Synthesis of Phospholipids by Phosphoramidite Methodology

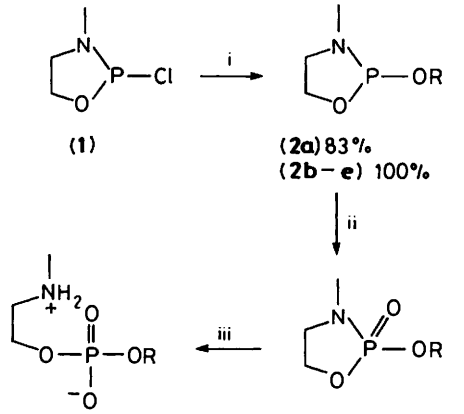
Christopher McGuigan * and Bernadette Swords
Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ

Novel phospholipids have been prepared by a rapid three-step procedure. The oxazaphosphacyclopentane (**1**) reacts with alcohols to yield cyclic phosphoramidites in high yield. These are oxidised to the corresponding phosphates with N_2O_4. The phosphates undergo P–N cleavage simply by treatment with water at ambient temperature, to yield phospholipids. Full spectroscopic and analytical data are presented and discussed.

Phospholipids are major components of cell membranes,[1] and are involved in a myriad of biological processes.[2] Modified, phospholipid-type species are of great current chemotherapeutic interest;[3] for example, various lipid materials have recently been studied as anti-inflammatory agents,[4] as cancer chemotherapeutic agents,[5] and in the therapy of Acquired Immunodeficiency Syndrome (AIDS).[6] Whilst natural phospholipids may be isolated (although this may give heterogeneous materials), unnatural ones must be synthesized. Thus, there is considerable interest in the development of new synthetic routes to phospholipids. There have been many important developments in the back-bone chemistry, that of the diacylglycerol moiety,[7] but rather less in the phosphate chemistry. We,[8] and others,[9] have reported on the advantages of phosphite and in particular phosphoramidite chemistry; especially in terms of the speed and yield of reaction. We now report full details of the application of this methodology to the preparation of a series of phospholipid-like materials, including data detailing the remarkably acid-labile nature of the cyclic intermediates. Indeed, the major advantage of this route may be that the use of trimethylamine is obviated; this is frequently used in the final step of phospholipid syntheses to allow introduction of the terminal trimethylamino group.[10] However, this very strongly basic and rather nucleophilic reagent may cause unwanted modification of the labile diacylglycerol moiety, and attack at the phosphorus. In the synthesis reported herein, the amino group is present throughout in a protected (cyclic) form, and is easily freed at the end of the synthesis by aqueous treatment. A preliminary account of this work has appeared,[8] as has its application to diamino phospholipids.[11]

The desired phosphitylating agent, 2-chloro-3-methyl-1,3,2-oxazaphosphacyclopentane (**1**), was prepared by the low temperature reaction of phosphorus trichloride with *N*-methylethanolamine.[12] Phosphorus NMR[13] and other spectroscopic data fully support the structure of this product. Of particular interest is the ^{13}C NMR which displays three signals, each with two bond phosphorus coupling clearly visible. The OCH_2 resonance is shifted downfield *ca.* 10 ppm relative to the spectrum of the starting *N*-methylethanolamine,[14] whereas the other two resonances are shifted *ca.* 5 ppm upfield relative to this material.

Compound (**1**) was treated with hexanol and triethylamine at −60 °C to yield the phosphite triester (**2a**), which was fully characterised by spectroscopic data. Interestingly, whilst the chemical shift of the *N*-methyl signal in the ^1H NMR spectrum is almost unchanged relative to (**1**), the magnitude of the phosphorus coupling is considerably reduced (from 15 to 12 Hz). The ^{31}P NMR chemical shift is greatly altered from +167 for (**1**) to +137 for (**2a**); this is entirely consistent with previous reports on similar species [*e.g.* δ_P 143 ppm for $PNMe_2(OEt)_2$].[15]



a: R = C_6H_{13}
b: R = $C_{12}H_{25}$
c: R = $C_{18}H_{37}$
d: R = $\Delta^9 - C_{18}H_{35}$
e: R = CH_2CO_2Et

Scheme. *Reagents:* i, ROH, Et_3N, CH_2Cl_2, −40 to −60 °C; ii, N_2O_4, CH_2Cl_2, −60 °C; iii, D_2O–THF, or D_2O, or H_2O, room temp., 1–3 h.

In a similar manner, compound (**1**) was treated with dodecanol and triethylamine to yield the phosphite (**2b**). In this case, the reaction was better conducted at a slightly higher temperature (−30 to −40 °C) on account of the poorer solubility of the alcohol in dichloromethane. The product was characterised by ^{31}P and ^{13}C NMR spectroscopy. The shift in the former spectrum was, as expected, very similar to that of (**2a**). The ^{13}C NMR spectrum was of particular interest, and was assigned by reference to compound (**1**) and model compounds such as decanol.[14] The signals for the six methylene carbon atoms towards the centre of the alkyl chain were coincident, as the literature would suggest. It is notable that all carbon atoms within 3 bonds of the phosphorus are coupled to it and appear as doublets. The magnitude of the coupling constant varies, generally being larger through oxygen than nitrogen. It is also notable that the magnitude of the phosphorus to NMe coupling constant for (**2b**) is half that for compound (**1**).

The octadecyl (**2c**) and oleyl (**2d**) cyclic phosphite triesters were prepared by the same method. Initial proplems with the former were solved by crushing and desiccating the (granular) octadecyl alcohol before use, to give a quantitative yield of the

phosphite. Both (**2c**) and (**2d**) gave similar ^{31}P chemical shift values to the earlier phosphites, as expected. Compound (**2c**) was also characterised by its ^{13}C NMR spectrum; this displayed very similar characteristics to that of (**2b**) above, although now with considerable overlap of those signals corresponding to carbon atoms towards the centre of the alkyl chain. The reason for preparing the oleyl compound (**2d**) was to test the applicability of this route to compounds containing the structural features encountered in natural phospholipids; since unsaturated alcohols are frequently present at the 2-position of natural diacyl glycerols, oleyl alcohol was used as a model. Similarly, we investigated the possibility of using an ester-containing alcohol in the synthesis. Ethyl glycolate was prepared by standard methods,[16] and reacted with compound (**1**) to yield the phosphite triester (**2e**). In this case the standard isolation procedure based on an aqueous extraction gave a poor yield, presumably due to the high water solubility of (**2e**) relative to (**2a–d**). Thus, an alternative non-aqueous isolation procedure was employed for (**2e**), giving the product in quantitative yield. It is notable that the ^{31}P NMR resonance of (**2e**) is *ca.* 3 ppm further downfield than that of (**2a–d**), presumably due to the electron-withdrawing nature of the glycolyl moiety.

Oxidation of the phosphite triesters (**2a–e**) was conducted using dinitrogen tetroxide, which has been used previously for phosphite oxidation.[17,18] It seemed likely that an excess of reagent would be deleterious to the integrity of the (labile) phosphate heterocycle, and thus a standard solution of the oxidant was used.[19] By assuming 1:4 phosphite:oxidant stoicheiometry[18] it was possible to use only the required quantity of reagent. Dichloromethane was used as solvent, bearing in mind the observation[20] that oxygen-containing solvents facilitate N_2O_4 addition across double bonds, such as are present in (**2d**) and in many natural phospholipids. The reaction of (**2a**) under these conditions proceeded cleanly at low temperature, to give the phosphate (**3a**) in near quantitative yield. As anticipated, the oxidation was marked by a dramatic upfield shift of the ^{31}P resonance; compound (**3a**) displayed a resonance at *ca.* 19 ppm, close to that recorded for analogous phosphates [*e.g.* δ_P 11 ppm for $OPNMe_2(OEt)_2$].[21] Again, the major change in the proton NMR spectrum on proceeding from phosphite to phosphate is the change in the P–NCH_3 coupling constant, from 12 Hz for (**2a**) to 10 Hz for (**3a**). This parameter seems particularly receptive to the precise environment at the phosphorus atom. Further characterisation was not conducted on this, or other phosphates, on account of their susceptibility to hydrolysis.

Similarly prepared were the dodecyl (**3b**), octadecyl (**3c**), oleyl (**3d**), and ethyl glycolyl (**3e**) analogues. These displayed similar ^{31}P NMR spectral data to (**3a**), as expected.

We had viewed the major advantage of this route to phospholipids to lie in the final step, the acid-catalysed hydrolysis of the P–N bond to yield acylic phosphate diesters, similar in structure to natural phospholipids. Although the phosphoramidate bond is known to be acid labile,[22] the precise acid lability of the heterocycle here employed was unclear. We have previously reported successful hydrolysis of this ring using rather forcing conditions (refluxing THF/2M HCl),[8] and other workers have reported the use of acetic acid.[23] However, we now decided to carry out kinetic studies of the hydrolysis reaction, employing ^{31}P NMR spectroscopy to follow the course of the reaction, since it was likely that a major upfield shift would result from P–N cleavage.[24] Moreover, we had successfully used such methods on similar diazo heterocycles.[11] Thus, following the conditions developed for the latter species, compound (**3a**) was mixed with only 5 molar % of hydrochloric acid in THF–D_2O at ambient temperature with the intention of recording ^{31}P NMR spectra at various times. Surprisingly, however, the reaction was complete within 10 min, giving a single product with a chemical shift of *ca.* 0.39 ppm. Neutralisation, lyophilisation, and chloroform extraction from the salt gave the product (**4a**) in quantitative yield. This product was pure by spectroscopy, but not by microanalysis; analytical data were obtained following column chromatography on silica gel, but this led to a reduced yield of 65%. The ^1H NMR spectrum of (**4a**) is particularly revealing, since the *N*-methyl signal at *ca.* δ 2.67 now appears as a singlet, having lost phosphorus coupling; this is firm evidence of P–N cleavage. The cleavage is further confirmed by ^{31}P NMR spectroscopy, which reveals a (solvent dependent) signal close to 0 ppm, as anticipated for a phosphate diester (*cf.* δ_P 4 ppm for sodium diethyl phosphate).[24] Phosphorus–nitrogen bond cleavage is also apparent from the ^{13}C NMR data of (**4a**) (see Table), in which the *N*-methyl signal (δ_C 31.5) is clearly a singlet having lost phosphorus coupling. The target compound (**4a**) was also characterised by mass spectrometry; perhaps surprisingly for such a polar species, the electron impact technique was successful and led to observation of a protonated molecular ion, with confirmation of the proposed structure by exact mass measurement. Fragmentation was observed by successive loss of alkyl groups from the chain.

Having noted the marked acid lability of (**3a**), it was of interest to pursue the least acidic conditions that would bring about hydrolysis. Not only would this be advantageous when dealing with labile diacylglycerols, but also the potentially problematic neutralisation step would be obviated. On occasion we had experienced problems where over-neutralisation had led to unwanted P–O cleavage. Thus, compound (**3a**) was dissolved in deuteriated water–THF, and ^{31}P NMR spectra recorded at appropriate intervals. Remarkably, hydrolysis proceeded as above in the absence of added acid; the reaction was slower under these conditions, but was complete in just over 1 h at ambient temperature. Simple lyophilisation of the reaction mixture gave the product (**4a**) in quantitative yield. This was fully characterised as above. The mechanism of this 'acid free' hydrolysis is unclear, but it may be catalysed by traces of acid present from the oxidation stage. However, the method does appear to be generally applicable. In the case of (**3b**), even the organic solvent was omitted and the phosphate was simply dissolved in D_2O, leading to emulsion formation after 30 min which precluded further NMR observation. However, lyophilisation after 3 h gave the pure product (**4b**) in quantitative yield. This showed similar spectral data to (**4a**) above, although electron impact mass spectrometry was now unsuccessful presumably due to the combined mass and charge of (**4b**). However, using FAB mode, the protonated molecular ion was observed. The problem of emulsion formation was even more evident in the case of the octadecyl compound (**3c**), but yet again simple lyophilisation gave the pure product (**4c**) in near quantitative yield. This was characterised by spectroscopic and analytical methods; again, the ^{13}C NMR spectrum is particularly informative, being directly comparable to that of (**2c**). Thus, the *N*-methyl resonance is shifted downfield on P–N cleavage, as anticipated on *N*-protonation, and now appears as a singlet having lost the coupling to phosphorus. On the other hand, the resonances of the two carbon atoms nearest to the phosphorus display upfield shifts. Interestingly, whilst the 2-bond phosphorus–carbon couplings are greatly reduced on proceeding from (**2c**) to (**4c**), the 3-bond couplings are increased.

Similarly prepared were the oleyl (**4d**) and ethyl glycolyl (**4e**) analogues, though chromatographic purification of the crude hydrolysis products was required in these cases. This did not greatly affect the yield of (**4e**) (82%), but the yield of (**4d**) was considerably reduced (65%). This correlates with the well known[25] difficulties encountered in the chromatography of phospholipids; alternative isolation procedures for (**4d**) and other compounds of this type are being sought. Both (**4d**) and (**4e**) were fully characterised. The ^{13}C NMR spectra follow the

Table. ^{13}C NMR data for (**4a–e**), recorded at 100 MHz [50 MHz for (**4e**)] in $CDCl_3$: phosphorus–carbon couplings (in Hz) are given in parentheses. Signal assignments refer to the following structure;

$CH_3CH_2CH_2(CH_2)_nCH_2CH_2CH_2OP(O)(OH)OCH_2CH_2NHCH_3$
A B C D E F G H I

Signal	(**4a**) (n = 0)	(**4b**) (n = 6)	(**4c**) (n = 12)	(**4d**) (n = 12)[a]	(**4e**)[b]
A	13.9	14.1	14.2	14.1	—
B	22.4	22.6	22.7	22.7	—
C	31.4	31.8	32.0	31.9	14.2
D	25.3	25.8	25.7	25.9	60.9
E	30.5 (7.5)	30.7 (7.4)	30.7 (7.4)	30.8 (7.5)	169.8 (7.7)
F	60.6 (3.8)	60.7 (4.9)	61.0 (5.2)	60.9 (3.3)	62.7 (3.9)
G	65.8 (5.8)	66.0 (5.9)	66.4 (5.6)	66.0 (5.8)	61.2 (5.1)
H	49.8 (6.3)	50.0 (6.0)	50.0 (6.7)	50.2 (5.5)	49.8 (5.7)
I	33.5	33.6	33.7	33.6	33.6

[a] Includes: CH=CH δ_C 130.0 and 129.9, and CH_2CH= δ_C 27.23.

[b] $CH_3CH_2OC(O)CH_2OP$–
C D E F

same trends as above, with additional peaks being observed for the alkenyl and glycolyl carbons as appropriate. The ^{13}C NMR spectrum of (**4d**) is particularly interesting, because the two alkenyl carbon atoms are non-equivalent, and the chemical shift of these resonances (δ_C 130.0/129.9 ppm) is very similar to that recorded for *cis*-dec-5-ene (δ_C 130.2 ppm) and rather different to that recorded for *trans*-dec-5-ene (δ_C 130.8 ppm).[26] The resonances of the adjacent methylene groups for these three compounds show a similar trend (δ_C 27.2, 27.5, and 32.9 respectively). These data firmly support the entirely *cis* geometry of the alkenyl moiety in (**4d**).

In conclusion, the method herein reported provides a rapid and convenient route to phospholipid-like species. The reactions proceed in high yield under mild conditions. In particular, the cyclic phosphoramidates are remarkably sensitive to hydrolysis and yield the desired phosphate diesters by simple treatment with water and lyophilisation. We regard this as a major advantage of this route, and are currently applying this method to more complicated phospholipids of biological interest.

Experimental

All reactions, excluding hydrolyses, were carried out under scrupulously dry conditions. Dichloromethane, diethyl ether, *N*-methylethanolamine, and triethylamine were dried by distillation from calcium hydride at atmospheric pressure. All but triethylamine were further dried over activated 4A molecular sieves. For TLC, Merck 60 F_{254} pre-coated silica plates were employed. For flash column chromatography, Merck Kieselgel 60 silica was used. Proton NMR spectra were recorded on a Varian XL200 spectrometer operating at 200 MHz. ^{13}C NMR spectra were obtained on this instrument, operating at 50 MHz, and ^{31}P NMR spectra similarly, at 80 MHz, or on a Varian CFT20 spectrometer operating at 32 MHz. Proton and carbon spectra were referenced to TMS, and phosphorus spectra to 85% phosphoric acid; positive shifts are downfield of the reference. In carbon spectra, carbon atoms in alkyl chains are numbered from the terminus. Mass spectra were recorded on a VG7070H spectrometer, courtesy of Dr. M. Mruzek (EIMS), or on a VG Zab1F spectrometer using *m*-nitrobenzyl alcohol as matrix, courtesy of the University of London Mass Spectrometry Service (FAB MS). Microanalyses were performed at UCL, courtesy of Mr. A. T. T. Stones.

2-Chloro-3-methyl-1,3,2-oxazaphosphacyclopentane (**1**).—Dry *N*-methylethanolamine (16 ml, 0.2 mol) and triethylamine (32 ml, 0.23 mol) in dichloromethane (30 ml) were added dropwise with vigorous stirring to dichloromethane (40 ml) at −40 °C under an atmosphere of nitrogen. Separately, but simultaneously, phosphorus trichloride (20 ml, 0.23 mol) in dichloromethane (50 ml) was added dropwise. After warming to −30 °C, a further portion of triethylamine (32 ml, 0.23 mol) in dichloromethane (20 ml) was added dropwise with vigorous stirring. The solution was allowed to warm to ambient temperature and stirred for 2 h. The solvent was then removed under reduced pressure, and the residue extracted with diethyl ether (3 × 100 ml). The extract was filtered, and the filtrate was concentrated under reduced pressure to give a yellow oil, which was vacuum distilled. The product was collected as a clear, colourless oil (12 g, 49%), b.p. 30–35 °C, 0.1 mmHg; $\delta_H(CDCl_3)$ 4.34 (2 H, m, CH_2O), 3.08 (2 H, m, CH_2N), and 2.66 (3 H, d, CH_3N, J 15 Hz); $\delta_P(CDCl_3)$ +167.3; $\delta_C(CDCl_3)$ 70.9 (d, CH_2O, J 9.5 Hz), 48.7 (d, CH_2N, J 7.2 Hz), and 30.9 (d, CH_3N, J 13.6 Hz); *m/z* (EIMS) 141 (M^+, ^{37}Cl, 5%), 138.9963 (M^+, ^{35}Cl 15%, calc. for $C_3H_7NOP^{35}Cl$, M 138.9954), 104 (M^+ − Cl, base peak), 56 (25), 42 (34), 28 (33); ν_{max}(liquid film) 2 960, 2 420, 1 640, 1 245, 1 000, 700 cm^{-1}.

2-Hexyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane (**2a**).—Anhydrous hexanol (2.24 g, 21.9 mmol) and triethylamine (2.21 g, 21.9 mmol) in dichloromethane (50 ml) were added dropwise with vigorous stirring to compound (**1**) (3.0 g, 21.5 mmol) in dichloromethane (50 ml) at −60 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 30 min, and then extracted with saturated aqueous sodium hydrogen carbonate (100 ml), followed by saturated brine (100 ml). The solution was then dried ($MgSO_4$) and evaporated under reduced pressure, to yield the product as a clear, colourless oil (3.67 g, 83%); $\delta_H(CDCl_3)$ 4.23 (2 H, m, OCH_2CH_2N), 3.66 (2 H, m, OCH_2R), 2.95 (2 H, m, NCH_2), 2.67 (3 H, d, NCH_3, J 11.8 Hz), 1.36 (8 H, m, 4 × CH_2), and 0.81 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ +135.0.

2-Dodecyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane (**2b**).—Compound (**1**) (0.50 g, 3.58 mmol) in dichloromethane (15 ml) was added dropwise with vigorous stirring to anhydrous dodecanol (0.667 g, 3.58 mmol) and triethylamine (0.50 ml, 3.62 mmol) in dichloromethane (15 ml) at −30 to −40 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 1 h, and then extracted with saturated aqueous sodium hydrogen carbonate (50 ml) followed

by saturated brine (50 ml). The solution was then dried ($MgSO_4$) and evaporated under reduced pressure, to yield the product as a clear, colourless oil (1.03 g, 100%); $\delta_P(CDCl_3)$ + 135.8; $\delta_C(CDCl_3)$ 69.0 (d, CH_2O, *J* 10.6 Hz), 63.3 (d, C-12, *J* 11.7 Hz), 49.6 (d, CH_2N, *J* 5.5 Hz), 32.0 (C-3), 31.6 (d, NMe, *J* 7.1 Hz), 29.6–31.6 (m, C-4–C-9), 29.4 (d, C-11, *J* 2.7 Hz), 25.9 (C-10), 22.7 (C-2), and 14.1 (C-1).

3-*Methyl*-2-*octadecyloxy*-1,3,2-*oxazaphosphacyclopentane* (**2c**).—Compound (**1**) (0.50 g, 3.58 mmol) in dichloromethane (15 ml) was added dropwise with vigorous stirring to anhydrous octadecanol (0.968 g, 3.58 mmol) and triethylamine (0.50 ml, 3.62 mmol) in dichloromethane (30 ml) at −40 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 2 h, and then extracted with saturated aqueous sodium hydrogen carbonate (50 ml), followed by saturated brine (50 ml). The solution was then dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as a white solid (1.34 g, 100%); $\delta_P(CDCl_3)$ +135.7; $\delta_C(CDCl_3)$ 68.9 (d, CH_2O, *J* 10.5 Hz), 63.2 (d, C-18, *J* 11.7 Hz), 49.6 (d, CH_2N, *J* 5.7 Hz), 31.9 (C-3), 31.5 (d, NMe, *J* 7.3 Hz), 29.2–32.0 (m, C-4–C-15), 29.4 (d, C-17, *J* 4.0 Hz), 25.9 (C-16), 22.8 (C-2), and 14.2 (C-1).

3-*Methyl*-2-*oleyloxy*-1,3,2-*oxazaphosphacyclopentane* (**2d**).—Anhydrous oleyl alcohol (0.84 g, 3.25 mmol) and triethylamine (0.50 ml, 3.58 mmol) in dichloromethane (30 ml) were added dropwise with vigorous stirring to compound (**1**) (0.50 g, 3.58 mmol) in dichloromethane (10 ml) at −60 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 1 h, and then extracted with saturated aqueous sodium hydrogen carbonate (50 ml), followed by saturated brine (50 ml). The solution was then dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as a colourless oil (1.24 g, 100%); $\delta_P(CDCl_3)$ +136.2.

2-(*Ethoxycarbonyl*)*methoxy*-3-*methyl*-1,3,2-*oxazaphosphacyclopentane* (**2e**).—Anhydrous ethyl glycolate (0.34 g, 3.25 mmol) and triethylamine (0.46 ml, 3.28 mmol) in dichloromethane (15 ml) were added dropwise with vigorous stirring to compound (**1**) (0.50 g, 3.58 mmol) in dichloromethane (15 ml) at −60 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature, with stirring for 1 h, and then evaporated under reduced pressure. The residue was extracted with dry hexane (100 ml), and the extract evaporated under reduced pressure to yield the product as a colourless oil (0.67 g, 100%); $\delta_P(CDCl_3)$ +138.7.

2-*Hexyloxy*-3-*methyl*-1,3,2-*oxazaphosphacyclopentane* 2-*Oxide* (**3a**).—A portion of standard dinitrogen tetroxide solution (11 ml, containing 1.3 mmol oxidant, sufficient to oxidise 5.2 mmol of phosphite) was added dropwise with vigorous stirring to compound (**2a**) (1.0 g, 4.9 mmol) in dichloromethane (20 ml) at −60 °C. The solution was warmed to ambient temperature, with stirring for 30 min, and the solvent then removed under reduced pressure to yield the product as a clear, pale yellow oil (1.04 g, 96%); $\delta_H(CDCl_3)$ 4.21 (2 H, m, OCH_2CH_2N), 3.93 (2 H, m, OCH_2R), 3.25 (2 H, m, NCH_2), 2.62 (3 H, d, NCH_3, *J* 10.0 Hz), 1.40 (8 H, m, 4 × CH_2), and 0.82 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ +18.9.

2-*Dodecyloxy*-3-*methyl*-1,3,2-*oxazaphosphacyclopentane* 2-*oxide* (**3b**). From compound (**2b**) (1.9 g) was prepared, by an entirely analogous manner to compound (**3a**) above, the title compound (**3b**) (2.0 g, 100%); $\delta_H(CDCl_3)$ 4.20 (2 H, m, OCH_2CH_2N), 4.02 (2 H, m, OCH_2R), 3.40 (2 H, m, NCH_2), 2.70 (3 H, d, NCH_3, *J* 10.0 Hz), 1.43 (20 H, m, 10 × CH_2), and 0.87 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ +18.7.

3-*Methyl*-2-*octadecyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*oxide* (**3c**). This was prepared from compound (**2c**) in an entirely analogous manner to compound (**3a**) above, except that stirring at ambient temperature was continued for 1 h. Thus, from (**2c**) (1.34 g) was produced (**3c**) as a white solid (1.39 g, 100%); $\delta_P(CDCl_3)$ +18.7.

3-*Methyl*-2-*oleyloxy*-1,3,2-*oxazaphosphacyclopentane* 2-*oxide* (**3d**). This was prepared from compound (**2d**) in an entirely analogous manner to compound (**3a**) above, except that the solution was concentrated immediately upon reaching ambient temperature. Thus, from (**2d**) (1.27 g) was produced (**3d**) (1.30 g, 100%); $\delta_P(CDCl_3)$ +19.6.

2-*Ethoxycarbonylmethoxy*-3-*methyl*-1,3,2-*oxazaphosphacyclopentane* 2-*oxide* (**3e**). This was prepared from compound (**2e**) in an entirely analogous manner to compound (**3d**) above. Thus, from (**2e**) (0.67 g) was produced (**3e**) (0.73 g, 100%); $\delta_P(CDCl_3)$ +20.1.

Attempted Kinetic Study of Acid-catalysed Hydrolysis of (**3a**).—Compound (**3a**) (0.40 g, 1.81 mmol) was dissolved in THF (1 ml) and D_2O (1 ml), and treated with 0.2M hydrochloric acid (0.53 ml, 5 molar %). ^{31}P NMR spectra were recorded after 10 min at ambient temperature; δ_P(THF–D_2O) +0.39.

The reaction mixture was then neutralised by careful addition of 0.2M aqueous sodium hydroxide, to give a final pH of 6.94. The mixture was lyophilised, and the residue extracted with chloroform (100 ml), dried ($MgSO_4$), and evaporated under reduced pressure to yield a pale yellow oil (0.43 g, 99%); $\delta_H(CDCl_3)$ 10.0 (2 H, br s, NH_2), 4.12 (2 H, m, OCH_2CH_2N), 3.78 (2 H, m, OCH_2R), 3.08 (2 H, m, NCH_2), 2.63 (3 H, s, NCH_3), 1.39 (8 H, m, 4 × CH_2), and 0.81 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ −1.4.

Kinetic Study of Water Mediated Hydrolysis of (**3a**).—Compound (**3a**) (0.45 g, 2.04 mmol) was dissolved in D_2O (1 ml) and THF (1 ml) at ambient temperature, and ^{31}P NMR spectra recorded at appropriate intervals over a period of 75 min; t = 0, δ_P(D_2O–THF) +23.0; t = 75 min, δ_P +0.48.

The reaction mixture was lyophilised to yield (**4a**) as a white solid (0.49 g, 100%); *m/z* (EIMS) 240.1371 (MH^+, calc. for $C_9H_{23}NO_4P$ 240.1359, 3%), 224 (M^+ − Me), 210 (M^+ − Et), 196 (M^+ − Pr), 183 (MH^+ − Bu), 138 (M^+ − HexO), 57 ($MeNCH_2CH_2^+$, base peak) [Found: C, 44.6; H, 9.2; N, 5.8. $C_9H_{22}NO_4P$ requires C, 44.2; H, 9.3; N, 5.9% (from dichloromethane–hexane, 1:4)].

Water Mediated Hydrolysis of (**3b**).—Compound (**3b**) (0.50 g, 1.64 mmol) was dissolved in D_2O (1.2 ml) at ambient temperature, and ^{31}P NMR spectra were recorded at appropriate intervals over 30 min, at which point an emulsion formed precluding further spectroscopic investigation. After stirring for 3 h at ambient temperature, the reaction mixture was lyophilised, yielding (**4b**) as a white solid (0.53 g, 100%); $\delta_H(CDCl_3)$ 4.10 (2 H, m, OCH_2CH_2N), 3.79 (2 H, m, OCH_2R), 3.06 (2 H, m, NCH_2), 2.61 (3 H, s, NCH_3), 1.33 (20 H, m, 10 × CH_2), and 0.81 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ −1.2; *m/z* (EIMS) 183 (M^+ − $C_{10}H_{20}$, 2%), 138 (M^+ − $C_{12}H_{25}O$, 21), 43 ($MeNCH_2^+$, 70); *m/z* (FAB MS) 324 (MH^+, 41%), 138 (M^+ − dodecanol, 40), 58 ($MeNEt^+$, base peak) (Found: C, 55.4; H, 10.7; N, 4.0; P, 9.3. $C_{15}H_{34}NO_4P$ requires C, 55.7; H, 10.6; N, 4.3; P, 9.6%).

Water Mediated Hydrolysis of (**3c**).—Compound (**3c**) (1.39 g, 3.57 mmol) was suspended in water (20 ml), and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised, yielding (**4c**) as a white solid (1.40 g, 97%); $\delta_H(CDCl_3)$ 9.98 (2 H, br s, NH_2), 4.18 (2 H, m, OCH_2CH_2N), 3.87 (2 H, m, OCH_2R), 3.13 (2 H, m, NCH_2), 2.70 (3 H, s, NCH_3), 1.42 (32 H, m, 16 × CH_2), and 0.88 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ −1.7; *m/z* (EIMS) 390 (MH^+ − H_2O, 0.3%), 252

($M^+ - C_{11}H_{23}$, 0.4), 224 ($M^+ - C_{13}H_{27}$, 1.2), 196 ($M^+ - C_{15}H_{31}$, 0.2), 182 ($M^+ - C_{16}H_{33}$, 0.3), 168 ($M^+ - C_{17}H_{35}$, 0.6), 138 ($M^+ - C_{18}H_{37}O$, base peak), 57 ($MeNCH_2CH_2^+$, 70); *m/z* (FAB MS) 408 (MH^+, 42%), 138 (M^+ − octadecanol, 9), 58 ($MeNEt^+$, base peak) (Found: C, 58.8; H, 11.35; N, 3.6. $C_{21}H_{46}NO_4P \cdot H_2O$ requires C, 59.27; H, 11.37; N, 3.29%).

Water Mediated Hydrolysis of (**3d**).—Compound (**3d**) (1.30 g, 3.36 mmol) was suspended in water (15 ml), and stirred at ambient temperature for 1 h. The reaction mixture was then lyophilised, and the residue was extracted with chloroform (100 ml). The extracts were dried ($MgSO_4$) and evaporated under reduced pressure to yield the crude product (1.34 g, 99%). This was further purified by column chromatography on silica (100 g), eluting with 80% methanol in chloroform. Pooling and evaporation of appropriate fractions gave (**4d**) as a white solid (0.88 g, 65%); $\delta_H(CDCl_3)$ 5.34 (2 H, m, CH=CH), 4.17 (2 H, m, OCH_2CH_2N), 3.84 (2 H, m, OCH_2R), 3.10 (2 H, m, NCH_2), 2.68 (3 H, s, NCH_3), 2.01 (4 H, m, CH_2C=), 1.59 (2 H, m, OCH_2CH_2R), 1.26 (22 H, m, 11 × CH_2), and 0.88 (3 H, t, CH_3CH_2); $\delta_P(CDCl_3)$ −1.9; *m/z* (FAB MS) 406 (MH^+, 5%), 321 (6), 138 (M^+ − oleyl alcohol, 2), 107 (3), 58 ($MeNEt^+$, base peak) (Found: C, 55.9; H, 10.3; N, 4.05. $C_{21}H_{44}NO_4P \cdot 2.5H_2O$ requires C, 55.98; H, 10.96; N, 3.11%).

Water Mediated Hydrolysis of (**3e**).—Compound (**3e**) (1.02 g, 4.57 mmol) was dissolved in water (12 ml), and stirred at ambient temperature for 1 h. The reaction mixture was then lyophilised and the residue purified by column chromatography on silica (80 g), eluting with 50–75% methanol in chloroform. Pooling and evaporation of appropriate fractions gave (**4e**) (0.90 g, 82%); $\delta_H(CDCl_3)$ 8.6 (2 H, br s, NH_2), 4.45 (2 H, d, $POCH_2R$, *J* 9.2 Hz), 4.18 (4 H, m, OCH_2CH_2N, CH_3CH_2), 3.17 (2 H, m, NCH_2), 2.72 (3 H, s, NCH_3), and 1.26 (3 H, t, CH_3CH_2, *J* 7.1 Hz); $\delta_P(CDCl_3)$ −2.5; *m/z* (FAB MS) 242 (MH^+, base peak), 169 ($MH^+ - CO_2Et$, 2%), 58 ($MeNEt^+$, 93) (Found: C, 33.75; H, 6.45; N, 5.55; P, 12.7. $C_7H_{16}NO_6P \cdot 0.5H_2O$ requires C, 33.61; H, 6.85; N, 5.60; P, 12.38%).

Acknowledgements

We thank the SERC for the provision of a studentship (B. S.) and thank The Nuffield Foundation for an equipment grant.

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Paper 9/03434J
Received 10*th August* 1989
Accepted 11*th October* 1989