Preparation of Novel N-Substituted Phospholipids *via* Phosphoramidite Intermediates

Christopher McGuigan *[,a] **and Bernadette Swords**[b]
[a] *Department of Chemistry, University of Southampton, Highfield, Southampton, SO9 5NH*
[b] *Department of Chemistry, University College London, 20 Gordon Street, London, WC1H 0AJ*

Novel *N*-alkylated phospholipids have been prepared in high yield by a three-step procedure utilising phosphoramidite intermediates. 3-*tert*-Butyl-2-chloro-1,3,2-oxazaphosphacyclopentane **1** reacts with alcohols to give cyclic phosphoramidites in near-quantitative yield. These are oxidised to the corresponding phosphates in very high yield with N_2O_4. The phosphates are hydrolysed in water at ambient temperature, *via* exclusive P–N cleavage, to yield the target phospholipids. Spectral data on the intermediates and products are included and discussed.

Phospholipids, well known as major structural components of cell membranes,[1] are implicated in many physiological processes.[2] Common natural phospholipids, which contain a diacylglycerol moiety attached *via* a phosphate diester to a choline or ethanolamine unit, largely comprise the fluid bi-layer of the mammalian cell membrane. There is mounting evidence[3] that changes in the lipid bi-layer are intimately involved in the neoplastic state and in viral infection, and that a new, non-genotoxic approach to chemotherapy may be available in the field of phospholipid analogues.

Until recently, the synthesis of phospholipids achieved by phosphate chemistry, could be slow, and yields poor; especially for rather bulky diacyl glycerols.[4] More recently, phosphite chemistry has been employed, with greatly improved syntheses.[5] However, consistently, the problem of introducing the amino terminus has been a major limitation of most routes. We have developed a new route to phospholipids[6] which entirely removes this problem, whilst retaining the advantages of phosphite reactivity. In particular, the amino group is 'protected' from the start in a phosphoramidite heterocycle. The rationale behind using phosphoramidite intermediates was that final P–N cleavage would be achieved by simple acid hydrolysis of the heterocycle.[6] Indeed we have recently noted the facile cleavage of the cyclic intermediate; simply using water at room temperature leads to smooth hydrolysis to the desired product.[7] The route we have introduced is readily adapted to allow the synthesis of entirely novel phospholipid analogues, whose biological properties are entirely unknown and would be eagerly awaited. For example, we have noted that the use of the diamidate versions of the above-mentioned reagents yield entirely novel amino-linked phosphoramidate lipids.[8]

It has been known for some time that viruses must perturb membrane structure in order to penetrate (and exit from) host cells. Kinchington and co-workers have very clearly demonstrated that infection of H9 cells with HIV1 leads to a reproducible change in the ratio of saturated to unsaturated fatty acids in the host cell membrane,[9] probably leading to a more fluid membrane, and thus facilitating viral penetration. Moreover, these workers reported that exogenous saturated fatty acids, and their analogues had a pronounced anti-viral effect. Although the mechanism by which these compounds exert this effect remains unclear, action in the membrane is strongly implicated, and anabolism to the corresponding phospholipids is quite likely. Indeed, Lewis[10] has noted anti-HIV activity at *ca.* 15 μmol dm^{-3} for several phosphatidyl cholines, ethanolamines and inositols. Interestingly, this group noted polyunsaturation of the 2-acyl chain to be preferable; saturated phospholipids were inactive. Very recently, Kucera and co-workers have noted very marked anti-HIV activity for unnatural phospholipid analogues.[11]

It is known that certain phospholipid analogues, the alkyl lysophospholipids (ALPS), have anti-cancer action.[12] Whilst the mechanism of action of these compounds is currently unclear, a site of action in the cell membrane seems very likely. Moreover, the apparent resistance of malignant cells to lipid peroxidation[3] does not appear to be related to enhanced antioxidant defences, but rather relates to differences in the cell membranes. This may correspond to actual differences in membrane composition, or more likely, to the lipid organisation and the detailed architecture of the cell membrane of malignant cells. This latter alternative suggests an exciting possibility; whilst the overall lipid composition of a cell membrane may be difficult to alter by exposure to exogenous lipid analogues, the possibility that an organisational difference may be involved suggests that a significant modification of the biological behaviour of malignant cells may result from the introduction of lipid analogues.

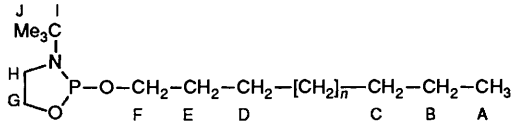
Thus, we sought the preparation of phospholipid analogues with small structural changes in the head-group region as potential anti-neoplastic or anti-viral agents. In this paper we describe the synthesis of some *N*-*tert*-butyl analogues using phosphoramidite chemistry.

The phosphorochloridite **1** was prepared by a method similar to that we have reported for the *N*-methyl analogue.[6] Thus, 2-*tert*-butylaminoethanol (TBAE) was treated with phosphorus trichloride in the presence of triethylamine. The solvent used was benzene, rather than dichloromethane, since this facilitates separation of the hydrochloride salt. Vacuum distillation gave the product **1** in moderate yield. This displays a single peak in the ^{31}P NMR, at δ *ca.* 166 and four (phosphorus-coupled) doublets, in its ^{13}C NMR spectrum.[13,14]

Compound **1** was treated with hexan-1-ol in dichloromethane containing triethylamine at low temperature to yield the phosphite triester **2a** in almost quantitative yield, after aqueous organic partition. This displayed a single resonance in its ^{31}P NMR spectrum, much further upfield (δ 134) than noted for compound **1**.

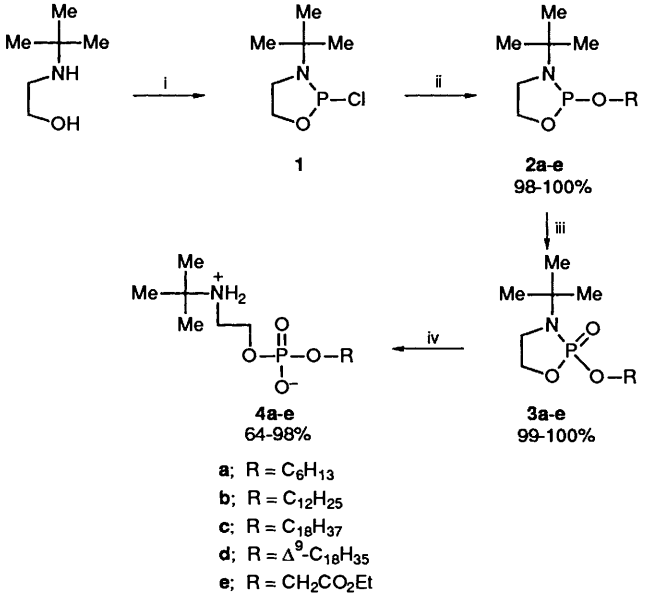
In a similar manner, compound **1** was treated with dodecanol and triethylamine to yield the phosphite triester **2b** in quantitative yield. Similarly prepared were the octadecyl **2c** and oleyl **2d** analogues. Compound **2c** was prepared at a slightly higher temperature since the long-chain alcohol was poorly soluble in dichloromethane at very low temperature. The isolation procedure was altered in the case of **2d**, because of difficulties encountered in the organic aqueous extraction. Thus, the reaction mixture was evaporated to dryness, the

Table 1 ^{13}C NMR data for **2a–e** recorded at 100 MHz in $CDCl_3$: phosphorus–carbon coupling constants (Hz) are given in parentheses. Signal assignments refer to the following structure:



Signal	**2a** (n = 0)	**2b** (n = 6)	**2c** (n = 12)	**2d** (n = 12)[a]	**2e**[b]
A	14.0	14.0	14.1	14.1	—
B	22.6	22.6	22.6	22.7	—
C	31.6	31.9	31.9	31.9	14.1
D	25.4	25.9	25.8	25.9	60.9
E	31.4(4.2)	31.5(4.6)	31.5(4.2)	31.5(4.3)	170.4(3.9)
F	62.7(12.2)	62.7(12.3)	62.7(11.9)	62.7(12.1)	60.1(13.7)
G	68.2(9.3)	68.2(9.1)	68.2(9.1)	68.2(9.3)	68.4(8.7)
H	42.4(5.3)	42.4(5.4)	42.4(5.3)	42.4(5.1)	42.1(5.4)
I	51.7(13.1)	51.7(13.5)	51.6(13.0)	51.5(13.0)	51.8(12.1)
J	29.9(9.7)	29.6(9.6)	29.9(9.6)	29.9(10.1)	29.8(9.7)

[a] Includes CH=CH δ_C 129.84 and 129.77 and CH_2CH= δ_C 27.2. [b] $CH_3CH_2OC(O)CH_2OP$ C D E F



Scheme 1 *Reagents and conditions:* i, Benzene, PCl_3, Et_3N, −10 °C; ii, ROH, CH_2Cl_2, Et_3N, −40 to −60 °C; iii, N_2O_4, CH_2Cl_2, −60 °C; iv, H_2O, room temp., 2–48 h.

residue treated with hexane, and the salt filtered off. Similarly, as a model ester, ethyl glycolate was treated with **1**. The non-aqueous isolation procedure was favoured here, on account of the significant water solubility of **2e**; this gave an almost quantitative yield. Carbon-13 NMR data for each of the phosphite triesters are listed in Table 1.

Thus, it appeared that **1** would react with a range of alcohols to give the corresponding phosphite triesters cleanly, and in very high yield. The reaction was equally successful with short, medium and long chain alcohols, and worked well with unsaturated and ester-containing alcohols.

Following our established synthetic route,[6–8] the next stage was oxidation of the phosphite heterocycles to the corresponding phosphates. This was achieved using a standard solution of dinitrogen tetroxide[15] in anhydrous dichloromethane at low temperature. Thus, the phosphites (**2a–e**) were converted into their corresponding phosphates (**3a–e**) in quantitative yield, the crude products being entirely pure by spectroscopy. The ^{31}P NMR data for the phosphates were very different from those of the phosphite precursors. Thus, resonances in the region of δ 16–17 were noted for **2a–e**, which is as expected for structures of this type.[14] Once again, the tetroxide oxidation method is seen to be highly successful for the conversion of phosphite heterocycles into their phosphates. The yields are very high, the crude products pure, and the isolation procedure facile.

The final step in the synthetic route is the hydrolytic cleavage of the P–N bond to yield the acyclic phospholipids (**4a–e**). We originally noted that the *N*-methyl analogues of compounds **3a–e** could be hydrolysed successfully using 2 mol dm^{-3} hydrochloric acid at reflux.[6] More recently,[7] we found that this hydrolysis could be conducted simply in water (often mixed with a co-solvent), in the absence of added acid and, moreover, at room temperature. It seemed likely that compounds **3a–e** would be hydrolysed less readily than the *N*-methyl analogues, on steric grounds at least. Thus, the hydrolysis of **3a** was followed by ^{31}P NMR spectroscopy, in water (D_2O) at ambient temperature. Acyclic product **4a** was noted after only 0.5 h, but the reaction was not complete until 8 h had elapsed. Simple lyophilisation gave **4a** in near-quantitative yield, the material being pure by both spectroscopy and microanalysis.

Thus, **4a** displayed one signal in its ^{31}P NMR spectrum at δ *ca.* −0.4. This shift is very different from that of the starting phosphate **3a**, and is entirely consistent with the cleavage of the P–N bond to yield a phosphate diester. The ^{13}C NMR spectrum is also informative (Table 2). Thus, phosphorus coupling to the carbon atoms of the *tert*-butyl moiety is now entirely removed, whilst that to other nearby carbon atoms is retained. This is strong evidence for P–N, rather than P–O cleavage. The FAB mass spectrum of **4a** further confirmed its structure, with peaks noted for the (protonated) molecular ion, and its dimer.

The dodecyl phosphate **3b** was similarly hydrolysed in water at ambient temperature, for 8 h. Lyophilisation gave **4b**, which was pure by spectroscopy but not by analysis. Pure material was readily obtained by flash column chromatography on silica, although the yield was somewhat reduced as a result of the chromatography.[16] The product **4b** showed very similar spectroscopic and other data to **4a** above. Similarly prepared were the octadecyl **4c** and oleyl **4d** compounds, which showed similar spectroscopic data to the earlier analogues. The former reaction was conducted for 8 h, as above, whilst the latter was noted to be slower, and extended to 48 h. Interestingly, both of these long-chain compounds displayed very broad ^{31}P NMR resonances, suggestive of the formation of micelles or other

Table 2 ^{13}C NMR data for **4a–e** recorded at 100 MHz in $CDCl_3$: phosphorus–carbon coupling constants (Hz) are given in parentheses. Signal assignments refer to the following structure:

$$\underset{A\ B\ C}{CH_3CH_2CH_2}[CH_2]_n\underset{D\ E\ F}{CH_2CH_2CH_2OP(O)(OH)OCH_2CH_2}\underset{G\ H\ \ I\ J}{NHCMe_3}$$

Signal	**4a** (n = 0)	**4b** (n = 6)	**4c** (n = 12)	**4d** (n = 12)[a]	**4e**[b]
A	14.0	14.1	14.1	14.1	—
B	22.5	22.6	22.6	22.6	—
C	31.4	31.8	31.9	31.8	14.0
D	25.2	25.7[c]	25.8	25.8	60.8
E	30.5(7.0)	30.6(7.5)	30.7(7.4)	30.7(7.4)	169.8(7.9)
F	61.0(4.6)	60.8(4.1)	61.0(4.1)	61.0(4.4)	62.6(4.6)
G	66.3(5.9)	66.2(6.2)	66.2(5.2)	66.1(4.4)	61.5(4.0)
H	42.6(1.5)	42.9[d]	42.5(2.3)	42.3(4.9)	42.2(4.2)
I	55.5	55.1	55.7	55.8	55.9
J	25.5	25.7[c]	25.7	25.7	25.6

[a] Includes CH=CH δ_C 129.9 and 129.7, and CH_2CH= δ_C 27.2. [b] $\underset{C\ D}{CH_3CH_2}\underset{E\ F}{OC(O)CH_2OP}$. [c] Signals coincide. [d] Coupling not resolved.

organised structures.[17] Interestingly, this was not observed with **4a–b** or with the previous[6] octadecyl or oleyl *N*-methyl analogues. The dependence of micelle formation on alkyl chain length might well be expected.[18]

Lastly, the glycolyl phosphate **3e** was hydrolysed to yield the acyclic phosphate **4e**. This reaction was rather rapid, being complete in under 1 h. The product displayed similar spectra to **4a–d** above, but not surprisingly did not display the apparent micelle formation noted for **4c–d**. Thus, the water-mediated hydrolysis method appears to be successful for the *N-tert*-butyl phosphates. The reactions are clean, and the crude yields high, although the necessity in most cases for chromatographic purification is troublesome, and lowers the isolated yield somewhat. We are currently seeking alternative purification methods to alleviate this problem.

Experimental

Commercially available Merck 60 F_{254} plates were used for the TLC and the components were visualised by iodine adsorbed on silica. Column chromatography was carried out using Merck Kieselgel 60 silica as the stationary phase. Phosphorus-31 NMR spectra were recorded on a Varian XL-200 spectrometer operating at 82 MHz or a Varian CFT20 instrument operating at 32 MHz and shifts are reported as δ values relative to 85% phosphoric acid as external standard, positive shifts are downfield. Carbon-13 NMR spectra were recorded on a Varian XL-200 spectrometer operating at 50 MHz, or a Varian VXR-400 spectrometer operating at 100 MHz and shifts are δ values relative to TMS; in the alkyl chains carbon atoms are numbered from the terminus. Both phosphorus-31 and carbon-13 NMR spectra were proton noise decoupled and all signals were singlets unless otherwise stated. ^1H NMR spectra were recorded on a Varian XL-200 spectrometer operating at 200 MHz and are reported δ values relative to internal TMS. All NMR spectra were recorded in $CDCl_3$ unless otherwise stated, and all coupling constants are reported in Hz. IR spectra were recorded on a Perkin-Elmer 983 spectrometer. Mass spectra were recorded on a VG7070H spectrometer, courtesy of Dr. M. Mruzek (EIMS) or on a VG Zab 1F spectrometer courtesy of the University of London Mass spectrometry group (FAB). Microanalyses were performed at University College London courtesy of the Group of Mr. A. T. T. Stones. Phospholipids were noted to be hygroscopic, and analytical data are presented appropriately. All experiments involving water-sensitive reagents were carried out under scrupulously dry conditions. Where needed, anhydrous solvents and reagents were obtained in the following ways. Triethylamine, benzene, hexane and dichloromethane were refluxed over CaH_2 for several hours and distilled. All but triethylamine were further dried over activated 4 Å molecular sieves. *tert*-Butylaminoethanol, and all other alcohols except octadecanol were distilled onto activated 4 Å molecular sieves. Octadecanol was crushed to a powder and then dried *in vacuo* at room temperature for several hours.

3-tert-*Butyl*-2-*chloro*-1,3,2-*oxazaphosphacyclopentane* **1**.—Dry TBAE (10.0 g, 85 mmol) and triethylamine (30 ml, 210 mmol) in benzene (80 ml) were added dropwise with vigorous stirring to benzene (150 ml) at −10 °C under an atmosphere of nitrogen. Separately, but simultaneously, phosphorus trichloride (12.8 g, 94 mmol) in benzene (60 ml) was added dropwise. The mixture was allowed to warm to ambient temperature, and stirred for 2 h. The precipitate was filtered off and washed with benzene (50 ml) and the combined filtrate and washings were evaporated under reduced pressure to give the crude product; this was distilled *in vacuo* to yield a colourless oil (9.7 g, 63%), b.p. 64–68 °C, 0.08 mmHg; δ_H 4.45 (2 H, m, CH_2O), 3.12 (2 H, m, CH_2N) and 1.33 (9 H, m, CH_3); δ_P 165.60; δ_C 70.3 (d, CH_2O, J 8.1), 53.3 (d, Me_3C, J 6.6), 41.8 (d, CH_2N, J 8.1) and 29.0 (d, Me, J 11.9); m/z (FAB) 544 (M_3H^+, 0.9%), 363 (M_2H^+, 17), 182 (MH^+, 93), 136 (10), 126 (15), 118 (57), 100 (21), 89 (7), 62 (26), 58 (22), 57 (38) and 44 (100).

3-tert-*Butyl*-2-*hexyloxy*-1,3,2-*oxazaphosphacyclopentane* **2a**.—Compound **1** (0.30 g, 1.65 mmol) in dry dichloromethane (15 ml) was added dropwise with vigorous stirring to hexan-1-ol (0.17 g, 1.67 mmol) and triethylamine (0.23 ml, 1.67 mmol) in dichloromethane (20 ml) at −60 °C under an atmosphere of nitrogen. The stirred solution was allowed to warm to ambient temperature over 1 h, and was then extracted with saturated aqueous sodium hydrogen carbonate (50 ml) followed by saturated brine (2 × 50 ml). The solution was then dried ($MgSO_4$) and evaporated under reduced pressure, to yield the product as a clear, colourless oil (0.40 g, 98%); δ_H 4.29 (2 H, m, CH_2O), 3.66 (2 H, m, OCH_2R), 3.00 (2 H, m, CH_2N), 1.51 (2 H, m, RCH_2CH_2O), 1.29 (15 H, m, Me_3C, $[CH_2]_3$) and 0.83 (3 H, t, CH_3); δ_P 134.17.

3-tert-*Butyl*-2-*dodecyloxy*-1,3,2-*oxazaphosphacyclopentane* **2b**.—This compound was prepared by a method entirely analogous to that used for **2a** above. Thus, from 0.30 g of compound **1** was isolated 0.55 g (100%) of **2b**; δ_H 4.25 (2 H, m, CH_2O), 3.67 (2 H, m, OCH_2R), 3.00 (2 H, m, CH_2N), 1.50 (2 H, m, RCH_2CH_2O), 1.24 (27 H, m, Me_3C, $[CH_2]_9$) and 0.86 (3 H, t, CH_3); δ_P 134.14.

3-tert-*Butyl*-2-*octadecyloxy*-1,3,2-*oxazaphosphacyclopentane* **2c**.—This compound was prepared by a method entirely analogous to that used for **2a** above, except that the reaction was conducted at −40 °C. Thus, from 0.20 g of compound **1** was isolated 0.45 g (98%) of **2c**; δ_H 4.1 (4 H, m, CH_2O, OCH_2R), 3.0 (2 H, m, CH_2N), 1.55 (2 H, m, CH_2CH_2OP), 1.26 (39 H, m, Me_3C, $[CH_2]_{15}$) and 0.86 (3 H, t, CH_3); δ_P 133.10.

3-tert-*Butyl*-2-*oleyloxy*-1,3,2-*oxazaphosphacyclopentane* **2d**.—Compound **1** (0.20 g, 1.10 mmol) in dry dichloromethane (15 ml) was added dropwise with vigorous stirring to oleyl alcohol (0.29 g, 1.10 mmol) and triethylamine (0.15 ml, 1.11 mmol) in dichloromethane (20 ml) at −60 °C under an atmosphere of nitrogen. The stirred solution was allowed to warm to ambient temperature over 1.5 h, and was then evaporated under reduced pressure. The residue was treated with hexane (100 ml), filtered, and the filtrate evaporated under reduced pressure to give the product as a colourless oil (0.45 g, 99%); δ_H 5.32 (2 H, m, CH=CH), 4.2 (2 H, m, CH_2O), 3.8 (2 H, m, OCH_2R), 3.0 (2 H, m, CH_2N), 2.01 (4 H, m, CH_2CH=), 1.23 (33 H, m, Me_3C, $[CH_2]_{12}$) and 0.86 (3 H, t, CH_3); δ_P 134.33.

3-tert-*Butyl*-2-*ethoxycarbonylmethoxy*-1,3,2-*oxazaphosphacyclopentane* **2e**.—This compound was prepared by a method entirely analogous to that used for **2d** above, except that the reaction time was 1 h. Thus, from 0.40 g of compound **1** was isolated 0.53 g (99%) of **2b**; δ_P 136.80.

3-tert-*Butyl*-2-*hexyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* **3a**.—A portion of standard dinitrogen tetroxide solution (3.0 ml; 0.48 mmol of oxidant, sufficient to oxidise 1.9 mmol of phosphite) was added dropwise with vigorous stirring to compound **2a** (0.40 g, 1.62 mmol) in dry dichloromethane (10 ml) −60 °C. The stirred solution was allowed to warm to ambient temperature over 45 min, and was then evaporated under reduced pressure, to yield the product as a clear oil (0.42 g, 99%); δ_P 16.10.

3-tert-*Butyl*-2-*dodecyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* **3b**.—This compound was prepared by a method entirely analogous to that used for **3a** above. Thus, from 0.55 g of compound **2b** was isolated 0.57 g (100%) of **3b**; δ_P 17.00.

3-tert-*Butyl*-2-*octadecyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* **3c**.—This compound was prepared by a method entirely analogous to that used for **3a** above. Thus, from 0.50 g of compound **2c** was isolated 0.52 g (100%) of **3c**; δ_P 16.30.

3-tert-*Butyl*-2-*oleyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* **3d**.—This compound was prepared by a method entirely analogous to that used for **3a** above. Thus, from 0.45 g of compound (**2d**) was isolated 0.46 g (100%) of **3d**; δ_P 17.09.

3-tert-*Butyl*-2-*ethoxycarbonylmethoxy*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* **3e**.—This compound was prepared by a method entirely analogous to that used for **3a** above. Thus, from 0.53 g of compound **2e** was isolated 0.56 g (100%) of **3e**; δ_P 16.60.

Water-mediated Hydrolysis of **3a**.—Compound **3a** (0.69 g, 2.62 mmol) was dissolved in D_2O (2 ml) at ambient temperature, and was lyophilised after 8 h at ambient temperature to yield **4a** as a white solid (0.72 g, 98%); δ_H 4.19 (2 H, m, CH_2O), 3.85 (2 H, m, OCH_2R), 3.05 (2 H, m, CH_2N), 1.41 (17 H, m, Me_3C, $[CH_2]_4$) and 0.81 (3 H, t, CH_3); δ_P −0.42; *m/z* (FAB) 563 (M_2H^+, 3%), 282 (MH^+, 44), 266 (M^+ − Me, 1), 118 (1), 101 (2), 99 (20), 57 (15) and 44 ($CH_2CH_2NH_2^+$, 100); ν_{max}(liquid film)/cm^{-1} 3037, 2947, 2930, 2858, 2642, 1613, 1463, 1376, 1200, 1065 and 1020 (Found: C, 49.15; H, 9.85; N, 4.95. $C_{12}H_{28}NO_4P$·$[H_2O]0.5$ requires C, 49.64; H, 10.07; N, 4.82%).

Hydrolysis of **3b**.—Compound **3b** (0.57 g, 1.64 mmol) suspended in water (12 ml) was stirred at ambient temperature, for 8 h. The reaction mixture was lyophilised to yield crude **4b**, which was further purified by flash column chromatography on silica (50 g). Elution with 20% methanol in chloroform, followed by pooling and evaporation of appropriate fractions, gave the product as a white solid (0.42 g, 71%); δ_H 4.25 (2 H, m, CH_2O), 3.88 (2 H, m, OCH_2R), 3.08 (2 H, m, CH_2N), 1.39 (29 H, m, Me_3C, $[CH_2]_{10}$) and 0.85 (3 H, t, CH_3); δ_P 0.98; *m/z* (FAB) 731 (M_2H^+, 1%), 366 (MH^+, 56), 350 (M^+ − Me, <1), 101 (0.5), 99 (2), 57 (14) and 44 ($CH_2CH_2NH_2^+$, 100); ν_{max}(liquid film)/cm^{-1} 2924, 2846, 2642, 1463, 1376, 1200, 1065 and 1026 (Found: C, 57.65; H, 10.7; N, 3.6. $C_{18}H_{40}NO_4P$·$[H_2O]_{0.5}$ requires C, 57.73; H, 11.04; N, 3.7%).

Hydrolysis of **3c**.—This compound was prepared by a method entirely analogous to that used for **3b** above, except that more water was used in the reaction (20 ml), and the column was eluted with 15% methanol in chloroform. Thus, from 0.52 g of compound **3c** was isolated 0.35 g (64%) of **4c**; δ_H 4.2 (2 H, m, CH_2O), 3.87 (2 H, m, OCH_2R), 3.1 (2 H, m, CH_2N), 1.37 (41 H, m, Me_3C, $[CH_2]_{16}$) and 0.85 (3 H, t, CH_3); δ_P −0.6 (br); *m/z* (FAB) 450 (MH^+, 10%), 101 (<1), 99 (1), 57 (37) and 44 ($CH_2CH_2NH_2^+$, 100); ν_{max}(liquid film)/cm^{-1} 2917, 2849, 1464, 1219, 1063 and 1026 (Found: C, 62.2; H, 11.25; N, 2.9. $C_{24}H_{52}NO_4P$·H_2O requires C, 61.64; H, 11.64; N, 2.99%).

Hydrolysis of **3d**.—This compound was prepared by a method entirely analogous to that used for **3b** above, except that the reaction mixture was stirred for 48 h. Thus, from 0.47 g of compound **3d** was isolated 0.36 g (74%) of **4d**; δ_H 5.31 (2 H, m, CH=), 4.2 (2 H, m, CH_2O), 3.85 (2 H, m, OCH_2R), 3.07 (2 H, m, CH_2N), 1.97 (4 H, m, CH_2CH=), 1.33 (33 H, m, Me_3C, $[CH_2]_{12}$) and 0.84 (3 H, t, CH_3); δ_P −3.9 (br); *m/z* (FAB) 448 (MH^+, 1%), 57 (<1), 56 (100) and 44 (53); ν_{max} (liquid film)/cm^{-1} 2925, 2849, 1613, 1461, 1373, 1221, 1201, 1081, 1067 and 1026 (Found: C, 60.25; H, 10.6; N, 3.15; P, 6.70. $C_{24}H_{50}NO_4P$·$[H_2O]_{1.5}$ requires C, 60.73; H, 11.26; N, 2.95; P, 6.53%).

Hydrolysis of **3e**.—This compound was prepared by a method entirely analogous to that used for **3b** above, except that the reaction mixture was stirred for only 2 h, and the column was eluted with a gradient (10 to 30%) of methanol in chloroform. Thus, from 0.56 g of compound **3e** was isolated 0.40 g (67%) of **4e**; δ_H 4.45 (2 H, m, glycolic CH_2), 4.2 (4 H, m, 2 × CH_2O), 3.13 (2 H, m, CH_2N), 1.35 (9 H, 3, Me_3C) and 1.23 (3 H, t, CH_3CH_2); δ_P −3.4; *m/z* (FAB) 284 (MH^+, 25%), 270 (2), 136 (8), 100 (44), 99 (<1), 57 (1) and 45 (100); ν_{max} (liquid film)/cm^{-1} 2966, 2931, 2651, 1753, 1613, 1379, 1215, 1078 and 1026 (Found: C, 39.65; H, 7.7; N, 4.75; P, 10.25. $C_{10}H_{22}NO_6P$·H_2O requires C, 39.87; H, 8.03; N, 4.65; P, 10.28%).

References

1 H. Eibl, *Angew. Chem., Int. Edn. Engl.*, 1984, **23**, 257.
2 D. J. Hanahan, *Annu. Rev. Biochem.*, 1986, **55**, 483.
3 K. H. Cheeseman, M. Collins, K. Proudfoot, T. F. Slater, G. W. Burton, A. C. Webb and K. U. Ingold, *Biochem. J.*, 1986, **235**, 507.
4 H. S. Hendrickson, E. K. Hendrickson and T. J. Rustad, *J. Lipid. Res.*, 1987, **28**, 864.
5 K. S. Bruzik, G. Salamonczyck and W. J. Stec, *J. Org. Chem.*, 1986, **51**, 2368.
6 C. McGuigan, *J. Chem. Soc., Chem. Commun.*, 1986, 533.
7 C. McGuigan and B. Swords, *J. Chem. Soc., Perkin Trans. 1*, 1990, 7893.
8 M. S. Anson and C. McGuigan, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1273.

9 K. Apostolov, W. Barker, S. A. Galpin, N. A. Habib, C. B. Wood and D. Kinchington, *FEBS Lett.*, 1989, **250**, 241.
10 D. Lewis, A. J. Baren, V. Knight and M. Jett, Presented at Vth International Conference on AIDS, Montreal, June 4th-9th 1989, Paper MCP124.
11 K. L. Meyer, C. J. Marasco, Jr., S. L. Morris-Natschke, K. S. Ishaq, C. Piantadosi and L. S. Kucera, *J. Med. Chem.*, 1991, **34**, 1377.
12 R. Andreesen, M. Modolell, H. U. Weltzien, H. Eibl, H. H. Common, G. W. Lohr and P. G. Munder, *Cancer Res.*, 1978, **38**, 3894.
13 The signals are assigned by reference to data on 2-methyl-1-heptylaminoethanol; see: L. F. Johnson and W. C. Jankowski, *C-13 NMR Spectra*, Wiley, New York, 1972.
14 V. Mark, C. H. Dungan, M. M. Crutchfield and J. R. Van Wazer, in *Topics in Phosphorus Chemistry*, vol. 5, eds. M. Grayson and E. J. Griffith, Wiley, New York, 1969.
15 J. R. Cox and F. H. Westheimer, *J. Am. Chem. Soc.*, 1958, **80**, 5441.
16 N. S. Chandrakumar and J. Hajdu, *J. Org. Chem.*, 1983, **48**, 1197; S. K. Bhatia and J. Hajdu, *Tetrahedron Lett.*, 1987, **28**, 1729.
17 P. L. Luisi, *Angew. Chem., Int. Edn. Engl.*, 1985, **24**, 439.
18 J. M. Seddon, *Biochim. Biophys. Acta*, 1990, **1031**, 1.

Paper 1/04093F
Received 6*th August* 1991
Accepted 16*th September* 1991