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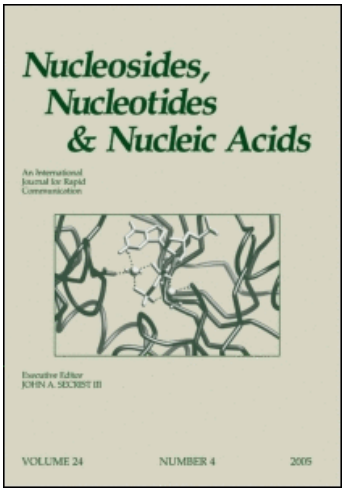

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Synthesis and Antiviral Activities of Some Novel Carbocyclic Nucleosides

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SYNTHESIS AND ANTIVIRAL ACTIVITIES OF SOME NOVEL CARBOCYCLIC NUCLEOSIDES

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Abstract: *cis*-3-Aminomethylcyclopentylmethanol (**4**), prepared from norbornene (**5**) in four steps and 51% overall yield, was used as a precursor in the synthesis of carbocylic nucleosides **13** - **18** containing guanine and 8-azaguanine bases. None of these compounds had appreciable activity against fourteen viruses in the concentration range tested. Compounds **13** and **16** showed cytotoxicity to all or part of the cell lines used.

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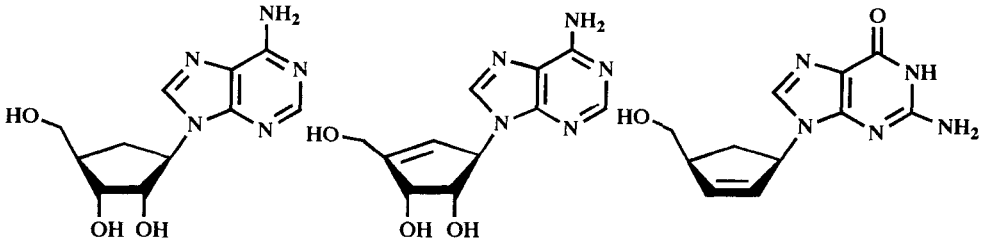
Spurred by the discovery of the natural antibiotics aristeromycin (**1**) and neplanocin A (**2**), both of which have antineoplastic and antiviral activities,[1] there has been an upsurge of interest in carbocyclic nucleosides in the last decade.[2] At the same time, the identification of retroviruses HIV-1 and HIV-2 as the causative agents of AIDS has likewise stimulated interest in the search for novel antiviral agents for treatment of this deadly disease.[3] As a result, a number of compounds with activities similar to **1** and **2** have been developed, one of the most outstandingly successful of these being carbovir (**3**), which has potent antiviral activity, particularly against HIV.[3,4] As yet there is no generally applicable system of rules relating the structure of a carbocyclic nucleoside to its therapeutic activity, although a partial study involving only a few structures has been carried out.[5,6]



1 **2** **3**

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4

In this work we synthesized a series of saturated 1'-homocarbovir analogues starting from the precursor amino alcohol, **4**. With regard to the known anti-HIV agent carbovir, these analogues have a fully saturated cyclopentane ring and an extra methylene between this ring and the base nitrogen, thus allowing evaluation of the effects of increased lipophilicity and molecular flexibility on the biological activity of carbovir.

Following the pioneering method for the synthesis of carbocyclic nucleosides,[7] two independent series of reactions were used for the preparation of these carbovir analogues. In the first series, the amino alcohol precursor **4** was synthesized in four steps and 51% overall yield from norbornene (**5**) (Scheme 1). In the second, a general method[4,8] of constructing guanine and 8-azaguanine rings was used to prepare carbocylic nucleoside analogues **13** - **18** from the amino alcohol **4** (Scheme 2).

The synthesis of **4** began with oxidative ring-opening of norbornene using $NaIO_4$ in the presence of RuO_2,[9] which afforded the diacid **6** after 4 days at room temperature. This diacid was converted into the anhydride **7** (not isolated) by treatment with acetic anhydride, and then reacted with gaseous ammonia to give amide **8**. Then, following esterification of **8** with diazomethane, the resulting amido ester **9** was reduced to the target precursor **4** using $BH_3{\cdot}S(CH_3)_2$.[10]

For preparation of the carbocyclic nucleosides, the general method[4,8] involved condensation of **4** with 2-amino-4,6-dichloropyrimidine, which afforded the amine **10**; reaction of **10** with 4-chlorobenzenediazonium chloride gave 5-(4-chlorophenylazo)-pyrimidine **11**; and reduction of **11** with Zn/acetic acid led to the triaminopyrimidine derivative **12**. Cyclization of **12** with triethyl orthoformate gave the 2-amino-6-chloropurine derivative **13**, which could be converted to the carbocyclic guanosine analogue **14** by treatment with 0.3N NaOH under reflux, or to the corresponding 2,6-diaminopurine analogue **15** by heating in liquid ammonia in a closed vessel. The 8-azaguanine analogues were obtained by reaction of **12** with $NaNO_2$/acetic acid, which gave the 8-aza compound **16**, and treatment of **16** with NaOH or ammonia gave the 8-azaguanine **17** and the 2,6-diamino-8-azapurine **18**, respectively.

The activities of compounds **13** - **18** against a variety of DNA and RNA viruses, and also their cytotoxicities for several host cell lines, were evaluated and compared

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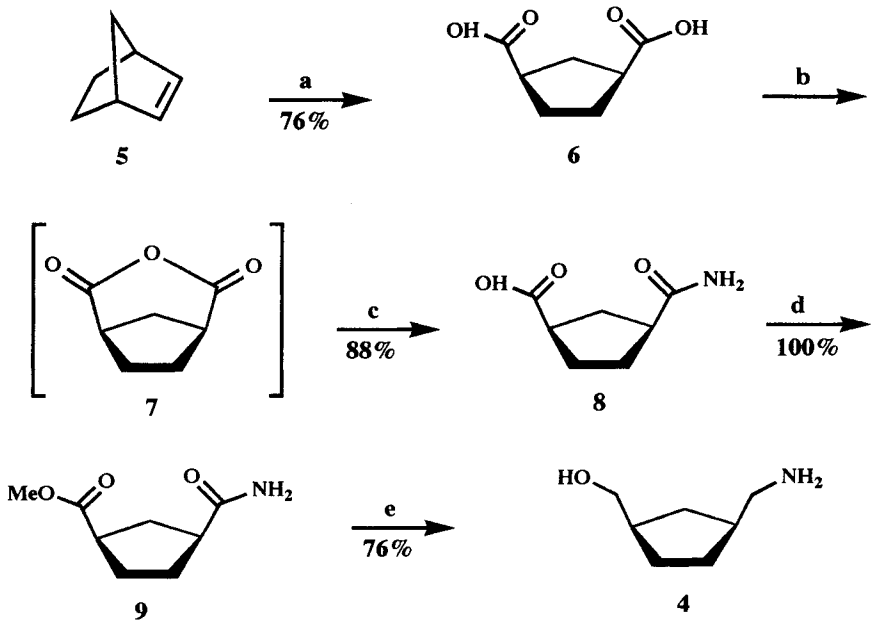

a: $NaIO_4/RuO_2$; b: Ac_2O; c: $NH_3(g)$; d: CH_2N_2; e: $BH_3{\cdot}S(CH_3)_2$

Scheme 1

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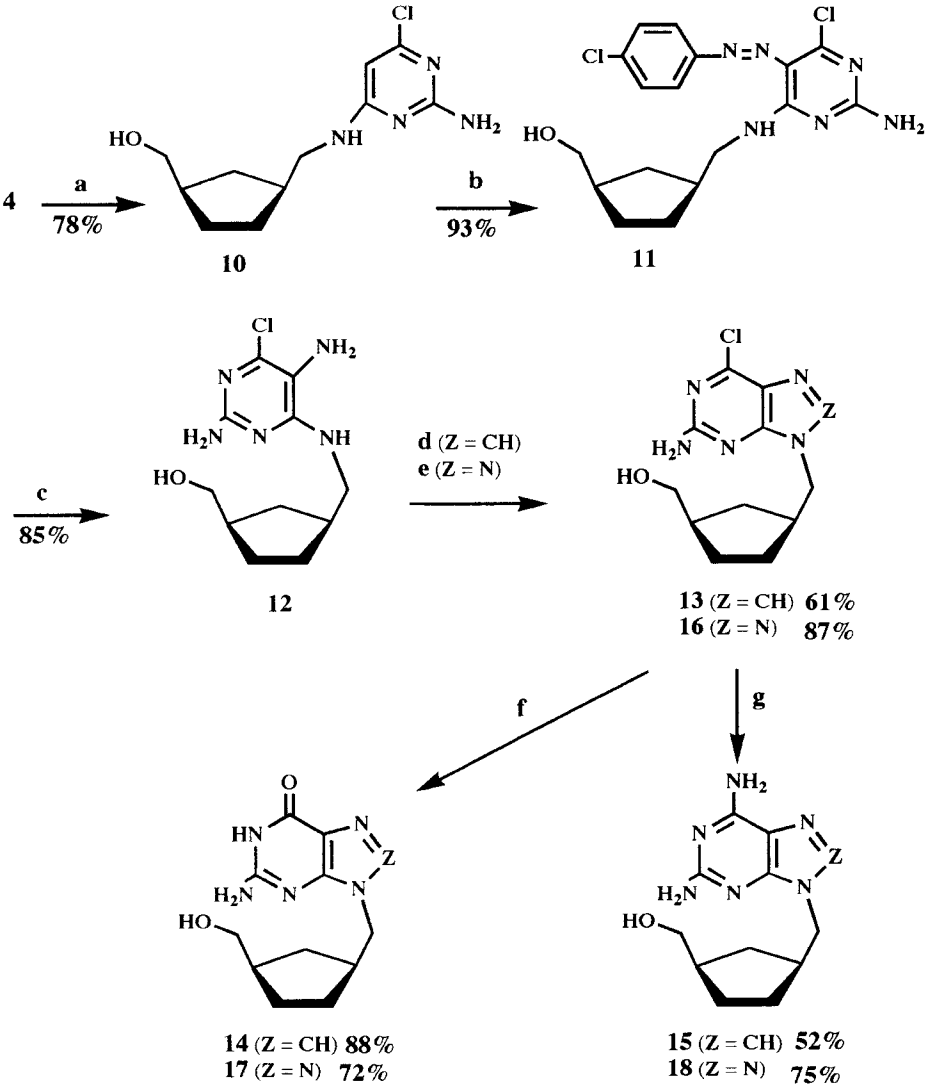
with the corresponding data for drug standards with known antiviral activities (Tables 1-3).

Compound **13**, which was the most cytotoxic of all the compounds that were evaluated, had no appreciable antiviral activity at subcytotoxic concentrations. For the remaining compounds (**14 - 18**), no antiviral activity was detected, not even at concentration up to 400 μg/mL.

Both compounds **13** and **16** were cytotoxic to human T-lymphocyte cells at a concentration of about 15 μg/mL. Neither these compounds nor the other newly synthesized compounds were active against HIV type 1 or 2 in the concentration range studied (Table 3). These data suggest, specially when compared with those available for carbovir,[4] that saturation of the carbocyclic sugar moiety coupled with the increased mobility brought about by the extra methylene group between the cyclopentane ring and the base nitrogen, either markedly decreased or abolished anti-HIV activity.



a: 2-amino-4,6-dichloropyrimidine; **b**: p-$ClC_6H_4N_2^+Cl^-$; **c**: Zn/AcOH; **d**: $HC(OEt)_3$; **e**: $NaNO_2$/AcOH; **f**: NaOH; **g**: NH_3

Scheme 2

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TABLE 1. Antiviral Activity* and Cytotoxicity** of Compounds **13-18.**

VIRUS (STRAIN)	CELL	13	14	15	16	17	18	BVDU[a]	RIBAV[b]	ACG[c]	DHPG[d]
HSV-1 (KOS)	E_6SM	>1	>400	>400	>200	>200	>400	0.04	300	0.07	0.004
HSV-2 (G)	E_6SM	>1	400	300	>200	>200	>400	200	150	0.07	0.007
Vaccinia	E_6SM	>1	>400	>400	>200	>200	>400	2	100	>400	>100
Vesicular stomatitis	E_6SM	>1	>400	>400	>200	>200	>400	>400	70	>400	>100
HSV-1 (TK^-B2006)	E_6SM	>1	>400	>400	>200	>200	>400	70	150	40	7
HSV-1(TK^-VMW1837)	E_6SM	>1	>400	300	>200	>200	>400	70	150	7	0.2
Cytotoxicity	E_6SM	≥1	>400	>400	≥400	>200	>400	>400	>400	>400	>100
										(*S*)-DHPA[e]	C-c^3 Ado[f]
Parainfluenza-3	Vero	>40	>200	>200	>200	>200	>100	>400	150	4	0.2
Reovirus-1	Vero	>40	>200	>200	>200	>200	>100	>400	70	2	2
Sindbis	Vero	>40	>200	>200	>200	>200	>100	>400	300	>400	>100
Coxsackie B4	Vero	>40	>200	>200	>200	>200	>100	>400	>400	>400	>100
Semliki forest	Vero	>40	>200	>200	>200	>200	>100	>400	>400	>400	>100
Cytotoxicity	Vero	≥40	≥400	≥400	≥400	≥400	≥200	>400	>400	>400	≥200
Vesicular stomatitis	HeLa	>10	>200	>200	>200	>200	>200	>400	20	>400	4
Coxsackie B4	HeLa	>10	>200	>200	>200	>200	>200	>400	70	>400	>400
Polio virus-1	HeLa	>10	>200	>200	>200	>200	>200	>400	70	>400	>400
Cytotoxicity	HeLa	≥10	≥400	≥400	400	≥400	400	>400	>400	>400	>400

*Minimum inhibitory concentration (μg/mL) required to reduce virus-induced cytopathogenicity by 50%.

**Minimum cytotoxic concentration (μg/mL) 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.

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TABLE 2. Antiviral activity (IC_{50})* and Cytotoxicity (CC_{50})** of Compounds **13-18** Against Several Strains of Varicella-Zoster Virus (VZV) and Cytomegalovirus (CMV) in Human Embryonic Lung (HEL) Cells.

VIRUS (STRAIN)	13	14	15	16	17	18	BVDU[a]	ACV[b]
VZV[e]								
TK^+ VZV (OKA strain)	>20	>200	>50	>200	>200	>50	0.0005	0.013
TK^+ VZV (YS strain)	>20	>200	>50	>200	>200	>50	0.0008	0.037
TK^- VZV (07/1 strain)	>20	200	>50	>200	>200	>50	10	4
TK^- VZV (YS/R strain)	20	130	>50	>50	>50	40	20	2
Cytotoxicity	2	80	100	100	150	50	-	100
CMV							**(*S*)-HPMPC**[c]	**DHPG**[d]
AD-169 strain (Assay-1)	>5	>200	>50	>200	>200	>50	0.02	0.15
Davis strain (Assay-1)	>5	>200	>50	>200	>200	>200	0.038	0.27
Cytotoxicity	2	80	100	100	150	50	100	>100

*50% Inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. Virus imput was 20 plaque forming units PFU for VZM and 100 PFU for CMV. **50% Cytotoxic concentration or concentration required to reduce cell growth by 50%. [a] Brivudin. [b] Acyclovir. [c] Cidofovir. [d] Ganciclovir. [e] TK^+, thimidine kinase efficient; TK^-, thimidine kinase deficient

TABLE 3. Anti-HIV-1 and HIV-2 Activity* and Cytotoxic Properties** of Compounds **13-18** in Human T-lymphocyte (CEM/0) Cells.

VIRUS	EC_{50} or CC_{50}	13	14	15	16	17	18
HIV-1	EC_{50} (μg/mL)	>4	>100	≥100	>0.8	>40	>40
HIV-2	EC_{50} (μg/mL)	>4	>100	108±11	>0.8	>40	>40
Cytotoxicity	CC_{50} (μg/mL)	16±0.71	>200	>200	15±7.0	>200	>200

*50% Effective concentration, or concentration required to protect CEM cell against the cytopathogenicity of HIV by 50%.

**50% Cytotoxic concentration, or concentration required to reduce CEM cell viability by 50%.

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Experimental

Melting points were determined in a Reichert Kofler thermopan. IR spectra of samples as KBr discs (solids) or liquid films between NaCl plates (oils) were recorded in a A Perkin-Elmer FTIR 1640 spectrometer. ^1H NMR spectra of solutions of samples in $CDCl_3$ or DMSO-d_6 were recorded in Bruker WM 250 or AMX 300 spectrometers. Column chromatography was done on Merck 60 silica gel (70-230 mesh); thin layer chromatography was performed on Merck GF_{254} chromatoplates.

***cis*-1,3-Cyclopentanedicarboxylic acid (6).** Oxidation of norbornene (**5**) to **6** was carried out using Clark's method;[9] yield 83 %. M.p. 120-121°C (Lit[9] 120-121°C). IR, ν (cm^{-1}): 1686 (acid CO). ^1H NMR ($CDCl_3$), δ (ppm): 1.91-2.12 (4H, m, 4-H_2+5-H_2), 2.27 (2H, t, J = 8.3 Hz, 2-H_2), 2.83-2.94 (2H, m, 1-H+3-H).

(±)-*cis*-3-(Aminocarbonyl)cyclopentanecarboxylic acid (8). A mixture of diacid **6** (5 g, 31.6 mmol) and acetic anhydride (28 mL) was refluxed for 25 h, whereupon the acetic acid and excess anhydride were evaporated *in vacuo*, until constant mass of the residue. The syrupy product, **7** (4.4 g), was dissolved in dry THF (140 mL), cooled to 0°C and treated with a stream of gaseous NH_3 for 35 min. The off-white suspension obtained was concentrated to dryness, taken in water (15 mL), and filtered. The filtrate was cooled to 0°C and acidified with conc. HCl and the precipitated product, **8** (4.36 g, 88%), was filtered out and dried. An analytical sample was obtained by recrystallization from isopropyl alcohol. M.p. 150-152°C. IR, ν (cm^{-}

^{1}): 3374, 3196, 1707 (acid CO), 1654 (amide I CO), 1584 (amide II). ^{1}H NMR (DMSO-d_6), δ (ppm): 1.63-1.78 (4H, m, 4-H_2+5-H_2), 1.79 (1H, dt, J_d = 12.6 Hz, J_t = 10.0 Hz, 2-HH), 2.01 (1H, dt, J_d = 12.6 Hz, J_t = 7.8 Hz, 2-HH), 2.57 (1H, dq, J_d = 9.9 Hz, J_q = 7.9 Hz, 3-H), 2.66 (1H, dq, J_d = 9.9 Hz, J_q = 7.9 Hz, 1-H), 6.72 and 7.23 (1H+1H, 2 br s, exchang. in D_2O, NH_2), 12.03 (1H, br s, exchang. in D_2O, OH).

(±)-*cis* Methyl 3-(aminocarbonyl)cyclopentanecarboxylate (9). A solution of **8** (2.82 g, 17.9 mmol) in dry THF (50 mL) was treated with 0.5 M ethereal diazomethane[11] (70 ml, 35 mmol). After 2 h the solvent was evaporated *in vacuo* to afford **9** (3.0 g, 100%) as a white solid. An analytical sample was obtained by recrystallization from toluene. M.p. 119-120°C. IR, ν (cm^{-1}): 3357, 3183, 1734 (ester CO), 1636 (amide I CO), 1432 (amide II). ^{1}H NMR ($CDCl_3$), δ (ppm): 1.91-2.06 (4H, m, 4-H_2+5-H_2), 2.09-2.28 (2H, m, 2-H_2), 2.74 (1H, quint, J = 8.4 Hz, 3-H), 2.88 (1H, dq, J_d = 6.8 Hz, J_q = 8.0 Hz, 1-H), 3.70 (3H, s, CH_3), 5.30 and 5.99 (1H+1H, 2 br s, exchang. in D_2O, NH_2).

(±)-*cis*-3-(Aminomethyl)cyclopentylmethanol (4). To a refluxing solution of amido ester **9** (1.93 g, 11.3 mmol) in dry THF (12 mL), 10M borane dimethylsulfide complex in DMS (2.5 mL, 25 mmol) was added dropwise. The mixture was refluxed for a further 2 h, and then left for 2 h at room temperature. Then, 6N HCl (2 mL) was added and the mixture was refluxed for 15 min, treated with solid NaOH (0.68 g, 17 mmol) and stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was dispersed in methanol and filtered. The filtrate was concentrated to an oil, which was column chromatographed (eluant: 1:1 ethyl acetate/methanol). Amino alcohol **4** (1.11 g, 76%) was isolated as a clear viscous liquid. IR, ν (cm^{-1}): 3356 (OH), 2939, 1047. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.80-0.92 (1H, m, 2-HH), 1.22-1.42 (2H, m), 1.53 (3H, br s, exchang. in D_2O, NH_2+OH), 1.72-1.82 (2H, m), 1.92-2.03 (2H, m), 2.15 (1H, sept, J = 6.9 Hz, 1-H), 2.65 (2H, d, J = 6.5 Hz, CH_2N), 3.54 (2H, d, J = 6.6 Hz, CH_2O).

(±)-*cis*-3-[(2-Amino-6-chloropyrimidin-4-yl)aminomethyl]cyclopentylmethanol (10). A solution of **4** (2.35 g, 18.2 mmol), 2-amino-4,6-dichloropyrimidine (4.48 g, 27.3 mmol) and triethylamine (11 mL) in dry 1-butanol (73 mL) was refluxed under a dry atmosphere for 48 h, whereupon the reaction mixture was cooled and the solvent was removed *in vacuo*. The residue remaining was column chromatographed (eluants: (i) 40:1 and (ii) 30:1 chloroform/methanol). The more polar mixture eluted **10**, which was isolated as an oil (3.64 g, 78%) after redissolving the crude residue in dry acetone, filtering and eliminating the solvent *in vacuo*. IR, ν (cm^{-1}): 3322, 1708, 1583, 1470. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.97 (1H, dt, J_d = 12.4 Hz, J_t = 8.9 Hz, 2-

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HH), 1.23-1.51 (2H, m), 1.71-1.86 (2H, m), 1.94-2.05 (1H, m), 2.12-2.23 (2H, m), 3.09-3.29 (3H, m, 1H exchang. in D_2O, CH_2N+OH), 3.54 and 3.58 (2H, AB system, J_{AB} = 9.2 Hz, both parts additionally split as d, J = 5.1 Hz, CH_2O), 4.96 (3H, br s, exchang. in D_2O, NH_2+NH), 5.78 (1H, s, 5'-H).

(±)-*cis*-3-{[2-Amino-6-chloro-5-(4-chlorophenylazo)pyrimidin-4-yl]amino-methyl}cyclopentylmethanol (11). 4-Chloroaniline (2.08 g, 16.3 mmol) in 3N HCl (35 mL) was treated at 0°C with $NaNO_2$ (1.2 g, 17.7 mmol) in water (14 mL). The diazonium salt obtained was added to a mixture of **10** (3.64 g, 14.2 mmol), acetic acid (70 mL), water (70 mL) and sodium acetate trihydrate (28 g, 200 mmol) and stirred overnight at room temperature. The precipitate was filtered out, washed with water until the washings were neutral, and dried to afford **11** (5.19 g, 93%) as a yellow solid. An analytical sample was obtained by double recrystallization from 1:2 acetone/methanol. M.p. 209-211°C. IR, ν (cm^{-1}): 3309, 3188, 1643, 1572, 1480. ^1H NMR ($CDCl_3$), δ (ppm): 1.05 (1H, dt, J_d = 12.6 Hz, J_t = 8.9 Hz, 2-HH), 1.39-1.53 (2H, m), 1.71 (1H, br s, exchang. in D_2O, OH), 1.78-1.89 (2H, m), 2.06 (1H, q, J = 4.2 Hz), 2.18-2.31 (2H, m), 3.45-3.52 (2H, m, CH_2N), 3.55-3.62 (3H, m, 1H exchang. in D_2O, CH_2O+NH), 5.30 (2H, br s, exchang. in D_2O, NH_2), 7.44 (2H, d, J = 8.8 Hz, 3''-H+5''-H), 7.71 (2H, d, J = 8.8 Hz, 2''-H+6''-H).

(±)-*cis*-3-[(2,5-Diamino-6-chloropyrimidin-4-yl)aminomethyl]cyclopentyl-methanol (12). A mixture of **11** (1.6 g, 4.1 mmol), Zn powder (2.65 g, 40.5 mmol), acetic acid (1.3 mL), water (61 mL) and ethanol (61 mL) was refluxed under argon for 3 h. Then, the reaction mixture was filtered, the solvent was removed *in vacuo* and the residue was column chromatographed (eluant: 15:1 chloroform/methanol). Compound **12** (0.94 g, 85%) was isolated as a reddish solid. M.p. 79-80°C. IR, ν (cm^{-1}): 3332, 1576, 1508, 1457. ^1H NMR ($CDCl_3$), δ (ppm): 1.04 (1H, dt, J_d = 12.8 Hz, J_t = 8.2 Hz, 2-HH), 1.28-1.56 (3H, m, 1H exchang. in D_2O), 1.70-1.88 (4H, m, 2H exchang. in D_2O), 1.98 (1H, dt, J_d = 12.8 Hz, J_t = 7.6 Hz, 2-HH), 2.14-2.25 (2H, m), 3.30 and 3.43 (2H, AB system, J_{AB} = 13.3 Hz, part A additionally split as a dd, J = 6.4 Hz and J = 5.7 Hz, and part B additionally split as a dd, J = 8.0 Hz and J = 6.4 Hz, CH_2N), 3.55 and 3.61 (2H, AB system, J_{AB} = 10.6 Hz, both parts additionally split as d, J = 6.1 Hz, CH_2O), 4.89 (2H, br s, exchang. in D_2O, NH_2), 5.56 (1H, br s, exchang. in D_2O, NH).

(±)-*cis*-3-(2-Amino-6-chloro-9*H*-purin-9-ylmethyl)cyclopentylmethanol (13). A mixture of **12** (1.3 g, 4.8 mmol), triethyl orthoformate (26 mL) and 12N HCl (1.2 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 one hour. The reaction mixture was adjusted to pH 8 using 1N NaOH, and the solvent

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was evaporated *in vacuo*. The crude product was column chromatographed (eluant: 20:1 chloroform/methanol). Compound **13** (0.42 g, 61%) was isolated as a white solid. An analytical sample was obtained by recrystallization from ethyl acetate. M.p. 149-150°C. ^{1}H NMR ($CDCl_3$), δ (ppm): 1.06 (1H, dt, J_d = 12.6 Hz, J_t = 9.5 Hz, 2-HH), 1.36-1.55 (3H, m, 1H exchang. in D_2O), 1.76-1.84 (2H, m), 1.93 (1H, dt, J_d = 12.6 Hz, J_t = 7.4 Hz, 2-HH), 2.16-2.26 (1H, m, 1-H), 2.47-2.57 (1H, m, 3-H), 3.55 and 3.62 (2H, AB system, J_{AB} = 10.1 Hz, part A additionally split as a d, J = 5.5 Hz, and part B additionally split as a d, J = 5.1 Hz, CH_2O), 4.15 (2H, d, J = 7.6 Hz, CH_2N), 7.98 (1H, s, 8'-H), 9.52 (1H, d, J = 10.6 Hz, exchang. in D_2O, NHH), 11.35 (1H, d, J = 10.6 Hz, exchang. in D_2O, NHH).

(±)-*cis*-2-Amino-6,9-dihydro-9-[3-(hydroxymethyl)cyclopentylmethyl]-1*H*-purin-6-one (14). A mixture of **13** (0.78 g, 2.7 mmol) and 0.33N NaOH (61 mL) was refluxed for 5 h, whereupon the solvent was removed *in vacuo*. The residue was column chromatographed (eluant: 5:1 chloroform/methanol) and compound **14** (0.63 g, 88%) was isolated as a white solid. An analytical sample was obtained by recrystallization of the crude from 1:2 methanol/water. M.p. 272-274°C. ^{1}H NMR (DMSO-d_6), δ (ppm): 0.87 (1H, dt, J_d = 12.5 Hz, J_t = 9.5 Hz, 2'-HH), 1.17-1.37 (2H, m), 1.46-1.62 (2H, m), 1.66 (1H, dt, J_d = 12.5 Hz, J_t = 7.3 Hz, 2'-HH), 1.92-2.02 (1H, m, 1'-H), 2.28-2.39 (1H, m, 3'-H), 3.25 (2H, t, J = 5.8 Hz, collapses to a d with J = 6.5 Hz after exchang. in D_2O, CH_2O), 3.85 (2H, d, J = 7.6 Hz, CH_2N), 4.52 (1H, t, J = 5.1 Hz, exchang. in D_2O, OH), 6.43 (2H, br s, exchang. in D_2O, NH_2), 7.67 (1H, s, 8-H), 10.49 (1H, s, exchang. in D_2O, NH).

(±)-*cis*-3-(2,6-Diamino-9*H*-purin-9-ylmethyl)cyclopentylmethanol (15). Compound **13** (0.78 g, 2.7 mmol) was suspended in methanol (8 mL) in a high pressure reactor equipped with a solid CO_2/acetone cooling bath. Liquid ammonia (30 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 was column chromatographed (eluant: 1:1 chloroform/methanol). Compound **15** (0.38 g, 52%) was isolated as a white solid. An analytical sample was obtained by recrystallization from methanol. M.p. 178-180°C. ^{1}H NMR (DMSO-d_6), δ (ppm): 0.90 (1H, dt, J_d = 12.3 Hz, J_t = 9.5 Hz, 2-HH), 1.20-1.40 (2H, m), 1.48-1.63 (2H, m), 1.67 (1H, dt, J_d = 12.3 Hz, J_t = 7.3 Hz, 2-HH), 1.93-2.03 (1H, m, 1-H), 2.34-2.44 (1H, m, 3-H), 3.25 (2H, m, d after exchang. in D_2O, J = 6.5 Hz, CH_2O), 3.86 (2H, d, J = 7.5 Hz, CH_2N), 4.47 (H, t, J = 4.5 Hz, exchang. in D_2O, OH), 5.75 (2H, s, exchang. in D_2O, 6'-NH_2), 6.61 (2H, s, exchang. in D_2O, 2'-NH_2), 7.75 (1H, s, 8'-H).

(±)-*cis*-3-(5-Amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-cyclopentylmethanol (16). Sodium nitrite (0.75 g, 10.9 mmol) in water (18 mL) was

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added to a cooled (0°C) solution of **12** (2.49 g, 9.1 mmol) in acetic acid (14 mL) and water (14 mL), and stirred for 3 h. Compound **16** (2.27 g, 87%) precipitated from the reaction mixture as a white solid, which was filtered out, washed with cold water and dried *in vacuo*. An analytical sample was obtained by recrystallization from methanol. M.p. 129-130°C. ^{1}H NMR ($CDCl_3$), δ (ppm): 1.09 (1H, dt, J_d = 13.0 Hz, J_t = 8.5 Hz, 2-HH), 1.45-1.57 (2H, m), 1.62 (1H, br s, exchang. in D_2O, OH), 1.73-1.82 (2H, m), 1.89 (1H, dt, J_d = 13.0 Hz, J_t = 7.4 Hz, 2-HH), 2.14-2.25 (1H, m, 1-H), 2.61-2.72 (1H, m, 3-H), 3.57 (2H, m, d after exchang. in D_2O, J = 6.0 Hz, CH_2O), 4.43 (2H, d, J = 7.8 Hz, CH_2N), 5.46 (2H, br s, exchang. in D_2O, NH_2).

(±)-*cis*-5-Amino-6,7-dihydro-3-[3-(hydroxymethyl)cyclopentylmethyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (17). A mixture of **16** (0.5 g, 1.8 mmol) and 0.25N NaOH (22 mL) was refluxed for 3 h, whereupon the reaction mixture was cooled and adjusted to pH 3 with 6N HCl. The precipitated solids were filtered out, washed with cold water until the washings were neutral, and dried *in vacuo* over phosphorus pentoxide to yield **17** as an off-white solid (0.33 g, 72%). An analytical sample was obtained by recrystallization from water. M.p. 229-230°C. ^{1}H NMR (DMSO-d_6), δ (ppm): 0.94 (1H, dt, J_d = 12.6 Hz, J_t = 9.4 Hz, 2'-HH), 1.27-1.39 (2H, m), 1.51-1.64 (2H, m), 1.70 (1H, dt, J_d = 12.6 Hz, J_t = 7.5 Hz, 2'-HH), 1.93-2.03 (1H, m, 1-H), 2.39-2.49 (1H, m, 3-H), 3.26 (2H, t, J = 5.8 Hz, collapses to a d with J = 6.6 Hz after exchang. in D_2O, CH_2O), 4.19 (2H, d, J = 7.3 Hz, CH_2N), 4.45 (1H, t, J = 5.1 Hz, exchang. in D_2O, OH), 6.90 (2H, br s, exchang. in D_2O, NH_2), 10.91 (1H, s, exchang. in D_2O, NH).

(±)-*cis*-3-(5,7-Diamino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)cyclopentylmethanol (18). Compound **16** (0.88 g, 3.1 mmol) was suspended in methanol (10 mL) in a high pressure reactor equipped with a CO_2/acetone cooling bath. Liquid ammonia (34 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 **18** (0.61 g, 75%) as a dark solid. A white, analytical sample was obtained by recrystallization from ethanol. M.p. 229-230°C. ^{1}H NMR (DMSO-d_6), δ (ppm): 0.95 (1H, dt, J_d = 12.6 Hz, J_t = 9.4 Hz, 2-HH), 1.28-1.39 (2H, m), 1.51-1.64 (2H, m), 1.70 (1H, dt, J_d = 12.6 Hz, J_t = 7.4 Hz, 2-HH), 1.93-2.03 (1H, m, 1-H), 2.42-2.52 (1H, m, 3-H), 3.26 (2H, dd, J = 6.2 Hz, J = 5.5 Hz, collapses to a d with J = 6.6 Hz after exchang. in D_2O, CH_2O), 4.19 (2H, d, J = 7.3 Hz, CH_2N), 4.46 (1H, t, J = 5.2 Hz, exchang. in D_2O, OH), 6.38 (2H, br s, exchang. in D_2O, 6'-NH_2), 7.39 and 7.65 (2H, 2 br s, exchang. in D_2O, 2'-NH_2).

Biological activity assays. For the methods used to assess antiviral activity and cytotoxicity, see ref. 12.

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