Novel Carbocyclic Nucleosides Containing a Cyclobutyl Ring: Adenosine Analogues

Antonio R. Hergueta,[a] Franco Fernández,[a] Carmen López,*[,a] Jan Balzarini,[b] and Eric De Clercq[b]

Departmento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago,[a] E-15706-Santiago de Compostela, Spain and Rega Institute for Medical Research, Katholieke Universiteit,[b] B-3000 Leuven, Belgium.
Received January 26, 2001; accepted May 14, 2001

(1*S*,1′*R*)-*cis*-1-(3′-aminomethyl-2′,2′-dimethylcyclobutyl)ethanol (1) and (1*S*,1′*R*)-*cis*-1-[3′-(2-aminoethyl)-2′,2′-dimethylcyclobutyl]ethanol (2) were used as precursors in the synthesis of cyclobutyl nucleoside analogues containing adenine and 8-azaadenine moieties, which were tested as antiviral and antitumoral agents in a variety of assay systems. Compounds 8 and 9 displayed significant activity against respiratory syncytial virus, and compounds 14 and 15 were moderately active against vaccinia virus.

Key words adenosine analogue; antitumoral; antiviral; carbocyclic nucleoside; respiratory syncytial virus; vaccinia virus

In recent years the search for new antitumoral and antiviral therapeutic agents has focused on carbocyclic analogues of nucleosides (CANs).[1—3] One such is the cyclobutane derivative lobucavir (I), a carbocyclic analogue of the natural antibiotic oxetanocin A (II), which has anti-HIV activity.[4] Lobucavir is active not only against HIV, but also against the herpes simplex viruses HSV-1 and HSV-2, cytomegalovirus, and hepatitis B virus.[5—9] This wide scope has prompted us to look for similar or improved antiviral properties in analogues of lobucavir with a modified cyclobutane moiety. Although certain limited structure–activity relationships have been inferred for CANs,[10,11] there are as yet no general rules of this kind.

Specifically, our aim has been to investigate the antiviral activity of a series of lobucavir analogues that i) lack the 2′-hydroxymethyl group of lobucavir, which makes them more like 3′-deoxynucleosides; ii) have a *gem*-dimethyl-substituted cyclobutane carbon that makes them more lipophilic and greatly restricts the conformational flexibility of the other substituents on the cyclobutane ring; and iii) have an extra methylene between the remaining hydroxymethyl group and the heterocyclic moiety, which further increases their lipophilicity but allows a certain degree of molecular flexibility.[12,13] Among our first findings was that some of the simplest lobucavir analogues of this kind (compounds III) have no activity against the viruses inhibited by lobucavir, but do have marked activity against respiratory syncytial virus (IIIa and IIIb) or, to a lesser extent, against vaccinia virus (IIIc).[13]

In addition, it has been reported that a 6′-*C*-methylneplanocin A (IVa) is more potent and/or selective than neplanocin A (IVb) against cytomegalovirus, vaccinia virus, parainfluenza virus, measles virus, respiratory syncytial virus, Junin virus, Tacaribe virus, vesicular stomatitis virus and reovirus.[14] This fact suggested to us to extend our research to compounds in which the cyclobutane ring bears a 1-hydroxyethyl group instead of the hydroxymethyl group of compounds III.

We have accordingly synthesized and assayed compounds of types V and VI, in the latter of which, additionally, lipophilicity and conformational flexibility have been further increased by inclusion of an extra methylene between the cyclobutane and heterocyclic moieties. The *S*-configuration for the 1-hydroxyethyl chain of V and VI was an unavoidable choice since the corresponding epimers were the only easily available ones *via* the reduction of the starting material, (−)-1*R*-*cis*-pinonic acid, with a variety of reagents.

Lobucavir analogues V or VI were synthesized by construction of an adenine or 8-azaadenine on the amino group of enantiomerically pure amino alcohols **1** or **2**,[15] using the classical approach to carbocyclic analogues of nucleosides (Chart 1).[16,17] Briefly, condensation of **1** or **2** with 5-amino-4,6-dichloropyrimidine afforded pyrimidinylamines **3** or **4**, respectively, and cyclization of **3** or **4** with triethyl orthoformate gave the 6-chloropurines **5** or **6**, which were converted to hypoxanthine analogues **7** or **8** by treatment with sodium hydroxide or to adenine analogues **9** or **10** by heating with concentrated aqueous ammonia. The 8-azaadenine derivatives were prepared by diazotizing **3** or **4** with sodium nitrite in hydrochloric acid and converting the resulting highly unstable 6-chloro-8-azapurines **11** or **12**, without their prior isolation, into the 8-azahypoxanthine analogue **13** (by simply heating the crude reaction mixture) or the 8-azaadenine analogues **14** or **15** (by treatment with concentrated aqueous ammonia).

Using procedures described elsewhere,[18,19] the antiviral activities of compounds **7**—**10** and **13**—**15** were evaluated *in vitro* against a variety of viruses. The viruses and host cells used were as follows: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), caccinia 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; 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/305757, B Hong Kong/5/72 and H3N2 (X31) in MDCK cells; and human immunodeficiency virus (HIV) types 1 and 2, in human T-lymphocyte (CEM/0) cells.

All the new compounds were inactive in most of the tests performed, but some of them showed some activity in tests against vaccinia virus (VV) or respiratory syncytial virus (RSV). Table 1 shows the results obtained with the new compounds, together with those obtained with their analogues of

* To whom correspondence should be addressed. e-mail: qomcls@uscmail.usc.es

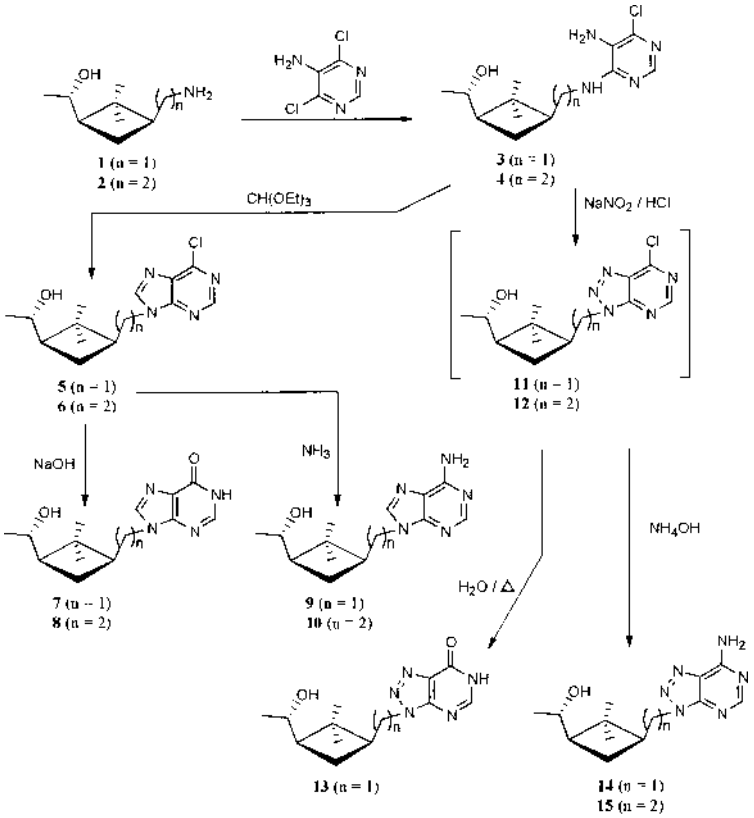
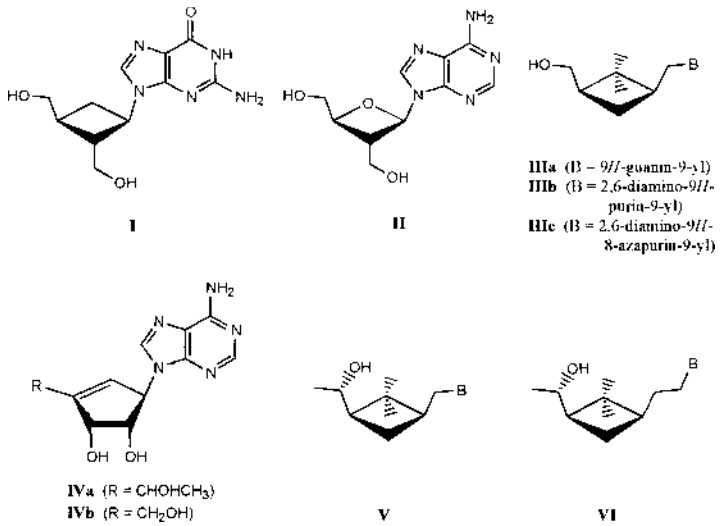


Chart 1



type III and with two well-known standards. With regard to VV, the moderate activities of compounds **14** and **15** against this virus are similar to that of IIIc (like **14** and **15** a 6-amino-8-azapurine derivative), which shows that an extra methyl group on the carbon bearing the hydroxyl of these compounds does not greatly alter their anti-VV activity, which remains of a magnitude similar to that of ribavirin and well below that of brivudin. With regard to RSV, the marked activity of the 6-aminopurine IIIb (about 15-fold less than those of neplanocin A and 6′-*C*-methylneplanocin A,[14] but of the same magnitude as that of ribavirin and more than 130-fold more active than brivudin) is retained by the type V analogue **9** but not by the type VI analogue **10**. Curiously, anti-RSV activity of the same magnitude as that of IIIb is displayed by the type VI hypoxanthine derivative **8**, whereas the corresponding type V analogue **7** is inactive. Thus although the replacement of the hydroxymethyl of III by a 1-hydroxyethyl does not greatly alter the antiviral profile of these compounds, introduction of an extra methylene between the carbocycle and the heterocyclic system can have a marked effect one way or the other, probably because of the resulting increase in the flexibility of the molecule in this region.

The antitumoral activities of compounds **7**—**10** and **13**—

Table 1. Antiviral Activity and Cytotoxicity of Compounds of Types III, V and VI against Vaccinia Virus (VV, in E_6SM Cell Cultures) and against Respiratory Syncytial Virus (RSV, in HeLa Cell Cultures)

Compound	Structural type	Anti-VV activity IC_{50}[a] (μg/ml)	Cytotoxicity MCC[b] (μg/ml)	Anti-RSV activity IC_{50}[a] (μg/ml)	Cytotoxicity MCC[b] (μg/ml)
IIIa	III	>400	>400	200	>400
IIIb	III	400	>400	3	≥400
IIIc	III	50	≥400	>400	>400
7	V	>300	≥400	>400	≥400
9	V	>300	400	2.4	≥400
13	V	>100	200	>200	≥400
14	V	70	≥200	>200	≥400
8	VI	>100	≥100	3	≥200
10	VI	>150	≥200	>400	>400
15	VI	40	400	>200	>400
Brivudin		1	≥400	>400	≥400
Ribavirin		33	>400	2	>400

a) Inhibitory concentration 50, required to reduce virus-induced cytopathogenicity by 50%. *b*) Minimum cytotoxic concentration, required to cause a microscopically detectable alteration of normal cell morphology.

15 were assayed against murine leukemia cells (L1210/0) and human T-lymphocytes (Molt4/C8 and CEM/0) using procedures described elsewhere.[19] Inhibitory concentrations 50 (IC_{50}), required to reduce cell growth by 50%, were >200 μg/ml for compounds with an oxygen at position 6 of the purine base (**7**, **13** and **8**) and type V derivative **14**. Highest cytostatic activities were measured for type VI derivatives **10** and **15**, with IC_{50} (Molt4/C8) of 101±62 and 110±6 μg/ml, respectively, and IC_{50} (CEM/0) of 85±59 and 128±11 μg/ml, 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 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 TMS as internal standard. Mass spectra were recorded on a Kratos MS-59 spectrometer. Silica gel (400 mesh) for flash chromatography (FC) was from Merck. Reagents and solvents were of commercial grade (Aldrich Chemical Co.).

(+)-(1*S*,1′*R*)-*cis*-1-[3′-(5-Amino-6-chloropyrimidin-4-ylaminomethyl)-2′,2′-dimethylcyclobutyl]ethanol (3) A solution of **1** (3.47 g, 22.10 mmol), 5-amino-4,6-dichloropyrimidine (4.30 g, 26.4 mmol) and triethylamine (14 ml) in dry 1-butanol (100 ml) was heated under reflux for 72 h. The reaction mixture was cooled and the solvent was removed *in vacuo.* Chromatography (3 : 2 CH_2Cl_2/EtOAc) gave **3** as a white solid (4.32 g, 69%). An analytical sample was obtained by recrystallization from cyclohexane/EtOAc, mp 154—156 °C. $[\alpha]_D^{25}$ +20.89° (*c*=0.78, MeOH). IR (KBr) 3437, 3230, 2955, 2868, 1653, 1636, 1587, 1506, 1456, 1169 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, d, *J*=6.02 Hz, C<u>H</u>$_3$CH<), 1.02 (3H, s, *trans*-2′-CH_3), 1.05 (3H, s, *cis*-2′-CH_3), 1.11 (1H, q, *J*=10.39 Hz, 4′-<u>H</u>H), 1.58 (1H, q, *J*=9.42 Hz, 1′-H), 1.79 (1H, dt, *J*=7.96, 10.27 Hz, 4′-H<u>H</u>), 2.03 (1H, ddd, *J*=7.37, 9.98, 15.22 Hz, 3′-H), 3.24 (1H, dd, *J*=7.05, 12.90 Hz, C<u>H</u>HN), 3.29—3.39 (1H, m, CH<u>H</u>N), 3.49 (1H, dq, *J*=6.02, 15.57 Hz, 1-H), 4.96 (1H, d, *J*=5.35 Hz, D_2O exchang., OH), 5.02 (2H, br s, D_2O exchang., NH_2), 6.51 (1H, br s, D_2O exchang., NH), 7.70 (1H, s, arom). ^{13}C-NMR (DMSO-d_6) δ: 16.53, 21.71, 25.09, 31.72, 39.36, 42.12, 49.71, 67.33, 123.76, 136.84, 145.97, 152.25. *Anal.* Calcd for $C_{13}H_{21}ClN_4O$: C, 54.83; H, 7.43; Cl, 12.45; N, 19.67. Found: C, 54.64; H, 7.30; Cl, 12.56; N, 19.47.

(+)-(1*S*,1′*R*)-*cis*-1-{3′-[2-(5-Amino-6-chloropyrimidin-4-ylamino)-ethyl]-2′,2′-dimethylcyclobutyl}ethanol (4) Starting from **2** (2.31 g, 13.50 mmol), 5-amino-4,6-dichloropyrimidine (2.65 g, 16.25 mmol) and dry triethylamine (8.50 ml), a procedure analogous to the above, including chromatography with 3 : 2 CH_2Cl_2/EtOAc as eluent, gave compound **4** as a white solid (1.84 g, 48%). An analytical sample was obtained by recrystallization from hexanes/diethyl ether, mp 126—127 °C. $[\alpha]_D^{25}$ +24.26° (*c*=0.54, MeOH). IR (KBr): 3338, 3250, 2955, 2880, 1662, 1583, 1507, 1421, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s, *trans*-2′-CH_3), 1.04 (3H, d, *J*=6.22 Hz, C<u>H</u>$_3$CH<), 1.12 (1H, q, *J*=10.17 Hz, 4′-<u>H</u>H), 1.13 (3H, s, *cis*-2′-CH_3), 1.49 (1H, q, *J*=7.88 Hz, 4′-H<u>H</u>), 1.61—1.78 (2H, m, C<u>H</u>$_2$CH$_2$N), 1.82 (1H, dt, *J*=6.10, 9.35 Hz, 3′-H), 1.94 (1H, dt, *J*=7.75, 9.88 Hz, 1′-H), 3.39 (4H, dt, *J*=5.68, 7.36 Hz, CH_2N+NH_2 (D_2O exchang.)), 3.70 (1H, dq, *J*=6.22, 9.88 Hz, 1-H), 4.90 (1H, br s, D_2O exchang., NH), 8.06 (1H, s, arom). ^{13}C-NMR ($CDCl_3$) δ: 17.11, 21.64, 26.91, 30.52, 31.79, 39.96, 40.10, 40.49, 50.65, 69.67, 121.90, 150.34, 155.76. *Anal.* Calcd for $C_{14}H_{23}ClN_4O$: C, 56.27; H, 7.76; Cl, 11.86; N, 18.75. Found: C, 56.12; H, 7.85; Cl, 12.05; N, 18.91.

(1*S*,1′*R*)-*cis*-1-[3′-(6-Chloro-9*H*-purin-9-ylmethyl)-2′,2′-dimethylcyclobutyl]ethanol (5) A mixture of **3** (2.40 g, 8.46 mmol), triethyl orthoformate (46.65 ml, 0.28 mol) and 12 N HCl (2.20 ml) was stirred for 36 h at room temperature and then concentrated to dryness *in vacuo.* Chromatography (1 : 1 CH_2Cl_2/EtOAc) gave **5** as a white foam (1.41 g, 57%) IR (KBr): 3430, 2958, 1714, 1590, 1557, 1444, 1372, 1334, 1183 cm^{-1}. FAB-HR-MS *m*/*z*: 294.1257 (Calcd for $C_{14}H_{19}ClN_4O$: 294.1248).

(1*S*,1′*R*)-*cis*-1-{3′-[2-(6-Chloro-9*H*-purin-9-yl)ethyl]-2′,2′-dimethylcyclobutyl}ethanol (6) Starting from **4** (0.85 g, 2.98 mmol), triethyl orthoformate (17 ml, 0.10 mol) and 12 N HCl (0.75 ml), a procedure analogous to the above, with final chromatography with 4 : 1 CH_2Cl_2/EtOAc as eluent, gave compound **6** as a white foam (0.42 g, 47%). IR (KBr): 3420, 2951, 1717, 1593, 1560, 1457, 1403, 1373, 1139 cm^{-1}. FAB-HR-MS *m*/*z*: 308.1417 (Calcd for $C_{15}H_{21}ClN_4O$: 308.1405).

(+)-(1*S*,1′*R*)-*cis*-1-[3′-(6-Hydroxy-9*H*-purin-9-ylmethyl)-2′,2′-dimethylcyclobutyl]ethanol (7) A mixture of **5** (0.53 g, 1.80 mmol) and 0.25 N NaOH (25 ml) was heated under reflux for 6 h, the reaction mixture was cooled, and the solvent was removed *in vacuo.* Chromatography (4 : 1 EtOAc/MeOH) gave **7** as a white solid (0.46 g, 92%). An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp 300—302 °C. $[\alpha]_D^{25}$ +33.96° (*c*=0.27, MeOH). IR (KBr): 3417, 3055, 2957, 1690, 1412, 1342 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.75 (3H, s, *trans*-2′-CH_3), 0.80 (3H, s, *cis*-2′-CH_3), 0.90 (1H, dt, *J*=6.13, 9.65 Hz, 4′-<u>H</u>H), 0.91 (3H, d, *J*=6.21 Hz, C<u>H</u>$_3$CH<), 1.63 (1H, q, *J*=10.24 Hz, 4′-H<u>H</u>), 1.64 (1H, m, 1′-H), 2.08 (1H, ddt, *J*=5.74, 9.17, 9.42 Hz, 3′-H), 2.92 (1H, dd, *J*=5.05, 7.90 Hz, 1-H), 3.93 (1H, dd, *J*=9.76, 13.50 Hz, C<u>H</u>HN), 4.18 (1H, dd, *J*=5.68, 13.50 Hz, CH<u>H</u>N), 4.66 (1H, d, *J*=5.43 Hz, D_2O exchang., OH), 8.02 (1H, s), 8.09 (1H, s), 12.23 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (DMSO-d_6) δ: 14.82, 19.86, 25.62, 32.81, 36.05, 43.16, 44.41, 44.66, 86.63, 119.08, 141.30, 145.71, 149.93, 156.26. *Anal.* Calcd for $C_{14}H_{20}N_4O_2$: C, 60.85; H, 7.30; N, 20.28. Found: C, 61.00; H, 7.33; N, 20.16.

(+)-(1*S*,1′*R*)-*cis*-1-{3′-[2-(6-Hydroxy-9*H*-purin-9-yl)ethyl]-2′,2′-dimethylcyclobutyl}ethanol (8) Starting from **6** (0.18 g, 0.61 mmol) and 0.25 N NaOH (25 ml), a procedure analogous to the above, including chromatography with 4 : 1 EtOAc/MeOH as eluent, gave compound **8** as a white solid (72 mg, 43%). An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp >310 °C. $[\alpha]_D^{25}$ +35.86° (*c*=0.15, MeOH). IR (KBr) 3412, 3050, 2947, 1686, 1590, 1413, 1225, 607 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.57 (3H, s, *trans*-2′-CH_3), 0.78 (3H, s, *cis*-2′-CH_3), 0.94 (3H, d, *J*=6.65 Hz, C<u>H</u>$_3$CH<), 1.25 (1H, dt, *J*=5.04, 12.58 Hz, 4′-<u>H</u>H), 1.26 (1H, q, *J*=9.96 Hz, 4′-H<u>H</u>), 1.37—1.47 (2H, m, C<u>H</u>$_2$CH$_2$N), 1.64 (1H, ddt, *J*=6.45, 8.02, 8.96 Hz, 3′-H), 1.90 (1H, ddd, *J*=6.45, 6.78, 12.41 Hz,

1′-H), 2.86 (1H, d, J=8.83 Hz, 1-H), 4.06 (1H, dt, J=5.35, 7.78 Hz, CHHN), 4.11 (1H, dt, J=5.35, 8.33 Hz, CHHN), 4.55 (1H, br s, D_2O exchang., OH), 8.02 (1H, s), 8.09 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 15.04, 20.11, 25.67, 31.14, 34.49, 36.40, 42.01, 42.91, 43.65, 86.73, 124.27, 140.27, 145.77, 148.70, 157.05. *Anal.* Calcd for $C_{15}H_{22}N_4O_2$: C, 62.05; H, 7.64; N, 19.30. Found: C, 62.24; H, 7.65; N, 19.17.

(+)-(1*S*,1′*R*)-*cis*-1-[3′-(6-Amino-9*H*-purin-9-ylmethyl)-2′,2′-dimethylcyclobutyl]ethanol (9) A mixture of **5** (0.75 g, 2.50 mmol) and concentrated NH_4OH (70 ml) was heated under reflux for 4 h, the reaction mixture was cooled, and the solvent was removed *in vacuo.* Chromatography (4 : 1 EtOAc/MeOH) gave **9** as a white solid (0.62 g, 88%). An analytical sample was obtained by recrystallization from EtOAc, mp 188—190 °C. $[\alpha]_D^{25}$ +20.38° (c=0.51, MeOH). IR (KBr): 3318, 3149, 2953, 1670, 1601, 1571, 1325, 721 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.76 (3H, s, *trans*-2′-CH_3), 0.79 (3H, s, *cis*-2′-CH_3), 0.86—0.93 (1H, m, 4′-HH), 0.90 (3H, d, J=10 Hz, CH_3CH<), 1.62—1.68 (2H, m, 4′-HH), 2.14 (1H, dd, J=5.90, 8.94 Hz, 3′-H), 2.92 (1H, dd, J=6.10, 7.89 Hz, 1-H), 3.92 (1H, dd, J=9.97, 13.33 Hz, CHHN), 4.18 (1H, dd, J=5.48, 13.33 Hz, CHHN), 4.65 (1H, d, J=5.61 Hz, D_2O exchang., OH), 7.14 (2H, br s, D_2O exchang., NH_2), 8.12 (1H, s), 8.14 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 14.83, 19.87, 25.64, 32.82, 36.06, 43.16, 44.41, 44.66, 86.66, 119.08, 141.31, 149.91, 152.69, 156.27. *Anal.* Calcd for $C_{14}H_{21}N_5O$: C, 61.07; H, 7.69; N, 25.43. Found: C, 61.22; H, 7.59; N, 25.40.

(+)-(1*S*,1′*R*)-*cis*-1-{3′-[2-(6-Amino-9*H*-purin-9-yl)ethyl]-2′,2′-dimethylcyclobutyl}ethanol (10) Starting from **6** (0.19 g, 0.64 mmol) and concentrated NH_4OH (25 ml), a procedure analogous to the above, including chromatography with 4 : 1 EtOAc/MeOH as eluent, gave compound **10** as a white solid (70 mg, 40%). An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp 143—145 °C. $[\alpha]_D^{25}$ +37.40° (c=0.27, MeOH). IR (KBr): 3367, 2951, 1655, 1598, 1456, 1416, 1309, 1245, 649 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.57 (3H, s, *trans*-2′-CH_3), 0.77 (3H, s, *cis*-2′-CH_3), 0.94 (3H, d, J=6.77 Hz, CH_3CH<), 1.27 (1H, q, J=10.28 Hz, 4′-HH), 1.30 (1H, q, J=10.07 Hz, 4′-HH), 1.36—1.53 (2H, m, CH_2CH_2N), 1.64 (1H, ddt, J=6.92, 9.12, 10.17 Hz, 3′-H), 1.90 (1H, ddd, J=6.80, 6.77, 12.87 Hz, 1′-H), 2.86 (1H, dd, J=5.58, 8.93 Hz, 1-H), 4.02 (1H, dt, J=6.06, 7.46 Hz, CHHN), 4.09 (1H, dt, J=6.06, 8.23 Hz, CHHN), 4.54 (1H, d, J=5.55 Hz, D_2O exchang., OH), 7.14 (2H, br s, D_2O exchang., NH_2), 8.13 (1H, s), 8.30 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 14.68, 19.35, 25.32, 30.56, 34.16, 36.03, 41.67, 42.10, 43.27, 86.40, 118.71, 140.77, 149.51, 152.31, 155.90. *Anal.* Calcd for $C_{15}H_{23}N_5O$: C, 62.26; H, 8.01; N, 24.20. Found: C, 62.00, H, 8.12; N, 24.23.

(+)-(1*S*,1′*R*)-*cis*-1-[3′-(7-Hydroxy-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-2′,2′-dimethylcyclobutyl]ethanol (13) A cooled solution (0 °C) of **3** (0.80 g, 2.82 mmol) in 1 N HCl (15.5 ml) was treated with a solution of $NaNO_2$ (0.27 g, 3.91 mmol) in water (6 ml). The mixture was heated under reflux for 1 h, the reaction mixture was cooled, and the solvent was removed *in vacuo.* Chromatography (10 : 1 EtOAc/MeOH) gave **13** as a white solid (0.54 g, 70%). An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp 177—179 °C. $[\alpha]_D^{25}$ +24.61° (c=0.78, MeOH). IR (KBr): 3338, 3138, 2967, 2956, 1662, 1601, 1579, 1505, 1265, 1088 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (3H, d, J=6.03 Hz, CH_3CH<), 0.89 (3H, s, *trans*-2′-CH_3), 1.11 (3H, s, *cis*-2′-CH_3), 1.32 (1H, q, J=10.20 Hz, 4′-HH), 1.61 (1H, dt, J=7.94, 9.78 Hz, 1′-H), 1.72 (1H, dt, J=7.79, 10.01 Hz, 4′-HH), 2.35 (1H, ddd, J=7.81, 10.14, 15.45 Hz, 3′-H), 3.51 (1H, dq, J=5.57, 14.93 Hz, 1-H), 4.15 (1H, d, J=5.16 Hz, D_2O exchang., OH), 4.30 (1H, dd, J=7.47, 13.97 Hz, CHHN), 4.51 (1H, dd, J=8.06, 13.97 Hz, CHHN), 8.23 (1H, s, arom), 12.23 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (DMSO-d_6) δ: 16.46, 21.61, 24.66, 30.94, 39.71, 40.95, 47.44, 49.42, 67.14, 129.76, 148.66, 149.83, 155.73. *Anal.* Calcd for $C_{13}H_{19}N_5O_2$: C, 56.30; H, 6.91; N, 25.25. Found: C, 56.13; H, 7.03; N, 25.34.

(+)-(1*S*,1′*R*)-*cis*-1-[(3′-(7-Amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-2′,2′-dimethylcyclobutyl]ethanol (14) A cooled solution (0 °C) of **3** (0.80 g, 2.82 mmol) in 1 N HCl (8 ml) was treated with a solution of $NaNO_2$ (0.27 g, 3.94 mmol) in water (10 ml). The mixture was stirred at 0 °C for 15 min, treated with concentrated NH_4OH (15 ml), and heated under reflux for 1 h. After cooling, the solvent was removed *in vacuo.* Chromatography (10 : 1 EtOAc/MeOH) gave **14** as a white solid (0.64 g, 82%). An analytical sample was obtained by recrystallization from EtOAc/MeOH, mp 204—205 °C $[\alpha]_D^{25}$ +19.08° (c=0.51, MeOH). IR (KBr): 3324, 3161, 2960, 1697, 1601, 1575, 1263, 725 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, s, *trans*-2′-CH_3), 0.87 (3H, d, J=6.03 Hz, CH_3CH<), 1.12 (3H, s, *cis*-2′-CH_3), 1.31 (1H, q, J=10.04 Hz, 4′-HH), 1.59 (1H, q, J=6.03 Hz, 1′-H), 1.71 (1H, dt, J=7.72, 9.79 Hz, 4′-HH), 2.39 (1H, ddd, J=7.33, 10.51, 15.26 Hz, 3′-H), 3.52 (1H, dq, J=6.03, 15.36 Hz, 1-H), 4.14 (1H, d, J=5.65 Hz, D_2O exchang., OH), 4.31 (1H, dd, J=7.16, 13.76 Hz, CHHN), 4.51 (1H, dd, J=8.10, 13.76 Hz, CHHN), 8.05 (1H, br s, D_2O exchang., NH), 8.28 (1H, s, arom), 8.35 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (DMSO-d_6) δ: 16.48, 21.62, 24.69, 30.94, 39.66, 40.84, 47.04, 49.45, 67.16, 124.05, 148.92, 156.55, 156.93. *Anal.* Calcd for $C_{13}H_{29}N_6O$: C, 56.50; H, 7.30; N, 30.41. Found: C, 56.67; H, 7.38; N, 30.19.

(+)-(1*S*,1′*R*)-*cis*-1-{3′-[2-(7-Amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)ethyl]-2′,2′-dimethylcyclobutyl}ethanol (15) Starting from a solution of **4** (0.35 g, 1.23 mmol) in 1 N HCl (3.20 ml), a solution of $NaNO_2$ (0.11 g, 1.59 mmol) in water (12 ml) and concentrated NH_4OH (7 ml), a procedure analogous to the above, with final chromatography using EtOAc as eluent, afforded compound **15** as a white solid (0.30 g, 88%). An analytical sample was obtained by recrystallization from EtOAc, mp 177—178 °C. $[\alpha]_D^{25}$ +35.86° (c=0.15, MeOH). IR (KBr): 3284, 2957, 1686, 1608, 1574, 1457, 1320 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, d, J=6.11 Hz, CH_3CH<), 0.94 (3H, s, *trans*-2′-CH_3), 0.95 (1H, q, J=10.47 Hz, 4′-HH), 0.98 (3H, s, *cis*-2′-CH_3), 1.53 (1H, dt, J=7.70, 9.97 Hz, 4′-HH), 1.61—1.69 (2H, m, CH_2CH_2N), 1.74 (1H, dt, J=7.07, 15.29 Hz, 3′-H), 1.91 (1H, ddd, J=6.92, 12.90, 13.21 Hz, 1′-H), 3.43 (1H, dq, J=5.11, 15.70 Hz, 1-H), 4.07 (1H, d, J=5.35 Hz, D_2O exchang., OH), 4.39 (2H, t, J=7.09 Hz, CH_2N), 4.07 (1H, br s, D_2O exchang., NH), 8.28 (1H, s, arom), 8.35 (1H, br s, D_2O exchang., NH). ^{13}C-NMR (DMSO-d_6) δ: 16.62, 21.68, 26.17, 29.91, 31.34, 39.04, 39.60, 45.19, 49.90, 67.25, 124.15, 149.03, 156.03, 156.99. *Anal.* Calcd for $C_{14}H_{22}N_6O$: C, 57.91; H, 7.64; N, 28.94. Found: C, 58.07; H, 7.68; N, 28.98.

Acknowledgment The authors thank the Spanish Ministry of Education and Science (DGICYT, PB94-0617) and the Xunta de Galicia (XUGA 20307B94) for financial support of this work.

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., Young M. G., Zahler R., *J. Med. Chem*., **34**, 1415—1421 (1991).
6) Nishiyama Y., Yamamoto N., Yamada Y., Daikoku T., Ichikawa Y., Takahashi K., *J. Antibiot*., **42**, 1854—1859 (1989).
7) Braitman A., Swerdel M. R., Olsen M. S., 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) Ireland C., Leeson P. A., Castañer J., *Drugs of the Future*, **22**, 359—370 (1997).
10) Agrofolio L., Suhas E., Farese A., Condom R., Challand S. R., Earl R. A., Gueldj R., *Tetrahedron*, **50**, 10611—10670 (1994).
11) Klopman G., Tu M., *J. Med. Chem*., **42**, 992—998 (1999).
12) Borges J. E. R., Fernández F., García X., Hergueta A. R., López C., Andrei G., Snoeck R., Witvrounw M., Balzarini J., De Clercq E., *Nucleosides and Nucleotides*, **17**, 1237—1253 (1998).
13) Blanco J. M., Caamaño O., Fernández F., García-Mera X., Hergueta A. R., López C., Rodríguez-Borges J. E., Balzarini J., De Clercq E., *Chem. Pharm. Bull*., **47**, 1314—1317 (1999).
14) Shuto S., Obara T., Toriya M., Hosoya M., Snoeck R., Andrei G., Balzarini J., De Clercq E., *J. Med. Chem*., **35**, 324—331 (1992).
15) *a*) Hergueta A. R., Ph. D. Dissertation, Univ. of Santiago de Compostela (1997); *b*) Hergueta A. R., Fernández F., López C., *Nucleosides and Nucleotides*, (in press).
16) Daluge S., Vince R., *J. Org. Chem*., **43**, 2311—2320 (1978).
17) Patil S. D., Koga M., Schneller S. W., *J. Med. Chem*., **35**, 2191—2195 (1992).
18) 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, France, 1994, pp. 108—125.
19) 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).