



**HAL**  
open science

# Synthesis and substrate properties towards HIV-1 reverse transcriptase of new diphosphate analogues of 9-[(2-phosphonmethoxy)ethyl]adenine

Wolfgang Hg Laux, Stephane Priet, Karine Alvarez, Suzanne Peyrottes,  
Christian Perigaud

► **To cite this version:**

Wolfgang Hg Laux, Stephane Priet, Karine Alvarez, Suzanne Peyrottes, Christian Perigaud. Synthesis and substrate properties towards HIV-1 reverse transcriptase of new diphosphate analogues of 9-[(2-phosphonmethoxy)ethyl]adenine. *Antiviral Chemistry and Chemotherapy*, 2018, 26, pp.204020661875763. 10.1177/2040206618757636 . hal-02094536

**HAL Id: hal-02094536**

<https://hal-amu.archives-ouvertes.fr/hal-02094536>

Submitted on 9 Apr 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial | 4.0 International License

# Synthesis and substrate properties towards HIV-1 reverse transcriptase of new diphosphate analogues of 9-[(2-phosphonmethoxy)ethyl]adenine

Antiviral Chemistry and Chemotherapy  
2018, Vol. 26: 1–8  
© The Author(s) 2018  
Reprints and permissions:  
sagepub.co.uk/journalsPermissions.nav  
DOI: 10.1177/2040206618757636  
journals.sagepub.com/home/avc



Wolfgang HG Laux<sup>1</sup>, Stéphane Priet<sup>2</sup>, Karine Alvarez<sup>2</sup>,  
Suzanne Peyrottes<sup>1</sup> and Christian Périgaud<sup>1</sup>

## Abstract

**Background:** The replacement of  $\beta,\gamma$ -pyrophosphate by  $\beta,\gamma$ -phosphonate moieties within the triphosphate chain of 5'-triphosphate nucleoside analogues was previously studied for various antiviral nucleoside analogues such as AZT and 2',3'-dideoxynucleosides. Thus, it has been shown that these chemical modifications could preserve, in some cases, the terminating substrate properties of the triphosphate analogue for HIV-RT. Herein, we aimed to study such 5'-triphosphate mimics based on the scaffold of the well-known antiviral agent 9-[(2-phosphonmethoxy)ethyl]adenine (PMEA, Adefovir).

**Methods:** Synthesis involved coupling of a morpholidate derivative of PME A with appropriate pyrophosphoryl analogues. The relative efficiencies of incorporation of the studied diphosphate phosphonates were measured using subtype B WT HIV-1 RT in an in vitro susceptibility assay, in comparison to the parent nucleotide analogue (PMEApp).

**Results:** Searching for nucleoside 5'-triphosphate mimics, we have synthesized and studied a series of diphosphate analogues of PME A bearing non hydrolysable bonds between the and phosphorus atoms. We also examined their relative inhibitory capacity towards HIV-1 reverse transcriptase in comparison to the parent nucleotide analogue (PMEApp). Only one of them appeared as a weak inhibitor ( $IC_{50} = 403.0 \pm 75.5 \mu M$ ) and proved to be less effective than PMEApp ( $IC_{50} = 6.4 \pm 0.8 \mu M$ ).

**Conclusion:** PME A diphosphoryl derivatives were designed as potential substrates and/or inhibitors of various viral polymerases. These modifications dramatically affect their ability to inhibit HIV-RT.

## Keywords

(phosphomethoxy)ethyl]adenine, phosphonate diphosphate analogues, HIV-1 reverse transcriptase, antiviral

## Introduction

The acyclic nucleoside phosphonate 9-[2-(phosphomethoxy)ethyl]adenine (PMEA, Figure 1) exhibits a broad-spectrum activity against different types of DNA viruses and retroviruses.<sup>1,2</sup> Its orally bioavailable form, the bis(pivaloyloxymethyl) prodrug (bis(POM)PMEA, Adefovir dipivoxil), has been approved for the treatment of chronic hepatitis B<sup>3</sup> and other types of prodrugs are still under investigations.<sup>4–6</sup> To achieve its inhibitory effect on viral synthesis, PME A must be converted intracellularly to its active diphosphorylated metabolite, PMEApp (Figure 1). PMEApp has been described to interact as an alternative substrate and as a competitive inhibitor of both herpes simplex type 1 (HSV-1) DNA

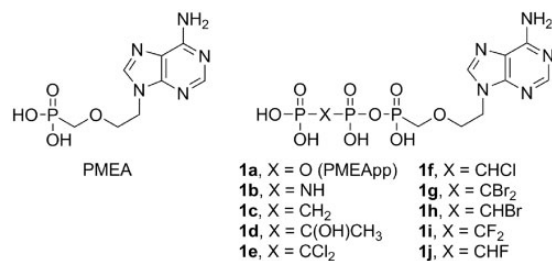
polymerase<sup>7,8</sup> and reverse transcriptases.<sup>9–11</sup> Variable inhibitory effects on human cellular DNA polymerases were observed, especially against DNA polymerase for which  $K_i$  value was in the same range as dATP,<sup>9,12–15</sup>

<sup>1</sup>Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS, Univ. Montpellier, ENSCM, Campus Triolet, Montpellier, Cedex, France  
<sup>2</sup>Laboratoire AFMB, AMU, CNRS, UMR 7257, Groupe "Chimie Médicinale Antivirale", Marseille, Cedex, France

### Corresponding author:

Christian Périgaud, Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 CNRS, Univ. Montpellier, ENSCM, Campus Triolet, cc1704, Place Eugène Bataillon, Montpellier 34095, Cedex 5, France.  
Email: christian.perigaud@umontpellier.fr





**Figure 1.** Chemical structures of PMEA, PMEApp 1a, and target diphosphate analogues 1b-j.

When compared to the affinity of PMEApp for HIV-RT (with  $K_i$  value in the nanomolar range), it may explain the antiviral selectivity of parent phosphonate.

As part of a research program, we decided to synthesize new nucleoside 5'-triphosphate mimics based on the PMEA scaffold and incorporating chemical modifications of the  $P\beta$ -O- $P\gamma$  phosphoester bonds. Replacement of the anhydride oxygen with isosteric groups leading to non-hydrolysable bonds, the resulting analogues were designed as biological tools for the study of substrate properties of cellular and/or viral enzymatic systems, as well as new potential therapeutic agents.<sup>16,17</sup> Based on previously published works on related topic,<sup>16</sup> requirements in the design of modified triphosphate analogues emerged: (i) the anhydride bond between  $\beta$ - and  $\gamma$ -phosphates, which is unaffected during the DNA biosynthesis, could be replaced with non-hydrolysable bond; (ii) similar modification could also be introduced between the 5'-position of sugar and the phosphorus atom; (iii) the anhydride bond between  $\alpha$ - and  $\beta$ -P atoms should be preserved in order to provide the possibility of the mimetic to interact with targeted polymerase as substrate.

In this respect, PMEApp constitutes an attractive model to study chemical modifications on the pyrophosphoryl residue due to its phosphonate structure, characterized by a stable P-C bond (toward phosphohydrolase-hydrolysis) between acyclic nucleoside moiety and  $\alpha$ -phosphorus atom, and its broad and high affinity for viral polymerases. Herein, we report the full accounts of the synthesis of compounds 1b-j and their study as terminating substrates in the DNA chain elongation catalyzed by human immunodeficiency virus (HIV) reverse transcriptase.

## Experimental section

### Material and methods

<sup>1</sup>H NMR (250 MHz) and <sup>13</sup>C NMR (100 MHz) spectra were recorded with proton decoupling at ambient temperature. Chemical shifts ( $\delta$ ) are quoted in parts per

million (ppm) referenced to the residual solvent peak chloroform (CDCl<sub>3</sub>) at 7.26 ppm and 77.0 ppm, deuterium oxide (D<sub>2</sub>O) at 4.63 ppm relative to tetramethylsilane (TMS). COSY experiments were performed in order to confirm proton assignments as well as 2D <sup>1</sup>H-<sup>13</sup>C heteronuclear COSY for the attribution of <sup>13</sup>C signals. <sup>31</sup>P NMR spectra were recorded at ambient temperature at 100 MHz. Chemical shifts are reported relative to external phosphoric acid (H<sub>3</sub>PO<sub>4</sub>). <sup>19</sup>F NMR spectra were recorded at ambient temperature at 235 MHz. Chemical shifts are reported relative to external trichlorofluoromethane (CFCl<sub>3</sub>). Coupling constants,  $J$ , are given in Hertz. FAB mass spectra were recorded in the negative-ion mode using glycerol/thioglycerol (1:1, v/v, G-T) as matrix. Only nominal mass of ions corresponding to the mass of the lightest chlorine or bromine isotopes is given. However, the halogen-isotope peak intensity patterns were ascertained and they agreed with the formula of the ions. Thin layer chromatography was performed on pre-coated aluminum sheets of Silica Gel 60 F<sub>254</sub> (Merck, Art. 5554), visualization of products was accomplished by UV absorbance followed by spraying with Hanes molybdate reagent. Column chromatography was carried out on Silica Gel 60 (Merck, Art. 9385). Analytical HPLC studies were performed using a reverse-phase analytical column (Nucleosil, C18, 150 × 4.6 mm, 5 m) equipped with a prefilter, a precolumn (Nucleosil, C18, 5 m), and a photodiode array detector. Compounds were eluted under isocratic conditions with 0.4% acetonitrile in 50 mM triethylammonium acetate buffer with a flow rate of 1 mL/min. All moisture sensitive reactions were carried out in anhydrous conditions under argon atmosphere using oven-dried glassware. Solvents were dried and distilled prior to use and solids were dried over P<sub>2</sub>O<sub>5</sub> under reduced pressure at rt.

### Chemistry

9-[(2-Phosphomethoxy)ethyl]-adenine (PMEA) and its phospho-

homorpholidate derivative **2** were synthesized according to a published procedure.<sup>18</sup>

The tributylammonium salts of pyrophosphate **3a** and diphosphonic acids **3b-d** were obtained from their commercially available forms: tetra sodium pyrophosphate decahydrate, tetra sodium imidodiphosphonate, methanediphosphonic acid and 1-hydroxyethylidene diphosphonic acid, respectively. The halomethylidene diphosphonic acids **3e-j** were obtained from their ethyl esters precursors following a usual way,<sup>19</sup> and stored as sodium forms after passage over a Dowex 50WX2 cation exchange resin column and freeze-drying. Tetraethyl

methylenediphosphonate 4 was commercially available. The halomethylene diphosphonate esters 5e, 5g, 6f and 6h were prepared using literature methods.<sup>20</sup>

Detailed description of experimental procedures and compound characterization are provided as supplementary data.

### *In vitro* drug susceptibility assays with recombinant subtype B WT HIV-1 RT

The p66RTB gene construct allowing the bacterial expression of the wild-type (WT) HIV-1 RT was described elsewhere.<sup>21,22</sup> The recombinant clade B WT HIV-1 RT was co-expressed with HIV-1 protease in *Escherichia coli* in order to obtain p66/p51 heterodimers, which were later purified using affinity chromatography. Enzymes were quantitated by active-site titration before biochemical studies.

Standard RT activity was assayed using 250 µg/mL of activated calf thymus DNA (GE Healthcare). To determine IC<sub>50</sub> values, reactions were performed with 10 nM enzyme and 5 µM each dNTP as a mixture (dATP, dCTP, dGTP, dTTP) containing 100 µCi/mmol of [<sup>3</sup>H]-labelled deoxythymidine 5'-triphosphate (Perkin Elmer), for 15 min with increasing amounts of phosphonate, compounds 1a (PMEApp as reference), 1c, 1e to 1j. Each aliquot was spotted in duplicate on DE81 ion-exchange paper discs. Paper discs were washed twice with 0.3 M ammonium formate, pH 8.0, twice with water and once with ethanol, and then dried and transferred into sample bags. Scintillation fluid was added and the radioactivity bound to the discs was determined by liquid scintillation counting with MicroBeta Trilux Counter. Values of IC<sub>50</sub> are the average from at least three independent experiments and were determined using Kaleidagraph data.

### Molecular modeling of HIV-1 RT in complex with diphosphate phosphonate analogues 1c, 1e, 1g, 1i

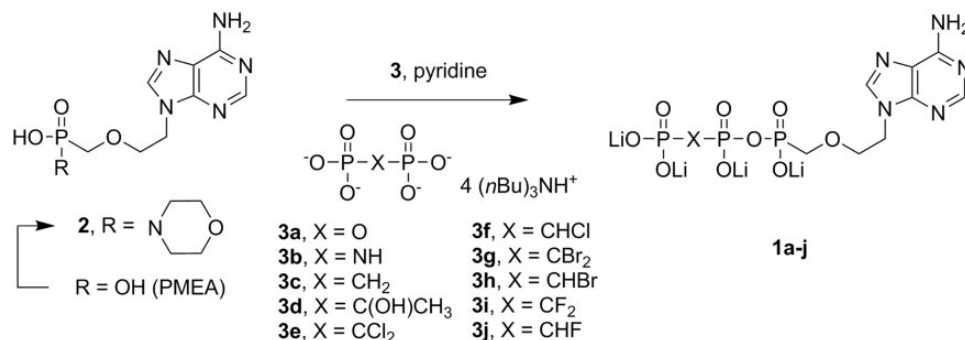
All models were based on the X-ray structure of the RT in complex with dsDNA and incoming PMPApp (PDB

code 1T05). The UCSF Chimera software (PMID: 15264254) was used to replace the PMP – moiety by the PME – equivalent. Moreover, the oxygen of the β,γ bridge of the diphosphate phosphonate PMPApp was replaced by CH<sub>2</sub> (compound 1c), CCl<sub>2</sub> (compound 1e), CBr<sub>2</sub> (compound 1g) and CF<sub>2</sub> (compound 1i) groups.

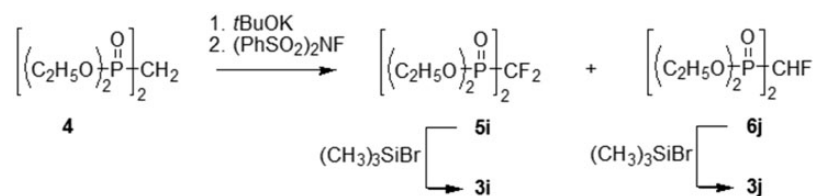
## Results

The synthesis of PME diphosphate 1a and its mimetics 1b-j was carried out according to a general procedure (Scheme 1),<sup>23,24</sup> which requires preliminary preparation of the phosphoromorpholidate derivative 2 of PME. This was accomplished by usual reaction of PME with morpholine and *N,N'*-dicyclohexylcarbodiimide as activating agent.<sup>18</sup> Isolated as its 4-morpholine *N,N'*-dicyclohexylcarboxamidinium salt, 2 was further condensed with the appropriate tributylammonium salts of the diphosphonic acids 3b-j. The imido- and methylenediphosphonate reagents 3b-d were commercially available as sodium or acidic forms. The halomethylidene diphosphonic acids 3e-h were prepared from the commercial tetraethyl methylenediphosphonate, following a published procedure.<sup>20</sup> Preparation of the fluorinated diphosphonic acids 3i, j has previously been described from direct halogenation of tetra alkyl methylenediphosphonates<sup>19</sup> or nucleophilic substitution of an appropriate halomethylphosphonate derivative by a phosphite anion.<sup>25-27</sup>

Moreover, in contrast to the dichloro- and dibromo-analogues (3e-h), nucleophilic dehalogenation of difluoromethylene-diphosphonates into the corresponding monofluoro esters by conventional methods<sup>28,29</sup> was unsuccessful. In such conditions, P–C bond cleavage was observed resulting in the formation of dialkyl difluoromethylphosphonates. Thus, we decided to select the first approach (i.e. direct halogenation) leading in one step to a mixture of the mono- and difluoro- compounds through reaction of electrophilic fluorinating reagents with the carbanions



**Scheme 1.** Final step in the synthesis of PMEApp 1a, and target diphosphate analogues 1b-j.



**Scheme 2.** Synthesis of the mono- and difluorobisphosphonic acids 3i, j.

of alkyl methylidenediphosphonates. Reported methodologies used perchloryl fluoride<sup>19</sup> or acetyl hypofluorite.<sup>30</sup> Herein, *N*-fluorobenzenesulfonimide was chosen as commercially available and easy handling fluorinating reagent.<sup>31</sup> Consequently, tetraethyl methylenediphosphonate 4 was deprotonated by the action of potassium *tert*-butoxide and treated with *N*-fluorobenzene-sulfonimide (Scheme 2). Purification of the resulting mixture on flash silica gel chromatography yielded 29% of the starting material 4 and 31% of each desired mono- and dihalogenated species 5i and 6j, respectively. Saponification of the tetraethyl fluoromethylenediphosphonate esters 5i and 6j was carried out using bromotrimethylsilane to give rise to the fluorinated diphosphonic acids 3i, j.

Crude reaction of the phosphoromorpholidate derivative of PMEAs 2 with pyrophosphate 3a or the appropriate tributylammonium salts of the diphosphonic acids 3b-j was firstly purified by a Dowex 1X2 chromatography using a gradient of aqueous lithium chloride in 0.01 M hydrochloric acid.<sup>18</sup> Then, DEAE-Sephadex A25 chromatography gave PMEAs diphosphate 1a and its mimetics 1b-j. The low yield obtained for derivative 1b (18%) was probably due to the chemical instability of the imido functionality<sup>32</sup> during purification step at acidic pH. Structures of the different mimetics of PMEAs diphosphate were assigned on the basis of their NMR data (Tables 1 and 2), MS and UV spectra. Purity was checked by analytical high pressure liquid chromatography (HPLC) and high resolution mass spectra (HRMS).

To evaluate the inhibitory activity of diphosphate phosphonates 1a, 1c, 1e to 1j on the reverse transcriptase (RT) of HIV-1, their relative efficiencies of incorporation were measured using subtype B WT HIV-1 RT in an *in vitro* susceptibility assay. The calculated 50% inhibitory concentration ( $IC_{50}$ ) values obtained in this assay showed that PMEApp 1a is active ( $IC_{50} = 6.4 \pm 0.8 \mu\text{M}$ ), this value was in agreement with literature data,<sup>33</sup> whereas the diphosphate phosphonate analogues are truly less potent ( $IC_{50} > 1000 \mu\text{M}$  or  $IC_{50} = 403.0 \pm 75.5 \mu\text{M}$  for compound 1i).

## Discussion

The synthesis of new mimics of PMEApp incorporating non hydrolysable bond between the  $\beta$ - and  $\gamma$ -P

**Table 1.** Selected <sup>31</sup>P NMR data of the new PMEAs diphosphate mimetics 1b-j.

Compound	Chemical shifts (ppm)			Coupling constants (Hz)	
	$\delta P\alpha$	$\delta P\beta$	$\delta P\gamma$	$^2J_{\alpha\beta}$	$^2J_{\beta\gamma}$
1a	9.4	-19.6	-4.3	24.9	18.1
1b	9.7	-6.2	0.7	25.6	5.2
1c	9.2	14.7	13.7	29.2	7.5
1d	9.9	16.4	17.6	36.1	32.2
1e	9.8	3.9	9.5	34.9	15.8
1f	9.4	7.9	10.3	31.1	5.2
1g	9.8	4.2	9.3	34.4	13.3
1h	9.3	7.1	9.9	31.2	3.9
1i	10.1	-3.8	3.9	33.8	59.0
1j	9.5	6.8	8.6	31.3	12.2

**Table 2.** Selected <sup>13</sup>C and <sup>19</sup>F NMR data of the new PMEAs diphosphate mimetics 1c-j.

Compound	Chemical shifts (ppm)		Coupling constants (Hz)		
	$\delta C$	$\delta F$	$^2J_{FC}$	$^2J_{FP\beta}$	$^2J_{FP\gamma}$
1c	30.8				
1d	72.4				
1e	78.0				
1f	49.5				
1g	57.4				
1h	38.2				
1i	118.7	-120.3	273.7	87.7	79.4
1j	89.3	-218.6	180.5	64.8	55.6

atoms has been carried out by reaction of the morpholidate derivative of PMEAs 2 with the appropriate diphosphonic acids 3b-j (Scheme 1). The target diphosphate analogues 1b-j were isolated as lithium forms in 18–65% yields.

The <sup>31</sup>P NMR spectra of the mimetic phosphonates 1b-j showed characteristic downfield shifts which permitted, from a straightforward comparison with literature data, the direct assignment of resonances for  $\alpha$ -

$\beta$ - and  $\gamma$ -phosphorus atoms (Table 1). The  $\alpha$ -P resonance was relatively independent of the substituent nature between  $\beta$  and  $\gamma$  phosphorus atoms. Compared to the parent phosphate, the phosphorus–phosphorus coupling constant  ${}^2J_{\alpha\beta}$  was generally increased through these changes and did not appear to correlate with the electronegativity of the substituents. The resonance for  $\beta$ -P was upfield from  $\alpha$ -P for all analogues excepted the methylene and hydroxyethylidene derivatives 1c,d. The difluoromethylene and the hydroxyethylidene derivatives 1d,i showed a large value for the  ${}^2J_{\beta\gamma}$  coupling constant. In contrast, the  ${}^2J_{\beta\gamma}$  coupling constants for the dihalogenomethylene analogues 1e,g were in the same range as the parent phosphate. Moreover, published  ${}^{31}\text{P}$  NMR data for diphosphate analogues bearing a  $\beta,\gamma$ -methylene bridge substituted with bulky and anionic functions did not show an increase of the  ${}^2J_{\beta\gamma}$  coupling constant.<sup>34</sup> This unusual degree of electronic interaction between  $\beta$  and  $\gamma$  the phosphorus atoms, previously reported in other series for difluoromethylene diphosphate analogues,<sup>35–37</sup> reflects a complex set of factors including the combined electronegativity and  $d\pi$ -bonding possibilities for a  $\beta$ -P ligand which may lead to conformational changes in the phosphoryl chain.<sup>35</sup>

The resonance of carbon atom between  $\beta$ -P and  $\gamma$ -P showed a normal dependence to the electronegativity of substituent (Table 2). The downfield shifts increased in the series  $\delta(\text{CF}_2) > \delta(\text{CHF}) > \delta(\text{CCl}_2) > \delta(\text{COHCH}_3) > \delta(\text{CBr}_2) > \delta(\text{CHCl}) > \delta(\text{CHBr}) > \delta(\text{CH}_2)$ . Finally, a single fluorine resonance was observed in  ${}^{19}\text{F}$  NMR spectra for both the difluoromethylene compound 1i ( $\delta -120.3$  ppm, dd) and the mixture of diastereoisomeric monofluoromethylene compounds 1j ( $\delta -218.6$  ppm, ddd) showing that the fluorine environments could be considered as magnetically equivalent. Coupling constants were different for the two phosphorus nuclei directly bonded to the fluorinated  $\beta,\gamma$ -methylene bridge.  ${}^2J_{\text{FP}}$  values for coupling with  $\text{P}\beta$  were greater than those for coupling with  $\text{P}\gamma$ .

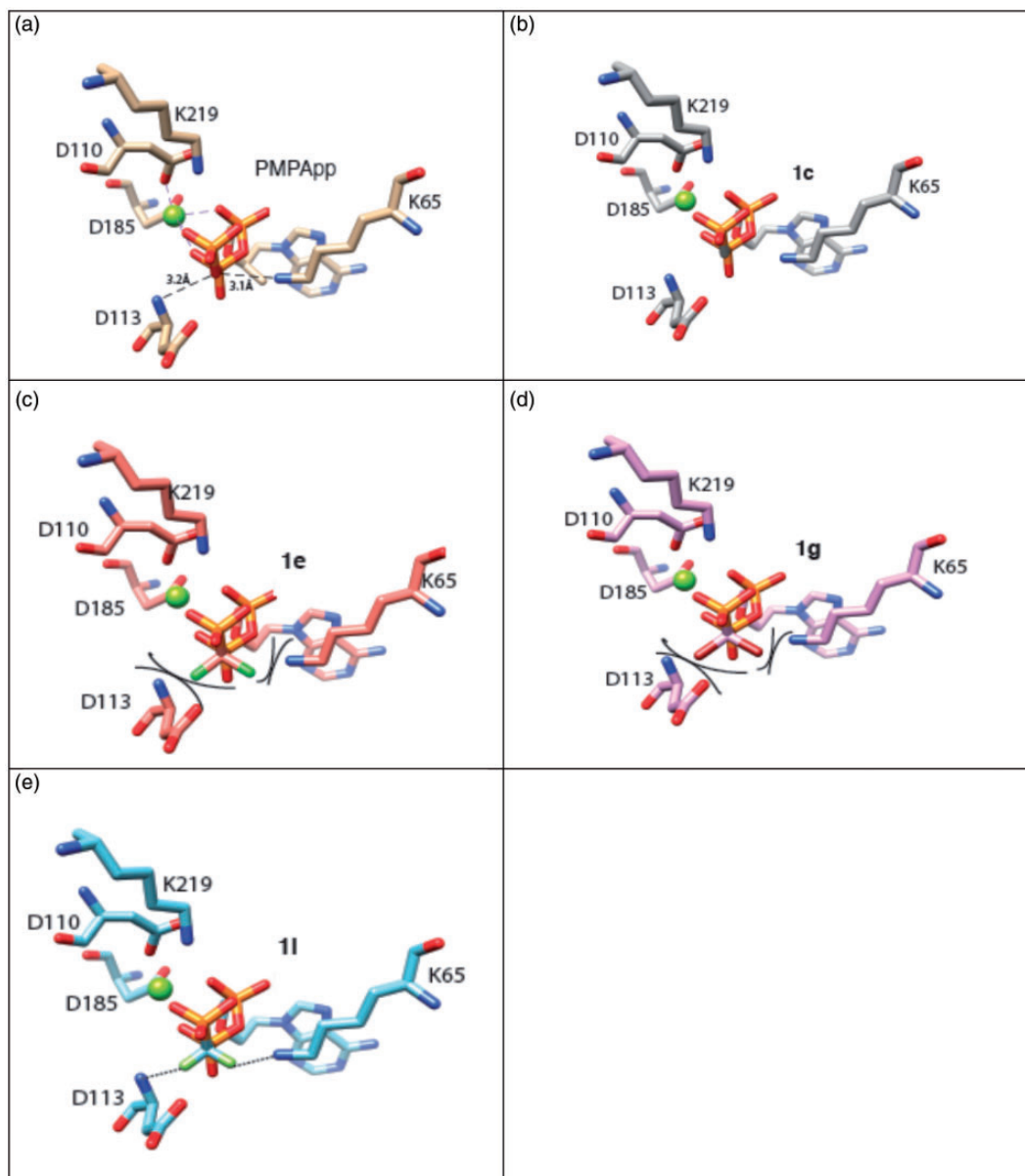
The substrate properties of these PMEAs diphosphoryl derivatives were comparatively studied, in cell free solutions, towards HIV-1 reverse transcriptase. Indeed, it has been previously shown in various nucleotide series that chemical modifications on 5'-phosphate residues could preserve, in some cases, the terminating substrate properties of the triphosphate analogue in the DNA biosynthesis catalyzed by different polymerases.<sup>17,38–40</sup> The substitution of  $\beta,\gamma$ -pyrophosphate by  $\beta,\gamma$ -phosphonates was rather systematically studied for a series of antiviral nucleoside analogues (AZT and 2',3'-dideoxynucleosides)<sup>41,42</sup> including modification of the triphosphate chain at the - position and presents some similarity with the compounds under study. Thus, in the particular case of (R)

P- boranonucleotide analogues of AZT, the order of activity towards the inhibition of HIV-1 reverse transcriptase is  $\text{CF}_2=\text{O} \gg \text{CHF} > \text{CCl}_2 > \text{NH} > \text{CH}_2$ ,<sup>41</sup> showing that the  $\beta,\gamma$ -difluoromethylene modification is effective and comparable to that of a natural bridge. However, the results obtained here demonstrate that none of the compounds 1b-j showed substrate properties towards HIV-1 reverse transcriptase till the concentration of 100 M (as example  $\text{IC}_{50}$  values for 1a and 1i were  $6.4 \pm 0.8 \mu\text{M}$  and  $403 \pm 75 \mu\text{M}$ , respectively). Even so, it is unpredictable, modifications of the nucleotide (either the sugar, base or 5'-triphosphate moieties) may affect its substrate activity through the modulation of its binding in the active site of the target enzyme or its incorporation.

Several factors including size, polarity, and electronegativity may modulate the activity, even so a certain tolerance of the HIV-1 reverse transcriptase to the  $\gamma$ -P-substituents was demonstrated in literature.<sup>42</sup>

To understand why diphosphate phosphonates analogues 1c, 1e, 1g and 1j are poor substrates of HIV-1 RT in comparison to PMEApp 1a, we performed modeling replacing the oxygen of the  $\beta,\delta$  bridge of the diphosphate phosphonate by  $\text{CH}_2$ ,  $\text{CCl}_2$ ,  $\text{CBr}_2$  or  $\text{CF}_2$  groups, with respect to specific geometry and bond distances. According to Tuske et al.,<sup>43</sup> amino acids R72 and K65, and D113 to a lesser extent, play a key role in the binding of the nucleotide in the active site and in their incorporation by HIV-1 RT (Figure 2(a)). If the substitution of oxygen atom of the,  $\beta,\gamma$ -pyrophosphate bond by a methylene group ( $\text{CH}_2$ ), *i.e.* compound 1c (Figure 2(b)), does not cause steric hindrance. However, the interaction with K65 (protonated form in the catalytic site) is lost and is responsible for nucleotide destabilization and discrimination. Indeed, the modification of its binding at the RT active site misaligns reactive centers and hampers the nucleophilic attack at the catalytic step of incorporation into viral DNA. When the O of the  $\beta,\gamma$  bridge is substituted by  $\text{CCl}_2$  or  $\text{CBr}_2$ , respectively, in compounds 1e and 1g, the major drawback observed is the steric hindrance (Figure 2(c) and (d)). Indeed, distances between analogues 1e and 1g and amino acids K65 and D113 are less than 1.8 Å and this close vicinity is prompted to destabilize complex between the nucleotide and HIV-1 RT. When the O of the bridge is replaced by a  $\text{CF}_2$ , *i.e.* compound 1i, the activity is somewhat restored. The steric hindrance is rather acceptable (Figure 2(c)) and distances with amino acids K65 and D113 are around 2.4 Å. The main benefit is that  $\text{CF}_2$  modification can establish electrostatic interactions with K65, counterbalancing the negative effect of the  $\beta,\gamma$  bridge modification.

Another important remark is the difference that can be observed when comparing the crystallographic



**Figure 2.** (a) Structure of HIV-1 RT active site in complex with PMPApp. (b), (c), (d) and (e) models for the putative positioning of acyclic diphosphate phosphonates 1c, 1e, 1g, 1i in the active site of HIV-1 RT. The atomic coordinates (PDB 1T05 – HIV-1 RT in complex with PMPApp) were used to visualize the complex HIV-1 RT- diphosphate phosphonate, after modeling, replacing PMP-moiety by PME- one and the oxygen of the, bridge of the diphosphate phosphonate by  $\text{CH}_2$ ,  $\text{CCl}_2$ ,  $\text{CBr}_2$  or  $\text{CF}_2$  groups, respecting specific geometry and bond distances. One magnesium ion is represented as green sphere. Amino acid R72 and the second magnesium ion are intentionally omitted for figure clarity. (a) Structure 1T05: distances between the O of the, bridge and amino acids K65 and D113 are mentioned in dotted line. (c) and (d) steric hindrance is mentioned in full line. (e) Interactions are mentioned in dotted line.

data from complexes of HIV-RT with purine or pyrimidine nucleotides. With purine nucleotides, as PMPApp, the amino acid R72 plays a crucial role in stacking the nucleobase, while only the amino acid R65 interacts with the O of the  $\beta,\gamma$  bridge. With pyrimidine nucleotides, their binding in the RT active site is slightly different, both amino acids R72 and K65 interact

with the O of the  $\alpha,\beta$  bridge and consequently the binding of the nucleotide is less sensible to the chemical modification of the bridge. This may explain why the modified phosphonate analogues of AZT are recognized and substrates for HIV-1 RT.<sup>41</sup> Thus, they are efficiently incorporated in the growing nucleic acid chain.

## Conclusion

Nine diphosphate analogues of PMEAs have been designed as isostere of the parent diphosphorylated form, PMEApp. The benefit of stable P–C bonds replacing scissile P–O–C linkage constitutes an attractive interest to evaluate the electronic and stereochemical requirements for binding to relevant proteins. It was observed that HIV-RT does not extend the DNA primer with the synthesized compounds. Within this acyclic series, the replacement of the  $\beta,\gamma$  bridge of the diphosphate phosphonate by  $\text{CH}_2$ ,  $\text{CHCl}$ ,  $\text{CCl}_2$ ,  $\text{CHBr}$ ,  $\text{CBr}_2$ ,  $\text{CHF}$  or  $\text{CF}_2$  groups has a drastic effect on the recognition by the HIV-RT.

## Authors' note

This article is dedicated to Dr. Gilles GOSSELIN on the occasion of his retirement.

## Acknowledgments

We thank the “Fonds der Chemischen Industrie” for a post-doctoral fellowship (W. L.). We thank the “Agence Nationale de Recherche sur le Sida et les Hépatites Virales” for its financial support.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

## Supplementary material

Supplementary material (synthetic procedure and compounds characterization) is provided as a separate electronic file (PDF format) and was submitted along with the manuscript.

## References

1. Cundy KC. Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir. *Clin Pharmacokinet* 1999; 36: 127–143.
2. Naesens L, Snoeck R, Andrei G, et al. HPMPC (cidofovir), PMEAs (adefovir) and related acyclic nucleoside phosphonate analogues: a review of their pharmacology and clinical potential in the treatment of viral infections. *Antivir Chem Chemother* 1997; 8: 1–23.
3. De Clercq E. Antiviral drugs in current clinical use. *J Clin Virol* 2004; 30: 115–133.
4. Fu XZ, Jiang SH, Li C, et al. Design and synthesis of novel bis(L-amino acid) ester prodrugs of 9- 2-(phosphonomethoxy)ethyl adenine (PMEAs) with improved anti-HBV activity. *Bioorg Med Chem Lett* 2007; 17: 465–470.
5. Lu P, Liu JX, Wang YY, et al. Design, synthesis and evaluation of novel oxazaphosphorine prodrugs of 9-(2-phosphonomethoxyethyl)adenine (PMEAs, adefovir) as potent HBV inhibitors. *Bioorg Med Chem Lett* 2009; 19: 6918–6921.
6. Pertusati F, Hinsinger K, Flynn AS, et al. PMPA and PMEAs prodrugs for the treatment of HIV infections and human papillomavirus (HPV) associated neoplasia and cancer. *Eur J Med Chem* 2014; 78: 259–268.
7. Merta A, Votruba I, Rosenberg I, et al. Inhibition of herpes-simplex virus-DNA polymerase by diphosphates of acyclic phosphonylmethoxyalkyl nucleotide analogs. *Antiviral Res* 1990; 13: 209–218.
8. Foster SA, Cerny J and Cheng YC. Herpes-simplex virus-specified DNA-polymerase is the target for the antiviral action of 9-(2-phosphonylmethoxyethyl)adenine. *J Biol Chem* 1991; 266: 238–244.
9. Cherrington JM, Allen SJW, Bischofberger N, et al. Kinetic interaction of the diphosphates of 9-(2-phosphonylmethoxyethyl)adenine and other anti-HIV active purine congeners with HIV reverse-transcriptase and human DNA polymerase-alpha, polymerase-beta and polymerase-gamma. *Antivir Chem Chemother* 1995; 6: 217–221.
10. Cherrington JM, Fuller MD, Mulato AS, et al. Comparative kinetic analyses of interaction of inhibitors with rauscher murine leukemia virus and human immunodeficiency virus reverse transcriptases. *Antimicrob Agents Chemother* 1996; 40: 1270–1273.
11. Votruba I, Travnicek M, Rosenberg I, et al. Inhibition of avian-myeloblastosis virus reverse-transcriptase by diphosphates of acyclic phosphonylmethyl nucleotide analogs. *Antiviral Res* 1990; 13: 287–293.
12. Birkus G, Votruba I, Holy A, et al. 9- 2-(phosphonomethoxy)ethyl adenine diphosphate (PMEApp) as a substrate toward replicative DNA polymerases alpha, delta, epsilon, and epsilon\*. *Biochem Pharmacol* 1999; 58: 487–492.
13. Cihlar T and Chen MS. Incorporation of selected nucleoside phosphonates and anti-human immunodeficiency virus nucleotide analogues into DNA by human DNA polymerases alpha, beta and gamma. *Antivir Chem Chemother* 1997; 8: 187–195.
14. Kramata P, Votruba I, Otova B, et al. Different inhibitory potencies of acyclic phosphonomethoxyalkyl nucleotide analogs toward DNA polymerases alpha, delta, and epsilon. *Mol Pharmacol* 1996; 49: 1005–1011.
15. Kramata P, Birkus G, Otmar M, et al. Structural features of acyclic nucleotide analogs conferring inhibitory effects on cellular replicative DNA polymerases. *Collect Czech Chem Commun* 1996; 61: S188–S191.
16. Krayevsky AA. Molecular bases of drug design for AIDS treatment: achievements and prospects. *Mol Biol* 1999; 33: 295–303.
17. Krayevsky A, Arzumanov A, Shirokova E, et al. dNTP modified at triphosphate residues: substrate properties towards DNA polymerases and stability in human serum. *Nucleosides Nucleotides* 1998; 17: 681–693.



18. Holy A and Rosenberg I. Synthesis of 9-(2-phosphonylmethoxyethyl)adenine and related-compounds. *Collect Czech Chem Commun* 1987; 52: 2801–2809.
19. McKenna CE and Shen PD. Fluorination of methanediphosphonate esters by perchloryl fluoride – synthesis of fluoromethanediphosphonic acid and difluoromethanediphosphonic acid. *J Org Chem* 1981; 46: 4573–4576.
20. McKenna CE, Khawli LA, Ahmad WY, et al. Synthesis of alpha-halogenated methanediphosphonates. *Phosphorus Sulfur Silicon Relat Elem* 1988; 37: 1–12.
21. Boretto J, Longhi S, Navarro JM, et al. An integrated system to study multiply substituted human immunodeficiency virus type 1 reverse transcriptase. *Anal Biochem* 2001; 292: 139–147.
22. Selmi B, Boretto J, Navarro JM, et al. The valine-to-threonine 75 substitution in human immunodeficiency virus type 1 reverse transcriptase and its relation with stavudine resistance. *J Biol Chem* 2001; 276: 13965–13974.
23. Moffatt JG. General synthesis of nucleoside-5' triphosphates. *Can J Chem* 1964; 42: 599–604.
24. Moffatt JG and Khorana HG. Nucleoside polyphosphates.10. The synthesis and some reactions of nucleoside-5' phosphoromorpholidates and related compounds. Improved methods for preparation of nucleoside-5' polyphosphates. *J Am Chem Soc* 1961; 83: 649–658.
25. Burton DJ and Flynn RM. Preparation of F-methylene bis phosphonates. *J Fluorine Chem* 1980; 15: 263–266.
26. Blackburn GM, England DA and Kolkmann F. Monofluoro-methylenebisphosphonic and difluoro-methylenebisphosphonic acids – isopolar analogs of pyrophosphoric acid. *J Chem Soc Chem Commun* 1981; 15: 930–932.
27. Hutchinson DW and Thornton DM. A simple synthesis of monofluoromethylene bisphosphonic acid. *J Organomet Chem* 1988; 340: 93–99.
28. Hutchinson DW and Semple G. Synthesis of alkylated methylene bisphosphonates via organothallium intermediates. *J Organomet Chem* 1985; 291: 145–151.
29. Hutchinson DW and Semple G. The dehalogenation of dihalogenomethylenebisphosphonates. *Phosphorus Sulfur Silicon Relat Elem* 1984; 21: 1–4.
30. Hebel D, Kirk KL, Kinjo J, et al. Synthesis of a difluoromethylenephosphonate analog of AZT-5'-triphosphate and its inhibition of HIV-1 reverse-transcriptase. *Bioorg Med Chem Lett* 1991; 1: 357–360.
31. Differding E and Ofner H. N-Fluorobenzenesulfonimide – a practical reagent for electrophilic fluorinations. *Synlett* 1991; 187–189.
32. Li RS, Muscate A and Kenyon GL. Synthesis, characterization, and inhibitory activities of nucleoside alpha, beta-imido triphosphate analogues on human immunodeficiency virus-1 reverse transcriptase. *Bioorg Chem* 1996; 24: 251–261.
33. Priet S, Roux L, Saez-Ayala M, et al. Enzymatic synthesis of acyclic nucleoside thiophosphonate diphosphates: effect of the alpha-phosphorus configuration on HIV-1 RT activity. *Antiviral Res* 2015; 117: 122–131.
34. Liu XH, Zhang XR and Blackburn GM. Synthesis of three novel supercharged beta, gamma-methylene analogues of adenosine triphosphate. *Chem Commun* 1997; 1: 87–88.
35. Blackburn GM, Kent DE and Kolkmann F. The synthesis and metal-binding characteristics of novel, isopolar phosphonate analogs of nucleotides. *J Chem Soc Perkin Trans 1* 1984; ■: 1119–1125.
36. Blackburn GM, Kent DE and Kolkmann F. 3 New beta, gamma-methylene analogs of adenosine-triphosphate. *J Chem Soc Chem Commun* 1981; 22: 1188–1190.
37. Arabshahi L, Khan NN, Butler M, et al. (Difluoromethylene)phosphates of guanine nucleosides as probes of DNA-polymerases and G-proteins. *Biochemistry* 1990; 29: 6820–6826.
38. Dyatkina N, Shirokova E, Theil F, et al. Modified triphosphates of carbocyclic nucleoside analogues: synthesis, stability towards alkaline phosphatase and substrate properties for some DNA polymerases. *Bioorg Med Chem Lett* 1996; 6: 2639–2642.
39. Shipitsin AV, Victorova LS, Shirokova EA, et al. New modified nucleoside 5' -triphosphates: synthesis, properties towards DNA polymerases, stability in blood serum and antiviral activity. *J Chem Soc Perkin Trans 1* 1999; 8: 1039–1050.
40. Alexandrova LA, Skoblov AY, Jasko MV, et al. 2' -Deoxynucleoside 5' -triphosphates modified at alpha-, beta- and gamma-phosphates as substrates for DNA polymerases. *Nucleic Acids Res* 1998; 26: 778–786.
41. Wang GY, Boyle N, Chen F, et al. Synthesis of AZT 5' -triphosphate mimics and their inhibitory effects on HIV-1 reverse transcriptase. *J Med Chem* 2004; 47: 6902–6913.
42. Boyle NA, Rajwanshi VK, Prhac M, et al. Synthesis of 2',3' -dideoxynucleoside 5' -alpha-P-borano-beta, gamma-(difluoromethylene)triphosphates and their inhibition of HIV-1 reverse transcriptase. *J Med Chem* 2005; 48: 2695–2700.
43. Tuske S, Sarafianos SG, Clark AD, et al. Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. *Nat Struct Mol Biol* 2004; 11: 469–474.