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Original article

6-(Hetero)Arylpurine nucleotides as inhibitors of the oncogenic target DNPH1: Synthesis, structural studies and cytotoxic activities

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ABSTRACT

The 2′-deoxynucleoside 5′-phosphate *N*-hydrolase 1 (DNPH1) has been proposed as a new molecular target for cancer treatment. Here, we describe the synthesis of a series of novel 6-aryl- and 6-heteroarylpurine riboside 5′-monophosphates *via* Suzuki–Miyaura cross-coupling reactions, and their ability to inhibit recombinant rat and human DNPH1. Enzymatic inhibition studies revealed competitive inhibitors in the low micromolar range. Crystal structures of human and rat DNPH1 in complex with one nucleotide from this series, the 6-naphthylpurine derivative, provided detailed structural information, in particular regarding the possible conformations of a long and flexible loop wrapping around the large hydrophobic substituent. Taking advantage of these high-resolution structures, we performed virtual docking studies in order to evaluate enzyme–inhibitor interactions for the whole compound series. Among the synthesized compounds, several molecules exhibited significant *in vitro* cytotoxicity against human colon cancer (HCT15, HCT116) and human promyelocytic leukemia (HL60) cell lines with IC_{50} values in the low micromolar range, which correlated with *in vitro* DNPH1 inhibitory potency.

1. Introduction

Deregulated expression of *c-myc* plays a significant role in human cancer development [1]. The mechanisms by which c-Myc induces neoplastic transformation and apoptosis have been the subject of several studies. As a result, a growing number of genes with different functional classes have been identified as targets for regulation by c-Myc [1–3]. Among the putative *c-Myc*-responsive *genes*, *rcl* (C6orf108) has been recognized as a growth-related gene [4,5]. Up-regulation of *rcl* has been observed in various human cancers, including breast [4,6], prostate [7] and colon carcinoma [8], as well as chronic lymphocytic leukemia [9]. In addition, *rcl* expression increases significantly with the tumor grade, strongly connecting *rcl* to breast tumorigenesis [6]. However, direct evidence for its role in cancer development is still lacking [5].

The *rcl* encoded protein, recently renamed DNPH1, was identified as an original nucleotide hydrolase that catalyzes the *N*-glycosidic cleavage of a 2′-deoxynucleoside 5′-monophosphate into a free base and 2-deoxyribose-5-phosphate, while the corresponding ribonucleotide acts as competitive inhibitor [10]. The NMR solution structures of rat DNPH1, in the *apo* form [11] and in complex with guanosine 5′-monophosphate GMP [12], gave the first structural insights into the substrate recognition. In addition, the substrate specificity was analyzed using rational site-directed mutagenesis and a first set of nucleotide nucleotide analogues [13,14].

We previously studied a series of N^6-substituted adenosine 5′-monophosphate (AMP) derivatives including some cytokinin ribosides known to inhibit proliferation of cancer cells and to induce apoptosis [15–17]. The pro-apoptotic effect of some N^6-substituted adenosine derivatives has been related to their intracellular conversion and accumulation as the corresponding nucleosides 5′-

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monophosphates in HL60 cells [18]. Our results indicated that N^6-substituted AMP derivatives inhibit DNPH1 in the micromolar range, supporting a possible implication of DNPH1 in their activity [19]. The crystallographic data of an engineered variant of rat DNPH1 (shortened and inactive form) bound to three N^6-substituted AMP derivatives (N^6-cyclopentyl, N^6-isopentenyl, N^6-furfuryl) [19,20] revealed tight interactions with the phosphate group and the ribose moiety of the co-crystallized ligands, while the modified nucleobases showed little interactions with the protein, the 6-alkyl substituents being mobile or in alternating conformations with apparently few van der Waals contacts [19]. Moreover the active site appeared to be largely open as the central loop (residues 47–59 in rat enzyme) lying at the entrance of the cavity was disordered.

With the aim of developing effective chemical tools for studying the biological role of DNPH1, we pursued the structure–activity relationships at the 6 position of the purine core by introducing less flexible substituents such as arenes and heteroarenes of various ring size and bearing diverse functional groups or different electronic characteristics to maximize additional interactions. In this paper, we describe the synthetic access to a series of C-6 aryl- and heteroarylpurine nucleosides as 5′-monophosphate derivatives (**1a**–**x**, Fig. 1), their inhibitory activities against recombinant rat and human DNPH1 and their cytotoxicity *in vitro*. Finally, we report the first crystal structure of human DNPH1 bound to a nucleotide from this series.

2. Results and discussion

2.1. Chemistry

The target nucleotides **1** were obtained following the synthetic routes illustrated in Scheme 1. Our initial strategy (route A) involved the palladium-catalyzed Suzuki–Miyaura cross-coupling reaction of 5′-phosphorylated 6-chloropurine derivative **3** with appropriate commercially available aryl- and heteroarylboronic acids. This key intermediate **3** was readily obtained in two steps from 6-chloropurine riboside (**2**) by isopropylidenation of the 2′,3′-diol [21], followed by 5′-phosphorylation with diethylchlorophosphate in anhydrous pyridine (65% overall yield).

Cross-coupling reactions of **3** with arylboronic acids were first attempted under conditions reported by Hocek et al. using 2′,3′,5′-triacetylated 6-chloropurine riboside [22–24]. As anticipated, reaction of **3** with 2-naphthylboronic acid in anhydrous conditions using $Pd(PPh_3)_4/K_2CO_3$ in toluene afforded compound **4a** in good yield (Table 1, entry 1). In the case of 3-cyanophenylboronic acid, the desired product **4b** was not formed under these conditions and only the oxygen-insertion product [25,26] **4c** was isolated (entry 2), while under aqueous conditions using dioxane/1 M aq. Cs_2CO_3 compound **4b** was obtained in 50% yield (entry 3). The use of a more efficient catalytic system based on $Pd(OAc)_2$ and 2-(dicyclohexylphosphino)biphenyl ligand (CyJohnPhos) [27] in dioxane/K_3PO_4 reduced the reaction time but gave similar yields in **4b** and **4c** (entry 4). When this catalytic system was used in aqueous conditions, both reaction time and yield were significantly improved and only the cross-coupling product **4b** was isolated in 70% yield (entry 5). A similar observation was done with 3-biphenylboronic acid as the reaction was complete in 80 min and **4d** formed in 71% yield (entries 6 & 7). These optimized conditions were successfully applied to the preparation of diverse substituted 6-phenylpurine derivatives **4e**–**k** (entries 8–14).

We next examined the Suzuki–Miyaura reaction of chloride **3** with heteroarylboronic acids. Reactions did not proceed well since compound **4l** was only isolated in 37% yield, even under an active catalyst system (Table 1, entry 15). Indeed, the intermediate **3** appeared to be substantially less reactive toward cross-coupling reaction with heteroarylboronic acids than triacetylated 6-chloropurine riboside [28]. The introduction of heteroarylboronic acids was successfully achieved starting from unprotected 6-chloropurine riboside (**2**) under aqueous Suzuki coupling

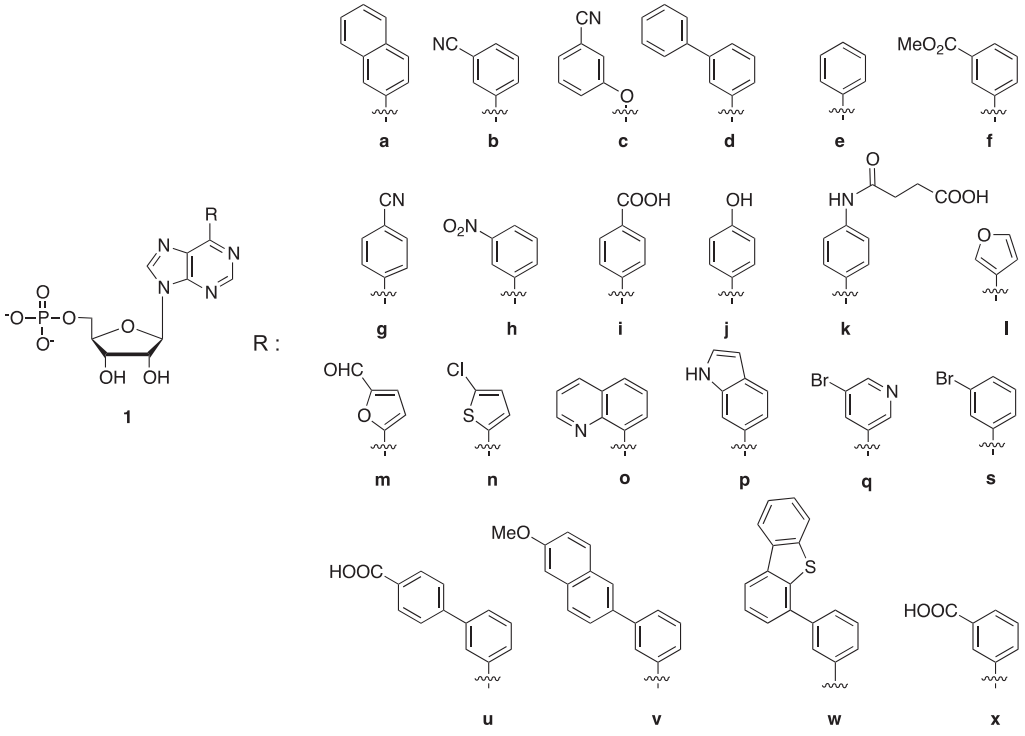
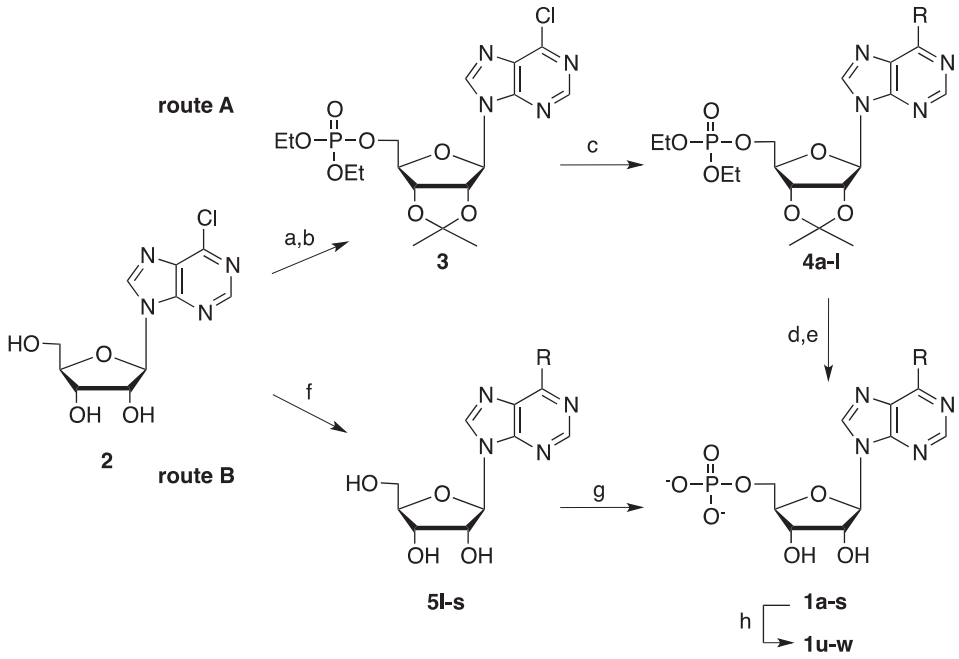


Fig. 1. Structures of 6-aryl and 6-heteroarylpurine ribonucleotides **1a**–**x**.



Scheme 1. Synthetic routes to the title nucleotides **1**. *Reagents and conditions*: (a) 2,2-dimethoxypropane, PTSA, acetone, rt, 3 h (85%); (b) chloro-diethylphosphate, pyridine, 0 °C, 2 h (76%); (c) R–$B(OH)_2$, catalyst, base, solvent, 100 °C, see Table 1 for conditions and yields; (d) TFA/H_2O (90/10), rt, 1 h (62–95%); (e) TMSBr, DMF, rt, 36 h (31–67%); (f) R–$B(OH)_2$, $Pd(OAc)_2$, TPPTS, H_2O/CH_3CN, 100 °C, 1–5 h (57–75%); (g) $POCl_3$, $PO(OEt)_3$, 4 °C, overnight (38–57%); (h) from **1s**, R–$B(OH)_2$, $Pd(OAc)_2$, TPPTS, H_2O/CH_3CN, 80 °C, 1–2 h (47–66%).

conditions [29,30] using $Pd(OAc)_2$ and a water-soluble phosphine (triphenylphosphine-3,3′,3″-trisulfonic acid trisodium salt, TPPTS) in the presence of aqueous Na_2CO_3 (Scheme 1, route B). The 6-heteroarylpurine ribosides **5l–q** were thus isolated in 40–75% yields (Table 2). While 3-bromophenylboronic acid failed to react with intermediate **3** under the conditions used above, reaction with **2** afforded the desired **5s** as the major product together with **5t** resulting from the self-condensation of the boronic acid.

Table 1
Suzuki–Miyaura cross-coupling reactions of **3** with R–$B(OH)_2$.

Entry	R	Condition[a]	Reaction time	Product	Yield %
1	2-Naphthyl	A	12 h	**4a**	62
2	3-Cyanophenyl	A	66 h	**4b**/**4c**	0/49[b]
3	3-Cyanophenyl	B	24 h	**4b**/**4c**	50/21
4	3-Cyanophenyl	C	7 h	**4b**/**4c**	52/11
5	3-Cyanophenyl	D	4 h	**4b**/**4c**	70/0
6	3-Biphenyl	B	12 h	**4d**	44
7	3-Biphenyl	D	1 h 20	**4d**	71
8	Phenyl	D	1 h 30	**4e**	76
9	3-Methoxycarbonylphenyl	D	4 h	**4f**	74
10	4-Cyanophenyl	D	1 h 30	**4g**	79
11	3-Nitrophenyl	D	1 h	**4h**	75
12	4-Carboxyphenyl	D	2 h	**4i**	83
13	4-Hydroxyphenyl	D	5 h	**4j**	56
14	4-Succinamidophenyl	D	1 h 30	**4k**	49[c]
15	3-Furanyl	D	30 h	**4l**	37[d]

[a] Reagents and conditions: Method A: R–$B(OH)_2$ (1.5 equiv), $Pd(PPh_3)_4$ (0.06 equiv), K_2CO_3 (1.5 equiv), anhydrous toluene, 100 °C; Method B: R–$B(OH)_2$ (1.2 equiv), $Pd(PPh_3)_4$ (0.06 equiv), 1 M aq. Cs_2CO_3 (2.4 equiv), dioxane, 100 °C; Method C: R–$B(OH)_2$ (1.5 equiv), $Pd(OAc)_2$ (0.10 equiv), CyJohnPhos (0.15 equiv), K_3PO_4 (2.0 equiv), anhydrous dioxane, 100 °C; Method D: R–$B(OH)_2$ (1.5 equiv), $Pd(OAc)_2$ (0.10 equiv), CyJohnPhos (0.15 equiv), 1 M aq. Cs_2CO_3 (2.0 equiv), dioxane, 100 °C.
[b] With 34% recovered starting material **3**.
[c] With 20% recovered **3**.
[d] With 25% recovered **3**.

The next steps in the synthetic path from compounds **4a–k** (Scheme 1, route A) were the removal of isopropylidene group by treatment with 90% aqueous TFA, followed by the cleavage of the ethyl esters with an excess of TMSBr in DMF to give the target nucleotides **1a–k** in 27–61% overall yields. For compound **4f**, the methyl ester was first removed by saponification (KOH/CH_3OH) to afford in three steps the 6-(3-carboxyphenyl)purine derivative **1x**. Following route B, selective phosphorylation [31] of nucleosides **5l–s** with $POCl_3$ in $PO(OEt)_3$ at 4 °C afforded the corresponding 5′-monophosphate derivatives **1l–s** in 38–57% yields. In addition, compound **1s** having a bromine atom was used as substrate for Suzuki–Miyaura reactions with boronic acids of medium to large ring sizes, providing bulky nucleotides **1u–w** (Table 2, entries 8–10). All the synthesized nucleotides **1** were purified by reverse phase high-performance liquid chromatography and isolated as sodium salts. The purity of all tested compounds was greater than 97%.

Table 2
Suzuki–Miyaura cross coupling reactions of **2** and **1s** with R–$B(OH)_2$.

Entry	R	Starting material	Reaction time[a]	Product (yield)
1	3-Furanyl	**2**	4 h	**5l** (60%)
2	5-Formyl-2-furanyl	**2**	2 h	**5m** (57%)
3	5-Chloro-2-thienyl	**2**	5 h	**5n** (68%)
4	8-Quinolinyl	**2**	4 h	**5o** (75%)
5	6-Indolyl	**2**	1 h	**5p** (73%)
6	5-Bromo-3-pyridinyl	**2**	1 h 30[b]	**5q** (40%), **5r** (9%)
7	3-Bromophenyl	**2**	2 h[b]	**5s** (65%), **5t** (16%)
8	4-Carboxyphenyl	**1s**	1 h[b]	**1u** (47%)[c]
9	6-Methoxy-2-naphthyl	**1s**	2 h[b]	**1v** (66%)[c]
10	4-Dibenzothienyl	**1s**	2 h[b]	**1w** (50%)[c]

[a] Reagents and conditions: R–$B(OH)_2$ (1.25 equiv), $Pd(OAc)_2$ (0.05 equiv), TPPTS (0.15 equiv), Na_2CO_3 (3 equiv), H_2O/CH_3CN (2:1) at 100 °C.
[b] Reagents and conditions: R–$B(OH)_2$ (1.25 equiv), $Pd(OAc)_2$ (0.05 equiv), TPPTS (0.15 equiv), Na_2CO_3 (3 equiv), H_2O/CH_3CN (2:1) at 80 °C.
[c] Yields after HPLC purification.

2.2. Inhibition of human and rat DNPH1

The target 6-(hetero)aryl purine nucleotides **1a**–**x** (Fig. 1) were tested for their ability to inhibit both rat and human DNPH1 enzymes using 2′-deoxyguanosine 5′-monophosphate (dGMP) as substrate. The K_i values were determined according to previously reported procedures [10,19] and summarized in Table 3. Data obtained with two representative N^6-substituted AMP previously studied [19], N^6-phenyl and N^6-benzyl derivatives, were included for comparison. All tested compounds were found to be competitive inhibitors of dGMP binding with micromolar affinity (except for the *O*-arylated compound **1c**). The 6-(hetero)arylpurine derivatives were more potent than AMP (40 μM) [13] with K_i ranging from 0.9 to 12 μM for rat enzyme (Table 3). The K_i values obtained with human DNPH1 were in a similar range (0.5–26 μM), confirming that the rat enzyme is a relevant model for the determination of the kinetic parameters.

To assess whether the binding pocket can tolerate large substituents at the 6-position of the purine, we introduced different (hetero)aryls of various ring sizes. From this series, it appears that the phenyl group (compound **1e**) can be replaced with other aromatic systems such as 3-furanyl (**1l**), 2-naphthyl (**1a**), 3-biphenyl (**1d**) and 6-indolyl (**1p**) without significant loss of inhibitory potency (K_i ranging from 0.9 to 1.5 μM for rat enzyme and from 0.5 to 2.8 μM for human enzyme). The best compound of this series was the biphenyl derivative (**1d**), which was 40-fold more potent than AMP for both enzymes. The 8-quinolinyl derivative (**1p**) was less potent with K_i of 12 μM and 26 μM for rat and human enzymes, respectively. One possible explanation could be that the *ortho* position of the diaryl (compared to 2-naphthyl **1a**) and/or the relative position of the nitrogen is able to orient the 6-substituent in a less favorable conformation in the binding pocket compared to the other aryls (discussed later, docking studies). Moreover, the enlargement of the substituent size as for compounds **1u**–**w** led to still potent inhibitors (K_i ranging from 0.5 to 2.9 μM for human enzyme), suggesting that the binding pocket can adapt to bulkier hydrophobic substituents. These results extend those previously obtained in the N^6-substituted AMP series and underlined the favorable contribution of hydrophobic substituents [19].

We also evaluated the impact of substituents as proton donors or acceptors on the aryl. There was no further improvement in affinity by introducing functional groups (COOH, CN, OH, NO_2, Br …) in *meta* or *para* position of the phenyl ring, suggesting the absence of favorable interactions with the protein (discussed later, docking studies). It was noteworthy that the presence of an oxygen atom at position 6 of the purine in the *O*-phenyl derivative **1c** led to a significantly reduced inhibitory activity (K_i of 107 μM against rat DNPH1) compared to 3-cyanophenyl **1b** (K_i of 12.0 μM) and N^6-phenyl AMP (K_i of 10.6 μM [19]). This could be explained by steric hindrance between the *m*-cyano group on the phenyl ring and the two isoleucines (I18 and I65) in the vicinity of the purine moiety.

Table 3
K_i values of nucleotides **1a**–**x** against rat and human DNPH1.

Compound	R	K_i (μM) rat DNPH1	K_i (μM) human DNPH1
AMP	NH_2	40.0 ± 1.0 [13]	19.2 ± 3.9
BAMP [19]	$NHCH_2Ph$	1.8 ± 0.2	1.9 ± 0.1
N^6-Ph-AMP [19]	NHPh	10.6 ± 0.5	11.2 ± 0.5
1a	2-Naphthyl	1.5 ± 0.1	2.8 ± 0.1
1b	3-Cyanophenyl	12.0 ± 1.0	4.2 ± 1.0
1c	*O*-(3-Cyanophenyl)	107.0 ± 1.0	93.0 ± 4.0
1d	3-Biphenyl	1.2 ± 0.1	0.5 ± 0.1
1e	Phenyl	1.4 ± 0.1	1.5 ± 0.2
1f	3-Methoxycarbonylphenyl	4.8 ± 0.3	2.4 ± 0.1
1g	4-Cyanophenyl	4.5 ± 0.4	2.1 ± 0.4
1h	3-Nitrophenyl	4.2 ± 0.7	5.3 ± 1.2
1i	4-Carboxyphenyl	7.4 ± 4.7	2.7 ± 1.0
1j	4-Hydroxyphenyl	1.5 ± 0.2	1.5 ± 0.3
1k	4-Succinamidophenyl	1.7 ± 0.2	3.7 ± 0.5
1l	3-Furanyl	1.8 ± 0.5	1.7 ± 0.1
1m	5-Formyl-2-furanyl	5.9 ± 0.2	3.8 ± 1.2
1n	5-Chloro-2-thienyl	1.4 ± 0.4	1.3 ± 0.2
1o	8-Quinolinyl	12.0 ± 0.5	25.9 ± 1.5
1p	6-Indolyl	0.9 ± 0.1	2.5 ± 0.5
1q	5-Bromo-3-pyridinyl	3.9 ± 1.0	1.6 ± 0.4
1s	3-Bromophenyl	1.4 ± 0.1	1.1 ± 0.3
1u	3-(4-Carboxyphenyl)phenyl	3.0 ± 0.5	1.5 ± 0.3
1v	3-(6-Methoxy-2-naphthyl)phenyl	1.6 ± 0.4	0.5 ± 0.2
1w	3-(4-Dibenzothienyl)phenyl	2.3 ± 1.0	1.4 ± 0.3
1x	3-Carboxyphenyl	5.3 ± 0.6	2.9 ± 0.5

Enzyme assays according to published procedures [13,19]. K_i values are the average of three independent experiments with standard deviations.

2.3. Crystal structures of human and rat DNPH1 in complex with compound *1a*

In order to understand the binding mode of this new series of inhibitors, we have undertaken their co-crystallization with DNPH1. This was performed using a variant of rat DNPH1 harboring truncated N- and C-termini as well as a point mutation (D69N) of a catalytic aspartate as previously [19,20], and also a new recombinant variant of the human DNPH1. Indeed, the enhanced affinity and stability of the new inhibitors allowed us to attempt at crystallizing a shortened form of the human enzyme bearing no mutation in its active site.

High-resolution crystal structures of rat and human DNPH1 bound to compound **1a** (PDB 4P5D and PDB 4P5E, respectively) were solved by molecular replacement using a previous crystal structure of the rat DNPH1 (PDB 4FYI) and refined at high resolution (2.11 and 1.35 Å, respectively) (Table 4). As expected the overall structure of human DNPH1 closely resembles that of rat enzyme (RMSD ~ 0.6 Å over 134 aligned residues; Fig. 2A) and the observed binding mode of **1a** appears highly similar in both proteins (Fig. 2B). Despite their distinct crystal packing environment, the two independent monomers in each structure build up similar dimers (Fig. 2A) stabilized by a large interface (~1460 Å^2) made of the three helices α2, α3 and α4. The phosphate and ribose moieties are bound in the same orientation in these crystal structures despite the slight variations in protein sequence (rat *vs.* human), the distinct crystallization conditions and also the point mutation D69N in the active site of the rat protein. The high-resolution of the human enzyme structure allows a fine analysis of the protein-ligand contacts. As previously described for the rat counterpart [20], an intricate network of hydrogen bonding involving four backbone amino groups (I29, R30, G31 and G100 in the human enzyme; Fig. 2C), two serine hydroxyl groups (S98 and S128′, where the symbol ′ means residue from the second subunit) and two water molecules, maintains tightly the phosphate group. The ribose moiety is bound with its five-atom heterocyclic ring almost perfectly planar (Fig. 2D). Close contacts are currently observed between the ribose oxygen O4′ and the methylene group Cγ of the methionine M119′ (dist.: 3.18 Å) suggesting the formation of an unusual hydrogen bond. The catalytic glutamate points its side-chain carboxylate group toward the C1′ and C4′ atoms (dist.: 3.27 and 3.45 Å, respectively) and is also hydrogen bonded to the hydroxyl O2′ (dist.: 2.61 Å). Close contacts are also observed between the backbone nitrogen and oxygen atoms of G27 with the hydroxyl O3′ and the carbon C3′ of the ribose moiety (dist.: 3.03 and 3.19 Å, respectively). In parallel, the two other catalytic residues (tyrosine Y24 and aspartate D80) are in contact with glutamate E104 (dist.: 2.53 Å) and the ribose C1′ atom (dist.: 3.31 Å), respectively.

Table 4
Data collection, phasing, and refinement statistics for DNPH1 structures.

	Rat DNPH1-**1a**	Human DNPH1-**1a**
Beamline	ID29	Proxima 1
No. of crystals	1	1
Space group	$P6_122$	$P12_11$
Unit-cell parameters		
a (Å)	178.2	49.0
b (Å)	178.2	52.3
c (Å)	56.9	54.5
α, β, γ (°)	90, 90, 120	90, 104.8, 90
No. molecules in asymmetric unit	2	2
Wavelength (Å)	0.9793	0.8103
Resolution (Å)	2.11 (2.22–2.11)	1.35 (1.41–1.35)
Rmerge (%)[a]	6.5 (11.5)	8.1 (49.3)
$<I/\sigma I>$	15.9 (3.9)	9.6 (2.6)
Completeness (%)[b]	99.5 (99.4)	100.0 (100.0)
Multiplicity[b]	6.5 (6.9)	4.5 (4.5)
Wilson *B* factor (Å^2)	35.3	10.4
Refinement		
Resolution (Å)	38.59–2.11	47.36–1.35
No. of reflections	30,858	58,477
Rwork/Rfree (%)[c]	17.2/19.2	15.6/18.3
No. Atoms		
Protein	2230	2347
Ligand	64	64
Ions	5	1
Water	140	373
B factors (Å^2)		
Protein	46.9	15.8
Ligand	12.6	12.5
Ions	109.9	24.3
Water	52.0	31.6
R.m.s deviations[d]		
Bond lengths (Å)	0.008	0.008
Bond angles (°)	1.19	1.22
Ramachandran (%)		
Most favored	93.3	97.0
Additional allowed	5.0	2.6
Disallowed regions	1.8	0.4

[a] $\Sigma hkl\Sigma i|Ihkl,i - Iaverage, hkl|/|\Sigma hkl\Sigma i|Ihkl,i| \times 100$.
[b] Values in parenthesis stands for the outer shell of resolution range.
[c] Deviation from ideal values.
[d] Rfree is calculated on a subset of reflections that are not used in the refinement (5%).

The purine moiety is stacked into the active site and sandwiched by two isoleucines (in human, I29 and I74; Fig. 2C and D) as previously observed for N^6-alkyl-AMP derivatives bound to the rat enzyme [19,20]. In the vicinity, a leucine (L62 in human, L51 in rat) is now stacked onto the large and aromatic naphthyl ring. In the rat enzyme, additional van der Waals contacts are also observed between the naphthyl ring and the backbone atoms (mainly E56, E57 and A58 in the rat protein) of the flexible loop (residues 47–59 and 58–70 in rat and human proteins, respectively) as well as some side-chains including one conserved arginine (R19 and R30, in rat and human, respectively; Fig. 2E). Despite the higher local flexibility, some of the contacts observed in the rat structure, are present in at least one of the two independent monomers of the human structure (e.g., L62 in human). In fact, most of the residues neighboring the naphthyl moiety are rather well conserved in sequence although some substitutions distinguish the human and rat counterparts (e.g., P53 *vs*. A64 in rat and human, respectively). Accordingly, the recognition of the aryl moiety should be highly similar in both proteins. However, a variation exists in the orientation of the 6-aryl group in the crystallographically independent subunits. While this group adopts apparently only one orientation in rat structure, two orientations exist in the human enzyme. The detected orientations may be partially influenced by the crystal packing, but it is likely that they exist in equilibrium in solution. The residual electron density suggests that the two alternating orientations occur in each subunit (at least in the human counterpart; see Fig. 2C and D). Indeed, depending on the monomer, a different part of this loop is almost invisible due to its high flexibility (from 60 to 65 in monomer A and from 63 to 71 in monomer B of the human enzyme). In all previously solved structures of rat DNPH1, this long loop (residues 47–59) was too flexible to be modeled with only very weak electron density visible in the vicinity of the ligand [20]. This apparent flexibility was in agreement with our former NMR studies [12]. Accordingly, the large and hydrophobic naphthyl ring seems to stabilize this long loop that now covers up the active site. A similar phenomenon appeared in the human enzyme crystallized in the presence of **1a**, although the loop 58–70 is not fully resolved (especially in its central part, residues 63–65). In agreement with the important flexibility of this part of the protein, various or large substituents can be afforded at C6 position of the purine with little changes on the measured affinity.

2.4. Ligand docking in silico

These two new crystal structures were then used to dock the whole series of nucleotide analogues into their active site using the software PLANTS [32]. A primary and naive screening without any water molecules or pharmacophoric restraints suggested a major binding mode corresponding to a *syn*-conformation of the adenosine moiety. The phosphate group was perfectly positioned but the ribose was slightly shifted. Only a few ligands adopt an *anti*-conformation resembling the one observed for compound **1a** in the crystal structures (see Fig. 3A and B). The particular geometry of the active site in the crystal structure (e.g., a very short contact between the ribose O4′ of **1a** and the methionine M119′; see above) may explain in part the discrepancies between the virtual screening and the known mode of binding. The rigid conformation of the ribose (in North conformation) does not allow reproducing its perfectly planar heterocyclic moiety. Noteworthy, the alternate South conformation of the ribose does not allow correct docking in the active site of DNPH1, indicating a tight recognition of the ribose in agreement with results obtained from crystal structures analysis. Importantly, in most cases the predicted binding modes favor a conformation with the nucleobase and the aryl substituent almost coplanar. However, the mostly incorrect positioning of the ribose and the nucleobase prevents further analysis of the protein-ligand interactions. In order to circumvent these difficulties, we resumed the screening with a pharmacophoric restraint deduced from the shape of the bound conformation of compound **1a**. This led to oversampling the *anti*-conformation instead of the *syn*-one. The phosphate group, the ribose and the nucleobase of most ligands can perfectly match the crystal binding mode in this case (mostly as the best pose in this screen as shown in Fig. 3C and D). Surprisingly, two ligands, **1a** and **1c**, failed to behave properly and seemed to be attracted 'upward' by the wrapping-up loop. Nevertheless, the restrained docking allows a better analysis of the potential interactions of the variable aryl substitutions. Most of them appear to be forming weak van der Waals interactions with the few hydrophobic residues of the flexible loop (e.g., L51, P53 and A58 in the rat DNPH1). The polar substituents of the aryl rings attempt to form hydrogen bonds with the conserved arginine (R19 and R30 in rat and human enzyme, respectively). This is predicted to be the best binding mode for the phenyl-substituted compounds bearing a cyano (**1b**), a nitro (**1h**), a bromo (**1s**), a carboxyl (**1x**) in *meta* position or the furanyl- and thienyl-substituted compounds bearing a carbonyl (**1m**) or a chloro (**1n**) group, respectively. However stabilization of the flexible loop on top of the arginine R19, as observed in the crystal structure of rat DNPH1 bound to **1a**, may prevent proper orientation toward the guanidinium group of the conserved arginine and the formation of a favorable hydrogen bond. The large

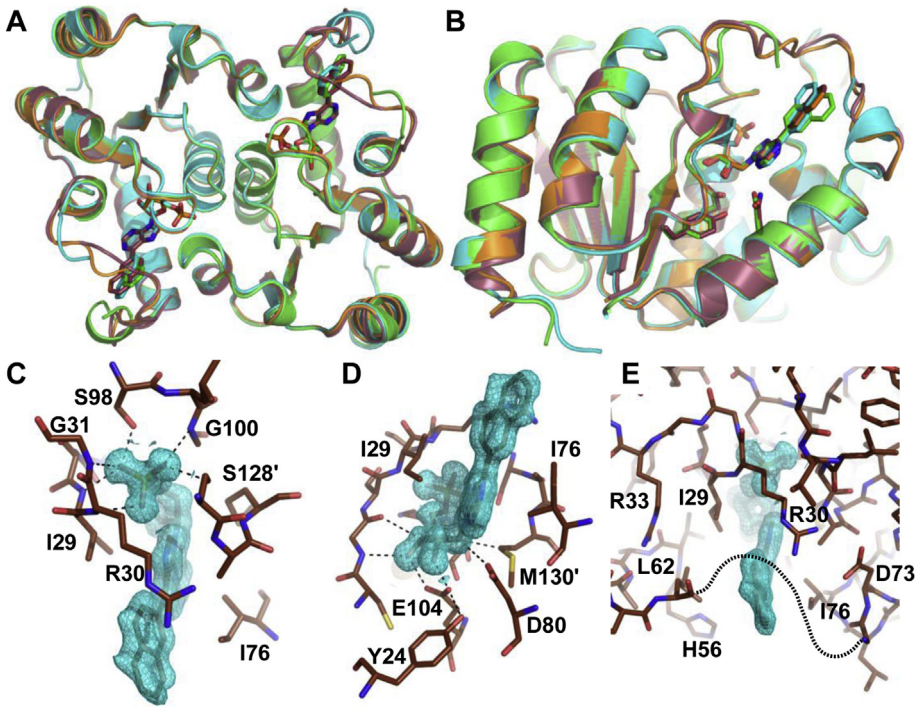


Fig. 2. Panels A and B: Superimposition of the rat and human DNPH1 structures bound to **1a**. Two independent subunits are observed in the crystal structures and the reconstituted dimers were superimposed in Coot (A: top view and B: side-view). The proteins are shown as colored ribbons with the ligand in sticks (cyan and green for the human enzyme; orange and brown for the rat enzyme). Panels C, D and E: Closer view of the ligand binding site of the human structure with a zoom on the phosphate group (C), the ribose moiety (D) and the naphthyl group (E). Both protein residues and the ligands are shown in sticks. Residues discussed in the text are labeled according to the human numbering. The $2Fo - Fc$ electron density map of the ligand molecule is shown in cyan mesh and surface. Map is contoured at 0.9 σ. These pictures are generated by the program PYMOL (http://pymol.sourceforge.net). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrophobic substitutions may form other interactions with the flexible loop without supporting further stabilizations in absence of a buried sub-pocket.

The screening of the human enzyme structure revealed a similar behavior compared to its rat orthologous enzyme. This suggested that the main variations observed in the ligand affinities might rather come from the general features of the long and flexible loop. Indeed, a slight variation in the local sequence (proline P53 *vs.* alanine A64 or leucine L54 *vs.* arginine R65 in the rat sequence versus the human one) certainly affects both the conformational stability of this loop and also its overall charge. Indeed, the negatively charged aryls show slightly better affinity for the human enzyme compared to the rat counterpart. However, the intrinsic flexibility of this loop and its particular conformation in the rat enzyme prevented further refinement in this analysis and additional works will be necessary.

2.5. Cytotoxicity evaluation

The 22 nucleotides **1a**–**x** (Fig. 1) as well as the nucleosides **5** synthesized in this study [33] were tested for their *in vitro* cytotoxic activity against five human cell lines from solid tumors including colon (HCT15 and HCT116), breast (MCF7), prostate (PC3) carcinomas, and promyelocytic leukemia cells (HL60). DNPH1 overexpression has been previously reported in these cancer cell lines [9]. N^6-benzyladenosine 5′-monophosphate (BAMP) and its corresponding nucleoside (BAR), known to exhibit strong cytotoxicity against HL60 cell lines and solid tumors [16,18,34], were included to these assays as internal control.

Cell viability was determined following a 3-day treatment using a quantitative metabolic assay with MTT. The complete data (% cell growth inhibition in the presence of nucleosides **5** or nucleotides **1** at 10 μM and 100 μM) are given as supplementary material (Fig. S1–S5). Similar levels of inhibition were observed for a given nucleoside and the corresponding nucleotide (Fig. S1–S5). For the most active molecules (>90% inhibition at 10 μM), the IC_{50} values (concentration inhibiting the cell growth by 50%) were determined from cell viability measurements at final concentrations ranging from 0.005 μM to 100 μM (ten dilutions). The data are summarized in Table 5. Thus, six nucleotides (**1e**, **1j**, **1l**, **1n**, **1p**, **1s**) exhibited significant cytotoxicity against HCT15 cells with IC_{50} values ranging from 0.3 to 1.4 μM (Table 5). These nucleotides were among the most potent inhibitors of human DNPH1 (Table 3) with the exception of **1d** and **1v** (<50% inhibition at 10 μM). As previously observed with a series of 6-phenylpurine nucleosides [24,28], nucleosides bearing bulky substituents at position 6 (compounds **1d** and **1u**–**1w**) were significantly less active (Fig. S1–S5). This might reflect a difference in the transport mechanism of these compounds and/or rephosphorylation efficiency. These nucleotides were also active on the other cancer cell lines, in particular on HCT116 and HL60 [28], but generally with a slightly reduced potency with IC_{50} values from 0.5 to 15.4 μM (Table 5). This apparent specificity could be explained in part by a well-known lower expression of DNPH1 in these cells [9] (and corroborated by Western-blot analysis, data not shown).

In order to gain insight into the mechanism of action of these compounds, the effect of an adenosine kinase (AK) inhibitor on the growth of cells treated with DNPH1 inhibitors was next examined. Treatment with the AK inhibitor A-134974 completely counteracted the inhibitory effect of all tested compounds, as illustrated in Fig. 4 with the most active molecules. These results indicate that the active metabolite is the monophosphate form. Thus, the 6-arylpurine nucleotides are converted to the corresponding nucleosides by extracellular nucleotidases, transported across the

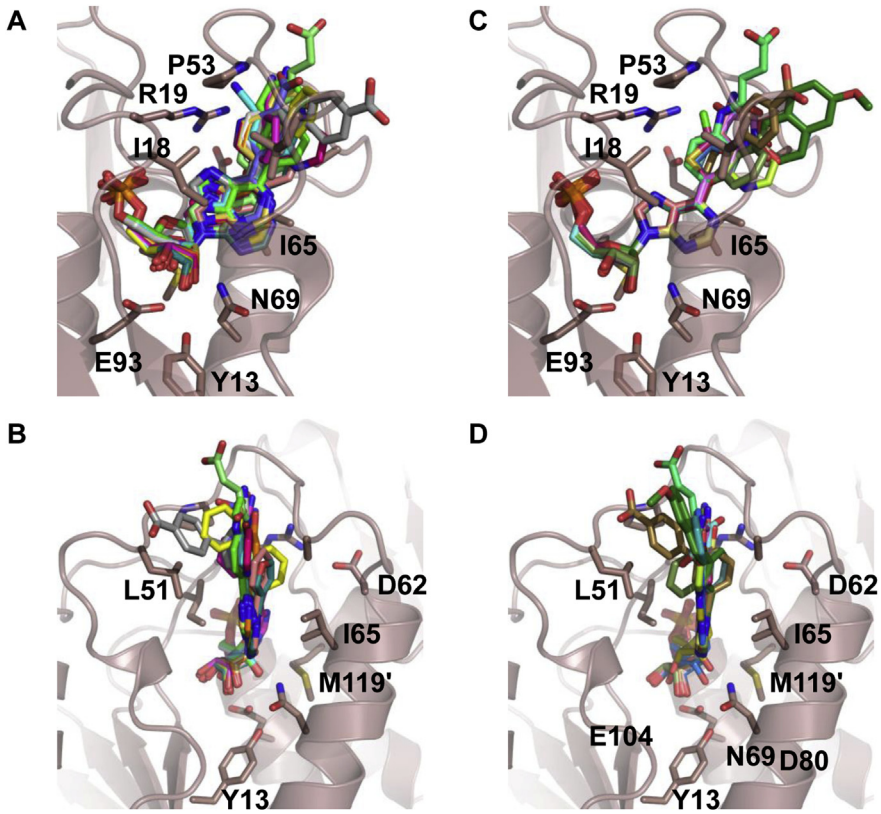


Fig. 3. Ligand docking into the rat DNPH1 structure solved in complex with **1a**. Results of naive docking are shown in panels A and B, while those obtained using a pharmacophoric restraint (compound **1a**) are shown in panels C and D. The protein is shown as colored ribbons with the important side-chains and the ligand in sticks. Residues discussed in the text are labeled according to the rat numbering. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

membrane and then re-phosphorylated in the cell by adenosine kinase. Comparable cytotoxicity patterns were obtained after treatment of cell lines by nucleosides **5** in the presence of adenosine kinase inhibitor (data not shown).

3. Conclusion

A series of 6-(hetero)arylpurine ribonucleotides was synthesized as potential DNPH1 inhibitors. Two synthetic routes based on Suzuki–Miyaura cross-coupling reactions were investigated providing the title compounds in good overall yields. Most of these nucleotides showed good inhibitory potency in the low to submicromolar range against both rat and human enzymes. One compound of this series, compound **1a**, was successfully co-crystallized with DNPH1 leading to the first crystal structure of the human enzyme (PDB 4P5E). This structure solved at atomic resolution in the absence of any mutation within the catalytic triad allowed very precise analysis of the ligand binding site. In addition, in the rat counterpart also co-crystallized with **1a**, we could also observe stabilization of the long loop wrapping over the active site (PDB 4P5D). The newly solved structures bring insights for the further development of inhibitors of DNPH1. Indeed the binding pocket affords large variation in the size and the nature of the 6-aryl substituent, and this novel series of inhibitors provides a slight improvement over the N^6-alkyl derivatives previously studied [19]. Virtual docking of the whole series of 6-aryl derivatives

Table 5
In vitro cytostatic activity of representative nucleotides **1** on cancer cell lines HCT15, HCT116 and HL60.

Compound	IC_{50} (μM)[a]		
	HCT15	HCT116	HL60
BAMP	0.8 ± 0.1	7.7 ± 0.8	5.0 ± 0.5
1e	0.7 ± 0.1	3.2 ± 0.1	1.7 ± 0.3
1j	0.3 ± 0.1	4.1 ± 1.0	0.5 ± 0.1
1l	1.3 ± 0.1	5.5 ± 2.5	15.4 ± 2.0
1n	1.0 ± 0.3	nd	6.5 ± 2.8
1p	1.4 ± 0.4	>80	0.5 ± 0.2
1s	0.6 ± 0.1	nd	3.9 ± 0.6

The values are averages of two independent experiments with standard deviations.
nd: not determined.
[a] Concentration of compound needed to reduce cell growth by 50% after 72 h incubation.

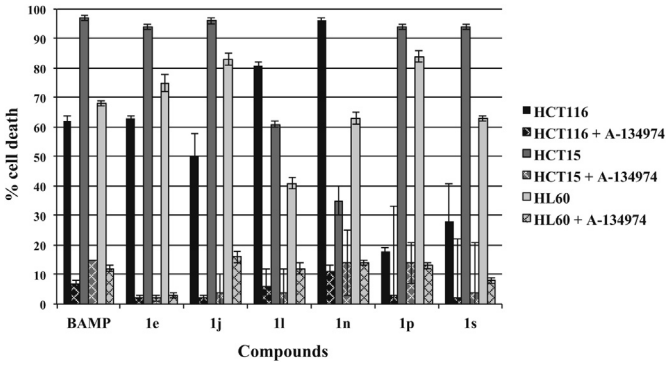


Fig. 4. Growth inhibition of HCT116, HCT15 and HL60 cells after 72 h treatment with DNPH1 inhibitors in the absence or in the presence of an adenosine kinase inhibitor (A-134974).

into the active site of rat and human DNPH1 was analyzed in view of the experimental affinities. This suggested that none of the electronegative substituent (cyano, carboxyl, bromo …) in *meta* position of the (hetero)aryl ring, managed to interact efficiently with the arginine (R30 and R19 in human and rat, respectively) despite the expected formation of a hydrogen bond. The knowledge of the major conformation of the long and flexible loop should help in deriving new aryl moieties to potentially better interact with this loop. Similarly, constraining the co-planarity of the aryl moiety and the nucleobase should enhance the affinity by reducing the entropy cost of ligand binding. The combination of these two derivatization strategies should bring better ligands but currently this stands beyond the scope of this article. More importantly, six potent inhibitors among this series of nucleotides showed significant cytotoxicity against human cancer cell lines that overexpressed DNPH1 [8]. Some discrepancies between measured DNPH1 inhibition and cytotoxic activity may be due to other properties of compounds such as cell penetration and enzyme activation.

Hocek et al. [35,36] have recently described the cytostatic activity of a series of 6-aryl and 6-heteroaryl-7-deazapurine ribonucleosides, compounds substituted with furan-2-yl and thienyl-2-yl being the most active with nanomolar cytostatic activity in multiple cancer cell lines. The authors have demonstrated intracellular phosphorylation to mono- and triphosphate metabolites, together with a potent inhibition of total RNA synthesis. Several 6-aryl and 6-heteroarylpurine nucleosides have already been reported to exhibit significant cytotoxic activity [28]. However little is known about the mechanism of action of these later 6-modified purines. Our results showed that the cytotoxicity of some molecules from these series relies on their phosphorylation by adenosine kinase, and probably their accumulation as monophosphorylated form, as shown previously for active cytokinins [18] and triciribine [37]. This common dependency (monophosphorylation) and the shared affinity for DNPH1, despite significant chemical variations, support that the anticancer effects observed *in vivo* could rely on common inhibition of DNPH1. This suggests that DNPH1 should be included in other evaluations as a new anticancer target.

4. Experimental section

4.1. Chemicals and general synthetic methods

All commercial chemicals and solvents, unless otherwise stated, were reagent grade and were used without further purification. Anhydrous reactions were carried out under argon atmosphere. Thin layer chromatography (TLC) was performed using silica gel $60F_{254}$ plates (Merck). Visualization was made with UV light (254 nm) and by spraying with a mixture of ethanol/anisaldehyde/sulfuric acid/acetic acid (90/5/4/1) followed by heating. Reactions were also monitored using an HPLC system (Agilent 1100 equipped with a C18 reverse phase column) coupled to a mass spectrometer (ESI source). Flash chromatography was performed using silica gel 60 (230–400 mesh, Merck). HPLC purification was carried out on an Agilent system (1100 Series) equipped with a diode array detector using a C18 reverse phase column (Kromasil, 5 μm, 100 Å, 150 × 4.5 mm) and a linear gradient of acetonitrile in 20 mM triethylammonium acetate (TEAA) buffer over 20 min at a flow rate of 4 mL/min. NMR spectra were recorded on Bruker Avance 400 (^{1}H at 400.13 MHz, ^{13}C at 100.62 MHz, ^{31}P at 161.62 MHz). The complete assignment of ^{1}H and ^{13}C signals was performed by analysis of the correlated homonuclear H,H-COSY and heteronuclear H,C-HMBC, H,C-HSQC spectra. Chemical shifts (δ) are reported in ppm relative to the solvent deuterated signals or to H_3PO_4 (0 ppm) as external standard for ^{31}P. Coupling constants (J values) are reported in Hertz. High-resolution mass spectra were measured on a Waters Q-Tof Micro MS instrument using a mobile phase of acetonitrile/water with 0.1% formic acid (positive ion mode) or 10 mM ammonium formate (negative ion mode). The purity of all tested compounds was greater than 97% as determined by HPLC using a Agilent 1100 system equipped with a diode array detector and a reverse phase column (C18 Kromasil, 3.5 μm, 30 × 4.6 mm) using a linear gradient of acetonitrile in 10 mM TEAA buffer over 20 min at a flow rate of 1 mL/min. Retention time (R_t) and gradient are given for each compound.

4.2. Synthesis of target nucleotides **1a**–**1k** and **1x** according to Scheme 1 route A

4.2.1. General procedures for Suzuki–Miyaura cross-coupling reactions

4.2.1.1. Method A. Arylboronic acid (1.5 equiv) and anhydrous potassium carbonate (1.5 equiv) were added to a stirred solution of **3** (1 equiv) in anhydrous toluene (10 mL/mmol). The reaction mixture was purged three times with argon, then $Pd(PPh_3)_4$ (0.06 equiv) was introduced. The mixture was warmed to 100 °C. When the reaction was judged complete (monitoring by LC–MS and TLC), the mixture was cooled to room temperature and filtered through a pad of Celite. Concentration of the filtrate and purification of the crude mixture by silica gel chromatography yielded the title compound.

4.2.1.2. Method B. Similar to method A except that aqueous cesium carbonate (1 M, 2.4 equiv) was used instead of potassium carbonate.

4.2.1.3. Method C. All solutions were deoxygenated before addition to the reaction mixture. $Pd(OAc)_2$ (0.10 equiv) and CyJohnPhos (0.15 equiv) in anhydrous dioxane (1 mL/mmol) were stirred at room temperature for 15 min under argon atmosphere. Then, a solution of **3** (1 equiv) in anhydrous dioxane (10 mL/mmol), arylboronic acid (1.5 equiv) and anhydrous tripotassium phosphate (2 equiv) were added. The reaction was warmed to 100 °C. When the reaction was judged complete (monitoring by LC–MS and TLC), the mixture was cooled to room temperature and filtered through a pad of celite. Concentration of the filtrate and purification of the crude mixture by silica gel chromatography yielded the title compound.

4.2.1.4. Method D. Similar to method C except that aqueous cesium carbonate (1 M, 2 equiv) was used instead of anhydrous tripotassium phosphate.

4.2.2. General procedure for removal of the isopropylidene group of compounds **4**

A solution of nucleotide **4** in TFA/H_2O (90/10, 10 mL/mmol) was stirred at room temperature. After completion of the reaction (1 h), the resulting solution was concentrated to dryness, coevaporated with toluene and purified by silica gel column chromatography to afford the unprotected compound **6**.

4.2.3. General procedure for removal of ethyl group of compounds **6**

To a stirred solution of the phosphotriester **6** (1 equiv) in anhydrous DMF (4 mL), bromotrimethylsilane (0.5 mL, ~20 equiv) was added at 0 °C under argon atmosphere. The mixture was then allowed to warm to room temperature and stirred for 36 h (monitoring by LC–MS). Then, the volatiles were removed and the mixture was diluted with NH_4OH (1.5 mL) and concentrated to dryness. The resulting white solid was taken up in 20 mM TEAA buffer (pH 7) and purified by preparative HPLC using a linear gradient of CH_3CN in 20 mM TEAA buffer as indicated. A final Dowex ion exchange led to the 5′-monophosphate derivative **1** as

sodium salt. For poorly-water-soluble compounds, no ion exchange was performed.

4.2.4. 6-Chloro-9-(5-O-diethylphosphate-2,3-O-isopropylidene-ß-D-ribofuranosyl)-9H-purine (**3**)

To a solution of 6-chloro-9-(2,3-*O*-isopropylidene-ß-D-ribofuranosyl)-9*H*-purine [21] (2.56 g, 7.83 mmol) in anhydrous pyridine (32 mL), diethylchlorophosphate (2.8 mL, 19.58 mmol) was added dropwise at 0 °C under argon atmosphere. The resulting solution was stirred at this temperature. After completion (2 h), MeOH (10 mL) was added and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (200 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) to afford compound **3** (2.76 g, 76%) as a yellow oil. ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.14 (2td, $^4J_{H,P} = 0.9$, $J = 7.1$, 2 × 3H, CH_3), 1.35 (s, 3H, $(CH_3)_2C$), 1.57 (s, 3H, $(CH_3)_2C$), 3.85–3.94 (m, 4H, O–CH_2), 4.05–4.19 (m, 2H, H-5′), 4.42–4.46 (m, 1H, H-4′), 5.08 (dd, $J_{3',4'} = 3.1$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.53 (dd, $J_{1',2'} = 2.0$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.38 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 8.84 (s, 2H, H-2, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 16.3 (d, $^3J_{C,P} = 6.4$, 2C, CH_3), 25.6 ($(CH_3)_2C$), 27.3 ($(CH_3)_2C$), 63.3 (d, $^2J_{C,P} = 5.7$, 2C, O–CH_2–CH_3), 66.8 (d, $^2J_{C,P} = 5.3$, C-5′), 81.2 (C-3′), 83.8 (C-2′), 85.6 (d, $^3J_{C,P} = 7.7$, C-4′), 90.5 (C-1′), 114.0 ($(CH_3)_2C$), 132.0 (C-5), 146.5 (C-8), 149.9 (C-6), 151.6 (C-4), 152.2 (C-2); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.90; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{17}H_{24}^{35}ClN_4O_7P + H]^+$ 463.1149, found 463.1168.

4.2.5. 6-(2-Naphthyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4a**)

Synthesized from compound **3** (350 mg, 0.76 mmol) and 2-naphtalene boronic acid (195 mg, 1.14 mmol) according to the General Procedure Method A. Compound **4a** (258 mg, 62%) was obtained as colorless crystals after 12 h of reaction and two successive flash column chromatographies (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 99/1). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.13 (2xtd, $^4J_{H,P} = 0.9$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.38 (s, 3H, $(CH_3)_2C$), 1.59 (s, 3H, $(CH_3)_2C$), 3.86–3.97 (m, 4H, O–CH_2–CH_3), 4.11–4.25 (m, 2H, H-5′), 4.44–4.48 (m, 1H, H-4′), 5.15 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.61 (dd, $J_{1',2'} = 2.1$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 7.58–7.67 (m, 2H, H-6 Arom., H-7 Arom.), 8.01 (dd, $J_{6\ Arom.,\ 8\ Arom.} = 1.3$, $J_{7\ Arom.,\ 8\ Arom.} = 7.6$, 1H, H-8 Arom.), 8.14–8.11 (m, 2H, H-4 Arom., H-5 Arom.), 8.88 (s, 1H, H-8), 8.91 (dd, $J_{1\ Arom.,\ 3\ Arom.} = 1.7$, $J_{3\ Arom.,\ 4\ Arom.} = 8.8$, 1H, H-3 Arom.), 9.08 (s, 1H, H-2), 9.49 (bs, 1H, H-1 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 ($^3J_{C,P} = 6.3$, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.4 ($^2J_{C,P} = 5.7$, O–CH_2–CH_3), 66.4 ($^2J_{C,P} = 5.3$, C-5′), 80.8 (C-3′), 83.4 (C-2′), 84.9 ($^3J_{C,P} = 7.8$, C-4′), 89.7 (C-1′), 113.5 ($(CH_3)_2C$), 125.6 (C-3 Arom.), 127.7 (2C, C-6 Arom., C-7 Arom.), 128.2 (C-8 Arom.), 128.8 (C-5 Arom.), 129.2 (C-4 Arom.), 130.2 (C-1 Arom.), 131.4 (C-5), 132.7 (C-8a Arom.), 133.3 (C-4a Arom.), 134.2 (C-2 Arom.), 145.3 (C-8), 151.7 (C-4), 152.0 (C-2), 153.0 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.08; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{27}H_{31}N_4O_7P + H]^+$ 555.2009, found 555.2021.

4.2.6. 6-(3-Cyanophenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4b**) and 6-(3-cyanophenoxy)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4c**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and 3-cyanophenylboronic acid (95 mg, 0.65 mmol) according to the General Procedure Method C. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4b** (116 mg, 52%) as a yellow oil together with the by-product **4c** (30 mg, 11%) as a brown oil. Synthesized from compound **3** (200 mg, 0.43 mmol) and 3-cyanophenylboronic acid (95 mg, 0.65 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4b** (159 mg, 70%).

4.2.6.1. Compound ***4b***. ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.13 (2xtd, $^4J_{H,P} = 0.9$, $J = 7.0$, 2 × 3H, O–CH_2–CH_3), 1.37 (s, 3H, $(CH_3)_2C$), 1.58 (s, 3H, $(CH_3)_2C$), 3.86–3.96 (m, 4H, O–CH_2–CH_3), 4.09–4.22 (m, 2H, H-5′), 4.43–4.47 (m, 1H, H-4′), 5.13 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.57 (dd, $J_{1',2'} = 2.1$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.42 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 7.82 (t, $J = 7.9$, 1H, H-5 Arom.), 8.04 (dt, $J = 1.3$, $J_{4\ Arom.,\ 5\ Arom.} = 7.7$, 1H, H-4 Arom.), 8.88 (s, 1H, H-8), 9.04–9.06 (m, 2H, H-2, H-6 Arom.), 9.14 (t, $J = 1.5$, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 25.1 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.3 (d, $^2J_{C,P} = 5.9$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.6$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 85.0 (d, $^3J_{C,P} = 7.6$, C-4′), 89.7 (C-1′), 112.0 (C-3 Arom.), 113.5 ($(CH_3)_2C$), 118.4 (CN), 130.1 (C-5 Arom.), 131.0 (C-5), 132.6 (C-2 Arom.), 133.4 (C-6 Arom.), 134.4 (C-4 Arom.), 136.1 (C-1 Arom.), 145.9 (C-8), 150.5 (C-6), 151.8 (C-4), 151.9 (C-2); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.07; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{24}H_{28}N_5O_7P + H]^+$ 530.1805, found 530.1831.

4.2.6.2. Compound ***4c***. ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.17 (2td, $^4J_{H,P} = 1.0$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.36 (s, 3H, $(CH_3)_2C$), 1.56 (s, 3H, $(CH_3)_2C$), 3.89–3.95 (m, 4H, O–CH_2–CH_3), 4.14–4.20 (m, 2H, H-5′), 4.40–4.46 (m, 1H, H-4′), 5.09 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.61 (dd, $J_{1',2'} = 2.3$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.37 (d, $J_{1',2'} = 2.3$, 1H, H-1′), 7.69–7.72 (m, 2H, H-4 Arom., H-5 Arom.), 7.79–7.82 (m, 1H, H-6 Arom.), 7.90–7.92 (m, 1H, H-2 Arom.) 8.54 (s, 1H, H-2), 8.70 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.3$, 2C, O–CH_2–CH_3), 25.1 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.4 (d, $^2J_{C,P} = 5.6$, 2C, O–CH_2–CH_3), 66.2 (d, $^2J_{C,P} = 5.7$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 84.8 (d, $^3J_{C,P} = 8.3$, C-4′), 89.6 (C-1′), 112.5 (C-3 Arom.), 113.6 ($(CH_3)_2C$), 117.9 (CN), 121.4 (C-5), 125.7 (C-2 Arom.), 127.3 (C-4 Arom.), 129.7 (C-6 Arom.), 131.1 (C-5 Arom.), 144.0 (C-8), 152.5 (C-2), 152.3 (C-1 Arom.), 152.5 (C-4), 158.9 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.05; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{24}H_{28}N_5O_8P + H]^+$ 546.1754, found 546.1739.

4.2.7. 6-(3-Biphenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4d**)

Synthesized from compound **3** (139 mg, 0.30 mmol) and 3-biphenylboronic acid (89 mg, 0.45 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4d** as a white powder (123 mg, 71%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.10–1.15 (m, 6H, O–CH_2–CH_3), 1.37 (s, 3H, $(CH_3)_2C$), 1.59 (s, 3H, $(CH_3)_2C$), 3.87–3.94 (m, 4H, O–CH_2–CH_3), 4.12–4.21 (m, 2H, H-5′), 4.45–4.46 (m, 1H, H-4′), 5.14 (dd, 1H, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, H-3′), 5.59 (dd, 1H, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.2$, H-2′), 6.43 (d, 1H, $J_{1',2'} = 2.2$, H-1′), 7.43 (dt, 1H, $J = 1.2$, $J = 7.3$, H-4′ Arom.), 7.55 (t, 2H, $J = 7.3$, H-3′ Arom., H-5′ Arom.), 7.69–7.77 (m, 3H, H-5 Arom., H-2′ Arom., H-6′ Arom.), 7.88 (dq, 1H, $J = 1.2$, $J_{5\ Arom.,\ 6\ Arom.} = 7.7$, H-6 Arom.), 8.80 (dt, 1H, $J = 1.3$, $J_{4\ Arom.,\ 5\ Arom.} = 7.8$, H-4 Arom.), 8.86 (s, 1H, H-8), 9.06 (s, 1H, H-2), 9.15 (t, 1H, $J = 1.7$, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.7$, 2C, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.3 (d, $^2J_{C,P} = 5.9$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.6$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 85.0 (d, $^3J_{C,P} = 6.9$, C-4′), 89.6 (C-1′), 113.5 ($(CH_3)_2C$), 126.7 (2C, C-2′ Arom., C-6′ Arom.), 127.7 (C-2 Arom.), 127.8 (C-4′ Arom.), 128.2 (C-4 Arom.), 129.1 (2C, C-3′ Arom., C-5′ Arom.), 129.4 (C-5 Arom.), 129.5 (C-6 Arom.), 131.0 (C-5), 135.8 (C-3 Arom.), 139.8 (C-1′ Arom.), 140.6 (C-1 Arom.), 145.3 (C-8), 151.7 (C-4), 151.9 (C-2), 152.9 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.09; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{29}H_{33}N_4O_7P + H]^+$ 581.2165, found 581.2180.

4.2.8. 6-Phenyl-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4e**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and phenylboronic acid (80 mg, 0.65 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4e** as a yellow oil (164 mg, 76%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.10 (2td, $^4J_{H,P} = 0.8$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.36 (s, 3H, $(CH_3)_2C$), 1.58 (s, 3H, $(CH_3)_2C$), 3.86–3.93 (m, 4H, O–CH_2–CH_3), 4.08–4.21 (m, 2H, H-5′), 4.47–4.51 (m, 1H, H-4′), 5.13 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.58 (dd, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.41 (d, $J_{1',2'} = 2.2$, 1H, H-1′), 7.57–7.64 (m, 3H, H-3 Arom., H-4 Arom., H-5 Arom.), 8.81–8.83 (m, 3H, H-8, H-2 Arom., H-6 Arom.), 9.03 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.3 (d, $^2J_{C,P} = 5.8$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.5$, C-5′), 80.7 (C-3′), 83.3 (C-2′), 84.8 (d, $^3J_{C,P} = 7.8$, C-4′), 89.6 (C-1′), 113.5 ($(CH_3)_2C$), 128.7 (C-4 Arom.), 129.3 (2C, C-2 Arom., C-6 Arom.), 130.9 (C-5), 131.7 (2C, C-3 Arom., C-5 Arom.), 135.1 (C-1 Arom.), 144.8 (C-8), 151.6 (C-4), 151.9 (C-2), 153.1 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.10; HRMS (ESI-TOF): m/z calcd for $[C_{23}H_{29}N_4O_7P + H]^+$ 505.1852, found 505.1870.

4.2.9. 6-(3-Methoxycarbonylphenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4f**)

Synthesized from compound **3** (300 mg, 0.65 mmol) and 3-methoxycarbonylphenylboronic acid (175 mg, 0.97 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 99/1) afforded **4f** as a white powder (270 mg, 74%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.13 (2td, $^4J_{H,P} = 0.9$, $J = 7.2$, 2 × 3H, O–CH_2–CH_3), 1.37 (s, 3H, $(CH_3)_2C$), 1.59 (s, 3H, $(CH_3)_2C$), 3.86–3.92 (m, 4H, O–CH_2–CH_3), 3.93 (s, 3H, CO_2CH_3), 4.15–4.18 (m, 2H, H-5′), 4.45–4.50 (m, 1H, H-4′), 5.13 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.59 (dd, $J_{1',2'} = 2.1$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 7.78 (t, $J = 7.8$, 1H, H-5 Arom.), 8.16 (dt, $J = 1.4$, $J_{4\ \text{Arom.},\ 5\ \text{Arom.}} = 7.8$, 1H, H-4 Arom.), 8.88 (s, 1H, H-8), 9.08 (s, 1H, H-2), 9.10 (dt, $J = 1.5$, $J_{5\ \text{Arom.},\ 6\ \text{Arom.}} = 8.0$, 1H, H-6 Arom.), 9.44 (t, $J = 1.8$, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 52.3 (CO_2CH_3), 63.3 (d, $^2J_{C,P} = 6.0$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.5$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 85.0 (d, $^3J_{C,P} = 8.0$, C-4′), 89.7 (C-1′), 113.5 ($(CH_3)_2C$), 129.3 (C-2 Arom.), 129.9 (C-5 Arom.), 130.2 (C-3 Arom.), 131.0 (C-5), 131.6 (C-4 Arom.), 133.8 (C-1 Arom.), 135.6 (C-6 Arom.), 145.6 (C-8), 151.7 (C-4), 151.8 (C-6), 152.0 (C-2), 165.9 (CO_2CH_3); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.09; HRMS (ESI-TOF): m/z calcd for $[C_{25}H_{31}N_7O_9P + H]^+$ 563.1907, found 563.1974.

4.2.10. 6-(4-Cyanophenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4g**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and 4-cyanophenylboronic acid (95 mg, 0.65 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4g** as a white powder (179 mg, 79%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.11 (2td, $^4J_{H,P} = 0.9$, $J = 7.0$, 2 × 3H, O–CH_2–CH_3), 1.36 (s, 3H, $(CH_3)_2C$), 1.58 (s, 3H, $(CH_3)_2C$), 3.85–3.95 (m, 4H, O–CH_2–CH_3), 4.11–4.19 (m, 2H, H-5′), 4.45–4.52 (m, 1H, H-4′), 5.12 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.58 (dd, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.2$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 8.08 (d, $J = 1.8$, 1H, H-3 Arom. or H-5 Arom.), 8.09 (d, $J = 1.8$, 1H, H-3 Arom. or H-5 Arom.), 8.90 (s, 1H, H-8), 8.96 (d, $J = 1.8$, 1H, H-2 Arom. or H-6 Arom.), 8.98 (d, $J = 1.7$, 1H, H-2 Arom. or H-6 Arom.), 9.10 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.6$, 2C, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.3 (d, $^2J_{C,P} = 5.9$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.4$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 84.9 (d, $^3J_{C,P} = 7.7$, C-4′), 89.7 (C-1′), 113.3 (C-4 Arom.), 113.5 ($(CH_3)_2C$), 118.5 (CN), 129.8 (2C, C-2 Arom., C-6 Arom.), 131.4 (C-5), 132.7 (2C, C-3 Arom., C-5 Arom.), 139.3 (C-1 Arom.), 146.0 (C-8), 150.8 (C-6), 152.0 (C-4), 152.1 (C-2); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.08; HRMS (ESI-TOF): m/z calcd for $[C_{24}H_{28}N_5O_7P + H]^+$ 530.1805, found 530.1818.

4.2.11. 6-(3-Nitrophenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4h**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and 3-nitrophenylboronic acid (108 mg, 0.65 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/2) afforded **4h** as an orange solid (176 mg, 75%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.12 (2td, $^4J_{H,P} = 0.9$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.37 (s, 3H, $(CH_3)_2C$), 1.58 (s, 3H, $(CH_3)_2C$), 3.86–3.95 (m, 4H, O–CH_2–CH_3), 4.11–4.23 (m, 2H, H-5′), 4.44–4.48 (m, 1H, H-4′), 5.13 (dd, $J_{3',4'} = 3.1$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.58 (dd, $J_{1',2'} = 2.1$, $J_{2',3'} = 6.3$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 7.92 (t, $J = 8.0$, 1H, H-5 Arom.), 8.43 (ddd, $J_{4\ \text{Arom.},\ 6\ \text{Arom}} = 1.1$, $J_{2\ \text{Arom.},\ \text{H-4 Arom.}} = 2.4$, $J_{4\ \text{Arom.},\ 5\ \text{Arom.}} = 8.3$, 1H, H-4 Arom.), 8.92 (s, 1H, H-8), 9.10 (s, 1H, H-2), 9.22 (dt, $J = 1.3$, $J_{5\ \text{Arom.},\ 6\ \text{Arom.}} = 7.8$, 1H, H-6 Arom.), 9.68 (t, $J = 2.0$, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.3$, 2C, O–CH_2–CH_3), 25.2 ($(CH_3)_2C$), 26.9 ($(CH_3)_2C$), 63.7 (d, $^2J_{C,P} = 5.7$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.5$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 85.0 (d, $^3J_{C,P} = 7.7$, C-4′), 89.8 (C-1′), 113.5 ($(CH_3)_2C$), 123.7 (C-2 Arom.), 125.6 (C-4 Arom.), 130.5 (C-5 Arom.), 131.1 (C-5), 135.1 (C-6 Arom.), 136.6 (C-1 Arom.), 146.0 (C-8), 148.2 (C-3 Arom.), 150.2 (C-6), 152.0 (2C, C-2, C-4); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.07; HRMS (ESI-TOF): m/z calcd for $[C_{23}H_{28}N_5O_9P + H]^+$ 550.1703, found 550.1705.

4.2.12. 6-(4-Carboxyphenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4i**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and 4-carboxyphenylboronic acid (108 mg, 0.65 mmol) according to the General Procedure Method D. When the reaction was complete (2 h), the reaction mixture was acidified to pH 2 with 1 M HCl, then extracted with CH_2Cl_2 (3 times). Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 98/6 + 0.5% AcOH) gave **4i** as a white powder (196 mg, 83%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.11 (2td, $^4J_{H,P} = 0.9$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.36 (s, 3H, $(CH_3)_2C$), 1.58 (s, 3H, $(CH_3)_2C$), 3.89–3.93 (m, 4H, O–CH_2–CH_3), 4.06–4.22 (m, 2H, H-5′), 4.48–4.51 (m, 1H, H-4′), 5.13 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.58 (dd, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.1$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 8.16 (d, $J = 8.6$, 2H, H-3 Arom., H-5 Arom.), 8.87 (s, 1H, H-8), 8.92 (d, $J = 8.6$, 2H, H-2 Arom., H-6 Arom.), 9.08 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 16.2 (d, $^3J_{C,P} = 6.5$, 2C, O–CH_2–CH_3), 25.7 ($(CH_3)_2C$), 27.4 ($(CH_3)_2C$), 63.8 (d, $^2J_{C,P} = 5.6$, 2C, O–CH_2–CH_3), 66.8 (d, $^2J_{C,P} = 6.3$, C-5′), 81.3 (C-3′), 83.8 (C-2′), 84.5 (d, $^3J_{C,P} = 7.5$, C-4′), 90.2 (C-1′), 114.0 ($(CH_3)_2C$), 129.9 (2C, C-2 Arom., C-6 Arom.), 130.1 (2C, C-3 Arom., C-5 Arom.), 131.7 (C-5), 133.8 (C-4 Arom.), 139.6 (C-1 Arom.), 146.2 (C-8), 152.3 (C-4), 152.4 (C-2), 152.5 (C-6), 167.5 (COOH); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.09; HRMS (ESI-TOF): m/z calcd for $[C_{24}H_{29}N_4O_9P + H]^+$ 549.1750, found 549.1766.

4.2.13. 6-(4-Hydroxyphenyl)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4j**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and 4-hydroxyphenylboronic acid (108 mg, 0.65 mmol) according to the General Procedure Method D. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 96/4) afforded **4j** as a white powder (123 mg, 56%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.11 (2td, $^4J_{H,P} = 0.8$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.36 (s, 3H, $(CH_3)_2C$), 1.57 (s, 3H, $(CH_3)_2C$), 3.86–4.00 (m, 4H, O–CH_2–CH_3), 4.06–4.13 (m, 1H, H-5′), 4.15–4.20 (m, 1H, H-5″), 4.40–4.43 (m, 1H,

H-4′), 5.12 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.2$, 1H, H-3′), 5.56 (dd, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.1$, 1H, H-2′), 6.38 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 6.95–6.98 (m, 2H, H-3 Arom., H-5 Arom.), 8.73–8.76 (m, 3H, H-8, H-2 Arom., H-6 Arom.), 8.91 (s, 1H, H-2), 10.12 (s, 1H, OH); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.7 (d, $^3J_{C,P} = 6.5$, 2C, O–CH_2–CH_3), 25.2 ((CH_3)$_2$C), 26.9 ((CH_3)$_2$C), 63.3 (d, $^2J_{C,P} = 5.8$, 2C, O–CH_2–CH_3), 66.3 (d, $^2J_{C,P} = 5.4$, C-5′), 80.8 (C-3′), 83.3 (C-2′), 84.8 (d, $^3J_{C,P} = 7.7$, C-4′), 89.5 (C-1′), 113.5 ((CH_3)$_2$C), 115.5 (2C, C-3 Arom., C-5 Arom.), 126.1 (C-1 Arom.), 129.9 (2C, C-2. Arom, C-6 Arom.), 131.3 (C-5), 144.3 (C-8), 151.2 (C-4), 151.9 (C-2), 153.3 (C-6), 160.5 (C-4 Arom.); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ −0.11; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{23}H_{29}N_4O_8P + H]^+$ 521.1801, found 521.1800.

4.2.14. 6-(4-Anilino-4-oxobutanoic acid)-9-(5-O-diethylphosphate-2,3-O-isopropylidene-β-D-ribofuranosyl)-9H-purine (**4k**)

Synthesized from compound **3** (200 mg, 0.43 mmol) and *N*-(4-phenylboronic)succinamic acid (152 mg, 0.65 mmol) according to the General Procedure Method D. When the reaction was complete, the reaction mixture was acidified to pH 2 with 1 M HCl, then extracted with CH_2Cl_2 (3 times). Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 96/4 to 90/10/0.5% AcOH) afforded **4k** as an orange powder (130 mg, 49%) together with the starting material **3** (40 mg, 20%). ^{1}H NMR (400.13 MHz, CD_3OD): δ 1.26 (2td, $^4J_{H,P} = 1.0$, $J = 7.1$, 2 × 3H, O–CH_2–CH_3), 1.48 (s, 3H, (CH_3)$_2$C), 1.69 (s, 3H, (CH_3)$_2$C), 2.73–2.80 (m, 4H, NHCO-(CH_2)$_2$–COOH), 4.01–4.08 (m, 4H, O–CH_2–CH_3), 4.26–4.36 (m, 2H, H-5′), 4.54–4.58 (m, 1H, H-4′), 5.26 (dd, $J_{3',4'} = 3.2$, $J_{2',3'} = 6.3$, 1H, H-3′), 5.65 (dd, $J_{1',2'} = 2.2$, $J_{2',3'} = 6.3$, 1H, H-2′), 6.43 (d, $J_{1',2'} = 2.1$, 1H, H-1′), 7.80–7.85 (m, 2H, H-3 Arom., H-5 Arom.), 8.63 (s, 1H, H-8), 8.73–8.76 (m, 2H, H-2 Arom., H-6. Arom.), 8.98 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, CD_3OD): δ 16.3 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 25.7 ((CH_3)$_2$C), 27.7 ((CH_3)$_2$C), 30.6 (NHCO–CH_2), 32.8 (CH_2–COOH), 65.8 (d, $^2J_{C,P} = 5.9$, 2C, O–CH_2–CH_3), 68.3 (d, $^2J_{C,P} = 5.7$, C-5′), 83.0 (C-3′), 85.4 (C-2′), 87.0 (d, $^3J_{C,P} = 7.9$, C-4′), 92.3 (C-1′), 115.9 ((CH_3)$_2$C), 120.6 (2C, C-3 Arom., C-5 Arom.), 129.9 (C-1 Arom.), 131.8 (2C, C-2 Arom., C-6 Arom.), 132.3 (C-5), 140.1 (C-4 Arom.), 146.2 (C-8), 153.0 (C-2), 153.5 (C-4), 155.8 (C-6), 173.5 (COOH), 177.2 (NHCO); ^{31}P NMR (161.62 MHz, CD_3OD): δ −0.36; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{27}H_{34}N_5O_{10}P + H]^+$ 620.2122, found 620.2047.

4.2.15. 6-(2-Naphthyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6a**)

Starting from **4a** (65 mg, 0.12 mmol), **6a** (54 mg, 90%) was obtained as a white powder after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 96/4). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19–1.22 (m, 6H, O–CH_2–CH_3), 3.97–4.04 (m, 4H, O–CH_2–CH_3), 4.18–4.21 (m, 2H, H-5′), 4.23–4.30 (m, 1H, H-4′), 4.33–4.37 (m, 1H, H-3′), 4.78 (q, $J = 5.2$, 1H, H-2′), 5.47 (d, $J_{3',OH} = 5.5$, 1H, OH-3′), 5.71 (d, $J = 5.6$, 1H, OH-2′), 6.17 (d, $J_{1',2'} = 4.9$, 1H, H-1′), 7.60–7.67 (m, 2H, H-6 Arom., H-7 Arom.), 8.02 (dd, $J_{6\ Arom.,8\ Arom.} = 1.4$, $J_{7\ Arom.,8\ Arom.} = 7.5$, 1H, H-8 Arom.), 8.12–8.15 (m, 2H, H-4 Arom., H-5 Arom.), 8.90 (s, 1H, H-8), 8.92 (dd, $J_{1\ Arom.,\ 3\ Arom} = 1.6$, $J_{3\ Arom.,\ 4\ Arom.} = 8.8$, 1H, H-3 Arom.), 9.08 (s, 1H, H-2), 9.50 (bs, 1H, H-1 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P} = 6.0$, 2C, O–CH_2–CH_3), 63.3 (d, $^2J_{C,P} = 5.3$, 2C, O–CH_2–CH_3), 66.6 (d, $^2J_{C,P} = 5.6$, C-5′), 69.9 (C-3′), 73.0 (C-2′), 82.6 (d, $^3J_{C,P} = 7.5$, C-4′), 88.0 (C-1′), 125.6 (C-3 Arom.), 126.7 (2C, C-6 Arom., C-7 Arom.), 127.8 (C-8 Arom.), 128.2 (C-5 Arom.), 129.2 (C-4 Arom.), 130.2 (C-1 Arom.), 131.1 (C-8a Arom.), 132.6 (C-5), 132.7 (C-4a Arom.), 134.1 (C-2 Arom.), 145.0 (C-8), 152.0 (C-2), 152.2 (C-4), 152.9 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.13; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{24}H_{27}N_4O_7P + H]^+$ 515.1696, found 515.1694.

4.2.16. 6-(3-Cyanophenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6b**)

Starting from **4b** (125 mg, 0.24 mmol), **6b** (69 mg, 62%) was obtained as a brown oil after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 97/3). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.17–1.21 (m, 6H, O–CH_2–CH_3), 3.94–4.03 (m, 4H, O–CH_2–CH_3), 4.15–4.34 (m, 4H, H-3′, H-4′, H-5′), 4.75 (q, $J = 5.1$, 1H, H-2′), 5.46 (d, $J_{3',OH} = 5.5$, 1H, OH-3′), 5.69 (d, $J_{2',OH} = 5.1$, 1H, OH-2′), 6.15 (d, $J_{1',2'} = 4.9$, 1H, H-1′), 7.85 (t, $J = 7.8$, 1H, H-5 Arom.), 8.06 (dt, $J = 1.5$, $J_{4\ Arom.,\ 5\ Arom.} = 7.6$, 1H, H-4 Arom.), 8.92 (s, 1H, H-8), 9.08–9.10 (m, 2H, H-2, H-6 Arom.), 9.18 (t, $J = 1.39$, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P} = 5.6$, 2C, O–CH_2–CH_3), 66.5 (d, $^2J_{C,P} = 5.8$, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P} = 7.4$, C-4′), 88.0 (C-1′), 112.0 (C-3 Arom.), 118.5 (CN), 119.5 (C-5 Arom.), 130.2 (C-5), 131.0 (C-6), 132.6 (C-2 Arom.), 133.5 (C-6 Arom.), 134.4 (C-4 Arom.), 136.3 (C-1 Arom.), 145.7 (C-8), 150.5 (C-2), 152.0 (C-4), 152.4 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.12; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{21}H_{24}N_5O_7P + H]^+$ 490.1492, found 490.1492.

4.2.17. 6-(3-Cyanophenoxy)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6c**)

Starting from **4c** (104 mg, 0.19 mmol), **6c** (67 mg, 70%) was obtained as a white solid after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 97/3). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19–1.23 (m, 6H, O–CH_2–CH_3), 3.96–4.06 (m, 4H, O–CH_2–CH_3), 4.13–4.20 (m, 2H, H-5′), 4.22–4.25 (m, 1H, H-4′), 4.27–4.30 (m, 1H, H-3′), 4.69 (q, $J = 5.2$, 1H, H-2′), 5.44 (d, $J_{3',OH} = 5.4$, 1H, OH-3′), 5.65 (d, $J_{2',OH} = 5.2$, 1H, OH-2′), 6.09 (d, $J_{1',2'} = 5.2$, 1H, H-1′), 7.69–7.72 (m, 2H, H-4 Arom., H-5 Arom.), 7.79–7.81 (m, 1H, H-6 Arom.), 7.91–7.93 (m, 1H, H-2 Arom.), 8.53 (s, 1H, H-2), 8.72 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P} = 6.4$, 2C, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P} = 3.1$, O–CH_2–CH_3), 66.6 ($^2J_{C,P} = 5.4$, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P} = 7.5$, C-4′), 88.1 (C-1′), 112.4 (C-3 Arom.), 117.9 (CN), 121.3 (C-5), 125.7 (C-2 Arom.), 127.4 (C-4 Arom.), 129.7 (C-6 Arom.), 131.1 (C-5 Arom.), 143.7 (C-8), 151.5 (C-2), 152.4 (C-1 Arom.), 153.1 (C-4), 158.9 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.12; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{21}H_{24}N_5O_8P + H]^+$ 506.1441, found 506.1425.

4.2.18. 6-(3-Biphenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6d**)

Starting from **4d** (85 mg, 0.15 mmol), **6d** (70 mg, 89%) was obtained as a pale yellow oil after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 97/3). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.20 (td, $^4J_{H,P} = 0.9$, $J = 7.1$, 6H, O–CH_2–CH_3), 3.95–4.03 (m, 4H, O–CH_2–CH_3), 4.17–4.23 (m, 2H, H-4′, H-5′), 4.26–4.35 (m, 2H, H-3′, H-5″), 4.76 (q, 1H, $J = 5.0$, H-2′), 5.46 (d, 1H, $J_{3',OH} = 5.3$, OH-3′), 5.70 (d, 1H, $J_{2',OH} = 5.5$, OH-2′), 6.17 (d, 1H, $J_{1',2'} = 4.8$, H-1′), 7.41–7.45 (m, 1H, H-4′ Arom.), 7.51–7.55 (m, 2H, H-3′ Arom., H-5′ Arom.), 7.69–7.77 (m, 3H, H-5 Arom., H-2′ Arom., H-6′ Arom.), 7.87–7.89 (m, 1H, H-6 Arom.), 8.80 (dt, 1H, $J = 1.4$, $J_{4\ Arom.,\ 5\ Arom.} = 7.8$, H-4 Arom.), 8.88 (s, 1H, H-8), 9.06 (s, 1H, H-2), 9.16 (t, 1H, $J = 1.8$, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P} = 6.5$, 2C, O–CH_2–CH_3), 63.3 (d, $^2J_{C,P} = 4.0$, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P} = 4.1$, O–CH_2–CH_3), 66.5 (d, $^2J_{C,P} = 5.6$, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P} = 7.5$, C-4′), 88.0 (C-1′), 126.7 (2C, C-2′ Arom., C-6′ Arom.), 127.7 (2C, C-2 Arom., C-4′ Arom.), 128.2 (C-4 Arom.), 128.3 (2C, C-3′ Arom., C-5′ Arom.), 129.1 (C-5 Arom.), 129.4 (C-6 Arom.), 131.0 (C-5), 135.8 (C-3 Arom.), 139.8 (C-1′ Arom.), 140.6 (C-1 Arom.), 145.0 (C-8), 152.0 (C-2), 152.2 (C-4), 152.8 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.12; HRMS (ESI-TOF): *m*/*z* calcd for $[C_{26}H_{29}N_4O_7P + H]^+$ 541.1852, found 541.1804.

*4.2.19. 6-Phenyl-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6e**)*

Starting from **4e** (149 mg, 0.30 mmol), **6e** (122 mg, 89%) was obtained as a yellow oil after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19 (t, J = 7.1, 6H, O–CH_2–CH_3), 3.94–4.02 (m, 4H, O–CH_2–CH_3), 4.16–4.22 (m, 2H, H-5′), 4.25–4.33 (m, 2H, H-3′, H-4′), 4.75 (q, J = 5.2, 1H, H-2′), 5.45 (d, $J_{3',OH}$ = 5.4, 1H, OH-3′), 5.68 (d, $J_{2',OH}$ = 5.6, 1H, OH-2′), 6.14 (d, $J_{1',2'}$ = 4.9, 1H, H-1′), 7.56–7.64 (m, 3H, H-3 Arom., H-4 Arom., H-5 Arom.), 8.81–8.84 (m, 2H, H-2 Arom., H-6 Arom.), 8.87 (s, 1H, H-8), 9.02 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 6.3, 2C, O–CH_2–CH_3), 63.4 (2d, $^2J_{C,P}$ = 4.1, 2C, O–CH_2–CH_3), 66.6 (d, $^2J_{C,P}$ = 5.3, C-5′), 69.9 (C-3′), 73.0 (C-2′), 82.6 (d, $^3J_{C,P}$ = 7.1, C-4′), 87.9 (C-1′), 128.7 (C-4 Arom.), 129.4 (2C, C-2 Arom., C-6 Arom.), 130.8 (C-5), 131.1 (2C, C-3 Arom., C-5 Arom.), 135.2 (C-1 Arom.), 144.9 (C-8), 152.0 (C-4), 152.2 (C-2), 153.0 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.11; HRMS (ESI-TOF): m/z calcd for $[C_{20}H_{25}N_4O_7P + H]^+$ 465.1539, found 465.1561.

*4.2.20. 6-(3-Methoxycarbonylphenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6f**)*

Starting from **4f** (200 mg, 0.36 mmol), **6f** (153 mg, 83%) was obtained as a white powder after a purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 97/3). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19 (t, J = 7.0, 6H, O–CH_2–CH_3), 3.94 (s, 3H, CO_2CH_3), 3.94–4.03 (m, 4H, O–CH_2–CH_3), 4.16–4.33 (m, 4H, H-3′, H-4′, H-5′), 4.75 (q, J = 5.0, 1H, H-2′), 5.47 (d, $J_{3',OH}$ = 5.4, 1H, OH-3′), 5.70 (d, $J_{2',OH}$ = 5.4, 1H, OH-2′), 6.15 (d, $J_{1',2'}$ = 4.8, 1H, H-1′), 7.79 (t, J = 7.8, 1H, H-5 Arom.), 8.17 (dt, J = 1.5, $J_{4\ Arom.,\ 5\ Arom.}$ = 7.8, 1H, H-4 Arom.), 8.90 (s, 1H, H-8), 9.07 (s, 1H, H-2), 9.10 (dt, J = 1.5, $J_{5\ Arom.,\ 6\ Arom.}$ = 8.0, 1H, H-6 Arom.), 9.45 (t, J = 1.8, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 5.5, 2C, O–CH_2–CH_3), 52.3 (CO_2CH_3), 63.4 (d, $^2J_{C,P}$ = 4.1, 2C, O–CH_2–CH_3), 66.5 (d, $^2J_{C,P}$ = 5.3, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P}$ = 7.7, C-4′), 88.0 (C-1′), 129.4 (C-2 Arom.), 129.9 (C-5 Arom.), 130.2 (C-3 Arom.), 130.9 (C-5), 131.5 (C-4 Arom.), 133.8 (C-1 Arom.), 135.7 (C-6 Arom.), 145.4 (C-8), 151.7 (C-4), 152.0 (C-6), 152.4 (C-2), 165.9 (CO_2CH_3); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.11; HRMS (ESI-TOF): m/z calcd for $[C_{22}H_{27}N_4O_9P + H]^+$ 523.1594, found 523.1586.

*4.2.21. 6-(4-Cyanophenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6g**)*

Starting from **4g** (164 mg, 0.31 mmol), **6g** (109 mg, 72%) was obtained as a white powder after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 96/4). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19 (t, J = 7.0, 6H, O–CH_2–CH_3), 3.94–4.02 (m, 4H, O–CH_2–CH_3), 4.15–4.33 (m, 4H, H-3′, H-4′, H-5′), 4.75 (q, J = 5.2, H-2′), 5.46 (d, $J_{3',OH}$ = 5.5, 1H, OH-3′), 5.69 (d, $J_{2',OH}$ = 5.6, OH-2′), 6.15 (d, $J_{1',2'}$ = 4.9, 1H, H-1′), 8.07–8.10 (m, 2H, H-3 Arom., H-5 Arom.), 8.92 (s, 1H, H-8), 8.96–8.99 (m, 2H, H-2 Arom., H-6 Arom.), 9.09 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 6.4, 2C, O–CH_2–CH_3), 63.3 (d, $^2J_{C,P}$ = 4.1, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P}$ = 4.2, O–CH_2–CH_3), 66.5 (d, $^2J_{C,P}$ = 5.3, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P}$ = 7.6, C-4′), 88.0 (C-1′), 113.2 (C-4 Arom.), 118.5 (CN), 129.8 (2C, C-2 Arom., C-6 Arom.), 131.3 (C-5), 132.6 (2C, C-3 Arom., C-5 Arom.), 139.3 (C-1 Arom.), 145.7 (C-8), 150.8 (C-6), 152.0 (C-4), 152.5 (C-2);^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.12; HRMS (ESI-TOF): m/z calcd for $[C_{21}H_{24}N_5O_7P + H]^+$ 490.1492, found 490.1468.

*4.2.22. 6-(3-Nitrophenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6h**)*

Starting from **4h** (152 mg, 0.28 mmol), **6h** (128 mg, 91%) was obtained as a white powder after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 96/4). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19 (td, $^4J_{H,P}$ = 0.9, J = 7.1, 6H, O–CH_2–CH_3), 3.95–4.03 (m, 4H, O–CH_2–CH_3), 4.16–4.21 (m, 2H, H-4′, H-5′), 4.22–4.28 (m, 1H, H-5″), 4.31 (q, J = 4.9, 1H, H-3′), 4.74 (q, J = 4.9, 1H, H-2′), 5.46 (d, $J_{3',OH}$ = 5.4, 1H, OH-3′), 5.70 (d, $J_{2',OH}$ = 5.4, 1H, OH-2′), 6.15 (d, $J_{1',2'}$ = 4.7, 1H, H-1′), 7.92 (t, J = 8.0, 1H, H-5 Arom.), 8.44 (ddd, $J_{4\ Arom.,\ 6\ Arom}$ = 1.1, $J_{2\ Arom.,\ 4\ Arom.}$ = 2.4, $J_{4\ Arom.,\ 5\ Arom.}$ = 8.3, 1H, H-4 Arom.), 8.95 (s, 1H, H-8), 9.09 (s, 1H, H-2), 9.23 (dt, J = 1.3, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.9, 1H, H-6 Arom.), 9.68 (t, J = 2.3, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.9 (d, $^3J_{C,P}$ = 6.5, 2C, O–CH_2–CH_3), 63.3 (d, $^2J_{C,P}$ = 3.9, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P}$ = 4.1, O–CH_2–CH_3), 66.6 (d, $^2J_{C,P}$ = 5.8, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.7 (d, $^3J_{C,P}$ = 7.5, C-4′), 88.1 (C-1′), 123.7 (C-2 Arom.), 125.6 (C-4 Arom.), 130.5 (C-5 Arom.), 131.0 (C-5), 135.1 (C-6 Arom.), 136.7 (C-1 Arom.), 145.8 (C-8), 148.2 (C-3 Arom.), 150.2 (C-6), 152.1 (C-4), 152.5 (C-2); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.12; HRMS (ESI-TOF): m/z calcd for $[C_{20}H_{24}N_5O_9P + H]^+$ 510.1390, found 510.1420.

*4.2.23. 6-(4-Carboxyphenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6i**)*

Starting from **4i** (148 mg, 0.27 mmol), **6i** (140 mg, 95%) was obtained as a white powder after a purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 98/2 to 88/12). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.18 (t, J = 7.1, 6H, O–CH_2–CH_3), 3.94–4.02 (m, 4H, O–CH_2–CH_3), 4.14–4.29 (m, 3H, H-4′, H-5′), 4.32 (t, J = 4.6, 1H, H-3′), 4.75 (t, J = 5.0, 1H, H-2′), 6.15 (d, $J_{1',2'}$ = 4.8, 1H, H-1′), 8.17 (d, J = 8.3, 2H, H-3 Arom. H-5 Arom.), 8.87–8.89 (m, 3H, H-8, H-2 Arom, H-6 Arom.), 9.05 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 6.3, 2C, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P}$ = 4.3, 2C, O–CH_2–CH_3), 66.6 (d, $^2J_{C,P}$ = 5.6, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.7 (d, $^3J_{C,P}$ = 7.4, C-4′), 88.0 (C-1′), 129.1 (2C, C-2 Arom., C-6 Arom.), 129.4 (2C, C-3 Arom., C-5 Arom.), 131.1 (C-5), 136.1 (C-4 Arom.), 138.4 (C-1 Arom.), 145.2 (C-8), 152.0 (C-2), 152.2 (C-4), 152.4 (C-6), 169.7 (COOH); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.09; HRMS (ESI-TOF): m/z calcd for $[C_{21}H_{25}N_4O_9P + H]^+$ 509.1437, found 509.1435.

*4.2.24. 6-(4-Hydroxyphenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6j**)*

Starting from **4j** (108 mg, 0.21 mmol), **6j** (89 mg, 89%) was obtained as a white powder after a purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.19 (t, J = 7.0, 6H, O–CH_2–CH_3), 3.94–4.02 (m, 4H, O–CH_2–CH_3), 4.14–4.20 (m, 2H, H-5′), 4.24–4.31 (m, 2H, H-3′, H-4′), 4.72 (t, J = 4.9, 1H, H-2′), 5.44 (bs, 1H, OH-3′), 5.66 (bs, 1H, OH-2′), 6.10 (d, $J_{1',2'}$ = 4.9, 1H, H-1′), 6.94–6.98 (m, 2H, H-3 Arom., H-5 Arom.), 8.73–8.76 (m, 3H, H-8, H-2 Arom., H-6 Arom.), 8.90 (s, 1H, H-2), 10.13 (s, 1H, OH); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 6.1, 2C, O–CH_2–CH_3), 63.3 (d, $^2J_{C,P}$ = 3.9, O–CH_2–CH_3), 63.4 (d, $^2J_{C,P}$ = 4.0, O–CH_2–CH_3), 66.6 (d, $^2J_{C,P}$ = 5.7, C-5′), 69.9 (C-3′), 73.0 (C-2′), 82.5 (d, $^3J_{C,P}$ = 7.7, C-4′), 87.8 (C-1′), 115.5 (2C, C-3 Arom., C-5 Arom.), 126.1 (C-1 Arom.), 129.9 (2C, C-2. Arom, C-6 Arom.), 131.3 (C-5), 144.0 (C-8), 151.8 (C-4), 151.9 (C-2), 153.2 (C-6), 160.5 (C-4 Arom.); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.09; HRMS (ESI-TOF): m/z calcd for $[C_{20}H_{25}N_4O_8P + H]^+$ 481.1488, found 481.1467.

*4.2.25. 6-(4-Anilino-4-oxobutanoic acid)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6k**)*

Starting from **4k** (112 mg, 0.18 mmol), **6k** (78 mg, 75%) was obtained as a white powder after a purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 90/10 + 0.5% AcOH). ^{1}H NMR (400.13 MHz, CD_3OD): δ 1.27 (t, J = 7.1, 6H, O–CH_2–CH_3), 2.62–2.73 (m, 4H, NHCO-(CH_2)$_2$–COOH), 4.04–4.12

(m, 4H, O–C*H*$_2$–CH$_3$), 4.27–4.41 (m, 3H, H-4′, H-5′), 4.51 (t, J = 5.0, 1H, H-2′), 4.82–4.85 (m, 1H, H-2′), 6.19 (d, $J_{1',2'}$ = 4.4, 1H, H-1′), 7.74–7.78 (m, 2H, H-3 Arom., H-5 Arom.), 8.58 (s, 1H, H-8), 8.65–8.68 (m, 2H, H-2 Arom., H-6. Arom.), 8.88 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, CD$_3$OD): δ 16.4 (d, $^3J_{C,P}$ = 6.7, 2C, O–CH$_2$–*C*H$_3$), 32.0 (NHCO–*C*H$_2$), 33.5 (*C*H$_2$–COOH), 65.7 (d, $^2J_{C,P}$ = 6.2, 2C, O–*C*H$_2$–CH$_3$), 68.1 (d, $^2J_{C,P}$ = 5.7, C-5′), 71.5 (C-3′), 75.1 (C-2′), 84.2 (d, $^3J_{C,P}$ = 7.6, C-4′), 90.5 (C-1′), 120.4 (2C, C-3 Arom., C-5 Arom.), 131.7 (2C, C-2 Arom., C-6 Arom.), 131.8 (C-1 Arom.), 132.2 (C-5), 143.0 (C-4 Arom.), 145.4 (C-8), 153.3 (C-2), 153.4 (C-4), 155.5 (C-6), 173.7 (COOH), 175.4 (NHCO); ^{31}P NMR (161.62 MHz, CD$_3$OD): δ −0.20; HRMS (ESI-TOF): m/z calcd for [C$_{24}$H$_{30}$N$_5$O$_{10}$P + H]$^+$ 580.1808, found 580.1819.

4.2.26. 6-(3-Carboxyphenyl)-9-(5-O-diethylphosphate-β-D-ribofuranosyl)-9H-purine (**6x**)

To a stirred solution of **4f** (130 mg, 0.23 mmol) in MeOH (0.40 mL) was added KOH (26 mg, 0.46 mmol, 2 equiv) and the reaction mixture was warmed for 10 min at 35 °C. MeOH was then evaporated and the crude mixture was diluted in CH_2Cl_2. Water was added and acidified (pH = 2). The layers were separated and the aqueous layer was then extracted three times with CH_2Cl_2. The combined organic layers were dried over sodium sulfate, filtered and concentrated to dryness to afford a crude oil. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6 + 0.5% AcOH) afforded **4x** (90 mg) as a white powder. Removal of isopropylidene according to the General Procedure afforded **6x** (73 mg, 63%) as a white powder after purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 96/4 to 87/13 + 0.5% AcOH). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.16 (t, J = 7.1, 6H, O–CH$_2$–*CH*$_3$), 3.94–4.02 (m, 4H, O–*CH*$_2$–CH$_3$), 4.14–4.26 (m, 3H, H-4′, H-5′), 4.32 (t, J = 4.6, 1H, H-4′), 4.75 (t, J = 4.9, 1H, H-2′), 6.14 (d, $J_{1',2'}$ = 4.9, 1H, H-1′), 7.66 (t, J = 7.7, 1H, H-5 Arom), 8.16 (d, $J_{4\ Arom.,\ 5\ Arom.}$ = 7.8, 1H, H-4 Arom.), 8.86 (s, 1H, H-8), 8.95–8.97 (m, 1H, H-6 Arom.), 9.04 (s, 1H, H-2) 9.42 (bs, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 15.8 (d, $^3J_{C,P}$ = 6.5, 2C, O–CH$_2$–*C*H$_3$), 63.3 (d, $^2J_{C,P}$ = 3.4, O–*C*H$_2$–CH$_3$), 63.4 (d, $^2J_{C,P}$ = 4.4, O–*C*H$_2$–CH$_3$), 66.7 (d, $^2J_{C,P}$ = 5.4, C-5′), 69.9 (C-3′), 73.1 (C-2′), 82.6 (d, $^3J_{C,P}$ = 7.4, C-4′), 88.0 (C-1′), 128.4 (C-2 Arom.), 130.3 (C-5 Arom.), 130.9 (C-3 Arom.), 131.8 (C-5), 132.0 (C-4 Arom.), 132.1 (C-1 Arom.), 135.0 (C-6 Arom.), 145.0 (C-8), 152.0 (C-4), 152.3 (C-2), 152.8 (C-6), 168.5 (COOH); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.09; HRMS (ESI-TOF): m/z calcd for [C$_{21}$H$_{25}$N$_4$O$_9$P + H]$^+$ 509.1437, found 509.1420.

4.2.27. 6-(2-Naphthyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1a**)

Starting from **6a** (45 mg, 0.09 mmol), **1a** was obtained as a white powder (28 mg, 63%) after HPLC purification (10–50% of CH_3CN in 20 mM TEAA). R_t = 12.32 min (10–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.15 (t, J = 7.1, 9H, $^+$NH–(CH$_2$–*CH*$_3$)$_3$), 2.97 (q, J = 7.1, 6H, $^+$NH-(*CH*$_2$–CH$_3$)$_3$), 3.93–3.96 (m, 2H, H-5′), 4.14–4.16 (m, 1H, H-4′), 4.30–4.32 (m, 1H, H-3′), 4.81 (t, J = 5.3, 1H, H-2′), 6.17 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 7.57–7.65 (m, 2H, H-6 Arom., H-7 Arom.), 8.00 (dd, $J_{6\ Arom.,8\ Arom.}$ = 1.4, $J_{7\ Arom.,8\ Arom.}$ = 7.5, 1H, H-8 Arom.), 8.10 (d, J = 8.9, 1H, H-4 Arom.), 8.14 (d, J = 7.9, 1H, H-5 Arom.) 8.89 (dd, $J_{1\ Arom.,\ 3\ Arom.}$ = 1.7, $J_{3\ Arom.,\ 4\ Arom.}$ = 8.7, 1H, H-3 Arom.), 9.03 (s, 1H, H-2), 9.10 (s, 1H, H-8), 9.49 (bs, 1H, H-1 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 8.6 ($^+$NH–(CH$_2$–*C*H$_3$)$_3$), 45.2 ($^+$NH-(*C*H$_2$–CH$_3$)$_3$), 64.1 (d, $^2J_{C,P}$ = 5.2, C-5′), 71.2 (C-3′), 74.4 (C-2′), 84.7 (d, $^3J_{C,P}$ = 7.1, C-4′), 87.0 (C-1′), 125.6 (C-3 Arom.), 126.7 (C-6 Arom. or C-7 Arom.), 127.6 (C-6 Arom. or C-7 Arom.), 127.8 (C-8 Arom.), 128.1 (C-5 Arom.), 129.2 (C-4 Arom.), 130.2 (C-1 Arom.), 130.7 (C-8a Arom.), 132.7 (C-5), 132.8 (C-4a Arom.), 134.1 (C-2 Arom.), 144.9 (C-8), 152.0 (C-2), 152.6 (C-4), 152.7 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 1.07; HRMS (ESI-TOF): m/z calcd for [C$_{20}$H$_{19}$N$_4$O$_7$P + H]$^+$459.1070, found 459.1038.

4.2.28. 6-(3-Cyanophenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1b**)

Starting from **6b** (52 mg, 0.11 mmol), **1b** was obtained as a white powder (32 mg, 63%) after HPLC purification (10–50% of CH_3CN in a 20 mM TEAA). R_t = 8.36 min (10–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d$_6$): δ 1.19 (t, J = 7.1, 16H, $^+$NH–(CH$_2$–CH$_3$)$_3$), 3.07 (q, J = 7.1, 11H, $^+$NH–(CH$_2$–CH$_3$)$_3$), 3.91–4.00 (m, 2H, H-5′), 4.14–4.15 (m, 1H, H-4′), 4.28 (t, J = 5.4, 1H, H-3′), 4.72 (t, 1H, J = 5.4, H-2), 6.15 (d, J = 5.4, 1H, H-1′), 7.82 (t, 1H, J = 7.9, H-5 Arom.), 8.05 (dt, J = 1.3, J = 7.7, 1H, H-4 Arom.), 9.04 (s, 1H, H-8), 9.07–9.09 (m, 2H, H-2, H-6 Arom.), 9.14 (t, J = 1.3, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d$_6$): δ 8.5 ($^+$NH–(CH$_2$–CH$_3$)$_3$), 45.5 ($^+$NH–(CH$_2$–CH$_3$)$_3$), 64.3 (d, $^2J_{C,P}$ = 4.8, C-5′), 70.8 (C-3′), 74.2 (C-2′), 84.3 (d, $^3J_{C,P}$ = 7.6, C-4′), 87.3 (C-1′), 111.9 (C-3 Arom.), 118.5 (CN), 130.1 (C-5 Arom.), 130.7 (C-5), 132.5 (C-2 Arom.), 133.5 (C-6 Arom.), 134.2 (C-4 Arom.), 136.3 (C-1 Arom.), 145.4 (C-8), 150.2 (C-4), 152.0 (C-2), 152.7 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d$_6$): δ 0.78; HRMS (ESI-TOF): m/z calcd for [C$_{17}$H$_{16}$N$_5$O$_7$P + H]$^+$ 434.0866, found 434.0881.

4.2.29. 6-(3-Cyanophenoxy)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1c**)

Starting from **6c** (58 mg, 0.11 mmol), **1c** was obtained as a white powder (29 mg, 54%) after HPLC purification (10–50% of CH_3CN in 20 mM TEAA). R_t = 7.49 min (10–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d$_6$): δ 1.19 (t, J = 7.1, 15H, $^+$NH–(CH$_2$–CH$_3$)$_3$), 3.08 (q, J = 7.1, 10H, $^+$NH–(CH$_2$–CH$_3$)$_3$), 3.93–4.02 (m, 2H, H-5′), 4.14 (q, J = 3.4, 1H, H-4′), 4.25 (dd, $J_{3',4}$ = 3.3, $J_{2',3'}$ = 5.0, 1H, H-3′), 4.69 (t, J = 5.0, 1H, H-2′), 6.08 (d, $J_{1',2'}$ = 5.0, 1H, H-1′), 7.69–7.71 (m, 2H, H-4 Arom., H-5 Arom.), 7.78–7.81 (m, 1H, H-6 Arom.), 7.92–7.93 (m, 1H, H-2 Arom.), 8.52 (s, 1H, H-2), 8.79 (s, 1H, H-8);^{13}C NMR (100.62 MHz, DMSO-d$_6$): δ 8.5 ($^+$NH–(CH$_2$–CH$_3$)$_3$), 45.4 ($^+$NH–(CH$_2$–CH$_3$)$_3$), 64.5 (d, $^2J_{C,P}$ = 5.4, C-5′), 70.8 (C-3′), 74.0 (C-2′), 84.1 (d, $^3J_{C,P}$ = 7.5, C-4′), 87.4 (C-1′), 112.4 (C-3 Arom.), 118.0 (CN), 121.0 (C-5), 125.7 (C-2 Arom.), 127.4 (C-4 Arom.), 129.6 (C-6 Arom.), 131.1 (C-5 Arom.), 143.4 (C-8), 151.5 (C-2), 152.5 (C-1 Arom.), 153.4 (C-4), 158.8 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d$_6$): δ 0.89; HRMS (ESI-TOF): m/z calcd for [C$_{17}$H$_{16}$N$_5$O$_8$P + H]$^+$ 450.0815, found 450.0807.

4.2.30. 6-(3-Biphenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1d**)

Starting from **6d** (28 mg, 0.05 mmol), **1d** was obtained as a white powder (13 mg, 50%) after HPLC purification (10%–50% of CH_3CN in 20 mM TEAA). R_t = 12.07 min (10–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 1.16 (t, J = 6.8, 10H, $^+$NH–(CH$_2$–*CH*$_3$)$_3$), 3.06 (q, J = 6.8, 7H, $^+$NH-(*CH*$_2$–CH$_3$)$_3$), 3.97–4.09 (m, 2H, H-5′), 4.15 (bs, 1H, H-4′), 4.26 (bs, 1H, H-3′), 4.71 (t, J = 5.9, 1H, H-2′), 6.15 (d, $J_{1',2'}$ = 5.9, 1H, H-1′), 7.41–7.44 (m, 1H, H-4′ Arom.), 7.54 (t, J = 7.8, 2H, H-3′ Arom., H-5′ Arom.), 7.70 (t, $J_{4\ Arom.,\ 5\ Arom.}$ = 7.8, 1H, H-5 Arom.), 7.75 (d, J = 7.3, 2H, H-2′ Arom., H-6′ Arom.), 7.86 (d, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.8, 1H, H-6 Arom.), 8.81 (d, $J_{4\ Arom.,\ 5\ Arom.}$ = 7.7, 1H, H-4 Arom.), 8.93 (s, 1H, H-8), 9.04 (s, 1H, H-2), 9.12 (bs, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 8.5 ($^+$NH–(CH$_2$–*C*H$_3$)$_3$), 45.5 ($^+$NH-(*C*H$_2$–CH$_3$)$_3$), 64.9 (d, J = 4.9, C-5′), 70.7 (C-3′), 74.0 (C-2′), 84.8 (d, J = 8.0, C-4′), 87.3 (C-1′), 126.8 (2C, C-2′ Arom., C-6′ Arom.), 127.6 (C-4′ Arom.), 127.7 (C-2 Arom.), 128.4 (C-4 Arom.), 129.1 (2C, C-3′ Arom., C-5′ Arom.), 129.4 (2C, C-5 Arom., C-6 Arom.), 130.8 (C-5), 135.9 (C-3 Arom.), 139.9 (C-1′ Arom.), 140.6 (C-1 Arom.), 144.7 (C-8), 152.0 (C-2), 152.5 (C-4), 152.7 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.49; HRMS (ESI-TOF): m/z calcd for [C$_{22}$H$_{21}$N$_4$O$_7$P + H]$^+$ 485.1226, found 485.1190.

4.2.31. 6-Phenyl-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1e**)

Starting from **6e** (98 mg, 0.21 mmol), **1e** was obtained as a white powder (44 mg, 47%) after HPLC purification (10–40% of CH_3CN in 20 mM TEAA). R_t = 10.06 min (10–40% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, DMSO-d_6): δ 3.85–3.95 (m, 2H, H-5′), 4.14 (bs, 1H, H-4′), 4.35 (bs, 1H, H-3′), 4.85 (t, J = 5.4, 1H, H-2′), 6.14 (d, $J_{1',2'}$ = 6.1, 1H, H-1′), 7.56–7.59 (m, 3H, H-3 Arom., H-4 Arom., H-5 Arom.), 8.80–8.82 (m, 2H, H-2 Arom., H-6 Arom.), 8.97 (s, 1H, H-2), 9.06 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 63.9 (d, $^2J_{C,P}$ = 4.9, C-5′), 71.4 (C-3′), 74.3 (C-2′), 85.0 (d, $^3J_{C,P}$ = 6.2, C-4′), 86.8 (C-1′), 128.6 (C-4 Arom.), 129.3 (2C, C-2 Arom., C-6 Arom.), 130.4 (C-5), 130.9 (2C, C-3 Arom., C-5 Arom.), 135.3 (C-1 Arom.), 144.9 (C-8), 151.9 (C-2), 152.7 (2C, C-4, C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 2.27; HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{17}N_4O_7P + H]^+$ 409.0913, found 409.0906.

4.2.32. 6-(3-Methoxycarbonylphenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1f**)

Starting from **6f** (105 mg, 0.20 mmol), **1f** was obtained as a white powder (68 mg, 67%) after HPLC purification (10–50% of CH_3CN in 20 mM of TEAA). R_t = 9.60 min (10–50% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.04 (s, 3H, CO_2CH_3), 4.18–4.32 (m, 2H, H-5′), 4.21–4.30 (m, 1H, H-4′), 4.62 (dd, $J_{3',4'}$ = 4.0, $J_{2',3'}$ = 5.1, 1H, H-3′), 4.88 (t, J = 5.2, 1H, H-2′), 6.32 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 7.66 (t, J = 7.8, 1H, H-5 Arom.), 8.09 (dt, J = 1.3, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.9, 1H, H-6 Arom.), 8.43 (d, $J_{4\ Arom.,\ 5\ Arom.}$ = 7.8, 1H, H-4 Arom.), 8.74 (t, J = 1.7, 1H, H-2 Arom.), 8.85 (s, 1H, H-8), 8.90 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, D_2O): δ 55.3(CO_2CH_3), 66.9 (d, $^2J_{C,P}$ = 4.8, C-5′), 72.9 (C-3′), 77.2 (C-2′), 86.3 (d, $^3J_{C,P}$ = 8.6, C-4′), 90.2 (C-1′), 131.1 (C-2 Arom.), 131.3 (C-5 Arom.), 132.1 (C-3 Arom.), 132.2 (2C, C-4 Arom., C-5), 133.9 (C-1 Arom.), 135.9 (C-6 Arom), 146.6 (C-8), 153.7 (C-6), 153.9 (C-2), 154.7 (C-4), 170.1 (CO_2CH_3); ^{31}P NMR (161.62 MHz, D_2O): δ 1.10; HRMS (ESI-TOF): m/z calcd for $[C_{18}H_{19}N_4O_9P + H]^+$ 467.0968, found 467.0936.

4.2.33. 6-(4-Cyanophenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1g**)

Starting from **6g** (95 mg, 0.19 mmol), **1g** was obtained as a white powder (35 mg, 38%) after HPLC purification (10–40% of CH_3CN in 20 mM TEAA). R_t = 9.46 min (10–40% of CH_3CN in 10 mM aq TEAA buffer); ^1H NMR (400.13 MHz, DMSO-d_6): δ 3.84–3.94 (m, 2H, H-5′), 4.13 (bs, 1H, H-4′), 4.34 (bs, 1H, H-3′), 4.85 (t, J = 5.4, 1H, H-2′), 6.14 (d, $J_{1',2'}$ = 6.0, 1H, H-1′), 8.03 (d, J = 8.1, 2H, H-3 Arom., H-5 Arom.), 8.96 (d, J = 8.1, 2H, H-2 Arom., H-6 Arom.), 9.02 (s, 1H, H-2), 9.15 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 63.8 (d, $^2J_{C,P}$ = 4.4, C-5′), 71.4 (C-3′), 74.5 (C-2′), 85.1 (d, $^3J_{C,P}$ = 7.0, C-4′), 87.0 (C-1′), 113.0 (C-4 Arom.), 118.6 (CN), 129.8 (2C, C-2 Arom., C-6 Arom.), 130.9 (C-5), 132.5 (2C, C-3 Arom., C-5 Arom.), 139.4 (C-1 Arom.), 145.7 (C-8), 150.3 (C-6), 152.0 (C-2), 153.0 (C-4); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 1.89; HRMS (ESI-TOF): m/z calcd for $[C_{17}H_{16}N_5O_7P + H]^+$ 434.0866, found 434.0883.

4.2.34. 6-(3-Nitrophenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1h**)

Starting from **6h** (108 mg, 0.21 mmol), **1h** was obtained as a white powder (70 mg, 67%) after HPLC purification (15–50% of CH_3CN in 20 mM TEAA). R_t = 8.91 min (15–50% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.18–4.28 (m, 2H, H-5′), 4.46–4.49 (m, 1H, H-4′), 4.60 (t, J = 4.6, 1H, H-3′), 4.82 (t, J = 5.2, 1H, H-2′), 6.20 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 7.58 (t, J = 8.0, 1H, H-5 Arom.), 8.17 (ddd, $J_{4\ Arom.,\ 6\ Arom.}$ = 1.1, $J_{2\ Arom.,\ 4\ Arom.}$ = 2.4, $J_{4\ Arom.,\ 5\ Arom.}$ = 8.2, 1H, H-4 Arom.), 8.37 (dt, J = 1.3, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.9, 1H, H-6 Arom.), 8.69 (s, 1H, H-2), 8.72 (t, J = 2.0, 1H, H-2 Arom.), 8.75 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.9 (d, $^2J_{C,P}$ = 4.8, C-5′), 73.0 (C-3′), 77.2 (C-2′), 86.8 (d, $^3J_{C,P}$ = 8.8, C-4′), 90.2 (C-1′), 126.2 (C-2 Arom.), 128.3 (C-4 Arom.), 132.6 (C-5 Arom.), 132.8 (C-5), 137.5 (C-6 Arom.), 137.7 (C-1 Arom.), 147.6 (C-8), 150.1 (C-3 Arom.), 154.0 (C-6), 154.3 (C-4), 154.4 (C-2); ^{31}P NMR (161.62 MHz, D_2O): δ 2.35; HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{16}N_5O_9P + H]^+$ 454.0764, found 454.0722.

4.2.35. 6-(4-Carboxyphenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1i**)

Starting from **6i** (90 mg, 0.18 mmol), **1i** was obtained as a white powder (35 mg, 44%) after HPLC purification (0–15% of CH_3CN in 20 mM TEAA). R_t = 14.47 min (0–15% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, DMSO-d_6): δ 3.84–3.93 (m, 2H, H-5′), 4.12 (bs, 1H, H-4′), 4.26–4.47 (m, 1H, H-3′), 4.84 (t, J = 5.5, 1H, H-2′), 6.14 (d, $J_{1',2'}$ = 6.1, 1H, H-1′), 8.06 (d, J = 8.0, 2H, H-3 Arom., H-5 Arom.), 8.78 (d, J = 8.0, 2H, H-2 Arom., H-6 Arom.), 8.98 (s, 1H, H-8), 9.08 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 64.0 (d, $^2J_{C,P}$ = 4.7, C-5′), 71.5 (C-3′), 74.4 (C-2′), 85.1 (d, $^3J_{C,P}$ = 7.3, C-4′), 86.8 (C-1′), 128.6 (2C, C-2 Arom., C-6 Arom.), 129.1 (2C, C-3 Arom., C-5 Arom.), 130.5 (C-5), 135.9 (C-4 Arom.), 141.9 (C-1 Arom.), 144.8 (C-8), 151.9 (C-2), 152.7 (2C, C-4, C-6), 169.1 (COO); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 1.92; HRMS (ESI-TOF): m/z calcd for $[C_{17}H_{17}N_4O_9P + H]^+$ 453.0811, found 453.0871.

4.2.36. 6-(4-Hydroxyphenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1j**)

Starting from **6j** (90 mg, 0.18 mmol), **1j** was obtained as a yellow powder (45 mg, 59%) after HPLC purification (10–30% of CH_3CN in 20 mM TEAA). R_t = 6.09 min (10–30% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.17–4.24 (m, 2H, H-5′), 4.46–4.47 (m, 1H, H-4′), 4.59 (t, J = 4.7, 1H, H-3′), 4.71 (overlapped peak with D_2O, 1H, H-2′), 6.22 (d, $J_{1',2'}$ = 5.0, 1H, H-1′), 6.88–6.91 (d, 2H, J = 8.7, H-3 Arom., H-5 Arom.), 7.96 (d, J = 8.6, 2H, H-2 Arom., H-6 Arom.), 8.65 (s, 1H, H-2), 8.75 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.4 (d, $^2J_{C,P}$ = 4.2, C-5′), 73.0 (C-3′), 77.2 (C-2′), 86.8 (d, $^3J_{C,P}$ = 8.8, C-4′), 90.1 (C-1′), 118.1 (2C, C-3 Arom., C-5 Arom.), 128.3 (C-1 Arom.), 132.2 (C-5), 133.9 (2C, C-2. Arom, C-6 Arom.), 146.4 (C-8), 153.7 (C-4), 154.2 (C-2), 157.1 (C-6), 161.5 (C-4 Arom.); ^{31}P NMR (161.62 MHz, D_2O): δ 3.51; HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{17}N_4O_8P + H]^+$ 425.0862, found 425.0861.

4.2.37. 6-(4-Anilino-4-oxobutanoic acid)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1k**)

Starting from **6k** (63 mg, 0.11 mmol), **1k** was obtained as a white powder (36 mg, 56%) after HPLC purification (0–30% of CH_3CN in 20 mM TEAA). R_t = 11.46 min (0–30% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 2.66–2.79 (m, 4H, NHCO-(CH_2)$_2$–COO), 4.19–4.29 (m, 2H, H-5′), 4.44–4.47 (m, 1H, H-4′), 4.58 (t, J = 4.7, 1H, H-3′), 4.78 (overlapped peak with D_2O, 1H, H-2′), 6.22 (d, $J_{1',2'}$ = 5.2, 1H, H-1′), 7.53–7.57 (m, 2H, H-3 Arom., H-5 Arom.), 8.10–8.14 (m, 2H, H-2 Arom., H-6 Arom.), 8.77 (s, 1H, H-2), 8.78 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 34.3 (NHCO–CH_2), 35.3 (CH_2–COO), 67.0 (d, $^2J_{C,P}$ = 4.8, C-5′), 72.9 (C-3′), 77.2 (C-2′), 86.5 (d, $^3J_{C,P}$ = 8.7, C-4′), 90.1 (C-1′), 122.8 (2C, C-3 Arom., C-5 Arom.), 132.3 (2C, C-2 Arom., C-6 Arom.), 132.6 (C-1 Arom.), 132.9 (C-5), 142.8 (C-4 Arom.), 146.7 (C-8), 154.0 (C-6), 154.4 (C-2), 156.8 (C-4), 176.8 (NHCO), 182.7 (COO); ^{31}P NMR (161.62 MHz, D_2O): δ 1.73; HRMS (ESI-TOF): m/z calcd for $[C_{20}H_{22}N_5O_{10}P + H]^+$ 524.1182, found 524.1228.

4.2.38. 6-(3-Carboxyphenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1x**)

Starting from **6x** (54 mg, 0.10 mmol), **1x** (16 mg, 31%) was obtained as a white powder after HPLC purification (0–20% of CH_3CN in 20 mM TEAA). R_t = 13.66 min (0–20% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, DMSO-d_6): δ 3.92 (bs, 2H, H-5′), 4.13 (bs, 1H,

H-4′), 4.35 (bs, 1H, H-3′), 4.82 (bs, 1H, H-2′), 6.13 (d, $J_{1',2'} = 5.7$, 1H, H-1′), 7.50 (t, $J = 7.8$, 1H, H-5 Arom.), 8.05 (d, $J_{4\ Arom.,\ 5\ Arom.} = 7.6$, 1H, H-4 Arom.), 8.75 (bs, 1H, H-6 Arom.), 8.94 (s, 1H, H-2), 9.06 (s, 1H, H-8), 9.42 (s, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 63.9 (d, $^2J_{C,P} = 4.8$, C-5′), 71.2 (C-3′), 74.5 (C-2′), 84.9 (C-4′), 87.0 (C-1′), 127.7 (C-5 Arom.), 130.4 (2C, C-2 Arom., C-6 Arom.), 130.7 (C-4 Arom.), 131.5 (C-5), 131.6 (C-3 Arom.), 134.6 (C-1 Arom.), 144.7 (C-8), 151.8 (C-4), 152.6 (C-2), 152.9 (C-6), 169.3 (COO); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 2.18; HRMS (ESI-TOF): m/z calcd for $[C_{17}H_{17}N_4O_9P + H]^+$ 453.0811, found 453.0835.

4.3. Synthesis of target nucleotides **1l**–**1w** according to Scheme 1 route B

4.3.1. General procedures for Suzuki–Miyaura cross-coupling reactions

Method E. To a stirred solution of 6-chloropurine riboside (**2**) (1 equiv) in H_2O/CH_3CN (2:1, 6 mL/mmol), $Pd(OAc)_2$ (0.05 equiv), TPPTS (0.15 equiv), arylboronic acid (1.25 equiv) and sodium carbonate (3 equiv) were added under argon atmosphere. The reaction mixture was warmed to 100 °C (or 80 °C when mentioned) and monitored by LC–MS and TLC. When the reaction was complete, the mixture was cooled to room temperature, neutralized by addition of 1 M aqueous HCl, and concentrated under reduce pressure. The residue was purified by silica gel chromatography or by preparative HPLC on a C18 reverse-phase column to yield the title compound **5**.

4.3.2. General procedure for phosphorylation of nucleosides **5**

To a stirred solution of nucleoside **5** (1 equiv) in triethylphosphate (1 mL), phosphoryl chloride (3 equiv) was added at 0 °C under argon atmosphere. The reaction mixture was stirred at 4 °C overnight (monitoring by LC–MS).

The reaction was quenched by addition of 10 mM TEAA buffer at 0 °C, neutralized by addition of Et_3N and diluted with water. The resulting solution was purified by preparative HPLC on a C18 reverse-phase column using a linear gradient of CH_3CN in 20 mM TEAA buffer and then exchanged on a Dowex ion resin to afford the nucleoside 5′-monophosphate **1** as a sodium salt. For poorly-water-soluble compounds, no ion exchange was performed.

4.3.3. 6-(Furan-3-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5l**)

Synthesized from **2** (200 mg, 0.70 mmol) and 3-furanylboronic acid (98 mg, 0.87 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 96/4) afforded **5l** (134 mg, 60%) as a white powder [23]. R_t = 12.66 min (10–40% of CH_3CN in a 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.57–3.62 (m, 1H, H-5′), 3.69–3.74 (m, 1H, H-5″), 4.00 (q, $J = 3.9$, 1H, H-4′), 4.22 (td, $J = 3.7$, $J_{3',OH} = 5.0$, 1H, H-3′), 4.65 (q, $J = 5.5$, 1H, H-2′), 5.10 (t, $J_{5',OH} = 5.6$, 1H, OH-5′), 5.21 (d, $J_{3',OH} = 5.0$, 1H, OH-3′), 5.52 (d, $J_{2',OH} = 5.9$, 1H, OH-2′), 6.06 (d, $J_{1',2'} = 5.5$, 1H, H-1′), 7.39 (d, $J_{4\ Arom.,\ 5\ Arom.} = 2.0$, 1H, H-4 Arom.),7.93 (t, $J = 1.7$, 1H, H-5 Arom.),8.78–8.84 (m, 1H, H-2 Arom.),8.86 (s, 1H, H-8), 8.89 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.8 (C-5′), 70.8 (C-3′), 74.2 (C-2′), 86.2 (C-4′), 88.2 (C-1′), 109.7 (C-4 Arom.), 123.4 (C-3 Arom.), 130.4 (C-5), 145.1 (C-8), 145.2 (C-5 Arom.),146.8 (C-2 Arom.), 149.3 (C-6), 151.7 (C-4), 152.6 (C-2); HRMS (ESI-TOF): m/z calcd for $[C_{14}H_{14}N_4O_5+H]^+$ 319.1042, found 319.1028.

4.3.4. 6-(5-Formylfuran-2-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5m**)

Synthesized from **2** (200 mg, 0.70 mmol) and 5-formyl-2-furanylboronic acid (122 mg, 0.87 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 90/10) afforded **5m** (137 mg, 57%) as a brown powder. R_t = 8.72 min (10–20% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.58–3.63 (m, 1H, H-5′), 3.70–3.75 (m, 1H, H-5″), 4.01 (q, $J = 4.9$, 1H, H-4′), 4.22 (q, $J = 4.8$, 1H, H-3′), 4.66 (q, $J = 5.4$, 1H, H-2′), 5.09 (t, $J_{5',OH} = 5.5$, 1H, OH-5′), 5.23 (d, $J_{3',OH} = 5.1$, 1H, OH-3′), 5.55 (d, $J_{2',OH} = 5.8$, 1H, OH-2′), 6.10 (d, $J_{1',2'} = 5.4$, 1H, H-1′), 7.77 (d, $J_{3\ Arom.,\ 4\ Arom.} = 3.8$, 1H, H-3 Arom.), 8.02 (d, $J_{3\ Arom.,\ 4\ Arom.} = 3.7$, 1H, H-4 Arom.), 8.99 (s, 1H, H-8), 9.03 (s, 1H, H-2), 9.81 (s, 1H, CHO); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.6 (C-5′), 70.6 (C-3′), 74.3 (C-2′), 86.2 (C-4′), 88.3 (C-1′), 118.9 (C-4 Arom.), 124.0 (C-3 Arom.), 130.3 (C-5), 144.0 (C-6), 146.6 (C-8), 152.6 (2C, C-2, C-4), 153.3 (C-5 Arom.), 153.7 (C-2 Arom.), 179.8 (CHO); HRMS (ESI-TOF): m/z calcd for $[C_{15}H_{14}N_4O_6+H]^+$ 347.0992, found 347.0962.

4.3.5. 6-(5-Chlorothienyl-2-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5n**)

Synthesized from **2** (200 mg, 0.70 mmol) and 5-chloro-2-thiopheneboronic acid (125 mg, 0.87 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 95/5) afforded **5n** (152 mg, 68%) as a yellow powder. R_t = 6.47 min (40–60% of CH_3CN in a 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.57–3.61 (m, 1H, H-5′), 3.69–3.74 (m, 1H, H-5″), 4.00 (q, $J = 3.9$, 1H, H-4′), 4.19–4.23 (m, 1H, H-3′), 4.64 (q, $J = 5.3$, 1H, H-2′), 5.09 (t, $J_{5',OH} = 5.6$, 1H, OH-5′), 5.22 (bs, 1H, OH-3′), 5.53 (d, $J_{2',OH} = 4.1$, 1H, OH-2′), 6.07 (d, $J_{1',2'} = 5.3$, 1H, H-1′), 7.37 (d, $J_{3\ Arom.,\ 4\ Arom.} = 4.1$, 1H, H-4 Arom.), 8.45 (d, $J_{3\ Arom.,\ 4\ Arom.} = 4.1$, 1H, H-3 Arom.), 8.88 (s, 1H, H-2), 8.93 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.2 (C-5′), 70.2 (C-3′), 73.8 (C-2′), 85.7 (C-4′), 87.8 (C-1′), 128.6 (C-5), 129.1 (C-4 Arom.), 132.2 (C-3 Arom.), 134.2 (C-5 Arom.), 138.6 (C-2 Arom.), 145.4 (C-8), 147.5 (C-6), 151.7 (C-4), 152.0 (C-2); HRMS (ESI-TOF): m/z calcd for $[C_{14}H_{13}ClN_4O_4S + H]^+$ 369.0424, found 369.0459.

4.3.6. 6-(Quinolin-8-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5o**)

Synthesized from **2** (287 mg, 1.00 mmol) and quinoline-8-boronic acid (216 mg, 1.25 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6) afforded **5o** (283 mg, 75%) as a white powder. R_t = 9.00 min (20–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.60 (dd, $J_{4',5'} = 4.1$, $J_{5',5''} = 12.0$, 1H, H-5′), 3.71 (dd, $J_{4',5''} = 4.2$, $J_{5',5''} = 12.0$, 1H, H-5″), 4.02 (q, $J = 3.9$, 1H, H-4′), 4.23 (dd, $J_{3',4'} = 3.4$, $J_{2',3'} = 4.9$, 1H, H-3′), 4.75 (dd, $J_{2',3'} = 4.9$, $J_{1',2'} = 5.8$, 1H, H-2′), 5.09 (t, $J_{5',OH} = 5.7$, 1H, OH-5′), 5.26 (d, $J_{3',OH} = 4.6$, 1H, OH-3′), 5.57 (d, $J_{2',OH} = 5.8$, 1H, OH-2′), 6.12 (d, $J_{1',2'} = 5.8$, 1H, H-1′), 7.67 (dd, $J_{2\ Arom.,\ 3\ Arom.} = 4.3$, $J_{3\ Arom.,\ 4\ Arom.} = 8.3$, 1H, H-3 Arom.), 7.85 (dd, $J = 7.1$, $J = 8.2$, 1H, H-6 Arom.), 8.14–8.27 (m, 2H, H-5 Arom., H-7 Arom.), 8.62 (dd, $J_{2\ Arom.,\ 4\ Arom.} = 1.8$, $J_{3\ Arom.,\ 4\ Arom.} = 8.4$, 1H, H-4 Arom.), 8.80 (s, 1H, H-8), 8.78 (dd, $J_{2\ Arom.,\ 4\ Arom.} = 1.8$, $J_{2\ Arom.,\ 3\ Arom.} = 4.4$, 1H, H-2 Arom.), 9.05 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.4 (C-5′), 70.5 (C-3′), 73.6 (C-2′), 85.9 (C-4′), 87.6 (C-1′), 121.7 (C-3 Arom.), 126.1 (C-6 Arom.), 127.9 (C-4a Arom.), 129.8 (C-5 Arom. or C-7 Arom.), 130.6 (C-5 Arom. or C-7 Arom.), 133.6 (C-5), 135.2 (C-8 Arom.), 136.2 (C-4 Arom.), 144.8 (C-8a Arom.), 145.7 (C-8), 150.5 (C-2 Arom.), 150.9 (C-4), 151.7 (C-2), 157.4 (C-6); HRMS (ESI-TOF): m/z calcd for $[C_{19}H_{17}N_5O_4+H]^+$ 380.1359, found 380.1306.

4.3.7. 6-(Indol-6-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5p**)

Synthesized from **2** (287 mg, 1.00 mmol) and indole-6-boronic acid (200 mg, 1.25 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 90/10) afforded **5p** (269 mg, 73%) as a white powder. R_t = 10.50 min (20–40% of CH_3CN in a 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.60–3.64 (m, 1H, H-5′),

3.72–3.76 (m, 1H, H-5″), 4.04 (q, J = 3.8, 1H, H-4′), 4.25 (td, $J_{3',4'}$ = 3.7, J = 5.0, 1H, H-3′), 4.69 (q, J = 5.5, 1H, H-2′), 5.17 (bs, 1H, OH-5′), 5.32 (bs, 1H, OH-3′), 5.62 (bs, 1H, OH-2′), 6.11 (d, $J_{1',2'}$ = 5.5, 1H, H-1′), 6.52–6.54 (m, 1H, H-3 Arom.), 7.55 (t, J = 2.7, 1H, H-2 Arom.), 7.71 (d, $J_{4\ \text{Arom.},\ 5\ \text{Arom.}}$ = 8.5, 1H, H-4 Arom.), 8.57 (dd, $J_{5\ \text{Arom.},\ 7\ \text{Arom.}}$ = 1.5, $J_{4\ \text{Arom.},\ 5\ \text{Arom.}}$ = 8.5, 1H, H-5 Arom.), 8.90 (s, 1H, H-8), 8.96 (s, 1H, H-2), 9.17 (t, J = 1.1, 1H, H-7 Arom.), 11.50 (bs, 1H, NH); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.3 (C-5′), 70.3 (C-3′), 73.7 (C-2′), 85.6 (C-4′), 87.7 (C-1′), 101.5 (C-3 Arom.), 114.0 (C-7 Arom.), 119.9 (2C, C-4 Arom., C-5 Arom.), 128.0 (C-6 Arom.), 128.4 (C-2 Arom.), 130.1 (C-3a Arom.), 130.4 (C-5), 135.9 (C-7a Arom.), 144.1 (C-8), 151.8 (C-2), 151.9 (C-4), 154.6 (C-6); HRMS (ESI-TOF): m/z calcd for $[C_{18}H_{17}N_5O_4+H]^+$ 368.1359, found 368.1328.

4.3.8. 6-(5-Bromopyridin-3-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5q**) and 6-(5′-bromo-3,3′-bipyridin-5-yl)-9-(β-D-ribofuranosyl)-9H-purine (**5r**)

Synthesized from **2** (200 mg, 0.70 mmol) and 5-bromopyridine-3-boronic acid (175 mg, 0.87 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6) afforded a mixture of **5q** (110 mg, 40%) and the by-product **5r** (32 mg, 9%). Compounds **5q** and **5r** were further separated by preparative HPLC (20%–40% of CH_3CN in 20 mM TEAA).

4.3.8.1. Compound **5q**.

R_t = 11.5 min (20–40% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.58–3.64 (m, 1H, H-5′), 3.70–3.76 (m, 1H, H-5″), 4.02 (q, J = 3.9, 1H, H-4′), 4.23 (q, J = 4.6, 1H, H-3′), 4.66 (q, J = 5.3, 1H, H-2′), 5.10 (t, $J_{5',OH}$ = 5.5, 1H, OH-5′), 5.24 (d, $J_{3',OH}$ = 5.1, 1H, OH-3′), 5.56 (d, $J_{2',OH}$ = 5.8, 1H, OH-2′), 6.12 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 8.91 (d, J = 2.3, 1H, H-6 Arom), 9.02 (s, 1H, H-8), 9.08 (s, 1H, H-2), 9.26 (t, J = 2.1, 1H, H-4 Arom), 9.88 (d, J = 1.8, 1H, H-2 Arom); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.1 (C-5′), 70.1 (C-3′), 73.8 (C-2′), 85.7 (C-4′), 87.8 (C-1′), 120.3 (C-5 Arom), 131.2 (C-5), 132.7 (C-3 Arom), 138.4 (C-4 Arom), 145.9 (C-8), 148.4 (C-2 Arom), 149.1 (C-6), 152.0 (C-2 or C-6 Arom), 152.1 (C-2 or C-6 Arom), 152.3 (C-4); HRMS (ESI-TOF): m/z calcd for $[C_{15}H_{14}^{79}BrN_5O_4+H]^+$ 408.0307, found 408.0318.

4.3.8.2. Compound **5r**.

R_t = 10.2 min (20–40% of CH_3CN in 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.62 (m, 1H, H-5′), 3.74 (m, 1H, H-5″), 4.03 (q, J = 3.9, 1H, H-4′), 4.24 (q, J = 4.3, 1H, H-3′), 4.67 (q, J = 5.0, 1H, H-2′), 5.11 (t, $J_{5',OH}$ = 5.5, 1H, OH-5′), 5.26 (d, $J_{3',OH}$ = 5.0, 1H, OH-3′), 5.57 (d, $J_{2',OH}$ = 5.7, 1H, OH-2′), 6.13 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 8.53 (t, J = 2.0, 1H, H-4′ Arom), 8.80 (d, J = 2.2, 1H, H-6′ Arom), 9.01 (m, 2H, H-2′ Arom, H-8), 9.09 (s, 1H, H-2), 9.14 (d, J = 2.3, 1H, H-2 Arom), 9.30 (t, J = 2.2, 1H, H-4 Arom), 9.93 (d, J = 2.0, 1H, H-6 Arom); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.1 (C-5′), 70.2 (C-3′), 73.9 (C-2′), 85.7 (C-4′), 87.8 (C-1′), 120.7 (C-5′ Arom), 131.1 (C-5), 131.2 (C-5 Arom), 131.4 (C-3 Arom), 134.3 (C-3′ Arom), 134.7 (C-4 Arom), 136.9 (C-4′ Arom), 145.7 (C-8), 146.4 (C-2′ Arom), 149.9 (C-2 Arom, or C-6 Arom or C-6′ Arom), 150.0 (C-2 Arom, or C-6 Arom or C-6′ Arom), 150.1 (C-2 Arom, or C-6 Arom or C-6′ Arom), 150.4 (C-6), 152.1 (C-2), 152.3 (C-4); HRMS (ESI-TOF): m/z calcd for $[C_{20}H_{17}^{79}BrN_6O_4+H]^+$ 485.0573, found 485.0594.

4.3.9. 6-(3-Bromophenyl)-9-(β-D-ribofuranosyl)-9H-purine (**5s**) and 6-[3-(3-bromobiphenyl)]-9-(β-D-ribofuranosyl)-9H-purine (**5t**)

Synthesized from **2** (200 mg, 0.70 mmol) and 3-bromophenylboronic acid (175 mg, 0.87 mmol) according to the General Procedure Method E. Purification by flash column chromatography (30 g SiO_2, CH_2Cl_2/MeOH: 100/0 to 94/6) afforded a mixture of **5s** (185 mg, 65%) and **5t** (55 mg, 16%). Compounds **5s** and **5t** were further separated by preparative HPLC (40%–60% of CH_3CN in 20 mM TEAA buffer).

4.3.9.1. Compound **5s**.

R_t = 6.58 min (40–60% of CH_3CN in 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.59–3.64 (m, 1H, H-5′), 3.71–3.75 (m, 1H, H-5″), 4.01 (q, J = 3.9, 1H, H-4′), 4.23 (t, J = 4.3, 1H, H-3′), 4.66 (t, J = 5.1, 1H, H-2′), 5.10 (bs, 1H, OH-5′), 5.24 (bs, 1H, OH-3′), 5.55 (bs, 1H, OH-2′), 6.11 (d, $J_{1',2'}$ = 5.4, 1H, H-1′), 7.58 (t, J = 7.9, 1H, H-5 Arom.), 7.79 (ddd, J = 1.0, J = 2.1, $J_{4\ \text{Arom.},\ 5\ \text{Arom.}}$ = 8.0, 1H, H-4 Arom.), 8.83 (dt, J = 1.3, $J_{5\ \text{Arom.},\ 6\ \text{Arom.}}$ = 7.9, 1H, H-6 Arom.), 8.97 (s, 1H, H-8), 9.02 (t, J = 1.7, 1H, H-2 Arom.), 9.04 (s, 1, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.1 (C-5′), 70.2 (C-3′), 73.8 (C-2′), 85.7 (C-4′), 87.8 (C-1′), 122.0 (C-5), 128.1 (C-6 Arom.), 130.9 (C-5 Arom.), 131.7 (C-2 Arom.), 133.7 (C-4 Arom.), 137.4 (C-1 Arom.), 145.4 (C-8), 151.0 (C-6), 151.9 (C-2), 152.4 (C-4); HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{15}^{79}BrN_4O_4+H]^+$ 407.0355, found 407.0322.

4.3.9.2. Compound **5t**.

R_t = 14.74 min (40–60% of CH_3CN in 10 mM TEAA buffer); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.58–3.66 (m, 1H, H-5′), 3.68–3.76 (m, 1H, H-5″), 4.02 (q, J = 3.6, 1H, H-4′), 4.24 (dd, $J_{3',4'}$ = 3.8, $J_{2',3'}$ = 5.0, 1H, H-3′), 4.68 (q, J = 4.9, 1H, H-2′), 5.12 (t, 1H, $J_{5',OH}$ = 5.5, 1H, OH-5′), 5.24 (d, $J_{3',OH}$ = 5.0, 1H, OH-3′), 5.55 (d, $J_{2',OH}$ = 5.7, 1H, OH-2′), 6.13 (d, $J_{1',2'}$ = 5.5, 1H, H-1′), 7.51 (t, J = 7.9, 1H, H-5′ Arom.), 7.62–7.67 (m, 1H, H-4′ Arom.), 7.74 (t, J = 7.8, 1H, H-5 Arom.), 7.79 (dt, J = 1.3, $J_{5'\ \text{Arom.},\ 6'\ \text{Arom.}}$ = 7.7, 1H, H-6′ Arom.), 7.91–7.95 (m, 2H, H-2′ Arom., H-4 Arom.), 8.85 (dt, J = 1.3, $J_{5\ \text{Arom.},\ 6\ \text{Arom.}}$ = 7.8, 1H, H-6 Arom.), 8.97 (s, 1H, H-8), 9.06 (s, 1H, H-2), 9.13 (t, J = 1.8, 1H, H-2 Arom.); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 61.1 (C-5′), 70.2 (C-3′), 73.7 (C-2′), 85.6 (C-4′), 87.7 (C-1′), 122.5 (C-3′ Arom.), 125.9 (C-6′ Arom.), 127.8 (C-2 Arom.), 128.9 (C-6 Arom.), 129.3 (2C, C-4 Arom., C-2′ Arom.) 129.6 (C-5 Arom.), 131.0 (C-5), 131.3, (2C, C-4′ Arom., C-5′ Arom.), 136.0 (C-3 Arom.), 139.1 (C-1 Arom.), 142.3 (C-1′ Arom.), 145.2 (C-8), 152.0 (C-2), 152.3 (C-4), 152.6 (C-6); HRMS (ESI-TOF): m/z calcd for $[C_{22}H_{19}^{79}BrN_4O_4+H]^+$ 483.0668, found 483.0666.

4.3.10. 6-(Furan-3-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1l**)

Starting from **5l** (75 mg, 0.24 mmol), **1l** (54 mg, 52%) was obtained as a yellow powder after HPLC purification (5–20% of CH_3CN in 20 mM TEAA). R_t = 15.38 min (5–20% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, D_2O): δ 4.17–4.27 (m, 2H, H-5′), 4.46–4.49 (m, 1H, H-4′), 4.59 (t, J = 4.4, 1H, H-3′), 4.84 (t, J = 5.1, 1H, H-2′), 6.24 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 7.04 (d, J = 1.8, 1H, H-4 Arom.), 7.71 (t, J = 1.7, H-5 Arom.), 8.41 (bs, 1H, H-2 Arom.), 8.66 (s, 1H, H-2), 8.75 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.8 (d, $^2J_{C,P}$ = 4.7, C-5′), 73.0 (C-3′), 77.1 (C-2′), 86.7 (d, $^3J_{C,P}$ = 8.1, C-4′), 90.0 (C-1′), 111.0 (C-4 Arom.), 123.8 (C-3 Arom.), 131.8 (C-5), 146.6 (C-8), 147.2 (C-5 Arom.), 149.3 (C-2 Arom.), 152.0 (C-6), 153.3 (C-4), 154.5 (C-2); ^{31}P NMR (161.62 MHz, D_2O): δ 2.23; HRMS (ESI-TOF): m/z calcd for $[C_{14}H_{15}N_4O_8P + H]^+$ 399.0706, found 399.0711.

4.3.11. 6-(5-Formylfuran-2-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1m**)

Starting from **5m** (88 mg, 0.25 mmol), **1m** (54 mg, 52%) was obtained as a brown powder after HPLC purification (5–20% of CH_3CN in 20 mM TEAA). R_t = 13.19 min (5–20% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, D_2O): δ 4.18–4.27 (m, 2H, H-5′), 4.47–4.49 (m, 1H, H-4′), 4.61 (t, J = 4.5, 1H, H-3′), 4.87 (t, J = 5.1, 1H, H-2′), 6.27 (d, $J_{1',2'}$ = 5.1, 1H, H-1′), 7.59 (d, $J_{3\ \text{Arom.},\ 4\ \text{Arom.}}$ = 3.7, 1H, H-3 Arom.), 7.65 (d, $J_{3\ \text{Arom.},\ 4\ \text{Arom.}}$ = 3.7, 1H, H-4 Arom.), 8.80 (s, 1H, H-2), 8.88 (s, 1H, H-8), 9.67 (s, 1H, CHO); ^{13}C NMR (100.62 MHz, D_2O): δ 66.8 (d, $^2J_{C,P}$ = 3.6, C-5′), 73.1 (C-3′), 77.2 (C-2′), 86.9 (d, $^3J_{C,P}$ = 8.7, C-4′), 90.3 (C-1′), 120.7 (C-3 Arom.), 127.7 (C-4 Arom.), 131.3 (C-5), 145.9 (C-6), 148.4 (C-8), 154.5 (C-2), 154.6 (C-4), 155.5 (C-2 Arom.), 155.7 (C-5 Arom.), 183.9 (CHO); ^{31}P NMR (161.62 MHz, D_2O): δ 1.74; HRMS (ESI-TOF): m/z calcd for $[C_{15}H_{15}N_4O_9P + H]^+$ 427.0655, found 427.0668.

4.3.12. 6-(5-Chlorothien-2-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1n**)

Starting from **5n** (138 mg, 0.37 mmol), **1n** (104 mg, 57%) was obtained as a yellow powder after HPLC purification (20–40% of CH_3CN in a 20 mM TEAA). R_t = 10.20 min (10–40% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.20–4.31 (m, 2H, H-5′), 4.46–4.49 (m, 1H, H-4′), 4.60 (t, J = 4.7, 1H, H-3′), 4.80 (overlapped peak with D_2O, 1H, H-2′), 6.19 (d, $J_{1',2'}$ = 5.0, 1H, H-1′), 6.78 (d, $J_{3\ Arom.,\ 4\ Arom.}$ = 4.1, 1H, H-4 Arom.), 7.55 (d, $J_{3\ Arom.,\ 4\ Arom.}$ = 4.1, 1H, H-3 Arom.), 8.44 (s, 1H, H-2), 8.68 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.9 (d, $^2J_{C,P}$ = 5.3, C-5′), 73.0 (C-3′), 77.3 (C-2′), 86.5 (d, $^3J_{C,P}$ = 8.3, C-4′), 90.2 (C-1′), 130.1 (C-5), 130.8 (C-4 Arom.), 134.7 (C-3 Arom.), 138.5 (C-2 Arom.), 138.9 (C-5 Arom.), 146.6 (C-8), 150.3 (C-6), 153.4 (C-4), 154.1 (C-2); ^{31}P NMR (161.62 MHz, D_2O): δ 1.95; HRMS (ESI-TOF): m/z calcd for $[C_{14}H_{14}ClN_4O_7PS + H]^+$ 449.0088, found 449.0056.

4.3.13. 6-(Quinolin-8-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1o**)

Starting from **5o** (220 mg, 0.56 mmol), **1o** (34 mg, 12%) was obtained as a white powder after HPLC purification (15–30% of CH_3CN in 20 mM TEAA), together with starting compound **5o** (75 mg, 34%). R_t = 4.33 min (15–30% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, DMSO-d_6): δ 3.99–4.04 (m, 1H, H-5′), 4.08–4.14 (m, 1H, H-5″), 4.16 (q, J = 4.0, 1H, H-4′), 4.25 (dd, $J_{3',4'}$ = 4.0, $J_{2',3'}$ = 5.5, 1H, H-3′), 4.75 (t, J = 5.5, 1H, H-2′), 6.16 (d, $J_{1',2'}$ = 5.5, 1H, H-1′), 7.58 (dd, $J_{2\ Arom.,\ 3\ Arom.}$ = 4.2, $J_{3\ Arom.,\ 4\ Arom.}$ = 8.3, 1H, H-3 Arom.), 7.79 (dd, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.1, $J_{6\ Arom.,\ 7\ Arom.}$ = 8.2, 1H, H-6 Arom.), 7.93 (dd, J = 1.5, $J_{5\ Arom.,\ 6\ Arom.}$ = 7.1, 1H, H-5 Arom.), 8.19 (dd, $J_{5\ Arom.,\ 6\ Arom.}$ = 1.5, $J_{6\ Arom.,\ 7\ Arom.}$ = 8.2, 1H, H-7 Arom.), 8.49 (dd, J = 1.8, $J_{3\ Arom.,\ 4\ Arom.}$ = 8.3, 1H, H-4 Arom.), 8.68 (s, 1H, H-8), 8.79 (dd, $J_{2\ Arom.,\ 4\ Arom.}$ = 1.8, $J_{2\ Arom.,\ 3\ Arom.}$ = 4.2, 1H, H-2 Arom.), 9.06 (s, 1H, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 65.3 (d, $^2J_{C,P}$ = 4.8, C-5′), 70.6 (C-3′), 73.3 (C-2′), 83.5 (d, $^3J_{C,P}$ = 8.0, C-4′), 87.2 (C-1′), 121.7 (C-3 Arom.), 126.0 (C-6 Arom.), 127.9 (C-4a Arom.), 129.8 (C-7 Arom.), 130.6 (C-5 Arom.), 133.4 (C-5), 135.1 (C-8 Arom.), 136.2 (C-4 Arom.), 144.4(C-8), 145.6 (C-8a Arom.), 150.4 (C-2 Arom.), 151.0 (C-4), 151.8 (C-2), 157.3 (C-6); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 0.06; HRMS (ESI-TOF): m/z calcd for $[C_{19}H_{18}N_5O_7P + H]^+$ 460.1022, found 460.1024.

4.3.14. 6-(Indol-6-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1p**)

Starting from **5p** (70 mg, 0.19 mmol) and $POCl_3$ (53 μL, 0.57 mmol), LC–MS showed only 50% of conversion after one night of reaction at 4 °C. $POCl_3$ (53 μL, 0.38 mmol) was added and the mixture was stirred for 18 h at 4 °C. Compound **1p** (38 mg, 41%) was obtained as a yellow powder after HPLC purification (10–50% of CH_3CN in 20 mM TEAA). R_t = 9.88 min (10–50% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.07–4.19 (m, 2H, H-5′), 4.32–4.35 (m, 1H, H-4′), 4.41 (t, J = 4.9, 1H, H-3′), 4.51 (t, J = 4.8, 1H, H-2′), 5.89 (d, $J_{1',2'}$ = 4.5, 1H, H-1′), 6.27 (d, $J_{2\ Arom.,\ NH}$ = 2.9, 1H, NH), 7.18 (d, $J_{4\ Arom.,\ 5\ Arom.}$ = 8.3, 1H, H-4 Arom.), 7.26 (d, J = 3.0, 1H, H-2 Arom.), 7.44 (dd, $J_{5\ Arom.,\ 7\ Arom.}$ = 1.6, $J_{4\ Arom.,\ 5\ Arom.}$ = 8.4, 1H, H-5 Arom.), 7.79 (s, 1H, H-7 Arom.), 8.24 (s, 1H, H-2), 8.45 (s, 1H, H-8). Peak missing : H-3 Arom; ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 66.7 (d, $^2J_{C,P}$ = 4.5, C-5′), 72.7 (C-3′), 77.1 (C-2′), 86.1 (d, $^3J_{C,P}$ = 8.7, C-4′), 90.1 (C-1′), 115.7 (C-7 Arom.), 122.2 (2C, C-4 Arom., C-5 Arom.), 129.0 (C-6 Arom.), 131.0 (C-2 Arom.), 131.9 (C-5), 132.2 (C-3a Arom.), 137.4 (C-7a Arom.), 145.4 (C-8), 152.9 (C-4), 153.5 (C-2), 157.8 (C-6). Peak missing C-3 Arom; ^{31}P NMR (161.62 MHz, D_2O): δ 2.22; HRMS (ESI-TOF): m/z calcd for $[C_{18}H_{18}N_5O_7P + H]^+$ 448.1022, found 448.1035.

4.3.15. 6-(5-Bromopyridin-3-yl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1q**)

Starting from compound **5q** (82 mg, 0.20 mmol), **1q** (37 mg, 35%) was obtained as a white powder after HPLC purification (15–30% of CH_3CN in 20 mM TEAA). R_t = 10.9 min (10–40% of CH_3CN in 10 mM aq TEAA buffer); ^1H NMR (400.13 MHz, D_2O): δ 4.14–4.24 (m, 2H, H-5′), 4.43–4.46 (m, 1H, H-4′), 4.57 (dd, $J_{3',4'}$ = 3.9, $J_{2',3'}$ = 5.1, 1H, H-3′), 4.82 (t, J = 5.2, 2H, H-2′), 6.23 (d, $J_{1',2'}$ = 5.4, 1H, H-1′), 8.52 (t, J = 2.0, 1H, H-6 Arom), 8.55 (d, J = 2.2, 1H, H-4 Arom), 8.76 (s, 1H, H-2), 8.81 (s, 1H, H-8), 9.05 (d, J = 1.9, 1H, H-2 Arom); ^{13}C NMR (100.62 MHz, D_2O): δ 66.8 (d, $^2J_{C,P}$ = 5.1, C-5′), 73.1 (C-3′), 77.2 (C-2′), 86.9 (d, $^3J_{C,P}$ = 8.5, C-4′), 90.1 (C-1′), 123.1 (C-5 Arom), 132.9 (C-5), 134.0 (C-3 Arom), 142.0 (C-6 Arom), 148.0 (C-8), 149.6 (C-2 Arom), 152.3 (C-6), 154.2 (C-4 Arom), 154.3 (C-4), 154.5 (C-2); ^{31}P NMR (161.62 MHz, D_2O): δ 2.40; HRMS (ESI-TOF): m/z calcd for $[C_{15}H_{15}^{79}BrN_5O_7P + H]^+$ 487.9971, found 487.9949.

4.3.16. 6-(3-Bromophenyl)-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1s**)

Starting from **5s** (80 mg, 0.20 mmol), **1s** (39 mg, 38%) was obtained as a white powder after HPLC purification (20–30% of CH_3CN in 20 mM TEAA). R_t = 12.12 min (20–30% of CH_3CN in 10 mM TEAA); ^1H NMR (400.13 MHz, D_2O): δ 4.19–4.23 (m, 2H, H-5′), 4.47–4.48 (m, 1H, H-4′), 4.61 (t, J = 4.6, 1H, H-3′), 4.86 (t, J = 5.2, 1H, H-2′), 6.27 (d, $J_{1',2'}$ = 5.3, 1H, H-1′), 7.29 (t, J = 7.9, 1H, H-5 Arom.), 7.53–7.55 (m, 1H, H-4 Arom.), 8.00 (dt, J = 1.3, $J_{5\ Arom.,\ 6\text{-}Arom.}$ = 8.0, 1H, H-6 Arom.), 8.13 (t, J = 1.8, 1H, H-2 Arom.), 8.79 (s, 1H, H-2), 8.84 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.7 (d, $^2J_{C,P}$ = 4.8, C-5′), 73.0 (C-3′), 77.1 (C-2′), 86.8 (d, $^3J_{C,P}$ = 8.7, C-4′), 90.0 (C-1′), 124.5 (C-3 Arom.), 130.4 (C-6 Arom.), 132.7 (C-5 Arom.), 132.8 (C-5), 134.2 (C-2 Arom.), 136.5 (C-4 Arom.), 138.0 (C-1 Arom.), 147.3 (C-8), 154.2 (C-6), 154.4 (C-2), 155.5 (C-4); ^{31}P NMR (161.62 MHz, D_2O): δ 2.90; HRMS (ESI-TOF): m/z calcd for $[C_{16}H_{16}^{79}BrN_4O_7P + H]^+$ 487.0018, found 487.0030.

4.3.17. 6-[3-(4′-Carboxy)-biphenyl]-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1u**)

Synthesized from **1s** (55 mg, 0.09 mmol) and 4-carboxyphenylboronic acid (20 mg, 0.12 mmol) according to the General Procedure Method E. Purification by reverse-phase HPLC (10–40% of CH_3CN in 20 mM TEAA) followed by an exchange on a sodium Dowex resin, afforded **1u** (26 mg, 47%) as a white powder. R_t = 9.50 min (10–40% of CH_3CN in 10 mM aq TEAA buffer); ^1H NMR (400.13 MHz, D_2O): δ 4.16–4.26 (m, 2H, H-5′), 4.44 (bs, 1H, H-4′), 4.55 (t, J = 4.4, 1H, H-3′), 4.72 (t, J = 5.4, 1H, H-2′), 6.14 (d, $J_{1',2'}$ = 4.9, 1H, H-1′), 7.35–7.49 (m, 4H, H-2′ Arom., H-5 Arom., H-6 Arom., H-6′ Arom.), 7.81 (d, J = 7.9, 2H, H-3′ Arom., H-5′ Arom.), 7.96–7.99 (m, 2H, H-2 Arom., H-4 Arom.), 8.61 (s, 1H, H-2), 8.67 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, D_2O): δ 66.9 (d, $^2J_{C,P}$ = 4.7, C-5′), 73.0 (C-3′), 77.1 (C-2′), 86.4 (d, $^3J_{C,P}$ = 8.1, C-4′), 90.3 (C-1′), 128.7 (2C, C-2′ Arom., C-6′ Arom.), 129.5 (C-2 Arom.), 131.7 (C-4 Arom.), 131.7, 131.9, 132.0 (4C, C-3′ Arom., C-5′ Arom., C-5 Arom., C-6 Arom.), 132.8 (C-5), 136.7 (C-1 Arom.), 137.4 (C-4′ Arom.), 141.7 (C-3 Arom.), 144.1 (C-1′ Arom.), 146.8 (C-8), 153.8 (C-4), 154.2 (C-2), 157.0 (C-6), 177.3 (COO); ^{31}P NMR (161.62 MHz, D_2O): δ 2.13; HRMS (ESI-TOF): m/z calcd for $[C_{23}H_{21}N_4O_9P + H]^+$ 529.1124, found 529.1132.

4.3.18. 6-[3-(6-Methoxy-2-naphtyl)phenyl]-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1v**)

Synthesized from **1s** (32 mg, 0.07 mmol) and 6-methoxy-2-naphtalene boronic acid (21 mg, 0.11 mmol) according to the General Procedure Method E. Purification by reverse-phase HPLC (20–40% of CH_3CN in 20 mM TEAA), followed by an exchange on a sodium Dowex resin, afforded **1v** (28 mg, 66%) as a white powder. R_t = 14.20 min (20–40% of CH_3CN in 10 mM TEAA); ^1H NMR

(400.13 MHz, DMSO-d_6): δ 3.90 (s, 3H, O–CH_3), 3.92–3.95 (m, 2H, H-5′), 4.14–4.16 (m, 1H, H-4′), 4.35 (t, J = 3.8, 1H, H-3′), 4.88 (t, J = 5.4, 1H, H-2′), 6.17 (d, $J_{1',2'}$ = 5.8, 1H, H-1′), 7.21 (dd, J = 2.5, J = 8.9, 1H, H-7 Arom.$_{Naph}$), 7.37 (d, J = 2.6, 1H, H-5 Arom.$_{Naph}$), 7.71 (t, J = 7.8, 1H, H-5 Arom.), 7.88 (dd, J = 1.9, J = 8.5, 1H, H-3 Arom.$_{Naph}$), 7.95–7.97 (m, 3H, H-4 Arom.$_{Ph}$, H-4 Arom.$_{Naph}$, H-8 Arom.$_{Naph}$), 8.23 (bs, 1H, H-1 Arom.$_{Naph}$), 8.85 (d, J = 7.9, 1H, H-6 Arom.$_{Ph}$), 9.03 (s, 1H, H-2), 9.09 (s, 1H, H-8), 9.17 (bs, 1H, H-2 Arom.$_{Ph}$); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 55.2 (O–CH_3), 64.2 (d, $^2J_{C,P}$ = 4.2, C-5′), 71.2 (C-3′), 74.3 (C-2′), 84.7 (d, $^3J_{C,P}$ = 7.2, C-4′), 87.0 (C-1′), 105.8 (C-5 Arom.$_{Naph}$), 119.0 (C-7 Arom.$_{Naph}$), 125.2 (C-1 Arom.$_{Naph}$), 125.4 (C-3 Arom.$_{Naph}$), 127.4, 127.6 (2C, C-Arom), 128.3 (C-6 Arom.$_{Naph}$), 128.8 (C-8a Arom.$_{Naph}$), 129.4, 129.5 (2C, C-Arom.), 129.8 (C-Arom.), 130.6 (C-5), 133.7 (C-4a Arom.$_{Naph}$), 134.9 (C-2 Arom.$_{Naph}$), 136.0 (C-1 Arom.$_{Ph}$), 140.6 (C-3 Arom.$_{Naph}$), 145.0 (C-8), 152.0 (C-2), 152.6 (C-4), 152.8 (C-6), 157.5 (C-6 Arom.$_{Naph}$); ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 1.96; HRMS (ESI-TOF): m/z calcd for $[C_{27}H_{25}N_4O_8P + H]^+$ 565.1488, found 565.1510.

4.3.19. 6-[3-(Dibenzothiophenyl)phenyl]-9-(β-D-ribofuranosyl)-9H-purine 5′-monophosphate (**1w**)

Synthesized from **1s** (28 mg, 0.05 mmol) and 4-dibenzothienylboronic acid (0.06 mmol, 14 mg) according to the General Procedure Method E. Purification by preparative HPLC (30%–50% of CH_3CN in 20 mM TEAA), followed by an ion exchange on a sodium Dowex resin, afforded **1w** (16 mg, 50%) as a white powder. R_t = 12.71 min (30–50% of CH_3CN in 10 mM TEAA); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 3.85–3.87 (m, 2H, H-5′), 4.11 (bs, 1H, H-4′), 4.31 (bs, 1H, H-3′), 4.81–4.84 (m, 1H, H-2′), 6.15 (d, $J_{1',2'}$ = 6.0, 1H, H-1′), 7.52–7.75 (m, 6H, H-Arom), 8.40–8.44 (m, 5H, H-Arom), 9.09 (s, 1H, H-2), 9.23 (s, 1H, H-8); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ 64.4 (C-5′), 72.2 (C-3′), 75.3 (C-2′), 85.7 (C-4′), 87.3 (C-1′), 121.1, 122.8, 125.5, 128.1, 129.1 (11C–H Arom.), 141.1 (C-8), 152.1 (C-2) *Peaks missing*: C_q; ^{31}P NMR (161.62 MHz, DMSO-d_6): δ 1.68; HRMS (ESI-TOF): m/z calcd for $[C_{28}H_{23}N_4O_7PS + H]^+$ 591.1104, found 591.1094.

5. Expression and purification of recombinant rat and human DNPH1s

Wild type rat and human DNPH1s as well as truncated rat DNPH1 D69N were expressed and purified as described [19]. A truncated human DNPH1 was amplified using oligonucleotides AGCTCATATGCGCCCGGCCCTGTACTTCTGC and AGCTCTCGAGAGGATCAGCCTCGAAGTATCGATCCAG with plasmid pET28a human dnph1 as DNA template in a standard PCR reaction. The amplified DNA fragment was digested with NcoI and XhoI restriction enzymes, gel purified and ligated to pET24a digested with the same restriction enzymes. The ligation mixture was used to transform strain DH5α. Plasmid with the correct DNA sequence was used to transform strain Bli5. Expression and purification of the truncated human DNPH1-His was as described previously [19]. The resulting protein contains a His tag at its C terminus and is deleted from 19 to 12 amino acids at the N and C terminus, respectively.

6. Inhibitory potency against rat and human DNPH1s

The K_i values were determined as described previously [10,19]. Briefly, enzymatic activity of rat DNPH1 (5 μM) was measured by incubating the enzyme with dGMP (100 μM) and by following the production of 2-deoxyribose 5-phosphate spectrophotometrically [10]. The activity of human DNPH1 was determined by incubating the enzyme (14 μM) with dGMP (200 μM) and by following the production of guanine (G) by RR-HPLC on a C18 reverse phase column (ZORBAX Eclipse XDB-C18, 2.1 × 50 mm, 1.8 μm) using a flow rate of 0.25 mL/min and a 1–12% linear gradient of acetonitrile in 20 mM TEAA buffer at pH 7 over 3.50 min. The retention times of G and dGMP are 1 min and 2.8 min, respectively. The initial velocity of the reaction was measured at a fixed concentration of dGMP and variable concentrations of inhibitors.

7. Crystallization and data collection

Crystal growth conditions for rat DNPH1 have been described previously [20] and are briefly reported here. Crystals of liganded DNPH1 were grown in high-salt concentrations (1.1–1.3 M $LiSO_4$ or NH_4SO_4) in mild basic buffer (100 mM Tris pH 7.4–8.0) at a protein concentration of 12 mg/mL in 50 mM citrate buffer pH 7.2. It gave rise to diffracting crystals of rat DNPH1 in complex with compound **1a** (Synchrotron beamlines at the ESRF, Grenoble). Identification of crystallization conditions for human DNPH1 was carried out using the vapor diffusion method with a Mosquito (TTP Labtech) nanolitre dispensing system. Sitting drops were set using 400 nL of 1:1 mixture of truncated human DNPH1 protein at 7.5 mg/mL in complex with compound **1a** at 5 mM and a crystallization solution (672 different conditions, commercially available), equilibrating against 150 μL reservoir in a Greiner plate. The best crystals appeared directly in Greiner plate within one week and had dimensions of up to 0.1 mm × 0.1 mm × 0.2 mm. The condition of crystallization was 20% (w/v) PEG-3000, 0.1 M Tris–HCl pH 7 and 0.2 M calcium acetate at 18 °C (condition F6 from commercial kit wizard II, Emerald Biosystems). The single crystals of this complex were flash-frozen in liquid nitrogen using paratone-paraffin oil (50%/50%) as a cryoprotectant. X-ray diffraction data were collected from human DNPH1 in complex with **1a** crystal on the beamline PROXIMA 1 at synchrotron SOLEIL (St Aubin, France). Diffraction images were collected with PILATUS detector 6 M with a rotation of 0.2° per image at a wavelength of 0.81035 Å (see Table 4).

8. Structure refinement

The new crystal structures were solved by molecular replacement using Molrep [38] and the recently solved structure of rat DNPH1 (PDB4FYI [20]) as a template. The first refinement steps of the structures of rat DNPH1 and human in complex with **1a** were performed using Refmac5 with iterative model building of the protein alone using Coot [38]. The ligand chemical structures were designed using the PRODRG server [39] and were placed in the density. Water molecules were added automatically using Coot and the structures of the complexes were further refined using Phenix. Space groups and refinement statistics are given in Table 4.

9. Ligand docking in silico

The new crystal structures afforded the docking of the series of compounds described in this series using the program PLANTS [32]. The ligand 3D coordinates in mol2 format were generated from 1D smile files using the software OpenBabel [40]. The binding site was defined as a sphere of 14 Å diameter around the N7 atom of bound ligand in the crystal structure (or, alternatively with a 12 Å diameter sphere around the C6 atom). The docking was run into the two crystallographically independent monomers for both the human and rat enzymes. In absence of pharmacophoric restraint, the ligands bind frequently into the pocket with the purine moiety in the *syn*-conformation while the phosphate is perfectly placed as in the crystal structures. In between, the ribose ring is slightly distorted compared to the crystal structure. However, among the ten best poses, *anti*-conformation is also observed and shows a similar

mode of binding as that observed in the known crystal structures. Nevertheless, in most of these cases, the ribose and the nucleobase are shifted out (by 1 or 2 Å). To better characterize the possible impact of each chemical substitution, we used the known binding mode of **1a** to restrain the docking searches (using default value of −3.0 for the pharmacophoric restraint). Taking advantage of the web server @TOME-2 [41], we resumed also the docking onto models deduced from the various crystal structures of the rat DNPH1 previously published [19]. This allowed us to evaluate the impact of slight conformational changes and of the variation in the co-crystallized ligands used a pharmacophoric restraint (data not shown). In general, this computation reproduced the results obtained on the two crystal structures described in this study.

10. Cytotoxicity assays

Cytotoxicity of synthesized compounds was assessed on a panel of human tumor cell lines using a quantitative metabolic assay with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) (Imagif, CNRS, Gif/Yvette, France). Cell viability was determined using MTT assay following a 3-day treatment. Measurements were performed in triplicate with a Biomek workstation (Beckman) in 96-well plates. HCT15 and HCT116 cells (colon adenocarcinoma) and HL60 cells (human promyelocytic leukemia cell lines) were grown in RPMI 1640 with L-Glutamine medium supplemented with 10% (v/v) fetal calf serum, 100 UI penicillin, 100 μg/mL streptomycin and 1.5 μg/mL fungizone and kept under 5% CO_2 at 37 °C. 96 well plates were seeded with 2000 cells per well for HL60 and HCT116, or with 600 cells per well for HCT15, in 200 μL medium. After 24 h, compounds dissolved in dimethylsulfoxide (DMSO) were added at a final concentration of 100 μM or 10 μM in a fixed volume of DMSO (1% final concentration). Controls received an equal volume of DMSO. After 72 h incubation, the number of viable cells was measured at 490 nm with the MTS reagent (Promega, Madison, WI). IC_{50} (concentration required to inhibit cell proliferation by 50%) was determined for the most active compounds at final concentrations ranging from 0.005 μM to 100 μM (10 different dilutions in duplicate). For the AK assay, adenosine kinase inhibitor (A-134974, Aldrich) was added to the cells at a final concentration of 5 μM before incubation with tested compounds (at a final concentration of 100 μM or 10 μM). Incubation in the presence of A-134974 alone in DMSO was used as a control. After 72 h incubation, the number of viable cells was measured at 490 nm and IC_{50} was determined as above.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.110.

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