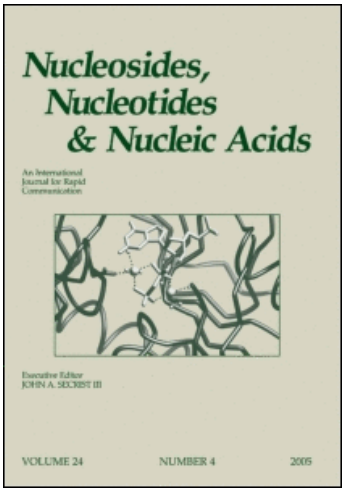

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Synthesis, Antiviral and Cytostatic Activities of Carbocyclic Nucleosides Incorporating a Modified Cyclopentane Ring. Part 2:[1] Adenosine and Uridine Analogues

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SYNTHESIS, ANTIVIRAL AND CYTOSTATIC ACTIVITIES OF CARBOCYCLIC NUCLEOSIDES INCORPORATING A MODIFIED CYCLOPENTANE RING. PART 2:[1] ADENOSINE AND URIDINE ANALOGUES.

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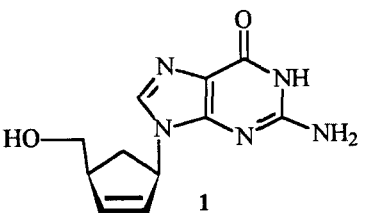
Abstract: Six new carbocyclic nucleosides were prepared by mounting a purine (compounds **5-7**), 8-azapurine (compounds **9** and **10**) or pyrimidine (compound **13**) base on the amino group of (1*R,cis*)-3-(aminomethyl)-1,2,2-trimethylcyclopentylmethanol (**2**). The antiviral activity of compounds **5-7**, **10** and **13**, and their cytostatic activity, were evaluated. At subtoxic concentrations, the compounds showed no or marginal antiviral activity. Compound **5** showed moderate inhibition on tumor cell proliferation.

In the search for new antitumor and antiviral therapeutic agents, much recent attention has been focused on carbocyclic nucleosides.[2] The potent antiviral properties displayed by carbovir (**1**)[3] and aristeromycin (**2**)[4] prompted us to search for congeners of these compounds with a modified cyclopentane moiety that might have similar or improved antiviral properties. Previously, we have prepared carbocyclic analogues of guanine and 8-azaguanine homo-nucleosides with a trimethylcyclopentane ring and evaluated their antiviral and antineoplasic activities.[1] In the present work, we report the synthesis and biological evaluation of parallel carbocyclic analogues of adenine, 8-azaadenine and uracil homo-nucleosides.



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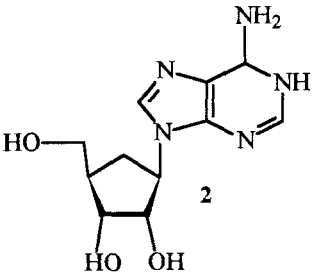
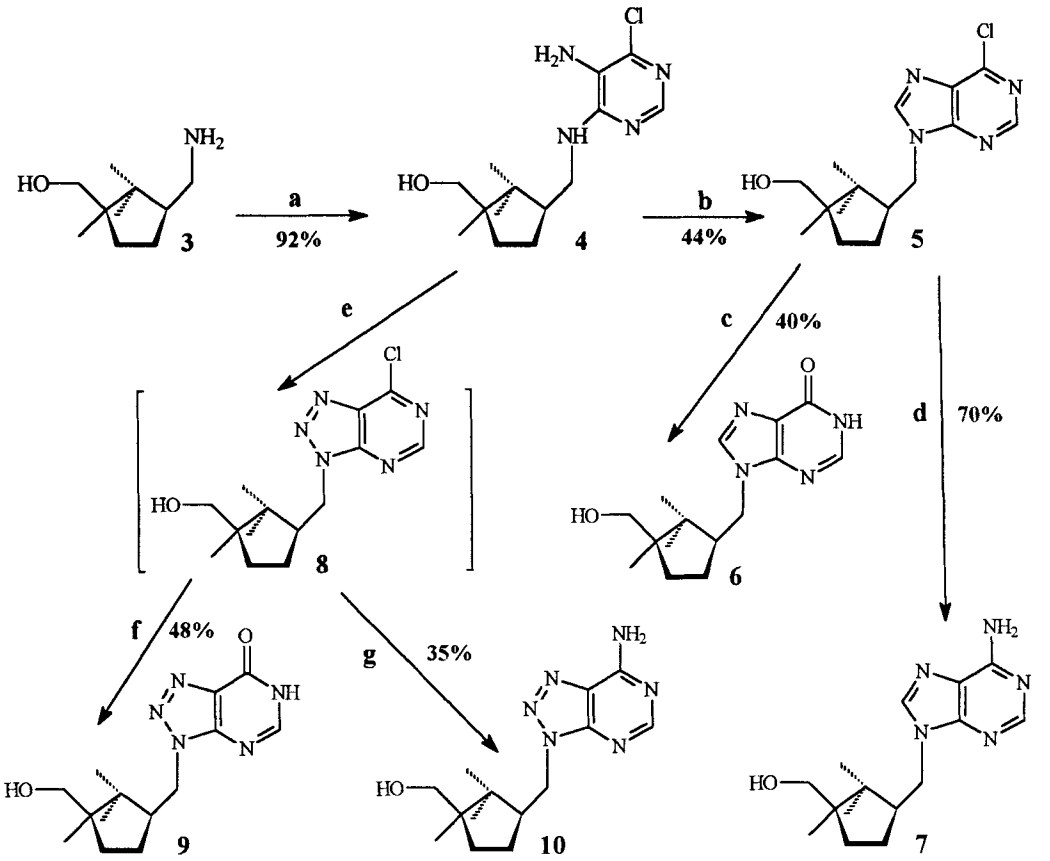


2

The synthesis of adenosine and 8-azaadenosine analogues is detailed in Scheme 1. In both cases, the base was constructed on the amino group of the precursor (1*R*,*cis*)-3-(aminomethyl)-1,2,2-trimethylcyclopentylmethanol (**3**).[5] Briefly, **3** was condensed with 5-amino-4,5-dichloropyrimidine following already described methods;[6] then cyclization of the resulting pyrimidinylamino compound **4** in triethyl orthoformate gave the 9-substituted-6-chloropurine **5**, which was hydrolysed in dilute sodium hydroxide to the inosine analogue **6** or aminated to the adenine derivative **7** in liquid ammonia. To obtain the corresponding 8-aza analogues, the triazole ring was formed by diazotation of **4** with sodium nitrite in hydrochloric acid[7] or acetic acid, to afford the 6-chloro-8-azapurine analogue **8** (not isolated), which was left overnight in the aqueous medium to form the 8-azainosine analogue **9**, or converted to **10**, by reaction with boiling aqueous ammonia.

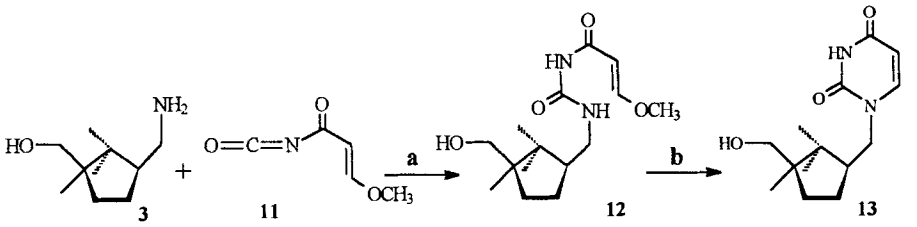
The uridine analogue was obtained by a route based on the acryloylurea variant[8] of the Shaw synthesis of 2,4-(1*H*,3*H*)-pyrimidinediones (Scheme 2). Briefly, 3-methoxypropenoyl isocyanate (**11**) (prepared and used under rigorously anhydrous conditions)[9] was reacted with **3** to obtain the acryloylurea **12**, which was then cyclized in basic solution by a conventional method,[10] affording **13** in 86% yield.

The activities of compounds **5-7**, **9**, **10** and **13** against a variety of DNA and RNA viruses, and also their cytotoxicities for several host cell lines, were evaluated, and the results were compared to the corresponding data for standard drugs with known antiviral activities (Tables 1 and 2). At subtoxic concentrations the compounds generally showed no activity or only marginal activity against the viruses tested. However, compound **5** showed a moderate activity against influenza virus type B (Table 2). Similarly, at subtoxic concentrations none of the compounds were active against HIV-1 or HIV-2 in CEM cells (data not shown).



a) 5-Amino-4,6-dichloropyrimidine, Et_3N, butanol, reflux, 44 h; b) $CH(OEt)_3$, 12 N HCl, r. t., 18 h; c) 0.33 N NaOH, reflux, 6.5 h; d) NH_3, MeOH, 78°C, 84 h; e) $NaNO_2$, AcOH or 1N HCl, 0°C;f) H_2O, r.t., 18 h; g) NH_4OH, reflux, 5 min.

Scheme 1



a) C_6H_6, DMF, r.t., overnight; b) 30%NH_4OH, reflux, 15 h.

Scheme 2

TABLE 1. Antiviral activity* and cytotoxicity** of compounds **5-7, 9, 10** and **13**

VIRUS (STRAIN)	CELL	5	6	7	9	10	13	Brivudine	Ribavirine	Ganciclovir	Acyclovir
HSV-1 (KOS)	E_6SM	>200	>200	>200	≥200	>100	>400	0.02	70	0.002	0.07
HSV-2 (G)	E_6SM	>200	>200	200	>200	>100	>400	100	100	0.001	0.07
Vaccinia	E_6SM	70	300	100	>200	70	400	2	20	>100	>100
Vesicular stomatitis	E_6SM	>200	>400	>200	>200	>100	>400	>400	7	>100	>100
TK^- HSV-1 (B2006)	E_6SM	40	>200	>200	>200	>100	>400	10	10	0.1	1
TK^-/TK^+ HSV-1 (VMW1837)	E_6SM	40	>200	40	>200	70	300	40	10	0.07	1
Cytotoxicity	E_6SM	400	>400	≥400	≥200	200	>400	>400	>400	>100	≥200
										(S)-DHPA[a]	C-c[3] Ado[b]
Vesicular stomatitis	Hela	40	>100	>40	>200	>40	>200	>200	20	>70	4
Coxsackie B4	Hela	>40	>100	>40	>200	>40	>200	>200	20	>400	>400
Respiratory syncytial	Hela	>40	>100	>40		>40	>200	>200	0.7	>400	>400
Cytotoxicity	Hela	100	≥200	≥100	≥200	>100	≥400	≥400	>400	>400	>400
Parainfluenza-3	Vero	>40	>200	>10	>400	>40	>400	>400	150	70	2
Reovirus-1	Vero	>40	>200	>10	>400	>40	>400	>400	70	70	0.7
Sindbis	Vero	>40	>200	>10	>400	>40	>400	>400	>400	>400	>400
Coxsackie B4	Vero	>40	>200	>10	>400	>40	>400	>400	>400	>400	>400
Punta Toro	Vero	>40	>200	>10	>400	>40	>400	>400	70	>400	>400
Cytotoxicity	Vero	≥100	≥400	≥40	>400	≥100	>400	>400	>400	>400	>400

* MIC_{50} or Minimun inhibitory concentration (μg/mL) required to reduce virus-induced cytopathogenicity 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 **5-7**, **10** and **13**

Influenza Virus Strain	CELL	5	6	7	10	13	Ribavirine	Rimantadine	Amantadine
H2N2 A2 Japan/305/57	MDCK	>250	>250	>250	>50	100	4.5	1.2	1.3
B Hong Kong/5/72	MDCK	10	>250	>250	>50	>250	4.9	>50	>250
H3N2 (X31)	MDCK	>50	>250	>250	>50	>250	5	4.9	3.3
Cytotoxicity	MDCK	> 250	> 250	> 250	250	200	> 250	> 250	> 250

*MIC_{50} or Minimum inhibitory concentration (μg/mL) required to reduce virus-induced cytopathogenicity by 50%.
**MCC or Minimumcytotoxic concentration (μg/mL) required to cause the microscopically detectable alteration of the normal cells morphology.
Cell line used: Madin-Darby canine kidney cells (MDCK).

TABLE 3. Inhibitory effects of compounds **5-7**, **9**, **10** and **13** on the proliferation of murine leukemia cells (L1210/0 and human T-lymphocyte (Molt4/C8) cells.

	IC_{50} (μg/mL)*					
Cell line	**5**	**6**	**7**	**9****	**10**	**13**
L1210/0	23 ± 1.9	> 200	108 ± 10	1.3 ± 0.8	≥ 200	> 200
Molt4/C8	3.8 ± 0.48	> 200	39 ± 6.8	1.6 ± 0.0	87 ± 13.8	> 200

*50% Inhibitory concentration, or concentration required to inhibit cell proliferation during the linear growth phase by 50%.
**At compound concentrations that ranged between 8 and 40 μg/mL the inhibitory effect became much less predominent, but started to increase again at 200 μg/mL.

Compounds **5-7**, **9**, **10** and **13** were also tested for cytostatic activity against two tumor cell lines. The most active compound was compound **5**, which was moderately effective in inhibiting the proliferation of human T-lymphocytes (Table 3).

Experimental

Silica gel (230 mesh) was purchased from Merck. 3-Methoxypropenoyl chloride was prepared from methyl 3-methoxypropenoate following a procedure similar to that used by Shaw to prepare the analogous 3-ethoxy derivative.[8,9] 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; ^{1}H NMR and ^{13}C NMR spectra were recorded in a Bruker AMX 300 spectrometer and mass spectra were recorded on a Kratos MS-59 spectrometer.

(1*R,cis*)-3-[(5-Amino-6-chloropyrimidin-4-yl)aminomethyl]-1,2,2-trimethylcyclopentylmethanol (4). Freshly prepared **3** (1.67 g, 9.76 mmol), 5-amino-4,6-dichloropyrimidine (2 g, 12.19 mmol), triethylamine (10.6 mL) and 1-butanol (53 mL) were refluxed under argon for 44 h. After evaporation of the volatile solvents, the residue (5.16 g) was pre-adsorbed on silica gel, packed on top of a silica gel column (155 g) and chromatographed with 7.5:1 CH_2Cl_2/MeOH as eluant, to isolate **4** (2.69 g, 92%) as a solid. An analytical sample was obtained by recrystallization from 9:1 EtOAc/hexane. M.p. 212-214°C. $[\alpha]_D^{25}$ +53.52 (*c* 1.05, MeOH). IR (KBr): 3345, 3255, 2956, 2870, 1654, 1586, 1458, 1424, 1017 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.77 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.15-1.27 (m, 2H), 1.44-1.49 (m, 1H), 1.85-1.89 (m, 1H), 2.09-2.14 (m, 1H), 3.17-3.26 (m, 2H), 3.31-3.43 (m, 2H), 4.34 (t, 1H, J = 4.9 Hz, D_2O exchang., OH) 5.03 (s, 2H, D_2O exchang., NH_2), 6.56 (t, 1H, J = 4.6 Hz, D_2O exchang., NH), 7.70 (s, 1H, H-2'). ^{13}C NMR (DMSO-d_6) δ: 18.28, 21.50, 23.88, 26.80, 33.91, 43.32, 43.95, 47.22, 48.56, 67.53, 123.71, 136.80, 146.02, 152.40. Anal. Calcd. for $C_{14}H_{23}ClN_4O$: C, 56.27; H, 7.76; N, 18.75. Found: C, 56.19; H, 7.93; N, 18.69.

(1*R,cis*)-3-(6-Chloro-9*H*-purin-9-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (5). A mixture of **4** (2.23 g, 7.49 mmol), triethyl orthoformate (41.3 mL, 368 mmol), and

12N HCl (0.50 mL) was stirred overnight. The resulting suspension was evaporated to dryness *in vacuo*, and the residue was treated with 0.5N HCl (156 mL) for 3 h at room temperature, whereupon the mixture was adjusted to pH 8 with 1N NaOH. The solvents were evaporated, and the crude product (7.56 g) was triturated in EtOH (30 mL), which was then filtered to remove undissolved NaCl. The EtOH was evaporated, to leave a pale yellow solid (3.15 g), which was chromatographed on silica gel (50 g) eluting with 9:1 EtOAc/hexane to isolate **5** (1.02 g, 44%) as a white solid. An analytical sample was obtained by recrystallization from 4:1 EtOAc/hexane. M.p. 139-141°C. $[\alpha]_D^{25}$ +35.86 (*c* 1.01, MeOH). IR (KBr) : 3412, 2961, 2872, 1593, 1560, 1402, 1329, 1184, 1026, 947 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.86 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.11-1.18 (m, 1H), 1.38-1.51 (m, 3H), 2.44-2.50 (m, 1H), 3.19 and 3.37 (AB part of ABX system, 2H, J_{AB} = 10.7 Hz, J_{AX} = 4.7 Hz, J_{BX} = 5.1 Hz, simplifies to a AB system on D_2O exchange, CH_2OH), 4.11 and 4.32 (AB part of a ABX system, 2H, J_{AB} = 13.5 Hz, J_{AX} = 10.4 Hz, J_{BX} = 4.8 Hz, CH_2N), 4.38 (t, 1H, J = 4.9 Hz, D_2O exchang., OH), 8.77 (s, 2H, purine H-2 + H-8). ^{13}C NMR (DMSO-d_6) δ: 18.16, 21.41, 23.52, 25.91, 33.53, 44.13, 45.96, 47.93, 48.62, 67.41, 131.17, 147.98, 149.30, 151.78, 152.32. Anal. Calcd. for: $C_{15}H_{21}ClN_4O$: C, 58.34; H, 6.85; N, 18.14. Found: C, 58.61; H, 7.02; N, 18.32.

(1*S,cis*)-6,9-Dihydro-9-[3-(hydroxymethyl)-2,2,3-trimethylcyclopentylmethyl]-1*H*-purin-6-one (6). A mixture of **5** (0.4 g, 1.30 mmol) and 0.33 N NaOH (27 mL) was refluxed for 6.5 h, whereupon the solvent was evaporated. The pale yellow foam obtained (1.27 g) was chromatographed on silica gel (28 g), eluting with 6:4 CH_2Cl_2/MeOH. Compound **6** (0.21 g, 40%) was isolated as white solid with m.p. 295-298°C. Recrystallization of this material from MeOH afforded an analytical sample with m.p. 297-299°C. $[\alpha]_D^{25}$ +39.67 (*c* 0.52, MeOH). IR (KBr): 3213, 2963, 2873, 1679, 1577, 1547, 1517, 1340, 1022 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.85 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.09-1.22 (m, 1H), 1.37-1.53 (m, 3H), 2.36-2.45 (m, 1H), 3.20 and 3.35 (m, 2H, AB system with J_{AB} = 10.7 Hz after D_2O exchange, CH_2OH), 3.95 and 4.17 (AB part of ABX system, 2H, J_{AB} = 13.4 Hz, J_{AX} = 10.1 Hz, J_{BX} = 4.9 Hz, CH_2N), 4.38 (t, 1H, D_2O exchang., OH), 8.03 and 8.12 (2s, 2H, purine H-2 + H-8), 12.21 (br. s, 1H, D_2O exchang., purine H-1). ^{13}CNMR (DMSO-d_6) δ: 18.14, 21.46, 23.48, 25.88, 33.51,

44.05, 45.33, 48.26, 48.63, 67.43, 124.23, 140.82, 145.74, 148.74, 157.07. Anal. Calcd. for: $C_{15}H_{22}N_4O_2$: C, 62.05; H, 7.64; N, 19.30. Found: C, 62.22, H, 7.53; N, 19.47.

(1*R,cis*)-3-(6-Amino-9*H*-purin-9-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (7). A solution of **5** (0.42 g, 1.36 mmol) in MeOH (4 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 78°C for 84 h. Evaporation of the ammonia and MeOH afforded crude **7** (0.44 g) as yellow crystals. Recrystallization of the crude product from H_2O afforded (0.26 g, 70%) of pure **7**. M.p. 251-253°C. $[\alpha]_D^{25}$ +32.92 (*c* 1.14, MeOH). IR (KBr): 3316, 3132, 2962, 1663, 1596, 1490, 1303, 1024 cm^{-1}. ^{1}NMR (DMSO-d_6) δ: 0.86 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.13-1.18 (m, 1H), 1.37-1.51, (m, 3H), 2.41-2.46 (m, 1H), 3.19 and 3.35 (m, 2H, AB system with J_{AB} = 10.8 Hz after D_2O exchange, CH_2OH), 3.95 and 4.16 (AB part of a ABX system, 2H, J_{AB} = 13.4 Hz, J_{AX} = 10.0 Hz, J_{BX} = 5.0 Hz, CH_2N), 4.37 (t, 1H, J = 4.8 Hz, D_2O exchang., OH), 7.15 (s, 2H, D_2O exchang., NH_2), 8.12 and 8.17 (2s, 2H, purine H-2 + H-8). ^{13}C NMR (DMSO-d_6) δ: 18.16, 21.44, 23.53, 26.00, 33.55, 44.02, 44.90, 48.05, 48.66, 67.45, 119.07, 141.32, 149.94, 152.68, 156.26. Anal. Calcd. for: $C_{15}H_{23}N_5O$: C, 62.26; H, 8.01; N, 24.20. Found: C, 62.42, H, 8.06; N, 24.12.

(1*S,cis*)-6,7-Dihydro-3-[3-(hydroxymethyl)-2,2,3-trimethylcyclopentylmethyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one (9). A solution of **4** (0.19 g, 0.64 mmol) in AcOH (2.13 mL) and H_2O (2 mL) cooled in on ice bath was treated with $NaNO_2$ (0.06 g, 0.86 mmol) in H_2O (1.5 mL). After 10 min the ice bath was removed and the suspension was left stirring 18 h at room temperature. The solvents were evaporated, and the pale yellow solid obtained (1.1 g) was chromatographed on silica gel (30 g), eluting with 1:0.5 EtOAc/hexane, to isolate **9** (90 mg, 48%) as a white solid. M.p. 220-222°C. $[\alpha]_D^{25}$ +30.00 (*c* 0.66, MeOH). IR (KBr): 2955, 1718, 1588, 1558, 1458, 1363, 1274, 1025 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.85 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.97-1.23 (m, 1H), 1.35-1.55 (m, 3H), 2.47-2.50 (m, 1H), 3.20 and 3.33 (m, 2H, AB system with J_{AB} = 10.6 Hz after D_2O exchange, CH_2OH), 4.31 and 4.54 (AB part of a ABX system, 2H, J_{AB} = 13.6 Hz, J_{AX} = 9.8 Hz, J_{BX} = 5.2 Hz, CH_2N), 4.39 (br. s, 1H, D_2O exchang., OH), 8.24 (s, 1H, H-5), 12.60 (br.s, 1H, D_2O exchang., H-6). ^{13}C NMR (DMSO-d_6) δ: 18.06, 21.36, 23.35, 25.99, 33.49, 44.11, 48.15, 48.56, 48.73, 67.42, 129.82, 148.88,

149.85, 155.83. EIMS *m/z* (%): 291 (M^+, 13), 274 (M^+-OH, 45), 261 (14), 260 (15), 219 (26), 204 (31), 178 (60), 176 (23), 162 (16), 151 (34), 150 (61), 139 (21), 138 (100), 124 (33), 123 (38), 96 (46), 95 (58), 93 (27), 91 (18), 81 (43), 79 (95), 67 (99), 55 (63). HR-EIMS *m/z*: Calcd for $C_{14}H_{21}N_5O_2$: 291.1696. Found: 291.1687.

(1*R,cis*)-3-(7-Amino-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-3-ylmethyl)-1,2,2-trimethylcyclopentylmethanol (10). A suspension of **4** (0.70 g, 2.34 mmol) in 1N HCl (5.1 mL) was treated with $NaNO_2$ (0.19 g, 2.80 mmol) at 0°C and stirred for 15 min. 30% NH_4OH (10.2 mL) was added, and the resulting suspension was refluxed for 5 min. The precipitate was filtered out, washed with H_2O and then air-dried in a fume hood to yield a solid (0.48 g), which was purified on silica gel (10 g) with 6:4 EtOAc/hexane as eluant to isolate **10** (0.24 g, 35%) as a white solid; an analytical sample was obtained by recrystallization of this material from EtOH/H_2O. M.p. 252-254°C. $[\alpha]_D^{25}$ +30.94 (*c* 0.50, MeOH). IR (KBr): 3353, 3119, 2960, 1664, 1603, 1580, 1329 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.83 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.14-1.19 (m, 1H), 1.40-1.52 (m, 3H), 2.53-2.56 (m, 1H), 3.20 and 3.38 (AB part of a ABX system, 2H, J_{AB} = 10.5 Hz, J_{AX} = 4.7 Hz, J_{BX} = 5.2 Hz, simplifies to a AB system on D_2O exchange, CH_2OH), 4.33 and 4.54 (AB part of a ABX system, 2H, J_{AB} = 13.6 Hz, J_{AX} = 9.7 Hz, J_{BX} = 5.4 Hz, CH_2N), 4.38 (t, 1H, J = 4.9 Hz, D_2O exchang., OH), 8.06 and 8.38 (2 br s, 2H, D_2O exchang., NH_2), 8.29 (s, 1H, H-5'). ^{13}C NMR (DMSO-d_6) δ: 18.10, 21.42, 23.44, 26.08, 33.56, 44.09, 48.04, 48.28, 48.61, 67.45, 124.14, 149.16, 156.57, 156.98. EIMS *m/z* (%): 290 (M^+, 7), 260 (M^+-[2 [CH_3], 16), 245 (M^+-[3 CH_3], 6), 177 (8), 161 (9), 160 (8), 149 (11), 137 (100), 123 (13), 95 (37), 94 (24), 81 (14), 79 (25), 69 (13), 67 (31), 55 (24). Anal. Calcd. for: $C_{14}H_{22}N_6O$: C, 57.91; H, 7.64; N, 28.94. Found: C, 58.03, H, 7.43; N, 29.12.

(1*S,cis*)-*N*-[(3-Hydroxymethyl-2,2,3-trimethyl)cyclopentylmethyl]-*N'*-(3-methoxypropenoyl)urea (12). Previously dried (at 100°C under vacuum over P_2O_5) silver cyanate (48.0 g, 320 mmol) was added to dry benzene (321 mL) in the dark under an argon atmosphere and the suspension was refluxed with vigorous stirring for 0.5 h. A solution of 3-methoxypropenoyl chloride (19.28 g, 160 mmol) in dry benzene (60 mL) was then added dropwise, and the resulting suspension was vigorously stirred while heating under reflux for a further 0.5 h. The mixture was allowed to settle at room

temperature for 3 h, and a portion of the supernatant (33 mL, theoretically containing 14.56 mmol of **11**) was transferred to a dry dropping funnel and added dropwise to a solution of **3** (2.49 g, 14.56 mmol) in dry dimethylformamide (68 mL) at -15°C. The mixture was allowed to warm to room temperature over 1 h, then stirred overnight at room temperature and concentrated under reduced pressure (oil pump) at a temperature below 40°C, driving off the solvent by repeated co-evaporation with EtOH. The residue (4.41 g) was chromatographed on silica gel (80 g) with 9:1 CH_2Cl_2/MeOH as eluant, and the **12**-containing fractions were pooled and re-chromatographed on a silica gel column (40 g) with 19:1 CH_2Cl_2/MeOH as eluant, to give compound **12** (1.39 g, 32 %) as a vitreous yellowish foam. IR (KBr): 3293, 2940, 2873, 1681, 1622, 1542, 1507, 1438, 1387, 1255, 1152, 1095 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.73 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.15-1.31 (m, 2H), 1.42-1.50 (m, 1H), 1.70-1.77 (m, 1H), 1.92-1.98 (m, 1H), 2.99 (ddd, 1H, J = 12.9, 8.9, 5.4 Hz, simplifies to the part A of a ABX system, J_{AB} = 12.9 Hz, J_{AX} = 8.9 Hz, after D_2O exchange, C*H*HNH), 3.15-3.37 (m, 3H) [after D_2O exchange it is resolved to 3.23 (B part of a ABX system, J_{AB} = 12.9 Hz, J_{BX} = 5.6 Hz, CH*H*NH), 3.21 and 3.35 (AB system, J_{AB} = 10.5 Hz, C*H*$_2$OH)], 3.67 (s, 3H, OCH_3), 4.31 (t, 1H, J = 4.9 Hz, D_2O exchang., OH), 5.51 (d, 1H, J = 12.3 Hz, COCH), 7.56 (d, 1H, J = 12.3 Hz, C*H*OCH_3), 8.44 (t, 1H, J = 5.4 Hz, D_2O exchang., CON*H*CH_2), 9.99 (s, 1H, D_2O exchang., CONHCO). ^{13}C NMR (DMSO-d_6) δ: 18.34, 21.38, 24.09, 26.24, 33.75, 38.98, 43.79, 48.00, 48.66, 58.33, 67.49, 98.26, 154.04, 163.12, 167.97. HR-EIMS *m/z*: Calcd for $C_{15}H_{26}N_2O_4$: 298.1893. Found: 298.1884.

(1*S*,*cis*)-1-[(3-Hydroxymethyl-2,2,3-trimethyl)cyclopentylmethyl]-1,2,3,4-tetrahydropyrimidine-2,4-dione (13). A suspension of **12** (0.30 g, 1.01 mmol) in 30% NH_4OH (13 mL) was heated under reflux for 15 h. The reaction mixture was concentrated *in vacuo* to a yellow solid (0.34 g), which was purified on silica gel (8 g) with 9:1 CH_2Cl_2/MeOH as eluant to isolate **13** (0.23 g, 86%) as a greenish solid. An analytical sample was obtained by recrystallization from EtOAc. M.p. 203-205°C. $[\alpha]_D^{25}$ +75.70 (*c* 1.1, MeOH). IR (KBr): 2962, 2872, 1711, 1670, 1459, 1377, 1335, 1235, 1029, 884, 549 cm^{-1}. ^1H NMR (Cl_3CD) δ: 0.86 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.32-1.52 (m, 2H), 1.57-1.66 (m, 1H), 1.77-1.84 (m, 1H), 2.17-2.22 (m, 1H), 3.47 and 3.62 (AB system, 2H, J = 10.7 Hz, C*H*$_2$OH), 3.51 and 3.87 (AB part of a ABX system, J_{AB} =

13.4 Hz, J_{AX} = 10.4 Hz, J_{BX} = 3.9 Hz, 2H, CH_2N), 3.66 (br. s, 1H, D_2O exchang., OH), 5.68 (d, 1H, J = 7.9 Hz, H-5), 7.14 (d, 1H, J = 7.9 Hz, H-6), 8.66 (br. s, 1H, D_2O exchang., NH). ^{13}C NMR (Cl_3CD) δ: 18.62, 21.40, 23.62, 26.50, 33.90, 44.80, 48.14, 48.78, 50.99, 69.59, 102.38, 144.87, 150.99, 163.56. Anal. Calcd. for: $C_{14}H_{22}N_2O_3$: C, 63.13; H, 8.33; N, 10.52. Found: C, 63.35, H, 8.43; N, 10.37.

Biological activity assays. Antiviral activity and cytotoxicity assays were carried out according to established procedures.[11]

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