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FLUORINE IN ENZYME CHEMISTRY PART 2[1]
THE PREPARATION OF DIFLUOROMETHYLENEPHOSPHONATE ANALOGUES OF GLYCOLYTIC PHOSPHATES APPROACHING AN ISOSTERIC AND ISOELECTRONIC PHOSPHATE MIMIC

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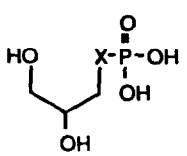
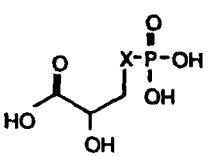
Abstract - The preparation of difluoromethylenephosphonate analogues of the glycolytic intermediates, glycerol-3-phosphate and 3-phosphoglycerate are described Attempts to prepare the corresponding analogue of dihydroxyacetone phosphate failed due the facile elimination of hydrogen fluoride from the target molecule. Finally the synthesis of a difluoromethylenephosphonate possessing inhibitory activity against RNA transcriptase from the influenza virus is described

The difluoromethylenephosphonate moiety has attracted much attention[2] in recent years largely due to a series of preliminary investigations on analogues of pyro- and tri-phosphates where the bridging oxygen atoms were replaced by a CF_2 group Notably the investigations by Blackburn[3] and co workers on CF_2 analogues of ATP and Poulter and co-workers[4] on CF_2 analogues of geranyl pyrophosphate indicate a closely analogous steric and electronic profile to that of the parent functionality There are wide implications of a potential, readily available, phosphate 'mimic' and we are beginning to study the enzymatic activity of a variety difluoromethylenephosphonates as isosteric and isoelectronic analogues of monophosphates as part of a wider programme of explorations on the effects of fluorine incorporation on enzymic activity

The glycolytic phosphates are suitable targets for investigation since these molecules are key metabolites and the enzymes of the glycolytic pathway are accessible from commercial sources The synthetic challenge is considerable as few efficient methods[5] exist for the introduction of the difluoromethylenephosphonate moiety into organic



X = CF_2 (1)
X = O (2)



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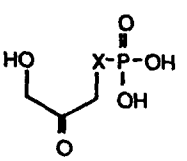
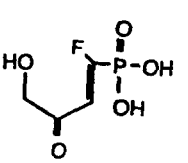
X = O (4)



X = CF_2 (5)
X = O (6)



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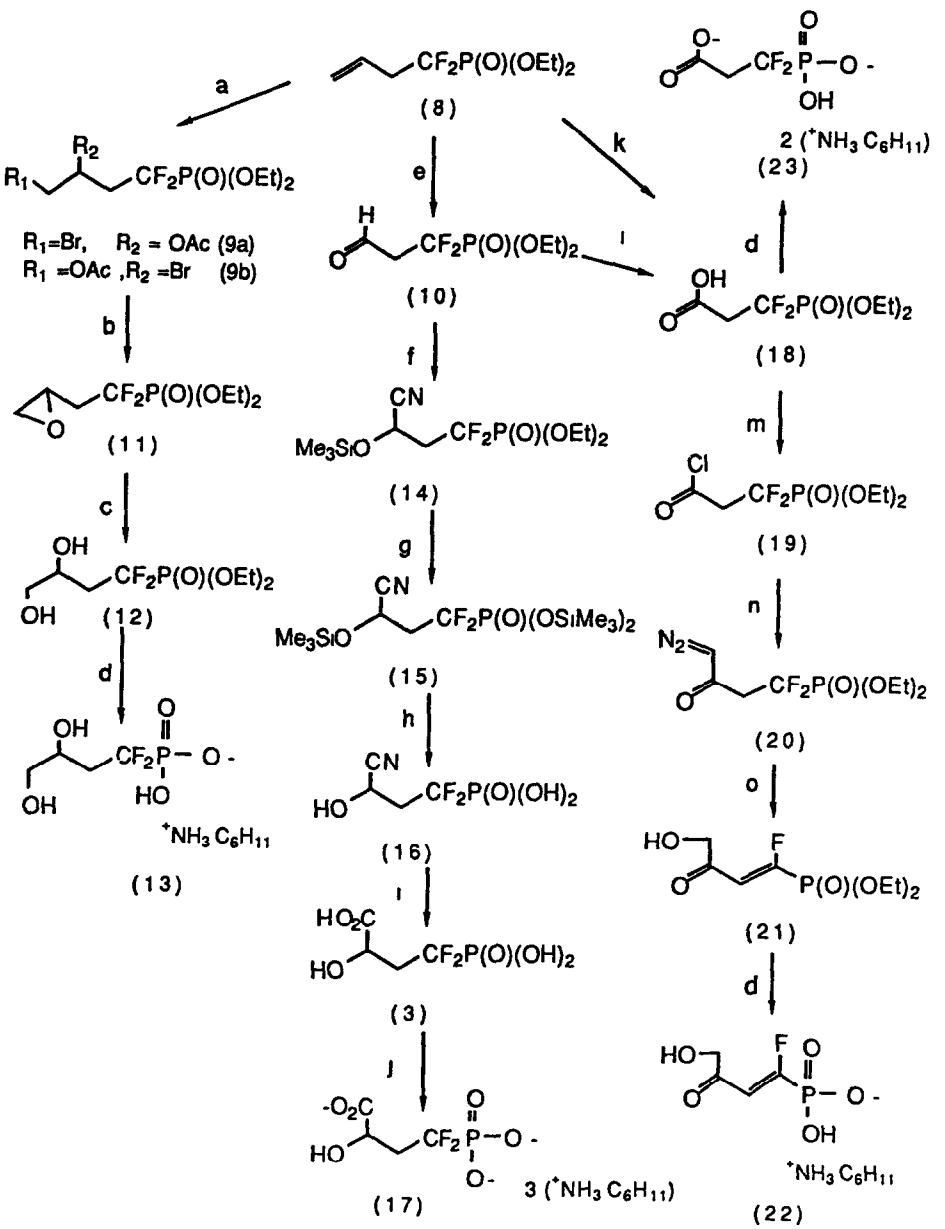


Reagents and Conditions

a $Hg(OAc)_2$,Br_2,AcOH, 10°C/8h, b KOH,CH_3OH, 18°C/8h, c 5%HCl/DMSO (2 1), d Me_3SiBr,H_2O,$C_6H_{11}NH_2$. e O_3,CH_3OH,CH_2Cl_2 then DMS, f. Me_3SiCN,18-crown-6/KCN(cat), g Me_3SiBr, h 3N HCl, 30°C/24h, i conc HCl, 85°C/20h, j $C_6H_{11}NH_2$; k $RuCl_3$ $3H_2O$,KIO_4,CH_3CN,CCl_4,H_2O, 18°C/30h, l $RuCl_3$ $3H_2O$,KIO_4, CH_3CN,CCl_4,H_2O, 18°C/6h, m $SOCl_2$, 60°C/12h, n CH_2CN_2, o Dowex "H".

Scheme 1

systems We have described the scope of diethyl difluoromethylphosphonylcadmium bromide, a reagent first reported[6,7] by Burton and co-workers Although this organocadmium reagent has limitations we favour it over the equivalent organolithium derivative, due to the inherent instability of the latter and in the present paper we describe full details of our synthetic procedures for the synthesis of difluoromethylenephosphonate analogues of the glycolytic intermediates[1] using this approach

Analogues (1) and (3) of glycerol-3-phosphate (2) and 3-phosphoglycerate (4) respectively, have been successfully prepared but, to date, we are unable to isolate the analogue (5), of dihydroxyacetone phosphate (6) without elimination of hydrogen fluoride, giving rise only to the fluorovinylphosphonate (7) Our routes to all of the analogues start from allyl phosphonate (8) (Scheme 1) which can be obtained in 64% yield after treatment of allyl bromide with diethyl difluoromethylphosphonylcadmium bromide in THF [1] As a consequence of the synthetic manipulations which can be carried out on (8), this allyl phosphonate occupies a pivotal role in the preparation of the target analogues In particular treatment of (8) with mercuric acetate and bromine gave rise to a 47 53 mixture of the isomeric bromo-acetates (9a and 9b) as the first transformation towards analogue (1) Ozonolysis of (8) afforded aldehyde (10) in high yield which opened up routes to the remaining two analogues (3) and (5)

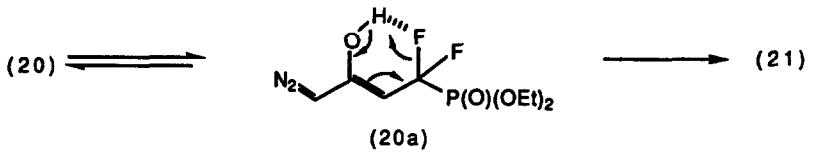
The mixture of bromo-acetates (9a and 9b) after treatment with methanolic KOH afforded epoxide (11), which was converted efficiently to the diol (12) under acid catalysed conditions. The diethyl phosphonate ester (12) was then hydrolysed by addition of bromotrimethylsilane[8] and neutralised to provide (13), the cyclohexylammonium salt of (1)

Aldehyde (10) was transformed smoothly into the protected cyanohydrin (14) after treatment with trimethylsilyl cyanide [9] Phosphate ester hydrolysis afforded the trisilylated intermediate (15) and then treatment with 3N hydrochloric acid gave (16), contaminated with (3) the product of the nitrile hydrolysis The nitrile was then completely hydrolysed in 6N HCl to give the 3-phosphoglycerate analogue (3) which was isolated after neutralisation as the tricyclohexylammonium salt (17)

Our route to (5), the difluoromethylenephosphonate analogue of dihydroxyacetone phosphate (6), involved generating the carboxylic acid (18) by oxidising[10] allyl phosphonate (8) with RuO_4 ($RuCl_3$, KIO_4, in CCl_4 H_3CCN H_2O,1 1 2) This could be accomplished directly in moderate yield (45%),[1] or more efficiently (85% overall) after oxidation of aldehyde (10) with RuO_4 under similar conditions Compound (18) was easily converted with thionyl chloride into the corresponding acid chloride (19) which gave, after treatment with diazomethane, the diazoketone (20) Subsequent conversion into the hydroxyketone under a variety of acidic conditions proved problematic In acidic methanol

[1] A preliminary account relating to the synthesis of (3) has been communicated[7]

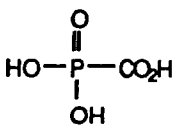
for example no identifiable products were isolated The smoothest transformation was accomplished using Dowex 'H⁺' form however, concomitant hydrogen fluoride elimination resulted affording fluorovinylphosphonate (21) as the only product. This compound was hydrolysed and isolated as the cyclohexylammonium salt (22).



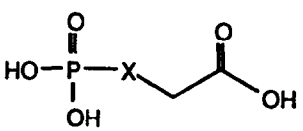
(20a)

The carboxylic acid (18) was also hydrolysed and converted into its dicyclohexylammonium salt (23) Unlike the transformation of (20) into (21), the conversion of (18) into (23) proceeded without competing elimination The ability of (20) to enolise to (20a) under acidic conditions presumably increases its capacity to eliminate hydrogen fluoride when compared to (18). Although not an analogue of a glycolytic metabolite this compound (23) has potential as an antiviral agent[11] acting as an analogue of inorganic pyrophosphate Indeed preliminary investigations indicate inhibitory activity against RNA transcriptase from the influenza virus but at half the level of activity of phosphonoformic acid (24), the most successful of the series, (ID_{50} s of 75μm and 35μm respectively)

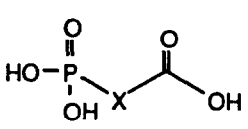
The salt of the difluorophosphonate (18) is a much more potent inhibitor of the enzyme than the corresponding methylene analogue (25) (ID_{50}>500μm) This can be contrasted with the analogous modification in the phosphonoacetic acid series where the opposite effect is apparent, i e (27) is a more effective inhibitor against Herpes Simplex Virus than (26) [2b,2c]



(24)



X = CF_2 (18)
X = CH_2 (25)



X = CF_2 (26)
X = CH_2 (27)

The pKa values of the final deprotonation of the two glycolytic analogues (1) and (3) were found to be 5 64 and 5 44 respectively This can be contrasted with pka values of 6 45 and 6 25 for the parent phosphate compounds (2)[13] and (4) [14] The increased acidity of the phosphonates inevitably weakens the electronic similarity to the phosphate group However in enzyme transformations where the phosphate is bound in the dianionic form this may not amount to a severe deviation We have in fact demonstrated[7] that (13) is a

substrate for glycerol-3-phosphate dehydrogenase and further work on the enzymology is proceeding

EXPERIMENTAL

IR spectra were recorded on a Perkin Elmer 257 Spectrometer Mass spectra were recorded on a VG-7070E instrument NMR spectra were obtained on a Bruker AC-250 and a Varian EM360L 60 MHz instruments in $CDCl_3$ or D_2O. Chemical shifts are quoted relative to TMS for ^1H- and ^{13}C- NMR spectra, ^{19}F chemical shifts are quoted as negative and relative to fluorotrichloromethane and ^{31}P chemical shifts relative to phosphoric acid Cadmium powder-100 mesh, 99.5% was obtained from Aldrich Chemicals and vacuum dried prior to use (70°C, 0 01 mmHg) Allyl bromide was freshly distilled and solvents were dried and distilled prior to use Reactions requiring anhydrous conditions were carried out under an atmosphere of nitrogen

Diethyl 1 1-difluoro-3,4-epoxybutylphosphonate (11)

A solution of (9a) and (9b)[1] (9 54 g, 26 mmol) in methanol was added dropwise to a solution of potassium hydroxide (3 02 g, 53 9 mmol) in methanol (25 ml) at 18°C The reaction was slightly exothermic and warmed initially and was stirred for 8 h by which time a precipitate of potassium bromide had formed Water (25 ml) was added and the epoxide extracted into ether (4 x 25 ml) The organic extracts were combined and dried over $MgSO_4$ and the solvent removed under reduced pressure The clear oil was distilled (60-64°C, 0 01 mmHg) to afford (11) (1 8 g, 7 8 mmol), 30% yield
^1H-NMR ($CDCl_3$) · 1 39 (6H, t, 7Hz, $C\underline{H}_3CH_2O$), 2.1-2 42 (2H, m, $C\underline{H}_2CF_2$), 2 56 (2H, m, $C\underline{H}_2OCH$), 2 87 (1H, m, $CH_2OC\underline{H}$), 4 32 (4H, p, CH_3CH_2O) ^{19}F-NMR ($CDCl_3$) -111 41 (d.t, J_{F-P} = 105 76 Hz, J_{F-H} = 20 Hz) ^{31}P-NMR ($CDCl_3$) : 5 37 (t).

Diethyl 1 1-difluoro-3,4-dihydroxybutylphosphonate (12)

A solution of (11) (0 3 g, 1 22 mmol) in DMSO (1 ml) and 5% aqueous HCl (2 ml) was stirred for 20 h at 18°C The solvents were removed at reduced pressure and the diol was used without further purification
^1H-NMR ($CDCl_3$) 1 2 (6H, t, 6.75Hz, $C\underline{H}_3CH_2O$), 2.15-2 26 (2H, m, $C\underline{H}_2CF_2$), 2 54 (2H, m, $O\underline{H}$, disappeared after shaking with D_2O), 3 42-3 55 (1H, m, $C\underline{H}OH$), 3 88-3 95 (2H, m, $C\underline{H}_2OH$), 4.10 (4H, p, $CH_3C\underline{H}_2O$) ^{19}F-NMR ($CDCl_3$) -111 6 (d t, J_{F-P} = 105 21 Hz, J_{F-H} = 18 9 Hz) ^{31}P-NMR ($CDCl_3$) 5 61 (t). IR (neat) 3395, 1265 cm^{-1}

Cyclohexylammonium 1 1-difluoro-3 4-dihydroxybutylphosphonate (13)

Bromotrimethylsilane (0 29 g, 1 90 mmol) was added dropwise to (12) (0 1 g, 0 38 mmol) and stirred for 12 h, after which volatiles were removed under reduced pressure (0.01 mmHg) The crude trimethylsilyl ester (0 08 g, 0 3 mmol) was then dissolved in ether (18 ml) and extracted into water (3 x 10 ml) The acidic aqueous extracts were combined and neutralised by dropwise addition of cyclohexylamine to pH 8 Lyophilisation afforded a white powder which was recrystallised from methanol/acetone (1/5) to give the cyclohexylammonium salt (13) (0 085 g 0 278 mmol), 75% yield
^1H NMR (D_2O) 2 16 (2H, m, CH_2CF_2), 3 5 (1H, d d, 11 6 Hz, 6 4 Hz, $CH_a\underline{H}_bOH$), 3 6 (1H, d d, 11 6 Hz, 4 2 Hz, $CH_a\underline{H}_bOH$) ^{19}F-NMR (D_2O) -108 (1F, d d t, J_{F-F} = 293 50 Hz, J_{F-P} = 87 0 Hz, J_{F-H} = 23 06 Hz); -110 (1F, d d t , J_{F-F} = 293 50 Hz, J_{F-P} = 87 0 Hz, J_{F-H} = 23 06 Hz) ^{31}P-NMR (D_2O) 5 05 (t, J_{F-P} = 86 Hz) (Found C, 39 8 , H, 7 27 ; N, 4 55 $C_{10}H_{22}NF_2PO_5$ requires C, 39 38 , H, 7 21 , N, 4 59%)

Ditrimethylsilyl 4 4-difluoro-2-trimethylsilyloxy-4-phosphonobutyrlnitrile (15)

Trimethylsilyl cyanide (2 g. 20 2 mmol) was added to a mixture containing (10) (3 0 g, 13 2 mmol). 18-crown-6 (18 mg) and potassium cyanide (15 mg) and stirring was continued for 16 h at 45°C Excess trimethylsilyl cyanide was removed under reduced pressure to afford crude silyated cyanohydrin (14) (3.3 g).

^{1}H-NMR ($CDCl_3$) : 0 35 (9H, s, $(CH_3)_3Si$); 1.18 (6H, t, 7 1 Hz, CH_3CH_2O); 2 43 (2H, t.t, CH_2CF_2), 4 13 (4H, p, CH_3CH_2O), 4 87 (1H, t, 7.4Hz, CHCN) ^{19}F-NMR ($CDCl_3$) -112 51 (d t, J_{F-P} = 102.9 Hz, J_{F-H} = 18 6 Hz) ^{31}P-NMR ($CDCl_3$) . 4 72 (t)

Bromotrimethylsilane (5 8 g, 37 9 mmol) was added dropwise over 15 min to (14) (3 25 g, 9 87 mmol) and the mixture stirred for 20 h at 20°C, then for 2 h at 35°C. Excess bromotrimethylsilane was removed under reduced pressure to leave the trisilylated product (15) (4.08 g, 9.8 mmol) as an oil in quantitative yield.
^{1}H-NMR ($CDCl_3$) : 0 42 (18H, s, 2x $(CH_3)_3SiOP$); 0 52 (9H, s,$(CH_3)_3SiOC$); 2.67 (2H, t.t, CH_2CF_2); 4 96 (1H, t, 5.5Hz, CHCN). ^{19}F-NMR ($CDCl_3$) : -111 62 (d t, J_{F-P} = 104.48 Hz, J_{F-H} = 15 93 Hz) ^{31}P-NMR ($CDCl_3$) 3 75 (t)

4 4-Difluoro-2-hydroxyphosphonobutyric acid (3)

3N HCl (60 ml) was added to a solution of (15) (4 08 g, 9 8 mmol) in ether and the biphasic reaction stirred at 30°C for 24 h. After cooling the aqueous layer was separated and the solvent removed under reduced pressure to afford a mixture of the nitrile (16) and the carboxylic acid (3). Further acidic hydrolysis with conc. HCl (10ml) at 85°C for 20 h afforded an oil after evaporation The product was washed by successive addition and then evaporation of three portions (10 ml) of distilled water to provide (3) (1.81 g, 9 mmol) as a clear oil in 68% overall yield from (10).
^{1}H-NMR (D_2O) · 2 45 (2H, t.t, CH_2CF_2), 4 85 (1H, t, CHOH) ^{13}C-NMR (D_2O/H_2O) : 36 75 (t, CHOH, J^3_{C-F} = 15 5 Hz), 64 68 (t, CH_2CF_2, J^2_{C-F} = 58.10 Hz), 122.10 (t.d, CF_2, J^1_{C-F} = 271.2 Hz, J^1_{C-P} = 185 1 Hz) ^{19}F-NMR · -96 26 (d t, J_{F-P} = 119.5 Hz, J_{F-H} = 18 5 Hz) ^{31}P NMR : 4 35 (t)

Dicyclohexylammonium 4 4-difluoro-3-hydroxyphosphonobutyrate (17)

Cyclohexylamine was added to a solution of (3) (1.5 g, 7 35 mmol) in water (20 ml) to pH 8 and then the mixture was stirred for 3 h at 20°C The solvent was removed at reduced pressure and the remaining solid recrystallised from methanol/acetone (1/5) to afford the tricyclohexylammonium salt (17) as a white amorphous powder (3 02 g, 5.84 mmol), 80% yield
^{1}H-NMR (D_2O) : 2 31 (2H, t t, CH_2CF_2), 3.42 (1H, m, CHOH). ^{19}F-NMR (D_2O) . -110.5 (1F, d d.m, J_{F-F} = 294 3 Hz, J_{F-P} = 87.5 Hz), -111 4 (1F, d d m, J_{F-F} = 294 3 Hz, J_{F-P} = 87.5 Hz) ^{31}P-NMR (D_2O) : 4 43 (t, J_{F-P} = 87 Hz). (Found · C, 51 21, H, 9 25, N, 8 36. $C_{22}H_{46}N_3F_2O_6P$ requires C, 51 06, H, 8 89, N, 8 12%)

Diethyl 3 3-difluoro-3-phosphonopropionic acid (18)

Potassium periodate (13 79 g, 60 mmol) and reuthenium(III) chloride trihydrate (70 mg, 0 26 mmol) were added to a biphasic solution of (10)[1] (3 22 g, 15 mmol) in carbontetrachloride (20 ml) acetonitrile (20 ml) and water (60 ml) and the entire mixture stirred vigorously for 6 h at 18°C Dichloromethane (150 ml) was added and the solution filtered The organic phase was separated and dried over $MgSO_4$ and the solvent removed at reduced pressure Purification by silica gel chromatography (EtOAc CH_2Cl_2/4 1) gave 18 (3 05 g, 13 mmol) as a clear oil, 87% yield (b p 96-101°C/0 01 mmHg) ^{1}H-NMR ($CDCl_3$) 1 3 (6H, t, CH_3CH_2), 3 04 (2H, d t, 18 82 Hz, CH_2CF_2), 4 19 (4H, q, CH_2O), 7 37 (1H, s, OH), ^{19}F-NMR ($CDCl_3$) -111 16 (d t, 105 19 Hz), ^{31}P-NMR ($CDCl_3$) . 4 99(t) IR (neat) 3350, 1745, 1275 cm^{-1} (Found C, 34 28, H, 5 38 $C_7H_{13}F_2O_5P$ requires C, 34 14, H, 5 28%)

Diethyl 3 3-difluoro-3-phosphonopropionyl chloride (19)

Thionyl chloride (3 26 g, 36 47 mmol) was added dropwise over 20 min to (18) (3 5 g, 14 58 mmol) and the reaction stirred at 60°C for 12 h Excess thionyl chloride was removed by distillation and the remaining traces were removed by addition and removal of benzene (50 ml) at reduced pressure After vacuum drying (0 01 mmHg) (19) (3 8 g, 14 36 mmol) was recovered as a residual oil in quantitative yield and was used without further

purification
^{1}H-NMR ($CDCl_3$) : 1 14 (6H, t, 7Hz, $C\underline{H}_3CH_2O$), 3 31 (2H , d t , $C\underline{H}_2CF_2$), 4 12 (4H, p, $CH_3C\underline{H}_2O$) ^{19}F-NMR . -111 96 (d t, J_{F-P} = 104.49 Hz, J_{F-H} =17 41 Hz). ^{31}P-NMR · 1 2 (t) IR (neat) : 1805, 1275 cm^{-1}

Diethyl 1,1-difluoro-3-oxo-4-diazobutylphosphonate (20)

A solution of (19) (5 1 g, 19 28 mmol) in ether (40 ml) was added dropwise over 60 min to an ethereal solution (50 ml) of diazomethane (53 mmol) at -10°C. Once addition was complete the solution was left to stir at 0°C for 15 h and then 18°C for 5 h The solvent was removed at reduced pressure to afford the diazoketone (20) as a clear oil (4 0 g, 14 8 mmol), 76% yield
^{1}H-NMR ($CDCl_3$) . 1.24 (6H, t, 7.5Hz, $C\underline{H}_3CH_2O$), 2 95 (2H, d t, J_{H-P} = 3 96 Hz, $C\underline{H}_2CF_2$), 4 14 (4H, p, $CH_3C\underline{H}_2O$), 5.53 (1H, s,$C\underline{H}N_2$) ^{19}F-NMR ($CDCl_3$) . -105 41 (d t, J_{F-P} = 102 13 Hz, J_{F-H} = 19.76 Hz) ^{31}P-NMR ($CDCl_3$) · 4 88 (t) IR (neat) . 2110 (CHN_2), 1735,1645, 1275 cm^{-1}.

Diethyl-trans 4-hydroxy-3-oxo-1-fluorobut-1-enylphosphonate (21)

Dowex 50x8-200 hydrogen 'H$^+$' form (10 g) was added to an aqueous solution (15 ml) of (20) (2 0 g, 7 4 mmol) and the mixture was shaken vigorously for 48 h After filtration the solution was extracted with ether (3 x 15 ml) The ether extracts were combined, dried over $MgSO_4$, and the solvent removed under reduced pressure The residual oil was purified by silica gel chromatography (hexane CH_2Cl_2/1 1) to afford (21) as a clear oil (0 5 g, 2.3 mmol), 31% yield
^{1}H-NMR ($CDCl_3$) 1 31 (6H, t, 7 5Hz, $C\underline{H}_3CH_2O$), 3 69 (1H, s, $O\underline{H}$, removed by shaking with D_2O), 4 08 (4H, p, $CH_3C\underline{H}_2O$), 4 38 (2H, s, $C\underline{H}_2OH$), 6 36 (1H, d d, J_{H-F} = 40 34 Hz, J_{H-P} = 8 44 Hz, $C\underline{H}$=) ^{13}C-NMR ($CDCl_3$) · 15 53 (s, $\underline{C}H_3CH_2O$), 63 62 (s, $CH_3\underline{C}H_2O$), 63 65 (s, $\underline{C}H_2OH$), 116 31 (d, J^2_{C-F} = 25 97 Hz, $\underline{C}H$=CF), 159 16 (d d, J^1_{C-F} = 306 93 Hz, J^1_{C-P} = 225 92 Hz, $\underline{C}F$), 187 3 (d, J^3_{C-F} = 10 18Hz, $\underline{C}O$) ^{19}F-NMR ($CDCl_3$) -104 89 (d d, J_{F-P} = 99 78 Hz, J_{F-H} = 40 34 Hz) ^{31}P-NMR ($CDCl_3$) 1 45 (d) IR (neat) · 3445, 1710, 1640, 1275 cm^{-1} (Found C, 39 76, H, 5 89 $C_8H_{14}FO_5P$ requires C, 40 0, H, 5 83%)

Cyclohexylammonium-trans 4-hydroxy-3-oxo-1-fluorobut-1-enylphosphonate (22)

Bromotrimethylsilane (1.0 g, 6 53 mmol) was added dropwise at 0°C over 5 min to (21) (0 5 g, 2 3 mmol) and the reaction was allowed to stir for 6 h at 18°C Volatiles were removed under vacuum and the residual oil was used directly ^{1}H-NMR indicated a new resonance at 0 3 ppm (18H, $(CH_3)_3SiO$) with no signals corresponding to the CH_3CH_2O-groups The oil was dissolved in ether (50 ml), treated with water (50ml) and the biphasic solution stirred for 2 h at 18°C The aqueous layer was separated and neutralised to pH 8 with cyclohexylamine After solvent removal (22) (1 35 g, 4 76 mmol) was recrystallisation (ether/methanol) and isolated as a white amorphous solid in 73% yield
^{1}H-NMR ($CDCl_3$) 4 56 (2H, s, $C\underline{H}_2OH$), 6 02 (1H, d d, $C\underline{H}$=) ^{19}F-NMR (D_2O) -102 44 (d d, J_{F-P} = 106 45 Hz, J_{F-H} = 44 52 Hz), ^{31}P NMR (D_2O) 0 84 (d) (Found C, 42 3 H, 6 75 $C_{10}H_{19}NFO_5P$ requires C, 42 4, H, 6 71%)

Dicyclohexylammonium 3,3-difluoro-3-phosphonopropionate (23)

The dicyclohexylammonium salt (23) was prepared from (18) according to the procedure outlined above for (22)
^{1}H-NMR (D_2O) 2 85 (2H, d t, $C\underline{H}_2CF_2$) ^{19}F-NMR (D_2O) -108 52 (d t, J_{F-P} = 88 95 Hz, J_{F-H} = 20 70 Hz). ^{31}P-NMR (D_2O) 2 54 (t) IR (nujol) 1650, 1265 cm^{-1} (Found . C, 46 52, H, 7 92, N, 7 33, P, 7 98 $C_{15}H_{21}N_2F_2O_5P$ requires C, 46 39, H,7 98, N, 7 21, P, 8 06%)

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