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Synthesis and anti-HIV activity of GS-9148 (2′-Fd4AP), a novel nucleoside phosphonate HIV reverse transcriptase inhibitor

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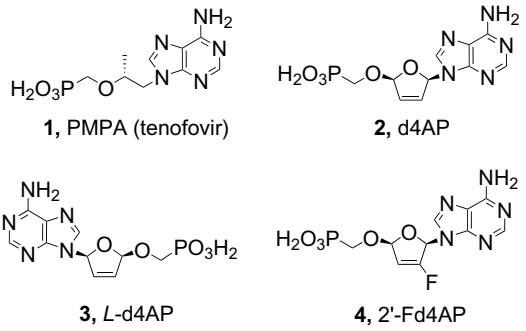
Abstract—GS-9148 (2′-Fd4AP, **4**) has been identified as a nucleoside phosphonate reverse transcriptase (RT) inhibitor with activity against wild-type HIV (EC_{50} = 12 μM). Unlike many clinical RT inhibitors, relevant reverse transcriptase mutants (M184V, K65R, 6-TAMs) maintain a susceptibility to 2′-Fd4AP that is similar to wild-type virus. The 2′-fluorine group was rationally designed into the molecule to improve the selectivity profile and in preliminary studies using HepG2 cells, compound **4** showed no measurable effect on mitochondrial DNA content indicating a low potential for mitochondrial toxicity.

Over the past decade, great strides have been made in the treatment of HIV infection through use of drug combinations known as highly active anti-retroviral therapy (HAART). HAART regimens usually include three or more drugs with inhibitory activity against either HIV reverse transcriptase (RT) or HIV protease.[1] Currently, most HAART regimens include a backbone of two nucleos(t)ide reverse-transcriptase inhibitors (N(t)RTIs) with a third agent added from the non-nucleoside reverse-transcriptase inhibitor (NNRTI) or protease inhibitor classes (PI).[2]

Tenofovir-DF (Viread®) is an orally available prodrug of PMPA (**1**, Fig. 1) and is currently the only clinically approved NtRTI for treatment of HIV infection. While regimens containing Viread have demonstrated excellent efficacy and safety profiles,[3] a subset of treatment-experienced patients harbor tenofovir-resistant virus either through presence of the K65R RT mutation or multiple thymidine-analog mutations (TAMs) in RT (4.3% and 8–31%, respectively).[4] The cyclic nucleoside phosphonate, d4AP (**2**, Fig. 1), identified earlier,[5] demonstrated a superior resistance profile compared to tenofovir and other NRTIs (Table 1).[6]



1, PMPA (tenofovir) **2,** d4AP **3,** *L*-d4AP **4,** 2′-Fd4AP

Figure 1. Tenofovir (**1**); cyclic analog, d4AP (**2**); and targets L-d4AP (**2**) and 2′-Fd4AP (**3**).

The N(t)RTIs are metabolized to their corresponding triphosphates (in the case of NRTIs) and diphosphophosphonates (in the case of NtRTIs). These phosphorylated species inhibit RT through incorporation into the growing DNA chain and subsequent termination. Unfortunately, many of the same triphosphates exhibit mitochondrial toxicity mediated through inhibition of DNA polymerase-γ (pol-γ).[7] As a result, patients treated with N(t)RTIs may experience changes in body fat distribution, hyperlipidemia, peripheral neuropathy, and lactic acidosis. Unfortunately, d4AP **2**, despite its excellent resistance profile, was shown to have mitochondrial toxicity commensurate with its anti-HIV activity, limiting any further development of this

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Table 1. Anti-HIV activity, enzymatic potency, cytotoxicity, and resistance profile of nucleoside phosphonates **1**–**4**

Compound	WT HIV[a] EC_{50}/μM	WT RT[b] IC_{50}/μM	MT-2[c] CC_{50}/μM	K65R[d] Fold res.	6TAMs[d] Fold res.	M184V[d] Fold res.
PMPA (**1**)	3.6 (1.5)	0.38 (0.20)	>500	4.3 (1.5)	8.8 (3.7)	0.7 (0.2)
d4AP (**2**)	2.1 (1.0)	0.60 (0.16)	>1000	2.9 (1.1)	2.9 (1.0)	0.9 (0.6)
L-d4AP (**3**)	5.9 (0.3)	—	>1000	3.6 (2.0)	13.7 (2.3)	7.1
2′-Fd4AP (**4**)	12.3 (3.4)	1.9 (0.8)	>1000	1.1 (0.2)	4.3 (0.4)	0.8 (0.3)
2′-Fd4A[e]	2.2 (1.3)	0.4 (0.2)	—	2.2 (0.4)	3.0 (0.4)	1.2 (0.7)
AZT[f]	0.16 (0.12)	0.05 (0.03)	>200	1.2 (0.7)	>50	0.7 (0.2)

[a] Antiviral activity in MT-2 cells infected with HIV-1 IIIb. Values are results of at least 2 experiments, standard deviation is given in parentheses.
[b] Data were obtained for the corresponding diphosphophosphonate or triphosphate of compound shown.
[c] Cytotoxicity in uninfected MT-2 cells.
[d] Resistance determined in MT-2 cell lines.
[e] See Ref. 17.
[f] AZT = 3′-azido-2′,3′-dideoxythymidine.

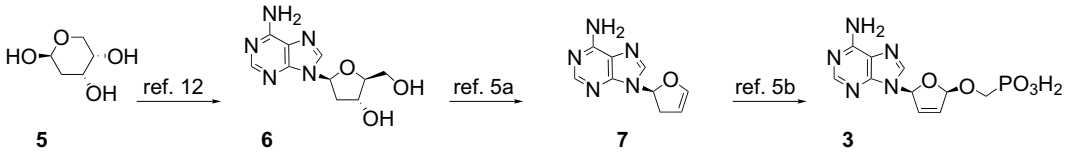
nucleoside phosphonate as an anti-HIV drug.[8] Therefore, we sought to design analogs of **2** that preserve the favorable resistance profile, while abrogating the mitochondrial toxicity.

Two strategies have been reported in the literature with regard to mitigating the toxicity of nucleosides. First, L-nucleosides demonstrate improved toxicological profiles over their D-nucleoside counterparts.[9] Second, 2′-β-F nucleosides appear to be weaker inhibitors of DNA polymerase-γ than their non-fluorinated analogs,[10] and thus are expected to have reduced mitochondrial toxicity.[11] Consequently, our targets became the L isomer of d4AP (**3**), and the 2′-fluorine substituted derivative, 2′-Fd4AP (**4**). Since *F* is a relatively conservative substitution for *H*, we reasoned that **4** was likely to preserve the desirable resistance profile of **2**. Herein we disclose the chemistry developed for synthesis of **3** and **4**, and our initial biological characterization.

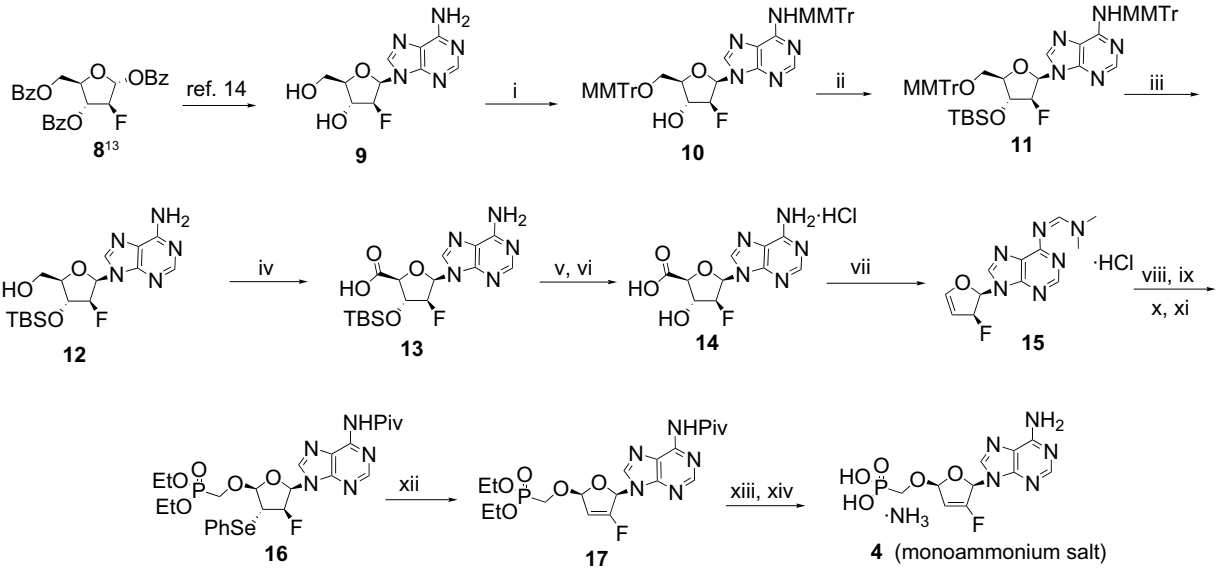
The synthesis of analog **3** (L-d4AP, Scheme 1) proceeded analogously to **2**, reported earlier.[5] Since 2′-deoxy-L-adenosine (**6**) was not available in substantial amounts, it was synthesized according to literature procedure[12] from 2′-deoxy-L-ribose (**5**). The identical synthetic sequence to that reported for **2** was then carried out, with **7** being the pivotal intermediate for subsequent introduction of the phosphonate functionality. L-d4AP (**3**) was obtained in 3% overall yield over 12 steps. C-18 gravity purification was necessary to obtain salt-free material after phosphonic ester deprotection.

Our strategy toward **4** exploited a similar glycal intermediate to **7**, except with the fluorine atom incorporated (compound **15**, Scheme 2). Starting from **8**,[13] compound **9** was obtained employing the method of Schaerer et al.[14] Selective oxidation of the 5′-carbon of **9** in the presence of the unprotected 3′-OH via known methods (catalytic oxidation[15] or $KMnO_4$[5a]) did not proceed smoothly. Thus, a sequential protection/deprotection sequence was required. Compound **9** was 5′-OH protected as a bis-monomethoxytrityl derivative **10**, followed by 3′-OH silylation to give **11**, and then the trityl groups were removed under acidic conditions. Optimal yields were obtained when the deprotection mixture was diluted with a high-boiling alcohol, such as 1-butanol, before concentration in vacuo; this ensured complete scavenging of the monomethoxytrityl cation. The resulting monomethoxytrityl-butyl ether could then be removed by trituration with Et_2O. Alcohol **12** was then oxidized by treatment with TEMPO/BAIB[16] to give carboxylic acid **13**. We attempted to remove the silyl ether of **13** under mineral acid conditions in order to simplify isolation of this polar, water-soluble product. Unfortunately, the material was refractory to deprotection with acid and required treatment with excess TBAF in THF. Fortunately, tetrabutylammonium carboxylate salt of this product precipitated from the deprotection mixture allowing for isolation of TBAF-free product. A salt exchange was then performed to remove the tetrabutylammonium salts by coevaporating three times from 1 N HCl in THF followed by trituration with THF. Compound **14** (HCl salt) was obtained in 35% yield from **9** over the six steps.

It was serendipitous that **14** was transformed to its HCl salt for characterization since attempts to prepare the glycal **15** from the tetrabutylammonium carboxylate salt of **14** by decarboxylative dehydration led to formation of 9-(furan-2-yl)-9*H*-purin-6-amine, presumably via elimination of HF. However, when care was taken to ensure complete conversion of **14** to the HCl salt, clean conversion to the desired glycal ensued (determined by LC–MS). Glycal **15** was activated with phenylselenyl chloride and underwent subsequent $AgClO_4$-mediated glycosylation with diethyl hydroxymethylphospho-



Scheme 1. Synthesis of L-d4AP. Glycal **7** was a pivotal intermediate in synthesis of **3**.



Scheme 2. Reagents and conditions: (i) MMTrCl, pyridine, 93%; (ii) TBSCl, imidazole, DMF, 84%; (iii) HCOOH/MeOH/CH_2Cl_2 (2:1:1) 2.5 h then excess *n*-butanol, 74%; (iv) TEMPO, BAIB, CH_2Cl_2, H_2O, 80%; (v) TBAF, THF; (vi) 1 N HCl in THF, 76% from **13**; (vii) DMF-dineopentylacetal, DMF, 130 °C, 3–4 min; (viii) PhSeCl, THF, −78 °C to rt; (ix) $HOCH_2PO_3Et_2$ then $AgClO_4$ followed by filtration and evaporation; (x) NH_3/MeOH, then evaporation; (xi) PivCl, pyridine, 13% from **14**; (xii) O_3, CCl_4, −15 °C then purge excess O_3 and 50 °C for 3 h, 78%; (xiii) NaOMe, MeOH, 100%; (xiv) TMSBr, DMF, 55 °C then excess 2,6-lutidine, MeOH and evaporation, then conc'd NH_4OH, 72% from **17**.

nate,[5b] in a sequence that proceeded stereoselectively to yield the 4′-β-phosphonomethoxy-3′-α-phenylselenide isomer.

Under the conditions of glycal formation, the nucleobase was transformed into its formamidine derivative. This functional group was cleaved in situ with NH_3 in MeOH immediately after the phosphonate was introduced. The resulting product was protected by treatment with PivCl in pyridine to provide **16** in 13% overall yield from **14**. It was noteworthy that the transformation of **14** to **15** was unusually sensitive to reaction time and internal temperature. As a consequence, the reaction was performed by submersing a thin-walled, round-bottom flask into a preheated oil bath (130 °C) for 3–4 min and then rapidly transferring it into a cooling bath. Successful transformations could be performed on up to 100-mg scale, whereas larger scale reactions led to the elimination of HF, likely due to the inability to precisely control the internal reaction temperature.

Formation of the d4 functionality from the phenyl selenide **16** was also non-trivial. Standard methods such as H_2O_2 did not efficiently oxidize the selenium and less well-known methods (e.g., Ti(O–*i*-Pr)$_4$ and *t*-BuO_2H)[18] led to complex mixtures. The oxidized phenylselenide of **16**, which ultimately formed with O_3[19] in CCl_4 at −15 °C, was stable and could be isolated at room temperature. This oxidized product was forced to eliminate to the desired d4 product by heating the CCl_4 solution to 50 °C, after completely purging residual O_3 from the reaction flask. Only the desired d4 derivative was isolated with no evidence for formation of the 3′–4′ regioisomer. Finally, standard deprotection conditions were used to cleave the pivaloyl group and dealkylate the phosphonic acid. The free phosphonic acid was converted to its monoammonium salt with excess aqueous NH_3 and the final product isolated by gravity C-18 chromatography eluting with neat H_2O. In order to determine the inhibition of isolated RT, the diphosphophosphonate of this compound was synthesized according to literature procedure.[20]

The compounds were tested for their whole-cell anti-HIV activity in MT-2 cells infected with wild-type, 6-TAMs or K65R RT mutants (Table 1). The panel also included the M184V RT mutant, which is found in 45% of the HAART treatment-experienced population.[4] While L-d4AP, which to the best of our knowledge represents the first reported antiviral L-nucleoside phosphonate, retained good wild-type anti-HIV activity relative to PMPA and d4AP, it was considerably less active against the M184V mutant. Given the prevalence of this mutation clinically, our interest shifted to 2′-Fd4AP (**4**).

While compound **4** was 6-fold less potent against wild-type virus than d4AP itself and 3-fold less potent than PMPA (Table 1, column 2), it retained excellent activity against several RT mutants. The marginal loss in WT anti-viral potency (EC_{50}) compared to phosphonates **1** and **2** compares nicely with the 3- to 5-fold reduced inhibition of RT by the active metabolites (Table 1, column 3). Thus, for this series of adenine phosphonate analogs, it would appear that the permeability and phosphorylation properties are similar.

Compound **4** experienced no loss of potency against the K65R RT mutant compared with wild-type virus,

whereas d4AP (**2**) was 3-fold, and PMPA (**1**) 4-fold less active. The loss of activity toward the 6TAMs mutant was comparable between the fluorinated **4** and non-fluorinated **1** analogs (~3- to 4-fold), but slightly improved over PMPA (8.8-fold). No loss of potency was observed for the natural D analogs toward the M184V RT mutant. Overall the resistance profile of the fluorinated analog **4** is improved over both PMPA and d4AP.

For comparison, 2′-Fd4A, the nucleoside analog of phosphonate **4**, was tested and found to be approximately 6-fold more potent against wild-type HIV. Much of this difference in potency is once again due to the approximate 5-fold improvement in potency toward RT inhibition (IC_{50} = 1.9 μM vs 0.4 μM for the active metabolites of **4** and 2′-Fd4A, respectively).[6] In earlier work from this group, the d4 nucleoside phosphonate series of analogs, when compared to their d4 nucleoside counterparts, consistently demonstrate a small loss in RT inhibition of <10-fold. In this respect, d4 nucleoside phosphonates are quite good 'bioisosteres' of the d4 nucleoside monophosphates. This is also reflected in the similar resistance profiles between 2′-Fd4A and **4** noted in Table 1.

Initial toxicological studies have yielded promising results. Compound **4** showed no cytotoxicity to MT-2 cells up to 1 mM. Perhaps most importantly, compound **4** demonstrated no depletion of mitochondrial DNA from HepG2 cells when tested at up to 300 μM for 14 days.[8] Complete biological and toxicological profiling of this compound will be published concomitant with this work.

Compound **4**, 2′-Fd4AP, is a new nucleoside phosphonate derivative with a good resistance profile (no resistance by M184V and K65R, minimal resistance from 6-TAMs) and promising toxicological profile compared to its non-fluorinated analog d4AP (**2**) and PMPA (**1**). A derivative of **4** is currently undergoing clinical investigation.

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