Synthesis and Antiviral and Cytostatic Activities of Carbocyclic Nucleosides Incorporating a Modified Cyclobutane Ring

Part 1: Guanosine Analogues

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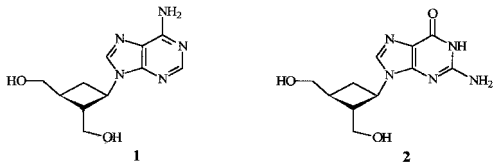
Summary

Five new carbocyclic nucleosides were prepared by constructing a guanine (compounds **3, 5**) or 8-azaguanine (compounds **4**, **6**, and **7**) base on the amino group of (1′*S*,3′*R*)-3-(3′-amino-2′,2′-dimethylcyclobutyl)propan-1-ol (**8**), and their activities against a variety of viruses and tumor cell lines were determined. Only compounds **3** and **7** showed a detectable activity at subtoxic concentrations against some viruses tested.

Introduction

There is growing interest in carbocyclic analogues of nucleosides (CANs) owing to their potential antineoplastic and antiviral properties [1]. For example, oxetanocin analogues **1** (Cyclobut-A) and **2** (Cyclobut-G) inhibit the replication of herpes simplex virus type 1 and type 2, varicella-zoster virus, and human cytomegalovirus, and have some activity against human immunodeficiency virus [2–6].



1 **2**

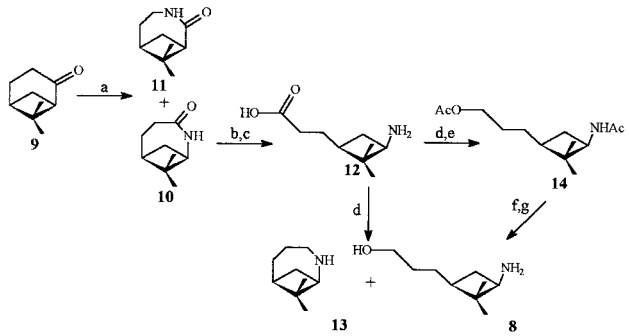
The synthesis of CANs usually involves construction of a natural or modified purine or pyrimidine base on an appropriate amino alcohol [7]. We are currently engaged in a research programme aimed at assessing the effects of structural parameters of the amino alcohol moiety on the biological activity of CANs [8]. In this work we report the preparation and biological evaluation of a series of guanosine analogues with a dimethylcyclobutane ring, **3–7**. These compounds are structurally related to Cyclobut-G (**2**).

Antiviral and/or cytotoxic activity of nucleoside antimetabolites is usually shown after they have undergone phosphorylation, and this step seems to be essential for activation of 2′,3′-didehydro-2′,3′-dideoxynucleosides, and their analogues with regard to anti-HIV action [9]. Although such activities are not observed after elongation of the 4′-hydroxymethyl group by carbons in normal nucleosides, cases are known of 4′-*homo*-carbocyclic nucleosides showing significant anti-HSV-1 activity [10].

Chemistry

Precursor amino alcohol **8** was prepared from nopinone (**9**) by modification of the previously established synthetic route [11]. Treatment of nopinone (**9**) with hydroxylamine-*O*-sulfonic acid in acetic acid led to a crude product that was chromatographed to afford lactam **10** (49% yield) and a small amount of the isomeric lactam **11** (5% yield).

Although the hydrolysis of **10** required forcing conditions, it produced amino acid hydrochloride salt in 99% yield, which was converted into free **12** by ion-exchange chromatography. In our modified approach, (Scheme 1) the carboxylic acid **12** was reduced directly, with $LiAlH_4$ in refluxing THF, rather than its methyl ester, thus obviating the use of diazomethane for preparation of the latter. Customary work-up of the crude product gave amino alcohol **8** in 28% yield, together with a small amount of amine **13** (12% yield) [12]. However, the yield of **8** could be improved by using an alternative work-up in which the crude product was first converted to the diacetyl derivative **14** (87%). This was then hydrolyzed in refluxing 2N HCl (22 h) to afford **8** in 83% yield.



Scheme 1. a) H_2NOSO_3H, AcOH, reflux; b) 12N HCl, reflux; c) Dowex 50Wx8-200, 14M NH_4OH; d) $LiAlH_4$, THF, reflux; e) Ac_2O, py., r.t.; f) 2N HCl, reflux; g) Amberlite IRA-400 (OH).

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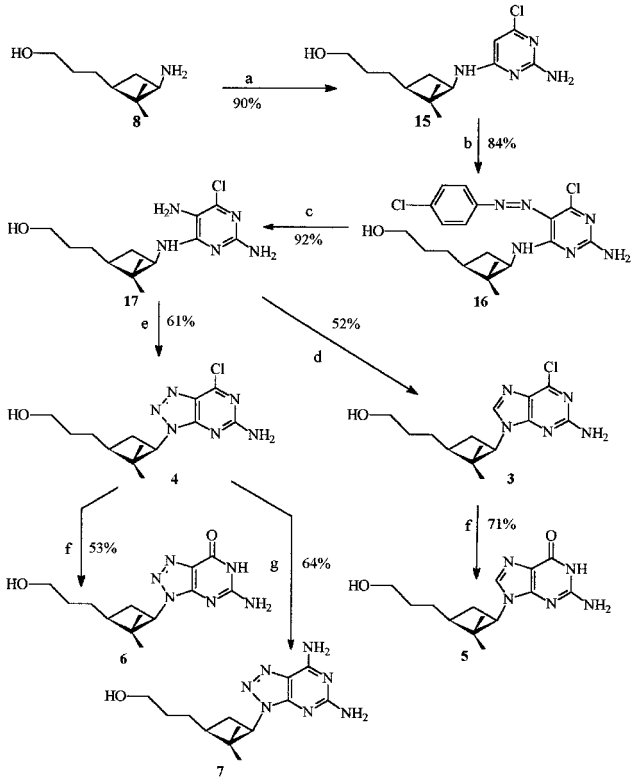

Scheme 2. a) 2-Amino-4,6-dichloropyrimidine, Et_3N, *n*-butanol, reflux; b) 4-chlorobenzenediazonium chloride, H_2O, AcOH, NaOAc, r.t.; c) Zn, AcOH, H_2O, EtOH, reflux; d) $CH(OEt)_3$,12N HCl, r.t.; e) $NaNO_2$, AcOH, H_2O, r.t.; f) 0.33N NaOH, reflux; g) NH_3, MeOH, 75 °C.

The synthesis of analogues **3–7** is detailed in Scheme 2; in all cases, well established methods [13] were used to construct a guanine or a modified guanine base on the amino group of (1′*S*,3′*R*)-3-(3′-amino-2′,2′-dimethylcyclobutyl)propan-1-ol (**8**). Briefly, **8** was condensed with 2-amino-4,6-dichloropyrimidine; the resulting pyrimidinylamino compound **15** was condensed with 4-chlorobenzenediazonium chloride to afford the 5-(4-chlorophenyl-azo)pyrimidine **16**, which was reduced with zinc in acetic acid to give the triaminopyrimidine **17**.

Then **17** was cyclized, either in triethyl orthoformate, which gave the 9-substituted 2-amino-6-chloropurine **3**, or in sodium nitrite/acetic acid, which gave the 5-amino-7-chloro-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine derivative **4** in good yield. The guanosine analogue **5**, and the corresponding 8-azaguanosine analogue **6**, were obtained from **3** and **4**, respectively, by hydrolysis in dilute sodium hydroxide**.** The 2,6-diamino-8-azapurinyl compound **7** was obtained by treatment of **4** with ammonia in a pressurized vessel.

Biological Evaluation

The new carbocyclic nucleosides **3**–**7** were evaluated for their antiviral activity in a wide variety of assay systems:

- At compound concentrations up to 400 μg/mL: herpes simplex virus type 1 (strain KOS), herpes simplex virus type 2 (strain G), thymidine kinase-deficient (TK^-) herpes simplex virus type 1 (strains B 2006 and VMW 1837), vaccinia virus and vesicular stomatitis virus in human embryonic skin-muscle fibroblasts (E_6SM); vesicular stomatitis virus, respiratory syncytial virus and Coxsackie B4 virus in human epithelial (HeLa) cells; parainfluenza virus type 3, reovirus type 1, sindbis virus and punta toro virus in African green monkey (Vero) kidney cells. Brivudine and ribavirin as well as acyclovir, ganciclovir and/or (*S*)-9-(2,3-dihydroxypropyl)adenine were used in parallel tests as reference drugs.
- At compound concentrations up to 250 μg/mL: influenza virus (strains H2N2 A2 Japan/305/57, B Hong Kong/5/72, H3N2 (X31) in Madin-Darby canine kidney (MDCK) cells. Ribavirin, rimantadine, and amantadine were used in parallel tests as reference drugs.
- At compound concentrations up to 200 μg/mL: human immunodeficiency virus (HIV) types 1 and 2 in T-lymphocyte (CEM/0) cells; cytotoxicity against host cells was correspondingly evaluated.
- At compound concentrations up to 50 μg/mL: cytomegalovirus (CMV, strains AD-169 and DAVIS) and varicella-zoster virus (VZV, strains OKA, YS, 07/1, and YS/R) in human embryonic lung (HEL) cells. Cidofovir and ganciclovir or brivudine and acyclovir were used in parallel tests as reference drugs.

Results and Discussion

The great majority of the compounds did not show an appreciable antiviral activity in these assays. However, the compounds **3** and **7** showed some activity against vaccinia virus, compound **3** (IC_{50}, 50 μg/mL) and compound **7** (IC_{50}, 60 μg/mL), TK HSV-1 (VMW 1837), compound **3** (IC_{50}, 40 μg/mL) and compound **7** (IC_{50}, 70 μg/mL), and Coxsackie B4 virus, compound **3** (IC_{50}, 60 μg/mL), at subtoxic concentrations. Activity found for some of the compounds tested against TK^- viruses suggests that, as in the case of their lower homologues [8], phosphorylation by a viral kinase is not a previous requisite for their activity.

In connection with anti-HIV tests, a weak cytotoxic activity against the host cells was detected for compounds **3** (CC_{50}, 64 ± 4 μg/mL), **4** (CC_{50}, 82 ± 1 μg/mL), **6** (CC_{50}, 116 ± 2 μg/mL) and **7** (CC_{50}, 87 ± 4 μg/mL). Compounds **3–7** were also tested for cytostatic activity against two tumor cell lines,

Table 1. Inhibitory effects of compounds **3–7** on the proliferation of murine leukemia (L1210/0) cells and human T-lymphocyte (Molt4/C8) cells.

Compounds	IC_{50} (g/mL)[a] L1210/0	Molt4/C8
3	87.5 ± 1.5	49.4 ± 15.6
4	106 ± 14	72.5 ± 6.9
5	>200	>200
6	178 ± 31	88.2 ± 5.3
7	96.8 ± 11.7	89.2 ± 1.1

[a] 50% Inhibitory concentration, or concentration required to inhibit cell proliferation during the linear growth phase by 50%

and except for compound **5**, showed some weak inhibition of cell proliferation (Table 1).

Acknowledgements

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Experimental Part

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 FT-IR 1640 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in a Bruker AMX 300 spectrometer, at 300 and 75 MHz, respectively. Microanalyses were performed by a Perkin-Elmer 240B Elemental Analyser. Biological assays on antiviral and cytotoxic activities and/or cytostatic activity against tumor cell lines were carried out using standard testing protocols [14].

(1R)-7,7-Dimethyl-2-azabicyclo[4.1.1]octan-3-one ***(10)*** *and (1R)-7,7-dimethyl-3-azabicyclo[4.1.1]octan-2-one* ***(11)***

A mixture of **9** (10 g, 72.35 mmol), glacial AcOH (260 mL) and hydroxylamine-*O*-sulfonic acid (12.68 g, 112.15 mmol) was stirred at 90 °C for 5 h. Once this mixture had cooled, H_2O (260 mL) was added and the organic layer was separated, washed with 10% $NaHCO_3$ and then brine, and dried over anhydrous Na_2SO_4. This solution was concentrated to a brown oil (8.06 g), which was column chromatographed on silica gel with 2:3 hexane/EtOAc as eluent. First to elute from the chromatography column was **10** (5.38 g, 49%), which was isolated as a white solid [11]. Next eluted **11** (0.60 g, 5%), which was also isolated as a white solid.

Compound **11**. An analytical sample of **11** was obtained by recrystallization from cyclohexane. Mp 69–71 °C. $[\alpha]_D^{25}$ 48.22 (*c* 0.62, MeOH). IR (KBr) cm^{-1}: 3286, 2925, 1644, 1472, 1359, 1142, 782. ^{1}H NMR ($CDCl_3$) δ: 1.04 and 1.35 (2 s, 3H+3H, >C(CH_3)$_2$), 1.78–1.91 (m, 1H), 2.15–2.29 (m, 2H), 2.24 (d, 1H, *J* = 12.41 Hz), 2.39 (dt, 1H, *J* = 7.48, 12.41 Hz,), 2.64 (dddd, 1H, *J* = 1.03, 2.18, 4.47, 6.41 Hz), 3.12 (dddd, 1H, *J* = 1.76, 6.22, 6.84, 14.14 Hz), 3.73 (dt, 1H, *J* = 4.48, 13.43 Hz,), 6.23 (br s, 1H, D_2O exch., NH). ^{13}C NMR ($CDCl_3$) δ: 20.00 (CH_2), 20.76 (CH_3), 26.80 (CH_2), 29.31 (CH_3), 39.68 (CH_2), 40.37 (C), 41.55 (CH), 53.57 (CH), 178.24 (C). *Anal.* $C_9H_{15}NO$: C, H, N.

(1′S,3′R)-3-(3′-Amino-2′,2′-dimethylcyclobutyl)propanoic Acid ***(12)***

Amino acid hydrochloride salt **12·HCl** was prepared from **10** [11]. A solution of **12·HCl** (0.65 g, 3.13 mmol) in water (27 mL) was deposited on top of a column of Dowex 50wX8-200 (11 mL of resin, 20 mL of water) and the column was eluted with water until the eluate had pH 6, and then with 14 M NH_4OH (100 mL). Concentration of the ammoniacal eluate under reduced pressure gave **12** (0.51 g, 95%). An analytical sample was prepared by recrystallization from an H_2O/acetone solvent pair. Mp 235–237 °C. IR (KBr) cm^{-1}: 2962, 2227, 1637, 1558, 1508, 1457, 1390, 781, 520. ^{1}H NMR (D_2O) δ: 0.87 and 0.93 (2 s, 3H+3H, >C(CH_3)$_2$), 1.30–1.53 (m, 3H), 1.58–1.66 (m, 1H), 1.88–1.93 (m, 2H), 2.14 (dt, 1H, *J* = 7.58, 10.91 Hz, C1′-H), 3.15 (dd, 1H, *J* = 7.79, 9.50 Hz, C3′-H). ^{13}C NMR (D_2O) δ: 14.97 (CH_3), 26.33 (CH_2), 27.76 (CH_3), 28.64 (CH_2), 35.81 (CH_2), 39.22 (CH), 41.16 (C), 51.26 (CH), 183.70 (C). *Anal*. $C_9H_{17}NO_2$: C, H, N.

(1′S,3′R)-3-(3′-Acetylamino-2′,2′-dimethylcyclobutyl)propyl Acetate ***(14)***

Amino acid **12** (0.50 g, 2.92 mmol) was added in two portions to a cooled (0 °C) suspension of $LiAlH_4$ (0.28 g, 7.30 mmol) in dry THF (7.50 mL) stirring under argon. The suspension was heated under reflux for 7 h and then stirred vigorously, cooled to 0 °C and quenched by slow, successive addition from a dropping funnel of water (12 mL) and 1N NaOH (6 mL). After a further 30 min stirring the solvents were evaporated and the resulting residue was dried by successive dissolution-evaporation cycles using absolute ethanol (25 mL) and then toluene (2 × 25 mL). The residue was stirred in Ac_2O (1.62 mL) and pyridine (1.62 mL) at room temperature for 20 h, and then the solvent was evaporated, the oil remaining was dissolved in CH_2Cl_2 (75 mL), washed with saturated $NaHCO_3$, and then H_2O, and dried over anhydrous Na_2SO_4. Concentration of this solution gave **14** (0.58 g, 87%) as an oil. An analytical sample was obtained by bulb-bulb distillation in a Kugelrohr apparatus (oven temp. 130–135 °C/0.01 Torr) (ref.[11] 133–135 °C).

(1′S,3′R)-3-(3′-Amino-2′,2′-dimethylcyclobutyl)propan-1-ol ***(8)***

A solution of **14** (0.56 g, 2.32 mmol) in 2 N HCl (60 mL) was heated under reflux for 22 h. The solvent was evaporated and the solid remaining was dried by three dissolution-evaporation cycles, using absolute ethanol (20 mL) and then toluene (2 × 20 mL) to form the corresponding azeotropes. The white solid obtained was the hydrochoride of **8** (0.47 g). Mp 160–164 °C (ref.[11] mp 162–164 °C).

The solid **8**HCl was dissolved in MeOH (40 mL) and passed through a column packed with Amberlite IRA-400 resin in OH^- form (16 mL). Concentration of the basic eluate under reduced pressure gave **8** (0.32 g, 83%) as a colorless oil that spontaneously crystallized. An analytical sample was obtained by recrystallization of this material from EtOH/Et_2O. Mp 79–81 °C (ref.[11] mp 79–81 °C).

(1′S,3′R)-3-[3′-(2-Amino-6-chloropyrimidin-4-yl)amino-2′,2′-dimethylcyclobutylpropan-1-ol ***(15)***

Freshly prepared **8** (3.34 g, 21.27 mmol), 2-amino-4,6-dichloropyrimidine (5.26 g, 32.07 mmol), triethylamine (18 mL), and *n*-butanol (88 mL) were heated under reflux in an argon atmosphere for 71 h. After evaporation of the volatile solvents, the residue was pre-adsorbed on silica gel, packed on top of a silica gel column (300 g), and chromatographed with 20:1 CH_2Cl_2/MeOH as eluant. The fractions containing product were concentrated to a syrup that crystallized spontaneously, affording **15** (5.53 g, 92%) as a white crystalline solid: Mp 48–49 °C. IR (KBr) cm^{-1}: 3322, 2936, 1582, 1459, 1367, 1245, 1158, 1055. ^{1}H NMR (DMSO-d_6) δ: 0.90 and 1.17 (2 s, 3H+3H, >C(CH_3)$_2$), 1.21–1.38 (m, 3H), 1.40–1.55 (m, 3H), 1.65–1.76 (m, 1H), 1.87 (br s, 1H, D_2O exch., OH), 2.39 (dt, 1H, *J* = 7.67, 10.65 Hz, C3′-H), 3.62 (t, 2H, *J* = 6.26 Hz, OCH_2), 4.94 (br s, 3H, D_2O exch., NH_2 + NH), 5.77 (s, 1H, pyrimidine C5-H). *Anal.* $C_{13}H_{21}ClN_4O$: C, H, N.

(1′S,3′R)-3-{3′-[2-Amino-6-chloro-5-(4-chlorophenylazo)pyrimidin-4-ylamino-2′,2′-dimethylcyclobutyl}propan-1-ol ***(16)***

4-Chlorobenzenediazonium chloride was prepared by mixing 4-chloroaniline (2.84 g, 22.27 mmol), 3 N HCl (44 mL) and $NaNO_2$ (1.70 g, 24.64 mmol) in cold H_2O (18 mL). This solution was added to a mixture of **15** (5.53 g, 19.57 mmol), AcOH (109 mL), H_2O (89 mL), and NaOAc3 H_2O (35.50 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 under a fume hood to yield **16** (6.93 g, 84 %). An analytical sample was obtained by recrystallization of the crude product from acetone. Mp 222–223.5 °C. IR (KBr) cm^{-1}: 3187, 2934, 1638, 1568, 1475, 1369, 1059, 835, 782. ^{1}H NMR (DMSO-d_6) δ: 0.97 and 1.12 (2 s, 3H+3H, >C(CH_3)$_2$), 1.24–1.41 (m, 4H), 1.47–1.57 (m, 1H), 1.64–1.69 (m, 1H), 2.29–2.37 (m, 1H), 3.34–3.37 (m, 2H), 4.23–4.31 (m, 1H), 4.37 (t, 1H, D_2O exch., *J* = 5.06 Hz, OH), 7.46 (s, 1H, D_2O exch., N*H*H), 7.56 (d, 2H, *J* = 8.64 Hz, C3-H + C5-H of 4-ClC_6H_4), 7.64 (s, 1H, D_2O exch., NH*H*), 7.71 (d, 2H, *J* = 8.64 Hz, C2-H + C6-H of 4-ClC_6H_4), 10.36 (d, 1H, D_2O exch., *J* = 8.06 Hz, NH). ^{13}C NMR (DMSO-d_6) δ: 16.67 (CH_3), 26.32 (CH_2), 29.22 (CH_3), 31.09 (CH_2), 32.05 (CH_2), 43.23 (C), 50.67 (CH), 61.02 (CH_2), 118.63 (C), 122.95 (CH), 129.70 (CH), 133.58 (C), 150.85 (C), 154.64 (C), 161.32 (C), 165.07(C) . *Anal*. $C_{19}H_{24}Cl_2N_6O$: C, H, N.

(1′S,3′R)-3-[3′-(2,5-Diamino-6-chloropyrimidin-4-yl)amino-2′,2′-dimethylcyclobutyl]propan-1-ol ***(17)***

A mixture of **16** (7.07 g, 16.71 mmol), zinc dust (11.66 g), AcOH (5.73 mL), H_2O (250 mL), and EtOH (250 mL) was heated under reflux in an argon atmosphere for 7 h. The zinc was filtered off, and the solvents were

evaporated to leave a solid residue (15.22 g), which was pre-adsorbed on silica gel, packed on top of a silica gel column (340 g), and chromatographed with 9:1 CH_2Cl_2/MeOH as eluant. The fractions containing product were concentrated to a pink solid (4.3 g, 86%). An analytical sample was obtained by recrystallization of the crude product from H_2O. Mp 128–130 °C. IR (KBr) cm^{-1}: 3509, 3308, 2929, 1574, 1500, 1437, 1248, 1055, 863. ^1H NMR ($CDCl_3$) δ: 0.89 and 1.17 (2 s, 3H+3H, >C(CH_3)$_2$), 1.22–1.55 (m, 5H, one of them D_2O exch., OH), 1.63–1.77 (m, 2H), 2.38 (dt, 1H, *J* = 7.68, 10.61 Hz, C1′-H), 2.70 (s, 2H, D_2O exch., NH_2), 3.63 (t, 2H, *J* = 6.22 Hz, OCH_2), 4.08 (dt, 1H, *J* = 8.05, 9.71 Hz, C3′-H), 4.59 (s, 2H, D_2O exch., NH_2), 5.40 (d, 1H, D_2O exch., *J* = 7.91 Hz, NH). ^{13}C NMR ($CDCl_3$) δ: 16.15 (CH_3), 26.63 (CH_2), 29.57 (CH_3), 31.27 (CH_2), 32.24 (CH_2), 39.51 (CH), 43.95 (C), 51.61 (CH), 63.21 (CH_2), 111.38 (C), 148.71 (C), 158.01 (C), 159.22 (C). *Anal*. $C_{13}H_{22}ClN_5O$: C, H, N.

(1′S,3′R)-3-[3′-(2-Amino-6-chloro-9H-purin-9-yl)-2′,2′-dimethylcyclobutyl]propan-1-ol ***(3)***

A mixture of **17** (1.50 g, 5.00 mmol), triethyl orthoformate (28.85 mL) and 12 N HCl (1.35 mL) was stirred overnight. The resulting suspension was evaporated to dryness *in vacuo*, and the residue was treated with 0.5 N HCl (39 mL) for 4 h at room temperature, whereupon the mixture was adjusted to pH 8 with 0.5 N NaOH and the solvents were evaporated. The crude product (4.62 g) was triturated in $CHCl_3$ (10 mL), undissolved NaCl was filtered off, and the $CHCl_3$ was evaporated. The pale yellow residue (1.36 g) was chromatographed on silica gel (35 g) using 20:1 $CHCl_3$/MeOH as eluant, and **3** (0.81 g, 52%) was isolated as a white solid. An analytical sample was obtained by recrystallization of this material from EtOH/H_2O. Mp 161–163 °C. $[\alpha]_D^{25}$ 139.65 (*c* 0.58, MeOH). IR (KBr) cm^{-1}: 3328, 2933, 1612, 1562, 1465, 1403, 1239, 1058, 914. ^1H NMR (DMSO-d_6) δ: 0.67 and 1.23 (2 s, 3H+3H, >C(CH_3)$_2$), 1.29–1.41 (m, 4H), 1.83–1.85 (m, 1H), 2.35–2.42 (m, 2H), 3.34–3.40 (m, 2H), 4.36–4.42 (m, 2H, one of them D_2O exch., OH + C3′-H), 6.83 (s, 2H, D_2O exch., NH_2), 8.26 (s, 1H, C8-H). ^{13}C NMR (DMSO-d_6) δ: 16.11 (CH_2), 26.31 (CH_3), 27.99 (CH_3), 29.35 (CH_2), 31.16 (CH), 38.91 (CH_2), 44.35 (C), 54.33 (CH_2), 61.13 (CH), 123.91 (C), 142.57 (C), 149.57 (C), 154.81 (C), 160.06 (C). *Anal*. $C_{14}H_{20}ClN_5O$: C, H, N.

(1′S,3′R)-3-[3′-(5-Amino-7-chloro-3H-1,2,3-triazolo[4,5-d]pyrimidin-3-yl)-2′,2′-dimethylcyclobutyl]propan-1-ol ***(4)***

A cooled solution of **17** (2 g, 6.67 mmol) in AcOH (10.64 mL) and H_2O (53 mL) was treated with $NaNO_2$ (0.60 g, 8.69 mmol) in H_2O (35 mL) and stirred for 2 h at 0 °C and then 2 h at room temperature. The resulting solution was evaporated to dryness, and the solid residue (2.88 g) was chromatographed on silica gel (170 g) with 6:1 $CHCl_3$/MeOH as eluant. Compound **4** (1.27 g, 61%) was isolated as a pale yellow solid that after recrystallization from 1:1 hexane/EtOAc, had mp 106–108 °C. $[\alpha]_D^{25}$ 86.47 (*c* 0.51, MeOH). IR (KBr) cm^{-1}: 3320, 3204, 2939, 1647, 1606, 1564, 1515, 1450, 1430, 1391, 1245, 1192, 1004. ^1H NMR ($CDCl_3$) δ: 0.76 and 1.30 (2 s, 3H+3H, >C(CH_3)$_2$), 1.39–1.63 (m, 5H, one of them D_2O exch., OH), 1.91–2.04 (m, 1H, C4′-H), 2.55 (dt, 1H, *J* = 7.84, 11.21 Hz, C4′-H), 2.92 (virtual q, 1H, *J* = 10.66 Hz, C1′-H), 3.67–3.70 (m, 2H, OCH_2), 4.69 (dd, 1H, *J* = 8.13, 10.00 Hz, C3′-H), 5.43 (s, 2H, D_2O exch., NH_2). ^{13}C NMR ($CDCl_3$) δ: 16.64 (CH_2), 26.48 (CH_3), 27.77 (CH_3), 29.44 (CH_2), 31.07 (CH), 39.75 (CH_2), 45.53 (C), 58.33 (CH_2), 63.16 (CH), 130.13 (C), 152.65 (C), 154.28 (C), 161.00 (C). *Anal*. $C_{13}H_{19}ClN_6O$: C, H, N.

(1′R,3′S)-2-Amino-6,9-dihydro-9-[3′-(3-hydroxypropyl)-2′,2′-dimethylcyclobutyl]-1H-purin-6-one ***(5)***

Compound **3** (0.30 g, 0.97 mmol) was heated under reflux for 5 h in 0.33 N NaOH (20 mL), and then the solvent was evaporated. The resulting pale yellow foam (0.71 g) was chromatographed on silica gel (30 g), and eluted with 5:1 $CHCl_3$/MeOH. Compound **5** (0.20 g, 71%) was isolated as white solid that, after recrystallization from H_2O, had mp 303–305 °C. $[\alpha]_D^{25}$ 107.72 (*c* 0.61, MeOH). IR (KBr) cm^{-1}: 3386, 2932, 1705, 1636, 1601, 1560, 1474, 1361. ^1H NMR (DMSO-d_6) δ: 0.66 and 1.19 (2 s, 3H+3H, >C(CH_3)$_2$), 1.24–1.42 (m, 4H), 1.76–1.81 (m, 1H, C3′-H), 2.26–2.33 (m, 2H), 3.31–3.41 (m, 2H, OCH_2), 4.24–4.30 (m, 1H, C1′-H), 4.39 (t, 1H, D_2O exch., *J* = 5.12 Hz, OH), 6.32 (s, 2H, D_2O exch., NH_2), 7.70 (s, 1H, C8-H), 10.50 (s, 1H, D_2O exch., C1-H). ^{13}C NMR (DMSO-d_6) δ: 16.12 (CH_2), 26.33 (CH_3), 28.15 (CH_3), 29.41 (CH_2), 31.18 (CH), 39.02 (CH_2), 44.19 (C), 53.94 (CH_2), 61.16 (CH), 117.52 (C), 136.53 (C), 152.06 (C), 153.58 (C), 156.97 (C). *Anal*. $C_{14}H_{21}N_5O_2$: C, H, N.

(1′R,3′S)-5-Amino-6,7-dihydro-3-[3′-(3-hydroxypropyl)-2′,2′-dimethylcyclobutyl]-3H-1,2,3-triazolo[4,5-d]pyrimidin-7-one ***(6)***

A mixture of **4** (0.36 g, 1.16 mmol) and 0.33 N NaOH (14.5 mL) was heated under reflux for 3 h, whereupon its pH was adjusted to 3 with 6 N HCl. A gelatinous precipitate that had been formed was filtered off, washed with cold water, and then dried *in vacuo* over P_2O_5 to afford **6** (0.18 g, 52%) as an off-white solid. Recrystallization of this material from H_2O afforded an analytical sample with mp 261–263 °C. $[\alpha]_D^{25}$ 97.40 (*c* 0.50, MeOH). IR (KBr) cm^{-1}: 3286, 2926, 1716, 1639, 1602, 1573, 1458, 1365, 1305, 786, 680. ^1H NMR (DMSO-d_6) δ: 0.65 and 1.22 (2 s, 3H+3H, >C(CH_3)$_2$), 1.27–1.47 (m, 4H), 1.81–1.92 (m, 1H, C3′-H), 2.42 (dt, 1H, *J* = 7.9, 10.76 Hz, C4′-H), 2.63 (virtual c, 1H, *J* = 10.48 Hz, C4′-H), 3.34-3.39 (m, 2H, OCH_2), 4.39 (t, 1H, D_2O exch., *J* = 4.96 Hz, OH), 4.52 (dd, 1H, *J* = 8.25, 9.69 Hz, C1′-H), 6.83 (s, 2H, D_2O exch., NH_2), 10.89 (s, 1H, D_2O exch., NH). ^{13}C NMR (DMSO-d_6): δ: 16.11 (CH_2), 26.24 (CH_3), 27.27 (CH_3), 29.10 (CH_2), 30.59 (CH), 39.58 (CH_2), 44.49 (C), 56.77 (CH_2), 60.88 (CH), 124.43 (C), 151.61 (C), 155.16 (C), 156.18 (C). *Anal*. $C_{13}H_{20}N_6O_2$: C, H, N.

(1′S,3′R)-3-[3′-(5,7-Diamino-3H-1,2,3-triazolo[4,5-d]pyrimidin-3-yl)-2′,2′-dimethylcyclobutyl]propan-1-ol ***(7)***

A solution of **4** (0.24 g, 0.77 mmol) in MeOH (10 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 75 °C for 48 h. Evaporation of the ammonia and MeOH afforded crude **7** (0.21 g) as brown crystals, which were recrystallized from H_2O to afford pure **7** (0.17 g, 76%). Mp 174–176 °C. $[\alpha]_D^{25}$ 120.94 (*c* 0.64, MeOH). IR (KBr) cm^{-1}: 3338, 3200, 2937, 1657, 1593, 1494, 1421, 1355, 1049. ^1H NMR (DMSO-d_6) δ: 0.65 and 1.23 (2 s, 3H+3H, >C(CH_3)$_2$), 1.28–1.48 (m, 4H), 1.81–1.90 (m, 1H, C1′-H), 2.42 (dt, 1H, *J* = 7.90, 10.74 Hz, C4′-H), 2.70 (virtual q, 1H, *J* = 10.49 Hz, C4′-H), 3.39 (dd, 2H, *J* = 5.62, 10.88 Hz, OCH_2, on addition of D_2O, this signal simplifies to a triplet), 4.39 (t, 1H, D_2O exch., *J* = 5.16 Hz, OH), 4.55 (dd, 1H, *J* = 8.22, 9.87 Hz, C3′-H), 6.31 (s, 2H, D_2O exch., NH_2), 7.50 (br s, 2H, D_2O exch., NH_2). ^{13}C NMR (DMSO-d_6) δ: 16.24 (CH_2), 26.25 (CH_3), 27.34 (CH_3), 29.21 (CH_2), 30.87 (CH), 38.89 (CH_2), 44,49 (C), 56.33 (CH_2), 60.83 (CH), 120.50 (C), 152.01 (C), 156.19 (C), 162.77 (C). *Anal*. $C_{13}H_{21}N_7O$: C, H, N.

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[12] (1*R*)-7,7-Dimethyl-2-azabicyclo[4.1.1]octane (**13**). IR (film) cm^{-1}: 3352, 2950, 1558, 1464, 1364, 1319, 1015. ^{1}H NMR ($CDCl_3$) δ: 0.93 and 1.07 (2 s, 3H+3H, >C(CH_3)$_2$), 1.10–1.36 (m, 2H), 1.42–1.58 (m, 2H one of then D_2O exch., NH), 1.75–1.86 (m, 1H), 1.93–2.05 (m, 2H), 2.57 (t, 2H, *J* = 7.30 Hz), 3.50 (dd, 1H, *J* = 6.25, 10.84 Hz), 3.58 (ddd, 1H, *J* = 3.57, 7.79, 10.82 Hz). ^{13}C NMR ($CDCl_3$) δ: 16.90 (CH_2), 26.93 (CH_3), 31.38 (CH_3), 34.96 (CH_2), 39.95 (C), 40.52 (CH_2), 40.98 (CH), 44.93 (CH_2), 64.04 (CH). *Anal.* $C_9H_{17}N$: C, H, N.

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