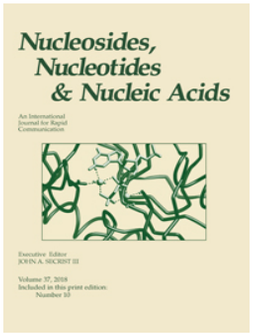

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Efficient one-pot, three-component procedure to prepare new α-aminophosphonate and phosphonic acid acyclic nucleosides

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ABSTRACT

An efficient one-pot three-component Kabachnik–Fields reaction of aldehydes (acyclic nucleosides), amines (or amino acid), and triethyl phosphite proceeded for the synthesis of aminophosphonates using natural phosphate coated with iodine (I_2@NP) as a catalyst. The novel α-aminophosphonate and phosphonic acid acyclic nucleosides were tested for their anti-HCV and anti-HIV activities. The molecular docking showed that the non-activity of these compounds could be due to the absence of hydrophobic pharmacophores.

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KEYWORDS
Acyclic nucleosides; α-aminophosphonates; Kabachnik–Fields reaction; catalysis; natural phosphate

1. Introduction

Viral diseases are a major health problem worldwide. Indeed, there is still a need to discover new potent, safe, and selective antiviral drugs. Among these, α-aminophosphonates have attracted the attention of medicinal chemists due to their potential antiviral activity.[1] The α-aminophosphonates are structurally similar to α-amino acids, known to have good cell permeability,[2] and physiological stability as the phosphorus–carbon bond, which is not susceptible to enzymatic degradation by phosphatases.[3–5] Moreover, acyclic nucleoside phosphonates, such as adefovir and tenofovir, are now in clinical use for the treatment of viral infections (HIV, HBV, CMV). These drugs possess a phosphonomethyl ether moiety allowing them to bypass the first phosphorylation step.[6,7] The after-mentioned properties prompted many research groups, including ours, to synthesize acyclic nucleoside phosphonates varying notably the length and substitution pattern of the phosphonate link.[8–12]

The synthesis of α-aminophosphonates has gained more attention due to their broad spectrum of biological activity like anticancer, antiviral,

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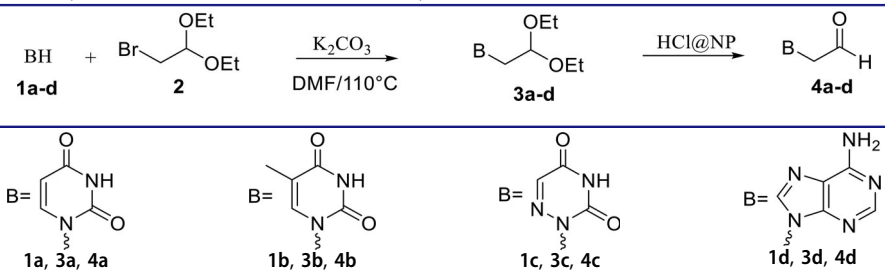
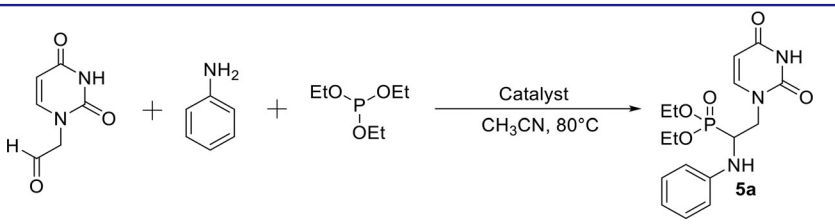
Table 1. Synthesis of nucleoside N-acetaldehydes **4a–d**.



herbicides, fungicides, bactericides, peptide mimetic, and enzyme inhibitor.[13–18] Three-component one-pot Kabachnik–Fields condensation of carbonyl compounds with amines and phosphites is perhaps the most exploited reaction for the preparation of structurally diverse α-aminophosphonates.[19,20] While previous procedures were limited to simple starting materials and newer wide-scope syntheses catalyzed by Lewis acids, have been reported.[2,21–26] Recently, several solid catalysts are used in the preparation of α-aminophosphonates such as $SbCl_3/Al_2O_3$,[27] $Mg(ClO_4)_2$,[28] $Na_2CaP_2O_7$,[29] KF-doped natural phosphate (NP),[30] and PEG-SO_3H.[31] In general, these three-component reactions may take place via an imine or an α-hydroxy-phosphonate intermediate.[32] During the formation of the imine, water formed which could either deactivate or decompose the Lewis acid catalyst, thereby limiting the scope of carrying out this reaction in one-pot.[33] Among supports reported in the literature, NP found[30] to be an efficient Lewis acid to catalyze the synthesis of α-aminophosphonates in one-pot due to its ability to tolerate the water generated during the course of the reaction. Moreover, it was used widely as support because of its ionic substitution ability, structural stability, and high adsorption capacity, making it an attractive cost-effective catalyst for several chemical transformations.[34–36] Meanwhile, iodine has emerged as a very efficient Lewis acid catalyst for various organic transformations and is relatively inexpensive compared to other Lewis acids, including rare earth metal triflates, and is more tolerant in comparison to typical Lewis acids/bases.[37–39] Furthermore, Zahouily et al. showed that iodine supported on NP could be effectively employed for the protection of carbonyl compounds as their thioacetals in good yield at ambient temperature and mild conditions.[40]

In the light of these successes, here we report a one-pot three-component reaction of α-aminophosphonates using a small amount of natural phosphate coated with iodine (I_2@NP) as a catalyst. A simple protocol followed in the preparation of novel α-aminophosphonate and phosphonic acid acyclic nucleosides with both pyrimidine and purine nucleobases. Finally, all newly synthesized compounds were evaluated for their anti-HCV and anti-HIV activities.

Table 2. Optimal conditions for the preparation of aminophosphonates **5a**.



Entry	Catalyst	Yield (%)[a]
1	–	20
2	NP	0
3	I_2	30
4	I_2@NP	77
5	$ZnCl_2$@NP	65
6	$ZnBr_2$@NP	40
7	CF_3SO_3H@NP	25
8	$Zn(OTf)_2$@NP	51
9	$SnCl_4$@NP	38

[a]Isolated yield after gel chromatography purification.

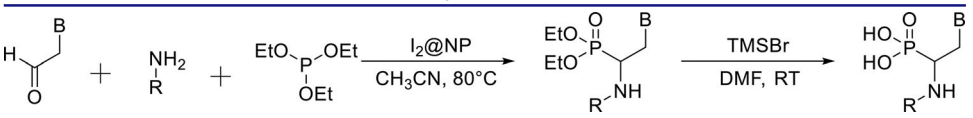
2. Results and discussion

The strategy for the synthesis of a novel conjugated acyclic nucleoside-α-amino acid phosphonate **5–9** and their phosphonic analogs **10–14** is reported in Table 1. Our study started with the preparation of various acyclic nucleoside N-acetaldehydes, which were obtained by treatment of the unprotected nucleobases with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate. Then, aldehydes **4a–d** obtained by hydrolysis of the acetal group **3a–d** using NP/HCl as an acidic medium (Table 1).[41]

To evaluate the influence of the catalyst, we prepared NPs coated with different Lewis acids using aldehyde **4a**, aniline, and triethyl phosphite as a model experiment (Table 2). We notice that when we used the iodine alone as a catalyst, the acyclonucleoside was obtained only with 30%, but when we applied heterogeneous catalysis conditions using NP as support and iodine as catalyst (I_2@NP) the yield increase to 77%. Thus, the use of the NP allows to improve the yield and to reused this catalyst many times. In summary, the best results were obtained with I_2@NP in refluxing acetonitrile, which yielded α-aminophosphonate **5a** in 77% yields (Table 2, entry 4).

As presented in Table 3, the optimized conditions were generalized using acyclic nucleoside acetaldehydes **4a–d**, triethyl phosphite, and various amines, including amino acids. The acyclic nucleoside diethyl α-aminophosphonates obtained **5–9(a–d)** were further treated with TMSBr (TMSBr) in DMF (dimethylformamide) to yield the corresponding α-amino phosphonic acids **10–14(a–d)**. The reaction tolerates aromatic and aliphatic amines as well as amino acids. The results provide the first examples of conjugated nucleoside-α-amino acids phosphonates and pave the way for the design of new potential bioactive compounds through a simple and eco-friendly transformation.

Table 3. Results of the preparation of novel conjugated nucleoside-α-amino acid phosphonates **5–9(a–d)** and their phosphonic analogs **10–14(a–d)**.



Entry	B	R	Phosphonate, yield (%[a])	Phosphonic, yield (%[a])
1	U	Ph	**5a**, 77	**10a**, 60
2	T	Ph	**5b**, 45	**10b**, 60
3	6-AzaU	Ph	**5c**, 40	**10c**, 50
4	A	Ph	**5d**, 40	**10d**, 70
5	U	Ph-CH_2	**6a**, 50	**11a**, 60
6	T	Ph-CH_2	**6b**, 60	**11b**, 35
7	6-AzaU	Ph-CH_2	**6c**, 60	**11c**, 35
8	A	Ph-CH_2	**6d**, 62	**11d**, 25
9	U	CH_3-CH-CH_2-CH_3	**7a**, 66	**12a**, 25
10	T	CH_3-CH-CH_2-CH_3	**7b**, 60	–[b]
11	6-AzaU	CH_3-CH-CH_2-CH_3	**7c**, 50	**12c**, 20
12	A	CH_3-CH-CH_2-CH_3	**7d**, 50	–[b]
13	U	CH_2-CO_2Et	**8a**, 55	**13a**, 40
14	T	CH_2-CO_2Et	**8b**, 60	**13b**, 20
15	6-AzaU	CH_2-CO_2Et	**8c**, 50	**13c**, 20
16	A	CH_2-CO_2Et	**8d**, 55	**13d**, 20
17	U	CH_3-CH-CO_2Me	**9a**, 40	**14a**, 35
18	T	CH_3-CH-CO_2Me	**9b**, 40	**14b**, 25
19	6-AzaU	CH_3-CH-CO_2Me	**9c**, 41	**14c**, 30
20	A	CH_3-CH-CO_2Me	**9d**, 42	**14d**, 15

[a]Isolated yield after gel chromatography purification. [b]A mixture undefined compounds.

Table 4. Antiviral activity against hepatitis C virus (genotype **1b**) in Huh 5.2 cells.

Products	EC50	CC50
10a	50	50
10b	50	50
10c	50	50
10d	50	50
11a	50	50
11b	46.6	50
11c	50	50
11d	50	50
12a	50	50
12c	47.7	50
13a	50	50
13b	50	50
13c	50	50
13d	50	50
14a	50	50
14b	46.6	50
14c	50	50
14d	50	50
Ribavirin	21	7

CC_{50} concentrations of compound required for 50% extinction of Huh 5.2 cells.
EC_{50} concentrations of compound achieving 50% inhibition of the replicon system.

Next, we evaluated the antiviral activity of these new phosphonic acids in an HCV sub-genomic RNA replicon using Huh 5.2 cells (Table 4). Unfortunately, none of the compounds exhibited any significant antiviral activity. On the other hand, these new phosphonic acids were subjected to

Table 5. Antiviral activity against HIV-1 and 2.

Products	Strains	IC_{50} (μg/mL)	CC_{50} (μg/mL)	IS
10a	III_B	>81.5	=81.5	<1
	ROD	>84	=84.3	<1
10b	III_B	>87	=87	<1
	ROD	>86.9	=86.9	<1
10d	III_B	>65.7	=65.7	<1
	ROD	>77.5	77.5	<1
11a	III_B	>65.1	=65.1	<1
	ROD	>78.3	=78.3	<1
11b	III_B	>76.5	=76.5	<1
	ROD	>85.1	=85.1	<1
11c	III_B	>100	>100	X
	ROD	>100	>100	X
11d	III_B	>100	>100	X1
	ROD	>100	>100	X1
12a	III_B	>50	>50	X1
	ROD	>50	>50	X1
12c	III_B	>100	>100	X1
	ROD	>97.7	=97.7	<1
13b	IIIB	>87	=87	<1
	ROD	>86.9	=86.9	<1
13c	IIIB	>65.7	=65.7	<1
	ROD	>77.5	77.5	<1
13d	IIIB	>81.5	=81.5	<1
	ROD	>84.	=84.3	<1
14a	IIIB	>65.1	=65.1	<1
	ROD	>78.3	=78.3	<1
14b	IIIB	>76.5	=76.5	<1
	ROD	>85.1	=85.1	<1
14c	IIIB	>100	>100	X
	ROD	>100	>100	X
14d	IIIB	>100	>100	X1
	ROD	>100	>100	X1
D4T	IIIB	0.0586	>20	341
	ROD	0.0929	>20	215
AZT	IIIB	0.00143	>25	17,482
	ROD	0.00113	>25	22,202

Strain III for VIH-1 strain ROD for VIH-2; IC_{50}: inhibitory concentration, permitting the protection of 50% of the cells against the VIH; CC_{50}: cytotoxic concentration, reducing 50% of the cells non-infected; IS: index of selectivity or CC_{50}/CI_{50}.

a standard in vitro antiviral screening using HIV-1 and HIV-2 (Table 5). None of the compounds exhibited any significant antiviral activity. Several factors could be responsible for the inactivity of these nucleoside derivatives. For instance, their inability to enter cells or to serve as substrates for intracellular enzymes catalyzing triphosphorylation, as well as a lack of inhibition of viral polymerase by their triphosphate forms, would all account for their inactivity against these viruses.

In the aim to understand the lack of activity of synthesized compounds and suggests some modifications to improve the antiviral activity, molecular docking carried out. The HCV NS3 protease plays a pivotal role in the replication of the HCV virus. Its inhibition has proven effective in reducing viral loads in humans.[42] Furthermore, several phosphonate derivatives were reported as potential inhibitors of HCV NS3 protease.[43–46] Meanwhile, HIV reverse transcriptase (RT) is an attractive target for the

Table 6. Detail of binding interactions.

Protein	Ligand	Energy of binding (kcal/mol)	RMSD (Å)
1W3C	DN1	−8.79	2.37
	14a	−3.55	–
2RF2	MRX	−10.55	0.27
	14a	−6.63	–

treatment of HIV disease. Over the past years, various nucleoside phosphonates described as inhibitors of RT.[47–49] Considering these facts, the compound **14a** docked into the active sites of HCV NS3 protease (Protein Data Bank [PDB]: 1W3C)[50] and HIV RT (PDB: 2RF2).[51] Before, to determine the validity of the docking protocol, first, the self-docking experiments were carried out. The root-mean-square deviation (RMSD) between the predicted and the native poses was found to be 2.37 Å (1W3C; DN1) and 0.27 Å (2RF2; MRX). These results indicated that the adopted docking protocol is good for the reproduction of the native poses.

The docking results are summarized in Table 6 and Figures 1–4. Regarding HCV NS3 protease, the compound **14a** showed a low affinity with an estimated binding energy of −3.55 kcal/mol. It reported that the hydrophobic interactions with amino acid residues Val132, Cys159, and Val158 enhanced the inhibitory effect of HCV NS3 protease inhibitors.[52] As shown in Figure 2, the hydrophile phosphonic acid group of ligand **14a** is located in the hydrophobic cavity (brown region) of protease protein, leading to a decrease in the inhibitory effect (absence of hydrophobic interactions). Thus, the introduction of aromatic hydrophobic pharmacophore (such as biphenyl)[52] will increase the hydrophobic interactions, especially with CYS159, which improve the antiviral activity against HCV. On the other hand, HIV RT also showed a low affinity for compound **14a** with the estimated free energy of binding −6.63 kcal/mol. This could be explained by the lack of hydrophobic and aromatic interactions. Also, the hydroxyl group (phosphonate) is located in the hydrophobic region, leading to repulsion between the protein and the ligand.

3. Conclusion

In conclusion, I_2@NP was found to be an efficient catalyst for the one-pot three-component reaction of acyclic nucleoside acetaldehydes, amines, and triethyl phosphites. New α-aminophosphonates and α-aminophosphonic acids were obtained in good yields with an eco-friendly, inexpensive, and efficient catalyst. This work opens a way to explore the chemical diversity offered by this methodology.

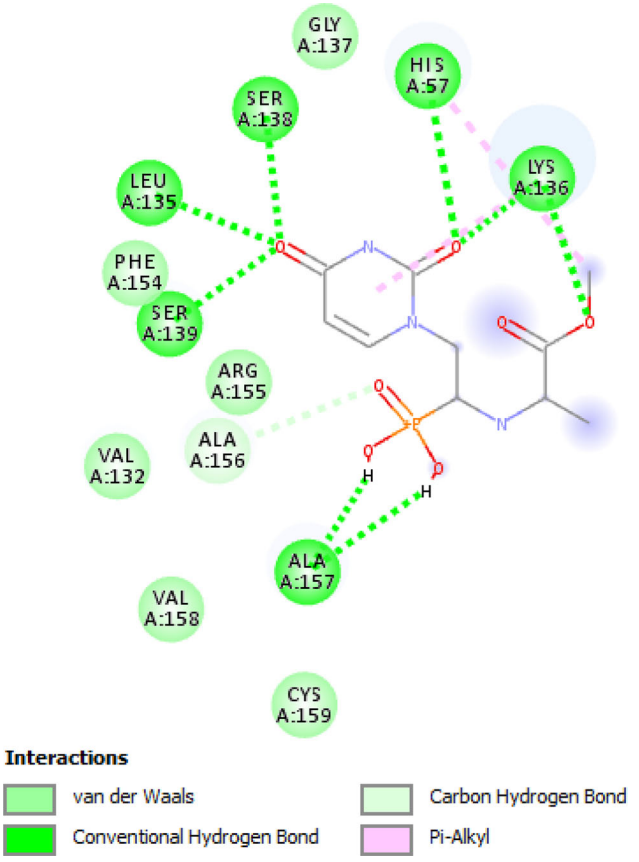


Figure 1. 2D interactions of compound **14a** in the binding sites of HCV NS3 protease.

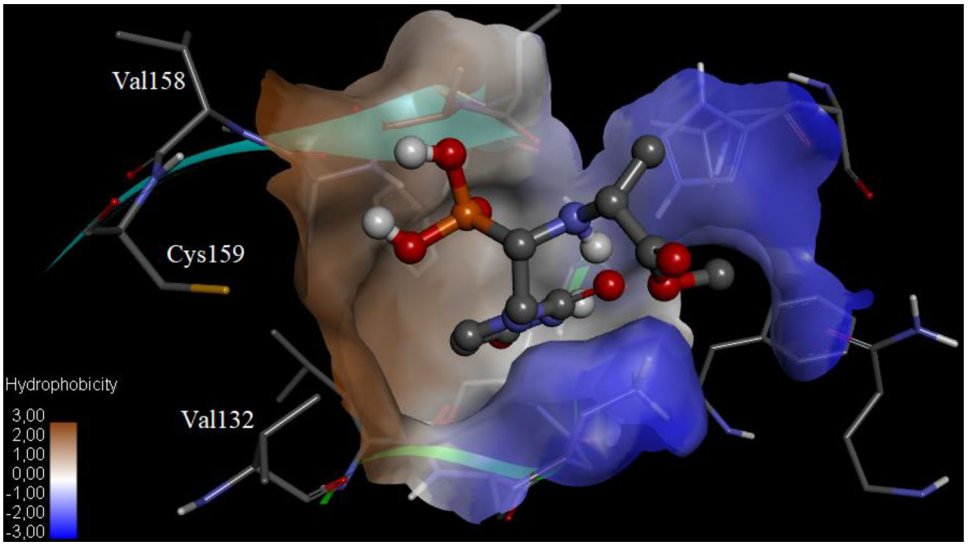


Figure 2. The position of ligand **14a** in the hydrophobic cavity of HCV NS3 protease.

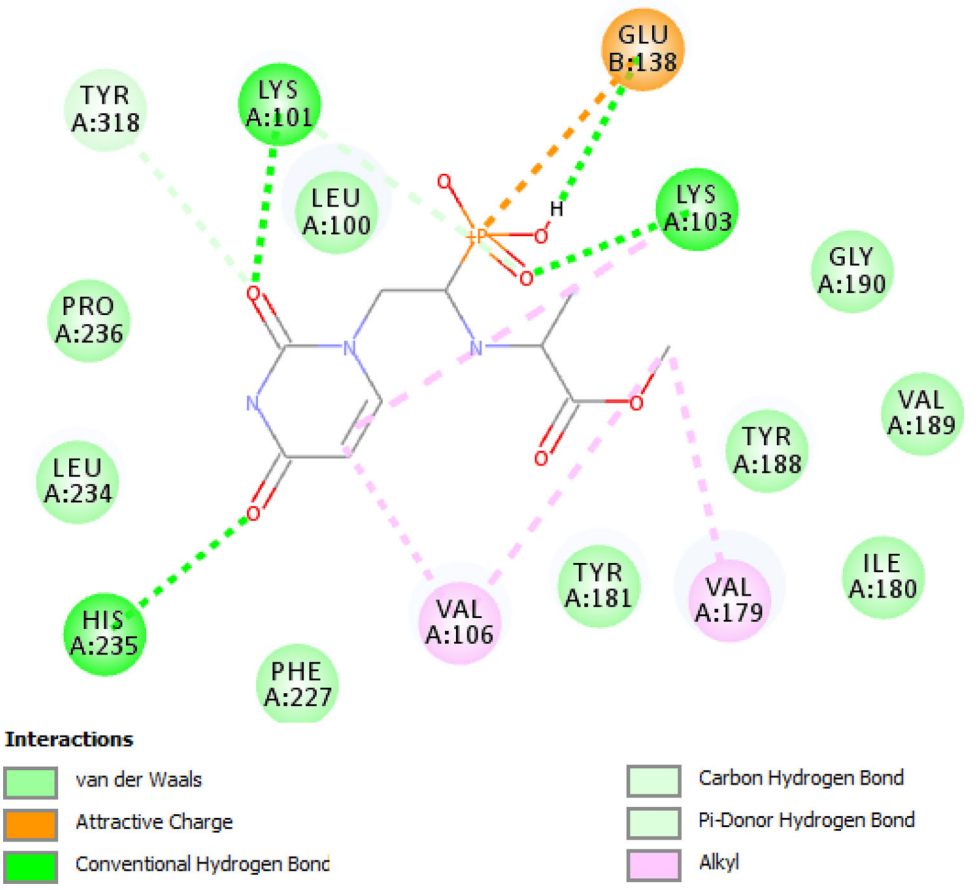


Figure 3. 2D interactions of compound **14a** in the binding sites of HIV-1 RT.

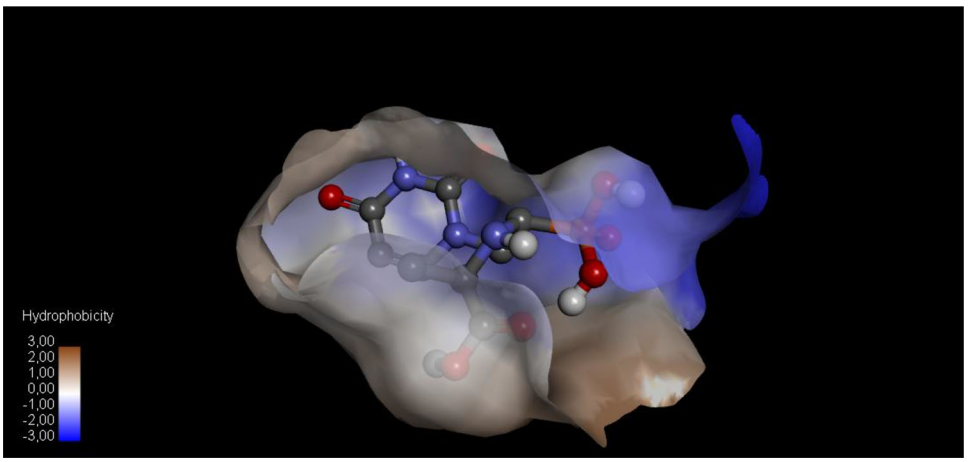


Figure 4. The position of ligand **14a** in the hydrophobic cavity of HIV-1 RT.

4. Experimental

4.1. Chemistry

Melting points were measured using a Büchi B-545 digital capillary melting point apparatus and used without correction. Reactions were checked with TLC using aluminum sheets with silica gel 60 F254 from Merck. The spectra of ^{1}H NMR and ^{13}C NMR were recorded in solution in DMSO-d_6 or $CDCl_3$ on a Bruker Advance 300 spectrometer at 300 and 75 MHz, respectively. The chemical shifts are expressed in parts per million (ppm) by using DMSO-d_6 as internal reference. The multiplicities of the signals are indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet, and coupling constants are expressed in Hertz. FAB (fast atom bombardment) mass spectra were recorded on a Varian MAT 311A spectrometer. Mass spectra were collected using an API 3200 LC/MS/MS system, equipped with an ESI source. The chemical reagents used in synthesis were purchased from Fluka, Sigma-Aldrich. Column chromatography was performed on silica gel (30–60 mm). All solvents were distilled and dried before using them.

5. General procedure for the preparation of aldehydes 3a–d

To a solution of the base (0.80 mmol) and K_2CO_3 (0.5 eq) in DMF (15 mL) was added 1.5 eq. of bromoacetaldehyde diethyl acetal and the resulting mixture was refluxed for a time ranging from 3 to 4 h. After completion of the reaction as indicated by TLC, the reaction mixture was quenched with acetic acid in water (10% v/v) and filtered. Evaporation of the solvent followed by purification (chromatography column on silica gel, CH_2Cl_2/MeOH) afforded the corresponding aldehydes.

1,1-diethoxy-2-(thymin-1-yl) acetaldehyde ***3a***

Yield: 56%; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.10 (t, $J = 7.03$ Hz, 6H), 1.85 (s, 3H, CH_3), 3.50 (q, $J = 7.03$ Hz, 2H), 3.65 (q, $J = 7.04$ Hz, 2H), 3.65 (d, $J = 5.23$ Hz, 2H), 4.55 (t, $J = 5.25$ Hz, 1H), 7.05 (s, 1H), 9.55 (sb, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.17, 15.27, 50.83, 64.27, 100.35, 109.89, 142.07, 151.30, 164.62. MS (FAB+), $m/z = 243$ $[M + H]^+$. HRMS Calcd for $C_{11}H_{18}N_2O_4$ $[M + H]^+$: 243.2716 found 243.2722.

1,1-diethoxy-2-(uracil-1-yl) acetaldehyde ***3b***

Yield: 60%; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 6H, $J = 7.02$ Hz, $2CH_3$), 3.50 (q, 2H, $J = 7.03$ Hz, OCH_2), 3.65 (q, 2H, $J = 7.03$ Hz, OCH_2), 3.70 (d, 2H, $J = 5.23$ Hz, N-CH_2), 4.55 (t, 1H, $J = 5.25$ Hz, CH-CH_2), 5.50 (d, 1H, $J = 7.93$ Hz, H-5), 7.20 (d, 1H, $J = 7.9$ Hz, H-6), 9.55 (sl, 1H, N3-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.07–14.27 (2$\underline{C}H_3$), 50.09 ($\underline{C}H_2$-CH), 63.22 (2$\underline{C}H_2$-CH_3), 99.30 ($\underline{C}H$-CH_2), 100.59 (C5), 145.09 (C6), 150.33

(C4), 163.06 (C2). MS (FAB+), $m/z = 229$ $[M + H]^+$. HRMS Calcd for $C_{10}H_{16}N_2O_4$ $[M + H]^+$: 229.2450 found 229.2458.

1,1-diethoxy-2-(azauracil-1-yl) acetaldehyde ***3c***

Yield: 60%; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 6H, $J = 7.02$ Hz, $2CH_3$), 3.50 (q, 2H, $J = 7.02$ Hz, OCH_2), 3.65 (q, 2H, $J = 7.02$ Hz, OCH_2), 4.00 (d, 2H, $J = 5.78$ Hz, N-CH_2), 4.85 (t, 1H, $J = 5.80$ Hz, CH-CH_2), 7.35 (s, 1H, H-5), 9.70 (sl, 1H, N-3H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 15.17 ($2CH_3$), 41.36 (CH_2-CH), 62.00 ($2CH_2$-CH_3), 97.80 (CH-CH_2), 135.36 (C5), 149.26 (C4), 155.93 (C2). MS (FAB+), $m/z = 230$ $[M + H]^+$. HRMS Calcd for $C_9H_{15}N_3O_4$ $[M + H]^+$: 230.2331 found 230.2325.

1,1-diethoxy-2-(adenin-9-yl) acetaldehyde ***3d***

Yield: 60%; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 6H, 7.02 Hz, $2CH_3$), 3.48 (q, 2H, $J = 7.03$ Hz, OCH_2-CH_3), 3.70 (q, 2H, $J = 7.03$ Hz, OCH_2-CH_3), 4.20 (d, 2H, $J = 5.22$ Hz, N-CH_2), 4.65 (t, 1H, J = 5.23 Hz, CH_2-CH-O), 5.50 (s, 2H, NH_2), 7.80 (s, 1H, H-2), 8.30 (s, 1H, H-8). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 15.22 ($2CH_3$), 46.28 (CH_2-CH), 63.96 ($2CH_2$-CH_3), 100.37 (CH-CH_2), 119.26 (C5), 141.77 (C6), 150.28 (C4), 153.02 (C2), 155.29 (C8). MS (FAB+), $m/z = 252$ $[M + H]^+$. HRMS Calcd for $C_{11}H_{17}N_5O_2$ $[M + H]^+$: 252.2849 found 252.2858.

5.1. Catalyst preparation

Natural phosphate[53]

NP used in this work was obtained in the Khouribga region (Morocco). Prior to use, this material requires initial treatments such as crushing and washing. For use in organic synthesis, the NP is treated by techniques involving attrition, sifting, calcination (900 °C), washing, and recalcination. These treatments lead to a fraction between 100 and 400 micron that is rich in phosphate and has following chemical composition: P_2O_5 (34.24%), CaO (54.12%), F^- (3.37%), SiO_2 (2.42%), SO_3 (2.21%), CO_2 (1.13%), Na_2O (0.92%), MgO (0.68%), Al_2O_3 (0.46%), Fe_2O_3 (0.36%), K_2O (0.04%), and several metals (Zn, Cu, Cd, V, U, Cr) in the range of ppm. The structure of the material is similar to that of fluorapatite ($Ca_{10}(PO_4)_6F_2$). In sedimentary rocks, phosphates are formed from compounds derived from apatite by partial isomorphic substitution of: Na^+, Mg^{2+}, Co^{2+}, Fe^{3+}, or Al^{3+} for Ca^{2+} ions, VO_4^{3-}, SO_4^{2-}, CO_3^{2-} or MnO_4^- for PO_4^{3-} ions, and OH^- or Cl^- for F^-. These different substitutions cause distortions of the structure which depends on the nature and the radii of the ions involved. This solid presented a very low surface area (BET) at ca. 1 $m^2\ g^{-1}$.

NP coated with iodine (I_2@NP)

To a solution of iodine (759 mg) in CH_2Cl_2 (5 mL) was added NP (3 g) and stirred for 15 min and evaporated to dryness.

NP coated with $ZnBr_2$

To a solution of $ZnBr_2$ (25 mg) in water (5 mL) was added NP (175 mg). The mixture was stirred for 15 min and evaporated to dryness.

NP coated with $ZnCl_2$

To a solution of $ZnCl_2$ (25 mg) in water (5 mL) was added NP (175 mg). The mixture was stirred for 15 min and evaporated to dryness.

NP coated with CF_3SO_3H

To a solution of CF_3SO_3H (1 mL) in methylene chloride (5 mL) was added NP (3 g). The mixture was stirred for 15 min and evaporated to dryness.

NP coated with $SnCl_4$

A mixture of 1 g of $SnCl_4$ (0.45 mL) in 5 mL water was stirred for 5 min, and then 1 g of NP was added. The mixture was stirred for 15 min and evaporated to dryness.

6. General procedure for the synthesis of α-amino, amino acid phosphonate acyclonucleosides

6.1. Method A

To a suspension of the desired acetal (0.41 mmol) in acetonitrile (8 mL), was added 400 mg of NP/HCl and water (2 mL) and the mixture was heated (80 °C) for 2 h. After filtration, the solid residue was washed by CH_3CN and the filtrate was evaporated to yield the corresponding aldehyde **4(a–d)** quantitatively. To the crude aldehyde was added acetonitrile (5 mL), 1 equivalent of the amine/amino acid, triethyl phosphite (0.9 eq, 0.06 mL), and 0.2 equivalent of natural doped phosphate (I_2@NP, 104 mg; ($ZnCl_2$@NP, 92 mg; $ZnBr_2$@NP, 160 mg; CF_3SO_3H @NP, 40 mg; $Zn(OTf)_2$@NP, 280 mg; $SnCl_4$@NP, 44 mg). The mixture was refluxed for 3 h and the resulting suspension was filtered and washed with acetonitrile. The filtrate was then evaporated and the residue was dissolved in CH_2Cl_2, washed with a solution of $Na_2S_2O_3$ (1 M), and the organic phase was dried over Na_2SO_4. After filtration and evaporation, the crude product was purified by silica gel chromatography (eluent: CH_2Cl_2/MeOH).

6.2. Method B

To a suspension of the desired acetal (0.41 mmol) in acetonitrile (8 mL), was added 400 mg of HCl@NP and water (2 mL) and the mixture was heated (80 °C) for 2 h. After filtration, the solid residue was washed by CH_3CN and the filtrate was evaporated to yield the corresponding aldehyde quantitatively. To the crude aldehyde was added acetonitrile (5 mL), 1 equivalent of the amine/amino acid, triethyl phosphite (0.9 eq, 0.06 mL),

and 0.2 equivalent of natural doped phosphate (I_2@NP, 104 mg). The mixture was refluxed for 3 h and the resulting suspension was filtered and washed with acetonitrile. The filtrate was then evaporated and the residue was dissolved in CH_2Cl_2, washed with a solution of $Na_2S_2O_3$ (1 M), and the organic phase was dried over Na_2SO_4. After filtration and evaporation, the crude product was purified by silica gel chromatography (eluent: CH_2Cl_2/MeOH).

N^1 (2-anilino-2-diethoxyphosphinyl-ethan-1-yl) uracil ***5a***

Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 3H, $J = 7.02$ Hz, CH_3), 1.20 (t, 3H, $J = 7.02$ Hz, CH_3), 3.80–4.10 (m, 4H, 2 OCH_2-CH_3) , 4.18 (m, 2H, N1-CH_2-CH), 4.40 (m, 1H, CH-CH_2); 4.60 (s, 1H, NH-Ph), 5.50 (d, 1H, $J = 7.87$ Hz, H5), 6.50–7.00 (m, 5H, Ph), 7.05 (d, 1H, $J = 7.87$ Hz, H6), 10.10 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 15 ($2CH_3$), 48.91 (CH_2-CH), 61.80–63.12 ($2CH_2$-CH_3), 100.69 (CH-CH_2), 112.28 (C5), 128.41–144 (Ph), 145.23 (C6), 150.39 (C4) 163.24 (C2). MS (FAB+), $m/z = 368$ $[M + H]^+$. HRMS Calcd for $C_{16}H_{22}N_3O_5P$ $[M + H]^+$: 368.3367 found 368.3375.

N^1 (2-anilino-2-diethoxyphosphinyl-ethan-1-yl) thymine ***5b***

Yield: 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 3H, CH_3), 1.20 (t, 3H, $J = 7.03$ Hz, CH_3), 1.70 (s, 3H, CH_3), 3.80–4.10 (m, 4H, 2 OCH_2-CH_3), 4.20 (m, 2H, N1-CH_2-CH), 4.40 (m, 1H, CH-CH_2); 4.60 (s, 1H, NH-Ph), 6.50–7.00 (m, 5H, Ph), 7.05 (s, 1H, H6), 9.80 (s, 1H, N3-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 12.11 (CH_3); 16.35 ($2CH_3$), 48.91 (CH_2-CH), 62.74 (CH_2-CH_3), 63.99 (CH_2-CH_3), 100.01 (CH-CH_2), 113.35 (C5), 129.32–141 (Ph), 146.43 (C6), 151.46 (C4), 164.56 (C2). MS (FAB+), $m/z = 382$ $[M + H]^+$. HRMS Calcd for $C_{17}H_{24}N_3O_5P$ $[M + H]^+$: 382.3633 found 382.3640.

N^1 (2-anilino-2-diethoxyphosphinyl-ethan-1-yl) azauracil ***5c***

Yield: 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 3H, CH_3), 1.22 (t, 3H, $J = 7.02$ Hz, CH_3), 3.90–4.10 (m, 4H, 2 OCH_2-CH_3), 4.15 (m, 2H, N1-CH_2-CH), 4.40 (m, 1H, CH-CH_2), 4.50 (s, 1H, NH-Ph), 7.20 (s, 1H, H5), 6.50–7.10 (m, 5H, Ph), 10.60 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.38 ($2CH_3$), 38.22 (CH_2-CH), 60.83 (CH_2-CH_3), 62.00 (CH_2-CH_3); 111.45 (CH-CH_2), 113.37 (C5), 127.27–144.53 (Ph), 147.59 (C4), 154.57 (C2). MS (FAB+), $m/z = 369$ $[M + H]^+$. HRMS Calcd for $C_{15}H_{21}N_4O_5P$ $[M + H]^+$: 369.3248 found 369.3239.

N^9 (2-anilino-2-diethoxyphosphinyl-ethan-1-yl) adenine ***5d***

Yield: 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.10 (t, 3H, $J = 7.06$ Hz, CH_3); 1.20 (t, 3H, CH_3), 3.90–4.10 (m, 4H, 2 OCH_2-CH_3), 4.30 (m, 2H, N9-CH_2-CH), 4.40 (m, 1H, CH-CH_2), 4.60 (s, 1H, NH-Ph), 5.60 (s, 2H, NH_2), 6.40–7.05 (m, 5H, Ph), 7.75 (s, 1H, H2), 8.35 (s, 1H, H8).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 16.34 ($2CH_3$), 44.66 (CH_2-CH), 62.83–63.96 ($2CH_2$-CH_3), 113.52 (CH-CH_2), 119.52 (C5), 129.22–140 (Ph), 141.34 (C6),

150.28 (C4), 153.02 (C2), 155.35 (C8). MS (FAB+), $m/z = 391$ $[M+H]^+$. HRMS Calcd for $C_{17}H_{23}N_6O_3P$ $[M+H]^+$: 391.3766 found 391.3773.

N^1 (2-benzylamino-2-diethoxyphosphinyl-ethan-1-yl) uracil ***6a***

Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.20 (t, 3H, $J = 7.02$ Hz, CH_3), 1.30 (t, 3H, CH_3), 3.20 (m, 1H, Ha), 3.5 (m, 1H, Hb), 3.80–3.90 (m, 2H, N1-CH_2-CH), 4.20 (m, 4H, 2 OCH_2-CH_3) , 4.25 (m, 1H, CH-CH_2), 4.30 (s, 1H, N-H-CH_2-Ph), 5.60 (d, 1H, $J = 7.87$ Hz, H5), 6.50–7.00 (m, 5H, ph), 7.20 (d, 1H, $J = 7.87$, H6), 9.20 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 16.6 ($2CH_3$), 49.72 (CH_2-CH), 52.55 (CH_2-Ph), 62.61–62.77 ($2CH_2$-CH_3), 101.11 (CH-CH_2), 127.52 (C5), 128–139 (Ph), 145.25 (C6), 150.83 (C4), 163.96 (C2). MS (ES+), $m/z = 382$ $[M+H]^+$. HRMS Calcd for $C_{17}H_{24}N_3O_5P$ $[M+H]^+$: 382.3633 found 382.3640.

N^1 (2-benzylamino-2-diethoxyphosphinyl-ethan-1-yl) thymine ***6b***

Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.20 (t, 3H, $J = 7.03$ Hz, CH_3), 1.30 (t, 3H, $J = 7.03$ Hz, CH_3), 1.80 (s, 3H, CH_3), 3.10 (m, 1H, Ha) , 3.50 (m, 1H, Hb), 3.80–3.90 (m, 2H, N1-CH_2-CH), 4.00–4.10 (m, 4 H, 2 OCH_2-CH_3), 4.10 (m, 1H, CH-CH_2), 4.25 (s, 1H, NH-Ph), 7.05 (s, 1H, H6), 7.20 (m, 5H, Ph), 9.80 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 12.19 (CH_3); 16.44 ($2CH_3$), 48.02 (CH_2-CH), 52.31 (CH_2-Ph), 62.57 (CH_2-CH_3), 62.77 (CH_2-CH_3), 109 (CH-CH_2), 127 (C5), 141.9 (C6), 128–139 (Ph), 151.1 (C4), 164.64 (C2). MS (ES+), $m/z = 396$ $[M+H]^+$. HRMS Calcd for $C_{18}H_{26}N_3O_5P$ $[M+H]^+$: 396.3899 found 396.3906.

N^1 (2-benzylamino-2-diethoxyphosphinyl-ethan-1-yl) azauracil ***6c***

Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.30 (t, 3H, $J = 7.02$ Hz, CH_3), 1.40 (t, 3H, $J = 7.02$ Hz, CH_3), 3.40 (m, 1H, Ha), 3.80 (m, 1H, Hb), 3.90–4.10 (m, 2H, N1-CH_2-CH), 4.30 (m, 4H, 2 OCH_2-CH_3), 4.50 (m, 1H, CH-CH_2), 4.55 (s, 1H, NH-CH_2-Ph), 7.20 (s, 1H, H5), 7.40 (m, 5H, Ph), 10.60 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 16.60 ($2CH_3$), 39.80 (CH_2-CH), 51.77 (CH_2-Ph), 62.51 (CH_2-CH_3), 62.72 (CH_2-CH_3), 127.23 (CH-CH_2), 135.14 (C5), 128–135 (Ph), 149.48 (C4), 156.26 (C2). MS (ES+), $m/z = 383$ $[M+H]^+$. HRMS Calcd for $C_{16}H_{23}N_4O_5P$ $[M+H]^+$: 383.3513 found 383.3506.

N^9 (2-benzylamino-2-diethoxyphosphinyl-ethan-1-yl) adenine ***6d***

Yield: 62%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.20 (t, 3H, $J = 7.06$ Hz, CH_3), 1.30 (t, 3H, $J = 7.06$ Hz, CH_3), 3.30 (m, 1H, Ha), 3.90 (m, 1H, Hb), 3.70–3.90 (m, 2H, N9-CH_2-CH), 4.00 (m, 1H, CH-CH_2), 4.20 (m, 4H, 2 OCH_2-CH_3), 4.25 (s, 1H, NH-CH_2-Ph), 5.90 (s, 2H, NH_2), 7.30 (m, 5H, Ph), 7.90 (s, 1H, H2), 8.35 (s, 1H, H8).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 16.44 ($2CH_3$), 44.11 (CH_2-CH), 62.74–62.83 ($2CH_2$-CH_3), 119.26 (CH-CH_2), 52.18 (CH_2-Ph), 127.19 (C5), 128–138 (Ph), 141.86 (C6), 150.16 (C4), 152.75 (C2), 155.28 (C8). MS (ES+), $m/z = 405$ $[M+H]^+$. HRMS Calcd for $C_{18}H_{25}N_6O_3P$ $[M+H]^+$: 405.4032 found 405.4040.

N^1 (2-butylamino-2-diethoxyphosphinyl-éthan-1-yl) uracil ***7a***

Yield: 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.80 (t, 3H, CH_3-CH_2), 1.20 (m, 6H, 2CH_3-CH_2), 1.30 (q, 2H, CH_2-CH_3), 1.40 (m, 2H, CH_2-CH_2-CH_3), 2.60–2.80 (m, 2H, NH-CH_2), 3.00–3.60 (m, 2H, Ha and Hb), 4.10 (m, 1H, CH-CH_2), 4.25 (m, 4H, 2 OCH_2-CH_3) , 4.30 (m, 1H, N-H), 5.60 (d, 1H, $J = 7.87$ Hz, H5), 7.30 (d, 1H, $J = 7.87$ Hz, H6), 9.00 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.87 (CH_3-CH_2), 16.45 (CH_3), 16.50 (CH_3), 20.08 (CH_2-CH_3), 28.92 (CH_2-CH_2-CH_3), 48.82 (CH2-CH_2-CH_2-CH_3), 49.84 (CH_2-CH), 62.47–62.88 (2CH_2-CH_3), 100.92 (CH-CH_2), 128.81 (C5), 146.48 (C6), 150.92 (C4), 163.91 (C2). MS (ES+), $m/z = 348$ $[M + H]^+$. HRMS Calcd for $C_{14}H_{26}N_3O_5P$ $[M + H]^+$: 348.3471 found 348.3480.

N^1 (2-butylamino-2-diethoxyphosphinyl-ethan-1-yl) thymine ***7b***

Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.80 (t, 3H, CH_3-CH_2), 1.30 (t, 6H, 2 CH_3-CH_2), 1.80 (s, 3H, CH_3), 1.35 (m, 2H, CH_2-CH_3), 1.40 (m, 2H, CH_2-CH_2-CH_3), 2.90 (m, 2H, NH-CH_2), 3.10 (m, 2H, Ha and Hb), 3.90–4.10 (m, 6H, 2 OCH_2-CH_3, CH-CH_2 and N-H), 7.20 (s, 1H, H6), 8.70 (s, 1H, N3-H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 12.19 (CH_3), 13.87 (CH_3-CH_2), 16.45 (CH_3), 16.50 (CH_3), 20.08 (CH_2-CH_3), 28.92 (CH_2-CH_2-CH_3), 48.82 (CH_2CH_2-CH_2-CH_3), 49.84 (CH_2-CH), 62.47–62.88 (2CH_2-CH_3), 100.92 (CH-CH_2), 128.81 (C5), 146.48 (C6), 150.92 (C4), 163.91 (C2). MS (ES+), $m/z = 362$ $[M + H]^+$. HRMS Calcd for $C_{15}H_{28}N_3O_5P$ $[M + H]^+$: 362.3736 found 362.3742.

N^1 (2-butylamino-2-diethoxyphosphinyl-ethan-1-yl) azauracil ***7c***

Yield: 47%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (t, 3H, CH_3), 1.20 (m, 6H, 2CH_3-CH_2), 1.30 (q, 2H, CH_2-CH_3), 1.40 (m, 2H, CH_2-CH_2-CH_3), 2.50–2.80 (m, 2H, NH-CH_2), 3.40 (m, 2H, Ha and Hb), 4.00–4.30 (m, 6H, 2 OCH_2, CH-CH_2, N-H), 7.30 (s, 1H, H5), 10.60 (s, 1H, N3-H) .^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.87 (CH_3-CH_2), 16.49–16.54 (2CH_3), 48 (CH_2-CH), 20.01 (CH_2-CH_3), 32.35 (CH_2-CH_2-CH_3), 39.69 (CH_2-CH_2-CH_2-CH_3), 62.22 (CH_2-CH_3), 62.67 (CH_2-CH_3), 100 (CH-CH_2), 135.06 (C5), 149.51 (C4), 156.42 (C2) . MS (ES+), $m/z = 349$ $[M + H]^+$. HRMS Calcd for $C_{13}H_{25}N_4O_5P$ $[M + H]^+$: 349.3351 found 349.3360.

N^9 (2-butylamino-2-diethoxyphosphinyl-ethan-1-yl) adenine ***7d***

Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.80 (t, 3H, CH_3), 1.20 (m, 6H, 2 CH_3-CH_2), 1.20–1.40 (q, 2H, CH_2-CH_3), 1.40–1.50 (m, 2H, CH_2-CH_2-CH_3), 2.50–2.70 (m, 2H, NH-CH_2), 3.10 (m, 2H, Ha and Hb), 4.10 (m, 4H, 2OCH_2-CH_3), 4.25 (m, 1H, N-H), 4.30 (m, 1H, CH-CH_2), 6.10 (s, 2H, NH_2), 8.10 (s, 1H, H2), 8.30 (s, 1H, H8).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 13.53 (CH_3-CH_2), 16.44 (2CH_3), 20.04 (CH_2-CH_3), 29.35 (CH_2-CH_2-CH_3), 40.02 (CH_2-CH2-CH_2-CH_3)44.17 (CH_2-CH), 63.02 (2CH_2-CH_3), 118.99 (CH-CH_2), 120 (C5), 142.31 (C6), 150.08 (C4), 152.7 (C2),

155.2 (C8). MS (ES+), $m/z = 371$ $[M + H]^+$. HRMS Calcd for $C_{15}H_{27}N_6O_3P$ $[M + H]^+$: 371.3870 found 371.3865.

N^1 (2-glycinoethylester-2-diethoxyphosphinyl-ethan-1-yl) uracil ***8a***

Yield: 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.13 (t, 3H, $J = 7.13$ Hz, CH_3), 1.30 (t, 6H, $J = 7.06$ Hz, $2CH_3$), 3.25 (t, 1H, $J = 4.6$ Hz, CH), 3.50–3.60 (m, 2H, CH_2), 3.70 (d, 2H, $J = 4.6$ Hz, CH_2), 4.10 (m, 7H, $3CH_2$, NH), 5.60 (d, 1H, $J = 7.9$ Hz, H-5), 7.20 (d, 1H, $J = 7.9$ Hz, H-6), 9.55 (sd, 1H, NH).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.15 (CH_3), 16.45 (CH_3), 16.49 (CH_3), 49.40 (CH), 52.98 (CH_2), 54.45 (CH_2), 60.90 (CH_2), 62.77 (CH_2), 62.73 (CH_2), 101.39 (C5), 146.06 (C6), 151.35 (C4), 164.11 (C2), 173.63 (CO, ester). MS (ES+), $m/z = 378$ $[M + H]^+$. HRMS Calcd for $C_{14}H_{24}N_3O_7P$ $[M + H]^+$: 378.3300 found 378.3310.

N^1 (2-glycinoethylester-2-diethoxyphosphinyl-ethan-1-yl) thymine ***8b***

Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.15 (t, 3H, $J = 7.15$ Hz, CH_3), 1.30 (t, 6H, $J = 7.05$ Hz, $2CH_3$), 1.80 (s, 3H, CH_3), 3.25 (t, 1H, $J = 4.5$ Hz, CH), 3.50–3.60 (m, 2H, CH_2), 3.70 (d, 2H, $J = 4.5$ Hz, CH_2), 4.10 (m, 7H, $3CH_2$, NH), 7.10 (s, 1H, H-6), 9.20 (sd, 1H, NH).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.15 (CH_3), 16.45 (CH_3), 16.67 (CH_3), 44.70 (CH_2), 44.80 (CH_2), 54.46 (CH_2), 56.48 (CH_2), 61.81 (CH_2), 64.27 (CH), 119.82 (C5), 143.71 (C6), 151.28 (C4), 153.63 (C2), 157.11 (C8), 173.19 (CO, ester). MS (ES+), $m/z = 392$ $[M + H]^+$. HRMS Calcd for $C_{15}H_26N_3O_7P$ $[M + H]^+$: 392.3566 found 392.3574.

N^1 (2-glycinoethylester-2-diethoxyphosphinyl-ethan-1-yl)-6-azauracil ***8c***

Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.15 (t, 3H, $J = 7.13$ Hz, CH_3), 1.30 (t, 6H, $J = 7.06$ Hz, $2CH_3$), 3.25 (t, 1H, $J = 3.6$ Hz, CH), 3.50–3.60 (dd, 2H, $J = 3.6$ Hz, CH_2), 4.00–4.20 (m, 9H, $4CH_2$, NH), 7.20 (s, 1H, H-5), 10.50 (sd, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.15 (CH_3), 16.53 (CH_3), 16.48 (CH_3), 39.50 (CH), 49.01 (CH_2), 52.02 (CH_2), 54.07 (CH_2), 60.72 (CH_2), 62.70 (CH_2), 135.00 (C5), 150.09 (C4), 156.97 (C2), 172.65 (CO, ester). MS (ES+), $m/z = 379$ $[M + H]^+$. HRMS Calcd for $C_{13}H_{23}N_4O_7P$ $[M + H]^+$: 379.3181 found 379.3175.

N^9 (2-glycinoethylester-2-diethoxyphosphinyl-ethan-1-yl) adenine ***8d***

Yield: 55%; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 1.20 (t, 3H, $J = 7.15$ Hz, CH_3), 1.30 (t, 6H, $J = 7.05$ Hz, $2CH_3$), 3.20 (t, 1H, $J = 4.5$ Hz, CH), 3.30 (d, 2H, $J = 4.5$ Hz, CH_2), 3.70 (m, 2H, CH_2), 4.00 (q, 2H, $J = 7.15$ Hz, CH_2), 4.35 (q, 4H, $J = 7.05$ Hz, $2CH_2$), 8.20 (s, 2H, H-2, H-8).^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 9.31 (CH_3), 14.46 (CH_3), 16.67 (CH_3), 44.70 (CH_2), 44.80 (CH_2), 54.46 (CH_2), 56.48 (CH_2), 61.81 (CH_2), 64.27 (CH), 119.82 (C5), 143.71 (C6), 151.28 (C4), 153.63 (C2), 157.11 (C8), 173.19 (CO, ester). MS (ES+), $m/z = 401$ $[M + H]^+$. HRMS Calcd for $C_{15}H_{25}N_6O_5P$ $[M + H]^+$: 401.3700 found 401.3710.

N^1 (2-alaninomethylester-2-diethoxyphosphinyl-ethan-1-yl) uracil ***9a***

Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.11 (d, 3H, $J = 7.13$ Hz, CH_3), 1.16 (m, 9H, $3CH_3$), 3.25 (t, 1H, $J = 4.6$ Hz, CH), 3.50–3.60 (q, 1H, $J = 7.13$ Hz, CH), 3.70 (d, 2H, $J = 4.6$ Hz, CH_2), 4.01 (m, 5H, $2CH_2$, NH), 5.60 (d, 1H, $J = 7.9$ Hz, H-5), 7.20 (d, 1H, $J = 7.9$ Hz, H-6), 9.55 (sd, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 16.42 (CH_3), 18.71 (CH_3), 19.06 (CH_3), 46.41 (CH_2), 49.30 (CH), 50.70 (CH_3), 51.90 (CH), 62.60 (CH_2), 62.70 (CH_2), 101.30 (C5), 146.10 (C6), 151.10 (C4), 164.40 (C2), 174.70 (CO, ester). MS (ES+), $m/z = 392[M + H]^+$. HRMS Calcd for $C_{15}H_{26}N_3O_7P$ $[M + H]^+$ 392.3566 found 392.3572.

N^1 (2-alaninomethylester-2-diethoxyphosphinyl-ethan-1-yl) thymine ***9b***

Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.00 (d, 3H, $J = 7.10$ Hz, CH_3), 1.30 (m, 12H, $4CH_3$) 3.25 (t, 1H, $J = 4.60$ Hz, CH), 3.50–3.60 (q, 1H, $J = 7.10$ Hz, CH), 3.70 (d, 2H, $J = 4.6$ Hz, CH_2), 4.01 (m, 5H, $2CH_2$, NH), 7.10 (s, 1H, H-6), 9.60 (sd, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)11.00 (CH_3), 16.60 (CH_3), 18.80 (CH_3), 19.20 (CH_3), 49.10 (CH_2), 49.60 (CH), 50.90 (CH_3), 52.09 (CH), 55.30 (CH_2), 62.70 (CH_2), 109.20 (C5), 141.90 (C6), 151.30 (C4), 164.90 (C2), 174.80 (CO, ester). MS (ES+), $m/z = 406[M + H]^+$. HRMS Calcd for $C_{16}H_{28}N_3O_7P$ $[M + H]^+$: 406.3832 found 406.3842.

N^1 (2-alaninomethylester-2-diethoxyphosphinyl-ethan-1-yl)-6-azauracil ***9c***

Yield: 52%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.00 (d, 3H, $J = 7.10$ Hz, CH_3), 1.30 (m, 9H, $3CH_3$) 3.25 (t, 1H, $J = 4.6$Hz, CH), 3.50–3.60 (q, 1H, $J = 7.10$Hz, CH), 3.70 (d, 2H, $J = 4.60$Hz, CH_2), 4.01 (m, 5H, $2CH_2$, NH), 7.20 (s, H-5), 9.60 (sd, 1H, NH).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)16.42 (CH_3), 19.06 (CH_3), 19.32 (CH_3), 46.00 (CH_2), 50.50 (CH), 51.80 (CH_3), 54.13 (CH), 62.60 (CH_2), 63.70 (CH_2), 135.00 (C5), 150.20 (C4), 157.10 (C2), 176.50 (CO, ester). MS (ES+), $m/z = 393[M + H]^+$. HRMS Calcd for $C_{14}H_{25}N_4O_7P$ $[M + H]^+$: 393.3447 found 393.3440.

N^9 (2-alaninomethylester-2-diethoxyphosphinyl-ethan-1-yl) adenine ***9d***

Yield: 55%; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 1.00 (d, 3H, $J = 7.13$ Hz, CH_3), 1.40–1.60 (m, 9H, $3CH_3$), 3.10 (t, 1H, $J = 4.5$ Hz, CH), 3.50–3.70 (q, 1H, $J = 7.13$ Hz, CH), 4.01 (d, 2H, $J = 4.50$ Hz, CH_2), 4.35 (m, 5H, $2CH_2$, NH), 8.20 (s, 2H, H-2, H-8). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm)10.00 (CH_3), 16.74 (CH_3), 19.18 (CH_3), 45.01 (CH_2), 48.00 (CH_2), 52.23 (CH_3), 53.07 (CH), 56.17 (CH) 119.82 (C5), 143.75 (C6), 151.08 (C4), 153.70 (C2), 157.17 (C8), 176.24 (CO, ester). MS (ES+), $m/z = 415[M + H]^+$. HRMS Calcd for $C_{16}H_{27}N_6O_5P$ $[M + H]^+$. 415.3965 found 415.3975.

7. Deprotection of α-amino and α-amino acid phosphonate acyclonucleosides

7.1. General procedure

To a suspension of α-aminophosphonate acyclonucleosides **5–9** (0.15 mmol) in acetonitrile (5 mL) was added TMSBr (10 eq), and the resulting mixture was stirred overnight at room temperature. After evaporation of the solvent, water (5 mL) was added and the resulting suspension was neutralized with an 28% ammonia solution. The water was then removed in vacuo and the residue was purified with on preparative plate (eluent: isopropanol/NH_4OH/H_2O) and reverse-phase HPLC (C18). The final product was obtained after a cation exchange chromatography on a Na^+ DOWEX50WX2. The residue obtained is dried, the purity of products was controlled by HPLC (eluent: CH_3CN/H_2O, 50/50 v/v).

N^1 (2-anilino-2-dihydroxyphosphinyl-ethan-1-yl) uracil ***10a***

Yield: 64%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.60 (m, 1H, Ha), 3.85 (m, 1H, Hb), 4.35 (m, 1H, CH-CH_2), 5.50 (d, 1H, $J = 7.87$ Hz, H5), 6.50–7.00 (m, 5H, Ph), 7.45 (d, 1H, $J = 7.87$ Hz, H6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 51.6 (CH_2-CH), 100.69 (CH-CH_2), 112.48 (C5), 129–144 (Ph), 148.23 (C6), 152.73 (C4), 167.24 (C2). MS (ES+), $m/z = 312$ $[M + H]^+$. HRMS Calcd for $C_{12}H_{14}N_3O_5P$ $[M + H]^+$: 312.2304 found 312.2312.

N^1 (2-anilino-2-dihydroxyphosphinyl-ethan-1-yl) thymine ***10b***

Yield: 60%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.50 (s, 3H, CH_3), 3.70 (m, 1H, Ha), 4.20 (m, 1H, Hb), 4.35 (m, 1H, CH-CH_2), 6.50–7.00 (m, 5H, Ph), 7.30 (s, 1H, H6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 12.11 (CH_3), 51.53 (CH_2-CH), 110.01 (CH-CH_2), 113.42 (C5), 129.32–141.00 (Ph), 143.95 (C6), 152 (C4), 164.56 (C2). MS (ES+), $m/z = 326$ $[M + H]^+$. HRMS Calcd for $C_{13}H_{16}N_3O_5P$ $[M + H]^+$: 326.2570 found 326.2580.

N^1 (2-anilino-2-dihydroxyphosphinyl-ethan-1-yl) azauracil ***10c***

Yield: 50%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 4.05 (m, 1H, CH-CH_2), 4.20 (m, 1H, Ha), 4.40 (m, 1H, Hb), 7.15 (s, 1H, H5), 6.50–7.00 (m, 5H, Ph).^{13}C NMR (100 MHz, D_2O) δ (ppm) 42.24 (CH_2-CH), 113.17 (CH-CH_2), 129.39–141 (Ph), 135.05 (C5), 150.42 (C4), 158.24 (C2). MS (ES+), $m/z = 313$ $[M + H]^+$. HRMS Calcd for $C_{11}H_{13}N_4O_5P$ $[M + H]^+$: 313.2184 found 313.2178.

N^9 (2-anilino-2-dihydroxyphosphinyl-ethan-1-yl) adenine ***10d***

Yield: 70%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 4.35 (m, 1H, Ha), 4.50 (m, 1H, Hb), 4.90 (m, 1H, CH-CH_2), 6.30–7.10 (m, 5H, Ph), 7.75 (s, 1H, H2), 8.35 (s, 1H, H8). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 46.48 (CH_2-CH), 112.49 (CH-CH_2), 117.30 (C5), 128.40–144 (Ph), 142.88 (C6), 149.06 (C4), 151.79 (C2), 154.92 (C8). MS (ES+), $m/z = 363$ $[M + H]^+$. HRMS Calcd for $C_{13}H_{15}N_8O_3P$ $[M + H]^+$. 363.2837 found 363.2845.

N^1 (2-benzylamino-2-dihydroxyphosphinyl-ethan-1-yl) uracil ***11a***

Yield: 60%; ^{1}H NMR (400 MHz, D_2O), δ (ppm) 3.20 (m, 1H, Ha), 3.90 (m, 1H, Hb), 4.00 (m, 2H, N1-CH_2-CH), 4.30 (m, 1H, CH-CH_2), 5.60 (d, 1H, $J = 7.87$, H5), 7.30 (m, 5H, Ph), 7.45 (d, 1H, $J = 7.87$, H6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 48.81 (CH_2-CH), 51.60 (CH_2-Ph), 102.12 (CH-CH_2), 129.29 (C5), 129–131.11 (Ph), 146.84 (C6), 153 (C4), 166.45 (C2). MS (ES+), $m/z = 326$ $[M + H]^+$. HRMS Calcd for $C_{13}H_{16}N_3O_5P$ $[M + H]^+$: 326.25.70 found 326.2564.

N^1 (2-benzylamino-2-dihydroxyphosphinyl-ethan-1-yl) thymine ***11b***

Yield: 35%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.70 (s, 3H, CH_3), 3.30 (m, 1H, Ha), 3.90 (m, 1H, Hb), 4.10–4.30 (m, 2H, N1-CH_2-CH), 4.50 (m, 1H, CH-CH_2), 7.40 (m, 5H, Ph), 7.20 (s, 1H, H6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 11.19 (CH_3), 48.50 (CH_2-CH), 111.39 (CH-CH_2), 127 (C5), 129–130 (Ph), 142.22 (C6), 153.14 (C4), 166.64 (C2). MS (ES+), $m/z = 340$ $[M + H]^+$. HRMS Calcd for $C_{14}H_{18}N_3O_5P$ $[M + H]^+$: 340.2835 found 340.2841.

N^1 (2-benzylamino-2-dihydroxyphosphinyl-ethan-1-yl) azauracil ***11c***

Yield: 35%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.40 (m, 1H, Ha), 4.10 (m, 1H, Hb), 4.20 (m, 2H, N1-CH_2-CH), 4.50 (m, 1H, CH-CH_2), 7.30 (s, 1H, H5), 7.40 (m, 5H, Ph). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 39.63 (CH_2-CH), 50.13 (CH_2-Ph), 128.21 (CH-CH_2), 129–131 (Ph), 135.51 (C5), 150.59 (C4), 157.93 (C2). MS (ES+), $m/z = 327$ $[M + H]^+$. HRMS Calcd for $C_{12}H_{15}N_4O_5P$ $[M + H]^+$. 327.2450 found 327.2457.

N^9 (2-benzylamino-2-dihydroxyphosphinyl-éthan-1-yl) adenine ***11d***

Yield: 24%; ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.30 (m, 1H, Ha), 3.90 (m, 1H, Hb), 4.00–4.30 (m, 2H, N9-CH_2-CH), 4.60 (m, 1H, CH-CH_2), 7.00 (m, 5H, Ph), 7.90 (s, 1H, H2), 7.95 (s, 1H, H8). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 44.11 (CH_2-CH), 52.18 (CH_2-Ph), 119.26 (CH-CH_2), 127.15 (C5), 128–130 (Ph), 141.86 (C6), 150.16 (C4), 152.75 (C2), 155.28 (C8). MS (ES+), $m/z = 349$ $[M + H]^+$. HRMS Calcd for $C_{14}H_{17}N_6O_3P$ $[M + H]^+$: 349.2969 found 349.2977.

N^1 (2-butylamino-2-dihydroxyphosphinyl-éthan-1-yl) uracil ***12a***

Yield: 25%; ^{1}H NMR (400 MHz, D_2O), δ (ppm) 0.80 (t, 3H, CH_3-CH_2), 1.20 (q, 2H, CH_2-CH_3), 1.50 (m, 2H, CH_2-CH_2-CH_3), 3.10 (m, 2H, CH_2-$(CH_2)_2$-CH_3), 3.40–4.10 (m, 2H, N1-CH_2), 4.30 (m, 1H, CH-CH_2), 5.60 (d, 1H, $J = 7.87$ Hz, H5), 7.30 (d, 1H, $J = 7.87$ Hz, H6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 12.74 (CH_3-CH_2), 19.04 (CH_2-CH_3), 28.03 (CH_2-CH_2-CH_3), 46.42 (CH_2-CH_2-CH_2-CH_3), 47.90 (CH_2-CH), 102.05 (CH-CH_2), 128.81 (C5), 147.21 (C6), 153.21 (C4), 166.72 (C2). MS (ES+), $m/z = 292$ $[M + H]^+$. HRMS Calcd for $C_{10}H_{18}N_3O_5P$ $[M + H]^+$: 292.2407 found 292.2415.

N^1 (2-butylamino-2-dihydroxyphosphinyl-éthan-1-yl) azauracil ***12c***

Yield: 20%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 0.80 (t, 3H, CH_3), 1.20 (q, 2H, CH_2-CH_3), 1.50 (m, 2H, CH_2-CH_2-CH_3), 3.10 (m, 2H, CH_2-

$(CH_2)_2$-CH_3), 3.70 (m, 1H, CH-CH_2), 4.30 (m, 2H, N1-CH_2) , 7.50 (s, 1H, H5). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 12.75 (CH_3-CH_2), 19.04 (CH_2-CH_3), 28.03 (CH_2-CH_2-CH_3), 46.42 (CH_2-CH_2-CH_2-CH_3), 47.90 (CH_2-CH), 100 ($CHCH_2$), 135.06 (C5), 147.21 (C4); 153.21 (C2). MS (ES+), *m/z* = 293 $[M+H]^+$. HRMS Calcd for $C_9H_{17}N_4O_5P$ $[M+H]^+$ 293.2288 found 293.2295.

N^1 (2-glycinoethylester-2-dihydroxyphosphinyl-ethan-1-yl) uracil ***13a***

Yield: 40%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.15 (t, 3H, *J* = 7.10 Hz, CH_3), 3.25 (t, 1H, *J* = 3.50 Hz, CH), 3.70 (m, 2H, CH_2), 3.80 (d, 2H, *J* = 3.5 Hz, CH_2), 4.10 (q, 4H, *J* = 7.10 Hz, 2CH_2), 5.70 (d, 2H, *J* = 7.9 Hz, 1H, H-5), 7.60 (d, 2H, *J* = 7.90 Hz, 1H, H-6). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 16.45 (CH_3), 49.40 (CH), 54.79 (CH_2), 56.50 (CH_2), 57.39 (CH_2), 101.37 (C5), 147.18 (C6), 152.20 (C4), 166.67 (C2), 171.42 (CO, ester). MS (ES+), *m/z* = 322 $[M+H]^+$. HRMS Calcd for $C_{10}H_{16}N_3O_7P$ $[M+H]^+$: 322.2237 found 322.2246.

N^1 (2-glycinoethylester-2-dihydroxyphosphinyl-ethan-1-yl) thymine ***13b***

Yield: 20%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.15 (t, 3H, *J* = 7.10 Hz, CH_3), 1.70 (s, 3H, CH_3), 3.25 (t, 1H, *J* = 3.50 Hz, CH), 3.70 (m, 2H, CH_2), 3.80 (d, 1H, *J* = 3.25 Hz, CH_2), 4.10 (q, 4H, *J* = 7.10 Hz, 2CH_2), 7.60 (s, 1H, H-6).^{13}C NMR (100 MHz, D_2O) δ (ppm) 13.20 (CH_3), 16.45 (CH_3), 49.40 (CH), 54.79 (CH_2), 56.50 (CH_2), 57.39 (CH_2), 101.37 (C5), 147.18 (C6), 152.20 (C4), 166.67 (C2), 171.42 (CO, ester). MS (ES+), *m/z* = 336 $[M+H]^+$. HRMS Calcd for $C_{11}H_{18}N_3O_7P$ $[M+H]^+$: 336.2502 found 336.2512.

N^1 (2-glycinoethylester-2-dihydroxyphosphinyl-ethan-1-yl) azauracil ***13c***

Yield: 20%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.15 (t, 3H, *J* = 7.10 Hz, CH_3), 3.25 (t, 1H, *J* = 3.5 Hz, CH), 3.70 (m, 2H, CH_2), 3.80 (d, 2H, *J* = 3.5 Hz, CH_2), 4.10 (q, 4H, *J* = 7.10 Hz, 2CH_2), 7.50 (s, 1H, H-5). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 13.16 (CH_3), 39.50 (CH), 49.01 (CH_2), 52.02 (CH_2), 62.57 (CH_2), 135.55 (C5), 150.77 (C4), 158.23 (C2), 172.65 (CO, ester). MS (ES+), *m/z* = 323$[M+H]^+$. HRMS Calcd for $C_9H_{15}N_4O_7P$ $[M+H]^+$: 323.2117 found 323.212.

N^9 (2-glycinoethylester-2-dihydroxyphosphinyl-ethan-1-yl) adenine ***13d***

Yield: 20%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.00 (t, 3H, *J* = 7.13 Hz, CH_3), 3.20 (t, 1H, *J* = 3.6 Hz, CH), 3.40 (m, 2H, CH_2), 3.70 (d, 2H, *J* = 3.6 Hz, CH_2), 4.20 (q, 2H, *J* = 7.13 Hz, CH_2), 8.10 (s, 2H, H-2, H-8). ^{13}C NMR (100 MHz D_2O) δ (ppm) 12.99 (CH_3), 44.80 (CH_2), 54.46 (CH_2), 56.48 (CH_2), 62.71 (CH), 119.82 (C5), 143.71 (C6), 152.28 (C4), 153.63 (C2), 157.11 (C8), 173.19 (CO, ester). MS (ES+), *m/z* = 345 $[M+H]^+$. HRMS Calcd for $C_{11}H_{17}N_6O_5P$ $[M+H]^+$: 345.2636 found 345.2628.

N^1 (2-alaninometylester-2-dihydroxyphosphinyl-ethan-1-yl) uracil ***14a***

Yield: 35%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.15 (d, 3H, *J* = 7.10 Hz, CH_3), 2.75 (t, 1H, *J* = 4.6 Hz, CH), 3.40 (q, 1H, *J* = 7.10 Hz, CH), 3.60 (s,

3H, CH_3), 4.00 (d, 2H, $J = 4.6$ Hz, CH_2), 5.65 (d, $J = 7.90$ Hz, 1H, H-5), 7.50 (d, $J = 7.90$ Hz, 1H, H-6).^{13}C NMR (100 MHz, D_2O) δ (ppm) 17.84 (CH_3), 48.84 (CH), 52.7 (CH), 55.03 (CH_3), 58.49 (CH_2), 101 (C5), 147.07 (C6), 158.50 (C4), 174.64 (C2), 181.70 (CO, ester). MS (ES+), *m/z* = 326[M + H]$^+$. ^{31}P NMR (75 MHz, D_2O) δ (ppm) 14.93, 16.22. HRMS Calcd for $C_{11}H_{18}N_3O_7P$ [M + H]$^+$: 326.2502 found 326.2509.

N^1 (2-alaninometylester-2-dihydroxyphosphinyl-ethan-1-yl) thymine ***14b***

Yield: 25%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.15 (d, 3H, $J = 7.10$ Hz, CH_3), 1.80 (s, 3H, CH_3) 2.75 (t, 1H, $J = 4.60$ Hz, CH), 3.40 (q, 1H, $J = 7.10$ Hz, CH), 3.60 (s, 3H, CH_3), 4.00 (d, 2H, $J = 4.6$ Hz, CH_2), 7.44 (s, 1H, H-6).^{13}C NMR (100 MHz, D_2O) δ (ppm)13.20 (CH_3), 17.83 (CH_3), 48.84 (CH), 52.62 (CH), 53.54 (CH_3), 58.54 (CH_2), 101.55 (C5), 147.06 (C6), 158.50 (C4), 174.60 (C2), 181.65 (CO, ester). MS (ES+), *m/z* = 350 (M + H+). ^{31}P NMR (75 MHz, D_2O) δ (ppm): 14.09, 16.06. HRMS Calcd for $C_{12}H_{20}N_3O_7P$ [M + H]$^+$: 350.2768 found 350.2774.

N^1 (2-alaninometylester-2-dihydroxyphosphinyl-ethan-1-yl) azauracil ***14c***

Yield: 30%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.19 (d, 3H, $J = 7.13$ Hz, CH_3), 3.30 (t, 1H, $J = 4.4$ Hz, CH), 3.40 (q, 1H, $J = 7.13$ Hz, CH), 3.60 (s, 3H, CH_3), 4.00 (d, 2H, $J = 4.40$ Hz, CH_2), 7.50 (s, 1H, H-5).^{13}C NMR (100 MHz, D_2O) δ (ppm) 13.20 (CH_3), 39.85 (CH), 47.30 (CH), 53.79 (CH_3), 62.63 (CH_2), 135.59 (C5), 150.78 (C4), 158.31 (C2), 181.60 (CO, ester). MS (ES+), *m/z* = 337 (M + H+). ^{31}P NMR (75 MHz, D_2O) δ (ppm) 14.34, 16.25. HRMS Calcd for $C_{10}H_{17}N_4O_7P$ [M + H]$^+$: 337.2383 found 337.2376.

N^9 (2-alaninometylester-2-dihydroxyphosphinyl-ethan-1-yl) adenine ***14d***

Yield: 15%; ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.19 (d, 3H, $J = 7.10$ Hz, CH_3), 3.30 (t, 1H, $J = 4.5$ Hz, CH), 3.40 (q, 1H, $J = 7.10$ Hz, CH), 3.60 (s, 3H, CH_3), 4.00 (d, 2H, $J = 4.50$ Hz, CH_2), 8.00 (s, 2H, H-8, H-2). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 13.20 (CH_3), 39.85 (CH), 47.30 (CH), 53.79 (CH_3), 62.63 (CH_2), 119.82 (C5), 143.71 (C6), 152.28 (C4), 153.63 (C2), 157.11 (C8), 173.19 (CO, ester). MS (ES+), *m/z* = 359 (M + H+). ^{31}P NMR (75 MHz, D_2O) δ (ppm) 15.06, 15.90. HRMS Calcd for $C_{12}H_{19}N_6O_5P$ [M + H]$^+$: 359.2902 found 359.2912.

7.2. Molecular docking

In silico computational docking studies were performed using AutoDock4.2.[54] The X-ray crystallographic structures of HCV NS3 protease and HIV RT were downloaded from the RCSB PDB 1W3C[50] and 2RF2,[51] respectively. The proteins were prepared separately by removing water and co-crystallized ligands bound with the proteins to make receptor free of any ligand before docking. Then, polar hydrogen and Gasteiger

charges were added using the MGL Tools and proteins saved in PDBQT format.[55,56] Ligand **14a** was created separately using Chem Draw Ultra 12.0, energy minimized in Chem3D, torsional bonds of ligand were set flexible and saved in PDBQT format. Next, the receptor was kept rigid, the grid covering all the amino acid residues present inside the active site of proteins was built for 1W3C (grid box size of 40 Å × 46Å × 52 Å with a spacing of 0.375 Å between the grid points and centered at 66.685 (x), 18.626 (y), and 0.736 (z)) and for 2RF2 (grid box size of 50 Å × 40 Å × 56 Åwith a spacing of 0.375 Å between the grid points and centered at 7.845 (x), 13.204 (y), and 15.671 (z)). The best conformers were searched by the Lamarckian genetic algorithm (LGA), the population size was set to 150, and the maximum number of energy evaluation was set to 250,000,00. Finally, the results were analyzed and visualized by the Discovery Studio.

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