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Synthesis of Novel *N*-Branched Acyclic Nucleoside Phosphonates As Potent and Selective Inhibitors of Human, *Plasmodium falciparum* and *Plasmodium vivax* 6-Oxopurine Phosphoribosyltransferases

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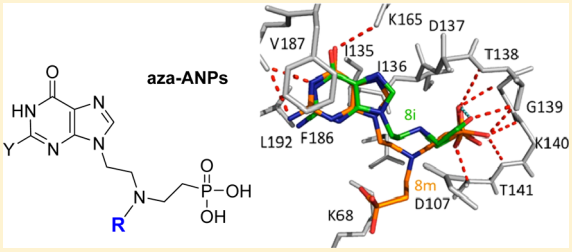
ABSTRACT: Hypoxanthine−guanine−(xanthine) phosphoribosyltransferase (HG(X)PRT) is crucial for the survival of malarial parasites *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*). Acyclic nucleoside phosphonates (ANPs) are inhibitors of HG(X)-PRT and arrest the growth of *Pf* in cell culture. Here, a novel class of ANPs containing trisubstituted nitrogen (aza-ANPs) has been synthesized. These compounds have a wide range of K_i values and selectivity for human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT. The most selective and potent inhibitor of *Pf*HGXPRT is 9-[*N*-(3-methoxy-3-oxopropyl)-*N*-(2-phosphonoethyl)-2-aminoethyl]-hypoxanthine (K_i = 100 nM): no inhibition could be detected against the human enzyme. This compound exhibits the highest ever reported selectivity for *Pf*HGXPRT compared to human HGPRT. For *Pv*HGPRT, 9-[*N*-(2-carboxyethyl)-*N*-(2-phosphonoethyl)-2-aminoethyl]guanine has a K_i of 50 nM, the best inhibitor discovered for this enzyme to date. Docking of these compounds into the known structures of human HGPRT in complex with ANP-based inhibitors suggests reasons for the variations in affinity, providing insights for the design of antimalarial drug candidates.



INTRODUCTION

Acyclic nucleoside phosphonates (ANPs) are reverse transcriptase inhibitors, and several ANP-based drugs are in current clinical use for the treatment of serious viral infections (e.g., Cidofovir, Adefovir, Tenofovir).[1,2] These compounds consist of a nucleobase, either 6-aminopurine or pyrimidine, linked to a phosphonate group by an acyclic linker. These nucleotide analogues are excellent templates for drug design because of the absence of the labile glycosidic bond of the nucleotides and the stability of the phosphonate moiety compared with the phosphate ester bond of the nucleotides. Such bonds can be enzymatically hydrolyzed within the cell to yield inactive compounds. The chemical structure of the ANPs also allows the synthesis of prodrugs to increase membrane permeability and bioavailability. Another structural advantage of this class of molecules is the flexibility of the acyclic chain, which enables the compounds to adopt a range of conformations to facilitate binding to not only reverse transcriptases and DNA polymerases but potentially to other enzymes using nucleotides as substrates or products.

Some ANPs with 6-oxopurine bases have been found to arrest parasitemia in *Plasmodium falciparum* (*Pf*) grown in culture with IC_{50} values as low as 1 μM.[3,4] These compounds are potent inhibitors of hypoxanthine−guanine−(xanthine) phosphoribosyltransferase (HG(X)PRT), and it is proposed that this is how they exert their antimalarial effect.[4−7] The only other class of HG(X)PRT inhibitors known so far are the transition state analogues, (1*S*)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate (immucillinHP) and (1*S*)-1-(9-deazaguanin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol 5-phosphate (immucillinGP).[8] The activity of HG(X)PRT is necessary for both survival and reproduction of many protozoan parasites.[9] This is because, unlike mammalian cells, protozoan parasites such as *Plasmodium*, the causative agent of malaria, are auxotrophic for the purine ring. Thus, they depend on HG(X)PRT for the synthesis of the 6-oxopurine nucleoside monophosphates required for RNA/DNA production.[10,11] The reaction catalyzed by HG(X)PRT is given in Figure 1.

There are four major species of *Plasmodium* that infect humans and result in symptoms of malaria: *falciparum*, *vivax*, *malariae*, and *ovale*. *Pf* and *Plasmodium vivax* (*Pv*) are the most lethal and widespread, with both species infecting around 500 million people per year, resulting in at least one million deaths per annum, mainly among children.[12] Drugs such as artemisinin and combination therapies, quinine, chloroquine, and primaquine, are the only known treatments for malaria but, because of increasing resistance[13] as well as cost-effectiveness, there is an urgent need for the discovery of new targets and therapeutic leads for the development of potent antimalarials.

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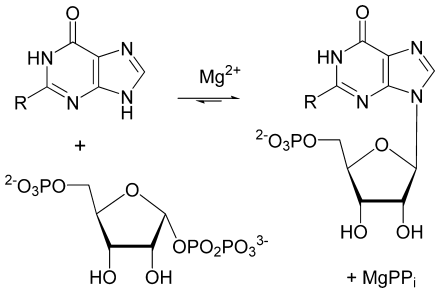


Figure 1. The reaction catalyzed by HG(X)PRT. R = −H (hypoxanthine), $-NH_2$ (guanine), −OH (xanthine).

The subject of this paper is the design and synthesis of a new series of ANPs with novel linkers designed to increase affinity and/or selectivity for *Pf*HGXPRT and *Pv*HGPRT over the corresponding human HGPRT. These compounds are based on the 2-(phosphonoethoxy)ethyl (PEE) ANPs but with a nitrogen atom replacing oxygen in the phosphonate tail (Figure 2). This scaffold was chosen as both 2-(phosphonoethoxy)ethyl

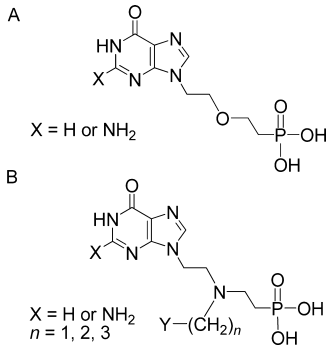


Figure 2. Comparison of the structure of PEE compounds (A) with the aza-ANPs (B). Y and *n* denote the variety of functional groups in the side chain.

guanine (PEEG) and 2-(phosphonoethoxy)ethyl hypoxanthine (PEEHx) are good inhibitors of human HGPRT and *Pf*HGXPRT.[4] They have K_i values for human HGPRT of 1 and 3.6 μM, respectively, and 0.1 and 0.3 μM for *Pf*HGXPRT. In all of the new compounds (aza-ANPs), there are five atoms between the N-9 atom in the purine ring and the phosphonate phosphorus atom. Different side chains were attached to the linker nitrogen atom in attempts to improve the affinity and/or selectivity for the *Plasmodium* enzymes. Herein is reported the synthesis of 24 new ANPs and a comparison of their inhibition constants for human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT. Docking of the aza-ANPs into the known crystal structures[4] of human HGPRT in complex with 2-(phosphonoethoxy)ethyl guanine or hypoxanthine (PEEG or PEEHx) and 3-hydroxy-2-(phosphonomethoxy)propyl guanine (HPMPG) provides insights into how these compounds may bind in the active site.

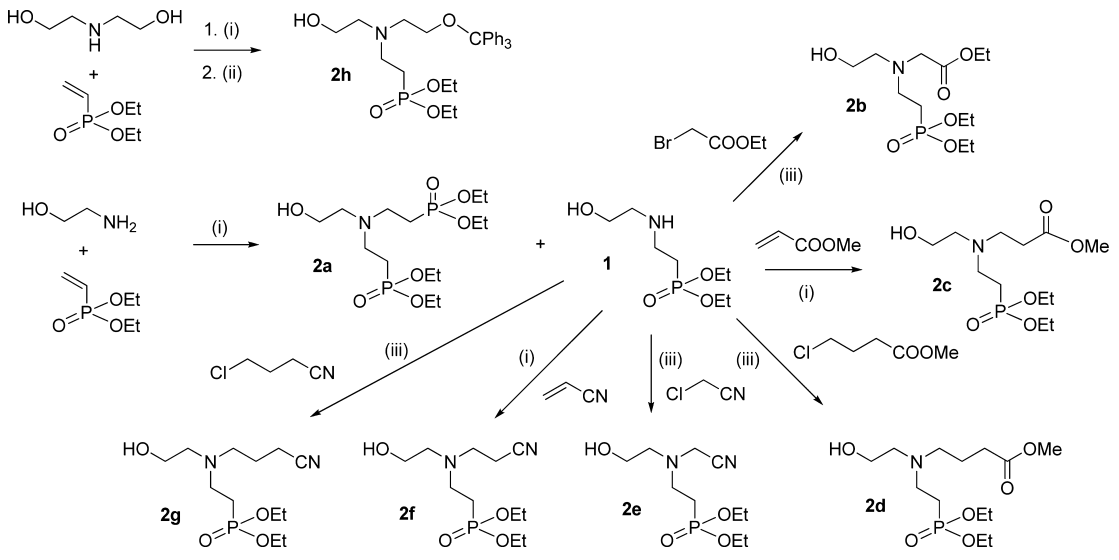
RESULTS AND DISCUSSION

Chemical Synthesis. The design of the aza-ANPs is based on previous results from SAR studies.[4−7] ANPs containing a nitrogen atom in the acyclic chain (aza-ANPs, Figure 2B) have three main advantages for inhibitor development: (1) derivatives with different linker lengths and nitrogen position (determining the location of the side chain) can be easily synthesized, (2) a variety of synthetic methods can be applied to attach a side chain with various functional groups to the NH-moiety, and (3) difficulties with the synthesis and characterization of stereoisomers, typical for C-branched ANPs, are avoided.

Synthesis of several compounds containing a nucleobase as well as an acyclic chain with substituted nitrogen has recently been published.[14−16] However, only one type of acyclic nucleoside phosphonate (with an unsubstituted NH-moiety) has previously been reported.[17]

A number of different synthetic procedures can be used for carbon−nitrogen bond formation. One of these is the aza-Michael addition, and thus reaction of vinylphosphonate with an amino group was chosen for the synthesis of the β-aminophosphonate moiety. The use of various solvents and catalysts for the reaction has been described in the literature, but we applied the recently published methodology using only

Scheme 1[a]



[a](i) H_2O, rt; (ii) TrCl, Et_3N, DMAP, CH_2Cl_2; (iii) K_2CO_3, MeCN.

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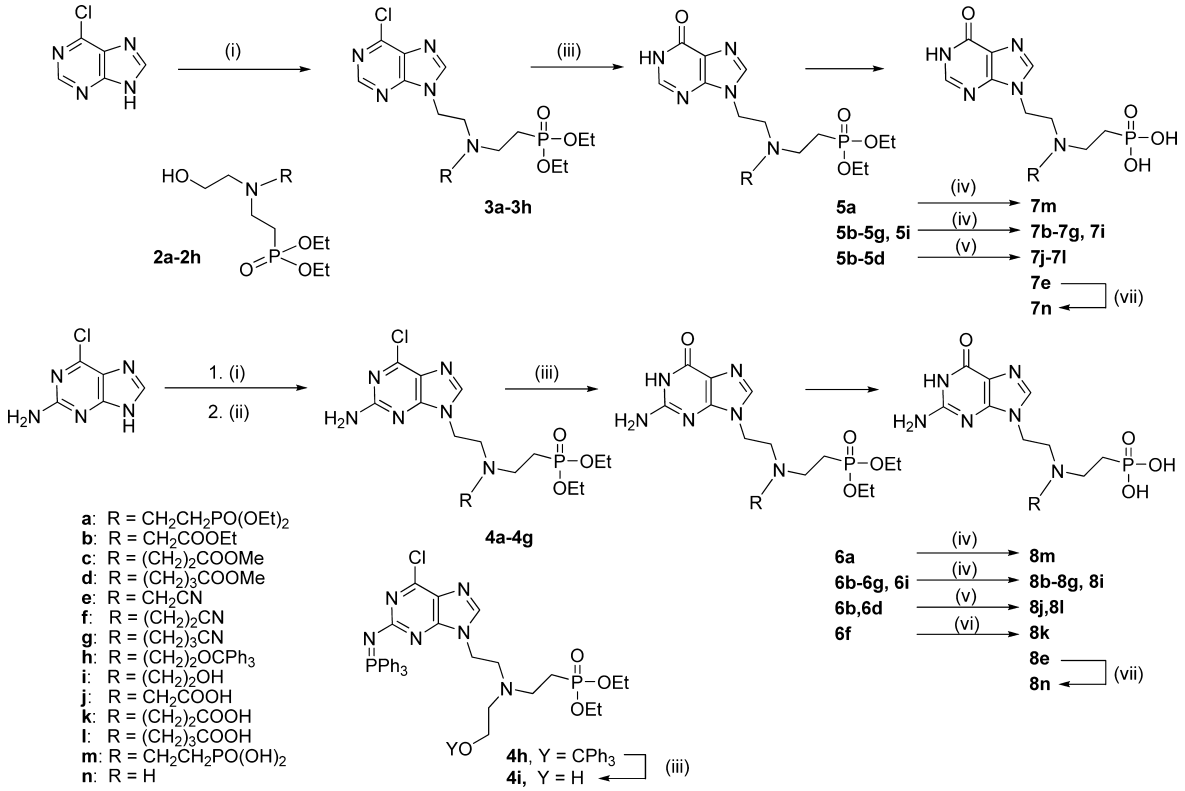
Scheme 2[a]



[a](i) PPh_3, DIAD, THF; (ii) addition of H_2O, heating; (iii) 75% aq trifluoroacetic acid; (iv) Me_3SiBr, MeCN, DMF, 2,6-lutidine; (v) (1) aq NaOH, THF, MeOH, (2) Me_3SiBr, MeCN, DMF, 2,6-lutidine; (vi) (1) aq NaOH, MeOH, (2) Me_3SiBr, MeCN, DMF, 2,6-lutidine; (vii) DMF, heating.

water without any catalyst or organic cosolvent.[18] Thus, aza-Michael addition of diethyl vinylphosphonate to 1 equiv of 2-aminoethanol produced desired phosphonate **1** (77% yield) together with bisphosphonate **2a** (8% yield).

Diethyl 2-(2-hydroxyethylamino)ethylphosphonate **1** was used as the starting material for the synthesis of a series of branched phosphonates **2b**−**2g** (Scheme 1). Two types of reactions were used to introduce suitable substituents to the NH-group of the phosphonate **1**: (a) alkylation with the corresponding halogenoderivative was used to attach a one-carbon or three-carbon chain bearing a cyano or carboxylic ester group (compounds **2b**, **2d**, **2e**, and **2g**, isolated yields 64−77%), (b) Another aza-Michael addition of methyl acrylate or acrylonitrile to the phosphonate **1** was employed to prepare compounds **2c** and **2f** with a two-carbon chain (isolated yields 50% and 78%, respectively). To complement the series, a protected hydroxyethyl derivative **2h** was synthesized by aza-Michael addition of diethyl vinylphosphonate to diethanolamine followed by tritylation of one of the hydroxy groups (Scheme 1).

The series of hydroxyderivatives **2a**−**2h** prepared by the above-mentioned straightforward approach was used to introduce branched acyclic moieties to the N^9-position of 6-chloropurine or 2-amino-6-chloropurine (Scheme 2) via Mitsunobu reaction.[19,20] 6-Chloropurine phosphonates **3a**−**3h** were produced in good yields (about 80%). In the case of 2-amino-6-chloropurine phosphonates **4a**−**4g**, the Mitsunobu reaction had to be followed by heating in water/tetrahydrofuran to decompose the triphenylphosphoranylidene intermediate rising from the presence of the free amino group.[17] Anomalous behavior was observed for the tritylated derivative **4h**: the triphenylphosphoranylidene intermediate was very stable when the trityl group was present. After deprotection under acidic conditions (the 6-chloro group was unexpectedly completely preserved), the triphenylphosphoranylidene **4i** was decomposed using standard procedures.

The chlorine atom of phosphonates **3a**−**3h** and **4a**−**4g**,**4i** was next displaced with hydroxyl by nucleophilic aromatic substitution in acidic conditions (75% aqueous trifluoroacetic acid) to form hypoxanthine and guanine phosphonates **5a**−**5g** and **6a**−**6g**,**6i**. The tritylated derivative **3h** was simultaneously deprotected to give the hydroxyderivative **5i**. Instead of direct Mitsunobu reaction of 6-oxopurines with poor regioselectivity, this two-step approach was used for the preparation of 9-substituted hypoxanthine and guanine derivatives to avoid the separation of N^7 and N^9 regioisomers.

To form free phosphonic acids **7b**−**7g**,**7i** and **8b**−**8g**, **8i**, ester groups of diethyl phosphonates **5b**−**5g**,**5i** and **6b**−**6g**,**6i** were cleaved under standard conditions using Me_3SiBr/acetonitrile followed by hydrolysis; 2,6-lutidine was added to neutralize contamination of Me_3SiBr by HBr. In the tetraethyl bisphosphonates **5a** and **6a**, both phosphonate moieties were simultaneously deprotected and free bisphosphonic acids **7m** and **8m** were obtained. Alkaline hydrolysis of carboxylic acid esters **5b**−**5d**, **6b**, and **6d** with aqueous sodium hydroxide followed by cleavage of phosphonate esters under the above-mentioned conditions afforded the corresponding phosphonic acids **7j**−**7l**, **8j**, and **8l** with free carboxylic acid moiety in the side chain. The phosphonic acid **8k** was prepared by the alkaline hydrolysis of the cyanoderivative **6f** again followed by cleavage of the phosphonate ester (Scheme 2).

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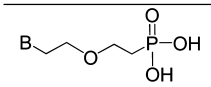
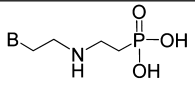
Table 1. Comparison of the K_i Values of the PEE Compounds with Aza-ANP

Compound	B = Guanine				B = Hypoxanthine			
		K_i (μM)				K_i (μM)		
		human	*Pf*	*Pv*		human	*Pf*	*Pv*
	PEEG	1±0.5	0.1±0.02	ND[a]	PEEHx	3.6±0.2	0.3±0.04	ND[a]
	**8n**	3±0.6	16±1	11±0.6	**7n**	40±2	≥200	≥250

[a]ND = not determined.

During the purification of the cyanomethyl phosphonic acids **7e** and **8e**, partial cleavage of the side-chain at higher temperature was observed. To complement the series of target ANPs, we refluxed these unstable cyanomethyl derivatives in dimethylformamide for several days to obtain compounds **7n** and **8n** with an unsubstituted NH-moiety (Scheme2). All target ANPs described above, **7b**−**7g**, **7i**−**7n**, **8b**−**8g**, and **8i**−**8n**, were purified by preparative HPLC or ion-exchange chromatography.

The cytotoxicity of the prepared aza-ANPs was tested on several cell lines (HEL, Vero, CRFK), and none of the compounds exhibited any significant toxicity (CC_{50} > 100 μg/mL) except for the cyano derivative **8e** (CC_{50} = 53 μg/mL, HEL cells). The title compounds were also tested for their in vitro inhibition of the cell growth in mouse leukemia L1210 cells, human T-lymphoblastoid CCRF-CEM cell line, human promyelocytic leukemia HL-60 cells, and human cervix carcinoma HeLa S3 cells (Dr. H. Kaiserová-Mertlíková, IOCB, Prague), and no cytostatic activity was observed.

Inhibition of Human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT by the Aza-ANPs. *Effect of the Substitution of the Oxygen Atom with Nitrogen.* The substitution of the oxygen atom in the acyclic linker (as in the PEE compounds;[4] Figure 2A) by −NH− (compounds **7n** and **8n**) resulted in an increase in the K_i values for human HGPRT and *Pf*HGPRT (Table 1).

These new ANPs are also weak inhibitors for *Pv*HGPRT. The K_i for **8n** is 11 μM, similar to that found for *Pf*HGXPRT, while for the hypoxanthine derivative, **7n**, no inhibition was detected (K_i is ≥200 μM). One possible explanation for these increased K_i values is that the substitution of the partially negatively charged oxygen atom by the positively charged nitrogen changes the conformation of the linker, resulting in different interactions between the ligand and the active site amino acid residues. These aza-ANPs are competitive inhibitors with *P*Rib-*PP*. Thus, they bind in the same location in the active site as the naturally occurring substrates or products of the reaction. However, they may not be able to induce the conformational changes which occur in the enzyme on the binding of the transition state analogue inhibitors.[8] When immucillinGP 5′-phosphate, PP_i and, Mg^{2+} bind to human HGPRT, a large flexible loop closes over the active site, firmly anchoring them in position. ANPs are analogues of the nucleoside monophosphate products of the reaction, IMP and GMP. In the crystal structure of GMP bound to human HGPRT, this loop is only partially visible. The K_i values[4] for GMP are similar to that found for this new aza-ANP **8n**: 5.8, 10, and 26.2 μM for human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT, respectively. The K_i values for IMP for these three enzymes are 5.4, 3.6, and 62 μM for human HGPRT, *Pf*HGXPRT and *Pv*HGPRT, respectively, not significantly different from those found for GMP. When PEEG or PEEHx bind to human HGPRT, the loop does become visible and partially closes.[4] Thus, it is likely that one of the contributing factors leading to the weaker binding of **7n** and **8n** compared with PEEG and PEEHx is the failure of the loop to become ordered. Therefore, when **7n** or **8n** bind to the enzymes, the structure is likely to be similar to that when GMP or IMP are present and commencing to leave the active site.

Addition of a Side Chain with a Second Phosphonate Group to the Basic Linker. In an attempt to improve the affinity of these new ANPs for the *Plasmodium* enzymes, a second phosphonate group was attached via a two-carbon chain to the linker nitrogen atom (**7m** and **8m**). The hypothesis was that this second phosphonate group may be able to occupy part of the pyrophosphate binding site, increasing the affinity. The K_i values for these new compounds are given in Table 3. For human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT, these values are 3, 0.4, and 13 μM, respectively (**7m**, hypoxanthine as the purine base). When hypoxanthine is replaced by guanine, K_i values are lower for all three enzymes. For human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT, they are 0.1. 0.3 and 0.9 μM, respectively (**8m**). Thus, the addition of this second phosphonate group does affect the affinity of these ANPs for all three enzymes, decreasing the K_i for all these enzymes significantly. The decrease in the K_i values when the hydrogen on the nitrogen atom in the linker is replaced by a phosphonate group is reflected by comparison of their K_i ratios (Table 2).

However, as the K_i values for all three enzymes are similar (**8m** and **7m**), selectivity is lost (Table 3). A series of compounds with different substituents at the nitrogen atom

Table 2. Decrease in the K_i Value When the Hydrogen Atom Attached to the Nitrogen Atom in the Linker Is Replaced by a Phosphonate Group

enzyme	K_i ratio (**8n**/**8m**) guanine as the base	K_i ratio (**7n**/**7m**) hypoxanthine as the base
humanHGPRT	15-fold	13-fold
*Pf*HGXPRT	53-fold	>500-fold
*Pv*HGPRT	12-fold	>20-fold

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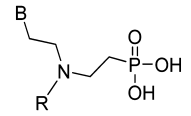
Table 3. K_i Values of Aza-ANPs for Human HGPRT, *Pf*HGXPRT, and *Pv*HGPRT

 Substituent R		B = Guanine				B = Hypoxanthine		
		K_i (μM)				K_i (μM)		
		human	*Pf*	*Pv*		human	*Pf*	*Pv*
COOEt	**8b**	0.1±0.02	1.7±0.3	1.5±0.4	**7b**	2.6±0.5	1.9±0.4	11±1
COOMe	**8c**	0.5±0.1	0.1±0.02	4±0.5	**7c**	≥200[b]	0.1±0.03	21±2
COOMe	**8d**	0.1±0.02	1.3±0.1	≥200[b]	**7d**	22±3	≥220[b]	≥250[b]
CN	**8e**	ND[a]	ND[a]	ND[a]	**7e**	35±5	≥250[b]	47±5
CN	**8f**	0.07±0.02	0.2±0.03	0.3±0.03	**7f**	2.4±0.2	0.2±0.05	0.7±0.06
CN	**8g**	0.6±0.1	4±0.05	8±1	**7g**	29±2	6±0.06	≥230
COOH	**8j**	1.4±0.2	0.7±0.1	28±1	**7j**	32±2	0.6±0.06	178±10
COOH	**8k**	0.15±0.04	0.2±0.03	0.05±0.004	**7k**	≥240[b]	0.4±0.03	41±5
COOH	**8l**	0.1±0.02	0.1±0.01	25±4	**7l**	5.4±0.2	≥210	1.6±0.2
OH	**8i**	0.07±0.005	0.2±0.04	1.4±0.1	**7i**	5.5±0.4	2.6±0.4	21±3
$P(O)(OH)_2$	**8m**	0.2±0.006	0.3±0.05	0.9±0.1	**7m**	3.0±0.6	0.4±0.1	13±3

[a]ND, not determined. [b]No inhibition could be observed under conditions where measurable inhibition would have been detected if the K_i value had been less than or equal to the specified value.

were thus synthesized to try to increase both potency and selectivity.

Aza-ANPs with Various Substituents to the Linker Nitrogen Atom. The basic structure of aza-ANPs was modified by attaching various substituents to the nitrogen atom of the linker. These substituents differed in the chemical nature of the functional groups and the number of carbon atoms between the trisubstituted nitrogen and the functional group. The structures and K_i values for the three enzymes are given in Table 3.

Effect of the Purine Base on the K_i Values for All Three Enzymes. For human HGPRT, guanine is always the preferred base, with selectivity ratios in favor of guanine over hypoxanthine of 23−220 (compounds **8b**−**8m**, **7b**−**7n**). As a substrate, guanine binds slightly more tightly than hypoxanthine (cf. K_m for guanine is 1.9 μM and for hypoxanthine is 3.4 μM),[21] perhaps because guanine has the ability to form an extra hydrogen bond between the 2-amino group and the active site amino acid residues. However, this K_m difference is only 2-fold, suggesting that it is the structure or chemical nature of the linker and the groups attached to the linker that contribute to the increase of the K_i ratio in favor of guanine-containing aza-ANPs. *Pf*HGXPRT exhibited similar K_i values for the majority of these compounds irrespective of whether guanine or hypoxanthine was the base. There were two exceptions: **8i**/**7i** (13-fold in favor of guanine) and **8d**/**7d** (1.3 μM for guanine and ≥220 μM for hypoxanthine). *Pv*HGPRT favored the ANPs with guanine as the purine base, with ratios between 2 and 820. The exception was compounds **8l** and **7l**, where *Pv*HGPRT preferred the hypoxanthine derivative by 17-fold. Thus, it can be deduced that for this class of ANPs, human HGPRT and *Pv*HGPRT usually prefer guanine as the purine base while *Pf*HGXPRT is not as discriminatory.

Effect of the Nature of the Linker and the Different Attachments. For the parasite enzymes, the optimal number of carbon atoms between the trisubstituted nitrogen and the substituent functional group was usually two (Table 3). Compounds where the functional group was −COOMe or −CN or −COOH or −OH were all effective inhibitors of *Pf*HGXPRT. *Pv*HGPRT is different in that it has a general preference for cyano derivatives although its lowest K_i value was found when the base was guanine and the functional group was −COOH (**8f**; 50 nM). The most selective compounds tested for the malarial enzymes were **7c** and **7k** as no inhibitory activity could be observed against human HGPRT (K_i ≥200 μM), but they were good inhibitors of *Pf*HGXPRT (0.1 and 0.4 μM, respectively).

Docking of the ANPs into the Active Site of Human HGPRT. Attempts were made to dock all of the ANPs in Tables 1 and 3 into the active site of the known crystal structures of human HGPRT. Docking into the free structure of human HGPRT or into the enzyme in complex with immucillinGP and Mg^{2+}-pyrophosphate was unsuccessful. These structures are not good models for the binding of the ANPs because a number of conformational changes occur when substrates enter the active site, products of the reaction are released or when the transition state analogues bind. Thus, these two structures differ too

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dramatically from the conformation expected when the aza-ANPs bind.

The structures which did provide good solutions were human HGPRT in complex with 2-(phosphonoethoxy)ethyl (PEE) guanine or PEE hypoxanthine (Figure 2A) and 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) guanine.[4] These structures are expected to most closely resemble that of the enzyme when in complex with the aza-ANPs. In general, the docking poses with the highest scores locate the purine ring in the purine binding pocket and the phosphonate group in the 5′-phosphate binding site.

*Comparison of the Docked Structure of **8n** (Guanine As the Base) with the Crystal Structure of PEEG in Complex with Human HGPRT.* Superimposition of the docked structure of **8n** in complex with human HGPRT onto the known structure of PEEG in complex with this enzyme showed that the phosphonate groups were virtually superimposable. Differences in the superimposition of the two ANPs in the active site lay in the position of the linker. The replacement of a partially negatively charged oxygen atom with a positively charged nitrogen atom may explain the apparent difference between these two structures (Figure 3). The disubstituted nitrogen of

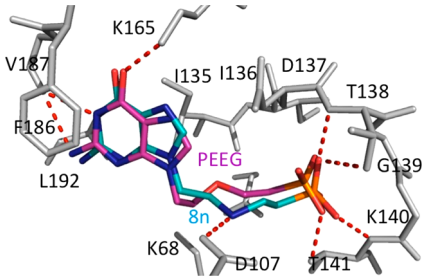

Figure 3. Comparison of the crystal structure[4] of PEEG in complex with human HGPRT with that of the docked structure of **8n**. This figure shows the change in the location of the disubstituted nitrogen and the movement of the purine ring.

8n is located 1.4 Å from the corresponding oxygen atom in the PEEG structure (Figure 3). This moves the nitrogen atom in the linker closer to the OD2 atom of D107 (2.91 Å compared with 4.69 Å between the oxygen atom in the linker in the PEEG structure and this atom) and away from the back surface of the active site. D107 is located in the large flexible loop (residues 100−120), which closes over the active site during catalysis. In the PEEG structure, this mobile loop might be less hindered and better able to open and close over the active site explaining, in part, the lower K_i values of PEEG compared with **8n**. The changes in the linker structure also results in a subtle change in the predicted orientation of the purine ring with the 6-oxo group being located further away (2.8 Å compared to 2.4 Å) from the NZ atom of K165. This difference may also contribute to the observed decrease in affinity of the aza-ANPs. For the parasite enzymes, the change in the structure of the linker has a much more drastic effect on the affinity with these aza-ANPs binding extremely weakly. Thus, for *Pf*HGXPRT and *Pv*HGPRT, the orientation of the purine ring in the active site may be more compromised.

*The Docked Structure of **8m** (Guanine As the Base).* The addition of a second phosphonate group to the nitrogen atom in the linker (as in **8m**) resulted in a decrease in K_i for human HGPRT by 15-fold (**8m** cf. **8n**). This could be attributed to the extra interactions between this group and the enzyme's active site residues. The docked structure of **8m** (Figure 4) suggests that this second phosphonate group does not, however, make

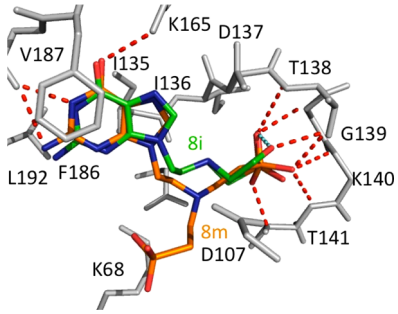

Figure 4. Comparison of the docked structures of **8i** with **8m**. In these two structures, the trisubstituted nitrogen is differently oriented, resulting in the phosphonate group of **8m** being located near the NZ atom of K68 while the hydroxyl group of **8i** is located in the 5′-phosphate binding pocket, forming a hydrogen bond with the phosphorus oxygen atoms.

the same interactions with the amino acid residues as does pyrophosphate when it is bound in the active site in complex with immucillinGP and magnesium ions. The reason for this may be that, when PP_i binds, the structure of the enzyme is very different from that in its absence. The phosphorus oxygen atoms of the second phosphonate group in **8m** are further away from the NE1 of R199 (11 Å compared with 2.8 and 2.7 Å when PP_i is bound) and 6.6 Å from the main chain nitrogen atom of S103 compared with 2.7 Å. Probably the biggest structural change between the immucillinGP complex and the ANP complexes with human HGPRT is the position of the large flexible loop which, in the former, is firmly closed over the active site while, in the latter, it is only partially closed. The other difference between these two structures is the location of the K68 side chain. When PP_i binds, this side chain has to rotate by 180° to allow room for this compound. In the crystal structures where ANPs are bound (PDB codes 3GGJ, 3GEP), this rotation does not occur as there are no groups attached to the ANPs to occupy this site. Because **8m** is unable to dock into the transition state analogue structure, it is deduced that this movement does not occur and that the second phosphonate group does not occupy this site. Mg^{2+} has not been modeled into the **8m** structure. A hypothesis to explain the lower K_i for **8m**, compared with **8n**, is that the oxygen atoms of the phosphonate group interact with the enzyme via magnesium ion(s).

*Comparison of the Docked Structure of **8m** with **8i** (Guanine As the Base).* In the pose which gave the best docking score for all of these compounds, the hydroxyl group is located close to the phosphonate group (1.8 Å: Figure 4). This compound is a good inhibitor of human HGPRT (K_i = 0.07 μM) and also of the parasite enzymes (0.2 μM for *Pf*HGXPRT and 1.4 μM for *Pv*HGPRT). The docking studies suggest that, for **8i**, the hydroxyl group of the side chain can make internal hydrogen bonds with the phosphonate group. As a result of this, the PP_i binding site is largely unoccupied. The docking studies show that when **8i** is bound there still remains unoccupied space in the 5′-phosphate binding pocket. Thus, there is a possibility that this region could be exploited to decrease the K_i value.

*Comparison of the Docked Structures of **8f** and **7f** (Same Linker, Different Base).* In these two docked structures, no

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difference was observed in the location of the purine base and the phosphonate group (Figure 5). There is, however, a large

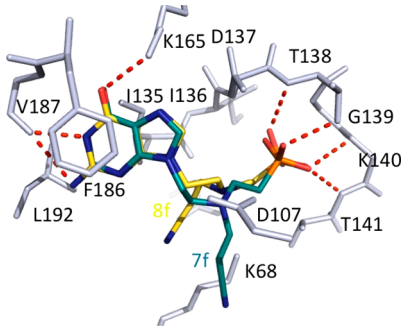

Figure 5. Comparison of the docked structures of **8f** with **7f**. In these two structures, the purine ring and the phosphonate group are located in the same place and the difference in the positioning of the cyano group is shown.

difference in the K_i values for the human enzyme depending on the attached base [cf. 0.07 μM with guanine (**8f**) and 2.4 μM with hypoxanthine (**7f**)]. In contrast, the nature of the base did not have any effect on the K_i values for either *Pf*HGXPRT or *Pv*HGPRT. Examination of the two docked structures in the human enzyme suggests that the 34-fold difference in the K_i values may be due to the cyano group being located in different regions in the active site (Figure 5). Comparison of the docked structures of **7f** and **8f** show that the cyano substituents in these two poses are 4.6 Å apart. This structural change is due to the slightly different conformations adopted by the linker. The docked structure of **8f** into the human HGPRT−3-hydroxy-2-(phosphonomethoxy)propyl guanine complex shows that the nitrogen in the cyano group is further away from the NZ atom of K68 (4.6 Å) but is only 2.8 Å, if the NZ adopts the same structure as it does in human HGPRT−PEEG complex. This situation is reversed for **7f**, i.e., the cyano nitrogen is closer to the NZ atom in the human HGPRT−3-hydroxy-2-(phosphonomethoxy)propyl guanine complex (3.63 Å) but 5.5 Å distant in the human HGPRT−PEEG structure. As the K_i values do not differ for the parasite enzymes irrespective of the base, one deduction that could be made is that, in these two ANPs, the linker adopts the structure found when attached to guanine. Thus, it would be expected that the cyano group would be located closer to the corresponding lysine residue (K68 in human).

Docked Structure of ***8d.*** This compound is a good inhibitor of human HGPRT (0.1 μM) and is a reasonable inhibitor of *Pf*HGXPRT (1.3 μM) but binds very weakly, if at all, ($K_i \geq 200$ μM) to *Pv*HGPRT. Docking suggests that its side chain functional group (−COOMe) does not bind in the same location as the phosphonate group in **8m** (cf. Figures 4 and 6). This new orientation could be attributed to the longer side chain in **8d**. The functional group is predicted to bind in a hydrophobic pocket which is not located in the same area as PP_i. Therefore, attachment of other functional groups able to occupy the PP_i site may lead to increased potency.

CONCLUSION

An efficient method has been developed for the synthesis of a new type of ANPs containing a di- or trisubstituted nitrogen in the acyclic moiety. In the disubstituted compounds, the affinity for all the enzymes is decreased, possibly because of the new

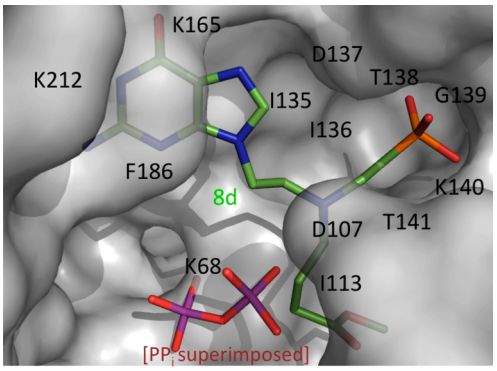

Figure 6. The docking of **8d** containing a −COOMe attachment to the active site of HGPRT. The location of PP_i when it binds in the enzyme has been superimposed from the structure of human HGPRT in complex with immucillinHP, PP_i, and Mg^{2+}.

positive charge in the linker due to the substitution of −NH− for −O−. However, the trisubstituted nitrogen compounds exhibit low K_i values and, in some instances, excellent selectivity. The low K_i values of some of these compounds for *Pf*HGXPRT and *Pv*HGPRT and the high selectivity in favor of these enzymes compared with human HGPRT suggest that they are lead compounds for development as antimalarial drugs. Docking of these compounds suggests how these compounds bind in the active site of human HGPRT and provides preliminary explanations for their K_i values. Further chemical modifications can now be made to improve both potency and selectivity. Work is ongoing to obtain crystal structures of the three enzymes in complex with the aza-ANPs to verify the docking studies and to determine more precisely how these molecules bind in the active site.

EXPERIMENTAL SECTION

Synthesis and Analytical Chemistry. Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and the compounds were dried over P_2O_5 at 2 kPa. NMR spectra were recorded on Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) and Bruker Avance 400 (^{1}H at 400 MHz, ^{13}C at 100.6 MHz) spectrometers with TMS as internal standard or referenced to the residual solvent signal. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer. The purity of the tested compounds was determined by the combustion analysis (C, H, N) and was higher than 95%. The chemicals were obtained from commercial sources (Sigma-Aldrich) or prepared according to the published procedures. Dimethylformamide and acetonitrile were distilled from P_2O_5 and stored over molecular sieves (4 Å). THF was distilled from sodium/benzophenone under argon. Preparative HPLC purifications were performed on columns packed with 7 μm C18 reversed phase resin (Waters Delta 600 chromatograph column), 17 mm × 250 mm, in ca. 200 mg batches of mixtures using gradient MeOH/H_2O as eluent. Chromatography on Dowex 1 × 2 (acetate form) was as follows: after application of the aqueous solution of the crude product onto the column, it was washed with water until the UV absorption was low. The column was then washed with a gradient of acetic (0−1 M) followed by a gradient of formic acid (0.125−2 M).

Synthesis of Diethyl 2-(2-Hydroxyethylamino)-ethylphosphonate (1) and Tetraethyl 2,2′-(2-Hydroxyethylazanediyl)bis(ethane-2,1-diyl)diphosphonate (2a). The mixture of 2-aminoethanol (10.7 g, 175 mmol), H_2O (250 mL), and diethyl vinylphosphonate (16.4 g, 100 mmol) was stirred for 2 days at roomtemperature. Solvent was evaporated and the residue codistilled with ethanol. The resulting compound was purified by chromatography on silica gel ($CHCl_3$−MeOH). Monophosphonate **1**

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was obtained as a major product, bisphosphonate **2a** as a minor product.

1: oil, 17.22 g, 77%. ^{1}H NMR (DMSO-d_6): 3.97 m, 4 H (Et); 3.42 t, 2 H, J = 5.7 (CH_2O); 2.70 m, 2 H (CH_2N); 2.55 t, 2 H, J = 5.7 (CH_2N); 1.89 m, 2 H (CH_2P); 1.22 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 60.48 d, 2 C, J(P,C) = 6.2 (Et); 59.95 (C−O); 50.85 (C−N); 42.54 d, J(P,C) = 2.5 (C-NP); 25.48 d, J(P,C) = 136.4 (C−P); 15.91 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 226 [M + H]$^+$.

2: oil, 3.05 g, 8%. ^{1}H NMR (DMSO-d_6): 4.42 t, 1 H, J = 5.6 (OH); 3.97 m, 8 H (Et); 3.42 q, 2 H, J = 5.9 (CH_2O); 2.67 m, 4 H (CH_2N); 2.47 t, 2 H, J = 6.0 (CH_2N); 1.87 m, 4 H (CH_2P); 1.22 t, 12 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 60.72 d, 4 C, J(P,C) = 6.3 (Et); 58.90 (C−O); 54.53 (C−N); 46. 27, 2 C (C−N); 22.34 d, 2 C, J(P,C) = 135.2 (C−P); 16.11 d, 4 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 390 [M + H]$^+$.

Synthesis of 2b−2g from Diethyl 2-(2-Hydroxyethylamino)-ethylphosphonate (1). *Method A.* Alkylation: The mixture of **1** (6 g, 26.7 mmol) and K_2CO_3 (3.7 g, 26.7 mmol) in dry acetonitrile (80 mL) was cooled to −10 °C and a corresponding halogenoderivative (40 mmol) was added. The reaction mixture was stirred at −10 °C for 1 h and then at room temperature for 2 days. The solvent was then removed by evaporatation. Water and $CHCl_3$ were added, and the organic layer was separated, washed with brine, and dried over anhydrous $MgSO_4$. After filtration, solvent was evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$−MeOH), the product was obtained as yellow oil.

Method B. Michael addition: A corresponding vinylderivative (45 mmol) was added to the solution of **1** (6.75 g, 30 mmol) in water (70 mL). The reaction mixture was stirred overnight at room temperature, evaporated, and the residue codistilled with toluene. The resulting compound was purified by column chromatography on silica gel ($CHCl_3$−MeOH), and the product was obtained as yellow oil.

*Ethyl 2-((2-(Diethoxyphosphoryl)ethyl)(2-hydroxyethyl)amino)-acetate (**2b**).* Method A, starting from ethyl bromoacetate, yield 64%. ^{1}H NMR (DMSO-d_6): 4.43 t, J = 5.5 (OH); 4.07 q, 2 H, J = 7.1 (Et); 3.97 m, 4 H (Et); 3.43 m, 2 H (CH_2O); 3.41 s, 2 H (CH_2CO); 2.83 m, 2 H (CH_2N); 2.63 t, 2 H, J = 6.0 (CH_2N); 1.90 m, 2 H (CH_2P); 1.21 t, 6 H, J = 7.1 (Et); 1.18 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 60.73 d, 2 C, J(P,C) = 6.3 (Et); 59.59 (Et); 59.22 (C−O); 55.36, 54.19, and 47.34 (C−N); 23.53 d, J(P,C) = 134.1 (C−P); 16.12 d, 2 C, J(P,C) = 5.8 (Et); 14.01 (Et). MS (ESI): m/z = 312 [M + H]$^+$.

*Methyl 3-((2-(Diethoxyphosphoryl)ethyl)(2-hydroxyethyl)amino)-propanoate (**2c**).* Method B, starting from methyl acrylate, yield 50%. ^{1}H NMR (DMSO-d_6): 4.35 t, J = 5.4 (OH); 3.97 m, 4 H (Et); 3.58 s, 3 H (Me); 3.40 q, 2 H, J = 5.8 (CH_2O); 2.71 t, 2 H, J = 7.0 (CH_2CO); 2.66 m, 2 H (CH_2N); 2.46 t, 2 H, J = 6.2 (CH_2N); 2.40 t, 2 H, J = 7.0 (CH_2N); 1.86 m, 2 H (CH_2P); 1.22 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 172.36 (CO); 60.70 d, 2 C, J(P,C) = 6.2 (Et); 59.01 (C−O); 54.98, 48.64, and 46.75 (C−N); 51.06 (Me); 31.86 (C−CO); 22.34 d, J(P,C) = 134.9 (C−P); 16.12 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 312[M + H]$^+$.

*Methyl 3-((2-(Diethoxyphosphoryl)ethyl)(2-hydroxyethyl)amino)-butanoate (**2d**).* Method A, additional heating 70 h, 80 °C, starting from methyl 4-chlorobutyrate, yield 77%. ^{1}H NMR (DMSO-d_6): 4.36 t, J = 5.4 (OH); 3.97 m, 4 H (Et); 3.57 s, 3 H (Me); 3.40 q, 2 H, J = 6.0 (CH_2O); 2.65 m, 2 H (CH_2N); 2.43 t, 2 H, J = 6.2 (CH_2N); 2.39 t, 2 H, J = 7.0 (CH_2N); 2.32 t, 2 H, J = 7.3 (CH_2N); 1.85 m, 2 H (CH_2P); 1.60 p, 2 H, J = 7.2 (CH_2); 1.22 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 60.76 d, 2 C, J(P,C) = 6.2 (Et); 59.03 (C−O); 55.16, 52.21, and 46.83 (C−N); 51.06 (Me); 30.87 (C−CO); 22.04 d, J(P,C) = 134.8 (C−P); 22.07; 16.19 d, 2 C, J(P,C) = 2.8 (Et). MS (ESI): m/z = 326 [M + H]$^+$.

*Diethyl 2-(2-Cyanomethylamino)ethylphosphonate (**2e**).* Method A, starting from chloroacetonitrile, yield 65%. ^{1}H NMR (DMSO-d_6): 4.61 t, J = 5.4 (OH); 3.98 m, 4 H (Et); 3.82 s, 3 H (CH_2CN); 3.48 q, 2 H, J = 5.5 (CH_2O); 2.66 m, 2 H (CH_2N); 2.53 t, 2 H, J = 5.8 (CH_2N); 1.97, 2 H (CH_2P); 1.23 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 116.13 (CN); 60.86 d, 2 C, J(P,C) = 6.2 (Et); 58.92 (C−O); 55.03 (C−N); 51.51 (C−N); 46.89 (C−N); 22.87 (C); 22.20 d, J(P,C) = 135.0 (C−P); 16.11 d, 2 C, J(P,C) = 5.8 (Et); 16.64 (C−CN). MS (ESI): m/z = 293 [M + H]$^+$.

*Diethyl 2-(2-Cyanoethylamino)ethylphosphonate (**2f**).* Method B, starting from acrylonitrile, yield 78%. ^{1}H NMR (DMSO-d_6): 4.44 t, J = 5.4 (OH); 3.98 m, 4 H (Et); 3.44 q, 2 H, J = 6.0 (CH_2O); 2.75 t, 2 H, J = 6.8(CH_2N); 2.72 m, 2 H (CH_2N); 2.57 t, 2 H, J = 6.8 (CH_2CN); 2.53 t, 2 H, J = 6.1 (CH_2N); 1.91 m, 2 H (CH_2P); 1.22 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 119.94 (CN); 60.76 d, 2 C, J(P,C) = 6.2 (Et); 59.14 (C−O); 54.66 (C−N); 48.83 (C−N); 46.77 (C−N); 22.60 d, J(P,C) = 133.9 (C−P); 16.10 d, 2 C, J(P,C) = 5.3 (Et); 15.51 (C−CN). MS (ESI): m/z = 279 [M + H]$^+$.

*Diethyl 2-(2-Cyanopropylamino)ethylphosphonate (**2g**).* Method A, KI (0.4 g, 2.6 mmol) added, additional heating 70 h, 80 °C, starting from 4-chlorobutyronitrile, yield 69%. ^{1}H NMR (DMSO-d_6): 4.40 t, J = 5.4 (OH); 3.98 m, 4 H (Et); 3.42 dd, 2 H, J = 11.3 and 5.7 (CH_2O); 2.66 m, 2 H (CH_2N); 2.50 m, 6 H, J = 6.8 (CH_2N and CH_2CN); 1.89 m, 2 H (CH_2P); 1.64 m, 2 H, (CH_2); 1.23 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 120.62 (CN); 60.71 d, 2 C, J(P,C) = 6.3 (Et); 58.92 (C−O); 55.46 (C−N); 47.47 (C−N); 41.65 (C−N); 22.26 d, J(P,C) = 136.6 (C−P); 16.11 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 287 [M + Na]$^+$.

*Diethyl 2-((2-Hydroxyethyl)(2-(trityloxy)ethyl)amino)-ethylphosphonate (**2h**).* The mixture of diethanolamine (2.1 g, 20 mmol), H_2O (50 mL), and diethyl vinylphosphonate (3.3 g, 20 mmol) was stirred at room temperature overnight. The solvent was evaporated and the residue codistilled with ethanol. The residue was purified by chromatography on silica gel ($CHCl_3$−MeOH) to obtain diethyl 2-(bis(2-hydroxyethyl)amino)ethylphosphonate, yield 5.03 g (93%). ^{1}H NMR (DMSO-d_6): 4.35 t, 2 H, J = 5.5 (OH); 3.98 m, 4 H (Et); 3.41 q, 4 H, J = 6.0 (CH_2O); 2.71 m, 2 H (CH_2N); 2.49 m, 4 H (CH_2N); 1.89 m, 2 H (CH_2P); 1.22 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 60.71 d, 2 C, J(P,C) = 6.3 (Et); 58.97, 2 C (C−O); 55.68, 2 C (C−N); 47.43 d, J(P,C) = 1.5 (C−N); 22.42 d, J(P,C) = 134.9 (C−P); 16.12 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 270 [M + H]$^+$. This intermediate (3.4 g, 12.6 mmol) was dissolved in CH_2Cl_2 (50 mL), a catalytical amount of dimethylaminopyridine was added followed by Et_3N (1.2 mL), and then tritylchloride (3.5 g, 14.5 mmol) dissolved in CH_2Cl_2 (30 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature and solvent evaporated. The residue was purified by column chromatography on silica gel ($CHCl_3$−MeOH) to afford the product as yellowish oil (6.01 g, 93%). ^{1}H NMR (DMSO-d_6): 7.38 m, 6 H, 7.33 m, 6 H and 7.25 m, 3 H (Ar); 4.35 t, J = 5.5 (OH); 3.92 m, 4 H (Et); 3.38 m, 2 H (CH_2O); 3.01 t, 2 H, J = 6.0 (CH_2O); 2.70 m, 2 H (CH_2N); 2.66 t, 2 H, J = 6.0 (CH_2N); 2.47 t, 2 H, J = 6.3 (CH_2N); 1.87 m, 2 H (CH_2P); 1.17 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 143.83, 3 C (Ar); 128.07, 6 C (Ar); 127.68, 6 C (Ar); 126.78, 3 C (Ar); 85.98 (Tr); 62.11 (C−O-Tr); 60.65 d, 2 C, J(P,C) = 6.2 (Et); 59.1 (C−O); 55.88, 2 C (C−N); 53.06 (C−N); 47.73 (C−N); 22.62 d, J(P,C) = 135.2 (C−P); 16.09 d, 2 C, J(P,C) = 5.4 (Et). MS (ESI): m/z = 512 [M + H]$^+$.

Synthesis of N^9-Substituted 6-Chloropurines 3a−3h via Mitsunobu Reaction, General Procedure. To a solution of triphenylphosphine (6.3 g, 24 mmol) in dry THF (100 mL) cooled to −30 °C under argon atmosphere, diisopropylazadicarboxylate (DIAD, 4.4 mL, 23 mmol) was added slowly. The mixture was stirred for 30 min, and this preformed complex was added to chloropurine (3.5 g, 22.6 mmol), dry THF (70 mL), and corresponding diethyl phosphonate **2a−2h** (11.2 mmol) at −30 °C under argon. The resulting mixture was slowly warmed to room temperature and stirred for 48 h. Solvent was evaporated, and the crude mixture was purified by chromatography on silica gel (MeOH−$CHCl_3$). The pure product was obtained as yellowish foam.

*Tetraethyl 9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3a**).* Starting from diethyl phosphonate **2a**, yield 80%. ^{1}H NMR (DMSO-d_6): 8.78 s, 1 H and 8.77 s, 1 H (H-2 and H-8); 4.33 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.92 m, 8 H (Et); 2.85 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.64 dd, 4 H, J = 9.4, J_g = 15.6 (H-3′ and H-5′); 1.70 m, 4 H (H-4′ and H-6′); 1.19 t, 12 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 151.86 (C-4); 151.14 (C-2); 148.70 (C-6); 147.93 (C-8); 130.58 (C-

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5); 60.73 d, 4 C, J(P,C) = 6.3 (Et); 51.06 (C-2′); 46.04, 2 C (C-3′ and C-5′); 42.00 (C-1′); 22.26 d, 2 C, J(P,C) = 135.0 (C-4′and C-6′); 16.05 d, 4 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 526 $[M + H]^+$.

*Diethyl 9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3b**).* Starting from diethyl phosphonate **2b**, yield 79%. ^{1}H NMR (DMSO-d_6): 8.77 s, 1 H and 8.72 s, 1 H (H-2 and H-8); 4.34 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.90 m, 4 H (Et); 3.41 s, 2 H (H-5′); 3.01 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.22 m, 2 H (H-3′); 1.67 m, 2 H (H-4′); 1.17 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 170.64 (CO); 151.99 (C-4); 151.19 (C-2); 149.11 (C-6); 147.99 (C-8); 130.60 (C-5); 60.79 d, 2 C, J(P,C) = 6.1 (Et); 59.78 (Et); 53.45, 51.83, and 47.04 (C-2′, C-5′ and C-3′); 41.94 (C-1′); 22.97 d, J(P,C) = 134.2 (C-4′); 16.15 d, 2 C, J(P,C) = 5.7 (Et); 14.11 (Et). MS (ESI): m/z = 448 $[M + H]^+$.

*Diethyl 9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3c**).* Starting from diethyl phosphonate **2c**, yield 56%. ^{1}H NMR (DMSO-d_6): 8.77 s, 1 H and 8.64 s, 1 H (H-2 and H-8); 4.32 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.94 m, 4 H (Et); 3.45 s, 3 H (Me); 2.86 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.66 m, 4 H (H-3′ and H-5′); 2.19 t, 2 H, J(6′,5′) = 6.7 (H-6′); 1.74 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 171.98 (CO); 151.85 (C-4); 151.12 (C-2); 148.66 (C-6); 147.78 (C-8); 130.62 (C-5); 60.74 d, 2 C, J(P,C) = 6.3 (Et); 51.33, 48.06, and 46.15 (C-2′, C-3′ and C-5′); 50.95 (Me); 41.90 (C-1′); 31.67 (C-6′); 21.93 d, J(P,C) = 134.7 (C-4′); 16.09 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 448 $[M + H]^+$.

*Diethyl 9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3d**).* Starting from diethyl phosphonate **2d**, yield 83%. ^{1}H NMR (DMSO-d_6): 8.76 s, 1 H and 8.71 s, 1 H (H-2 and H-8); 4.32 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.95 m, 4 H (Et); 3.50 s, 3 H (Me); 2.83 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.65 dd, 2 H, J = 15.8 and 8.5 (H-3′); 2.35 t, 2 H, J(5′,6′) = 6.8 (H-5′); 1.96 t, 2 H, J(7′,6′) = 7.4 (H-7′); 1.76 m, 2 H (H-4′); 1.33 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 172.94 (CO); 151.83 (C-4); 151.13 (C-2); 148.75 (C-6); 147.79 (C-8); 130.61 (C-5); 60.73 d, 2 C, J(P,C) = 6.3 (Et); 51.42, 51.40, and 46.22 (C-2′, C-3′ and C-5′); 50.93 (Me); 42.01 (C-1′); 30.29 (C-6′); 21.80 d, J(P,C) = 134.6 (C-4′); 21.77 (C-7′); 16.08 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 462 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3e**).* Starting from diethyl phosphonate **2e**, yield 87%. ^{1}H NMR (DMSO-d_6): 8.78 s, 1 H and 8.70 s, 1 H (H-2 and H-8); 4.41 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.92 m, 4 H (Et); 3.85 s, 2 H(H-5′); 2.95 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.67 dd, 2 H, J = 9.8 and 16.00 (H-3′); 1.78 m, 2 H (H-4′); 1.18 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 151.93 (C-4); 151.20 (C-2); 148.72 (C-6); 147.65 (C-8); 130.57 (C-5); 115.95 (CN); 60.84 d, 2 C, J(P,C) = 6.2 (Et); 52.03 and 47.07 (C-2′ and C-3′); 41.27 and 40.99 (C-1′ and C-5′); 23.02 d, J(P,C) = 136.4 (C-4′); 16.04 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 401 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3f**).* Starting from diethyl phosphonate **2f**, yield 85%. ^{1}H NMR (DMSO-d_6): 8.77 s, 1 H and 8.71 s, 1 H (H-2 and H-8); 4.35 t, 2 H, J(1′,2′) = 5.9 (H-1′); 3.93 m, 4 H (Et); 2.91 t, 2 H, J(2′,1′) = 5.9 (H-2′); 2.74 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.67 dd, 2 H, J = 8.5 and 16.0 (H-3′); 2.47 t, 2 H, J(6′,5′) = 7.0 (H-6′); 1.73 m, 2 H (H-4′); 1.19 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 151.93 (C-4); 151.17 (C-2); 148.74 (C-6); 147.65 (C-8); 130.65 (C-5); 119.52 (CN); 60.79 d, 2 C, J(P,C) = 6.2 (Et); 51.26, 48.02, and 46.14 (C-2′, C-5′ and C-3′); 41.85 (C-1′); 22.22 d, J(P,C) = 134.6 (C-4′); 16.09 d, 2 C, J(P,C) = 5.7 (Et); 15.42 (C-6′). MS (ESI): m/z = 415 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3g**).* Starting from diethyl phosphonate **2g**, yield 81%. ^{1}H NMR (DMSO-d_6): 8.89 s, 1 H and 8.85 s, 1 H (H-2 and H-8); 4.45 t, 2 H, J(1′,2′) = 6.0 (H-1′); 4.06 m, 4 H (Et); 2.97 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.77 dd, 2 H, J = 9.0 and 15.7 (H-3′); 2.55 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.33 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.89 m, 2 H (H-4′); 1.56 m, 2 H (H-6′); 1.32 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 152.36 (C-4); 151.20 (C-2); 148.82 (C-6); 147.77 (C-8); 129.17 (C-5); 120.23 (CN); 60.75 d, 2 C, J(P,C) = 6.3 (Et); 51.51, 51.05, and 46.20 (C-2′, C-5′ and C-3′); 41.97 (C-1′); 22.57 (C-6′); 16.09 d, 2 C, J(P,C) = 5.7 (Et); 13.43 (C-7′). MS (ESI): m/z = 429 $[M + H]^+$.

*Diethyl 9-[(N-(2-(Trityloxy)ethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-6-chloropurine (**3h**).* Starting from diethyl phosphonate **2h**, yield 76%. ^{1}H NMR (DMSO-d_6): 8.65 s, 1 H and 8.62 s, 1 H (H-2 and H-8); 7.25 m, 15 H (Tr); 4.29 t, 2 H, J(1′,2′) = 5.7(H-1′); 3.87 m, 4 H (Et); 2.87 t, 2 H, J(2′,1′) = 5.7 (H-2′); 2.77 t, 2 H, J(6′,5′) = 5.8 (H-6′); 2.67 m, 4 H (H-3′ and H-5′); 1.73 m, 2 H (H-4′); 1.13 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 151.73 (C-4); 151.01 (C-2); 148.65 (C-6); 147.70 (C-8); 143.59, 3 C (Tr); 130.50 (C-5); 127.92, 6 C (Tr); 127.61, 6 C (Tr); 126.75, 3 C (Tr); 86.02 (Tr); 61.96 (C-6′); 60.66 d, 2 C, J(P,C) = 6.2 (Et); 52.42, 52.18, and 47.23 (C-2′, C-3′ and C-5′); 41.98 (C-1′); 22.28 d, J(P,C) = 134.0 (C-4′); 16.04 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 648 $[M + H]^+$.

Synthesis of N^9-Substituted 2-Amino-6-Chloropurines 4a−4i via Mitsunobu Reaction, General Procedure. Starting from 2-amino-6-chloropurine and diethyl phosphonates **2a**−**2h** the procedure was identical as described above for 6-chloropurine. Then after the stirring of reaction mixture overnight, water (30 mL) was added and the mixture was heated at 80 °C for 30 h. Solvent was evaporated, the residue was codistilled with toluene or ethanol, and the crude mixture purified by chromatography on silica gel (MeOH−$CHCl_3$). The pure product was isolated as yellow foam.

*Tetraethyl 9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4a**).* Starting from diethyl phosphonate **2a**, yield 79%. ^{1}H NMR (DMSO-d_6): 8.17 s, 1 H (H-8); 6.89 s, 2 H (NH_2); 4.07 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.93 m, 8 H (Et); 2.76 t, 2 H, J(2′,1′) = 5.7 (H-2′); 2.63 dd, 4 H, J = 15.6 and 9.2 (H-6′ and H-5′); 1.72 m, 4 H (H-4′ and H-6′); 1.19 t, 12 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 159.54 (C-2); 153.95 (C-4); 149.05 (C-6); 143.78 (C-8); 123.15 (C-5); 60.77 d, 4 C, J(P,C) = 6.3 (Et); 51.03 (C-2′); 46.12, 2 C (C-3′ and C-5′); 41.43 (C-1′); 22.33 d, 2 C, J(P,C) = 134.8 (C-4′and C-6′); 16.09 d, 4 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 541 $[M + H]^+$.

*Diethyl 9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4b**).* Starting from diethyl phosphonate **2b**, yield 47%. ^{1}H NMR (DMSO-d_6): 8.14 s, 1 H (H-8); 6.87 s, 2 H (NH_2); 4.08 t, 2 H, J(1′,2′) = 5.4 (H-1′); 4.03 dd, 2 H, J = 14.2 and 7.2 (Et); 3.91 m, 4 H (Et); 3.41 s, 1 H (H-5′); 2.94 t, 2 H, J(2′,1′) = 5.4 (H-2′); 2.76 dd, 2 H, J = 15.92 and 8.4 (H-3′); 1.68 m, 2 H (H-4′); 1.18 t, 6 H, J = 7.0 (Et); 1.15 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 170.70 (CO); 159.59 (C-2); 154.05 (C-4); 149.05 (C-6); 143.78 (C-8); 123.17 (C-5); 60.84 d, 2 C, J(P,C) = 6.1 (Et); 59. 79 (Et); 53.52, 51.76, and 47.10 (C-2′, C-3′ and C-5′); 41.32 (C-1′); 23.34 d, J(P,C) = 133.9 (C-4′); 16.13 d, 2 C, J(P,C) = 5.7 (Et); 13.98 (Et). MS (ESI): m/z = 463 $[M + H]^+$

*Diethyl 9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4c**).* Starting from diethyl phosphonate **2c**, yield 59%. ^{1}H NMR (DMSO-d_6): 8.05 s, 1 H (H-8); 6.87 s, 2 H (NH_2); 4.05 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.94 m, 4 H (Et); 3.51 s, 3 H (Me); 2.75 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.67 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.63 m, 2 H (H-3′); 2.26 t, 2 H, J(6′,5′) = 6.8 (H-6′); 1.73 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 172.13 (CO); 159.52 (C-2); 153.92 (C-4); 149.00 (C-6); 143.61 (C-8); 123.18 (C-5); 60.77 d, 2 C, J(P,C) = 6.1 (Et); 51.03 (Me); 51.35, 48.08, and 46.24 (C-2′, C-3′ and C-5′); 41.26 (C-1′); 31.71 (C-6′); 22.12 d, J(P,C) = 135.2 (C-4′); 16.11 d, 2 C, J(P,C) = 5.6 (Et). MS (ESI): m/z = 463 $[M + H]^+$.

*Diethyl 9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4d**).* Starting from diethyl phosphonate **2d**, yield 80%. ^{1}H NMR (DMSO-d_6): 8.11 s, 1 H (H-8); 6.86 s, 2 H (NH_2); 4.06 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.95 m, 4 H (Et); 3.53 s, 3 H (Me); 2.74 t, 2 H, J(2′,1′) = 5.7 (H-2′); 2.74 dd, 2 H, J = 15.8 and 8.4 (H-3′); 2.36 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.07 t, 2 H, J(7′,6′) = 7.4 (H-7′); 1.75 m, 2 H (H-4′); 1.42 m, 2 H, (H-6′); 1.21 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 173.08 (CO); 159.51 (C-2); 153.92 (C-4); 149.05 (C-6); 143.60 (C-8); 123.19 (C-5); 60.76 d, 2 C, J(P,C) = 6.3 (Et); 50.97 (Me); 51.43, 51.40, and 46.28 (C-2′, C-3′ and C-5′); 41.32 (C-1′); 30.42 (C-6′); 21.94 d, J(P,C) = 134.5 (C-4′); 21.84 (C-7′); 16.10 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 477 $[M + H]^+$.

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*Diethyl 9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4e**).* Starting from diethyl phosphonate **2e**, yield 73%. ^{1}H NMR (DMSO-d_6): 8.12 s, 1 H (H-8); 6.90 s, 2 H (NH_2); 4.15 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.92 m, 4 H (Et); 3.86 s, 2 H (H-5′); 2.86 t, 2 H, J(2′,1′) = 5.7 (H-2′); 2.67 dd, 2 H, J = 15.9 and 9.6 (H-3′); 1.79 m, 2 H (H-4′); 1.19 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 159.56 (C-2); 154.03 (C-4); 149.08 (C-6); 143.40 (C-8); 124.07 (C-5); 115.91 (CN); 60.90 d, 2 C, J(P,C) = 6.2 (Et); 52.02, 47.03, 40.98, and 40.48 (C-2′, C-3′, C-5′ and C-1′); 23.13 d, J(P,C) = 136.1 (C-4′); 16.07 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 416 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4f**).* Starting from diethyl phosphonate **2f**, yield 78%. ^{1}H NMR (DMSO-d_6): 8.14 s, 1 H (H-8); 6.89 s, 2 H (NH_2); 4.08 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.93 m, 4 H (Et); 2.83 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.76 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.66 dd, 2 H, J = 16.0 and 8.3 (H-3′); 2.53 t, 2 H, J(6′,5′) = 6.6 (H-6′); 1.73 m, 2 H (H-4′); 1.19 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 159.55 (C-2); 153.96 (C-4); 149.08 (C-6); 143.49 (C-8); 123.17 (C-5); 119.73 (CN); 60.84 d, 2 C, J(P,C) = 6.3 (Et); 51.23, 48.15, and 46.31 (C-2′, C-3′ and C-5′); 41.30 (C-1′); 22.41 d, J(P,C) = 134.5 (C-4′); 16.09 d, 2 C, J(P,C) = 5.7 (Et); 15.45 (C-6′). MS (ESI): m/z = 430 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-2-amino-6-chloropurine (**4g**).* Starting from diethyl phosphonate **2g**, yield 66%. ^{1}H NMR (DMSO-d_6): 8.14 s, 1 H (H-8); 6.88 s, 2 H (NH_2); 4.08 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.95 m, 4 H (Et); 2.76 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.64 dd, 2 H, J = 15.7 and 8.8 (H-3′); 2.44 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.27 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.77 m, 2 H (H-4′); 1.49 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 159.53 (C-2); 153.94 (C-4); 149.14 (C-6); 143.55 (C-8); 123.17 (C-5); 120.35 (CN); 60.78 d, 2 C, J(P,C) = 6.3 (Et); 51.48, 51.02, and 46.33 (C-2′, C-3′ and C-5′); 41.28 (C-1′); 22.63 (C-6′); 21.95 d, J(P,C) = 136.2 (C-4′); 16.11 d, 2 C, J(P,C) = 5.7 (Et); 13.49 (C-7′). MS (ESI): m/z = 444 $[M + H]^+$.

*Diethyl 9-[(N-(2-Trityloxyoxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]2-amino-6-chloropurine (**4h**).* **4h** was isolated as the triphenylphosphoranylidene intermediate MS (ESI) m/z = 923 $[M + H]^+$, starting from diethyl phosphonate **2h**, yield 73%. This compound was detritylated to enable cleavage of the triphenylphosphoranylidene moiety and hydrolysis of the 6-chlorogroupduring the next steps (see synthesis of **4i** and **6i**).

*Diethyl 9-[(N-(2-Hydoxyoxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]2-amino-6-chloropurine (**4i**).* Intermediate **4h** (5 g, 5.4 mmol) in 75% aqueous trifluoroacetic acid was stirred overnight at room temperature, solvents were evaporated and the residue was codistilled with water and ethanol. The crude mixture was purified by chromatography on silica gel (MeOH−$CHCl_3$) to obtain 2.2 g (60%) of detritylated compound **4i** in the form of triphenylphosphoranylidene derivative. ^{1}H NMR (DMSO-d_6): 8.37 s, 1 H (H-8); 7.95 m, 6 H, 7.86 m, 3 H and 7.22 m, 6 H (Ar); 4.41 t, 2 H, J(1′,2′) = 6.2 (H-1′); 4.06 m, 4 H (Et); 23.70 t, 2 H, J(6′,5′) = 5.0 (H-6′); 3.28 m, 4 H, (H-2′ and H-3′); 3.09 m, 2 H (H-5′); 2.16 m, 2 H (H-4′); 1.29 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 162.70 d, J(P,C) = 35.3 (C-2); 154.71 d, J(P,C) = 2.8(C-4); 151.34 (C-6); 147.52 (C-8); 136.13 d, J(P,C) = 2.2, 134.95 d, J(P,C) = 11.2 and 131.00 d, J(P,C) = 13.6 (PPh_3); 128.33 (C-5); 122.27 d, J(P,C) = 104.9 (PPh_3); 63.84 d, 2 C, J(P,C) = 6.6 (Et); 58.26 (C-6′); 56.01 (C-5′); 53.82 (C-2′); 49.31 (C-3′); 41.73 (C-1′); 22.43 d, J(P,C) = 139.4 (C-4′); 16.68 d, 2 C, J(P,C) = 6.0 (Et). MS (ESI): m/z = 681 $[M + H]^+$.

Synthesis of Hypoxanthine Derivatives 5a−5g and 5i and Guanine Derivatives 6a−6g and 6i, General Procedure. The corresponding 6-chloropurine derivative **3a−3h** (2 mmol) or the corresponding 2-amino-6-chloropurine derivative **4a−4g**,**4i** was dissolved in trifluoroacetic acid (aqueous, 75%, 20 mL) and stirred overnight. The solvent was evaporated and the residue codistilled with water (3×) and ethanol. After chromatography on silica gel (MeOH−$CHCl_3$), the pure products were colorless foams.

*Tetraethyl 9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]-hypoxanthine (**5a**).* Starting from diethyl phosphonate **3a**, yield 67%. ^{1}H NMR (DMSO-d_6): 12.29 s, 1 H (NH); 8.14 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.15 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.94 m, 8 H (Et); 2.79 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.63 dd, 4 H, J = 15.6 and 9.16 (H-3′ and H-5′); 1.73 m, 4 H (H-4′ and H-6′); 1.20 t, 12 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.43 (C-6); 148.20 (C-4); 145.31 (C-2); 140.54 (C-8); 123.66 (C-5); 60.97 d, 4 C, J(P,C) = 6.1 (Et); 51.14 (C-2′); 46.28, 2 C (C-3′ and C-5′); 41.02 (C-1′); 21.75 d, 2 C, J(P,C) = 135.9 (C-4′and C-6′); 16.08 d, 4 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 508 $[M + H]^+$.

*Diethyl 9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5b**).* Starting from diethyl phosphonate **3b**, yield 75%. ^{1}H NMR (DMSO-d_6): 12.26 s, 1 H (NH); 8.09 s, 1 H and 8.02 s, 1 H (H-2 and H-8); 4.17 t, 2 H, J(1′,2′) = 6.0 (H-1′); 4.04 q, 2 H, J(1′,2′) = 7.1 (Et); 3.91 m, 4 H (Et); 3.42 s, 2 H (H-5′); 2.96 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.76 dd, 2 H, J = 8.4 and 16.2 (H-3′); 1.69 m, 2 H (H-4′); 1.18 t, 6 H, J = 7.1 (Et);1.16 t, 3 H, J = 7.2 (Et). ^{13}C NMR (DMSO-d_6): 170.72 (CO); 156.59 (C-6); 148.31 (C-4); 145.20 (C-2); 140.72 (C-8); 123.66 (C-5); 60.82 d, 2 C, J(P,C) = 6.2 (Et); 59.78 (Et); 53.51, 52.22, and 47.19 (C-2′, C-5′ and C-3′); 41.59 (C-1′); 23.40 d, J(P,C) = 134.3 (C-4′); 16.14 d, 2 C, J(P,C) = 5.8 (Et); 14.01 (Et). MS (ESI): m/z = 430 $[M + H]^+$.

*Diethyl 9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5c**).* Starting from diethyl phosphonate **3c**, yield 66%. ^{1}H NMR (DMSO-d_6): 12.25 s, 1 H (NH); 8.02 s, 2 H (H-2 and H-8); 4.14 t, 2 H, J(1′,2′) = 5.9 (H-1′); 3.94 m, 4 H (Et); 3.51 s, 3 H (Me); 2.78 t, 2 H, J(2′,1′) = 5.9 (H-2′); 2.68 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.63 m, 2 H (H-3′); 2.27 t, 2 H, J(6′,5′) = 6.7 (H-6′); 1.74 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 172.14 (CO); 156.50 (C-6); 148.17 (C-4); 145.09 (C-2); 140.53 (C-8); 123.69 (C-5); 60.75 d, 2 C, J(P,C) = 6.2 (Et); 51.02 (Me); 51.89, 48.15, and 46.31 (C-2′, C-5′ and C-3′); 41.51 (C-1′); 31.77 (C-6′); 22.11 d, J(P,C) = 134.7 (C-4′); 16.08 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 430 $[M + H]^+$.

*Diethyl 9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5d**).* Starting from diethyl phosphonate **3d**, yield 42%. ^{1}H NMR (DMSO-d_6): 12.26 s, 1 H (NH); 8.07 s, 1 H and 8.02 s, 1 H (H-2 and H-8); 4.15 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.95 m, 4 H (Et); 3.53 s, 3 H (Me); 2.77 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.63 dd, 2 H, J = 8.4 and 15.9 (H-3′); 2.36 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.06 t, 2 H, J(7′,6′) = 7.4 (H-7′); 1.76 m, 2 H (H-4′); 1.42 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 173.11 (CO); 156.52 (C-6); 148.21 (C-4); 145.11 (C-2); 140.57 (C-8); 123.74 (C-5); 60.75 d, 2 C, J(P,C) = 6.3 (Et); 51.91, 51.50, and 46.32 (C-2′, C-5′ and C-3′); 50.97 (Me); 41.60 (C-1′); 30.40 (C-7′); 21.92 d, J(P,C) = 134.7 (C-4′); 21.90 (C-6′); 16.11 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 444 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5e**).* Starting from diethyl phosphonate **3e**, yield 95%. ^{1}H NMR (DMSO-d_6): 12.30 s, 1 H (NH); 8.08 s, 1 H and 8.04 s, 1 H (H-2 and H-8); 4.24 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.93 m, 4 H (Et); 3.86 s, 2 H (H-5′); 2.88 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.65 dd, 2 H, J = 9.4 and 15.9 (H-3′); 1.78 m, 2 H (H-4′); 1.19 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 156.51 (C-6); 148.30 (C-4); 145.27 (C-2); 140.45 (C-8); 123.66 (C-5); 115.99 (CN); 60.91 d, 2 C, J(P,C) = 6.2 (Et); 52.38 and 46.34 (C-2′ and C-3′); 41.05 and 40.85 (C-5′ and C-1′); 23.12 d, J(P,C) = 136.3 (C-4′); 16.08 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 383 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5f**).* Starting from diethyl phosphonate **3f**, yield 95%. ^{1}H NMR (DMSO-d_6): 12.27 s, 1 H (NH); 8.10 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.17 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.94 m, 4 H (Et); 2.85 t, 2 H, J(2′,1′) = 6.1 (H-2′); 2.76 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.66 dd, 2 H, J = 8.3 and 15.9 (H-3′); 2.49 m, 2 H (H-6′); 1.74 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.57 (C-6); 148.24 (C-4); 145.21 (C-2); 140.49 (C-8); 123.71 (C-5); 119.70 (CN); 60.81 d, 2 C, J(P,C) = 6.2 (Et); 51.80, 48.22, and 46.38 (C-2′, C-5′ and C-3′); 41.54 (C-1′); 22.41 d, J(P,C) = 134.4 (C-4′); 16.11 d, 2 C, J(P,C) = 5.8 (Et); 15.49 (C-6′). MS (ESI): m/z = 397 $[M + H]^+$.

*Diethyl 9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5g**).* Starting from diethyl phosphonate

3g, yield 76%. ^{1}H NMR (DMSO-d_6): 12.28 s, 1 H (NH); 8.10 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.17 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.96 m, 4 H (Et); 2.79 t, 2 H, J(2′,1′) = 6.1 (H-2′); 2.65 dd, 2 H, J = 8.7 and 15.8 (H-3′); 2.44 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.24 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.79 m, 2 H (H-4′); 1.50 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.48 (C-6); 148.23 (C-4); 145.19 (C-2); 140.55 (C-8); 123.73 (C-5); 120.34 (CN); 60.77 d, 2 C, J(P,C) = 6.2 (Et); 51.90, 51.06, and 46.34 (C-2′, C-5′ and C-3′); 41.53 (C-1′); 22.66 (C-6′); 21.91 d, J(P,C) = 134.7 (C-4′); 16.12 d, 2 C, J(P,C) = 5.7 (Et); 13.41 (C-7′). MS (ESI): m/z = 411 [M + H]$^+$.

*Diethyl 9-[(N-(2-Hydroxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**5i**).* Starting from diethyl phosphonate **3h**, during the hydrolysis of 6-chloro group, the trityl moiety was simultaneously cleaved to form hydroxydetivative **5i**. The crude product was without chromatography used directly in the next step. MS (ESI): m/z = 388 [M + H]$^+$.

*Tetraethyl 9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]-guanine (**6a**).* Starting from diethyl phosphonate **4a**, yield 85%. ^{1}H NMR (DMSO-d_6): 10.76 s, 1 H (NH); 7.86 s, 1 H (H-8); 6.58 s, 2 H (NH$_2$); 4.07 t, 2 H, J(1′,2′) = 5.6 (H-1′); 3.97 m, 8 H (Et); 2.95 t, 2 H, J(2′,1′) = 5.6 (H-2′); 2.83 m, 4 H (H-6′ and H-5′); 1.91 m, 4 H (H-4′ and H-6′); 1.22 t, 12 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.30(C-6); 153.60 (C-2); 150.74 (C-4); 137.76 (C-8); 116.25 (C-5); 61.01 d, 4 C, J(P,C) = 6.2 (Et); 51.07 (C-2′); 46.31, 2 C (C-3′ and C-5′); 41.02 (C-1′); 21.26 d, 2 C, J(P,C) = 134.2 (C-4′and C-6′); 16.10 d, 4 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 523 [M + H]$^+$.

*Diethyl 9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6b**).* Starting from diethyl phosphonate **4b**, yield 95%. ^{1}H NMR (DMSO-d_6): 10.60 s, 1 H (NH); 7.71 s, 1 H (H-8); 6.46 s, 2 H (NH$_2$); 4.05 q, 2 H, J = 7.1 (Et); 3.97 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.92 m, 4 H (Et); 3.41 s, 1 H (H-5′); 2.90 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.76 dd, 2 H, J = 16.2 and 8.3 (H-3′); 1.718 m, 2 H (H-4′); 1.19 t, 6 H, J = 7.0 (Et); 1.16 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 170.76 (CO); 156.69 (C-8); 153.42 (C-2); 151.03 (C-4); 137.94 (C-8); 116.11 (C-5); 60.87 d, 2 C, J(P,C) = 6.2 (Et); 59. 81 (Et); 53.58, 52.10, and 47.20 (C-2′, C-3′ and C-5′); 41.14 (C-1′); 23.40 d, J(P,C) = 136.4 (C-4′); 16.15 d, 2 C, J(P,C) = 6.1 (Et); 14.03 (Et). MS (ESI): m/z = 445 [M + H]$^+$.

*Diethyl 9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6c**).* Starting from diethyl phosphonate **4c**, yield 73%. ^{1}H NMR (DMSO-d_6): 10.74 s, 1 H (NH); 7.79 s, 1 H (H-8); 6.56 s, 2 H (NH$_2$); 4.07 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.57 s, 3 H (Me); 3.99 m, 4 H (Et); 3.51 m, 4 H, (H-2′ and H-5′); 2.90 m, 4 H (H-3′ and H-6′); 1.91 m, 2 H (H-4′); 1.22 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 171.89 (CO); 156.29 (C-6); 153.58 (C-2); 150.72 (C-4); 137.61 (C-8); 117.13 (C-5); 61.01 d, 2 C, J(P,C) = 6.1 (Et); 51.25 (Me); 51.52, 48.05, and 46.55 (C-2′, C-3′ and C-5′); 39.22 (C-1′); 30.99 (C-6′); 22.94 d, J(P,C) = 134.4 (C-4′); 16.10 d, 2 C, J(P,C) = 5.8 (Et). MS (ESI): m/z = 445 [M + H]$^+$.

*Diethyl 9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6d**).* Starting from diethyl phosphonate **4d**, yield 57%. ^{1}H NMR (DMSO-d_6): 10.60 s, 1 H (NH); 7.65 s, 1 H (H-8); 6.47 s, 2 H (NH$_2$); 3.95 m, 6 H (H-1′ and Et); 3.55 s, 3H (Me); 2.70 m, 2 H, (H-2′); 2.62 m, 2 H (H-5′); 2.37 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.14 t, 2 H, J(7′,6′) = 7.3 (H-7′); 1.76 m, 2 H (H-4′); 1.48 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 173.20 (CO); 156.67 (C-6); 153.33 (C-2); 150.96 (C-4); 137.75 (C-8); 116.38 (C-5); 60.78 d, 2 C, J(P,C) = 6.2 (Et); 51.00 (Me); 51.84, 51.52, and 46.36 (C-2′, C-3′ and C-5′); 41.05 (C-1′); 30.51 (C-7′); 16.11 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 459 [M + H]$^+$.

*Diethyl 9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6e**).* Starting from diethyl phosphonate **4e**, yield 95%. ^{1}H NMR (DMSO-d_6): 10.66 s, 1 H (NH); 7.63 s, 1 H (H-8); 6.52 s, 2 H (NH$_2$); 4.04 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.94 m, 4 H (Et); 3.87 s, 2 H(H-5′); 2.82 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.65 dd, 2 H, J = 9.3 and 16.0 (H-3′); 1.81 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.67 (C-6); 153.42 (C-2); 151.05 (C-4); 137.55 (C-8); 116.28 (C-5); 115.94 (CN); 60.93 d, 2 C, J(P,C) = 6.2 (Et); 51.75 and 46.43 (C-2′ and C-3′); 41.01 and 40.20 (C-5′ and C-1′); 23.17 d, J(P,C) = 136.2 (C-4′); 16.08 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 398 [M + H]$^+$.

*Diethyl 9-[(N-(2-Cyanoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6f**).* Starting from diethyl phosphonate **4f**, yield 95%. ^{1}H NMR (DMSO-d_6): 10.63 s, 1 H (NH); 7.71 s, 1 H (H-8); 6.50 s, 2 H (NH$_2$); 3.94 m, 6 H (H-1′ and Et); 2.77 m, 4 H(H-2′ and H-5′); 2.66 dd, 2 H, J = 8.3 and 15.9 (H-3′); 2.54 t, 2 H, J(6′,5′) = 6.8 (H-3′); 1.75 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 156.66 (C-6); 153.41 (C-2); 150.96 (C-4); 137.69 (C-8); 119.82 (CN); 116.27 (C-5); 60.84 d, 2 C, J(P,C) = 6.3 (Et); 51.68, 48.30, and 46.48 (C-2′, C-3′ and C-5′); 22.46 d, J(P,C) = 134.5 (C-4′); 16.11 d, 2 C, J(P,C) = 5.8 (Et); 15.53 (C-6′). MS (ESI): m/z = 412 [M + H]$^+$.

*Diethyl 9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6g**).* Starting from diethyl phosphonate **4g**, yield 95%. ^{1}H NMR (DMSO-d_6): 10.66 s, 1 H (NH); 7.70 s, 1 H (H-8); 6.51 s, 2 H (NH$_2$); 3.96 m, 6 H (H-1′ and Et); 2.74 m, 2 H(H-2′); 2.66 m, 2 H (H-3′); 2.48 m, 2 H(H-5′); 2.29 t, 2 H, J(7′,6′) = 7.1 (H-7′); 1.82 m, 2 H (H-4′); 1.54 m, 2 H (H-6′); 1.21 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 156.65 (C-6); 153.42 (C-2); 150.98 (C-4); 137.79 (C-8); 120.41 (CN); 116.28 (C-5); 60.84 d, 2 C, J(P,C) = 6.3 (Et); 54.22, 51.75, and 46.43 (C-2′, C-3′ and C-5′); 40.90 (C-1′); 22.04 d, J(P,C) = 155.6 (C-4′); 22.58 (C-6′); 16.12 d, 2 C, J(P,C) = 5.7 (Et); 13.48 (C-6′). MS (ESI): m/z = 426 [M + H]$^+$.

*Diethyl 9-[(N-(2-Hydroxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**6i**).* Prior the above-mentioned general procedure, diethyl phosphonate **4i** had to be heated for 24 h at 80 °C in the mixture of THF (60 mL) and water (30 mL) to cleave the triphenylphosphoranylidene moiety. Solvents were evaporated and the residue treated with trifluoroacetic acid, yield 85%. ^{1}H NMR (DMSO-d_6): 10.84 s, 1 H (NH); 7.72 s, 1 H (H-8); 6.65 s, 2 H (NH$_2$); 4.01 t, 2 H, J(1′,2′) = 5.9 (H-1′); 3.95 m, 4 H (Et); 2.85 m, 2 H(H-2′); 2.79 m, 2 H(H-5′); 2.69 m, 2 H (H-3′); 1.83 m, 2 H (H-4′); 1.20 t, 6 H, J = 7.0 (Et). ^{13}C NMR (DMSO-d_6): 156.85 (C-6); 153.58 (C-2); 151.01 (C-4); 137.90 (C-8); 116.26 (C-5); 61.00d, 2 C, J(P,C) = 6.2 (Et); 58.92 (C-6′); 55.12 (C-5′); 52.62 (C-2′); 48.50 (C-3′); 22.08 d, J(P,C) = 134.4 (C-4′); 16.12 d, 2 C, J(P,C) = 5.7 (Et). MS (ESI): m/z = 403 [M + H]$^+$.

Synthesis of the Free Phosphonic Acids 7b−7g, 7i, 8b−8g, and 8i: General Procedure. A mixture of the corresponding diethyl ester **5a−5g,5i** or **6a−6g,6i** (1 mmol), acetonitrile (20 mL), 2,6-lutidine (0.1 mL), and $BrSiMe_3$ (1 mL) was stirred for 2 days at room temperature. After evaporation and codistillation with acetonitrile, the residue was treated with aqueous methanol (2: 1, 30 mL) for 1 h and evaporated. The residue was purified by preparative HPLC (water−methanol) or by chromatography on Dowex 1 × 2 (acetate form).

*9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7b**).* Starting from diethyl phosphonate **5b**, yield 39%, white foam. ^{1}H NMR (DMSO-d_6): 12.26 s, 1 H (NH); 8.08 s, 1 H and 8.02 s, 1 H (H-2 and H-8); 4.17 t, 2 H, J(1′,2′) = 6.0 (H-1′); 4.03 q, 2 H, J = 7.1 (Et); 3.34 s, 2 H (H-5′); 2.94 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.76 m, 2 H (H-3′); 1.48 m, 2 H (H-4′); 1.16 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 170.78 (CO); 156.61 (C-6); 148.30 (C-4); 145.21 (C-2); 140.71 (C-8); 123.60 (C-5); 59.77 (Et); 53.79, 52.16, and 48.34 (C-2′, C-5′ and C-3′); 41.50 (C-1′); 14.02 (Et). Anal. Calcd for $C_{13}H_{20}N_5O_6P$·1/2MeOH: C, 41.65; H, 5.70; N, 17.99. Found: C, 41.53; H, 5.45; N, 17.78. MS (ESI−): m/z = 372 [M − H]$^-$.

*9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7c**).* Chromatography on Dowex 1 × 2 (acetate form, elution by 0.25 M acetic acid). Starting from diethyl phosphonate **5c**, yield 60%, white foam. ^{1}H NMR (DMSO-d_6): 12.29 s, 1 H (NH); 8.05 s, 1 H and 8.04 s, 1 H (H-2 and H-8); 4.26 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.54 s, 3 H (Me); 2.97 t, 2 H, J(2′,1′) = 6.1 (H-2′); 2.86 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.81 m, 2 H (H-3′); 2.42 t, 2 H, J(6′,5′) = 6.8 (H-6′); 1.67 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 171.68 (CO); 156.51 (C-6); 148.21 (C-4); 145.29 (C-2); 140.47 (C-8); 123.72 (C-5); 51.24 (Me); 51.61, 48.02, and 47.48 (C-2′, C-5′ and C-3′); 40.64 (C-1′); 30.89 (C-6′); 24.16 d, J(P,C) = 132.2 (C-4′). Anal.

Calcd for $C_{13}H_{20}N_5O_6P·3/2H_2O$: C, 39.00; H, 5.79; N, 17.49. Found: C, 39.00; H, 5.50; N, 17.57. MS (ESI−): m/z = 372 $[M - H]^-$.

*9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7d**).* Starting from diethyl phosphonate **5d**, yield 41%, white solid. ^{1}H NMR (DMSO-d_6): 12.30 s, 1 H (NH); 8.08 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.26 m, 2 H, (H-1′); 3.55 s, 3 H (Me); 2.96 m, 2 H (H-2′); 2.82 m, 2 H (H-5′); 2.55 m, 2 H (H-3′); 2.14 t, 2 H, J(7′,6′) = 6.8 (H-7′); 1.65 m, 2 H (H-4′); 1.53 m, 2 H (H-6′). ^{13}C NMR (DMSO-d_6): 172.93 (CO); 156.50 (C-6); 148.23 (C-4); 145.26 (C-2); 140.50 (C-8); 123.76 (C-5); 51.07 (Me); 51.58, 51.31, and 47.57 (C-2′, C-5′ and C-3′); 40.82 (C-1′); 30.32 (C-7′); 24.19 d, J(P,C) = 129.4 (C-4′); 21.16 (C-6′). Anal. Calcd for $C_{14}H_{22}N_5O_6P·H_2O$: C, 41.48; H, 5.97; N, 17.28. Found: C, 41.79; H, 5.81; N, 17.04. MS (ESI−): m/z = 386 $[M - H]^-$.

*9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-hypoxanthine (**7e**).* To improve solubility, dimethylformamide (2 mL) was added to the reaction mixture. Starting from diethyl phosphonate **5e**, yield 59%, white solid. ^{1}H NMR (DMSO-d_6): 12.29 s, 1 H (NH); 8.06 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.25 t, 2 H, J(1′,2′) = 5.7 (H-1′); 3.83 s, 2 H (H-5′); 2.85 t, 2 H, J(2′,1′) = 5.7 (H-2′); 2.66 dd, 2 H, J = 7.5 and 15.8 (H-3′); 1.52 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 156.51 (C-6); 148.28 (C-4); 145.27 (C-2); 140.44 (C-8); 123.59 (C-5); 116.11 (CN); 52.07 and 48.33 (C-2′ and C-3′); 41.22 and 40.82 (C-5′ and C-1′); 24.83 d, J(P,C) = 134.1 (C-4′). Anal. Calcd for $C_{11}H_{15}N_6O_4P·2/3H_2O$: C, 39.06; H, 4.87; N, 24.84. Found: C, 38.92; H, 4.68; N, 24.56. MS (ESI−): m/z = 298 $[M - H]^-$.

*9-[(N-(2-Cyanoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-hypoxanthine (**7f**).* Starting from diethyl phosphonate **5f**, yield 67%, white foam. ^{1}H NMR (DMSO-d_6): 12.26 s, 1 H (NH); 8.09 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.18 t, 2 H, J(1′,2′) = 6.2 (H-1′); 2.82 t, 2 H, J(2′,1′) = 6.2 (H-2′); 2.74 t, 2 H, J(5′,6′) = 6.7 (H-5′); 2.68 m, 2 H (H-3′); 2.60 m, 2 H (H-6′); 1.55 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 156.53 (C-6); 148.24 (C-4); 145.20 (C-2); 140.47 (C-8); 123.68 (C-5); 119.66 (CN); 51.87, 48.23, and 47.13 (C-2′, C-5′ and C-3′); 41.47 (C-1′); 24.98 d, J(P,C) = 131.9 (C-4′); 15.53 (C-6′). Anal. Calcd for $C_{12}H_{17}N_6O_4P·4/3H_2O$: C, 39.56; H, 5.44; N, 23.07. Found: C, 39.70; H, 5.27; N, 23.07. MS (ESI−): m/z = 339 $[M - H]^-$.

*9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-hypoxanthine (**7g**).* Starting from diethyl phosphonate **5g**, yield 51%, white solid. ^{1}H NMR (DMSO-d_6): 12.34 s, 1 H (NH); 8.12 s, 1 H and 8.05 s, 1 H (H-2 and H-8); 4.32 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.10 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.92 dd, 2 H, J = 7.5 and 15.2 (H-3′); 2.75 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.38 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.74 m, 2 H (H-4′); 1.68 m, 2 H(H-6′). ^{13}C NMR (DMSO-d_6): 156.45 (C-6); 148.26 (C-4); 145.40 (C-2); 140.40 (C-8); 123.76 (C-5); 120.04 (CN); 51.48, 50.75, and 47.57 (C-2′, C-5′ and C-3′); 40.23 (C-1′); 23.75 d, J(P,C) = 131.5 (C-4′); 21.46 (C-6′); 13.52 (C-6′). Anal. Calcd for $C_{13}H_{19}N_6O_4P·2H_2O$: C, 40.00; H, 5.94; N, 21.54. Found: C, 40.44; H, 5.85; N, 21.31. MS (ESI−): m/z = 353 $[M - H]^-$.

*9-[(N-(2-Hydroxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-hypoxanthine (**7i**).* To improve solubility, dimethylformamide (2.5 mL) was added to the reaction mixture. Chromatography on Dowex 1 × 2 (acetate form, elution by 0.25 M acetic acid). Starting from diethyl phosphonate **5i**, yield 36%, white foam. ^{1}H NMR (DMSO-d_6): 12.39 s, 1 H (NH); 8.16 s, 1 H and 8.07 s, 1 H (H-2 and H-8); 4.45 t, 2 H, J(1′,2′) = 5.8 (H-1′); 3.60 m, 2 H (H-6′); 3.37 t, 2 H, J(2′,1′) = 5.8 (H-2′); 3.28 m, 2 H (H-3′); 3.04 t, 2 H(H-5′); 1.86 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 156.48 (C-6); 148.25 (C-4); 145.52 (C-2); 140.41 (C-8); 123.82 (C-5); 56.48, 54.51, 51.63, and 48.47 (C-6′, C-2′, C-5′ and C-3′); 23.53 d, J(P,C) = 131.2 (C-4′). Anal. Calcd for $C_{11}H_{18}N_5O_5P$: C, 39.88; H, 5.48; N, 21.14. Found: C, 40.23; H, 5.524; N, 20.92. MS (ESI−): m/z = 330 $[M - H]^-$.

*9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7m**).* Chromatography on Dowex 1 × 2 (acetate form, elution by 0.125 M formic acid). Starting from diethyl phosphonate **5a**, yield 85%, white foam. ^{1}H NMR (DMSO-d_6): 12.64 s, 1 H (NH); 8.51 s, 1 H and 8.15 s, 1 H (H-2 and H-8); 4.64 t, 2 H, J(1′,2′) = 6.8 (H-1′); 3.67 t, 2 H, J(2′,1′) = 6.8 (H-2′); 3.35 m, 4 H (H-3′ and H-5′); 2.10 m, 4 H (H-4′ and H-6′). ^{13}C NMR (DMSO-d_6): 155.78 (C-6); 148.08 (C-4); 146.40 (C-2); 140.15 (C-8); 122.37 (C-5); 49.84 (C-2′); 47.51, 2 C (C-3′ and C-5′); 38.17 (C-1′); 22.30 d, 2 C, J(P,C) = 132.4 (C-4′and C-6′). Anal. Calcd for $C_{11}H_{19}N_5O_7P$: C, 33.43; H, 4.85; N, 17.72. Found: C, 33.96; H, 4.71; N, 17.46. MS (ESI−): m/z = 394 $[M - H]^-$.

*9-[(N-(2-Ethoxy-2-oxoethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8b**).* Starting from diethyl phosphonate **6b**, yield 35%, white solid. ^{1}H NMR (DMSO-d_6): 10.54 s, 1 H (NH); 7.70 s, 1 H (H-8); 6.43 s, 2 H (NH_2); 4.05 q, 2 H, J = 7.1 (Et); 3.97 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.39 s, 2 H (H-5′); 2.88 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.79 dd, 2 H, J = 7.4 and 16.0 (H-3′); 1.56 m, 2 H (H-4′); 1.17 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 170.78 (CO); 156.74 (C-6); 153.38 (C-2); 151.06 (C-4); 137.89 (C-8); 116.11 (C-5); 59.82 (Et); 53.81, 52.28, and 48.05 (C-2′, C-5′ and C-3′); 40.94 (C-1′); 26.23, J(P,C) = 131.7 (C-4′); 14.05 (Et). Anal. Calcd for $C_{13}H_{21}N_6O_6P·2/3H_2O$: C, 39.00; H, 5.62; N, 20.99. Found: C, 38.85; H, 5.55; N, 20.92. MS (ESI−): m/z = 387 $[M - H]^-$.

*9-[(N-(3-Methoxy-3-oxopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8c**).* To improve solubility, dimethylformamide (6 mL) was added to the reaction mixture. Starting from diethyl phosphonate **6c**, yield 31%, white solid. ^{1}H NMR (DMSO-d_6): 10.55 s, 1 H (NH); 7.62 s, 1 H (H-8); 6.46 s, 2 H (NH_2); 3.98 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.54 s, 3 H (Me); 2.75 m, 6 H (H-2′, H-3′, H-5′); 2.36 t, 2 H, J(6′,5′) = 6.9 (H-6′); 1.67 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 172.04 (CO); 156.66 (C-6); 153.32 (C-2); 150.93 (C-4); 137.66 (C-8); 116.24 (C-5); 51.14 (Me); 51.94, 48.13, 47.31 (C-2′, C-5′ and C-3′); 40.59 (C-1′); 31.45 (C-6′); 24.58 d, J(P,C) = 131.5 (C-4′). Anal. Calcd for $C_{13}H_{21}N_6O_6P·H_2O$: C, 38.43; H, 5.71; N, 20.68. Found: C, 38.13; H, 5.86; N, 20.48. MS (ESI−): m/z = 387 $[M - H]^-$.

*9-[(N-(4-Methoxy-4-oxobutyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8d**).* Starting from diethyl phosphonate **6d**, yield 58%, white foam. ^{1}H NMR (DMSO-d_6): 10.66 s, 1 H (NH); 7.69 s, 1 H (H-8); 6.56 s, 2 H (NH_2); 4.11 t, 2 H, J(1′,2′) = 5.4 (H-1′); 3.56 s, 3 H (Me); 3.02 t, 2 H, J(2′,1′) = 5.4 (H-2′); 2.96 dd, 2 H, J = 15.0 and 8.3 (H-3′); 2.68 t, 2 H, J(5′,6′) = 6.6 (H-5′); 2.23 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.71 m, 2 H (H-4′), 1.63 m, 2 H (H-6′). ^{13}C NMR (DMSO-d_6): 172.88 (CO); 156.65 (C-6); 153.46 (C-2); 150.96 (C-4); 137.54 (C-8); 116.28 (C-5); 51.13 (Me); 51.50, 51.26, and 47.83 (C-2′, C-3′ and C-5′); 30.30 (C-7′); 24.10 d, J(P,C) = 129.3 (C-4′), 20.61 (C-6′). Anal. Calcd for $C_{14}H_{23}N_6O_6P·H_2O$: C, 40.00; H, 5.99; N, 19.99. Found: C, 39.99; H, 5.91; N, 19.77. MS (ESI): m/z = 401 $[M - H]^-$.

*9-[(N-(2-Cyanomethyl)-N-(2-phosphonoethyl))-2-aminoethyl]-guanine (**8e**).* To improve solubility, dimethylformamide (10 mL) was added to the reaction mixture. Starting from diethyl phosphonate **6e**, yield 89%, white solid. ^{1}H NMR (DMSO-d_6): 11.39 s, 1 H (NH); 9.05 s, 1 H (H-8); 7.07 s, 2 H (NH_2); 4.19 t, 2 H, J(1′,2′) = 5.2 (H-1′); 3.82 s, 2 H (H-5′); 4.19 t, 2 H, J(2′,1′) = 5.2 (H-2′); 2.71dd, 2 H, J = 8.5 and 16.8 (H-3′); 1.65 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 155.02 (C-6); 153.73 (C-2); 149.81 (C-4); 137.49 (C-8); 116.08 (CN and C-5); 51.68 and 47.78 (C-2′ and C-3′); 41.80 and 41.27 (C-1′ and C-5′); 25.74 d, J(P,C) = 134.2 (C-4′). Anal. Calcd for $C_{11}H_{16}N_7O_4P·MeOH$: C, 38.61; H, 5.40; N, 26.26. Found: C, 38.12; H, 5.15; N, 26.39. MS (ESI−): m/z = 340 $[M - H]^-$.

*9-[(N-(2-Cyanoethyl)-N-(2- phosphonoethyl))-2-aminoethyl]-guanine (**8f**).* To improve solubility, dimethylformamide (4 mL) was added to the reaction mixture. Starting from diethyl phosphonate **6f**, yield 36%, white foam. ^{1}H NMR (DMSO-d_6): 10.53 s, 1 H (NH); 7.70 s, 1 H (H-8); 6.44 s, 2 H (NH_2); 3.97 t, 2 H, J(1′,2′) = 6.3 (H-1′); 2.73 m, 6 H (H-2′, H-3′ and H-5′); 2.53 t, 2 H, J(6′,5′) = 6.5 (H-6′); 1.59 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 156.69 (C-6); 153.34 (C-2); 150.99 (C-4); 137.65 (C-8); 119.94 (CN); 116.26 (C-5); 51.90, 48.31, and 47.24 (C-2′, C-5′ and C-3′); 40.89 (C-1′); 25.14 d, J(P,C) = 131.4 (C-4′); 15.56 (C-6′). Anal. Calcd for $C_{12}H_{18}N_7O_4P·MeOH$: C, 40.31; H, 5.72; N, 25.31. Found: C, 40.22; H, 5.78; N, 25.09. MS (ESI−): m/z = 354 $[M - H]^-$.

*9-[(N-(2-Cyanopropyl)-N-(2-phosphonoethyl))-2-aminoethyl]-guanine (**8g**).* To improve solubility, dimethylformamide (14 mL) was added to the reaction mixture. Starting from diethyl phosphonate **6g**, yield 58%, white solid. ^{1}H NMR (DMSO-d_6): 10.61 s, 1 H (NH); 7.71 s, 1 H (H-8); 6.50 s, 2 H (NH_2); 4.06 t, 2 H, J(1′,2′) = 5.8 (H-1′);

2.89 m, 2 H(H-2′); 2.86 m, 2 H (H-3′); 2.62 t, 2 H, J(5′,6′) = 6.8 (H-5′); 2.34 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.70 m, 2 H (H-4′); 1.67 m, 2 H (H-6′). ^{13}C NMR (DMSO-d_6): 156.60 (C-6); 153.41 (C-2); 150.96 (C-4); 137.60 (C-8); 120.27 (CN); 116.21 (C-5); 51.74, 50.86, and 47.47 (C-2′, C-3′ and C-5′); 40.23 (C-1′); 24.23 d, J(P,C) = 130.73 (C-4′); 21.96 (C-6′); 13.51 (C-6′). Anal. Calcd for $C_{13}H_{20}N_7O_4P{\cdot}3/2H_2O$: C, 39.40; H, 5.85; N, 24.74. Found: C, 39.12; H, 5.46; N, 24.33. MS (ESI): m/z = 368 $[M + H]^+$.

*9-[(N-(2-Hydroxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8i**).* To improve solubility, dimethylformamide (14 mL) was added to the reaction mixture. Starting from diethyl phosphonate **6i**, yield 62%, white solid. ^{1}H NMR (DMSO-d_6): 10.74 s, 1 H (NH); 7.75 s, 1 H (H-8); 6.62 s, 2 H (NH_2); 4.27 t, 2 H, J(1′,2′) = 6.2 (H-1′); 3.64 t, 2 H, J(6′,5′) = 5,4 (H-6′); 3.38 t, 2 H, J(2′,1′) = 6.2 (H-2′); 3.27 m, 2 H (H-3′); 3.11 t, 2 H, J = 5.4 (H-5′); 1.90 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 157.01 (C-6); 153.95 (C-2); 151.26 (C-4); 137.87 (C-8); 116.58 (C-5); 56.29 (C-6′), 54.62, 51.98, and 59.11 (C-5′, C-2′ and C-3′); 38.89 (C-1′); 23.64 d, J(P,C) = 130.8 (C-4′). Anal. Calcd for $C_{11}H_{19}N_6O_5P{\cdot}H_2O$: C, 36.27; H, 5.81; N, 23.07. Found: C, 35.99; H, 5.65; N, 22.72. MS (ESI−): m/z = 345 $[M - H]^-$.

*9-[(N,N-(Bis-2-phosphonoethyl))-2-aminoethyl]guanine (**8m**).* Starting from diethyl phosphonate **6a**, yield 51%, white solid. ^{1}H NMR (DMSO-d_6): 8.36 s, 1 H (H-8); 7.98 s, 4.62 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.73 t, 2 H, J(2′,1′) = 6.3 (H-2′); 3.52 dd, 4 H, J = 8.2 and 16.4 (H-3′ and H-5′); 2.02 m, 4 H (H-4′ and H-6′). ^{13}C NMR (DMSO-d_6): 156.74 (C-6); 155.08 (C-2); 151.22 (C-4); 138.5 (C-8); 116.32 (C-5); 51.21 (C-2′); 49.44, 2 C (C-3′ and C-5′); 39.56 (C-1′); 22.78 d, 2 C, J(P,C) = 129.41 (C-4′and C-6′). Anal. Calcd for $C_{15}H_{23}N_6O_4P{\cdot}2H_2O$: C, 29.60; H, 5.42; N, 18.83. Found: C, 29.36; H, 5.37; N, 19.14. MS (ESI−): m/z = 409 $[M - H]^-$.

Synthesis of Carboxylic Acids 7j−7l, 8j: and 8l, General Procedure. A mixture of corresponding ester **5b−5d**, **6b**, or **6d** (1 mmol), tetrahydrofurane (10 mL), methanol (10 mL), and aqueous NaOH (10 M, 0.2 mL) was refluxed for 2 h and then stirred at room temperature overnight. After evaporation and codistillation with toluene/ethanol and acetonitrile, the residue was dissolved in acetonitrile (20 mL) and dimethylformamide (8 mL). 2,6-Lutidine (0.1 mL) and $BrSiMe_3$ (1 mL) were added, and the mixture was stirred for 2 days at room temperature. After evaporation and codistillation with acetonitrile, the residue was treated with aqueous methanol (2: 1, 30 mL) for 1 h, evaporated, and codistilled with water. The residue was purified by preparative HPLC (water−methanol).

*9-[(N-(Carboxymethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7j**).* Starting from diethyl phosphonate **5b**, yield 35%, white solid. ^{1}H NMR (DMSO-d_6): 12.29 s, 1 H (NH); 8.11 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.20 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.35 s, 2 H (H-5′); 2.97 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.80 m, 2 H (H-3′); 1.54 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 172.22 (CO); 156.61 (C-6); 148.30 (C-4); 145.32 (C-2); 140.72 (C-8); 123.63 (C-5); 53.86, 52.34, and 48.25 (C-2′, C-5′ and C-3′); 41.39 (C-1′); 26.05 d, J(P,C) = 131.4 (C-4′). Anal. Calcd for $C_{11}H_{16}N_5O_6P{\cdot}1/2H_2O$: C, 37.29; H, 4.84; N, 19.77. Found: C, 37.48; H, 4.65; N, 19.49. MS (ESI−): m/z = 344 $[M - H]^-$.

*9-[(N-(2-Carboxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7k**).* Starting from diethyl phosphonate **5c**, yield 60%, white foam. ^{1}H NMR (DMSO-d_6): 12.26 s, 1 H (NH); 8.05 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.21 t, 2 H, J(1′,2′) = 6.1 (H-1′); 2.86 t, 2 H, J(2′,1′) = 6.1 (H-2′); 2.77 t, 2 H, J(5′,6′) = 6.9 (H-5′); 2.74 m, 2 H (H-3′); 2.30 t, 2 H, J(6′,5′) = 6.9 (H-6′); 1.56 m, 2 H (H-4′). ^{13}C NMR (DMSO-d_6): 173.12 (CO); 156.52 (C-6); 148.21 (C-4); 145.24 (C-2); 140.54 (C-8); 123.68 (C-5); 51.99, 48.29 and 47.54 (C-2′, C-5′ and C-3′); 41.16 (C-1′); 31.58 (C-6′); 24.59 d, J(P,C) = 131.6 (C-4′). Anal. Calcd for $C_{12}H_{18}N_5O_6P{\cdot}5/3H_2O$: C, 37.02; H, 5.52; N, 17.99. Found: C, 37.18; H, 5.55; N, 17.75. MS (ESI−): m/z = 358 $[M - H]^-$.

*9-[(N-(Carboxypropyl)-N-(2-phosphonoethyl))-2-aminoethyl]hypoxanthine (**7l**).* Starting from diethyl phosphonate **5d**, yield 56%, white solid. ^{1}H NMR (DMSO-d_6): 12.31 s, 1 H (NH); 8.10 s, 1 H and 8.03 s, 1 H (H-2 and H-8); 4.28 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.00 t, 2 H, J(2′,1′) = 6.0 (H-2′); 2.84 m, 2 H (H-3′); 2.60 t, 2 H, J(5′,6′) = 6.9 (H-5′); 2.12 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.65 m, 2 H (H-4′); 1.56 m, 2 H (H-6′). ^{13}C NMR (DMSO-d_6): 174.03 (CO); 156.49 (C-6); 148.25 (C-4); 145.29 (C-2); 140.49 (C-8); 123.74 (C-5); 51.68, 51.48, and 47.72 (C-2′, C-5′ and C-3′); 40.68 (C-1′); 30.65 (C-7′); 24.20 d, J(P,C) = 131.5 (C-4′); 21.12 (C-6′). Anal. Calcd for $C_{13}H_{20}N_5O_6P{\cdot}3/2H_2O$: C, 40.59; H, 6.39; N, 17.75. Found: C, 40.64; H, 6.21; N, 17.62. MS (ESI−): m/z = 372 $[M - H]^-$.

*9-[(N-(Carboxymethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8j**).* Starting from diethyl phosphonate **6b**, yield 54%, white solid. ^{1}H NMR (DMSO-d_6): 10.58 s, 1 H (NH); 7.73 s, 1 H (H-8); 6.46 s, 2 H (NH_2); 4.00 t, 2 H, J(1′,2′) = 6.1 (H-1′); 3.34 s, 2 H (H-5′); 2.93 t, 2 H, J(2′,1′) = 6.1 (H-2′); 2.84 dd, 2 H, J = 8.1 and 16.1 (H-3′); 1.61 m, 2 H (H-4′); 1.17 t, 3 H, J = 7.1 (Et). ^{13}C NMR (DMSO-d_6): 172.13 (CO); 156.70 (C-6); 153.43 (C-2); 151.00 (C-4); 137.88 (C-8); 116.06 (C-5); 54.01, 52.51, and 48.18 (C-2′, C-5′ and C-3′); 40.78 (C-1′); 25.97, J(P,C) = 133.6 (C-4′). Anal. Calcd for $C_{11}H_{17}N_6O_6P{\cdot}3/2H_2O$: C, 34.11; H, 5.21; N, 21.70. Found: C, 33.82; H, 5.19; N, 21.29. MS (ESI−): m/z = 359 $[M - H]^-$.

*9-[(N-(Carboxypropyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8l**).* Starting from diethyl phosphonate **6d**, yield 58%, white solid. ^{1}H NMR (DMSO-d_6): 10.61 s, 1 H (NH); 7.68 s, 1 H (H-8); 6.54 s, 2 H (NH_2); 4.08 t, 2 H, J(1′,2′) = 5.8 (H-1′); 2.95 t, 2 H, J(2′,1′) = 5.8 (H-2′); 2.89 m, 2 H (H-3′); 2.63 t, 2 H, J(5′,6′) = 7.0 (H-5′); 2.17 t, 2 H, J(7′,6′) = 7.2 (H-7′); 1.68 m, 2 H (H-4′), 1.60 m, 2 H (H-6′). ^{13}C NMR (DMSO-d_6): 174.08 (CO); 156.64 (C-6); 153.42 (C-2); 151.00 (C-4); 137.53 (C-8); 116.27 (C-5); 51.78, 51.50, and 47.85 (C-2′, C-3′ and C-5′); 39.99 (C-1′); 30.87 (C-7′); 24.30 d, J(P,C) = 132.91 (C-4′), 20.96 (C-6′). Anal. Calcd for $C_{13}H_{21}N_6O_6P{\cdot}H_2O$: C, 38.43; H, 5.71; N, 20.68. Found: C, 38.51; H, 5.64; N, 20.53. MS (ESI−): m/z = 387 $[M - H]^-$.

*9-[(N-(2-Carboxyethyl)-N-(2-phosphonoethyl))-2-aminoethyl]guanine (**8k**).* A mixture of cyanoderivative 6f (0.41 g, 1 mmol), methanol (15 mL), and aqueous NaOH (25%, 3 mL) was refluxed for 4 h and then stirred at room temperature overnight. After evaporation and codistillation with toluene/ethanol and acetonitrile, the residue was dissolved in acetonitrile (20 mL). 2,6-Lutidine (0.1 mL) and $BrSiMe_3$ (1 mL) were added and the mixture stirred for 2 days at room temperature. After evaporation and codistillation with acetonitrile, the residue was treated with aqueous methanol (2: 1, 30 mL) for 1 h and evaporated. The residue was purified by preparative HPLC (water−methanol), yield 45%, white solid. ^{1}H NMR (D_2O): 7.64 s, 1 H (H-8); 4.73 t, 2 H, J(1′,2′) = 6.0 (H-1′); 3.84 t, 2 H, J(2′,1′) = 6.0 (H-2′); 3.62 t, J(5′,6′) = 6.5,2 H (H-5′); 3.58 m, 2 H (H-3′); 2.92 t, 2 H, J(6′,5′) = 6.5 (H-6′); 2.08 m, 2 H (H-4′). ^{13}C NMR (D_2O): 174.21 (CO); 156.44 (C-6); 155.32 (C-2); 151.01 (C-4); 138.57 (C-8); 116.21 (C-5); 51.80, 50.29, and 49.72 (C-2′, C-3′ and C-5′); 40.29 (C-1′); 28.54 (C-6′); 22.62 d, J(P,C) = 129.3 (C-4′). Anal. Calcd for $C_{12}H_{19}N_6O_6P{\cdot}4/3H_2O$: C, 36.18; H, 5.48; N, 21.10. Found: C, 36.41; H, 5.23; N, 20.86. MS (ESI−): m/z = 373 $[M - H]^-$.

Synthesis of Phosphonic Acids 7n and 8n, General Procedure. The corresponding cyanomethyl derivative **7e** or **8e** (0.25 mmol) in dimethylformamide (10 mL) was heated at 120 °C for 3 days. The solvent was then evaporated and the residue codistilled with toluene. The residue was purified by preparative HPLC (water−methanol).

*9-[(N-(2-Phosphonoethyl))-2-aminoethyl]hypoxanthine (**7n**).* Starting from diethyl phosphonate **7e**, yield 49%, white solid. ^{1}H NMR (D_2O + NaOD): 8.04 s, 1 H and 7.88 s, 1 H (H-2 and H-8); 4.21 t, 2 H, J(1′,2′) = 6.2(H-1′); 2.96 t, 2 H, J(2′,1′) = 6.2 (H-2′); 2.69 dd, 2 H, J = 7.3 and 16.3 (H-3′); 1.50 m, 2 H (H-4′). ^{13}C NMR (D_2O + NaOD): 168.34 (C-6); 154.10 (C-2); 150.33 (C-4); 123.76 (C-5); 47.80, 45.21, and 43.89 (C-2′, C-3′ and C-1′); 29.99 d, J(P,C) = 127.2 (C-4′). Anal. Calcd for $C_9H_{14}N_5O_4P{\cdot}H_2O$: C, 35.41; H, 5.28; N, 22.94. Found: C, 35.06; H, 5.29; N, 23.39. MS (ESI−): m/z = 286 $[M - H]^-$.

*9-[(N-(2-Phosphonoethyl))-2-aminoethyl]guanine (**8n**).* Starting from diethyl phosphonate **8e**, yield 30%, white solid. ^{1}H NMR (D_2O + NaOD): 7.59 s, 1 H (H-8); 4.05 t, 2 H, J(1′,2′) = 6.3 (H-1′); 2.88 t, 2 H, J(2′,1′) = 6.3 (H-2′); 2.66 dd, 2 H, J = 7.2 and 16.3 (H-3′); 1.470 m, 2 H (H-4′). ^{13}C NMR (D_2O + NaOD): 168.68 (C-6); 161.54 (C-2); 151.81 (C-4); 138.94 (C-8); 117.99 (C-5); 47.63, 45.02, and 43.25

(C-2′, C-3′ and C-1′); 29.82 d, J(P,C) = 127.6 (C-4′). Anal. Calcd for $C_{13}H_{21}N_6O_6P·H_2O$: C, 38.43; H, 5.71; N, 20.68. Found: C, 38.51; H, 5.64; N, 20.53. HRMS calcd for $C_9H_{14}N_6O_4P$, 301.08196; found, 301.08196. MS (ESI−): m/z = 301 $[M - H]^-$.

Determination of K_i Values. The K_i values were determined using a spectrophotometric assay at 25 °C, 0.1 M Tris-HCl, 10 mM $MgCl_2$, and pH 7.4 with *P*Rib-*PP* as the variable substrate and guanine as the fixed substrate.[22] The K_i values are $K_{i(app)}$ as they were measured at a single concentration of the second substrate. The concentration of the second substrate (guanine) was saturating: 60 μM. $K_{i(app)}$ was calculated using the equation $K_{m(app)} = K_m (1 + [I]/K_{i(app)})$.

Docking of the AzaANPs into the Active Site of Human HGPRT. Models for the ANPs were generated using the Dundee PRODRUG server.[23] Coordinates for the human HGPRT structure were obtained from the Protein Data Bank. Only Chain A from the coordinate files were used for docking. All of the water molecules and ligands were removed prior to docking. Compounds **7f** and **8f** had the highest docking scores when docked into 3GEP (human HGPRT in complex with 3-hydroxy-2-(phosphonomethoxy)propyl guanine).[4] All of the other compounds had the highest docking scores when docked into 3GGJ (human HGPRT in complex with 2-(phosphonoethoxy)-ethyl guanine or hypoxanthine).[4] The program GOLD[24] was used for all the docking calculations. All hydrogen atoms were added to the protein within GOLD. The active site center was defined by the coordinates of a carboxylate oxygen atom on the side chain of D13. The search radius around this point was 15 Å. For each ligand and protein, 20 independent docking searches were undertaken. Scoring was made with the default setting, ChemPLP.[25]

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ANPs, acyclic nucleoside phosphonates; aza-ANPs, acyclic nucleoside phosphonates with build-in nitrogen atom in the acyclic moiety; CRFK, feline renal cells; HEL, human embryonic lung cells; HG(X)PRT, hypoxanthine-guanine-(xanthine) phosphoribosyltransferase; PEEG, 2-(phosphonoethoxy)ethyl guanine; PEEHx, 2-(phosphonoethoxy)ethyl hypoxanthine; *Pf*, *Plasmodium falciparum*; PP_i, pyrophosphate; PRib-PP, 5-phosphoribosyl-1-pyrophosphate; *Pv*, *Plasmodium vivax*; Vero, kidney epithelial cells

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