Prodrugs of Phosphonoformate: The Effect of *para*-Substituents on the Products, Kinetics and Mechanism of Hydrolysis of Dibenzyl Methoxycarbonylphosphonate

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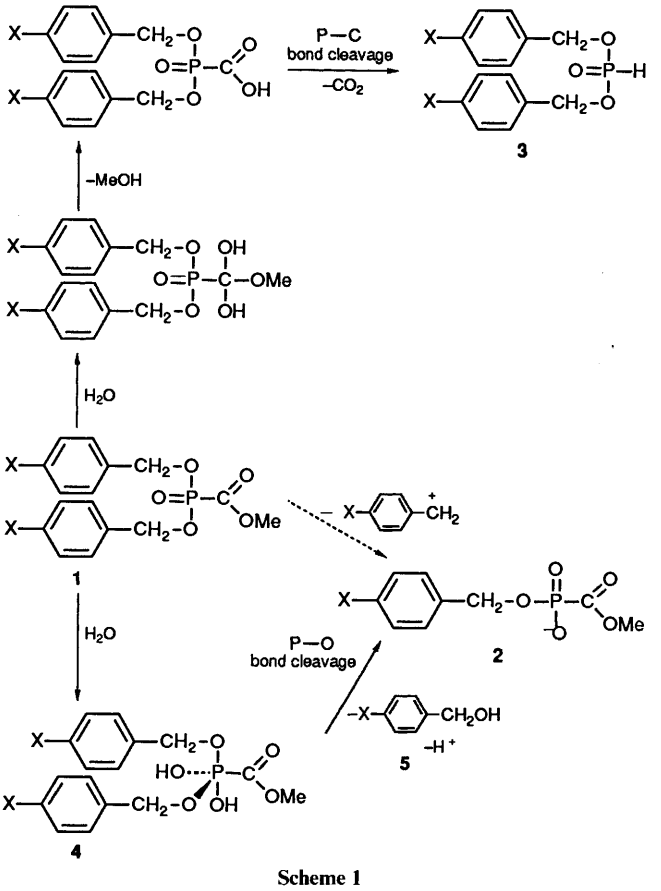
The *para*-substituted analogues of dibenzyl methoxycarbonylphosphonate (**1**; X = N_3, NO_2, CF_3) have been prepared in *ca.* 60% yield and their product and kinetic profiles of hydrolysis have been evaluated. Relative to the unsubstituted analogue **1** (X = H) which has a half-life of 64 min, the *para*-substituted triesters of phosphonoformate undergo rapid hydrolysis at pH 7.4 and 36.4 °C with half-lives of 2.5 min (X = N_3), 5.6 min (X = NO_2) and 15 min (X = CF_3). In contrast to **1** (X = H), which on hydrolysis gives *ca.* 30% C–P bond cleavage with dibenzyl phosphite formation, the *para*-substituted analogues hydrolyse predominantly to the *para*-substituted benzyl methoxycarbonylphosphonate diesters (**2**). Triester **1** (X = NO_2) hydrolyses to the diester **2** (X = NO_2) with P–O bond cleavage, consistent with nucleophilic attack of water at phosphorus and identical to that observed for the unsubstituted analogue. In contrast, triester **1** (X = N_3) hydrolyses to the diester **2** (X = N_3) with C–O bond cleavage presumably *via* an intermediate with benzyl carbonium ion character.

The effective delivery of a drug that contains a phospho group [$-PO(OH)_2$] as part of its structure continues to present a challenge in prodrug design. At physiological pH the phospho group is ionised and therefore the drug is only poorly transported across cell membranes and other physiological barriers, for example the blood-brain barrier.[1] The antiviral agent phosphonoformate (FOSCARNET) falls into this category.[2–4] A range of *P,P*-di(alkyl) triesters[5] and more recently *P,P*-di(acyloxymethyl) triesters[6] of phosphonoformate have been prepared as lipophilic prodrugs. These compounds do not show improved antiviral activity[5,6] and, in the case of the acyloxymethyl derivatives, unspecified 'hydrolytic instability' was given as a reason for their unsuitability as prodrugs.[6]

Benzyl esters of carbamates have been shown to be useful prodrugs for compounds containing an amino group.[7,8] As part of our interest in targeting antiviral agents containing a phospho group to the central nervous system we have extended this approach to phosphonoformate. Recently we reported the synthesis and hydrolytic fate of dibenzyl methoxycarbonylphosphonate (**1**; X = H), the unsubstituted dibenzyl triester of phosphonoformate.[9] The hydrolysis of **1** (X = H) was rapid with a half-life of 64 min at 36.4 °C and resulted in a complex product profile with benzyl methoxycarbonylphosphonate (**2**; X = H) and dibenzyl phosphite (**3**; X = H) as the major products. These products arise from competition between initial nucleophilic attack by water at phosphorus and at the carbonyl carbon resulting in P–O and P–C bond cleavage, respectively. There was no evidence to indicate C–O bond cleavage with concomitant formation of a benzyl carbonium ion intermediate (Scheme 1). These results are in agreement with the recent study of Krol *et al.* who reported that triesters of phosphonoformate were hydrolysed to a mixture of hydrogen phosphite and phosphonoformate esters.[10] Together, these studies have highlighted the overall hydrolytic instability of phosphonoformate triesters and the significant problem of hydrolytic P–C bond cleavage which results in the destruction of the parent drug structure.

An important goal in the design of useful phosphonoformate triester prodrugs is, therefore, to direct hydrolysis away from abortive P–C bond cleavage and towards P–O bond cleavage in which the integrity of the parent drug is maintained. In this report we describe the synthesis of a series of *para*-substituted dibenzyl methoxycarbonylphosphonates and report on the effect of the *para*-substituent on the products, kinetics and mechanism of hydrolysis.

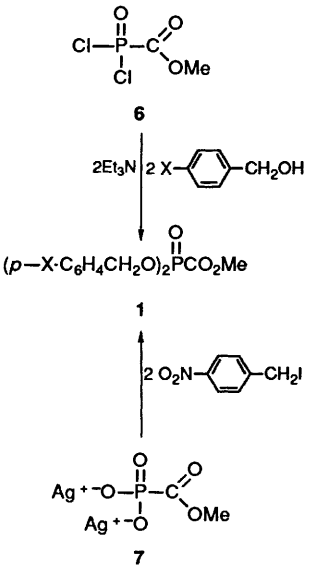


Scheme 1

Results and Discussion

The *P,P*-di(*para*-substituted benzyl) triesters of phosphono-

formate **1** (X = CF_3 and N_3) were readily prepared in *ca.* 60% yield from the reaction of two equivalents of the appropriate *para*-substituted benzyl alcohol **5** (X = CF_3 and N_3) with methoxycarbonylphosphonic dichloride[11] (**6**) in the presence of triethylamine. The *p*-nitrobenzyl triester **1** (X = NO_2) was prepared in 67% yield by the reaction of two equivalents of *p*-nitrobenzyl iodide[12] with the di-silver salt of methoxycarbonylphosphonate (**7**).[13] The triesters were readily purified by flash



column chromatography[14] with the exception of the *p*-azidobenzyl triester **1** (X = N_3), which degraded on silica. The di(*p*-methoxybenzyl) triester **1** (X = OMe) could not be prepared by either route. On treatment of **6** with *p*-methoxybenzyl alcohol the only product isolated by flash column chromatography was di(*p*-methoxybenzyl) ether, the structure of which was confirmed by comparison with an authentic sample prepared by the reaction of *p*-methoxybenzyl chloride with *p*-methoxybenzyl alcohol. Presumably, the ether arises by nucleophilic substitution of the *p*-methoxybenzyl alcohol on the benzyl carbon of either the triester product **1** (X = OMe) or the intermediate product after substitution of one chloride with the alcohol. The fact that the formation of the corresponding ethers was not observed during the synthesis of the triesters **1** (X = NO_2, CF_3 and N_3) suggests that the reaction proceeds by an 'S_N1-type' mechanism *via* a resonance-stabilised *p*-methoxybenzyl carbonium ion. To test this hypothesis the synthesis of di(*m*-methoxybenzyl) methoxycarbonylphosphonate was attempted. Comparison of the Hammett constants[15] for the *meta*-methoxy group (σ_m + 0.12) with that for the *para*-methoxy group (σ_p − 0.27) shows that the *meta*-methoxy group is electron-withdrawing and hence is unable to stabilise a benzyl carbonium ion. Therefore the *meta*-methoxybenzyl triester should be more stable, and in support of this, the reaction of two equivalents of *m*-methoxybenzyl alcohol with methoxycarbonylphosphonic dichloride (**6**) was found to give the appropriate triester in good yield. This result supports the involvement of a benzyl carbonium ion in the formation of di(*p*-methoxybenzyl) ether during the attempted synthesis of the triester **1** (X = OMe).

To assist in the identification of the hydrolysis products from the triesters, authentic samples of the *para*-substituted benzyl methoxycarbonylphosphonates **2** (X = NO_2, CF_3 and N_3) were prepared by the reaction of the appropriate triester **1** (X = NO_2, CF_3 and N_3) with sodium iodide.[16] The triesters and diesters were characterised by ^1H, ^{13}C and ^{31}P NMR spectroscopy, mass spectrometry and elemental analysis.

The triesters **1** (X = NO_2, CF_3 and N_3) were subjected to hydrolysis in potassium phosphate buffer (pH 7.4, 0.1 mol dm^{-3})–CD_3CN (1:1, v/v) at 36.4 °C and the reaction was monitored by ^{31}P NMR (^1H decoupled and coupled) spectroscopy. Hydrolysis of the *para*-nitrobenzyl triester **1** (X = NO_2) proceeded rapidly and after 14 min there was one major peak observed at δ_P −4.75 ppm (tq, J_{PH} 7.3, 0.9 Hz), corresponding to potassium *p*-nitrobenzyl methoxycarbonylphosphonate (**2**; X = NO_2), and four unknown minor peaks at δ_P 11.53, 5.94, 0.82 and −5.27 ppm, together with a minor peak at δ_P −3.54 ppm corresponding to unreacted triester. Comparison of the ^{31}P NMR (^1H coupled) spectra of the four unknown products with those spectra obtained from the products of hydrolysis of the unsubstituted dibenzyl methoxycarbonylphosphonate **1** (X = H)[9] allowed the hydrolysis products to be identified as di(*p*-nitrobenzyl) phosphite (**3**; X = NO_2) (δ_P 11.53 ppm, dpent, J_{PH} 733, 10 Hz), potassium *p*-nitrobenzyl phosphite (**8**; X = NO_2) (δ_P 5.94 ppm, dt, J_{PH} 629, 8.4 Hz), potassium *p*-nitrobenzyl *p*-nitrobenzyloxycarbonylphosphonate (**9**; X = NO_2) (δ_P −5.27 ppm, t, J_{PH} 7.9 Hz) and potassium di(*p*-nitrobenzyl) phosphate (**10**; X = NO_2) (δ_P 0.82 ppm). Inspection of the ^{31}P NMR after 24 h showed no new peaks.

Hydrolysis of the *para*-trifluoromethylbenzyl triester **1** (X = CF_3) proceeded more slowly than the nitro analogue and, after 45 min, there were three major peaks corresponding to unreacted triester **1** (X = CF_3) (δ_P −3.54 ppm), potassium *p*-trifluoromethylbenzyl methoxycarbonylphosphonate (**2**; X = CF_3) (δ_P −4.78 ppm, tq, J_{PH} 7.2, 0.9 Hz) and an unknown at δ_P 11.37 ppm which, when ^1H coupled, was identified as di(*p*-trifluoromethylbenzyl) phosphite (**3**; X = CF_3) (δ_P 11.37 ppm, dpent, J_{PH} 728, 9.9 Hz). Three minor peaks were observed at δ_P 5.87, −5.21 and 0.96 ppm which were identified as potassium *p*-trifluoromethylbenzyl phosphite (**8**; X = CF_3) (δ_P 5.87 ppm, dt, J_{PH} 627, 8.2 Hz), potassium *p*-trifluoromethylbenzyl *p*-trifluoromethylbenzyloxycarbonylphosphonate (**9**; X = CF_3) (δ_P −5.21 ppm, t, J_{PH} 7.0 Hz) and potassium di(*p*-trifluoromethylbenzyl) phosphate (**10**; X = CF_3) (δ_P 0.96 ppm, pent, J_{PH} 7.7 Hz). After 24 h no new peaks were observed.

Interestingly, the hydrolysis of the *para*-azidobenzyl triester **1** (X = N_3) was extremely rapid giving rise to one major peak corresponding to potassium *p*-azidobenzyl methoxycarbonylphosphonate (**2**; X = N_3) (δ_P −4.78 ppm, tq, J_{PH} 7.3, 0.9 Hz) after 5 min. There were also four minor products which were identified as di(*p*-azidobenzyl) phosphite (**3**; X = N_3) (δ_P 10.94 ppm, dpent, J_{PH} 730, 10 Hz), potassium *p*-azidobenzyl phosphite (**8**; X = N_3) (δ_P 5.85 ppm, dt, J_{PH} 620, 8.4 Hz), potassium *p*-azidobenzyl *p*-azidobenzyloxycarbonylphosphonate (**9**; X = N_3) (δ_P −5.09 ppm) and potassium di(*p*-azidobenzyl) phosphate (**10**; X = N_3) (δ_P 2.23 ppm, pent, J_{PH} 5.1 Hz).

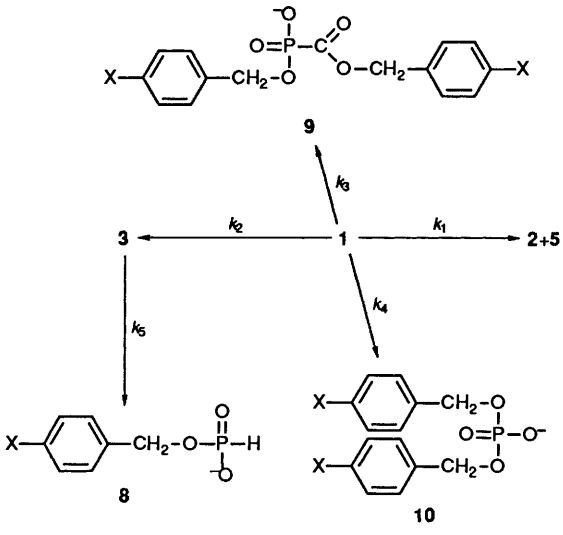
Inspection of the ^{31}P NMR spectra over 24 h showed that the products of hydrolysis for the *para*-substituted benzyl triesters **1** (X = NO_2, CF_3 and N_3) were similar and could be described by the pathways previously reported for the unsubstituted benzyl triester (Scheme 2), where k_1–k_5 are the first-order rate constants.[9] The rate constants, calculated as previously described using the ^{31}P NMR peak areas corrected for the differing response of the components,[9] are shown in Table 1. The rate constants for the simple unsubstituted benzyl triester **1** (X = H) are also shown for comparison.

Further insight into the mechanism of hydrolysis of the *p*-nitrobenzyl triester **1** (X = NO_2) was obtained by repeating the hydrolysis in an 80% enriched $H_2{}^{18}O$ sodium phosphate buffered water–acetonitrile mixture (1:1 v/v). After 4 h the reaction mixture was concentrated, *para*-nitrobenzyl alcohol (**5**; X = NO_2) was extracted with dichloromethane and the diester **2** (X = NO_2) with water. The diester **2** (X = NO_2) was

Table 1 First-order rate constants and half-lives for the hydrolysis of *para*-substituted benzyl triesters of phosphonoformate in phosphate buffer (pH 7.4, 0.1 mol dm^{-3})–CD_3CN (1:1, v/v)

Substituent X	First-order rate constants/10^{-2} min^{-1}							
	k_1	k_2	k_3	k_4	k_5	k_6[a]	k_1/k_2	$t_{\frac{1}{2}}$/min[b]
NO_2	10.4	1.44	0.485	0.133	1.53	12.5	7.22	5.6
CF_3	3.38	0.874	0.264	0.133	0.566	4.65	3.87	15
H[c]	0.656	0.355	0.036	0.042	0.090	1.18	1.85	64
N_3	26.9	0.314	0.321	0.349	0.010	27.9	85.6	2.5

[a] Overall rate constant for the hydrolysis of parent triester **1**, $k_6 = k_1 + k_2 + k_3 + k_4$. [b] Calculated half-life. [c] Rate data from reference 9.



Scheme 2

analysed by FAB mass spectrometry for the incorporation of ^{18}O-label and a comparison with unlabelled material showed peaks at 298 (^{16}O, 30%) and 300 (^{18}O, 70%) for an ion corresponding to sodium *p*-nitrobenzyl methoxycarbonylphosphonate plus a hydrogen cation. This result was confirmed by the ^{31}P NMR spectrum of the diester **2** (X = NO_2) which gave singlets at −4.292 (^{16}O, 20%) and −4.326 (^{18}O, 80%) ppm indicating the attachment of a single ^{18}O-label to phosphorus. In addition, the CI mass spectrum of the *p*-nitrobenzyl alcohol (**5**; X = NO_2) showed a molecular ion at *m/z* 171 ($^{16}O + NH_4^+$) indicating the absence of an ^{18}O-label in this molecule. These results are similar to those obtained previously for the unsubstituted dibenzyl methoxycarbonylphosphonate.[9] This suggests that the *p*-nitrobenzyl and unsubstituted benzyl triesters are hydrolysed by the same mechanism *via* P–O bond cleavage with nucleophilic substitution of water at phosphorus to give the diesters **2** (X = H and NO_2) (Scheme 1).

The rate constants k_1 and k_5 indicate the effect of the *para*-substituent on the rate of cleavage of the triesters **1** to the diesters **2** and of the *para*-substituted dibenzyl phosphites **3** to the benzyl phosphites **8**, respectively. The rate constants k_1 and k_5 increase in the order H < CF_3 < NO_2 indicating that the rate of benzyl cleavage increases as the electron-withdrawing ability of the *para*-substituent increases. We have previously suggested that the hydrolysis of the unsubstituted triester **1** (X = H) proceeds *via* the pentacoordinate intermediate **4** with subsequent loss of benzyl alcohol.[9] Therefore, the increased magnitude of k_1 and k_5 for the *para*-nitrobenzyl and *para*-trifluoromethylbenzyl triesters must either be a result of the increased electrophilicity at phosphorus or the enhanced leaving group ability of the *para*-substituted benzyloxy groups. In addition, comparison of k_1 with k_5 for X = H, NO_2 and CF_3 shows that k_1 is 6–7 times larger than k_5. This can be attributed to the electron-withdrawing effect of the methoxycarbonyl group in **1** which makes the phosphorus of the triesters more sensitive to nucleophilic attack than the phosphites **3**.

The rate constant k_2 indicates the influence of the *para*-substituent on the rate of P–C bond cleavage to the *para*-substituted dibenzyl phosphites **3** *via* nucleophilic attack of water at the carbonyl carbon (Scheme 1). The magnitude of k_2 increases in the order H < CF_3 < NO_2 indicating that the rate of P–C cleavage increases in parallel with the electron-withdrawing ability of the *para*-substituent. This result is consistent with the electron-withdrawing effect of the *para*-substituent enhancing the susceptibility of the carbonyl carbon to nucleophilic attack. These data show that as the electron-withdrawing ability of the *para*-substituent increases, both k_1 and k_2 increase. However, more significantly, the ratio of k_1/k_2 increases with the increasing electron-withdrawing ability of the *para*-substituent (Table 1). This indicates that electron-withdrawing substituents direct hydrolysis towards P–O bond cleavage and away from P–C bond cleavage. The increase in the ratio k_1/k_2 is accompanied by an increase in the overall rate constant, k_6, for hydrolysis of the parent triester **1**. Therefore, although hydrolysis can be diverted away from abortive P–C bond cleavage and towards P–O benzyl cleavage, the strategy is compromised by the increase in the rate of hydrolysis of the parent triester.

The *p*-azidobenzyl triester **1** (X = N_3) was studied because it possessed a *para*-substituent with mixed electronic properties. This triester was rapidly hydrolysed at a rate comparable to the *p*-nitrobenzyl triester **1** (X = NO_2) giving the diester **2** (X = N_3) as the major product. The mechanism of hydrolysis of the *p*-azidobenzyl triester **1** (X = N_3) was investigated using 80% enriched $H_2{}^{18}O$. FAB mass spectrometry of the diester **2** (X = N_3) showed peaks at *m/z* 294 (^{16}O, 96%) and 296 (^{18}O, 4%) and also at 316 (^{16}O, 96%) and 318 (^{18}O, 4%) corresponding to sodium *p*-azidobenzyl methoxycarbonylphosphonate plus a hydrogen cation and a sodium cation respectively. EI mass spectrometry of the *p*-azidobenzyl alcohol **5** (X = N_3) isolated from the reaction mixture showed peaks at *m/z* 149 (^{16}O, 26%) and 151 (^{18}O, 74%) indicating the incorporation of an ^{18}O-label into the alcohol. These results are in direct contrast to those obtained for the unsubstituted benzyl **1** (X = H) and *p*-nitrobenzyl **1** (X = NO_2) triesters. This result, coupled with the high value of k_1 for X = N_3, indicates a change in mechanism involving possible loss of the azidobenzyl group as a benzyl carbonium ion by C–O bond cleavage. This conclusion is supported by our recent observations on the bioactivation of di(*p*-acetoxybenzyl) methylphosphonate in which an intermediate with substantial *p*-hydroxybenzyl carbonium ion character was implicated.[17] The Hammett constant for the azido group (σ_p +0.08) lies close to that for hydrogen and, as such, the loss of benzyl alcohol from the *p*-azidobenzyl triester **1** (X = N_3) may have been expected to proceed by P–O bond cleavage. However, the σ_p^- constant which describes the

resonance contribution of the azido group to the stabilisation of a positive charge has a value of −0.54 suggesting a strong mesomeric electron-releasing effect.[18] This strong resonance effect would serve to stabilise a *p*-azidobenzyl carbonium ion and hence promote degradation of the triester *via* C–O bond cleavage. In support of this, Hoz *et al.* have recently shown that the azido group has the ability to stabilise an α-carbonium ion.[19] That it was not possible to synthesise the *p*-methoxybenzyl triester (**1**, X = OMe) [σ_p·(OMe) −0.78], but possible to synthesise the *p*-azidobenzyl triester (**1**, X = N_3) [σ_p·(N_3) −0.54] raises the question: at what point does the electron-donating ability of the *para*-substituent prevent the synthesis of a stable triester?

The primary aim of this study was to design lipophilic prodrugs of phosphonoformate which, unlike dibenzyl methoxycarbonylphosphonate (**1**; X = H), are resistant to P–C bond cleavage upon hydrolysis. This aim has been partially fulfilled as the hydrolysis of the electron-donating or electron-withdrawing *para*-substituted dibenzyl methoxycarbonylphosphonates **1** (X = N_3 and NO_2) proceeds mainly *via* the benzyl methoxycarbonylphosphonate diesters **2** (X = N_3 and NO_2) with minimal dibenzyl phosphite **3** (X = N_3 and NO_2) formation. Although hydrolysis can be diverted away from abortive P–C bond cleavage, the *para*-substituted triesters **1** (X = N_3 and NO_2) are, however, very unstable with half-lives of only 2.5 and 5.6 min, respectively. The difficulty in designing triesters of phosphonoformate which have both the required stability and product profile on hydrolysis suggests that triesters are unlikely to be suitable prodrug forms of phosphonoformate.

Experimental

^{1}H NMR spectra were recorded on a Varian EM-360 60 MHz spectrometer with tetramethylsilane (Me_4Si) as the reference; high resolution ^{1}H (300 and 250.1 MHz), ^{31}P (121.5 and 101.3 MHz) and ^{13}C (75.5 and 62.9 MHz) NMR were recorded on Bruker AC spectrometers. ^{31}P and ^{13}C spectra were referenced to 85% H_3PO_4 and ethylbenzene respectively: positive chemical shifts are downfield from the reference. ^{31}P and ^{13}C NMR were ^{1}H-decoupled (composite pulse decoupling) unless otherwise stated. All *J* values are quoted in Hz. Mass spectra were obtained on a V.G. Micromass 12 instrument at 70 eV and a source temperature of 300 °C; accurate mass data were obtained on a V.G. 7070E instrument under EI, CI(NH_3), and positive ion FAB (thioglycerol or nitrobenzyl alcohol matrix) techniques. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer. UV spectra were recorded on a Unicam SP 800 UV recording spectrophotometer. Melting points were measured on a Gallenkamp Electrothermal Digital apparatus and are not corrected. Flash column chromatography[14] was performed using Sorbsil C60 silica gel. TLC was performed using plastic-backed Kieselgel 60 silica gel plates containing a fluorescent indicator. Spots were visualised under 254 nm UV light or with the aid of iodine. All R_f values quoted were obtained by TLC using the same eluent as stated for flash chromatography. Elemental analyses were performed by Butterworths Laboratories, Middlesex. All chemicals were obtained from Aldrich Chemical Company. The following solvents were dried by heating under reflux followed by distillation over the appropriate drying reagent: dichloromethane (P_2O_5), acetone (4 Å molecular sieve), toluene (Na) and triethylamine (KOH). The phosphate buffer (0.1 mol dm^{-3}; pH 7.4) was prepared by mixing aqueous solutions of disodium (or dipotassium) hydrogen phosphate (0.2 mol dm^{-3}, 40.5 cm^3) and sodium (or potassium) dihydrogen phosphate (0.2 mol dm^{-3}, 9.5 cm^3), and the volume was adjusted to 100 cm^3 with water.

p-*Azidobenzyl Alcohol*[8] (**5**; X = N_3).—A solution of sodium nitrite (3.39 g, 0.049 mol) in water (30 cm^3) was added dropwise to a stirred solution of *p*-aminobenzyl alcohol (5.50 g, 0.045 mol) in 5 mol dm^{-3} HCl (200 cm^3) at 0–5 °C. After 30 min the presence of excess nitrosonium ion, NO^+, was checked with starch–iodide paper. Sodium azide (11.70 g, 0.18 mol) was added in portions over 30 min and stirring continued for a further 2 h. The mixture was added to iced water (100 cm^3), neutralised with 10 mol dm^{-3} NaOH (90 cm^3) and extracted with chloroform. The extracts were dried (Na_2SO_4) and concentrated to give **5** (X = N_3) as a dark orange solid (5.09 g, 0.03 mol, 74%), m.p. 26–28 °C; λ_{max}/nm($CHCl_3$) 251; ν/cm^{-1}(Nujol) 3305 (OH) and 2100 (N_3); δ_H($CDCl_3$; 60 MHz) 4.6 (2 H, s, CH_2), 6.8 (2 H, d, J_{HH} 8.0, Ar) and 7.15 (2 H, d, J_{HH} 8.0, Ar); *m*/*z* (EI) 149 ($M^{\cdot+}$, 42%), 122 (100), 91 (52), 28 (77).

Synthesis of Di(para-*substituted Benzyl*) *Triesters of Phosphonoformate* (**1**; X = CF_3 *and* N_3).—These were prepared from methoxycarbonylphosphonic dichloride (**6**) and the appropriate *para*-substituted benzyl alcohol as described previously for dibenzyl methoxycarbonylphosphonate.[9]

Di(*p*-azidobenzyl) methoxycarbonylphosphonate (**1**; X = N_3) was isolated without flash column chromatography as a brown solid (63%), m.p. 28–30 °C (Found: C, 47.5; H, 3.9; N, 20.7. $C_{16}H_{15}N_6O_5P$ requires C, 47.77; H, 3.76; N, 20.89%); λ_{max}/nm($CHCl_3$) 251 and 212; ν/cm^{-1}(thin film) 2100 (N_3), 1700 (C=O) and 1280 (P=O); δ_H($CDCl_3$; 300 MHz) 3.91 (3 H, s, OCH_3), 5.32 (4 H, d, J_{PH} 9.1, 2 × CH_2), 6.90 (4 H, d, J_{HH} 8.3, Ar) and 7.05 ppm (4 H, d, J_{HH} 8.5, Ar); δ_P(121.5 MHz) −4.1 ppm (s), (pent, J_{PH} 8.6, ^{1}H coupled); δ_C(62.9 MHz) 52.56 (d, J_{PC} 5.4, OCH_3), 69.04 (d, J_{PC} 6.3, CH_2), 118.92 (s, aromatic CH), 129.89 (s, aromatic CH), 131.53 (d, J_{PC} 6.4, aromatic C), 140.64 (s, aromatic C) and 166.60 ppm (d, J_{PC} 272.2, C=O); *m*/*z* (CI) 420 ($M + NH_4^+$, 13%), 375 (32), 149 (59) and 132 (100). Observed accurate mass 420.1187 ($M + NH_4^+$), $C_{16}H_{15}N_6O_5P{\cdot}NH_4^+$ requires 420.1185.

Di(*p*-trifluoromethylbenzyl) methoxycarbonylphosphonate (**1**; X = CF_3) was isolated by flash chromatography as a colourless oil. Traces of trifluoromethylbenzyl alcohol which co-eluted were removed by Kugel distillation at 90 °C (1 mm Hg) to give **1** (X = CF_3) (54%) (Found: C, 47.3; H, 3.3. $C_{18}H_{15}O_5PF_6$ requires C, 47.39; H, 3.31%); ν/cm^{-1}(thin film) 1725 (C=O) and 1325 cm^{-1} (P=O); δ_H($CDCl_3$; 300 MHz) 3.81 (3 H, s, OCH_3), 5.25 (4 H, d, J_{PH} 8.4, 2 × CH_2), 7.45 (4 H, d, J_{HH} 8.3, Ar) and 7.58 ppm (4 H, d, J_{HH} 8.3, Ar); δ_P(121.5 MHz) −3.9 ppm (s), (pent, J_{PH} 8.0, ^{1}H coupled); δ_C(75.5 MHz) 52.79 (d, J_{PC} *ca.* 6, OCH_3), 68.64 (d, J_{PC} 6.0, CH_2), 125.65 (s, aromatic CH), 128.32 (s, aromatic CH), 131.00 (q, J_{CF} 34.0, aromatic C) and 138.76 (d, J_{PC} *ca.* 6, aromatic C), carbonyl not observed; *m*/*z* (FAB; thioglycerol matrix) 457 ($M + H^+$, 40%), 297 (6), 159 (100), 140 (17), 109 (10) and 91 (4). Observed accurate mass 457.0639 ($M + H^+$), $C_{18}H_{16}F_6O_5P$ requires 457.0640.

Di(p-*nitrobenzyl*) *Methoxycarbonylphosphonate* (**1**; X = NO_2).—A solution of *p*-nitrobenzyl iodide[12] (0.38 g, 1.44 mmol) in toluene (25 cm^3) was added dropwise over 30 min to a stirred suspension of di-silver methoxycarbonylphosphonate (**7**) (0.27 g, 0.76 mmol) in toluene which was protected from light and under argon. After 24 h, the precipitate of silver iodide was removed by filtration and the filtrate washed with water (2 × 30 cm^3), dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography [EtOAc–hexane (2:1), R_f 0.60]. Recrystallisation from toluene–light petroleum (b.p. 60–80 °C) gave **1** (X = NO_2) as a colourless solid (0.21 g, 0.51 mmol, 67%); m.p. 58–62 °C (Found: C, 47.05; H, 3.75; N, 6.75. $C_{16}H_{15}N_2O_9P$ requires C, 46.83; H, 3.69; N, 6.83%); ν/cm^{-1}(Nujol) 1700 (C=O), 1520 (NO_2, asymmetrical), 1340 (NO_2, symmetrical) and 1280 (P=O); δ_H($CDCl_3$; 300 MHz) 3.92

(3 H, s, OCH_3), 5.47 (4 H, d, J_{PH} 9.1, 2 × CH_2), 7.96 (4 H, d, J_{HH} 8.3, Ar) and 8.13 ppm (4 H, d, J_{HH} 8.3, Ar); δ_P(101.3 MHz) −3.91 ppm (s), (pent q, J_{PH} 8.2, 1.0, ^{1}H coupled); δ_C(62.9 MHz) 52.95 (d, J_{PC} 5.5, OCH_3), 68.03 (d, J_{PC} 5.7, CH_2), 123.83 (s, aromatic CH), 128.17 (s, aromatic CH), 141.72 (d, J_{PC} 6.5, aromatic C) and 147.98 ppm (s, aromatic C), carbonyl not detected; *m/z* (CI) 428 (M + NH_4^+, 100%), 411 (3), 274 (6), 166 (98), 136 (50). Observed accurate mass 428.0859 (M + NH_4^+), $C_{16}H_{15}N_2O_9P{\cdot}NH_4^+$ requires 428.0859.

Attempted Synthesis of Di(p-*methoxybenzyl*) *Methoxycarbonylphosphonate* (**1**; X = OMe).—The synthesis of **1** (X = OMe) was attempted by the reaction of *p*-methoxybenzyl alcohol (**5**; X = OMe) with methoxycarbonylphosphonic dichloride (**6**) as described previously for **1** (X = H).[9] The triester could not be isolated and the only characterised product was di(*p*-methoxybenzyl) ether which was purified by flash column chromatography [EtOAc–hexane (1:1), R_f 0.70] as a colourless oil (42%); δ_H($CDCl_3$; 300 MHz) 3.79 (6 H, s, 2 × OCH_3), 4.46 (4 H, s, CH_2), 6.88 (4 H, d, J_{HH} 8.0, Ar) and 7.29 ppm (4 H, d, J_{HH} 8.0, Ar); δ_C(75.5 MHz) 46.24 (s, CH_2), 55.23 (s, OCH_3) and 113.72 (s, aromatic CH) 130.00 ppm (s, aromatic CH), aromatic C not detected; *m/z* (EI) 258 (M^+, 80%), 228 (100), 197 (57), 137 (60), 121 (33), 107 (49) and 91 (46).

Di(*m*-methoxybenzyl) methoxycarbonylphosphonate was prepared in a similar way to **1** (X = CF_3 and N_3) from *m*-methoxybenzyl alcohol and methoxycarbonylphosphonic dichloride (**6**). Flash column chromatography [EtOAc–hexane, (2:1), R_f 0.57] afforded the title compound as a colourless oil (Found: C, 56.3; H, 5.65. $C_{18}H_{21}O_7P$ requires C, 56.84; H, 5.57%); ν/cm^{-1}(thin film) 1720 (C=O) and 1280 (P=O); δ_H($CDCl_3$; 300 MHz) 3.78 (6 H, s, 2 × CH_3OAr), 3.80 (3 H, d, J_{PH} 0.9, $COOCH_3$), 5.19 (4 H, d, J_{PH} 8.3, 2 × CH_2) and 6.86–7.29 ppm (8 H, m, Ar); δ_P(101.3 MHz) −4.27 ppm (s), (pent q, J_{PH} 8.2, 1.1, ^{1}H coupled); *m/z* (FAB, thioglycerol matrix) 380 (M^+, 9%), 241 (100) and 121 (100). Observed accurate mass 380.1025 (M^+), $C_{18}H_{21}O_7P$ requires 380.1025.

Synthesis of Sodium para-*Substituted Benzyl Methoxycarbonylphosphonates* (**2**; X = NO_2, CF_3 *and* N_3).—These were prepared by treating the appropriate triester **1** (X = NO_2, CF_3 and N_3) with sodium iodide as previously described.[9]

Sodium *p*-azidobenzyl methoxycarbonylphosphonate (**2**; X = N_3) was obtained as a colourless solid (58%), m.p. 154–156 °C (Found: C, 36.7; H, 2.9; N, 14.4. $C_9H_9N_3O_5PNa$ requires C, 36.87; H, 3.09; N, 14.33%); λ_{max}/nm(water) 251 and 207sh; ν/cm^{-1}(Nujol) 2100 (N_3) and 1700 (C=O); δ_H(D_2O; 300 MHz) 3.53 (3 H, s, OCH_3), 4.88 (2 H, d, J_{PH} 9.6, CH_2), 7.05 (2 H, d, J_{HH} 8.3, Ar) and 7.39 ppm (2 H, d, J_{HH} 8.6, Ar); δ_P(121.5 MHz) −4.38 ppm (s), (tq, J_{PH} 8.0, 0.8, ^{1}H coupled); δ_C(75.5 MHz) 44.67 (d, J_{PC} *ca.* 6 Hz, OCH_3), 60.68 (d, J_{PC} *ca.* 6, CH_2), 111.89 (s, aromatic CH), 122.56 (s, aromatic CH), 126.25 (d, J_{PC} *ca.* 6, aromatic C) and 132.74 (s, aromatic C), carbonyl not detected; *m/z* (FAB, thioglycerol matrix) 316 (M + Na^+, 100%), 294 (M + H^+, 43), 245 (86), 185 (55), 125 (76) and 104 (26). Observed accurate mass 294.0256 (M + H^+), $C_9H_{10}N_3O_5PNa$ requires 294.0256.

Sodium *p*-trifluoromethylbenzyl methoxycarbonylphosphonate (**2**; X = CF_3). The reaction mixture was stirred for 2 h at room temperature to give **2** (X = CF_3) as a colourless solid (56%), m.p. >225 °C; ν/cm^{-1}(Nujol) 1700 (C=O); δ_H(D_2O; 300 MHz) 3.48 (3 H, s, OCH_3), 4.88 (2 H, d, J_{PH} 8.3, CH_2), 7.37 (2 H, d, J_{HH} 8.7, Ar) and 7.53 ppm (2 H, d, J_{HH} 8.5, Ar); δ_P(121.5 MHz) −4.3 ppm (s), (t, J_{PH} 9.0, ^{1}H coupled).

Sodium *p*-nitrobenzyl methoxycarbonylphosphonate (**2**; X = NO_2) was obtained as a colourless solid (73%), m.p. >300 °C (Found: C, 36.7; H, 3.2; N, 4.5. $C_9H_9NO_7PNa$ requires C, 36.55; H, 3.05; N, 4.70%); ν/cm^{-1}(Nujol) 1700 (C=O); δ_H(D_2O; 300 MHz) 3.62 (3 H, s, OCH_3), 5.01 (2 H, d, J_{PH} 9.8, CH_2), 7.91 (2 H, d, J_{HH} 8.3, Ar) and 8.07 ppm (2 H, d, J_{HH} 8.3, Ar); δ_P(121.5 MHz) −4.26 ppm (s), (tq, J_{PH} 8.7, 0.8, ^{1}H coupled); δ_C(75.5 MHz) 44.76 (s, OCH_3), 59.60 (d, J_{PC} *ca.* 6, CH_2), 116.45 (s, aromatic CH), 120.59 (s, aromatic CH), 137.67 (d, J_{PC} *ca.* 6, aromatic C) and 140.08 ppm (s, aromatic C), carbonyl not detected; *m/z* (FAB, thioglycerol matrix) 298 (M + H^+, 43%), 267 (4), 184 (84) and 115 (100). Observed accurate mass 298.0093 (M + H^+), $C_9H_{10}NO_7PNa$ requires 298.0093.

^{18}O *Hydrolysis Studies with the Triesters of Phosphonoformate* **1** (X = NO_2 *and* N_3).—A solution of the triester (15 mg) in dry acetonitrile (1.0 cm^3) was added to 80% [^{18}O], 20% [^{16}O] water (1.0 cm^3) buffered with NaH_2PO_4/Na_2HPO_4 (0.002 85 g/0.0138 g, pH 7.4) and left to stand in the dark for 4 h. The solution was concentrated to dryness. The *para*-substituted benzyl alcohol (**5**) was extracted into dichloromethane (3 cm^3) and the diester salt **2** (X = N_3, NO_2) into water (3 cm^3). The dichloromethane extract was dried (Na_2SO_4), concentrated and the benzyl alcohol analysed for ^{18}O incorporation by mass spectrometry (CI and EI). The aqueous fraction containing the diester salt was concentrated on a freeze-drier and then analysed by ^{31}P NMR spectroscopy and/or FAB (thioglycerol matrix) mass spectrometry.

Hydrolysis of the Triesters of Phosphonoformate **1** (X = NO_2, N_3 *and* CF_3) *Monitored by* ^{31}P *NMR Spectroscopy*.—The triesters (15 μmol) were dissolved in CD_3CN (0.5 cm^3) and their ^{31}P NMR (101.3 MHz) spectra recorded with the NMR probe at 36.4 °C [**1** (X = NO_2), δ_P −3.54 ppm (s); **1** (X = N_3), δ_P −3.71 ppm (s); **1** (X = CF_3), δ_P −3.60 ppm (s)]. Potassium phosphate buffer (0.1 mol dm^{-3}, pH 7.4, 0.5 cm^3, pre-equilibrated at 37 °C) was added to initiate the hydrolysis and the ^{31}P NMR spectra were recorded at regular time intervals using a sweep width of 2702 Hz, a data block size of 32K, an acquisition time of 3.03 s, 80 scans and 3.3 data points Hz^{-1}. Each FID was transformed with a line broadening of 1.0 Hz and the spectrum plotted on a wide expansion. The area of each peak was determined from its width and height. The areas for each component were corrected for peak response using the factors measured previously for the hydrolysis products of dibenzyl methoxycarbonylphosphonate (**1**; X = H).[9]

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