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From norbornane-based nucleotide analogs locked in South conformation to novel inhibitors of feline herpes virus

Milan Dejmek [a], Hubert Hřebabecký [a], Michal Šála [a], Martin Dračínský [a], Eliška Procházková [a], Pieter Leyssen [b], Johan Neyts [b], Jan Balzarini [b], Radim Nencka [a,*]

[a] Gilead Sciences & IOCB Research Centre, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i, 166 10 Prague 6, Czech Republic
[b] Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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ABSTRACT

A synthetic route toward a series of unique cyclic nucleoside phosphonates locked in South conformation is described. The desired conformation is stabilized by a substitution of the sugar moiety by bicyclo[2.2.1]heptane (norbornane) bearing a purine or pyrimidine nucleobase in the bridgehead position. Although the final phosphonate derivatives are devoid of any significant antiviral activity probably due to the unfavorable conformational properties, several intermediates and their analogs exhibit surprising activity against feline herpes virus. Since these compounds do not possess an appropriate hydroxymethyl function allowing phosphorylation and subsequent incorporation into the polynucleotide chain, it seems to be likely that these compounds act by a novel unknown mechanism of action and may represent a new possible alternative for nucleoside and nucleotide therapeutics of this widely spread feline infection. A number of derivatives exerted also a significant antiviral activity against Coxsackievirus B3 and B4.

1. Introduction

Modern antiviral treatment relies on nucleoside and nucleotide derivatives, a fundamental element of so-called 'cocktail therapy', which is the most successful in the current therapy of HIV infection. The development of anti-HIV therapeutics has brought nucleoside phosphonates to the spotlight and allowed their full recognition as the most effective class of nucleoside and nucleotide reverse-transcriptase inhibitors (NRTIs).[1] Although the acyclic nucleoside phosphonates (ANPs), represented by tenofovir **1**, have obtained most of the fame, there is a number of other interesting cyclic nucleoside phosphonates being developed including GS-9148 (**2**) and its carbocyclic derivatives (e.g., **3**), which combine the strengths of the nucleoside phosphonates, the didanosine or abacavir-like skeleton and rational design-based optimization, leading to their significant anti-HIV potency and favorable resistance profile.[2] Another cyclic nucleoside phosphonate that has gained considerable attention in the literature is PMDTA (**4**), reported by Herdewijn and co-workers (Fig. 1).[3]

Although conformational constriction has proven to be a vital modification of nucleosides leading to an enhancement of desired antiviral properties, conformationally locked derivatives of cyclic nucleoside phosphonate have been reported rather rarely so far.

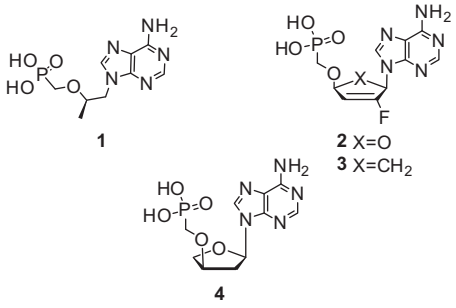

Figure 1. The structures of important acyclic and cyclic nucleoside phosphonates.

Saneyoshi et al. have prepared several locked derivatives of PMDTA,[4] whereas Gilead Sciences, Inc. has filed a patent concerning various conformationally locked phosphonate analogs as antiviral agents.[5]

In both of these cases, however, the conformation was locked using bicyclo[3.1.0]hexane as a substitute of the natural tetrahydofuran sugar ring. Recently, we have introduced bicyclo[2.2.1]heptane (norbornane)[6] as a potentially attractive surrogate of the sugar moiety and shown that some derivatives bearing the 6-chloropurine nucleobase can possess significant antiviral activities against (+)ssRNA viruses from the *Picornaviridae*

* Corresponding author. Tel.: +420 220 183 265; fax: +420 220 183 560.
E-mail address: nencka@uochb.cas.cz (R. Nencka).

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family.[7] This pseudosugar motif was adopted also by other authors recently.[8]

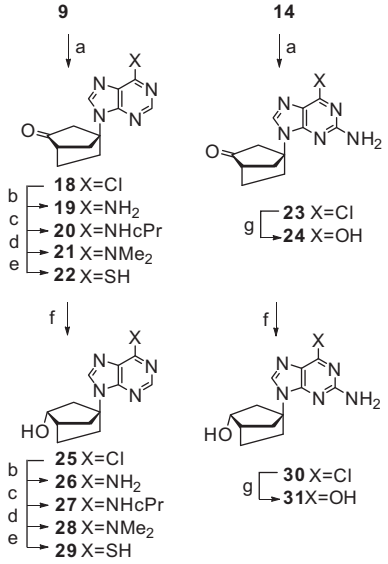
Herein, we examine the use of norbornane skeleton for the stabilization of the South conformation of racemic 5′-nornucleosides and the corresponding nucleoside phosphonates. Although the findings of Marquez and co-workers suggest that the nucleosides locked in South conformation are not suitable substrates for polymerases, which results in their inactivity against herpes viruses,[9] we decided to investigate this trend further by employing a structurally different type of bridges locking nucleoside phosphonates in this particular conformation. To our surprise, several precursors of this synthesis exhibit significant activity against feline herpes virus (FHV-1), which is the most widely spread infection of household cats, causing typically severe respiratory and ophthalmological problems in newborn kittens and resulting in a mortality of up to 50%.[10] The treatment of this disease has been traditionally connected with nucleoside derivatives (trifluridine, ganciclovir) together with adjuvant therapy by interferons and L-lysine.[11]

2. Results and discussion

2.1. Chemistry

Using a methodology reported elsewhere, we were able to obtain large quantities of norbornane intermediate **5**,[12] which was selected as a suitable starting material for the further synthesis of derivatives locked in South conformation. The preparation of the crucial intermediate **6** was achieved using a six-step procedure described in detail in Supplementary material. The essential step, the introduction of a carboxylic moiety into the bridgehead position, was performed by the Hell–Volhard–Zelinsky bromination connected with the skeleton rearrangement.[13]

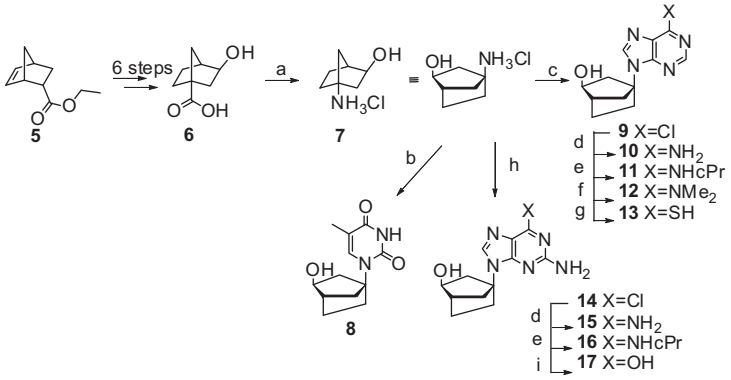
Since the target structures bear the nucleobase in the bridgehead position of the norbornane bicycle, the only way to introduce purine or thymine functionality is the nucleobase construction on a primary amino group. In the case of 6-chloropurine, the classical two-step procedure using triethyl orthoformate to close the imidazole ring was used; in the case of 2-amino-6-chloropurine, however, we advantageously employed a recently published one-pot methodology, which uses a formylated pyrimidine precursor.[14] All constructions of nucleobases proceeded smoothly with moderate yields, which were expected with a highly sterically demanding substrate such as **7** with the amino group on the tertiary carbon. The amino group-containing key intermediate **7** was prepared from **6** using Curtius rearrangement, which afforded **7** in good yield (Scheme 1).



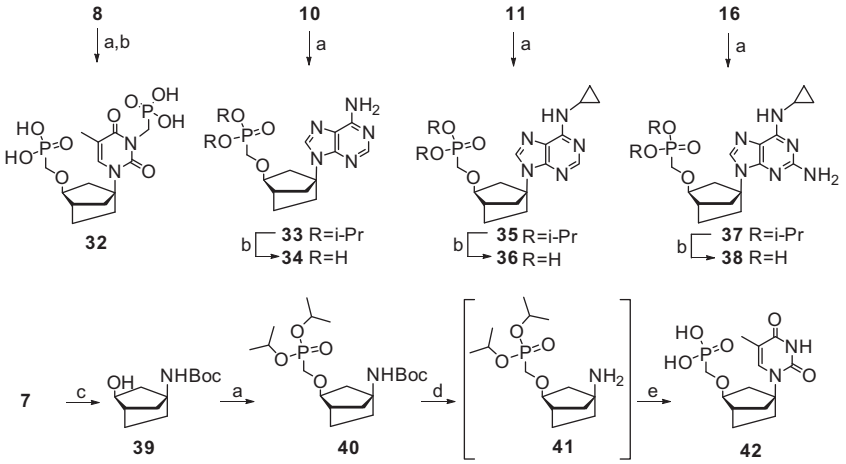
Scheme 2. Reagents and conditions: (a) PDC, DCM, 7 d, 82% for **18**, or PDC, DMF, 12 h, 56% for **23**; (b) NH_3, EtOH, 120 °C, 30 min, 77% for **19**, 70% for **26**; (c) cyclopropylamine, EtOH, MW, 140 °C, 30 min, 77% for **20**, 85% for **27**; (d) DMF, MW, 200 °C, 2 min, 86% for **21**, 77% for **28**; (e) thiourea, EtOH, 105 °C, overnight, 89% for **22**, 68% for **29**; (f) $NaBH_4$, MeOH, 5 h, 89% for **25**, 51% for **30**; (g) TFA, H_2O, 24 h, 86% for **24**, 64% for **31**.

To explore the relationship between the structure and biological activity further, and in order to enrich our library of compounds, additional derivatization of **9** and **14** was performed—first, the configuration of the C-2 hydroxy group was inverted using a standard oxidation–reduction sequence employing PDC as the oxidant and $NaBH_4$ as the reductant (Scheme 2).

Subsequently, the nucleophilic substitution of the C-6 chlorine atom of the purine nucleobase of **9**, **14**, **18**, **23**, **25** and **30** gave rise to a small library of final compounds. It is noteworthy that although the reduction of the C-2 keto group of **18** with sodium borohydride afforded the *endo* derivative almost exclusively (<3% of the *exo* derivative in the reaction mixture, which was separable by column chromatography), the same reaction of **23** resulted in a chromatographically only partially separable mixture of *endo*/*exo* 4:1, which had to be purified by crystallization. Dimethylamino derivatives **12**, **21** and **28** were prepared by a recently described method using dimethylamine generated in situ by microwave-assisted decomposition of DMF.[15]



Scheme 1. Reagents and conditions: (a) (1) ClCOOEt, TEA, acetone, 0 °C, 1 h, (2) NaN_3, H_2O, 0 °C, 1 h, (3) dioxane, HCl, reflux, 5 h, 74%; (b) (1) ethyl [(2*E*)-3-ethoxy-2-methylprop-2-enoyl]carbamate, dioxane, 100°, 3 h, (2) Dowex 50W (H^+), dioxane, 100 °C overnight, 61%; (c) 4,6-dichloro-5-formamidopyrimidine, DIPEA, *n*-BuOH, MW, 160 °C, 2 h, 55%; (d) NH_3, EtOH, 120 °C, 30 min, 83% for **10**, 83% for **15**; (e) cyclopropylamine, EtOH, MW, 140 °C, 30 min, 88% for **11**, 76% for **16**; (f) DMF, MW, 200 °C, 2 min, 86%; (g) thiourea, EtOH, 105 °C, overnight, 76%; (h) 2-amino-4,6-dichloro-5-formamidopyrimidine, DIPEA, *n*-BuOH, MW, 160 °C, 2 h, 63%; (i) TFA, H_2O, 24 h, 56%.



Scheme 3. Reagents and conditions: (a) (*t*-BuO)$_2$Mg, TsCH$_2$P(O)(O*i*-Pr)$_2$, DMF, 60 °C, 48 h, 89% for **33**, 25% for **35**, 81% for **37**, 58% for **40**; (b) TMSBr, DCM, 24 h, 60% for **32**, 95% for **34**, 92% for **36**, 90% for **38**; (c) Boc$_2$O, DIPEA, DCM, overnight, 89%; (d) TFA, DCM, 2 h, 56%; (e) (1) ethyl [(2*E*)-3-ethoxy-2-methylprop-2-enoyl]carbamate, dioxane, 100°, 3 h, (2) Dowex 50W (H$^+$), dioxane, 100 °C, 12 h, 43%.

Phosphonate functionality was introduced to **10**, **11** and **16** using tosylmethanphosphonate as the alkylating agent. The use of (*t*-BuO)$_2$Mg as a base and DMF as a solvent afforded significantly better yields than the use of *t*-BuONa in THF (Scheme 3). Direct alkylation of the thymine-based compound **8** afforded a product alkylated on both the hydroxyl group of the norbornane and the N-3 position of the thymine nucleobase. Therefore, the phosphonate moiety was introduced first to the Boc-protected **7** and the thymine was constructed afterwards. An interesting feature of this reaction is that during the Dowex 50 (H$^+$)-catalyzed ring-closure reaction, the phosphonate diester is converted to a free phosphonate **42**, which was isolated on the C-18 reverse phase.

2.2. Biological activity

The antiviral activities of all 5′-nornucleoside derivatives as well as the nucleoside phosphonates were evaluated against a broad panel of viruses containing RNA-, DNA- and retroviruses. In accordance with the observation of Marquez et al.,[9] these compounds locked in South conformation were devoid of any activity against HIV and human herpes viruses.

Several compounds lacking the phosphonate moiety exerted activity against feline herpes virus (Table 1). Since the activity was observed for unphosphorylated derivatives rather than nucleoside phosphonates, we can speculate that it is not based on the incorporation of these compounds into the polynucleotide chain. The activity seems to be rather related to the 9-norbornyl-6-aminopurine skeleton, with the most active compound being the adenine derivative **19**.

Table 1
The antiviral activity of selected compounds against feline herpes virus (in μg mL^{-1}) in Crandell-Rees feline kidney cell cultures

	EC$_{50}$[a]	CC$_{50}$[b]
10	14 ± 3	>100
19	7 ± 2	>100
20	52 ± 7	>100
26	51 ± 1	>100
27	42 ± 15	>100
Ganciclovir	0.22 ± 0.01	>100

[a] 50% effective concentration or compound concentration causing 50% inhibition of the virus-induced cytopathic effect as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Cytotoxic concentration as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

Table 2
The antiviral effect of selected compounds on the replication of Coxsackievirus in Vero cell cultures (in μg mL^{-1})

	CVB3 EC$_{50}$[a]	CVB4 EC$_{50}$	CC$_{50}$[b]
9	9 ± 1	9 ± 2	>100
14	16 ± 2	9 ± 0	>100
18	7 ± 1	39 ± 5	>100
23	15 ± 2	12 ± 0	>100
25	22 ± 5	9 ± 0	56 ± 8
30	10 ± 1	12 ± 0	>100

[a] 50% effective concentration or compound concentration causing 50% inhibition of the virus-induced cytopathic effect as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% cytotoxic concentration as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.

Moreover, a number of compounds exerted activity against Coxasckieviruses B3 and B4 (Table 2). In addition, compounds **20** and **23** inhibited the replication of Varicella zoster virus in human embryonic lung cells with EC$_{50}$ = 41 μg mL^{-1} and EC$_{50}$ = 20 μg mL^{-1}, respectively. The nucleoside phosphonates were also screened for inhibitory activity against adenylate cyclase toxin from *Bordetella pertussis* in our cell-based assay, but none of them exerted significant a reduction of the activity at 10 μM concentrations.

3. Conclusion

We report on an efficient synthesis of a novel series of norbornane-based conformationally locked nucleoside and nucleotide analogs. Firstly, we prepared a number of 5′-nornucleoside derivatives locked in South conformation via a substitution of the natural tetrahydrofuran sugar ring with norbornane, bearing the purine or pyrimidine nucleobase in the bridgehead position. Secondly, we introduced a phosphonate moiety onto the C-3 hydroxygroup, which led to the cyclic nucleoside phosphonates, stabilized by bicyclo[2.2.1]heptane in the desired South conformation.

Our observation supports the findings of Marquez et al.,[9] because none of the prepared nucleoside phosphonates exerted significant activity against any of the viruses evaluated, although this might be caused not only by the disfavored South conformation but also by the enhanced steric hindrance of the inferior nucleotide side incurred by the ethylene bridge.

Several compounds obtained in the first part of our study, lacking the phosphonate moiety, significantly inhibited the replication of feline herpes virus. The most efficient compound was norborne-3-on derivative **19** with $EC_{50} = 7 \pm 2\ \mu g\ mL^{-1}$. Since this derivative cannot be phosphorylated and incorporated into a polynucleotide chain by viral DNA polymerases, it seems to be plausible that this type of compounds acts via a new, as yet unknown mechanism of action and presents the first step towards the development of a novel type of therapeutics complementary to the current nucleoside-based treatment of this widespread feline infection.

4. Experimental section

The reagents and solvents were purchased and used as received, or prepared according to published procedures. The 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 the solvent signal as a reference. The 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 utilized in the 2D experiments. The mass spectra were measured on an LTQ Orbitrap XL (Thermo Fisher Scientific) using electrospray ionization (ESI). The elemental analyses were measured on Perkin Elmer CHN Analyzer 2400, Series II Sys (Perkin Elmer, Norwolk, CT) or on SPECTRO iQ II (Spectro Analytical Instruments, Germany). The melting points are uncorrected and were determined on a Büchi Melting Point B-540 apparatus. The microwave syntheses were carried out in a CEM Discover instrument with a single-mode cavity and focused microwave heating (a microwave power supply of 0–300 W, 1W increments, IR temperature sensor, sealed-vessel mode, a pressure range of 0–20 bar, 10 or 60 mL vials). The column chromatography was performed on a 40–60 μm silica gel using the ISCO flash chromatography system or standard glass columns. The purity of all of the compounds prepared was higher than 98% unless stated otherwise.

4.1. (1*S**,2*S**,4*S**)-4-Aminobicyclo[2.2.1]heptan-2-ol hydrochloride (7)

Triethylamine (3.2 mL, 23 mmol) and ethyl chloroformate (1.9 mL, 20.1 mmol) were added to a solution of **6** (3 g, 19.2 mmol) in dry acetone (50 mL) at 0 °C. After 1 h at 0 °C, a solution of sodium azide (3.7 g, 57.6 mmol) in water (50 mL) was added. After 1 h at 0 °C, the reaction mixture was diluted with water (400 mL) and extracted with ethyl acetate (4 × 250 mL). The combined organic extracts were washed with satd $NaHCO_3$ (2 × 200 mL) and water (200 mL), dried over sodium sulfate and evaporated. The oily residue was dissolved in dioxane (50 mL) and 2M HCl (150 mL), heated to reflux for 5 h, evaporated and codistilled with toluene (3 × 200 mL) to afford crude amine, which was further purified by precipitation as hydrochloride from the ethanol–diethylether mixture to afford **7** (2.35 g, 74%) as white crystals.

^{1}H NMR (500 MHz, DMSO): δ 1.01 (ddt, 1H, J_{gem} = 8.9, $J_{7a\text{-}3en}$ = 2.5, $J_{7a\text{-}1}$ = $J_{7a\text{-}2}$ = 1.5, H-7a), 1.05–1.12 (m, 2H, H-5endo, H-6endo), 1.16 (ddd, 1H, J_{gem} = 12.4, $J_{3ex\text{-}5ex}$ = 3.6, $J_{3ex\text{-}2}$ = 2.6, H-3exo), 1.27 (m, 1H, H-5exo), 1.43–1.55 (m, 2H, H-6exo, H-7b), 1.62 (ddd, 1H, J_{gem} = 12.4, $J_{3en\text{-}2}$ = 7.0, $J_{3en\text{-}7a}$ = 2.4, H-3endo), 1.84 (dm, 1H, $J_{1\text{-}6ex}$ = 5.1, H-1), 3.62 (dm, 1H, $J_{2\text{-}3en}$ = 7.0, H-2). ^{13}C NMR (125.8 MHz, DMSO): δ 25.22 (C-6), 35.37 (C-5), 41.75 (C-7), 43.63 (C-1), 48.87 (C-3), 61.41 (C-4), 73.80 (C-2). HRMS ($C_7H_{14}NO$): calculated: 128.1075; found: 128.1070 (M+H).

4.2. 1-[(1*R**,3*R**,4*R**)-3-Hydroxybicyclo[2.2.1]hept-1-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (8)

Free amine **7** (225 mg, 1.38 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 (305 mg, 1.5 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. The Dowex resin was filtered off, the crude product was adsorbed on silica, and purification by flash chromatography (1–5% methanol in ethyl acetate) and crystallization from toluene afforded **8** (200 mg, 61%) as white crystals (mp = 256–257 °C). ^{1}H NMR (500 MHz, DMSO): δ1.22 (m, 1H, H-6endo), 1.29 (dm, 1H, J_{gem} = 12.8, H-3exo), 1.44 (m, 1H, H-5exo), 1.66 (tdd, 1H, J_{gem} = $J_{6ex,5ex}$ = 12.5, $J_{6ex,1}$ = 5.3, $J_{6ex,5en}$ = 4.2, H-6exo), 1.74 (dm, 1H, J_{gem} = 9.1, H-7a), 1.77 (d, 3H, $J_{CH3,6'}$ = 1.2, CH_3), 2.01–2.07 (m, 2H, H-1, H-5endo), 2.15 (dm, 1H, J_{gem} = 9.1, H-7b), 2.58 (ddd, 1H, J_{gem} = 12.8, $J_{3en,2}$ = 7.0, $J_{3en,7a}$ = 2.4, H-3endo), 3.73 (m, 1H, H-2), 4.76 (d, 1H, $J_{OH,2}$ = 3.6, OH), 7.51 (q, 1H, $J_{6',CH3}$ = 1.2, H-6′), 11.08 (br s, 1H, H-3′). ^{13}C NMR (125.8 MHz, DMSO): δ 12.18 (CH_3), 24.20 (C-6), 31.41 (C-5), 38.00 (C-7), 41.53 (C-1), 44.97 (C-3), 68.97 (C-4), 72.71 (C-2), 107.82 (C-5′), 139.99 (C-6′), 151.06 (C-2′), 164.30 (C-4′). ESI MS m/z (%): 259.1 (100) [M+H], 281.1 (55) [M+Na]; HRMS ESI ($C_{12}H_{17}N_2O_3$): calculated: 237.12337; found: 237.12339. For $C_{12}H_{16}N_2O_3$ (236.27): calculated: 61.00; C, 6.83; H, 11.86; N; found: 61.27; C, 6.98; H, 11.72; N.

4.3. (1*S**,2*S**,4*S**)-4-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (9)

A solution of **7** (2.3 g, 14 mmol), 4,6-dichloro-5-aminopyrimidine (3.4 g, 21 mmol) and DIPEA (7.9 mL, 56 mmol) in *n*-butanol (80 mL) was heated in a sealed microwave vessel at 160 °C for 4 h. The resulting reaction intermediate was purified by chromatography on silica gel (toluene–ethyl acetate = 1:4), dissolved in a mixture of triethyl ortoformate (300 mL) and concd HCl (4 mL) and stirred at room temperature for 3–5 d, and then evaporated. This oily residue was dissolved in a mixture of THF and 1M hydrochloric acid (1:1, 125 mL) and stirred at rt for 4 h. After neutralization with sodium hydrogencarbonate, all volatiles were evaporated and the product was purified by column chromatography (ethyl acetate—toluene—acetone—ethanol 17:4:3:1) and crystallization from a toluene–cyclohexane mixture to afford **9** (2.1 g, 55%) as white crystals (mp = 155–157 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.35 (dddd, 1H, J_{gem} = 12.4, $J_{6en\text{-}5en}$ = 9.2, $J_{6en\text{-}5ex}$ = 4.8, $J_{6en\text{-}7b}$ = 2.1, H-6endo), 1.75 (dm, 1H, J_{gem} = 12.4, H-3exo), 1.79 (tdd, 1H, J_{gem} = $J_{6ex\text{-}5ex}$ = 12.3, $J_{6ex\text{-}1}$ = 5.1, $J_{6ex\text{-}5en}$ = 4.2, H-6exo), 1.90 (m, 1H, H-5exo), 2.01 (dddd, 1H, J_{gem} = 11.3, $J_{5en\text{-}6en}$ = 9.1, $J_{5en\text{-}6ex}$ = 4.1, $J_{5en\text{-}7b}$ = 2.3, H-5endo), 2.05 (dm, 1H, J_{gem} = 9.1, H-7a), 2.19 (dm, 1H, $J_{1\text{-}6ex}$ = 5.0, H-1), 2.49 (m, 1H, H-7b), 2.54 (ddd, 1H, J_{gem} = 12.5, $J_{3en\text{-}2}$ = 6.9, $J_{3en\text{-}7a}$ = 2.4, H-3endo), 3.87 (m, 1H, H-2), 4.93 (d, 1H, $J_{OH\text{-}2}$ = 3.5, OH), 8.71 (s, 1H, H-8′), 8.77 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.04 (C-6), 32.65 (C-5), 37.57 (C-7), 42.69 (C-1), 45.85 (C-3), 65.17 (C-4), 72.67 (C-2), 131.72 (C-5′), 146.59 (C-8′), 149.37 (C-6′), 151.28 (C-2′), 152.43 (C-4′). ESI MS m/z (%): 265.2 (100) [M+H]. For $C_{12}H_{13}N_4OCl$ (262.69): calculated: 54.45; C, 4.95; H, 21.17; N, 13.39; Cl; found: 54.49; C, 5.08; H, 21.26; N, 12.99; Cl.

4.4. (1*R**,2R*,4*R**)-4-(2-Amino-6-chloro-9*H*-purin-9-yl) bicyclo[2.2.1]heptan-2-ol (14)

2-Amino-4,6-dichloro-5-formamidopyrimidine (2.4 g, 11.5 mmol) and DIPEA (5 mL, 28.8 mmol) were added to a solution of the **7** (2.2 g, 9.6 mmol) in *n*-butanol (40 mL), and the reaction mixture was

heated in a sealed microwave vessel at 160 °C for 2 h. Flash chromatography (2–10% methanol in ethyl acetate) followed by crystallization from toluene afforded **14** (1.4 g, 63%) as off-white crystals (mp = 218–219 °C (decomp.)). ^{1}H NMR (500 MHz, DMSO): δ 1.29 (m, 1H, H-6endo), 1.62 (dm, 1H, J_{gem} = 12.5, H-3exo), 1.70–1.83 (m, 2H, H-5exo, H-6exo), 1.93 (m, 1H, H-5endo), 2.01 (dm, 1H, J_{gem} = 9.1, H-7a), 2.15 (m, 1H, H-1); 2.35 (dm, 1H, J_{gem} = 9.2, H-7b), 2.51 (ddd, 1H, J_{gem} = 12.5, $J_{3en,2}$ = 6.9, $J_{3en,7}$ = 2.6, H-3endo), 3.82 (m, 1H, H-2), 4.87 (t, 1H, J_{OH-CH2} = 3.5, OH), 6.79 (br s, 2H, NH_2), 8.11 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.13 (C-6), 32.41 (C-5), 37.38 (C-7), 42.64 (C-1), 45.79 (C-3), 64.41 (C-4), 72.69 (C-2), 124.26 (C-5′), 142.21 (C-8′), 149.66 (C-6′), 154.65 (C-4′), 159.49 (C-2′). ESI MS *m*/*z* (%): 280.1 (97) [M+H], 302.1 (100) [M+Na]; HRMS ESI ($C_{12}H_{14}N_5OCl$): calculated: 280.09596, found: 280.09595. For $C_{12}H_{14}ClN_5O$ (279.73): calculated: 51.52; C, 5.04; H, 12.67; Cl, 25.04; N; found: 51.55; C, 5.05; H, 12.59; Cl, 25.10; N.

4.5. (1*S**,4*S**)-4-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (18)

A solution of **9** (1 g, 3.8 mmol) in dichlormethane (50 mL) was added dropwise to a suspension of PDC (2.84 g, 7.6 mmol) and crushed molecular sieves (3 g) in dichlormethane (50 mL). The reaction mixture was stirred at rt for 7 d, the solid parts were filtered off and thoroughly washed with chloroform. The resulting brown oil was adsorbed on silica gel and chromatographed on a short column of silica (ethyl acetate—toluene—acetone—ethanol 17:4:3:1). Crystallization from a water–methanol mixture afforded **18** (830 mg, 82%) as white crystals (mp = 167–168 °C).

^{1}H NMR (500 MHz, DMSO): δ 1.58 (m, 1H, H-6endo), 2.12 (tt, 1H, J_{gem} = $J_{6ex-5ex}$ = 12.6, J_{6ex-1} = $J_{6ex-5en}$ = 4.9, H-6exo), 2.27 (m, 1H, H-5exo), 2.34 (dddd, 1H, J_{gem} = 11.7, $J_{5en-6en}$ = 9.0, $J_{5en-6ex}$ = 4.8, J_{5en-7} = 2.0, H-5endo), 2.52–2.60 (m, 2H, H-7), 2.79 (dm, 1H, J_{1-6ex} = 5.0, H-1), 2.85–2.86 (m, 2H, H-3), 8.77 (s, 1H, H-8′), 8.80 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 23.31 (C-6), 31.68 (C-5), 39.95 (C-7), 48.60 (C-3), 49.00 (C-1), 63.47 (C-4), 131.74 (C-5′), 146.37 (C-8′), 149.49 (C-6′), 151.48 (C-2′), 152.35 (C-4′) 211.07 (C-2). ESI MS *m*/*z* (%): 263.1 (100) [M+H], 285.1 (4) [M+Na]. For $C_{12}H_{11}N_4OCl$ (262.69): calculated: 54.87; C, 4.22; H, 21.33; N, 13.50; Cl; found: 54.70; C, 4.21; H, 20.99; N, 13.39; Cl.

4.6. (1*R**,4*R**)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (23)

A solution of **14** (1.11 g, 4 mmol) in DMF (10 mL) was added dropwise to a solution of PDC (3 g, 8 mmol) in DMF (70 mL). The reaction mixture was stirred at rt for 12 h, the volatiles were evaporated and the product was purified by flash chromatography (1–3% methanol in ethyl acetate). Crystallization from toluene afforded **23** (620 mg, 56%) as white crystals (mp = 209–210 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.53 (m, 1H, H-6endo), 2.07 (m, 1H, H-6exo), 2.18–2.25 (m, 2H, H-5), 2.44 (ddd, 1H, J_{gem} = 9.8, $J_{7b,3en}$ = 3.9, $J_{7b,1}$ = 1.3, H-7b), 2.49 (m, 1H, H-7a), 2.71–2.84 (m, 3H, H-1, H-3), 6.90 (br s, 2H, NH_2), 8.19 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 23.27 (C-6), 31.35 (C-5), 39.7 (C-7), 48.25 (C-3), 48.96 (C-1), 62.78 (C-4), 124.17 (C-5′), 141.83 (C-8′), 149.80 (C-6′), 154.50 (C-4′), 159.59 (C-2′), 211.54 (C-2). ESI MS *m*/*z* (%): 278.1 (43) [M+H], 300.0 (100) [M+Na]; HRMS ESI ($C_{12}H_{13}ON_5Cl$): calculated: 278.08031; found: 278.08041.

4.7. (1*S**,2*R**,4*S**)-4-(6-Chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (25)

$NaBH_4$ (52 mg, 1.4 mmol) was added portionwise to a solution of **18** (600 mg, 2.3 mmol) in dry methanol (15 mL) at 0 °C and the reaction mixture was stirred at this temperature for one hour. A saturated solution of ammonium chloride (30 mL) was added and the product was extracted with ethyl acetate (5 × 30 mL). The combined organic extracts were dried over sodium sulfate and chromatographed on silica gel (ethyl acetate—toluene—acetone—ethanol 17:4:3:1) to afford **25** (540 mg, 89%) as white powder. For analytical purposes, 100 mg of the product were crystallized from a toluene–cyclohexane mixture (white crystals, mp = 154–155 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.65 (m, 1H, H-6exo), 1.80 (dm, 1H, J_{gem} = 12.2, H-3endo), 1.97 (m, 1H, H-5exo), 2.11–2.21 (m, 4H, H-5endo, H-6endo, H-7), 2.29 (m, 1H, H-1), 2.31 (ddd, 1H, J_{gem} = 12.3, J_{3ex-2} = 10.4, $J_{3ex-5ex}$ = 3.8, H-3exo), 4.28 (m, 1H, H-2), 4.99 (d, 1H, J_{OH-2} = 4.1, OH), 8.66 (s, 1H, H-8′), 8.76 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 20.81 (C-6), 34.16 (C-5), 40.15 (C-7), 41.00 (C-1), 43.61 (C-3), 66.11 (C-4), 69.74 (C-2), 131.71 (C-5′), 146.37 (C-8′), 149.34 (C-6′), 151.26 (C-2′), 152.32 (C-4′). ESI MS *m*/*z* (%): 263.2 (100) [M+H]. For $C_{12}H_{13}N_4OCl$ (262.69): calculated: 54.45; C, 4.95; H, 21.17; N, 13.39; Cl; found: 54.28; C, 5.02; H, 21.10; N, 13.39; Cl.

4.8. (1*R**,2*S**,4*R**)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (30)

$NaBH_4$ (39 mg, 1 mmol) was added portionwise to a solution of **23** (470 mg, 1.7 mmol) in dry methanol (30 mL) at 0 °C and the reaction mixture was stirred at this temperature for 5 h. A saturated solution of ammonium chloride (30 mL) and water (100 mL) was added and the product was extracted into ethyl acetate (5 × 50 mL). The organic extracts were combined and dried with sodium sulfate. Flash chromatography (1–10% methanol in ethyl acetate) and multiple crystallization from a toluene–ethyl acetate mixture afforded **30** (430 mg, 51%) as white powder (mp = 213–214 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.59 (m, 1H, H-6exo), 1.74 (dm, 1H, J_{gem} = 12.3, H-3endo), 1.89 (m, 1H, H-5exo), 2.07–2.15 (m, 4H, H-5endo, H-6endo, H-7), 2.21 (ddd, 1H, J_{gem} = 12.3, $J_{3ex,2}$ = 10.4, $J_{3ex,5ex}$ = 3.7, H-3exo), 2.23 (m, 1H, H-1), 4.24 (m, 1H, H-2), 4.93 (d, 1H, J_{OH-2} = 3.9, OH), 6.81 (br s, 2H, NH_2), 8.06 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.74 (C-6), 33.91 (C-5), 40.00 (C-7), 40.93 (C-1), 43.37 (C-3), 65.33 (C-4), 69.76 (C-2), 124.25 (C-5′), 141.98 (C-8′), 149.60 (C-6′), 154.53 (C-4′), 159.46 (C-2′). ESI MS *m*/*z* (%): 280.1 (73) [M+H], 302.1 (100) [M+Na]; HRMS ESI ($C_{12}H_{14}ON_5ClNa$): calculated: 302.07791; found: 302.07789.

4.9. Microwave-assisted ammonolysis of the C-6 chlorine atom of the purine nucleobase (compounds 10, 15, 19 and 26)

A solution of a 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 the reaction completion). The volatiles were evaporated and the crude compound was adsorbed on silica gel and purified by flash chromatography and subsequent crystallization from aqueous methanol.

4.9.1. (1*S**,2*S**,4*S**)-4-(6-Amino-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (10)

From **9** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—acetone—ethanol—water 100:15:6:4; crystallization from water; yield: 100 mg, 83%, colorless needles (mp = 253–254 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.31 (dddd, 1H, J_{gem} = 12.4, $J_{6en-5en}$ = 9.1, $J_{6en-5ex}$ = 4.7, J_{6en-7b} = 2.1, H-6endo), 1.69 (dm, 1H, J_{gem} = 12.4, H-3exo), 1.75 (tdd, 1H, J_{gem} = $J_{6ex-5ex}$ = 12.3, J_{6ex-1} = 5.0, $J_{6ex-5en}$ = 4.2, H-6exo), 1.85 (m, 1H, H-5exo), 1.94 (dddd, 1H, J_{gem} = 11.3, $J_{5en-6en}$ = 9.1, $J_{5en-6ex}$ = 4.1, J_{5en-7b} = 2.2, H-5endo), 2.00 (dm, 1H, J_{gem} = 9.1, H-7a), 2.15 (dm, 1H, J_{1-6ex} = 5.0, H-1), 2.39 (dq, 1H, J_{gem} = 9.1, J_{7b-1} = J_{7b-5en} = J_{7b-6en} = 2.0, H-7b), 2.49 (ddd,

1H, J_{gem} = 12.6, J_{3en-2} = 6.8, J_{3en-7a} = 2.4, H-3endo), 3.84 (m, 1H, H-2), 4.88 (d, 1H, J_{OH-2} = 3.5, OH), 7.19 (br s, 2H, NH_2), 8.116 and 8.120 (s, 2H, H-2′, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.22 (C-6), 32.73 (C-5), 37.61 (C-7), 42.75 (C-1), 46.02 (C-3), 64.38 (C-4), 72.81 (C-2), 119.82 (C-5′), 139.74 (C-8′), 150.24 (C-4′), 152.27 (C-2′), 156.31 (C-6′). ESI MS *m*/*z* (%): 246.2 (100) [M+H], 268.1 (21) [M+Na]. For $C_{12}H_{15}N_5O$ (245.28): calculated: 58.76; C, 6.16; H, 28.55; N; found: 58.48; C, 6.17; H, 28.28; N.

4.9.2. (1*S**,2*S**,4*S**)-4-(2,6-Diamino-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (15)

From **14** (220 mg, 0.79 mmol); mobile phase: 10–15% methanol in ethyl acetate; crystallization from a water–methanol mixture; yield: 170 mg, 83%, pale-orange crystals (mp = 295–296 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.27 (m, 1H, H-6endo), 1.59 (dm, 1H, J_{gem} = 12.6, H-3exo), 1.68–1.81 (m, 2H, H-5exo, H-6exo), 1.89 (m, 1H, H-5endo), 2.00 (dm, 1H, J_{gem} = 9.1, H-7a), 2.12 (m, 1H, H-1); 2.28 (dm, 1H, J_{gem} = 9.1, H-7b), 2.49 (m, 1H, H-3endo), 3.80 (m, 1H, H-2), 4.82 (m, 1H, OH), 5.63 (br s, 2H, 2′-NH_2), 6.60 (br s, 2H, 6′-NH_2), 7.68 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.27 (C-6), 32.51 (C-5), 37.44 (C-7), 42.66 (C-1), 45.98 (C-3), 63.82 (C-4), 72.79 (C-2), 114.28 (C-5′), 136.27 (C-8′), 152.53 (C-4′), 156.33 (C-6′), 159.91 (C-2′). ESI MS *m*/*z* (%): 261.3 (100) [M+H], 283.3 (14) [M+Na]; HRMS ESI ($C_{12}H_{17}ON_6$): calculated: 261.14584; found: 261.14578. For $C_{12}H_{16}N_6O$ (260.30): calculated: 55.37; C, 6.20; H, 32.29; N; found: 55.21; C, 6.29; H, 32.59; N.

4.9.3. (1*S**,4*S**)-4-(6-Amino-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (19)

From **18** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—acetone—ethanol—water 100:15:6:4; crystallization from a water–methanol mixture; yield: 93 mg, 77%, colorless crystals (mp = 239–240 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.54 (m, 1H, H-6endo), 2.09 (m, 1H, H-6exo), 2.21–2.30 (m, 2H, H-5), 2.46–2.52 (m, 2H, H-7), 2.71 (m, 1H, H-1), 2.75–2.85 (m, 2H, H-3), 7.25 (br s, 2H, NH_2), 8.14 (s, 1H, H-2′), 8.19 (s, 1H, H-8′).[13]C NMR (125.8 MHz, DMSO): δ 23.40 (C-6), 31.64 (C-5), 39.99 (C-7), 48.64 (C-3), 49.08 (C-1), 62.79 (C-4) 119.73 (C-5′), 139.44 (C-8′), 150.08 (C-4′), 152.44 (C-2′), 156.35 (C-6′), 211.68 (C-2). ESI MS *m*/*z* (%): 244.1 (100) [M+H]. For $C_{12}H_{13}N_5O$ (243.26): calculated: 59.25; C, 5.39; H, 28.79; N; found: 58.96; C, 5.35; H, 28.45; N.

4.9.4. (1*S**,2R*,4*S**)-4-(6-Amino-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (26)

From **25** (110 mg, 0.4 mmol); mobile phase: ethyl acetate—acetone—ethanol—water 100:15:6:4; crystallization from water; yield: 71 mg, 70%, colorless crystals (mp = 252–253 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.61 (m, 1H, H-6exo), 1.73 (dm, 1H, J_{gem} = 12.3, H-3endo), 1.93 (m, 1H, H-5exo), 2.08–2.16 (m, 4H, H-5endo, H-6endo, H-7), 2.24 (m, 1H, H-1), 2.58 (ddd, 1H, J_{gem} = 12.3, J_{3ex-2} = 10.5, $J_{3ex-5ex}$ = 3.1, H-3exo), 4.26 (m, 1H, H-2), 4.93 (d, 1H, J_{OH-2} = 4.1, OH), 7.15 (br s, 2H, NH_2), 8.06 (s, 1H, H-8′), 8.11 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 20.84 (C-6), 34.25 (C-5), 40.23 (C-7), 41.07 (C-1), 43.69 (C-3), 65.30 (C-4), 69.89 (C-2), 119.81 (C-5′), 139.50 (C-8′), 150.11 (C-4′), 152.23 (C-2′), 156.28 (C-6′). NegESI MS *m*/*z* (%): 244.2 (100) [M−H]. For $C_{12}H_{15}N_5O$ (245.28): calculated: 58.76; C, 6.16; H, 28.55; N; found: 58.39; C, 6.25; H, 28.65; N.

4.10. Microwave-assisted nucleophilic displacement of the C-6 chlorine atom of the purine nucleobase with cyclopropylamine (compounds 11, 16, 20 and 27)

A solution of a 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 the reaction completion). The volatiles were evaporated and the crude product was adsorbed on silica and purified by column chromatography and crystallization.

4.10.1. (1*S**,2*S**,4*S**)-4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]bicyclo [2.2.1]heptan-2-ol (11)

From **9** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene–cyclohexane mixture; yield: 123 mg, 88%, white crystals (mp = 155–156 °C). ^{1}H NMR (500 MHz, DMSO): δ 0.60 and 0.71 (m, 4H, CH_2-cyclop), 1.31 (dddd, 1H, J_{gem} = 12.3, $J_{6en-5en}$ = 9.0, $J_{6en-5ex}$ = 4.8, J_{6en-7b} = 2.0, H-6endo), 1.69 (dm, 1H, J_{gem} = 12.5, H-3exo), 1.75 (tdd, 1H, J_{gem} = $J_{6ex-5ex}$ = 12.3, J_{6ex-1} = 5.0, $J_{6ex-5en}$ = 4.2, H-6exo), 1.85 (m, 1H, H-5exo), 1.94 (dddd, 1H, J_{gem} = 11.3, $J_{5en-6en}$ = 9.2, $J_{5en-6ex}$ = 4.1, J_{5en-7b} = 2.2, H-5endo), 2.00 (dm, 1H, J_{gem} = 9.1, H-7a), 2.15 (dm, 1H, J_{1-6ex} = 4.9, H-1), 2.39 (dm, 1H, J_{gem} = 9.1, H-7b), 2.50 (ddd, 1H, J_{gem} = 12.5, J_{3en-2} = 6.9, J_{3en-7a} = 2.4, H-3endo), 2.99 (br s, 1H, CH-cyclop), 3.84 (m, 1H, H-2), 4.88 (d, 1H, J_{OH-2} = 3.5, OH), 7.86 (br s, 1H, NH), 8.12 (s, 1H, H-8′), 8.22 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 6.61 (CH_2-cyclop), 24.23 (C-6), 32.75 (C-5), 37.63 (C-7), 42.75 (C-1), 46.03 (C-3), 64.40 (C-4), 72.81 (C-2), 120.20 (C-5′), 139.59 (C-8′), 149.69 (C-4′), 152.18 (C-2′), 155.88 (C-6′). ESI MS *m*/*z* (%): 286.2 (100) [M+H], 308.2 (12) [M+Na]. For $C_{15}H_{19}N_5O$ (285.16): calculated: 63.14; C, 6.71; H, 24.54; N; found: 62.86; C, 6.83; H, 24.24; N.

4.10.2. (1*R**,2R*,4*R**)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]bicyclo[2.2.1]heptan-2-ol (16)

From **14** (490 mg, 1.75 mmol). The poorly soluble product was filtered off and thoroughly washed with water and methanol. Yield: 400 mg, 76%, pink crystals (mp = 258 °C (decomp.)). ^{1}H NMR (500 MHz, DMSO): δ 0.57 and 0.65 (m, 4H, CH_2-cyclop), 1.27 (m, 1H, H-6endo), 1.58 (dm, 1H, J_{gem} = 12.5, H-3exo), 1.68–1.80 (m, 2H, H-5exo, H-6exo), 1.89 (m, 1H, H-5endo), 2.00 (m, 1H, J_{gem} = 9.1, H-7a), 2.12 (m, 1H, H-1), 2.28 (dm, 1H, J_{gem} = 9.2, H-7b), 2.50 (m, 1H, H-3endo), 3.01 (br s, 1H, CH-cyclop), 3.80 (m, 1H, H-2), 4.81 (d, 1H, $J_{OH,2}$ = 3.6, OH), 5.69 (br s, 2H, NH_2), 7.21 (br s, 1H, NH), 7.66 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 6.58 (CH_2-cyclop), 24.27 (C-6), 32.51 (C-5), 37.44 (C-7), 42.65 (C-1), 45.98 (C-3), 63.80 (C-4), 72.78 (C-2), 114.54 (C-5′), 135.96 (C-8′), 152.1 (C-4′), 156.08 (C-6′), 159.82 (C-2′). ESI MS *m*/*z* (%): 301.3 (100) [M+H], 323.3 (10) [M+Na]; HRMS ESI ($C_{15}H_{21}ON_6$): calculated: 301.17714; found: 301.17697. For $C_{15}H_{20}N_6O$ (300.36): calculated: 59.98; C, 6.71; H, 27.98; N; found: 60.11; C, 6.80; H, 27.80; N.

4.10.3. (1*S**,4*S**)-4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]bicyclo[2.2.1] heptan-2-one (20)

From **18** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene-cyclohexane mixture; yield: 104 mg, 77%, white crystals (mp = 173–174 °C). ^{1}H NMR (500 MHz, DMSO): δ 0.61 and 0.72 (m, 4H, CH_2-cyclop), 1.55 (m, 1H, H-6endo), 2.10 (m, 1H, H-6exo), 2.20–2.30 (m, 2H, H-5), 2.47–2.52 (m, 2H, H-7), 2.72 (dm, 1H, J_{1-6ex} = 5.2, H-1), 2.75–2.85 (m, 2H, H-3), 3.02 (br s, 1H, CH-cyclop), 7.91 (br s, 1H, NH), 8.19 (s, 1H, H-8′), 8.24 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 6.58 (CH_2-cykclop), 23.41 (C-6), 31.65 (C-5), 39.99 (C-7), 48.66 (C-3), 49.07 (C-1), 62.80 (C-4), 120.12 (C-5′), 139.29 (C-8′), 149.63 (C-4′), 152.34 (C-2′), 155.85 (C-6′), 211.67 (C-2). ESI MS *m*/*z* (%): 284.2 (100) [M+H]. For $C_{15}H_{17}N_5O$ (283.32): calculated: 63.59; C, 6.05; H, 24.72; N; found: 63.33; C, 6.04; H, 24.36; N.

4.10.4. (1*S**,2R*,4*S**)-4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]bicyclo [2.2.1]heptan-2-ol (27)

From **25** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene–cyclohexane mixture; yield: 117 mg, 85%, white crystals (mp = 201–202 °C). ^{1}H NMR (500 MHz, DMSO): δ 0.59 (m, 2H, CH_2-cyclop), 0.70 (m, 2H, CH_2-cyclop), 1.61 (m, 1H, H-6exo), 1.73 (dm, 1H, J_{gem} = 12.4, H-3endo), 1.93 (m, 1H, H-5exo), 2.08–2.16 (m, 4H, H-5endo, H-6endo, H-7), 2.22–2.30 (m, 2H, H-3exo, H-1), 3.00 (br s, 1H, CH-cyclop), 4.25 (m, 1H, H-2), 4.94 (d, 1H, $J_{OH\text{-}2}$ = 4.1, OH), 7.85 (br s, 1H, NH), 8.07 (s, 1H, H-8′), 8.22 (br s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 6.57 (CH_2-cyclop), 20.86 (C-6), 34.27 (C-5), 40.24 (C-7), 41.08 (C-1), 43.71 (C-3), 65.32 (C-4), 69.90 (C-2), 120.21 (C-5′), 139.35 (C-8′), 149.49 (C-4′), 152.15 (C-2′), 155.83 (C-6′). ESI MS *m*/*z* (%): 286.3 (100) [M+H]. For $C_{15}H_{19}N_5O$ (285.16): calculated: 63.14; C, 6.71; H, 24.54; N; found: 63.12; C, 6.74; H, 24.69; N.

4.11. Microwave-assisted nucleophilic displacement of a C-6 chlorine atom of the purine nucleobase with in-situ generated dimethylamine (compounds 12, 21, and 28)

A solution of substrate (0.5 mmol) in DMF (3 mL) was subjected to microwave irradiation (sealed vessel, 200 °C, 2 min). The volatiles were evaporated, and the crude product was adsorbed on silica and purified by column chromatography and crystallization.

4.11.1. (1*S**,2*S**,4*S**)-4-[6-(Dimethylamino)-9*H*-purin-9-yl]bicyclo[2.2.1] heptan-2-ol (12)

From **9** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene—cyclohexane mixture; yield: 116 mg, 86%, white crystals (mp = 143–144 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.31 (dddd, 1H, J_{gem} = 12.3, $J_{6en\text{-}5en}$ = 9.2, $J_{6en\text{-}5ex}$ = 4.7, $J_{6en\text{-}7b}$ = 2.1, H-6endo), 1.68 (dm, 1H, J_{gem} = 12.4, H-3exo), 1.75 (tdd, 1H, J_{gem} = $J_{6ex\text{-}5ex}$ = 12.3, $J_{6ex\text{-}1}$ = 5.1, $J_{6ex\text{-}5en}$ = 4.0, H-6exo), 1.84 (m, 1H, H-5exo), 1.95 (dddd, 1H, J_{gem} = 11.3, $J_{5en\text{-}6en}$ = 9.4, $J_{5en\text{-}6ex}$ = 4.1, $J_{5en\text{-}7b}$ = 2.2, H-5endo), 2.01 (dm, 1H, J_{gem} = 9.0, H-7a), 2.15 (dm, 1H, $J_{1\text{-}6ex}$ = 5.0, H-1), 2.39 (dq, 1H, J_{gem} = 9.1, $J_{7b\text{-}1}$ = $J_{7b\text{-}5en}$ = $J_{7b\text{-}6en}$ = 2.0, H-7b), 2.51 (ddd, 1H, J_{gem} = 12.4, $J_{3en\text{-}2}$ = 6.9, $J_{3en\text{-}7a}$ = 2.4, H-3endo), 3.44 (br s, 6H, CH_3), 3.84 (m, 1H, H-2), 4.88 (d, 1H, $J_{OH\text{-}2}$ = 3.6, OH), 8.12 (s, 1H, H-8′), 8.19 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.21 (C-6), 32.59 (C-5), 37.54 (C-7), 42.69 (C-1), 45.91 (C-3), 64.42 (C-4), 72.78 (C-2), 120.36 (C-5′), 138.58 (C-8′), 151.07 (C-4′), 151.55 (C-2′), 154.53 (C-6′). ESI MS *m*/*z* (%): 274.2 (100) [M+H], 296.1 (43) [M+Na]. For $C_{14}H_{19}N_5O$ (273.33): calculated: 61.52; C, 7.01; H, 25.62; N; found: 61.43; C, 7.04; H, 25.26; N.

4.11.2. (1*S**,4*S**)-4-[6-(Dimethylamino)-9*H*-purin-9-yl]bicyclo[2.2.1] heptan-2-one (21)

From **18** (130 mg, 0.5 mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene–cyclohexane mixture; yield: 116 mg, 86%, white crystals (mp = 160–161 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.55 (m, 1H, H-6endo), 2.10 (m, 1H, H-6exo), 2.19–2.31 (m, 2H, H-5), 2.28 (m, 2H, H-3), 2.49 (m, 2H, H-7), 2.71 (dm, 1H, $J_{1\text{-}6ex}$ = 5.1, H-1), 3.46 (br s, 6H, CH_3), 8.19 (s, 1H, H-8′), 8.22 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 23.40 (C-6), 31.53 (C-5), 38.16 (CH_3), 39.91 (C-7), 48.55 (C-3), 49.03 (C-1), 62.82 (C-4), 120.28 (C-5′), 138.29 (C-8′), 150.91 (C-4′), 151.73 (C-2′), 154.55 (C-6′), 211.65 (C-2). ESI MS *m*/*z* (%): 272.2 (100) [M+H]. For $C_{14}H_{17}N_5O$ (271.31): calculated: 61.98; C, 6.32; H, 25.81; N; found: 61.55; C, 6.33; H, 25.52; N.

4.11.3. (1*S**,2R*,4*S**)-4-[6-(Dimethylamino)-9*H*-purin-9-yl]bicyclo[2.2.1] heptan-2-ol (28)

From **25** (110 mg, 0.4 mmol mmol); mobile phase: ethyl acetate—toluene—acetone—ethanol 17:4:3:1; crystallization from a toluene–cyclohexane mixture; yield: 87 mg, 77%, white crystals (mp = 156–157 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.60 (m, 1H, H-6exo), 1.74 (dm, 1H, J_{gem} = 12.4, H-3endo), 1.91 (m, 1H, H-5exo), 2.08–2.16 (m, 4H, H-5endo, H-6endo, H-7), 2.22–2.30 (m, 2H, H-1, H-3exo), 3.44 (br s, 6H, CH_3), 4.25 (m, 1H, H-2), 4.94 (d, 1H, $J_{OH\text{-}2}$ = 4.0, OH), 8.07 (s, 1H, H-8′), 8.19 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 20.84 (C-6), 34.15 (C-5), 40.17 (C-7), 41.03 (C-1), 43.61 (C-3), 65.35 (C-4), 69.88 (C-2), 120.38 (C-5′), 138.38 (C-8′), 150.95 (C-4′), 151.55 (C-2′), 154.54 (C-6′). ESI MS *m*/*z* (%): 274.3 (100) [M+H]. For $C_{14}H_{19}N_5O$ (273.33): calculated: 61.52; C, 7.01; H, 25.62; N; found: 61.75; C, 7.05; H, 25.69; N.

4.12. Nucleophilic displacement of a C-6 chlorine atom of the purine nucleobase with a sulfanyl group (compounds 13, 22, and 29)

A solution of a 6-chloropurine derivative (0.5 mmol) and thiourea (0.6 mmol) in dry ethanol (4 mL) was heated in a pressure vessel at 105 °C overnight. The poorly soluble product was collected on a paper filter and washed thoroughly with ethanol and diethylether.

4.12.1. (1*S**,2*S**,4*S**)-4-(6-Sulfanyl-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (13)

From **9** (130 mg, 0.5 mmol); yield: 98 mg, 76%, white powder (mp >320 °C (decomp.)). ^{1}H NMR (500 MHz, DMSO): δ 1.31 (dddd, 1H, J_{gem} = 12.1, $J_{6en\text{-}5en}$ = 8.8, $J_{6en\text{-}5ex}$ = 4.6, $J_{6en\text{-}7b}$ = 2.1, H-6endo), 1.67 (dm, 1H, J_{gem} = 12.5, H-3exo), 1.75 (tdd, 1H, J_{gem} = $J_{6ex\text{-}5ex}$ = 12.1, $J_{6ex\text{-}1}$ = 5.1, $J_{6ex\text{-}5en}$ = 4.0, H-6exo), 1.83 (m, 1H, H-5exo), 1.95 (m, 1H, H-5endo), 1.98 (dm, 1H, J_{gem} = 9.1, H-7a), 2.16 (dm, 1H, $J_{1\text{-}6ex}$ = 5.0, H-1), 2.40 (dq, 1H, J_{gem} = 9.2, $J_{7b\text{-}1}$ = $J_{7b\text{-}5en}$ = $J_{7b\text{-}6en}$ = 2.0, H-7b), 2.49 (ddd, 1H, J_{gem} = 12.4, $J_{3en\text{-}2}$ = 6.8, $J_{3en\text{-}7a}$ = 2.4, H-3endo), 3.84 (dm, 1H, $J_{2\text{-}3en}$ = 6.5, H-2), 4.92 (br s, 1H, OH), 8.18 (d, 1H, $J_{2'\text{-}1'}$ = 3.8, H-2′), 8.29 (s, 1H, H-8′), 13.74 (br s, 1H, H-1′).^{13}C NMR (125.8 MHz, DMSO): δ 24.14 (C-6), 32.94 (C-5), 37.70 (C-7), 42.71 (C-1), 46.13 (C-3), 64.87 (C-4), 72.73 (C-2), 136.05 (C-5′), 142.17 (C-8′), 144.47 (C-2′), 144.78 (C-4′), 176.07 (C-6′). ESI MS *m*/*z* (%): 263.1 (12) [M+H], 285.1 (100) [M+Na]. For $C_{12}H_{14}N_4OS$ (262.33): calculated: 54.94; C, 5.38; H, 21.36; N, 12.22; S; found: 54.64; C, 5.37; H, 21.09; N, 12.12; S.

4.12.2. (1*S**,4*S**)-4-(6-Sulfanyl-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-one (22)

From **18** (130 mg, 0.5 mmol); yield: 115 mg, 89%, white powder (mp >320 °C (decomp.)). ^{1}H NMR (500 MHz, DMSO): δ 1.55 (m, 1H, H-6endo), 2.09 (tt, 1H, J_{gem} = $J_{6ex\text{-}5ex}$ = 12.5, $J_{6ex\text{-}1}$ = $J_{6ex\text{-}5en}$ = 4.9, H-6exo), 2.20 (m, 1H, H-5exo), 2.27 (m, 1H, J_{gem} = 11.7, $J_{5en\text{-}6en}$ = 9.0, $J_{5en\text{-}6ex}$ = 4.8, $J_{5en\text{-}7}$ = 1.9, H-5endo), 2.46–2.50 (m, 2H, H-7), 2.73 (dm, 1H, $J_{1\text{-}6ex}$ = 4.9, H-1), 2.78–2.79 (m, 2H, H-3), 8.19 (bd, 1H, $J_{2'\text{-}1'}$ = 1.8, H-2′), 8.34 (s, 1H, H-8′), 13.77 (br s, 1H, H-1′). ^{13}C NMR (125.8 MHz, DMSO): δ 23.37 (C-6), 31.92 (C-5), 40.05 (C-7), 48.78 (C-3), 49.00 (C-1), 63.18 (C-4), 136.04 (C-5′), 141.82 (C-8′), 144.64 (C-4′), 144.72 (C-2′), 176.20 (C-6′), 211.22 (C-2). ESI MS *m*/*z* (%): 261.1 (18) [M+H], 283.1 (100) [M+Na]. For $C_{12}H_{12}N_4OS$ (260.31): calculated: 55.37; C, 4.65; H, 21.52; N, 12.32; S; found: 55.16; C, 4.66; H, 21.17; N, 12.39; S.

4.12.3. (1*S**,2R*,4*S**)-4-(6-Sulfanyl-9*H*-purin-9-yl)bicyclo[2.2.1]heptan-2-ol (29)

From **25** (110 mg, 0.4 mmol); yield: 74 mg, 68%, white powder (mp >320 °C (decomp.)). ^{1}H NMR (500 MHz, DMSO): δ 1.62 (m, 1H, H-6exo), 1.75 (m, 1H, J_{gem} = 12.4, H-3endo), 1.90 (m, 1H,

H-5exo), 2.07–2.16 (m, 4H, H-5endo, H-6endo, H-7), 2.20–2.26 (m, 2H, H-1, H-3exo), 4.25 (m, 1H, H-2), 4.97 (br s, 1H, OH), 8.17 (s, 1H, H-2′), 8.23 (s, 1H, H-8′), 13.70 (br s, 1H, NH). ^{13}C NMR (125.8 MHz, DMSO): δ 20.88 (C-6), 34.44 (C-5), 40.29 (C-7), 41.02 (C-1), 43.89 (C-3), 65.81 (C-4), 69.80 (C-2), 136.05 (C-5′), 141.90 (C-8′), 144.46 (C-2′), 144.67 (C-4′), 176.07 (C-6′). EI MS *m*/*z* (%): 262.1 (100) [M]. For $C_{12}H_{14}N_4OS$ (262.33): calculated: 54.94; C, 5.38; H, 21.36; N, 12.22; S; found: 54.61; C, 5.40; H, 21.60; N, 12.26; S.

4.13. Acid-catalyzed hydrolysis of 2-amino-6-chloropurine substrates to guanine derivatives (compounds 17, 24, and 31)

A solution of a 2-amino-6-chloropurine derivative (0.5 mmol) in a TFA–water mixture (2:1, 6 mL) was stirred at rt overnight. The volatiles were evaporated and the crude product was codistilled with ethanol (3 × 10 mL), NH_4OH (10 mL) and ethanol (2 × 10 mL). The poorly soluble product was shortly boiled in a water–methanol mixture (1:1), collected on a filter and thoroughly washed with water, ethanol and diethylether.

4.13.1. 2-Amino-9-[(1*R**,3*R**,4*R**)-3-hydroxybicyclo[2.2.1]hept-1-yl]-1,9-dihydro-6*H*-purin-6-one (17)

From **14** (100 mg, 0.36 mmol); yield: 52 mg, 56%, pale-orange powder (mp >360 °C). ^{1}H NMR (500 MHz, DMSO): δ 1.25 (m, 1H, H-5endo), 1.56 (dm, 1H, J_{gem} = 12.6, H-2exo), 1.67–1.77 (m, 2H, H-6exo, H-5exo), 1.89 (m, 1H, H-6endo), 1.97 (dm, 1H, J_{gem} = 9.2, H-7a), 2.11 (m, 1H, H-4); 2.27 (dm, 1H, J_{gem} = 9.2, H-7b), 2.48 (ddd, 1H, J_{gem} = 12.5, $J_{2en,3}$ = 6.9, $J_{2en,7a}$ = 2.3, H-2endo), 3.79 (m, 1H, H-3), 4.82 (m, 1H, OH), 6.36 (br s, 2H, NH_2), 7.66 (s, 1H, H-8′), 10.10 (br s, 1H, H-1′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.24 (C-5), 32.64 (C-6), 37.50 (C-7), 42.62 (C-4), 46.04 (C-2), 64.08 (C-1), 72.76 (C-3), 117.83 (C-5′), 136.25 (C-8′), 151.88 (C-4′), 152.94 (C-2′), 157.08 (C-6′). ESI MS *m*/*z* (%): 262.2 (52) [M+H], 284.2 (100) [M+Na]; HRMS ESI ($C_{12}H_{16}O_2N_5$): calculated: 262.12985, found: 262.12983. For $C_{12}H_{15}N_5O_2$ (261.28): calculated: 55.16; C, 5.79; H, 26.80; N; found: 55.09; C, 5.77; H, 26.71; N.

4.13.2. 2-Amino-9-[(1*R**,4*R**)-3-oxobicyclo[2.2.1]hept-1-yl]-1,9-dihydro-6*H*-purin-6-one (24)

From **23** (150 mg, 0.54 mmol); yield: 120 mg, 86%, brown powder (mp >360 °C (decomp)). ^{1}H NMR (500 MHz, DMSO): δ 1.50 (m, 1H, H-5endo), 2.05 (m, 1H, H-5exo), 2.13–2.22 (m, 2H, H-6), 2.37 (ddd, 1H, J_{gem} = 9.8, $J_{7b,3en}$ = 4.0, $J_{7b,1}$ = 1.4, H-7b), 2.46 (dm, 1H, J_{gem} = 9.8, H-7a), 2.68 (m, 1H, H-4), 2.69 (dd, 1H, J_{gem} = 17.2, $J_{2en,7b}$ = 4.0, H-2endo), 2.77 (dm, 1H, J_{gem} = 17.2, H-2exo), 6.39 (br s, 2H, NH_2), 7.74 (s, 1H, H-8′), 10.61 (br s, 1H, NH). ^{13}C NMR (125.8 MHz, DMSO): δ 23.37 (C-5), 31.55 (C-6), 39.8 (C-7), 48.48 (C-2), 49.00 (C-4), 62.54 (C-1), 117.71 (C-5′), 135.95 (C-8′), 151.76 (C-4′), 153.16 (C-2′), 156.99 (C-6′), 211.90 (C-3). ESI MS *m*/*z* (%): 260.2 (40) [M+H], 282.2 (100) [M+Na]; HRMS ESI ($C_{12}H_{13}O_2N_5Na$): calculated: 282.09615; found: 282.09613. For $C_{12}H_{13}N_5O_2$. 0.5 H_2O (268.27): calculated: 53.72; C, 5.26; H, 26.11; N; found: 53.65; C, 5.19; H, 25.96; N.

4.13.3. 2-Amino-9-[(1*R**,3*R**,4*R**)-3-hydroxybicyclo[2.2.1]hept-1-yl]-1,9-dihydro-6*H*-purin-6-one (31)

From **30** (100 mg, 0.36 mmol); yield: 60 mg, 64%, pale-orange powder (mp >360 °C (decomp)). ^{1}H NMR (500 MHz, DMSO): δ 1.56 (m, 1H, H-6exo), 1.70 (dm, 1H, J_{gem} = 12.5, H-3endo), 1.82 (m, 1H, H-5exo), 2.02–2.18 (m, 4H, H-5endo, H-6endo, H-7), 2.15 (m, 1H, H-3exo), 2.19 (m, 1H, H-1), 4.21 (m, 1H, H-2), 4.88 (d, 1H, J_{OH-2} = 3.7, OH), 6.30 (br s, 2H, NH_2), 7.60 (s, 1H, H-8′), 10.52 (br s, 1H, H-1′). ^{13}C NMR (125.8 MHz, DMSO): δ 24.71 (C-6), 33.80 (C-5), 40.05 (C-7), 40.88 (C-1), 42.80 (C-3), 66.48 (C-4), 69.91 (C-2), 117.45 (C-5′), 133.01 (C-8′), 152.44 (C-4′), 153.88 (C-2′), 155.13 (C-6′). ESI MS *m*/*z* (%): 262.2 (61) [M+H], 284.2 (100) [M+Na]; HRMS ESI ($C_{12}H_{16}O_2N_5$): calculated: 262.12985, found: 262.12988. For $C_{12}H_{15}N_5O_2$ (261.28): calculated: 55.16; C, 5.79; H, 26.80; N; found: 55.32; C, 5.85; H, 26.50; N.

4.14. *tert*-Butyl [(1*R**,3*R**,4*R**)-3-hydroxybicyclo[2.2.1]hept-1-yl] carbamate (39)

Boc_2O (1.31 g, 6 mmol) was added to a solution of **7** (655 mg, 4 mmol) and DIPEA (2.09 mL, 12 mmol) in DCM (25 mL), and the reaction mixture was stirred at rt overnight. DCM was evaporated, and the resulting slurry was dissolved in ethyl acetate and washed with water (2 × 50 mL). The organic layer was dried over sodium sulfate, evaporated, and the crude product was purified by flash chromatography (30–50% ethyl acetate in hexane) and crystallized from hexane to afford **39** (807 mg, 89%) as white crystals.

^{1}H NMR (500 MHz, DMSO): δ 1.12 (m, 1H, H-5endo), 1.34–1.40 (m, 2H, H-6endo, H-7b), 1.40 (s, 9H, CH_3), 1.47 (dm, 1H, J_{gem} = 12.5, H-2exo), 1.52–1.61 (m, 2H, H-5exo, H-6exo), 1.84 (dm, 1H, J_{gem} = 9.1, H-7a), 1.89–1.92 (m, 2H, H-4, H-2endo), 3.66 (dm, 1H, $J_{3,2en}$ = 7.0, H-3), 6.46 (br s, 1H, NH). ^{13}C NMR (125.8 MHz, DMSO): δ 24.07 (C-5), 28.09 (CH_3), 31.99 (C-6), 37.39 (C-7), 42.17 (C-4), 45.80 (C-2), 59.99 (C-1), 72.77 (C-3), 77.03 (CH_3), 154.51 (COO). ESI MS *m*/*z* (%): 250.1 (100) [M+Na]; HRMS ESI ($C_{12}H_{21}O_3NNa$): calculated: 250.14136; found: 250.14125. For $C_{12}H_{21}NO_3$ (227.30): calculated: 63.41; C, 9.31; H, 6.16; N; found: 63.43; C, 9.33; H, 6.23; N.

4.15. The method of secondary-hydroxyl-group alkylation with diisopropyl tosylmethanphosphonate (compounds 33, 35, 37 and 40)

A solution of diisopropyl tosylmethanphosphonate (1.5 mmol) in DMF (5 mL) was added to a solution of a substrate (1 mmol) and (*t*-BuO)$_2$Mg (1.5 mmol) in dry DMF (25 mL), and the mixture was heated to 60 °C for 3 d. The volatiles were evaporated and the crude product was adsorbed on silica and purified by flash chromatography to afford phosphonate diisopropyl ester.

4.15.1. Diisopropyl [({4-[6-amino-9*H*-purin-9-yl]bicyclo[2.2.1]hept-2-yl}oxy)methyl]phosphonate (33)

From **10** (231 mg, 0.94 mmol); mobile phase: 3–10% methanol in ethyl acetate; yield: 350 mg, 89%, clear oil. ^{1}H NMR (500 MHz, DMSO): δ 1.24 (m, 12H, CH_3-*i*-Pr); 1.30 (m, 1H, H-6endo), 1.81 (m, 1H, H-6exo), 1.84 (m, 1H, H-3exo), 1.91 (m, 1H, H-5exo), 1.97 (m, 1H, H-5endo); 2.03 (dm, 1H, J_{gem} = 9.4, H-7b), 2.28 (dm, 1H, J_{gem} = 9.4, H-7a), 2.5 (m, 2H, H-1, H-3endo), 3.68–3.79 (m, 3H, CH_2P, H-2), 4.60 (m, 2H, CH-*i*-Pr), 7.18 (s, 2H, NH_2), 8.11 (s, 1H, H-2′), 8.14 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 23.87 (C-6), 23.89–24.06 (m, CH_3-*i*-Pr), 32.61 (C-5), 37.83 (C-7), 38.61 (C-1), 43.11 (C-3), 62.54 (d, J_{C-P} = 165.7, CH_2-P), 64.15 (C-4), 70.35 (d, J_{C-P} = 6.2, *CH*-*i*-Pr), 83.40 (d, J_{2-P} = 13.1, C-2), 119.79 (C-5′), 139.70 (C-8′), 150.19 (C-4′), 152.26 (C-2′), 156.30 (C-6′). ESI MS *m*/*z* (%): 424.3 (100) [M+H], 446.3 (95) [M+Na]; HRMS ESI ($C_{19}H_{30}O_4N_5NaP$): calculated: 446.19276; found: 446.19254. For $C_{19}H_{30}N_5O_4P$ (423.45): calculated: 53.89; C, 7.14; H, 16.54; N; 7.31; P; found: 53.94; C, 7.23; H, 16.15; N, 7.38; N.

4.15.2. Diisopropyl [({4-[6-(cyclopropylamino)-9*H*-purin-9-yl]bicyclo[2.2.1]hept-2-yl}oxy)methyl]phosphonate (35)

From **11** (280 mg, 0.8 mmol); the mobile phase: 1–3% methanol in ethyl acetate; yield: 70 mg, 25%, clear oil.

^{1}H NMR (500 MHz, DMSO): δ 0.66 and 0.92 (m, 4H, CH_2-cyclop), 1.32–1.34 (m, 12H, CH_3-*i*Pr), 1.90–1.98 (m, 4H, H-3exo, H-5a, H-6), 2.09–2.16 (m, 2H, H-5b, H-7a), 2.40 (dm, 1H, J_{gem} = 9.2, H-7b), 2.57 (m, 1H, H-1), 2.69 (ddd, 1H, J_{gem} = 12.8, J_{3en-2} = 6.9, J_{3en-7a} = 2.6,

H-3endo), 3.02 (br s, 1H, CH-cyclop), 3.69 (dd, 1H, J_{gem} = 13.4 $J_{H\text{-}C\text{-}P}$ = 9.5) and 3.74 (dd, 1H, J_{gem} = 13.4, $J_{H\text{-}C\text{-}P}$ = 9.4, CH_2P), 3.79 (dm, 1H, $J_{2\text{-}3en}$ = 7.0, H-2), 4.71–4.80 (m, 2H, CH-*i*Pr), 6.04 (br s, 1H, NH), 7.76 (s, 1H, H-8′), 8.46 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, DMSO): δ 7.31 (CH_2-cyclop), 23.98–24.09 (CH_3-*i*Pr), 24.20 (C-6), 33.02 (C-5), 38.16 (C-7), 38.90 (C-1), 43.35 (C-3), 63.26 (d, $J_{C\text{-}P}$ = 169.8, CH_2P), 64.49 (C-4), 71.07 (d, $J_{C\text{-}O\text{-}P}$ = 6.7, CH-iPr), 83.90 (d, $J_{3\text{-}P}$ = 12.7, C-2), 120.70 (C-5′), 138.35 (C-8′), 149.90 (C-4′), 152.64 (C-2′), 155.74 (C-6′). HRMS ESI ($C_{22}H_{35}N_5O_4P$): calculated: 464.2421; found: 464.2420.

4.15.3. Diisopropyl [({4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]bicyclo[2.2.1]hept-2-yl}oxy)methyl] phosphonate (37)

From **16** (250 mg, 0.83 mmol); mobile phase: 1–2% methanol in ethyl acetate; yield: 320 mg, 81%, clear oil. ^{1}H NMR (500 MHz, DMSO): δ 0.57 and 0.65 (m, 4H, CH_2-cyclop), 1.22–1.26 (m, 12H, CH_3-*i*-Pr), 1.26 (m, 1H, H-6endo), 1.71 (dm, 1H, J_{gem} = 12.8, H-3exo), 1.75–1.85 (m, 2H, H-5exo, H-6exo), 1.93 (m, 1H, H-5endo), 2.07 (dm, 1H, J_{gem} = 9.4, H-7b), 2.12 (dm, 1H, J_{gem} = 9.4, H-7a), 2.45 (m, 1H, H-1), 2.54 (ddd, 1H, J_{gem} = 12.8, $J_{3en,2}$ = 6.8, $J_{3en,7}$ = 2.2, H-3endo), 3.01 (br s, 1H, CH-cyclop), 3.69 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 9.2, CH_2Pa), 3.72 (m, 1H, H-2), 3.75 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 8.9, CH_2Pb), 4.55–4.64 (m, 2H, *CH*-*i*-Pr), 5.69 (br s, 2H, NH_2), 7.25 (m, 1H, NH), 7.69 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, DMSO): δ 6.58 (CH_2-cyclop), 23.88–24.04 (m, CH_3-*i*-Pr), 23.99 (C-6), 32.43 (C-5), 37.75 (C-7), 38.60 (C-1), 43.07 (C-3), 62.51 (d, $J_{C\text{-}P}$ = 165.8, CH_2-P), 63.62 (C-4), 70.28 (m, *CH*-*i*-Pr), 83.37 (d, $J_{2\text{-}P}$ = 13.0, C-2), 114.52 (C-5′), 135.91 (C-8′), 152.0 (C-4′), 156.11 (C-6′), 159.87 (C-2′). NegESI MS *m*/*z* (%): 423.1 (100) [M−H]; HRMS negESI ($C_{14}H_{21}O_9N_2P_2$): calculated: 423.07278; found: 423.07269. For $C_{22}H_{35}N_6O_4P$ (478.52): calculated: 55.22; C, 7.37; H, 17.56; N; 6.47; P; found: 55.28; C, 7.47; H, 17.30; N, 6.78; P.

4.15.4. Diisopropyl [({(1*R**,3*R**,4*R**)-4-[(*tert*-butoxycarbonyl) amino]bicyclo[2.2.1]hept-2-yl}oxy)methyl]phosphonate (40)

From **39** (770 mg, 3.39 mmol); mobile phase: 60–100% ethyl acetate in hexane; yield: 800 mg, 58%, clear oil. ^{1}H NMR (500 MHz, DMSO): δ 1.09 (m, 1H, H-6endo), 1.22–1.25 (m, 12H, CH_3CH), 1.37 (s, 9H, CH_3C), 1.33–1.40 (m, 2H, H-5endo, H-7b), 1.50–1.62 (m, 3H, H-3exo, H-6exo, H-5exo), 1.67 (m, 1H, H-7a), 1.88 (m, 1H, H-3endo), 2.20 (m, 1H, H-1), 3.51 (m, 1H, H-2), 3.60 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 9.3, CH_2Pb), 3.68 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 9.0, CH_2Pa), 4.54–4.63 (m, 2H, CH_3CH), 7.03 and 7.32 (br s, 1H, NH). ^{13}C NMR (125.8 MHz, DMSO): δ 23.87–24.05 (m, CH_3CH), 24.06 (C-6), 28.46 (CH_3C), 32.07 (C-5), 37.83 (C-7), 38.13 (C-1), 42.74 (C-3), 60.09 (C-4), 62.48 (d, $J_{C\text{-}P}$ = 166.0, CH_2-P), 70.21–70.29 (m, CH_3CH), 77.48 (CH_3C), 83.89 (d, $J_{2\text{-}P}$ = 13.3, C-2), 154.80 and 154.97 (COO). ESI MS *m*/*z* (%): 406.3 (17) [M+H], 428.3 (100) [M+Na]; HRMS ESI ($C_{19}H_{36}O_6NNaP$): calculated: 428.21725; found: 428.21728.

4.16. The method of hydrolysis of phosphonate diesters (compounds 32, 34, 36 and 38)

TMSBr (0.7 mL) was added to a solution of diisopropyl phosphonate (0.5 mmol) in dry DCM (10 mL) and the reaction mixture was stirred at rt for 24 h. The volatiles were evaporated and the crude product was codistilled with dry ethanol (3 × 15 mL) to afford free phosphonate as a clear solid or white lyophilizate. Further purification was possible on a short C18 column.

4.16.1. ({5-methyl-2,6-Dioxo-3-[(1*R**,3*R**,4*R**)-3-(phosphonomethoxy)bicyclo[2.2.1]hept-1-yl]-3,6-dihydropyrimidin-1(2*H*)-yl}methyl)phosphonic acid (32)

From **8** (100 mg, 0.42 mmol); the mobile phase: 1–2% methanol in ethyl acetate; after hydrolysis of ester groups: the mobile phase: 30–80% methanol in water, C18 column; yield: 107 mg, 60%, clear solid or white lyophilizate. ^{1}H NMR (500 MHz, D_2O): δ 1.34 (m, 1H, H-5endo), 1.57–1.66 (m, 2H, H-2exo, H-6exo), 1.82–1.91 (m, 2H, H-5exo, H-7b), 1.90 (d, 3H, $J_{CH3,6'}$ = 1.1, CH_3), 2.13–2.24 (m, 2H, H-6endo, H-7a), 2.71 (ddd, 1H, J_{gem} = 13.0, $J_{2en,3}$ = 6.9, $J_{2en,7b}$ = 2.4, H-2endo), 3.68 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 9.3, OCH_2Pb), 3.73 (dd, 1H, J_{gem} = 13.7, $J_{H,C,P}$ = 9.1, OCH_2Pa), 3.82 (m, 1H, H-3), 4.33 (d, 2H, $J_{H,C,P}$ = 12.4, NCH_2Pb), 7.61 (br s, 1H, H-6′). ^{13}C NMR (125.8 MHz, D_2O): δ 12.72 (CH_3), 24.36 (C-5), 31.59 (C-6), 38.69 (C-7), 39.02 (d, $J_{C\text{-}P}$ = 149.4, NCH_2-P), 42.02 (C-2), 63.63 (d, $J_{C\text{-}P}$ = 159.8, OCH_2-P), 71.44 (C-1), 84.79 (d, $J_{2\text{-}P}$ = 11.6, C-3), 108.96 (C-5′), 140.35 (C-6′), 152.18 (C-2′), 165.68 (C-4′). NegESI MS *m*/*z* (%): 423.1 (100) [M−H]; HRMS negESI ($C_{14}H_{21}O_9N_2P_2$) calculated: 423.07278; found: 423.07269. For $C_{14}H_{22}N_2O_9P_2$ (424.28): calculated: 39.63; C, 5.23; H, 6.60; N; 14.60; P; found: 39.81; C, 5.21; H, 6.40; N, 14.52; P.

4.16.2. [({4-[6-Amino-9*H*-purin-9-yl]bicyclo[2.2.1]hept-2-yl}oxy) methyl]phosphonic acid (34)

From **33** (250 mg, 0.59 mmol); yield: 190 mg, 95%, poorly-soluble white powder (mp = 289–290 °C). ^{1}H NMR (500 MHz, D_2O): δ 1.42 (m, 1H, H-6endo), 1.82 (m, 1H, H-5exo), 1.87–1.94 (m, 2H, H-3exo, H-6exo), 1.97–2.03 (m, 2H, H-5endo, H-7b), 2.38 (dm, 1H, J_{gem} = 9.1, H-7a), 2.55 (m, 1H, H-3endo), 2.60 (bd, 1H, $J_{1,6ex}$ = 5.0, H-1), 3.43 (dd, 1H, J_{gem} = 12.5, $J_{H,C,P}$ = 9.3, CH_2Pb), 3.49 (dd, 1H, J_{gem} = 12.5, $J_{H,C,P}$ = 9.0, CH_2Pa), 3.87 (m, 1H, H-2), 8.12–8.14 (m, 2H, H-2′, H-8′). ^{13}C NMR (125.8 MHz, D_2O): δ 24.52 (C-6), 32.90 (C-5), 38.51 (C-7), 39.33 (C-1), 43.30 (C-3), 65.44 (C-4), 66.65 (d, $J_{C\text{-}P}$ = 151.0, CH_2-P), 83.98 (d, $J_{2\text{-}P}$ = 11.0, C-2), 119.87 (C-5′), 141.95 (C-8′), 149.86 (C-4′), 152.38 (C-2′), 156.05 (C-6′). NegESI MS *m*/*z* (%): 338.1 (100) [M−H]; HRMS negESI ($C_{13}H_{17}O_4N_5P$): calculated: 338.10236; found: 338.10268. For $C_{13}H_{18}N_5O_4P$ (339.29): calculated: 46.02; C, 5.35; H, 20.64; N; 9.13; P; found: 46.37; C, 5.21; H, 20.29; N, 9.02; P.

4.16.3. [({4-[6-(Cyclopropylamino)-9*H*-purin-9-yl]bicyclo[2.2.1] hept-2-yl}oxy)methyl]phosphonic acid (36)

From **35** (70 mg, 0.15 mmol); yield: 53 mg, 92%, white solid. ^{1}H NMR (500 MHz, D_2O): δ 0.90 and 1.12 (m, 4H, CH_2-cyclop), 1.62 (m, 1H, H-6endo), 1.94–2.01 (m, 3H, H-3exo, H-5endo, H-6exo), 2.11–2.16 (m, 2H, H-5exo, H-7a), 2.47 (dm, 1H, J_{gem} = 9.6, H-7b), 2.65 (m, 1H, H-1), 2.68 (ddd, 1H, J_{gem} = 12.7, $J_{3en\text{-}2}$ = 6.8, $J_{3en\text{-}7a}$ = 2.5, H-3endo), 2.91 (br s, 1H, CH-cyclop), 3.73 (dd, 1H, J_{gem} = 13.7, $J_{H\text{-}C\text{-}P}$ = 9.4, CH_2Pa), 3.80 (dd, 1H, J_{gem} = 13.7, $J_{H\text{-}C\text{-}P}$ = 9.1, CH_2Pb), 3.94 (dm, 1H, $J_{2\text{-}3en}$ = 6.8, H-2), 8.36 (s, 1H, H-8′), 8.43 (s, 1H, H-2′). ^{13}C NMR (125.8 MHz, D_2O): δ 7.31 (CH_2-cyclop), 23.33 (CH-cyclop), 24.25 (C-4), 33.15 (C-5), 38.43 (C-7), 39.45 (C-1), 43.41 (C-3), 63.93 (d, $J_{C\text{-}P}$ = 159.9, (CH_2P), 66.14 (C-4), 71.07 (d, $J_{C\text{-}O\text{-}P}$ = 6.7, CH-*i*Pr), 83.90 (d, $J_{3\text{-}P}$ = 12.7, C-3), 120.70 (C-5′), 138.35 (C-8′), 149.90 (C-4′), 152.64 (C-2′), 155.74 (C-6′). HRMS ESI ($C_{16}H_{22}N_5O_4P$): calculated: 380.1482; found: 380.1482.

4.16.4. [({4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]bicyclo [2.2.1]hept-2-yl}oxy)methyl]phosphonic acid (38)

From **37** (220 mg, 0.46 mmol); the mobile phase: (30–80% methanol in water); yield: 164 mg, 90%, clear solid or white lyophilizate. ^{1}H NMR (500 MHz, D_2O): δ 0.83 and 1.05 (m, 4H, CH_2-cyclop), 1.36 (m, 1H, H-6endo), 1.79–1.98 (m, 5H, H-5, H-3exo, H-6exo, H-7b), 2.30 (dm, 1H, J_{gem} = 9.4, H-7a), 2.54 (ddd, 1H, J_{gem} = 12.6, $J_{3en,2}$ = 6.9, $J_{3en,7b}$ = 2.3, H-3endo), 2.56 (bd, 1H, $J_{1,6ex}$ = 4.8, H-1), 2.84 (br s, 1H, CH-cyclop), 3.59 (dd, 1H, J_{gem} = 13.3, $J_{H,C,P}$ = 9.5, CH_2Pb), 3.67 (dd, 1H, J_{gem} = 13.3, $J_{H,C,P}$ = 9.0, CH_2Pa), 3.83 (m, 1H, H-2), 7.92 (s, 1H, H-8′). ^{13}C NMR (125.8 MHz, D_2O): δ 7.53 (CH_2-cyclop), 24.14 (C-6), 32.85 (C-5), 38.13 (C-7), 39.19 (C-1), 43.02 (C-3), 64.81 (d, $J_{C\text{-}P}$ = 156.9, CH_2-P), 65.43 (C-4), 84.36

(d, $J_{2\text{-P}}$ = 11.9, C-2), 112.50 (C-5′), 141.51 (C-8′), 151.4 (C-4′), 150.63 and 152.47 (C-2′, C-6′). NegESI MS *m*/*z* (%): 393.1 (100) [M−H]; HRMS negESI ($C_{16}H_{22}O_4N_6P$): calculated: 393.14456; found: 393.14462. For $C_{16}H_{23}N_6O_4P$ (394.37): calculated: 48.73; C, 5.88; H, 21.31; N; 7.85; P; found: 48.91; C, 5.76; H, 21.33; N, 7.72; P.

4.17. ({[(1*R**,3*R**,4*R**)-4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)bicyclo[2.2.1]hept-2-yl]oxy}methyl) phosphonic acid (42)

TFA (2 mL) was added to the solution **40** (800 mg) in DCM (10 mL) and the reaction mixture was stirred at rt for 2 h. The volatiles were evaporated, and the product was codistilled with dry ethanol (3 × 15 mL) and purified on Dowex 50 (H^+) to afford **41** (320 mg, 56%) as brownish oil, which was directly used in the next reaction. Ethyl [(2*E*)-3-ethoxy-2-methylprop-2-enoyl]carbamate (203 mg, 1 mmol) was added to a solution of **41** (280 mg, 0.92 mmol) in dioxane (15 mL) and the reaction mixture was heated at 100 °C for 3 h. Dowex 50 (H^+, 5 g) was then added and the reaction mixture was heated to 100 °C overnight. The Dowex resin was filtered off, the crude product was adsorbed on C18 silica and purified by flash chromatography on the reverse-phase column (30–80% methanol in water) **42** (130 mg, 43%) as white lyophilizate. ^{1}H NMR (500 MHz, D_2O): δ 1.21 (m, 1H, H-6endo), 1.41–1.51 (m, 2H, H-3exo, H-5exo), 1.77 (d, 3H, CH_3), 1.69–1.80 (m, 2H, H-6exo, H-7b), 2.02–2.11 (m, 2H, H-5exo, H-7a), 2.34 (d, 1H, $J_{1,6ex}$ = 5.2, H-1), 2.59 (ddd, 1H, J_{gem} = 12.9, $J_{3en,2}$ = 2.1, $J_{3en,7a}$ = 2.1, H-3endo), 3.44 (dd, 1H, J_{gem} = 13.2, $J_{H,C,P}$ = 9.6, CH_2Pb), 3.49 (dd, 1H, J_{gem} = 13.2, $J_{H,C,P}$ = 9.7, CH_2Pa), 3.63 (d, 1H, $J_{2,3en}$ = 6.6, H-2), 4.67 (br s, 2H, POH), 7.50 (d, 1H, $J_{6',CH3}$ = 1.1, H-6′), 11.11 (s, 1H, NH). ^{13}C NMR (125.8 MHz, D_2O): δ 12.18 (CH_3), 23.99 (C-6), 31.47 (C-5), 37.46 (C-1), 38.31 (C-7), 42.19 (C-3), 63.96 (d, $J_{C\text{-P}}$ = 162.5, CH_2-P), 68.78 (C-4), 83.06 (d, $J_{2\text{-P}}$ = 12.4, C-2), 107.93 (C-5′), 139.92 (C-6′), 151.09 (C-2′), 164.31 (C-4′). NegESI MS *m*/*z* (%): 329.2 (100) [M−H]; HRMS NegESI ($C_{13}H_{18}O_6N_2P$): calculated: 329.08970; found: 329.08997.

4.18. Antiviral-activity assays

The compounds were evaluated against the following viruses: herpes simplex virus type 1 (HSV-1) KOS strain, thymidine kinase-deficient (TK^-) HSV-1 KOS strain resistant to ACV (ACV^r), herpes simplex virus type 2 (HSV-2) strains Lyons and G, varicella-zoster virus (VZV) Oka strain, TK^- VZV strain 07−1, human cytomegalovirus (HCMV) strains AD-169 and Davis, vaccinia virus Lederle strain, respiratory syncytial virus (RSV) Long strain, vesicular stomatitis virus (VSV), Coxsackie B4, parainfluenza 3, influenza virus A (subtypes H1N1, H3N2), influenza virus B, Sindbis, reovirus-1, Punta Toro, human immunodeficiency virus type 1 III_B strain and human immunodeficiency virus type 2 strain ROD. The antiviral assays, other than anti-HIV assays, were based on the 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) or with 20 plaque-forming units (PFU) (VZV) 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 had not been treated with the test compounds. Antiviral activity was expressed as the EC_{50} or the compound concentration required to reduce virus-induced cytopathogenicity or viral plaque formation by 50%.

4.19. Anti-HIV activity assays

The inhibition of HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in CEM cell cultures was measured in microtiter 96-well plates containing ~3 × 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 d 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%.

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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.2014.04.004.

References and notes

1. Cihlar, T.; Ray, A. S. *Antiviral Res.* **2010**, *85*, 39.
2. (a) Boojamra, C. G.; Mackman, R. L.; Markevitch, D. Y.; Prasad, V.; Ray, A. S.; Douglas, J.; Grant, D.; Kim, C. U.; Cihlar, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1120; (b) Mackman, R. L.; Lin, K. Y.; Boojamra, C. G.; Hui, H.; Douglas, J.; Grant, D.; Petrakovsky, O.; Prasad, V.; Ray, A. S.; Cihlar, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1116.
3. Wu, T. F.; Froeyen, M.; Kempeneers, V.; Pannecouque, C.; Wang, J.; Busson, R.; De Clercq, E.; Herdewijn, P. *J. Am. Chem. Soc.* **2005**, *127*, 5056.
4. Saneyoshi, H.; Deschamps, J. R.; Marquez, V. E. *J. Org. Chem.* **2010**, *75*, 7659.
5. Boojamara, C. G.; Cannizzaro, C. E.; Chen, J. M.; Chen, X.; Cho, A.; Chong, L. S.; Fardis, M.; Jin, H.; Hirschmann, R. F.; Huang, A. X.; Kim, C. U.; Kirschberg, T. A.; Lee, C. P.; Lee, W. A.; Mackman, R. L.; Markevitch, D. Y.; Oare, D.; Prasad, V. K.; Pyun, H.-J.; Ray, A. S.; Sherlock, R.; Swaminathan, S.; Watkins, W.; Zhang, J. R.; WO2004096286A2.
6. Šála, M.; Hřebabecký, H.; Dračínský, M.; Masojídková, M.; De Palma, A. M.; Neyts, J.; Holý, A. *Tetrahedron* **2009**, *65*, 9291.
7. (a) Šála, M.; De Palma, A. M.; Hřebabecký, H.; Dejmek, M.; Dračínský, M.; Leyssen, P.; Neyts, J.; Mertlíková-Kaiserová, H.; Nencka, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4271; (b) Šála, M.; Hřebabecký, H.; Leyssen, P.; Dejmek, M.; Dračínský, M.; De Palma, A. M.; Neyts, J.; Nencka, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1963.
8. Tănase, C. I.; Drăghici, C.; Căproiu, M. T.; Shova, S.; Mathe, C.; Cocu, F. G.; Enache, C.; Maganu, M. *Bioorg. Med. Chem.* **2014**, *22*, 513.
9. (a) Marquez, V. E.; Ben-Kasus, T.; Barchi, J. J.; Green, K. M.; Nicklaus, M. C.; Agbaria, R. *J. Am. Chem. Soc.* **2004**, *126*, 543; (b) Marquez, V. E.; Hughes, S. H.; Sei, S.; Agbaria, R. *Antiviral Res.* **2006**, *71*, 268.
10. Field, H. J.; Biswas, S.; Mohammad, I. T. *Antiviral Res.* **2006**, *71*, 127.
11. Thiry, E.; Addie, D.; Belak, S.; Boucraut-Baralon, C.; Egberink, H.; Frymus, T.; Gruffydd-Jones, T.; Hartmann, K.; Hosie, M. J.; Lloret, A.; Lutz, H.; Marsilio, F.; Pennisi, M. G.; Radford, A. D.; Truyen, U.; Horzinek, M. C. *J. Feline Med. Surg.* **2009**, *11*, 547.
12. Dejmek, M.; Hřebabecký, H.; Šála, M.; Dračínský, M.; Nencka, R. *Synthesis-Stuttgart* **2011**, 4077.
13. (a) Boehme, W. R. *J. Am. Chem. Soc.* **1959**, *81*, 2762; (b) Kwart, H.; Null, G. *J. Am. Chem. Soc.* **1959**, *81*, 2765.
14. Dejmek, M.; Kovačková, S.; Zborníková, E.; Hřebabecký, H.; Šála, M.; Dračínský, M.; Nencka, R. *RSC Adv.* **2012**, *2*, 6970.
15. Čechová, L.; Jansa, P.; Šála, M.; Dračínský, M.; Holý, A.; Janeba, Z. *Tetrahedron* **2011**, *67*, 866.