Carbocyclic Oxetanocins Lacking the C-3′ Methylene

Jayu Wu,[†] Stewart W. Schneller,*[,‡] and Katherine L. Seley[‡]

Department of Chemistry, University of South Florida, Tampa, Florida 33620, and Department of Chemistry, Auburn University, Auburn, Alabama 36849-5312

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 November 7, 1996[⊗]

Using the observation that the side effects of aristeromycin (carbocyclic adenosine) were reduced by removing the methylene at the center in aristeromycin where phosphorylation occurs, derivatives of carbocyclic oxetanocin A (**4a**), oxetanocin G (**4b**), and 2-aminooxetanocin A (**16**) lacking the 3′-methylene have been prepared in racemic form. The only viruses for which an appreciable inhibitory effect of the compounds (minimum inhibitory concentration ranging from 1 to 40 μg/mL) was noted were herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV). However, when directly compared for their antiviral potency against HSV-1 with their parents oxetanocin A and oxetanocin G, compounds **4a** and **4b** proved clearly less active.

Nucleosides possessing the oxetanosyl N-glycoside feature are structurally an interesting class of compounds. The parent compound of this series is the naturally occurring adenine derivative oxetanocin A (**1a**, Figure 1),[1a] which was isolated from a culture filtrate of *Bacillus megaterium*.[1b] The ability of oxetanocin A to display antiviral properties[2] led to the synthesis of the guanine (oxetanocin G, **1b**), hypoxanthine, and xanthine analogs[3] and to the carbocyclic adenine[4] and guanine[4b−d] derivatives (**2a** and **2b**, respectively). Among this latter group of compounds, **1b**, **2a**, and **2b** have displayed meaningful antiviral properties.[5] For example, **1b** has shown activity toward HCMV,[6a] varicella-zoster virus,[6b] and hepatitis B virus[6c] while **2a** and **2b** have shown a broad range of activity toward the herpes viruses, hepatitis B virus, and HIV.[4c,d,7] Evidence exists that suggests that the mechanism by which **1b** and **2b** exert their antiviral effects requires conversion to their 3′-triphosphate derivatives, which act at the level of viral DNA synthesis.[6,8] Even though it is less clear[8e] whether the same transformation is necessary for the antiviral properties of **2a**, nucleotide formation often leads to unacceptable side effects with nucleoside-derived agents.[9]

We recently found[10] that the mechanism of antiviral action of carbocyclic nucleosides that depend on nucleotide derivative formation can be altered toward a more favorable selectivity index by removal of the methylene unit at the center in the nucleoside analog where nucleotide formation occurs (as illustrated by the structural alteration of aristeromycin (**3a**) to 5′-noraristeromycin (**3b**)).[10] Extending this same strategy to **2a** and **2b** gives rise to **4a** and **4b**, which are carbocyclic oxetanocins lacking the C-3′ methylene moiety and are the focus of this report.

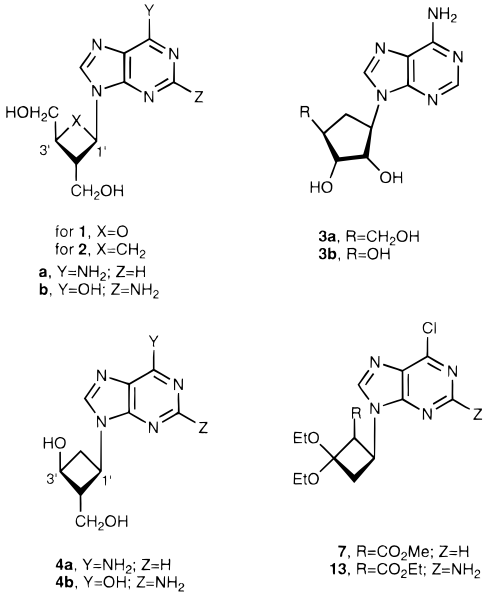


Figure 1.

Chemistry

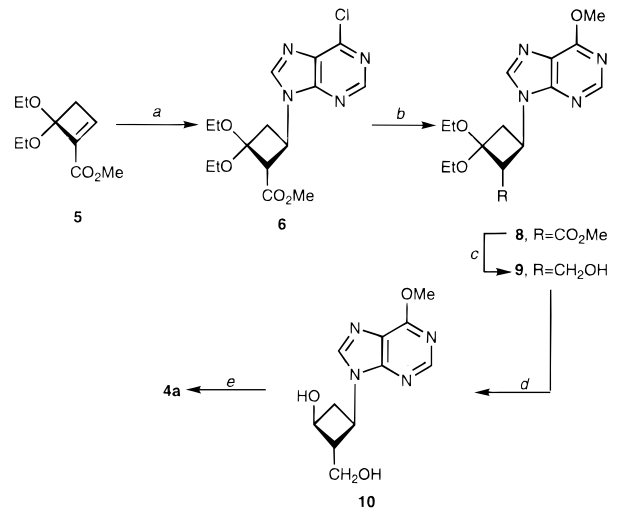
The synthesis of **4a** began with the Michael addition[8d] of 6-chloropurine to methyl 4,4-diethoxycyclobut-1-ene-1-carboxylate (**5**) (Scheme 1).[11] This process gave almost exclusively the *trans* N-9 adduct **6** with only a trace of the *cis* isomer **7**. Methanolysis of **6** to **8** was followed by reduction with lithium aluminum hydride to provide the corresponding alcohol **9**. Hydrolysis of the ketal function of **9** yielded a hydroxy ketone that was not fully characterized but was reduced with sodium borohydride in methanol to give diol **10** as the only product. It is believed that the stereoselectivity of this latter reduction resulted because sodium borohydride first removed the proton from the methylene hydroxy group to form a boron complex which then delivered the hydride to the carbonyl carbon from the bottom face of the cyclobutyl ring. Amination of the diol **10** was accomplished by heating in methanol saturated with ammonia to yield the target (±)-9-[(1α,2β,3α)-3-hydroxy-2-(hydroxymethyl)cyclobutyl]adenine (**4a**).

Applying a similar strategy, the Michael addition[8d] of 2-amino-6-chloropurine to ethyl 4,4-diethoxycyclobut-1-ene-1-carboxylate (**11**)[12] gave the N-9 purine derivative **12** as the major product with trace amounts of the

* Address correspondence to this author at the Department of Chemistry, Auburn University, Auburn, AL 36849-5312.
[†] University of South Florida.
[‡] Auburn University.
[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1997.

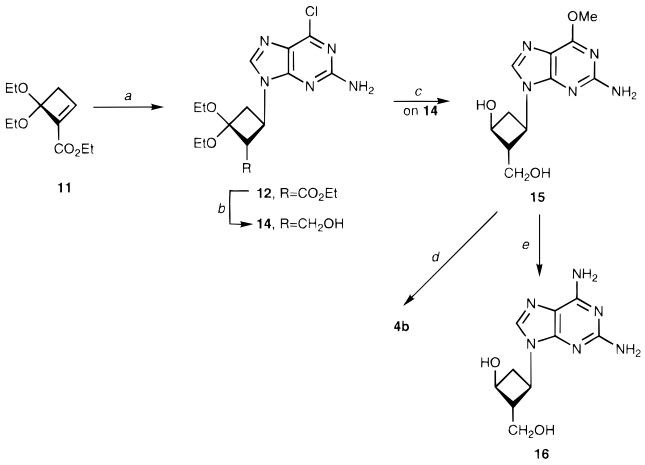
S0022-2623(96)00770-4 CCC: $14.00

Scheme 1[a]



[a] Reaction conditions: (a) DBU in DMF, 6-chloropurine, room temperature; (b) NaOMe in MeOH, room temperature; (c) $LiAlH_4$ in THF; (d) (i) 1 N HCl in acetone, room temperature; (ii) $NaBH_4$ in MeOH, 0 °C; (e) NH_3 in MeOH, 120 °C.

Scheme 2[a]



[a] Reaction conditions: (a) DBU in DMF, 2-amino-6-chloropurine, room temperature; (b) $LiAlH_4$ in THF, 0 °C; (c) (i) 1 N HCl in acetone, room temperature; (ii) $NaBH_4$ in MeOH, 0 °C; (d) 1 N HCl, reflux; (e) NH_3 in MeOH, 120 °C.

cis isomer **13**. Reduction of the ester group of **12** to the alcohol **14** was followed by hydrolysis of the ketal of **14** and then reduction with accompanying methanolysis to result in **15**. Acidic hydrolysis of **15** yielded the guanine derivative **4b**.

With **15** available, it was also converted into (±)-9-[(1α,2β,3α)-3-hydroxy-2-(hydroxymethyl)cyclobutyl]-2,6-diaminopurine (**16**) (Scheme 2) by heating **15** with ammonia-saturated methanol in a sealed tube.

Structure Assignments

The structural assignments of nucleosides **4a**, **4b**, and **16** were accomplished using 2D NMR techniques. The analysis began with a DEPT 135 experiment to establish assignment of the carbon atoms of the cyclobutyl moiety. Following this, a standard COSY 90 experiment allowed assignments of the cyclobutyl protons, which, together with subsequent HMQC experiments, permitted assignment of all the protonated cyclobutyl carbons. A 2D NOESY analysis was then performed to assign the relative cyclobutyl stereochemistry of the compounds. As a consequence, **4a** showed strong NOEs between H-1′ (δ 4.35) and H-3′ (δ 3.86), $H_α$-4′ (δ 2.75), and H-5′ (δ 3.59) (see Figure 2 for reference to cyclobutyl position numbers). Strong NOEs were also observed between H-3′ and H-5′, and $H_α$-4′. These observations indicate that H-1′, H-3′, $H_α$-4′, and H-5′ must be related in a *syn* manner on the α-face of the cyclobutyl moiety. With this information for **4a**, it was not surprising to find that there was no significant NOE between H-1′ and H-2′ as a result of their *anti* relationship. NOEs were also observed between 3′-OH (δ 5.32), H-2′ (δ 2.85), and $H_β$-4′ (δ 2.33). Thus, these hydrogens must be related in a *syn* manner on the β-face of the cyclobutyl unit. Furthermore, the heterocyclic H-8 (δ 8.25) displayed strong NOEs to H-2′ and to $H_β$-4′. This data proves conclusively that the H-1′ and H-3′ are located on the α-face of the cyclobutane ring, while H-2′ is located on the β-face. These results place the 3′-OH *syn* to the heterocyclic base and 2′-CH_2OH *anti* to the heterocyclic base. Similar results were recorded for **4b** and **16**.

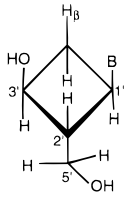


Figure 2.

Antiviral Results

Compounds **4a**, **4b**, and **16** were evaluated against a wide variety of both DNA viruses and RNA viruses (Table 1). Appreciable antiviral activity (within the concentration range of 2−40 μg/mL) was noted with compounds **4b** and **16** against herpes simplex virus type 1 (HSV-1) and with compounds **4a** and **4b** against varicella-zoster virus (VZV). The compounds were clearly less active against the thymidine kinase-deficient (TK^-) HSV-1 and VZV strains. This indicates that for their anti-HSV and anti-VZV activity, the compounds must depend on phosphorylation by the virus-induced thymidine kinase. This was confirmed in additional experiments with clinical VZV isolates[13] where compound **4b** proved inhibitory to a TK^+ VZV isolate at 8 μg/mL, while being inactive at >50 μg/mL against a TK^- VZV isolate (data not shown).

Compounds **4a**, **4b**, and **16** were inactive against vaccinia virus and cytomegalovirus (CMV). They were also evaluated, but found ineffective, against a broad range of RNA viruses, including vesicular stomatitis virus (VSV), Coxsackie B4, polio-1, parainfluenza-3, reo-1, Sindbis, Semliki forest, and human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2).

In additional experiments, in which compounds **4a**, **4b**, and **16** were directly compared with oxetanocin A and G for their activity against clinical HSV-1 and HSV-2 isolates, i.e. Hu-3, Hu-5, HS-47[14] in HEL cells, compounds **4a**, **4b**, and **16** did not prove inhibitory to the different virus isolates at 50 μg/mL, while oxetanocins A and G inhibited the cytopathicity of these viruses at concentrations as low as 1−3 and 0.002−0.004 μg/mL, respectively (data not shown). This clearly demonstrates that the derivatization of compounds **1a** (oxetanocin A) and **1b** (oxetanocin G) to **4a** and **4b**,

Table 1. Antiviral Activity of Compounds **4a**, **4b**, and **16**

		minimum inhibitory concentration[a] (μg/mL)				
virus	cell	**4a**	**4b**	**16**	acyclovir (ganciclovir) ((ribavirin))	brivudin (cidofovir) ((C-c[3] Ado))
HSV-1 (KOS)	E_6SM	>200	8	7	0.04	0.004
HSV-1 (F)	E_6SM	>200	7	7		0.01
HSV-1 (McIntyre)	E_6SM	>200	40	2		0.007
HSV-2 (G)	E_6SM	300	300	175	0.07	>200
HSV-2 (196)	E_6SM	>200	>200	400		>200
HSV-2 (Lyons)	E_6SM	200	200	40		100
vaccinia	E_6SM	150	125	100	>200	0.07
vesicular stomatitis	E_6SM	>200	>400	100	>200	>200
TK HSV-1 (B2006)	E_6SM	40	55	>400	70	7
morphology	E_6SM	400	>400	>400	400	≥400
VZV (OKA)	HEL	9	0.9	35	0.1	0.0008
VZV (YS)	HEL	30	4	35	0.1	0.002
TK$^-$ (VZV (07/1)	HEL	20	20	28	5	5
TK$^-$ VZV (YS/R)	HEL	35	>50	>50	5	>50
CMV (AD-169)	HEL	>50	>50	>50	(1.5)	(0.06)
CMV (Davis)	HEL	>50	35	>50	(0.13)	(0.07)
growth	HEL	120	170	100	>200	150
vesicular stomatitis	HeLa	>400	>200	300	((4))	((0.1))
coxsackie B4	HeLa	>400	>200	>200	((70))	((>400))
Polio-1	HeLa	>400	>200	150	((70))	((>400))
morphology	HeLa	>400	>400	>400	((>200))	((>400))
Parainfluenza-3	Vero	>200	>200	>200	((70))	((7))
Reo-1	Vero	>200	>200	>200	((70))	((2))
Sindbis	Vero	>200	>200	>200	((150))	((>400))
Semliki forest	Vero	>200	>200	>200	((300))	((>400))
morphology	Vero	>400	>400	>400	((>400))	((>400))
HIV-1(III_B)	CEM	>100	>100	≥100		
HIV-1(ROD)	CEM	>100	>100	≥100		
viability	CEM	>100	>100	≥100		

[a] Required to inhibit by 50%: virus-induced cytopathicity in E_6SM, HeLa, Vero cells; virus plaque formation in HEL cells, virus-induced syncytium formation in CEM cells; HEL cell growth; CEM cell viability; or to cause a microscopic alteration of cell morphology (E_6SM, HeLa, Vero cells).

respectively, resulted in a considerable decrease of anti-HSV activity.

Experimental Section

Materials and Methods. The glassware used in the reactions was dried overnight in an oven at 100 °C. The reactions were carried out using freshly distilled solvents under anhydrous conditions in an argon or nitrogen atmosphere. Diethyl ether (Et_2O) was dried from sodium benzophenone; dimethylformamide (DMF) was treated with potassium hydroxide and distilled over $MgSO_4$; methanol (MeOH) was distilled from and stored over molecular sieves (4 Å); tetrahydrofuran (THF) was freshly distilled over lithium aluminum hydride.

Unless otherwise noted, reagents were used as received from the supplier. 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 or exposure to iodine vapor. The column chromatography purifications were performed on Aldrich flash chromatography silica gel 60 (particle size 0.035−0.07 mm; 220−440 mesh ASTM) by eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C) homogeneous materials.

The ^{1}H and ^{13}C NMR spectra and the other 1D and 2D spectra were recorded on either a JEOL FX90Q or Bruker AMX-360 spectrometer in $CDCl_3$ or DMSO-d_6 referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Pulse programs that were used in the 2D NMR experiments are as follows: COSY 90 2D homonuclear shift correlation;[15,16] INVBDGTP 2D, H-/X correlation via heteronuclear zero and double quantum coherence using BIRD sequence, phase selective using TPPI, decoupling during acquisition using GARP1;[17] NOESYTP 2D, homonuclear correlation via dipolar coupling may be due to NOE or chemical exchange, phase sensitive using TPPI.

Melting point data was obtained using a Mel-Temp capillary melting point apparatus and is uncorrected. The microanalyses were performed by M-H-W Laboratories, Phoenix, AZ, on samples that were homogeneous by TLC analysis.

(±)-9-[(1α,2β)-3,3-Diethoxy-2-(methoxycarbonyl)cyclobutyl]-6-chloropurine (6). A cold (0 °C) solution of methyl 4,4-diethoxycyclobut-1-ene-1-carboxylate (**5**)[11] (1.37 g, 6.4 mmol) and 6-chloropurine (660 mg, 4.27 mmol) in anhydrous DMF (30 mL) was treated with DBU (1 equiv). The resulting solution was stirred overnight at room temperature; this was followed by removal of the solvent under vacuum. The residue was dissolved in CH_2Cl_2 (100 mL) and this solution washed with aqueous $NaHCO_3$ solution and dried (Na_2SO_4). The CH_2Cl_2 was removed under reduced pressure to give a new residue, which was purified by flash chromatography (hexane:EtOAc, 3:2) to give **6** (1.29 g, 81.8%) as a white solid that was purified by recrystallization from Et_2O: mp 132−133 °C; R_f = 0.4 (hexane:EtOAc, 1:1); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.22 (t, J = 7.0 Hz, 3 H), 1.29 (t, J = 7.0 Hz, 3 H), 2.99 (d, J = 8.5 Hz, 2 H), 3.53 (m, 2 H), 3.67 (m, 1 H), 3.76 (s, 3 H), 3.80 (m, 1 H), 4.13 (d, J = 8.5 Hz, 1 H), 5.27 (dt, J = 8.5 Hz, 1 H), 8.76 (s, 1 H), 8.24 (s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 15.0, 15.1, 37.3, 45.0, 52.2, 56.3, 57.6, 58.1, 98.3, 132.1, 146.5, 151.8, 151.9, 168.3. Anal. ($C_{15}H_{19}ClN_4O_4$) C, H, N.

(±)-9-[(1α,2β)-3,3-Diethoxy-2-(methoxycarbonyl)cyclobutyl]-6-methoxypurine (8). To a solution of **6** (850 mg, 2.3 mmol) in anhydrous MeOH (20 mL) was added, dropwise, a solution of NaOMe in MeOH (17 mL, 25% wt NaOMe). The resulting mixture was then stirred at room temperature for 1 h. Diethyl ether was added to this mixture and the precipitated NaOMe removed by filtration. The filtrate was concentrated under reduced pressure to a residue that was dissolved in H_2O (5 mL). This solution was neutralized with concentrated HCl and then extracted with CH_2Cl_2 (3 × 20 mL). The

combined CH_2Cl_2 extracts were washed with saturated $NaHCO_3$ solution and brine and then dried (Na_2SO_4). Following removal of the CH_2Cl_2, the residue was purified by flash chromatography (hexane:EtOAc, 4:5) to give **8** (750 mg, 92.9%) as a syrup: $R_f = 0.33$ (hexane:EtOAc, 1:1); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.21 (t, $J = 7.1$ Hz, 3 H), 1.29 (t, $J = 7.1$ Hz, 3 H), 2.95 (d, $J = 8.9$ Hz, 2 H), 3.55−3.49 (m, 2 H), 3.62−3.71 m, 1 H), 3.75 (s, 3 H), 3.78−3.85 (m, 1 H), 4.12 (d, $J = 8.2$ Hz, 1 H), 4.18 (s, 3 H), 5.22 (m, 1 H), 8.02 (s, 1 H), 8.54 (s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 15.0, 15.1, 37.4, 44.4, 52.1, 56.3, 57.5, 58.0, 98.3, 122.0, 141.3, 152.0, 152.1, 161.0, 168.5. Anal. ($C_{16}H_{22}N_4O_5$) C, H, N.

(±)-9-[(1α,2β)-3,3-Diethoxy-2-(hydroxymethyl)cyclobutyl]-6-methoxypurine (9). To a solution of **8** (700 mg, 2.0 mmol) in anhydrous THF (30 mL) at 0 °C was added slowly $LiAlH_4$ (120 mg, 3.16 mmol). The resultant mixture was stirred for 3 h. After adding H_2O (0.3 mL), 6 N NaOH solution (0.3 mL), and H_2O (1 mL) to this mixture, it was stirred vigorously at room temperature for 20 min. After filtration to remove insoluble material, the filtrate was evaporated with the aid of a rotary evaporator to give a residue that was dissolved in H_2O (5 mL) and this solution then extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure, and the residue purified by flash column chromatography (5% MeOH in CH_2Cl_2) to give **9** (620 mg, 96.3%) as a syrup: $R_f = 0.58$ (MeOH:CH_2Cl_2, 5:95); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.24 (dt, $J = 7.2$ Hz, 6 H), 2.67−2.72 (m, 1 H), 3.02−3.11 (m, 2 H), 3.5−3.6 (m 4 H), 3.93 (m, 1 H), 4.04 (m, 1 H), 4.19 (s, 3 H), 4.77 (dt, $J = 8.2$ Hz, 1 H), 8.07 (s, 1 H), 8.52 (s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 15.0, 15.1, 37.0, 44.9, 50.7, 54.2, 55.1, 57.8, 60.6, 99.0, 121.9, 140.6, 151.7, 151.8, 161.1. Anal. ($C_{15}H_{22}N_4O_4$) C, H, N.

(±)-9-[(1α,2β,3α)-3-Hydroxy-2-(hydroxymethyl)cyclobutyl]-6-methoxypurine (10). To a solution of **9** (360 mg, 1.12 mmol) in acetone (38 mL) was added slowly 1 N HCl (6 mL). The resulting solution was stirred at room temperature for 2 days. The solvent was then removed under reduced pressure and the residue treated with 6 N NaOH. This new mixture was extracted with EtOAc (3 × 25 mL). The EtOAc extracts were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give a foam, which was used in the next reaction without further purification.

The crude material obtained in the previous step was dissolved in dry MeOH (35 mL). The resulting solution was cooled to 0 °C and treated with $NaBH_4$ (100 mg) in three portions. This new solution was stirred for 1 h and quenched with acetone (1 mL), and this new mixture was stirred for another 20 min. The solvent was removed under reduced pressure by using a rotary evaporator and the residue purified by flash column chromatography (CH_2Cl_2:MeOH, 9:1) to give **10** (170 mg, 61% from **9**) as a white foam: $R_f = 0.79$ (MeOH:CH_2Cl_2, 1:6); ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.37 (m, 1 H), 2.79 (m, 1 H), 2.92 (m, 1 H), 3.59 (br s, 2 H), 3.89 (m, 1 H), 4.10 (s, 3 H), 4.45 (dt, $J = 8.0$ Hz, 1 H), 4.74 (br s, 1 H), 5.37 (br s, 1 H), 8.23 (s, 1 H), 8.53 (s, 1 H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 37.4, 43.1, 53.8, 55.0, 59.4, 60.8, 124.5, 142.7, 145.4, 151.3, 156.7; low-resolution mass spectrum (FAB), *m/e* 369 (MH^+); high-resolution mass spectrum (FAB), *m/e* 369.1341 (MH^+), $C_{16}H_{21}ClN_4O_4$ requires 369.1330.

(±)-9-[(1α,2β,3α)-3-Hydroxy-2-(hydroxymethyl)cyclobutyl]adenine (4a). A solution of **10** (100 mg, 0.4 mmol) in dry MeOH (20 mL) was saturated with NH_3 at 0 °C. The resulting mixture was heated to 120 °C in a sealed tube for 2 days. The reaction mixture was then cooled to room temperature and the solvent removed with the aid of a rotary evaporator. The residue was purified by flash column chromatography (CH_2Cl_2:MeOH, 5:1) to give **4a** (85 mg, 91%) that was recrystallized from EtOAc:MeOH as a white solid: mp 199−201 °C; $R_f = 0.35$ (CH_2Cl_2:MeOH, 5:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.33 (m, 1 H, H_β-4′), 2.75 (m, 1 H, H_α-4′), 2.85 (m, 1 H, H-2′), 3.59 (m, 2 H, H-5′), 3.86 (m, 1 H, H-3′), 4.35 (m, 1 H, H-1′), 4.86 (t, $J = 4.8$ Hz, 1 H, 5′-OH), 5.32 (d, $J = 6.5$ Hz, 1 H, 3′-OH), 7.21 (br s, 2 H, NH_2), 8.13 (s, 1 H, H-2), 8.25 (s, 1 H, H-8); ^{13}C NMR (90 MHz, DMSO-d_6) δ 37.4, 42.9, 55.7, 59.7, 61.0, 119.2, 139.7, 149.6, 152.2, 156.0. Anal. ($C_{10}H_{12}N_5O_2 \cdot 0.5H_2O$) C, H, N.

(±)-9-[(1α,2β)-3,3-Diethoxy-2-(ethoxycarbonyl)cyclobutyl]-2-amino-6-chloropurine (12). Applying the same procedure used in the preparation of compound **6**, a cold (0 °C) solution of **11**[12] (950 mg, 4.44 mmol) and 2-amino-6-chloropurine (500 mg, 2.95 mmol) in anhydrous DMF (15 mL) was treated with DBU (0.015 mL). The resulting solution was stirred for 20 min at room temperature and the solvent removed with the aid of a rotary evaporator. The residue was dissolved in CH_2Cl_2 (60 mL), washed with $NaHCO_3$, and dried (Na_2SO_4). The CH_2Cl_2 was removed under reduced pressure and the residue purified by flash column chromatography (hexane:EtOAc, 1:1) to give **12** (0.97 g, 86%), which was recrystallized from Et_2O as a white solid: mp 177−178 °C; $R_f = 0.59$ (hexane:EtOAc, 1:2); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.20 (t, $J = 7.1$ Hz, 3 H), 1.28 (t, $J = 7.0$ Hz, 3 H), 1.29 (t, $J = 7.0$ Hz, 3 H), 2.91 (m, 2 H), 3.50 (m, 2 H), 3.67 (m, 1 H), 3.81 (m, 1 H), 3.99 (d, $J = 7.9$ Hz, 1 H), 4.19 (m, 1 H), 4.26 (m, 1 H), 5.10 (dt, $J = 8.2$ Hz, 1 H), 5.57 (br s, 2 H), 7.89 (s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 14.1, 14.9, 15.0, 37.1, 43.9, 56.4, 57.4, 58.0, 61.0, 98.2, 125.6, 141.4, 151.3, 153.9, 168.9, 167.9. Anal. ($C_{16}H_{12}ClN_5O_4$) C, H, N.

(±)-9-[(1α,2β)-3,3-Diethoxy-2-(hydroxymethyl)cyclobutyl]-2-amino-6-chloropurine (14). A procedure similar to that used for preparing **9** was employed. To a solution of **12** (450 mg, 1.17 mmol) in anhydrous THF (20 mL) at 0 °C was added slowly $LiAlH_4$ (70 mg, 1.84 mmol). The resultant reaction mixture was stirred for 1.5 h. After the sequential addition of H_2O (0.2 mL), 6 N NaOH solution (0.2 mL), and H_2O (0.5 mL), the mixture was stirred vigorously at room temperature for 2 min. Filtration and evaporation of the filtrate with the aid of a rotary evaporator yielded a residue, which was dissolved in H_2O (0.5 mL), and this new solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure, and the residue was purified by flash column chromatography (MeOH:CH_2Cl_2, 5:95) to give analytically pure **14** (380 mg, 94.5%) as a white solid: mp 185.5−186.5 °C; $R_f = 0.21$ (hexane:EtOAc, 1:2); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.16 (m, 6 H), 2.49 (m, 1 H), 2.77 (m, 1 H), 3.10 (m, 1 H), 3.40 (m, 1 H), 3.45 (m, 2 H), 3.53 (m, 2 H), 3.71 (m, 1 H), 4.34 (dt, $J = 8.5$ Hz, 1 H), 4.52 (br s, 1 H), 6.91 (br s, 2 H), 8.32 (s, 1 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 15.0, 15.2, 36.9, 44.9, 56.6, 57.2, 58.3, 98.2, 123.6, 141.8, 149.3, 154.1, 159.5. Anal. ($C_{14}H_{12}ClN_5O_3$) C, H, N.

(±)-9-[(1α,2β,3α)-(3-Hydroxy-2-(hydroxymethyl)cyclobutyl]-2-amino-6-methoxypurine (15). To a solution of **14** (800 mg, 2.34 mmol) in acetone (80 mL) was added slowly 1 N HCl (15 mL). The resultant solution was stirred at room temperature for 2 days. The solvent was then removed with the aid of a rotary evaporator and the residue neutralized with 6 N NaOH solution, and this mixture was extracted with EtOAc. The EtOAc extracts were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give, after purification using flash column chromatography (CH_2Cl_2:MeOH, 12:1), (±)-9-[(1α,2β)-2-(hydroxymethyl)-3-oxocyclobutyl]-2-amino-6-chloropurine as a white solid: mp 174 °C; $R_f = 0.64$ (CH_2Cl_2:MeOH, 8:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 3.43 (m, 2 H), 3.69 (m, 1 H), 3.76 (m, 2 H), 4.25 (br s, 1 H), 5.18 (m, 1 H), 6.93 (br s, 2 H), 8.40 (s, 1 H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 42.4, 51.6, 56.9, 68.2, 123.7, 142.2, 149.4, 154.2, 159.5, 205.3.

A solution of the compound described above (350 mg, 1.31 mmol) in dry MeOH (40 mL) at 0 °C was treated with $NaBH_4$ (90 mg) in three portions. The solution was stirred for 1 h, quenched with acetone (1 mL), and stirred vigorously for another 20 min. The solvent was removed with the aid of a rotary evaporator and the residue purified by flash column chromatography (CH_2Cl_2:MeOH, 9:1) to give **15** (290 mg, 83%), which was recrystallized from MeOH as a white solid: mp 200−202 °C; $R_f = 0.36$ (CH_2Cl_2:MeOH, 8:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.15 (m, 1 H), 2.69 (m, 1 H), 3.54 (d, $J = 4.4$ Hz, 2 H), 3.80 (m, 1 H), 3.95 (s, 3 H), 4.20 (dt, $J = 8.2$ Hz, 1 H), 4.71 (br s, 1 H), 5.25 (br s, 1 H), 6.37 (br s, 2 H), 8.00 (s, 1 H); ^{13}C NMR (90 MHz, DMSO-d_6) δ 37.9, 42.0, 53.1, 55.4, 59.4, 60.9, 85.7, 138.2, 154.1, 159.6, 160.9; low-resolution mass spectrum (FAB), *m/e* 266 (MH^+); high-

resolution mass spectrum (FAB), *m/e* 266.1253 (MH^+), $C_{11}H_{15}N_5O_3$ requires 266.1253.

(±)-9-[(1α,2β,3α)-3-Hydroxy-2-(hydroxymethyl)cyclobutyl]guanine (4b). A solution of **15** (200 mg, 0.74 mmol) in 1 N HCl was heated under reflux for 6 h. The H_2O was then removed under reduced pressure and the residue azeotroped with absolute EtOH. The new residue was dissolved in a small amount of H_2O and this solution neutralized with 6 N NaOH solution. A white precipitate formed immediately. This mixture was refrigerated overnight and the solid material collected by filtration, washed with cold H_2O, and purified by flash column chromatography (CH_2Cl_2:MeOH, 4:1) to provide **4b** (170 mg, 91%) as a white solid: mp 299−301 °C; $R_f = 0.36$ (CH_2Cl_2:MeOH, 3:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.15 (m, 1 H, H_β-4′), 2.69 (m, 1 H, H_α-4′), 2.78 (m, 1 H, H-2′), 3.54 (m, 2 H, H-5′), 3.79 (m, 1 H, H-3′), 4.12 (m, 1 H, H-1′), 4.72 (t, J = 4.7 Hz, 2 H, 5′-OH), 5.25 (d, J = 6.8 Hz, 1 H, 3′-OH), 6.71 (s, 2 H, NH_2), 7.86 (s, 1 H, H-8), 10.85 (s, 1 H, NH); ^{13}C NMR (90 MHz, DMSO-d_6) δ 38.0, 41.8, 55.6, 59.4, 60.9, 116.7, 135.7, 151.0, 153.6, 156.7. Anal. ($C_{10}H_{13}N_5O_3$) C, H, N.

(±)-9-[(1α,2β,3α)-3-Hydroxy-2-hydroxymethyl)cyclobutyl]-2,6-diaminopurine (16). Using the same procedure that led to **4a**, amination of **15** (150 mg, 0.56 mmol) in MeOH (20 mL) in a sealed tube at 120 °C for 2 days gave **16** (100 mg, 71%) as a while solid following recrystallization from MeOH: mp 247−249 °C; $R_f = 0.20$ (CH_2Cl_2:MeOH, 5:1); ^{1}H NMR (360 MHz, DMSO-d_6) δ 2.18 (m, 1 H, H_β-4′), 2.71 (m, 1 H, H_α-4′), 3.55 (d, J = 4.5 Hz, 2 H, H-5′), 3.79 (m, 1 H, H-3′), 4.11 (dt, J = 8.4 Hz, 1 H, H-1′), 4.88 (br s, 1 H, 3′-OH), 5.25 (br s, 2 H, 5′-OH), 5.77 (br s, 2 H, NH_2), 6.70 (br s, 2 H, NH_2), 7.85 (s, 1 H, H-8); ^{13}C NMR (90 MHz, DMSO-d_6) δ 37.6, 42.1, 55.7, 59.8, 61.0, 113.5, 135.9, 151.6, 156.0, 159.9. Anal. ($C_{10}H_{14}N_6O_2$) 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, or HEL cell cultures, following previously established procedures.[18−20] 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.

Cytotoxicity Assays. The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero) or inhibition of normal cell growth (HEL), as previously described[21] or inhibition of cell viability (CEM). For all cell viability determinations, 100-μL aliquots of the cell suspension (7.5×10^5 CEM cell/mL) were added to the wells of a microtiter plate containing 100 μL of varying concentrations of the test compounds. After a 3-day incubation period at 37 °C in a humidified CO_2-controlled incubator, the number of viable cells was determined with a Coulter Counter.

Inhibition of HIV-Induced Giant Cell Formation. CEM cell cultures were suspended at 250000−300000 cells/mL of culture medium and infected with HIV-1 (III_B) or HIV-2 (ROD) at 100 $CCID_{50}$/mL. Then, 100 μL of the infected cell suspension were transferred to 200 μL microtiter plate wells containing 100 μL of serial dilutions of the test compound solutions. After 4 days of incubation at 37 °C, cell cultures were examined for syncytium formation as previously described.[22]

Acknowledgment. This research was supported by funds from the Department of Health and Human Services (NO1-AI-72645 and U19-A131718), and this is appreciated. The assistance of Dr. Tony Gambino in obtaining some of the NMR spectral data is gratefully acknowledged. These investigations were also supported by the Biomedical Research Programme of the European Commission and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (project number 3.0180.95) and the Belgian Geconcerteerde Onderzoeksacties (project number 95/5). 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) (a) Nakamura, H.; Hasegawa, S.; Shimada, N.; Fuji, A.; Takita, T.; Itaka, Y. The X-Ray Structure Determination of Oxetanocin. *J. Antibiot.* **1986**, *39*, 1626−1629. (b) Shimada, N.; Hasegawa, S.; Harada, T.; Tomisawa, T.; Fuji, A.; Takita, T. Oxetanocin, A Novel Nucleoside from Bacteria. *J. Antibiot.* **1986**, *39*, 1623−1625.

(2) Hoshino, H.; Shimizu, N.; Shimada, N.; Takita, T.; Takeuchi, T. Inhibition of Ineffectivity of Human Immunodeficiency Virus by Oxetanocin. *J. Antibiot.* **1987**, *40*, 1077−1078.

(3) Shimada, N.; Hasegawa, S.; Saito, S.; Nishikiori, T.; Fuji, A.; Takita, T. Derivatives of Oxetanocin: Oxetanocins H, X and G, and 2-Aminooxetanocin A. *J. Antibiot.* **1987**, *40*, 1788−1790.

(4) (a) Honjo, M.; Maruyama, T.; Sato, Y.; Horii, T. Synthesis of the Carbocyclic Analogue of Oxetanocin A. *Chem. Pharm. Bull.* **1989**, *37*, 1413−1415. (b) Ichikawa, Y.-I.; Narita,, A.; Shiozawa, A.; Hayashi, Y.; Narasaka, K. Enantio- and Diasteroselective Synthesis of Carbocyclic Oxetanocin Analogues. *J. Chem. Soc., Chem. Commun.* **1989**, 1919−1921. (c) Norbeck, D. W.; Kern, E.; Hayashi, S.; Rosenbrook, W.; Sham, H.; Herrin, T.; Plattner, J. J.; Erickson, J.; Clement, J.; Swanson, R.; Shipkowitz, N.; Hady, D.; Marsh, K.; Arnett, G.; Shannon, W.; Broder, S.; Mitsuya, H. Cyclobut-A and Cyclobut-G: Broad-Spectrum Antiviral Agents with Potential Utility for the Therapy of AIDS. *J. Med. Chem.* **1990**, *33*, 1281−1285. (d) Bisacchi, G.; Braitman, A.; Cianci, C. W.; Clark, J. M.; Field, A. K.; Hagen, M. E.; Hockstein, D. R.; Malley, M. F.; Mitt, T.; Slusarchyk, W. A.; Sundeen, J. E.; Terry, B. J.; Tuomari, A. V.; Weaver, E. R.; Young, M. G.; Zahler, R. Synthesis and Antiviral Activity of Enantiomeric Forms of Cyclobutyl Nucleoside Analogues. *J. Med. Chem.* **1991**, *34*, 1415−1421. (e) Huryn, D. M.; Okabe, M. AIDS-Driven Nucleoside Chemistry. *Chem Rev.* **1992**, *92*, 1745−1768. (f) Norbeck, D. W.; Plattner, J.; Rosen, T. J.; Pariza, R. J.; Sowin, T. J.; Garmaise, D. L.; Hannich, S. M. European Patent 0366059 A-2, May 2, 1990.

(5) Slusarchyk, W. A.; Young, M. G.; Bisacchi, G.; Hockstein, D. R.; Zahler, R. Synthesis of SQ-33,054, A Novel Cyclobutane Nucleoside with Potent Antiviral Activity. *Tetrahedron Lett.* **1989**, *30*, 6453−6456.

(6) (a) Nishiyama, Y.; Yamamoto, N.; Takahash, K. ; Shimada, N. Selective Inhibition of Human Cytomegalovirus Replication by a Novel Nucleoside, Oxetanocin G. *Antimicrob. Agents Chemother.* **1988**, *32*, 1053−1056. (b) Sakuma, T.; Saijo, M.; Suzutani, T.; Yoshida, I.; Saito, S.; Kitagawa, M.; Hasegawa, S.; Azuma, M. Antiviral Activity of Oxetanocins against Varicella-Zoster Virus. *Antimicrob. Agents Chemother.* **1991**, *35*, 1512−1514. (c) Nagahata, T.; Kitagawa, M.; Matsubara, K. Effect of Oxetanocin G, a Novel Nucleoside Analog, on DNA Synthesis by Hepatitis B. Virus Virions. *Antimicrob. Agents Chemother.* **1994**, *38*, 707−712.

(7) (a) Field, A. K.; Tuomari, A. V.; McGeever-Rubin, B.; Terry, B. J.; Mazina, K. E.; Haffey, M. L.; Hagen, M. E.; Clark, J. M.; Braitman, A.; Slusarchyk, W. A.; Zahler, R. (±)-(1α,2β,3α)-9-[2,3-*bis*(hydroxymethyl)cyclobutyl]guanine [(±)-BHCG or SQ 33054]: a Potent and Selective Inhibitor of Herpesviruses. *Antiviral Res.* **1990**, *13*, 41−52. (b) Hayashi, S.; Norbeck, D. W.; Rosenbrook, W.; Fine, R. L.; Matsukura, M.; Plattner, J. J.; Broder, S.; Mitsuya, H. Cyclobut-A and Cyclobut-G, Carbocyclic Oxetanocin Analogs That Inhibit the Replication of Human Immunodeficiency Virus in T Cells and Monocytes and Macrophages *In Vitro*. *Antimicrob. Agents Chemother.* **1990**, *34*, 287−294. (c) Clement, J. J.; Kern, E. R. Cyclobutyl Compounds as Antiviral Agents. *Trans. Proceed.* **1991**, *23*, 159−161.

(8) (a) Daikoku, T.; Yamamoto, N.; Saito, S.; Kitagawa, M.; Shimada, N.; Nishiyama, Y. Mechanism of Inhibition of Human Cytomegalovirus Replication by Oxetanocin G. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 805−812. (b) Yamanaka, G.; Tuomai, A. V.; Hagen, M.; McGeever-Rubin, B.; Terry, W.; Haffey, M.; Bisacchi, G. S.; Field, A. K. Selective Activity and Cellular Pharmacology of (1*R*-1α,2β,3α)-9-[2,3-*bis*(Hydroxymethyl)cyclobutyl]guanine in Herpesvirus-Infected Cells. *Mol. Pharmacol.* **1991**, *40*, 446−453. (c) Terry, B. J.; Cianci, C. W.; Hagen, M. E. Inhibition of Herpes Simplex Virus Type 1 DNA Polymerase by (1*R*-1α,2β,3α)-9-[2,3-*bis*(Hydroxymethyl)cyclobutyl]guanine. *Mol. Pharmacol.* **1991**, *40*, 591−596. (d) Norbeck, D. W.; Sham, H. L.; Rosenbrook, W.; Herrin, T.; Plattner, J. J. Synthesis and Activities of the Phosphonate Isostere of the Monophosphate of (±)-Cyclobut-G. *Nucleosides Nucleotides* **1992**, *11*, 1383−1391.

Maruyama, T.; Hanai, Y.; Sato, Y.; Snoeck, R.; Andrei, G.; Hosoya, M.; Balzarini, J.; De Clercq, E. Synthesis and Antiviral Activity of Carbocyclic Oxetanocin Analogues (C-OXT-A, C-OXT-G) and Related Compounds. II. *Chem. Pharm. Bull.* **1993**, *41*, 516−521.

(9) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosylhomocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521−1530.

(10) (a) 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. (b) Siddiqi, S. M.; Chen, X.; Schneller, S. W.; Ikeda, S.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. Antiviral Enantiomeric Preference for 5′-Noraristeromycin. *J. Med. Chem.* **1994**, *37*, 551−554.

(11) Semmelhack, M. F.; Tomoda, S.; Hiroto, N.; Boettger, S. D.; Hurst, K. M. Synthesis of Racemic Formannosin and Illudol Using a Biosynthetically Patterned Common Intermediate. *J. Am. Chem. Soc.* **1982**, *104*, 747−759.

(12) Semmelhack, M. F.; Tomoda, S.; Hurst, K. M. Synthesis of (±)-Illudol. *J. Am. Chem. Soc.* **1980**, *102*, 7567−7568.

(13) Snoeck, R.; Gérard, M.; Sadzot-Delvaux, C.; Andrei, G.; Balzarini, J.; Reymen, D.; Ahadi, N.; De Bruyn, J. M.; Piette, J.; Rentier, B.; Clumeck, N.; De Clercq, E. Meningoradiculoneuritis Due to Acyclovir-resistant Varicella Zoster Virus in an Acquired Immune Deficiency Syndrome Patient. *J. Med. Virol.* **1994**, *42*, 338−347.

(14) Snoeck, R.; Andrei, G.; Gérard, M.; Silverman, A.; Hedderman, A.; Balzarini, J.; Sadzot-Delvaux, C.; Tricot, G.; Clumeck, N.; De Clercq, E. Successful Treatment of Progressive Mucocutaneous Infection Due to Acyclovir- and Foscarnet-resistant Herpes Simplex Virus with (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC). *Clin. Infect. Dis.* **1994**, *18*, 570−578.

(15) Aue, W. P.; Bartholdi, E.; Ernst, R. R. Two-Dimensional Spectroscopy. Application to Nuclear Magnetic Resonance. *J. Chem. Phys.* **1976**, *64*, 2229−2246.

(16) Nagayama, K.; Kumar, A.; Wuthrich, K.; Ernst, R. R. Experimental Techniques of Two-Dimensional Correlated Spectroscopy. *J. Magn. Reson.* **1980**, *40*, 321−334.

(17) Bax, A.; Subramanian, S. Sensitivity-Enhanced Two-Dimensional Heteronuclear Shift Correlation NMR Spectroscopy. *J. Magn. Reson.* **1986**, *67*, 565−569.

(18) 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.; Löw, 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.

(19) 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.

(20) 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.

(21) 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.

(22) Balzarini, J.; Naesens, L.; Schlachmuylders, J.; Niphuis, H.; Rosenberg, I.; Holy, A.; Schellekens, H.; De Clercq, E. 9-(2-Phosphonylmethoxyethyl)adenine (PMEA) Effectively Inhibits Retrovirus Replication *In Vitro* and Simian Immunodeficiency Virus Infection in Rhesus Monkeys. *AIDS* **1991**, *5*, 21−28.

JM960770E