 1381, 1326, 1250, 1225, 1176, 1144, 1116, 1089, 1051, 1004, 927, 788. ^{1}H NMR (DMSO-d_6): δ 7.60 (br s, 2, NH_2), 4.97 (m, 1, CHN), 4.76 (d, J = 6.4 Hz, 1, CHOH), 4.57 (d overlaps next resonance, J = 6.0 Hz, CHOH), 4.55 [partially overlapped t, J = 5.0 Hz, 2 (includes resonance at δ 4.57), CH_2OH], 4.22 [m (approximate q), 1, CHOH], 4.09 (unsymmetrical q, 1, CHOH), 3.50–3.73 (pair of m centered at 3.55 and 3.68, total 2, CH_aH_bOH), 2.61 (unsymmetrical q, 1, $CHCH_aH_bCHN$), 2.11–2.28 (m, 2, $CHCH_aH_bCHN$); the signals at 7.60, 4.76, 4.57, and 4.55 disappear upon exchange with D_2O, while the signals at 4.22, 4.09, 3.68, and 3.55 simplify. Anal. ($C_{10}H_{13}N_6O_3Cl$): C, H, N.

(±)-5-Amino-3,6-dihydro-3-[(1α,2α,3α,4α)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (12). A solution of 0.196 g (0.652 mmol) of **11** in 25 mL of 1 N HCl was heated to reflux for 5 h. The solvent was removed in vacuo to leave a yellow solid. This was recrystallized from H_2O to give 0.0694 g (37.7%) of **12** as a light pink powder. Mp: (shrivels 175–178 °C) 226–228 °C dec; TLC: R_f = 0.44 (solvent C). UV: λ_{max} 273 (sh), 203 nm in 0.1 N HCl; 271 (sh), 253, 203 nm in H_2O; 278, 252 (sh), 223 nm in 0.1 N NaOH; FABMS (thioglycerol): m/z 283 [(M + H)$^+$], 281 [(M – H)$^-$]. IR (KBr): 3600–3000 (br) with maxima at 3536, 3389 (s) and 3163 (s), 2945, 2896, 1704 (s), 1661 (s), 1629 (s), 1582 (s), 1537, 1454, 1367, 1301, 1204, 1143, 1117, 1056, 1023, 976, 813, 793, 739, 720, 684, 636. ^{1}H NMR (DMSO-d_6): δ 10.90 (br s, 1, HNC=O purine), 6.86 (br s, 2, NH_2), 4.86 [m (approximate q), 1, CHN], 4.73 (d, J = 6.4 Hz, 1, CHOH), 4.63 (d, J = 6.9 Hz, 1, CHOH), 4.54 (t, J = 5.1 Hz, 1, CH_2OH), 4.17 [m (approximate q), 1, CHOH], 4.04 (unsymmetrical q, 1, CHOH), 3.47–3.72 (pair of m centered at 3.52 and 3.66, total 2, CH_aH_bOH), 2.45 (partially obscured by solvent, $CHCH_aH_bCHN$), 2.08–2.23 (m, 2, $CHCH_aH_bCHN$); the signals at 10.90, 6.86, 4.73, 4.63, and 4.54 disappear upon exchange with D_2O, while the signals at 4.17, 4.04, 3.66, and 3.52 simplify and the signal at 2.45 appears separate from the solvent as an unsymmetrical quartet. Anal. ($C_{10}H_{14}N_6O_4 \cdot H_2O$): C, H, N.

(±)-(1α,2α,3α,5α)-3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-5-(hydroxymethyl)-1,2-cyclopentanediol (13). As the solid, 0.0737 g (0.245 mmol) of **11** was added to a stainless steel bomb and then treated with excess liquid ammonia. The bomb was sealed and heated to 75 °C for 46 h. Evaporation of the ammonia left a generally white, solid residue which was dissolved in hot H_2O and filtered and the filtrate was adjusted to approximately 10 mL. The light yellow solid that separated was the desired product, **13** (0.0374 g, 54.2%). Mp: 263–266 °C dec. TLC: R_f = 0.48 (solvent C). UV: λ_{max} 298 (sh), 278, 253, 213 nm in 0.1 N HCl; 287, 259, 223 nm in H_2O; 286, 259 (sh), 226 nm in 0.1 N NaOH. EIMS (330 °C, 30 eV): m/z 281 (M^+, 10.2), 264 (M – OH, 4.7), 224 (9.2), 206 (10.8), 194 (46.0), 176 (16.3), 152 (P + 2 H, 100), 151 (P + H, 29.2), 150 (P, 32.3), 126 (26.8), 125 (21.8), 110 (17.0). FABMS (thioglycerol): m/z 282 [(M + H)$^+$], 280 [(M – H)$^-$]. IR (KBr): 3485, 3445, 3318, 3149 (br s,), 2938, 2910, 1677, 1630, 1605 (s), 1495, 1421, 1357, 1330, 1303, 1149, 1092, 1020, 923, 786, 747, 685, 664, 643, 563. ^{1}H NMR (DMSO-d_6): δ 7.20–7.80 (br s, 2, NH_2), 6.33 (br s, 2, NH_2), 5.05 (d, J = 7.9 Hz, 1, C_3HOH), 4.97 (unsymmetrical q, 1, CHN), 4.73 (d, J = 6.2 Hz, 1, C_2HOH), 4.48 (t, J = 5.2 Hz, 1, CH_2OH), 4.20 (br q, J = 6.1 Hz, 1, C_2HOH), 4.02 (m, 1, C_3HOH), 3.47–3.74 (pair of m centered at 3.52 and 3.69, total 2, CH_aH_bOH), 2.38 (br unsymmetrical q, 1, $CHCH_aH_bCHN$), 2.18 (m, 1, $CHCH_aH_bCHN$), 2.06 (m, 1, $CHCH_aH_bCHN$); the signals at 7.20–7.80, 6.33, 5.05, 4.73, and 4.48 disappear upon exchange with D_2O, while the signals at 4.20, 4.02, 3.69, and 3.52 simplify. Anal. ($C_{10}H_{15}N_7O_3$): C, H, N.

Antiviral Evaluation. HSV-1 and IV-A. The methods and procedures used to evaluate compounds **8–13** for antiviral activity against these viruses have been described previously.[25,26] These compounds were tested for inhibition of cytopathogenic effects (CPE) produced by strain E-377 of HSV-1 replicating in African green monkey kidney (Vero) cells of influenza virus type A_0/PR/8/34 using Madin–Darby canine kidney host cell cultures.

HCMV. Compound **9** was evaluated for its ability to selectively inhibit HCMV replication in vitro by utilizing a virus yield reduction assay. Details of the method, which were followed with only slight modification, were communicated earlier.[9] The challenge virus, the Ad169 strain of HCMV, was passaged, titrated, and assayed in human diploid embryonic lung (MRC5) cells. The positive control was the known, active compound 9-1,3-dihydroxy-2-(propoxymethyl)guanine (DHPG). Drug cytotoxicity was determined quantitatively by a method based on the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial enzymes of viable host cells to MTT formazan.[30]

HIV. Compounds **8–13** were tested for their ability to inhibit the growth of the virus in both CEM-V and MT-2 cell lines by using a microculture tetrazolium assay method to quantitate viral pathogenicity. Details of this procedure have already been reported.[13] Concentrations from 1.25 × 10^{-5} to 125 μg/mL were investigated for compounds **8–12**, and concentrations from 6.25 × 10^{-6} to 62.5 μg/mL were tested for compound **13**.

Acknowledgment. We gratefully acknowledge the support of this work by the National Cancer Institute, Grant CA 23263. We wish to thank Jay Brownell for the large-scale preparation of synthetic starting materials and for conducting the P-388 mouse leukemia cytotoxicity studies. We also thank Drs. William M. Shannon and Gussie Arnett of Southern Research Institute for the antiviral screening of these compounds against HSV-1, IV-A, and CMV. Testing against HIV was carried out under the auspices of the NCI's Developmental Therapeutics Program.

Registry No. 1, 49805-30-3; 2, 87419-91-8; 3, 56-05-3; 4, 125073-22-5; 5, 125073-23-6; 6, 50796-89-9; 7, 125073-24-7; 8, 125073-25-8; 9, 125073-26-9; 10, 125073-27-0; 11, 125073-28-1; 12, 125073-29-2; 13, 125073-30-5.

(30) Mosman, T. *J. Immunol. Methods* **1983**, *65*, 55.