Monofluorophosphonates as Phosphate Mimics in Bioorganic Chemistry: a Comparative Study of CH_2-, CHF- and CF_2-Phosphonate Analogues of *sn*-Glycerol-3-phosphate as Substrates for *sn*-Glycerol-3-phosphate Dehydrogenase

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The synthesis of the cyclohexylammonium salts of 3-(*S*),4-dihydroxy-1(*R,S*)-fluorobutylphosphonic acid **3** and 1,1-difluoro-3-(*S*),4-dihydroxybutylphosphonic acid **4** is reported; **3** is a better substrate for NADH linked *sn*-glycerol-3-phosphate dehydrogenase than the difluoromethylenephosphonate **4**; a comparative study of the CH_2-, CHF and CF_2- phosphonate analogues of *sn*-glycerol-3-phosphate is reported.

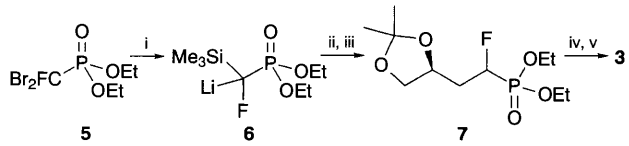
Difluoromethylenephosphonates (CF_2-phosphonate) have,[1] and are currently being[2] widely explored as phosphatase stable phosphate mimics for biological systems, however, their monofluoromethylenephosphonate (CHF-phosphonate) counterparts are only just being evaluated[3] in biological systems despite some obvious advantages. The pK_a of the second deprotonation of a phosphate group is *ca.* 6.4.[4] Any phosphonate mimic should ideally emulate this as it is generally considered to be an important electronic factor in the binding of such analogues to enzymes. The CH_2-phosphonate has a pK_a of *ca.* 7.6[4] and is clearly less acidic. The electron withdrawing effect of the two fluorine atoms on the CF_2-phosphonate significantly lowers the pK_a to *ca.* 5.4,[5b] however, the introduction of only one fluorine atom for the CHF-phosphonate results in a pK_a of *ca.* 6.5,[6] almost identical to that of the natural phosphate. A recent theoretical study[7] also suggests that the electrostatic profile of a CHF-phosphonate is close in magnitude to that of the phosphate.

Earlier work from this laboratory[5a] has shown that 1,1-difluoro-3-(*R,S*),4-dihydroxybutylphosphonate **4**, an analogue of *sn*-glycerol-3-phosphate **1**, is a substrate for NADH linked *sn*-glycerol-3-phosphate dehydrogenase. However, our preliminary studies suggested that the CF_2-phosphonate analogue, albeit in racemic form, was a poorer substrate than *sn*-glycerol-3-phosphate **1** and the corresponding CH_2-phosphonate analogue **2** for the dehydrogenase. In view of this we decided to compare both the CHF-phosphonate **3** and the CF_2-phosphonate **4** now in homochiral form, as analogues of **1** to assess the significance of the sequential addition of one and two fluorine atoms onto the phosphonate carbon.

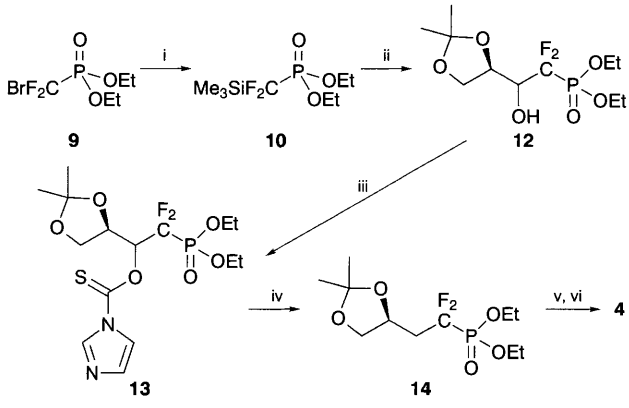
Our synthetic approach to 3-(*S*),4-dihydroxy-1-(*R,S*)-fluorobutylphosphonate **3** exploited recent methodology[8] employing the α-lithiated-α-fluorotrimethylsilylmethylphosphonate carbanion **6**. This organo-lithium reagent is readily prepared by double halogen exchange in the presence of chlorotrimethylsilane, from diethyl dibromofluoromethylphosphonate **5**. The protected phosphonate **7** was efficiently accessed by alkylation of **6** with 2,2-dimethyl-1,3-dioxolane-4-(*R*)-methyl triflate **8**,[9] followed by desilylation and aqueous workup. Treatment with bromotrimethylsilane and subsequent addition of water provided the desired monofluorophosphonic acid, as a diastereomeric mixture (1 : 1) epimeric at the CHF stereogenic centre. This compound was isolated after neutralisation as its biscyclohexylammonium salt **3** (Scheme 1).†

Our route to the CF_2-phosphonate analogue **4** exploited methodology for the formation of 1,1-difluoro-2-hydroxy alkylphosphonates by addition of difluoro(trimethylsilyl)-methylphosphonate **10** to carbonyl compounds under fluoride catalysis.[10] The silylated phosphonate **10** was easily accessible by direct silylation of the bromodifluoromethylphosphonate **9** using *n*-butyllithium and chlorotrimethylsilane.[11] Thus, treatment of **10** with (*S*)-2,3-*O*-isopropylideneglyceraldehyde **11** in the presence of tetrabutylammonium fluoride gave after hydrolysis, the 1,1-difluoro-2-hydroxybutylphosphonate **12**. In order to carry out a Barton deoxygenation,[12] compound **12** was treated with thiocarbonylbisimidazole in refluxing THF to give thioimidazolide **13**. Reduction of **13** with tri-*n*-butyltin hydride in the presence of AIBN in refluxing toluene delivered the desired phosphonate **14**[13] which was deprotected in the usual manner and isolated as its biscyclohexylammonium salt (Scheme 2).‡

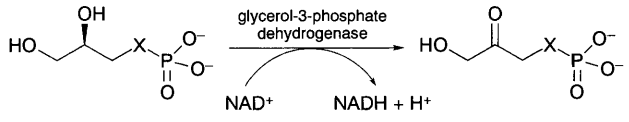
Evaluation of the Michaelis-constants (K_m) and relative V_{max} values[14,15] of **3** and **4** (Scheme 3) revealed that the CHF-phosphonate is a significantly better substrate for NADH linked



Scheme 1 *Reagents and conditions*: i, 2.2 equiv. Bu^nLi, Me_3SiCl, THF, −78 °C, 10 min; ii, **8**, −78 °C, 40 min; iii, LiOEt–EtOH, 0 °C, 1 h then aq. NH_4Cl–diethyl ether, 83%; iv, Me_3SiBr, room temp., 3 h then H_2O, room temp., 8 h; v, $C_6H_{11}NH_2$, 63%.

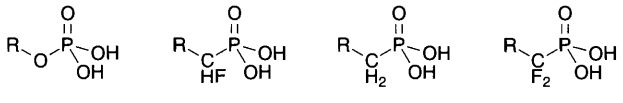


Scheme 2 *Reagents and conditions*: i, Bu^nLi, Me_3SiCl, THF, −78 °C, 20 min, 92%; ii, **11**, 0.05 equiv. Bu_4NF, 3 Å mol sieves, THF, room temp., 24 h then sat. aq. $NaHCO_3$, 2 h, 36%; iii, $Im_2C{=}S$, THF, reflux, 3 h, 81%; iv, Bu_3SnH, AIBN, toluene, reflux, 2 h, 66%; v, Me_3SiBr, room temp., 3 h then H_2O, 15 h; vi, $C_6H_{11}NH_2$, 30%.



	K_m / mmol dm^{-3}	V_{rel}
1 X = O	0.2	1.0
2 X = CH_2	0.18	0.8
3 X = CHF	0.17	0.9
4 X = CF_2	0.73	0.8

Scheme 3



sn-glycerol-3-phosphate dehydrogenase (Sigma Type I, rabbit muscle) than the CF_2-phosphonate (K_m **3** = 0.17 mmol dm^{-3} K_m **4** = 0.73 mmol dm^{-3}). Both diastereoisomers of **3** were processed by the dehydrogenase at different but comparable rates as determined by ^{19}F NMR, and the K_m value must therefore be an average of that of the two diastereoisomers. In fact, **3** shows the same K_m value as the CH_2-phosphonate **2**[4,14] and both have a lower K_m values than glycerol-3-phosphate itself. Thus, from this comparative study only the CF_2-phosphonate deviates significantly from the parent phosphate. It is unlikely that the bridging oxygen atom of the phosphate group is involved in a hydrogen bond to the enzyme, as this interaction would be lost in the CH_2-phosphonate case and would be expected to increase that K_m. The performance of the phosphonate analogues (CH_2- ~ CHF- > CF_2-) cannot obviously be attributed to ionisation as the CH_2-phosphonate would be expected to perform least well on this basis, assuming that the enzyme binds the phosphate and phosphonates in their dianionic form. On the other hand, the trend is consistent with the general view that a hydrogen atom can be substituted by a fluorine atom without introducing a significant steric perturbation. Substitution of the second fluorine atom is deleterious possibly due to adverse steric interactions but is more likely due to a greater electrostatic potential associated with the CF_2 group over that of CHF in **3** or oxygen in **1**. This analysis is consistent with the theoretical study[7] where the CHF-phosphonate and the phosphate group are predicted to have a similar polar electrostatic profile. The directionality of the electrostatic potential will differ for the diastereomers of **3** at the CHF stereogenic centre and this could account for the small rate difference observed between them during the enzymatic oxidation.

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Footnotes

† All intermediates gave satisfactory spectral data. *Selected data* for **3**: ^1H NMR (D_2O) δ 1.03–1.26 (8H, m), 1.40–1.86 (14H, m), 2.84–3.00 (2H, m), 3.25–3.50 (2H, m), 3.62–3.81 (1H, m), 4.26–4.41 and 4.44–4.61 (1H, dm, $^2J_{HF}$ 47.5 Hz). ^{19}F NMR (D_2O) δ −201.58 (dddd, $^2J_{FP}$ 63, $^2J_{FH}$ 47.5, $^3J_{FH}$ 35.7, $^3J_{FH}$ 23 Hz), −204.48 (dddd, $^2J_{FH}$ 63, $^2J_{FH}$ 47.9, $^3J_{FH}$ 42.3, $^3J_{FH}$ 14.8 Hz). ^{31}P NMR (D_2O) δ 12.09, 12.41 (d, $^2J_{PF}$ 63 Hz).

‡ *Selected data* for **4**: ^1H NMR (D_2O) δ 1.03–1.25 (8H, m), 1.40–1.83 (10H, m), 1.89–2.07 (2H, m), 2.84–3.00 (2H, m), 3.33 (1H, dd, $^2J_{HH}$ 11.7, $^3J_{HH}$ = 6.4 Hz), 3.42 (1H, dd, $^2J_{HH}$ 11.7, $^3J_{HH}$ 4.3 Hz), 3.87–3.94 (1H, m). ^{19}F NMR (D_2O) δ −108.51 (ddt, $^2J_{FF}$ 282, $^2J_{FP}$ 85.2, $^3J_{FH}$ 21.4 Hz), −111.50 (ddt, $^2J_{FF}$ 282, $^2J_{FP}$ 85.2, $^3J_{FH}$ 21.4 Hz). ^{31}P NMR (D_2O) δ 5.55 (t, $^2J_{PF}$ 85.9 Hz).

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