Notes

Antiviral Enantiomeric Preference for 5'-Noraristeromycin

Suhaib M. Siddiqi, Xing Chen, and Stewart W. Schneller*

Department of Chemistry, University of South Florida, Tampa, Florida 33620-5250

Satoru Ikeda, Robert Snoeck, Graciela Andrei, Jan Balzarini, and Erik De Clercq

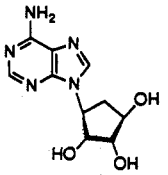
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Received October 1, 1993®

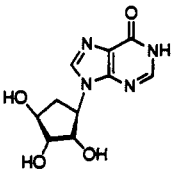
In order to determine if the potent antiviral properties of (±)-5'-noraristeromycin reside in one of its enantiomers, an analysis of each enantiomer has been carried out. A five-step route to the (+)-stereoisomer is described from (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate, whereas the synthesis of the (−)-enantiomer had been reported previously from the same starting material. The (−)-**2** and (+)-**2** enantiomers were evaluated for antiviral activity against a large number of viruses and found to display an antiviral activity spectrum characteristic of (*S*)-adenosyl-L-homocysteine hydrolase inhibitors. The (−)-enantiomer retained the significant anticytomegalovirus properties previously reported for the racemic **2** and was, on the average, 10-fold more potent than (+)-**2** in inhibiting virus replication, tumor cell growth, and (*S*)-adenosyl-L-homocysteine hydrolase activity.

The antiviral potential of carbocyclic adenosine (aristeromycin, **1**)[1] has been limited by the toxicity that arises from the biochemical consequence of its metabolic 5'-triphosphate product.[2] As part of an effort to modify the C-5' center of **1** with the intention to circumvent the undesirable nucleotide outcome, we recently reported racemic 5'-noraristeromycin ((±)-**2**) as a potent, nontoxic antiviral agent.[3] In order to ascertain if this activity resided in one of the enantiomers of (±)-**2**, we undertook an antiviral analysis of each enantiomer. The preparation of the D-like enantiomer (−)-**2** was reported previously,[4] and the synthesis of the L-like enantiomer (+)-**2** is reported here.[5] It should be noted that, in addition to its relevance for the bioenantiomeric preference associated with (±)-**2**, compound (+)-**2** also provides an entry into the L-carbocyclic nucleoside series, whose biological properties have not been studied[6] to the same extent as the L-ribofuranosyl series.[7] For this reason, the effect of (−)-**2**, (+)-**2**, and (±)-**2** on tumor cell proliferation is also reported.

(·)-**1**, R=CH_2OH
(·)-**2**, R=OH



(+)-**2**

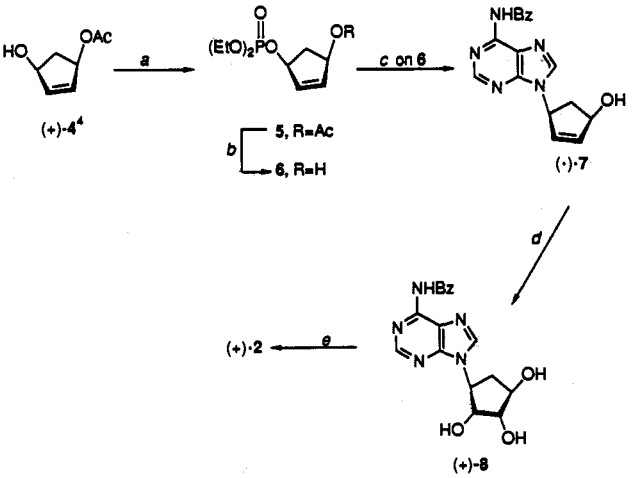


(·)-**3**

Chemistry

Recent work with aristeromycin[6b] and carbocyclic 2,6-diaminopurine ribofuranosides[6b,d] suggested that (±)-**2**[3] could be resolved with the use of adenosine deaminase. Thus, treating (±)-**2**[3] with this enzyme led, after 4 days, to a mixture of the partially resolved (+)-**2** and the inosine analogue (−)-**3**. In view of the length of time this enzymatic reaction was foreseen as requiring to provide enantiomerically pure (+)-**2**, attention turned to a chemical means of preparing this enantiomer. Before describing that method, however, it is pertinent to note that adenosine deaminase acted on the enantiomer of **2** that has a configurational resemblance to the other carbocyclic nucleosides[6b,d] that are substrates for this enzyme. It is also noteworthy that the rate of deamination of (±)-**2** appears to be slower than for (±)-**1**.[6b] This suggests that an important aspect of the antiviral potency of **2** may be due to its relative stability toward adenosine deaminase.

Scheme 1[a]



[a] Reaction conditions: (a) $(EtO)_2P(=O)Cl$ and pyridine in CH_2Cl_2; (b) NH_3/MeOH; (c) N^6-benzoyladenine and NaH in DMSO and then catalytic $Pd(PPh_3)_4$, PPh_3, and **6** in THF; (d) OsO_4, 60% aqueous *N*-methylmorpholine *N*-oxide in THF–H_2O; (e) NH_4OH/MeOH.

A more useful synthesis of (+)-**2** (Scheme 1) began with the conversion of (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate (**4**)[4] into its phosphate derivative **5** using diethyl chlorophosphate in the presence of pyridine. Selective ammonolysis of **5** to **6** was followed by a

® Abstract published in *Advance ACS Abstracts*, January 1, 1994.

0022-2623/94/1837-0551$04.50/0

Table 1. Activity of (−)-2 and (+)-2 against Different Viruses in Different Cell Systems

virus	cell[b]	MIC_{50} (μg/mL)[a] (−)-2	(+)-2	neplanocin A
HSV-1 (KOS)	E_6SM	>400	>400	70
HSV-2 (G)	E_6SM	>400	>400	>100
VV	E_6SM	0.04	0.7	0.2
VSV	E_6SM	0.1	2.0	2
VSV	HeLa	0.2	2	2
coxsackie B4	HeLa	>400	>400	>10
polio-1	HeLa	>400	>400	>10
RSV (strain Long)	HeLa	>200	>200	>1.2
parainfluenza-3	HeLa	0.07	0.2	0.2
reovirus-1	Vero	0.7	7	0.7
sindbis	Vero	>400	>400	>10
semliki forest	Vero	>400	>400	>10
junin	Vero	6	30	0.4
tacaribe	Vero	8	50	0.4
HIV-1	CEM	>0.16	>4	
HIV-2	CEM	>0.16	>4	
influenza A	MDCK	>100	>200	1.2
influenza B	MDCK	>100	>200	≥2.8
CMV (AD-169)	HEL	0.01–0.05[c]	5–20	0.2–0.5
CMV (Davis)	HEL	0.03–0.07[d]	3–20	0.2–0.3
VZV (OKA)	HEL	>50	>50	6
VZV (YS)	HEL	>50	>50	3.5

[a] Required to reduce virus-induced cytopathicity by 50%. [b] Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal (E_6SM, HeLa, Vero, or MDCK) cell morphology was >400 μg/mL for both (−)-2 and (+)-2. For neplanocin A, the MIC_{50} for (E_6SM, HeLa, Vero, or MDCK) cell morphology was ≥200, ≥40, 20, 40, and 6 μg/mL, respectively. Compounds (−)-2 and (+)-2 inhibited HEL cell growth by 50% at a concentration (IC_{50}) of 50 μg/mL. Their IC_{50}s for CEM cell growth are indicated in Table 2. [c] MIC_{50} versus AD-169 for ganciclovir = 0.7–4 μg/mL and for (S)-HPMPC = 0.07–0.2 μg/mL.[22] [d] MIC_{50} versus Davis for ganciclovir = 0.5–1 μg/mL and for (S)-HPMPC = 0.1–0.4 μg/mL.[22]

palladium-catalyzed coupling[4,9] of N^6-benzoyladenine with 6 to give 7. Standard glycolization of 7 to 8 was followed by debenzoylation of 8 using ammonium hydroxide to provide the desired (1*R*,2*S*,3*R*,4*S*)-4-(6-amino-9*H*-purin-9-yl)cyclopentane-1,2,3-triol ((+)-2). With this means to (+)-2, it is now possible to prepare both the (−)-2[4] and (+)-2 enantiomers from the common precursor 4.

Antiviral Results

Compounds (−)-2 and (+)-2 were evaluated against a wide variety of both DNA viruses and RNA viruses (Table 1). The compounds showed a marked activity against vaccinia virus (VV), vesicular stomatitis virus (VSV), parainfluenza-3 virus, and reovirus-1 (MIC_{50} values ranging from 0.07 to 7 μg/mL).

Of particular interest was the observation that the previously noted significant activity of (±)-2 toward cytomegalovirus (CMV)[3] was found to be due to the (−)-2 enantiomer. Current interest in the development of new anti-CMV agents is due to several reasons. First, CMV has been increasingly recognized as an important and often life-threatening pathogen in immunocompromised patients, leading to various organ infections such as pneumonitis, retinitis, colitis, encephalitis, and hepatitis. Also, the two compounds which are licensed and currently used in the treatment of CMV infections [that is, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (ganciclovir) and trisodium phosphonoformate (foscarnet)][10] exhibit a number of undesirable properties: (i) toxicity (that is, neutropenia and nephrotoxicity, respectively), (ii) disease relapses after cessation of therapy, and (iii) emergence of drug-resistant virus strains upon prolonged treatment. Thus, to evaluate the potential of (−)-2 as a new anti-CMV agent, its MIC_{50} values were compared to those of ganciclovir, which is particularly useful in treating CMV retinitis,[11] and (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)-propyl]cytosine (HPMPC), for which clinical trials have been started in HIV-positive patients.[12,13] Compound (−)-2 was more potent (see footnotes c and d of Table 1) than either of these compounds.

Table 2. Activity of (−)-2, (+)-2, and (±)-2 against Proliferation of Different Tumor Cells

cell	IC_{50} (μg/mL)[a] (−)-2	(+)-2	(±)-2
murine leukemia L1210/0	0.50 ± 0.15	5.70 ± 0.18	0.63 ± 0.09
human T-lymphocyte Molt 4 (clone 8)	0.39 ± 0.23	4.65 ± 0.96	0.58 ± 0.21
human T-lymphocyte CEM/0	0.42 ± 0.21	3.99 ± 0.86	0.68 ± 0.45

[a] Concentration required to inhibit tumor cell proliferation by 50%.

Table 3. Inhibitory Effects of (−)-2, (+)-2, (±)-2, and Neplanocin A on L929 Cell AdoHcy Hydrolase Activity

compound	K_i (μM)	IC_{50} (μM)
(−)-2		0.032 ± 0.0048
(+)-2		0.59 ± 0.25
(±)-2	0.0111 ± 0.0048	
neplanocin A	0.003 ± 0.001	0.0052 ± 0.0018

Toward the other viruses, the (−)-2 enantiomer was invariably (5–20)-fold more potent as an antiviral compound than the (+)-2 enantiomer. Also, the (−)-enantiomer was about 10-fold more cytostatic to the proliferation of murine leukemia L1210 and human T lymphocyte (Molt-4 and CEM) cells than the (+)-enantiomer (Table 2). When evaluated for their inhibitory effect on (*S*)-adenosyl-L-homocysteine (AdoHcy) hydrolase activity, (−)-2 again proved almost 20-fold more potent than (+)-2 (Table 3). Thus, the AdoHcy hydrolase inhibiting effect previously noted for (±)-2[3] could be attributed primarily to the (−)-enantiomer.

The antiviral activity spectrum shown by the (−)- and (+)-enantiomers of 5′-noraristeromycin is compatible with their action targeted at AdoHcy hydrolase. Also, their cytostatic activity can be attributed to AdoHcy hydrolase inhibition. Their relative (about 10-fold difference) potencies in inhibiting AdoHcy hydrolase activity, virus replication, and tumor cell proliferation also point to a possible relationship between the antiviral and cytostatic effects of the (−)- and (+)-5′-noraristeromycins and their inhibitory effects on AdoHcy hydrolase.

Experimental Section

Materials and Methods. 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. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q spectrometer (operated at 90 MHz and 22.5 MHz, respectively) in $CDCl_3$ or DMSO-d_6 referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols d (doublet), t (triplet), and m (multiplet). Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm E. Merck Silica gel 60-F_{254} precoated silica gel plates (Aldrich) with visualization by irradiation with a Mineralight UVGL-25 lamp, exposure to iodine vapor, or spraying with 3% phenol in 5% ethanolic H_2SO_4 and subsequent heating at 200 °C. The column chromatographic purifications were performed using Davidson Chemical silica gel (60–200 mesh) or Aldrich silica gel (230–400 mesh, 60 Å), eluting with the indicated solvent system. Yields refer to chromato-

graphically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials. The reactions were generally carried out in a Ar atmosphere under anhydrous conditions. The data reported in Tables 1, 2, and 3 were determined from two to four independent assays.

Reaction of (±)-5′-Noraristeromycin with Adenosine Deaminase. (±)-5′-Noraristeromycin ((±)-**2**)[3] (350 mg, 1.39 mmol) was suspended in 0.2 M phosphate buffer (60 mL), and this mixture was refluxed until complete dissolution occurred. After the mixture was cooled to room temperature, adenosine deaminase (1000 units, Sigma Type VIII) was added and the resulting mixture was stirred at room temperature for 4 days. After refluxing for 30 min, the clear solution was filtered while hot through a pad of Celite and the celite pad washed with hot H_2O (2 × 5 mL). The combined filtrates were evaporated to dryness, and the residue was subjected to flash column chromatography (MeOH–CH_2Cl_2, 1:4) to give 203 mg (58%) of partially resolved 5′-noraristeromycin: mp 227 °C dec; $[\alpha]^{25}_D$ +18.6° (c 1, DMF). The ^{1}H and ^{13}C NMR spectral data for this product were identical to those for (±)-**2**[3] and (−)-**2**.[4]

Further elution of the silica gel column (MeOH–CH_2Cl_2, 1:4) gave (−)-**3** (105 mg, 30%) as a white solid: mp 233–235 °C (lit.[3] mp 230–232 °C for (±)-**3**); $[\alpha]^{25}_D$ −39.4 (c 1, DMF). The ^{1}H and ^{13}C NMR spectral data were identical with those for (±)-**3**.[3]

(1*S*,4*R*)-*N*-[9-(4-Hydroxy-2-cyclopenten-1-yl)-9*H*-purin-6-yl]benzamide ((−)-7). Diethyl chlorophosphate (9.25 g, 53.6 mmol) was added to a 0 °C solution of (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate[4] (5.68 g, 40.0 mmol) in CH_2Cl_2 (40 mL) and pyridine (6 mL). After being stirred for 30 min at 0 °C, the resulting white slurry was stirred for 4 h at room temperature. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and washed with ice-cold 5% aqueous HCl solution (3 × 30 mL), saturated aqueous $NaHCO_3$ solution (3 × 30 mL), and brine (3 × 30 mL). The organic phase was dried ($MgSO_4$) and evaporated to dryness with the aid of a rotary evaporator to give crude (1*S*,4*R*)-4-acetoxy-2-cyclopenten-1-yl diethyl phosphate (**5**) (11.5 g, 100%) as a yellow oil. This substance was used in the next step without purification and characterization.

A solution of crude **5** (11.5 g, 41.37 mmol) in NH_3-saturated MeOH (150 mL) was placed in a sealed tube. This mixture was stirred at 40 °C for 4 h and the solvent removed by rotary evaporation. The residue was purified by silica gel chromatography (MeOH–CH_2Cl_2, 0.5:9.5) to give (1*S*,4*R*)-4-hydroxy-2-cyclopenten-1-yl diethyl phosphate (**6**) (9.36 g, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.32 (t, 6 H, J = 7 Hz, 2 × CH_3), 1.83 (dt, 1 H, J_1 = 15.5 Hz, J_2 = 4.5 Hz, H-5), 2.67 (dt, 1 H, J_1 = 15.5 Hz, J_2 = 4.5 Hz, H-5), 4.25 (m, 5 H, 2 × CH_2 and OH), 4.68 (m, 1 H, H-4), 5.26 (m, 1 H, H-1), 6.05 (m, 2 H, H-2 a nd H-3). This material was used in the next step without further characterization.

To a solution of N^6-benzoyladenine (6.08 g, 25.42 mmol) in dry DMSO (40 mL) was added sodium hydride (60% suspension in mineral oil, 1.02 g, 25.42 mmol). The reaction mixture was then stirred at room temperature for 30 min followed by the addition of tetrakis(triphenylphosphine)palladium (2.9 g, 2.54 mmol), triphenylphosphine (1 g, 3.81 mmol), and a solution of **6** (5.16 g, 36.34 mmol) in dry THF (150 mL). This mixture was stirred at 50 °C for 20 h. The volatiles were removed by rotary evaporation *in vacuo* at 18 mmHg and 50 °C. The residue was slurried in CH_2Cl_2 (250 mL) and filtered to remove the insoluble solids. The filtrate that resulted was washed with brine (3 × 200 mL), dried ($MgSO_4$), and evaporated to dryness. The residual oil was purified by flash chromatography on silica gel by eluting first with AcOEt to remove the nonpolar impurities and then with AcOEt–MeOH (9:1). The product-containing fractions were evaporated to dryness with the aid of a rotary evaporator, and the residue was recrystallized from AcOEt to give (−)-**7** as an off-white solid (4.2 g, 51.5%): mp 160–162 °C (lit.[4] mp for (+)-**7**, 159–161 °C); $[\alpha]^{25}_D$ −70.8° (c 0.555, MeOH) [lit.[4] $[\alpha]^{25}_D$ for (+)-**7**, +70.27° (c 0.555, MeOH)]. The ^{1}H and ^{13}C NMR spectral data were identical with those for (+)-**7**.[4] Anal. ($C_{17}H_{15}N_5O_2$) C, H, N.

(1*R*,2*S*,3*R*,4*S*)-*N*-[9-(2,3,4-Trihydroxy-2-cyclopenten-1-yl)-9*H*-purin-6-yl]benzamide ((+)-8). To a solution of (−)-**7** (4.0 g, 12.46 mmol) in THF–H_2O (10:1, 50 mL) was added 60% aqueous *N*-methylmorpholine *N*-oxide (3.65 g, 18.69 mmol) followed by osmium tetroxide (100 mg). The reaction mixture was then stirred at room temperature for 24 h. The solvent was removed by rotary evaporation and the residue treated with saturated aqueous $NaHSO_3$ solution (10 mL). This new mixture was evaporated to dryness and the residue coevaporated with EtOH (3 × 50 mL) to give a gummy material, which was purified by flash column chromatography on silica gel (MeOH–CH_2Cl_2, 1:9) to give (+)-**8** (3.8 g, 86%) as a white solid: mp 150 °C dec, (lit.[4] mp for (−)-**8**, 150 °C); $[\alpha]^{25}_D$ +31.4° (c 0.5, MeOH) [lit.[4] $[\alpha]^{25}_D$ for (−)-**8**, −31.7° (c 0.5, MeOH)]. The ^{1}H and ^{13}C NMR spectral data were identical with those for (−)-**8**.[4] Anal. ($C_{17}H_{17}N_5O_4$) C, H, N.

(1*R*,2*S*,3*R*,4*S*)-4-(6-Amino-9*H*-purin-9-yl)cyclopentane-1,2,3-triol ((+)-2). A solution of (+)-**8** (3.6 g, 10.14 mmol) in MeOH–NH_4OH (1:1, 100 mL) was heated, in a sealed tube, at 100 °C for 20 h. After cooling to room temperature, the reaction mixture was evaporated to dryness. The residue was triturated with a small amount of MeOH to give a white solid, which was filtered and purified by flash column chromatography on silica gel (MeOH–CH_2Cl_2, 4:6). The product-containing fractions were evaporated to dryness with the aid of a rotary evaporator, and the residue was recrystallized from ethanol to give (+)-**2** (2.1 g, 83%) as an off-white solid: mp 227 °C dec (lit.[4] mp for (−)-**2**, 227 °C dec); $[\alpha]^{25}_D$ +44.2° (c 1.0, DMF) [lit.[4] $[\alpha]^{25}_D$ for (−)-**2**, −45.3° (c 1.0, DMF)]. The ^{1}H and ^{13}C NMR spectral data were identical with those for (±)-**2**[3] and (−)-**2**.[4] Anal. ($C_{10}H_{13}N_5O_3 \cdot H_2O$) C, H, N.

Antiviral Activity Assays. The antiviral assays, other than the anti-HIV-1 assays, were based on an inhibition of virus-induced cytopathicity in either E_6SM, HeLa, Vero, MDCK, or HEL cell cultures, following previously established procedures.[14-16] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$[17] of virus, 1 $CCID_{50}$[17] being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Cytostatic Activity Assays. The cytostatic assays were performed as previously described.[18] Briefly, 100-μL aliquots of the cell suspensions (5 × 10^5 murine leukemia L1210 or 7.5 × 10^5 human T-lymphocyte Molt-4 or CEM cells/mL) were added to the wells of a microtiter plate containing 100 μL of varying concentrations of the test compounds. After a 2-day (L1210) or 3-day (Molt-4 and CEM) incubation period at 37 °C in a humidified CO_2-controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the compound concentration that reduced the number of viable cells by 50% (CC_{50}). The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero, and MDCK) or inhibition of normal cell growth (HEL), as previously described.[18]

Inhibition of HIV-1-Induced Cytopathicity in MT-4 Cells. Human 5 × 10^5 MT-4 cells were infected with 100 $CCID_{50}$[17] of HIV-1/mL (strain HTLV-III_B) and seeded in 200-μL microtiter plate wells, containing appropriate dilutions of the test compounds.[19] After 5 days of incubation at 37 °C, the number of viable cells was determined in a blood-cell counting chamber by trypan blue exclusion.

Inhibition of AdoHcy Hydrolase Activity. Measurements of L929 cell AdoHcy hydrolase activity inhibition were carried out as described by us.[20,21] The AdoHcy hydrolase activity was measured in the direction of AdoHcy synthesis, using as substrates [8-^{14}C]Ado at various concentrations and D,L-homocysteine at a concentration of 2 mM. The K_m of the purified L929 cell AdoHcy hydrolase for [8-^{14}C]Ado was 0.51 μM.

Acknowledgment. This research was supported by funds from the Department of Health and Human Services (NO1-AI-72645), and this is appreciated. These investigations were also supported by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (project number 3.0026.91) and the

Belgian Geconcerteerde Onderzoeksacties (project number 90/94-2). We thank Anita Van Lierde, Frieda De Meyer, Anita Camps, Lizette van Berckelaer, Ann Absillis, and Ria Van Berwaer for excellent technical assistance and Christiane Callebaut for fine editorial help.

References

(1) De Clercq, E. S-Adenosylhomocysteine Hydrolase Inhibitors as Broad Spectrum Antiviral Agents. *Biochem. Pharmacol.* **1987**, *36*, 2567–2575.

(2) Wolfe, M. S.; Borchardt, R. T. S-Adenosyl-L-homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521–1530.

(3) Patil, S. D.; Schneller, S. W.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Synthesis and Antiviral Properties of (±)-5′-Noraristeromycin and Related Purine Carbocyclic Nucleosides. A New Lead for Anti-Human Cytomegalovirus Agent Design. *J. Med. Chem.* **1992**, *35*, 3372–3377.

(4) Siddiqi, S. M.; Chen, X.; Schneller, S. W. Enantiospecific Synthesis of 5′-Noraristeromycin and Its 7-Deaza Derivative and a Formal Synthesis of (−)-5′-Homoaristeromycin. *Nucleosides Nucleotides* **1993**, *12*, 267–278.

(5) During the preparation of this manuscript, an alternative synthesis of (−)-**2** and (+)-**2** was described without synthetic details: Merlo, V.; Reece, F. J.; Roberts, S. M.; Gregson, M.; Storer, M. Synthesis of Optically Active 5′-Noraristeromycin: Enzyme Catalyzed Kinetic Resolution of 9-(4-Hydroxycyclopent-2-enyl)purines. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1717–1718.

(6) (a) Herdewijn, P.; Balzarini, J.; De Clercq, E.; Vanderhaeghe, H. Resolution of Aristeromycin Enantiomers. *J. Med. Chem.* **1985**, *28*, 1385–1386. (b) Secrist, J. A., III; Montgomery, J. A.; Shealy, Y. F.; O'Dell, C. A.; Clayton, S. J. Resolution of Racemic Carbocyclic Analogues of Purine Nucleosides through the Action of Adenosine Deaminase. Antiviral Activity of the Carbocyclic 2′-Deoxyguanosine Enantiomers. *J. Med. Chem.* **1987**, *30*, 746–749. (c) Bennett, L. L., Jr.; Shealy, Y. F.; Allan, P. W.; Rose, L. M.; Shannon, W. M.; Arnett, G. Phosphorylation of the Carbocyclic Analog of 2′-Deoxyguanosine in Cells Infected with Herpes Viruses. *Biochem. Pharmacol.* **1990**, *40*, 1515–1522. (d) Vince, R.; Brownell, J. Resolution of Racemic Carbovir and Selective Inhibition of Human Immunodeficiency Virus by the (−) Enantiomer. *Biochem. Biophys. Res. Commun.* **1990**, *168*, 912–916. (e) Miller, W. H.; Daluge, S. M.; Garvey, E. P.; Hopkins, S.; Reardon, J. E.; Boyd, F. L.; Miller, R. L. Phosphorylation of Carbovir Enantiomers by Cellular Enzymes Determines the Stereoselectivity of Antiviral Activity. *J. Biol. Chem.* **1992**, *267*, 21220–21224.

(7) See, for example: (a) Beach, J. W.; Jeong, L. S.; Alves, A. J.; Pohl, D.; Kim, H. O.; Chang, C.-N.; Doong, S.-L.; Schinazi, R. F.; Cheng, Y.-C.; Chu, C. K. Synthesis of Enantiomerically Pure (2′*R*,5′*S*)-(−)-1-[2-(Hydroxymethyl)oxathiolan-5-yl]cytosine as a Potent Antiviral Agent against Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV). *J. Org. Chem.* **1992**, *57*, 2217–2219. (b) Spadari, S.; Maga, G.; Focher, F.; Ciarrocchi, G.; Manservigi, R.; Arcamone, F.; Capobianco, M.; Carcuro, A.; Colonna, F.; Iotti, S.; Garbesi, A. L-Thymidine Is Phosphorylated by Herpes Simplex Virus Type I Thymidine Kinase and Inhibits Viral Growth. *J. Med. Chem.* **1992**, *35*, 4214–4220. (c) Kim, H. O.; Shanmuganathan, K.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Schinazi, R. F.; Chang, C.-N.; Cheng, Y.-C.; Chu, C. K. Potent Anti-HIV and Anti-HBV Activities of (−)-L-β-Dioxolane-C and (+)-L-β-Dioxolane-T and Their Asymmetric Syntheses. *Tetrahedron Lett.* **1992**, *33*, 6899–6902. (d) Kim, H. O.; Schinazi, R. F.; Nampalli, S.; Shanmuganathan, K.; Cannon, D. L.; Alves, A. J.; Jeong, L. S.; Beach, J. W.; Chu, C. K. 1,3-Dioxolanylpurine Nucleosides (2*R*,4*R*) and (2*R*,4*S*) with Selective Anti-HIV-1 Activity in Human Lymphocytes. *J. Med. Chem.* **1993**, *36*, 30–37.

(8) (a) Tanigawa, Y.; Nishimura, K.; Kawasaki, A.; Murahashi, S.-I. Palladium(0)-Catalyzed Allylic Alkylation and Amination of Allylic Phosphates. *Tetrahedron Lett.* **1982**, *23*, 5549–5552. (b) Murahashi, S.-I.; Taniguchi, Y.; Imada, Y.; Tanigawa, Y. Palladium-(0)-Catalyzed Azidation of Allyl Esters. Selective Synthesis of Allyl Azides, Primary Allylamines, and Related Compounds. *J. Org. Chem.* **1989**, *54*, 3292–3303. (c) Murahashi, S.-I.; Imada, Y.; Taniguchi, Y.; Higashiura, S. Palladium(0)-Catalyzed Alkoxycarbonylation of Allyl Phosphates and Acetates. *J. Org. Chem.* **1993**, *58*, 1538–1545.

(9) See, for example: (a) Deardorff, D. R.; Linde, R. G., II; Martin, A. M.; Shulman, M. J. Enantioselective Preparation of Functionalized Cyclopentanoids via a Common Chiral (π-Allyl)palladium Complex. *J. Org. Chem.* **1989**, *54*, 2759–2762. (b) Peel, M. R.; Sternbach, D. D.; Johnson, M. R. A Short Enantioselective Synthesis of the Carbocyclic Nucleoside Carbovir. *J. Org. Chem.* **1991**, *56*, 4990–4993.

(10) Neyts, J.; De Clercq, E. Strategies for the Treatment and Prevention of Cytomegalovirus Infections. *Int. J. Antimicrob. Agents* **1993**, in press.

(11) Faulds, D.; Heel, R. C. Ganciclovir. A Review of Its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy in Cytomegalovirus Infections. *Drugs* **1990**, *39*, 597–638.

(12) Drew, W. L.; Lalezari, J. P.; Glutzer, E.; Lynch, T.; Flaherty, J.; Martin, J. C.; Fisher, P. E.; Jaffe, H. S. The Safety, Pharmacokinetics, and Anti-CMV Activity of Weekly HPMPC in HIV-positive Patients Excreting CMV. *Multidisciplinary Approach to Understanding Cytomegalovirus Disease*; Michelson, S., Plotkin, S. A., Eds.; Excerpta Medica, International Congress Series 1032; Elsevier Science Publishers, B. V.: Amsterdam, 1993; pp 287–292.

(13) De Clercq, E. Therapeutic Potential of HPMPC as an Antiviral Drug. *Rev. Med. Virol.* **1993**, *3*, 85–96.

(14) Schols, D.; De Clercq, E.; Balzarini, J.; Baba, M.; Witvrouw, M.; Hosoya, M.; Andrei, G.; Snoeck, R.; Neyts, J.; Pauwels, R.; Nagy, M.; Györgyi-Edelényi, J.; Machovich, R.; Horváth, I.; Lőw, M.; Görög, S. Sulphated Polymers are Potent and Selective Inhibitors of Various Enveloped Viruses, including Herpes Simplex Virus, Cytomegalovirus, Vesicular Stomatitis Virus, Respiratory Syncytial Virus, and Toga-, Arena-, and Retroviruses. *Antiviral Chem. Chemother.* **1990**, *1*, 233–240.

(15) De Clercq, E.; Descamps, J.; Verhelst, G.; Walker, R. T.; Jones, A. S.; Torrence, P. F.; Shugar, D. Comparative Efficacy of Antiherpes Drugs against Different Strains of Herpes Simplex Virus. *J. Infect. Dis.* **1980**, *141*, 563–574.

(16) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A Novel Selective Broad-spectrum anti-DNA Virus Agent. *Nature* **1986**, *323*, 464–467.

(17) $CCID_{50}$ stands for 50% cell culture infective dose (amount required to infect 50% of the cells).

(18) De Clercq, E.; Balzarini, J.; Torrence, P. F.; Mertes, M. P.; Schmidt, C. L.; Shugar, D.; Barr, P. J.; Jones, A. S.; Verhelst, G.; Walker, R. T. Thymidylate Synthetase as a Target Enzyme for the Inhibitory Activity of 5-Substituted 2′-Deoxyuridines on Mouse Leukemia L-1210 Cell Growth. *Mol. Pharmacol.* **1981**, *19*, 321–330.

(19) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. Marked *in vivo* Antiretrovirus Activity of 9-(2-Phosphonylmethoxyethyl)adenine, a Selective Anti-Human Immunodeficiency Virus Agent. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332–336.

(20) Cools, M.; Balzarini, J.; De Clercq, E. Mechanism of Antiviral and Cytotoxic Action of (±)-6′-β-fluoroaristeromycin, a Potent Inhibitor of S-Adenosylhomocysteine Hydrolase. *Mol. Pharmacol.* **1991**, *39*, 718–724.

(21) Cools, M.; De Clercq, E. Correlation Between the Antiviral Activity of Acyclic and Carbocyclic Adenosine Analogues in Murine L929 Cells and Their Inhibitory Effect on L929 Cell S-Adenosylhomocysteine Hydrolase. *Biochem. Pharmacol.* **1989**, *38*, 1061–1067.

(22) Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine, a Potent and Selective Inhibitor of Human Cytomegalovirus Replication. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839–1844.