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# 4-Substituted-1,2,3-triazolo nucleotide analogues as CD73 inhibitors, their synthesis, *in vitro* screening, kinetic and *in silico* studies

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## Abstract

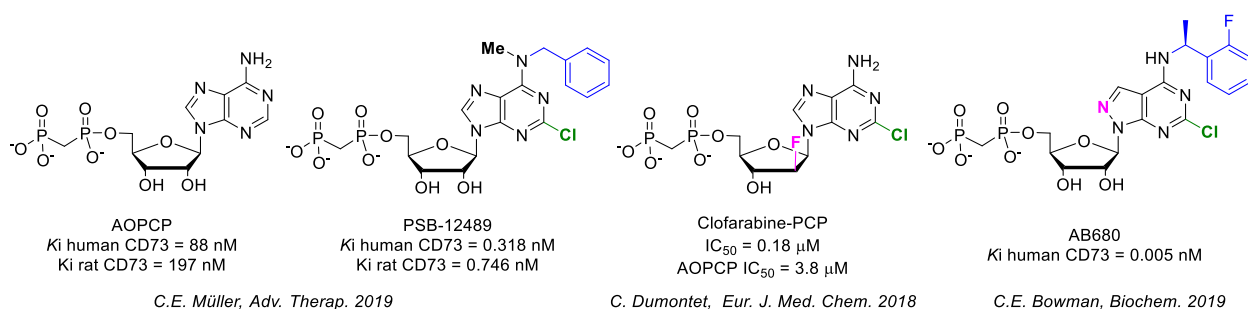
Three series of nucleotide analogues were synthesized and evaluated as potential CD73 inhibitors. Nucleobase replacement consisted in connecting the appropriate aromatic or purine residues through a triazole moiety that is generated from 1,3-dipolar cycloaddition. The first series is related to 4-substituted-1,2,3-triazolo- $\beta$ -hydroxyphosphonate ribonucleosides. Additional analogues were also obtained, in which the phosphonate group was replaced by a bisphosphonate pattern (P-C-P-C, series 2) or the ribose moiety was removed leading to acyclic derivatives (series 3). The  $\beta$ -hydroxyphosphonylphosphonate ribonucleosides (series 2) were found to be potent inhibitors of CD73 using both purified recombinant protein and cell-based assays. Two compounds (**2a** and **2b**) that contained a bis(trifluoromethyl)phenyl or a naphthyl substituents proved to be the most potent inhibitors, with  $IC_{50}$  values of  $4.8 \pm 0.8 \mu\text{M}$  and  $0.86 \pm 0.2 \mu\text{M}$ , compared to the standard AOPCP ( $IC_{50}$  value of  $3.8 \pm 0.9 \mu\text{M}$ ), and were able to reverse the adenosine-mediated immune suppression on human T cells. This series of compounds illustrates a new type of CD73 inhibitors.

## Keywords

Nucleotide, bis-phosphonate, click chemistry, 5'-ectonucleotidase, enzyme inhibitor, immunology

## 1. Introduction

Human ecto-5'-nucleotidase CD73 is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein belonging to the family of 5'-nucleotidases (3.1.3.5). It is mainly involved in the hydrolysis of extracellular adenosine 5'-monophosphate (AMP) into adenosine and inorganic phosphate [1], and consequently regulates the production of immunosuppressive and pro-tumoral adenosine in the tumor microenvironment [2]. CD73 also exists as a soluble form (likely issued upon cleavage of the GPI anchor) which contributes to the immune suppressive response [3, 4]. Many studies have shown that CD73 blockade leads to the decrease of tumor growth and metastasis [5–9]. Thus, CD73 is a validated target in oncology [10, 11] and recently lead to clinical trials with both small molecules [12, 13] and monoclonal antibodies (mAbs) [14] targeting this enzyme [15]. However, aside from their production cost, the use of mAbs requires the injection of large amounts and, in case of solid tumors, they suffer from a poor penetration and retention in the targeted tissue [16, 17]. Thus, it is of great interest to pursue the development of small molecules targeting CD73 [13, 18].



**Fig 1.** Selection of CD73 inhibitors (substrate analogues) reported in the literature [19–21].

Historically, one of the most potent inhibitors of CD73 is AOPCP (5'-( $\alpha,\beta$ -methylene)diphosphate adenosine, Fig. 1), that has been source of inspiration for us [20] and others [19, 21] in the search for more potent inhibitors. Although these compounds showed good inhibitory potential toward CD73, they can suffer from poor bioavailability as they are negatively charged at physiological pH, and they can undergo intensive metabolism through the enzymatic cleavage of either the P-O-C5' linkage and/or depurination. Indeed, stability studies in rat liver microsomes showed a complete degradation of AOPCP within ~10 min compared to 5% degradation for compound PSB-12489 within 8 hours [19, 22]. Nevertheless, these results highlighted the fact that the nucleobase modification can readily improve the metabolic stability of these derivatives. Among those, researchers from Arcus Biosciences have proposed the replacement of the purine by a pyrazolopyridine scaffold [21] and reported the unique properties of AB680 [23], which recently entered Phase 1 clinical trials in metastatic pancreatic tumors.

As a part of our work targeting 5'-nucleotidases, we reported new compounds combining CD73 inhibition and intrinsic cytotoxicity of nucleoside analogues [20]. Thus, clofarabine-PCP (Fig. 1) exhibited higher activity than AOPCP. We also proposed another type of analogues where the 5'-aminoadenosine moiety was used as a scaffold allowing us to perform di-substitution at the 5' position [20, 24] and to replace the labile P-O-C5' linkage. However, these modifications failed to give significant inhibition of CD73 compared to AOPCP and previously reported analogues.

Recently, the co-crystal structure of PSB-12489 in the active site has been reported [19] and shown some similarity with the binding mode of AOPCP reported by Knapp K. *et al.* [25]. Phenylalanine residues (F500 and F417) exert hydrophobic  $\pi$ -stacking interactions with the nucleobase of PSB-12489 while the aspartate residue (D506) showed hydrogen interactions with both hydroxyl groups of the ribose moiety (Fig. S1). Aside from these interactions, the phosphonate groups exhibit various interactions with the residues N245, R354, G447, N117 and H118 but also with two zinc ions located in the active site. In addition, the  $N^6$  benzyl moiety shows hydrophobic interactions with the carbon atoms of the residues D121, S185 and N186 located in the *N*-terminal domain (Fig. S1). These results suggest that additional space is available in the hydrophobic pocket where the nucleobase is located. Beside our research on CD73, we previously reported a series of nucleoside 5'-monophosphate analogues targeting the 5'-cytosolic nucleotidase II (cN-II), also referred as  $\beta$ -hydroxyphosphonate ribonucleosides [26]. Given the structural similarity between the two preferred substrates of cN-II and CD73, IMP and AMP respectively, we decided to reposition this series of compounds and these latter have been tested as CD73 substrate analogues and inhibitors.

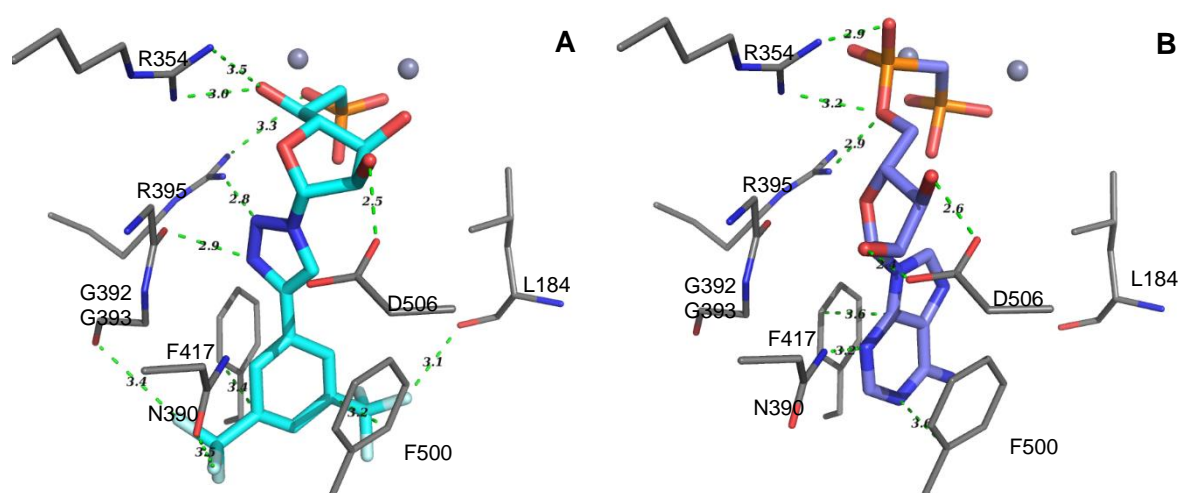
## 2. Results and Discussion

### 2.1. Preliminary dataset and novel analogues

Our initial library of  $\beta$ -hydroxyphosphonate ribonucleosides including 4-substituted-1,2,3-triazoles [25] as nucleobases was assayed for their ability to inhibit 5'-ectonucleotidase activity using a rapid *in vitro* cell-based assay at a single concentration (100  $\mu$ M). None of them provided convincing evidence with regard to CD73 inhibition with the exception of the derivative including a 3,5-(bistrifluoromethyl)phenyl residue as substituent of the triazole ring and reaching  $22 \pm 5\%$  inhibition (compound **1a**, Table 1).

*In silico* studies of **1a** as potential CD73 inhibitor were performed (Fig. 2) and showed that this derivative has a docking score value (DS) of 122 that is higher than AMP (DS = 110) but lower than AOPCP (DS = 152). In addition, predicted binding poses (Fig. 2A) indicated the loss of one hydrogen

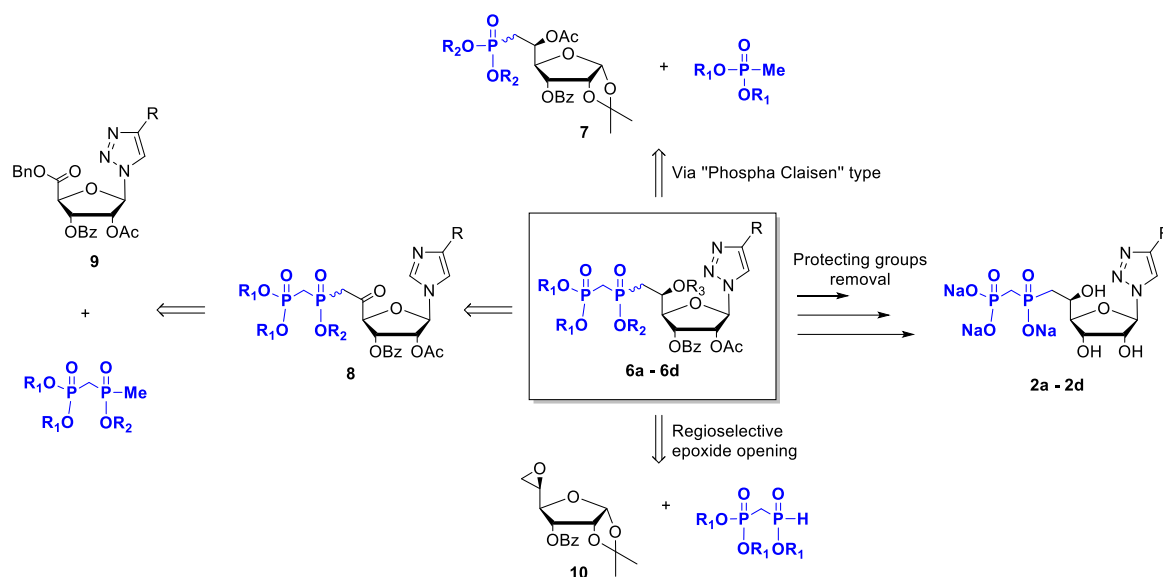
bond with ribose-D506 in comparison to the AOPCP (Fig. 2B) but additional interactions were observed. They mainly concerned: (i) an H-bond between the hydroxyl group in the  $\beta$ -position related to the phosphorus atom and R354, (ii) the ionic interaction between the triazole nitrogen and R395, (iii) closer van der Waals contacts with F417 and F500 and (iv) two halogen bonds observed with F atom and N390/G393/L184 (Fig. 2A).



**Fig. 2.** Close-up views of main interactions between **1a** (panel A) or AOPCP (panel B) with CD73. (A) Binding poses of **1a** (cyan sticks) in the catalytic pocket of the targeted protein (CD73, PDB 4H2I) showing key-interactions (labelled as green dashed lines). (B) Same view of the binding site with AOPCP (blue sticks).

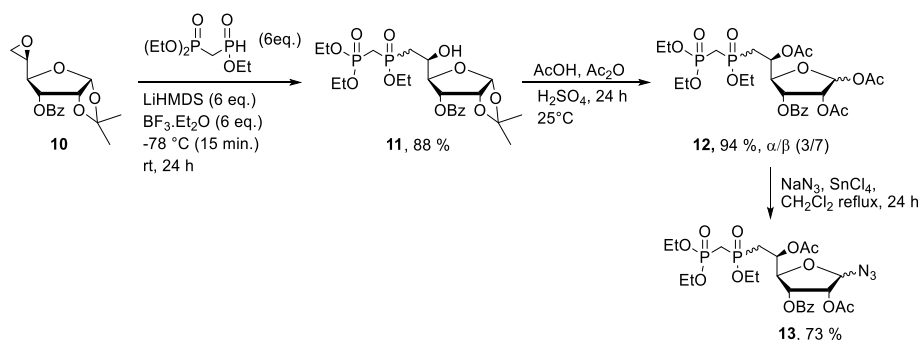
In light of the above considerations, we decided to further investigate  $\beta$ -hydroxyphosphonate ribonucleoside analogues as well as two original series of compounds derived from **1a** (Fig. 3) for targeting CD73. In order to maintain the hydrophobic  $\pi$ -stacking interactions, a triazole incorporating aromatic groups was used for nucleobase replacement. Thus, the first series (Fig. 3, **1b-d**) corresponds to the **1a** skeleton where the aromatic moiety is extended. In the second series (Fig. 3, **2a-d**), we replaced the phosphonate group by a bisphosphonate pattern (P-C-P-C) similar to AOPCP but where the oxygen atom in 5'-position was replaced by a carbon atom. Finally, in the third series of compounds (Fig. 3, **3a-e**), we removed the ribosyl moiety and synthesized new compounds in which the bisphosphonate chain and the substituted triazole are directly linked. We also substituted the aromatic group by an adenylyl residue (Fig. 3, **3f-h**) to restore the interactions observed with the potent CD73 inhibitors (AOPCP, PSB-12489...) previously reported in the literature [19] (Fig. 1).





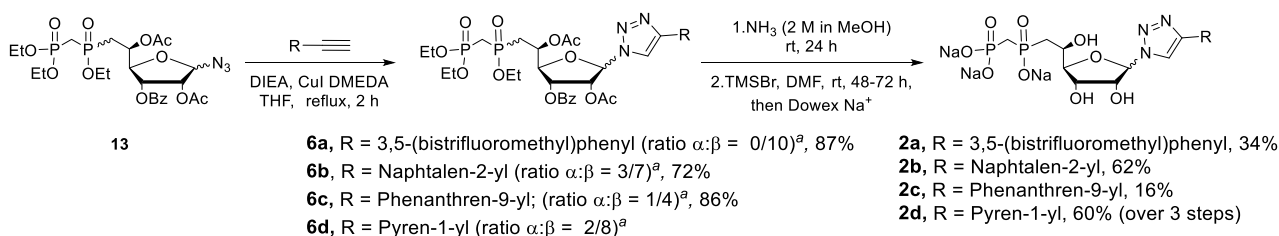
**Scheme 2.** Retrosynthetic approach to reach the P-C-P-C chaining;  $R_1 = i$ -Propyl,  $R_2 =$  Ethyl.

The first approach involved a phosphorus Claisen type reaction between derivative **7** (already available in our laboratory [26, 28]) and the commercially available diisopropyl-methylphosphonate, following the procedure reported by Gavara L. *et al.*, [29, 30]. Despite several attempts, this approach did not give rise to the desired compound and we only observed the removal of basolabile protecting groups from **7**. Thus, the second approach based on the synthesis of the  $\beta$ -cetophosphonylphosphonate **8** was tested. This strategy has been previously used for the preparation of pyrimidine containing nucleoside  $\beta$ -(*R/S*)-hydroxyphosphonate analogues [31]. However, this last failed to give satisfaction (formation of compound **8** was not observed and degradation of starting material **9** occurred after 18h in presence of *n*-BuLi). Therefore, the third strategy relying on the regioselective ring opening of the epoxide **10** was implemented. The latter is inspired from the synthesis of  $\beta$ -hydroxyphosphonate ribonucleosides [26] and the preparation of the Bissret reagent [32] was required beforehand. Optimization of this step led to the use of a large excess of each reactant (LiHMDS,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ , and Bissret reagent) to isolate the  $\beta$ -hydroxyphosphonylphosphonate derivative **11** (Scheme 3) in 88% yield.



**Scheme 3.** Synthesis of the key intermediate **13**.

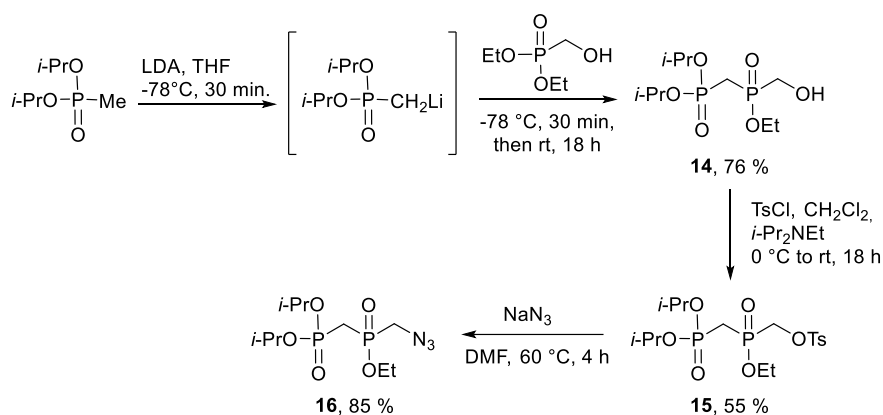
Concomitant removal of the 1,2-isopropylidene group and acetolysis was accomplished through the treatment of compound **11** with acetic acid and acetic anhydride in the presence of a catalytic amount of sulfuric acid. Compound **12** was obtained with 94% yield as a mixture of  $\alpha$ - and  $\beta$ -anomers (ratio  $\alpha$ :  $\beta$  = 3:7 determined by  $^1\text{H}$  NMR spectroscopy). Azidation of this latter gave rise to the desired key intermediate **13** with 73% yield as a mixture of  $\alpha$ / $\beta$  anomers which ratio was not possible to determine by NMR spectroscopy at this stage. Then, 1,3-dipolar cycloaddition reaction was performed using derivative **13** and a selection of commercially available alkynes (2-ethynyl-naphthalene, 9-ethynylphenanthrene, 1-ethynylpyrene and 1-ethynyl (3,5-bistrifluoromethyl)phenyl), in presence of CuI as catalyst, and afforded the fully protected 1,4-triazolo-ribonucleotides **6a-c** (72-87%) as mixtures of  $\alpha$ - and  $\beta$ -anomers (Scheme 4).



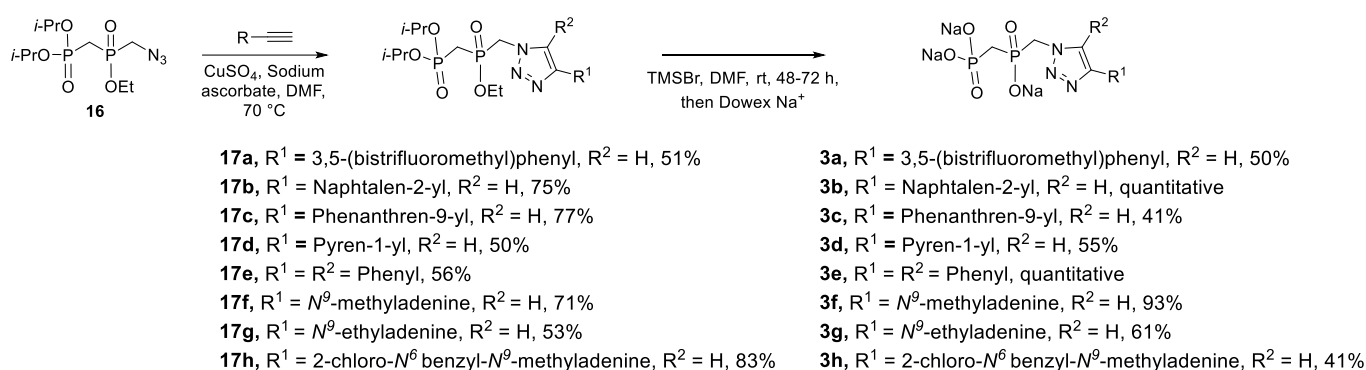
**Scheme 4.** Synthetic pathway of compounds **2a-d** belonging to the  $\beta$ -hydroxyphosphonyl-phosphonate derivatives, series 2. <sup>a</sup> ratio determined by  $^1\text{H}$  NMR spectroscopy.

Removal of sugar protecting groups was carried out using a methanolic ammonia solution (2 M) and the resulting adducts were treated with TMSBr to provide the corresponding phosphonic acids that were isolated as sodium salt **2a-c** (16-62% over 2 steps). For compound **2d**, the reaction sequence was performed from **13**, without isolation of intermediate **6d** as pure compound, affording the expected material in 60% overall yield.  $\beta$ -anomer was the major anomer present in all final compounds (**2a-d**). In order to study the relevance of the ribosyl moiety, we developed the third series of compounds. The proposed synthetic pathway required the prior synthesis of the key intermediate **16** (Scheme 5). This latter was obtained in three steps with 35% overall yield starting from the commercially available diisopropylmethylphosphonate and following the methodology described by Gavara L. *et al.*, [30] (Scheme 5).





**Scheme 5.** Synthesis of the key intermediate **16**.



**Scheme 6.** Synthetic pathway of compounds **3a-h** belonging to acyclic bis-phosphonate derivatives, series 3.

CuAAC reactions were carried out by reacting the intermediate **16** with the appropriate and commercially available alkynes in the presence of CuSO<sub>4</sub> (Scheme 6) to afford compounds **17a-e** (50-77%). The introduction of the purine residues was successfully performed using synthetic alkynes (obtained following described procedures [33,34]) and compounds **17f-h** (53-83%) were isolated. Final deprotection of the phosphonic acids was carried out in the presence of TMSBr and they were isolated as their sodium salt **3a-h** (41-100%).

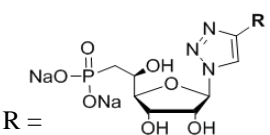
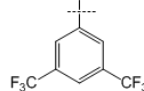
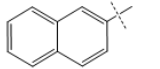
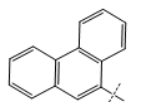
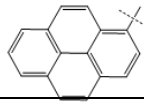
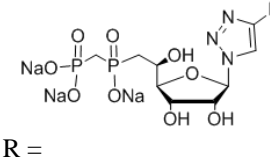
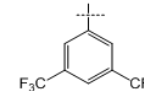
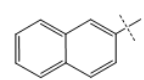
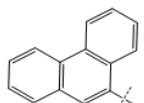

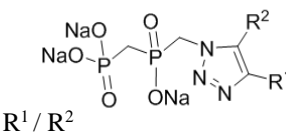
### 2.3. Inhibitory activity of synthesized compounds toward hCD73

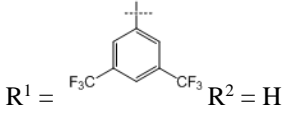
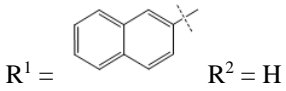
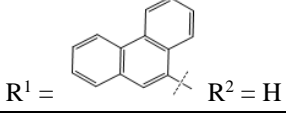
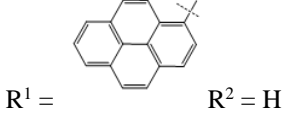
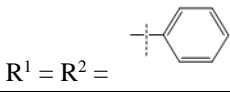
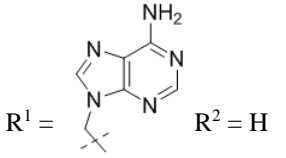
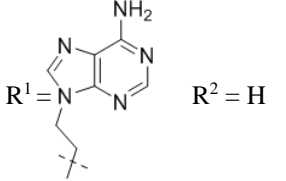
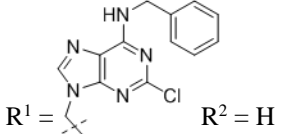
All synthesized compounds were evaluated for their inhibitory potential toward purified recombinant human CD73 protein as well as in a cell-based assay (Table 1). Comparing results with the recombinant enzyme and the three compounds (**1a**, **2a**, and **3a**) containing the bis(trifluoromethyl)phenyl moiety; **1a** and **3a** showed a weak inhibition at the two tested concentrations (10 and 100 μM) compared to the bis-phosphonate derivative **2a**, which induced a strong inhibition of the hCD73 activity from 10 μM and a full inhibition at higher concentrations. The results from the cell-based assay confirmed these

data (91% for **2a** vs 22% and 37% for **1a** and **3a**, respectively) that highlight the crucial importance of the bis-phosphonate for an optimal binding orientation (and consequently enzymatic inhibition). The contribution of the bis(trifluoromethyl)phenyl group may also play an important role for additional interactions with CD73 amino acids (aromatic substituent in place of the nucleobase and halogen bonding with CF<sub>3</sub>). Similarly, the derivatives carrying a naphthalene group led to a strong (81-99%) inhibition in the range 10-100 μM and moderately (25%) at 5 μM in the case of **2b**, while **1b** and **3b** exhibited only weak enzyme inhibition. Compound **2b** could not be evaluated in the cell-based assay due to technical interferences, but **1b** and **3b** were only weak inhibitors here as well. When substituting the naphthalene group by phenanthrene or pyrene (to increase aromaticity), a similar behavior was observed with a 86-97% inhibition (at 100 μM) induced only by bis-phosphonate derivatives **2c**, **2d**, **3c** and **3d**. A better efficacy of pyrene versus phenanthrene was observed using the recombinant protein whereas in the cellular assay, these compounds were ranked in the following order: **3d**<**2c**<**3c**<**2d** with inhibitions between 53 and 101%. The introduction of the purine nucleobase within the third series (**3f-h**) promoted an improvement of the enzyme inhibition exclusively for **3h** harboring a N<sup>6</sup>-benzyl substituent on the 2-chloropurine moiety, and this was true using both the recombinant protein and the cell-based assays. Unfortunately, the absence of ribose moiety is highly detrimental for conserving a strong inhibition. We also assessed the intrinsic toxicity of the studied compounds by the means of their antiproliferative activity at 100 μM using breast cancer cells (MDA-MB-231), and found that compounds in series 1, as well as compounds **2b**, **3e**, **3f** and **3g** had no or weak toxicity (>80% cell survival). All other compounds showed at least moderate toxicity with cell survival between 13 and 44% survival after three days.

For compounds exhibiting a strong inhibition of the *in vitro* hCD73 activity (>50% at 10 μM), IC<sub>50</sub> curves were computed from kinetics inhibition assays in presence of varying inhibitor concentrations. Inhibition curves are shown in Fig. 4 for the selected compounds, with IC<sub>50</sub> values (also reported in Table 1) of 4.8 ± 0.8, 0.86 ± 0.2, 3.6 ± 0.6 and 4.2 ± 0.7 μM for compounds **2a**, **2b**, **2d** and **3d**, respectively. Inhibition was found in the same range of that observed for AOPCP, used as standard, and the strongest inhibition (IC<sub>50</sub> = 0.8 μM) was induced by compound **2b**, the derivative wearing a naphthyl group as substituent on the triazole.

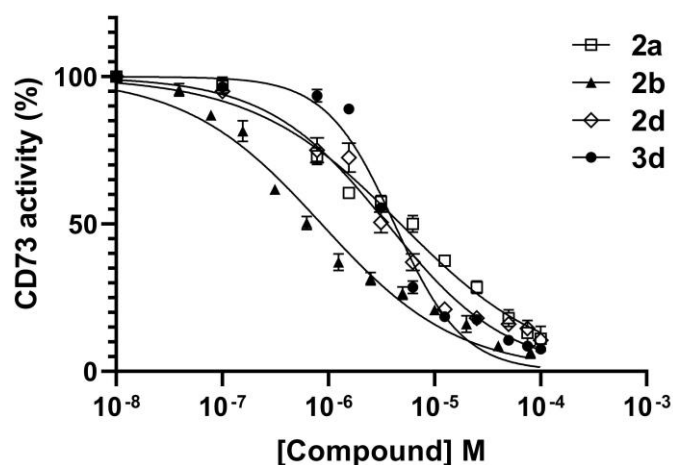
**Table 1.** Inhibitory activities and intrinsic cytotoxicity of studied compounds. The inhibitory activities are represented as percentage inhibition of the AMPase activity of CD73, and the intrinsic cytotoxicity as percentage cell survival as compared to unexposed cells.

Compound	Structure	Docking score	Inhibition (%)			Cell-based assay**	Cell survival (%)
			Purified recombinant <i>h</i> CD73				
			100 $\mu$ M	10 $\mu$ M	IC <sub>50</sub> ( $\mu$ M)	100 $\mu$ M	100 $\mu$ M
<b>AOPCP</b>		<b>152</b>	98 $\pm$ 5	85 $\pm$ 5	3.8 $\pm$ 0.9*	84 $\pm$ 2	99.7 $\pm$ 2.8
<b>Series 1</b>							
<b>1a</b>		122	10 $\pm$ 1	3 $\pm$ 4		22 $\pm$ 5	108 $\pm$ 16
<b>1b</b>		120	37 $\pm$ 7	29 $\pm$ 5		30 $\pm$ 4	107 $\pm$ 3
<b>1c</b>		130	6 $\pm$ 7	13 $\pm$ 6		60 $\pm$ 6	114 $\pm$ 13
<b>1d</b>		129	<b>75 <math>\pm</math> 2</b>	13 $\pm$ 2		38 $\pm$ 6	99 $\pm$ 14
<b>Series 2</b>							
<b>2a</b>		147	<b>94 <math>\pm</math> 4</b>	<b>70 <math>\pm</math> 6</b>	<b>4.8 <math>\pm</math> 0.8</b>	<b>91 <math>\pm</math> 6</b>	37 $\pm$ 4
<b>2b</b>		141	<b>98 <math>\pm</math> 2</b>	<b>81 <math>\pm</math> 3</b>	<b>0.86 <math>\pm</math> 0.2</b>	<i>n.d.</i>	80 $\pm$ 5
<b>2c</b>		143	<b>88 <math>\pm</math> 3</b>	38 $\pm$ 5		60 $\pm$ 5	42 $\pm$ 6
<b>2d</b>		144	<b>97 <math>\pm</math> 3</b>	<b>76 <math>\pm</math> 3</b>	<b>3.6 <math>\pm</math> 0.6</b>	<b>101 <math>\pm</math> 11</b>	27 $\pm$ 5
<b>Series 3</b>							

<b>3a</b>		<b>127</b>	$10 \pm 2$	$22 \pm 7$		$37 \pm 8$	$26 \pm 6$
<b>3b</b>		<b>127</b>	$12 \pm 5$	$18 \pm 3$		$38 \pm 6$	$29 \pm 9$
<b>3c</b>		<b>137</b>	<b><math>86 \pm 6</math></b>	$11 \pm 5$		$71 \pm 13$	$33 \pm 5$
<b>3d</b>		<b>139</b>	<b><math>92 \pm 7</math></b>	$60 \pm 5$	<b><math>4.2 \pm 0.7</math></b>	$53 \pm 14$	$13 \pm 2$
<b>3e</b>		<b>117</b>	$4 \pm 7$	$2 \pm 2$		$-1 \pm 3$	$106 \pm 6$
<b>3f</b>		<b>134</b>	$29 \pm 7$	$2 \pm 6$		$30 \pm 3$	$97 \pm 2$
<b>3g</b>		<b>136</b>	$47 \pm 8$	$3 \pm 2$		$20 \pm 1$	$108 \pm 7$
<b>3h</b>		<b>148</b>	$56 \pm 6$	$3 \pm 5$		$74 \pm 4$	$44 \pm 5$

n.d., due to interference between the coloration of compound **2b** and the Green Malachite reagent. \* from reference [20].

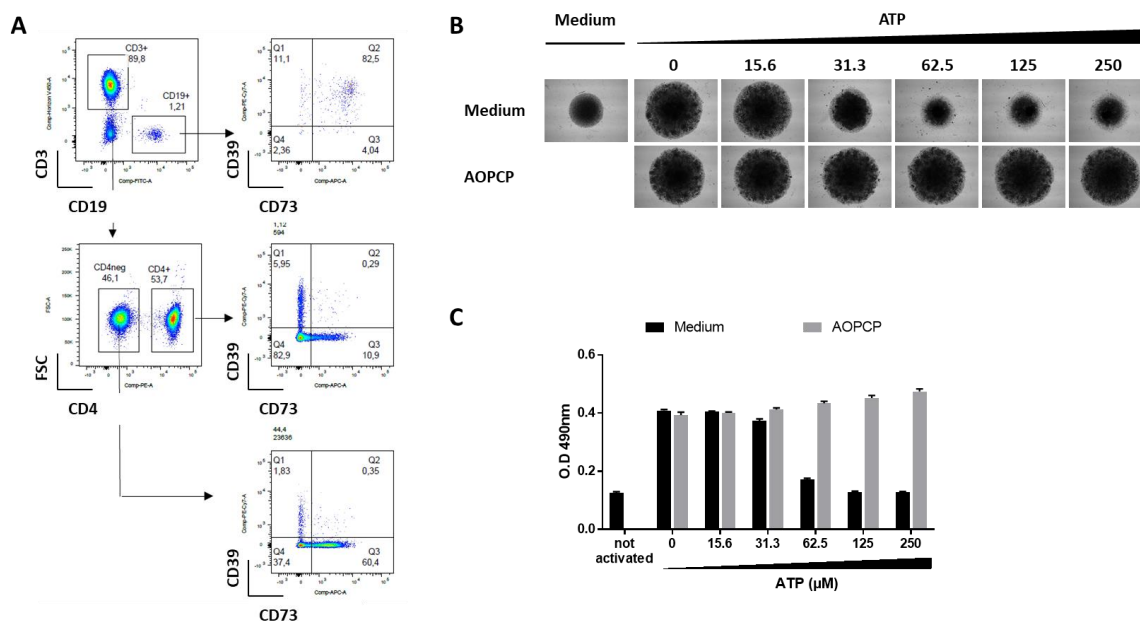
\*\* The inhibition activities are represented as percentage inhibition of the production of phosphate by CD73.



**Fig. 4.** *In vitro* enzymatic activity (recombinant purified *h*CD73) measured in presence of the most active compounds, **2a** (□), **2b** (▲), **2d** (◇) and **3d** (●). Data are represented as mean + SD from three independent kinetics reactions.

#### 2.4. Inhibitory function in immune cell-based assay

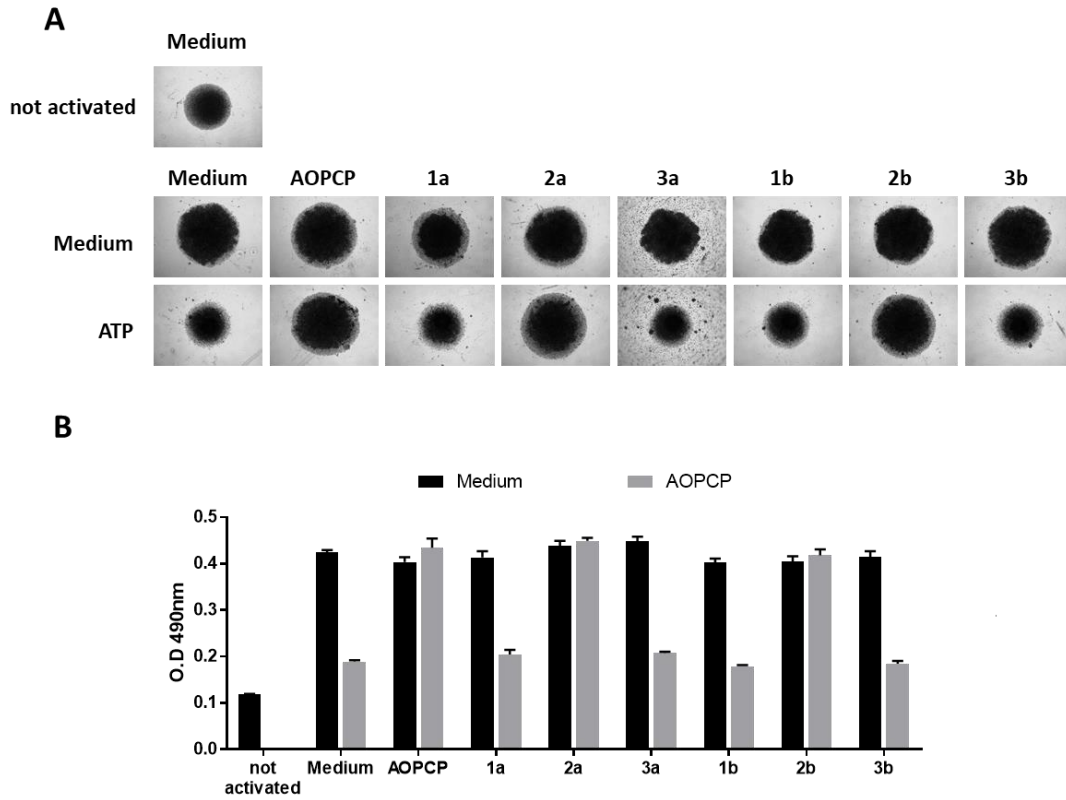
As indicated, adenosine generated from AMP dephosphorylation by CD73 presents suppressive effects on immune cells including T cells through binding to the adenosine receptor A2AR. Indeed, we recently demonstrated that AMP (*via* its dephosphorylation into adenosine by CD73) and adenosine block *in vitro* TCR-induced proliferation of CD4 and CD8 T cells [35, 36]. To investigate the capacity of the compounds containing either bis(trifluoromethylphenyl) (compounds **1a**, **2a** and **3a**) or naphthyl (compounds **1b**, **2b** and **3b**) substituents in each series, in comparison with AOPCP, to antagonize this immunosuppressive effect mediated by CD73 enzymatic activity, we developed an *in vitro* immunoassay (Fig. 5). Briefly, human B and T cells were cultured in the presence of TCR signal (anti-CD3/anti-CD28 beads) to favor T cell proliferation and we added ATP that, through the combined action of CD39 and CD73 enzymes expressed by B and T cells (Fig. 5A), is degraded into AMP and then adenosine. The latter blocks T cell proliferation and this change may be visualized either by pellet size at the end of the culture (Fig. 5B) and by MTS assay (Fig. 5C).



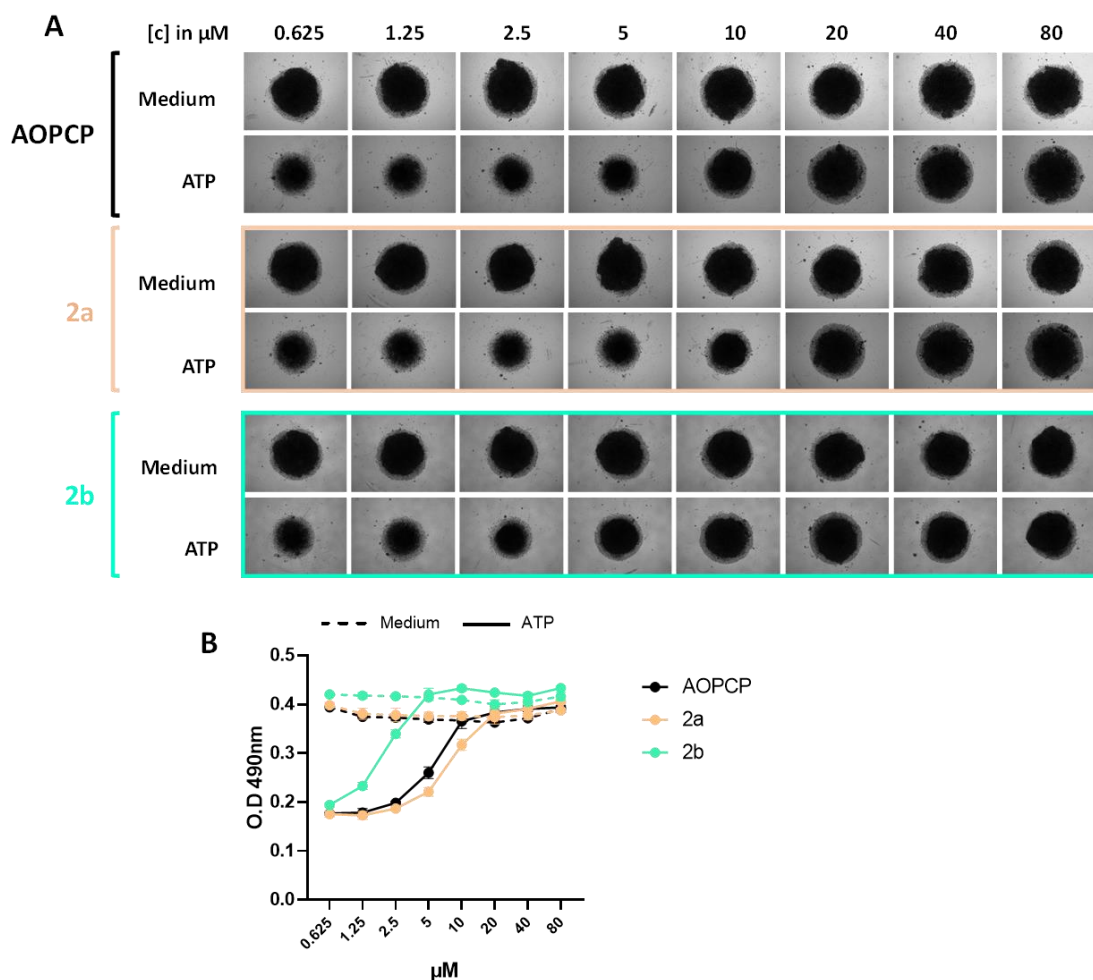
**Fig. 5.** Principle of the *in vitro* immuno-assay. B cells (CD19<sup>+</sup>), T cells (CD3<sup>+</sup>), and their subsets (CD4 and CD8) from human blood express CD39 and/or CD73 (A). Addition of exogenous ATP, through the combined action of CD39 and CD73 enzymes, that is successively degraded into AMP and adenosine, which strongly inhibits TCR-induced T cell proliferation evaluated at day 3 either by microscopic evaluation of pellet size (B) or MTS assay (C). In (C), data are represented as mean values of optimal density at 490 nm (O.D) + standard deviation (SD) from technical triplicates.

Tested at 100 μM, AOPCP completely reversed the inhibitory impact of exogenous ATP on T cell proliferation (Fig. 6A and 6B). Among the different compounds from the three series tested, only **2a** and **2b** were able to antagonize completely (**2b**) or partly (**2a**) the inhibition of T cell proliferation (Fig. 6A and 6B). Compounds of series 2, but not those from other series, present an efficiency demonstrating their ability to interfere with the production of extracellular adenosine.

To go further, we demonstrated in a dose-response study (0.625-80 μM) that **2b** completely restores T cell proliferation at 5 μM, whereas a loss of activity of **2a** was noticed below 15 μM (Fig. 7A and 7B). It is also important to note that AOPCP efficacy was lost below 10 μM. These results strongly suggest that these two compounds are as efficient as AOPCP, and more potent for compound **2b**, in the blockage of the hydrolysis of extracellular ATP into adenosine through the inhibition of the CD73 enzymatic activity.



**Fig. 6.** Evaluation of the efficacy of selected CD73 inhibitors in a functional assay on immune cells. The capacity of selected CD73 inhibitors to antagonize the blockade of T cells proliferation after TCR triggering induced by CD73 activity was assessed after 3 days by microscopy (A) and MTS assay (B). In (B), data are represented as mean values of optimal density at 490 nm (O.D) + standard deviation (SD) from technical triplicates.



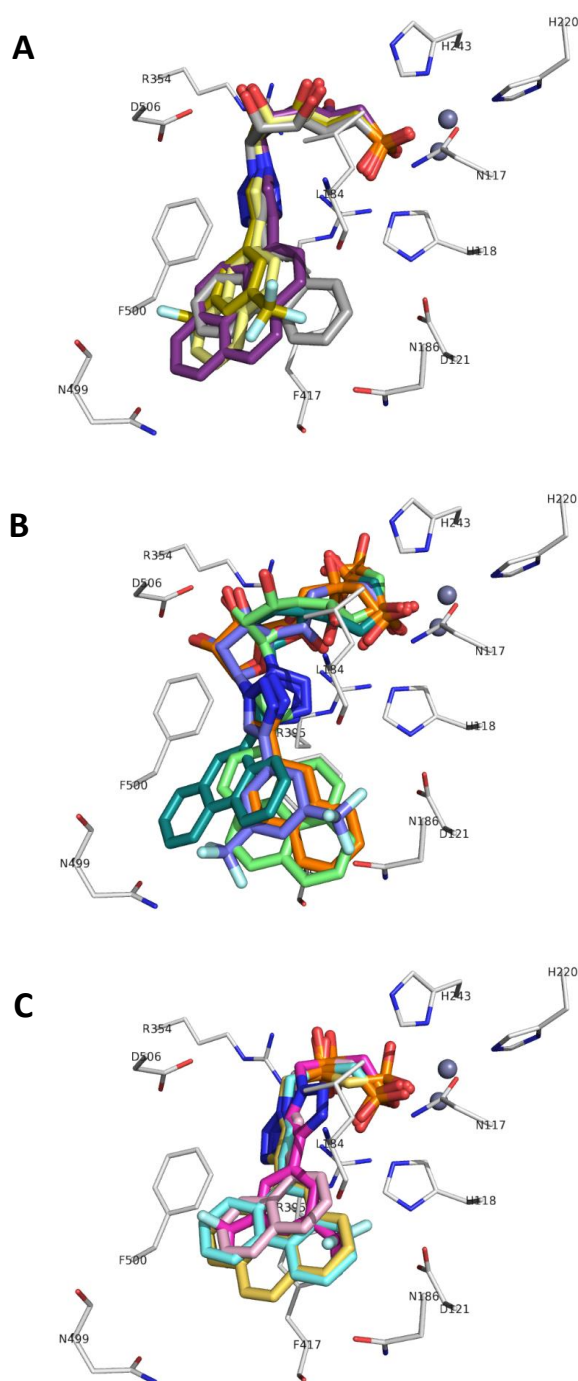
**Fig. 7.** Dose response studies comparing the efficacy of **2a** and **2b**, as CD73 chemical inhibitors, with AOPCP to restore T cell proliferation. Concentration range (0.625-80  $\mu\text{M}$ ) of chemical inhibitors were used for evaluating their capacity to antagonize the blockade of T cells proliferation after TCR triggering induced by CD73, assessed after 3 days by microscopy (A) or MTS assay (B). In (B), data are represented as mean values of optimal density at 490 nm (O.D) + standard deviation (SD) from technical triplicates.

### 2.5. Molecular modeling and Docking Studies

In order to better understand the factors governing the efficacy of CD73 inhibitors, the overall set of compounds was docked on CD73 active site (corresponding to the substrate binding when CD73 is in closed conformation). We first compared binding modes within the same series of compounds (Fig. 8) to appreciate the alignment or the misalignment of chemical structures belonging to the same family. A perfect overlay is observed for triazole, ribose moiety and  $\beta$ -hydroxyphosphonate chain with the first series (Fig. 8A), while the binding was more dispersed for series 2 with changes in the triazole /

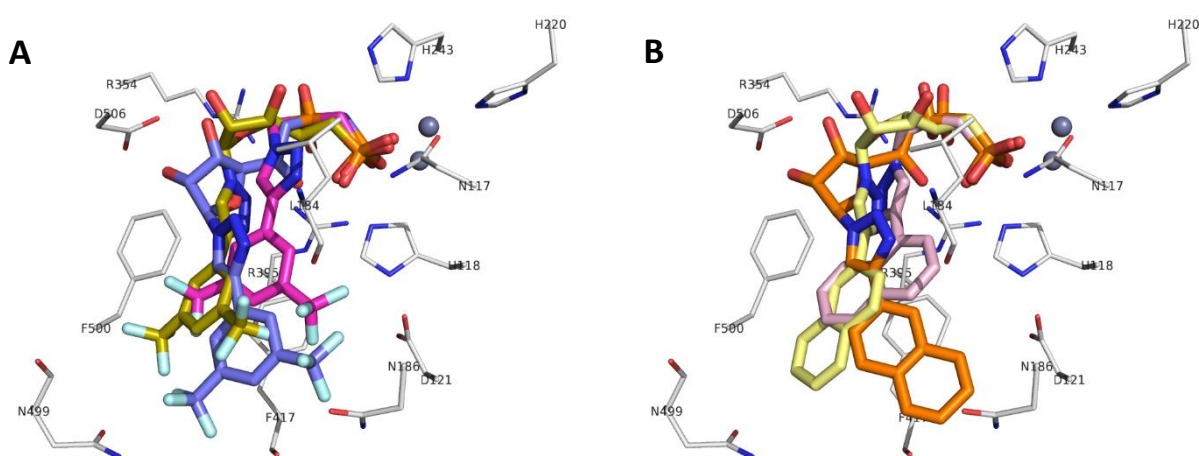


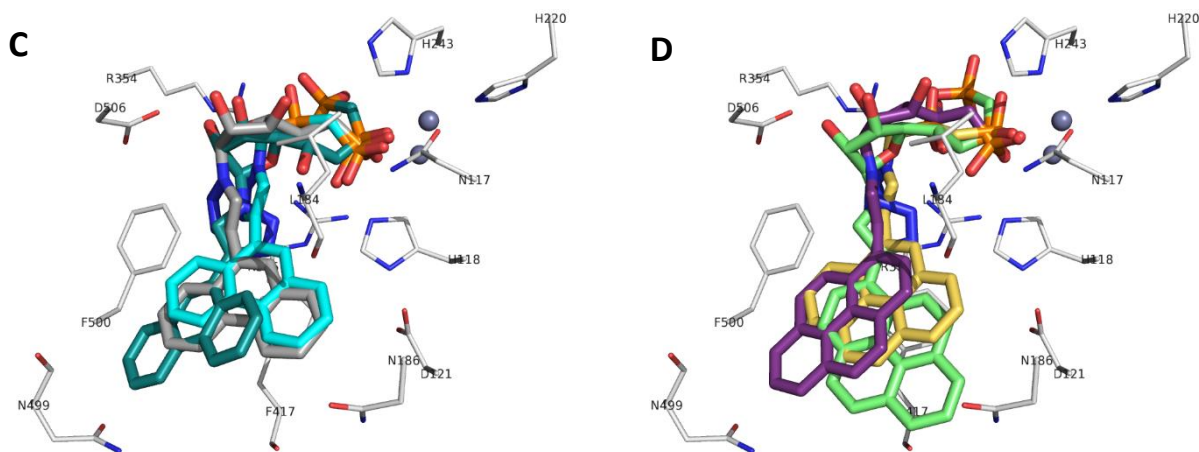
bis-phosphonate chain orientation (Fig. 8B). Compounds belonging to series 3 bound very similarly (except **3a** for which triazole group is shifted) as shown in Fig. 8C.



**Fig. 8.** Docking poses obtained for compounds (A) from series 1, (B) from series 2 and (C) for series 3. Compounds are depicted in thick sticks and color code is as follow: **1a** (gold), **1b** (pale yellow), **1c** (grey), **1d** (purple), **2a** (blue), **2b** (orange), **2c** (dark cyan), **2d** (green), **3a** (pink), **3b** (pale rose), **3c** (light blue), and **3d** (dark yellow). Amino acids from the CD73 binding site are depicted in thin grey sticks and zinc ions as grey sphere.

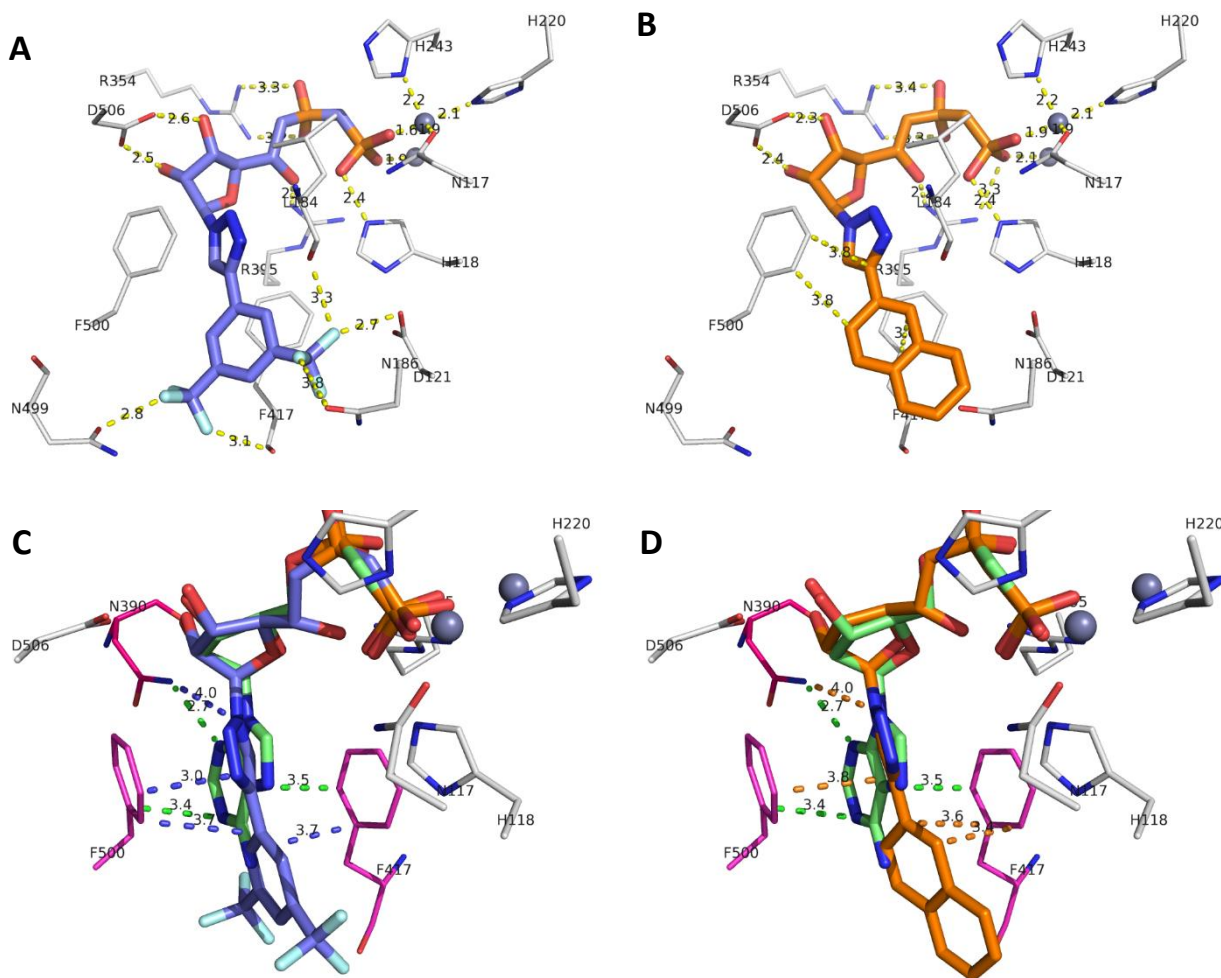
Then, we compared binding prediction of derivatives not within the same series but between series and for compounds wearing the same substituent, as for instance, CF<sub>3</sub> group (**1a**, **2a** and **3a**) and a displacement of the bis(trifluoromethyl)phenyl moiety is observed as shown in Fig. 9A. Therefore, the binding to CD73 seems first governed by the electrostatic interactions between negatively charged oxygen atoms of the phosphonate chain as main driving interactions. The same behavior is observed for compounds **1b**, **2b** and **3b**, as shown in Fig 9B. The length of the phosphonate / phosphate chain dictates the positioning of the ribose when present allowing the formation of hydrogen bonds between hydroxyl group and D506, which are only possible with compounds from series 2. The modification of the natural nucleobase by another aromatic substituent is well accommodated by the enzyme when the previous prerequisites are fulfilled (distance long enough between aromatic group and phosphate oxygen atoms). An increase in the aromaticity was a feature already described as an important contribution in the binding of nucleoside analogues like for instance the stacking between adenine of AOPCP (Fig. 2B) and the two Phe residues (F417 and F500) of CD73, and this contribution could be observed for most of the derivatives (Fig. 9). However, even with the correct orientation of this group, several derivatives (**1b-d**, **2c**, **3b-c**, and **3e-h**) did not promote a strong enzymatic inhibition at 10 μM. The main reason likely lies in the imperfect distance separating both ends of the molecule to form all interactions with protein residues (in addition to the absence of ribose moiety for series 3). This conclusion is also supported by the lack of inhibition observed with compounds **3f** and **3g** for which the linker between nucleobase and triazole has been adjusted to one or two carbons. Nevertheless, a stronger inhibition was detected for derivative **2b** (naphthalene) in comparison to derivatives **2c** (phenanthrene) or **2d** (pyrene) highlighting the importance of van der Waals contacts in this area but with moderation.





**Fig. 9.** Comparison of docking poses obtained between compounds wearing same substituent. (A) Binding orientations for compounds **1a** (dark yellow), **2a** (blue) and **3a** (pink sticks) from series 1. Amino acids from CD73 binding site are depicted in thin grey sticks and zinc ions as grey spheres. Binding predictions for compounds **1b**, **2b** and **3b** (B), for compounds **1c**, **2c** and **3c** (C) and for compounds **1d**, **2d** and **3d** (D). Color codes are identical to the ones used in previous figure.

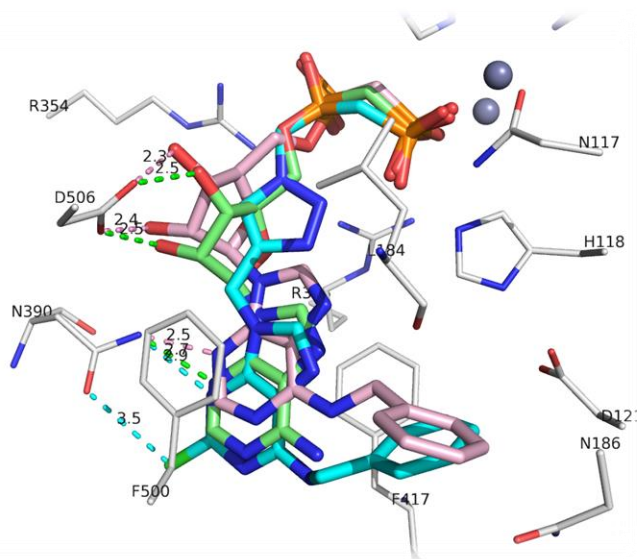
We then studied in details, the interaction of compounds **2a** and **2b** to decipher the molecular mechanism that would be responsible of such inhibitory activity (Fig. 10). For compound **2a**, the numerous halogen bonds observed between fluor atoms and protein residues (such as D121, L184, N186, F417 and N499) reinforce its binding affinity and may contribute to its biological activity in addition to the phosphonate / ribose connection with the active site residues. For compound **2b** and quite surprisingly, only few contacts were observed between the naphthalene group and aromatic residues of CD73 (Fig. 10B). However, the stacking of this group between the two phenylalanine residues was observed and the participation of triazole for **2b** may increase the stability of the compound in the active site. This feature may explain the difference observed in activity when comparing AOPCP with compounds **2a** (Fig. 10C) or **2b** (Fig. 10D). Indeed, the distances measured for van der Waals interactions between the two phenylalanine residues were greater for **2a** and **2b** than for AOPCP (Fig. 10C). Moreover, for **2b** the naphthyl group makes an additional interaction with F417 which may compensate the binding strength. Finally, a hydrogen bond formed between N390 and a nitrogen atom from either the nucleobase of AOPCP or the triazole moiety is changed with a larger distance for compounds **2a** and **2b** (2.7 versus 4.0 Å for AOPCP and **2a** or **2b**, respectively).



**Fig. 10.** Close-up views of main interactions between **2a** (blue sticks, panel A) or **2b** (orange sticks, panel B) with CD73 residues. Comparison between binding mode of AOPCP (green sticks) and **2a** (panel C) or **2b** (panel D). Amino acids from the CD73 binding site are depicted in thin grey / pink sticks, zinc ions as grey spheres and interactions are coded by the same color as those of compounds.

Comparison of derivatives **3f-h** with AOPCP suggested that the ribose moiety is crucial for an optimal binding as shown in Fig. 11 in which structures of derivative **3h** was superimposed to AOPCP and its analogue wearing a *N*<sup>6</sup>-benzyl group. The important hydrogen bonds formed between hydroxyl groups and residue D506 were found to be optimal with this last derivative (shorter distances, 2.3/2.4 Å with *N*<sup>6</sup>-benzyl AOPCP as compared to 2.5/2.5 Å for AOPCP) with a slight change in the positioning of benzyl group that may increase hydrophobic interactions with N186. Hydrogen bonding between N390 and nucleobase (nitrogen atom) is detected with variations in distance, increased from 2.5, 2.7 and up to 2.9 Å for compounds, *N*<sup>6</sup>-benzyl-AOPCP, AOPCP and **3h**, respectively. Interestingly, the halogen bond formed between the nucleobase of compound **3h** and N390 was observed despite the imperfect

binding due to the absence of ribose moiety (Fig. 11). This halogen bond was previously described with other CD73 inhibitors such as PSB12489 [19].



**Fig. 11.** Comparison of binding mode of derivative **3h** (absence of ribose, cyan sticks) and AOPCP (green) or *N*<sup>6</sup>-benzyl-AOPCP (light rose).

### 3. Conclusion

Until very recently, the blockade of adenosine release through the inhibition of ecto-nucleotidase CD73 was essentially associated with the clinical development of mAbs, whereas small-molecule inhibitors did not reach the clinical phases. Thus, the discovery of AB680 opens new horizons for small molecules, especially for nucleotide-based analogues. Herein, we have designed and studied three series of AMP or ADP mimics including various modifications of the nucleobase (series 1, 2 and 3), the phosphonate (series 2 and 3) and /or the ribose (series 3) counterparts on the basis of a  $\beta$ -hydroxyphosphonate ribonucleoside, used as a starting point. For all derivatives, the 1,3-dipolar cycloaddition reaction was used to generate the triazolo linkage between the sugar residue or a surrogate. Several synthetic strategies were explored to obtain  $\beta$ -hydroxyphosphonylphosphonate derivatives and the successful one involves the ring opening of an epoxide intermediate. The overall set of compounds was evaluated towards the purified recombinant *h*CD73 (10-100  $\mu$ M) as well as cell-based assays (CD73 activity of MDA-MB-231 cancer cells at 100  $\mu$ M). Series 1 (4-substituted-1,2,3-triazolo- $\beta$ -hydroxyphosphonate ribonucleosides) failed to inhibit the CD73 activity in a significant manner except for compound **1d** (75% inhibition at 100  $\mu$ M), including a pyrenyl substituent. This same trend can be observed for series 3 that corresponds to the acyclic bisphosphonate analogues. Among these, compounds **3c** and **3d**, incorporating respectively a phenanthryl or a pyrenyl substituent,

appeared as modest CD73 inhibitors in both assays. Finally, series 2 brings the best results with all compounds (corresponding to  $\beta$ -hydroxyphosphonylphosphonate ribonucleoside analogues) showing inhibition of the CD73 activity in the 10 to 100  $\mu$ M concentration range. This result clearly highlights the crucial roles of the bisphosphonate and the ribose residues. On the basis of the CD73-inhibition assays and molecular modeling studies, derivatives belonging to the three series and bearing a bis(trifluoromethyl)phenyl or a naphthyl substituents (**1a**, **2a** and **3a** and **1b**, **2b** and **3b**) were selected for further biological evaluation on possible reversion of the inhibitory impact of exogenous ATP on T cell proliferation in comparison to AOPCP, used as a reference inhibitor of the CD73-mediated adenosine production.

In conclusion, we have identified new potent and metabolically stable nucleotide-like CD73 inhibitors (compounds **2a** and **2b**). Our lead compound (**2b**) is able to block the biological effect related to extracellular pro-tumoral adenosine production in a dose-dependent manner, being more potent than AOPCP, and thus to potentially restore immune cell functionality. Further studies are now required to investigate the ability of this lead compound to restore immune cell function in the tumor environment and characterize the therapeutic potential of this derivative that deserves our attention.

## 4. Experimental

### 4.1. Chemistry

$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and  $^{31}\text{P}$  spectra were recorded with proton decoupling at ambient temperature on the following spectrometers: Bruker Avance III (600, 500 or 400 MHz). Chemical shifts ( $\delta$ ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak:  $\text{CDCl}_3$  at 7.26 ppm and 77.16 ppm,  $\text{DMSO-}d_6$  at 2.50 ppm and 39.52 ppm,  $\text{CD}_3\text{OD}$  at 3.31 ppm and 49.00 ppm, and  $\text{D}_2\text{O}$  at 4.79 ppm relative to TMS. For  $^{31}\text{P}$  NMR spectra, chemical shifts are reported relative to external  $\text{H}_3\text{PO}_4$ . COSY experiments were performed in order to confirm proton assignments as well as 2D  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear COSY for the attribution of  $^{13}\text{C}$  signals. Coupling constants,  $J$ , are given in Hertz (Hz). Multiplicity are indicated by: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), br (broad), dd (doublet of doublet).

Thin layer chromatography was performed on pre-coated aluminum sheets of Silica Gel 60 F254 (Merck), and visualization of products was accomplished by UV absorbance followed by spraying with Hanes molybdate reagent. Purifications were carried out on Silica Gel 60 (Merck). Reverse Phase purifications were performed using an automated system Puriflash 430 from Interchim using a C-18 column (PF-C13AQ). Ion exchange resin Dowex 50WX2 ( $\text{Na}^+$  form) was freshly prepared prior to use. Mass spectra were recorded on a Micromass Q-TOF mass spectrometer using electrospray ionization. HRMS were obtained with a Waters Synapt G2S (Waters, SN: UEB205) spectrometer



equipped with positive electrospray source ionization (ESI). The capillary voltage was set to 2 kV and the sampling cone voltage was set to 30 V.

All moisture sensitive reactions were carried out in anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use. Solids were dried over P<sub>2</sub>O<sub>5</sub> under reduced pressure at room temperature.

#### 4.1.1. Method A. General procedure for the preparation of compounds **5b–5d** and **6a–6c**.

To a solution of the required azido sugar **4** or **13** (1 eq.) in dry THF (45 mL/mmol) were added the appropriate alkyne (5.4 eq.), diisopropylethylamine (1.9 eq.), CuI (0.57 eq.), and DMEDA (5.2 eq.). The resulting mixture was heated to reflux until TLC showed completion of the reaction. Volatiles were removed under reduced pressure and EtOAc was added. The organic layer was washed twice with water and once with an aqueous solution EDTA (1%, m/v). The organic layer was then dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification of the crude was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc) to give the desired compound.

##### 4.1.1.1. 3'-O-Benzoyl-6'-deoxy-2',5'-di-O-acetyl-6'-diethylphosphono-1-(4-(2 naphthalen)-1H-1,2,3-triazol-1-yl)-β-D-allofuranose (**5b**)

Compound **5b** (290 mg, 66%) was obtained as a white solid from compound **4** (338 mg, 0.66 mmol) using general procedure A. *R<sub>f</sub>* (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, 1/1) 0.26; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.37 (s, 1H, H<sub>triazole</sub>), 8.09 (dd, *J* = 7.7, 6.3 Hz, 3H, H-Ar), 7.95 – 7.79 (m, 4H, H-Ar), 7.68 – 7.56 (m, 1H, H-Ar), 7.54 – 7.41 (m, 4H, H-Ar), 6.30 (d, *J* = 4.8 Hz, 1H, H-1'), 6.09 (t, *J* = 5.1 Hz, 1H, H-2'), 6.06 – 5.98 (m, 1H, H-3'), 5.54 (dtd, *J* = 10.5, 6.7, 3.8 Hz, 1H, H-5'), 4.79 (t, *J* = 4.0 Hz, 1H, H-4'), 4.18 – 3.95 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.26 (dq, *J* = 19.2, 15.5, 6.7 Hz, 2H, H-6', H-6''), 2.07 (s, C(O)CH<sub>3</sub>), 2.06 (s, C(O)CH<sub>3</sub>), 1.28 (td, *J* = 7.1, 2.9 Hz, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 169.9, 169.4 (C(O)CH<sub>3</sub>), 165.1 (C(O)-Ar), 148.5 (C=CH<sub>triazole</sub>), 134.0, 133.6, 133.5, 130.0, 129.0, 128.8, 128.4, 127.9, 127.5, 126.7, 126.5, 124.9, 123.9 (C-Ar), 119.6 (C=CH<sub>triazole</sub>), 89.9 (C-1'), 84.6 (d, *J* = 9.6 Hz, C-4'), 74.4 (C-2'), 70.8 (C-3'), 67.8 (C-5'), 62.3 (t, *J* = 6.9 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 27.5 (d, *J* = 141.6 Hz, C-6'), 21.1, 20.5 (C(O)CH<sub>3</sub>), 16.5 (d, *J* = 6.1 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ = 25.2; Q-TOF MS E<sup>+</sup>: *m/z* 666.22 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>33</sub>H<sub>37</sub>N<sub>3</sub>O<sub>10</sub>P: 666.2217 [M+H]<sup>+</sup>, found: 666.2211.

##### 4.1.1.2. 3'-O-Benzoyl-2',5'-di-O-acetyl-6'-diethylphosphono-1-(4-(phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)-β-D-allofuranose (**5c**)

Compound **5c** (386 mg, 72%) was obtained as a pale yellow solid from compound **4** (384 mg, 0.75 mmol) using general procedure A. *R<sub>f</sub>* (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, 1/1): 0.36; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.82 – 8.69 (m, 2H, H-Ar), 8.35 (dd, *J* = 8.1, 1.2 Hz, 1H, H-Ar), 8.16 – 8.08 (m, 2H, H-Ar), 8.03 (d, *J*

= 4.8 Hz, 2H, H-Ar, H<sub>triazole</sub>), 7.93 (dd,  $J = 7.8, 1.2$  Hz, 1H, H-Ar), 7.75 – 7.59 (m, 5H, H-Ar), 7.51 (t,  $J = 7.6$  Hz, 2H, H-Ar), 6.36 (d,  $J = 4.9$  Hz, 1H, H-1'), 6.17 (t,  $J = 5.2$  Hz, 1H, H-2'), 6.12 – 6.04 (m, 1H, H-3'), 5.57 (dtd,  $J = 10.4, 6.7, 3.6$  Hz, 1H, H-5'), 4.83 (t,  $J = 3.9$  Hz, 1H, H-4'), 4.30 – 3.91 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.51 – 2.12 (m, 2H, H-6', H-6''), 2.09 (d,  $J = 5.1$  Hz, 6H, 2 x C(O)CH<sub>3</sub>), 1.28 (td,  $J = 7.1, 2.7$  Hz, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta = 169.5, 169.0$  (C(O)CH<sub>3</sub>), 164.6 (C(O)-Ar), 146.9 (C=CH<sub>triazole</sub>), 133.5, 130.9, 130.4, 130.2, 129.7, 129.5, 128.7, 128.5, 128.4, 127.0, 126.7, 126.6, 126.5, 125.8, 125.7, 122.7 (C-Ar), 122.25 (C=CH<sub>triazole</sub>), 122.2 (C-Ar), 89.5 (C-1'), 84.2 (d,  $J = 9.5$  Hz, C-4'), 74.1 (C-2'), 70.4 (C-3'), 67.3 (C-5'), 61.8 (dd,  $J = 9.5, 6.5$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 27.1 (d,  $J = 141.6$  Hz, C-6'), 20.7, 20.08 C(O)CH<sub>3</sub>), 16.0 (d,  $J = 6.0$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta = 25.1$ ; Q-TOF MS E<sup>+</sup>:  $m/z$  716.24 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>37</sub>H<sub>39</sub>N<sub>3</sub>O<sub>10</sub>P: 716.2373 [M+H]<sup>+</sup>, found: 716.237.

4.1.1.3. *3'-O-Benzoyl-6'-deoxy-2',5'-di-O-acetyl-6'-diethylphosphono-1-(4-pyren-1H-1,2,3-triazol-1-yl)- $\beta$ -D-allofuranose (5d)*

Compound **5d** (220 mg, 62%) was obtained as a yellow solid from compound **4** (250 mg, 0.48 mmol) using general procedure A.  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, 1/1): 0.32; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta = 8.67$  (d,  $J = 9.3$  Hz, 1H, H-Ar), 8.24 (dt,  $J = 23.0, 9.0$  Hz, 5H, H-Ar), 8.16 – 8.09 (m, 5H, H<sub>triazole</sub>, H-Ar), 8.05 (dd,  $J = 13.8, 6.3$  Hz, 1H, H-Ar), 7.65 (dd,  $J = 10.5, 4.3$  Hz, 1H, H-Ar), 7.52 (t,  $J = 7.6$  Hz, 2H, H-Ar), 6.40 (d,  $J = 4.9$  Hz, 1H, H-1'), 6.21 (t,  $J = 5.1$  Hz, 1H, H-2'), 6.14 – 6.07 (m, 1H, H-3'), 5.67 – 5.51 (m, 1H, H-5'), 4.85 (t,  $J = 3.9$  Hz, 1H, H-4'), 4.22 – 4.00 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.31 (dq,  $J = 19.3, 15.5, 6.8$  Hz, 2H, H-6', H-6''), 2.10 (d,  $J = 2.8$  Hz, 6H, 2 x C(O)CH<sub>3</sub>), 1.29 (td,  $J = 7.1, 2.8$  Hz, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta = 170.4, 169.9$  (C(O)CH<sub>3</sub>), 165.5 (C(O)-Ar), 148.5 (C=CH<sub>triazole</sub>), 134.4, 132.1, 131.9, 131.5, 130.4, 129.4, 129.3, 129.0, 128.6, 127.91, 127.8, 126.7, 126.1, 125.8, 125.6, 125.5, 125.3, 125.1, 125.1 (C-Ar), 123.1 (C=CH<sub>triazole</sub>), 90.4 (C-1'), 85.1 (d,  $J = 9.3$  Hz, C-4'), 75.0 (C-2'), 71.3 (C-3'), 68.2 (C-5'), 62.7 (t,  $J = 6.9$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 28.0 (d,  $J = 141.9$  Hz, C-6'), 21.6, 21.01 C(O)CH<sub>3</sub>), 16.9 (d,  $J = 5.9$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta = 25.1$ ; Q-TOF MS E<sup>+</sup>:  $m/z$  740.24 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>39</sub>H<sub>39</sub>N<sub>3</sub>O<sub>10</sub>P: 740.2373 [M+H]<sup>+</sup>, found: 740.2374.

4.1.1.4. *3'-O-Benzoyl-6'-deoxy-2',5'-O-diacetyl-6'-(triethylphosphonomethylphosphonate)-1'-(4-(3,5-bis(trifluoro-methyl)phenyl)1H-1,2,3-triazol-1-yl)- $\beta$ -D-allofuranose (6a)*

Diastereoisomers **6a** (302.5 mg, 87%) were obtained as a brown solid in 2 h from compound **13** (251.2 mg, 0.4 mmol) using general procedure A.  $R_f$  (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.5; Analytical data for dial and dia2: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta = 8.74$  (s, 1H, H<sub>triazole</sub>, dial or dia2), 8.73 (s, 1H, H<sub>triazole</sub>, dial or dia2) 8.41 (d,  $J = 4.3$  Hz, 4H, H-Ar, dial + dia2), 8.09 – 8.07 (m, 4H, H-Ar, dial + dia2), 7.84 (s, 2H, H-Ar, dial + dia2), 7.66 – 7.63 (m, 2H, H-Ar, dial + dia2), 7.52 – 7.49 (m, 4H, H-Ar, dial +



dia2), 6.41 – 6.38 (m, 2H, H-1', dia1 + dia2), 5.87 – 5.84 (m, 4H, H2' and H3', dia1 + dia2), 5.73 – 5.66 (m, 2H, H-5', dia1 + dia2), 4.61 – 4.56 (m, 2H, H-4', dia1 + dia2), 4.20 – 4.07 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.80 – 2.62 (m, 4H CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 2.53 – 2.28 (m, 4H, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 2.12 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2), 2.11 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2) 2.03 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2), 2.02 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2), 1.35 – 1.25 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 170.6 (C(O)CH<sub>3</sub>, dia1 + dia2), 169.3 (C(O)CH<sub>3</sub>, dia1 + dia2), 165.0 (C(O)-Ar, dia1 + dia2), 146.0 (CH-Ar, dia1 + dia2), 134.0 (CH-Ar, dia1 + dia2), 132.8 (CH-Ar, dia1 + dia2), 132.8 – 132.2 (C-Ar, dia1 + dia2), 130.0 (CH-Ar, dia1 + dia2), 128.9 (CH-Ar, dia1 + dia2), 126.0 (CH-Ar, dia1 + dia2), 124.5 – 120.8 (m, CF<sub>3</sub>, dia1 + dia2), 121.9 (CH-Ar, dia1 + dia2), 120.7 (C=CH<sub>triazole</sub>, dia1 + dia2), 90.1 (C-1', dia1 + dia2), 85.0 (C-4', dia1 + dia2), 74.3 (C-2', dia1 + dia2), 71.2 (C-3', dia1 + dia2), 67.2 (C-5', dia1 + dia2), 62.7 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 31.8 – 26.5 (m, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 21.2 – 20.4 (m, C(O)CH<sub>3</sub>, dia1 + dia2), 16.3 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ = 43.2 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, dia1 or dia2), 42.4 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, dia1 or dia2), 19.0 (P(O)(OEt)<sub>2</sub>, dia1 or dia2), 18.9 (P(O)(OEt)<sub>2</sub>, dia1 or dia2); Q-TOF MS E<sup>+</sup>: m/z 858.20 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>34</sub>H<sub>40</sub>N<sub>3</sub>O<sub>12</sub>F<sub>6</sub>P<sub>2</sub>: 858.1991 [M+H]<sup>+</sup>, found: 858.2003.

4.1.1.5. *3'-O-Benzoyl-2',5'-di-O-acetyl-6'-(triethylphosphonomethylphosphonate)-1'-(4-(2-naphthalen)-1H-1,2,3-triazol-1-yl)-(α,β)-D-allofuranose (6b)*

Diastereoisomers **6b** (207.0 mg, 72%) were obtained as brown solid in 2 h from compound **13** (230.0 mg, 0.37 mmol) using general procedure A. The ratio of α/β anomers was determined as 30/70 based on <sup>1</sup>H NMR analysis. R<sub>f</sub> = (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.60; Analytical data for α-anomer (dia1 + dia2): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.34 (s, 2H, H-Ar, dia1 + dia2), 8.27 (2s, 2H, H<sub>triazole</sub>, dia1 + dia2), 8.10 – 8.07 (m, 2H, H-Ar, dia1 + dia2) 8.02 – 8.00 (m, 1H, H-Ar, dia1 or dia2), 7.98 – 7.94 (5H, H-Ar, dia1 + dia2), 7.93 – 7.81 (5H, H-Ar, dia1 + dia2), 7.51 – 7.48 (m, 5H, H-Ar, dia1 + dia2), 7.29 – 7.27 (m, 4H, H-Ar, dia1 + dia2), 6.77 (2d, J = 6.2 Hz, 2H, H-1', dia1 + dia2), 5.90 (dd, J = 6.0, 1.9 Hz, 2H, H-3', dia1 + dia2), 5.82 – 5.78 (m, 2H, H-2', dia1 + dia2), 5.75 – 5.56 (m, 2H, H5', dia1 + dia2), 5.09 – 5.07 (m, 1H, H-4', dia1 or dia2), 5.01 – 4.99 (m, 1H, H-4', dia1 or dia2), 4.23 – 4.07 (m, 12H, O-CH<sub>2</sub>-CH, dia1 + dia2<sub>3</sub>), 2.79 – 2.26 (m, 8H, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 2.21 – 2.20 (2s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.91 (s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.40 – 1.25 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 170.4 (C(O)CH<sub>3</sub>, dia1 or dia2), 169.4 (C(O)CH<sub>3</sub>, dia1 or dia2), 165.1 (C(O)-Ar, dia1 + dia2), 148.5 – 147.6 (C=CH<sub>triazole</sub>, dia1 + dia2), 134.0 (CH-Ar dia1 or dia2), 133.7 (C-Ar, dia1 or dia2), 133.4 (C-Ar, dia1 or dia2), 130.0 (CH-Ar dia1 or dia2), 129.8 (CH-Ar dia1 or dia2), 128.9 (CH-Ar dia1 or dia2), 128.8 (CH-Ar dia1 or dia2), 128.7 (CH-Ar dia1 or dia2), 128.4 (CH-Ar dia1 or dia2), 128.3 (CH-Ar dia1 or dia2), 127.9 (CH-Ar dia1 or dia2), 127.6 (CH-Ar dia1 or

dia2), 126.6 (CH-Ar dia1 or dia2), 126.4 (CH-Ar dia1 or dia2), 126.4 (CH-Ar dia1 or dia2) , 124.5 (CH-Ar dia1 + dia2), 124.0 (C-Ar dia 1 or dia2), 123.9 (C-Ar, dia1 or dia2), 119.1 (CH<sub>triazole</sub>, dia1 + dia2), 87.9 (C-1', dia1 + dia2), 86.6 (C-4', dia1 or dia2), 86.5 (C-4' dia1 or dia2), 71.3 – 71.1 (m, C-2', dia1 + dia2), 70.5 – 70.4 (m, C-3', dia1 + dia2), 68.0 (C-5', dia1 + dia2), 63.1 – 61.4 (m, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 31.9 – 26.7 (m, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 21.3 - 20.3 (m, C(O)CH<sub>3</sub>, dia1 + dia2), 16.6 – 16.4 (m, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2). Analytical data for β-anomer (dia1 + dia2): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 8.40 (s, 2H, H-Ar, dia1 + dia2), 8.34, (s, 1H, H<sub>triazole</sub> dia1 or dia2) 8.31 (1H, 1H, H<sub>triazole</sub> dia1 or dia2), 8.10 – 8.07 (m, 4H, H-Ar, dia1 + dia2), 7.93 – 7.81 (m, 8H, H-Ar, dia1 + dia2), 7.66 – 7.62 (m, 2H, H-Ar, dia1 + dia2), 7.51 – 7.48 (m, 8H, H-Ar, dia1 + dia2), 6.36 (d, *J* = 5.5 Hz, 1H, H-1', dia1 or dia2), 6.33 (d, *J* = 5.0 Hz, dia1 or dia2), 6.04 – 5.94 (m, 4H, H-2' and H-3', dia1 + dia2), 5.75 – 5.56 (m, 2H, H5', dia1 + dia2), 4.69 (dt, *J* = 13.5, 4.2 Hz, 2H, H-4', dia1 + dia2), 4.23 – 4.07 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.79 (s, 7H, dia1 + dia2), 2.79 – 2.26 (m, 8H, CH<sub>2</sub>PCH<sub>2</sub>P, dia1 + dia2), 2.11 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2), 2.07 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2 ), 2.05 – 2.04 (2s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.40 – 1.25 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 170.4 (C(O)CH<sub>3</sub>, dia1 + dia2), 169.4 (C(O)CH<sub>3</sub>, dia1 or dia2), 165.1 (C(O)-Ar, dia1 + dia2), 148.5 – 147.6 (C=CH<sub>triazole</sub>, dia1 + dia2), 134.0 (CH-Ar dia1 or dia2), 133.7 (C-Ar, dia1 or dia2), 133.4 (C-Ar, dia1 or dia2), 130.0 (CH-Ar dia1 or dia2), 129.8 (CH-Ar dia1 or dia2), 128.9 (CH-Ar dia1 or dia2), 128.8 (CH-Ar dia1 or dia2), 128.7(CH-Ar dia1 or dia2), 128.4 (CH-Ar dia1 or dia2) , 128.3 (CH-Ar dia1 or dia2), 127.9 (CH-Ar dia1 or dia2), 127.6 (CH-Ar dia1 or dia2), 126.6 (CH-Ar dia1 or dia2), 126.4 (CH-Ar dia1 or dia2), 126.4 (CH-Ar dia1 or dia2), 124.8 (CH-Ar, dia1 + dia2), 124.0 (C-Ar dia 1 or dia2), 123.9 (C-Ar, dia1 or dia2), 119.8 (CH<sub>triazole</sub>, dia1 or dia2) , 119.7 (CH<sub>triazole</sub>, dia1 or dia2), 89.9 (C-1', dia1 + dia2), 85.1 (C-4', dia1 or dia2), 84.8 (C-4', dia1 or dia2), 74.3 (C-2', dia1 + dia2), 70.8 (C-3', dia1 + dia2), 67.3 (C-5', dia1 + dia2), 63.1 – 61.4 (m, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2) , 31.9 – 26.7 (m, CH<sub>2</sub>PCH<sub>2</sub>P, dia1 + dia2), 21.3 - 20.3 (m, C(O)CH<sub>3</sub>, dia1 + dia2), 16.6 – 16.4 (m, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>): δ = 42.8 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 42.3 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 42.0 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 41.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 19.4 (P(O)(OEt)<sub>2</sub>, α or β-anomer); Q-TOF MS E<sup>+</sup>: m/z 772.24 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>36</sub>H<sub>44</sub>N<sub>3</sub>O<sub>12</sub>P<sub>2</sub>: 772.2400 [M+H]<sup>+</sup>, found: 772.2399.

4.1.1.6. *3'-O-Benzoyl-2',5'-di-O-acetyl-6'-(triethylphosphonomethylphosphonate)-1'-(4-phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)-(α,β)-D-allofuranose (6c)*

Diastereoisomers **6c** (323.6 mg, 86%) were obtained as an orange solid in 2 h from compound **13** (284.5 mg, 0.46 mmol) using general procedure **A**. The ratio of α/β anomers was determined as 1/4 based on <sup>1</sup>H NMR analysis. *R<sub>f</sub>* = (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.62; Analytical data for α-anomer (dia1 + dia2): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 8.78 (d, *J* = 8.2 Hz, 2H, H-Ar, dia1 + dia2), 8.72 (d, *J* = 8.3

Hz, 2H, H-Ar, dia1 + dia2), 8.39 – 8.37 (m, 2H, H-Ar, dia1 + dia2), 8.24 (s, 1H, H<sub>triazole</sub>, dia1 or dia2), 8.23 (s, 1H, H<sub>triazole</sub>, dia1 or dia2), 7.94 – 7.91 (m, 2H, H-Ar, dia1 + dia2), 7.84 – 7.81 (m, 6H, H-Ar, dia1 + dia2), 7.72 – 7.61 (m, 4H, H-Ar, dia1 + dia2), 7.55 – 7.45 (m, 4H, H-Ar, dia1 + dia2), 7.42 – 7.37 (m, 2H, H-Ar, dia1 + dia2), 7.14 – 7.10 (m, 4H, H-Ar, dia1 + dia2), 6.85 – 6.83 (m, 2H, H-1', dia1 + dia2), 5.92 – 5.90 (m, 2H, H-3', dia1 + dia2), 5.86 – 5.83 (m, 2H, H-2', dia1 + dia2), 5.68 – 5.60 (m, 2H, H-5', dia1 + dia2), 5.06 – 5.05 (m, 1H, H4' dia1 or dia2) 4.99 – 4.98 (m, 1H, H-4' dia1 or dia2), 4.22 – 3.97 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.73 – 2.34 (m, 8H, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 2.21 (s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.97 (s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.38 – 1.17 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 170.4 (C(O)CH<sub>3</sub>, dia1 + dia2), 169.4 (C(O)CH<sub>3</sub>, dia1 + dia2), 165.1 (C(O)-Ar, dia1 + dia2), 134.0 (CH-Ar dia1 or dia2), 131.4 (C-Ar dia1 or dia2), 130.8 (C-Ar dia1 or dia2), 130.6 (C-Ar, dia1 or dia2), 130.1 (C-Ar dia1 or dia2), 130.0 (CH-Ar, dia1 or dia2), 129.7 (C-Ar, dia1 or dia2), 129.1 (CH-Ar, dia1 or dia2), 128.8 (CH-Ar, dia1 or dia2), 128.5 (CH-Ar, dia1 or dia2), 127.4 (CH-Ar, dia1 or dia2), 127.2 (CH-Ar, dia1 or dia2), 127.1 (CH-Ar, dia1 or dia2), 126.9 (CH-Ar dia1 or dia2), 126.3 (CH-Ar, dia1 or dia2), 123.1 – 122.8 (CH-Ar, dia1 or dia2), 122.7 (CH<sub>triazole</sub>, dia1 + dia2), 87.90 (C-1', dia1 + dia2), 86.3 (C-4', dia1 + dia2), 71.3 (C-2', dia1 + dia2), 70.3 (C-3', dia1 + dia2), 67.9 (C-5', dia1 + dia2), 62.8 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 62.6 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 61.5 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 31.7 – 26.9 (m, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 21.2 (C(O)CH<sub>3</sub>, dia1 + dia2), 20.6 (C(O)CH<sub>3</sub>, dia1 + dia2), 20.4 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 16.4 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2); Analytical data for β-anomer (dia1 + dia2): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 8.78 (d, *J* = 8.2 Hz, 2H, H-Ar, dia1 + dia2), 8.72 (d, *J* = 8.3 Hz, 2H, H-Ar, dia1 + dia2), 8.39 – 8.37 (m, 2H, H-Ar, dia1 + dia2), 8.30 (s, 1H, H<sub>triazole</sub>, dia1 or dia2), 8.25 (s, 1H, H<sub>triazole</sub>, dia1 or dia2), 8.11 – 8.09 (m, 4H, H-Ar, dia1 + dia2), 8.04 (2s, 2H, H-Ar, dia1 + dia2), 7.94 – 7.91 (m, 2H, H-Ar, dia1 + dia2), 7.72 – 7.61 (m, 10H, H-Ar, dia1 + dia2), 7.52 – 7.49 (m, 4H, H-Ar, dia1 + dia2), 6.40 (dd, *J* = 17.6, 5.3 Hz, 2H, H-1', dia1 + dia2), 6.15 – 6.11 (m, 2H, H-2', dia1 + dia2), 6.06 – 6.01 (m, 2H, H-3', dia1 + dia2), 5.77 – 5.69 (m, 2H, H5', dia1 + dia2), 4.76 – 4.74 (m, 1H, H-4', dia1 or dia2), 4.73 – 4.71 (m, 1H, H-4', dia1 or dia2), 4.22 – 3.97 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.72 – 2.34 (m, 8H, CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 2.13 (s, 3H, C(O)CH<sub>3</sub>, dia1 + dia2), 2.10 (s, 3H, C(O)CH<sub>3</sub>, dia1 or dia2), 2.08 (s, 6H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.38 – 1.17 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 170.4 (C(O)CH<sub>3</sub>, dia1 + dia2), 169.4 (C(O)CH<sub>3</sub>, dia1 + dia2), 165.1 (C(O)-Ar, dia1 + dia2), 147.4, 134.0 (CH-Ar, dia1 + dia2), (C-Ar dia1 or dia2), 130.8 (C-Ar dia1 or dia2), 130.6 (C-Ar dia1 or dia2), 130.1 (C-Ar dia1 or dia2), 129.9 (CH-Ar, dia1 or dia2), 129.7 (C-Ar, dia1 or dia2), 129.1 (C-Ar, dia1 or dia2), 128.8 (CH-Ar, dia1 + dia2), 128.5 (CH-Ar, dia1 + dia2), 127.4 (CH-Ar, dia1 + dia2), 127.2 (CH-Ar, dia1 + dia2), 127.0 (CH-Ar, dia1 + dia2), 126.9 (CH-Ar, dia1 + dia2), 126.3 (CH-Ar, dia1 + dia2), 123.1 – 122.7 (CH-Ar, dia1 + dia2), 122.7 (CH<sub>triazole</sub>, dia1 +

dia2), 89.90 (C-1', dia1 + dia2), 85.2 – 85.0 (C4', dia1 + dia2), 74.4 (C-2', dia1 + dia2), 71.1 (C-3', dia1 or dia2), 70.8 (C-3', dia1 or dia2); 67.3 (C-5', dia1 + dia2), 62.8 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 62.6 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 61.5 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 31.7 – 26.9 (CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 21.2 (C(O)CH<sub>3</sub>, dia1 + dia2), 20.6 (C(O)CH<sub>3</sub>, dia1 + dia2), 16.4 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ = 42.8 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 42.3 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 42.0 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 41.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, α or β-anomer), 19.4 (19.0 (P(O)(OEt)<sub>2</sub>, α and β-anomer); Q-TOF MS E<sup>+</sup>: m/z 822.26 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>40</sub>H<sub>36</sub>N<sub>3</sub>O<sub>12</sub>P<sub>2</sub>: 822.2557 [M+H]<sup>+</sup>, found: 822.2559.

#### 4.1.2. Preparation of Bisseret reagent: diethyl 1-(ethoxyphosphinyl)methylphosphonate [32]

To a solution of chlorodiethylphosphite (796 μL, 5.5 mmol) in freshly distilled THF (30 mL), at room temperature under an argon atmosphere, was added dropwise a borane tetrahydrofuran complex solution (5.5 mL, 1 eq.) and the resulting mixture was stirred for 2 h.

To a solution of diethyl methylphosphonate (2 mL, 2.5 eq.) in freshly distilled THF was added dropwise at -78 °C a solution of *n*-BuLi in hexane (6 mL, 2.7 eq., 2.5 M). The resulting mixture was stirred for 1 h at -78 °C before addition of previously prepared borane tetrahydrofuran complex by cannula. Then, the reaction mixture was allowed to warm to room temperature in 1.5 h and stirred for an additional 30 min. Saturated solution of NH<sub>4</sub>Cl was added dropwise at -20 °C and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/Acetone: 9/1) to provide the desired intermediate. This later (1.126 g, 3.9 mmol) was introduced in a round bottomed flask equipped with a condenser and dissolved with 40 mL of absolute ethanol (0.1 M, final concentration). The mixture was freeze using liq. nitrogen and degassed under high vacuum for 5 min. The flask was backfilled with argon and the mixture allowed warming to room temperature for 10 min. This degas / thaw procedure was repeated twice and the solution heated in refluxing Ethanol under argon for 4 days, TLC showing completion of the reaction (EtOAc/Ethanol, 9/1, v/v). Volatiles were removed under high vacuum to provide the desired compound (941 mg, 70% overall yield). The crude was directly used for the next step without purification. Characterizations (<sup>1</sup>H, <sup>31</sup>P NMR) were in agreement with the literature [31]. R<sub>f</sub> = (EtOAc/EtOH: 9/1) 0.3; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.3 (dt, *J* = 580.2 Hz, 1H, H-P) 4.25 – 4.12 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.51 – 2.40 (m, 2H, P-CH<sub>2</sub>-P), 1.41 – 1.34 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>): δ = 26.0 (d, *J* = 5.8 Hz, P(O)H), 18.5 (d, *J* = 5.8 Hz, P(O)(OEt)<sub>2</sub>).

#### 4.1.3. 3-O-Benzoyl-6-deoxy-6-(tetraethylphosphonomethylphosphonate)-1,2-O-isopropylidene-D allofuranose (**11**)

A solution of the Bissereet reagent (5.5 g, 6 eq.) in freshly distilled THF (11 mL) was freeze-dried using liquid nitrogen and degassed under high vacuum for 10 min. The flask was backfilled with argon and the mixture allowed to warm to room temperature. The mixture was cooled to -78 °C and a solution of LiHMDS in THF (22.5 mL, 6 eq., 1 M) was added dropwise. After 20 min, a solution of the epoxide **10** (1.1 g, 3.7 mmol) in freshly distilled THF (11 mL) was added, followed by the addition of BF<sub>3</sub>·Et<sub>2</sub>O (2.8 mL, 6 eq.). The mixture was stirred at -78 °C for 20 min then allowed to warm to room temperature overnight. The mixture was cooled to -20 °C and the reaction quenched by adding a saturated solution of NH<sub>4</sub>Cl. The aqueous layer was extracted with EtOAc (3 x 30 mL) then the combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification was first performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) to provide the desired compound **11** contaminated with the Bissereet reagent, this last was then purified once again on silica gel (EtOAc/EtOH, 9/1) to afford a mixture of diastereoisomers **11** as a colorless oil (1.8 g, 88%). *R<sub>f</sub>* = (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.4; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 8.07 – 8.05 (m, 4H, H-Ar, dia1 + dia2), 7.58 – 7.55 (m, 2H, H-Ar, dia1 + dia2), 7.45 – 7.42 (m, 4H, H-Ar, dia1 + dia2), 5.88 – 5.87 (m, 2H, H-1, dia1 + dia2), 5.11 – 5.05 (m, 2H, H-3, dia1 + dia2), 4.98 – 4.96 (m, 2H, H-2, dia1 + dia2), 4.28 – 4.06 (m, 16H, H-4, H-5 and O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.65 – 2.21 (m, 8H, CH<sub>2</sub>PCH<sub>2</sub>P, dia1 + dia2), 1.52 (2s, 6H, CH<sub>3</sub> isopropylidene, dia1 + dia2), 1.35 – 1.23 (m, 24H, CH<sub>3</sub> isopropylidene and O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 165.8 (CO, dia1 + dia2), 133.4 (CH-Ar, dia1 + dia2), 130.0 (CH-Ar, dia1 + dia2), 129.6 (C-Ar, dia1 + dia2), 128.6 (CH-Ar, dia1 + dia2), 113.3 (C(CH<sub>3</sub>)<sub>2</sub>, dia1 + dia2), 104.5 (C-1, dia1 + dia2), 81.3 – 81.1 (m, C-4, dia1 + dia2), 78.3 (C-2, dia1 + dia2), 73.5 (C-3, dia1 or dia2), 73.1 (C-3, dia1 or dia2), 66.8 (C-5, dia1 or dia2), 66.6 (C-5, dia1 or dia2), 63.1 – 62.7 (m, 3 x O-CH<sub>2</sub>-CH<sub>3</sub> dia1 or dia2), 61.4 – 61.3 (m, O-CH<sub>2</sub>-CH<sub>3</sub> dia1 or dia2), 33.5 (d, *J* = 11.1 Hz, P-CH<sub>2</sub>-P, dia1 + dia2), 32.7 (CH-CH<sub>2</sub>-P, dia1 + dia2), 27.0 (CH<sub>3</sub> isopropylidene, dia1 or dia2), 26.9 (CH<sub>3</sub> isopropylidene, dia1 or dia2), 16.6 – 16.4 (m, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>): δ = 45.8 (CH<sub>2</sub>-P(O)-CH<sub>2</sub> dia1 or dia2), 44.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>, dia1 or dia2), 20.5 (P(O)(OEt)<sub>2</sub>, dia1 or dia2), 16.4 (P(O)(OEt)<sub>2</sub> dia1 or dia2); Q-TOF MS E<sup>-</sup>: *m/z* 549.17 [M-H]<sup>-</sup>; HRMS Q-TOF MS E<sup>-</sup>: calculated for C<sub>23</sub>H<sub>35</sub>O<sub>11</sub>P<sub>2</sub>: 549.1655 [M-H]<sup>-</sup>, found: 549.1664.

#### 4.1.4. 1,2,5-tri-O-Acetyl-3-O-benzoyl-6-deoxy-6-(triethylphosphonomethyl-phosphonate)-(α,β)-ribo-(5S)-hexofuranose (**12**)

To a solution of compound **11** (1.8 g, 3.33 mmol) in AcOH (13 mL), were added Ac<sub>2</sub>O (4.7 mL) and sulfuric acid (470 μL). The mixture was stirred at 25 °C for 24 h and cooled at 0 °C before dropwise

addition of a saturated solution of NaHCO<sub>3</sub>. The aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) to provide a mixture of the 4 diastereoisomers of compound **12** (2.0 g, 94%). The ratio of  $\alpha/\beta$  anomers was determined as 30/70 based on <sup>1</sup>H NMR analysis. *R<sub>f</sub>* = (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.45; Analytical data for  $\alpha$ -anomer (dia1 + dia2): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.09 – 8.06 (m, 4H, H-Ar, dia1 or dia2), 7.62 – 7.59 (m, 2H, H-Ar, dia1 or dia2), 7.49 – 7.45 (m, 4H, H-Ar, dia1 or dia2), 6.48 (d, *J* = 4.5 Hz, 2H, H-1, dia1 + dia2), 5.73 – 5.68 (m, 2H, H-3), 5.57 – 5.47 (m, 2H, H-5, dia1 + dia2), 5.27 – 5.24 (m, 2H, H-2, dia1 + dia2), 4.68 – 4.67 (m, 1H, H-4, dia1 or dia2), 4.62 – 4.61 (m, 1H, H-4, dia1 or dia2), 4.21 – 4.06 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 2.71 – 2.23 (m, 8H, CH<sub>2</sub>-P-CH<sub>2</sub>P, dia1 + dia2), 2.15 – 1.97 (m, 18H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.36 – 1.28 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 169.9 (C(O)CH<sub>3</sub>, dia1 or dia2), 169.2 (C(O)CH<sub>3</sub>, dia1 or dia2), 165.2 (C(O)-Ar, dia1 + dia2), 133.8 (CH-Ar, dia1 + dia2), 129.9 (CH-Ar, dia1 + dia2), 128.7 (CH-Ar, dia1 + dia2), 94.0 (C-1, dia1 + dia2), 85.7 (C-4, dia1 + dia2), 70.5 (C-2, dia1 + dia2), 69.1 (C-3, dia1 + dia2), 68.0 (C-5, dia1 + dia2), 62.7 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 61.5 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 31.9 – 30.5 (CH<sub>2</sub>PCH<sub>2</sub>P, dia1 + dia2), 21.2 (C(O)CH<sub>3</sub>, dia1 or dia2), 20.6 (C(O)CH<sub>3</sub>, dia1 or dia2), 16.6 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 16.4 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2); Analytical data for  $\beta$ -anomer (dia1 + dia2): <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.03 – 8.01 (m, 4H, H-Ar, dia1 or dia2), 7.62 – 7.59 (m, 2H, H-Ar, dia1 or dia2), 7.49 – 7.45 (m, 4H, H-Ar, dia1 or dia2), 6.23 (dd, *J* = 7.1, 1.4 Hz, 2H, H-1, dia1 + dia2), 5.73 – 5.68 (m, 2H, H-3, dia1 + dia2), 5.57 – 5.47 (m, 4H, H-5 and H-2, dia1 + dia2), 4.58 – 4.53 (m, 2H, H-4, dia1 + dia2), 4.21 – 4.06 (m, 12H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2), 2.71 – 2.23 (m, 8H, CH<sub>2</sub>-P-CH<sub>2</sub>P, dia1 + dia2), 2.15 – 1.97 (m, 18H, C(O)CH<sub>3</sub>, dia1 + dia2), 1.36 – 1.28 (m, 18H, O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 + dia2); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 169.9 (C(O)CH<sub>3</sub>, dia1 or dia2), 169.2 (C(O)CH<sub>3</sub>, dia1 or dia2), 165.2 (C(O)-Ar, dia1 or dia2), 133.8 (CH-Ar, dia1 + dia2), 129.9 (CH-Ar, dia1 + dia2), 128.7 (CH-Ar, dia1 + dia2), 98.5 (C-1, dia1 + dia2), 83.6 (C-4, dia1 and dia2), 74.9 (C-2, dia1 + dia2), 71.5 (C-3, dia1 or dia2), 71.2 (C-3, dia1 or dia2), 68.0 (C-5, dia1 + dia2), 62.7 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 61.5 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 31.9 – 30.5 (CH<sub>2</sub>-P-CH<sub>2</sub>-P, dia1 + dia2), 21.2 (C(O)CH<sub>3</sub>, dia1 or dia2), 20.6 (C(O)CH<sub>3</sub>, dia1 or dia2), 16.6 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2), 16.4 (O-CH<sub>2</sub>-CH<sub>3</sub>, dia1 or dia2); Analytical data for  $\alpha$  and  $\beta$ -anomers: <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>):  $\delta$  = 42.7 – 41.9 (m CH<sub>2</sub>-P(O)-CH<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer), 19.6 – 19.5 (P(O)(OEt)<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer); Q-TOF MS E<sup>+</sup>: *m/z* 637.18 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>26</sub>H<sub>39</sub>O<sub>14</sub>P<sub>2</sub>: 637.1815 [M+H]<sup>+</sup>, found: 637.1823.

4.1.5. *1-Azido-2,5-di-O-acetyl-3-O-benzoyl-6-deoxy-6-(triethylphosphono-methyl-phosphonate)-( $\alpha,\beta$ )-ribo-(5S)-hexofuranose (13)*

To a solution of the compound **12** (1.2 g, 1.97 mmol) in dry dichloromethane (10 mL) was added sodium azide (281.7 mg, 2.2 eq.) and SnCl<sub>4</sub> (460  $\mu$ L, 4 eq.) under an argon atmosphere. After refluxing for 6 h, the reaction was quenched by adding a saturated solution of NaHCO<sub>3</sub> and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) to provide a mixture of the 4 diastereoisomers of compound **13** (889.1 mg, 73%). Analytical data for  $\alpha$  and  $\beta$ -anomers:  $R_f$  = (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5) 0.45; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 8.07 – 7.98 (m, 8H, H-Ar,  $\alpha$  and  $\beta$ -anomers), 7.59 – 7.56 (m, 4H, H-Ar,  $\alpha$  and  $\beta$ -anomers), 7.47 – 7.42 (m, 8H, H-Ar,  $\alpha$  and  $\beta$ -anomers), 5.68 – 5.57 (m, 8H, H-1, H-5,  $\alpha$  and  $\beta$ -anomers), 5.52 – 5.42 (m, 4H, H-3,  $\alpha$  and  $\beta$ -anomers), 5.22 – 5.12 (m, 4H, H-2,  $\alpha$  and  $\beta$ -anomers), 4.60 – 4.53 (m, 4H, H-4,  $\alpha$  and  $\beta$ -anomers), 4.16 – 4.07 (m, 24H, O-CH<sub>2</sub>-CH<sub>3</sub>,  $\alpha$  and  $\beta$ -anomers), 2.65 – 2.21 (m, 16H, CH<sub>2</sub>-P-CH<sub>2</sub>-P), 2.09 (s, 12H, C(O)CH<sub>3</sub>,  $\alpha$  and  $\beta$ -anomers), 2.01 (s, 12H, C(O)CH<sub>3</sub>,  $\alpha$  and  $\beta$ -anomers), 1.33 – 1.25 (m, 36H, O-CH<sub>2</sub>-CH<sub>3</sub>,  $\alpha$  and  $\beta$ -anomers); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  = 170.2 (C(O)CH<sub>3</sub>), 169.4 (C(O)CH<sub>3</sub>), 165.0 (C(O)-Ar), 133.8 (CH-Ar), 130.0 (CH-Ar), 129.8 (CH-Ar), 128.7 (CH-Ar), 93.2 (C-3), 89.7 (C-1), 83.3 (C-4), 74.9 (C-2), 71.4 (C-2), 70.9 (C-2), 70.6 (C-2), 67.4 (C-5), 67.3 (C-5), 62.6 (O-CH<sub>2</sub>-CH<sub>3</sub>), 61.4 (O-CH<sub>2</sub>-CH<sub>3</sub>), 31.1 – 26.9 (m, CH<sub>2</sub>PCH<sub>2</sub>P), 21.1 – 20.4 (m, C(O)CH<sub>3</sub>), 16.4 (O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>):  $\delta$  = 42.5 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer), 42.2 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer), 41.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer), 41.8 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>,  $\alpha$  or  $\beta$ -anomer), 19.5 – 19.4 (m, P(O)(OEt)<sub>2</sub>,  $\alpha$  and  $\beta$ -anomer); Q-TOF MS E<sup>+</sup>: m/z 620.18 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>24</sub>H<sub>36</sub>N<sub>3</sub>O<sub>12</sub>P<sub>2</sub>: 620.1774 [M+H]<sup>+</sup>, found: 620.1768.

4.1.6. *Method B. General procedure for the removal of sugar protecting groups.*

The protected compound was dissolved in a methanolic ammonia solution (2 M, 20 mL/mmol) and the resulting mixture stirred overnight at room temperature before concentration in vacuum. Purification of the crude was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) to give the desired compounds.

4.1.7. *Method C. General procedure for the removal of phosphonic acids protecting groups.*

To a solution of protected phosphonate (1 eq.) in DMF (20 mL/mmol) at 0 °C was added dropwise TMSBr (20 eq.). The reaction mixture was stirred at room temperature until TLC (*i*-PrOH/NH<sub>4</sub>OH/H<sub>2</sub>O, 7/1/2, v/v/v) showed completion of the reaction. The mixture was cooled to 0 °C then neutralized with an aqueous triethylammonium bicarbonate solution (TEAB, 1 M, pH 7) and concentrated to dryness under reduce pressure. Reverse-phase column chromatography of the residue

with H<sub>2</sub>O/MeOH (100/0 to 0/100) followed by ion exchange on Dowex 50WX2 (Na<sup>+</sup> form) and freeze-drying afforded the desired compound as sodium salt.

4.1.7.1. *6'-Deoxy-6'-phosphono-1-(4-naphthalen-1H-1,2,3-triazol-1-yl)-β-D-allofuranose (disodium salt) (1b)*

Compound **5b** (250 mg, 0.38 mmol) was first treated using general procedure **B** to give the intermediate (167 mg, 93%). Treatment of this last (127 mg, 0.27 mmol) following general procedure **C** gave rise to the desired compound **1b** (96 mg, 77%, white solid). *R<sub>f</sub>* (*i*-PrOH/NH<sub>4</sub>OH/ H<sub>2</sub>O, 7/2/1) 0.14; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 8.38 (s, 1H, H<sub>triazole</sub>), 7.98 (s, 1H, H-Ar), 7.82 (dd, *J* = 7.7, 4.1 Hz, 3H, H-Ar), 7.66 (dd, *J* = 8.6, 1.6 Hz, 1H, H-Ar), 7.58 – 7.42 (m, 2H, H-Ar), 6.08 (d, *J* = 4.9 Hz, 1H, H-1'), 4.69 (t, *J* = 5.1 Hz, 1H, H-2'), 4.57 (t, *J* = 4.9 Hz, 1H, H-3'), 4.34 – 4.28 (m, 1H, H-4'), 4.22 (dt, *J* = 12.8, 4.4 Hz, 1H, H-5'), 2.13 – 1.33 (m, 2H, H-6', H-6''); <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O): δ = 147.6 (C=CH<sub>triazole</sub>), 133.0, 132.9, 128.7, 128.2, 127.7, 126.8, 126.6, 126.5, 124.3, 123.5 (C-Ar), 120.7 (C=C<sub>Htriazole</sub>), 91.7 (C-1'), 88.3 (d, *J* = 13.4 Hz, C-4'), 75.2 (C-2'), 69.3 (C-3'), 67.6 (C-5'), 31.6 (d, *J* = 127.4 Hz, C-6'); <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O): δ = 18.9; Q-TOF MS E<sup>+</sup>: *m/z* 422.11 [M-2Na+3H]<sup>+</sup>, 444.09 [M-Na+2H]<sup>+</sup>, 843.22 [2M-4Na+5H]<sup>+</sup>; Q-TOF MS E<sup>-</sup>, *m/z* 420.08 [M-2Na+H]<sup>-</sup>, 841.18 [2M-4Na+3H]<sup>-</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>7</sub>Na<sub>2</sub>P: 466.0756 [M+H]<sup>+</sup>, found: 466.0759; UV (H<sub>2</sub>O) λ<sub>max</sub> = 243 nm (ε<sub>max</sub> = 59843).

4.1.7.2. *6'-Deoxy-6'-phosphono-1-(4-(phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)-β-D-allofuranose (disodium salts) (1c)*

Compound **5c** (303 mg, 0.42 mmol) was first treated using general procedure **B** to give the intermediate (237 mg, 97%). Treatment of this last (200 mg, 0.38 mmol) following general procedure **C** gave rise to the desired compound **1c** (153 mg, 78%, white solid). *R<sub>f</sub>* (*i*-PrOH/NH<sub>4</sub>OH/ H<sub>2</sub>O, 7/2/1) 0.17; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 7.71 (d, *J* = 8.2 Hz, 1H, H-Ar), 7.62 (d, *J* = 12.7 Hz, 2H, H-Ar, H<sub>triazole</sub>), 7.55 (d, *J* = 8.1 Hz, 1H, H-Ar), 7.21 – 7.05 (m, 3H, H-Ar), 6.97 (dd, *J* = 15.5, 7.8 Hz, 2H, H-Ar), 6.86 (t, *J* = 7.3 Hz, 1H, H-Ar), 5.89 (d, *J* = 4.0 Hz, 1H, H-1'), 4.61 – 4.51 (m, 2H, H-2', H-3'), 4.24 (d, *J* = 3.2 Hz, 2H, H-4', H-5'), 2.02 – 1.75 (m, 2H, H-6', H-6''); <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O): δ = 145.9 (C=CH<sub>triazole</sub>), 130.2, 129.6, 129.39, 128.7, 128.5, 128.0, 126.8, 126.7, 126.5, 126.4, 125.2, 124.7, 123.3 (C-Ar), 122.4 (C=C<sub>Htriazole</sub>), 121.8 (C-Ar), 91.44 (C-1'), 87.9 (d, *J* = 13.4 Hz, C-4'), 75.0 (C-2'), 69.1 (C-3'), 67.2 (d, *J* = 1.8 Hz, C-5'), 31.2 (d, *J* = 129.9 Hz, C-6'); <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O): δ = 20.2; Q-TOF MS E<sup>+</sup>: *m/z* 381.30 [M-6Na+5H]<sup>+</sup>, 415.21 [M-3Na-9H]<sup>+</sup>, 472.13 [M-2Na+3H]<sup>+</sup>, 473.13 [M-2Na+4H]<sup>+</sup>, 494.11 [M-Na+2H]<sup>+</sup>, 943.25 [2M-4Na+5H]<sup>+</sup>. Q-TOF MS E<sup>-</sup>: *m/z* 394.89 [M-5Na-6H]<sup>-</sup>, 398.89 [M-5Na-2H]<sup>-</sup>, 470.11 [M-2Na+H]<sup>-</sup>, 471.11 [M-2Na+2H]<sup>-</sup>, 538.10 [M+Na]<sup>-</sup>, 606.09 [M+4Na-  
H]<sup>-</sup>, 941.23 [2M-4Na+3H]<sup>-</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>7</sub>P: 472.1274 [M-



$2\text{Na}+3\text{H}]^+$ , found: 472.1276; UV ( $\text{H}_2\text{O}$ )  $\lambda_{1\text{max}} = 211 \text{ nm}$  ( $\epsilon_{1\text{max}} = 32193$ ),  $\lambda_{2\text{max}} = 254 \text{ nm}$  ( $\epsilon_{2\text{max}} = 54248$ ),  $\lambda_{3\text{max}} = 298 \text{ nm}$  ( $\epsilon_{3\text{max}} = 12356$ ).

4.1.7.3. *6'-Deoxy-6'-phosphono-1-(4-pyren-1H-1,2,3-triazol-1-yl)- $\beta$ -D-allofuranose (disodium salts) (1d)*

Compound **5d** (209 mg, 0.28 mmol) was first treated using general procedure **B** to give the intermediate (139 mg, 90%). Treatment of this last (100 mg, 0.38 mmol) following general procedure **C** gave rise to the desired compound **1d** (89 mg, 92%, white solid).  $R_f$  (iPrOH/ $\text{NH}_4\text{OH}$ / $\text{H}_2\text{O}$ : 7/2/1) 0.12;  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 7.58$  (s, 1H,  $\text{H}_{\text{triazole}}$ ), 7.43 (d,  $J = 9.0 \text{ Hz}$ , 1H, H-Ar), 7.24 – 6.82 (m, 6H, H-Ar), 6.65 (s, 2H, H-Ar), 5.92 (s, 1H, H-1'), 4.63 (s, 2H, H-2', H-3'), 4.30 (s, 2H, H-4', H-5'), 2.05 – 1.81 (m, 2H, H-6', H-6'');  $^{13}\text{C}$  NMR (101 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 146.3$  ( $\underline{\text{C}}=\text{CH}_{\text{triazole}}$ ), 130.1, 129.9, 129.5, 127.2, 126.7, 126.6, 126.0, 125.8, 125.1, 124.4, 124.3, 123.9, 123.2, 123.0, 122.9 (C-Ar), 122.4 ( $\text{C}=\underline{\text{C}}\text{H}_{\text{triazole}}$ ), 91.5 (C-1'), 87.8 (d,  $J = 13.5 \text{ Hz}$ , C-4'), 75.2 (C-2'), 69.1 (C-3'), 67.2 (C-5'), 31.5 (d,  $J = 130.3 \text{ Hz}$ , C-6');  $^{31}\text{P}$  NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 20.2$ ; Q-TOF MS  $\text{E}^+$ :  $m/z$  496.13 [ $\text{M}-2\text{Na}+3\text{H}]^+$ , 497.13 [ $\text{M}-2\text{Na}+4\text{H}]^+$ , 518.11 [ $\text{M}-\text{Na}+2\text{H}]^+$ , 719.35 [ $\text{M}+8\text{Na}-4\text{H}]^+$ ; Q-TOF MS  $\text{E}^-$ :  $m/z$  494.11 [ $\text{M}-2\text{Na}+\text{H}]^-$ , 495.11 [ $\text{M}-2\text{Na}+2\text{H}]^-$ ; HRMS Q-TOF MS  $\text{E}^+$ : calculated for  $\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_7\text{P}$ : 494.1117 [ $\text{M}-2\text{Na}+\text{H}]^+$ , found: 494.1117; UV ( $\text{H}_2\text{O}$ )  $\lambda_{1\text{max}} = 243 \text{ nm}$  ( $\epsilon_{1\text{max}} = 46608$ ),  $\lambda_{2\text{max}} = 267 \text{ nm}$  ( $\epsilon_{2\text{max}} = 21788$ ),  $\lambda_{3\text{max}} = 277 \text{ nm}$  ( $\epsilon_{3\text{max}} = 32682$ ),  $\lambda_{4\text{max}} = 346 \text{ nm}$  ( $\epsilon_{4\text{max}} = 26224$ ).

4.1.7.4. *6'-deoxy-6'-phosphonomethylphosphonate-1'-(4-(3,5-bis-trifluoromethyl phenyl)-1H-1,2,3-triazol-1-yl)- $\beta$ -D-allofuranose (trisodium salt) (2a)*

Compound **6a** (292.5 mg, 0.34 mmol) was first treated using general procedure **B** to give the intermediate. Treatment of this last following general procedure **C** in 48 h gave rise to the desired compound **2a** (72 mg, 34%, overall yield, white solid).  $R_f$  (i-PrOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$ , 7/2/1) 0.20;  $^1\text{H}$  NMR (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.75$  (s, 1H,  $\text{H}_{\text{triazole}}$ ), 8.38 (s, 2H, H-Ar), 8.07 (s, 1H, H-Ar), 6.19 (s, 1H, H-1'), 4.74 (s, 1H, H-2'), 4.58 (s, 1H, H-3'), 4.21 – 4.10 (m, 2H, H-4' and H-5'), 2.41 – 2.34 (m, 1H,  $\underline{\text{C}}\text{H}_2\text{-P}$ ), 2.22 – 1.91 (m, 2H,  $\underline{\text{C}}\text{H}_2\text{-P-CH}_2\text{-P}$ ), 1.87 – 1.75 (m, 1H  $\text{P-CH}_2\text{-P}$ );  $^{13}\text{C}$  NMR (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 145.7$  ( $\underline{\text{C}}=\text{CH}_{\text{triazole}}$ ), 131.6 (C-Ar), 131.4 (C-Ar), 131.4 (C-Ar), 126.1 (CH-Ar), 124.4 ( $\text{CF}_3$ ), 122.4 (CH-Ar), 121.3 ( $\text{C}=\underline{\text{C}}\text{H}_{\text{triazole}}$ ), 91.7 (C-1'), 90.2 (d,  $J = 15.2 \text{ Hz}$ , C-4'), 75.4 (C-2'), 69.4 (C-3'), 67.0 (C-5'), 33.9 – 32.7 (m,  $\underline{\text{C}}\text{H}_2\text{-P-CH}_2\text{-P}$ );  $^{31}\text{P}$  NMR (202 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 36.6$  ( $\text{CH}_2\text{-P(O)-CH}_2$ ), 12.7 ( $\text{P(O)(ONa)}_2$ ); Q-TOF MS  $\text{E}^+$ :  $m/z$  586.05 [ $\text{M}-3\text{Na}+4\text{H}]^+$ ; HRMS Q-TOF MS  $\text{E}^+$ : calculated for  $\text{C}_{17}\text{H}_{20}\text{F}_6\text{N}_3\text{O}_9\text{P}_2$ : 586.0573 [ $\text{M}-3\text{Na}+4\text{H}]^+$ , found: 586.0554.

4.1.7.5. *6'-Deoxy-6'-(triethylphosphonomethylphosphonate)-1'-(4-(2-naphthalen)-1H-1,2,3-triazol-1-yl)-( $\alpha,\beta$ )-D-allofuranose (trisodium salt) (2b)*

Compound **6b** (202 mg, 0.26 mmol) was first treated using general procedure **B** to give the intermediate. Treatment of this last following general procedure **C** in 72 h gave rise to the desired

compound **2b** (86.7 mg, 62%, overall yield, white solid) as a mixture of  $\alpha/\beta$  anomers (ratio: 30/70).  $R_f = (i\text{-PrOH}/\text{H}_2\text{O}/\text{NH}_4\text{OH}, 7/2/1) 0.22$ ; Analytical data for  $\alpha$ -anomer:  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.50$  (s, 1H,  $\text{H}_{\text{triazole}}$ ), 8.08 (s, 1H, H-Ar), 7.88 – 7.84 (m, 3H, H-Ar), 7.76 – 7.73 (m, 1H, H-Ar), 7.54 – 7.49 (m, 2H), 6.49 (d,  $J = 5.6$  Hz, 1H, H-1'), 4.75 – 4.70 (m, 1H, 1H, H-2'), 4.59 – 4.54 (m, 1H, H-3'), 4.46 – 4.45 (m, 1H, H-4'), 4.27 – 4.19 (m, 1H, H-5'), 2.29 – 1.91 (m, 4H,  $\text{CH}_2\text{-P-CH}_2\text{-P}$ );  $^{13}\text{C NMR}$  (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 147.0$  ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 132.9 (C-Ar), 132.8 (C-Ar) 128.6 (CH-Ar), 128.1 (CH-Ar) 127.6 (CH-Ar), 126.6 (CH-Ar), 126.5 (CH-Ar), 124.3 (CH-Ar), 124.2 (C-Ar), 123.4 (CH-Ar), 121.8 ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 89.9 (C-4'), 89.8 (C-1'), 71.6 (C-2'), 69.1 (C-3'), 67.0 (C-5'), 34.2 – 31.8 (m,  $\text{CH}_2\text{-P-CH}_2\text{-P}$ );  $^{31}\text{P NMR}$  (202 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 35.1$  ( $\text{CH}_2\text{-P(O)-CH}_2$ ), 14.6 ( $\text{P(O)(ONa)}_2$ ); Analytical data for  $\beta$ -anomer:  $^1\text{H NMR}$  (500 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.47$  (s, 1H,  $\text{H}_{\text{triazole}}$ ), 8.08 (s, 1H, H-Ar), 7.88 – 7.84 (m, 3H, H-Ar), 7.76 – 7.73 (m, 1H, H-Ar), 7.54 – 7.49 (m, 2H, H-Ar), 6.10 (d,  $J = 5.4$  Hz, 1H, H-1'), 4.75 – 4.70 (m, 1H, 1H, H-2'), 4.59 – 4.54 (m, 1H, H-3'), 4.27 – 4.19 (m, 2H, H-4' and H-5'), 2.29 – 1.91 (m, 4H,  $\text{CH}_2\text{PCH}_2\text{P}$ );  $^{13}\text{C NMR}$  (126 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 147.6$  ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 132.9 (C-Ar), 132.8 (C-Ar), 128.6 (CH-Ar), 128.1 (CH-Ar), 127.6 (CH-Ar), 126.7 (CH-Ar), 126.6 (CH-Ar), 126.5 (CH-Ar), 124.3 (CH-Ar), 124.2 (C-Ar), 123.4 (CH-Ar), 120.5 ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 91.6 (C-1'), 89.1 (d,  $J = 14.7$  Hz, C-4'), 75.1 (C-2'), 69.3 (C-3'), 66.8 (C-5'), 34.2 – 31.8 (m,  $\text{CH}_2\text{-P-CH}_2\text{-P}$ );  $^{31}\text{P NMR}$  (202 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 34.9$  ( $\text{CH}_2\text{-P(O)-CH}_2$ ), 14.6 ( $\text{P(O)(ONa)}_2$ ); Q-TOF MS  $\text{E}^+$ :  $m/z$  566.04 [ $\text{M-3Na+4H}$ ] $^+$ ; HRMS Q-TOF MS  $\text{E}^+$ : calculated for  $\text{C}_{19}\text{H}_{21}\text{N}_3\text{O}_9\text{Na}_3\text{P}_2$ : 566.0446 [ $\text{M-3Na+H}$ ] $^+$ , found: 566.0443.

4.1.7.6. *6'-Deoxy-6'-(phosphonomethylphosphonate)-1'-(4-(phenanthren-9-yl)-1H-1,2,3-triazol-1-yl)- $\beta$ -D-allofuranose (trisodium salt) (2c)*

Compound **6c** (202 mg, 0.26 mmol) was first treated using general procedure **B** to give the intermediate. Treatment of this last following general procedure **C** in 72 h gave rise to the desired compound **2c** (34.2 mg, 16%, overall yield, white solid) contaminated with less than 10% of  $\alpha$  anomers.  $R_f = (i\text{-PrOH}/\text{H}_2\text{O}/\text{NH}_4\text{OH}, 7/2/1) 0.23$ ;  $^1\text{H NMR}$  (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 8.57$  (d,  $J = 8.3$  Hz, 1H, H-Ar), 8.51 (d,  $J = 8.3$  Hz, 1H, H-Ar), 8.30 (s, 1H,  $\text{H}_{\text{triazole}}$ ), 7.95 (d,  $J = 8.1$  Hz, 1H, H-Ar), 7.74 (d,  $J = 7.7$  Hz, 1H, H-Ar), 7.63 – 7.60 (m, 3H, H-Ar), 7.56 – 7.53 (m, 2H, H-Ar), 6.19 (d,  $J = 5.4$  Hz, 1H, H-1'), H-2' signal in  $\text{D}_2\text{O}$  peak, 4.66 – 4.62 (m, 1H, H-3'), 4.31 – 4.25 (m, 2H, H4' and H5'), 2.26 – 1.97 (m, 4H,  $\text{CH}_2\text{-P-CH}_2\text{-P}$ );  $^{13}\text{C NMR}$  (151 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 146.3$  ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 130.5 (C-Ar), 129.9 (C-Ar), 129.8 (C-Ar), 129.0 (C-Ar), 128.8 (CH-Ar), 128.5 (CH-Ar), 127.5 (CH-Ar), 127.1 (2 x CH-Ar), 127.0 (CH-Ar), 125.4 (CH-Ar), 125.0 (C-Ar), 123.4 ( $\text{C}=\text{CH}_{\text{triazole}}$ ), 122.9 (CH-Ar), 122.4 (CH-Ar), 91.6 (C-1'), 89.1 (d,  $J = 14.5$  Hz, C-4'), 75.1 (C-2'), 69.2 (C-3'), 66.7 (C-5'), 34.2 – 31.8 (m,  $\text{CH}_2\text{PCH}_2\text{P}$ );  $^{31}\text{P NMR}$  (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 34.7$  ( $\text{CH}_2\text{-P(O)-CH}_2$ ), 14.8 ( $\text{P(O)(ONa)}_2$ ); Q-TOF

MS E<sup>+</sup>: m/z 550.11 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>23</sub>H<sub>26</sub>N<sub>3</sub>O<sub>9</sub>P<sub>2</sub>: 550.1139 [M-3Na+4H]<sup>+</sup>, found: 550.1123.

4.1.7.7. *6'-Deoxy-6'-(phosphonomethylphosphonate)-1'-(4-pyren-1H-1,2,3-triazol-1-yl)-β-D-allofuranose (trisodium salt) (2d)*

Treatment of compound **13** (302.8 mg, 0.49 mmol) using general procedure **A** gave rise to the desired intermediate **6d** (337.5 mg). This later was subsequently treated following general procedure **B** in 24 h and then **C** for 72 h. To give compound **2d** (202.5 mg, 60% overall, brown solid) contaminated with less than 10% of the α-anomer. *R*<sub>f</sub> = (*i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, 7/2/1) 0.26; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ = 8.05 (s, 1H, H<sub>triazole</sub>), 7.76 (s, 1H, H-Ar), 7.70 (s, 2H, H-Ar), 7.52 (d, *J* = 8.9 Hz, 1H, H-Ar), 7.46 (s, 3H, H-Ar), 7.39 (d, *J* = 8.6 Hz, 1H, H-Ar), 7.33 (d, *J* = 9.3 Hz, 1H, H-Ar), 6.16 (d, *J* = 5.0 Hz, 1H, H-1'), H-2' signal in D<sub>2</sub>O peak, 4.68 – 4.66 (m, 1H, H-3'), 4.36 (s, 1H, H-5'), 4.30 (s, 1H, H-4'), 2.30 – 2.03 (m, 4H, CH<sub>2</sub>-P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O): δ = 146.5 (C=CH<sub>triazole</sub>), 130.4 (C-Ar), 130.3 (C-Ar), 129.7 (C-Ar), 127.3 (CH-Ar), 126.6 (CH-Ar), 125.9 (CH-Ar), 124.9 (CH-Ar), 124.2 (CH-Ar), 123.2 (C=CH<sub>triazole</sub>), 123.0 (C-Ar), 122.8 (C-Ar), 122.4 (C-Ar), 91.5 (C-1'), 88.8 (d, *J* = 14.3 Hz, C-4'), 75.2 (C-2'), 69.2 (C-3'), 66.6 (C-5'), 34.4 – 31.4 (m, CH<sub>2</sub>-P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O): δ = 34.1 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 15.4 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: m/z 574.11 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>25</sub>H<sub>26</sub>N<sub>3</sub>O<sub>9</sub>P<sub>2</sub>: 574.1139 [M-3Na+4H]<sup>+</sup>, found: 574.1126.

4.1.7.8. *[(1H-1,2,3-Triazole-4-(3,5-bis-trifluoromethylphenyl))phosphonyl]-methyl phosphonate (trisodium salt) (3a)*

The title compound was obtained as a white solid (116.8 mg, 50%) in 24 h from compound **17a** (255 mg, 0.44 mmol) following general procedure **C**. *R*<sub>f</sub> = (*i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, 7/2/1) 0.20; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ = 8.40 (s, 1H, H<sub>triazole</sub>), 8.26 (s, 2H, H-Ar), 7.97 (s, 1H, H-Ar), N-CH<sub>2</sub>P signal in D<sub>2</sub>O peak, 2.08 (t, *J* = 18.2 Hz, 2H, P-CH<sub>2</sub>-P). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O): δ = 145.0 (C=CH<sub>triazole</sub>), 131.5 (C-Ar), 131.3 (q, *J* = 33.3 Hz, C-Ar), 125.9 (CH-Ar), 123.5 (C=CH<sub>triazole</sub>), 123.2 (q, *J* = 272.0 Hz, CF<sub>3</sub>), 122.1 (CH-Ar), 50.7 (d, *J* = 94.4 Hz, P-CH<sub>2</sub>-P), 30.6 – 28.9 (m, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O): δ = 25.0 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 13.7 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>-</sup>: m/z 452.00 [M-3Na+2H]<sup>-</sup>; HRMS Q-TOF MS E<sup>-</sup>: calculated for C<sub>12</sub>H<sub>10</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>F<sub>6</sub>: 452.0000 [M-3Na+2H]<sup>-</sup>, found: 451.9994.

4.1.7.9. *[(1H-1,2,3-Triazole-4-(naphthalen))phosphinyl]-methylphosphonate (trisodium salt) (3b)*

The title compound was obtained as a white solid (320 mg, 100%) from compound **17b** (355 mg, 0.74 mmol) following general procedure **C** in dichloromethane. *R*<sub>f</sub> (*i*-PrOH /NH<sub>4</sub>OH/H<sub>2</sub>O 7/2/1) 0.05. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 8.23 (s, 1H, H<sub>triazole</sub>), 8.16 (s, 1H, H-Ar), 7.93 (t, *J* = 7.0 Hz, 3H, H-Ar), 7.81 (d, *J* = 8.3 Hz, 1H, H-Ar), 7.63 – 7.51 (m, 2H, H-Ar), 4.74 (d, *J* = 9.1 Hz, 2H, N-CH<sub>2</sub>-P), 2.43 – 1.90 (dd, *J* = 18.6, 17.6 Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O): δ = 147.2 (C=CH<sub>triazole</sub>), 133.0,

132.8, 128.6, 128.1, 127.7, 126.9, 126.8, 126.6, 124.2, 123.6 (C-Ar), 122.8 (C=CH<sub>triazole</sub>), 50.8 (d,  $J = 94.7$  Hz, N-CH<sub>2</sub>-P), 29.8 (dd,  $J = 121.6, 84.8$  Hz, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O):  $\delta = 25.0$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 14.0 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>-</sup>:  $m/z$  340.02 [M-4Na]<sup>-</sup>, 362.00 [M-3Na-H]<sup>-</sup>, 368.03 [M-3Na+5H]<sup>-</sup>, 390.01 [M-2Na+4H]<sup>-</sup>. Q-TOF MS E<sup>+</sup>: 348.03 [M-4Na+8H]<sup>+</sup>, 366.04 [M-3Na+3H]<sup>+</sup>, 388.02 [M-2Na+2H]<sup>+</sup>, 411.99 [M-Na+2H]<sup>+</sup>, 433.97 [M+H]<sup>+</sup>, 456.01 [M+Na+H]<sup>+</sup>, 733.09 [2M-6Na+7H]<sup>+</sup>, 777.05 [2M-4Na+5H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>14</sub>H<sub>16</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 368.0565 [M-3Na+5H]<sup>+</sup>, found: 368.0568; UV (EtOH abs)  $\lambda_{1\max} = 217$  nm ( $\epsilon_{1\max} = 20409$ ),  $\lambda_{2\max} = 246$  nm ( $\epsilon_{2\max} = 38586$ ),  $\lambda_{3\max} = 289$  nm ( $\epsilon_{3\max} = 9156$ ).

4.1.7.10. [(1*H*-1,2,3-Triazole-4-(phenanthrene-9-yl))-phosphonyl]-methylphosphonate (trisodium salt) (**3c**)

The title compound was obtained as a white solid (38.3 mg, 41%) 5 days from compound **17c** (101.6 mg, 0.20 mmol) using general procedure C.  $R_f = (i\text{-PrOH}/\text{H}_2\text{O}/\text{NH}_4\text{OH}, 7/2/1) 0.23$ ; <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta = 8.71$  (d,  $J = 8.3$  Hz, 1H, H-Ar), 8.65 (d,  $J = 8.2$  Hz, 1H, H-Ar), 8.26 (s, 1H, H<sub>triazole</sub>), 8.13 (d,  $J = 8.1$  Hz, 1H, H-Ar), 7.89 (d,  $J = 7.7$  Hz, 1H, H-Ar), 7.84 (s, 1H, H-Ar), 7.72 – 7.69 (m, 2H, H-Ar), 7.64 (t,  $J = 7.4$  Hz, 2H, H-Ar), 4.92 (d,  $J = 9.0$  Hz, 2H, N-CH<sub>2</sub>P), 2.26 (t,  $J = 18.1$  Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O):  $\delta = 148.5$  (C=CH<sub>triazole</sub>), 133.2 (C-Ar), 132.6 (C-Ar), 132.3 (C-Ar), 131.9 (C-Ar), 131.4 (CH-Ar), 131.0 (CH-Ar), 130.1 (CH-Ar), 129.8 (2 x CH-Ar), 129.6 (CH-Ar), 128.3 (CH-Ar), 128.1 (C-Ar & C=CH<sub>triazole</sub>), 125.5 (CH-Ar), 125.0 (CH-Ar), 53.4 (d,  $J = 94.7$  Hz, N-CH<sub>2</sub>P), 33.3 – 31.9 (P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O):  $\delta = 25.1$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 14.0 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  418.07 [M-3Na+4H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>18</sub>H<sub>18</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 418.0722 [M-3Na+4H]<sup>+</sup>, found: 418.0726.

4.1.7.11. [(1*H*-1,2,3-Triazole-4-(pyrenyl))-phosphonyl]-methylphosphonate (trisodium salt) (**3d**)

The title compound was obtained as a white solid (74.5 mg, 55%) in 5 days from compound **17d** (146.3 mg, 0.26 mmol) following general procedure C.  $R_f = (i\text{-PrOH}/\text{H}_2\text{O}/\text{NH}_4\text{OH}, 7/2/1) 0.25$ ; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta = 8.07$  (s, 1H, H<sub>triazole</sub>), 7.85 (s, 8H, H-Ar), 7.54 (d,  $J = 8.8$  Hz, 2H, H-Ar), N-CH<sub>2</sub>P signal in D<sub>2</sub>O peak, 2.19 (t,  $J = 18.1$  Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O):  $\delta = 146.2$  (C=CH<sub>triazole</sub>), 130.4 (C-Ar), 130.3 (C-Ar), 129.8 (C-Ar), 127.5 (CH-Ar), 127.2 (CH-Ar), 127.0 (C-Ar), 126.7 (CH-Ar), 126.3 (CH-Ar), 125.8 (CH-Ar), 125.2 (CH-Ar), 125.0 (C-Ar), 124.8 (C=CH<sub>triazole</sub>), 124.3 (CH-Ar), 123.2 (CH-Ar), 123.2 (CH-Ar), 123.1 (C-Ar), 123.0 (C-Ar), 50.7 (d,  $J = 94.9$  Hz, N-CH<sub>2</sub>P) 30.7 – 29.0 (m, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O):  $\delta = 25.0$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 14.0 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  442.07 [M-3Na+4H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>20</sub>H<sub>18</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 442.0722 [M-3Na+4H]<sup>+</sup>, found: 442.0725.

4.1.7.12. [(1*H*-1,2,3-Triazole-4,5-diphenyl)phosphinyl]-methylphosphonate (trisodium salt) (**3e**)

The title compound was obtained as a white solid (247 mg, 100%) from compound **17e** (258 mg, 0.538 mmol) following general procedure **C** in dichloromethane.  $R_f$  (*i*-PrOH /NH<sub>4</sub>OH/H<sub>2</sub>O, 7/2/1) 0.03; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 7.65 – 7.52 (m, 3H, H-Ar), 7.52 – 7.47 (m, 4H, H-Ar), 7.42 – 7.33 (m, 3H, H-Ar), 4.64 (d,  $J$  = 8.9 Hz, 2H, N-CH<sub>2</sub>-P), 2.16 (dd,  $J$  = 18.5, 17.7 Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (101 MHz, D<sub>2</sub>O):  $\delta$  = 144.2 (C-4), 135.79 (C-5), 130.5, 130.1, 130.0, 129.3, 128.8, 128.41, 127.3, 126.47 (C-Ar), 48.6 (d,  $J$  = 95.6 Hz, N-CH<sub>2</sub>-P), 30.70 (dd,  $J$  = 120.6, 84.3 Hz, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (162 MHz, D<sub>2</sub>O):  $\delta$  = 24.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 14.5 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  374.05 [M-4Na+7H]<sup>+</sup>, 394.07 [M-3Na+4H]<sup>+</sup>, 395.08 [M-3Na+5H]<sup>+</sup>, 416.05 [M-2Na+3H]<sup>+</sup>, 438.04 [M-Na+2H]<sup>+</sup>, 787.14 [2M-6Na+7H]<sup>+</sup>; Q-TOF MS E<sup>-</sup>: 392.06 [M-3Na+2H]<sup>-</sup>, 414.04 [M-2Na+H]<sup>-</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>16</sub>H<sub>18</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 394.0722 [M-3Na+4H]<sup>+</sup>, found: 394.0722; UV (EtOH abs)  $\lambda_{max}$  = 249 nm ( $\epsilon_{max}$  = 12529).

4.1.7.13. [1*H*-1,2,3-Triazole-4-(9-methyladenine)phosphinyl]-methylphosphonate (trisodium salt) (**3f**)

The title compound was obtained as a white solid (223 mg, 93%) from compound **17f** (266 mg, 0.53 mmol) following general procedure **C**. Purification was performed by column chromatography on reverse phase (gradient: A = TEAB 0.1M, B= ACN/H<sub>2</sub>O 8/2, gradient 100% A to 10% B).  $R_f$  (*i*-PrOH /NH<sub>4</sub>OH/H<sub>2</sub>O 7/2/1) 0.04; <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta$  = 8.24 (s, 1H, H-4), 8.21 (s, 1H, H-8), 8.12 (s, 1H, H<sub>triazole</sub>), 5.58 (s, 2H, CH<sub>2</sub>), 4.80 (d,  $J$  = 8.9 Hz, 2H, N-CH<sub>2</sub>-P), 2.13 (t,  $J$  = 18.2 Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (151 MHz, D<sub>2</sub>O):  $\delta$  = 155.5 (C-2), 152.6 (C-4), 148.9 (C-6), 142.3 (C-8), 142.2 (C=CH<sub>triazole</sub>), 125.0 (C=CH<sub>triazole</sub>), 118.4 (C-1), 50.7 (d,  $J$  = 94.3 Hz, N-CH<sub>2</sub>-P), 38.6 (s, CH<sub>2</sub>), 30.0 (dd,  $J$  = 121.4, 84.6 Hz, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (243 MHz, D<sub>2</sub>O):  $\delta$  = 25.1 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 13.7 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  369.04 [M-4Na+7H]<sup>+</sup>, 389.06 [M-3Na+4H]<sup>+</sup>, 411.05 [M-2Na+3H]<sup>+</sup>, 433.03 [M-Na+2H]<sup>+</sup>, 455.01 [M+H]<sup>+</sup>, 477.02 [M+Na]<sup>+</sup>, 777.12 [2M-6Na+7H]<sup>+</sup>, 887.03 [2M-Na+2H]; Q-TOF MS E<sup>-</sup>: 387.05 [M-3Na+2H]<sup>-</sup>, 409.03 [M-2Na+H]<sup>-</sup>; HRMS Q-TOF MS E<sup>-</sup>: calculated for C<sub>10</sub>H<sub>13</sub>N<sub>8</sub>O<sub>5</sub>P<sub>2</sub>: 387.0484 [M-3Na+2H]<sup>-</sup>, found: 387.0486; UV (EtOH abs)  $\lambda_{max}$  = 259 nm ( $\epsilon_{max}$  = 4614).

4.1.7.14. [(1*H*-1,2,3-Triazole-4-(9-ethyladenine))phosphonyl]-methylphosphonate (trisodium salt) (**3g**)

The title compound was obtained as a white solid (85.2 mg, 61%) in 5 days from compound **17g** (153 mg, 0.29 mmol) using general procedure **C**.  $R_f$  = (*i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, (5/1/1) 0.10; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta$  = 8.07 (s, 1H, H-2), 7.81 (s, 1H, H-8), 7.67 (s, 1H, H<sub>triazole</sub>), 4.60 (d,  $J$  = 9.1 Hz, 2H, N-CH<sub>2</sub>P), 4.42 (t,  $J$  = 6.4 Hz, 2H, N-CH<sub>2</sub>-CH<sub>2</sub>), 3.19 (t,  $J$  = 6.4 Hz, 2H, CH<sub>2</sub>-C<sub>triazole</sub>), 1.97 (t,  $J$  = 18.1

Hz, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O): δ = 154.2 (C-6), 150.7 (C-2), 148.4 (C-4), 143.5 (C=CH<sub>triazole</sub>), 142.6 (C-8), 124.5 (C=CH<sub>triazole</sub>), 118.1 (C-5), 50.41 (d, J = 94.5 Hz, N-CH<sub>2</sub>P), 43.7 (N<sup>9</sup>-CH<sub>2</sub>), 30.3 – 28.7 (m, P-CH<sub>2</sub>-P), 25.2 (CH<sub>2</sub>-C<sub>triazole</sub>); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O): δ = 24.9 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 13.9 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: m/z 403.08 [M-3Na+4H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>11</sub>H<sub>17</sub>N<sub>8</sub>O<sub>5</sub>P<sub>2</sub>: 403.0797 [M-3Na+4H]<sup>+</sup>, found: 403.0805.

#### 4.1.7.15. [1H-1,2,3-Triazole-4-(9-methyl-2-chloro-N<sup>6</sup>benzyladenine))phosphonyl]-methylphosphonate (trisodium salt) (**3h**)

Compound **16** (280 mg, 0.85 mmol) was first treated using general procedure **D** for 3 h to give the protected intermediate **17h** (444.9 mg, 83%). This intermediate (71 mg, 0.11 mmol) was converted into desired compound **3h** (27 mg, 41%, white solid) following general procedure **C** in 3 days. R<sub>f</sub> = (*i*-PrOH/H<sub>2</sub>O/NH<sub>4</sub>OH, 5/1/1) 0.15; <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ = 8.03 (s, 1H, H<sub>triazole</sub>), 8.00 (s, 1H, H-8), 7.27 – 7.18 (m, 5H, H-Ar), 5.39 (s, 2H, N-CH<sub>2</sub>), 4.70 (d, J = 9.0 Hz, 2H, N-CH<sub>2</sub>-P), 4.60 (s, 2H, NH-CH<sub>2</sub>), 2.08 – 2.01 (m, 2H, P-CH<sub>2</sub>-P); <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O): δ = 154.9 (C-2), 153.9 (C-6), 148.9 (C-4), 141.7 (C-8), 141.7 (C=CH<sub>triazole</sub>), 137.7 (C-Ar), 128.5 (CH-Ar), 127.3 (CH-Ar), 127.1 (CH-Ar), 125.0 (C=CH<sub>triazole</sub>), 117.6 (C-5), 50.5 (d, J = 94.5 Hz, N-CH<sub>2</sub>-P), 43.8 (NH-CH<sub>2</sub>), 38.4 (N-CH<sub>2</sub>), 30.7 – 29.0 (m, P-CH<sub>2</sub>-P); <sup>31</sup>P NMR (202 MHz, D<sub>2</sub>O): δ = 25.0 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 13.6 (P(O)(ONa)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: m/z 513.07 [M-3Na+4H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>17</sub>H<sub>20</sub>N<sub>8</sub>O<sub>5</sub>P<sub>2</sub>Cl: 513.0720 [M-3Na+4H]<sup>+</sup>, found: 513.0725.

#### 4.1.8. Diisopropyl 1-[ethyl(hydroxymethyl)phosphinyl]methylphosphonate (**14**) [30]

Diisopropyl methyl phosphonate (1.85 mL, 10 mmol, 1 eq.) was added to lithium diisopropylamide solution 2 M (16 mL, 32 mmol, 3.2 eq.) dissolved in dry THF (30 mL) at –78 °C and stirred for 30 min. Diethyl hydroxymethylphosphonate (1.8 mL, 12 mmol, 1.2 eq.) was then added via syringe. The mixture was stirred 30 min at –78 °C and then 4h at room temperature. Acetic acid (2.9 mL, 50 mmol, 5 eq.) was added and a white precipitate appeared. After concentration, NaHCO<sub>3</sub> and ethyl acetate was added and the mixture extracted with water. The product being in the aqueous layer, it was extracted with dichloromethane after saturating the aqueous layer with sodium chloride. After drying over MgSO<sub>4</sub>, filtration and concentration under vacuum, the desired product was obtained as a yellow oil (2.3 g) contaminated with 3% of (*i*-PrO)<sub>2</sub>P(O)CH<sub>3</sub> and 5% of (EtO)<sub>2</sub>P(O)-CH<sub>2</sub>OH, thus corresponding to 7.6 mmol of **14**, 76%). R<sub>f</sub> (EtOAc/Acetone, 7/3) 0.13; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 4.85 – 4.58 (m, 3H, OH, CH(CH<sub>3</sub>)<sub>2</sub>), 4.29 – 4.07 (m, 4H, CH<sub>2</sub>-OH, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.70 – 2.33 (m, 2H, P-CH<sub>2</sub>-P), 1.57 – 1.17 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 72.5 (d, J = 6.7 Hz, CH), 72.3 (d, J = 6.7 Hz, CH), 61.7 (d, J = 6.5 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 59.7 (d, J = 107.6 Hz, CH<sub>2</sub>OH), 28.2 (dd, J = 134.8, 77.2 Hz, P-CH<sub>2</sub>-P), 24.1 (s, CH<sub>3</sub>), 24.0 (d, J = 4.3 Hz, CH<sub>3</sub>), 16.6 (d, J = 5.5 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR

(121 MHz, CDCl<sub>3</sub>):  $\delta$  = 43.1, 19.8; Q-TOF MS E<sup>+</sup>: m/z 303.11 [M+H]<sup>+</sup>, 627.20 [2M+ Na]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup> calculated for C<sub>10</sub>H<sub>25</sub>O<sub>6</sub>P<sub>2</sub>: 303.1126 [M+H]<sup>+</sup>, found: 303.1126.

#### 4.1.9. Diisopropyl 1-[ethyl(tosyloxymethyl)phosphinyl]-methylphosphonate (**15**) [30]

To a solution of **14** (2.8 g, 9.27 mmol, 1 eq.) in dry dichloromethane (31 mL) under argon was added *N,N*-diisopropylethylamine (3.94 mL, 23.18 mmol, 2.5 eq.). The mixture was cooled at 0 °C and then a solution of tosyl chloride (3.53 g, 18.54 mmol, 2 eq.) in dichloromethane (15 mL) was added. The temperature was allowed to increase to room temperature overnight. The solvent was then removed under reduced pressure and the residue obtained was purified by flash chromatography on silica gel using a mixture of EtOAc/acetone (9/1 to 0/1) to give the expected compound **15** as a yellow oil (2.33 g, 55%). *R<sub>f</sub>* (EtOAc/acetone 7/3) 0.27; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.80 (d, *J* = 8.4 Hz, 2H, H-Ar), 7.36 (d, *J* = 8.1 Hz, 2H, H-Ar), 4.95 – 4.53 (m, 2H, CH), 4.53 – 3.99 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub>, O-CH<sub>2</sub>-P), 2.56 – 2.27 (m, 5H, P-CH<sub>2</sub>-P, Ar-CH<sub>3</sub>), 1.38 – 1.26 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 145.7, 131.9, 130.2, 128.4 (C-Ar), 72.0 (d, *J* = 6.5 Hz, CH), 71.8 (d, *J* = 6.4 Hz, CH), 63.4 (d, *J* = 113.1 Hz, O-CH<sub>2</sub>-P), 62.4 (d, *J* = 6.6 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 27.1 (dd, *J* = 136.8, 89.0 Hz, P-CH<sub>2</sub>-P), 24.4 – 23.6 (m, CH<sub>3</sub>), 21.8 (CH<sub>3</sub>-Ar), 16.5 (d, *J* = 6.2 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta$  = 35.6 (d, *J* = 3.1 Hz), 15.9 (d, *J* = 3.1 Hz); Q-TOF MS E<sup>+</sup>: m/z 457.12 [M+H]<sup>+</sup>, 458.12 [M+2H]<sup>+</sup>, 913.23 [2M+ H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>17</sub>H<sub>31</sub>O<sub>8</sub>P<sub>2</sub>S: 457.1215 [M+H]<sup>+</sup>, found: 457.1215.

#### 4.1.10. Diisopropyl 1-[ethyl(azidomethyl)phosphinyl]methylphosphonate (**16**) [30]

To a solution of **15** (2.83 g, 6.3 mmol, 1 eq.) in DMF (12.6 mL) under argon was added sodium azide (615 g, 9.46 mmol, 1.5 eq.). The reaction was stirred at 60 °C for 4 h. After cooling the mixture at 0 °C, cold water (25 mL) was added. The organic layer was extracted three times with dichloromethane and concentrated under reduced pressure. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under vacuum. The crude obtained was purified by column chromatography on silica gel using a mixture of CH<sub>2</sub>Cl<sub>2</sub> and acetone (9/1 to 5/5) to afford the desired compound **16** as a yellow oil (1.7 g, 85%). *R<sub>f</sub>* (CH<sub>2</sub>Cl<sub>2</sub>/Acetone, 1/1) 0.30; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 5.02 – 4.51 (m, 2H, CH), 4.38 – 4.13 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.86 – 3.50 (m, 2H, CH<sub>2</sub>-N<sub>3</sub>), 2.55 – 2.32 (m, 2H, P-CH<sub>2</sub>-P), 1.49 – 1.23 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 72.0 (d, *J* = 6.5 Hz, CH), 71.7 (d, *J* = 6.6 Hz, CH), 62.17 (d, *J* = 6.7 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 47.9 (d, *J* = 107.1 Hz, P-CH<sub>2</sub>-N<sub>3</sub>), 27.3 (dd, *J* = 136.2, 82.8 Hz, P-CH<sub>2</sub>-P), 24.2 – 23.9 (m, CH<sub>3</sub>), 16.62 (d, *J* = 5.8 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta$  = 39.6, 16.4; Q-TOF MS E<sup>+</sup>: m/z 328.12 [M+H]<sup>+</sup>, 350.10 [M+Na]<sup>+</sup>, 677.21 [2M+Na]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>10</sub>H<sub>24</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 328.1191 [M+H]<sup>+</sup>, found: 328.1190.

#### 4.1.11. Method D. General procedure for the preparation of compounds **17a** – **17h**

To a solution of the azide **16** (1 eq.) in DMF (0.8 mL/mmol) were added the appropriate alkyne (5.1 eq.), CuSO<sub>4</sub> (0.03 eq.) and sodium ascorbate (0.1 eq.). The mixture was heated at 70 °C until TLC showed completion of the reaction. Water and EtOAc were added and the aqueous layer was extracted 3 times with EtOAc (20 mL). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to dryness. Purification of the crude was performed on silica gel with the appropriate eluent.

##### 4.1.11.1. Diisopropyl-1-[ethyl(1H-1,2,3-triazole-4-(3,5-bis-trifluoromethylphenyl))phosphonyl]-methylphosphonate (**17a**)

Compound **17a** (270 mg, 51%) was obtained as a white solid from compound **16** (307 mg, 0.93 mmol) using general procedure **D** (5 h). Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3). *R*<sub>f</sub> = (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3) 0.37; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 8.37 (s, 1H, H<sub>triazole</sub>), 8.30 (s, 2H, 2 x H-Ar), 7.83 (s, 1H, H-Ar), 5.14 (d, *J* = 11.2 Hz, 2H, N-CH<sub>2</sub>P), 4.92 – 4.83 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.82 – 4.73 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.25 – 4.17 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.53 – 2.32 (m, 2H, P-CH<sub>2</sub>-P), 1.41 -1.32 (m, 15H, 5 x CH<sub>3</sub>); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 145.6 (C=CH<sub>triazole</sub>), 132.6 (C-Ar) 132.5 (q, *J* = 33.6 Hz C-Ar), 125.8 (CH-Ar), 123.3 (q, *J* = 272.8 Hz, CF<sub>3</sub>), 122.6 (C=CH<sub>triazole</sub>), 121.9 (CH-Ar), 72.8 (d, *J* = 6.5 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 72.0 (d, *J* = 6.6 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 63.0 (d, *J* = 6.7 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 48.7 (d, *J* = 100.7 Hz, N-CH<sub>2</sub>P), 28.7 – 26.9 (m, P-CH<sub>2</sub>P), 24.3 (CH<sub>3</sub>), 24.0 (CH<sub>3</sub>), 16.6 (CH<sub>3</sub>); <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ = 35.0 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 15.6 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>). Q-TOF MS E<sup>+</sup>: *m/z* 566.14 [M+H]<sup>+</sup>. HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>20</sub>H<sub>28</sub>F<sub>6</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 566.1408 [M+H]<sup>+</sup>, found: 566.1407.

##### 4.1.11.2. Diisopropyl 1-[ethyl(1H-1,2,3-triazole-4-naphthalen)phosphinyl]-methyl-phosphonate (**17b**)

Compound **17b** (373 mg, 75% and containing ~ 10% of starting material **16**) was obtained as a white solid from compound **16** (340 mg, 1.04 mmol) using general procedure **D**. Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 1/1). *R*<sub>f</sub> (CH<sub>2</sub>Cl<sub>2</sub>/Acetone, 7/3) 0.20; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ = 8.35 (s, 1H, H<sub>triazole</sub>), 8.28 (s, 1H, H-Ar), 8.01 – 7.73 (m, 4H, H-Ar), 7.66 – 7.36 (m, 2H, H-Ar), 5.11 (dd, *J* = 9.8, 2.1 Hz, 2H, N-CH<sub>2</sub>-P), 5.01 – 4.64 (m, 2H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.40 – 4.00 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.68 – 2.23 (m, 2H, P-CH<sub>2</sub>-P), 1.48 – 1.11 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ = 148.3 (C=CH<sub>triazole</sub>), 133.7, 133.4, 128.8, 128.4, 127.9, 127.7, 126.6, 126.4, 124.7, 123.9 (C-Ar), 121.7 (C=CH<sub>triazole</sub>), 72.6 (d, *J* = 6.6 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 72.0 (d, *J* = 6.5 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 62.9 (d, *J* = 6.8 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 48.6 (d, *J* = 101.2 Hz, N-CH<sub>2</sub>-P), 27.7 (dd, *J* = 135.8, 86.7 Hz, P-CH<sub>2</sub>-P), 24.1 (td, *J* = 11.0, 4.5 Hz, CH<sub>3</sub>), 16.6 (d, *J* = 6.1 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ = 35.4 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 15.9 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: *m/z* 368.08 [M-5Na+4H]<sup>+</sup>, 438.13 [M-2Na+5H]<sup>+</sup>, 480.18 [M+H]<sup>+</sup>, 481.19 [M+2H]<sup>+</sup>, 502.16 [M+Na]<sup>+</sup>, 503.17 [M+Na+H]<sup>+</sup>, 959.36 [2M+H]<sup>+</sup>,



960.36 [2M+2H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>22</sub>H<sub>32</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 480.1817 [M+H]<sup>+</sup>, found: 480.1819.

4.1.11.3. *Diisopropyl-1-[ethyl(1H-1,2,3-triazole-4-(phenanthrene-9-yl))phosphonyl]-methylphosphonate (17c)*

Compound **17c** (363 mg, 77%) was obtained as a white solid from compound **16** (290 mg, 0.88 mmol) using general procedure **D** (10 days). Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3). *R*<sub>f</sub> = (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3) 0.5; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ = 8.78 (d, *J* = 8.1 Hz, 1H, H-Ar), 8.72 (d, *J* = 8.3 Hz, 1H, H-Ar), 8.42 (d, *J* = 7.6 Hz, 1H, H-Ar), 8.25 (s, 1H, H<sub>triazole</sub>), 8.04 (s, 1H, H-Ar), 7.92 (d, *J* = 7.8 Hz, 1H, H-Ar), 7.72 – 7.68 (m, 2H, H-Ar), 7.64 – 7.61 (m, 2H, H-Ar), 5.19 (dd, *J* = 9.8, 3.2 Hz, 2H, N-CH<sub>2</sub>P), 4.91 – 4.86 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.81 – 4.76 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.30 – 4.25 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.59 – 2.44 (m, 2H, P-CH<sub>2</sub>-P), 1.42 – 1.34 (m, 15H, 5 x CH<sub>3</sub>); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>): δ = 147.3 (C=CH<sub>triazole</sub>), 131.5 (C-Ar), 130.9 (C-Ar), 130.6 (C-Ar), 130.2 (C-Ar), 129.1 (CH-Ar), 128.7 (CH-Ar), 127.4 (CH-Ar), 127.2 (CH-Ar), 127.1 (CH-Ar), 126.9 (CH-Ar), 126.5 (C-Ar), 126.2 (CH-Ar), 124.6 (C=CH<sub>triazole</sub>), 123.2 (CH-Ar), 122.7 (CH-Ar), 72.6 (d, *J* = 6.4 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 72.0 (d, *J* = 6.5 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 62.9 (d, *J* = 6.7 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 48.7 (d, *J* = 101.7 Hz, N-CH<sub>2</sub>P), 28.6 – 27.1 (m, P-CH<sub>2</sub>-P), 24.3 – 24.0 (CH<sub>3</sub>), 16.6 (d, *J* = 6.2 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ = 41.1 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 18.4 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: *m/z* 530.20 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>26</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 530.1974 [M+H]<sup>+</sup>, found: 530.1973.

4.1.11.4. *Diisopropyl-1-[ethyl(1H-1,2,3-triazole-4-pyrenyl)phosphonyl]-methyl phosphonate (17d)*

Compound **16** (289.5 mg, 0.88 mmol) was treated following general procedure **D** (3 days). The crude material was purified by flash chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone: 7/3) to provide compound **17d** contaminated with compound **16**. To a solution of this mixture in absolute ethanol (5 mL) was added 10% Pd/C (3 mg) and the residue was stirred for 24 h under a hydrogen atmosphere. Filtration through a celite pad followed by concentration to dryness and purification on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3) provided the desired compound **17d** as a yellow solid (146.3 mg, 50%). *R*<sub>f</sub> = (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3) 0.54; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ = 8.72 (d, *J* = 9.3 Hz, 1H, H-Ar), 8.36 (s, 1H, H<sub>triazole</sub>), 8.29 (d, *J* = 7.9 Hz, 1H, H-Ar), 8.24 – 8.20 (m, 3H, H-Ar), 8.15 – 8.10 (m, 3H, H-Ar), 8.03 (t, *J* = 7.6 Hz, 1H, H-Ar), 5.23 (dd, *J* = 9.8, 2.9 Hz, 2H, N-CH<sub>2</sub>P), 4.93 – 4.86 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.82 – 4.76 (m, 1H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.32 – 4.27 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.62 – 2.45 (m, P-CH<sub>2</sub>-P), 1.42 – 1.34 (m, 15H, 5 x CH<sub>3</sub>); <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ = 147.9 (C=CH<sub>triazole</sub>), 131.6 (C-Ar), 131.5 (C-Ar), 131.0 (C-Ar), 128.7 (C-Ar), 128.4 (CH-Ar), 128.1 (CH-Ar), 127.5 (CH-Ar), 127.3 (CH-Ar), 126.3 (CH-Ar), 125.6 (CH-Ar), 125.4 (CH-Ar), 125.2 (C-Ar), 125.0 (CH-Ar), 124.9 (2s, C-Ar), 124.8 (CH-Ar), 124.7 (C=CH<sub>triazole</sub>), 72.6 (d, *J* = 6.5 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 72.0 (d, *J* = 6.6 Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 62.95

(d,  $J = 6.7$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 48.7 (d,  $J = 101.6$  Hz, N-CH<sub>2</sub>P), 28.6 – 26.9 (m, P-CH<sub>2</sub>P), 24.3 – 24.0 (m, CH<sub>3</sub>), 16.6 (d,  $J = 6.2$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta = 32.9$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 13.3 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  554.20 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>28</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 554.1974 [M+H]<sup>+</sup>, found: 554.1979.

4.1.11.5. *Diisopropyl 1-[ethyl(1H-1,2,3-triazole-4,5-diphenyl)phosphinyl]-methyl-phosphonate (17e)*

Compound **17e** (271 mg, 56%) was obtained as a white solid from compound **16** (310 mg, 0.94 mmol) using general procedure **D** (7 days). Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/acetone: 10/0 to 7/3).  $R_f$ (CH<sub>2</sub>Cl<sub>2</sub>/acetone, 7/3) 0.21; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 7.55 - 7.45$  (m, 7H, H-Ar), 7.30 – 7.24 (m, 3H, H-Ar), 4.90 – 4.72 (m, 4H, N-CH<sub>2</sub>-P, CH(CH<sub>3</sub>)<sub>2</sub>), 4.21 – 4.06 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.81 – 2.71 (m, 2H, P-CH<sub>2</sub>-P), 1.38 – 1.26 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta = 144.4$  (C-4), 135.1 (C-5), 130.8, 130.6, 130.1, 129.5, 128.6, 128.0, 127.3, 127.1 (C-Ar), 72.0 (d,  $J = 6.3$  Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 71.7 (d,  $J = 6.4$  Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 62.6 (d,  $J = 6.8$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 46.4 (d,  $J = 103.2$  Hz, N-CH<sub>2</sub>-P), 28.6 (dd,  $J = 134.7, 85.5$  Hz, P-CH<sub>2</sub>-P), 24.1 (dt,  $J = 8.8, 3.9$  Hz, CH<sub>3</sub>), 16.5 (d,  $J = 6.2$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (162 MHz, CDCl<sub>3</sub>):  $\delta = 35.9$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 16.4 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  464.15 [M-2Na+5H]<sup>+</sup>, 506.20 [M+H]<sup>+</sup>, 507.20 [M+2H]<sup>+</sup>, 1011.39 [2M+H]<sup>+</sup>, 1012.39 [2M+2H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>24</sub>H<sub>34</sub>N<sub>3</sub>O<sub>5</sub>P<sub>2</sub>: 506.1974 [M+H]<sup>+</sup>, found: 506.1971.

4.1.11.6. *Diisopropyl 1-[ethyl(1H-1,2,3-triazole-4-(9-methyladenine) phosphinyl)-methyl phosphonate (17f)*

Compound **17f** (485 mg, 71%) was obtained as a white solid from compound **16** (450 mg, 1.37 mmol) and *N*<sup>9</sup>-propargyladenine (371 mg, 1.56 mmol) using general procedure **D**. Purification was performed on silica gel (isocratic EtOAc/MeOH, 3/1).  $R_f$ (EtOAc/MeOH, 3/1) 0.43; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta = 8.33$  (s, 1H, H-4), 7.99 (s, 1H, H-8), 7.97 (s, 1H, H<sub>triazole</sub>), 5.91 (s, 2H, NH<sub>2</sub>), 5.47 (s, 2H, CH<sub>2</sub>), 5.00 (d,  $J = 9.8$  Hz, 2H, N-CH<sub>2</sub>-P), 4.83 – 4.66 (m, 2H, CH(CH<sub>3</sub>)<sub>2</sub>), 4.18 – 4.04 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.53 – 2.27 (m, 2H, P-CH<sub>2</sub>-P), 1.35 – 1.21 (m, 15H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta = 155.7$  (C-2), 153.3 (C-4), 149.9 (C-6), 142.7 (C=CH<sub>triazole</sub>), 140.4 (C-8), 124.8 (C=CH<sub>triazole</sub>), 119.6 (C-1), 72.5 (d,  $J = 6.5$  Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 72.0 (d,  $J = 6.6$  Hz, CH(CH<sub>3</sub>)<sub>2</sub>), 62.8 (d,  $J = 6.6$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>), 48.6 (d,  $J = 101.4$  Hz, N-CH<sub>2</sub>-P), 27.8 (dd,  $J = 135.6, 86.7$  Hz, P-CH<sub>2</sub>-P), 24.2 (m, CH<sub>3</sub>), 16.4 (d,  $J = 6.1$  Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>):  $\delta = 35.0$  (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 15.8 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>:  $m/z$  501.19 [M+H]<sup>+</sup>, 502.19 [M+2H]<sup>+</sup>, 523.17 [M+Na]<sup>+</sup>, 1001.37 [2M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>18</sub>H<sub>30</sub>N<sub>8</sub>O<sub>5</sub>P<sub>2</sub>: 501.1893 [M+H]<sup>+</sup>, found: 501.1891.

4.1.11.7. *Diisopropyl-1-[ethyl-(1H-1,2,3-triazole-4-(9-ethyladenine))phosphonyl]-methylphosphonate (17g)*

Compound **17g** (294 mg, 53%) was obtained as a white solid from compound **16** (347 mg, 1.06 mmol) and *N*<sup>9</sup>-(but-3-yn-1-yl)adenine (224 mg, 1.2 mmol) using general procedure **D** (4 h). Purification was performed on silica gel (AcOEt/MeOH: 6/3). *R*<sub>f</sub> = (AcOEt/MeOH, 6/3) 0.34; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ = 8.20 (s, 1H, H-2), 7.57 (s, 1H, H-8), 7.49 (s, 1H, H<sub>triazole</sub>), 6.73 (s, 2H, NH<sub>2</sub>), 4.93 – 4.91 (m, 2H, N-CH<sub>2</sub>P), 4.74 – 4.64 (m, 2H, 2 x CH(CH<sub>3</sub>)<sub>2</sub>), 4.49 (t, *J* = 6.3 Hz, 2H, N-CH<sub>2</sub>), 4.07 – 3.99 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.21 (t, *J* = 5.5 Hz, 2H, CH<sub>2</sub>-C<sub>triazole</sub>), 2.47 – 2.33 (m, 2H, P-CH<sub>2</sub>-P), 1.25 – 1.22 (m, 12H, 2 x CH(CH<sub>3</sub>)<sub>2</sub>), 1.16 (t, *J* = 7.0 Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>): δ = 155.8 (C-6), 152.7 (C-2), 149.6 (C-4), 143.6 (C=CH<sub>triazole</sub>), 140.5 (C-8), 123.6 (C=CH<sub>triazole</sub>), 119.3 (C-5), 72.1 (CH(CH<sub>3</sub>)<sub>2</sub>), 71.6 (CH(CH<sub>3</sub>)<sub>2</sub>), 62.5 (d, *J* = 6.5 Hz O-CH<sub>2</sub>-CH<sub>3</sub>), 48.3 (d, *J* = 101.6 Hz, N-CH<sub>2</sub>P), 43.0 (N<sup>9</sup>-CH<sub>2</sub>), 28.1 – 26.7 (m, P-CH<sub>2</sub>-P), 26.0 (CH<sub>2</sub>-C<sub>triazole</sub>), 23.9 – 23.7 (m, CH<sub>3</sub>), 16.2 (d, *J* = 6.0 Hz, O-CH<sub>2</sub>-CH<sub>3</sub>); <sup>31</sup>P NMR (202 MHz, CDCl<sub>3</sub>): δ = 35.4 (CH<sub>2</sub>-P(O)-CH<sub>2</sub>), 15.9 (P(O)(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>); Q-TOF MS E<sup>+</sup>: *m/z* 515.21 [M+H]<sup>+</sup>; HRMS Q-TOF MS E<sup>+</sup>: calculated for C<sub>19</sub>H<sub>33</sub>N<sub>8</sub>O<sub>5</sub>P<sub>2</sub>: 515.2049 [M+H]<sup>+</sup>, found: 515.2056.

4.2. *Computational methodology*

All compounds were modelled using the DataWarrior program (v5.2.1) and tridimensional conformer was generated for each molecule using initial torsions from crystallographic database and the random low-energy bias algorithm [37]. Molecular docking was carried out on the crystal structure of CD73 (PDB 4H2I, closed conformation or crystal form III from Knapp K. *et al.*, [25]). The Gold program v5.6 (Genetic Optimization for Ligand Docking) [38] from the Cambridge Crystallographic Data Centre (CCDC, Software Limited) was used to dock all compounds by targeting the CD73 substrate binding site and defined as a sphere of 10 Å radius around the CZ atom of Arg395 with the option for scrutiny of cavity activated and Water molecules from the crystal were preserved (free of translation / rotation within a 2 Å window) during the docking. The search of best docking poses was performed by executing 50 runs of genetic algorithms (search-based optimization technique based on genetics and natural selection) and the ranking of docking solutions was computed by the GoldScore fitness function by using the complete linkage clustering method from the RMSD matrix of generated solutions. The GoldScore scoring function was selected as it outperformed other ones and could better predict control compounds such as AMP or AOPCP. This function accounts for factors such as H-bonding energy, van der Waals energy, metal interaction and ligand torsion strain (hence, the score reflects the theoretical affinity and is expressed as arbitrary units). The highest-ranked poses were

selected for structural analysis and interaction measurements with CD73 amino acids using the PyMol Molecular Graphics System (v1.8, Schrödinger, LLC).

#### 4.3. Kinetics inhibition assays

Adenosine 5'-monophosphate sodium salt (AMP) and adenosine 5'-( $\alpha,\beta$ -methylene) diphosphate (AOPCP) used as positive control were purchased from Sigma-Aldrich. Kinetics inhibition assays were carried out on the purified recombinant CD73 (soluble form, residues 27-549 expressed in Sf9 cells and purified as previously described [3]) at 5 nM (final concentration) by quantifying the substrate (AMP) and the product (adenosine) of the CD73-catalyzed reaction. Briefly, CD73 activity was determined by steady-state kinetics in a thermostatically controlled beaker under magnetic stirring at 37 °C in a buffer containing Tris-HCl 50 mM pH 7, NaCl 100 mM, MgCl<sub>2</sub> 1 mM, CaCl<sub>2</sub> 1 mM. Reaction was allowed to occur upon addition of the substrate (100  $\mu$ M) and quenched every 10 s by addition of 10% of perchloric acid. The same procedure was repeated in presence of each inhibitor (at 10 or 100  $\mu$ M pre-incubated with the enzyme). Reaction products were quantified by HPLC chromatography (Waters Alliance) using a Partisphere 5-SAX column (AIT France) and 10 mM ammonium phosphate buffer pH 5.5 as mobile phase. Quantification was achieved by integrating AMP and adenosine peaks, further normalized in respect to the initial amount of AMP, allowing to calculate steady-state rate constants and inhibition percentage. For IC<sub>50</sub> determination, a concentration range (from 0.01 to 100  $\mu$ M) was used for each inhibitor to perform steady-state kinetics assays (results are expressed as mean  $\pm$  SD of three independent experiments). IC<sub>50</sub> values were computed from non-linear curve fitting in GraphPad Prism.

#### 4.4. Cancer cell-based assays

CD73 activity of MDA-MB-231 cancer cells was determined in absence and presence of studied compounds. Briefly, cells (25,000 cells per plate) were seeded one day before the assay in 96-well plates respectively. Cells were then washed 2-4 times with CD73-buffer (MgCl<sub>2</sub> 2 mM, NaCl 120 mM, KCl 5 mM, glucose 10 mM, Hepes 20 mM pH 7.4) before incubation with 100 or 500  $\mu$ l of the same buffer with or without AMP 200  $\mu$ M and indicated compounds for 10 or 30 minutes at 37 °C. Free inorganic phosphate (produced by AMP by CD73) was finally quantified using Green Malachite Phosphate Assay Kit (Gentaur). The produced amount of inorganic phosphate in each condition was calculated using an external calibration curve and considering eventual intrinsic absorbance of each compound, and the inhibition was calculated by  $(1 - \text{activity}_{\text{inhibitor}} / \text{activity}_{\text{control}}) \times 100\%$ . Results are means of indicated number of replicates  $\pm$  SEM. For cytotoxicity, MDA-MB-231 cells (3,000 per well) were seeded in 96 well plates and incubated with 100  $\mu$ M of studied compounds. After three days,

metabolically active cells were quantified with MTT reagent, and the percentage calculated in comparison to unexposed cells.

#### *4.5. Immune cells functional assay*

Adenosine generated from AMP dephosphorylation by CD73 blocks T cell proliferation after TCR triggering [35, 36]. We developed a biological immuno-assay based on this principle. Briefly, B cells and T cells were purified from healthy donors' blood by sequential Ficoll (d= 1.077, Eurobio) and Percoll (d=1.0-1.3, 51%, GE Healthcare) density gradients. B and T cells (50,000 per well) were preincubated for 1 hour at 37 °C under 5% CO<sub>2</sub>, with chemical compounds selected as CD73 inhibitors (10 or 100 µM) or APCP (50 µM, Sigma-Aldrich) as internal control in 96-round-bottom well plates (Falcon) in complete RPMI medium (cRPMI) [supplemented with antibiotics, L-glutamine (Life Technologies) and 5% of human serum AB (EFS)]. Then, we added anti-CD3/anti-CD28 beads (Expand beads; Life Technologies, ratio 1 bead to 4 cells) for 3 days to induce T cell proliferation. The impact of the enzymatic activity of CD73 inducing generation of adenosine was assessed by addition of exogenous ATP (62.5 µM, Sigma-Aldrich) in a total volume of 100 µl of cRPMI at the beginning of the culture. Each condition was performed in triplicate. At the end of the experimental time course, the proliferation was assessed by capture of photomicrographs for each condition and the addition of MTS substrate (Promega) for two hours at 37 °C under 5% CO<sub>2</sub>. The enzymatic activity of cells that is correlated with living cells number, allowed the degradation of MTS substrate into formazan that was quantified by optical density at 490 nm on a Tecan (ThermoScientific). A negative control of proliferation was obtained by culture of cells without the Expand beads.

#### **Author contributions**

All authors contributed to the experimentations and/or to the writing and preparation of the manuscript. All authors have given approval to the final version of the manuscript.

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## Appendix A. Supplementary data

Supplementary figure (Fig. S1) and spectral data (NMR) of final compounds and intermediates (file type, PDF).

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