Synthesis of fluorinated phosphonoacetate derivatives of carbocyclic nucleoside monophosphonates and activity as inhibitors of HIV reverse transcriptase

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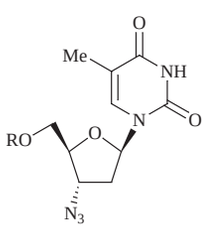
The syntheses of compounds **8**–**10** are described; the compounds showed some activity as inhibitors of HIV reverse transcriptase ($IC_{50} \geqslant 365$ μM).

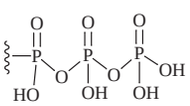
Introduction

The Human Immunodeficiency Virus (HIV) was first isolated in 1982 and since then has been a major focal point in the search for effective therapies to treat retroviral diseases. The life cycle of HIV is typical of a retrovirus where, following cell infection, the viral genome must first be translated from RNA to DNA before the expression of viral proteins can proceed *via* the usual sequence. The DNA polymerase which co-ordinates this transcription process, HIV reverse transcriptase (HIV-rt), has long been a key biological target for the design of anti-retroviral chemotherapies.



AZT **1** R=H

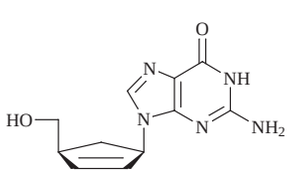
AZTTP **2** R=



Substrate analogue

All of the currently approved agents which target HIV-rt are dideoxynucleoside analogues such as 3′-azido-3′-deoxythymidine (AZT).[1] These nucleoside surrogates are metabolised in cells to the corresponding dideoxynucleoside-5′-triphosphate derivatives (ddNTP) [*e.g.* AZT triphosphate (AZTTP) **2**] which are utilised by HIV-rt as mimetics of the natural substrates for DNA chain polymerisation. Since these NTP analogues lack a 3′-hydroxy group, then once they are incorporated into DNA, chain growth is effectively terminated.

In addition to nucleoside analogues such as AZT, carbocyclic nucleosides, such as carbovir **3**, have been shown to possess potent anti-HIV activity.[2] Carbovir itself is too toxic for use in the clinic but a closely related compound, **4**, developed by Burroughs Wellcome and later Glaxo Wellcome[3] is safe and effective and is scheduled to be launched, as abacavir, into the clinics in the USA early in 1999. The carbocyclic nucleoside **4** and AZT **1** will be used as a dual therapy for HIV-infected patients.



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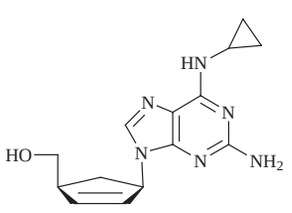

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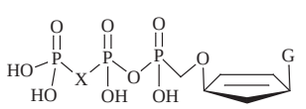
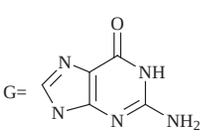
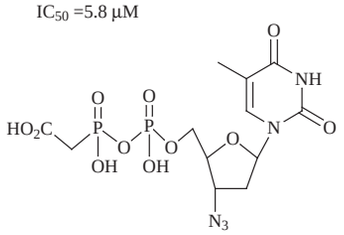
We have been engaged in the search for other carbocyclic nucleosides and nucleotides with potent anti-HIV activity (through the inhibition of HIV-rt) and low toxicity. For example, we have reported that the pyrophosphoryl phosphonate **5**[4] is a potent inhibitor of HIV-rt (IC_{50}, 0.5 μM; *cf.* AZTTP, 1.0 μM). Interestingly the P_β,P_γ-difluoromethylenediphosphonate derivative **6** is only one order of magnitude less potent as an inhibitor of HIV-rt (IC_{50}, 5.8 μM).[5] In view of the fact, reported elsewhere, that the 5′-phosphorylphosphonoacetate derivative of AZT (**7**) is only 30-fold less active than AZTTP as an inhibitor of avian myeloblastosis virus reverse transcriptase,[6] we felt that it would be equally interesting to prepare and test this type of analogue in our series (*i.e.* compound **8**). In addition, the fluoro-substituted compounds **9** and **10** were added to our list of targets in anticipation that fluoro-substitution would improve bioisosterism with the parent pyrophosphorylphosphonate **5**. Not only were these compounds interesting to test as HIV-rt inhibitors *per se* but it was noted that their intracellular metabolism would release the corresponding phosphonoacetic acids **11**–**13**. Phosphonoacetic acid (PAA) is known to be a



5 X=O
6 X=CF_2

IC_{50} =0.5 μM [5]
IC_{50} =5.8 μM





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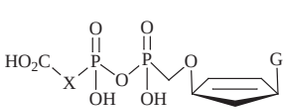
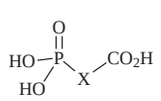


8 X=CH_2
9 X=CFH
10 X=CF_2



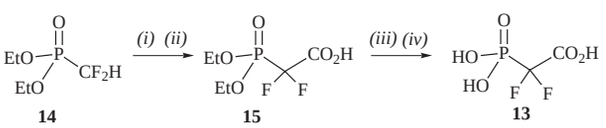
11 X=CH_2
12 X=CFH
13 X=CF_2

(PAA)

reversible non-competitive inhibitor of the DNA polymerases of herpes simplex virus, cytomegalovirus and HIV[7,8] hence compounds of type **8**–**10** could potentially provide dual-action anti-viral agents, the synergistic activity giving an opportunity to thwart the build up of viral resistance.

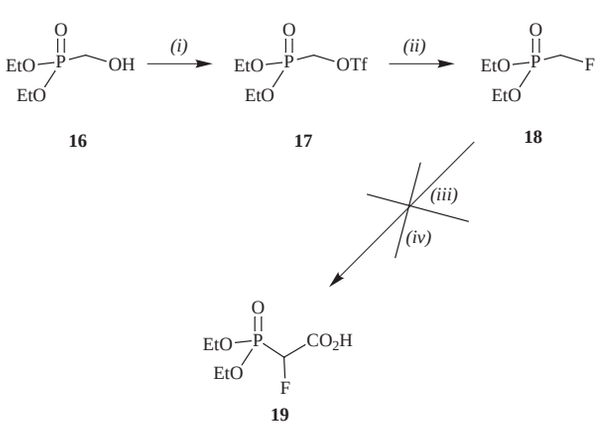
Results

Diethylphosphoryldifluoroacetic acid **15** was prepared, according to the method of Blackburn *et al.*,[9] by carboxylation of the lithiated anion of the commercially available difluoromethylphosphonate **14**. This was an efficient procedure as, following an aqueous acid–base work-up, compound **15** was isolated in good yield and required no further purification. De-esterification by treatment with bromotrimethylsilane gave the free phosphonic acid **13** (Scheme 1).



Scheme 1 (*i*) LDA, THF, −78 °C; (*ii*) CO_2; (*iii*) TMSBr, 24 h; (*iv*) H_2O.

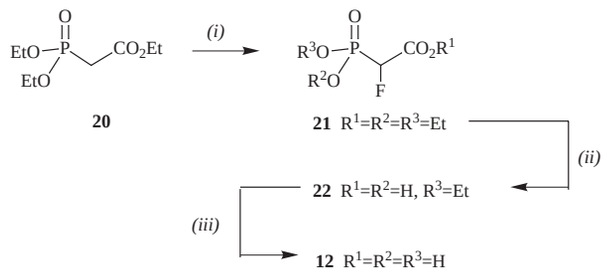
Attempted preparation of phosphonate **18** by treating the alcohol **16** with DAST at −78 °C was unsuccessful giving poor product conversion and low yields (<2%). In contrast, activation of the alcohol, by first forming the triflate **17**,[10] followed by nucleophilic displacement with tetrabutylammonium fluoride (TBAF) proved to be a safe and simple route to the monofluorinated product **18** (Scheme 2). Carboxylation of the



Scheme 2 (*i*) NaH, CF_3SO_2Cl, Et_2O, −20 °C 30 min; (*ii*) TBAF, THF, room temperature 1 h; (*iii*) LDA, THF, −78 °C; (*iv*) CO_2.

lithiated anion of **18** proved to be difficult, furnishing the monofluorinated phosphonoacetic acid **19** in very poor yield (<4%).

The published experimental procedure, upon which this reaction was based,[9] reports isolated yields of 63% when using the lithiated anion of diisopropyl fluoromethylphosphonate; preparations using the diethyl ester **18** were not reported. It was suspected that the difficulties encountered in the attempted carboxylation of **18** were due to a reduction in the stability of the lithiated anion compared to the diisopropyl ester derivative. In order to assess the relative thermal stability of the lithiated species, a sample of **18** was deprotonated using LDA and kept at −78 °C for 60 minutes before quenching with a saturated solution of potassium dihydrogenphosphate from which only 49% of starting material was recovered (as determined by ^1H and ^{31}P NMR studies). A similar stability assay has been reported for the diisopropyl fluoromethylphosphonate where 70% of the starting material was recovered after stirring the lithiated anion at 20 °C for 60 minutes[11] indicating that the lithiated anion of the diethyl phosphonate **18** is a lot less stable in comparison. It would appear that the preparation of **19** using this procedure was constrained by the instability of the deprotonated phosphonate **18**. Since the diisopropyl derivative was not readily available, the monofluorination of ethyl diethoxyphosphorylacetate **20**, was explored as an alternative route to *α*-fluorophosphonoacetic acid **12** (Scheme 3).



Scheme 3 (*i*) NaH, THF, 0 °C to room temperature 90 min, then F-TEDA-BF_4, THF–DMF, room temperature 140 min; (*ii*) $NaOH_{(aq)}$, reflux 20 h; (*iii*) TMSBr, 48 h then H_2O.

For the requisite fluorination step the NF "electrophilic" fluorinating agents were chosen for reasons of safety and ease of handling. *N*-Fluoro-*o*-benzenedisulfonimide (NFOBS) has previously been used for the monofluorination of the sodium ester enolate of **20**, reportedly giving **21** in good yield (78%).[12] Unfortunately, the preparation of NFOBS requires the treatment of *o*-benzenedisulfonimide with molecular fluorine, a procedure requiring specialist apparatus.[13] Hence the alternative, commercially available, fluorinating agents *N*-fluorobenzenesulfonimide[14] (NFSI) and 1-(chloromethyl)-4-fluoro-1,4-diazabicyclo[2.2.2]octane bis(tetrafluoroborate) (F-TEDA-BF_4)[15] were chosen for investigation. A number of procedures for deprotonation of **20**, and subsequent monofluorination, were carried out under various conditions. The best result was obtained on fluorination of the sodium enolate of **20** using F-TEDA-BF_4 in a THF–DMF solvent mixture at room temperature, though product yields were still quite poor (17%) (Scheme 3).

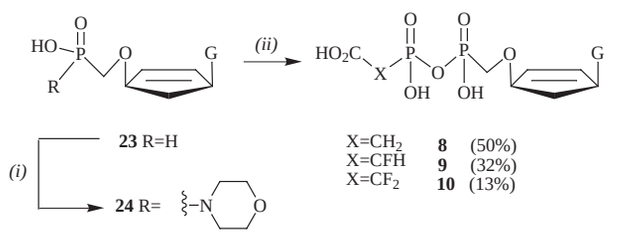
Numerous attempts were made to improve the yield of **21** but without success. Neither changing the counter cation (K^+, Li^+) nor the use of an appropriate crown ether offered any improvement in yield. The root of the problem in this procedure was believed to be the poor solubility of the F-TEDA-BF_4 in THF. Even the use of DMF co-solvent failed to fully solvate the fluorinating agent. In the absence of DMF, F-TEDA-BF_4 remained unsolvated and no product formation was observed (by TLC). Whilst DMF was necessary to enable fluorination to take place it was also found to limit product recovery by retaining some of the reaction products in the aqueous layer during work up. The uncharged NFSI reagent was more soluble in THF, but the amount of fluorinated product formed was negligible (by TLC). The reduction in product formation, observed when using NFSI, was due to its lower chemical reactivity in comparison with F-TEDA-BF_4.[16] Treatment of **21** with dilute sodium hydroxide under reflux gave the di-acid **22** in excellent yield. This compound could be carried through the final de-esterification step without further purification, the remaining phosphonate ester group being removed by treatment with a large excess of bromotrimethylsilane, followed by hydrolysis to give the free acid **12**.

For the final coupling step the known phosphonate **23**[4] was converted into the morpholidate **24**.[17] Contemporaneously, the phosphonoacetic acids **11**–**13** were converted into the corresponding tributylammonium salts. The corresponding tris-ammonium salts of **11**–**13** were also prepared for inclusion in the HIV-rt enzyme assay. The tributylammonium salts of **11**–**13** were coupled with the morpholidate **24** to give the respective triphosphate analogues **8**–**10** which were isolated as their tris-ammonium salts (Scheme 4).

Table 1 Relative efficacy of NTP analogues **8**–**10** as inhibitors of HIV-rt[a] and comparative activity of the phosphonoacetic acid side chain units **11**–**13** to assess the contribution of synergistic inhibitory effects towards the overall activity of **8**–**10**

Compound (tris-ammonium salt)	IC_{50}/μM	Compound (tris-ammonium salt)	IC_{50}/μM
8	2208	**11**	1556
9	4447	**12**	1590
10	365	**13**	1808
5	1.5		
AZTTP **2**	1.6		

[a] Testing was carried out using the DuPont RT-Detect™ Reverse Transcript Assay.



Scheme 4 (*i*) Morpholine, DCC, *t*-BuOH–H_2O reflux 4 h; (*ii*) **11**, **12** or **13** (NBu_3 salt), DMSO 14–39 h room temperature.

Biological results

The three NTP mimics **8**–**10** (as tris-ammonium salts) were tested as inhibitors of HIV-rt in an enzyme assay (Table 1). The phosphonoacetic acids **11**–**13** (as tris-ammonium salts) were tested in the same assay. Both AZTTP **2** and the pyrophosphoryl phosphonate **3** were used as standards.

Both **8** and the monofluorinated analogue **9** showed poor activity as inhibitors of HIV-rt, being three orders of magnitude less active than the parent compound **5**. The difluorinated analogue **10** was markedly more effective than the monofluorinated substrate **9** but was still two hundred times less potent than **5**. The phosphonoacetic acids salts **11**–**13** all showed poor inhibitory properties; the degree of fluorosubstitution had no significant influence on the potency of these pyrophosphate analogues as inhibitors of HIV-rt.

In order to achieve appreciable synergistic activity, the phosphonoacetic acid component of compounds **8**–**10** must be a potent inhibitor of HIV-rt. None of the above substrates was able to satisfy this criterion, as indicated by the activity of compounds **11**–**13**. The disappointing activity of the acids **8**–**10** may be due to the fact that the carboxy group is a poor mimic of the terminal phosphonate group in compound **6**. The planarity of this carboxy functionality might badly affect the binding interactions of the triphosphate side chain in the active site of the enzyme, interactions which are essential to enable the efficient incorporation of monophosphonate **24** into the elongating DNA chain.

In conclusion, we have shown that compounds **8**–**10** are relatively poor inhibitors of HIV-rt. However, even though the IC_{50} value of compound **10** is at least 200-fold greater than that of the parent triphosphate **5**, these results demonstrate the ability of HIV-rt to recognise NTP isosteres bearing only a single acidic hydroxy group at the γ-position.[6,18] As reverse transcriptases are generally less substrate-specific than their host cell DNA polymerases, the development of more potent mono-γ-hydroxylated NTP derivatives could lead to a range of DNA polymerase inhibitors which are highly selective for HIV-rt leading to reduced cytotoxicity.[18] Our investigations into the synthesis and biological properties of such inhibitors will be reported in due course.

Experimental

Petroleum ether had bp 40–60 °C (referred to as petrol). TLC was performed on pre-coated silica gel glass plates (Merck silica gel 60F 254). For the TLC assay of the highly polar phosphate and triphosphate analogues 1 M NH_4HCO_3(aq)–EtOH–NH_4OH(dilute) (20 : 50 : 1) was used as eluent. Flash chromatography was performed over silica (Merck silica gel 60 40–63 μm). Anion exchange chromatography was performed on a column of Fractogel EMD DEAE-650 (M) (Merck, Art. 16886) (40 × 100 mm) eluting with a linear gradient of aqueous ammonium hydrogen carbonate at a flow rate of *ca*. 200 ml h^{-1}. The column was connected to a UV detector (254 nm, LKB 2238, Uvicord SII) and a fraction collector. NMR spectra were recorded on a Bruker AM300 and a Bruker Avance DRX 400 spectrometer at the following frequencies: 300 or 400 MHz for ^{1}H NMR, 75 or 100 MHz for ^{13}C NMR, 162 MHz for ^{31}P NMR (H_3PO_4 external reference) and 376 MHz for ^{19}F NMR (C_6F_6 external reference); *J* values are given in Hz. Infrared spectra were recorded on a Perkin Elmer 881 Fourier Transform Spectrophotometer. UV spectra were recorded on a UV4-200 Unicam UV–VIS spectrometer. Mass spectra were obtained on either a Kratos Profile HV3 or a VG7070E/DEC VAX 4000.60 spectrometer. Samples of AZT **(1)** were received as generous donations from C. McGuigan and P. Sutton (Welsh School of Pharmacy, University of Wales, Cardiff) and Wellcome research laboratories through the MRC AIDS Reagent Programme.

(1′S,4′R)-9-(4′-{[(Carboxymethyl)hydroxyphosphoryloxy](hydroxy)phosphorylmethoxy}cyclopent-2′-enyl)guanine (tris-ammonium salt) 8

A solution of the morpholidate **24** (0.06 mmol) in anhydrous DMSO (1.5 cm^3) was added dropwise to a solution of **11** (tributylammonium salt, 0.2 mmol) in DMSO (1.5 cm^3). This solution was stirred at room temperature for 16 h. The reaction was diluted with water (400 cm^3) before purification by anion exchange chromatography eluting with a linear gradient of $NH_4HCO_{3(aq)}$ (0–0.3 M, 800 cm^3). The product was eluted at 0.23 M NH_4HCO_3. Product fractions were coevaporated several times with water before being lyophilised to give **8** as a white lyophilate (14 mg, 50%). ν_{max}(KBr disc)/cm^{-1} 3600–2600 (OH, NH_2), 1697 (C=O), 1617 (C=C, C=N), 1203 (P=O); λ_{max}(H_2O)/nm 253.7; δ_H(400 MHz, D_2O) 1.87 (1H, dt, *J* 14.5, 4.3, 5′-βH), 2.81 (2H, d, *J* 19.9, PC$H_2$$CO_2H$), 2.97 (1H, dt, *J* 14.4, 7.7, 5′-αH), 3.81 (2H, d, *J* 8.3, PCH_2O), 5.27–5.33 (1H, m, 1′-H), 6.08–6.12 (1H, m, 2′-H), 6.35–6.38 (1H, m, 3′-H), 7.70–7.95 (1H, br, 8-H); δ_C(100 MHz, D_2O) 37.69 (C-5′), 57.46 (C-1′), 65.12 (d, *J* 161.9, PCH_2O), 84.56 (d, *J* 11.1, C-4′), 132.79 (C-2′), 136.14 (C-3′), 153.72 (C), 158.93 (C); δ_P(162 MHz, D_2O) 10.05 (d, *J* 29.7, P_α), 11.47 (d, *J* 29.0, P_β); *m*/*z* (ES) 450 (100%, MH^+), 328 (M − $HO_2CCH_2PO_2$).

(1′S,4′R)-9-(4′-{[(Carboxyfluoromethyl)hydroxyphosphoryloxy](hydroxy)phosphorylmethoxy}cyclopent-2′-enyl)guanine (tris-ammonium salt) 9

A solution of the morpholidate **24** (0.072 mmol) in anhydrous DMSO (1.5 cm^3) was added dropwise to a solution of **12** (tributylammonium salt, 0.324 mmol) in DMSO (1.5 cm^3). The solution was stirred at room temperature for 39 h. The reaction mixture was dissolved in water (300 cm^3) before purification by anion exchange chromatography eluting with a linear gradient of $NH_4HCO_{3(aq)}$ (0–0.3 M, 800 cm^3). The product was eluted at 0.20 M. Product fractions were coevaporated several times with water before being lyophilised to give the product ammonium salt **9** as a white lyophilate (12 mg, 32%). ν_{max}(KBr)/cm^{-1} 3600–2700 (br, NH_2, OH), 1697, 1618 (C=N, C=O, C=C), 1236 (P=O); λ_{max}(H_2O)/nm 240.0; δ_H(400 MHz, D_2O) 1.90 (1H, dt, *J* 14.4, 4.0, 5′-βH), 2.97 (1H, dt, *J* 14.8, 7.2, 5′-αH), 3.82 (2H, d, *J* 9.2,

PCH_2), 4.75–4.81 (1H, br, 4′-H), 5.06 (1H, br d, *J* 47.6, PCFH), 5.30–5.36 (1H, br, 1′H), 6.11 (1H, d, *J* 5.4, 2′-H), 6.38 (1H, d, *J* 5.3, 3′-H), 7.75–8.00 (1H, br, 8-H); δ_C(100 MHz, D_2O) 37.58 (C-5′), 57.46 (C-1′), 64.99 (d, *J* 164.6, PCH_2), 84.56 (d, *J* 11.5, C-4′), 132.79 (C-2′), 136.15 (C-3′), 154.0, 158.66 (C); δ_F(376 MHz, D_2O) −31.0 to −35.0 (br, CFH); δ_P(162 MHz, D_2O) 2.36 (br, P_β), 10.48 (d, *J* 24.0, P_α); *m*/*z* (ES) 468 (100%, MH^+), 328 (10, M − $HO_2CCFHPO_2$).

(1′S,4′R)-9-(4′-{[(Carboxydifluoromethyl)hydroxyphosphoryloxy](hydroxy)phosphorylmethoxy}cyclopent-2′-enyl)guanine (tris-ammonium salt) 10

A solution of the morpholidate **24** (0.072 mmol) in anhydrous DMSO (1.5 cm^3) was added dropwise to a solution of **13** (tributylammonium salt, 0.324 mmol) in DMSO (1.5 cm^3). The solution was stirred at room temperature for 15 h. The reaction mixture was dissolved in water (250 cm^3) before purification by anion exchange chromatography eluting with a linear gradient of $NH_4HCO_{3(aq)}$ (0–0.4 M, 800 cm^3). The product was eluted at 0.20 M NH_4HCO_3. Product fractions were coevaporated several times with water before being lyophilised to give a white lyophilate (15 mg). Further attempts to separate the product from the coeluting impurity **13**, by anion exchange [linear gradient of $NH_4HCO_{3(aq)}$ (0.1–0.3 M, 800 cm^3)], gave the title compound still not completely pure (7 mg, 13%). Approximately 30 mol% of the isolated product consisted of the difluorophosphonoacetic acid **13** (by ^{19}F and ^{31}P NMR). No further attempts at purification were made. ν_{max}(KBr)/cm^{-1} 3600–2800 (br, NH_2, OH), 1696, 1600 (C=N, C=O, C=C), 1269 (P=O); λ_{max}(H_2O)/nm 252.0; δ_H(400 MHz, D_2O) 1.87 (1H, dt, *J* 14.0, 4.0, 5′-βH), 2.95 (1H, dt, *J* 14.0, 7.0, 5′-αH), 3.82 (2H, d, *J* 9.0, PCH_2), 4.75–4.79 (1H, m, 4′-H), 5.27–5.32 (1H, m, 1′H), 6.11 (1H, d, *J* 5.0, 2′-H), 6.36 (1H, dt, *J* 5.0, 1.5, 3′-H), 7.75–7.90 (1H, br, 8-H); δ_F(376 MHz, D_2O) 49.82 (d, *J* 97.0); δ_P(162 MHz, D_2O) −4.98 (dt, *J* 99.0, 31, P_β), 10.87 (d, *J* 31, P_α); *m*/*z* (ES) 286 (4%, MH^+), 328 (42, M − $HO_2CCF_2PO_2$), 260 (100).

2-Fluoro-2-phosphonoacetic acid (12)

Bromotrimethylsilane (5 cm^3, 37.9 mmol) was added dropwise to a solution of **22** (393 mg, 2.13 mmol) in anhydrous DMF (20 cm^3), giving a small exotherm, and the solution was stirred at room temperature for 48 h. The excess bromosilane was removed *in vacuo* and the residue cooled to 0 °C before the dropwise addition of distilled water (6 cm^3) to give an off-white emulsion which eventually gave a clear solution after stirring at 0–20 °C for 1 h. The reaction mixture was then washed with ether (3 × 10 cm^3) and the aqueous layer was further diluted in water (250 cm^3) before loading onto an anion exchange column (DEAE). This was washed through with water (300 cm^3) followed by $NH_4HCO_{3(aq)}$ (0.2 M, 250 cm^3). Water was removed from the hydrogen carbonate fractions *in vacuo* and volatile salts were removed by repeated coevaporation with water. This was finally lyophilised to give **(12)** as a white lyophilate (303 mg, 69%). ν_{max}(neat)/cm^{-1} 2600–3400 (OH), 2150–2350 (P-OH), 1604 (C=O), 1256 (P=O); δ_H(400 MHz, D_2O) 4.87, (1H, dd, *J* 48.0, 11.6, PCFH); δ_P(162 MHz, D_2O) 9.90 (d, *J* 67.8); δ_F(376 MHz, D_2O) −31.68 (br); *m*/*z* (EI) 209 (0.8%, $M(NH_3)_3^+$), 158 (1.6, M^+), 77 (100, $CFHCO_2H^+$).

2,2-Difluoro-2-phosphonoacetic acid (13)

Bromotrimethylsilane (2.8 cm^3, 21.21 mmol) was added dropwise to a neat sample of **15** (443 mg, 1.91 mmol) under an inert atmosphere and was stirred at room temperature for 24 h. The excess bromosilane was removed *in vacuo* and the residue cooled to 0 °C before the dropwise addition of distilled water (5 cm^3) which was then stirred at 0–20 °C for 30 min. Fine solids were removed by filtration, the filtrate was washed with ethyl acetate (3 × 2 cm^3) and the solvents were removed *in vacuo* to give the title compound **13** as a very thick yellow–brown oil (307 mg, 91%). δ_C(100 MHz, D_2O) 112.81 (dt, *J* 267.4, 181.1, CF_2), 166.73 (dt, *J* 27.0, 15.1, C=O); δ_P(162 MHz, D_2O) 1.91 (t, *J* 87.0) (lit.,[19] 0.8, t); δ_F(376 MHz, D_2O) 45.48 (d, *J* 86.9) (lit.,[19] −117.6, *J* 89 ($CFCl_3$ external standard)) [Found: (EI) M^+, 175.96750. $C_2H_3F_2O_5P$ requires 175.96862]; *m*/*z* 177 (0.7%, MH^+), 132 (41.9, M − CO_2), 82 (100, M − $(EtO)_2P(O)$).

General procedure for the preparation of the phosphonoacetic acid ammonia salts

The tris-ammonium salts of compounds **11**–**13** were prepared as outlined below for phosphonoacetic acid **11**. Compound **11** (17 mg, 0,12 mmol) was dissolved in an aqueous solution of NH_4HCO_3 (0.75 M, 5 cm^3) and stirred at room temperature for 30 min. The excess volatile salts were then removed by repeated coevaporation with water *in vacuo* before lyophilising to give the tris-ammonium salt of **11**, as a white lyophilate, in quantitative yield.

General procedure for the preparation of the phosphonoacetic acid tributylammonium salts

All the tributylammonium salts of the pyrophosphate analogues were prepared as outlined below for the monofluorophosphonoacetic acid **12**. To a solution of the free acid **12** (0.37 mmol) in 50% aqueous ethanol (2 cm^3) was added tributylamine (1.5 equiv., 0.56 mmol). This was stirred at room temperature for 30 min before removing the solvents to give the tributylammonium salt of **12** as a viscous brown oil. This was stored as a solution in DMF (0.6 M). Before being used in the subsequent phosphorylation reaction, solvents were removed from an appropriately sized aliquot and residual water was removed *in vacuo* as an azeotropic mixture (3 × 1 cm^3 of pyridine followed by 1 cm^3 of benzene).

Diethyl phosphoryl-2,2-difluoroacetic acid (15)

A solution of diethyl difluoromethylphosphonate **14** (1.0 g, 5.32 mmol) in THF (8 cm^3) was added dropwise to a solution of LDA (5.85 mmol) in THF (20 cm^3), cooled to −78 °C, and the solution was stirred for 30 min. Carbon dioxide gas (dried over concentrated H_2SO_4) was bubbled through the reaction mixture for 20 min whilst stirring at −78 °C. The reaction mixture was allowed to warm to 0 °C before the dropwise addition of 2 M H_2SO_4 (3.2 cm^3, 6.4 mmol). Once the effervesence had stopped the reaction was neutralised with saturated $NaHCO_{3(aq)}$. The aqueous layer was separated and washed (Et_2O, 2 × 5 cm^3) and then acidified with 2 M H_2SO_4 (pH 1). This was then extracted with ethyl acetate (5 × 10 cm^3). The combined organics were dried ($MgSO_4$) and the solvents removed *in vacuo* to give the title compound as a yellow oil (0.85 g, 69%). ν_{max}(neat)/cm^{-1} 1762 (C=O), 1269 (P=O); δ_H(300 MHz, $CDCl_3$) 1.42 (6H, t, *J* 6.0, CH_3), 4.35–4.45 (4H, m, 2 × CH_2), 9.87 (1H, s, CO_2H); δ_C(75 MHz, $CDCl_3$) 16.19 (d, *J* 5.7, CH_3), 66.54 (d, *J* 6.8, CH_2); δ_F(376 MHz, $CDCl_3$) 47.05 (d, *J* 96.8) (lit.,[10] −117.3 ($CFCl_3$ external standard)); δ_P(162 MHz, $CDCl_3$) 3.78 (t, *J* 96.8) (lit.,[10] 3.2); *m*/*z* (EI) 233 (3.5%, MH^+), 188 (15.3, M − CO_2), 132 (100).

Diethyl fluoromethylphosphonate (18)

A solution of the triflate **17** (5.08 g, 0.015 mol) in THF (22 cm^3) was cooled to 0 °C before 20 cm^3 (0.02 mol) of tetrabutylammonium fluoride (1 M in THF) was added dropwise . The solution was stirred at 0 °C for 60 min. Solvents were then removed and dichloromethane (35 cm^3) was added. The organic layer was washed (H_2O, 3 × 8 cm^3), dried ($MgSO_4$) and evaporated to a crude oil. This was purified by flash chromatography, using EtOAc–petrol (1 : 1) as eluent, to give **18** as a pale yellow oil (1.67 g, 67%). ν_{max}(neat)/cm^{-1} 1269 (P=O) (lit.,[12] 1265), 1031 (P–OEt); δ_H(300 MHz, $CDCl_3$) 1.37 (6H, t, *J* 7.1, 2 × CH_3), 4.22

(4H, dq, *J* 8.3, 7.1, 2 × CH_2O), 4.67 (2H, dd, *J* 46.9, 4.8, PCH_2F); δ_C(100 MHz, $CDCl_3$) 16.40 (d, *J* 5.7, CH_3), 63.01 (d, *J* 6.3, CH_2O), 76.59 (dd, *J* 180.7, 169.3, PCH_2F); δ_P(162 MHz, $CDCl_3$) 17.01 (d, *J* 63.2) (lit.,[12] 16.3); δ_F(376 MHz, $CDCl_3$) −85.77 (dt, *J* 63.2, 46.7) (lit.,[12] −250.4 ($CFCl_3$ external standard)) [Found: (EI) M^+, 170.05076. $C_5H_{12}FO_3P$ requires 170.05081]; *m*/*z* 170 (1.9%, M^+), 149 (38.3), 86 (50.7), 57 (100).

Assessment of the thermal stability of lithiated diethyl fluoromethylphosphonate (18)[11]

To a solution of LDA (1.20 mmol) in anhydrous THF (1.5 cm^3), pre-cooled to −78 °C, was added a solution of **18** (186 mg, 1.09 mmol) in THF (1 cm^3) dropwise. This was stirred at −78 °C for 60 min before allowing to warm to 0 °C and quenching with a saturated solution of aqueous KH_2PO_4. The mixture was extracted (EtOAc × 3) and the combined organics were dried ($MgSO_4$) before solvents were removed to give a crude sample of **18** as a yellow–brown oil (103 mg). The crude mixture was approximately 88 mol% pure (by ^{31}P NMR) showing that only 49% of the starting material **18** was recovered.

Ethyl 2-fluoro 2-(diethoxyphosphoryl)acetate (21)

To a slurry of sodium hydride (pre-washed with Et_2O) in anhydrous THF (20 cm^3) at 0 °C was added a solution of ethyl diethoxyphosphorylacetate **20** (1.02 g, 4.5 mmol) in THF (20 cm^3) dropwise which was stirred at 0 °C for 30 min and at room temperature for 60 min. Following the addition of anhydrous DMF (13 cm^3), (F-TEDA-BF_4) (145 mg, 4.83 mmol) was added as one portion and the solution was stirred at room temperature for 140 min. The reaction mixture was then poured into ether (30 cm^3) and the organics were washed with 5% aqueous sulfuric acid (25 cm^3) followed by saturated aqueous sodium hydrogen carbonate (25 cm^3). The organic layer was then dried ($MgSO_4$) and solvents were removed. The crude material was purified by chromatography using EtOAc–petrol (1 : 1) as eluent to give **21** as a colourless oil (188 mg, 17%). ν_{max}(neat)/cm^{-1} 1769 (C=O), 1278 (P=O); δ_H(300 MHz, $CDCl_3$) 1.34 (3H, t, *J* 7.2, CH_3), 1.37 (6H, ddt, *J* 7.0, 3.0, 0.5, 2 × CH_3), 4.19–4.30 (4H, m, 2 × CH_2OP), 4.35, (2H, q, *J* 7.5, CO_2CH_2), 5.19, (1H, dd, *J* 47.7, 12.0, PCFH) (lit.,[13] 5.2, *J* 48, α-proton); δ_C(100 MHz, $CDCl_3$) 14.03, (CH_3), 16.31 (d, *J* 5.7, CH_3), 62.42 (CH_2), 64.19 (d, *J* 6.7, CH_2), 64.22 (d, *J* 6.7, CH_2), 85.04 (dd, *J* 196.6, 158.6, CFH), 164.81 (d, *J* 20.9, C=O); δ_P(162 MHz, $CDCl_3$) 10.77 (d, *J* 72.0); δ_F(376 MHz, $CDCl_3$) −46.50 (dd, *J* 72.0, 47.0) (lit.,[13] −210, *J* 70.5 ($CFCl_3$ external standard)) [Found: (EI) M^+, 242.07218. $C_8H_{16}FO_5P$ requires 242.07194]; *m*/*z* 242 (3.1%, M^+), 214 (31.6, MH − Et), 197 (39.2, M − OEt), 137 (26.2, M − $CFHCO_2Et$), 109 (100).

2-Fluoro 2-(ethoxy)(hydroxy)phosphorylacetic acid (22)

A solution of **21** (522 mg, 2.16 mmol) in aqueous sodium hydroxide (2 M, 10 cm^3) was heated under reflux for 20 h. The reaction mixture was allowed to cool and the fine solids were filtered off. Excess sodium hydroxide was removed by the addition of portions of Dowex 50W-X8 (H) to the filtrate whilst stirring until the mixture was sufficiently acidified (pH 1). The Dowex resin was removed and the filtrate was lyophilised to give **22** as a sticky yellow–white lyophilate (393 mg, 98%). Trace impurities were evident by ^1H NMR but the highly polar nature of the monoester made further purification very difficult so the isolated material was carried through, to be purified at a later step. δ_H(300 MHz, D_20) 1.37 (3H, t, *J* 7.5, CH_3), 4.14 (2H, dt, *J* 6.6, 6.6, CH_2), 5.40 (1H, dd, *J* 47.1, 12.3, PCFH); δ_C(100 MHz, D_2O) 15.96 (d, *J* 5.5, CH_3), 62.97 (t, *J* 6.3, CH_2), 86.68 (dd, *J* 187.6, 143.3, CFH), 170.57 (C=O); δ_P(162 MHz, D_2O) 8.33 (d, *J* 64.0); δ_F(376 MHz, D_2O) −42.53 (dd, *J* 64.0, 47.0) [Found: (FAB) M − H^+, 185.00135. $C_4H_7FO_5P$ requires 185.00152]; *m*/*z* 185 (97%, M − H^+), 153 (100).

General procedure for the preparation of the morpholidate (24)[4,17]

This compound was made up immediately prior to coupling with the bisphosphonate residues. A typical procedure for the preparation of the morpholidate **24** is as follows: To a solution of the phosphonate **23**[4] (17.1 mg, 0.052 mmol) in 50% aqueous 2-methylpropan-2-ol (1.5 cm^3) was added morpholine (0.036 cm^3, 0.41 mmol) which was stirred at room temperature for 1 h. This was brought to reflux and a solution of DCC (84.7 mg, 0.41 mmol) in 2-methylpropan-2-ol (3 cm^3) was added dropwise over 4 h *via* a syringe pump and was left under reflux until the reaction had gone to completion (by TLC). After allowing the mixture to cool, solvents were evaporated and water was added (5 cm^3). The suspension was filtered and the residue washed (H_2O, 8 cm^3). Aqueous extracts were combined and washed (ether, 3 × 5 cm^3) and solvents were then removed from the combined aqueous layers to give a pale brown gum. Residual water was removed as an azeotropic mixture (pyridine 3 × 1 cm^3 followed by benzene 1 cm^3) to give the morpholidate **24** as an off white solid.

Enzyme assays

Testing of compounds as inhibitors of HIV-rt catalysed DNA synthesis was carried out using the DuPont RT-Detect™ assay (cat. No. NEK-070A). Recombinant HIV-1-rt was also purchased from DuPont (cat. no. NEI-490) . All the reference standards, inhibitor-free controls and test compound concentrations were assayed in at least two parallel runs and the results were averaged. Activity values are expressed as the concentration of test substrate (μM) which is required to inhibit HIV-rt catalysed cDNA synthesis by 50% (IC_{50}). The IC_{50} values are relative to the values of the external standards, AZTTP **2** and **5**, which were also tested in the same assay. AZTTP **2**[20] and **5**[4] were prepared according to literature procedures.

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