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Synthesis and antiviral activity of N^9-[3-fluoro-2-(phosphonomethoxy)propyl] analogues derived from N^6-substituted adenines and 2,6-diaminopurines

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ABSTRACT

An efficient method for the synthesis of N^9-[3-fluoro-2-(phosphonomethoxy)propyl] (FPMP) derivatives of purine bases has been developed. Both (*R*)- and (*S*)-enantiomers of the N^6-substituted FPMP derivatives of adenine and 2,6-diaminopurine were prepared and their anti-human immunodeficiency virus (HIV) and anti-Moloney murine sarcoma virus (MSV) activity was evaluated. Whereas none of the 6-substituted FPMPA derivatives showed any antiviral activity, several FPMPDAP derivatives had a moderate antiretroviral activity. Moreover, the data obtained from the study of the substrate activity of the active derivatives towards N^6-methyl-AMP aminohydrolase support the notion that the studied N^6-substituted FPMPDAP derivatives act as prodrugs of the antiretroviral FPMPG analogues.

1. Introduction

Acyclic nucleoside phosphonates (ANPs) are nucleotide analogs with phosphorous atom attached to the side aliphatic chain through a stable P–C bond.[1] ANPs exhibit various antiviral,[2] cytostatic,[3] antiparasitic,[4] and immunomodulatory properties.[5] Three ANPs (cidofovir, adefovir, tenofovir) are active components of potent antivirals used in human medicine for treatment of hepatitis B, AIDS, and other diseases caused by DNA viruses.[6]

An interesting subclass of ANPs is represented by the purine N^9-[3-fluoro-2-(phosphonomethoxy)propyl] (FPMP) derivatives **1** (Fig. 1).[2,7] In contrast to the purine N^9-[3-hydroxy-2-(phosphonylmethoxy)propyl] (HPMP) derivatives **2** (Fig. 1), which are active against a broad spectrum of DNA viruses,[1] the FPMP compounds **1** exhibit potent and selective activity against retroviruses (HIV-1 and HIV-2).[7,8] Thus, replacement of the hydroxyl group at the C′-3 position of an aliphatic chain by fluorine leads to a completely different pattern of antiviral activity where the loss of activity against DNA viruses is compensated by high and selective antiretroviral activity. In addition, (*S*)-FPMPA also showed interesting activity (EC_{50}: 1.2 μM) against hepatitis B virus (HBV).[9]

Moreover, fluorinated nucleoside analogs also drew attention from the pharmaceutical industry due to improved pharmacokinetic properties (absorption, distribution, metabolism, and excretion) and diminished side effects (toxicity).[10]

In analogy with the other ANPs, the virus-inhibitory activity of the FPMP analogs is based on their intracellular phosphorylation to give their diphosphates, which subsequently act as terminators of the growing DNA chain.[7,11] In regard of the biological properties of the fluorinated ANPs, main attention was aimed at the derivatives containing adenine, guanine, and 2,6-diaminopurine moieties (Table 1).[12] The anti-HIV effects of both the (*R*) and (*S*) enantiomers of the corresponding guanine (FPMPG) and diaminopurine (FPMPDAP) derivatives are comparable, whereas the activity of the adenine analogue (FPMPA) is strictly enantiospecific.[12] (*S*)-FPMPA is 30-fold more effective an inhibitor of HIV-1 and HIV-2 replication than its (*R*) counterpart and the difference in the antiviral activity of the enantiomers in the adenine series is probably caused by preferential phosphorylation of (*S*)-FPMPA by cellular AMP kinases.[12,13]

In order to improve the antiviral properties of the FPMP derivatives we have decided to perform structure–activity relationship (SAR) study focused on the enantiomeric N^6-substituted FPMP derivatives of adenine and 2,6-diaminopurine represented by the general structure **3** (Fig. 1). Our reasoning was based on the fact that N^6-substitution can considerably increase the antiviral and cytostatic activity[14] of ANPs or enhance their immunostimulatory and immunomodulatory activity.[5e] It has been found that the N^6-substituted 2,6-diaminopurine analogues are metabolized to

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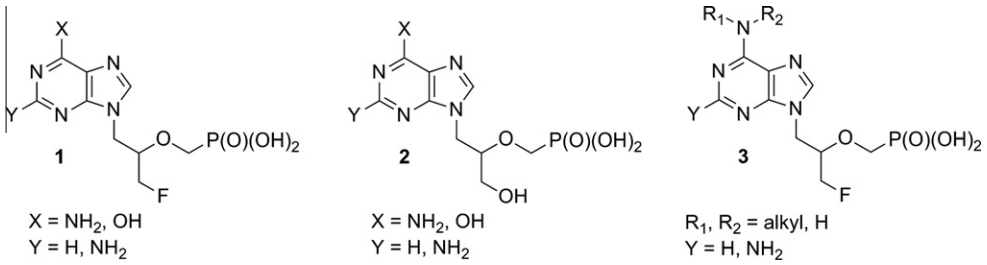


Figure 1. Acyclic nucleoside phosphonates.

Table 1
Anti-HIV-1 activity [EC_{50} (μM)] of purine FPMP analogues in MT-4 cells[12]

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)
(*R*)-FPMPA	272 ± 23.0	>300
(*S*)-FPMPA	8.9 ± 0.03	>300
(*R*)-FPMPG	5.9 ± 1.2	>300
(*S*)-FPMPG	3.9 ± 1.1	103 ± 90
(*R*)-FPMPDAP	4.3 ± 0.9	>300
(*S*)-FPMPDAP	15.0 ± 9.0	>300

[a] Compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cells by 50%.
[b] Compound concentration required to reduce MT-4 cell viability by 50%.

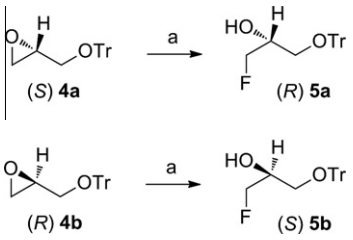
the corresponding guanine counterparts by N^6-methyl-AMP aminohydrolase and thus can be considered as prodrugs.[15]

From a chemical viewpoint, an improved synthetic strategy for the convenient preparation of the purine FPMP analogues has been developed since the original approach was quite laborious and rather expensive.[8b]

2. Results and discussion

2.1. Chemistry

Enantiomeric 3-fluoro-1,2-propanediols[16] were used in the original synthesis of the FPMP derivatives.[8b] In the present work, commercially available enantiomeric O-tritylated glycidols **4a** and **4b** were selected as a convenient starting material (Scheme 1). Glycidols **4** were converted regioselectively and in high yields to the corresponding fluorohydrines **5** by the treatment with potassium hydrogendifluoride and catalytic amounts of tetrabutylammonium dihydrogentrifluoride[17] under solid–liquid PTC (phase transfer catalysis) conditions.[18] Although the stereocenter at the C-2 position of glycidols **4** is not attacked during the reaction,[18] the formal configuration of fluorohydrines **5** is changed to the opposite one (Scheme 1). This fact was confirmed by the X-ray structure of fluorohydrine **5a** (Fig. 2). The data from X-ray crystallography analysis also showed that intramolecular O–H···O hydrogen bonds of the C-2 hydroxyl group play a dominant role in the crystal packing of the compound **5a** (Fig. 3).



Scheme 1. Synthesis of the fluorohydrines **5a** and **5b**. Reagents and conditions: (a) KHF_2, PhCl, cat. $Bu_4NH_2F_3$, 135 °C.

Alkylation of the fluorohydrines **5** with diisopropyl *O*-(*p*-toluensulphonyloxy)methylphosphonate[19] (**6**) with excess of NaH in DMF under standard conditions afforded phosphonates **8** in good yields (Scheme 2). Enantiomer **8a** was also obtained in the same yield (67%) by an alternative procedure using bromomethylphosphonate **7** instead of tosylate **6** under similar reaction conditions.[20]

Detritylation of compounds **8** was carried out in 80% aqueous acetic acid and gave acceptable yields of the derivatives **9**.

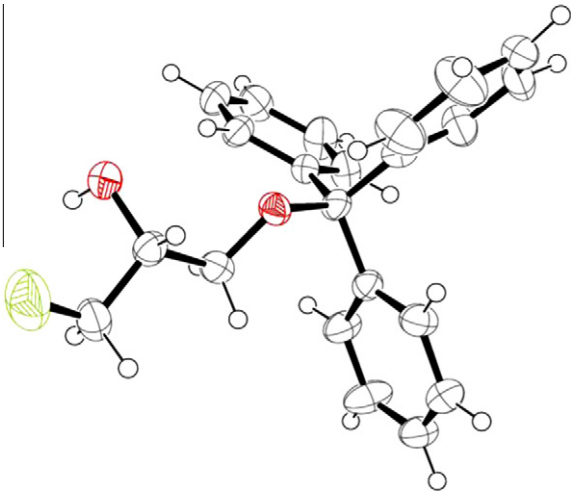


Figure 2. X-ray structure of fluorohydrine **5a**.

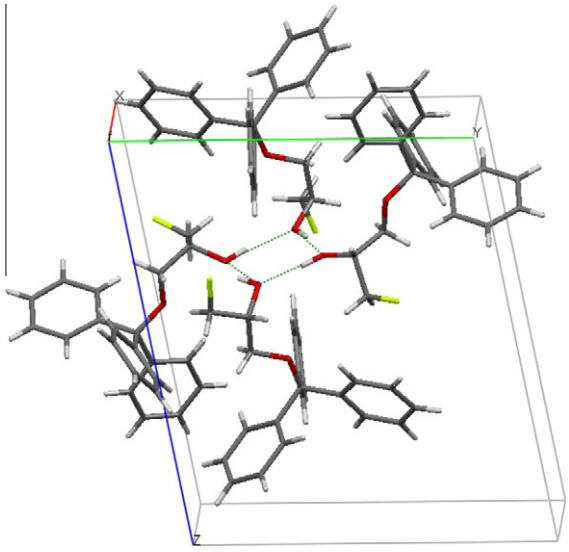


Figure 3. Crystal packing of fluorohydrine **5a**.