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Synthesis and anti-HIV activity of 2′-fluorine modified nucleoside phosphonates: Analogs of GS-9148

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Abstract—Modified purine analogs of GS-9148 [5-(6-amino-purin-9-yl)-4-fluoro-2,5-dihydro-furan-2-yloxymethyl]-phosphonic acid (2′-Fd4AP) were synthesized and their anti-HIV potency evaluated. The antiviral activity of guanosine analog (2′-Fd4GP) was comparable that of to 2′-Fd4AP in MT-2 cells, but selectivity was reduced.

There are currently eight approved nucleos(t)ide HIV reverse transcriptase (RT) inhibitors (N(t)RTI), but due to cross resistance and long term toxicities there remains a clinical need to develop novel n(t)RTI that can expand the current treatment options, especially for HIV patients harboring resistant virus.[1] In the preceding paper, we described the synthesis and anti-HIV evaluation of GS-9148 (**1**, [5-(6-amino-purin-9-yl)-4-fluoro-2,5-dihydro-furan-2-yloxymethyl]-phosphonic acid, 2′-Fd4AP), a novel nucleoside phosphonate RT inhibitor (Fig. 1).[2] GS-9148 has a promising antiviral resistance profile, retaining potency toward HIV RT resistant virus containing M184V, multiple thymidine analog mutations (TAMs), and K65R resistance mutations.[3] GS-9131 (**2**), an ethylalaninyl phosphonoamidate prodrug of GS-9148, demonstrated excellent potency toward multiple subtypes of HIV-1 clinical isolates (mean EC_{50} = 37 nM), several fold better than 3′-azido-2′,3′-dideoxythymidine (**3**, AZT).[3] The amidate prodrug is readily hydrolyzed inside lymphoid cells to parent **1** and subsequently anabolized to the active diphosphate metabolite.[4] The diphosphate metabolite has demonstrated a long intracellular half-life in vitro (activated CD4+ cells of 19 h) and in vivo (circulating dog PBMCs of >24 h), a property that would support once-daily dosing.[3,4] Based on these data we have continued to explore

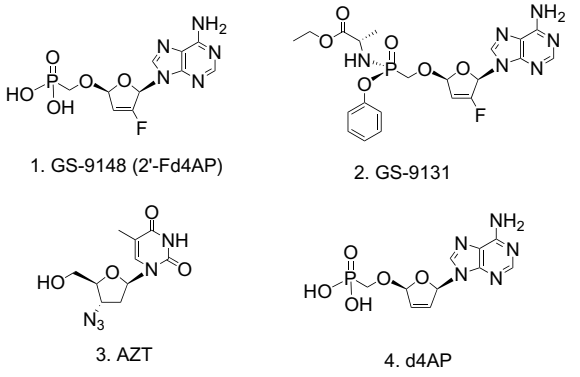

Figure 1. HIV RT inhibitors.

the SAR around GS-9148 with the aim of further improving the potency and resistance profile.

The 2′-fluorine group of **1** was rationally designed into d4AP (**4**) to address undesirable activity toward host mitochondrial DNA γ polymerase.[2,3,5] The success of this modification demanded that it be retained in future analogs. Hence the next generation analogs of **1** focused on modifications of the purine base and reducing the saturated ring. The saturated *beta*-fluorine analog **6** was easily prepared by radical deselanation of **5**, an intermediate in the synthesis of **1**, followed by hydrolysis of the ester and pivaloyl groups (Scheme 1).[2,6] The synthetic route that yielded **1** also formed the basis for the preparation of additional purine analogs **19**, **24**, and **26**

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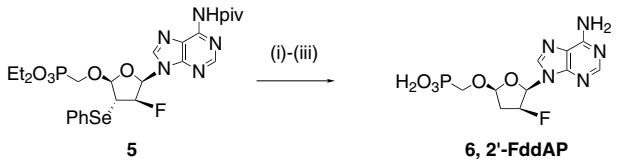
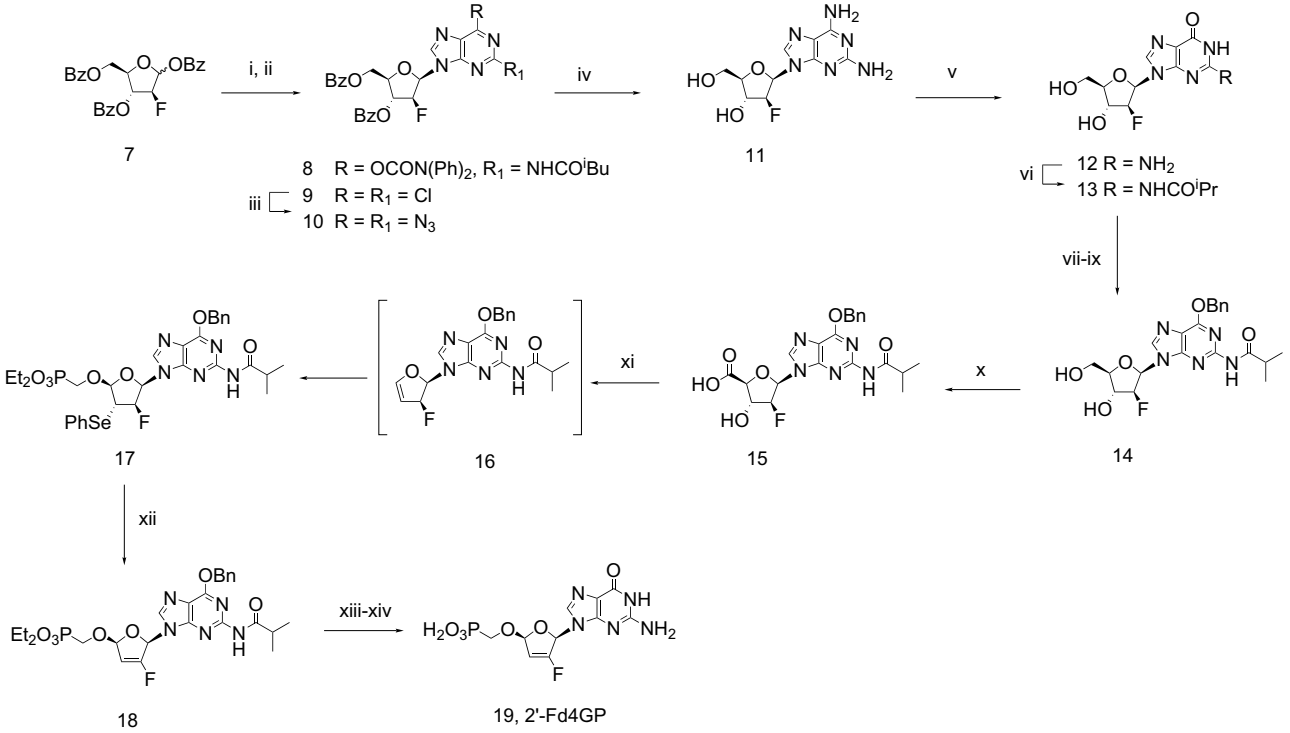


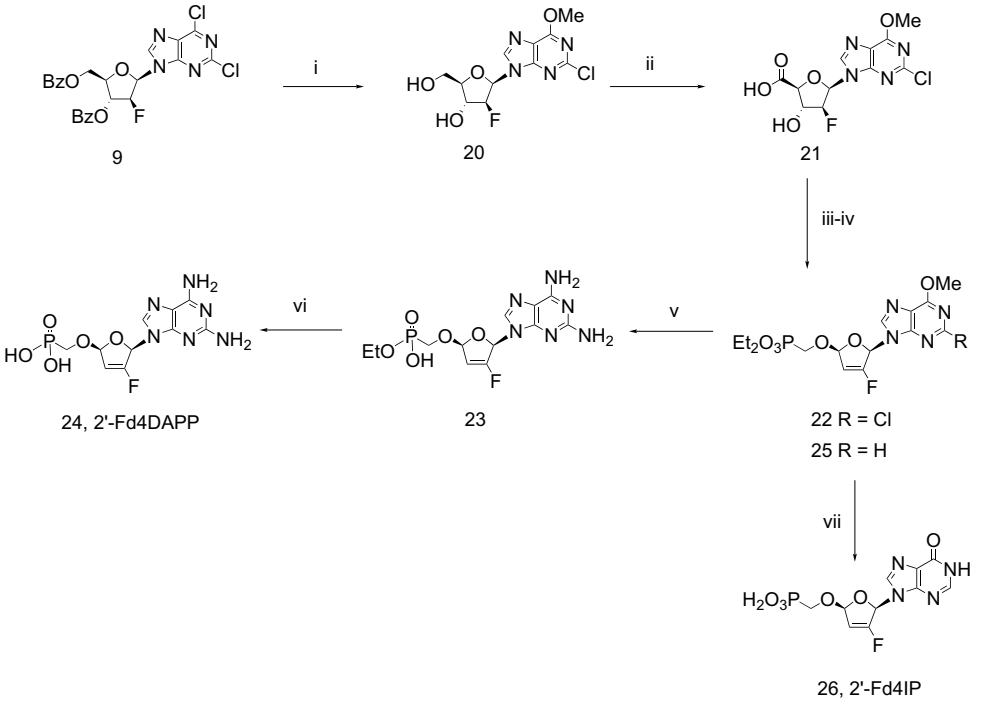
Scheme 1. Reagents and conditions: (i) *n*-Bu_3SnH, AIBN, benzene; (ii) NaOMe, MeOH, 100%; (iii) TMSBr, DMF, lutidine, 50 °C, then NH_4OH.

(Scheme 2 and 3). The focus was directed specifically to purine analogs since earlier pyrimidine analogs of **4** demonstrated poor resistance profiles toward key HIV mutations.[7] Guanosine analog **19** was prepared from the fluoro sugar **7** according to Scheme 2. Initially, conversion of **7** to the anomeric bromide, by treatment with HBr,[2] was followed by the attempted addition of 2-*N*-isobutyryl amide, 6-*O* diphenylcarbamoyl protected guanine to provide **8**. Unfortunately, the addition was low-yielding and also resulted in significant amounts of the *N*-7 substituted product that made efficient isolation of the desired *N*-9 product problematic. Exploration of alternative guanine precursors identified 2,6-dichloropurine as the optimal precursor, which coupled with the anomeric bromide to yield intermediate **9** in 64% yield.[8] Treatment of **9** with sodium azide in refluxing EtOH resulted in **10** which was then reduced and debenzoylated using $NaBH_4$ in ethanol to provide diaminopurine nucleoside **11**.[9,10] Diaminopurine **11** was then enzymatically converted to guanosine analog **12**, by adenosine deaminase type II.[9] Attempts to oxidize **12** to the 5′-carboxylic acid without protection of the guanosine base proved difficult. Therefore, the 2-amino group was protected once again as the 2-*N*-isobutyryl amide **13**. Acetylation of the ribose hydroxyl groups, followed by Mitsunobu mediated installation of the 6-*O* benzyl ether, and subsequent removal of the acetyl groups yielded the base protected intermediate **14**.[11] **14** was converted to acid **15**, using platinum-catalyzed oxidation in 37% yield.[12] Decarboxylative dehydration of acid **15** by heating in the presence of DMF-dineopentylalcohol formed the intermediate glycal **16**, which, without purification, was activated with PhSeCl and converted to phosphonate **17**.[13] The instability of the glycal intermediate was also a challenge in this synthetic route and care had to be used to avoid extensive depurination and furan formation. Oxidation of selenium by O_3 followed by elimination provided olefin derivative **18**. Finally, **18** was treated with TMSI in DMF and 2,4,6-collidine at 45 °C to simultaneously remove both the phosphonate ester groups and the 6-*O* benzyl ether. Treatment with concentrated ammonium hydroxide to remove the isobutyryl group then yielded the ammonium salt of guanosine phosphonate **19**.

A logical approach to the 2,6-diaminopurine phosphonate analog **24** was to utilize the 2,6-diaminopurine nucleoside **11**, but attempts to form the corresponding glycal intermediate, after oxidiation to the 5′-carboxylic acid, were unsuccessful. Therefore, an alternative route from 2,6-dichloro intermediate **9** was pursued (Scheme



Scheme 2. Reagents and conditions: (i) HBr, AcOH; (ii) NaH, CH_3CN, 2,6-dichloropurine, 64%; (iii) NaN_3, EtOH, reflux, 1 h, 100%; (iv) $NaBH_4$, EtOH , dioxane; (v) adenosine deaminase type II, rt, 16 h, 100%; (vi) TMSCl, pyridine, $(^iPrCO)_2O$, concd NH_4OH, 70%; (vii) Ac_2O, Py, 71%; (viii) Ph_3P, BnOH, DIAD, dioxane; (ix) NaOMe, MeOH, rt, 44% (two steps); (x) O_2, 10% Pt/C, H_2O, 60 °C, 24 h, 37%; (xi) a—$Me_2NCH(OCH_2{}^tBu)_2$, CH_3SO_3H, 100 °C; b—PhSeCl, $HOCH_2P(O)(OEt)_2$, $AgClO_4$; (xii) O_3, CH_2Cl_2, 0–45 °C; (xiii) TMSI, 2,4,6-collidine, DMF, 45 °C; (xiv) concd NH_4OH, 45 °C, 5 h.



Scheme 3. Reagents and conditions: (i) NaOMe, MeOH, 85%; (ii) O_2, 10% Pt/C, H_2O, $NaHCO_3$, 70 °C, 24 h, 57%; (iii) a—$Me_2NCH(OCH_2{}^tBu)_2$, CH_3SO_3H, 110 °C; b—PhSeCl, $HOCH_2P(O)(OEt)_2$, $AgClO_4$; (iv) O_3, CH_2Cl_2, 0–45 °C; (v) concd NH_4OH–EtOH (5:1 ratio), 140 °C; (vi) TMSBr, 2,4,6-collidine, DMF, 45 °C, 5 h; (vii) TMSBr, DMF, 50 °C; then NH_4OH, 45%.

3). Treatment of **9** with NaOMe in methanol generated nucleoside **20**. Platinum-catalyzed oxidation provided the 5′-carboxylic acid **21**, and conversion to the phosphonate **22** via the glycal proceeded smoothly. Ammonolysis in a steel bomb at 140 °C overnight displaced the chlorine, methoxy group, and one phosphonate ethyl group to afford the 2,6 diaminopurine phosphonic acid monoester **23**. Subsequent treatment with TMSBr then generated the target 2,6 diaminopurine phosphonate **24**. Inosine nucleoside phosphonate **26** was easily obtained from **25**[14] in 45% yield by initial deprotection of the phosphonate ester groups using TMSBr, followed by treatment with ammonium hydroxide.

Guanosine phosphonate, **19** (2′-Fd4GP), displayed antiviral activity (EC_{50} = 17.3 μM) in a similar range to that of adenosine analog, **1** (2′-Fd4AP), and ~5-fold less potent than tenofovir (Table 1). However, in the MT-2 cell cytotoxicity assay there was a clear difference in selectivity, in favor of **1**. It is interesting to note that the corresponding nucleoside analogs of **1** and **19** (i.e., 2′-Fd4A and 2′-Fd4G) have been reported and the guanosine nucleoside analog was also marginally less potent than the corresponding adenine analog (5-fold).[15] The hypoxanthine and 2,6-diaminopurine nucleoside phosphonate analogs, **26** and **24**, respectively, were inactive up to 300 μM. This is in contrast to the corresponding nucleoside hypoxanthine and 2,6-diaminopurine analogs, 2′-Fd4I and 2′-Fd4DAP, respectively, which were both reported to be quite potent (EC_{50} < 5 μM).[15] These results suggest that these base modified nucleoside phosphonates may be limited in their intracellular metabolic pathways to generate the active diphospho-phosphonates (triphosphate equivalent). The reduced 2′-F-2′,3′-dideoxy adenine phosphonate analog **6** demonstrated weak antiviral activity. Previous studies on non-fluorinated 2′,3′-dideoxy phosphonate analogs have shown that the saturated ribose ring is inferior in potency toward HIV RT compared to the unsaturated 2′,3′-dideoxy-2′,3′-didehydro ribose ring, potentially due to the saturated ribose ring adopting a more southern conformation.[7] The 2′-*beta*-fluorine group would be expected to also favor a southern conformation. Therefore, the diminished antiviral activity of **6** is likely due, in part, to reduced potency toward HIV RT.

Table 1. Anti-HIV activity and cytotoxicty

Compound	Code	WT HIV[a] EC_{50} (μM)	MT-2[b] CC_{50} (μM)
	Tenofovir	3.6 (1.5)	>1000
1	2′-Fd4AP	12.3 (3.4)	>1000
19	2′-Fd4GP	17.4 (5.3)	700
26	2′-Fd4IP	>300	—
24	2′-Fd4DAPP	>300	—
6	2′-FddAP	283	>1000

[a] Antiviral activity in MT-2 cells infected with HIV-1 IIIb. Values are the results of at least 2 experiments, standard deviation given in parentheses.
[b] Cytotoxicity in uninfected MT-2 cells.

Synthesis of several purine and ribose modified analogs of the 2′-fluoro modified nucleoside phosphonate GS-9148, **1**, identified the guanosine analog, **19**, as a potent but less selective nucleoside phosphonate inhibitor of HIV.

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