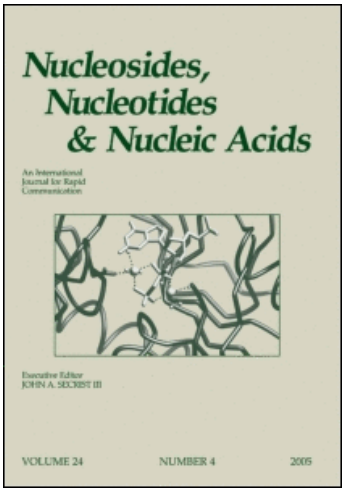

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Synthesis and Antiviral and Cytostatic Activities of Carbocyclic Nucleosides Incorporating a Modified Cyclopentane Ring. I: Guanosine Analogues

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SYNTHESIS AND ANTIVIRAL AND CYTOSTATIC ACTIVITIES OF CARBOCYCLIC NUCLEOSIDES INCORPORATING A MODIFIED CYCLOPENTANE RING. 1: GUANOSINE ANALOGUES.

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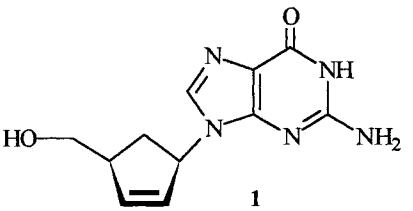
Abstract: Six new carbocyclic nucleosides were prepared by constructing a guanine (compounds **6**, **8** and **10**) or 8-azaguanine (compounds **7**, **9** and **11**) base on the amino group of (1*R*, *cis*)-3-(aminomethyl)-1,2,2-trimethylcyclopentylmethanol (**2**), and their activities against 13 viruses and 3 tumor cell lines were determined. Compounds **9**, **10** and **11** showed activity against human immunodeficiency virus type 1 (HIV-1), and compound **11** also against vaccina virus, whereas compounds **6** and **7** showed some inhibition of tumor cell proliferation.

Over the last ten years, carbocyclic analogues of nucleosides (CANs)[1] have attracted growing interest due to the stability *in vivo* and powerful antiviral and/or antitumour activities of some of these compounds. (-)-Carbovir (**1**), for example, is a potent inhibitor of the HIV reverse transcriptase following its conversion to the 5'-triphosphate,[2] and thus of potential interest for the treatment of AIDS.



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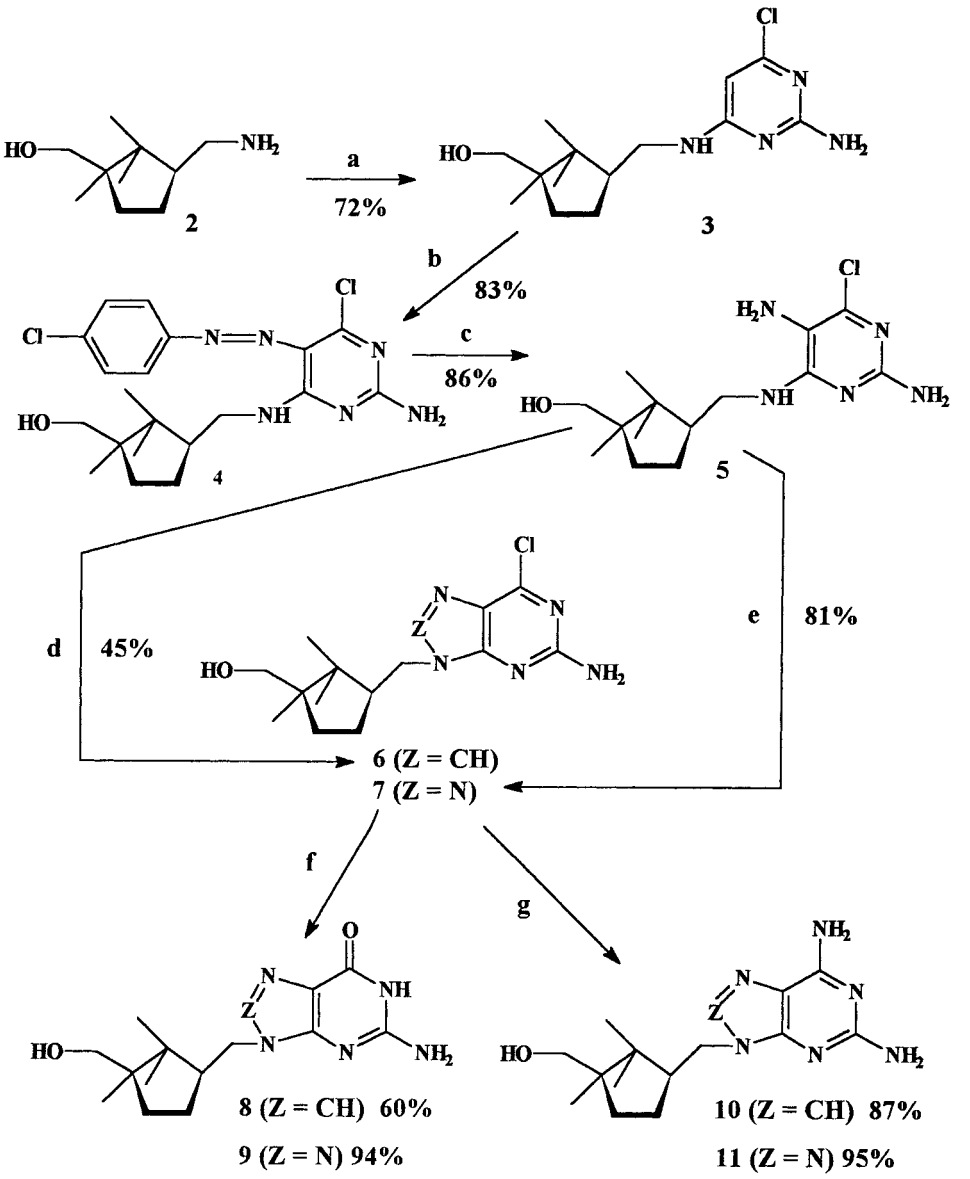
With a view to developing potent and selective antiviral agents, we are examining the influence of the structural characteristics of CANs on their biological activity. Amoung the structural characteristics considered are conformational mobility, the distance separating the hydroxymethyl oxygen and pseudo-glycosidic nitrogen, the presence of lipophilic and hydrophilic groups, and the absolute and relative stereochemistries of these groups.[3] In this work we report the preparation and biological evaluation of a series of carbocyclic analogues of guanosine that are structurally related to carbovir.[4]

The synthesis of these analogues is detailed in Scheme 1; in all cases, the guanine or modified guanine base was constructed[5,6] on the amino group of the precursor (1*R*, *cis*)-3-(aminomethyl)-1,2,2-trimethylcyclopentylmethanol (**2**).[7] Briefly, **2** was condensed with 2-amino-4,6-dichloropyrimidine; the resulting pyrimidinylamino compound **3** was condensed[6] with 4-chlorobenzenediazonium chloride to afford the 5-(4-chlorophenyl-azo)pyrimidine **4**; and **4** was reduced with Zn in acetic acid to give the triaminopyrimidine **5**. Then **5** was cyclized, either in triethyl orthoformate, which gave the 9-substituted 2-amino-6-chloropurine **6**, or in sodium nitrite/ acetic acid, which gave (1*R*, *cis*)-3-(5-amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-yl)methyl-1,2,2-trimethylcyclopentylmethanol (**7**) in good yield. The guanosine analogue **8** was obtained by hydrolysis of **6** in dilute sodium hydroxide; the 2,6-diaminopurinyl compound **10** was obtained by amination of **6** in liquid ammonia; and the corresponding 8-azaguanosine analogues **9** and **11** were obtained by applying the latter treatments to **7**.

The activities of compounds **6** -**11** against a variety of DNA and RNA viruses, and also their cytotoxicities for several host cell lines, were evaluated and compared with the corresponding data for standard drugs with known antiviral activities (Tables 1, 2 and 3).

The compounds **6**-**11** showed no antiviral activity, at subtoxic concentrations against various DNA viruses, except for compound **11** against vaccinia virus (MIC_{50}: 30 μg/mL) (Table 1). Likewise, none of the compounds proved inhibitory to influenza A or B virus (Table 2). Compounds **9**, **10** and **11** showed activity against HIV-1 at a concentration that was about 5-fold lower than the concentration needed to impair cell viability (Table 3). One or several of the structural differences between the carbocyclic



a) 2-Amino-4,6-dichloropyrimidine, Et_3NH_2, butanol, reflux, 48 h; b) 4-chlorobenzenediazonium chloride, H_2O, AcOH, NaOAc, r. t.; c) Zn, AcOH, H_2O, EtOH, 6 h, reflux; d) $CH(OEt)_3$, 12 N HCl, 1 h, r. t.; e) $NaNO_2$, AcOH, H_2O, 2 h, 0°C; f) 0.33 N NaOH, 5 h, reflux; g) NH_3, MeOH, 48 h, 175°C.

Scheme 1

TABLE 1. Antiviral Activity* and Cytotoxicity** of Compounds **6-11**

VIRUS (STRAIN)	CELL	6	7	8	9	10	11	Brivubin	Ribavirin	Ganciclovir	Acyclovir
HSV-1 (KOS)	E_6SM	>100	>100	>400	>200	150	>400	0.004	>100	0.0004	0.004
HSV-2 (G)	E_6SM	>100	>100	>400	>200	150	300	>100	>100	0.0004	0.004
Vaccinia	E_6SM	≥100	>100	>400	>200	150	30	1	20	>100	>200
Vesicular stomatitis	E_6SM	>100	>100	>400	>200	>400	>400	>400	20	>100	>400
HSV-1 (TK^- B2006)	E_6SM	>200	>400	>400	>400	>100	150	10	7	0.7	20
Cytotoxicity	E_6SM	≥200	200	>400	400	>400	>400	200	≥200	>100	>400
										(S)-DHPA[a]	C-c^{3}Ado[b]
Vesicular stomatitis	Hela	>400	>400	>400	>400	>400	>400	>400	20	70	4
Coxsackie B4	Hela	200	>400	>400	>400	40	300	>400	70	>400	>400
Respiratory syncytial	Hela	>200	>400	>400	>400	40	>400	>400	1	>400	150
Cytotoxicity	Hela	>400	>400	>400	>400	>400	>400	>400	≥400	>400	>400
Parainfluenza-3	Vero	>200	>100	>400	>200	>40	>200	>400	70	40	2
Reovirus-1	Vero	>200	>100	>400	>200	>40	>200	>400	70	100	2
Sindbis	Vero	>200	>100	>400	>200	>40	>200	>400	200	>400	>400
Coxsackie B4	Vero	>200	>100	>400	>200	>40	>200	>400	>400	>400	>400
Punta Toro	Vero	>200	>100	>400	>200	>40	>200	>400	70	>400	>400
Cytotoxicity	Vero	400	200	>400	400	≥100	400	>400	>400	>400	>400

* MIC_{50} **or** Minimun inhibitory concentration (μg/mL) required to reduce virus-induced cytopatogenicity by 50%.

** MCC or Minimum cytotoxic concentration (μg/mL) required to cause a microscopically detectable alteration of normal cell morphology.

[a] (*S*)-9-(2,3-Dihydroxypropyl)adenine. [b] Carbocyclic 3-deazaadenosine.

Cell lines used: human embryonic skin-muscle (E_6 SM) fibroblasts, human epithelial (Hela) cells and African green monkey (Vero) kidney cells.

TABLE 2. Anti-influenza virus activity* and cytotoxicity** of Compounds **6-11**

Influenza Virus Strain	CELL	6	7	8	9	10	11	Ribavirin	Rimantadine	Amantadine
H2N2 A2 Japan/305/57	MDCK	>50	>50	>50	>50	>50	>50	102	0.2	6.8
B Hong Kong/5/72	MDCK	>50	>50	>50	>50	>50	>50	4.5	>50	>250
H3N2 (X31)	MDCK	>50	>50	>50	>50	>50	>50	4.9	10	29
Cytotoxicity	MDCK	250	250	250	250	250	250	>250	250	>250

*MIC_{50} or Minimun inhibitory concentration (μg/mL) required to reduce virus-induced cytopatogenicity by 50%.
**MCC or Minimun cytotoxic concentration (μg/mL) required to cause a microscopically detectable alteration of normal cell morphology.
Cell line used: Madin-Darby canine kidney.

TABLE 3. Activity of Compounds **6-11** against HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells.

	EC_{50} *(μg/mL)		CC_{50}** (μg/mL)
Compounds	**HIV-1**	**HIV-2**	**Cell viability**
6	> 4	> 4	17.5 ± 3
7	> 0.8	> 0.8	17 ± 2.5
8	> 100	> 100	> 200
9	26 ± 22	≥ 100	156 ± 61
10	7.0 ± 3.0	14 ± 6.5	28 ± 13
11	22 ± 8	80 ± 28	108 ± 20

*50% Effective concentration, or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%
**50% Cytotoxic concentration, or conentration required to reduce viability of the cells by 50%.

moiety of this family of compounds and that of carbovir[2], namely the saturation of the cyclopentane ring, and the greater conformational mobility brought about by inclusion of the methylene bridge between this ring and the base nitrogen may be responsible for their relative inactivity.

Compounds **6-11** were also tested for cytostatic activity against tumor cell lines. Compounds **6** and 7 showed moderate inhibition of the proliferation of murine leukemia L 1210 and human T-lymphocyte MOLT4/C8 cells (Table 4).

Experimental

Silica gel (230 mesh) was purchased from Merck. All other chemicals used were of reagent grade and were obtained from Aldrich Chemical Co. Melting points were measured on a Reichert Kofler thermopan and are uncorrected; Na-D line polarimetry was carried out at 25°C in a Perkin-Elmer 241 polarimeter; infrared spectra were recorded in a Perkin-Elmer FTIR 1640 spectrometer; and ^{1}H NMR and ^{13}C NMR spectra were recorded in a Bruker AMX 300 spectrometer.

TABLE 4. Inhibitory effects of compounds **6-11** on the proliferation of murine leukemia cells (L1210/0 and human T-lymphocyte (Molt4/C8) cells.

	IC_{50} (μg/mL)*	
Compound	**L1210/0**	**Molt4/C8**
6	16.2 ± 2.6	13.2 ± 0.8
7	14.5 ± 0.6	16.1 ± 1.5
8	195 ± 7	> 200
9	130 ± 5	113 ± 19
10	94.0 ± 5.6	51.7 ± 6.7
11	80.7 ± 1.8	97.5 ± 13.4

* 50% Inhibitory concentration, or concentration required to inhibit cell proliferation during the linear growth phase by 50%.

(1*R,cis*)-3-[(2-Amino-6-chloropyrimidin-4-yl)aminomethyl]-1,2,2-trimethylcyclopentylmethanol (3). Freshly prepared **2** (4.32 g, 25.3 mmol), 2-amino-4,6-dichloropyrimidine (5.32 g, 32.6 mmol), triethylamine (13 mL) and 1-butanol (86 mL) were refluxed under argon for 48 h. After evaporation of the volatile solvents, the residue was pre-adsorbed on silica gel, packed on top of a silica gel column (250 g) and chromatographed with 40:1 $CHCl_3$/ MeOH as eluant. The fractions containing product were concentrated to a syrup (5.46 g) and acetone was added to precipitate the triethylamine salt, which was filtered out. Addition of ethyl acetate to the filtrate afforded **3** as a white crystalline solid (5.39 g, 72%); m.p. 145-149 °C. An analytical sample was obtained by recrystallization of this material from 7:3 EtOAc/hexane: m.p. 148-50°C. $[\alpha]_D^{25}$ + 49.59 (*c* 0.36, MeOH). IR (KBr): 3503, 3286, 3171, 2962, 1654, 1586, 1547, 1450, 906, 789, 665 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.83 (s, 3H, C*H*$_3$), 1.01 (s, 3H, C*H*$_3$), 1.03 (s, 3H, C*H*$_3$), 1.23-1.43 (m, 2H), 1.56-1.70 (m, 1H), 1.91-2.10 (m, 2H), 3.02 (m, 1H, NH-C*H*$_2$), 3.31 (m, 1H, NH-C*H*$_2$), 3.61 and 3.47 (AB system, 2H, *J* = 10.73 Hz, HOC*H*$_2$), 4.87 (br s, 4H, D_2O exchangeable, N*H*$_2$ + N*H* + O*H*), 5.78 (s, 1H, *H*-5').

(1*R,cis*)-3-{[2-Amino-6-chloro-5-(4-chlorophenylazo)pyrimidin-4-yl]amino-methyl}-1,2,2-trimethylcyclopentylmethanol (4). 4-Chlorobenzenediazonium chloride

was prepared by mixing 4-chloroaniline (3.03 g, 23.76 mmol), 3N HCl (52 mL) and $NaNO_2$ (1.77 g, 25.65 mmol) in cold H_2O (21.5 mL). This solution was added to a mixture of **3** (6.17 g, 20.63 mmol), AcOH (103 mL), H_2O (103 mL), and NaOAc. 3 H_2O (41.3 g), and stirred overnight at room temperature. The yellow precipitate was filtered out and washed with cold H_2O until the washings were neutral, and then air-dried in a fume hood to yield **4** (7.47 g, 83 %). An analytical sample was obtained by recrystallization of the crude **4** from acetone: m.p. 269-71°C. $[\alpha]_D^{25}$ - 1.57 (*c* 0.97, DMF). IR (KBr): 3454, 3308, 3193, 2958, 2868, 1644, 1568, 1483, 1368, 1336, 1321, 1055, 1294, 1086, 783 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.79 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.23-1.37 (m, 2H), 1.46-1.52 (m, 1H), 1.81-1.84 (m, 1H), 2.12-2.17 (m, 1H, *H-3*), 3.23-3.32 (m, 1H, NH-CH_2), 3.20 and 3.36 (AB system, 2H, *J* = 10.52 Hz, OHCH_2), 3.57-3.66 (m, 1H, NH-CH_2), 7.62 (br s, 2H, D_2O exchangeable, NH_2), 7.56 (d, 2H, *J* = 8.77 Hz, *H*-3'+*H*-5'), 7.69 (d, 2H, *J* = 8.78 Hz, *H*-2'-+*H*-6'), 10.19 (t, 1H, *J* = 5.05 Hz, N*H*). ^{13}C NMR (DMSO-d_6) δ: 165.00, 161.28, 155.02, 150.97, 133.43, 129.88, 123.11, 118.73, 67.48, 48.46, 47.40, 43.69, 42.01, 33.83, 26.42, 24.38, 21.39, 18.60.

(1*R,cis*)-3-[(2,5-Diamino-6-chloropyrimidin-4-yl)aminomethyl]-1,2,2-trimethylcyclopentylmethanol (5). A mixture of **4** (2.62 g, 5.99 mmol), zinc dust (3.91 g, 65.27 mmol), AcOH (1.88 mL), H_2O (89 mL) and EtOH (89 mL) was refluxed under argon for 6 h. The zinc was removed, and the solvents were evaporated to leave a solid residue (5.02 g). This was pre-adsorbed on silica gel, packed onto the top of a silica gel column (130 g) and chromatographed with 15:1 CH_3Cl/MeOH as eluant. The fractions containing product were concentrated to a pink syrup which crystallized from 1:4 EtOH/H_2O to afford **5** as pink crystals (1.62 g, 86 %). M.p. 165-167.5 °C. IR (KBr): 3314, 2966, 2872, 1618, 1588, 1445, 1026 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.76 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.17-1..25 (m, 2H), 1.44-1.49 (m, 1H), 1.85-1.89 (m, 1H), 2.07-2.12 (m, *H*-3), 3.14-3.27 (m, 2H), 3.28-3.39 (m, 2H), 3.91 (s, 2H, D_2O exchangeable, NH_2), 4.33 (t, 1H, D_2O exchangeable, *J* = 4.85 Hz, O*H*), 5.56 (s, 2H, D_2O exchangeable, NH_2), 6.18 (t, 1H, *J* = 4.45, D_2O exchangeable, N*H*). ^{13}C NMR

(DMSO-d_6) δ: 155.96, 155.49, 140.85, 113.63, 67.55, 48.55, 47.36, 43.94, 43.11, 33.96, 26.85, 23.94, 21.54, 18.39.

(1*R,cis*)-3-(2-Amino-6-chloro-9*H*-purin-9-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (6). A mixture of **5** (1.62 g, 5.17 mmol), triethyl orthoformate (27.5 mL), and 12N HCl (1.27 mL), was stirred overnight. The resulting suspension was evaporated to dryness *in vacuo*, and the residue was treated with 0.5N HCl (38 mL) for 1h at room temperature, whereupon the mixture was adjusted to pH 8 with 1N NaOH. The solvents were evaporated, and the crude product (4.62 g) was triturated in $CHCl_3$ (30 mL), and filtered to remove undissolved NaCl. The $CHCl_3$ was evaporated, to leave a pale yellow solid, which was purified on silica gel (60 g) eluting with 20:1 $CHCl_3$/MeOH. Crude **6** (0.75 g, 45%) was isolated as a white solid; an analytical sample was obtained by recrystallization of this crude material from MeOH. M.p. 227-229°C. $[\alpha]_D^{25}$ + 49.86 (*c* 1.01, MeOH). IR (KBr): 3311, 3208, 2946, 1609, 1556, 1520, 1469, 1404, 1357, 1309, 909 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.94 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 1.30-1.43 (m, 2H), 1.45-1.75 (m, 2H), 2.33-2.44 (m, 1H, *H*-3), 3.49 (dd, 1H, *J* = 10.62, 2.33 Hz, OHCH_2), 3.63 (dd, 1H, *J* = 10.64, 4.18 Hz, OHCH_2), 3.88 (dd, 1H, *J* = 13.56, 10.51 Hz, NCH_2), 4.16 (dd, 1H, *J* = 13.56, 4.38 Hz, NCH_2), 5.11 (s, 2H, NH_2), 7.76 (s, 1H, *H*-8 purine). ^{13}C NMR ($CDCl_3$) δ: 159.19, 154.17, 151.45, 142.63, 125.58, 69.38, 48.85, 48.54, 45.84, 44.63, 33.62, 26.77, 23.66, 21.09, 18.54.

(1*R,cis*)-3-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (7). A cooled solution of **5** (2.11 g, 6.73 mmol) in AcOH (10.7 mL) and H_2O (53 mL) treated with $NaNO_2$ (0.60 g, 8.7 mmol) in H_2O (35 mL), and stirred for 2 h at 0°C and then 1 h at room temperature. The resulting solution was evaporated to dryness, and the solid residue (3.12 g) was purified on silica gel column (80 g) with 7.5:1 CH_2Cl_2/MeOH as eluant. Compound **7** (1.77 g, 81%) was isolated as a pale yellow solid with mp 179-182°C. Recrystallization from toluene afforded an analytical sample with m.p. 183-85°C. $[\alpha]_D^{25}$ + 32.64 (*c* 0.8, MeOH). IR (KBr): 3331, 3198, 2948, 1654, 1610, 1560, 1518, 1028, 1007 cm^{-1}. ^1H NMR (CD_3Cl) δ: 0.97 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.38-1.28 (m, 1H), 1.42-1.46 (m, 1H), 1.67-

1.49 (m, 3 H), 2.62-2.66 (m, 1H), 3.48 (dd, 1H, J = 10.73, 4.67 Hz, OHCH_2), 3.62 (dd, 1H, J = 10.72, 5.76 Hz, OHCH_2), 4.31 (dd, 1H, J = 13.47, 10.13 Hz, NCH_2), 4.48 (dd, 1H, J = 13.46, 4.98 Hz, NHCH_2), 5.52 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ: 162.05, 152.53, 152.49, 128.32, 67.43, 48.58, 48.18, 47.75, 44.65, 33.54, 26.08, 23.49, 21.44, 18.13.

(1*S,cis*)-2-Amino-6,9-dihydro-9-[3-(hydroxymethyl)-2,2,3-trimethylcyclopentylmethyl]-1*H*-purin-6-one (8). A mixture of **6** (0.57g, 1.76 mmol) and 0.33 N NaOH (33 mL) was refluxed for 5 h, whereupon the solvent was evaporated leaving a pale yellow foam (1.26 g) that was chromatographed on silica gel (50 g), eluting with 5:1 $CHCl_3$/MeOH. Compound **8** (0.32 g, 60%) was isolated as white solid with m.p. 322-324°C (dec.). Recrystallization of this material from 1:1.1 H_2O/MeOH afforded an analytical sample with mp 324-326°C (dec.). $[\alpha]_D^{25}$ + 50.60 (*c* 1.025, MeOH). IR (KBr): 3321, 3157, 2944, 1685, 1607, 1569, 1546, 1388, 1026 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.82 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.12-1.28 (m, 1H), 1.33-1.53 (m, 3H), 2.31-2.42 (m, 1H, H-1'), 3.19 (dd, 1H, J = 10.38, 4.63 Hz, OHCH_2), 3.34 (dd, 1H, the residual H_2O signal overlaps this signal; addition of D_2O simplifies both this signal and the signal at 3.19 to doublets, and J = 10.74 Hz can be discerned, OHCH_2), 3.75 (dd, 1H, J = 13.33, 9.96 Hz, NCH_2), 3.94 (dd, 1H, J = 13.40, 5.18 Hz, NCH_2), 4.36 (t, 1H, D_2O exchangeable, J = 4.88 Hz, O*H*), 7.72 (s, 1H, H-8 purine), 10.50 (s, 1H, D_2O exchangeable, O*H*-6). ^{13}CNMR (DMSO-d_6) δ: 156.76, 153.52, 151.23, 137.52, 116.78, 67.09, 48.25, 47.61, 44.11, 43.62, 33.18, 25.61, 23.15, 21.11, 17.83.

(1*S,cis*)-5-Amino-6,7-dihydro-3-[3-(hydroxymethyl)-2,2,3-trimethylcyclopentylmethyl]-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (9). A mixture of **7** (0.35 g, 1.08 mmol) and 0.25N NaOH (22 mL) was refluxed for 6.5 h, whereupon its pH was adjusted to 3 with 6N HCl, causing a gelatinous precipitate to form. This was filtered out and washed with cold water, and then dried *in vacuo* over P_2O_5 to afford **9** (0.31 g, 94%) as an off-white solid with m.p. 313-316°C (dec.). Recrystallization of this material from EtOH afforded an analytical sample with m.p. 316-318°C (dec.). $[\alpha]_D^{25}$ + 34.70 (*c* 0.86, MeOH). IR (KBr): 3392, 3311, 3164, 2943, 1718, 1687, 1640, 1604, 1574, 1533, 1374,

1027 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.82 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.22-1.13 (m, 1H), 1.35-1.56 (m, 4H), 3.19 (dd, 1H, J = 10.62, 4.73 Hz, $OHCH_2$), 3.37 (dd, 1H, J = 10.73, 5.35 Hz, $OHCH_2$), 4.06 (dd, 1H, J = 13.58, 9.54 Hz, NCH_2), 4.28 (dd, 1H, J = 13.64, 5.61 Hz, NCH_2), 4.37 (t, 1H, D_2O exchangeable, J = 4.92 Hz, *OH*), 6.88 (s, 2H, D_2O exchangeable, NH_2), 10.89 (s, 1H, D_2O exchangeable, *OH*). ^{13}C NMR (DMSO-d_6) δ: 156.10, 155.68, 151.53, 124.49, 67.45, 48.60, 47.74, 47.50, 44.02, 33.54, 26.00, 23.43, 21.46, 18.12.

(1*R,cis*)-3-(2,6-Diamino-9*H*-purin-9-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (10). A solution of **6** (0.32 g, 0.99 mmol) in MeOH (20 mL) was cooled to -60°C in a reaction bomb, liquid ammonia was passed into the solution and the bomb was sealed and then heated at 175°C for 48 h. Evaporation of the ammonia and MeOH afforded crude **10** (0.31 g) as yellow crystals; this was adsorbed onto silica gel (2 g) and packed onto a silica gel column (14 g), which was then eluted with 9:1 CH_2Cl_2/ MeOH. Recrystallization of the chromatographed product (0.26 g, 87%) from H_2O afforded 0.16 g of pure **10**. M.p. 274-276°C. $[\alpha]_D^{25}$ + 42.32 (*c* 1.03, MeOH). IR (KBr): 3334, 3165, 2959, 2871, 1668, 1592, 1521, 1474, 1404, 1022 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.83 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 1.12-1.18 (m, 1H), 1.34-1.50 (m, 3H), 2.38-2.42 (m, 1H, *H*-3), 3.19 (dd, 1H, J = 10.48, 4.75 Hz, $OHCH_2$), 3.36 (dd, 1H, J = 10.57, 5.23 Hz $OHCH_2$), 3.76 (dd, 1H, J = 13.36, 9.91 Hz, NCH_2), 3.93 (dd, 1H, J = 13.40, 5.21 Hz, NCH_2), 4.35 (t, 1H, J = 4.92 Hz, D_2O exchangeable, *OH*), 5.71 (s, 2H, D_2O exchangeable, NH_2), 6.59 (s, 2H, D_2O exchangeable, NH_2), 7.73 (s, 1H, *H*-8). ^{13}C NMR (DMSO-d_6) δ: 160.50, 156.36, 152.24, 137.96, 113.48, 67.48, 48.65, 47.87, 44.19, 43.97, 33.59, 26.09, 23.59, 21.47, 18.24.

(1*R,cis*)-3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (11). Compound **7** (0.35 g, 1.07 mmol) was aminated as described for compound **9**, but with a reaction time of 84 h at 78 °C. After removal of the methanol and NH_3, the residue was dissolved in MeOH and re-evaporated to dryness to leave **11** (0.37 g, 95%) as a yellow solid, with m.p. 245-250°C. The crude product was recrystallized from 8:2 EtOH/AcOEt to afford pure **11**. M.p. 249-251°C. $[\alpha]_D^{25}$ + 27.94

(*c* 0.9, MeOH). IR (KBr): 3381, 2964, 1684, 1601, 1497, 1423, 1026, 995, 793 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.82 (s, 3H, CH_3), 0.88 (s, 6H, 2 CH_3), 1.13-1.22 (m, 1H), 1.36-1.55 (m, 4H), 3.20 (dd, 1H, J = 10.51, 4.73 Hz, OHCH_2), 3.37 (dd, 1H, J = 10.58, 5.23 Hz, OHCH_2), 4.08 (dd, 1H, J = 13.60, 9.47 Hz, NCH_2), 4.29 (dd, 1H, J = 13.63, 5.70 Hz, NCH_2), 4.37 (virtual s, 1H, D_2O exchangeable, O*H*), 6.36 (s, 2H, D_2O exchangeable, NH_2), 7.0-7.34 (br, 2H, D_2O exchangeable, NH_2). ^{13}C NMR (DMSO-d_6) δ: 163.03, 156.48, 151.84, 120.54, 67.46, 48.63, 47.72, 47.15, 44.00, 33.57, 26.06, 23.47, 21.45, 18.16.

Biological activity assays. Antiviral activity and cytotoxicity assays were carried out according to previously established procedures.[8]

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