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Synthesis and biological evaluation of phosphonated carbocyclic 2′-oxa-3′-aza-nucleosides

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Abstract—The synthesis of carbocyclic 2′-oxa-3′-aza-nucleosides has been described, based on the 1,3-dipolar cycloaddition of a new phosphonated nitrone with vinyl acetate followed by coupling with silylated nucleobases. The obtained compounds have been evaluated for their ability to inhibit the reverse transcriptase of avian myeloblastosis retrovirus: no significant activity has been observed.

1. Introduction

Since the identification of HIV as the causative agent of AIDS more than 20 years ago, many efforts have been made to keep this disease under control and actually 19 compounds have been formally approved as anti-HIV drugs.[1]

These therapeutic agents act at different steps of the viral replication cycle and belong to five different categories: the nucleoside reverse transcriptase inhibitors (NRTIs), the nucleotide reverse transcriptase inhibitors (NtRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), the protease inhibitors (PIs), and fusion inhibitors (FI).[2]

Diverse strategies combining and alternating NRTIs, NNRTIs and PIs have been developed and are object of ongoing experimentation.[3]

The combination of inhibitors of viral proteases and of the RT determined a quantum leap in potency and efficacy of anti-HIV therapy in protocols of 'highly active anti-retroviral therapy (HAART)'.[4]

NRTIs may be considered as prodrugs; to become active, these nucleosides must be phosphorylated by cellular kinases to give successively the corresponding 5′-mono-, di-, and triphosphates. The efficacy of this process is extremely low (e.g., 0.3% for AZT). Therefore, many efforts have been made to improve therapeutic properties by shortening this cascade and bypassing at least the first phosphorylation step. This approach has resulted in the synthesis of numerous nucleotide analogues (NtRTIs).[5]

A nucleoside 5-phosphonate is essentially a nucleoside monophosphate analogue. However, a phosphonate has the advantage over its phosphate counterpart of being metabolically stable, as its phosphorus–carbon bond is not susceptible to phosphatase hydrolysis. More importantly, the presence of a 5′-phosphonate moiety allows the first phosphorylation step required for nucleoside activation to be skipped, therefore bypassing this inefficient and often rate-limiting step in the conversion to 5′-triphosphate. Like a nucleoside monophosphate, a nucleoside phosphonate can be further phosphorylated by cellular nucleotide kinases.[6] The concept of nucleoside phosphonate has been applied to design chain terminators for anti-HIV chemotherapy and proved to be valid. 9-(2-Phosphonylmethoxypropyl) adenine (PMPA) and 9-(2-phosphonylmethoxyethyl) adenine (PMEA) are two effective and potent nucleoside phosphonate chain terminators for HIV reverse transcriptase (RT).[7]

Keywords: Modified nucleosides; 1,3-Dipolar cycloaddition; Antiviral activity, Phosphonated nucleosides.
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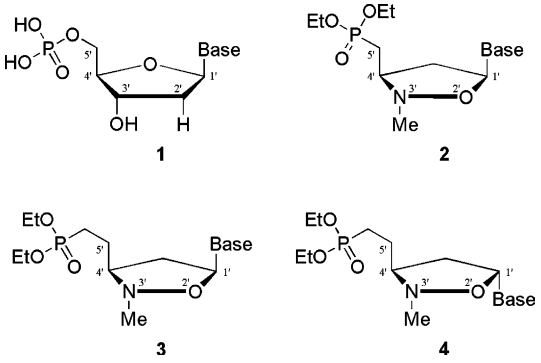
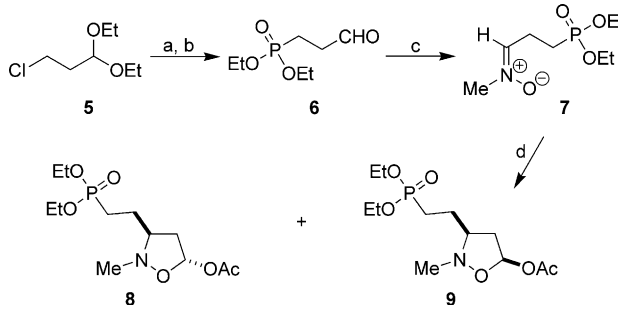


Figure 1.



Scheme 1. Reagents and conditions: (a) $(EtO)_3P$, reflux, 12h; (b) HCl 10%, 90 °C, 2 h; (c) $CH_3NHOH{\cdot}HCl$, CH_2Cl_2; (d) vinyl acetate, microwave irradiation, 100 W, 60 °C, 20 min.

Recently, we have described the potent reverse transcriptase (RT) inhibitors **2** which represent the lower phosphonate homologous of monophosphate compounds **1** where the oxygen atom in C-5′ is eliminated.[8] Compounds of type **2** show low levels of cytotoxicity and exert, on RT from almost two different retroviruses, a specific inhibitory activity, which is comparable with that of AZT, thus opening a new perspective on their possible use as therapeutic agents, in anti-retroviral and anti-HBV chemotherapy.

In this paper, we describe the synthesis of isoxazolidinyl nucleotide type **3** and **4** analogues of compound **1** where the 5′-oxygen atom is substituted by the isosteric methylene moiety (Fig. 1).

It is interesting to speculate that the biological effects exhibited by nucleoside analogues depend importantly on the relative disposition of the phosphate or phosphonate moiety and the heterocyclic base. In particular, it seems to be important to preserve the distance generated from the sugar moiety (spacer unit) between these two key elements.[9]

Phosphonates **3** preserve the five atom chain length between the heterocyclic base and the phosphonate moiety of natural nucleosides. As a consequence, this modification could improve the anti-viral activity of **2**.

2. Chemistry

The strategy of the synthetic approach is based on the construction of the new phosphonated nitrone **7**, which has been prepared from the commercially available 3-chloro propanaldehyde diethyl acetal **5**. The Arbuzov reaction of **5** with triethyl phosphite followed by efficient acidic hydrolysis furnished the corresponding phosphonated aldehyde **6**. The subsequent reaction with *N*-methyl hydroxylamine afforded nitrone **7** in *E*/*Z* mixture and in good yields (85%). Nitrone **7** was purified through aluminum oxide chromatographic column (chloroform–methanol, 95:5) and immediately used for the further reactions. Compound **7** is light stable and, after some days, decomposes into an unidentifiable mixture of products.

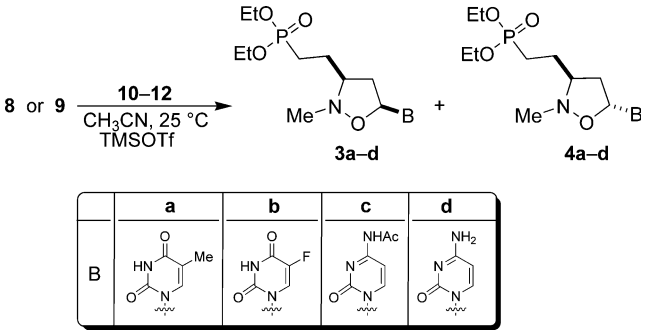
Table 1. AM1 calculations results on transition state structures for isoxazolidines **8** and **9**

Transition state	ΔH_f (kcal/mol)	Percent calcd
(*E*)-*exo*	−248.326	39.95
(*E*)-*endo*	−248.551	58.27
(*Z*)-*exo*	−246.053	0.88
(*Z*)-*endo*	−246.058	0.89

Nitrone **7** and vinyl acetate in excess were heated at 70 °C for 12 h to give, isoxazolines **8** and **9** in 50% global yield. Better results were obtained when the 1,3-dipolar cycloaddition reaction was performed under microwave irradiation: the reaction time was reduced to 20 min and the yields increased to 65% (Scheme 1).

The ^{1}H NMR spectrum of the crude cycloaddition reaction shows the presence of two 5-epimeric isoxazolidines in a 1.85:1 ratio. Isoxazolidines **8** and **9** were sensitive to water in neutral and acidic conditions and rapidly decomposed into a complex mixture products. Consequently, they were promptly used for the successive nucleosidation reaction to give the corresponding more stable nucleotides **3** and **4**. Information on the stereochemical outcome of the cycloaddition process was tentatively inferred by the aid of theoretical supports. Nitrone **7** exists as a mixture of *E*/*Z* isomers (*Z*/*E* ratio, 6:1). AM1 calculations[10] suggest the (*E*)-isomer as the more reactive form of nitrone **7**: the (*E*)-*endo* transition state leading to cis isomer is about 0.22 kcal/mol more stable than the (*E*)-*exo* one leading to trans stereoisomer (Table 1). According to these data, we may assume that the major product of the cycloaddition reaction is compound **9** in agreement with the results reported for similar α-alkoxynitrones.[11] Moreover, these values agree satisfactorily with the results already reported for their lower homologues.[8]

The mixture of two cycloadducts was coupled with silylated nucleobases according to the Vorbrüeggen methodology.[12] The condensation with silylated thymine **10**, *N*-acetylcytosine **11**, and 5-fluorouracil **12**, performed in acetonitrile at 55 °C in the presence of 0.4 equiv of trimethylsilyltriflate (TMSOTf) as catalyst, proceeded with good yields: a mixture of β- and α-nucleosides **3** and **4** were obtained (Scheme 2). The compounds **3c** and **4c** were further treated with 5%



Scheme 2.

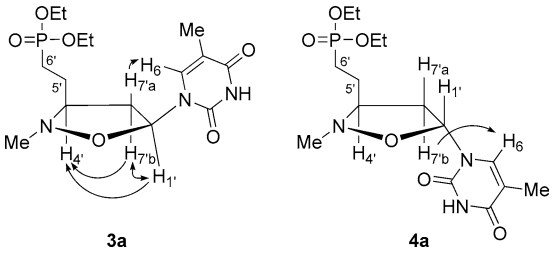


Figure 2. Relevant NOE effects on compounds **3a** and **4a**.

aqueous sodium carbonate to give the deacetylated derivatives **3d** and **4d**.

Pure anomers were isolated by chromatography on MPLC Büchi C-601 using $CHCl_3$–MeOH (99:1) as solvent (10 mL/min). The relative configurations were assigned on the basis of ^1H NMR and NOE experiments. In particular for compound **3a**, chosen as model, the irradiation of $H_{1'}$ increased the resonance of $H_{7'b}$ (the downfield resonance of methylene protons at 7′), and $H_{4'}$ and H_6. Conversely, when $H_{7'b}$ was irradiated, enhancements of the $H_{1'}$, $H_{4'}$, and $H_{7'a}$ signals were observed; while, irradiation of $H_{7'a}$ gave rise to a strong positive NOE effect for H_6 and $H_{7'b}$. These data unambiguously indicate a β-configuration where $H_{4'}$ and the pyrimidine base are in a trans relationship. Furthermore, the α-configuration or anomers **4** was supported by the strong NOE effect observed for H_6 when irradiating $H_{7'b}$ (Fig. 2).

Several attempts to optimize the reaction yields and conditions, in favor of the formation of the β-isomer **3a**, were carried out by varying the temperature and the amounts of catalyst. The best results have been obtained using 0.4 equiv of TMSOTf and a temperature of 70 °C. For *N*-acetylcytosine, in these experimental conditions, the β-anomer clearly predominates as the nearly exclusive product (1:9, α/β ratio), while in the case of thymine and 5-fluorouracil a significant amount of α-anomers has been observed (3:7, α/β ratio).

3. Biological assays

The synthesized compounds, **3a**–**d** and **4a**–**d**, were tested in vitro for their cytotoxicity, on Molt-3 cells. Moreover, the inhibitory activity versus retroviral RT, with reference to known anti-viral compounds (AZT and compound **2**), was assayed. None of the above-mentioned synthetized phosphonated nucleosides show any significant cytotoxicity up to 1000 μM at the higher concentration tested.

The ability to inhibit the reverse transcriptase activity of avian myeloblastosis retrovirus was tested in a cell free assay as previously reported.[8] No retroviral activity was observed at the higher concentration tested, that is, at 1000 μM. As expected, the references, AZT and compound **2**, completely inhibit the RT activity at the 10 nM.

Surprisingly, the insertion of methylene in compounds **2** not only does not potentiate but determines the complete lack of biological effects. However, the synthesis of compounds **3** provides a contribution to the research on the structural features which concur to determine the biological activity of the *N*,*O*-nucleoside system.

The insertion of nucleotides in the growing nucleic acid chain, operated by polymerases, is assisted by bivalent metallic ions that facilitate the transfer of nucleotide units.[13] Although we do not know the exact interaction of compound of type **2** with the RT polymerase, we speculate that the nitrogen atom of the isoxazolidine ring would facilitate the breaking between P_α and P_β in the nucleotides triphosphate and, in this way, allows the transfer of a diphosphoryl or a nucleotidyl group. The nitrogen atom is probably responsible for metal–ion coordinations and, in this context, while the distance P_α–N_3 in compounds **2** allows the formation of 6-membered chelates, for compounds **3** the distance P_α–N_3 is not compatible with anyone stable 7-membered chelate and, consequently, any anti-viral activity is forbidden.

4. Experimental

4.1. General

Commercially available chemicals and solvents were reagent grade and used as received. All solvents were dried according to the literature methods. Elemental analyses were performed on a Perkin-Elmer 220B micro analyzer. NMR spectra were recorded on a Varian instrument at 300 or 500 MHz (^1H) and at 75 MHZ (^{13}C) using deuterochloroform as solvent; chemical shifts are given in parts per million from TMS as internal standard. NOE experiments were performed by a modified cycle NOE sequence, implemented in Darmstadt, which does alternate scan subtraction of two FIDs in which the saturation frequency is moved on-resonance and off-resonance. Power may be reduced from ordinary NOE experiments because the irradiation is cycled through the lines of the multiplet; it is a steady-state NOE in which a single resonance is saturated at low power for approximately 5× T1 (5s in our case) before acquiring the FID. Thin-layer chromatographic separations were achieved through Merck silica gel 60-F_{254} precoated aluminum plates. Preparative separations were carried out

by MPLC Büchi C-601 using Merck silica gel 0.040–0.063 mm.

4.2. Starting materials

3-Chloro propanaldehyde diethyl acetal **5**, thymine **10**, 5-fluorouracil **11**, and *N*-acetylcytosine **12** were purchased from Aldrich Co.

4.2.1. Diethyl {(3*Z*) and (3*E*)-3-[methyl (oxido) imino]propyl} phosphonate 7. A solution of diethyl 2-formylethylphosphonate **6**[14] (20 mmol), *N*-methylhydroxylamine hydrochloride (20 mmol), and trietylamine (20 mmol) in toluene (70 mL) was stirred at room temperature for 5 h. The reaction mixture was filtered, the solvent was evaporated under reduced pressure, and the residue was purified through aluminum oxide chromatographic column (chloroform–methanol, 95:5) to give an mixture *E*/*Z* (1:6) of diethyl {(3*Z*) and (3*E*)-3-[methyl (oxido) imino]propyl} phosphonate **7** as a colorless oil (85% yield). *Z*-isomer: δ_H (300 MHz, $CDCl_3$): 1.35 (t, 3H, J = 5.9 Hz), 1.95 (m, 2H), 2.85 (m, 2H), 3.60 (s, 3H, *N*-CH_3), 4.10 (q, 4H, J = 5.9 Hz), 6.90 (t, 1H, J = 6.8 Hz); δ_C (75 MHz, $CDCl_3$): 16.48, 16.56, 20.46, 26.82, 52.36, 61.70, 61.74, 102.22. *E*-isomer: δ_H (300 MHz, $CDCl_3$): 1.35 (t, 3H, J = 5.9 Hz), 1.90 (m, 2H), 2.85 (m, 2H), 3.49 (s, 3H, *N*-CH_3), 4.10 (q, 4H, J = 5.9 Hz), 6.85 (t, 1H, J = 6.3 Hz); δ_C (75 MHz, $CDCl_3$): 15.92, 16.50, 20.01, 26.50, 39.72, 61.16, 61.70, 101.90. Anal. Calcd for $C_6H_{18}NO_4P$: C, 36.18; H, 9.10; N, 7.03. Found: C, 36.12; H, 9.05; N, 6.98.

4.2.2. Diethyl 2-(3*RS*,5*SR*)-5-acetoxy-2-methylisoxazolidin-3-yl-ethylphosphonate and diethyl 2-(3*RS*,5*RS*)-5-acetoxy-2-methylisoxazolidin-3-yl-ethylphosphonate 8 and 9. *Method A*: a solution of nitrone **7** (5.7 mmol) in vinyl acetate (30 mL) was heated at 70 °C for 12 h. The reaction mixture was then evaporated under reduced pressure and after rapid purification through aluminum oxide chromatographic column (chloroform), afforded an oil (50% yield) identified as a mixture of isoxazolidines **8** and **9**.

Method B: the solution of nitrone **7** (5.7 mmol) and vinyl acetate (10 mL) was irradiated under microwave conditions at 100 W and 60 °C for 20 min; isoxazolidines **8** and **9** as a mixture have been obtained in 65% yield and after a rapid purification on aluminum oxide (chloroform), they were promptly used for the successive reactions. First eluted fractions gave an inseparable mixture of **8** and **9** (1.85:1) as a colorless oil. Major isomer **9**: δ_H (300 MHz, $CDCl_3$): 1.2 (t, 6H, J = 6.2 Hz), 1.73 (m, 2H), 1.75 (m, 2H), 2.03 (m, 1H, H_{4a}), 2.05 (s, 3H), 2.79 (s, 3H, N-CH_3), 2.80 (m, 1H, H_{4b}), 3.55 (m, 1H), 4.10 (q, 4H, J = 6.2 Hz), 6.35 (dd, 1H J = 5.9 and 1.5 Hz). Minor isomer **8**: δ_H (300 MHz, $CDCl_3$): 1.2 (t, 6H, J = 6.2 Hz), 1.73 (m, 2H), 1.75 (m, 2H), 2.03 (s, 3H), 2.52 (m, 2H, H_4), 2.75 (s, 3H, N-CH_3), 3.55 (m, 1H), 4.12 (q, 4H, J = 6.2 Hz), 6.30 (dd, 1H J = 5.7 and 2.3 Hz).

4.2.3. General procedure for the preparation of nucleotides 3a–c and 4a–c. A suspension of nucleobase **10**–**12** (0.62 mmol) in dry acetonitrile (3 mL) was treated with bis(trimethylsilyl)-acetamide (BSA) (2.54 mmol) and refluxed for 15 min under stirring. To the clear solution obtained were added a solution of the epimeric isoxazolidines **8** and **9** (0.52 mmol) in dry acetonitrile (3 mL) and trimethylsilyltriflate (TMSOTf) (0.21 mmol) dropwise, and the reaction mixture was stirred at 70 °C for 6 h. After being cooled at 0 °C, the solution was neutralized by careful addition of aqueous 5% sodium bicarbonate and then concentrated in vacuo. After the addition of dichloromethane (8 mL), the organic phase was separated, washed with water (2× 10 mL), dried over sodium sulfate, filtered, and evaporated to dryness. The residue was purified by chromatography on MPLC Büchi C-601 using $CHCl_3$–MeOH (99:1) as solvent (10 mL/min).

4.2.4. Diethyl {(1′*SR*,4′*RS*)-1′-[[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl]}ethylphosphonate 3a. (Sticky oil, 49%); δ_H (500 MHz, $CDCl_3$): 1.31 (t, 6H, J = 7 Hz), 1.7 (m, 2H, $H_{5'}$), 1.93 (m, 2H, $H_{6'}$), 1.94 (d, 3H, J = 1.0 Hz), 1.99 (ddd, 1H, J = 4.0, 9.5 and 13.5, $H_{7'a}$), 2.75 (m, 1H, $H_{4'}$), 2.76 (s, 3H, N-CH_3), 3.07 (ddd, 1H, J = 6, 7.5, and 13.5, $H_{7'b}$), 4.13 (q, 4H, J = 7 Hz), 6.1 (dd, 1H, J = 4.0 and 7.5 Hz, $H_{1'}$), 7.61 (q, 1H, J = 1 Hz, H_6), 8.91 (br s, 1H, NH); δ_C (125 MHz, $CDCl_3$): 12.67, 12.68, 16.42, 20.88, 21.79, 43.32, 44.04, 61.67, 61.85, 68.14, 82.35, 110.56, 135.78, 150.39, 163.89. Anal. Calcd for $C_{15}H_{26}N_3O_6P$: C, 47.91; H, 6.97; N, 11.17. Found: C, 47.86; H, 6.90; N, 11.13.

4.2.5. Diethyl {(1′*RS*,4′*RS*)-1′-[[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl]}ethylphosphonate 4a. (Sticky oil, 21%); δ_H (500 MHz, $CDCl_3$): 1.22 (t, 6H, J = 7 Hz), 1.65 (m, 2H, $H_{5'}$), 1.92 (m, 2H, $H_{6'}$), 1.94 (d, 3H, J = 1.0 Hz), 2.40 (m, 1H, $H_{7'a}$), 2.76 (m, 1H, $H_{7'b}$), 2.78 (m, 1H, H_4), 2.79 (s, 3H, N-CH_3), 4.15 (q, 4H, J = 7 Hz), 6.13 (m, 1H, $H_{1'}$), 7.35 (q, 1H, J = 1 Hz, H_6), 9.10 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 12.61, 12.66, 16.36, 20.50, 22.70, 40.70, 44.00, 61.73, 61.86, 68.00, 83.03, 111.04, 135.35, 150.46, 164.05. Anal. Calcd for $C_{15}H_{26}N_3O_6P$: C, 47.91; H, 6.97; N, 11.17. Found: C, 47.88; H, 6.89; N, 11.10.

4.2.6. Diethyl [(1′*SR*,4′*RS*)-1′-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl] ethylphosphonate 3b. (Sticky oil, 55%); δ_H (500 MHz, $CDCl_3$): 1.20 (t, 6H, J = 6.5 Hz), 1.60 (m, 2H, $H_{5'}$), 1.82 (m, 1H, $H_{6'}$),1.95 (ddd, 1H, J = 4.2, 10.1 and 13.6, $H_{7'a}$), 2.70 (ddd, 1H, J = 6.0, 7.1 and 10.1 Hz, $H_{4'}$), 2.74 (s, 3H, N-CH_3), 3.10 (ddd, 1H, J = 7.1, 7.7, and 13.6, $H_{7'b}$), 4.10 (q, 4H, J = 6.5 Hz), 6.10 (dd, 1H, J = 4.2 and 7.7 Hz, $H_{1'}$), 7.99 (d, 1H, J = 6.1 Hz, H_6), 9.40 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 16.38, 16.40, 19.40, 29.60, 44.26, 46.52, 63.78, 63.75, 69.01, 82.71, 127.92, 141.30, 148.91, 160.28. Anal. Calcd for $C_{14}H_{23}N_3O_6PF$: C, 44.33; H, 6.11; N, 11.07. Found: C, 44.30; H, 6.05; N, 11.02.

4.2.7. Diethyl [(1′*RS*,4′*RS*)-1′-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl] ethylphosphonate 4b. (Sticky oil, 15%); δ_H

(500 MHz, $CDCl_3$): 1.32 (t, 6H, J = 7.1 Hz), 1.68 (m, 2H, $H_{5'}$), 2.35 (m, 2H, $H_{6'}$), 2.52 (m, 1H, J = $H_{4'}$), 2.80 (s, 3H, N-CH_3), 2.98 (ddd, 1H, J = 7.4, 9 and 15 Hz, $H_{7'a}$), 3.39 (ddd, 1H, J = 3.24, 8.4, and 15 Hz, $H_{7'b}$), 4.12 (q, 4H, J = 7.1 Hz), 6.14 (dd, 1H, J = 3.24 and 7.4 Hz, $H_{1'}$), 7.93 (d, 1H, J = 6.28 Hz, H_6), 9.70 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 16.38, 16.40, 20.50, 29.63, 44.15, 44.97, 63.06, 63.73, 69.84, 84.11, 127.90, 141.30, 148.93, 157.22. Anal. Calcd for $C_{14}H_{23}N_3O_6PF$: C, 44.33; H, 6.11; N, 11.07. Found: C, 44.28; H, 6.07; N, 11.01.

4.2.8. Diethyl {(1′*SR*,4′*RS*)-1′-[4-(acetylamino)-2-oxopyrimidin-1(2*H*)-yl]-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl} ethylphosphonate 3c. (Sticky oil, 70%); δ_H (500 MHz, $CDCl_3$): 1.31 (t, 6H, J = 7 Hz), 1.7 (m, 2H, $H_{5'}$), 1.95 (m, 2H, H'_6), 1.98 (ddd, 1H, $H_{7'a}$, J = 3.4, 9.4 and 13.8), 2.10 (s, 3H), 2.74 (ddd, 1H, J = 2.74, 7.6 and 9.4 $H_{4'}$), 2.76 (s, 3H, N-CH_3), 3.19 (ddd, 1H, J = 7.6, 9.4 and 13.8, $H_{7'b}$), 4.13 (q, 4H, J = 7 Hz), 6.1 (dd, 1H, J = 3.4 and 7.6 Hz, $H_{1'}$), 7.40 (d, 1H, J = 7.4 Hz), 8.15 (d, 1H, J = 7.4 Hz), 9.14 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 14.80, 14.82, 14.99, 23.44, 24.92, 44.68, 44.70, 61.62, 62.81, 62.85, 84.59, 96.74, 141.49, 148.71, 157.80, 171.58. Anal. Calcd for $C_{16}H_{27}N_4O_6P$: C, 48.53; H, 6.66; N, 13.72. Found: C, 44.49; H, 6.70; N, 13.51.

4.2.9. Diethyl {(1′*RS*,4′*RS*)-1′-[4-(acetylamino)-2-oxopyrimidin-1(2*H*)-yl]-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl} ethylphosphonate 4c (sticky oil, 8%). (Sticky oil, 70%); δ_H (500 MHz, $CDCl_3$): 1.31 (t, 6H, J = 7 Hz), 1.72 (m, 2H, $H_{5'}$), 2.08 (s, 3H), 2.10 (m, 2H, H'_6), 2.74 (m, 1H, $H_{7'a}$), 2.76 (s, 3H, N-CH_3), 3.78 (m, 1H, $H_{4'}$), 3.10 (m, 1H, $H_{7'b}$), 4.13 (q, 4H, J = 7 Hz), 6.02 (dd, 1H, J = 7.5 and 9.8 Hz, $H_{1'}$), 7.46 (d, 1H, J = 7.3 Hz), 7.82 (d, 1H, J = 7.3 Hz), 9.14 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 14.80, 14.82, 14.99, 23.57, 24.16, 43.33, 44.72, 62.81, 62.85, 62.89, 84.60, 95.89, 144.49, 148.71, 157.80, 171.58. Anal. Calcd for $C_{16}H_{27}N_4O_6P$: C, 48.53; H, 6.66; N, 13.72. Found: C, 44.46; H, 6.75; N, 13.65.

4.2.10. Synthesis of nucleotides 3d and 4d. A solution of **3c** or **4c** (100 mg), in a mixture of aqueous K_2CO_3 (5%, 5 mL) and methanol (5 mL) was left to stir for 4 h; solvent was then evaporated under reduced pressure, and the residue was purified by flash chromatography ($CHCl_3$–MeOH, 95:5) to give the deacetylated compounds **3d** or **4d**.

4.2.11. Diethyl [(1′*SR*,4′*RS*)-1′-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl] ethylphosphonate 3d. (Sticky oil, 95%); δ_H (500 MHz, $CDCl_3$): 1.32 (t, 6H, J = 7 Hz), 1.60 (m, 2H, $H_{5'}$), 1.95 (m, 2H, H'_6), 2.00 (br s, 2H, NH_2), 2.05 (ddd, 1H, $H_{7'a}$, J = 3.4, 9.4 and 13.8), 2.74 (ddd, 1H, J = 3.0, 7.6 and 9.4 Hz, $H_{4'}$), 2.70 (s, 3H, N-CH_3), 3.20 (ddd, 1H, J = 7.5, 9.4 and 13.8 Hz, $H_{7'b}$), 4.13 (q, 4H, J = 7 Hz), 6.1 (dd, 1H, J = 3.4 and 7.5 Hz, $H_{1'}$), 6.40 (d, 1H, J = 7.4 Hz), 7.30 (d, 1H, J = 7.4 Hz), 9.00 (br s, 1H, NH); δ_C (125 MHz, $CDCl_3$): 14.80, 14.82, 14.99, 21.40, 42.60, 43.40, 61.50, 62.70, 62.80, 70.20, 95.33, 141.49, 150.22, 165.20. Anal. Calcd for $C_{14}H_{25}N_4O_5P$: C, 46.66; H, 6.99; N, 15.55. Found: C, 46.72; H, 6.78; N, 15.51.

4.2.12. Diethyl [(1′*RS*,4′*RS*)-1′-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-3′-methyl-2′-oxa-3′-azacyclopent-4′-yl] ethylphosphonate 4d. (Sticky oil, 95%); δ_H (500 MHz, $CDCl_3$): 1.40 (t, 6H, J = 7 Hz), 1.65 (m, 2H, $H_{5'}$), 1.97 (m, 2H, H'_6), 2.00 (br s, 2H, NH_2), 2.01 (ddd, 1H, $H_{7'a}$, J = 3.5, 9.0 and 14.0 Hz), 2.74 (ddd, 1H, J = 3.0, 7.5 and 9.0 $H_{4'}$), 2.76 (s, 3H, N-CH_3), 3.20 (ddd, 1H, J = 7.5, 9.0 and 14.0, $H_{7'b}$), 4.15 (q, 4H, J = 7 Hz), 6.2 (dd, 1H, J = 3.5 and 9.0 Hz, $H_{1'}$), 6.50 (d, 1H, J = 7.4 Hz), 7.10 (d, 1H, J = 7.4 Hz), 9.00 (br s, 1H NH); δ_C (125 MHz, $CDCl_3$): 14.85, 14.88, 15.02, 21.45, 42.50, 44.0, 62.50, 62.68, 62.80, 70.10, 96.01, 141.50, 150.20, 165.20. Anal. Calcd for $C_{14}H_{25}N_4O_5P$: C, 46.66; H, 6.99; N, 15.55. Found: C, 46.70; H, 6.82; N, 15.50.

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