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Synthesis and Evaluation of Novel Acyclic Nucleoside Phosphonates as Inhibitors of *Plasmodium falciparum* and Human 6-Oxopurine Phosphoribosyltransferases

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Acyclic nucleoside phosphonates (ANPs) are a promising class of antimalarial therapeutic drug leads that exhibit a wide variety of K_i values for *Plasmodium falciparum* (*Pf*) and human hypoxanthine-guanine-(xanthine) phosphoribosyltransferases [HG(X)PRTs]. A novel series of ANPs, analogues of previously reported 2-(phosphonoethoxy)ethyl (PEE) and (*R*,*S*)-3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) derivatives, were designed and synthesized to evaluate their ability to act as inhibitors of these enzymes and to extend our ongoing antimalarial structure–activity relationship studies. In this series, (*S*)-3-hydroxy-2-(phosphonoethoxy)propyl (HPEP), (*S*)-2-(phosphonomethoxy)-propanoic acid (CPME), or (*S*)-2-(phosphonoethoxy)propanoic acid (CPEE) are the acyclic moieties. Of this group, (*S*)-3-hydroxy-2-(phosphonoethoxy)propylguanine (HPEPG) exhibits the highest potency for *Pf*HGXPRT, with a K_i value of 0.1 μM and a K_i value for human HGPRT of 0.6 μM. The crystal structures of HPEPG and HPEPHx (where Hx = hypoxanthine) in complex with human HGPRT were obtained, showing specific interactions with active site residues. Prodrugs for the HPEP and CPEE analogues were synthesized and tested for in vitro antimalarial activity. The lowest IC_{50} value (22 μM) in a chloroquine-resistant strain was observed for the bis-amidate prodrug of HPEPG.

Introduction

Malaria remains one of the most serious infectious diseases in the world today with forty percent of the world's population living in areas of risk resulting in more than 200 million infected individuals and 500 000 fatalities each year.[1] *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) are the most widespread species that cause malaria in humans.[2,3] While *Pf* is reputed to be the most lethal, *Pv* is responsible for serious illness with relapsing infections. This is due to the fact that the *Pv* parasite can lie dormant in the liver of the host.[3] The current standard treatment for uncomplicated *Pf* and *Pv* malaria are artemisinin-based combination therapies and chloroquine plus primaquine, respectively.[4] However, antimalarial drug resistance has developed and spread for these antimalarial medications, particularly in Southeast Asia, where both *Plasmodium* species are prevalent.[5–7] Therefore, alternative drug candidates with novel mechanisms of action are urgently needed.

A promising approach for the development of new antimalarials is based on the inhibition of *Pf* hypoxanthine-guanine-xanthine phosphoribosyltransferase (HGXPRT).[8,9] HG(X)PRT is a key enzyme of the purine salvage pathway where it catalyzes the formation of the *N*-glycosidic bond between the N^9 atom of the 6-oxopurine base and the C^1 atom of 5-phospho-α-D-ribosyl-1-pyrophosphate (*P*Rib-*PP*) (Figure 1).[10]

A significant difference between the metabolic pathways of *Plasmodium* and its human host cell is the ability to synthesize the purine nucleoside monophosphates essential for the production of DNA/RNA. Mammalian cells are able to produce these metabolites either by de novo synthesis or by salvage of the preformed purine bases. In contrast, *Pf* and *Pv* parasites do not possess the de novo pathway, relying on salvage of the 6-

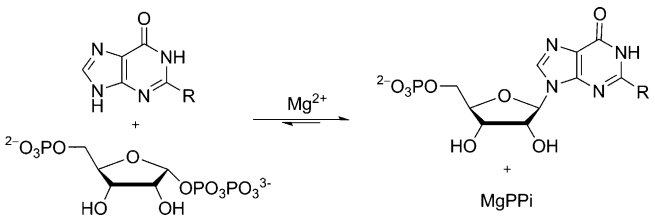

Figure 1. Reaction catalyzed by HG(X)PRT. The naturally occurring bases are hypoxanthine (R = H), guanine (R = NH_2) and xanthine (R = OH).

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oxopurines transported from their host cell. Thus, the activity of HG(X)PRT is crucial in the replication and survival of these parasites making this enzyme a promising target for the design of novel antiparasitic drugs.[3]

Acyclic nucleoside phosphonates (ANPs)[11] containing either a pyrimidine or 6-amino purine base constitute a distinguished class of successful therapeutic antiviral agents inhibiting either RNA reverse transcriptases or DNA polymerases.[12] Three of them have been approved worldwide for clinical use (adefovir, cidofovir and tenofovir).[12] In this type of nucleotide analogues, the heterocyclic base and phosphonate group are linked by various acyclic chains that mimic the sugar moiety. There are several advantages that the ANPs possess with regard to their potential as therapeutic drugs. These include:[12] 1) resistance toward degradation by enzymes in vivo because of the stable carbon-phosphorus bond; 2) attractive conformational flexibility; and 3) low cost of synthesis making them suitable therapeutics, especially for third world countries. Several ANPs containing hypoxanthine or guanine as the nucleobase (e.g., 2-(phosphonoethoxy)ethyl (PEE) hypoxanthine, PEE guanine and (*R*,*S*)-3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) guanine, Figure 2, Table S1) have proven to be good inhibitors of *Pf*HGXPRT and *Pv*HGPRT.[13] These compounds were the first

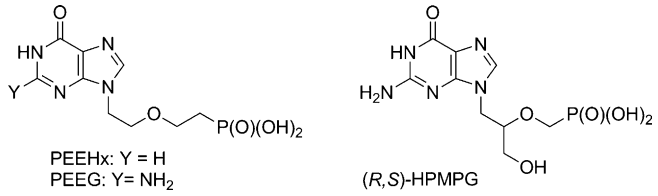


Figure 2. Structures of two types of acyclic nucleoside phosphonates (ANPs) shown to be potent inhibitors of human HGPRT and *Pf*HGXPRT.[13]

HG(X)PRT inhibitors found to selectively discriminate between the human and parasitic enzymes. Subsequently, a broad spectrum of ANPs as inhibitors of human, *Escherichia coli* (*Ec*), *Mycobacterium tuberculosis* (*Mt*), *Pf* and *Pv* HG(X)PRTs has been investigated.[14,15]

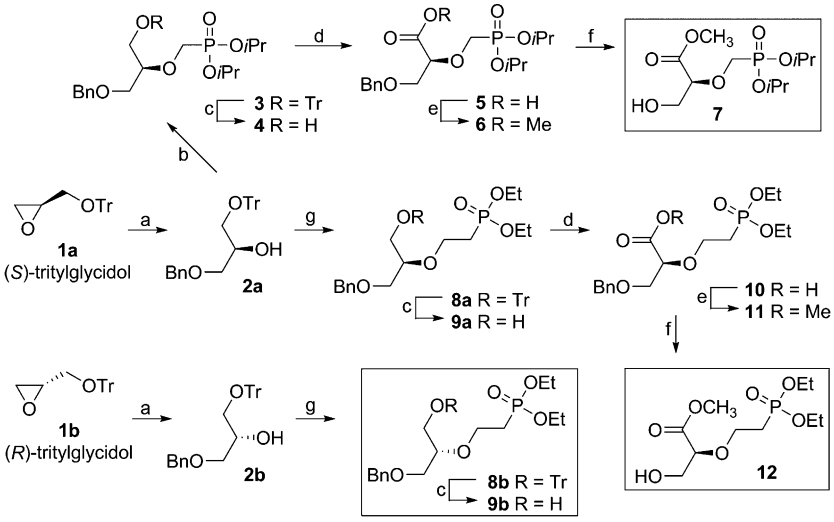
The ANPs in Figure 2 are submicromolar inhibitors of *Pf*HGXPRT.[13] Therefore, a computational study based on the crystal structures of human HGPRT in complex with PEEHx, PEEG or (*S*)-HPMPG was undertaken. The results suggested that if a hydroxymethyl, carboxy or methoxycarbonyl group could be attached to the acyclic linker at the second carbon atom from the purine ring, these compounds may have enhanced affinity. Herein, we report the synthesis and inhibitory properties of ANPs containing (*S*)-2-(phosphonomethoxy)propanoic acid (or 2′-carboxy-PME, CPME, **17**, **18**),[16] (*S*)-3-hydroxy-2-(phosphonoethoxy)propyl (HPEP, **25**–**27**) or (*S*)-2-(phosphonoethoxy)propanoic acid (or 2′-carboxy-PEE, CPEE, **32**, **33**, **36**, **37**) as the acyclic moieties together with the synthesis of the corresponding prodrugs of HPEP and CPEE analogues and their antimalarial activities.

Results and Discussion

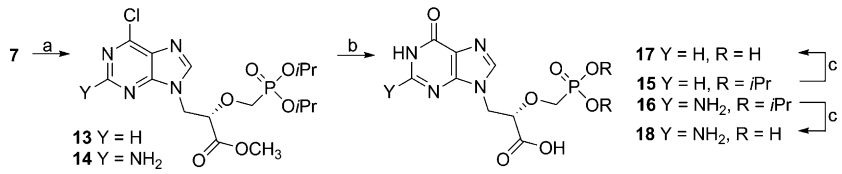
Chemistry

(*S*)- and (*R*)-tritylglycidol **1a** and **1b** (Scheme 1) were chosen as the starting materials for the synthesis of the target ANP series. Intermediate **4** (Scheme 1), prepared through **2a** and **3**, was oxidized using TEMPO/$NaClO_2$/NaClO under mild reaction conditions to give **5** (in a 34% overall yield from **1a**),[16,17] which, under treatment with diazomethane, quantitatively produced the methyl ester **6** (Scheme 1). The key intermediate **7** was then obtained in a 95% yield after removal of the benzyl group from compound **6**.

Modification of the previously described oxa-Michael addition of the secondary alcohol to diethyl vinylphosphonate (DEVP)[18] was used for introducing the ethylphosphonate moiety. Treatment of alcohols **2a** and **2b** with DEVP and a catalytic amount of KOH in dioxane,[19] to give compounds **8a** and **8b** (Scheme 1), followed by the removal of the trityl groups by holding at reflux in acetic acid, gave derivatives **9a** and **9b**, respectively, in 37–39% overall yields (from compounds **2**). Compound **9a** was further oxidized using TEMPO/$NaClO_2$/NaClO system to give carboxyl derivative **10** (83%) which was then quantitatively converted with diazomethane to its methyl ester **11** (Scheme 1). Finally, the removal of the benzyl group from



Scheme 1. Synthesis of key intermediates **7**, **9b** and **12**. *Reagents and conditions*: a) BnOH, NaH, DMF, 100 °C, 2 h; b) 1. NaH, DMF, 0 °C, 15 min, 2. $BrCH_2P(O)(OiPr)_2$, DMF, RT, 48 h; c) 80% AcOH, reflux, 2 h; d) TEMPO, $NaClO_2$, NaClO, phosphate buffer, MeCN, 40 °C, 48 h; e) CH_2N_2, EtOAc, RT, 0.5 h; f) H_2/Pd/C, MeOH, RT, 72 h; g) KOH, $CH_2{=}CHP(O)(OEt)_2$, dioxane, RT, 70 h.



Scheme 2. Preparation of (*S*)-CPMEHx (**17**) and (*S*)-CPMEG (**18**). *Reagents and conditions*: a) Mitsunobu conditions: 1. 6-chloro or 2-amino-6-chloropurine, compound **7**, PPh_3, dioxane, 2. DIAD, RT, 24 h; b) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3–5 h; c) Me_3SiBr, MeCN, RT, overnight.

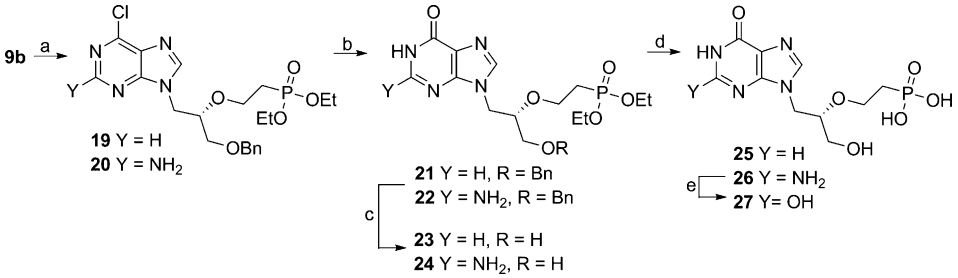
compound **11** afforded the desired derivative **12** in a 93% yield.

Mitsunobu conditions[20] were employed for introduction of the acyclic moieties, namely alcohols **7**, **9b** and **12**, to the N^9-position of 6-chloropurine and 2-amino-6-chloropurine in satisfactory yields (40–80%; Schemes 2–4). For 2-amino-6-chloropurine derivatives **14**, **20** and **29**, the crude Mitsunobu reaction mixtures were heated in water/dioxane to decompose triphenylphosphoranylidene intermediates which were formed due to presence of free amino groups in the C2 position.[21] The resulting 6-chloropurine and 2-amino-6-chloropurine derivatives can be, in general, transformed into the corresponding 6-oxopurine analogues using nucleophilic aromatic substitution in either basic or acidic conditions. When the basic procedure (DABCO/K_2CO_3) was applied, the methyl ester group in compounds **13**, **14**, **28** and **29** was simultaneously cleaved to produce carboxylic acids **15** (59%), **16** (56%), **30** (57%) and **31** (55%), respectively (Scheme 2 and 4). Treatment of compounds **28** and **29** (Scheme 4) under acidic conditions (75% aqueous trifluoroacetic acid at room temperature) led preferentially to the formation of methyl esters **34** (84%) and **35** (81%), respectively. Analogous acidic hydrolysis (75% aqueous trifluoroacetic acid) at elevated temperature (50 °C) and prolonged time (overnight) also leads to a cleavage of the ester moiety and treatment of compound **29** by this method gave the carboxylic acid **31** in a 78% yield (Scheme 4).

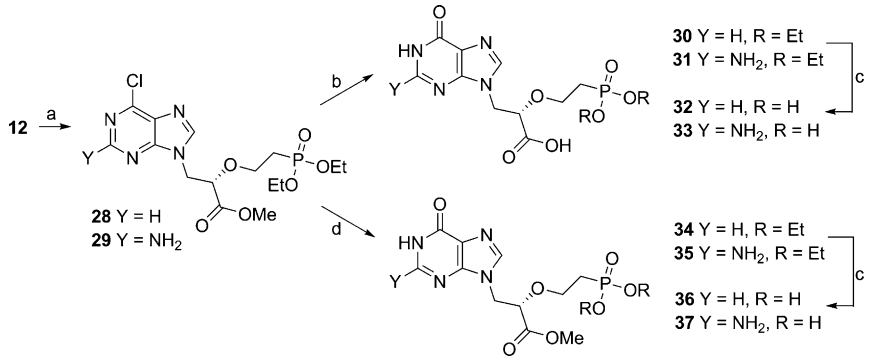
In the HPEP series, 6-chloropurine derivatives **19** and **20** (Scheme 3) were converted into 6-oxopurine analogues **21** and **22**, respectively, by the basic procedures (DABCO/K_2CO_3) described above. Subsequently, compounds **23** and **24** (Scheme 3) were prepared by hydrogenolysis of the protecting *O*-benzyl groups in derivatives **21** and **22**, respectively.

To obtain desired free phosphonic acids **17**, **18**, **25**, **26**, **32**, **33**, **36** and **37**, the ester functions on the phosphonate moiety were removed under standard reaction conditions using Me_3SiBr/MeCN followed by hydrolysis (Schemes 2–4).[22] The guanine derivative **26** was then converted by treatment with isoamylnitrite in AcOH to xanthine analogue **27** to give a 35% yield (Scheme 3).

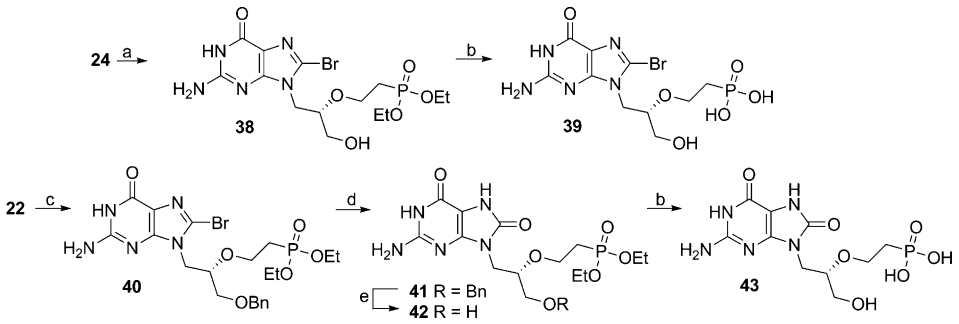
The current SAR study of the (*S*)-HPEP analogues was also extended for derivatives containing a bromo or oxo group at the C8 position of guanine. Two methods were employed for bromination of guanine containing ANPs: bromination of compound **24** with a bromine/CCl_4/DMF mixture to yield 85% of 8-bromoguanine derivative **38** (Scheme 5); bromination of *O*-benzyl derivative **22** using $NaBrO_3/Na_2S_2O_4$ to afford brominated product **40** in a 67% yield (the anticipated simultaneous bromination and benzyl moiety removal[23] was not achieved). Compound **40** was subsequently heated in a AcONa/AcOH mixture to afford 8-oxoguanine derivative **41** (80% yield),[24] which was then converted by hydrogenolysis to compound **42** in an 82% yield (Scheme 5). Finally, desired ANPs **39** (77%) and **43** (77%) were obtained in good yields after removal of phosphonate ethyl ester moieties (Scheme 5).



Scheme 3. Preparation of (*S*)-HPEPHx (**25**), (*S*)-HPEPG (**26**) and (*S*)-HPEPX (**27**). *Reagents and conditions*: a) Mitsunobu conditions: 1. 6-chloro or 2-amino-6-chloropurine, PPh_3, dioxane, 2. DIAD, RT, 24 h; b) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3 h; c) H_2/Pd/C, AcOH, RT, 72 h; d) Me_3SiBr, MeCN, RT, overnight; e) isoamylnitrite, 80% AcOH, RT, overnight.



Scheme 4. Preparation of (*S*)-CPEEHx (**32**), (*S*)-CPEEG (**33**) and their methyl esters **36** and **37**. *Reagents and conditions*: a) Mitsunobu conditions: 1. 6-chloro or 2-amino-6-chloropurine, PPh_3, dioxane, 2. DIAD, RT, 24 h; b) DABCO, K_2CO_3, dioxane/H_2O (4:1), 90 °C, 3–5 h; c) Me_3SiBr, MeCN, RT, overnight; d) 75% CF_3COOH, RT, 24 h.



Scheme 5. Preparation of 8-substituted (*S*)-HPEPG analogues **39** and **43**. *Reagents and conditions*: a) Br_2/CCl_4, DMF, RT, 3 h; b) Me_3SiBr, MeCN, RT, overnight; c) $NaBrO_3$, $Na_2S_2O_4$, EtOAc/H_2O (5:4), RT, 1 h; d) AcONa, AcOH, 130 °C, 4 h; e) H_2/Pd/C, AcOH, RT, 24 h.

The above described 6-chloropurine intermediates **19** (Scheme 3) and **28** (Scheme 4) were also used for the preparation of the corresponding adenine containing ANPs bearing CH_2OH, COOH and $CONH_2$ moieties at C2 side chain position, that is, compounds **46**, **48** and **50**, respectively (Scheme S1, Supporting Information). These compounds have been designed, in an analogy to previously reported adenine based ANPs,[25] as potential inhibitors of *Bordetella pertussis* adenylate cyclase toxin (ACT, CyA), but no activity was observed in the ACT inhibitory assay[25] with these compounds (the assay details in Supporting Information).

Several bis-amidate prodrugs of the HPEP and CPEE analogues, namely compounds **51**–**56** (Scheme S2, Supporting Information), were then prepared by the previously reported methodology[26] (see Supporting Information for details) to test their effectiveness as antimalarial compounds in cell based assays.

Inhibition studies of novel 6-oxopurine acyclic nucleoside phosphonates

The synthesized ANPs analogues containing (*S*)-3-hydroxy-2-(phosphonoethoxy)propyl (HPEP), (*S*)-2-(phosphonomethoxy)-propanoic acid (CPME) or (*S*)-2-(phosphonoethoxy)propanoic acid (CPEE) moieties were tested as potential inhibitors of human HGPRT and *Pf*HGXPRT.

(S)-3-Hydroxy-2-(phosphonoethoxy)propyl (HPEP) derivatives

Table 1 compares the K_i values for human HGPRT and *Pf*HGXPRT when HPEP is the acyclic linker and is attached to five different 6-oxopurine bases. In this series of ANPs, if the purine base has a high K_M for the enzymes,[10] or does not bind at all, then the ANP containing that particular purine base exhibits high K_i values. This data, therefore, emphasizes the important contribution of the purine base to the degree of inhibition for these compounds. For example, xanthine is not a substrate for human HGPRT and its K_M for *Pf*HGXPRT is 189 μM.[10] Therefore, this ANP has an extremely high K_i value for the enzymes and, at the concentration of inhibitor added in the assay, no inhibition could be detected. Thus, >30 μM is likely to be a low estimate of its true K_i value.

The K_i value for HPEPG (**26**) is similar to that of PEEG for both enzymes (0.6 μM *vs* 1 μM for human HGPRT and 0.1 μM (both compounds) for *Pf*HGXPRT, Tables 1 and S1). Thus, the addition of the CH_2OH group to the acyclic linker does not significantly affect the K_i value.

In contrast, when the base is hypoxanthine, the K_i value for human HGPRT decreases for the HPEP compound relative to PEEHx (from 3 to 0.5 μM). However, for *Pf*HGXPRT, the reverse occurs and the K_i value increases from 0.3 μM to 2 μM (Tables 1 and S1). This results in the K_i value for HPEPHx for human HGPRT becoming similar to HPEPG, suggesting that the contribution of the exocylic amino group of the purine base may not be as important in human HGPRT as that of the ANP moiety itself. For *Pf*HGXPRT, HPEPG has the highest affinity from this group of ANPs and has selectivity in favor of this enzyme over human HGPRT of 6.

Table 1. Comparison of the K_i values of (*S*)-3-hydroxy-(2-phosphonoethoxy)propyl (HPEP) ANPs for human HGPRT and *Pf*HGXPRT.

Attachment to C2 in the linker	Compd	Base	K_i [μM]	
			human	*Pf*
$-CH_2OH$	**43**	8-oxoguanine	>30[a]	>30[a]
$-CH_2OH$	**27**	xanthine	>30[b]	>30[b]
$-CH_2OH$	**39**	8-bromoguanine	13 ± 2	>30[c]
$-CH_2OH$	**26**	guanine	0.6 ± 0.1	0.1 ± 0.05
$-CH_2OH$	**25**	hypoxanthine	0.5 ± 0.2	2 ± 0.8

[a] Assay [inhibitor]: 31 μM. [b] Assay [inhibitor]: 49 μM. [c] Assay [inhibitor]: 33 μM.

(S)-2-(Phosphonoethoxy)propanoic acid (CPEE) derivatives and their methyl esters

The K_i values for human HGPRT and *Pf*HGXPRT when CPEE (or its corresponding methyl ester) is the acyclic moiety are given in Table 2. For human HGPRT, CPEEHx (**32**) and its methyl ester **36** show a significant decrease in affinity compared with the corresponding HPEP analogue **25** (K_i values increase from 0.5 μM to 6 μM). However, when the base is guanine, the K_i value is virtually unchanged with values of 0.6, 0.8 and 0.3 μM for the three ANPs (Tables 1 and 2). Once again, the reverse is true for *Pf*HGXPRT, with the K_i values for CPEEHx and its ester being similar to HPEPHx (1–2 μM cf. 1 μM; Tables 1 and 2). When guanine is the base, the K_i value for CPEEG (**33**) compared with HPEPG (**26**) increases from 0.1 μM to 0.5 μM and the K_i value for its methyl ester **37** increases to 5 μM with a resultant decrease in affinity of ~50-fold.

Table 2. Comparison of the K_i values for the (*S*)-2-carboxy-(phosphonoethoxy)ethyl (CPEE) derivatives for human HGPRT and *Pf*HGXPRT.

Attachment to C2 in the linker	Compd	Base	K_i [μM]	
			human	*Pf*
–COOH	**33**	guanine	0.8 ± 0.2	0.5 ± 0.2
–COOH	**32**	hypoxanthine	6.3 ± 1.5	2 ± 1
–COOMe	**37**	guanine	0.3 ± 0.1	5 ± 1
–COOMe	**36**	hypoxanthine	6 ± 2	1 ± 0.9

This data shows the importance of the character of the purine base and the chemical structure of the acyclic moiety in positioning the inhibitor in the active site of these two enzymes to obtain the highest affinity and selectivity. In some cases, these two moieties work in synergy but, in other cases, the contribution of one of them is more influential. Thus, it can be hypothesized that it is the chemical structure of the acyclic moiety that is responsible for positioning both the purine base and the phosphonate moiety in the active site of either of these two enzymes to obtain optimal binding.

(S)-2-(Phosphonomethoxy)propanoic acid (CPME) derivatives

Compounds **17** and **18** are very weak inhibitors with K_i values of much greater than 30 μM as no inhibition was observed at the concentration of these compounds in the assay (32.4 and 46.7 μM for compounds **17** and **18**, respectively). It has been shown that when the acyclic linker connecting the phosphorus and N^9 atoms is only four atoms long, the affinity for such ANPs in the active site decreases significantly.[27] This has been attributed to the fact that the phosphonate group cannot reach into the 5′-phosphate binding pocket to form maximal interactions with the amino acid side chains or the backbone atoms of those residues that surround this group (residues D137-T141 in human HGPRT and D148-T152 in *Pf*HGXPRT). However, (*S*)-3-hydroxy-2-(phosphonomethoxy)propyl guanine [(*S*)-HPMPG] does bind to both the human and *Pf* enzymes, albeit weakly, with K_i values of 177 and 28 μM, respectively.[13] The crystal structure of (*S*)-HPMPG in complex with human HGPRT suggests that one of the reasons for this affinity is a hydrogen bond between the hydroxy group and the NZ atom of K68 which occurs in one of the two subunits. However, it may be that, for the CPMEG compound, the conformation of the acyclic moiety has changed the orientation of the ANP in the active site and that the side chain of this residue is not in a position to form this hydrogen bond. Also, for (*S*)-HPMPG the phosphonate group does indeed reach into the 5′-phosphate binding pocket due to its location in the active site.[13a] This may not be the case for inhibitors containing CPME as the acyclic moiety.

IC_{50} values of the prodrugs of novel ANPs for Pf *in cell culture*

Table 3 shows the activity of six prodrugs of the HPEP and CPEE compounds against *Pf* in cell culture (synthesis and structures are given in Supporting Information).

Table 3. In vitro antimalarial activity of ANP prodrugs against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) *P. falciparum* lines.

ANP moiety	Base	Compound	Parent	IC_{50} [μM]	
				D6	W2
HPEP	hypoxanthine	**51**	**25**	103	76
HPEP	guanine	**52**	**26**	70	22
HPEP	hypoxanthine	**53**	**25**	695	523
HPEP	guanine	**54**	**26**	274 ± 37	261 ± 25
CPEE	hypoxanthine	**55**	**32**	60 ± 12	60 ± 11
CPEE	guanine	**56**	**33**	54 ± 8	51 ± 5
–	–	chloroquine	–	0.012 ± 0.004	0.169 ± 0.075

Compounds **51**, **52**, **55** and **56** (Scheme S2) are bisphosphoramidate prodrugs with two ethyl L-phenylalanyl moieties attached to the parent compound. Compounds **53** and **54** are monophosphoramidates where the remaining phosphonate oxygen is replaced by NH_2 group (Scheme S2). The second strategy was not successful as it resulted in a dramatic increase in the IC_{50} value (7-fold when the base is hypoxanthine and 12-fold when the base is guanine). One suggestion for this increase is that the amino group replacing the phosphonate oxygen is not easily hydrolyzed in vivo and the resulting ANP could be a weak inhibitor of the *Pf* enzyme. In all cases, the prodrugs containing guanine as the base have lower IC_{50} values than when the purine base is hypoxanthine. This is consistent with the fact that the K_i values for the parent compound are lower for guanine than for the ANPs with hypoxanthine as the nucleobase.

Crystal structures of compounds 25 and 26 in complex with human HGPRT

These two structures contain a tetramer in the asymmetric unit. The overall fold of each subunit is similar to that of PEEG (PDB code: 3GGJ) and PEEHx (PDB code: 3GGC).[13a] In these latter two structures, the asymmetric unit is a dimer though the normal tetrameric molecule for human HGPRT is observed when adjoining asymmetric units are considered. Thus, the quaternary structure is the same in all four structures. Data collection and refinement statistics for the two complexes are given in Table S2 (Supporting Information). The electron density for both inhibitors bound in the active site is shown in Figure 3, unequivocally demonstrating their location together with those of a magnesium ion and water molecules in the active site.

The movement of flexible loops and the specific interactions surrounding the purine base and phosphonate group when HPEPG (compound **26**) and HPEPHx (compound **25**) bind in the active site of human HGPRT (Figure 4). The chemical nature of the five atoms connecting the N^9 of the purine ring to the phosphorus atom in the phosphonate group is the same in the HPEP and PEE compounds, that is, $-(CH_2)_2O(CH_2)_2-$. The design of this linker places the purine base under the aromatic ring of F186, forming a Π-stacking arrangement, and the phosphonate group in the 5′-phosphate binding pocket (Figure 4). The figure also demonstrates the movement of two

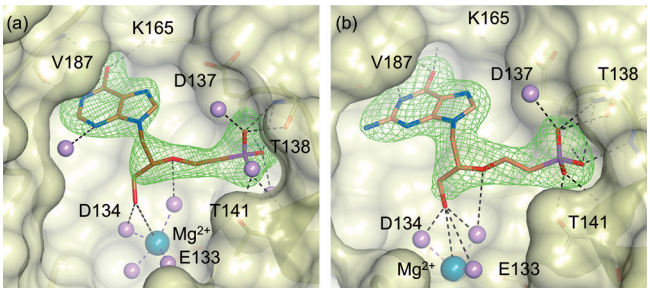


Figure 3. The crystal structure of the active site of human HGPRT in complex with a) HPEPHx (**25**) and b) HPEPG (**26**). The F_o-F_c omit electron density is shown as green wire. Human HGPRT is presented as a Connolly surface. Hydrogen bonds to the inhibitors are shown as black dashed lines. Coordination to the Mg^{2+} ions is shown as purple dashed lines. Water molecules within hydrogen bonding distance or that coordinate to the Mg^{2+} ions are depicted as pink spheres.

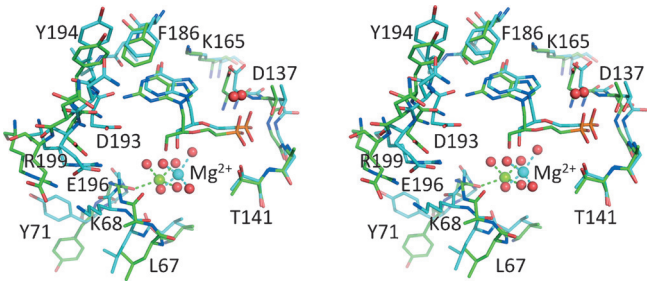


Figure 4. Stereoview of the superimposition of the active site of human HGPRT in complex with HPEPHx (**25**) (cyan carbon atoms) and HPEPG (**26**) (green carbon atoms).

flexible loops that occurs when these inhibitors bind and, in particular, the loops that comprise the amino acid residues between F186–196E and L67–71Y.

The mobile loops between residues 187–193 and 67–71

When the transition state analogue, (1*S*)-1-(9-deazaguanin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate guanine (ImmGP),[28] binds to human HGPRT, the nitrogen atom of the exocyclic amino group attached to the C2 atom in the purine ring forms two hydrogen bonds: to the carbonyl oxygen atoms of V187 (2.6–2.8 Å) and D193 (2.7 Å but 3.1 Å in one subunit). These residues are located on the flexible loop (187–193). When PEEG binds, there is also a hydrogen bond to the carbonyl of V187 (3.1 Å) but the carbonyl of D193 is too far away for such bonds to form (4 Å in one subunit and 3.5 Å in the second). However, when compound **26** binds in the active site, the location of this loop is altered so that the distances between these two carbonyl oxygen atoms and the exocyclic nitrogen atom in the purine ring (3.2 −3.9 Å to the carbonyl oxygen of V187 and 3–3.3 Å to the carbonyl oxygen of D193) are altered. In the presence of compound **26**, the loop containing these two residues moves by ~2.4 Å compared with when HPEPHx binds (Figure 5). This is very different to that found when PEEG and PEEHx bind as there is no change in the structure of the loop between residues 187–193. This is attributed to the fact that the location in the active site of these two inhibitors is virtually identical except for that addition of the exocyclic amino group. The movement observed when compound **25** binds appears to cause a cascade effect on the structure of the enzyme in this region (Figure 5). Thus, though the integrity of the β-sheets and α-helices is maintained, there is a shift so that the areas in this region are no longer completely superimposable (Figure 5).

This structural change also affects the association between subunits in the two structures of human HGPRT in complex with compounds **25** and **26**. Thus, in the complex with compound **26**, the shift of the flexible loop (residues 187-193) results in the formation of two hydrogen bonds between subunits (A with C and B with D). In this structure, the hydroxy group of the side chain Y71 (subunit B) forms a hydrogen bond with the carbonyl oxygen of Y71 in the adjoining subunit (subunit D). The hydroxy group of this amino acid residue (Y71; subunit D) can then form a hydrogen bond with the NZ atom of K82 in subunit B. These two hydrogen bonds assist in stabilizing the interface between these two subunits. These two hydrogen bonds cannot form in the complex with compound **25** because the side chain of Y71 is pointing in the opposite direction (Figure 4). The difference in the movement of the flexible loop between residues 67–71 and, in particular K68, is discussed later.

How these structural changes influence the K_i value cannot be determined but they do demonstrate the difficulties in predicting precisely how these compounds could bind and how binding affects the positioning of the mobile loops in the active site.

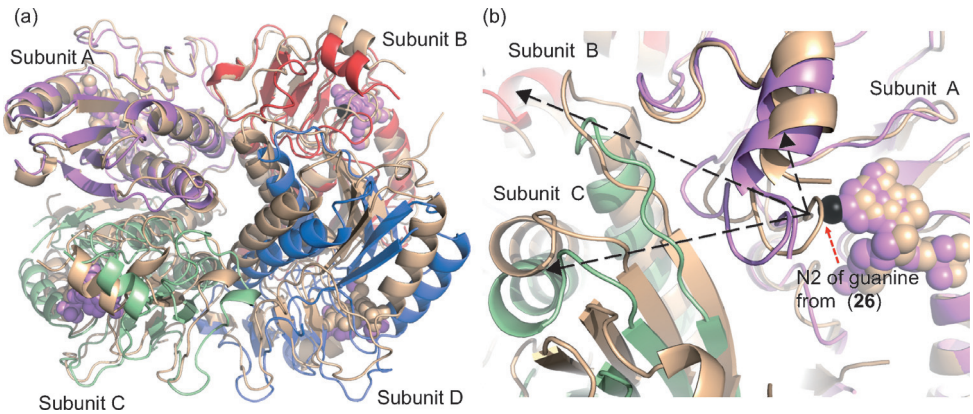


Figure 5. Superimposition of subunit A of the human HGPRT–compound **25** complex (brown) with subunit A of the human HGPRT–compound **26** complex (pink, red, green, blue). a) Overall view showing the relative movements of the subunits. b) The origin of the movement is the presence of the amino group in C2 position of compound **26** when the closest loop moves by 2.7 Å. This transmits a movement of the helix in subunit C by 4.1 Å and the upper helix in subunit A by 2.7 Å. However, the lower helix in subunit A, bordered by the 5′-phosphate binding pocket does not relocate.

K165

In both the HPEP structures, the invariant lysine residue (completely conserved across all the 6-oxopurine PRTases) forms a hydrogen bond with the 6-oxo group (Figure 4). This confers specificity for compounds containing an oxygen, halogen or thiol at this position of the purine ring in contrast with an amino group, which abolishes binding.

The 5′-phosphate binding pocket

The phosphonate group of the two structures is locked into position by hydrogen bonds to the D137-T141 amino acid residues that surround the 5′-phosphate binding pocket (the amides of D137, T138, G139 and T141 together with bonds to the side chains of T138 and T141) (Figure 4).[13a]

Two water molecules can be observed in the binding pocket of the phosphonate group in both structures. The first forms a bridge between one of the phosphonate group oxygen atoms and the OD1 atom of D137 residue (complex with **26**, subunits A and C; and complex with **25**, subunits A, C and D). These bonds assist in anchoring the phosphonate group in position and also help to restrict the movement of the side chain of D137. This is another of the flexible regions of the enzyme and demonstrates how different network of interactions contribute to the affinity of the inhibitor. There is a second water molecule located at the "bottom" of this pocket (Figure 4). This molecule forms a hydrogen bond with the amide of M142 (complex with **26**, subunit B; and complex with **25**, subunits A, C and D). In the other subunits, no other hydrogen bond is formed except in subunit B (complex with **25**) where there is an interaction with the carbonyl oxygen atoms of I135 and E133. This is also another flexible part of the enzyme. This arrangement of water molecules is the same as in the structure of PEEG and PEEHx in complex with human HGPRT.[13a] In the transition state structure, the water molecule which forms a hydrogen bond with the side chain of D137 is absent. This is due to the fact that this region of the enzyme has moved so that the OD1 atom of D137 forms a hydrogen bond with the N^7 atom of the purine ring. This is an important bond as it has been proposed that it is necessary to promote catalysis. Thus, the variable network of hydrogen bonds in this area of the active site seems to be another factor that can influence the affinity of an inhibitor for these enzymes. The electron density for all the water molecules is unequivocal and, thus, this strongly suggests that these molecules play a crucial role in the binding of the inhibitors.

The effect of a water molecule found within hydrogen bonding distance to the N^3 atom of the purine ring

The positioning of a water molecule around the N^3 atom of the purine ring differs between the structures in complex with compounds **25** and **26**. As shown in Figure 3, there is a bridging water molecule between the N^3 atom of the purine ring and the carbonyl oxygen of D193 in the complex with compound **25**. This water molecule is not observed in the complex with compound **26**.

A water molecule is also found in this location when ANPs, containing a second phosphonate group, are bound in the active site.[14d–f] In the majority of instances, this water molecule is coordinated to the second magnesium and acts as a bridge to the phosphonate oxygen. However, on some occasions, it has been found to form a hydrogen bond with the side chain of D193. A water molecule has also been found in hydrogen bond distance to the N^3 atom when ImmGP binds together with pyrophosphate.[28] Here, it is coordinated to the magnesium ion coordinated to the phosphoryl oxygen atoms of pyrophosphate. The complex with compound **25** is the first instance that the presence of a water molecule linking the N^3 atom to the D193 side chain is observed when a second divalent ion, together with pyrophosphate or its mimic, are not found in the active site.

The large mobile loop (residues 100–120)

One difference between the structures of PEEG, PEEHx and compounds **25** and **26**, is found in the large mobile loop. In the former two structures,[13a] this loop is visible though some residues are missing. In the PEEG structure, the loop is complete in chain A but residues D107 and T110 are not visible in chain B. For PEEHx, residues N106 and D107 are missing chain A and G117 in chain B. The structure of this loop is mainly random coil and it adopts a position that appears to be intermediate between that found in the ImmGP complex with human HGPRT[28] and when this enzyme is in complex with the nucleoside monophosphate, GMP. However, unlike the complex with ImmGP, there are no interactions between the amino acids in this loop and atoms in either PEEG or PEEHx. This suggests that, for these ANPs, the positioning of the loop may not affect their affinity. In comparison, there is no electron density for residues 100–120 in the structures of human HGPRT in complex with compounds **25** and **26** (Table S2) indicating that, when these two ANPs bind, the loop is mobile and not fixed in any one position. It is deduced, however, that like the PEEG and PEEHx complexes, this loop does not play a direct role in the binding of either of the two new ANPs. However, it cannot be negated that the positioning of this loop may influence the movement of other flexible loops that surround the active site of the enzyme(s). The influence of the CH_2OH group and its interactions with magnesium and water ions and the resultant location of the carbonyl, amide and side chain of K68 in this complex.

Interactions with Mg^{2+} and water molecules

There is one magnesium ion present in the structure of human HGPRT in complex with compounds **25** and **26**. This ion adopts regular near octahedral geometry and is coordinated to the side chains of the two acidic amino acid residues, E133 and D134, and four waters. These two amino acids are completely conserved in all the 6-oxopurine PRTases known to date suggesting that their role is of high importance in the

binding of substrates/products of the catalytic reaction (Figure 3). Crystal structures of both the human and *Pf* enzymes suggest that a magnesium ion can enter this region of the active site by two ways: 1) coordinated to the hydroxy groups of the ribose ring of *P*Rib-*PP*; or 2) entering independently to assist in preparing the structure of the active site to accept substrates or inhibitors.[29] For compounds **25** and **26**, only the latter can apply. It has been purported that when magnesium is found in this area of the active site, the side chain of K68 (K77 in *Pf*) rotates by 180° where it can then set up communication with the adjacent subunit of the tetrameric structure (the carbonyl of V96 in human HGPRT and the carbonyl of E108 in *Pf*HGXPRT).

A second magnesium ion has been found in the active site of other structures of the human and *Pf* structures in complex with inhibitors but this only occurs in two instances: 1) when it is coordinated to PP_i either as it is entering or leaving the active site;[28,30] and 2) in the case of some ANP inhibitors when a second phosphonate group (mimicking PP_i) is bound.[14d–f] Therefore, a second divalent metal ion is not found in the complex with **25** or **26** as there are no phosphoryl oxygen atoms present.

The magnesium ion that is coordinated to the side chains of E133 and D134 does have a role in binding of compounds **25** and **26** though this is slightly different in both complexes (Figure 3). This is either by direct coordination to the inhibitor or via bridging water molecules to the inhibitor. Two water molecules are found in the vicinity of the hydroxy group of the inhibitor and the magnesium ion coordinated to the side chains of E133 and D134. For the complex with compound **25**, one of these water molecules forms a hydrogen bond with the carbonyl of G69 (subunit A) or a hydrogen bond with D134 (subunit C) and in two subunits (B and D) with the carbonyl of K68. This network helps to keep the flexible loop that contains K68 close to the active site (167–172). K68 is located at the top of this loop that connects a β-sheet (residues 61–65) to an α-helix (residues 70–86). In contrast, such a bonding pattern does not exist in the complex with compound **26** and this water molecule does not partake in any other interactions at the active site. In the complex with compound **25**, the arrangement is slightly different due to the fact that the hydroxy group of the inhibitor is in a slightly different position. In this case, it still forms a hydrogen bond with a water molecule coordinated to magnesium (subunits A and B) but, in subunits C and D, it has moved closer to magnesium (~3 Å distant). As a result, there is insufficient room to accommodate a water molecule in this location. One of the biggest differences between the two structures is the positioning of the flexible loop containing K68. In the complex with compound **25**, it has moved further away from the active site and too far for the oxygen of the carbonyl group to form a hydrogen bond to the water molecule which is coordinated to magnesium and also forms a hydrogen bond to the hydroxy group of the inhibitor.

The second water molecule performs an interesting function though it appears to be absent in one of the subunits in complex **26** (subunit B) and in complex **25** (subunit D). In the latter structure, this second water interacts with the oxygen atom in the acyclic linker as it is only 3–3.3 Å away. This interaction is not as definitive in the structure with compound **26.** In subunits A and C, it is ~3.8 Å away from the oxygen in the linker though, in subunit D, it forms a strong interaction (2.5 Å).

There are no magnesium ions in the PEE structures and the bonding pattern in the HPEPHx with magnesium could be part of the reason that the K_i for compound **25** is lower for human HGPRT compared with PEEHx (*cf.* 0.5 with 3.6 μM). It is speculated that the role of the divalent metal ion could be different in *Pf*HGXPRT accounting, in some part, for the differences in affinity for these ANPs.

Communication between subunits: the loop containing K68 (residues 67–72)

When compound **26** binds in the active site, the side chain of K68 does not form any interactions with amino acid residues either within its own subunit or with those in an adjacent subunit. This is because the side chain in this complex is flexible (i.e., there is no electron density) and is therefore modeled in as far as the Cβ atom. This is not the case for compound **25** where there is strong density for this side chain and it forms hydrogen bonds not only within its own subunit (the OE1 atom of E196) but also with the carbonyl oxygen of V96, thus assisting in stabilizing the two dimers which constitute the biologically active tetramer. The structure of this mobile loop is thus different in these two structures (Figure 4 and 5). The contribution to the dimeric interface for compound **25** is similar to that found in the complex of *Pf*HGXPRT with ImmHP and $PP_i{\cdot}Mg^{2+}$.[30] The side chain of K77 in *Pf*HGXPRT forms a hydrogen bond to the carbonyl oxygen of E108 (V96 in human HGPRT). The side chain of E108 is then available to form a hydrogen bond with the side chain of R80 which is found in the same adjacent subunit as K77.

Thus, two of the contributing factors in stabilizing the dimeric interface differ in the structures with compounds **25** and **26**. For compound **25**, one of these is the mobile loop between 67–71 while, for compound **26**, it appears to be the loop containing residues 187-194.

The CPEE compounds and their methyl esters

For the CPEEHx compound (**32**) and its methyl ester **36**, there is a 10-fold increase in the K_i value compared with HPEPHx (**25**). From the crystal structure of HPEPHx, it could be speculated that this could be due to the fact that the network of hydrogen bonds that holds the hydroxy group in place are no longer able to form. The K_i values when guanine is the base are similar for all three ANPs. Thus, the positioning of the hydroxy group may not be as influential for these ANPs. It is unknown if the binding of magnesium ions effects the affinity of this group of ANPs for *Pf*HGXPRT as the only available structures have pyrophosphate bound as well as a second ligand.[28,30]

Thus, a number of factors come into play when these ANPs bind. One of the most critical appears to be the movement of the flexible loops surrounding the active site. At this stage, it

cannot be determined if these occur in synchrony or consecutively or what is the trigger(s), that prompts these movements. What is known from the crystal structures is that these movements do occur. Further structures of the enzymes and, in particular, that of *Pf*HGXPRT should assist in explaining this interesting phenomenon.

Conclusions

A novel series of acyclic nucleoside phosphonates (ANPs) containing either (*S*)-3-hydroxy-2-(phosphonoethoxy)propyl (HPEP) or (*S*)-2-(phosphonomethoxy)propanoic acid (CPME) or (*S*)-2-(phosphonoethoxy)propanoic acid (CPEE) moieties have been synthesized. For the HPEP compounds, the nature of the purine base has a strong influence on the K_i values as only ANPs containing bases that are good substrates for either enzyme, that is, guanine and hypoxanthine, exhibit low K_i values. The chemical nature of the acyclic linker also contributes to the affinity of these inhibitors as shown for the three different acyclic phosphonate moieties, HPEP, CPEE and its methyl ester. None of the CPME derivatives exhibited inhibition of either enzyme at the concentrations used in the assay ($K_i >$ 30 μM) indicating again the importance of the nature and length of the acylic phosphonate moiety in conferring affinity. Crystal structures of HPEPG and HPEPHx demonstrate the mobility of a number of loops surrounding the active site. The flexibility of these loops which contain amino acid residues directly involved in the binding of the ANPs is a factor which has to be considered in effective selective and potent drug design. Thus, to accomplish inhibition with sub-nanomolar K_i values, one goal should be to "lock" these flexible loops firmly in place to achieve more interactions with the inhibitors. The importance of water molecules at key locations in the active site cannot be overlooked. The ability of these inhibitors to maximize subunit interactions at the interface of each pair of dimers may also contribute to stabilizing the structure of enzyme(s) and could be another factor leading to tight binding inhibitors. Finally, phosphoramidate prodrugs of HPEPG, HPEPHx, CPEEG and CPPEHx were synthesized and tested for their ability to arrest the growth of *Pf* lines in cell culture. This data shows that the chemical nature of the prodrug has a significant effect on the IC_{50} values and highlights the importance of further prodrug design in improving their biological activity.

Experimental Section

General

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa, and compounds were dried at 2 kPa over P_2O_5. TLC was performed on TLC aluminum sheets (silica gel 60 F_{254}, Merck), chromatographic systems are described in the text. Column chromatography was performed on silica gel 230–400 mesh, 60 Å (Merck). Reverse phase HPLC separation were performed on a Waters Delta 600 instrument with a Waters 486 Tunable Absorbance Detector using column Phenomenex Gemini C_{18} (10 μm, 250×21.2 mm, flow 10 mL min^{-1} preparative column). ^1H and ^{13}C NMR spectra were measured on a Bruker Avance II 600 (^1H at 600 MHz and ^{13}C at 151 MHz) and/or Bruker Avance II 500 (^1H at 500 MHz and ^{13}C at 126 MHz) spectrometers in $CDCl_3$, DMSO or D_2O (NaOD additive) and referenced to TMS, ^{13}C chloroform signal (δ 77.0) or dioxane used as internal standard ($\delta = 3.75$ and $\delta = 67.19$). The numbering system for assignment of NMR signals is outlined in Figure S1 (Supporting Information). ^1H and ^{13}C NMR spectra of the corresponding (*S*)-isomers, as well as racemates, are identical and are given for one isomer only. Mass spectra were measured on a ZABEQ (VG Analytical) spectrometer using FAB (ionization by Xe, accelerating voltage 8 kV, glycerol matrix) or by ESI technique. Optical rotations were measured on an AUTOPOL IV polarimeter (Rudolph research analytical) at 20 °C; $[\alpha]_D$ values are given [10^{-1} deg cm^2 g^{-1}] and concentrations *c* are [g/100 mL]. Microwave experiments were carried out in 10 mL or 80 mL vial in CEM Discover (Explorer) microwave apparatus operated at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W. Chemicals were purchased from Sigma–Aldrich (Prague, Czech Republic). Diethyl vinylphosphonate was purchased from Epsilon Chemie (France). Solvents were dried by standard procedures and stored over molecular sieves (4 Å). L-Phenylalanine ethyl ester hydrochloride was dried in vacuo at 30 °C and 0.1 mbar for 1 day. (*S*)- and (*R*)-tritylglycidole (**1a** and **1b**, respectively) were purchased from DAISO Co. Ltd., Japan. Chromatographic system H1: ethyl acetate/acetone/ethanol/water = 15:3:4:3.

Procedures

Purification of the phosphonic acids (procedure A): Crude phosphonic acid was dissolved in water and purified by preparative HPLC. Product was eluted by linear gradient from water to 50% methanol in water. UV absorbing fractions containing product were collected and evaporated in vacuo. Compounds were crystallized from the water/ethanol mixture.

Hydrolysis of the phosphonate diester to corresponding phosphonic acids (procedure B): The appropriate diester (0.5 mmol) was dissolved in acetonitrile (10 mL) and bromotrimethylsilane (1 mL) was added dropwise. The mixture was stirred overnight and evaporated to dryness. Residue was co-distilled with acetonitrile (3×20 mL) and water (2×10 mL). Crude product was crystallized from the ethanol/water mixture or further purified by procedure A.

Basic hydrolysis of the 6-chloropurine derivatives to corresponding 6-oxopurine phosphonates (procedure C): The 6-chloropurine derivative (1 mmol) was dissolved in mixture of dioxane and water (5:1) (120 mL), DABCO (0.8 mmol) and potassium carbonate (1 mmol) were added. The reaction mixture was heated at 90 °C for 3–5 h. After cooling down, volatiles were evaporated and a crude residue was purified by silica gel chromatography to give the corresponding products.

Acidic hydrolysis of the 6-chloropurine derivatives to corresponding 6-oxopurine phosphonates (procedure D): The 6-chloropurine derivative (1 mmol) was dissolved in trifluoroacetic acid (75%, 15 mL) and the mixture was stirred overnight. Volatile materials were evaporated in vacuo, the residue co-distilled with water (3 times) and neutralized with ammonia (1:10 with water) to pH 6–7. Volatiles were evaporated and a crude residue was purified by silica gel chromatography (gradient from 1–12% methanol in chloroform) to give the corresponding products.

Synthesis of the 6-chloropurine derivatives from phosphonate alcohols under Mitsunobu conditions (procedure E): A solution of DIAD (2.95 mL, 15 mmol) in dioxane (50 mL) was added dropwise under argon atmosphere to a mixture of alcohol (**7**, **9b**, **12**) (10 mmol), 6-chloropurine (2.0 g, 13 mmol), and Ph_3P (3.41 g,

13 mmol) in dioxane (150 mL) and the reaction mixture was stirred at RT for 24 h. When the reaction was finished, the solvent was removed in vacuo and the residue was purified by silica gel chromatography (gradient from 20–100% ethyl acetate in *iso*-hexanes and 0-6% methanol in ethyl acetate).

Synthesis of the 2-amino-6-chloropurine derivatives from phosphonate alcohols under the Mitsunobu conditions (procedure F): The procedure is identical with the procedure E, but 2-amino-6-chloropurine was used instead of 6-chloropurine. When the reaction was finished, water was added (50 mL) into reaction mixture and stirred at 80 °C for 6 h (to remove triphenylphosphine adducts). The mixture was evaporated and the residue was purified by silica gel chromatography (gradient from 20–100% ethyl acetate in *iso*-hexanes and 0–12% methanol in ethyl acetate).

Hydrogenolysis of benzyl group (procedure G): The corresponding derivative (2 mmol) was dissolved in glacial acetic acid (120 mL) and a flask was purged with argon and evacuated (3 times). After that catalytic amount of 10% Palladium on carbon under argon atmosphere was added. Then the flask was evacuated and purged with hydrogen (3 times) and the mixture was vigorously stirred at RT until the reaction was complete (~3 days). The reaction mixture was filtered through short silica gel column and the filter pad was washed with acetic acid (50 mL) and methanol (50 mL). The filtrate was evaporated and a crude product was purified by silica gel chromatography (gradient from 2–12% methanol in chloroform) to afford the corresponding products.

Synthesis and compound characterization

(*S*)- and (*R*)-1-(Benzyloxy)-3-trityloxypropan-2-ol (2a and 2b): The compounds **2a** and **2b** were prepared according to a previously described procedure[31] in 95 and 97% yields, respectively.

Diisopropyl (*S*)-(((1-(benzyloxy)-3-(trityloxy)propan-2-yl)oxy)methyl)phosphonate (3): Compound **3** was prepared according to a previously described procedure[31] and was used in the next reaction step without further purification.

Diisopropyl (*R*)-(((1-benzyloxy)-3-hydroxypropan-2-yl)oxy)methyl)phosphonate (4): Compound **4** was prepared according to a previously described procedure[31] in a 40% yield (from **2a**).

(*S*)-3-(Benzyloxy)-2-((diisopropoxyphosphoryl)methoxy)propanoic acid (5): To a solution of compound **4** (14.4 g, 40 mmol), TEMPO (625 mg, 4 mmol), sodium chlorite (80%) (9.0 g, 80 mmol), acetonitrile (200 mL) and 0.67 M sodium phosphate buffer (160 mL, pH 6.7) was added dropwise a solution of dilute sodium hypochlorite (40 mL of household bleach SAVO (40.72 mg mL^{-1}) with 40 mL of water). The reaction mixture was heated at 40 °C for 48 h. After cooling the reaction to RT, water (300 mL) was added and the pH was adjusted to 8.0 by addition 48 mL of 2 M NaOH. Then the reaction mixture was cooled to 0 °C (ice bath) and solution of sodium sulfite (12 g Na_2SO_3 in 200 mL H_2O) was added. After stirring at RT for 0.5 h, the solution was acidified with 2 M HCl to pH 3–4 and extracted with ethyl acetate (3×300 mL). The combined organic phases were washed with water (2×200 mL), brine (2×200 mL), dried over $MgSO_4$ overnight, and then concentrated to give (after evaporation) the crude product as a yellowish oil. This was subjected to silica gel chromatography (gradient from 0–10% methanol in ethyl acetate) to afford (after evaporation) compound **5** as a colorless oil which solidified (13.2 g, yield 88%). ^{1}H NMR (MeOD): δ = 7.25–7.35 (m, 5H, H-2′, 3′, 4′), 4.70–4.79 (m, 2H, CH-*i*Pr), 4.60 (d, J_{gem} = 12.0 Hz, 1H, H-5a), 4.55 (d, J_{gem} = 12.0 Hz, 1H, H-5b), 4.30 (m, 1H, H-2), 4.11 (dd, J_{gem} = 13.7 Hz, J_{4a-P} = 8.8 Hz, 1H, H-4a), 3.84 (dd, J_{gem} = 13.7 Hz, J_{4b-P} = 9.2 Hz, 1H, H-4b), 3.82 (m, 2H, H-3), 1.31–1.34 ppm (m, 12H, CH_3-*i*Pr); ^{13}C NMR (MeOD): δ = 172.73 (C1), 139.35 (C1′), 129.32 (C3′), 128.86 (C2′), 128.70 (C4′), 81.55 (d, $J_{C-O-C-P}$ = 12.7 Hz, C2), 74.36 (C5), 73.33–73.46 (m, CH-*i*Pr), 71.52 (C3), 65.40 (d, J_{C-P} = 167.5 Hz, C4), 24.23–24.38 ppm (m, CH_3-*i*Pr); MS-ESI$^+$ *m/z* (%): 375 (10) $[M+H]^+$, 397 (100) $[M+Na]^+$) 419 (40) $[M+2Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{27}O_7NaP$ $[M+Na]^+$ 397.1387, found 397.1388; Anal. calcd for $C_{17}H_{27}O_7P$: C 54.54, H 7.27, P 8.27, found: C 54.37, H 7.32, P 8.09; $[\alpha]_D^{20} = -14.2$ (*c* = 0.435 g/100 mL, $CHCl_3$).

Methyl (*S*)-3-(benzyloxy)-2-((diisopropoxyphosphoryl)methoxy)-propanoate (6): Compound **5** (11.2 g, 30 mmol) was dissolved in ethyl acetate (150 mL) and solution of diazomethane (1 M CH_2N_2 in dry Et_2O) was added dropwise until the solution wasn't yellowish. The reaction was stirred at RT for 0.5 h. Excess of diazomethane was decomposed by adding of several drops of ethanol. The solution was concentrated in vacuo and a crude product was purified by silica gel chromatography (gradient from 40–60% ethyl acetate in *iso*-hexanes) to give (after evaporation) the title compound as a colorless oil (11.4 g, yield 98%). ^{1}H NMR ($CDCl_3$): δ = 7.26–7.36 (m, 5H, H-2′, 3′, 4′), 4.72–7.79 (m, 2H, CH-*i*Pr), 4.61 (d, J_{gem} = 12.1 Hz, 1H, H-5a), 4.55 (d, J_{gem} = 12.1 Hz, 1H, H-5b), 4.38 (t, J_{2-3} = 4.3 Hz, 1H, H-2), 4.06 (dd, J_{gem} = 13.9 Hz, J_{4a-P} = 8.7 Hz, 1H, H-4a), 3.76–3.80 (m, 3H, H-3, 4b), 3.76 (s, 3H, O-CH_3), 1.31–1.34 ppm (m, 12H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$): δ = 170.19 (C1), 137.72 (C1′), 128.31 (C3′), 127.70 (C2′), 127.66 (C4′), 80.00 (d, $J_{C-O-C-P}$ = 9.6 Hz, C2), 73.40 (C5), 71.41 (d, J_{C-O-P} = 6.5 Hz, CH-*i*Pr), 71.20 (d, J_{C-O-P} = 6.5 Hz, CH-*i*Pr), 70.21 (C3), 64.93 (d, J_{C-P} = 166.4 Hz, C4), 52.08 (O-CH_3), 23.89–24.10 ppm (m, CH_3-*i*Pr); MS-ESI$^+$ *m/z* (%): 389 (100) $[M+H]^+$, 411 (95) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{18}H_{30}O_7P$ $[M+H]^+$ 389.1535, found 389.1536; $[\alpha]_D^{20} = -20.3$ (*c* = 0.370 g/100 mL, $CHCl_3$).

Methyl (*S*)-2-((diisopropoxyphosphoryl)methoxy)-3-hydroxypropanoate (7): Compound **6** (9.7 g, 25 mmol) from the previous reaction step was dissolved in methanol (250 mL) and a flask was purged with argon and evacuated (3-times). After that catalytic amount of 10% palladium on carbon under argon atmosphere was added. Then the flask was evacuated and purged with hydrogen (3-times) and the mixture was vigorously stirred at RT until the reaction was complete (~3 days). The reaction mixture was filtered through short silica gel column and the filter pad was washed with methanol (100 mL). The filtrate was evaporated and a crude product was purified by silica gel chromatography (gradient from 60-80% ethyl acetate in *iso*-hexanes) to afford (after evaporation) the title compound as a colorless oil (7.1 g, yield 95%). ^{1}H NMR ($CDCl_3$): δ = 4.72–4.82 (m, 2H, CH-*i*Pr), 4.23 (dd, J_{2-3b} = 6.4 Hz, J_{2-3a} = 3.3 Hz, 1H, H-2), 4.11 (dd, J_{gem} = 14.0 Hz, J_{4a-P} = 8.2 Hz, 1H, H-4a), 3.93 (dd, J_{gem} = 12.2 Hz, J_{3a-2} = 3.3 Hz, 1H, H-3a), 3.85 (dd, J_{gem} = 12.2 Hz, J_{3b-2} = 6.4 Hz, 1H, H-3b), 3.77 (s, 3H, O-CH_3), 3.77 (dd, J_{gem} = 14.0 Hz, J_{4b-P} = 7.7 Hz, 1H, H-4b), 3.22 (bs, 1H, OH), 1.31–1.37 ppm (m, 12H, CH_3-*i*Pr); ^{13}C NMR ($CDCl_3$): δ = 170.12 (C1), 81.54 (d, $J_{C-O-C-P}$ = 8.7 Hz, C2), 71.69 (d, J_{C-O-P} = 6.7 Hz, CH-*i*Pr), 71.42 (d, J_{C-O-P} = 6.7 Hz, CH-*i*Pr), 64.97 (d, J_{C-P} = 167.8 Hz, C4), 62.84 (C3), 52.08 (O-CH_3), 23.85–24.05 ppm (m, CH_3-*i*Pr); MS-ESI$^+$ *m/z* (%): 299 (5) $[M+H]^+$, 321 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{24}O_7P$ $[M+H]^+$ 299.1254, found 299.1255; $[\alpha]_D^{20} = -23.3$ (*c* = 0.510 g/100 mL, $CHCl_3$).

Diethyl (*S*)-(2-((1-benzyloxy)-3-(trityloxy)propan-2-yl)oxy)ethyl)-phosphonate (8a): A mixture of compound **2a** (17 g, 40 mmol) and catalytic amount of KOH (560 mg, 10 mmol) in dry dioxane (200 mL) was stirred at RT for 15 min. Diethyl vinylphosphonate (8 mL, 52 mmol) was added dropwise and the reaction was vigo-

rously stirred at RT for 70 h. The mixture was poured into water and extracted with ethyl acetate (3×300 mL). The combined organic phases were washed with brine (2×200 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude mixture was subjected to silica gel chromatography (gradient from 20–70% ethyl acetate in *iso*-hexanes with 0.1% Et_3N) to give (after evaporation) the crude product as a yellowish oil (13 g, yield 55% of the crude product, used in the next step without further purification). MS-ESI$^+$ *m/z* (%): 611 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{35}H_{41}O_6NaP$ $[M+Na]^+$ 611.2533, found 611.2530.

Diethyl (*R*)-(2-((1-(benzyloxy)-3-hydroxypropan-2-yl)oxy)ethyl)-phosphonate (9a): Compound **8a** (13 g of the crude product) was dissolved in 80% acetic acid (150 mL) and heated to reflux for 2 h. After cooling down, the reaction mixture was diluted with 100 mL of water/dioxane (1:1) and white crystals of trityl alcohol were filtered off. The solution was extracted with ethyl acetate (3× 150 mL), the combined organics were washed with water (2× 50 mL), brine (2×50 mL), dried over $MgSO_4$ and concentrated in vacuo to give a crude oil. This was subjected to silica gel chromatography (gradient from 40–100% ethyl acetate in *iso*-hexanes and 0–5% methanol in ethyl acetate) to afford (after evaporation) the title compound as a colorless oil (5.1 g, 37% overall yield from **2a**). ^{1}H NMR ($CDCl_3$): δ = 7.27–7.35 (m, 5H, H-2′, 3′, 4′), 4.53 (s, 2H, H-6), 4.05–4.16 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.98 (m, 1H, H-4a), 3.81 (m, 1H, H-4b), 3.72 (dd, J_{gem} = 11.7 Hz, J_{3a-2} = 2.8 Hz, H-3a), 3.62 (m, 1H, H-2), 3.49–3.59 (m, 3H, H-1, 3b), 2.02–2.16 (m, 2H, H-5), 1.32 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**), 1.31 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ($CDCl_3$): δ = 137.98 (C1′), 128.34 (C3′), 127.62 (C4′), 127.54 (C2′), 80.02 (C2), 73.40 (C6), 70.19 (C1), 63.93 (d, J_{C-C-P} = 5.9 Hz, C4), 62.17 (C3), 61.87 (d, J_{C-O-P} = 6.4 Hz, P-O-**C**H$_2$-CH$_3$), 61.57 (d, J_{C-O-P} = 6.5 Hz, P-O-**C**H$_2$-CH$_3$), 27.08 (d, J_{C-P} = 141.5 Hz, C5), 16.32 ppm (d, $J_{C-C-O-P}$ = 6.2 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 347 (20) $[M+H]^+$, 369 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{28}O_6P$ $[M+H]^+$ 347.1618, found 347.1617; Anal. calcd for $C_{16}H_{28}O_6P$ H_2O: C 52.74, H 8.02, P 8.50, found: C 52.95, H 7.98, P 8.40; $[\alpha]_D^{20}$ = +8.3 (*c* = 0.289 g/100 mL, $CHCl_3$).

(*S*)-3-(Benzyloxy)-2-(2-(diethoxyphosphoryl)ethoxy)propanoic acid (10): Compound **9a** (4.2 g, 12 mmol) was treated analogously compound **4** (chromatography: gradient from 0-3% methanol in chloroform) to give the title compound as a colorless oil (3.6 g, yield 83%). ^{1}H NMR ($CDCl_3$): δ = 7.27–7.36 (m, 5H, H-2′, 3′, 4′), 4.57 (s, 2H, H-6), 4.08–4.18 (m, 5H, H-2, P-O-C**H$_2$**-CH$_3$), 3.99 (m, 1H, H-4a), 3.91 (dd, J_{gem} = 10.5 Hz, J_{1a-2} = 2.7 Hz, 1H, H-1a), 3.85 (m, 1H, H-4b), 3.78 (dd, J_{gem} = 10.5 Hz, J_{1b-2} = 6.6 Hz, 1H, H-1b), 2.25 (m, 1H, H-5a), 2.10 (m, 1H, H-5b), 1.33 (t, $J_{CH3-CH2}$ = 7.0 Hz, 3H, P-O-CH$_2$-C**H$_3$**), 1.29 ppm (t, $J_{CH3-CH2}$ = 7.0 Hz, 3H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ($CDCl_3$): δ = 171.39 (C3), 137.64 (C1′), 128.30 (C3′), 127.65 (C4′), 127.55 (C2′), 79.29 (C2), 73.45 (C6), 70.90 (C1), 65.42 (d, J_{C-C-P} = 6.4 Hz, C4), 62.50 (d, J_{C-O-P} = 6.3 Hz, P-O-**C**H$_2$-CH$_3$), 62.00 (d, J_{C-O-P} = 6.7 Hz, P-O-**C**H$_2$-CH$_3$), 26.51 (d, J_{C-P} = 141.7 Hz, C5), 16.23 ppm (d, $J_{C-C-O-P}$ = 5.9 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 361 (45) $[M+H]^+$, 383 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{26}O_7P$ $[M+H]^+$] 361.1411, found 361.1409; Anal. calcd for $C_{16}H_{26}O_7P$ 0.3H_2O: C 52.54, H 7.06, P 8.01, found: C 52.68, H 7.08, P 7.83; $[\alpha]_D^{20}$ = −23.4 (*c* = 0.295 g/100 mL, $CHCl_3$).

Methyl (*S*)-3-(benzyloxy)-2-(2-(diethoxyphosphoryl)ethoxy)propanoate (11): Compound **10** (3.6 g, 10 mmol) was treated analogously compound **5** to give the title compound as a colorless oil (3.7 g, yield 99%). MS-ESI$^+$ *m/z* (%): 375 (15) $[M+H]^+$, 397 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{17}H_{27}O_7NaP$ $[M+Na]^+$ 397.1387, found 397.1386.

Methyl (*S*)-2-(2-(diethoxyphosphoryl)ethoxy)-3-hydroxypropanoate (12): Compound **11** (3.56 g, 9.5 mmol) was dissolved in the mixture of methanol and acetic acid (3:1, 120 mL) and treated analogously compound **6** (chromatography: gradient from 0–5% methanol in chloroform) to give the title compound as a colorless oil (2.5 g, yield 93%). ^{1}H NMR ($CDCl_3$): δ = 4.04–4.19 (m, 6H, H-2, 4a, P-O-C**H$_2$**-CH$_3$), 3.90 (dd, J_{gem} = 12.0 Hz, J_{1a-2} = 3.2 Hz, 1H, H-1a), 3.69–3.80 (m, 5H, H-1b, 4b, O-C**H$_3$**), 2.05–2.24 (m, 2H, H-5), 1.34 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**), 1.33 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ($CDCl_3$): δ = 170.63 (C3), 80.49 (C2), 64.79 (d, J_{C-C-P} = 5.9 Hz, C4), 62.76 (C1), 62.09 (d, J_{C-O-P} = 6.5 Hz, P-O-**C**H$_2$-CH$_3$), 61.73 (d, J_{C-O-P} = 6.5 Hz, P-O-**C**H$_2$-CH$_3$), 52.08 (O-**C**H$_3$), 26.73 (d, J_{C-P} = 142.1 Hz, C5), 16.35 ppm (d, $J_{C-C-O-P}$ = 6.2 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 285 (5) $[M+H]^+$, 307 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{21}O_7NaP$ $[M+Na]^+$ 307.0917, found 307.0916; Anal. calcd for $C_{10}H_{21}O_7P$ H_2O: C 39.74, H 7.67, P 10.25, found: C 39.91, H 7.69, P 9.98; $[\alpha]_D^{20}$ = −41.7 (*c* = 0.281 g/100 mL, $CHCl_3$).

Diethyl (*R*)-(2-((1-benzyloxy)-3-(trityloxy)propan-2-yl)oxy)ethyl)-phosphonate (8b): Compound **2b** (42.5 g, 100 mmol) was placed into 2 reaction flasks and treated analogously compound **2a** to give the title compound as a yellowish oil (33 g, yield 56% of the crude product, used in the next step without further purification). MS spectrum is identical to that of compound **8a**.

Diethyl (*S*)-(2-((1-(benzyloxy)-3-hydroxypropan-2-yl)oxy)ethyl)-phosphonate (9b): Compound **8b** (33 g) was treated analogously compound **8a** to give the title compound as a colorless oil (13.5 g, 39% overall yield from **2b**). ^{1}H NMR, ^{13}C NMR and MS spectra are identical to those of compound **9a**; $[\alpha]_D^{20}$ = −7.4 (*c* = 0.298 g/100 mL, $CHCl_3$).

Methyl 3-(6-chloropurin-9-yl)-2-((diisopropoxyphosphoryl)methoxy)propanoate (13): From alcohol **7** (1.19 g, 4 mmol) and 6-chloropurine (804 mg, 5.2 mmol), applied procedure E, obtained the title compound as a yellowish oil (835 mg, yield 48%). ^{1}H NMR ($CDCl_3$): δ = 8.75 (s, 1H, H-2), 8.28 (s, 1H, H-8), 4.75 (dd, J_{gem} = 14.2 Hz, $J_{1'a-2'}$ = 3.3 Hz, 1H, H-1′a), 4.62–4.72 (m, 2H, CH-*i*Pr), 4.61 (dd, J_{gem} = 14.2 Hz, $J_{1'b-2'}$ = 6.8 Hz, 1H, H-1′b), 4.57 (dd, $J_{2'-1'b}$ = 6.8 Hz, $J_{2'-1'a}$ = 3.3 Hz, 1H, H-2′), 4.06 (dd, J_{gem} = 14.0 Hz, $J_{4'a-P}$ = 8.8 Hz, 1H, H-4′a), 3.77 (s, 3H, O-CH$_3$), 3.67 (dd, J_{gem} = 14.0 Hz, $J_{4'b-P}$ = 7.9 Hz, 1H, H-4′b), 1.31 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.30 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.26 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.23 ppm (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr); ^{13}C NMR ($CDCl_3$): δ = 168.81 (COOH), 151.97 (C2), 151.82 (C4), 151.06 (C6), 146.34 (C8), 131.26 (C5), 77.50 (d, $J_{C-O-C-P}$ = 9.5 Hz, C2′), 71.46 (d, J_{C-O-P} = 6.8 Hz, CH-*i*Pr), 65.20 (d, J_{C-P} = 166.7 Hz, C4′), 52.73 (O-CH$_3$), 45.31 (C1′), 23.87–24.00 ppm (m, CH$_3$-*i*Pr); MS-ESI$^+$ *m/z* (%): 435 (40) $[M+H]^+$, 457 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{25}O_6N_4ClP$ $[M+H]^+$ 435.1195, found 435.1194; FTIR ($CHCl_3$): $\tilde{\nu}$ = 3126, 3002, 1754, 1594, 1567, 1499, 1438, 1339, 1233, 1145, 998 cm^{-1}; $[\alpha]_D^{20}$ = −7.8 (*c* = 0.275 g/100 mL, DMSO).

Methyl 3-(2-amino-6-chloropurin-9-yl)-2-((diisopropoxyphosphoryl)methoxy) propanoate (14): Compound **7** (1.19 g, 4 mmol) and 2-amino-6-chloropurine (882 mg, 5.2 mmol), applied procedure F to give the title compound as a yellowish oil which solidified (722 mg, yield 40%). ^{1}H NMR ([D$_6$]DMSO): δ = 7.99 (s, 1H, H-8), 6.93 (bs, 2H, NH$_2$), 4.56 (dd, $J_{2'-1'b}$ = 7.9 Hz, $J_{2'-1'a}$ = 3.6 Hz, 1H, H-2′), 4.40–4.52 (m, 3H, H-1′a, CH-*i*Pr), 4.33 (dd, J_{gem} = 14.7 Hz, $J_{1'b-2'}$ = 7.9 Hz, 1H, H-1′b), 3.91 (dd, J_{gem} = 13.8 Hz, $J_{4'a-P}$ = 8.7 Hz, 1H, H-4′a), 3.76 (dd, J_{gem} = 13.8 Hz, $J_{4'b-P}$ = 9.3 Hz, 1H, H-4′b), 3.69 (s, 3H, O-CH$_3$), 1.18 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.15 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.14 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH$_3$-*i*Pr), 1.09 ppm (d,

J_{CH3-CH} = 6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ([D$_6$]DMSO): δ = 169.42 (C3′), 160.04 (C2), 154.32 (C4), 149.49 (C6), 143.77 (C8), 123.15 (C5), 77.56 (d, $J_{C-O-C-P}$ = 12.4 Hz, C2′), 70.57 (m, CH-*i*Pr), 64.21 (d, J_{C-P} = 164.2 Hz, C4′), 52.52 (O-CH_3), 44.29 (C1′), 23.66–23.91 ppm (m, CH_3-*i*Pr); MS-ESI$^+$ *m/z* (%): 450 (40) $[M+H]^+$, 472 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{16}H_{26}O_6N_5ClP$ $[M+H]^+$ 450.1304, found 450.1308; FTIR ($CHCl_3$): $\tilde{\nu}$ = 3532, 3424, 3205, 2987, 2958, 2939, 1753, 1612, 1568, 1438, 1388, 1234, 1180, 1104 cm^{-1}; $[\alpha]_D^{20}$ = −10.5 (*c* = 0.357 g/100 mL, DMSO).

(*S*)-2-((Diisopropoxyphosphoryl)methoxy)-3-(hypoxanthin-9-yl)-propanoic acid (15): Treatment of compound **13** (435 mg, 1 mmol) by procedure C (chromatography: linear gradient of H1 in ethyl acetate) gave the title compound as a yellowish solid (237 mg, yield 59%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.46 (bs, 1H, NH), 8.04 (s, 1H, H-2), 7.99 (s, 1H, H-8), 4.50 (dd, J_{gem} = 14.4 Hz, $J_{1'a-2'}$ = 3.1 Hz, 1H, H-1′a), 4.36–4.50 (m, 2H, CH-*i*Pr), 4.21 (ddm, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 9.2 Hz, 1H, H-1′b), 4.11 (bdd, J_{gem} = 14.4 Hz, $J_{4'a-P}$ = 8.1 Hz, 1H, H-4′a), 3.97 (dm, $J_{2'-1'b}$ = 9.2 Hz, 1H, H-2′), 3.57 (dd, J_{gem} = 13.7 Hz, $J_{4'b-P}$ = 8.8 Hz, 1H, H-4′b), 1.16 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH_3-*i*Pr), 1.11 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH_3-*i*Pr), 1.10 (d, J_{CH3-CH} = 6.2 Hz, 3H, CH_3-*i*Pr), 1.07 ppm (d, J_{CH3-CH} = 6.2 Hz, 3H, CH_3-*i*Pr); ^{13}C NMR ([D$_6$]DMSO): δ = 170.77 (C3′), 157.06 (C6), 148.73 (C4), 145.44 (C2), 140.72 (C8), 123.77 (C5), 81.15 (d, $J_{C-O-C-P}$ = 12.5 Hz, C2′), 70.26 (d, J_{C-O-P} = 6.3 Hz, CH-*i*Pr), 70.16 (d, J_{C-O-P} = 6.3 Hz, CH-*i*Pr), 63.23 (d, J_{C-P} = 163.6 Hz, C4′), 46.18 (C1′), 23.65–23.94 ppm (m, CH_3-*i*Pr); MS-ESI$^-$ *m/z* (%): 401 (100) $[M-H]^+$; HRMS-ESI$^-$: *m/z* calcd for $C_{15}H_{22}O_7N_4P$ $[M-H]^+$ 401.1232, found 401.1231; FTIR (KBr): $\tilde{\nu}$ = 3423, 2981, 2936, 1699, 1611, 1550, 1465, 1386, 1376, 1243, 1126, 993 cm^{-1}; $[\alpha]_D^{20}$ = −14.9 (*c* = 0.356 g/100 mL, MeOH).

(*S*)-3-(Guanin-9-yl)-2-((diisopropoxyphosphoryl)methoxy) propanoic acid (16):[16] Treatment of compound **14** (450 mg, 1 mmol) by procedure C (chromatography: linear gradient of H1 in ethyl acetate) gave compound **16** as a yellowish solid (234 mg, yield 56%). ^{1}H NMR, ^{13}C NMR and MS spectra are identical with the original spectroscopic data.[16]

(*S*)-3-(Hypoxanthin-9-yl)-2-(phosphonomethoxy)propanoic acid (17): Treatment of compound **15** (201 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a white solid (110 mg, 69%). ^{1}H NMR (D_2O): δ = 8.17 (s, 2H, H-2, 8), 4.60 (dd, J_{gem} = 14.8 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.54 (dd, J_{gem} = 14.8 Hz, $J_{1'b-2'}$ = 5.6 Hz, 1H, H-1′b), 4.21 (dd, $J_{2'-1'b}$ = 5.6 Hz, $J_{2'-1'a}$ = 4.1 Hz, 1H, H-2′), 3.72 (dd, J_{gem} = 12.9 Hz, $J_{4'a-P}$ = 8.8 Hz, 1H, H-4′a), 3.48 ppm (dd, J_{gem} = 12.9 Hz, $J_{4'b-P}$ = 9.9 Hz, 1H, H-4′b); ^{13}C NMR (D_2O): δ = 177.04 (C3′), 159.31 (C6), 149.78 (C4), 146.15 (C2), 143.70, (C8), 123.47 (C5), 81.63 (d, $J_{C-O-C-P}$ = 12.4 Hz, C2′), 67.06 (d, J_{C-P} = 155.2 Hz, C4′), 46.43 ppm (C1′);MS-ESI$^-$ *m/z* (%): 317 (100) $[M-H]^+$, 339 (25) $[M+Na]^+$, 355 (15) $[M+2Na]^+$; HRMS-ESI$^-$: *m/z* calcd for $C_9H_{10}O_7N_4P$ $[M-H]^+$ 317.0293, found 317.0293; FTIR (KBr): $\tilde{\nu}$ = 1700, 1586, 1552, 1480, 1414, 1349, 1289, 1152, 1059, 968, 906, 788 cm^{-1}; $[\alpha]_D^{20}$ = −14.9 (*c* = 0.470 g/100 mL, H_2O).

(*S*)-3-(Guanin-9-yl)-2-(phosphonomethoxy)propanoic acid (18): Treatment of compound **16** (209 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a white solid (112 mg, yield 67%). ^{1}H NMR (D_2O): δ = 7.84 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.7 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.32 (dd, J_{gem} = 14.7 Hz, $J_{1'b-2'}$ = 6.1 Hz, 1H, H-1′b), 4.17 (dd, $J_{2'-1'b}$ = 6.1 Hz, $J_{2'-1'a}$ = 4.1 Hz, 1H, H-2′), 3.73 (dd, J_{gem} = 13.0 Hz, $J_{4'a-P}$ = 8.7 Hz, 1H, H-4′a), 3.51 ppm (dd, J_{gem} = 13.0 Hz, $J_{4'b-P}$ = 9.8 Hz, 1H, H-4′b); ^{13}C NMR (D_2O): δ = 177.21 (C3′), 159.57 (C6), 154.24 (C2), 152.37 (C4), 141.38 (C8), 116.08 (C5), 81.73 (d, $J_{C-O-C-P}$ = 12.5 Hz, C2′), 66.85 (d, J_{C-P} = 156.1 Hz, C4′), 45.82 ppm (C1′); MS-ESI$^-$ *m/z* (%): 332 (100) $[M-H]^+$, 354 (60) $[M+Na]^+$, 376 (15) $[M+2Na]^+$; HRMS-ESI$^-$: *m/z* calcd for $C_9H_{11}O_7N_5P$ $[M-H]^+$ 332.0401, found 332.0404; FTIR (KBr): $\tilde{\nu}$ = 1693, 1607, 1540 1481, 1409, 1376, 1176, 1108, 1069, 914 cm^{-1}; $[\alpha]_D^{20}$ = −20.2 (*c* = 0.316 g/100 mL, H_2O).

Diethyl (*S*)-(2-((1-(benzyloxy)-3-(6-chloropurin-9-yl)propan-2-yl)oxy)ethyl)phosphonate (19): From alcohol **9b** (3.46 g, 10 mmol) and 6-chloropurine (2.0 g, 13 mmol), applied procedure E, obtained the title compound as a yellowish oil (3.85 g, yield 80%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.76 (s, 1H, H-2), 8.66 (s, 1H, H-8), 7.26–7.35 (m, 5H, H-2″, 3″, 4″), 4.38–4.52 (m, 4H, H-1′, 4′), 3.99 (m, 1H, H-2′), 3.87–3.93 (m, 4H, P-O-C**H$_2$**-CH_3), 3.68 (m, 1H, H-5′a), 3.53 (m, 1H, H-5′b), 3.50 (d, $J_{3'-2'}$ = 5.0 Hz, 2H, H-3′), 1.89 (dt, $J_{6'-5'}$ = 7.4 Hz, $J_{6'-P}$ = 18.3 Hz, 2H, H-6′), 1.15 ppm (t, $J_{CH3-CH2}$ = 7.0 Hz, 6H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 152.42 (C4), 151.68 (C2), 149.12 (C6), 148.33 (C8), 138.22 (C1″), 130.78 (C5), 128.42 (C3″), 127.72 (C2″), 127.71 (C4″), 76.12 (C2′), 72.63 (C4′), 69.46 (C3′), 63.99 (C5′), 61.15 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H_2-CH_3), 44.96 (C1′), 26.30 (d, J_{C-P} = 136.9 Hz, C6′), 16.37 ppm (d, $J_{C-C-O-P}$ = 5.9 Hz, P-O-CH_2-**C**H_3); MS-ESI$^+$ *m/z* (%): 483 (100) $[M+H]^+$, 505 (65) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_5N_4ClP$ $[M+H]^+$ 483.1559, found 483.1558; $[\alpha]_D^{20}$ = −11.7 (*c* = 0.403 g/100 mL, DMSO).

Diethyl (*S*)-(2-((1-(2-amino-6-chloropurin-9-yl)-3-(benzyloxy)propan-2-yl)oxy)ethyl) phosphonate (20): Compound **9b** (1.73 g, 5 mmol) and 2-amino-6-chloropurine (1.1 g, 6.5 mmol), applied procedure F to give the title compound as a yellowish oil (1.4 g, yield 55%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.07 (s, 1H, H-8), 7.27–7.36 (m, 5H, H-2″, 3″, 4″), 6.91 (bs, 2H, NH_2), 4.49 (s, 2H, H-4′), 4.22 (dd, J_{gem} = 14.5 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.12 (dd, J_{gem} = 14.5 Hz, $J_{1'b-2'}$ = 7.5 Hz, 1H, H-1′b), 3.87–3.94 (m, 5H, H-2′, P-O-C**H$_2$**-CH_3), 3.66 (m, 1H, H-5′a), 3.45–3.52 (m, 3H, H-3′, 5′b), 1.84–1.91 (m, 2H, H-6′), 1.20 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-C**H$_3$**), 1.16 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 159.99 (C2), 154.53 (C4), 149.48 (C6), 144.01 (C8), 138.24 (C1″), 128.47 (C3″), 127.77 (C2″), 127.59 (C4″), 123.28 (C5), 76.18 (C2′), 72.67 (C4′), 69.66 (C3′), 64.00 (C5′), 61.19 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H_2-CH_3), 44.36 (C1′), 26.37 (d, J_{C-P} = 136.2 Hz, C6′), 16.39 ppm (d, $J_{C-C-O-P}$ = 5.9 Hz, P-O-CH_2-**C**H_3); MS-ESI$^+$ *m/z* (%): 498 (75) $[M+H]^+$, 520 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_5N_5ClNaP$ $[M+Na]^+$ 520.1487, found 520.1487; Anal. calcd for $C_{21}H_{29}O_5N_5ClP$: C 50.66, H 5.87, N 14.07, P 6.22, Cl 7.12, found: C 50.48, H 5.99, N 14.10, P 5.97, Cl 7.03; $[\alpha]_D^{20}$ = −19.0 (*c* = 0.415 g/100 mL, DMSO).

Diethyl (*S*)-(2-((1-benzyloxy)-3-(hypoxanthin-9-yl)propan-2-yl)-oxy)ethyl)phosphonate (21): Treatment of compound **19** (966 mg, 2 mmol) by procedure C (chromatography: gradient from 1-5% methanol in chloroform) gave the title compound as a yellowish oil (761 mg, yield 82%). ^{1}H NMR ([D$_6$]DMSO): δ = 12.29 (s, 1H, NH), 8.04 (s, 2H, H-2, 8), 7.27–7.36 (m, 5H, H-2″, 3″, 4″), 4.49 (s, 2H, H-4′), 4.32 (dd, J_{gem} = 14.3 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.21 (dd, J_{gem} = 14.3 Hz, $J_{1'b-2'}$ = 7.3 Hz, 1H, H-1′b), 3.88–3.95 (m, 5H, H-2′, P-O-C**H$_2$**-CH_3), 3.65 (m, 1H, H-5′a), 3.43–3.56 (m, 3H, H-3′, 5′b), 1.88 (dt, $J_{6'-P}$ = 18.3 Hz, 2H, H-6′), 1.16 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 156.88 (C6), 148.78 (C4), 145.68 (C2), 141.15 (C8), 138.30 (C1″), 128.46 (C3″), 127.75 (C2″), 127.72 (C4″), 123.86 (C5), 76.61 (C2′), 72.63 (C4′), 69.62 (C3′), 64.07 (C5′), 61.18 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H_2-CH_3), 61.17 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H_2-CH_3), 44.54 (C1′), 26.35 (d, J_{C-P} = 136.5 Hz, C6′), 16.39 ppm (d, $J_{C-C-O-P}$ = 5.9 Hz, P-O-CH_2-**C**H_3); MS-ESI$^+$ *m/z* (%): 465 (30) $[M+H]^+$, 487 (100) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{29}O_6N_4NaP$ $[M+Na]^+$ 487.1717, found 487.1716; Anal. calcd for $C_{21}H_{29}O_6N_4P$: C 54.31, H 6.29, N 12.06, P 6.67, found: C 54.11, H 6.35, N 11.89, P 6.51; $[\alpha]_D^{20}$ = −10.3 (*c* = 0.455 g/100 mL, DMSO).

Diethyl (*S*)-(2-((guanin-9-yl)-3-(benzyloxy)propan-2-yl)oxy)ethyl)-phosphonate (22): Treatment of compound **20** (996 mg, 2 mmol) by procedure C (chromatography: gradient from 1-7% methanol in chloroform) gave the title compound as a yellowish solid (631 mg, yield 66%). ^{1}H NMR ([D$_6$]DMSO): δ = 10.57 (s, 1H, NH), 7.63 (s, 1H, H-8), 7.27–7.37 (m, 5H, H-2″, 3″, 4″), 6.44 (s, 2H, NH$_2$), 4.49 (s, 2H, H-4′), 3,97–4.12 (m, 2H, H-1′), 3.84–3.95 (m, 5H, H-2′, P-O-C**H$_2$**-CH$_3$), 3.64 (m, 1H, H-5′a), 3.38–3.52 (m, 3H, H-3′, 5′b), 1.88 (dt, $J_{6'-5'}$ = 7.5 Hz, $J_{6'-P}$ = 18.3 Hz, 2H, H-6′), 1.17 ppm (t, $J_{CH3-CH2}$ = 7.0 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 157.03 (C6), 153.72 (C2), 151.56 (C4), 138.30 (C1″), 138.27 (C8), 128.49 (C3″), 127.80 (C2″), 127.74 (C4″), 116.49 (C5), 76.56 (C2′), 72.66 (C4′), 69.83 (C3′), 64.05 (C5′), 61.21 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H$_2$-CH$_3$), 61.20 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H$_2$-CH$_3$), 44.09 (C1′), 26.36 (d, J_{C-P} = 136.2 Hz, C6′), 16.40 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 480 (55) [*M*+H]$^+$, 502 (100) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{21}$H$_{31}$O$_6$N$_5$P [*M*+H]$^+$ 480.2007, found 480.2006; Anal. calcd for C$_{21}$H$_{31}$O$_6$N$_5$P: C 52.61, H 6.31, N 14.61, P 6.46, found: C 52.37, H 6.32, N 14.47, P 6.37; $[\alpha]_D^{20}$ = −10.5 (*c* = 0.342 g/100 mL, DMSO).

Diethyl (*S*)-(2-((1-hydroxy-3-(hypoxanthin-9-yl)propan-2-yl)oxy)-ethyl)phosphonate (23): Compound **21** (720 mg, 1.55 mmol) was treated with procedure G to give the title compound as a yellowish oil which solidified (465 mg, yield 80%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.04 (m, 2H, H-2, 8), 4.90 (bs, 1H, OH), 4.30 (dd, J_{gem} = 14.3 Hz, $J_{1'a-2'}$ = 3.9 Hz, 1H, H-1′a), 4.14 (dd, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 7.6 Hz, 1H, H-1′b), 3.88–3.96 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.58–3.71 (m, 2H, H-2′, 4′a), 3.39–3.51 (m, 3H, H-3′, 4′b), 1.85–1.92 (m, 2H, H-5′), 1.18 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 156.90 (C6), 148.77 (C4), 145.68 (C2), 141.19 (C8), 123.87 (C5), 78.64 (C2′), 63.86 (C4′), 61.22 (m, P-O-**C**H$_2$-CH$_3$), 60.91 (C3′), 44.48 (C1′), 26.34 (d, J_{C-P} = 136.7 Hz, C5′), 16.41 ppm (d, $J_{C-C-O-P}$ = 6.0 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 375 (100) [*M*+H]$^+$, 397 (50) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{14}$H$_{24}$O$_6$N$_4$P [*M*+H]$^+$ 375.1428, found 375.1427; FTIR (KBr): $\tilde{\nu}$ = 3052, 1696, 1593, 1551, 1414, 1341, 1221, 1118, 1100, 1054, 1042, 1028, 961, 789 cm^{-1}; Anal. calcd for C$_{14}$H$_{24}$O$_6$N$_4$P: C 44.92, H 6.19, N 14.97, P 8.27, found: C 45.13, H 6.33, N 14.75, P 8.13; $[\alpha]_D^{20}$ = −12.5 (*c* = 0.208 g/100 mL, MeOH).

Diethyl (*S*)-(2-((guanin-9-yl)-3-hydroxypropan-2-yl)oxy)ethyl)-phosphonate (24): Compound **22** (580 mg, 1.21 mmol) was treated with procedure G to give the title compound as a white solid (367 mg, yield 78%). ^{1}H NMR ([D$_6$]DMSO): δ = 7.62 (s, 1H, H-8), 4.10 (dd, J_{gem} = 14.3 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 3.91–3.99 (m, 5H, H-1′b, P-O-C**H$_2$**-CH$_3$), 3.61–3.68 (m, 2H, H-2′, 4′a), 3.53 (m, 1H, H-4′b), 3.38–3.41 (m, 2H, H-3′), 1.86–1.95 (m, 2H, H-5′), 1.21 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**); 1.20 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 156.82 (C6), 153.58 (C2), 151.38 (C4), 138.10 (C8), 116.40 (C5), 78.45 (C2′), 63.64 (C4′), 61.07 (m, P-O-**C**H$_2$-CH$_3$), 60.87 (C3′), 43.70 (C1′), 26.36 (d, J_{C-P} = 136.7 Hz, C5′), 16.20 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 390 (100) [*M*+H]$^+$, 412 (15) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{14}$H$_{25}$O$_6$N$_5$P [*M*+H]$^+$ 390.1537, found 390.1536; FTIR (KBr): $\tilde{\nu}$ = 3423, 3123, 2982, 1691, 1657, 1609, 1583, 1540, 1481, 1412, 1229, 1167, 1117, 1051, 1027, 961, 782 cm^{-1}; Anal. calcd for C$_{14}$H$_{25}$O$_6$N$_5$P: C 43.19, H 6.21, N 17.99, P 7.96, found: C 42.95, H 6.25, N 17.91, P 7.83; $[\alpha]_D^{20}$ = −22.6 (*c* = 0.261 g/100 mL, MeOH).

(*S*)-(2-((1-Hydroxy-3-(hypoxanthin-9-yl)propan-2-yl)oxy)ethyl)-phosphonic acid (25): Treatment of compound **23** (187 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a white solid (116 mg, yield 73%). ^{1}H NMR (D$_2$O): δ = 8.12 (s, 1H, H-2), 8.10 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.7 Hz, $J_{1'a-2'}$ = 3.8 Hz, 1H, H-1′a), 4.26 (dd, J_{gem} = 14.8 Hz, $J_{1'b-2'}$ = 7.7 Hz, 1H, H-1′b), 3.81 (m, 1H, H-2′), 3.63–3.70 (m, 2H, H-3′a, 4′a), 3.47–3.54 (m, 2H, H-3′b, 4′b), 1.60–1.76 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O): δ = 158.46 (C6), 149.02 (C4), 145.79 (C2), 142.75 (C8), 122.92 (C5), 78.08 (C2′), 65.90 (C4′), 60.52 (C3′), 44.68 (C1′), 28.86 ppm (d, J_{C-P} = 129.9 Hz, C5′); MS-ESI$^+$ *m/z* (%): 319 (100) [*M*+H]$^+$, 341 (80) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{10}$H$_{16}$O$_6$N$_4$P [*M*+H]$^+$ 319.0802, found 319.0802; FTIR (KBr): $\tilde{\nu}$ = 3050, 1689, 1592, 1552, 1521, 1414, 1343, 1211, 1097, 1055, 1041, 909, 789 cm^{-1}; $[\alpha]_D^{20}$ = −5.3 (*c* = 0.338 g/100 mL, H$_2$O).

(*S*)-(2-((Guanin-9-yl)-3-hydroxypropan-2-yl)oxy)ethyl) phosphonic acid (26): Treatment of compound **24** (195 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a white solid (127 mg, yield 76%). ^{1}H NMR (D$_2$O): δ = 7.74 (s, 1H, H-8), 4.19 (dd, J_{gem} = 14.8 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.04 (dd, J_{gem} = 14.8 Hz, $J_{1'b-2'}$ = 7.1 Hz, 1H, H-1′b), 3.75 (m, 1H, H-2), 3.52–3.69 (m, 3H, H-3′a, 4′), 3.45 (dd, J_{gem} = 12.4 Hz, $J_{3'b-2'}$ = 5.5 Hz, 1H, H-3′b), 1.62–1.77 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O): δ = 158.54 (C6), 153.46 (C2), 151.47 (C4), 140.24 (C8), 115.16 (C5), 65.61 (d, J_{C-C-P} = 1.8 Hz, C4′), 60.22 (C3′), 43.46 (C1′), 28.61 ppm (d, J_{C-P} = 130.0 Hz, C5′); MS-ESI$^+$ *m/z* (%): 334 (100) [*M*+H]$^+$, 356 (20) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{10}$H$_{17}$O$_6$N$_5$P [*M*+H]$^+$ 334.0911, found 334.0910; FTIR (KBr): $\tilde{\nu}$ = 3379, 3320, 3222, 1688, 1655, 1611, 1580, 1539, 1480, 1411, 1097, 1049, 909, 781 cm^{-1}; $[\alpha]_D^{20}$ = −15.4 (*c* = 0.240 g/100 mL, H$_2$O).

(*S*)-(2-((1-(Xanthin-9-yl)-3-hydroxypropan-2-yl)oxy)ethyl)phosphonic acid (27): Compound **26** (250 mg, 0.75 mmol) was dissolved in 80% acetic acid (50 mL) and *iso*-amyl nitrite (1.5 mL) was added. The reaction mixture was stirred at RT overnight. Volatiles were evaporated and a residue was co-distilled with water (3× 10 mL) and toluene (3×20 mL). The crude product was purified by procedure A to give the title compound as a white solid (87 mg, yield 35%). ^{1}H NMR (D$_2$O + NaOD): δ = 7.73 (s, 1H, H-8), 4.23 (dd, J_{gem} = 14.8 Hz, $J_{1'b-2'}$ = 4.3 Hz, 1H, H-1′b), 4.10 (dd, J_{gem} = 14.8 Hz, $J_{1'a-2'}$ = 6.9 Hz, 1H, H-1′a), 3.85 (ddt, $J_{2'-1'a}$ = 6.9 Hz, $J_{2'-3'a}$ = 5.2 Hz, $J_{2'-1'b}$ = $J_{2'-3'b}$ = 4.4 Hz, 1H, H-2′), 3.78 (m, 1H, H-4′b), 3.69 (m, 1H, H-4′a), 3.59 (dd, J_{gem} = 12.4 Hz, $J_{3'b-2'}$ = 4.5 Hz, 1H, H-3′b), 3.52 (dd, J_{gem} = 12.4 Hz, $J_{3'a-2'}$ = 5.3 Hz, 1H, H-3′a), 1.68–1.81 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O + NaOD): δ = 161.65 (C6), 160.15 (C2), 154.03 (C4), 140.63 (C8), 115.07 (C5), 78.40 (C2′), 67.49 (C4′), 60.99 (C3′), 43.98 (C1′), 30.46 ppm (d, J_{C-P} = 126.2 Hz, C5′); MS-ESI$^-$ *m/z* (%): 333 (100) [*M*−H]$^+$, 355 (75) [*M*+Na]$^+$; HRMS-ESI$^-$: *m/z* calcd for C$_{10}$H$_{14}$O$_7$N$_4$P [*M*−H]$^+$ 333.0606, found 333.0607; FTIR (KBr): $\tilde{\nu}$ = 3500, 3193, 3124, 2294, 1720, 1695, 1612, 1580, 1393, 1149, 1100, 1051, 1010, 747 cm^{-1}; $[\alpha]_D^{20}$ = −4.7 (*c* = 0.363 g/100 mL, H$_2$O).

Methyl (*S*)-3-(6-chloropurin-9-yl)-2-(2-(diethoxyphosphoryl)-ethoxy)propanoate (28): From alcohol **12** (1.71 g, 6 mmol) and 6-chloropurine (1.21 g, 7.8 mmol), applied procedure E, obtained the title compound as a yellow oil (1.1 g, yield 44%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.75 (s, 1H, H-2), 8.32 (s, 1H, H-8), 4.72 (dd, J_{gem} = 14.5 Hz, $J_{1'a-2'}$ = 3.9 Hz, 1H, H-1′a), 4.58 (dd, J_{gem} = 14.6 Hz, $J_{1'b-2'}$ = 6.7 Hz, 1H, H-1′b), 4.31 (dd, $J_{2'-1'a}$ = 3.8 Hz, $J_{2'-1'b}$ = 6.8 Hz, 1H, H-2′), 4.03–4.14 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.96 (m, 1H, H-4′a), 3.75 (s, 3H, O-CH$_3$), 3.62 (m, 1H, H-4′b), 2.00–2.14 (m, 2H, H-5′), 1.31 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 169.32 (C3′), 151.98 (C2), 151.71 (C4), 151.02 (C6), 146.53 (C8), 131.13 (C5), 76.55 (C2′), 65.43 (C4′), 61.87 (m, P-O-**C**H$_2$-CH$_3$), 52.67 (O-CH$_3$), 45.43 (C1′), 26.87 (d, J_{C-P} = 140.8 Hz, C5′), 16.37 ppm (d, $J_{C-C-O-P}$ = 6.0 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 421 (35) [*M*+H]$^+$, 443 (100) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{15}$H$_{23}$O$_6$N$_4$ClP [*M*+H]$^+$ 421.1038, found 421.1037; Anal. calcd for C$_{15}$H$_{22}$ClN$_4$O$_6$P: C 42.82, H 5.27, Cl 8.43, N 13.31, P 7.36, found: C 42.65, H 5.37, Cl 8.25, N 13.03, P 7.15; $[\alpha]_D^{20}$ = −5.3 (*c* = 0.316 g/100 mL, DMSO).

Methyl (*S*)-3-(2-amino-6-chloropurin-9-yl)-2-(2-(diethoxyphosphoryl)ethoxy)propanoate (29): Compound **12** (1.99 g, 7 mmol) and 2-amino-6-chloropurine (1.54 g, 9.1 mmol), applied procedure F to give the title compound as a yellowish oil (1.16 g, yield 38%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.05 (s, 1H, H-8), 6.93 (s, 2H, NH$_2$), 4.46 (dd, $J_{2'-1'a}$ = 4.1 Hz, $J_{2'-1'b}$ = 7.1 Hz, 1H, H-2′), 4.41 (dd, J_{gem} = 14.5 Hz, $J_{1'a-2'}$ = 4.0 Hz, 1H, H-1′a), 4.31 (dd, J_{gem} = 14.5 Hz, $J_{1'b-2'}$ = 7.1 Hz, 1H, H-1′b), 3.90–3.99 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.72 (m, 1H, H-4′a), 3.66 (s, 3H, O-CH$_3$), 3.54 (m, 1H, H-4′b), 1.91–2.05 (m, 2H, H-5′), 1.19 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 170.01 (C3′), 160.02 (C2), 154.35 (C4), 149.48 (C6), 144.00 (C8), 123.08 (C5), 76.03 (C2′), 64.50 (C4′), 61.29 (d, J_{C-O-P} = 6.1 Hz, P-O-**C**H$_2$-CH$_3$), 52.40 (O-CH$_3$), 44.46 (C1′), 26.11 (d, J_{C-P} = 137.3 Hz, C5′), 16.41 ppm (d, $J_{C-C-O-P}$ = 5.7 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 436 (40) [*M*+H]$^+$, 458 (100) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{15}$H$_{24}$O$_6$N$_5$ClP [*M*+H]$^+$ 436.1147, found 436.1146; $[\alpha]_D^{20}$ = −7.4 (*c* = 0.398 g/100 mL, DMSO).

(*S*)-2-(2-(Diethoxyphosphoryl)ethoxy)-3-(hypoxanthin-9-yl)propanoic acid (30): Treatment of compound **28** (421 mg, 1 mmol) by procedure C (chromatography: linear gradient of H1 in ethyl acetate) gave the title compound as a yellowish solid (221 mg, yield 57%). ^{1}H NMR ([D$_6$]DMSO): δ = 12.48 (bs, 1H, NH), 8.022 and 8.017 (2 s, 2×1H, H-2, 8), 4.47 (dd, J_{gem} = 14.2 Hz, $J_{1'a-2'}$ = 3.5 Hz, 1H, H-1′a), 4.21 (dd, J_{gem} = 14.2 Hz, $J_{1'b-2'}$ = 8.5 Hz, 1H, H-1′b), 3.88–3.97 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.86 (dd, $J_{2'-1'a}$ = 3.5 Hz, $J_{2'-1'b}$ = 8.4 Hz, 1H, H-2′), 3.80 (m, 1H, H-4′a), 3.39 (m, 1H, H-4′b), 1.86–1.97 (m, 2H, H-5′), 1.17 ppm (t, J_{CH3CH2} = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 172.00 (C3′), 157.01 (C6), 148.73 (C4), 145.53 (C2), 141.06 (C8), 123.70 (C5), 79.92 (C2′), 63.17 (C4′), 61.23 (d, J_{C-O-P} = 6.2 Hz, P-O-**C**H$_2$-CH$_3$), 61.22 (d, J_{C-O-P} = 6.2 Hz, P-O-**C**H$_2$-CH$_3$), 46.07 (C1′), 26.19 (d, J_{C-P} = 136.8 Hz, C5′), 16.39 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^-$ *m/z* (%): 387 (100) [*M*−H]$^+$; HRMS-ESI$^-$: *m/z* calcd for C$_{14}$H$_{20}$O$_7$N$_4$P [*M*−H]$^+$ 387.1075, found 387.1074; FTIR (KBr): $\tilde{\nu}$ = 2982, 1698, 1608, 1549, 1522, 1390, 1350, 1229, 1124, 1052, 1028, 963 cm^{-1}; $[\alpha]_D^{20}$ = −14.0 (*c* = 0.377 g/100 mL, MeOH).

(*S*)-3-(Guanin-9-yl)-2-(2-(diethoxyphosphoryl)ethoxy)propanoic acid (31): Treatment of compound **29** (436 mg, 1 mmol) by procedure C (chromatography: linear gradient of H1 in ethyl acetate) gave the title compound as a yellowish solid (222 mg, yield 55%). An alternative procedure: The compound **29** (436 mg, 1 mmol) was dissolved in 15 mL of 75% CF$_3$COOH and the solution was heated at 50 °C overnight. Volatiles were evaporated and a residue was purified by silica gel chromatography (gradient from 10–50% methanol in chloroform) to afford (after evaporation) the title compound as a yellowish solid (314 mg, yield 78%). ^{1}H NMR ([D$_6$]DMSO): δ = 11.49 (bs, 1H, NH), 7.60 (s, 1H, H-8), 6.98 (bs, 2H, NH$_2$), 4.32 (dd, J_{gem} = 14.1 Hz, $J_{1'a-2'}$ = 3.2 Hz, 1H, H-1′a), 3.87–3.99 (m, 5H, H-1′b, P-O-C**H$_2$**-CH$_3$), 3.75–3.84 (m, 2H, H-2′, 4′a), 3.29–3.42 (m, 1H, H-4′b), 1.88–1.96 (m, 2H, H-5′), 1.18 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 172.66 (C3′), 157.46 (C6), 154.18 (C2), 151.50 (C4), 138.21 (C8), 116.26 (C5), 80.02 (C2′), 63.26 (C4′), 61.24 (d, J_{C-O-P} = 6.2 Hz, P-O-**C**H$_2$-CH$_3$), 45.57 (C1′), 26.74 (d, J_{C-P} = 136.5 Hz, C5′), 16.41 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 404 (25) [*M*+H]$^+$, 426 (100) [*M*+Na]$^+$, 448 (85) [*M*+2Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{14}$H$_{23}$O$_7$N$_5$P [*M*+H]$^+$ 404.1330, found 404.1329; FTIR (KBr): $\tilde{\nu}$ = 3413, 3319, 3128, 2983, 2930, 2775, 1740, 1693, 1621, 1538, 1481, 1369, 1101 cm^{-1}; $[\alpha]_D^{20}$ = −49.8 (*c* = 0.283 g/100 mL, DMSO).

(*S*)-3-(6-oxo-1,6-dihydro-9H-purin-9-yl)-2-(2-phosphonoethoxy)-propanoic acid (32): Treatment of compound **30** (194 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a yellowish solid (125 mg, yield 75%). ^{1}H NMR (D$_2$O): δ = 8.02 (s, 1H, H-2), 8.01 (s, 1H, H-8), 4.40 (dd, J_{gem} = 14.4 Hz, $J_{1'a-2'}$ = 3.2 Hz, 1H, H-1′a), 4.19 (dd, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 8.4 Hz, 1H, H-1′b), 3.83 (dd, $J_{2'-1'a}$ = 3.2 Hz, $J_{2'-1'b}$ = 8.4 Hz, 1H, H-2′), 3.56 (m, 1H, H-4′a), 3.31 (m, 1H, H-4′b), 1.45–1.58 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O): δ = 175.67 (C3′), 158.67 (C6), 150.00 (C4), 146.78 (C2), 143.17 (C8), 124.16 (C5), 80.58 (C2′), 67.39 (C4′), 47.30 (C1′), 31.2 and 30.2 ppm (C5′); MS-ESI$^-$ *m/z* (%): 331 (100) [*M*−H]$^+$, 353 (30) [*M*+Na]$^+$; HRMS-ESI$^-$: *m/z* calcd for C$_{10}$H$_{12}$O$_7$N$_4$P [*M*−H]$^+$ 331.0449, found 331.0450; FTIR (KBr): $\tilde{\nu}$ = 3058, 1692, 1614, 1590, 1551, 1520, 1415, 1349, 1291, 1112, 1063, 900 cm^{-1}; $[\alpha]_D^{20}$ = −5.0 (*c* = 0.301 g/100 mL, H$_2$O).

(*S*)-3-(Guanin-9-yl)-2-(2-phosphonoethoxy)propanoic acid (33): Treatment of compound **31** (202 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a yellowish solid (127 mg, yield 73%). ^{1}H NMR (D$_2$O): δ = 7.69 (s, 1H, H-8), 4.32 (dd, J_{gem} = 14.7 Hz, $J_{1'a-2'}$ = 3.9 Hz, 1H, H-1′a), 4.16 (dd, J_{gem} = 14.7 Hz, $J_{1'b-2'}$ = 7.1 Hz, 1H, H-1′b), 4.03 (dd, $J_{2'-1'a}$ = 3.9 Hz, $J_{2'-1'b}$ = 7.0 Hz, 1H, H-2′), 3.65 (m, 1H, H-4′a), 3.50 (m, 1H, H-4′b), 1.68–1.81 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O): δ = 176.79 (C3′), 158.87 (C6), 153.54 (C2), 151.72 (C4), 140.44 (C8), 115.47 (C5), 78.98 (C2′), 65.86 (C4′), 43.25 (C1′), 28.54 ppm (d, J_{C-P} = 129.5 Hz, C5′); MS-ESI$^-$ *m/z* (%): 346 (100) [*M*−H]$^+$, 368 (35) [*M*+Na]$^+$; HRMS-ESI$^-$: *m/z* calcd for C$_{10}$H$_{13}$O$_7$N$_5$P [*M*−H]$^+$ 346.0558, found 346.0556; FTIR (KBr): $\tilde{\nu}$ = 3388, 3142, 1696, 1601, 1540, 1478, 1410, 1299, 1107, 1063, 906, 781 cm^{-1}; $[\alpha]_D^{20}$ = −23.7 (*c* = 0.207 g/100 mL, H$_2$O).

Methyl (*S*)-2-(2-(diethoxyphosphoryl)ethoxy)-3-(hypoxanthin-9-yl)propanoate (34): Treatment of compound **28** (421 mg, 1 mmol) by procedure D gave the title compound as a colorless oil which solidified (338 mg, yield 84%). ^{1}H NMR ([D$_6$]DMSO): δ = 8.04 (s, 1H, H-2), 8.01 (s, 1H, H-8), 4.38–4.52 (m, 3H, H-1′, 2′), 3.90–3.98 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.72 (m, 1H, H-4′a), 3.64 (s, 3H, O-CH$_3$), 3.53 (m, 1H, H-4′b), 1.91–2.05 (m, 2H, H-5′), 1.19 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 169.95 (C3′), 156.83 (C6), 148.64 (C4), 145.81 (C2), 141.16 (C8), 123.72 (C5), 76.38 (C2′), 64.52 (C4′), 61.29 (d, J_{C-O-P} = 6.2 Hz, P-O-**C**H$_2$-CH$_3$), 52.35 (O-CH$_3$), 44.78 (C1′), 26.08 (d, J_{C-P} = 139.8 Hz, C5′), 16.41 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 403 (30) [*M*+H]$^+$, 425 (100) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{15}$H$_{24}$O$_7$N$_4$P [*M*+H]$^+$ 403.1377, found 403.1376; FTIR (KBr): $\tilde{\nu}$ = 3056, 2980, 2955, 1752, 1695, 1587, 1550, 1441, 1415, 1391, 1220, 1121, 1090, 1045, 1029, 964 cm^{-1}; $[\alpha]_D^{20}$ = −8.0 (*c* = 0.263 g/100 mL, MeOH).

Methyl (*S*)-3-(guanin-9-yl)-2-(2-(diethoxyphosphoryl)ethoxy)propanoate (35): Treatment of compound **29** (436 mg, 1 mmol) by procedure D gave the title compound as a white solid (338 mg, yield 81%). ^{1}H NMR ([D$_6$]DMSO): δ = 10.69 (bs, 1H, NH), 7.60 (s, 1H, H-8), 6.57 (bs, 2H, NH$_2$), 4.40 (dd, $J_{2'-1'a}$ = 4.1 Hz, $J_{2'-1'b}$ = 7.3 Hz, 1H, H-2′), 4.28 (dd, J_{gem} = 14.4 Hz, $J_{1'a-2'}$ = 4.1 Hz, 1H, H-1′a), 4.18 (dd, J_{gem} = 14.4 Hz, $J_{1'b-2'}$ = 7.3 Hz, 1H, H-1′b), 3.89–4.00 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.71 (m, 1H, H-4′a), 3.66 (s, 3H, O-CH$_3$), 3.51 (m, 1H, H-4′b), 1.92–2.02 (m, 2H, H-5′), 1.19 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 170.09 (C3′), 156.99 (C6), 153.90 (C2), 151.46 (C4), 138.26 (C8), 116.28 (C5), 76.43 (C2′), 64.50 (C4′), 61.32 (d, J_{C-O-P} = 6.2 Hz, P-O-**C**H$_2$-CH$_3$), 52.31 (O-CH$_3$), 44.18 (C1′), 26.13 (d, J_{C-P} = 136.5 Hz, C5′), 16.40 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$), 16.39 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 418 (50) [*M*+H]$^+$, 440 (100) [*M*+Na]$^+$, 462 (45) [*M*+2Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for C$_{15}$H$_{25}$O$_7$N$_5$P [*M*+H]$^+$ 418.1486, found 418.1485; FTIR (KBr): $\tilde{\nu}$ = 3434, 3140, 2756, 1681, 1605, 1539, 1470, 1459, 1414, 1358, 1301, 1212, 1143, 1026, 971, 801 cm^{-1}; $[\alpha]_D^{20}$ = −13.5 (*c* = 0.310 g/100 mL, MeOH).

(*S*)-(2-((1-Methoxy-1-oxo-3-(hypoxanthin-9-yl)propan-2-yl)oxy)-ethyl)phosphonic acid (36): Treatment of compound **34** (201 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a yellowish solid (123 mg, yield 71%). ^{1}H NMR (D_2O): δ = 8.12 (s, 1H, H-2), 8.06 (s, 1H, H-8), 4.56–4.66 (m, 2H, H-1′), 4.50 (m, 1H, H-2′), 3.45–3.81 (m, 5H, H-4′, O-CH_3), 1.72–1.99 ppm (m, 2H, H-5′); ^{13}C NMR (D_2O): δ = 172.23 (C3′), 158.54 (C6), 148.89 (C4), 145.83 (C2), 142.87 (C8), 123.03 (C5), 76.35 (C2′), 66.95 (C4′), 53.01 (O-CH_3), 45.27 (C1′), 28.80 ppm (d, J_{C-P} = 129.4 Hz, C5′); MS-ESI$^+$ *m/z* (%): 347 (100) $[M+H]^+$, 369 (95) $[M+Na]^+$, 391 (55) $[M+2Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{11}H_{16}O_7N_4P$ $[M+H]^+$ 347.0751, found 347.0751; FTIR (KBr): $\tilde{\nu}$ = 1742, 1677, 1587, 1551, 1434, 1402, 1348, 1123, 1045, 900 cm^{-1}; $[\alpha]_D^{20}$ = −5.2 (*c* = 0.278 g/100 mL, H_2O).

(*S*)-(2-((Guanin-9-yl)-1-methoxy-1-oxopropan-2-yl)oxy)ethyl)-phosphonic acid (37): Treatment of compound **35** (209 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a white solid (125 mg, yield 69%). ^{1}H NMR (D_2O): δ = 7.77 (s, 1H, H-8), 4.48–4.53 (m, 2H, H-1′a, 2′), 4.42 (dd, J_{gem} = 15.6 Hz, $J_{1'b-2'}$ = 6.0 Hz, 1H, H-1′b), 3.83 (m, 1H, H-4′a), 3.74 (s, 3H, O-CH_3), 3.73 (m, 1H, H-4′b), 1.85–1.95 ppm (m, 2H, H-5′); ^{13}C NMR (D_2O): δ = 173.03 (C3′), 159.49 (C6), 154.31 (C2), 152.26 (C4), 141.18 (C8), 116.14 (C5), 76.98 (C2′), 67.44 (C4′), 53.62 (O-CH_3), 45.20 (C1′), 29.41 ppm (d, J_{C-P} = 129.9 Hz, C5′); MS-ESI$^-$ *m/z* (%): 360 (100) $[M-H]^+$, 382 (60) $[M+Na]^+$; HRMS-ESI$^-$: *m/z* calcd for $C_{11}H_{15}O_7N_5P$ $[M-H]^+$ 360.0715, found 360.0714; FTIR (KBr): $\tilde{\nu}$ = 3344, 3158, 2954, 1630, 1598, 1482, 1438, 1412, 1362, 1108, 1074, 900, 783 cm^{-1}; $[\alpha]_D^{20}$ = −3.1 (*c* = 0.285 g/100 mL, H_2O).

Diethyl (*S*)-(2-((1-(8-bromoguanin-9-yl)-3-hydroxypropan-2-yl)-oxy)ethyl)phosphonate (38): Compound **24** (779 mg, 2 mmol) was dissolved in DMF (10 mL) and a solution of bromine (0.15 mL, 3 mmol) in carbon tetrachloride (10 mL) was added. The reaction mixture was stirred at RT for 3 h. Volatiles were evaporated and a residue was co-distilled with toluene (3×20 mL). This was subjected to silica gel chromatography (gradient from 5-12% methanol in chloroform) to afford (after evaporation) the crude product. Crystallization from ethyl acetate gave the title compound as a yellowish solid (800 mg, yield 85%). ^{1}H NMR ([D_6]DMSO): δ = 10.71 (s, 1H, NH), 6.63 (bs, 2H, NH_2), 3.84-4.05 (m, 6H, H-1′, P-O-C**H$_2$**-CH_3), 3.71 (m, 1H, H-2′), 3.57 (m, 1H, H-4′a), 3.47 (dd, J_{gem} = 11.8 Hz, $J_{3'a-2'}$ = 4.5 Hz, 1H, H-3′a), 3.40 (dd, J_{gem} = 11.8 Hz, $J_{3'b-2'}$ = 5.5 Hz, 1H, H-3′b), 3.39 (m, 1H, H-4′b), 1.73–1.91 (m, 2H, H-5′), 1.18 ppm (t, $J_{CH3-CH2}$ = 7.0 Hz, 6H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D_6]DMSO): δ = 155.72 (C6), 154.02 (C2), 152.81 (C4), 121.74 (C8), 116.88 (C5), 78.26 (C2′), 64.18 (C4′), 61.45 (C3′), 61.30 (d, J_{C-O-P} = 6.2 Hz, P-O-C**H$_2$**-CH_3), 61.22 (d, J_{C-O-P} = 6.2 Hz, P-O-C**H$_2$**-CH_3), 45.20 (C1′), 26.38 (d, J_{C-P} = 135.9 Hz, C5′), 16.39 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-C**H$_3$**), 16.38 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-C**H$_3$**); MS-ESI$^+$ *m/z* (%): 468 (10) $[M+H]^+$, 470 (9) $[M+H]^+$, 490 (100) $[M+Na]^+$, 492 (97) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{23}O_6N_5BrNaP$ $[M+Na]^+$ 490.0462, found 490.0461; FTIR (KBr): $\tilde{\nu}$ = 3417, 3311, 3215, 3149, 2983, 2933, 1690, 1633, 1599, 1523, 1465, 1365, 1221, 1117, 1029, 964 cm^{-1}; $[\alpha]_D^{20}$ = −19.9 (*c* = 0.371 g/100 mL, DMSO).

(*S*)-(2-((1-(8-Bromoguanin-9-yl)-3-hydroxypropan-2-yl)oxy)ethyl)-phosphonic acid (39): Treatment of compound **38** (234 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a yellowish solid (159 mg, yield 77%). ^{1}H NMR (D_2O): δ = 4.13–4.20 (m, 2H, H-1′), 3.88 (m, 1H, H-2′), 3.72–3.80 (m, 2H, H-3′a, 4′a), 3.60 (dd, J_{gem} = 12.4 Hz, $J_{3'b-2'}$ = 5.3 Hz, 1H, H-3′b), 3.57 (m, 1H, H-4′b), 1.79–1.96 ppm (m, 2H, H-5′); ^{13}C NMR (D_2O): δ = 158.07 (C6), 154.30 (C2), 153.14 (C4), 125.48 (C8), 116.67 (C5), 78.52 (C2′), 65.90 (C4′), 61.38 (C3′), 45.37 (C1′), 28.61 ppm (d, J_{C-P} = 132.6 Hz, C5′); MS-ESI$^-$ *m/z* (%): 410 (100) $[M-H]^+$, 412 (97) $[M-H]^+$; HRMS-ESI$^-$: *m/z* calcd for $C_{10}H_{14}O_6N_5BrP$ $[M-H]^+$ 409.9871, found 409.9871; FTIR (KBr): $\tilde{\nu}$ = 3561, 3463, 3328, 3162, 2311, 1689, 1636, 1599, 1410, 1236, 1117, 1043, 931, 776 cm^{-1}; $[\alpha]_D^{20}$ = −4.3 (*c* = 0.257 g/100 mL, H_2O).

Diethyl (*S*)-(2-((1-(8-bromoguanin-9-yl)-3-(benzyloxy)propan-2-yl)oxy)ethyl)phosphonate (40): Compound **22** (1.92 g, 4 mmol) was dissolved in ethyl acetate (20 mL) and aqueous solution (15 mL) of sodium bromate (1.81 g, 12 mmol) was added. To a vigorously stirred two-phase system a solution of sodium hydrosulfite (2.6 g, 12 mmol) in water (30 mL) was added dropwise. The mixture was vigorously stirred at RT for 2 h. The reaction was diluted with water and extracted with ethyl acetate (3×150 mL). The combined organic layers were washed with brine (2×100 mL), dried over $MgSO_4$ and concentrated in vacuo. A residue was purified by silica gel chromatography (gradient from 1-8% methanol in chloroform) to give (after evaporation) the title compound as a yellow foam (1.50 g, yield 67%). ^{1}H NMR ([D_6]DMSO): δ = 10.71 (s, 1H, NH-1), 7.27–7.37 (m, 5H, H-2″, 3″, 4″), 6.59 (bs, 2H, NH_2), 4.51 (s, 2H, H-4′), 3.99–4.07 (m, 2H, H-1′), 3.83–3.95 (m, 5H, H-2′, P-O-C**H$_2$**-CH_3), 3.61 (m, 1H, H-5′a), 3.55 (dd, J_{gem} = 10.5 Hz, $J_{3'a-2'}$ = 4.2 Hz, 1H, H-3′a), 3.49 (dd, J_{gem} = 10.5 Hz, $J_{3'b-2'}$ = 5.4 Hz, 1H, H-3′b), 3.38 (m, 1H, H-5′b), 1.72–1.88 (m, 2H, H-6′), 1.16 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D_6]DMSO): δ = 155.75 (C6), 154.02 (C2), 152.84 (C4), 138.27 (C1″), 128.46 (C3″), 127.75 (C2″), 127.71 (C4″), 121.61 (C8), 116.94 (C5), 76.19 (C2′), 72.67 (C4′), 70.22 (C3′), 64.35 (C5′), 61.23 (d, J_{C-O-P} = 6.1 Hz, P-O-C**H$_2$**-CH_3), 61.18 (d, J_{C-O-P} = 6.1 Hz, P-O-C**H$_2$**-CH_3), 45.41 (C1′), 26.42 (d, J_{C-P} = 135.8 Hz, C6′), 16.36 (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-C**H$_3$**), 16.35 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-C**H$_3$**); MS-ESI$^+$ *m/z* (%): 558 (100) $[M+H]^+$, 560 (95) $[M+H]^+$, 580 (45) $[M+Na]^+$, 582 (43) $[M+Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{30}O_6N_5BrP$ $[M+H]^+$ 558.1112, found 558.1114; $[\alpha]_D^{20}$ = −20.7 (*c* = 0.304 g/100 mL, DMSO).

Diethyl (*S*)-(2-((1-(8-oxoguanin-9-yl)-3-(benzyloxy)propan-2-yl)-oxy)ethyl)phosphonate (41): Compound **40** (860 mg, 1.54 mmol) was dissolved in a solution of sodium acetate (1.26 g, 15.4 mmol) in glacial acetic acid (100 mL). The reaction mixture was heated at 130 °C for 4 h. Acetic acid was evaporated and a residue was co-distilled with water (1×20 mL), toluene (3×20 mL) and purified by silica gel chromatography (gradient from 2-8% methanol in chloroform) to afford (after evaporation) the title compound as a white foam (608 mg, yield 80%). ^{1}H NMR ([D_6]DMSO): δ = 10.72 (bs, 1H, NH-1), 10.56 (s, 1H, NH-7), 7.25–7.36 (m, 5H, H-2″, 3″, 4″), 6.48 (bs, 2H, NH_2), 4.47 (bs, 2H, H-4′), 3.86–3.98 (m, 5H, H-2′, P-O-C**H$_2$**-CH_3), 3.74 (dd, J_{gem} = 14.0 Hz, $J_{1'a-2'}$ = 7.2 Hz, 1H, H-1′a), 3.67 (m, 1H, H-5′a), 3.63 (dd, J_{gem} = 14.0 Hz, $J_{1'b-2'}$ = 5.6 Hz, 1H, H-1′b), 3.58 (m, 1H, H-5′b), 3.52 (dd, J_{gem} = 10.6 Hz, $J_{3'a-2'}$ = 3.8 Hz, 1H, H-3′a), 3.44 (dd, J_{gem} = 10.6 Hz, $J_{3'b-2'}$ = 5.9 Hz, 1H, H-3′b), 1.81–1.96 (m, 2H, H-6′), 1.18 (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-C**H$_3$**), 1.17 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 3H, P-O-CH_2-C**H$_3$**); ^{13}C NMR ([D_6]DMSO): δ = 153.71 (C6), 152.57 (C8), 151.36 (C2), 148.35 (C4), 138.44 (C1″), 128.42 (C3″), 127.71 (C2″), 127.64 (C4″), 98.45 (C5), 75.67 (C2′), 72.58 (C4′), 70.81 (C3′), 63.90 (C5′), 61.22 (d, J_{C-O-P} = 6.2 Hz, P-O-C**H$_2$**-CH_3), 61.19 (d, J_{C-O-P} = 6.2 Hz, P-O-C**H$_2$**-CH_3), 40.64 (C1′), 26.52 (d, J_{C-P} = 135.6 Hz, C6′), 16.41 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH_2-C**H$_3$**); MS-ESI$^+$ *m/z* (%): 496 (15) $[M+H]^+$, 518 (100) $[M+Na]^+$, 540 (15) $[M+2Na]^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{21}H_{30}O_7N_5NaP$ $[M+Na]^+$ 518.1775, found 518.1775; FTIR (KBr): $\tilde{\nu}$ = 3423, 3318, 3025, 1716, 1682, 1596, 1455, 1387, 1365, 1219, 1099, 1027, 962, 736 cm^{-1}; $[\alpha]_D^{20}$ = −14.6 (*c* = 0.274 g/100 mL, MeOH).

Diethyl (*S*)-(2-((1-(8-oxoguanin-9-yl)-3-hydroxypropan-2-yl)oxy)-ethyl)phosphonate (42): Compound **41** (533 mg, 1.1 mmol) was treated with procedure G to give the title compound as a white

solid (361 mg, yield 82%). ^{1}H NMR ([D$_6$]DMSO): δ = 10.67 (bs, 1H, NH-1), 10.60 (s, 1H, NH-7), 6.49 (bs, 2H, NH$_2$), 4.75 (t, $J_{OH-3'}$ = 6.0 Hz, OH), 3.88–4.01 (m, 4H, P-O-C**H$_2$**-CH$_3$), 3.57–3.72 (m, 5H, H-1′, 2′, 4′), 3.42 (ddd, J_{gem} = 12.0 Hz, $J_{3'a-OH}$ = 6.1 Hz, $J_{3'a-2'}$ = 3.9 Hz, 1H, H-3′a), 3.34 (dt, J_{gem} = 12.0 Hz, $J_{3'b-OH}$ = $J_{1'b-2'}$ = 5.9 Hz, 1H, H-3′b), 1.85–1.97 (m, 2H, H-5′), 1.20 ppm (t, $J_{CH3-CH2}$ = 7.1 Hz, 6H, P-O-CH$_2$-C**H$_3$**); ^{13}C NMR ([D$_6$]DMSO): δ = 153.63 (C6), 152.69 (C8), 151.21 (C2), 148.36 (C4), 98.41 (C5), 77.77 (C2′), 63.61 (C4′), 61.75 (C3′), 61.28 (d, J_{C-O-P} = 6.3 Hz, P-O-**C**H$_2$-CH$_3$), 61.22 (d, J_{C-O-P} = 6.3 Hz, P-O-**C**H$_2$-CH$_3$), 40.14 (C1′), 26.46 (d, J_{C-P} = 135.9 Hz, C5′), 16.42 ppm (d, $J_{C-C-O-P}$ = 5.8 Hz, P-O-CH$_2$-**C**H$_3$); MS-ESI$^+$ *m/z* (%): 406 (20) [*M*+H]$^+$, 428 (100) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{14}H_{25}O_7N_5P$ [*M*+H]$^+$ 406.1486, found 406.1486; FTIR (KBr): $\tilde{\nu}$ = 3169, 1712, 1685, 1593, 1539, 1460, 1382, 1360, 1248, 1226, 1100, 1050, 960 cm^{-1}; $[\alpha]_D^{20}$ = −4.0 (*c* = 0.276 g/100 mL, MeOH).

(*S*)-(2-((1-(8-oxoguanin-9-yl)-3-hydroxypropan-2-yl)oxy)ethyl)-phosphonic acid (43): Treatment of compound **42** (203 mg, 0.5 mmol) by procedure B followed by procedure A gave the title compound as a yellowish solid (135 mg, yield 77%). ^{1}H NMR (D$_2$O): δ = 3.91 (dd, J_{gem} = 14.6 Hz, $J_{1'a-2'}$ = 5.1 Hz, 1H, H-1′a), 3.87 (dd, J_{gem} = 14.6 Hz, $J_{1'b-2'}$ = 6.0 Hz, 1H, H-1′b), 3.73–3.87 (m, 3H, H-2′, 4′), 3.71 (dd, J_{gem} = 12.3 Hz, $J_{3'a-2'}$ = 3.9 Hz, 1H, H-3′a), 3.55 (dd, J_{gem} = 12.3 Hz, $J_{3'b-2'}$ = 6.0 Hz, 1H, H-3′b), 1.74–1.91 ppm (m, 2H, H-5′); ^{13}C NMR (D$_2$O): δ = 154.55 (C8), 154.21 (C6), 153.39 (C2), 150.09 (C4), 99.77 (C5), 78.23 (C2′), 66.44 (d, J_{C-C-P} = 1.6 Hz, C4′), 61.75 (C3′), 40.59 (C1′), 29.57 ppm (d, J_{C-P} = 129.8 Hz, C5′); MS-ESI$^+$ *m/z* (%): 350 (100) [*M*+H]$^+$, 372 (65) [*M*+Na]$^+$; HRMS-ESI$^+$: *m/z* calcd for $C_{10}H_{17}O_7N_5P$ [*M*+H]$^+$ 350.0860, found 350.0860; FTIR (KBr): $\tilde{\nu}$ = 3444, 3366, 3205, 2646, 1684, 1652, 1607, 1538, 1461, 1222, 1126, 1047, 1014, 978, 964 cm^{-1}; $[\alpha]_D^{20}$ = −7.3 (*c* = 0.399 g/100 mL, H$_2$O).

Biological assays

Determination of K_i values: Human HGPRT was stored in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4, 200 μM *P*Rib-*PP*, 1 mM DTT, −80 °C. *Pf*HGXPRT was stored in 0.01 M phosphate, 60 μM Hx, 200 μM *P*Rib-*PP*, pH 7.2, 1 mM DTT as previously described.[9] This difference is because the *Pf* enzyme is completely inactive under the conditions used to store the human enzyme. The human and *Pf* enzymes contain a hexa-histidine tag at the N-terminal. For enzyme assays for human HGPRT, the buffer was 0.1 M Tris-HCl, 0.01 M $MgCl_2$, pH 7.4. For the *Pf* enzyme, the assays were performed in 0.01 M phosphate, 0.01 M $MgCl_2$. The K_i values were calculated by Hanes' plots at a fixed concentration of guanine (60 μM) and at variable concentrations of *P*Rib-*PP* (14–1000 μM) depending on the $K_{M(app)}$ in the presence of the inhibitor.

Crystallization and structure determination: For crystallization experiments, human HGPRT was concentrated and stored as previously described.[9] The hanging drop method was used where 1 μL of enzyme in complex with the inhibitor was added to 1 μL of well solution. The trays were then incubated at 18 °C. The reservoir solution for compound **25** was 20% PEG3000, 0.2 M calcium acetate, 0.1 M Tris-HCl, pH 7.4. For compound **26**, the reservoir solution was the same except that the concentration of PEG3000 was decreased to 15%. The crystals were placed in a cryostream (100 K). X-ray data were collected remotely by BLU-ICE[32] using beamline MX1 at the Australian synchrotron. Data were scaled and merged using XDS.[33] The structure was solved by molecular replacement with the program PHASER[34] within PHENIX 1.8-1069[35] with the protein coordinates of the tetramer of human HGPRT in complex with ([(2-[(guanine-*9H*-yl)methyl]propane-1,3-diyl)bis(oxy)bis(methylene))diphosphonic acid (PDB code: 4IJQ). Subsequent refinement of the coordinates was with PHENIX1.8-1069[35] and model building was with COOT 0.7.[36] The structural restraints files for the inhibitors were generated by use of the PRODRG2 Dundee server. The atomic coordinates and structure factors of human HGPRT in complex with the two inhibitors have been deposited in the Protein Data Bank. The PDB accession code for (*S*)-(2-((1-hydroxy-3-(hypoxanthin-9-yl)propan-2-yl)oxy)ethyl)phosphonic acid (compound **25**) is 5BRN and, for the guanine derivative **26**, the code is 5BSK.

In vitro antimalarial activity of ANP prodrugs: *P. falciparum* D6 (Sierra-Leone) laboratory line, sensitive to most antimalarial drugs and W2 (Indochina) line, resistant to chloroquine and pyrimethamine, were maintained in RPMI-1640-LPLF complete medium, containing 10% human plasma, at 4% hematocrit and 1% to 8% parasitemia.[37] Cultures were routinely synchronized using D-sorbitol.[38] To evaluate the antimalarial activity of the ANPs, the [^{3}H]-hypoxanthine growth inhibition assay[39] was used, where the uptake of [^{3}H]-hypoxanthine by malaria parasites is used as a surrogate marker for parasite growth. For these assays, stock solutions of ANPs were made to concentrations of 20–40 mM in DMSO or water and subsequently diluted in hypoxanthine-free complete media prior to assay. The assays (in 96-well plate format) were initiated when the majority of parasites (>90%) at early trophozoite (ring) stage. Parasite cultures (100 μL per well) at 0.5% initial parasitemia and 2% hematocrit in hypoxanthine-free RPMI1640-LPLF medium were exposed to ten 2-fold serial dilutions of the ANPs and chloroquine (reference drug) for 96 h, with [^{3}H]-hypoxanthine (0.2 μCi well^{-1}) added ~48 h after beginning of the experiment. The [^{3}H]-hypoxanthine incorporation data were analyzed and sigmoidal growth inhibition curves were produced by non-linear regression analysis of the [^{3}H]-hypoxanthine incorporation data versus log-transformed concentrations of the compounds using GraphPad Prism V5.0 software (GraphPad Software Inc. USA), from which the inhibitory concentration (IC50) that cause 50% of parasite growth were determined. The IC_{50} values were based on at least two independent experiments with mean ± SD calculated.

Abbreviations: ANP: acyclic nucleoside monophosphonate, *P*Rib-*PP*: 5-phospho-α-D-ribosyl-1-pyrophosphate, PEE: 2-(phosphonoethoxy)ethyl, HPMP: (*S*)-3-hydroxy-2-(phosphonomethoxy)propyl, ImmGP: (1*S*)-1-(9-deazaguanin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate, HPEP: (*S*)-3-hydroxy-2-(phosphonoethoxy)propyl, CPME: (*S*)-2-(phosphonomethoxy)propanoic acid, CPEE: (*S*)-2-(phosphonoethoxy)propanoic acid, HG(X)PRTs: hypoxanthine-guanine-(xanthine) phosphoribosyltransferases.

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