Bioorganic & Medicinal Chemistry 17 (2009) 7008–7014

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

δ-Di-carboxybutyl phosphoramidate of 2′-deoxycytidine-5′-monophosphate as substrate for DNA polymerization by HIV-1 reverse transcriptase

Ivan Zlatev [a], Anne Giraut [b], François Morvan [a,*], Piet Herdewijn [b], Jean-Jacques Vasseur [a]

[a] Institut des Biomolécules Max Mousseron, UMR 5247, Université Montpellier 2, CC1704, Place E. Bataillon, 34095 Montpellier Cedex 5, France
[b] Rega Institute for Medical Research, K.U.Leuven, Laboratory of Medicinal Chemistry, Minderbroedersstraat 10, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 18 June 2009
Revised 30 July 2009
Accepted 3 August 2009
Available online 8 August 2009

Keywords:
Polymerase
Modified nucleotide
Nucleotide analog
Phosphoramidate
HIV

ABSTRACT

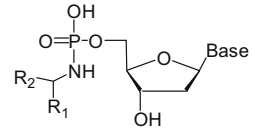
The replacement of the pyrophosphate moiety of 2′-deoxynucleoside triphosphates by non natural δ-dicarboxylic butyl amino acid allows incorporation of natural 2′-deoxycytidine into DNA using HIV-1 reverse transcriptase (RT) as enzyme. In contrast, the 3′-deoxycytidine analogue was not a substrate of the HIV-1 RT.

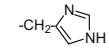
1. Introduction

For the past decades, nucleoside analogues have revealed an outstanding potential in therapeutics as efficient antiviral or anti-metabolic agents. These compounds act like prodrugs, as they need intracellular phosphorylation by kinases before interacting with cellular or viral polymerases. However, the intracellular metabolisation to their nucleoside 5′-triphosphate (NTP) entities is cumbersome and thus a limiting factor for their therapeutic activity. Modified nucleoside 5′-monophosphate/phosphonate analogues (phosphotriesters or phosphoramidates) have been synthesized[1] and can be introduced in the cell, where the removal of the masking group(s) leads to the corresponding free nucleoside 5′-monophosphate/phosphonate (NMP) entities to be transformed by kinases to the target NTPs. Yet, the two remaining steps of kinase phosphorylation remain a problem due to substrate specificity of cellular enzymes.[2,3] Hence, the use of NTP analogues, that would serve as direct substrates/inhibitors to polymerases, bypassing all the kinase activation pathways, would be of great interest.

Herdewijn et al. recently showed that amino acid phosphoramidates of 2′-deoxynucleoside-5′-monophosphates (AA-dNMPs) can serve as substrates for polymerization by the HIV Reverse Transcriptase (RT) or other thermostable cellular polymerases.[4–6] In fact, interestingly, a natural α-L-amino acid moiety, attached to the NMP by a 5′-phosphoramidate linkage is a successful pyrophosphate mimic and can efficiently act as a leaving group in a template-directed, polymerase-catalyzed DNA oligomerization.[4]

In particular, among various AA-dNMPs, only L-Asp-dNMPs (**11**, Fig. 1) and L-His-dNMPs (**12**, Fig. 1) exhibited efficient incorporation by HIV-1 RT, suggesting that Asp and His amino acids posses the required structural and electronic features for efficient coordination with the catalytic metal cations inside the polymerase active site.[5] L-Asp-dNMPs demonstrated by far the most efficient incorporation and their kinetic analysis indicated K_m values much higher than those measured for natural dNTPs, and, however,



	R_1	R_2
11	-COOH	$-CH_2COOH$
12	-COOH	
13	-COOH	$-CH_2CH_2COOH$
14	-H	$-CH_2COOH$
1	-H	$-(CH_2)_2CH(COOH)_2$

Base = C for **1**, A for **11-14**

Figure 1. Structures of amino acid phosphoramidates of nucleoside 5′-monophosphates; used in previous studies (**11**, **12**, **13** and **14**), and in the present study (**1**).

* Corresponding author. Tel.: +33 0 467 144 961; fax: +33 0 467 042 029.
E-mail address: morvan@univ-montp2.fr (F. Morvan).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.08.001

importantly, V_{max} values only 3.1-fold lower.[6] This latter observation indicates efficient nucleophilic displacement of the amino acid moiety, but poor selectivity/affinity of the AA-dNMP analogue for the polymerase active site. Therefore, the design of novel phosphoramidate analogues with higher affinity for the polymerization active site would be of significant interest towards the efficiency improvement of this type of compounds.

Along this line, we present herein the synthesis and the incorporation properties of a novel phosphoramidate conjugate of 2′-deoxycytidine-5′-monophosphate (dCMP), exhibiting a non-natural amino acid moiety, namely the δ-di-carboxylic amino acid moiety (compound **1**, Fig. 1). This non-natural amino acid was designed as a novel pyrophosphate mimic, featuring the closely situated double negative charge, as for aspartic residue in **11**, of its malonate moiety, attached via the relatively long and thus flexible propyl chain. The structure concept was based on the previous findings for AA-dNMP conjugates, assessing the poor HIV-1 RT substrate nature for the Glu analogue[5] (**13**, Fig. 1) and the β-Ala one[11] (**14**, Fig. 1). Hence, the design of a non-natural amino acid side chain taking into account these observations, in the aim to improve polymerase affinity, seemed of particular interest (vide infra for further discussion). On the other hand, the malonate dianion represents good bidentate chelating features, as it is well known for forming six membered chelate rings with metal ions, and was hence chosen in the aim to achieve coordination of a magnesium cation within the polymerization site.

2. Results and discussion

The target δ-dicarboxylic-butyl phosphoramidate 2′-deoxycytidine derivative **1** was prepared using a strategy involving the amidative oxidation of the di-3′-*O*,N^4-benzoylated 2′-deoxycytidin-5′-yl-*H*-phosphonate monoester **4** by CCl_4 in the presence of the appropriate amino diethyl ester derivative **10** (Scheme 1).[7]

First, compound **10** was prepared in a three-step sequence, starting from commercially available 3-bromopropylamine hydrobromide (Scheme 1A). After protection of the amino function with a *tert*-butoxycarbonyl (Boc) group, the di-carboxylic moiety was introduced as its diethyl ester, affording compound **9** after the nucleophilic substitution of the bromine atom in **8** by the in situ generated diethyl malonate carbanion.[8] Deprotection of the Boc group was accomplished by treating **9** with trifluoroacetic acid, affording the desired di-carboxylic aminobutyric acid diethyl ester **10** as its trifluoroacetate salt (Scheme 1A). On the other hand, the conveniently protected 2′-deoxycytidine 5′-*H*-phosphonate (**4**) was prepared from commercially available N^4-benzoyl-2′-deoxy-5′-*O*-dimethoxytritylcytidine, in three steps, including benzoylation of the 2′-hydroxyl group, subsequent detritylation and then introduction of the *H*-phosphonate monoester on the 5′ position of **3** using diphenyl phosphite[9] (Scheme 1B). Compound **4** was then oxidized by CCl_4 in presence of a silylating agent, triethylamine and amine **10**, affording the fully protected diethylester phosphoramidate derivative, which was further deprotected firstly by treatment with aqueous 0.4 M sodium hydroxide and secondly with 28% aqueous ammonia (Scheme 1B). Target dicarboxylic phosphoramidate **1** was obtained after a final purification by chromatography and sodium ion exchange.

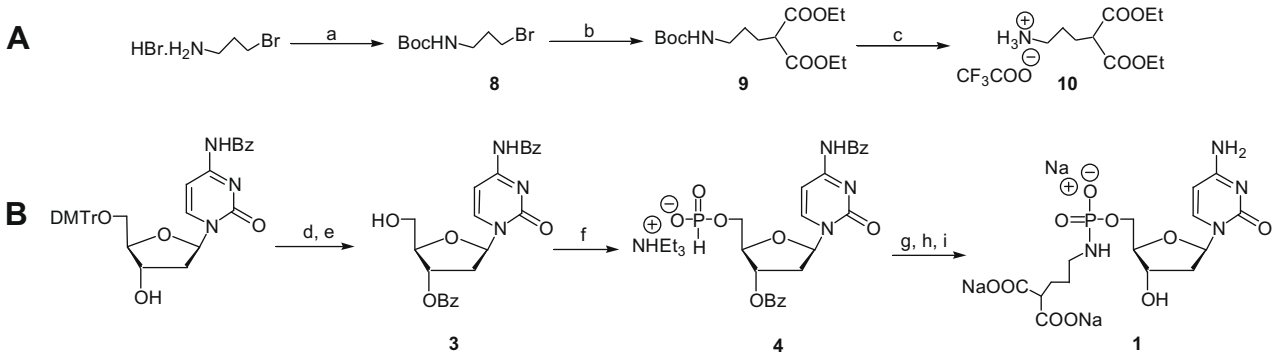
The 3′-deoxy isomer of the title compound **1**, compound **2** (Fig. 2), was synthesized in a similar way (see Supplementary data), in order to be used as a negative control in the polymerase assay, since 2′-hydroxyl nucleotides are not substrates of HIV-1 RT.

3. Single nucleotide incorporation assay

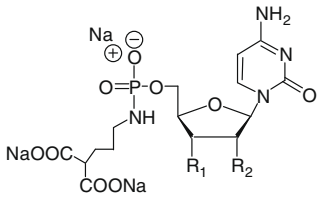
The essential role of the HIV-1 RT in HIV replication renders this viral polymerase the main target enzyme in treating HIV infection. The HIV-1 RT is an error-prone polymerase with a high mutation rate, exhibiting important flexibility and high tolerance towards chemically modified nucleotide substrates.[10] In previous studies,[4–6] it was showed that HIV-1 RT was able to successfully incorporate AA-dNMPs instead of natural dNTPs, during polymerase-catalyzed, template-directed DNA synthesis. Here, interestingly, compound **1**, bearing the newly introduced, non-natural δ-di-carboxylic amino moiety, was smoothly recognized as a substrate by HIV-1 RT, and was efficiently incorporated into the growing DNA primer, resulting in a $(n+1)$ elongation of the primer P affording P+1 (Fig. 3, Panel I), up to 54% in 60 min at 500 μM nucleotide concentration. No significant improvement of the elongation was observed when 1 mM concentration of the nucleotide was used (Fig. 3). As expected, no incorporation was observed when the 3′-deoxyribocytidine phosphoramidate **2** was used in the experiment (Fig. 3, Panel II) demonstrating that the incorporation of **1** was indeed performed by HIV-1 RT.

Compound **1** was also substrate for elongation of P complementary to a three-deoxyguanosine sequence. At 500 μM and 1 mM, 60% of the primer was elongated up to a P+3 product, while the rest remained unreacted showing that three successive non natural phosphoramidite 2′-deoxycytidine **1** could be incorporated (Fig. 4).

When these results were compared to the ones obtained with the incorporation of the L-Asp-dCMP analogue (Fig. 1, 8 **11**, Base = Cytidinyl),[6] a 1.5-fold decrease in the amount of the $(n+1)$ product was observed (54% for **1** vs 82% for **11**, for 60 min of reaction at 500 μM concentration of nucleotide). This demonstrates lesser efficiency for the non-natural AA-dCMP conjugate **1**, sug-



Scheme 1. (A) Synthesis of amino acid **10**. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, NEt_3, 90%; (b) diethyl malonate, sodium, EtOH, reflux, 77%; (c) CF_3COOH, CH_2Cl_2, 98%; (B) Synthesis of amino acid phosphoramidate **1**. Reagents and conditions: (d) benzoyl chloride, pyridine; (e) 10% benzene sulfonic acid, CH_2Cl_2, MeOH, 93%; (f) diphenyl phosphite, pyridine; then H_2O/NEt_3, 90%; (g) *N*,*O*-bis-trimethylsilylacetamide, pyridine, CCl_4, NEt_3, **10**; (h) 0.4 M NaOH, CH_3CN; then DOWEX-50WX8-pyridinium; (i) 28% NH_4OH; then DOWEX-50WX8-sodium, 38%.



	R_1	R_2
1	-OH	-H
2	-H	-OH

Figure 2. Chemical structures of the prepared 4,4-dicarboxybutyl phosphoramidates of 2′-deoxy (**1**) and 3′-deoxy (**2**) cytidine.

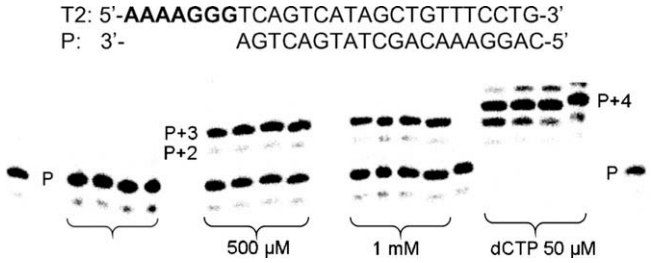


Figure 4. Elongation of P primer hybridized to T2 template with 1 as substrate and HIV-1 RT as enzyme. Conditions: primer P was 5′-labeled with ^{33}P, followed by annealing to template T2. Reactions were carried out with 125 nM primer·template (P·T2), [HIV-1 RT] = 0.025 U μL^{-1}, time points: 30, 60, 90, 120 min. Blank: no nucleotide analogue—500 µM: concentration of **1**—1 mM: concentration of **1**—50 µM concentration of dCTP.

gesting a lower affinity of compound **1** for the polymerase active site and/or less efficient displacement of the non-natural amino acid, acting as leaving group. This could be due to a poorer pyrophosphate mimic character for this non-natural amino acid moiety, compared to the Asp residue. The relatively important distance between the first negative charge borne by the 5′-phosphorus group and the charges of the malonate moiety might be pointed out as a negative feature of this pyrophosphate mimic. However, interestingly, compound **1** was more efficiently incorporated by HIV-1 RT than the Glu phosphoramidate conjugate (**13**, Fig. 1),[5] suggesting that the distance between the phosphoramidate monoester group and the pending carboxylate group(s) is not the sole parameter encountered. Likewise, one might suggest that the flexible propyl chain allows efficient positioning of the dicarboxylic moiety inside the polymerization site and thus a smooth nucleophilic displacement of the leaving group. However, the exact mechanism of nucleotidyl transfer using AA-dNMP conjugates remains unknown. The successful incorporation of compound **1**, implying that the non-natural amino acid moiety acts indeed as a pyrophosphate mimic, replacing the β- and γ-phosphate groups, remains interesting in regards of the flexibility of HIV-1 RT, able to accept modified nucleotide analogues as substrates, and pointing out the possibility of designing new AA-dNMP conjugates, exhibiting a large variety of natural or non-natural amino acid moieties. Another interesting point in this study is the successful incorporation of a phosphoramidate conjugate containing δ-carboxylic amino acid moiety. Indeed, so far, all the phosphoramidates used in the previous studies exhibit an α-carboxy group adjacent to the amino group linked with the 5′ phosphorus atom (Fig. 1). Moreover, the β-Ala dNMP conjugate (**14**, Fig. 1) was recently designed and evaluated by us, without observing any incorporation by the HIV-1 RT, indicating the importance of the carboxy group being fixed at the α-position of the amino acid.[11] Interestingly, the compound **1** presented herein represents an alternative AA-dNMP conjugate lacking the α-carboxy group and successfully incorporated into the DNA chain by the polymerase.

4. Conclusion

In conclusion, a newly designed phosphoramidate conjugate of 2′-deoxycytidine-5′-monophosphate **1**, bearing a non-natural δ-dicarboxybutylamino moiety was prepared. As previously demonstrated for the aspartyl phosphoramidate moiety, the δ-dicarboxylic butyl-amino moiety linked to the phosphorous atom acts as a mimic of a pyrophosphate group and successfully behaves as a leaving group in a nucleotidyl transfer reaction, allowing the extend of a DNA primer when the non-natural AA-dCMP phosphoramidate **1** was used as a substrate for template-directed DNA synthesis, catalyzed by HIV-1 RT and allowed a partial elongation of several non-natural AA-dCMP phosphoramidates **1**. This result should contribute to the development of this new type of NTP analogues, which can find future applications in therapeutics and biotechnology, serving as direct substrates/inhibitors to polymerases, bypassing all the kinase activation pathways.

5. Experimental

5.1. General chemistry procedures

TLC was performed on *Merck* silica coated plates $60F_{254}$ (art. 5554). Compounds were revealed on UV light (254 nm) and after

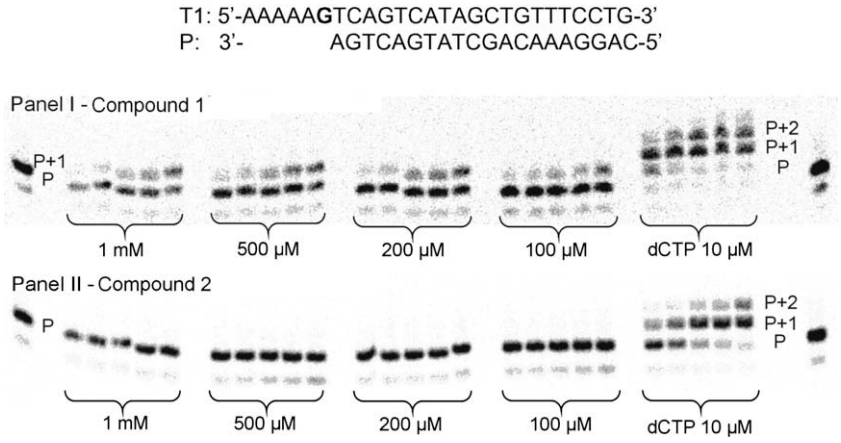


Figure 3. Panel I: incorporation of phosphoramidate **1** by HIV-1 RT. Panel II: incorporation of phosphoramidate **2** by HIV-1 RT. Conditions: primer P was 5′-labeled with ^{33}P, followed by annealing to template T1. Reactions were carried out with 125 nM primer·template (P·T1), [HIV-1 RT] = 0.025 U μL^{-1}, time points: 5, 10, 20, 30 and 60 min.

spraying with 10% sulfuric acid ethanol solution and heating. Phosphorus containing compounds were revealed after spraying with the Hanes molybdate reagent[12] and heating. Amines were revealed after spraying with saturated ninhydrine solution in ethanol (7.0 g/L) and heating. Column chromatography was performed on silica gel *Merck* 60 (art. 9385). Flash chromatography was performed using a *Biotage* SP1 apparatus and *Biotage* silica gel columns. Ion exchange purifications were carried using *Pharmacia* Sephadex A-25 resin (art. 170), *ISCO* TRIS Pump and UA-6 detector (254 nm). Triethylammonium bicarbonate (TEAB) 1 M, pH 7.5 was prepared from triethylamine, milliQ water (18.2 MΩ cm) and CO_2 gas. Ion exchange reactions were performed using DOWEX 50WX8-200 H^+ resin (*Aldrich*), different ion forms were obtained after washing with corresponding 2 M aqueous solutions and then water until neutrality. Analytical HPLC were performed using a Nucleosil C_{18} column, (150 × 4.6 mm, 5 μm) equipped with a prefilter and a precolumn (Nucleosil, C_{18}, 5 μm) on a *Waters* apparatus using a *Waters*-616 Pump and a *Waters* 966 Photodiode Array Detector. Compounds were eluted using a linear gradient 0–80% of acetonitrile in a 50 mM triethylammonium acetate buffer (pH 7), with a 1 mL/min flow rate. All moist-sensitive reactions were carried under anhydrous conditions using dry glassware, anhydrous solvents and Argon atmosphere. Acetonitrile was distilled from CaH_2 and stored over activated 3 Å molecular sieves. Pyridine and triethylamine were distilled from CaH_2. Dichloromethane and carbon tetrachloride were distilled from P_2O_5. Ethanol was distilled from sodium. All commercially available reagents were used as received. 3 Å molecular sieves were dried in a 300 °C oven during 3 h prior to use. ESI and ESI-HRMS spectra were recorded on a Q-Tof *Micromass* spectrometer. ^1H and ^{13}C NMR spectra were recorded at room temperature on *Brüker* spectrometers at 200 or 300 MHz (^1H) and 75 or 100 MHz (^{13}C). Chemical shifts are given in ppm referenced to the solvent residual peak ($CDCl_3$—7.27 and 77.0 ppm; DMSO-d_6—2.49 and 39.5 ppm; D_2O—4.79 ppm + drop of CH_3OH at 49.5 ppm as internal reference).[13] Coupling constants are given in hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), broad signal (br), doublet of doublet (dd), doublet of triplet (dt), doublet of quartet (dq), pseudo (p), multiplet (m). Signal assignments were based on 2D homo-$^1H/^1H$, NOE or heteronuclear-$^1H/^{13}C$ correlations and D_2O exchange. ^{31}P NMR spectra were recorded at 121 MHz on proton-decoupled mode and on proton-coupled mode for *H*-Phosphonates. Chemical shifts are given in ppm referenced to external H_3PO_4-80%; coupling constants are given in hertz. UV spectra are recorded on a *Varian* Cary 300 Bio UV/Vis spectrometer.

5.1.1. Synthesis of *N*-(*tert*-butoxycarbonyl)-3-bromopropylamine (8)

To a solution of 3-bromopropylamine hydrobromide (1.1 mg, 5 mmol, 1 equiv) in 30 mL of anhydrous DCM, were added: 2 g of Boc_2O (10 mmol, 2 equiv), in solution in 20 mL of anhydrous DCM, and 0.8 mL of anhydrous triethylamine. The mixture was stirred at room temperature for 1 h, then diluted with 20 mL of DCM, and washed twice with saturated bicarbonate solution, then once with brine. The organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography (gradient: 100% of cyclohexane to 15% of AcOEt), affording pure **8** as a colorless oil (1.08 g, 90%). R_f: 0.35—cyclohexane/AcOEt—8:2 (v/v). ^1H NMR: ($CDCl_3$, 200 MHz) δ 4.70 (1H, s, l, NHBoc), 3.46 (2H, t, CH_2Br, J = 6.5 Hz), 3.31 (2H, q, CH_2NHBoc, J = 6.4 Hz), 2.07 (2H, qn, $-CH_2-$, J = 6.5 Hz), 1.46 (9H, s, CH_3-Boc). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 155.9 (OC(O)NH), 79.4 ($C(CH_3)_3$), 39.0 (CH_2NHBoc), 32.7 ($-CH_2-$), 30.8 (CH_2Br), 28.4 (CH_3-Boc). ESI$^+$ m/z 238, 240 $(M+H)^+$, 182, 184 $(M-t\text{Bu}+H)^+$.

5.1.2. Synthesis of 2-[3-(*tert*-butoxycarbonylamino)propyl]-diethyl malonate (9)

10 mL of freshly distilled ethanol were introduced into a two-necked flask equipped with a water condenser. 270 mg of sodium (12 mmol, 2 equiv) were added and the mixture was stirred for 5 min. 1.9 mL of diethyl malonate (12 mmol, 2 equiv) were then introduced and the mixture was stirred at room temperature until consumption of sodium. 1.5 g of compound **8** (6.2 mmol, 1 equiv), dissolved in 10 mL of freshly distilled ethanol, were then introduced to the reaction mixture over 5 min.[8] The mixture was stirred under reflux for 1 h, cooled to room temperature, diluted with 20 mL of AcOEt and hydrolyzed with 10 mL of water. It was then washed twice with water, and then the aqueous layers were back-extracted with AcOEt. The combined organic layers were dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography (gradient: 100% of cyclohexane to 15% of AcOEt), affording pure **9** as a colorless oil (1.55 g, 77%). R_f: 0.34—cyclohexane/AcOEt—7:3 (v/v). ^1H NMR: ($CDCl_3$, 200 MHz) δ 4.60 (1H, pt, br, NHBoc), 4.22 (4H, q, OCH_2CH_3, J = 7.0 Hz), 3.36 (1H, t, $-CH(COOEt)_2$, J = 7.4 Hz), 3.16 (2H, q, CH_2NHBoc, J = 6.4 Hz), 1.94 (2H, q, $CH_2CH(COOEt)_2$, J = 7.6 Hz), 1.50 (2H, m, $-CH_2-$), 1.46 (9H, s, CH_3-Boc), 1.29 (6H, t, OCH_2CH_3, J = 7.0 Hz). ^{13}C NMR: ($CDCl_3$, 100 MHz) δ 169.3 (COOEt), 155.9 (OC(O)NH), 79.2 ($C(CH_3)_3$), 61.4 (OCH_2CH_3), 51.6 ($CH(COOEt)_2$), 40.0 (CH_2-NHBoc), 28.4 (CH_3-Boc), 27.8 ($-CH_2-$), 25.9 ($CH_2CH(COOEt)_2$), 14.1 (OCH_2CH_3). ESI$^+$ m/z 635 $(2\ M+H)^+$, 318 $(M+H)^+$, 262 $(M-t\text{Bu}+H)^+$.

5.1.3. Synthesis of 2-(3-amino-propyl)-diethyl malonate, TFA salt (10)

Compound **9** (1.4 g, 4.4 mmol, 1 equiv) was dissolved in 40 mL of anhydrous DCM, 10 mL of TFA (30 equiv) were added and the mixture was stirred at room temperature for 10 min. Solvents were then removed under reduced pressure, the residue was coevaporated three times with 95 ethanol and dried on an oil pump. Compound **10** was a yellow oil (1.43 g, 98%). R_f: 0.44—DCM/MeOH/AcOH—75:20:5 (v/v/v). ^1H NMR: ($CDCl_3$, 300 MHz) δ 7.85 (3H, s, br, NH_3^+), 4.20 (4H, q, OCH_2CH_3, J = 7.1 Hz), 3.37 (1H, t, $-CH(COOEt)_2$, J = 7.1 Hz), 3.05 (2H, q, CH_2NH_3), 1.97 (2H, q, systA_2B_2, $CH_2CH(COOEt)_2$, J = 7.3 Hz), 1.76 (2H, m, systA_2B_2, $-CH_2-$), 1.27 (6H, t, OCH_2CH_3, J = 7.1 Hz). ^{13}C NMR: ($CDCl_3$, 75 MHz) δ 169.3 (COOEt), 61.9 (OCH_2CH_3), 51.1 ($CH(COOEt)_2$), 39.5 (CH_2NH_3), 25.3 ($CH_2CH(COOEt)_2$), 24.9 ($-CH_2-$), 13.8 (OCH_2CH_3). ESI$^+$ m/z 435 $(2(M-\text{TFA})+H)^+$, 218 $(M-\text{TFA}+H)^+$.

5.1.4. Synthesis of N^4-acetyl-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine (5)

To a solution of 3′-deoxycytidine[14] (0.54 g, 2.4 mmol, 1 equiv) in anhydrous DMF (12 mL) was added acetic anhydride (0.25 mL, 2.6 mmol, 1.1 equiv). The mixture was stirred under Argon for 24 h.[15] DMF was removed under reduced pressure (oil pump) and the residue was dried by azeotropic distillation with anhydrous pyridine (three times). It was then dissolved in 24 mL of dry pyridine and DMTrCl was added (1 g, 2.9 mmol, 1.2 equiv). Mixture was stirred for 1 h at room temperature. 5 mL of methanol were added; the mixture was reduced to half volume, diluted with dichloromethane and washed with saturated bicarbonate solution. The organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. Crude product was then purified by column chromatography (gradient: DCM 99%, NEt_3 1–5% of MeOH) affording pure **5** as white foam (1.1 g, 80%). R_f: 0.52—DCM/MeOH—9:1 (v/v). ^1H NMR: ($CDCl_3$, 300 MHz) δ 9.06 (1H, s, br, NH_{Ac}), 8.31 (1H, d, H_6, J = 7.4 Hz), 7.33–7.17 (9H, m, H_{DMTr}), 7.12 (1H, d, H_5, J = 7.4 Hz), 6.79–6.76 (4H, m, H_{DMTr}), 5.70 (1H, d, H-1′, J = 0.9 Hz), 4.63 (1H, m, $H_{4'}$), 4.38 (2H, m, br, $OH_{2'}$, $H_{2'}$), 3.73 (6H, 2s, O-CH_3DMTr), 3.73–3.23 (2H, m, $H_{5',5''}$), 2.17 (3H, s, NHC(O)CH_3), 2.10–1.90 (2H, m, $H_{3',3''}$). ^{13}C NMR: ($CDCl_3$, 75 MHz) δ 170.1

(C(O)CH_3), 162.5 (C_4), 158.7, 158.6 (COCH_3DMTr), 156.1 (C_2), 144.7 (C_6), 144.3 (C_{DMTr}), 135.5, 135.4, 130.1, 130.0, 128.1, 128.0, 127.1, 113.3 (CH_{DMTr}, C_{DMTr}), 96.3 (C_5), 95.2 ($C_{1'}$), 86.8 (CAr_3-DMTr), 81.3 ($C_{4'}$), 77.0 ($C_{2'}$), 63.7 ($C_{5'}$), 55.3 (O-CH_3DMTr), 33.1 ($C_{3'}$), 25.0 (C(O)CH_3). FAB$^+$ (GT) *m/z* 572 (M+H)$^+$, 330 (DMTr)$^+$, 154 $(BH_2)^+$; FAB$^-$ (GT) *m/z* 570 (M-H)$^-$, 152 (B)$^-$.

5.1.5. Synthesis of N^4-acetyl-3′-deoxy-2′-*O*-palmitoylcytidine (6)

To a solution of **5** (1.3 g, 2.3 mmol, 1 equiv) dried by azeotropic distillation with anhydrous pyridine, in 10 mL of dry pyridine, was added palmitoyl chloride (3.3 mL, 11.5 mmol, 5 equiv). Mixture was stirred for 2 h at room temperature. 5 mL of methanol were added, and then the reaction mixture was concentrated to half volume, diluted with 30 mL of dichloromethane and washed with concentrated bicarbonate solution. The organic layers were combined, dried over Na_2SO_4 and evaporated to dryness. The resulting residue was coevaporated with dry toluene to remove pyridine, and was then dissolved in 35 mL of CH_2Cl_2/MeOH—7:3 (v/v). Solution was chilled at 0 °C and benzene sulfonic acid (1.1 g, 7 mmol, 3 equiv), dissolved in 11 mL of CH_2Cl_2/MeOH—7:3 (v/v), was added dropwise. Mixture was stirred for 30 min at 0 °C, and then neutralized with 30 mL of saturated bicarbonate solution. After extraction with dichloromethane, the organic layers were washed with $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness. Crude product was then purified by column chromatography (gradient: CH_2Cl_2 100–5% of methanol) affording pure **6** as a yellow gel (0.77 g, 70%). R_f: 0.22—DCM/MeOH—95:5 (v/v). ^{1}H NMR: (DMSO-d_6, 300 MHz, 50 °C) δ 10.77 (1H, s, br, NH_{Ac}), 8.38 (1H, d, H_6, J = 7.5 Hz), 7.18 (1H, d, H_5, J = 7.5 Hz), 5.82 (1H, d, $H_{1'}$, J = 1.2 Hz), 5.27 (1H, pd, $H_{2'}$, J = 5.5 Hz), 5.09 (1H, s, br, $OH_{5'}$), 4.35–4.30 (1H, m, $H_{4'}$), 3.83–3.58 (2H, 2dd, $H_{5',5''}$, ^2J = 12.0 Hz), 2.36 (2H, t, C(O)CH_2–, J = 7.3), 2.22–2.14 (1H, ddd, $H_{3'}$, ^2J = 14.0 Hz, ^3J = 10.3 Hz, ^3J = 5.7 Hz), 2.12 (3H, s, NHC(O)CH_3), 1.95–1.90 (1H, ddd, $H_{3''}$, ^2J = 14.0 Hz, ^3J = 5.5 Hz, ^3J = 1.5 Hz), 1.56 (2H, qn, C(O)CH_2CH_2–, J = 7.1), 1.26 (24H, s, br, 12 × –CH_2–), 0.87 (3H, t, –CH_2CH_3, J = 6.6 Hz). FAB$^+$ (GT) *m/z* 508 (M+H)$^+$.

5.1.6. Synthesis of N^4-acetyl-3′-deoxy-2′-*O*-palmitoylcytidine-5′-yl hydrogenophosphonate, triethylammonium salt (7)

Nucleoside **6** (0.76 g, 1.5 mmol, 1 equiv) was dried by azeotropic distillation with anhydrous pyridine and then dissolved in 7.5 mL of dry pyridine. 2 mL of diphenylphosphite (10.5 mmol, 7 equiv) were added and the mixture was stirred under Argon for 30 min.[9] Reaction was hydrolyzed by addition of 6 mL of water/triethylamine—1:1 (v/v) and stirred for 10 more min. Solvents were removed under reduced pressure, the resulting oil was dissolved in CH_2Cl_2 and washed twice with saturated bicarbonate solution. The organic layers were dried over Na_2SO_4 and evaporated to dryness. Crude product was then purified by column chromatography (gradient: CH_2Cl_2 95%, NEt_3 5–6% of methanol) affording pure **7** as a yellow oil (0.84 g, 84%). R_f: 0.27—DCM/MeOH—9:1 (v/v). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ 12.32 (1H, s, br, NH_{TEAH}), 9.86 (1H, s, br, NH_{Ac}), 8.48 (1H, d, H_6, J = 7.5 Hz), 7.31 (1H, d, H_5, J = 7.5 Hz), 6.86 (1H, d, $^1J_{H-P}$ = 682.2 Hz), 5.91 (1H, s, $H_{1'}$), 5.25 (1H, pd, $H_{2'}$, J = 4.8 Hz), 4.45 (1H, m, $H_{4'}$), 4.31–3.96 (2H, 2dd, $H_{5',5''}$), 3.03 (6H, q, CH_{2TEAH}), 2.35–2.21 (3H, t, C(O)CH_2–, $H_{3'}$), 2.18 (3H, s, NHC(O)CH_3), 1.98–1.90 (1H, m, $H_{3''}$), 1.57 (2H, qn, C(O)CH_2CH_2–, J = 7.5), 1.28 (9H, t, CH_{3TEAH}, J = 7.5 Hz), 1.18 (24H, s, br, 12 × –CH_2–), 0.81 (3H, t, –CH_2CH_3, J = 6.7 Hz). ^{13}C NMR: ($CDCl_3$, 75 MHz) δ 172.4 (CO-Pal), 170.9 (C(O)CH_3), 162.7 (C-4), 155.1 (C_2), 145.3 (C_6), 96.3 (C_5), 91.2 ($C_{1'}$), 80.6 ($C_{4'}$, $^3J_{C-P}$ = 8.0 Hz), 62.7 ($C_{5'}$), 53.4 (C(O)CH_2–), 52.9 (C(O)CH_2CH_2–), 45.6 (CH_{2TEAH}), 34.2 ($C_{3'}$), 31.9, 31.1, 29.7, 29.5, 29.3, 29.2, 29.1, 25.0, 24.8 (–CH_2–), 22.7 (C(O)CH_3), 14.1 (CH_3CH_2–), 8.6 (CH_3-TEA). ^{31}P NMR: ($CDCl_3$, 121 MHz) δ 4.7 (dt, $^1J_{H-P}$ = 621.4 Hz, $^3J_{H-P}$ = 5.9 Hz). FAB$^+$ (GT) *m/z* 673 (M+H)$^+$, 594 (M−TEAH+2H)$^+$, 154 $(BH_2)^+$, 102 (TEAH)$^+$; FAB$^-$ (GT) *m/z* 570 (M−TEAH)$^-$, 152 (B)$^-$. HRMS: FAB$^+$ (GT) *m/z* calcd for $(C_{27}H_{45}N_3O_8P)^+$: 570.2944, found: 570.2953.

5.1.7. Synthesis of *O*-(3′-deoxycytidin-5′-yl)-*N*-[(3-malon-2-yl)-propyl]-phosphoramidate, sodium salt (2)

5.1.7.1. Oxidation. The *H*-phosphonate monoester **7** (35 mg, 0.05 mmol, 1 equiv), was dried by azeotropic distillation with anhydrous pyridine (2×) and vacuum dried under P_2O_5. It was then dissolved in 0.1 mL of anhydrous pyridine and 0.15 mL of *N*,*O*-bistrimethylsilyl acetamide (BSA) and the solution was stirred 30 min at room temperature under Argon. 0.5 mL of anhydrous CCl_4 (50 equiv), 0.2 mL of anhydrous triethylamine (10 equiv) and 200 mg (0.6 mmol, 12 equiv) of **10**, dissolved in 0.1 mL of anhydrous pyridine, were simultaneously added. The reaction mixture was stirred at room temperature for 2 h, the solvents were removed under vacuum, and the residue was coevaporated with acetonitrile.

5.1.7.2. Deprotection. The crude product was dissolved in 2 mL of THF/ACN—1:1 (v/v) and 5 mL of 0.4 M aqueous NaOH were added, then the mixture was stirred for 10 h at room temperature. The mixture was quenched by adding some DOWEX-50WX8 pyridinium resin, then the resin was filtered off and well rinsed. The filtrates were evaporated to dryness and coevaporated three times with ethanol. The crude was then dissolved in 10^{-3} M TEAB and purified by ion exchange DEAE-A25 Sephadex chromatography (linear gradient of 10^{-3} M TEAB to 0.4 M). The fractions containing the product were gathered and evaporated to dryness, then the residue was further purified by RP-18 flash chromatography (linear gradient of ACN: 0–5% in 20 mM TEAB). The appropriate fraction were gathered and evaporated to dryness. The final target compound was eluted on an ion exchange DOWEX-Na^+ column and after lyophilization from water compound **2** was a white powder (8 mg, 32%). t_R—HPLC: 5.9 min (>99%)—0 to 40% ACN in 30 min. UV: (H_2O) λ_{max} = 272 nm (ε = 7 500). ^{1}H NMR: (D_2O, 300 MHz) δ 8.06 (1H, d, H_6, $^3J_{H6-H5}$ = 7.5 Hz), 6.06 (1H, d, H_5, $^3J_{H5-H6}$ = 7.5 Hz), 5.82 (1H, s, $H_{1'}$), 4.65–4.60 (1H, m, $H_{4'}$), 4.44 (1H, pd, $H_{2'}$), 4.20–4.14 (1H, ddd, systAB, $H_{5'}$, $^2J_{H5'-H5''}$ = 11.7 Hz, ^3J = 3.9 Hz, ^3J = 2.1 Hz), 3.96–3.90 (1H, m, systAB, $H_{5''}$), 3.07 (1H, t, $CH(COO)_2$, ^3J = 7.2 Hz), 2.80 (2H, pq, PNHCH_2, ^3J = 7.8 Hz), 2.20–2.10 (1H, m, systAB, $H_{3'}$), 2.04–1.97 (1H, ddd, systAB, $H_{3''}$, $^2J_{H3''-H3'}$ = 14.7 Hz, ^3J = 6.0 Hz, ^3J = 2.4 Hz), 1.76–1.38 (4H, m, 2 × –CH_2–). ^{31}P NMR: (D_2O, 121 MHz) δ 9.5 (P–N). ESI$^-$ *m/z* 449 (M−3Na+2H)$^-$. HRMS: ESI$^-$ *m/z* calcd for $(C_{15}H_{22}N_4O_{10}P)^-$: 449.1074, found: 449.1078.

5.1.8. Synthesis of N^4,3′-*O*-dibenzoyl-2′-deoxycytidine (3)

To a solution of commercial N^4-benzoyl-5′-*O*-DMTr-2′-deoxycytidine (1.3 g, 2 mmol, 1 equiv), dried by azeotropic distillation with anhydrous pyridine (3×), in 6.5 mL of anhydrous pyridine, at 0 °C and under Argon atmosphere, were added 0.5 mL of benzoyl chloride (4 mmol, 4 equiv). The ice bath was removed and the mixture was stirred for 30 min. 5 mL of methanol were added and the mixture was concentrated to half volume, diluted with 30 mL of dichloromethane (DCM) and washed 3 × 20 mL with a saturated bicarbonate solution. The organics were gathered, dried over Na_2SO_4, filtered and evaporated to dryness. The crude residue was then coevaporated three times with acetonitrile, and dissolved in 40 mL of DCM/MeOH—7:3 (v/v) and cooled to 0 °C. Benzene sulfonic acid 10% solution was then added dropwise (1 g of BSA, 6 mmol, 3 equiv, dissolved in 10 mL of DCM/MeOH—7:3 (v/v)) and the mixture was stirred for 30 min at 0 °C. The reaction mixture was finally neutralized with 20 mL of a saturated $NaHCO_3$ solution, and stirred for 20 more min. The neutralized mixture was pored into a separating funnel and extracted with dichloromethane. The organics were then washed three times with a saturated bicarbonate solution, dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by silica gel chro-

matography (linear gradient: 0–5% of MeOH in DCM). Compound **3** was a white foam (0.81 g, 93%). R_f: 0.52—DCM/MeOH—9:1 (v/v). ^{1}H NMR: (DMSO-d_6, 300 MHz) δ 11.29 (1H, s, br, NH_{Bz}), 8.43 (1H, d, H_6, $^3J_{H6-H5}$ = 7.5 Hz), 8.04–8.01 (4H, m, H_{ortho}), 7.73–7.49 (6H, m, H_{para}, H_{meta}), 7.40 (1H, d, H_5, $^3J_{H5-H6}$ = 6.6 Hz), 6.30 (1H, pt, $H_{1'}$, $^3J_{H1'-H2'}$ = 7.4 Hz, $^3J_{H1'-H2''}$ = 6.0 Hz), 5.51 (1H, m, $H_{3'}$), 5.30 (1H, pt, br, $OH_{5'}$), 4.32 (1H, m, $H_{4'}$), 3.75 (2H, m, $H_{5',5''}$), 2.71–2.64 (1H, dd, systAB, $H_{2'}$, $^2J_{H2'-H2''}$ = 14.8 Hz, $^3J_{H2'-H1'}$ = 5.7 Hz), 2.44–2.35 (1H, m, systAB, $H_{2''}$). ^{13}C NMR: (DMSO-d_6, 75 MHz) δ 165.2 (CO_{Bz}), 163.1 (C_4), 154.3 (C_2), 144.8 (C_6), 133.6, 133.1, 132.7, 129.3, 128.8, 128.4 (CH_{Bz}), 128.3 (C_{Bz}), 96.3 (C_5), 86.4 ($C_{1'}$), 85.5 ($C_{4'}$), 75.6 ($C_{3'}$), 61.8 ($C_{5'}$), 38.3 ($C_{2'}$). ESI$^+$ *m/z* 871 (2 M+H)$^+$, 436 (M+H)$^+$.

5.1.9. Synthesis of N^4,3′-*O*-dibenzoyl-2′-deoxycytidin-5′-yl hydrogenophosphonate, triethylammonium salt (4)

To a solution of **3** (0.37 g, 0.85 mmol, 1 equiv), dried by azeotropic distillation with anhydrous pyridine (2×), in 5 mL of anhydrous pyridine at room temperature and under Argon atmosphere, were added 1.5 mL of diphenylphosphite (7 mmol, 7 equiv). The mixture was stirred at room temperature for 1 h, and then hydrolyzed by adding 4 mL of water/triethylamine—1:1 (v/v) solution. The solvents were removed under vacuum, and the residue was dissolved in dichloromethane, then washed twice with a saturated bicarbonate solution. The organics were dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by silica gel chromatography (linear gradient: 0 to 5% of MeOH in DCM/Net_3—95:5). Compound **4** was a white foam (0.46 g, 90%). R_f: 0.35—DCM/MeOH—9:1 (v/v). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ 12.35 (1H, s, br, NH_{TEAH}), 9.10 (1H, s, br, NH_{Bz}), 8.53 (1H, d, H_6, $^3J_{H6-H5}$ = 7.5 Hz), 8.04–7.88 (4H, m, H_{ortho}), 7.60–7.42 (7H, m, H_{para}, H_5, H_{meta}), 6.91 (1H, d, H-P, $^1J_{H-P}$ = 621.3 Hz), 6.48 (1H, pt, $H_{1'}$, $^3J_{H1'-H2'}$ = 5.1 Hz), 5.61 (1H, m, $H_{3'}$), 4.43 (1H, m, $H_{4'}$), 4.21 (2H, m, $H_{5',5''}$), 3.09 (6H, q, $CH_{2\text{-}TEAH}$, ^3J = 7.5 Hz), 2.86–2.80 (1H, m, $H_{2'}$), 2.41–2.31 (1H, m, $H_{2''}$), 1.33 (9H, $CH_{3\text{-}TEAH}$, ^3J = 7.5 Hz). ^{13}C NMR: ($CDCl_3$, 75 MHz) δ 165.9 (CO_{Bz}), 162.3 (C_4), 155.1 (C_2), 145.2 (C_6), 133.4, 133.2, 133.1, 129.7, 129.3, 128.9, 128.5, 127.7 (CH_{Bz}), 97.0 (C_5), 87.2 ($C_{1'}$), 84.8 ($C_{4'}$, $^3J_{C-P}$ = 7.7 Hz), 76.3 ($C_{3'}$), 63.4 ($C_{5'}$, $^2J_{C-P}$ = 4.1 Hz), 45.5 ($CH_{2\text{-}TEAH}$), 39.2 ($C_{2'}$), 8.6 ($CH_{3\text{-}TEAH}$). ^{31}P NMR: ($CDCl_3$, 121 MHz) δ 4.3 (dt, $^1J_{H-P}$ = 621.3 Hz, $^3J_{H5',5''-P}$ = 6.0 Hz). ESI$^-$: *m/z* 498 (M-TEAH)$^-$. HRMS: ESI$^-$ *m/z* calcd for $(C_{23}H_{21}N_3O_8P)^+$: 498,1066, found: 498,1089.

5.1.10. Synthesis of *O*-(2′-deoxycytidin-5′-yl)-*N*-[(3-malon-2-yl)propyl]-phosphoramidate, sodium salt (1)

5.1.10.1. Oxidation. The *H*-phosphonate monoester **4** (15 mg, 0.025 mmol, 1 equiv), was dried by azeotropic distillation with anhydrous pyridine (2×) and vacuum dried under P_2O_5. It was then dissolved in 0.1 mL of anhydrous pyridine and 0.15 mL of *N*,*O*-bistrimethylsilyl acetamide (BSA) and the solution was stirred 30 min at room temperature under Argon. 0.25 mL of anhydrous CCl_4 (50 equiv), 0.2 mL of anhydrous triethylamine (20 equiv) and 100 mg (0.3 mmol, 12 equiv) of **10**, dissolved in 0.1 mL of anhydrous pyridine, were simultaneously added. The reaction mixture was stirred at room temperature for 2 h, the solvents were removed under vacuum, and the residue was coevaporated with acetonitrile.

5.1.10.2. Deprotection. The crude product was dissolved in 1.5 mL of ACN and 4 mL of 0.4 M aqueous NaOH were added, then the mixture was stirred for 10 h at room temperature. The mixture was quenched by adding some DOWEX-50WX8 pyridinium resin, then the resin was filtered off and well rinsed. The filtrates were evaporated to dryness and coevaporated three times with ethanol. The residue was dissolved in 5 mL of 28% ammonia solution in a hermetically closed flask and was stirred for 5 h at room temperature. Solvent was removed under vacuum and the crude was then dissolved in 10^{-3} M TEAB and purified by ion exchange DEAE-A25 Sephadex chromatography (linear gradient of 10^{-3} M TEAB to 0.4 M). The fractions containing the product were gathered and evaporated to dryness, then the residue was further purified by RP-18 flash chromatography (linear gradient of ACN: 0–5% in 20 mM TEAB). The appropriate fraction were gathered and evaporated to dryness. The final target compound was eluted on an ion exchange DOWEX-Na^+ column and after lyophilization from water compound **1** was a white powder (5 mg, 38%). t_R—HPLC: 6.2 min (>98%)—0 to 40% ACN in 30 min. UV: (H_2O) λ_{max} = 272 nm (ε = 7300). ^{1}H NMR: (D_2O, 300 MHz) δ 7.97 (1H, d, H_6, $^3J_{H6-H5}$ = 7.5 Hz), 6.32 (1H, t, $H_{1'}$, $^3J_{H1'-H2'}$ = 6.9 Hz), 6.10 (1H, d, H_5, $^3J_{H5-H6}$ = 7.5 Hz), 4.56–4.52 (1H, m, $H_{4'}$), 4.18 (1H, pt, $H_{3'}$), 4.02–3.93 (2H, m, $H_{5'}$, $H_{5''}$), 3.04 (1H, t, $CH(COO)_2$, ^3J = 7.8 Hz), 2.79 (2H, pq, $PNHCH_2$, ^3J = 7.8 Hz), 2.46–2.38 (1H, ddd, systAB, $H_{2'}$, $^2J_{H2'-H2''}$ = 14.1 Hz, ^3J = 6.3 Hz, ^3J = 3.9 Hz), 2.34–2.25 (1H, m, systAB, $H_{2''}$), 1.74–1.37 (4H, m, 2 × $-CH_2-$). ^{31}P NMR: (D_2O, 121 MHz) δ 9.4 (P–N). ESI$^-$: *m/z* 449 (M−3Na+2H)$^-$. HRMS: ESI$^-$ *m/z* calcd for $(C_{15}H_{22}N_4O_{10}P)^-$: 449,1074, found: 449,1056.

5.2. Single nucleotide incorporation and elongation tests

Chemicals: Highly purified 2'-deoxycytidine triphosphate used in DNA polymerase reactions was purchased from *Pharmacia.*

Oligodeoxyribonucleotides: Oligodeoxyribonucleotides P, T1 and T2 were purchased from *Sigma Genosys or Eurogentec*. The concentrations were determined with a *Varian Cary-300-Bio* UV Spectrophotometer.

The lyophilized oligonucleotides were dissolved in diethylpyrocarbonate (DEPC)-treated water and stored at −20 °C. The primer oligonucleotides were 5′-^{33}P-labeled with 5′-[γ^{33}P]-ATP (*Perkin Elmer*) using T4 polynucleotide kinase (*NEB*) according to standard procedures. The labeled oligonucleotide was further purified using *Illustra™ Microspin™* G-25 columns (*GE Healthcare*).

DNA polymerase reactions: End-labeled primer was annealed to its template by combining primer P and template (T1 for incorporation and T2 for elongation) in a molar ratio of 1:2 and heating the mixture to 70 °C for 10 min followed by slow cooling to room temperature over a period of 1.5 h. For the incorporation of compounds **1** and **2**, a series of 20 μL-batch reactions was performed with the enzyme HIV-1 RT (*Ambion*, 10 U/μL stock solution). The final mixture contained 125 nM primer·template complex, RT buffer (250 mM Tris·HCl, 250 mM KCl, 50 mM $MgCl_2$, 2.5 mM spermidine, 50 mM dithiothreitol (DTT); pH 8.3), 0.03 U/μL HIV-1 RT, and different concentrations of phosphoramidate building blocks (1 mM, 500 μM, 200 μM and 100 μM). In the control reaction with the natural nucleotide, a 10 μM dCTP concentration was used. The mixture was incubated at 37 °C and aliquots were quenched after 5, 10, 20, 30 and 60 min. For the elongation with **1**, the same mixture containing the appropriate P:T2 hybrid was incubated at 37°C and aliquots were quenched after 30, 60, 90 and 120 min.

Electrophoresis: All polymerase reaction aliquots (2.5 μL) were quenched by the addition of 10 μL of loading buffer (90% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol and 50 mM ethylenediaminetetraacetic acid (EDTA)). Samples were heated at 85 °C for 3 min prior to resolution by electrophoresis for 3 h at 2000 V on a 0.4 mm 20% denaturing gel in the presence of a 100 mM *Tris*-borate, 2.5 mM EDTA buffer; pH 8.3. Products were visualized by phosphorimaging. The amount of radioactivity in the bands corresponding to the products of enzymatic reactions was determined by using the Cyclone imaging apparatus and its Optiquant image analysis software (both *Perkin Elmer*).

Acknowledgments

This work was supported by grant from the French 'Agence Nationale de Recherche Contre le Sida' (ANRS). I.Z. thanks the 'Min-

istère National de la Recherche et de la Technologie' for the award of a research studentship. F.M. is from Inserm.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.08.001.

References and notes

1. Hecker, S. J.; Erion, M. D. *J. Med. Chem.* **2008**, *51*, 2328.
2. Kukhanova, M.; Krayevsky, A.; Prusoff, W.; Cheng, Y. C. *Curr. Pharm. Des.* **2000**, *6*, 585.
3. Lavie, A.; Konrad, M. *Mini-Rev. Med. Chem.* **2004**, *4*, 351.
4. Adelfinskaya, O.; Herdewijn, P. *Angew. Chem., Int. Ed.* **2007**, *46*, 4356.
5. Adelfinskaya, O.; Terrazas, M.; Froeyen, M.; Marliere, P.; Nauwelaerts, K.; Herdewijn, P. *Nucleic Acids Res.* **2007**, *35*, 5060.
6. Terrazas, M.; Marliere, P.; Herdewijn, P. *Chem. Biodiversity* **2008**, *5*, 31–39.
7. Atherton, F. R.; Openshaw, H. T.; Todd, A. R. *J. Chem. Soc.* **1945**, 660.
8. Nelson, P. S.; Kent, M.; Muthini, S. *Nucleic Acids Res.* **1992**, *20*, 6253.
9. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355.
10. Bebenek, K.; Abbotts, J.; Wilson, S. H.; Kunkel, T. A. *J. Biol. Chem.* **1993**, *268*, 10324.
11. Giraut, A.; Song X.-P.; Dyubankova, N.; Herdewijn, P. *Chembiochem 2009 early view* (DOI: 10.1002/cbic.200900185).
12. Hanes, C. S.; Isherwood, F. A. *Nature* **1949**, *164*, 1107–1112.
13. Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. *J. Org. Chem.* **1997**, *62*, 7512–7515.
14. Zlatev, I.; Vasseur, J. J.; Morvan, F. O. *Tetrahedron Lett.* **2008**, *49*, 3288–3290.
15. Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides Nucleotides* **1989**, *8*, 179.