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Enantiomeric radiochemical synthesis of R and S (1-(6-amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)methylphosphonic acid (FPMPA)

Dale O. Kiesewetter,[a]* Kathleen Knudson,[a] Matt Collins,[b] Sharat Srinivasula,[c] Esther Lim,[d] and Michele Di Mascio[e]

Therapy for human immunodeficiency virus (HIV)-infected patients requires chronic multidrug administration. The eventual failure of therapy in some patients has brought into question the tissue concentration of the drugs. With an appropriately radiolabeled compound, we could utilize positron emission tomography to provide quantitative time–activity curves for various tissues. We have developed a fluorine-18 labeled analog of Tenofovir, the active metabolite of Tenofovir DF, a commonly prescribed component of multidrug therapy. Because (1-(6-amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)methylphosphonic acid (FPMPA) has a chiral center, we prepared both enantiomers and confirmed that the S-isomer exhibited significantly higher antiviral activity than the R-isomer. In viral replication inhibition assays in human MT4 cells infected with $SHIV_{DH12R}$, S-FPMPA had an IC_{50} of 1.85 μM (95% CI; 0.8–5.53), while the R-isomer was inactive. An appropriate chiral precursor was prepared to allow the incorporation of fluorine-18. The [^{18}F]FPMPA in racemic, R, or S form was prepared in a 50 min synthesis in 38 ± 5% yield ($n = 23$, corrected for decay). The product was of high radiochemical and enantiomeric purity. The specific activity of the final product was 4.0 ± 1.8 Ci/μmol at EOB (end of bombardment). This product may provide information about drug tissue distribution in animal models under chronic drug treatment.

Keywords: fluorine-18; Tenofovir; antiretroviral drugs; HAART

Introduction

Since the introduction of highly active antiretroviral therapy (HAART), there has been a dramatic decrease in human immunodeficiency virus type 1 (HIV-1)-related mortality, and HIV-1 infection has been transformed into a chronic or subacute disease in substantial numbers of treated patients[1]. HAART treatment successfully suppresses plasma viremia in many patients below the threshold of detection of the commonly used viral determination assays in clinical practice (25 virions per mL of plasma). However, more sensitive assays have demonstrated that $CD4^+$ T cells, the major cell target for HIV-1, retain replication-competent viral DNA and may be reactivated to produce virus even after years of viral suppression[2]. Thus, long-term anti-HIV treatment does not permit the eradication of HIV-1 infection and continues to be associated with substantial toxicity[3], adherence difficulties, and drug resistance evolution[4]. Our current knowledge on antiviral efficacy, dosing, and toxicity of available HAART regimens are mostly derived from plasma or blood kinetics of anti-HIV drugs. However, the blood comprises only 2% of total target cells in the body. Tissue drug levels may substantially differ from corresponding plasma levels, and drug distribution processes may be characterized by high intertissue variability, leading to suboptimal target site concentrations and the potential risk for therapeutic failures[5]. Biopsies, for certain lymphoid organs, have frequently been applied to measure drug concentrations in tissues. However, due to the invasive nature of these procedures, it is difficult, for obvious ethical reasons, to perform longitudinal analysis in the same subject, which sets a limit on our knowledge of the *in vivo* kinetics of antiretroviral drugs during the years of chronic treatment in the population of HIV-1-infected patients. One component often included in HAART regimens is Tenofovir disoproxil fumarate (Figure 1), which is rapidly converted *in vivo* to Tenofovir (PMPA {1-(6-amino-9H-purin-9-yl)- propan-2-yloxy)methylphosphonic acid}). This drug is a member of a class of nucleotide analog reverse transcriptase (RT) inhibitors characterized by low metabolism in

[a]*Positron Emission Tomography Radiochemistry Group, National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, USA*

[b]*Bioqual, Rockville, MD, USA*

[c]*Biostatistics Research Branch, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA*

[d]*Philadelphia VA Medical Center, University of Pennsylvania, Philadelphia, PA, USA*

[e]*Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA*

**Correspondence to: Dale O. Kiesewetter, PRG/NIBIB, 10 Center Drive MSC 1180, 10/1C401, Bethesda, MD 20892, USA.*
E-mail: dk7k@nih.gov

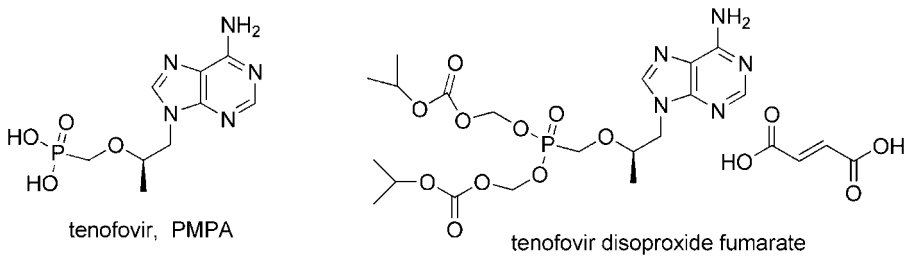

Figure 1. The structures of Tenofovir and its orally active prodrug.

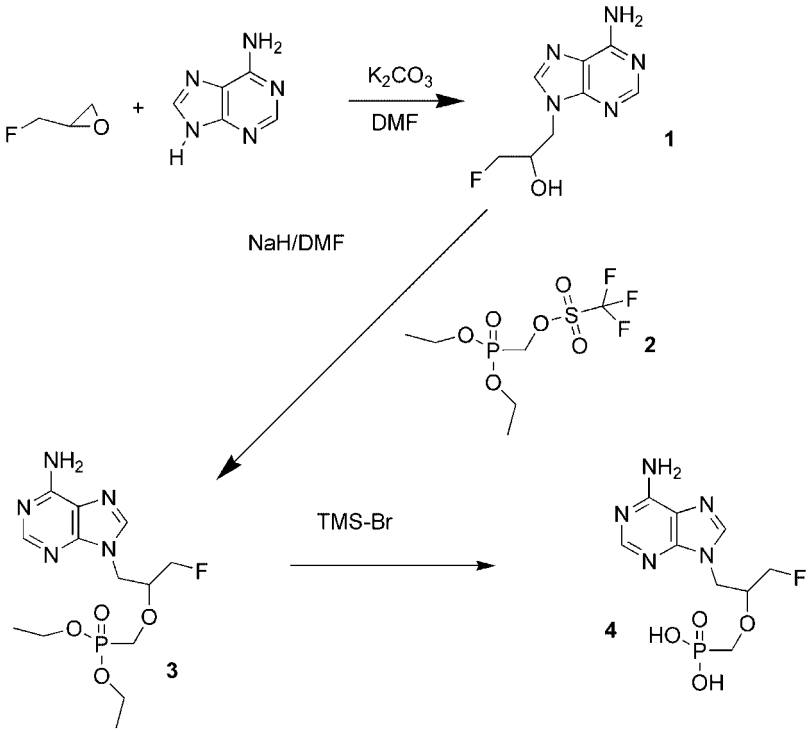

Figure 2. Chemical synthesis of authentic (RS) FPMPA.

the body[6]. The historical development of this class of compounds was recently the subject of a review article[7]. The molecule contains a phosphonate moiety to mimic the 5′-phosphate of the normal nucleotide. Thus, it is converted intracellularly, by infected and uninfected cells, into Tenofovir diphosphate, an acyclic analog of ATP that inhibits viral RT[8]. Positron emission tomography (PET) has greatly expanded the scope of the pharmacokinetic measurements that can be performed noninvasively in humans. Thus, we sought to identify an analog of Tenofovir to be labeled with a positron emitting radionuclide as a candidate PET agent to study antiretroviral tissue kinetics *in vivo* with noninvasive imaging.

The chemical structure of PMPA precludes the rapid incorporation of carbon-11 (*t* 1/2 20 min) into the molecule. There has been a significant amount of work in the development of nucleotide analog RT inhibitors. Fortunately, a fluorinated analog (1-(6-amino-94-purin-9-yl)-3-fluoropropan-2-yloxy (FPMPA), Figure 2, compound 4) had already been prepared[9]. The hydroxyl analog (HPMPA) is also a known antiviral compound. The prior synthesis of HPMPA provided the necessary knowledge for the preparation of a suitable precursor for radiofluorination.

HPMPA, which contains a 3′-hydroxy moiety, was more active against DNA viruses while PMPA, which is unsubstituted, was more active against RNA viruses. FPMPA was also more active against RNA viruses[10]. These molecules possess a chiral center at the second position of the propyl chain. The S enantiomer of FPMPA and R enantiomer of PMPA showed the highest inhibition of retroviral replication[10].

In this article, we report the inhibition of viral replication of the two isomers in rhesus monkey peripheral blood mononuclear cells (PBMC) and human MT4 cells. We also report the successful radiochemical synthesis of racemic [^{18}F]FPMPA as well as of the pure enantiomers (R)- and (S)-[^{18}F]FPMPA.

Results and discussion

The ability of antiretroviral compounds to penetrate into tissue target cells at sufficient levels to prevent residual viral replication is crucial for the long-term control of viremia in HIV-1-treated patients. It is known that antiretroviral compounds have differential penetrations in certain anatomic compartments[11,12] and between subjects[13], raising the possibility that localized sub-optimal concentrations of one drug of the

combination increases the probability of drug resistance strain generation[14], a major cause of drug therapy failure in the population of HIV-1-infected patients. In order to be effective these drugs need to penetrate within cells, the intracellular concentration is the ultimate driving force to define antiviral efficacy. The tissue and intracellular accumulation of HIV drugs is determined primarily by their physicochemical characteristics (e.g. lipophilicity, charge), by plasma protein binding and active transporters, such as P-glycoprotein, and the more recently discovered family of multidrug resistance proteins[15]. It is currently unknown whether differential penetration of drugs in certain anatomic compartments is associated with long-term control of viremia in HIV-1-infected treated patients, mostly because of the lack of techniques that can noninvasively interrogate local drug-level concentrations throughout the body. Indeed, our current knowledge on antiretroviral drug kinetics comes from studies mostly in blood compartments, along with a few studies in cerebrospinal fluid, seminal, and extra-vaginal fluid kinetics from which inference is made on the central nervous system and genital tract compartments. It is also unknown whether, in tissues, changes in active efflux pumps due to prolonged exposure to antiretroviral therapy may explain the waning HAART efficacy during chronic antiviral therapy, thus, mimicking known paradigms of drug failure in anticancer and antibacterial chemotherapy[16,17]. We here describe a radiolabeled derivative of a commonly used antiretroviral compound, PMPA, as a model to noninvasively study biodistribution with PET imaging. PMPA itself was not readily amenable to radiolabeling, but a fluorine-containing analog, FPMPA, was already known that displayed antiviral activity[10,18,19]. Furthermore, of the two enantiomers, S-FPMPA displayed greater inhibitor activity in the viral systems studied[10].

We prepared racemic FPMPA[19] using the literature procedure as a guideline (Figure 2). Adenine was treated with epifluorohydrin to provide 3-fluoro-2-hydroxypropyl adenine. The phosphonate functionality was introduced by coupling (diethoxyphosphoryl)methyl trifluoromethanesulfonate (**2**)[20] with the secondary alcohol of **1** without protection of the 2-amino functional group of adenine. Treatment of **3** with bromotrimethylsilane yielded racemic FPMPA. The synthesis of the respective enantiomers of authentic FPMPA was achieved ultimately by preparation of chiral methanesulfonate precursors (described below) and conducting a fluorination.

For purposes of incorporating fluorine-18 (t 1/2 = 110 min), we required a precursor activated for nucleophilic fluorination. Following fluorination any and all protective groups must be easily removable to allow total synthesis time to be minimized as required by the short half-life of the radionuclide. We prepared the bis(trityl) diethylphosphonate analog of HPMPA (**5**) in three steps from adenine. Following synthesis of the 2, 3-dihydroxypropyl adenine, two trityl groups were introduced at the primary alcohol and the amine using the procedures described by Webb[21] or Jokic and Skaric[22]. We obtained slightly better yields of the bis(trityl) intermediate using the procedure conducted in pyridine[22]. The phosphonate functionality was introduced using the same procedure as for FPMPA. Removal of the trityl protecting groups with acetic acid provided the diethyl phosphonate **6**. Treatment of **6** with methanesulfonyl chloride in dimethyl formamide (DMF) and triethyl amine gave a small amount of a product in which the methanesulfonate had formed at only the primary alcohol. The yield of **7** was greatly enhanced by conducting the reaction in pyridine.

Preparation of the pure enantiomers of the methanesulfonate was achieved using identical chemistry. The enantiomeric diethylphosphonates of HPMPA were prepared by first reacting adenine with the commercially available R or S glycidyl butyrate in DMF in an analogous procedure to that previously described[23]. The enantiomeric purity of the intermediates was not determined. However, as none of the synthetic steps involve cleavage of chemical bonds at the chiral center, we expect the chiral purity to be unchanged from that of the commercial precursors. The butyrates were methanolized to provide the single enantiomer of the diol. This diol was converted to the bis(trityl) compound, **6**, as a single enantiomer. The rest of the procedure follows that described above.

This methanesulfonate, **7**, as a racemic mixture or as individual R or S enantiomers provided for efficient fluorine-18 incorporation under conditions employing Kryptofix 2.2.2 and K_2CO_3 in acetonitrile (Figure 3). Protection of the 2-amino group of adenine was not required. The deprotection of the diethyl phosphonate proved to be rapid using trimethylsilyl bromide, requiring only 5 min (Figure 4). Purification was accomplished by high-performance liquid chromatography (HPLC), which provided the desired radiolabeled [^{18}F]FPMPA. The radiochemical synthesis and purification were accomplished in 38 ± 5% yield (n = 23, corrected for decay) with a synthesis time of about 50 min. Thus, from about 30 mCi of [^{18}F]fluoride one can obtain 6–8 mCi of [^{18}F]FPMPA in less than 1 h. The product was of high radiochemical purity (> 98%) (Figure 5). The specific activity of the product was measured for eight batches and was 4.0 ± 1.8 Ci/μmol at EOB. The HPLC eluate (2% ethanol in 50 mM NaH_2PO_4) may only require adjustment for isotonicity and pH prior to injection for *in vivo* studies. Identity was confirmed by co-elution of the obtained radiochemical product with the unlabeled standard on HPLC. Chiral HPLC of the products demonstrated a single enantiomer in each case (Figure 6).

Inhibitory activity of R-PMPA, (RS)-FPMPA, R-FPMPA, and S-FPMPA

We conducted viral replication inhibition tests in two systems, rhesus monkey PBMC infected with SIVmac239 and human MT4 cells infected with $SHIV_{DH12R}$, which are of interest for our subsequent work. Our assay of R-PMPA inhibition of SIVmac239 provided an IC_{50} of 2.32 μM (95% CI; 1.51–3.34] that was consistent with previously reported data[24] (Figure 7 (a)). Two separate assays with (RS)-FPMPA provided viral growth IC_{50}s of 4.85 μM (95% CI; 3.8–6.67) and 6.66 μM (95% CI; 5.1–8.9) (Figure 7 (b)), which were not statistically significantly different. However, the observed increase in IC_{50} for the racemic (RS)-FPMPA of approximately 2-3-fold compared with the IC_{50} of R-PMPA was highly statistically significant ($P < 0.001$) and consistent with the racemic antiretroviral drug being a 1:1 ratio of two enantiomers, of which only one is active on the RT[10]. Thereafter, the two isolated enantiomers were subsequently tested for viral replication in human MT4 cells infected with $SHIV_{DH12R}$. As expected the S-FPMPA, but not the R-FPMPA, showed inhibitory activity of viral replication with an estimated IC_{50} of 1.85 μM (95% CI; 0.8–5.53) as shown in Figure 7(c) and (d).

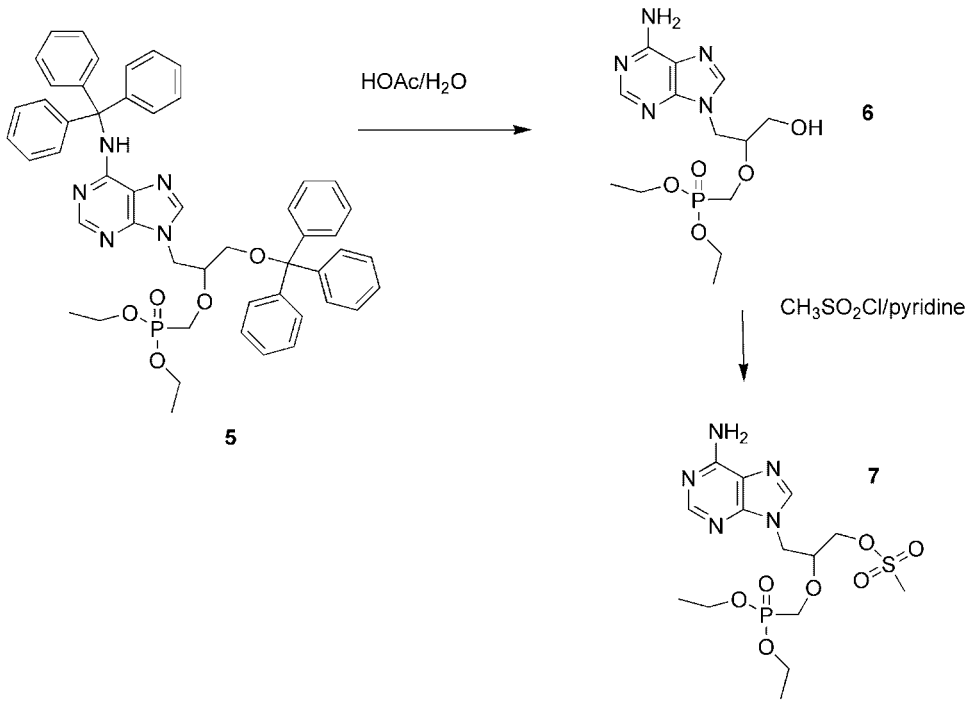

Figure 3. Chemical synthesis of methanesulfonate precursor for radiolabeling. Compound **5** can be prepared in each enantiomeric form.

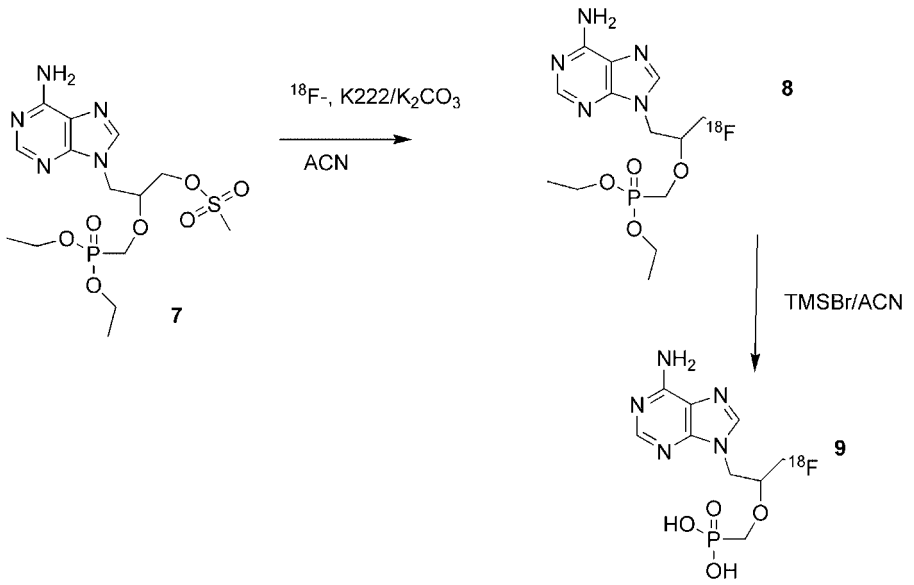

Figure 4. Radiochemical synthesis scheme.

Materials and methods

General: Unless otherwise indicated all chemicals were purchased from commercial sources and used as received. (Diethoxyphosphoryl)methyl trifluoromethanesulfonate (**2**) was prepared by Xu *et al.*'s[20] method. ^{1}H-NMR spectra were obtained on a Varian Gemini 2000 spectrometer at 200 MHz or a Bruker Avance 300 at 300 MHz. ^{13}C-NMR were obtained at 75 MHz on the Bruker Avance 300. Signals are reported downfield from internal tetramethylsilane. Semipreparative HPLC employed a Perkin-Elmer Series 200 pump coupled with an HP1100 detector and data system. Analytical HPLC used an HP1100 system.

1-(6-Amino-9H-purin-9-yl)-3-fluoropropan-2-ol (**1**) was prepared by the reaction of adenine with epifluorohydrin according to the published procedure[19]. Instead of crystallization, we used silica gel chromatography to isolate the title compound.

Diethyl (1-(6-amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)-methylphosphonate (3)

1 (292 mg, 1.39 mmol) was dissolved in DMF (5 mL) and treated with NaH (110 mg, 60% emulsion, 2.76 mmol) and the mixture stirred at room temperature for 80 min. **2** (470 mg, 1.57 mmol)

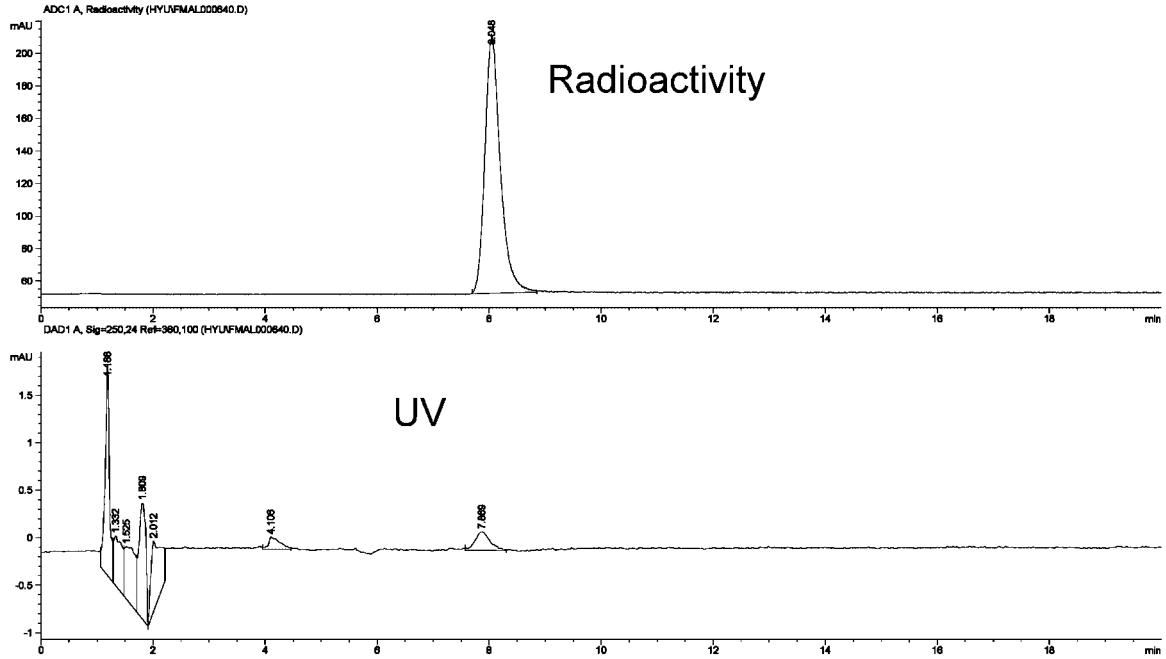

Figure 5. Sample quality control chromatogram of S-FPMPA. Column: Agilent Eclipse XDB-C-18 4.6 × 150 mm. Eluate: 15% CH_3CN, 85% 5 mM $nBu_4NH_2PO_4$, 1 mL/min.

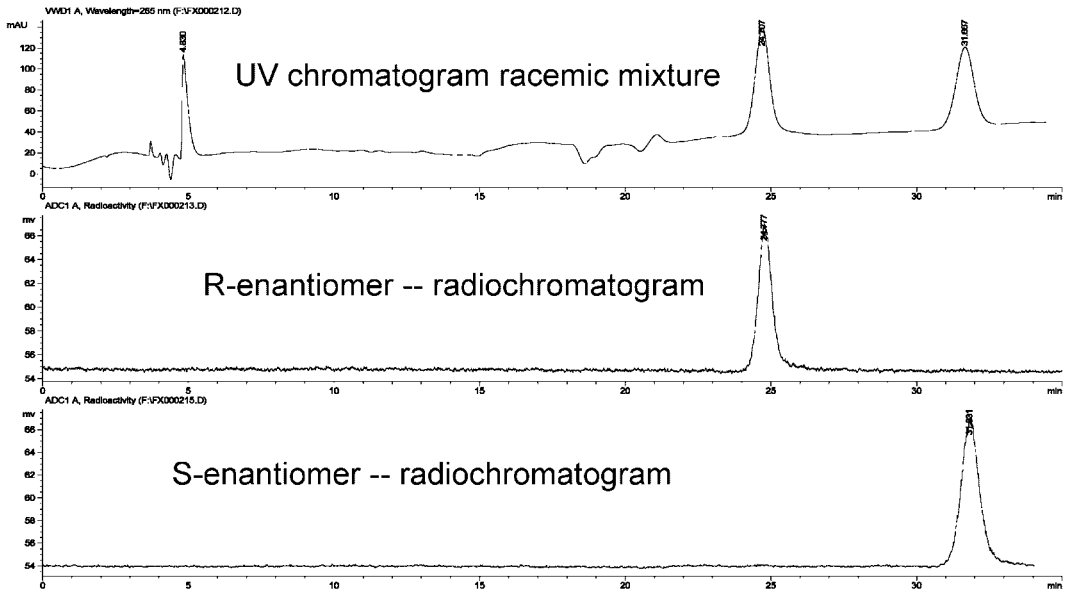

Figure 6. Radiochromatograms of the isolated enantiomers. Column: Luna C-18 4.6 × 250 mm. Eluate: 4 mM $CuSO_4$, 4 mM L-phenyl alanine, 1 mL/min.

was added dropwise over about 5 min and stirred at room temperature for 3 h. At the end of the reaction, the DMF was removed on a rotary evaporator (15 T). Toluene (40 mL) was added and evaporated on the rotary evaporator. The resulting slurry was preabsorbed on silica gel for flash chromatography. Chromatography was conducted on an Analogix system using an Analogix RS-12 cartridge and eluting with 90% chloroform, 9% methanol, 1% ammonium hydroxide. The product was collected (252 mg, 50.2%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.27 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.4 Hz, 3H), 3.7–3.9 (m, 2H), 4.0–4.2 (m, 4H), 4.2–4.2 (m, 2H), 4.4–4.65 (m, 2H), 4.65–4.81 (dd, J = 10, 3.4 Hz, 1H), 5.59 (brs, 2H), 7.94 (s, 1H), 8.36 (s, 1H).

(1-(6-Amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)methylphosphonic acid (4)

3 (204 mg, 0.565 mmol) was dissolved in 3 mL DMF and placed under argon. Bromotrimethylsilane (722 μL, 5.65 mmol) was added via a syringe. The reaction was stirred overnight. The DMF was evaporated on a rotary evaporator; toluene was added and evaporated. The residue was dissolved in 3 mL water; 3 mL acetone was added and the resulting solution cooled. The precipitate was collected and recrystallized from ethanol/ether to give the product (112 mg, 65%). ^{1}H-NMR (200 MHz, DMSO-d6) δ 3.50–3.75 (m, 2H), 3.95–4.20 (dm, J = 21.5, 1H), 4.20–4.60 (m,

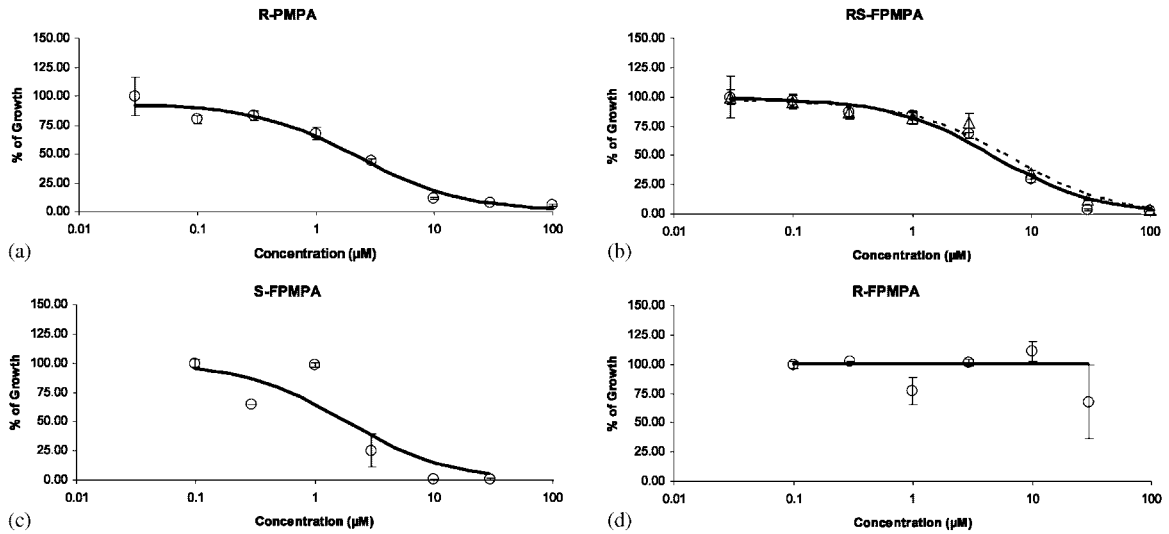

Figure 7. Inhibitory activity of R-PMPA (a) and *RS*-FPMPA (b) in macaque PBMC cells infected with SIVmac239; and of *S*-FPMPA (c) and *R*-FPMPA (d) in human MT4 cells infected with $SHIV_{DH12R}$.

3.5 H), 4.60–4.75 (dd, $J = 10.2$, 3.4 Hz, 0.5H), 7.29 (s, 2H), 8.14 (s, 1H), 8.15 (s, 1H). ^{19}F-NMR (internal TFA) −232.5 (dt, $J = 47.2$, 18.4 Hz). LCMS (ESI), 306 M+H.

(R)-Diethyl (1-(6-(tritylamino)-9H-purin-9-yl)-3-(trityloxy)-propan-2-yloxy)methylphosphonate (5)

(R)-1-(6-(Tritylamino)-9H-purin-9-yl)-3-(trityloxy)propan-2-ol (2.28 g, 3.29 mmol) in DMF (15 mL) was treated with NaH (263 mg (60% in oil)) under argon for 2 h. The mixture was placed in an ice bath and **2** was added dropwise. The solution was stirred at ice temperature for 1 h then set in a refrigerator overnight. The DMF was evaporated under vacuum and the residue absorbed onto 6 g of silica gel. The product was purified by chromatography eluting with 1:1 ethyl acetate:hexane for 7 min then over 1 min transitioning to 100% ethyl acetate. Yield 1.89 g, 68%. The S-isomer was prepared with the same procedure starting from the S precursor in 78% yield. ^{1}H-NMR for R isomer (300 MHz, acetone d-6) δ 1.17 (t, $J = 7.1$ Hz, 3H), 1.20 (t, $J = 7.4$ Hz, 3H), 3.10 (dd, $J = 10.5$, 5.0 Hz, 1H), 3.28 (dd, $J = 10.4$, 4.2 Hz, 1H), 3.7–3.9 (m, 1H), 3.9–4.15 (m, 6H), 4.3–4.6 (m, 2H), 6.97 (s, 1H), 7.1–7.3 (m, 18H), 7.3–7.5 (m, 12H), 7.82 (s, 1H), 8.02 (s, 1H).

(R)-Diethyl (1-(6-amino-9H-purin-9-yl)-3-hydroxypropan-2-yloxy)methylphosphonate (6)

Intermediate **5** (1.89 g, 2.24 mmol) was treated with 50 mL of 80% HOAc in water. The mixture was heated at 80°C for 2 h. The mixture was allowed to stand at room temperature for 2 h then placed in a refrigerator overnight. The mixture was filtered to collect some of the triphenylmethanol side product. The filtrate was evaporated and purified by chromatography. The sample was preabsorbed on 3 g silica gel and eluted with 90:9:1 ($CHCl_3$:MeOH:NH_4OH) to give the product in 66% yield. The S-isomer was similarly obtained in 69% yield ^{1}H-NMR for R-isomer δ 1.11 (t, $J = 7.0$ Hz, 3H), 1.16, (t, $J = 7.0$ Hz, 3H), 3.47 (t, $J = 5.3$ Hz, 2H), 3.7–3.9 (m, 7H), 4.20 (dd, $J = 14.5$, 7.4 Hz, 1H), 4.35 (dd, $J = 14.5$, 3.6 Hz, 1H), 4.98 (t, $J = 5.7$, 1H, OH), 7.20 (s, 2H), 8.04 (s, 1H), 8.13 (s, 1H). ^{13}C-NMR for S-isomer, 16.1, 43.4, 59.9, 61.4, 61.5, 61.5, 61.6, 61.7, 63.6, 80.0 (d, $J = 11.4$ Hz), 118.3, 141.3, 149.6, 152.2, 155.8.

(R)-3-(6-Amino-9H-purin-9-yl)-2-((diethoxyphosphoryl)-methoxy)propyl methanesulfonate (7)

Alcohol **6** (500 mg, 1.39 mmol) was dissolved in 10 mL pyridine and treated with methanesulfonyl chloride (191 mg, 1.67 mmol). The reaction was followed by thin layer chromatography (TLC). After 1 h, an additional equivalent of methanesulfonyl chloride was added, followed an hour later by a third equivalent. The pyridine was evaporated; the residue dissolved in ethanol and preabsorbed onto 3.5 g of silica gel. The material was chromatographed under the following program: 100% ethyl acetate for 1 min followed by a gradient over 20 min to 100% (20% (10% NH_4OH in ethanol) 80% EtOAc). The yield was 455 mg, 75 %. The S-isomer was similarly obtained in 88% yield. ^{1}H-NMR for R isomer (300 MHz, DMSO d-6) δ 1.13 (t, $J = 7.1$ Hz, 3H), 1.18 (t, $J = 7.1$ Hz, 3H), 3.34, (s, 3H), 3.8–4.1 (m, 6H), 4.2–4.6 (m, 5H), 7.24 (s, 2H), 8.10 (s, 1H), 8.16 (s, 1H). ^{13}C for S-isomer (75 MHz, DMSO d-6) δ 16.1 (t, $J = 5.4$ Hz), 36.7, 42.9, 61.6 (d, $J = 6.4$ Hz), 61.6, 61.8 (d, $J = 6.4$ Hz), 77.1 (d, $J = 13.1$ Hz), 118.5, 149.6, 152.4, 155.9.

R- or S- (1-(6-Amino-9H-purin-9-yl)-3-fluoropropan-2-yloxy)-methylphosphonic acid (FPMPA, 9)

The corresponding chiral methanesulfonates **7** were suspended in CH_3CN and treated with 3-fold excess of tetramethylammonium bifluoride and heated at 80°C for 3 h until TLC indicated consumption of starting material. The product was loaded onto a silica gel column and eluted with 90:9:1 ($CHCl_3$:MeOH:NH_4OH). The major component was dissolved in 0.5 mL DMF and treated with 10 equivalents of bromotrimethylsilane overnight. The DMF and bromotrimethylsilane were removed under vacuum, the residue taken up in water and the product precipitated with acetone. The products were about 85% pure by HPLC (Agilent XDB C-18, 85% (5 mM tetrabutylammonium phosphate, TBAP) 15% CH_3CN). Higher purity (98%) was obtained after preparative HPLC (Luna C-18 2% ethanol in

50 mM NH_4OAc) of part of the sample. NMR indicated that the samples contained 1–2 equivalents of acetate after drying under vacuum.

^{1}H-NMR for R isomer (300 MHz, D_2O) δ 3.51 (dd, $J = 13.1$, 9.7 Hz, 1H), 3.69 (dd, $J = 13.1$, 9.2 Hz, 1H), 4.04 (dm, $J = 22$ Hz, 1H), 4.3–4.60 (m, 3.5H), 4.71 (dd, $J = 10.6$, 3.6 Hz, 0.5 H—part of $C\underline{H}_2F$). R-, S- or RS (1-(6-Amino-9H-purin-9-yl)-3-[^{18}F]fluoropropan-2-yloxy)methylphosphonic acid ([^{18}F]FPMPA, **9**). A reaction tube (13 × 100 mm) was charged with Kryptofix 2.2.2 (4.5 mg, 12 μmol), K_2CO_3 (60 μL of 0.1 M in water, 6 μmol), and F-18 fluoride. The solution evaporated to dryness. Three portions of CH_3CN were added and each in turn evaporated to dryness. A solution of methanesulfonate **7** (3 mg, 8 μmol) in 300 μL of CH_3CN was added to the dried residue. The resulting mixture was heated at 105°C for 5 min. The solution was loaded onto a short column of silica gel. The reaction tube was rinsed with 200 μL of 15% ethanol in CH_3CN; this solution was also added to the silica gel column. The combined liquids were pushed through the silica gel column and the column was eluted with an additional 600 μL of 15% ethanol in CH_3CN. The combined elute was assayed and evaporated to dryness. CH_3CN (100 μL) and bromotrimethylsilane (100 μL) were added. The resulting solution was heated at 75°C for 1 min then allowed to stand at room temperature for 4 additional minutes. The solution was evaporated to dryness; the residue was taken up in 200 μL of HPLC eluant and injected onto the HPLC (Luna C-18(2) 10 × 250, eluted with 2% ethanol in 10 mM NaH_2PO_4). The radioactive peak eluting at ~15 min was collected. The eluant, after adjusting for isotonicity and pH, may be suitable for direct injection into animals. Specific activity was measured by re-injection onto an Agilent XDB-C18 column, eluting with 15% CH_3CN in 5 mM TBAP and comparing the mass peak at 250 nm with a standard curve ($t_R = 7.7$ min).

Determination of enantiomeric purity: A Phenomenex Luna C-18 (4.6 mm × 250 mm × 5 μm) was eluted with 4 mM CuSO4 and 4 mM L-phenylalanine at 1 mL/min. UV absorbance was monitored at 260 nm (retention time for S enantiomer 32 min; for R enantiomer 24 min).

Assay of virus replication in rhesus monkey PBMC and human MT4 cells

The inhibitory activity of nonradiolabeled (RS)-FPMPA and R-PMPA was assessed in macaque PBMC infected with SIVmac239. The preparation and infection of macaque PBMC has been previously described[25,26]. Briefly, PBMC were stimulated with concavalin A and cultured in the presence of recombinant human IL-2 for 3 days prior to viral inoculation. Subsequently, PBMC were cultured in 96 well plates in the presence of different concentrations of (RS)-FPMPA or R-PMPA and spinoculated (1200 × *g* for 1 h)[27] with virus stocks at a multiplicity of infection (MOI) of 10^{-2}. Each assay was performed in six replicates. On day 5 from viral inoculation, culture supernatants were collected from PBMC to assess virus replication by virion-associated RT assay[28]. The inhibitory activity of R-FPMPA and S-FPMPA was determined in human T- leukemic cells (MT4)[29] infected with $SHIV_{DH12R}$. Briefly, MT4 cells were cultured in RPMI 1640 enriched with 10% fetal bovine serum and infected with $SHIV_{DH12R}$ with virus stocks at an MOI of 10^{-3}. After 2 h incubation, the cells were washed to remove unbound virions. The infected cells were cultured for 7 days in the presence of different concentrations of R-FPMPA or S-FPMPA. Each assay was performed in triplicate. On day 7, p24 ELISA assay was performed on culture supernatant to determine viral burden[30]. For both assays, results were expressed as a percentage of growth of the virus.

Statistical methods

The IC_{50} values are determined by nonlinear regression analysis using the equation of a sigmoid plot. For each assay, the mean value of the replicates at different concentrations is fitted to the sigmoid curve using the Levenberg–Marquardt Algorithm[31].

The best fit theoretical curve is then used to locate the IC_{50}, the concentration of the antiviral compound needed to inhibit 50% of viral growth. The 95% confidence interval of IC_{50} is calculated by a bootstrap method[32]. With this method, the best fit theoretical curve is challenged by the original observed data (mean value of the replicates) by generating surrogate data, obtained by shuffling without replacing the residual difference between the theoretical and the observed data and adding the residual to the observed data. This procedure is repeated 1000 times, and the 95% bootstrap percentile interval is calculated from these bootstrap IC_{50}'s. The *P*-value of the difference between estimates of IC_{50} in two independent plates was obtained from a permutation test on the distribution of the bootstrap values.

Conclusions

We have confirmed in our system of retroviral replication that the S-FPMPA is the more potent enantiomer. We have developed a robust radiochemical synthesis method for the preparation of R- or S-[^{18}F]FPMPA that is suitable for preclinical studies of the utility for imaging drug biodistribution. The radiochemical synthesis provides the desired product in 38 ± 5% yield and requires about 50 min. This product is suitable for further studies of biodistribution. Potential pre-clinical and clinical applications of this novel PET tracer are (1) tissue drug kinetics during chronic treatment; (2) drug interactions; and (3) generalization of the concept of antiviral efficacy of HAART regimens.

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