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Synthesis, conformational studies, and biological properties of phosphonomethoxyethyl derivatives of nucleobases with a locked conformation *via* a pyrrolidine ring†

Radek Pohl,[a] Lenka Poštová Slavětínská,[a] Wai Soon Eng,[b] Dianne T. Keough,[b] Luke W. Guddat[b] and Dominik Rejman*[a]

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Systematic structure–activity studies on a diverse family of nucleoside phosphonic acids has led to the development of potent antiviral drugs such as HPMPC (CidofovirTM), PMEA (AdefovirTM), and PMPA (TenofovirTM), which are used in the treatment of CMV-induced retinitis, hepatitis B, and HIV, respectively. Here, we present the synthesis of a novel class of acyclic phosphonate nucleotides that have a locked conformation *via* a pyrrolidine ring. NMR analysis of these compounds revealed that the pyrrolidine ring has a constrained conformation when in the *cis*-form at pD < 10 *via* hydrogen bonding. Four of these compounds were tested as inhibitors of the human and *Plasmodium falciparum* 6-oxopurine phosphoribosyltransferases. The most potent has a K_i of 0.6 μM for *Plasmodium falciparum* HGXPRT.

Introduction

Over the past few decades, there has been an enormous effort devoted to the synthesis and biochemical and biological evaluation of analogs of natural nucleosides and nucleotides. This has largely been due to their usefulness as tools in investigations aimed at a thorough understanding of metabolic processes. Such analogues exhibit their biological properties either *via* inhibition of enzymes of the nucleoside/nucleotide metabolism pathway or in the triphosphorylated form, where they can become incorporated into DNA or RNA *via* the action of polymerases or transcriptases. Among the most successful classes of nucleotide analogs are those which possess the enzymatically and chemically stable phosphonate moiety[1] as a replacement for the phosphate group. The advantage of this modification is that it improves the stability by preventing hydrolysis of the phosphate ester bond.

Systematic investigation of the structurally diverse nucleoside phosphonic acids has led to the development of potent antiviral drugs. Their chemistry is based on both the acyclic phosphonate nucleotides **1** [2,3] and cyclic counterparts **2** and **3** (Fig. 1).[4] Specifically, acyclic compounds **1a–c** were shown to inhibit the replication of DNA viruses and retroviruses, whereas cyclic compounds **2a–d** and **3a–d** exhibited favorable antiviral profiles against HIV strains. Thus, the modification of the sugar-phosphate moiety of the nucleotides is a successful approach in contributing to the pool of potential antivirals.

Furthermore, several types of aza-sugar nucleoside phosphonates, such as the pyrrolidine **4**,[5,6] isoxazolidine **5** [7] and **6**,[8] and aziridine **7** [9] ring-containing compounds, have been reported. However, among these only analogues **6a–e** exerted significant inhibition of HIV reverse transcriptase comparable to AZT as well as maintained a low level of cytotoxicity. Pyrrolidine nucleosides **8** and **9** [10] served as the starting point for the synthesis of pyrrolidine phosphonate analogs of nucleotides **10a–12**, recently described by our group.[11] Phosphonate derivative **13** has been found to be a potent inhibitor of thymidine phosphorylase from spontaneous SD-rat lymphoma cells exhibiting an IC_{50} of 11 nM[12] and guanine derivative **14** exhibited nanomolar activity against human purine nucleoside phosphorylase.[13]

Herein, we present the synthesis and biological evaluation of phosphonomethoxyethyl derivatives of nucleobases **15a–e** and **16a–c** (Fig. 2) that are conformationally locked *via* a pyrrolidine ring. The conformational lock may, in principle, increase the entropy contribution to the binding energy of the ligand to its biological target. Herein we attempted to compare inhibition properties of PMEG and PMEHx with their conformationally locked counterparts **15b–c** and **16b–c** towards oxopurineribosyltransferases.

[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic. E-mail: rejman@uochb.cas.cz*
[b]*The School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072 QLD, Australia. E-mail: luke.guddat@uq.edu.au*
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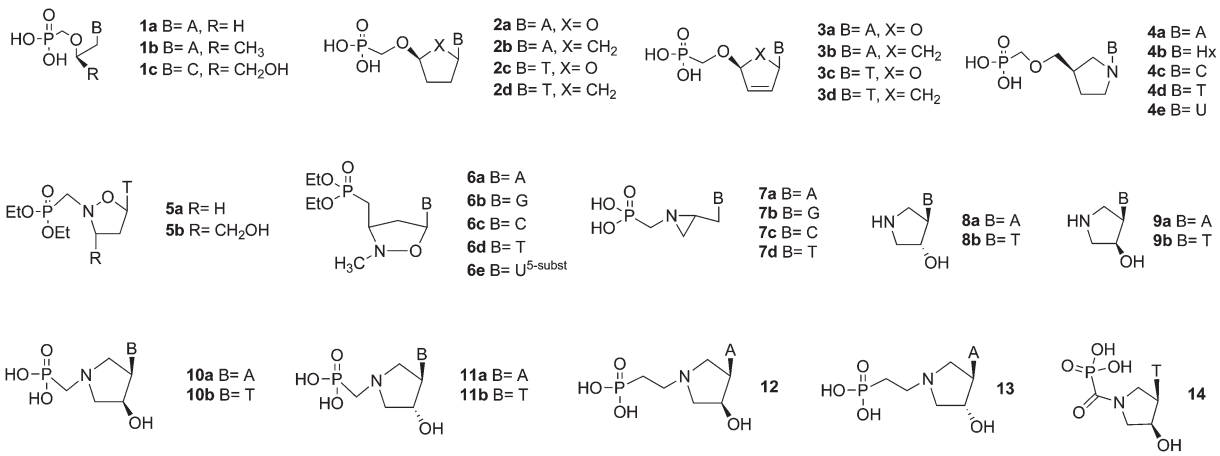


Fig. 1 Structures of nucleotide analogs.

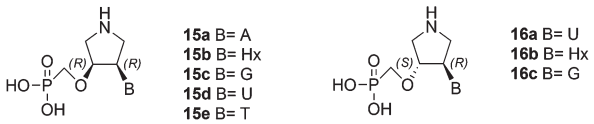


Fig. 2 Structures of target compounds **15a–e** and **16a–c**.

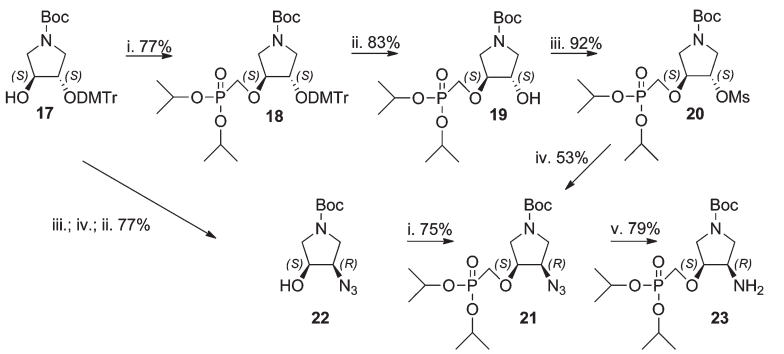
Results and discussion

Synthesis

The synthesis of the title compounds could be divided into two parts: (A) synthesis of the pyrrolidine phosphonate intermediate containing either a hydroxyl (**19**) or primary amino group (**23**), and (B) attaching the nucleobase to the intermediate (*via* the Mitsunobu reaction in the case of the hydroxyl derivative or nucleobase assembly procedure on the amino moiety). Two routes to the synthesis of the amino intermediate **23** were evaluated (Scheme 1).

Monodimethoxytrityl derivative **17** was reacted with diisopropyl tosyloxymethanephosphonate to afford phosphonate **18** that was treated with 1.5% TFA in DCM to yield the first intermediate **19**. Compound **19** was mesylated and treated with sodium azide giving azido derivative **21**. This reaction was accompanied by removal of one isopropyl ester group decreasing the yield of **21**. Thus, a different route to azido derivative **21** was explored. Monodimethoxytrityl derivative **17** was first converted to the azido derivative **22** that subsequently reacted with diisopropyl tosyloxymethanephosphonate. The obtained azido derivative **21** was finally converted to the amino derivative **23** by catalytic hydrogenation over a palladium catalyst. The chloropurine intermediate **25** was prepared by the Mitsunobu reaction of hydroxy derivative **19** with 6-chloropurine (**24**) (Scheme 2). Adenine derivative **15a** was prepared from **25** by aminolysis with conc. aqueous ammonia and dioxane followed by stirring with 20% TFA in DCM (removal of the Boc protecting group) and finally by bromotrimethylsilane treatment (to remove isopropyl esters). The hypoxanthine derivative **15b** was prepared from the same intermediate **25** by bromotrimethylsilane treatment followed by heating with aq. 3 M HCl.

The guanine nucleobase was formed on the amino moiety of **23** using a standard procedure employing 2,5-diamino-4,6-dichloropyrimidine (**26**) according to Scheme 3.[14]



Scheme 1 Synthesis of precursors **19** and **23**.

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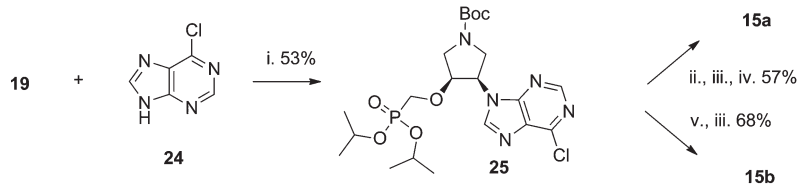
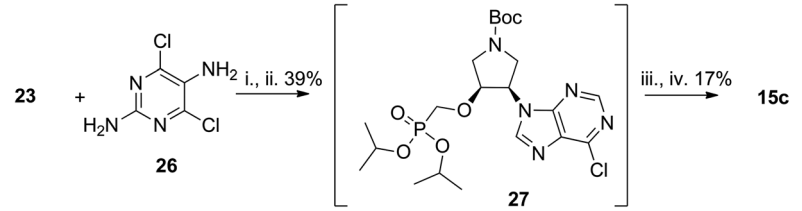


i. Ph_3P, DIAD, THF; ii. conc. aq. NH_3, dioxane; iii. Me_3SiBr, MeCN; iv. 20% TFA/DCM; v. 3M aq. HCl

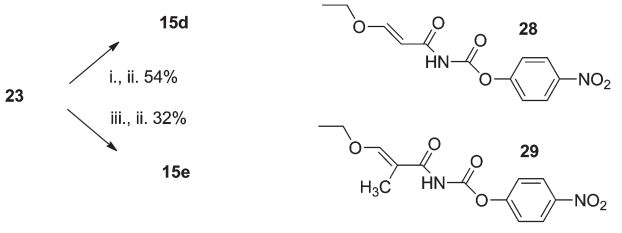
Scheme 2 Synthesis of adenine and hypoxanthine derivatives **15a** and **15b**.



i. DIPEA, nBuOH, 140 °C; ii. $(EtO)_2CHOAc$, DMF; iii. Me_3SiBr, MeCN; 1.5M aq. HCl, 80 °C

Scheme 3 Synthesis of guanine derivative **15c**.

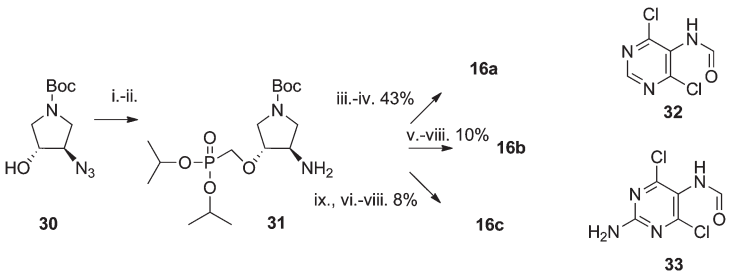
The reaction of amine **23** with reagent **28** [15] leads to the formation of a linear intermediate with a high yield (Scheme 4). This intermediate, after silica gel chromatography purification, was dissolved in dioxane and heated with Dowex 50 in H^+ for 5 h. The treatment with Dowex accomplished the cyclisation of the uracil moiety, removal of the Boc protecting group and, surprisingly, removal of both isopropyl ester groups, thus leading to the final uracil derivative **15d**. The thymine derivative **15e** was prepared by the same procedure except that reagent **28** was replaced by reagent **29** [15] (Scheme 4).



i. **28**, dioxane, rt; ii. Dowex 50, dioxane 80 °C; iii. **29**, dioxane, rt

Scheme 4 Synthesis of uracil and thymine derivatives **15d** and **15e**.

The uracil derivative **16a** with a *trans* configuration was prepared using the same synthetic procedure as for derivative **15d** (Scheme 5). The starting azido derivative **30** was prepared according to our previously published procedure.[13] Hypoxanthine derivative **16b** and guanine derivative **16c** were prepared using the nucleobase assembly approach adopted from ref. 16 (employing 4,6-dichloro-5-formamidopyrimidine (**32**) and 2-amino-4,6-dichloro-5-formamidopyrimidine (**33**) respectively) followed by bromotrimethylsilane promoted iso-



i. $TsOCH_2P(O)(OiPr)_2$, NaH, THF; ii. H_2, Pd/C, EtOH; iii. **28**, dioxane; iv. Dowex 50 H^+, dioxane, 80 °C; v. **32**, DIPEA, nBuOH, 110 °C; vi. $(EtO)_2CHOAc$, DMF, 120 °C; vii. 1.5 M aq. HCl, 70 °C; viii. Me_3SiBr, MeCN; ix. **33**, DIPEA, nBuOH

Scheme 5 Synthesis of uracil, hypoxanthine, and guanine derivatives **16a–c**.

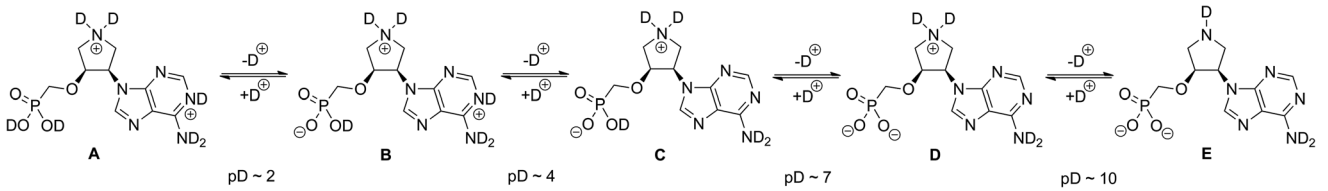


Fig. 3 Deuteration/dedeuteration transitions of **15a** in D_2O at different pD values.

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propyl ester group removal. The reaction of amino derivative **31** with **32** and **33** did not lead to purine ring closure so additional treatment with diethoxymethyl acetate in DMF at elevated temperatures was required. It appears that the nucleobase assembly on a primary amino group is the preferred procedure for introduction of thymine or uracil but for purine bases the Mitsunobu alkylation is the method of choice.

NMR conformational analysis

The final compounds **15a–e** and **16a–c** were fully characterized by ^{1}H, ^{13}C and ^{31}P NMR in D_2O solutions. The *cis* and *trans* relative configuration of uracil derivatives **15d** and **16a** was determined by inspection of the H,H-ROESY spectrum. Thus, a strong NOE cross-peak of H-6 from the uracil nucleobase and H-3′ from the pyrrolidine moiety can be found in the H,H-ROESY spectrum of *trans*-derivative **16a**. This NOE interaction is missing in the case of *cis*-derivative **15d**. In addition, *cis* and *trans* isomers differ significantly in the magnitudes of ^3J(H,H) coupling constants of pyrrolidine protons. Characteristic values of ^3J(3′,4′) that can be used for determination of the relative configuration directly from ^{1}H NMR spectra are 4.1–5.3 Hz for *cis*-derivatives **15a–e** and 1.2–2.0 Hz for *trans*-derivatives **16a–c**.

Since the original acyclic phosphonate moiety in **15a–e** and **16a–c** is conformationally restricted by the five-membered pyrrolidine ring we were interested in conformation preferences of such pyrrolidine derivatives. Taking into account that molecules contain both acidic (phosphonic acid) and basic (pyrrolidine component) moieties, we first examined at which pD deuteration/dedeuteration transitions take place (Fig. 3).

Therefore, D_2O solutions of **15a** and **16c** were titrated with diluted solutions of DCl in D_2O or NaOD in D_2O and ^{1}H, ^{13}C and ^{31}P NMR spectra were acquired (see ESI†). Based on the titration curves five different deuterated/dedeuterated forms A–E of **15a** can be observed at different pD values (Fig. 3). The pyrrolidine nitrogen remains in positively charged deuterated form C until pD ~ 10. This is manifested by the H-2′ and H-5′ ^{1}H chemical shift changes or C-2′ and C-5′ ^{13}C chemical shift changes. At pD ~ 4, dedeuteration of adenine nitrogen N-1 was observed by the changes in the ^{13}C chemical shift of C-2 and C-6. We have also found that deuteration/dedeuteration of other derivatives **15b–e** and **16a–b** follows the same trends resulting in dedeuteration of positively charged pyrrolidine nitrogen at pD ~ 10.

The protonation/deprotonation or deuteration/dedeuteration of the pyrrolidine nitrogen can influence the conformation of the five-membered pyrrolidine ring (Fig. 4).

The particular conformation is described by two pseudorotation parameters: by the phase angle (P) and by the maximum puckering amplitude (ϕ_{max}).[17] The phase angle is a periodic variable indicating which ring atoms are situated outside the ring plane and can reach 0°–360°. The maximum puckering amplitude describes the degree of distortion of the five-membered ring out of the plane and its value is usually in the range of 35°–45°. Therefore, we examined conformation preferences of the pyrrolidine ring in *cis*-adenine derivative (**15a**) and *trans*-guanine derivative (**16c**) at low (<2.0) and high (>12.0) pD values. The conformation analysis based on the concept of pseudorotation[17] was performed using ^3J(H,H) spin–spin couplings of pyrrolidine ring protons within the Matlab Pseudorotation GUI program[18] and the methodology developed for the conformational analysis of pyrrolidine nucleotide analogues we have published previously.[19] In *trans*-derivative **16c**, we observed only negligible changes in ^3J(H,H) of pyrrolidine protons upon pD change indicating little or no change in the conformation of the pyrrolidine ring. This assumption was later confirmed by the conformation analysis

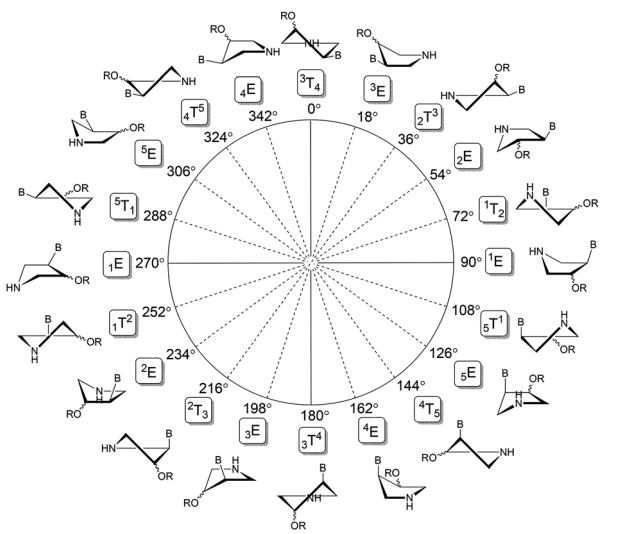

Fig. 4 Pyrrolidine pseudorotation pathway (P = 0° to 360°) of PME derivatives **15a–e** and **16a–c**. The sign B stands for a nucleobase and R means a phosphonomethyl moiety.

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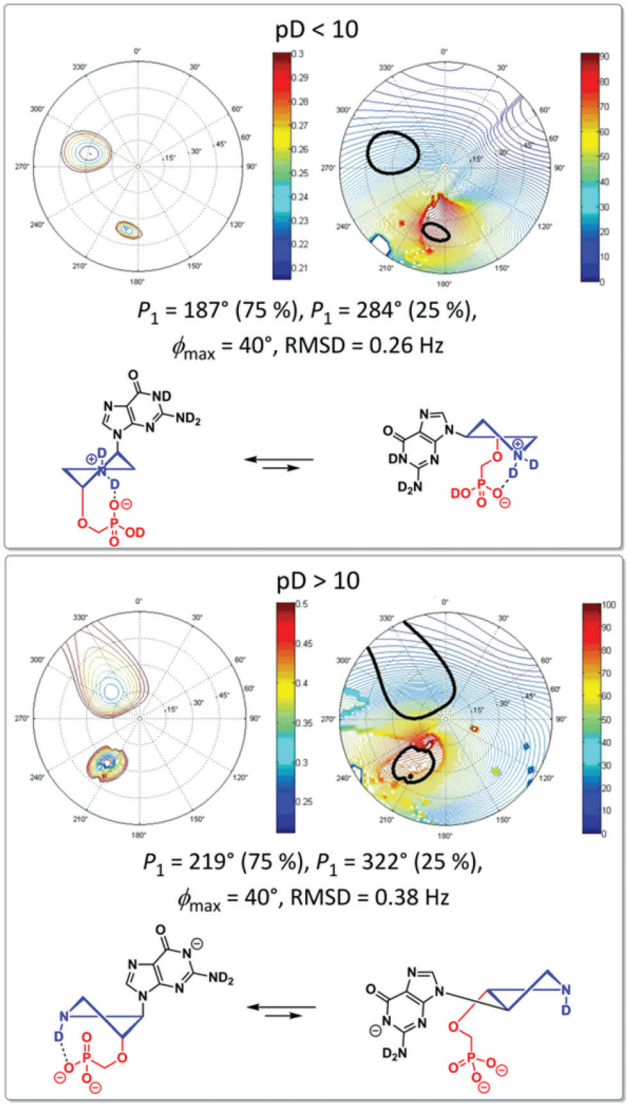

Fig. 5 Conformation of the pyrrolidine ring of derivative **16c** at different pD values in a D_2O solution examined by NMR.

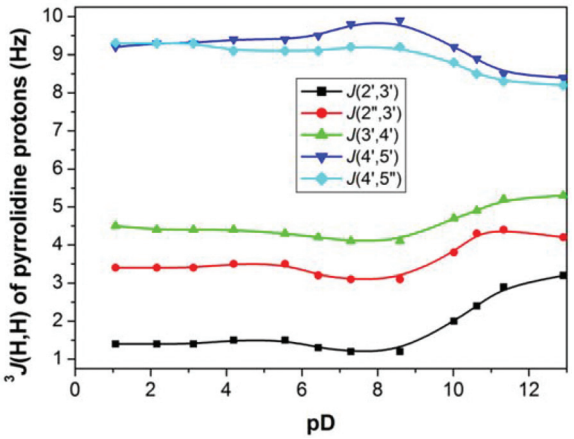

Fig. 6 Changes in the values of ^3J(H,H) coupling constants of derivative **15a** upon pD change.

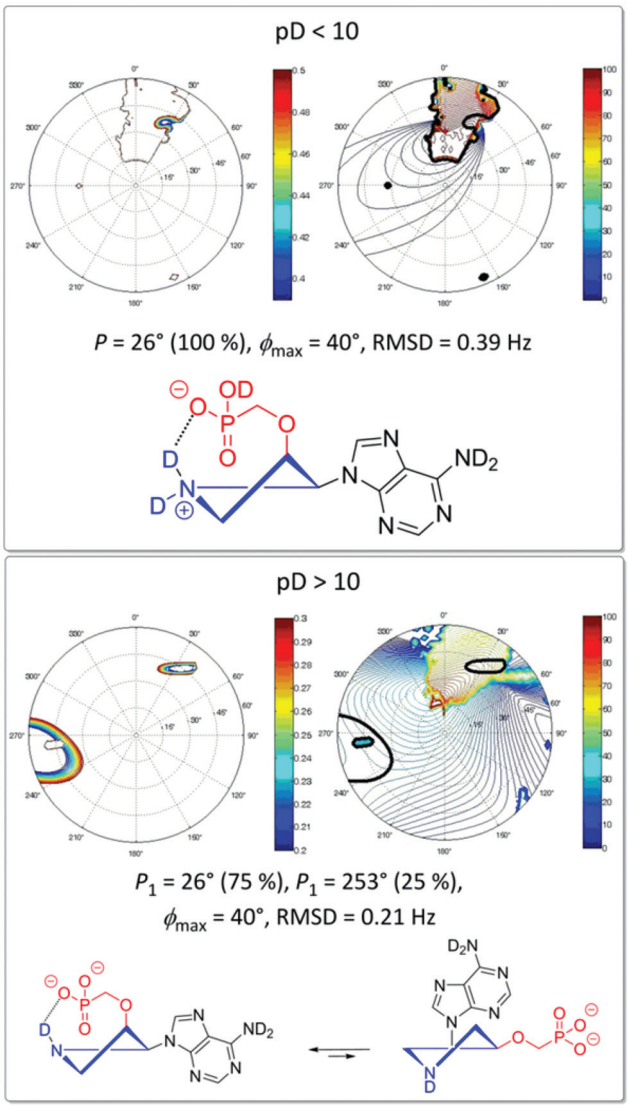

Fig. 7 Conformation of the pyrrolidine ring of derivative **15a** at different pD values in a D_2O solution examined by NMR.

of **16c** (Fig. 5) that revealed the existence of very similar conformations at both high and low pD.

Changes in ^3J(H,H) of pyrrolidine protons of *cis*-derivative **15a** upon pD change (Fig. 6) on the other hand suggest that the dedeuteration of the pyrrolidine ring at pD ~ 10 may result in changes of the pyrrolidine ring conformation.

The conformation analysis of *cis*-derivative **15a** at pD < 10 revealed the exclusive existence of one conformer (P = 26°) constrained by strong hydrogen bonding between the phosphonate moiety and the deuterated positively charged pyrrolidine nitrogen (Fig. 7). This hydrogen bonding is weakened as a consequence of dedeuteration at pD > 10, which results in an equilibrium of two conformers (P_1 = 26° (75%), P_2 = 253° (25%)) in D_2O solution. Similar behavior was also observed for hypoxanthine and guanine derivatives **15b** and **15c**, respectively.

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Biological activity

Compounds **15a–e** and **16a–c** were tested for their cytostatic (HepG2, HL60, HeLa S3, CCRF-CEM), antimicrobial, and antifungal (*Escherichia coli* CCM 3954, *Enterococcus faecalis* CCM 4224, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* CCM 4223, *Bacillus subtilis*, *Streptococcus agalactiae*, *Candida albicans*, and *Candida krusei*) activities but no significant effects were observed.

Compounds **15a–e** and **16a–b** did not exhibit any inhibitory activity against human mitochondrial (mdN) and cytosolic (cdN) 5′(3′)-deoxynucleotidases.[20] Compounds **15a** and **15e** were tested in a HCV replicon assay and did not exhibit any activity at a concentration below 50 μM.

Inhibition of human HGPRT and *Pf*HGXPRT by pyrrolidine derivatives of PME derivatives of the acyclic nucleoside phosphonates

The K_i values of four compounds were determined for human hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and *P. falciparum* (*Pf*) hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT) – a potential drug target for treatment of malaria (Table 1). There are two chemical differences between these compounds: (i) the purine base is either guanine or hypoxanthine; and (ii) there are two isomers. One has the *S* configuration at the carbon atom of the five membered ring and the second has the *R* configuration.

The data show that compounds as the *S*-isomer have lower K_i values for the parasite enzyme while those that are the *R*-isomer have lower K_i values for the human enzyme. The inhibitors containing hypoxanthine (**15b** *vs.* **15c** and **16b** *vs.* **16c**) as the base have lower K_i values for the parasite enzyme but the reverse is true for the human enzyme as it favours compounds with guanine as the base.

Structural analysis

Docking studies were undertaken to try to understand how the pyrrolidine derivatives bind in the active site.[22] The crystal structures of human HGPRT in complex with 9-2-[-2(phosphonoethoxy)ethyl]guanine (PEEG) and 9-2-[-2(phosphonoethoxy)ethyl]hypoxanthine (PEEHx) (PDB: 3GGC and 3GGJ, respectively) were used as the model template.[21] The PEE compounds contain an extra carbon atom in the linker connecting the N^9 atom of the purine ring with the phosphorus atom of the phosphonate group compared with **15b**, **15c**, **16b** and **16c**. However, they are similar in that they both contain an oxygen atom two atoms distal to the N^9. The acyclic nucleoside phosphonates (PEEG and PEEHx) bind to two key regions in the active site of human HGPRT: the purine binding site and the 5′-phosphate binding pocket (D137-T141).[21] To validate this approach, we first docked PEEG and PEEHx into the protein devoid of the ligand. The results showed that all the highest scoring docking poses correlated with the position observed in the crystal structure. The rmsd for all atoms in the ligands was <0.2 Å (Fig. 8a and b). The docked structures of **15b** and **16b** are compared in Fig. 8c and those of **15c** and **16c** are compared in Fig. 8d.

These data show that the phosphonate group in all four compounds is found in the 5′-phosphate binding pocket, but it can have two different orientations depending on the isomer. Thus, the chemical structure of the two isomers appears to be responsible for the location of the phosphonate group. Therefore, the nature of the base itself does not affect the location of the phosphonate group (*cf.* **15b** with **15c** and **16b** with **16c**). The differences in affinity for each isomer with either guanine or Hx as the base only relates to the binding of the base itself as human HGPRT has a higher affinity for guanine over hypoxanthine but *Pf*HGXPRT binds hypoxanthine better than guanine.[23] For the weaker inhibitors of human HGPRT (**15b** and **15c**), the phosphonate group does

Table 1 HG(X)PRT inhibitory activity of compounds **15b**, **15c**, **16b**, and **16c**

	K_i (μM)	
Compound	Hu	*Pf*
15b	72	0.6
15c	29	2
16b	5.7	80
16c	0.3	NI
PMEG[21]	29	1.6
PEEG[21]	1.0	0.1
PEEHx[21]	3.6	0.3

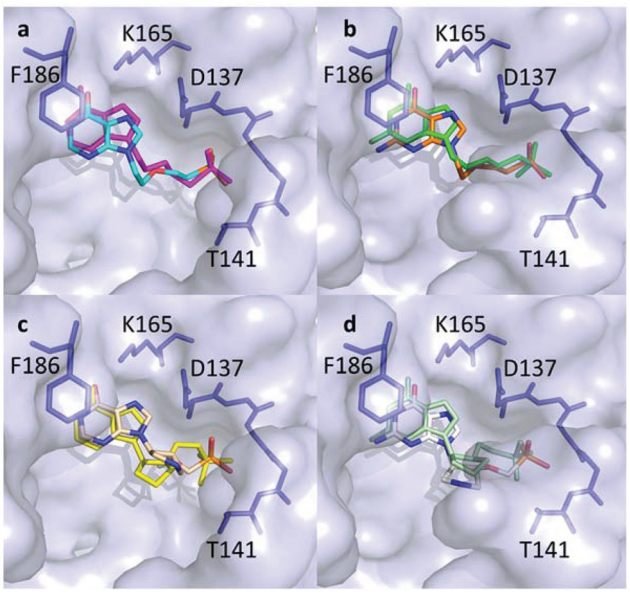

Fig. 8 Molecular docking models and the crystal structure of the nucleoside phosphonates in the active site of human HGPRT. (a) Comparison of the location of PEEHx in the crystal structure (carbon atoms in cyan) with that of the docked compound (magenta). (b) Comparison of the location of PEEG in the crystal structure (carbon atoms in tan) with that of the docked compound (bright green). (c) **15b** (yellow) and **16b** (carbon atoms in cream). (d) **15c** (pale green) and **16c** (carbon atoms in white).

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not reach as far into the 5′-phosphate binding pocket as it does in **16b** and **16c**. This location of the phosphonate group in the active site could be one of the contributing factors for the differences in the K_i values between the human and *Pf* enzymes for the two isomers. In comparison, *Pf*HGXPRT favours the *S*-isomers of the pyrrolidine nucleoside phosphonates over their *R*-isomer counterparts. The docking studies suggest that the "open" structure of these pyrrolidine derivatives is preferred to the "locked" structure when these compounds bind in the active site of the 6-oxopurine phosphoribosyltransferases.

Conclusions

Eight PME derivatives of nucleobases with a locked conformation *via* a pyrrolidine ring have been synthesized. Pyrimidine derivatives were prepared in good yields *via* nucleobase construction on a primary amine. In the case of purine bases, Mitsunobu coupling with a hydroxy derivative appeared to be a better approach than construction of the nucleobase on a primary amino group. NMR conformation analysis revealed that the conformation of the pyrrolidine ring in *cis*-series **15** is pH dependent. It was found that protonation of the pyrrolidine ring at pH < 10 is responsible for the constraining of the conformation and the exclusive existence of one conformer. Derivatives bearing hypoxanthine and guanine nucleobases were tested as inhibitors of the human and *Plasmodium falciparum* 6-oxopurine phosphoribosyltransferases. The most potent compound **15b** has a K_i of 0.6 μM for *Pf* HGXPRT with the selectivity in favour of the *Pf* enzyme over its human counterpart of approx. 120-fold (*S*-isomer). However, when the purine base is the same but the isomer is different (*R*-isomer), this selectivity changes in favour of the human enzyme (14-fold in favour of the human enzyme). Docking studies suggest that the *R*-isomer is favoured for the human HGPRT because the phosphonate group reaches further into the 5′-phosphate binding pocket. This allows the phosphoryl oxygens to form more hydrogen bonds with the amino acid side chain or main chain atoms in the flexible loop surrounding this group. These findings will help us in designing better and more selective inhibitors of parasite HGXPRT.

Experimental

Unless stated otherwise, all used solvents were anhydrous. The final products were lyophilized from water, and dried over phosphorus pentoxide at 50–70 °C and 13 Pa. TLC was performed on silica gel pre-coated aluminium plates silica gel/TLC-cards, UV 254 (Fluka), and compounds were detected with UV light (254 nm), by heating (detection of the dimethoxytrityl group; orange color), by spraying with a 1% solution of ninhydrin to visualize amines, and by spraying with a 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (blue color of mono- and diesters of phosphonic acid). Preparative column chromatography was carried out on silica gel (40–60 μm; Fluka) neutralized with triethylamine (1 ml 100 g^{-1}), and elution was performed at the flow rate of 40 ml min^{-1}. The following solvent systems were used for TLC and preparative chromatography: toluene–ethyl acetate 1 : 1 (T); chloroform–ethanol 9 : 1 (C1); ethyl acetate–acetone–ethanol–water 6 : 1 : 1 : 0.5 (H3); ethyl acetate–acetone–ethanol–water 4 : 1 : 1 : 1 (H1). The concentrations of solvent systems are stated as volume percentages (%, v/v). Analytical RP HPLC was performed on an LC5000 Liquid Chromatograph (INGOS-PIKRON, CR) using a Luna C18 (2) column (4.6 × 150 mm) at a flow rate of 1 ml min^{-1} by gradient elution of methanol in 0.1 M TEAA pH 7.5 (*A* = 0.1 M TEAA; *B* = 0.1 M TEAA in 50% aqueous methanol; *C* = methanol). Mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument, using FAB (ionization with Xe, accelerating voltage 8 kV). Glycerol and thioglycerol were used as matrices. NMR spectra were measured on Bruker AVANCE 600 (^{1}H at 600.1 MHz, ^{13}C at 150.9 MHz), Bruker AVANCE 500 and Varian UNITY 500 (^{1}H at 500.0 and 499.8 MHz, ^{13}C at 125.7 MHz, ^{31}P at 202.3 MHz) spectrometers. Chemical shifts (in ppm, δ scale) were referenced to the solvent signal ($CDCl_3$, ^{1}H: 7.26 ppm, ^{13}C: 77.0 ppm; DMSO-d_6, ^{1}H: 2.50 ppm, ^{13}C 39.7 ppm), or to dioxane as the external standard when D_2O solutions were used (^{1}H: 3.75 ppm, ^{13}C: 69.3 ppm). Coupling constants (*J*) are given in Hz. Complete assignment of protons and carbons was done by analysis of correlated homonuclear 2D-COSY and heteronuclear ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra. The relative configuration was checked using DPFGSE-NOE and 2D-ROESY techniques.

[3*S*,4*R*]-(4-(Adenin-9-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 15a

A mixture of **25** (0.72 g, 1.38 mmol), dioxane (14 ml) and conc. aq. ammonia (50 ml) was stirred at 50 °C in a sealed flask for five days. The mixture was concentrated *in vacuo*. The protected adenine intermediate (HRMS (FAB+) for $C_{21}H_{36}N_6O_6P$ $(M + H)^+$ calcd 499.2428, found 499.2429) was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform.

This intermediate (0.69 g, 1.384 mmol) was without further characterisation dissolved in 20% TFA in DCM (20 ml). The reaction mixture was stirred at rt overnight. The reaction mixture was diluted with chloroform (50 ml) and extracted with water (2 × 50 ml). The aqueous phase was applied to a column of Dowex 50 in H^+ form. Dowex was washed with water (200 ml) and eluted with 3% aq. ammonia. The yellowish solution was evaporated. The residue was co-evaporated with ethanol (1 × 20 ml) and acetonitrile (2 × 20 ml) and dissolved in DMF (15 ml). Bromotrimethylsilane (1 ml, 7 mmol) was added under an argon atmosphere and the reaction mixture was stirred at rt for two days. The reaction mixture was concentrated *in vacuo*. 2 M aq. TEAB (5 ml) and ethanol (10 ml) were added. The solution was concentrated *in vacuo*. The title compound was obtained by preparative HPLC on the reversed phase using a linear gradient of methanol in 0.1 M aq. TEAB.

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After conversion to sodium salt by passing through a column of Dowex 50 in Na^+ form (30 ml) the title compound was obtained in 58% (0.27 g, 0.803 mmol) yield as a fluffy solid (after lyophilisation from water).

^{1}H NMR (499.8 MHz, D_2O, 25 °C): 3.35 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 8.2, CH_aH_bP); 3.48 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 10.3, CH_aH_bP); 3.54 (dd, 1H, J_{gem} = 12.9, $J_{2'b,3'}$ = 3.0, H-2′b); 3.80 (dd, 1H, J_{gem} = 12.0, $J_{5'b,4'}$ = 9.7, H-5′b); 3.86 (dd, 1H, J_{gem} = 12.9, $J_{2'a,3'}$ = 1.2, H-2′a); 3.91 (dd, 1H, J_{gem} = 12.0, $J_{5'a,4'}$ = 9.1, H-5′a); 4.43 (ddd, 1H, $J_{3',4'}$ = 4.1, $J_{3',2'}$ = 3.0, 1.2, H-3′); 5.37 (ddd, 1H, $J_{4',5'}$ = 9.7, 9.1, $J_{4',3'}$ = 4.1, H-4′); 8.21 (s, 1H, H-2); 8.58 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 48.39 (CH_2-5′); 51.32 (CH_2-2′); 57.10 (CH-4′); 69.95 (d, $J_{C,P}$ = 150.7, CH_2P); 80.73 (d, $J_{C,P}$ = 12.1, CH-3′); 120.57 (C-5); 144.96 (CH-8); 152.08 (C-4); 155.29 (CH-2); 158.27 (C-6).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 12.86.

IR ν_{max}(KBr) 2370 (w, vbr), 1644 (s), 1605 (s), 1576 (m), 1509 (w), 1477 (m), 1418 (w), 1374 (w), 1333 (w), 1301 (w), 1254 (w), 1224 (vw), 1115 (m, br, sh), 1075 (m, br), 970 (m), 798 (w), 648 (w).

HRMS (ESI+) for $C_{10}H_{16}N_6O_4P$ $(M + H)^+$: calcd 315.09652, found 315.09648.

$[\alpha]^{20}$ = +43.2 (*c* 0.389, H_2O).

[3*S*,4*R*]-(4-(Hypoxanthin-9-yl)pyrrolidin-3-yl)-oxymethanephosphonic acid 15b

Bromotrimethylsilane (0.66 ml, 5 mmol) was added to a solution of compound **25** (0.54 g, 1.04 mmol) in acetonitrile (10 ml) under an argon atmosphere. The reaction mixture was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*, co-evaporated with toluene (2 × 10 ml) and dissolved in 3 M aq. HCl (30 ml). The mixture was stirred at 80 °C overnight, diluted with water (100 ml) and applied on a column of Dowex 50 in H^+ form (100 ml). The resin was washed with water (150 ml) and the crude product was eluted with 3% aq. ammonia. The title compound was obtained in pure form by preparative HPLC, and converted to sodium salt by passing through a column of Dowex 50 in Na^+ form. After lyophilisation from water 68% yield (0.24 g, 0.71 mmol) of the title compound was obtained in the form of a white amorphous solid.

^{1}H NMR (600.1 MHz, D_2O, 25 °C): 3.05 (dd, 1H, J_{gem} = 12.5, $J_{H,P}$ = 8.3, $CH_a\mathbf{H_b}P$); 3.25 (dd, 1H, J_{gem} = 12.5, $J_{H,P}$ = 9.6, $\mathbf{CH_a}H_bP$); 3.26 (d, 2H, $J_{2',3'}$ = 3.8, H-2′); 3.30 (dd, 1H, J_{gem} = 11.8, $J_{5'b,4'}$ = 8.7, H-5′b); 3.45 (dd, 1H, J_{gem} = 11.8, $J_{5'a,4'}$ = 8.2, H-5′a); 4.27 (dt, 1H, $J_{3',4'}$ = 5.3, $J_{3',2'}$ = 3.8, H-3′); 5.06 (ddd, 1H, $J_{4',5'}$ = 8.7, 8.2, $J_{4',3'}$ = 5.3, H-4′); 8.15 (s, 1H, H-2); 8.30 (s, 1H, H-8).

^{13}C NMR (150.9 MHz, D_2O, 25 °C): 51.09 (CH_2-5′); 52.68 (CH_2-2′); 58.65 (CH-4′); 70.37 (d, $J_{C,P}$ = 151.1, CH_2P); 82.60 (d, $J_{C,P}$ = 10.5, CH-3′); 125.17 (C-5); 143.35 (CH-8); 152.93 (C-4); 155.86 (CH-2); 170.01 (C-6).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 13.60.

IR ν_{max}(KBr) 3415 (vs, br), 3260 9 (vs, vbr, sh), 3137 (vs, vbr), 1685 (s), 1559 (s), 1520 (m), 1470 (s), 1415 (m, sh), 1383 (m), 1335 (m), 1119 (s, br), 1051 (m, sh), 912 (w, sh), 896 (w, sh), 793 (vw), 652 (m).

HRMS (ESI-) for $C_{10}H_{13}N_5O_5P$ $(M - H)^-$: calcd 314.06598, found 314.06611.

$[\alpha]^{20}$ = +19.6 (*c* 0.73, H_2O).

[3*S*,4*R*]-(4-(Guanin-9-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 15c

A mixture of amino derivative **23** (1.95 g, 5.13 mmol), 2,5-diamino-4,6-dichloropyrimidine (1.8 g, 10.26 mmol) and TEA (3 ml, 22 mmol) in *n*BuOH (50 ml) was stirred in a sealed reactor at 150 °C overnight. The pyrimidine intermediate was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform (66% yield, 1.77 g, 3.37 mmol), dissolved in diethoxymethylacetate (20 ml) and stirred at rt for 20 h. The reaction mixture was stirred at 80 °C for an additional 2 h and at 110 °C overnight. Chloroaminopurine intermediate **27** was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform in 39% yield (0.77 g, 1.33 mmol) in the form of a gray amorphous solid and used without further characterisation.

TMSBr (0.88 ml, 6.65 mmol) was added to a solution of the intermediate **27** in DMF (15 ml) at rt under an argon atmosphere. The reaction mixture was stirred overnight. The mixture was concentrated *in vacuo*. The residue was dissolved in 1.5 M aq. HCl (50 ml) and stirred at 80 °C overnight. The reaction mixture was diluted with water (100 ml) and applied on a column of Dowex 50 in H^+ form (80 ml). The Dowex was washed with water (150 ml) and the crude product was eluted with 3% aq. ammonia. The solvent was removed *in vacuo* and the title compound was obtained using preparative reversed phase HPLC, converted to its sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilized from water in 17% overall yield (81 mg, 0.23 mmol) in the form of a white amorphous solid.

^{1}H NMR (499.8 MHz, D_2O, 25 °C): 3.20 (dd, 1H, J_{gem} = 12.6, $J_{H,P}$ = 8.2, $CH_a\mathbf{H_b}P$); 3.29 (dd, 1H, J_{gem} = 12.9, $J_{2'b,3'}$ = 4.4, H-2′b); 3.33 (dd, 1H, J_{gem} = 12.6, $J_{H,P}$ = 9.3, $\mathbf{CH_a}H_bP$); 3.37 (m, 2H, H-2′a,5′b); 3.52 (dd, 1H, J_{gem} = 11.9, $J_{5'a,4'}$ = 8.4, H-5′a); 4.32 (ddd, 1H, $J_{3',4'}$ = 5.0, $J_{3',2'}$ = 4.4, 3.0, H-3′); 4.97 (td, 1H, $J_{4',5'}$ = 8.4, $J_{4',3'}$ = 5.0, H-4′); 8.07 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 50.47 (CH_2-5′); 52.25 (CH_2-2′); 57.95 (CH-4′); 70.25 (d, $J_{C,P}$ = 150.8, CH_2P); 82.02 (d, $J_{C,P}$ = 10.5, CH-3′); 118.60 (C-5); 141.99 (CH-8); 154.55 (C-4); 159.62 (C-2); 165.61 (C-6).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 13.85.

IR ν_{max}(KBr) 3431 (vs, br), 1682 (m, br), 1634 (s, br), 1571 (m), 1536 (w), 1480 (w), 1412 (w, br), 1111 (w, br, sh), 1080 (m, br), 973 (w), 802 (vw), 783 (w), 639 (w).

HRMS (ESI+) for $C_{10}H_{15}N_6O_5PNa$ $(M + Na)^+$: calcd 353.07338, found 353.07343.

$[\alpha]^{20}$ = +51.5 (*c* 0.307, H_2O).

[3*S*,4*R*]-(4-(Uracil-1-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 15d

Reagent **28** (0.36 g, 1.3 mmol) was added to a solution of amino derivative **23** (0.45 g, 1.18 mmol) in dioxane (12 ml). The reaction mixture was stirred at rt overnight. The mixture

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was concentrated *in vacuo* and a linear intermediate was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform in the form of yellowish foam. Dowex 50 in H^+ form (10 g) was added to a solution of the intermediate in dioxane (15 ml). The suspension was stirred at 85 °C for 5 h. The reaction mixture was filtered; the resin was washed with ethanol (50 ml) and eluted with 3% aq. ammonia (100 ml). The filtrate was concentrated and the desired product was obtained by preparative reverse phase HPLC with 54% overall yield (0.2 g, 0.64 mmol) after conversion to sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilisation from water in the form of a white amorphous solid.

^{1}H NMR (500.0 MHz, D_2O, 25 °C): 3.45 (dd, 1H, J_{gem} = 12.8, $J_{2'b,3'}$ = 2.8, H-2′b); 3.46 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 8.4, $CH_a\mathbf{H_b}P$); 3.50 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 10.3, $C\mathbf{H_a}H_bP$); 3.59 (dd, 1H, J_{gem} = 12.2, $J_{5'b,4'}$ = 9.9, H-5′b); 3.73 (dd, 1H, J_{gem} = 12.2, $J_{5'a,4'}$ = 9.9, H-5′a); 3.79 (dd, 1H, J_{gem} = 12.8, $J_{2'a,3'}$ = 0.7, H-2′a); 4.33 (ddd, 1H, $J_{3',4'}$ = 4.1, $J_{3',2'}$ = 2.8, 0.7, H-3′); 5.36 (td, 1H, $J_{4',5'}$ = 9.9, $J_{4',3'}$ = 4.1, H-4′); 5.83 (d, 1H, $J_{5,6}$ = 8.1, H-5); 8.05 (d, 1H, $J_{6,5}$ = 8.1, H-6).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 46.54 (CH_2-5′); 51.46 (CH_2-2′); 57.26 (CH-4′); 69.83 (d, $J_{C,P}$ = 150.8, CH_2P); 80.57 (d, $J_{C,P}$ = 12.5, CH-3′); 104.30 (CH-5); 148.21 (CH-6); 155.48 (C-2); 169.22 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 12.89.

IR ν_{max}(KBr) 3189 (m, br), 2980 (s), 2936 (m), 1696 (vs, br), 1628 (m), 1480 (m, sh), 1457 (s), 1408 (s), 1387 (s), 1377 (s, sh), 1365 (s, sh), 1279 (s), 1244 (s, br), 1225 (s, sh), 1175 (s), 1142 (s), 1104 (s), 1011 (s, sh), 991 (vs), 888 (m), 768 (m).

HRMS (ESI+) for $C_9H_{14}O_6N_3PNa$ $(M + Na)^+$ calcd 314.05124, found 314.05123.

$[\alpha]^{20}$ = +88.9 (*c* 0.189, H_2O).

[3*S*,4*R*]-(4-(Thymin-1-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 15e

Reagent **29** (0.75 g, 2.56 mmol) was added to a solution of amino derivative **23** (0.65 g, 1.7 mmol) in dioxane (15 ml). The reaction mixture was stirred at rt overnight. The mixture was concentrated *in vacuo* and a linear intermediate was obtained by column chromatography on silica gel using a linear gradient of ethanol in chloroform in the form of yellowish foam. Dowex 50 in H^+ form (15 g) was added to a solution of the intermediate in dioxane (25 ml). The suspension was stirred at 85 °C for 5 h. The reaction mixture was filtered; the resin was washed with ethanol (50 ml) and eluted with 3% aq. ammonia (100 ml). The filtrate was concentrated and the desired product was obtained by preparative reverse phase HPLC in 32% overall yield (175.6 mg, 0.54 mmol) after conversion to sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilisation from water in the form of a white amorphous solid.

^{1}H NMR (500.0 MHz, D_2O, 25 °C): 1.90 (d, 1H, ^4J = 1.1, CH_3); 3.45 (dd, 1H, J_{gem} = 12.9, $J_{2'b,3'}$ = 3.1, H-2′b); 3.52 (dd, 1H, J_{gem} = 12.4, $J_{H,P}$ = 8.6, $CH_a\mathbf{H_b}P$); 3.55 (dd, 1H, J_{gem} = 12.4, $J_{H,P}$ = 9.7, $C\mathbf{H_a}H_bP$); 3.68 (dd, 1H, J_{gem} = 12.5, $J_{5'b,4'}$ = 10.2, H-5′b); 3.74 (dd, 1H, J_{gem} = 12.5, $J_{5'a,4'}$ = 9.6, H-5′a); 3.81 (d, 1H, J_{gem} = 12.9, H-2′a); 4.38 (bdd, 1H, $J_{3',4'}$ = 4.1, $J_{3',2'}$ = 3.1, H-3′); 5.31 (ddd, 1H, $J_{4',5'}$ = 10.2, 9.6, $J_{4',3'}$ = 4.1, H-4′); 7.83 (q, 1H, ^4J = 1.1, H-6).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 14.23 (CH_3); 46.50 (CH_2-5′); 51.72 (CH_2-2′); 57.70 (CH-4′); 69.67 (d, $J_{C,P}$ = 152.7, CH_2P); 80.67 (d, $J_{C,P}$ = 11.9, CH-3′); 113.32 (C-5); 143.71 (CH-6); 155.31 (C-2); 169.14 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 13.78.

IR ν_{max}(KBr) 3260 (w, vbr, sh), 2831 (w, vvbr), 1695 (vs), 1663 (s, sh), 1521 (w, br), 1473 (w), 1442 (w), 1394 (w), 1375 (w, sh), 1283 (m), 1126 (m), 1072 (m, br), 970 (w), 789 (w), 769 (w).

HRMS (ESI+) for $C_{10}H_{15}O_6N_3P$ $(M + H)^+$: calcd 304.07039, found 304.06983.

$[\alpha]^{20}$ = +69.5 (*c* 0.364, H_2O).

[3*R*,4*R*]-(4-(Uracil-1-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 16a

The compound was prepared according to the experimental procedure for compound **15d** starting from amino derivative **31** (0.37 g, 0.97 mmol) in 43% overall yield (0.132 g, 0.42 mmol) in the form of a white amorphous colorless solid.

^{1}H NMR (500.0 MHz, D_2O, 25 °C): 3.47 (dd, 1H, J_{gem} = 11.8, $J_{H,P}$ = 10.0, $CH_a\mathbf{H_b}P$); 3.54 (dd, 1H, J_{gem} = 11.8, $J_{H,P}$ = 9.7, $C\mathbf{H_a}H_bP$); 3.57 (dd, 1H, J_{gem} = 12.7, $J_{2'b,3'}$ = 2.4, H-2′b); 3.71 (dd, 1H, J_{gem} = 13.1, $J_{5'b,4'}$ = 5.3, H-5′b); 3.80 (dd, 1H, J_{gem} = 12.7, $J_{2'a,3'}$ = 5.4, H-2′a); 3.91 (dd, 1H, J_{gem} = 13.1, $J_{5'a,4'}$ = 8.9, H-5′a); 4.53 (ddd, 1H, $J_{3',2'}$ = 5.4, 2.4, $J_{3',4'}$ = 2.0, H-3′); 4.76 (dd, 1H, $J_{4',5'}$ = 8.9, 5.3, $J_{4',3'}$ = 2.0, H-4′); 5.85 (d, 1H, $J_{5,6}$ = 8.0, H-5); 7.72 (d, 1H, $J_{6,5}$ = 8.0, H-6).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 49.39 (CH_2-5′); 53.29 (CH_2-2′); 68.82 (CH-4′); 69.51 (d, $J_{C,P}$ = 151.4, CH_2P); 85.66 (d, $J_{C,P}$ = 13.6, CH-3′); 104.78 (CH-5); 149.73 (CH-6); 154.55 (C-2); 169.26 (C-4).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 13.25.

IR ν_{max}(KBr) 3500–3000 (m, vbr), 2792 (m, vbr), 2630 (m, br, sh), 2630 (m, br, sh), 2454 (m, vbr), 1695 (vs, br), 1628 (m, br, sh), 1461 (m, sh), 1440 (m), 1389 (m), 1277 (m), 1160 (m, br, sh), 1105 (s, br, sh), 1063 (s, br), 971 (m), 914 (m, br), 767 (m).

HRMS (ESI+) for $C_9H_{14}O_6N_3PNa$ $(M + Na)^+$ calcd 314.05124, found 314.05124.

$[\alpha]^{20}$ = −66.0 (*c* 0.053, H_2O).

[3*R*,4*R*]-(4-(Hypoxanthin-9-yl)pyrrolidin-3-yl)-oxymethanephosphonic acid 16b

A mixture of amino derivative **31** (0.23 g, 0.61 mmol), 4,6-dichloro-5-formamidopyrimidine (**32**) (0.14 g, 0.73 mmol), and DIPEA (0.52 ml, 3.05 mmol) in *n*BuOH (10 ml) was stirred at 110 °C overnight. The reaction mixture was concentrated *in vacuo*, dissolved in DMF (5 ml), diethoxymethyl acetate (3 ml) was added, and the mixture was stirred at 120 °C overnight. The chloropurine intermediate was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform and was used in the next step without further characterisation (except for LCMS). The intermediate (0.31 g, 0.6 mmol) was stirred in 1.5 M aq. HCl (50 ml) at 75 °C over-

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night, applied on a column of Dowex 50 in H^+ form (100 ml), washed with 50% aq. ethanol (150 ml) and eluted with 3% NH_3 in 50% aq. ethanol (300 ml). The obtained yellow solution was evaporated and purified using preparative HPLC on the reversed phase. The obtained hypoxanthine diisopropyl ester (80 mg, 0.2 mmol) was co-evaporated with MeCN (3 × 10 ml), dissolved in the same solvent, and Me_3SiBr (0.13 ml, 1 mmol) was added under an argon atmosphere. The reaction mixture was stirred under an argon atmosphere at rt overnight. The title compound was obtained by preparative reverse phase HPLC in 10% overall yield (21.8 mg, 65 μmol) – calculated from **31** – after conversion to sodium salt by passing through a column of Dowex 50 in Na^+ form and lyophilisation from water in the form of a white amorphous solid.

^{1}H NMR (600.1 MHz, D_2O, 25 °C): 3.60 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 10.0, $CH_a\mathbf{H_b}P$); 3.69 (dd, 1H, J_{gem} = 12.1, $J_{H,P}$ = 9.5, $C\mathbf{H_a}H_bP$); 3.76 (dt, 1H, J_{gem} = 13.3, $J_{2'b,3'} = J_{2'b,4'}$ = 1.2, H-2′b); 3.84 (dd, 1H, J_{gem} = 13.3, $J_{2'a,3'}$ = 4.2, H-2′a); 4.07 (dd, 1H, J_{gem} = 13.6, $J_{5'b,4'}$ = 3.2, H-5′b); 4.15 (dd, 1H, J_{gem} = 13.6, $J_{5'a,4'}$ = 7.7, H-5′a); 4.51 (dt, 1H, $J_{3',2'}$ = 4.2, 1.2, $J_{3',4'}$ = 1.2, H-3′); 5.47 (ddt, 1H, $J_{4',5'}$ = 7.7, 3.2, $J_{4',3'} = J_{4',2'b}$ = 1.2, H-4′); 8.18 (s, 1H, H-2); 8.25 (s, 1H, H-8).

^{13}C NMR (150.9 MHz, D_2O, 25 °C): 50.33 (CH_2-5′); 52.42 (CH_2-2′); 62.19 (CH-4′); 69.49 (d, $J_{C,P}$ = 152.9, CH_2P); 86.50 (d, $J_{C,P}$ = 13.3, CH-3′); 126.74 (C-5); 144.33 (CH-8); 148.42 (CH-2); 151.25 (C-4); 161.39 (C-6).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 13.57.

IR ν_{max}(KBr) 3434 (vs, br), 3264 (m, br, sh), 2923 (m), 2853 (m), 2790 (m, vbr, sh), 1695 (s), 1588 (m), 1550 (w), 1515 (w), 1470 (w, sh), 1418 (w), 1382 (vw), 1346 (vw), 1216 (w), 1190 (w, br), 1146 (w, vbr), 1112 (w, sh), 1051 (m, br), 912 (w, br), 896 (w, sh), 790 (w), 646 (w).

HRMS (ESI-) for $C_{10}H_{13}N_5O_5P$ $(M - H)^-$: calcd 314.06598, found 314.06585.

$[\alpha]^{20}$= −30.4 (*c* 0.184, H_2O).

[3*R*,4*R*]-(4-(Guanin-9-yl)pyrrolidin-3-yl)oxymethanephosphonic acid 16c

The title compound was prepared from amino derivative **31** (0.23 g, 0.61 mmol) and 2-amino-4,6-dichloro-5-formamidopyrimidine (**33**) (0.15 g, 0.73 mmol) using the same procedure as for compound **16b** in 8% overall yield (17.3 mg, 49 μmol) in the form of a white amorphous solid.

^{1}H NMR (500.0 MHz, D_2O, 25 °C): 3.71 (dt, 1H, J_{gem} = 13.3, $J_{2'b,3'} = J_{2'b,4'}$ = 1.4, H-2′b); 3.71 (dd, 1H, J_{gem} = 12.5, $J_{H,P}$ = 9.9, $CH_a\mathbf{H_b}P$); 3.79 (dd, 1H, J_{gem} = 12.5, $J_{H,P}$ = 9.6, $C\mathbf{H_a}H_bP$); 3.90 (dd, 1H, J_{gem} = 13.3, $J_{2'a,3'}$ = 4.6, H-2′a); 4.04 (dd, 1H, J_{gem} = 13.5, $J_{5'b,4'}$ = 3.4, H-5′b); 4.08 (dd, 1H, J_{gem} = 13.5, $J_{5'a,4'}$ = 6.7, H-5′a); 4.52 (ddd, 1H, $J_{3',2'}$ = 4.6, 1.4, $J_{3',4'}$ = 1.9, H-3′); 5.29 (m, 1H, H-4′); 7.89 (s, 1H, H-8).

^{13}C NMR (125.7 MHz, D_2O, 25 °C): 50.19 (CH_2-5′); 52.53 (CH_2-2′); 61.61 (CH-4′); 68.89 (d, $J_{C,P}$ = 155.7, CH_2P); 86.68 (d, $J_{C,P}$ = 13.1, CH-3′); 119.04 (C-5); 141.77 (CH-8); 153.73 (C-4); 156.3 (C-2); 161.56 (C-6).

^{31}P{^{1}H} NMR (202.3 MHz, D_2O, 25 °C): 14.71.

IR ν_{max}(KBr) 3311 (m, vbr), 3117 (m, br), 3022 (m, br, sh), 2749 (m, vbr), 2440 (w, vbr), 1690 (vs), 1658 (s), 1607 (m), 1580 (m, sh), 1536 (w), 1486 (w), 1415 (w), 1374 (w, br), 1117 (m), 1064 (m, br), 970 (w), 779 (w), 691 (vw), 640 (vw).

HRMS (ESI+) for $C_{10}H_{15}N_6O_5PNa$ $(M + Na)^+$: calcd 353.07338, found 353.07346.

$[\alpha]^{20}$= +48.8 (*c* 0.172, H_2O).

[3*S*,4*S*] Diisopropyl 1-*N*-*tert*-butyloxycarbonyl-4-dimethoxytrityloxypyrrolidin-3-yloxymethylphosphonate 18

[3*S*,4*S*]-1-*N*-Boc-3-dimethoxytrityloxy-4-hydroxypyrrolidine (12 g, 34.42 mmol) diisopropyl tosyloxymethanephosphonate (17.19 g, 49 mmol) was dissolved in THF (300 ml). The solution was cooled to 0 °C and sodium hydride (2.8 g, 70 mmol) was added. The reaction mixture was stirred at rt for 3 days and then cooled to 0 °C and acetic acid (1.8 ml, 29 mmol) was added slowly. The temperature was allowed to rise to rt and the solvent was evaporated *in vacuo*. The title compound was obtained by chromatography on silica gel using a linear gradient of ethyl acetate in toluene as a viscous colorless oil in 77% yield (14.5 g, 22.11 mmol).

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (500.0 MHz, $CDCl_3$, 25 °C): 1.24, 1.27, 1.28, 1.29 (4 × d, 4 × 6H, J_{vic} = 6.2, $(C\mathbf{H_3})_2CH$); 1.41, 1.46 (2 × s, 2 × 9H, $(CH_3)_3C$); 2.92 (d, 1H, J_{gem} = 12.0, H-5b); 3.07 (dd, 1H, $J_{3,4}$ = 4.7, $J_{3,2a}$ = 4.1, H-3); 3.08 (dd, 1H, J_{gem} = 12.0, $J_{5a,4}$ = 4.7, H-5a); 3.25, 3.29 (2 × dd, 2 × 1H, J_{gem} = 13.4, $J_{H,P}$ = 9.7, CH_2P); 3.30–3.33 (m, 3H, H-3,5); 3.34 (dd, 1H, J_{gem} = 13.2, $J_{H,P}$ = 9.7, $CH_a\mathbf{H_b}P$); 3.37, 3.41 (2 × d, 2 × 1H, J_{gem} = 12.1, H-2b); 3.42 (dd, 1H, J_{gem} = 13.2, $J_{H,P}$ = 9.7, $C\mathbf{H_a}H_bP$); 3.49, 3.56 (2 × dd, 2 × 1H, J_{gem} = 12.1, $J_{2a,3}$ = 4.1, H-2a); 3.786, 3.789 (2 × s, 2 × 6H, CH_3O-DMTr); 4.10 (bt, 2H, $J_{4,3} = J_{4,5a}$ = 4.7, H-4); 4.60–4.70 (m, 4H, $C\mathbf{H}(CH_3)_2$); 6.83, 6.84 (2 × m, 2 × 4H, H-*m*-C_6H_4-DMTr); 7.13–7.36 (m, 14H, H-*o*-C_6H_4-DMTr, H-*m*,*p*-C_6H_5-DMTr); 7.43 (m, 4H, H-*o*-C_6H_5-DMTr).

^{13}C NMR (125.7 MHz, $CDCl_3$, 25 °C): 23.85, 23.97, 24.01 (d, $J_{C,P}$ = 4.0, $(\mathbf{C}H_3)_2CH$); 28.39, 28.45 ($(\mathbf{C}H_3)_3C$); 48.68, 49.48 (CH_2-2); 50.72, 50.82 (CH_2-5); 55.17 (CH_3O-DMTr); 63.92, 64.03 (d, $J_{C,P}$ = 170.0, CH_2P); 70.95, 71.03, 71.05, 71.13 (d, $J_{C,P}$ = 7.0, $\mathbf{C}H(CH_3)_2$); 74.27, 75.06 (CH-4); 79.14, 79.24 ($\mathbf{C}(CH_3)_3$); 83.65, 84.37 (d, $J_{C,P}$ = 13.0, CH-3); 87.10, 87.25 (C-DMTr); 113.22, 113.25 (CH-*m*-C_6H_4-DMTr); 126.99 (CH-*p*-C_6H_5-DMTr); 127.91 (CH-*m*-C_6H_5-DMTr); 128.18, 128.21 (CH-*o*-C_6H_5-DMTr); 130.10, 130.14 (CH-*m*-C_6H_4-DMTr); 136.11, 136.20, 136.30 (C-i-C_6H_4-DMTr); 144.97, 145.03 (C-i-C_6H_5-DMTr); 154.54, 154.66 (CO); 158.67, 158.70 (C-*p*-C_6H_4-DMTr).

^{31}P{^{1}H} NMR (202.3 MHz, $CDCl_3$, 25 °C): 19.00, 19.05.

HRMS (FAB+) for $C_{37}H_{50}NO_9PNa$ $(M + H + Na)^+$: calcd 706.3121, found 706.3146.

[3*S*,4*S*] Diisopropyl 1-*N*-*tert*-butyloxycarbonyl-4-hydroxypyrrolidin-3-yloxymethylphosphonate 19

2% TFA in DCM (200 ml) was added to compound **18** (14.5 g, 22.11 mmol) and the reaction mixture was stirred until the DMTr group was cleaved completely (followed by TLC,

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~20 min). $NaHCO_3$ (20 g) and MeOH (50 ml) were added and the suspension was vigorously stirred until neutral pH. The suspension was filtered over celite and the filtrate was evaporated. The title compound was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in 83% yield (6.97 g, 18.28 mmol) in the form of colorless oil that upon keeping in a refrigerator (at 4 °C) starts to crystallize after several days.

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (499.8 MHz, DMSO-d_6, 25 °C): 1.22, 1.23, 1.24 (3 × d, 24H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 1.38, 1.39 (2 × s, 2 × 9H, (CH$_3$)$_3$C); 3.14, 3.15 (2 × d, 2 × 1H, J_{gem} = 11.4, H-5b); 3.26, 3.29 (2 × dd, 2 × 1H, J_{gem} = 11.4, $J_{5a,4}$ = 4.4, H-5a); 3.30–3.34 (m, 3H, 2 × H-2b, H-2a); 3.37 (dd, 1H, J_{gem} = 12.2, $J_{2a,3}$ = 4.1, H-2a); 3.77 (dd, 1H, J_{gem} = 13.1, $J_{H,P}$ = 9.0, CH$_a$**H$_b$**P); 3.79 (d, 2H, $J_{H,P}$ = 9.0, CH$_2$P); 3.81 (dd, 1H, J_{gem} = 13.1, $J_{H,P}$ = 9.0, C**H$_a$**H$_b$P); 3.83 (m, 2H, H-3); 4.08, 4.10 (2 × m, 2 × 1H, H-4); 4.58 (m, 4H, C**H**(CH$_3$)$_2$); 5.249, 5.253 (2 × d, 2 × 1H, $J_{OH,3}$ = 3.6, OH).

^{13}C NMR (125.7 MHz, DMSO-d_6, 25 °C): 23.84, 23.86 (d, $J_{C,P}$ = 4.4, (**C**H$_3$)$_2$CH); 24.00, 24.01 (d, $J_{C,P}$ = 3.6, (**C**H$_3$)$_2$CH); 28.34 ((**C**H$_3$)$_3$C); 48.85, 49.23 (CH$_2$-2); 51.97, 52.26 (CH$_2$-5); 63.05, 63.19 (d, $J_{C,P}$ = 165.4, CH$_2$P); 70.44, 70.46, 70.47 (d, $J_{C,P}$ = 6.3, **C**H(CH$_3$)$_2$); 70.99, 71.81 (CH-4); 78.52, 78.55 (**C**(CH$_3$)$_3$); 83.92 (d, $J_{C,P}$ = 12.3, CH-3); 84.83 (d, $J_{C,P}$ = 12.0, CH-3); 153.97, 153.99 (CO).

^{31}P{^{1}H} NMR (202.3 MHz, DMSO-d_6,CDCl$_3$, 25 °C): 20.12, 20.17.

IR ν_{max}(CHCl$_3$) 3610 (w), 3363 (m, vbr), 2983 (vs), 2936 (s), 1689 (vs), 1679 (vs, sh), 1478 (s), 1467 (m), 1455 (s), 1415 (vs, br), 1389 (s), 1377 (s), 1368 (s), 1245 (s, br), 1170 (vs), 1143 (s), 1105 (vs), 1002 (vs, vbr), 889 (m).

HRMS (ESI+) for $C_{16}H_{32}NO_7PNa$ (M + Na)$^+$ calcd 404.18141, found 404.18153.

[3*S*,4*S*] Diisopropyl 1-*N*-*tert*-butyloxycarbonyl-4-mesyloxypyrrolidin-3-yloxymethylphosphonate 20

Mesyl chloride (4.26 ml, 55 mmol) was added to a solution of **19** (6.97 g, 18.28 mmol) and DMAP (6.7 g, 55 mmol) in DCM (130 ml) at 0 °C. The reaction mixture was stirred at rt for 2 h. The reaction mixture was washed with a saturated solution of sodium bicarbonate. The organic phase was evaporated and the title compound was obtained by chromatography on silica gel using a linear gradient of ethyl acetate in toluene as a colorless syrup in 92% yield (7.71 g, 16.78 mmol).

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (499.9 MHz, CDCl$_3$, 25 °C): 1.33, 1.34, 1.35 (3 × d, 24H, J_{vic} = 6.2, (C**H**$_3$)$_2$CH); 1.47 (s, 18H, (CH$_3$)$_3$C); 3.09, 3.11 (2 × bs, 2 × 3H, CH$_3$-Ms); 3.48–3.73 (m, 8H, H-2,5); 3.75–3.84 (m, 4H, CH$_2$P); 4.27 (bm, 2H, H-3); 4.68–4.82 (m, 4H, C**H**(CH$_3$)$_2$); 5.13 (m, 2H, H-4).

^{13}C NMR (125.7 MHz, CDCl$_3$, 25 °C): 23.91, 23.94 (d, $J_{C,P}$ = 4.2, (**C**H$_3$)$_2$CH); 23.99, 24.01 (d, $J_{C,P}$ = 3.5, (**C**H$_3$)$_2$CH); 28.35 ((**C**H$_3$)$_3$C); 38.63 (CH$_3$-Ms); 48.61 (CH$_2$-2); 49.31 (CH$_2$-2,5); 49.83 (CH$_2$-5); 64.57, 64.63 (d, $J_{C,P}$ = 169.3, CH$_2$P); 71.26–71.51 (**C**H(CH$_3$)$_2$); 79.45 (CH-4); 80.08 (**C**(CH$_3$)$_3$); 80.13 (CH-4); 82.10, 83.29 (d, $J_{C,P}$ = 10.4, CH-3); 154.02, 154.08 (CO).

^{31}P{^{1}H} NMR (202.3 MHz, CDCl$_3$, 25 °C): 18.28, 18.30.

HRMS (ESI+) for $C_{17}H_{34}NO_9PSNa$ (M + Na)$^+$ calcd 482.15896, found 482.15880.

[3*S*,4*R*] Diisopropyl 4-azido1-1-*N*-*tert*-butyloxycarbonylpyrrolidin-3-yloxymethylphosphonate 21

Method A. Sodium azide (1.3 g, 20 mmol) was added to a solution of **20** (4.63 g, 10.08 mmol) in DMF (50 ml). The reaction mixture was stirred at 95 °C overnight. Sodium azide (1.3 g, 20 mmol) was added and the reaction mixture was stirred at 120 °C for an additional 12 h. The reaction mixture was filtered, and the filtrate was evaporated. The title compound was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in 53% yield (2.17 g, 5.34 mmol) in the form of colorless oil.

Method B. Sodium hydride (1.16 g, 29 mmol) was added to a solution of compound **22** (3.31 g, 14.5 mmol) and diisopropyl tosyloxymethanephosphonate (7.62 g, 21.75 mmol) in THF (150 ml). The reaction mixture was stirred at rt overnight. The reaction mixture was cooled to −5 °C and acetic acid (1.66 ml, 29 mmol) was added (hydrogen is formed!). The reaction mixture was stirred at rt for 10 min, concentrated *in vacuo* and the title compound was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in 75% yield (4.4 g, 10.83 mmol) in the form of colorless oil.

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (500.0 MHz, CDCl$_3$, 25 °C): 1.35, 1.35 (2 × d, 2 × 12H, J_{vic} = 6.1, (CH$_3$)$_2$CH); 1.45 (s, 18H, (CH$_3$)$_3$C); 3.40 (dd, 1H, J_{gem} = 11.0, $J_{2b,3}$ = 4.6, H-2b); 3.41 (dd, 1H, J_{gem} = 10.6, $J_{5b,4}$ = 4.6, H-5b); 3.48 (m, 2H, H-2b,5b); 3.54–3.60 (m, 4H, H-2a,5a); 3.79–3.89 (m, 4H, CH$_2$P); 3.91, 3.95 (2 × m, 2 × 1H, H-4); 4.28, 4.29 (2 × m, 2 × 1H, H-3); 4.71–4.83 (m, 4H, C**H**(CH$_3$)$_2$).

^{13}C NMR (125.7 MHz, CDCl$_3$, 25 °C): 23.94 (d, $J_{C,P}$ = 4.5, (**C**H$_3$)$_2$CH); 24.03, 24.04 (d, $J_{C,P}$ = 3.9, (**C**H$_3$)$_2$CH); 28.37 ((**C**H$_3$)$_3$C); 47.59, 47.75, 48.16 (CH$_2$-2,5); 59.73, 60.30 (CH-4); 64.64, 64.92 (d, $J_{C,P}$ = 167.9, CH$_2$P); 71.36 (d, $J_{C,P}$ = 6.7, **C**H(CH$_3$)$_2$); 76.75, 77.00 (**C**(CH$_3$)$_3$); 80.08 (d, $J_{C,P}$ = 8.7, CH-3); 80.86 (d, $J_{C,P}$ = 9.2, CH-3); 154.10 (CO).

^{31}P{^{1}H} NMR (202.3 MHz, CDCl$_3$, 25 °C): 18.41, 18.44.

HRMS (ESI+) for $C_{16}H_{31}N_4O_6PNa$ (M + H + Na)$^+$: calcd 429.1879, found 429.1876.

[3*S*,4*R*] 4-Azido-1-*N*-*tert*-butyloxycarbonyl-3-hydroxypyrrolidine 22

MsCl (20 ml, 260 mmol) was added dropwise to a solution of dimethoxytrityl derivative **17** (64.6 g, 127.77 mmol) and DMAP (32 g, 260 mmol) in DCM (1 L) at 0 °C. The reaction mixture was stirred at rt overnight. The mixture was washed with sat. aq. $NaHCO_3$ (2 × 400 ml), 10% aq. citric acid (2 × 400 ml) and water (2 × 400 ml). The organic phase was dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was dissolved in DMF (1 L). Sodium azide (26 g, 400 mmol) was added and the reaction mixture was stirred at 110 °C overnight. The reaction mixture was concentrated *in vacuo*. Ethyl acetate

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(500 ml) was added to the residue. The slurry was filtered and concentrated *in vacuo*. 1.5% TFA in DCM (600 ml) was added and the mixture was stirred until complete removal of the DMTr group (followed by TLC in 50% EtOAc/toluene and 10% EtOH in chloroform) for *ca.* 2 h. Solid $NaHCO_3$ (40 g) and MeOH (100 ml) were added in portions. The mixture was vigorously stirred until the pH was neutral (~30 min). The suspension was filtered and the filtrate was concentrated *in vacuo*. The title compound was obtained by chromatography on silica gel using a linear gradient of ethyl acetate in toluene with 77% yield (over three steps) (22.6 g, 99 mmol) in the form of colorless oil that solidified on keeping in the refrigerator.

^{1}H NMR (499.8 MHz, DMSO-d_6, 80 °C): 1.41 (s, 9H, $(CH_3)_3C$); 3.13 (dd, 1H, J_{gem} = 10.9, $J_{2b,3}$ = 5.5, H-2b); 3.23 (dd, 1H, J_{gem} = 11.3, $J_{5b,4}$ = 5.0, H-5b); 3.42 (dd, 1H, J_{gem} = 10.9, $J_{2a,3}$ = 6.0, H-2a); 3.46 (dd, 1H, J_{gem} = 11.3, $J_{5a,4}$ = 6.0, H-5a); 3.92 (ddd, 1H, $J_{4,5}$ = 6.0, 5.0, $J_{4,3}$ = 4.4, H-4); 4.33 (m, 1H, H-3); 5.36 (bs, 1H, OH).

^{13}C NMR (125.7 MHz, DMSO-d_6, 80 °C): 28.00 ($(\mathbf{C}H_3)_3C$); 47.45 (CH_2-5); 50.44 (CH_2-2); 61.24 (CH-4); 70.52 (CH-3); 78.50 ($(CH_3)_3\mathbf{C}$); 153.40 (CO).

IR ν_{max}(KBr) 3340 (m), 2980 (m), 2942 (w), 2138 (m), 2124 (s, sh), 2095 (s), 1682 (s), 1479 (m), 1470 (m), 1455 (m, sh), 1420 (vs), 1391 (m, sh), 1369 (m), 1217 (w), 1257 (m), 1163 (s), 1093 (m, sh), 552 (w).

HRMS (ESI+) for $C_9H_{16}N_4O_3Na$ $(M + Na)^+$ calcd 251.1115, found 251.1114.

[3*S*,4*R*] Diisopropyl 4-amino-1-*N*-*tert*-butyloxycarbonylpyrrolidin-3-yloxymethylphosphonate 23

A solution of **21** (2.17 g, 5.34 mmol) in ethanol (50 ml) was hydrogenated in the presence of Pd/C (0.2 g) overnight. The suspension was filtered over celite and this was evaporated. The title compound was obtained without further purification in 79% yield (1.61 g, 4.232 mmol) in the form of colorless oil.

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (500.0 MHz, DMSO-d_6, 25 °C): 1.21–1.26 (m, 24H, $(C\mathbf{H_3})_2CH$); 1.375, 1.378 (2 × s, 2 × 9H, $(CH_3)_3C$); 2.82, 2.84 (2 × dd, 2 × 1H, J_{gem} = 9.9, $J_{5b,4}$ = 7.5, H-5b); 3.25 (dd, 2H, J_{gem} = 12.1, $J_{2b,3}$ = 4.3, H-2b); 3.29–3.43 (m, 6H, H-2a,4,5a); 3.77–3.85 (m, 4H, H-3, $CH_a\mathbf{H_b}P$); 3.88 (dd, 2H, J_{gem} = 13.9, $J_{H,P}$ = 8.9, $C\mathbf{H_a}H_bP$); 4.54–4.66 (m, 4H, $C\mathbf{H}(CH_3)_2$).

^{13}C NMR (125.7 MHz, DMSO-d_6, 25 °C): 23.87 (d, $J_{C,P}$ = 4.0, $(\mathbf{C}H_3)_2CH$); 24.00, 24.05 (d, $J_{C,P}$ = 3.6, $(\mathbf{C}H_3)_2CH$); 28.32 ($(\mathbf{C}H_3)_3C$); 48.99, 49.40 (CH_2-2); 50.47, 50.87 (CH_2-5); 52.34, 53.18 (CH-4); 63.78, 64.01 (d, $J_{C,P}$ = 164.5, CH_2P); 70.44–71.54 ($\mathbf{C}H(CH_3)_2$); 78.51 ($\mathbf{C}(CH_3)_3$); 80.94 (d, $J_{C,P}$ = 12.0, CH-3); 81.67 (d, $J_{C,P}$ = 11.6, CH-3); 153.69, 153.79 (CO).

^{31}P{^{1}H} NMR (202.3 MHz, DMSO-d_6, 25 °C): 20.32, 20.36.

HRMS (ESI+) for $C_{16}H_{33}N_2O_6PNa$ $(M + H + Na)^+$: calcd 403.1968, found 403.1971.

[3*S*,4*R*] Diisopropyl 4-(6-chloropurin-9-yl)-1-*N*-*tert*-butyloxycarbonylpyrrolidin-3-yloxymethylphosphonate 25

DIAD (1.81 ml, 9.36 mmol) was added to a solution of triphenylphosphine (1.52 g, 9.36 mmol) in THF (30 ml). The mixture was stirred at rt for 3 h. A mixture of **19** (1.19 g, 3.12 mmol) and 6-chloropurine (0.58 g, 3.74 mmol) in THF (30 ml) was added. The suspension turned to a clear red-brown solution in 5 min. The reaction mixture was stirred at rt for an additional 2 h. The solvent was removed *in vacuo* and the title compound was obtained by column chromatography on silica gel using a linear gradient of ethyl acetate in toluene in 53% yield (0.85 g, 1.64 mmol).

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (600.1 MHz, DMSO-d_6, 25 °C): 0.98, 1.00, 1.05, 1.07, 1.13 (5 × d, 24H, J_{vic} = 6.1, $(C\mathbf{H_3})_2CH$); 1.42, 1.44 (2 × s, 2 × 9H, $(CH_3)_3C$); 3.51–3.71 (m, 6H, H-2′, $CH_a\mathbf{H_b}P$); 3.83–3.90 (m, 4H, H-5′b, $C\mathbf{H_a}H_bP$); 3.956, 3.962 (2 × dd, 2 × 1H, J_{gem} = 9.9, $J_{5'a,4'}$ = 8.9, H-5′a); 4.29–4.44 (m, 4H, H-3′, $CH_a\mathbf{H_b}P$); 5.37, 5.38 (2 × td, 2 × 1H, $J_{3',4'}$ = 8.9, $J_{3',2'}$ = 8.9, 4.2, H-3′); 8.71, 8.72 (2 × s, 2 × 1H, H-8); 8.80 (s, 2H, H-2).

^{13}C NMR (150.9 MHz, DMSO-d_6, 25 °C): 23.62, 23.71, 23.73 (d, $J_{C,P}$ = 4.4, $(\mathbf{C}H_3)_2CH$); 23.81, 23.87 (d, $J_{C,P}$ = 3.8, $(\mathbf{C}H_3)_2CH$); 28.37 ($(\mathbf{C}H_3)_3C$); 4.69, 46.90 (CH_2-5′); 48.87, 49.34 (CH_2-2′); 54.54, 55.02 (CH-3′); 63.08, 63.30 (d, $J_{C,P}$ = 164.0, CH_2P); 70.36, 70.38 (d, $J_{C,P}$ = 6.0, $\mathbf{C}H(CH_3)_2$); 70.49 (d, $J_{C,P}$ = 6.3, $\mathbf{C}H(CH_3)_2$); 78.31, 79.10 (d, $J_{C,P}$ = 12.2, CH-3′); 79.34, 79.37 ($\mathbf{C}(CH_3)_3$); 130.85, 130.90 (C-5); 147.06 (CH-8); 149.27 (C-6); 151.77 (CH-2); 152.52 (C-4); 153.76, 153.79 (CO).

^{31}P{^{1}H} NMR (202.3 MHz, DMSO-d_6, 25 °C): 19.45, 19.54.

IR ν_{max} (KBr) 2976 (s), 1705 (s, sh), 1679 (vs), 1596 (s), 1558 (m), 1492 (m), 1479 (m), 1465 (m), 1435 (s), 1415 (vs), 1399 (vs), 1388 (s, sh), 1372 (s), 1365 (s, sh), 1340 (s), 1259 (s), 1232 (s), 1228 (s), 1212 (m, sh), 1175 (s, sh), 1167 (s), 1140 (s), 1105 (s), 1008 (vs), 987 (vs), 933 (m), 884 (m), 790 (m), 644 (w, sh), 636 (m).

HRMS (ESI+) for $C_{21}H_{34}N_5O_6ClP$ $(M + H)^+$: calcd 518.1930, found 518.1928.

[3*R*,4*R*] Diisopropyl 4-amino-1-*N*-*tert*-butyloxycarbonylpyrrolidin-3-yloxymethylphosphonate 31

Starting from azido derivative **30** [13] (3.12 g, 13.67 mmol) the same synthetic procedures as for compound **23** were used. The title compound was obtained in 50% (2.76 g, 6.8 mmol) overall yield.

NMR – (1 : 1 mixture of amide rotamers)

^{1}H NMR (500.0 MHz, DMSO-d_6, 25 °C): 1.222, 1.23, 1.24 (3 × d, 24H, J_{vic} = 6.2, $(C\mathbf{H_3})_2CH$); 1.38, 1.39 (2 × s, 2 × 9H, $(CH_3)_3C$); 3.00 (bd, 2H, J_{gem} = 10.7, H-5b); 3.23–3.33 (m, 4H, H-2b,5a); 3.34 (bm, 2H, H-4); 3.44, 3.47 (2 × dd, 2 × 1H, J_{gem} = 12.0, $J_{2a,3}$ = 4.4, H-2a); 3.68 (bm, 2H, H-3); 3.73–3.82 (m, 4H, CH_2P); 4.53–4.63 (m, 4H, $C\mathbf{H}(CH_3)_2$).

^{13}C NMR (125.7 MHz, DMSO-d_6, 25 °C): 23.85, 23.87 (d, $J_{C,P}$ = 4.4, $(\mathbf{C}H_3)_2CH$); 24.01, 24.02 (d, $J_{C,P}$ = 3.6, $(\mathbf{C}H_3)_2CH$);

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28.37 ($(\mathbf{C}H_3)_3C$); 48.94, 49.32 (CH_2-2); 51.99, 52.32 (CH_2-5); 53.82, 54.67 (CH-4); 63.10, 63.23 (d, $J_{C,P}$ = 165.5, CH_2P); 70.36–70.47 ($\mathbf{C}H(CH_3)_2$); 78.37, 78.39 ($\mathbf{C}(CH_3)_3$); 85.25 (d, $J_{C,P}$ = 12.5, CH-3); 86.14 (d, $J_{C,P}$ = 12.0, CH-3); 153.95, 153.99 (CO).

$^{31}P\{^1H\}$ NMR (202.3 MHz, DMSO-d_6, 25 °C): 20.24, 20.39.

HRMS (ESI+) for $C_{16}H_{33}N_2O_6PNa$ $(M + H + Na)^+$: calcd 403.1968, found 403.1971.

Determination of kinetic constants

The K_i values for the ANPs were determined using a spectrophotometric method (*J. Med. Chem.* (2009) 52: 4390) with the concentration of the invariable substrate (guanine) being 60 μM and the concentration of the variable substrate (PRibPP) ranging between 20 and 580 μM. The values were calculated from Hanes plots using the equations for either competitive or non-competitive inhibition.

Docking studies

The three dimensional structures of **15c**, **15d**, **16c** and **16d** were obtained using the PRODRG2 server.[24] The program GOLD was used for all the docking calculations.[22] Coordinates for human HGPRT were obtained using chain A of the complex with 2-(phosphonoethoxy)ethyl guanine (PEEG, PDB: 3GGJ) and 2-(phosphonoethoxy)ethyl hypoxanthine (PEEHx, PDB: 3GGC).[21] For setting up the protein template, the inhibitor, the water molecules and the magnesium ions were removed from the coordinates. Prior to docking simulations the enzyme was protonated using the GOLD default settings. The active site centre was defined by the coordinates of the phosphorus atom of PEEG or PEEHx when bound to human HGPRT. The search radius for all calculations was 15 Å. For each ligand twenty independent docking searches were performed. The GoldScore fitness function (default settings) was used to rank the poses. This algorithm takes into account the H-bonding energy, van der Waals energy and ligand torsion strain.

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