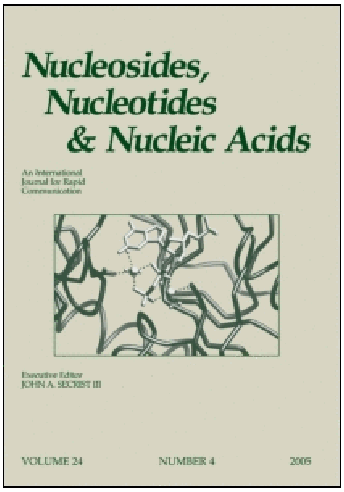

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Novel Carbocyclic Nucleosides Containing A Cyclobutyl Ring. Guanosine and Adenosine Analogues

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To cite this Article Borges, José E. R. , Fernández, Franco , García, Xerardo , Hergueta, Antonio R. , López, Carmen , Andrei, Graciela , Snoeck, Robert , Witvrounw, Myriam , Balzarini, Jan and De Clercq, Erik(1998) 'Novel Carbocyclic Nucleosides Containing A Cyclobutyl Ring. Guanosine and Adenosine Analogues', Nucleosides, Nucleotides and Nucleic Acids, 17: 7, 1237 — 1253

To link to this Article: DOI: 10.1080/07328319808004236

URL: http://dx.doi.org/10.1080/07328319808004236

NUCLEOSIDES & NUCLEOTIDES, 17(7), 1237-1253 (1998)

NOVEL CARBOCYCLIC NUCLEOSIDES CONTAINING A CYCLOBUTYL RING. GUANOSINE AND ADENOSINE ANALOGUES

José E. R. Borges,[1] Franco Fernández,[1] Xerardo García,[1] Antonio R. Hergueta,[1] Carmen López,*[1] Graciela Andrei,[2] Robert Snoeck,[2] Myriam Witvrounw,[2] Jan Balzarini[2] and Erik De Clercq[2]

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Abstract: (1*R*,*cis*)-2-(3-Amino-2,2-dimethylcyclobutyl)ethanol (**4**) was used as a precursor in the synthesis of cyclobutyl nucleoside analogues containing guanine, 8-azaguanine, adenine or 8-azaadenine. All the compounds were evaluated as antiviral agents in a variety of assay systems. Some activity was noted for compound **13, 17, 19** and **20** against vaccinia virus and for compounds **11, 12, 13, 17, 19** and **20** against herpes simplex virus, at concentrations that were up to 10-fold below the cytotoxic concentrations for the host cells.

Nucleoside analogues form a major part of the large number of antiviral agents developed for treatment of AIDS since the identification of its causative agent, the human immunodeficiency virus (HIV).[1,2] Typical examples are the natural antibiotic derived from oxetane, oxetanocin A (**1**), which is active against the HIV *in vitro*,[3] and its carbocyclic analogues, cyclobut-A (**2**) and cyclobut-G (**3**),[4,5] which are not only active agains HIV but also against herpes simplex virus (HSV-1, HSV-2) and cytomegalovirus.[4,6,7,8]

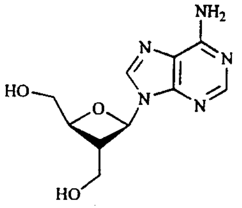
We have now synthesized a variety of cyclobutyl analogues of nucleosides related to **2** and **3**, starting from amino alcohol **4**.[9] These analogues differ from cyclobut-G in that they bear a *gem*-dimethyl group instead of a hydroxymethyl group at C2, and have an extra methylene between the carbocycle and the hydroxymethyl group at C1.

These structural modifications allow evaluation of the effects of increased lipophilicity and molecular flexibility on the pharmacological activity of cyclobut-G.

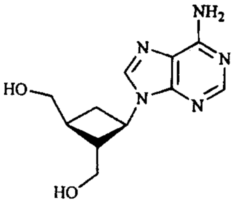
The analogues were prepared by construction of a guanine, 8-azaguanine, adenine or 8-azaadenine on the amino group of amino alcohol **4**, using the classical approach to carbocyclic nucleosides of nucleosides.[10-14] The first step in the preparation of the guanosine and 8-azaguanosine analogues (Scheme 1) was condensation of **4** with 2-amino-4,6-dichloropyrimidine, which afforded the amine **5**. Then, amine **5** was coupled with 4-chlorobenzenediazonium chloride to afford 5-(4-chlorophenylazo)pyrimidine **6**, which was reduced to triaminopyrimidine **7** with zinc in acetic acid. Cyclization of **7** with triethyl orthoformate gave 2-amino-6-chloropurine **8**, which was converted into the guanosine analogue **9** by refluxing it in sodium hydroxide or to 2,6-diaminopurine analogue **10** by heating it with liquid ammonia under pressure. To obtain the 8-azaguanine carbonucleosides, triaminopyrimidine **7** was diazotized with sodium nitrite in hydrochloric acid, and then the cyclized product **11** was converted to **12** by treating it with sodium hydroxide, or to **13** by treating it with liquid ammonia.

Preparation of the adenine and 8-azaadenine carbonucleosides (Scheme 2) began with condensation of **4** with 5-amino-4,6-dichloropyrimidine, which afforded diamine **14**. Cyclization of this diamine with triethyl orthoformate gave 6-chloropurine **15**, which was converted to inosine analogue **16** by refluxing it in sodium hydroxide, or to adenosine analogue **17** by heating it with liquid ammonia under pressure. The 8-azaadenine carbonucleosides were prepared by diazotizing **14** with sodium nitrite in hydrochloric acid, which affords the highly unstable cyclization product 6-chloro-8-azapurine **18** (not isolated). This was converted into the 8-azainosine analogue **19** by simply heating the crude reaction mixture, and into the 8-azaadenine analogue **20** upon treatment with concentrated aqueous ammonia followed by 5 min under reflux conditions.

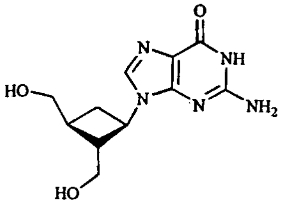
The activities of compounds **9** - **13** and **16**, **17**, **19** and **20** against a variety of DNA and RNA viruses, and their cytotoxicities against several types of host cells, were evaluated and compared with the corresponding data for reference compounds with known antiviral activities. Most of the new analogues showed no antiviral activity at concentration up to 400 μg/mL (Tables 1-3). Compounds **13**, **17**, **19** and **20** showed activity against vaccinia virus, and compounds **11**, **12**, **13**, **17**, **19** and **20** showed


NH2
HO
HO

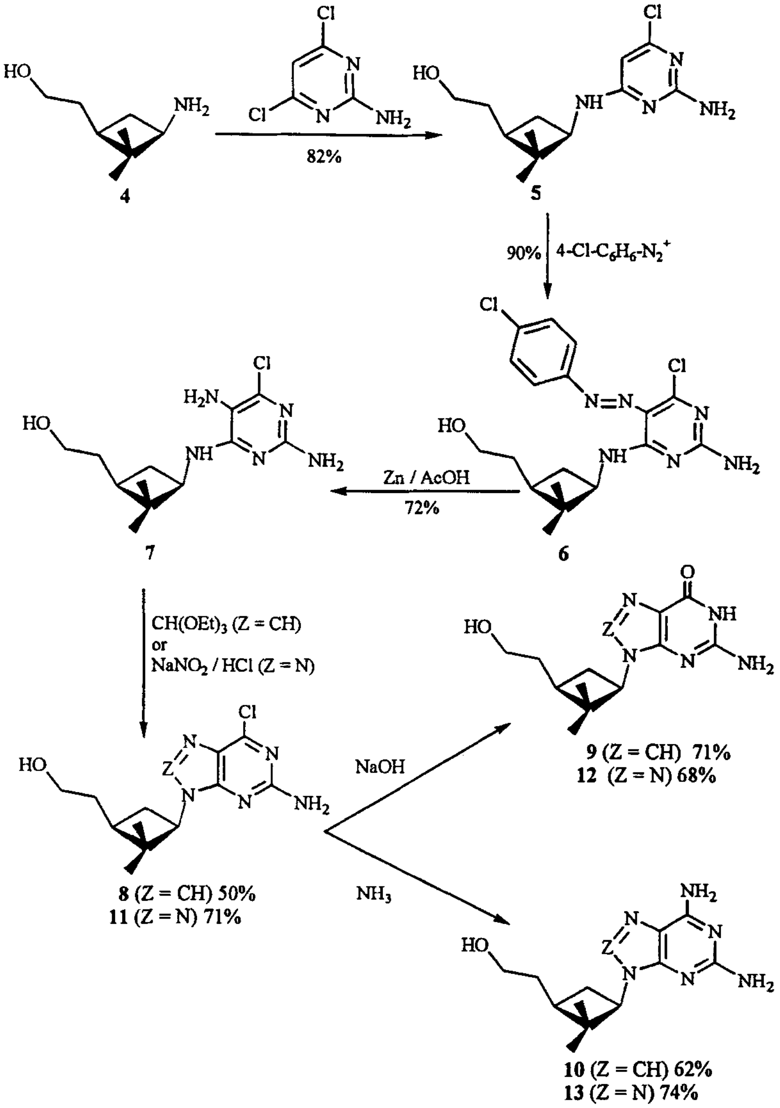
1


NH2
HO
HO

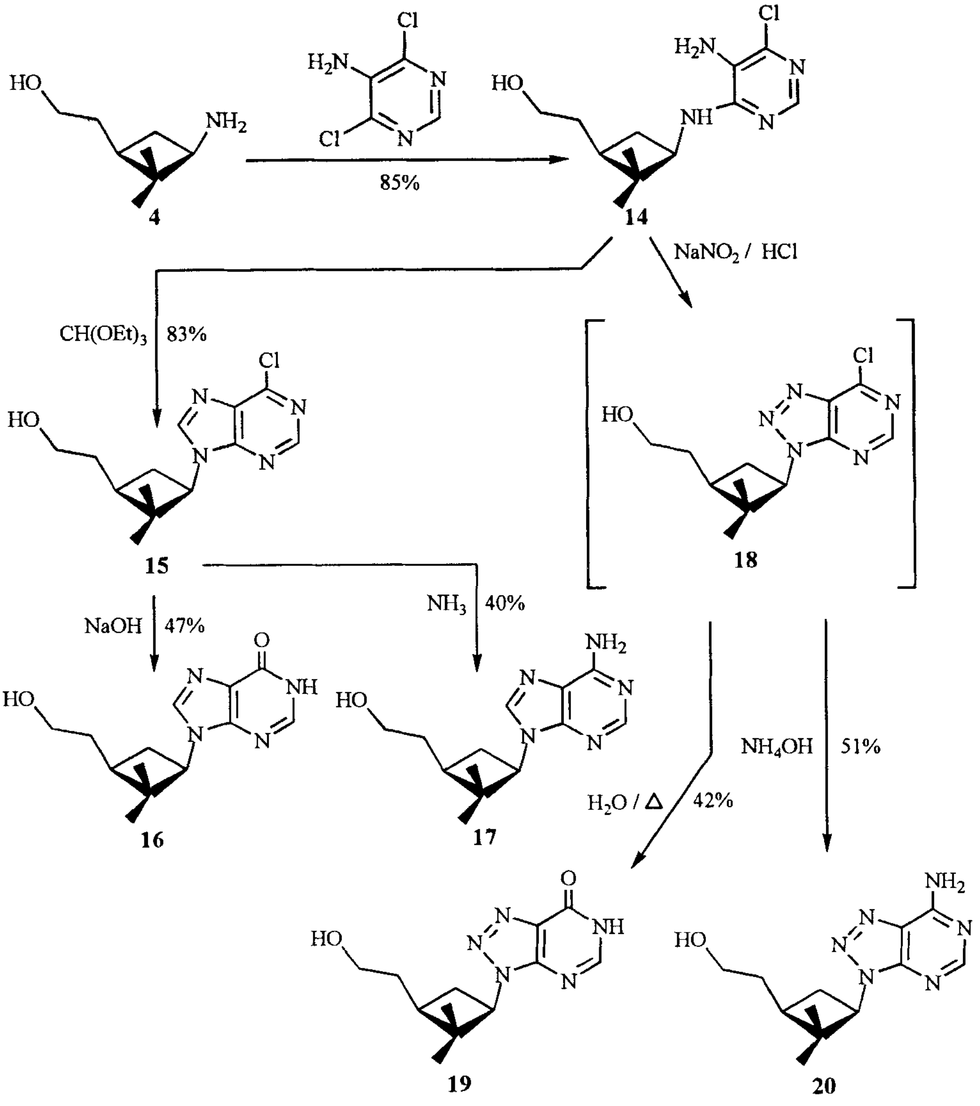
2


O
NH
NH2
HO
HO

3


4
82%
5
90%
4-Cl-C_6H_6-N_2^+
6
Zn / AcOH
72%
7
CH(OEt)3 (Z = CH)
or
NaNO2 / HCl (Z = N)
8 (Z = CH) 50%
11 (Z = N) 71%
NaOH
NH3
9 (Z = CH) 71%
12 (Z = N) 68%
10 (Z = CH) 62%
13 (Z = N) 74%

Scheme 1



Scheme 2

activity against herpes simplex virus type 1 and type 2 (including thymidine kinase-deficient HSV-1) at concentrations of 20 μg/mL (or higher), that is, at concentrations that were 10-fold (or less) lower than the cytotoxic concentrations.

Given the lack of activity of these nucleoside analogues against HIV-1 and HIV-2 at the concentrations studied (Table 1), it would appear that replacement of one of the

TABLE 1. Anti-HIV-1 activity and cytotoxicity of test compounds in human MT-4 and CEM cell cultures

Compound	EC_{50}[a](mg/mL)				CC_{50}[b] (mg/mL)
	HIV-1		HIV-2		
	MT-4	CEM	MT-4	CEM	MT-4
9	>250	>100	>250	>100	>250
10	>250	>20	>250	>20	≥250
11	>200	>4	>200	>4	190
12	>250	>100	>250	>100	≥250
13	>100	>20	>100	>20	180
16	>100	>100	>100	>100	108
17	>250	>20	>250	>20	≥250
19	>100	>100	>100	>20	112
20	>100	>100	>100	>20	83

[a] EC_{50} or 50% effective concentrations, required to inhibit virus-induced cytopathicity in MT-4 and CEM cells.

[b] CC_{50} or 50% cytotoxic concentration, required to reduced MT-4 cell viability by 50%.

hydroxymethyl groups of cyclobut-G with a *gem*-dimethyl group, and/or the addition of an extra methylene between the carbocycle and the other hydroxymethyl group, interferes with the anti-HIV activity of this drug. This interference may occur at the level of the activating (phosphorylating) enzymes and/or the HIV reverse transcriptase.

EXPERIMENTAL PART

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. ^1H and ^{13}C NMR spectra were recorded in a Bruker AMX-300 spectrometer, at 300 and 75 MHz,

TABLE 2. Antiviral Activity (EC$_{50}$)* and Cytotoxicity (MCC)** of Compounds **9**, **10**, **11**, **12**, **13**, **16**, **17**, **19** and **20** Against DNA and RNA Viruses.

VIRUS (STRAIN)	CELL	9	10	11	12	13	16	17	19	20	BVD[a]	RBV[b]	ACV[c]	GCV[d]
HSV-1 (KOS)	E$_6$SM	300	>400	>100	300	>400	>400	>400	>200	>400	0.004	>100	0.004	0.0004
HSV-2 (G)	E$_6$SM	>400	>400	>100	300	150	>400	100	70	100	>100	>100	0.004	0.0004
HSV-1 TK$^-$(B2006)	E$_6$SM	300	>400	20	40	70	>400	70	40	70	4	40	0.07	0.004
HSV-1 TK$^-$(VMW1837)	E$_6$SM	>400	>400	>100	300	100	>400	100	70	150	4	>100	0.1	0.2
Vaccinia	E$_6$SM	>400	200	>100	200	20	>400	70	70	70	2	20	200	>100
Vesicular stomatitis	E$_6$SM	>400	>400	>100	>400	>400	>400	>400	>200	>400	>400	20	>400	>100
<u>Cytotoxicity</u>	E$_6$SM	>400	>400	200	>400	>400	>400	>400	400	>400	200	≥200	>400	>100
													DHPA[e]	**C-c^{3}Ado[f]**
Parainfluenza-3	Vero	>400	>400	>200	>400	300	>400	>400	100	>100	>400	70	40	2
Reovirus-1	Vero	>400	>400	>200	>400	200	>400	>400	150	>100	>400	70	100	2
Sindbis	Vero	>400	>400	>200	>400	>400	>400	>400	>100	>100	>400	200	>400	>400
Coxsackie B4	Vero	>400	>400	>200	>400	>400	>400	>400	>100	>100	>400	>400	>400	>400
Punta Toro	Vero	>400	>400	>200	>400	>400	>400	>400	>100	>100	>400	70	>400	>400
<u>Cytotoxicity</u>	Vero	>400	>400	400	>400	>400	400	≥400	≥200	200	>400	>400	>400	>400
Vesicular stomatitis	HeLa	>400	>400	200	>400	>400	>400	300	>200	>400	>400	20	70	4
Coxsackie B4	HeLa	>400	100	>200	200	200	>400	150	>200	>400	>400	70	>400	>400
Respiratory Syncytial	HeLa	>400	>400	>200	>400	>400	70	70	>200	>400	>400	1	>400	150
<u>Cytotoxicity</u>	HeLa	>400	>400	400	>400	>400	>400	>400	≥400	>400	>400	≥400	>400	>400

* 50% Effective concentration (mg/mL), or concentration required to reduce virus-induced cytopathicity by 50%.

** Minimum cytotoxic concentration (mg/mL), or concentration required to cause a microscopically detectable alteration of normal cell morphology. [a] Brivudin.

[b] Ribavirin. [c] Acyclovir. [d] Ganciclovir. [e] *S*)-9-(2,3-Dihydroxypropyl)adenine. [f] Carbocyclic 3-deazaadenosine.

TABLE 3. Antiviral Activity (EC_{50})* and Cytotoxicity (CC_{50})** of Compounds **9**, **10**, **11**, **12**, **13**, **16**, **17**, **19** and **20** Against Several Strains of Varicella-Zoster Virus (VZV) and Cytomegalovirus (CMV) in Human Embryonic Lung (HEL) Cells.

VIRUS (STRAIN)	9	10	11	12	13	16	17	19	20	ACV[a]	BVD[b]
VZV											
TK^+ VZV (OKA strain)	>50	>50	>20	>50	20	>50	>20	>20	>20	0.2	0.0007
TK^+ VZV (YS strain)	>50	>50	>20	>50	>20	>50	>20	>20	>20	0.4	0.0001
TK^- VZV (07/1 strain)	>50	50	>20	>50	>20	>50	>20	>20	>20	14	-
TK^- VZV (YS/R strain)	>50	>50	>20	>50	>20	>50	>20	>20	>20	13	50
Cytotoxicity	>50	>50	>50	>50	30	>50	40	37	34	>200	>200
CMV										**GCV[c]**	**CDV[d]**
AD-169 strain	>50	>50	>50	>50	>20	>50	20	>20	>20	0.4	0.1
Davis strain	>50	>50	>50	>50	>20	>50	>20	>20	>20	0.31	0.2
Cytotoxicity	>50	>50	>50	>50	30	>50	40	37	34	>50	>50

* 50% Effective concentration (mg/mL), or concentration required to reduce virus-induced plaque formation by 50%. Virus Imput was 20 plaque-forming units (PFU) for VZV and 100 PFU for CMV.

** 50% Cytotoxic concentration (mg/mL), or concentration required to reduce cell growth by 50%.

[a] Acyclovir. [b] Brivudin. [c] Ganciclovir. [d] Cidofovir. TK^+, thymidine kinase-competent. TK^-, thymidine kinase-deficient.

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*R*,3*R*)-2-[3-(2-Amino-6-chloropyrimidin-4-ylamino)-2,2-dimethylcyclobutyl]ethanol (5). A solution of **4** (4.9 g, 34.0 mmol), 2-amino-4,6-dichloropyrimidine (8.0 g, 48.7 mmol) and triethylamine (20 mL) in dry 1-butanol (73 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, (eluant 1:1 CH_2Cl_2/MeOH), and then redissolved in dry acetone, and the solution was filtered and the solvent was eliminated *in vacuo* to afford compound **5** as a colorless foam (7.5 g, 82%). $[\alpha]_D^{25}$ +111.19 (*c* 1.135, MeOH). IR (KBr), ν (cm^{-1}): 3749, 3313, 1576, 1457, 1354, 1179, 907. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.90 (3H, s, *t*-2-CH_3), 1.17 (3H, s, *c*-2-CH_3), 1.40 (1H, q, J = 10.20 Hz, 4-$\underline{\mathrm{H}}$H), 1.51 (1H, dt, J_d = 8.72 Hz, J_t = 6.68 Hz, 4-H$\underline{\mathrm{H}}$), 1.66 (1H, sex, J = 6.67 Hz, C$\underline{\mathrm{H}}$HCH$_2$O), 1.82 (1H, q, J = 8.34 Hz, CH$\underline{\mathrm{H}}$CH$_2$O), 2.42 (1H, dt, J_d = 7.71 Hz, J_t = 3.02 Hz, 1-H), 3.58 (3H, t, J = 6.73 Hz, CH_2O + 3-H), 4.97 (3H, bs, D_2O exch., NH_2 + NH), 5.76 (1H, s, arom). ^{13}C NMR ($CDCl_3$), δ (ppm): 16.53, 29.64, 30.09, 32.33, 33.53, 36.29, 44.23, 52.79, 61.70, 92.83, 162.70, 164.28. HRMS *m/z*: Calcd for $C_{12}H_{19}ClN_4O$: 270.1248. Found: 270.1239.

(-)-(1*R*,3*R*)-2-{3-[(2-Amino-6-chloro-5-(4-chlorophenylazo)pyrimidin-4-yl-amino]-2,2-dimethylcyclobutyl}ethanol (6). 4-Chloroaniline (3.70 g, 30.0 mmol) in 3N HCl (25 mL) was treated at 0°C with $NaNO_2$ (2.10 g, 30.0 mmol) in water (25 mL). The diazonium salt obtained was added to a mixture of **5** (7.05 g, 26.0 mmol), $NaOAc{\cdot}3H_2O$ (48.0 g), acetic acid (120 mL) and water (120 mL) and stirred overnight at room temperature. The precipitate was filtered out, washed with water until the washings were neutral, and dried to afford **6** (9.58 g, 90%) as a yellow solid. An analytical sample was obtained by double recrystallization from acetone. M.p. 243-245°C. $[\alpha]_D^{25}$ -12.66 (*c* 0.15, MeOH). IR (KBr), ν (cm^{-1}): 3750, 3310, 1734, 1699, 1458. ^{1}H NMR ($CDCl_3$), δ (ppm): 1.03 (3H, s, *t*-2-CH_3), 1.19 (3H, s, *c*-2-CH_3), 1.52 (1H, q, J = 10.19 Hz, 4-$\underline{\mathrm{H}}$H), 1.58 (1H, dt, J_d = 8.31 Hz, J_t = 6.69 Hz, 4-H$\underline{\mathrm{H}}$), 1.72 (1H, q, J = 6.72 Hz, C$\underline{\mathrm{H}}$HCH$_2$O), 1.88-1.91 (1H, m, CH$\underline{\mathrm{H}}$CH$_2$O), 2.49 (1H, dt, J_d = 10.64 Hz, J_t = 7.62 Hz, 1-H), 3.63 (2H, t, J = 6.72 Hz, CH_2O), 4.32 (1H, dt, J_d = 9.56

Hz, J_t = 7.82 Hz, 3-H), 5.25 (2H, bs, D_2O exch., NH_2), 7.43 (2H, dt, J_d = 8.74 Hz, J_t = 2.70 Hz, arom (3-H + 5-H)), 7.70 (2H, dt, J_d = 8.74 Hz, J_t = 2.70 Hz, arom (2-H + 6-H)), 10.41 (1H, d, J = 7.61 Hz, D_2O exch., NH). ^{13}C NMR ($CDCl_3$), δ (ppm): 17.37, 29.39, 30.09, 32.71, 33.68, 36.84, 44.05, 51.86, 61.94, 123.39, 129.80, 135.60, 151.38, 155.34, 161.13. Anal. Calcd. for $C_{18}H_{22}Cl_2N_6O$: C, 52.82; H, 5.42; N, 20.53. Found: C, 52.64, H, 5.39; N, 20.37.

(+)-(1*R*,3*R*)-2-[3-(2,5-Diamino-6-chloropyrimidin-4-ylamino)-2,2-dimethylcyclobutyl]ethanol (7). A mixture of **6** (9 g, 22.05 mmol), Zn powder (12.27 g, 184.3 mmol), acetic acid (6.50 mL), water (140 mL) and ethanol (140 mL) was refluxed under argon for 3 h. Then, the reaction mixture was filtered, the solvent was removed *in vacuo* and the residue was purified by FC (eluant EtOAc). Compound 7 (4.53 g, 72 %) was isolated as a reddish foam. $[\alpha]_D^{25}$ +100.58 (*c* 0.51, MeOH). IR (KBr), ν(cm^{-1}): 2947, 2602, 1560, 1036, 851. ^1H NMR ($CDCl_3$), δ (ppm): 0.90 (3H, s, *t*-2-CH_3), 1.17 (3H, s, *c*-2-CH_3), 1.43 (1H, q, J = 10.29 Hz, 4-$\underline{H}$H), 1.51 (1H, dt, J_d = 6.73 Hz, J_t = 1.97 Hz, 4-H$\underline{H}$), 1.67 (1H, quint, J = 6.66 Hz, C$\underline{H}$HCH$_2$O), 1.79-1.85 (1H, m, CH$\underline{H}$CH$_2$O), 2.40 (1H, dt, J_d = 10.65 Hz, J_t = 7.66 Hz, 1-H), 2.71 (2H, bs, D_2O exch., NH_2), 3.59 (2H, t, J = 6.83 Hz, CH_2O), 4.09 (1H, dt, J_d = 9.82 Hz, J_t = 8.02 Hz, 3-H), 4.67 (3H, bs, D_2O exch., NH_2 + OH), 5.47 (1H, d, J = 7.61 Hz, D_2O exch., NH). ^{13}C NMR ($CDCl_3$), δ (ppm): 16.47, 29.56, 32.19, 33.73, 36.45, 44.19, 52.03, 61.76, 111.57, 148.43, 158.18, 159.24. HRMS *m/z*: Calcd for $C_{12}H_{20}ClN_5O$: 285.1357. Found: 285.1363.

(1*R*,3*R*)-2-[3-(2-Amino-6-chloro-9*H*-purin-9-yl)-2,2-dimethylcyclobutyl]-ethanol (8). A mixture of 7 (2.70 g, 9.50 mmol), triethyl orthoformate (52 mL) and 12N HCl (2.5 mL) under argon was stirred overnight at room temperature. The mixture was concentrated to dryness *in vacuo*, and 0.5N HCl was added to the residue and stirred for 1 hour. Then the reaction mixture was adjusted to pH 8 with 1N NaOH, and the solvent was evaporated *in vacuo*. The crude product was purified by FC (eluant 10:1 CH_2Cl_2/MeOH). Compound **8** (1.42 g, 50%) was isolated as a yellow solid. HRMS *m/z*: Calcd for $C_{13}H_{18}ClN_5O$: 295.1200. Found: 295.1204.

(+)-(1*R*,3*R*)-2-Amino-6,9-dihydro-9-[3-(2-hydroxyethyl)-2,2-dimethylcyclobutyl]-1*H*-purin-6-one (9). A mixture of **8** (0.50 g, 1.70 mmol) and 0.33N NaOH (30 mL) was refluxed for 6 h, whereupon the solvent was removed *in vacuo*. The residue was purified by FC (eluant 5:1 CH_2Cl_2/MeOH) to afford compound **9** (0.33 g, 71 %) as a white solid. An analytical sample was obtained by recrystallization of the crude product from ethanol. M.p. 307-309°C. $[\alpha]_D^{25}$ +108.40 (*c* 0.25, MeOH). IR (KBr), ν (cm^{-1}): 3275, 2950, 1703, 1599, 1528, 1047, 784. ^1H NMR (DMSO-d_6), δ (ppm): 0.66 (3H, s, *t*-2-CH_3), 1.17 (3H, s, *c*-2-CH_3), 1.41-1.46 (1H, m, 4-$\underline{H}$H), 1.55 (1H, dt, J_d = 13.41 Hz, J_t = 6.69 Hz, 4-H$\underline{H}$), 1.86 (1H, q, J = 8.14 Hz, 3-H), 2.31 (2H, t, J = 9.09 Hz, $C\underline{H}_2CH_2O$), 3.33 (2H, m (t, after D_2O exch., J = 6.73 Hz), CH_2O), 4.27 (1H, dd, J = 10.00 Hz, J = 8.20 Hz, 1-H), 4.41 (1H, t, J = 5.00 Hz, D_2O exch., OH), 6.32 (2H, bs, D_2O exch., NH_2), 7.80 (1H, s, arom), 10.50 (1H, bs, D_2O exch., NH). ^{13}C NMR ($CDCl_3$), δ (ppm): 16.34, 28.05, 29.28, 33.33, 35.99, 44.28, 54.12, 59.64, 117.18, 136.73, 151.78, 153.71, 157.21. Anal. Calcd. for $C_{13}H_{19}N_5O_2$: C, 56.30; H, 6.91; N, 25.25. Found: C, 56.44, H, 6.79; N, 25.07.

(+)-(1*R*,3*R*)-2-[3-(2,6-Diamino-9*H*-purin-9-yl)-2,2-dimethylcyclobutyl]ethanol (10). Compound **8** (0.50 g, 1.70 mmol) was suspended in methanol (17 mL) in a high pressure reactor equipped with a solid CO_2/acetone cooling bath. Liquid ammonia (20 mL) was added and the vessel was closed and heated at 75°C for 48 h. Then, the mixture was concentrated to dryness *in vacuo* and the residue recrystallized from EtOAc/MeOH. M.p. 236-238°C. $[\alpha]_D^{25}$ +53.4 (*c* 0.50, MeOH). IR (KBr), ν (cm^{-1}): 3493, 3138, 1669, 1596, 1287, 1049. ^1H NMR (DMSO-d_6), δ (ppm): 0.68 (3H, s, *t*-2-CH_3), 1.19 (3H, s, *c*-2-CH_3), 1.43 (1H, dt, J_d = 13.96 Hz, J_t = 6.54 Hz, 4-$\underline{H}$H), 1.56 Hz, 4-$\underline{H}$H), 1.56 (1H, dt, J_d = 13.53 Hz, J_t = 6.54 Hz, 4-H$\underline{H}$), 1.90 (1H, q, J = 8.20 Hz, 1-H), 2.35 (2H, dt, J_d = 7.99 Hz, J_t = 7.77 Hz, $C\underline{H}_2CH_2O$), 3.33 (2H, m (t, after D_2O exch., J = 6.02 Hz), CH_2O), 4.33 (1H, t, J = 9.18 Hz, 3-H), 4.45 (1H, bs, D_2O exch., OH), 6.68 (2H, bs, D_2O exch., NH_2), 7.83 (2H, bs, D_2O exch., NH_2), 8.04 (1H, s, arom). ^{13}C NMR (DMSO-d_6), δ (ppm): 15.97, 27.63, 28.87, 32.91, 35.61, 43.96, 53.88, 59.21, 112.09, 138.52, 151.76, 152.58, 155.72. Anal. Calcd. for $C_{13}H_{20}N_6O$: C, 56.50; H, 7.30; N, 30.41. Found: C, 56.64, H, 7.09; N, 30.47.

(+)-(1*R*,3*R*)-2-[3-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2,2-dimethylcyclobuthyl]ethanol(11). Sodium nitrite (0.51 g, 7.43 mmol) in water (16 mL) was added to a cooled (0°C) solution of 7 (1.80 g, 6.30 mmol) in acetic acid (10 mL) and water (10 mL), and stirred for 3 h. After working-up, an oil (2 g) was obtained, which was purified by FC (eluant 7:1 CH_2Cl_2/MeOH), to afford compound **11** as a white solid (1.32 g, 71%). An analytical sample was obtained by recrystallization of the crude from Hex/Et_2O. M.p. 70-71°C. $[\alpha]_D^{25}$ +100.40 (*c* 0.25, MeOH). IR (KBr), ν (cm^{-1}): 3324, 2954, 1639, 1565, 1388, 1251. ^1H NMR (DMSO-d_6), δ (ppm): 0.66 (3H, s, *t*-2-CH_3), 1.23 (3H, s, *c*-2-CH_3), 1.48 (1H, dt, J_d = 13.55 Hz, J_t = 8.01 Hz, 4-$\underline{H}$H), 1.58 (1H, q, J = 6.73 Hz, 4-H$\underline{H}$), 2.02 (1H, q, J = 7.86 Hz, 1-H), 2.40 (1H, dt, J_d = 10.84 Hz, J_t = 7.90 Hz, C$\underline{H}$HCH_2O), 2.73 (1H, q, J = 7.90 Hz, CH$\underline{H}$$CH_2O$), 3.38 (2H, dt, J_d = 6.65 Hz, J_t = 5.45 Hz, CH_2O), 4.43 (1H, t, J = 5.06 Hz, D_2O exch., OH), 4.68 (1H, dd, J = 9.84 Hz, J = 8.08 Hz, 3-H), 7.61 (2H, bs, D_2O exch., NH_2). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.64, 27.55, 29.28, 33.52, 36.20, 44.75, 57.06, 59.61, 124.75, 151.81, 155.74, 156.05. Anal. Calcd. for $C_{12}H_{17}ClN_6O$: C, 48.57; H, 5.77; N, 28.32. Found: C, 48.64, H, 5.99; N, 28.46.

(+)-(1*R*,3*R*)-5-Amino-6,7-dihydro-3-[3-(2-hydroxyethyl)-2,2-dimethylcyclobutyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (12). A mixture of **11** (0.50 g, 1.68 mmol) and 0.25N NaOH (21 mL) was refluxed for 5 h, whereafter the reaction mixture was cooled and adjusted to pH 3 with 6N HCl. The precipitated solid was filtered out, washed with cold water until the washings were neutral, and dried in vacuo over phosphorus pentoxide to yield **12** as an off-white solid (0.31 g, 68%). An analytical sample was obtained by recrystallization of the crude product from water. M.p. 280-281°C. $[\alpha]_D^{25}$ +66.20 (*c* 0.50, MeOH). IR (KBr), ν (cm^{-1}): 3291, 2943, 1718, 1636, 1602, 784. ^1H NMR (DMSO-d_6), δ (ppm): 0.65 (3H, s, *t*-2-CH_3), 1.20 (3H, s, *c*-2-CH_3), 1.44 (1H, dt, J_d = 13.84 Hz, J_t = 6.50 Hz, 4-$\underline{H}$H), 1.58 (1H, dt, J_d = 13.84 Hz, J_t = 6.81 Hz, 4-H$\underline{H}$), 1.98 (1H, sex, J = 6.62 Hz, 3-H), 2.41 (1H, dt, J_d = 10.82 Hz, J_t = 7.91 Hz, C$\underline{H}$HCH_2O), 2.66 (1H, q, J = 7.87 Hz, CH$\underline{H}$$CH_2O$), 3.35 (2H, m (t, after D_2O exch., J = 6.55 Hz), CH_2O), 4.41 (1H, t, J = 5.03 Hz, D_2O exch., OH), 4.53 (1H, dd, J = 9.81 Hz, J = 8.11 Hz, 1-H), 6.83 (2H, bs, D_2O exch., NH_2), 10.88 (1H, s, D_2O exch., NH). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.65, 27.55, 29.28, 33.52, 36.21, 44.76, 57.07, 59.61, 124.75, 151.86, 155.63, 156.13. Anal. Calcd. for $C_{12}H_{18}N_6O_2$: C, 51.79; H, 6.52; N, 30.20. Found: C, 51.61, H, 6.59; N, 30.06.

(+)-(1*R*,3*R*)-2-{3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)-2,2-dimethylcyclobutyl}ethanol (13). Compound **11** (0.50 g, 1.68 mmol) was suspended in methanol (10 mL) in a high pressure reactor equipped with a CO_2/acetone cooling bath. Liquid ammonia (20 mL) was added and the vessel was closed and heated at 75°C for 48 h. Then, the mixture was concentrated to dryness *in vacuo* to afford compound **13** (0.48 g) as a dark solid, which gave a white solid (0.34 g, 74%) after recrystallization from MeOH. M.p. 265-266°C. $[\alpha]_D^{25}$ +38.20 (*c* 0.25, MeOH). IR (KBr), ν (cm^{-1}): 3493, 3288, 1625, 1490, 1103, 1057. ^1H NMR (DMSO-d_6), δ (ppm): 0.65 (3H, s, *t*-2-CH_3), 1.21 (3H, s, *c*-2-CH_3), 1.45 (1H, dt, J_d = 14.04 Hz, J_t = 6.53 Hz, 4-$\underline{H}$H), 1.59 (1H, dt, J_d = 13.48 Hz, J_t = 6.80 Hz, 4-H$\underline{H}$), 1.98 (1H, q, J = 8.27 Hz, 1-H), 2.41 (1H, dt, J_d = 10.78 Hz, J_t = 7.90 Hz, C$\underline{H}$HCH_2O), 2.73 (1H, q, J = 10.51 Hz, CH$\underline{H}$$CH_2O$), 3.36 (2H, t, J = 6.78 Hz, CH_2O), 4.41 (1H, t, J = 5.05 Hz, D_2O exch., OH), 4.55 (1H, dd, J = 9.83 Hz, J = 8.16 Hz, 3-H), 6.30 (2H, bs, D_2O exch., NH_2), 7.44 (2H, bs, D_2O exch., NH_2). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.74, 27.55, 29.39, 33.57, 36.24, 44.80, 56.87, 59.66, 120.80, 152.30, 156.50, 163.07. Anal. Calcd. for $C_{12}H_{19}N_7O$: C, 51.97; H, 6.91; N, 35.35. Found: C, 51.71, H, 7.02; N, 35.16.

(+)-(1*R*,3*R*)-2-[3-(5-Amino-6-chloropyrimidin-4-ylamino)-2,2-dimethylcyclobutyl]ethanol (14). A suspension of **4** (1.38 g, 9.70 mmol) and 5-amino-4,6-dichloropyrimidine (2.00 g, 12.2 mmol) in dry triethylamine (11 mL) and 1-butanol (55 mL) was heated under reflux conditions for 48 h. Then, the reaction mixture was cooled, the solvent was removed *in vacuo* and the resulting gum (4.70 g) was purified by FC (eluant 25:1 CH_2Cl_2/MeOH). Aminochloropyridine **14** (2.18 g, 85%) was isolated as a dark solid. An analytical sample was obtained by recrystallization of the crude product from Hex/CH_2Cl_2. M.p. 158-159°C. $[\alpha]_D^{25}$ +46.26 (*c* 0.50, MeOH). IR (KBr), ν (cm^{-1}): 3365, 2941, 1663, 1578, 1420. ^1H NMR (DMSO-d_6), δ (ppm): 0.77 (3H, s, *t*-2-CH_3), 1.13 (3H, s, *c*-2-CH_3), 1.36 (1H, q, J = 6.70 Hz, 4-$\underline{H}$H), 1.50 (1H, quint, J = 6.73 Hz, 4-H$\underline{H}$), 1.58 (1H, q, J = 10.11 Hz, C$\underline{H}$HCH_2O), 1.68-1.76 (1H, m, CH$\underline{H}$$CH_2O$), 2.22 (1H, dt, J_d = 10.28 Hz, J_t = 7.69 Hz, 1-H), 3.29-3.33 (2H, m,

CH_2O), 4.12 (1H, dt, J_d = 9.27 Hz, J_t = 7.73 Hz, 3-H), 4.37 (1H, t, J = 5.06 Hz, D_2O exch., OH), 5.09 (2H, s, D_2O exch., NH_2), 6.54 (1H, d, J = 2.36 Hz, D_2O exch., NH), 7.66 (1H, s, arom). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.56, 29.96, 30.19, 33.91, 35.95, 43.92, 52.11, 59.79, 123.45, 137.07, 145.81, 151.92. Anal. Calcd. for $C_{12}H_{19}ClN_4O$: C, 53.23; H, 7.07; N, 20.69. Found: C, 53.31, H, 7.01; N, 20.96.

(+)-(1*R*,3*R*)-2-[3-(6-Chloro-9*H*-purin-9-yl)-2,2-dimethylcyclobutyl]ethanol (15). A mixture of **14** (1.85 g, 6.80 mmol), triethylorthoformate (40 mL) and 12N HCl (1.8 mL) was stirred at room temperature for 36 h. Then, the solvent was removed *in vacuo,* and the solid residue (2.40 g) was purified by FC (eluant 50:1 CH_2Cl_2/MeOH), affording chloropurine **15** (1.58 g, 83%) as a yellow oil. $[\alpha]_D^{25}$ +37.76 (*c* 0.76, MeOH). IR (film), ν (cm^{-1}): 3385, 3103, 2956, 1718, 1654, 1590, 1489, 1396, 1026. ^1H NMR (DMSO-d_6), δ (ppm): 0.62 (3H, s, *t*-2-CH_3), 1.20 (3H, s, *c*-2-CH_3), 1.43 (1H, dt, J_d = 12.28 Hz, J_t = 6.96 Hz, 4-$\underline{H}$H), 1.57 (1H, quint, J = 6.72 Hz, 4-H$\underline{H}$), 1.98 (1H, quint, J = 7.72 Hz, 1-H), 2.38-2.64 (2H, m, $C\underline{H}_2CH_2O$), 3.37 (2H, dq, J_d = 11.02 Hz, J_q = 6.45 Hz, CH_2O), 4.13 (1H, bs, D_2O exch., OH), 4.64 (1H, dd, J = 10.12 Hz, J = 8.12 Hz, 3-H), 8.72 and 8.81 (2H, 2s, arom). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.18, 27.80, 28.89, 33.20, 35.91, 44.47, 55.41, 59.59, 131.42, 146.72, 149.34, 151.79, 152.53. HRMS *m/z*: Calcd for $C_{13}H_{17}ClN_4O$: 280.1091. Found: 280.1086.

(+)-(1*R*,3*R*)--6,9-Dihydro-9-[3-(2-hydroxyethyl)-2,2-dimethylcyclobutyl]-1*H*-purin-6-one (16). A mixture of **15** (0.60 g, 2.14 mmol) and 0.25N NaOH (25 mL) was heated under reflux conditions for 5 h, whereafter the reaction mixture was cooled and the solvent was removed *in vacuo.* The solid residue (0.64 g) was purified by FC (eluant 4:1 EtOAc/MeOH), affording purinone **16** (0.26 g; 47%) as a white solid, which was recrystallized from EtOAc/MeOH. M.p. 238-240°C. $[\alpha]_D^{25}$ +59.96 (*c* 0.245, MeOH). IR (KBr), ν (cm^{-1}): 3416, 2953, 1683, 1474, 1415, 1050. ^1H NMR (DMSO-d_6), δ (ppm): 0.63 (3H, s, *t*-2-CH_3), 1.18 (3H, s, *c*-2-CH_3), 1.44 (1H, dt, J_d = 13.75 Hz, J_t = 6.65 Hz, 4-$\underline{H}$H), 1.57 (1H, dt, J_d = 13.75 Hz, J_t = 6.80 Hz, 4-H$\underline{H}$), 1.88-1.99 (1H, m, 3-H), 2.32-2.46 (2H, m, $C\underline{H}_2CH_2O$), 3.30-3.42 (2H, m, $C\underline{H}_2O$), 4.49 (2H, dd, J = 10.12 Hz, J = 8.21 Hz, 1-H + OH (D_2O exch.)), 8.00 and 8.19 (2H, 2s, arom), 12.35

(1H, bs, D_2O exch., NH). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.22, 27.87, 28.95, 33.27, 35.89, 44.34, 54.84, 59.57, 124.57, 139.59, 145.73, 148.93, 157.12. Anal. Calcd. for $C_{13}H_{18}N_4O_2$: C, 59.53; H, 6.92; N, 21.36. Found: C, 59.43, H, 7.04; N, 21.06.

(+)-(1*R*,3*R*)-2-[3-(6-Amino-9*H*-purin-9-yl)-2,2-dimethylcyclobutyl]ethanol (17). Liquid ammonia (30 mL) was added to a cooled (CO_2/acetone) suspension of **16** (0.50 g, 1.78 mmol) in MeOH (5 mL) in a pressure reactor, and the mixture was heated at 75°C for 48 h. The solvent was evaporated at reduced pressure, and the solid residue obtained was purified by FC (eluant 10:1 EtOAc/MeOH), affording the compound **17** (0.18 g, 40%) as a white solid, which was recrystallized from Et_2O/MeOH. M.p. 178-180°C. $[\alpha]_D^{25}$ +27.92 (*c* 0.50, MeOH). IR (KBr), ν (cm^{-1}): 3853, 2952, 1654, 1602, 1329, 1041, 649. ^1H NMR (DMSO-d_6), δ (ppm): 0.64 (3H, s, *t*-2-CH_3), 1.20 (3H, s, *c*-2-CH_3), 1.46 (1H, dt, J_d = 13.75 Hz, J_t = 6.50 Hz, 4-$\underline{H}$H), 1.58 (1H, dt, J_d = 13.75 Hz, J_t = 6.79 Hz, 4-H$\underline{H}$), 1.95 (1H, quint, J = 8.13 Hz, 1-H), 2.33-2.43 (2H, m, C$\underline{H}_2$CH_2O), 3.37 (2H, s, C$\underline{H}_2$O), 4.41 (1H, bs, D_2O exch., OH), 4.52 (1H, dd, J = 10.01 Hz, J = 8.28 Hz, 3-H), 7.18 (2H, s, D_2O exch., NH), 8.11 and 8.24 (2H, 2s, arom). ^{13}C NMR (DMSO-d_6), δ (ppm): 16.28, 27.85, 29.13, 33.34, 35.94, 44.35, 54.55, 59.65, 119.36, 140.23, 150.24, 152.53, 156.14. Anal. Calcd. for $C_{13}H_{19}N_5O$: C, 59.75; H, 7.33; N, 26.80. Found: C, 59.53, H, 7.24; N, 27.00.

(+)-(1*R*,3*R*)-6,7-Dihydro-3-[3-(2-hydroxyethyl)-2,2-dimethylcyclobutyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (19). A cooled (0°C) solution of aminochloropyrimidine **14** (0.30 g; 1.11 mmol) in 1N HCl (4 mL) was treated with a solution of sodium nitrite (0.11 g; 1.48 mmol) in water (10 mL). The mixture was stirred and allowed to warm up to room temperature, refluxed for 1 hour, and, then, the solvent was removed *in vacuo* and the solid residue (0.73 g) was purified by FC (eluant 10:1 EtOEt/MeOH); affording 8-azapurinone **19** (0.16 g, 52%) as a white solid, which was recrystallized from Hex/EtOAc/MeOH. M.p. 209-211°C. $[\alpha]_D^{25}$ +49.71 (*c* 0.20, MeOH). IR (KBr), ν (cm^{-1}): 3308, 2887, 1716, 1591, 1458, 1277, 1036. ^1H NMR (DMSO-d_6), δ (ppm): 0.60 (3H, s, *t*-2-CH_3), 1.23 (3H, s, *c*-2-CH_3), 1.48 (1H, dt, J_d = 8.20 Hz, J_t = 6.52 Hz, 4-$\underline{H}$H), 1.58 (1H, quint, J = 6.72 Hz, 4-H$\underline{H}$), 2.02-2.08

(1H, m, 3-H), 2.45-2.54 (1H, m, C<u>H</u>HCH$_2$O), 2.75 (1H, q, J = 10.54 Hz, CH<u>H</u>CH$_2$O), 3.36-3.41 (2H, m, C<u>H</u>$_2$O), 4.43 (1H, t, J = 4.88 Hz, D$_2$O exch., OH), 4.81 (1H, dd, J = 9.86 Hz, J = 8.02 Hz, 1-H), 8.22 (1H, s, arom), 12.67 (1H, bs, D$_2$O exch., NH). ^{13}C NMR (DMSO-*d*$_6$), δ (ppm): 16.48, 27.44, 28.86, 33.43, 36.15, 44.92, 58.08, 59.58, 130.14, 149.19, 149.80, 155.79. Anal. Calcd. for $C_{12}H_{17}N_5O_2$: C, 54.74; H, 6.51; N, 26.60. Found: C, 54.54, H, 6.38; N, 26.40.

(+)-(1*R*,3*R*)-2-[3-(7-Amino-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidin-3-yl)-2,2-dimethylcyclobutyl]ethanol (20). To a cooled (0°C) mixture of **14** (0.80 g, 2.95 mmol) and 1N HCl (8 mL), was added a solution of sodium nitrite (0.27 g, 3.94 mmol) in water (30 mL). The mixture was stirred at 0°C for 15 min, and then concentrated NH_4OH (16 mL) was added and heated under reflux conditions for 5 min. The solvent was evaporated at reduced pressure, and the solid residue was purified by FC (eluant 10:1 EtOAc/MeOH), affording compund **20** (0.39 g, 51%) as a white solid, which was recrystallized from CH_2Cl_2/MeOH. M.p. 225-227°C. $[\alpha]_D^{25}$ +43.24 (*c* 0.25, MeOH). IR (KBr), ν (cm^{-1}): 3750, 3279, 1693, 1613, 1572, 1321. ^{1}H NMR (DMSO-*d*$_6$), δ (ppm): 0.60 (3H, s, *t*-2-CH$_3$), 1.24 (3H, s, *c*-2-CH$_3$), 1.47 (1H, dt, J_d = 7.80 Hz, J_t = 5.84 Hz, 4-<u>H</u>H), 1.60 (1H, t, J = 6.72 Hz, 4-H<u>H</u>), 2.01-2.07 (1H, m, 1-H), 2.45-2.54 (1H, m, C<u>H</u>HCH$_2$O), 2.82 (1H, q, J = 10.53 Hz, CH<u>H</u>CH$_2$O), 3.36-3.42 (2H, m, CH$_2$O), 4.40 (1H, t, J = 6.65 Hz, D$_2$O exch., OH), 4.81 (1H, dd, J = 9.81 Hz, J = 8.12 Hz, 3-H), 8.03 (1H, bs, D$_2$O exch., NH), 8.26 (1H, s, arom), 8.33 (1H, s, D$_2$O exch., NH). ^{13}C NMR (DMSO-*d*$_6$), δ (ppm): 16.55, 27.38, 29.02, 33.47, 36.20, 44.92, 57.85, 59.62, 124.40, 149.59, 156.55, 156.97. Anal. Calcd. for $C_{12}H_{18}N_6O$: C, 54.95; H, 6.92; N, 32.04. Found: C, 54.84, H, 6.78; N, 32.15.

Biological activity assays. The methods used to assess antiviral activity and cytotoxicity have been described previously.[15-17]

Acknowledgements: The authors thank the Spanish Ministry of Education and Science (MEC-DGICYT, PB94-0617) and the Xunta de Galicia (XUGA 20307B94) for financial support for this work. Work at the Rega Institute was supported by the Belgian "Geconcerteerde Onderzoeksacties" (No.95/5), the Belgian "Fonds voor

Wetenschappelijk Onderzoek" (No. G.3304.96) and the Biomedical Health Programme of the European Commission. We thank Ann Absillis, Anita Camps, Frieda De Meyer, Katrien Saelen, Ria Van Berwaer and Anita Van Lierde for excellent technical assistance.

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