# Preparation of phospholipid analogues using the phosphoramidite route

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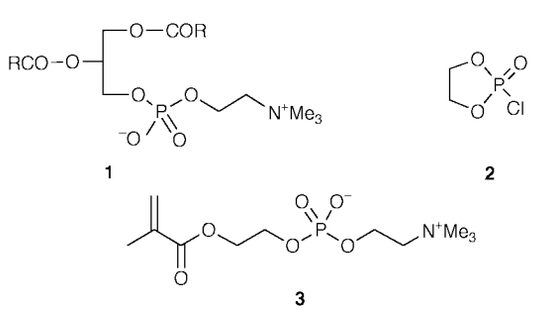
The phosphoramidite route has been used to prepare phospholipid analogues possessing biocompatible properties and the monomer 2-(methacryloyloxy)ethylphosphorylcholine, utilised in the preparation of biocompatible polymers. Modifications to established methodology include, as an alternative to the thermally unstable tetrazole, the use of 4,5-dichloroimidazole as an acid catalyst for preparing phosphite esters from the corresponding phosphoramidites, and the use of trimethylamine *N*-oxide as oxidant for the conversion of the phosphite esters into the corresponding phosphates.

Although the use of phosphite triesters[1] and their preparation from phosphoramidite esters[2] is well established for the formation of the phosphate linkages in deoxyribonucleic acid and ribonucleic acid synthesis, relatively little work has been reported on their use in the preparation of phospholipids.[3] One reason for this gap is that relatively few variants in the hydrophilic head groups of the phospholipids occur in nature and established routes to these have been developed. For example, for the phosphorylcholine group **1**, phospholane intermediates such as **2** are used.[4]

In our work aimed at exploring structure–activity relationships in the biocompatibility behaviour of phospholipids,[4] a variety of analogues of the trimethylammonium and glycerol units in **1** were required for which the established methods were not always easily adapted. Furthermore, use of the traditional methods for making monomers of the type **3**, utilised in the preparation of biocompatible polymers,[5] were often found unsuitable because of the attendant uncontrolled polymerisation of the methacrylyl ester intermediates. A study of the use of the phosphoramidite approach to prepare **3** was therefore made.

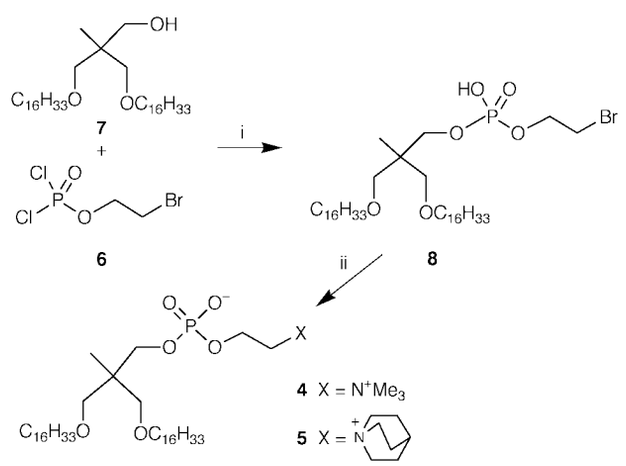



## Results and discussion

In order to establish the phosphoramidite methodology in this area, initial targets selected were the phospholipid analogues **4** and **5**, which had been previously prepared from the corresponding dichlorophosphate **6** and the alcohol **7**[4] by the route depicted in Scheme 1, *via* the bromoalkyl derivative **8**. Overall yields from the starting bromo alcohols were modest, at best, mainly owing to the difficulty in obtaining the dichlorophosphate intermediate **6** in a pure state and the subsequent problem of isolation of the pure products from the crude reaction mixtures obtained (Table 1).

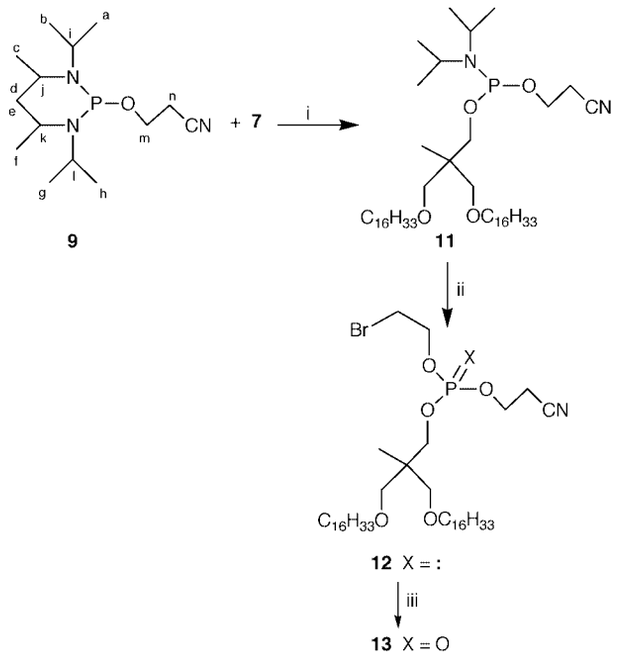
**Table 1** Typical overall yield for compounds prepared by either chlorophosphate or phosphoramidite chemistry

| Compound | Phosphorylating agent | Yield (%) |
|---|---|---|
| **4** | **6** | 38 |
| **4** | **11** | 48 |
| **5** | **6** | 1 |
| **5** | **11** | 59 |
| **15** | hexyl analogue of **6** | 3 |
| **15** | **11** | 40 |




**Scheme 1** *Reagents*: i, $NEt_3$; ii, $Me_3N$.

The phosphoramidite reagent, 2-cyanoethoxybis(diisopropylamino)phosphine **9**, was prepared by the literature method.[6] Coupling of the phosphoramidite **9** to the tris(hydroxymethyl)ethane bishexadecyl ether **7** in the presence of diisopropylammonium tetrazolide **10** as catalyst,[7] in dichloromethane, was accomplished within minutes, under rigorously anhydrous conditions, to form the mixed diester **11** (Scheme 2).



**Scheme 2** *Reagents*: i, diisopropylammonium tetrazolide **10**; ii, 1*H*-tetrazole and 2-bromoethanol; iii, MCPBA.

The diester was isolated and immediately converted to the mixed triester **12** (86% yield), by reaction with 2-bromoethanol, this reaction again being catalysed by 1*H*-tetrazole. Oxidation of the phosphite ester **12** with 3-chloroperbenzoic acid (MCPBA) in dichloromethane afforded the corresponding phosphate **13**, which was readily deprotected to form the dialkyl phosphate **8**, shown to be much purer than material obtained by the dichlorophosphate route (Scheme 1). Subsequent reaction of **8** with trimethylamine in a sealed tube afforded the phosphorylcholine analogue **4**. The overall yield by this route, although involving more steps, was 48%, higher than that obtained by the standard route (Table 1). Similar quaternisation of the bromide **8** with quinuclidine also gave a good yield of the analogue **5**.

The generality of the method was further exemplified by the synthesis of the triphenylphosphonium analogue **15**. For this preparation, 6-bromohexan-1-ol was initially quaternised with triphenylphosphine to give the salt **14**. Reaction of this alcohol with the phosphoramidite **11**, using tetrazole as catalyst, afforded the corresponding phosphite, which was immediately oxidised with MCPBA to the phosphate and treated with ammonia as base to effect elimination of the cyanoethyl group to afford the desired phosphate **15** in reasonable overall yield (Table 1).

These three syntheses illustrated the usefulness of the phosphoramidite route in making phospholipid analogues. Attention was then turned to the more challenging target of preparing the unstable 2-(methacryloyloxy)ethylphosphorylcholine monomer (HEMA-PC) **3**.

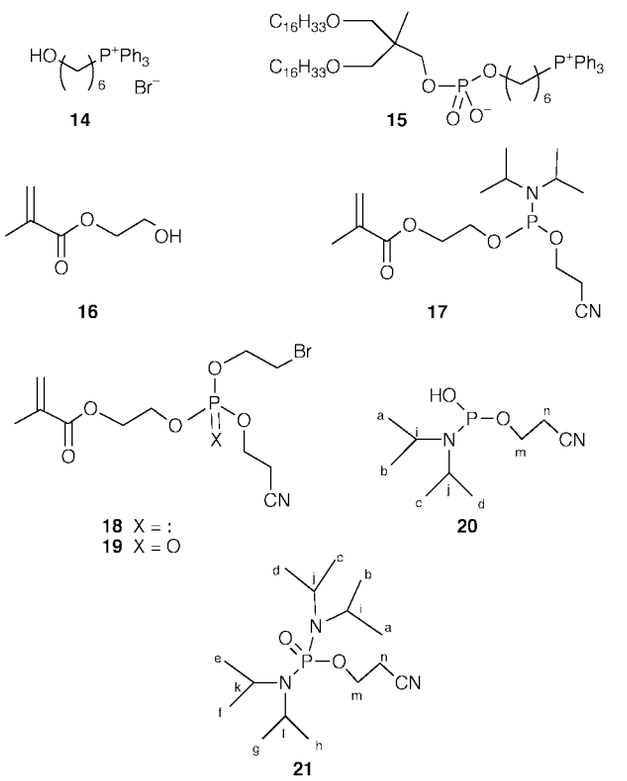
A route similar to that described in Scheme 2 was initially investigated. Thus the methacrylate ester **16** was treated with the phosphoramidite **9** using the tetrazolium salt **10** as catalyst. The initial ester **17** produced was then coupled with bromoethanol to give the triester **18**, which was oxidised with MCPBA, in dichloromethane, to give the corresponding phosphate **19** before deprotection and quaternisation with trimethylamine to give the required ester **3**. The overall yield was poor (≈10%), the crude material often undergoing rapid gelation. Means for improving on this overall conversion and the purity of the HEMA-PC were therefore required.

Two problems were encountered in this synthesis. First, the phosphoramidite **9** was partly hydrolysed by the ingress of water, despite vigorous drying of solvents and reagents by

**Table 2** $^1$H NMR spectra[a] ($\delta$, integration) of compounds **9**, **20** and **21**

| | Compound | | |
|---|---|---|---|
| Protons | **9** | **20** | **21** |
| a–h | 1.18, 24 H | 1.30, 12 H | 1.25, 24 H |
| n | 2.6, 2 H | 2.8, 2 H | 2.75, 2 H |
| i–l | 3.5, 4 H | 3.55, 2 H | 3.5, 4 H |
| m | 3.8, 2 H | 4.2, 2 H | 4.15, 2 H |

[a] Spectra run at 200 MHz, using $CDCl_3$ as solvent with TMS as internal standard. Letters refer to the proton assignments (see structures).



standard methods,[8] during the attempted coupling to 2-hydroxyethyl methacrylate **16**, to produce the corresponding phosphite **20**, a process catalysed by the tetrazolium salt **10** and which itself, because of its thermal instability,[6] was extremely difficult to obtain in a completely anhydrous state. The second problem was that complete removal of the trimethylamine hydrobromide, produced during quaternisation, from the HEMA-PC **3** proved difficult and the presence of this salt created instability in the target methacrylate ester, causing uncontrolled polymerisation.

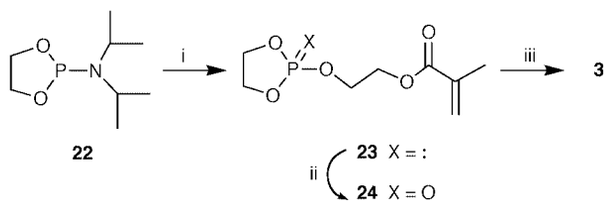
The phosphite **20** was readily identified by its characteristic $^1$H NMR spectrum (Table 2). Exposure of the phosphoramidite **9** to air resulted in absorption of water to give mainly the phosphite **20** rather than aerial oxidation to the phosphate **21**.

Reduction in the undesired hydrolytic side reaction was achieved by using smaller volumes of acetonitrile and by replacing the tetrazolium salt **10** as the acid catalyst. Several alternative acid catalysts were tested, including acetic acid, 1,2,4-triazole and 4,5-dichloroimidazole. The last acid catalyst proved to be equally effective as the tetrazole system as the acid catalyst, giving cleaner reaction products. Acetonitrile as solvent also had another advantage since the product diisopropylammonium dichloroimidazolide produced during the coupling is almost completely insoluble in this solvent and could be removed by simple filtration rather than, as in the original process, by repeated aqueous extractions when using dichloromethane as solvent.

Whilst the hydrolysis problem had been largely overcome, the second problem, removal of trimethylammonium hydro-

bromide produced in the last step, proved difficult and, as a consequence, the HEMA-PC produced had limited stabilty on storage, undergoing uncontrolled polymerisation. Use of ion-exchange resins, 4 Å molecular sieves, or sodium trimethylsilanate failed to give purer material. Although column chromatography through silica gel, using dichloromethane as eluent, was successful, recoveries of HEMA-PC were still low (<30% overall) and column chromatography was not acceptable for use on an industrial scale.

A modified route to HEMA-PC was finally adopted. In this route, the cyclic ethylene phosphite ester **22** was initially prepared, and reacted with 2-hydroxyethyl methacrylate **16** in acetonitrile, using 4,5-dichloroimidazole as catalyst, with cooling of the mixture, and filtering off of the diisopropylammonium dichlorimidazolide salt that precipitated out, to produce the cyclic phosphite **23** (Scheme 3), followed by oxid-




**Scheme 3** *Reagents*: i, **16** and 4,5-dichloroimidazole; ii, $Me_3NO$; iii, $NMe_3$.

ation to the phosphate **24** and then reaction with trimethylamine to form the phosphorylcholine unit, thus avoiding the formation of trimethylammonium bromide as a side product. The HEMA-PC product **3** formed in this manner was contaminated only with a small amount of residual dichloroimidazolide salt (<3%), a quantity that did not cause the rapid deterioration in the monomeric product.

One further modification of the process was also investigated. This concerned the oxidant used for the conversion of the intermediate phosphite **23** into the corresponding cyclic phosphate ester **24**. Whilst the use of MCPBA for this step was efficient, giving clean yields of the phosphate intermediate, removal of the spent reagent by extraction was required.

As alternatives the use of either *tert*-butyl hydroperoxide[9] or dinitrogen tetraoxide[10] was investigated. Both reagents gave clean conversions of the phosphite intermediate **23** into the corresponding phosphate **24** but, in order to produce high-quality HEMA-PC **3**, in both cases an extraction of the cyclic phosphate **24** from the side products and unchanged oxidants was required and gave no advantage over the use of the peracid.

Finally, the use of trimethylamine *N*-oxide[8] was examined. It was hoped that this reagent would both oxidise the cyclic phosphite **23** and that the product trimethylamine could be used as the reagent for opening of the cyclic phosphate to produce the phosphorylcholine unit in a one-pot process.

The oxidation of the phosphite **23** with trimethylamine *N*-oxide was exothermic in both acetonitrile or dichloromethane solution so dropwise addition of the reagent to a solution of the phosphite in acetonitrile was used. In order to avoid residual oxidant in the product, slightly less than one equivalent of the oxidant was used and, before warming, further trimethylamine was added to form the HEMA-PC **3**. Using this method, stable batches of HEMA-PC could be prepared on a large scale (>100 g) in overall yields of >45%.

## Experimental

Diisopropylammonium tetrazolide was freshly prepared as required from 1*H*-tetrazole and freshly distilled diisopropylamine and dried under reduced pressure at temperatures <40 °C. 4,5-Dichloroimidazole was purchased from Aldrich Chemical Co, Poole, Dorset. TLC and preparative layer chromatography (PLC) were performed on silica gel 60 $F_{254}$ pre-coated glass plates from BDH. All solvents were purified and dried before use using standard methods.[8] 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 of solvent under reduced pressure using a rotary evaporator.

Elemental analyses were carried out by MEDAC Ltd, Brunel Science Centre, Egham, Surrey. $^1$H NMR spectroscopy was carried out on either a Bruker AC360 spectrometer or a JEOL FX200 instrument; FAB mass spectra were obtained on a Kratos Concept 1S instrument, and low-resolution EI and CI spectra using a VG Trio 2000 machine.

### 2-Cyanoethoxybis(diisopropylamino)phosphine 9

This was prepared by the literature method.[6] Diisopropylamine (75.7 g, 0.75 mol) was added dropwise over a period of 1 h to dichloro-2-cyanoethoxyphosphine (13.2 g, 0.08 mol) as a solution in anhydrous diethyl ether (180 cm$^3$) at −10 °C under nitrogen. The reaction mixture was allowed to warm to room temperature and was stirred for 16 h, filtered, the solvent was evaporated off, and the residue fractionally distilled, the fraction of boiling range 96–110 °C (0.05–0.2 mmHg) being collected as the title compound (13.2 g, 57%).

### 2,2-Bis(hexadecyloxymethyl)propyl-2-cyanoethoxy(diisopropylamino)phosphine 11

Dichloromethane was distilled prior to use and diisopropylammonium tetrazolide **10** and the bis(hexadecyl ether) alcohol **7** were dried over phosphorus pentaoxide before use. The alcohol **7** (1.7 g, 3 mmol) and tetrazolide salt (0.46 g, 2.7 mmol) were dissolved in dry dichloromethane (40 cm$^3$) under nitrogen and the phosphoramidite **9** (0.90 g, 3.0 mmol) was added with stirring at room temperature. After 40 min the reaction mixture was extracted successively with aq. sodium hydrogen carbonate (2 × 40 cm$^3$; 2% *w*/*v*) and brine (40 cm$^3$). The aqueous solutions were back-extracted with dichloromethane (2 × 60 cm$^3$), and the organic phases combined, dried, and evaporated to give the title compound (2.3 g, 100%) which was not further purified. The obtained material showed $\delta_H$ ($CDCl_3$) 0.9 (6 H, t, *J* 7 Hz, 2 × *Me*$CH_2$), 0.94 (3 H, s, *Me*C), 1.2 (12, d, *J* 7 Hz, 4 × *Me*CH), 1.3 (52 H, m, 26 × $CH_2$), 1.5 (4 H, m, 2 × O*C*$H_2$C$H_2$), 2.61 (2 H, t, *J* 7 Hz, $CH_2CN$), 3.21 (4 H, s, 2 × $CCH_2O$), 3.38 (4 H, t, *J* 7 Hz, 2 × O*C*$H_2$C$H_2$), 3.5 (4 H, m, $POCH_2$ and 2 × C*H*$Me_2$), 3.81 (2 H, m, $POCH_2$).

### 2,2-Bis(hexadecyloxymethyl)propyl-2-bromoethoxy-2-cyanoethoxyphosphine 12

The phosphoramidite **11** (0.98 g, 1.27 mmol) was added to a mixture of 2-bromoethanol (0.28 g, 2.23 mmol) and 1*H*-tetrazole (0.06 g, 0.95 mmol) in acetonitrile (20 cm$^3$) containing dichloromethane (1 cm$^3$) and the solution was stirred at room temperature for 16 h. The mixture was concentrated and the residue was dissolved in dichloromethane (30 cm$^3$) and extracted successively with 2% aq. sodium hydrogen carbonate (2 × 30 cm$^3$) and brine (30 cm$^3$), with back-washing with more dichloromethane (30 cm$^3$). After combining of the organic extracts the solution was dried, filtered, and evaporated to give the title compound (0.87 g, 86%); $\delta_H$ ($CDCl_3$) 0.9 (6 H, t, *J* 7 Hz), 0.95 (3 H, s), 1.22 (52 H, m), 1.50 (4 H, m), 2.7 (2 H, t, *J* 7 Hz), 3.3 (4 H, s), 3.4 (4 H, t, *J* 7 Hz), 3.5 (2 H, t, *J* 6 Hz), 3.7 (2 H, d, *J* 3 Hz), 4.1 (4 H, m). This was characterised as the phosphate **13**.

### 2,2-Bis(hexadecyloxymethyl)propyl 2-bromoethyl 2-cyanoethyl phosphate 13

A solution of the phosphite **12** (0.87 g, 1.1 mmol) and MCPBA (99%; 0.19 g, 1.1 mmol) in dichloromethane (10 cm$^3$) was

stirred at room temperature for 30 min. The solution was extracted successively with aq. sodium hydrogen carbonate (2% w/v; 2 × 10 $cm^3$) and brine (10 $cm^3$). The aqueous layers were back-washed with dichloromethane (30 $cm^3$), and the organic phases were combined, dried, filtered, and evaporated to small bulk, and the concentrate filtered through a short column of silica gel, using dichloromethane as eluent, before drying to afford the title phosphate (0.74 g, 83%); $\delta_H$ ($CDCl_3$) 0.9 (6 H, t, *J* 7 Hz, 2 × *Me*$CH_2$), 0.99 (3 H, s, MeC), 1.22 (52 H, m, 26 × $CH_2$), 1.5 (4 H, m, 2 × $OCH_2CH_2$), 2.8 (2 H, t, *J* 7 Hz, $CH_2CN$), 3.22 (4 H, s, 2 × $CCH_2O$), 3.4 (4 H, t, *J* 7 Hz, 2 × $OCH_2CH_2$), 3.55 (2 H, t, *J* 7 Hz, $CH_2Br$), 4.0 (2 H, d, *J* 3 Hz, $POCH_2C$), 4.3 (4 H, m, 2 × $POCH_2CH_2$) (Found: C, 58.5; H, 9.8; N, 1.6; P, 3.8. $C_{42}H_{83}BrNO_6P{\cdot}3H_2O$ requires C, 58.4; H, 9.7; N, 1.6; P, 3.6%).

### 2,2-Bis(hexadecyloxymethyl)propyl 2-(trimethylammonioethyl) hydrogen phosphate 4

The bromoethyl phosphate **13** (0.25 g, 0.31 mmol) was treated with a solution of trimethylamine (0.66 g, 11.2 mmol) in dry acetonitrile (5 $cm^3$) at 48 °C in a sealed tube for 48 h. A solid precipitated, which was collected by filtration, washed with a little acetonitrile and then purified by column chromatography on silica gel (10 g), with chloroform–methanol–0.88 ammonia (690 : 270 : 64) as eluent, to give the title compound (0.13 g, 56%) (Found: $MH^+$, 735.6507. Calc. for $C_{42}H_{91}NO_6P$, *m*/*z*, 735.6506); this material showed the same $R_f$ value as an authentic sample.[4]

### Preparation of the quinuclidinium analogue 5

The preparation of this analogue was carried out in a similar manner to that used for the trimethylammonium derivative **4**, except that quinuclidine (0.77 g, 6.9 mmol) was used with the bromoethyl ester **13** (0.56 g, 0.69 mmol). The precipitated solid was collected, and purified by column chromatography through silica gel (15 g) with chloroform–methanol–water (65 : 25 : 4) as eluent to give the title analogue[4] (0.54 g, 69%) (Found: $MH^+$, 786. Calc. for $C_{46}H_{93}NO_6P$, *m*/*z*, 786).

### 6-(Triphenylphosphonio)hexanol bromide 14

A mixture of 6-bromohexan-1-ol (18.5 g, 0.1 mol) and triphenylphosphine (26.85 g, 0.1 mol) in dry acetonitrile (360 $cm^3$) was heated in a sealed vessel at 70 °C for 184 h. The reaction mixture was cooled and concentrated and the resulting residue was dissolved in chloroform (270 $cm^3$) and added to dry diethyl ether (910 $cm^3$) to precipitate out the product phosphonium salt. After stirring of the precipitate for 1 h it was collected by filtration, washed with more diethyl ether, and dried *in vacuo* to give the title salt (35.6 g, 79%).

### 2,2-Bis(hexadecyloxymethyl)propyl 6-(triphenylphosphonio)-hexyl phosphate 15

The phosphoramidite **11** (0.5 g, 0.65 mmol) was added to a mixture of 6-(triphenylphosphonio)hexan-1-ol bromide (0.29 g, 0.65 mmol) in dichloromethane (4 $cm^3$) and 1*H*-tetrazole (45 mg, 0.65 mmol) in acetonitrile (10 $cm^3$) and the mixture was stirred at room temperature for 16 h. The resulting solution was immediately treated with a solution of MCPBA (0.12 g, 0.65 mmol) in dichloromethane (10 $cm^3$) and the mixture was stirred at room temperature for 15 min, after which time the bulk of the solvent was evaporated off under reduced pressure, dichloromethane (50 $cm^3$) was added, and the solution washed with saturated aq. sodium hydrogen carbonate (3 × 50 $cm^3$), the aqueous layers being back-washed with more dichloromethane (50 $cm^3$). The organic phases were combined, dried, and filtered and the solvent was removed. The crude product (0.53 g) was dissolved in a mixture of methanol (40 $cm^3$) and chloroform (7 $cm^3$) and treated with 5% w/v aq. ammonia (9 $cm^3$) at room temperature for 4 h before concentration of the solution to small bulk, the residual water being removed by azeotroping with benzene. The crude residual product was dissolved in a small quantity of chloroform and then chromatographed through silica gel, using gradient elution from chloroform to 1 : 3 methanol–chloroform as eluent. The title salt was obtained (0.26 g, 40%) and showed $\delta_H$ ($CDCl_3-CD_3OD$, 3 : 1) 0.85 (9 H, m, 3 × Me), 1.25 (52 H, m, 26 × $CH_2$), 1.35–1.7 (12 H, m, 6 × $CH_2$), 3.2 (4 H, s, 2 × $CCH_2O$), 3.29 (4 H, t, *J* 7 Hz, 2 × $OCH_2CH_2$), 3.5–3.8 (4 H, m, $POCH_2C$ and $Ph_3P^+CH_2$), 3.85 (2 H, m, $CHCH_2OP$), 7.6–7.9 (15 H, m, ArH), identical to the spectrum of authentic material.[4]

### 2-(Methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate 3 [hydroxyethylmethacryloylphosphorylcholine, HEMA-PC]

**2-Cyanoethoxy(diisopropylamino)-2-(methacryloyloxy)-ethoxyphosphine 17.** Freshly prepared hydroxyethyl methacrylate **16** (0.22 g, 1.66 mmol) and diisopropylammonium tetrazolide **10** (0.26 g, 1.49 mmol) were dissolved in dry dichloromethane (6 $cm^3$) under argon and bis(diisopropylamino)-2-cyanoethoxyphosphine **9** (0.5 g, 1.66 mmol) was added with stirring. After 20 min, the reaction mixture was diluted with dichloromethane (14 $cm^3$) and the solution was extracted with saturated aq. sodium hydrogen carbonate (3 × 20 $cm^3$), the aqueous washings being back-extracted with more dichloromethane (60 $cm^3$). The combined organic phase was dried, filtered, and concentrated under reduced pressure to give the phosphine **17** (0.57 g), which was used without further purification. The crude material showed $\delta_H$ ($CDCl_3$) 1.22 (12 H, m, 4 × *Me*CH), 2.0 (3 H, s, MeC=C), 2.7 (2 H, t, *J* 7 Hz, $CH_2CN$), 3.62 (2 H, m, 2 × C*H*$Me_2$), 3.9 (4 H, m, 2 × $POCH_2$), 4.35 (2 H, t, *J* 7 Hz, $CH_2OCO$), 5.62 (1 H, s, HC=C), 6.18 (1 H, s, HC=C).

**2-Bromoethoxy-(2-cyanoethoxy)-2-(methacryloyloxy)ethoxy-phosphine 18.** The phosphoramidite **17** (0.55 g, 1.66 mmol) was added to a solution of bromoethanol (0.36 g, 2.9 mmol) and 1*H*-tetrazole (90 mg, 1.24 mmol) in acetonitrile (8 $cm^3$) and the mixture was stirred at room temperature for 16 h before being concentrated. The residue was dissolved in dichloromethane (20 $cm^3$) and extracted with saturated aq. sodium hydrogen carbonate (2 × 20 $cm^3$), back-washing with more dichloromethane (40 $cm^3$). The combined organic phase was dried, filtered, and concentrated to give the crude ester **18** (0.48 g), $\delta_H$ ($CDCl_3$) 1.96 (3 H, s, MeC=C), 2.7 (2 H, t, *J* 7 Hz, $CH_2CN$), 3.5 (2 H, t, *J* 7 Hz, $CH_2Br$), 4.1 (6 H, m, 3 × $CH_2OP$), 4.3 (2 H, m, $CH_2OCO$), 5.6 (1 H, s, HC=C), 6.15 (1 H, s, HC=C).

**2-Bromoethyl 2-cyanoethyl 2-(methacryloyloxy)ethyl phosphate 19.** MCPBA (freshly prepared from commercial peracid; 0.21 g, 1.22 mmol) was added to a solution of the phosphine **17** (0.45 g, 1.21 mmol) in dichloromethane (6 $cm^3$) and the mixture was stirred at room temperature for 10 min. The reaction mixture was diluted with more dichloromethane (14 $cm^3$) and washed with saturated aq. sodium hydrogen carbonate (2 × 20 $cm^3$), back-washing the aqueous layers with dichloromethane (40 $cm^3$). The organic portions were combined, dried, filtered, and evaporated to dryness under reduced pressure before finally drying over $P_2O_5$ to afford the title ester (0.38 g, 62%) (Found: C, 35.3; H, 5.0; N, 3.7; P, 8.3. $C_{11}H_{17}BrNO_6P$ requires C, 35.7; H, 4.6; N, 3.8; P, 8.4%); $\delta_H$ ($CDCl_3$) 2.0 (3 H, s, Me), 2.8 (2 H, t, *J* 7 Hz, $CH_2CN$), 3.56 (2 H, t, *J* 7 Hz, $CH_2Br$), 4.4 (8 H, m, 4 × $CH_2O$), 5.62 (1 H, s, HC=C), 6.19 (1 H, s, HC=C).

**Amination of the phosphate 19.** A stirred solution of the phosphate triester **19** (0.38 g, 1.03 mmol) and trimethylamine (0.17 g, 10.3 mmol) in acetonitrile (10 $cm^3$) was heated to 75 °C, in a sealed tube, for 24 h. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in methanol (1 $cm^3$) before passage through a silica gel column

(10 g), and elution with methanol, to produce HEMA-PC **3** (0.06 g, 12% overall) (Found: $MH^+$, 296.1274. $C_{11}H_{23}NO_6P$ requires *m*/*z*, 296.1263); $\delta_H$ 1.97 (3 H, s, MeC=C), 3.2 (9 H, s, $Me_3N^+$), 3.65 (2 H, m, $CH_2N^+$), 4.15 (2 H, m, $CH_2OP$), 4.3 (2 H, m, $CH_2OP$), 4.4 (2 H, m, $CH_2OCO$), 5.75 (1 H, s, HC=C), 6.18 (1 H, s, HC=C).

Repetition of this synthesis, using 4,5-dichloroimidazole in place of tetrazole, gave an improvement to 30% overall yield.

### Preparation of HEMA-PC 3 from diisopropylaminoethylene phosphite 22

**Ethylenechlorophosphite.** To a solution of freshly distilled phosphorus trichloride (100 g, 0.73 mol) in dichloromethane (150 $cm^3$) was added, slowly, re-distilled ethylene glycol (44.65 g, 0.72 mol) at such a rate that the evolution of gas did not become excessive. On completion of addition the mixture was stirred at room temperature for 1 h before the solvent was removed by distillation at atmospheric pressure. The residue was distilled under reduced pressure and the distillate collected to yield the title chloride (44.43 g, 48%), distillation range 22–25 °C/0.4 mmHg; $\delta_H$ 4.25 (2 H, m, $CH_2OP$), 4.45 (2 H, m, $CH_2OP$).

**Ethylene diisopropylphosphoramidite 22.** Diisopropylamine (80 g, 0.79 mol) was added dropwise over a period of 2 h to a solution of ethylene chlorophosphite (20 g, 0.16 mol) in dichloromethane (250 $cm^3$) at −10 °C under nitrogen. The reaction mixture was allowed to equilibrate at room temperature and was stirred for 16 h. The mixture was filtered, evaporated to ≈150 $cm^3$ volume, cooled in an ice-bath and then refiltered. The volume was further reduced to ≈75 $cm^3$, recooled and refiltered to remove most of the diisopropylammonium chloride. The remaining solution was fractionally distilled under reduced pressure to give the title ester (28 g, 93%), bp 44 °C/0.15 mmHg; $\delta_H$ (200 MHz; $CDCl_3$) 1.2 (12 H, d, *J* 6 Hz, 4 × Me), 3.45 (2 H, m, 2 × CH), 3.9 (2 H, m, $CH_2OP$), 4.1 (2 H, m, $CH_2OP$); *m*/*z* (CI) 192 ($M + H^+$).

**Ethylenedioxy-2-(methacryloyloxy)ethoxyphosphine 23.** To a solution of 4,5-dichloroimidazole (7.16 g, 52 mmol) in acetonitrile (80 $cm^3$) was added a solution of 2-hydroxyethyl methacrylate **16** (7.16 g, 52 mmol), under an atmosphere of nitrogen. The phosphoramidite **22** (10 g, 52 mmol) was then added and the reaction mixture stirred under nitrogen for 40 min, then filtered through Celite to remove the precipitated solids, including the diisopropylammonium dichloroimidazolide. The solvent was removed under reduced pressure to afford the title phosphite, which was not further purified but which was stored at −15 °C before use. The phosphite showed $\delta_H$ (200 MHz; $CDCl_3$) 1.98 (3 H, s, MeC=C), 4.0 (4 H, m, 2 × $CH_2OP$), 4.3 (4 H, m, 2 × $CH_2OP$), 5.61 (1 H, s, HC=C), 6.2 (1 H, s, HC=C).

**Ethylene 2-(methacryloyloxy)ethyl phosphate 24.** To the phosphite **23** (10.9 g, 49 mmol) was added, dropwise with stirring over a period of 15 min, a solution of trimethylamine *N*-oxide (3.73 g, 49 mmol) in acetonitrile (20 $cm^3$). The clear solution was stirred for a further 40 min. A sample of the solution was evaporated under reduced pressure for NMR analysis. This showed $\delta_H$ (200 MHz; $CDCl_3$) 1.98 (3 H, s, MeC=C), 4.4 (8 H, m, 3 × $CH_2OP$, $CH_2O_2C$), 5.61 (1 H, s, HC=C), 6.2 (1 H, s, HC=C). This crude material was not further purified before use in the next stage.

**HEMA-PC 3.** The ethylene phosphate **24** (11.3 g, 48 mmol) as a solution in acetonitrile (20 $cm^3$) and trimethylamine (4.64 g, 78 mmol) were heated together in a sealed tube at 50 °C for 48 h. The resulting mixture was filtered through Celite, whilst still warm, and the solvent removed from the filtrate by evaporation under reduced pressure. The product (volume ≈25 $cm^3$) was cooled to −20 °C for 16 h before the mixture was allowed to reach ambient temperature. The solid precipitate was collected by filtration under nitrogen and washed successively with cold acetonitile (5 $cm^3$) and ethyl acetate (10 $cm^3$) and then dried *in vacuo* over $P_2O_5$ to afford the title monomer (5.68 g, 37%). A second crop (0.26 g, 1.7%) was recovered from the filtrate. The product showed $\delta_H$ (200 MHz; $D_2O$) 1.97 (3 H, s, MeC=C), 3.2 (9 H, s, $Me_3N^+$), 3.65 (2 H, m, $CH_2N^+$), 4.15 (2 H, m, $CH_2OP$), 4.3 (2 H, m, $CH_2OP$), 4.4 (2 H, m, $CH_2OCO$), 5.75 (1 H, s, HC=C), 6.18 (1H, s, *H*C=C) (Found: C, 44.9; H, 7.5; N, 4.7; P, 10.4. Calc. for $C_{11}H_{22}NO_6P$: C, 44.7; H, 7.5, N, 4.7, P, 10.5%) (Found: $MH^+$, 296.1262. $C_{11}H_{23}NO_6P$ requires *m*/*z*, 296.1263).

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## References

1 R. L. Letsinger, J. L. Finnan, G. A. Heavner and W. B. Lunsford, *J. Am. Chem. Soc.*, 1975, **97**, 3278; R. L. Letsinger and W. B. Lunsford, *J. Am. Chem. Soc.*, 1976, **98**, 3655.
2 S. L. Beaucage and M. H. Caruthers, *J. Am. Chem. Soc.*, 1981, **103**, 3185; L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.*, 1983, **24**, 245; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 1925.
3 See P. Klotz, L. A. Cuccia, N. Mohamed, G. Just and R. B. Lennox, *J. Chem. Soc.*, *Chem. Commun.*, 1994, 2043 and references therein; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 10441; T. Desai, J. Gigg, R. Gigg and E. Martin Zamora, in *Synthesis in Lipid Chemistry*, ed. J. H. P. Tyman, Royal Society of Chemistry, London, 1996, pp. 67–92; D. M. Brown and P. R. Hammond, *J. Chem. Soc.*, 1960, 4232.
4 *cf.* J. E. Browne, R. T. Freeman, J. C. Russell and P. G. Sammes, *J. Chem. Soc.*, *Perkin Trans. 1*, 1999, preceding paper.
5 Y. Kadoma, N. Nakabayashi, E. Masuhara and J. Yamauchi, *Kobunshi Ronbunshu*, 1978, **35**, 423 (*Chem. Abstr.*, 1978, **89**, 186031b); K. Ishihara, T. Ueda and N. Nakabayashi, *Polym. J.*, 1990, **22**, 355; M. Kojima, K. Ishihara, A. Watenabe and N. Nakabayashi, *Biomaterials*, 1991, **12**, 121; R. W. J. Bowers, S. A. Jones, P. W. Stratford and S. A. Charles, *GB Pat.*, 9 201 215, 1990 (*Chem. Abstr.*, 1993, **119**, 162502t).
6 M. H. Caruthers, A. D. Barone, S. L. Beaucage, D. R. Dodds, E. F. Fisher, L. J. McBride, M. Matteucci, Z. Stabinsky and J. Y. Tang, *Methods Enzymol.*, 1987, **154**, 287.
7 A. D. Barone, J. Y. Tang and M. H. Caruthers, *Nucleic Acids Res.*, 1984, **12**, 4051.
8 D. D. Perrin, W. L. F. Amarego and D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1966.
9 Y. Hayakawa, M. Uchiyama and R. Noyori, *Tetrahedron Lett.*, 1986, **27**, 4191.
10 J. R. Cox and F. H. Westheimer, *J. Am. Chem. Soc.*, 1958, **80**, 5441.

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