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Research paper

# Purine containing carbonucleoside phosphonate analogues as novel chemotype for *Plasmodium falciparum* Inhibition

Bemba Sidi Mohamed<sup>a</sup>, Minh Chau Nguyen<sup>b</sup>, Sharon Wein<sup>c</sup>, Jean-Pierre Uttaro<sup>a</sup>, Xavier Robert<sup>b</sup>, Sébastien Violot<sup>b</sup>, Lionel Ballut<sup>b</sup>, Vinesh Jugnarain<sup>b</sup>, Christophe Mathé<sup>a,\*\*</sup>, Rachel Cerdan<sup>c</sup>, Nushin Aghajari<sup>b</sup>, Suzanne Peyrottes<sup>a,\*</sup>

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## ABSTRACT

The nucleotidase ISN1 is a potential therapeutic target of the purine salvage pathway of the malaria parasite *Plasmodium falciparum*. We identified *Pf*ISN1 ligands by *in silico* screening of a small library of nucleos(t)ide analogues and by thermal shift assays. Starting from a racemic cyclopentyl carbocyclic phosphonate scaffold, we explored the diversity on the nucleobase moiety and also proposed a convenient synthetic pathway to access the pure enantiomers of our initial hit (compound (±)-2). 2,6-Disubstituted purine containing derivatives such as compounds 1, (±)-7e and β-L-(+)-2 showed the most potent inhibition of the parasite *in vitro*, with low micromolar IC<sub>50</sub> values. These results are remarkable considering the anionic nature of nucleotide analogues, which are known to lack activity in cell culture experiments due to their scarce capacity to cross cell membranes. For the first time, we report the antimalarial activity of a carbocyclic methylphosphonate nucleoside with an L-like configuration.

## 1. Introduction

*Plasmodium falciparum* (*Pf*) is responsible for the most lethal form of malaria, which is a major health threat in tropical and subtropical countries. In 2021, the WHO estimated the number of malaria deaths at 619 000 and 247 million cases of malaria worldwide, the latter having increased for the second consecutive year [1]. In October 2021, the RTS,S (Mosquirix®) malaria vaccine was approved and recommended by the WHO for children in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission [1,2]. Unfortunately, the vaccine has a limited efficacy [3] and treatment is still complicated by the emergence of drug resistance [4] and the development of new drugs is urgently required.

Unlike the human host which has both *de novo* and salvage pathways for purine nucleotide synthesis, the purine salvage pathway is the sole source of purine nucleotides for the rapidly multiplying parasite. This pathway is therefore essential for parasite survival and *Plasmodium* enzymes involved in the purine salvage pathway are considered as potential targets for antimalarial chemotherapy [5].

Among the *Pf* purine metabolism enzymes, adenosine deaminase (ADA), hypoxanthine guanine-xanthine phosphoribosyl transferase (HGXPRT) and purine nucleoside phosphorylase (PNP) have been and are still being extensively explored as chemotherapeutic targets [5b,c, 6], but other enzymes as e.g. the GMP synthetase (GMPS) [7] and the IMP specific nucleotidase (ISN1) [7,8], are also of interest. Briefly, *Pf*ISN1 is responsible for the catabolism of inosine 5'-monophosphate (IMP) into inosine whereas *Pf*GMPS catalyzes the conversion of xanthosine 5'-monophosphate (XMP) into guanosine 5'-monophosphate (GMP) (Fig. 1). Considering the discrete structural differences of the respective substrates and products of hydrolysis within this metabolic process (Fig. 1), we envisaged an *in silico* and *in vitro* screening of our in-house library of nucleos(t)ide analogues to identify small molecules that could serve as hits for further development of potential inhibitors. Herein, we focus on the *Pf* 5'-nucleotidase, henceforward referred to as *Pf*ISN1, which has been recently characterized from a functional and a structural point of view [8]. Thus, the biochemical characterization of this enzyme revealed that it has substrate specificity for IMP with ATP as an activator, and that this activation was observed only at physiolog-

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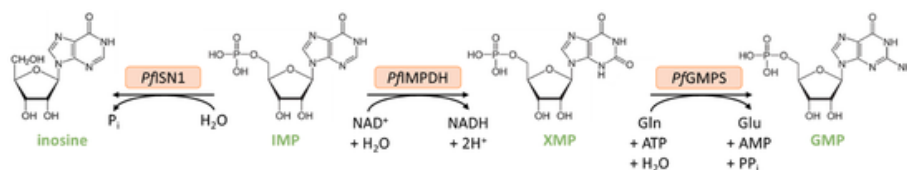


Fig. 1. Catalytic reactions of the three enzymes (*PfISN1*, Inosine-5'-monophosphate dehydrogenase or *PfIMPDH*, and *PfGMPS*) involved in the conversion of IMP into GMP and catabolism of IMP into inosine.

ical pH of 7–8 and not at lower pH values [8]. Hence, *PfISN1* accepts purine containing nucleoside 5'-monophosphates as substrates and this knowledge formed the starting point for the design and the screening of new potential anti-malarial drug candidates.

## 2. Results and discussion

A total of 133 compounds comprising purine containing nucleos(t)ide analogues from our in-house library, compounds previously synthesized by us (Fig. 2) [9], were selected prior to virtual- and experimental screening for their potential interaction with *PfISN1*.

### 2.1. Virtual screening

Structure-based virtual “blind” screening against *PfISN1* was performed on one of the monomers from the structural and functional tetramer, due to calculation limits related to the big size of the tetramer. 133 Molecules were screened and displayed  $\Delta G$ 's between  $-11.8$  and  $-5.8$  (Table S1) kcal/mol, with a majority interaction with residues within a large binding cleft in the native structure of *PfISN1* (without any bound ligand), and involving residues from the so-called motifs I, II, III and IV of the haloacid dehydrogenase (HAD) family enzymes (Fig. 3). One of these, motif I, involves the active site in the catalytic domain and motif IV residues in the Cap-domain. Indeed, when mutating these residues, the hydrolytic activity is totally abolished [8]. Interestingly, the results from this general screening on the native *PfISN1* monomer varied slightly when the screening was performed on the three-dimensional structure to which ATP was bound in the so-called ATP-activator binding site [8] and equally when screening was performed on the three-dimensional structure of IMP (substrate) bound *PfISN1* in which the IMP molecule had been deleted from the structure.

An example hereof was the identification of compounds 1 and 2 (Fig. 2) which were bound to the active site in the two latter structures, whereas they were slightly displaced in the docking studies using the native structure (Fig. S1). These only displayed  $\Delta G$ 's of  $-8.3$ , and  $-8.2$  kcal/mol, respectively, when screening the native monomer

whereas when screening against the structure to which ATP (PDB entry 6RMD) and to which IMP were bound (PDB entry 6RME),  $\Delta G$ 's of  $-8.8$  ( $\pm$ )-1, and  $-7.5$  kcal/mol ( $\pm$ )-2, and  $-9.6$  ( $\pm$ )-1 and  $-9.1$  kcal/mol ( $\pm$ )-2 were found, respectively. However, these two ligands bind to the large binding cleft as the vast majority of molecules screened, and more precisely at the level of motif IV. Details of the interactions with these two ligands are given in Table S3.

### 2.2. Experimental screening

Experimental screening was performed in Thermal Shift Assays (TSA) using Differential Scanning Fluorimetry (DSF). Usage of dyes to monitor the thermal denaturation of proteins with sensitive fluorescence detection enables rapid and inexpensive determination of the stability of the studied protein by using real-time PCR instruments. Hence, this approach can be used as a low cost, initial screen for discovering new ligands by measuring their interactions with a protein of interest by the detection of increases in protein stability (or in certain cases decreases when unfavorable binding) that typically occur upon ligand binding. Thus, the previous set of purine nucleos(t)ide molecules was tested on *PfISN1* and after elimination of non-specific or miscellaneous compounds (including products inducing aggregation), the screening indicated two compounds (Fig. 2, framed derivatives) with putative binding to *PfISN1*. Interestingly, these two carbocyclic nucleoside phosphonate analogues have either stabilizing (compound ( $\pm$ )-1) or destabilizing (compound ( $\pm$ )-2) effects with  $\Delta T_m$ 's of  $4.8$  °C, and  $-5.8$  °C for *PfISN1*, respectively (Fig. 4). Thus, these two derivatives were the most interesting hits and were further tested for their interaction with *PfISN1* by micro-scale thermophoresis (Fig. 5). These experiments indicated a  $K_d$  of  $\sim 19.5$   $\mu$ M with compound ( $\pm$ )-1 and a  $K_d$  of  $\sim 24$   $\mu$ M with compound ( $\pm$ )-2.

It should be mentioned that TSA screening, besides indicating potential stabilizing ligand candidates on this enzyme, also allowed to have insights into molecules which cause protein aggregation, which is also a quite valid information. This latter information was also held up

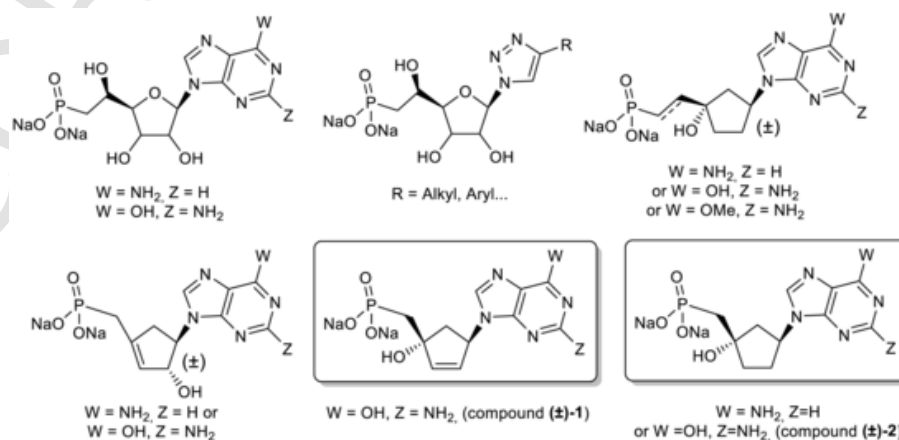
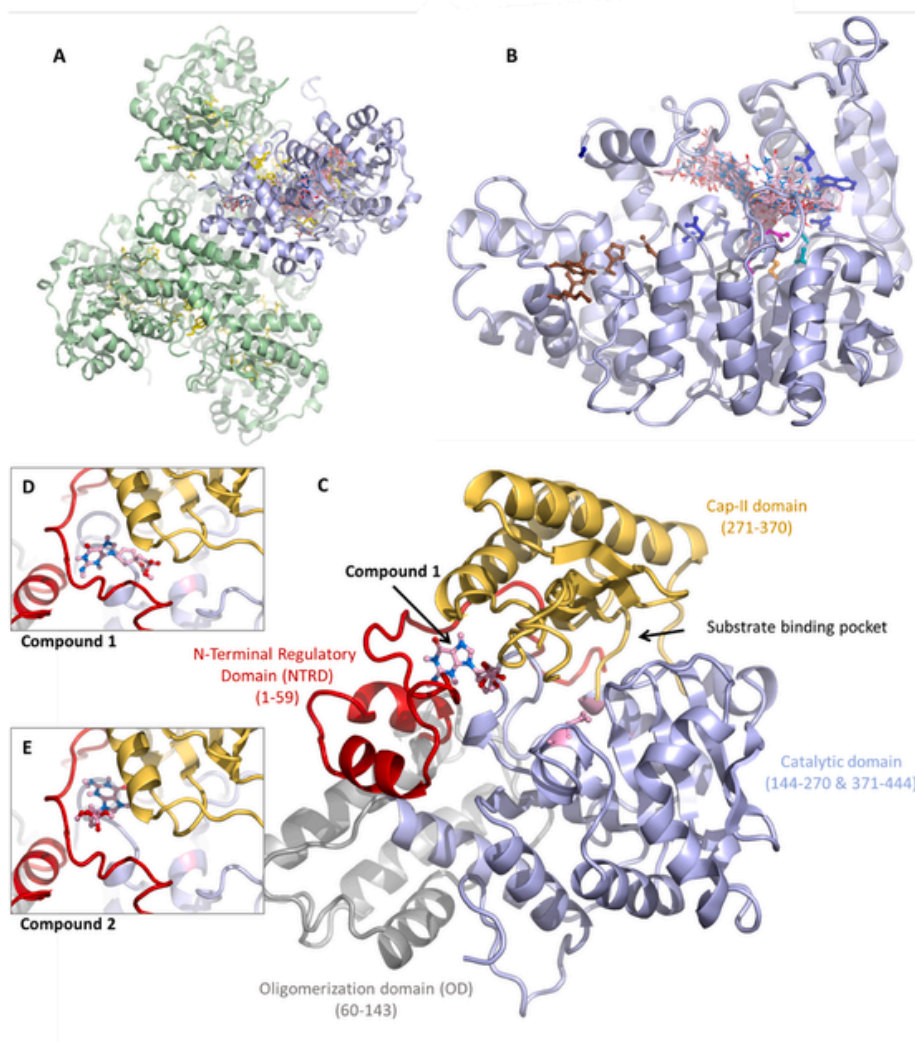


Fig. 2. Examples of the structural diversity explored within our in-house library and selected derivatives (compounds (( $\pm$ )-1 and (( $\pm$ )-2 in framed structures) for further investigations.



**Fig. 3. Selected poses observed during the virtual screening.** (A) Quaternary structure of the *PfISN1* tetramer (PDB entry 6RMO). The monomer used in docking studies is presented in light violet, and amino acid residues from motifs I to IV are highlighted in yellow. Small molecules docked to the monomer are shown in pink. (B) Close-up on the *PfISN1* monomer with docked small molecules in pink. (C) Close-up on the *PfISN1* monomer with compound ( $\pm$ )-1. Corresponding docking poses of compound ( $\pm$ )-1 (D) and ( $\pm$ )-2 (E) in pink. Motifs and binding sites are colored with the following color codes: motif I, dark pink; motif II teal; motif III, orange; motif IV, tv-blue; ATP binding site, brown.

against results from virtual screening of the library of ligands by docking.

As compounds ( $\pm$ )-1 and ( $\pm$ )-2 (Fig. 2) showed high structure similarity and similar behavior during TSA and thermophoresis experiments, we decided to use the compound ( $\pm$ )-2 scaffold for further exploration of the structural diversity. In addition, corresponding starting material and synthetic intermediates were also easier to handle in synthesis [9c], while we have previously observed side-reaction with unsaturated intermediates.

### 2.3. Chemistry

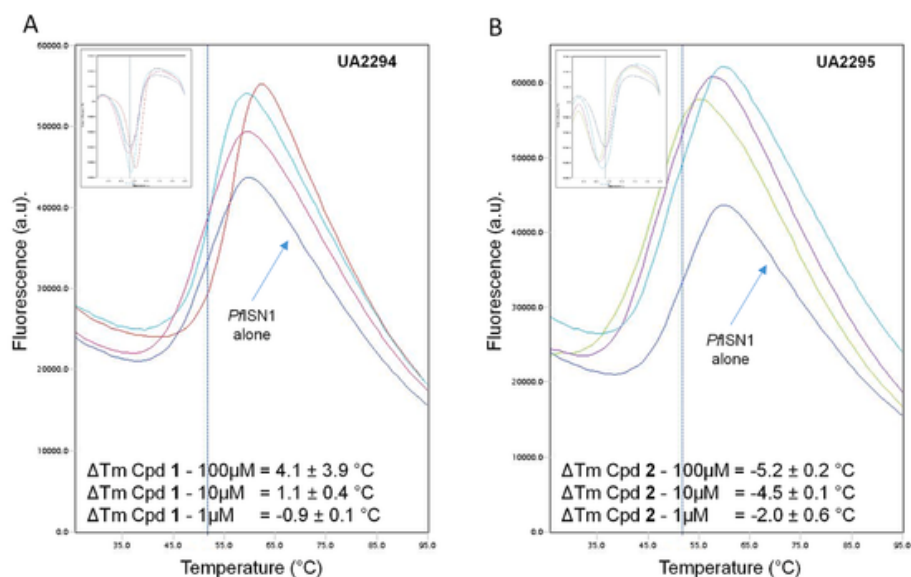
Carbocyclic nucleosides are analogues in which the oxygen in the furanose ring has been replaced by a methylene group [10]. This modification confers chemical and metabolic stability and the addition of a phosphonate group allows these derivatives to behave as nucleoside 5'-monophosphate mimics able to interfere with enzymes belonging to the nucleotide metabolism [11,12].

The synthesis of the carbocyclic derivatives depicted in Fig. 2 has been previously reported [9c,d] and all compounds were obtained as racemates for preliminary evaluation. Thus, we initially decided to ex-

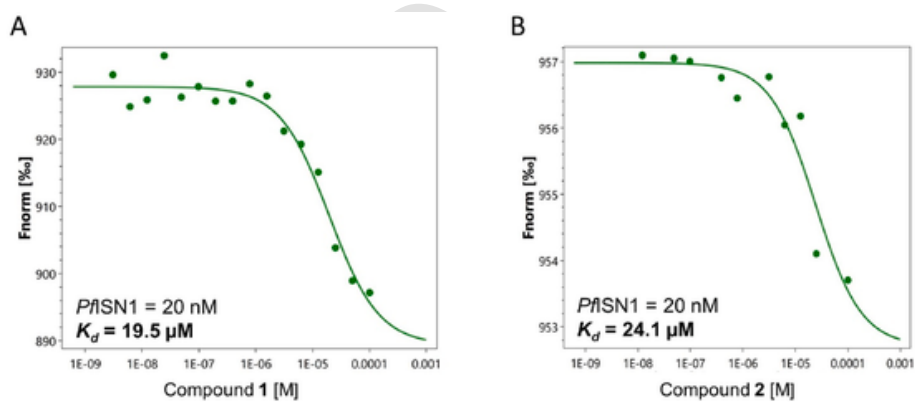
plore the modification of the purine ring on the basis of compound 2' scaffold using the ( $\pm$ )-1-((dimethoxyphosphoryl)methyl)-3-hydroxycyclopentyl acetate (**3**) as starting material (Scheme 1). Coupling of ( $\pm$ )-**3** and various purine derivatives commercially available (2,6-dichloropurine) or obtained beforehand (N<sup>6</sup>-Benzyl-2-chloropurine, 2-amino-N<sup>6</sup>-Benzyl-purine, N<sup>2,6</sup>-BocBenzyl-purine, N<sup>6</sup>-diBoc-2-fluoropurine and N<sup>2,6</sup>-diBoc-purine) under Mitsunobu conditions [9c] provided the fully protected carbocyclic phosphonate nucleoside ( $\pm$ )-**4a-f** which underwent either deacetylation affording compounds ( $\pm$ )-**5a-c** or acidic treatment leading to compounds ( $\pm$ )-**5e-f**. Then, ( $\pm$ )-**4d** and ( $\pm$ )-**5e-f** were treated by K<sub>2</sub>CO<sub>3</sub> in methanol to afford derivatives ( $\pm$ )-**6d-f**.

Finally, conversion of the dimethylphosphonate group into the corresponding phosphonic acid was performed by using TMSBr or TMSCl with NaI in DMF and the desired compounds ( $\pm$ )-**6a-c** and ( $\pm$ )-**7d-f** were isolated as their monosodium salts.

In parallel, we embarked on the development of a synthetic pathway for the preparation of enantiopure forms of our initial hit (Scheme 2, compound 2). After several attempts of chiral resolution of intermediate ( $\pm$ )-**3**, we then considered *cis*-( $\pm$ )-4-*O*-tert-butyl dimethylsilylcyclopent-2-enol ( $\pm$ )-**8** as starting material (Scheme 2). This latter is



**Fig. 4. Screening by Thermal Shift Assays.** Fluorescence melting curves of *PflSN1* with compound 1 (A) and compound ( $\pm$ )-2 (B). The color codes of the  $\Delta T_m$  shifts are for (A) compound ( $\pm$ )-1 at a concentration of 100  $\mu\text{M}$  in red, 10  $\mu\text{M}$  in light blue, 1  $\mu\text{M}$  in pink and *PflSN1* without ligand in dark blue, (B) compound 2 at a concentration of 100  $\mu\text{M}$  in light green, 10  $\mu\text{M}$  in pink, 1  $\mu\text{M}$  in cyan and *PflSN1* without ligand in dark blue. The respective first derivative curves are shown as an inset for each panel. Number of assays,  $n$ , were  $n = 3$  for each compound and  $n = 7$  for reference  $\text{H}_2\text{O}$ .



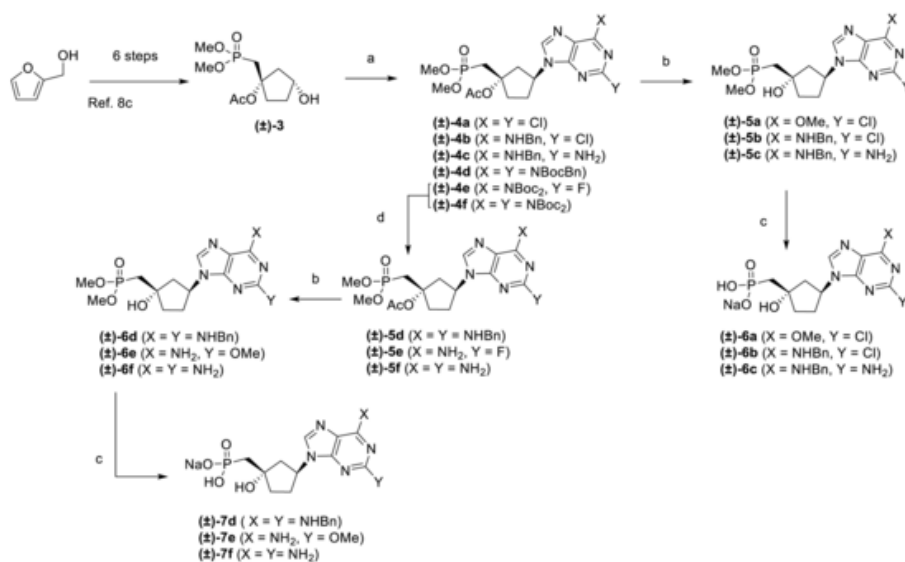
**Fig. 5. Microscale Thermophoresis analysis of protein-ligand interactions.** (A) Interaction between labeled *PflSN1* and compound ( $\pm$ )-1 as ligand. Changes in thermophoresis of a titration of compound ( $\pm$ )-1 at concentrations from 0.1 mM to 10 nM against 20 nM *PflSN1* are shown. The fit yielded a  $K_d$  of 19.5  $\mu\text{M}$ . (B) Interaction between labeled *PflSN1* and compound ( $\pm$ )-2. Changes in thermophoresis of a titration of compound 2 at concentrations from 0.1 mM to 10 nM against 20 nM *PflSN1* are shown. The fit yielded a  $K_d$  of 24.1  $\mu\text{M}$ .

readily prepared in three steps and in multi-gram scale from commercially available bio-sourced furfuryl alcohol following earlier reported procedures [9c,13]. In addition to the synthesis of carbonucleoside analogues [10c,14], the preparation of optically active *cis*-(+)- and *cis*-(-)-4-*O*-protected-cyclopent-2-enol has a wide interest because of their potential use as chiral building blocks for the total synthesis of natural products [15] and prostaglandin derivatives [16]. The synthesis of enantiomeric pure *cis*-2-cyclopentene-1,4-diols is already reported in the literature and mainly involves the palladium-catalyzed desymmetrization of meso-cyclopentenoids [17] or the enzymatic resolutions of 4-*O*-protected derivatives [13,18]. However, these procedures requiring expensive (bio)-catalysts may not be considered as user-friendly, and are quite difficult to implement on a large scale to access these chiral building blocks in large quantities.

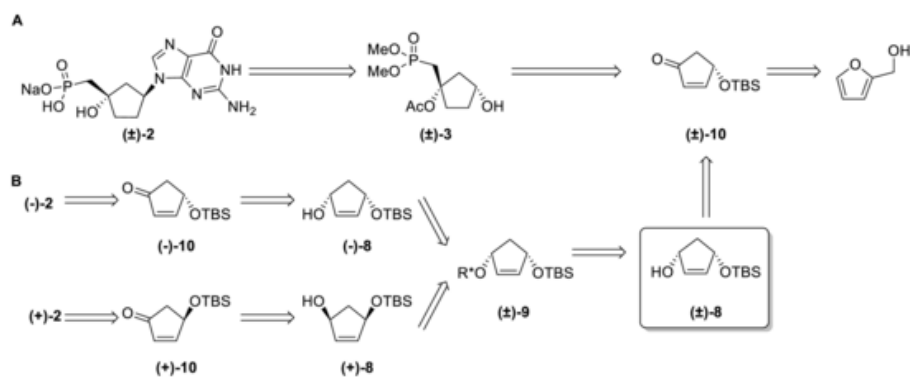
Thus, we set-up a convenient procedure based on the use of resolving agents and the corresponding racemic mixture of ( $\pm$ )-8, and then chromatographic separation of *cis*-(+)- and *cis*-(-)-4-*O*-protected-cyclopent-2-enols. Among the common resolving agents tested (see supporting information for details) [19], (*R*)-(-)-*O*-acetyl-mandelic acid

and DCC/DMAP mediated coupling (Steglich esterification) [7] were selected as efficient method for the introduction of the resolving agent and the corresponding diastereoisomeric mixture of 9 was isolated in 93% yield (Scheme 3).

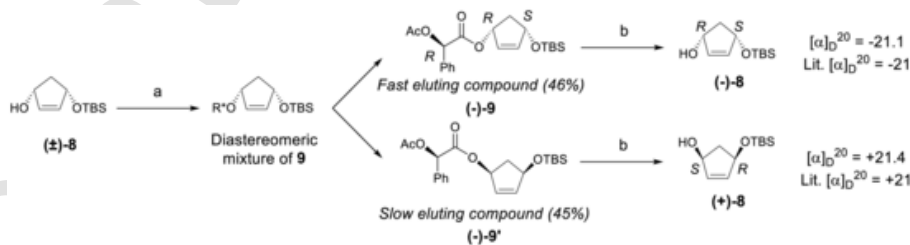
The best results for TLC separation were obtained with petroleum ether/dichloromethane and this eluent was used to determine the best parameters to perform the flash chromatography purification. Thus, the highest ratio of separation between the fast and the slow eluting diastereoisomers allowed to isolate 49% and 48% of both compounds (Scheme 3), respectively. In order to identify each diastereoisomers, removal of the resolving agent was accomplished with LiOH in aqueous THF (Scheme 3) and gave rise, after a rapid purification on silica gel, to the corresponding pure enantiomer of *cis*-4-*O*-TBS-cyclopent-2-enol (+)-8 and (-)-8, in high yield (95%). Finally, the specific rotatory power value of each enantiomer was determined and compared with the previously reported data from the literature for (-)-(1*R*,4*S*)-4-((tert-butyl)dimethylsilyl)oxycyclopent-2-enol [13] and (+)-(1*S*,4*R*)-4-((tert-butyl)dimethylsilyl)oxycyclopent-2-enol [20]. Thus, the fast eluting di-



**Scheme 1.** Synthetic route to carbocyclic methylphosphonate nucleoside analogues including nucleobase diversity. Reagents and conditions (a) 2,6-dichloropurine or N<sup>6</sup>-Benzyl-2-chloropurine or 2-amino-N<sup>6</sup>-Benzyl-purine or N<sup>2,6</sup>-BocBenzyl-purine or N<sup>6</sup>-Boc-2-fluoropurine or N<sup>2,6</sup>-diBoc-purine, PPh<sub>3</sub>, DIAD, THF, 0 °C to rt, 22–78%; (b) K<sub>2</sub>CO<sub>3</sub>, MeOH, rt, 24 h, 55–71%; (c) For **5a**, TMSBr, DMF, 0 °C, 1 h then rt, 24h, then Dowex Na<sup>+</sup>, 71%; For **5b-c** and **6d-f**, TMSCl, NaI, CH<sub>3</sub>CN, DMF, 40 °C, 1 h then rt, overnight, then Dowex Na<sup>+</sup>, 51–81%; (d) For **4e-f**, TFA, Cl(CH<sub>2</sub>)<sub>2</sub>Cl, rt, overnight, >98%.



**Scheme 2.** A) Main intermediates in the synthesis of (±)-2. B) Retro-synthetic approach envisaged to access enantiopure forms of compound 2.



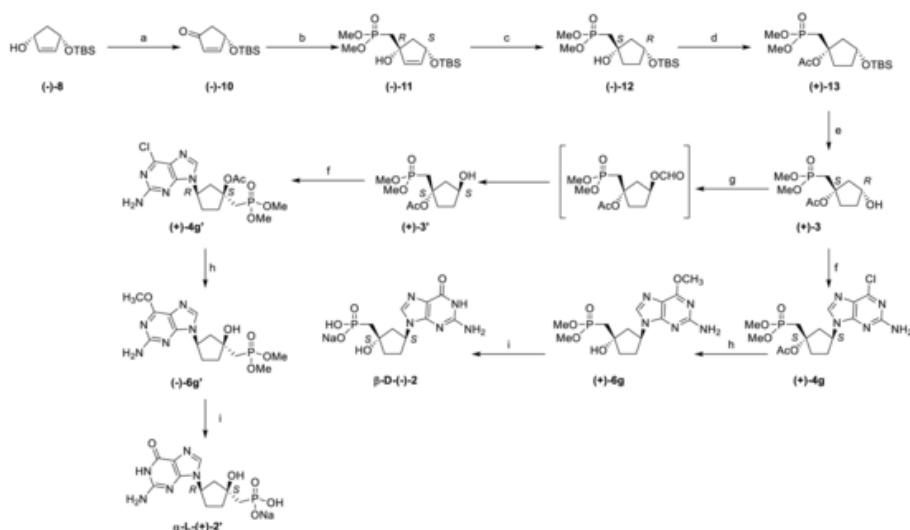
**Scheme 3.** Coupling reaction with resolving agents and identification of the pure enantiomers of *cis*-4-*O*-TBScyclopent-2-enol (+)-8 and (-)-8. Reagents and conditions: (a) (*R*)-(-)-*O*-Acetyl-mandelic acid, DCC, DMAP, DCM, rt, 2 h, 93%; (b) LiOH, THF, H<sub>2</sub>O, rt, 1 h, 95%.

astereoisomer led after deprotection to compound (-)-8 while the slow eluting one afforded after identical treatment to compound (+)-8.

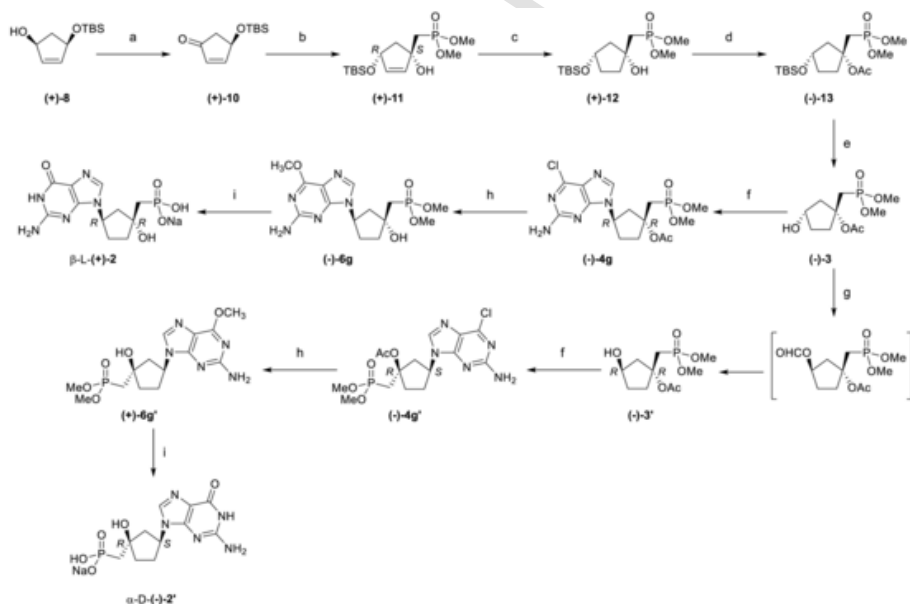
With these two enantiomers in hands, we engaged into the preparation of the four stereoisomers of compound 2 (Schemes 4 and 5). Briefly, Dess-Martin oxidation of (-)-8 led to the 4-*O*-TBS-2-cyclopentanone (-)-10, which was submitted to the addition of dimethyl methylphosphonate carbanion to isolate the desired compound (-)-11 (Scheme 4). As previously observed [9c], the nucleophilic attack occurred on the less-hindered face due to the presence of the silyl protecting group. Then, reduction of the double bond afforded the saturated derivative (-)-12, which was acetylated and subsequent removal

of the TBS group gave rise to the required carbocyclic precursor, namely, (1*S*,3*R*)-1-((dimethoxyphosphoryl)methyl)-3-hydroxycyclopentyl acetate (+)-3.

Intermediate (+)-3 may either be coupled to 2-amino-6-chloropurine, leading to the carbonucleotide (+)-4g or treated under Mitsunobu conditions to obtain the opposite stereochemistry of the carbon atom in position 3, namely, (1*S*,3*S*)-1-((dimethoxyphosphoryl)methyl)-3-hydroxycyclopentyl acetate (+)-3'. This latter was also coupled to 2-amino-6-chloropurine under Mitsunobu conditions leading to the diastereoisomer (+)-4g'. Finally, both (+)-4g and (+)-4g' were treated with K<sub>2</sub>CO<sub>3</sub> in methanol affording 2-amino-6-methoxypurine



**Scheme 4.** Synthetic route to  $\alpha$ -L-(+)-2' and  $\beta$ -D(-)-2 from starting material (-)-8 and common intermediate (+)-3. Reagents and conditions (a) Dess-Martin periodinane, DCM, rt, 2 h, 87%; (b)  $(\text{CH}_3\text{O})_2\text{P}(\text{O})\text{CH}_3$ , n-BuLi, THF,  $-78^\circ\text{C}$  to rt, 2 h, 85%; (c)  $\text{H}_2$ , Pd/C, MeOH, rt, 6 h, 97%; (d)  $\text{Ac}_2\text{O}$ ,  $\text{NEt}_3$ , DMAP, THF, rt, 6 days, 87%; (e)  $\text{NEt}_3\cdot 3\text{HF}$ , MeOH, rt, overnight, 96%; (f) 2-amino-6-chloro-purine,  $\text{PPh}_3$ , DIAD, THF,  $0^\circ\text{C}$  to rt, 2 h, 72–81%; (g)  $\text{PPh}_3$ , DIAD,  $\text{HCO}_2\text{H}$ , THF,  $0^\circ\text{C}$ , 1 h, then  $\text{NEt}_3$ , MeOH, rt, 4h, 74% over the two steps; (h)  $\text{K}_2\text{CO}_3$ , MeOH, rt, 24 h; 73–74%; (i) TMSBr, THF,  $0^\circ\text{C}$  to rt, then Dowex  $\text{Na}^+$  49–79%.



**Scheme 5.** Synthetic route to  $\alpha$ -D(-)-2' and  $\beta$ -L(+)-2 from starting material (+)-8 and common intermediate (-)-3. Reagents and conditions (a) Dess-Martin periodinane, DCM, rt, 2 h, 87%; (b)  $(\text{CH}_3\text{O})_2\text{P}(\text{O})\text{CH}_3$ , n-BuLi, THF,  $-78^\circ\text{C}$  to rt, 2 h, 85%; (c)  $\text{H}_2$ , Pd/C, MeOH, rt, 6 h, 97%; (d)  $\text{Ac}_2\text{O}$ ,  $\text{NEt}_3$ , DMAP, THF, rt, 6 days, 87%; (e)  $\text{NEt}_3\cdot 3\text{HF}$ , MeOH, rt, overnight, 96%; (f) 2-amino-6-chloro-purine,  $\text{PPh}_3$ , DIAD, THF,  $0^\circ\text{C}$  to rt, 2 h, 72–81%; (g)  $\text{PPh}_3$ , DIAD,  $\text{HCO}_2\text{H}$ , THF,  $0^\circ\text{C}$ , 1 h, then  $\text{NEt}_3$ , MeOH, rt, 4h, 74% over the two steps; (h)  $\text{K}_2\text{CO}_3$ , MeOH, rt, 24 h; 73–74%; (i) TMSBr, THF,  $0^\circ\text{C}$  to rt, then Dowex  $\text{Na}^+$ , 49–79%.

derivatives, respectively (-)-6g' and (+)-6g and then in the presence of TMSBr in THF to remove the dimethylphosphonoester protecting groups. The first pair of diastereoisomers (+)-2' and (-)-2 were isolated as sodium salts after RP-chromatography and percolation onto ion-exchange resin (Dowex  $\text{Na}^+$  form).

Thus, considering that endogenous nucleosides are defined as D-enantiomer with  $\beta$ -configuration, the levogyre carbonucleoside phosphonate (-)-2 (Scheme 4) may be viewed as a natural enantiomer and named  $\beta$ -D(-)-2, whereas the dextrogyre enantiomer (+)-2' may be viewed as the non-natural L-enantiomer with  $\alpha$ -configuration and therefore named as  $\alpha$ -L(+)-2'.

A similar sequence of reactions was repeated using 4-O-TBS-cyclopent-2-enol (+)-8 as starting material (Scheme 5) and affording

the common intermediate (-)-3 in ~60% yield over five steps. This last was then converted into the carbonucleotide derivatives (-)-4g and (-)-4g' by using Mitsunobu procedures and allowed to obtain the second pair of enantiomers (+)-2 and (-)-2', as their sodium salts, after removal of the various protecting groups. As previously discussed, the levogyre carbonucleoside phosphonate (-)-2' (Scheme 5) may be viewed as a natural D-enantiomer but with  $\alpha$ -configuration, leading to  $\alpha$ -D(-)-2'. Whereas the dextrogyre enantiomer (+)-2 may be viewed as the non-natural L-enantiomer with  $\beta$ -configuration and therefore named as  $\beta$ -L(+)-2.

All final compounds were fully characterized by NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{31}\text{P}$ ), MS and were subjected to biological evaluation in order to determine their ability to inhibit the growth of *P. falciparum* in RBC.



#### 2.4. *In vitro* antimalarial activity

*In vitro* antimalarial activities of compounds ( $\pm$ )-1 and ( $\pm$ )-2, of a selection of structurally related analogues (Fig. 2) from our in-house library, and of the newly synthesized compounds were determined in *P. falciparum* (3D7 strain) infected red-blood cells (RBCs) (Table 1). Among the 15 compounds challenged, 6 compounds (UA2299, UA2300, compounds ( $\pm$ )-1 and ( $\pm$ )-2, ( $\pm$ )-7e and ( $\pm$ )-7f) exhibited  $IC_{50}$  in the 1–10  $\mu$ M range. These all include a 2,6-disubstituted purine as nucleobase and the carbocyclic methylphosphonate nucleoside analogues. Compounds ( $\pm$ )-1, ( $\pm$ )-2 and ( $\pm$ )-7e exhibited the most potent antimalarial activity. These results are particularly encouraging considering the anionic nature of these nucleotide analogues, which are known to lack activity in cell culture experiments due to their limited ability to cross cell membranes.

One should note that compounds ( $\pm$ )-7a-f did not show any interaction with *Pf*ISN1 when tested in TSA, whereas when performing the docking studies, these derivatives displayed  $\Delta G$  values between  $-8.1$  and  $-10.5$  kcal mol $^{-1}$ . Compounds ( $\pm$ )-7b, ( $\pm$ )-7c and ( $\pm$ )-7d showing highest binding potential with  $\Delta G$ 's of  $-10.5$ ,  $9.6$  and  $9.5$  kcal mol $^{-1}$ , respectively (Table S2).

Then, the newly obtained 4 diastereomers of compound ( $\pm$ )-2 were evaluated (Table 2). Among the pairs of D- and L-like carbonucleotide derivatives, the L-like enantiomer with  $\beta$ -configuration ( $\beta$ -L-(+)-2) exhibited the highest anti-plasmodial activity, with an  $IC_{50}$  value of  $1.3$   $\mu$ M, whereas the  $\beta$ -D- and  $\alpha$ -D-like enantiomers are modest inhibitors of the *Pf* growth and the  $\alpha$ -L-like enantiomer is totally devoid of activity.

The enantiomeric pure forms listed in Table 2 were tested by docking studies as well. Nevertheless, the results from the docking (Table S3) were not in agreement with the *in vitro* studies. Despite the fact that compound (+)-2 was shown to display the highest anti-plasmodial activity *in vitro*, docking studies of the enantiomeric pure forms indicated that (–)-2 was the strongest interacting ligand.

Even so, the different behavior of D- and L-like antiviral nucleoside analogues is known in the literature [21,22], it has been less often reported for nucleotide derivatives [12a]. To our knowledge, it is the first time that a significant antiplasmodial activity is observed for a carbocyclic methylphosphonate nucleoside with an L-like configuration.

### 3. Conclusion

We have synthesized new carbocyclic methylphosphonate nucleoside analogues bearing 2,6-disubstituted purines as nucleobase using ( $\pm$ )-1-((dimethoxyphosphoryl)methyl)-3-hydroxycyclopentyl acetate (3) as starting material. On the basis of the results of the biochemical assays, compound ( $\pm$ )-2 was selected and we have developed an efficient and straightforward procedure for the chemical desymmetrization of *cis*-( $\pm$ )-4-*O*-TBS-cyclopent-2-enol (( $\pm$ )-8) in order to access the corresponding four enantiomerically pure stereoisomers. Using (*R*)-(–)-*O*-acetyl mandelic acid as resolving agent, we afforded in two steps from compound ( $\pm$ )-8 the corresponding *cis*-(–) and *cis*-(+) enantiomers, in 43% and 42% overall yield, respectively. Our methodology has been readily used to access compounds of interest such as the D- and L-like enantiomers of carbocyclic methylphosphonate nucleoside ( $\pm$ )-2.

Among the 19 compounds evaluated for their antimalarial activity, the derivatives incorporating the guanine or the 6-amino, 2-methoxypurine as nucleobases exhibited significant inhibition of the parasite growth in the low micromolar range.

Comparison of the results from the *in silico* docking studies (Tables S1 and S2) and TSA studies with *in vitro* anti-plasmodial activity indicated a divergence of the results. Indeed, when testing the compounds for their effect on the activity of purified recombinant *Pf*ISN1 (10 mM IMP and 2 mM compound at pH 8.0 (in duplicate), data not shown), the studied derivatives seemed to have neither inhibitory nor activating ef-

**Table 1**  
Inhibition of *P. falciparum* growth by racemic mixture of purine containing carbonucleotides.

Compound	Structure	$IC_{50}$ ( $\mu$ M) <sup>a</sup>
( $\pm$ )-UA2297		$\gg 100$
( $\pm$ )-UA2298		$50.0 \pm 2.0$
( $\pm$ )-UA2299		$7.0 \pm 1.0$
( $\pm$ )-UA2300		$10.2 \pm 2.0$
( $\pm$ )-UA2301		$\gg 100$
( $\pm$ )-UA2327		$\gg 100$
( $\pm$ )-1		$1.0 \pm 0.3$
( $\pm$ )-2		$4.4 \pm 1.1$
UA2296		$14.7 \pm 2.0$
( $\pm$ )-7a		$\gg 100$
( $\pm$ )-7f		$8.8 \pm 1.6$
( $\pm$ )-7e		$2.2 \pm 0.1$
( $\pm$ )-7b		$68.3 \pm 10.9$

(continued on next page)

Table 1 (continued)

Compound	Structure	IC <sub>50</sub> (μM) <sup>a</sup>
(±)-7c		79.0 ± 11.0
(±)-7d		41.0 ± 5.5
CQ		0.020 ± 0.003

<sup>a</sup> IC<sub>50</sub> values (concentration inhibiting 50% of the parasite growth) are the mean of at least three independent experiments performed in duplicate (± SEM). Chloroquine (CQ) is used as reference antimalarial compound.

Table 2

Inhibition of *P. falciparum* growth by compound 2 and its enantiomeric pure forms.

Compound	Structure	IC <sub>50</sub> (μM) <sup>a</sup>
(±)-2		4.4 ± 1.1
β-D(-)-2		44 ± 7.4
β-L(+)-2		1.3 ± 0.2
α-D(-)-2'		42 ± 11.6
α-L(+)-2'		> 100
CQ		0.020 ± 0.003

<sup>a</sup> IC<sub>50</sub> values (concentration inhibiting 50% of the parasite growth) are the mean of at least three independent experiments performed in duplicate (± SEM). Chloroquine (CQ) is used as reference antimalarial compound.

fect on the enzyme. Thus, we speculate that the effect of the compounds on the parasite in culture may be associated with their interference with some of the other enzymes involved in the purine salvage/AMP, GMP synthesis.

Observing the antimalarial activity of nucleoside phosphonate analogues usually requires their conversion into the corresponding prodrugs in order to temporarily mask the negative charge and allow cell penetration, therefore our results are particularly remarkable. In addition, this is the first report on a carbocyclic methylphosphonate nucleoside with an L-like configuration. Further modifications of this new chemotype represents a promising strategy for developing more powerful derivatives.

## 4. Experimental section

### 4.1. Virtual screening of molecules

Prior to the computer-aided screening, each entry of the chemo library, encoded as SMILES strings corresponding to the enantiomeric pure form, was converted to 3D structures in the MOL2 format using the

Open Babel software (<http://openbabel.org>), and thereafter in the PDBQT format with the AutoDockTools 1.5.6 package (<https://autodocksuite.scripps.edu/adt>), leading to a virtual library of 197 distinct small-molecule compounds. The crystal structures [7] of native *Pf*ISN1 (PDB entry 6RMO), IMP bound inactive *Pf*ISN1 (PDB entry 6RME) and ATP bound *Pf*ISN1 (PDB entry 6RMD) were prepared using AutoDockTools: polar hydrogen atoms were added, non-polar hydrogens were merged with these latter, and the Gasteiger partial atomic charges were calculated. The docking experiments were then carried out with the program AutoDock Vina v1.1.2 [23]. In all calculations, the small-molecule compounds were treated as being flexible whereas the target protein was treated as being rigid. Due to the large size of the physiological tetramer, not compatible with a docking calculation, the search space was defined in order to encompass only one monomer (chain I of PDB 6RMO, chain D of PDB 6RMD and chain A of 6RME), and an exhaustiveness value of 500 was used considering the wide search area (68 × 68 × 58 Å<sup>3</sup>). Finally, the resulting docking poses were sorted according to their predicted binding energy (ΔG). A visual examination of the best poses was performed using PyMOL (Schrödinger, LLC - <https://pymol.org>).

### 4.2. Protein production and purification of *Pf*ISN1

The expression and purification of recombinant *Pf*ISN1 was performed as described earlier [8]. Briefly, the plasmid carrying the different constructs of *Pf*ISN1 gene was transformed into *E. coli* BL21-CodonPlus (DE3)-RIL cells. A single colony was inoculated into 10 mL of Luria Broth containing 50 μg/mL of ampicillin and grown overnight at 37 °C. The overnight culture was added to 2 L of LB with antibiotics and grown at 37 °C until the OD600 reached ~0.6, hereafter induced with 0.3 mM IPTG (isopropyl-β-D-thiogalactopyranoside) and grown for further 18 h at 17 °C. The cells were pelleted by centrifugation at 6000g, 10 min at 4 °C and stored at -80 °C.

The pellet was resuspended in lysis buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl - buffer A) and disrupted using a microfluidizer (3 cycles at 15 000 psi). The lysate was centrifuged at 14 000 g, 30 min at 8 °C and the supernatant was filtered using a 0.45 μm cutoff filter before injection onto a 5 mL HisTrap FF crude using an ÄKTA purifier (GE Healthcare). The column was washed with Buffer A containing 1 M NaCl followed by an elution in buffer A containing 500 mM imidazole. After 12% SDS-PAGE (SDS-polyacrylamide gel electrophoresis) analysis, fractions with relatively pure protein were pooled and concentrated (30 kDa cut-off Amicon Ultra-15centrifugal filter units, EMD Millipore). Further purification by size-exclusion chromatography using a Superdex 200 10/300 GL column (GE Healthcare) was done in buffer A for preparation of samples at physiological pH and in 50 mM MES pH 5.0, 100 mM NaCl for samples at acidic pH. Aliquots of the purified protein were flash frozen and stored at -80 °C.

### 4.3. Fluorescence-based screening

A real-time PCR device (StepOnePlus™ Real-Time PCR System, Applied Biosystems) was used to monitor protein unfolding by the increase in fluorescence of the fluorophore SYPRO™ Orange (ThermoFisher). Protein samples (1.5 μM) in 50 mM Na<sub>3</sub>PO<sub>4</sub> pH 8.0, 100 mM NaCl and each of the ligands in a reaction volume of 25 μL were incubated in 96-well PCR microplates (MicroAmp™ Fast Optical 96-Well Reaction Plate, ThermoFisher) sealed with an optical foil (QPCR MicroAmp™ optical adhesive film, ThermoFisher). Studied compounds were dissolved in water (as for compounds 1 and 2) or in DMSO with their concentrations varying from 10 μM to 1 mM. After 5 min of incubation in the RT-PCR device at 25 °C, the samples were heated 0.9 °C per min from 25 °C to 95 °C. The fluorescence intensity was measured every 1–3 °C, and plotted as a function of temperature by using the StepOnePlus Real-Time PCR System software (Applied Biosystems).

First derivative curves allowing the determination of melting temperatures,  $T_m$ , were calculated using the same software.

#### 4.4. Microscale Thermophoresis

PfISN1 was labeled using the Monolith NT™ Protein Labeling Kit RED-NHS (NanoTemper Technologies GmbH, Germany) according to the supplier's recommendations. Measurements were carried out by using the Monolith NT.115 (NanoTemper Technologies GmbH, Germany) in an assay buffer consisting of 50 mM PBS pH 8.0, 100 mM NaCl and Monolith NT.115 premium capillaries. Labeled PfISN1 was kept at a constant concentration of 20 nM, whereas the unlabeled binding ligands were titrated in 1:1 dilutions, to a highest concentration of 0.1 mM. The Monolith Control software (v1.6.1) was employed for analysis of all MST data.

#### 4.5. In vitro antimalarial activity

Drug effects on *in vitro* *P. falciparum* growth (3D7 strain) were measured in microtiter plates according to a modified Desjardins test [24]. *P. falciparum* infected RBCs suspension (1.5% final hematocrit and 0.6% parasitemia) were grown in complete medium (HEPES-buffered RPMI 1640 + 10% AB human serum) with or without drug. The compounds were dissolved in RPMI 1640 or DMSO and then further diluted in culture medium. Parasites growth was assessed by measuring the incorporation of [<sup>3</sup>H] hypoxanthine into nucleic acids as previously described [24b]. Suspensions of *P. falciparum* -infected RBCs were incubated with various concentrations of analogues during 48 h. Then 30 µL of [<sup>3</sup>H] hypoxanthine (0.5 µCi/well) was added for an additional 24h-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 Microbeta2 (PerkinElmer). Radioactivity background was obtained from incubation of non-infected RBCs under the same conditions and the value obtained was deduced. Results were expressed as IC<sub>50</sub>, which is the drug concentration leading to 50% inhibition of parasite growth.

#### 4.6. Chemistry

<sup>1</sup>H NMR, <sup>13</sup>C NMR and <sup>31</sup>P spectra were recorded with proton decoupling at ambient temperature on the following spectrometers: Bruker Avance III (600, 500 or 400 MHz). Chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak: CDCl<sub>3</sub> at 7.26 ppm and 77.16 ppm, CD<sub>3</sub>OD at 3.41 ppm and 49.00 ppm, and D<sub>2</sub>O at 4.63 ppm relative to TMS. For <sup>31</sup>P NMR spectra, chemical shifts are reported relative to external H<sub>3</sub>PO<sub>4</sub>. Multiplicity of signals are indicated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). MS and HR-MS were recorded in the positive or negative mode on a Waters Synapt G2S spectrometer equipped with electrospray source ionization (ESI). Thin layer chromatography was performed on pre-coated aluminum sheets of Silica Gel 60 F<sub>254</sub> (Merck), and visualization of products was accomplished by UV absorbance and ethanolic acidic *p*-anisaldehyde solution or 5% ethanolic sulfuric acid solution, followed by heating. Purification by column chromatography was performed with silica gel 60 (230–400 mesh) or with reversed phase silica gel (RP-18, 25–40 µm). Ion exchange chromatography was performed on DOWEX 50WX8-200 resins (Na<sup>+</sup> form). All moisture sensitive reactions were carried out in anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use. Solids were dried over P<sub>2</sub>O<sub>5</sub> under reduced pressure at room temperature. Commercially available reagents were used as purchased without further purification.

##### 4.6.1. Protocol A (Mitsunobu conditions for Nucleobase coupling)

To a solution of triphenylphosphine (2.0 eq) and heterocyclic base (2.0 eq) in tetrahydrofuran (THF) (10 mL/mmol) at 0 °C was added dropwise diisopropyl azodicarboxylate (DIAD) (2.0 eq). The reaction mixture was stirred at rt for 1 h. Then a solution of alcohol (1.0 eq) in THF was added to the reaction mixture. The reaction was stirred at rt for 1 h before filtration and evaporation of the solvent. Purification by flash silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 1:0 to 97:3) gave rise to the desired product as white foam.

4.6.1.1. (±)-1-((Dimethoxyphosphoryl)methyl)-3-(2,6-dichloro-9H-purin-9-yl)cyclopentyl acetate (±)-4a. The product was obtained (1.15 g, 70%) according to the Protocol A using compound (±)-3 (1 g).

R<sub>f</sub> (DCM/MeOH: 95/5) = 0.42. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.23 (s, 1H, H<sub>8</sub>), 5.17–5.09 (m, 1H, H<sub>1</sub>), 3.76 (d, *J* = 11.0 Hz, OCH<sub>3</sub>), 3.75 (d, *J* = 11.0 Hz, OCH<sub>3</sub>), 3.03 (ddd, *J* = 14.5, 8.1, 1.4 Hz, 1H, H<sub>5a</sub>), 2.91 (dd, *J* = 19.0, 15.5 Hz, 1H, H<sub>6a</sub>), 2.69 (dd, *J* = 19.8, 15.5 Hz, 1H, H<sub>6b</sub>), 2.57–2.45 (m, 4H, H<sub>2a</sub> + H<sub>3</sub> + H<sub>5b</sub>), 2.22–2.14 (m, 1H, H<sub>2b</sub>), 2.08 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 170.8 (C=O), 152.8 (C<sub>4</sub>), 152.6 (C<sub>9</sub>), 151.9 (C<sub>8</sub>), 144.7 (C<sub>6</sub>), 131.3 (C<sub>5</sub>), 86.1 (d, *J* = 3.7 Hz, C<sub>1</sub>), 55.2 (C<sub>1</sub>), 52.5 (d, *J* = 7.0 Hz, 2xOCH<sub>3</sub>), 44.0 (d, *J* = 5.5 Hz, C<sub>5</sub>), 36.9 (d, *J* = 8.7 Hz, C<sub>3</sub>), 31.8 (d, *J* = 139.4 Hz, C<sub>6</sub>), 30.6 (C<sub>2</sub>), 22.1 (CH<sub>3</sub>). <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ 27.9. MS (ESI<sup>+</sup>): *m/z* = 437.0 [M+H]<sup>+</sup> HRMS (ESI<sup>+</sup>) calculated for C<sub>15</sub>H<sub>20</sub>N<sub>4</sub>O<sub>5</sub>Cl<sub>2</sub>P [M+H]<sup>+</sup>: 437.0550; found: 437.0548.

4.6.1.2. (±)-1-((Dimethoxyphosphoryl)methyl)-3-(2-dichloro-6-aminobenzyl-9H-purin-9-yl)cyclopentyl acetate (±)-4b. The product was obtained (230 mg, 30%) according to the Protocol A using compound (±)-3 (400 mg).

R<sub>f</sub> (DCM/MeOH: 96/4) = 0.31. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.81 (s, 1H, H<sub>8</sub>), 7.39–7.27 (m, 5H, H<sub>Ar</sub>), 6.56 (s, 1H, NH), 5.08–5.00 (m, 1H, H<sub>1</sub>), 4.81 (s, 2H, CH<sub>2</sub>), 3.76 (d, *J* = 11.0 Hz, OCH<sub>3</sub>), 3.75 (d, *J* = 11.0 Hz, OCH<sub>3</sub>), 2.98 (dd, *J* = 14.8, 8.3 Hz, 1H, H<sub>5a</sub>), 2.91 (dd, *J* = 18.9, 15.5 Hz, 1H, H<sub>6a</sub>), 2.72 (dd, *J* = 19.7, 15.5 Hz, 1H, H<sub>6b</sub>), 2.54–2.37 (m, 4H, H<sub>2a</sub> + H<sub>3</sub> + H<sub>5b</sub>), 2.17–2.08 (m, 1H, H<sub>2b</sub>), 2.07 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 171.0 (C=O), 138.8 (C<sub>8</sub>), 128.9 (2xCH), 128.8 (C<sub>9</sub>), 128.1 (CH), 127.8 (2xCH), 86.3 (d, *J* = 3.8 Hz, C<sub>1</sub>), 54.5 (C<sub>1</sub>), 52.6 (d, *J* = 6.7 Hz, 2xOCH<sub>3</sub>), 44.9 (CH<sub>2</sub>), 44.5 (d, *J* = 5.8 Hz, C<sub>5</sub>), 37.2 (d, *J* = 8.4 Hz, C<sub>3</sub>), 32.0 (d, *J* = 139.4 Hz, C<sub>6</sub>), 31.1 (C<sub>2</sub>), 22.3 (CH<sub>3</sub>). <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ 28.2. MS (ESI<sup>+</sup>): *m/z* = 508.1 [M+H]<sup>+</sup> HRMS (ESI<sup>+</sup>) calculated for C<sub>22</sub>H<sub>28</sub>N<sub>5</sub>O<sub>5</sub>Cl<sub>2</sub>P [M+H]<sup>+</sup>: 508.1519; found: 508.1517.

4.6.1.3. (±)-1-((Dimethoxyphosphoryl)methyl)-3-(2-amino-6-aminobenzyl-9H-purin-9-yl)cyclopentyl acetate (±)-4c. The product was obtained (200 mg, 22%) according to the Protocol A using compound (±)-3 (500 mg).

R<sub>f</sub> (DCM/MeOH: 96/4) = 0.25. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.49 (s, 1H, H<sub>8</sub>), 7.37–7.24 (m, 5H, H<sub>Ar</sub>), 6.16 (s, 1H, NH), 4.90–4.80 (m, 5H, NH<sub>2</sub> + CH<sub>2</sub> + H<sub>1</sub>), 3.74 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 3.74 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 2.93–2.79 (m, 3H, H<sub>6</sub> + H<sub>5a</sub>), 2.50–2.43 (m, 3H, H<sub>3</sub> + H<sub>5b</sub>), 2.39–2.31 (m, 1H, H<sub>2a</sub>), 2.16–2.10 (m, 1H, H<sub>2b</sub>), 2.07 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 171.0 (C=O), 138.8 (C<sub>9</sub>), 136.4 (C<sub>8</sub>), 132.9 (C<sub>4</sub>), 128.8 (2xCH), 128.0 (2xCH), 127.5 (CH), 86.4 (d, *J* = 4.9 Hz, C<sub>1</sub>), 54.0 (C<sub>1</sub>), 52.6 (d, *J* = 6.7 Hz, 2xOCH<sub>3</sub>), 44.5 (CH<sub>2</sub>), 44.0 (d, *J* = 6.1 Hz, C<sub>5</sub>), 37.4 (d, *J* = 9.0 Hz, C<sub>3</sub>), 32.1 (d, *J* = 139.2 Hz, C<sub>6</sub>), 30.6 (C<sub>2</sub>), 22.3 (CH<sub>3</sub>). <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ 28.7. MS (ESI<sup>+</sup>): *m/z* = 489.2 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>22</sub>H<sub>30</sub>N<sub>6</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 489.2008; found: 489.2015.

4.6.1.4. (±)-1-((Dimethoxyphosphoryl)methyl)-3-(6-(benzyl(tert-butoxycarbonyl)amino)-2-((tert-butoxy-carbonyl)(benzyl)amino)-9H-purin-9-yl)cyclopentyl acetate (±)-4d. The product was obtained

(593 mg, 83%) according to the Protocol A using compound ( $\pm$ )-3 (480 mg).

$R_f$  (DCM/MeOH: 97/3) = 0.48.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.94 (s, 1H,  $\text{H}_8$ ), 7.34–7.30 (m, 2H,  $\text{H}_{11}$ ), 7.25–7.14 (m, 8H,  $\text{H}_{11}$ ), 5.04 (s, 2H,  $\text{CH}_2$ ), 5.04 (m, 1H,  $\text{H}_1$ ), 3.72 (d,  $J = 11.0$  Hz,  $\text{OCH}_3$ ), 3.72 (d,  $J = 10.9$  Hz,  $\text{OCH}_3$ ), 2.88 (ddd,  $J = 14.3, 8.0, 1.9$  Hz, 1H,  $\text{H}_{5a}$ ), 2.80 (dd,  $J = 18.7, 15.6$  Hz, 1H,  $\text{H}_{6a}$ ), 2.51 (dd,  $J = 19.6, 15.7$  Hz, 1H,  $\text{H}_{6b}$ ), 2.49–2.45 (m, 1H,  $\text{H}_{3a}$ ), 2.41 (dd,  $J = 14.2, 9.6$  Hz, 1H,  $\text{H}_{5b}$ ), 1.36–2.21 (m, 2H,  $\text{H}_{2a} + \text{H}_{2b}$ ), 2.18–2.07 (m, 1H,  $\text{H}_{2b}$ ), 2.05 (s, 3H,  $\text{CH}_3$ ), 1.42 (s, 9H,  $3 \times \text{CH}_3$ ), 1.39 (s, 9H,  $3 \times \text{CH}_3$ ).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  171.0 (C=O), 154.5 (C), 153.5 (C), 153.4 (C), 153.4 (C), 153.1 (C), 142.4 (C), 142.0 (C), 138.5 (C), 128.9 (2xCH), 128.2 (2xCH), 128.0 (2xCH), 127.8 (2xCH), 126.9 (CH), 126.6 (CH), 125.0 (C), 86.9 (d,  $J = 6.1$  Hz,  $\text{C}_4$ ), 82.3 (C), 81.7 (C), 55.2 (C), 52.5 (d,  $J = 4.3$  Hz,  $\text{OCH}_3$ ), 52.5 (d,  $J = 4.2$  Hz,  $\text{OCH}_3$ ), 51.0 ( $\text{CH}_2$ ), 43.8 (d,  $J = 6.4$  Hz,  $\text{C}_5$ ), 37.1 (d,  $J = 9.9$  Hz,  $\text{C}_3$ ), 31.6 (d,  $J = 139.9$  Hz,  $\text{C}_6$ ), 30.2 (C), 28.2 ( $3 \times \text{CH}_3$ ), 28.0 ( $3 \times \text{CH}_3$ ), 22.3 ( $\text{CH}_3$ ).  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  28.6. MS (ESI<sup>+</sup>):  $m/z = 765.3$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{38}\text{H}_{50}\text{N}_6\text{O}_9\text{P}$  [M+H]<sup>+</sup>: 765.3358; found: 765.3377.

4.6.1.5. ( $\pm$ )-1-((Dimethoxyphosphoryl)methyl)-3-(6-(di-tert-butoxycarbonyl)amino-2-fluoro-9H-purin-9-yl) cyclopentyl acetate ( $\pm$ )-4e. The product was obtained (350 mg, 78%) according to the Protocol A using compound ( $\pm$ )-3 (200 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.4.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.11 (s, 1H,  $\text{H}_8$ ), 5.09–5.02 (m, 1H,  $\text{H}_1$ ), 3.75 (d,  $J = 10.9$  Hz, 3H,  $\text{OCH}_3$ ), 3.74 (d,  $J = 11.1$  Hz, 3H,  $\text{OCH}_3$ ), 3.00 (ddd,  $J = 14.7, 7.9, 1.9$  Hz, 1H,  $\text{H}_{5a}$ ), 2.91 (dd,  $J = 19.0, 15.6$  Hz, 1H,  $\text{H}_{6a}$ ), 2.68 (dd,  $J = 19.8, 15.5$  Hz, 1H,  $\text{H}_{6b}$ ), 2.58–2.42 (m, 4H,  $\text{H}_{2a} + \text{H}_{2b} + \text{H}_{2c}$ ), 2.22–2.16 (m, 1H,  $\text{H}_{2b}$ ), 2.08 (s, 3H,  $\text{CH}_3$ ), 1.47 (s, 18H,  $3 \times \text{CH}_3$ ).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9 (C=O), 157.6 (d,  $j = 216.2$  Hz,  $\text{C}_2$ ), 154.8 (d,  $j = 17.2$  Hz,  $\text{C}_1$ ), 152.2 (d,  $j = 17.1$  Hz,  $\text{C}_1$ ), 150.2 (2x $\text{C}^{\text{g-Boc}}$ ), 144.2 (d,  $J = 3.0$  Hz,  $\text{C}_8$ ), 127.6 (d,  $J = 4.9$  Hz,  $\text{C}_5$ ), 86.2 (d,  $J = 4.1$  Hz,  $\text{C}_4$ ), 84.5 (2xOC( $\text{CH}_3$ )), 50.1 (C), 52.6 (d,  $J = 6.4$  Hz, 2xOCH<sub>3</sub>), 43.9 (d,  $J = 5.8$  Hz,  $\text{C}_5$ ), 37.0 (d,  $J = 9.0$  Hz,  $\text{C}_3$ ), 31.9 (d,  $J = 139.5$  Hz,  $\text{C}_6$ ), 30.3 (C), 27.9 ( $3 \times \text{CH}_3$ ), 22.3 ( $\text{CH}_3$ ).  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  27.9. MS (ESI<sup>+</sup>):  $m/z = 602.2$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{25}\text{H}_{38}\text{N}_6\text{O}_9\text{FP}$  [M+H]<sup>+</sup>: 602.2389; found: 602.2391.

4.6.1.6. ( $\pm$ )-1-((Dimethoxyphosphoryl)methyl)-3-(2,6-(tetra-tert-butoxycarbonyl)diamino-9H-purin-9-yl) cyclopentyl acetate ( $\pm$ )-4f. The product was obtained (1.1 g, 74%) according to the Protocol A using compound ( $\pm$ )-3 (500 mg).

$R_f$  (DCM/MeOH: 96/4) = 0.54.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.15 (s, 1H,  $\text{H}_8$ ), 5.12–5.09 (m, 1H,  $\text{H}_1$ ), 3.74 (d,  $J = 10.9$  Hz, 6H, 2xOCH<sub>3</sub>), 3.03 (dd,  $J = 14.2, 7.7$  Hz, 1H,  $\text{H}_{5a}$ ), 2.88 (dd,  $J = 19.0, 15.5$  Hz, 1H,  $\text{H}_{6a}$ ), 2.68 (dd,  $J = 19.6, 15.6$  Hz, 1H,  $\text{H}_{6b}$ ), 2.58–2.42 (m, 4H,  $\text{H}_{2a} + \text{H}_{2b} + \text{H}_{2c}$ ), 2.22–2.16 (m, 1H,  $\text{H}_{2b}$ ), 2.07 (s, 3H,  $\text{CH}_3$ ), 1.43 (s, 18H,  $3 \times \text{CH}_3$ ), 1.42 (s, 18H,  $3 \times \text{CH}_3$ ).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  170.9 (C=O), 153.9 (C), 151.8 (C), 151.1 (C), 150.9 (C), 150.3 (C), 144.4 (C), 127.9 (C), 86.4 (d,  $J = 4.5$  Hz,  $\text{C}_4$ ), 83.9 (2xOC( $\text{CH}_3$ )), 83.3 (2xOC( $\text{CH}_3$ )), 50.0 (C), 52.6 (d,  $J = 6.7$  Hz, 2xOCH<sub>3</sub>), 44.1 (d,  $J = 6.2$  Hz,  $\text{C}_5$ ), 37.0 (d,  $J = 8.8$  Hz,  $\text{C}_3$ ), 31.9 (d,  $J = 139.8$  Hz,  $\text{C}_6$ ), 30.5 (C), 28.0 ( $3 \times \text{CH}_3$ ), 27.9 ( $3 \times \text{CH}_3$ ), 22.2 ( $\text{CH}_3$ ).  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  28.0. MS (ESI<sup>+</sup>):  $m/z = 799.3$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{35}\text{H}_{56}\text{N}_6\text{O}_{13}\text{P}$  [M+H]<sup>+</sup>: 799.3644; found: 799.3637.

4.6.1.7. (1S,3S)-3-(2-Amino-6-chloro-9H-purin-9-yl)-1-((dimethoxyphosphoryl)methyl)cyclopentyl acetate (+)-4g. The product was obtained (631 mg, 81%) according to the Protocol A using compound (1S,3R)-(+)-3 (500 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.32.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.89 (s, 1H), 4.92 (dq,  $J = 9.3, 8.3$  Hz, 1H,  $\text{H}_1$ ), 3.75 (d,  $J = 11.0$  Hz, 3H, OCH<sub>3</sub>), 3.74 (d,  $J = 11.0$  Hz, 3H, OCH<sub>3</sub>), 2.94–2.87 (m, 2H, CHP + H), 2.81 (dd,  $J = 20.1, 15.5$  Hz, 1H, CHP), 2.55 (dd,

$J = 14.4, 9.5$  Hz, 1H), 2.49 (t,  $J = 7.6$  Hz, 2H), 2.42–2.35 (m, 1H), 2.16 (dq,  $J = 13.4, 7.7$  Hz, 1H), 2.07 (s, 3H, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (126 MHz,  $\text{CDCl}_3$ )  $\delta$  171.0 (C=O), 158.8 (C), 153.4 (C), 151.5 (C), 141.6 (C), 125.7 (C), 86.3 (d,  $J = 4.5$  Hz,  $\text{C}_4$ ), 54.7 (C), 52.6 (t,  $J = 5.7$  Hz, 2OCH<sub>3</sub>), 43.4 (d,  $J = 5.4$  Hz,  $\text{C}_5$ ), 37.3 (d,  $J = 9.1$  Hz,  $\text{C}_3$ ), 31.9 (d,  $J = 139.8$  Hz,  $\text{C}_6$ ), 30.3 (C), 22.3 (CH<sub>3</sub>).  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ )  $\delta$  28.5.  $[\alpha]_D^{20} = +31.1$  (c = 1.00,  $\text{CHCl}_3$ ). MS (ESI<sup>+</sup>):  $m/z = 418.1$  (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for  $\text{C}_{15}\text{H}_{22}\text{ClN}_5\text{O}_5\text{P}$  [M+H]<sup>+</sup>: 418.1042; Found: 418.1060.

4.6.1.8. (1S,3R)-3-(2-Amino-6-chloro-9H-purin-9-yl)-1-((dimethoxyphosphoryl)methyl)cyclopentyl acetate (+)-4g'. The product was obtained (434 mg, 72%) according to the Protocol A using compound (1S,3S)-(+)-3' (370 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.34.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  7.95 (s, 1H,  $\text{H}_8$ ), 5.01–4.96 (m, 1H,  $\text{H}_1$ ), 3.75 (d,  $J = 11.1$  Hz, 3H, OCH<sub>3</sub>), 3.75 (d,  $J = 11.1$  Hz, 3H, OCH<sub>3</sub>), 2.80 (dd,  $J = 19.2, 15.5$  Hz, 1H, CHP), 2.75–2.67 (m, 2H,  $\text{H}_{3a} + \text{H}_{5a}$ ), 2.64 (ddd,  $J = 15.5, 5.7, 2.0$  Hz, 1H,  $\text{H}_{5b}$ ), 2.58 (dd,  $J = 19.7, 15.6$  Hz, 1H, CHP), 2.42 (dtd,  $J = 13.2, 7.8, 2.9$  Hz, 1H,  $\text{H}_{2a}$ ), 2.19–2.13 (m, 1H,  $\text{H}_{2b}$ ), 2.08 (s, 3H, CH<sub>3</sub>), 2.03 (ddd,  $J = 14.3, 11.1, 7.7$  Hz, 1H,  $\text{H}_{5c}$ ).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  170.7 (C=O), 158.9 (C), 153.6 (C), 151.4 (C), 140.6 (C), 125.4 (C), 86.1 (d,  $J = 4.3$  Hz,  $\text{C}_4$ ), 53.5 (C), 52.7 (d,  $J = 4.1$  Hz, OCH<sub>3</sub>), 52.62 (d,  $J = 4.0$  Hz, OCH<sub>3</sub>), 45.1 (d,  $J = 6.2$  Hz,  $\text{C}_5$ ), 37.6 (d,  $J = 8.3$  Hz,  $\text{C}_3$ ), 32.1 (d,  $J = 139.6$  Hz,  $\text{C}_6$ ), 31.3 (C), 22.2 (CH<sub>3</sub>).  $^{31}\text{P}$  NMR (202 MHz,  $\text{CDCl}_3$ )  $\delta$  27.9.  $[\alpha]_D^{20} = +25.8$  (c = 1.00,  $\text{CHCl}_3$ ). MS (ESI<sup>+</sup>):  $m/z = 418.1$  (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for  $\text{C}_{15}\text{H}_{22}\text{ClN}_5\text{O}_5\text{P}$  [M+H]<sup>+</sup>: 418.1042; Found: 418.1059.

4.6.1.9. (1R,3R)-3-(2-Amino-6-chloro-9H-purin-9-yl)-1-((dimethoxyphosphoryl)methyl)cyclopentyl acetate (-)-4g. The product was obtained (580 mg, 82%) according to the Protocol A using compound (1R,3S)-(-)-3 (450 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.32.  $[\alpha]_D^{20} = -30.7$  (c = 1.00,  $\text{CHCl}_3$ ).

4.6.1.10. (1R,3S)-3-(2-Amino-6-chloro-9H-purin-9-yl)-1-((dimethoxyphosphoryl)methyl)cyclopentyl acetate (1R,3S)-(-)-4g'. The product was obtained (420 mg, 63%) according to the Protocol A using compound (1R,3R)-(-)-3' (430 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.34.  $[\alpha]_D^{20} = -26.7$  (c = 1.00,  $\text{CHCl}_3$ ).

#### 4.6.2. Protocol B

To a solution of carbonucleoside phosphonate (1.0 eq) in methanol (15 mL/mmol) was added  $\text{K}_2\text{CO}_3$  (3.5 eq). The reaction mixture was stirred at rt for 24 h before filtration and evaporation of the solvent. Purification by silica gel column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  1:0 to 97/3) gave the desired product as white foam.

4.6.2.1. ( $\pm$ )-Dimethyl((3-(2-chloro-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phosphonate ( $\pm$ )-5a. The product was obtained (600 mg, 67%) according to the Protocol B using compound ( $\pm$ )-4a (1 g).

$R_f$  (DCM/MeOH: 95/5) = 0.4.  $^1\text{H}$  NMR (400 MHz, Methanol- $d_4$ )  $\delta$  8.38 (s, 1H,  $\text{H}_8$ ), 5.30–5.22 (m, 1H,  $\text{H}_1$ ), 4.18 (s, 3H, OCH<sub>3</sub>), 3.79 (d,  $J = 11.0$  Hz, OCH<sub>3</sub>), 3.78 (d,  $J = 11.0$  Hz, OCH<sub>3</sub>), 2.61–2.48 (m, 2H,  $\text{H}_{2a} + \text{H}_{2b}$ ), 2.45–2.33 (m, 4H,  $\text{H}_{3a} + \text{H}_{5a} + \text{H}_{6a}$ ), 2.24–2.10 (m, 1H,  $\text{H}_{2b}$ ), 2.08–2.01 (m, 1H,  $\text{H}_{3a}$ ).  $^{13}\text{C}$  NMR (101 MHz, Methanol- $d_4$ )  $\delta$  162.5 (COCH<sub>3</sub>), 154.2 (C), 153.9 (C), 144.2 (C), 121.4 (C), 78.7 (d,  $J = 5.3$  Hz,  $\text{C}_4$ ), 56.7 (C), 55.5 (OCH<sub>3</sub>), 53.7 (t,  $J = 7.1$  Hz, 2xOCH<sub>3</sub>), 47.1 (d,  $J = 8.6$  Hz,  $\text{C}_5$ ), 39.9 (d,  $J = 9.5$  Hz,  $\text{C}_3$ ), 36.6 (d,  $j = 138.5$  Hz,  $\text{C}_6$ ), 31.2 (C).  $^{31}\text{P}$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.6. MS (ESI<sup>+</sup>):  $m/z = 391.0$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{14}\text{H}_{21}\text{N}_4\text{O}_5\text{ClP}$  [M+H]<sup>+</sup>: 391.0938; found: 391.0938.

4.6.2.2. ( $\pm$ )-Dimethyl ((3-(2-chloro-6-aminobenzyl-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate ( $\pm$ )-5b. The product was obtained (90 mg, 55%) according to the Protocol B using compound ( $\pm$ )-4b (180 mg).

$R_f$  (DCM/MeOH: 96/4) = 0.3.  $^1H$  NMR (400 MHz, Methanol- $d_4$ )  $\delta$  8.13 (s, 1H,  $H_8$ ), 7.41–7.39 (m, 2H,  $H_{Ar}$ ), 7.35–7.31 (m, 2H,  $H_{Ar}$ ), 7.28–7.23 (m, 2H,  $H_{Ar}$ ), 5.21–5.13 (m, 1H,  $H_1$ ), 4.76 (s, 2H,  $CH_2$ ), 3.78 (d,  $J$  = 11.0 Hz,  $OCH_3$ ), 3.78 (d,  $J$  = 11.0 Hz,  $OCH_3$ ), 2.57–2.45 (m, 2H,  $H_{2a}$  +  $H_{5a}$ ), 2.44–2.30 (m, 4H,  $H_{3a}$  +  $H_{5a}$  +  $H_6$ ), 2.17–2.07 (m, 1H,  $H_{2a}$ ), 2.08–1.99 (m, 1H,  $H_{3a}$ ).  $^{13}C$  NMR (101 MHz, Methanol- $d_4$ )  $\delta$  141.2 (C<sub>9</sub>), 129.5 (2xCH), 128.8 (CH), 128.3 (2xCH), 78.7 (d,  $J$  = 5.2 Hz, C<sub>4</sub>), 56.3 (C<sub>1</sub>), 53.1 (t,  $J$  = 6.6 Hz, 2xOCH<sub>3</sub>), 47.2 (d,  $J$  = 8.6 Hz, C<sub>5</sub>), 45.2 (CH<sub>2</sub>), 40.0 (d,  $J$  = 9.5 Hz, C<sub>3</sub>), 36.6 (d,  $J$  = 138.4 Hz, C<sub>6</sub>), 31.4 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.7. MS (ESI<sup>+</sup>):  $m/z$  = 466.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>20</sub>H<sub>26</sub>N<sub>5</sub>O<sub>4</sub>ClP [M+H]<sup>+</sup>: 466.1411; found: 466.1411.

4.6.2.3. ( $\pm$ )-Dimethyl ((3-(2-amino-6-aminobenzyl-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate ( $\pm$ )-5c. The product was obtained (105 mg, 64%) according to the Protocol B using compound ( $\pm$ )-4c (180 mg).

$R_f$  (DCM/MeOH: 96/4) = 0.18.  $^1H$  NMR (400 MHz, Methanol- $d_4$ )  $\delta$  7.85 (s, 1H,  $H_8$ ), 7.40–7.38 (m, 2H,  $H_{Ar}$ ), 7.35–7.31 (m, 2H,  $H_{Ar}$ ), 7.28–7.24 (m, 2H,  $H_{Ar}$ ), 5.12–5.03 (m, 1H,  $H_1$ ), 4.77 (s, 2H,  $CH_2$ ), 3.79 (d,  $J$  = 11.0 Hz,  $OCH_3$ ), 3.79 (d,  $J$  = 11.0 Hz,  $OCH_3$ ), 2.55–2.29 (m, 6H,  $H_{carbocycle}$  +  $H_6$ ), 2.11–1.98 (m, 2H,  $H_{carbocycle}$ ).  $^{13}C$  NMR (101 MHz, Methanol- $d_4$ )  $\delta$  161.9 (C<sub>9</sub>), 156.4 (C<sub>carbocycle</sub>), 152.2 (C<sub>9</sub>), 140.6 (C<sub>9</sub>), 137.5 (C<sub>8</sub>), 129.5 (2xCH), 128.5 (2xCH), 128.1 (CH), 114.9 (C<sub>9</sub>), 78.6 (d,  $J$  = 5.1 Hz, C<sub>4</sub>), 55.2 (C<sub>1</sub>), 53.0 (t,  $J$  = 6.6 Hz, 2xOCH<sub>3</sub>), 47.2 (d,  $J$  = 8.5 Hz, C<sub>5</sub>), 45.0 (CH<sub>2</sub>), 39.9 (d,  $J$  = 9.5 Hz, C<sub>3</sub>), 36.7 (d,  $J$  = 138.2 Hz, C<sub>6</sub>), 31.4 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.9. MS (ESI<sup>+</sup>):  $m/z$  = 447.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>20</sub>H<sub>28</sub>N<sub>6</sub>O<sub>4</sub>P [M+H]<sup>+</sup>: 447.1913; found: 447.1910.

4.6.2.4. ( $\pm$ )-Dimethyl((3-(6-(aminobenzyl)-2-(aminobenzyl)-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl) phosphonate ( $\pm$ )-6d. The product was obtained (213 mg, 66%) according to the Protocol B using compound ( $\pm$ )-5d (350 mg).

$R_f$  (DCM/MeOH: 97/3) = 0.28.  $^1H$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  7.83 (s, 1H,  $H_8$ ), 7.66–7.63 (m, 2H,  $H_{Ar}$ ), 7.38–7.37 (m, 2H,  $H_{Ar}$ ), 7.17–7.28 (m, 2H,  $H_{Ar}$ ), 7.23–7.19 (m, 3H,  $H_{Ar}$ ), 5.13–5.07 (m, 1H,  $H_1$ ), 4.78 (s, 2H,  $CH_2$ ), 3.74 (d,  $J$  = 11.1 Hz,  $OCH_3$ ), 3.73 (d,  $J$  = 10.9 Hz,  $OCH_3$ ), 2.53–2.45 (m, 2H,  $H_{2a}$  +  $H_{5a}$ ), 2.39 (ddd,  $J$  = 13.1, 7.4, 2.1 Hz, 1H,  $H_{5b}$ ), 2.35 (dd,  $J$  = 18.3, 2.0 Hz, 2H,  $H_6$ ), 2.35–2.31 (m, 1H,  $H_3$ ), 2.23–2.17 (m, 1H,  $H_{2b}$ ), 2.04–2.00 (m, 1H,  $H_3$ ), 1.98–1.94 (m, 1H,  $H_{2b}$ ).  $^{13}C$  NMR (126 MHz, Methanol- $d_4$ )  $\delta$  157.9 (C<sub>9</sub>), 156.0 (C<sub>9</sub>), 151.7 (C<sub>9</sub>), 142.5 (C<sub>9</sub>), 140.8 (C<sub>9</sub>), 138.7 (C<sub>8</sub>), 129.5 (2xCH), 128.4 (2xCH), 128.4 (2xCH), 128.0 (CH), 122.1 (CH), 120.0 (2xCH), 116.0 (C<sub>9</sub>), 78.8 (d,  $J$  = 5.2 Hz, C<sub>4</sub>), 56.2 (C<sub>1</sub>), 53.0 (t,  $J$  = 5.7 Hz, 2xOCH<sub>3</sub>), 46.8 (d,  $J$  = 9.0 Hz, C<sub>5</sub>), 45.0 (CH<sub>2</sub>), 40.1 (d,  $J$  = 9.5 Hz, C<sub>3</sub>), 36.7 (d,  $J$  = 138.4 Hz, C<sub>6</sub>), 30.9 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.8. MS (ESI<sup>+</sup>):  $m/z$  = 523.2 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>26</sub>H<sub>32</sub>N<sub>6</sub>O<sub>4</sub>P [M+H]<sup>+</sup>: 523.2227; found: 523.2223.

4.6.2.5. ( $\pm$ )-Dimethyl((3-(6-amino-2-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phosphonate ( $\pm$ )-6e. The product was obtained (120 mg, 62%) according to the Protocol B using compound ( $\pm$ )-5e (208 mg).

$R_f$  (DCM/MeOH: 9/1) = 0.33.  $^1H$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  7.99 (s, 1H,  $H_8$ ), 5.14–5.09 (m, 1H,  $H_1$ ), 3.92 (s, 3H,  $OCH_3$ ), 3.75 (d,  $J$  = 11.1 Hz, 3H,  $OCH_3$ ), 3.74 (d,  $J$  = 10.9 Hz, 3H,  $OCH_3$ ), 2.52–2.32 (m, 6H,  $H_{2a}$  +  $H_{3a}$  +  $H_5$  +  $H_6$ ), 2.15–2.09 (m, 1H,  $H_{2b}$ ), 2.03–1.98 (m, 1H,  $H_{3b}$ ).  $^{13}C$  NMR (151 MHz, Methanol- $d_4$ )  $\delta$  163.5 (C<sub>9</sub>), 158.1 (C<sub>9</sub>), 152.4 (C<sub>9</sub>), 140.2 (C<sub>8</sub>), 116.7 (C<sub>5</sub>), 78.8 (d,  $J$  = 5.1 Hz, C<sub>4</sub>), 56.0 (C<sub>1</sub>), 55.1 (OCH<sub>3</sub>), 53.1 (d,  $J$  = 6.3 Hz, OCH<sub>3</sub>), 53.0 (d,  $J$  = 6.3 Hz,

OCH<sub>3</sub>), 47.0 (d,  $J$  = 8.4 Hz, C<sub>5</sub>), 40.0 (d,  $J$  = 9.3 Hz, C<sub>3</sub>), 36.6 (d,  $J$  = 138.4 Hz, C<sub>6</sub>), 31.2 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.7. MS (ESI<sup>+</sup>):  $m/z$  = 372.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>14</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 372.1441; found: 372.1437.

4.6.2.6. ( $\pm$ )-Dimethyl((3-(2,6-diamino-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phosphonate ( $\pm$ )-6f. The product was obtained (350 mg, 71%) according to the Protocol B using compound ( $\pm$ )-5f (550 mg).

$R_f$  (DCM/MeOH: 9/1) = 0.4.  $^1H$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  7.89 (s, 1H,  $H_8$ ), 5.09–5.04 (m, 1H,  $H_1$ ), 3.78 (d,  $J$  = 10.9 Hz, OCH<sub>3</sub>), 3.77 (d,  $J$  = 10.9 Hz, OCH<sub>3</sub>), 2.51–2.29 (m, 6H,  $H_{carbocycle}$  +  $H_6$ ), 2.09–1.98 (m, 2H,  $H_{carbocycle}$ ).  $^{13}C$  NMR (151 MHz, Methanol- $d_4$ )  $\delta$  161.5 (C<sub>9</sub>), 157.4 (C<sub>9</sub>), 152.8 (C<sub>9</sub>), 138.3 (C<sub>8</sub>), 114.6 (C<sub>5</sub>), 78.6 (d,  $J$  = 5.0 Hz, C<sub>4</sub>), 55.3 (C<sub>1</sub>), 53.1 (d,  $J$  = 7.2 Hz, 2xOCH<sub>3</sub>), 47.1 (d,  $J$  = 8.4 Hz, C<sub>5</sub>), 39.9 (d,  $J$  = 9.3 Hz, C<sub>3</sub>), 36.6 (d,  $J$  = 138.7 Hz, C<sub>6</sub>), 31.4 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.9. MS (ESI<sup>+</sup>):  $m/z$  = 357.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>13</sub>H<sub>22</sub>N<sub>6</sub>O<sub>4</sub>P [M+H]<sup>+</sup>: 357.1431; found: 357.1435.

4.6.2.7. Dimethyl (((1S,3S)-3-(2-amino-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate (+)-6g. The product was obtained (380 mg, 73%) according to the Protocol B using compound (1S,3S)-(+)-4g (610 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.1.  $^1H$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  7.96 (s, 1H,  $H_8$ ), 5.13 (tt,  $J$  = 9.6, 7.2 Hz, 1H,  $H_1$ ), 4.08 (s, 3H,  $CH_3$ ), 3.80 (d,  $J$  = 11.1 Hz, 3H,  $OCH_3$ ), 3.80 (d,  $J$  = 10.9 Hz, 3H,  $OCH_3$ ), 2.55–2.34 (m, 6H), 2.11 (dddd,  $J$  = 13.2, 10.3, 6.7, 4.3 Hz, 1H), 2.05–2.00 (m, 1H).  $^{13}C$  NMR (151 MHz, Methanol- $d_4$ )  $\delta$  162.6 (C<sub>9</sub>), 161.6 (C<sub>9</sub>), 154.9 (C<sub>9</sub>), 139.7 (C<sub>8</sub>), 115.7 (C<sub>5</sub>), 78.7 (d,  $J$  = 5.2 Hz, C<sub>4</sub>), 55.7 (C<sub>1</sub>), 54.1 (OCH<sub>3</sub>), 53.1 (t,  $J$  = 7.3 Hz, 2OCH<sub>3</sub>), 47.0 (d,  $J$  = 8.2 Hz, C<sub>5</sub>), 39.9 (d,  $J$  = 9.5 Hz, C<sub>3</sub>), 36.6 (d,  $J$  = 138.3 Hz, C<sub>6</sub>), 31.2 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  31.9. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +19.5 (c = 1.00, MeOH). MS (ESI<sup>+</sup>):  $m/z$  = 372.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>14</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 372.1431; Found: 372.1457.

4.6.2.8. Dimethyl (((1S,3R)-3-(2-amino-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate (-)-6g'. The product was obtained (252 mg, 74%) according to the Protocol B using compound (1S,3R)-(-)-4g' (400 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.2.  $^1H$  NMR (600 MHz, Methanol- $d_4$ )  $\delta$  7.97 (s, 1H,  $H_8$ ), 5.04 (dtd,  $J$  = 12.2, 8.6, 3.7 Hz, 1H,  $H_1$ ), 4.09 (s, 3H,  $OCH_3$ ), 3.81 (d,  $J$  = 11.0 Hz, 3H,  $OCH_3$ ), 3.80 (d,  $J$  = 10.9 Hz, 3H,  $OCH_3$ ), 2.56 (dd,  $J$  = 15.2, 11.2 Hz, 1H,  $H_5$ ), 2.42–2.30 (m, 4H,  $H_2$  +  $H_6$ ), 2.25 (dt,  $J$  = 15.3, 3.3 Hz, 1H,  $H_{5b}$ ), 2.12 (ddt,  $J$  = 13.1, 6.5, 2.2 Hz, 1H,  $H_{3a}$ ), 1.87 (td,  $J$  = 12.9, 8.1 Hz, 1H,  $H_{3b}$ ).  $^{13}C$  NMR (151 MHz, Methanol- $d_4$ )  $\delta$  162.9 (C<sub>9</sub>), 161.2 (C<sub>9</sub>), 153.7 (C<sub>9</sub>), 140.7 (C<sub>8</sub>), 115.8 (C<sub>5</sub>), 78.6 (d,  $J$  = 5.5 Hz, C<sub>4</sub>), 55.3 (C<sub>1</sub>), 54.2 (OCH<sub>3</sub>), 53.1 (d,  $J$  = 6.8 Hz, OCH<sub>3</sub>), 53.0 (d,  $J$  = 6.9 Hz, OCH<sub>3</sub>), 46.8 (d,  $J$  = 8.3 Hz, C<sub>5</sub>), 41.5 (d,  $J$  = 10.3 Hz, C<sub>3</sub>), 36.4 (d,  $J$  = 139.4 Hz, C<sub>6</sub>), 32.1 (C<sub>2</sub>).  $^{31}P$  NMR (162 MHz, Methanol- $d_4$ )  $\delta$  32.1. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -26.3 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>):  $m/z$  = 372.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>14</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 372.1431; Found: 372.1452.

4.6.2.9. Dimethyl (((1R,3R)-3-(2-amino-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate (-)-6g. The product was obtained (354 mg, 74%) according to the Protocol B using compound (1R,3R)-(-)-4g (545 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.1. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -16.7 (c = 1.00, MeOH).

4.6.2.10. Dimethyl (((1R,3S)-3-(2-amino-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl) phospho-nate (+)-6g'. The product was obtained (150 mg, 46%) according to the Protocol B using compound (1R,3S)-(+)-4g' (370 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.2.  $[\alpha]_D^{20} = +18.5$  ( $c = 1.00$ , MeOH).

#### 4.6.3. Protocol C (Removal of the Boc protecting group)

To a stirred solution of protected phosphonate (1.0 eq) in  $\text{ClCH}_2\text{CH}_2\text{Cl}$  (20 mL/mmol), trifluoroacetic acid (8 mL/mmol) was added dropwise and the reaction was stirred at room temperature overnight. The volatile solvents were removed under reduced pressure, and the residue was purified by column chromatography on silica gel (DCM/MeOH: 1/0 to 95/5) to give the deprotected compound as a white foam.

4.6.3.1. ( $\pm$ )-1-((Dimethoxyphosphoryl)methyl)-3-(6-(aminobenzyl)-2-(aminobenzyl)-9H-purin-9-yl) cyclopentyl acetate ( $\pm$ )-5d. The product was obtained (360 mg, 98%) according to the Protocol C using compound ( $\pm$ )-4d (500 mg).

$R_f$  (DCM/MeOH: 97/3) = 0.45.  $^1\text{H NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta$  7.62–7.61 (m, 2H,  $\text{H}_{\text{Ar}}$ ), 7.55 (s, 1H,  $\text{H}_8$ ), 7.40–7.38 (m, 2H,  $\text{H}_{\text{Ar}}$ ), 7.35–7.32 (m, 2H,  $\text{H}_{\text{Ar}}$ ), 7.30–7.26 (m, 3H,  $\text{H}_{\text{Ar}}$ ), 7.14 (s, 1H, NH), 6.18 (s, 1H, NH), 4.93–4.87 (m, 1H,  $\text{H}_{1a}$ ), 4.83 (s, 2H,  $\text{CH}_2$ ), 3.73 (d,  $J = 11.0$  Hz,  $\text{OCH}_3$ ), 3.72 (d,  $J = 10.9$  Hz,  $\text{OCH}_3$ ), 2.93 (ddd,  $J = 14.1, 7.7, 1.7$  Hz, 1H,  $\text{H}_{5a}$ ), 2.89 (dd,  $J = 18.8, 15.6$  Hz, 1H,  $\text{H}_{6a}$ ), 2.73 (dd,  $J = 19.9, 15.5$  Hz, 1H,  $\text{H}_{6b}$ ), 2.60 (dd,  $J = 14.1, 10.0$  Hz, 1H,  $\text{H}_{5b}$ ), 2.56–2.51 (m, 1H,  $\text{H}_{3a}$ ), 2.48–2.43 (m, 1H,  $\text{H}_{3b}$ ), 2.41–2.35 (m, 1H,  $\text{H}_{2a}$ ), 2.32–2.26 (m, 1H,  $\text{H}_{2b}$ ), 2.09 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ )  $\delta$  171.1 (C=O), 154.8 (C), 140.6 (C), 138.8 (C), 137.2 (C), 128.8 (2xCH), 128.8 (2xCH), 127.8 (2xCH), 127.5 (CH), 121.8 (CH), 119.0 (2xCH), 116.0 (C), 86.5 (d,  $J = 5.7$  Hz,  $\text{C}_4$ ), 54.8 (C), 52.6 (d,  $J = 7.2$  Hz,  $2 \times \text{OCH}_3$ ), 44.7 (CH<sub>2</sub>), 43.7 (d,  $J = 6.1$  Hz,  $\text{C}_5$ ), 37.3 (d,  $J = 9.8$  Hz,  $\text{C}_3$ ), 32.1 (d,  $J = 139.5$  Hz,  $\text{C}_6$ ), 29.9 (C<sub>2</sub>), 22.3 (CH<sub>3</sub>).  $^{31}\text{P NMR}$  (162 MHz,  $\text{CDCl}_3$ )  $\delta$  28.5. MS (ESI<sup>+</sup>):  $m/z = 565.2$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{28}\text{H}_{34}\text{N}_6\text{O}_5\text{P}$  [M+H]<sup>+</sup>: 565.2344; found: 565.2323.

4.6.3.2. ( $\pm$ )-1-((Dimethoxyphosphoryl)methyl)-3-(6-amino-2-fluoro-9H-purin-9-yl) cyclopentyl acetate ( $\pm$ )-5e. The product was obtained (220 mg, quantitative) according to the Protocol C using compound ( $\pm$ )-4e (300 mg).

$R_f$  (DCM/MeOH: 9/1) = 0.26.  $^1\text{H NMR}$  (600 MHz, Methanol- $d_4$ )  $\delta$  8.13 (s, 1H,  $\text{H}_8$ ), 5.06–5.01 (m, 1H,  $\text{H}_{1a}$ ), 3.76 (d,  $J = 11.1$  Hz, 6H,  $2 \times \text{OCH}_3$ ), 2.96 (dd,  $J = 18.8, 15.6$  Hz, 1H,  $\text{H}_{6a}$ ), 2.94 (td,  $J = 6.8, 1.6$  Hz, 1H,  $\text{H}_{5a}$ ), 2.66 (dd,  $J = 19.4, 15.8$  Hz, 1H,  $\text{H}_{6b}$ ), 2.52–2.36 (m, 4H,  $\text{H}_{2a} + \text{H}_{3a} + \text{H}_{5b}$ ), 2.23–2.17 (m, 1H,  $\text{H}_{2b}$ ), 2.07 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C NMR}$  (151 MHz, Methanol- $d_4$ )  $\delta$  172.6 (C=O), 160.3 (d,  $J = 208.3$  Hz,  $\text{C}_2$ ), 159.0 (d,  $J = 20.6$  Hz, C), 152.0 (d,  $J = 19.7$  Hz, C), 141.6 (d,  $J = 2.7$  Hz,  $\text{C}_8$ ), 118.8 (d,  $J = 4.0$  Hz,  $\text{C}_5$ ), 87.5 (d,  $J = 6.7$  Hz,  $\text{C}_4$ ), 55.9 (C<sub>1</sub>), 53.3 (d,  $J = 7.0$  Hz,  $2 \times \text{OCH}_3$ ), 45.0 (d,  $J = 7.2$  Hz,  $\text{C}_5$ ), 38.4 (d,  $J = 9.3$  Hz,  $\text{C}_3$ ), 32.1 (d,  $J = 139.7$  Hz,  $\text{C}_6$ ), 30.8 (C<sub>2</sub>), 22.1 (CH<sub>3</sub>).  $^{31}\text{P NMR}$  (162 MHz, Methanol- $d_4$ )  $\delta$  29.5. MS (ESI<sup>+</sup>):  $m/z = 402.1$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{15}\text{H}_{22}\text{N}_5\text{O}_5\text{FP}$  [M+H]<sup>+</sup>: 402.1347; found: 402.1343.

4.6.3.3. ( $\pm$ )-1-((Dimethoxyphosphoryl)methyl)-3-(2,6-diamino-9H-purin-9-yl)cyclopentylacetate ( $\pm$ )-5f. The product was obtained (490 mg, quantitative) according to the Protocol C using compound ( $\pm$ )-4f (1 g).

$R_f$  (DCM/MeOH: 9/1) = 0.22.  $^1\text{H NMR}$  (400 MHz, Methanol- $d_4$ )  $\delta$  8.04 (s, 1H,  $\text{H}_8$ ), 5.06–4.97 (m, 1H,  $\text{H}_{1a}$ ), 3.77 (d,  $J = 11.0$  Hz,  $\text{OCH}_3$ ), 3.77 (d,  $J = 11.1$  Hz,  $\text{OCH}_3$ ), 2.96 (dd,  $J = 18.8, 15.8$  Hz, 1H,  $\text{H}_{6a}$ ), 2.98–2.92 (m, 1H,  $\text{H}_{5a}$ ), 2.83 (dd,  $J = 19.4, 15.8$  Hz, 1H,  $\text{H}_{6b}$ ), 2.55–2.38 (m, 4H,  $\text{H}_{2a} + \text{H}_{3a} + \text{H}_{5b}$ ), 2.23–2.14 (m, 1H,  $\text{H}_{2b}$ ), 2.07 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C NMR}$  (101 MHz, Methanol- $d_4$ )  $\delta$  172.5 (C=O), 154.1 (C), 153.4 (C), 152.1 (C), 141.6 (C), 113.2 (C), 87.6 (d,  $J = 5.9$  Hz,  $\text{C}_4$ ), 55.6 (C<sub>1</sub>), 53.3 (d,  $J = 6.8$  Hz,  $2 \times \text{OCH}_3$ ), 44.9 (d,  $J = 7.1$  Hz,  $\text{C}_5$ ), 38.1 (d,  $J = 9.7$  Hz,  $\text{C}_3$ ), 32.2 (d,  $J = 140.1$  Hz,  $\text{C}_6$ ), 30.6 (C<sub>2</sub>), 22.1 (CH<sub>3</sub>).  $^{31}\text{P NMR}$  (162 MHz, Methanol- $d_4$ )  $\delta$  29.6. MS (ESI<sup>+</sup>):

$m/z = 399.1$  [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{15}\text{H}_{24}\text{N}_6\text{O}_5\text{P}$  [M+H]<sup>+</sup>: 399.1553; found: 399.1546.

#### 4.6.4. Protocol D

To a solution of carbonucleoside phosphonate (1.0 eq) in acetonitrile or dimethylformamide (10 mL/mmol) was added sodium iodide (3.0 eq). The reaction mixture is heated at 40 °C and trimethylsilyl chloride (3.0 eq) was added. After 1 h, the reaction mixture was cooled to rt and stirred overnight. The reaction mixture was neutralized with TEAB 1 M to obtain pH 7. The solvent was evaporated and the residue was purified by reverse phase chromatography ( $\text{H}_2\text{O}/\text{MeOH}$ : 1/0 to 1/1) followed by Dowex ion-exchange chromatography ( $\text{Na}^+$ ) to give a desired product as white solid.

4.6.4.1. ( $\pm$ )-((3-(2-Chloro-6-aminobenzyl-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt, ( $\pm$ )-6b. The product was obtained (41 mg, 60%) according to the Protocol D using compound ( $\pm$ )-5b (70 mg).

$R_f$  (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 7/2/1): 0.24.  $^1\text{H NMR}$  (400 MHz, D<sub>2</sub>O)  $\delta$  8.09 (s, 1H,  $\text{H}_8$ ), 7.38–7.00 (m, 5H,  $\text{H}_{\text{Ar}}$ ), 4.97–4.89 (m, 1H,  $\text{H}_{1a}$ ), 4.61 (s, 2H,  $\text{CH}_2$ ), 2.56–2.44 (m, 2H), 2.25–2.16 (m, 2H), 2.08 (dd,  $J = 1.4, 16.5$  Hz, 2H,  $\text{H}_6$ ), 2.01–1.89 (m, 2H).  $^{13}\text{C NMR}$  (101 MHz, D<sub>2</sub>O)  $\delta$  154.7 (C), 153.5 (C), 139.8 (C<sub>8</sub>), 137.7 (C), 128.5 (C), 128.5 (2xCH), 127.3 (CH), 127.1 (2xCH), 78.9 (d,  $J = 4.9$  Hz,  $\text{C}_4$ ), 54.2 (C<sub>1</sub>), 46.1 (d,  $J = 8.0$  Hz,  $\text{C}_5$ ), 43.9 (CH<sub>2</sub>), 38.5 (d,  $J = 127.2$  Hz,  $\text{C}_6$ ), 38.1 (d,  $J = 8.2$  Hz,  $\text{C}_3$ ), 30.5 (C<sub>2</sub>).  $^{31}\text{P NMR}$  (162 MHz, D<sub>2</sub>O)  $\delta$  19.4. MS (ESI<sup>-</sup>):  $m/z = 436.0$  [M - Na]<sup>-</sup>. HRMS (ESI<sup>-</sup>) calculated for  $\text{C}_{18}\text{H}_{20}\text{N}_5\text{O}_4\text{ClP}$  [M - Na]<sup>-</sup>: 436.0942; found: 436.0942.

4.6.4.2. ( $\pm$ )-((3-(2-Amino-6-aminobenzyl-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt, ( $\pm$ )-6c. The product was obtained (35 mg, 51%) according to the Protocol D using compound (+/-)-5c (70 mg).

$R_f$  (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 7/2/1): 0.26.  $^1\text{H NMR}$  (400 MHz, D<sub>2</sub>O)  $\delta$  7.89 (s, 1H,  $\text{H}_8$ ), 7.33–7.24 (m, 5H,  $\text{H}_{\text{Ar}}$ ), 4.93–4.86 (m, 1H,  $\text{H}_{1a}$ ), 4.68 (s, 2H,  $\text{CH}_2$ ), 2.49–2.41 (m, 2H,  $\text{H}_{2a} + \text{H}_{3a}$ ), 2.21–2.15 (m, 2H,  $\text{H}_{3a} + \text{H}_{5b}$ ), 2.01 (d,  $J = 1.6, 16.3$  Hz, 2H,  $\text{H}_6$ ), 1.98–1.88 (m, 2H,  $\text{H}_{3b} + \text{H}_{2b}$ ).  $^{13}\text{C NMR}$  (101 MHz, D<sub>2</sub>O)  $\delta$  160.1 (C), 157.0 (C), 155.2 (C), 138.7 (C), 137.4 (C<sub>8</sub>), 128.7 (2xCH), 127.2 (CH), 127.1 (2xCH), 113.4 (C), 79.1 (d,  $J = 4.8$  Hz,  $\text{C}_4$ ), 53.6 (C<sub>1</sub>), 46.3 (d,  $J = 8.1$  Hz,  $\text{C}_5$ ), 43.8 (CH<sub>2</sub>), 38.7 (d,  $J = 126.0$  Hz,  $\text{C}_6$ ), 38.3 (d,  $J = 8.7$  Hz,  $\text{C}_3$ ), 30.5 (C<sub>2</sub>).  $^{31}\text{P NMR}$  (162 MHz, D<sub>2</sub>O)  $\delta$  18.9. MS (ESI<sup>+</sup>):  $m/z = 417.1$  [M - H]<sup>-</sup>. HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{18}\text{H}_{22}\text{N}_6\text{O}_4\text{P}$  [M - H]<sup>-</sup>: 417.1444; found: 417.1440.

4.6.4.3. ( $\pm$ )-((3-(6-Aminobenzyl-2-aminobenzyl-9H-purin-9-yl)-1-hydroxycyclopentyl)methyl)-phosphonate, sodium salt, ( $\pm$ )-7d. The product was obtained (146 mg, 81%) according to the Protocol D using compound ( $\pm$ )-6d (185 mg).

$R_f$  (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 7/2/1): 0.68.  $^1\text{H NMR}$  (600 MHz, D<sub>2</sub>O)  $\delta$  7.72 (s, 1H,  $\text{H}_8$ ), 7.14 (d,  $J = 7.8$  Hz, 2H,  $\text{H}_{\text{Ar}}$ ), 6.91 (d,  $J = 7.6$  Hz, 2H,  $\text{H}_{\text{Ar}}$ ), 6.86 (d,  $J = 8.8$  Hz, 2H,  $\text{H}_{\text{Ar}}$ ), 6.83 (d,  $J = 7.8$  Hz, 2H,  $\text{H}_{\text{Ar}}$ ), 6.77 (t,  $J = 7.4$  Hz, 1H,  $\text{H}_{\text{Ar}}$ ), 6.59 (t,  $J = 7.5$  Hz, 1H,  $\text{H}_{\text{Ar}}$ ), 4.84–4.75 (m, 1H,  $\text{H}_{1a}$ ), 4.26 (s, 2H,  $\text{CH}_2$ ), 2.35–2.27 (m, 2H,  $\text{H}_{2a} + \text{H}_{3a}$ ), 2.12–2.02 (m, 2H,  $\text{H}_{3a} + \text{H}_{5b}$ ), 1.99 (d,  $J = 16.4$  Hz, 2H,  $\text{H}_6$ ), 1.88–1.85 (m, 1H,  $\text{H}_{3b}$ ), 1.76–1.71 (m, 1H,  $\text{H}_{2b}$ ).

$^{13}\text{C NMR}$  (151 MHz, D<sub>2</sub>O)  $\delta$  157.8 (C), 156.1 (C), 151.7 (C), 141.8 (C), 140.6 (C), 139.0 (C<sub>8</sub>), 130.3 (2xCH), 130.1 (2xCH), 128.6 (CH), 128.5 (2xCH), 123.3 (CH), 120.6 (2xCH), 115.8 (C), 80.8 (d,  $J = 5.3$  Hz,  $\text{C}_4$ ), 55.7 (C<sub>1</sub>), 47.9 (d,  $J = 8.6$  Hz,  $\text{C}_5$ ), 45.6 (CH<sub>2</sub>), 40.5 (d,  $J = 127.2$  Hz,  $\text{C}_6$ ), 40.2 (d,  $J = 8.9$  Hz,  $\text{C}_3$ ), 32.2 (C<sub>2</sub>).  $^{31}\text{P NMR}$  (162 MHz, D<sub>2</sub>O)  $\delta$  19.8. MS (ESI<sup>+</sup>):  $m/z = 495.1$  [M+H]<sup>+</sup>.

HRMS (ESI<sup>+</sup>) calculated for  $\text{C}_{24}\text{H}_{28}\text{N}_6\text{O}_4\text{P}$  [M+H]<sup>+</sup>: 495.1898; found: 495.1904.

4.6.4.4. ( $\pm$ )- ((3-(6-Amino-2-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl) phosphonate, sodium salt, ( $\pm$ )-7e. The product was obtained (68 mg, 77%) according to the Protocol D using compound ( $\pm$ )-6e (90 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 7/2/1): 0.28. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  7.99 (s, 1H, H<sub>8</sub>), 5.00–4.94 (m, 1H, H<sub>1</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 2.50–2.41 (m, 2H, H<sub>2a</sub> + H<sub>5a</sub>), 2.27–2.20 (m, 2H, H<sub>3a</sub> + H<sub>5b</sub>), 2.04 (dd, *J* = 16.4, 1.3 Hz, 2H, H<sub>6</sub>), 2.00–1.92 (m, 2H, H<sub>2b</sub> + H<sub>3b</sub>). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  161.7 (C<sub>4</sub>), 156.1 (C<sub>2</sub>), 150.8 (C<sub>6</sub>), 139.5 (C<sub>8</sub>), 114.8 (C<sub>5</sub>), 79.2 (d, *J* = 5.1 Hz, C<sub>1</sub>), 54.6 (OCH<sub>3</sub>), 54.3 (C<sub>1</sub>), 45.9 (d, *J* = 8.2 Hz, C<sub>5</sub>), 38.5 (d, *J* = 126.6 Hz, C<sub>6</sub>), 38.3 (d, *J* = 8.7 Hz, C<sub>3</sub>), 30.1 (C<sub>2</sub>). <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O)  $\delta$  19.4. MS (ESI<sup>+</sup>): *m/z* = 344.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>12</sub>H<sub>19</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 344.1132; found: 344.1124.

4.6.4.5. ( $\pm$ )- ((3-(2,6-Diamino-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl) phosphonate, sodium salt, ( $\pm$ )-7f. The product was obtained (47 mg, 51%) according to the Protocol D using compound ( $\pm$ )-6f (150 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/2/2): 0.49. <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  8.11 (s, 1H, H<sub>8</sub>), 5.10–5.05 (m, 1H, H<sub>1</sub>), 2.62–2.57 (m, 2H, H<sub>2a</sub> + H<sub>5a</sub>), 2.35–2.30 (m, 2H, H<sub>3a</sub> + H<sub>5b</sub>), 2.13 (d, *J* = 16.0 Hz, 2H, H<sub>6</sub>), 2.11–2.05 (m, 2H, H<sub>2b</sub> + H<sub>3b</sub>). <sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O)  $\delta$  160.0 (C<sub>4</sub>), 156.2 (C<sub>2</sub>), 151.3 (C<sub>6</sub>), 138.4 (C<sub>8</sub>), 113.3 (C<sub>5</sub>), 79.3 (C<sub>1</sub>), 53.9 (C<sub>1</sub>), 46.4 (d, *J* = 7.7 Hz, C<sub>5</sub>), 39.0 (d, *J* = 127.5 Hz, C<sub>6</sub>), 38.6 (d, *J* = 9.1 Hz, C<sub>3</sub>), 30.6 (C<sub>2</sub>). <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O)  $\delta$  19.3. MS (ESI<sup>+</sup>): *m/z* = 351.1 [M+H]<sup>+</sup>. HRMS (ESI<sup>+</sup>) calculated for C<sub>11</sub>H<sub>17</sub>N<sub>6</sub>O<sub>4</sub>NaP [M+H]<sup>+</sup>: 351.0956; found: 351.0947.

#### 4.6.5. Protocol E

To a solution of carbonucleoside phosphonate (1.0 eq) in dimethylformamide (10 mL/mmol) at 0 °C was added dropwise trimethylsilyl bromide (10 eq). The reaction was stirred 1 h at 0 °C and then 23h at rt. After addition of TEAB (1 M) until pH 7, evaporation of solvents and purification by reversed phase chromatography (H<sub>2</sub>O/MeOH 1:0 to 1:1) followed by Dowex chromatography (Na<sup>+</sup>). The desired product obtained after lyophilization as white foam.

4.6.5.1. ( $\pm$ )-((3-(2-Chloro-6-methoxy-9H-purin-9-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt, ( $\pm$ )-6a. The product was obtained (140 mg, 71%) according to the Protocol E using compound ( $\pm$ )-5a (200 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/2/2): 0.43. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O)  $\delta$  8.37 (s, 1H, H<sub>8</sub>), 5.26–4.97 (m, 1H, H<sub>1</sub>), 4.12 (s, 3H, OCH<sub>3</sub>), 2.63–2.50 (m, 2H), 2.36–2.26 (m, 2H), 2.17 (d, *J* = 17.1 Hz, 2H, H<sub>6</sub>), 2.11–1.98 (m, 2H). <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O)  $\delta$  160.9 (COCH<sub>3</sub>), 152.2 (C<sub>4</sub>), 152.0 (C<sub>2</sub>), 142.7 (C<sub>8</sub>), 119.5 (C<sub>5</sub>), 78.8 (d, *J* = 4.5 Hz, C<sub>1</sub>), 55.4 (OCH<sub>3</sub>), 54.7 (C<sub>1</sub>), 45.8 (d, *J* = 7.9 Hz, C<sub>5</sub>), 38.3 (d, *J* = 128.6 Hz, C<sub>6</sub>), 38.0 (d, *J* = 8.7 Hz, C<sub>3</sub>), 30.2 (C<sub>2</sub>). <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O)  $\delta$  20.2. MS (ESI<sup>-</sup>): *m/z* = 361.0 [M<sup>-</sup> Na<sup>+</sup>]. HRMS (ESI<sup>-</sup>) calculated for C<sub>12</sub>H<sub>15</sub>N<sub>4</sub>O<sub>5</sub>ClP [M<sup>-</sup> Na<sup>+</sup>]: 361.0938; found: 361.0938.

4.6.5.2. (((1S,3R)-3-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt, (+)-2'. The product was obtained (168 mg, 79%) according to the Protocol E using compound (-)-6g\* (224 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/2/2): 0.46. <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  8.02 (s, 1H, H<sub>8</sub>), 4.89 (dtd, *J* = 10.2, 8.3, 4.6 Hz, 1H, H<sub>1</sub>), 2.61 (dd, *J* = 14.9, 10.2 Hz, 1H, H<sub>2a</sub>), 2.41 (dtd, *J* = 13.0, 7.8, 2.1 Hz, 1H, H<sub>2a</sub>), 2.22–2.16 (m, 1H, H<sub>5a</sub>), 2.15–2.05 (m, 4H, H<sub>3a</sub> + H<sub>5b</sub> + H<sub>6</sub>), 1.96 (ddd, *J* = 13.4, 11.9, 7.8 Hz, 1H, H<sub>3b</sub>). <sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O)  $\delta$  158.8 (C=O), 153.4 (C<sub>2</sub>), 151.1 (C<sub>4</sub>), 138.8 (C<sub>8</sub>), 115.9 (C<sub>5</sub>), 78.7 (d, *J* = 4.2 Hz, C<sub>1</sub>), 53.7 (C<sub>1</sub>), 45.7 (d, *J* = 7.8 Hz, C<sub>5</sub>), 39.2 (d, *J* = 8.7 Hz, C<sub>3</sub>), 38.5 (d, *J* = 128.8 Hz, C<sub>6</sub>), 31.2 (C<sub>2</sub>). <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O)  $\delta$  20.18. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +7 (c = 1.00, H<sub>2</sub>O). MS (ESI<sup>+</sup>):

*m/z* = 330.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>11</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 330.0962; Found: 330.0978.

4.6.5.3. (((1S,3S)-3-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt, (-)-2. The product was obtained (169 mg, 49%) according to the Protocol E using compound (+)-6g (350 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/3/1): 0.35. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  7.86 (s, 1H, C<sub>8</sub>), 4.90–4.82 (m, 1H, H<sub>1</sub>), 2.45–2.35 (m, 2H), 2.23–2.16 (m, 2H), 2.08 (d, *J* = 17.0 Hz, 2H, H<sub>6</sub>), 1.96–1.87 (m, 2H). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  158.8 (C=O), 153.3 (C<sub>2</sub>), 151.3 (C<sub>4</sub>), 138.0 (C<sub>8</sub>), 115.9 (C<sub>5</sub>), 78.8 (d, *J* = 4.5 Hz, C<sub>1</sub>), 53.8 (C<sub>1</sub>), 45.8 (d, *J* = 7.7 Hz, C<sub>5</sub>), 38.2 (d, *J* = 129.0 Hz, C<sub>6</sub>), 37.9 (d, *J* = 8.7 Hz, C<sub>3</sub>), 30.1 (C<sub>2</sub>). <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O)  $\delta$  20.4. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -4.5 (c = 1.00, H<sub>2</sub>O). MS (ESI<sup>+</sup>): *m/z* = 330.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>11</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>P [M+H]<sup>+</sup>: 330.0962; Found: 330.0980.

4.6.5.4. (((1R,3S)-3-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt (-)-2'. The product was obtained (90 mg, 87%) according to the Protocol E using compound (+)-6g\* (110 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/2/2): 0.46. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -6.9 (c = 1.00, H<sub>2</sub>O)

4.6.5.5. (((1R,3R)-3-(2-Amino-6-oxo-1H-purin-9(6H)-yl)-1-hydroxycyclopentyl) methyl)phosphonate, sodium salt (+)-2. The product was obtained (245 mg, 85%) according to the Protocol E using compound (-)-6g (307 mg).

R<sub>f</sub> (iPrOH/NH<sub>4</sub>OH/H<sub>2</sub>O: 6/3/1): 0.35. [ $\alpha$ ]<sub>D</sub><sup>20</sup> = +4.6 (c = 1.00, H<sub>2</sub>O).

#### 4.6.6. Procedure for the deracemization of cis-4-O-TBScyclopent-2-enol ( $\pm$ )-8

To a mixture of racemic 4-((tert-butyl)dimethylsilyloxy)cyclopent-2-enol (+/-)-8 (1 g, 4.66 mmol), dicyclohexylcarbodiimide (1.45g, 7 mmol) and dimethylamino-pyridine (57 mg, 0.46 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> under atmosphere of argon was added (R)-O-acetyl mandelic acid (1.36 g, 7 mmol). After 2 h, a total conversion was observed by TLC. Filtration of the DCU, gave a filtrate which was concentrated and preliminary purification was performed using petroleum ether and CH<sub>2</sub>Cl<sub>2</sub> (1/1: v/v) as isocratic, the mixture of the 2 diastereoisomers was obtained with an excellent yield (1.7 g, 93%). The best conditions of purification were obtained with pre-packed silica gel column from Biotage (40  $\mu$ m, 80 g) using as eluent petroleum ether and dichloromethane (2 CV at 0%, 7 CV at 20%, 10 CV at 30% and 15 CV at 50% of dichloromethane). When 500 mg of the mixture of diastereoisomers were injected, three fractions (F<sub>1</sub>: 230 mg; F<sub>2</sub>: 17 mg(mixture); F<sub>3</sub>: 226 mg) were isolated.

4.6.6.1. Fast eluting compound (F<sub>1</sub>): (R)-(1R,4S)-4-((tert-butyl)dimethylsilyloxy)cyclopent-2-en-1-yl 2-acetoxy-2-phenylacetate (-)-9. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.46 (dd, *J* = 6.7, 2.9 Hz, 2H, H<sub>Ar</sub>), 7.40–7.37 (m, 3H, H<sub>Ar</sub>), 5.94 (dt, *J* = 5.7, 1.6 Hz, 1H, H<sub>alcene</sub>), 5.89 (s, 1H, H<sub>ester</sub>), 5.72 (dt, *J* = 5.6, 1.6 Hz, 1H, H<sub>alcene</sub>), 5.50 (ddt, *J* = 7.4, 5.0, 1.4 Hz, 1H, CHO), 4.72–4.68 (m, 1H, CHO), 2.80 (dt, *J* = 13.9, 6.9 Hz, 1H, H<sub>5</sub>), 2.19 (s, 3H, CH<sub>3</sub>), 1.66 (dt, *J* = 13.9, 5.2 Hz, 1H, H<sub>5b</sub>), 0.89 (s, 9H, 3CH<sub>3</sub>), 0.08 (s, 6H, 2CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  170.5 (C<sub>ester</sub>), 168.8 (C<sub>ester</sub>), 139.5 (C<sub>alcene</sub>), 133.9 (C<sub>Ar</sub>), 130.6 (C<sub>alcene</sub>), 129.3 (CH<sub>Ar</sub>), 128.9 (2CH<sub>Ar</sub>), 127.7 (2CH<sub>Ar</sub>), 78.3 (CHO), 74.9 (CHO), 74.8 (CHOAc), 41.1 (CH<sub>2</sub>), 26.0 (3CH<sub>3</sub>), 20.9 (CH<sub>3</sub>), 18.3 (C<sub>q</sub>), -4.5 (CH<sub>3</sub>), -4.6 (CH<sub>3</sub>). [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -21.1 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 413.2 (M + Na)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>21</sub>H<sub>31</sub>O<sub>5</sub>Si [M+H]<sup>+</sup>: 391.1935; Found: 391.1939.

4.6.6.2. *Slow eluting compound (F<sub>3</sub>): (R)-(1S,4R)-4-((tert-butylidimethylsilyloxy)cyclopent-2-en-1-yl 2-acetoxy-2-phenylacetate (-)-9'*. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.48–7.45 (m, 2H, H<sub>Ar</sub>), 7.41–7.36 (m, 3H, H<sub>Ar</sub>), 5.97 (dt, *J* = 5.7, 1.6 Hz, 1H, H<sub>alcene</sub>), 5.90–5.88 (m, 2H, H<sub>alcene</sub> + H<sub>α-ester</sub>), 5.54–5.50 (m, 1H, CHO), 4.67 (dddd, *J* = 8.0, 4.5, 2.2, 1.1 Hz, 1H, CHO), 2.70 (dt, *J* = 13.8, 7.3 Hz, 1H, H<sub>5a</sub>), 2.19 (s, 3H, CH<sub>3</sub>), 1.41 (dt, *J* = 13.9, 5.0 Hz, 1H), 0.86 (s, 9H, 3CH<sub>3</sub>), 0.05 (s, 3H, CH<sub>3</sub>), 0.03 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 170.5 (C<sub>ester</sub>), 168.7 (CH<sub>ester</sub>), 139.5 (C<sub>alcene</sub>), 133.8 (C<sub>Ar</sub>), 130.8 (CH<sub>alcene</sub>), 129.3 (CH<sub>Ar</sub>), 128.9 (2CH<sub>Ar</sub>), 127.7 (2CH<sub>Ar</sub>), 78.3 (CHO), 74.9 (CHO), 74.7 (CHOAc), 40.8 (CH<sub>2</sub>), 26.0 (3CH<sub>3</sub>), 20.9 (CH<sub>3</sub>), 18.3 (C<sub>q</sub>), -4.5 (CH<sub>3</sub>), -4.6 (CH<sub>3</sub>). [α]<sub>D</sub><sup>20</sup> = -60.8 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 413.2 (M + Na)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>21</sub>H<sub>31</sub>O<sub>5</sub>Si [M+H]<sup>+</sup>: 391.1935; Found: 391.1937.

To a solution of each diastereoisomer previously isolated F<sub>1</sub> or F<sub>3</sub> (400 mg, 1.02 mmol) in THF/H<sub>2</sub>O (1/1: v/v), at rt, was added lithium hydroxide (123 mg, 5.12 mmol). After 1 h stirring, completion of the reaction was observed by TLC. A saturated solution of NH<sub>4</sub>Cl (20 mL) and CH<sub>2</sub>Cl<sub>2</sub> (20 mL) were added, the layers were separated and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4x20 mL). The organic layers were combined, dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. After flash silica gel chromatography (PE/EtOAc: 1/0 to 4/1), the products were obtained with 98% (from F<sub>1</sub>, 210 mg) and 92% (from F<sub>3</sub>, 201 mg).

4.6.6.3. *From fast eluting compound ((-)-9): (1R,4S)-4-((tert-butylidimethylsilyloxy)cyclopent-2-enol, (-)-8 [13]*. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.94 (dt, *J* = 5.6, 1.5 Hz, 1H), 5.88 (dt, *J* = 5.6, 1.6 Hz, 1H), 4.69–4.62 (m, 1H), 4.60–4.57 (m, 1H), 2.68 (dt, *J* = 13.9, 7.1 Hz, 1H), 1.51 (dt, *J* = 13.7, 4.5 Hz, 1H), 0.89 (s, 9H), 0.09 (s, 6H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 137.1 (CH<sub>alcene</sub>), 135.7 (CH<sub>alcene</sub>), 75.3 (CHO), 75.3 (CHO), 44.8 (CH<sub>2</sub>), 26.0 (3CH<sub>3</sub>), 18.3 (C<sub>q</sub>), -4.5 (CH<sub>3</sub>). [α]<sub>D</sub><sup>20</sup> = -21.1 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 215.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>11</sub>H<sub>23</sub>O<sub>2</sub>Si [M+H]<sup>+</sup>: 215.1469; Found: 215.1462.

4.6.6.4. *From slow eluting compound ((-)-9'): (1S,4R)-4-((tert-butylidimethylsilyloxy)cyclopent-2-enol (+)-8 [13]*. [α]<sub>D</sub><sup>20</sup> = +21.4 (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.7. Protocol F (Oxidation with DMP)

To a solution of the alcohol (1.0 eq) in dichloromethane (14 mL/mmol) were added DESS-MARTIN-Periodinane (1.25eq.) at rt, and the reaction mixture was stirred for 2h. The reaction was stopped by adding a 1:1 mixture of sodium thiosulfate (1 M) and a saturated aqueous solution of sodium hydrogen carbonate. The aqueous layer was extracted with dichloromethane; the combined organic layers were dried over MgSO<sub>4</sub> and concentrated. Purification on silica gel (PE/EtOAc: 1.0 to 9/1) provided the desired product.

4.6.7.1. *(S)-4-((tert-Butylidimethylsilyloxy)cyclopent-2-enone (-)-10*. The product was obtained (1.29 g, 87%), as colorless oil, according to Protocol F using 1.5 g of (-)-8.

R<sub>f</sub> (PE/EtOAc: 9/1) = 0.4. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.45 (dd, *J* = 5.7, 2.3 Hz, 1H, H<sub>3</sub>), 6.18 (dd, *J* = 5.7, 1.3 Hz, 1H, H<sub>3</sub>), 4.98 (dtd, *J* = 5.9, 2.2, 1.3 Hz, 1H, H<sub>4</sub>), 2.70 (dd, *J* = 18.2, 6.0 Hz, 1H, H<sub>5</sub>), 2.24 (dd, *J* = 18.2, 2.2 Hz, 1H, H<sub>5b</sub>), 0.91 (s, 9H, 3CH<sub>3</sub>), 0.13 (s, 3H, CH<sub>3</sub>), 0.12 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 206.7 (C<sub>1</sub>), 164.0 (C<sub>3</sub>), 134.6 (C<sub>2</sub>), 71.0 (C<sub>4</sub>), 45.1 (C<sub>5</sub>), 25.9 (3CH<sub>3</sub>), 18.2 (C<sub>q</sub>), -4.6 (CH<sub>3</sub>), -4.6 (CH<sub>3</sub>). [α]<sub>D</sub><sup>20</sup> = -49.6 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 213.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>11</sub>H<sub>21</sub>O<sub>2</sub>Si [M+H]<sup>+</sup>: 213.1305; Found: 213.1319.

4.6.7.2. *(R)-4-((tert-Butylidimethylsilyloxy)cyclopent-2-enone (+)-10*. The product was obtained (1.23 g, 94%), as colorless oil, according to the Protocol F using compound (+)-8 (1.32 g).

R<sub>f</sub> (PE/EtOAc: 9/1) = 0.48. [α]<sub>D</sub><sup>20</sup> = +46.1 (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.8. Protocol G (Preparation of the carbocyclic phosphonate)

To a solution of dimethyl methylphosphonate (1.0 eq) in THF (4 mL/mmol) at -78 °C was added dropwise a solution of *n*-BuLi (1.0 eq 2.5 M in THF). The mixture was then stirred at -78 °C for 20 min and a solution of cyclopentenone (1.0 eq, in THF) was added dropwise. The reaction mixture was stirred for 2 h at rt, and then quenched by the addition of a saturated aqueous solution of ammonium chloride. The aqueous phase was extracted with diethyl ether twice, the combined organic layers were dried over MgSO<sub>4</sub> and concentrated. The crude was purified by silica gel column chromatography (PE/EtOAc 100:0 to 0:100) giving rise to the desired product.

4.6.8.1. *Dimethyl (((1R,4S)-4-((tert-butylidimethylsilyloxy)-1-hydroxycyclopent-2-en-1-yl)methyl) phosphonate (-)-11*. The product was obtained (1.69 g, 85%), as colorless oil, according to the Protocol G using compound (-)-10 (1.26 g).

R<sub>f</sub> (EtOAc) = 0.38. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.99 (dd, *J* = 5.7, 1.2 Hz, 1H, H<sub>alcene</sub>), 5.83 (dd, *J* = 5.6, 1.9 Hz, 1H, H<sub>alcene</sub>), 4.71 (ddt, *J* = 6.4, 4.5, 1.5 Hz, 1H), 3.75 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 3.74 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 2.54 (ddd, *J* = 13.7, 6.7, 0.6 Hz, 1H, H<sub>5a</sub>), 2.17–2.06 (m, 2H, CH<sub>2</sub>P), 1.90 (ddd, *J* = 13.6, 4.5, 2.3 Hz, 1H, H<sub>5b</sub>), 0.87 (s, 9H, 3CH<sub>3</sub>), 0.07 (s, 6H, 2CH<sub>3</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 138.2 (d, *J* = 8.8 Hz, C<sub>2</sub>), 136.0 (C<sub>3</sub>), 80.2 (d, *J* = 5.0 Hz, C<sub>1</sub>), 75.1 (C<sub>4</sub>), 52.5 (d, *J* = 6.3 Hz, OCH<sub>3</sub>), 52.5 (d, *J* = 6.4 Hz, OCH<sub>3</sub>), 50.1 (d, *J* = 9.5 Hz, C<sub>5</sub>), 36.0 (d, *J* = 136.4 Hz, CH<sub>2</sub>P), 26.0 (3CH<sub>3</sub>), 18.2 (C<sub>q</sub>), -4.5 (2CH<sub>3</sub>). <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>) δ 31.0. [α]<sub>D</sub><sup>20</sup> = -57.4 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 337.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>14</sub>H<sub>30</sub>O<sub>5</sub>PSi [M+H]<sup>+</sup>: 337.1595; Found: 337.1604.

4.6.8.2. *Dimethyl (((1S,4R)-4-((tert-butylidimethylsilyloxy)-1-hydroxycyclopent-2-en-1-yl)methyl) phosphonate (+)-11*. The product was obtained (1.69 g, 88%), as colorless oil, according to the Protocol G using compound (+)-10 (1.21 g). R<sub>f</sub> (EtOAc) = 0.38. [α]<sub>D</sub><sup>20</sup> = +50.1 (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.9. Protocol H (Reduction of the cyclopentenyl alcohol)

To a solution of the cyclopentenyl methylphosphonate (1 eq) in methanol (4 mL/mmol) was added Pd/C (10%, 0.5% eq). The reaction mixture was stirred under hydrogen at atmospheric pressure overnight at rt. The reaction mixture was filtrated through Celite and the filtrate was concentrated under vacuum. Chromatography on silica gel (PE/EtOAc: 1/0 to 0/1) give rise to the desired product.

4.6.9.1. *Dimethyl (((1S,3R)-3-((tert-butylidimethylsilyloxy)-1-hydroxycyclopentyl)methyl) phosphonate (-)-12*. The product was obtained (1.59 g, 97%), as colorless oil, according to the Protocol g using compound (-)-11 (1.65 g).

R<sub>f</sub> (DCM/MeOH: 95/5) = 0.4. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.39–4.36 (m, 1H, H<sub>3</sub>), 4.07 (s, 1H, OH), 3.75 (d, *J* = 10.9, 3H, OCH<sub>3</sub>), 3.75 (d, *J* = 10.9, 3H, OCH<sub>3</sub>), 2.16 (ddd, *J* = 18.4, 1.3, 0.7 Hz, 2H, CH<sub>2</sub>P), 2.11 (ddt, *J* = 10.0, 5.0, 2.4 Hz, 1H), 2.00 (dq, *J* = 13.9, 2.0 Hz, 1H), 1.96–1.80 (m, 4H), 0.87 (s, 9H, 3CH<sub>3</sub>), 0.06 (s, 6H, 2CH<sub>3</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 79.0 (d, *J* = 4.5 Hz, C<sub>1</sub>), 74.7 (C<sub>3</sub>), 52.4 (d, *J* = 6.3 Hz, OCH<sub>3</sub>), 52.3 (d, *J* = 6.3 Hz, OCH<sub>3</sub>), 49.2 (d, *J* = 8.5 Hz, C<sub>2</sub>), 39.6 (d, *J* = 8.7 Hz, C<sub>5</sub>), 36.1 (d, *J* = 138.5 Hz, CH<sub>2</sub>P), 34.6 (C<sub>4</sub>), 25.9 (3CH<sub>3</sub>), 18.1 (C<sub>q</sub>), -4.8 (2CH<sub>3</sub>). <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>) δ 31.4. [α]<sub>D</sub><sup>20</sup> = -1.4 (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 339.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>14</sub>H<sub>32</sub>O<sub>5</sub>PSi [M+H]<sup>+</sup>: 339.1751; Found: 339.1761.



4.6.9.2. *Dimethyl ((1R,3S)-3-((tert-butyl dimethylsilyl)oxy)-1-hydroxycyclopentyl)methyl phosphonate (+)-12*. The product was obtained (1.55 g, 93%), as colorless oil, according to the Protocol H using compound (+)-11 (1.67 g).

$R_f$  (DCM/MeOH: 95/5) = 0.4.  $[\alpha]_D^{20} = +1.1$  (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.10. Protocol I (Acetylation of the tertiary alcohol)

To a solution of cyclopentyl methylphosphonate (1 eq) in dry THF (3 mL/mmol) at rt was successively added DMAP (0.1 eq), triethylamine (1.5 eq) and acetic anhydride (2.5 eq). The reaction mixture was stirred for 6 days and then concentrated. The crude was diluted with ethyl acetate and washed twice with saturated sodium carbonate aqueous solution, and then brine. The organic layer was dried over MgSO<sub>4</sub> and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (DCM/MeOH 1:0 to 98:2) to give rise to the desired product.

##### 4.6.10.1. (1S,3R)-3-((tert-Butyldimethylsilyl)oxy)-1-

((dimethoxyphosphoryl)methyl)cyclopentyl acetate (+)-13. The product was obtained (1.52 g, 87%), as colorless oil, according to the Protocol I using compound (-)-12 (1.56 g).

$R_f$  (DCM/MeOH: 95/5) = 0.28. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 4.27–4.23 (m, 1H, H<sub>3</sub>), 3.72 (d, *J* = 10.9 Hz, 6H, 2OCH<sub>3</sub>), 2.67 (dd, *J* = 18.9, 15.5 Hz, 1H, CH<sub>2</sub>P), 2.46 (dd, *J* = 19.6, 15.5 Hz, 1H, CH<sub>2</sub>P), 2.38–2.32 (m, 1H, H<sub>5a</sub>), 2.30–2.26 (m, 1H, H<sub>2a</sub>), 2.19 (dd, *J* = 14.9, 6.8 Hz, 1H, H<sub>2b</sub>), 2.00 (s, 3H, CH<sub>3</sub>), 1.93–1.86 (m, 2H, H<sub>4a</sub> + H<sub>5b</sub>), 1.77–1.70 (m, 1H, H<sub>4b</sub>), 0.86 (s, 9H, 3CH<sub>3</sub>), 0.02 (s, 3H, CH<sub>3</sub>), 0.02 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 171.2 (C=O), 85.8 (d, *J* = 4.4 Hz, C<sub>1</sub>), 72.3 (C<sub>3</sub>), 52.4 (d, *J* = 6.2 Hz, OCH<sub>3</sub>), 52.4 (d, *J* = 6.8 Hz, OCH<sub>3</sub>), 48.3 (d, *J* = 8.8 Hz, C<sub>2</sub>), 37.7 (d, *J* = 6.1 Hz, C<sub>5</sub>), 34.5 (C<sub>4</sub>), 32.9 (d, *J* = 138.5 Hz, CH<sub>2</sub>P), 25.9 (3CH<sub>3</sub>), 22.3 (CH<sub>3</sub>), 18.2 (C<sub>9</sub>), -4.7 (CH<sub>3</sub>), -4.7 (CH<sub>3</sub>). <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>) δ 29.1.  $[\alpha]_D^{20} = +2.7$  (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 381.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>16</sub>H<sub>34</sub>O<sub>6</sub>PSi [M + H]<sup>+</sup>: 381.1857; Found: 381.1884.

##### 4.6.10.2. (1R,3S)-3-((tert-Butyldimethylsilyl)oxy)-1-

((dimethoxyphosphoryl)methyl)cyclopentyl acetate (-)-13. The product was obtained (1.59 g, 93%), as colorless oil, according to the Protocol I using compound (+)-12 (1.53 g).

$R_f$  (DCM/MeOH: 95/5) = 0.28.  $[\alpha]_D^{20} = -4.1$  (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.11. Protocol J (Removal of the TBS protecting group)

To a solution of the cyclopentyl dimethyl phosphonate (1.0 eq) in MeOH (2.5 mL/mmol) at rt was added slowly a solution of Et<sub>3</sub>N·3HF (2.0 eq). The reaction mixture was stirred overnight and the volatiles were evaporated. Purification of the residue by silica gel column chromatography (DCM/MeOH 1:0 to 96:4) gave the desired product as a white solid.

##### 4.6.11.1. (1S,3R)-1-((Dimethoxyphosphoryl)methyl)-3-

hydroxycyclopentyl acetate (+)-3. The product was obtained (1 g, 96%) according to the Protocol J using compound (+)-13 (1.49 g).

$R_f$  (DCM/MeOH: 95/5) = 0.2. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.34 (tq, *J* = 6.6, 3.2 Hz, 1H, H<sub>3</sub>), 3.72 (d, *J* = 10.9 Hz, 6H, 2OCH<sub>3</sub>), 2.61 (dd, *J* = 19.1, 15.5 Hz, 1H, CHP), 2.52 (dd, *J* = 19.4, 15.5 Hz, 1H, CHP), 2.51–2.45 (m, 1H), 2.35 (dp, *J* = 15.3, 1.5 Hz, 1H), 2.20 (dd, *J* = 15.3, 6.7 Hz, 1H), 2.03 (s, 3H, CH<sub>3</sub>), 2.04–1.87 (m, 2H), 1.78 (dddd, *J* = 12.6, 11.3, 5.7, 3.3 Hz, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 171.1 (C=O), 86.8 (d, *J* = 5.1 Hz, C<sub>1</sub>), 72.4 (C<sub>3</sub>), 52.5 (d, *J* = 6.7 Hz, 2OCH<sub>3</sub>), 48.0 (d, *J* = 6.9 Hz, C<sub>2</sub>), 37.3 (d, *J* = 8.0 Hz, C<sub>5</sub>), 34.2 (C<sub>4</sub>), 32.8 (d, *J* = 139.4 Hz, CH<sub>2</sub>P), 22.3 (CH<sub>3</sub>). <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>) δ 28.7.  $[\alpha]_D^{20} = +3.6$  (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 267.1

(M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>10</sub>H<sub>20</sub>O<sub>6</sub>P [M + H]<sup>+</sup>: 267.0992; Found: 267.1016.

##### 4.6.11.2. (1R,3S)-1-((Dimethoxyphosphoryl)methyl)-3-

hydroxycyclopentyl acetate (-)-3. The product was obtained (1.02 g, 96%) according to the Protocol J using compound (-)-13 (1.52 g).

$R_f$  (DCM/MeOH: 95/5) = 0.2.  $[\alpha]_D^{20} = -5.1$  (c = 1.00, CHCl<sub>3</sub>).

#### 4.6.12. Protocol K (Two-step one pot inversion of stereochemistry via Mitsunobu reaction)

To a solution of alcohol (1.0 eq), triphenylphosphine (2.0 eq) and formic acid (2.0 eq) in THF (3 mL/mmol), and at 0 °C, was slowly added DIAD (2.0 eq). The resulting mixture was stirred at this temperature for 1 h. After completion of the reaction a saturated solution of NaHCO<sub>3</sub> was added. The resulting mixture was extracted with ethyl acetate and the combined organic layers were dried over MgSO<sub>4</sub> and concentrated, affording the corresponding intermediate. After filtration on a pad of silica gel, the mixture of this last and Et<sub>3</sub>N (5% eq) in MeOH (2 mL/mmol) was stirred for 1.5h at rt. The resulting reaction mixture was concentrated affording a crude, which was purified by chromatography on silica gel using CH<sub>2</sub>Cl<sub>2</sub> to CH<sub>2</sub>Cl<sub>2</sub>/MeOH (96/4) as gradient.

##### 4.6.12.1. (1S,3S)-1-((Dimethoxyphosphoryl)methyl)-3-

hydroxycyclopentyl acetate (+)-3'. The product was obtained (400 mg, 74%), as white solid, according to the Protocol K using compound (+)-3 (540 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.2. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.38 (tt, *J* = 5.7, 2.7 Hz, 1H, H<sub>3</sub>), 3.72 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 3.72 (d, *J* = 11.0 Hz, 3H, OCH<sub>3</sub>), 2.78 (dd, *J* = 19.1, 15.5 Hz, 1H, CHP), 2.69 (dd, *J* = 19.3, 15.5 Hz, 1H, CHP), 2.44 (dd, *J* = 15.3, 6.1 Hz, 1H, H<sub>2a</sub>), 2.24–2.16 (m, 3H, H<sub>2b</sub> + H<sub>5</sub>), 1.99 (s, 3H, CH<sub>3</sub>), 2.03–1.92 (m, 1H, H<sub>4a</sub>) 1.71 (dddt, *J* = 13.4, 6.7, 4.3, 2.0 Hz, 1H, H<sub>4b</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 171.1 (C=O), 87.0 (d, *J* = 3.7 Hz, C<sub>1</sub>), 72.2 (C<sub>3</sub>), 52.5 (t, *J* = 7.2 Hz, 2OCH<sub>3</sub>), 48.7 (d, *J* = 6.7 Hz, C<sub>2</sub>), 36.9 (d, *J* = 6.5 Hz, C<sub>5</sub>), 34.0 (C<sub>4</sub>), 32.9 (d, *J* = 138.1 Hz, CH<sub>2</sub>P), 22.2 (CH<sub>3</sub>). <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>) δ 29.5.  $[\alpha]_D^{20} = +1$  (c = 1.00, CHCl<sub>3</sub>). MS (ESI<sup>+</sup>): *m/z* = 267.1 (M + H)<sup>+</sup>. HRMS (ESI<sup>+</sup>): calculated for C<sub>10</sub>H<sub>20</sub>O<sub>6</sub>P [M + H]<sup>+</sup>: 267.0992; Found: 267.1024.

##### 4.6.12.2. (1R,3R)-1-((Dimethoxyphosphoryl)methyl)-3-

hydroxycyclopentyl acetate (-)-3'. The product was obtained (465 mg, 89%), as white solid, according to the Protocol K using compound (1R,3S)-(-)-3 (527 mg).

$R_f$  (DCM/MeOH: 95/5) = 0.2.  $[\alpha]_D^{20} = -1$  (c = 1.00, CHCl<sub>3</sub>).

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nushin Aghajari reports financial support was provided by French National Research Agency.

#### Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2023.115581>.

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