Pergamon

Tetrahedron, Vol. 52, No. 47, pp. 14937-14950, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00906-4

Acyloxymethyl and 4-Acyloxybenzyl Diester Prodrugs of Phosphonoformate

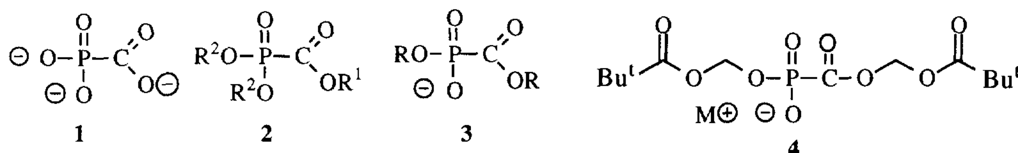
Andrew D. Briggs,[a] Michel Camplo,[a] Sally Freeman,[a*] Jan Lundström[b] and Brian G. Pring[b]

[a]Department of Pharmacy, University of Manchester, Oxford Road, Manchester, M13 9PL, U.K.

[b]Astra Arcus AB, Forskargatan 20, S-151 85 Södertälje, Sweden.

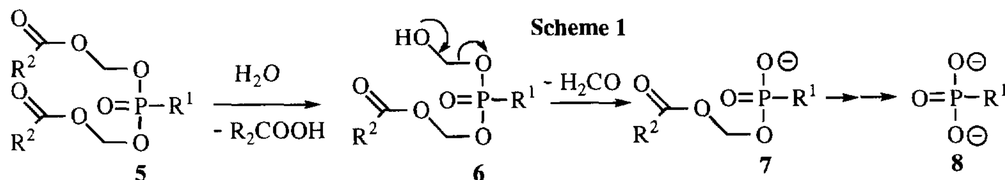
Abstract: Sodium pivaloyloxymethyl (pivaloyloxymethoxycarbonyl)phosphonate **4**, sodium 4-acyloxybenzyl phenoxycarbonylphosphonates **14a-c** and sodium 4-acyloxybenzyl benzyloxycarbonylphosphonates **15a,b** have been prepared as bioreversible prodrugs of the antiviral phosphonoformate **1**. Their hydrolyses, *in vivo* systemic bioavailability and antiviral activity are reported. Of the compounds evaluated **4** was the best prodrug. Copyright © 1996 Elsevier Science Ltd

Trisodium phosphonoformate (foscarnet, Foscavir®, **1**) is a broad spectrum antiviral agent with activity against herpes simplex virus (HSV), human immunodeficiency virus (HIV) and cytomegalovirus (CMV), which is a prevalent opportunistic infection in AIDS patients.[1] It is approved as an intravenous drug for the treatment of CMV retinitis in AIDS patients and for the therapy of acyclovir-unresponsive HSV in AIDS patients. However, due to its highly ionic nature **1** shows poor penetration into cells and intravenous doses of 200 mg/kg/day are required to achieve a therapeutic effect.[1] An oral formulation with improved transport properties would be of significant benefit, and here prodrugs of **1** are considered towards this goal. Simple alkyl triesters **2** and diesters **3** have previously been prepared. However apart from the phenyl analogues, they were not active against HSV, presumably because of the lack of hydrolysis to **1**.[2] Attention was focused on bioreversible prodrugs of **1**.



Acyloxymethyl esters are the most promising bioreversible prodrug modifications for phosphates and phosphonates: The first study by Farquhar and coworkers[3,4] entailed the preparation of bis(acyloxymethyl) esters of phenyl and benzyl phosphate **5** (R^1 = OPh, OBn, R^2 = Me, Pr^i, Bu^t). After cell penetration, the triesters are designed to undergo esterase-catalysed cleavage of one acyloxymethyl ester to give the hydroxymethyl analogue **6**. This intermediate is labile and spontaneously eliminates formaldehyde to give diester **7**. A repeat of this bioactivation gives phenyl or benzyl phosphate **8** (R^1 = OPh, OBn) (Scheme 1).

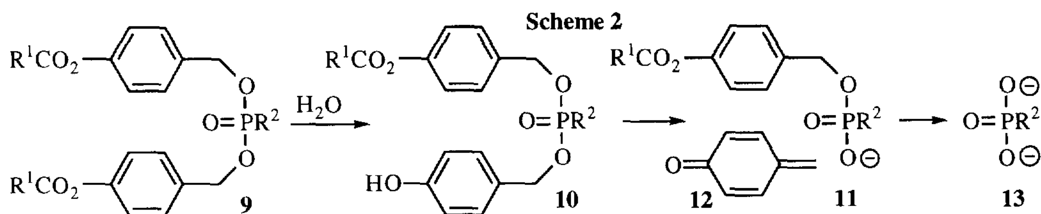
Scheme 1



This approach has been extended to a range of anticancer and antiviral nucleotides, including ddU 5'-monophosphate,[5] FdU 5'-monophosphate,[6] AZT 5'-monophosphate,[7] N^6,2'-O-dibutyryl cAMP,[8] PMEA[9-11] and 9-[2-(phosphonomethoxy)ethoxy]adenine,[12] D-*myo*-inositol 3,4,5,6-tetrakisphosphate[13] and oligonucleotides.[14,15] The acyloxymethyl group has also been incorporated into prodrugs of phosphonoformate, with the synthesis of triesters **5** (R^2 = Me, Pr^i, Bu^t; R^1 = $-CO_2Me$, $-CO_2Et$, $-CO_2Ph$, $-CO_2C_6H_3Cl_2$).[16,17] However, their instability prevented them from being suitable prodrug forms. In contrast, diesters of phosphonoformate are more stable towards chemical hydrolysis and here attention focused on the bis(pivaloyloxymethyl) diester **4** bearing a bioreversible group on the carboxyester. Although some success was achieved, this compound proved difficult to prepare, and attention was turned to other biolabile modifications.

The 4-acyloxybenzyl ester has been developed by our group as an alternative bioreversible protecting group: The bis(4-acetoxybenzyl) ester of methylphosphonate **9**[18] (R^1 = R^2 = Me) hydrolyses in the presence of porcine liver carboxyesterase (PLCE), to give intermediate **10**, the electron donating 4-hydroxybenzyl group of which promotes C-O bond cleavage leading to the formation of the monoester **11** and quinone methide **12** (which ultimately gives 4-hydroxybenzyl alcohol). Hydrolysis of the second 4-acetoxybenzyl group was slower, but formation of methylphosphonate **12** (R^2 = Me) was observed (Scheme 2).

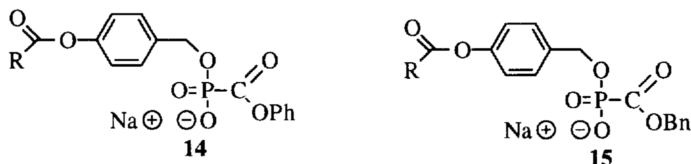
Scheme 2



The 4-acyloxybenzyl group has been incorporated into prodrugs of the antiviral, phosphonoacetate **9** (R^2 = MeO_2CCH_2, R^1 = Me, Et, Pr, Bu, Pr^i, Bu^t),[19] AZT 5'-monophosphate **9** (R^2 = 5'-AZT)[20] and 5',5'-nucleotide dimers containing AZT.[21] In related studies, a range of substituted benzyl esters of the 5',5'-nucleotide dimer[22] and prodrugs of methylphosphonate and 5'-monophosphate of AZT bearing a substituted benzyl group, which upon bioactivation liberates ethyl 4-hydroxycinnamate,[23,24] have been prepared and evaluated. Meier has also reported cyclic prodrugs of nucleotide monophosphates utilising salicyl alcohols.[25]

The initial intention was to prepare *P,P*-bis(4-acyloxybenzyl) triesters of phosphonoformate. However studies with the unsubstituted dibenzyl analogue **2** (R^1 = Me, R^2 = Bn) showed that this triester was unstable towards chemical hydrolysis with a half-life of 1 h at physiological pH and temperature.[26] Decomposition to a complex set of products was observed, some in which the P-C bond had been cleaved, making triesters of phosphonoformate unsuitable prodrug modifications.[26-29] Here attention focused on diesters: previously phosphonoformate has been coupled to a variety of nucleosides[30-33] and tyrosine.[34] However, the drug was

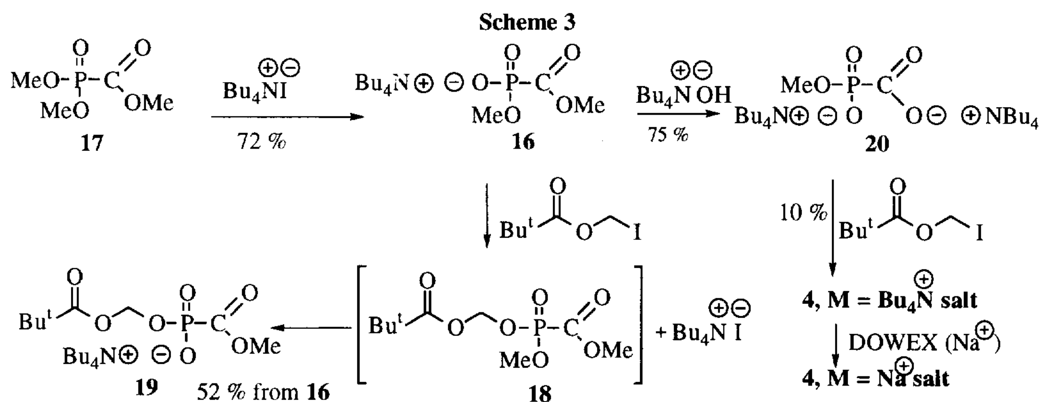
not released from these derivatives. Most recently a lipid prodrug of phosphonoformate has been reported, which showed enhanced antiviral activity *in vitro*.[35] Bioreversible *P*-mono(4-acyloxybenzyl) esters of phosphonoformate with phenyl **14** and benzyl **15** groups on the carboxyester are reported here.



Results and Discussion

Acyloxymethyl esters are typically prepared by the alkylation of a phosphate or phosphonate anion with a halomethyl acylate, usually the iodo derivative. The chemistry to prepare diester **4** was first developed using a model system. Tetra-*n*-butylammonium methyl methoxycarbonylphosphonate **16,** prepared by the reaction of dimethyl methoxycarbonylphosphonate **17** with tetra-*n*-butylammonium iodide, was treated with iodomethyl pivalate. The expected product, triester **18**, was not observed, and the isolated product was the desired diester **19**, the formation of which can be rationalised by the demethylation of **18** with the by-product, tetra-*n*-butylammonium iodide (Scheme 3). This model chemistry provides a short route to diester **4** by the alkylation of methyl bis(tetra-*n*-butylammonium) carboxyphosphonate **20,** which was prepared by the reaction of **16** with tetra-*n*-butylammonium hydroxide. Alkylation of **20** with iodomethyl pivalate gave the tetra-*n*-butylammonium salt of **4**, however it was difficult to purify and was only isolated in low yield. Passage of the tetra-*n*-butylammonium salt of **4** through a DOWEX-50 (Na^+ form) cation exchange column gave the sodium salt (Scheme 3). Difficulties in purification meant that the preparation of larger quantities of **4** was unsuccessful, and attention was switched to the 4-acyloxybenzyl derivatives **14** and **15,** which do not require alkylation chemistry for their construction.

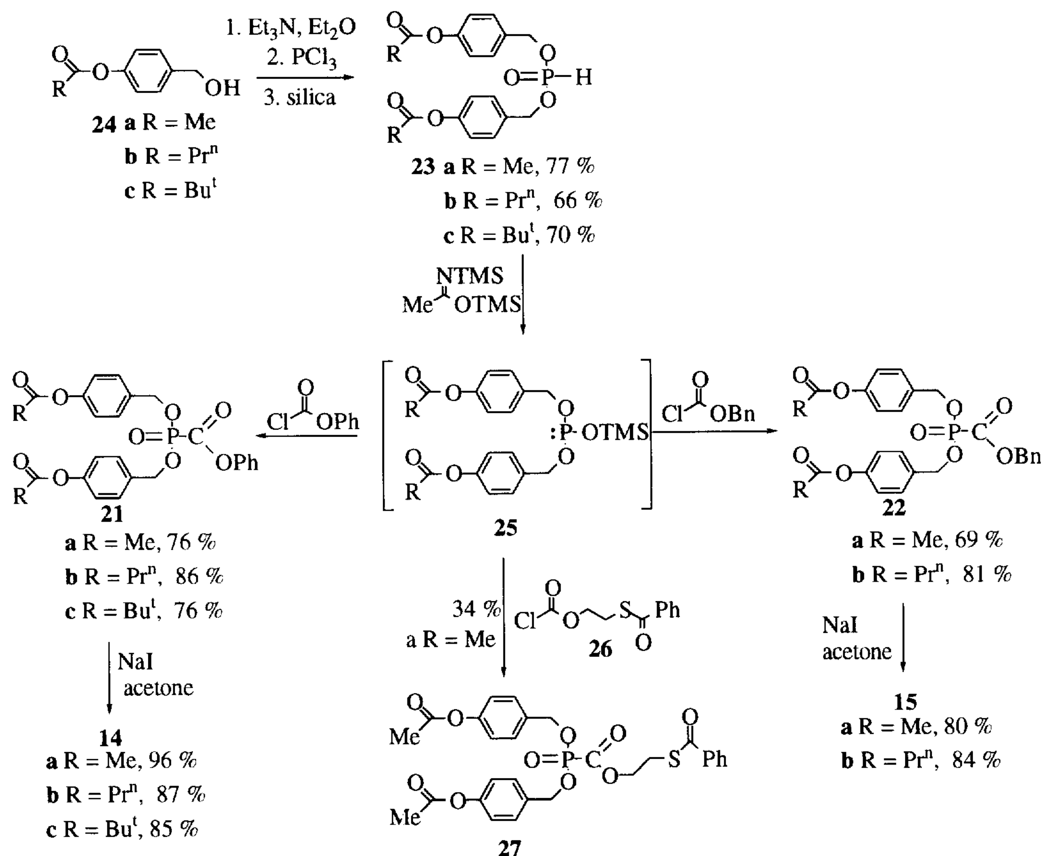
Scheme 3



The preparation of 4-acyloxybenzyl diesters **14** and **15** is best approached by the monodebenzylation of the corresponding triesters **21** and **22**, our first targets. In a previous study, the unsubstituted dibenzyl analogue **2** (R^1 = Me, R^2 = Bn) was prepared by reaction of benzyl alcohol with methoxycarbonylphosphonic dichloride.[25] In our experience, it has not been possible to prepare the phosphonic dichloride derivative with groups other than a short alkyl (Me, Et) substituent on the carboxyester; therefore we focused on the syntheses

of **21** and **22** using Perkov chemistry. The di(4-acyloxybenzyl) phosphites **23a-c** were prepared by reaction of the corresponding 4-acyloxybenzyl alcohols **24a-c** with PCl_3, followed by flash column chromatography on silica (Scheme 4). Any tris(4-acyloxybenzyl) phosphite or P-Cl intermediate formed was hydrolysed by water present in the silica. The diphosphites **23a-c** were silylated using *N,O*-bis(trimethylsilyl)acetamide to give the trisphosphites **25a-c**. Without isolation, these were reacted with phenyl chloroformate to give di(4-acyloxybenzyl) phenoxycarbonylphosphonates **21a-c** (Scheme 4).

Scheme 4



The intention was to prepare prodrugs of phosphonoformate, bearing a bioreversible 4-acyloxybenzyl group on the carboxyester. Towards this goal, model triesters **22a,b** were prepared in good yields by reaction of **25** with benzyl chloroformate (Scheme 4). 4-Acetoxybenzyl chloroformate was then prepared from 4-acetoxybenzyl alcohol and phosgene, and although its NMR spectra were consistent with the required chloroformate, surprisingly it failed to react with trisphosphite **25**. In an attempt to obtain a prodrug of phosphonoformate bearing a bioreversible carboxyester group, 2-(*S*-benzoylthio)ethyl chloroformate **26**, prepared from 2-(*S*-benzoylthio)ethanol and phosgene, was reacted with **25** to give di(4-acetoxybenzyl) [2-(*S*-benzoylthio)ethoxycarbonyl]phosphonate **27** (Scheme 4).

Diesters **14** and **15** were prepared in good yields by the reaction of triesters **21** and **22** with NaI.[2]

Hydrolysis and Antiviral Activity

The *in vitro* hydrolyses of the triesters and diesters of phosphonoformate were conducted in homogenates of rat liver and rat intestine as single determinations. HPLC was used to selectively detect the formation of phosphonoformate, and the results are given in Table 1. Only low levels of phosphonoformate were released from triesters **21a,b** and **22a,b** in both liver and intestine. It is interesting that hydrolysis of triester **27**, with the 2-(*S*-benzoylthio)ethyl carboxyester, showed no release of phosphonoformate (above the detection level). These results are not unexpected, principally because the hydrolyses of triesters of phosphonoformate are complex.[26-29]

Table 1 Phosphonoformate release (%) from the *in vitro* hydrolysis of phosphonoformate triesters and diesters in rat tissue.

Analogue	Tissue	0 min	5 min	15 min	60 min	240 min
4 (Na^+ salt)	liver	<1	2	7	34	50
4 (Na^+ salt)	intestine	2	2.5	5	21	43
14a	liver	7.5	7.5	7.5	8	7.5
14a	intestine	<4	<4	<4	<4	4.5
14b	liver	7	11	10	11	10
14b	intestine	<3	<3	<3	<3	7.5
14c	liver	<1	2	6	23	25
14c	intestine	<3	<3	3	11	20.5
15a	liver	6	11	9	6.5	8
15a	intestine	<3	<3	<3	<3	3.5
15b	liver	12	3	29.5	32	30
15b	intestine	<3	<3	<3	<3	8
21a	liver	5.5	5	5	7	6.5
21a	intestine	<4	4.5	4.5	4	<4
21b	liver	1	5.5	7	8	11
21b	intestine	<3	<3	3	4	8
22a	liver	2	3	3.5	3.5	3
22a	intestine	<3	<3	4	3.5	7
22b	liver	1	6	8	12	13.5
22b	intestine	<3	<3	<3	<3	5
27	liver	<1	<1	<1	<1	<1
27	intestine	<3	<3	<3	<3	<3

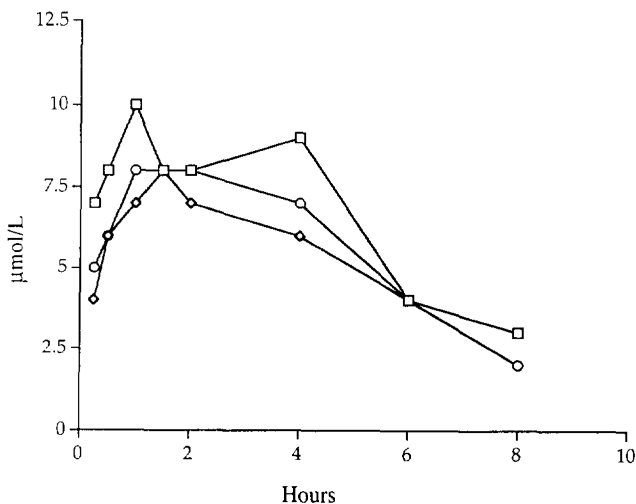
The study was designed to evaluate diesters of phosphonoformate, and the 4-acyloxybenzyl diesters **14c** and **15b** showed an increased release of phosphonoformate, with 25 and 30% formed, respectively, after 4 h in rat liver homogenate. By far the greatest release of phosphonoformate was observed with the pivaloyloxymethyl analogue **4**, with 50% conversion after 4 h in rat liver homogenate.

The hydrolysis of diester **14b** was also monitored by ^1H and ^{31}P NMR spectroscopy in a methylphosphonate buffer (0.1 M, pD 8.0) at 37 °C. In the absence of PLCE, **14b** hydrolysed with a half-life of ~12 h, the principal route (~90 %) of hydrolysis being cleavage of the phenyl carboxyester to give 4-

butanoyloxybenzyl disodium carboxyphosphonate. Some hydrolysis of the 4-butanoyloxybenzyl group was also observed. In the presence of a high concentration of PLCE (170 units), hydrolysis of the 4-butanoyloxybenzyl group occurred rapidly and the only phosphorus containing product was disodium phenoxycarbonylphosphonate, which did not cleave to phosphonoformate. With ten-fold less enzyme, 80% disodium phenoxycarbonylphosphonate was formed. The other 20% of product was 4-butanoyloxybenzyl disodium carboxyphosphonate which arose from competing chemical hydrolysis of **14b** and its slow esterase-catalysed hydrolysis to phosphonoformate **1** was observed.

The tetra-*n*-butylammonium and sodium salts of **4** and diester **14a** were also tested in rats *in vivo* by oral administration at doses of 180 μmol/kg. Phosphonoformate was detected in plasma of the rats administered with either salt of **4**, however with **14a**, only trace quantities of phosphonoformate were observed. The systemic availability of phosphonoformate following oral administration of the tetra-*n*-butylammonium and sodium salts of **4** could be estimated at 25% (data shown for sodium salt, Figure 1) by comparison with plasma levels obtained after intravenous administration of 180 μmol/kg of phosphonoformate. Following oral administration of the trisodium salt of phosphonoformate to rats, the degree of absorption has been estimated at 20 - 30%.[36]

Figure 1 Plasma levels of phosphonoformate after oral administration of **4** (sodium salt) to three male rats.



The antiviral activity of the phosphonoformate diesters against HSV-1 was measured in human lung fibroblast cell culture as single determinations. The sodium salt of **4** gave an IC_{50} value of 295 μM, being ~4-fold less active than phosphonoformate (IC_{50} 80 μM) and cell toxicity was observed at 400 μM. Diester **14a** had an IC_{50} of 343 μM, showing slightly lower activity than phosphonoformate (IC_{50} 173 μM). Some of the diesters were more active than phosphonoformate (IC_{50} value of 227 μM in this series of tests): **14b** (IC_{50} 108 μM), **14c** (IC_{50} 287 μM), **15a** (IC_{50} 196 μM) and **15b** (IC_{50} 126 μM).

In summary, based upon *in vitro* hydrolysis studies and *in vivo* oral bioavailability, the acyloxymethyl ester **4** was the best prodrug of the compounds prepared. However, the synthesis of this analogue proceeded in a low yield and it was difficult to purify. In contrast, the 4-acyloxybenzyl diesters **14a-c** and **15a,b** were

readily prepared in high yield. However, only a maximum of ~30% phosphonoformate was released during *in vitro* hydrolysis studies and little phosphonoformate was detected in plasma following oral administration of **14a** to rats. NMR studies on the hydrolysis of **14b** suggest that release of phosphonoformate from such analogues first requires chemical hydrolysis of a labile carboxyester, prior to the esterase-catalysed hydrolysis of the benzyl phosphonate ester.

Acknowledgements: We thank Astra Arcus AB, Södertälje, Sweden for financial support. The mass spectra and elemental analyses were recorded at the Department of Chemistry, University of Manchester. Antiviral testing was carried out at the Swedish Institute for Infectious Disease Control, Stockholm.

Experimental section

NMR spectra were recorded on a Jeol EX-270 MHz spectrometer at ^{1}H (270.0 MHz), ^{31}P (109.2 MHz) and ^{13}C (67.8 MHz). ^{1}H and ^{13}C (^{1}H decoupled) NMR spectra were referenced to tetramethylsilane at 0 ppm unless otherwise stated, and ^{31}P NMR spectra were referenced to 85 % H_3PO_4 at 0 ppm. J-values are given in Hz. Mass spectra were recorded on a Kratos Concept 1-S mass spectrometer using xenon as a carrier gas and 3-nitrobenzyl alcohol as a matrix for FAB, or a Fisons VG Trio spectrometer for CI (ammonia carrier gas). Elemental analysis gave poor results (apart from **14b**) which can be attributed to the triesters of phosphonoformate being thick oils which retained traces of solvent, and the salts of the diesters of phosphonoformate being hygroscopic. Melting points were measured on a Gallenkamp digital capillary apparatus. Flash column chromatography was performed using Sorbsil C60 40/60H silica gel. TLC was performed using Merck aluminium backed silica gel 60 plates containing a fluorescent indicator. Spots were visualised under 254 nm UV light or with a phosphomolybdic acid or molybdic acid dip. The following solvents were purified by heating under reflux, followed by distillation over the appropriate drying reagent: dichloromethane (P_2O_5), THF (Na / benzophenone), Et_3N (CaH_2), toluene (Na / benzophenone), acetone (B_2O_3) and Et_2O (Na / benzophenone). PCl_3 was distilled prior to use. All chemicals were obtained from Aldrich Chemical Company. Porcine liver carboxyesterase (PLCE), as a suspension in ammonium sulphate, was obtained from Sigma Chemical Company: One unit of esterase will hydrolyse 1 µmol of ethyl butyrate to butyric acid and ethanol per minute at pH 8.0 at 25°C. The 4-acyloxybenzyl alcohols were prepared from the appropriate acyl anhydride and 4-hydroxybenzyl alcohol.

Tetra-*n*-butylammonium methyl methoxycarbonylphosphonate (16). To dimethyl methoxycarbonylphosphonate (370 mg, 2.8 mmol) in dry THF (10 mL), tetra-*n*-butylammonium iodide (1.0 g, 2.8 mmol) was added and stirred at room temp. for 24 h. The mixture was filtered and the filtrate concentrated under reduced pressure to give **16** (0.8 g, 72 %). - ^{1}H NMR (D_2O, referenced to HOD at 4.7 ppm): δ 0.8 (t, J = 7.3, 12H, 4 x CH_3CH_2), 1.21 (sextet, J = 7.3, 8H, 4 x CH_2CH_3), 1.50 (pentet, J = 8.2, 8H, 4 x CH_2CH_2N), 3.05 (br.t, J = 8.6, 8H, 4 x NCH_2), 3.52 (d, $J_{P,H}$ = 10.9, 3H, $POCH_3$), 3.64 (s, 3H, $COCH_3$). - ^{13}C NMR ($CDCl_3$): δ 13.3 (s, 4C, 4 x CH_3), 19.3 (s, 4C, 4 x CH_3CH_2), 23.6 (s, 4C, 4 x CH_2CH_2N), 50.0 (d, $J_{P,C}$ = 3.7, 1C, $COOCH_3$), 52.6 (d, $J_{P,C}$ = 6.1, 1C, $POCH_3$), 58.2 (s, 4C, 4 x CH_2N), 173.2 (d, $J_{P,C}$ = 238.0, 1C, C=O). - ^{31}P NMR (D_2O): δ -5.48 (q, $J_{P,H}$ = 10.0, ^{1}H coupled). - IR (KBr): υ_{max} 1050, 1080, 1700. - MS (FAB^-): m/z 152.9954 (M^-), $C_3H_6O_5P$ requires 152.9953.

Methyl bis(tetra-*n*-butylammonium) carboxyphosphonate (20). To **16** (100 mg, 0.25 mmol) in water (2 mL) at room temp., tetra-*n*-butylammonium hydroxide (162 mL, 0.25 mmol) was added and stirred for 16 h. Water was removed by freeze drying to give **20** (116 mg, 75 %). - ^{1}H NMR ($CDCl_3$): δ 0.98 (t, *J* = 6.9, 12H, 4 x CH_3CH_2), 1.45 (sextet, *J* = 7.3, 8H, 4 x CH_3CH_2), 1.61 (pentet, *J* = 7.3, 8H, 4 x NCH_2CH_2), 3.35 (br t, *J* = 7.9, 8H, 4 x NCH_2), 3.66 (3H, d, $J_{P,H}$ = 9.9, $POCH_3$). - ^{13}C NMR ($CDCl_3$): δ 13.6 (s, 4C, 4 x CH_3CH_2), 19.5 (s, 4C, CH_3CH_2), 24.0 (s, 4C, 4 x NCH_2CH_2), 51.8 (d, $J_{P,C}$ = 4.9, 1C, $COOCH_3$), 58.3 (s, 4C, 4 x NCH_2), 177.7 (d, $J_{P,C}$ = 222.0, 1C, *C*=O). - ^{31}P NMR ($CDCl_3$): δ 2.84 (q, $J_{P,H}$ = 10.2, ^{1}H coupled). - MS (FAB$^-$): m/z 138.9798 (M$^-$), $C_2H_3O_5P$ requires 138.9796.

Tetra-*n*-butylammonium pivaloyloxymethyl methoxycarbonylphosphonate (19). To iodomethyl pivalate[6] (115 mg, 0.75 mmol) in dry toluene (4 mL) at room temp., **16** (200 mg, 0.5 mmol) was added and stirred for 16 h. Tetra-*n*-butylammonium iodide was removed by filtration, the filtrate was concentrated under reduced pressure and purified by preparative layer chromatography, eluting with ethyl acetate, to give **19** (125 mg, 52 %). - ^{1}H NMR (D_2O, referenced to HOD at 4.7 ppm): δ 0.92 (t, *J* = 7.3, 12H, 4 x CH_3), 1.17 (s, 9H, CMe_3), 1.33 (sextet, *J* = 7.3, 8H, 4 x CH_2CH_3), 1.62 (pentet, *J* = 8.2, 8H, CH_2CH_2N), 3.05 (br. t, *J* = 8.6, 8H, 4 x NCH_2), 4.76 (s, 3H, $COOCH_3$), 5.56 (d, J_{PH} = 13.5, 2H, CH_2O). - ^{13}C NMR (D_2O): δ 13.8 (s, 3C, CMe_3), 20.1 (s, 4C, 4 x CH_3CH_2), 24.1 (s, 4C, 4 x CH_3CH_2), 27.0 (s, 4C, 4 x CH_2CH_2N), 39.4 (s, 1C, CMe_3), 52.3 (d, $J_{P,C}$ = 4.6, 1C, $COOCH_3$), 59.1 (s, 4C, 4 x NCH_2), 84.1 (d, $J_{P,C}$ = 6.1, 1C,CH_2O), 173.4 (d, $J_{P,C}$ = 251.4, 1C, $COOCH_3$), 181.1 (1C, Bu^{t}CO). - ^{31}P NMR (D_2O): δ -6.8 (q, J_{PH} = 10.0, ^{1}H coupled). - IR (Nujol): υ_{max} 1040, 1080, 1720, 1760. - MS (FAB$^-$): m/z 253.0476 (M$^-$), $C_8H_{14}O_7P$ requires 253.0477.

Tetra-*n*-butylammonium pivaloyloxymethyl (pivaloyloxymethoxycarbonyl)phosphonate (4). Iodomethyl pivalate[6] (156 mg, 1 mmol) was added to **20** (210 mg, 0.33 mmol) in dry toluene (4 mL) at room temp. The mixture was stirred for 3 days. Tetra-*n*-butylammonium iodide was removed by filtration, the filtrate was concentrated under reduced pressure and purified by preparative layer chromatography ($MeOH:CH_2Cl_2$, 1:9) to give the tetra-*n*-butylammonium salt of **4** as a pale yellow solid (19 mg, 10 %). - ^{1}H NMR ($CDCl_3$): δ 1.00 (t, *J* = 7.3, 12H, 4 x CH_3), 1.18 (s, 9H, Me_3C), 1.19 (s, 9H, Me_3C), 1.45 (sextet, *J* = 7.3, 8H, 4 x CH_2CH_3), 1.66 (pentet, *J* = 7.9, 8H, 4 x CH_2CH_2N), 3.32 (br. t, *J* = 8.6, 8H, 4 x CH_2N), 5.66 (d, $J_{P,H}$ = 11.5, 2H, CH_2OP), 5.78 (s, 2H, CH_2OC). - ^{13}C NMR ($CDCl_3$): δ 13.7 (s, 4C, 4 x CH_3CH_2), 19.7 (s, 4C, 4 x CH_2CH_3), 24.0 (s, 4C, 4 xCH_2CH_2N), 26.9 (s, 3C, Me_3C), 27.0 (s, 3C, Me_3C), 38.6 (s, 1C, Me_3C), 38.7 (s, 1C, Me_3C), 58.8 (s, 4C, 4 x CH_2N), 79.2 (d, $J_{P,C}$ = 4.8, 1C, CH_2OC), 83.6 (d, $J_{P,C}$ = 4.9, 1C, CH_2OP), 172.3 (d, $J_{P,C}$ = 236.0, 1C, $COOCH_2$), 177.2 (s, 1C, Bu^{t}CO), 177.5 (s, 1C, Bu^{t}CO). - ^{31}P NMR ($CDCl_3$): δ -9.15 (t, J_{PH} = 11.4, ^{1}H coupled). - IR (Nujol): υ_{max} 1030, 1070, 1740. - MS (FAB$^-$): m/z 153.1003 (M$^-$), $C_{29}H_{31}O_9P$ requires 153.1001.

Sodium pivaloyloxymethyl (pivaloyloxymethoxycarbonyl)phosphonate (4). The tetra-*n*-butylammonium salt of **4** (234 mg, 0.4 mmol) was dissolved in water (5 mL) and applied to a cation exchange resin column (Dowex 50-X8, 20-50 mesh, 7 mL). The column was eluted with water (15 mL) and the filtrate was concentrated under reduced pressure to give the sodium salt of **4**. - ^{1}H NMR (D_2O): δ 1.18 (s, 18H, 6 x Me), 5.55 (d, $J_{P,H}$ = 13.2, 2H, CH_2OP), 5.84 (s, 2H, CH_2OC).

Di(4-acetoxybenzyl) phosphite (23a). To **24a** (0.83 g, 5 mmol) in dry Et_2O (15 mL), Et_3N (0.70 mL, 5 mmol) was added at 0 °C. Phosphorus trichloride (0.15 mL, 1.67 mmol) was added, dropwise, at that temp. The mixture was heated at reflux for 2 h, allowed to cool to room temp. and stirred for 16 h. The mixture was quenched with H_2O (10 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give a pale yellow oil. The crude product was dissolved in CH_2Cl_2 (20 mL), silica (2 g) was added and the mixture was stirred for 16 h. Removal of solvent under reduced pressure gave the product absorbed onto silica which was purified by flash column chromatography (gradient: hexane-EtOAc, 3:1 to 1:1 to 1:3) to give **23a** as an oil (0.485 g, 77 %). - R_f (hexane-EtOAc, 1:1): 0.15. - ^1H NMR ($CDCl_3$): δ 2.29 (s, 6H, 2 x CH_3), 4.96-5.10 (m, 4H, 2 x CH_2O), 6.94 (d, $J_{P,H}$ = 708.9, 1H, P-*H*), 7.08 (d, *J* = 8.6, 4H, Ar), 7.36 (d, *J* = 8.3, 4H, Ar). - ^{13}C NMR ($CDCl_3$): δ 20.9 (s, 2C, 2 x CH_3), 66.5 (d, $J_{P,C}$ = 4.9, 2C, 2 x CH_2O), 121.8 (s, 4C, Ar), 129.1 (s, 4C, Ar), 132.9 (d, $J_{P,C}$ = 6.1, 2C, Ar), 150.7 (s, 2C, Ar), 169.1 (s, 2C, 2 x Me*C*=O). - ^{31}P NMR ($CDCl_3$): δ 8.35 (d.pentet, $J_{P,H}$ = 709.0, $J_{P,H}$ = 9.6, ^1H coupled). - MS (CI): m/z 379.0954 (M + H^+), $C_{18}H_{20}O_7P$ requires 379.0947.

Compounds **23b** and **23c** were prepared from the appropriate 4-acyloxybenzyl alcohol using a method similar to that described for **23a**:

Di(4-*n*-butanoyloxybenzyl) phosphite (23b). Purified by flash column chromatography (gradient: hexane-EtOAc, 3:1 to 1:1) as an oil (66 %), R_f (hexane-EtOAc, 1:1) 0.30. - ^1H NMR ($CDCl_3$): δ 1.04 (t, *J* = 7.5, 6H, 2 x CH_3), 1.78 (sextet, *J* = 7.5, 4H, 2 x CH_2Me), 2.54 (t, *J* = 7.5, 4H, 2 x CH_2C=O), 5.04 (dd, J_{gem} = 16.3, $J_{P,H}$ = 9.7, 4H, 2 x CH_2O), 6.94 (d, $J_{P,H}$ = 708.7, 1H, P-*H*), 7.08 (d, *J* = 8.6, 4H, Ar), 7.36 (d, *J* = 8.3, 4H, Ar). - ^{13}C NMR ($CDCl_3$): δ 13.6 (s, 2C, 2 x CH_3), 18.4 (s, 2C, 2 x CH_2Me), 36.1 (s, 2C, 2 x CH_2C=O), 66.6 (d, $J_{P,C}$ = 4.9, 2C, 2 x CH_2O), 121.9 (s, 4C, Ar), 129.2 (s, 4C, Ar), 132.9 (d, $J_{P,C}$ = 6.1, 2C, Ar), 150.9 (s, 2C, Ar), 171.9 (s, 2C, 2 x *C*=O). - ^{31}P NMR ($CDCl_3$): δ 8.37 (d.pentet, $J_{P,H}$ = 708.7, $J_{P,H}$ = 9.4, ^1H coupled). - MS (CI): m/z 452.1839 (M + NH_4^+), $C_{22}H_{31}NO_7P$ requires 452.1838.

Di(4-pivaloyloxybenzyl) phosphite (23c). Purified by flash column chromatography (gradient: hexane-EtOAc, 3:1 to 1:1) as an oil (70 %), R_f (hexane-EtOAc, 1:1) 0.35. - ^1H NMR ($CDCl_3$): δ 1.36 (s, 18H, 6 x CH_3), 5.04 (dd, $J_{P,H}$ = 9.7, *J* = 3.1, 4H, 2 x CH_2O), 6.94 (d, $J_{P,H}$ = 708.3, 1H, P-*H*), 7.06 (d, *J* = 8.6, 4H, Ar), 7.36 (d, *J* = 8.8, 4H, Ar). - ^{13}C NMR ($CDCl_3$): δ 27.0 (s, 6C, 6 x CH_3), 39.0 (s, 2C, 2 x CMe_3), 66.6 (d, $J_{P,C}$ = 4.8, 2C, 2 x CH_2O), 121.8 (s, 4C, Ar), 129.1 (s, 4C, Ar), 132.8 (d, $J_{P,C}$ = 6.1, 2C, Ar), 151.2 (s, 2C, Ar), 176.8 (s, 2C, 2 x *C*=O). - ^{31}P NMR ($CDCl_3$): δ 8.34 (d.pentet, $J_{P,H}$ = 708.5, $J_{P,H}$ = 9.3, ^1H coupled). - MS (CI): m/z 462.1802 (M^+), $C_{24}H_{31}O_7P$ requires 462.1805.

Di(4-acetoxybenzyl) phenoxycarbonylphosphonate (21a). To **23a** (200 mg, 0.53 mmol) in CH_2Cl_2 (3 mL), *N,O*-bis(trimethylsilyl)acetamide (0.16 mL, 0.64 mmol, 1.2 equiv.) was added and the mixture was stirred for 3 h. Phenyl chloroformate (0.13 mL, 1.06 mmol, 2 equiv.) in CH_2Cl_2 (3 mL) was added and the mixture was stirred for a further 16 h. Removal of solvent under reduced pressure gave the crude product as a yellow oil. Purification by flash column chromatography (gradient: hexane-EtOAc, 5:1 to 1:1) gave **21a** as an oil (200 mg, 76 %), R_f (hexane-EtOAc, 1:1) 0.60. - ^1H NMR ($CDCl_3$): δ 2.30 (s, 6H, 2 x CH_3), 5.28 (d, $J_{P,H}$

= 8.3, 4H, 2 x CH_2O), 7.01-7.04 (m, 2H, Ar), 7.20-7.44 (m, 11H, Ar). - ^{13}C NMR ($CDCl_3$): δ 21.0 (s, 2C, 2 x CH_3), 69.3 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2O), 121.1 (s, 2C, Ar), 121.9 (s, 4C, Ar), 126.7 (s, 2C, Ar), 129.5 (s, 4C, Ar), 129.6 (s, 1C, Ar), 132.4 (d, $J_{P,C}$ = 6.1, 2C, Ar), 149.5 (d, $J_{P,C}$ = 8.5, 1C, Ar), 151.0 (s, 2C, Ar), 164.8 (d, J_{PC} = 275.9, 1C, PC=O), 169.2 (s, 2C, MeC=O). - ^{31}P NMR ($CDCl_3$): δ -4.38 (pentet, $J_{P,H}$ = 8.4, ^1H coupled). - MS (CI): m/z 499.1143 (M + H^+), $C_{25}H_{24}O_9P$ requires 499.1158.

The following compounds were prepared from appropriate di(4-acyloxybenzyl) phosphites and chloroformates using a method similar to that described for **21a**:

Di(4-*n*-butanoyloxybenzyl) phenoxycarbonylphosphonate (21b). Purified by flash column chromatography (gradient: hexane-EtOAc, 5:1 to 3:1 to 1:1) as an oil (86 %), R_f (hexane-EtOAc, 1:1) 0.40. - ^1H NMR ($CDCl_3$): δ 1.04 (t, J = 7.5, 6H, 2 x CH_3), 1.78 (sextet, J = 7.5, 4H, 2 x CH_2Me), 2.54 (t, J = 7.5, 4H, 2 x CH_2C=O), 5.28 (d, $J_{P,H}$ = 8.3, 4H, 2 x CH_2O), 7.02-7.11 (m, 6H, Ar), 7.25-7.43 (m, 7H, Ar). - ^{13}C NMR ($CDCl_3$): δ 13.7 (s, 2C, 2 x CH_3), 18.5 (s, 2C, 2 x CH_2Me), 36.2 (s, 2C, 2 x CH_2C=O), 69.5 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2O), 121.0 (s, 4C, Ar), 121.9 (s, 4C, Ar), 126.6 (s, 1C, Ar), 129.5 (s, 4C, Ar), 132.2 (d, $J_{P,C}$ = 6.1, 2C, Ar), 149.5 (d, $J_{P,C}$ = 8.6, 1C, Ar), 151.0 (s, 2C, Ar), 164.8 (d, $J_{P,C}$ = 275.9, 1C, P-C=O), 171.8 (s, 2C, 2 x C=O). - ^{31}P NMR ($CDCl_3$): δ -4.40 (pentet, $J_{P,H}$ = 8.4, ^1H coupled). - MS (CI): m/z 572.2060 (M + NH_4^+), $C_{29}H_{35}NO_9P$ requires 572.2049.

Di(4-pivaloyloxybenzyl) phenoxycarbonylphosphonate (21c). Purified by flash column chromatography (gradient: hexane-EtOAc, 5:1 to 3:1 to 1:1) as an oil (76 %), R_f (hexane-EtOAc, 1:1) 0.50. - ^1H NMR ($CDCl_3$): δ 1.35 (s, 18H, 6 x CH_3), 5.28 (d, $J_{P,H}$ = 8.5, 4H, 2 x CH_2O), 7.01-7.15 (m, 6H, Ar), 7.25-7.42 (m, 7H, Ar). - ^{13}C NMR ($CDCl_3$): δ 27.0 (s, 6C, 6 x CH_3), 38.9 (s, 2C, 2 x CCH_3), 69.4 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2O), 121.0 (s, 2C, Ar), 121.3 (s, 2C, Ar), 121.8 (s, 4C, Ar), 126.6 (s, 1C, Ar), 129.5 (s, 4C, Ar), 132.1 (d, J_{PC} = 6.1, 2C, Ar), 149.5 (d, $J_{P,C}$ = 8.5, 1C, Ar), 151.4 (s, 2C, Ar), 164.8 (d, $J_{P,C}$ = 275.8, 1C, P-C=O), 172.2 (s, 2C, 2 x C=O). - ^{31}P NMR ($CDCl_3$): δ -4.45 (pentet, $J_{P,H}$ = 8.4, ^1H coupled). - MS (CI): m/z 600.2370 (M + NH_4^+), $C_{31}H_{39}NO_9P$ requires 600.2362.

Di(4-acetoxybenzyl) benzyloxycarbonylphosphonate (22a). Purified by flash column chromatography (gradient: hexane-EtOAc, 3:1 to 1:1) as an oil (69 %), R_f (hexane-EtOAc, 1:1) 0.45. - ^1H NMR ($CDCl_3$): δ 2.30 (s, 6H, 2 x CH_3), 5.16 (d, $J_{P,H}$ = 7.9, 4H, 2 x CH_2OP), 5.24 (d, $J_{P,H}$ = 1.0, 2H, CH_2O), 7.05 (d, J = 8.6, 4H, Ar), 7.31-7.35 (m, 9H, Ar). - ^{13}C NMR ($CDCl_3$): δ 21.1 (s, 2C, 2 x CH_3), 67.7 (d, $J_{P,C}$ = 4.9, 1C, CH_2Ph), 69.1 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2OP), 121.8 (s, 6C, Ar), 128.7 (s, 4C, Ar), 129.5 (s, 4C, Ar), 132.5 (d, $J_{P,C}$ = 6.1, 2C, Ar), 134.2 (s, 1C, Ar), 150.9 (s, 1C, Ar), 166.2 (d, $J_{P,C}$ = 271.0, 1C, P-C=O), 169.2 (s, 2C, 2 x MeC=O). - ^{31}P NMR ($CDCl_3$): δ -4.14 (pentet, J_{PH} = 7.3, ^1H coupled). - MS (CI): m/z 530.1586 (M + NH_4^+), $C_{26}H_{29}NO_9P$ requires 530.1586.

Di(4-*n*-butanoyloxybenzyl) benzyloxycarbonylphosphonate (22b). Purified by flash column chromatography (gradient: hexane-EtOAc, 5:1 to 3:1 to 1:1) as an oil (81 %), R_f (hexane-EtOAc, 1:1) 0.50. - ^1H NMR ($CDCl_3$): δ 1.03 (t, J = 7.5, 6H, 2 x CH_3), 1.76 (sextet, J = 7.4, 4H, 2 x CH_2Me), 2.52 (t, J = 7.3, 4H, 2 x CH_2C=O), 5.15 (d, $J_{P,H}$ = 8.2, 4H, 2 x CH_2OP), 5.21 (d, $J_{P,H}$ = 1.0, 2H, CH_2O), 7.02-7.05 (m, 4H, Ar), 7.30-7.33 (m, 9H, Ar). - ^{13}C NMR ($CDCl_3$): δ 13.4 (s, 2C, 2 x CH_3), 18.2 (s, 2C, 2 x

CH_2Me), 35.9 (s, 2C, 2 x $CH_2C=O$), 67.4 (d, $J_{P,C}$ = 4.9, 1C, CH_2Ph), 68.9 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2O), 121.6 (s, 6C, Ar), 128.4 (s, 2C, Ar), 128.5 (s, 1C, Ar), 129.2 (s, 4C, Ar), 132.2 (d, $J_{P,C}$ = 6.1, 2C, Ar), 134.1 (s, 1C, Ar), 150.8 (s, 2C, Ar), 166.0 (d, $J_{P,C}$ = 269.8, 1C, P-C=O), 171.6 (s, 2C, 2 x C=O). - ^{31}P NMR ($CDCl_3$): δ -4.16 (pentet, $J_{P,H}$ = 8.1, ^1H coupled). - MS (CI): m/z 586.2190 (M + NH_4^+), $C_{30}H_{37}NO_9P$ requires 586.2206.

Di(4-acetoxybenzyl) [2-(S-benzoylthio)ethyloxycarbonyl]phosphonate (27). Purified by flash column chromatography (gradient: hexane-EtOAc, 5:1 to 3:1) as an oil (34 %), R_f (hexane-EtOAc, 1:1) 0.35. - ^1H NMR ($CDCl_3$): δ 2.28 (s, 6H, 2 x CH_3), 3.32-3.36 (m, 2H, CH_2S), 4.41 (t, J = 5.5, 2H, CH_2O), 5.19 (d, $J_{P,H}$ = 8.3, 4H, 2 x CH_2OP), 7.05-7.09 (m, 4H, Ar), 7.36-7.58 (m, 7H, Ar), 7.91-7.94 (m, 2H, Ar). - ^{13}C NMR ($CDCl_3$): δ 20.9 (s, 2C, 2 x CH_3), 27.0 (s, 1C, CH_2S), 64.1 (d, $J_{P,C}$ = 4.9, 1C, CH_2O), 69.0 (d, $J_{P,C}$ = 6.1, 2C, 2 x CH_2OP), 121.7 (s, 4C, Ar), 127.1 (s, 2C, Ar), 128.6 (s, 2C, Ar), 129.3 (s, 4C, Ar), 132.2 (d, $J_{P,C}$ = 6.1, 2C, Ar), 133.6 (s, 1C, Ar), 136.2 (s, 1C, Ar), 150.8 (s, 2C, Ar), 165.9 (d, $J_{P,C}$ = 270.9, 1C, P-C=O), 169.1 (s, 2C, 2 x C=O), 190.4 (s, 1C, PhC=O). - ^{31}P NMR ($CDCl_3$): δ -4.35 (pentet, $J_{P,H}$ = 8.2, ^1H coupled). - MS (CI): m/z 604.1403 (M + NH_4^+), $C_{28}H_{31}NO_{10}PS$ requires 604.1406.

Sodium 4-acetoxybenzyl phenoxycarbonylphosphonate (14a). To **21a** (400 mg, 0.803 mmol) in dry acetone (6 mL) at room temp., NaI (221 mg, 0.803 mmol) was added and stirred for 2 h, after which TLC showed complete disappearance of starting material. H_2O (8 mL) was added, the layers were separated, the aqueous layer was washed with Et_2O/MeOH (20:1) (2 x 20 mL) and concentrated under reduced pressure to give **14a** as a solid (287 mg, 96 %), m.p. >220 °C. - ^1H NMR (D_2O, referenced to MeCN): δ 2.21 (s, 3H, CH_3), 5.04 (d, $J_{P,H}$ = 9.2, 2H, CH_2O), 6.88-7.05 (m, 4H, Ar), 7.20-7.43 (m, 5H, Ar). - ^{13}C NMR (D_2O, referenced to MeCN): δ 21.1 (s, 1C, CH_3), 68.7 (d, $J_{P,C}$ = 4.8, 1C, CH_2O), 122.1 (s, 2C, Ar), 122.6 (s, 2C, Ar), 127.6 (s, 1C, Ar), 130.2 (s, 2C, Ar), 130.7 (s, 2C, Ar), 135.7 (d, $J_{P,C}$ = 4.9, 1C, Ar), 150.2, (d, $J_{P,C}$ = 6.1, 1C, Ar), 150.8 (s, 1C, Ar), 172.2 (d, $J_{P,C}$ = 294.1, 1C, P-C=O), 174.0 (s, 1C, C=O). - ^{31}P NMR (D_2O): δ -5.15 (t, $J_{P,H}$ = 9.2, ^1H coupled). - MS (FAB^+): m/z 373.0462 (M + H^+), $C_{16}H_{15}O_7PNa$ requires 373.0453.

The following compounds were prepared from the appropriate phosphonoformate triester and NaI using a method similar to that described for **14a**:

Sodium 4-*n*-butanoyloxybenzyl phenoxycarbonylphosphonate (14b). 87 %, m.p. >220 °C. - (Found C, 53.3; H, 4.5. $C_{18}H_{18}O_7PNa$ requires C, 54.0; H, 4.5%). - ^1H NMR (D_2O, referenced to MeCN): δ 0.90 (t, J = 7.4, 3H, CH_3), 1.63 (sextet, J = 7.4, 2H, CH_2Me), 2.51 (t, J = 7.3, 2H, $CH_2C=O$), 5.04 (d, $J_{P,H}$ = 9.2, 2H, CH_2O), 6.87-6.90 (m, 2H, Ph), 7.04 (d, 2H, J = 8.6, Ar), 7.23-7.38 (m, 3H, Ph), 7.42 (d, J = 8.6, 2H, Ar). - ^{13}C NMR (D_2O, referenced to MeCN): δ 13.5 (s, 1C, CH_3), 18.7 (s, 1C, CH_2Me), 36.4 (s, 1C, $CH_2C=O$), 68.6 (d, $J_{P,C}$ = 6.1, 1C, CH_2O), 122.2 (s, 2C, Ar), 122.6 (s, 2C, Ar), 127.6 (s, 1C, Ar), 130.3 (s, 2C, Ar), 130.7 (s, 2C, Ar), 135.7 (d, $J_{P,C}$ = 4.9, 1C, Ar), 150.3 (d, $J_{P,C}$ = 7.3, 1C, Ar), 150.9 (s, 1C, Ar), 172.7 (d, $J_{P,C}$ = 244.1, 1C, P-C=O), 176.7 (s, 1C, C=O). - ^{31}P NMR (D_2O): δ -5.18 (t, $J_{P,H}$ = 9.4, ^1H coupled). - MS (FAB^+): m/z 401.0769 (M + H^+), $C_{18}H_{19}O_7PNa$ requires 401.0766.

Sodium 4-pivaloyloxybenzyl phenoxycarbonylphosphonate (14c). 85 %, m.p. >220 °C. - ^1H NMR (D_2O, referenced to MeCN): δ 1.25 (s, 9H, 3 x CH_3), 5.04 (d, $J_{P,H}$ = 9.2, 2H, CH_2O), 6.86-7.05 (m,

4H, Ar), 7.23-7.45 (m, 5H, Ph). - ^{13}C NMR (D_2O, referenced to MeCN): δ 31.4 (s, 3C, 3 x CH_3), 44.0 (s, 1C, CMe_3), 73.2 (d, $J_{P,C}$ = 6.1, 1C, CH_2O), 126.6 (s, 2C, Ar), 126.9 (s, 2C, Ar), 132.0 (s, 1C, Ar), 134.7 (s, 2C, Ar), 135.1 (s, 2C, Ar), 139.9 (d, $J_{P,C}$ = 4.9, 1C, Ar), 154.7 (d, $J_{P,C}$ = 7.3, 1C, Ar), 155.6 (s, 1C, Ar), 177.1 (d, $J_{P,C}$ = 242.9, 1C, P-*C*=O), 186.1 (s, 1C, *C*=O). - ^{31}P NMR (D_2O): δ -5.21 (t, $J_{P,H}$ = 9.4, ^1H coupled). - MS (FAB+): m/z 415.0927 (M + H^+), $C_{19}H_{20}O_7PNa$ requires 415.0923.

Sodium 4-acetoxybenzyl benzyloxycarbonylphosphonate (15a). 80%, m.p. >220 °C. - ^1H NMR (D_2O, referenced to MeCN): δ 2.12 (s, 3H, CH_3), 4.82 (d, $J_{P,H}$ = 7.6, 2H, CH_2O), 4.99 (s, 2H, CH_2Ph), 6.82 (d, J = 8.3, 2H, Ar), 7.14 (d, J = 8.6, 2H, Ar), 7.16-7.18 (m, 5H, Ph). - ^{13}C NMR (D_2O, referenced to MeCN): δ 21.1 (s, 1C, CH_3), 67.4 (d, $J_{P,C}$ = 3.7, 1C, CH_2Ph), 68.1 (d, $J_{P,C}$ = 6.1, 1C,CH_2O), 122.3 (s, 2C, Ar), 129.0 (s, 2C, Ar), 129.2 (s, 1C, Ar), 129.4 (s, 2C, Ar), 129.8 (s, 2C, Ar), 135.5 (d, $J_{P,C}$ = 6.1, 1C, Ar), 135.9 (s, 1C, Ar), 150.5 (s, 1C, Ar), 173.3 (d, $J_{P,C}$ = 241.7, 1C, P-*C*=O), 173.5 (s, 1C, *C*=O). - ^{31}P NMR (D_2O): δ -4.76 (br. s, ^1H coupled). - MS (FAB+): m/z 387.0619 (M + H^+), $C_{17}H_{17}O_7PNa$ requires 387.0610.

Sodium 4-*n*-butanoyloxybenzyl benzyloxycarbonylphosphonate (15b). 84 %, m.p. >220 °C. - ^1H NMR (D_2O, referenced to MeCN): δ 0.76 (t, J = 7.6, 3H, CH_3), 1.46 (sextet, J = 7.4, 2H, CH_2Me), 2.23 (t, J = 7.3, 2H, CH_2C=O), 4.78 (d, $J_{P,H}$ = 8.2, 2H, CH_2O), 4.79 (2H, s, CH_2O), 6.66 (d, J = 8.6, 2H, Ar), 6.98-7.05 (m, 7H, Ar). - ^{13}C NMR (D_2O, referenced to MeCN): δ 13.8 (s, 1C, CH_3), 18.8 (s, 1C, CH_2Me), 36.4 (s, 1C, CH_2C=O), 67.1 (d, $J_{P,C}$ = 4.9, 1C, CH_2Ph), 68.0 (d, $J_{P,C}$ = 4.9, 1C, CH_2O), 122.0 (s, 2C, Ar), 128.7 (s, 1C, Ar), 129.1 (s, 4C, Ar), 129.6 (s, 2C, Ar), 135.5 (d, $J_{P,C}$ = 7.3, 1C, Ar), 136.0 (s, 1C, Ar), 150.5 (s, 1C, Ar), 173.4 (d, $J_{P,C}$ = 241.7, 1C, P-*C*=O), 174.2 (s, 1C, *C*=O). - ^{31}P NMR (D_2O): δ -4.88 (br. s, ^1H coupled). - MS (FAB+): m/z 415.0930 (M + H^+), $C_{19}H_{21}O_7PNa$ requires 415.0922.

Antiviral activity. The antiviral activity of the compounds was determined essentially according to the method described by Wahren and coworkers.[37] Confluent human lung fibroblast cells were infected with herpes simplex virus type 1 (HSV-1). After absorption for one hour at 37 °C, virus was removed and antiviral drugs diluted in cell media were added, at concentrations of 800 μM down to 3 μM, as single determinations. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air until characteristic cytopathic effect was seen in control wells (24-48 h). Cells were then lysed by addition of Triton X-100, and viral antigen content of the supernatants measured by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody.

Hydrolysis in tissue homogenates. Male Sprague Dawley rats (body weight 250 - 350 g; B & K Universal, Sollentuna, Sweden) were killed by a blow on the neck and bled. The liver and a segment of the small intestine (approximately 50 cm descending from the stomach) were excised and put on ice. Intestinal contents were removed by flushing with approximately 20 mL of ice cold saline solution. The tissues were homogenised in three (liver) or two (intestine) times their weight of Tris-HCl buffer (0.05 M, pH 7.5) using a Polytron® homogeniser. The resulting mixtures were not centrifuged to remove cellular and nuclear debris but used as such. Portions of the homogenates (15 - 30 mL) were transferred to glass stoppered 50 mL test tubes

and placed in a rocking water bath at 37 °C. The reaction was initiated by adding solutions of the test substances (100 mMol) to final concentrations of 1.0 mM. (Compounds **4**, **14a-c**, **15b** and **27** were dissolved in Tris-HCl buffer solution. Compounds **15a**, **21a-b** and **22a-b** were dissolved in a 50/50 mixture of polyethyleneglycol and ethanol). Samples (0.5 mL) were withdrawn immediately after addition of test compounds and after 5, 15, 60 and 240 min. and transferred to test tubes containing methanol (0.5 mL). The resulting mixtures were centrifuged at ~2000 g for 15 min. The supernatants were analysed as single determinations for phosphonoformate by an HPLC method.[38]

***In vivo* experiments in rats.** Male Sprague-Dawley rats (body weight 250 - 350 g) received the test compounds in single oral doses of 180 μmol/kg. Test compounds were dissolved in distilled water immediately before dosing. Concentrations of administered test solutions were 80 mM. Oral administration was by gavage using a steel cannula with a ball-shaped tip connected to a plastic syringe. Serial blood samples (~ 0.3 mL) were collected from the orbital venous plexus or from a tail vein at the following times after administration: 15 and 30 min, 1, 1.5, 2, 4 , 6 and 8 h. Plasma was separated and analysed for phosphonoformate by HPLC.[38]

Chemical hydrolysis of diester 14b. A solution of **14b** (16 μmol) in aqueous sodium methylphosphonate buffer [Buffer: ^{31}P NMR: δ 23.7. - ^{1}H NMR: δ 1.15 (d, $J_{P,H}$ = 16.1) - used to reference subsequent spectra] (0.1 M, pD 8.0) (0.8 mL) was first recorded by ^{1}H and ^{31}P NMR spectroscopy [^{31}P NMR: δ -5.08. - ^{1}H NMR: δ 0.93 (t, J = 7.4, CH_3, 3H), 1.67 (sextet, J = 7.4, 2H, CH_2Me), 2.55 (t, J = 7.4, 2H, CH_2C=O), 5.07 (d, $J_{P,H}$ = 9.2, 2H, CH_2O), 6.91-7.48 (m, 9H, aromatic)]. The sample was monitored by ^{1}H NMR spectroscopy at 37 °C at regular time intervals over 7 days. The rate constant was 9.3 x 10^{-4} min^{-1} (R^2 0.996). Approximately 90% of the hydrolysis occurred at the phenoxycarbonyl group with the formation of 4-butanoyloxybenzyl disodium carboxyphosphonate [^{1}H NMR: δ including 4.85 (d, $J_{P,H}$ = 6.9, 2H, CH_2O] and phenol [detected by tlc, R_f (hexane-ethyl acetate, 1:1) 0.8]. Some hydrolysis (~10%) of the 4-butanoyloxybenzyl group was also observed, with the formation of sodium butanoate [^{1}H NMR: δ 0.79 (t, J = 7.4, 3H), 1.45 (sextet, J = 7.3, 2H), 2.05 (t, J = 7.3, 2H)] and 4-hydroxybenzyl alcohol [^{1}H NMR: δ including 4.45 (s, 2H)].

Esterase-catalysed hydrolysis of diester 14b. PLCE (17 or 170 units) was added to a solution of **14b** (16 μmol) in aqueous sodium methylphosphonate buffer (0.1 M, pD 8.0) (0.8 mL). The samples were monitored by ^{1}H and ^{31}P NMR spectroscopy at 37 °C at regular time intervals. With 170 units, hydrolysis was rapid giving rise only to disodium phenoxycarbonylphosphonate, ^{31}P NMR: δ -4.23, together with 4-hydroxybenzyl alcohol [^{1}H NMR: δ including 4.42 (s, 2H), R_f (hexane-ethyl acetate, 1:1) 0.45] and sodium butanoate [^{1}H NMR: δ 0.76 (t, J = 7.4, 3H), 1.42 (sextet, J = 7.4, 2H), 2.03 (t, J = 7.4, 2H)]. Phenol could not be detected by tlc. With 17 units of PLCE, ~80% of **14b** cleaved to disodium phenoxycarbonylphosphonate, ^{31}P NMR: δ -4.4, whereas ~20% of the reaction proceeded by chemical hydrolysis to 4-butanoyloxybenzyl disodium carboxyphosphonate, which underwent slow esterase-catalysed hydrolysis to phosphonoformate, **1**, ^{31}P NMR: δ 0.3.

References

1. Wagstaff, A. J.; Bryson, H. M. *Drugs* **1994**, *48*, 199-226.
2. Norén, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stening, G. *J. Med. Chem.* **1983**, *26*, 264-270.
3. Farquhar, D.; Srivastva, D. N.; Kuttesch, N. J.; Saunders, P. P. *J. Pharm. Sci.* **1983**, *72*, 324-325.
4. Srivastva, D. N.; Farquhar, D. *Bioorg. Chem.* **1984**, *12*, 118-129.
5. Sastry, J. K.; Nehete, P. N.; Khan, S.; Nowak, B. J.; Plunkett, W.; Arlinghaus, R. B.; Farquhar, D. *Molecular Pharmacology* **1991**, *41*, 441-445.
6. Farquhar, D.; Khan, S.; Srivastva, D. N.; Saunders, P. P. *J. Med. Chem.* **1994**, *37*, 3902-3909.
7. Pompon, A.; Lefebvre, I.; Imbach, J.-L.; Kahn, S.; Farquhar, D. *Antiviral Chem. Chemother.* **1994**, *5*, 91-98.
8. Schultz, C.; Vajanaphanich, M.; Harootunian, A. T.; Sammak, P. J.; Barrett, K. E.; Tsien, R. Y. *J. Biol. Chem.* **1993**, *268*, 6316-6322.
9. Starrett Jr., J. E.; Tortolani, D. R.; Russell, J.; Hitchcock, M. J. M.; Whiterock, V.; Martin, J. C.; Mansuri, M. M. , *J. Med. Chem.* **1994**, *37*, 1857-1864.
10. Starrett Jr., J. E.; Tortolani, D. R.; Hitchcock, M. J. M.; Martin, J. C.; Mansuri, M. M. *Antiviral Res.* **1992**, *19*, 267-273.
11. Srinivas, R. V.; Robbins, B. L.; Connelly, M. C.; Gong, Y.-F.; Bischofberger, N.; Fridland, A. *Antimicrob. Agents Chemother.* **1993**, *37*, 2247-2250.
12. Serafinowska, H. T.; Ashton, R. J.; Bailey, S.; Harnden, M. R.; Jackson, S. M.; Sutton, D. *J. Med.Chem.* **1995**, *38*, 1372-1379.
13. Vajanaphanich, M.; Schultz, C.; Rudolf, M. T.; Wasserman, M.; Enyedi, P.; Craxton, A.; Shears, S. B.; Tsien, R. Y.; Barrett, K. E.; Traynor-Kaplan, A. *Nature* **1994**, *371*, 711-714.
14. Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2471-2476.
15. Barber, I.; Rayner, B.; Imbach, J.-L. *Nucleosides Nucleotides* **1995**, *14*, 1083-1085.
16. Iyer, R. P.; Phillips, L. R.; Biddle, J. A.; Thakker, D. R.; Egan, W. *Tetrahedron Lett.* **1989**, *30*, 7141-7144.
17. Iyer, R. P.; Boal, J. H.; Phillips, L. R.; Thakker, D. R.; Egan, W. *J. Pharm. Sci.* **1994**, *83*, 1269-1273.
18. Freeman, S.; Irwin, W. J.; Mitchell, A. G.; Nicholls, D.; Thomson, W. *J. Chem. Soc. Chem. Commun.* **1991**, 875-877.
19. Mitchell, A. G.; Thomson, W.; Nicholls, D.; Irwin, W. J.; Freeman, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2345-2353.
20. Thomson, W.; Nicholls, D.; Irwin, W. J.; Al-Mushadani, J. S.; Freeman, S.; Karpas, A.; Petrik, J.; Mahmood, N.; Hay, A. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1239-1245.
21. Routledge, A.; Walker, I.; Freeman, S.; Hay, A.; Mahmood, N. *Nucleosides Nucleotides* **1995**, *14*, 1545-1558.
22. Meier, C.; Habel, L. W.; Balzarini, J.; De Clercq, E. *Liebigs Ann.* 1995, 2203-2208.
23. Glazier, A. Phosphorus Prodrugs, International Patent Publication Number WO/91/19721, Dec. 1991.
24. Glazier, A.; Kwong, C.; Rose, J.; Buckheit, R.; Korba, B.; Abou-Donia, M.; Smith, E.; Wright, G. E. *Antiviral Res.* **1992**, Supplement 1, *17*, Abstract 66, 77.
25. Meier, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 70-72.
26. Mitchell, A. G.; Nicholls, D.; Walker, I.; Irwin, W. J.; Freeman, S. *J. Chem Soc., Perkin Trans. 2* **1991**, 1297-1303.
27. Krol, E. S.; Davis, J. M.; Thatcher, G. R. J. *J. Chem. Soc., Chem. Commun.* **1991**, 118-119.
28. Mitchell, A. G.; Nicholls, D.; Irwin, W. J.; Freeman, S. *J. Chem Soc., Perkin Trans. 2* **1992**, 1145-1150.
29. Krol, E. S.; Thatcher, G. R. J. *J. Chem. Soc., Perkin Trans. 2* **1993**, 793-794.
30. Vaghefi, M. M.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1986**, *29*, 1389-1393.
31. Griengl, H.; Hayden, W.; Penn, G.; De Clercq, E.; Rosenwirth, B. *J. Med. Chem.* **1988**, *31*, 1831-1839.
32. Lambert, R. W.; Martin, J.; Thomas, G. J.; Duncan, I. B.; Hall, M. J.; Heimer, E. P. *J. Med. Chem.* **1989**, *32*, 367-374.
33. Charvet, A.-S.; Camplo, M.; Faury, P.; Graciet, J.-C.; Mourier, N.; Chermann, J.-C.; Kraus, J.-L. *J. Med. Chem.* **1994**, *37*, 2216-2223.
34. Walker, I.; Nicholls, D.; Irwin, W. J.; Freeman, S. *Int. J. Pharm.* **1994**, *104*, 157-167.
35. Hostetler, K. Y.; Kini, G. D.; Beadle, J. R.; Aldern, K. A.; Gardner, M. F.; Border, R.; Kumar, R.; Barshak, L.; Sridhar, C. N.; Wheeler, C. J.; Richman, D. D. *Antiviral Res.* **1996**, *31*, 59-67.
36. Lundström, J. (unpublished results).
37. Wahren, B.; Harmenberg, J.; Sundqvist, V. A.; Leven, B.; Skoldenberg, B. *J. Virological Methods* **1983**, *6*, 141-149.
38. Pettersson, K. J.; Nordgren, T.; Westerlund, D. *J. Chromat.* **1989**, *488*, 447-455.

(*Received in UK* 22 *July* 1996; *revised* 27 *September* 1996; *accepted* 3 *October* 1996)