Synthesis and Antiviral Activity of Enantiomeric Forms of Cyclobutyl Nucleoside Analogues

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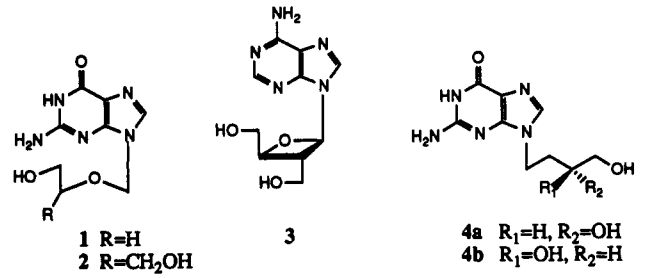
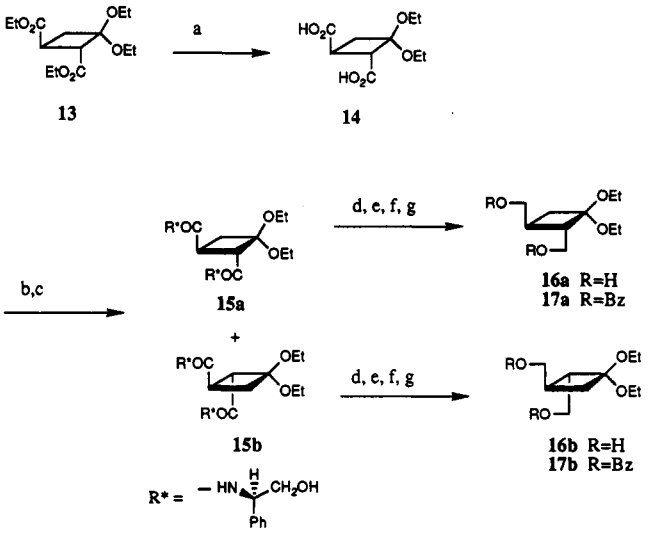
The syntheses of the enantiomeric cyclobutyl guanine nucleoside analogues [1*R*-1α,2β,3α]- and [1*S*-1α,2β,3α]-2-amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-6*H*-purin-6-one (**7** and **8**, respectively) and the enantiomeric cyclobutyl adenine analogues [1*R*-1α,2β,3α]- and [1*S*-1α,2β,3α]-6-amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]purine (**9** and **10**, respectively) are described. *trans*-3,3-Diethoxy-1,2-cyclobutanedicarboxylic acid (**14**) was coupled with *R*-(−)-2-phenylglycinol to provide a mixture of diastereomeric bis-amides, **15a** and **15b**, which was readily separated by crystallization. Conversion of each bis-amide to the corresponding diol enantiomer, **16a** and **16b**, respectively, was effected by a facile three-step sequence in high overall yield. Homochiral diol **16a** was converted in a straightforward manner to **7** and **9**, and homochiral diol **16b** was similarly converted to the corresponding optical isomers **8** and **10**. Compounds **7** and **9**, which mimic the absolute configuration of natural nucleosides, are highly active against a range of herpesviruses in vitro while the isomers of opposite configuration, **8** and **10**, are devoid of antiherpes activity. The corresponding triphosphates of **7** and **8** (**7**-TP and **8**-TP) were prepared enzymatically. Compound **7**-TP selectively inhibits HSV-1 DNA polymerase, compared to human (HeLa) DNA polymerase, while **8**-TP is much less inhibitory than **7**-TP against both types of enzymes. Compounds **7** and **9** are efficacious in a mouse cytomegalovirus model infection.

The design of potent and selective antiviral agents remains one of the central goals of antiviral medicinal chemistry.[1] In the search for therapeutic agents against herpesviruses and HIV (human immunodeficiency virus), much attention has focused on the preparation of analogues of natural nucleosides as antimetabolites. Such analogues have been modified either in the carbohydrate portion of the molecule, the base portion, or both, and display large variations in antiviral activities and toxicities to the host.[1-3] Certain nucleosides that have been modified solely in the carbohydrate portion demonstrate selective and potent antiviral activity. Examples include acyclovir (**1**), which has proven value in the therapy of several classes of herpes infections,[3] ganciclovir (**2**), which is used to treat cytomegalovirus infections in immunocompromised patients,[4] and the recently isolated natural product oxetanocin (**3**), which shows activity against HIV (Chart I).[5] Not surprisingly, chirality also plays a role in the antiviral activity of nucleoside analogues that contain one or more asymmetric centers. For example, the *R* isomer of 9-(3,4-dihydroxybutyl)guanine (**4a**) is 5-fold more active than the corresponding *S* isomer (**4b**) in in vitro assays.[6] Other descriptions of the dependence of activity

Chart I



Scheme I[a]



[a] (a) KOH, MeOH, H_2O; (b) *R*-(−)-2-phenylglycinol, DCC; (c) crystallize from CH_2Cl_2 to obtain **15a** and then from Et_2O/CH_2Cl_2 to obtain **15b**; (d) *tert*-butyldimethylsilyl chloride, imidazole; (e) N_2O_4, NaOAc, CCl_4; (f) $LiBH_4$, THF; (g) benzoyl chloride, pyridine.

(1) (a) Hirsch, M. S.; Kaplan, J. C. In *Fields Virology*, 2nd ed.; Fields, B. N., Knipe, D. M., Eds.; Raven: New York, 1990; pp 441–468. (b) Rachlis, A. R. *Clin. Dermatol.* **1989**, *7*, 69–79. (c) Crowe, S.; Mills, J. *Dermatologic Clinics* **1988**, *6*, 521–537.

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(3) (a) Thiers, B. H. *Dermatologic Clinics* **1990**, *8*, 583–587. (b) O'Brien, J. J.; Campolir, D. M. *Drugs* **1989**, *37*, 233–309. (c) Reines, E. D.; Gross, P. A. *Medical Clinics of North America* **1988**, *72*, 691–715.

(4) Fanhavar, P.; Nahata, M. C.; Brady, M. T. *J. Clin. Pharm. Ther.* **1989**, *14*, 329–340.

(5) (a) Hoshino, H.; Shimizu, N.; Shimada, N.; Takita, T.; Takeuchi, T. *J. Antibiot.* **1987**, *40*, 1077–1078. (b) Shimada, N.; Saito, S.; Hasegawa, S.; Takahashi, K.; Fujii, A.; Takita, T.; Seki, J.; Hoshino, H.; Nishiyama, Y.; Nagahata, T.; Matsubara, K. 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, 1988; Abstract 1008.

(6) Datema, R.; Johansson, N. G.; Oberg, B. *Chem. Scr.* **1986**, *26*, 49–53.

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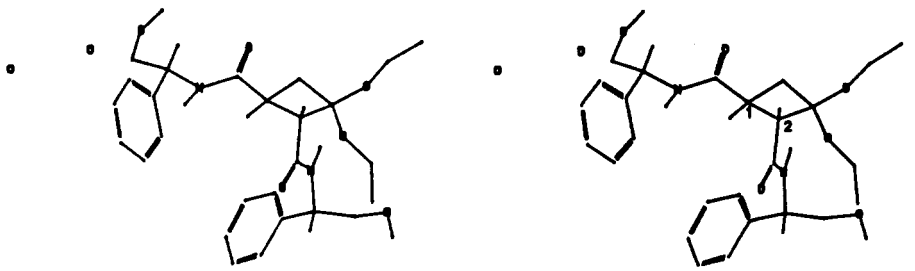
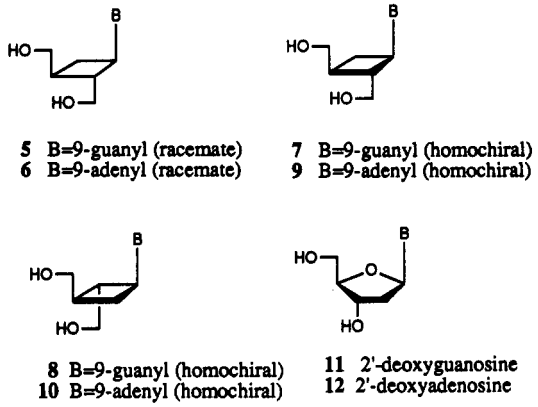


Figure 1. X-ray crystal structure of compound **15a**.

Table I. Antiherpes Activities of Enantiomers 7–10, Racemates 5 and 6, and Ganciclovir (2) in Tissue Culture

virus (strain)	ID_{50}, μM						
	7	8	5	9	10	6	2 (ganciclovir)
HSV-1 (Schooler)	0.02–0.04	>377	0.08–0.2	0.8–2	>400	8	0.02–0.04
HSV-2 (186)	0.02–0.04	>377	0.04–0.08	0.8–2	>400	4–8	0.04
VZV (Ellen)	0.02–0.08	>377	0.2	0.8–2	>400	0.8–2	0.8–2
HCMV (AD 169)	0.8–2	>377	2–4	≤0.4	>400	0.8	0.4–2
MCMV (Smith)	0.2–0.4		1–2	0.4–0.8		2	4

Chart II



on the enantiomeric form of nucleoside analogues have been reported.[7] Recently we reported the synthesis and in vitro antiherpes activity of the novel racemic cyclobutyl nucleosides **5** (SQ-33,054) and **6** (SQ-33,242) (Chart II).[8,9]

We felt it important to synthesize and test separately for antiviral activity each enantiomer corresponding to these racemates, i.e. the enantiomers **7** and **8** that comprise racemate **5** and the enantiomers **9** and **10** that comprise racemate **6**.[10] The relationship of the hydroxy and base moieties in enantiomers **7** and **9** corresponds to that in the naturally occurring nucleosides 2′-deoxyguanosine (**11**) and 2′-deoxyadenosine (**12**), respectively. In the optical antipodes, **8** and **10**, the hydroxymethyl group at the 2-position of the cyclobutyl ring does not spatially correspond with the 3′-hydroxy group of the natural nucleosides. Herein we wish to describe an efficient synthetic route to the individual enantiomers **7–10** and a comparison of the in vitro antiherpes activity of these enantiomers. In addition, the evaluation of the corresponding triphosphates of **7** and **8** as inhibitors of herpes simplex virus type 1 (HSV-1) DNA polymerase and of human DNA polymerase is described. Finally, efficacy studies of enantiomers **7** and **9** in a murine cytomegalovirus (MCMV) model infection are presented.

Chemistry

Racemic cyclobutane diester **13**[8,11] (Scheme I) served as starting material in the synthesis of compounds **7–10**. This diester was saponified to provide the corresponding diacid **14** in 92% yield. The diacid was then coupled with *R*-(−)-2-phenylglycinol in the presence of DCC to afford a mixture (ca. 1:1) of diastereomeric bis-amides **15a** and **15b** in 67% yield. The diastereomeric mixture was then separated by crystallization to afford good recoveries of each

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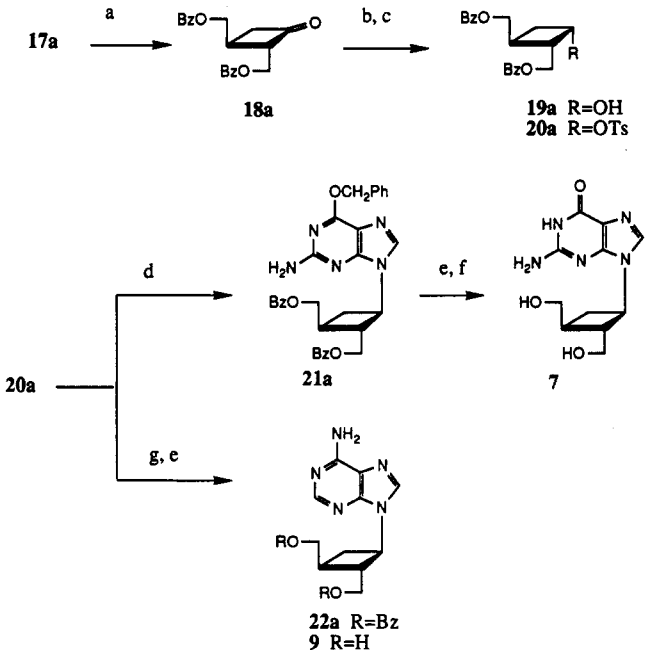
(8) (a) Slusarchyk, W. A.; Young, M. G.; Bisacchi, G. S.; Hockstein, D. R.; Zahler, R. *Tetrahedron Lett.* **1989**, 6453–6456. (b) Slusarchyk, W. A.; Bisacchi, G. S.; Hockstein, D. R.; Young, M. G.; Field, A. K.; McGeever-Rubin, B.; Tuomari, A. V.; Zahler, R. 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, 1989; Abstract 1330. (c) Field, A. K.; Toumari, A. V.; McGeever-Rubin, B.; Terry, B. J.; Mazina, K. E.; Haffey, M. L.; Hagen, M. E.; Clark, J. M.; Braitman, A.; Slusarchyk, W. A.; Young, M. G.; Zahler, R. *Antiviral Res.* **1990**, *13*, 41–52.

(9) Recently, other laboratories have independently described the synthesis of the racemic compounds **5** and/or **6**: (a) Norbeck, D. W.; Kern, E.; Hayashi, S.; Rosenbrook, W.; Sham, H.; Herrin, T.; Plattner, J. J.; Erickson, J.; Clement, J.; Swanson, R.; Shipkowitz, N.; Hardy, D.; Marsh, K.; Arnett, G.; Shannon, W.; Broder, S.; Mitsuya, H. *J. Med. Chem.* **1990**, *33*, 1281–85. (b) Honjo, M.; Maruyama, T.; Sato, Y.; Horii, T. *Chem. Pharm. Bull.* **1989**, *37*, 1413–1415. (c) Katagiri, N.; Sato, H.; Kaneko, C. *Chem. Pharm. Bull.* **1990**, *38*, 288–290.

(10) Recently, another laboratory has described the synthesis and in vitro antiviral activity of the enantiomers **7** and **9**: Ichikawa, Y.; Narita, A.; Shiozawa, A.; Hayashi, Y.; Narasaka, K. *J. Chem. Soc., Chem. Commun.* **1989**, 1919–1921. A recent patent also has described the preparation of enantiomer **7** (no biological data): Norbeck, D. W.; Plattner, J. J.; Rosen, T. J.; Pariza, R. J.; Sowin, T. J.; Garmaise, D. L.; Hannick, S. M. Eur. Pat. Appl. EP 366059, 1990.

(11) Brannock, K. C.; Burpitt, R. D.; Thweatt, J. G. *J. Org. Chem.* **1964**, *29*, 940.

Scheme II[a,b]



[a] H_2SO_4, acetonitrile/water; (b) LS-Selectride; (c) TsCl, pyridine; (d) 2-amino-6-(benzyloxy)purine, K_2CO_3, 18-crown-6; (e) NaOMe, MeOH; (f) 1 N HCl; (g) adenine, K_2CO_3, 18-crown-6. [b] The corresponding enantiomeric series of compounds, **18b–22b**, **8**, and **10** was prepared analogously starting from **17b**.

pure diastereomer **15a** and **15b**. The configuration of diastereomer **15a** was ascertained by X-ray crystallographic analysis (Figure 1). The absolute stereochemistry at C1,C2 was assigned as *S,R* relative to the known *R* configuration of the (−)-2-phenylglycinol used in the synthesis. Both amide moieties of **15a** were converted to hydroxymethyl groups by an efficient three-step procedure. First, the hydroxy groups of **15a** were protected as *tert*-butyldimethylsilyl ethers. Treatment of this crude silyl-protected bis-amide with dinitrogen tetroxide in carbon tetrachloride buffered with sodium acetate[12] afforded the corresponding bis-*N*-nitrosoamide which was then reduced immediately with lithium borohydride in THF to afford homochiral diol **16a** (78% overall yield for 3 steps). Initial attempts to reduce the bis-*N*-nitrosoamide with sodium borohydride in glyme according to the procedure of Saavreda[13] gave only poor yields of the desired diol **16a**. It was speculated that the low yields were due to a slow rate of reduction of the *N*-nitrosoamide moieties by the heterogeneous mixture of sodium borohydride in glyme, which allowed for competing thermal decomposition.[14] The use of the soluble lithium borohydride in THF provided dramatically higher yields of **16a**. Subsequent treatment of **16a** with benzoyl chloride in pyridine provided homochiral dibenzoate ester **17a**.

By employing similar chemistry, bis-amide diastereomer **15b** was converted to the homochiral dibenzoate ester **17b**. The dibenzoate esters **17a** and **17b** were assessed for enantiomeric purity by HPLC, and each was found to have an enantiomeric excess of greater than 99%. Dibenzoate **17a** was then converted (Scheme II) to the homochiral guanine and adenine nucleoside analogues, **7** and **9**, respectively, employing chemistry similar to that described for the racemates **5** and **6**.[8] The dibenzoate **17b** was likewise converted (Scheme II) to the corresponding homochiral guanine and adenine nucleoside analogues **8** and **10**, respectively.

Table II. Subcutaneous Efficacy of **7** and **9** in a Mouse Cytomegalovirus Infection

compd	mg/kg per day	survivors, alive/total	PD_{50},[a] mg/kg per day	mean day of death for total dead ± SD
7				
	100.0	10/10	10 ± 5	
	50.0	9/10		6.0 ± 0.0
	25.0	9/10		4.0 ± 0.0
	12.5	5/10		5.4 ± 1.1[b]
9				
	100.0	10/10	9 ± 3	
	50.0	10/10		
	25.0	10/10		
	12.5	4/10		8.8 ± 5.0[b]
	6.3	4/9		4.4 ± 0.5
2 (ganciclovir)				
	100.0	10/10	<12.5	
	50.0	10/10		
	25.0	10/10		
	12.5	7/10		5.7 ± 0.6[b]
placebo				
		2/10		3.4 ± 1.1

[a] Probit analysis (±SE). [b] $P < 0.05$. Sample is significantly different from placebo. Statistical analysis was computed only for groups with 2 or more deaths.

Biological Results and Discussion

The results of plaque-reduction assays against herpesviruses for enantiomers **7–10** are displayed in Table I and are compared to previous results[8] for the racemates **5** and **6** and for ganciclovir (**2**). As shown, the enantiomer of "natural configuration" (**7** or **9**), is as active or slightly more active than the corresponding racemate whereas the enantiomer of opposite configuration (**8** or **10**) displays no detectable antiviral activity. Compound **7** is comparable in activity to ganciclovir against HSV-1, HSV-2, and human cytomegalovirus (HCMV) and is more active than ganciclovir against varicella zoster virus (VZV). Compound **9** is comparable in activity to ganciclovir against VZV and HCMV. Also, both **7** and **9** are somewhat more active than ganciclovir against MCMV. None of the compounds described were toxic to intact cell monolayers at the highest concentrations tested (300 μM). The spatial configuration of the hydroxymethyl group at the 2-position of the cyclobutyl ring in these nucleoside analogues is seen as a critical determinant for antiherpes activity. The required configuration is that which more closely mimics the configuration of the 3′-hydroxy group of the natural nucleosides.

The antiherpetic effect of a number of guanine-containing nucleoside analogues is believed to be due to the inhibition of viral DNA polymerase by the corresponding nucleoside analogue triphosphate metabolites.[15] The triphosphates of the "natural" enantiomer **7** and its an-

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(13) Saavreda, J. E. *J. Org. Chem.* **1979**, *44*, 860–861.
(14) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6014–6022.
(15) (a) Reardon, J. E.; Spector, T. *J. Biol. Chem.* **1989**, *264*, 7405–7411. (b) Field, A. K.; Davies, M. E.; DeWitt, C.; Perry, H. C.; Liou, R.; Germershausen, J.; Karkas, J. D.; Ashton, W. T.; Johnston, D. B. R.; Tolman, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4139–4143. (c) Smee, D. F.; Martin, J. C.; Verheyden, J. P. H.; Matthews, T. R. *Antimicrob. Agents Chemother.* **1983**, *23*, 676–682. (d) Larsson, A.; Stenberg, K.; Ericson, A.-C.; Haglund, U.; Yisak, W.-A.; Johansson, N. G.; Oberg, B.; Datema, R. *Antimicrob. Agents Chemother.* **1986**, *30*, 598–605. (e) Datema, R.; Ericson, A.-C.; Field, H. J.; Larsson, A.; Stenberg, K. *Antiviral Res.* **1987**, *7*, 303–316. (f) Karkas, J. D.; Ashton, W. T.; Canning, L. F.; Liou, R.; Germershausen, J.; Bostedor, R.; Arison, B.; Field, A. K.; Tolman, R. L. *J. Med. Chem.* **1986**, *29*, 842–848.

Table III. Oral Efficacy of 9 in a Mouse Cytomegalovirus Infection

compd	mg/kg per day	survivors, alive/total	PD_{50},[a] mg/kg per day	mean day of death for total dead ± SD
9				
	100.0	10/10[b]	36 ± 6	
	50.0	6/10[b]		6.3 ± 3.9
	25.0	3/10		3.6 ± 1.4
	12.5	1/10		3.9 ± 0.8
2 (ganciclovir)				
	50.0	5/10	57 ± 24	4.4 ± 1.5
	25.0	1/10		4.8 ± 0.8[b]
	12.5	1/10		3.6 ± 0.5
placebo				
		0/10		3.5 ± 1.3

[a] Probit analysis (±SE). [b] $P < 0.05$. Sample is significantly different from placebo. Statistical analysis was computed only for groups with 2 or more deaths.

tipode 8 were prepared enzymatically and were evaluated as inhibitors of HSV-1 DNA polymerase and of human DNA polymerase derived from HeLa cells. The triphosphate of 7 is a selective inhibitor of the HSV-1 DNA polymerase, displaying a K_i of 17 ± 3 nM, compared to a K_i of 410 ± 40 nM against human DNA polymerase activity. The triphosphate of 8 was much less inhibitory against both enzymes, displaying K_i values of 400 μM and >250 μM against the HSV-1 polymerase and human polymerase, respectively. The large difference in antiherpes activities between the enantiomers 7 and 8 is reflected on a molecular level by the large difference in the inhibition of HSV-1 DNA polymerase by their corresponding triphosphates. More extensive studies on the enzymatic phosphorylation behavior of 7 and 8 and the interactions of their corresponding triphosphates with herpes DNA polymerase will be published elsewhere.[16]

Tables II and III display the results of treatment with compounds 7, 9, and ganciclovir (2) in a mouse cytomegalovirus infection. When administered subcutaneously (Table II), both 7 and 9 are efficacious in the therapy of a lethal MCMV infection as indicated by the PD_{50} values of 10 and 9 mg/kg per day, respectively. Ganciclovir (PD_{50} of <12.5 mg/kg per day) was also efficacious in this infection. PD_{50} values for subcutaneously administered ganciclovir in three earlier MCMV model infections ranged from 8–13 mg/kg per day (data not shown). The efficacies of 7 and 9 are similar to that of 2, although a quantitative comparison was not determined from these data. When administered orally (Table III), 9 is also efficacious in the treatment of this infection, displaying a PD_{50} of 36 mg/kg per day. Ganciclovir (PD_{50} of 57 mg/kg per day) was also efficacious. PD_{50} values for orally administered ganciclovir in two earlier MCMV model infections ranged from 50–65 mg/kg per day (data not shown). No overt toxic manifestations were noted in the groups treated with the highest level of drug (100 mg/kg for 5 days). More extensive in vivo efficacy studies of 7 will be published elsewhere.[17]

Experimental Section

Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were obtained with a JEOL GX-270 or GSX-270 270MHz spectrometer with TMS as internal reference, unless otherwise specified. Chemical shifts are expressed in δ units (parts per million). Ultraviolet spectra were recorded on a Shimadzu UV-265. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Melting points (uncorrected) were determined on a Thomas-Hoover capillary apparatus. Diaion CHP20P is a reverse-phase resin for chromatography and was obtained from Mitsubishi Chemical Industries Limited. Analytical data for new compounds are listed in Table IV.

***trans*-3,3-Diethoxy-1,2-cyclobutanedicarboxylic Acid, Diethyl Ester (13).** A modification of a published procedure[11] was employed. A mixture of diethylketene acetal[18] (38.35 g, 0.331 mol) and diethyl fumarate (56.28 g, 0.327 mol) in 100 mL of *tert*-butyl alcohol was heated at 84 °C under argon for 72 h. The reaction mixture was distilled (bp 113–125 °C, 1 mmHg; lit.[11] bp 103–109 °C, 0.2 mmHg) to afford 50.4 g (54%) of 13: ^1H NMR ($CDCl_3$) δ 1.20 (m, 12 H), 2.24 (ddd, J = 1.1, 8.8, 12.1 Hz, 1 H), 2.58 (dd, J = 11, 12.1 Hz, 1 H), 3.3–3.8 (m, 6 H), 4.16 (m, 4 H).

***trans*-3,3-Diethoxy-1,2-cyclobutanedicarboxylic Acid (14).** To a solution of 13 (288 g, 1.0 mol) in 2 L of MeOH was added 2 L of 2 N KOH. The mixture warmed to 43 °C upon addition and was kept at this temperature for 1 h. The mixture was concentrated in vacuo to 2 L, extracted with Et_2O (2 × 0.5 L), and then cooled to 10 °C with an ice bath. This temperature was maintained while the pH was lowered to 1.8 with use of 3 N HCl. The cold slurry was extracted with EtOAc (2 × 1 L), saturated with NaCl, and extracted again with 1 L of EtOAc. The combined extracts were washed with 0.5 L of brine, dried (Na_2SO_4), and concentrated to give 213.5 g (92%) of 14 as a white solid: ^1H NMR (CD_3CN, reference CHD_2CN = 1.93 ppm) δ 1.11 (t, J = 7.4 Hz, 1 H), 1.14 (t, J = 7.4 Hz, 1 H), 2.14 (dd, J = 9.0, 12.7 Hz, 1 H), 2.55 (dd, J = 9.5, 12.7 Hz, 1 H), 3.15 (ddd, each J = 9 Hz, 1 H), 3.3–3.7 (m, 5 H), 9.0 (br s, 2 H).

[1*S*-[1α(*S),2β(*S**)]]-3,3-Diethoxy-*N*,*N*′-bis(2-hydroxy-1-phenylethyl)-1,2-cyclobutanedicarboxamide (15a) and [1*R*-[1α(*R**),2β(*R**)]]-3,3-Diethoxy-*N*,*N*′-bis(2-hydroxy-1-phenylethyl)-1,2-cyclobutanedicarboxamide (15b).** To a solution of 30 g (129 mmol) of 14 in 250 mL of CH_2Cl_2 was added 46 g (336 mmol) of *R*-(−)-2-phenylglycinol under argon. The mixture was stirred until the *R*-(−)-2-phenylglycinol had largely dissolved and then cooled to 5 °C, and 60 g (291 mmol) of 1,3-dicyclohexylcarbodiimide was added. After stirring overnight at room temperature, the reaction mixture was poured into 1 L of Et_2O and the insolubles were removed by filtration. The filtrate was washed with 1 N HCl, brine, saturated aqueous $NaHCO_3$, and brine. After drying ($MgSO_4$) the solvent was removed to yield an oily solid residue which was chromatographed on silica gel. Sequential elution with EtOAc/hexane (7:3), EtOAc, and MeOH/EtOAc (5:95), afforded 40.6 g (67%) of a mixture (ca. 1:1) of diastereomers **15a** and **15b**. Separation of **15a** and **15b** was accomplished by sequential crystallization from CH_2Cl_2 to afford **15a** and CH_2Cl_2 (or MeOH)/Et_2O to yield **15b**. Thus, the above mixture was taken up in 300 mL of warm CH_2Cl_2 and was cooled to 5 °C. Filtration of the resulting solid yielded 11.7 g of **15a** (>97% diastereomeric purity by HPLC) as a white powder. Removal of solvent from the filtrate gave a foam which was taken up in 50 mL of CH_2Cl_2 and diluted to 1.5 L with Et_2O. The clear colorless solution was then reduced in volume to 1 L on the steam bath and allowed to stand. Filtration yielded 18.0 g of **15b** (>87% purity by HPLC) as a white powder. After one additional cycle of crystallization, second crops of **15a** and **15b** were obtained (purities comparable to those described above). Approximately 85% of the total diastereomeric mixture was thereby separated.

Recrystallization of 11.7 g of **15a** (>97% diastereomeric purity) from CH_2Cl_2 yielded, in two crops, 11.0 g of **15a** of 100% diastereomeric purity (by HPLC): ^1H NMR ($CDCl_3$) δ 1.16 (t, J = 7.0 Hz, 3 H), 1.27 (t, J = 7.0 Hz, 3 H), 2.36 (dd, J = 1.8, 4.7 Hz, 1 H), 2.55 (dd, J = 8.2, 12.3 Hz, 1 H), 2.65 (dd, J = 5.9, 7.0 Hz, 1 H), 2.72 (t, J = 5.9 Hz, 1 H), 3.13 (m, 2 H), 3.42 (m, 2 H), 3.59 (q, J = 7.0 Hz, 2 H), 4.85 (m, 4 H), 5.10 (m, 2 H), 7.2–7.6 (m, 10 H), 8.08 (d, J = 7.6 Hz, 1 H), 8.25 (d, J = 7.6 Hz, 1 H).

Similarly, recrystallization of the combined fractions of **15b** (>87% diastereomeric purity) from MeOH/Et_2O gave material of 100% diastereomeric purity: ^1H NMR ($CDCl_3$) δ 1.28 (t, J = 7.0 Hz, 3 H), 1.35 (t, J = 7.0 Hz, 3 H), 2.55 (m, 3 H), 3.00 (q, J

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Table IV. Analytical Data

compd	formula	anal.	mp, °C	$[\alpha]^{22}_D$	purification[a]
7	$C_{11}H_{15}N_5O_3 \cdot 1.10H_2O$	C,H,N	>270	-27.6 (c = 1.01, DMSO)	H_2O
8	$C_{11}H_{15}N_5O_3 \cdot 0.85H_2O$	C,H,N	313-315	+25.8 (c = 1.03, DMSO)	H_2O/CH_3CN
9	$C_{11}H_{15}N_5O_2$	C,H,N	149-151	-13.5 (c = 1.0, H_2O)	b
10	$C_{11}H_{15}N_5O_2$	C,H,N	152-153	+14.0 (c = 1.03, H_2O)	b
14	$C_{10}H_{16}O_6$	C,H	118-120		EtOAc
15a	$C_{26}H_{34}N_2O_6 \cdot 0.25H_2O$	C,H,N	128-129	-16.8 (c = 1.00, MeOH)	CH_2Cl_2
15b	$C_{26}H_{34}N_2O_6$	C,H,N	129-130	-11.1 (c = 0.637, MeOH)	$MeOH/Et_2O$
16a	$C_{10}H_{20}O_4 \cdot 0.10H_2O$	C,H	oil	-17.3 (c = 1.06, $CHCl_3$)	c
16b	$C_{10}H_{20}O_4$	d	oil	+17.9 (c = 5.79, $CHCl_3$)	c
17a	$C_{24}H_{28}O_6$	C,H	oil	+15.0 (c = 2.16, $CHCl_3$)	e
17b	$C_{24}H_{28}O_6$	d	oil	-15.7 (c = 2.26, $CHCl_3$)	e
18a	$C_{20}H_{18}O_5$	C,H	97-98	+24.9 (c = 1.04, $CHCl_3$)	CH_2Cl_2/Et_2O
18b	$C_{20}H_{18}O_5$	C,H	97-98	-24.7 (c = 2.03, $CHCl_3$)	CH_2Cl_2/Et_2O
19a	$C_{20}H_{20}O_5$	C,H	73-74	-13.9 (c = 1.18, $CHCl_3$)	Et_2O/hexane
19b	$C_{20}H_{20}O_5$	C,H	71-72	+12.6 (c = 1.00, $CHCl_3$)	Et_2O/hexane
20a	$C_{27}H_{26}O_7S$	C,H	90-91	+15.4 (c = 1.10, $CHCl_3$)	Et_2O/hexane
20b	$C_{27}H_{26}O_7S$	C,H	93-94	-13.7 (c = 1.03, $CHCl_3$)	Et_2O/hexane
21a	$C_{32}H_{29}N_5O_5$	C,H,N	oil	-12.5 (c = 1.16, $CHCl_3$)	c
21b	$C_{32}H_{29}N_5O_5$	d	oil	d	
22a	$C_{25}H_{23}N_5O_4$	C,H,N	150-152	-25.9 (c = 1.15, DMSO)	e
22b	$C_{25}H_{23}N_5O_4$	d	153-155	+24.4 (c = 1.01, DMSO)	e

[a] Recrystallization solvent for analytical sample, unless otherwise specified. [b] Triturated with CH_3CN. [c] Analytical sample was purified by semipreparative HPLC (Whatman partisil 10 M9/50 column), with EtOAc as eluent for **16a** and **16b**, and with EtOAc/hexane as eluent for **21a**. [d] Not determined. [e] Analytical sample was purified by flash chromatography on silica gel with EtOAc/hexane as eluent for **17a** and **17b**, and with $EtOH/CH_2Cl_2$ for eluent for **22a** and **22b**.

= 9.4 Hz, 1 H), 3.33 (t, J = 6.5 Hz, 1 H), 3.40 (d, J = 10.0, 1 H), 3.60 (m, 4 H), 3.95 (m, 4 H), 5.20 (m, 2 H), 7.2-7.6 (m, 10 H), 7.93 (d, J = 7.6 Hz, 1 H), 8.21 (d, J = 7.6 Hz, 1 H).

HPLC analyses of compounds **15a** and **15b** were performed by using a μ-porasil (Waters Assoc) column, eluting with 4% MeOH in chloroform (**15b** eluted before **15a**).

Crystal Structure Analysis. Crystallization from water afforded crystals of **15a** as the dihydrate. Unit cell parameters were obtained through a least-squares analysis of the experimental diffractometer settings of 25 high-angle reflections by using Cu Kα monochromatic radiation (λ = 1.5418 Å): a = 10.964 (1), b = 9.593 (1), c = 13.917 (1) Å, β = 106.49 (1)°, V = 1403.5 (4) Å^3. Space group $P2_1$ was assigned on the basis of systematic absences, $0k0$ ($k = 2n + 1$). The crystal density, D_{obs} = 1.22 g cm^{-3} was measured by flotation in carbon tetrachloride/hexane mixtures (D_{calc} = 1.20 for Z = 2, $C_{26}H_{34}N_2O_6 \cdot 2H_2O$). A total of 2796 reflections were measured on a CAD4 diffractometer at 23 °C with the $\theta = 2\theta$ variable scan technique and were corrected only for Lorentz polarization factors. Background counts were collected at the extremes of the scan for half the time of the scan. Two standard reflections were monitored for decay; no decrease of intensity was observed during the course of the measurements. All calculations utilized the SDP program package with minor local modifications.[19] The structure was solved by direct methods and refined on the basis of 1377 "observed" reflections with $I \geq 3\sigma(I)$. Although some hydrogen positions were evident on difference maps, they were introduced in idealized positions and their scattering was taken into account in the terminal stages of refinement. Least-squares weights, $w = \sigma^{-2}(F_o)$ were calculated with the assumption that $\sigma^2 = \epsilon^2 + (\rho I)^2$ where ϵ is the statistical counting error and ρ = 0.04. The function minimized in the least-squares refinements was $\sum_w(|F_o| - |F_c|)^2$. R is defined as $\sum||F_o| - |F_c||/\sum|F_o|$ while $R_w = [\sum_w(|F_o| - |F_c|)^2/\sum_w|F_o|^2]^{1/2}$. The refinements (assuming the absolute configuration shown in Figure 1, holding the y coordinate of O100 fixed and assuming anisotropic motion of all C,N,O atoms) converged at R = 0.071, R_w = 0.079. The final difference map contained no significant features. Tables of atomic coordinates, thermal parameters, and bond distances and angles are included as supplementary material.

(1*S*-*trans*)-3,3-Diethoxy-1,2-cyclobutanedimethanol (16a). To a solution of 23.5 g (50 mmol) of **15a** and 13.6 g (200 mmol) of imidazole in 100 mL of dry DMF at 5 °C was added 15.8 g (105 mmol) of *tert*-butyldimethylsilyl chloride under argon. After stirring at 5 °C for 1.5 h, the mixture was diluted to 600 mL with EtOAc and washed with 3% HCl, water, and brine. Drying (Na_2SO_4) and removal of solvent gave an oily solid which was taken up in 50 mL of EtOAc and diluted with 200 mL of hexane. The resulting slurry was filtered and the cake washed with 100 mL of EtOAc/hexane (1:4). Concentration of the filtrate in vacuo gave 33.6 g of crude product as a clear glass.

To a stirred, 5 °C solution of the above crude product (33.6 g) in 250 mL of dry CCl_4 containing 35 g (595 mmol) of anhydrous NaOAc was added 75 mL (207 mmol) of a 2.76 M solution of dinitrogen tetroxide in CCl_4 over 15 min. The resulting yellow mixture was stirred for another 15 min at 5 °C and then poured into a mixture of ice (500 mL), water (200 mL), sodium acetate trihydrate (100 g), and CH_2Cl_2 (500 mL). The mixture was shaken and the resulting yellow organic layer separated and washed with brine. Drying ($MgSO_4$) and concentration in vacuo at <15 °C gave 46.6 g of a thick yellow oil.

A solution of the entire sample of the above crude material in 200 mL of dry THF at 5 °C was transferred by cannula over 15 min into a 5 °C solution of lithium borohydride in THF (150 mL of a 2 M solution, 300 mmol) under argon. The clear orange mixture was allowed to stir at room temperature overnight. The resulting nearly colorless solution was cooled in an ice bath and was treated dropwise with 25 mL of water. The resulting slurry was diluted with 500 mL of Et_2O, and 100 mL of water was added to dissolve most of the solid. The layers were separated, and the aqueous layer was extracted with Et_2O and with EtOAc. The organic layers were combined, dried (Na_2SO_4), and concentrated to afford 33.8 g of a crude oil. Column chromatography on silica gel eluting with a hexane/EtOAc gradient afforded 8.0 g of **16a** as a colorless oil (78% overall from **15a**): ^{1}H NMR ($CDCl_3$) δ 1.186 (t, J = 7.1 Hz, 3 H), 1.190 (t, J = 7.1 Hz, 3 H), 1.73 (ddd, J = 1.1, 7.7, 12.1 Hz, 1 H), 2.10 (m, 1 H), 2.33 (m, 2 H), 3.04 (br s, 1 H), 3.18 (br s, 1 H), 3.47 (m, 5 H), 3.74 (m, 3 H).

(1*S*-*trans*)-3,3-Diethoxy-1,2-cyclobutanedimethanol, Dibenzoate Ester (17a). To a solution of **16a** (35.1 g, 172 mmol) in 250 mL of dry pyridine at 5 °C was added benzoyl chloride (59.7 mL, 514 mmol) over 0.5 h, and the mixture was stirred at room temperature for 2.5 h under argon. The mixture was cooled to 5 °C, 125 mL of water was added over 5 min, and the mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo, and the residue was concentrated from water (×3) and from toluene (×2) and was partitioned between EtOAc and water. The organic layer was washed with 10% sodium bisulfate (2 × 250 mL), water (4 × 250 mL), saturated sodium bicarbonate (2 × 250 mL), and water (3 × 250 mL). Drying (Na_2SO_4) and removal of solvent in vacuo afforded 83 g of crude **17a**, sufficiently pure for use in the next step: ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7 Hz, 3 H), 1.20 (t, J = 7 Hz, 3 H), 1.98 (ddd, J =

(19) SDP, Structure Determination Package, A. Frenz & Associates, College Station, TX.

1.5, 7, 11.6 Hz, 1 H), 2.38 (m, 1 H), 2.48 (dd, J = 9.5, 11.5 Hz, 1 H), 2.76 (ddd, each J = ca. 7 Hz, 1 H), 3.49 (m, 4 H), 4.43 (m, 3 H) 4.60 (dd, J = 7, 11.5 Hz, 1 H), 7.38 (m, 4 H), 7.52 (m, 2 H), 8.01 (m, 4 H).

The enantiomeric purity of **17a** was assessed by HPLC by using a Chiralcel OD column (Daicel Chemical Industries, Ltd.), eluting with 1.5% 2-propanol in hexane at a flow rate of 1.0 mL/min. An enantiomeric excess of >99% was found.

(2*S*-*trans*)-2,3-Bis[(benzoyloxy)methyl]cyclobutanone (18a). To a solution of crude **17a** (83 g) in 1.75 L of CH_3CN was added 660 mL of 0.5 N H_2SO_4. The mixture was stirred at room temperature for 17 h and was diluted with 5 L of EtOAc, washed with water (2 × 1 L), saturated sodium bicarbonate (1 L), water (2 × 1 L), and brine (1 L). The organic phase was dried (Na_2SO_4) and concentrated to a white solid in vacuo. Trituration with 400 mL of Et_2O and cooling at −30 °C for 2 h gave a solid which was filtered, washed with cold Et_2O, and dried in air to give 46.4 g (80% overall from **16a**) of **18a**: ^{1}H NMR ($CDCl_3$) δ 2.97 (m, 1 H), 3.05 (ddd, J = 3.3, 7.1, 17.6 Hz, 1 H), 3.24 (ddd, J = 2.2, 9.3, 17.6 Hz, 1 H), 3.66 (m, 1 H), 4.57 (m, 4 H), 7.42 (m, 4 H), 7.56 (m, 2 H), 8.00 (m, 4 H).

[1*S*-(1α,2β,3β)]-3-Hydroxy-1,2-cyclobutanedimethanol, 1,2-Dibenzoate Ester (19a). To a solution of **18a** (33.81 g, 100 mmol) in 440 mL of dry THF at −78 °C under argon was added 100 mL of 1 M LS-Selectride in THF (100 mmol) over 20 min. After stirring another 10 min at −78 °C, the mixture was warmed to room temperature and treated with 100 mL of saturated $NaHCO_3$ over 5 min. The resultant mixture was cooled in an ice/acetone bath and treated with 36.5 mL of 30% H_2O_2 at a rate so as to maintain the temperature at 25–30 °C. After the addition the mixture was diluted with 300 mL of water and extracted with 1.1 L of EtOAc. The organic phase was washed with water (×3), dried (Na_2SO_4), and concentrated to a colorless oil (35 g). The oil was chromatographed on a 1 L pad of silica gel eluting with EtOAc/hexane (2:1) to afford 27 g of pure **19a** as a colorless oil. An additional 3.4 g of pure **19a** was obtained following rechromatography of 4.4 g of slightly impure **19a** for a total of 30.4 g (90%): ^{1}H NMR ($CDCl_3$) δ 2.17 (m, 2 H), 2.72 (m, 2 H), 3.11 (d, J = 3.3 Hz, 1 H), 4.35 (m, 3 H), 4.48 (m, 1 H), 4.83 (dd, J = 8.8, 11.5 Hz, 1 H), 7.43 (m, 4 H), 7.56 (m, 2 H), 8.02 (m, 4 H).

[1*S*-(1α,2β,3β)]-3-[[(4-Methylphenyl)sulfonyl]oxy]-1,2-cyclobutanedimethanol, Dibenzoate Ester (20a). A solution of 7.14 g (21 mmol) of **19a** and 12.0 g (63 mmol) of tosyl chloride was heated at 57 °C for 7 h under argon, after which another 4 g (21 mmol) of tosyl chloride was added and heating continued for 1 h. Water (1.5 mL) was added, and the mixture was heated with stirring at 40–45 °C for 1.5 h. The volatiles were removed in vacuo, and the residue was concentrated from toluene, affording a solid. The solid was partitioned between water and EtOAc, and the organic layer was washed with water, aqueous $NaHCO_3$, and water. The organic solution was dried (Na_2SO_4) and concentrated to afford a thick oil which was triturated with pentane to afford 8.69 g (84%) of **20a** as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.32 (m, 1 H), 2.39 (s, 3 H), 2.52 (m, 1 H), 2.76 (m, 1 H), 2.99 (m, 1 H), 4.34 (m, 2 H), 4.51 (m, 2 H), 5.15 (m, 1 H), 7.2–8.0 (m, 12 H).

[1*S*-(1α,2β,3α)]-3-[2-Amino-6-(phenylmethoxy)-9*H*-purin-9-yl]-1,2-cyclobutanedimethanol, 1,2-Dibenzoate Ester (21a). A mixture of 2-amino-6-(benzyloxy)purine (13.4 g, 55.6 mmol), **20a** (18.33 g, 37.1 mmol), powdered anhydrous K_2CO_3 (10.22 g, 74.05 mmol), and 18-crown-6 (9.8 g, 37.1 mmol) in 495 mL of dry DMF was stirred at 110 °C for 21 h under argon. The mixture was cooled to room temperature and filtered, and the filtrate was concentrated in vacuo to an oil. This was partitioned between EtOAc and water, and the organic phase was washed twice with water, dried (Na_2SO_4) and concentrated to a foam. Chromatography on silica gel in EtOAc/hexane (1:1) gave 10.7 g (51%) of **21a** as a foam: ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 3 H), 3.34 (m, 1 H), 4.52 (m, 4 H), 4.68 (ddd, each J = ca. 8.8 Hz, 1 H), 4.84 (s, 2 H), 5.53 (s, 2 H), 7.68 (s, 1 H), 7.26–8.07 (m, 15 H).

[1*R*-1α,2β,3α]-2-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-6*H*-purin-6-one (7). To a solution of **21a** (20.0 g, 35.46 mmol) in 550 mL of MeOH was added 5 mL of 25% sodium methoxide in MeOH, and the mixture was heated with stirring at 40 °C for 2 h under argon. HCl (3N, 275 mL) was added, and the mixture was heated at 50 °C for 2 h. The mixture was concentrated to 100 mL and diluted with 100 mL of water. The solution was washed with Et_2O (3 × 100 mL), and the pH was adjusted to 8.5 by the slow addition of 360 mL of 2 N KOH, resulting in the precipitation of crude product. The solid was filtered and chromatographed on Diaion CHP20P resin, eluting with a CH_3CN/water gradient. The combined product fractions were concentrated in vacuo until turbid. Cooling this turbid solution for 1 h at 5 °C gave crystals which were filtered and dried in vacuo affording 10.5 g of **7** (63%): ^{1}H NMR (DMSO-d_6) δ 2.04 (m, 2 H), 2.36 (m, 1 H), 2.68 (m, 1 H), 3.49 (m, 4 H), 4.42 (ddd, each J = ca. 8.5 Hz, 1 H), 4.57 (t, J = 5.3 Hz, 1 H), 4.62 (t, J = 5 Hz, 1 H), 6.36 (s, 1 H), 7.82 (s, 1 H), 10.50 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 29.56, 33.05, 46.53, 47.65, 61.41, 63.46, 116.76, 135.71, 150.91, 153.28, 156.85; UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 253.3 nm (ϵ 11 600).

[1*R*-1α,2β,3α]-6-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]purine 2,3-Dibenzoate Ester (22a). A mixture of **20a** (29.39 g, 55.4 mmol), adenine (11.2 g, 83.2 mmol), finely ground anhydrous potassium carbonate (7.65 g, 55.4 mmol) and 18-crown-6 (14.64 g, 55.4 mmol) in 500 mL of dry DMF was stirred at 110 °C for 15 h under argon. The mixture was then cooled to room temperature and concentrated to dryness in vacuo. The residue was partitioned between EtOAc (1.2 L) and water (0.5 L), and the organic phase was washed with water (2 × 100 mL) and dried (Na_2SO_4). The solution was concentrated to ca. 100 mL, the resulting slurry was filtered, and the filter cake was washed with EtOAc. Chromatography of this solid on silica gel eluting with EtOH/CH_2Cl_2 (5:95) afforded 10.6 g of **22a** as a white solid. An additional 2.3 g was obtained by chromatography of the filtrate from the isolation of crude **22a**, for a total of 12.9 g (51%) of pure **22a**: ^{1}H NMR ($CDCl_3$) δ 2.6 (m, 1 H), 2.8 (m, 2 H), 3.5 (m, 1 H), 4.5 (m, 4 H), 4.81 (apparent q, J = ca. 8.8 Hz, 1 H), 5.58 (s, 1 H), 7.35–8.2 (m, 10 H), 8.22 (s, 1 H).

[1*R*-1α,2β,3α]-6-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]purine (9). To a slurry of 10.6 g (23.2 mmol) of **22a** in 500 mL of dry MeOH was added 3 mL of 25% sodium methoxide in MeOH. Stirring at 43 °C under nitrogen gave a homogeneous mixture within 1.5 h. After a total of 2 h heating, the mixture was concentrated in vacuo to a semisolid. This material was slurried in water, the pH was adjusted from 12.85 to 7 with 10% HCl, and the slurry was chromatographed on 800 mL of CHP20P resin. Elution with water and then MeOH/water (3:7) afforded 6.0 g of a viscous oil which was triturated with 200 mL of CH_3CN to give 4.72 g (82%) of **9** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.09 (m, 1 H), 2.23 (dd, J = 9.5, 20.1 Hz, 1 H), 2.47 (m, 1 H), 2.78 (m, 1 H), 3.52 (m, 4 H), 4.58 (t, J = 5.3 Hz, 1 H), 4.62 (m, 1 H), 4.73 (t, J = 5.3 Hz, 1 H), 7.15 (s, 1 H), 8.14 (s, 1 H), 8.24 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 29.2, 33.2, 47.43, 47.75, 61.6, 63.6, 119.1, 139.6, 149.2, 152.2, 156.0; UV (H_2O, pH 7.2, phosphate buffer) λ_{max} 261.4 nm (ϵ 14 720).

(1*R*-*trans*)-3,3-Diethoxy-1,2-cyclobutanedimethanol (16b) was prepared from **15b** in the same manner as described for **16a** to afford **16b** in 73% overall yield: ^{1}H NMR.[20]

(1*R*-*trans*)-3,3-Diethoxy-1,2-cyclobutanedimethanol, dibenzoate ester (17b) was prepared from **16b** in the same manner as described for **17a** to afford **17b** in 94% yield: ^{1}H NMR.[20]

The enantiomeric purity of **17b** was assessed by HPLC by using a Chiracel OD column, eluting with 1.5% 2-propanol in hexane at a flow rate of 1.0 mL/min. An enantiomeric excess of >99% was found.

(2*R*-*trans*)-2,3-Bis[(benzoyloxy)methyl]cyclobutanone (18b) was prepared from **17b** in the same manner as described for **18a** to afford **18b** in 95% yield: ^{1}H NMR.[20]

[1*R*-(1α,2β,3β)]-3-Hydroxy-1,2-cyclobutanedimethanol, 1,2-dibenzoate ester (19b) was prepared from **18b** in the same manner as described for **19a** to afford **19b** in 70% yield: ^{1}H NMR.[20]

[1*R*-(1α,2β,3β)]-3-[[(4-Methylphenyl)sulfonyl]oxy]-1,2-cyclobutanedimethanol, dibenzoate ester (20b) was prepared from **19b** in the same manner as described for **20a** to afford **20b** in 84% yield: ^{1}H NMR.[20]

[1*R*-(1α,2β,3α)]-3-[2-Amino-6-(phenylmethoxy)-9*H*-purin-9-yl]-1,2-cyclobutanedimethanol, 1,2-dibenzoate ester

(20) Spectral data were identical with those of the corresponding enantiomer.

(21b) was prepared from **20b** in the same manner as described for **21a** to afford **21b** in 51% yield: ^{1}H NMR.[20]

[1*S*-1α,2β,3α]-2-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-6*H*-purin-6-one (8) was prepared from **21b** in the same manner as described for **7** to afford **8** in 57% yield: ^{1}H NMR.[20]

[1*S*-1α,2β,3α]-6-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]purine, 2,3-dibenzoate ester (22b) was prepared from **20b** in the same manner as described for **22a** to afford **22b** in 31% yield: ^{1}H NMR.[20]

[1*S*-1α,2β,3α]-6-Amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]purine (10) was prepared from **22b** in the same manner as described for **9** to afford **10** in 89% yield: ^{1}H NMR, ^{13}C NMR, UV.[20]

Antiviral Assays in Cell Culture. Viruses, cells, and assays have been described in detail previously.[8c] In brief, herpes simplex virus type 1 (HSV-1) strain Schooler and HSV-2 strain 186 were prepared as extracts from infected Vero cell cultures. Human cytomegalovirus (HCMV) strain AD169, varicella-zoster virus (VZV) strain Ellen, and mouse cytomegalovirus (MCMV) strain Smith were prepared as suspensions of infected WI-38 or mouse embryo (ME) cells. WI-38 (CCL75) cells were obtained from ATCC and were grown in Eagles minimum essential medium with Earle's salts (EMEM) supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% FBS (Gibco Laboratories, Grand Island, NY). ME cells were prepared from primary cultures of mouse embryos and grown in Hams-F10 medium supplemented as described above.

HSV-1, HSV-2, HCMV, and VZV were assayed on WI-38 cell monolayers. MCMV was assayed on ME cells. Viruses were adsorbed to cell culture monolayers in 6-well culture plates (Costar, Cambridge, MA) for 1–2 h prior to addition of maintenance medium containing duplicate dilutions of the test compound (EMEM plus supplements, 1% carboxymethyl cellulose, 2.5% FBS ± drug). Inhibition of plaque development for all viruses was evaluated on monolayers stained after 4–6 days incubation at 37 °C. ID_{50} values were determined from the drug concentration which conferred 50% plaque reduction compared to virus controls. All titrations were done in duplicate.

Triphosphate Synthesis. The triphosphates of **7** and **8** were prepared by incubating 2 mg/mL nucleoside with HSV-1 thymidine kinase in 50 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 30 mM KCl, 2 mM DTT, 5 mM ATP, 30 mM creatine phosphate, 0.075 mg/mL BSA, and 8 units/mL creatine kinase at 37 °C. After 24 h HSV-1 thymidine kinase (1 unit/mL), guanylate kinase, (1 unit/mL), nucleoside-5′-diphosphate kinase (2 units/mL), 10 mM phosphoenolpyruvate, pyruvate kinase (4 units/mL), and creatine kinase (8 units/mL) were added and incubation continued for 72 h at 37 °C. The reaction products were separated by HPLC with use of a Synchropak AX100 column with a linear gradient of 0–100% 1 M KH_2PO_4, pH 5.6. Triphosphates were then desalted on DEAE-Sephadex A-25 by using a 0.2–2 M NH_4HCO_3 (pH 7.5) gradient, lyophilized several times and quantitated by UV. The overall yields were 2% and 20% for **7** triphosphate and **8** triphosphate, respectively.

DNA Polymerase Inhibition Studies. HeLa S3 cells were infected with HSV-1 (KOS) at a multiplicity of infection of 10 plaque-forming units per cell and harvested 20 h post infection. Extracts were prepared from infected and uninfected HeLa cells as described previously[21] and stored at −70 °C. HSV-1 DNA polymerase was purified by DEAE-cellulose, ssDNA cellulose, MonoS (Pharmacia), and ssDNA-cellulose chromatography as described previously.[22]

Inhibition of purified HSV-1 DNA polymerase by the triphosphates of **7** and **8** were determined in 50 mM Tris-HCl pH 8; 5 mM $MgCl_2$; 1 mM DTT; 0.1 M $(NH_4)_2SO_4$; 5 μM (each) dATP, dCTP, and [^{3}H]dTTP (250 cpm/pmol); 30 μg/mL activated calf thymus DNA; 0.1 mg/mL BSA; HSV-1 DNA polymerase; and varying dGTP. Incubation was for 20 min at 37 °C, and incorporation of [^{3}H]dTTP into DNA was quantitated by Cl_3CCO_2H precipitation and scintillation counting.[22]

Inhibition of HeLa DNA polymerase activity was studied under the following conditions: 50 mM Hepes pH 7; 5 mM $Mg(OAc)_2$; 1 mM DTT; 30 μg/mL activated calf thymus DNA; 0.1 mg/mL BSA; 5 μM (each) dATP, dCTP, and [^{3}H]dTTP (500 cpm/pmol); varying dGTP; and cell extracts from HeLa S3 cells. Incubation was for 30 min at 37 °C, and incorporation of radiolabel into DNA was quantitated by Cl_3CCO_2H precipitation and scintillation counting.

In Vivo Antiviral Assays. MCMV stocks for animal infections were prepared by four serial infections with 10% infected salivary gland extracts used as inocula. The final virus stocks were stored at −70 °C. Female Swiss-Webster mice weighing 20–23 g, obtained from Taconic Farms, Germantown, NY, were employed for all studies. Mice were infected intraperitoneally with 2×10^6 PFU of MCMV contained in 0.5 mL 0.3% BSA/PBS. Clinical signs of infection appeared 2–3 days after infection. Death usually occurred within 6 days or recovery started at 7 days post infection. Compounds were prepared for animal studies in either phosphate buffered (0.05M) saline or in sodium phosphate buffer (0.05M) both adjusted to pH 11. Antiviral therapy was initiated at 1 h post infection and continued twice daily for 5 days.

Animal survival was assessed daily for 21 days at which time remaining animals were sacrificed. Mean day of death (MDD) was calculated to determine extended survival times for mice which responded to therapy but eventually died by 21 days. The protective dose 50% (PD_{50}) based on survival was calculated by probit analysis ± standard error (SE) (Finney, D. *Probit Analysis*; Cambridge University Press: New York, NY, 1971). Comparisons were made for statistical analysis between treatment groups and placebo-treated infected controls. Fisher's exact test was used to analyze survivor (mortality) results. Student's *t* test was used to analyze MDD (extended survival).

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Registry No. **7**, 127759-89-1; **7** triphosphate derivative, 132435-15-5; **8**, 132077-79-3; **8** triphosphate derivative, 132435-16-6; **9**, 126187-01-7; **10**, 132294-13-4; **13**, 127696-10-0; **14**, 132178-71-3; **15a**, 132178-72-4; **15a** *O*-TBS derivative, 132178-73-5; **15a** *O*-TBS-*N*-nitroso derivative, 132178-74-6; **15b**, 132294-21-4; **15b** *O*-TBS derivative, 132294-29-2; **15b** *O*-TBS-*N*-nitroso derivative, 132294-30-5; **16a**, 132294-14-5; **16b**, 132294-22-5; **17a**, 132294-15-6; **17b**, 132294-23-6; **18a**, 132294-16-7; **18b**, 132294-24-7; **19a**, 132294-25-8; **20a**, 132294-18-9; **20b**, 132294-26-9; **21a**, 132294-19-0; **21b**, 132294-27-0; **22a**, 132294-20-3; **22b**, 132294-28-1; (*R*)-(−)-2-phenylglycinol, 56613-80-0; polymerase, 9012-90-2; ketene di-*O*-ethylacetal, 2678-54-8; diethyl fumarate, 623-91-6; 2-amino-6-(benzyloxy)purine, 19916-73-5; adenine, 73-24-5.

Supplementary Material Available: All further information concerning the X-ray analysis (positional parameters and their estimated standard deviations, general temperature factor expressions, bond distances, and bond angles) (5 pages). Ordering information is given on any current masthead page.

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