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5′,6′-Nucleoside Phosphonate Analogues Architecture: Synthesis and Comparative Evaluation towards Metabolic Enzymes

Franck Gallier,[a] Julie A. C. Alexandre,[b] Chahrazade El Amri,[b] Dominique Deville-Bonne,[b] Suzanne Peyrottes,*[a] and Christian Périgaud[a]

Nucleoside phosphonates have been designed as stable 5′-mononucleotide mimics and are nowadays considered a potent class of antiviral agents. Within cells, they must be metabolised to the corresponding diphosphate to exert their biological activity. In this process, the first phosphorylation step, catalysed by nucleoside monophosphate kinases (NMP kinases), has been proposed as a bottleneck. Herein, we report the synthesis of a series of ribonucleoside phosphonate derivatives isosteric to 5′-mononucleotides, with different degrees of flexibility within the 5′,6′-C–C bond, as well as different polarities, through the introduction of hydroxy groups. The influence of these modifications on the capacity of the compounds to act as substrates for appropriate human NMP kinases, involved in nucleic acids metabolism, has been investigated. Low flexibility, as well as an absence of hydroxy groups within the ribose–phosphorus architecture, is critical for efficient phosphotransfer. Among the series of pyrimidine analogues, one derivative was shown to be phosphorylated by human UMP-CMP kinase, with rates similar to those of dUMP and even better than dCMP.

Introduction

In the search for new and effective antiviral/anticancer agents, various modifications to nucleosides have been proposed, that is, the nucleobase and/or sugar moiety.[1] However, to interfere with the nucleic acid biosynthesis, nucleoside analogues must be converted to their corresponding triphosphate derivatives.[2] The first step in this process is the phosphorylation of the nucleoside analogue to the 5′-monophosphate (NMP) by a nucleoside kinase. This step is often considered to be rate limiting in the overall formation of the active metabolite, the nucleoside 5′-triphosphate. In this context, acyclic nucleoside phosphonates (ANPs) have emerged as a key class of antiviral agents[3] because, unlike nucleoside analogues, they do not require the initial phosphorylation step.[4] Their efficacy was proven against various infections, including human immunodeficiency virus (HIV), hepatitis B virus (HBV) or cytomegalovirus (CMV). While many nucleoside analogues have been successfully developed for DNA viruses, this class of compounds is still under investigation for RNA viruses, for which hepatitis C is the archetype. Over the past few years, a significant effort has been devoted to the discovery and the development of nucleoside analogues to treat infection by hepatitis C virus (HCV) with the aim of improving currently available therapies. Several classes of ribonucleoside analogues that include modifications on the ribose moiety have been shown to inhibit HCV replication. Among them, analogues containing a methyl group at the 2′-C position have been shown to be potent inhibitors and to exert their activity as functional chain terminators of RNA synthesis. Recently, beta-D-2′-ethynyl-7-deaza-adenosine was reported as a promising scaffold for the development of dengue virus polymerase inhibitors.

Herein, we describe the development of an original series of nucleoside 5′-monophosphate analogues (NMP, mononucleotides) focusing on phosphonate derivatives containing a ribofuranose ring.[5] Particularly, we explored a series of NMP isosteres, α,β-modified phosphonate nucleosides (Figure 1), presenting

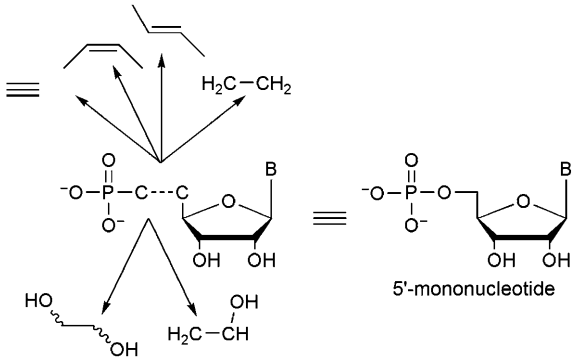


Figure 1. Generic structures of the targeted mononucleotide isosteres.

[a] Dr. F. Gallier, Dr. S. Peyrottes, Prof. C. Périgaud
Institut des Biomolécules Max Mousseron (IBMM)
UMR 5247 CNRS-UM1&2, Université Montpellier 2, cc1705
Place E. Bataillon, 34095 Montpellier Cedex 5 (France)
Fax: (+33) 467-042-029
E-mail: suzanne.peyrottes@univ-montp2.fr

[b] Dr. J. A. C. Alexandre, Dr. C. El Amri, Dr. D. Deville-Bonne
Groupe d'Enzymologie Moléculaire et Fonctionnelle
Unité de Recherches 4, Université Paris 6, cc 256
7 Quai Saint Bernard, 75252 Paris Cedex 5 (France)

different degrees of flexibility within the 5′,6′-C–C bond. Furthermore, we examined whether these modifications affect their phosphorylation by human NMP kinases.

Results and Discussion

Synthesis

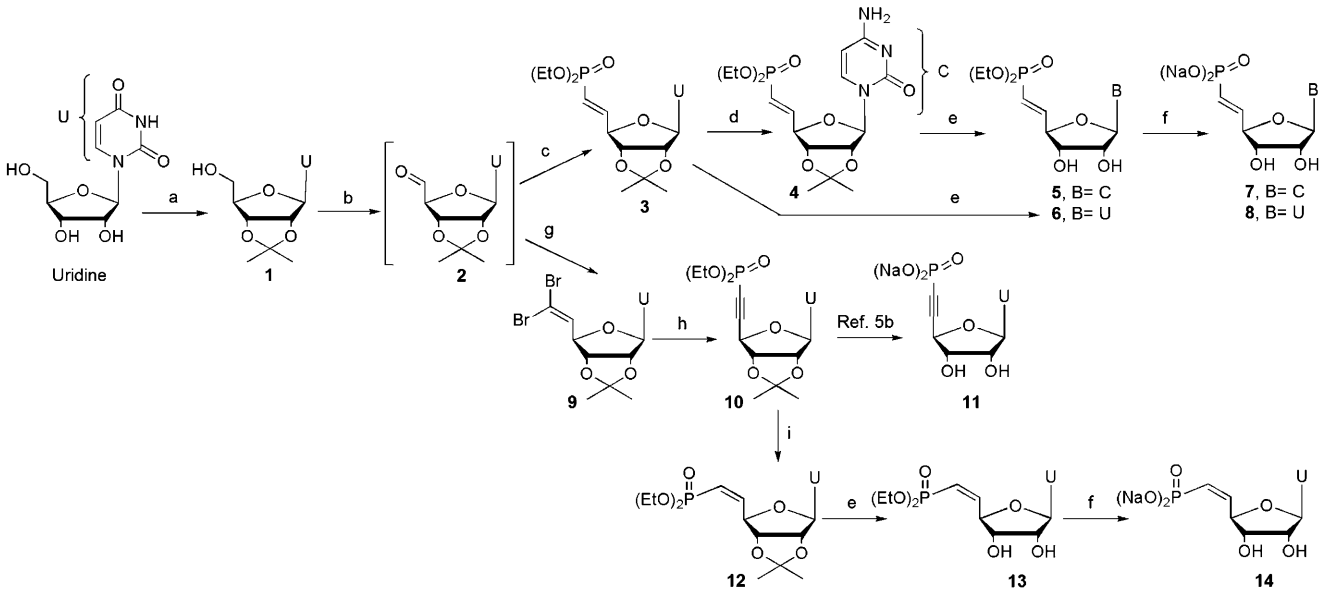
Both osidic (and further condensation of the nucleobases) and nucleosidic strategies may lead to the targeted derivatives (Figure 1). The latter option was selected and applied to commercially available and inexpensive uridine (Scheme 1) in order to access model compounds for the assays in only a few steps of chemical synthesis.

We first investigated the preparation of vinylphosphonate derivatives **7**, **8** and **14** (Scheme 1), and alkynylphosphonate **11** was obtained as previously described.[5b] The acetonide **1** was obtained after crystallisation and submitted to oxidation according to previously published procedures.[6] The common 5′-aldehyde intermediate **2** was directly used either in a Wittig or a Corey–Fuchs[7] type reaction, leading to the *E*-vinylphosphonate **3** or the vinyldibromo derivative **9**, respectively. Then, alkynylphosphonate **10** was obtained in 61% yield from **9** using a palladium-catalysed phosphonylation procedure described by Lera and Hayes.[8] The cytosine-containing *E*-vinylphosphonate derivative **4** was obtained in good yield from its corresponding uridine analogue **3** following previously published procedure.[9]

Removal of the 2′,3′-isopropylidene protecting group was performed upon acidic treatment of **3** and **4**, and gave derivatives **5** and **6** in moderate yields. Finally, hydrolysis of the phosphonate esters was carried out using trimethylsilyl bromide (TMSBr) under standard conditions, and the corresponding phosphonic acids **7** and **8** were obtained after purification on reverse-phase chromatography and subsequent ion exchange.

In order to obtain the vinylphosphonate **14** with *Z* stereochemistry, two synthetic pathways involving either the Horner–Wadsworth–Emmons (HWE) procedure modified by Still and Gennari[10] or the reduction of an alkynyl phosphonate[11] can be used. As the stereoselectivity of the HWE reaction depends largely on the substrate, we embarked on the reduction of the previously prepared alkynyl intermediate **10**. Formation of the *Z* alkene can be achieved using the hydrometallation of simple alkynes,[12] and a modification of the initial procedure following Xiong et al.[13] allows the use of a commercially available zirconium complex. In contrast, Cristau et al.[14] reported that, depending on the nature of the substrates, the reduction of an alkyne by a simple hydrogenation procedure may lead to the formation of the alkene of inverse stereochemistry *E* (~5–10%), as well as the fully saturated derivative (<5%). Thus, we firstly explored the reduction (hydrozirconation) of alkynylphosphonate **10** under the conditions described in the literature,[13] and the starting material was recovered unchanged. Among the modifications of the experimental conditions (time, temperature, amount of the reagent) that were tested, the use of a two-fold excess of the zirconium complex led to the formation of a vinylphosphonate derivative in 60% yield. However, analysis of the ^{1}H and ^{31}P NMR spectra and comparison with literature data[6c,15] (Table 1) showed that only the *E* stereoisomer was isolated.

Finally, synthesis of *Z*-vinylphosphonate **12** was performed using poisoned hydrogenation (Lindlar catalyst) of alkyne **10**. The ^{31}P NMR spectra of the reaction mixture clearly showed the formation of a major product with a peak at 14.7 ppm corresponding to the *Z* isomer; minor signals were attributed to the *E* isomer ($\delta P = 16.9$ ppm, <5%), the alkane derivative



Scheme 1. Synthetic routes to compounds **7**, **8**, **11** and **14** from uridine. *Reagents and conditions*: a) $Me_2C(OMe)_2$, *p*-toluenesulfonic acid (PTSA), acetone, 81%; b) CrO_3, Ac_2O, pyridine, CH_2Cl_2; c) $(EtO)_2POCHP\Phi_3$, DMSO, 50%; d) N-Me-pyrrolidine, $(CF_3CO)_2O$, *p*-nitrophenol, CH_3CN, 60%; e) TFA_{aq} 7/3, 60%; f) TMSBr, DMF then H_2O and Dowex Na^+, 70–90%; g) CBr_4, PΦ3, CH_2Cl_2, 25%; h) $(EtO)_2POH$, $PdAc_2$, 1,1′-bis(diphenylphosphino)ferrocene (dppf), 61%; i) H_2, Lindlar catalyst, quinoline, EtOH, 90%.

Table 1. Comparison of the coupling constants obtained from the literature data[14,16] and from compounds isolated using hydrozirconation or hydrogenation reactions.

	Coupling constants (*J*) [Hz]			
	Z isomer	*E* isomer	Hydrozirconation	Hydrogenation[a]
$J_{cis-H,H}$	8–15	–	–	13.2
$J_{trans-H,H}$	–	14–18	17.1	–
$J_{cis-H,P}$	–	10–30	22.5	–
$J_{trans-H,P}$	30–50	–	–	51.0
$J_{gem-H,P}$	12–20	12–20	20.3	17.1

[a] $J_{P-C4'}$ (8.1 Hz) was determined by ^{13}C NMR and was in agreement with the data reported by Quntar et al. for *Z*-vinylphosphonates.[12]

($\delta P = 31.2$ ppm, $< 5\%$), and the initial substrate ($\delta P = -8.4$ ppm, $< 5\%$). Derivative **12** was isolated in 91% yield and the values of the coupling constants were in agreement with the literature data (Table 1). Removal of the 2′,3′-isopropylidene protecting group and hydrolysis of the phosphonate esters were performed as described earlier and afforded derivatives **13** and **14** in good yields (Scheme 1).

Then, we considered modification of the double bond of compound **3** using standard hydrogenation, dihydroxylation or epoxydation conditions (Scheme 2). Hydrogenation of *E*-vinylphosphonate **3** in the presence of Pd/C following a previously published procedure[17] led to the fully saturated derivative **15** in 88% yield. Deprotection steps were performed as described earlier, and compounds **16** and **17** were isolated in high yields. The Sharpless dihydroxylation procedure[18] usually reported calls for the use of a catalytic amount of osmium tetraoxyde, however, we performed the dihydroxylation of compound **3** under the conditions (almost stoichiometric) described by Jung et al.[19] for similar substrates. We observed the formation of the two diastereoisomers **18a**,**b**, which were separated by reverse-phase chromatography. The use of AD-mix α or β led to a modest diastereoisomeric excess of 68% and 46%, respectively. The same major compound was obtained for both attempts, and the stereochemistry was ascertained on the basis of literature data.[19] This result illustrates the fact that the substrate is driving the reaction when a chiral starting material is used. In agreement with the Kishi rules,[20] the major isomer probably results from attack of the osmium tetraoxyde on the less bulky face of the nucleoside and with an anti orientation towards the allylic oxygen atom. Deprotected derivatives **19a**,**b** and **20a**,**b** were obtained following general procedures described earlier.

Finally, various attempts for the direct epoxydation of the *E*-vinylphosphonate were unsuccessful,[21] and derivative **22** was isolated in low yield (15% over two steps) via an alternative route.[22] From diol **18a**, a tosylated intermediate was made that underwent intramolecular cyclisation. However, due to the low availability of the starting diol, this synthesis was not pursued.

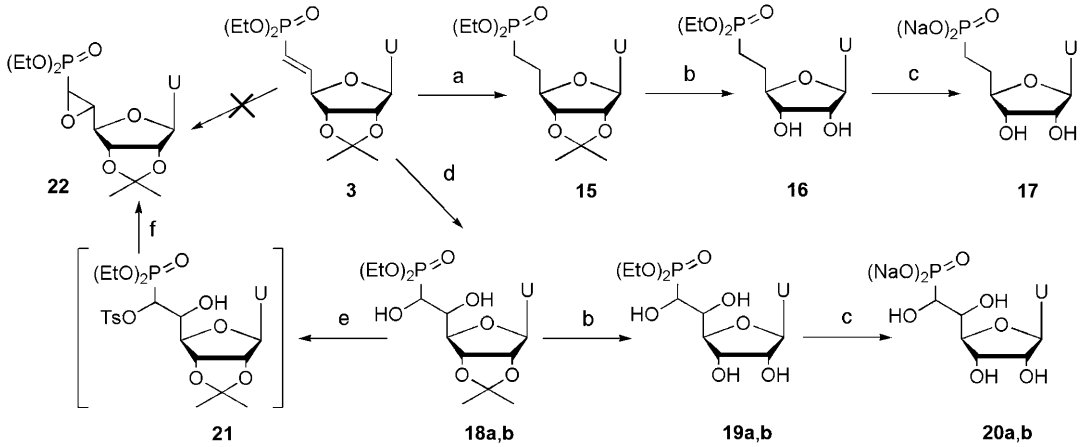
Biological activity in cell culture experiments

All compounds were subjected to standard in vitro antiviral assays against a panel of RNA viruses, unfortunately none of them showed remarkable activity (up to 75 μM). They were also tested in cell culture experiments (L1210, Messa, and MCF7 cell lines) for their ability to inhibit cell growth, but none of them exhibited significant cytostatic or cytotoxic activity.

The lack of biological activity of these derivatives might be attributed to several features, including 1) their inability to diffuse through the cell membrane; 2) their effective conversion from the parent compound to the corresponding triphosphate analogue; 3) their affinity for the target polymerases and/or efficiency of incorporation into viral RNA. Therefore, we engaged in the study of their capacity to act as substrates for appropriate human NMP kinases.

Reaction of 5′,6′-nucleoside phosphonate analogues with human NMP kinases

All of the structurally modified nucleoside phosphonic acids (**7**, **8**, **11**, **14**, **17**, **20a**,**b**), a shorter analogue (**23**, Figure 2),[23] as well as a series of ribonucleoside β-hydroxyphosphonate analogues (**24**–**27**, Figure 2)[5a] previously obtained, were tested



Scheme 2. Synthetic routes to compounds **17** and **20** from intermediate **3**. *Reagents and conditions*: a) H_2, Pd/C, EtOH, 88%; b) aq TFA (70%), 95%; c) 1. TMSBr, DMF; 2. H_2O, Dowex Na^+, 65–90%; d) K_2OsO_4, aq *t*-BuOH, Ad-mix α or β, $MeSO_2NH_2$, 50%; e) TsCl, pyridine; f) K_2CO_3, acetone, 15%.

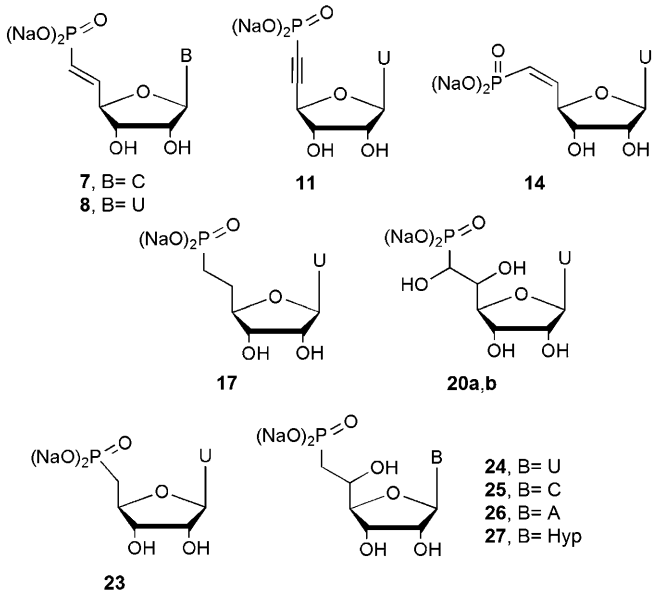


Figure 2. Structures of the studied compounds towards hNMP kinases.

against hUMP-CMPK (Table 2) and hAMPKs (Table 3) and compared to natural NMPs, as well as some analogues of biological interest.

As shown in Table 2, within the uridine phosphonate analogues, removal of the oxygen atom bridging the carbon in 5′-position (C-5′) and the phosphorus atom prevented any reaction of **23** with the enzyme. Replacement of this oxygen by a methylene group (derivative **17**) restored the reaction but without saturation of the enzyme.

The presence of a double bond between C-5′ and C-6′ with a *Z* configuration forced the phosphonate group towards the alpha face of the nucleoside analogue, and this was not tolerated by the enzyme; compound **14** is not a substrate. In contrast, the *E* isomer of **8** was a substrate with an excellent rate constant ($k_{cat}=50\ s^{-1}$), with approximately 40% of the UMP reaction rate, however, a lower affinity ($K_m=3.8$ mM) was observed. Furthermore, a triple bond in the C5′–C6′ position was also favourable; alkyne **11** had a better affinity ($K_m=1.3$ mM) than alkene **8**, and despite a slower rate constant, it showed improved catalytic efficiency ($k_{cat}/K_m=1.8\times10^4\ M^{-1}s^{-1}$, 0.6% of UMP catalytic efficiency). The presence of a hydroxy group at C-5′ (**24**) was tolerated by hUMP-CMPK, as well as hydroxy groups at both C-5′ and C-6′ in derivative **20a**. However, this analogue was found to be a poor substrate for the enzyme, with weak affinity and a slow phosphorylation rate.

The best modification in the uridine series translated into the cytosine series, as compound **7** (which includes an *E* double bond between C-5′ and C-6′) was the best substrate for hUMP-CMPK, with a catalytic efficiency about 3% that of CMP (k_{cat}/K_m in the $10^5\ M^{-1}s^{-1}$ range). Although the addition of a hydroxy group at C-5′ on the cytosine derivative **25** was less well tolerated, this compound was still phosphorylated by hUMP-CMPK ($5000\ M^{-1}s^{-1}$, 0.2% of CMP catalytic efficiency), with an efficiency far superior to that of cidofovir ($CE=60\ M^{-1}s^{-1}$), the broad-spectrum ANP analogue used in clinics.[24]

Adenosine phosphonate **26** bearing a hydroxy group in the C-5′ position was also found to be phosphorylated by hAMPK1 and hAMPK2 (Table 3), contrary to the inosine analogue **27**. The cytoplasmic (hAMPK1) and mitochondrial (hAMPK2) AMP kinases both recognised the analogue with a K_m value only ten-times higher than AMP. The phosphorylation rates were quite good, particularly with hAMPK2, resulting in high catalytic efficiency ($5\times10^4\ M^{-1}s^{-1}$, 5% the catalytic efficiency for AMP), far above that of tenofovir ($800\ M^{-1}s^{-1}$), a slow substrate for the enzyme.[25] Additionally, analogues that are not NMP kinase substrates were examined for inhibitory activity with regard to the target enzymes, but none of them led to any inhibition.

Table 2. Catalytic parameters of pyrimidine phosphonate analogues with hUMP-CMPK compared to natural substrates and biologically relevant analogues.

Substrate[a]	K_m [mM]	V [U mg^{-1}]	% V	k_{cat} [s^{-1}]	k_{cat}/K_m [M^{-1}s^{-1}]	% k_{cat}/K_m for CMP	% k_{cat}/K_m for UMP
D-CMP	0.020±0.005	350±30	100	130	6.5×10^6	100	232
D-UMP	0.05±0.01	350±30	100	130	2.8×10^6	43	100
D-dCMP	1.1±0.2	198±13	57	80	7×10^4	1	2.5
D-dUMP	1.3±0.3	18±1	5	7.3	6×10^3	0.09	0.2
L-3TCMP	0.15±0.02	100±10	29	36	2.8×10^5	4.3	10
araCMP	0.26±0.05	400±40	114	150	5.8×10^5	8.9	2.1
Cidofovir	1.0±0.3	0.14±0.05	0.04	0.06	60	0.0009	0.002
23	ND	ND	–	–	–	–	–
17	ND	ND	–	–	3.2×10^3	0.05	0.11
8	3.8±1.3	129±19	37	52	1.3×10^4	0.2	0.42
14	ND	ND	–	–	–	–	–
11	1.3±0.2	58±3	17	24	1.8×10^4	0.3	0.64
24	4±1	4.2±0.4	1.2	1.7	395	0.006	0.014
20a	6±2	0.13±0.03	0.04	0.05	9	0.0001	0.0003
7	0.52±0.09	215±12	61	87	1.7×10^5	2.6	6.1
25	3.3±0.5	40±2	11	16	5×10^3	0.08	0.18

[a] Kinetic parameters of hUMP-CMPK for natural and biologically relevant analogues taken from the report by Pasti et al.[26] and Alexandre et al.;[27] ND: not detectable.

Structure-based molecular docking of 5′-6′-nucleoside phosphonates within the NMP site of hUMP-CMPK and hAMPK1

In order to rationalise the findings of the enzymatic assays, molecular docking was performed to predict an atomic-level picture of the interactions between the most efficiently phosphorylated derivatives (**7** and **11** for pyrimidine derivatives and **26** for purine derivatives) and key residues in the NMP site (Figure 3a–c). Although NMP kinases have a conserved three-dimensional structure, their NMP binding sites differ in certain residues

Table 3. Catalytic parameters of purine β-hydroxyphosphonate analogues **26** and **27** with hAMPK1 and hAMPK2 compared to natural substrates and biologically relevant analogues.

Enzyme	Substrate[a]	K_m [mM]	V [U mg^{-1}]	% V	k_{cat} [s^{-1}]	k_{cat}/K_m [M^{-1} s^{-1}]	% k_{cat}/K_m
hAMPK1	D-AMP	0.14 ± 0.02	1240 ± 20	100	500	3×10^6	100
	PMPA (Tenofovir)	3.0 ± 0.3	0.60 ± 0.03	0.05	0.22	75	0.003
	PMEA Adefovir)	6 ± 1	0.21 ± 0.03	0.02	0.08	14	0.0005
	26	1.9 ± 0.3	11.0 ± 0.7	0.9	4.5	2.3×10^3	0.08
	27	ND	ND	–	–	–	–
hAMPK2	D-AMP	0.08 ± 0.02	170 ± 25	100	80	1×10^6	100
	PMPA (Tenofovir)	3.0 ± 0.3	5.0 ± 0.1	3	2.4	800	0.08
	PMEA (Adefovir)	6.0 ± 0.5	3.2 ± 0.1	2	1.5	250	0.03
	26	1.0 ± 0.1	122 ± 7	72	50	5.2×10^4	5.2
	27	ND	ND	–	–	–	–

[a] Kinetic parameters of AMP, PMPA and PMEA are taken from the report by Alexandre et al.[27] and Topalis et al.;[25c] ND: not detectable.

accounting for substrate specificities. Despite their 40% sequence identity, hAMPK1 and hUMP-CMCK vary substantially in their substrate repertoires.[4b] In particular, we and others have previously reported that cytosolic and mitochondrial AMP kinases are strictly enantioselective, while UMP-CMP kinase is able to phosphorylate L-CMP and L-3TCMP.[26–28] Additionally, hUMP-CMPK plays a key role in the activation of cidofovir, the well-established ANP with broad-spectrum anti-DNA virus activities. Furthermore, hAMPK has been shown to slowly phosphorylate ANPs that are dAMP mimics, such as 9-(2-(phosphonylmethoxy)ethyl)adenine (adevofir, PMEA) approved for use against HBV in 2002, and (*R*-9-(2-(phosphonomethoxyl)propyl)adenine (tenofovir, PMPA), approved for use against HIV-1 in 2001 and HBV in 2008.[3]

Figure 3 illustrates how our best substrates could potentially occupy the NMP site of the corresponding NMP kinases. The positioning of the rigid-linker-containing compounds **7** and **11** (Figure 3a and b) within the active site agrees well with their size. Compound **11** being shorter is predicted to have a weaker affinity for hUMP-CMPK than derivative **7**, and to be less efficiently phosphoryled (Table 2). The positioning of compound **7** is predicted to occupy the NMP pocket in a similar manner to CMP or UMP, namely the pyrimidine part is mostly involved in hydrophobic interactions with residues R42, I45, A37, V65 which form a hydrophobic patch, while the phosphate sugar moiety is securely anchored in the arginine pocket (Figure 3a).

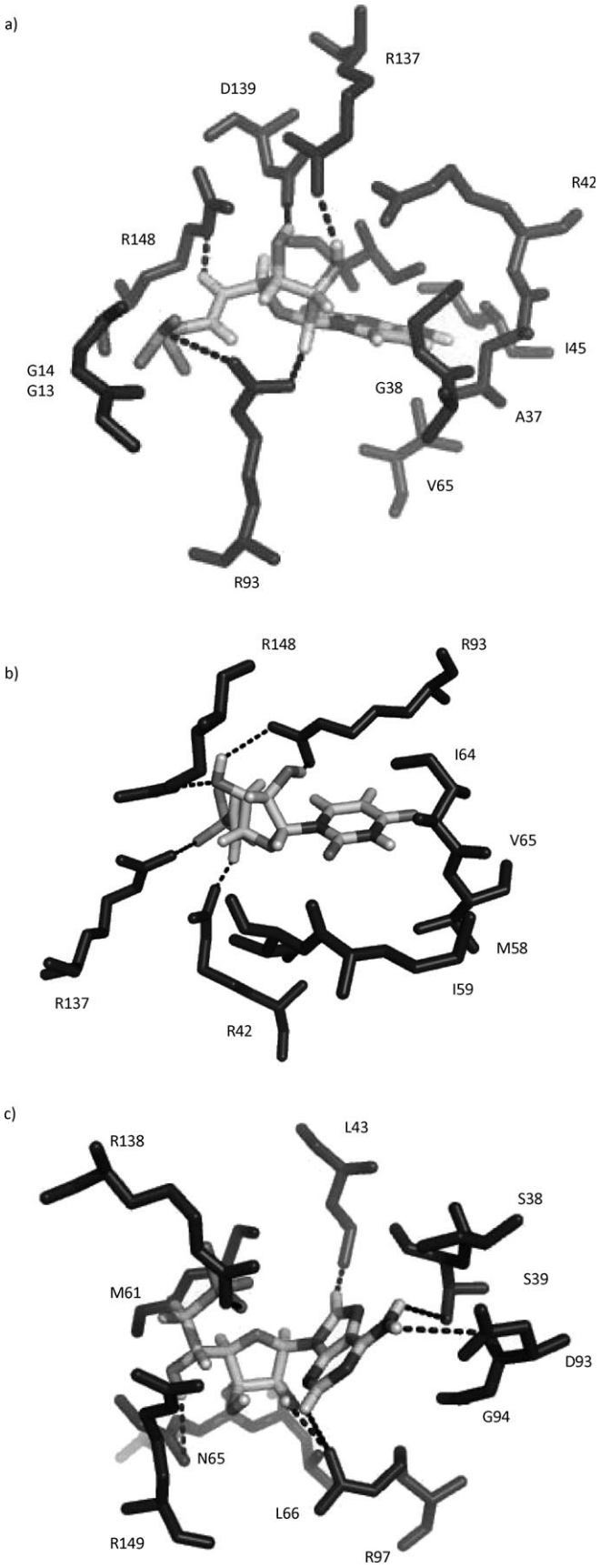
Despite its shorter size, analogue **11** is predicted to establish a number of key interactions within the active site. Most of these interactions involve the phosphate group and critical arginine residues, namely R93, R148 and R137. The nucleobase is, however, not as densely packed in the NMP hydrophobic pocket as expected, nor is it predicted to form hydrogen bonds with vicinal residue side chains as compound **7** is.

Purine derivative **26** was shown to be more than 100-fold more efficiently phosphorylated than the clinically approved acyclic nucleoside phosphonates, tenofovir and adefovir. We previously provided insight on the structural basis accounting for the phosphorylation of these molecules by hAMPK1 and hAMPK2. Both PMPA and PMEA are less bulky than the native substrate D-AMP and thus lack moieties that facilitate their optimal accommodation into the NMP site.[25c] However, PMPA is slightly bulkier than PMEA as it contains an extra methyl group, and this explains its improved affinity for both hAMPK1 and hAMPK2. Concerning analogue **26** (Figure 3c), it can be noticed that the molecule is well maintained within the hAMPK1 acceptor site through both hydrogen bonds between the purine moiety and polar residues (T39, R97, D93) and hydrophobic contacts with L33 and 66. The sugar phosphate counterpart of **26** is associated via a CH_2CH_2OH moiety, which provides some flexibility as seen in Figure 3c. The geometry of the $CH_2PO_3^-$ moiety is not extended, as in the case of native substrates and compounds **7** and **11**. The geometry of the latter is maintained by charge neutralisation with arginine residues (R138, R149), hydrophobic contacts with M61, as well as hydrogen bonds involving the hydroxy groups of the linker at the C5′ position, the ribose and residues R149 and R 97. It is also noteworthy that attempts to produce models with compounds bearing flexible linkers, such as **17** and **23**, failed.

The impact of the presence of hydroxy groups in the C5′–C6′ hinge bond is an important finding in the present study. This is exemplified by comparing compounds **24** and **20a**: the first one bearing a single hydroxy and the second two, for both of which molecular docking tentative resulted in nonproductive positioning, agreeing with the low rates of phosphotransfer measured (Table 2), thus validating our molecular docking comparative study. However, when adenine is used as a nucleobase within the β-hydroxyphosphonate series, the resulting compound is better recognised than tenofovir (PMPA), and is shown to accommodate the NMP pocket in a similar manner to AMP. The gain in flexibility in the sugar phosphate linkage compared to the parent ribose phosphate accounts for the lower rate of phosphorylation measured for this analogue compared to D-AMP.

Conclusions

We examined whether flexibility of the 5′,6′-C–C bond of several 5′-mononucleotide analogues affected their potential to be substrates for human nucleotide kinases. The lack of affinity of highly flexible and shorter derivatives, such as **17** and **23**, for hUMP-CMPK is in agreement with the absence of biological activity. However, we have shown that hUMP-CMPK was able to activate some nucleoside phosphonates presenting a certain degree of rigidity within the 5′,6′-C–C bond but keeping the orientation of the phosphonate moiety close to the nucleobase and on the β-face of the sugar.



Human UMP-CMP kinase and AMP kinases possess structural similarities, but differ in their dynamic behaviour, especially in the mechanics of their NMP and P-loop closure, which is a crucial element determining their substrate specificity. However, both enzymes were shown to activate certain nucleoside phosphonates presenting modifications in C5′–C6′, paving the way for the design of a new class of ribonucleotide analogues with isosteric character. Among the series of pyrimidine analogues, one derivative was phosphorylated by hUMP-CMPK with rates similar to those of dUMP and even better than dCMP. Additionally, a single adenosine β-hydroxyphosphonate analogue was also found to be phosphorylated by hAMPK1 and hAMPK2, with rates ranging those of well-known adenine containing ANPs, such as adefovir (PMEA) and tenofovir (PMPA). These two later constructs could constitute a promising template for further development as antiviral agents against RNA viruses.

Experimental Section

Chemistry

General remarks: Unless otherwise stated, ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75 MHz with proton decoupling at ambient temperature. Chemical shifts (δ) are referenced to the residual solvent peak ([D_6]DMSO at 2.49 ppm and 39.5 ppm) relative to TMS. Deuterium exchange, decoupling and COSY experiments were performed in order to confirm proton assignments. Coupling constants (*J*) are reported in Hertz (Hz). Two-dimensional ^{1}H–^{13}C heteronuclear COSY experiments were used to assign ^{13}C signals. Unless otherwise stated, ^{31}P NMR spectra were recorded at ambient temperature at 121 MHz with proton decoupling. Chemical shifts (δ) are reported relative to external H_3PO_4. Fast atom bombardment (FAB) mass spectra were recorded in the positive-ion or negative-ion mode using thioglycerol/glycerol (1:1, *v/v*, G-T) as the matrix. Specific rotations were measured with on a Perkin–Elmer model 241 spectropolarimeter (path length = 1 cm) and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were carried out by the Service de Microanalyses du CNRS, Division de Vernaison (France). Thin-layer chromatography (TLC) was performed on precoated aluminium sheets of silica gel 60 F_{254} (Merck), and visualisation of products was accomplished under UV light followed by charring with 5% ethanolic sulfuric acid with heating for carbohydrates and nucleotides. Flash chromatography was carried out using 63–100 μm silica gel (Merck; article: 115101) or 40–63 μm silica gel (Merck; article: 109385). Solvents were reagent grade or purified by distillation prior to use, and solids were dried over P_2O_5 under reduced pressure at room temperature (RT). Moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware. All aqueous (aq) solutions were saturated with the specified salt unless otherwise indicated. Organic solutions were dried over anhyd Na_2SO_4 after work-up and solvents were removed by evaporation at reduced pressure.

Figure 3. Predicted spatial positioning and hydrogen bond of the most efficient pyrimidine and purine phosphonates within the NMP site of hUMP-CMPK (model generated from PDB: 2UKD) and hAMPK1 (PDB: 1Z83), respectively. a) hUMP-CMPK/compound **7**; b) hUMP-CMPK/compound **11**; c) hAMPK1/compound **26**. Residues from the NMP binding site are shown as grey sticks, C5′–C6′ phosphonate analogues are shown as sticks in CPK nomenclature colours. Hydrogen bonds are indicated by black dashed lines.

Removal of the isopropylidene protecting group (method A): The protected nucleotide derivative was dissolved at RT in aq trifluoroacetic acid (TFA; 70%, *v/v*, 8 mL mmol^{-1}) and stirred until completion of the reaction was indicated by TLC. The solution was evaporated under reduced pressure and coevaporated with absolute ethanol.

Removal of phosphonic ester protecting groups (method B): The nucleoside diethylphosphonate was dissolved in anhyd *N*,*N*-dimethylformamide (DMF; 20 mL mmol^{-1}), and treated with trimethylsilyl bromide (10 equiv) at 0 °C. The mixture was stirred at RT until completion of the reaction was indicated by TLC. The reaction mixture was neutralised with aq triethyl ammonium bicarbonate (TEAB; 1 M) and concentrated under high vacuum.

2′,3′-*O*-Isopropylideneuridine (1):[6b] A suspension of uridine (12 g, 49 mmol) in acetone was treated with 2,2-dimethoxypropane (18 mL, 148 mmol) and paratoluenesulfonic acid monohydrate (940 mg, 4.9 mmol). The mixture was stirred for 2 h at 60 °C. The solution was evaporated under reduced pressure; the residue was dissolved in EtOAc and washed with saturated $NaHCO_3$. The organic layer was dried over anhyd Na_2SO_4, filtered and evaporated under reduced pressure to give a white solid. Recrystallisation from acetone gave compound **1** as white crystals (11.3 g, 81%): R_f = 0.4 (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.40 (br s, 1H, NH), 7.79 (d, J = 8.1 Hz, 1H, H-6), 5.83 (d, J = 2.4 Hz, 1H, H-1′), 5.63 (d, J = 8.0 Hz, 1H, H-5), 5.12 (br s, 1H, OH-5′), 4.89 (dd, J = 2.5, 6.2 Hz, 1H, H-2′), 4.74 (dd, J = 3.6, 6.2 Hz, 1H, H-3′), 4.07 (dd, J = 4.0, 8.1 Hz, 1H, H-4′), 3.57 (sl, 2H, H-5′, H-5″), 1.48, 1.29 ppm (2 s, 6H, C(CH$_3$)$_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.2 (C-4), 150.3 (C-2), 141.8 (C-6), 112.9 (C(CH$_3$)$_2$), 101.7 (C-5), 91.1 (C-1′), 86.5 (C-4′), 83.6 (C-2′), 80.4 (C-3′), 61.2 (C-5′), 27.0, 25.1 ppm (C(CH$_3$)$_2$); MS (FAB > 0, GT): *m/z* 569 [2*M*+H]$^+$, 285 [*M*+H]$^+$, 113 [B+2H]$^+$; MS (FAB < 0, GT): *m/z* 567 [2*M*−H]$^-$, 284 [*M*−H]$^-$.

1-(2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-6′-diethylphosphono-β-D-ribo-5′(*E*)hexenofuranosyl)uracil (3):[19] Chromium(VI) oxide (17.0 g, 170 mmol) was suspended in anhyd CH_2Cl_2 (170 mL) and DMF (40 mL). After stirring for 15 min, the reaction was cooled to 0 °C and treated dropwise with acetic anhydride (16 mL) and then pyridine (28 mL). A solution of **1** (12.1 g, 43 mmol) in CH_2Cl_2 (170 mL) and DMF (40 mL) was added at 0 °C over 30 min. The reaction mixture was stirred for 1 h, then the chromium salts were precipitated in cold EtOAc (2 L), and the resulting suspension was filtered through silica gel. The crude solution was concentrated under reduced pressure, coevaporated with toluene and dried over P_2O_5 to give the aldehyde intermediate.

A solution of [(diethoxyphosphonyl)methylidene]triphenylphosphorane[16] (30 g, 50 mmol) in dimethyl sulfoxide (DMSO; 100 mL) was added to a solution of the aldehyde intermediate (~12 g) in DMSO (100 mL). This mixture was stirred for 20 h at RT. Water (700 mL) and CH_2Cl_2 were added. The two phases were separated and the organic layer was washed with water, dried over anhyd Na_2SO_4, filtered and concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH, 99:1→97:3, *v/v*) gave compound **3** as a white foam (10.1 g, 48%): R_f = 0.3 (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{20}$ = +34.3 (c = 1.08 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.45 (br s, 1H, NH), 7.74 (d, J = 8.0 Hz, 1H, H-6), 6.70 (ddd, J = 5.8, 17.1, 22.5 Hz, 1H, H-5′), 6.00 (dd, J = 17.1, 20.3 Hz, 1H, H-6′), 5.94 (s, 1H, H-1′), 5.64 (d, J = 8.0 Hz, 1H, H-5), 5.14 (d, J = 6.3 Hz, 1H, H-2′), 4.86 (m, 1H, H-3′), 4.60 (m, 1H, H-4′), 4.05–3.95 (m, 4H, OCH$_2$CH$_3$), 1.51, 1.30 (2 s, 6H, C(CH$_3$)$_2$), 1.23 ppm (t, J = 7.0 Hz, 6H, OCH$_2$CH$_3$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.2 (C-4), 150.2 (C-2), 148.2 (C-5′, d, J = 5.4 Hz), 143.6 (C-6), 119.2 (C-6′, d, J = 183.3 Hz), 113.3 (C(CH$_3$)$_2$), 101.8 (C-5), 92.9 (C-1′), 86.8 (C-4′, d, J = 23.9 Hz), 83.7, 83.5 (C-2′, C-3′), 61.4, 61.3 (OCH$_2$CH$_3$), 2d, J = 5.4 Hz), 26.9, 25.1 (C(CH$_3$)$_2$), 16.2, 16.1 ppm (OCH$_2$CH$_3$); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ = 16.9 ppm; UV/Vis (EtOH 95): λ_{max} (ε) = 256 (9700), λ_{min} (ε) = 227 nm (2500); MS (FAB>0, GT): *m/z* 833 [2*M*+H]$^+$, 417 [*M*+H]$^+$; MS (FAB < 0, GT): *m/z* 831 [2*M*−H]$^-$, 415 [*M*−H]$^-$, 111 [B]$^-$; Anal. calcd for $C_{17}H_{25}N_2O_8P$: C 49.04, H 6.05, N 6.73; found : C 48.81, H 6.00, N 6.52.

1-(2′,3′-*O*-Isopropylidene-5′,6′-Dideoxy-6′-diethylphosphono-β-D-ribo-5′(*E*)-hexenofuranosyl)cytosil (4): An ice-cooled solution of compound **3** (200 mg, 0.48 mmol) in anhyd CH_3CN (5 mL) was treated with *N*-methylpyrrolidine (0.45 mL, 4.33 mmol) and stirred for 1 h. Trifluoroacetic anhydride (0.22 mL, 1.58 mmol) was then added and stirring was continued at 0 °C for an additional 1 h. Finally, *p*-nitrophenol (200 mg, 1.44 mmol) was added and the reaction was stirred for 4 h. The solvent was then evaporated and the oily residue redissolved in EtOAc. The resulting organic layer was washed with saturated aq $NaHCO_3$ and water, dried over anhyd Na_2SO_4, filtered and concentrated under reduced pressure. The crude was dissolved in dioxane (15 mL) and a concentrated NH_4OH solution (7 M, 3 mL) was added. The mixture was stirred at RT overnight and then concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH, 95:5→90:10, *v/v*) gave compound **4** as a white foam (0.120 g, 60%): R_f = 0.36 (CH_2Cl_2/MeOH, 90:10); $[\alpha]_D^{20}$ = +41.8 (c = 0.91 in MeOH) ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.66 (d, J = 7.4 Hz, 1H, H-6), 7.31 (br s, 2H, NH), 6.75 (ddd, J = 6.7, 17.2, 21.7 Hz, 1H, H-5′), 5.96 (ddd, J = 1.1, 17.2, 20.3 Hz, 1H, H-6′), 5.72 (d, J = 1.0 Hz, 1H, H-1′), 5.70 (d, J = 7.3 Hz, 1H, H-5), 5.07 (dd, J = 1.1, 6.3 Hz, 1H, H-2′), 4.86 (dd, J = 6.3, 4.1 Hz 1H, H-3′), 4.56 (m, 1H, H-4′), 3.96 (q, 4H, OCH$_2$CH$_3$), 1.50, 1.29 (2 s, 6H, C(CH$_3$)$_2$), 1.23 ppm (t, J = 7.1 Hz, 6H, OCH$_2$CH$_3$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 166.1 (C-4), 154.7 (C-2), 149.0 (C-5′, d, J = 5.4 Hz), 118.9 (C-6′, d, J = 183.0 Hz), 113.0 (C(CH$_3$)$_2$), 94.5 (C-5), 94.1 (C-1′), 87.4 (C-4′, d, J = 24.3 Hz), 84.3, 84.0 (C-2′, C-3′), 61.4, 61.3 (OCH$_2$CH$_3$), 2d, J = 5.1 Hz), 26.9, 25.1 (C(CH$_3$)$_2$), 16.2, 16.1 ppm (OCH$_2$CH$_3$); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ = 17.1; UV/Vis (EtOH 95): λ_{max} (ε) = 240 (8300), λ_{min} (ε) = 225 nm (7400); MS (FAB > 0, GT): *m/z* 831 [2*M*+H]$^+$, 416 [*M*+H]$^+$; MS (FAB < 0, GT): *m/z* 829 [2*M*−H]$^-$, 414 [*M*−H]$^-$, 110 [B]$^-$; Anal. calcd for $C_{17}H_{26}N_3O_7P$,0.1H_2O: C 48.94, H 6.33, N 10.07, P 7.42; found : C 48.69, H 6.16, N 9.84, P 7.14.

1-(5′,6′-Dideoxy-6′-diethylphosphono-β-D-ribo-5′(*E*)-hexenofuranosyl)cytosil (5): Compound **4** (450 mg, 1.0 mmol) was treated using method A. Column chromatography of the crude materials on reverse phase (H_2O/CH_3CN, 0→30%) and freeze drying gave the titled compound as a white solid (400 mg, 97%): R_f = 0.4 (CH_2Cl_2/MeOH, 8:2); $[\alpha]_D^{20}$ = +66.3 (c = 0.92 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.66 (br s, 1H, NH), 7.61 (d, J = 7.5 Hz, 1H, H-6), 7.44 (br s, 1H, NH), 6.75 (ddd, J = 5.7, 16.7, 22.4 Hz, 1H, H-5′), 6.02 (ddd, J = 1.2, 17.2, 20.4 Hz, 1H, H-6′), 5.80 (d, J = 7.5 Hz, 1H, H-), 5.76 (d, J = 3.1 Hz, 1H, H-1′), 5.51 (br s, 1H, OH-2′), 5.38 (br s, 1H, OH-3′), 4.34 (m, 1H, H-4′), 3.99 (m, 6H, H-2′, H-3′, OCH$_2$CH$_3$), 1.24 ppm (t, J = 6.9 Hz, 6H, (OCH$_2$CH$_3$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 165.7 (C-4), 155.0 (C-2), 150.0 (C-5′, d, J = 5.3 Hz), 143.7 (C-6), 119.8 (C-6′, d, J = 183.7 Hz), 95.6 (C-5), 92.4 (C-1′), 83.5 (C-4′, d, J = 23.0 Hz), 74.4 (C-3′), 74.3 (C-2′), 62.6 (OCH$_2$CH$_3$, d, J = 5.5 Hz), 17.5, 17.4 ppm (OCH$_2$CH$_3$); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ = 17.2; UV/Vis (EtOH 95): λ_{max} (ε) = 270 nm (7900), λ_{min} (ε) = 252 nm (6800); MS (FAB > 0, GT): *m/z* 751 [2*M*+H]$^+$, 376 [*M*+H]$^+$; MS (FAB < 0, GT): *m/z* 374 [*M*−H]$^-$; Anal. calcd for $C_{14}H_{22}N_3O_7P$, 1.2H_2O: C 42.36, H 6.20, N 10.59, P 7.80; found : C 42.75, H 5.85, N 10.13, P 7.55.

1-(5′,6′-Dideoxy-6′-diethylphosphono-β-D-ribo-5′(*E*)-hexenofuranosyl)uracil (6): Compound **3** (800 mg, 1.92 mmol) was treated using method A. Column chromatography of the crude materials on reverse phase (H_2O/CH_3CN, 0→30%) and freeze drying gave the titled compound as a white solid (500 mg, 70%): R_f=0.2 (CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{20}$=+31.2 (*c*=0.93 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.40 (d, *J*=1.6 Hz, 1H, NH), 7.63 (d, *J*=8.1 Hz, 1H, H-6), 6.74 (ddd, *J*=5.8, 17.1, 22.5 Hz, 1H, H-5′), 6.01 (ddd, *J*=1.2, 17.1, 20.3 Hz, 1H, H-6′), 5.74 (d, *J*=4.7 Hz, 1H, H-1′), 5.65 (dd, *J*=2.1, 8.0 Hz, 1H, H-5), 5.54 (br s, 1H, OH-2′), 5.47 (br s, 1H, OH-3′), 4.34 (m, 1H, H-4′), 4.15 (t, *J*=4.8 Hz, 1H, H-2′), 4.05–3.95 (m, 5H, H-3′, OCH_2CH_3), 1.24 ppm (t, *J*=7.0 Hz, 6H, (OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=163.0 (C-4), 150.5 (C-2), 148.4 (C-5′, d, *J*=5.4 Hz), 141.5 (C-6), 118.6 (C-6′, d, *J*=183.6 Hz), 102.0 (C-5), 89.9 (C-1′), 82.6 (C-4′, d, *J*=23.0 Hz), 73.0 (C-3′), 72.3 (C-2′), 61.4, 61.3 (OCH_2CH_3, 2d, *J*=5.5 Hz), 16.2, 16.1 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ=17.2 ppm; UV/Vis (EtOH 95): λ_{max} (ε)= 259 (10200), λ_{min} (ε)=230 nm (2600); MS (FAB>0, GT): *m/z* 753 [2M+H]$^+$, 377 [M+H]$^+$; MS (FAB<0, GT): *m/z* 751 [2*M*−H]$^-$, 375 [M−H]$^-$; Anal. calcd for $C_{14}H_{21}N_2O_8P$, 0.3H_2O: C 44.05, H 5.70, N 7.34, P 8.11; found : C 43.73, H 5.83, N 7.20, P 7.74.

1-(5′,6′-Dideoxy-6′-phosphono-β-D-ribo-5′(*E*)-hexenofuranosyl)cytosil (disodium salt) (7): Compound **5** (172 mg, 0.46 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, then ion exchange on DOWEX Na^+ and freeze drying gave the title compound as a white solid (145 mg, 87%): R_f=0.12 (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20}$=+42.6 (*c*=0.94 in H_2O); ^{1}H NMR (300 MHz, D_2O): δ=7.58 (d, *J*=7.6 Hz, 1H, H-6), 6.36 (ddd, *J*=6.0, 17.2, 19.8 Hz, 1H, H-5′), 6.07 (t, *J*=1.1, 17.0 Hz, 1H, H-6′), 5.96 (d, *J*=7.6 Hz, 1H, H-5), 5.82 (d, *J*=73.3 Hz, 1H, H-1′), 4.46 (m, 1H, H-4′), 4.22 (dd, *J*=3.4, 5.1 Hz, 1H, H-2′), 4.05 ppm (dd, *J*=5.2, 6.7 Hz, 1H, H-3′); ^{13}C NMR (75 MHz, D_2O): δ=166.1 (C-4), 157.5 (C-2), 141.4 (C-6), 138.7 (C-5′, d, *J*=4.8 Hz), 128.3 (C-6′, d, *J*=172.2 Hz), 96.3 (C-5), 90.7 (C-1′), 83.2 (C-4′, d, *J*=21.7 Hz), 73.7, 72.8 ppm (C-3′, C-2′); ^{31}P NMR (100 MHz, D_2O): δ=10.4 ppm; UV/Vis (H_2O): λ_{max} (ε)= 269 ppm (8900), λ_{min} (ε)=249 nm (5500); MS (FAB>0, GT): *m/z* 342 [*M*+2H-Na]$^+$; MS (FAB<0, GT): *m/z* 318 [*M*−2Na+H]$^-$; Anal. calcd for $C_{10}H_{12}N_3Na_2O_7P$: C 33.07, H 3.33, N 11.57, P 8.53; found : C 33.67, H 3.16, N 11.63, P 8.35.

1-(5′,6′-Dideoxy-6′-phosphono-β-D-ribo-5′(*E*)-hexenofuranosyl)uracil (disodium salt) (8):[19] Compound **6** (180 mg, 0.48 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, then ion exchange on DOWEX Na^+ and freeze drying gave the title compound as a white solid (90 mg, 52%): R_f=0.13 (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20}$=+34.9 (*c*=0.86 in H_2O); ^{1}H NMR (300 MHz, D_2O): δ=7.58 (d, *J*=8.1 Hz, 1H, H-6), 6.33 (ddd, *J*=5.9, 17.2, 19.8 Hz, 1H, H-5′), 6.04 (t, *J*=16.9 Hz, 1H, H-6′), 5.79 (d, *J*=4.1 Hz, 1H, H-1′), 5.78 (d, *J*=7.9 Hz, 1H, H-5), 4.44 (dd, *J*=5.8, 6.0 Hz, 1H, H-4′), 4.26 (dd, *J*=4.3, 4.8 Hz, 1H, H-2′), 4.06 ppm (t, *J*=5.7 Hz, 1H, H-3′); ^{13}C NMR (75 MHz, D_2O): δ=166.1 (C-4), 151.5 (C-2), 141.8 (C-6), 138.7 (C-5′, d, *J*=4.8 Hz), 128.2 (C-6′, d, *J*=172.3 Hz), 102.4 (C-5), 90.1 (C-1′), 83.5 (C-4′, d, *J*=21.7 Hz), 73.1, 72.8 ppm (C-3′, C-2′); ^{31}P NMR (100 MHz, D_2O): δ=10.6 ppm; UV/Vis (H_2O): λ_{max} (ε)= 260 nm (10400), λ_{min} (ε)=229 nm (2300); MS (FAB>0, GT): *m/z* 365 [*M*+H]$^+$, 343 [*M*+2H-Na]$^+$; MS (FAB<0, GT): *m/z* 341 [*M*−Na]$^-$, 319 [M−2Na+H]$^-$; Anal. calcd for $C_{10}H_{11}N_2Na_2O_8P$: C 32.98, H 3.04, N 7.69, P 8.51; found: C 33.40, H 3.81, N 7.58, P 8.15.

1-(2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-6′,6′-dibromo-β-D-ribo-5′-hexenofuranosyl)uracil (9):[29] A suspension of chromium(VI) oxide (17.0 g, 170 mmol) in anhyd CH_2Cl_2 (170 mL) and DMF (40 mL) was stirred for 15 min, then treated at 0 °C with acetic anhydride (16 mL) and then pyridine (28 mL). A solution of 2′,3′-isopropylideneuridine (**1**; 12.1 g, 43 mmol) in CH_2Cl_2 (170 mL) and DMF (40 mL) was added at 0 °C over 30 min. The reaction mixture was stirred for 1 h, the chromium salts were precipitated in cold EtOAc (2 L), and the resulting suspension was filtered over silica gel. The crude solution was concentrated under reduced pressure, coevaporated with toluene, and dried over P_2O_5. A solution of crude aldehyde (6.1 g, 21.6 mmol) in CH_2Cl_2 (180 mL) and CBr_4 (14.3 g, 43.2 mmol) was treated with triphenylphosphine (22.6 g, 86.4 mmol), and the mixture stirred at RT for 22 h. The mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude was twice purified by column chromatography (toluene/EtOAc, 7:3, *v/v*) to give compound **9** as a white solid (1.85 g, 25%): R_f=0.3 (Toluene/AcOEt, 1:1); $[\alpha]_D^{20}$=+76 (*c*=0.5 in CH_2Cl_2); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.48 (br s, 1H, NH), 7.75 (d, *J*=8.0 Hz, 1H, H-6), 6.83 (d, *J*=8.2 Hz, 1H, H-5′), 5.77 (d, *J*=1.1 Hz, 1H, H-1′), 5.64 (dd, *J*=2.1, 8.0 Hz, 1H, H-5), 5.14 (dd, *J*=1.3, 6.3 Hz, 1H, H-2′), 4.87 (dd, *J*=3.5, 6.3 Hz, 1H, H-3′), 4.61 (dd, *J*=3.5, 8.1 Hz, 1H, H-4′), 1.50, 1.29 ppm (2 s, 6H, $C(CH_3)_2$); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ= 163.3 (C-4), 150.4 (C-2), 143.8 (C-6), 137.0 (C-5′), 113.2 ($C(CH_3)_2$), 101.7 (C-5), 94.0 (C-1′), 93.1 (C-6′), 87.3 (C-4′), 84.0, 83.9 (C-2′,C-3′), 26.8, 25.0 ppm ($C(CH_3)_2$); UV/Vis (EtOH 95): λ_{max} (ε)=255 nm (11500), λ_{min} (ε)=230 nm (4200); MS (FAB>0, GT): *m/z* (%): 441 (33), 439 (66), 437 (33) [*M*+H]$^+$; MS (FAB<0, GT): *m/z* (%): 439 (33), 437 (66), 435 (33) [*M*−H]$^-$; Anal. calcd for $C_{13}H_{14}Br_2N_2O_5$: C 35.64, H 3.22, N 6.39; found: C 35.76, H 3.34, N 6.13.

1-(2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-6′-diethylphosphono-β-D-ribo-5′-hexyno-furanosyl)uracil (10): Palladium(II) acetate (78 mg, 0.35 mmol) and diphenylphosphinoferrocene (383 mg, 0.7 mmol) were dissolved in DMF (9 mL). The mixture was stirred at RT for 20 min. A solution of **9** (760 mg, 1.7 mmol), diethylphosphite (0.45 mL, 3.5 mmol) and propylene oxide (0.22 mL, 5.2 mmol) in DMF (24 mL) was added dropwise at RT. The solution was stirred for 16 h at 90 °C. The solution was evaporated under high vacuum and coevaporated with abs EtOH. Column chromatography (EtOAc/cyclohexane, 3:1, *v/v*) gave the desired compound as a yellow/orange foam (450 mg, 61%): R_f=0.2 (AcOEt); $[\alpha]_D^{20}$=+18.8 (*c*=0.85 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=11.48 (br s, 1H, NH), 7.73 (d, *J*=8.0 Hz, 1H, H-6), 5.84 (s, 1H, H-1′), 5.62 (d, *J*=8.0 Hz, 1H, H-5), 5.28 (d, *J*=6.1 Hz, 1H, H-2′), 5.18 (dd, *J*=2.9, 6.0 Hz, 1H, H-3′), 5.06 (t, *J*=3.2 Hz, 1H, H-4′), 4.1–4.0 (m, 4H, OCH_2CH_3), 1.48, 1.31 (2 s, 6H, $C(CH_3)_2$), 1.26 ppm (2t, *J*=7.0 Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO) δ=163.3 (C-4), 150.2 (C-2), 143.6 (C-6), 113.19 ($C(CH_3)_2$), 101.5 (C-5), 96.8 (C-5′, d, *J*=48.0 Hz), 93.9 (C-1′), 84.3 (C-4′, d, *J*=70.4 Hz), 76.5 (C-6′, d, *J*=286.3 Hz), 76.6 (C-2′), 76.5 (C-3′), 63.2–63.1 (OCH_2CH_3), 26.4, 24.8 ($C(CH_3)_2$), 15.8, 15.7 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ=−8.4 ppm; UV/Vis (EtOH 95): λ_{max} (ε)=256 nm (9500), λ_{min} (ε)=225 nm (1500); MS (FAB>0, GT): *m/z* 829 [2*M*+H]$^+$, 415 [*M*+H]$^+$; MS (FAB<0, GT): *m/z* 827 [2*M*−H]$^-$, 413 [*M*−H]$^-$, 111 [B]$^-$; Anal. calcd for $C_{17}H_{23}N_2O_8P$: C 49.28, H 5.59, N 6.76, P 7.48; found: C 49.38, H 5.62, N 6.61, P 7.54.

1-(2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-6′-diethylphosphono-β-D-ribo-5′(*Z*)-hexenofuranosyl)uracil (12): A solution of **10** (400 mg, 1 mmol) in abs EtOH (40 mL) was treated with Lindlar catalyst (10% weight of alkyne, 40 mg) at 0 °C and quinoline (20% weight of palladium, 0.008 mL). The solution was stirred vigorously under H_2 at atmospheric pressure for 5 h at RT. The suspension was filtered through celite and concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH, 95:5, *v/v*) gave compound

12 as a white foam (365 mg, 91%): $R_f = 0.3$ (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{20} = +78$ ($c = 1.00$ in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta =$ 11.45 (br s, 1H, NH), 7.76 (d, $J = 8.0$ Hz, 1H, H-6), 6.56 (ddd, $J = 9.7$, 13.2, 51.0 Hz, 1H, H-5′), 5.90 (dd, $J = 13.2$, 17.0 Hz, 1H, H-6′), 5.78 (d, $J = 1.1$ Hz, 1H, H-1′), 5.64 (d, $J = 8.0$ Hz, 1H, H-5), 5.40 (dd, $J =$ 4.2, 9.5 Hz, 1H, H-4′), 5.11 (dd, $J = 1.2$, 6.2 Hz, 1H, H-2′), 4.78 (dd, $J = 4.6$, 6.0 Hz, 1H, H-3′), 4.05–3.90 (m, 4H, OCH_2CH_3), 1.51, 1.29 (2 s, 6H, $C(CH_3)_2$), 1.24, 1.23 ppm (2t, $J = 7.0$ Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 163.3$ (C-4), 150.2 (C-2), 148.0 (C-5′), 143.6 (C-6), 120.6 (C-6′, d, $J = 177.8$ Hz), 113.2 ($C(CH_3)_2$), 101.7 (C-5), 92.9 (C-1′), 84.0 (C-2′), 83.9 (C-3′), 82.2 (C-4′, d, $J = 8.1$ Hz), 61.2, 61.1 (OCH_2CH_3), 26.9, 25.3 ($C(CH_3)_2$), 16.1, 16.0 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): $\delta = 14.7$ ppm; UV/Vis (EtOH 95): λ_{max} (ε) = 256 nm (10900), λ_{min} (ε) = 228 nm (3100); MS (FAB > 0, GT): *m/z* 833 $[2M+H]^+$, 417 $[M+H]^+$; MS (FAB < 0, GT): *m/z* 831 $[2M-H]^-$, 415 $[M-H]^-$, 111 $[B]^-$; Anal. calcd for $C_{17}H_{25}N_2O_8P$: C 49.04, H 6.05, N 6.73, P 7.44; found: C 49.01, H 6.28, N 6.48, P 7.10.

1-(5′,6′-Dideoxy-6′-diethylphosphono-β-D-ribo-5′(*Z*)-hexenofuranosyl)uracil (13): Compound **12** (365 mg, 0.88 mmol) was treated using method A. Column chromatography on reverse phase (H_2O/CH_3CN, 0→30%) and freeze drying gave the titled compound as a white solid (300 mg, 90%): $R_f = 0.1$ (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{20} = +$ 23.4 ($c = 1.07$ in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 11.38$ (br s, 1H, NH), 7.72 (d, $J = 8.1$ Hz, 1H, H-6), 6.60 (ddd, $J = 9.7$, 13.1, 50.9 Hz, 1H, H-5′), 5.88 (dd, $J = 13.1$, 17.0 Hz, 1H, H-6′), 5.76 (d, $J =$ 5.0 Hz, 1H, H-1′), 5.65 (d, $J = 8.0$ Hz, 1H, H-5), 5.51 (d, $J = 4.8$ Hz, 1H, OH-2′), 5.30 (d, $J = 5.4$ Hz, 1H, OH-3′), 5.14 (dd, $J = 4.1$, 9.6 Hz, 1H, H-4′), 4.21 (pseudo-q, $J = 4.8$ Hz, 1H, H-2′), 4.07–3.93 (m, 4H, OCH_2CH_3), 3.89 (pseudo-q, $J = 5.0$ Hz, 1H, H-3′), 1.24 ppm (2t, $J =$ 7.0 Hz, 6H, (OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 163.0$ (C-4), 150.7 (C-2), 148.8 (C-5′), 141.4 (C-6), 119.9 (C-6′, d, $J = 178.4$ Hz), 102.0 (C-5), 88.9 (C-1′), 79.6 (C-4′, d, $J = 8.2$ Hz), 73.9 (C-3′), 72.5 (C-2′), 61.3, 61.2 (OCH_2CH_3), 16.1, 16.0 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): $\delta = 15.0$ ppm; UV/Vis (EtOH 95): λ_{max} (ε) = 259 nm (9600), λ_{min} (ε) = 230 nm (2800); MS (FAB > 0, GT): *m/z* 753 $[2M+H]^+$, 377 $[M+H]^+$; MS (FAB < 0, GT): *m/z* 751 $[2M-H]^-$, 375 $[M-H]^-$, 111 $[B]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{14}H_{22}N_2O_8P$: 377.1114; found: 377.1101; Anal. calcd for $C_{14}H_{21}N_2O_8P$, $0.3H_2O$: C 44.05, H 5.70, N 7.34, P 8.11; found: C 44.31, H 5.73, N 7.41, P 7.50.

1-(5′,6′-Dideoxy-6′-phosphono-β-D-ribo-5′(*Z*)-hexenofuranosyl)uracil (disodium salt) (14): Compound **13** (175 mg, 0.47 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, and subsequent ion exchange on DOWEX Na^+ and freeze drying gave the titled compound as a white solid (100 mg, 59%): $R_f = 0.13$ (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20} = +16.2$ ($c = 1.11$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta = 7.68$ (d, $J = 8.1$ Hz, 1H, H-6), 6.13 (ddd, $J = 8.7$, 13.4, 50.1 Hz, 1H, H-5′), 6.04 (dd, $J = 13.4$, 28.3 Hz, 1H, H-6′), 5.84 (d, $J = 8.2$ Hz, 1H, H-5), 5.83 (d, $J = 3.7$ Hz, 1H, H-1′), 5.36–5.30 (m, 1H, H-4′), 4.36 (dd, $J = 4.1$, 5.8 Hz, 1H, H-2′), 4.07 ppm (t, $J = 5.7$ Hz, 1H, H-3′); ^{13}C NMR (75 MHz, D_2O): $\delta = 166.2$ (C-4), 151.5 (C-2), 142.0 (C-6), 138.3 (C-5′), 130.3 (C-6′, d, $J = 166.4$ Hz), 102.2 (C-5), 90.5 (C-1′), 80.4 (C-4′, d, $J = 8.3$ Hz), 73.9 (C-3′), 73.5 ppm (C-2′); ^{31}P NMR (100 MHz, D_2O): $\delta = 8.6$ ppm; UV/Vis (H_2O) λ_{max} (ε) = 260 nm (10700), λ_{min}(ε) = 229 nm (2300); MS (FAB > 0, GT): *m/z* 365 $[M+H]^+$, 343 $[M+2H-Na]^+$; MS (FAB < 0, GT): *m/z* 341 $[M-Na]^-$, 319 $[M-2Na+H]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{10}H_{12}N_2Na_2O_8P$: 365.0127; found: 365.0132; Anal. calcd for $C_{10}H_{11}N_2Na_2O_8P$: C 32.98, H 3.04, N 7.69, P 8.51; found: C 32.30, H 3.65, N 7.26, P 7.30.

1-(2′,3′-*O*-Isopropylidene-5′,6′-dideoxy-6′-diethylphosphono-β-D-ribofuranosyl)uracil (15):[30] A solution of **3** (800 mg, 1.9 mmol) in abs EtOH (40 mL) was treated with 5% Pd/C (100 mg mmol^{-1}) at 0 °C. The solution was stirred vigorously under H_2 at atmospheric pressure for 12 h at RT. The suspension was filtered through celite and concentrated under reduced pressure. Column chromatography (CH_2Cl_2/MeOH, 95:5, *v/v*) gave the titled compound as a white foam (700 mg, 88%): $R_f = 0.4$ (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{20} = +16.0$ ($c = 1.00$ in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO) $\delta = 11.42$ (br s, 1H, NH), 7.71 (d, $J = 8.0$ Hz, 1H, H-6), 5.75 (d, $J = 2.2$ Hz, 1H, H-1′), 5.64 (d, $J = 8.0$ Hz, 1H, H-5), 5.01 (dd, $J = 2.2$, 6.5 Hz, 1H, H-2′), 4.64 (dd, $J = 4.8$, 6.4 Hz, 1H, H-3′), 4.05–3.85 (m, 5H, OCH_2CH_3, H-4′), 1.90–1.65 (m, 4H, H-5′, H-5″, H-6′,H-6″), 1.48, 1.28 (2 s, 6H, $C(CH_3)_2$), 1.22 ppm (t, $J = 7.0$ Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO) $\delta = 163.2$ (C-4), 150.2 (C-2), 142.9 (C-6), 113.5 ($C(CH_3)_2$), 101.9 (C-5), 91.2 (C-1′), 85.2 (C-4′, d, $J = 17.6$ Hz), 83.4 (C-2′), 82.6 (C-3′), 61.0, 60.9 (($OCH_2CH_3)_2$, d, $J = 6.8$ Hz), 27.0 ($C(CH_3)_2$), 25.8 (C-5′, d, $J = 4.1$ Hz), 25.2 ($C(CH_3)_2$), 20.7 (C-6′, d, $J = 140.6$ Hz), 16.3, 16.2 ppm ($OCH_2CH_3)_2$; ^{31}P NMR (100 MHz, [D$_6$]DMSO): $\delta = 31.2$ ppm; UV/Vis (EtOH 95): $\lambda_{max}(\varepsilon) = 257$ nm (9800), $\lambda_{min}(\varepsilon) = 227$ nm (2300); MS (FAB > 0, GT): *m/z* 837 $[2M+H]^+$, 419 $[M+H]^+$; MS (FAB < 0, GT): *m/z* 835 $[2M-H]^-$, 417 $[M-H]^-$.

1-(5′,6′-Dideoxy-6′-diethylphosphono-β-D-ribofuranosyl)uracil (16): Compound **15** (350 mg, 0.83 mmol) was treated using method A. Column chromatography (CH_2Cl_2/MeOH, 9:1, *v/v*) and freeze drying gave the titled compound as a white solid (300 mg, 95%): $R_f = 0.1$ (CH_2Cl_2/MeOH, 95:5); $[\alpha]_D^{20} = +14.4$ ($c = 0.90$ in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 11.36$ (br s, 1H, NH), 7.60 (d, $J =$ 8.1 Hz, 1H, H-6), 5.69 (d, $J = 4.9$ Hz, 1H, H-1′), 5.64 (d, $J = 8.0$ Hz, 1H, H-5), 5.37 (d, $J = 5.1$ Hz, 1H, OH-2′), 5.14 (d, $J = 5.3$ Hz, 1H, OH-3′), 4.13–4.05 (m, 1H, H-2′), 4.05–3.90 (m, 4H, OCH_2CH_3), 3.85–3.70 (m, 2H, H-3′, H4′), 1.90–1.70 (m, 4H, H-5′, H-5″, H-6′, H-6″), 1.23 ppm (t, $J = 7.0$ Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 163.0$ (C-4), 150.6 (C-2), 141.4 (C-6), 102.0 (C-5), 88.9 (C-1′), 82.6 (C-4′, d, $J = 16.6$ Hz), 72.5 (C-2′), 72.4 (C-3′), 60.9 (OCH_2CH_3, 2d, $J = 6.0$ Hz), 25.9 (C-5′, d, $J = 3.0$ Hz), 20.9 (C-6′, d, $J =$ 140.4 Hz), 16.3, 16.2 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): $\delta = 31.5$ ppm; UV/Vis (EtOH 95): $\lambda_{max}(\varepsilon) = 259$ nm (9800), $\lambda_{min}(\varepsilon) = 228$ nm (2000); MS (FAB > 0, GT): *m/z* 757 $[2M+H]^+$, 379 $[M+H]^+$; MS (FAB < 0, GT): *m/z* 755 $[2M-H]^-$, 377 $[M-H]^-$, 111 $[B]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{14}H_{24}N_2O_8P$: 379.1270; found: 379.1283; Anal. calcd for $C_{14}H_{23}N_2O_8P$: C 44.45, H 6.13, N 7.40, P 8.19; found: C 43.90, H 6.38, N 7.26, P 7.20.

1-(5′,6′-Dideoxy-6′-phosphono-β-D-ribofuranosyl)uracil (disodium salt) (17):[31] Compound **16** (200 mg, 0.53 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, and ion exchange on DOWEX Na^+ and freeze drying gave the titled compound as a white solid (130 mg, 67%): $R_f = 0.16$ (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20} = +11.7$ ($c = 1.03$ in H_2O); ^{1}H NMR (300 MHz, D_2O): $\delta =$ 7.60 (d, $J = 8.1$ Hz, 1H, H-6), 5.81 (d, $J = 8.0$ Hz, 1H, H-5), 5.77 (d, $J =$ 4.7 Hz, 1H, H-1′), 4.26 (t, $J = 4.7$ Hz, 1H, H-2′), 4.01–3.92 (m, 2H, H-3′, H-4′), 2.00–1.77 (m, 2H, H-5′, H-5″), 1.73–1.50 ppm (m, 2H, H-6′, H-6″); ^{13}C NMR (75 MHz, D_2O): $\delta = 166.2$ (C-4), 151.9 (C-2), 141.9 (C-6), 102.6 (C-5), 87.7 (C-1′), 84.3 (C-4′, d, $J = 10.3$ Hz), 73.8 (C-2′), 71.0 (C-5′, d, $J = 2.1$ Hz), 70.0 (C-3′), 68.1 ppm (C-6′, d, $J = 148.9$ Hz); ^{31}P NMR (100 MHz, D_2O): $\delta = 24.4$ ppm; UV/Vis (H_2O): $\lambda_{max}(\varepsilon) =$ 260 nm (9400), $\lambda_{min}(\varepsilon) = 229$ nm (1800); MS (FAB > 0, GT): *m/z* 367 $[M+H]^+$, 345 $[M+2H-Na]^+$; MS (FAB < 0, GT): *m/z* 343 $[M-Na]^-$, 321 $[M-2Na+H]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{10}H_{14}N_2Na_2O_8P$: 367.0283; found: 367.0261; Anal. calcd for

$C_{10}H_{13}N_2Na_2O_8P$: C 32.80, H 3.58, N 7.65, P 8.46; found: C 31.55, H 4.41, N 7.22, P 7.20.

Dihydroxylation procedure: A stirred suspension of AD-mix α or β (700 mg) in 50% aq *t*-BuOH (6 mL) was treated with $K_2OsO_4{\cdot}2H_2O$ (144 mg, 0.4 mmol) at RT. The yellow mixture was stirred until two phases were observed (~10–20 min), and then $CH_3SO_2NH_2$ (448 mg, 0.5 mmol) was added. The mixture was cooled to 0 °C and treated with **3** (200 mg, 0.5 mmol). After 2 h, the resulting mixture was allowed to warm to RT and then stirred for 5 h. The solution was quenched by addition of sodium sulfite (1.5 g, 24 mmol), and the reaction mixture was stirred for 1 h. The solution was filtered, and the precipitate was washed with EtOAc. The filtrate was dried over Na_2SO_4, filtered and concentrated under reduced pressure. According to ^{1}H NMR, AD-mix α gave 83% of the major diol, whereas using AD-mix β, the same product was formed in 70%. Column chromatography on reverse phase (H_2O/CH_3CN, 10→20%, *v/v*; major product eluted first) gave the titled compound (50% yield of first eluted).

1-(2′,3′-*O*-Isopropylidene-6′-diethylphosphono-β-D-ribo-5′S,6′S-hexofuranosyl)uracil (18a): R_f = 0.3 (CH_2Cl_2/MeOH, 9:1); $[\alpha]_D^{20}$ = + 2.1 (*c* = 0.94 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.20 (brs, 1H, NH), 7.75 (d, *J* = 8.1 Hz, 1H, H-6), 5.77 (d, *J* = 2.3 Hz, 1H, H-1′), 5.62 (d, *J* = 8.0 Hz, 1H, H-5), 5.41 (t, *J* = 7.0 Hz, 1H, OH-6′), 5.18 (d, *J* = 7.7 Hz, 1H, OH-5′), 5.03 (dd, *J* = 2.5, 6.5 Hz, 1H, H-2′), 4.95 (dd, *J* = 3.1, 6.5 Hz, 1H, H-3′), 4.10–3.90 (m, 5H, H-4′,OCH_2CH_3), 3.90–3.70 (m, 2H, H-5′, H-6′), 1.47, 1.29 (2 s, 6H, $C(CH_3)_2$), 1.21 ppm (t, *J* = 7.1 Hz, 6H, (OCH_2CH_3)). (300 MHz, $CDCl_3$): δ = 9.73 (brs, 1H, NH), 7.32 (d, *J* = 8.1 Hz, 1H, H-6), 5.75 (d, *J* = 8.1 Hz, 1H, H-5), 5.57 (d, *J* = 1.6 Hz, 1H, H-1′), 5.18 (dd, *J* = 2.8, 6.5 Hz, 1H, H-3′), 5.12 (dd, *J* = 1.6, 6.5 Hz, 1H, H-2′), 4.44 (brs, 1H, OH-5′), 4.30–4.10 (m, 6H, H-4′, H-5′, OCH_2CH_3), 4.00 (d, *J* = 10.7 Hz, 1H, H-6′), 3.54 (brs, 1H, OH-6′), 1.16 (s, 3H, $C(CH_3)_2$), 1.34 (m, 9H, $C(CH_3)_2$, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.2 (C-4), 150.2 (C-2), 142.9(C-6), 112.6 ($C(CH_3)_2$), 101.8 (C-5), 92.2 (C-1′), 85.1 (C-4′, d, *J* = 11.9 Hz), 83.2 (C-2′), 81.2 (C-3′), 69.7 (C-5′), 66.9 (C-6′, d, *J* = 161.7 Hz), 61.8 (OCH_2CH_3, d, *J* = 6.5 Hz), 61.2 (OCH_2CH_3, d, *J* = 6.8 Hz), 27.0, 25.1 ($C(CH_3)_2$), 16.4, 16.3 ppm OCH_2CH_3). (75 MHz, $CDCl_3$): δ = 163.0, 150.8 (C-2, C-4), 143.1 (C-6), 114.2 ($C(CH_3)_2$), 103.0 (C-5), 96.2 (C-1′), 87.2 (C-4′, d, *J* = 14.4 Hz), 84.4 (C-2′), 81.8 (C-3′), 69.5 (C-5′), 67.6 (C-6′, d, *J* = 162.2 Hz), 63.7, 62.7 (OCH_2CH_3, 2d, *J* = 6.8 Hz), 27.1, 25.2 ($C(CH_3)_2$), 16.4 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO) δ = 24.1; (100 MHz, $CDCl_3$): δ = 22.6 ppm; UV/vis (EtOH 95): $\lambda_{max}(\varepsilon)$ = 258 (9900), $\lambda_{min}(\varepsilon)$ = 227 nm (2300); MS (FAB > 0, GT): *m/z* 901 $[2M+H]^+$, 451 $[M+H]^+$, 339 $[M-B]^+$; MS (FAB < 0, GT): *m/z* 899 $[2M-H]^-$, 449 $[M-H]^-$, 111 $[B]^-$; Anal. calcd for $C_{17}H_{27}N_2O_{10}P$, 0.3H_2O: C 44.80, H 6.10, N 6.15, P 6.80; found: C 44.42, H 6.21, N 6.02, P 6.85.

1-(2′,3′-*O*-Isopropylidene-6′-diethylphosphono-β-D-ribo-5′R,6′R-hexofuranosyl)uracil (18b): R_f = 0.3 (CH_2Cl_2/MeOH, 2:1); $[\alpha]_D^{20}$ = −2.1 (*c* = 0.96 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.40 (brs, 1H, NH), 7.89 (d, *J* = 8.1 Hz, 1H, H-6), 5.90 (d, *J* = 1.1 Hz, 1H, H-1′), 5.80 (brs, 1H, OH-5′ or OH-6′), 5.60 (brs, 1H, OH-5′ or OH-6′), 5.64 (d, *J* = 8.0 Hz, 1H, H-5), 4.86–4.78 (m, 2H, H-2′, H-3′), 4.32 (t, *J* = 3.0 Hz, H-4′), 4.12–3.97 (m, 4H, OCH_2CH_3), 3.9–3.7 (m, 2H, H-5′, H-6′), 1.49, 1.29 (2 s, 6H, $C(CH_3)_2$), 1.23 ppm (t, *J* = 7.0 Hz, 6H, OCH_2CH_3). (300 MHz, $CDCl_3$): δ = 9.60 (brs, 1H, NH), 7.58 (d, *J* = 8.1 Hz, 1H, H-6), 5.80 (d, *J* = 3.2 Hz, 1H, H-1′), 5.66 (d, *J* = 8.1 Hz, 1H, H-5), 4.91 (dd, *J* = 3.2, 6.3 Hz, 1H, H-3′), 4.80 (dd, *J* = 3.2, 6.2 Hz, 1H, H-2′), 4.39 (t, *J* = 2.8 Hz, H-4′), 4.3–3.9 (m, 8H, $(OCH_2CH_3)_2$, H-5′, H-6′, OH-5′, OH-6′), 1.52 (s, 3H, $C(CH_3)$), 1.31–1.25 ppm (m, 9H, $C(CH_3)$, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.1 (C-4), 150.4 (C-2), 141.3 (C-6), 113.1 ($C(CH_3)_2$), 101.9 (C-5), 89.8 (C-1′), 84.9 (C-4′, d, *J* = 8.1 Hz), 83.3, 80.6 (C-2′, C-3′), 69.7 (C-5′, d, *J* = 9.7 Hz), 67.2 (C-6′, d, *J* = 159.1 Hz), 62.1, 61.4 (OCH_2CH_3, 2d, *J* = 6.8 Hz), 27.1, 25.3 ($C(CH_3)_2$), 16.4, 16.3 ppm (OCH_2CH_3). (75 MHz, $CDCl_3$): δ = 163.3 (C-4), 150.4 (C-2), 142.0 (C-6), 114.6 ($C(CH_3)_2$), 102.9 (C-5), 93.4 (C-1′), 86.2 (C-4′, d, *J* = 9.4 Hz), 83.3 (C-2′), 80.6 (C-3′), 70.2 (C-5′, d, *J* = 4.4 Hz), 68.2 (C-6′, d, *J* = 162.7 Hz), 63.7, 63.0 (OCH_2CH_3, 2d, *J* = 7.0 Hz), 27.3, 25.4 ($C(CH_3)_2$), 16.5, 16.4 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ = 23.0 ppm. (100 MHz, $CDCl_3$): δ = 21.9 ppm; UV/Vis (EtOH 95) $\lambda_{max}(\varepsilon)$ = 259 nm (9300), λ_{min} (ε) = 228 nm (2200); MS (FAB > 0, GT): *m/z* 901 $[2M+H]^+$, 451 $[M+H]^+$, 339 $[M-B]^+$; MS (FAB < 0, GT): *m/z* 899 $[2M-H]^-$, 449 $[M-H]^-$, 111 $[B]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{17}H_{28}N_2O_{10}P$: 451.1482; found: 451.1498; Anal. calcd for $C_{17}H_{27}N_2O_{10}P$: C 45.34, H 6.04, N 6.22, P 6.88; found: C 44.49, H 6.13, N 6.12, P 6.20.

1-(6′-Diethylphosphono-β-D-ribo-5′S,6′S-hexofuranosyl)uracil (19a): Compound **18a** (605 mg, 1.34 mmol) was treated using method A. Column chromatography on reverse phase (H_2O/CH_3CN, 0→15%) and freeze drying gave the titled compound as a white solid (525 mg, 93%): R_f = 0.4 (CH_2Cl_2/MeOH, 8:2); $[\alpha]_D^{20}$ = −18.8 (*c* = 1.01 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.29 (brs, 1H, NH), 7.65 (d, *J* = 8.1 Hz, 1H, H-6), 5.72 (m, 1H, H-1′), 5.57 (d, *J* = 8.1 Hz, 1H, H-5), 5.40 (brs, 1H, OH-2′), 5.26 (brs, 1H, OH-3′), 5.15 (brs, 1H, OH-5′), 4.98 (brs, 1H, OH-6′), 4.12–4.04 (m, 2H, H-2′, H-3′), 4.04–3.86 (m, 4H, OCH_2CH_3), 3.86–3.82 (m, 1H, H-4′), 3.82–3.67 (m, 2H, H-5′, H-6′), 1.17, 1.16 ppm (2t, *J* = 7.0 Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.0 (C-4), 150.9 (C-2), 140.9 (C-6), 102.0 (C-5), 86.6 (C-1′), 84.3 (C-4′, d, *J* = 11.2 Hz), 72.3, 70.0 (3 s, C-2′, C-3′, C-5′), 66.8 (C-6′, d, *J* = 161.4 Hz), 62.0, 61.2 (OCH_2CH_3, 2d, *J* = 6.8 Hz), 16.3, 16.2 ppm (OCH_2CH_3); ^{31}P NMR (100 MHz, [D$_6$]DMSO): δ = 24.1; UV/Vis (EtOH 95): λ_{max} (ε) = 260 (9700), λ_{min} (ε) = 228 nm (2000); MS (FAB > 0, GT): *m/z* 821 $[2M+H]^+$, 411 $[M+H]^+$, 299 $[M-B]^+$; MS (FAB < 0, GT): *m/z* 819 $[2M-H]^-$, 409 $[M-H]^-$, 111 $[B]^-$; Anal. calcd for $C_{14}H_{23}N_2O_{10}P$ 0.3H_2O: C 40.45, H 5.72, N 6.74, P 7.45; found: C 40.18, H 5.85, N 6.57, P 7.27.

1-(6′-Diethylphosphono-β-D-ribo-5′R,6′R-hexofuranosyl)uracil (19b): Compound **18b** (450 mg, 1.0 mmol) was treated using method A. Column chromatography on reverse phase (H_2O/CH_3CN, 0 to 15%) and freeze drying gave the titled compound as a white solid (400 mg, 97%): R_f = 0.3 (CH_2Cl_2/MeOH, 8:2); $[\alpha]_D^{20}$ = + 13.8 (*c* = 1.09 in MeOH); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.32 (brs, 1H, NH), 7.99 (d, *J* = 8.1 Hz, 1H, H-6), 5.78 (d, *J* = 5.3 Hz, 1H, H-1′), 5.78 (brs, 1H, OH-5′ or OH-6′), 5.64 (d, *J* = 8.1 Hz, 1H, H-5), 5.59 (brs, 1H, OH-5′ or OH-6′), 5.36 (d, *J* = 5.4 Hz, 1H, OH-2′), 5.13 (d, *J* = 4.6 Hz, 1H, OH-3′), 4.15 (m, 1H, H-4′), 4.11–3.96 (m, 6H, H-2′, OCH_2CH_3, H-3′), 3.77 (m, 2H, H-5′, H-6′), 1.23 ppm (t, *J* = 7.0 Hz, 6H, OCH_2CH_3); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 163.1 (C-4), 150.7 (C-2), 140.6 (C-6), 101.7 (C-5), 87.5 (C-1′), 84.0 (C-4′, d, *J* = 2.3 Hz), 73.6 (C-2′), 71.0 (C-3′), 69.7 (C-5′, d, *J* = 11.6 Hz), 67.2 (C-6′, d, *J* = 159.0 Hz), 62.1, 61.4 (OCH_2CH_3, 2d, *J* = 6.9 Hz), 16.3, 16.2 ppm (OCH_2CH_3, 2d, *J* = 5.6 Hz); ^{31}P NMR (100 MHz, [D$_6$]DMSO) δ = 22.9 ppm; UV/Vis (EtOH 95): $\lambda_{max}(\varepsilon)$ = 260 (9900), $\lambda_{min}(\varepsilon)$ = 229 nm (1900); MS (FAB > 0, GT): *m/z* 843 $[2M+Na]^+$, 821 $[2M+H]^+$, 433 $[M+Na]^+$, 411 $[M+H]^+$; MS (FAB < 0, GT): *m/z* 819 $[2M-H]^-$, 409 $[M-H]^-$; HRMS-FAB: *m/z* $[M+H]^+$ calcd for $C_{14}H_{24}N_2O_{10}P$: 411.1169, found: 411.1186; Anal. calcd for $C_{14}H_{23}N_2O_{10}P$: C 40.98, H 5.65, N 6.83, P 7.55; found: C 40.55, H 5.85, N 6.69, P 6.70.

1-(6′-Phosphono-β-D-ribo-5′S,6′S-hexofuranosyl)uracil (disodium salt) (20a): Compound **19a** (204 mg, 0.5 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, and subsequent ion exchange on DOWEX Na^+ and freeze drying gave the titled compound as a white solid (90 mg, 45%): R_f = 0.09 (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20}$ = −15.6 (*c* = 0.90 in H_2O); ^{1}H NMR (300 MHz, D_2O): δ = 7.76 (d,

J=8.1 Hz, 1H, H-6), 5.87 (d, J=5.8 Hz, 1H, H-1′), 5.81 (d, J=8.0 Hz, 1H, H-5), 4.36–4.23 (m, 2H, H-3′, H-2′), 4.13 (m, 1H, H-4′), 3.99 (m, 1H, H-5′), 3.70 ppm (dd, J=1.8, 10.8 Hz, 1H, H-6′); ^{13}C NMR (75 MHz, D_2O): δ=166.2 (C-4), 151.9 (C-2), 141.9 (C-6), 102.6 (C-5), 87.7 (C-1′), 84.3 (C-4′, d, J=10.3 Hz), 73.8 (C-2′), 71.0 (C-5′, d, J=2.1 Hz), 70.0 (C-3′), 68.1 ppm (C-6′, d, J=148.9 Hz); ^{31}P NMR (100 MHz, D_2O): δ=16.3 ppm; UV/Vis (H_2O): $\lambda_{max}(\varepsilon)$=260 (9600), $\lambda_{min}(\varepsilon)$=229 nm (2100); MS (FAB>0, GT): m/z 399 $[M+H]^+$; MS (FAB<0, GT): m/z 375 $[M-Na]^-$, 353 $[M-2Na+H]^-$; Anal. calcd for $C_{10}H_{13}N_2Na_2O_{10}P$ 1.6H_2O: C 28.13, H,3.82, N 6.56, P 7.25; found: C 28.02, H 3.89, N 6.56, P 7.32.

1-(6′-Phosphono-β-D-ribo-5′*R*,6′*R*-hexofuranosyl)uracil (disodium salt) (20b): Compound **19b** (185 mg, 0.45 mmol) was treated using method B. Column chromatography on reverse phase (H_2O) gave the corresponding phosphonic acid, and subsequent ion exchange on DOWEX Na^+ and freeze drying gave the titled compound as a white solid (55 mg, 31%): R_f=0.1 (*i*PrOH/NH_4OH 30%/H_2O, 7:1:2); $[\alpha]_D^{20}$=+12.1 (c=0.91 in H_2O); ^{1}H NMR (300 MHz, D_2O): δ=7.84 (d, J=8.1 Hz, 1H, H-6), 5.76 (d, J=4.1 Hz, 1H, H-1′), 5.74 (d, J=8.0 Hz, 1H, H-5), 4.24–4.09 (m, 3H, H-2′, H-4′, H-3′), 3.93 (q, J=4.5 Hz, H-5′), 3.68 ppm (dd, J=4.3, 10.2 Hz, 1H, H-6′); ^{13}C NMR (75 MHz, D_2O): δ=166.3 (C-4), 151.7 (C-2), 148.8 (C-5′), 142.2 (C-6), 102.2 (C-5), 89.4 (C-1′), 84.7 (C-4′, d, J=9.5 Hz), 73.6 (C-2′), 70.8 (C-5′, d, J=3.6 Hz), 70.2 (C-3′), 69.2 ppm (C-6′, d, J=148.4 Hz); ^{31}P NMR (100 MHz, D_2O): δ=15.9 ppm; UV/Vis (H_2O); $\lambda_{max}(\varepsilon)$=260 (8900), $\lambda_{min}(\varepsilon)$=228 nm (1500); MS (FAB>0, GT): m/z 399 $[M+H]^+$, 377 $[M+2H-Na]^+$; MS (FAB<0, GT): m/z 375 $[M-Na]^-$, 353 $[M-2Na+H]^-$; HRMS-FAB: m/z $[M+H]^+$ calcd for $C_{10}H_{14}N_2Na_2O_{10}P$: 399.0181; found: 399.0163.

1-(2′,3′-*O*-Isopropylidene-5′,6′-epoxy-6′-diethylphosphono-β-D-ribofuranosyl)uracil (22): A stirred solution of diol **18** (130 mg, 0.29 mmol) in anhyd CH_2Cl_2 (1.5 mL) was treated with Et_3N (0.073 mL, 0.52 mmol) and *p*-nitrosulfonyl chloride (84 mg, 0.38 mmol) at 0 °C and stirred at 0 °C for 26 h. The resulting mixture was diluted with EtOAc and washed with aq NH_4Cl. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was dissolved in acetone (3 mL) and K_2CO_3 (120 mg, 0.87 mmol) was added at RT. The mixture was stirred for 24 h and K_2CO_3 (120 mg, 0.87 mmol) was added a second time. After 3 h, the reaction was still incomplete. The mixture was filtered through celite and the filtrate was evaporated. Column chromatography (CH_2Cl_2/Acetone, 7:3, *v/v*) gave the titled compound (30 mg, 15%): R_f=0.3 (CH_2Cl_2/Acetone, 7:3); ^{1}H NMR (300 MHz, [D_6]DMSO): δ=9.61 (brs, 1H, NH), 7.16 (d, J=8.1 Hz, 1H, H-6), 5.66 (d, J=8.0 Hz, 1H, H-5), 5.19 (s, 1H, H-1′), 5.20–5.11 (m, 2H, H-2′, H-3′), 4.57 (dd, J=3.0, 8.7 Hz, 1H, H-4′), 4.20–4.06 (m, 4H,OCH_2CH_3), 3.51 (m, 1H, H-5′), 2.97 (dd, J=4.2, 25.8 Hz, 1H, H-6′), 1.47 (s, 3H, $C(CH_3)$), 1.28 ppm (m, 9H, ($C(CH_3)$, OCH_2CH_3); ^{31}P NMR (100 MHz, [D_6]DMSO): δ=17.3 ppm; MS (FAB>0, GT): m/z 865 $[2M+H]^+$, 433 $[M+H]^+$, 321 $[M-B]^+$; MS (FAB<0, GT): m/z 863 $[2M-H]^-$, 431 $[M-H]^-$, 111 $[B]^-$.

Biology

Antiviral activity measurements: Compounds were evaluated in cell-based assays against a number of representative viruses from $ssRNA^+$ flaviviridae and $ssRNA^-$ families, such as yellow fever (YFV) and west nile (WNV) viruses for flavivirus, hepatitis C virus (HCV) for hepacivirus, and respiratory syncytial virus (RSV) for pneumovirus. All experiments were carried out following previously described methods.[32]

Cytotoxicity studies: Methylthiazoletetrazolium (MTT), isopropanol and NaCl were purchased from Sigma–Aldrich (St. Quentin Fallavier, France) and HCl from Merck (Strasbourg, France). RPMI and DMEM cell culture media were purchased from Invitrogen (Cergy Pontoise, France), L-glutamine and penicillin streptomycin from Gibco (Cergy Pontoise, France), and fetal bovine serum (FBS) from PAN Biotech GmbH (California, USA). Human breast adenocarcinoma cells (MCF7) were grown in DMEM media containing L-glutamine, penicillin (200 UI mL^{-1}), streptomycin (200 μg mL^{-1}) and 10% FBS. Human sarcoma cells (Messa) and murine leukaemia cells (L1210) were grown in RPMI media containing penicillin (200 UI mL^{-1}), streptomycin (200 μg mL^{-1}) and 10% FBS. All cells were grown at 37 °C in the presence of 5% CO_2. Development and characterisation of deoxynucleoside-resistant sub-cell lines of MCF7 (MCF7 1K), Messa (Messa 10K) and L1210 (L1210 10K) have been reported elsewhere.[33]

For adherent cells (MCF7 and Messa), 1000–3000 cells were plated per well in 96-well plates (Becton Dickinson) in a volume of 100 μL and incubated for 24 h at 37 °C before drugs were added at different concentrations. For suspension cells (L1210), 100 μL containing 10000 cells were added to each well of the 96-well plates containing 100 μL media with different concentrations of drugs. After incubation at 37 °C for 6 d, MTT (100 μg) was added and, after 2 h of incubation at 37 °C, the supernatant was replaced with 100 μL isopropanol/HCl/H_2O (90:9:1) to solubilise the formazan crystals. Spectro-photometric determination of optical density was performed using a microplate reader (Labsystem Multiskanner RC). The 50% inhibitory concentration (IC_{50}) was defined as the concentration inhibiting proliferation to a level equal to 50% of that of controls, and the resistance ratio (RR) was the ratio between the IC_{50} value of the deoxynucleoside analogue in the resistant cell line and the IC_{50} value determined in the sensitive parent cell line. The statistical significance between IC_{50} values and RR values was determined using a Student's t-test.

Protein expression and purification: Human his-tagged UMP-CMP kinase was expressed and purified to homogeneity as described previously.[26,34] The recombinant hAMPK1 and hAMPK2 were prepared as described.[25c]

Enzymatic activity measurements: The activities of hNMP kinases were followed using a coupled spectro-photometric assay.[35] The reaction mixture contained 50 mM Tris/HCl (pH 7.4), 50 mM KCl, 5 mM $MgCl_2$, 1 mM ATP, 0.2 mM NADPH, 1 mM phosphophenolpyruvate, 1 mM DTT, 4 U μL^{-1} pyruvate kinase and 4 U μL^{-1} lactate deshydrogenase. The enzyme stock solutions were hUMP-CMPK 26 mg mL^{-1}; hAMPK1 16 mg mL^{-1}; hAMPK2 1.23 mg mL^{-1}. Briefly, assays were carried out at 37 °C, the reaction started by adding the enzyme, and then the nucleotide analogue at the desired concentration and the decrease in absorbance at 340 nm was measured. The kinase concentrations were 4 nM to 8 μM in order to measure initial rates below 0.2 ΔA min^{-1}. The absence of inhibition of the coupled system was checked by measuring the reaction with 20 μM ADP with and without the test compounds at 1 mM. No inhibition was observed. Assays were performed in duplicate or triplicate. Results (initial rates) were analysed using the KALEIDAGRAPH software. Inhibitory potential of phosphonate analogues that did not behave as NMP kinase substrates was evaluated at 1 mM concentration against the selected NMP kinases. Thus, the given NMP kinase is preincubated with the potential inhibitor for 5–15 min, and then the native substrate is added allowing the reaction to start.

Structure-based molecular docking

Nucleotide analogues were drawn using Chemsketch software (http://www.nmrsoftware.com/products/chem_dsn_lab/chemsketch/) to produce two-dimensional molecules subsequently translates to pdb files. Bond chemistry and sugar dihedral angles were checked using ArgusLab software (http://www.arguslab.com/arguslab40.htm) and visualised within the PyMOL graphic system if necessary. Docking of nucleotide/nucleoside analogues was performed using ArgusLab software. As only the open structure of hUMP-CMPK (nonfunctional dimer) is available, the closed form was generated from the coordinates of the dictyoselium enzyme (PDB: 2UKD) as previously described.[34] HMM-HMM comparison using the HHpred server (http://toolkit.tuebingen.mpg.de/hhpred) on the Protein Data Bank database was used to search for structural templates suitable for comparative modelling;[36] in this case, it was the dictyoselium enzyme (PDB: 2UKD). The model of hUMP-CMP kinase was then generated using Modeller v.8.1, and the model exhibiting the best consensus between Verify3D and Prosa2003[37] evaluation functions was selected for analysis.

The coordinates of the closed form (PDB: 1Z83) was used for hAMPK1. Docking precision was set to high, and the flexible ligand docking mode was used for each docking run. The geometry of each investigated analogue was checked and optimised using ArgusLab options to avoid the occurrence of bad interactions. Resulting complexes from poses of lowest energy were visualised within Molegro molecular viewer.[38] Positioning in the NMP site pockets and the hydrogen-bond network for each nucleotide analogue/NMP kinase model were examined and figures subsequently generated.

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