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Norbornane-based nucleoside and nucleotide analogues locked in North conformation

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ABSTRACT

We report on the synthesis of novel conformationally locked nucleoside and nucleotide derivatives, which are structurally closely related to clinically used antivirals such as didanosine and abacavir. As a suitable conformationally rigid substitute of the sugar/pseudosugar ring allowing a permanent stabilization of the nucleoside in North conformation we employed bicyclo[2.2.1]heptane (norbornane) substituted in the bridgehead position with a hydroxymethyl group and in the C-3 position with a nucleobase. Prepared nucleoside derivatives were also converted into appropriate phosphoramidate prodrugs (ProTides) in order to increase delivery of the compounds in the cells. All target compounds were evaluated in a broad antiviral and cytostatic assay panel.

1. Introduction

Carbocyclic nucleoside and nucleotide derivatives have acquired enormous attention during recent years not only due to their success in the field of anti-HIV[1] and anti-HBV treatment,[2] represented by the clinically used antivirals abacavir and entecavir, but also promising results in the preclinical trials on several other viral diseases caused by HCV[3] and influenza virus.[4–6] Furthermore, Marquez and coworkers proved that the conformation of carbocyclic nucleosides can significantly influence their antiviral activity due to different conformational preferences of enzymes involved in the phosphorylation[7] of these nucleoside analogues to their triphosphates, and of polymerases[8] responsible for their incorporation into the polynucleotide chain. They also showed that several carbocyclic nucleosides with the pseudosugar ring locked in 2′-*exo* conformation, so called North conformation according to the position on the pseudorotational cycle,[9] exert significant antiviral activities. The most interesting compounds from the bicyclo[3.1.0]hexane series seems to be *N*-MCT (**1**), which possesses a highly selective inhibitory activity against herpes viruses (Fig. 1).[10] Lately, this type of locked nucleosides also found a new application as rigid ligands for adenosine receptors.[11] Several interesting locked carbocyclic nucleoside derivatives were also prepared as monomers for LNA technology.[12,13]

The antiviral activities of various nucleoside derivatives can be significantly enhanced by conversion to their monophosphate prodrugs.[14] Usefulness of the phosphoramidate (or ProTide) approach has recently been demonstrated on a number of various examples, for instance, abacavir's *L* counterpart, which possesses significant potency against HIV and HBV when converted into appropriate phosphoramidate prodrug.[15]

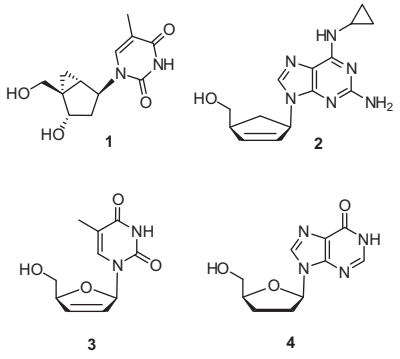

Figure 1. Structure of N-MCT **1**, abacavir **2**, stavudine **3** and didanosine **4**.

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Recently we have introduced bicyclo[2.2.1]heptane (norbornane) as a perspective novel pseudosugar pattern[16] and showed that 9-norbornylpurine derivatives dramatically reduce replication of picornaviruses, namely Coxsackievirus B3.[17–19] However, the most attractive norbornane derivatives mimicking anti-HIV compounds such as abacavir **2**, stavudine **3** or didanosine **4** locked in the North conformation have not been prepared yet. In this study we therefore explored possible synthetic routes toward nucleoside derivatives bearing a hydroxymethyl group in the norbornane's bridgehead position and a nucleobase in the 3-*exo* position. Besides these nucleosides we also prepared their phosphoramidate prodrugs.

2. Results and discussion

2.1. Chemistry

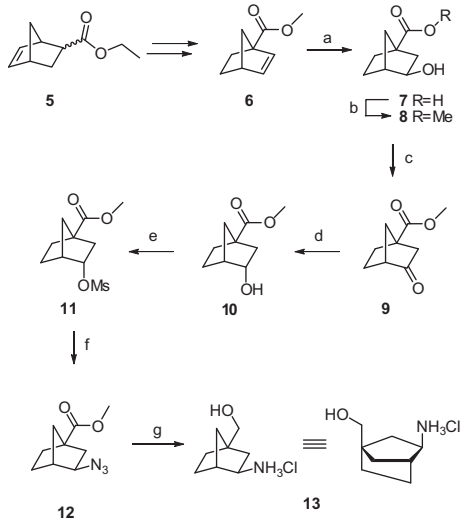
The main challenge in the synthesis of the bridgehead substituted norbornanes is the introduction of almost any functional group into the connection of the two rings and then modifying this functionality to our needs.

The whole synthetic pathway started from hydroxyester **8**, which was prepared in 7 steps from easily available derivative **5**.[20,21]

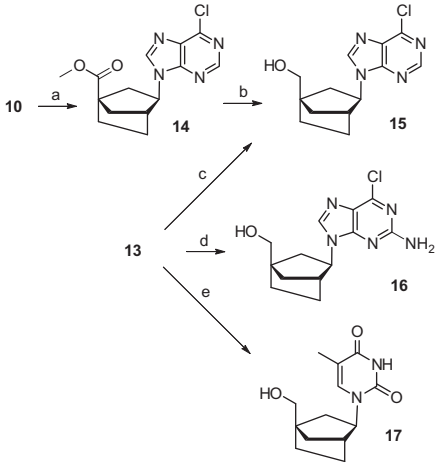
Configuration of the compound **8** at the C-3 hydroxy group was inverted by the means of a standard oxidation-reduction procedure and from the alcohol **10** the key amine substrate **13** was prepared in three simple steps. The *endo* hydroxyl group was mesylated and nucleophilically exchanged for an azido group, which was then reduced to an amino function with $LiAlH_4$. The C-1 methylester group was reduced to hydroxymethyl in this reaction step.

In spite of a literature precedent stating that azidomercuration reaction can be used to introduce an azido group directly into the *exo* position of a norbornane bicycle,[22] which would shorten this reaction sequence dramatically, in our case a similar reaction of compound **6** afforded only poor yields (10–20%) and led mostly to recovered starting material.

This fact, along with our original intent to introduce the nucleobase into the molecule via the Mitsunobu reaction using **10** as a substrate led us to choose this rather lengthy linear approach.



Scheme 1. (a) (1) $Hg(OAc)_2$, THF–H_2O, 2 h. (2) 3 M NaOH, 30 min, (3) $NaBH_4$, 3 M NaOH, 10 min, 67%; (b) CH_2N_2, 10 min, 99%; (c) PDC, DCM, overnight, 82%; (d) $NaBH_4$, MeOH, overnight, 88%; (e) MsCl, pyridine, 3 h, 99%; (f) NaN_3, DMF, 115 °C, overnight, 92%; (g) LAH, THF, 2 h, 59%.

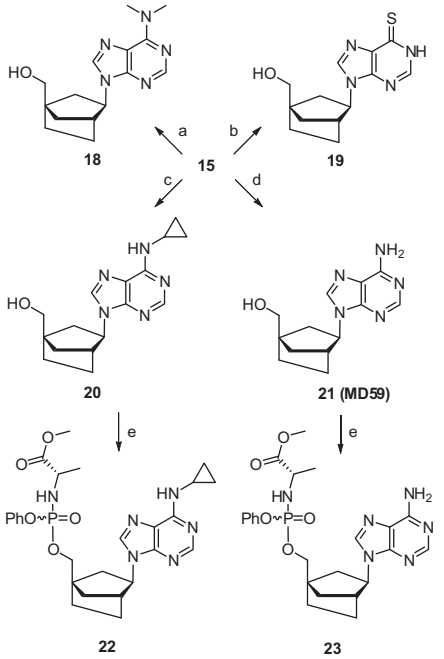


Scheme 2. (a) PPh_3, DIAD, 6-chloropurine, THF, reflux, 5 h, 67%; (b) DIBAL-H, DCM, −78 °C, 45 min, 72%; (c) 4,6-dichloro-5-formamidopyrimidine, DIPEA, *n*-BuOH, MW, 160 °C, 2 h, 69% (d) 2-amino-4,6-dichloro-5-formamidopyrimidine, DIPEA, EtOH/H_2O, MW, 140 °C, 1 h, 83%; (e) (1) ethyl [(2*E*)-3-ethoxy-2-methylprop-2-enoyl]carbamate, dioxane, 100°, 3 h, (2) Dowex 50 W (H^+), dioxane, 100 °C overnight, 69%.

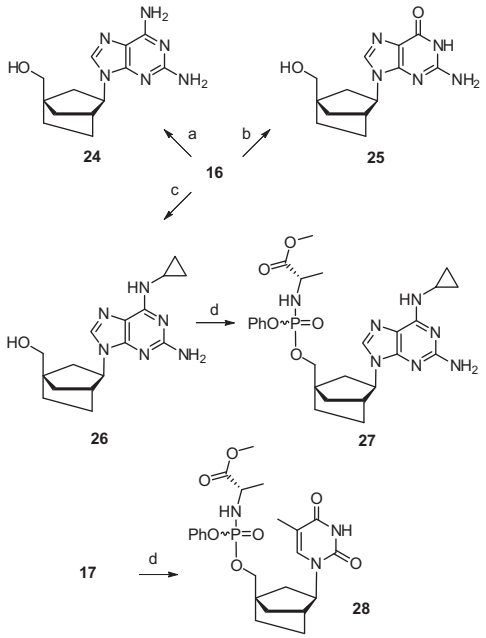
However, it must be stressed that none of the above listed intermediates had to be purified chromatographically (Scheme 1).

Two different approaches are in principle useable for the introduction of a purine nucleobase to the skeleton. Although the Mitsunobu reaction of **10** with 6-chloropurine was successful and subsequent reduction of the ester function with DIBAL-H afforded **15** in reasonable overall yield, similar reactions with 2,6-dichloropurine, 2-amino-6-chloropurine, thymine or *N*-3-benzoyl thymine either failed completely or their yields were lower than 10% and therefore unacceptable.

Much better results were achieved employing a nucleobase construction approach. A recently described one-pot microwave



Scheme 3. (a) $(CH_3)_2NH_2^+(CH_3)_2NCOO^-$, 24 h, 69%; (b) thiourea, EtOH, 105 °C, overnight, 77%; (c) cyclopropylamine, EtOH, 140 °C, 30 min, 80%; (d) NH_3, EtOH, MW. 120 °C, 30 min, 67%; (e) *t*-BuMgCl, phenylmethoxyalaninyl phosphochloridate, THF, 3 days, 38% for **22**, 59% for **23**.



Scheme 4. (a) NH_3, EtOH, MW, 120 °C, 30 min, 95%; (b) TFA, H_2O, 24 h, 60%; (c) cyclopropylamine, EtOH, MW, 140 °C, 30 min, 83%; (d) *t*-BuMgCl, phenylmethoxyalaninyl phosphochloridate, THF, 3 days, 55% for **27**, 29% for **28**.

assisted procedure[23] afforded good to very good yields of purine-based nucleoside analogues **15** and **16**, starting from the key amine **13**. Thymine nucleobase was prepared using the same substrate and standard construction protocol (Scheme 2).[24]

The C-6 position of purines **15** and **16** was derivatized to obtain a diverse spectrum of compounds, which might provide interesting leads on the structure-activity relationship.[25] Apart from the obvious adenosine **21**, and guanosine **25** analogues, also diaminopurine derivative **24**, 6-cyclopropylamino derivatives **20** and **26**, 6-dimethylamino derivative **18** and 6-thio derivative **19** were prepared (Scheme 3 and 4).

From the 3-cyclopropylamino derivative **20**, adenosine derivative **21**, 2-amino-6-cyclopropylamino derivative **26** and thymidine analogue **17**, phosphoramidate prodrugs **22**, **23**, **27** and **28** have been prepared using phenylmethoxyalaninyl phosphochloridate as a reagent (Scheme 3 and 4).

2.2. Biological activity

All prepared compounds (nucleosides **15** to **21**(MD59), **24**–**26** and phosphoramidates **22**, **23** and **27**, **28**) were evaluated in the broad antiviral screening focused on a number of viruses including retroviruses (HIV-1, HIV-2), DNA viruses (i.e., herpetic viruses and poxviruses) and also a variety of RNA viruses. Furthermore, the compounds were also evaluated for their cytostatic activity against murine and human tumor cell lines.

Compounds **15 (MD53)** and **16 (MD279)** inhibited replication of Coxsackievirus B3 at an EC_{50} = 25 ± 3 μM, CC_{50} >359 μM and EC_{50} = 31 ± 7 μM, CC_{50} >359 μM. This activity might be related to the presence of the chlorine atom at the 6-position of the purine nucleobase since none of the other compounds, including the closely related **18**, **19**, **20** and **21** derivatives were endowed with anti-Coxsackievirus activity, which is in agreement with our recently published data.[26] The adenine derivative **21** (MD59) exerted modest activity against HIV (HIV-1 EC_{50} = 100 ± 10 μM, CC_{50} >386 μM; HIV-2 EC_{50} = 231 ± 46 μM, CC_{50} >386 μM), feline herpes virus (EC_{50} = 58 ± 5 μM, CC_{50} >386 μM) and influenza A virus (H1N1 subtype) (EC_{50} = 179 ± 5 μM; CC_{50} >386 μg mL^{-1}). However,

Table 1
Inhibitory effects of selected compounds on the proliferation of murine leukemia cells (L1210), human T-lymphocyte cells (CEM) and human cervix carcinoma cells (HeLa)

	IC_{50} (μM)[a]		
	L1210	CEM	HeLa
16	139 ± 17	88 ± 20	>340
21 (MD59)	408 ± 81	>386	609 ± 227
22	129 ± 59	78 ± 0	136 ± 9
23	160 ± 2	⩾200	>200
24	179 ± 11	174 ± 40	190 ± 15
26	146 ± 10	124 ± 32	101 ± 16
5-FU[b]	0.33 ± 0.17	18 ± 5	2.6 ± 0.4

[a] 50% inhibitory concentration. Data are the mean (±SD) of at least 2 to 3 independent experiments.
[b] 5-FU—5-fluorouridine was used as a control.

the corresponding phosphoramidate prodrug lacked activity at 100 μM and was poorly cytostatic (CC_{50} = 223 ± 9 μM). These findings suggest that the compound was not able to inhibit virus (i.e., HIV) replication in cell culture, presumably due to an unsuccessful delivery of the 5′-monophosphate derivative. In fact, none of the protide derivatives were endowed with significant antiviral activity.

Several compounds were shown to be endowed with limited cytostatic activity against several tumor cell lines (Table 1). However, only derivatives **16** and **22** exerted activities lower than 100 μM in CEM cell line. Although the antiviral activity of **21** (MD59) is moderate it is clearly distinguishable from the cytostatic effect in all our assays. Both the antiviral and cytostatic mechanisms of action are so far unclear.

Compound **21** (MD59) inhibited also PI4KIIα (data and results of docking studies based on our recent crystal structure[27] are summarized in Supplementary information).

3. Conclusion

We report on an efficient synthesis of novel norbornane-based nucleoside and nucleotide analogues locked in North conformation. Placement of the hydroxymethyl substituent into the bridgehead position, although synthetically challenging, allowed its appropriate location in respect to the nucleobase in the position 3-*exo* of the norbornane skeleton. In this study we have also clearly demonstrated the advantages of our new single step variant of the Traube synthesis that brings a significant facilitation for the syntheses of carbocyclic nucleosides, where the purine nucleobase cannot be introduced by other means (e.g., Mitsunobu or Tsuji–Trost reaction).

Although the target nucleoside and nucleotide derivatives exerted only modest or low antiviral activity, this study fills the gap in our understanding of possibilities and limitations of effective use of various restriction patterns for the stabilization of carbocyclic nucleosides in North conformation. Our data show that the North conformation of these nucleoside and nucleotide derivatives itself does not guarantee enhancement of the antiviral effect and, from this perspective, it seems to be clear that a small change in the selected bridge can crucially influence the recognition of the compound by enzymes essential for virus replication.

4. Experimental section

- Reagents and solvents were purchased and used as received, or prepared according to published procedures. NMR spectra were recorded on Bruker Avance I 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) and Bruker Avance II 600 (^{1}H at 600 MHz, ^{13}C at 150 MHz) spectrometers using DMSO-d_6 or $CDCl_3$ as a solvent and using solvent signal as a reference. Chemical shifts

(δ) and coupling constants (J) were expressed in ppm and Hz, respectively. All structures were confirmed and ^{1}H and ^{13}C signals were assigned by a combination of 1D and 2D NMR (H,H-COSY, H,C-HSQC, H,C-HMBC, ROESY) techniques. Standard pulse programs from the library of the spectrometer were used; gradient selection was used in the 2D experiments. Mass spectra were measured on an LTQ Orbitrap XL (Thermo Fisher Scientific) using electrospray ionization (ESI). GC–MS analyses were performed on a 6890 N gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a Phenomenex ZB-5 HT capillary column. Elemental analyses were measured on Perkin Elmer CHN Analyzer 2400, Series II Sys (Perkin Elmer, Norwolk, CT, USA) or on SPECTRO iQ II (Spectro Analytical Instruments, Germany). Melting points are uncorrected and were determined on Büchi Melting Point B-540 apparatus. Microwave syntheses were carried out in a CEM Discover instrument with a single-mode cavity and focused microwave heating (microwave power supply 0–300 W, 1 W increments, IR temperature sensor, sealed vessel mode, pressure range 0–20 bar, 10 or 60 mL vials). Column chromatography was performed on a 40–60 μm silica gel using ISCO flash chromatography system or standard glass columns. Purity of all prepared compounds was higher than 98% unless stated otherwise.

4.1. Methyl (1*S**,4*S**)-3-oxobicyclo[2.2.1]heptane-1-carboxylate (9)

Alcohol **8** (6.8 g, 40 mmol) was dissolved in dichloromethane (20 mL) and added dropwise to a vigorously stirred suspension of PDC (22.7 g, 60 mmol) and crushed molecular sieves (23 g) in dichloromethane (200 mL). Reaction mixture was stirred at RT overnight, solids were filtered off on a cellite pad and solvent was evaporated. Resulting dark-brown slurry was filtered through a plug of silica gel (toluene/ethyl acetate 4:1) to afford ketone **|9** (5.57 g, 82%) as a colorless oil. [Found: C, 64.18; H, 7.24; $C_9H_{12}O_3$ requires C, 64.27; H, 7.19%]; δ_H (500 MHz, DMSO): 3.66 (s, 3H, CH_3), 2.59 (dm, 1H, J_{4-6ex} = 4.7, H-4), 2.38 (dm, 1H, J_{gem} = 17.8, H-2exo), 2.12 (dm, 1H, J_{gem} = 17.8, H-2endo), 2.01 (m, 1H, H-6endo), 1.96 (m, 1H, J_{gem} = 12.8, H-5exo), 1.93 (dm, 1H, J_{gem} = 10.1, H-7a), 1.83 (ddd, 1H, J_{gem} = 10.1, $J_{7b,4}$ = 4.2, $J_{7b,3en}$ = 1.2, H-7b), 1.70 (m, 1H, H-6exo), 1.43 (m, 1H, H-5endo). δ_C (125.8 MHz, DMSO): 213.4 (COO), 173.7 (C-3), 52.0 (CH_3), 50.6 (C-4), 50.5 (C-1), 46.7 (C-2), 40.3 (C-7), 30.6 (C-6), 24.0 (C-5). ESI MS m/z (%): 169.1 (2) [MH^+], 191.1 (100) [MNa^+]; HRMS (ESI, MNa^+) calculated: 193.08352 found: 193.08358.

4.2. Methyl (1*R**,3*S**,4*R**)-3-hydroxybicyclo[2.2.1]heptane-1-carboxylate (10)

$NaBH_4$ was added portionwise to a solution of ketone **9** (5.57 g, 33 mmol) in dry methanol (100 mL) at 0 °C and this solution was stirred at RT overnight. Methanol was evaporated and the residue was partitioned between brine and ethyl acetate. Water phase was further extracted with ethyl acetate (2 × 100 mL), collected organic layers were dried over sodium sulfate and evaporated. After codistillation with methanol (3 × 50 mL), **10** (4.96 g, 88%) was acquired as a colorless oil. [Found: C, 63.56; H, 8.41; $C_9H_{14}O_3$ requires C, 63.51; H, 8.29%]; δ_H (500 MHz, DMSO): 4.69 (d, 1H, OH′), 3.66 (dm, 1H, J_{3-2en} = 6.6, H-3), 3.60 (s, 3H, CH_3), 2.07 (d, 1H, J_{4-5ex} = 4.8, H-4), 1.82 (dm, 1H, J_{gem} = 9.5, H-7a), 1.80 (ddd, 1H, J_{gem} = 12.8, J_{2en-3} = 6.8, J_{2en-7a} = 2.3, H-2endo), 1.68 (m, 1H, H-6exo), 1.56 (dm, 1H, J_{gem} = 12.2, H-5exo), 1.51 (dm, 1H, J_{gem} = 12.8, H-2exo), 1.35 (dm, 1H, J_{gem} = 9.5, H-7b), 1.29 (dddd, 1H, J_{gem} = 11.6, $J_{6en-5en}$ = 9.2, $J_{6en-5ex}$ = 4.0, J_{6en-7} = 2.3, H-6endo), 1.03 (dm, 1H, J_{gem} = 12.5, H-2endo). δ_C (125.8 MHz, DMSO): 175.5 (COO), 73.1 (C-3), 51.6 (CH_3), 50.9 (C-1), 44.8 (C-4), 44.7 (C-2), 38.2 (C-7), 31.7 (C-6), 24.4 (C-5). ESI MS m/z (%): 171.1 (5) [MH^+], 193.1 (100) [MH^+]; HRMS (ESI, MNa^+) calculated: 193.08352 found: 193.08358.

4.3. Methyl (1*R**,3*S**,4*S**)-3-azidobicyclo[2.2.1]heptane-1-carboxylate (12)

Mesylchloride (4.2 mL, 53 mmol) was added to a solution of **10** (6.9 g, 41 mmol) in dry pyridine at 0 °C. Reaction mixture was stirred at rt for 3 h, quenched with water and partitioned between water (100 mL) and ethyl acetate (300 mL). Organic phase was then washed with dil. HCl (2 × 50 mL), $NaHCO_3$ (2 x 50 mL) and water, dried with sodium sulfate and evaporated. Acquired **11** (10 g, 99%, >98% pure on GCMS analysis) was directly used to the next reaction.

Sodium azide (7.8 g, 120 mmol) was added to a solution of mesylate **11** (10 g, 40 mmol) in DMF (150 mL) and the reaction mixture was heated to 115 °C overnight. After evaporation of DMF, crude product was partitioned between water (100 mL) and ethyl acetate (300 mL). Organic phase was washed with water (2 × 100 mL), dried with sodium sulfate and evaporated. Acquired **12** (6.9 g, 92%, >95% pure on GCMS analysis) was directly used to the next reaction, analytical sample was purified by column chromatography (hexane/ethyl acetate 22:3). [Found: C, 55.32; H, 6.76; N, 21.30; $C_9H_{13}N_3O_2$ requires C, 55.37; H, 6.71; N, 21.52%]; δ_H (600 MHz, $CDCl_3$): 3.70 (s, 3H, CH_3), 3.64 (dm, 1H, $J_{3,2en}$ = 7.5, H-3), 2.40 (dm, 1H, $J_{4,5ex}$ = 4.8, H-4), 1.96 (ddd, 1H, J_{gem} = 13.3, $J_{2en,3}$ = 7.5, $J_{2en,7a}$ = 2.7, H-2endo), 1.83–1.92 (m, 3H, H-2exo, H-6exo, H-7b),1.77 (tt, 1H, J_{gem} = $J_{5ex,6ex}$ = 12.6, $J_{5ex,4}$ = $J_{5ex,6en}$ = 4.5, H-5exo), 1.58 (dm, 1H, J_{gem} = 10.1, H-7a), 1.47 (m, 1H, H-6endo), 1.28 (m, 1H, H-5endo). δ_C (150 MHz, $CDCl_3$): 175.2 (COO), 64.3 (C-3), 51.8 (CH_3), 51.6 (C-1), 42.8 (C-4), 40.7 (C-2), 39.1 (C-7), 32.1 (C-6), 26.0 (C-5). ESI MS m/z (%): 218.2 (100) [MH^+]; HRMS (ESI, MNa^+) calculated: 218.09000, found: 218.08981.

4.4. ((1*R**,3*S**,4*S**)-3-Aminobicyclo[2.2.1]heptan-1-yl)methanol (13)

A solution of LAH (1 M THF solution, 25 mL) was added dropwise to a stirred solution of **12** (2 g, 10 mmol) in dry THF (100 mL) under argon atmosphere at 0 °C. After 2 h at RT the reaction was quenched with carefull addition of water, solids were filtered off on a cellite pad and thoroughly washed with ethanol. Crude **13** was purified on Dowex 50 (H^+) and then precipitated as a hydrochloride (1.05 g, 59%) with 1 M HCl/Et_2O solution from its ethanolic solution (>94% pure on LC–MS analysis). Sample for analytical purposes was obtained by benzoylation of the crude amine and subsequent chromatography of tribenzoylated product on silica gel. [Found: C, 76.83; H, 6.08; N, 3.28; $C_{29}H_{27}NO_4$ requires C, 76.80; H, 6.00; N, 3.09%]; δ_H (500 MHz, DMSO): 7.95 (m, 2H, H-o′), 7.64 (tt, 1H, $J_{p'-m'}$ = 7.5, $J_{p'-o'}$ = 1.3, H-p′), 7.49 (m, 2H, H-m′), 7.32–7.37 (m, 6H, H-o, H-p), 7.23 (m, 4H, H-m), 4.58 (ddd, 1H, J_{3-2en} = 8.2, J_{3-2ex} = J_{3-4} = 5.5, J_{3-7} = 1.0, H-3), 4.41 (m, 2H, CH_2-O), 2.61 (dm, 1H, J_{4-5ex} = 4.4, H-4), 2.06 (m, 1H, H-2exo), 2.02 (m, 1H, H-2endo), 1.98 (dm, 1H, J_{gem} = 9.3, H-7a), 1.72 (m, 1H, H-5exo), 1.55 (m, 1H, H-6endo), 1.38–1.47 (m, 2H, H-5endo, H-6exo), 1.24 (dm, 1H, J_{gem} = 9.3, H-7b). δ_C (125.8 MHz, DMSO): 173.5 (CONH), 165.8 (COO), 137.5 (C-i), 133.5 (C-p′), 132.2 (C-p), 129.9 (C-i′), 129.3 (C-o′), 128.9 (C-m′), 128.7 (C-m), 128.6 (C-o), 67.3 (CH_2-O), 61.8 (C-3), 47.5 (C-1), 42.4 (C-4), 39.7 (C-2), 38.9 (C-7), 30.6 (C-6), 29.3 (C-5). ESI MS m/z (%): 454.2 (26) [MH^+], 476.2 (100) [MNa^+]; HRMS (ESI, MNa^+) calculated: 476.18378; found: 476.18371.

4.5. [(1*R**,3*R**,4*R**)-3-(6-Chloro-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (15)

To a solution of amine **13** (355 mg, 2 mmol) in *n*-BuOH (10 mL), was added 4,6-dichloro-5-formamidopyrimidine (460 mg, 2.4 mmol) and DIPEA (1.05 mL, 6 mmol) and the reaction mixture was microwave irradiated in a sealed vessel on 160 °C for 2 h. Flash chromatography (1–2% methanol in ethyl acetate) followed by crystallization from toluene/cyclohexane mixture afforded **15** (384 mg, 69%) as white crystals (mp = 150–151 °C). [Found: C, 56.29; H, 5.61; N, 19.85; Cl, 12.60; $C_{13}H_{15}N_4OCl$ requires C, 56.02; H, 5.42; N, 20.10; Cl, 12.72%]; δ_H (500 MHz, DMSO): 8.75 (s, 1H, H-2′), 8.28 (s, 1H, H-8′), 4.77 (ddd, 1H, J_{3-2en} = 8.3, J_{3-2ex} = 4.5, J_{3-7a} = 1.4, H-3), 3.86 (m, 2H, CH_2O), 2.66 (bd, 1H, J_{4-5ex} = 4.7, H-4), 2.16 (ddd, 1H, J_{gem} = 13.5, J_{2en-3} = 8.3, J_{2en-7a} = 1.4, H-2endo), 2.10 (dm, 1H, J_{gem} = 13.5, H-2exo), 1.91 (m, 1H, H-5exo), 1.80 (dm, 1H, J_{gem} = 10.4, H-7b), 1.57-1.69 (m, 2H, H-5endo, H-6exo), 1.38-1.44 (m, 2H, H-6endo,H-7a). δ_C (125.8 MHz, DMSO): 151.78 (C-4′), 151.65 (C-2′), 150.96 (C-6′), 142.92 (C-8′), 131.94 (C-5′), 65.08 (CH_2O), 59.38 (C-3), 50.47 (C-1), 43.02 (C-4), 40.0 (C-2), 38.2 (C-7), 30.6 (C-6), 27.8 (C-5). ESI MS *m*/*z* (%): 279.1 (100) [MH^+]. HRMS (ESI, MH^+) calculated: 279.10126; found: 279.10122.

4.6. [(1*R**,3*R**,4*R**)-3-(2-Amino-6-chloro-9H-purin-9-yl) bicyclo[2.2.1]hept-1-yl]methanol (16)

To a solution of amine **13** (355 mg, 2 mmol) in water-EtOH mixture (1:1, *v*/*v*, 10 mL), was added 2-amino-4,6-dichloro-5-formamidopyrimidine (500 mg, 2.4 mmol) and DIPEA (1.05 mL, 6 mmol) and the reaction mixture was microwave irradiated in a sealed vessel on 140 °C for 1 h. Flash chromatography (1–5% methanol in ethyl acetate) followed by crystallization from toluene afforded **14** (486 mg, 83%) as white crystals (mp = 201–202 °C). [Found: C, 53.21; H, 5.59; N, 23.60; Cl, 12.19; $C_{13}H_{16}ClN_5O$ requires C, 53.15; H, 5.49; N, 23.84; Cl, 12.07%]; δ_H (500 MHz, DMSO): 8.23 (s, 1H, H-8′), 6.89 (bs, 2H, NH_2), 4.57 (t, 1H, J_{OH-CH2} = 5.3, OH), 4.40 (m, 1H, H-3), 3.56–3.62 (m, 2H, CH_2O), 2.45 (m, 1H, H-4), 1.84–1.93 (m, 2H, H-2); 1.70 (m, 1H, H-5exo), 1.60 (dm, 1H, J_{gem} = 10.3, H-7a), 1.40–1.52 (m, 2H, H-5endo, H-6exo), 1.19–1.26 (m, 2H, H-6endo, H-7b). δ_C (125.8 MHz, DMSO): 159.8 (C-2′), 154.2 (C-4′), 149.5 (C-6′), 140.8 (C-8′), 123.9 (C-5′), 63.6 (CH_2O), 58.1 (C-3), 50.4 (C-1), 42.5 (C-4), 38.0 (C-7), 30.4 (C-6), 27.7 (C-5). ESI MS *m*/*z* (%): 294.1 (42) [MH^+], 316.1 (100) [MNa^+]. HRMS (ESI, MH^+) calculated: 294.11161, found: 294.11170.

4.7. 1-[(1*R**,2*R**,4*R**)-4-(Hydroxymethyl)bicyclo[2.2.1]hept-2-yl]-5-methylpyrimidine-2,4(1H,3H)-dione (17)

Free amine **13** (264 mg, 1.87 mmol, amine freed from the corresponding hydrochloride using Dowex 50 (H^+)) was dissolved in dioxane (20 mL), ethyl [(2*E*)-3-ethoxy-2-methylprop-2-enoyl]carbamate (402 mg, 2 mmol) was added and the reaction mixture was heated to 100 °C for 3 h. Dowex 50 (H^+, 5 g) was added and the reaction mixture was heated to 100 °C overnight. Dowex resin was filtered off, crude product was adsorbed on silica and purification by flash chromatography (1–5% methanol in ethyl acetate) and crystallization from toluene/ethyl acetate mixture afforded **17** (326 mg, 69%) as white crystals (mp = 204–205 °C). [Found: C, 62.12; H, 7.20; N, 11.37; $C_{13}H_{18}N_2O_3$ requires C, 62.38; H, 7.25; N, 11.19%]; δ_H (500 MHz, DMSO): 11.20 (bs, 1H, H-3′), 7.48 (q, 1H, $J_{6',CH3}$ = 1.2, H-6′), 4.54 (t, 1H, $J_{OH,CH2}$ = 5.4, OH), 4.22 (bdd, 1H, $J_{2,3en}$ = 8.2, $J_{2,3ex}$ = 4.8, H-2), 3.52 (d, 2H, $J_{CH2,OH}$ = 5.4, CH_2O), 2.34 (bd, 1H, $J_{1,6ex}$ = 4.6, H-1), 1.79 (d, 3H, $J_{CH3,6'}$ = 1.2, CH_3), 1.78 (ddd, 1H, J_{gem} = 13.1, $J_{3en,2}$ = 8.3, $J_{3en,7a}$ = 2.4, H-3endo), 1.65 (m, 1H, H-6exo), 1.34–1.51 (m, 4H, H-3exo, H-6endo, H-5exo, 7b), 1.13–1.19 (m, 2H, H-5endo, 7a). δ_C (125.8 MHz, DMSO): 163.9 (C-4′), 151.3 (C-2′), 137.2 (C-6′), 108.3 (C-5′), 63.6 (CH_2O), 59.4 (C-2), 50.2 (C-4), 41.0 (C-3), 40.9 (C-1), 38.4 (C-7), 30.2 (C-5), 28.6 (C-6), 12.4 (CH_3). ESI MS *m*/*z* (%): 273.1 (100) [MH^+], 295.1 (56) [MNa^+]. HRMS (ESI, MNa^+) calculated: 273.12096, found: 273.12098.

4.8. Microwave assisted ammonolysis of C-6 chlorine atom of purine nucleobase (compounds 21 and 24)

A solution of 6-chloropurine or 2-amino-6-chloropurine derivative (up to 1 mmol) in ethanolic ammonia (3.5 M, 5 mL) was heated in a microwave reactor at 120 °C for 20–40 min (TLC determination of reaction completion). Volatiles were evaporated and crude compound was adsorbed on silica gel and purified by flash chromatography and subsequent crystallization from aqueous methanol.

4.8.1. [(1*R**,3*R**,4*R**)-3-(6-Amino-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (21, MD59)

From **15** (140 mg, 0.5 mmol); mobile phase: 5–15% methanol in ethyl acetate; yield 100 mg, 77%; colorless crystals (mp = 199–200 °C). [Found: C, 59.98; H, 6.67; N, 26.75; $C_{13}H_{17}ON_5$ requires C, 60.21; H, 6.61; N, 27.01%]; δ_H (500 MHz, DMSO): 8.22 (s, 1H, H-8′), 8.13 (s, 1H, H-2′), 7.19 (bs, 2H, NH), 4.57 (t, 1H, J_{OH-CH2} = 5.4, OH), 4.52 (m, 1H, H-3), 3.59 (m, 2H, CH_2O), 2.45 (dm, 1H, J_{4-5ex} = 4.6, H-4), 1.91-1.94 (m, 2H, H-2), 1.70 (m, 1H, H-5exo), 1.63 (dm, 1H, J_{gem} = 10.2, H-7b), 1.46-1.52 (m, 2H, H-5endo,H-6exo), 1.24 (m, 1H, H-6endo), 1.20 (dm, 1H, J_{gem} = 10.2, H-7a). δ_C (125.8 MHz, DMSO): 156.2 (C-6′), 152.4 (C-2′), 149.7 (C-4′), 138.4 (C-8′), 119.3 (C-5′), 63.7 (CH_2O), 58.0 (C-3), 50.5 (C-1), 42.8 (C-4), 40.0 (C-2), 38.0 (C-7), 30.4 (C-6), 27.8 (C-5). ESI MS *m*/*z* (%): 260 (100) [MH^+], 282 (17) [MNa^+]. HRMS (ESI, MH^+) calculated: 260.15114, found: 260.15117.

4.8.2. [(1*R**,3*R**,4*R**)-3-(2,6-diamino-9H-purin-9-yl)bicyclo [2.2.1]hept-1-yl]methanol (24)

From **16** (115 mg, 0.39 mmol); mobile phase: 10-25% methanol in ethyl acetate; yield 101 mg, 95%, light orange crystals (mp = 272–273 °C). [Found: C, 57.16; H, 6.59; N, 30.59; $C_{13}H_{18}N_6O$ requires C, 56.92; H, 6.61; N, 30.64%]; δ_H (500 MHz, DMSO): 7.78 (s, 1H, H-8′), 6.63 (bs, 2H, 6′-NH_2), 5.74 (bs, 2H, 2′-NH_2), 4.57 (t, 1H, J_{OH-CH2} = 5.7, OH), 4.33 (m, 1H, H-3), 3.59 (d, 2H, $J_{CH2,OH}$ = 5.4, CH_2O), 2.36 (dm, 1H, J_{4-5ex} = 4.7, H-4), 1.82–1.88 (m, 2H, H-2), 1.68 (m, 1H, H-5exo), 1.62 (dm, 1H, J_{gem} = 10.2, H-7a), 1.36–1.50 (m, 2H, H-5endo, H-6exo), 1.22 (m, 1H, H-6endo), 1.17 (dm, 1H, J_{gem} = 10.2, H-7b). δ_C (125.8 MHz, DMSO): 160.3 (C-2′), 156.3 (C-6′), 152.0 (C-4′), 134.8 (C-8′), 113.7 (C-5′), 63.7 (CH_2O), 57.1 (C-3), 50.3 (C-1), 42.8 (C-4), 40.0 (C-2), 37.9 (C-7), 30.5 (C-6), 27.8 (C-5). ESI MS *m*/*z* (%): 275.3 (100) [MH^+], 297.3 (18) [MNa^+]. HRMS (ESI, MH^+) calculated: 275.16149; found: 275.16144.

4.9. Microwave assisted nucleophilic displacement of C-6 chlorine atom of purine nucleobase with cyclopropylamine (compounds 20 and 26)

A solution of 6-chloropurine or 2-amino-6-chloropurine derivative and cyclopropylamine (10 equiv) in ethanol (5 mL per 1 mmol of substrate) was heated in a microwave reactor at 140 °C for 10–40 min (TLC determination of reaction completion). Volatiles were evaporated, crude product was adsorbed on silica and purified by column chromatography and crystallization.

4.9.1. {(1*R**,3*R**,4*R**)-3-[6-(Cyclopropylamino)-9H-purin-9-yl] bicyclo[2.2.1]hept-1-yl}methanol (20)

From **15** (140 mg, 0.5 mmol); mobile phase: 1–10% methanol in ethyl acetate; crystallization from toluene/cyclohexane mixture;

yield 120 mg, 80%, white crystals (mp = 146–146.5 °C). [Found: C, 63.98; H, 7.21; N, 23.31; $C_{16}H_{21}N_5O$ requires C, 64.19; H, 7.07; N, 23.39%]; δ_H (500 MHz, DMSO): 8.22 (s, 2H, H-2′, H-8′), 7.85 (bs, 1H, NH), 4.57 (t, 1H, J_{OH-CH2} = 5.4, OH), 4.54 (m, 1H, H-3), 3.59 (m, 2H, CH_2O), 3.02 (bs, 1H, CH-cyklop), 2.45 (bd, 1H, J_{4-5ex} = 4.7, H-4), 1.91-1.94 (m, 2H, H-2), 1.70 (m, 1H, H-5exo), 1.63 (dm, 1H, J_{gem} = 10.2, H-7b), 1.46-1.53 (m, 2H, H-5endo,H-6exo), 1.25 (m, 1H, H-6endo), 1.20 (dm, 1H, J_{gem} = 10.1, H-7a), 0.60 and 0.71 (m, 4H, CH_2-cyklop). δ_C (125.8 MHz, DMSO): 155.7 (C-6′), 152.3 (C-2′), 149.0 (C-4′), 138.3 (C-8′), 119.7 (C-5′), 63.7 (CH_2O), 58.0 (C-3), 50.5 (C-1), 42.8 (C-4), 40.0 (C-2), 38.0 (C-7), 30.5 (C-6), 27.8 (C-5), 6.6 (CH_2-cyklop). ESI MS *m*/*z* (%): 300 (100) [MH^+], 322 (41) [MNa^+]. HRMS (ESI, MH^+) calculated: 300.18244; found: 300.18140.

4.9.2. (1*R**,2*R**,4*R**)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]bicyclo[2.2.1]heptan-2-ol (26)

From **16** (500 mg, 1.53 mmol); mobile phase: 5-15% methanol in ethyl acetate; crystallization from water; yield 400 mg, 83%, white crystals (mp = 134–135 °C). [Found: C, 61.29; H, 7.12; N, 26.59; $C_{16}H_{22}N_6O$ requires C, 61.13; H, 7.05; N, 26.73%]; δ_H (500 MHz, DMSO): 7.77 (s, 1H, H-8′), 7.24 (bs, 1H, NH), 5.79 (bs, 2H, NH_2), 4.56 (t, 1H, $J_{OH,CH2}$ = 5.4, OH), 4.34 (m, 1H, H-3), 3.58 (d, 2H, $J_{CH2,OH}$ = 5.4, CH_2O), 3.03 (bs, 1H, CH-cyclop), 2.35 (dm, 1H, J_{4-5ex} = 4.9, H-4), 1.81–1.88 (m, 2H, H-2), 1.68 (m, 1H, H-5exo), 1.61 (dm, 1H, J_{gem} = 10.1, H-7a), 1.39–1.50 (m, 2H, H-5endo, H-6exo), 1.22 (m, 1H, H-6endo), 1.17 (m, 1H, H-7b), 0.57 and 0.64 (m, 4H, CH_2-cyclop). δ_C (125.8 MHz, DMSO): 160.2 (C-2′), 156.0 (C-6′), 151.5 (C-4′), 134.5 (C-8′), 113.9 (C-5′), 63.7 (CH_2O), 57.1 (C-3), 50.3 (C-1), 42.8 (C-4), 40.0 (C-2), 37.9 (C-7), 30.5 (C-6), 27.8 (C-5), 6.6 (CH_2-cyclop), ESI MS *m*/*z* (%): 315.3 (100) [MH^+], 337.3 (11) [MNa^+]. HRMS (ESI, MH^+) calculated: 315.19279; found: 315.19276.

4.10. {(1*R**,3*R**,4*R**)-3-[6-(Dimethylamino)-9H-purin-9-yl]bicyclo [2.2.1]hept-1-yl}methanol (18)

A solution **15** (140 mg, 0.5 mmol) in dimethylamino dimethylcarbamate (2 mL) was stirred at RT overnight. Volatiles were evaporated, crude product was adsorbed on silica and purification by flash chromatography (1–5% methanol in ethyl acetate) and crystallization from toluene/cyclohexane mixture afforded **18** (100 mg, 69%) as white crystals (mp = 120–121 °C). [Found: C, 62.60; H, 7.32; N, 24.25; $C_{15}H_{21}N_5O$ requires C, 62.70; H, 7.37; N, 24.37%]; δ_H (500 MHz, DMSO): 8.23 (s, 1H, H-8′), 8.20 (s, 1H, H-2′), 4.57 (t, 1H, J_{OH-CH2} = 5.3, OH), 4.54 (ddd, 1H, J_{3-2en} = 8.5, J_{3-2ex} = 4.6, J_{3-7a} = 1.2, H-3), 3.59 (m, 2H, CH_2O), 3.45 (bs, 6H, N-CH_3), 2.44 (bd, 1H, J_{4-5ex} = 4.7, (H-4), 1.94 (ddd, 1H, J_{gem} = 13.2, J_{2en-3} = 8.4, J_{2en-7a} = 2.3, H-2endo), 1.87 (dm, 1H, J_{gem} = 13.2, H-2exo), 1.70 (m, 1H, H-5exo), 1.60 (dm, 1H, J_{gem} = 10.2, H-7b), 1.45-1.53 (m, 2H, H-5endo, H-6exo), 1.25 (m, 1H, H-6en), 1.21 (dm, 1H, J_{gem} = 10.2, H-7a). δ_C (125.8 MHz, DMSO): 154.4 (C-6′), 151.8 (C-2′), 150.5 (C-4′), 137.2 (C-8′), 119.9 (C-5′), 63.7 (CH_2O), 57.9 (C-3), 50.5 (C-1), 42.7 (C-4), 40.1 (C-2), 38.5 (N-CH_3), 38.0 (C-7), 30.4 (C-6), 27.8 (C-5). ESI MS *m*/*z* (%): 288 (100) [MH^+], 310 (44) [MNa^+]. HRMS (ESI, MH^+) calculated: 288.18244; found: 288.18238.

4.11. [(1*R**,3*R**,4*R**)-3-(6-Thio-9H-purin-9-yl)bicyclo[2.2.1] hept-1-yl]methanol (19)

A solution of **16** (140 mg, 0.5 mmol) and thiourea (0.6 mmol) in dry ethanol (4 ml) was heated in a pressure vessel at 105 °C overnight. Poorly soluble product was collected on a paper filter and washed thoroughly with ethanol and diethylether to afford pure **19** (94 mg, 68%) as white powder (mp >320 °C (decomp.)). [Found: C, 56.28; H, 5.85; N, 20.04; S, 11.65; $C_{13}H_{16}ON_4S$ requires C, 56.50; H, 5.84; N, 20.27; S, 11.60%]; δ_H (500 MHz, DMSO): 13.70 (bs, 1H, SH), 8.39 (s, 1H, H-8′), 8.18 (d, 1H, $J_{2'-SH}$ = 3.8, H-2′), 4.56 (bs, 1H, OH), 4.53 (ddd, 1H, J_{3-2en} = 8.4, J_{3-2ex} = 4.7, J_{3-7a} = 1.3, H-3), 3.59 (m, 2H, CH_2O), 2.47 (bd, 1H, J_{4-5ex} = 4.6, H-4), 1.87-1.97 (m, 2H, H-2), 1.70 (m, 1H, H-5exo), 1.59 (dm, 1H, J_{gem} = 10.3, H-7b), 1.45-1.53 (m, 2H, H-5endo, H-6exo), 1.20-1.27 (m, 2H, H-6endo, H-7a). δ_C (125.8 MHz, DMSO): 176.0 (C-6′), 144.8 (C-2′), 144.2 (C-4′), 140.7 (C-8′), 135.5 (C-5′), 63.6 (CH_2O), 58.6 (C-3), 50.5 (C-1), 42.8 (C-4), 40.0 (C-2), 38.0 (C-7), 30.4 (C-6), 27.7 (C-5). ESI MS *m*/*z* (%): 277 (6) [MH^+], 299 (31) [MNa^+]. HRMS (ESI, MH^+) calculated: 277.11231; found: 277.11238.

4.12. 2-Amino-9-[(1*R**,2*R**,4*R**)-4-(hydroxymethyl)bicyclo [2.2.1]hept-2-yl]-1,9-dihydro-6H-purin-6-one (25)

A solution of **16** (100 mg, 0.53 mmol) in TFA/water mixture (2:1, 6 mL) was stirred at RT overnight. Volatiles were evaporated and crude product was codistilled with ethanol (3 × 10 mL), NH_4OH (10 mL) and ethanol (2 × 10 ml). Poorly soluble product was shortly boiled in water/methanol mixture (1:1), collected on a paper filter and thoroughly washed with water, ethanol and diethylether to afford **25** (56 mg, 60%) as pale orange powder (mp >335 °C (decomp.)). [Found: C, 56.93; H, 6.11; N, 25.20; $C_{13}H_{17}N_5O_2$ requires C, 56.71; H, 6.22; N, 25.44%]; δ_H (500 MHz, DMSO): 10.58 (bs, 1H, H-1′), 7.77 (s, 1H, H-8′), 6.47 (bs, 2H, NH_2), 4.56 (t, 1H, J_{OH-CH2} = 5.3, OH), 4.29 (m, 1H, H-2), 3.54–3.60 (m, 2H, CH_2O), 2.35 (dm, 1H, J_{1-6ex} = 4.7, H-1), 1.78–1.87 (m, 2H, H-3), 1.67 (m, 1H, H-6exo), 1.58 (dm, 1H, J_{gem} = 10.2, H-7a), 1.37–1.49 (m, 2H, H-6endo, H-5exo), 1.21 (m, 1H, H-5endo), 1.17 (dm, 1H, J_{gem} = 10.2, H-7b). δ_C (125.8 MHz, DMSO): 157.0 (C-6′), 153.6 (C-2′), 151.3 (C-4′), 134.8 (C-8′), 117.0 (C-5′), 63.6 (CH_2O), 57.5 (C-2), 50.4 (C-4), 42.8 (C-1), 37.9 (C-7), 30.5 (C-5), 27.8 (C-6). ESI MS *m*/*z* (%): 276.2 (15) [MH^+], 298.2 (100) [MNa^+]. HRMS (ESI, MH^+) calculated: 276.14550, found: 244.14557.

4.13. Method of preparation of phosphoramidate prodrugs (compounds 22, 23, 27 and 28)

To a solution of substrate (0.5 mmol) in dry THF (10 mL) under argon atmosphere was added *t*-BuMgCl (1 M solution, 1 mL) and a solution of phenylmethoxyalaninyl phosphochloridate (1 mmol) in dry THF (5 mL). Reaction mixture was stirred at RT for 3 days and then quenched with sat. solution of ammonium chloride. Volatiles were evaporated and flash chromatography of the residue (methanol/ethyl acetate) afforded product as white lyophilizate.

4.13.1. Methyl (2S)-2-{[({(1*R**,3*R**,4*R**)3-[6-(cyclopropylamino)-9H-purin-9-yl]bicyclo[2.2.1]heptan-1-yl}methoxy)(phenoxy)phosphoryl]amino}propanoate (22)

From **20** (200 mg, 0.67 mmol); mobile phase: 5–15% methanol in ethyl acetate; yield 136 mg, 38%. [Found: C, 57.81; H, 6.15; N, 15.40; P, 5.49; $C_{26}H_{33}N_6O_5P$ requires C, 57.77; H, 6.15; N, 15.55; P, 5.73]; δ_H (500 MHz, DMSO): 8.22–8.25 (m, 2H, H-2′, H-8′), 7.87 (bs, 1H, 6′-NH), 7.33 (m, 2H, H-3″), 7.11–7.20 (m, 3H, H-2″, H-4″), 5.98 (m, 1H, P-NH), 4.56 (m, 1H, H-3), 4.16–4.24 (m, 2H, CH_2-O), 3.84 (m, 1H, CH-COO), 3.57 and 3.59 (s, 3H, OCH_3), 3.04 (bs, 1H, CH-cyclop), 2.48 (m, 1H, H-4), 1.88–2.06 (m, 2H, H-2), 1.63–1.75 (m, 2H, H-5exo, H-7b), 1.46–1.57 (m, 2H, H-5endo, H-6exo), 1.20–1.37 (m, 5H, CH-*CH*$_3$, H-6endo, H-7a), 0.61 and 0.71 (m, 4H, CH_2-cyclop). δ_C (125.8 MHz, DMSO): 173.9 and 173.9 (COO), 155.7 (C-6′), 152.4 (C-2′), 151.0 and 151.0 (C-1″), 149.0 (C-4′), 138.3 (C-8′), 129.7 (C-3″), 124.7 (C-4″), 120.5 and 120.5 (C-2″), 119.7 (C-5′), 68.7 (CH_2O), 57.6 and 57.7 (C-3), 52.0 (O-CH_3), 49.9 and 52.0 (*CH*-COO), 48.5 (C-1), 42.8 (C-4), 39.6 (C-2), 37.9 and 38.0 (C-7), 30.1 and 30.2 (C-6), 27.5 (C-5), 19.9 (CH-*CH*$_3$), 6.6

(CH_2-cyclop). 541.2 (100) [MH^+]; HRMS ESI (MH^+) calculated: 541.2323, found: 541.2322.

4.13.2. Methyl (2*S*)-2-{[({(1*R**,3*R**,4*R**)3-[6-amino-9H-purin-9-yl] bicyclo[2.2.1]heptan-1-yl}methoxy)(phenoxy) phosphoryl]amino}propanoate (23)

From **21** (105 mg, 0.41 mmol); mobile phase: 5–15% methanol in ethyl acetate; yield 120 mg, 59%. [Found: C, 55.39; H, 5.88; N, 16.66; P, 6.31; $C_{23}H_{29}N_6O_5P$ requires C, 55.20; H, 5.84; N, 16.79; P, 6.19]; δ_H (500 MHz, DMSO): 8.23–8.25 (m, 1H, H-8′), 8.13 (m, 1H, H-2′), 7.33 (m, 2H, H-3″), 7.11–7.21 (m, 5H, NH_2, H-2″, H-4″), 5.98 (m, 1H, NH), 4.54 (m, 1H, H-3), 4.16–4.25 (m, 2H, 1-CH_2-O), 3.85 (m, 1H, NH-*CH*), 3.58 and 3.59 (m, 3H, O-CH_3), 2.48 (m, 1H, H-4), 1.88–2.05 (m, 2H, H-2en, H-2ex), 1.64–1.73 (m, 2H, H-5ex, H-7a), 1.48–1.55 (m, 2H, H-5en, H-6en), 1.25–1.36 (m, 2H, H-6ex, H-7b), 1.22–1.24 (m, 3H, CH-CH_3). δ_C (125.8 MHz, DMSO): 173.9 and 173.9 (COO), 156.2 (C-6′), 152.4 (C-2′), 151.0 (dm, $J_{1'',P}$ = 6.7, C-1″), 149.7 (C-4′), 138.4 and 138.5 (C-8′), 129.7 and 129.7 (C-3″), 124.6 (C-4″), 120.4 and 120.5 (d, $J_{2'',P}$ = 4.5, C-2″), 119.3 (C-5′), 68.6 and 68.7 (m, 1-CH_2-O), 57.6–57.7 (C-3), 52.0 and 52.0 (O-CH_3), 49.9 and 50.0 (NH-*CH*), 48.5 and 48.5 (d, J_{1-P} = 8.2, C-1), 42.8 (m, C-4), 39.5 (C-2), 37.9 and 39.0 (C-7), 30.1–30.2 (m, C-6), 27.5 and 27.5 (C-5), 19.8 and 19.9 (d, $J_{CH3,P}$ = 7.3, CH-CH_3). ESI MS *m*/*z* (%): 501.2 (100) [MH^+], 523.2 (60) [MNa^+]. HRMS (ESI, MH^+) calculated: 501.20098; found: 501.20091.

4.13.3. Methyl (2*S*)-2-{[({(1*R**,3*R**,4*R**)3-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]bicyclo[2.2.1]heptan-1-yl}methoxy)(phenoxy)phosphoryl]amino}propanoate (27)

From **26** (307 mg, 0.98 mmol); mobile phase: 5–20% methanol in ethyl acetate; yield 300 mg, 55%. [Found: C, 56.33; H, 6.21; N, 17.53; P, 5.85; $C_{26}H_{34}N_7O_5P$ requires C, 56.21; H, 6.17; N, 17.65; P, 5.58]; δ_H (500 MHz, DMSO): 7.79–7.80 (m, 1H, H-8′), 7.34 (m, 2H, H-3″), 7.27 (bs, 1H, NH-cyclop), 7.13–7.21 (m, 3H, H-2″, H-4″), 5.97 (m, 1H, NH), 5.81 (bs, 1H, NH_2), 4.36 (m, 1H, H-3), 4.16–4.24 (m, 2H, 1-CH_2-O), 3.85 (m, 1H, NH-*CH*), 3.58–3.59 (m, 3H, O-CH_3), 3.03 (bs, 1H, CH-cyclop), 2.38 (m, 1H, H-4), 1.81–1.98 (m, 2H, H-2en, H-2ex), 1.61–1.73 (m, 2H, H-5ex, H-7a), 1.41–1.55 (m, 2H, H-5en, H-6en), 1.21–1.32 (m, 5H, CH-CH_3, H-6ex, H-7b), 0.56–0.67 (m, 4H, CH_2-cyclop). δ_C (125.8 MHz, DMSO): 173.9 and 173.9 (COO), 160.2 (C-2′), 156.1 (C-6′), 151.0 (m, C-4′, C-1″), 134.6 (C-8′), 129.7 and 129.7 (C-3″), 124.6 (C-4″), 120.4 and 120.5 (d, $J_{2'',P}$ = 4.7, C-2″), 113.9 (C-5′), 68.7 and 68.8 (m, 1-CH_2-O), 56.8–56.9 (m, C-3), 52.0 and 52.0 (O-CH_3), 49.9 and 50.0 (NH-*CH*), 48.4 and 48.4 (d, J_{1-P} = 8.2, C-1), 42.8 and 42.8 (C-4), 39.8 (C-2), 37.8 and 37.9 (C-7), 30.2–30.3 (m, C-6), 27.6 (C-5), 23.9 (CH-cyclop), 19.8 and 19.9 (d, $J_{CH3,P}$ = 7.5, CH-CH_3), 6.6 (CH_2-cyclop). ESI MS *m*/*z* (%): 556.3 (100) [MH^+], 578.3 (35) [MNa^+]. HRMS (ESI, MH^+) calculated: 556.24318; found: 556.24292.

4.13.4. Methyl (2*S*)-2-[({[(1*R**,3*R**,4*R**)-3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)bicyclo[2.2.1]heptan-1-yl] methoxy}(phenoxy)phosphoryl)amino]propanoate (28)

From **17** (326 mg, 1.3 mmol); mobile phase: 1-5% methanol in ethyl acetate; yield 185 mg, 29%. [Found: C, 56.04; H, 6.20; N, 8.14; P, 5.95; $C_{23}H_{30}N_3O_7P$ requires C, 56.21; H, 6.15; N, 8.55; P, 6.30]; δ_H (500 MHz, DMSO): 11.22 (bs, 1H, H-3′), 7.50 (m, 1H, H-6′), 7.32–7.37 (m, 2H, H-3″), 7.13–7.19 (m, 3H, H-2″, H-4″), 5.96 (m, 1H, NH), 4.22 (m, 1H, H-3), 4.10–4.17 (m, 2H, 1-CH_2-O), 3.83 (m, 1H, NH-*CH*), 3.58 and 3.59 and 3.60 and 3.60 (s, 3H, O-CH_3), 2.39 (m, 1H, H-4), 1.86 (m, 1H, H-2a), 1.79 (m, 3H, 5′-CH_3), 1.66 (m, 1H, H-5a), 1.35–1.54 (m, 4H, H-2b, H-5b, H-6a, 7a), 1.21–1.26 (m, 5H, H-6b, 7b, NH-CH-CH_3). δ_C (125.8 MHz, DMSO): 173.9 and 173.9 (COO), 163.9 (C-4′), 151.3 and 151.3 (C-1″), 151.0 and 151.0 (C-2′), 137.2 (C-6′), 126.7–129.7 (C-3″), 124.6 (C-4″), 120.4 and 120.5 (C-2″), 108.3 and 108.3 (C-5), 68.4–68.7 (m, 1-CH_2), 59.2 (C-3), 52.0 (O-CH_3), 49.9 and 50.0 (NH-*CH*), 48.2 (C-1), 40.8 (C-2), 40.3 (C-4), 38.3 and 38.4 (C-7), 29.9–30.1 (m, C-6), 28.3–28.7 (m, C-5), 19.8–19.9 (m, NH-CH-CH_3), 12.4 (5′-CH_3). ESI MS *m*/*z* (%): 492.4 (3) [MH^+], 514.3 (100) [MNa^+]. HRMS (ESI, MNa^+) calculated: 514.17136; found: 514.17144.

4.14. Antiviral activity assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) strain KOS, thymidine kinase-deficient (TK−) HSV-1 KOS strain resistant to ACV (ACVr), herpes simplex virus type 2 (HSV-2) strains Lyons and G, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) strain Long, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, Influenza virus A (subtypes H1N1, H3N2), influenza virus B, reovirus-1, Sindbis and Punta Toro. The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) fibroblasts, African green monkey cells (Vero), human epithelial cells (HeLa) or Madin–Darby canine kidney cells (MDCK). Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations of the test compounds. Viral cytopathicity or plaque formation was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

Inhibition of HIV-1(IIIB)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing $\sim 3 \times 10^5$ CEM cells/mL infected with 100 $CCID_{50}$ of HIV per milliliter and containing appropriate dilutions of the test compounds. After 4−5 days of incubation at 37 °C in a CO_2-controlled humidified atmosphere, CEM giant (syncytium) cell formation was examined microscopically. The EC_{50} (50% effective concentration) was defined as the compound concentration required to inhibit HIV-induced giant cell formation by 50%.

4.15. Cytostatic activity assays

All assays were performed in 96-well microtiter plates. To each well were added $(5 - 7.5) \times 10^4$ tumor cells and a given amount of the test compound. The cells were allowed to proliferate for 48 h (murine leukemia L1210 cells) or 72 h (human lymphocytic CEM and human cervix carcinoma HeLa cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that inhibited cell proliferation by 50%.

4.16. The EC_{50} and CC_{50} calculations

The EC_{50} and CC_{50} calculations were performed using following formula: 100-ratio of [50% − % activity at the nearest tested concentration that results in higher than 50% inhibition]/[% activity at the nearest concentration that results in lower than 50% inhibition − % activity at the nearest concentration that results in higher than 50% inhibition] multiplied by (higher tested concentration − lower tested concentration).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.11.011.

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