

evaporated under reduced pressure, and the solution was neutralized to pH 7 with diluted CH_3COOH . The precipitate was filtered and recrystallized in appropriate solvent.

Compound **9g** displayed the following: $^1\text{H NMR}$ (CF_3COOD) δ 3.2 (d, 2 H), 3.7-4 (m, 3 H), 6.8 (s, 1 H), 7.25-7.7 (m, 4 H).

Biochemical Assays. Crude synaptic membranes (CSM) were prepared from whole rat brain according to the method of Enna and Snyder.²⁰ Male Wistar rats (250 g) were killed by decapitation. Membranes from rat cerebral cortex were homogenized in ice-cold 0.32 M sucrose (20 mL for one brain) with a laboratory mixer (Silverston) for 60 s. The crude nuclear pellet (P_1) was isolated by centrifugation (4 °C) at 1000g for 10 min and discarded. The supernatant was recentrifuged (4 °C) for 20 min at 20000g. The crude mitochondrial pellet (P_2) from this centrifugation was lysed by resuspension in ice-cold water (20 mL for one brain). After homogenization with a laboratory mixer, the mixture was centrifuged (4 °C) for 20 min at 8000g. The supernatant and soft upper "buffy coat" of the pellet were collected and centrifuged (4 °C) at 48000g for 10 min to yield the crude synaptic membranes.

[^3H]GABA Binding Assay (GABA_A Assay). CSM (P_4) were stored at -20 °C for at least 18 h before use (up to 2 months). After thawing, the membranes were resuspended in 50 mM Tris-citrate buffer, pH 7.1, containing Triton X-100 (0.05% v/v), and the homogenate was incubated at 37 °C for 30 min. The suspension was centrifuged (4 °C) for 10 min at 48000g. The resultant pellet was homogenized in ice-cold 50 mM Tris-citrate buffer, pH 7.1 (4.5 mL for one brain), with a Potter-Elvehjem homogenizer fitted with a Teflon pestle.

For the binding assay procedures, aliquots of synaptic membranes (0.5 mg of protein) were incubated at 4 °C for 5 min in 2 mL of Tris-citrate buffer containing 0.4 μCi of [^3H]GABA (4-amino-*n*-[2,3- ^3H]butyric acid, Amersham) with a specific activity of 78 Ci/mM. Various concentrations of compounds to be tested were added. At the end of the incubation, the mixture was quickly filtered under vacuum through premoistened Whatman GF/C filters and washed with 10 mL of ice-cold Tris-citrate buffer.

Filters were transferred to a scintillation vial containing 5 mL of HP/b Beckman scintillation fluid. The tritium content of each sample was estimated by liquid scintillation spectrometry. Nonspecific binding determined in the presence of 100 μM of muscimol represented less than 10.8% of the total binding and was subtracted from the total binding to give specific binding.

The IC_{50} values for tested compounds were estimated by measuring the inhibition of different concentrations and performing log prohibit analyses of the results.

[^3H]Baclofen Binding Assay (GABA_B Assay). Interaction with the GABA_B receptors was examined with [^3H]baclofen as described by Hill and Bowery.¹¹ CSM (P_4) were washed with ice-cold distilled water (20 mL for one brain) by centrifugation (4 °C) for 10 min at 48000g. The resulting pellet was stored frozen at -20 °C for at least 18 h prior to use (up to 2 months). After decongelation for 15 min at 20 °C, membranes were resuspended in 50 mM Tris-HCl, pH 7.4, buffer with 2.5 mM CaCl_2 (10 mL for one brain) and incubated for 45 min at 20 °C. This suspension was centrifuged (4 °C) at 7000g for 10 min and the resultant pellet incubated in Tris-HCl buffer (10 mL for one brain). These centrifugations and incubations were started against three times. In a final time, the suspension was centrifuged (4 °C) at 7000g for 10 min and the pellet resuspended in Tris-HCl buffer (4.5 mL for one brain).

Membranes equivalent to 0.5 mg of protein were incubated in triplicate in 1 mL of 50 mM Tris-HCl, pH 7.4, buffer with 2.5 mM CaCl_2 containing the drugs to be tested and 0.6 μCi of [^3H]baclofen (DL-[butyl-4- ^3H (N)]baclofen, NEN) with a specific activity of 45 Ci/mM. These homogenates were incubated for 30 min at room temperature in conical microcentrifuge tubes and the assay terminated by centrifugation at 7000g for 10 min. The supernatant was discarded, the pellet was carefully rinsed two times with 1 mL of Tris-HCl buffer, and remaining fluid blotted from the surface of the pellet was aspirated under vacuum. The pellet was solubilized with ultrasonic bath for 10 min in 1 mL of HP/b Beckman scintillation fluid. The radioactivity was measured 12 h later in a liquid scintillation counter. Nonspecific binding was determined with 1 mM GABA and presented 66.6% of total binding. IC_{50} values were estimated as described elsewhere.

(20) Enna, S. J.; Snyder, S. H. *Brain Res.* 1975, 100, 81.

Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides through the Action of Adenosine Deaminase. Antiviral Activity of the Carbocyclic 2'-Deoxyguanosine Enantiomers

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The action of adenosine deaminase on racemic carbocyclic analogues of 6-aminopurine nucleosides was investigated. When either racemic carbocyclic adenosine [(±)-C-Ado] or the racemic carbocyclic analogue [(±)-C-2,6-DAP-2'-dR] of 2,6-diaminopurine 2'-deoxyribofuranoside was incubated with this enzyme, approximately half of the material was deaminated rapidly. From the resulting solution, the D isomers of the deaminated carbocyclic analogues (D-carbocyclic inosine, D-C-Ino, or D-carbocyclic 2'-deoxyguanosine, D-2'-CDG) and the L isomers of the undeaminated carbocyclic analogues were isolated. At higher concentrations of the enzyme, deamination of L-C-Ado and L-C-2,6-DAP-2'-dR proceeded slowly, thus also making the other enantiomers accessible. In tests in vitro against herpes simplex virus, types 1 and 2, D-2'-CDG was as active and potent as (±)-2'-CDG, whereas L-2'-CDG displayed only modest activity. In contrast to the previously reported high activity and potency of (±)-C-2,6-DAP-2'-dR against these two viruses, L-C-2,6-DAP-2'-dR was inactive.

The general synthetic routes¹⁻⁹ to the requisite cyclopentane precursors of carbocyclic analogues of nucleosides

lead to the racemic forms of the target nucleoside analogues. It has been assumed that the various biological

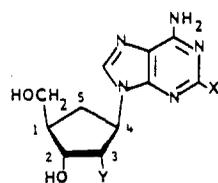
- (1) Shealy, Y. F.; Clayton, J. D. *J. Am. Chem. Soc.* 1966, 88, 3885-3887; *J. Am. Chem. Soc.* 1969, 91, 3075-3083.
- (2) Shealy, Y. F.; O'Dell, C. A. *Tetrahedron Lett.* 1969, 2231-2234. O'Dell, C. A.; Shealy, Y. F. *Nucleic Acid Chemistry*, Townsend, L. B., Tipson, R. S., Ed.; Wiley: New York, 1978; Part 1, pp 161-167.
- (3) Daluge, S.; Vince, R. *Tetrahedron Lett.* 1976, 3005-3008; *J. Org. Chem.* 1978, 43, 2311-2320.
- (4) Vince, R.; Daluge, S. *J. Org. Chem.* 1980, 45, 531-533.

- (5) Just, G.; Reader, G.; Chalard-Faure, B. *Can. J. Chem.* 1976, 54, 849-860. Just, G.; Ouellet, R. *Can. J. Chem.* 1976, 54, 2925-2934.
- (6) Saksena, A. K. *Tetrahedron Lett.* 1980, 133-136.
- (7) Paulsen, H.; Maas, U. *Chem. Ber.* 1981, 114, 346-348.
- (8) Kam, B. L.; Oppenheimer, N. J. *J. Org. Chem.* 1981, 46, 3268-3272.
- (9) Bindu Madhavan, G. V.; Martin, J. C. *J. Org. Chem.* 1986, 51, 1287-1293.

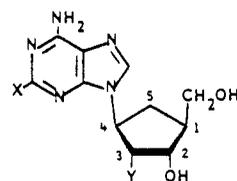
activities reported for carbocyclic analogues of nucleosides are exerted by the enantiomer that is analogous to the β -D-nucleoside structure, but there have been only a few reports of optically active carbocyclic analogues. Most of these reported studies deal with the syntheses and biological activities of the natural products aristeromycin (**1a**)¹⁰ and neplanocin (**3**)¹¹ and their transformation products. Aristeromycin was shown by Kishi and co-workers^{12,13} to be an enantiomer of the carbocyclic analogue of adenosine (C-Ado, **1a** + **2a**), which had been synthesized by a regiospecific and stereospecific route.¹ Arita et al.¹⁴ synthesized (-)-aristeromycin and (-)-neplanocin A by employing an esterase to hydrolyze selectively one of the ester groups of a bicyclic diester precursor of the required aminocyclopentane and aminocyclopentene. Lim and Marquez synthesized (-)-neplanocin from D-(+)-ribonic acid lactone,¹⁵ and Bindu Madhavan and Martin⁹ formally synthesized (-)-aristeromycin by resolving an azidocyclopentane precursor of (-)-aristeromycin. Recently, Herdewijn et al.¹⁶ obtained both of the enantiomers of C-Ado through enzymatic hydrolysis of (\pm)-C-AMP with 5'-ribonucleotide phosphohydrolase.¹⁷ In this paper, we describe a different biochemical resolution of synthetic carbocyclic analogues.

Enzymatic Deamination. Soon after C-Ado (**1a** + **2a**) was first synthesized, Bennett et al.¹⁸ demonstrated that it is a substrate for adenosine kinase and for adenosine deaminase. It seemed likely that the substrate (or most effective substrate) for these enzymes was the C-Ado enantiomer that corresponds to natural adenosine (β -D-adenosine). If so, the use of these enzymes would provide a means of obtaining at least one of the enantiomers of carbocyclic analogues of 6-aminopurine nucleosides. In addition, if adenosine deaminase acts in the postulated manner, an enantiomer of the deamination product, a C-6-oxopurine nucleoside, should be obtainable. Because the racemic carbocyclic analogues of 2'-deoxyguanosine (**4b** + **5b**) and 2,6-diaminopurine 2'-deoxyribofuranoside (**1b** + **2b**) are highly effective antiviral agents,¹⁹ application of the deaminase method to the resolution of these compounds appeared to be especially attractive.

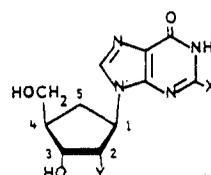
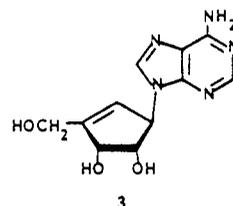
Synthetic (\pm)-C-Ado was treated with commercial adenosine deaminase (ADA)²⁰ in aqueous solution or phosphate buffer. The deamination was monitored by



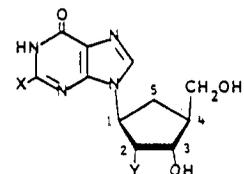
1a: X = H; Y = OH (1R, 2S, 3R, 4R), D-C-Ado
b: X = NH₂; Y = H (1R, 2S, 4R), D-C-2,6-DAP-2'-dR



2a: X = H; Y = OH (1S, 2R, 3S, 4S), L-C-Ado
b: X = NH₂; Y = H (1S, 2R, 4S), L-C-2,6-DAP-2'-dR



4a: X = H; Y = OH (1R, 2S, 3R, 4R), D-C-Ino
b: X = NH₂; Y = H (1R, 3S, 4R), D-2'-CDG



5a: X = H; Y = OH (1S, 2R, 3S, 4S), L-C-Ino
b: X = NH₂; Y = H (1S, 3R, 4S), L-2'-CDG

following the increase in carbocyclic inosine (C-Ino) as well as the decrease in C-Ado by HPLC analysis of aliquots of the reaction mixture. As has been previously demonstrated, the deamination is slow relative to the deamination of adenosine itself,¹⁸ and a plateau was reached in about 3 h at ambient temperature. At this plateau the reaction mixture contained approximately equal amounts of C-Ado and C-Ino. Isolation of the C-Ado showed it to have an optical rotation of +51.1°, comparing favorably to the literature values for aristeromycin,^{13,16} though opposite in sign. Thus, the enantiomer with the same absolute configuration as adenosine deaminates first, leaving the (+) enantiomer of C-Ado largely untouched and also allowing the isolation of the D isomer of C-Ino. D-C-Ino isolated from the same reaction mixture had a rotation of -48.9°. Yields of L-C-Ado and D-C-Ino after separation and recrystallization were 68% and 48%, respectively. Incubation of the racemic C-Ado with the enzyme for prolonged periods did result in the deamination of the L isomer, and therefore either isomer is accessible by this procedure.

The carbocyclic analogue of racemic 2,6-diaminopurine 2'-deoxyribofuranoside ((\pm)-C-2,6-DAP-2'-dR, **1b** + **2b**) was treated with ADA in phosphate buffer solution. The deamination reaction was monitored in the same manner as described for C-Ado. With this compound, deamination essentially ceased within 80–120 min at ambient temperature when the ratio of ADA to (\pm)-C-2,6-DAP-2'-dR was one-half unit per micromole. The HPLC analyses indicated that the reaction solution contained approximately equal amounts of C-2,6-DAP-2'-dR and 2'-CDG. After the enzyme had been deactivated thermally, 2'-CDG crystallized from the chilled solution. Since only about one-half of racemic C-2,6-DAP-2'-dR had been deaminated, the enzyme must have acted almost entirely on the enantiomer that is the analogue of β -D-2'-deoxyribofuranosyl-2,6-DAP

- (10) Kusaka, T.; Yamamoto, H.; Shibata, M.; Muroi, M.; Kishi, T.; Mizuno, K. *J. Antibiot.* **1968**, *21*, 255–263.
(11) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 360–366. Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675–680.
(12) Kishi, T.; Muroi, M.; Kusaka, T.; Nishikawa, M.; Kamiya, K.; Mizuno, K. *Chem. Commun.* **1967**, 852–853.
(13) Kishi, T.; Muroi, M.; Kusaka, T.; Nishikawa, M.; Kamiya, K.; Mizuno, K. *Chem. Pharm. Bull.* **1972**, *20*, 940–946.
(14) Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1983**, *105*, 4049–4055.
(15) Lim, M.-I.; Marquez, V. E. *Tetrahedron Lett.* **1983**, *24*, 5559–5562.
(16) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Vanderhaeghe, H. *J. Med. Chem.* **1985**, *28*, 1385–1386.
(17) Shealy, Y. F.; Clayton, J. D. *J. Pharm. Sci.* **1973**, *62*, 1252–1257.
(18) Bennett, L. L., Jr.; Allan, P. W.; Hill, D. L. *Mol. Pharmacol.* **1968**, *4*, 208–217.
(19) Shealy, Y. F.; O'Dell, C. A.; Shannon, W. M.; Arnett, G. *J. Med. Chem.* **1984**, *27*, 1416–1421.
(20) Adenosine aminohydrolase (EC 3.5.4.4) from calf intestinal mucosa, purchased from Sigma Chemical Co., St. Louis, MO 63178; Product No. A-1030, Type VIII. One unit of this enzyme preparation will deaminate 1.0 μ mol of adenosine to inosine per minute at pH 7.5 at 25 °C.

(1b). The isolated 2'-CDG, therefore, is the enantiomer (D-2'-CDG, 4b) that is the analogue of β -D-2'-deoxyguanosine. The yields of isolated D-2'-CDG, based on the conversion of only one of the DAP enantiomers, were 60–70%.

The relative amounts of C-2,6-DAPdR and 2'-CDG in the filtrate obtained from one of the deamination experiments were about 85% and 15%, respectively. The deoxyguanosine analogue was adsorbed on an anion-exchange resin, and the filtrate from the resin treatment was chromatographed on a cation-exchange resin to furnish the enantiomer [L-C-2,6-DAP-2'-dR, 2b] of racemic C-2,6-DAP-2'-dR that was not deaminated. Preliminary studies of the deamination of (\pm)-C-2,6-DAP-2'-dR (1b + 2b) or of L-C-2,6-DAP-2'-dR (2b) with much larger amounts of ADA showed that 2b is slowly deaminated. When the racemic compound was incubated with 10 units of ADA/ μ mol, the formation of D-2'-CDG reached a plateau in approximately 20 min; HPLC of aliquots removed during 4–94 h showed a slow decrease in the C-2,6-DAP-2'-dR peak and a corresponding slow increase in the 2'-CDG peak. In order to isolate L-2'-CDG (5b), L-C-2,6-DAP-2'-dR was then treated with 4 units of ADA/ μ mol. After 20 h under ambient conditions (20 ± 2 °C), the reaction solution was maintained at 37 °C to increase the rate of deamination. At this temperature, the deamination was essentially complete in the interval 48–72 h, and L-2'-CDG was isolated.

The optical rotations of D-2'-CDG and L-2'-CDG were of comparable magnitude and opposite in sign at the sodium D line and at the mercury 546 and 578 lines. The optical rotations of L-C-2,6-DAP-2'-dR at the same lines were similar in magnitude and sign to those of L-2'-CDG. It is worthy of note that the isomer corresponding to 2'-deoxyguanosine (2'-DG) has a positive rotation at the sodium D line, while 2'-DG has a negative rotation, as does aristeromycin.

Antiviral Evaluation.²¹ Evaluation of D-2'-CDG, L-2'-CDG, and L-C-2,6-DAP-2'-dR against herpes simplex type 1 (HSV-1) and type 2 (HSV-2) replicating in Vero cells was carried out as previously described.²³ For comparison, (\pm)-2'-CDG, (\pm)-C-2,6-DAP-2'-dR, and *ara*-A were also tested and gave values consistent with previous experiments.¹⁹ The virus ratings (VR) and values of MIC₅₀ of D-2'-CDG in two tests against HSV-1 were 4.8–5.9 and 0.2–0.3 μ g/mL, respectively. These results are similar to the data from simultaneous tests of (\pm)-2'-CDG (VR = 5.6–7.0 and MIC₅₀ = 0.2–0.3 μ g/mL) and are similar, also, to the earlier results against HSV-1 in rabbit kidney cells.¹⁹ The activity of D-2'-CDG vs. HSV-2 (VR = 3.8, MIC₅₀ = 0.7 μ g/mL) likewise is the same as the activity of (\pm)-2'-CDG (VR = 3.7, MIC₅₀ = 0.8 μ g/mL¹⁹).

In two tests against HSV-1 in Vero cells, L-2'-CDG (5b) showed modest activity (VR = 0.8, 1.1). L-C-2,6-DAP-2'-dR (2b) was not active against HSV-1 or HSV-2. In these tests with Vero cells as host cells and in earlier tests with rabbit kidney cells,¹⁹ (\pm)-C-2,6-DAP-2'-dR was highly active against both HSV-1 and HSV-2.

These results demonstrate that the antiviral activity expressed by (\pm)-2'-CDG resides principally in the enantiomer that is analogous to natural β -D-2'-deoxyguanosine. The analogue of β -L-2,6-DAP-2'-dR is devoid of activity in these tests, and, therefore, the activity of (\pm)-C-2,6-DAP-2'-dR must be exerted by the enantiomer that is analogous to β -D-2,6-DAP-2'-dR. Because the L-C-2,6-DAP-2'-dR used to prepare L-2'-CDG exhibited no antiviral activity and because the antiviral activity of racemic C-2,6-DAP-2'-dR and 2'-CDG are comparable in our system, it is clear that the modest antiviral activity of the L-2'-CDG is not attributable to a minor impurity.

Summary. These results demonstrate that the action of ADA on racemic carbocyclic analogues of 6-aminopurine nucleosides is a practical method of obtaining one of the enantiomers of the aminopurine and both enantiomers of the corresponding 6-oxopurine. Because synthetic methods are available for converting a 6-oxopurine to a 6-aminopurine,²⁴ the ADA method also can serve as the basis for obtaining the other 6-aminopurine enantiomer.

Experimental Section

General Methods. Melting temperatures (mp) were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (UV) were recorded with a Cary Model 17 spectrophotometer, and absorption maxima are reported in nanometers; sh = shoulder. Solutions for ultraviolet determinations were prepared by diluting a 5-mL aliquot of a water solution of the carbocyclic analogue to 50 mL with 0.1 N hydrochloric acid, phosphate buffer (pH 7), or 0.1 N sodium hydroxide. Absorption maxima of these solutions are reported as being determined at pH 1, 7, or 13, respectively. Mass spectra were determined at 70 eV by the fast-atom-bombardment (FAB) method and with a Varian/MAT 311 A spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and the Molecular Spectroscopy Section of Southern Research Institute. Thin-layer chromatography (TLC) was performed on plates of silica gel, and developed plates were examined with ultraviolet light. High-pressure liquid chromatography (HPLC) was performed with a Hewlett-Packard 1084B liquid chromatograph equipped with a variable-wavelength detector set at 280 nm, an automatic injector, and a C₁₈ μ -Bondapak ODS 10- μ m column. Isolated specimens were dissolved in water at a concentration of 1 mg/mL. In order to determine accurately the progress of the deamination reactions with an ultraviolet detector, standards of racemic starting materials and products were run in order to calibrate peak areas. Unless indicated otherwise, aliquots of reaction solutions and most isolated specimens were assayed by using a gradient eluting solvent of water-acetonitrile (9:1 \rightarrow 1:9 over 20 min); flow rate, 1 mL/min. Some of the aliquots and the analytical samples of D-2'-CDG, L-2'-CDG, and C-L-2,6-DAP-2'-dR were also assayed by using a gradient eluting solvent of 0.01 M NH₄H₂PO₄ (pH 5.1)-MeOH (9:1 \rightarrow 1:9 over 20 min). Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Yield calculations are based upon conversion of one-half of the racemic starting materials.

[1R,2S,3R,4R-(1 α ,2 β ,3 β ,4 α)]-D-1,9-Dihydro-9-[2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]-6H-purin-6-one (D-C-Ino, 4a) and [1S,2R,3S,4S-(1 α ,2 β ,3 β ,4 α)]-L-4-(6-Amino-9H-purin-9-yl)-2,3-dihydroxycyclopentane-methanol (L-C-Ado, 2a). A solution of 400 mg (1.51 mmol) of carbocyclic adenosine in 80 mL of hot water was cooled to room temperature before the addition of 100 μ L (250 units) of ADA.²⁰ After being stirred for 3 h at room temperature, the solution was boiled for

- (21) The antiviral activity of each compound is expressed as a virus rating (VR), and the potency is given as a minimum inhibitory concentration (MIC₅₀). The VR, determined by the general method of Ehrlich et al.,²² is a weighted measurement of antiviral activity that takes into account both the degree of inhibition of virus-induced cytopathogenic effects and the degree of cytotoxicity produced by the test compound. A virus rating (VR) equal to or greater than 1.0 indicates definite and significant antiviral activity, a VR of 0.5–0.9 indicates marginal to moderate antiviral activity, and a VR less than 0.5 usually indicates no significant antiviral activity. MIC₅₀ is the concentration of the tested compound required to inhibit cytopathogenic effects by 50%.
- (22) Ehrlich, J.; Sloan, B. J.; Miller, F. A.; Machamer, H. E. *Ann. N.Y. Acad. Sci.* **1965**, *130*, 5–16.
- (23) Shealy, Y. F.; O'Dell, C. A.; Arnett, G.; Shannon, W. M.; Thorpe, M. C.; Riordan, J. M.; Coburn, W. C., Jr. *J. Med. Chem.* **1986**, *29*, 1720.

- (24) Vorbruggen, H.; Krolkiewicz, K. *Liebigs Ann. Chem.* **1976**, *745*–761.

5 min, filtered through Celite, and examined by TLC and HPLC, which shows 52.3% carbocyclic inosine (0.79 mmol, 210 mg), leaving 0.72 mmol, 200 mg of carbocyclic adenosine. The solution was applied to an ion-exchange column (diameter 1 cm) containing 3.3 mL (3 equiv) of Amberlite IRA 400(OH⁻). The column was then eluted with 500 mL of water to remove the adsorbed C-Ado (no further C-Ado was observed by TLC). Evaporation of this solution to dryness followed by recrystallization from water gave 143 mg (71.5%) of L-C-Ado: $[\alpha]_D^{23} +51.1^\circ$ (c 0.3, DMF); mp 208–210 °C (racemic C-Ado, 244 °C¹); HPLC 98.67%; TLC, homogeneous in (3:1 CHCl₃-MeOH) containing 5% acetic acid. Anal. (C₁₁H₁₅N₅O₃·0.25H₂O) C, H, N.

The ion-exchange column was eluted with 200 mL of 3 N acetic acid. Evaporation of the eluate followed by recrystallization from water gave 90.7 mg (43.2%) of chromatographically pure D-carbocyclic inosine, which was 100% pure by HPLC, mp 240 °C with shrinking from 237 °C (lit. mp (racemic) 225–227 °C,¹ 235 °C²⁵), $[\alpha]_D^{23} -48.9^\circ$ (c 0.2, DMF). Anal. (C₁₁H₁₄N₄O₄) C, H, N.

[1R,3S,4R-(1 α ,3 β ,4 α)]-D-2-Amino-1,9-dihydro-9-[3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6H-purin-6-one (D-2'-CDG, 4b). Racemic carbocyclic 2,6-diaminopurine 2'-deoxyribofuranoside (350 mg, 1.32 mmol) was dissolved in 70 mL of 0.05 M phosphate buffer (pH 7.4) at 50 °C. The solution was cooled to room temperature, ADA²⁰ [250 μ L containing 625 units (0.5 unit/ μ mol of (\pm)-C-2,6-DAPdR)] was added in one portion, and the progress of the reaction was monitored by HPLC. The deamination reaction had essentially stopped within 2 h, and the HPLC data indicated that the reaction solution contained approximately equal amounts of 4b and 2b. The reaction solution was heated at 100 °C for 3 min to deactivate the enzyme, the mixture was filtered through Celite to remove agglutinated protein, and the filtrate was refrigerated when crystals began to form. D-2'-CDG (4b) was filtered off, washed with cold water, and dried in vacuo at 78 °C: yield, 80 mg (45%); mp 244–247 °C (inserted at 100 °C, 3 °C/min). Additional 4b crystallized when the filtrate (including water washings) from the first crop was concentrated in vacuo to a final volume of 8 mL: yield after drying in vacuo at 78 °C for 2 h, 30 mg (17%, 2 crops); mp 244–246 °C (inserted at 100 °C, 3 °C/min). The analytical sample was obtained by combining the three crops of 4b and recrystallizing (twice) from water: mp 242–245 °C (inserted at 100 °C, 3 °C/min); HPLC,²⁶ $t_R = 6.3$ min (99.8%); TLC, 1 spot (5:2:3 BuOH-HOAc-H₂O and 4:1 2-propanol-1 M NH₄OAc); MS (FAB), m/e 266 (M + 1); UV_{max} 254 nm (ϵ 11800) and 279 (8000) at pH 1, 253 (13000) and 270–275 sh at pH 7, 255–260 sh and 268 (11300) at pH 13; $[\alpha]_D^{23} +5.5^\circ$, $[\alpha]_{546}^{23} +4.8^\circ$, $[\alpha]_D^{23} +4.9 \pm 0.1^\circ$ (c 1.0, 0.1 N NaOH). Anal. (C₁₁H₁₅N₅O₃·1.5H₂O) C, H, N.

[1S,3R,4S-(1 α ,3 β ,4 α)]-L-2-Amino-1,9-dihydro-9-[3-hydroxy-4-(hydroxymethyl)cyclopentyl]-6H-purin-6-one (L-CDG, 5b). A solution of 40 mg (0.15 mmol) of L-C-2,6-DAPdR (2b) and 240 μ L of ADA (600 units, 4 units/ μ mol of 2b) in 80 mL of phosphate buffer (pH 7.5) was stirred at room temperature (18–22 °C) for 20 h. The deamination reaction was monitored by using the ammonium dihydrogen phosphate gradient eluting system. After 20 h, the ratio of 2b to 5b was about 2:1, and the reaction solution was then stirred at 37 °C to increase the reaction rate. After 72 h at 37 °C, HPLC indicated that the relative amounts of 5b and 2b in the reaction solution were 98% and 0.4%, respectively. The reaction mixture was heated at 100 °C for 3 min to deactivate the enzyme, the mixture was filtered through

Celite to remove suspended protein, and the filtrate and water washings were combined and concentrated in vacuo to a volume of 7 mL. After crystals began to form, the mixture was refrigerated. The white crystalline product was filtered away, washed with cold water, and dried in vacuo at 78 °C: yield, 28 mg (70%); mp 238–241 °C (inserted at 135 °C, 3 °C/min). Recrystallization of this specimen from 2.2 mL of water afforded the analytical sample of 5b: recovery, 26 mg (93%); mp 243–246 °C (inserted at 100 °C, 3 °C/min); HPLC,²⁶ $t_R = 6.3$ min (99.6%); TLC, 1 spot (5:2:3 BuOH-HOAc-H₂O and 4:1 2-propanol-1 M NH₄OAc); MS (FAB), m/e 266 (M + 1); UV_{max} 254 nm (ϵ 11900), 279 (8100) at pH 1, 253 (12800) and 270–275 sh at pH 7, 255–260 sh and 268 (11200) at pH 13; $[\alpha]_{546}^{16} -6.0^\circ$, $[\alpha]_{578}^{16} -5.5^\circ$, $[\alpha]_D^{16} -5.2^\circ$ (c 1.0, 0.1 N NaOH). Anal. (C₁₁H₁₅N₅O₃·1.5H₂O) C, H, N.

[1S,2R,4S-(1 α ,2 β ,4 α)]-L-4-(2,6-Diamino-9H-purin-9-yl)-2-hydroxycyclopentanemethanol (L-C-2,6-DAP-2'-dR, 2b). The filtrate from the isolation of D-2'-CDG (4b) was shown by HPLC analysis to contain 2b and 4b in a ratio of about 85:15. The solution was diluted to 75 mL with water and was stirred for 1.5 h with 5 mL of an anion-exchange resin (Dowex 1-X8, OH⁻ form). HPLC analysis of the supernatant solution indicated that all of the remaining 4b had been absorbed on the resin. The mixture was filtered, and the filtrate (combined with the water washings) was chromatographed on a column that contained 30 mL of a cation-exchange resin (Bio-Rad AG 50W-X4, H⁺ form). The column was washed thoroughly with water and was then eluted with 1 N aqueous ammonia. Product-containing fractions were identified by UV analysis of the column effluent and were concentrated in vacuo to crystalline 2b: yield, 100 mg (57%); HPLC, 99%.

Additional 2b was obtained by extracting the Dowex 1-X8 resin used above with three 25-mL portions of boiling water: weight, 15 mg (9%); HPLC, 99.3%. The analytical sample was obtained as a white, crystalline solid by recrystallizing the combined crops of 2b: recovery, 90 mg (78%); mp 210–213 °C dec (inserted at 100 °C, 3 °C/min); HPLC,²⁶ $t_R = 7.5$ min (99.7%); TLC, 1 spot (5:2:3 BuOH-HOAc-H₂O and 4:1 2-propanol-1 M NH₄OAc); MS (FAB), m/e 265 (M + 1); UV_{max} 217 nm (ϵ 22300), 253 (9500), and 291 (9900) at pH 1, 215 (29000) and 245–250 sh, 255 (8200), and 280 (10500) at pH 7 and 13; $[\alpha]_{546}^{23} -5.9^\circ$, $[\alpha]_{578}^{23} -5.5^\circ$, $[\alpha]_D^{23} -4.8^\circ$ (c 1.0, H₂O). Anal. (C₁₁H₁₆N₆O₂·0.25H₂O) C, H, N.

Antiviral Evaluations In Vitro. The compounds reported were tested for inhibition of the cytopathogenic effects produced by strain 377 (TK⁺) of HSV-1 or strain MS of HSV-2 replicating in Vero cells. The data summarized were acquired by methods and procedures described previously for the evaluation of compounds for antiviral activity in vitro.²³ The general assay method was described by Ehrlich et al.,²² but some modifications were incorporated.

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Registry No. 2a, 99395-42-3; 2b, 106863-56-3; 4a, 16975-94-3; 4b, 100018-53-9; 5b, 106863-55-2; adenosine deaminase, 9026-93-1; (\pm)-carbocyclic adenosine, 13190-75-5; (\pm)-carbocyclic 2,6-diaminopurine 2'-deoxyribofuranoside, 91296-15-0.

(25) Marumoto, R.; Yoshioka, Y.; Furukawa, Y.; Honjo, M. *Chem. Pharm. Bull.* 1976, 24, 2624–2628.

(26) HPLC of the analytical samples of 2b, 4b, and 5b were performed on the same day, with the same column, and under the same conditions. (See General Methods.)