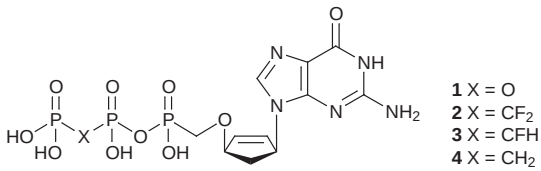
Synthesis of a potent inhibitor of HIV reverse transcriptase

Chris J. Hamilton,[a] Stanley M. Roberts[b]† and Alexander Shipitsin[c]

[a] *Department of Chemistry, Exeter University, Stocker Road, Exeter, Devon, UK EX4 4QD*
[b] *Robert Robinson Laboratories, Department of Chemistry, Liverpool University, Liverpool, UK L69 3BX*
[c] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov Street, Moscow 117984, Russia*

The newly synthesised P_β,–P_γ-difluoromethylenebisphosphonate analogue 2 of *nor*-carbovir triphosphate is a potent inhibitor of HIV reverse transcriptase; it also exhibits a greatly enhanced stability to dephosphorylation, in foetal blood serum, relative to AZTTP and other nucleoside triphosphates.

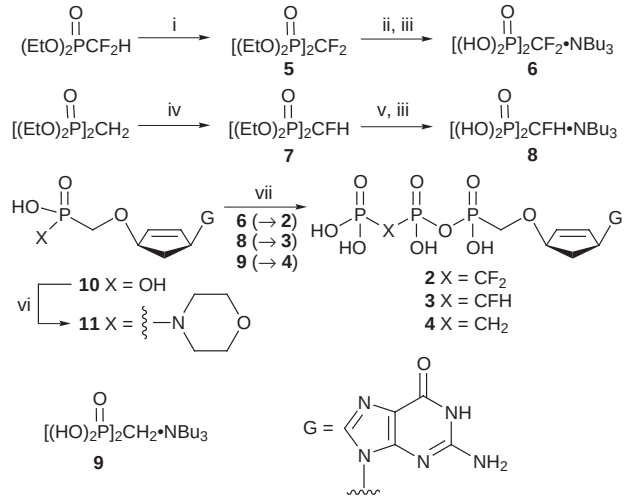
The *in vivo* lability of the P–O–P phosphate ester bonds in nucleoside triphosphates (NTPs) possessing interesting biological activity *in vitro* has prompted the search for more stable analogues. A great deal of effort has centred on the substitution of the phosphate ester oxygen atoms with carbon to give the corresponding phosphonates. These phosphonates are generally more stable to hydrolytic cleavage whilst being isosteric with their parent phosphates. The lower electronegativity of the methylene group, compared to oxygen, leads to a significant decrease in the acid dissociation constants of these phosphonic acids, which is often reflected in a reduction of biological activity. Through the seminal work of Blackburn and co-workers, halogenoalkylphosphonates have been shown to have improved potential as phosphate mimetics, as they more closely resemble the steric and electronic features of their parent units.[1]



We have previously shown that the pyrophosphoryl phosphonate **1** shows potent inhibition of HIV reverse transcriptase (HIV-rt), comparable to that of both AZT and carbovir triphosphates.[2] It is noteworthy that the active compound **1** has an absolute configuration that is the mirror image of that expected for a natural nucleotide.[3] Previous assessments of other such 'unnatural' nucleosides have shown them to exhibit reduced cytoxicity relative to their natural enantiomers.[4]

Further progress in this area would result from structural modifications of the diphosphate unit in **1** to further enhance its stability *in vivo* whilst still retaining good biological activity. To these ends we have prepared a series of bisphosphonates **2**–**4** with progressive fluoro-substitution within the P_β,P_γ-methylene linker group, as described in Scheme 1.

Noteworthy features of the chemical syntheses include the preparation of the tetraethyl difluoromethylenebisphosphonate **5** by coupling diethyl difluoromethylphosphonate with diethyl chlorophosphate,[5] and the preparation of tetraethyl fluoromethylenebisphosphonate **7** using the easy to handle and readily available fluorinating reagent *N*-fluorodibenzenesulfonimide.[6] The tetraesters were hydrolysed using bromotrimethylsilane and subsequently converted into their respective tributylammonium salts **6**, **8** and **9**. The bisphosphonates were coupled to the nucleoside monophosphonate **10** *via* the activated morpholidate **11**.[2,7] Products were purified by anion



Scheme 1 *Reagents and conditions*: i, LDA, THF, −70 °C, 1 h, then $ClP(O)(OEt)_2$, −70 °C, 1 h (34%); ii, Me_3SiBr, room temp., 18 h (83%); iii, NBu_3, EtOH, H_2O, room temp., 90 min; iv, KHMDS, THF, −78 °C, 1 h, then *N*-fluorodibenzenesulfonimide, THF–toluene, −78 °C, 90 min (26%); v, Me_3SiBr, room temp., 72 h (98%); vi, morpholine, DCC, Bu^tOH, H_2O, reflux, 5 h; vii, DMSO, room temp., 0.5–7 days (24–32%)

exchange, followed by reverse phase chromatography, and isolated as their ammonium salts.[8]

The efficacies of **2**–**4** as inhibitors of recombinant HIV-1-rt (Du Pont cat. No. NEI-490) were examined using the Du Pont RT-Detect™ Reverse Transcriptase Assay (cat. No. NEK-070) and the results are shown in Table 1.

Compounds **2**–**4** showed an expected correlation of increased activity with an increase in fluoro-substitution, culminating in the CF_2 analogue **2** being just an order of magnitude less active than the parent compound **1**.

The stability of the CF_2 compound **2** in human foetal blood serum was assessed and compared with AZTTP, some natural nucleoside triphosphates, and the pyrophosphoryl phosphonates' natural enantiomer, **1**-ent (Table 2). Blood serum is an appropriate medium in which to perform this assay as it contains numerous dephosphorylating enzymes and so provides a good model system of the extracellular environment *in vivo*. The half-

Table 1 Relative efficacy of substrates **1**–**4** as inhibitors of HIV-rt[a]

Compound	IC_{50}[b]/μM
1	0.5
2	5.8
3	34.8
4	> 100
AZTTP	1.0

[a] The IC_{50} values shown are the results from enzyme assays carried out in reaction mixtures containing 0.1 units ml^{-1} of HIV-rt, which were incubated at 37 °C for 110 min. [b] IC_{50} = substrate concentration required to inhibit to HIV-rt by 50%.

Table 2 Half-life time in fetal blood serum at 37 °C

Compound	$t_{1/2}$
1-ent	65 min[a]
2	45 h[a]
dGTP	< 30 min[b]
dATP	< 30 min[b]
AZTTP	5 min[b]

[a] As determined by the appearance of the phosphonate **10**. [b] As determined by the disappearance of the nucleoside triphosphate.

life of compound **2** was found to be 90 times greater than those of the natural purine NTPs, dGTP and dATP and also significantly greater than that of the pyrophosphoryl phosphonate **1**-ent. Evidently, the P_β,P_γ-difluoromethylene group greatly enhances the stability of compound **2** towards enzymatic dephosphorylation of the terminal phosphate group. Cleavage of the phosphate ester linkage at the P_α,P_β position could also be reduced as a result of the CF_2 analogue **2** being a poorer substrate for those dephosphorylating enzymes which function to hydrolyse NTPs at this position.

In conclusion, we have shown that it is possible to incorporate two stabilising phosphonate linkages in the 5′-side chain of the 'unnatural' enantiomer of carbovir triphosphate with only a modest compromise in biological activity whilst significantly enhancing biological stability. The use of more lipophilic derivatives of compound **2**, in order to facilitate drug delivery into whole cells, is currently under investigation and will be reported in due course.

We thank the MRC for a research studentship (to C. J. H.) and Wellcome Research Laboratories for the generous donation of AZT, made available through the MRC AIDS Reagent Project.

Notes and References

† E-mail: sj11@liverpool.ac.uk

1 G. M. Blackburn, D. Kent, F. Eckstein and T. Perée, *Nucleosides Nucleotides*, 1985, **4**, 165; G. M. Blackburn and D. Kent, *J. Chem. Soc., Perkin Trans. 1*, 1986, 913; G. M. Blackburn and D. Kent, *J. Chem. Soc., Chem. Commun.*, 1981, 930.

2 D. Coe, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans. 1*, 1992, 2695; V. Merlo, S. M. Roberts, R. Storer and R. Bethell, *J. Chem. Soc., Perkin Trans. 1*, 1994, 1477.

3 Both enantiomers of carbovir triphosphate are equally effective as inhibitors of HIV-rt; W. Miller, S. Daluge, E. Garvey, S. Hopkins, J. Reardon, F. Boyd and F. Miller, *J. Biol. Chem.*, 1992, **267**, 21220.

4 T. Lin, M. Luo, M. Liu, Y. Zhu, E. Gullen, G. Dutschman and Y. Cheng, *J. Med. Chem.*, 1996, **39**, 1757.

5 M. Obayashi, E. Ito, K. Matsui and K. Kondo, *Tetrahedron Lett.*, 1982, **23**, 2323.

6 E. Differding, R. Duthaler, A. Krieger, R. Rüegg and C. Schmit, *Synlett*, 1991, 395; previous methodology employed perchloryl fluoride (C. McKenna and P. Shen, *J. Org. Chem.*, 1981, **46**, 4573) or acetyl hypofluorite (D. Hebel, K. Kirk, J. Kinjo, T. Kovács, L. Lesiak, J. Balzarini, E. De Clercq and P. Torrence, *Bioorg. Med. Chem. Lett.*, 1991, **7**, 357).

7 J. Moffatt and H. Khorana, *J. Am. Chem. Soc.*, 1961, **83**, 649; J. Moffatt, *Can. J. Chem.*, 1964, **42**, 599.

8 *Selected data* for **2**: ν_{max}(KBr)/cm^{-1} 1269 (P=O); λ_{max}(H_2O)/nm 252.0; δ_H(400 MHz, D_2O) 1.89 (1 H, dt, *J* 15.6, 4.4, 5′-βH), 2.97 (1 H, dt, *J* 14.0, 7.2, 5′-αH), 3.84 (2 H, d, *J* 9.2, PCH_2), 4.76–4.81 (1 H, br, 4′-H), 5.28–5.0 (1 H, br, 1′-H), 6.07–6.11 (1 H, m, 2′-H), 6.33–6.38 (1 H, m, 3′-H), 7.75–7.87 (1 H, br, 8-H); δ_F(376 MHz, D_2O) 42.40–42.90 (br, CF_2); δ_P(162 MHz, D_2O) −1.69 (br, P_β), 3.90 (br, P_γ), 11.44 (d, *J* 31.0, P_α); *m/z* (ES) 522 (6%, MH^+), 328 (15, M − $CH_2F_2O_4P_2$). For **3**: ν_{max}(KBr)/cm^{-1} 1236 (P=O); δ_P(162 MHz, D_2O) 2.44 (br, P_β), 9.61 (br, P_γ), 10.34 (d, *J* 29.0, P_α); *m/z* (ES) 504 (57%, MH^+), 328 (100, M − $CFH_3O_4P_2$). For **4**: ν_{max}(KBr)/cm^{-1} 1216 (P=O); δ_P(162 MHz, D_2O) 15.97 (1P, br, P_β), 10.08 (2P, br, P_α and P_γ); *m/z* (ES) 486 (35%, MH^+), 328 (100, M − $CH_4O_4P_2$).

Received in Cambridge, UK, 9th February 1998; 8/01127C