Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

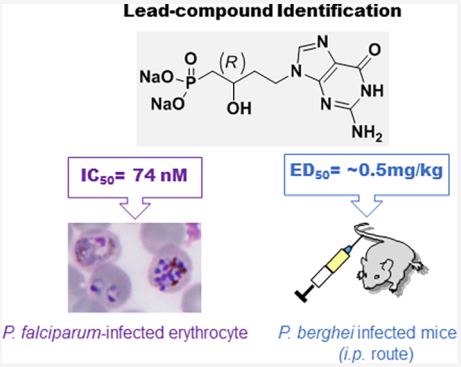
β-Hydroxy- and β-Aminophosphonate Acyclonucleosides as Potent Inhibitors of *Plasmodium falciparum* Growth

Thomas Cheviet, Sharon Wein, Gabriel Bourchenin, Manon Lagacherie, Christian Périgaud, Rachel Cerdan,* and Suzanne Peyrottes*

Cite This: https://dx.doi.org/10.1021/acs.jmedchem.0c00131 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Malaria is an infectious disease caused by a parasite of the genus *Plasmodium*, and the emergence of parasites resistant to all current antimalarial drugs highlights the urgency of having new classes of molecules. We developed an effective method for the synthesis of a series of β-modified acyclonucleoside phosphonate (ANP) derivatives, using commercially available and inexpensive materials (i.e., aspartic acid and purine heterocycles). Their biological evaluation in cell culture experiments and SAR revealed that the compounds' effectiveness depends on the presence of a hydroxyl group, the chain length (four carbons), and the nature of the nucleobase (guanine). The most active derivative inhibits the growth of *Plasmodium falciparum in vitro* in the nanomolar range (IC_{50} = 74 nM) with high selectivity index (SI > 1350). This compound also showed remarkable *in vivo* activity in *P. berghei*-infected mice (ED_{50} ~ 0.5 mg/kg) when administered by the ip route and is, although less efficient, still active via the oral route. It is the first ANP derivative with such potent antimalarial activity and therefore has considerable potential for development as a new antimalarial drug.

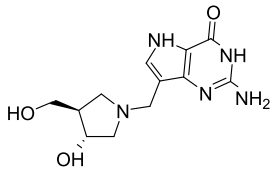


INTRODUCTION

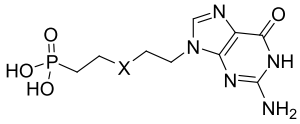
Drug discovery is mainly based on the complementarity of target-based (protein screening) and phenotypic (whole cell screening) approaches, and both strategies have been used to identify novel antimalarials.[1]

Cell-based phenotypic screening has proven to be a valuable approach for drug candidate identification, and it is used for decades to identify new chemotypes. One of its limitations is that it fails to provide information on the molecular target and/or the mechanism of action. On the contrary, target-based approaches involve the screening of a library of compounds against a defined protein followed by structural optimization of a hit-compound for potency and selectivity. In the case of parasitic diseases, there is a rather limited number of validated molecular targets if we consider the strict definition that a validated target is associated with a registered drug, for which the corresponding mode of action has been proven. As example, several proteins from the purine and pyrimidine synthesis pathways have been proposed as chemotherapeutic targets for various parasites.[2] Special attention was given to the purine salvage pathway as many parasitic protozoa lack the *de novo* pathway for the biosynthesis of nucleoside 5′-monophosphates. Indeed, they rely exclusively on the enzymes from the purine salvage pathway and related transporters for the supply of purines and purine-containing nucleotides. In the case of malaria, one of the most important human parasitic diseases and a major cause of mortality worldwide, parasite resistance to traditional antimalarial drugs is now a serious concern. Thus, increasing efforts have been made to develop new drugs with novel mechanisms of action.[1c,3] We have recently reviewed the literature on inhibitors of the purine salvage pathway and highlighted two main families of potential drugs (Figure 1),[4] namely, immucillin derivatives and acyclonucleoside phosphonates (ANPs), which target the purine nucleoside phosphorylase (PNP)[5] and the hypoxanthine−guanine−xanthine phosphoryl transferase (HGXPRT),[6] respectively. Several generations of immucillin have been studied as transition state inhibitors of PNP and have exhibited potent antimalarial activities *in vitro* and *in vivo*.[7] Some have already been the subject of preclinical studies,[7b] but no further development has been reported to date. In addition, ANPs are also promising as a class of antimalarial agents because they inhibit HGXPRT.[8] This enzyme is crucial for the synthesis of inosine 5′-monophosphate (IMP) from hypoxanthine, the central and unique precursor for all purine-containing nucleotides required during plasmodial nucleic acid biosynthesis. These compounds were designed on the basis of the crystal structure of 2-(phosphonoethoxy)ethylguanine (PEEG, Figure 1, X = O) in complex with *Hs*HGPRT[9] and incorporate a purine base (preferably guanine or hypoxanthine), a five atoms

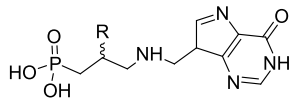
Received: January 23, 2020



DADMe-Immucillin-G
Ki 0.16 nM / Ki* 7 pM on *Hs* PNP
Ki 0.89 nM on *Pf* PNP



PEEG, X=O, K_i 0.1 μM on *Pf* HGXPRT
K_i 1 μM on *Hs* HGPRT
IC_{50} (D6) = 242 μM
Aza-ANP, X=NH, K_i 16 μM on *Pf* HGXPRT
K_i 3 μM on *Hs* HGPRT
Thia-ANP, X=S, K_i 0.1 μM on *Pf* HGXPRT
K_i 0.4 μM on *Hs* HGPRT
$X=CH_2$, K_i 2 μM on *Pf* HGXPRT
K_i 0.5 μM on *Hs* HGPRT

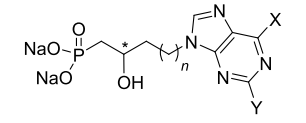


Aza-C-ANP, R=H, K_i 27.6 nM on *Pf* HGXPRT
K_i >10 μM on *Hs* HGPRT
R=OH, K_i 9.1 nM on *Pf* HGXPRT
K_i >10 μM on *Hs* HGPRT
$R=CH_2OH$, K_i 490 nM on *Pf* HGXPRT
K_i >10 μM on *Hs* HGPRT

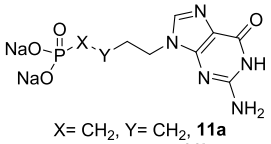
Figure 1. Examples of immucilin,[7b] ANP (X = O,[9] NH,[11] S,[12] CH_2[13]), and aza-C-ANP[14] derivatives described in the literature[4] as potent inhibitors of PNP and HG(X)PRT.

linker (preferably including an heteroatom), and the phosphonate group. The selectivity of these series of ANPs is usually modest with the exception of the Aza-C-ANPs. In addition, due to the anionic nature of ANPs at physiological pH, these compounds generally require conversion to neutral prodrugs to observe effective biological activity in cell culture experiments.[8b,10] This observation points to the fact that drugs must be able to enter the targeted cell, in this case the infected red blood cell, in order to block parasite processes.

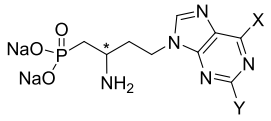
Herein, we describe the synthesis of a new series of ANPs (Figure 2). Their antimalarial potency was evaluated by a



n=0, X= NH_2, Y= H, **1** (*R*) and **1** (*S*)
X= OH, Y= NH_2, **2** (*R*) and **2** (*S*)
X= Y= NH_2, **3** (*R*) and **3** (*S*)
n=1, X= NH_2, Y= H, **4** (*R*) and **4** (*S*)
X= OH, Y= NH_2, **5** (*R*) and **5** (*S*)
X= Y= NH_2, **6** (*R*) and **6** (*S*)
X= OH, Y= H, **7** (*R*) and **7** (*S*)
X= OH, Y= OH, **8** (*R*) and **8** (*S*)
X= H, Y= NH_2, **9** (*R*)
X= NH_2, Y= F, **10** (*R*)



X= CH_2, Y= CH_2, **11a**
X= CH, Y= CH, **11b**



X= OH, Y= NH_2, **12** (*R*) and **12** (*S*)
X= Y= NH_2, **13** (*R*) and **13** (*S*)

Figure 2. Structures of the studied compounds.

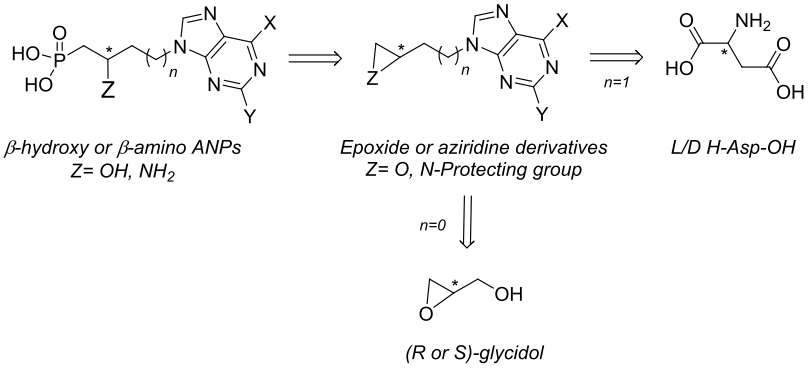
phenotypic approach against *P. falciparum* (*in vitro*) and *P. berghei* (*in vivo*) and revealed different properties from those of immucilins and aza-C-ANP.

RESULTS AND DISCUSSION

Chemistry. We focused specifically on the synthesis of α/β-hydroxyphosphonate derivatives as structural analogues of hydroxycarboxylic acids[15] (the latter are involved in various biological processes). Thus, we describe a limited number of regio- and stereoselective synthetic approaches to obtain these derivatives as pure enantiomers under mild conditions.[16] We previously reported on the synthesis of β-hydroxyphosphonate ribonucleoside derivatives using the efficient ring-opening reaction of epoxides with phosphorus nucleophile, catalyzed by BF_3·etherate.[17] In this case, chirality at the β-position (relative to the phosphorus atom) is controlled and originated from the naturally occurring chiral pool. Similarly, our general approach to synthesis (Scheme 1) involved the use of D- or L-aspartic acid or (*R*)- or (*S*)-glycidol, as cheap and/or naturally occurring chiral starting materials. Thus, propyl derivatives (compounds **1**−**3**, Figure 2, *n* = zero, namely, C3 series) may be obtained by the direct coupling of glycidol with the appropriate nucleobase precursors, followed by a ring-opening reaction. For butyl derivatives (compounds **4**−**10**, **12**, and **13**, Figure 2, *n* = 1, namely, C4 series), the required epoxide or aziridine should be obtained in a few steps, through intramolecular nucleophilic substitution from aspartic acid, in accordance with previously described procedures.[18] To avoid side reactions during the coupling steps, exocyclic functions of the purines were protected either by a *tert*-butyloxycarbonyl (Boc) group or a methoxy group (for 6-oxopurines).

Thus, Boc protected adenine, 2-amino-6-*O*-methylpurine and 2,6-diaminopurine (obtained beforehand using previously reported protocols[19]) were coupled either with (*R*)- or (*S*)-

Scheme 1. Proposed Retrosynthesis



β-hydroxy or β-amino ANPs
Z= OH, NH_2

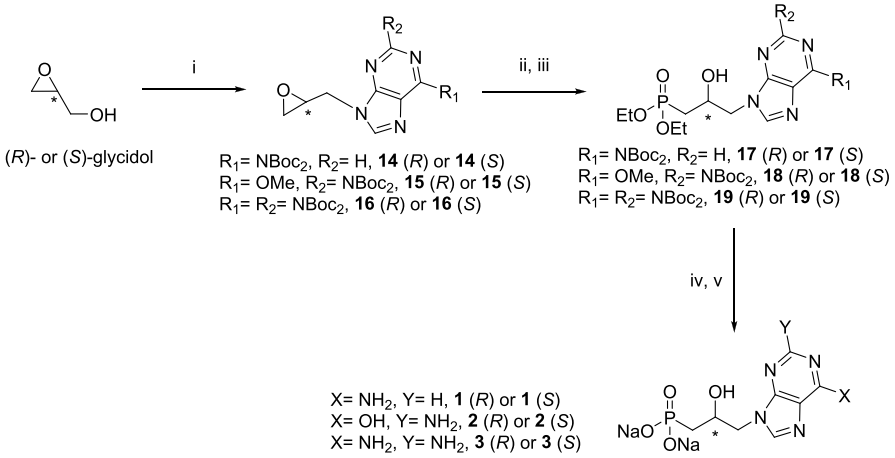
Epoxide or aziridine derivatives
Z= O, N-Protecting group

L/D H-Asp-OH

(R or S)-glycidol

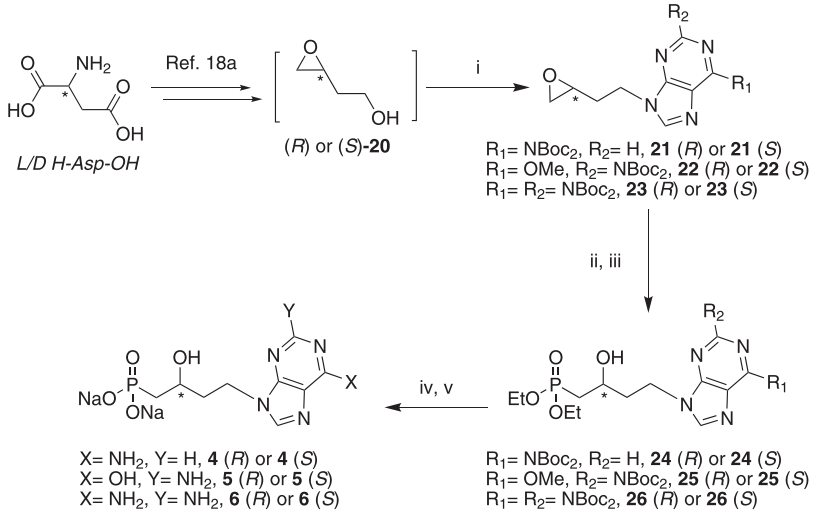
https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthetic Route to C3-β-Hydroxy-ANPs Containing Adenine, Guanine, and 2,6-Diaminopurine as Nucleobases[a]



[a]Reagents and conditions: (i) bis(Boc)adenine or bis(Boc)-2-amino-6-*O*-methylpurine or tetra(Boc)-2,6-diaminopurine, PPh_3, DEAD, THF, 0 °C to rt, 24 h; (ii) diethyl phosphite, *N*,*O*-BSA, CH_2Cl_2, reflux, 3−4 h, then addition of **14**−**16** in CH_2Cl_2, −60°C; (iii) $BF_3{\cdot}Et_2O$, −60 °C to rt, overnight; (iv) trifluoroacetic acid in CH_2Cl_2, 0 °C to rt, 5 h; (v) TMSBr in CH_3CN, rt, 1−2 d, then H_2O, 0 °C to rt, 1 h.

Scheme 3. Synthetic Route to C4-β-Hydroxy-ANPs Containing Adenine, Guanine, and 2,6-Diaminopurine as Nucleobases[a]



[a]Reagents and conditions: (i) bis(Boc)adenine or bis(Boc)-2-amino-6-*O*-methylpurine or tetra(Boc)-2,6-diaminopurine, PPh_3, DEAD, THF, 0 °C to rt, 24 h; (ii) diethyl phosphite, *N*,*O*-BSA, CH_2Cl_2, reflux, 3−4 h; **21**−**23** were added in CH_2Cl_2, −60°C; (iii) $BF_3{\cdot}Et_2O$, −60 °C to rt, overnight; (iv) trifluoroacetic acid in CH_2Cl_2, 0 °C to rt, 5 h; (v) TMSBr in CH_3CN, rt, 1−2 d, then H_2O, 0 °C to rt, 1 h.

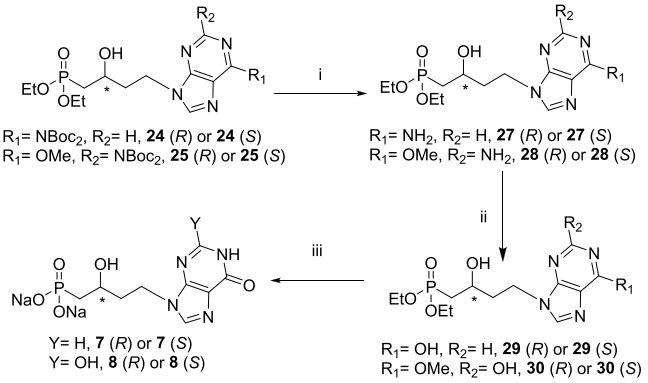
glycidol under Mitsunobu conditions[20] and yielded the enantiomeric pairs of epoxides **14**−**16** (Scheme 2). Then, the ring-opening step was carried out in the presence of an excess of silylated diethyl phosphite and $BF_3{\cdot}Et_2O$ and generated fully protected β-hydroxyphosphonate derivatives **17**−**19**. Removal of the various protecting groups was performed using a standard protocol with trifluoroacetic acid and then bromotrimethylsilane. The resulting phosphonic acids were eventually converted into their sodium salts, compounds **1**−**3**, by percolation onto ion-exchange resin (Dowex Na^+ form).

We then envisaged obtaining the corresponding β-hydroxyphosphonate derivatives, including a butyl chain, compounds **4**−**6** (Figure 2). The synthesis required the (*R*)- and (*S*)-2-hydroxyethyloxirane **20** (Scheme 3), which were prepared in three steps starting from D- or L-aspartic acid according to previously reported procedures.[18a] Isolation of epoxide **20** was problematic (due to its volatility and solubility in water). Therefore, we decided to use it as a crude after filtration of the reaction media. The solution of **20** in dichloromethane was added directly in the Mitsunobu coupling step with the required nucleobases, providing the enantiomeric pairs of epoxides **21**−**23**. The above-mentioned steps (ring-opening reaction and removal of protecting groups) were performed on these substrates and yielded isolated intermediates **24**−**26** and then the desired final compounds **4**−**6** as sodium salts.

As xanthine and hypoxanthine derivatives are important substrates during the biosynthesis of *P. falciparum* nucleic acids (through the salvage pathway), we chose to synthesize these analogues in the C4 series (compounds **7** and **8**, Figure 2) from derivatives **24** and **25** (Scheme 4). Briefly, acidic treatment allowed the removal of Boc protecting groups and yielded intermediates **27** and **28** quantitatively, which were then subjected to a diazotization reaction.[13] Thus, β-hydroxyphosphonate derivatives **29** and **30**, including hypoxanthine and 2-oxo-6-*O*-methylpurine, were converted into their phosphonic acids (**7** and **8** isolated as sodium salts) with trimethylsilyl bromide.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Synthetic Route to C4-β-Hydroxy-ANPs Containing Xanthine and Hypoxanthine as Nucleobases[a]



[a]Reagents and conditions: (i) trifluoroacetic acid 20% in CH_2Cl_2, 0 °C to rt, 45 min; (ii) $NaNO_2$, H_2O, AcOH 1 M, 70 °C, 1 h; (iii) TMSBr in CH_3CN, rt, 1−2 d, then TEAB, 0 °C to rt, 1 h.

We showed that compounds with (*R*)-stereochemistry and four-carbon linker had the best activities (see *in vitro* data section, Table 1). We thus extended our SAR study to non-natural purines (Figure 2, compounds **9** and **10**), incorporating 2-amino- and 6-amino-2-fluoropurines.

Compounds **9** and **10** were prepared following the same synthetic approach as described for derivatives **4**−**6** (Scheme 5). Compound **20**(*R*) was coupled under Mitsunobu conditions to protected *N*-$(Boc)_2$-2-aminopurine and *N*-$(Boc)_2$-6-amino-2-fluoropurine in order to produce the corresponding epoxides **31** and **32**. The ring-opening reaction yielded the expected β-hydroxyphosphonate derivatives **33** and **34**. The concomitant removal of Boc and diethyl phosphoester groups yielded the targeted phosphonic acids **9**(*R*) and **10**(*R*) as sodium salts.

We then probed the essentiality of the hydroxyl group at the β-position relative to the phosphorus atom by either removing it (Figure 2, compounds **11a** and **11b** including a butyl or butenyl linker) or replacing it by an amino group (Figure 2, compounds **12** and **13**, corresponding to β-aminophosphonate derivatives).

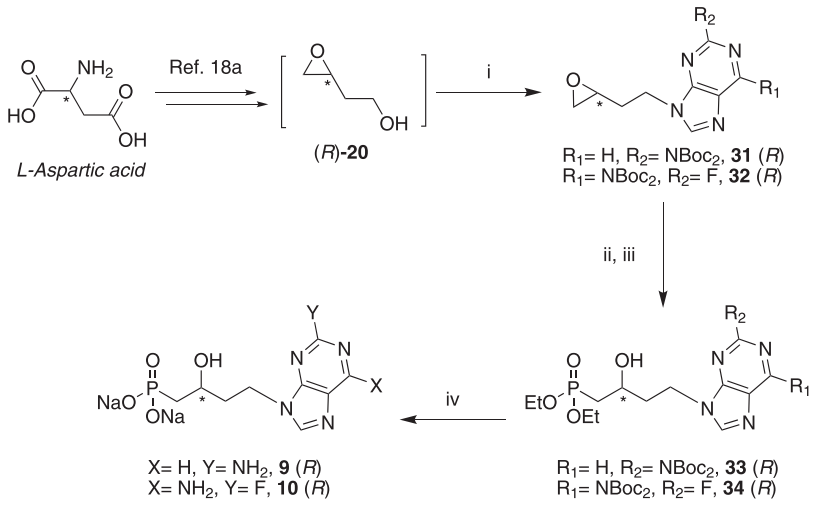
The guanine derivatives **11a** and **11b** were obtained by adapting the existing procedure for **11a**[13,21] and using an alternative route for **11b**[22] (cf. Supporting Information for details).

To access the β-aminophosphonate in the C4-series (Scheme 6), we developed an original synthetic route. The preparation of enantiomerically pure *N*-Boc-aziridine **35**(*S*) or **35**(*R*) was previously reported in a few steps from L- or D-aspartic acid, respectively.[18d] Briefly, quantitative synthesis of aspartic acid dimethyl esters was carried out using $SOCl_2$ in methanol, followed by the introduction of a *tert*-butyloxycarbonyl protecting group on the amine. The subsequent reduction of the diesters in the presence of $NaBH_4$ and $CaCl_2$ yielded the corresponding (*S*)- or (*R*)-*N*-Boc-amino diols.[18b] The latter compounds were converted to the 1,4-ditosylated intermediates. *In situ* this led to the intramolecular cyclization in the presence of potassium hydroxide and provided the enantiomeric pair of *N*-Boc-aziridine **35** in 45% overall yields (over five steps).[18d] Then, nucleophilic displacement of the remaining tosyl group by the appropriate purine precursors was accomplished in the presence of potassium carbonate and led to the isolation of the *N*-9-regioisomers **36** and **37** (sole products).

The ring-opening of activated aziridines was somewhat puzzling. It is highly dependent on both the nature of the protecting groups on the aziridine ring and the phosphorus nucleophilic species (data not shown). Best conditions involved the use of a Boc group and the addition of the lithium phosphite generated *in situ* from diethyl phosphite and LiHMDS at low temperature. After overnight stirring, satisfactory yields of the desired β-aminophosphonate derivatives **38** and **39** were isolated and we observed concomitant and partial removal of the Boc group on the exocyclic amino group of the nucleobases. Final removal of the remaining Boc groups was performed by treating derivatives **38** and **39** with trifluoroacetic acid in dichloromethane yielding compounds **40** and **41**. On hydrolysis of the diethyl phosphonate moiety (using TMSBr in DMF), this led to the expected derivatives **12** and **13.**

Enantiomeric Excess Characterization. ^{31}P NMR spectroscopy was used in combination with a chiral solvating agent

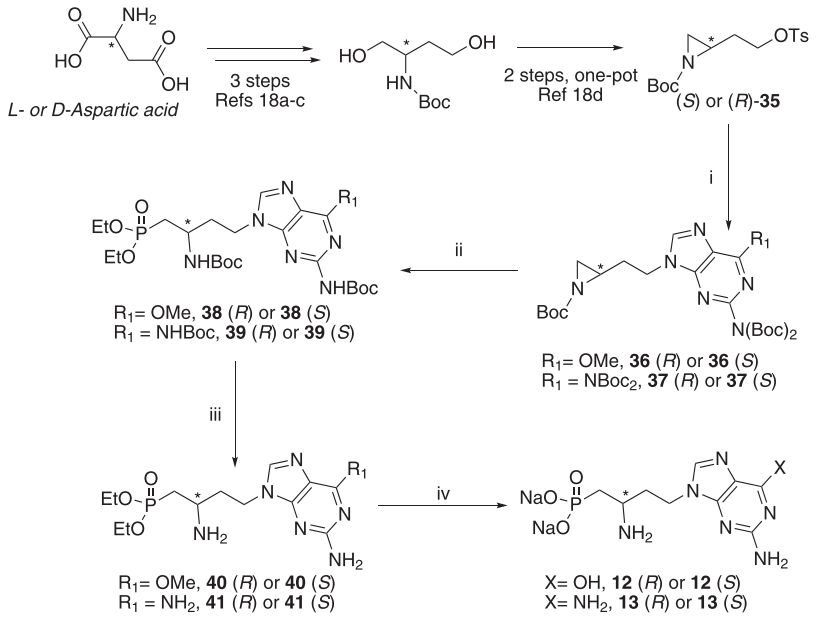
Scheme 5. Synthetic Route to C4-β-Hydroxy-ANPs Containing 2-Amino- and 2-Fluoro-6-aminopurines as Nucleobases[a]



[a]Reagents and conditions: (i) bis(Boc)-2-amino-purine or bis(Boc)-6-amino-2-fluoropurine, PPh_3, DEAD, THF, 0 °C to rt, 24 h; (ii) diethyl phosphite, *N*,*O*-BSA, CH_2Cl_2, reflux, 3−4 h; **31** or **32** was added in CH_2Cl_2, −60 °C; (iii) $BF_3.Et_2O$, −60 °C to rt, overnight; (iv) TMSBr in CH_3CN, rt, 1−2 d, then H_2O, 0 °C to rt, 1 h.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 6. Synthetic Route to C4-β-Amino-ANPs Containing Guanine and 2,6-Diaminopurine as Nucleobases[a]



[a]Reagents and conditions: (i) bis(Boc)-2-amino-6-*O*-methylpurine or tetra(Boc)-2,6-diaminopurine, K_2CO_3, DMF, rt, 3 d; (ii) diethyl phosphite, LiHMDS, THF, −78 °C, 15 min, then **36** or **37** was cannulated in THF, −78 °C to rt, then rt overnight; (iii) trifluoroacetic acid in CH_2Cl_2, 0 °C to rt, 5 h; (iv) TMSBr in DMF, rt, 1−2 d, then TEAB, 0 °C to rt, 1 h.

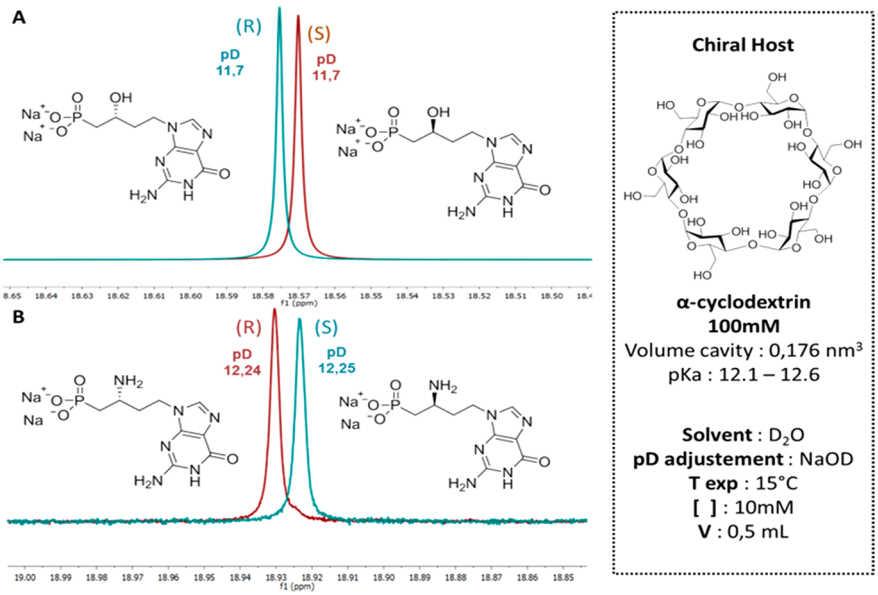


Figure 3. Separation of pair of enantiomers of two families of ANPs by ^{31}P NMR: (A) comparison of ^{31}P NMR of enantiomers of β-hydroxyphosphonates; (B) comparison of ^{31}P NMR of enantiomers of β-aminophosphonates. Characteristics of α-cyclodextrin are drawn from the literature.[24]

(CSA) to stereodiscriminate a pair of isomers and check the enantiopurity of our compounds. This simple rapid approach requires a small amount of compound. It has already been reported for chiral α-amino and α-hydroxyphosphonic acids using α-cyclodextrin as CSA.[23] Thus, several factors (temperature, pD, etc..) were tested to determine the best conditions for our derivatives based on β-hydroxyphosphonates (Supporting Information Figures S1−S3). The best separation was observed at T = 15 °C, the lowest tested temperature. This may be because it slows the conformational exchange rate of both enantiomers inside the cyclodextrin cavity and, thus, enhances their differentiation. The optimal pD was slightly different (always under basic conditions), depending on the studied compounds (β-amino and β-hydroxyphosphonates). This suggests that the ionization of the hydroxyl and amino group plays an important role in the complexation of the compound inside the cavity. The two enantiomers of each compound were tested separately and showed only one peak at a specific δ, at the same pD (Figure 3). The procedure made it possible to differentiate the two peaks from a racemic mixture. Thus, we demonstrate the enantiopurity of our final compounds and the reliability of our synthesis.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. Comparative Effects of ANP Derivatives on the Growth of *P. falciparum* (3D7 Strain) and Erythroblast (K562) Human Cell Line[a]

				IC_{50} (μM)[b]		
compd	base (X, Y)	chain length (n)	polar group	*P. falciparum*	K562	SI[c]
1(R)	adenine (X = NH_2, Y = H)	C3 (n = 0)	OH	>1000	>1000	NA
1(S)				>1000	>1000	NA
2(R)	guanine (X = OH, Y = NH_2)	C3 (n = 0)	OH	>1000	>1000	NA
2(S)				595	>1000	>1.7
3(R)	2,6-diaminopurine (X = Y= NH_2)	C3 (n = 0)	OH	327 ± 97	>1000	>2.6
3(S)				510	>1000	>2
4(R)	adenine (X = NH_2, Y = H)	C4 (n = 1)	OH	>1000	>1000	NA
4(S)				>1000	>1000	NA
5(R)	guanine (X = OH, Y = NH_2)	C4 (n = 1)	OH	0.074 ± 0.007	>1000	>1351
5(S)				6.1 ± 1.1	>1000	164
6(R)	2,6-diaminopurine (X = Y= NH_2)	C4 (n = 1)	OH	48	>1000	>21
6(S)				>1000	>1000	NA
7(R)	hypoxanthine (X = OH, Y = H)	C4 (n = 1)	OH	>1000	>1000	NA
7(S)				>1000	>1000	NA
8(R)	xanthine (X = OH, Y = OH)	C4 (n = 1)	OH	590	>1000	NA
8(S)				910	>1000	>1
9(R)	2-aminopurine(X = H, Y= NH_2)	C4 (n = 1)	OH	67 ± 11	>1000	>16
10(R)	2-fluoro-6-aminopurine (X = NH_2, Y = F)	C4 (n = 1)	OH	26.3 ± 5.3	>1000	>38
11a	guanine	C4	X = Y = CH_2	74 ± 8.6	>1000	>17
11b			X = Y = CH	≫100	>1000	NA
12(R)	guanine (X = OH, Y = NH_2)	C4	NH_2	>100	>1000	NA
12(S)				>100	>1000	NA
13(R)	2,6-diaminopurine (X = Y = NH_2)	C4	NH_2	≫100	>1000	NA
13(S)				≫100	>1000	NA
chloroquine				0.014 ± 0.002	25.3 ± 3.3	1807

[a]Values for X, Y, and n are related to formulas shown in Figure 2. [b]Values are the mean of at least three independent experiments performed in duplicate (±SEM). For other values, the mean of two independent experiments (each performed in duplicate) and differing by <30% are given. [c]SI (selectivity index) is the ratio of IC_{50} value for K562 cells to the IC_{50} value for *P. falciparum*. NA: not applicable. Parasite growth was assessed after 48 h of contact with the compound by measuring the incorporation of [^{3}H] hypoxanthine into nucleic acids from 48 to 66 h.[25]

***In Vitro* Antimalarial Activities.** The ability of our novel derivatives to interfere with parasite growth in cell culture was monitored by adding the compounds to a *P. falciparum*-infected erythrocyte suspension for a complete 48 h parasite cycle before assessing parasite viability (Table 1). The C3-series analogues **1**−**3** showed low antimalarial activities with IC_{50} higher than 300 μM for both R and S enantiomers, irrespective of the nature of the nucleobase. In this series, the most potent analogue is the 2,6-diaminopurine. In the C4-series with a hydroxyl group in the β-position relative to the phosphorus atom, we tested both enantiomers (compounds **4**−**8**) and different nucleobases (compounds **4**−**10**). The best activity was always observed for the R-enantiomer (Table 1) when the analogue was active (compounds **5**, **6**, and **8**). Concerning the nature of the nucleobase, the guanine derivatives **5**(R) and **5**(S) were the most interesting compounds with an IC_{50} value of 74 nM and 6.1 μM, respectively. Compounds **6**(R), **9**(R), **10**(R) exhibited modest antiplasmodial activities with IC_{50} values between 26 and 67 μM. The use of hypoxanthine (compounds **7**, both R and S isomers) and xanthine (compounds **8**, both R and S isomers) resulted in total loss of the activity indicating the important role of the amino substitution at position 2.

Finally, concerning the nature of the polar group in the β-position relative to the phosphorus atom, the hydroxyl group remained the best option (Table 1). Its replacement by an NH_2 group (compounds **12** and **13**, with IC_{50} values of >100 μM) or removal (compounds **11a**,**b**) led to a significant decrease of the antiplasmodial activity, at least 1000-fold (in the μM range) compared to compound **5**(R) (in the nM range).

In order to assess the effect of the analogues on the development of *P. falciparum* parasites, an infected-erythrocyte culture treated with **5**(R) was followed over 69 h. Thin blood smears showed that treated parasites developed normally for 48 h until the end of the cycle at the schizont stage (Figure 4A). However, only few new parasites were detected at the following cycle when treated with 2 μM compound **5**(R), and they died before 69 h. Immunofluorescence assays on mature parasites at late schizont stage showed that the daughter cells developed normally in the presence of compound **5**(R) and that the DNA content of the newly formed merozoites seemed unaffected by the treatment (Figure 4B). This suggests that the mode of action of compound **5**(R) could be different from other classes of ANPs that have been described to target DNA synthesis (Keough et al.[9,26]). This hypothesis is supported by the comparison of our data with the literature. It has been reported that both guanine and hypoxanthine ANP derivatives equally inhibited recombinant *Pf*HGXPRT.[12,13] In contrast, our guanine derivatives (compounds **5**) have an antimalarial activity between nM and low μM range, whereas our hypoxanthine derivatives (compounds **7**) were not active at 1 mM. Additionally, Cesnek et al.[13] showed that the guanine derivative corresponding to our compound **11a** has no affinity for *Pf*HGXPRT with K_i > 100 μM. In our experiments, this compound **11a** showed a modest inhibition of *P. falciparum* growth *in vitro* with an IC_{50} value of 74 μM. Altogether, these data suggest that our series of

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

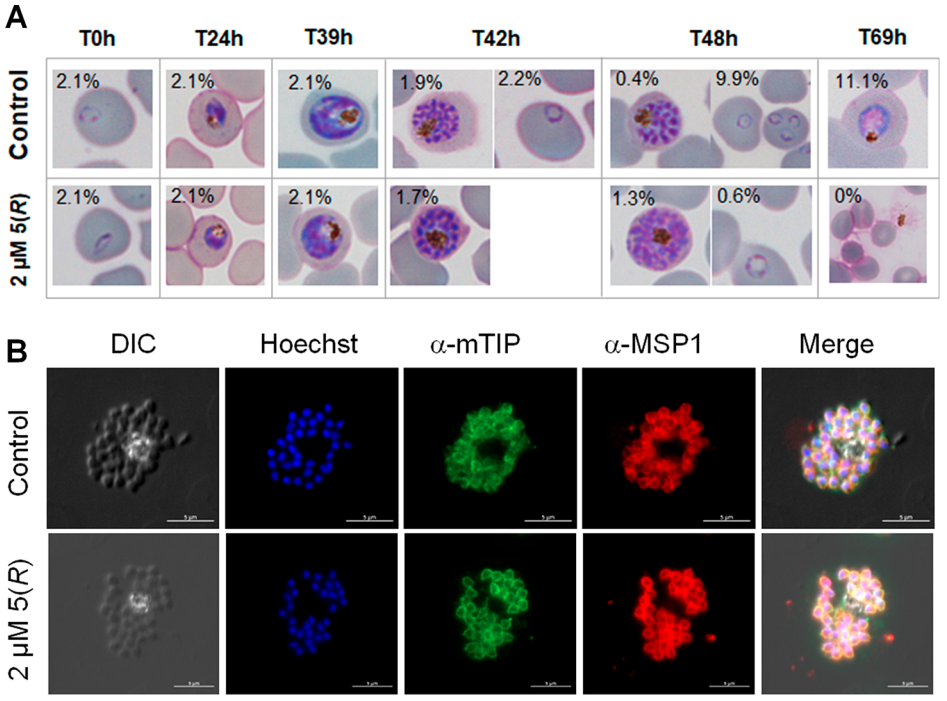


Figure 4. Effect of compound **5**(*R*) on the development of *P. falciparum*. A tightly synchronized culture of *P. falciparum* at ring stage was treated or not with 2 μM **5**(*R*). (A) Follow-up of parasite development on Giemsa-stained smears. Parasitemia is indicated for each parasite stage of the culture. Treatment with **5**(*R*) showed no morphological effect until the late schizont stage (48 h). At time 69 h, all parasites visible at time 48 h have disappeared. (B) Immunofluorescence assay on infected erythrocytes at time 45 h, at segmented schizont stage. Formation of merozoites membrane was visualized by labeling the internal membrane complex (α-mTIP) and the membrane surface (α-MSP1). The labeling of nuclei with Hoechst showed that all merozoites, in control and treated parasites, possessed a nucleus.

analogues have a different mode of action from other classes of ANPs and a target that is unlikely to be the *Pf*HGXPRT.

We then evaluated the compounds for toxicity on human blood cell lines (K562 cells) and calculated selectivity indexes (SI) (Table 1). None of the derivatives inhibited human blood cell growth below a concentration of 1 mM indicating a high selectivity for infected red blood cells. All ANPs tested are of anionic character which generally limits their passive diffusion across biological membranes, explaining their low toxicity toward human cell lines. Our data showed that the ANPs developed in this study affected parasite growth and were therefore likely able to specifically enter into infected red blood cells. This could be due to the presence of channels transporting negatively charged molecules on the infected erythrocyte membrane that are induced by the parasite.[27]

Altogether, these data revealed a unique antimalarial pharmacophore and the compound **5**(*R*) shows considerable potential with an antimalarial activity of 74 nM and a SI higher than 1350.

Evaluation of Compounds 5(*R*) and 5(*S*) on the Drug-Resistant Strains of *P. falciparum*. Compounds **5**(*R*) and **5**(*S*) were selected for further studies with the multidrug-resistant W2 strain and the chloroquine-resistant *FcM29* strain of *P. falciparum* (Table 2). Both derivatives showed equivalent potency in the three strains 3D7, W2, and FcM29.

Antimalarial Activity in *P. berghei* Infected Mice. According to the results obtained *in vitro*, compound **5**(*R*) was evaluated in an *in vivo* assay using *P. berghei*-infected mice (Figure 5). After 4 days of treatment by intraperitoneal (ip) route, the parasitemia was quantified at day 5 and the dose required to decrease the parasitemia by 50% (ED_{50}) was determined. In these conditions, the derivative **5**(*R*) showed a potent efficacy *in vivo* with an ED_{50} value of 0.56 mg/kg (Figure 5A). Parasites were not detected after a treatment with 30 mg/kg at day 5, but we observed a parasite recrudescence for two mice after day 11 (6.9% and 16.7% of parasitemia) and for one mouse after day 14 (1.7% of parasitemia). In parallel, we tested the effect of a single dose of 30 mg/kg administered at day 1. This treatment showed a decrease of the parasitemia by 95% as compared to the controls. The efficacy of the derivative may enable the dosage regimens to be modified for further development. The activity was also evaluated by oral administration after 4 days of treatment. In these conditions, the ED_{50} was found to be 30 mg/kg indicating a low bioavailability likely due to the polarity and anionic character of the compound (Figure 5B).

In comparison, artesunate, an artemisinin derivative, showed an ED_{50} value of ~0.1 mg/kg by ip route (Figure 5C) and a treatment of 27 mg/kg did not allow a complete cure. This suggests that the derivative **5**(*R*) showed a potent activity comparable to this widely used antimalarial drug.

Table 2. Comparative Effects of Derivatives 5(*R*) and 5(*S*) on the Growth of *P. falciparum* Strains, Sensitive (3D7) or Resistant (FcM29 and W2) to Chloroquine[a]

	IC_{50} (μM)		
compd	3D7	FcM29	W2
5(*R*)	0.074 ± 0.007	0.03 ± 0.003	0.045 ± 0.006
5(*S*)	6.1 ± 1.1	4.5 ± 1.3	5.6 ± 1.4
chloroquine	0.016 ± 0.001	0.117 ± 0.010	0.131 ± 0.022

[a]Values (±SEM) are the mean of at least three independent experiments performed in duplicate.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

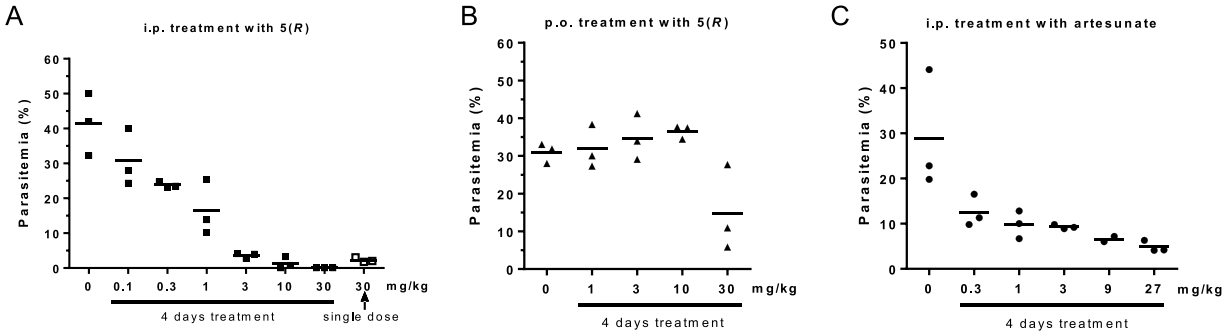


Figure 5. *In vivo* antimalarial activity of **5**(*R*) and artesunate in *P. berghei*-infected mice. **5**(*R*) antimalarial activity was determined after intraperitoneal (ip) (A) or oral (po) (B) administration. Mice were treated with one daily dose for 4 consecutive days from day 1 or with a single dose of 30 mg/kg on day 1 after infection. (C) Artesunate antimalarial activity after intraperitoneal administration. Mice were treated with one daily dose for 4 consecutive days from day 1. In all cases, parasitemia was determined at day 5. In all cases, controls received vehicle only.

CONCLUSION

We reported and tested 24 derivatives, including 22 novel acyclic nucleoside phosphonates, as potential inhibitors of *P. falciparum* growth. Synthesis of β-hydroxyphosphonates with different chain lengths (three to four carbon atoms) bearing various nucleobases was performed. An original synthetic pathway to β-aminophosphonates was developed and constitutes a new method for accessing compounds of this class with therapeutic potential. Biological assays of phosphonic acid compounds (as sodium salts) on cell cultures revealed that the compounds' effectiveness depends on the hydroxyl group, the chain length, and the nature of the base. Of all compounds, derivative **5**(*R*) appears to have the most suitable characteristics (i.e., guanine as nucleobase, a butyl chain, a hydroxy group in the β-position and with *R* stereochemistry). It shows remarkable *in vitro* activity against *P. falciparum* infected red blood cells (IC_{50} = 74 nM), and it has a high selectivity index (SI > 1350). No toxicity on human cell lines was found. The derivative **5**(*R*) also showed a considerable activity *in vivo* ($ED_{50} \sim 0.5$ mg/kg by ip route) in the same range as the currently used artesunate. To our knowledge, this is the first report on the antiplasmodial activity (*in vitro* and *in vivo*) of an acyclonucleoside phosphonate derivative. Given these encouraging results, we will pursue our research on this family of β-substituted ANPs to improve oral bioavailability using a prodrug strategy and to decipher the mechanism of action of the compound **5**(*R*) which is likely different from the other ANPs.

EXPERIMENTAL SECTION

Chemistry. General Information. Solvents were dried by standard procedures. Tetrahydrofuran (THF) was freshly distilled from Na under argon and in the presence of benzophenone. All reagents and chemicals were obtained from commercial sources. Thin layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F_{254} (Merck, no. 5554). Product spots on TLC plate were visualized with a UV lamp (254 nm). Flash chromatography on normal phase (silica gel) and on reversed phase (C18AQ) was performed on Biotage or Interchim Flash Chromatography Systems, respectively. NMR spectra were recorded on Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) or 300 (^{1}H at 300 MHz, ^{13}C at 125.8 MHz) spectrometer with TMS as internal standard or referenced to the residual solvent signal ($CDCl_3$, DMSO-d_6 or D_2O) for ^{1}H and ^{13}C. H_3PO_4 was used as external standard for ^{31}P NMR, and corresponding spectra were recorded with proton decoupling at ambient temperature. Chemical shifts (δ) are quoted in parts per million (ppm). Mass spectra (ESI) and high-resolution mass spectroscopy (HR-MS) results were recorded in the negative and/or positive-ion mode using a SYNAPT G2 mass spectrometer. Optical rotation was determined by a Krüss P8000 polarimeter. Specific rotations $[\alpha]^D_{20}$ are given in deg cm^3 g^{-1} dm^{-1}. Purity was determined by high performance liquid chromatography (HPLC). Purity of all final compounds was 95% or higher. The instrument was a Waters HPLC system (separation module 2695, 996 photodiode array detector 2996). The column was a Waters Symmetry Shield (50 mm × 4.6 mm, 3.5 μm) RP-18-column. The elution solvents were water containing 0.1% (v/v) of TFA (solvent A) and acetonitrile containing 0.1% (v/v) TFA (solvent B). A linear gradient was applied from 0% to 100% of solvent B in 7 or 15 min with a flow rate of 1 mL/min.

General Procedure A. *Preparation of Epoxide Derivatives in the C3 Series.* To a solution of commercially available (*R*)- or (*S*)-glycidol (1.1 equiv) in freshly distilled tetrahydrofuran (1.5 mL/mmol of nucleobase) were added under argon atmosphere triphenylphosphine (1.2 equiv) and the nucleobase (1 equiv). The reaction mixture was cooled to 0 °C, and diethyl azodicarboxylate (1.2 equiv) was added dropwise. After 10 min, the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was concentrated under vacuum, dissolved in a small amount of diethyl ether, and stored at 4 °C overnight. The triphenylphosphine oxide precipitate was filtrated off and washed with cold diethyl ether. The filtrate was then concentrated under vacuum and the crude purified by flash chromatography on silica gel, using either an isocratic of AcOEt or CH_2Cl_2/MeOH (0 to 5%) to isolate the desired product.

*N,N-6-Bis(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)methyl)-adenine (**14**(R)).* Compound **14**(*R*) (1.86 g) was obtained in 87% yield as an oil, according to procedure A, from (*R*)-glycidol (362 μL). R_f = 0.41 in AcOEt. $[\alpha]_D^{20}$ = +22.5° (*c* 1.02, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.86 (s, 1H, H_{-2}), 8.60 (s, 1H, H_{-8}), 4.67 (dd, 1H, ^2J = 15.0 Hz, ^3J = 2.7 Hz, CH_2N), 4.40 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.0 Hz, CH_2N), 3.47 (m, 1H, CHO), 2.83 (m, 1H), 1.37 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 152.4 (CH_{-2}), 145.1 (CH_{-8}), 128.6 (C_{-5}), 83.9 ($C(CH_3)_3$), 49.9 (CHO), 45.5 (OCH_2), 45.4 (CH_2N), 27.9 (CH_3). MS (ESI) *m*/*z* 392.2 $[M + H]^+$. HRMS: calcd $C_{18}H_{26}N_5O_5$ $[M + H]^+$ 392.1934, obs 392.1921.

*N,N-6-Bis(tert-butoxycarbonyl)-N-9-(2S-(oxiran-2-yl)methyl)-adenine (**14**(S)).* Compound **14**(*S*) (1.01 g) was obtained in 86% yield as a solid, according to procedure A, from (*S*)-glycidol (181 μL). R_f = 0.41 in AcOEt. $[\alpha]_D^{20}$ = −16.8° (*c* 1.01, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.86 (s, 1H, H_{-2}), 8.60 (s, 1H, H_{-8}), 4.65 (dd, 1H, ^2J = 15.0 Hz, ^3J = 2.7 Hz, CH_2N), 4.40 (dd, 1H, ^2J = 15.0 Hz, ^3J = 6.0 Hz, CH_2N), 3.47 (m, 1H, CHO), 2.83 (m, 1H), 1.38 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 153.3 (C_{-4}), 151.6 (CH_{-2}), 150.0, 149.1 (C_{-6}, C=O), 147.0 (CH_{-8}), 127.5 (C_{-5}), 83.3 ($C(CH_3)_3$), 49.4 (CHO), 45.1 (OCH_2), 45.0 (CH_2N), 27.3 (CH_3). MS (ESI) *m*/*z* 392.3 $[M + H]^+$. HRMS: calcd $C_{18}H_{26}N_5O_5$ $[M + H]^+$ 392.1934, obs 392.1941.

*N,N-2-Bis(tert-butoxycarbonyl)-6-methoxy-N-9-(2R-(oxiran-2-yl)methyl)-2-aminopurine (**15**(R)).* Compound **15**(*R*) (1.72 g) was obtained in 74% yield as a solid, according to procedure A, from (*R*)-glycidol (336 μL). R_f = 0.2 in AcOEt. $[\alpha]_D^{20}$ = +20.5° (*c* 1.05, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.39 (s, 1H, H_{-8}), 4.54−4.34 (m, 2H,

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

CH_2N), 4.07 (s, 3H, OCH_3), 3.40 (m, 1H, CHO), 2.81 (m, 1H, OCH_2), 2.50 (m, 1H partially covered by DMSO, OCH_2), 1.39 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 160.6 (C_{-6}), 152.7 (C_{-4}), 151.0 (C_{-2}), 150.3 (C=O), 144.9 (CH_{-8}), 118.7 (C_{-5}), 82.6 ($\underline{C}(CH_3)_3$), 54.3 (OCH_3), 49.3 (CHOH), 44.8 (OCH_2), 44.7 (CH_2N), 27.3 (CH_3). MS (ESI) m/z 422.2 $[M + H]^+$. HRMS: calcd $C_{19}H_{28}N_5O_6$ $[M + H]^+$ 422.2040, obs 422.2034.

*N,N-2-Bis(tert-butoxycarbonyl)-6-methoxy-N-9-(2S-(oxiran-2-yl)-methyl)-2-aminopurine (**15**(S)).* Compound **15**(*S*) (1.75 g) was obtained in 76% yield as a solid, according to procedure A, from (*S*)-glycidol (332 μL). R_f = 0.2 in AcOEt. $[\alpha]_D^{20}$ = −22.1° (*c* 0.98, MeOH). ^1H NMR (DMSO-d_6): δ = 8.39 (s, 1H, H_{-8}), 4.54−4.33 (m, 2H, CH_2N), 4.07 (s, 3H, OCH_3), 3.41 (m, 1H, CHO), 2.81 (m, 1H, OCH_2), 2.50 (m, 1H partially covered by DMSO, OCH_2), 1.39 (s, 18H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 160.6 (C_{-6}), 152.7 (C_{-4}), 151.0 (C_{-2}), 150.3 (C=O), 144.9 (CH_{-8}), 118.7 (C_{-5}), 82.7 ($\underline{C}(CH_3)_3$), 54.3 (OCH_3), 49.3 (CHOH), 44.9 (OCH_2), 44.7 (CH_2N), 27.3 (CH_3). MS (ESI) m/z 422.2 $[M + H]^+$. HRMS: calcd $C_{19}H_{28}N_5O_6$ $[M + H]^+$ 422.2040, obs 422.2047.

*N,N-2,6-Tetra(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)-methyl)-2,6-diaminopurine (**16**(R)).* Compound **16**(*R*) (1.40 g) was obtained in 84% yield as a solid, according to procedure A, from (*R*)-glycidol (165 μL). R_f = 0.55 in AcOEt. $[\alpha]_D^{20}$ = +22.2° (*c* 1.01, MeOH). ^1H NMR (DMSO-d_6): δ = 8.66 (s, 1H, H_{-8}), 4.67−4.36 (m, 2H, CH_2N), 3.45 (m, 1H, CHO), 2.83 (m, 1H, OCH_2), 2.44 (m, 1H, OCH_2), 1.36 (s, 36H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 154.0 (C_{-4}), 151.0, 150.2 (C_{-6}, C_{-2}), 148.0, 149.5, 149.6, 150.2 (C=O), 125.9 (CH_{-8}), 83.4, 82.8 ($\underline{C}(CH_3)_3$), 49.2 (CHOH), 44.7 (OCH_2, CH_2N), 27.2, 27.0 (CH_3). MS (ESI) m/z 607.3 $[M + H]^+$. HRMS: calcd $C_{28}H_{43}N_6O_9$ $[M + H]^+$ 607.3092, obs 607.3088.

*N,N-2,6-Tetra(tert-butoxycarbonyl)-N-9-(2S-(oxiran-2-yl)-methyl)-2,6-diaminopurine (**16**(S)).* Compound **16**(*S*) (2.31 g) was obtained in 84% yield as a solid, according to procedure A, from (*S*)-glycidol (275 μL). R_f = 0.42 in AcOEt/petroleum ether (6/4, v/v). $[\alpha]_D^{20}$ = −21.8° (c 1.0, MeOH). ^1H NMR (DMSO-d_6): δ = 8.66 (s, 1H, H-8), 4.70−4.36 (m, 2H, CH_2N), 3.45 (m, 1H, CHO), 2.83 (m, 1H, OCH_2), 2.44 (m, 1H, OCH_2), 1.36 (s, 36H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 154.0 (C-4), 151.0, 150.2 (C-6, C-2), 148.0, 149.5, 149.6, 150.2 (C=O), 125.9 (CH-8), 83.4, 82.8 ($C(CH_3)_3$), 49.2 (CHOH), 44.7 (OCH_2, CH_2N), 27.2, 27.0 (CH_3). MS (ESI) m/z 607.3 $[M + H]^+$. HRMS: calcd $C_{28}H_{43}N_6O_9$ $[M + H]^+$ 607.3092, obs 607.3082.

General Procedure B. *Preparation of Epoxide Derivatives in the C4 Series.* To a solution of enantiomerically pure 2-bromo-1,4-butanediol[28] (2 equiv) in freshly distilled dichloromethane (6.5 mL/mmol of nucleobase) was added under argon atmosphere anhydrous cesium carbonate (Cs_2CO_3, 3.6 equiv). The reaction mixture was stirred at room temperature and followed by TLC (CH_2Cl_2/MeOH 95/5 v/v, with sulfuric acid revelation) until completion. After 24 h, the suspension was filtered and the precipitate washed with 3 mL of anhydrous dichloromethane. The filtrate was then transferred to a round-bottom flask flushed with argon, and anhydrous tetrahydrofuran (3.8 mL/mmol of nucleobase), triphenylphosphine (1.2 equiv), and the nucleobase (1 equiv) were successively added. The reaction mixture was cooled to 0 °C, and diethyl azodicarboxylate (1.2 equiv) was added dropwise. After 10 min, the reaction mixture was allowed to warm to room temperature and stirred for 24 h with TLC monitoring (CH_2Cl_2/MeOH 95/5 v/v). The reaction mixture was concentrated under vacuum, dissolved in a small amount of diethyl ether, and stored at 4 °C overnight. The triphenylphosphine oxide precipitate was removed by filtration and washed with cold diethyl ether. The filtrate was then concentrated under vacuum and the crude purified by flash chromatography on silica gel, using $CHCl_3$/AcOEt (0 to 100%) to remove the remaining triphenylphosphine oxide, and then CH_2Cl_2/MeOH (0 to 10%) to isolate the desired product, which was obtained as a pale yellow oil.

*N,N-6-Bis(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)ethyl)-adenine (**21**(R)).* Compound **21**(*R*) (637 mg) was obtained in 53% yield, according to procedure B, from 2-(*R*)-bromo-1,4-butanediol (1 g). R_f = 0.50 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ = +18.52° (*c* 0.8, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.82 (s, 1H, H_{-2}), 8.09 (s, 1H, H_{-8}), 4.48−4.39 (m, 2H, CH_2N), 2.93−2.90 (m, 1H, CHO), 2.72−2.70 (m, 1H, $\underline{CH_2}CH$), 2.43−2.41 (m, 1H, $O\underline{CH_2}CH$), 2.38−2.32 (m, 1H, $\underline{CH_2}CH_2N$), 1.95−1.88 (m, 1H, $\underline{CH_2}CH_2N$), 1.41 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 153.4 (C_{-4}), 152.1 (CH_{-2}), 150.5 (C_{-6}), 150.4 (C=O), 144.9 (CH_{-8}), 128.9 (C_{-5}), 83.8 ($C(CH_3)_3$), 49.4 (CHOH), 46.8 ($O\underline{CH_2}CH$), 41.4 (s, CH_2N), 32.7 ($\underline{CH_2}CH_2N$), 27.8 (CH_3). HRMS (TOF ESI > 0) found: 406.2055. Calculated for $C_{19}H_{28}N_5O_5$: 406.2050 $(M + H)^+$.

*N,N-6-Bis(tert-butoxycarbonyl)-N-9-(2S-(oxiran-2-yl)ethyl)-adenine (**21**(S)).* Compound **21**(*S*) (1 g) was obtained in 63% yield, according to procedure B, from 2-(*S*)-bromo-1,4-butanediol (1.32 g). R_f = 0.50 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ −20.98 (*c* 0.4, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.83 (s, 1H, H_{-2}), 8.10 (s, 1H, H_{-8}), 4.49−4.40 (m, 2H, CH_2N), 2.93−2.91 (m, 1H, CHO), 2.73−2.71 (m, 1H, $O\underline{CH_2}CH$), 2.43−2.42 (m, 1H, $O\underline{CH_2}CH$), 2.39−2.32 (m, 1H, $\underline{CH_2}CH_2N$), 1.95−1.88 (m, 1H, $\underline{CH_2}CH_2N$), 1.41 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 153.4 (C_{-4}), 152.1 (C_{-2}), 150.5 (C_{-6}), 150.4 (C=O), 144.9 (C_{-8}), 128.9 (C_{-5}), 83.8 ($\underline{C}(CH_3)_3$), 49.4 (CHOH), 46.8 ($O\underline{CH_2}CH$), 41.4 (CH_2N), 32.7 ($\underline{CH_2}CH_2N$), 27.8 (CH_3). HRMS (TOF ESI > 0) found: 406.2055. Calculated for $C_{19}H_{28}N_5O_5$: 406.2050 $(M + H)^+$.

*N,N-2-Bis(tert-butoxycarbonyl)-6-methoxy-N-9-(2R-(oxiran-2-yl)ethyl)-2-aminopurine (**22**(R)).* Compound **22**(*R*) (1.57 g) was obtained in 66% yield, according to procedure B, from 2-(*R*)-bromo-1,4-butanediol (1.85 g). R_f = 0.52 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ +20.86° (*c* 0.9, CH_2Cl_2). ^1H NMR (DMSO-d_6) δ 8.45 (s, 1H, H_{-8}), 4.39−4.35 (m, 2H, CH_2N), 4.06 (s, 3H, OCH_3), 2.94−2.93 (m, 1H, CHO), 2.60−2.57 (m, 1H, $O\underline{CH_2}CH$), 2.29−2.27 (m, 1H, OCH_2), 2.10−2.09 (m, 1H, $\underline{CH_2}CH_2N$), 1.98−1.95 (m, 1H, $\underline{CH_2}CH_2N$), 1.43 (s, 18H, CH_3). ^{13}C NMR(DMSO-d_6) δ 160.3 (C_{-6}), 152.4 (C_{-4}), 150.5 (C_{-2}), 150.2 (C=O), 144.7 (CH_{-8}), 118.8 (C_{-5}), 82.4 ($\underline{C}(CH_3)_3$), 54.1 (OCH_3), 49.0 (CHOH), 45.1 ($O\underline{CH_2}CH$), 40.6 (CH_2N), 31.9 ($\underline{CH_2}CH_2N$), 27.1 (CH_3). HRMS (TOF ESI > 0) found: 436.2199. Calculated for $C_{20}H_{30}N_5O_6$: 436.2196 $(M + H)^+$.

*N,N-2-Bis(tert-butoxycarbonyl)-6-methoxy-N-9-(2S-(oxiran-2-yl)-ethyl)-2-aminopurine (**22**(S)).* Compound **22**(*S*) (787 mg) was obtained in 66% yield, according to procedure B, from 2-(*S*)-bromo-1,4-butanediol (926 mg). R_f = 0.52 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ −22.53° (*c* 1.4, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 7.96 (s, 1H, H_{-8}), 4.43−4.35 (m, 2H, CH_2N), 4.14 (s, 3H, OCH_3), 2.93−2.90 (m, 1H, CHO), 2.73−2.72 (m, 1H, $O\underline{CH_2}CH$), 2.41−2.40 (m, 1H, $O\underline{CH_2}CH$), 2.32−2.26 (m, 1H, $\underline{CH_2}CH_2N$), 1.97−1.92 (m, 1H, $\underline{CH_2}CH_2N$), 1.43 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 161.6 (C_{-6}), 152.8 (C_{-4}), 152.1 (C_{-2}), 151.1 (C=O), 143.0 (CH_{-8}), 120.2 (C_{-5}), 83.2 ($\underline{C}(CH_3)_3$), 54.7 (OCH_3), 49.4 (CHOH), 46.9 ($O\underline{CH_2}CH$), 41.4 (CH_2N), 32.9 ($\underline{CH_2}CH_2N$), 28.0 (CH_3). HRMS (TOF ESI > 0) found: 436.2200. Calculated for $C_{20}H_{30}N_5O_6$: 436.2196 $(M + H)^+$.

*N,N-2,6-Tetra(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)ethyl)-2,6-diaminopurine (**23**(R)).* Compound **23**(*R*) (625 mg) was obtained in 90% yield, according to procedure B, from 2-(*R*)-bromo-1,4-butanediol (376 mg). R_f = 0.34 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ +20.5° (*c* 0.9, CH_2Cl_2). ^1H NMR (DMSO-d_6): δ = 8.72 (s, 1H, H_{-8}), 4.45 (t, 2H, CH_2N), 2.93−2.96 (m, 1H, CHO), 2.56 (m, 1H, $\underline{CH_2}CH$), 2.27 (m, 1H, $O\underline{CH_2}CH$), 2.20−2.13 (m, 1H, $\underline{CH_2}CH_2N$), 2.16−1.95 (m, 1H, $\underline{CH_2}CH_2N$), 1.35 (s, 36H, CH_3). ^{13}C NMR (DMSO-d_6) δ 153.9 (C_{-4}), 150.7 (C_{-6}), 148.0, 149.4, 149.5, 150.3 (C=O), 126.3 (C_{-5}), 82.8, 83.4 ($C(CH_3)_3$), 49.2 (CHOH), 45.1 ($O\underline{CH_2}CH$), 41.4 (CH_2N), 31.9 ($\underline{CH_2}CH_2N$), 27.2, 27.0 (CH_3). MS (ESI) m/z 621.3 $[M + H]^+$; 643.3 $[M + Na]^+$; 1263.6 $[M + 2Na]^+$.

*N,N-2,6-Tetra(tert-butoxycarbonyl)-N-9-(2S-(oxiran-2-yl)ethyl)-2,6-diaminopurine (**23**(S)).* Compound **23**(*S*) (3.01 g) was obtained in 89% yield, according to procedure B, from 2-(*S*)-bromo-1,4-butanediol (1.84 g). R_f = 0.23 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ −19.8° (*c* 0.85, CH_2Cl_2). ^1H NMR (DMSO-d_6): δ = 8.71 (s, 1H, H_{-8}), 4.44 (t, 2H, CH_2N), 2.94 (m, 1H, CHO), 2.56 (m, 1H, $\underline{CH_2}CH$), 2.26 (m, 1H, $O\underline{CH_2}CH$), 2.20−2.13 (m, 1H, $\underline{CH_2}CH_2N$), 2.04−1.97 (m, 1H, $\underline{CH_2}CH_2N$), 1.35 (s, 36H, CH_3). ^{13}C NMR (DMSO-d_6) δ 153.9 (C_{-4}), 150.7 (C_{-6}), 148.0, 149.4, 149.5, 150.2 (C=O), 126.3 (C_{-5}), 82.8,

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

83.4 ($C(CH_3)_3$), 49.1 (CHOH), 45.1 (OCH_2CH), 41.1 (CH_2N), 31.9 (CH_2CH_2N), 27.2, 27.0 (CH_3). MS (ESI) m/z 621.33 $[M + H]^+$; 643.31 $[M + Na]^+$.

*N,N-2-Bis(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)ethyl)-2-aminopurine (**31**(R)).* Compound **31**(*R*) (1.90 g) was obtained in 71% yield, according to procedure B, from 2-(*R*)-bromo-1,4-butanediol (2.2 g). R_f = 0.40 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ +20.80° (*c* 0.9, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1H, H_{-6}), 8.16 (s, 1H, H_{-8}), 4.45 (m, 2H, CH_2N), 2.94 (m, 1H, CHO), 2.76 (m, 1H, OCH_2CH), 2.45 (m, 1H, OCH_2CH), 2.42−2.30 (m, 1H, CH_2CH_2N), 2.05−1.93 (m, 1H, CH_2CH_2N), 1.43 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 149.6 (C═O), 146.2 (CH_{-8}), 83.3 ($C(CH_3)_3$), 49.3 (CHOH), 46.8 (OCH_2CH), 41.2 (CH_2N), 32.6 (CH_2CH_2N), 29.7, 27.9 (CH_3). MS (ESI) m/z 406.21 $[M + H]^+$.

*N,N-6-Bis(tert-butoxycarbonyl)-N-9-(2R-(oxiran-2-yl)ethyl)-2-fluoroadenine (**32**(R)).* Compound **32**(*R*) (725 mg) was obtained in 50% yield, according to procedure B, from 2-(*R*)-bromo-1,4-butanediol (1.16 g). R_f = 0.43 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ = +18.25° (*c* 0.8, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H, H_{-8}), 4.39 (m, 2H, CH_2N), 2.96−2.92 (m, 1H, CHO), 2.76 (m, 1H, CH_2CH), 2.47 (m, 1H, OCH_2CH), 2.42−2.27 (m, 1H, CH_2CH_2N), 1.96−1.85 (m, 1H, CH_2CH_2N), 1.46 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 157.7 (C_{-4}), 156.0 (C_{-6}), 154.1, 154.0 (C_{-2}), 151.0, 151.1 (C═O), 144.9 (C_{-8}), 126.0, 125.9 (C_{-5}), 83.3 ($C(CH_3)_3$), 48.2 (CHOH), 45.8 (OCH_2CH), 40.5 (CH_2N), 31.4 (CH_2CH_2N), 26.7 (CH_3). MS (ESI) m/z 424.2.

General Procedure C. *Ring-Opening Reaction Leading to β-Hydroxyphosphonates Derivatives.* To a solution of diethyl phosphite (6 equiv) in freshly distilled dichloromethane (3 mL/mmol of epoxide) was added under argon *N,O*-BSA (6.4 equiv). The reaction mixture was stirred and refluxed for 4 h. Reaction progress was followed by ^{31}P NMR to check the disappearance of diethyl phosphite signal ($\delta \sim 7$ ppm), while silylated diethyl phosphite appeared ($\delta \sim 127$ ppm). The reaction mixture was allowed to reach to room temperature and then cooled to −60 °C. A solution of the epoxide (1 equiv) in freshly distilled dichloromethane (6.6 mL/mmol of epoxide) was added dropwise. Then, boron trifluoride diethyl etherate (6 equiv) was added dropwise, and the reaction mixture was stirred for 3 h and then allowed to warm to room temperature overnight. Volatiles were removed under reduced pressure; the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. Purification by flash chromatography on silica gel using CH_2Cl_2/MeOH (0 to 10%) gave rise to the desired product as a white solid.

*Diethyl (R)-(3-(6-(Bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**17**(R)).* Compound **17**(*R*) (1.37 g) was obtained in 62% yield, according to procedure C, from **14**(*R*) (1.62 g). R_f = 0.42 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +7.8° (*c* 1.02, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.83 (s, 1H, H_{-2}), 8.52 (s, 1H, H_{-8}), 5.50 (d, 1H, OH), 4.46 (m, 1H, CHOH), 4.24 (m, 2H, CH_2N), 3.90−4.04 (m, 4H, OCH_2CH_3), 1.84−2.08 (m, 2H, CH_2P), 1.38 (s, 18H, CH_3), 1.22 (t, 6H, *J* = 6.9 Hz, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 153.7 (C_{-4}), 152.0 (C_{-2}), 150.6 (C_{-6}), 150.4 (C═O), 146.4 (C_{-8}), 128.7 (C_{-5}), 84.0 ($C(CH_3)_3$), 65.5 (CHOH), 62.5, 62.4 (OCH_2CH_3), 49.9, 49.7 (CH_2N), 31.6, 30.2 (CH_2P), 27.9 (CH_3), 16.5, 16.4 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): δ = 28.1. MS (ESI) m/z 530.3 $[M + H]^+$. HRMS: calcd $C_{22}H_{37}N_5O_8P$ $[M + H]^+$ 530.2380, obs 530.2391.

*Diethyl (S)-(3-(6-(Bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**17**(S)).* Compound **17**(*S*) (0.95 g) was obtained in 64% yield, according to procedure C, from **14**(*S*) (1.14 g). R_f = 0.42 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −6.9° (*c* 1.01, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.83 (s, 1H, H_{-2}), 8.51 (s, 1H, H_{-8}), 5.48 (d, 1H, OH), 4.46 (m, 1H, CHOH), 4.24 (m, 2H, CH_2N), 4.01 (m, 4H, OCH_2CH_3), 1.85−2.24 (m, 2H, CH_2P), 1.39 (s, 18H, CH_3), 1.23 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 153.8 (C_{-4}), 152.1 (C_{-2}), 150.7 (C_{-6}), 150.6 (C═O), 146.5 (C_{-8}), 128.8 (C_{-5}), 87.0 ($C(CH_3)_3$), 65.6 (CHOH), 62.6, 62.5 (OCH_2CH_3), 50.0, 49.8 (CH_2N), 31.7, 30.3 (CH_2P), 28.0 (CH_3), 16.7, 16.6 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): δ = 28.1. MS (ESI) m/z 530.3 $[M + H]^+$. HRMS: calcd $C_{22}H_{37}N_5O_8P$ $[M + H]^+$ 530.2380, obs 530.2375.

*Diethyl (R)-(3-(2-(Bis(tert-butoxycarbonyl)amino)-6-methoxy-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**18**(R)).* Compound **18**(*R*) (107 mg) was obtained in 41% yield, according to procedure C, from **15**(*R*) (200 mg). R_f = 0.16 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ +8° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6): δ 8.30 (s, 1H, H_{-8}), 5.41 (d, 1H, J = 5.1 Hz, OH), 4.39 (m, 1H, CHOH), 4.05 (m, 9H, CH_2N, OCH_3, OCH_2CH_3), 1.98 (m, 2H, CH_2P), 1.38 (s, 18H, CH_3), 1.22 (6H, OCH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 160.5 (C_{-6}), 152.8 (C_{-4}), 150.3 (C═O), 145.5 (CH_{-8}), 118.8 (C_{-5}), 82.5 ($C(CH_3)_3$), 72.7 (CHOH), 64.0, 61.1 (OCH_2CH_3), 54.2 (OCH_3), 27.3 ($CH_{3,\ Boc}$), 16.0, 16.1 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): 28.1. MS (ESI) m/z 560.3 $[M + H]^+$. HRMS: calcd $C_{23}H_{39}N_5O_9P$ $[M + H]^+$ 560.2485, obs 560.2481.

*Diethyl (S)-(3-(2-(Bis(tert-butoxycarbonyl)amino)-6-methoxy-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**18**(S)).* Compound **18**(*S*) (1.01 g) was obtained in 43% yield, according to procedure C, from **15**(*S*) (1.75 g). R_f = 0.16 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ −6.8° (*c* 0.95, MeOH). ^{1}H NMR ($CDCl_3$): δ = 8.08 (s, 1H, H_{-8}), 5.23 (m, 1H, OH), 4.72 (m, 1H, CHOH), 4.40−4.20 (m, 2H, CH_2N), 4.10−3.95 (m, 7H, OCH_3, OCH_2CH_3), 2.00−1.67 (2m, 2H, CH_2P), 1.37 (2s, 18H, CH_3), 1.23 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 161.4 (C_{-6}), 152.9 (C_{-4}), 151.7 (C_{-2}), 150.9 (C═O), 144.5 (CH_{-8}), 119.5 (C_{-5}), 83.1 ($C(CH_3)_3$), 65.3 (CHOH), 62.2, 62.1 (OCH_2CH_3), 54.6 (OCH_3), 49.9, 49.7 (NCH_2), 31.5, 30.1 (PCH_2), 27.8 (CH_3), 16.3, 16.2 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): 28.1. MS (ESI) m/z 560.3 $[M + H]^+$; 582.23 $[M + Na]^+$.

*Diethyl (R)-(3-(2,6-(Tetra(tert-butoxycarbonyl)diamino)-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**19**(R)).* Compound **19**(*R*) (482 mg) was obtained in 36% yield, according to procedure C, from **16**(*R*) (1.08 g). R_f = 0.18 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ +11.8° (*c* 1.2, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.56 (s, 1H, H_{-8}), 5.51 (1H, OH), 4.47 (m, 1H, CHOH), 4.21 (m, 2H, CH_2N), 3.99 (m, 4H, OCH_2CH_3), 2.00 (m, 2H, CH_2P), 1.36 (s, 36H, CH_3), 1.22 (pt, 6H, OCH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 154.0 (C_{-4}), 150.7 (C_{-2}), 150.2, 149.6, 149.3, 148.5 (C_{-6}, C═O), 126.1 (C_{-5}), 83.4, 82.7 ($C(CH_3)_3$), 64.0 (CHOH), 61.1, 60.9 (OCH_2CH_3), 32.1 (CH_2N), 30.3 (CH_2P), 27.0, 27.2 (CH_3), 16.0, 16.1 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): 27.9. MS (ESI) m/z 745.3 $[M + H]^+$. HRMS: calcd $C_{32}H_{54}N_6O_{12}P$ $[M + H]^+$ 745.3537, obs 745.3543.

*Diethyl (S)-(3-(2,6-(Tetra(tert-butoxycarbonyl)diamino)-9H-purin-9-yl)-2-hydroxypropyl)phosphonate (**19**(S)).* Compound **19**(*S*) (1.76 g) was obtained in 66% yield, according to procedure C, from **16**(*S*) (2.18 g). R_f = 0.21 in CH_2Cl_2/MeOH (95/5, v/v). $[\alpha]_D^{20}$ −8° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6): δ = 8.56 (s, 1H, H_{-8}), 5.52 (d, 1H, J = 5.1 Hz, OH), 4.47 (m, 1H, CHOH), 4.20 (m, 2H, CH_2N), 3.99 (m, 4H, OCH_2CH_3), 2.00 (m, 2H, CH_2P), 1.36 (s, 36H, CH_3), 1.23 (pt, 6H, OCH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 154.0 (C_{-4}), 150.7 (C_{-2}), 150.2, 149.6, 149.3, 148.5 (C_{-6}, C═O), 126.1 (C_{-5}), 83.4, 82.7 ($C(CH_3)_3$), 63.9 (CHOH), 61.1, 60.9 (OCH_2CH_3), 32.1 (CH_2N), 30.3 (CH_2P), 27.1, 27.2 (CH_3), 16.0, 16.1 (OCH_2CH_3). ^{31}P NMR (DMSO-d_6): 27.9. MS (ESI) m/z 745.3 $[M + H]^+$. HRMS: calcd $C_{32}H_{54}N_6O_{12}P$ $[M + H]^+$ 745.3537, obs 745.3531.

*Diethyl (R)-(4-(6-(Bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**24**(R)).* Compound **24**(*R*) (886 mg) was obtained in 52% yield, according to procedure C, from **21**(*R*) (1.28 g). R_f = 0.5 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ +17.05° (*c* 0.09, CH_2Cl_2). ^{1}H NMR($CDCl_3$) δ 8.85 (s, 1H, H_{-2}), 8.13 (s, 1H, H_{-8}), 4.54−4.44 (m, 2H, CH_2N), 4.09 (m, 4H, OCH_2CH_3), 3.95−3.84 (m, 1H, CHOH), 2.18, 2.01 (2m, 2H, CH_2CH_2N), 1.93 (m, 2H, CH_2P), 1.46 (s, 18H, CH_3), 1.33−1.28 (m, 6H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 153.6 (C_{-4}), 152.1 (C_{-2}), 150.7 (C_{-6}), 150.5 (C═O), 145.5 (C_{-8}), 129.0 (C_{-5}), 83.9 ($C(CH_3)_3$), 63.4 (CHOH), 62.2 (OCH_2CH_3), 40.9 (CH_2N), 38.0−37.8 (d, *J* = 17.5 Hz, CH_2CH_2N), 34.1, 33.0 (CH_2P), 28.0 (CH_3), 16.6, 16.5 (OCH_2CH_3). ^{31}P NMR ($CDCl_3$) δ 29.2 (s). HRMS (TOF ESI > 0) found: 544.2534. Calculated for $C_{23}H_{39}N_5O_8P$: 544.2536 $(M + H)^+$.

*Diethyl (S)-(4-(6-(Bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**24**(S)).* Compound **24**(*S*) (746 mg) was obtained in 54% yield, according to procedure C, from **21**(*S*) (1 g). R_f = 0.5 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ −19.3° (*c* 0.3, CH_2Cl_2).

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

^{1}H NMR($CDCl_3$) δ 8.85 (s, 1H, H_{-2}), 8.13 (s, 1H, H_{-8}), 4.55−4.43 (m, 2H, CH_2N), 4.20 (s, 1H, OH), 4.15−4.05 (m, 4H, $\underline{CH_2}CH_3$), 3.96−3.85 (m, 1H, $\underline{CH}OH$), 2.22−1.97 (2m, 2H, $\underline{CH_2}CH_2N$), 1.97−1.90 (m, 2H, CH_2P), 1.46 (s, 18H, CH_3), 1.31 (6H, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 153.6 (C_{-4}), 152.1 (C_{-2}), 150.7 (C_{-6}, C=O), 145.5 (C_{-8}), 129.0 (C_{-5}), 83.9 ($\underline{C}(CH_3)_3$), 63.4 (CHOH), 62.2 ($O\underline{C}H_2CH_3$), 40.9 (CH_2N), 37.9 ($\underline{C}H_2CH_2N$), 34.1, 33.0 (CH_2P), 28.0 (CH_3), 16.5, 16.6 ($\underline{C}H_3CH_2$). ^{31}P NMR ($CDCl_3$) δ 29.2 (s). HRMSTOF ESI+ found: 544.2540. Calculated for $C_{23}H_{39}N_5O_8P$: 544.2536 $(M + H)^+$.

*Diethyl (R)-(4-(2-(Bis(tert-butoxycarbonyl)amino)-6-methoxy-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**25**(R)).* Compound **25**(*R*) (761 mg) was obtained in 37% yield, according to procedure C, from **22**(*R*) (1.57 g). R_f = 0.60 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ +15.24° (*c* 0.1, CH_2Cl_2). ^{1}H NMR($CDCl_3$) δ 7.97 (s, 1H, H_{-8}), 4.50−4.29 (2m, 2H, CH_2N), 4.14 (s, 3H, OCH_3), 4.13−4.00 (m, 4H, $O\underline{CH_2}CH_3$), 3.83 (m, 1H, $\underline{CH}OH$), 2.17−1.88 (2m, 4H, $\underline{CH_2}CH_2N$, CH_2P), 1.46 (s, 18H, CH_3), 1.29 (t, *J* = 7.1 Hz, 6H, $OCH_2\underline{CH_3}$). ^{13}C NMR ($CDCl_3$) δ 161.6 (C_{-6}), 153.0 (C_{-4}), 152.0 (C_{-2}), 151.1 (C=O), 143.5 (CH_{-8}), 120.0 (C_{-5}), 83.3 ($\underline{C}(CH_3)_3$), 63.1 (CHOH), 62.2 ($O\underline{C}H_2CH_3$), 54.7 (OCH_3), 40.8 (CH_2N), 38.2, 38.1 ($\underline{C}H_2CH_2N$), 34.3, 33.2 (CH_2P), 28.1 (CH_3), 16.8 ($OCH_2\underline{C}H_3$). ^{31}P NMR ($CDCl_3$) δ 29 (s). HRMS (TOF ESI > 0) found: 574.2642. Calculated for $C_{24}H_{41}N_5O_9P$: 574.2642 $(M + H)^+$.

*Diethyl (S)-(4-(2-(Bis(tert-butoxycarbonyl)amino)-6-methoxy-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**25**(S)).* Compound **25**(*S*) (742 mg) was obtained in 45% yield, according to procedure C, from **22**(*S*) (1.25 g). R_f = 0.53 in CH_2Cl_2/MeOH (9/1, v/v). $[\alpha]_D^{20}$ −18.2° (*c* 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H, H_{-8}), 4.50−4.30 (2m, 2H, CH_2N), 4.15 (s, 1H, OCH_3), 4.13−4.03 (m, 4H, $O\underline{CH_2}CH_3$), 3.88−3.78 (m, 1H, CHOH), 2.18−1.85 (2m, 4H, $\underline{CH_2}CH_2N$, CH_2P), 1.46 (s, 18H, CH_3), 1.29 (t, 6H, $OCH_2\underline{CH_3}$). ^{13}C NMR ($CDCl_3$) δ 161.6 (C_{-6}), 153.0 (C_{-4}), 152.0 (C_{-2}), 151.1 (C=O), 143.5 (CH_{-8}), 120.0 (C_{-5}) 83.3 ($\underline{C}(CH_3)_3$), 63.1 ($\underline{CH}OH$), 62.2, 62.1 ($O\underline{C}H_2CH_3$), 54.7 (OCH_3), 40.8 (CH_2N), 38.2, 38.1 ($\underline{C}H_2CH_2N$), 34.3, 33.2 (CH_2P), 28.0 (CH_3), 16.5 ($OCH_2\underline{C}H_3$). ^{31}P NMR($CDCl_3$) δ 29 (s). HRMS (TOF ESI > 0) found: 574.2640. Calculated for $C_{24}H_{41}N_5O_9P$: $(M + H)^+$.

*Diethyl (R)-(4-(2,6-(Tetra(tert-butoxycarbonyl)diamino)-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**26**(R)).* Compound **26**(*R*) (2.04 g) was obtained in 70% yield, according to procedure C, from **23**(*R*) (2.38 g). R_f = 0.42 in CH_2Cl_2/MeOH (90/10, v/v). ^{1}H NMR (DMSO-d_6): δ = 8.67 (s, 1H, H_{-8}), 5.11 (d, 1H, OH), 4.36 (m, 1H, $\underline{CH}OH$), 3.96−3.75 (2m, 6H, CH_2N, $O\underline{CH_2}CH_3$), 2.10−1.90 (m, 2H, CH_2P), 1.37 (2s, 36H, CH_3), 1.18 (6H, $OCH_2\underline{CH_3}$). ^{13}C NMR (DMSO-d_6) δ 153.8 (C_{-4}), 150.7 (C_{-2}), 150.3, 149.6, 149.4 (C=O), 147.9 (CH_{-8}), 126.2 (C_{-5}), 83.3, 82.8 ($\underline{C}(CH_3)_3$), 63.1 (CHOH), 60.9, 60.8 ($O\underline{C}H_2CH_3$), 34.4 (CH_2N), 32.6 (CH_2P), 27.2, 27.0 (CH_3), 16.1, 16.0 ($OCH_2\underline{C}H_3$). ^{31}P NMR (DMSO-d_6): 28.4. MS (ESI) *m*/*z* 759.37 $[M + H]^+$. HRMS: calcd $C_{33}H_{56}N_6O_{12}P$ $[M + H]^+$ 759.3694, obs 759.3687.

*Diethyl (S)-(4-(2,6-(Tetra(tert-butoxycarbonyl)diamino)-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**26**(S)).* Compound **26**(*S*) (1.57 g) was obtained in 70% yield, according to procedure C, from **23**(*S*) (1.83 g). R_f = 0.24 in CH_2Cl_2/MeOH (95/5, v/v). ^{1}H NMR (DMSO-d_6): δ = 8.67 (s, 1H, H_{-8}), 5.11 (d, 1H, OH), 4.36 (m, 1H, $\underline{CH}OH$), 4.0−3.80 (2m, 6H, CH_2N, $O\underline{CH_2}CH_3$), 2.20−1.85 (2m, 4H, $O\underline{CH_2}CH_3$, CH_2P), 1.36 (2s, 36H, CH_3), 1.18 (m, 6H, $OCH_2\underline{CH_3}$). ^{13}C NMR (DMSO-d_6) δ 153.8 (C_{-4}), 150.7 (C_{-2}), 150.3, 149.6, 149.4 (C=O), 147.9 (CH_{-8}), 126.2 (C_{-5}), 83.3, 82.8 ($\underline{C}(CH_3)_3$), 63.1 (CHOH), 60.9, 60.8 ($O\underline{C}H_2CH_3$), 34.4 (CH_2N), 32.6 (CH_2P), 27.2, 27.0 (CH_3), 16.1, 16.0 ($OCH_2\underline{C}H_3$). ^{31}P NMR (DMSO-d_6): 28.4. MS (ESI) *m*/*z* 759.37 $[M + H]^+$.

*Diethyl (R)-(4-(2-(Bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**33**(R)).* Compound **33**(*R*) (598 mg) was obtained in 73% yield, according to procedure C, from **31**(*R*) (609 mg). R_f = 0.25 in CH_2Cl_2/MeOH (95/5, v/v). ^{1}H NMR($CDCl_3$) δ 9.10 (s, 1H, H_{-6}), 8.23 (s, 1H, H_{-8}), 4.46 (m, 2H, CH_2N), 4.09 (m, 4H, $O\underline{CH_2}CH_3$), 3.86 (m, 1H, $\underline{CH}OH$), 2.17−1.85 (2m, 4H, $\underline{CH_2}CH_2N$, CH_2P), 1.45 (s, 18H, CH_3), 1.29 (m, 6H, $OCH_2\underline{CH_3}$). ^{13}C NMR ($CDCl_3$) δ 153.1 (C_{-4}), 151.4 (C_{-2}), 149.8 (C=O), 147.2 (C_{-8}), 83.6 ($\underline{C}(CH_3)_3$), 63.4 (CHOH), 62.4 ($O\underline{C}H_2CH_3$), 40.9 (CH_2N), 37.9−37.8 ($\underline{C}H_2CH_2N$), 34.5, 33.1 (CH_2P), 28.2 (CH_3), 16.7, 16.6 ($OCH_2\underline{C}H_3$). ^{31}P NMR ($CDCl_3$) δ 29.0 (s). MS (ESI) *m*/*z* 566.24 $[M + Na]^+$.

*Diethyl (R)-(4-(6-(Bis(tert-butoxycarbonyl)amino)-9H-2-fluoro-purin-9-yl)-2-hydroxybutyl)phosphonate (**34**(R)).* Compound **34**(*R*) (830 mg) was obtained in 89% yield, according to procedure C, from **32**(*R*) (703 mg). R_f = 0.25 in CH_2Cl_2/MeOH (95/5, v/v). ^{1}H NMR($CDCl_3$) δ 8.35 (s, 1H, H_{-8}), 4.45 (m, 2H, CH_2N), 4.12−4.05 (m, 4H, $O\underline{CH_2}CH_3$), 3.95 (m, 1H, $\underline{CH}OH$), 2.20−1.90 (2m, 4H, $\underline{CH_2}CH_2N$, CH_2P), 1.49 (s, 18H, CH_3), 1.30 (m, 6H, $OCH_2\underline{CH_3}$). ^{13}C NMR ($CDCl_3$) δ 155.8 (C_{-6}), 154.1 (C_{-4}), 150.9 (C_{-2}), 149.0 (C=O), 144.8 (C_{-8}), 83.4 ($\underline{C}(CH_3)_3$), 62.4 (CHOH), 61.2 ($O\underline{C}H_2CH_3$), 40.1 (CH_2N), 36.4, 36.3 ($\underline{C}H_2CH_2N$), 33.2, 31.8 (CH_2P), 26.8 (CH_3), 15.4, 15.3 ($OCH_2\underline{C}H_3$). ^{31}P NMR ($CDCl_3$) δ 29.1 (s). MS (ESI) *m*/*z* 562.25 $[M + H]^+$.

General Procedure D. *Removal of Boc Protecting Groups.* To a stirred solution of β-hydroxyphosphonate (1 equiv) in anhydrous dichloromethane (14 mL/mmol) under argon and at room temperature was added dropwise a solution of trifluoroacetic acid in anhydrous dichloromethane (19 mL/mmol, 3/7, v/v). The reaction mixture was stirred at room temperature for 5 h. Volatiles were removed under reduced pressure, and the crude was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (0 to 10%). The desired compound was obtained as a white foam.

*Diethyl (R)-(4-(4-(6-Amino-9H-purin-9-yl)-2-hydroxybutyl)-phosphonate (**27**(R)).* The compound **27**(*R*) (530 mg) was obtained in quantitative yield, according to procedure D, from **24**(*R*) (840 mg). R_f = 0.42 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +15° (*c* 0.2, CH_2Cl_2). ^{1}H NMR($CDCl_3$) δ 8.31 (s, 1H, H_{-2}), 7.91 (s, 1H, H_{-8}), 6.87 (s, 2H, NH_2), 4.50−4.32 (2m, 2H, CH_2N), 4.13−4.03 (m, 4H, $\underline{CH_2}CH_3$), 3.90−3.85 (m, 1H, CHOH), 2.18−2.12 (m, 1H, CH_2CH_2N), 2.02−1.88 (m, 3H, $\underline{CH_2}CH_2N$, CH_2P), 1.31−1.27 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 154.5 (C_{-6}), 150.3 (C_{-2}), 149.9 (C_{-4}), 142.0 (CH_{-8}), 119.5 (C_{-5}), 63.2 (CHOH), 62.3−62.1 ($\underline{CH_2}CH_3$), 40.9 (CH_2N), 38.2, 38.1 ($\underline{CH_2}CH_2N$), 34.3, 33.0 (CH_2P), 16.5 ($\underline{CH_3}CH_2$). ^{31}P NMR ($CDCl_3$) δ 28.7 (s). HRMS (TOF ESI > 0) found: 344.1487. Calculated for $C_{13}H_{23}N_5O_4P$: 344.1488 $(M + H)^+$.

*Diethyl (S)-(4-(6-Amino-9H-purin-9-yl)-2-hydroxybutyl)-phosphonate (**27**(S)).* The compound **27**(*S*) (127 mg) was obtained in quantitative yield, according to procedure D, from **24**(*S*) (200 mg). R_f = 0.39 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −16.2° (*c* 0.1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H, H_{-2}), 7.91 (s, 1H, H_{-8}), 6.87 (s, 2H, NH_2), 4.51−4.31 (2m, 2H, CH_2N), 4.15−4.00 (m, 4H, $\underline{CH_2}CH_3$), 3.96−3.83 (m, 1H, CHOH), 2.20−1.98 (m, 1H, CH_2CH_2N), 1.96−1.89 (m, 2H, CH_2P), 1.29 (m, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 154.5 (C_{-6}), 150.3 (C_{-2}), 149.9 (C_{-4}), 142.0 (CH_{-8}), 119.5 (C_{-5}), 63.2 (CHOH), 62.3, 62.2 ($\underline{CH_2}CH_3$), 40.9 (CH_2N), 38.2 ($\underline{CH_2}CH_2N$), 34.3, 33.0 (CH_2P), 16.5 ($\underline{CH_3}CH_2$). ^{31}P NMR ($CDCl_3$) δ 28.7 (s). HRMS (TOF ESI > 0) found: 344.1490. Calculated for $C_{13}H_{23}N_5O_4P$: 344.1488 $(M + H)^+$.

*Diethyl (R)-(4-(2-Amino-6-methoxy-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**28**(R)).* Compound **28**(*R*) (470 mg) was obtained in quantitative yield, according to procedure D, from **25**(*R*) (720 mg). R_f = 0.50 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +20.95° (*c* 0.5, CH_2Cl_2). ^{1}H NMR($CDCl_3$) δ 7.81 (s, 1H, H_{-8}), 4.43−4.26 (m, 2H, CH_2N), 4.13 (s, 3H, OCH_3), 4.12−4.05 (m, 4H, $\underline{CH_2}CH_3$), 4.0−3.92 (m, 1H, $\underline{CH}OH$), 2.14−1.88 (m, 4H, CH_2CH_2N, CH_2P), 1.30 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 162.6 (C_{-6}), 157.8 (C_{-2}), 149.3 (C_{-4}), 140.2 (CH_{-8}), 114.7 (C_{-5}), 63.4 (CHOH), 62.4, 62.3 ($\underline{CH_2}CH_3$), 55.1 (OCH_3), 41.4 (CH_2N), 38.1, 38.0 ($\underline{CH_2}CH_2N$), 33.9, 32.8 (CH_2P), 16.5 ($\underline{CH_3}CH_2$). ^{31}P NMR($CDCl_3$) δ 29.1 (s). HRMS (TOF ESI > 0) found: 374.1589. Calculated for $C_{14}H_{25}N_5OP$: 374.1593 $(M + H)^+$.

*Diethyl (S)-(4-(2-Amino-6-methoxy-9H-purin-9-yl)-2-hydroxybutyl)phosphonate (**28**(S)).* Compound **28**(*S*) (280 mg) was obtained in quantitative yield, according to procedure D, from **25**(*R*) (430 mg). R_f = 0.50 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −17.62° (*c* 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.81 (s, 1H, H_{-8}), 4.42−

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

4.25 (m, 2H, CH_2N), 4.13 (s, 3H, OCH_3), 4.12−4.05 (m, 4H, $\underline{CH_2}CH_3$), 4.00−3.92 (m, 1H, $\underline{CH}OH$), 2.13−1.92 (m, 4H, CH_2CH_2N, CH_2P), 1.31 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 162.6 (C_{-6}), 157.8 (C_{-2}), 149.3 (C_{-4}), 140.2 (CH_{-8}), 114.7 (C_{-5}), 63.4 (CHOH), 62.4, 62.3 ($\underline{CH_2}CH_3$), 55.1 (OCH_3), 41.4 (CH_2N), 38.1, 38.0 ($\underline{CH_2}CH_2N$), 33.9, 32.8 (CH_2P), 16.5, 16.4 ($\underline{CH_3}CH_2$). ^{31}P NMR ($CDCl_3$) δ 29.2 (s). HRMS (TOF ESI > 0) found: 374.1597. Calculated for $C_{14}H_{25}N_5O_5P$: 374.1593 $(M + H)^+$.

General Procedure E. *Preparation of Oxopurines β-Hydroxyphosphonate Derivatives.* A solution of β-hydroxyphosphonate (1 equiv) in acetic acid 1 M (15 mL/mmol) was heated at 65 °C. Sodium nitrite (15 equiv) dissolved in water (2.22 mL/mmol of $NaNO_2$) was added portionwise over a 30 min period. The reaction mixture was stirred for 2 h at 65 °C, then cooled to 0 °C and quenched by addition of aqueous sodium 1 M until pH = 7. Volatiles were removed under reduced pressure and the crude was purified by flash chromatography silica gel on C18 reverse phase using water/MeOH gradient (0 to 100% MeOH) to afford the product as a white solid after freeze-drying.

*Diethyl (R)-(2-Hydroxy-4-(6-oxo-1,6-dihydro-9H-purin-9-yl)-butyl)phosphonate (**29**(R)).* Compound **29**(*R*) (156 mg) was obtained in 66% yield, according to procedure E, from **27**(*R*) (236 mg). R_f = 0.30 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +14.77° (*c* 1.2, CH_2Cl_2). ^1H NMR ($CDCl_3$) δ 8.14 (s, 1H, H_{-2}), 7.91 (s, 1H, H_{-8}), 4.36 (m, 2H, CH_2N), 4.11−4.08 (m, 4H, $\underline{CH_2}CH_3$), 3.94 (m, 1H, CHOH), 2.18−1.93 (2m, 4H, $\underline{CH_2}CH_2N$, CH_2P), 1.30 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR ($CDCl_3$) δ 159.2 (C_{-6}), 149.2 (C_{-4}), 145.4, 145.3 (CH_{-2}) 140.9 (CH_{-8}), 124.7 (C_{-5}), 63.2 (CHOH), 62.3−62.1 ($\underline{CH_2}CH_3$), 40.9 (CH_2N), 38.4, 38.3 ($\underline{CH_2}CH_2N$), 34.3, 33.2 (CH_2P), 16.5 ($\underline{CH_3}CH_2$). ^{31}P NMR (D_2O) δ 29.4 (s). HRMS (TOF ESI > 0) found: 345.1326. Calculated for $C_{13}H_{22}N_4O_5P$: 345.1328 $(M + H)^+$.

*Diethyl (S)-(2-Hydroxy-4-(6-oxo-1,6-dihydro-9H-purin-9-yl)-butyl)phosphonate (**29**(S)).* Compound **29**(*S*) (250 mg) was obtained in 83% yield, according to procedure E, from **27**(*S*) (300 mg). R_f = 0.32 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −16.53° (*c* 0.5, CH_2Cl_2). ^1H NMR (D_2O) δ 8.22 (s, 1H, H_{-2}), 8.14 (s, 1H, H_{-8}), 4.43−4.40 (m, 2H, CH_2N), 4.09−4.0 (m, 4H, $\underline{CH_2}CH_3$), 3.86−3.80 (m, 1H, CHOH), 2.27−2.23 (m, 1H, $\underline{CH_2}CH_2N$), 2.20−2.14 (m, 2H, CH_2P), 2.08−2.00 (m, 1H, $\underline{CH_2}CH_2N$), 1.23 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR (D_2O) δ 146.1 (CH_{-2}), 142.3 (CH_{-8}), 63.4 (CHOH), 63.3, 63.2 ($\underline{CH_2}CH_3$), 41.0 (CH_2N), 37.0, 36.9 ($\underline{CH_2}CH_2N$), 33.2, 31.8 (CH_2P), 15.5, 15.4 ($\underline{CH_3}CH_2$). ^{31}P NMR(D_2O) δ 31 (s). HRMS (TOF ESI > 0) found: 345.1329. Calculated for $C_{13}H_{22}N_4O_5P$: 345.1328 $(M + H)^+$.

*Diethyl (R)-(2-Hydroxy-4-(6-methoxy-2-oxo-2,3-dihydro-9H-purin-9-yl)butyl)phosphonate (**30**(R)).* Compound **30**(*R*) (231 mg) was obtained in 49% yield, according to procedure E, from **28**(*R*) (469 mg). R_f = 0.40 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +19.6° (*c* 0.6, CH_2Cl_2). ^1H NMR (D_2O) δ 7.79 (s, 1H, H_{-8}), 4.24−4.20 (m, 2H, CH_2N), 4.04 (s, 3H, OCH_3), 4.06−3.97 (m, 4H, $\underline{CH_2}CH_3$), 3.84−3.66 (m, 1H, CHOH), 2.24−2.17 (m, 1H, $\underline{CH_2}CH_2N$), 2.17−2.11 (m, 2H, CH_2P), 1.98−1.91 (m, 1H, $\underline{CH_2}CH_2N$), 1.21−1.17 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR (D_2O) δ 166.8 (C_{-6}), 162.0 (C_{-2}), 154.3 (C_{-4}), 140.1 (CH_{-8}), 112.4 (C_{-5}), 63.2−63.1 ($\underline{CH_2}CH_3$, CHOH), 53.8 (OCH_3), 39.8 (CH_2N), 37.0, 36.9 ($\underline{CH_2}CH_2N$), 32.9, 31.8 (CH_2P), 15.4, 15.3 ($\underline{CH_3}CH_2$). ^{31}P NMR (D_2O) δ 31.2 (s). HRMS (TOF ESI > 0) found: 375.1431. Calculated for $C_{14}H_{24}N_4O_6P$: 375.1433 $(M + H)^+$.

*Diethyl (S)-(2-Hydroxy-4-(6-methoxy-2-oxo-2,3-dihydro-9H-purin-9-yl)butyl)phosphonate (**30**(S)).* Compound **30**(*S*) (70 mg) was obtained in 53% yield, according to procedure E, from **28**(*S*) (130 mg). R_f = 0.40 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −20.20° (*c* 1.1, CH_2Cl_2). ^1H NMR (D_2O) δ 7.79 (s, 1H, H_{-8}), 4.25−4.20 (m, 2H, CH_2N), 4.04 (s, 3H, OCH_3), 4.07−3.97 (m, 4H, $\underline{CH_2}CH_3$), 3.84−3.46 (m, 1H, CHOH), 2.20−2.11 (m, 3H, $\underline{CH_2}CH_2N$, CH_2P), 1.98−1.91 (m, 1H, $\underline{CH_2}CH_2N$), 1.21−1.16 (m, 6H, $\underline{CH_3}CH_2$). ^{13}C NMR (D_2O) δ 166.9 (C_{-6}), 162.0 (C_{-2}), 154.4 (C_{-4}), 140.1 (CH_{-8}), 112.4 (C_{-5}), 63.2, 63.1 ($\underline{CH_2}CH_3$, CHOH), 53.8 (OCH_3), 39.7 (CH_2N), 37.0, 36.9 ($\underline{CH_2}CH_2N$), 32.9, 31.8 (CH_2P), 15.4, 15.3 (s, $\underline{CH_3}CH_2$). ^{31}P NMR (D_2O) δ 31.2 (s). HRMS (TOF ESI > 0) found: 375.1434. Calculated for $C_{14}H_{24}N_4O_6P$: 375.1433 $(M + H)^+$

General Procedure F. *Removal of the Diethyl Phosphonate Groups.* To a stirred solution of diethyl-β-hydroxyphosphonate (1 equiv) in anhydrous acetonitrile (20 mL/mmol), cooled at 0 °C, was added dropwise bromotrimethylsilane (6.6 equiv). The reaction mixture was stirred at room temperature until completion of the reaction was indicated by TLC monitoring (isopropanol/water/ammoniac 7/2/1 v/v/v). When *in situ* removal of Boc groups was required, water was added to the reaction mixture and stirring was pursued 1 h at rt. Then, triethylammonium bicarbonate solution (TEAB 1M) was added to the reaction mixture until pH = 7, and the volatiles were evaporated under high vacuum and the aqueous residue was freeze-dried. The resulting lyophilizate was purified by flash chromatography silica gel on C18 reverse phase using water/MeOH gradient (0 to 100%) and freeze-dried, giving rise to the desired phosphonic acid. Eventually, the latter was dissolved in a small amount of water and percolated through a Dowex resin column (Na^+ form) to afford after freeze-drying the corresponding compound as sodium salt.

*(R)-(3-(6-Amino-9H-purin-9-yl)-2-hydroxypropyl)phosphonic Acid (**1**(R)).* Compound **1**(*R*) (216 mg) was obtained in 45% yield, according to procedure F, from **17**(*R*) (805 mg). R_f = 0.66 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 96%. ^1H NMR (D_2O): δ = 8.00 (s, 1H, H_{-2}), 7.96 (s, 1H, H_{-8}), 4.38−4.21 (m, 2H, CH_2N), 4.16−4.05 (m, 1H, $\underline{CH}OH$), 1.95−1.83 (m, 2H, CH_2P). ^{13}C NMR (D_2O) δ 154.8 (C_{-6}), 151.7 (CH_{-2}), 148.4 (C_{-4}), 142.7 (CH_{-8}), 117.7 (C_{-5}), 66.2 (CHOH), 49.9 (CH_2N), 34.1, 32.4 (CH_2P). ^{31}P NMR (D_2O): δ = 19.8. MS (ESI) *m*/*z* 272.06 $[M - H]^-$. HRMS: calcd $C_8H_{11}N_5O_4P$ $[M - H]^-$ 272.0549, obs 272.0551.

*(S)-(3-(6-Amino-9H-purin-9-yl)-2-hydroxypropyl)phosphonic Acid (**1**(S)).* Compound **1**(*S*) (256 mg) was obtained in 47% yield, according to procedure F, from **17**(*S*) (898 mg). R_f = 0.54 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 99%. ^1H NMR (D_2O): δ = 8.02 (s, 1H, H_{-2}), 8.00 (s, 1H, H_{-8}), 4.40−4.21 (m, 2H, CH_2N), 4.17−4.06 (m, 1H, $\underline{CH}OH$), 1.97−1.77 (2m, 2H, CH_2P). ^{13}C NMR (D_2O) δ 155.0 (C_{-6}), 151.9 (CH_{-2}), 148.6 (C_{-4}), 142.8 (CH_{-8}), 117.8 (C_{-5}), 66.3 (CHOH), 50.0, 49.8 (CH_2N), 34.1, 32.4 (CH_2P). ^{31}P NMR (D_2O): δ = 19.7. MS (ESI) *m*/*z* 318.04 $[M + H]^+$; 296.05 $[M - Na + 2H]^+$; 274.07 $[M - 2Na + 3H]^+$. HRMS: calcd $C_8H_{11}N_5O_4Na_2P$ $[M + H]^+$ 318.0344, obs 318.0349.

*(R)-(3-(2-Amino-6-oxo-9H-purin-9-yl)-2-hydroxypropyl)-phosphonic Acid (**2**(R)).* Compound **2**(*R*) (370 mg) was obtained in 68% yield, according to procedure F, from **18**(*R*) (878 mg). R_f = 0.44 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 99%. ^1H NMR (D_2O): δ = 7.76 (s, 1H, H_{-8}), 4.33−4.17 (m, 2H, CH_2N), 4.05−3.93 (m, 1H, $\underline{CH}OH$), 2.00−1.80 (m, 2H, CH_2P). ^{13}C NMR (D_2O) δ 158.8 (C_{-6}), 153.6 (C_{-4}), 140.6 (CH_{-8}), 115.7 (C_{-5}), 66.2 (CHOH), 49.7, 49.6 (CH_2N), 33.9, 32.6 (CH_2P). ^{31}P NMR (D_2O): δ = 20.1. MS (ESI) *m*/*z* 288.05 $[M - H]^-$, 577.11 $[2M - H]^-$. HRMS: calcd $C_8H_{11}N_5O_5P$ $[M - H]^-$ 288.0498, obs 288.0498.

*(S)-(3-(2-Amino-6-oxo-9H-purin-9-yl)-2-hydroxypropyl)-phosphonic Acid (**2**(S)).* Compound **2**(*S*) (176 mg) was obtained in 70% yield, according to procedure F, from **18**(*S*) (421 mg). R_f = 0.47 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 96%. ^1H NMR (D_2O): δ = 7.76 (s, 1H, H_{-8}), 4.32−4.19 (m, 2H, CH_2N), 4.05−3.93 (m, 1H, $\underline{CH}OH$), 1.97−1.78 (m, 2H, CH_2P). ^{13}C NMR (D_2O) δ 158.8 (C_{-6}), 153.5 (C_{-4}), 151.6 (C_{-2}), 140.6 (CH_{-8}), 115.6 (C_{-5}), 66.2 (CHOH), 49.7, 49.5 (CH_2N), 34.1, 32.3 (CH_2P). ^{31}P NMR (D_2O): δ = 19.9. MS (ESI) *m*/*z* 290.07 $[M - 2Na + 3H]^+$, 312.05 $[M - Na + 2H]^+$; 334.03 $[M + H]^+$. HRMS: calcd $C_8H_{11}N_5O_5Na_2P$ $[M + H]^+$ 334.0293, obs 334.0302.

*(R)-(3-(2,6-Diamino-9H-purin-9-yl)-2-hydroxypropyl)phosphonic Acid (**3**(R)).* Compound **3**(*R*) (206 mg) was obtained in 64% yield, according to procedure F, from **19**(*R*) (719 mg). R_f = 0.54 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 95%. ^1H NMR (D_2O): δ = 7.78 (s, 1H, H_{-8}), 4.32−4.20 (m, 2H, CH_2N), 4.10−3.96 (m, 1H, $\underline{CH}OH$), 1.87−1.67 (m, H, CH_2P). ^{13}C NMR (D_2O) δ 159.5 (C_{-6}), 155.7 (C_{-4}), 140.6 (CH_{-8}), 66.6 (CHOH), 49.6, 49.4 (CH_2N), 34.2, 32.5 (CH_2P). ^{31}P NMR (D_2O): δ = 18.7. MS (ESI) *m*/*z* 289.08 $[M - 2Na + 3H]^+$, 311.06 $[M - Na + 2H]^+$, 333.05 $[M + H]^+$. HRMS: calcd $C_8H_{12}N_6O_4Na_2P$ $[M + H]^+$ 333.0453, obs 333.0460.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

*(S)-(3-(2,6-Diamino-9H-purin-9-yl)-2-hydroxypropyl)phosphonic Acid (**3**(S)).* Compound **3**(*S*) (705 mg) was obtained in 61% yield, according to procedure F, from **19**(*S*) (193 mg). R_f = 0.56 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 98%. ^{1}H NMR (D_2O): δ = 7.80 (s, 1H, H_{-8}), 4.28−4.17 (m, 2H, CH_2N), 4.03−3.93 (m, 1H, CHOH), 1.80−1.54 (m, H, CH_2P). ^{13}C NMR (D_2O) δ 159.7 (C_{-6}), 155.8 (C_{-4}), 151.0 (C_{-2}), 140.6 (CH_{-8}), 112.7 (C_{-5}), 67.1 (CHOH), 49.8, 49.6 (CH_2N), 34.4, 32.8 (CH_2P). ^{31}P NMR (D_2O): δ = 17.5. MS (ESI) *m*/*z* 289.08 $[M - 2Na + 3H]^+$, 311.06 $[M - Na + 2H]^+$, 333.05 $[M + H]^+$, 355.03 $[M + Na]^+$. HRMS: calcd $C_8H_{12}N_6O_4Na_2P$ $[M + H]^+$ 333.0453, obs 333.0450.

*(R)-(4-(6-Amino-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid (**4**(R)).* Compound **4**(*R*) (216 mg) was obtained in 95% yield, according to procedure F, from **24**(*R*) (358 mg). R_f = 0.21 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. ^{1}H NMR (D_2O): δ = 7.99 (s, 1H, H_{-2}), 7.96 (s, 1H, H_{-8}), 4.26−4.16 (m, 2H, CH_2N), 4.00−3.87 (m, 1H, CHOH), 2.24−2.11 (m, 1H, CH_2CH_2N), 1.99−1.79 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 154.9 (C_{-6}), 151.8 (C_{-4}), 148.3 (CH_{-2}), 142.2 (CH_{-8}), 117.9 (C_{-5}), 65.5 (CHOH), 40.9 (CH_2N), 37.0, 36.9 (CH_2CH_2N), 36.7, 35.5 (CH_2P). ^{31}P NMR (D_2O): δ = 20.7. MS (ESI) *m*/*z* 332.02 $[M + H]^+$; 310.04 $[M - Na + 2H]^+$; 288.07 $[M - 2Na + 3H]^+$. HRMS: calcd $C_9H_{15}N_5O_4P$ $[M - 2Na + 3H]^+$ 288.0862, obs 288.0867.

*(S)-(4-(6-Amino-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid (**4**(S)).* Compound **4**(*R*) (167 mg) was obtained in 69% yield, according to procedure F, from **24**(*S*) (398 mg). R_f = 0.26 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 95%. ^{1}H NMR (D_2O): δ = 7.98 (s, 1H, H_{-2}), 7.96 (s, 1H, H_{-8}), 4.25−4.15 (m, 2H, CH_2N), 3.98−3.87 (m, 1H, CHOH), 2.22−2.11 (m, 1H, CH_2CH_2N), 1.98−1.80 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 154.9 (C_{-6}), 151.8 (C_{-4}), 148.3 (CH_{-2}), 142.2 (CH_{-8}), 117.9 (C_{-5}), 65.5 (CHOH), 40.9 (CH_2N), 37.0, 36.9 (CH_2CH_2N), 36.7, 35.5 (CH_2P). ^{31}P NMR (D_2O): δ = 20.6. MS (ESI) *m*/*z* 310.04 $[M + H]^+$; 288.07 $[M - Na + 2H]^+$. HRMS: calcd $C_9H_{15}N_5O_4P$ $[M - Na + 2H]^+$ 288.0862, obs 288.0860.

*(R)-(4-(2-Amino-6-oxo-9H-purin-9-yl)-2-hydroxybutyl)-phosphonic Acid (**5**(R)).* Compound **5**(*R*) (165.6 mg) was obtained in 68% yield, according to procedure F, from **25**(*R*) (404 mg). R_f = 0.60 in *i*PrOH/H_2O/NH_4OHc (7/4/2, v/v/v). Purity (HPLC) > 95%. $[\alpha]_D^{20}$ +7° (*c* 0.0097, H_2O). ^{1}H NMR (D_2O): δ = 7.75 (s, 1H, H_{-8}), 4.17−4.05 (m, 2H, CH_2N), 3.98−3.87 (m, 1H, CHOH), 2.21−2.08 (m, 1H, CH_2CH_2N), 1.96−1.77 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 158.7 (C_{-6}), 153.5 (C_{-4}), 151.3 (CH_{-2}), 140.7 (CH_{-8}), 115.7 (C_{-5}), 65.4 (CHOH), 40.6 (CH_2N), 37.1, 37.0 (CH_2CH_2N), 36.7, 35.4 (CH_2P); ^{31}P NMR (D_2O): δ = 20.5. MS (ESI) *m*/*z* 304.08 $[M - Na + 2H]^+$, 326.06 $[M + H]^+$. HRMS: calcd $C_9H_{15}N_5O_5P$ $[M + H]^+$ 304.0811, obs 304.0808.

*(S)-(4-(2-Amino-6-oxo-9H-purin-9-yl)-2-hydroxybutyl)-phosphonic Acid (**5**(S)).* Compound **5**(*S*) (171 mg) was obtained in 51% yield, according to procedure F, from **25**(*S*) (558 mg). R_f = 0.10 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. ^{1}H NMR (D_2O): δ = 7.77 (s, 1H, H_{-8}), 4.15−4.05 (m, 2H, CH_2N), 3.98−3.87 (m, 1H, CHOH), 2.23−2.09 (m, 1H, CH_2CH_2N), 1.96−1.80 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 158.7 (C_{-6}), 153.5 (C_{-4}), 151.3 (CH_{-2}), 140.0 (CH_{-8}), 115.7 (C_{-5}), 65.2 (CHOH), 40.5 (CH_2N), 37.0, 36.9 (CH_2CH_2N), 36.8, 35.0 (CH_2P). ^{31}P NMR (D_2O): δ = 21.0. MS (ESI) *m*/*z* 348.04 $[M + H]^+$, 326.06 $[M - Na + 2H]^+$, 304.08 $[M - 2Na + 3H]^+$. HRMS: calcd $C_9H_{15}N_5O_5P$ $[M - 2Na + 3H]^+$ 304.0811, obs 304.0813.

*(R)-(4-(2,6-Diamino-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid (**6**(R)).* Compound **6**(*R*) (72 mg) was obtained in 31% yield, according to procedure F, from **26**(*R*) (501 mg). R_f = 0.19 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. ^{1}H NMR (D_2O): δ = 7.76 (s, 1H, H-8), 4.14−4.05 (m, 2H, CH_2N), 3.97−3.90 (m, 1H, CHOH), 2.19−2.07 (m, 1H, CH_2CH_2N), 1.96−1.75 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 159.7 (C-6), 156.5 (C-4), 155.8 (CH-2), 140.2 (CH-8), 115.2 (C-5), 65.6 (CHOH), 40.4 (CH_2N), 37.1, 37.0 (CH_2CH_2N), 36.6, 35.4 (CH_2P). ^{31}P NMR (DMSO-d_6): 20.2. MS (ESI) *m*/*z* 303.10 $[M + H]^+$. HRMS: calcd $C_9H_{16}N_6O_4P$ $[M + H]^+$ 303.0968, obs 303.0971.

*(S)-(4-(2,6-Diamino-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid (**6**(S)).* Compound **6**(*S*) (220 mg) was obtained in 70% yield, according to procedure F, from **26**(*S*) (693 mg). R_f = 0.20 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. ^{1}H NMR (D_2O): 7.77 (s, 1H, H_{-8}), 4.17−4.06 (m, 2H, CH_2N), 4.0−3.90 (m, 1H, CHOH), 2.20−2.08 (m, 1H, CH_2CH_2N), 1.98−1.73 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 159.7 (C_{-6}), 155.9 (C_{-4}), 151.0 (CH_{-2}), 140.3 (CH_{-8}), 112.9 (C_{-5}), 65.4 (CHOH), 40.3 (CH_2N), 37.0, 36.9 (CH_2CH_2N), 36.4, 35.5 (CH_2P). ^{31}P NMR (D_2O): 20.2. MS (ESI) *m*/*z* 303.12 $[M + H]^+$. HRMS: calcd $C_9H_{16}N_6O_4P$ $[M + H]^+$ 303.0971, obs 303.0971.

*(R)-(2-Hydroxy-4-(6-oxo-1,6-dihydro-9H-purin-9-yl)butyl)-phosphonic Acid Disodic Salt (**7**(R)).* Compound **7**(*R*) (169 mg) was obtained in 70% yield, according to procedure F, from **29**(*R*) (250 mg). R_f = 0.19 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. $[\alpha]_D^{20}$ +16.28° (*c* 6.45, H_2O). ^{1}H NMR(D_2O) δ 8.19 (s, 1H, H_{-2}), 8.16 (s, 1H, H_{-8}), 4.40−4.37 (t, *J* = 7.3 Hz, 2H, CH_2N), 4.00−3.93 (m, 1H, CHOH), 2.28−2.21 (m, 1H, CH_2CH_2N), 2.03−1.99 (m, 1H, CH_2CH_2N), 1.81−1.76 (m, 2H, CH_2P). ^{13}C NMR (D_2O) δ 158.7 (C_{-6}), 148.9 (C_{-4}), 145.5 (CH_{-2}), 142.4 (CH_{-8}), 123.3 (C_{-5}), 65.6 (CHOH), 41.3 (CH_2N), 37.2, 37.1 (CH_2CH_2N), 36.5, 35.5 (CH_2P). ^{31}P NMR(D_2O) δ 19.8 (s). HRMS (TOF ESI < 0) found: 287.0547. Calculated for $C_9H_{12}N_4O_5P$: 287.0545 $(M - H)^-$.

*(S)-(2-Hydroxy-4-(6-oxo-1,6-dihydro-9H-purin-9-yl)butyl)-phosphonic Acid Disodic Salt (**7**(S)).* Compound **7**(*S*) (77 mg) was obtained in 52% yield, according to procedure F, from **29**(*S*) (155 mg). R_f = 0.19 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. $[\alpha]_D^{20}$ −14.19° (*c* 4.65, H_2O). ^{1}H NMR (D_2O) δ 8.19 (s, 1H, H_{-2}), 8.16 (s, 1H, H_{-8}), 4.41−4.38 (t, *J* = 7.2 Hz, 2H, CH_2N), 3.98−3.92 (m, 1H, CHOH), 2.30−2.24 (m, 1H, CH_2CH_2N), 2.05−1.99 (m, 1H, CH_2CH_2N), 1.86−1.81 (dd, *J* = 17.3, 6.6 Hz, 2H, CH_2P). ^{13}C NMR(D_2O) δ 158.7 (C_{-6}), 148.9 (C_{-4}), 145.4 (CH_{-2}), 142.4 (CH_{-8}), 123.3 (C_{-5}), 65.4 (CHOH), 41.3 (CH_2N), 37.1, 37.0 (CH_2CH_2N), 36.5, 35.5 (CH_2P). ^{31}P NMR(D_2O) δ 20.2. HRMS (TOF ESI > 0) found: 289.0703. Calculated for $C_9H_{14}N_4O_5P$: 289.0702 $(M + H)^+$.

*(R)-(4-(2,6-Dioxo-1,2,3,6-tetrahydro-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid Disodic Salt (**8**(R)).* Compound **8**(*R*) (90 mg) was obtained in 46% yield, according to procedure F, from **30**(*R*) (206 mg). R_f = 0.17 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. $[\alpha]_D^{20}$ +17.5° (*c* 4.4, H_2O). ^{1}H NMR(D_2O) δ 7.79 (s, 1H, CH_{-8}), 4.25−4.21 (t, *J* = 7.3 Hz, 2H, CH_2N), 4.01−3.94 (m, 1H, CHOH), 2.27−2.20 (m, 1H, CH_2CH_2N), 2.00−1.84 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 160.0 (C_{-6}), 153.5 (C_{-2}), 143.2 (C_{-4}), 139.1 (CH_{-8}), 115.2 (C_{-5}), 65.1 (CHOH), 41.6 (CH_2N), 36.5 (CH_2CH_2N), 36.4, 35.4 (CH_2P). ^{31}P NMR (D_2O) δ 20.4. HRMS (TOF ESI < 0) found: 303.0492. Calculated for $C_9H_{12}N_4O_6P$: 303.0494 $(M + H)^+$.

*(S)-(4-(2,6-Dioxo-1,2,3,6-tetrahydro-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid Disodic Salt (**8**(S)).* Compound **8**(*S*) (97 mg) was obtained in 40% yield, according to procedure F, from **30**(*S*) (261 mg). R_f = 0.15 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 98%. $[\alpha]_D^{20}$ −17.58° (*c* 4.95, H_2O). ^{1}H NMR (D_2O) δ 7.78 (s, 1H, CH_{-8}), 4.23−4.20 (t, *J* = 7.3 Hz, 2H, CH_2N), 4.01−3.94 (m, 1H, CHOH), 2.26−2.19 (m, 1H, CH_2CH_2N), 1.98−1.85 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 160.2 (C_{-6}), 154.5 (C_{-2}), 144.9 (C_{-4}), 139.2 (C_{-8}), 115.1 (C_{-5}), 65.1 (CHOH), 41.3 (CH_2N), 36.6, 36.5 (CH_2CH_2N), 36.4, 35.4 (CH_2P). ^{31}P NMR (D_2O) δ 20.4. HRMS (TOF ESI > 0) found: 349.0291. Calculated for $C_9H_{12}N_4O_6Na_2P$: 349.0290 $(M + H)^+$.

*(R)-(4-(2-Amino-9H-purin-9-yl)-2-hydroxybutyl)phosphonic Acid (**9**(R)).* Compound **9**(*R*) (190 mg) was obtained in 40% yield, according to procedure F. R_f = 0.32 in *i*PrOH/H_2O/NH_4OHc (7/2/1, v/v/v). Purity (HPLC) > 99%. $[\alpha]_D^{20}$ +2.8° (*c* 0.0124, H_2O). ^{1}H NMR (D_2O) δ 8.64 (s, 1H, H_{-6}), 8.18 (s, 1H, H_{-8}), 4.32 (m, 2H, CH_2N), 4.00−3.95 (m, 1H, CHOH), 2.28−2.26 (m, 1H, CH_2CH_2N), 2.11−1.85 (2m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR (D_2O) δ 158.9 (C_{-6}), 152.9 (C_{-2}), 147.5 (C_{-4}), 145.2 (CH_{-8}), 126.5 (C_{-5}), 65.3 (CHOH), 40.3 (CH_2N), 36.6, 36.5 (CH_2CH_2N), 36.5, 35.3 (CH_2P). ^{31}P NMR (D_2O): δ = 20.8. MS (ESI) *m*/*z* 286.07 $[M - H]^-$, 573.15 $[2M - H]^-$. HRMS: calcd $C_9H_{15}N_5O_4P$ $[M + H]^+$ 288.0862, obs 288.0861.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

*(R)-(4-(6-Amino-2-fluoro-9H-purin-9-yl)-2-hydroxybutyl)-phosphonic Acid (**10**(R)).* Compound **10**(*R*) (144 mg) was obtained in 26% yield, according to procedure F. R_f = 0.39 in *i*PrOH/H_2O/NH_4OH_c (7/2/1, v/v/v). Purity (HPLC) > 96%. $[\alpha]_D^{20}$ +3.5 (*c* 0.007, H_2O). ^{1}H NMR δ 7.85 (s, 1H, H_{-8}), 4.15 (m, 2H, CH_2N), 3.97−3.93 (m, 1H, CHOH), 2.21−2.17 (m, 1H, CH_2CH_2N), 1.96−1.80 (m, 3H, CH_2CH_2N, CH_2P). ^{13}C NMR(D_2O) δ 157.2 (C_{-6}), 153.1 (C_{-2}), 150.8 (C_{-4}), 141.6 (CH_{-8}), 141.5 (C_{-5}), 65.2 (CHOH), 40.3 (CH_2N), 36.8, 36.7 (CH_2CH_2N), 36.7, 35.4 (CH_2P). ^{31}P NMR (DMSO-d_6): 20.7. MS (ESI) *m*/*z* 326.2 $[M - H]^-$.

N-Boc tosylated aziridines **35**(*R*) and **35**(*S*) were prepared in 5 steps following reported procedures.[18d,29]

*(R)-tert-Butyl-(2-(2-tosyloxy)ethyl)aziridine-N-carboxylate (**35**(R)).* R_f = 0.3 in hexane/AcOEt (5/5, v/v). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 4.23−4.14 (m, 2H, CH_2O), 2.45 (s, 3H, CH_3), 2.42−2.39 (m, 1H, CHN), 2.26 (d, *J* = 7 Hz, 1H, CH_2N), 1.92 (d, *J* = 3.7 Hz, 1H, CH_2N), 1.90−1.84 (m, 1H, CH_2CH_2O), 1.78−1.73 (m, 1H, CH_2CH_2O), 1.43 (s, 9H, CH_3). HRMS (TOF ESI > 0) found: 364.1194. Calculated for $C_{16}H_{23}N_5O_6SNa$: 364.1195 $(M + Na)^+$.

*(S)-tert-Butyl-(2-(2-tosyloxy)ethyl)aziridine-N-carboxylate(**35**(S)).* R_f = 0.3 in hexane/AcOEt (5/5, v/v). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 1H, H), 7.35 (d, *J* = 8.2 Hz, 1H, H), 4.23−4.14 (m, 2H, CH_2O), 2.45 (s, 3H, CH_3), 2.43−2.38 (m, 1H, CHN), 2.26 (d, J = 6.5 Hz, 1H, CH_2N), 1.92 (d, J = 3.4 Hz, 1H, CH_2N), 1.90−1.84 (m, 1H, CH_2CH_2O), 1.79−1.72 (m, 1H, CH_2CH_2O), 1.42 (s, 9H, CH_3). HRMS (TOF ESI > 0) found: 342.1377. Calculated for $C_{16}H_{24}N_5O_6S$: 342.1375 $(M + H)^+$.

General Procedure G. *Preparation of N-Boc Purinyl Aziridines Derivatives.* To a solution of protected purine nucleobase (1 equiv) in anhydrous DMF (1.5 mL/mmol), and under argon atmosphere, was added anhydrous K_2CO_3 (2.6 equiv), and the mixture was heated at 60 °C for 1 h. The mixture was then cooled at room temperature, and a solution of *N*-Boc tosylated aziridine **35** (1.1 equiv) in anhydrous DMF (1.5 mL/mmol) was added dropwise over a 30 min period. The reaction mixture was kept under stirring for 3 days at room temperature with TLC monitoring (hexane/ethyl acetate, 5/5, v/v). Then, the volatiles were evaporated under high vacuum, and the resulting residue was dissolved in ethyl acetate, sonicated, and the undissolved salts were filtered on a sintered glass (porosity 4). The filtrate was then evaporated and purified on silica gel by flash chromatography (petroleum ether/ethyl acetate gradient) to afford the *N*-Boc purinyl aziridine as a white foam.

*(R)-tert-Butyl-(2-(2-amino-di(N-tert-butyloxycarbonyl)-6-methoxy-N-9-purine)ethyl)aziridine-N-carboxylate (**36**(R)).* Compound **36**(*R*) (983 mg) was obtained in 69% yield, according to procedure G, from **35**(*R*) (1 g). R_f = 0.3 in hexane/AcOEt (5/5, v/v). $[\alpha]_D^{20}$ −38.2 (*c* 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 1H, H_8), 4.49−4.36 (m, 2H, CH_2N), 4.15 (s, 3H, CH_3O), 2.37−2.25 (m, 4H, CHN, CH_2CH_2N, $CHCH_2N$), 1.90 (d, *J* = 3.5 Hz, 1H, $CHCH_2N$), 1.75−1.68 (m, 1H, CH_2CH_2N), 1.47 (s, 9H, CH_3), 1.44 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 162.2 (C═O), 161.6 (C_{-6}), 152.8 (C_{-4}), 152.0 (C_{-2}), 151.2 (C═O), 143.7 (C_{-8}), 120.3 (C_{-5}), 83.2, 81.9 ($C(CH_3)_3$), 54.7 (OCH_3), 42.4 (CH_2N), 35.0 (CHN), 32.8 (CH_2CH_2N), 31.9 ($CHCH_2N$), 28.1 ($C(CH_3)_3$). HRMS (TOF ESI > 0) found: 535.2880. Calculated for $C_{25}H_{39}N_6O_7$: 535.2880 $(M + H)^+$.

*(S)-tert-Butyl-(2-(2-amino-di(N-tert-butyloxycarbonyl)-6-methoxy-N-9-purine)ethyl)aziridine-N-carboxylate (**36**(S)).* Compound **36**(*S*) (893 mg) was obtained in 44% yield, according to procedure G, from **35**(*S*) (1.3 g). R_f = 0.3 in hexane/AcOEt (5/5, v/v). $[\alpha]_D^{20}$ +38.8° (*c* 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1H, H_8), 4.48−4.35 (m, 2H, CH_2N), 4.14 (s, 3H, CH_3O), 2.35−2.23 (m, 4H, CHN, $CHCH_2N$, CH_2CH_2N), 1.88 (d, *J* = 3.5 Hz, 1H, $CHCH_2N$), 1.75−1.68 (m, 1H, CH_2CH_2N), 1.46 (s, 9H, CH_3), 1.43 (s, 18H, CH_3). ^{13}C NMR ($CDCl_3$) δ 162.2 (C═O), 161.6 (C_{-6}), 152.8 (C_{-4}), 152.0 (C_{-2}), 151.1 (C═O), 143.7 (C_{-8}), 120.3 (C_{-5}), 83.1, 81.9 ($C(CH_3)_3$), 54.6 (OCH_3), 42.4 (CH_2N), 35.0 (CHN), 32.8 (CH_2CH_2N), 31.9 ($CHCH_2N$), 28.0 ($C(CH_3)_3$). HRMS (TOF ESI > 0) found: 535.2882. Calculated for $C_{25}H_{39}N_6O_7$: 535.2880 $(M + H)^+$.

*(R)-tert-Butyl-(2-(2,6-diamino-di(N-tert-butyloxycarbonyl)-N-9-purine)ethyl)aziridine-N-carboxylate (**37**(R)).* Compound **37**(*R*) (1 g) was obtained in 87% yield, according to procedure G, from **35**(*R*) (600 mg). R_f = 0.3 in hexane/AcOEt (5/5, v/v). $[\alpha]_D^{20}$ −41.5° (*c* 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, H_8), 4.53−4.41 (m, 2H, CH_2N), 2.38−2.29 (m, 3H, CH_2CH_2N, CHN), 1.91 (d, *J* = 3.3 Hz, 1H, $CHCH_2N$), 1.75−1.68 (m, 1H, CH_2CH_2N), 1.47 (s, 9H, CH_3), 1.42 (s, 36H, CH_3). ^{13}C NMR($CDCl_3$) δ 162.3 (C═O), 154.3 (C_{-4}), 151.1 (C_{-2}), 151.0 (C═O), 150.3 (C_{-6}), 146.3 (C_{-8}), 127.6 (C_{-5}), 83.8, 83.3, 81.9 ($C(CH_3)_3$), 42.53 (CH_2N), 34.9 (CHN), 32.7 (CH_2CH_2N), 31.9 ($CHCH_2N$), 28.1, 28.0, 27.8 ($C(CH_3)_3$). HRMS (TOF ESI > 0) found: 720.3937. Calculated for $C_{34}H_{54}N_7O_{10}$: 720.3932 $(M + H)^+$.

*(S)-tert-Butyl-(2-(2,6-diamino-di(N-tert-butyloxycarbonyl)-N-9-purine)ethyl)aziridine-1-carboxylate (**37**(S)).* Compound **37**(*S*) (1.07 g) was obtained in 93% yield, according to procedure G, from **35**(*S*) (600 mg). R_f = 0.3 in hexane/AcOEt (5/5, v/v). $[\alpha]_D^{20}$ +40.6° (c 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, H8), 4.55−4.39 (m, 2H, CH_2N), 2.39−2.25 (m, 3H, CH_2CH_2N, CHN), 1.91 (d, *J* = 3.3 Hz, 1H, $CHCH_2N$), 1.76−1.67 (m, 1H, CH_2CH_2N), 1.47 (s, 9H, CH_3), 1.42 (s, 36H, CH_3). ^{13}C NMR ($CDCl_3$) δ 162.2 (C═O), 154.3 (C-4), 152.1 (C-2), 151.1, 151.0 (C═O), 150.3 (C-6), 146.3 (C-8), 127.6 (C-5), 83.8, 83.3, 81.9 ($C(CH_3)_3$), 42.5 (CH_2N), 35.0 (CHN), 32.7 (CH_2CH_2N), 32.0 ($CHCH_2N$), 28.1, 28.0, 27.9 ($C(CH_3)_3$). HRMS (TOF ESI > 0) found: 720.3928. Calculated for $C_{34}H_{54}N_7O_{10}$: 720.3932 $(M + H)^+$.

General Procedure H. *Preparation of β-Aminophosphonates **38**(R) or **38**(S) from N-Boc Aziridine Derivatives **36**(R) and **36**(S).* Diethyl phosphite (5 equiv) was dissolved in anhydrous THF (1 mL/mmol DEP) under argon atmosphere and cooled at −78 °C. LiHMDS (5 equiv) was added dropwise to the mixture. The reaction mixture was kept under stirring at −78 °C for 15 min. Then, the solution of aziridine **36**(*R*) or **36**(*S*) (1 equiv) in anhydrous THF (2.8 mL/mmol aziridine) was added dropwise. After 5 min, the cooled bath was removed and the mixture slowly warmed to room temperature. The reaction was stirred overnight and followed by TLC monitoring (CH_2Cl_2/MeOH 95/5 v/v). After 24 h, the mixture was quenched by addition of saturated aqueous solution of NH_4Cl, until dissolution of the previously formed salts. The resulting solution was extracted twice by ethyl acetate and the organic layers were gathered, dried over $MgSO_4$, concentrated under vacuum, and finally purified on silica gel by flash chromatography (dichloromethane/methanol gradient up to 10% methanol) to afford the expected *N*-Boc *β*-aminophosphonate **38**(*R*) or **38**(*S*) as a white foam.

*(R)-2-Amino-(N-tert-butoxycarbonyl)-6-methoxy-N-9-(3-amino-4-diethylphosphonobutyl)purine (**38**(R)).* Compound **38**(*R*) (915 mg) was obtained in 62% yield, according to procedure H, from **36**(*R*) (1.37 g). R_f = 0.4 in CH_2Cl_2/MeOH 95/5 (v/v). $[\alpha]_D^{20}$ −20.8° (*c* 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, H_8), 7.33 (s, 1H, NH), 6.26 (d, *J* = 7.4 Hz, 1H, CHNH), 4.33−4.28 (m, 1H, CH_2N), 4.23−4.17 (m, 1H, CH_2N), 4.11 (s, 3H, OCH_3), 4.06−4.0 (m, 4H, CH_2CH_3), 3.78−3.66 (m, 1H, CHNH), 2.41−2.29 (m, 2H, CH_2P, CH_2CH_2N), 2.09−1.99 (m, 2H, CH_2P, CH_2CH_2N), 1.54 (s, 9H, CH_3), 1.44 (s, 9H, CH_3), 1.28−1.22 (m, 6H, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 161.5 (C_{-6}), 155.8 (C═O), 153.4 (C_{-4}), 152.4 (C_{-2}), 150.7 (C═O), 141.7 (C_{-8}), 117.9 (C_{-5}), 81.3, 79.3 ($C(CH_3)_3$), 61.8 (CH_2CH_3), 54.4 (OCH_3), 44.7 (CHNH), 41.0 (CH_2N), 35.5 (CH_2CH_2N), 31.0, 30.0 (CH_2P), 28.6, 28.4 ($C(CH_3)_3$), 16.5 (CH_2CH_3). ^{31}P NMR($CDCl_3$) δ 28.6 (s). HRMS (TOF ESI > 0) found: 573.2800. Calculated for $C_{24}H_{42}N_6O_8P$: 573.2802 $(M + H)^+$.

*(S)-2-Amino-(N-tert-butoxycarbonyl)-6-methoxy-N-9-(3-amino-4-diethylphosphonobutyl)purine (**38**(S)).* Compound **38**(*S*) (84 mg) was obtained in 40% yield, according to procedure H, from **36**(*S*) (193 mg). R_f = 0.4 in CH_2Cl_2/MeOH 95/5 (v/v) $[\alpha]_D^{20}$ +21.2° (c 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, H8), 7.32 (s, 1H, NH), 6.26 (d, *J* = 7.4 Hz, 1H, CHNH), 4.33−4.28 (m, 1H, CH_2N), 4.23−4.17 (m, 1H, CH_2N), 4.11 (s, 3H, OCH_3), 4.07−4.00 (m, 4H, CH_2CH_3), 3.76−3.67 (m, 1H, CHNH), 2.41−2.29 (m, 2H, CH_2P, CH_2CH_2N), 2.12−1.99 (m, 2H, CH_2P, CH_2CH_2N), 1.54 (s, 9H, CH_3), 1.44 (s, 9H, CH_3), 1.28−1.22 (m, 6H, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 161.5 (C-6), 155.8 (C═O), 153.4 (C-4), 152.4 (C-2), 150.6 (C═O), 141.7 (C-8), 118.0

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

(C-5), 81.3, 79.3 (C(CH_3)$_3$), 61.8 (CH_2CH_3), 54.4 (OCH_3), 44.7 (CHNH), 41.0 (CH_2N), 35.5 (CH_2CH_2N), 31.0, 30.1 (CH_2P), 28.6, 28.4 (C(CH_3)$_3$), 16.5 (CH_2CH_3). ^{31}P NMR ($CDCl_3$) δ 28.6 (s). HRMS (TOF ESI > 0) found: 573.2808. Calculated for $C_{24}H_{42}O_8N_6P$: 573.2802 $(M + H)^+$.

General Procedure I. *Preparation of β-Aminophosphonates **39**(R) or **39**(S) from N-Boc Aziridine Derivatives **37**(R) and **37**(S).* Diethyl phosphite (3 equiv) was dissolved in anhydrous THF (1 mL/mmol DEP) under argon atmosphere and cooled at −78 °C. LiHMDS (3 equiv) was added dropwise to the mixture. The mixture was kept under stirring at −78 °C for 15 min. Then, a solution of aziridine **37**(R) or **37**(S) (1 equiv) in anhydrous THF (2.8 mL/mmol of aziridine) was added dropwise. After 5 min, the cooled bath was removed and the mixture slowly warmed to room temperature. The reaction was stirred overnight followed by TLC monitoring (CH_2Cl_2/MeOH 95/5 v/v). After 24 h, the mixture was quenched by addition of saturated aqueous solution of NH_4Cl until dissolution of the previously formed salts. The resulting solution was extracted twice by ethyl acetate and the organic layers were gathered, dried over $MgSO_4$, concentrated under vacuum, and finally purified on silica gel by flash chromatography (dichloromethane/methanol gradient up to 10% methanol) to afford the expected *N*-Boc *β*-aminophosphonate **39**(R) or **39**(S) as a white foam.

*(R)-2,6-Diamino-(N-tert-butoxycarbonyl)-N-9-(3-amino-4-diethylphosphonobutyl)purine (**39**(R)).* Compound **39**(R) (1.47 g) was obtained in 76% yield, according to procedure I, from **37**(R) (2.13 g). R_f = 0.4 in CH_2Cl_2/MeOH 95/5 (v/v)). $[\alpha]_D^{20}$ −20.9° (c 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H, H_8), 7.87 (s, 1H, NH), 7.74 (s, 1H, NH), 6.30 (d, J = 7.6 Hz, 1H, CHNH), 4.34−4.18 (2m, 2H, CH_2N), 4.07−3.99 (m, 4H, CH_2CH_3), 3.75−3.65 (m, 1H, CHNH), 2.42−2.25 (m, 2H, CH_2CH_2N, CH_2P), 2.15−2.00 (m, 2H, CH_2CH_2N, CH_2P), 1.53 (s, 9H, CH_3), 1.51 (s, 9H, CH_3), 1.44 (s, 9H, CH_3), 1.28−1.20 (m, 6H, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 153.0 (C_{-4}), 152.6 (C_{-2}), 150.8, 150.2 (C═O), 149.8 (C_{-6}), 142.1 (C_{-8}), 118.2 (C_{-5}), 82.3, 81.0, 79.3 (C(CH_3)$_3$), 61.8 (CH_2CH_3), 44.6 (CHNH), 41.0 (CH_2N), 35.5, 35.4 (CH_2CH_2N), 31.1, 30.0 (CH_2P), 28.6, 28.3 (C(CH_3)$_3$), 16.5 (CH_2CH_3). ^{31}P NMR($CDCl_3$) δ 28.54 (s). HRMS (TOF ESI > 0) found: 658.3333. Calculated for $C_{28}H_{49}N_7O_9P$: 658.3329 $(M + H)^+$.

*(S)-2,6-Diamino-(N-tert-butoxycarbonyl)-N-9-(3-amino-4-diethylphosphonobutyl)purine (**39**(S)).* Compound **39**(S) (1.58 g) was obtained in 78% yield, according to procedure I, from **37**(S) (2.22 g). R_f = 0.4 in CH_2Cl_2/MeOH 95/5 (v/v). $[\alpha]_D^{20}$ +20.1° (c 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H, H8), 7.87 (s, 1H, NH), 7.81 (s, 1H, NH), 6.32 (m, 1H, CHNH), 4.32−4.18 (2 m, 2H, CH_2N), 4.03−4.00 (m, 4H, CH_2CH_3), 3.75−3.64 (m, 1H, CHNH), 2.41−1.98 (2m, 4H, CH_2CH_2N, CH_2P), 1.52 (s, 9H, CH_3), 1.50 (s, 9H, CH_3), 1.43 (s, 9H, CH_3), 1.27−1.20 (m, 6H, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ 155.8 (C-4), 152.6 (C-2), 150.8, 150.2 (C═O), 149.8 (C-6), 142.1 (C-8), 118.2 (C-5), 82.3, 81.0, 79.3 (C(CH_3)$_3$), 61.7 (CH_2CH_3), 44.6 (CHNH), 41.0 (CH_2N), 35.5, 35.4 (CH_2CH_2N), 31.0, 29.9 (CH_2P), 28.6, 28.3, 28.2 (C(CH_3)$_3$, 16.5 (CH_2CH_3). ^{31}P NMR ($CDCl_3$) δ 28.5 (s). HRMS (TOF ESI > 0) found: 658.3339. Calculated for $C_{28}H_{49}N_7O_9P$: 658.3329 $(M + H)^+$.

General Procedure D′. *Removal of Boc Protecting Groups.* To a stirred solution of *β*-aminophosphonate (1 equiv) in anhydrous dichloromethane (14 mL/mmol) under argon, and at room temperature, was added dropwise a solution of trifluoroacetic acid in anhydrous dichloromethane (19 mL/mmol, 3/7, v/v). The reaction mixture was stirred at room temperature for 5 h. Volatiles were removed under reduced pressure, and the crude was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH (0 to 10%). The desired compound was obtained as a white foam.

*Diethyl (R)-(4-(2-Amino-6-methoxy-9H-purin-9-yl)-2-aminobutyl)phosphonate (**40**(R)).* Compound **40**(R) (594 mg) was obtained in quantitative yield, according to procedure D′, from **38**(R) (915 mg). R_f = 0.25 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −12.9° (c 1, MeOH). ^{1}H NMR (MeOD) δ 7.91 (s, 1H, H_8), 4.40−4.25 (m, 2H, CH_2N), 4.14−4.07 (m, 4H, CH_2CH_3), 4.06 (s, 1H, OCH_3), 3.43−3.36 (m, 1H, $CHNH_2$), 2.45−2.18 (m, 4H, CH_2CH_2N, CH_2P), 1.27 (m, 6H, CH_2CH_3). ^{13}C NMR (MeOD) δ 162.9 (C_{-6}), 162.0 (C_{-2}), 155.1 (C_{-4}), 140.7 (CH_{-8}), 114.7 (C_{-5}), 64.1 (OCH_2CH_3), 54.4 (OCH_3), 46.2 (CHNH), 40.1 (CH_2N), 35.4, 35.3 (CH_2CH_2N), 30.0, 28.8 (CH_2P), 16.6 (OCH_2CH_3). ^{31}P NMR (MeOD) δ 26.0 (s). HRMS (TOF ESI > 0) found: 373.1761. Calculated for $C_{14}H_{26}N_6O_4P$: 373.1753 $(M + H)^+$.

*Diethyl (S)-(4-(2-Amino-6-methoxy-9H-purin-9-yl)-2-aminobutyl)phosphonate (**40**(S)).* Compound **40**(S) (290 mg) was obtained in quantitative yield, according to procedure D′, from **38**(S) (447 mg). R_f = 0.25 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +13.5° (c 1, MeOH). ^{1}H NMR(MeOD) δ 7.92 (s, 1H, H_8), 4.39−4.23 (2m, 2H, CH_2N), 4.13−4.07 (m, 4H, CH_2CH_3), 4.06 (s, 3H, OCH_3), 3.42−3.35 (m, 1H, $CHNH_2$), 2.44−2.18 (m, 4H, CH_2CH_2N, CH_2P), 1.28 (m, 6H, CH_2CH_3). ^{13}C NMR (MeOD) δ 162.9 (C_{-6}), 162.1 (C_{-2}), 140.7 (CH_{-8}), 64.1 (CH_2CH_3), 54.4 (OCH_3), 46.2 (CHNH), 40.1 (CH_2N), 35.4, 35.3 (CH_2CH_2N), 29.9, 28.8 (CH_2P), 16.6 (CH_2CH_3). ^{31}P NMR(MeOD) δ 26.0 (s). HRMS (TOF ESI > 0) found: 373.1756. Calculated for $C_{14}H_{26}N_6O_4P$: 373.1753 $(M + H)^+$.

*Diethyl (R)-(4-(2,6-Diamino-9H-purin-9-yl)-2-aminobutyl)-phosphonate (**41**(R)).* Compound **41**(R) (663 mg) was obtained in quantitative yield, according to procedure D′, from **39**(R) (1.47 g). R_f = 0.2 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ −12.8° (c 1, MeOH). ^{1}H NMR (MeOD) δ 7.90 (s, 1H, H_8), 4.35−4.22 (m, 2H, CH_2N), 4.15−4.07 (m, 4H, CH_2CH_3), 3.46−3.38 (m, 1H, $CHNH_2$), 2.43−2.19 (m, 4H, CH_2CH_2N, CH_2P), 1.30 (m, 6H, CH_2CH_3). ^{13}C NMR (MeOD) δ 154.8 (C_{-2}), 153.2 (C_{-4}), 141.0 (CH_{-8}), 117.0 (C_{-6}), 113.2 (C_{-5}), 64.2 (CH_2CH_3), 46.1 (CHNH), 40.1 (CH_2N), 35.2 (CH_2CH_2N), 29.9, 28.7 (CH_2P), 16.6 (CH_2CH_3). ^{31}P NMR(MeOD) δ 26.0 (s). HRMS (TOF ESI > 0) found: 358.1761. Calculated for $C_{13}H_{25}N_7O_3P$: 358.1756 $(M + H)^+$.

*Diethyl (S)-(4-(2,6-Diamino-9H-purin-9-yl)-2-aminobutyl)-phosphonate (**41**(S)).* Compound **41**(S) (685 mg) was obtained in quantitative yield, according to procedure D′, from **39** (S) (1.58 g). R_f = 0.2 in CH_2Cl_2/MeOH (90/10, v/v). $[\alpha]_D^{20}$ +12.4° (c 1, MeOH). ^{1}H NMR (MeOD) δ 7.86 (s, 1H, H8), 4.34−4.22 (2m, 2H, CH_2N), 4.14−4.07 (m, 4H, CH_2CH_3), 3.44−3.36 (m, 1H, $CHNH_2$), 2.44−2.17 (m, 4H, CH_2CH_2N, CH_2P), 1.29 (m, 6H, CH_2CH_3). ^{13}C NMR (MeOD) δ 155.9 (C-2), 153.1 (C-4), 140.4 (CH-8), 117.2 (C-6), 113.4 (C-5), 64.3, 64.1 (CH_2CH_3), 46.1 (CHNH), 40.0 (CH_2N), 35.3 (CH_2CH_2N), 29.9, 29.0 (CH_2P), 16.6 (CH_2CH_3). ^{31}P NMR (MeOD) δ 26.0 (s). HRMS (TOF ESI > 0) found: 358.1758. Calculated for $C_{13}H_{25}N_7O_3P$: 358.1767 $(M + H)^+$.

General Procedure F′. *Removal of the Diethyl Phosphonate Groups.* To a stirred solution of diethyl-*β*-aminophosphonate (1 equiv) in anhydrous dimethylformamide (20 mL/mmol), cooled at 0 °C, was added dropwise bromotrimethylsilane (6.6 equiv). The reaction mixture was stirred at room temperature until completion of the reaction was indicated by TLC monitoring (isopropanol/water/ammoniac 7/2/1 v/v/v). When *in situ* removal of Boc groups was required, water was added to the reaction mixture and stirring was pursued 1 h at rt. Then, triethylammonium bicarbonate solution (TEAB 1 M) was added to the reaction mixture until pH = 7. The volatiles were evaporated under high vacuum, and the aqueous residue was freeze-dried. The resulting lyophilizate was purified by flash chromatography silica gel on C18 reverse phase using water/MeOH gradient (0 to 100%) and freeze-dried, giving rise to the desired phosphonic acid. Eventually, the latter was dissolved in a small amount of water and percolated through a Dowex resin column (Na^+ form) to afford after freeze-drying the corresponding compound as sodium salt.

*(R)-(4-(2-Amino-6-oxo-9H-purin-9-yl)-2-aminobutyl)phosphonic Acid (**12**(R)).* Compound **12**(R) (520 mg) was obtained in 82% yield, according to procedure F′, from **40**(R) (679 mg). R_f = 0.15 in *i*PrOH/H_2O/NH_4OH_c (7/2/1, v/v/v). Purity (HPLC) > 98%. $[\alpha]_D^{20}$ −8.8° (c 0.1, H_2O). ^{1}H NMR (D_2O) δ 7.82 (s, 1H, H_8), 4.21 (t, J = 7.4 Hz, 2H, CH_2N), 3.47−3.37 (m, 1H, $CHNH_2$), 2.23−2.17 (m, 2H, CH_2CH_2N), 1.89−1.79 (m, 1H, CH_2P), 1.67−1.54 (m, 1H, CH_2P). ^{13}C NMR (D_2O) δ 158.9 (C_{-6}), 153.8 (C_{-2}), 151.5 (C_{-4}), 139.7 (CH_{-8}), 115.8 (C_{-5}), 46.6 ($CHNH_2$), 39.6 (CH_2N), 34.7, 34.6 (CH_2CH_2N), 31.5, 30.7 (CH_2P). ^{31}P NMR(D_2O) δ 16.36. HRMS (TOF ESI > 0) found: 347.0611. Calculated for $C_9H_{14}N_6O_4PNa_2$: 347.0610 $(M + H)^+$.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

*(S)-(4-(2-Amino-6-oxo-9H-purin-9-yl)-2-aminobutyl)phosphonic Acid (**12**(S))*. Compound **12**(*S*) (162 mg) was obtained in 64% yield, according to procedure F′, from **40**(*S*) (270 mg). R_f = 0.15 in *i*PrOH/H_2O/NH_4OH_c (7/2/1, v/v/v). Purity (HPLC) > 98%. $[\alpha]_D^{20}$ +9.7° (*c* 0.1, H_2O). ^{1}H NMR (D_2O) δ 7.77 (s, 1H, H_8), 4.22−4.09 (m, 2H, CH_2N), 3.49−3.36 (m, 1H, $\underline{CH}NH_2$), 2.22−2.18 (m, 2H, $\underline{CH_2}CH_2N$), 1.91−1.85 (m, 1H, CH_2P), 1.70−1.64 (m, 1H, CH_2P). ^{13}C NMR (D_2O) δ 159.0 (C_{-6}), 153.9 (C_{-2}), 151.6 (C_{-4}), 139.8 (C_{-8}), 115.8 (C_{-5}), 46.6 ($CHNH_2$), 39.7 (CH_2N), 34.7, 34.6 ($\underline{CH_2}CH_2N$), 31.5, 30.7 (CH_2P). ^{31}P NMR (D_2O) δ 16.3. HRMS (TOF ESI > 0) found: 347.0605. Calculated for $C_9H_{14}N_6O_4PNa_2$: 347.0610 $(M + H)^+$.

*(R)-(4-(2,6-Diamino-9H-purin-9-yl)-2-aminobutyl)phosphonic Acid (**13**(R))*. Compound **13**(*R*) (471 mg) was obtained in 73% yield, according to procedure F′, from **41**(*R*) (663 mg). R_f = 0.15 in *i*PrOH/H_2O/NH_4OH_c (7/2/1, v/v/v). Purity (HPLC) > 97%. $[\alpha]_D^{20}$ −8.0° (*c* 0.1, H_2O). ^{1}H NMR (D_2O) δ 7.87 (s, 1H, H_8), 4.23−4.14 (m, 2H, CH_2N), 3.35−3.28 (m, 1H, $\underline{CH}NH_2$), 2.17−2.04 (m, 2H, $\underline{CH_2}CH_2N$), 1.80−1.73 (m, 1H, CH_2P), 1.60−1.50 (m, 1H, CH_2P). ^{13}C NMR (D_2O) δ 156.1 (C_{-2}), 151.0 (C_{-4}), 140.2 (CH_{-8}), 117.3 (C_{-6}), 113.0 (C_{-5}), 45.3 ($CHNH_2$), 40.8 (CH_2N), 38.1, 38.0 ($\underline{CH_2}CH_2N$), 37.0, 36.1 (CH_2P). ^{31}P NMR(D_2O) δ 17.2. HRMS (TOF ESI < 0) found: 346.0770. Calculated for $C_9H_{15}N_7O_3PNa_2$: 346.0769 $(M + H)^+$.

*(S)-(4-(2,6-Diamino-9H-purin-9-yl)-2-aminobutyl)phosphonic Acid (**13**(S))*. Compound **13**(*S*) (435 mg) was obtained in 71% yield, according to procedure F′, from **41**(*S*) (630 mg). R_f = 0.15 in *i*PrOH/H_2O/NH_4OH_c (7/2/1, v/v/v). Purity (HPLC) > 96%. $[\alpha]_D^{20}$ +8.9° (*c* 0.1, H_2O). ^{1}H NMR (D_2O) δ 7.89 (s, 1H, H_8), 4.19−4.10 (m, 2H, CH_2N), 3.16−3.09 (m, 1H, $\underline{CH}NH_2$), 2.04−1.98 (m, 1H, $\underline{CH_2}CH_2N$), 1.88−1.84 (m, 1H, $\underline{CH_2}CH_2N$), 1.67−1.61 (m, 1H, CH_2P), 1.48−1.41 (m, 1H, CH_2P). ^{13}C NMR (D_2O) δ 156.1 (C_{-2}), 151.0 (C_{-4}), 140.1 (C_{-8}), 117.3 (C_{-6}), 113.0 (C_{-5}), 45.3 ($CHNH_2$), 40.8 (CH_2N), 38.1, 38.0 ($\underline{CH_2}CH_2N$), 37.0, 36.1 (CH_2P). ^{31}P NMR (D_2O) δ 17.2. HRMS (TOF ESI > 0) found: 346.0769. Calculated for $C_9H_{15}N_7O_3PNa_2$: 346.0769 $(M + H)^+$.

*N-9-[4-Phosphonic acid butyl]guanine (**11a**).*[13,21,30] Compound **11a** was obtained in 58% yield according to literature. R_f = 0.18 (*i*PrOH/H_2O/NH_4OH 7/2/1). Purity (HPLC) > 98%. ^{1}H NMR (D_2O): δ = 1.3 (t, 9H), 1.55−1.66 (m, 4H), 1.84−1.95 (m, 2H), 3.20 (m, 6H), 4.07 (m, 2H), 7.84 (s, 1H). ^{13}C NMR (D_2O): δ = 20.22, 20.25, 26.7, 27.7 (CH_2), 30.2, 30.3, (CH_2P), 43.2, 46.6 (CH_2N), 115.8 (C_{-5}), 140.1 (C_{-8}), 151.4 (C_{-4}), 153.6 (C_{-2}), 158.8(C_{-6}). ^{31}P NMR (D_2O): 25.7. MS (ESI) *m*/*z* 286.07 $[M - HNEt_3]^-$; 288.09 $[M + 2H\text{-}HNEt_3]^+$. HRMS: calcd $C_9H_{15}N_5O_4P$ $[M + 2H - HNEt_3]^+$ 288.0862, obs 288.0864.

*(Z)-(4-(2-Amino-6-hydroxy-9H-purin-9-yl)but-1-en-1-yl)-phosphonic Acid Disodic Salt (**11b**)*. The diethyl-(*Z*)-(4-(2-amino-6-methoxy-9*H*-purin-9-yl)-but-1-en-1-yl)phosphonate (261 mg, 1 equiv) was dissolved under argon atmosphere in anhydrous DMF (20 mL/mmol) at 0 °C. TMSBr (6.6 equiv) was added dropwise to the solution, and the mixture was kept at 0 °C for 5 min, then allowed to slowly warm to room temperature and stirred for 3 days. The reaction progress was followed by TLC monitoring (isopropanol/water/ammoniac 7/2/1 v/v/v). The reaction was quenched by addition of a triethylbutylammonium solution (1 M, pH 7). The volatiles were removed under vacuum, and the resulting aqueous solution was freeze-dried. The crude was purified on reverse phase flash chromatography (water/methanol gradient, 0 to 100% methanol), leading to the phosphonate triethylammonium salts as a white powder. The compound was percolated through a Na^+ Dowex resin, and after freeze-drying of the require fractions the sodium salts were obtained as a white lyophilizate. The desired compound (130 mg) was obtained in 54% yield. R_f = 0.18 (*i*PrOH/H_2O/NH_4OH 7/2/1). Purity (HPLC) > 96%. ^{1}H NMR (500 MHz, D_2O) δ 7.82 (s, 1H, H_{-8}), 6.11 (ddt, *J* = 45.8, 13.0, 7.4 Hz, 1H, $C\underline{H}CH_2$), 5.83 (ddt, *J* = 17.7, 13.1, 1.6 Hz, 1H, CHP), 4.15 (t, *J* = 7.1 Hz, 2H, CH_2N), 2.93 (qdd, *J* = 7.0, 2.8, 1.5 Hz, 2H, $C\underline{H_2}CH$). ^{13}C NMR (126 MHz, D_2O) δ 158.9 (C_{-6}), 153.5 (C_{-2}), 151.4 (C_{-5}), 141.1 ($\underline{C}HCH_2$), 140.1 (CH_{-8}), 126.3 (d, *J* = 171.7 Hz, CHP), 115.9 (C_{-4}), 42.8 (CH_2N), 30.2 (d, *J* = 21.8 Hz, 1C, $\underline{C}H_2CH$). ^{31}P NMR (202 MHz, D_2O) δ 10.5. HMRS TOF ESI+ found: 286.0708. Calculated for $C_9H_{13}N_5O_4P$: 286.0705 $(M + H)^+$.

Biological Experiments. *In Vitro* Antimalarial Activity. Drug effects on *in vitro P. falciparum* growth (3D7, W2, and FcM29 strains) were measured in microtiter plates according to a modified Desjardins test.[25,31] *P. falciparum* infected erythrocyte suspension (1.5% final hematocrit and 0.6% parasitemia) was grown in complete medium (Hepes-buffered RPMI 1640 + 10% AB human serum) with or without ANP analogues. The compounds were dissolved in RPMI 1640 and then further diluted in culture medium. Parasite growth was assessed by measuring the incorporation of [^{3}H]hypoxanthine into nucleic acids as previously described.[25] Suspensions of *P. falciparum* infected erythrocytes were incubated with various concentrations of analogues during 48 h. Then 30 μL of [^{3}H]hypoxanthine (0.5 μCi/well) was added for an additional 18 h period. The reactions were stopped by freezing at −80 °C. Cells were lysed by thawing, and the parasite macromolecules including nucleic acids were recovered by harvesting the lysate on glass-fiber filter plates (Unifilter 96 GF/C, PerkinElmer) using a FilterMate cell harvester (Packard Instruments). The radioactivity was counted on a TopCount microplate scintillation counter (Packard Instruments). Radioactivity background was obtained from incubation of noninfected erythrocytes under the same conditions, and the value obtained was deduced. Results were expressed as IC_{50}, which is the drug concentration leading to 50% inhibition of parasite growth. A nonlinear regression model (sigmoidal dose−response/variable slope; GraphPad Prism 6) was used to calculate the IC_{50} values.

***In Vitro* Toxicity against Erythroblast K562 Cell Line.** K-562 cells were cultured in complete medium (RPMI 1640 complemented with 10% fetal calf serum in 96-well microplates (8000 cells per well) and incubated 24 h at 37 °C and under 5% CO_2 in the presence of various concentrations of tested analogues as above. After 24 h incubation at 37 °C, 30 μL of complete medium containing 0.5 μCi [^{3}H]thymidine was added to each well for an additional 6 h period. The reactions were stopped by freezing at −80 °C. Cell macromolecules including nucleic acids were recovered as above. Radioactivity background was obtained from the incubation of culture medium alone.

Effect of Compounds 5 on *P. falciparum* Development. Drug effects on parasite development were determined on a tightly synchronized culture at ring stage. Infected red blood cells were treated or not with 2 μM **5**(*R*) during 69 h at 2.5% hematocrit and 1−2% parasitemia. Culture medium with or without drug was changed every 24 h during the entire treatment period. Thin blood smears were realized every 6 h to determine the parasitemia and the morphological changes of parasites. Determination of parasitemia and morphological observations were done on smears fixed in methanol and stained with Diff-Quick (pH 7.2; Dade Behring, France). Immunofluorescence assays (IFAs) were performed on smeared infected erythrocytes fixed with 4% formaldehyde in phosphate saline buffer (PBS) for 30 min at room temperature, quenched with 0.1 M glycine (4 min) in PBS, and permeabilized with 0.1% (v/v) Triton X-100 in PBS (5 min). Smears were then blocked in PBS containing 1.5% BSA during 30 min and then incubated with primary antibodies (α-mTIP (myosin Tail Domain Interacting Protein)[32] or mouse α-MSP1(Merozoite Surface Protein 1)[33] diluted in PBS containing 0.15% BSA for 1 h. After three washes, appropriate secondary antibodies were added for 1 h before staining of nuclei with Hoechst (5 min). Observations were performed with a Zeiss Axioimager epifluorescence microscope equipped with a Zeiss Axiocam MRmCCD camera at Montpellier RIO imaging facility. Images were processed using Zen Blue 2.3 (Zeiss) for optical sectioning, luminosity, and contrast adjustment.

***In Vivo* Antimalarial Activity.** Female Swiss NMRI mice (Charles River Laboratories, France) were infected on day 0 by intravenous injection into the caudal vein of 10^7 *P. berghei ANKA*-infected erythrocytes (clone 2.34). Mice were treated once a day for four consecutive days from day 1 by ip or oral route. Compound **5**(*R*) was dissolved in 0.9% NaCl or water (100 μL per mouse and 3 mice per group) for ip or oral administration, respectively. Artesunate was dissolved in DMSO (100 μL per mouse and 3 mice per group). Doses were 0, 0.1, 0.3, 1, 3, 10, and 30 mg/kg for compound **5**(*R*) and 0, 0.3, 1, 3, 9, and 27 mg/kg for artesunate by ip. By oral route, doses were 0, 1, 3, 10, and 30 mg/kg for compound **5**(*R*). Control (untreated) group

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

received only 0.9% NaCl or water. On day 5, parasitemia was counted on Giemsa-stained thin blood smears and the dose leading to the reduction of 50% of the parasitemia (ED_{50}) was determined. Parasitemia was followed for 30 days.

The experiments done here conformed to the national and European regulations and to French laws (EU Directive No. 86/609 modified by the Directive 2010/63 regarding the Protection of Animals used for Experimental and Other Scientific Purposes). The animal studies were performed at the "Centre d'Elevage et de Conditionnement Experimental des Modèles Animaux", Montpellier, under Permission No. E 34-172-23 (University of Montpellier) after approval by the Animal Experimenting Commission (Project No. 01230.02).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00131.

Synthesis information and spectral data (NMR, MS and HPLC) for final compounds and additional figures for NMR studies (PDF)

Spectral data (NMR) for intermediates (PDF)

Molecular formula strings and some data for final compounds (CSV)

■ AUTHOR INFORMATION

Corresponding Authors

Rachel Cerdan − *Laboratory of Pathogen Host Interactions (LPHI), UMR 5235 UM-CNRS, Université de Montpellier, 34095 Montpellier, France*; Email: rachel.cerdan@umontpellier.fr

Suzanne Peyrottes − *Team Nucleosides & Phosphorylated Effectors, Institute for Biomolecules Max Mousseron (IBMM), UMR 5247 UM-CNRS-ENSCM, Université de Montpellier, 34095 Montpellier, France*; orcid.org/0000-0003-1705-0576; Email: suzanne.peyrottes@umontpellier.fr

Authors

Thomas Cheviet − *Team Nucleosides & Phosphorylated Effectors, Institute for Biomolecules Max Mousseron (IBMM), UMR 5247 UM-CNRS-ENSCM, Université de Montpellier, 34095 Montpellier, France*

Sharon Wein − *Laboratory of Pathogen Host Interactions (LPHI), UMR 5235 UM-CNRS, Université de Montpellier, 34095 Montpellier, France*

Gabriel Bourchenin − *Team Nucleosides & Phosphorylated Effectors, Institute for Biomolecules Max Mousseron (IBMM), UMR 5247 UM-CNRS-ENSCM, Université de Montpellier, 34095 Montpellier, France*

Manon Lagacherie − *Team Nucleosides & Phosphorylated Effectors, Institute for Biomolecules Max Mousseron (IBMM), UMR 5247 UM-CNRS-ENSCM, Université de Montpellier, 34095 Montpellier, France*

Christian Périgaud − *Team Nucleosides & Phosphorylated Effectors, Institute for Biomolecules Max Mousseron (IBMM), UMR 5247 UM-CNRS-ENSCM, Université de Montpellier, 34095 Montpellier, France*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.0c00131

Author Contributions

The manuscript was written through contributions of all authors. S.P., T.C., and C.P. designed the synthesis and NMR studies. T.C., G.B., and M.L. performed the chemical experiments. S.W. and R.C. designed the biological studies. S.W. performed the biological experiments. All authors have given approval to the final version of the manuscript.

Funding

This work is part of the research project "NAN-Pal" supported by the Région Occitanie and SATT AxLR.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is part of the research project "NAN-Pal" supported by the Région Occitanie and SATT AxLR. We thank the Centre d'Elevage et de Conditionnement Experimental des Modèles Animaux (University of Montpellier, France) for providing access to their facilities for animal studies, as well as the imaging facility Montpellier Ressources Imagerie, member of the national infrastructure France-Bioimaging supported by the French National Research Agency (ANR-10-INBS-04, "Investments for the future"). We thank the technical excellence of Mathilde Gens and Natacha Venturini.

■ ABBREVIATIONS USED

AcOH, acetic acid; ANP, acyclonucleosides phosphonate; Boc, *tert*-butyloxycarbonyl; *NO*-BSA, *N*,*O*-bis(trimethyl)-silylacetamide; CSA, chiral solvating agent; DEAD, diethylazodicarboxylate; DEP, diethyl phosphite; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; ED_{50}, dose required to decrease parasitemia by 50%; ESI, electrospray ionization; H-Asp-OH, aspartic acid; HGXPRT, hypoxanthine−guanine−xanthine phosphoryl transferase; HPLC, high performance liquid chromatography; HR-MS, high resolution mass spectrometry; IC_{50}, concentration required for 50% of inhibition of the parasite growth; IMP, inosine 5′-monophosphate; ip, intraperitoneal; LiHMDS, lithium hexamethyldisilazane; MeOH, methanol; NMR, nuclear magnetic resonance; pD, pH in deuterated media; *Pf*, *Plasmodium falciparum*; PNP, purine nucleoside phosphorylase; RP, reverse phase chromatography; rt, room temperature; SI, selectivity index; TEAB, triethylammonium bicarbonate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMSBr, trimethylsilyl bromide; UV, ultraviolet

■ REFERENCES

(1) (a) Fernández, E.; Castellote, M. I.; Chaparro, M. J.; Díaz, B.; Fernández, J.; Gordo, M.; de las Heras, L.; León, M. L.; Rueda, L.; Calderón, F. Chapter One - A Decade of Malaria Phenotypic Screenings: Key Lessons on the Discovery and Development of New Antimalarial Drugs. In *Annual Reports in Medicinal Chemistry*; Chibale, K., Ed.; Academic Press, 2019; Vol. *53*, pp 1−23. (b) Guiguemde, W. A.; Shelat, A. A.; Garcia-Bustos, J. F.; Diagana, T. T.; Gamo, F.-J.; Guy, R. K. Global Phenotypic Screening for Antimalarials. *Chem. Biol.* **2012**, *19* (1), 116−129. (c) Okombo, J.; Chibale, K. Insights into Integrated Lead Generation and Target Identification in Malaria and Tuberculosis Drug Discovery. *Acc. Chem. Res.* **2017**, *50* (7), 1606−1616.

(2) Berg, M.; Van der Veken, P.; Goeminne, A.; Haemers, A.; Augustyns, K. Inhibitors of the Purine Salvage Pathway: A Valuable Approach for Antiprotozoal Chemotherapy? *Curr. Med. Chem.* **2010**, *17* (23), 2456−2481.

(3) Chaparro, M. J.; Calderón, F.; Castañeda, P.; Fernández-Alvaro, E.; Gabarró, R.; Gamo, F. J.; Gómez-Lorenzo, M. G.; Martín, J.; Fernández, E. Efforts Aimed to Reduce Attrition in Antimalarial Drug Discovery: A Systematic Evaluation of the Current Antimalarial Targets Portfolio. *ACS Infect. Dis.* **2018**, *4* (4), 568−576.

(4) Cheviet, T.; Lefebvre-Tournier, I.; Wein, S.; Peyrottes, S. Plasmodium Purine Metabolism and Its Inhibition by Nucleoside and Nucleotide Analogues. *J. Med. Chem.* **2019**, *62* (18), 8365−8391.
(5) Ringia, E. A. T.; Schramm, V. L. Transition States and Inhibitors of the Purine Nucleoside Phosphorylase Family. *Curr. Top. Med. Chem.* **2005**, *5* (13), 1237−1258.
(6) de Jersey, J.; Holy, A.; Hockova, D.; Naesens, L.; Keough, D. T.; Guddat, L. W. 6-Oxopurine Phosphoribosyltransferase: A Target for the Development of Antimalarial Drugs. *Curr. Top. Med. Chem.* **2011**, *11* (16), 2085−2102.
(7) (a) Evans, G. B.; Tyler, P. C.; Schramm, V. L. Immucillins in Infectious Diseases. *ACS Infect. Dis.* **2018**, *4* (2), 107−117. (b) Cassera, M. B.; Hazleton, K. Z.; Merino, E. F.; Obaldia, N., III; Ho, M.-C.; Murkin, A. S.; DePinto, R.; Gutierrez, J. A.; Almo, S. C.; Evans, G. B.; Babu, Y. S.; Schramm, V. L. Plasmodium Falciparum Parasites are Killed by a Transition State Analogue of Purine Nucleoside Phosphorylase in a Primate Animal Model. *PLoS One* **2011**, *6* (11), No. e26916. (c) Ting, L.-M.; Shi, W.; Lewandowicz, A.; Singh, V.; Mwakingwe, A.; Birck, M. R.; Ringia, E. A. T.; Bench, G.; Madrid, D. C.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H.; Schramm, V. L.; Kim, K. Targeting a Novel Plasmodium Falciparum Purine Recycling Pathway with Specific Immucillins. *J. Biol. Chem.* **2005**, *280* (10), 9547−9554.
(8) (a) Spacek, P.; Keough, D. T.; Chavchich, M.; Dracinsky, M.; Janeba, Z.; Naesens, L.; Edstein, M. D.; Guddat, L. W.; Hockova, D. Synthesis and Evaluation of Asymmetric Acyclic Nucleoside Bisphosphonates as Inhibitors of Plasmodium falciparum and Human Hypoxanthine-Guanine-(Xanthine) Phosphoribosyltransferase. *J. Med. Chem.* **2017**, *60* (17), 7539−7554. (b) Keough, D. T.; Hockova, D.; Janeba, Z.; Wang, T. H.; Naesens, L.; Edstein, M. D.; Chavchich, M.; Guddat, L. W. Aza-acyclic Nucleoside Phosphonates Containing a Second Phosphonate Group As Inhibitors of the Human, Plasmodium Falciparum and Vivax 6-Oxopurine Phosphoribosyltransferases and Their Prodrugs As Antimalarial Agents. *J. Med. Chem.* **2015**, *58* (2), 827−846. (c) Keough, D. T.; Spacek, P.; Hockova, D.; Tichy, T.; Vrbkova, S.; Slavetinska, L.; Janeba, Z.; Naesens, L.; Edstein, M. D.; Chavchich, M.; Wang, T. H.; de Jersey, J.; Guddat, L. W. Acyclic Nucleoside Phosphonates Containing a Second Phosphonate Group Are Potent Inhibitors of 6-Oxopurine Phosphoribosyltransferases and Have Antimalarial Activity. *J. Med. Chem.* **2013**, *56* (6), 2513−2526.
(9) Keough, D. T.; Hocková, D.; Holý, A.; Naesens, L. M. J.; Skinner-Adams, T. S.; Jersey, J. d.; Guddat, L. W. Inhibition of Hypoxanthine-Guanine Phosphoribosyltransferase by Acyclic Nucleoside Phosphonates: A New Class of Antimalarial Therapeutics. *J. Med. Chem.* **2009**, *52* (14), 4391−4399.
(10) Spacek, P.; Keough, D. T.; Chavchich, M.; Dracinsky, M.; Janeba, Z.; Naesens, L.; Edstein, M. D.; Guddat, L. W.; Hockova, D. Synthesis and Evaluation of Symmetric Acyclic Nucleoside Bisphosphonates as Inhibitors of the Plasmodium Falciparum, Plasmodium Vivax and Human 6-Oxopurine Phosphoribosyltransferases and the Antimalarial Activity of Their Prodrugs. *Bioorg. Med. Chem.* **2017**, *25* (15), 4008−4030.
(11) Hocková, D.; Keough, D. T.; Janeba, Z.; Wang, T.-H.; de Jersey, J.; Guddat, L. W. Synthesis of Novel N-Branched Acyclic Nucleoside Phosphonates As Potent and Selective Inhibitors of Human, Plasmodium Falciparum and Plasmodium Vivax 6-Oxopurine Phosphoribosyltransferases. *J. Med. Chem.* **2012**, *55* (13), 6209−6223.
(12) Klejch, T.; Keough, D. T.; Chavchich, M.; Travis, J.; Skácel, J.; Pohl, R.; Janeba, Z.; Edstein, M. D.; Avery, V. M.; Guddat, L. W.; Hocková, D. Sulfide, Sulfoxide and Sulfone Bridged Acyclic Nucleoside Phosphonates as Inhibitors of the Plasmodium Falciparum and Human 6-Oxopurine Phosphoribosyltransferases: Synthesis and Evaluation. *Eur. J. Med. Chem.* **2019**, *183*, 111667.
(13) Cesnek, M.; Hockova, D.; Holy, A.; Dracinsky, M.; Baszczynski, O.; de Jersey, J.; Keough, D. T.; Guddat, L. W. Synthesis of 9-Phosphonoalkyl and 9-Phosphonoalkoxyalkyl Purines: Evaluation of their Ability to Act as Inhibitors of Plasmodium Falciparum, Plasmodium Vivax and Human Hypoxanthine-Guanine-(Xanthine) Phosphoribosyltransferases. *Bioorg. Med. Chem.* **2012**, *20* (2), 1076−1089.
(14) Clinch, K.; Crump, D. R.; Evans, G. B.; Hazleton, K. Z.; Mason, J. M.; Schramm, V. L.; Tyler, P. C. Acyclic Phosph(on)ate Inhibitors of Plasmodium Falciparum Hypoxanthine-Guanine-Xanthine Phosphoribosyltransferase. *Bioorg. Med. Chem.* **2013**, *21* (17), 5629−5646.
(15) Blad, C. C.; Ahmed, K.; Ijzerman, A. P.; Offermanns, S., 7 - Biological and Pharmacological Roles of HCA Receptors. In *Advances in Pharmacology*; Neubig, R. R., Ed.; Academic Press, 2011; Vol. *62*, pp 219−250.
(16) (a) Li, Z.; Racha, S.; Dan, L.; El-Subbagh, H.; Abushanab, E. A General and Facile Synthesis of Beta- and Gamma-Hydroxy Phosphonates from Epoxides. *J. Org. Chem.* **1993**, *58* (21), 5779−5783. (b) Demmer, C. S.; Krogsgaard-Larsen, N.; Bunch, L. Review on Modern Advances of Chemical Methods for the Introduction of a Phosphonic Acid Group. *Chem. Rev.* **2011**, *111* (12), 7981−8006.
(17) Hospital, A.; Meurillon, M.; Peyrottes, S.; Périgaud, C. An Alternative Pathway to Ribonucleoside β-Hydroxyphosphonate Analogues and Related Prodrugs. *Org. Lett.* **2013**, *15* (18), 4778−4781.
(18) (a) Volkmann, R. A.; Kelbaugh, P. R.; Nason, D. M.; Jasys, V. J. 2-Thioalkyl Penems: An Efficient Synthesis of Sulopenem, a (5R,6S)-6-(1(R)-hydroxyethyl)-2-[(cis-1-oxo-3-thiolanyl)thio]-2-penem Antibacterial. *J. Org. Chem.* **1992**, *57* (16), 4352−4361. (b) Hou, D.-R.; Reibenspies, J. H.; Burgess, K. New, Optically Active Phosphine Oxazoline (JM-Phos) Ligands: Syntheses and Applications in Allylation Reactions. *J. Org. Chem.* **2001**, *66* (1), 206−215. (c) Molinaro, C.; Bulger, P. G.; Lee, E. E.; Kosjek, B.; Lau, S.; Gauvreau, D.; Howard, M. E.; Wallace, D. J.; O'Shea, P. D. CRTH2 Antagonist MK-7246: A Synthetic Evolution from Discovery through Development. *J. Org. Chem.* **2012**, *77* (5), 2299−2309. (d) Aaseng, J. E.; Gautun, O. R. Synthesis of (S)-2-Amino-7-methoxytetralin and Isoindolo[1,2-a]-isoquinolinone Derivatives from l-Aspartic Acid. *Tetrahedron* **2014**, *70* (34), 5057−5063.
(19) (a) Dey, S.; Garner, P. Synthesis of tert-Butoxycarbonyl (Boc)-Protected Purines. *J. Org. Chem.* **2000**, *65* (22), 7697−7699. (b) Porcheddu, A.; Giacomelli, G.; Piredda, I.; Carta, M.; Nieddu, G. A Practical and Efficient Approach to PNA Monomers Compatible with Fmoc-Mediated Solid-Phase Synthesis Protocols. *Eur. J. Org. Chem.* **2008**, *2008* (34), 5786−5797.
(20) (a) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, *1981* (01), 1−28. (b) Hughes, D. L. Progress in the Mitsunobu Reaction. A Review. *Org. Prep. Proced. Int.* **1996**, *28* (2), 127−164.
(21) Kim, C. U.; Luh, B. Y.; Misco, P. F.; Bronson, J. J.; Hitchcock, M. J. M.; Ghazzouli, I.; Martin, J. C. Acyclic Purine Phosphonate Analogs as Antiviral Agents. Synthesis and Structure-Activity Relationships. *J. Med. Chem.* **1990**, *33* (4), 1207−1213.
(22) Geant, P.-Y.; Mohamed, B. S.; Périgaud, C.; Peyrottes, S.; Uttaro, J.-P.; Mathé, C. Probing the Reactivity of H-Phosphonate Derivatives for the Hydrophosphonylation of Various Alkenes and Alkynes Under Free-Radical Conditions. *New J. Chem.* **2016**, *40* (6), 5318−5324.
(23) Kmiecik, N.; Serafin, M.; Olszewski, T. K.; Zymanczyk-Duda, E. Rapid Enantiodifferentation of Chiral Organophosphorus Compounds by P-31 NMR Spectroscopy in the Presence of alpha-Cyclodextrin as the Chiral Solvating Agent. *Spectroscopy* **2017**, *32* (6), 36−39.
(24) Schurig, V.; Nowotny, H.-P. Gas Chromatographic Separation of Enantiomers on Cyclodextrin Derivatives. *Angew. Chem., Int. Ed. Engl.* **1990**, *29* (9), 939−957.
(25) Ancelin, M. L.; Calas, M.; Vidal-Sailhan, V.; Herbuté, S.; Ringwald, P.; Vial, H. J. Potent Inhibitors of Plasmodium Phospholipid Metabolism with a Broad Spectrum of in Vitro Antimalarial Activities. *Antimicrob. Agents Chemother.* **2003**, *47* (8), 2590−2597.
(26) Keough, D. T.; Rejman, D.; Pohl, R.; Zborníková, E.; Hocková, D.; Croll, T.; Edstein, M. D.; Birrell, G. W.; Chavchich, M.; Naesens, L. M. J.; Pierens, G. K.; Brereton, I. M.; Guddat, L. W. Design of Plasmodium Vivax Hypoxanthine-Guanine Phosphoribosyltransferase Inhibitors as Potential Antimalarial Therapeutics. *ACS Chem. Biol.* **2018**, *13* (1), 82−90.
(27) (a) Kirk, K.; Horner, H. A.; Elford, B. C.; Ellory, J. C.; Newbold, C. I. Transport of Diverse Substrates into Malaria-Infected

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX

Erythrocytes via a Pathway Showing Functional Characteristics of a Chloride Channel. *J. Biol. Chem.* **1994**, *269* (5), 3339−3347. (b) Staines, H. M.; Alkhalil, A.; Allen, R. J.; De Jonge, H. R.; Derbyshire, E.; Egée, S.; Ginsburg, H.; Hill, D. A.; Huber, S. M.; Kirk, K.; Lang, F.; Lisk, G.; Oteng, E.; Pillai, A. D.; Rayavara, K.; Rouhani, S.; Saliba, K. J.; Shen, C.; Solomon, T.; Thomas, S. L. Y.; Verloo, P.; Desai, S. A. Electrophysiological Studies of Malaria Parasite-Infected Erythrocytes: Current Status. *Int. J. Parasitol.* **2007**, *37* (5), 475−482.

(28) Zurwerra, D.; Gertsch, J.; Altmann, K.-H. Synthesis of (−)-Dactylolide and 13-Desmethylene-(−)-Dactylolide and Their Effects on Tubulin. *Org. Lett.* **2010**, *12* (10), 2302−2305.

(29) Jörres, M.; Schiffers, I.; Atodiresei, I.; Bolm, C. Asymmetric Michael Additions of α-Nitrocyclohexanone to Aryl Nitroalkenes Catalyzed by Natural Amino Acid-Derived Bifunctional Thioureas. *Org. Lett.* **2012**, *14* (17), 4518−4521.

(30) Harnden, M. R.; Parkin, A.; Parratt, M. J.; Perkins, R. M. Novel Acyclonucleotides: Synthesis and Antiviral Activity of Alkenylphosphonic Acid Derivatives of Purines and a Pyrimidine. *J. Med. Chem.* **1993**, *36* (10), 1343−1355.

(31) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative Assessment of Antimalarial Activity in Vitro by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother.* **1979**, *16* (6), 710−718.

(32) Green, J. L.; Martin, S. R.; Fielden, J.; Ksagoni, A.; Grainger, M.; Yim Lim, B. Y. S.; Molloy, J. E.; Holder, A. A. The MTIP-Myosin A Complex in Blood Stage Malaria Parasites. *J. Mol. Biol.* **2006**, *355* (5), 933−941.

(33) Burghaus, P. A.; Holder, A. A. Expression of the 19-Kilodalton Carboxy-Terminal Fragment of the Plasmodium Falciparum Merozoite Surface Protein-1 in Escherichia Coli as a Correctly Folded Protein. *Mol. Biochem. Parasitol.* **1994**, *64* (1), 165−169.

https://dx.doi.org/10.1021/acs.jmedchem.0c00131
J. Med. Chem. XXXX, XXX, XXX−XXX