On the preparation of some phospholipid analogues

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Several structural analogues of the biocompatible diacyl glycerol phosphatidylcholine family have been prepared. Considerable variation in structure is allowed without impairing the desirable biocompatible properties. In particular, derivatives in which the fatty ester link is replaced by ether links and in which the central glycerol group is replaced by simple variants such as the symmetrical tris(hydroxymethyl)ethane group retain biocompatible properties.

Phosphatidylcholine-containing compounds and polymers are of importance since they have been shown to render surfaces 'biocompatible'.[1] Biocompatibility, in this context, refers to the property of surfaces *not* to interact with body tissues or fluids, such as blood, in order to avoid adhesion of cells and proteins,[2] platelet activation[3] and activation of the blood-clotting cascade. Materials that possess this biocompatibility property are of importance in a variety of medical dressings and devices in order to avoid the potentially fatal formation of thrombi or infection.[4]

The outer surfaces of erythrocytes and platelet cells are dominated (>90%) by the presence of phosphorylcholine head groups where the phosphate and trimethylammonium entities are separated by two methylene groups. The biocompatibility properties of typical phosphorylcholine esters, such as **1**, have been linked to its ability to bind water in a hydration shell that mimics bulk water but through which proteins are unable to penetrate.[1f] Water binds tightly to the phosphatidylcholine head group, with up to twelve molecules of water being tightly bound, as determined by various techniques.[5] Little work, however, has been carried out to explore the extent to which the chemical structure of the zwitterion determines its ability to render surfaces biocompatible. Previous studies have concentrated on the pharmacological effects of synthetic phosphorylcholine analogues.[6]

In this paper we describe some recent attempts to modify the phosphorylcholine structure, by the preparation of a series of analogues, in order to investigate biocompatible structure–efficacy relationships, using some standard *in vitro* tests for biocompatibility.

Results and discussion

In selecting possible structural variations, cognisance was taken of the ability of the phospholipase enzymes to hydrolyse phospholipids (see arrows in Fig. 1) and the preference for compounds with increased resistance to such enzyme-mediated hydrolysis. In the first instance, in order to limit the possible number of structural variations, modifications to the organic groups were made rather than trying to prepare analogues of the phosphate ester group.

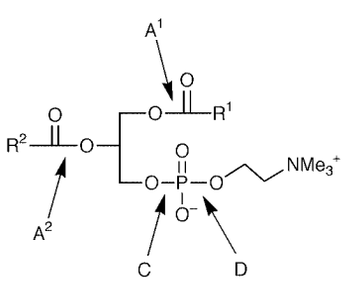
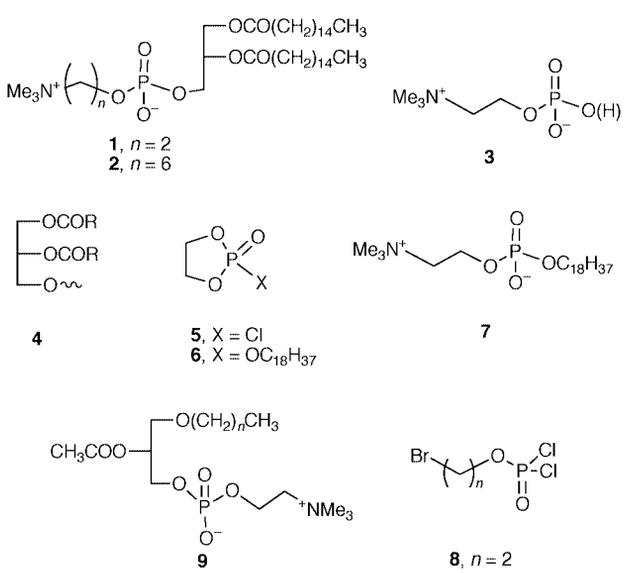


Fig. 1 Ester-cleavage points for the phospholipases.

Ester variations

Our initial approach was to retain the phosphatidylcholine group **3** and explore structural variations to the ester portion, normally represented by the di-*O*-acylglycerol group, *e.g.* **4**. The latter group poses synthetic and stability problems in that optically active 1,2-di-*O*-acylglycerol esters readily equilibrate with the 1,3-di-*O*-acyl isomers, thus losing their chirality. The ester groups are also subject to enzymic attack. Nature has recognised this 'stability' problem in that certain *Archaebacteria* utilise fatty ether derivatives of glycerol in place of fatty esters, in order to withstand some of the extreme conditions experienced, *e.g.* thermophilic bacteria.[7]



The first analogue studied was the known octadecyl ester of phosphatidylcholine, **7**,[8] produced by reaction of octadecan-1-ol with the dioxaphospholane **5**[9] to give the phosphate ester **6** and then ring opening of the dioxophospholane group by reaction with trimethylamine. However, the product **7** proved to be an oversimplification and surfaces coated with this derivative gave only a very poor biological performance (see Table 1).

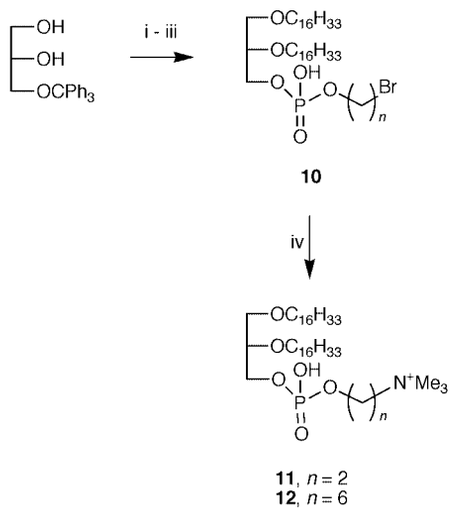
Table 1 Biological results[a]

Compound	Concn./ mmol dm^{-3}	% Fibrinogen reduction[b]	% Reduction, platelet adhesion[c]	% Reduction, platelet activation[d]
7	23	10		
11	14	90		91
12	14	92		
17	14	94		100
18	14	31		
19	14	88		
20	4.2		105	96
20	14	77		98
21	4.2		−42	66
21	14	28		
26	14	80		
27	14	31	77	77
28	4.2	86		
29	14	89		
30	4.2		107	94
30	14	71		

[a] Carried out at Biocompatibles Ltd. Average values given. Standard errors within the range ±10%. See Experimental section for details. [b] Decrease indicates reduced fibrinogen binding to coated polyethylene ribbons. [c] % Reduction in platelet adhesion to coated poly(vinyl chloride) ribbon quoted; −ve figure indicates an increase in platelet adhesion. [d] % Reduction in platelet activation quoted, using coated poly(vinyl chloride) ribbon.

Effort was then concentrated on analogues bearing at least two alkyl side chains.

The racemic diether derivative **11** was next prepared (Scheme 1) in high overall yield from 1-*O*-tritylglycerol,[10] using the



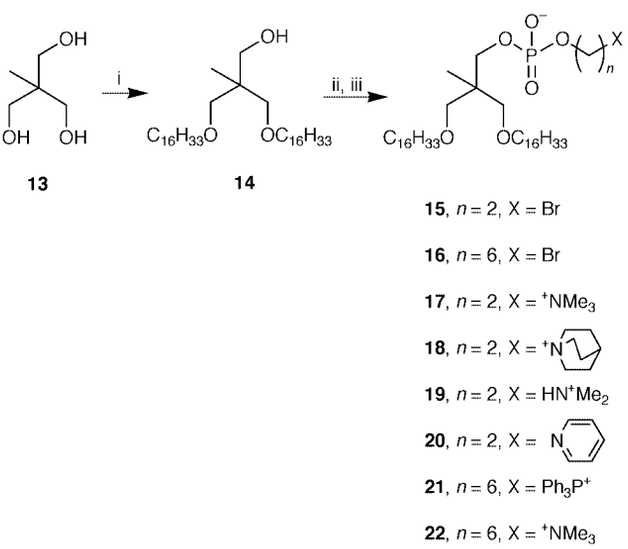
Scheme 1 *Reagents*: i, $C_{16}H_{33}Br$; ii, toluene-4-sulfonic acid, MeOH; iii, **8**, then H_2O; iv, Me_3N.

ester **8** *via* amination of the intermediate **10**. Compound **11**, which is similar to the platelet activation factor **9**,[11] performed in a similar manner to the corresponding ester control **1** in coated surface tests.†,[1] Thus replacement of the ester functions by the more stable ether groups does not appear to affect the biocompatible properties of this series of derivatives.

Spacer variations

Alongside the choline derivative **11**, the homologue **12** was prepared, in which the spacer group between the zwitterionic groups is increased to six atoms, using the bromohexyl analogue of **8**. Earlier work had shown that such a variation in structure in the parent **2** had only marginally affected its ability to form liposomes but little work on the effect of the chain-length variation on biocompatibility had been done.[12] Compound **12** was shown to possess similar biocompatible efficacy to the parent system **1**.†

These analogues, **11** and **12**, were prepared as racemates. In order to avoid the use of the glycerol ether backbone and problems with selective primary *vs*. secondary alcohol reactivity and racemate formation, use of the novel glycerol analogue tris(hydroxymethyl)ethane **13** was next explored. The bis-hexadecyl ether **14** was produced by direct alkylation of the triol (Scheme 2) and this was converted into the bromoalkyl



Scheme 2 *Reagents*: i, $C_{16}H_{33}Br$; ii, either **8** or hexyl congener; iii, nucleophiles, sealed-tube conditions.

phosphate derivatives **15** and **16**, the halogeno group of which could be replaced by reaction with various nucleophiles.

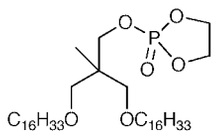
Variation in the cationic group

The intermediate **15** was aminated to form the series of ammonium derivatives **17**–**20**; the homologue **16** was used to prepare the derivatives **21** and **22**.

The intermediate **15** proved to be unstable to storage and difficult to obtain pure, even after repeated chromatographic separations. It was postulated that this instability might be due to intramolecular bromine displacement to form the cyclic

† Tests carried out in house at Biocompatibles Ltd.

intermediate **23**, which would be expected to readily undergo hydrolysis. In order to find out if such neighbouring group participation was of importance in the quaternisation reactions, the ^{13}C-labelled material **25** was prepared (Scheme 3).



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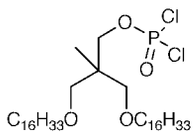

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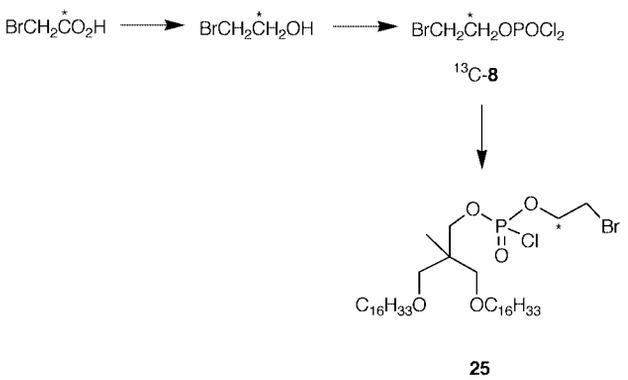
Reaction of this with trimethylamine under the standard conditions afforded ^{13}C-labelled **17** in which no scrambling of the label was observed, indicating that, in this case, intermediate formation of the cyclic ester **23** was *not* occurring. Possibly, a general neighbouring group effect, due to the hydrated phosphate function, explains the lability of the bromoethyl group.

Reaction of more hindered nucleophiles with the intermediate **15** often proved low yielding or abortive and, therefore, as an alternative method, the dichlorophosphate **24** was initially prepared, and reacted with a range of substituted alcohols under conditions favouring monoester formation. In this manner the derivatives **26**–**28** were prepared.



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Scheme 3

Non-alkyl zwitterionic spacers

Simple extension of the ethyl spacer, in the zwitterionic phosphatidylcholine group, from C_2 to C_6 had only a marginal effect on the behaviour of these analogues in *in vitro* tests. Some examples of non-alkyl spacers were therefore prepared, including compounds **29** and **30**. The former was obtained by reaction of 3-(dimethylamino)phenyl dichlorophosphate with the alcohol **14**, followed by hydrolysis to the phosphate and then methylation of the amine with methyl iodide. The 3-hydroxypyridine derivative **30** was prepared by initial preparation of 1-methyl-3-oxidopyridinium **31** and reaction of this with the dichlorophosphate **8**.

Biological results

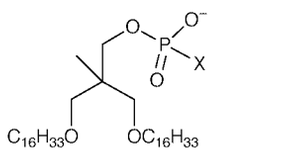
Table 1 lists representative biocompatibility results obtained with some of these tris(hydroxymethyl)ethane derivatives. The surface properties of the prepared compounds were determined after coating from an ethanolic solution onto either polyethylene tubing, for fibrinogen adsorption, or onto poly(vinyl chloride) ribbon for determination of the adherence of activated platelets. All results are presented as % reductions when compared with untreated controls.

The quinuclidinium **18** and dimethylsulfonium **27** derivatives both gave reductions in the fibrinogen assay of 31% and the latter surprisingly large reductions on both platelet adhesion (77%) and activation (77%) assays. The hexyl-spaced triphenylphosphonium compound **21** showed only a moderate reduction

Table 2 Activities against phospholipases[a]

Compound	Phospholipase C. % Extent of cleavage	Phospholipase D. % Extent of cleavage
1	100	100
11	0	100
12	0	90
17	0	100
19	0	50
20	0	50
22	0	95
26	0	5
29	0	30
30	0	95

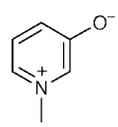
[a] See Experimental section for details. Results are averages of multiple runs; standard errors within ±10%.



26, X = $OCH_2CH_2N^+Et_3$

27, X = $OCH_2CH_2S^+Me_2$

28, X = $OCH_2CH_2N^+Me_2C_6H_{13}$



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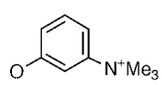
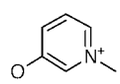
29, X =



30, X =



in fibrinogen adsorption and platelet activation but a very large platelet-adhesion effect. This result suggests that these coated surfaces are preferentially adsorbed *without* the normal activation, a property that is of potential utility in certain applications.

Some initial studies on the stability of these analogues to enzymic degradation were also carried out; specifically, resistance to phospholipases C and D. These are known to hydrolyse at the phosphate ester junctions indicated in Fig. 1 and the results are included in Table 2. Dipalmitoylphosphatidylcholine (DPPC) **1** was used as the reference compound and a literature assay method was used for the lipase C.[13] Lipase D was indirectly estimated by oxidation of the liberated choline using a commercially available assay kit (see Experimental section).

As can be seen from the results listed in Table 2, all the analogues tested were resistant to hydrolysis by phospholipase C, indicating that the diether linkage was unable to interact with the enzyme. Phospholipase D was much less specific and was capable of hydrolysing a wide variety of substrates. Of these, the triethylammonium analogue **26** was the least affected, indicating that the cationic site on the enzyme is not able to accept such a large entity.

Conclusions

The above results show that *considerable* latitude is possible in the design of effective biocompatible analogues of normal phosphatidylcholine esters, such as DPPC **1**. Thus, (i) the zwitterionic groups can be separated by a spacer length of up to six atoms without undue loss of biocompatibility; (ii) two long-chain alkyl groups are required but these may be linked by an ether link rather than an ester group; (iii) the central glyceryl group may be replaced by simple variants such as the tris(hydroxymethyl)ethane structure. The modified structures,

such as compounds **17** and **26**, retain biocompatibility but are much more resistant to enzymic degradation than the natural phosphatidylcholine system.

Experimental

Microanalyses were carried out by MEDAC Ltd, Brunel Science Park, Egham, Surrey. TLC used silica gel 60 F_{254}-coated glass plates, 0.25 mm, from BDH or, for preparative TLC (PLC), glass plates precoated with 1 mm of the same material. NMR spectroscopy was carried out on either a Bruker AC300 spectrometer (^{1}H at 300 MHz, ^{13}C at 75 MHz) or a JEOL FX200 instrument (^{1}H at 200 MHz); FAB mass spectra were obtained on a Kratos Concept 1S instrument, and EI and CI spectra using a VG Trio 2000 machine. Accurate mass measurements were carried out at the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea. Where necessary, solvents were dried and distilled before use.[14] Light petroleum refers to the fraction of boiling range 40–60 °C; for chromatography analytical grade solvents were used as supplied from Romil Chemicals (Cambridge, UK). Solutions were generally dried over anhydrous sodium sulfate before removal under reduced pressure using a rotary evaporator. Bromoethyl dichlorophosphate **8** was prepared by the method of Hirt and Berchtold.[15] Where relevant, derivatives are generally named as esters of the standard phosphatidylcholine head group, **4**.

Biological assays

The % fibrinogen-reduction, platelet-adhesion and platelet-activation studies were carried out in house by Biocompatibles Ltd, using standard protocols. Materials were dissolved in ethanol before coating onto either polyethylene tubing for the fibrinogen-adsorption studies or, for the platelet-adhesion and -activation assays, onto poly(vinyl chloride) ribbon at a concentration of 14 mmol dm^{-3} for the former and 4.2 mmol dm^{-3} for the latter.

The activity of phospholipase C (from *Clostridium perfringens*) followed the method of Zwaal and Roelofsen,[13] using DPPC **1** as the control, and employing either a colorimetric determination of the liberated phosphate monoester or TLC to follow the formation of the liberated alcohol.

Phospholipase D from *Streptomyces chromofuscus* was employed using a modification of the method described by Yang.[16] This involves a colorimetric determination (estimation) of hydrogen peroxide formed during the oxidation of the released choline. The method was also monitored by TLC detection of the liberated hydrolysis products.

Octadecyl phosphatidylcholine[8] 7

To a mixture of octadecan-1-ol (10 g, 36.9 mmol) and triethylamine (3.74 g, 1 equiv.) in diethyl ether (100 cm^3), at room temperature, was added dropwise a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane[9] **5** (5.27 g, 36.9 mmol) in diethyl ether (10 cm^3). The reaction mixture was stirred for 2 h, then filtered and the solvent removed. The residue was dissolved in 1:1 v/v acetonitrile–chloroform (150 cm^3) before addition of trimethylamine (6 g, 0.101 mol) in acetonitrile (15 cm^3) and the mixture was stirred at 50 °C for 16 h in a pressure bottle. On cooling of the reaction mixture to room temperature, a solid precipitated, which was filtered off and recrystallised from dichloromethane to yield the title compound (3.13 g, 19%); mp >220 °C (decomp.) (lit.[8] 235 °C decomp.) (Found: C, 59.1; H, 11.4; N, 2.9, P, 6.7. Calc. for $C_{23}H_{50}NO_4P{\cdot}2H_2O$: C, 58.7; H, 11.5; N, 3.0; P, 6.6%); δ_H (200 MHz; $CDCl_3$–CD_3OD) 0.87 (3 H, m, $MeCH_2$), 1.25 (30 H, br s, aliphatic H), 1.6 (2 H, m, CH_2CH_2 OP), 3.25 (9 H, s, Me_3N^+), 3.65 (2 H, m, CH_2N^+), 3.8 (2 H, q, CH_2OP), 4.25 (2 H, m, $POCH_2CH_2N$); *m/z* (FAB) 436 (M + H$^+$; 100%).

1-*O*-(Cholinephosphoryl)-2,3-di-*O*-hexadecylglycerol 11

1,2-Di-*O*-hexadecyl-3-*O*-tritylglycerol. 1-*O*-Tritylglycerol (5.0 g, 15 mmol prepared as per the literature[10]) was dissolved in dry DMF (50 cm^3), sodium hydride (1.32 g as a 60% w/w dispersion in paraffin oil, prewashed with hexane; 33 mmol) was added and the mixture was stirred at room temperature for 15 min before addition of 1-bromohexadecane (9.13 g, 30 mmol). The mixture was stirred at 40 °C for 16 h, cooled, and partitioned between chloroform (100 cm^3) and water (100 cm^3). The organic phase was washed successively with saturated aq. $NaHCO_3$ (100 cm^3) and brine (100 cm^3) before drying, filtering, and removal of solvent under reduced pressure. The product was purified by chromatography through silica and eluted with 2:23 ethyl acetate–light petroleum, to give pure 1,2-di-*O*-hexadecyl-3-*O*-tritylglycerol (8.0 g, 68%) as a waxy solid [Found (FAB MS): MH$^+$, 747. $C_{51}H_{87}O_3$ requires *m/z* 747].

1,2-Di-*O*-hexadecylglycerol. 1,2-Di-*O*-hexadecyl-3-*O*-tritylglycerol (8.0 g, 10.2 mmol) was dissolved in methanol (100 cm^3) with toluene-4-sulfonic acid (0.6 g) and the solution was stirred at room temperature for 16 h. The bulk of the solvent was removed under reduced pressure and the residue was partitioned between chloroform (100 cm^3) and water (100 cm^3). The organic phase was washed successively with water (2 × 100 cm^3), and brine (100 cm^3) before drying, filtering, and evaporating to dryness. The residue was chromatographed through silica and eluted with light petroleum and light petroleum–ethyl acetate (23:2) to give 1,2-di-*O*-hexadecylglycerol (3.0 g, 54%) as a white solid (Found: MH$^+$, 541. $C_{35}H_{73}O_3$ requires *m/z* 541); δ_H (300 MHz) 0.88 (6 H, t, *J* 7 Hz, 2 × CH_3), 1.26 [52 H, m, 2 × $(CH_2)_{13}$], 1.56 (4 H, m, 2 × CH_2CH_2O), 3.4–3.75 (9 H, m, CHO and CH_2O).

1,2-Di-*O*-hexadecylglycerol (0.50 g, 0.924 mmol) was dissolved in dry diethyl ether (10 cm^3) under an atmosphere of dry nitrogen and treated with triethylamine (0.22 cm^3, 1.57 mmol) followed by the dropwise addition of a solution of 2-bromoethyl dichlorophosphate **8** (0.33 g, 1.57 mmol) in diethyl ether (5 cm^3) and the mixture was stirred at room temperature for 5 h. Further portions of triethylamine (0.22 cm^3) and the dichlorophosphate (0.66 g) were added and the mixture was heated to reflux for 16 h. A final addition of triethylamine (0.22 cm^3) and the dichlorophosphate (0.33 g) was made and the mixture was heated for a final 1 h. The reaction mixture was cooled, triethylamine (1.8 cm^3) and water (1 cm^3) were added, and the mixture was stirred for a further 2 h. The bulk of the ether was removed under reduced pressure and the residue was dissolved in dichloromethane (100 cm^3) and washed successively with water (2 × 100 cm^3) and brine (100 cm^3) before drying and filtering. Removal of the solvent afforded compound **10** as an oil, which was immediately dissolved in acetonitrile (28 cm^3) and chloroform (40 cm^3). The solution was heated with trimethylamine (1.5 g, 25 mmol) in a sealed vessel at 50 °C for 14 h. The solvents were then removed and the residue chromatographed through silica, and eluted with chloroform–methanol–25% ammonia in the proportions 690:270:64 to afford one major product. Fractions containing the product were combined, evaporated to dryness and azeotroped with benzene before drying over P_2O_5 *in vacuo* for 3 h to afford the title compound **11** (393 mg, 61%) as a hygroscopic solid (Found: MH$^+$, 706. $C_{40}H_{85}NO_6P$ requires, *m/z* 706); δ_H (1:20 CD_3OD–$CDCl_3$) 0.88 (6 H, t, *J* 7 Hz, 2 × CH_3), 1.26 (52 H, m), 1.54 (4 H, m), 3.42 [9 H, s, $(CH_3)_3N^+$], 3.57 (6 H, m), 3.88 (5 H, m), 4.33 (2 H, m); δ_C (1:20 CD_3OD–$CDCl_3$) 14.1, 22.7, 26.0, 29.9, 32.2, 54.6 [$(CH_3)_3N^+$], 58.8, 66.3, 68.6, 70.2, 71.9, 78.9.

2,3-Bis-*O*-(hexadecyloxy)propyl 6-(trimethylammonio)hexyl phosphate 12

This was prepared in a similar manner to the choline derivative

11, using 6-bromohexyl dichlorophosphate in place of the ethyl homologue. The title compound was obtained in 51% yield (Found: MH^+, 763. $C_{44}H_{93}NO_6P$ requires *m*/*z*, 763); δ_H (1:20 CD_3OD–$CDCl_3$) 0.88 (6 H, t *J* 7 Hz) 1.25 (52 H, m), 1.50 (8 H, m), 1.60 (2 H, m), 1.80 (2 H, m), 3.25 [9 H, s, $(CH_3)_3N^+$], 3.39–3.60 (9 H, m), 3.63 (4 H, m).

2,2-Bis(hexadecyloxymethyl)propan-1-ol 14

1,1,1-Tris(hydroxymethyl)ethane **13** (30 g, 0.25 mol) was added in portions to a stirred suspension of fresh sodium hydride (12 g, from 60% w/w suspension in mineral oil washed with hexane and dried under nitrogen; 0.5 mol) in DMF (200 cm^3). The mixture was stirred at room temperature for 1 h before the addition of bromohexadecane (167.7 g, 0.55 mol). The mixture was then heated, with stirring, at 50 °C for 44 h. After the mixture had cooled, water was added to destroy the residual sodium hydride and the mixture was then partitioned between dichloromethane (200 cm^3) and water (200 cm^3). The organic layer was washed successively with more water, dil. HCl (0.1 M) and brine before drying, filtering, and removal of solvent under reduced pressure to produce the product as a waxy solid (150 g, 98%). Portions of the product were purified as required by column chromatography through silica gel, and elution with 9:1 light petroleum–ethyl acetate. The *title product* showed δ_H ($CDCl_3$) 0.85 (3 H, s, MeC), 0.9 (6 H, t, *Me*CH_2), 1.26 (52 H, aliphatic H), 1.53 (4 H, m, OCH_2CH_2), 2.06 (1 H, s, *O*H), 3.41 (8H, OCH_2), 3.55 (2 H, s, $HOCH_2$); δ_C 13.8, 17.2, 22.3, 25.8, 29.0, 29.3, 31.5, 40.0, 69.5, 71.4, 75.1 (Found: C, 78.4; H, 14.0. $C_{37}H_{76}O_3$ requires C, 78.2; H, 13.5%).

2,2-Bis(hexadecyloxymethyl)propyl 2-bromoethyl phosphate 15

To a solution of the alcohol **14** (1 g, 1.75 mmol) in diethyl ether (20 cm^3), kept under an atmosphere of dry nitrogen, was added triethylamine (0.60 g, 6 mmol) and then, dropwise, bromoethyl dichlorophosphate **8** (1.26 g, 6 mmol). The mixture was heated to 50 °C for 16 h before cooling and the addition of triethylamine (0.6 g, 6 mmol) and water (4.09 g, 227 mmol); the mixture was then heated, with stirring, for a further 2 h at 50 °C. After cooling, the water layer was removed and the residue was dried by azeotroping with benzene (3 × 20 cm^3). The residue was dissolved in diethyl ether (20 cm^3) and the solution was filtered, and evaporated under reduced pressure to afford the crude product, which was further purified by column chromatography, and elution with chloroform and chloroform–methanol (5:1) mixtures to give the desired product **15** (0.32 g, 34%) as a waxy solid, δ_H (200 MHz, $CDCl_3$) 0.9 (9 H, m, 3 × Me), 1.25 (52 H, m, aliphatic H), 1.50 (4 H, m, OCH_2CH_2), 3.25 (4 H, s, OCH_2C), 3.38 (4 H, t, OCH_2CH_2), 3.51 (2 H, t, $BrCH_2$), 3.75 (2 H, s, $POCH_2$), 4.2 (2 H, m, $BrCH_2CH_2OP$). The bromoethyl ester was used, without further characterisation, to prepare the derivatives **17–20**.

General method for reaction of 15 with nucleophiles

The nucleophile (20–30 mmol) was added to a solution of the bromoethyl ester **15** (1 mmol) in dry chloroform (10 cm^3) and the solution was stirred at 60 °C for 16 h, following the disappearance of the starting halide by TLC. The reaction mixture was cooled, the solvent and excess of nucleophile removed by evaporation under reduced pressure, and the residue chromatographed through silica gel using chloroform–methanol–water mixtures, generally in the range 65:25:4 respectively.

Trimethylammonium derivative 17. Carried out on 0.33 mmol scale, acetonitrile (10 cm^3) was used as a co-solvent with chloroform (2.5 cm^3) for the trimethylamine (0.52 g, 9.9 mmol). The *product* (240 mg, 38%) showed δ_H ($CDCl_3$–CD_3OD) 0.88 (6 H, t, CH_2Me), 0.95 (3 H, s, *Me*C), 1.25 (52 H, m, aliphatic H), 1.42–1.52 (4 H, m, OCH_2CH_2), 3.26 (4 H, s), 3.34 (4 H, t, *J* 6 Hz), 3.42 [9 H, s, $(CH_3)_3N^+$], 3.67 (2 H, d, *J* 5 Hz), 3.84 (2 H, s), 4.3 (2 H, s); δ_C 14.1, 17.1, 22.6, 26.2, 29.3, 29.7, 31.9, 40.7, 54.4, 59.0, 66.4, 68.6, 71.6, 72.9 (Found: MH^+, 735.6507. $C_{42}H_{89}NO_6P$ requires *m*/*z*, 735.6506).

Quinuclidinium derivative 18. Elution from the column with chloroform–methanol–water (70:25:5, v/v) afforded the *product* (23%); δ_H ($CDCl_3$–CD_3OD), 0.88 (6 H, t, *Me*CH_2), 0.95 (3 H, s, MeC), 1.25 (53 H, m, aliphatic H), 1.49 (4 H, m, OCH_2CH_2), 2.02 (6 H, m, $CH_2CH_2N^+$), 3.29 (4 H, s, OCH_2C), 3.36 (4 H, t, OCH_2CH_2), 3.46 (2 H, m, $N^+CH_2CH_2$), 3.59 (6 H, t, CH_2N^+), 3.71 (2 H, d, $POCH_2C$), 4.21 (2 H, m, $N^+CH_2CH_2OP$); δ_C ($CDCl_3$) 14.09 (CH_3CH_2), 17.13 (CH_3C), 19.65 ($HCCH_2CH_2N^+$), 22.66 (CH_2CH_3), 24.05 ($HCCH_2CH_2N^+$), 26.19 ($OCH_2CH_2CH_2$), 26.94 [$(CH_2)_{10}$ + OCH_2CH_2], 31.90 ($CH_2CH_2CH_3$), 40.7 (C), 55.50 ($HCCH_2CH_2N^+$), 58.29 (CH_2CH_2OP), 64.63 ($POCH_2C$), 68.46 ($N^+CH_2CH_2OP$), 71.60 (OCH_2CH_2) and 73.03 (CCH_2O) (Found: C, 66.9; H, 11.9; N. 1.7; P, 4.2. $C_{46}H_{92}NO_6P{\cdot}2H_2O$ requires C, 67.1; H, 11.8; N, 1.7; P, 3.8%).

Dimethylammonium derivative 19. The product was chromatographed twice, eluting from the column with 9:1 chloroform–methanol, to give the *dimethylammonium derivative* (22%); δ_H ($CDCl_3$–CD_3OD) 0.88 (6 H, t, *Me*CH_2), 0.95 (3 H, s, MeC), 1.25 (52 H, m, aliphatic H), 1.49 (4 H, OCH_2CH_2), 2.89 (6 H, s, Me_2NH^+), 3.25 (2 H, m, CH_2N^+), 3.28 (4 H, s, OCH_2C), 3.35 (4 H, t, OCH_2CH_2), 3.75 (2 H, $POCH_2C$), 4.2 (2 H, m, CH_2CH_2OP) (Found: M^+, 719.6192. $C_{41}H_{86}NO_6P$ requires *M*, 719.6208).

Pyridinium derivative 20. The product was chromatographed using 70:25:5 chloroform–methanol–water as eluent, to give the *product* (9%); δ_H ($CDCl_3$) 0.88 (6 H, t, *Me*CH_2), 0.93 (3 H, s, *Me*), 1.25 (52 H, m, aliphatic H), 1.48 (4 H, t, OCH_2CCH_2), 3.27 (4 H, s, OCH_2C), 3.35 (4 H, t, OCH_2CH_2), 3.67 (2 H, d, $POCH_2C$), 4.26 (2 H, m, $N^+CH_2CH_2C$), 4.82 (2 H, t, $N^+CH_2CH_2OP$), 8.04 (2 H, t, $CHCHN^+$), 8.44 (1 H, t, $CHCHCHN^+$), 9.06 (2 H, d, CHN^+); δ_C ($CDCl_3$–CD_3OD) 14.05 (CH_2CH_3), 16.97 (CH_3C), 22.61 (CH_2CH_3), 26.11 ($OCH_2CH_2CH_2$), 29.64 [$(CH_2)_{10}$ + OCH_2CH_2), 31.85 ($CH_2CH_2CH_3$), 40.6 (C), 62.18 ($N^+CH_2CH_2OP$), 68.5 ($POCH_2C$), 71.6 (OCH_2CH_2), 72.8 (CCH_2O), 128.06 (CH:CHN^+) and 145.5 (CHN^+ + $HCCH$:CHN^+); *m*/*z* (FAB) 754 (M + H^+, 56%) (Found: C, 67.1; H, 11.4; N, 1.8; P, 3.8. $C_4H_{84}NO_6P{\cdot}2H_2O$ requires C, 66.9; H, 11.2; N, 1.8; P, 3.9%).

6-(Triphenylphosphonio)hexyl phosphate analogue 21

6-Bromohexyl dichlorophosphate 8 (*n* = 6). Distilled phosphoryl trichloride (0.85 g, 5.5 mmol) in dichloromethane (10 cm^3) was added dropwise to a solution of 6-bromohexan-1-ol (1 g, 5.5 mmol) in dichloromethane (10 cm^3) under nitrogen. The mixture was left for 16 h at room temperature before removal of the solvent and excess of phosphoryl trichloride under reduced pressure. The crude product was used in subsequent steps.

6-Bromohexyl 2,2-bis(hexadecyloxymethyl)propyl hydrogen phosphate 16. 2,2-Bis(hexadecyloxymethyl)propan-1-ol **14** (1.00 g, 1.76 mmol) was dissolved in dry diethyl ether (50 cm^3) and to this was added a mixture of triethylamine (0.35 g, 3.5 mmol) and 6-bromohexyl dichlorophosphate **8** (*n* = 6) (1.05 g, 3.6 mmol) in dry diethyl ether (20 cm^3). The reaction mixture was stirred for 48 h at room temperature before addition of more triethylamine (0.35 g, 3.5 mmol) and water (1 cm^3) and the mixture was heated to reflux for 3 h before being cooled to room temperature. The aqueous layer was removed and the organic layer evaporated under reduced pressure. The residue was azeotroped with benzene before removal of the solvent and drying of the residue under reduced pressure. Trituration

of the crude product with diethyl ether afforded, as a precipitate, the *title phosphate* (1.40 g, 98%). This was used directly in the next step. The obtained material showed δ_H (200 MHz; $CDCl_3$) 0.9 (9 H, m, Me), 1.25 (52 H, m, aliphatic H), 1.4–1.6 (8 H, m, CH_2), 1.9 (4 H, m), 3.28 (4 H, s, CCH_2O), 3.3–3.6 (8 H, m, CH_2), 4.05 (2 H, m, CH_2OP).

The bromide **16** (1.66 g, 1.9 mmol) was dissolved in dry chloroform (40 cm^3) and a solution of triphenylphosphine (2.32 g, 8.8 mmol) in acetonitrile (20 cm^3) was added. The solution was sealed in a pressure vessel under nitrogen and heated at 70 °C for 16 h. More triphenylphosphine (2.32 g, 8.8 mmol) was added and the solution reheated for a further 16 h. The crude material obtained after removal of solvent was chromatographed through silica gel, and eluted with chloroform and then 690:270:64 chloroform–methanol–conc. ammonia to afford the crude major product. The material was further purified by PLC to afford the pure *triphenylphosphonium analogue* **21** (17 mg, 3.1%) (Found: MH^+, 993.7229. $C_{61}H_{103}O_6P_2$ requires *m*/*z*, 993.7199); δ_H ($CDCl_3$–CD_3OD) 0.88 (9 H, 3 × Me), 1.25 [52 H, br s, 2 × $(CH_2)_{13}$], 1.36–1.48 [8 H, m, 2 × OCH_2CH_2 + $(CH_2)_2CH_2CH_2OP$], 1.61 [4 H, m, $CH_2(CH_2)_2CH_2CH_2OP$], 3.20 (4 H, s, 2 × CCH_2O), 3.29 (4 H, t, 2 × OCH_2CH_2), 3.47 (2 H, m, P^+CH_2), 3.67 (2 H, d, $POCH_2$), 3.80 (2 H, m, CH_2CH_2OP), 7.7–7.8 (15 H, ArH); δ_C ($CDCl_3$–CD_3OD) 13.34 (CH_2CH_3), 16.48 (CH_3C), 18.21 (CH_2P^+), 21.20 ($CH_2CH_2P^+$), 22.0 (CH_2CH_3), 24.46 [$(CH_2)_2(CH_2)_2OP$], 25.69 ($OCH_2CH_2CH_2$), 29.18 [$(CH_2)_{10}$ + OCH_2CH_2 + CH_2CH_2OP], 31.42 ($CH_2CH_2CH_3$), 40.2 (C), 64.4 (CH_2OP), 67.83 ($POCH_2C$), 71.19 (OCH_2CH_2), 72.48 (CCH_2O), 117 (CP^+), 130.0 ($CHCHCP^+$), 132.9 ($CHCP^+$) and 134.9 [$CH(CH_2)_2CP^+$]; δ_P (121 MHz, $CDCl_3$–CD_3OD) −0.99 (1 P, s), 25.01 (1 P, s).

Preparation of the parent 2-(triphenylphosphonio)ethyl derivative was accomplished in very small quantity and the material was not fully characterised.

^{13}C-Labelled trimethylammonium derivative 17

2-Bromo[1-^{13}C]ethanol. To a stirred solution of bromoacetic-[^{13}C] acid (0.5 g, 3.6 mmol; 99% isotopic abundance) in dry tetrahydrofuran (5 cm^3), at 0 °C under nitrogen, was added borane–tetrahydrofuran complex (1 M; 4.3 cm^3, 4.3 mmol) dropwise during 10 min. After stirring for a further 20 min at 0 °C the solution was warmed to room temperature and stirred for a further 64 h. Aq. HCl (3 M; 1.5 cm^3) was added and the solution stirred for a further 90 min before addition of more water (10 cm^3) and extraction into diethyl ether (4 × 10 cm^3); the extract was dried, filtered, and the volume reduced by distillation under atmospheric pressure to ≈10 cm^3. The solution was diluted with unlabelled bromoethanol (1.35 g) and the rest of the solvent was removed before microdistillation of the product to give labelled bromoethanol (1.03 g; 24% isotopic abundance); δ_C (50 MHz; $CDCl_3$) 35.5 (H_2CBr), 62.5 (H_2COH).

2-Bromo[1-^{13}C]ethyl dichlorophosphate ^{13}C-8. A solution of the labelled bromoethanol (1.03 g, 8.2 mmol) in dichloromethane (5 cm^3) was added, dropwise, to a solution of freshly distilled phosphoryl trichloride in dichloromethane (5 cm^3) and the solution was stirred for 16 h under nitrogen. The solvent was removed and the residue distilled to give the dichlorophosphate as a pale yellow liquid (0.551 g; 26% isotopic abundance).

Preparation of ^{13}C-labelled ester 25. To the labelled chloride ^{13}C-**8** (0.27 g, 1.2 mmol) was added dropwise, over a period of 1 min, a solution of the alcohol **14** (0.40 g, 0.71 mmol) and triethylamine (0.12 g, 1.2 mmol) in diethyl ether (10 cm^3). The mixture was stirred, whilst warming to 40 °C, for 2 h. After cooling, the solvent was removed and the residue azeotroped with benzene. The dry residue was triturated with diethyl ether and filtered, when evaporation afforded the required ester **25** (0.50 g, 93%), δ_C ($CDCl_3$) 14.12 (H_2CMe), 17.62 (*C*Me), 22.71 ($MeCH_2$), 26.18, 29.71 and 31.93 (CH_2), 45.72 (CH_2Br), 65.71 and 66.81 (CH_2OP), 71.84 and 75.56 (CH_2O).

The labelled bromide **25** (0.25 g, 0.33 mmol) as a solution in chloroform (2.5 cm^3) was treated with a solution of trimethylamine (0.59 g, 9.9 mmol) in acetonitrile (10 cm^3) in a pressure bottle and heated to 60 °C for 16 h. After cooling to room temperature some of the solvent was removed under reduced pressure until a precipitate just started to form. The mixture was then cooled to −20 °C for 20 min and the precipitate collected (0.43 g) before purification by column chromatography through silica gel, and elution with chloroform and then 690:270:64 chloroform–methanol–ammonia to afford the *labelled ester* **17** (32 mg, 13%; 24% isotopic abundance). The ^{13}C NMR spectrum indicated all the label was in the starting position, δ_C ($CDCl_3$) 14.0 ($MeCH_2$), 17.0 (*Me*C), 22.6 ($MeCH_2$), 26.1, 29.6 and 31.9 (CH_2), 40.7 (C), 54.3 (Me_3N^+), 59 (CH_2OP), 66.5 (CCH_2OP), 68.5 ($Me_3N^+CH_2$), 71.6 and 72.8 (CCH_2O) [Found: M^+(^{13}C), 736.6524. $^{12}C_{41}{}^{13}CH_{90}NO_6P$ requires *M*, 736.6539].

2,2-Bis(hexadecyloxymethyl)propyl dichlorophosphate 24

To a solution of freshly distilled phosphorus oxychloride (0.4 g, 2.64 mmol) dissolved in ether (10 cm^3) was added, at room temperature, a solution of triethylamine (0.18 g, 1.76 mmol) and 2,2-bis(hexadecyloxymethyl)propanol (1.00 g, 1.76 mmol) in ether (10 cm^3). The mixture was then stirred at 40 °C for 16 h in a sealed vessel before cooling to room temperature, filtering off the precipitated triethylammonium chloride and concentrating the filtrate under reduced pressure. The labile acid halide **24** (0.85 g, 70%) was used without further purification. The viscous oil showed δ_H (200 MHz, $CDCl_3$) 0.85 (6 H, t, $MeCH_2$), 1.0 (3 H, s, *Me*), 1.25 (52 H, m, CH_2), 1.5 (4 H, m, OCH_2CH_2), 3.25 (4 H, s, CCH_2O), 3.35 (4 H, t, OCH_2CH_2), 4.26 (2 H, d, $POCH_2$).

Triethylammonioethyl phosphate analogue 26

2-Bromoethanol (2.0 g, 16 mmol) and triethylamine (1.62 g, 16 mmol) were heated in dichloromethane (40 cm^3) at reflux for 16 h. The resultant precipitate was filtered off and dried over phosphorus pentaoxide *in vacuo* to afford 2-triethylammonioethanol bromide (1.47 g, 41%), δ_H (D_2O) 1.3 (9 H, t, Me), 3.4 (8 H, m, CH_2N^+), 4.0 (2 H, m, CH_2OH).

The salt (0.18 g, 1.23 mmol) and triethylamine (0.12 g, 1.23 mmol) in diethyl ether (10 cm^3) were added to a solution of the dichlorophosphate **24** (0.843 g, 1.23 mmol) in diethyl ether (5 cm^3). The solvent was removed and replaced with acetonitrile (20 cm^3), and the solution heated to reflux for 16 h. Water (1.33 g, 74 mmol) and triethylamine (0.12 g, 1.23 mmol) were added and the mixture was heated to reflux for a further 2 h, before being evaporated to dryness, with azeotroping with benzene to remove the water. The residue was chromatographed through silica gel, and eluted with 690:270:64 chloroform–methanol–ammonia, to give the required *title product* (14 mg, 1.5%); δ_H ($CDCl_3$) 0.88 (6 H, t, $MeCH_2$), 0.93 (3 H, s, Me), 1.26 (52 H, m, aliphatic H), 1.36 (9 H, m, $MeCH_2N^+$), 1.50 (4 H, t, OCH_2CH_2), 3.26 (4 H, s, CCH_2O), 3.35 (4 H, t, OCH_2CH_2), 3.48 (6 H, m, CH_2N^+), 3.66 (4 H, m, CH_2N^+, CCH_2OP), 4.29 (2 H, m, $N^+CH_2CH_2OP$) (Found: MH^+, 776.6865. $C_{45}H_{95}NO_6P$ requires *m*/*z*, 776.6897).

2-(Dimethylsulfonio)ethyl phosphate analogue 27

2-(Dimethylsulfonio)ethyl toluene-4-sulfonate (0.49 g, 1.75 mmol), the dichlorophosphate **24** (1.20 g, 1.75 mmol) and triethylamine (0.18 g, 1.75 mmol) were heated in acetonitrile (10 cm^3) at 50 °C for 16 h. The reaction mixture was cooled, and concentrated in a stream of nitrogen, before chromatography through silica gel, and elution with chloroform and then

690:270:64 chloroform–methanol–ammonia. The fractions containing the product were evaporated and the solid residue was triturated with hexane (5 × 10 cm^3) and acetone (10 × 10 cm^3) before the solid precipitate was collected by centrifugation. The solid was dried over phosphorus pentaoxide to afford the *sulfonium product* (40 mg, 3%), δ_H ($CDCl_3$–CD_3OD) 0.88 (6 H, t, *Me*), 0.95 (3 H, s, Me), 1.25 (52 H, m, aliphatic H), 1.49 (4 H, m, OCH_2CH_2), 3.04 (6 H, s, Me_2S^+), 3.28 (4 H, s, C CH_2O), 3.36 (4 H, t, OCH_2CH_2), 3.58 (2 H, m, S^+CH_2), 3.71 (2 H, d, $POCH_2C$), 4.26 (2 H, m, $POCH_2CH_2$); δ_C (CD_3OD–$CDCl_3$) 14.0, 17.0, 22.9, 26.5, 27.0, 30.0, 32.0, 41.2, 46.4, 59.5, 69.0, 72.3, 73.5 [Found: (M + H^+), 737.5904; C, 66.8; H, 11.6; S, 4.3. $C_{41}H_{85}O_6SP$ requires *M* + H, 737.5882; C, 66.8; H, 11.6; S, 4.35%].

2-(Hexyldimethylammonio)ethyl phosphate analogue 28

2-(Hexyldimethylammonio)ethyl toluene-4-sulfonate (0.302 g, 0.44 mmol), prepared from 2-(4-tolylsulfonyloxy)ethanol and hexyldimethylamine, the chlorophosphate ester **24** (0.302 g, 0.44 mmol) and triethylamine (0.044 g, 0.44 mmol) were heated in dichloromethane (5 cm^3) in a pressure bottle at 50 °C for 16 h. Water (1 cm^3) was then added together with triethylamine (0.044 g, 0.44 mmol) and the mixture was heated at 50 °C for a further 6 days. The reaction mixture was evaporated and the residue chromatographed through silica gel, eluting with, first, chloroform and then with 690:270:64 chloroform–methanol–ammonia. The fractions containing the phosphatidylcholine analogue were combined, dried and the residual solid triturated with acetone to give the title product (7 mg, 2%), δ_H ($CDCl_3$–CD_3OD) 0.80–0.95 (12 H, m), 1.25 (52 H, aliphatic H), 1.35 (6 H, m, CH_2), 1.51 (4 H, OCH_2CH_2), 1.65 (2 H, m CH_2), 3.15 (6 H, s, Me_2N^+), 3.22 (4 H, s, CH_2O), 3.35 (6 H, m, CH_2O and CH_2N^+), 3.60 (2 H, m, N^+CH_2), 3.70 (2 H, m, $POCH_2OP$), 4.23 (2 H, m, CH_2OP) (Found: MH^+, 804.7203. $C_{47}H_{99}NO_6P$ requires *m*/*z*, 804.7210).

3-(Trimethylammonio)phenyl phosphate analogue 29

3-(Dimethylamino)phenol was purified by recrystallisation from boiling water several times before use; activated charcoal was used to decolourise the material.

A solution of the phenol (2 g, 14.6 mmol) in dichloromethane (10 cm^3) was added to a stirred solution of phosphoryl trichloride (2.24 g, 14.6 mmol) in dichloromethane (20 cm^3) over a period of 30 min and the mixture stirred under nitrogen for 16 h. A further portion of phosphoryl trichloride was then added (2.24 g, 14.6 mmol) and stirring was continued for a further 2 h before removal of the solvent and excess of the reagent under reduced pressure. The residue was dried at a high vacuum pump before triturating with light petroleum and drying (under reduced pressure) to produce 3-(dimethylamino)-phenyl dichlorophosphate as the hydrochloride salt (2.37 g, 56%), δ_H ($CDCl_3$) 2.9 (6 H, s, Me_2N), 6.2, 6.35 and 7.1 (4 H, ArH). This material was unstable to storage in air and so was used immediately after preparation.

To a solution of the ether alcohol **14** (2.00 g, 3.5 mmol) in dichloromethane (10 cm^3) containing triethylamine (0.71 g, 7.0 mmol) was added 3-(dimethylamino)phenyl dichlorophosphate (3.55 g, 12.2 mmol), described above. The reaction mixture was heated under nitrogen at 50 °C for 20 h. Water (3.78 g, 210 mmol) and triethylamine (0.71 g, 7 mmol) were added and the mixture reheated to 50 °C for a further 5 h. The organic layer was collected, the solvent removed under reduced pressure, and the residue azeotroped with benzene to give a gelatinous product. The solvent was removed, the residue was dissolved in chloroform (100 cm^3), and the solution washed with water (2 × 10 cm^3); emulsions formed during this step and were left to settle for 16 h. The organic layer was dried, the solvent removed, and the residue again azeotroped with benzene before drying (under reduced pressure) to afford a waxy residue (1.86 g). This was chromatographed through silica gel with 5:1 chloroform–methanol as eluent to give 2,2-bis(hexadecyloxymethyl)propyl 3-(dimethylamino)phenyl hydrogen phosphate (350 mg, 13%); δ_H 0.85 (9 H, m), 1.25 (52 H, m), 1.4 (4 H, m), 2.8 (6 H, s), 2.9–3.35 (8 H, m), 3.75 (2 H, m), 6.35 (1 H, m), 6.65 (2 H, m), 7.0 (1 H, s). This material was used in the next step without further purification.

Potassium carbonate (0.08 g, 0.64 mmol) and 18-crown-6 (0.17 g, 0.64 mmol) were stirred at room temperature for 20 min in dichloromethane (4 cm^3) and to this mixture was added a solution of the above dimethylaminophenyl phosphate (0.35 g, 0.45 mmol) in dichloromethane (4 cm^3), followed by iodomethane (8 cm^3). The reaction mixture was sealed under nitrogen and heated at room temperature for 40 h. The solvent and excess of iodomethane were removed under reduced pressure; the residue was dissolved in acetonitrile (10 cm^3) and the solution was filtered and then dried. The solid product was purified by PLC on silica gel with chloroform–methanol–water (65:25:4) as eluent. The major band was collected by extraction with chloroform–methanol and evaporated to give a solid (50 mg), which was re-dissolved in chloroform (1 cm^3), and acetone was added dropwise to precipitate the solid. The solid was collected, and dried over phosphorus pentaoxide as the title compound (14.9 mg, 4.2%); δ_H 0.88 (9 H, m), 1.25 (52 H, m), 1.48 (4 H, m), 3.25 (4 H, s), 3.31 (4 H, t, *J* 7 Hz), 3.71 (9 H, s), 3.86 (2 H, d, *J* 3 Hz), 7.25–7.5 (3 H, m) and 7.92 (1 H, s); δ_C 14.10, 17.10, 22.66, 26.16m, 29.69, 31.90, 40.70, 57.22, 69.01, 71.55, 72.89, 112.40, 122.63, 130.56, 147.30, 155.40 (Found: MH^+, 782.6421. $C_{46}H_{89}NO_6P$ requires *m*/*z*, 782.6427).

N-Methylpyridinium-3-yl phosphate analogue 30

3-Hydroxypyridine (0.5 g, 5.2 mmol) and methyl toluene-4-sulfonate (1.47 g, 7.9 mmol) were heated in acetonitrile (15 cm^3) at 50 °C for 16 h in a sealed vessel. The resulting precipitate was collected, and dried over phosphorus pentaoxide *in vacuo* for 48 h. The solid was suspended in acetonitrile (20 cm^3) and the suspension shaken with Amberlite IRA 401 ion-exchange resin [6 g, preactivated and washed with 1 M aq. sodium hydroxide (1 dm^3), distilled water (1 dm^3) and acetone (1 dm^3)] for 4 h. The mixture was filtered and the filtrate evaporated to give *N*-methyl-3-oxidopyridinium betaine **31** as a solid. This was stored *in vacuo* over phosphorus pentaoxide and used as soon as possible after preparation.

Triethylamine (0.18 g, 1.73 mmol) and the betaine **31** (0.29 g, 2.6 mmol) were dissolved in a mixture of dry acetonitrile (10 cm^3) and diethyl ether (10 cm^3) and the solution was added dropwise to a solution of the dichlorophosphate **24** (1.19 g, 1.78 mmol) in acetonitrile (10 cm^3) at room temperature, producing an immediate precipitate. The reaction mixture was heated to 40 °C in a sealed vessel for 16 h before addition of water (5 ml) and triethylamine (0.18 g, 1.73 mmol) and re-heating the mixture to 40 °C for a further 2 h. The solvents were removed under reduced pressure and the residue azeotroped with benzene, before removal of the solvent and drying the residue *in vacuo* to give crude product (1.8 g). This was chromatographed through silica and eluted with chloroform–methanol–water (65:25:4). The initially enriched fractions containing the product were rechromatographed, using chloroform–methanol–0.88 ammonia (690:270:64) as eluent, to yield the *title compound* (242 mg, 19%) δ_H (CD_3OD–$CDCl_3$) 0.88 (6 H, t, *J* 7 Hz), 0.94 (3 H, s), 1.25 (52 H, m), 1.49 (4 H, m), 3.28 (4 H, s), 3.34 (4 H, t, *J* 7 Hz), 3.86 (2 H, d *J* 3 Hz), 4.41 (3 H, s), 7.80 (1 H, m), 8.20 (1 H, m), 8.27 (1 H, m), 8.87 (1 H, s); δ_C (CD_3OD–$CDCl_3$) 14.0, 16.99, 22.58, 26.08, 29.27–29.6, 31.85, 40.7, 48.63, 69.51, 71.57, 72.76, 127.94, 135.75, 137.96, 154.11 (Found: C, 67.9; H, 11.1; N, 2.0; P, 3.9. $C_{43}H_{82}NO_6P\cdot H_2O$ requires C, 68.1; H, 11.2; N, 1.85; P, 4.0%).

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