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Structure–activity relationships of β-hydroxyphosphonate nucleoside analogues as cytosolic 5′-nucleotidase II potential inhibitors: Synthesis, *in vitro* evaluation and molecular modeling studies

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ABSTRACT

The cytosolic 5′-nucleotidase II (cN-II) has been proposed as an attractive molecular target for the development of novel drugs circumventing resistance to cytotoxic nucleoside analogues currently used for treating leukemia and other malignant hemopathies. In the present work, synthesis of β-hydroxyphosphonate nucleoside analogues incorporating modifications either on the sugar residue or the nucleobase, and their *in vitro* evaluation towards the purified enzyme were carried out in order to determine their potency towards the inhibition of cN-II. In addition to the biochemical investigations, molecular modeling studies revealed important structural features for binding affinities towards the target enzyme.

1. Introduction

Enzymes are attractive biological targets for small-molecule drug discovery, especially those involved in the nucleic acid biosynthesis. Polymerases are key enzymes in these processes and they have been extensively studied as targets for the treatment of viral infections and cancers [1]. Thus, nucleos(t)ide analogues are widely used as therapeutic agents because they mimic physiological metabolites and interfere with key steps in viral particle production and/or in cancer cell proliferation, respectively [2]. To obtain these effects, the phosphorylated forms (nucleotides) must compete with endogenous nucleotides. While much effort has been carried out for the identification of novel analogues that are intrinsically virotoxic or cytotoxic, very little is known regarding the relationship between the size of the pools of endogenous nucleos(t)ides and the therapeutic effect of these drugs. It is expected that their biological effect is directly related to the ability of unnatural nucleotides to compete with the physiological ones, thereby emphasizing the importance of mechanisms regulating nucleotide pools [3,4]. Although the individual steps involved in nucleoside transport and metabolism have been dissected and the majority of the related proteins identified, the overall regulation of nucleos(t)ide pools in mammalian cells remains unclear. Thus, intracellular monophosphorylation of nucleoside analogues is balanced by a family of enzymes called 5′-nucleotidases (5′-NTs: EC 3.1.3.5), which catalyze the hydrolysis of deoxyribo- and ribonucleoside 5′-monophosphates into the corresponding nucleosides and inorganic phosphate [3,5,6]. Among the eight members of this family, our interest for the cytosolic 5′-nucleotidase II (cN-II, EC 3.1.3.5) arose from the fact that its expression level is of crucial

Abbreviations: AML, acute myeloid leukemia; cN-II, cytosolic 5′-nucleotidase II; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; IMP, inosine 5′-monophosphate; 5-NTs, 5′-nucleotidases; RNA, ribonucleic acid; TMSBr, trimethylsilyl bromide; TMSCl, trimethylsilyl chloride; TPPS, 4,4′,4″,4‴-(porphine-5,10,15,20-tetrayl)tetrakis benzenesulfonic acid; Ts, *p*-toluenesulfonyl.

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interest for patients treated with nucleoside analogue-based chemotherapy [7,8]. Indeed, a high level of expression of cN-II mRNA in blasts is predictive of worse outcome in patients receiving cytarabine-based regimens (a well-known nucleoside analogue used to treat acute myeloid leukemia, AML) [9,10]. In addition, the inhibition of cN-II expression by short hairpin RNA was associated with the induction of apoptosis in human astrocytoma cells, suggesting that cN-II could be a therapeutic target in brain tumors [11]. Finally, recent reports showed that hyperactive mutated cN-II in relapsed children with acute lymphoblastic leukemia, and treated with the antimetabolite 6-mercaptopurine, was associated with a worse survival [12,13].

In light of the various structural, functional and regulatory properties of this enzyme, we envisaged cN-II as an attractive target for developing different types of inhibitors that could interfere with protein function or regulation [14,15]. It must be highlighted that cN-II is called high-K_m nucleotidase indicating that K_m value for its favorite substrate, IMP (inosine 5′-monophosphate) is in the millimolar range. Therefore, potential competitive inhibitors are expected to exhibit K_i values in the same range. In addition, cN-II acts as a tetramer (dimer of dimer) and possesses two regulatory or effector sites. The effector site 1 is located closed to the IMP binding site at the interface between two monomers and regulates the enzyme activity (for instance, in presence of ATP, the k_{cat} is increased and K_m is lowered for IMP [16]. Several attempts to target this site have been performed to find other type of inhibitors (such as non-competitive ones) but yet not successful. Our previous studies led to the selection of a first series of 5′-monophosphate nucleoside analogues, such as β-(S)-hydroxyphosphonate derivatives (compounds **2** and **4**, Fig. 1), able to interfere with the hydrolysis of IMP by purified recombinant cN-II [15]. Thus, these two derivatives were considered as a starting point for further optimization and we elaborated several computer-based strategies to improve their efficiency. First, we investigated the effect of the following sugar modifications: (i) inversion of the C2′ and C3′-configurations (Fig. 1, compounds **5**–**16**) or of the C5′ stereochemistry *i.e.* the (*R*)-isomer of the β-hydroxyphosphonate (Fig. 1, compounds **17**–**18**), (ii) opening of the sugar ring (Fig. 1, compounds **19**–**22**).

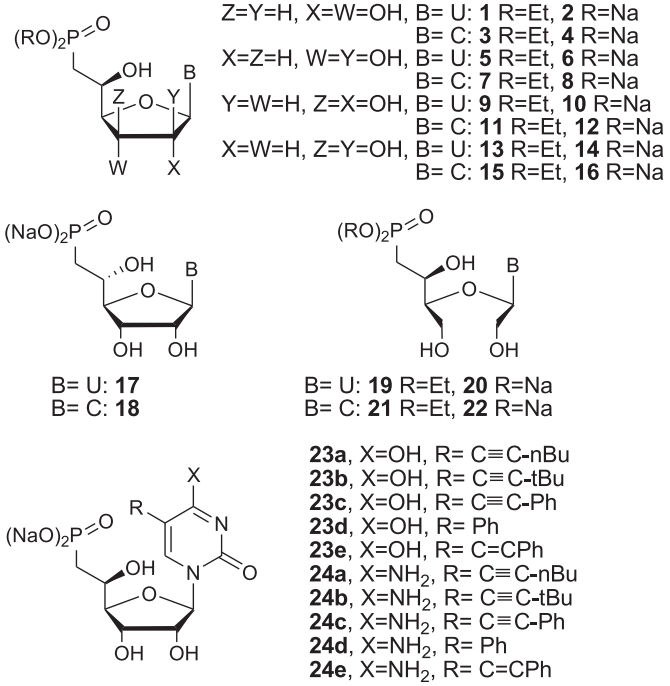


Fig. 1. Structure of the studied compounds.

As we previously reported the crucial role of a hydrophobic clamp around the nucleobase for substrate recognition, that is promoted by three protein residues Phe157, His209 and Tyr210 (Fig. 2) [15], we performed further modeling studies of compounds **2** and **4** within the active site of the enzyme. It revealed that a large hydrophobic pocket was available near the nucleobase and therefore, we envisaged a second series of derivatives including non-natural pyrimidines. The volume of this cavity has been estimated to be ~1200 Å^3 (Fig. 2) allowing various kind of modifications at the C5 position of uracil and cytosine. Thus, we decided to append lipophilic or aromatic substituents on the nucleobase (Fig. 1, compounds **23a**–**e** and **24a**–**e**), that should allow additional Π–Π and/or hydrophobic interactions between the ligand and the protein residues (especially Phe157 and farther Phe354, see Fig. 2).

We have synthesized and evaluated a series of thirty-two β-hydroxyphosphonate derivatives as potential cN-II ligands to determine their activity against cN-II and possible mechanisms of inhibition. In addition, molecular modeling and docking studies were performed to identify the interaction profile of these compounds, as well as key structural parameters responsible for their properties.

2. Results and discussion

2.1. Chemistry

Synthesis of altrofuranoside derivatives **5**–**8** and acyclophosphonates **19**–**22** was initiated from the previously obtained 1-[6-deoxy-6-diethylphosphono-β-D-allofuranosyl] uracil **1** (Scheme 1) [17]. Inversion of the C2′ configuration was envisaged through a well-known two-steps sequence [18] involving the formation of the 2,2′-anhydro intermediate **29** and subsequent opening of the nucleobase-sugar ring. Thus, diethylphosphoester analogue **5** was reacted with trimethylsilyl bromide affording the corresponding phosphonic acid (as sodium salt after percolation on a Dowex ion-exchange resin) **6** in low yield, due to purification losses. From intermediate **5**, the

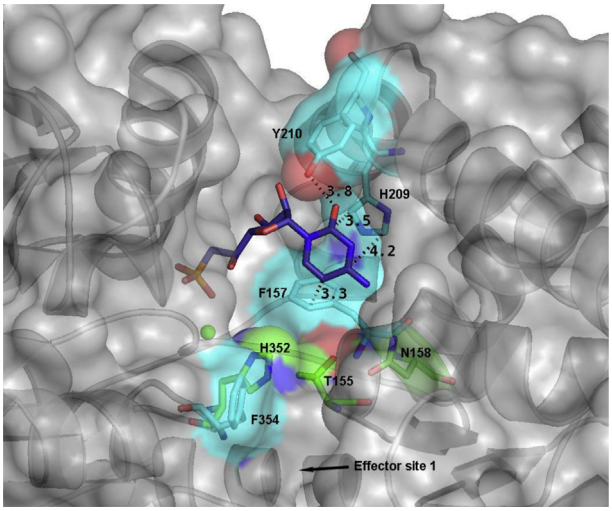
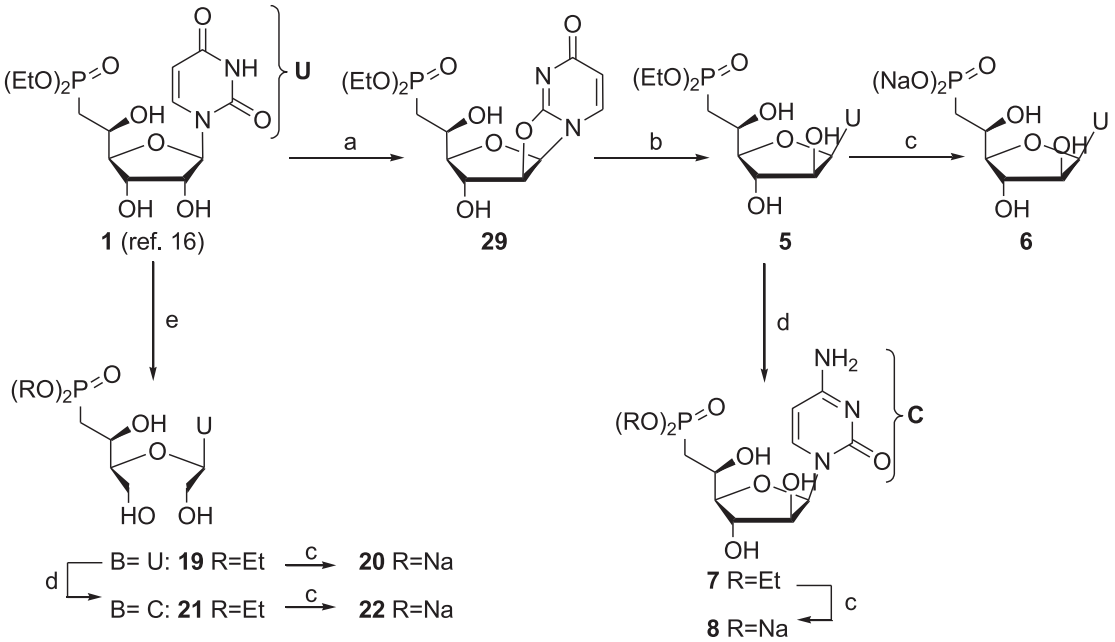


Fig. 2. Surface representation of the IMP binding site (PDB: 2XCW) in presence of the cytosine derivative **4** obtained by molecular docking (starting point for optimization) and showing a large cavity (delineated by the three residues F157, H209 and Y210) surrounding the nucleobase. The magnesium ion is depicted as a green sphere and secondary elements of cN-II are shown in gray. The contacts between derivative **4** and the hydrophobic clamp are indicated in Å. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

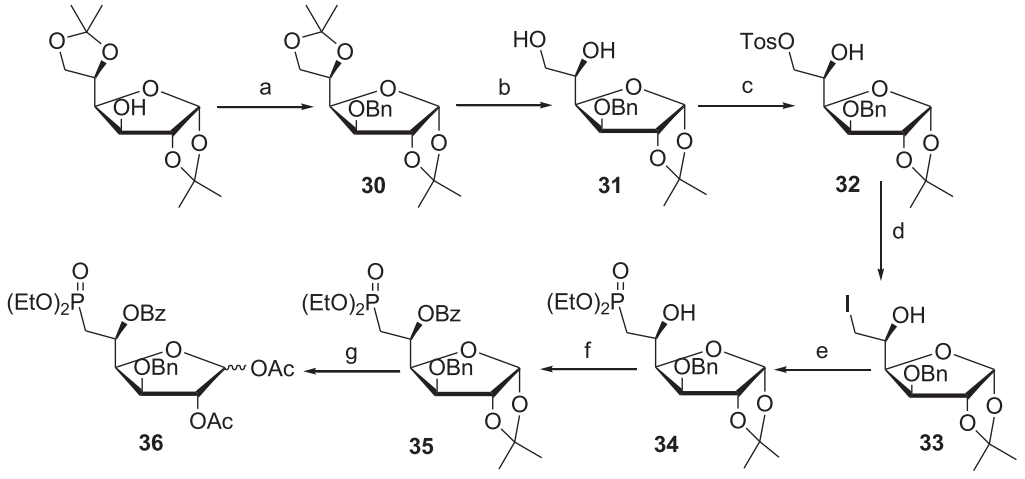


Scheme 1. Synthesis of β-D-altrofuranoside derivatives **5**–**8** and acyclophosphonates **19**–**22**. *Reagents & Conditions* : a) Diphenylcarbonate, DMF then Na_2CO_3; b) NaOH, MeOH/water; c) TMSBr, DMF then Dowex exchange (Na^+ form); d) 1) *N*-Methyl pyrrolidine, TMSCl, $(CF_3CO)_2O$, CH_3CN then 4-nitrophenol 2) NH_4OH, dioxan; e) $NaIO_4$, dioxan/water then $NaBH_4$.

cytosine analogue **7** was generated using a previously published procedure [19,20] involving activation of the 4-keto group by 4-nitrophenol in presence of *N*-methyl pyrrolidine, trimethylsilyl chloride and trifluoroacetic anhydride, and then treatment with ammoniacal solution. Removal of the phosphonate protecting groups and isolation of derivative **8** was carried out using the same procedure followed for **6**.

Scission of the 2′,3′-bond of allofuranoside derivative **1** by sodium periodate oxidation followed by sodium borohydride reduction of the dialdehyde intermediate (not isolated) resulted in the formation of the acyclic analogue **19** in quantitative yield [21]. The latter was converted to the corresponding cytosine derivative **21** by using the nitrophenylation–aminolysis procedure previously described for compound **7**. Finally, the ethyl phosphonate protecting groups of compounds **19** and **21** were eliminated using TMSBr and purification by reverse phase chromatography and ionic exchange afforded acyclic analogues **20** and **22** in 54% and 24% yields, respectively.

Synthesis of the β-hydroxyphosphonate nucleoside analogues with β-D-gluco- and β-D-mannofuranoside configurations (Fig. 1, compounds **9**–**12** and **13**–**16**, respectively) were obtained using a similar ex-chiral pool pathway as previously reported for the corresponding β-D-allofuranoside derivatives [17]. This approach involved the preparation of an appropriate phosphonylated sugar intermediate **36** (Scheme 2) and further glycosylation reaction with the nucleobase. Thus, commercially available diacetone D-glucose was converted into 3-benzylated derivative **30**, which upon acidic treatment provided the corresponding 5,6-diol **31** in 93% yield. Then, selective tosylation of the primary alcohol was carried out, followed by *in situ* nucleophilic substitution of this good leaving group by iodine using sodium iodide in acetone, and compound **33** was obtained in 70% yield over the two steps.



Scheme 2. Synthetic pathways for intermediate **36**. *Reagents & Conditions*: a) BnBr, KOH, DMSO; b) AcOH aq; c) TsCl, DMAP, pyridine; d) NaI, acetone; e) $P(OEt)_3$; f) BzCl, pyridine; g) Ac_2O, H_2SO_{4cat}, AcOH.

The diethylphosphonate group was installed through a Michaelis–Arbuzov reaction leading to derivative **34**, which was first benzoylated on position 5 and then peracetylated to afford **36** (Scheme 2), this two-step procedure being necessary to obtain the required intermediate in 83% yield.

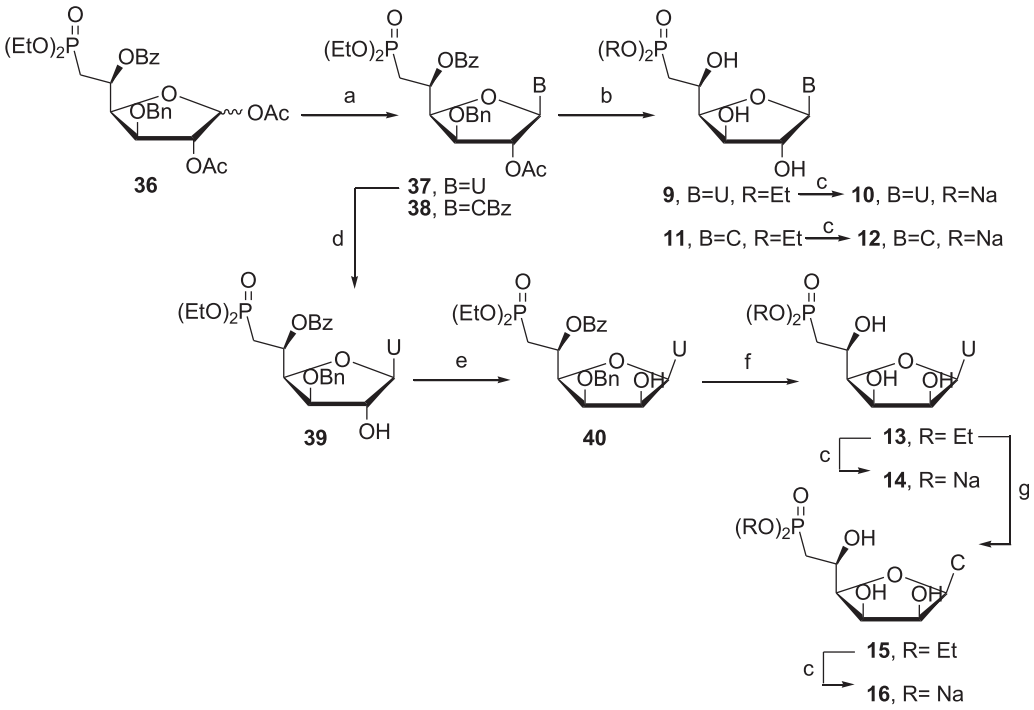
Compound **36** was successively engaged in glycosylation reaction with either uracil or *N*-4-benzoylcytosine (Scheme 3), in presence of an excess of tin(IV) chloride as previously reported [17], and phosphonate nucleoside derivatives **37** and **38** were isolated in 73% and 65% yields, respectively. Removal of sugar protecting groups was performed in one step from compound **37** using hydrogenation in presence of ammonium formate, leading to uracil derivative **9**. Whereas for cytosine analogue **38**, two steps were required due to the presence of the benzoyl group on the nucleobase, which necessitate the use of methanolic ammonia. Finally, phosphonic acids **10** and **12** were obtained using the same procedure as described above.

Synthesis of phosphonate nucleoside analogues **13** and **14** (Scheme 3) including D-mannose as sugar residue was performed with a two-steps oxydation–reduction procedure. First, selective elimination of the 2′-acetyl group of compound **37** was carried out in presence of aqueous hydrazine [22]. Thus, oxidation of the secondary alcohol of intermediate **39** was achieved using the Dess–Martin periodinane reagent. The 2′-keto-intermediate was not isolated but directly reduced in presence of sodium borohydride, affording derivative **40** in 55% yields over the two steps. Due to the steric hindrance of the β-face of the nucleoside derivative, a complete stereoselectivity of the reaction was observed. The final deprotection steps were carried out, leading successively to compounds **13** and **14** in the uracil series. The corresponding cytosine analogues **15** and **16** were obtained as reported above after nitrophenylation–aminolysis and deprotection steps, respectively.

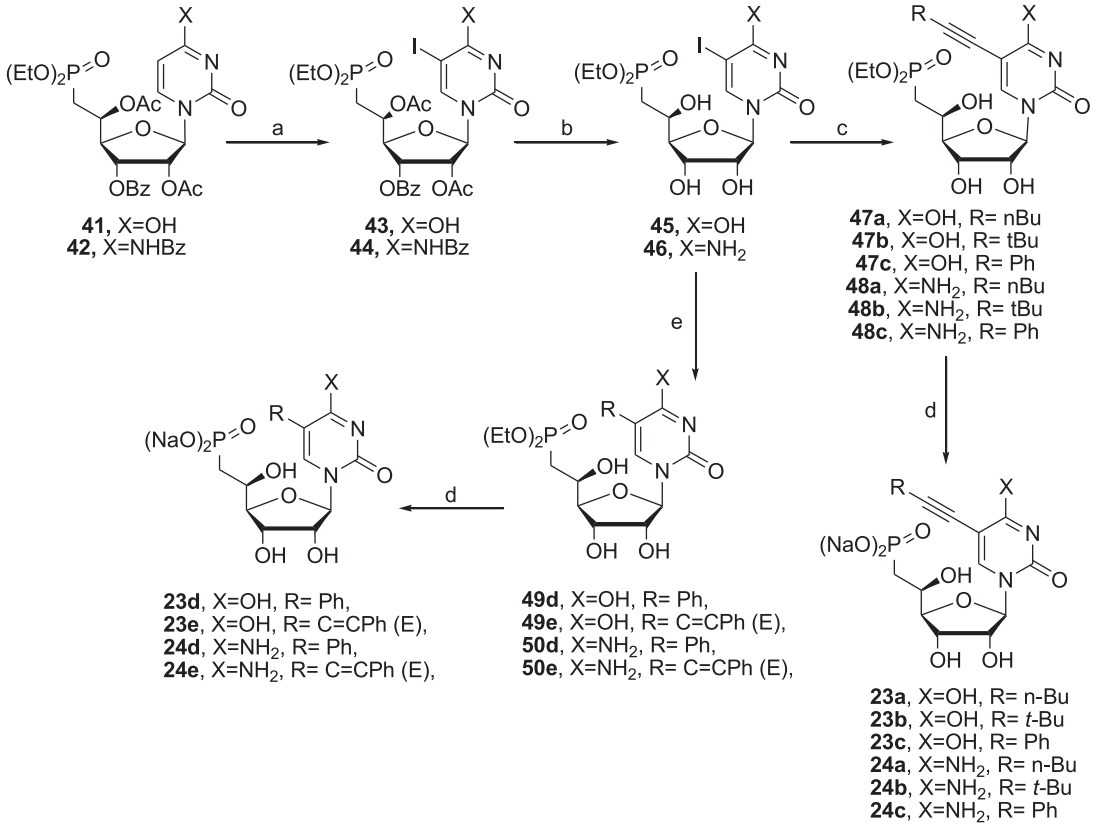
The second part of our work was devoted to the synthesis of β-hydroxyphosphonate nucleoside analogues incorporating modified pyrimidines. As the substrate cavity was larger than the space occupied by the natural nucleobase of the previously studied ligands (Fig. 2), we decided to introduce aromatic and hydrophobic substituents in the C5 position of both compounds **2** and **4**. Access to such a kind of derivatives may readily be achieved using either Sonogashira [23] or Suzuki [24] coupling reactions, that could be carried out from the corresponding β-hydroxyphosphonate C5-iodo-nucleoside intermediate. Furthermore, this synthetic approach allows to introduce an aromatic group either directly in the C5 position or through a two-methylene spacer that can be rigid (triple bond) or somehow flexible (double bond). Thus, treatment of fully protected β-hydroxyphosphonate analogues **41** [17] with ICl in dichloromethane afforded the corresponding protected 5-iodouracil derivative **43** [25], whereas I_2/HIO_3 in acetic acid and tetrachloromethane [26] was used for cytosine derivative **42** and gave rise to the intermediate **44** (Scheme 4). Removal of the sugar protecting groups under basic conditions afforded compounds **45** and **46** in 84% and 97% yields, respectively. Under Sonogashira conditions [23,27], reaction of the latter ones with *n*-butyl, *tert*-butyl- or phenyl-acetylene, in the presence of Et_3N, CuI and $Pd(PPh_3)_2$, produced derivatives **47a–c** and **48a–c**, respectively.

Following removal of the phosphonate protecting groups with TMSBr, the corresponding 5-(*n*-butyl, or *tert*-butyl, or phenyl)-ethynyl-nucleoside phosphonic acids **23a–c** and **24a–c** were obtained, as their sodium salts, in yields ranging from 23% to 95%.

After the successful introduction of alkynyl groups at the C5 position, we extended the study to other hydrophobic phenyl and (E)-phenylethenyl derivatives. Synthesis of the corresponding compounds was accomplished using a Suzuki coupling reaction from intermediates **45 and 46** [24]. Thus, treatment of these compounds with phenyl and (E)-1-(2-phenyl)ethylene boronic acids in presence of TPPS and $PdCl_4Na_2$ led to the formation of the required C5-substituted derivatives **49d,e** and **50d,e** in yields on a range of 25–59%. Final step of deprotection of phosphonic acid



Scheme 3. Synthesis of β-D-gluco- and β-D-mannofuranoside derivatives, **9–12** and **13–16**. *Reagents & Conditions* : a) Uracil or *N*-4-benzoylcytosine, *N,O*-bis(trimethylsilyl)acetamide, CH_3CN then $SnCl_4$; b) NH_4CO_2H, Pd/C, MeOH from **37** to 1) BCl_3, CH_2Cl_2 at −78 °C, 2) NH_3sat, MeOH from **38**; c) TMSBr, DMF then Dowex exchange (Na^+); d) NH_2NH_2aq, AcOH, pyridine; e) 1) Dess Martin reagent, CH_2Cl_2, 2) $NaBH_4$, CH_2Cl_2; f) BCl_3, CH_2Cl_2 at −78 °C; g) 1) *N*-Methyl pyrrolidine, TMSCl, $(CF_3CO)_2O$, CH_3CN then 4-nitrophenol 2) NH_4OH, dioxan.



Scheme 4. Synthesis of C5-substituted derivatives **23a**–**e** and **24a**–**e**. *Reagents & Conditions* : a) Icl, CH_2Cl_2 from **41**; I_2, HIO_3, AcOH, CCl_4 from **42**; b) NH_3/MeOH; c) Alkynes, $Pd(PPh_3)_4$, CuI, NEt_3, DMF; d) TMSBr, DMF then Dowex exchange (Na^+ form); e) TPPS, $PdNa_2Cl_4$, KOH, boronic acids, H_2O/DMF.

functionality was performed as previously and targeted derivatives **23d,e**, **24d,e** were isolated as their sodium salts.

2.2. Nucleotidase activity inhibition assays

The potential inhibitory effect of the β-hydroxyphosphonate derivatives was evaluated using an enzyme activity assay with the recombinant purified cN-II and IMP as substrate (Tables 1 and 2). Best inhibitors, for which inhibition parameters are shown in bold characters in Tables 1 and 2, were selected using the following thresholds: inhibition at 1 mM > 50% or K_i < 2.5 mM. Fittings and double reciprocal representations are shown in Fig. S1. As observed in our previous studies [15], the inhibition was very weak (<20%, Supporting Information) for derivatives bearing phosphonoester groups such as compounds **1**, **3**, **5**, **7**, **9**, **19** and **21**. This result supported that interactions between the two oxygens of the phosphonate functionality and the magnesium ion present in the active site are required for the enzyme inhibition.

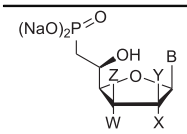
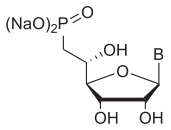
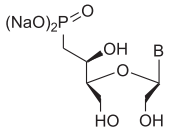
In general, the cytosine-based analogues were more efficient cN-II inhibitors than their uracil-based counterparts. The parent compounds **2** and **4** exhibited more than 70% of inhibition of the nucleotidase activity at 1 mM. Their kinetics evaluation corroborated the inhibition observed with the rapid screening assay with K_i values of 2.08 and 1.74 mM, respectively.

When comparing compounds within the uracil series (Table 1), all derivatives **6** (altrofuranose), **10** (glucofuranose) and **14** (mannofuranose) showed a much lower activity than compound **2** (allofuranose). A similar tendency of the effect of sugar modifications was also observed in the cytosine series. The modification of the C3′-stereocenter (either to glucofuranose or to mannofuranose) seemed to be less advantageous as the inhibition of compounds **12** and **16** was weaker (48% and 35%, respectively) than the corresponding allofuranose derivative **4** (85% at 1 mM). Interestingly, the activity of compound **8** (altrofuranose) was slightly better than compound **4,** with K_i values of 0.98 and 1.74 mM respectively, despite the inversion of the C2′-configuration.

Surprisingly, the inversion of the C5′-configuration (compounds **17** and **18**) was also detrimental for the protein interaction as well as the scission of the C2′–C3′ bond (compounds **19**–**22**), and all those compounds showed a percentage of inhibition of the nucleotidase activity below 45%.

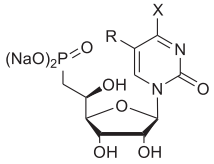
We then evaluated the effect of substituents at the C5 position (Table 2). For several compounds, including phenyl-ethynyl or ethenyl groups, the inhibition could not be measured using the rapid assay as the green malachite reagent interfered with this type of compounds. Consequently, kinetic studies were performed allowing the determination of the inhibition constants for most of the compounds and a few of them exhibited an inhibition below 45% (**23a, 23e** and **24a,b**). Among this series of derivatives, marked differences were observed between uracil- and cytosine-based analogues. Within the uracil-based series, the presence of hindered ethynyl groups (*i.e. tert*-butyl and phenyl) seemed to improve the affinity of the related derivatives for cN-II as compared to the reference compound **2**. Indeed, derivatives **23b**, **23c** and **23e** were able to inhibit the nucleotidase activity with K_i ranging from 2.18 to 6 mM. In contrast, for cytosine-based compounds, almost all modifications were detrimental when including an ethynyl spacer (compounds **24a**–**c**). Whereas, derivative **24d** (where the phenyl group is directly attached to the C5 position) exhibited a K_i of 2 mM similar to the reference compound **4**. Only, compound **24e** (with a phenyl-ethenyl group) exhibited a lower K_i value (1.14 mM) than the other compounds. It should be noted that this constitutes an

Table 1
Evaluation of the β-hydroxyphosphonate derivatives incorporating sugar-modification as cN-II inhibitors.

Generic structure	Compound #	Inhibition at 1 mM (%)	K_i (mM)
			
Z=Y=H, X=W=OH, B=U	**2**	**72 ± 6**	**2.08 ± 0.63**
B=C	**4**	**85 ± 5**	**1.74 ± 1.06**
X=Z=H, W=Y=OH, B=U	**6**	25 ± 1	>100
B=C	**8**	**80 ± 10**	**0.98 ± 0.24**
Y=W=H, Z=X=OH, B=U	**10**	25 ± 5	n.d.
B=C	**12**	48 ± 5	8.90 ± 3.34
X=W=H, Z=Y=OH, B=U	**14**	25 ± 5	>50
X=W=H, Z=Y=OH, B=C	**16**	35 ± 2	11.1 ± 10.9
			
B=U	**17**	43 ± 6	n.d.
B=C	**18**	42 ± 8	n.d.
			
B=U	**20**	20 ± 4	n.d.
B=C	**22**	45 ± 5	4.06 ± 1.32

Bold value highlights the compounds of interest.

Table 2
Evaluation of the β-hydroxyphosphonate derivatives incorporating nucleobase-modifications as cN-II inhibitors.

Generic structure	Compound #	clog*P*[a]	Inhibition at 1 mM (%)	K_i (mM)
				
X=OH, R=H	**2**	**−3.36**	**72 ± 6**	**2.08 ± 0.63**
R=C≡C-nBu	**23a**	−0.97	45 ± 3	n.d.
R=C≡C-tBu	**23b**	−1.21	n.d.[b]	5.94 ± 3.64
R=C≡C-Ph	**23c**	−1.68	n.d.[b]	**2.18 ± 0.51**
R=Ph	**23d**	−1.31	24 ± 7	n.d.
R=C=C-Ph	**23e**	−0.73	n.d.[b]	3.36 ± 1.74
X=NH$_2$, R=H	**4**	**−3.20**	**85 ± 5**	**1.74 ± 1.06**
R=C≡C-nBu	**24a**	−0.81	40 ± 10	n.d.
R=C≡C-tBu	**24b**	−1.05	46 ± 20	7.33 ± 2.28
R=C≡C-Ph	**24c**	−1.52	n.d.[b]	8.85 ± 5.50
R=Ph	**24d**	**−1.16**	**60 ± 10**	**2.01 ± 0.54**
R=C=C-Ph	**24e**	−0.57	n.d.[b]	**1.14 ± 0.42**

Bold value highlights the compounds of interest.
[a] clog*P* values were calculated using the Mol Inspiration software program.
[b] Precipitate in presence of the chemical reagent used (green malachite).

improvement in terms of inhibition as this derivative was found to be more active than its parent compound **4** and as efficient as derivative **8** (altrofuranose series).

2.3. Molecular modeling studies

Molecular docking of the studied derivatives was carried out to define the essential structural elements leading to enzyme inhibition and compared to the ones already reported for parent compounds **2** and **4** [15]. Briefly, key residues of cN-II are involved in the binding of the β-hydroxyphosphonate nucleoside analogues such as: (i) Phe157, His209 and Tyr210 acting as tweezers around the nucleobase, (ii) Glu161, Arg202 and Asn158 binding either the oxo or the amino group of the pyrimidine bases, (iii) Asp54 and Asn250 bridging the β-hydroxyl group and finally the negatively charged oxygens of the phosphonate group interacting with the magnesium ion present in the active site.

When comparing the docking poses of compounds within the uracil series (Fig. 3A), we clearly observed that for compounds **6** and **10** the β-hydroxyl group is oriented in the opposite direction than for **2** and **14**, thus weakening the hydrogen bonding with Asp54 and Asn250. For compounds **2** and **14**, the β-hydroxyl group is located at the same place but the distance between the C4-oxygen of the nucleobase and Arg202 increased (*i.e.* 3.18 Å *vs.* 4.43 Å, Fig. 3B) and a stronger interaction is favored for compound **2**. These two observations corroborated the decreased efficiency of all sugar-modified uracil derivatives.

Concerning the cytosine-based derivatives (Fig. 3C and D), a specific interaction can be observed between Ser251 and the 3′-hydroxyl group of the sugar moiety, especially for compound **8**. Similarly, an electrostatic interaction between Lys215 and the 2′-hydroxyl group may highlight the potential role of Lys215 in stabilizing the cis orientated 2′,3′-diol of parent compound **4** (Fig. 3C). Few structural features may explain the observed difference in terms of efficiency between compounds **4** and **8**. All interactions were found to be stronger for derivatives **8** compared to **4** according to the distances measured between the 2′- and the 3′-hydroxyl groups and either Ser251 or Lys215, in addition these interactions are strengthened by a better stacking with His209 for the analogue **8** (Fig. 3C). We hypothesized that the inversion of the C2′-configuration led to a change in the sugar puckering with concomitant rotation of the nucleobase around the glycosidic bound, thus moving this closer to His209. When comparing compounds **8** and **16**, both compounds were found to superimpose except the 3′-hydroxyl group, owing to the inversion of the C3′-configuration (Fig. 3D). This observation highlighted the importance of the stabilization of the sugar counterpart through the interaction of the 3′-hydroxyl group with Ser251. Finally, due to the increased flexibility of the acyclic derivative **22**, the nucleobase (cytosine) is oriented in a very different way than for the corresponding parent analogue **4** (Fig. 4A) weakening all interactions, except the hydrogen bond between the 2′-hydroxyl and Lys215 that is preserved for both compounds.

Then, we examined the effect of the nucleobase modifications introduced in order to fill in the protein cavity observed in the vicinity of the substrate. Compound **23a** (including an *n*-butyl group) is accommodated by the protein without any steric hindrance, but the end of the cavity being more polar (presence of Thr155 and Asn158 residues) it probably resulted in less favored contacts (Fig. 4B and C). In contrast, the compounds **23b** and **23c** (including a shorter tail as a *tert*-butyl and phenyl groups, respectively) interacted only with the hydrophobic part of the cavity (Fig. 4C and D). The length of the "tail" anchored to the C5-position of the nucleobase must be fine-tuned in order to fill perfectly the hydrophobic part of the cavity without extending too much, and avoiding to

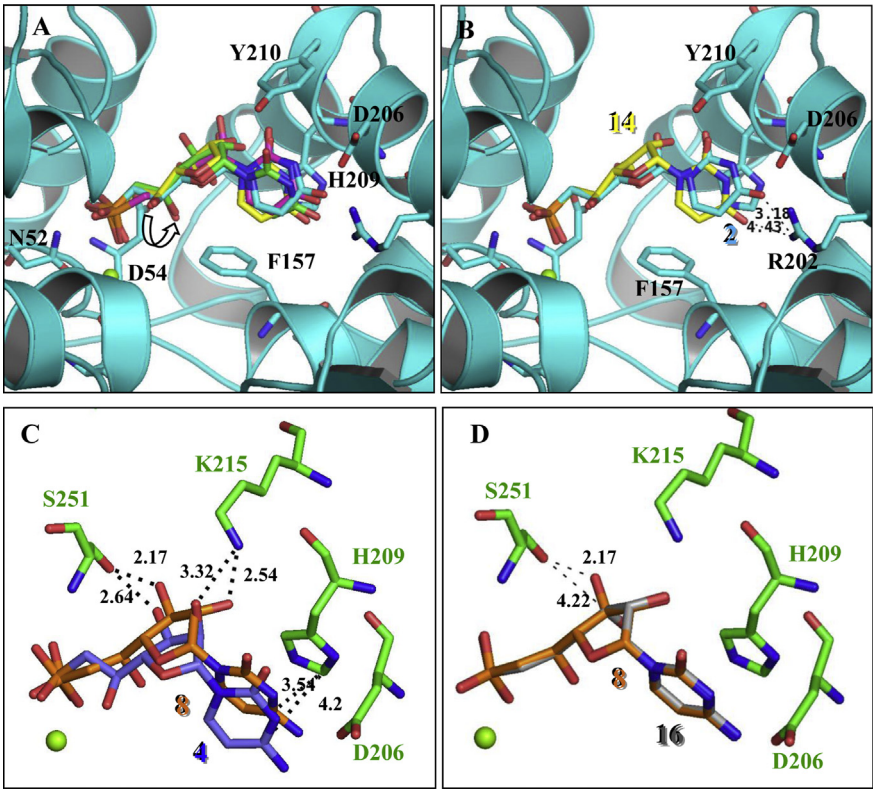


Fig. 3. (A) Docking poses obtained by docking uracil-based compounds onto cN-II enzyme; namely compounds **2** (cyan), **6** (green), **10** (pink) and **14** (yellow), the black arrow highlights the rocking motion of the β-hydroxyl group. (B) Difference in the binding modes of compounds **2** (cyan) and **14** (yellow) and resulting interactions with R202 (weaker interaction with **14**). (C) Predicted binding for cytosine-based compounds **4** (blue) and **8** (orange) showing stronger interactions of compound **8** with protein residues S251 and K215. (D) Comparison of the interactions of with S251 for analogues **8** (orange) and **16** (gray). Secondary structure of cN-II is shown as cartoon representation and phosphonate analogues in sticks. The active site Mg^{2+} ion is depicted as a green sphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reach the polar environment present further down the cavity. In this respect, interactions of compounds **23c**–**d** were carefully studied, as various positions of the aromatic substituent were envisaged by introducing the phenyl group directly on the C5-position and through a double or a triple bond. Interestingly, a nice superimposition of the aromatic ring for compounds **23c** and **23e** was observed (Fig. 4D), fitting the overall cavity and corroborating the improved efficacy of such derivatives compared to other uracil-based compounds.

As cytosine-based compounds were generally found to be more efficient in terms of enzyme inhibition than the uracil ones, the C5-substitution of the nucleobase was performed with the same groups and the resulting compounds **24a**–**e** were also analyzed by docking. The compounds **24a** (40% of inhibition) and **24c** (K_i value of 8.85 mM) modestly inhibited cN-II activity and seemed to behave like **23e**, showing the extension of the tail close to the polar end of the cavity (Fig. 5A). In contrast, the derivative **24b** exhibited a K_i value of 7.33 mM, despite occupying only the apolar part of the binding pocket as observed for **23b** (K_i value of 5.94 mM). This feature was not found with a phenyl-ethenyl group and binding modes of the uracil-based compound **23e** compared to the cytosine analogue **24e** were clearly different, confirming that the nucleobase location is also driven by the amino group of cytosine.

Interestingly, within the cytosine-based series the smallest base modification (compound **24d** including a phenyl group directly attached to the C5-position) appears to be advantageous for a tighter binding (Fig. 5B). Indeed, the phenyl group was found to fit the small hydrophobic cavity without overlapping to the polar side. This last result corroborates the *in vitro* inhibition observed with this compound (K_i about 2 mM).

Remarkably, the maximum size of an "extended" binding group for the nucleobase was accomplished with compound **24e** (Fig. 5B) that is shown to be able to interact with another part of the binding pocket (without exiting). However, two alternatives binding poses were observed for this derivative either shifted to the left or the right side of the central groove connecting the active site with the effector site 1 (Fig. 6). Each binding mode was representative as a cluster of poses observed for both orientations and resulting docking scores were almost identical. Interestingly, the most important interactions with the protein were preserved (with Asp206 and Lys215). Since it was impossible to conclude about the most probable binding pose, a molecular dynamics simulation was carried out using as starting point the energy minimized docking poses. As shown in Fig. 6 (panels A–C) from snapshots of the simulation, one conformation (blue one) of **24e** exhibited higher mobility than the other one (red). The root-mean square deviation (RMSD) of all **24e** atoms confirmed this result as shown in Fig. 6D. Indeed, the RMSD for one conformer (blue) increased continuously with temperature without coming back to its basal level while it was smaller (less than 1 Å) for the other conformer (red). In addition, the high mobility of the blue conformer was also observed by monitoring the distance between one residue of the protein (Pro 293) and the benzyl group of **24e**. According to these simulations, the derivative **24e** should bind preferentially in the right side of central cavity. This result is in agreement with other derivatives bearing a phenyl group and that localize in these groove. Additional simulations included five rounds of heating and cooling (simulated annealing) provided very similar RMSD curves for the most mobile conformer of this compound (Fig. S3). Because of the two different binding poses predicted for this particular compound, we checked

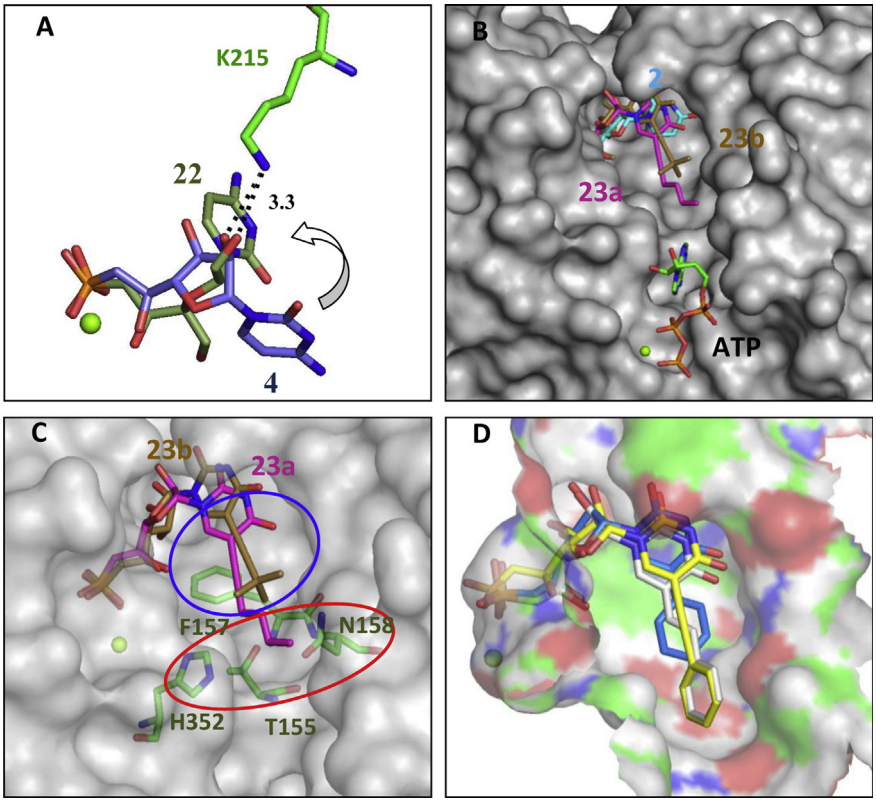


Fig. 4. (A) Comparison of binding poses obtained with compounds **4** (furanose ring) and **22** (acyclic). The arrow shows the flip motion of the nucleobase for compound **22**. (B) Binding mode obtained with nucleobase-modified derivatives **23a** and **23b**. The parent compound **2** (cyan) and ATP (bound to effector site 1) are shown in stick model. (C) Same derivatives as in (B) showing the hydrophobic pocket and including F157 (blue circle) and polar residues T155, N158 and H352 belonging to the same cavity (red circle). (D) Binding poses of derivatives **23c** (yellow), **23d** (blue) and **23e** (white) to compare the aromatic substitutions bearing a double or a triple bond. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

if the inhibition mode was purely competitive (Fig. S2). The two fitting models giving the lowest reduced Chi^2 value were competitive ($Chi^2 = 5.81$) and mixed type ($Chi^2 = 6.66$). However, the K_i' value determined in the mixed type model was unrealistic (540 ± 1430 mM). The competitive mode was therefore confirmed for **24e**.

The analysis of intermolecular interactions between cN-II residues and the derivatives was carried out by measuring all the hydrogen bonds and van der Waals contacts (Table 3). From these data, several key residues were always found like Asn 52, Asp54, Phe157, His209 and Tyr210. However, it remained complicated to extract a clear picture from this analysis like crucial residues as the size of compound is variable. Nevertheless, it seemed that (for this family of compounds) a few more interactions are favorable in respect to the activity (Asn250 and Lys 292). In contrast, two residues appeared to have an opposite effect (based on the appearance frequency in inactives derivatives), like Lys215 and Ser251.

3. Conclusion

We previously shown that derivative **4** can interfere with cN-II, the cytosine containing analogue being equipotent in terms of

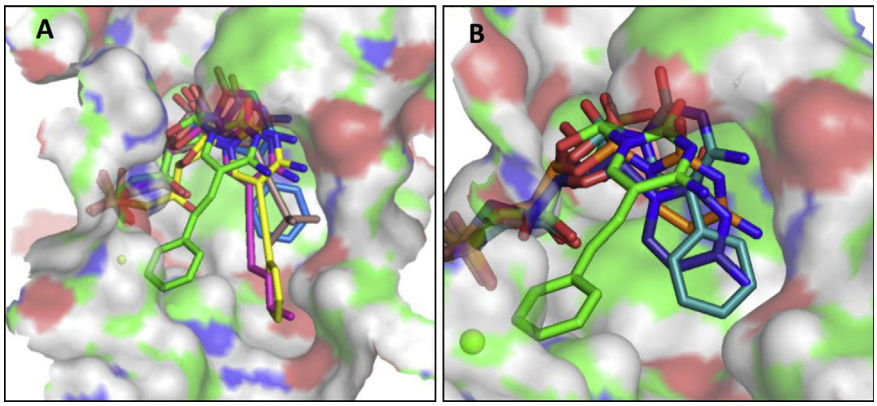


Fig. 5. (A) Overlay of binding modes obtained for the different cytosine-based modified compounds **24a** (pink), **24b** (brown), **24c** (yellow), **24d** (cyan) and **24e** (green). (B) Comparison of the best cytosine-based compounds **4** (dark blue), **8** (orange), **24d** (cyan) and **24e** (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

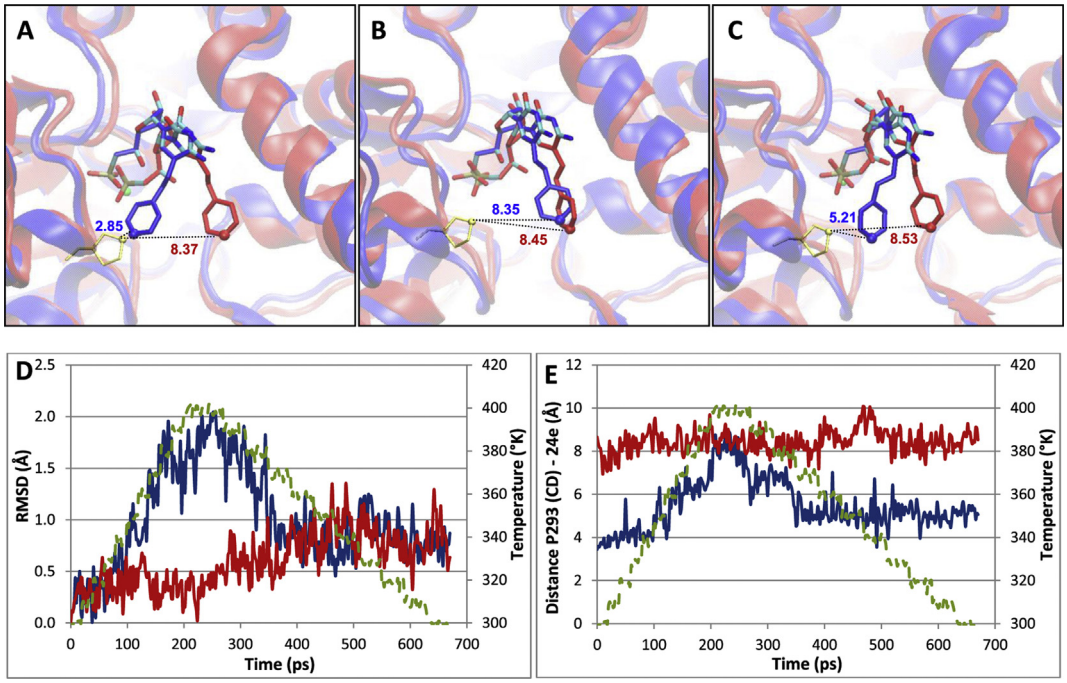


Fig. 6. Discerning between the two alternative binding modes observed for compound **24e**. Snapshots of the molecular dynamics simulations (simulated annealing) of the two docking poses (depicted in blue or red) are shown at different times, 0 ps (A), 250 ps (B) and 680 ps (C). Secondary structure elements of cN-II are colored according to the starting conformation of ligand (blue or red and depicted in stick model). Distances are indicated in angströms. (D) Root-mean square deviation of ligand atoms for each conformation (blue and red lines), temperature is indicated as a green dash line. (E) Motion of **24e** during simulation monitored by the distance between its terminal carbon atom and Pro 293 (as shown in panels A–C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

inhibition to the hypoxanthine and adenine counterparts [15]. Therefore, this derivative was used as starting point for an SAR study. Additional assays and present results obtained for compounds **1**–**22**, while modest, suggested that only minor modification of the sugar counterpart is accepted and confirmed the β-hydroxyphosphonate scaffold as a potential lead structure.

Modification of the nucleobase is tolerated but no real improvement in terms of inhibition efficiency could be observed with such compounds (K_i values ranging from 1.14 to 8.85 mM). Nevertheless, the size of the modification is clearly important to keep the binding inside the cavity without overlapping to the effector site. Compound **24d** (K_i value of 2.01 mM), including a single phenyl group as substituent and compound **24e** (phenylethenyl group, K_i value of 1.14 mM) were equally potent to derivative **4** (without any base modification) and found to be the most promising compounds for future drug developments. The reduction of the flexibility by introducing a double or triple bond between the nucleobase and the substituent was not really efficient except for compound **24e** for which the inhibition constant was around 1 mM with a constrained orientation near an aromatic area (privileged orientation according to the stronger interactions). In addition, the volume of the cavity is not extensible and may only accommodate small size substituent as several derivatives were shown to extend outside of the cavity. Even so the enzyme is able to undergo small domain motions during the nucleotidase reaction (molecular dynamics simulations, data not shown) and leading to little variations of the volume of the cavity, the size of substituent group is rather limited. Overall, the SAR data indicate that there are two exploitable regions near the nucleobase scaffold within the protein active site. The first is hydrophobic and the second is more polar including protein residues like Thr155 and Asn158. Therefore, the size and the nature of the residues forming the cavity are of interest and render possible to consider novel modifications of the nucleobase, considering as new osidic scaffold the best compound **8** for instance (K_i value of 0.98 mM).

In conclusion, we report an in-depth SAR study of β-hydroxyphosphonate nucleoside analogues as cN-II inhibitors. cN-II is also named high-K_m nucleotidase owing to the high substrate concentration required for activity (in the mM range), therefore the K_i values of about 1 mM obtained for derivatives **8** and **24e** are relevant in this particular context. We have already validated the concept of using cN-II inhibitors to sensitize cancer cells to cytotoxic nucleoside analogues with compounds identified through virtual screening (exhibiting a K_i of 1.25 mM), and being structurally different from substrate analogues [28]. In addition, the medical relevance for the development of such inhibitors has been confirmed by recent observations [12,13], thus comforting our hypothesis on the interest of cN-II inhibitors. As these molecules are too polar (clog *P* are shown in Table 2) and ionized at physiological pH to cross effectively biological membrane, their biological evaluation will need the development of corresponding prodrugs to temporarily masked phosphonate group [29], which constitutes the next step of this research project.

4. Experimental section

4.1. Molecular docking of the β-hydroxyphosphonates analogues

Molecular docking was carried out using Gold 5.1 program (Genetic Optimization for Ligand Docking, CCDC Software limited) installed on an IBM blade center. First of all, the different compounds were modeled using VegaZZ molecular modeling software [30] and the atomic charges were assigned using the Gasteiger–Marsili empirical atomic partial charges [31,32]. The potential energy for all compounds was minimized in two steps (500 of steepest descent followed by 5000 steps of conjugate gradient with a gradient tolerance of 0.01 kcal/mol Å). The crystal structure of human cN-II (2XCW) solved in presence of IMP [33] was used for docking and the other available structures with various ligands bound to the protein (pdb codes: 2XJC, 2XJB, 2XCV and 2XCW) were used for calibrating the docking procedure. During this step of

Table 3
Summary of the cN-II residues found to interact with the derivatives. The ionic interactions always presents between the phosphonate oxygens and magnesium ion are not included in the table for clarity. The cutoff values for hydrogen bonds and van der Waals contacts were 3.5 Å and 4.5 Å, respectively. Residues in green reflect the most encountered interactions with active compounds and in red the most unfavorable interactions found with inactive compounds.

Compound #	H-bonds	van der Waals contacts
2	**N52**, M53, **D54**, R202, **K215**, **T249**, **K292**	**D54**, **F157**, V205, D206, **H209**, **Y210**, **N250**
4	**N52**, **D54**, **K215**, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **N250**
6	**N52**, **D54**, Y210, **T249**, **N250**, S251, **K292**	**D54**, **F157**, V205, **H209**, **Y210**, **N250**, **S251**
8	**N52**, **D54**, **T249**, **N250**, **K292**	**D54**, **T155**, **F157**, **H209**, **N250**
10	**N52**, **D54**, R202, **T249**, **K292**	**D54**, **F157**, V205, **H209**, **Y210**, **K215**, **N250**
12	**N52**, **D54**, Y210, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, **N250**, **S251**
14	**N52**, **D54**, **K215**, **T249**, **N250**, **K292**	**D54**, **F157**, V205, **H209**, **Y210**, **N250**, **S251**
16	**N52**, **D54**, Y210, **K215**, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, **N250**, **S251**
17	**N52**, M53, **D54**, **K215**, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, T249, **N250**, **S251**
18	**N52**, **D54**, **K215**, **T249**, **N250**, S251, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, **N250**, **S251**
20	**N52**, **D54**, R202, **T249**, **N250**, **K292**	**D54**, **F157**, V205, **H209**, **Y210**, **K215**, **N250**
22	**N52**, **D54**, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, **N250**, **S251**, Y255
23a	**N52**, **D54**, **T249**, **N250**, S251, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, **H209**, **Y210**, **K215**, T249, **N250**, **S251**, H352
23b	**N52**, **D54**, **T249**, **N250**, S251, **K292**	**D54**, **T155**, **F157**, **N158**, **H209**, **Y210**, **K215**, **N250**
23c	**N52**, **D54**, R202, **K215**, **T249**, **N250**, S251, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, D206, **H209**, **Y210**, **N250**, H352
23d	**N52**, **D54**, **T249**, **N250**, S251, K292	**D54**, **T155**, **F157**, **N158**, V205, D206, **H209**, **Y210**, **N250**
23e	**N52**, R202, **K215**, **T249**, **N250**, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, **H209**, **Y210**, **N250**, H352
24a	**N52**, **D54**, D206, **K215**, **T249**, **N250**, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, **H209**, **Y210**, **N250**, S251, H352
24b	**N52**, **D54**, **T249**, **N250**, **K292**	**D54**, **F157**, **N158**, R202, V205, **H209**, **Y210**, **K215**, **N250**, S251
24c	**N52**, D206, **K215**, **T249**, **N250**, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, D206, **H209**, **Y210**, **N250**, H352
24d	**N52**, **D54**, **T249**, **N250**, **K292**	**D54**, **F157**, **N158**, V205, **H209**, **Y210**, **K215**, **N250**, S251
24e	**N52**, **D54**, **K215**, **T249**, **N250**, **K292**	**D54**, **F157**, **H209**, **Y210**, **K215**, **N250**, S251, K292, P293, H352, G355
24e	**N52**, **D54**, **K215**, **T249**, S251, **N250**, **K292**	**D54**, Y151, N154, **T155**, **F157**, **N158**, **H209**, **Y210**, **N250**, H352

structure selection and comparison, we noted that the Mg^{2+} ion was perfectly coordinated with aspartate residues, water molecules and oxygen from IMP or GMP (in regard to the previous structure 2JC9 for instance). Hydrogen atoms were added using Gold prior to flexible docking of the chemical compounds. Then, 50 genetic algorithms (GA) were performed with a radius of 15 Å around the magnesium ion used as target atom for docking. The water molecules present in the crystal structures were retained and allowed to spin around their oxygen atom. For each docked molecule, two independent docking runs (50 GA, each) were performed using Goldscore as scoring function. The different docking poses were analyzed by the clustering method (complete linkage) from the RMSD matrix of ranking solutions. The structural analysis and visualization of docking poses was carried out using the Pymol software [34]. The determination of the intermolecular interactions between cN-II and the derivatives was achieved using LigPlot+ [35] program with an upper cutoff value of 3.5 and 4.5 Å for hydrogen bonds and van der Waals contacts, respectively.

4.2. Molecular dynamics simulations of the derivative ***24e***

Simulations were carried out on an IBM cluster in explicit water (TIP3P model) using the NAMD 2.9 software package [36] with periodic boundary conditions. The two docking poses of derivative **24e** obtained by docking were immersed in a cubic box filled with water molecules with a distance between protein and box edges of at least 10 Å and the system was neutralized by adding Na^+ or Cl^- ions. Temperature (300 K) and pressure (1 atm) were kept constants during the simulation using Langevin dynamics and Nosé-Hoover Langevin piston [37,38]. A 12 Å cutoff was used for the van der Waals interactions with a switching function starting from 10 Å. The PME (Particle Mesh Ewald) algorithm [39] was used to calculate long-range electrostatics with a grid spacing of 1 Å. The potential energy of the entire system was minimized for 50,000 steps (time step of 1 fs) with the conjugate gradient method and the CHARMM all-atom parameter set 27 [40] followed by a gradual heating from 0 K to 300 K in 30 ps (increment of 10 K) under constant temperature and pressure. The system was further equilibrated for 100,000 steps before simulated annealing (from 300 K to 400 K) using a two steps "heating–cooling" procedure (increment or decrement of 10 K by reinitializing the atom velocities with the Langevin temperature and piston). The cooling step was achieved two times slower than the heating step with an equilibration of 50 ps between both and a time step of 2 fs in combination with the shake algorithm for water molecules. The simulation trajectories were analyzed using VMD [41].

4.3. Evaluation of the inhibition of the nucleotidase activity by β-hydroxyphosphonate analogues

The nucleotidase activity was determined in the presence or in the absence of β-hydroxyphosphonate analogues using the ΔN30

recombinant purified cN-II. Briefly, the pET24b plasmid encoding for cN-II with a 30 aminoacids deletion in N-terminal and a His tag in C-terminal was used to transform *E. coli* BL21 cells. After growth and induction, cN-II was purified using an ÄKTA-purifier fast protein liquid chromatography equipment (GE Healthcare) connected to an His-Trap FF 5 mL column followed by a HiLoad 16/60 Superdex 75 gel filtration column according to the procedure described previously [15]. Two different enzyme activity assays were used, either using the Green Malachite Phosphate Assay Kit (Gentaur) in order to quantify the inorganic phosphate release upon hydrolysis of IMP by cN-II, or the measurement of the kinetics rate constants of cN-II at different IMP concentrations. For the first assay, cN-II was added to a final concentration of 0.1 μM in 80 μL of buffer containing 50 mM imidazole pH 6.5, 500 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT on ice and the reaction was started by addition of the substrate (50 μM of IMP with an equimolar concentration of Mg^{2+}) and incubated at 37 °C for 5 min. The reaction was stopped by adding 20 μL of Green Malachite Reagent and free phosphate was quantified by measuring the absorbance above 630 nm using a Tecan plate reader (Sunrise) and comparison with a 0–50 μM phosphate calibration curve. A second kinetics assay was required for confirming the inhibition observed with the Green Malachite Phosphate assay kit and also to evaluate the inhibition of compounds that react with the Green colorant. Therefore, the enzyme (0.1 μM) and substrate (IMP, varying from 200 μM to 3 mM) freshly prepared in the same buffer as above were mixed in a thermostatically controlled beaker with magnetic stirring. The reaction was stopped at different times by mixing with 10% perchloric acid. Quantification of inosine and IMP present in the reaction mixtures quenched at different times was performed by HPLC (Waters) using a Partisphere 5-SAX column (Whatman) and 15 mM ammonium phosphate buffer pH 5.5 as mobile phase. The raw data were fitted with an equation for competitive inhibition using GraFit 7 (Erithacus software) in order to determine the K_i values.

4.4. Chemical synthesis

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use, DMF and methanol from CaH_2, dichloromethane from P_2O_5, pyridine and Et_3N from KOH. Solids were dried over P_2O_5 under reduced pressure at rt. Moisture sensitive reactions were performed under argon atmosphere using oven-dried glassware. ^1H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz with proton decoupling at 25 °C using a Bruker 300 Advance. Chemical shifts are given in δ values referenced to the residual solvent peak ($CHCl_3$ at 7.26 and 77 ppm or DMSO-d_5 at 2.49 and 39.5 ppm) relative to TMS. COSY experiments were performed in order to confirm proton assignments. Coupling constants, *J*, are reported in Hertz (Hz). 2D ^1H–^3C heteronuclear COSY spectra were recorded for the attribution of ^{13}C signals. ^{31}P NMR spectra were recorded at ambient temperature at 121 MHz with proton decoupling. Chemical shifts are reported relative to external H_3PO_4. FAB mass spectra were recorded in the positive-ion or negative-ion mode on a JEOL JMS DX 300 using thioglycerol/glycerol (1:1, v/v, G-T) or NBA (nitrobenzylalcohol) as matrix. ESI Mass and High Resolution Mass spectra were recorded in the positive or negative-ion mode on a Micromass Q-TOF. UV spectra were recorded on an Uvikon 931 (Kontron). Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). TLC was performed on pre-coated aluminum sheets of silica gel 60 F_{254} (Merck, Art. 9385), visualization of products being accomplished by UV absorbance followed by charring with 5% ethanolic sulfuric acid and then heating. The purity of all tested compounds was determined by elemental analysis or for few compounds by HR-MS and HPLC analysis (at 254 nm, purity proved to be ≥ 95%).

Starting nucleotides **1**, **41** and **42** were obtained according previously published procedure [17].

4.4.1. General procedure for diethylphosphonate removal (compounds ***6***, ***8***, ***10***, ***12***, ***14***, ***16***, ***20***, ***22***, ***23a–e***, ***24a–e***)

The protected phosphonate (1 eq.) was dissolved in anhydrous DMF (20 mL/mmol) and trimethylsilyl bromide (10–15 eq.) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature until completion of the reaction was indicated by TLC (7% Isopropanol: 2% water: 1% ammonia). Then, the reaction was stopped by adding triethylammonium bicarbonate (TEAB 1M, pH 7) and concentrated to dryness under high vacuum. Column chromatography of the crude materials on reverse phase (gradient: water to acetonitrile 50%) gave the expected phosphonic acid (as triethylammoium salt), which was passed through a Dowex Na^+ ion exchange column, the desired fractions were collected and lyophilized leading to the title compound as sodium salt.

*4.4.1.1. 1-[6-Deoxy-6-phosphono-β-D-altrofuranosyl]uracil (diacid salt) (**6**).* Treatment of compound **5** (350 mg, 0.89 mmol) following general procedure gave rise to derivative **6** contaminated by triethylammonium salts. HPLC purification using a Hypercarb analytical column only gave rise to a small quantity of the expected product (9 mg, 26%). ^1H NMR (400 MHz, D_2O) $\delta = 7.75$ (d, $J = 8.13$ Hz, 1H, H-6), 6.05 (d, $J = 4.68$ Hz, 1H, H-1′), 5.75 (d, $J = 8.11$ Hz, 1H, H-5), 4.25–4.15 (m, 2H, H-2′, H-3′), 4.15–4.05 (m, 1H, H-5′), 3.7 (t, $J = 4.55$ Hz, 1H, H-4′), 1.9–1.6 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 166.3$ (C-4), 15.4 (C-2), 143.3 (C-6), 101.0 (C-5), 85.8–85.6 (C-4′, d, $J = 8$ Hz), 85.1 (C-1′), 75.8 (C-2′), 74.6 (C-3′), 67.0 (C-5′), 32.2–30.9 (C-6′, d, $J = 130$ Hz). ^{31}P NMR (121 MHz, D_2O) $\delta = 20.6$. MS FAB > 0 (NBA) *m/z* 339 $(M\text{-}2Na + 3H)^+$. FAB<0 (NBA) *m/z* 383 $(M-H)^-$, 359 $(M - Na)^-$, 337 $(M - 2Na-H)-$ UV $\lambda_{max} = 263$ nm ($\varepsilon_{max} = 9500$) (EtOH 95). Calculated for $C_{10}H_{13}N_2Na_2O_9P$, $2H_2O$: C, 28.72; H, 4.4; N, 6.7. Found: C, 28.65; H, 4.80; N, 6.64.

*4.4.1.2. 1-[6-Deoxy-6-phosphono-β-D-altrofuranosyl]cytosine (disodium salt) (**8**).* Treatment of compound **7** (174 mg, 0.442 mmol) following general procedure gave rise to the expected derivative **8** (90 mg, 53%). R*f* (iPrOH/H_2O/NH_4OH, 7/2/1, v/v) 0.09. ^1H NMR (400 MHz, D_2O) $\delta = 7.75$ (d, $J = 7.56$ Hz, 1H, H-6), 6.10 (d, $J = 4.32$ Hz, 1H, H-1′), 5.95 (d, $J = 7.55$ Hz, 1H, H-5), 4.3–4.2 (m, 2H, H-2′, H-3′), 4.2–4.1 (m, 1H, H-5′), 3.85 (t, $J = 4.54$ Hz, 1H, H-4′), 1.85–1.2 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 168.1$ (C-4), 157.2 (C-2), 143.0 (C-6), 95.2 (C-5), 86.0–85.9 (C-4′, d, $J = 9$ Hz), 85.9 (C-1′), 75.6 (C-2′), 75.2 (C-3′), 67.0 (C-5′, d, $J = 3$ Hz), 32.3–31.0 (C-6′, d, $J = 131$ Hz). ^{31}P NMR (81 MHz, D_2O) $\delta = 21.9$ MS FAB > 0 (GT) *m/z* 382 $(M + 3H)^+$, 338 $(M - 2Na + 3H)^+$. FAB < 0 (GT) *m/z* 358 $(M - Na)^-$, 336 $(M - 2Na)^-$. UV $\lambda_{max} = 273$ nm ($\varepsilon_{max} = 14{,}100$) (EtOH 95). Calculated for $C_{10}H_{14}N_3O_8PNa_2$, $1.5H_2O$: C, 29.42; H, 4.60; N, 10.29. Found: C, 29.51; H, 4.90; N, 9.93.

*4.4.1.3. 1-[6-Deoxy-6-phosphono-β-D-glucofuranosyl]uracil (disodium salt) (**10**).* Treatment of compound **9** (290 mg, 0.736 mmol) following general procedure gave rise to the expected derivative (200 mg, 71%). R*f* (iPrOH/H_2O/NH_4OH, 7/2/1, v/v) 0.11. ^1H NMR (400 MHz, D_2O) $\delta = 7.87$ (d, $J = 8.16$ Hz, 1H, H-6), 5.75 (d, $J = 8.13$ Hz, 1H, H-5), 5.7 (d, $J = 0.74$ Hz, 1H, H-1′), 4.35–4.25 (m, 1H, H-5′), 4.2 (dd, $J = 1.03$–3.06 Hz, 1H, H-3′), 4.15 (s, 1H, H-2′), 4.1 (dd, $J = 3.04$–7.72 Hz, 1H, H-4′), 2.1–1.7 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 166.5$ (C-4), 151.5 (C-2), 142.4 (C-6), 100.9 (C-5), 91.7 (C-1′), 85.9–85.8 (C-4′, d, $J = 14$ Hz), 80.4 (C-2′), 74.1 (C-3′), 65.3–65.2 (C-5′, d, $J = 4$ Hz), 33.4–32.1 (C-6′, d, $J = 131$ Hz). ^{31}P NMR (81 MHz,

D_2O) δ = 20.9. MS FAB > 0 (GT) *m/z* 383 $(M + 3H)^+$, 361 $(M - 2Na + 2H)^+$. FAB < 0 (GT) *m/z* 359 $(M - Na)^-$, 337 $(M - 2Na + H)^-$. UV λ_{max} = 263 nm (ε_{max} = 10,200) (EtOH 95). Analysis Calculated for $C_{10}H_{13}N_2O_9PNa_2$, $3.5H_2O$: C, 26.98; H, 4.53; N, 6.29. Found: C, 26.91; H, 4.10; N, 5.91.

*4.4.1.4. 1-[6-Deoxy-6-phosphono-β-D-glucofuranosyl]cytosine (monosodium salt) (**12**).* Treatment of compound **11** (350 mg, 0.89 mmol) following general procedure gave rise to the expected derivative (90 mg, 27%). R*f* (iPrOH/H_2O/NH_4OH, 7/2/1, v/v) 0.16. ^{1}H NMR (400 MHz, D_2O) δ = 7.60 (d, *J* = 8.1 Hz, 1H, H-6), 5.68 (d, *J* = 8.0 Hz, 1H, H-5), 5.42 (s, 1H, H-1′), 4.0–3.8 (m, 4H, H-5′, H-3′, H-2′, H-4′), 2.0–1.5 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 165.6 (C-4), 156.5 (C-2), 142.4 (C-6), 94.9 (C-5), 92.5 (C-1′), 86.0–85.8 (C-4′, d, *J* = 14 Hz), 80.5 (C-2′), 74.1 (C-3′), 65.2–65.1 (C-5′, d, *J* = 4.7 Hz), 33.7–31.9 (C-6′, d, *J* = 132 Hz). ^{31}P NMR (81 MHz, D_2O) δ = 21.7. MS FAB > 0 (GT) *m/z* 360 $(M + H)^+$, 338 $(M - Na + H)^+$. FAB<0 (GT) *m/z* 336 $(M - Na)^-$. HR-MS Calculated for $C_{10}H_{17}N_3O_8P$: 338.0753; Found: 338.0759. UV λ_{max} = 2671 nm (ε_{max} = 10,700) (H_2O). Analysis Calculated for $C_{10}H_{15}N_3O_8PNa$: C, 26.98; H, 4.53; N, 6.29. Found: C, 19.16; H, 2.95; N, 6.62.

*4.4.1.5. 1-[6-Deoxy-6-phosphono-β-D-mannofuranosyl]uracil (disodium salt) (**14**).* Treatment of compound **13** (200 mg, 0.507 mmol) following general procedure gave rise to the expected derivative (92 mg, 51%). R*f* (iPrOH/H_2O/NH_4OH, 7/2/1, v/v) 0.12. ^{1}H NMR (400 MHz, D_2O) δ = 7.96 (d, *J* = 8.4 Hz, 1H, H-6), 6.13 (d, *J* = 6.9 Hz, 1H, H-1′), 5.80 (d, *J* = 8.1 Hz, 1H, H-5), 4.65–4.58 (m, 1H, H-2′), 4.38–4.31 (m, 2H, H-3′, H-5′), 3.90–3.82 (m, H-4′), 2.1–1.7 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 166.4 (C-4), 151.9 (C-2), 143.8 (C-6), 100.8 (C-5), 84.4 (C-1′), 82.8–82.6 (C-4′, d, *J* = 14 Hz), 71.7 (C-2′), 69.0 (C-3′), 65.8–65.7 (C-5′, d, *J* = 4 Hz), 33.2–31.5 (C-6′, d, *J* = 130 Hz). ^{31}P NMR (81 MHz, D_2O) δ = 21.2. MS FAB > 0 (GT) *m/z* 361 $(M - Na + 2H)^+$, 339 $(M - 2Na + 3H)^+$. FAB < 0 (GT) *m/z* 359 $(M - Na)^-$, 337 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{10}H_{16}N_2O_9P$: 339.0593; Found: 339.0586. UV λ_{max} = 263 nm (ε_{max} = 10,600) (H_2O). Analysis Calculated for $C_{10}H_{13}N_2O_9PNa_2$, $3.5H_2O$: C, 26.98; H, 4.53; N, 6.29. Found: C, 19.75; H, 3.10; N, 4.35; P, 4.92.

*4.4.1.6. 1-[6-Deoxy-6-phosphono-β-D-mannofuranosyl]cytosine (disodium salt) (**16**).* Treatment of crude compound **15** (46 mg, 0.09 mmol) following general procedure gave rise to the expected derivative (14 mg, 37%). R*f* (iPrOH/H_2O/NH_4OH, 7/2/1, v/v/v) 0.12. ^{1}H NMR (400 MHz, D_2O) δ = 8.01 (d, *J* = 7.57 Hz, 1H, H-6), 6.22 (d, *J* = 6.74 Hz, 1H, H-1′), 6.06 (d, *J* = 7.55 Hz, 1H, H-5), 4.67 (m, 1H, H-2′), 4.44 (m, 2H, H-3′, H-5′), 3.92 (m, H-4′), 2.01–1.71 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 166.4 (C-4), 158.1 (C-2), 143.7 (C-6), 95.3 (C-5), 85.0 (C-1′), 83.0 (C-4′, d, *J* = 14 Hz), 71.9 (C-2′), 69.6 (C-3′), 66.4 (C-5′, d, *J* = 4 Hz), 33.3 (C-6′, d, *J* = 130 Hz). ^{31}P NMR (81 MHz, D_2O) δ = 19.4. MS ESI-QT of >0 *m/z* 338.2 $(M - 2Na + 3H)^+$, 360.0 $(M - Na + 2H)^+$. MS ESI-QT of <0 *m/z* 336.3 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{10}H_{16}N_3O_8PNa$: 360.0573; Found: 360.0559. UV λ_{max} = 270 nm (ε_{max} = 7900) (H_2O).

*4.4.1.7. 1-(5S)-[6-Deoxy-6-phosphono-2,3-seco-β-D-ribohexofuranosyl]uracil (disodium salt) (**20**).* Treatment of compound **19** (300 mg, 0.75 mmol) following general procedure gave rise to the derivative **20** as hygroscopic lyophilisate (138 mg, 54%). R*f* (EtOAc/MeOH, 5/5, v/v) 0.12. ^{1}H NMR (400 MHz, D_2O) δ = 7.80 (d, *J* = 8.05 Hz, 1H, H-6), 5.95 (t, *J* = 5.63 Hz, 1H, H-1′), 5.87 (d, *J* = 8.06 Hz, 1H, H-5), 3.95 (m, 1H, H-5′), 3.85–3.65 (m, 4H, H-3′, H-3″, H-2′, H-2″), 3.55 (m, 1H, H-4′), 1.8–1.5 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 166.3 (C-4), 152.3 (C-2), 142.4 (C-6), 102.4 (C-5), 83.9 (C-1′), 82.5–82.3 (C-4′, d, *J* = 13 Hz), 67.2 (C-5′, d, *J* = 4 Hz), 61.7, 59.6 (C-2′ and C-3′), 31.5, 30.2 (C-6′, d, *J* = 130 Hz). ^{31}P NMR (121 MHz, D_2O) δ = 21.7. MS FAB > 0 (NBA) *m/z* 341 $(M - 2Na + H)^+$. FAB < 0 (NBA) *m/z* 339 $(M - 2Na + H)^-$. UV λ_{max} = 262 nm (ε_{max} = 10,100) (EtOH 95). Calculated for $C_{10}H_{15}N_2O_9PNa_2$, $0.7H_2O$: C, 30.41; H, 4.43; N, 7.09. Found: C, 30.25; H, 4.79; N, 6.87.

*4.4.1.8. 1-(5S)-[6-deoxy-6-phosphono-2,3-seco-β-D-ribohexofuranosyl]cytosine (disodium salt) (**22**).* Treatment of compound **21** (520 mg, 1.31 mmol) following general procedure gave rise to the derivative **22** as hygroscopic lyophilisate (120 mg, 24%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.13. ^{1}H NMR (400 MHz, D_2O) δ = 7.49 (d, *J* = 7.5 Hz, 1H, H-6), 5.80 (d, *J* = 7.5 Hz, 1H, H-5), 5.70 (t, *J* = 5.37 Hz, 1H, H-1′), 3.58 (m, 1H, H-5′), 3.50 (m, 4H, H-3′, H-3″, H-2′, H-2″), 3.20 (m, 1H, H-4′), 1.2–1.6 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 165.8 (C-4), 157.8 (C-2), 142.4 (C-6), 96.4 (C-5), 84.1 (C-1′), 82.2–82.0 (C-4′, d, *J* = 14 Hz), 67.0 (C-5′, d, *J* = 7 Hz), 62.0, 59.4 (C-2′ and C-3′), 31.7, 30.4 (C-6′, d, *J* = 140 Hz). ^{31}P NMR (121 MHz, D_2O) δ = 20.5. MS FAB > 0 (NBA) *m/z* 679 $(2M - 2Na + H)^+$, 340 $(M - 2Na + H)^+$. FAB<0 (NBA) *m/z* 339 $(M - 2Na + H)^-$. UV λ_{max} = 272 nm (ε_{max} = 9500) (EtOH 95) Calculated for $C_{14}H_{26}N_3O_8P$, $0.55H_2O$: C, 41.49; H, 6.74; N, 10.37. Found: C, 41.49; H, 6.71; N, 10.30.

*4.4.1.9. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-hexyn-1-yl-uracil (disodium salt) (**23a**).* Treatment of compound **47a** (80 mg, 0.18 mmol) following general procedure gave rise to the expected derivative (73 mg, 88%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.10. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.38 (s, 1H, H-6), 5.85 (d, *J* = 5.70 Hz, 1H H-1′), 4.07 (m, 3H, H-2′, H-3′, H-5′), 3.89 (m, 1H, H-4′), 2.42 (t, *J* = 6.75 Hz, 2H, CH_2), 1.56 (m, 6H, CH_2, H-6′, H-6″), 0.90 (t, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 162.1 (C-4), 150.2 (C-2), 143.3 (C-6), 99.6 and 93.6 (C≡C), 89.2 (d, *J* = 14.42 Hz, C-4′), 87.6 (C-1′), 75.0 (C-2′), 73.1 (C-3′), 68.7 (C-5′), 67.2 (C-5), 30.6 (C-6′, CH_2). 21.7 (CH_2), 18.8 (CH_2), 13.8 (CH_3). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 18.6. MS ESI-QT of >0 *m/z* 837 $(2M + H)^+$, 419 $(M + H)^+$. HR-MS Calculated for $C_{16}H_{24}N_2O_9P$: 419.1219; Found: 419.1208. UV (EtOH 95) λ_{max} = 293 nm (ε_{max} = 9200), λ_{max} = 238 nm (ε_{max} = 9300).

*4.4.1.10. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-(3,3-dimethyl-1-butyn-1-yl)-uracil (disodium salt) (**23b**).* Treatment of compound **47b** (114 mg, 0.26 mmol) following general procedure gave rise to the expected derivative (112 mg, 95%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.07. ^{1}H NMR (300 MHz, D_2O) δ = 8.04 (s, 1H, H-6), 5.75 (d, *J* = 5.43 Hz, 1H, H-1′), 4.18 (m, 1H, H-3′), 4.09 (m, 2H, H-2′, H-5′), 3.99 (m, 1H, H-4′), 1.66 (m, 2H, H-6′, H-6″), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75 MHz, D_2O) δ = 164.7 (C-4), 151.0 (C-2), 143.6 (C-6), 104.5 and 100.1 (C≡C), 88.1 (C-1′), 87.3 (d, *J* = 13.13 Hz, C-4′), 73.9 (C-2′), 69.4 (C-5′), 68.1 (C-5), 7.2 (C-3′), 31.8 (d, *J* = 127.5 Hz, C-6′), 29.9 ($C(\underline{C}H_3)_3$). ^{31}P NMR (81 MHz, D_2O) δ = 19.0. MS ESI-QT of >0 *m/z* 419 $(M + H)^+$, 441 $(M + Na)^+$. MS ESI-QT of <0 *m/z* 417 $(M-H)^-$. HR-MS Calculated for $C_{16}H_{24}N_2O_9P$: 419.1219; Found: 419.1228. UV (EtOH 95) λ_{max} = 292 nm (ε_{max} = 6600), λ_{max} = 228 nm (ε_{max} = 6700).

*4.4.1.11. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-phenylethynyl-uracil (disodium salt) (**23c**).* Treatment of compound **47c** (175 mg, 0.35 mmol) following general procedure gave rise to the expected derivative (133 mg, 77%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.18. ^{1}H NMR (400 MHz, D_2O) δ = 8.29 (s, 1H, H-6), 7.53 (m, 2H, H–Ar), 7.39 (m, 3H, H–Ar), 5.90 (d, *J* = 5.26 Hz, 1H, H-1′), 4.37–4.25 (m, 3H, H-2′, H-3′, H-5′), 4.14 (m, 1H, H-4′), 1.90 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) δ = 164.2 (C-4), 150.7 (C-2), 144.1 (C-6), 131.5, 129.1, 128.7, 121.8 (C–Ar), 100.0 and 94.0 (C≡C), 88.6 (C-1′), 87.4 (d, *J* = 13.48 Hz, C-4′), 79.9 (C-5), 74.2 (C-2′), 68.4 (C-3′), 67.2 (d, *J* = 2.69 Hz, C-5′), 32.7 (d, *J* = 132.0 Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) δ = 20.7. MS ESI-QT of >0 *m/z* 439.1 $(M - 2Na + 3H)^+$. MS ESI-QT of

<0 *m/z* 437.2 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{18}H_{18}N_2O_9P$: 437.0750; Found: 437.0770. UV (H_2O) $\lambda_{max} = 263$ nm ($\varepsilon_{max} = 15{,}195$), $\lambda_{max} = 277$ nm ($\varepsilon_{max} = 14{,}182$), $\lambda_{max} = 305$ nm ($\varepsilon_{max} = 18{,}041$).

*4.4.1.12. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-phenyl-uracil (disodium salt) (**23d**).* Treatment of compound **49d** (97 mg, 0.21 mmol) following general procedure gave rise to the expected derivative (84 mg, 88%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.31. ^{1}H NMR (400 MHz, D_2O) $\delta = 8.11$ (s, 1H, H-6), 7.46 (m, 5H, H–Ar), 6.00 (d, $J = 4.26$ Hz, 1H, H-1′), 4.36 (m, 2H, H-2′, H-3′), 4.25–4.17 (m, 2H, H-4′, H-5′), 1.87 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 164.6$ (C-2), 151.4 (C-4), 138.9 (C-6), 131.8, 128.6, 128.4, 128.3 (C–Ar), 115.8 (C-5), 88.4 (C-1′), 87.1 (d, $J = 13.09$ Hz, C-4′), 73.9 (C-2′), 68.3 (C-3′), 67.0 (d, $J = 2.30$ Hz, C-5′), 32.3 (d, $J = 130.7$ Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.4$. MS ESI-QT of >0 *m/z* 459.0 $(M + H)^+$, 437.1 $(M - Na + 2H)^+$, 415.1 $(M - 2Na + 3H)^+$. MS ESI-Qt of <0 *m/z* 413.2 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{16}H_{18}N_2Na_2O_9P$: 459.0545; Found: 459.0532. UV (H_2O) $\lambda_{max} = 235$ nm ($\varepsilon_{max} = 12{,}191$), $\lambda_{max} = 279$ nm ($\varepsilon_{max} = 10{,}092$).

*4.4.1.13. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-(2-(E)-phenylvinyl)-uracil (disodium salt) (**23e**).* Treatment of compound **49e** (71 mg, 0.14 mmol) following general procedure gave rise to the expected derivative (39 mg, 57%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.27. ^{1}H NMR (400 MHz, D_2O) $\delta = 8.14$ (s, 1H, H-6), 7.50–7.29 (m, 5H, H–Ar), 7.23 (d, $J = 16.51$ Hz, HC═C), 6.85 (d, $J = 16.54$ Hz, 1H, HC═C), 5.93 (d, $J = 5.03$ Hz, 1H, H-1′), 4.38–4.30 (m, 3H, H-2′, H-3′, H-5′), 4.17 (m, 1H, H-4′), 1.90 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 164.1$ (C-2), 150.8 (C-4), 137.4 (C-6), 136.8, 129.5, 128.8, 127.9, 126.3 (C–Ar, C═C), 119.0 (C═C), 112.7 (C-5), 88.3 (C-1′), 87.0 (d, $J = 13.5$ Hz, C-4′), 74.0 (C-2′), 68.2 (C-3′), 67.1 (C-5′), 32.3 (d, $J = 129.2$ Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.0$. MS ESI-QT of >0 *m/z* 463.1 $(M - Na + 2H)^+$, 441.1 $(M - 2Na + 3H)^+$. MS ESI-Qt of <0 *m/z* 439.2 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{18}H_{20}N_2Na_2O_9P$: 485.0702; Found: 485.0710. UV $\lambda_{max} = 271$ nm ($\varepsilon_{max} = 8281$) (H_2O).

*4.4.1.14. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-hexyn-1-yl-cytosine (disodium salt) (**24a**).* Treatment of compound **48a** (135 mg, 0.29 mmol) following general procedure gave rise to the expected derivative (30 mg, 23%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.35. ^{1}H NMR (400 MHz, D_2O) $\delta = 8.14$ (s, 1H, H-6), 5.93 (d, $J = 5.08$ Hz, 1H, H-1′), 4.33–4.23 (m, 3H, H-2′, H-3′, H-5′), 4.13 (m, 1H, H-4′), 2.44 (t, $J = 7.07$ Hz, 2H, CH_2C≡C), 1.87 (m, 2H, H-6′, H-6″), 1.55–1.40 (m, 4H, CH_2), 0.90 (t, $J = 7.27$ Hz, CH_3). ^{13}C NMR (100 MHz, D_2O) $\delta = 165.1$ (C-2), 156.3 (C-4), 143.8 (C-6), 98.3 and 93.5 (C≡C), 89.1 (C-1′), 86.9 (d, $J = 13.78$ Hz, C-4′), 74.2 (C-2′), 70.0 (C-3′), 68.2 (C-5), 67.0 (C-5′), 32.2 (d, $J = 130.05$ Hz, C-6′), 29.8 ($\underline{CH_2}$C≡C), 214 ($\underline{CH_2}CH_2$–C≡C), 18.3 ($CH_3\underline{CH_2}$), 12.8 (CH_3). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.2$. MS ESI-QT of >0 *m/z* 440.1 $(M - Na + 2H)^+$, 418.1 $(M - 2Na + 3H)^+$. MS ESI-QT of<0 *m/z* 416.2 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{16}H_{25}N_3O_8P$: 418.1379; Found: 418.1386. UV (H_2O) $\lambda_{max} = 209$ nm ($\varepsilon_{max} = 20{,}758$), $\lambda_{max} = 235$ nm ($\varepsilon_{max} = 15{,}324$), $\lambda_{max} = 296$ nm ($\varepsilon_{max} = 7593$).

*4.4.1.15. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-(3,3-dimethyl-1-butyn-1-yl)-cytosine (disodium salt) (**24b**).* Treatment of compound **48b** (145 mg, 0.31 mmol) following general procedure gave rise to the expected derivative (103 mg, 73%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.38. ^{1}H NMR (400 MHz, D_2O) $\delta = 8.15$ (s, 1H, H-6), 5.92 (d, $J = 5.09$ Hz, 1H, H-1′), 4.33–4.13 (m, 4H, H-2′, H-3′, H-4′, H-5′), 1.90 (m, 2H, H-6′, H-6″), 1.29 (s, 9H, CH_3). ^{13}C NMR (100 MHz, D_2O) $\delta = 164.8$ (C-2), 156.2 (C-4), 144.0 (C-6), 106.0 and 93.2 (C≡C), 89.2 (C-1′), 86.8 (d, $J = 13.76$ Hz, C-4′), 74.2 (C-2′), 68.8 (C-3′), 68.2 (C-5), 66.9 (d, $J = 2.69$ Hz, C-5′), 32.2 (d, $J = 131.1$ Hz, C-6′), 29.8 (CH_3), 27.7 (Cq). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.6$. MS ESI-QT of >0 *m/z* 440.0 $(M + 2H - Na)^+$, 418.0 $(M + 3H - 2Na)^+$ MS ESI-QT of <0 *m/z* 416.2 $(M + H - 2Na)^-$. HR-MS Calculated for $C_{16}H_{23}N_3O_8Na_2P$: 462.1018; Found: 462.0995. UV (H_2O) $\lambda_{max} = 208$ nm ($\varepsilon_{max} = 20{,}574$), $\lambda_{max} = 235$ nm ($\varepsilon_{max} = 14{,}768$), $\lambda_{max} = 297$ nm ($\varepsilon_{max} = 7454$).

*4.4.1.16. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-phenylethynyl-cytosine (disodium salt) (**24c**).* Treatment of compound **48c** (162 mg, 0.33 mmol) following general procedure gave rise to the expected derivative (126 mg, 80%). R*f* (iPrOH/NH_4OH/H_2O, 9/9/2, v/v/v) 0.39. ^{1}H NMR (400 MHz, D_2O) $\delta = 8.25$ (s, 1H, H-6), 7.55–7.35 (2m, 5H, H–Ar), 5.85 (d, $J = 4.78$ Hz, 1H, H-1′), 4.35–4.05 (2m, 4H, H-2′, H-3′, H-5′, H-4′), 2.05–1.80 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 164.4$ (C-4), 155.9 (C-2), 144.5 (C-6), 131.3, 129.0, 128.6, 121.5 (C–Ar), 95.4 and 92.7 (C≡C), 89.5 (C-1′), 86.6 (d, $J = 13.63$ Hz, C-4′), 79.1 (C-2′), 74.4 (C-3′), 68.2 (C-5), 67.0 (C-5′), 32.3 (d, $J = 131.0$ Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.4$. MS ESI-QT of >0 *m/z* 482.0 $(M + H)^+$, 460.0 $(M - Na + 2H)^+$, 438.0 $(M - 2Na + 3H)^+$. MS ESI-Qt of<0 *m/z* 436.1 $(M - 2Na + H)^+$. HR-MS Calculated for $C_{18}H_{19}N_3O_8Na_2P$: 482.0705; Found: 482.0694. UV $\lambda_{max} = 210$ nm ($\varepsilon_{max} = 15{,}161$), $\lambda_{max} = 283$ nm ($\varepsilon_{max} = 8125$) (H_2O).

*4.4.1.17. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-phenyl-cytosine (disodium salt) (**24d**).* Treatment of compound **50d** (109 mg, 0.23 mmol) following general procedure gave rise to the expected derivative (85 mg, 80%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.15. ^{1}H NMR (400 MHz, D_2O) $\delta = 7.96$ (s, 1H, H-6), 7.52–7.41 (m, 5H, H–Ar), 5.98 (d, $J = 4.31$ Hz, 1H, H-1′), 4.35–4.21 (m, 3H, H-2′, H-3′, H-5′), 4.16 (m, 1H, H-4′), 1.87 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 162.9$ (C-4), 155.6 (C-2), 138.7 (C-6), 130.5, 127.7, 127.5, 127.2 (C–Ar), 109.0 (C-5), 87.7 (C-1′), 85.1 (d, $J = 13.3$ Hz, C-4′), 72.5 (C-2′), 66.6 (C-3′), 65.4 (d, $J = 2.4$ Hz, C-5′), 30.7 (d, $J = 130.5$ Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) $\delta = 20.3$. MS ESI-QT of >0 *m/z* 458.2 $(M + H)^+$, 436.2 $(M - Na + 2H)^+$, 414.2 $(M - 2Na + 3H)^+$. MS ESI-Qt of<0 *m/z* 412.1 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{16}H_{20}N_3NaO_8P$: 436.0886; Found: 436.0866. UV (H_2O) $\lambda_{max} = 232$ nm ($\varepsilon_{max} = 7489$), 282 nm ($\varepsilon_{max} = 4110$).

*4.4.1.18. 1-[6-Deoxy-6-phosphono-β-D-allofuranosyl]-5-(2-(E)-phenylvinyl)-cytosine (disodium salt) (**24e**).* Treatment of compound **50e** (95 mg, 0.19 mmol) following general procedure gave rise to the expected derivative (22 mg, 24%). R*f* (iPrOH/NH_4OH/H_2O, 7/2/1, v/v/v) 0.13 ^{1}H NMR (400 MHz, D_2O) $\delta = 8.31$ (s, 1H, H-6), 7.55–7.25 (m, 6H, H–Ar, HC═C), 6.84 (d, $J = 4.1$ Hz, 1H, HC═C), 5.92 (d, $J = 3.8$ Hz, 1H, H-1′), 4.48–4.11 (m, 4H, H-2′, H-3′, H-4′, H-5′), 1.90–1.62 (m, 2H, H-6′, H-6″). ^{13}C NMR (100 MHz, D_2O) $\delta = 165.6$ (C-4), 158.3 (C-2), 139.4 (C-6), 137.9, 132.6, 130.3, 129.6, 127.9 (C–Ar, HC═C), 119.4 (HC═C), 108.8 (C-5), 90.6 (C-1′), 88.0 (d, $J = 12.7$ Hz, C-4′), 76.0 (C-2′), 69.2 (C-3′), 68.85 (C-5′), 33.7 (d, $J = 125.2$ Hz, C-6′). ^{31}P NMR (81 MHz, D_2O) $\delta = 19.2$. MS ESI-QT of >0 *m/z* 440.2 $(M - 2Na + 3H)^+$. MS ESI-Qt of <0 *m/z* 438.2 $(M - 2Na + H)^-$. HR-MS Calculated for $C_{18}H_{23}N_3NaO_8P$: 440.1223; Found: 440.1234. UV (H_2O) $\lambda_{max} = 278$ nm ($\varepsilon_{max} = 9351$).

*4.4.2. 1-(5S)-[6-deoxy-6-diethylphosphono-2,3-seco-β-D-ribohexofuranosyl]uracil (**19**)*

Compound **1** (2 g, 5.07 mmol) was dissolved in a mixture of dioxane (53 mL) and water (10 mL) and a solution of $NaIO_4$ (1.21 g, 5.57 mmol, 1.1 eq.) in water (10 mL) was added. The reaction mixture was stirred for 1.5 h at rt, diluted with dioxane, and then filtered. The solid residue was washed with dioxane, and then sodium borohydride (0.192 g, 5.07 mmol, 1 eq.) was added to the filtrate. The reaction mixture was stirred for 15 min, concentrated and freeze-dried. Column chromatography of the crude materials on reverse phase (gradient: water to acetonitrile) gave the expected

product **19** as a white solid (2.0 g, 99%). R*f* (EtOAc/MeOH, 5/5, v/v) 0.65. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.25 (s, 1H exchangeable, NH), 7.78 (d, J = 8.06 Hz, 1H, H-6), 5.91 (t, J = 5.81 Hz, 1H, H-1′), 5.88 (d, J = 8.04 Hz, 1H, H-5), 4.10–3.90 (m, 5H, H-5′, OC$\underline{H}_2$CH$_3$), 3.85–3.65 (m, 4H, H-3′, H-3″, H-2′, H-2″), 3.57 (m, 1H, H-4′), 2.15–1.80 (m, 2H, H-6′, H-6″), 1.22–1.18 (m, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 166.1 (C-4), 152.3 (C-2), 142.3 (C-6), 102.6 (C-5), 84.1 (C-1′), 82.2–82.0 (C-4′, d, J = 13 Hz), 65.6–65.5 (C-5′, d, J = 7 Hz), 63.3 (m, O$\underline{C}$H$_2$CH$_3$), 59.3 (C-2′), 57.4 (C-3′), 29.0–27.5 (C-6′, d, J = 140 Hz), 15.6–15.5 (OCH$_2$$\underline{C}H_3$, d, J = 6 Hz). ^{31}P (121 MHz, DMSO-d_6) δ = 32.1. MS FAB>0 (GT) m/z 793 (2M + H)$^+$, 397 (M + H)$^+$. FAB<0 (GT) m/z 791 (2M − H)$^-$, 395 (M − H)$^-$. UV λ_{max} = 263 nm (ε_{max} = 11,800) (EtOH 95).

4.4.3. 1-(5S)-[6-Deoxy-6-diethylphosphono-2,3-seco-β-D-ribohexofuranosyl]cytosine (**21**)

Compound **19** (0.30 g, 0.76 mmol) was suspended in anhydrous acetonitrile (7.8 mL), *N*-methyl pyrrolidine (0.76 mL, 7.27 mmol) and trimethylsilyl chloride (0.29 mL, 2.27 mmol) were added to the reaction mixture. After 1 h of stirring, trifluoroacetic anhydride (0.32 mL, 2.27 mmol) was added at 0 °C, the reaction mixture was stirred for 1 h more at 0 °C and followed by the addition of 4-nitrophenol (0.315 g, 2.27 mmol). Three hours later, the volatiles were evaporated under reduced pressure, the crude was diluted in dioxane (20 mL) and NH_4OH 28% (7.57 mL) was added. The reaction mixture was stirred overnight at 50 °C, neutralized with a solution of acetic acid 10% aqueous, freeze-dried and then purified twice on reverse phase column chromatography (gradient: water to acetonitrile) to give the cytosine containing acycloderivative **21** as a yellow solid (105 mg, 35%). R*f* (EtOAc/MeOH, 5/5, v/v) 0.34. ^{1}H NMR (400 MHz, D$_2$O) δ = 7.70 (d, J = 7.5 Hz, 1H, H-6), 6.05 (d, J = 7.49 Hz, 1H, H-5), 5.95 (d, J = 5.61 Hz, 1H, H-1′), 4.15–3.95 (m, 4H, OC$\underline{H}_2$CH$_3$), 3.95–3.88 (m, 1H, H-5′), 3.85–3.65 (m, 4H, H-3′, H-3″, H-2′, H-2″), 3.45 (m, 1H, H-4′), 2.15–1.85 (m, 2H, H-6′, H-6″), 1.22–1.15 (m, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, D$_2$O) δ = 166.0 (C-4), 158.1 (C-2), 142.1 (C-6), 96.6 (C-5), 84.4 (C-1′), 82.0–81.9 (C-4′, d, J = 14 Hz), 65.7–65.6 (C-5′, d, J = 7 Hz), 63.3 (m, O$\underline{C}$H$_2$CH$_3$), 61.8 (C-2′), 59.2 (C-3′), 29.1–27.7 (C-6′, d, J = 140 Hz), 15.6–15.5 (OCH$_2$$\underline{C}H_3$, d, J = 6 Hz). ^{31}P NMR (121 MHz, D$_2$O) δ = 29.4. MS FAB > 0 (NBA) m/z 791 (2M + H)$^+$, 396 (M + H)$^+$. FAB < 0 (NBA) m/z 789 (2M − H)$^-$, 394 (M − H)$^-$. UV λ_{max} = 272 nm (ε_{max} = 10,200) (EtOH 95). Calculated for $C_{14}H_{26}N_3O_8P$, 0.55H_2O: C, 41.49; H, 6.74; N, 10.37. Found: C, 41.49; H, 6.71; N, 10.30.

4.4.4. 1-[2,2′-Anhydro-6-deoxy-6-diethylphosphono-β-D-altrofuranosyl]uracil (**29**)

Diphenyl-carbonate (0.956 g, 4.46 mmol) was added to a solution of the nucleotide **1** (1.6 g, 4.06 mmol) in anhydrous DMF (20 mL) under argon. The reaction mixture was heated at 90 °C, the addition of Na_2CO_3 (16 mg, 10% weight) was performed and stirring was pursued at 110 °C during 1.5 h. The solution was concentrated under high vacuum. Column chromatography of the crude materials on silica gel (dichloromethane/methanol, 9/1 then 8/2, v/v) gave the anhydro-derivative as a light yellow powder (1.06 g, 70%). R*f* (EtOAc/MeOH, 5/5, v/v) 0.44. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.91 (d, J = 7.47 Hz, 1H, H-6), 6.50 (d, J = 5.78 Hz, 1H, H-1′), 6.15 (d, J = 7.45 Hz, 1H, H-5), 5.42 (d, J = 5.72 Hz, 1H, H-2′), 4.71 (s, 1H, H-3′), 4.09 (d, J = 8.11 Hz, 1H, H-4′), 4.05–3.9 (m, 4H, OC$\underline{H}_2$CH$_3$), 3.55–3.41 (m, 1H, H-5′), 2.11–1.86 (m, 2H, H-6′, H-6″), 1.30 (m, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 175.3 (C-4), 160.8 (C-2), 138.3 (C-6), 109.2 (C-5), 91.6–91.5 (C-4′, d, J = 17 Hz), 91.2 (C-1′), 89.3 (C-2′), 74.1 (C-3′), 65.5–65.45 (C-5′, d, J = 5 Hz), 63.4, 63.37 (2d, J = 6.0 Hz, O$\underline{C}$H$_2$CH$_3$), 29.6–28.2 (C-6′, d, J = 141 Hz), 15.6–15.5 (OCH$_2$$\underline{C}H_3$, d, J = 5 Hz). ^{31}P NMR (121 MHz, DMSO-d_6) δ = 30.8. MS FAB > 0 (NBA) m/z 753 (2M + H)$^+$, 377 (M + H)$^+$. FAB<0 (NBA) m/z 375 (M − H)$^-$. UV λ_{max} = 248 nm (ε_{max} = 7100), 224 nm (ε_{max} = 8700), λ_{min} = 235 nm (ε_{min} = 6500), (EtOH 95). Calculated for $C_{14}H_{23}N_2O_8P$, 0.3H_2O: C, 44.69; H, 5.62; N, 7.44. Found: C, 44.45; H, 6.10; N, 7.13.

4.4.5. 1-[6-Deoxy-6-diethylphosphono-β-D-altrofuranosyl]uracil (**5**)

The intermediate **29** (1 g, 2.65 mmol) was dissolved in a mixture of methanol and water (27 mL, 1/1, v/v). A solution of NaOH 1 N was added (26.5 mL) and the reaction mixture was stirred for 1 h, neutralized with AcOH, concentrated, diluted in water and freeze-dried. Column chromatography of the crude materials on reverse phase (water/acetonitrile, 9/1 then 5/5, v/v) gave the expected product as a white powder (1 g, 96%). R*f* (EtOAc/MeOH, 7/3, v/v) 0.47. ^{1}H NMR (400 MHz, D$_2$O) δ = 7.7 (d, J = 8.12 Hz, 1H, H-6), 6.07 (d, J = 4.57 Hz, 1H, H-1′), 5.67 (d, J = 8.11 Hz, 1H, H-5), 4.28 (dd, J = 3.42–4.53 Hz, 1H, H-2′), 4.22 (dd, J = 3.5–4.53 Hz, 1H, H-3′), 4.17 (m, 1H, H-5′), 4.1–4.0 (m, 4H, OC$\underline{H}_2$CH$_3$), 3.75 (dd, J = 4.53–5.99 Hz, 1H, H-4′), 2.3–2.05 (m, 2H, H-6′, H-6″), 1.21 (m, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, D$_2$O) δ = 166.4 (C-4), 151.5 (C-2), 143.0 (C-6), 101.0 (C-5), 85.5 (C-1′), 85.5–85.3 (C-4′, d, J = 15 Hz), 75.6 (C-2′), 75.3 (C-3′), 65.6–65.5 (C-5′, d, J = 5 Hz), 63.4 (m, O$\underline{C}$H$_2$CH$_3$), 29.5–28.1 (C-6′, d, J = 140 Hz), 15.6–15.5 (OCH$_2$$\underline{C}H_3$, d, J = 6 Hz). ^{31}P NMR (121 MHz, D$_2$O) δ = 32.0. MS FAB > 0 (NBA) m/z 789 (2M + H)$^+$, 395 (M + H)$^+$. FAB < 0 (NBA) m/z 393 (M − H)$^-$. UV λ_{max} = 263 nm (ε_{max} = 10,000) (EtOH 95). Calculated for $C_{14}H_{23}N_2O_9P$, 0.5H_2O: C, 41.69; H, 6.00; N, 6.95. Found: C, 41.42; H, 5.76; N, 6.73.

4.4.6. 1-[6-Deoxy-6-diethylphosphono-β-D-altrofuranosyl]cytosine (**7**)

The derivative **5** (300 mg, 0.761 mmol) was suspended in anhydrous acetonitrile (7.6 mL). *N*-methyl pyrrolidine (0.76 mL, 7.30 mmol) and trimethylsilyl chloride (0.29 mL, 2.28 mmol) were added to the reaction mixture. After 1 h of stirring, TFA anhydride (0.32 mL, 2.28 mmol) was added at 0 °C, the reaction mixture was stirred for 1 h more at 0 °C and followed by the addition of 4-nitrophenol (0.316 g, 2.28 mmol). Three hours later, the mixture was concentrated under reduced pressure, the crude was diluted in dioxane (23 mL) and NH_4OH 28% (7.61 mL) was added. The reaction mixture was stirred overnight, neutralized with a solution of acetic acid 10% aqueous, freeze-dried and then purified twice on reverse phase column chromatography (gradient:water to acetonitrile) to give the desired product **7** (130 mg, 43%). R*f* (CH_2Cl_2/MeOH, 8/2, v/v) 0.09. ^{1}H NMR (400 MHz, D$_2$O) δ = 7.65 (d, J = 7.56 Hz, 1H, H-6), 6.1 (d, J = 4.23 Hz, 1H, H-1′), 5.9 (d, J = 7.55 Hz, 1H, H-5), 4.29 (dd, J = 2.83–4.19 Hz, 1H, H-2′), 4.21 (dd, J = 2.9–3.93 Hz, 1H, H-3′), 4.20–4.15 (m, 1H, H-5′), 4.15–4.05 (m, 4H, OC$\underline{H}_2$CH$_3$), 3.75 (dd, J = 4–6.31 Hz, 1H, H-4′), 2.4–2.0 (m, 2H, H-6′, H-6″), 1.3–1.2 (m, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, D$_2$O) δ = 166.1 (C-4), 157.3 (C-2), 142.7 (C-6), 95.2 (C-5), 86.3 (C-1′), 85.8–85.6 (C-4′, d, J = 17 Hz), 75.6 (C-2′), 74.5 (C-3′), 65.7 (C-5′, d, J = 5 Hz), 63.5–63.4 (O$\underline{C}$H$_2$CH$_3$), 29.6–28.2 (C-6′, d, J = 140 Hz), 15.6–15.9 (OCH$_2$$\underline{C}H_3$, d, J = 5 Hz). ^{31}P NMR (81 MHz, D$_2$O) δ = 33.2. MS FAB > 0 (NBA) m/z 787 (2M − H)$^+$, 394 (M − H)$^+$, 112 (base-H)$^+$, FAB<0 (NBA) m/z 785 (2M − H)$^-$, 392 (M − H)$^-$. UV λ_{max} = 273 nm (ε_{max} = 10,300) (EtOH 95). Calculated for $C_{14}H_{24}N_3O_8P$, 1.3H_2O: C, 40.35; H, 6.43; N, 10.08. Found: C, 40.09; H, 6.24; N, 9.86.

4.4.7. 3-O-Benzyl-1,2-5,6-O-diisopropylidene-α-D-glucofuranose (**30**) [42,43]

Diacetonide-D-glucose (5 g, 19.2 mmol) was added to a suspension of KOH (4.3 g, 76.8 mmol) in anhydrous DMSO (32 mL). After 30 min stirring, BnBr (4.6 mL, 38.4 mmol) was added. The reaction mixture was stirred for 14 h at room temperature, diluted

with water (20 mL), extracted three times with diethyl ether. The organic layers were dried over Na_2SO_4, concentrated under reduced pressure. The oil obtained was purified on silica gel chromatography column (hexane/ethyl acetate, 9/1 then 8/2, v/v) to give the corresponding protected compound as yellow oil (6.4 g, 95%). R*f* (Hexane/EtOAc, 8/2, v/v) 0.28. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.25 (m, 5H, H–Ar), 5.85 (d, J = 3.76 Hz, 1H, H-1), 4.72 (d, J = 3.8 Hz, 1H, H-2), 4.69 (d, J = 11.9 Hz, 1H, H-7), 4.54 (d, J = 11.86 Hz, 1H, H-7′), 4.28 (dd, J = 6.14–6.78 Hz, 1H, H-5), 4.1 (dd, J = 3.14–6.78 Hz, 1H, H-4), 3.98 (dd, J = 6.14–8.35 Hz, 1H, H-6), 3.93 (d, J = 3.14 Hz, 1H, H-3), 3.8 (dd, J = 6.38–8.35 Hz, 1H, H-6′), 1.4–1.1 (4s, 12H, C(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 138.3, 128.7, 128.1, 128.0 (C–Ar), 111.3 (C(CH_3)$_2$), 108.5 (C(CH_3)$_2$), 105.2 (C-1), 82.0 (C-2), 81.6 (C-3), 80.9 (C-4), 72.7 (C-5), 71.5 (C-7), 66.6 (C-6), 27.1, 27.0, 26.5, 25.7 (C(CH_3)$_2$).

4.4.8. 3-O-Benzyl-1,2-O-isopropylidene-α-D-glucofuranose (**31**)

A solution of 3-*O*-benzyl-1,2-5,6-*O*-diisopropylidene-D-glucose (26.9 g, 76.8 mmol) in aqueous acetic acid (9/1, v/v, 385 mL) was warmed for 1 h at 60 °C. The solution was then concentrated and co-evaporated three times with toluene. Column chromatography of crude materials on silica gel (dichloromethane/ethyl acetate, 8/2 to 0/10, v/v) gave the titled compound as colorless oil (22.35 g, 93%). R*f* (CH_2Cl_2/EtOAc, 8/2, v/v) 0.12. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.25 (m, 5H, H–Ar), 5.8 (d, J = 3.7 Hz, 1H, H-1), 4.75 (d, J = 6.08 Hz, 1H exchangeable, OH-5), 4.7 (d, J = 11.6 Hz, 1H, H-7), 4.67 (d, J = 3.7 Hz, 1H, H-2), 4.6 (d, J = 11.4 Hz, 1H, H-7′), 4.45 (d, J = 5.58 Hz, 1H exchangeable, OH-6), 3.95 (m, 2H, H-3, H-4), 3.75 (m, 1H, H-5), 3.6 (m, H-6), 3.4 (m, 1H, H-3), 1.4–1.2 (2s, 6H, C(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 138.2, 128.2, 127.5, 127.4 (C–Ar), 110.5 (C(CH_3)$_2$), 104.6 (C-1), 81.4 (C-2), 81.1 (C-3), 79.7 (C-4), 71.2 (C-7), 68.1 (C-5), 63.7 (C-6), 26.5, 26.1 (C(CH_3)$_2$). MS FAB > 0 (NBA) m/z 621 (2M + H)$^+$, 311 (M + H)$^+$. FAB < 0 (NBA) m/z 619 (2M − H)$^-$, 309 (M − H)$^-$.

4.4.9. 3-O-Benzyl-6-deoxy-6-iodo-1,2-O-isopropylidene-α-D-glucofuranose (**33**) [44]

3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucose (1 g, 3.2 mmol) was dissolved in anhydrous pyridine (32 mL). *N,N*-dimethylaminopyridine (0.393 g, 3.2 mmol) was added at 0 °C, and then the solution was stirred for 15 min before adding the tosylchloride (1.22 g, 6.4 mmol). The solution was allowed to room temperature and stirred overnight. The reaction mixture was cooled to 0 °C; water and ethyl acetate were added. After decantation and extraction with ethyl acetate, the combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and co-evaporated with toluene. The oil obtained (containing tosyl intermediate **32**) was partially dissolved into acetone and the insoluble salts were removed by filtration. The filtrate was concentrated and the residue dissolved in anhydrous acetone (10 mL) under argon, sodium iodide (623 mg, 4.16 mmol) was added and the mixture was refluxed overnight and then concentrated. Column chromatography of the crude materials on silica gel (cyclohexane/ethyl acetate, 8/2, v/v) gave the iodo derivative as a yellow oil (0.93 g, 70%). R*f* (Cyclohexane/EtOAc, 8/2, v/v) 0.29. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.40–7.25 (m, 5H, H–Ar), 5.81 (d, J = 3.7 Hz, 1H, H-1), 5.4 (l, 1H exchangeable, OH-5), 4.7 (d, J = 3.9 Hz, 1H, H-2), 4.68 (d, J = 11.9 Hz, 1H, H-7), 4.59 (d, J = 11.6 Hz, 1H, H-7′), 3.97 (d, J = 2.8 Hz, 1H, H-3), 3.89 (dd, J = 2.8 and 8.66 Hz, 1H, H-4), 3.5–3.3 (m, 3H, H-5, H-6, H-6′), 1.4–1.25 (2s, 6H, C(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 138.0, 128.2–127.4 (C–Ar), 111.0 (C(CH_3)$_2$), 104.5 (C-1), 82.5 (C-4), 81.5 (C-2), 80.5 (C-3), 71.2 (C-7), 65.4 (C-5), 26.7, 26.1 (C(CH_3)$_2$), 16.7 (C-6). MS FAB > 0 (NBA) m/z 421 (M + H)$^+$, 405 (M − OH + H)$^+$, 91 (Bn)$^+$.

4.4.10. 3-O-Benzyl-6-deoxy-6-diethylphosphono-1,2-O-isopropylidene-α-D-glucofuranose (**34**) [45]

A solution of iodo derivative **33** (0.80 g, 2 mmol) in triethylphosphite (4 mL) was heated at 110 °C under argon for two days. The solution was evaporated under high vacuum at 70 °C. Column chromatography of the crude mixture on silica gel (ethyl acetate) gave the expected phosphonate derivative as colorless oil (0.692 g, 83%). R*f* (CH_2Cl_2/EtOAc, 8/2, v/v) 0.10. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.35–7.25 (m, 5H, H–Ar), 5.8 (d, J = 3.75 Hz, 1H, H-1), 5.07 (d, J = 7.11 Hz, 1H exchangeable, OH-5), 4.7 (d, J = 3.76 Hz, 1H, H-2), 4.67 (d, J = 11.5 Hz, 1H, H-7), 4.58 (d, J = 11.5 Hz, 1H, H-7′), 4.15–4.05 (m, 1H, H-3), 4.0–3.85 (m, 6H, H-4, H-5, O$CH_2$$CH_3$), 2.05–1.9 (m, 2H, H-6, H-6′), 1.4–1.25 (2s, 6H, C(CH_3)$_2$), 1.24–1.19 (m, 6H, OCH$_2$CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 138.6, 128.6, 128.1, 128.0 (C–Ar), 111.2 (C(CH_3)$_2$), 104.9 (C-1), 83.4–83.3 (C-4, d, J = 15 Hz), 82.0 (C-2), 81.0 (C-3), 71.6 (C-7), 63.2 (C-5, d, J = 6 Hz), 61.4–61.1 (OCH_2CH$_3$, m), 31.7–30.3 (C-6, d, J = 138.9 Hz), 27.1, 26.6 (C(CH_3)$_2$), 16.8–16.7 (OCH$_2$CH_3, m). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 27.2. MS FAB > 0 (NBA) m/z 861 (2M + H)$^+$, 431 (M + H)$^+$, 91 (Bn)$^+$. FAB < 0 (NBA) m/z 859 (2M − H)$^-$, 429 (M − H)$^-$.

4.4.11. 5-O-Benzoyl-3-O-benzyl-6-deoxy-6-diethylphosphono-1,2-O-isopropylidene-α-D-gluco-furanose (**35**)

Benzoyl chloride (0.88 mL, 7.54 mmol) was added at room temperature to a solution of intermediate **34** (2.5 g, 5.8 mmol) in anhydrous pyridine (15 mL) under argon. The mixture was stirred overnight, then iced-water (100 mL) was added and the resulting aqueous phase was extracted with ethyl acetate. The organic layers were successively washed with HCl 1 N, saturated $NaHCO_3$, and water, then dried over Na_2SO_4 and concentrated. Column chromatography of the crude mixture on silica gel (dichloromethane/ethyl acetate, 7/3, v/v) gave the expected phosphonate derivative as a yellow oil (2.9 g, 93%). R*f* (CH_2Cl_2/EtOAc, 7/3, v/v) 0.22. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.00–7.90 (m, 2H, H–Ar, Bz), 7.70–7.60 (m, 1H–Ar, Bz), 7.50–7.40 (m, 2H–Ar, Bz), 7.2–7.1 (m, 5H, H–Ar, Bn), 5.9 (d, J = 3.73 Hz, 1H, H-1), 5.52 (m, J = 3.9–19.9 Hz, 1H, H-5), 4.8 (d, J = 3.73 Hz, 1H, H-2), 4.65 (d, J = 11.4 Hz, 1H, H-7), 4.51 (dd, J = 3.22–7.17 Hz, 1H, H-4), 4.35 (d, J = 11.4 Hz, 1H, H-7′), 4.0 (d, J = 3.27 Hz, 1H, H-3), 3.95–3.80 (m, 4H, O$CH_2$$CH_3$), 2.45–2.23 (m, 2H, H-6, H-6′), 1.4–1.27 (2s, 6H, C(CH_3)$_2$), 1.1–1.0 (2t, J = 7.05 Hz, 6H, OCH$_2$CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.9 (C=O), 137.8–125.8 (C–Ar), 111.6 (C(CH_3)$_2$), 105.4 (C-1), 81.5 (C-2), 80.9 (C-3), 80.1–80.0 (C-4, d, J = 10 Hz), 71.4 (C-7), 66.9–66.8 (C-5, d, J = 6 Hz), 61.6–61.5 (OCH_2CH$_3$, m), 27.8–26.4 (C-6, d, J = 140 Hz), 27.1, 26.6 (C(CH_3)$_2$), 16.5–16.4 (OCH$_2$CH_3, m). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 26.9. MS FAB>0 (NBA) m/z 1069 (2M + H)$^+$, 535 (M + H)$^+$, 91 (Bn)$^+$.

4.4.12. 5-O-Benzoyl-3-O-benzyl-6-deoxy-1,2-O-diacetyl-6-diethylphosphono-α,β-D-glucofuranose (**36**)

A solution of the fully protected sugar phosphonate **35** (8.0 g, 14.9 mmol) in glacial acetic acid (56.7 mL), acetic anhydride (20.3 mL) and concentrated sulfuric acid (7.25 mL added at 0 °C) was stirred overnight at room temperature. The reaction was diluted in dichloromethane, washed with aqueous saturated $NaHCO_3$ solution. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Silica gel column chromatography of the crude materials (toluene/acetone, 7/3, v/v) gave the acetylated derivative as a yellow oil (8.0 g, 90%). R*f* (Toluene/Acetone, 7/3, v/v) 0.44. ^{1}H NMR(400 MHz, DMSO-d_6) δ = 8.00–7.90 (m, 2H, H–Ar, Bz), 7.70–7.60 (m, 1H–Ar, Bz), 7.55–7.45 (m, 2H–Ar, Bz), 7.35–7.2 (m, 5H, H–Ar, Bn), 6.35 (d, J = 4.61 Hz, 1H, H-1 anomer min), 6.05 (s, 1H, H-1 anomer maj), 5.7–5.5 (m, 1H, H-5), 5.25 (m, 1H, H-2), 4.8–4.7 (m, 1H, H-4), 4.7–4.6 (m, 1H, H-7), 4.6–4.4 (m, 2H, H-3 anomer min, H-7′), 4.3 (d, J = 5.34 Hz, 1H, H-3

anomer maj), 3.9–3.8 (m, 4H, OCH_2CH_3), 2.45–2.2 (m, 1H, H-6, H-6′), 2.2–1.9 (m, 6H, $C(CH_3)_2$), 1.1–0.95 (m, 6H, OCH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 169.5–164.4 (C=O), 137.4–125.3 (C–Ar), 98.6 (C-1 anomer maj), 93.0 (C-1 anomer min), 83.1–83.0 (C-4 anomer maj, d, J = 11 Hz), 79.5–79.3 (C-3 anomer maj, d, J = 16 Hz), 78.8–78.7 (C-3 anomer min, d, J = 12 Hz and C-4 anomer min), 78.0 (C-2 anomer maj), 76.0 (C-2 anomer min), 71.7–71.3 (C-7), 61.1–61.0 (OCH_2CH_3), 20.6–20.3 (CH_3, Ac, m), 16.0–15.9 (OCH_2CH_3, m). ^{31}P NMR (121 MHz, DMSO-d_6) δ = 27.0. MS FAB > 0 (NBA) m/z 1157 $(2M + H)^+$, 579 $(M + H)^+$, 105 $(Bz)^+$, 91 $(Bn)^+$. FAB < 0 (NBA) m/z 535 $(M - Ac)^-$.

4.4.13. 1-[2-O-Acetyl-5-O-benzoyl-3-O-benzyl-6-deoxy-6-diethylphosphono-β-D-glucofuranosyl] uracil (**37**)

The dried uracil (1.55 g, 13.84 mmol) was dissolved in anhydrous acetonitrile (36 mL) and *N,O*-bis(trimethylsilyl)acetamide (6.8 mL, 27.68 mmol) was added under argon. The solution was refluxed 2 h and then the reaction mixture was allowed to cool to room temperature. A solution of sugar-phosphonate **36** (4 g, 6.92 mmol) in anhydrous acetonitrile (36 mL) was added, followed by tin (IV) chloride (3.24 mL, 27.68 mmol). The resulting mixture was stirred overnight at room temperature, poured into a cold saturated $NaHCO_3$ solution and filtered over celite. The celite cake was washed with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 97/3, v/v) gave the expected product (3.2 g, 73%). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.56. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.3 (s, 1H, exchangeable, NH), 7.90–7.80 (m, 2H, H–Ar, Bz), 7.70–7.60 (m, 1H, H–Ar, Bz), 7.5 (d, J = 8.13 Hz, 1H, H-6), 7.50–7.40 (m, 2H, H–Ar, Bz), 7.3–7.2 (m, 5H, H–Ar, Bn), 6.00 (d, J = 1.5 Hz, 1H, H-1′), 5.8–5.6 (m, 1H, H-5′), 5.3 (d, J = 1.4 Hz, 1H, H-2′), 5.2 (m, 1H, H-5), 4.7 (d, J = 10.99 Hz, 1H, H-7″), 4.5 (dd, J = 3.66–6.61 Hz, 1H, H-4′), 4.4 (d, J = 10.97 Hz, 1H, H-7‴), 4.2 (d, J = 3.7 Hz, 1H, H-3′), 3.95–3.8 (m, 4H, OCH_2CH_3), 2.5–2.25 (m, 2H, H-6′, H-6″), 2.1 (s, 3H, Ac), 1.1–0.9 (2t, 6H, OCH_2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.9 (C=O, Ac), 164.8 (C=O, Bz), 163.4 (C-4), 150.6 (C-2), 140.8 (C-6), 137.3–128.4 (C–Ar), 102.2 (C-5), 88.4 (C-1′), 81.8 (C-4′), 79.7 (C-3′), 79.0 (C-2′), 71.5 (C-7′), 66 (C-5′), 61.7–61.6 (OCH_2CH_3, m), 21.2 (CH_3, Ac), 16.52, 16.46, 16.40.16.34 (OCH_2CH_3). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 27.5. MS FAB>0 (NBA) m/z 631 $(M + H)^+$, 105 $(Bz)^+$, 91 $(Bn)^+$. FAB<0 (NBA) m/z 629 $(M - H)^-$.

4.4.14. 1-[6-Deoxy-6-diethylphosphono-β-D-glucofuranosyl]uracil (**9**)

The Pd/C (520 mg, 100% weight) was added to a solution of nucleotide compound **37** (520 mg, 0.825 mmol) in methanol (36 mL) at 0 °C under argon. Ammonium formate (261 mg, 4.2 mmol) was added at room temperature. The solution was refluxed for 4.5 h, filtered over celite. The celite cake was washed with methanol and the filtrate was concentrated under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 95/5, v/v) and then reverse phase chromatography gave the expected product (154 mg, 50%). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.16. ^1H NMR (400 MHz, D_2O) δ = 7.75 (d, J = 8.15 Hz, 1H, H-6), 5.75 (d, J = 8.15 Hz, 1H, H-5), 5.70 (d, J = 0.78 Hz, 1H, H-1′), 4.4–4.3 (m, 1H, H-5′), 4.2 (m, 2H, H-2′, H-3′), 4.15–4.05 (m, 5H, H-4′, OCH_2CH_3), 2.4–2.1 (m, 2H, H-6′, H-6″), 1.3–1.1 (m, 6H, OCH_2CH_3). ^{13}C NMR (75 MHz, D_2O) δ = 166.5 (C-4), 151.5 (C-2), 142.2 (C-6), 101.1 (C-5), 91.9 (C-1′), 85.4–85.3 (C-4′, d, J = 14 Hz), 80.4 (C-2′), 73.8 (C-3′), 63.5–63.3 (C-5′, OCH_2CH_3, m), 30.5–29.1 (C-6′, d, J = 139 Hz), 15.6–15.5 (OCH_2CH_3, m). ^{31}P NMR (121 MHz, D_2O) δ = 29.2. MS FAB > 0 (NBA) m/z 789 $(2M + H)^+$, 395 $(M + H)^+$. FAB < 0 (NBA) m/z 787 $(2M - H)^-$, 393 $(M - H)^-$. UV λ_{max} = 262 nm (ε_{max} = 11,400) (EtOH 95). Analysis Calculated for $C_{14}H_{23}N_2O_9P$, 0.9H_2O: C, 40.96; H, 6.09; N, 6.82. Found: C, 40.93; H, 6.23; N, 6.80.

4.4.15. 1-[2-O-Acetyl-5-O-benzoyl-3-O-benzyl-6-deoxy-6-diethylphosphono-β-D-glucofuranosyl] N-4-benzoyl-cytosine (**38**)

The dried protected nucleobase (2.97 g, 13.84 mmol) was dissolved in anhydrous acetonitrile (35 mL) and *N,O*-bis(trimethylsilyl) acetamide (6.76 mL, 27.6 mmol) was added under argon. The solution was refluxed for 2 h, and then the reaction mixture was allowed to cool to room temperature. A solution of phosphonate intermediate **36** (4 g, 6.92 mmol) in anhydrous acetonitrile (35 mL) was added, followed by tin (IV) chloride (3.24 mL, 27.68 mmol). The resulting mixture was stirred overnight at room temperature, poured into a cold saturated $NaHCO_3$ solution and filtered over celite. The celite cake was washed with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 97/3, v/v) gave the expected product (3.25 g, 65%). R*f* (CH_2Cl_2/MeOH, 95/5, v/v) 0.48. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.2 (s, 1H, exchangeable, NH), 8.0 (d, J = 7.49 Hz, 1H, H-6), 8.0–7.4 (m, 10H, Bz), 7.2–7.1 (m, 5H, Bn), 7.05 (d, J = 7.27 Hz, 1H, H-5′), 6.0 (s, 1H, H-1′), 5.85–5.75 (m, 1H, H-5), 5.40 (s, 1H, H-2′), 4.7 (dd, J = 3.4–7.35 Hz, 1H, H-4′), 4.6, 4.4 (2d, J = 11.2 Hz, 2H, H-7′, H-7″), 4.2 (d, J = 3.61 Hz, 1H, H-3′), 4.0–3.8 (m, 4H, OCH_2CH_3), 2.6–2.3 (m, 2H, H-6′, H-6″), 2.2 (s, 3H, Ac), 1.2–1.0 (2t, 6H, OCH_2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 169.3 (C=O, Ac), 164.3 (C=O, Bz), 163.2 (C-4), 154.3 (C-2), 144.8 (C-6), 136.7–127.8 (C–Ar), 95.9 (C-5), 89.7 (C-1′), 82.4–82.3 (C-4′, d, J = 10 Hz), 78.8 (C-2′), 78.19 (C-3′), 71.0 (CH_2, Bn), 65.6 (C-5′, d, J = 6 Hz), 61.3–61.2 (m, OCH_2CH_3, C-6′), 20.7 (Ac), 16.0–15.8 (OCH_2CH_3). ^{31}P NMR (121 MHz, DMSO-d_6) δ = 28.9. MS FAB > 0 (NBA) m/z 734 $(M + H)^+$. FAB < 0 (NBA) m/z 732 $(M - H)^-$.

4.4.16. 1-[6-Deoxy-6-diethylphosphono-β-D-glucofuranosyl] cytosine (**11**)

The nucleotide compound **38** (300 mg, 0.517 mmol) was dissolved in anhydrous dichloromethane (44 mL) under argon. The reaction mixture was cooled to −78 °C and BCl_3 1 M in heptane (18.74 mL, 18.74 mmol) was added. The solution was stirred overnight, heated slowly to room temperature. Then methanol (80 mL) and NH_4OH (28 mL) were added at 0 °C and the reaction mixture was concentrated under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 9/1 then 0/1, v/v) gave the desired compound (153 mg, 75%). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.16. ^1H NMR (400 MHz, D_2O) δ = 7.7 (d, J = 7.59 Hz, 1H, H-6), 5.9 (d, J = 7.58 Hz, 1H, H-5), 5.7 (s, 1H, H-1′), 4.4–4.3 (m, 1H, H-5′), 4.2 (s, 1H, H-2′), 4.15–4.05 (m, 6H, H-3′, H-4′, OCH_2CH_3), 2.5–2.15 (m, 2H, H-6′, H-6″), 1.3–1.2 (m, 6H, OCH_2CH_3). ^{13}C NMR (100 MHz, D_2O) δ = 166.3 (C-4), 157.4 (C-2), 142.0 (C-6), 95.1 (C-5), 92.8 (C-1′), 85.5–85.3 (C-4′, d, J = 15 Hz), 80.6–74.0 (C-2′, C-3′), 63.6–63.3 (C-5′, OCH_2CH_3, m), 30.5–29.1 (C-6′, d, J = 140 Hz), 15.6–15.5 (OCH_2CH_3, d, J = 6 Hz). ^{31}P NMR (121 MHz, D_2O) δ = 30.8. MS ESI-QT of >0 m/z 394.2 $(M + H)^+$. HR-MS Calculated for $C_{14}H_{25}N_3O_8P$: 394.1379; Found: 394.1401.

4.4.17. 1-[5-O-Benzoyl-3-O-benzyl-6-deoxy-6-diethylphosphono-β-D-glucofuranosyl]uracil (**39**)

The uracil derivative **37** (1.3 g, 2.06 mmol) was dissolved in a mixture of pyridine and glacial acetic acid (22 mL, 4/1, v/v), then hydrated hydrazine (0.14 mL, 28.06 mmol) was added and the reaction mixture was heated 5 h at 100 °C. The solution was allowed to room temperature, diluted with acetone, dichloromethane and water. The resulting organic layer was washed with a saturated $NaHCO_3$ solution, brine, then dried over Na_2SO_4 and concentrated

under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 9/1, v/v) gave the expected product (0.820 g, 70%). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.42. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.35 (s, 1H exchangeable, NH), 7.9–7.4 (m, 5H–Ar, Bz), 7.6 (d, J = 8.13 Hz, 1H, H-6), 7.2 (sl, 5H–Ar, Bn), 6.1 (l, 1H exchangeable, OH), 5.8 (d, J = 1.07 Hz, 1H, H-1′), 5.7 (m, 1H, H-5′), 5.2 (d, J = 8.13 Hz, 1H, H-5), 4.6 (m, 2H, H-4′, H-7′), 4.4 (m, 2H, H-2′, H-7′′), 4.0 (d, 1H, J = 3.7 Hz, H-3′), 3.95–3.8 (m, 4H, OCH_2CH_3), 2.6–2.3 (m, 2H, H-6′, H-6′′), 1.1–0.9 (m, 6H, OCH_2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 164.3 (C=O), 163.0 (C-4), 150.4 (C-2), 140.7 (C-6), 140.7–127.8 (C–Ar), 101.0 (C-5), 90.8 (C-3′), 81.8 (C-1′), 81.4–81.3 (C-4′, d, J = 11 Hz), 76.6 (C-2′), 70.8 (C-7), 66.0–65.9 (C-5′, d, J = 6.0 Hz), 61.2–61.1 (m, OCH_2CH_3), 27.3–25.9 (C-6′, d, J = 140 Hz), 16.0–15.9 (m, OCH_2CH_3). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 27.7. MS FAB > 0 (NBA) m/z 1177 $(2M + H)^+$, 589 $(M + H)^+$. FAB < 0 (NBA) m/z 1175 $(2M - H)^-$, 587 $(M - H)^-$.

4.4.18. 1-[5-O-Benzoyl-3-O-benzyl-6-deoxy-6-diethylphosphono-β-D-mannofuranosyl]uracil (**40**)

A solution of compound **39** (1.13 g, 1.92 mmol) in dry dichloromethane (20 mL), at 0 °C, was treated by Dess-Martin periodinane (1.63 g, 3.84 mmol, 2 eq.). The reaction mixture was stirred for 2 h at room temperature, diluted with ethyl acetate, and quenched by adding a saturated solution of $NaHCO_3$ containing Na_2SO_3. After decantation, the organic layer was dried over Na_2SO_4, concentrated under reduced pressure and coevaporated with anhydrous toluene to afford the keto-intermediate. This last was dissolved in absolute ethanol (20 mL) under argon. A solution of sodium borohydride (0.227 g, 6.14 mmol) in absolute ethanol (20 mL) was added dropwise at 0 °C. The mixture was stirred for 1 h at room temperature, and neutralized with HCl 1 N. After adding dichloromethane and water, the resulting organic layer was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. Silica gel column chromatography of the crude materials (dichloromethane/methanol, 97/3, v/v) gave the expected product (0.510 g, 45%). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.41. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.15 (s, 1H exchangeable, NH), 7.9–7.3 (m, 5H–Ar, Bz), 7.7 (d, J = 8.12 Hz, 1H, H-6), 7.3–7.1 (m, 5H–Ar, Bn), 5.95 (d, J = 6.69 Hz, 1H, H-1′), 5.75 (d, J = 5.56 Hz, 1H exchangeable, OH), 5.6–5.5 (m, 1H, H-5′), 5.1 (dd, J = 1.98–8.1 Hz, 1H, H-5), 4.7 (d, J = 10.69 Hz, 1H, H-7′), 4.5 (m, 1H, H-2′), 4.3 (d, J = 10.69 Hz, 1H, H-7′′), 4.15 (m, 1H, H-4′), 4.1 (m, 1H, H-3′), 3.80–3.65 (m, 4H, OCH_2CH_3), 2.4–2.1 (m, 2H, H-6′, H-6′′), 1.0–0.8 (m, 6H, OCH_2CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 164.9 (C=O), 163.5 (C-4), 151.3 (C-2), 144.0 (C-6), 138.3–128.2 (C–Ar), 100.4 (C-5), 83.4 (C-1′), 77.0, 73.6, 71.183.4 (C-4′, C-2′, C-3′), 61.6–61.5 (d, J = 6 Hz, C-7), 16.5–16.4 (m, OCH_2CH_3). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 27.6. MS FAB > 0 (NBA) m/z 589 $(M + H)^+$. FAB < 0 (NBA) m/z 587 $(M - H)^-$.

4.4.19. 1-[6-Deoxy-6-diethylphosphono-β-D-mannofuranosyl]uracil (**13**)

The nucleotide compound **40** (0.675 g, 1.14 mmol) was dissolved in anhydrous dichloromethane (50 mL) under argon. The reaction mixture was cooled to −78 °C and BCl_3 1 M in heptane (22.8 mL, 22.8 mmol) was added. The solution was stirred overnight, heated slowly to room temperature. Then methanol (100 mL) and NH_4Cl (27 mL) were added at 0 °C and the middle was concentrated under reduced pressure. Two silica gel column chromatographies of the crude materials (dichloromethane/methanol, 8/2 then 9/1, v/v) and reverse phase chromatography gave the expected product (0.290 g, 65%). R*f* (CH_2Cl_2/MeOH, 9.1, v/v) 0.22. ^{1}H NMR (400 MHz, D_2O) δ = 7.85 (d, J = 8.16 Hz, 1H, H-6), 6.10 (d, J = 6.87 Hz, 1H, H-1′), 5.75 (d, J = 8.15 Hz, 1H, H-5), 4.6 (dd, J = 4.78–6.86 Hz, 1H, H-2′), 4.4–4.35 (m, 1H, H-5′), 4.3 (dd, J = 3.14–4.78 Hz, 1H, H-3′), 4.15–4.0 (m, 4H, OCH_2CH_3), 3.8 (dd, J = 3.09–8.32 Hz, 1H, H-4′), 2.4–2.05 (m, 2H, H-6′, H-6′′), 1.3–1.2 (m, 6H, OCH_2CH_3). ^{13}C NMR (100 MHz, D_2O) δ = 166.4 (C-4), 151.8 (C-2), 143.6 (C-6), 100.9 (C-5), 84.5 (C-1′), 82.3–82.2 (C-4′, d, J = 15 Hz), 71.6 (C-2′), 68.8 (C-3′), 63.9–63.8 (C-5′, d, J = 6 Hz), 63.3 (m, OCH_2CH_3), 30.0–28.6 (C-6′, d, J = 140 Hz), 15.6–15.5 (OCH_2CH_3, d, J = 6 Hz). ^{31}P NMR (121 MHz, D_2O) δ = 29.4. MS FAB > 0 (NBA) m/z 395 $(M + H)^+$. FAB<0 (NBA) m/z 393 $(M - H)^-$. UV λ_{max} = 262 nm (ε_{max} = 16,900) (EtOH 95). Analysis Calculated for $C_{14}H_{23}N_2O_9P$, $0.8H_2O$: C, 41.14; H, 6.07; N, 6.85. Found: C, 41.09; H, 6.44; N, 6.65.

4.4.20. 1-[6-Deoxy-6-diethylphosphono-β-D-mannofuranosyl] cytosine (**15**)

The nucleotide compound **13** (282 mg, 0.566 mmol) was suspended in anhydrous acetonitrile (5.7 mL). *N*-methyl pyrrolidine (0.56 mL, 5.436 mmol) and trimethylsilyl chloride (0.22 mL, 1.70 mmol) were added to the reaction mixture. After 1 h stirring, TFA anhydride (0.24 mL, 1.70 mmol) was added at 0 °C, the reaction mixture was stirred for 1 h more at 0 °C and followed by the addition of 4-nitrophenol (236 mg, 1.70 mmol). After 3 h, the reaction mixture was concentrated under reduced pressure, the crude was diluted in dioxane (17 mL) and NH_4OH 28% (5.7 mL) was added. The reaction mixture was stirred overnight, neutralized with an aqueous solution of acetic acid (10%, v/v) and freeze-dried. Due to the small amount of compound obtained (46 mg, 16%), the crude was directly engaged in the next step without further purification. R*f* (CH_2Cl_2/MeOH, 9.1, v/v) 0.12. ^{31}P NMR (81 MHz, D_2O) δ = 27.5.

4.4.21. 1-[3-O-Benzoyl-6-deoxy-2,5-O-diacetyl-6-diethylphosphono-β-D-allofuranosyl]-5-iodo-uracil (**43**)

The nucleotide **41** (1 g, 1.72 mmol) was dissolved in anhydrous dichloromethane (20 mL), iodine chloride (0.51 g, 3.18 mmol) was added, the mixture was refluxed for 15 h. The organic layer was washed with aqueous $NaHSO_3$ (2% w/v), dried over $MgSO_4$ and concentrated. The crude product was purified on silica gel (DCM/EtOAc, 1/2, v/v) yielding derivative **43** (1.00 g, 82%) as a pale yellow solid. R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.55. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.93 (bs, 1H, NH), 8.08 (m, 2H, H–Ar), 7.70 (s, 1H, H-6), 7.62 (m, 1H, H–Ar), 7.48 (m, 2H, H–Ar), 6.08 (d, J = 6.43 Hz, 1H, H-1′), 5.78 (dd, J = 4.00, 6.34 Hz, 1H, H-3′), 5.49 (m, 1H, H-5′), 5.40 (t, J = 6.40 Hz, 1H, H-2′), 4.60 (t, J = 3.62 Hz, 1H, H-4′), 4.12 (m, 4H, OCH_2CH_3), 2.31 (m, 2H, H-6′, H-6′′), 2.22, 1.99 (2s, 6H, Ac), 1.30 (td, J = 7.06 and 1.82 Hz, 6H, OCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.7, 169.6 (C=O, Ac), 165.0 (C=O, Bz), 159.3 (C-4), 149.6 (C-2), 144.1 (C-6), 133.8, 129.8 (Cq, C–Ar), 128.7 (C–Ar), 87.4 (C-1′), 83.0 (d, J = 7.64 Hz, C-4′), 72.6 (C-2′), 69.7 (C-5), 69.6 (C-3′), 68.2 (C-5′), 62.2 (dd, J = 6.14 and 15.03 Hz, OCH_2CH_3), 28.1 (d, J = 140.81 Hz, C-6′), 21.3, 20.3 (CH_3, Ac), 16.3 (d, J = 5.54 Hz, OCH_2CH_3). ^{31}P NMR (81 MHz, $CDCl_3$) δ = 24.7. MS ESI-QT of >0 m/z 709.0 $(M + H)^+$. HR-MS Calculated for $C_{25}H_{31}N_2O_{12}PI$: 709.0659; Found: 709.0659.

4.4.22. 1-[3-O-Benzoyl-6-deoxy-2,5-O-diacetyl-6-diethylphosphono-β-D-allofuranosyl]-N-4-benz-oyl-5-iodo-cytosine (**44**)

Iodine (0.75 g, 2.92 mmol) was added to a suspension of **42** (3.34 g, 4.87 mmol) in a mixture of CH_3COOH and CCl_4 (20 mL, 1/1, v/v). After heating to 40 °C, iodic acid (0.77 g, 4.38 mmol) was added and the reaction mixture was stirred at 40 °C overnight. The suspension was concentrated in vacuo and the residue taken up in dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and aqueous $NaHSO_3$ (2%, w/v), dried with $MgSO_4$ and concentrated. Column chromatography of the crude product (dichloromethane/ethyl acetate, 1/2, v/v) yielded compound **44** (2.39 g, 61%) as a pale yellow solid. R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.64. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 13.14 (bs, 1H, NH), 8.38 and 8.09 (2m,

4H, H–Ar), 7.81 (s, 1H, H-6), 7.49 (m, 6H, H–Ar), 6.13 (d, J = 6.57 Hz, 1H, H-1′), 5.81 (dd, J = 3.75 and 6.25 Hz, 1H, H-3′), 5.51 (m, 1H, H-5′), 5.41 (t, J = 6.41 Hz, 1H, H-2′), 4.63 (t, J = 3.45 Hz, 1H, H-4′), 4.12 (m, 4H, OC$\underline{H}_2$CH$_3$), 2.34 (m, 2H, H-6′, H-6″), 2.23 and 2.00 (2s, 6H, CH$_3$, Ac), 1.30 (t, J = 7.06 Hz, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, CDCl$_3$) δ = 180.0 (C = 0, Bz), 169.7, 169.8 (C═O, Ac), 165.0 (C═O, Bz), 156.1 (C-4), 147.4 (C-2), 144.8 (C-6), 136.4, 133.9, 133.1, 130.4, 129.8, 128.7 (Cq, C–Ar), 128.3 (C–Ar), 87.5 (C-1′), 83.4 (d, J = 7.60 Hz, C-4′), 72.7 (C-2′), 70.9 (C-5), 69.6 (C-3′), 68.3 (C-5′), 62.4 (dd, J = 15.38 and 6.22 Hz, O$\underline{C}$H$_2$CH$_3$), 28.2 (d, J = 140.8 Hz, C-6′), 21.3 and 20.3 (CH$_3$, Ac), 16.3 (d, J = 5.87 Hz, OCH$_2\underline{C}$H$_3$). ^{31}P NMR (81 MHz, CDCl$_3$) δ = 24.7. MS ESI-QT of >0 m/z 812.0 (M + H)$^+$. HR-MS Calculated for $C_{32}H_{36}N_3O_{12}PI$: 812.1081; Found: 812.1066.

4.4.23. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-iodo-uracil (**45**)

The nucleotide **43** (1.4 g, 2.40 mmol), was dissolved in methanolic ammonia (48 mL) and stirred at room temperature until completion of the reaction was indicated by TLC. Purification by column chromatography (dichloromethane/methanol, 9/1, v/v) gave the expected compound **45** (0.79 g, 84%) as a white powder. Rf (CH$_2$Cl$_2$/MeOH, 9/1, v/v) 0.38. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.30 (s, 1H, H-6), 5.74 (d, J = 5.81 Hz, 1H, H-1′), 5.61 (d, J = 5.32 Hz, 1H, OH), 5.41 (d, J = 5.30 Hz, 1H, OH), 5.07 (d, J = 4.65 Hz, 1H, OH), 4.01 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.84 (m, 1H, H-4′), 1.99 (m, 2H, H-6′, H-6″), 1.24 (t, J = 7.05 Hz, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 160.4 (C-4), 150.4 (C-2), 145.0 (C-6), 87.4 (C-1′), 86.9 (d, J = 13.13 Hz, C-4′), 73.4 (C-2′), 69.7 (C-5), 68.1 (C-3′), 65.4 (d, J = 2.38 Hz, C-5′), 61.1 (dd, J = 15.12 and 6.19 Hz, O$\underline{C}$H$_2$CH$_3$), 30.3 (d, J = 139.06 Hz, C-6′), 16.2 (d, J = 5.99 Hz, OCH$_2\underline{C}$H$_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 28.9. MS ESI-QT of >0 m/z 520.9 (M + H)$^+$. HR-MS Calculated for $C_{14}H_{23}N_2O_9PI$: 521.0186; Found: 521.0196.

4.4.24. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-iodo-cytosine (**46**)

Derivative **46** (1.48 g, 2.85 mmol, 97%), was obtained by treatment of **44** (2.39 g, 2.95 mmol) as described for **45**. Rf (CH$_2$Cl$_2$/MeOH, 9/1, v/v) 0.44. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.23 (s, 1H, H-6), 7.88 (bs, 1H, NH), 6.68 (bs, 1H, NH), 5.71 (d, J = 5.14 Hz, 1H, H-1′), 5.62 (d, J = 5.10 Hz, 1H, OH), 5.35 (d, J = 5.61 Hz, 1H, OH), 5.02 (d, J = 5.16 Hz, 1H, OH), 4.00 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.82 (m, 1H, H-4′), 1.97 (m, 2H, H-6′, H-6″), 1.23 (t, J = 7.4 Hz, 6H, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 163.6 (C-4), 154.1 (C-2), 147.8 (C-6), 88.9 (C-1′), 86.3 (d, J = 13.34 Hz, C-4′), 73.6 (C-2′), 68.0 (C-3′), 65.3 (d, J = 2.56 Hz, C-5′), 61.0 (dd, J = 6.23 and 19.75 Hz, O$\underline{C}$H$_2$CH$_3$), 56.76 (C-5), 29.9 (d, J = 137.55 Hz, C-6′), 16.2 (d, J = 5.97 Hz, OCH$_2\underline{C}$H$_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.0. MS ESI-QT of >0 m/z 520.0 (M + H)$^+$. HR-MS Calculated for $C_{14}H_{24}N_3O_8PI$: 520.0346; Found: 520.0319.

4.4.25. General procedure for Sonogashira coupling reaction (compounds **47a**–**c** and **48a**–**c**)

The C5-iodonucleotide (1 eq.) was dissolved in anhydrous DMF (30 mL/mmol), Pd(PPh$_3$)$_4$ (0.2 eq.) and CuI (0.4 eq.) were added and then the required alcyne derivative (6 eq.) and Et$_3$N (4 eq.) were added dropwise under Ar atmosphere. The reaction mixture was stirred at room temperature in the dark, until completion of the reaction was indicated by TLC. Then, the reaction was concentrated to dryness under high vacuum. Column chromatography of the crude mixture on silica gel (dichloromethane/methanol, 9/1, v/v) and eventually reverse phase (gradient: water to methanol, 30%) gave the expected derivative.

4.4.25.1. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-hexyn-1-yl-uracil (**47a**).

According to general procedure, the title compound (39 mg, 35%) was obtained, as yellow solid, from **45** (120 mg, 0.24 mmol) and n-hexyne (223 μL, 1.94 mmol). Rf (EtOAc/MeOH, 8/2, v/v) 0.38. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.58 (bs, 1H, NH), 8.03 (s, 1H, H-6), 5.79 (d, J = 4.20 Hz, 1H, H-1′), 5.58 (d, J = 5.75 Hz, 1H, OH), 5.41 (d, J = 6.01 Hz, 1H, OH), 5.09 (d, J = 4.77 Hz, 1H, OH), 4.03–3.95 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.79 (dd, J = 3.25 Hz, 1H, H-4′), 2.37 (t, J = 6.73 Hz, 2H, CH$_2$), 2.01 (m, 2H, H-6′, H-6″), 1.45 (m, 4H, CH$_2$), 1.22 (td, J = 7.06 and 1.96 Hz, OCH$_2$C$\underline{H}_3$), 0.85 (t, 3H, CH$_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 161.1 (C-4), 149.8 (C-2), 142.8 (C-6), 95.4 and 93.4 (C≡C), 87.2 (C-1′), 87.1 (d, J = 13.98 Hz, C-4′), 73.1 (C-2′), 72.6 (C-3′), 68.3 (C-5′), 65.4 **(C-5)**, 61.9 (dd, J = 23.73 and 5.95 Hz, O$\underline{C}$H$_2$CH$_3$), 30.2 (CH$_2$), 28.4 (d, J = 137.67 Hz, C-6′), 21.3 and 18.5 (CH$_2$), 16.2 (OCH$_2\underline{C}$H$_3$), 13.4 (CH$_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 31.8. MS ESI-QT of >0 m/z 447 (M + H)$^+$. HR-MS Calculated for $C_{18}H_{28}N_2O_9P$: 447.1532; Found: 447.1535.

4.4.25.2. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-(3,3-dimethyl-1-butyn-1-yl)-uracil (**47b**).

According to general procedure, the title compound (114 mg, 42%) was obtained, as a yellow solid, from **45** (300 mg, 0.6 mmol) and 3,3-dimethyl-1-butyne (448 μL, 3.6 mmol). Rf (EtOAc/MeOH, 8/2, v/v) 0.31. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.60 (bs, 1H, NH), 8.00 (s, 1H, H-6), 5.75 (d, J = 6.24 Hz, 1H, H-1′), 5.58 (d, J = 5.69 Hz, 1H, OH), 5.39 (d, J = 5.59 Hz, 1H, OH), 5.09 (d, J = 4.83 Hz, 1H, OH), 4.05–3.97 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.80 (m, 1H, H-4′), 2.02 (m, 2H, H-6′, H-6″), 1.25 (s, 9H, C(CH$_3$)$_3$), 1.22 (td, J = 7.06 and 1.96 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 162.7 (C-4), 151.0 (C-2), 144.1 (C-6), 102.3 and 100.5 (C≡C), 88.5 (C-1′), 88.2 (d, J = 13.89 Hz, C-4′), 74.3 (C-2′), 72.3 (C-3′), 69.4 (C-5′), 66.6 **(C-5)**, 61.0 (dd, J = 23.73 and 5.95 Hz, O$\underline{C}$H$_2$CH$_3$), 31.9 (C($\underline{C}$H$_3$)$_3$), 29.7 (d, J = 137.60 Hz, C-6′), 16.2 (OCH$_2\underline{C}$H$_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 31.8. MS ESI-QT of >0 m/z 447 (M + H)$^+$. HR-MS Calculated for $C_{18}H_{28}N_2O_9P$: 447.1532; Found: 447.1535.

4.4.25.3. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-phenylethynyl-uracil (**47c**).

According to general procedure, the title compound (184 mg, 94%) was obtained, as a yellow solid, from **45** (205 mg, 0.39 mmol) and phenylacetylene (260 μL, 2.34 mmol). Rf (CH$_2$Cl$_2$/MeOH, 9/1, v/v) 0.20. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.74 (bs, 1H, NH), 8.31 (s, 1H, H-6), 7.50–7.40 (m, 5H, H–Ar), 5.80 (d, J = 5.62 Hz, 1H, H-1′), 5.67 (d, J = 5.46 Hz, 1H, OH), 5.44 (d, J = 5.48 Hz, 1H, OH), 5.09 (d, J = 5.07 Hz, 1H, OH), 4.04–3.97 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.86 (m, 1H, H-4′), 2.14–1.86 (m, 2H, H-6′, H-6″), 1.23 (td, J = 7.06 and 1.96 Hz, OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 161.2 (C-4), 149.7 (C-2), 143.9 (C-6), 131.1, 128.6, 122.3 (C–Ar), 98.4 and 91.8 (C≡C), 87.5 (C-1′), 86.9 (d, J = 13.32 Hz, C-4′), 82.2 (C-5), 73.5 (C-2′), 68.1 (C-3′), 65.3 (C-5′), 61.0 (dd, J = 23.73 and 5.95 Hz, O$\underline{C}$H$_2$CH$_3$), 30.1 (d, J = 137.50 Hz, C-6′), 16.2 (OCH$_2\underline{C}$H$_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.0. MS ESI-QT of >0 m/z 495.3 (M + H)$^+$. HR-MS Calculated for $C_{22}H_{28}N_2O_9P$: 495.1532; Found: 495.1540.

4.4.25.4. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-hexyn-1-yl-cytosine (**48a**).

According to general procedure, the title compound (135 mg, 68%) was obtained, as a white solid from **46** (217 mg, 0.42 mmol) and n-hexyne (286 μL, 2.52 mmol). Rf (CH$_2$Cl$_2$/MeOH, 9/1, v/v) 0.32. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.03 (s, 1H, H-6), 7.73 (bs, 1H, NH), 6.77 (bs, 1H, NH), 5.77 (d, J = 5.47 Hz, 1H H-1′), 5.54 (d, J = 5.20 Hz, 1H, OH), 5.31 (d, J = 5.39 Hz, 1H, OH), 5.01 (d, J = 4.92 Hz, 1H, OH), 4.01 (m, 7H, OC$\underline{H}_2$CH$_3$, H-2′, H-3′, H-5′), 3.80 (m, 1H, H-4′), 2.40 (t, J = 6.98 Hz, 2H, C$\underline{H}_2$–C≡C), 1.96 (m, 2H, H-6′, H-6″), 1.48 (m, 4H, CH$_2$), 1.23 (t, J = 7.05 Hz, 6H, OCH$_2$C$\underline{H}_3$), 0.89 (t,

J = 7.20 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.3 (C-4), 153.8 (C-2), 144.2 (C-6), 95.7 and 90.7 (C≡C), 88.6 (C-1′), 86.6 (d, J = 14.32 Hz, C-4′), 73.6 (C-2′), 71.7 (C-3′), 68.1 (C-5), 65.4 (d, J = 2.97 Hz, C-5′), 61.1 (dd, J = 17.63, 6.07 Hz, O$\underline{C}H_2CH_3$), 30.2 (d, J = 137.79 Hz, C-6′), 30.1 ($\underline{C}H_2C{\equiv}C$), 21.5, 18.7 ($CH_2$), 16.2 (d, J = 5.91 Hz, OCH$_2\underline{C}H_3$), 13.4 (CH_3). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.1. MS ESI-QT of >0 m/z 474.0 $(M + H)^+$. HR-MS Calculated for $C_{20}H_{33}N_3O_8P$: 474.2005; Found: 474.1999.

*4.4.25.5. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-(3,3-dimethyl-1-butyn-1-yl)-cytosine (**48b**).* According to general procedure, the title compound (145 mg, 80%) was obtained, as a white solid, from **46** (200 mg, 0.39 mmol) and 3,3-dimethyl-1-butyne (283 μL, 2.34 mmol). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.17. ^1H NMR (400 MHz, DMSO-d_6) δ = 8.02 (s, 1H, H-6), 7.75 (bs, 1H, NH), 6.49 (bs, 1H, NH), 5.76 (d, J = 5.52 Hz, 1H, H-1′), 5.53 (d, J = 5.29 Hz, 1H, OH), 5.29 (d, J = 5.75 Hz, 1H, OH), 4.98 (d, J = 5.16 Hz, 1H, OH), 3.99 (m, 7H, O$\underline{C}H_2CH_3$, H-2′, H-3′, H-5′), 3.81 (m, 1H, H-4′), 2.05 (m, 2H, H-6′, H-6″), 1.27 (s, 9H, CH_3), 1.23 (t, J = 7.05 Hz, 6H, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.0 (C-4), 153.8 (C-2), 144.4 (C-6), 103.1 and 90.5 (C≡C), 88.6 (C-1′), 86.4 (d, J = 13.82 Hz, C-4′), 73.5 (C-2′), 70.2 (C-3′), 68.1 (C-5), 65.4 (d, J = 2.73 Hz, C-5′), 61.1 (dd, J = 26.42 and 5.99 Hz, O$\underline{C}H_2CH_3$), 30.5 (CH_3), 30.0 (d, J = 137.97 Hz, C-6′), 27.8 (Cq), 16.2 (d, J = 6.05 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.2. MS ESI-QT of >0 m/z 474.2 $(M + H)^+$. HR-MS Calculated for $C_{20}H_{33}N_3O_8P$: 474.2005; Found: 474.2017.

*4.4.25.6. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-phenylethynyl-cytosine (**48c**).* According to general procedure, the title compound (165 mg, 80%) was obtained, as a white solid, from **46** (217 mg, 0.42 mmol) and phenylacetylene (275 μL, 2.52 mmol). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.17. ^1H NMR (400 MHz, DMSO-d_6) δ = 8.30 (s, 1H, H-6), 7.83 (bs, 1H, NH), 7.59 (m, 2H, H–Ar), 7.39 (m, 3H, H–Ar), 7.09 (bs, 1H, NH), 5.80 (d, J = 5.23 Hz, 1H, H-1′), 5.64 (d, J = 5.27 Hz, 1H, OH), 5.36 (d, J = 5.70 Hz, 1H, OH), 5.01 (d, J = 5.37 Hz, 1H, OH), 3.99 (m, 7H, O$\underline{C}H_2CH_3$, H-2′, H-3′, H-5′), 3.85 (m, 1H, H-4′), 1.99 (m, 2H, H-6′, H-6″), 1.23 (td, J = 7.04 and 2.24 Hz, 6H, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 163.7 (C-4), 153.7 (C-2), 145.6 (C-6), 131.2, 128.4, 122.5 (C–Ar), 93.7 and 89.8 (C≡C), 88.7 (C-1′), 86.4 (d, J = 13.92 Hz, C-4′), 81.4 (C-2′), 73.9 (C-3′), 68.0 (C-5), 65.3 (C-5′), 61.1 (dd, J = 26.11 and 6.14 Hz, O$\underline{C}H_2CH_3$), 30.0 (d, J = 137.41 Hz, C-6′), 16.2 (d, J = 5.98 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.1. MS ESI-QT of >0 m/z 494.2 $(M + H)^+$. HR-MS Calculated for $C_{22}H_{29}N_3O_8P$: 494.1692; Found: 494.1708.

*4.4.26. General procedure for Suzuki coupling reaction (compounds **49d**, **49e** and **50d**, **50e**)*

A round bottom flask containing C5-iodonucleotide (1 eq.), boronic acid derivative (1.3 eq.) and KOH (2 eq.) was purged with Ar. Upon addition of TPPS (0.025 eq.), $PdNa_2Cl_4$ (0.01 eq.) and degazed H_2O/DMF (1/1, v/v, 5 mL/mmol), the reaction mixture was stirred at 100 °C until completion of the reaction was indicated by HPLC. Then, the reaction mixture was concentrated to dryness under high vacuum. Column chromatography of the crude materials on silica gel (gradient: dichloromethane/methanol, 9/1, v/v) and eventually by reverse phase chromatography (water to methanol 50%) gave the expected derivative.

*4.4.26.1. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-phenyl-uracil (**49d**).* According to general procedure, the title compound (97 mg, 35%) was obtained, as a white solid, from **45** (305 mg, 0.59 mmol) and phenyl-boronic acid (93 mg, 0.77 mmol). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.35. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.56 (bs, 1H, NH), 8.07 (s, 1H, H-6), 7.53 (m, 2H, H–Ar), 7.37 (m, 3H, H–Ar), 5.87 (d, J = 5.19 Hz, 1H, H-1′), 5.54 (d, J = 5.51 Hz, 1H, OH), 5.43 (d, J = 5.41 Hz, 1H, OH), 5.09 (d, J = 4.78 Hz, 1H, OH), 4.13 (m, 2H, H-2′, H-3′), 3.99 (m, 5H, O$\underline{C}H_2CH_3$, H-5′), 3.87 (m, 1H, H-4′), 2.00 (m, 2H, H-6′, H-6″), 1.21 (td, J = 7.05 and 2.96 Hz, 6H, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 162.0 (C-4), 150.2 (C-2), 138.0 (C-6), 132.9 (Cq), 128.0, 127.2 (C–Ar), 113.7 (C-5), 87.5 (C-1′), 86.8 (d, J = 12.85 Hz, C-4′), 73.4 (C-2′), 68.3 (C-3′), 65.5 (d, J = 2.28 Hz, C-5′), 61.1 (dd, J = 23.60 and 6.05 Hz, O$\underline{C}H_2CH_3$), 30.1 (d, J = 137.39 Hz, C-6′), 16.2 (d, J = 5.87 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 28.9. MS ESI-QT of >0 m/z 471.2474 $(M + H)^+$. HR-MS Calculated for $C_{20}H_{28}N_2O_9P$: 471.1532; Found: 471.1518.

*4.4.26.2. 1-[6-Deoxy-6-diethylphosphono-β-D-allofuranosyl]-5-(2-(E)-phenylvinyl)-uracil (**49e**).* According to general procedure, the title compound (71 mg, 25%) was obtained, as a white solid, from **45** (301 mg, 0.58 mmol) and 2-(E)-phenylvinyl-boronic acid (111 mg, 0.75 mmol). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.30. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.41 (bs, 1H, NH), 8.10 (s, 1H, H-6), 7.49–7.23 (m, 6H, H–Ar, HC=C), 6.94 (d, J = 16.47 Hz, 1H, HC=C), 5.84 (d, J = 5.61 Hz, 1H, H-1′), 5.73 (bs, 1H, OH), 5.45 (bs, 1H, OH), 5.13 (bs, 1H, OH), 4.02 (m, 7H, O$\underline{C}H_2CH_3$, H-2′, H-3′, H-5′), 3.85 (m, 1H, H-4′), 2.02 (m, 2H, H-6′, H-6″), 1.24 (q, J = 6.80 Hz, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 162.1 (C-4), 149.8 (C-2), 138.3 (C-6), 137.5 (Cq), 128.6, 127.6, 127.3, 125.9 (C–Ar, $\underline{C}$=C), 121.2 (C=C), 110.9 (C-5), 87.4 (C-1′), 87.0 (d, J = 14.42 Hz, C-4′), 73.5 (C-2′), 68.4 (C-3′), 65.5 (C-5′), 61.1 (dd, J = 31.48 and 6.0 Hz, O$\underline{C}H_2CH_3$), 30.1 (d, J = 138.0 Hz, C-6′), 13.2 (d, J = 5.77 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.3. MS ESI-QT of >0 m/z 497.2 $(M + H)^+$. HR-MS Calculated for $C_{22}H_{30}N_2O_9P$: 497.1689; Found: 497.1682.

*4.4.26.3. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-phenyl-cytosine (**50d**).* According to general procedure, the title compound (203 mg, 59%) was obtained, as a white lyophilisate, from **46** (203 mg, 0.39 mmol) and phenyl-boronic acid (62 mg, 0.51 mmol). R*f* (CH_2Cl_2/MeOH, 9/1, v/v) 0.17. ^1H NMR (400 MHz, D_2O) δ = 7.75 (s, 1H, H-6), 7.49–7.41 (m, 5H, H–Ar), 5.94 (d, J = 4.10 Hz, 1H, H-1′), 4.34–4.17 (m, 3H, H-2′, H-3′, H-5′), 4.10 (m, 4H, O$\underline{C}H_2CH_3$), 3.98 (t, J = 4.41 Hz, 1H, H-4′), 2.18 (m, 2H, H-6′, H-6″), 1.26 (t, J = 7.09 Hz, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, D_2O) δ = 162.4 (C-4), 154.9 (C-2), 138.0 (C-6), 130.0, 127.2, 127.0, 126.7 (C–Ar), 108.5 (C-5), 87.9 (C-1′), 83.9 (d, J = 17.87 Hz, C-4′), 71.6 (C-2′), 66.8 (C-3′), 63.6 (d, J = 4.58 Hz, C-5′), 61.3 (d, J = 7.16 Hz, O$\underline{C}H_2CH_3$), 27.0 (d, J = 140.95 Hz, C-6′), 13.4 (d, J = 5.74 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, D_2O) δ = 31.68. MS ESI-QT of >0 m/z 470.0 $(M + H)^+$. HR-MS Calculated for $C_{20}H_{29}N_3O_8P$: 470.1692; Found: 470.1705.

*4.4.26.4. 1-[6-Deoxy-diethylphosphono-β-D-allofuranosyl]-5-(2-(E)-phenylvinyl)cytosine (**50e**).* According to general procedure, the title compound (86 mg, 45%) was obtained, as a white solid, from **46** (202 mg, 0.39 mmol) and 2-(E)-phenylvinylboronic acid (75 mg, 0.51 mmol). R*f* (CH_2Cl_2/MeOH, 7/2/1, v/v/v) 0.57. ^1H NMR (400 MHz, DMSO-d_6) δ = 8.28 (s, 1H, H-6), 7.58–7.21 (m, 7H, H–Ar, NH_2), 7.09 (d, J = 16.11 Hz, 1H, HC=), 6.89 (d, J = 16.04 Hz, 1H, HC=), 5.81 (d, J = 4.56 Hz, 1H, H-1′), 5.70 (d, J = 5.29 Hz, 1H, OH), 5.38 (d, J = 5.22 Hz, 1H, OH), 5.01 (d, J = 5.33 Hz, 1H, OH), 4.13–3.99 (m, 7H, O$\underline{C}H_2CH_3$, H-2′, H-3′, H-5′), 3.87 (m, 1H, H-4′), 2.18–1.89 (m, 2H, H-6′, H-6″), 1.22 (m, OCH$_2\underline{C}H_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 164.0 (C-4), 155.0 (C-2), 138.4 (C-6), 137.7, 128.9, 127.8, 127.7, 126.9 (C–Ar, C=C), 119.9 (C=C), 104.7 (C-5), 89.9 (C-1′), 86.5 (d, J = 13.89 Hz, C-4′), 74.4 (C-2′), 68.6 (C-3′), 65.9 (C-5′), 61.6 (dd, J = 29.58 and 6.18 Hz, O$\underline{C}H_2CH_3$), 30.5 (d, J = 137.36 Hz, C-6′), 16.7 (d, J = 5.95 Hz, OCH$_2\underline{C}H_3$). ^{31}P NMR (81 MHz, DMSO-d_6) δ = 29.2. MS ESI-QT of>0 m/z 496.1 $(M + H)^+$. HR-MS Calculated for $C_{22}H_{31}N_3O_8P$: 496.1849; Found: 496.1848.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.055.

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