Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 3448

www.rsc.org/obc

PAPER

Synthesis of a new family of acyclic nucleoside phosphonates, analogues of TPases transition states†

Bénédicte Dayde,[a,b] Samira Benzaria,[b] Claire Pierra,[b] Gilles Gosselin,[b,c] Dominique Surleraux,[b] Jean-Noël Volle,[a] Jean-Luc Pirat[a] and David Virieux*[a]

Received 17th January 2012, Accepted 29th February 2012
DOI: 10.1039/c2ob25131k

A 6-step procedure was developed for the synthesis of a new family of acyclic nucleoside phosphonates (ANPs), "PHEEPA" [(2-pyrimidinyl-2-(2-hydroxyethoxy)ethyl)phosphonic acids] in overall yields ranging from 4.5% to 32%. These compounds, which possess on one side a hydroxy function and on the other side a phosphonate group, can be considered either as potential antiviral agents or as transition state analogues of nucleoside phosphorylases such as thymidine phosphorylase.

Introduction

For several decades, nucleoside and nucleotide analogues have proved to be promising therapeutic agents, in particular as antivirals.[1] Among them, the acyclic nucleoside phosphonate family (ANPs) represents a prominent class of nucleotide analogues with a broad-spectrum of antiviral activity (HIV, HBV, papilloma-, adeno-, herpes- and poxviruses).[2] Some of them have been approved worldwide by regulatory agencies for clinical uses.[3] Indeed, one of the first ANP derivatives, (*S*)-9-(3-hydroxy-2-phosphonomethoxypropyl)adenine [(*S*)-HPMPA] **1**, was developed 30 years ago, and exhibited a notable activity against virtually all DNA viruses. This structural motif is shared by the group of acyclic nucleoside phosphonates for which Tenofovir **2**, Adefovir **3** and Cidofovir **4** are the well-known blockblusters. Adefovir **3** (9-(2-phosphonylmethoxyethyl)adenine, PMEA, Adefovir dipivoxil: Hepsera®) is a large-spectrum nucleotide analogue active against herpes-, hepadna- and retroviruses used in standard therapy of chronic HBV infection. Tenofovir **2** ((*R*)-9-(2-phosphonylmethoxypropyl)adenine), PMPA, Tenofovir disoproxil fumarate: Viread®) is prescribed for the treatment of both AIDS and HBV infections, whereas Cidofovir **4** (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, HPMPC, Vistide®) is a commonly prescribed drug for treatment of cytomegalovirus virus infections in immunosuppressed patients (Fig. 1).

Their modes of action are similar: in cell, the phosphonate group is transformed into di- and/or triphosphate bioconjugates which inhibit DNA polymerase and/or reverse transcriptase.

We present herein the synthesis of a new family of ANPs in the pyrimidinyl series **5** (Fig. 2). The acyclic sugar chain of these new compounds is terminated, on one side by a hydroxy function and, on the other side, by a phosphonate group. The latter is less polar than the phosphate part and should prevent

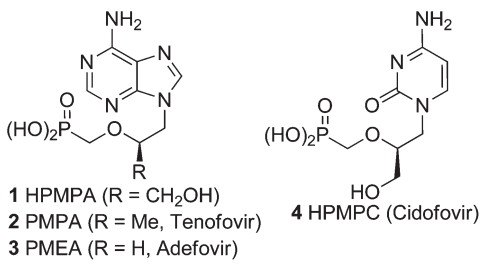


Fig. 1 Marketed acyclic nucleoside analogues.

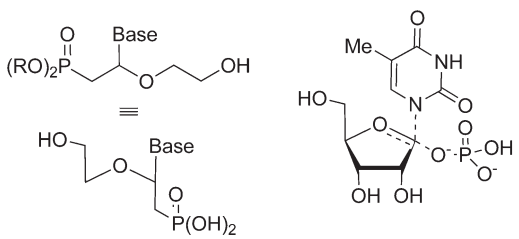
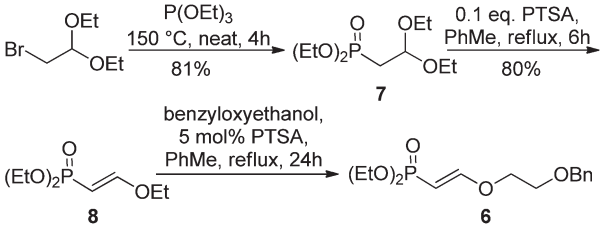


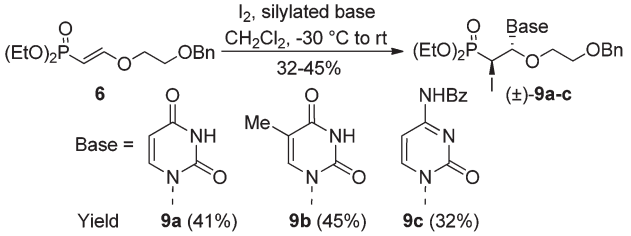
Fig. 2 Targeted acyclic nucleoside analogues and transition state derivative of thymidine phosphorylase.

[a]*AM2N, Institut Charles Gerhardt, UMR 5253, ENSCM, 8 rue de l'Ecole Normale, F-34296 Montpellier, France. E-mail: david.virieux@enscm.fr; Fax: +33 (0)467 14 43 19; Tel: +33 (0)467 14 43 14*
[b]*Idenix Pharmaceuticals, Medicinal Chemistry Laboratory, Cap Gamma, 1682 rue de la Valsière, BP50001, 34189 Montpellier Cedex 4, France*
[c]*UMR5247 CNRS-UM1-UM2 (IBMM), Université Montpellier 2, 34095 Montpellier Cedex 5, France*

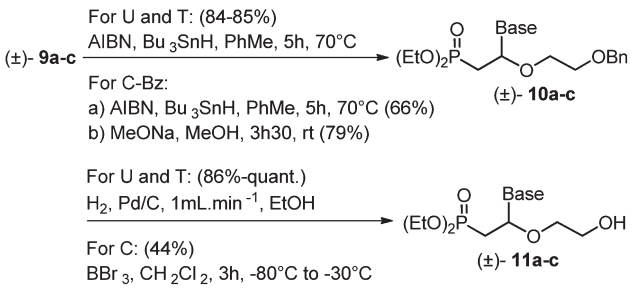
†Electronic supplementary information (ESI) available: Copies of the relevant spectra or analyses (^{1}H NMR, ^{13}C NMR, ^{31}P NMR, MS, HRMS). See DOI: 10.1039/c2ob25131k



Scheme 1 Synthesis of benzyloxyethyloxyethylenylphosphonate **6**.



Scheme 2 Iodo-base introduction on **6**.



Scheme 3 Reduction and debenzylation steps.

enzymatic degradation and/or facilitate the nucleoside activation step by avoiding problems during the intracellular first phosphorylation step. Moreover, the pseudo-anomeric carbon is preserved and the central oxygen of the aminal function could behave as the classical ether function of ANPs. Indeed, it has been suggested that the oxygen atom would contribute to the formation of reactive species in the active site of the nucleic acid polymerases by a metal ion binding.[4] Another interest can be found in the structural analogy with the transition state involved in pyrimidine or purine nucleoside phosphorylase enzymes as illustrated with TPase (Fig. 2).[5]

Results and discussion

The strategy to access these new compounds **5** was based on the introduction of the nucleobase on a key intermediate, the diethyl 2-[2-(benzyloxyl)ethoxy]ethenylphosphonate **6** (Scheme 1). This vinylphosphonate derivative **6** was synthesized from the (*E*)-diethyl 2-ethoxyvinylphosphonate **8**, accessible in two steps starting from the reaction of triethyl phosphite with bromoacetaldehyde diethylacetal.[6] The second step was scarcely described and currently uses strong bases and/or high temperature.[7] In this work, the elimination reaction was performed under milder conditions, with 0.1 equivalent of *p*-toluenesulfonic acid. Ethanol was removed during the reaction by azeotropic distillation. Under these new conditions, the expected vinylphosphonate **8** was obtained quantitatively after 6 h heating and isolated in large scale in 80% yield after distillation. The ethenylphosphonate **6** was finally prepared by an addition–elimination step using benzyloxyethanol. Despite a substoichiometric amount of this reagent, 15–20% of the bis(benzyloxyethyl)acetal was observed which was probably in equilibrium with the expected product **6** in the reaction mixture. Nevertheless, the key precursor **6** was synthesized in 65% yield and with purity higher than 95% by ^{31}P NMR after distillation.

The key step for the synthesis of targets **5** was the addition of appropriate nucleobases to the unsaturated intermediate **6**. The reactivity of this unusual double bond was not described before. Our first attempts at the addition of nucleobase used Michael-type conditions. Unfortunately, no reaction was observed either in basic[8] or acidic conditions.[9] In order to get a better knowledge of the reactivity and the behavior of this alkene function, we tried to perform dihydroxylation[10] and epoxidation reactions.[11] Whatever the reagent, no reaction was observed under the tested conditions.

The α,β-unsaturated phosphonate **6** was successfully reacted in the presence of iodine and *O*-silyl uracil (Scheme 2).[12] By contrast, the addition of purines such as adenine appeared inefficient.

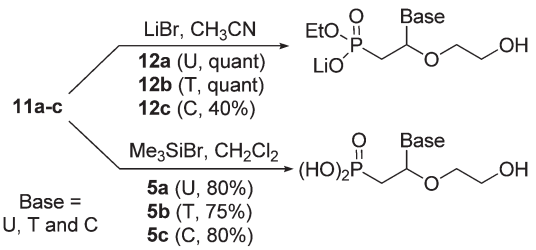
Using these conditions, the addition of pyrimidine nucleobases was carried out using uracil, thymine and *N*-benzoyl-cytosine. The reaction was fully stereo- and regioselective affording only one regio- and diastereomer **9a–c**. Formerly, the stereochemical outcome of this addition led to a racemic mixture where iodine and nucleobase were introduced relatively in the *trans* position (Scheme 2).

Further, removal of the iodine was effective using the radical deiodination mediated by tributyltin hydride (Scheme 3).[13] The expected products **10a–c** (U, T and C-Bz) were obtained in yields ranging from 66 to 85%. Benzoyl protection of the cytosine base was removed using sodium methoxide to afford the free cytosine derivative **10c** in 79% yield.[14] The following step was the debenzylation of the terminal hydroxyl group which occurred selectively and completely with uracil **11a** and thymine **11b** using a continuous flow reactor (H-Cube apparatus) and a Pd/C 10% cartridge in full H_2 mode with a flow of 1 mL min^{-1} in ethanol. Unfortunately the debenzylation failed under the same conditions with the unprotected cytosine **10c**. After optimization, the best conditions for the debenzylation of cytosine derivative **10c** were found using boron tribromide in dichloromethane (1.0 M) at −30 °C.[15] The expected product **11c** was isolated in 44% yield along with a partial P-ester cleavage.

Finally, the phosphonate ester deprotection of this new series was achieved using either LiBr[16] for an access to the monophosphonate esters **12a–c**, or TMSBr[17] to get the free phosphonic acids **13a–c** (Scheme 4).

Conclusions

In summary, we successfully developed a methodology for the access to a new family of ANPs in the pyrimidinyl series, keeping the aminal function. It allowed the preparation of original nucleotide analogues in only 6–7 steps and in overall yields ranging from 4.5% and 32%. The activity evaluations of such compounds will be presented in a dedicated paper.



Scheme 4 Synthesis of free phosphonic acid and mono-lithium salt derivatives **5a–c** and **12a–c**.

Experimental section

General considerations: Before use, commercial reagents were purified by distillation or sublimation. All manipulations were carried out using standard Schlenck techniques. Solvents were dried according to current methods, distilled and stored under nitrogen atmosphere. All reactions involving air or moisture sensitive reagents or intermediates were carried out under dry nitrogen in flame-dried glassware. Melting points were measured on a Büchi B-540 apparatus and are uncorrected. All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR using a Bruker DRX 400 MHz NMR spectrometer or a Bruker Avance 250 MHz NMR spectrometer. All NMR experiments performed on phosphorus were recorded with proton-decoupled. All NMR recorded during reaction were done with a closed capillary DMSO-d_6 probe in the NMR tube. Low and high resolution mass spectra were determined on an electrospray ionization (RSI) WATERS Micromas Q-Tof spectrometer with as internal reference H_3PO_4 (0.1% in water–acetonitrile, 1 : 1).

Diethyl (2,2-diethoxyethyl)phosphinate (7)

In a 100 mL two-necked flask under nitrogen equipped with a Dean Stark apparatus were successively introduced 2-bromoacetaldehyde diethyl acetal (20.00 g, 101 mmol) and triethyl phosphite (33.70 g, 202 mmol). The reaction mixture was heated at 165 °C for 4 h. The resulting bromoethane was distilled off in the Dean Stark (~6 mL). Then, the reaction mixture was cooled to room temperature and distilled under vacuum (bp = 78–81 °C, $P = 93 \times 10^{-3}$ mbar) affording **7** as a colorless oil (21.01 g, 81%).‡ ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 26.4; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 4.80 (dt, $^{3}J_{PH}$ = 5.5 Hz, $^{3}J_{HH}$ = 5.3 Hz, 1H, OCHO), 4.06–3.99 (m, 4H, 2 CH_2), 3.61–3.54 (m, 1H, CH_2), 3.50–3.42 (m, 1H, CH_2), 2.11 (dd, $^{2}J_{PH}$ = −18.7 Hz, $^{3}J_{HH}$ = 5.7 Hz, 2H, PCH_2), 1.26 (t, $^{3}J_{HH}$ = 7.1 Hz, 6H, CH_3), 1.12 (t, $^{3}J_{HH}$ = 7.2 Hz, 6H, CH_3).

(*E*)-Diethyl (2-ethoxy)vinylphosphonate (8)

In a 100 mL two-necked flask under nitrogen equipped with a Dean Stark apparatus were introduced diethyl 2,2-diethoxyethylphosphonate **7** (20.97 g, 82 mmol) in toluene (100 mL) and *p*-toluenesulfonic acid (1.37 g, 8 mmol). The reaction mixture was heated 6 h in order to remove the toluene–ethanol azeotrope. After return to rt, the reaction mixture was concentrated under vacuum and distilled (bp = 84–87 °C, $P = 32 \times 10^{-3}$ mbar) to afford **8** as a colorless oil (13.72 g, 80%).§ ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 22.6; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 7.14 (dd, $^{3}J_{HH}$ = 13.6 Hz, $^{3}J_{PH}$ = 11.5 Hz, 1H, OCH), 4.64 (dd, $^{3}J_{HH}$ = 13.6 Hz, $^{2}J_{PH}$ = −10.0 Hz, 1H, PCH), 4.05–3.93 (m, 4H, 2 CH_2), 3.83 (q, $^{3}J_{HH}$ = 13.6 Hz, 2H, OCH_2), 1.27 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, CH_3), 1.12 (t, $^{3}J_{HH}$ = 7.1 Hz, 6H, 2 CH_3).

(*E*)-Diethyl 2-[2-(benzyloxy)ethoxy]vinylphosphonate (6)

In a 100 mL two-necked flask under nitrogen equipped with a Dean Stark apparatus were introduced (*E*)-diethyl (2-ethoxy) vinylphosphonate **8** (8.00 g, 38 mmol) in toluene (360 mL), benzyloxyethanol (5.67 g, 37 mmol) and *p*-toluenesulfonic acid (0.32 g, 1.9 mmol). The reaction mixture was heated 6 h in order to remove the toluene–ethanol azeotrope. After return to rt, the reaction mixture was concentrated under vacuum. The impurities were removed by distillation (b.p. = 150–170 °C, 60–70 × 10^{-3} mbar) affording a yellow oil as residue **6** (9.09 g, 79%). ^{31}P ($CDCl_3$, 161.97 MHz) δ = 22.0; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 7.45–7.23 (m, 6H, CH and OCH), 4.74 (dd, $^{3}J_{HH}$ = 13.6 Hz, $^{2}J_{PH}$ = −9.7 Hz, 1H, PCH), 4.56 (s, 2H, $PhCH_2$), 4.10–3.97 (m, 6H, 3 CH_2), 3.72–3.69 (m, 2H, CH_2), 1.30 (t, $^{3}J_{HH}$ = 6.9 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 163.4 (d, $^{2}J_{CP}$ = 21.5 Hz, OCH), 137.6 (s, C), 128.4 (s, CH), 127.8 (s, CH), 127.7 (s, CH), 88.5 (d, $^{1}J_{CP}$ = 200.5 Hz, PCH), 73.3 (s, $PhCH_2$), 69.9 (s, OCH_2), 67.8 (s, OCH_2), 61.8 (d, $^{2}J_{CP}$ = 5.2 Hz, OCH_2), 16.3 (d, $^{3}J_{CP}$ = 6.7 Hz, CH_3); MS-ESI$^+$ *m*/*z* = 315.1 (31%) [M + H]$^+$; HRMS-ESI$^+$ (*m*/*z*): [M + H]$^+$ calcd for $C_{15}H_{23}O_5P$: 315.1361; found 315.1359.

General procedure for the synthesis of 9a–9c

In a 100 mL two-necked flask under nitrogen were introduced (*E*)-diethyl 2-[2-(benzyloxy)ethoxy]vinylphosphonate **6** (1 eq.) and CH_2Cl_2 (1 volume, concentration 0.1 mol × L^{-1}). The reaction mixture was cooled at −30 °C. The appropriate trimethylsilyl nucleobase (3 eq.) and iodine (1.8 eq.) were added while maintaining the temperature at −30 °C for 5 h. Then, the reaction mixture was allowed to reach rt for the night. After addition of CH_2Cl_2 (5 volumes), the organic layer was washed with a solution of 10% $Na_2S_2O_3$ (2.5 volumes) until it became colorless and then the organic layer was washed with water. The organic layers were dried over $MgSO_4$ and concentrated under vacuum giving the crude material which was purified by column chromatography on silica gel.

(±)-(1*R*,2*R*)-Diethyl 2-[2-(benzyloxy)ethoxy]-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-1-iodoethylphosphonate (9a). Ester **6** (0.50 g, 1.60 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (25 g, gradient CH_2Cl_2–AcOEt (80 : 20 to 20 : 80)) affording **9a** as a clear oil (0.35 g, 40%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 16.6; ^{1}H NMR ($CDCl_3$,

‡ The phosphonate **7** was already described by Varlet *et al.*[6]

§ The formation of **8** was already described in literature using basic conditions (LDA): Kouno *et al.*[7a]

400.13 MHz) δ = 9.55 (s, 1H, NH), 7.55 (d, $^3J_{HH}$ = 8.1 Hz, 1H, NCH), 7.31–7.20 (m, 5H, CH), 5.57 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, CH), 5.41 (t, $^3J_{HH}$ = $^3J_{PH}$ = 3.2 Hz, 1H, OCHN), 4.49 and 4.45 (2 d, 2H, $^2J_{HH}$ = −11.9 Hz, $PhCH_2$), 4.35 (dd, $^2J_{PH}$ = 14.3 Hz, $^3J_{HH}$ = 3.2 Hz, 1H, PCH), 4.24–4.05 (m, 4H, 2 OCH_2), 3.92–3.50 (m, 4H, CH_2CH_2), 1.28 and 1.27 (2 t, $^3J_{HH}$ = 4.1 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 162.6 (s, C═O), 149.3 (s, C═O), 139.0 (s, NCH), 136.6 (s, C), 126.8 (s, CH), 126.6 (s, CH), 127.5 (s, CH), 100.2 (s, CH), 82.8 (s, OCHN), 72.2 (s, $PhCH_2$), 69.3 (s, OCH_2), 67.4 (s, OCH_2), 63.2, 62.9 (2 d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 18.1 (d, $^1J_{CP}$ = 145.6 Hz, PCH), 15.3 (d, $^3J_{CP}$ = 5.9 Hz, CH_3); MS-ESI$^+$ *m/z* = 553.0 (100%) $[M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{27}IN_2O_7P$: 553.0601; found 553.0607.

(±)-(1*R*,2*R*)-Diethyl 2-[2-(benzyloxy)ethoxy]-2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-1-iodoethylphosphonate (9b). Ester **6** (1.02 g, 3.20 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (25 g, gradient 100% CH_2Cl_2 to 100% AcOEt) affording **9b** as a clear oil (0.81 g, 45%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 16.6; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 8.03 (s, 1H, NH), 7.36 (s, 1H,═CH–N), 7.30–7.20 (m, 5H, CH), 5.42 (t, $^3J_{HH}$ = $^3J_{PH}$ = 3.3 Hz, 1H, OCHN), 4.48 and 4.46 (2 d, 2H, $^2J_{HH}$ = −11.8 Hz, $PhCH_2$), 4.31 (dd, $^2J_{PH}$ = −14.1 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, PCH), 4.18–4.09 (m, 4H, $POCH_2$), 3.84–3.50 (m, 4H, 2 CH_2), 1.28 and 1.27 (2 t, $^3J_{HH}$ = 7.3 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 163.7 (s, C═O), 150.2 (s, C═O), 139.0 (s, CHN), 137.7 (s, ^{Ph}C), 128.5 (s, CH), 127.9 (s, CH), 127.7 (s, CH), 109.8 (s, CMe), 83.6 (s, OCHN), 73.3 (s, CH_2Ph), 70.1 (s, OCH_2), 68.5 (s, OCH_2), 64.2 (d, $^2J_{CP}$ = 5.9 Hz, OCH_2), 63.9 (d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 18.9 (d, $^1J_{CP}$ = 145.6 Hz, PCH), 16.4 (d, $^3J_{CP}$ = 5.9 Hz, CH_3), 12.5 (s, CH_3); MS-ESI$^+$ *m/z* = 567.2 (100%) $[M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{29}N_2O_7IP$: 567.0757; found 567.0757.

(±)-(1*R*,2*R*)-Diethyl 2-[2-(benzyloxy)ethoxy]-2-[2-(4-benzoylamino-2-oxo-2*H*-pyrimidin-1-yl)]-1-iodoethylphosphonate (9c). Ester **6** (0.94 g, 3.00 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (40 g, gradient 100% CH_2Cl_2 to 100% AcOEt) affording **9c** as a yellow solid (0.62 g, 32%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 17.1; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 8.99 (s, 1H, NH), 8.03 (d, $^3J_{HH}$ = 7.5 Hz, 1H, NCH), 7.87–7.83 (m, 2H, CH), 7.56–7.40 (m, 4H, CH and CH), 7.32–7.19 (m, 5H, CH), 5.45 (t, $^3J_{HH}$ = $^3J_{PH}$ = 2.6 Hz, 1H, OCHN), 4.62 (dd, $^2J_{PH}$ = −14.6 Hz, $^3J_{HH}$ = 2.6 Hz, 1H, PCH), 4.48 and 4.45 (2 d, $^2J_{HH}$ = −10.0 Hz, 2H, $PhCH_2$), 4.20–4.08 (m, 4H, 2 $POCH_2$), 3.88 (ddd, $^2J_{HH}$ = −9.9 Hz, $^3J_{HH}$ = 4.3 Hz, $^3J_{HH}$ = 2.0 Hz, 1H, OCH_2), 3.75–3.62 (m, 2H, OCH_2), 3.54 (ddd, $^3J_{HH}$ = 6.7 Hz, $^2J_{HH}$ = 4.3 Hz, $^3J_{HH}$ = 2.0 Hz, 1H, OCH_2), 1.26 (t, $^3J_{HH}$ = 7.0 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 162.8 (s, C═O), 145.1 (s, CH), 137.7 (s, *C*), 133.1, 128.9, 128.5, 127.8, 127.6, 127.5 (6 s, CH), 95.8 (s, CH), 84.5 (s, OCHN), 73.2 (s, $PhCH_2$), 70.5 (s, OCH_2), 68.4 (s, OCH_2), 64.1 (d, $^2J_{CP}$ = 6.3 Hz, OCH_2), 63.8 (d, $^2J_{CP}$ = 6.8 Hz, OCH_2), 19.8 (d, $^1J_{CP}$ = 145.6 Hz, PCH), 16.4 (d, $^3J_{CP}$ = 6.1 Hz, CH_3); MS ESI$^+$ *m/z* = 656 (100%) $[M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{26}H_{32}N_3O_7IP$: 656.1023; found 656.1017.

General procedure for the deiodination reaction – synthesis of 10a–c

Iodophosphonate **9a–c** (1 eq, concentration 0.024 mol L^{-1} in toluene – 1 volume) was dissolved in a two-necked round-bottom flask with a condenser and under nitrogen. AIBN (0.8 eq) and nBu_3SnH (1.8 eq) were added to the solution and the mixture was heated for 5 h at 70 °C. After cooling at room temperature, saturated NH_4Cl solution was added (1 volume) and the resulting phases separated. The aqueous layer was extracted 3 times by AcOEt (1 volume) and the organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The crude mixture was purified by chromatography on silica gel.

(±)-Diethyl 2-[2-(benzyloxy)ethoxy]-2-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)ethylphosphonate (10a). Iodophosphonate **9a** (0.61 g, 1.10 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (gradient 100% AcOEt–50%–50% AcOEt–(AcOEt–EtOH 9 : 1)) affording **10a** as a colorless oil (0.398 g, 85%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 22.9; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 8.78 (s, 1H, NH), 7.34 (d, $^3J_{HH}$ = 8.1 Hz, 1H, NCH), 7.31–7.18 (m, 5H, CH), 5.95 (ddd, $^3J_{PH}$ = 7.80 Hz, $^3J_{HH}$ = 6.6 Hz, $^3J_{HH}$ = 6.1 Hz, 1H, OCHN), 5.64 (dd, $^3J_{HH}$ = 8.1 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, CHC(O)), 4.45 (s, 2H, $PhCH_2$), 4.07–3.99 (m, 4H, 2 OCH_2), 3.73–3.47 (m, 4H, CH_2CH_2), 2.30 (ddd, $^3J_{PH}$ = −18.3 Hz, $^2J_{HH}$ = −15.4 Hz, $^3J_{HH}$ = 6.1 Hz, 1H, PCH_2), 2.23 (ddd, $^3J_{PH}$ = −18.9 Hz, $^2J_{HH}$ = −15.4 Hz, $^3J_{HH}$ = 6.6 Hz, 1H, PCH_2), 1.22 and 1.23 (2 t, $^3J_{HH}$ = 7.0 Hz, 6H, 2 CH_3), ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 162.9 (s, C═O), 150.4 (s, C═O), 139.2 (s, NCH), 137.7 (s, C), 128.5 (s, CH), 127.8 (s, CH), 127.7 (s, CH), 102.9 (s, CH), 81.6 (s, OCHN), 73.3 (s, CH_2), 68.9 (s, CH_2), 68.6 (s, CH_2), 62.4, 62.1 (2 d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 32.4 (d, $^1J_{CP}$ = 142.0 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 6.6 Hz, 2 CH_3); MS ESI$^+$ *m/z* = 427.2 (100%) $[M + H]^+$, *m/z* = 853.5 (92%) $[2M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{28}N_2O_7P$: 427.1634; found 427.1633.

(±)-Diethyl 2-(2-(benzyloxy)ethoxy)-2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)ethylphosphonate (10b). Iodophosphonate **9b** (0.64 g, 1.10 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (gradient 100% AcOEt–50%–50% AcOEt–(AcOEt–EtOH 9 : 1)) affording **10b** as a colorless oil (0.416 g, 84%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 23.2; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 8.92 (s, 1H, NH), 7.30–7.14 (m, 6H, CH and ^{Ph}CH), 5.96 (ddd, $^3J_{PH}$ = 7.6 Hz, $^3J_{HH}$ = 6.7 Hz, $^3J_{HH}$ = 6.2 Hz, 1H, OCHN), 4.44 (s, 2H, CH_2), 4.07–3.99 (m, 4H, OCH_2), 3.70–3.47 (m, 4H, CH_2CH_2), 2.30 (ddd, $^2J_{PH}$ = −20.3 Hz, $^2J_{HH}$ = −16.8 Hz, $^3J_{HH}$ = 6.7 Hz, 1H, PCH_2), 2.23 (ddd, $^2J_{PH}$ = −19.7 Hz, $^2J_{HH}$ = −16.8 Hz, $^3J_{HH}$ = 6.2 Hz, 1H, PCH_2), 1.79 (s, 3H, CH_3), 1.21 and 1.23 (2 t, $^3J_{HH}$ = 7.2 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 163.7 (s, C═O), 150.6 (s, C═O), 137.8 (s, C), 134.7 (s, CH), 128.4 (s, CH), 127.8 (s, CH), 127.6 (s, CH), 111.5 (s, C), 81.0 (s, OCHN), 73.3 (s, $PhCH_2$), 68.7 (s, 2 CH_2), 62.3 (d, $^2J_{CP}$ = 5.9

Hz, OCH_2), 62.0 (d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 32.4 (d, $^1J_{CP}$ = 142.0 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 5.9 Hz, CH_3), 12. 5 (s, CH_3); MS ESI$^+$ *m/z* = 441.2 (100%) $[M + H]^+$, *m/z* = 881.5 (92%) $[2M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{20}H_{30}N_2O_7P$: 441.1791; found 441.1790.

(±)-Diethyl 2-[2-(benzyloxy)ethoxy]-2-[2-(4-benzoylamino-2-oxo-2*H*-pyrimidin-1-yl)]ethylphosphonate (Bz-10c). Iodophosphonate **9c** (0.33 g, 0.50 mmol, 1 eq.) was reacted according to the general procedure. The crude reaction mixture was purified by chromatography on silica gel (gradient 100% AcOEt–50%–50% AcOEt–(AcOEt–EtOH 9 : 1)) affording a white solid (0.177 g, 66%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 23.5; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 9.05 (s, 1H, NH), 7.91 (d, $^3J_{HH}$ = 7.5 Hz, 1H, NCH), 7.88–7.83 (m, 2H, CH), 7.55–7.50 (m, 2H, CH), 7.47–7.39 (m, 3H, ^{Ph}CH and CH), 7.30–7.17 (m, 5H, CH), 6.03 (ddd, $^3J_{PH}$ = 9.8 Hz, $^3J_{HH}$ = 7.7 Hz, $^3J_{HH}$ = 4.3 Hz, 1H, OCHN), 4.44 (s, 2H, $PhCH_2$), 4.09–3.98 (m, 4H, OCH_2), 3.78–3.69 (m, 1H, OCH_2), 3.63–3.56 (m, 2H, OCH_2), 3.55–3.48 (m, 1H, OCH_2), 2.43 (ddd, $^2J_{PH}$ = −18.8 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 4.3 Hz, 1H, PCH_2), 2.27 (ddd, $^2J_{PH}$ = −18.0 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 7.7 Hz, 1H, PCH_2), 1.22 and 1.21 (2 t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 162.4 (s, C═O), 144.1 (s, NCH), 137. 8 (s, ^{Ph}C), 133.1, 128.9, 128.4, 128.9, 127.8, 127.7, 127.6 (7 s, CH), 97.2 (s, CH), 83.5 (s, OCHN), 73.2 (s, CH_2), 69.9 (s, OCH_2), 68.6 (s, OCH_2), 62.0, 61.7 (2 d, $^2J_{CP}$ = 6.2 Hz, 2 OCH_2), 32.5 (d, $^1J_{CP}$ = 140.9 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 6.2 Hz, CH_3); MS ESI$^+$ *m/z* = 530.8 (100%) $[M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{26}H_{33}N_3O_7P$: 530.2056; found 530.2060.

(±)-Diethyl 2-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-2-[2-(benzyloxy)ethoxy]ethylphosphonate (10c). To a solution of benzoyl phosphonate **Bz-10c** (378 mg, 0.71 mmol, 1 eq.) in anhydrous methanol (9 mL) was added a solution of sodium methoxide (0.5 M, 9 mL, 4.5 mmol, 6.3 eq.). The reaction mixture was stirred at rt for 3h 30 min. Then methanol (25 mL) was added and the resulting solution was acidified to pH = 2 by a hydrochloric acid (1 N). Methanol was removed under vacuum and the aqueous phase extracted by AcOEt (2 × 25 mL). The aqueous phase was basified using NaOH (1 M) until pH > 12 and then extracted by AcOEt (4 × 25 mL). The organic phases were dried over $MgSO_4$, filtered and concentrated under vacuum giving **10c** as a colorless oil (240 mg, 79%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 24.0; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 7.45 (d, $^3J_{HH}$ = 7.4 Hz, 1H, NCH), 7.32–7.14 (m, 5H, ^{Ph}CH), 6.01 (ddd, $^3J_{PH}$ = 8.8 Hz, $^3J_{HH}$ = 7.8 Hz, $^3J_{HH}$ = 4.7 Hz, 1H, OCHN), 5.76 (d, $^3J_{HH}$ = 7.5 Hz, 1H), 4.44 (s, 2H, $PhCH_2$), 4.07–3.99 (m, 4H, 2 OCH_2), 3.70–3.47 (m, 4H, CH_2CH_2), 2.27 (ddd, $^2J_{PH}$ = −18.7 Hz, $^2J_{HH}$ = 15.5 Hz, $^3J_{HH}$ = 4.7 Hz, 1H, PCH_2), 2.23 (ddd, $^2J_{PH}$ = −18.1 Hz, $^2J_{HH}$ = 15.5 Hz, $^3J_{HH}$ = 7.8 Hz, 1H, PCH_2), 1.21 and 1.22 (2 t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 165.5 (s, C═O), 155.9 (s, CNH_2), 140.3 (s, CHN), 138.0 (s, ^{Ph}C), 128.4, 127.7, 127.6 (s, ^{Ph}CH), 95.3 (s, CH), 82.1 (s, OCHN), 73.1 (s, $PhCH_2$), 68.8, 68.7 (2 s, CH_2CH_2), 62.2, 61.9 (d, $^2J_{CP}$ = 6.3 Hz, 2 CH_2), 32. 6 (d, $^1J_{CP}$ = 140.7 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 6.2 Hz, CH_3); MS ESI$^+$ *m/z* = 426.3 (100%) $[M + H]^+$, *m/z* = 851.6 (42%) $[2M + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{29}N_3O_6P$: 426.1794; found 426.1791.

(±)-Diethyl 2-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonate (11a). *O*-Benzyl phosphonate **10a** (52 mg, 0.12 mmol, 1 eq.) was dissolved in anhydrous ethanol (6 mL) and the solution was reacted using a H-cube (Pd/C 10%, 1 mL min^{-1}, full H_2 mode). The reaction mixture was concentrated under vacuum giving **11a** as a colorless oil (43 mg, quantitative). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 24.6; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 9.76 (s, 1H, NH), 7.40 (d, $^3J_{HH}$ = 8.1 Hz, 1H, NCH), 6.03 (ddd, $^3J_{HH}$ = 10.2 Hz, $^3J_{PH}$ = 5.7 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, OCHN), 5.75 (d, $^3J_{HH}$ = 8.1 Hz, 1H, CH), 4.18–4.00 (m, 4H, OCH_2), 3.80–3.40 (m, 4H, CH_2CH_2), 2.27 (ddd, $^2J_{PH}$ = −18.3 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 10.2 Hz, 1H, PCH_2), 2.25 (ddd, $^2J_{PH}$ = −18.8 Hz, $^2J_{HH}$ = 15.3 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, PCH_2), 1.28 and 1.29 (2 t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 163.5 (s, C═O), 150.5 (s, C═O), 138.5 (s, NCH), 103.4 (s, CH), 81.0 (s, OCHN), 71.5 (s, OCH_2), 62.7 (d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 62.5 (d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 60.3 (s, CH_2OH), 32.6 (d, $^1J_{CP}$ = 142.0 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 5.9 Hz, CH_3), 16.3 (d, $^3J_{CP}$ = 7.0 Hz, CH_3); MS ESI$^+$ *m/z* = 337.2 (100%) $[M + H]^+$, *m/z* = 673.4 (94%) $[2M + H]^+$, *m/z* = 359.5 (20%) $[M + Na]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}N_2O_7P$: 337.1165; found 337.1158.

(±)-Diethyl 2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonate (11b). *O*-Benzyl phosphonate **10b** (50 mg, 0.11 mmol, 1 eq.) was dissolved in anhydrous ethanol (5.5 mL) and the solution was reacted using a H-cube (Pd/C 10%, 1 mL min^{-1}, full H_2 mode). The reaction mixture was concentrated under vacuum giving **11b** as colorless oil (33 mg, 86%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 24.8; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 9.59 (s, 1H, NH), 7.19 (s, 1H, NCH), 6.04 (ddd, $^3J_{HH}$ = 9.9 Hz, $^3J_{PH}$ = 5.8 Hz, $^3J_{HH}$ = 3.4 Hz, 1H, OCHN), 4.40 (s, 1H, OH), 4.18–4.02 (m, 4H, OCH_2), 3.80–3.40 (m, 4H, CH_2CH_2), 2.29 (ddd, $^2J_{PH}$ = −18.2 Hz, $^2J_{HH}$ = −15.1 Hz, $^3J_{HH}$ = 9.8 Hz, 1H, PCH_2), 2.21 (ddd, $^2J_{PH}$ = 18.9 Hz, $^2J_{HH}$ = 15.1 Hz, $^3J_{HH}$ = 3.4 Hz, 1H, PCH_2), 1.88 (d, $^4J_{HH}$ = 0.8 Hz, 3H, CH_3), 1.28 and 1.29 (2 t, $^3J_{HH}$ = 6.9 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 164.0 (s, C═O), 150.6 (s, C═O), 134.0 (s, NCH), 112.0 (s, C), 80.5 (s, OCHN), 71.3 (s, OCH_2), 62.6 (d, $^2J_{CP}$ = 6.4 Hz, OCH_2), 62.4 (d, $^2J_{CP}$ = 6.6 Hz, OCH_2), 60.3 (s, CH_2OH), 32.5 (d, $^1J_{CP}$ = 141.5 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 3.3 Hz, CH_3), 16.3 (d, $^3J_{CP}$ = 3.3 Hz, CH_3); 12. 7 (s, CH_3); MS ESI$^+$ *m/z* = 351.2 (100%) $[M + H]^+$, *m/z* = 701.4 (86%) $[2M + H]^+$, *m/z* = 373.2 (28%) $[M + Na]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{23}N_2O_7P$: 351.1321; found 351.1317.

(±)-Diethyl 2-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonate (11c). To a two-necked round bottom flask under nitrogen containing benzyl phosphonate **10c** (0.12 g, 0.28 mmol, 1 eq.) dissolved in anhydrous CH_2Cl_2 (3 mL) at −80 °C was added dropwise boron tribromide (0.28 mL, 2.8 mmol, 10 eq.). The heterogeneous mixture was kept for 2h 30 min between −50 °C and −30 °C. Then methanol–CH_2Cl_2 (1 : 1, 6 mL) was added and the reaction mixture was allowed to return to rt. The reaction was concentrated under vacuum and purified by flash chromatography using C18 reverse

phase leading to **11c** as a viscous oil (35 mg, 37%). ^{31}P NMR ($CDCl_3$, 161.97 MHz) δ = 25.7; ^{1}H NMR ($CDCl_3$, 400.13 MHz) δ = 7.45 (d, $^3J_{HH}$ = 7.5 Hz, 1H, NCH), 6.07 (ddd, $^3J_{HH}$ = 9.6 Hz, $^3J_{PH}$ = 6.1 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, ^{3}CH), 5.99 (d, $^3J_{HH}$ = 7.5 Hz, 1H, CH), 4.21–3.96 (m, 4H, 2 OCH_2), 3.71 (ddd, $^2J_{HH}$ = −10.7 Hz, $^3J_{HH}$ = 6.5 Hz, $^3J_{HH}$ = 3.8 Hz, 1H, OCH_2), 3.65–3.56 (m, 2H, OCH_2), 3.44 (ddd, $^2J_{HH}$ = 11.3 Hz, $^3J_{HH}$ = 6.3 Hz, $^3J_{HH}$ = 3.0 Hz, 1H, OCH_2), 2.29 (ddd, $^2J_{PH}$ = −17.6 Hz, $^2J_{HH}$ = −15.2 Hz, $^3J_{HH}$ = 9.6 Hz, 1H, PCH_2), 2.18 (ddd, $^2J_{PH}$ = −18.5 Hz, $^2J_{HH}$ = −15.2 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, PCH_2), 1.27 and 1.26 (2 t, $^3J_{HH}$ = 7.1 Hz, 6H, 2 CH_3), ^{13}C NMR ($CDCl_3$, 100.61 MHz) δ = 165.9 (s, C), 156.2 (s, C═O), 139.3 (s, NCH), 96.3 (s, C), 81.4 (s, OCHN), 71.2 (s, OCH_2), 62.4 (d, $^2J_{CP}$ = 6.5 Hz, OCH_2); 60.4 (s, CH_2OH), 32.6 (d, $^1J_{CP}$ = 140.4 Hz, PCH_2), 16.4 (d, $^3J_{CP}$ = 2.3 Hz, CH_3), 16.3 (d, $^3J_{CP}$ = 2.4 Hz, CH_3); MS ESI$^+$ *m/z* = 336.0 (100%) $[M + H]^+$, *m/z* = 671.0 (12%) $[2M + H]^+$, *m/z* = 358.0 (15%) $[M + Na]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{23}N_3O_6P$: 336.1324; found 336.1317.

General procedure for the mono-deprotection of phosphonate ester – synthesis of compounds 12a–c

The appropriate diethyl phosphonate **11a–c** was dissolved in acetonitrile (1 volume, 0.07 mol L^{-1}). Then lithium bromide (2 eq.) was added and the reaction mixture was heated to 80 °C for 3.5 days. After cooling to rt, the suspension was filtered off and the solid dissolved in water and extracted by CH_2Cl_2 in order to remove apolar organic impurities. The aqueous phase was concentrated under vacuum affording the desired mono lithium salt.

(±)-Lithium ethyl 2-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonate (12a). Diethyl uracyl phosphonate **11a** (21.0 mg, 0.06 mmol, 1 eq.) was reacted according to the general procedure and afforded after concentration **12a** as a white solid 15.0 mg (76%). ^{31}P NMR (D_2O, 161.97 MHz) δ = 18.5; ^{1}H NMR (D_2O, 400.13 MHz) δ = 7.68 (d, $^3J_{HH}$ = 8.1 Hz, 1H, NCH), 5.84 (ddd, $^3J_{HH}$ = 7.1 Hz, $^3J_{PH}$ = 6.8 Hz, $^3J_{HH}$ = 5.9 Hz, 1H, OCHN), 3.85–3.74 (m, 2H, OCH_2), 3.72–3.48 (m, 4H, CH_2CH_2), 2.21 (ddd, $^2J_{PH}$ = −17.5 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 7.1 Hz, 1H, PCH_2), 2.12 (ddd, $^2J_{PH}$ = −17.4 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 5.9 Hz, 1H, PCH_2), 1.13 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 100.61 MHz) δ = 166.4 (s, C═O), 151.8 (s, CO), 141.9 (s, NCH), 102.6 (s, C), 83.0 (s, OCHN), 70.1 (s, OCH_2), 60.7 (d, $^2J_{CP}$ = 5.7 Hz, OCH_2), 60.1 (s, CH_2OH), 32.2 (d, $^1J_{CP}$ = 133.3 Hz, PCH_2), 15.9 (d, $^3J_{CP}$ = 6.4 Hz, CH_3); MS ESI$^+$ *m/z* = 315.2 (100%) $[M + Li + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + Li + H]^+$ calcd for $C_{10}H_{17}N_2O_7PLi$: 315.0933; found 315.0924.

(±)-Lithium ethyl 2-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethyl phosphonate (12b). Diethyl thymine phosphonate **11b** (25.0 mg, 0.07 mmol, 1 eq.) was reacted according to the general procedure and afforded after concentration **12b** as a white solid 20.0 mg (85%). ^{31}P NMR (D_2O, 161.97 MHz) δ = 18.7; ^{1}H NMR (D_2O, 400.13 MHz) δ = 7.48 (s, 1H, NCH), 5.80 (ddd, $^3J_{HH}$ = 6.8 Hz, $^3J_{PH}$ = 6.5 Hz, $^3J_{HH}$ = 6.2 Hz, 1H, OCHN), 3.80–3.68 (m, 2H, OCH_2), 3.66–3.42 (m, 4H, CH_2CH_2), 2.16 (ddd, $^2J_{PH}$ = −17.6 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 6.8 Hz, 1H, PCH_2), 2.21 (ddd, $^2J_{PH}$ = −17.3 Hz, $^2J_{HH}$ = −15.3 Hz, $^3J_{HH}$ = 6.2 Hz, 1H, PCH_2), 1.79 (s, 3H, CH_3), 1.07 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 100.61 MHz) δ = 166.6 (s, C═O), 151.9 (s, C═O), 137.4 (s, NCH), 111.7 (s, C), 82.5 (s, OCHN), 69.7 (s, OCH_2), 60.6 (d, $^2J_{CP}$ = 5.7 Hz, OCH_2), 60.0 (s, CH_2OH), 32.1 (d, $^1J_{CP}$ = 133.4 Hz, PCH_2), 15.8 (d, $^3J_{CP}$ = 6.4 Hz, CH_3), 11.5 (s, 3H, CH_3); MS ESI$^+$ *m/z* = 323.2 (100%) $[M + H]^+$, 329.2 (22%) $[M + Li + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + Li + H]^+$ calcd for $C_{11}H_{19}N_2O_7PLi$: 329.1090; found 329.1088.

(±)-Lithium ethyl 2-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonate (12c). Diethyl cytosine phosphonate **11c** (25.0 mg, 0.07 mmol, 1 eq.) was reacted according to the general procedure and afforded after concentration **12c** as a white solid 20.0 mg (85%, purity 80%). ^{31}P NMR (D_2O, 161.97 MHz) δ = 19.0; ^{1}H NMR (D_2O, 400.13 MHz) δ = 7.64 (d, $^3J_{HH}$ = 7.4 Hz, 1H, NCH), 6.01 (d, $^3J_{HH}$ = 7.4 Hz, 1H, CH), 5.87 (m, 1H, OCHN), 3.84–3.65 (m, 2H, OCH_2), 3.60–3.44 (m, 4H, CH_2CH_2), 2.23–2.09 (m, 2H, PCH_2), 1.79 (s, 3H, CH_3), 1.12 (t, $^3J_{HH}$ = 7.1 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 100.61 MHz) δ = 165.9 (s, C═O), 157.6 (s, C–N), 141.6 (s, NCH), 96.6 (s, C), 83.4 (s, OCHN), 69.9 (s, OCH_2), 60.7 (d, $^2J_{CP}$ = 5.7 Hz, OCH_2), 60.0 (s, CH_2OH), 32.4 (d, $^1J_{CP}$ = 132.6 Hz, PCH_2), 15.6 (d, $^3J_{CP}$ = 6.4 Hz, CH_3).

General procedure for double deprotection of phosphonate ester – synthesis of compounds 5a–c

The appropriate diethyl phosphonate **11a–c** was dissolved in CH_2Cl_2 (1 volume, 0.07 mol L^{-1}). Then TMS-Br (10 eq.) was added and the reaction mixture was stirred 16 h at rt. The reaction mixture was concentrated under vacuum and methanol was added. The reaction mixture was stirred for 30 min, then concentrated under vacuum and purified by chromatography on reverse phase.

(±)-2-(2,4-Dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonic acid triethylammonium salt (5a). Diethyl uracil phosphonate **11a** (162.0 mg, 0.48 mmol, 1 eq.) was reacted according to the general procedure. **5a** is obtained as the triethylammonium salt by trituration in a mixture of triethylamine–methanol and concentration under vacuum as a colorless oil (60.0 mg, 44%). ^{31}P NMR (D_2O, 161.97 MHz) δ = 20.5; ^{1}H NMR (D_2O, 400.13 MHz) δ = 7.73 (d, $^3J_{HH}$ = 8.1 Hz, 1H, NCH), 5.92 (ddd, $^3J_{HH}$ = 8.1 Hz, $^3J_{PH}$ = 5.9 Hz, $^3J_{HH}$ = 4.9 Hz, 1H, OCHN), 5.88 (d, $^3J_{HH}$ = 8.1 Hz, 1H, CH), 3.80–3.48 (m, 4H, CH_2CH_2), 3.16 (q, $^3J_{HH}$ = 7.3 Hz, 4H, $CH_{2(NEt3)}$), 2.22 (ddd, $^2J_{PH}$ = −17.1 Hz, $^2J_{HH}$ = −15.1 Hz, $^3J_{HH}$ = 8.1 Hz, 1H, PCH_2), 2.06 (ddd, $^2J_{PH}$ = −17.6 Hz, $^2J_{HH}$ = −15.1 Hz, $^3J_{HH}$ = 4.9 Hz, 1H, PCH_2), 1.24 (t, $^3J_{HH}$ = 7.3 Hz, 6H, $CH_{3(NEt3)}$); ^{13}C NMR (D_2O, 100.61 MHz) δ = 168.8 (s, C═O), 154.3 (s, C═O), 144.3 (s, NCH), 105.0 (s, CH), 85.8 (s, OCHN), 72.6 (s, OCH_2), 62.5 (s, CH_2OH), 49.1 (s, $CH_{2(NEt3)}$), 36.5 (d, $^1J_{CP}$ = 130.3 Hz, PCH_2), 10.7 (s, $CH_{3(NEt3)}$); MS ESI$^+$ *m/z* = 280.9 (100%) $[M + H]^+$, 560.8 (5%) $[2M + H]^+$, 382.0 (3%) $[M + NEt_3 + H]^+$; HRMS-ESI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_8H_{14}N_2O_7P$: 281.0539; found 281.0547.

(±)-2-(5-Methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)ethylphosphonic acid (5b). Diethyl thymine

phosphonate **11b** (187.0 mg, 0.53 mmol, 1 eq.) was reacted according to the general procedure. **5b** was isolated as a white solid (116 mg, 74%). ^{31}P NMR (D_2O, 161.97 MHz) δ = 20.9; ^{1}H NMR (D_2O, 250.13 MHz) δ = 7.35 (s, 1H, NCH), 5.75 (ddd, $^{3}J_{HH}$ = 7.1 Hz, $^{3}J_{PH}$ = 6.5 Hz, $^{3}J_{HH}$ = 6.0 Hz, 1H, OCHN), 3.65–3.24 (m, 4H, CH_2CH_2), 2.26 (ddd, $^{2}J_{PH}$ = −18.7 Hz, $^{2}J_{HH}$ = −15.7 Hz, $^{3}J_{HH}$ = 7.1 Hz, 1H, PCH_2), 2.13 (ddd, $^{2}J_{PH}$ = −18.1 Hz, $^{2}J_{HH}$ = −15.7 Hz, $^{3}J_{HH}$ = 6.0 Hz, 1H, PCH_2), 1.67 (d, $^{4}J_{HH}$ = 2.2 Hz, 3H, CH_3); ^{13}C NMR (D_2O, 62.90 MHz) δ = 166.3 (s, C═O), 151.7 (s, C═O), 136.7 (s, NCH), 111.9 (s, C), 81.6 (s, OCHN), 69.9 (s, OCH_2), 60.0 (s, CH_2OH), 30.8 (d, $^{1}J_{CP}$ = 133.8 Hz, PCH_2), 11.4 (s, CH_3); MS ESI$^+$ *m*/*z* = 295.0 (100%) $[M + H]^+$, 589.1 (28%) $[2M + H]^+$; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_9H_{16}N_2O_7P$: 295.0695; found 295.0685.

(±)-2-(4-Amino-2-oxo-2*H*-pyrimidin-1-yl)-2-(2-hydroxyethoxy)-ethylphosphonic acid (5c). Diethyl cytosine phosphonate **11c** (100.0 mg, 0.53 mmol, 1 eq.) was reacted according to the general procedure. **5c** was isolated as a colorless oil (81 mg, 97%). The product was also isolated as the triethylamine salt. ^{31}P NMR (D_2O, 161.97 MHz) δ = 20.3 (acidic form) or 19.1 (mono triethylammonium salt); ^{1}H NMR (D_2O, 400.13 MHz, triethylammonium salt) δ = 7.81 (d, $^{3}J_{HH}$ = 7.6 Hz, 1H, NCH), 6.14 (d, $^{3}J_{HH}$ = 7.6 Hz, 1H, CHOH), 5.98 (ddd, $^{3}J_{HH}$ = 8.2 Hz, $^{3}J_{PH}$ = 7.0 Hz, $^{3}J_{HH}$ = 4.6 Hz, 1H, OCHN), 3.84–3.51 (m, 4H, CH_2CH_2), 3.20 (q, $^{3}J_{HH}$ = 7.3 Hz, 2H, $CH_2(NEt_3)$), 2.56 (ddd, $^{2}J_{PH}$ = −17.1 Hz, $^{2}J_{HH}$ = −15.1 Hz, $^{3}J_{HH}$ = 8.2 Hz, 1H, PCH_2), 2.14 (ddd, $^{2}J_{PH}$ = −17.8 Hz, $^{2}J_{HH}$ = −15.1 Hz, $^{3}J_{HH}$ = 4.6 Hz, 1H, PCH_2), 1.27 (t, $^{3}J_{HH}$ = 7.3 Hz, 3H, $CH_3(NEt_3)$); ^{13}C NMR (D_2O, 100.61 MHz, acidic form) δ = 159.0 (s, C═O), 148.3 (s, C), 144.0 (s, NCH), 95.7 (s, ^{7}C), 83.5 (s, OCHN), 70.8 (s, OCH_2), 60.0 (s, CH_2OH), 32.6 (d, $^{1}J_{CP}$ = 135.4 Hz, PCH_2); MS ESI$^+$ *m*/*z* = 280.1 (70%) $[M + H]^+$, 281.1 (100%) $[M + D]^+$; HRMS-ESI$^+$ (*m*/*z*): $[M + H]^+$ calcd for $C_8H_{15}N_3O_6P$: 280.0698; found 280.0699.

Acknowledgements

The first author (B. Dayde) is particularly grateful to Idenix Pharmaceuticals and to the French National Association for Technical Research (Association Nationale de la Recherche Technique, ANRT) for a doctoral fellowship.

Notes and references

1 E. De Clercq, *Future Virol.*, 2008, **3**, 393.
2 (*a*) P. Doláková, M. Dracínský, J. Fanfrlík and A. Holý, *Eur. J. Org. Chem.*, 2009, 1082; (*b*) E. De Clercq, *Biochem. Pharmacol.*, 2007, **73**, 911; (*c*) A. Holý, *Curr. Pharm. Des.*, 2003, **9**, 2567; (*d*) E. De Clercq and A. Holý, *Nat. Rev. Drug Discovery*, 2005, **4**, 928; (*e*) A. Khandazhinskaya, M. Yasko and E. Shirokova, *Curr. Med. Chem.*, 2006, **13**, 2953.
3 E. De Clercq, *Med. Res. Rev.*, 2009, **29**, 571.
4 A. Fernández-Botello, A. Holý, V. Moreno and H. Sigel, *J. Inorg. Biochem.*, 2004, **98**, 2114.
5 A. Esteban-Gamboa, J. Balzarini and R. Esnouf, *J. Med. Chem.*, 2000, **43**, 971.
6 J. M. Varlet, G. Fabre, F. Sauveur, N. Collignon and P. Savignac, *Tetrahedron*, 1981, **37**, 1377.
7 (*a*) R. Kouno, T. Okauchi, M. Nakamura, J. Ichikawa and T. J. Minami, *J. Org. Chem.*, 1998, **63**, 6239; (*b*) B. Iorga, F. Eymery, V. Mouriès and P. Savignac, *Tetrahedron*, 1998, **54**, 14637.
8 (*a*) H. B. Lazrek, A. Rochdi and H. Khaider, *Tetrahedron*, 1998, **54**, 3807; (*b*) B. Shadid, H. C. van der Plas, W. H. J. Boesten and J. Kamphuis, *Tetrahedron*, 1990, **46**, 913.
9 (*a*) Z. Song, A. DeMarco and M. Zhao, *J. Org. Chem.*, 1999, **64**, 1859; (*b*) P. Y. F. Deghati, A. Borghini and A. M. C. H. van den Nieuwendijk, *Bioorg. Med. Chem.*, 2003, **11**, 899.
10 (*a*) D. S. Stoianova and P. R. Hanson, *Org. Lett.*, 2001, **3**, 3285; (*b*) M. Schulz, R. Kluge, S. Liebsch, J. Lessig, M. Halik and F. Gadissa, *Tetrahedron*, 1996, **52**, 13151; (*c*) C. Yuan, J. Li and W. J. Zhang, *J. Fluorine Chem.*, 2006, **127**, 44.
11 (*a*) G. Bellucci, C. Chiappe and F. D'Andrea, *Tetrahedron: Asymmetry*, 1995, **6**, 221; (*b*) P. Cheshev, A. Marra and A. Dondoni, *Carbohydr. Res.*, 2006, **341**, 2714; (*c*) M. Yamashita, V. Krishna Reddy, L. N. Rao, B. Haritha, M. Maeda, K. Suzuki, H. Totsuka, M. Takahashi and T. Oshikawa, *Tetrahedron Lett.*, 2003, **44**, 2339.
12 F. E. McDonald and M. M. Gleason, *J. Am. Chem. Soc.*, 1996, **118**, 6648.
13 S. Dong and L. A. Paquette, *J. Org. Chem.*, 2006, **71**, 1647.
14 K. A. Watanabe, K. Harada and J. Zeidler, *J. Med. Chem.*, 1990, **33**, 2145.
15 J. Wolf, J.-M. Jarrige, J.-C. Florent, D. S. Grierson and C. Monneret, *Synthesis*, 1992, 773.
16 H. Krawczyk, *Synth. Commun.*, 1997, **27**, 3151.
17 P. Shahgaldian, A. W. Coleman and V. I. Kalchenko, *Tetrahedron Lett.*, 2001, **42**, 577.

