An Epimer of 5′-Noraristeromycin and Its Antiviral Properties

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

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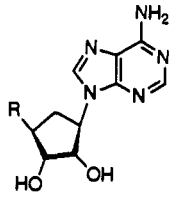
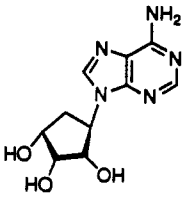
A derivative of 5′-noraristeromycin epimeric at the 5′-nor center ((−)-**3**) has been prepared enantiospecifically in three steps from (+)-((1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate. Compound (−)-**3** was evaluated for antiviral activity against a large number of viruses and found to display marked activity against varicella-zoster virus, vaccinia virus, vesicular stomatitis virus, parainfluenza virus, reovirus, and cytomegalovirus. A similar antiviral activity spectrum was shown by the *S*-adenosylhomocysteine hydrolase inhibitors neplanocin A and carbocyclic 3-deazaadenosine. While equally potent as neplanocin A against most of the viruses tested, compound (−)-**3** was significantly less cytotoxic. The results of this study suggest that (−)-**3** should be pursued for the treatment of those virus infections [that is, pox (VV), rhabdo (VSV), paramyxo (parainfluenza), and reo] that appear to be exquisitively sensitive to the compound.

Derivatives of adenosine capable of inhibiting *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase provide a potentially rich source of antiviral agents.[1,2] Carbocyclic adenosine (aristeromycin, 1) is one such derivative whose antiviral effectiveness is limited by its toxicity caused by metabolism to its 5′-monophosphate by adenosine kinase.[3] In exploring derivatives of 1 less likely[4,5] or unable[6,7] to undergo 5′-phosphorylation, we reported the synthesis and potent antiviral properties with no associated toxicity for (±)-5′-noraristeromycin ((±)-**2**).[4] We have since found that the antiviral properties of 5′-noraristeromycin reside in its (−)-**2** isomer, which is also a strong inhibitor of AdoHcy hydrolase.[5b] We have become particularly interested in the effect of (−)-**2** toward human cytomegalovirus[4] and are seeking a clearer picture of the structural features essential for its antiviral properties. To that end, *epi*-5′-noraristeromycin ((−)-**3**) is reported here[8] to ascertain the biological consequences of inversion of the hydroxyl substituent of **2** at the site previously occupied by the C-5′ hydroxymethyl group of 1.



(-)-1, R=CH_2OH
(-)-2, R=OH

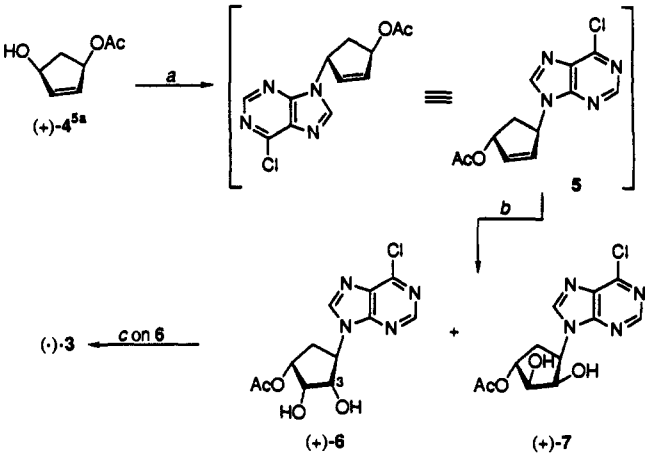


(-)-3

Chemistry

The synthesis of **3** (Scheme 1) began by subjecting (+)-4[5a] to the Mitsunobu reaction[9] with 6-chloropurine to provide **5**. Standard glycolization conditions on **5** led to a mixture of the desired **6** and its isomer **7** in approximately a 2:1 ratio. Following separation of this mixture using flash column chromatography, **6** and **7** were distinguished by a 1-D nuclear Overhauser enhancement determination.

Scheme 1[a]



[a] Reaction conditions: (a) 6-chloropurine/PPh_3/diethyl azodicarboxylate in THF, room temperature; (b) OsO_4/*N*-methylmorpholine *N*-oxide in THF–H_2O, room temperature; (c) NH_3 in MeOH, 100 °C.

In this regard, preirradiation of the cyclopentyl H-3 at δ 4.3 ppm in **6** resulted in enhancement of the H-1, H-2, and H-5_a protons; no enhancement was observed for H-4 and H-5_b. This confirmed the *cis* relationship between the hydroxyl groups and the acetate. Ammonolysis of **6** then gave **3**.

Antiviral Results

Compound (−)-**3** was evaluated against a wide variety of both DNA viruses and RNA viruses (Table 1) and showed activity against the same viruses as (−)-**2**[5b] but was, in general, slightly less potent. Compound (−)-**3** did prove particularly active against vaccinia virus (VV), vesicular stomatitis virus (VSV), parainfluenza-3 virus, and reovirus-1 [minimum inhibitory concentration (MIC): 0.04–0.3 μg/mL]. Also, (−)-**3** was markedly active against cytomegalovirus (CMV) and varicella-zoster virus (VZV). In its antiviral activity spectrum, (−)-**3** displayed many similarities with neplanocin A and carbocyclic 3-deazaadenosine (C-c^3Ado), two well-established AdoHcy hydrolase inhibitors. In fact, those viruses that are, as a rule, sensitive to AdoHcy hydrolase inhibitors (especially

* Address correspondence to this author at the Department of Chemistry, Auburn University, Auburn, AL 36849.

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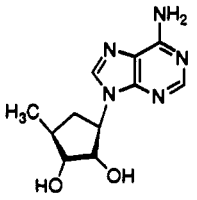
Table 1. Activity of Compound (−)-3 against Different Viruses in Different Cell Systems

virus	cell	MIC$_{50}$ (μg/mL)[a] (−)-3	neplanocin A	C-c^{3}Ado
HSV-1 (KOS)	E_6SM	300 (>400)	70 (100)	>400 (>400)
HSV-2	E_6SM	300	>100	>400
TK$^-$ HSV-1 (B2006)	E_6SM	200	20	>400
TK$^-$ HSV-1 (VMW 1837)	E_6SM	200	70	>400
VV	E_6SM	0.1	0.2	1
VSV	E_6SM	0.2	2	1
VSV	HeLa	0.2 (>400)	2 (40)	7 (>400)
coxsackie B4	HeLa	>400	>40	>400
polio-1	HeLa	>400	>40	>400
RSV (Long)	HeLa	100	>1 (1)	100
parainfluenza-3	Vero	0.04 (400)	0.2 (40)	1 (>400)
reovirus-1	Vero	0.3	0.7	2
sindbis	Vero	>200	2 (40)	4
semliki forest	Vero	>200	7 (40)	>400 (>400)
junin	Vero	30	1	7
tacaribe	Vero	27	1	7
HIV-1	MT-4	>0.8 (1)		
HIV-2	MT-4	>0.8		
influenza A	MDCK	>200 (>200)	1 (4)	100 (>200)
influenza B	MDCK	>200	>3	>30
CMV (AD-169)	HEL	5 (115)	0.6 (15)	7 (>50)
CMV (Davis)	HEL	3	0.4	7
VZV (OKA)	HEL	6	6	>40
VZV (YS)	HEL	1	1	>40

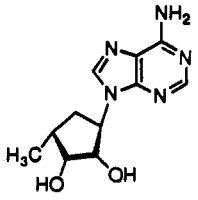
[a] Required to reduce virus-induced cytopathicity by 50%. In parentheses are the minimum cytotoxic concentrations required to cause a microscopically detectable alteration of normal (E_6SM, HeLa, Vero, or MDCK) cell morphology or to inhibit HEL cell growth by 50%.

VV, VSV, parainfluenza, and reovirus) were inhibited by (−)-3 at a lower MIC than by neplanocin A and C-c^{3}Ado.

The ability of (−)-3 to inhibit AdoHcy hydrolase was determined using enzyme from L929 cells. An IC_{50} value (0.042 μM) was found comparable to that of (−)-2 (0.032 μM)[5b] but 14-fold less than that of neplanocin A (0.0059 μM). The similarity in AdoHcy hydrolase inhibitory activity of (−)-2 and its epimer (−)-3 is in contrast to the epimeric pair (−)-8 and (−)-9 where the latter compound was considerably less inhibitory.[10]



(·)-8



(−)-9

It is noteworthy that (−)-3 was significantly less toxic to the host cells [human embryonic skin–muscle (E_6SM), human carcinoma (HeLa), African green monkey kidney (Vero), Madin–Darby canine kidney, and human embryonic lung (HEL)] than neplanocin A.

Experimental Section

Materials and Methods. 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 plates, with visualization by irradiation with a Mineralight UVGL-25 lamp, exposure to iodine vapor, or spraying 3% phenol in 5% ethanolic H_2SO_4 and subsequent heating at 200 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q or Bruker AMX-360 spectrometer in the solvents indicated with tetramethylsilane as internal standard. The spin multiplicities are indicated by the symbols s (singlet), m (multiplet), and br (broad). Optical rotations were measured on a Perkin-Elmer 241 MC 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.

(1*R*,2*S*,3*S*,4*R*)-Acetic Acid 4-(6-Chloro-9*H*-purin-9-yl)-2,3-dihydroxycyclopentyl Ester (6) and (1*R*,2*R*,3*R*,4*R*)-Acetic Acid 4-(6-Chloro-9*H*-purin-9-yl)-2,3-dihydroxycyclopentyl Ester (7). A solution of triphenylphosphine (10 g, 38.13 mmol) in dry THF (250 mL) was cooled to −20 °C (ice–MeOH bath), and diethyl azodicarboxylate (8 mL, 50.86 mmol) was added over a period of 10 min. This mixture was stirred at −20 °C for 20 min to yield a white precipitate of triphenylphosphine–diethyl azodicarboxylate complex. To this latter complex as a suspension were added 6-chloropurine (4.78 g, 36.93 mmol) and a solution of (+)-4[5a] (5 g, 35.21 mmol) in dry THF (50 mL). The cooling bath was removed, and the reaction mixture was stirred at room temperature for 20 h. After evaporation of the reaction mixture to dryness, the residue (5) was dissolved in THF–H_2O (11:1, 300 mL) and 60% aqueous *N*-methylmorpholine *N*-oxide (9 mL, 46.09 mmol) and osmium tetroxide (160 mg) were added to it. Following stirring at room temperature for 20 h, the solvent was removed by rotary evaporation, and the residue was purified by means of flash chromatography on silica gel. The fractions eluting with CH_2Cl_2–MeOH (95:5) gave a mixture of 6 (R_f = 0.3) and 7 (R_f = 0.25), which was isolated by gravity column chromatography on silica gel (Merck silica gel 60 Å) to give 6 (3.04 g, 32%) and 7 (1.4 g, 14.6%).

Physical data for 6: colorless foam; $[\alpha]^{25}_D$ +30.66° (c 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.76–1.90 (m, 1 H, H-5), 2.11 (s, 3 H, CH_3), 2.50–2.60 (m, 1 H, H-5), 3.86 (m, 1 H, H-1), 4.30 (m, 1 H, H-3), 4.50–5.01 (m, 2 H, H-2 and H-4), 5.30–5.52 (m, 2 H, OH), 8.55 (s, 1 H, H-2 of purine), 8.64 (s, 1 H, H-8 of purine); ^{13}C NMR ($CDCl_3$) δ 21.07, 34.56, 54.12, 72.21, 77.36, 78.66, 130.61, 138.96, 145.95, 149.56, 151.79, 171.62. Anal. ($C_{12}H_{13}ClN_4O_4$) C, H, N.

Physical data for 7: colorless foam; $[\alpha]^{25}_D$ +43.75° (c 0.8, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 2.35–2.89 (m, 2 H, H-5), 2.00 (s, 3 H, CH_3), 3.89 (m, 1 H, H-1), 4.10–4.49 (m, 2 H, H-2 and H-3), 4.51–4.90 (m, 1 H, H-4), 5.42 (m, 1 H, OH), 5.50 (m, 1 H, OH), 8.55 (s, 1 H, H-2 of purine), 8.72 (s, 1 H, H-8 of purine); ^{13}C NMR ($CDCl_3$) δ 19.55, 35.48, 55.20, 70.91, 75.57, 76.60, 132.58, 143.47, 150.17, 152.36, 161.53, 169.67. Anal. ($C_{12}H_{13}ClN_4O_4$) C, H, N.

(1*R*,2*S*,3*S*,4*R*)-4-(6-Amino-9*H*-purin-9-yl)cyclopentane-1,2,3-triol ((−)-3). A solution of 6 (310 mg, 0.1 mmol) in MeOH (20 mL) was saturated with NH_3. This mixture was heated in a sealed vessel at 100 °C for 24 h. After cooling to room temperature, the reaction mixture was evaporated to dryness, and the residue was loaded on a Dowex 50X8 (H^+) column (2 ×

15 cm²). The column was first eluted with deionized H_2O until the eluents were neutral to pH paper; this was followed by 50-mL portions of 2 N aqueous NH_4OH. The product containing fractions was evaporated to dryness to give **3** (209 mg, 88%) as a pale yellow solid: mp 220 °C dec; $[\alpha]^{25}_D$ −43.7° (c 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.92 (m, 1 H, H-5), 2.50–2.75 (m, 1 H, H-5), 3.20 (br, 1 H, OH), 3.45 (m, 1 H, H-1), 3.86 (m, 1 H, H-4), 4.15 (m, 2 H, H-2 and H-3), 5.09 (m, 2 H, OH), 7.19 (s, 2 H, NH_2), 8.13 (s, 1 H, H-2 of purine), 8.18 (s, 1 H, H-8 of purine); ^{13}C NMR (DMSO-d_6) δ 39.50, 52.77, 71.62, 73.52, 78.72, 140.59, 141.02, 151.37, 151.86, 155.70. Anal. ($C_{10}H_{13}N_5O_3 \cdot 0.5H_2O$) 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.[11-13] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ 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 cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero, MDCK) or inhibition of normal cell growth (HEL) as previously described.[14]

Inhibition of HIV-1-Induced Cytopathicity in MT-4 Cells. Human 5 × 10^5 MT-4 cells were infected with 100 $CCID_{50}$ of HIV-1 (strain HTLV-III_B) per milliliter and seeded in 200-μL microtiter plate wells, containing appropriate dilutions of the test compounds.[15] 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 Cools and co-workers.[16]

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