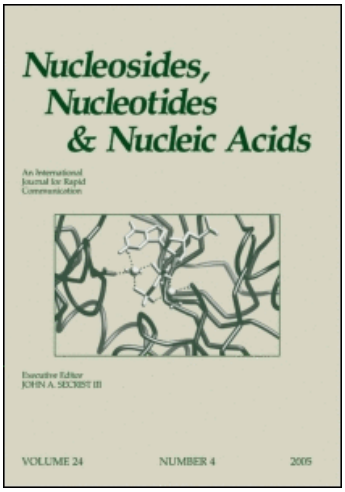

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S-Adenosyl-L-homocysteine Hydrolase Inhibitors as Anti-Viral Agents: 5'-Deoxyaristeromycin

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S-ADENOSYL-L-HOMOCYSTEINE HYDROLASE INHIBITORS AS ANTI-VIRAL AGENTS: 5'-DEOXYARISTEROMYCIN

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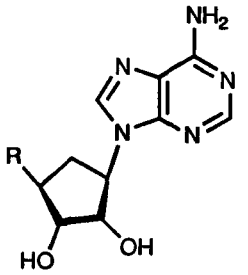
Abstract. (-)-5'-Deoxyaristeromycin (-)-(3) has been prepared in 13 steps beginning with cyclopentadiene. Compound (-)-3 showed an antiviral activity spectrum characteristic of that of *S*-adenosyl-L-homocysteine hydrolase inhibitors (such as carbocyclic 3-deazaadenosine and neplanocin A), and, in that regard, (-)-3 was found to possess an IC_{50} of 3.28 ± 0.48 μM towards this enzyme.

Human cytomegalovirus (HCMV) has become a prominent pathogen affecting individuals whose immune systems have been weakened as a result of organ transplantation therapy or HIV infection leading to AIDS.[1] To date, investigations seeking agents for treating this disease have principally focused on inhibition of the virally encoded DNA polymerase[2] and such efforts have produced 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG or ganciclovir).[3] However, due to the toxicity and emerging drug resistance associated with this latter compound,[4] more selective agents are desirable.

During our efforts to uncover less toxic anti-HCMV candidates that would not target DNA polymerase, we sought nucleoside derivatives less likely or unlikely to undergo nucleotide formation. From this approach, 5'-nor aristeromycin (**1**) was found[5] to possess potent activity towards HCMV (2.5 times that of DHPG) at non-toxic concentrations, possibly the result of the desired reduced phosphorylation (nucleotide formation).

In seeking insight into the mechanism by which **1** could have achieved its anti-HCMV effect, it was found to be inhibitory towards *S*-adenosyl-L-homocysteine (AdoHcy)hydrolase,[5] which is a frequently observed biological property of carbocyclic adenosine derivatives[6] for which aristeromycin (**2**) is the parent molecule. Inhibition of



(±)-**1**, R=OH
(-)-**2**, R=CH_2OH
(-)-**3**, R=CH_3

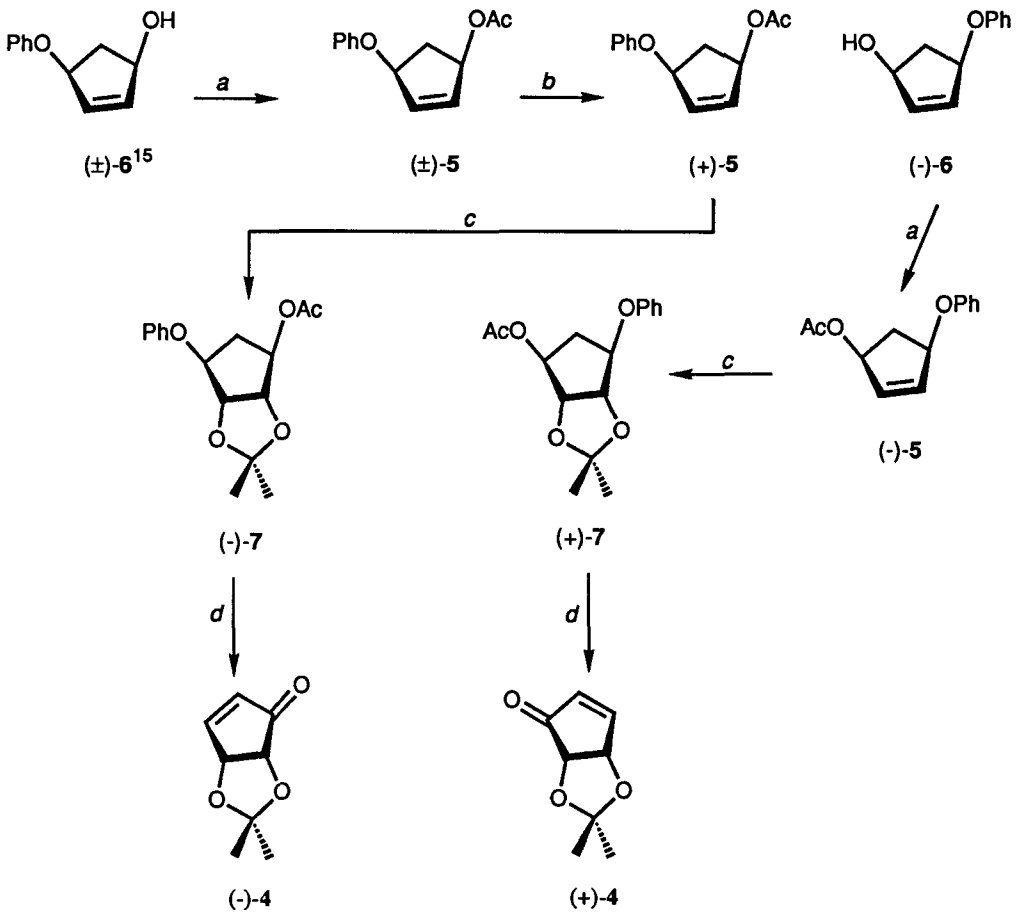
AdoHcy hydrolase by **1** would then have affected macromolecular methylation during HCMV processing.[7]

These observations prompted our consideration of 5'-deoxyaristeromycin (-)-(**3**) as another potential anti-HCMV candidate that would (i) act via inhibition of AdoHcy hydrolase and (ii) be incapable of nucleotide formation. Reported here are the results of this study, which, in addition to an anti-HCMV analysis, include a thorough antiviral study of (-)-**3**. [During the preparation of this manuscript, a synthesis of (-)-**3** and its inhibitory effect on AdoHcy hydrolase were reported;[8] however, an extensive antiviral analysis of (-)-**3**, as described here, was not available.[9]]

Chemistry

Prompted by the work from the laboratories of Johnson[10] and Borchardt[8,11a] our synthesis of (-)-**3** was designed to begin with the 1,4-conjugate addition reaction of lithium dimethylcuprate to the protected cyclopentenone (-)-**4** (Scheme 1), which we chose to use when difficulty was encountered in preparing the corresponding cyclohexylidene derivative of Borchardt,[11a] who has also reported a similar situation.[11b] In this regard, we desired a more convenient method to (-)-**4** than reported[12,13] that would employ an inexpensive starting material and would not only provide (-)-**4** for the purposes of this and related projects but would also allow concurrent access to the enantiomer (+)-**4** whose availability in significant quantities was important to the goals of the South Florida laboratory.

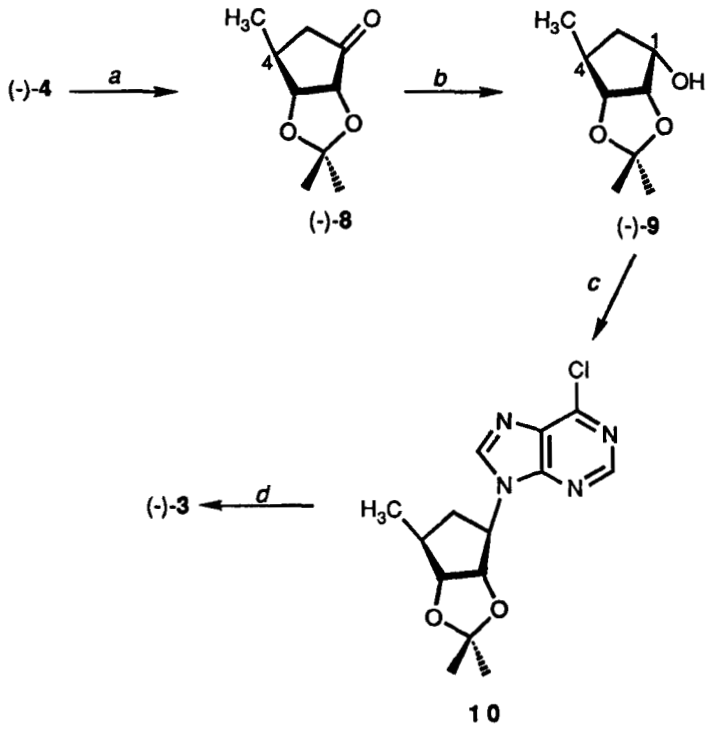
Thus, as a result of our observation that *Pseudomonas cepacia* lipase (PCL)[14] provides a convenient means for the enzymatic resolution of cyclic acetates, we chose to begin (Scheme 1) with the racemic phenoxyacetate **5** (the presence of the phenyl group was foreseen to provide a useful chromophore for chromatographic purposes throughout the early stages of this synthesis). Compound (±)-**5** was prepared by acetylation of (±)-**6** (readily available from cyclopentadiene in two steps).[15] The acetate (±)-**5** was treated with



Reaction conditions: *a*, Ac_2O/pyridine/catalytic DMAP in CH_2Cl_2; *b*, *Pseudomonas cepacia* lipase/phosphate buffer, 25 °C; *c*, (i) N-methylmorpholine N-oxide/catalytic OsO_4 in acetone/H_2O; (ii) 2,2-dimethoxypropane/acetone/catalytic *p*-TsOH; *d*, (i) KOH in MeOH; (ii) pyridinium chlorochromate/molecular sieves 4-Å powder in CH_2Cl_2

Scheme 1

PCL in phosphate buffer using 1 N sodium hydroxide solution to maintain a constant pH. After the consumption of 0.5 equivalent of the sodium hydroxide solution, the reaction stopped indicating a high selectivity of this enzyme for substrate (±)-**5**. The resulting compounds (+)-**5** and (-)-**6** were easily separated by column chromatography and distinguished by NMR spectroscopy. Conversion of (+)-**5** into (-)-**7** followed standard conditions (N-methylmorpholine N-oxide /catalytic amount of osmium tetroxide followed



Reaction conditions: *a*, Me_2CuLi in THF at 20 °C; *b*, BH_3-THF, 25 °C; *c*, 6-chloropurine/Ph_3P/diethyl azodicarboxylate in THF 60 °C; *d*, (i) NH_3 in MeOH, 70 °C; (ii) Dowex 50X8 (H^+) resin in MeOH, reflux

Scheme 2

by isopropylidenation). Saponification of (-)-7 and then treatment of the alcohol that resulted with pyridinium chlorochromate[16] in the presence of molecular sieves (4Å) powder proceeded via an oxidative elimination process to afford enone (-)-4 in high optical purity in a 41% overall yield from cyclopentadiene.

Enantiomer (+)-4 (Scheme 1) was synthesized from (-)-5 following a similar sequence of reactions (also in an overall yield from cyclopentadiene of 41%). The use of this enantiomer for preparing carbocyclic nucleosides and nucleotides will be the subject of future publications.

With (-)-4 available it was added to a solution of lithium dimethylcuprate (Scheme 2), formed by the procedure of Johnson and Chen.[10] Quenching the addition reaction with saturated ammonium chloride-ammonium hydroxide solution (1:1) gave the substituted

cyclopentanone (-)-**8** (Scheme 2). ^{1}H and ^{13}C NMR spectral data for this compound agreed with the literature for (±)-**8**.[10] Assurance that the methyl group had entered the top face of the cyclopentyl unit of (-)-**4** was obtained by a 1-D nuclear Overhauser enhancement (n.O.e.) determination. In this case, pre-irradiation of H-4 of (-)-**8** resulted in the enhancement of the methyl protons corresponding to the isopropylidene group, indicating that the H-4 is on the same side of the cyclopentane ring as the isopropylidene group. Pre-irradiation of H-4 did not produce an enhancement of the proton corresponding to H-3, which showed that H-3 is *trans* to H-4. All other enhancements were also consistent with the assigned stereochemistry. Further evidence for the structural assignment of (-)-**8** was provided by ^{1}H COSY NMR experiments, which lacked coupling between H-3 and H-4 supporting the *trans* orientation of these protons. These NMR results for (-)-**8** resemble the data reported by Borchardt's group[11a] for a similar compound.

Reduction of (-)-**8** with borane-tetrahydrofuran complex afforded alcohol (-)-**9**. This compound was 99% diastereomerically pure by ^{1}H and ^{13}C NMR analysis and the *trans* orientation of the methyl and hydroxyl groups was established by 1-D n.O.e. experiment in which pre-irradiation of H-1 resulted in enhancement of the methyl protons at C-4. Pre-irradiation of H-1 also showed an enhancement of the proton corresponding to H-2, which supports a *cis* relationship between H-2 and H-1. All other enhancements were consistent with the assigned stereochemistry. A ^{1}H COSY NMR analysis provided further support for structure (-)-**9**. In this case, there was no proton NMR coupling between H-3 and H-4, which confirmed the *trans* orientation of these protons. Also, the ^{1}H COSY experiment showed a strong coupling between H-1 and H-2, indicating a *cis* relationship of these atoms. As with (-)-**8** these observations are consistent with the studies performed on a similar compound that has been reported in the literature.[11a]

Alcohol (-)-**9** was coupled with 6-chloropurine under Mitsunobu conditions[17] to give **10**. The ^{13}C NMR chemical shift values of the purine carbon atoms of **10** indicated that it was exclusively the N-9 product with no contamination by the N-7 isomer.[18] Compound **10** was then treated with methanolic ammonia to give crude protected (-)-**3** that was subjected to deprotection in refluxing methanol containing Dowex 50X8 (H^{+}) resin. From this treatment, (-)-5'-deoxyaristeromycin (-)-(**3**) was obtained.

Antiviral Results

Compound (-)-**3** was evaluated (Table 1) against a wide variety of DNA (herpes including HCMV, pox) and RNA (picorna, toga, orthomyxo, paramyxo, arena, rhabdo, reo, and retro) viruses. Compound (-)-**3** was particularly active against pox (vaccinia), rhabdo (vesicular stomatitis), paramyxo (parainfluenza), reo and arena (Junin, Tacaribe)

Table 1. Inhibitory effect of compound (-)-3 on the replication of DNA and RNA viruses.

Virus (strain)	Cell	MIC[a] (μg/mL)				
		(-)-3	BVDU	Ribavirin	C-c^{3}Ado	Neplanocin A
HSV-1 (KOS)	E_6SM	300	0.015	300	200	
HSV-2 (G)	E_6SM	300	20	>400	200	
TK$^-$ HSV-1 (B2006)	E_6SM	10	100	200	20	
Vaccinia	E_6SM	2	2	70	2	
Vesicular stomatitis	E_6SM	0.7	>400	100	0.7	
VZV (Oka)	HEL	8				1
VZV (YS)	HEL	12				2.4
TK$^-$ VZV (YS-R)	HEL	4				1.9
CMV (AD-169)	HEL	>20				1.1
CMV (Davis)	HEL	>10				0.5
Vesicular stomatitis	HeLa	2	>400	20	4	
Coxsackie B4	HeLa	>200	>400	70	>400	
Polio-1	HeLa	>200	>400	150	>400	
Respiratory syncytial (Long)	HeLa	≥40		4		
Parainfluenza-3 (VR-93)	Vero	20	>400	20	0.7	
Reo-1	Vero	4	>400	150	1	
Sindbis	Vero	>100	>400	20		
Coxsackie B4	Vero	>100	300	>400	300	
Semliki forest	Vero	>100	>400	>400	>400	
Junin	Vero	10		6.5		6.5
Tacaribe	Vero	23		8		5.5
Influenza A (Ishikawa)	MDCK	>100		4		
Influenza B (Singapore)	MDCK	>100		4		
HIV-1	MT-4	>4		>5	>0.8	>0.006
HIV-2	MT-4	>4		>5		
Morphology	E_6SM	>400	>400	>400	>400	
Growth	HEL	>50				21
Morphology	HeLa	≥200	>400	>400	>400	
Morphology	Vero	≥100	>400	>400	>400	
Morphology	MDCK	≥100		> 200		
Viability	MT-4	13		6.5	1.1	0.016

[a]Minimum inhibitory concentration required to either reduce virus-induced cytopathicity (or, for VZV and CMV, plaque formation) by 50%, or to alter normal (microscopically detectable) cell morphology (E_6SM, HeLa, Vero, MDCK), or to inhibit cell growth (HEL) or cell viability (MT-4) by 50%.

viruses, as well as thymidine kinase-deficient (TK^-) herpes simplex virus (HSV) and TK^- and TK^+ varicella-zoster virus (VZV). This activity spectrum is similar to that of AdoHcy hydrolase inhibitors [such as carbocyclic 3-deazaadenosine (C-c^{3}Ado) and neplanocin A], which have broad-spectrum antiviral activity against vaccinia, vesicular stomatitis, reo, arena, parainfluenza, respiratory syncytial virus[6] and also TK^- herpesviruses[19] and human cytomegalovirus.[20] To confirm this, compound (-)-**3** was found to possess an IC_{50}=3.28 ± 0.48 μM, which is consistent with the data reported by the Borchardt group.[8]

While (-)-**3** was similarly inhibitory to vaccinia and vesicular stomatitis virus (in several cell lines) as was carbocyclic 3-deazaadenosine, it was less active against parainfluenza and respiratory syncytial virus, and also less active than neplanocin A against TK^- VZV and HCMV. As seen with other AdoHcy hydrolase inhibitors, (-)-**3** did not prove active against human immunodeficiency virus (HIV) at subtoxic concentrations for the host (MT-4) cells. In this system (exponentially growing cells), (-)-**3** was considerably less toxic (based on inhibition of cell viability) than carbocyclic 3-deazaadenosine and neplanocin A.

Experimental Section

Unless otherwise noted, the reactions were carried out using freshly distilled solvents under anhydrous conditions in an Ar or N_2 atmosphere. The glassware was dried overnight in an oven at 100 °C. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm E. Merck silica gel 60-F_{254} precoated silica gel glass plates, with visualization by irradiation with a Mineralight UVGL-25 lamp, exposure to iodine vapor or by spraying 3% phenol in 5% ethanolic H_2SO_4 and subsequent heating at 200 °C. The column chromatography purifications were performed with the solvents indicated using the silica gel indicated, Davidson Chemical silica gel (60-200 mesh) or Aldrich silica gel (230-400 mesh, 60 Å). ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q spectrometer in the solvents indicated with tetramethylsilane as internal standard. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Optical rotations were measured on Perkin-Elmer 241MC polarimeter. Melting points were recorded on a Mel-Temp capillary melting point apparatus and are uncorrected. The microanalyses were performed by M-H-W Laboratories, Phoenix, AZ.

Pseudomonas cepacia lipase (PCL, PS-30) was purchased from Amano International Enzyme Company, Troy, VA. The Cu(I)I was purchased from Aldrich Chemical Company, Milwaukee, WI (Cat. # 21,555-4) and used without further purification from a freshly opened bottle. The activated molecular sieves 4Å powder were also purchased from Aldrich.

(3*R*,5*S*)-3-Acetoxy-5-phenoxycyclopent-1-ene ((+)-5) and (3*S*,5*R*)-3-Hydroxy-5-phenoxycyclopent-1-ene ((-)-6). Compound (±)-6 was prepared by adaptation of a literature route[15] in the following way to permit scale-up. To a 0 °C cooled suspension of Na_2CO_3 (1 kg) and freshly cracked cyclopentadiene (200 g, 3.03 mol) in CH_2Cl_2 (2.5 L) was added 32% peracetic acid (500 mL, 2.17 mol, pretreated with 25 g of AcONa) dropwise over a period of 1.5 h. The reaction mixture was stirred at room temperature for 3 h. The inorganic salts were removed by filtration and washed with CH_2Cl_2 (1 L). The resultant filtrate was used immediately in the next step.

A solution of tetrakis(triphenylphosphine)palladium (6 g, 5.19 mmol) in dry THF (1 L) was prepared at 0 °C and to this solution phenol (235 g, 2.44 mol) was added. After stirring at 0 °C for 5 min, the CH_2Cl_2-cyclopentene monoepoxide solution (see above) was added over a 30 min period while maintaining the reaction at 0 °C. The reaction mixture was then stirred at the room temperature for 10 min followed by evaporation to dryness. The brown residue was dissolved in Et_2O (1 L) and washed with H_2O (2 x 500 mL), dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by silica gel column chromatography. The column was eluted first with pentane and then with hexane/AcOEt (8:2) to give (±)-3-hydroxy-5-phenoxycyclopent-1-ene ((±)-6)[15] (285 g, 72% based upon the initial cyclopentadiene consumed) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.68 (dt, *J*=15.4 and 4.4 Hz, 1 H, CH), 2.81 (dt, *J*=15.4 and 7.8 Hz, 1 H, CH), 3.35 (br, 1 H, OH), 4.75 (dd, *J*=7 and 4 Hz, 1 H, CHOH), 5.07 (dd, *J*=7 and 4 Hz, 1 H, CHOAr), 6.08 (s, 2 H, CH=CH), 7.34-6.78 (m, 5 H, Ar); ^{13}C NMR ($CDCl_3$) δ 39.56, 73.32, 78.62, 114.05, 119.47 128.14, 131.34, 136.81, 156.47.

To a solution of (±)-6 (221 g, 1.26 mol), 4-dimethylaminopyridine (1 g, 8.18 mmol) and pyridine (120 g, 1.562 mol) in dry CH_2Cl_2 (1 L) at 0 °C was added Ac_2O (150 g, 1.47 mol). After stirring at 0 °C for 2 h, the reaction mixture was stirred at the room temperature for 15 h. The ice-cold reaction mixture was stirred vigorously with saturated aqueous $NaHCO_3$ solution (3 x 500 mL). The organic phase was separated and washed with ice cold 1 N HCl (2 x 500 mL) and H_2O (500 mL), dried ($MgSO_4$) and evaporated to dryness. The residual brown oil was recrystallized from pentane to afford (±)-3-acetoxy-5-phenoxycyclopent-1-ene ((±)-5) (247 g, 89%) as a white solid: mp 56-58 °C; ^{1}H NMR ($CDCl_3$) δ 1.97 (dt, *J*=15.5 and 4 Hz, 1 H, CH), 2.05 (s, 3 H, Me), 2.96 (dt, *J*= 15.5 and 7.6 Hz, 1 H, CH), 5.15 (m, 1 H, CHOAr), 5.62 (m, 1 H, CHOAc), 6.22 (dd, *J*=8.2 and 6.4 Hz, 2 H,CH=CH), 7.05-7.88 (m, 3 H, Ar), 7.41-7.15 (m, 2 H, Ar); ^{13}C NMR ($CDCl_3$) δ 20.53, 38.56, 77.35, 80.16, 115.97, 121.61, 130.16, 134.66, 135.69, 158.44, 171.34.

To a 3 L three-necked flask (fitted with a Hirschberg stirrer, dropping funnel and a pH electrode) was added 237 g (1.09 mol) of (±)-5, 0.2 M phosphate buffer (pH 7, 1.5 L)

and acetone (300 mL). To the stirred suspension was added *Pseudomonas cepacia* lipase (5 g, 169000 units) in one portion. The rapidly decreasing pH was maintained between 7.0 and 7.3 by continuous addition of aqueous NaOH solution (22 g, 0.55 mol, in 500 mL of H_2O). After 2.5 h, more *Pseudomonas cepacia* lipase (1 g, 33800 units) was added. After consumption of the aqueous NaOH solution (approx. 8.5 h), the reaction mixture was diluted with AcOEt (1 L) and filtered through celite. The celite pad was washed with an additional amount of AcOEt (1 L). The organic layer was separated and the aqueous layer was extracted with AcOEt (5 x 300 mL). The combined organic phases were dried ($MgSO_4$) and evaporated to dryness. The residual brown oil was purified by silica gel column chromatography. The column was eluted with hexane/AcOEt (9:1) to give (+)-**5** (95.18 g, 80%), which was recrystallized from pentane: mp 65-66 °C; $[\alpha]_D^{25}$ +32.3 (*c* 1.0, acetone). The spectral data (^1H and ^{13}C NMR) was identical to (±)-**5**. Anal. Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H 6.47. Found: C, 71.49; H 6.38.

The column was next eluted with hexane/AcOEt (6:4) to give (-)-**6** (76.87 g, 80%) as a hygroscopic yellow oil: $[\alpha]_D^{25}$ -5.6 (*c* 1.0, acetone) [reference 15 reported $[\alpha]_D^{21}$ +13.4 (*c* 2.26, $CHCl_3$)]. The spectral data (^1H and ^{13}C NMR) was identical to (±)-**6** and agreed with the literature data.[15] Anal. Calcd. for $C_{11}H_{12}O_2 \cdot 0.5\ H_2O$: C, 70.64; H, 7.11. Found: C, 70.82; H, 6.86.

(1*R*, 2*S*, 3*R*, 4*S*)-1-Acetoxy-4-phenoxy-2,3-(isopropylidenedioxy)-cyclopentane ((-)-7). To a solution of (+)-**5** (95 g, 0.44 mol) and *N*-methylmorpholine *N*-oxide (170 mL of a 60% aqueous solution, 0.872 mol) in acetone (600 mL) and H_2O (200 mL) cooled to 0 °C was added OsO_4 (500 mg) in one portion. After stirring this solution at room temperature for 20 h, the acetone was removed by rotary evaporation. The aqueous residue was cooled to 0 °C and saturated aqueous $NaHSO_3$ solution (200 mL) was added. After stirring for 15 min at 0 °C, this new mixture was extracted with AcOEt (5 x 300 mL). The combined organic phases were washed with ice cold 1 N HCl (2 x 200 mL), dried ($MgSO_4$) and evaporated to dryness to give crude diol (112.0 g, 100%). Without further characterization, this product was used immediately in the next step.

To a solution of the crude diol (112.0 g) in dry acetone (350 mL) that contained 2,2-dimethoxypropane (250 mL) was added *p*-TsOH (2 g). After stirring for 15 h at room temperature, the reaction mixture was neutralized with saturated aqueous Na_2CO_3. The volatile materials were removed by rotary evaporation *in vacuo* and without heating. The residue was dissolved in AcOEt (1 L) and washed with brine (5 x 200 mL), dried ($MgSO_4$) and the AcOEt removed *in vacuo*. The residual yellow oil was purified by silica gel column chromatography and the fraction eluting with hexane/AcOEt (9.5:0.5) to afforded (-)-**7** (113.60 g, 89%), which solidified in the freezer and was recrystallized from pentane: mp

50-51 °C; $[\alpha]_D^{25}$ -11.6 (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 1.47 (s, 3 H, Me), 1.30 (s, 3 H, Me), 2.04 (s, 3 H, Me), 2.20 (dt, *J*=15.4 and 4.4 Hz, 1 H, CH), 2.41 (dt, *J*=15.7 and 5.5 Hz, 1 H, CH), 4.70 (m, 3 H, CHO), 5.13 (d, *J*=5.6 Hz, 1 H, CHOAr), 6.78-7.03 (m, 3 H, Ar), 7.42-7.16 (m, 2 H, Ar); ^{13}C NMR ($CDCl_3$) δ 20.85, 24.45, 26.83, 34.31, 78.95, 81.44, 84.21, 84.58, 111.24, 115.57, 121.26, 129.71, 157.12, 170.39. Anal. Calcd. for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.76; H, 6.80.

(4*R*,5*R*)-4,5-(Isopropylidenedioxy)-2-cyclopentenone ((-)-4). To a solution of (-)-**7** (113.6 g, 0.389 mol) in MeOH (150 mL) was added a solution of KOH (25 g, 0.45 mol) in MeOH (300 mL). The reaction mixture was stirred at room temperature for 3 h and then neutralized to pH 7 by the dropwise addition of concentrated HCl. The MeOH was removed *in vacuo* and the residue was dissolved in AcOEt (1 L). After washing with brine (3 x 200 mL), the organic phase was dried ($MgSO_4$) and evaporated to dryness. The residual colorless oil (95.35 g) was added slowly to a stirred suspension of molecular sieves (4Å powder, 200 g) and pyridinium chlorochromate (147 g, 0.68 mL) in dry CH_2Cl_2 (3 L). After stirring at room temperature for 15 h, the reaction mixture was diluted with Et_2O (1 L) and filtered through silica gel. The filtrate was evaporated to dryness and the residue was purified by silica gel column chromatography (eluent hexane/AcOEt, 8:2) to give (-)-**4** (53.91 g, 90% based on (-)-**7**) as a colorless crystalline solid, which was recrystallized from pentane/Et_2O (8:1): mp 65-66 °C (lit.[12] 66-67 °C); $[\alpha]_D^{25}$ -72.39 (*c* 0.92, $CHCl_3$) [lit.[13] $[\alpha]_D$ -70.8 (*c* 0.92 $CHCl_3$)]; ^1H NMR ($CDCl_3$) δ 1.39 (br s, 6 H, 2 x Me), 4.43 (d, *J*=5.5 Hz, 1 H, H-5), 5.24 (dd, *J*=2.2 and 5.5 Hz, 1 H, H-4), 6.19 (d, *J*=5.9 Hz, 1 H, H-2), 7.58 (dd, *J*=2.2 and 5.9 Hz, 1 H, H-3). This data agrees with the literature.[12,13]

(3*S*,5*R*)-3-Acetoxy-5-phenoxycyclopent-1-ene ((-)-5). Using the same procedure for converting (±)-**6** into (±)-**5**, compound (-)-**5** was obtained (18.4 g, 99%): mp 64-65 °C; $[\alpha]_D^{25}$ -32.5 (*c* 1.0, acetone)) from (-)-**6** (15.0 g, 85.22 mmol). The NMR spectral data for (-)-**5** was identical with (+)-**6**.

(1*S*, 2*R*, 3*S*, 4*R*)-1-Acetoxy-4-phenoxy-2,3-(isopropylidenedioxy)-cyclopentane ((+)-7). Using the same procedure for converting (+)-**5** into (-)-**7**, compound (+)-**7** was obtained (15.6 g, 90%; $[\alpha]_D^{25}$ +11.5 (*c* 1.0, CH_2Cl_2)) from (-)-**5** (13.0 g, 59.63 mmol). The physical data (mp, NMR) for (+)-**7** was identical with (-)-**7**.

(4*S*,5*S*)-4,5-(Isopropylidenedioxy)-2-cyclopentenone ((+)-4). Using the same procedure as that presented for converting (-)-**7** into (-)-**4**, compound (+)-**4** was obtained (2.4 g, 90%; $[\alpha]_D^{25}$ +71.3 (*c* 0.92, $CHCl_3$)) (lit.[13] $[\alpha]_D^{25}$ +71.8) from (+)-**7** (5 g, 17.12 mmol). The physical data (mp, NMR) for (+)-**4** was identical with that for (-)- **4**.

(1*S*, 2*S*, 3*R*, 4*S*)-4-Methyl-2,3-(isopropylidenedioxy)cyclopentan-1-ol (-)-(9). A slurry of CuI (4.4 g, 23.10 mmol) in dry THF (50 mL) was cooled to -20

°C in an ice/MeOH bath and 1.5 M methyl lithium in hexane (31 mL, 46.48 mmol) was added over a period of 5 min under an Ar atmosphere. After stirring 3 min at -20 °C, (-)-**4** (2 g, 18.18 mmol) dissolved in dry THF (50 mL) was added to the organocuprate solution at -20 °C and this new mixture stirred at this temperature for 30 min. The reaction mixture was quenched by pouring onto 200 mL of saturated aqueous NH_4Cl/NH_4OH solution (1:1) and this stirred for 15 min at the room temperature. The THF layer was separated and the aqueous layer extracted with Et_2O (3 x 30 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to dryness. The residual oil was dissolved in Et_2O and purified by silica gel column chromatography. Elution with Et_2O yielded (-)-(2*R*, 3*R*, 4*S*)-4-methyl-2,3-(isopropylidenedioxy)-1-cyclopentanone (-)-(**8**) (2.95 g, 96%) as a colorless oil: $[\alpha]_D^{25}$ -224.73 (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.06 (d, *J*=7.6 Hz, 3 H, Me), 1.35 (s, 3 H, Me), 1.43 (s, 3 H, Me), 1.97 (d, *J*=18.2 Hz, 1 H, CH), 2.52 (pentet, *J*=7.8 Hz, 1 H, CH), 2.81 (dd, *J*=18.2 and 8.4 Hz, 1 H, C<u>H</u>CH_3), 4.24 (d, *J*=5.2 Hz, 1 H, CHO), 4.50 (d, *J*=5.2 Hz, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 19.11, 24.79, 26.77, 31.08, 41.43, 78.00, 83.12, 112.16, 214.29. This spectral data agreed with that for (±)-**8** as reported in the literature.[10]

To a solution of (-)-**8** (2.9 g, 17.06 mmol) in dry THF (50 mL) cooled to -20 °C was added 1.0 M of BH_3•THF complex (40 mL, 40 mmol) under an Ar atmosphere. The reaction mixture was stirred at the room temperature for 3 h, and then evaporated to dryness. The residual oil was co-evaporated with dry MeOH (3 x 50 mL) to give crude product that was purified by silica gel column chromatography (AcOEt/hexane, 6:4) to provide (-)-**9** (2.64 g, 90%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 0.96 (d, *J*=7.2 Hz, 3 H, Me), 1.32 (s, 3 H, Me), 1.42 (s, 3 H, Me), 1.96 (m, 2 H, CH_2), 2.2 (m, 1 H, C<u>H</u>CH_3), 4.10 (br, 1 H, OH), 4.14 (d, *J*=7 Hz, 1 H, C<u>H</u>OH), 4.29 (d, *J*=5.8 Hz, 1 H, CHO), 4.52 (dd, *J*=5.6 and 5.5 Hz, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 18.96, 24.56, 26.29, 35.61, 38.48, 71.53, 79.22, 86.37, 111.51. Anal. Calcd. for $C_9H_{16}O_3$: C, 62.77; H, 9.36. Found: C, 62.73; H, 9.40.

5'-Deoxyaristeromycin (-)-(3). To suspension of 6-chloropurine (0.494 g, 3.19 mmol) and triphenyl phosphine (0.838 g, 3.19 mmol) in dry THF (20 mL) was added diethyl azodicarboxylate (0.557 g, 3.19 mmol). This was followed by vigorous stirring at the room temperature under an Ar atmosphere for 5 min. To this mixture was added a solution of **9** (0.50 g, 2.91 mmol) in dry THF (20 mL). After stirring at room temperature for 2 h, the mixture was heated at 60 °C for 48 h and then evaporated to dryness. The residue was purified by silica gel column chromatography by first eluting with CH_2Cl_2 to remove nonpolar impurities, and then with CH_2Cl_2/MeOH (98:2). The product containing fractions were combined and evaporated to dryness. The residue was subjected to a second chromatographic purification (Et_2O/hexane, 8:2) to give 9-[(1'*R*, 2'*S*, 3'*R*, 4'*R*)-4'-

methyl-2',3'-isopropylidenedioxy)cyclopentan-1'-yl]-6-chloropurine (**10**) (0.52 g, 58% with slight contamination by dicarbethoxyhydrazine) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.08 (d, *J*=7 Hz, Me), 1.33 (s, 3 H, Me), 1.59 (s, 3 H, Me), 2.5 (m, 3 H, H-4' and H-5'), 3.5 (dt, *J*=15.6 and 7 Hz, 1 H, 1'-H), 4.5 (m, 1 H, H-3'), 5.1 (m, 1 H, H-2'), 8.29 (s, 1 H, H-2), 8.75 (s, 1 H, H-8); ^{13}C NMR ($CDCl_3$) δ 12.96, 14.56, 16.38, 23.58, 25.91, 60.96, 82.20, 84.21, 111.53, 112.54, 130.74, 143.31, 150.14, 155.44.

Compound **10** (0.38 g, 1.23 mmol) was added to a saturated methanolic NH_3 solution (25 mL), which was then sealed in an autoclave. The autoclave was heated at 70 °C for 20 h. After cooling to 0 °C, the autoclave was opened and the reaction mixture was evaporated to dryness to afford a white solid residue (370 mg). This solid was dissolved in MeOH (100 mL) and refluxed for 4 h in the presence of Dowex 50X8 (H^+) resin (12 g). The solvent was removed by rotary evaporation and the residue was placed on a Dowex 50X8 (H^+) resin column (2 x 15 cm length). The column was eluted with H_2O until the eluents were neutral to pH paper, and then eluted with 75 mL portions of concentrated NH_4OH. The product containing fractions were evaporated to dryness and the residue recrystallized from acetone/MeOH to give pure (-)-**3** (0.183 g, 60% based upon (-)-**9**) as a white solid: mp 158-160 °C (lit.[8] 172-174 °C); $[\alpha]_D^{25}$ -40.0 (*c* 0.106, MeOH) [lit.[8] $[\alpha]_D$ -36.0 (*c* 0.994, 0.3 N HCl)]; ^{1}H NMR (DMSO-d_6) δ 1.15 (d, *J*=7 Hz, Me), 2.34-1.45 (m, 3 H, H-4' and H-5'), 3.68 (m, 1 H, H-1'), 4.36 (m, 2 H, OH), 4.62 (d, *J*=15 Hz, 1 H, H-3'), 4.96 (d, *J*=15 Hz, 1 H, H-2'), 7.23 (s, 2 H, NH_2), 8.15 (s, 1 H, H-2), 8.28 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 19.29, 34.57, 37.71, 60.57, 74.66, 76.50, 119.60, 140.48, 149.80, 152.29, 156.24. Anal. Calcd. for $C_{11}H_{15}N_5O_2 \cdot 0.50\ H_2O$: C, 51.15; H, 6.24; N, 27.12. Found: C, 51.48; H, 6.24; N, 26.99.

Antiviral Activity Assays. The antiviral assays were carried out as described in reference 21, except for the anti-VZV assays (see reference 22). The sources of the viruses have also been described in these previous publications. Abbreviations used for the viruses and cells are as follows: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VZV, varicella-zoster virus; HCMV, human cytomegalovirus; TK$^-$, thymidine kinase deficient; E_6SM, embryonic skin-muscle; HEL, human embryonic lung; MDCK, Madin-Darby canine kidney. Abbreviations used for the reference compounds are as follows: BVDU, (*E*)-5-(2-bromovinyl)-2'-deoxyuridine; C-c^{3}Ado, carbocyclic 3-deazaadenosine.

Cytotoxicity Assays. Cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero, MDCK) or inhibition of cell growth (HEL), as has been described previously.[21]

AdoHcy Hydrolase Assays. AdoHcy hydrolase was purified from murine L929 cells to apparent homogeneity by using affinity chromatography; enzymatic activity was measured in the direction of AdoHcy synthesis, as described previously.[6b]

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