The synthesis of novel bisphosphonates as inhibitors of phosphoglycerate kinase (3-PGK)

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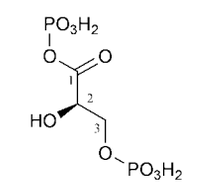
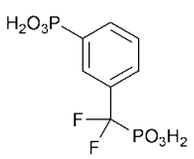
A series of conformationally-restrained analogues of 1,3-bisphospho-D-glyceric acid (1,3-BPG) **1** has been synthesised for use as inhibitors of 3-PGK (E.C. 2.7.2.3). These compounds have non-scissile phosphonate linkages and also incorporate α-halogen substituents to make them isopolar and isosteric mimics of the natural substrate. A monocyclic aryl core between the two phosphoryl centres provides both a rigid framework linking these moieties and loci for further substitution. The compounds were tested against human 3-PGK and found to be good competitive inhibitors. α-Fluorination of the phosphonic acids increased the affinity for the enzyme into the submicromolar range. Correlation of IC_{50} data with pK_{a^3} and pK_{a^4} values indicates that the acidity of the phosphoryl group exerts a strong influence on protein binding.

Introduction

The glycolytic pathway converts glucose into pyruvate and is the major source of energy in the citric acid cycle. Phosphoglycerate kinase, 3-PGK, is the sixth enzyme in the pathway and converts 1,3-bisphospho-D-glyceric acid, 1,3-BPG **1**, into



1,3-Bis-phospho-D-glyceric acid **1**



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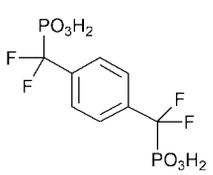

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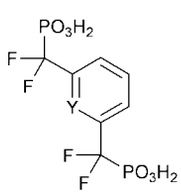

4a, Y = CH
4b, Y = N

3-phospho-D-glyceric acid (3-PGA) with concomitant conversion of ADP into ATP. There is a loop in the glycolytic pathway at this point whereby **1** is converted into 2,3-bisphospho-D-glyceric acid, 2,3-BPG, by 2,3-bisphosphoglycerate mutase (2,3-BPGM) which is then further converted into 3-PGA in a process that also generates ATP. The active site of the mutase enzyme is known to be smaller than that of 3-PGK.[1,2] Thus, 3-PGK is a significant therapeutic target, especially where there is a desire to impede glycolysis without shutting down the pathway completely. Simple modification of the routes used for the synthesis of these analogues could lead to bulkier bisphosphonates capable of selective inhibition of 3-PGK but not of 2,3-BPGM.

3-PGK is a bilobal enzyme and has been crystallised from a wide variety of sources.[3–7] It is known that 3-PGA is bound to the face of the N-terminal domain and that the nucleotide is bound to the face of the C-terminal domain. The fact that the two binding sites are too far apart for direct phosphoryl transfer to occur led to the proposal by Blake[8] that 3-PGK undergoes a conformational change on binding of both substrates in a mechanism termed 'hinge-bending'. The resultant 'closed' structure of 3-PGK was recently identified by Bernstein *et al.*[9] in a crystal structure that shows hinge-bending by 3-PGK only when two substrates, ADP and 3-PGA, are bound. Modelling based on conformational change locates the substrates ATP and 3-PGA some 6 Å apart, close enough for direct phosphoryl transfer to occur.

We have sought in this work to probe the influence of anionic charge on this conformational change and on the affinity of analogues of 1,3-BPG for 3-PGK primarily through the production of a series of conformationally restrained bisphosphonic acids whose acidities are modulated through α-halogenation. Syntheses of α-fluorophosphonates have usually employed one of three general strategies: the reaction of diethylaminosulfur trifluoride, DAST, with α-hydroxy or α-ketophosphonate esters,[10,11] the fluorination of phosphonate anions by "F^+" donors such as $FClO_3$[11] and, more recently, *N*-fluorobisbenzenesulfonimide (Accufluor™, NFSi),[12] and alkylation/acylation processes employing fluorohalomethylphosphonate esters.[13–16] Each of these methods has found application in the present work.

Results and discussion

Analogue design

Systematic design of inhibitors of 3-PGK has generally focused on analogues closely isosteric to the natural substrate[17,18] and in which the enzyme-labile phosphate linkage has been replaced by a non-scissile P–C bond. The mismatch resulting from replacement of the bridging oxygen with a methylene group has frequently been corrected by α-fluorination or α-chlorination of the phosphonic acid and much evidence has suggested that replacements of this nature maintain both their isosteric and isoelectronic character compared to the parent phosphate monoester.[19–25]

In order to incorporate specific features into the design, we embarked on the following approach. We focused on bisphosphonates with a separation of the two phosphoryl centres close

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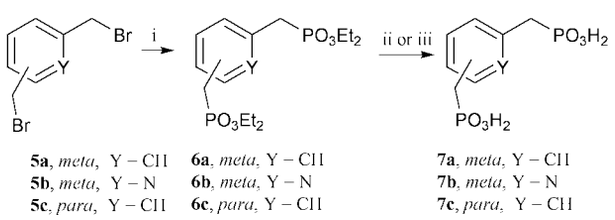
to that in the extended conformation of **1** and also having α-halogenation to provide closer mimicry to the polarity of 1,3-BPG. Secondly, we wished to restrict the large number of potential conformational states of pentane-1,5-bisphosphonates by introducing a rigid core. Finally, we sought analogues incorporating sites where additional functionality could be added without significant modification to the synthetic route. Such functionality *inter alia* could change the polarity of the bisphosphonates, increase their gross volume, and/or provide additional hydrogen bonding capacity. These considerations are satisfied through the use of a 1,3- or 1,4-disubstituted aromatic ring as a spacer, *e.g.* benzene or pyridine **4a**,**b**. Other studies have described good inhibition of 3-PGK by analogues of 1,3-BPG which place the phosphorus centres somewhat closer together than in the natural substrate.[17,18] We therefore also sought to synthesise bisphosphonates with four- and six-atom spacers between the phosphoryl centres (*e.g.* **2** and **3**) to compare with those having a five-carbon spacer (*e.g.* **4**).

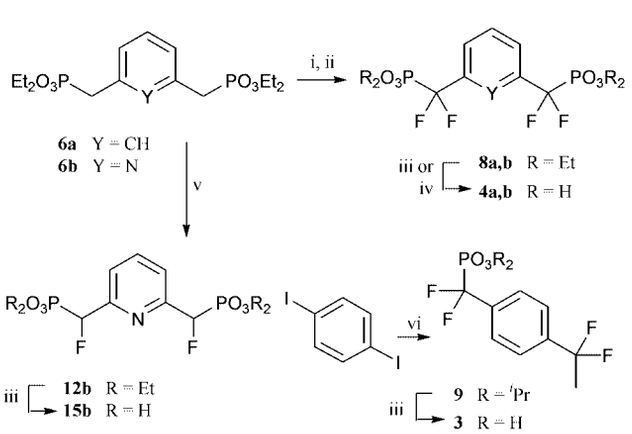
Synthesis of symmetrical bisphosphonates

The bis(bromomethyl) compounds **5a**–**c** (Scheme 1) were condensed with triethyl phosphite using an Arbuzov reaction to give the corresponding bismethylenephosphonates **6a**–**c**. The ester products were isolated in good yield, **6a** and **6b** as oils and **6c** as a white solid. De-esterification was achieved either using hot 6 M HCl (for **6a**,**c**) or bromotrimethylsilane[26] followed by methanolysis of the intermediate tetrakistrimethylsilyl ester (**6b**) to give the corresponding bisphosphonic acids **7a**,**b**,**c** respectively.



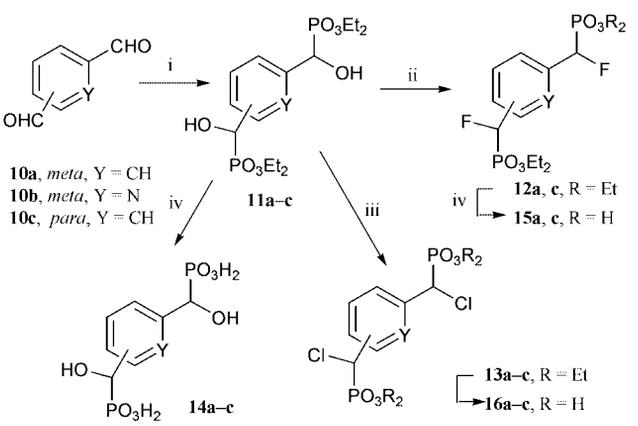
Scheme 1 *Reagents and conditions*: i, $(EtO)_3P$, 120 °C, 12–15 h, 61–82%; ii, 6 M HCl, 110 °C, 12 h, 71–77%; iii, TMSBr, rt, 24 h then MeOH, 61%.

Compounds **6a** and **6b** were fluorinated with excess NFSi using the procedure described by Taylor and co-workers[12] in yields of *ca.* 60% to give the corresponding bisdifluoromethylenephosphonates **8a**,**b** (Scheme 2). Attempts to fluorinate **6c** by this procedure failed. However, the Cu(I)-catalysed reaction of diisopropyl bromocadmium difluoromethylphosphonate with 1,4-diiodobenzene[14] was employed giving **9** in 65% yield (Scheme 2). The esters **8** and **9** were smoothly de-esterified with bromotrimethylsilane (for **8a** and **9**) or iodotrimethylsilane[27] (**8b**, where TMSBr did not work) to give the corresponding bisphosphonic acids **4a**,**b** and **3** respectively (Scheme 2).



Scheme 2 *Reagents and conditions*: i, **6a**,**b** added to NaHMDS, −78 °C, 1 h; ii, *N*-fluorobisbenzenesulfonimide (NFSi), −78 °C, 2 h, 59–60%; iii, TMSBr, rt, 72 h then MeOH, 65–73%; iv, TMSI, rt, 12 h then MeOH, 74%; v, NaHMDS–THF, −78 °C then NFSi (1.0 equiv.), −78 °C, 1 h; vi, $(^iPrO)_2P(O)CF_2CdBr$, Cu(I)Br, rt, 12 h, 65%.

The bis-α-hydroxymethylenephosphonates were prepared from the corresponding dialdehydes by H-phosphonate addition (Scheme 3). Isophthaldehyde **10a** and terephthaldehyde **10c** were commercially available while pyridine-2,6-dicarboxaldehyde **10b** was prepared from commercially available pyridine-2,6-dimethanol as described.[28] Each of the dialdehydes was phosphorylated using a typical Michaelis–Becker procedure with diethyl phosphonate and triethylamine to give the products in excellent yield. Compound **11a** was formed by heating **10a** for 17 h. Compound **11b** was generated from **10b** after stirring for 36 h at rt (Scheme 3). In the case of aldehyde **10c**, the reaction was extremely rapid and gave **11c** as a solid product within 10 min.

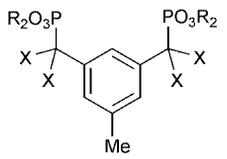


Scheme 3 *Reagents and conditions*: i, $(EtO)_2PHO$–NEt_3, rt or 100 °C, 10 min to 36 h, 77–92%; ii, DAST, rt, 10 min, 80–89%; iii, $SOCl_2$, rt, 2–8 h, 25–68%; iv, TMSBr, rt, 10–20 h then MeOH, 57–85%.

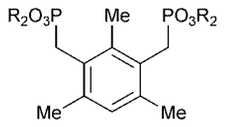
Fluorination and chlorination of hydroxyphosphonates **11a** and **11c** were achieved in good to excellent yields using diethylaminosulfur trifluoride (DAST) and thionyl chloride respectively giving **12a**,**c** and **13a**,**c** respectively. Attempts to fluorinate the pyridyl compound **11b** with DAST failed as also did the use of a wide variety of alternative fluorinating agents, which either led to decomposition or complete recovery of starting material **11b**. Compound **12b** was therefore isolated from the mixture of products formed in the fluorination of dilithio diethyl 2,6-bis-[(diethoxyphosphoryl)methyl]pyridine **6b** through the use of limiting NFSi (Scheme 2).[29,30]

Both oxalyl and thionyl chlorides failed to convert **11b** into **13b** in satisfactory yield under a wide range of conditions and only small amounts of the desired product mixed with decomposition products were produced. The best yield (25%) of **13b** was achieved using excess thionyl chloride at room temperature. Likewise, reactions of **11b** with reagents for *N*-oxidation gave similar mixtures of decomposition products. It is noteworthy that the α-chloro- and α-fluoro-bisphosphonates are themselves perfectly stable species. As before, all of the esters **11**, **12**, and **13** were smoothly de-esterified by the use of bromotrimethylsilane followed by methanolysis to give the corresponding bisphosphonic acids **14**, **15** and **16** respectively.

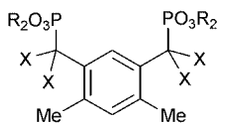
To support an exploration of the tolerance of the active site of 3-PGK to additional steric features in potential inhibitors, a series of bisphosphonates was prepared with one, two, or three methyl substituents on the aromatic ring. Compounds **18**, **20**, **22**, **24**, **26**, and **28** were prepared from the commercially available bis(bromo and chloromethyl)aromatics by the same routes as for compounds **4** and **7**.



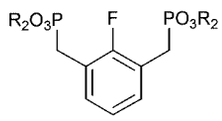
17 X = H, R = Et
18 X = H, R = H
19 X = F, R = Et
20 X = F, R = H



25 R = Et
26 R = H



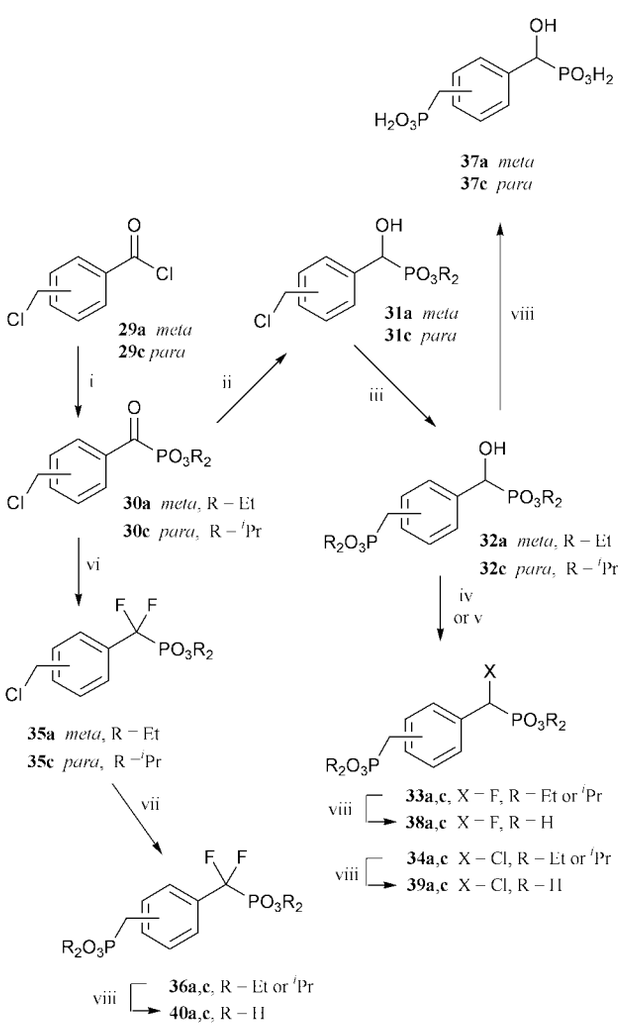
21 X = H, R = Et
22 X = H, R = H
23 X = F, R = Et
24 X = F, R = H



27 R = Et
28 R = H

Unsymmetrical bisphosphonates

These compounds were generally prepared by procedures similar to those used for the symmetrical bisphosphonates (Scheme 4). The need for bifunctional starting materials having



Scheme 4 *Reagents and conditions*: i, $(RO)_3P$, 0 °C, 5 h, 90–92%; ii, $NaBH_4$, 0 °C, 3 h, 50–77%; iii, $(RO)_3P$, 120 °C, 36 h, 61–75%; iv, DAST, rt, 10 min, X = F, 90–91%; v, $SOCl_2$, 40 °C, 12 h, X = Cl, 93–95%; vi, DAST, rt, 14 h, 73–76%; vii, $(RO)_2PONa$, 0 °C to rt, 12 h, 90–94%; viii, TMSBr, rt, 10–20 h then MeOH, 56–75%.

differential electrophilic reactivity at two sites was met by 3- and 4-(chloromethyl)benzoyl chlorides **29a**,**c**. Phosphorylation of **29a**,**c** to give the α-ketophosphonates **30a**,**c** was achieved using a modification of Berlin's procedure.[31] These products were reduced to the corresponding α-hydroxyphosphonates **31a**,**c** with sodium borohydride using Shahak's method.[32] A standard Arbuzov reaction gave the unsymmetrical α-hydroxybisphosphonates **32a**,**c**. α-Fluorination was achieved using DAST to give **33a**,**c** in 90% yield while α-chlorination with thionyl chloride in refluxing DCM gave **34a**,**c** with yields of 50–62% (93–95% based on unrecovered starting material). Use of more vigorous conditions led to increased side-product formation while milder conditions gave lower product yields.

For synthesis of α-difluorinated unsymmetrical bisphosphonates, the α-ketophosphonates **30a**,**c** were fluorinated using DAST in good yield to give **35a**,**c**. The Arbuzov reaction failed for **35a** and therefore a Michaelis–Becker procedure with dialkyl phosphonate and sodium hydride was used. The product bisphosphonates **36a**,**c** were formed in 90% yield, based on 50% recovery of starting material. As before, each of the unsymmetrical bisphosphonate esters **32**, **33**, **34**, and **36** were smoothly de-esterified with TMSBr followed by methanolysis to give the corresponding bisphosphonic acids **37**, **38**, **39**, and **40** respectively.

Arenebisphosphonates

The synthesis of these compounds called for the co-formation of arene and benzyl phosphonates and the palladium-catalysed coupling used by Hirao *et al.*[33,34] was chosen. 3-(Bromomethyl)-bromobenzene was reacted with triethyl phosphite in a standard Arbuzov reaction to give **41** and the product converted into the arylphosphonate **42** in 68% yield using palladium-catalysed coupling (Scheme 5). The tetraethyl ester **42** was efficiently converted into the bisphosphonic acid **43** with TMSBr as before. Methylenephosphonate **42** was fluorinated[12] in 53% yield to give **44**, which was converted into the free bisphosphonic acid **2** using TMSBr. By contrast to **41**, its α-fluoro-derivative did not undergo the palladium-catalysed reaction so the route was modified by reversing the sequence of introduction of the two phosphonates. The commercially available dioxolane **45** provided an ideal starting material for this modified route and gave **46** in good yield using the palladium-catalysed coupling conditions. The aldehyde function was unmasked under mild conditions[35] to give **47** and then phosphonate addition gave **48** in 53% yield. α-Fluorination and chlorination gave **49** and **50** respectively by usual procedures in good yields and the bisphosphonate esters **48**, **49**, and **50** were converted into the corresponding free phosphonic acids **51**, **52**, and **53** respectively using TMSBr (Scheme 5).

Other bisphosphonates

As an example of a bisphosphonate with the phosphoryl centres closer together than in the aromatic bisphosphonates, **54** was synthesised from methallyl chloride (3-chloro-2-methylpropene) (Scheme 5). The starting material was condensed with triethyl phosphite in a typical Arbuzov reaction and the product de-esterified as usual with TMSBr to give the free acid **55**.

Biological results and discussion

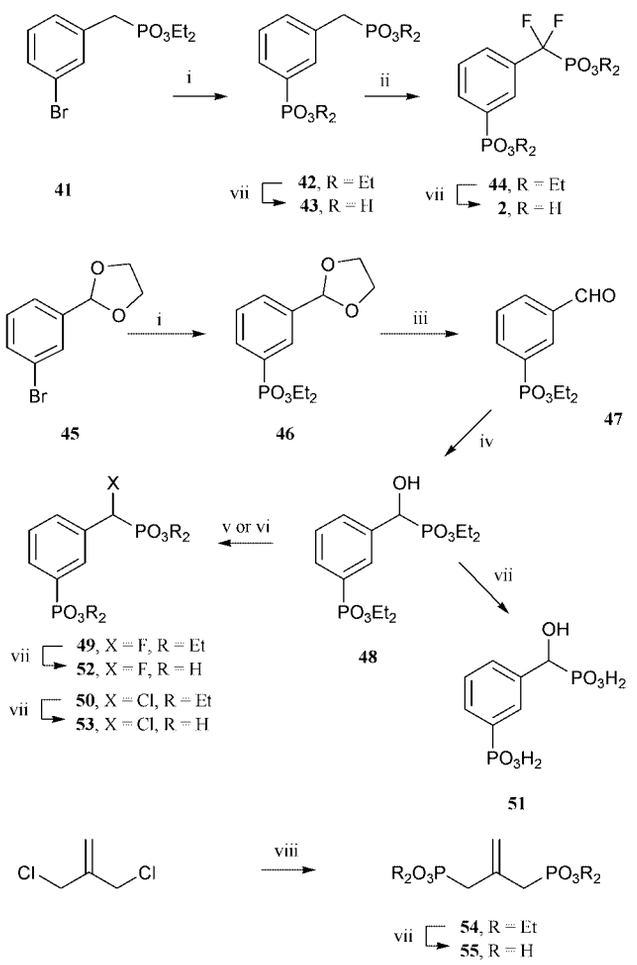
With a wide range of bisphosphonates available (Fig. 1), testing was undertaken to determine their mode of inhibition and activity against the enzyme and to relate these data to the physical characteristics of these ligands for 3-PGK.

Determination of 3-PGK inhibitory activity. 3-PGK can be assayed using either the forward or the reverse reaction (Fig. 2). The forward assay system detects ATP formed in a coupled reaction with firefly luciferase (FFL)[36] whereby the action of FFL on luciferin and ATP yields oxyluciferin and light (Fig. 2A). The reduction in light emission in the presence of an

Table 1 K_i values for selected bisphosphonate inhibition of 3-PGK

Compound	K_m [a,b]/μM	K_i [a]/μM	IC_{50} [a,c]/μM
7a	71 ± 7.1	120 ± 15	200
7b	77 ± 9	80 ± 10	182
14a	73 ± 7.5	75 ± 8.5	150
14b	95 ± 7	156 ± 17	163
15a	74 ± 15	0.69 ± 0.12	1.3
16b	84 ± 8	0.33 ± 0.05	1.34

[a] At 37 °C in 0.1 M NaCl, pH 7.1. [b] K_m for 1,3-BPG. [c] IC_{50} values ±5%. Results average of duplicate runs.



Scheme 5 *Reagents and conditions*: i, $(EtO)_2PHO$, NEt_3, $Pd(PPh_3)_4$, 95 °C, 12 h, 68%; ii, **42** added to NaHMDS, −78 °C, 1 h then NFBS, −78 °C, 2 h, 53%; iii, 15% H_2SO_4 on silica, rt, 4 h, 90%; iv, $(EtO)_2PHO$, NEt_3, rt, 36 h, 53%; v, DAST, rt, 10 min, X = F, 93%; vi, xs. $SOCl_2$, 40 °C, 8 h, X = Cl, 61%; vii, TMSBr, rt, 10–20 h, 58–81%; viii, $(EtO)_3P$, 120 °C, 17 h, 40%.

inhibitor enables determination of IC_{50} values for inhibitors. α-Halogenated bisphosphonates proved strong inhibitors of 3-PGK and were tested at 1 μM concentration while bis-α-hydroxy- and bismethylenephosphonates were used at 10 μM.

Kinetic analysis. The kinetics of inhibition of 3-PGK were determined by assaying the conversion of 3-PGA into 1,3-BPG, which is coupled to formation of glyceraldehyde 3-phosphate (GA3P) using GA3P dehydrogenase (GAPDH) (Fig. 2B), and monitored at 340 nm for formation of NADH.[37] A representative group of the above bisphosphonates was selected for kinetic analysis, namely compounds **7a**,**b**, **14a**,**b**, **15a**, and **16b**. Concentrations of inhibitors at 0.3, 1.0, and 2.5 times IC_{50} were used in kinetic runs for a range of six concentrations of 3-PGA spanning its K_m for 3-PGA (~150 μM). The data were analysed computationally to identify the nature of the enzyme inhibition

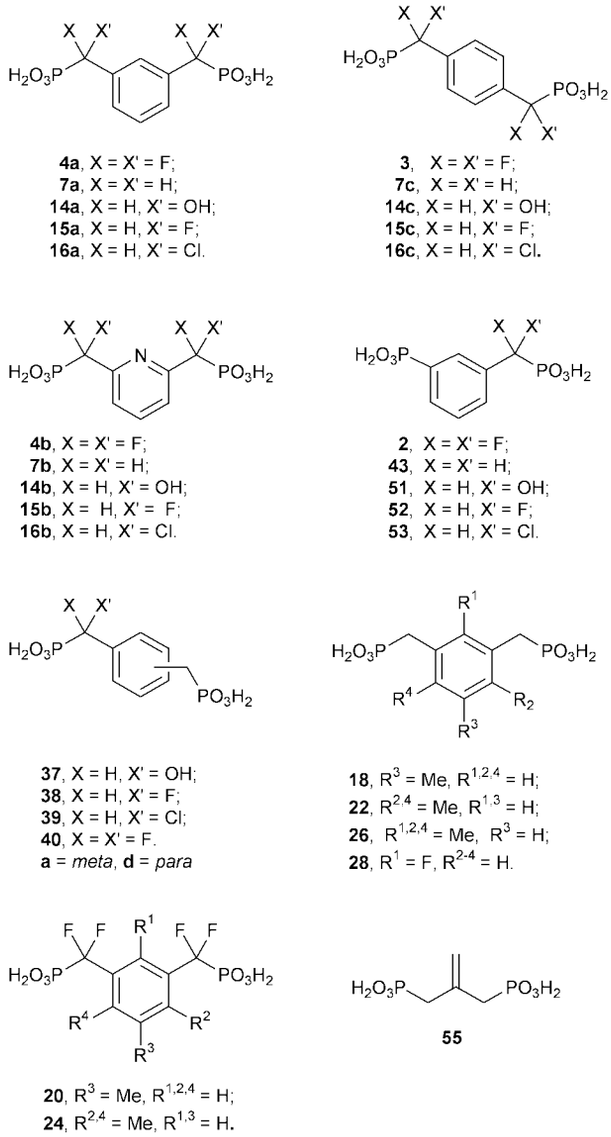


Fig. 1 Bisphosphonates synthesized as inhibitors of 3-PGK.

and to evaluate K_i (Table 1) and graphed as Lineweaver–Burk plots. In all cases, the quality of fit of the data (not shown) using analysis for competitive inhibition was clearly superior to fits based on uncompetitive or non-competitive inhibition.

Determination of pK_a values. pK_a Values were determined for 22 of the bisphosphonic acids by potentiometric titration in the range 11 > pH > 3.5. This range covers the observed values of pK_{a^3} and pK_{a^4} and of the pyridinium–NH (in the case of compounds **4b**, **7b** and **14b**). Data were analysed using a multiple pK_a analysis programme written for Kaleidagraph™. Experimental pK_a values so obtained were corrected for statistical factors[38–40] to take into account the differences in acidity between bisphosphonates having two equivalent phosphonic acid functions and those with non-equivalent phosphonic acids. For non-equivalent bisphosphonic acids, the assignment of observed pK_a values as pK_{a^3} and pK_{a^4} respectively was based on previous results for α-substituted bisphosphonic acids (Table 2).[18,21,41,42]

Discussion of results

Comparisons between different inhibitors based on their IC_{50} and pK_a values support a number of conclusions about the requirements of the active site of 3-PGK for inhibitor structure.

First, all of the compounds evaluated by K_i determination are *competitive* inhibitors of the enzyme, the best showing sub-

Table 2 IC_{50} values for inhibitors

Compound	IC_{50} [a]/μM	pK_{a^3}, pK_{a^4}	Compound	IC_{50} [a]/μM	pK_{a^3}, pK_{a^4}
2	1.0	5.16, 6.90	**22**	73	7.24, 7.78
3	0.98	4.82, 5.60	**24**	0.84	5.33, 5.92
4a	0.96		**26**	78	7.40, 8.16
4b	1.17	5.18, 5.30	**28**	147	
6a	200	7.11, 7.71	**37a**	68.8	6.98, 7.29
6b	182	6.70, 8.49	**37c**	89	
6c	193	7.03, 7.49	**38a**	8.35	
14a	150	6.78, 7.23	**38c**	10.7	6.30, 7.72
14b	163	6.57, 7.58	**39a**	8.0	
14c	181		**39c**	9.4	
15a	1.3	6.01, 6.37	**40a**	1.8	5.04, 5.84
15b	1.33		**40c**	1.98	5.27, 6.63
15c	1.15	6.02, 6.37	**43**	138	6.47, 7.61
16a	1.0		**51**	102	6.55, 7.46
16b	1.34		**52**	16	5.80, 6.99
16c	1.08		**53**	17	
18	99		**55**	87	6.45, 7.45
20	0.88				

[a] IC_{50} values are ±5%. [b] pK_a values were determined at 37 ± 0.1 °C in 0.152 M NaCl and are accurate to ±0.05.

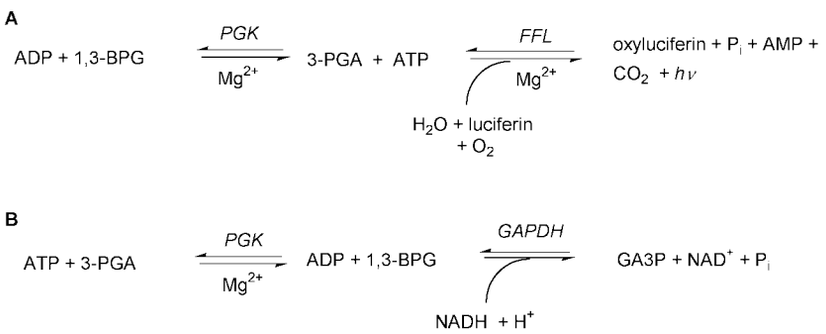


Fig. 2 Assay of the forward (**A**) and reverse (**B**) reactions catalysed by 3-PGK.

micromolar IC_{50} values (Table 1). The values of the inhibition constants K_i for the seven compounds determined correlate well with the corresponding values for IC_{50} (Table 1).[43] All of these values were determined at high sulfate concentration (40 mM) in order to achieve Michaelis–Menten kinetic behaviour for 3-PGK.[37]

Secondly, there appears to be a clear rank order of inhibitor strength as a function of the phosphonic acid α-substituent that is apparently independent of changes in ring-orientation and further substitution patterns of: $–CH_2P < –CH(OH)P \ll –CHClP \approx –CHFP < –CF_2P$ though differences are sometimes small for the last three groups. It is evident that α-hydroxylation of the phosphonic acids does not materially enhance their affinity for 3-PGK (Table 2, entries **7a** and **14a**, **7b** and **14b**, **43** and **51**, *etc.*). By contrast, α-halogenation *always* improves affinity of the bisphosphonic acids for 3-PGK, usually by one to two orders of magnitude and in the best cases leading to submicromolar values for K_i and IC_{50}. This shows the value of isopolar mimicry of the phosphate linkage of the natural substrate. In quantitative terms, there is a modest correlation of IC_{50} with pK_{a^3} ($R = 0.87$, Fig. 3a) and a similarly modest relationship with pK_{a^4} ($R = 0.85$) over the range of 21 bisphosphonates thus evaluated and with slopes close to unity for both curves. What is most clearly shown by these data is that to achieve high affinity for 3-PGK, the inhibitors must have a third phosphonic acid dissociation, pK_{a^3}, below 6.5 (Fig. 3a) and a fourth one, pK_{a^4}, below 7 (*cf.* **2**, **38c**, **52**, **53**) (Fig. 3b).

Thirdly, it appears that 3-PGK can tolerate bisphosphonates with additional steric components (*e.g.* the series **18**, **20**, **22**, **24**, **26**, **28**) where in some cases strain must force the two phosphoryl functions to lie outside the plane of the benzene ring (notably **26**). Also, 3-PGK shows little discrimination against compounds with a separation of phosphoryl centres greater than that of the natural substrate, notably in the series of *p*-substituted unsymmetrical *p*-xylylbisphosphonates (**37c**–**40c**). This kinase can, however, show good binding to inhibitors with fewer atoms linking these phosphoryl centres (the series **2**, **52**, **53**) though there appears to be a lower limit to this tolerance (**55**).

Lastly, the unsymmetrical benzenephosphonic acid inhibitors with one α-halo- and especially one α,α-difluoromethylenephosphonate substituent show activity at least as good as their bismethylenephosphonate counterparts with two such α-halomethylene centres. This observation is borne out by comparison of the pairs **16a** and **39a**, **15a** and **38a**, and **4** and **40a**. This analysis shows that 3-PGK reads out the charge on each phosphonic acid group independently, a feature that has yet to emerge from studies on α-fluorinated arylalkylbisphosphonates with other enzymes, notably protein tyrosine phosphatases.[44] Further studies will be necessary to determine whether these unsymmetrical inhibitors bind in one particular orientation to the basic patch of 3-PGK, though such behaviour has been detected in work on some unsymmetrical azapentane-1,5-bisphosphonates.[18,42] It may well emerge that appropriately designed inhibitors for 3-PGK (and perhaps other kinases) may differentiate between phosphonate mimicry of the transferable phosphate and the static one at C-3 of 3-PGA.

In conclusion, the present results provide one of the clearest cut examples yet observed of the value of α-fluorination of phosphonic acids to improve their affinity for proteins as non-hydrolysable enzyme inhibitors or substrate analogues of phosphates and demonstrate that sub-micromolar inhibition can be achieved for highly polar, water-soluble inhibitors of kinases.

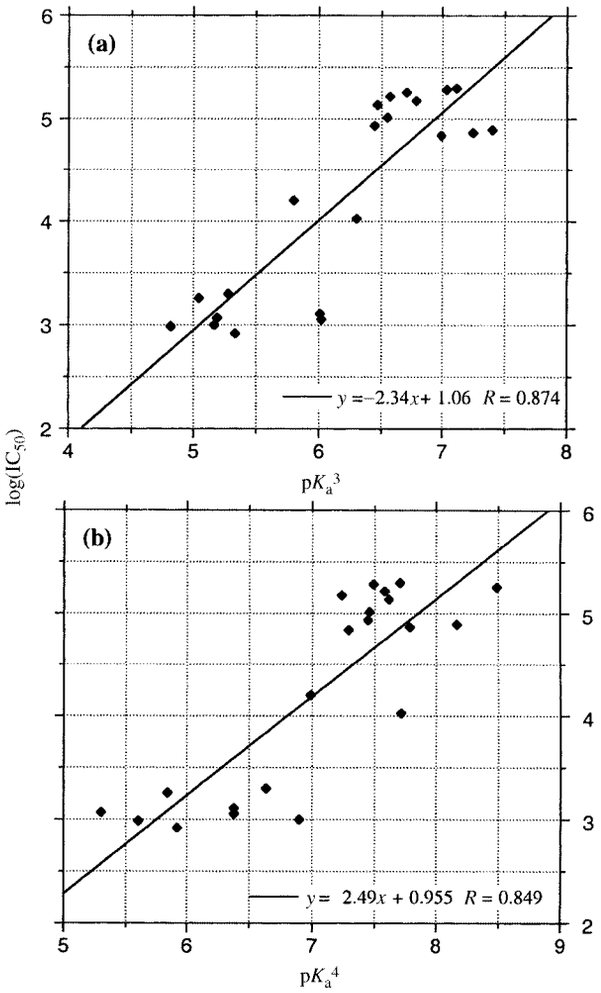


Fig. 3 Graph of $\log(IC_{50})$ *vs.* pK_a for bisphosphonate inhibitors of 3-PGK.

Experimental

Tetrahydrofuran (THF) was refluxed over sodium and benzophenone and distilled when the mixture turned blue. Dichloromethane and toluene were distilled off calcium hydride and toluene was stored over calcium hydride. Unless otherwise stated, petrol refers to petroleum ether bp 40–60 °C. Melting points were measured on a Kofler hot stage micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 457 grating infra-red spectrometer. NMR spectra were recorded on a Bruker AC-250 spectrometer sample changer with a quadronuclear probe. All spectra are reported using the δ scale in parts per million. Proton chemical shifts are accurate to +0.01 ppm. Coupling constant J values are recorded in Hz and refer to vicinal proton–proton coupling ($^3J_{H,H}$) unless otherwise stated. Phosphorus, fluorine and carbon chemical shifts are accurate to +0.1 ppm. Proton spectra have been calibrated by either external tetramethylsilane or *via* internal solvent reference except in cases where D_2O has been used as the solvent. In this case 2,2-dimethyl-2-silapentane-5-sulfonate was used as the reference sample. Phosphorus NMR spectra were recorded at 101 MHz and are referenced downfield from external 85% H_3PO_4. Fluorine NMR spectra were recorded at 235 MHz and are referenced downfield from external $CFCl_3$. Carbon NMR spectra were recorded at 62.9 MHz. Mass spectra were obtained on a Kratos MS80 mass spectrometer in conjunction with a Kratos DS90 data station using chemical ionisation with ammonia as the reagent gas (CI) or electron impact (EI). Fast atom bombardment (FAB) spectra were obtained on a Kratos MS80RF machine together with the DS90 data station. Electrospray (ES) mass spectra were recorded on a Fisons V.G. Platform. R_f values of the products were obtained by thin layer chromatography (TLC) using silica gel plates (Merck Art. 5554 DC-Alufolien Kieselgel 60 F_{254}) and spots were detected by UV light or by dipping the plate in either alkaline potassium permanganate or an ethanolic solution of phosphomolybdic acid with development at 120 °C.

Except where stated all intermediates were purified by flash column chromatography on Kieselgel 60, 230–400 mesh (Merck 9385) or Chromatographiegel 560, 40–63 mesh (Chemische Fabrik Uetikon CH-8707).

Lineweaver–Burk plots were determined using Origin™ and this programme was also used to calculate K_i values and error factors for the kinetic data. pH Titration profiles were obtained using a Radiometer (Copenhagen) TTT80 Titrator with ABU880 Autoburette and the pK_a values obtained from the data using software written for Kaleidagraph™.

(3-Phosphonophenyl)difluoromethylphosphonic acid (2)

Diethyl [3-(diethoxyphosphoryl)phenyl]difluoromethylphosphonate **44** (0.22 g, 0.53 mmol) was dissolved in DCM (20 ml) under nitrogen and TMSBr (0.58 g, 3.70 mmol, 7 eq., 0.49 ml) added *via* syringe. ^{31}P NMR spectroscopy showed the reaction to be complete after 22 h and the reaction was quenched with methanol (10 ml) and the solvent was removed under reduced pressure. The residue was co-evaporated with methanol (4 volumes) to yield the crude free acid (0.13 g). The crude free acid was dissolved in methanol, cyclohexylamine (2 eq.) added, and the solvent was removed under reduced pressure and the product purified by recrystallisation from methanol, with acetone to give the biscyclohexylammonium salt of the *title compound* as a white solid (0.22 g, 80%, mp >275 °C); δ_H (D_2O) 1.15–1.95 (22 H, m, cyclohexyl-H), 7.50–7.55 (1 H, m, ArH), 7.70–7.85 (3 H, m, 3 ArH); δ_P (D_2O) 4.65 (t, J_{FP} 102), 12.7 (s); δ_F (D_2O) −110.7 (d, J_{FP} 103); δ_C (D_2O) 23.8, 24.2, 30.3, 50.1, 126.3, 128.3, 130.9, 134.0, 136.3.

1,4-Bis(phosphonodifluoromethyl)benzene (3)

1,4-Bis[(diisopropoxyphosphoryl)difluoromethyl]benzene **9** (0.17 g, 0.33 mmol) was deprotected as for **2** with TMSBr (0.35 g, 2.31 mmol, 0.31 ml). The reaction was stirred at rt and followed by ^{31}P NMR spectroscopy. After 96 h the reaction was complete and was worked up as usual to yield the crude free acid (0.10 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.11 g, 65%, mp >275 °C) (Found: C 44.4, H 6.05, N 5.1. $C_{20}H_{34}F_4N_2O_6P_2$ (salt) requires C 44.7, H 6.4, N 5.2%); δ_H (D_2O) 1.25–1.95 (22 H, m, cyclohexyl-H), 7.30, 7.75 (2 H each, 2 d, J 8.0, 2 ArH each); δ_P (D_2O) 5.21 (t, J_{PF} 92.5); δ_F (d_4-MeOH) −109.4 (d, J_{PF} 91.8); free acid: δ_C (D_2O) 128.3, 134.0.

1,3-Bis(phosphonodifluoromethyl)benzene (4a)

1,3-Bis[(diethoxyphosphoryl)difluoromethyl]benzene **8a** (0.31 g, 0.70 mmol) was de-esterified as for **2** with TMSBr (0.75 g, 4.87 mmol, 0.64 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.27 g, 73%, mp >275 °C); δ_H (D_2O) 1.05–2.00 (22 H, m, cyclohexyl-H), 7.55–7.65 (4 H, m, ArH); δ_P (D_2O) 5.07 (t, J_{PF} 103); δ_F (D_2O) −110.1 (d, J_{PF} 105); δ_C (D_2O) 23.8, 24.2, 34.1, 50.2, 128.3, 128.8, 130.1, 134.0.

2,6-Bis(phosphonodifluoromethyl)pyridine (4b)

2,6-Bis[(diethoxyphosphoryl)difluoromethyl]pyridine **8b** (0.49 g, 1.08 mmol) was dissolved in DCM (20 ml) under argon. Iodotrimethylsilane (1.52 g, 7.58 mmol, 7 eq., 1.08 ml) was added *via* syringe and the reaction followed by ^{31}P NMR spectroscopy until de-esterification was complete then the reaction

was quenched by dropwise addition of methanol. Solvent was removed under reduced pressure and the residue co-evaporated with methanol (6 vol). The resulting yellow solid was dissolved in water (15 ml) and the solution neutralised with 0.5 M NaOH. The product was lyophilised and the *title compound* isolated as a white solid (0.22 g, 64%, mp 268 °C) (Found: C 42.5, H 5.9, N 8.1. $C_{19}H_{33}F_4N_3O_6P_2$ (salt) requires C 42.4, H 6.2, N 7.8%); δ_H (D_2O) 7.70–7.75 (2 H, m, 2 ArH), 7.95–8.05 (1 H, m, ArH); δ_P (D_2O) 3.53 (t, J_{PF} 95.3); δ_F (D_2O) −114.3 (d, J_{FP} 96.9); δ_C (D_2O) 117.5 (dt, J_{CP} 182, J_{CF} 260), 123.4, 139.3, 152.4.

1,3-Bis[(diethoxyphosphoryl)methyl]benzene[45] (6a)

1,3-Bis(bromomethyl)benzene **5a** (5.00 g, 18.94 mmol) and triethyl phosphite (4.62 g, 39.78 mmol, 4.81 ml) were stirred together at 120 °C overnight under a nitrogen atmosphere. The reaction was cooled and the volatiles removed *in vacuo*. The crude product was purified by flash column chromatography to yield the *title compound* as a colourless oil (5.94 g, 80%); R_f 0.22 (DCM–MeOH, 95:5) (Found: M^+ 378.135. $C_{16}H_{28}O_6P_2$ requires *M* 378.136); ν_{max}/cm^{-1} (film) 2909 (C–H), 1559 (C=C), 1213 (P=O), 1097 (P–O–C); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.1, 4 CH_3CH$_2$–), 3.10 (4 H, d, J_{HP} 20.0, 2 PCH$_2$–), 3.95–4.05 (8 H, m, 4 CH$_3$CH_2–), 7.05–7.15 (4 H, m, ArH); δ_P ($CDCl_3$) 26.7 (s); δ_C ($CDCl_3$) 16.0, 16.1, 33.2 (d, J_{CP} 125), 61.7, 61.8, 128.3, 128.7, 130.7, 131.5; *m/z* (EI^+) 379 (100%, MH^+), 351 (12, M − C_2H_4), 323 (6, M − 2C_2H_4), 295 (8, M − 3C_2H_4), 267 (13, M − 4C_2H_4), 185 (26, M − 4C_2H_4 − PO_3H_2).

2,6-Bis[(diethoxyphosphoryl)methyl]pyridine (6b)

2,6-Bis(bromomethyl)pyridine **5b** (1.52 g, 5.75 mmol) was dissolved in toluene (20 ml) and triethyl phosphite (1.40 g, 12.07 mmol, 2.00 ml) added *via* syringe. The reaction was heated at reflux for 15 h. Solvent was removed *in vacuo* and the crude product purified by flash column chromatography to give the *title compound* as a pale yellow liquid (1.33 g, 61%); R_f 0.45 (DCM–MeOH, 90:10); ν_{max}/cm^{-1} (film) 2909 (C–H), 1576 (C=C), 1222 (P=O), 1097 (P–O–C) (Found: M^+ 379.132. $C_{15}H_{27}NO_6P_2$ requires 379.131); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.2, 4 CH_3CH$_2$–), 3.35 (4 H, d, J_{HP} 21.5, 2 PCH$_2$–), 4.45–4.60 (8 H, m, *J* 6.2, 4 CH$_3$CH_2–), 7.20–7.25 (2 H, m, ArH), 7.50–7.60 (1 H, m, ArH); δ_P ($CDCl_3$) 25.5 (s); δ_C ($CDCl_3$) 16.1, 16.2, 36.6 (d, J_{CP} 135), 61.9, 62.0, 118.2, 122.2, 136.7; *m/z* (EI^+) 379 (55%, M^+), 323 (10, M − 2C_2H_4).

1,4-Bis[(diethoxyphosphoryl)methyl]benzene[45] (6c)

1,4-Bis(bromomethyl)benzene **5c** (10.00 g, 37.89 mmol) and triethyl phosphite (13.22 g, 79.58 mmol, 2.1 eq., 13.65 ml) were stirred together under an inert atmosphere at 120 °C for 15 h and volatiles were then removed *in vacuo*. The *title compound* was isolated as a white crystalline solid by filtration and was used without further purification (11.74 g, 82%); R_f 0.10 (DCM–MeOH, 95:5) (Found: M^+ 378.135. $C_{16}H_{28}O_6P_2$ requires 378.136); ν_{max}/cm^{-1} (Nujol mull) 2909 (C–H), 1654 (C=C), 1247 (P=O), 1025 (P–O–C); δ_H ($CDCl_3$) 1.05 (12 H, t, *J* 6.0, 4 CH_3CH$_2$–), 2.90 (4 H, d, J_{HP} 20.5, PCH$_2$–), 3.75–3.85 (8 H, m, 4 CH$_3$CH_2–), 7.05 (4 H, s, 4 ArH); δ_P ($CDCl_3$) 27.1 (s); δ_C ($CDCl_3$) 16.0, 16.1, 33.2 (d, J_{CP} 130), 61.7, 61.8, 128.9, 131.7; *m/z* (EI) 378 (15%, M^+), 241 (76, M − PO_3Et_2), 213 (10, M − PO_3Et_2 − C_2H_4), 185 (27, M − PO_3Et_2 − 2C_2H_4), 104 (100, M − 2PO_3Et_2).

1,3-Bis(phosphonomethyl)benzene (7a)

1,3-Bis[(diethoxyphosphoryl)methyl]benzene **6a** (2.00 g, 5.29 mmol) was dissolved in 6 M hydrochloric acid (30 ml) and the mixture heated to reflux for 18 h. Solvents were removed under reduced pressure and the product co-evaporated with 5 vol water. Some of this was dissolved in water (5 ml) and titrated to pH 7.5 with 1 M NaOH. Lyophilisation of this solution yielded the tetrasodium salt of the *title compound*, isolated as a glassy white solid. The remaining crude free acid (1.20 g) was lyophilised, dissolved in methanol, cyclohexylamine (2 eq.) added, and solvent removed under reduced pressure to yield a white solid. This was crystallised by precipitation from methanol with acetone. The biscyclohexylammonium salt of the *title compound* was isolated as a white solid (1.33 g, 71%, mp >275 °C) (Found: C 51.6, H 8.3, N 5.85. $C_{20}H_{38}N_2O_6P_2$ (salt) requires C 51.7, H 8.25, N 6.0%); free acid: δ_H (D_2O) 3.15 (4 H, d, J_{HP} 25.5, 2 PCH$_2$–), 7.05–7.30 (4 H, m, ArH); δ_P (D_2O) 25.8 (s); δ_C (D_2O) 34.1 (d, J_{CP} 131), 127.9, 128.9, 130.7, 132.9; *m/z* (ES^-) 267 (100%, MH^+), 185 (63, M − PO_3Et_2), 105 (48, M − 2PO_3Et_2).

2,6-Bis(phosphonomethyl)pyridine (7b)

2,6-Bis[(diethoxyphosphoryl)methyl]pyridine **6b** (1.25 g, 3.30 mmol) was deprotected as for **2** with TMSBr to afford the crude product as an off-white solid (0.57 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.94 g, 61%, mp >275 °C) (Found: C 48.9, H 7.9, N 8.85. $C_{19}H_{38}N_3O_6P_2$ (salt) requires C 48.9, H 8.2, N 9.0%. Found: M^+ 268.014. $C_7H_{12}NO_6P_2$ requires *M*H 268.014); free acid: δ_H (D_2O) 3.50 (4 H, d, J_{HP} 24.7, 2 PCH$_2$–), 7.65–7.75 (1 H, m, ArH), 8.30–8.35 (2 H, m, ArH); δ_P (D_2O) 14.4 (s); δ_C (D_2O) 34.1 (d, J_{CP} 124), 117.5, 125.6, 138.1; *m/z* (FAB^-) 266 (100%, M − 1); *m/z* (FAB^+) 268 (67%, MH^+), 367 (25, M + $C_6H_{11}NH_3^+$), 467 (M + 2$C_6H_{11}NH_3^+$).

1,4-Bis(phosphonomethyl)benzene[45]

1,4-Bis[(diethoxyphosphoryl)methyl]benzene **6c** (2.00 g, 5.29 mmol) was dissolved in 6 M hydrochloric acid (50 ml) and heated at reflux overnight then solvent removed under reduced pressure. Co-evaporation of the residue with water (5 × 50 ml) furnished the *title compound* as a white solid (1.08 g, 77%, mp 269–70 °C, lit.[46] 268–271 °C); δ_H (d_4-MeOH) 3.05 (4 H, d, J_{HP} 15.9, PCH$_2$–), 7.25 (4 H, s, 4 ArH); δ_P (d_4-MeOH) 24.8 (s); δ_C (d_4-MeOH) 33.2 (d, J_{CP} 134), 129.4, 131.1.

1,3-Bis[(diethoxyphosphoryl)difluoromethyl]benzene (8a)

Sodium hexamethyldisilazide (NaHMDS) (8.00 mmol, 1.0 M solution in THF, 8.00 ml) was added to THF (50 ml) under an inert atmosphere and the solution cooled to −78 °C. 1,3-Bis[(diethyoxyphosphoryl)methyl]benzene **6a** (0.55 g, 1.45 mmol) in THF (15 ml) was added dropwise at this temperature over 5 min and the resulting dark green solution stirred for 1 h. A solution of *N*-fluorobisbenzenesulfonimide (3.35 g, 10.62 mmol) in THF (10 ml) was added to the reaction during 5 min at −78 °C and the resulting solution stirred at −78 °C for 2 h. The solution was allowed to warm to −20 °C and then quenched with 0.01 M hydrochloric acid (50 ml). Volatiles were removed under reduced pressure and the residue extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with 5% sodium bicarbonate (50 ml) and brine (50 ml), dried over $MgSO_4$, filtered, and solvent removed under reduced pressure. The crude material was purified by flash column chromatography to yield the *title compound* as a yellow oil (0.42 g, 65%); R_f 0.15 (DCM–MeOH, 98:2) (Found: M^+ 451.106. $C_{16}H_{25}F_4O_6P_2$ requires 451.107); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.3, 4 CH_3CH$_2$–), 4.10–4.25 (8 H, m, 4 CH$_3$CH_2–), 7.50–7.55, 7.75–7.85 (2 H each, 2 m, 2 ArH); δ_P ($CDCl_3$) 6.20 (t, J_{PF} 113); δ_F ($CDCl_3$) −108.2 (t, J_{PF} 116); δ_C ($CDCl_3$), 16.1, 16.2, 64.9, 65.0, 117.7 (dt, J_{CP} 218, J_{CF} 263), 126.6, 128.1, 128.8, 133.2 (dt, $^2J_{CP}$ 12.4, $^2J_{CF}$ 21.0, *C*-CF_2P); *m/z* (EI^+) 451 (100%, MH^+), 423 (8, M − C_2H_4), 395 (5, M − 2C_2H_4).

2,6-Bis[(diethoxyphosphoryl)difluoromethyl]pyridine (8b)

The *title compound* was made by the same method as **8a** using 2,6-bis[(diethoxyphosphoryl)methyl]pyridine **6b** (0.31 g, 0.81 mmol), NaHMDS (4.47 mmol, 1.0 M solution in THF, 4.47 ml)

and NFSi (2.13 g, 6.77 mmol). The crude material was purified by flash column chromatography (EtOAc–MeOH, 98 : 2) to give the *title compound* as a colourless oil (0.22 g, 59%); R_f 0.25 (DCM–MeOH, 95 : 5) (Found: M^+ 451.095. $C_{15}H_{23}F_4NO_6P_2$ requires 451.094); ν_{max}/cm^{-1} (film) 2967 (C–H), 1614 (C=C), 1280 (P=O), 1040 (P–O–C); δ_H ($CDCl_3$) 1.10 (12 H, t, *J* 6.3, 4 CH_3CH$_2$–), 4.00–4.10 (8 H, m, 4 CH$_3$CH_2–), 7.25–7.30 (1 H, m, ArH), 7.60–7.65 (2 H, m, ArH); δ_P ($CDCl_3$) 5.03 (t, J_{PF} 107); δ_F ($CDCl_3$) −111.5 (t, J_{PF} 109); δ_C ($CDCl_3$) 15.7, 15.8, 65.2, 65.3, 115.7 (dt, J_{CP} 224, J_{CF} 266), 128.5, 132.6, 151.1 (dt, $^2J_{CP}$ 14.3, $^2J_{CF}$ 24.8, *C*-CF$_2$P); *m*/*z* (EI^+) 451 (100%, M^+), 423 (27, M − C_2H_4), 315 (45%, M − PO_3Et_2)$^+$, 178 (49, M − 2PO_3Et_2)$^+$.

1,4-Bis[(diisopropoxyphosphoryl)difluoromethyl]benzene[14] (9)

Diisopropyl bromodifluoromethanephosphonate (2.98 g, 10.10 mmol, 3.33 eq.) was dissolved in dry DMF (25 ml) under an inert atmosphere and acid-washed cadmium (1.25 g, 11.11 mmol, 3.67 eq.) added with cooling. The suspension was stirred at rt for 2 h and excess metal allowed to settle. The organometallic was transferred to a fresh flask under argon, the residue washed with DMF (10 ml) which was added to the solution. 1,4-Diiodobenzene (1.00 g, 3.03 mmol) in DMF (10 ml) and copper(I) chloride (0.70 g, 7.07 mmol, 2.67 eq.) were added at rt and the reaction was stirred overnight. Ether (100 ml) was added, the solution filtered, and the filtrate washed with ether. The ether layer was washed with ammonium chloride and water, dried over Na_2SO_4, filtered and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography to yield the title compound as a pale yellow oil (2.74 g, 60%); R_f 0.59 (DCM); ν_{max}/cm^{-1} (film) 2989 (C–H), 1641 (C=C), 1266 (P=O), 1001 (P–O–C); δ_H ($CDCl_3$) 1.20, 1.30 (12 H each, 2 d, *J* 6.0, 2 (CH_3)$_2$CH– each), 4.70–4.75 (4 H, m, 4 (CH_3)$_2$CH–), 7.70 (4 H, d, *J* 8.3, 4 ArH); δ_P ($CDCl_3$) 4.42 (t, J_{PF} 115); δ_F ($CDCl_3$) −105.2 (t, J_{PF} 112); δ_C ($CDCl_3$) 23.6, 24.2, 74.0, 74.1, 117.6 (dt, J_{CP} 218, J_{CF} 263) 128.1, 132.3 (dt, $^2J_{CP}$ 14.3, $^2J_{CF}$ 22.8, C–CF$_2$P), 137.5.

1,3-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene (11a)

Isophthalaldehyde **10a** (3.00 g, 22.82 mmol) and diethyl phosphonate (6.32 g, 45.85 mmol, 5.90 ml) were stirred together in a dry flask under an inert atmosphere. Triethylamine (2.32 g, 22.8 mmol) was added with cooling and the reaction was then heated and stirred at 100 °C for 17 h. The reaction was cooled, diluted with water (100 ml), and extracted with DCM (2 × 50 ml). The combined organics were washed with 1 M hydrochloric acid (50 ml) and brine (50 ml), dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The *title compound* was isolated as a viscous colourless oil after flash column chromatography (DCM–MeOH, 90 : 10) (7.02 g, 77%); R_f 0.43 (DCM–MeOH, 90 : 10) (Found: M^+ 411.134. $C_{16}H_{29}P_2O_8$ requires 411.134); ν_{max}/cm^{-1} (film) 3411 (O–H), 2987 (C–H), 1658 (C=C), 1266 (P=O), 1028 (P–O–C); δ_H ($CDCl_3$) 1.10–1.20 (12 H, m, 4 CH_3CH$_2$–), 2.60 (2 H, br, 2 –OH), 3.80–4.05 (8 H, m, 4 CH$_3$CH_2–), 4.90 (2 H, d, J_{HP} 9.5, 2 PC*H*(OH)–), 7.10–7.30 (4 H, m, ArH); δ_P ($CDCl_3$) 21.89, 21.95 (2 s); δ_C ($CDCl_3$) 16.4, 16.5, 68.1, 68.2, 71.1 (d, J_{CP} 161), 126.1, 126.9, 128.0, 128.1, 137.2; *m*/*z* (ES^+) 411 (100%, MH^+), 433 (67, M + Na), 821 (23, 2M + H), 843 (66, 2M + Na).

2,6-Bis[(diethoxyphosphoryl)hydroxymethyl]pyridine (11b)

2,6-Pyridinedicarbaldehyde[46] **10b** (3.49 g, 26.23 mmol) and diethyl phosphonate (7.25 g, 52.46 mmol, 6.80 ml) were stirred together in DCM (50 ml) under nitrogen. Triethylamine (2.65 g, 26.23 mmol, 3.65 ml) was added *via* syringe and the reaction stirred at rt for 36 h then water (100 ml) was added. The layers were separated and the aqueous layer extracted with DCM (2 × 50 ml). The combined organic extracts were washed with water (50 ml), dried over $MgSO_4$, filtered, and volatiles removed under reduced pressure. The crude product was purified by flash column chromatography and the *title compound* isolated as a pale yellow oil (9.13 g, 86%); R_f 0.37 (DCM–MeOH, 90 : 10); ν_{max}/cm^{-1} (film) 3382 (O–H), 2985 (C–H), 1641 (C=C), 1595 (C=C), 1257 (P=O), 1026 (P–O–C); δ_H ($CDCl_3$) 1.20–1.35 (12 H, m, 4 CH_3CH$_2$–), 4.00–4.15 (8 H, m, 4 CH$_3$CH_2–), 5.10 (2 H, 2 d, J_{HP} 8.0, 2 PC*H*(OH)–), 7.45–7.55 (2 H, m, ArH), 7.70–7.80 (1 H, m, ArH); δ_P ($CDCl_3$) 20.1, 20.2 (2 s); δ_C ($CDCl_3$) 16.3, 16.4, 63.0, 63.3, 70.6 (d, J_{CP} 159), 121.3, 137.3, 154.0; *m*/*z* (EI^+) 412 (72%, MH^+), 396 (25, M − OH), 380 (10, M − 2OH), 274 (98, M − PO_3Et_2), 136 (68, M − 2PO_3Et_2).

1,4-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene[46] (11c)

Terephthalaldehyde **10c** (10.00 g, 74.55 mmol) was placed in a flask under nitrogen and diethyl phosphonate (20.54 g, 149.11 mmol, 19.20 ml) added with stirring at rt. Triethylamine (7.54 g, 74.55 mmol, 10.40 ml) was added and an exothermic reaction occurred. After 10 min the mixture solidified, was allowed to cool, suspended in DCM (100 ml), filtered, and the solid washed with DCM (50 ml) to afford the *title compound* as a white solid (28.19 g, 92%, mp 207–208 °C, lit.[47] 204–205 °C; R_f 0.25 (DCM–MeOH, 90 : 10); ν_{max}/cm^{-1} (Nujol mull) 3246 (O–H), 2924 (C–H), 1616 (C=C), 1228 (P=O), 1020 (P–O–C); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.3, 4 CH_3CH$_2$–), 3.95–4.10 (8 H, m, 4 CH$_3$CH_2–), 5.00 (2 H, d, J_{HP} 10.1, PCH$_2$–), 7.50 (4 H, s, 4 ArH); δ_P ($CDCl_3$) 21.6 (s); δ_C ($CDCl_3$) 16.1, 16.2, 62.2, 74.1 (d, J_{CP} 162), 126.4, 135.4.

1,3-Bis[(diethoxyphosphoryl)fluoromethyl]benzene (12a)

1,3-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11a** (2.07 g, 5.05 mmol) was dissolved in DCM (40 ml) under argon and DAST (2.45 g, 15.15 mmol, 2.01 ml) was added *via* syringe. The reaction was stirred for 15 min, cooled to 0 °C, and quenched with sodium bicarbonate solution. The layers were separated and the aqueous layer washed with DCM (2 × 50 ml). The combined organics were washed with water (2 × 50 ml) and brine (50 ml), dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give the *title compound* as a yellow liquid (1.32 g, 80%); R_f 0.50 (DCM–MeOH, 90 : 10) (Found: M^+ 414.116. $C_{16}H_{26}P_2O_6F_2$ requires 414.117); δ_H ($CDCl_3$) 1.20–1.30 (12 H, t, *J* 6.2, 4 CH_3CH$_2$–), 3.90–4.10 (8 H, m, 4 CH$_3$CH_2–), 5.65 (2 H, dd, J_{HP} 7.5, J_{HF} 43.5, 2 PCHF–), 7.40–7.55 (4 H, m, ArH); δ_P ($CDCl_3$) 14.6, 15.4 (2 d, J_{PF} 83.3); δ_F ($CDCl_3$) −201.3, −201.8 (2 d); H-coupled −201.3, −201.9 (2 dd, J_{PF} 83.3, J_{HF} 43); δ_C ($CDCl_3$) 16.2, 16.3, 62.4, 62.5, 88.7 (dd, J_{CP} 164, J_{CF} 181), 125.1, 126.7, 128.0, 134.3; *m*/*z* (EI^+) 414 (51%, M^+), 137 (100, PO_3Et_2), 108 (70, PO_3HEt), 81 (52, PO_3Et_2).

2,6-Bis[(diethoxyphosphoryl)fluoromethyl]pyridine (12b)

This compound was prepared from **6b** by a similar reaction as for **8b** but using 1.0 equivalent of NFSi and chromatography of the crude product mixture on silica (eluent EtOAc); R_f 0.46 (EtOAc–MeOH, 90 : 10) (Found: M^+ 415.114. $C_{15}H_{25}F_2NO_6P_2$ requires 415.113); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.2, 4 CH_3CH$_2$–), 4.00–4.05 (8 H, m, 4 CH$_3$CH_2–), 5.95 (2 H, dd, J_{HP} 9.2, J_{HF} 43.6, 2 PCHF–), 7.60–7.65 (2 H, m, ArH), 7.90–7.95 (1 H, m, ArH); δ_P ($CDCl_3$) 13.7 (d, J_{PF} 79.8); δ_F ($CDCl_3$) −206.2 (d); H-coupled −206.3 (dd, J_{PF} 80.7, J_{HF} 43.6); δ_C ($CDCl_3$) 16.0, 16.1, 64.0, 64.2, 89.1 (dd, J_{CP} 166, J_{CF} 186), 128.1, 134.0, 152.7 (d, $^2J_{CF}$ 20.2); *m*/*z* (EI^+) 415 (86%, M^+), 141 (100, M − 2PO_3Et_2).

1,4-Bis[(diethoxyphosphoryl)fluoromethyl]benzene (12c)

1,4-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11c** (0.33 g, 0.57 mmol) was suspended in DCM (30 ml) under an inert atmosphere and DAST (0.37 g, 2.27 mmol, 4 eq., 0.30 ml) added *via* syringe, the resulting solution stirred for 10 min at rt

and then quenched with aqueous $NaHCO_3$. The reaction was worked up as usual and the crude product isolated as a yellow oil. Purification by flash column chromatography afforded the *title compound* as a colourless oil (0.30 g, 89%); R_f 0.24 (DCM–MeOH, 90:10) (Found: 414.117. $C_{16}H_{26}F_2O_6P_2$ requires *M* 414.117); ν_{max}/cm^{-1} (Nujol mull) 2984 (C–H), 1629 (C=C), 1265 (P=O), 1030 (P–O–C); δ_H ($CDCl_3$) 1.20–1.35 (12 H, m, 4 CH_3CH$_2$–), 3.95–4.05 (8 H, m, 4 CH$_3$CH_2–), 5.60 (2 H, dd, J_{HP} 7.2, J_{HF} 45.8, 2 PCHF–), 7.50–7.60 (4 H, m, 4 ArH); δ_P ($CDCl_3$) 15.0 (d, J_{FP} 88.8); δ_F ($CDCl_3$) −202.3; H-coupled −202.2 (J_{FP} 88.8, J_{HF} 45.8); δ_C ($CDCl_3$) 16.2, 16.3, 63.4, 63.8, 89.9 (dd, J_{CP} 169, J_{CF} 183), 126.7, 134.1; *m*/*z* (EI^+) 414 (5%, M^+).

1,3-Bis[(diethoxyphosphoryl)chloromethyl]benzene (13a)

1,3-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11a** (6.44 g, 15.08 mmol) was stirred with thionyl chloride (20 ml) at rt for 8 h then volatiles removed under reduced pressure. The residue was dissolved in DCM (50 ml) and washed with water (2 × 100 ml). The combined organics were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (DCM–MeOH, 98:2) to give the *title compound* as a white, crystalline solid (4.73 g, 68%); R_f 0.50 (DCM–MeOH, 90:10); ν_{max}/cm^{-1} (film) 2984, 2937 (C–H), 1654 (C=C), 1266 (P=O), 1001 (P–OEt), 737 (C–Cl); δ_H ($CDCl_3$) 1.15–1.30 (12 H, t, *J* 6.2, 4 CH_3-CH$_2$–), 3.90–4.10 (8 H, m, 4 CH$_3$CH_2–), 4.80 (2 H, d, J_{HP} 8.5, 2 PCHCl–), 7.30–7.60 (4 H, m, ArH); δ_P ($CDCl_3$) 15.4, 15.6 (2 s); δ_C ($CDCl_3$) 16.1, 16.2, 53.7 (d, J_{CP} 161), 62.4, 62.5, 128.8, 129.6, 129.6, 134.9; *m*/*z* (FAB^+) 445 (26%, M^+), 447 (25, M + 4), 335 (100, M − 4C_2H_4), 301 (82, M − 4C_2H_4 − Cl).

2,6-Bis[(diethoxyphosphoryl)chloromethyl]pyridine (13b)

2,6-Bis[(diethoxyphosphoryl)hydroxymethyl]pyridine **11b** (1.00 g, 2.36 mmol) was dissolved in thionyl chloride (10 ml), the reaction stirred for 8 h at rt, then excess thionyl chloride removed under reduced pressure. The residue was dissolved in DCM (50 ml) and washed with water (2 × 100 ml), the combined organics dried over $MgSO_4$, filtered, and solvent removed under reduced pressure. The crude product was purified by flash column chromatography (DCM–MeOH, 95:5) to give the *title compound* as a yellow oil (0.26 g, 24%); δ_H ($CDCl_3$) 1.15–1.30 (12 H, m, 4 CH_3CH$_2$–), 4.05–4.15 (8 H, m, 4 CH$_3$CH_2), 4.80 (2 H, 2 d, J_{HP} 9.0, 2 PCHCl–), 7.30–7.40 (2 H, m, ArH), 7.55–7.70 (1 H, m, ArH); δ_P ($CDCl_3$) 16.0, 16.2 (2 s); δ_C ($CDCl_3$) 16.1, 16.3, 53.7 (d, J_{CP} 165), 62.0, 62.2, 128.6, 129.6, 134.3; *m*/*z* (EI^+) 441 (32%, MH^+), 304 (37, M − PO_3Et_2), 166 (86, M − 2PO_3Et_2).

1,4-Bis[(diethoxyphosphoryl)chloromethyl]benzene (13c)

1,4-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11c** (4.83 g, 11.78 mmol, 2.1 eq., 1.80 ml) was reacted with thionyl chloride (2.95 g, 24.75 mmol) as above to furnish the *title compound* as a white solid (3.42 g, 65%); R_f 0.41 (DCM–MeOH, 90:10) (Found: C 43.3, H 5.85, Cl 15.45. $C_{16}H_{26}Cl_2O_6P_2$ requires C 43.0, H 5.9, Cl 15.7%. Found: M^+ 446.058. $C_{16}H_{26}Cl_2O_6P_2$ requires *M* 446.058); ν_{max}/cm^{-1} (Nujol mull) 2924 (C–H), 1612 (C=C), 1254 (P=O), 1039 (P–O–C); δ_H ($CDCl_3$) 1.15, 1.30 (6 H each, 2 t, *J* 6.4, 4 CH_3CH$_2$–), 4.00–4.10 (8 H, m, 4 CH$_3$CH_2–), 4.90 (2 H, d, J_{HP} 13.7, PCH$_2$–), 7.50 (4 H, s, 4 ArH); δ_P ($CDCl_3$) 17.3 (s); δ_C ($CDCl_3$, d_4-MeOH to dissolve) 16.3, 16.3, 53.1 (d, J_{CP} 160), 64.0, 64.2, 129.1, 135.0; *m*/*z* (EI^+) 446 (36%, M^+), 411 (35, M − Cl), 309 (25, M − PO_3Et_2), 172 (57, M − 2PO_3Et_2 − Cl), 138 (100, HPO_3Et_2).

1,3-Bis(phosphonohydroxymethyl)benzene (14a)

1,3-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11a** (2.53 g, 4.88 mmol) was deprotected as for **2** with TMSBr (5.23 g, 34.65 mmol, 4.52 ml) to yield the *title compound* (1.20 g, 74%). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (mp >275 °C) (Found: C 47.5, H 7.4, N 5.45. $C_{20}H_{38}N_2O_8P_2$ (salt) requires C 47.75, H 7.7, N 5.65%); δ_H (D_2O) 5.00 (2 H, d, J_{HP} 11.0, 2 PC*H*(OH)–), 7.40–7.50 (3 H, m, ArH), 7.50–7.55 (1 H, m, ArH); δ_P (D_2O) 19.5 (s); δ_C (D_2O) 69.7 (d, J_{CP} 159), 125.0, 126.4, 128.2, 136.3; *m*/*z* (ES^+) 299 (100%, MH^+), 281 (7, M − OH), 597 (20, 2M).

2,6-Bis(phosphonohydroxymethyl)pyridine (14b)

2,6-Bis[(diethoxyphosphoryl)hydroxymethyl]pyridine **11b** (0.70 g, 1.65 mmol) was deprotected as for **2** with TMSBr (1.77 g, 11.56 mmol, 7 eq., 1.50 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.58 g, 68%, mp >275 °C) (Found: C 45.9, H 7.7, N 8.1. $C_{19}H_{38}N_3O_8P_2$ (salt) requires C 45.75, H 7.7, N 8.4%); free acid: δ_H (D_2O) 3.50 (2 H, d, J_{HP} 11.7, PC*H*(OH)–), 7.75–7.90 (2 H, m, $H_{3,5}$), 7.50–7.60 (1 H, m, H_4); δ_P (D_2O) 14.0 (s); Biscyclohexylammonium salt: δ_C (D_2O) 23.7, 24.2, 30.3, 50.1, 70.9 (d, J_{CP} 154), 121.4, 139.9, 155.8; *m*/*z* (EI^+) 300 (38%, MH^+), 399 (22, M + $C_6H_{11}NH_3^+$).

1,4-Bis(phosphonohydroxymethyl)benzene (14c)

1,4-Bis[(diethoxyphosphoryl)hydroxymethyl]benzene **11c** (2.01 g, 4.88 mmol) was deprotected as for **2** with TMSBr (5.23 g, 34.15 mmol, 7 eq., 4.50 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (1.46 g, 60%, mp >275 °C) (Found: C 45.1, H 7.95, N 5.3. $C_{20}H_{42}N_2O_{10}P_2$ (salt + 2 H_2O) requires C 45.4, H 7.7, N 5.6); δ_H (d_4-MeOH) 1.10–2.00 (22 H, m, cyclohexyl-H), 5.10 (1 H, d, J_{HP} 9.8 Hz, PC*H*(OH)–), 7.10 (4 H, s, ArH); δ_P (d_4-MeOH) 17.1 (s); δ_C (d_4-MeOH) 23.8, 24.3, 30.3, 50.2, 91.6 (d, J_{CP} 153), 126.9, 138.5.

1,3-Bis(phosphonofluoromethyl)benzene (15a)

1,3-Bis[(diethoxyphosphoryl)fluoromethyl]benzene **12a** (0.67 g, 1.62 mmol) was deprotected as for **2** with TMSBr (1.74 g, 11.35 mmol, 1.50 ml) to yield the *title compound* as an off-white solid (0.42 g, 85%). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (Found: C 47.95, H 7.2, N 5.6. $C_{20}H_{36}F_2N_2O_6P_2$ (salt) requires C 48.0, H 7.25, N 5.6%); free acid: δ_H (D_2O) 5.65 (2 H, dd, J_{HP} 7.5, J_{HF} 48.1, 2 PCHF–), 7.30–7.35 (4 H, m, ArH); δ_P (D_2O) 12.7 (d, J_{PF} 82.2); δ_F (D_2O) −201.6 (d); H-coupled −201.6 (dd, J_{PF} 83.3, J_{HF} 43.0); δ_C (D_2O) 23.8, 24.3, 30.3, 50.3, 91.9 (dd, J_{CP} 156, J_{CF} 176), 124.3, 126.5, 128.7, 135.7; *m*/*z* (EI^+) 301 (100%, MH^+).

2,6-Bis(phosphonofluoromethyl)pyridine (15b)

2,6-Bis[(diethoxyphosphoryl)fluoromethyl]pyridine **12b** (0.29 g, 0.64 mmol) was de-esterified as for **2** with TMSBr (0.69 g, 4.51 mmol, 0.59 ml) to yield the *title compound* as a brown solid (0.19 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.22 g, 65%, mp >275 °C); δ_H (D_2O) 1.05–1.95 (22 H, m, cyclohexyl-H), 5.75 (2 H, dd, J_{HP} 9.6, J_{HF} 44.7, 2 PCHF–), 7.45–7.55 (2 H, m, ArH), 7.95–8.00 (1 H, m, ArH); δ_P (D_2O) 8.7 (d, J_{PF} 70.0); δ_C (D_2O) 23.7, 24.4, 30.2, 50.1, 88.7 (dd, J_{CP} 164, J_{CF} 183), 128.0, 134.0, 152.9.

1,4-Bis(phosphonofluoromethyl)benzene (15c)

1,4-Bis[(diethoxyphosphoryl)fluoromethyl]benzene **12c** (0.20 g, 0.47 mmol) was de-esterified as for **2** with TMSBr (0.51 g, 3.30 mmol, 7 eq., 0.44 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.15 g, 64%, mp >275 °C) (Found: C 44.7, H 7.8, N 5.3. $C_{20}H_{40}F_2N_2O_8P_2$ (salt + 2 H_2O); requires C 44.8, H 7.5,

N 5.2%); δ_H (D_2O) 1.10–2.00 (22 H, m, cyclohexyl-H), 5.60 (1 H, dd, J_{HP} 7.4, J_{HF} 45.4, PCHF–), 7.30–7.35 (4 H, m, ArH); δ_P (D_2O) 11.2 (d, J_{FP} 79.5); δ_F (D_2O) −200.8 (d, J_{FP} 79.5); H-coupled −201.0 (dd, J_{FP} 79.1, J_{HF} 45.2); δ_C (D_2O) 23.8, 24.3, 30.3, 50.2, 115.3 (dd, J_{CP} 171, J_{CF} 184), 126.4, 136.1.

1,3-Bis(phosphonochloromethyl)benzene (16a)

1,3-Bis[(diethoxyphosphoryl)chloromethyl]benzene **13a** (0.59 g, 1.80 mmol) was deprotected as for **2** with TMSBr (1.26 g, 8.25 mmol, 1.10 ml) to yield the *title compound* as a white solid (0.53 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.36 g, 57%, mp >275 °C) (Found: C 45.6, H 6.85, N 4.9, Cl 13.2. $C_{20}H_{36}Cl_2N_2O_6P_2$ (salt) requires C 45.2, H 6.8, N 5.2, Cl 13.1%); δ_H (D_2O) 1.50–2.05 (22 H, m, cyclohexyl-H), 5.00 (2 H, d, J_{HP} 12.3, 2 PCHCl–), 7.45–7.60 (4 H, m, ArH); δ_P (D_2O) 12.8 (s); δ_C (D_2O) 23.8, 24.3, 30.3, 50.2, 72.1 (d, J_{CP} 152), 125.6, 126.6, 128.2, 138.0.

2,6-Bis(phosphonochloromethyl)pyridine (16b)

2,6-Bis[(diethoxyphosphoryl)chloromethyl]pyridine **13b** (0.21 g, 0.42 mmol) was de-esterified as for **2** with TMSBr (0.45 g, 2.96 mmol, 0.39 ml) to yield the crude free acid (0.11 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.13 g, 69%, mp >275 °C); δ_H (D_2O) 1.10–2.00 (22 H, m, cyclohexyl-H), 4.80 (2 H, d, J_{HP} 9.1, 2 PCHCl–), 7.30–7.35 (2 H, m, ArH), 7.45–7.50 (1 H, m, ArH); δ_P (D_2O) 17.0 (s); δ_C (D_2O) 23.8, 24.2, 30.3, 50.3, 72.1 (d, J_{CP} 153), 125.7, 126.4, 139.0; *m/z* (ES^+) 336 (27%, MH^+).

1,4-Bis(phosphonochloromethyl)benzene (16c)

1,4-Bis[(diethoxyphosphoryl)chloromethyl]benzene **13c** (1.30 g, 2.90 mmol) was deprotected as for **2** with TMSBr (3.11 g, 20.33 mmol, 7 eq., 2.68 ml) and the crude free acid isolated as usual (1.22 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.82 g, 70%, mp >275 °C); δ_H (d_4-MeOH) 1.00–2.00 (22 H, m, cyclohexyl-H), 4.20 (2 H, d, J_{HP} 7.5, 2 PCHCl–), 7.20 (4 H, s, ArH); δ_P (d_4-MeOH) 13.1 (s); δ_C (d_4-MeOH) 25.4, 25.9, 31.9, 51.7, 54.9 (d, J_{CP} 159), 131.3, 140.0.

3,5-Bis[(diethoxyphosphoryl)methyl]toluene (17)

3,5-Bis(bromomethyl)toluene (10.00 g, 36.23 mmol) and triethyl phosphite (12.04 g, 72.47 mmol, 12.43 ml) were stirred together at 120 °C overnight under nitrogen then volatiles removed *in vacuo*. The crude product was purified by flash column chromatography (DCM–MeOH, 95 : 5) to yield the *title compound* as a colourless oil (12.57 g, 89%); R_f 0.22 (DCM–MeOH, 90 : 10); ν_{max}/cm^{-1} (film) 2982, 2910 (C–H), 1604 (C=C), 1249 (P=O), 1027 (P–O–C) (Found: M^+ 393.158. $C_{17}H_{30}O_6P_2$ requires *M* 393.159); δ_H ($CDCl_3$) 1.00 (12 H, t, *J* 6.4, 4 CH_3CH_2–), 2.15 (3 H, s, Me), 2.85 (4 H, d, J_{HP} 27.0, 2 PCH_2–), 3.70–3.85 (8 H, m, 4 CH_3CH_2–), 6.70–6.80 (4 H, m, ArH); δ_P ($CDCl_3$) 26.6 (s); δ_C ($CDCl_3$) 16.1, 16.2, 21.0, 33.2 (d, J_{CP} 137), 61.8, 62.0, 128.0, 129.0, 131.5, 137.8; *m/z* (FAB^+) 393 (87%, MH^+), 365 (32, M − C_2H_4), 337 (17, M − $2C_2H_4$), 309 (15, M − $3C_2H_4$), 281 (36, M − $4C_2H_4$), 199 (56, M − $4C_2H_4$ − PO_3H_2), 119 (100, M − $4C_2H_4$ − $2PO_3H_2$).

3,5-Bis(phosphonomethyl)toluene (18)

3,5-Bis[(diethoxyphosphoryl)methyl]toluene **17** (1.73 g, 4.41 mmol) was deprotected as for **2** with TMSBr (4.73 g, 30.89 mmol, 4.08 ml) to afford the crude product as an off-white solid (0.97 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (1.22 g, 77%, mp >275 °C) (Found: C 52.46, H 8.21, N 5.83. $C_{21}H_{40}N_2O_6P_2$ (salt) requires C 52.69, H 8.43, N 5.86%) (Found: M^+ 281.033. $C_9H_{15}O_6P_2$ requires *M* 281.034); biscyclohexylammonium salt: δ_H (D_2O) 1.50–2.00 (22 H, m, cyclohexyl-H), 2.25 (3 H, s, Me), 2.85 (4 H, d, J_{HP} 23.5 Hz, 2 PCH_2–), 6.95–7.00 (3 H, m, ArH); δ_P (D_2O) 20.6 (s); free acid: δ_C (D_2O) 20.0, 33.5 (d, J_{CP} 132), 127.4, 128.8, 131.4, 137.7; *m/z* (FAB^+) 281 (38%, MH^+), 479 (M − 1 + $2C_6H_{11}NH_3^+$), 578 (M − 2 + $3C_6H_{11}NH_3^+$), 677 (M − 3 + $4C_6H_{11}NH_3^+$).

3,5-Bis[(diethoxyphosphoryl)difluoromethyl]toluene (19)

The *title compound* was made by the same method as **8a** using tetraethyl 3,5-bis(phosphonomethyl)toluene **17** (0.38 g, 9.73 mmol), NaHMDS (5.36 mmol, 1.0 M solution in THF, 5.36 ml), and NFSi (2.25 g, 7.12 mmol). The crude material was purified by flash column chromatography (DCM–MeOH, 98 : 2 to 95 : 5) to give a colourless oil (1.22 g, 63%); R_f 0.13 (DCM–MeOH, 95 : 5); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 6.3 Hz, 4 CH_3CH_2–), 2.35 (3 H, s, Me), 4.05–4.15 (8 H, m, 4 CH_3CH_2–), 7.45–7.55 (2 H, m, ArH), 7.85–7.90 (1 H, m, ArH); δ_P ($CDCl_3$) 6.3 (t, J_{PF} 112); δ_F ($CDCl_3$) −108.9 (t, J_{PF} 114); δ_C ($CDCl_3$) 15.8, 15.9, 20.9, 65.0, 65.1, 117.1 (dt, J_{CP} 219, J_{CF} 263), 125.8, 128.6, 132.8 (dt, $^2J_{CP}$ 14.3, $^2J_{CF}$ 20.9), 139.1; *m/z* (FAB^+) 465 (100%, MH^+).

3,5-Bis(phosphonodifluoromethyl)toluene (20)

3,5-Bis[(diethoxyphosphoryl)difluoromethyl]toluene **19** (0.30 g, 0.65 mmol) was de-esterified as for **2** with TMSBr (0.23 g, 1.47 mmol, 0.20 ml) to yield the crude free acid (0.18 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.20 g, 70%, mp >275 °C); δ_H (D_2O) 1.05–2.00 (22 H, m, cyclohexyl-H), 2.20 (3 H, s, Me), 7.45–7.50 (2 H, m, 2 ArH), 7.75–7.80 (1 H, m, ArH); δ_P (D_2O) 5.00 (t, J_{PF} 104); δ_F (d_4-MeOH) −106.2 (d, J_{PF} 104); δ_C (D_2O) 20.6, 24.3, 24.8, 30.2, 50.3, 125.7, 129.4, 133.3, 138.2.

1,5-Dimethyl-2,4-bis[(diethoxyphosphoryl)methyl]benzene (21)

1,5-Bis(chloromethyl)-2,4-dimethylbenzene (5.00 g, 24.63 mmol) and triethyl phosphite (5.72 g, 49.26 mmol, 5.90 ml) were stirred together at 120 °C under nitrogen for 27 h and volatiles removed *in vacuo*. The crude yellow oil was freed from the corresponding monophosphonate by flash column chromatography (DCM–MeOH, 95 : 5) to furnish the *title compound* as a pale yellow oil (9.13 g, 91%); R_f 0.30 (DCM–MeOH, 90 : 10); ν_{max}/cm^{-1} (film) 2981 (C–H), 1618 (C=C), 1250 (P=O), 1029 (P–O–C) (Found: M^+ 406.167. $C_{18}H_{32}O_6P_2$ requires *M* 406.167); δ_H ($CDCl_3$) 1.20 (12 H, t, *J* 5.5, 4 CH_3CH_2–), 2.25 (6 H, s, 2 Me), 3.05 (4 H, d, J_{HP} 21.2, 2 PCH_2–), 3.85–4.00 (8 H, m, 4 CH_3CH_2–), 6.90 (1 H, s, ArH), 7.05 (1 H, s, ArH); δ_P ($CDCl_3$) 27.4 (s); δ_C ($CDCl_3$) 16.3, 16.4, 19.4, 19.5, 30.5 (d, J_{CP} 139), 61.8, 62.0, 127.4, 127.5, 132.5, 135.7; *m/z* (EI^+) 406 (97%, M^+), 378 (38, M^+ − C_2H_4).

1,5-Dimethyl-2,4-bis(phosphonomethyl)benzene (22)

1,5-Dimethyl-2,4-bis[(diethoxyphosphoryl)methyl]benzene **21** (2.00 g, 4.93 mmol) was dissolved in 6 M hydrochloric acid (25 ml) and the mixture heated at reflux for 30 h then volatiles removed under reduced pressure and the product co-evaporated with 5 vol water to give the crude free acid (mp 257 °C). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (1.09 g, 75%, mp >275 °C) (Found: M^+ C 51.01, H 7.71, N 5.80. $C_{22}H_{38}N_2O_6P_2$ (salt) requires C 50.82, H 7.79, N 5.74%); free acid: δ_H (D_2O) 2.25 (6 H, s, 2 Me), 3.15 (4 H, d, J_{HP} 21.3, 2 PCH_2–), 6.95 (1 H, s, ArH), 7.05 (1 H, s, ArH); δ_P (D_2O) 27.5 (s); δ_C (D_2O) 19.3, 30.6 (d, J_{CP} 133), 127.8, 128.1, 130.8, 133.2; *m/z* (ES) 295 (8%, MH^+), 394 (7, M + $C_6H_{11}NH_3^+$).

1,5-Dimethyl-2,4-bis[(diethoxyphosphoryl)difluoromethyl]-benzene (23)

The *title compound* was made by the same method as for **8a** using 1,5-dimethyl-2,4-bis[(diethoxyphosphoryl)methyl]benzene **21** (0.20 g, 0.50 mmol), NaHMDS (2.75 mmol, 1.0 M solution in THF, 2.75 ml), and NFSi (1.15 g, 3.65 mmol). The crude product was purified by flash column chromatography (DCM to DCM–MeOH, 99 : 1) to give a colourless oil (0.14 g, 59%); R_f 0.35 (DCM–MeOH, 90 : 10) (Found: M^+ 479.138. $C_{18}H_{29}F_4O_6P_2$ requires *M* 479.138); δ_H ($CDCl_3$) 1.20 (12 H, t, *J* 6.2, 4 CH_3CH$_2$–), 2.40 (6 H, s, 2 Me), 4.00–4.15 (8 H, m, 4 CH$_3$CH_2–), 7.40–7.45 (1 H, m, ArH), 7.70–7.75 (1 H, d, *J* 2.1, ArH); δ_P ($CDCl_3$) 6.94 (t, J_{PF} 117); δ_F ($CDCl_3$) −110.8 (t, J_{PF} 116); δ_C ($CDCl_3$) 16.2, 16.3, 20.3, 64.8, 65.0, 117.5 (dt, J_{CP} 219, J_{CF} 256), 126.9, 128.9, 136.3, 140.3; *m*/*z* (FAB^+) 478 (100%, MH^+), 451 (6, M − C_2H_4), 341 (10, M − PO_3Et_2), 319 (37, M − PO_3Et_2 − C_2H_4).

1,5-Dimethyl-2,4-bis(phosphonodifluoromethyl)benzene (24)

1,3-Dimethyl-2,4-bis[(diethoxyphosphoryl)difluoromethyl]-benzene **23** (0.35 g, 0.27 mmol) was deprotected as for **2** with TMSBr (0.13 g, 1.91 mmol, 0.25 ml) to yield the crude free acid (0.20 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.29 g, 71%, mp >275 °C); δ_H (D_2O) 1.05–1.95 (22 H, m, cyclohexyl-H), 2.40 (6 H, s, 2 Me), 7.00, 7.50 (1 H each, 2 s, 1 ArH each); δ_P (D_2O) 5.39 (t, J_{PF} 97.1); δ_F (d_4-MeOH) −100.3 (d, J_{PF} 95.9); δ_C (D_2O) 19.8, 23.7, 24.2, 30.3, 50.1, 118.1 (dt, J_{CP} 215, J_{CF} 252), 125.6, 135.2, 138.3.

1,3,5-Trimethyl-2,4-bis[(diethoxyphosphoryl)methyl]benzene (25)

1,3-Bis(chloromethyl)-2,4,6-trimethylbenzene (10.00 g, 23.81 mmol) and triethyl phosphite (7.91 g, 47.52 mmol, 16.00 ml) were stirred together at 120 °C overnight, volatiles removed *in vacuo*, and the *title compound* was obtained as a pale yellow oil which was used without further purification (15.49 g, 80%); R_f 0.10 (DCM–MeOH, 95 : 5); ν_{max}/cm^{-1} (film) 2907 (C–H), 1654 (C=C), 1193 (P=O), 1002 (P–O–C); δ_H ($CDCl_3$) 1.20 (12 H, t, *J* 6.2, 4 CH_3CH$_2$–), 2.25 (6 H, s, 2 Me), 2.40 (3 H, s, Me), 3.20 (4 H, d, J_{HP} 23.0, 2 PCH$_2$–), 3.80–3.90 (8 H, m, 4 CH$_3$CH_2–), 6.75 (1 H, s, ArH); δ_P ($CDCl_3$) 28.0 (s); δ_C ($CDCl_3$) 16.2, 16.3, 19.2, 19.3, 20.7, 28.6 (d, J_{CP} 139), 61.7, 61.8, 126.8, 130.2, 135.9, 136.7; *m*/*z* (FAB^+) 421 (99%, MH^+), 393 (6, M − C_2H_4).

1,3,5-Trimethyl-2,4-bis(phosphonomethyl)benzene (26)

1,3,5-Trimethyl-2,4-bis[(diethoxyphosphoryl)methyl]benzene **25** (2.00 g, 4.76 mmol) was dissolved in 6 M hydrochloric acid (30 ml) and the mixture heated at reflux for 20 h. Volatiles were removed *in vacuo* and the residue co-evaporated with 5 vol water to give the free acid of the *title compound* as a white solid (0.98 g, 67%, mp >275 °C) (Found: M^+ C 54.32, H 8.42, N 5.93. $C_{23}H_{41}N_2O_6P_2$ (salt) requires C 54.52, H 8.76, N 5.53%); δ_H (D_2O) 2.35 (6 H, s, 2 Me), 2.40 (3 H, s, Me), 3.25 (4 H, d, J_{HP} 26.5, 2 PCH$_2$–), 6.85 (1 H, m, ArH); δ_P (D_2O) 27.5 (s); δ_C (D_2O) 19.0, 20.4, 29.8 (d, J_{CP} 134), 126.6, 130.7, 133.0, 137.5; *m*/*z* (ES+) 306 (100%, M^+).

1-Fluoro-2,6-bis[(diethoxyphosphoryl)methyl]benzene (27)

1,3-Bis(bromomethyl)-2-fluorobenzene (2.50 g, 8.87 mmol) and triethyl phosphite (2.95 g, 17.73 mmol, 3.0 ml) were stirred together in a dry flask under nitrogen then heated to 120 °C for 24 h. The flask was cooled and volatiles removed *in vacuo* at 40 °C. The crude product was purified by flash column chromatography to yield the *title compound* as a colourless oil (2.80 g, 80%); R_f 0.28 (DCM–MeOH, 90 : 10); ν_{max}/cm^{-1} (film) 2987 (C–H), 1630 (C=C), 1266 (P=O), 1167 (C–F), 1029 (P–O–C) (Found: M^+ 396.126; $C_{16}H_{27}FO_6P_2$ requires *M* 396.127); δ_H ($CDCl_3$) 1.15–1.25 (12 H, t, *J* 6.0, 4 CH_3CH$_2$–), 3.15 (4 H, d, J_{HP} 24.5, 2 PCH$_2$–), 3.85–4.05 (8 H, m, 4 CH$_3$-CH_2–), 6.95–7.05 (1 H, m, ArH), 7.10–7.20 (2 H, m, ArH); δ_P ($CDCl_3$) 25.4 (s); δ_F ($CDCl_3$) −121.8 (s), H-coupled −121.9 (t, J_{HF} 5.0); δ_C ($CDCl_3$) 16.2, 16.2, 26.5 (dd, J_{CP} 140, $^4J_{CF}$ 3.0), 61.7, 61.8, 121.5, 123.7, 130.4, 156.7 (dt, J_{CF} 246, $^4J_{CP}$ 7.6, ArC–F); *m*/*z* (EI^+) 396 (77%, M^+), 260 (77, M − PO_3Et_2), 122 (77, M − 2PO_3Et_2).

1-Fluoro-2,6-bis(phosphonomethyl)benzene (28)

1-Fluoro-2,6-bis[(diethoxyphosphoryl)methyl]benzene **27** (1.38 g, 3.50 mmol) was deprotected as for **2** with TMSBr (3.75 g, 24.46 mmol, 3.23 ml) to yield the *title compound* as a pale yellow solid (0.68 g, 69%). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (Found: C 50.07, H 7.91, N 5.66. $C_{20}H_{37}FN_2O_6P_2$ (salt) requires C 49.77, H 7.73, N 5.81%) (Found: M^+ 285.009. $C_8H_{12}P_2O_6F$ requires *M* 285.009); free acid: δ_H (D_2O) 3.05 (4 H, d, J_{HP} 25.2, 2 PCH$_2$–), 6.90–7.00 (1 H, m, ArH), 7.05–7.20 (2 H, m, ArH); δ_P (D_2O) 24.6 (s); δ_F (D_2O) −121.2 (s), H-coupled −121.0 (s); δ_C (d_4-MeOH) 28.2 (dd, J_{CP} 136, $^4J_{CF}$ 4.0), 121.5, 125.3, 131.8, 158.4 (dt, J_{CF} 192, $^4J_{CP}$ 5.2, ArC–F); *m*/*z* (FAB^+) 285 (100%, MH^+).

Diethyl 3-chloromethylbenzoylphosphonate (30a)

3-Chloromethylbenzoyl chloride **29a** (5.00 g, 26.45 mmol) was dissolved in dry DCM under nitrogen at 0 °C and triethyl phosphite (4.40 g, 26.45 mmol, 4.54 ml) added dropwise over 2 h. The reaction was stirred at rt for 2 h then allowed to stand overnight. Volatiles were removed *in vacuo* and the resulting crude pale oil used without purification (7.07 g, 92%); δ_H ($CDCl_3$) 1.30 (6 H, t, *J* 6.5, 2 CH_3CH$_2$–), 4.15–4.25 (4 H, m, 2 CH$_3$CH_2–), 4.55 (2 H, s, CH_2Cl), 7.40–7.45 (1 H, m, H^5), 7.50–7.55 (1 H, m, H^6), 8.15–8.20 (1 H, m, $H^{2,4}$); δ_P ($CDCl_3$) −1.41 (s); δ_C ($CDCl_3$) 16.1, 16.2, 45.2, 63.8, 63.9, 129.2, 129.4, 131.2, 134.7, 136.3, 138.4, 197.1.

Diisopropyl 4-chloromethylbenzoylphosphonate (30c)

4-Chloromethylbenzoyl chloride **29c** (25.00 g, 132.25 mmol) and triisopropyl phosphonate (27.54 g, 132.25 mmol, 32.60 ml) were reacted in the same way as for **30a**. The *title compound* was recovered as a yellow oil (34.61 g, 90%); δ_H ($CDCl_3$) 1.25–1.35 (12 H, m, 2 $(CH_3)_2$CH–), 4.50 (2 H, s, CH_2Cl), 4.60–4.75 (2 H, m, 2 $(CH_3)_2$CH–), 7.40–7.50 (2 H, m, ArH), 8.15–8.20 (2 H, m, ArH); δ_P ($CDCl_3$) −2.42 (s); δ_C ($CDCl_3$) 23.6, 23.7, 23.9, 24.0, 45.2, 73.2, 73.3, 128.8, 130.3, 135.0, 143.8, 196.3.

Diethyl (3′-chloromethylphenyl)hydroxymethylphosphonate (31a)

Diethyl 3-chloromethylbenzoylphosphonate **30a** (2.00 g, 6.68 mmol) was dissolved in DCM (100 ml) and ice-cold water (100 ml) added. Sodium borohydride (0.65 g, 17.20 mmol, 2.5 eq.) added in portions over 5 min at 0 °C then the mixture was stirred for 3 h at rt. The reaction was partitioned, the aqueous layer extracted with DCM (50 ml), and the combined organics washed with water (100 ml), dried over $MgSO_4$, filtered, and solvents removed *in vacuo*. The *title compound* was isolated by flash column chromatography as a colourless oil (1.55 g, 77%); R_f 0.19 (DCM–MeOH, 95 : 5) (Found: M^+ 282.063. $C_{12}H_{18}ClO_4P$ requires *M* 282.063); δ_H ($CDCl_3$) 1.20 (6 H, t, 2 CH_3-CH$_2$–, *J* 6.4), 3.95–4.05 (4 H, m, 2 CH$_3$CH_2–), 4.55 (2 H, s, CH_2Cl), 5.00 (1 H, d, J_{HP} 11.6, PCH(OH)–), 7.20–7.45 (4 H, m, ArH); δ_P ($CDCl_3$) 19.7 (s); δ_C ($CDCl_3$) 16.2, 16.3, 45.2, 63.5, 63.6, 70.3 (d, J_{CP} 160), 126.9, 127.2, 128.0, 128.4, 137.3, 137.7; *m*/*z* (EI^+) 292 (42%, M^+).

Diisopropyl (4′-chloromethylphenyl)hydroxymethylphosphonate (31c)

Diisopropyl 4-chloromethylbenzoylphosphonate **30c** (20.47 g, 64.26 mmol) and sodium borohydride (6.08 g, 160.65 mmol) were reacted under the same conditions as for **31a**. The product was purified by flash column chromatography (gradient elution from DCM–light petrol, 80:20 to DCM–MeOH, 95:5) to yield the *title compound* as an off-white solid (10.51 g, 50%, mp 81–83 °C); R_f 0.07 (DCM–MeOH, 95:5) (Found: M^+ 320.094. $C_{14}H_{22}ClO_4P$ requires *M* 320.094); δ_H ($CDCl_3$) 1.20–1.35 (12 H, m, 2 $(CH_3)_2CH$–), 4.20 (2 H, s, CH_2Cl), 4.55–4.65 (2 H, m, 2 $(CH_3)_2CH$–), 4.95 (1 H, d, J_{HP} 9.3, PC*H*(OH)–), 7.45 (2 H, dd, $J_{HH'}$ 8.2, $J_{HH'}$ 2.2, ArH), 7.55 (2 H, dd, $J_{HH'}$ 8.2, $J_{HH'}$ 2.2, ArH); δ_P ($CDCl_3$) 19.9 (s); δ_C ($CDCl_3$) 23.6, 23.8, 24.0, 24.2, 46.0, 70.5 (d, J_{CP} 140), 127.5, 128.4, 137.0, 137.2; *m*/*z* (FAB^+) 320 (5%, M^+).

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]hydroxymethylphosphonate (32a)

Diethyl (3′-chloromethylphenyl)hydroxymethylphosphonate **31a** (5.99 g, 20.56 mmol) and triethyl phosphite (3.59 g, 21.59 mmol, 1.05 eq., 3.70 ml) were stirred together at 120 °C for 36 h and the reaction cooled. The crude product was purified by flash column chromatography (gradient elution, DCM to DCM–MeOH, 95:5) to yield the *title compound* as a pale yellow oil (6.06 g, 75%); R_f 0.46 (DCM–MeOH, 90:10) (Found: M^+ 394.131. $C_{16}H_{28}O_7P_2$ requires *M* 394.131); ν_{max}/cm^{-1} (film) 2984, 2910 (C–H), 1607 (C=C), 1243 (P=O), 1027 (P–O–C); δ_H ($CDCl_3$) 1.20, 1.25 (6 H each, 2 t, *J* 6.4, 2 CH_3CH_2– each), 2.20 (1 H, br, –OH), 3.10 (1 H, d, J_{HP} 16.4, PC*H*(OH)–), 3.95–4.10 (8 H, m, 4 CH_3CH_2–), 7.15–7.30 (4 H, m, ArH); δ_P ($CDCl_3$) 21.8, 26.8 (2 s); δ_C ($CDCl_3$) 16.2, 16.3, 33.5 (d, J_{CP} 138), 62.8, 63.4, 70.2 (d, J_{CP} 161), 125.7, 128.4, 130.4, 131.1, 131.2, 137.8; *m*/*z* (EI^+) 394 (16%, MH^+), 378 (97, M − OH), 257 (77, M − PO_3Et_2), 242 (98, M − PO_3Et_2 − OH).

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]hydroxymethylphosphonate (32c)

Diisopropyl (4′-chloromethylphenyl)hydroxymethylphosphonate **31c** (6.15 g, 21.03 mmol) and triisopropyl phosphite (4.60 g, 22.08 mmol, 1.05 eq., 5.45 ml) were reacted as for **32a** and the *title compound* was isolated by flash column chromatography as a white solid (5.28 g, 61%); R_f 0.11 (DCM–MeOH, 95:5) (Found: M^+ 450.194. $C_{20}H_{36}O_7P_2$ requires *M* 450.194); δ_H ($CDCl_3$) 1.15, 1.25 (12 H each, 2 s, 2 $(CH_3)_2CH$– each), 3.05 (2 H, d, J_{HP} 21.7, PCH_2–), 4.50–4.60 (4 H, m, 2 $(CH_3)_2CH$– each), 4.90 (1 H, d, J_{HP} 10.3, PC*H*(OH)–), 7.25 (2 H, dd, $J_{HH'}$ 6.4, $J_{HH'}$ 2.1, ArH), 7.35 (2 H, d, $J_{HH'}$ 6.4, $J_{HH'}$ 2.1, ArH); δ_P ($CDCl_3$) 24.5, 20.2 (2 s); δ_C ($CDCl_3$) 23.7, 24.1, 34.5 (d, J_{CP} 139), 69.5 (d, J_{CP} 162), 127.2, 129.7, 131.7, 135.3; *m*/*z* (EI^+) 450 (10%, M^+), 285 (52, M − $PO_3Pr^i_2$), 243 (20, M − $PO_3Pr^i_2$ − C_3H_6), 201 (100, M − $PO_3Pr^i_2$ − $2C_3H_6$), 120 (75, M − $2PO_3Pr^i_2$).

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]fluoromethylphosphonate (33a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]hydroxymethylphosphonate **32a** (0.17 g, 0.45 mmol) was dissolved in DCM (20 ml) under argon and DAST (0.12 g, 0.76 mmol, 1.7 eq., 0.10 ml) was added *via* syringe. The reaction was stirred for 10 min then cooled to 0 °C and quenched with aqueous sodium bicarbonate. After usual work-up the *title compound* was isolated as a yellow liquid (0.16 g, 91%); R_f 0.31 (DCM–MeOH, 90:10) (Found: M^+ 396.127. $C_{16}H_{27}FO_6P_2$ requires 396.127); ν_{max}/cm^{-1} (film) 2984, 2912 (C–H), 1645 (C=C), 1253 (P=O), 1027 (P–O–C); δ_H ($CDCl_3$) 1.20, 1.25 (6 H each, 2 t, *J* 6.1, 2 CH_3CH_2– each), 3.05 (2 H, d, J_{HP} 16.9, PCH_2–), 3.85–4.05 (8 H, m, 4 CH_3CH_2–), 5.70 (1 H, dd, J_{HP} 6.1, J_{HF} 34.5, PCHF–), 7.25–7.35 (4 H, m, ArH); δ_P ($CDCl_3$) 15.4 (d, J_{PF} 84.3), 26.3 (s); δ_F ($CDCl_3$) −200.5 (d); H-coupled −200.3 (dd, J_{PF} 84.8, J_{HF} 34.5); δ_C ($CDCl_3$) 16.3, 16.4, 33.6 (d, J_{CP} 138), 62.0, 62.3, 89.2 (dd, J_{CP} 169, J_{CF} 184), 125.3, 127.9, 128.7, 130.5, 131.2, 133.1; *m*/*z* (EI^+) 396 (100%, M^+), 260 (70, M − PO_3Et_2), 123 (55, M − $2PO_3Et_2$).

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]fluoromethylphosphonate (33c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]hydroxymethylphosphonate **33c** (0.73 g, 1.63 mmol) and DAST (0.53 g, 3.26 mmol, 2 eq., 0.43 ml) were reacted as for **33a**. Usual work-up gave the crude product as an orange oil, was purified by flash column chromatography (gradient elution, DCM to DCM–MeOH, 98:2) to give the *title compound* as a white crystalline solid (0.87 g, 90%); R_f 0.20 (DCM–MeOH, 95:5) (Found: C 53.2, H 7.8. $C_{20}H_{35}FO_6P_2$ requires C 53.1, H 7.8; M^+ 452.189. $C_{20}H_{35}FO_6P_2$ requires *M* 452.190); δ_H ($CDCl_3$) 1.10–1.25 (24 H, m, 4 $(CH_3)_2CH$–), 3.05 (2 H, d, J_{HP} 21.7, PCH_2–), 4.45–4.60 (4 H, m, 4 $(CH_3)_2CH$–), 5.50 (1 H, dd, J_{HP} 7.6, J_{HF} 34.8, PCHF–), 7.20–7.30 (4 H, m, ArH); δ_P ($CDCl_3$) 13.8 (d, J_{PF} 86.0), 24.6 (s); δ_F ($CDCl_3$) −200.5 (d); H-coupled −200.6 (dd, J_{PF} 85.0, J_{HF} 7.6); δ_C ($CDCl_3$) 23.5, 23.6, 23.8, 23.9, 34.4 (d, J_{CP} 139), 70.3, 70.4, 71.8, 71.9, 89.2 (dd, J_{CP} 172, J_{CF} 183), 126.8, 129.7, 131.5, 132.8; *m*/*z* (EI^+) 452 (60%, M^+), 287 (49%, M − $PO_3Pr^i_2$), 284 (77%, M − $4C_3H_6$), 204 (77%, M − $PO_3Pr^i_2$ − $2C_3H_6$), 122 (100%, M − $2PO_3Pr^i_2$).

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]chloromethylphosphonate (34a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]hydroxymethylphosphonate **32a** (1.30 g, 3.29 mmol) was dissolved in DCM (15 ml) under argon, thionyl chloride (5 ml) added, and the reaction refluxed for 14 h. After this time, TLC showed the reaction was still incomplete but considerable side-product formation was observed. The reaction was cooled and volatiles removed *in vacuo*. The resulting yellow oil was purified by flash column chromatography to afford the *title compound* as a pale yellow oil (0.80 g, 95%, 62% conversion); R_f 0.48 (DCM–MeOH, 95:5) (Found: M^+ 412.099. $C_{16}H_{27}ClO_6P_2$ requires *M* 412.097); ν_{max}/cm^{-1} (film) 2981 (C–H), 1654 (C=C), 1252 (P=O), 1024 (P–O–C); δ_H ($CDCl_3$) 1.15, 1.20 (6 H each, 2 t, *J* 6.4, 2 CH_3CH_2– each), 3.15 (2 H, d, J_{HP} 21.3, PCH_2–), 3.95–4.05, 4.10–4.20 (4 H each, 2 m, 2 CH_3CH_2– each), 4.85 (1 H, d, J_{HP} 14.4, PC*H*(OH)–), 7.25–7.30 (4 H, m, ArH); δ_P ($CDCl_3$) 17.6, 26.3 (2 s); δ_C ($CDCl_3$) 16.2, 16.3, 33.6 (d, J_{CP} 137.8), 53.5 (d, J_{CP} 158.9), 62.1, 62.2, 64.0, 64.1, 127.5, 128.8, 130.3, 130.5, 132.2, 134.5; *m*/*z* (EI^+) 412 (100%, M^+), 276 (58, M − PO_3Et_2).

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]chloromethylphosphonate (34c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]hydroxymethylphosphonate **32c** (2.13 g, 4.72 mmol) was dissolved in DCM (20 ml) and thionyl chloride (5.61 g, 47.20 mmol, 10 eq., 3.44 ml) added slowly *via* syringe. The reaction was refluxed for 12 h then volatiles removed *in vacuo*. The *title compound* was separated from ~50% starting material by flash column chromatography, affording a clear oil (1.03 g, 93%, 50% conversion); R_f 0.44 (DCM–MeOH, 90:10); δ_H ($CDCl_3$) 1.20, 1.30 (12 H each, 2 d, *J* 6.2, 2 $(CH_3)_2CH$– each), 3.05 (2 H, d, *J* 22.0, PCH_2–), 4.50–4.60 (4 H, m, 4 $(CH_3)_2CH$–), 4.75 (1 H, d, J_{HP} 14.4, PCHCl–), 7.25, 7.40 (2 H each, 2 d, *J* 7.4, ArH); δ_P ($CDCl_3$) 15.8, 24.3 (2 s); δ_C ($CDCl_3$) 23.6, 23.8, 34.5 (d, J_{CP} 139), 53.7 (d, J_{CP} 162), 70.5, 72.5, 128.4, 129.1, 131.4, 133.0; *m*/*z* (EI^+) 468 (54, M^+), 300 (100, M − PO_3Et_2).

Diethyl 3-(chloromethylphenyl)difluoromethylphosphonate (35a)

Diethyl 3-chloromethylbenzoylphosphonate **30a** (1.00 g, 3.44 mmol) was dissolved in DCM (15 ml) under an inert atmosphere. DAST (1.66 g, 10.32 mmol, 3 eq., 1.40 ml) was added *via*

syringe at 0 °C, the reaction stirred overnight at rt, then quenched by dropwise addition of aqueous $NaHCO_3$. The reaction was worked up as usual and the crude product purified by flash column chromatography (DCM–MeOH, 98 : 2) to give the *title compound* as a pale orange oil (1.55 g, 76%); R_f 0.36 (DCM–MeOH, 95 : 5) (Found: M^+ 313.057. $C_{12}H_{17}ClFO_3P$ requires *M* 313.057); ν_{max}/cm^{-1} (film) 2985 (C–H), 1611 (C=C), 1265 (P=O), 1183 (C–F), 1023 (P–O–C); δ_H ($CDCl_3$) 1.25 (6 H, t, 2 CH_3CH_2–), 3.95–4.10 (4 H, m, 2 CH_3CH_2–), 4.55 (2 H, s, CH_2Cl), 7.40–7.50 (4 H, m, ArH); δ_P ($CDCl_3$) 6.41 (t, J_{PF} 111.9); δ_F ($CDCl_3$) −109.5 (d, J_{FP} 112); δ_C ($CDCl_3$) 16.2. 16.3, 45.5, 64.8, 64.9, 117.8 (dt, J_{CP} 218, J_{CF} 263), 126.3, 127.7, 128.9, 129.2, 133.0, 137.9; *m*/*z* (FAB^+) 313 (30%, MH^+), 175 (27, M − PO_3Et_2), 137 (25, PO_3Et_2).

Diisopropyl 4-(chloromethylphenyl)difluoromethylphosphonate (35c)

Diisopropyl 4-chloromethylbenzoylphosphonate **30c** (0.85 g, 2.67 mmol) and DAST (1.29 g, 8.01 mmol, 3 eq., 1.06 ml) were reacted under the same conditions as for **35a**. The product was purified by flash column chromatography (DCM–MeOH, 98 : 2), affording the *title compound* as a yellow oil (0.67 g, 73%) (Found: M^+ 340.080. $C_{14}H_{20}F_2O_3P$ requires *M* 340.081); δ_H ($CDCl_3$) 1.15, 1.20 (6 H each, 2 s, $(CH_3)_2CH$– each), 4.60 (2 H, s, CH_2Cl), 4.70–4.80, 4.85–4.90 (1 H each, 2 m, $(CH_3)_2$-C*H*– each), 7.45 (2 H, d, *J* 7.7, ArH), 7.55 (2 H, d, *J* 7.7, ArH); δ_P ($CDCl_3$) 4.81 (t, J_{FP} 116); δ_F ($CDCl_3$) −109.3 (d, J_{PF} 117); δ_C ($CDCl_3$) 23.5, 23.6, 24.1, 24.2, 45.4, 73.8, 74.0, 118.2 (dt, J_{CP} 220, J_{CF} 263), 126.7, 128.5, 133.0, 140.0; *m*/*z* (EI^+) 340 (9%, M^+), 256 (18, M − $2C_3H_6$), 175 (67, M − $PO_3Pr^i_2$), 140 (100, M − Cl − $PO_3Pr^i_2$).

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]difluoromethylphosphonate (36a)

Sodium hydride (0.08 g, 2.80 mmol, 90% powder) was suspended in dry THF under nitrogen and diethyl phosphonate (0.43 g, 3.08 mmol, 1.1 eq., 0.40 ml) added dropwise at 0 °C then diethyl 3-(chloromethylphenyl)difluoromethylphosphonate **35a** (1.00 g, 2.80 mmol) in dry THF (15 ml) was added dropwise over 5 min. The reaction was stirred overnight at rt then quenched with ethanol. After standard work-up the crude product was purified (~50%) by flash column chromatography (DCM–MeOH, 98 : 2) to yield the *title compound* as a pale yellow oil (0.62 g, 94%, 50% conversion); R_f 0.23 (DCM–MeOH, 95 : 5); ν_{max}/cm^{-1} (film) 2986, 2913 (C–H), 1653 (C=C), 1273 (P=O), 1024 (P–O–C); δ_H ($CDCl_3$) 1.18, 1.25 (6 H each, 2 t, *J* 7.1, 2 CH_3CH_2– each), 3.15 (2 H, d, J_{HP} 21.6, PCH_2–), 3.80–4.00, 4.10–4.15 (4 H each, 2 m, 2 CH_3CH_2– each), 7.30–7.45 (4 H, m, ArH); δ_P ($CDCl_3$) 6.65 (t, J_{PF} 116), 25.9; δ_F ($CDCl_3$) −108.9 (d, J_{FP} 114.9); δ_C ($CDCl_3$) 16.2. 16.3, 33.1 (d, J_{CP} 138), 62.1, 62.2, 64.7, 64.8, 117.9 (dt, J_{CP} 217, J_{CF} 263), 127.4, 127.5, 128.6, 132.1, 132.4, 132.9.

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]difluoromethylphosphonate (36c)

Diisopropyl 4-(chloromethylphenyl)difluoromethylphosphonate **35c** (0.53 g, 1.57 mmol) and diisopropyl phosphonate (0.29 g, 1.72 mmol, 1.1 eq., 0.29 ml) and sodium hydride (0.04 g, 1.72 mmol, 90% powder) were reacted as for **36a**. The *title compound* was isolated by flash column chromatography (DCM–MeOH, 98 : 2) as a colourless oil (0.34 g, 90%, 52% conversion); R_f 0.14 (DCM–MeOH, 98 : 2) (Found: M^+ 470.181. $C_{20}H_{34}F_2O_6P_2$ requires *M* 470.180); ν_{max}/cm^{-1} (film) 2923 (C–H), 1618 (C=C), 1265 (P=O), 1024 (P–O–C); δ_H ($CDCl_3$) 1.05–1.25 (24 H, m, 4 $(CH_3)_2CH$–), 3.05 (2 H, d, J_{HP} 22.0, PCH_2–), 4.50–4.55, 4.60–4.65 (2 H each, 2 m, 2 $(CH_3)_2CH$– each), 7.25 (2 H, d, *J* 7.9, ArH), 7.45 (2 H, d, *J* 7.7, ArH); δ_P ($CDCl_3$) 4.93 (t, J_{FP} 119), 24.1 (s); δ_F ($CDCl_3$) −109.1 (d, J_{PF} 118); δ_C ($CDCl_3$) 23.7, 23.9, 34.2 (d, J_{CP} 139), 70.6, 73.7, 117.9 (dt, J_{CP} 221, J_{CF} 263), 126.5, 129.7, 133.0 (ddt, $^2J_{CP}$ 13.8, $^2J_{CF}$ 22.1, $^4J_{CP}$ 3.8), 134.9; *m*/*z* (EI^+) 470 (44%, M^+), 428 (7, M − C_3H_6), 386 (10, M − C_3H_6), 306 (97, M − $PO_3Pr^i_2$), 264 (62, M − $PO_3Pr^i_2$ − C_3H_6), 222 (87 M − $PO_3Pr^i_2$ − $2C_3H_6$), 140 (88, M − $2PO_3Pr^i_2$).

3-(Phosphonomethylphenyl)hydroxymethylphosphonic acid (37a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]hydroxymethylphosphonate **32a** (0.19 g, 0.48 mmol) was deprotected as for **2** with TMSBr (0.59 g, 3.82 mmol, 8 eq., 0.50 ml) and the free acid was isolated as usual (0.11 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.18 g, 56%, mp >275 °C) (Found: C 49.9, H 8.2, N 5.5. $C_{20}H_{38}N_2O_7P_2$ (salt) requires C 50.0, H 8.0, N 5.8%; Found: M^+ 382.118. $C_{14}H_{26}NO_7P_2$ requires *M* 382.118); δ_H (D_2O) 1.10–2.00 (22 H, m, cyclohexyl-H), 2.90 (2 H, d, J_{HP} 16.4, PCH_2–), 4.60 (1 H, d, J_{HP} 14.0, PC*H*(OH)–), 7.05–7.25 (4 H, m, ArH); δ_P (D_2O) 15.8, 19.6 (2 s); δ_C (D_2O) 23.8, 24.2, 30.3, 36.5 (d, J_{CP} 124), 50.2, 73.5 (d, J_{CP} 144), 124.4, 127.8, 128.0, 131.4, 136.5, 140.7; *m*/*z* (FAB^+) 283 (25%, MH^+), 382 (29, M + $C_6H_{11}NH_3^+$).

4-(Phosphonomethylphenyl)hydroxymethylphosphonic acid (37c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]-hydroxymethylphosphonate **32c** (0.62 g, 1.36 mmol) was de-esterified as for **2** with TMSBr (1.67 g, 10.90 mmol, 8 eq., 1.45 ml) and the free acid was isolated as usual (0.42 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.57 g, 66%, mp >275 °C) (Found: C 49.7, H 8.25, N 5.8. $C_{20}H_{38}N_2O_7P_2$ (salt) requires C 50.0, H 8.0, N 5.8%); δ_H (D_2O) 1.20–2.00 (22 H, m, cyclohexyl-H), 2.90 (2 H, d, J_{HP} 20.8, PCH_2–), 4.80 (1 H, d, J_{HP} 9.7, PC*H*(OH)–), 7.15–7.30 (4 H, m, ArH); δ_P (D_2O) 21.2, 17.1 (2 s); δ_C (D_2O) 23.8, 24.2, 30.3, 35.7 (d, J_{CP} 137), 50.3, 72.0 (d, J_{CP} 128), 126.9, 129.2, 131.0, 134.4; *m*/*z* (FAB^+) 283 (100%, MH^+), 201 (87, M − PO_3H_2).

3-(Phosphonomethylphenyl)fluoromethylphosphonic acid (38a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]fluoromethylphosphonate **33a** (0.07 g, 0.18 mmol) was deprotected as for **2** with TMSBr (0.19 g, 1.24 mmol, 7 eq., 0.17 ml) and the free acid isolated as usual (0.05 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.05 g, 62%, mp >300 °C) (Found: C 50.0, H 7.4, N 6.1. $C_{20}H_{37}FN_2O_6P_2$ (salt) requires C 49.8, H 7.4, N 6.1%. Found: M^+ 384.114. $C_{14}H_{25}FNO_6P_2$ requires *M* 384.114); δ_H (D_2O) 1.10–2.00 (22 H, m, cyclohexyl-H), 3.00 (2 H, d, J_{HP} 16.4, PCH_2–), 5.50 (1 H, dd, J_{HP} 7.0, J_{HF} 39.1, PCHF–), 7.15–7.40 (4 H, m, ArH); δ_P (D_2O) 11.1 (d, J_{PF} 73.0), 20.3 (s); δ_F (D_2O) −193.4 (d); H-coupled −193.6 (dd, J_{HF} 34.2, J_{PF} 73.0); δ_C (D_2O) 23.8, 24.3, 30.3, 35.9 (d, J_{CP} 127), 50.2, 91.6 (dd, J_{CP} 169, J_{CF} 184), 124.2, 127.5, 128.2, 129.1, 134.1, 135.9; *m*/*z* (FAB^+) 285 (27%, M^+), 384 (92, M + $C_6H_{11}NH_3^+$).

4-(Phosphonomethylphenyl)fluoromethylphosphonic acid (38c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]fluoromethylphosphonate **33c** (0.26 g, 0.56 mmol) was deesterified as for **2** with TMSBr (0.61 g, 3.95 mmol, 7 eq., 0.52 ml) and the free acid isolated as usual (0.18 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.12 g, 63%, mp >300 °C) (Found: C 49.5, H 8.1, N 5.8. $C_{20}H_{37}FNO_6P_2$ (salt) requires C 49.8, H 7.4, N 6.1%); δ_H (D_2O) 1.20–2.00 (22 H, m, cyclohexyl-H), 3.00 (2 H, d, J_{HP} 21.3, PCH_2–), 5.80 (1 H, d, J_{HP} 6.8, J_{HF} 42.0, PCHF–), 7.30, 7.40 (2 H each, 2 d, *J* 7.1, 2 ArH each); δ_P (D_2O) 11.5 (d, J_{FP} 72.6), 20.9 (s); δ_F (D_2O) −195.2 (d); H-coupled −195.3 (dd, J_{PF} 74.1, J_{HF} 42.6); δ_C (D_2O) 23.8, 24.3, 30.2, 50.2, 128.3, 130.6, 134.0, 135.9.

3-(Phosphonomethylphenyl)chloromethylphosphonic acid (39a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]chloromethylphosphonate **34a** (0.31 g, 0.75 mmol) was deprotected as for **2** with TMSBr (0.81 g, 5.26 mmol, 7 eq., 0.70 ml) and the free acid isolated as usual (0.26 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.27 g, 71%, mp >300 °C) (Found: C 48.0, H 7.8, N 5.4, Cl 6.9. $C_{14}H_{25}ClNO_6P_2$ (salt) requires C 48.2, H 7.5, N 5.6, Cl 7.0%. Found: M^+ 384.114. $C_{14}H_{25}ClNO_6P_2$ requires *M* 384.114); δ_H (D_2O) 1.25–2.00 (22 H, m, cyclohexyl-H), 3.00 (2 H, d, J_{HP} 19.8, PCH_2–), 4.85 (1 H, d, J_{HP} 11.3, PCHCl–), 7.20–7.35 (4 H, m, ArH); δ_P (D_2O) 12.9, 20.8 (2 s); δ_C (D_2O) 23.8, 24.2, 30.3, 35.8 (d, J_{CP} 128), 50.3, 56.8 (d, J_{CP} 141), 126.2, 128.4, 129.3, 129.3, 135.5, 137.7; *m*/*z* (FAB^+) 301 (50%, MH^+), 400 (50, M + $C_6H_{11}NH_3^+$).

4-(Phosphonomethylphenyl)chloromethylphosphonic acid (39c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]chloromethylphosphonate **34c** (0.44 g, 0.93 mmol) was deprotected as for **2** with TMSBr (1.00 g, 6.54 mmol, 7 eq., 0.86 ml) and the free acid isolated as usual (0.29 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.28 g, 60%) (Found: C 47.7, H 7.4, N 5.5, Cl 6.9. $C_{20}H_{37}ClN_2O_6P_2$ (salt) requires C 48.3, H 7.5, N 5.6, Cl 7.0%); δ_H (D_2O) 1.15–2.00 (22 H, m, cyclohexyl-H), 3.00 (2 H, d, J_{HP} 20.8, PCH_2–), 4.90 (1 H, d, J_{HP} 12.5, PCHCl–), 7.35, 7.45 (2 H each, 2 d, *J* 7.7, 2 ArH each); δ_P (D_2O) 13.4, 17.3 (2 s); δ_C (D_2O) 23.8, 24.2, 30.3, 34.8 (d, J_{CP} 136), 50.3, 58.4 (d, J_{CP} 143), 126.8, 128.1, 134.5, 136.8.

3-(Phosphonomethylphenyl)difluoromethylphosphonic acid (40a)

Diethyl 3-[(diethoxyphosphoryl)methylphenyl]difluoromethylphosphonate **36a** (0.38 g, 0.92 mmol) was de-esterified as for **2** with TMSBr (0.99 g, 6.42 mmol, 7 eq., 0.85 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.23 g, 57%) (Found: C 47.75, H 7.55, N 5.45. $C_{20}H_{36}FN_2O_6P_2$ (salt) requires C 48.0, H 7.3, N 5.6%; Found: M^+ 402.104. $C_{14}H_{24}FNO_6P_2$ requires 402.105); δ_H (D_2O) 1.25–2.00 (22 H, m, cyclohexyl-H), 2.95 (2 H, d, J_{HP} 21.7, PCH_2–), 7.40–7.50 (4 H, m, 4 ArH); δ_P (D_2O) 4.88 (t, J_{FP} 102), 20.4; δ_F (D_2O) −109.9 (d, J_{FP} 102); δ_C (D_2O) 23.7, 24.2, 30.2, 50.0, 128.2, 128.3, 128.8, 130.1, 133.1, 134.0; *m*/*z* (FAB^+) 303 (29%, MH^+), 402 (97, M + $C_6H_{11}NH_3^+$).

4-(Phosphonomethylphenyl)difluoromethylphosphonic acid (40c)

Diisopropyl 4-[(diisopropoxyphosphoryl)methylphenyl]-difluoromethylphosphonate **36c** (0.05 g, 0.11 mmol) was deprotected as for **2** with TMSBr (0.11 g, 0.74 mmol, 7 eq., 0.10 ml). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.04 g, 75%) (Found: C 48.15, H 7.4, N 5.3. $C_{20}H_{36}F_2N_2O_6P_2$ (salt) requires C 48.0, H 7.25, N 5.6%); δ_H (D_2O) 1.25–2.00 (22 H, m, H-cyclohexyl), 3.15 (2 H, d, J_{HP} 22.7, PCH_2–), 7.25, 7.45 (2 H each, 2 d, *J* 7.1, 2 ArH each); δ_P (D_2O) 4.30 (t, J_{FP} 113), 26.0 (s); δ_F (D_2O) −111.2 (d, J_{FP} 112); δ_C (D_2O) 35.5 (d, 136), 126.1, 129.6.

Diethyl (3-bromophenylmethyl)phosphonate (41)

3-Bromobenzyl bromide (10.19 g, 40.77 mmol) and triethyl phosphite (4.74 g, 40.77 mmol, 7.10 ml) were stirred together in a dry flask under argon and heated at 120 °C for 14 h. The crude product was purified by flash column chromatography (DCM–MeOH, 95:5), affording the *title compound* as a colourless liquid (11.19 g, 90%); R_f 0.30 (DCM–MeOH, 95:5); δ_H ($CDCl_3$) 1.20 (6 H, t, *J* 6.3, 2 CH_3CH_2–), 3.05 (2 H, d, J_{HP} 27.0, PCH_2–), 3.90–4.05 (4 H, m, 2 CH_3CH_2–), 7.10–7.45 (4 H, m, ArH); δ_P ($CDCl_3$) 25.7 (s); δ_C ($CDCl_3$) 16.0, 16.1, 33.1 (d, J_{CP} 139), 61.3, 62.1, 122.3, 128.4, 129.9, 132.6, 134.0; *m*/*z* (EI^+) 306 (43%, MH^+), 250 (35, M − C_2H_4), 227 (41, M − Br), 171 (87, M − C_2H_4 − Br).

Diethyl [3-(diethoxyphosphoryl)phenyl]methylphosphonate (42)

Diethyl (3-bromophenylmethyl)phosphonate **41** (1.00 g, 3.26 mmol) and diethyl phosphonate (0.50 g, 3.58 mmol) were placed in a 5 ml reaction vessel under argon and triethylamine (0.36 g, 3.58 ml) added *via* syringe. Tetrakis(triphenylphosphine)palladium(0) (190 mg, 0.16 mmol, 0.05 eq.) was added and the reaction heated at 100 °C for 22 h. The reaction mixture was filtered through Celite (ether–hexanes, 50:50 as eluent) and solvent removed *in vacuo*. The crude product was purified by flash column chromatography (DCM–MeOH, 98:2), furnishing the *title compound* as a yellow liquid (0.80 g, 67%); R_f 0.19 (DCM–MeOH, 90:10) (Found: M^+ 364.120. $C_{15}H_{26}O_6P_2$ requires *M* 364.121); ν_{max}/cm^{-1} (film) 2984 (C–H), 1630 (C=C), 1246 (P=O), 1026 (P–O–C); δ_H ($CDCl_3$) 1.20, 1.30 (6 H each, 2 t, *J* 6.1, 2 CH_3CH_2– each), 3.15 (2 H, d, J_{HP} 18.5, PCH_2–), 3.95–4.05, 4.05–4.15 (4 H each, 2 m, 2 CH_3CH_2– each), 7.45–7.60 (4 H, m, ArH); δ_P ($CDCl_3$) 18.8, 25.9 (2 s); δ_C ($CDCl_3$) 16.2, 16.3, 33.5 (d, J_{CP} 128), 62.0, 127.2, 128.8, 130.1, 132.1, 132.4, 133.6; *m*/*z* (EI^+) 364 (90%, M^+), 228 (100, M − PO_3Et_2), 200 (37, M − PO_3Et_2 − C_2H_4), 172 (37, M − PO_3Et_2 − $2C_2H_4$).

(3-Phosphonophenyl)methylphosphonic acid (43)

Diethyl [3-(diethoxyphosphoryl)phenyl]methylphosphonate **42** (0.45 g, 1.24 mmol) was deprotected as for **2** with TMSBr (1.33 g, 8.66 mmol, 1.20 ml) to yield the free acid as an off-white solid (0.26 g, mp 270 °C). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.32 g, 58%, mp >275 °C) (Found: C 50.5, H 8.05, N 5.8. $C_{19}H_{36}N_2O_6P_2$ (salt) requires C 50.7, H 8.05, N 6.2%); free acid: δ_H (D_2O) 3.00 (2 H, d, J_{HP} 19.4, PCH_2–), 7.50–7.65 (4 H, m, ArH); δ_P (D_2O) 17.0, 25.0 (2 s); biscyclohexylammonium salt: δ_C (D_2O) 23.7, 24.2, 30.2, 35.8 (d, J_{CP} 127), 50.1, 127.6, 128.3, 130.8, 131.5, 131.9, 135.6.

Diethyl [3-(diethoxyphosphoryl)phenyl]difluoromethylphosphonate (44)

NaHMDS (1.89 mmol, 1.0 M solution in THF, 1.89 ml) and diethyl [3-(diethoxyphosphoryl)phenyl]methylphosphonate **42** (0.25 g, 0.69 mmol) were reacted as for **8a** at −78 °C for 1 h then NFSi (0.79 g, 2.52 mmol) added dropwise and the reaction stirred for 2 h at −78 °C. After work-up, the crude product was purified by flash column chromatography to give the *title compound* as a colourless oil (0.15 g, 53%); R_f 0.15 (DCM–MeOH, 95:5); ν_{max}/cm^{-1} (film) 2985 (C–H), 1629 (C=C), 1265 (P=O), 1032 (P–O–C); δ_H ($CDCl_3$) 1.20 (12 H, t, *J* 6.1, 4 CH_3CH_2–), 4.00–4.15 (8 H, m, 4 CH_3CH_2–), 7.55–7.70 (4 H, m, ArH); δ_P ($CDCl_3$) 6.21 (t, J_{PF} 96.2), 17.5 (s); δ_F ($CDCl_3$) −114.8 (d, J_{PF} 96.5); δ_C ($CDCl_3$) 16.2, 16.3, 63.3, 63.5, 117.4 (dt, J_{CP} 218, J_{CF} 268), 128.8, 129.2, 130.1, 131.5, 131.8, 133.3.

Diethyl 3-(dioxolan-2′-yl)phenylphosphonate (46)

Diethyl phosphonate (1.20 g, 8.69 mmol, 1.1 eq., 1.12 ml) and triethylamine (0.88 g, 8.69 mmol, 1.21 ml) were added *via* syringe to 2-(3′-bromophenyl)-1,3-dioxolane **45** (1.81 g, 7.90 mmol) under argon. Tetrakis(triphenylphosphine) palladium(0) (0.46 g, 0.40 mmol, 0.05 eq.) was added and the reaction heated to 95 °C for 12 h. The crude product was filtered through Celite™ (ether–hexane, 50:50 as eluent) and volatiles evaporated to yield the crude product as a deep yellow oil, purified by flash column chromatography (DCM–MeOH, 98:2) to give the *title compound* as a yellow oil (1.54 g, 68%); R_f 0.46 (DCM–MeOH, 90:10); ν_{max}/cm^{-1} (film) 2983, 2901 (C–H), 1646 (Ar C=C), 1250 (P=O), 1023 (P–O–C); δ_H ($CDCl_3$) 1.27 (6 H, t, *J* 6.2, CH_3CH_2–), 3.95–4.10 (8 H, m, CH_3CH_2– and

–OCH_2CH_2– overlapping), 5.80 (1 H, s, C*H*(OR)), 7.30–7.90 (4 H, m, ArH); δ_P ($CDCl_3$) 18.8 (s); δ_C ($CDCl_3$) 16.2, 16.3, 62.1, 62.2, 65.3 (–O*C*H_2*C*H_2–), 103.0 (*C*H(OR)), 128.6, 127.0, 130.0, 131.9, 132.5, 138.3; *m*/*z* (EI^+) 285 (48%, M − 1), 241 (10, M − CH_2CH_2O–), 158 (M − 1 − PO_3Et_2).

Diethyl (3-carbonylphenyl)phosphonate (47)

Aqueous 15% sulfuric acid (0.35 ml) was adsorbed onto silica gel (3.50 g) suspended in DCM (10 ml). Diethyl 3-(dioxolan-2′-yl)-phenylphosphonate **46** (0.40 g) was then added in DCM (10 ml) and the reaction stirred for 24 h at rt. The mixture was filtered, washed with DCM (100 ml), and solvent removed *in vacuo*. The crude product was purified by flash column chromatography to afford the *title compound* as a yellow oil (0.30 g, 90%); R_f 0.28 (EtOAc–petrol, 70:30) (Found: M^+ 242.070. $C_{11}H_{15}O_4P$ requires *M* 242.071); ν_{max}/cm^{-1} (film) 2983, 2899 (C–H), 1712 (CHO), 1646 (C=C), 1250 (P=O), 1023 (P–O–C); δ_H ($CDCl_3$) 1.25 (6 H, t, *J* 6.7, 2 C$H_3$$CH_2$–), 4.00–4.20 (4 H, m, 2 CH_3CH_2–), 7.50–7.60 (1 H, m, H^5), 7.95–8.05 (2 H, m, $H^{4,6}$), 8.25 (1 H, ddt, J_{HH^5} 0.6, $J_{HH^{4,6}}$ 1.5, J_{HP} 12.8, H^2), 10.0 (1 H, s, CHO); δ_P ($CDCl_3$) 17.4 (s); δ_C ($CDCl_3$) 16.3, 16.4, 62.5, 62.6, 128.4, 129.3, 132.0, 132.6, 133.4, 137.3, 191.3; *m*/*z* (EI^+) 242 (27%, M^+), 214 (15, M − C_2H_4), 187 (32, M − $2C_2H_4$), 105 (M − PO_3Et_2).

Diethyl [3-(diethoxyphosphoryl)phenyl]hydroxymethylphosphonate (48)

Triethylamine (0.05 g, 0.52 mmol, 0.5 eq., 0.07 ml) was added to diethyl (3-carbonylphenyl)phosphonate **47** (0.25 g, 1.03 mmol) and diethyl phosphonate (0.14 g, 1.03 mmol, 0.13 ml) in dry DCM (20 ml) and the reaction stirred overnight. A second aliquot of triethylamine was then added and a third aliquot after 4 h and volatiles removed *in vacuo*. The crude mixture was purified by flash column chromatography (EtOAc–petrol, 50:50) to yield the *title compound* as a colourless oil (0.25 g, 53%); R_f 0.16 (EtOAc–petrol, 70:30) (Found: M^+ 380.116. $C_{15}H_{26}O_7P_2$ requires *M* 380.115); ν_{max}/cm^{-1} (film) 2980 (C–H), 1631 (C=C), 1262 (P=O), 1032 (P–O–C); δ_H ($CDCl_3$) 1.20, 1.25 (6 H each, 2 t, *J* 7.0, 2 C$H_3$$CH_2$– each), 2.70 (1 H, br, –OH), 3.90–4.10 (8 H, m, 4 CH_3CH_2–), 5.05 (1 H, d, J_{HP} 11.6, PC*H*(OH)–), 7.30–7.40 (1 H, m, H^5), 7.60–7.70 (2 H, m, $H^{4,6}$), 7.80–7.85 (1 H, app. d, H^2); δ_P ($CDCl_3$) 18.2, 20.4 (2 s); δ_C ($CDCl_3$) 16.2, 16.3, 63.4 (d, J_{CP} 122), 64.4, 64.5, 128.1, 128.8, 131.2, 131.4, 135.3, 137.1; *m*/*z* (EI^+) 380 (14%, M^+), 243 (100, M − PO_3Et_2).

Diethyl [3-(diethoxyphosphoryl)phenyl]fluoromethylphosphonate (49)

Diethyl [3-(diethoxyphosphoryl)phenyl]hydroxymethylphosphonic acid **48** (0.36 g, 0.94 mmol) in DCM (20 ml) under argon was treated with DAST (0.31 g, 1.87 mmol, 0.25 ml) and the reaction stirred at rt for 10 min then worked up as usual. The resulting pale yellow oil was purified by flash column chromatography (DCM–MeOH, 95:5) to afford the *title compound* as a pale yellow oil (0.34 g, 93%); R_f 0.63 (DCM–MeOH, 90:10) (Found: M^+ 382.111. $C_{15}H_{25}FO_6P_2$ requires *M* 382.111); ν_{max}/cm^{-1} (film) 2985 (C–H), 1629 (C=C), 1265 (P=O), 1032 (P–O–C); δ_H ($CDCl_3$) 1.25, 1.30 (6 H each, 2 t, *J* 6.3, 2 C$H_3$$CH_2$– each), 4.00–4.15 (8 H, m, 4 C$H_3$$CH_2$–), 5.65 (1 H, dd, J_{HP} 10.5, J_{HF} 39.5, PCHF–), 7.55–7.65 (3 H, m, ArH), 7.70–7.85 (1 H, m, ArH); δ_P ($CDCl_3$) 14.7 (d, J_{PF} 83.3), 18.1 (s); δ_F ($CDCl_3$) −202.5 (d); H-coupled −202.6 (dd, J_{PF} 83.3, J_{HF} 39.5); δ_C ($CDCl_3$) 16.3, 16.4, 63.5, 63.8, 89.0 (dd, J_{CP} 169, J_{CF} 185), 128.7, 129.7, 130.6, 132.3, 132.5, 133.6; *m*/*z* (EI^+) 382 (71%, M^+), 354 (22, M − C_2H_4).

Diethyl [3-(diethoxyphosphoryl)phenyl]chloromethylphosphonate (50)

Diethyl [3-(diethoxyphosphoryl)phenyl]hydroxymethylphosphonate **48** (0.65 g, 1.71 mmol) in DCM (15 ml) was treated with thionyl chloride (5 ml) under argon as for **34c** and worked up as usual. The resulting yellow oil was purified from excess starting material by flash column chromatography to afford the *title compound* as a pale yellow oil (0.41 g, 61% conversion); R_f 0.20 (DCM–MeOH, 95:5) (Found: M^+ 398.082. $C_{15}H_{25}ClO_6P_2$ requires *M* 398.082); δ_H ($CDCl_3$) 1.25 (12 H, t, *J* 7.0, 4 CH_3-CH_2–), 3.95–4.10 (8 H, m, 4 CH_3CH_2–), 4.85 (1 H, d, J_{HP} 14.3, PCHCl–), 7.40–7.45 (1 H, m, ArH), 7.70–7.85 (3 H, m, ArH); δ_P ($CDCl_3$) 16.9, 18.5 (s); δ_C ($CDCl_3$) 16.1, 16.2, 53.4 (d, J_{CP} 158), 62.7, 62.8, 127.3, 128.7, 128.9, 132.1, 132.8, 134.6; *m*/*z* (EI^+) 398 (30%, M^+), 370 (12, M − C_2H_4), 363 (19, M − Cl), 342 (11, M − $2C_2H_4$), 314 (12, M − $3C_2H_4$), 286 (32, M − $4C_2H_4$).

(3-Phosphonophenyl)hydroxymethylphosphonic acid (51)

Diethyl [3-(diethoxyphosphoryl)phenyl]hydroxymethylphosphonate **48** (0.37 g, 0.98 mmol) was deprotected as for **2** with TMSBr (1.20 g, 7.80 mmol, 8 eq., 1.03 ml) and the free acid isolated as usual (0.26 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.21 g, 80%, mp >275 °C) (Found: C 48.4, H 8.0, N 5.9. $C_{19}H_{36}N_2O_7P_2$ (salt) requires C 48.9, H 7.8, N 6.0%); M^+ 368.103. $C_{13}H_{24}NO_7P_2$ requires *M* 368.103); δ_H (D_2O) 1.20–2.00 (22 H, m, cyclohexyl-H), 4.50 (1 H, d, J_{HP} 9.7, PC*H*(OH)–), 7.05–7.25 (4 H, m, 4 ArH); δ_P (D_2O) 12.9, 15.8 (2 s); δ_C (D_2O) 23.7, 24.2, 30.2, 71.0 (d, J_{CP} 143), 128.0, 128.6, 129.9, 131.9, 134.1, 137.7; *m*/*z* (FAB^+) 267 (27%, M − 1), 368 (M + $C_6H_{11}NH_3^+$), 187 (77, M − PO_3H_2).

(3-Phosphonophenyl)fluoromethylphosphonic acid (52)

Diethyl [3-(diethoxyphosphoryl)phosphonophenyl]fluoromethylphosphonate **49** (0.31 g, 0.82 mmol) was deprotected as for **2** with TMSBr (0.87 g, 5.71 mmol, 7 eq., 0.75 ml) and the free acid isolated as usual (0.25 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.17 g, 78%, mp >275 °C) (Found: C 48.4, H 7.3, N 5.95. $C_{19}H_{35}FN_2O_6P_2$ (salt) requires C 48.7, H 7.5, N 6.0%. Found: M^+ 370.098. $C_{13}H_{24}FNO_6P_2$ requires *M* 370.099); δ_H (D_2O) 1.15–2.00 (22 H, m, cyclohexyl-H), 5.70 (1 H, d, J_{HP} 7.9, J_{HF} 44.8, PCHF–), 7.25–7.50 (4 H, m, ArH); δ_P (D_2O) 11.0 (d, J_{FP} 75.8), 15.8 (s); δ_F (D_2O) −198.4 (d); H-coupled (dd, J_{FP} 75.0, J_{HF} 45.2); δ_C (D_2O) 23.7, 24.2, 30.3, 50.2, 92.5 (dd, J_{CP} 153, J_{CF} 176), 128.0, 128.2, 129.7, 129.9, 134.1, 136.5; *m*/*z* (FAB^+) 370 (72%, M + $C_6H_{11}NH_3^+$).

(3-Phosphonophenyl)chloromethylphosphonic acid (53)

Diethyl [3-(diethoxyphosphoryl)phenyl]chloromethylphosphonate **50** (0.16 g, 0.41 mmol) was deprotected as for **2** with TMSBr (0.44 g, 2.85 mmol, 7 eq., 0.38 ml) and the free acid isolated as usual (0.13 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (0.15 g, 79%, mp >275 °C) (Found: C 46.9, H 7.7, N 5.5, Cl 7.0. $C_{19}H_{35}ClN_2O_6P_2$ (salt) requires C 47.1, H 7.3, N 5.8, Cl 7.3%. Found: M^+ 386.086. $C_{13}H_{24}ClNO_6P_2$ requires *M* 386.069); δ_H ($CDCl_3$) 1.20–2.00 (22 H, m, cyclohexyl-H), 4.95 (1 H, d, J_{HP} 12.2, PCHCl–), 7.35–7.40 (1 H, m, ArH), 7.50–7.60 (3 H, m, 3 ArH); δ_P ($CDCl_3$) 13.0, 16.8 (2 s); δ_C ($CDCl_3$) 23.7, 24.2, 30.2, 50.3, 53.7 (d, J_{CP} 161), 126.3, 128.2, 130.9, 133.6, 134.0, 136.3; *m*/*z* (FAB^+) 386 (37%, M + $C_6H_{11}NH_3^+$), 253 (87, M − Cl).

Tetraethyl 2-methylenepropane-1,3-bisphosphonate (54)

Methallyl dichloride (2.00 g, 16.00 mmol) was placed in a dry flask under nitrogen and triethyl phosphite (5.32 g, 32.02 mmol, 5.90 ml) added. The reaction was heated at 120 °C for 17 h and the resultant pale yellow liquid purified by flash column chromatography from its monophosphonate to afford the *title compound* as a colourless liquid (2.10 g, 40%); R_f 0.12 (DCM–MeOH, 98:2) (Found: M^+ 329.129, $C_{12}H_{27}O_6P_2$ requires *M*

329.128); ν_{max}/cm^{-1} (film) 2909 (C–H), 1645 (C=C), 1250 (P=O), 1064 (P–O–C); δ_H ($CDCl_3$) 1.30 (12 H, t, *J* 6.2, 4 CH_3CH_2–), 2.70 (4 H, d, J_{HP} 18.5, 2 PCH_2–), 4.00–4.10 (8 H, m, 4 CH_3-CH_2–), 5.10 (2 H, t, *J* 5.5, >C=CH_2); δ_P ($CDCl_3$) 26.9 (s); δ_C ($CDCl_3$) 16.2, 16.3, 33.7 (d, J_{CP} 107), 61.8, 61.9, 119.4, 130.8; *m*/*z* (EI^+) 328 (30%, M^+), 191 (98, $M^+ - PO_3Et_2$), 163 (45, $M - PO_3Et_2 - C_2H_4$), 135 (38, $M^+ - PO_3Et_2 - 2C_2H_4$).

2-Methylenepropane-1,3-bisphosphonic acid (55)

Tetraethyl 2-methylenepropane-1,3-bisphosphonate **54** (1.69 g, 5.61 mmol) was deprotected as for **2** with TMSBr (5.23 g, 36.63 mmol, 4.80 ml) to yield the free acid as an off-white solid (0.91 g). Salt formation and purification as for **2** gave the biscyclohexylammonium salt of the *title compound* as a white solid (1.50 g, 81%, mp >275 °C); δ_H (D_2O) 2.55 (4 H, d, 2 PCH_2–, *J* 23.6), 4.95 (2 H, t, *J* 4.5, >C=CH_2); δ_P (D_2O) 20.4 (s); δ_C (D_2O) 31.3 (d, J_{CP} 132), 119.2, 136.7.

Biological experimental

1,3-Bisphospho-D-glyceric acid[47] (1)

D-Glyceraldehyde 3-phosphate (100 mg) in water (3 ml) was neutralised with 1 M NaOH. Phosphate solution (3.4 ml, 0.5 M) and NAD^+ solution (2.3 ml, 0.004 M) were added to this and the volume brought to 24 ml with water. GAPDH (300 units) and ADH (680 units) were added and then acetaldehyde (3 × 0.23 ml) in 3 aliquots after 0, 2, and 4 min. After 25 min at 18 °C, the reaction was acidified with H_2SO_4 (4 ml, 1 M) and poured immediately into acetone at 0 °C (300 ml). The 1,3-BPG precipitate was collected by centrifugation, washed once with cold acetone, and dissolved in cold water (18.7 ml). To this solution, a solution of strychnine hydrochloride (18.7 ml, 0.1 M) was added and the solution left in the cold room overnight to crystallise. The salt was collected by filtration, washed with cold water (3 ml) and then dissolved in cold water (10 ml). The strychnine was removed by extraction with cold chloroform (8 × 15 ml), with enough NaOH (0.2 M) added each time to keep the pH slightly alkaline.

The strychnine-free solution was placed in a vacuum desiccator over silica to remove dissolved chloroform and aliquots of the solution frozen and stored at −80 °C.

Isolation of 3-PGK[48]

Whole human blood (100 ml) was centrifuged at 15 krpm for 20 min at 0 °C and the supernatant removed. The red blood cells were washed twice with two volumes of physiological (0.9%) sodium chloride and two volumes of water added to haemolyse. The solution was dialysed against ten volumes of water for 3 h at 0 °C and again overnight. After this time, the solution was removed from the dialysis tubing and cooled to −60 °C. Ethanol–chloroform (2 : 1, 50 ml) was added at this temperature all at once and the pH adjusted to 7.0 with 1 M KOH. This solution was stirred in an ice-bath for 20 min and then centrifuged at 15 krpm for 20 min at 0 °C. The precipitate was discarded and 2.5 volumes of ethanol added slowly to the resulting solution at −25 °C. The temperature was held below 0 °C and after 20 min the precipitate was collected by centrifugation at 15 krpm for 15 min at 0 °C. The precipitate was dissolved in Na–K phosphate buffer (15 ml, 10 mM) and the solution dialysed overnight in the cold against the same buffer.

After analysis, glycerol was added to the buffered 3-PGK solution to give a 3-PGK solution in 40% glycerol and 60% buffer and this was stored at −20 °C.

Assay of PGK activity

The isolated enzyme solution was assayed[48] spectrophotometrically at high 3-PGA concentration to determine the activity. Three 1 ml cuvettes were taken and TRISCl solution (pH 7.4, 635 µl, 100 mM stock solution), magnesium sulfate (100 µl, 80 mM), and 3-PGA (100 µl, 60 mM) added. These were allowed to reach room temperature over 5 min (stock solutions kept at 0 °C) and then dithiothreitol (5 µl, 1.0 mM) and ATP (100 µl, 60 mM) added. After a further 5 min, NADH (20 µl, 12 mM), glyceraldehyde 3-phosphate dehydrogenase (20 µl, 100 u per ml) and the 3-PGK (20 µl) under test added. The cuvettes were immediately analysed in the spectrophotometer at 25 °C. From the rates of reaction the enzyme activity was calculated and found to be 12.5 u per ml.

Estimation of inhibitor activity

Each of the inhibitors under test (10 µl, 10 mM solutions) was used in duplicate in the above assay at a 3-PGA concentration chosen to give a V_{max} rate (100 µl, 6 mM solution) with 3-PGK (20 µl, 5 u per ml solution). These results were compared to the enzyme reaction in the absence of inhibitor and from these a percentage inhibition result calculated for each. From these results an estimated IC_{50} value was calculated for each.

Determination of K_i values for inhibitors

Solutions of inhibitor were made up at 0.3, 1.0, and 2.5 times the IC_{50} values determined for the inhibitor above. Solutions of 20, 50, 100, 150, 200, and 400 µM 3-PGA were also made up. These were used in duplicated 6 × 3 experiments in 36 one ml cuvettes in the back reaction assay.[48] TRISCl solution (pH 7.4, 625 µl, 100 mM stock solution), magnesium sulfate (100 µl, 80 mM), and 3-PGA (100 µl, 6 mM) were added. These were allowed to reach room temperature over 5 min and then dithiothreitol (5 µl, 1.0 mM) and ATP (100 µl, 60 mM) added. After a further 5 min, NADH (20 µl, 12 mM), glyceraldehyde 3-phosphate dehydrogenase (20 µl, 100 u per ml) and 3-PGK (20 µl, 5 u per ml) were added. The cuvettes were immediately analysed in the spectrophotometer at 25 °C. The results were analysed graphically using Origin™.

ATP-free adenosine diphosphate (ADP)[49]

Commercial ADP (100 mg) was dissolved in TRIS buffer (6 ml, 0.1 M, pH 7.4), potassium chloride (6 ml, 50 mM), magnesium acetate (6 ml, 10 mM), and glucose (6 ml, 0.16 mM). Hexokinase (25 units) was added to this solution and the reaction was incubated for 30 min at rt. The solution was stored at −20 °C and when needed 50 ml was acidified with HCl (50 ml, 1 M) and the pH brought to 7.5 with saturated TRIS buffer. Aliquots of the solution were frozen and stored at −20 °C.

Determination of IC_{50} values by forward reaction assay

For the forward reaction assay, triethanolamine (570 µl, 100 mM), water (335 ml), inhibitor (10 µl, 2, 20, or 200 µM), magnesium sulfate (20 µl, 80 mM) previously prepared ADP (35 µl, 3 mM), and Ap_5A (10 µl, 125 µM) added. 3-PGK (10 µl, 5 mu per ml) and 1,3-BPG (**1**, 10 µl, 200 µM) were added and the cuvette incubated at 25 °C for 8 min. After this time, the reaction was stopped by boiling at 70 °C for 4 min and the cuvettes stored at −20 °C for further analysis. The luminometer was calibrated with solutions of commercial ATP from 100 to 500 mM strength. In a 1 ml cuvette, potassium dihydrogen phosphate solution (333 µl, 10 mM), magnesium sulfate (333 µl, 80 mM), and sodium arsenate (333 µl, 100 mM) were placed. The ATP solution (10 µl) was added and, after mixing, the firefly luciferase/luciferin solution (30 µl) was added. This had been made up from the commercial vials by adding 10 ml cold water and centrifuging at 150 rpm for 10 min and was stored at 0 °C during the analysis then discarded. The cuvettes were unfrozen and a sample (10 µl) used in the luminometric analysis as detailed above. By comparison with the reaction performed without inhibitor, a % inhibition result could be calculated and hence the IC_{50} for each inhibitor.

Protocol for pH titrations

The bisphosphonic acid (15 mg) was dissolved in NaCl (5 ml, 0.152 M) and incubated in a water bath at 25 °C. The sample was adjusted to pH 11.5 (4 M NaOH) and titrated against HCl (0.5 M) across the pH range under study.

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