Synthesis and Antiviral and Antineoplastic Activities of Some Novel Carbocyclic Guanosine Analogues with a Cyclobutane Ring

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Cyclobutyl nucleoside analogues containing guanine and 8-azaguanine (compounds 5—10) were prepared from (1*R*,*cis*)-3-aminomethyl-2,2-dimethylcyclobutylmethanol (1). All were evaluated as antiviral and antitumoral agents in a variety of assay systems. Compounds 6 and 7 showed a noteworthy activity against a respiratory syncytial virus and compound 10 was moderately active against vaccinia virus. Only compound 5 showed some cytostatic activity.

Key words antineoplastic; antiviral; carbocyclic nucleoside; guanosine analogue

Because of their crucial roles in the storage and transfer of metabolic energy and genetic information, nucleosides are among the most important of biomolecules. This has stimulated great interest in nucleoside analogues as potential antiviral and antineoplastic agents.[1—3] One of the example is oxetanocin A (I), a natural antibiotic that is derived from oxetane and which, following conversion to its 5-triphosphate, is a potent inhibitor of human inmunodeficiency virus (HIV-1) reverse transcriptase and is thus of potential value for the treatment of AIDS;[4] its carbocyclic analogue, cyclobut-G (II),[5] is not only active against HIV but also against herpes simplex virus (HSV-1 and HSV-2), cytomegalovirus[6,7] and hepatitis B virus.[8] As yet there are no general rules relating the structures of carbocyclic nucleosides to their therapeutic activity, although trends among certain kinds of structure have been tentatively put forward.[9]

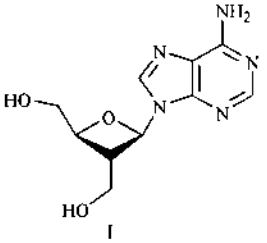
Among the structural characteristics to be considered are conformational mobility, the distance separating the hydroxymethyl oxygen and *pseudo*-glycosidic nitrogen, the presence of lipophilic groups, and the absolute and relative configurations of these molecules.[10]

In this work we synthesized a variety of dimethylcyclobutyl carbonucleosides, which are related to other 9-[*cis*-3-(hydroxymethyl)cyclobutyl]purines,[11] starting from enantiomerically pure aminoalcohol **1**.[12] Our analogues differ from cyclobut-G itself in that they have the opposite absolute configurations at their stereogenic centers, bear a *gem*-dimethyl group instead of hydroxymethyl group at C2, and have an extra methylene group between the carbocycle and the heterocyclic moieties.

Their synthesis is detailed in Chart 1; in all cases, the guanine or modified guanine base was constructed[13] on the amino group of the precursor (1*R*,*cis*)-3-aminomethyl-2,2-dimethylcyclobutylmethanol (**1**).

Briefly, **1** was condensed with 2-amino-4,6-dichloropyrimidine (72% yield), the resulting pyrimidinylamino compound **2** was reacted with 4-chlorobenzenediazonium chloride to afford the 5-(4-chlorophenylazo)pyrimidine **3** (87% yield), and this latter was reduced with Zn in acetic acid to give the triaminopyrimidine **4** (63% yield). Compound **4** was then cyclized, either with triethyl orthoformate, which gave (1*R*,*cis*)-3-(2-amino-6-chloro-9*H*-purin-9-ylmethyl)-2,2-dimethylcyclobutylmethanol (**5**) in 92% yield, or with sodium nitrite in acetic acid, which gave (1*R*,*cis*)-3-(5-amino-7-chloro-3*H*-[1,2,3]triazolo[4,5-*d*]-pyrimidin-3-ylmethyl)-2,2-dimethylcyclobutylmethanol (**8**) in 85% yield. The guanosine analogue **6** was obtained by hydrolysis of **5** in dilute sodium hydroxide (73% yield), and the 2,6-diaminopurinyl compound **7** was obtained by amination of **5** in aqueous ammonia (54% yield). Compound **8** was converted to **9** by treating it with sodium hydroxide (70% yield), and to the corresponding 8-azaguanosine analogue **10** by treatment with aqueous ammonia (83% yield).

The antiviral activities of compounds **5**—**10** were determined *in vitro* against a variety of viruses using previously established procedures.[14,15] The viruses and cells used were: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), vaccinia virus, vesicular stomatitis virus and thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B2006 and VMW1837) in E_6SM cells, parainfluenza virus type 3, reovirus type 1, Sindbis virus, Coxsackie B4 virus and Punta Toro virus in Vero cells, and vesicular stomatitis virus, Coxsackie B4 virus and respiratory syncytial virus in HeLa cells; cytomegalovirus (strains AD-169 and Davis) and varicella-zoster virus (strains TK^+ OKA, TK^+ YS, TK^- 07/1 and TK^- YS/R) in HEL cells; influenza virus (strains H2N2 A2 Japan/305/57, B Hong Kong/5/72 and H3N2 (X31)) in MDCK cells; and human inmunodeficiency virus (HIV) types 1 and 2, in human T-lymphocyte (CEM/0) cells. The antitumoral activities of compounds **5**—**10** were assayed also with murine leukemia cells (L1210/0) and human T-lymphocytes (Molt4/C8), using procedures described elsewhere.[15]



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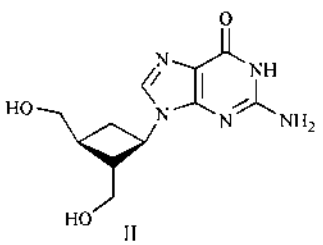

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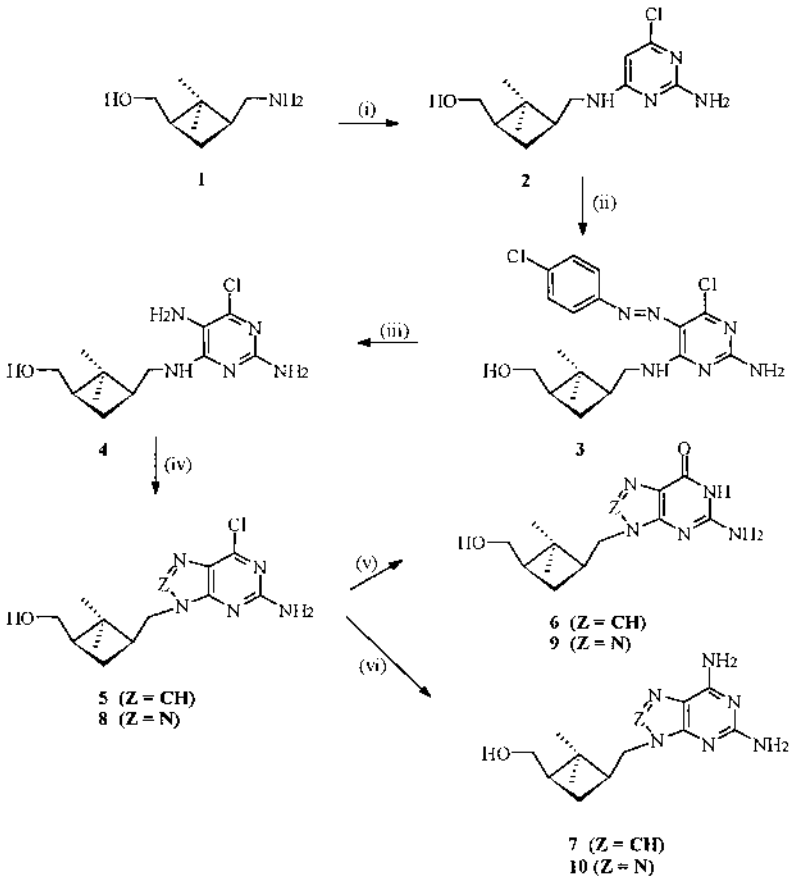

(i) 2-amino-4,6-dichloropyrimidine; (ii) 4-chlorobenzenediazonium chloride; (iii) Zn / AcOH; (iv) $CH(OEt)_3$ (Z=CH) or $NaNO_2$ / HOAc (Z=N); (v) NaOH; (vi) NH_4OH.

Chart 1

Though all the new compounds showed no detectable activity in most of the tests performed, it is noteworthy that compounds **6** and **7** showed activity against respiratory syncytial virus, with MIC_{50} values of 6 and 4 μg/ml respectively, (at 66 to 100 times below the cytotoxic concentration for the host cells). Compound **10** was also active against vaccinia virus, with an MIC_{50} value of 50 μg/ml (8 times below the cytotoxic concentration). Compound **5**, which showed moderate activity against Coxsackie B4 virus (MIC_{50} of 150 μg/ml), was the most active of our compounds in the cytostatic tests, with IC_{50} values of 111, 81 and 64 μg/ml against L1210/0, CEM/0 and Molt4/C8, respectively.

Experimental

Melting points were determined on a Reichert Kofler thermopan and are uncorrected. Sodium-D line polarimetry was carried out in a Perkin–Elmer 241 polarimeter. IR spectra of the samples in KBr discs (solids) or as films between NaCl plates (oils) were recorded in a Perkin–Elmer FTIR 1640 spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded in a Bruker AMX-300 spectrometer, at 300 and 75 MHz, respectively, with tetramethylsilane (TMS) as an internal standard. Microanalyses were done at the Microanalysis Service, University of Santiago, using a Perkin–Elmer 240B Elemental Analyzer. Silica gel (400 mesh) for flash chromatography (FC) was from Merck. Reagents and solvents were of commercial grade (Aldrich Chemical Co.).

(+)-(1*R*,*cis*)-3-(2-Amino-6-chloropyrimidin-4-ylaminomethyl)-2,2-dimethylcyclobutylmethanol (2) A solution of **1** (5.25 g, 36.7 mmol), 2-amino-4,6-dichloropyrimidine (9.20 g, 56.1 mmol) and triethylamine (22 ml) in dry 1-butanol (93 ml) was refluxed under a dry atmosphere for 48 h, whereafter the reaction mixture was cooled and the solvent was removed *in vacuo*. The residue was purified by FC (eluent CH_2Cl_2/MeOH 1 : 1), then redissolved in dry acetone. The solution was filtered and the solvent was evaporated *in vacuo* to afford compound **2** as a colorless foam (7.21 g, 72%). $[\alpha]_D^{25}$ +2.21° (*c*=0.66, MeOH). IR (KBr): 3311, 2953, 2242, 1578, 1458, 1362, 1237, 1002, 972 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s, *trans*-2-CH_3), 1.16 (3H, s, *cis*-2-CH_3), 1.22—1.30 (1H, m, 4-HH), 1.75 (1H, br s, D_2O exchang., OH), 1.99—2.14 (3H, m, 4-HH+1-H+3-H), 3.07—3.26 (2H, m, CH_2N), 3.54 (1H, dd, *J*=10.80, 6.23 Hz, CHHO), 3.62 (1H, dd, *J*=10.80, 7.80 Hz, CHHO), 4.71 (1H, br s, D_2O exchang., NH), 5.01 (2H, s, D_2O exchang., NH_2), 5.75 (1H, s, arom). ^{13}C-NMR ($CDCl_3$) δ: 16.88, 24.78, 31.67, 39.64, 41.62, 42.85, 44.38, 63.72, 160.13, 162.70, 164.56, 166.71.

(+)-(1*R*,*cis*)-3-[(2-Amino-6-chloro-5-(4-chlorophenylazo)pyrimidin-4-ylaminomethyl]-2,2-dimethylcyclobutylmethanol (3) 4-Chloroaniline (3.80 g, 30.8 mmol) in 3 N HCl (27 ml) was treated at 0 °C with $NaNO_2$ (2.25 g, 32.6 mmol) in water (25 ml). The diazonium salt obtained was added to a mixture of **2** (6.98 g, 25.8 mmol), $NaOAc \cdot 3H_2O$ (51.2 g), acetic acid (128 ml) and water (128 ml) and stirred overnight at room temperature. The precipitate was filtered out, washed with water until the washings were neutral, and dried to afford **3** (9.17 g, 87%) as a yellow solid. An analytical sample was obtained by double recrystallization from MeOH. mp 261—262 °C, $[\alpha]_D^{25}$ +29.6° (*c*=1.0, $CHCl_3$). IR (KBr): 3411, 3298, 3141, 2955, 1645, 1568, 1481, 1368, 1296 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, s, *trans*-2-CH_3), 1.20 (3H, s, *cis*-2-CH_3), 1.30—1.39 (1H, m, 4-HH), 2.08 (1H, q, *J*=7.76 Hz, 4-HH), 2.12—2.24 (2H, m, 1-H+3-H), 3.50 (2H, dd, *J*=5.50, 6.89 Hz, CH_2N), 3.56—3.69 (2H, m, CH_2O), 5.37 (2H, br s, D_2O exchang., NH_2), 7.42 (2H, d, *J*=8.78 Hz, Ar-*m*H, 7.69 (2H, d, *J*=8.78 Hz, Ar-*o*H), 10.13 (1H, br s, D_2O exchang., NH). ^{13}C-NMR ($CDCl_3$) δ: 16.98, 24.72, 31.76, 39.66, 41.58, 41.70, 44.54, 63.95, 120.45, 123.40, 129.79, 135.57, 151.43, 155.68, 161.17, 166.27. *Anal*. Calcd for $C_{18}H_{22}Cl_2N_6O$: C, 52.82; H, 5.42; Cl, 17,32; N, 20.53. Found: C, 52.57; H, 5.56; Cl, 17.03; N, 20.31.

(+)-(1*R*,*cis*)-3-(2,5-Diamino-6-chloropyrimidin-4-ylaminomethyl)-2,2-dimethylcyclobutylmethanol (4) A mixture of **3** (8.65 g, 21.2 mmol), Zn powder (11.80 g, 170 mmol), acetic acid (6.30 ml), water (135 ml) and ethanol (135 ml) was refluxed under argon for 5 h. Then, the reaction mixture was filtered, the solvent was removed *in vacuo* and the residue was purified by FC (eluent CH_2Cl_2/EtOAc 1 : 9). Compound **4** (3.81 g, 63%) was isolated as a reddish foam. $[\alpha]_D^{25}$ +4.51° (*c*=0.31, MeOH). IR (KBr): 3328, 2953, 2862, 1717, 1577, 1458, 1364, 1249, 865 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, s, *trans*-2-CH_3), 1.17 (3H, s, *cis*-2-CH_3), 1.23—1.31 (1H, m, 4-HH), 1.99—2.21 (4H, m, 3H after D_2O exchang., 4-HH, +1-H+3-H+OH), 2.39 (2H, s, D_2O exchang., NH_2), 3.28—3.41 (2H, m, CH_2N), 3.57 (1H, dd, *J*=6.05, 10.80 Hz, CHHO), 3.64 (1H, dd, *J*=7.82, 10.80 Hz, CHHO), 4.75 (2H, s, D_2O exchang., NH_2), 5.29 (1H, s, D_2O exchang., NH), ^{13}C-NMR ($CDCl_3$) δ: 16.96, 24.75, 31.81, 39.66, 41.87, 42.44, 44.48, 63.94, 111.64, 148.73, 158.31, 159.79.

(+)-(1*R*,*cis*)-3-(2-Amino-6-chloro-9*H*-purin-9-ylmethyl)-2,2-dimethylcyclobutylmethanol (5) A mixture of **4** (1.80 g, 6.30 mmol), triethyl orthoformate (25 ml) and 12 N HCl (1.6 ml) under argon was stirred overnight at room temperature. The mixture was concentrated to dryness *in vacuo*, and 0.5 N HCl (30 ml) was added to the residue and stirred for 1 h. Then, the reaction mixture was adjusted to pH 8 with 1 N NaOH, and the solvent was evaporated *in vacuo*. The crude product was purified by FC (eluent EtOAc). Compound **5** (1.72 g, 92%) was isolated as a yellow solid. An analytical sample was obtained by recrystallization from cyclohexane/Et_2O, mp 187—189 °C. $[\alpha]_D^{25}$ +12.00° (*c*=0.52, MeOH). IR (KBr): 3372, 3199, 1639, 1615, 1560, 1522, 1474, 1345, 1020, 907 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (3H, s, *trans*-2-CH_3), 1.01 (3H, s, *cis*-2-CH_3), 1.33 (1H, q, *J*=10.30 Hz, 4-HH), 1.74 (1H, dt, *J*=7.87, 10.38 Hz, 4-HH), 1.91, (1H, q, *J*=8.04 Hz, 1-H), 2.37 (1H, q, *J*=8.35 Hz, 3-H), 3.26 (1H, ddd, *J*=2.31, 6.26, 10.83 Hz, CHHO), 3.39 (1H, ddd, *J*=5.51, 8.02, 10.83 Hz, CHHO), 3.88 (1H, dd, *J*=8.63, 13.77 Hz, CHHN), 4.05 (1H, dd, *J*=7.29, 13.77 Hz, CHHN), 4.23 (1H, t, *J*=4.98 Hz, D_2O exchang., OH), 8.10 (2H, s, D_2O exchang., NH_2), 8.12 (1H, s, 8-H of purine). ^{13}C-NMR (DMSO-d_6) δ: 16.56, 24.57, 30.87, 39.14, 40.83, 43.87, 44.01, 61.68, 123.67, 143.50, 149.61, 154.39, 160.07. *Anal*. Calcd for $C_{13}H_{18}ClN_5O$: C, 52.79; H, 6.13; Cl, 11.99; N, 23.68. Found: C, 52.50; H, 6.15; Cl, 11.80; N, 24.00.

(+)-(1*R*,*cis*)-3-(2-Amino-1,6-dihydro-6-oxo-9*H*-purin-9-ylmethyl)-2,2-dimethylcyclobutylmethanol (6) A mixture of **5** (0.60 g, 2.03 mmol) and 0.33 N NaOH (48 ml) was refluxed for 6 h, whereupon the solvent was removed *in vacuo*. The residue was purified by FC (eluent EtOAc) to afford compound **6** (0.41 g, 73%) as a white solid. An analytical sample was obtained by recrystallization of the crude product from EtOH, mp 258—260 °C, $[\alpha]_D^{25}$ +15.16° (*c*=0.46, MeOH). IR (KBr): 3333, 2953, 2720, 1792, 1718, 1684, 1560, 1458, 1362, 1006 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (3H, s, *trans*-2-CH_3), 0.99 (3H, s, *cis*-2-CH_3), 1.31 (1H, q, *J*=10.29 Hz, 4-HH), 1.74 (1H, dt, *J*=7.85, 10.34 Hz, 4-HH), 1.90 (1H, q, *J*=8.08 Hz, 1-H), 2.30 (1H, q, *J*=8.38 Hz, 3-H), 3.26 (1H, m, dd after D_2O exchang., *J*=6.07, 10.93 Hz, CHHO), 3.36 (1H, m, dd after D_2O exchang., *J*=4.94, 10.93 Hz, CHHO), 3.75 (1H, dd, *J*=8.41, 13.70 Hz, CHHN), 3.93 (1H, dd, *J*=7.41, 13.70 Hz, CHHN), 4.23 (1H, t, *J*=4.96 Hz, D_2O exchang., OH), 6.66 (2H, s, D_2O exchang., NH_2), 7.62 (1H, s, 8-H of purine), 10.75 (1H, s, D_2O exchang., NH). ^{13}C-NMR (DMSO-d_6) δ: 16.57, 24.65, 30.89, 39.11, 41.18, 43.63, 43.87, 61.68, 116.86, 137.60, 151.39, 153.97, 157.17. *Anal*. Calcd for $C_{13}H_{19}N_5O_2$: C, 56.30; H, 6.91; N, 25.25. Found: C, 56.50; H, 6.75; N, 25.09.

(+)-(1*R*,*cis*)-3-(2,6-Diamino-9*H*-purin-9-ylmethyl)-2,2-dimethylcyclobutylmethanol (7) A suspension of **5** (0.50 g, 1.68 mmol) in concentrated NH_4OH (50 ml) was heated under reflux for 1 h. The reaction mixture was concentrated *in vacuo* to a yellow solid (0.60 g), which was purified by FC (eluent EtOAc/MeOH 7 : 3) to afford compound **7** (0.25 g, 54%) as a white solid. An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp >325 °C, $[\alpha]_D^{25}$ +7.67° (*c*=0.43, MeOH). IR (KBr): 3336, 2954, 1749, 1717, 1684, 1653, 1541, 1458, 1010, 789 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (3H, s, *trans*-2-CH_3), 1.01 (3H, s, *cis*-2-CH_3), 1.32 (1H, q, *J*=10.33 Hz, 4-HH), 1.74 (1H, dt, *J*=7.85, 10.49 Hz, 4-HH), 1.90 (1H, q, *J*=8.03 Hz, 1-H), 2.33 (1H, q, *J*=8.43 Hz, 3-H), 3.26 (1H, m, dd after D_2O exchang., *J*=6.34, 10.82 Hz, CHHO), 3.36 (1H, m, dd after D_2O exchang., *J*=8.19, 10.82 Hz, CHHO), 3.79 (1H, dd, *J*=8.42, 13.73 Hz, CHHN), 3.97 (1H, dd, *J*=7.39, 13.73 Hz, CHHN), 4.25 (1H, br s, D_2O exchang., OH), 6.09 (2H, br s, D_2O exchang., NH_2), 7.02 (2H, br s, D_2O exchang., NH_2), 7.75 (1H, s, 8-H of purine). ^{13}C-NMR (DMSO-d_6) δ: 16.59, 24.67, 30.93, 39.71, 41.05, 43.55, 43.89, 61.70, 112.97, 138.51, 151.87, 155.16, 159.01. *Anal*. Calcd for $C_{13}H_{20}N_6$: C, 56.50; H, 7.29; N, 30.41. Found: C, 56.56; H, 7.55; N, 30.09.

(−)-(1*R*,*cis*)-3-(5-Amino-7-chloro-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-2,2-dimethylcyclobutylmethanol (8) Sodium nitrite (0.43 g, 6.27 mmol) in water (13 ml) was added to a cooled (0 °C) solution of **4** (1.45 g, 5.10 mmol) in acetic acid (8 ml) and water (13 ml), then stirred for 3 h. After work-up, an oil (2.5 g) was obtained, which was purified by FC (eluent EtOAc) to afford compound **8** as a white solid (1.28 g, 85%). An analytical sample was obtained by recrystallization from hexane/Et_2O, mp 145—147 °C. $[\alpha]_D^{25}$ −0.89° (*c*=0.65, MeOH). IR (KBr): 3363, 3190, 1739, 1676, 1645, 1609, 1565, 1516, 1458, 1005 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, s, *trans*-2-CH_3), 1.05 (3H, s, *cis*-2-CH_3), 1.34 (1H, q, *J*=10.29 Hz, 4-HH), 1.79 (1H, dt, *J*=7.89, 10.46 Hz, 4-HH), 1.91 (1H, q, *J*=7.97 Hz, 1-H), 2.36 (1H, q, *J*=8.47 Hz, 3-H), 3.28 (1H, dd, *J*=6.17, 10.72 Hz, CHHO), 3.39 (1H, dd, *J*=8.19, 10.72 Hz, CHHO), 4.07 (1H, dd, *J*=7.19, 13.95 Hz, CHHN), 4.41 (1H, dd, *J*=8.21, 13.95 Hz, CHHN), 7.66 (1H, br s, D_2O exchang., NH), 8.31 (1H, br s, D_2O exchang., NH), 10.99 (1H, s, D_2O exchang., OH). ^{13}C-NMR (DMSO-d_6) δ: 16.44, 24.59, 30.70, 39.83, 41.07, 43.94, 46.47, 61.66, 124.45, 151.82, 155.83, 155.98. *Anal*. Calcd for $C_{12}H_{17}ClN_6O$: C, 48.57; H, 5.77; Cl, 11.95; N, 28.32. Found: C, 48.50; H, 5.55; Cl, 12.23; N, 28.09.

(−)-(1*R*,*cis*)-3-(5-Amino-6,7-dihydro-7-oxo-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-2,2-dimethylcyclobutylmethanol (9) A mixture of **8** (0.50 g, 1.68 mmol) and 0.25 N NaOH (21 ml) was refluxed for 5 h, whereafter the reaction mixture was cooled and adjusted to pH 3 with 6 N HCl. The precipitated solid was filtered out, washed with cold water until the washings were neutral, and dried *in vacuo* over P_2O_5 to yield **9** as an off-white solid (0.32 g, 70%). An analytical sample was obtained by recrystallization of the crude product from water. mp 284—285 °C, $[\alpha]_D^{25}$ −0.47° (*c*=0.49, MeOH). IR (KBr): 3355, 3183, 1715, 1661, 1575, 1368, 1059, 738, 563 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, s, *trans*-2-CH_3), 1.04 (3H, s, *cis*-2-CH_3), 1.34 (1H, q, *J*=10.31 Hz, 4-HH), 1.79 (1H, dt, *J*=7.85, 10.48 Hz, 4-HH), 1.92 (1H, q, *J*=8.14 Hz, 1-H), 2.36 (1H, q, *J*=8.38 Hz, 3-H), 3.28 (1H, dd, *J*=5.94, 10.82 Hz, CHHO), 3.38 (1H, ddd, *J*=5.39, 8.13, 10.82 Hz, CHHO), 4.06 (1H, dd, *J*=7.25, 13.93 Hz, CHHN), 4.24 (1H, d, *J*=4.89 Hz, D_2O exchang., OH), 4.26 (1H, dd, *J*=8.21, 13.93 Hz, CHHN), 7.16 (2H, s, D_2O exchang., NH_2). ^{13}C-NMR (DMSO-d_6) δ: 16.45, 24.59, 30.70, 39.28, 41.07, 43.95, 46.45, 61.65, 124.45, 151.21, 155.94, 161.75. *Anal*. Calcd for $C_{12}H_{18}N_6O_2$: C, 51.79; H, 6.52; N, 30.20. Found: C, 51.50; H, 6.55; N, 30.27.

(−)-(1*R*,*cis*)-3-(5,7-Diamino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-2,2-dimethylcyclobutylmethanol (10) A suspension of **8** (0.45 g, 1.52 mmol) in concentrated NH_4OH (40 ml) was heated under reflux for 7 h. The reaction mixture was concentrated *in vacuo* to a solid (0.54 g), which was purified by FC (eluent EtOAc/MeOH 4 : 1) to afford compound **10** (0.34 g, 83%). An analytical sample was obtained after recrystallization from EtOAc/MeOH, mp 212—214 °C, $[\alpha]_D^{25}$ −2.03° (*c*=0.45 MeOH). IR (KBr): 3347, 3198, 1665, 1624, 1595, 1491, 1421, 1095, 795 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, s, *trans*-2-CH_3), 1.06 (3H, s, *cis*-2-CH_3), 1.34 (1H, q, *J*=10.29 Hz, 4-HH), 1.78 (1H, dt, *J*=7.64, 10.46 Hz, 4-HH), 1.92 (1H, q, *J*=8.18 Hz, 1-H), 2.40 (1H, q, *J*=8.38 Hz, 3-H), 3.27 (1H, m, dd after D_2O exchang., *J*=6.34, 10.75 Hz, CHHO), 3.37 (1H, m, dd after D_2O exchang., *J*=8.03, 10.75 Hz, CHHO), 4.08 (1H, dd, *J*=7.30, 13.74 Hz, CHHN), 4.20 (1H, t, *J*=4.99 Hz, D_2O exchang., OH), 4.27 (1H, dd, *J*=8.38, 13.74 Hz, CHHN), 6.35 (2H, s, D_2O exchang., NH_2), 7.48 (2H, s, D_2O exchang., NH_2). ^{13}C-NMR (DMSO-d_6) δ: 16.48, 24.63, 30.73, 39.26, 41.04, 43.98, 46.17, 61.71, 120.51, 151.58, 156.53, 163.09. *Anal*. Calcd for $C_{12}H_{19}N_7O$: C, 51.97; H, 6.91; N, 35.35. Found: C, 51.60; H, 6.75; N, 35.27.

Biological Activity Assays The methods used to assess antiviral activity and cytotoxicity have been described previously.[14,15]

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References

1) De Clercq E., *Rev. Med. Virol*., **5**, 149—164 (1995).
2) Marquez V. E., *Adv. Antiviral Drug Des*., **2**, 89—146 (1996).
3) De Clercq E., *J. Med. Chem*., **38**, 2491—2517 (1995).
4) *a*) Jacobo-Molina A., Ding J., Nanni R. G., Clark A. D., Lu X., Tantillo C., Williams R. L., Kamer G., Ferris A. L., Clark P., Hizi A., Hughes S. H., Arnold E., *Proc. Natl. Acad. Sci. U.S.A*., **90**, 6320—

6324 (1993). *b*) Arnold E., Ding J., Hughes S. H., Hostomsky Z., *Curr. Opin. Struct. Biol.*, **5**, 27—38 (1995).
5) Bisacchi G. S., Braitman A., Cianci C. W., Clark J. M., Field A. K., Hagen M. E., Hockstein D. R., Malley M. F., Mitt T., Slursarchyk W. A., Sundeen J. E., Terry B. J., Tuomari A. V., Weaver E. R., Young M. G., Zahler R., *J. Med. Chem*., **34**, 1415—1421 (1991).
6) Nishiyama Y., Yamamoto Y., Yamada Y., Daikoku T., Ichikawa T., Takahashi K., *J. Antibiotics*, **52**, 1854—1859 (1989).
7) Braitman A., Swerdel M. R., Olsen S. J., Tuomari A. V., Lynch J. S., Blue B., Michalik T., Field A. K., Bonner D. P., Clark J. M., *Antimicrob. Agents Chemother*., **35**, 1464—1468 (1991).
8) Tenney D. J., Yamanaka G., Voss S. M., Cianci C. W., Tuomari A. V., Sheaffer A. K., Alam M., Colonno R. J., *Antimicrob. Agents Chemother*., **41**, 2680—2685 (1997).
9) Agrofoglio L., Suhas E., Farese A., Condom R., Challand S. R., Earl R. A., Guedj R., *Tetrahedron*, **50**, 10611—10670 (1994).
10) Nieto M. I., Blanco J. M., Caamaño O., Fernández F., García-Mera X., Balzarini J., Padalko E., Neyts J., De Clercq E., *Nucleosides and Nucleotides*, **17**, 1255—1266 (1998).
11) *a*) Maruyama T., Sato Y., Horii T., Shiota H., Nitta K., Shirasaka T., Mitsuya H., Honjo M., *Chem. Pharm. Bull*., **38**, 2719—2725 (1990); *b*) Maruyama T., Hanai Y., Sato Y., Snoeck R., Andrei G., Hosoya M., Balzarini J., De Clercq E., *Chem. Pharm. Bull.*, **41**, 516—521 (1993).
12) Fernández F., López C., Hergueta A. R., *Tetrahedron*, **51**, 10317—10322 (1995).
13) Balo M. C., Fernández F., Lens E., López C., De Clercq E., Andrei G., Snoeck R., Balzarini J., *Nucleosides and Nucleotides*, **15**, 1335—1346 (1996).
14) De Clercq E., "*In Vitro* and *Ex Vivo* Test Systems to Rationalize Drug Design and Delivery", ed. By Crommelin D., Couvreur P., Duchêne D., Editions de Santé, Paris, 1994, pp. 108—125.
15) De Clercq E., Descamps J., Verhelst G., Walter R. T., Jones A. S., Torrence P. F., Shugar D., *J. Infect. Dis*., **141**, 563—574 (1980).