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Synthesis of Analogues of Acyclic Nucleoside Diphosphates Containing a (Phosphonomethyl)phosphanyl Moiety and Studies of Their Phosphorylation

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Acyclic nucleoside diphosphonate derivatives of purines and pyrimidines were prepared by Mitsunobu reaction of suitably protected heterocyclic bases with alcohols containing the (phosphonomethyl)phosphanyl moiety. Furthermore, nonhydrolyzable acyclic analogues of dUDP were prepared as potential inhibitors of dUTPase. Their phosphorylation to analogues of dUTP, however, gave mixtures of linear and branched phosphates. The courses of the phosphorylation reactions were followed by ^{31}P NMR spectroscopy, and we discovered that both phosphonate and phosphinate moieties react with 1,1′-carbonyldiimidazole and tri-*n*-butylammonium phosphate. The pK_a values of the (phosphonomethyl)phosphanyl system were also determined by ^{31}P NMR spectroscopy

Introduction

Acyclic nucleoside phosphonates[1] (ANPs) represent a key class of nucleotide analogues with a broad spectrum of antiviral and cytostatic activity. Among ANPs, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir, Figure 1) in particular is active against DNA and retroviruses,[2] and its prodrug, adefovir dipivoxil (Hepsera),[3] has been approved for hepatitis B therapy.[4] 9-[2-(Phosphonomethoxy)-ethyl]-2,6-diaminopurine (PMEDAP) is active against DNA viruses and retroviruses[5] and also exhibits selective antitumor properties,[6] and the guanine derivative bearing the phosphonomethoxyethyl (PME) side chain possesses powerful antitumor activity.[7]

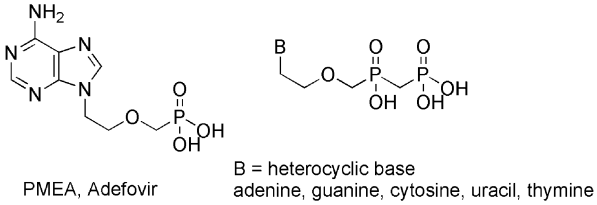

Figure 1. Acyclic analogues of nucleotides.

A second class of antiviral compounds is represented by 9-(*R*)-[2-(phosphonomethoxy)propyl]adenine (PMPA, tenofovir), a promising anti-HIV compound; its prodrug Viread has been approved for treatment of AIDS and chronic hepatitis B.[8] Substitution at the 2-position in the 2-(phosphonomethoxy)ethyl side chain by a hydroxymethyl group leads to a third structural type of antiviral compounds: 9-(*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine (HPMPC, cidofovir, Vistide) possesses general anti-DNA-viral activity.[9] Cidofovir has been approved for treatment of cytomegalovirus retinitis in AIDS patients, but was also successfully used in the treatment of various DNA-virus infections, in particular those caused by pox viruses.[10]

These nucleotide analogues each contain a stable isopolar phosphonomethyl ether moiety instead of the nucleotide phosphate ester group, which blocks their enzymatic degradation and/or eliminates problems with intracellular phosphorylation necessary for nucleoside activation. ANPs are phosphorylated in the cell to di- and triphosphates that inhibit viral DNA polymerase and/or reverse transcriptase.[11]

The (phosphonomethyl)phosphanyl unit (P–C–P–C–), in which the bridging oxygen atoms of the diphosphate moiety are replaced with methylene groups, can be regarded as a nonhydrolyzable mimic of diphosphate. Acyclic diphosphonate derivatives of adenine and guanine containing methoxyalkoxy side chains were previously reported to possess moderate antiviral activity against HSV-1, VZV, and visna virus.[12] Inhibitory activities of modified triphosphate analogues of carbocyclic nucleosides containing diphosphonate moieties against HIV reverse transcriptase and DNA polymerase have also been described.[13] Farnesyl diphosphate analogues in which the diphosphate unit is replaced by a (phosphonomethyl)phosphanyl moiety showed inhibitory activity against squalene synthetase[14] and farnesyl diphosphate synthetase.[15] Glutamine synthetase inhibitors[16] and transcarbamoylase inhibitors[17] containing diphosphonate moieties have also been described.

Here we describe syntheses of 2-[(hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl purines (A, G) and

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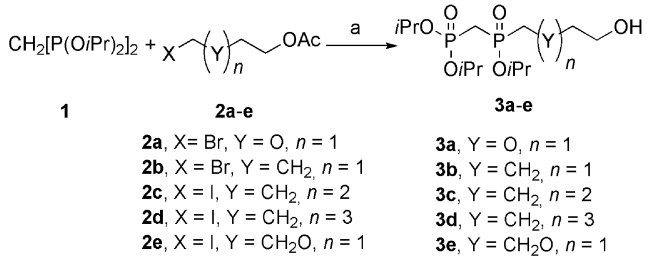
pyrimidines (C, U, T) (Figure 1) – chemically and enzymatically stable analogues of acyclic nucleoside diphosphates containing the PME side chain.

Furthermore, acyclic analogues of dUDP and dUTP containing the (phosphonomethyl)phosphanyl moiety were prepared as potential inhibitors of *Mycobacterium tuberculosis* dUTPase (deoxyuridine 5′-triphosphate nucleotidohydrolase).[18] dUTPase is essential in both eukaryotes and prokaryotes;[19] the enzyme catalyzes hydrolysis of dUTP into dUMP and diphosphate using Mg^{2+} as a cofactor.[20] The enzyme supplies the dUMP substrate for dTTP synthesis and, by maintaining low dUTP/dTTP ratios in the cell, minimizes uracil misincorporation into DNA.[21] dUTPase has been recognized as a valid target for drug design. Although some selective inhibitors of parasitic dUTPase have been described, the high degree of sequence and structural similarity between parasitic and human dUTPases may make development of drugs based on inhibition of dUTPase difficult. Previously described inhibitors of dUTPase include nonhydrolyzable analogues of nucleoside triphosphates in which the α,β bridging oxygen atom is replaced by a methylene[22] or an imido[23] group. 2′-Deoxyuridine derivatives containing either a triphenylmethyl or a triphenylsilyl substituent at their 5′-positions[24] and their acyclic analogues[25] selectively inhibit *Plasmodium falciparum* dUTPase.

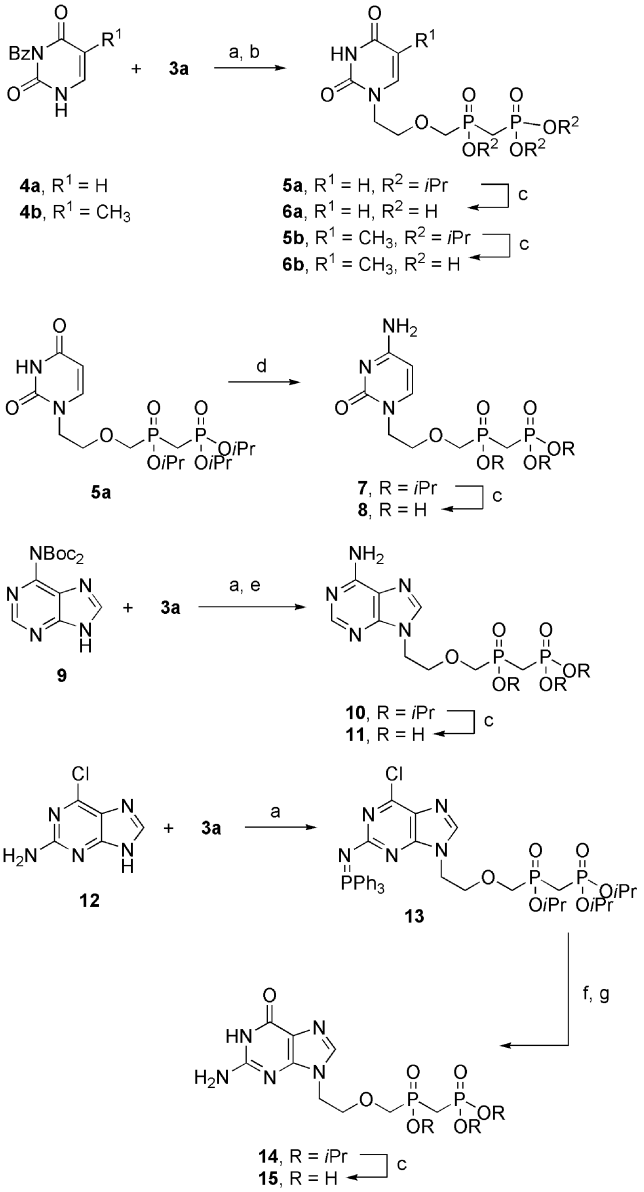
Results and Discussion

The diphosphate analogues were prepared by Mitsunobu reaction of suitably protected heterocyclic bases with the functionalized alcohols **3a**–**e**, each bearing the (phosphonomethyl)phosphanyl unit. Alcohols **3a**–**e** were prepared by Arbuzov reaction of the alkyl bromides **2a**[26] and **2b** or iodides **2c**–**e** with the air- and moisture-sensitive phosphonite **1** (Scheme 1).[27,16] The corresponding alkyl chlorides were unreactive at 120 °C, whereas heating to higher temperature led to the decomposition of the starting materials. Our attempts to perform the reaction under microwave heating conditions were unsuccessful. The terminal phosphonite moieties were oxidized to phosphonates with DMSO, and the acetyl protecting groups were removed by hydrolysis with hydrochloric acid. Alcohols **3a**–**e** were prepared in overall yields of 30–40%. This method proved in our hands to be better than the previously described Arbuzov reaction between (phosphonomethyl)phosphonite and alkyl halide,[12] due to the poor yields of the synthesis of the starting phosphonomethyl-phosphonite,[27,15] which was accompanied by formation of side products and complicated separation. Isopropyl esters were used instead of ethyl esters to suppress side Arbuzov reactions with ethyl bromide.



Scheme 1. Arbuzov reaction of **1** with alkyl halides **2a**–**e**. Reagents and conditions: a) 1. 120 °C, 2. DMSO, 3. HCl, EtOH.

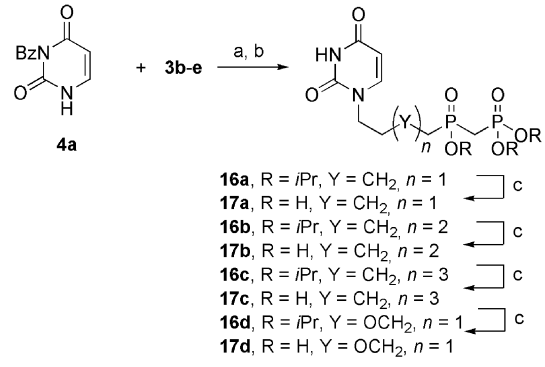
Uracil and thymine derivatives **6a** and **6b** were prepared by alkylation of N^3-benzoylated uracil **4a** and thymine **4b**[28] with alcohol **3a** under Mitsunobu conditions[29] in 79% and 52% yields, respectively (Scheme 2). Isopropyl esters were deprotected with trimethylsilyl bromide in acetonitrile by standard procedures. The uracil derivative **5a** was converted into the cytosine analogue **7** by treatment with 2,4,6-triiso-



Scheme 2. Mitsunobu reaction of heterocyclic bases with alcohol **3a**. Reagents and conditions: a) Ph_3P, DIAD, THF, b) MeONa, MeOH, c) $BrSiMe_3$, CH_3CN, d) 1. TPSCl, CH_3CN, 2. NH_4OH, e) HCl, CH_2Cl_2, f) H_2O, THF, g) TFAA, H_2O.

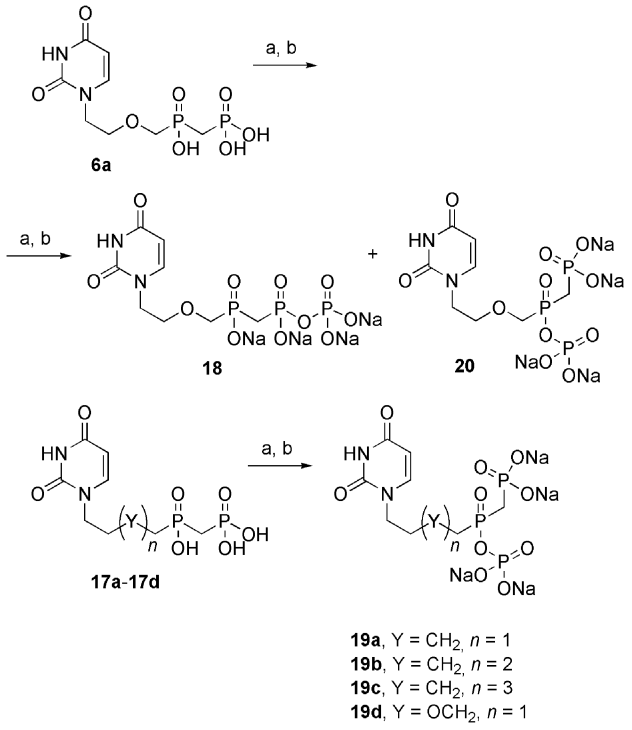
propylbenzenesulfonyl chloride (TPSCl) followed by amination by ammonium hydroxide;[30] treatment with trimethylsilyl bromide afforded **8** in 46% yield. Adenine derivative **10** was prepared by Mitsunobu reaction between **3a** and the bis-Boc-protected N^6-amino adenine **9**,[31] followed by hydrolysis of the bis-Boc protecting group with hydrochloric acid in dichloromethane.[32] The guanine counterpart **14** was prepared analogously by starting from 2-amino-6-chloropurine (**12**) by alkylation with **3a** to give predominantly the N^2-triphenylphosphoranylidene derivative **13**. Compound **13** was easily hydrolyzed by heating at reflux in a THF/H_2O mixture[33] to afford the 2-amino derivative, which was subsequently converted into guanine **14** by treatment with trifluoroacetic acid. Deprotection of **10** and **14** with trimethylsilyl bromide gave free acids **11** and **15**.

In addition to the 1-{2-[(hydroxy)(phosphonomethyl)-phosphorylmethoxy]ethyl}uracil (**6a**) with the methoxyethyl side chain, the series of uracil derivatives bearing carbon side chains of four, five, and six carbon atoms, as well as the ethoxyethyl side chain (Scheme 3), was prepared in order to study the potential inhibitory activities of these compounds towards dUTPase. Compounds **16** and **17** were prepared by the same procedure as described for compounds **5** and **6**: N^3-benzoylated uracil **4a** was alkylated with alcohols **3b–e** under Mitsunobu conditions, the benzoyl group was removed by treatment with sodium methoxide, and subsequent treatment with trimethylsilyl bromide gave diphosphonates **17a–d**.



Scheme 3. Synthesis of analogues of dUDP. Reagents and conditions: a) Ph_3P, DIAD, THF, b) MeONa, MeOH, c) $BrSiMe_3$, CH_3CN.

As the natural substrate of dUTPase is 2′-deoxyuridine triphosphate, we tried to convert the diphosphonates **6a** and **17a–d** into the corresponding triphosphate analogues (Scheme 4). Our attempts to prepare triphosphate mimics by the morpholidate method,[34] widely used for conversion of ANPs into phosphates, were unsuccessful, with the morpholidate of **6a** being formed only in low yield. Synthesis of triphosphate analogues by methods employing diphenyl chlorophosphate[35] or benzyl hydrogen phosphoramidate[36] failed as well. Phosphates were finally prepared by activation of phosphonate with 1,1′-carbonyldiimidazole (CDI), followed by addition of the tri-*n*-butylammonium phosphate in DMF.[37] The imidazolidate of **6a** was also prepared by treatment with imidazole in the presence of 2,2′-dithiodipyridine and triphenylphosphane,[38] but yields obtained by this method were lower than those of the reaction with CDI.



Scheme 4. Phosphorylation of compounds **6a** and **17a–d**. Reagents and conditions: a) 1. CDI, DMF, 2. $(Bu_3NH)H_2PO_4$, DMF, b) Dowex 50×8 (Na^+ form).

Treatment of **6a** with CDI followed by tri-*n*-butylammonium phosphate in DMF gave expected triphosphate analogue **18** in 5% yield (Scheme 4). Compound **18** was purified by anion-exchange chromatography (elution with TEAB), and the triethylammonium salt was finally converted into the corresponding sodium salt on DOWEX 50×8 (Na^+ form). Its identity was determined by ^1H and ^{31}P NMR, and its purity (determined by HPLC) exceeded 98%.

Compounds **17a–d** were similarly converted into their phosphate counterparts by the method described above and isolated as their sodium salts; however, only branched phosphates **19a–d** were isolated, as major products, in approximately 15–30% yields. The branched structures of compounds **19a–d** were confirmed by their ^{31}P NMR spectra. In the proton-decoupled spectra, P-β and P-γ appeared as doublets with coupling constants to P-α of 2 Hz and 28 Hz, respectively (see Table 1, Figure 2). The α position of P-α was confirmed by its chemical shift having the highest value (close to 50 ppm) and by the ^{31}P NMR spectrum without proton decoupling, in which P-α exhibited splitting due to coupling with two neighboring methylene groups.

Compound **18** is relatively stable: in neutral aqueous solution it decomposes into the starting diphosphonate **6a** and phosphate, but only 5% of diphosphonate **6a** had appeared in neutral aqueous solution over a 24 h period at room temperature. In contrast, very low stabilities of com-

Table 1. ^{31}P NMR chemical shifts (ppm) and interaction constants (Hz) of compounds **6a** and **17**–**20**.

	P-α	P-β	P-γ
6a	36.32 d, *J*(P-α,P-β) = 9.0	18.18 d, *J*(P-β,P-α) = 9.0	–
18	30.98 d, *J*(P-α,P-β) = 6.2	6.92 dd, *J*(P-β,P-α) = 6.2, *J*(P-β,P-γ) = 25.2	–5.51 d, *J*(P-γ,P-β) = 25.2
20	34.5 dd, *J*(P-α,P-γ) = 28.6, *J*(P-α,P-β) = 7.2	11.4 d, *J*(P-β,P-α) = 7.2	–5.4 d, *J*(P-γ,P-α) = 28.7
17a	49.0 d, *J*(P-α,P-β) = 7.0	15.6 d, *J*(P-β,P-α) = 7.0	–
19a	46.2 dd, *J*(P-α,P-γ) = 28.3, *J*(P-α,P-β) = 6.0	10.9 d, *J*(P-β,P-α) = 6.0	–5.1 d, *J*(P-γ,P-α) = 28.3
17b	49.8 d, *J*(P-α,P-β) = 6.7	14.56 d, *J*(P-β,P-α) = 6.7	–
19b	50.1 dd, *J*(P-α,P-γ) = 28.7, *J*(P-α,P-β) = 2.1	9.61 d, *J*(P-β,P-α) = 2.1	–4.4 d, *J*(P-γ,P-α) = 28.7
17c	50.53 d, *J*(P-α,P-β) = 7.8	14.80 d, *J*(P-β,P-α) = 7.8	–
19c	50.4 dd, *J*(P-α,P-γ) = 28.4, *J*(P-α,P-β) = 2.0	9.7 d, *J*(P-β,P-α) = 2.0	–4.3 d, *J*(P-γ,P-α) = 28.4
17d	44.3 d, *J*(P-α,P-β) = 9.2	15.8 d, *J*(P-β,P-α) = 9.2	–
19d	49.3 dd, *J*(P-α,P-γ) = 27.3, *J*(P-α,P-β) = 1.8	9.5 d, *J*(P-β,P-α) = 1.8	–4.4 d, *J*(P-γ,P-α) = 27.3

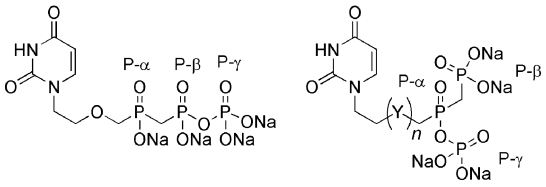


Figure 2. Labeling of phosphorus atoms for ^{31}P NMR spectroscopy.

pounds **19a**–**d** were observed; we managed to prepare them in 90–98% purities (the compounds tend to decompose into the starting diphosphonate and phosphate during purification and concentration).

To investigate whether the branched phosphates are formed immediately during the reaction or are formed by intramolecular migration of the terminal phosphate in its linear counterpart, we followed the course of the reaction of **6a** with CDI and tri-*n*-butylammonium phosphate by ^{31}P NMR spectroscopy. The signals of the starting material disappeared immediately after addition of CDI, but an unidentifiable mixture of several products was observed. After 2 h the spectrum was no longer changing, and tri-*n*-butylammonium phosphate was added to the reaction mixture (the imidazolide of **6a** was not isolated because of its instability). It was found that after 6 h both phosphates (branched and linear) had been formed in approximately 2:1 ratio. We used ^{31}P–^{31}P-COSY to identify the signals of linear and branched triphosphate analogues. Finally, the reaction mixture was separated, and the linear phosphate **18** was isolated in 7% yield together with the branched phosphate **20** in 13% yield (Scheme 4).

Identical experiments with compounds **19a** and **19d** showed the same results, with both branched and linear phosphates being formed. The ratio of branched and linear phosphate was from 2:1 to 10:1.

From NMR titration studies of **6a**, pK_a values for the (phosphonomethyl)phosphanyl residue were determined (Figures 3 and 4). The pK_a value of the phosphinate (P-α, Figure 3) and the pK_{a1} value of the phosphonate (P-β, Figure 4) are around 1.8 and 2.7, respectively. These pK_a values indicate that both groups are acidic enough to undergo reaction with CDI and phosphate and explain the formation of mixtures of branched and linear phosphates. This explanation is also supported by our observation of the formation of a mixture of products in the reaction of **6a** with CDI. No intramolecular migration of the phosphate group was observed.

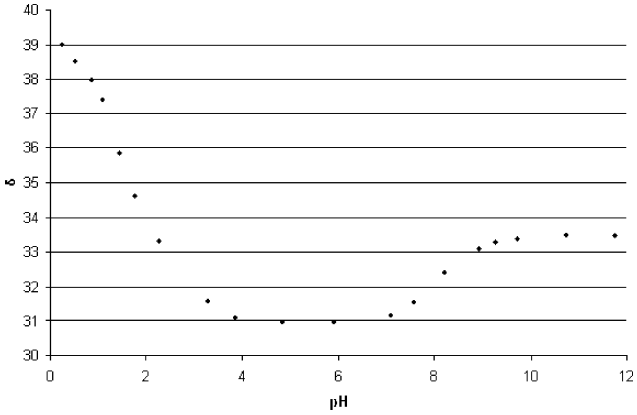


Figure 3. pH dependence of the chemical shifts δ (ppm) of P-α of compound **6a**.

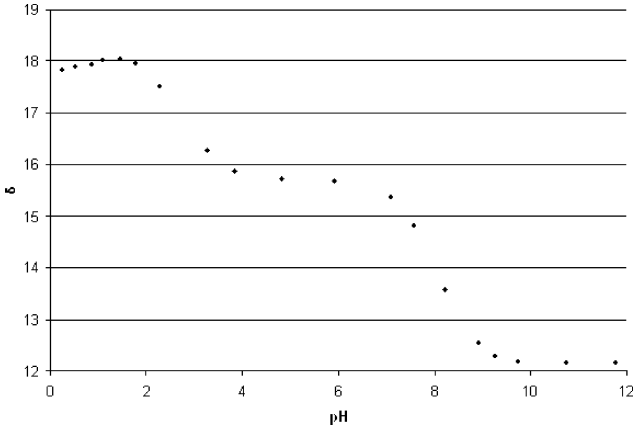


Figure 4. pH dependence of the chemical shifts δ (ppm) of P-β of compound **6a**.

The stabilities of linear and branched triphosphate analogues were also studied with ab initio quantum chemical calculations. We compared the thermodynamic stabilities of linear and branched phosphates (Figure 5) with carbon side chains (**21a** and **21b**) and methoxyethyl side chains (**22a** and **22b**). The triphosphate analogues were taken to be protonated for calculations (the molecule **21b** corresponds to com-

pound **19a**, compound **22a** corresponds to **18**, and **22b** corresponds to compound **20**). In the both cases the branched phosphates were more stable (see Table 2).

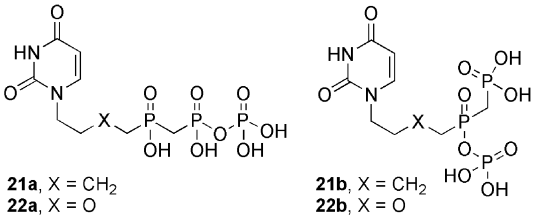


Figure 5. Molecules studied by ab initio quantum chemical calculations.

Table 2. Relative gas-phase energies (ΔE) and relative hydration free energies ($\Delta\Delta G_{HYD}$) in kcal mol^{-1}.

Molecule	ΔE	$\Delta\Delta G_{HYD}$	$\Delta E + \Delta\Delta G_{HYD}$
21a	0.0	0.0	0.0
21b	–9.1	4.9	–4.2
22a	0.0	0.0	0.0
22b	–10.5	4.5	–6.0

Compounds **6a**, **6b**, **8**, **11**, **15**, and **17a**–**d** were screened for cytostatic and antiviral activities: none of the tested compounds exhibited any significant biological activity or cytotoxicity. The dUDP and dUTP analogues **6a**, **17a**–**d**, **18**, **19a**–**d**, and **20** were tested for their potency to inhibit *Mycobacterium tuberculosis* dUTPase, but none of the analogues inhibited the enzyme.

Conclusions

In conclusion, a series of acyclic nucleoside diphosphate analogues of purine and pyrimidine nucleotides containing a stable (phosphonomethyl)phosphanyl unit were prepared by improved versions of previously described methods. Alcohols **3a**–**e** were prepared by Arbuzov reaction of diphosphonite **1** with acetyl alkyl bromides and alkyl iodides in overall 30–40% yields. Suitably protected heterocyclic bases were coupled with functionalized alcohols **3a**–**e** by Mitsunobu reaction and finally deprotected by standard procedures.

(Phosphonomethyl)phosphinates **6a** and **17a**–**d** were successfully transformed into their phosphate counterparts by conversion of (phosphonomethyl)phosphinates into the corresponding imidazolides by treatment with CDI and subsequently with tri-*n*-butylammonium phosphate. Interestingly, the branched phosphates **19a**–**d** and **20** were isolated as major products rather than the expected linear phosphates, which were present in lower yields. Detailed ^{31}P NMR studies of the courses of the reactions showed that both branched and linear phosphates are formed immediately during the reactions. No intramolecular phosphate migration was observed. The pK_a values of the phosphonate and the phosphinate moieties in compound **6a** were determined by ^{31}P NMR titration studies and are around 2.7 and 1.8, respectively; this explains the reactivities of both the phosphonate and the phosphinate residue with CDI. In addition, thermodynamic stability calculations showed the branched phosphates to be more stable.

All the prepared compounds were tested for antiviral and cytostatic activity and dUTPase inhibitory activity, but none exhibited any appreciable biological activity. These data indicate that the (phosphonomethyl)phosphanyl system is not an optimal analogue of the natural diphosphate in nucleotides. This effect may be due to the differences between the pK_a values of the (phosphonomethyl)phosphanyl analogues and the normal diphosphates, small geometric differences between C–P and O–P bonds, and differences in metal ion binding properties.[39,22]

Experimental Section

General: Solvents were dried by standard procedures. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone under argon. NMR spectra were recorded with Bruker Avance 500 (500 MHz for ^{1}H and 125.8 MHz for ^{13}C) and Bruker Avance 400 (^{1}H at 400, ^{13}C at 100.6 MHz) spectrometers in $CDCl_3$, [D_6]-DMSO, or D_2O. Chemical shifts (in ppm, δ scale) were referenced to TMS (for ^{1}H NMR spectra in $CDCl_3$) and/or to the solvent signal ($CDCl_3$ δ = 7.26 ppm for ^{1}H NMR and δ = 77.0 ppm for ^{13}C NMR; [D_6]DMSO δ = 2.5 ppm for ^{1}H NMR and δ = 39.7 ppm for ^{13}C). Chemical shifts in D_2O were referenced to 1,4-dioxane: δ = 3.75 ppm for ^{1}H NMR and δ = 67.19 ppm for ^{13}C NMR. Chemical shifts for ^{31}P NMR spectra were referenced to H_3PO_4 (δ = 0 ppm). For ^{31}P NMR spectroscopic data see Table 1. Melting points were determined on a Büchi Melting Point B-545 apparatus and are uncorrected. TLC was performed on plates of Kieselgel 60 F254 (Merck). Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer by FAB (ionization with Xe, accelerating voltage 8 kV, glycerol matrix) or on a LCQ classic spectrometer by electrospray ionization (ESI). Preparative HPLC purification was performed on a column packed with 10 μm C18 reversed phase (Luna), 250 × 21 mm; in ca 300 mg portions of mixtures with a linear gradient of triethylammonium hydrogen carbonate (TEAB, 0.1 M) in water and in 50% MeOH (linear gradient of TEAB in 50% MeOH, 0–100%). Preparative HPLC purification of triphosphate analogues was performed on a column packed with POROS® HQ 50 μm (50 mL) with use of a gradient of TEAB in water (0–0.4 M).

Tetraisopropyl methylenediphosphonite (**1**) was prepared by the previously described procedure[16] or purchased from Sigma–Aldrich. 5-Chloropentyl acetate, 4-bromobutyl acetate, and 6-chlorohexan-1-ol were purchased from Aldrich. 2-(2-Chloroethoxy)-ethanol was purchased from Janssen Chimica.

Method of Calculation: Gas-phase geometries and energies of the studied molecules were obtained with RI-MP2/cc-pVDZ.[40] These calculations were performed with Turbomole5.8.[41] Hydration free energies were calculated with the C-PCM implicit solvent model[42] implemented in the Gaussian03 code.[43] The recommended HF/6–31G* level combined with the united atom radii (UAHF) model was used.

General Procedure 1 (GP1). Arbuzov Reaction: An alkyl halide (**2a**–**e**, 1 mmol) was added dropwise at room temp. to tetraisopropyl methylenediphosphonite (**1**, 1 mmol), and the resulting mixture was heated at 120 °C under argon for 6 h. The reaction mixture was allowed to cool, DMSO (0.15 mL) was added, and the mixture was

heated at 60 °C for 2 h. The mixture was partitioned between water and $CHCl_3$. The organic fraction was washed with three portions of water and taken down in vacuo. The residue in EtOH (3 mL) was treated with HCl (2 M, 1.2 mL) and heated under reflux for 2 h. The mixture was cooled to room temp. and neutralized with aqueous ammonia (25%), and the solvents were evaporated. The residue in $CHCl_3$ was washed with water, dried with $MgSO_4$ and purified by chromatography on silica gel ($CHCl_3$/MeOH 0–5%).

General Procedure 2 (GP2). Mitsunobu Reaction: Diisopropyl azodicarboxylate (DIAD, 273 μL, 1.4 mmol) was added to a suspension of triphenylphosphane (394 mg, 1.6 mmol) in dry THF (6 mL), and the solution was stirred at 0 °C for 0.5 h. The prepared complex was added dropwise at –40 °C under argon to a suspension of the purine or pyrimidine base (**4a**, **4b**, **9**, or **12**, 1.1 mmol) and the appropriate alcohol (**3a**–**e**, 0.5 mmol) in dry THF (3 mL). The reaction mixture was allowed to warm to room temp. and stirred overnight, and the solvent was removed.

General Procedure 3 (GP3). Deprotection of Triisopropyl Esters: Me_3SiBr (3 mL) was added to a solution of the diphosphonate triisopropyl ester (1 mmol) in dry CH_3CN (30 mL) and the mixture was stirred at room temp. overnight. The solvent was removed in vacuo, and the residue was codistilled with water. The crude product was purified by preparative HPLC (linear gradient from H_2O to 50% CH_3OH in H_2O).

5-Iodopentyl Acetate (2c): 5-Chloropentyl acetate (10 g, 60 mmol) in acetone (500 mL) was treated with NaI (45 g, 0.3 mol) and heated under reflux for 32 h.[44] The mixture was evaporated to half of its volume and partitioned between H_2O and $CHCl_3$, the organic fraction was washed with H_2O, saturated $Na_2S_2O_3$, and H_2O and dried with $MgSO_4$, and the solvents were evaporated. Pale yellow oil, yield 11.4 g (73%). ^{1}H NMR ([D$_6$]DMSO): δ = 3.99 [t, J(1,2) = 6.57 Hz, 2 H, 1-H], 3.28 [t, J(5,4) = 6.88 Hz, 2 H, 5-H], 1.99 (s, 3 H, CH_3), 1.77 (m, 2 H, CH_2), 1.58 (m, 2 H, CH_2), 1.38 (m, 2 H, CH_2) ppm. MS (ESI): m/z (%) = 279.0 (26) [M + Na]$^+$. HRMS (ESI) calcd. for $C_7H_{13}INaO_2$ [M + Na]$^+$ 278.9858; found 278.9852.

6-Iodohexyl Acetate (2d): Acetic anhydride (10 mL) was added dropwise at 0 °C to 6-chlorohexan-1-ol (10 mL, 71.5 mmol) in pyridine (30 mL), and the mixture was allowed to warm slowly to room temp. and stirred for 4 h. EtOH (20 mL) was added, the mixture was concentrated, and the residue was codistilled with EtOH, diluted with $CHCl_3$, and washed with HCl (1 M), saturated $NaHCO_3$, and H_2O. The organic fraction was dried with $MgSO_4$ and concentrated to give 6-chlorohexyl acetate, yield 12.8 g (99%). ^{1}H NMR ($CDCl_3$): δ = 4.06 [t, J(1,2) = 6.67 Hz, 2 H, 1-H], 3.53 [t, J(6,5) = 6.67 Hz, 2 H, 6-H], 2.04 (s, 3 H, CH_3), 1.78 (m, 2 H, CH_2), 1.64 (m, 2 H, CH_2), 1.47 (m, 2 H, CH_2), 1.38 (m, 2 H, CH_2) ppm. MS (ESI): m/z (%) = 179 (15) [M + H]$^+$.

6-Chlorohexyl acetate was converted into its 6-iodo congener **2d** by the method described for **2c**, pale yellow oil, yield 90%. ^{1}H NMR ($CDCl_3$): δ = 4.05 [t, J(1,2) = 6.67 Hz, 2 H, 1-H], 3.18 [t, J(6,5) = 6.97 Hz, 2 H, 6-H], 2.04 (s, 3 H, CH_3), 1.84 (m, 2 H, CH_2), 1.63 (m, 2 H, CH_2), 1.46–1.32 (m, 4 H, 2 × CH_2) ppm. MS (ESI): m/z (%) = 293.1 (63) [M + Na]$^+$. HRMS (ESI) calcd. for $C_8H_{15}INaO_2$ [M + Na]$^+$ 293.0977; found 293.0976.

2-(2-Iodoethoxy)ethyl Acetate (2e): This compound was prepared from 2-(chloroethoxy)ethanol by the procedure described for **2d**.

2-(2-Chloroethoxy)ethyl Acetate: Colorless oil, yield 88%. ^{1}H NMR ($CDCl_3$): δ = 4.36 (m, 2 H, 1-H), 3.88 [t, J(3,4) = 5.76 Hz, 2 H, 3-H], 3.85 (m, 2 H, 2-H), 3.76 [t, J(4,3) = 5.76 Hz, 2 H, 4-H], 2.08 (s, 3 H, CH_3) ppm. MS (ESI): m/z (%) = 189.0 (94) [M + Na$^+$].

2-(2-Iodoethoxy)ethyl Acetate: Pale yellow oil, yield 65%. ^{1}H NMR ($CDCl_3$): δ = 4.22 (m, 2 H, 1-H), 3.74 (m, 2 H, 3-H), 3.69 (m, 2 H, 2-H), 3.25 (m, 2 H, 4-H), 2.08 (s, 3 H, CH_3) ppm. MS (ESI): m/z (%) = 280.9 (65) [M + Na]$^+$. HRMS (ESI) calcd. for $C_6H_{11}INaO_3$ [M + Na]$^+$ 280.9645; found 280.9644.

Diisopropyl [2-Hydroxyethoxymethyl(isopropoxy)phosphoryl]methyl-phosphonate (3a): This compound was prepared by GP1, colorless oil, yield 41%. ^{1}H NMR ($CDCl_3$): δ = 4.63–4.77 (m, 3 H, CHiPr), 3.97 [dd, J_{gem} = 12.9, J(H,P) = 7.6 Hz, 1 H] and 3.84 [dd, J_{gem} = 12.9, J(H,P) = 8.7 Hz, 1 H, OCH_2P], 3.57–3.72 (m, 4 H, 3-H, 4-H), 2.30–2.51 (m, 2 H, 1-H), 1.25–1.32 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 74.83 [d, J(C,P) = 6.5 Hz] and 71.24 [d, J(C,P) = 6.6 Hz] and 70.56 [d, J(C,P) = 5.6 Hz, CHiPr], 66.42 [d, J(C,P) = 119.2 Hz, C-2], 60.39 (C-4), 27.11 [dd, J(C,P) = 137.0, 83.3 Hz, PCH_2P], 23.9 (m, CH_3iPr) ppm. MS (ESI): m/z (%) = 359 (100) [M – H]$^-$. HRMS (ESI) calcd. for $C_{13}H_{30}NaO_7P_2$ [M + Na]$^+$ 383.1359; found 383.1359.

Diisopropyl [4-Hydroxybutyl(isopropoxy)phosphoryl]methylphosphonate (3b): This compound was prepared by GP1, colorless oil, yield 28%. ^{1}H NMR ($CDCl_3$): δ = 4.62–4.74 (m, 3 H, CHiPr), 3.61 [t, J(4,3) = 5.9 Hz, 2 H, 4-H], 2.22–2.35 (m, 2 H, PCH_2P), 1.85–1.98 (m, 2 H, 1-H), 1.74 (br. s, 1 H, OH), 1.66–1.73 (m, 2 H, 3-H), 1.53–1.64 (m, 2 H, 2-H), 1.26–1.29 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.73 [d, J(C,P) = 6.5 Hz, CHiPr], 71.27 [d, J(C,P) = 6.7 Hz, CHiPr], 69.70 [d, J(C,P) = 6.8 Hz, CHiPr], 60.30 (C-4), 32.58 [d, J(C,P) = 16.3 Hz, C-2], 28.74 [dd, J(C,P$_1$) = 135.7, J(C,P$_2$) = 77.6 Hz, PCH_2P], 28.42 [d, J(C,P) = 98.2 Hz, C-1], 24.44 [d, J(C,P) = 3.6 Hz, CH_3iPr], 24.25 [d, J(C,P) = 4.2 Hz, CH_3iPr], 24.05 [d, J(C,P) = 3.6 Hz, CH_3iPr], 23.98 [d, J(C,P) = 4.6 Hz, 2 × CH_3iPr], 23.84 [d, J(C,P) = 5.1 Hz, CH_3iPr], 17.64 [d, J(C,P) = 3.6 Hz, C-3] ppm. MS (ESI): m/z (%) = 381 (100) [M + Na]$^+$, 358.9 (52) [M + H]$^+$. HRMS (ESI) calcd. for $C_{14}H_{32}NaO_6P_2$ [M + Na]$^+$ 381.1566; found 381.1567.

Diisopropyl [5-Hydroxypentyl(isopropoxy)phosphoryl]methyl-phosphonate (3c): This compound was prepared by GP1, colorless oil, yield 30%. ^{1}H NMR ($CDCl_3$): δ = 4.68 (m, 3 H, CHiPr), 3.58 [t, J(1,2) = 6.4 Hz, 2 H, 1-H], 2.27 (m, 2 H, PCH_2P), 1.87 (m, 2 H, 5-H), 1.60 (m, 2 H, 4-H), 1.53 (m, 2 H, 2-H), 1.44 (m, 2 H, 3-H), 1.25–1.30 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.41 [d, J(C,P) = 6.7 Hz], 71.15 [d, J(C,P) = 6.5 Hz] and 69.66 [d, J(C,P) = 6.9 Hz, CHiPr], 62.32 (C-1), 31.94 (C-2), 29.82 [d, J(C,P) = 98.5 Hz, C-5], 29.32 [dd, J(C,P) = 7.69, 135.8 Hz, PCH_2P], 26.80 [d, J(C,P) = 16.3 Hz, C-3], 23.22–24.45 (m, CH_3iPr), 21.28 [d, J(C,P) = 6.6 Hz, C-4] ppm. MS (ESI): m/z (%) = 395.1 (82) [M + Na]$^+$. HRMS (ESI) calcd. for $C_{15}H_{34}NaO_6P_2$ [M + Na]$^+$ 395.1723; found 395.1721.

Diisopropyl [6-Hydroxyhexyl(isopropoxy)phosphoryl]methyl-phosphonate (3d): This compound was prepared by GP1, colorless oil, yield 40%. ^{1}H NMR ($CDCl_3$): δ = 4.70–4.80 (m, 3 H, CHiPr), 3.62 [t, J(1,2) = 6.5 Hz, 2 H, 1-H], 2.33 (m, 2 H, PCH_2P), 1.86–2.0 (m, 2 H, 6-H), 1.65 (m, 2 H, 5-H), 1.57 (m, 2 H, 2-H), 1.37–1.48 (m, 4 H, 3-H, 4-H), 1.32–1.36 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 71.32 [d, J(C,P) = 6.6 Hz], 71.09 [d, J(C,P) = 6.5 Hz] and 69.60 [d, J(C,P) = 6.8 Hz, CHiPr], 62.44 (C-1), 32.29 (C-2), 30.19 [d, J(4,P) = 16.4 Hz, C-4], 29.68 [d, J(6,P) = 98.5 Hz, C-6], 29.30 [dd, J(C,P) = 77.0, 135.9 Hz, PCH_2P], 25.01 (C-3), 23.82–24.40 (m, CH_3iPr), 21.42 [d, J(5,P) = 4.6 Hz, C-5] ppm. MS (ESI): m/z (%) = 387 (6) [M + H]$^+$, 409.1 (55) [M + H]$^+$. HRMS (ESI) calcd. for $C_{16}H_{36}NaO_6P_2$ [M + Na]$^+$ 409.1879; found 409.1880.

Diisopropyl [2-Hydroxyethoxyethyl(isopropoxy)phosphoryl]methylphosphonate (3e): This compound was prepared by GP1, colorless oil, yield 37%. ^{1}H NMR ($CDCl_3$): δ = 4.76 (m, 3 H, CHiPr), 3.85

(m, 2 H, 3-H), 3.71 (m, 2 H, 1-H), 3.59 (m, 2 H, 2-H), 2.51 (m, 2 H, PCH_2P), 2.44 and 2.18 (m, 2 H, 4-H), 1.33–1.37 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 72.41 (C-2), 71.45 [d, J(C,P) = 6.6 Hz], 71.18 [d, J(C,P) = 6.6 Hz], 70.15 [d, J(C,P) = 7.0 Hz, CH*i*Pr], 64.19 [d, J(C,P) = 5.9 Hz, C-3], 61.12 (C-1), 30.85 [dd, J(C,P) = 80.8, 135.9 Hz, PCH_2P], 29.36 [d, J(C,P) = 98.2 Hz, C-4], 23.82–24.34 (m, CH_3iPr) ppm. MS (ESI): *m*/*z* (%) = 375 (48) [M + H]$^+$, 397.1 (100) [M + Na]$^+$. HRMS (ESI) calcd. for $C_{14}H_{32}NaO_7P_2$ [M + Na]$^+$ 397.1515; found 397.1514.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}uracil (5a): This compound was prepared by GP2, the crude product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%) gave a colorless oil (180 mg, 79%). ^1H NMR ($CDCl_3$): δ = 9.29 (br. s, 1 H, NH), 7.42 [d, J(6,5) = 7.9 Hz, 1 H, 6-H], 5.65 [dd, J(5,6) = 7.9, J(5,NH) = 1.9 Hz, 1 H, 5-H], 4.79 [octet, J(CH,CH_3) = J(CH,P) = 6.3 Hz, 1 H, CH*i*Pr], 4.78 [dh, J(CH,CH_3) = 6.2, J(CH,P) = 7.0 Hz, 1 H, CH*i*Pr], 4.74 [dh, J(CH,CH_3) = 6.2, J(CH,P) = 8.0 Hz, 1 H, CH*i*Pr], 4.01 [ddd, J_{gem} = 14.5, J(1′a,2′a) = 5.8, J(1′a,2′b) = 3.2 Hz, 1 H, 1′a-H], 4.00 [dd, J_{gem} = 13.3, J(H,P) = 6.9 Hz, 1 H, OCH_2Pa], 3.92 (m, 2 H, 1′b, OCH_2Pb), 3.84 (m, 2 H, -H$^{2'}$), 2.40 (m, 2 H, PCH_2P), 1.36 [d, J(CH_3,CH) = 6.3 Hz, 3 H, CH_3iPr], 1.35 [d, J(CH_3,CH) = 6.3 Hz, 3 H, CH_3iPr], 1.35 [d, J(CH_3,CH) = 6.3 Hz, 3 H, CH_3iPr], 1.34 [d, J(CH_3,CH) = 6.3 Hz, 3 H, CH_3iPr], 1.31 [d, J(CH_3,CH) = 6.3 Hz, 3 H, CH_3iPr] ppm. ^{13}C NMR ($CDCl_3$): δ = 163.68 (C-4), 150.79 (C-2), 145.80 (C-6), 101.50 (C-5), 71.83 [d, J(C,P) = 6.4 Hz, CH*i*Pr], 71.36 [d, J(C,P) = 6.5 Hz, CH*i*Pr], 71.16 [d, J(2′,P) = 12.3 Hz, C-2′], 70.92 [d, J(C,P) = 6.7 Hz, CH*i*Pr], 67.63 [d, J(C,P) = 117.3 Hz, OCH_2P], 48.11 (C-1′), 27.33 [dd, J(C,P) = 136.6, 82.9 Hz, PCH_2P], 24.29 [d, J(C,P) = 3.8 Hz, CH_3iPr], 24.19 [d, J(C,P) = 3.5 Hz, CH_3iPr], 24.07 [d, J(C,P) = 3.1 Hz, CH_3iPr], 23.97 [d, J(C,P) = 4.1 Hz, 2 C, CH_3iPr], 23.83 [d, J(C,P) = 5.0 Hz, CH_3iPr] ppm. MS (ESI): *m*/*z* (%) = 477.0 (100) [M + Na]$^+$, 454.9 (19) [M + H]$^+$. $C_{17}H_{32}N_2O_8P_2$ (454.39): calcd. C 44.94, H 7.10, N 6.17, O 28.17, P 13.63; found C 45.19, H 7.38, N 5.81, P 13.58.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}thymine (5b): This compound was prepared by GP2, the crude product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%) to give a colorless oil (122 mg, 52%). ^1H NMR ($CDCl_3$): δ = 8.16 (br. s, 1 H, NH), 7.18 [d, J(6,CH_3) = 1.20 Hz, 1 H, 6-H], 4.77 (m, 3 H, CH*i*Pr), 3.95 (m, 4 H, 1′-H, 2′-H), 3.80 (m, 2 H, 3′-H), 2.37 (m, 2 H, PCH_2P), 1.36 [d, J(CH_3,CH) = 6.2 Hz, 3 H, CH_3iPr], 1.34 [d, J(CH_3,CH) = 6.2 Hz, 3 H, CH_3iPr], 1.33 [d, J(CH_3,CH) = 6.2 Hz, 3 H, CH_3iPr], 1.32 [d, J(CH_3,CH) = 6.2 Hz, 3 H, CH_3iPr], 1.31 [d, J(CH_3,CH) = 6.2 Hz, 3 H, CH_3iPr] ppm. MS (ESI): *m*/*z* (%) = 491.0 (100) [M + Na]$^+$, 469.0 (35) [M + H]$^+$. $C_{18}H_{34}N_2O_8P_2$ (454.39): calcd. C 46.15, H 7.32, N 5.98, O 27.32, P 13.22; found C 45.98, H 7.41, N 5.86, P 13.41.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}uracil (6a): This compound was prepared by GP3, white solid, yield 83%, m.p. 195 °C. ^1H NMR (D_2O): δ = 7.68 [d, J(6,5) = 7.9 Hz, 1 H, H-6], 5.82 [d, J(5,6) = 7.8 Hz, 1 H, 5-H], 4.01 (m, 2 H, H-1′), 3.81–3.84 (m, 4 H, H-2′, OCH_2P), 2.40 [dd, J(H,P_1) = 20.3, J(H,P_2) = 17.0 Hz, 2 H, PCH_2P] ppm. ^{13}C NMR (D_2O): δ = 167.54 (C-4), 152.87 (C-2), 148.64 (C-6), 101.77 (C-5), 71.07 [d, J(C,P) = 12.5 Hz, C-2′], 68.40 [d, J(C,P) = 117.6 Hz, OCH_2P], 48.93 (C-1′), 27.33 [dd, J(C,P_1) = 128.8, J(C,P_2) = 80.5 Hz, PCH_2P] ppm. MS (ESI): *m*/*z* (%) = 351.1 (66) [M + Na]$^+$. $C_8H_{14}N_2O_8P_2$ (328.15): calcd. C 29.28, H 4.30, N 8.54, O 39.00, P 18.88; found C 29.62, H 4.44, N 8.23, P 18.73.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}thymine (6b): This compound was prepared by GP3 and freeze dried, white solid, yield 82%, m.p. 238 °C. ^1H NMR (D_2O): δ = 7.52 [q, J(6,CH_3) = 1.2 Hz, 1 H, 6-H], 3.97 (m, 2 H, 1′-H), 3.81–3.83 (m, 4 H, 2′-H, OCH_2P), 2.38 [dd, J(H,P) = 16.9, J(H,P) = 20.5 Hz, 2 H, PCH_2P], 1.88 [d, J(CH_3,6) = 1.2 Hz, 3 H, 5-CH_3] ppm. ^{13}C NMR (D_2O): δ = 167.68 (C-4), 152.90 (C-2), 144.49 (C-6), 110.91 (C-5), 71.20 [d, J(C,P) = 12.6 Hz, C-2′], 68.38 [d, J(C,P) = 118.2 Hz, OCH_2P], 48.68 (C-1′), 27.20 [dd, J(C,P) = 81.2, J(C,P) = 129.2 Hz, PCH_2P], 11.89 (5-CH_3) ppm. MS (ESI): *m*/*z* (%) = 341 (100) [M – H]$^-$. $C_{10}H_{18}N_2O_7P_2$ (342.18): calcd. C 31.59, H 4.71, N 8.19, O 37.41, P 18.10; found C 31.39, H 4.72, N 7.96, P 18.41.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl-methoxy]ethyl}cytosine (7): Compound **5a** (310 mg, 0.68 mmol), Et_3N (0.3 mL), and TPSCl (0.63 g, 2.04 mmol) were stirred in CH_3CN (10 mL) at room temp. for 48 h. NH_4OH (25%, 5 mL) was added, the mixture was stirred for 24 h, and the solvents were evaporated. The residue in ethyl acetate was washed with brine, the aqueous fraction was than washed with five portions of $CHCl_3$, and the organic fractions were dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography to give a pale yellow foam (240 mg, 78%). ^1H NMR ([D_6]DMSO): δ = 7.60 [d, J(6,5) = 7.25 Hz, 1 H, 6-H], 7.49 (br. s, 1 H, NH-a), 7.23 (br. s, 1 H, NH-b), 5.67 [d, J(5,6) = 7.25 Hz, 1 H, 5-H], 4.60 (m, 6 H, CH*i*Pr), 3.83 (m, 4 H, 1′-H, 3′-H), 3.69 (m, 2 H, 2′-H), 2.40 (m, 2 H, PCH_2P), 1.24 (m, 9 H, CH_3iPr), 1.18 [d, J(CH_3,CH) = 6.14 Hz, 3 H, CH_3iPr], 1.16 [d, J(CH_3,CH) = 6.19 Hz, 3 H, CH_3iPr], 1.10 [d, J(CH_3,CH) = 6.19 Hz, 3 H, CH_3iPr] ppm. MS (ESI): *m*/*z* (%) = 454.0 (70) [M + H]$^+$, 496.0 (84) [M + Na]$^+$. $C_{17}H_{33}N_3O_7P_2$ (453.41): calcd. C 45.03, H 7.34, N 9.27, O 24.70, P 13.66; found C 45.12, H 7.31, N 9.12, P 13.52.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}cytosine (8): This compound was prepared by GP3 and freeze dried, white foam, yield 69%. ^1H NMR (D_2O): δ = 7.90 [d, J(6,5) = 7.6 Hz, 1 H, 6-H], 6.17 [d, J(5,6) = 7.7 Hz, 1 H, 5-H], 4.08 [t, J(1′,2′) = 4.7 Hz, 2 H, 1′-H], 3.83 [t, J(2′,1′) = 4.7 Hz, 2 H, 2′-H], 3.78 [d, J(H,P) = 7.6 Hz, 2 H, OCH_2P], 2.31 [dd, J(H,P) = 6.8, 10.3 Hz, 2 H, PCH_2P] ppm. ^{13}C NMR (D_2O): δ = 160.16 (C-4), 151.04 (C-6), 149.69 (C-2), 94.67 (C-5), 70.49 [d, J(2′,P) = 13.1 Hz, C-2′], 68.80 [d, J(C,P) = 119.9 Hz, OCH_2P], 49.87 (C-1′), 27.79 [dd, J(C,P) = 79.1, 125.9 Hz, PCH_2P] ppm. MS (ESI): *m*/*z* (%) = 326 (100) [M – H]$^-$. $C_8H_{15}N_3O_7P_2$ (327.17): calcd. C 29.37, H 4.62, N 12.84, O 34.23, P 18.93; found C 29.31, H 4.82, N 12.83, P 18.78.

9-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl-methoxy]ethyl}adenine (10): This compound was prepared by GP2, and the residue in dichloromethane (40 mL) was treated with HCl (6 M, 40 mL) and heated under reflux for 6 h. The pH of the aqueous phase was adjusted to 8 with a saturated aqueous solution of $NaHCO_3$. The mixture was then extracted with five portions of dichloromethane, the combined organic fractions were dried with $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/MeOH). Crystallization from ethyl acetate/light petroleum gave a white, crystalline product (28%), m.p. 82 °C. ^1H NMR ($CDCl_3$): δ = 8.35 (s, 1 H, 2-H), 7.97 (s, 1 H, 8-H), 5.84 (br. s, 2 H, NH_2), 4.68–4.80 (m, 3 H, CH*i*Pr), 4.42 (m, 2 H, 1′-H), 4.01 [dd, J_{gem} = 13.3, J(H,P) = 6.4 Hz, 1 H, OCH_2Pa], 3.98 (m, 2 H, 2′-H), 3.90

[dd, J_{gem} = 13.3, J(H,P) = 7.7 Hz, 1 H, OCH_2Pb], 2.31–2.42 (m, 2 H, PCH_2P), 1.31–1.35 (m, 15 H, CH_3iPr) and 1.24 [d, $J(CH_3,CH)$ = 6.2 Hz, 3 H, CH_3iPr] ppm. ^{13}C NMR ($CDCl_3$): δ = 155.39 (C-6), 152.89 (C-2), 149.90 (C-4), 141.37 (C-8), 119.39 (C-5), 71.71 [d, J(C,P) = 6.5 Hz, CH*i*Pr], 71.31 [d, $J(2',P)$ = 12.1 Hz, C-2′], 71.28 [d, J(C,P) = 7.0 Hz, CH*i*Pr], 70.81 [d, J(C,P) = 6.8 Hz, CH*i*Pr], 67.55 [d, J(C,P) = 116.8 Hz, OCH_2P], 43.31 (C-1′), 27.22 [dd, J(C,P) = 136.4, J(C,P) = 83.1 Hz, PCH_2P], 23.99 (m, CH_3iPr) ppm. MS (ESI): m/z (%) = 500.1 (100) $[M + Na]^+$. $C_{18}H_{33}N_5O_6P_2$ (477.43): calcd. C 45.28, H 6.97, N 14.67, O 20.11, P 12.98; found C 45.36, H 6.92, N 14.30, P 12.89.

9-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}adenine (11): This compound was prepared by GP3 and crystallized from water, white crystals, yield 78%, m.p. 196 °C. ^1H NMR (D_2O + NaOD): δ = 8.22 (s, 1 H, 8-H), 8.14 (s, 1 H, 2-H), 4.41 [t, $J(1',2')$ = 5.1 Hz, 2 H, 1′-H], 3.95 [t, $J(2',1')$ = 5.1 Hz, 2 H, 2′-H], 3.71 [d, J(H,P) = 6.5 Hz, 2 H, PCH_2O], 1.96 [t, J(H,P) = 18.0 Hz, 2 H, PCH_2P] ppm. ^{13}C NMR (D_2O + NaOD): δ = 155.91 (C-6), 152.75 (C-2), 149.27 (C-4), 143.63 (C-8), 118.76 (C-5), 71.15 [d, $J(2',P)$ = 10.4 Hz, C-2′], 69.99 [d, J(C,P) = 111.8 Hz, PCH_2O], 44.06 (C-1′), 30.98 [dd, J(C,P) = 80.9, J(C,P) = 118.4 Hz, PCH_2P] ppm. MS (ESI): m/z (%) = 350.1 (100) $[M - H]^-$. $C_9H_{15}N_5O_6P_2{\cdot}H_2O$ (369.21): calcd. C 29.28, H 6.64, N 18.97, O 30.33, P 16.78; found C 29.60, H 6.65, N 18.98, P 17.06.

***N*²-Triphenylphosphoranylidene 2-Amino-6-chloro-9-{2-[(diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylmethoxy]ethyl}purine (13):** This compound was prepared by GP2, and the residue was separated by flash chromatography ($CHCl_3$/MeOH), white foam, yield 24%. ^1H NMR ($CDCl_3$): δ = 7.87 (m, 5 H, arom.), 7.77 (s, 1 H, 8-H), 7.54 (m, 3 H, arom.), 7.44 (m, 7 H, arom.), 4.71 (m, 3 H, CH*i*Pr), 4.20 (m, 2 H, 2′-H), 3.94 [dd, J_{gem} = 13.3, J(H,P) = 6.2 Hz, 1 H, PCH_2a], 3.79 [dd, J_{gem} = 13.2, J(H,P) = 7.7 Hz, 1 H, PCH_2b], 3.72 (m, 2 H, 1′-H), 2.35 (m, 2 H, PCH_2P), 1.31 (m, 15 H, CH_3iPr), 1.23 [d, $J(CH_3,CH)$ = 6.2 Hz, 3 H, CH_3iPr] ppm. MS (ESI): m/z (%) = 772.2 (100) $[M + H]^+$. MS (ESI): m/z (%) = 770.2 (100) $[M - H]^-$.

9-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl-methoxy]ethyl}guanine (14): Compound **13** (350 mg, 0.45 mmol) in THF (10 mL) was treated with water (3 mL) and heated under reflux for 2 d. The mixture was evaporated and the crude product was treated with TFA in water (75%, 10 mL) at room temp. overnight. The solvent was evaporated and the residue was codistilled with water. The product was purified by flash chromatography (CH_3Cl/MeOH) and crystallized from EtOH/Et_2O mixture to afford white crystals, yield (two steps) 167 mg (74%), m.p. 108 °C. ^1H NMR ([D_6]-DMSO): δ = 10.56 (br. s, 1 H, NH), 7.65 (s, 1 H, 8-H), 6.44 (br. s, 2 H, NH_2), 4.53–4.63 (m, 3 H, CH*i*Pr), 4.12 (m, 2 H, 1′-H), 3.85 [d, J(H,P) = 6.7 Hz, 2 H, OCH_2P], 3.81 (m, 2 H, 2′-H), 2.38–2.51 (m, 2 H, PCH_2P), 1.23 (d, J = 6.2 Hz, 6 H), 1.22 (d, J = 6.2 Hz, 6 H), 1.20 (d, J = 6.2 Hz, 3 H) and 1.12 (d, J = 6.2 Hz, 3 H, CH_3iPr) ppm. ^{13}C NMR ([D_6]DMSO): δ = 157.05 (C-6), 153.74 (C-2), 151.38 (C-4), 137.93 (C-8), 116.61 (C-5), 70.88 [d, $J(2',P)$ = 11.3 Hz, C-2′], 70.61 [d, J(C,P) = 6.2 Hz], 70.44 [d, J(C,P) = 6.2 Hz] and 69.80 [d, J(C,P) = 6.5 Hz, CH*i*Pr], 66.85 [d, J(C,P) = 115.7 Hz, OCH_2P], 42.44 (C-1′), 25.99 [dd, J(C,P) = 81.7, 133.9 Hz], 23.78–24.24 (m, CH_3iPr) ppm. MS (ESI): m/z (%) = 494.1 (34) $[M + H]^+$, 516.1 (100) $[M + Na]^+$. $C_{18}H_{33}N_5O_7P_2$ (493.43): calcd. C 43.81, H 6.74, N 14.19, O 22.70, P 12.55; found C 43.87, H 6.77, N 13.76, P 12.59.

9-{2-[(Hydroxy)(phosphonomethyl)phosphorylmethoxy]ethyl}-guanine (15): This compound was prepared by GP3; DMF (1 mL) was added to the reaction mixture. White solid, yield 52%, m.p. 190 °C with dec. ^1H NMR (D_2O): δ = 7.88 (s, 1 H, 8-H), 4.25 [t, $J(1',2')$ = 5.1 Hz, 2 H, 1′-H], 3.92 [t, $J(2',1')$ = 5.1 Hz, 2 H, 2′-H], 3.71 [d, J(H,P) = 6.9 Hz, 2 H, OCH_2P], 2.02 [dd, J(H,P) = 17.0, 19.1 Hz, 2 H, PCH_2P] ppm. ^{13}C NMR (D_2O): δ = 159.54 (C-6), 154.28 (C-2), 152.03 (C-4), 141.12 (C-8), 116.30 (C-5), 71.31 [d, $J(2',P)$ = 10.9 Hz, C-2′], 69.82 [d, J(C,P) = 112.9 Hz, OCH_2P], 43.70 (C-1′), 30.33 [dd, J(C,P) = 80.1, 119.6 Hz, PCH_2P] ppm. MS (ESI): m/z (%) = 366.0 (100) $[M - H]^-$. $C_9H_{15}N_5O_7P_2{\cdot}H_2O$ (385.20): calcd. C 28.06, H 4.45, N 18.18, O 33.23, P 16.08; found C 28.11, H 4.39, N 17.77, P 15.77.

1-{4-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]-butyl}uracil (16a): This compound was prepared by GP2, the crude product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. Purification by flash chromatography ($CHCl_3$/MeOH 0–10%) afforded a colorless oil (225 mg, 96%). ^1H NMR ($CDCl_3$): δ = 9.16 [d, J(NH,5) = 6.5 Hz, 1 H, NH], 7.18 [d, J(6,5) = 7.9 Hz, 1 H, 6-H], 5.61 [dd, J(5,6) = 7.9, J(5,NH) = 2.0 Hz, 1 H, 5-H], 4.64–4.73 (m, 3 H, CH*i*Pr), 3.64–3.73 (m, 2 H, 1′a,1′b-H), 2.22–2.36 (m, 2 H, PCH_2P), 1.86–2.02 (m, 2 H, 4′-H), 1.74–1.79 (m, 2 H, 2′-H), 1.60–1.66 (m, 2 H, 3′-H), 1.25–1.29 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.68 (C-4), 150.71 (C-2), 144.57 (C-6), 102.06 (C-5), 71.57 [d, J(C,P) = 6.6 Hz, CH*i*Pr], 71.19 [d, J(C,P) = 6.7 Hz, CH*i*Pr], 69.93 [d, J(C,P) = 6.8 Hz, CH*i*Pr], 48.41 (C-1′), 29.63 [d, J(C,P) = 15.4 Hz, C-2′], 29.43 [dd, J(C,P) = 135.3, J(C,P) = 177.5 Hz, PCH_2P], 29.07 [d, J(C,P) = 99.2 Hz, C-4′], 24.42 [d, J(C,P) = 3.7 Hz, CH_3iPr], 24.16 [d, J(C,P) = 4.3 Hz, CH_3iPr], 24.05 [d, J(C,P) = 3.7 Hz, CH_3iPr], 23.97 [d, J(C,P) = 4.5 Hz, 2 × CH_3iPr], 23.86 [d, J(C,P) = 5.2 Hz, CH_3iPr], 18.58 [d, J(C,P) = 4.4 Hz, C-3′] ppm. MS (ESI): m/z (%) = 452.9 (100) $[M + H]^+$, 475.1 (67) $[M + Na]^+$. $C_{18}H_{34}N_2O_7P_2$ (452.42): calcd. C 47.79, H 7.57, N 6.19, O 24.75, P 13.69; found C 47.71, H 7.53, N 5.92, P 13.60.

1-{5-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]-pentyl}uracil (16b): This compound was prepared by GP2, the crude product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. The product was isolated by flash chromatography ($CHCl_3$/MeOH 0–10%) as a colorless oil (186 mg, 79%). ^1H NMR ($CDCl_3$): δ = 9.15 (br. s, 1 H, NH), 7.21 [d, J(6,5) = 7.9 Hz, 1 H, 6-H], 5.68 [dd, J(5,6) = 7.9, J(5,NH) = 1.5 Hz, 1 H, 5-H], 4.70–4.80 (m, 3 H, CH*i*Pr), 3.73 (m, 2 H, 1′-H), 2.35 (m, 2 H, PCH_2P), 1.95 (m, 2 H, 5′-H), 1.65–1.76 (m, 4 H, 2′-H, 4′-H), 1.46 (m, 2 H, 3′-H), 1.32–1.36 (m, 18 H, CH_3iPr) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.65 (C-4), 150.71 (C-2), 144.49 (C-6), 102.04 (C-5), 71.46 [d, J(C,P) = 6.7 Hz], 71.13 [d, J(C,P) = 6.4 Hz] and 69.76 [d, J(C,P) = 7.0 Hz, CH*i*Pr], 48.47 (C-1′), 29.37 [dd, J(C,P) = 77.7, 135.2 Hz, PCH_2P], 29.59 [d, J(C,P) = 99.0 Hz, C-5′], 28.37 (C-2′), 27.26 [d, $J(3',P)$ = 16.5 Hz, C-3′], 23.84–24.44 (m, CH_3iPr), 21.10 [d, J(C,P) = 4.5 Hz, C-4′] ppm. MS (ESI): m/z = 489.1 $[M + Na]^+$. HRMS (ESI) calcd. for $C_{19}H_{36}N_2NaO_7P_2$ $[M + Na]^+$ 489.1895; found 489.1891. $C_{19}H_{36}N_2O_7P_2$ (466.44): calcd. C 48.92, H 7.78, N 6.01, O 24.01, P 13.28; found C 48.81, H 7.72, N 5.83, P 13.11.

1-{6-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphoryl]-hexyl}uracil (16c): This compound was prepared by GP2, the product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. Flash chromatography ($CHCl_3$/MeOH 0–10%) gave **16c** as a colorless oil (187 mg, 78%). ^1H NMR ($CDCl_3$): δ = 9.45 (br. s, 1 H, NH), 7.19 [d, J(6,5) = 7.9 Hz, 1 H, 6-H], 5.70 [d, J(5,6) = 7.9 Hz, 5-H], 4.70–4.82 (m, 3 H, CH*i*Pr),

3.72 (m, 2 H, 1′-H), 2.36 (m, 2 H, PCH_2P), 1.86–2.02 (m, 2 H, 6′-H), 1.69 (m, 2 H, 2′-H), 1.64 (m, 2 H, 5′-H), 1.45 (m, 2 H, 4′-H), 1.37 (m, 2 H, 3′-H), 1.32–1.36 (m, 18 H, CH_3*i*Pr) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.72 (C-4), 150.76 (C-2), 144.38 (C-6), 102.02 (C-5), 71.34 [d, *J*(C,P) = 6.6 Hz], 71.07 [d, *J*(C,P) = 6.5 Hz] and 69.65 [d, *J*(C,P) = 6.8 Hz, CH*i*Pr], 48.61 (C-1′), 30.04 [d, *J*(4′,P) = 16.2 Hz, C-4′], 29.70 [d, *J*(6′,P) = 99.6 Hz, C-6′], 29.32 [dd, *J*(C,P) = 77.8, 134.3 Hz, PCH_2P], 28.68 (C-2′), 25.83 (C-3′), 23.83–24.43 (m, CH_3*i*Pr), 21.36 [d, *J*(5′,P) = 4.6 Hz, C-5′] ppm. MS (ESI): *m*/*z* (%) = 503 (100) $[M + Na]^+$. HRMS (ESI) calcd. for $C_{20}H_{38}N_2NaO_7P_2$ $[M + Na]^+$ 503.2051; found 503.2045. $C_{20}H_{38}N_2O_7P_2$ (480.47): calcd. C 50.00, H 7.97, N 5.83, O 23.31, P 12.89; found C 50.26, H 7.99, N 5.68, P 12.79.

1-{2-[(Diisopropoxyphosphorylmethyl)(isopropoxy)phosphorylethoxy]ethyl}uracil (16d): This compound was prepared by GP2, the crude product in dry MeOH (5 mL) was treated with MeONa/MeOH (1 M, 1 mL) at room temp. overnight and neutralized with acetic acid, and the solvents were evaporated. The product was purified by flash chromatography ($CHCl_3$/MeOH 0–10%), colorless oil (171 mg, 73%). ^{1}H NMR ($CDCl_3$): δ = 9.26 (br. s, 1 H, NH), 7.43 [d, *J*(6,5) = 7.9 Hz, 1 H, 6-H], 5.66 [dd, *J*(5,6) = 7.9, *J*(5,NH) = 1.8 Hz, 1 H, 5-H],4.72–4.79 (m, 3 H, CH*i*Pr), 3.92 (m, 2 H, 1′-H), 3.66–3.85 (m, 4 H, 2′-H, 3′-H), 2.35–2.46 (m, 3 H, PCH_2P, 4a-H), 2.21 (m, 1 H, 4b-H), 1.32–1.36 (m, 18 H, CH_3*i*Pr) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.76 (C-4), 150.85 (C-2), 146.06 (C-6), 101.48 (C-5), 71.48 [d, *J*(C,P) = 6.5 Hz], 71.26 [d, *J*(C,P) = 6.5 Hz] and 70.19 [d, *J*(C,P) = 6.9 Hz, CH*i*Pr], 68.63 (C-2′), 64.90 [d, *J*(C,P) = 2.4 Hz, C-3′], 48.21 (C-1′), 30.57 [dd, *J*(C,P) = 80.3, 135.4 Hz, PCH_2P], 30.51 [d, *J*(C,P) = 97.5 Hz, C-4′], 23.84–24.37 (m, CH_3*i*Pr) ppm. MS (ESI): *m*/*z* (%) = 491.1 (100) $[M + Na]^+$. HRMS (ESI) calcd. for $C_{18}H_{34}N_2NaO_8P_2$ $[M + Na]^+$ 491.1688; found 491.1686. $C_{18}H_{34}N_2O_8P_2$ (468.42): calcd. C 46.15, H 7.32, N 5.98, O 27.32, P 13.22; found C 45.91, H 7.29, N 5.79, P 13.12.

1-{4-[(Hydroxy)(phosphonomethyl)phosphoryl]butyl}uracil (17a): This compound was prepared by GP3, white solid, yield 74%, 182 °C. ^{1}H NMR (D_2O): δ = 7.65 [d, *J*(6,5) = Hz 7.9, 1 H, 6-H], 5.82 [d, *J*(5,6) = 7.8 Hz, 1 H, 5-H], 3.81 [t, *J*(1′,2′) = 7.2 Hz, 2 H, 1′-H], 2.47 [dd, *J*(H,P) = 16.8, *J*(H,P) = 20.4 Hz, 2 H, PCH_2P], 1.94–1.99 (m, 2 H, 4′a, 4′b-H), 1.78–1.83 (m, 2 H, 2′a, 2′b-H), 1.58–1.65 (m, 2 H, 3′a, 3′b-H) ppm. ^{13}C NMR (D_2O): δ = 167.55 (C-4), 152.98 (C-2), 147.98 (C-6), 102.09 (C-5), 48.96 (C-1′), 29.63 [d, *J*(C,P) = 16.5 Hz, C-2′], 29.32 [d, *J*(C,P) = 96.4 Hz, C-4′], 29.25 [dd, *J*(C,P_1) = 80.0, *J*(C,P_2) = 127.0 Hz, PCH_2P], 18.71 [d, *J*(C,P) = 4.1 Hz, C-3′] ppm. MS (ESI): *m*/*z* (%) = 325 (100) $[M - H]^-$. $C_9H_{16}N_2O_7P_2 \cdot 1/2\,H_2O$ (335.19): calcd. C 32.25, H 5.11, N 8.36, O 35.80, P 18.48; found C 32.07, H 5.29, N 8.02, P 18.37.

1-{5-[(Hydroxy)(phosphonomethyl)phosphoryl]pentyl}uracil (17b): This compound was prepared by GP3, white solid, yield 90%, m.p. 198–200 °C. ^{1}H NMR (D_2O): δ = 7.64 [d, *J*(6,5) = 7.9 Hz, 1 H, 6-H], 5.81 [d, *J*(5,6) = 7.8 Hz, 1 H, 5-H], 3.79 [t, *J*(1′,2′) = 7.2 Hz, 2 H, 1′-H], 2.46 [dd, *J*(H,P) = 16.7, 20.3 Hz, 2 H, PCH_2P], 1.92 (m, 2 H, 5′-H), 1.72 (m, 2 H, 2′-H), 1.62 (m, 2 H, 4′-H), 1.43 (m, 2 H, 3′-H) ppm. ^{13}C NMR (D_2O): δ = 167.57 (C-4), 153.01 (C-2), 148.09 (C-6), 101.98 (C-5), 49.37 (C-1′), 29.54 [d, *J*(C,P) = 95.8 Hz, C-5′], 29.25 [dd, *J*(C,P) = 79.6, 126.6 Hz, PCH_2P], 28.13 (C-2′), 27.24 [d, *J*(3′,P) = 16.7 Hz, C-3′], 21.24 [d, *J*(4′,P) = 4.1 Hz, C-4′] ppm. MS (ESI): *m*/*z* (%) = 339 (100) $[M - H]^-$. $C_{10}H_{18}N_2O_7P_2$ (340.21): calcd. C 35.30, H 5.33, N 8.23, O 32.92, P 18.21; found C 35.36, H 5.39, N 8.08, P 18.29.

1-{6-[(Hydroxy)(phosphonomethyl)phosphoryl]hexyl}uracil (17c): This compound was prepared by GP3, white solid, yield 73%, m.p. 185–186 °C. ^{1}H NMR (D_2O): δ = 7.64 [d, *J*(6,5) = 7.8 Hz, 1 H, 6-H], 5.81 [d, *J*(5,6) = 7.8 Hz, 1 H, 5-H], 3.77 (t, 2 H, 1′-H), 2.33 [dd, *J*(H,P) = 16.7, 19.8 Hz, 2 H, PCH_2P], 1.89 (m, 2 H, 6′-H), 1.69 (m, 2 H, 2′-H), 1.56 (m, 2 H, 5′-H), 1.43 (m, 2 H, 4′-H), 1.35 (m, 2 H, 3′-H) ppm. ^{13}C NMR (D_2O): δ = 167.54 (C-4), 152.98 (C-2), 148.13 (C-6), 101.92 (C-5), 49.57 (C-1′), 30.09 [d, *J*(4′,P) = 16.4 Hz, C-4′], 29.81 [dd, *J*(C,P) = 78.1, 123.4 Hz, PCH_2P], 29.70 [d, *J*(5′,P) = 95.7 Hz, C-6′], 28.42 (C-2′), 25.67 (C-3′), 21.51 [d, *J*(5′,P) = 4.3 Hz, C-5′] ppm. MS (ESI): *m*/*z* (%) = 353 (100) $[M - H]^-$. $C_{11}H_{20}N_2O_7P_2$ (354.23): calcd. C 37.30, H 5.69, N 7.91, O 31.62, P 17.49; found C 37.69, H 5.71, N 7.96, P 17.20.

1-{2-[(Hydroxy)(phosphonomethyl)phosphorylethoxy]ethyl}uracil (17d): This compound was prepared by GP3, white solid, yield 72%, m.p. 195 °C. ^{1}H NMR (D_2O): δ = 7.65 [d, *J*(6,5) = 7.9 Hz, 1 H, 6-H], 5.81 [d, *J*(5,6) = 7.9 Hz, 1 H, 5-H], 3.99 (m, 2 H, 1′-H), 3.80 [dt, *J*(3′,4′) = 6.9, *J*(3′,P) = 15.5 Hz, 2 H, 3′-H], 3.76 (m, 2 H, 2′-H), 2.45 [dd, *J*(H,P) = 17.1, 20.3 Hz, 2 H, PCH_2P], 2.23 [dt, *J*(H,P) = 14.7, *J*(4′,3′) = 6.9 Hz, 2 H, 4′-H] ppm. ^{13}C NMR (D_2O): δ = 167.53 (C-4), 152.92 (C-2), 148.45 (C-6), 101.87 (C-5), 68.40 (C-2′), 65.17 [d, *J*(C,P) = 2.7 Hz, C-3′], 48.84 (C-1′), 30.68 [d, *J*(C,P) = 96.3 Hz, C-4′], 30.42 [dd, *J*(C,P) = 81.1, 126.6 Hz, PCH_2P] ppm. MS (ESI): *m*/*z* (%) = 341 (100) $[M - H]^-$. $C_9H_{16}N_2O_8P_2$ (342.18): calcd. C 31.59, H 4.71, N 8.19, O 37.41, P 18.10; found C 31.48, H 4.83, N 8.15, P 17.82.

Triphosphate Analogues 18–20. General Procedure: Diphosphonate **6a** or **17a–d** (0.061 mmol) in MeOH (5 mL) was treated with tri-*n*-butylamine (29 μL, 0.122 mmol) and the mixture was heated until clear solution was obtained. The solvent was evaporated and the residue was dried with P_2O_5 in vacuo. CDI (49 mg, 0.305 mmol) in DMF (1 mL) was added dropwise under argon at 0 °C to the solution of the prepared bis(tri-*n*-butylammonium) salt in DMF (1 mL) and the resulting mixture was stirred at room temp. for 3 h. Methanol (10 μL, 0.244 mmol) was added, and after the system had been stirred for 1 h tri-*n*-butylammonium phosphate (0.5 M in DMF) was added and the reaction mixture was stirred for 6–7 h at room temp. The reaction solution was diluted with TEAB (2 mL, 0.025 M), placed on a column of POROS® HQ, and eluted with a linear gradient of TEAB (0–0.4 M). The fractions containing product were concentrated, the residue was applied onto DOWEX 50×8 (Na^+ form), eluted with water, and concentrated, and the residue in water (2 mL) was freeze-dried. The structures were elucidated by ^{1}H and ^{31}P NMR and the purities were determined by HPLC. For ^{31}P NMR spectroscopic data see Table 1.

Compound 18: White powder, yield 5%. ^{1}H NMR (D_2O): δ = 7.84 [d, *J*(6,5) = 7.9 Hz, 1 H, H-6], 5.93 [d, *J*(5,6) = 7.8 Hz, 1 H, H-5], 4.11 [t, *J*(1′,2′) = 5.0 Hz, 2 H, 1′-H], 3.93 [t, *J*(2′,1′) = 5.0 Hz, 2′-H], 3.83 [d, *J*(CH_2,P) = 6.8 Hz, PCH_2], 2.37 [dd, *J*(H,P_1) = 20.3, *J*(H,P_2) = 17.2 Hz, 2 H, PCH_2P] ppm.

Compound 19a: White powder, yield 16%. ^{1}H NMR (D_2O): δ = 7.8 [d, *J*(6,5) = 7.8 Hz, 1 H, 6-H], 5.93 [d, *J*(5,6) = 7.8 Hz, 1 H, 5-H], 3.9 [t, *J*(1′,2′) = 7.3 Hz, 2 H, 1′-H], 2.6 (m, 2 H, PCH_2P), 2.25–2.18 (m, 2 H, 4′-H), 1.96–1.88 (m, 2 H, 2′-H), 1.81–1.75 (m, 2 H, 3′-H) ppm.

Compound 19b: White powder, yield 22%. ^{1}H NMR (D_2O): δ = 7.74 [d, *J*(6,5) = 7.7 Hz, 1 H, 6-H], 5.9 [d, *J*(5,6) = 7.7 Hz, 1 H, 5-H], 3.89 [t, *J*(1′,2′) = 7.2 Hz, 2 H, 1′-H], 2.38 (dd, $J_1 = J_2$ = 17.7 Hz, PCH_2P), 2.17 (m, 2 H, 5′-H), 1.84–1.82 (m, 4 H, 2′-H, 4′-H), 1.54 (m, 2 H, 3′-H) ppm.

Compound 19c: White powder, yield 19%. ^{1}H NMR (D_2O): δ = 7.72 [d, *J*(6,5) = 7.7 Hz, 1 H, 6-H], 5.9 [d, *J*(5,6) = 7.7 Hz, 1 H, 5-H], 3.87 [t, *J*(1′,2′) = 6.6 Hz, 2 H, 1′-H], 2.38 (dd, $J_1 = J_2$ =

17.7 Hz, 2 H, PCH_2P), 2.16 (m, 2 H, 6′-H), 1.81–1.78 (m, 4 H, 2′-H, 5′-H), 1.54–1.44 (m, 4 H, 4′-H, 3′-H) ppm.

Compound 19d: White powder, yield 28%. ^1H NMR (D_2O): δ = 7.81 [d, J(6,5) = 7.9 Hz, 1 H, 6-H], 5.94 [d, J(5,6) = 7.9 Hz, 1 H, 5-H], 4.11 (m, 2 H, 1′-H), 4.0–3.95 (m, 2 H, 3′-H), 3.9 (m, 2 H, 2′-H), 2.45 [dd, J(H,P) = 18.2, 19.0 Hz, 2 H, PCH_2P], 2.23 (m, 2 H, 4′-H) ppm.

Compound 20: White powder, yield 13%. ^1H NMR (D_2O): δ = 7.84 [d, J(6,5) = 7.9 Hz, 1 H, H-6], 5.93 [d, J(5,6) = 7.8 Hz, 1 H, 5-H], 4.04 [t, J(1′,2′) = 4.8 Hz, 2 H, 1′-H], 3.91 [t, J(2′,1′) = 4.88 Hz, 2′-H], 3.66 [d, $J(CH_2,P)$ = 7.0 Hz, 2 H, PCH_2O], 2.56 [dd, $J(H,P_1)$ = 19.7, $J(H,P_2)$ = 17.8 Hz, 2 H, PCH_2P] ppm.

Determination of pK_a: Compound **6a** (10 mg) was dissolved in acetate buffer (0.025 M, 0.5 mL) and acidified with HCl (2 M), the pH was measured, and then the ^{31}P NMR spectrum was acquired. NaOH (0.1 M, one drop) was then added repeatedly, the sample was shaken, and pH and ^{31}P NMR spectrum were measured. The pH dependence of the ^{31}P chemical shifts was plotted and pK_a was estimated to be at the pH at which the phosphorus chemical shift is just in the middle between the chemical shifts of the protonated and unprotonated forms.

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