THE SYNTHESIS OF SOME NOVEL PHOSPHOLIPID ANALOGUES AS POTENTIAL CHEMOTHERAPEUTIC AGENTS

by

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Scientifically speakin', it's all a question of the accidental gatherin' together of mollycewels an' atoms.

"The Plough and the Stars"
Sean O'Casey

Acknowledgement.

I would like to take this opportunity to thank the Science and Engineering Research council for the provision of a research studentship and Professor M.L. McGlashan and Professor R.J.H. Clark for the provision of research facilities at UCL.

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I thank all my friends and colleagues in the department, past and present, for making my time here so enjoyable, with a special thanks to Vern for his assistance with endnote and Colin for his patience when teaching me how to use a word processor. I also thank Professor A.G. Davies and Dr P.J. Garratt for their assistance in my final year.

Finally, I thank my parents for all their support over the years and it is to them that I dedicate my thesis.

Abstract.

This thesis is concerned with the synthesis of a range of novel phospholipid analogues with a variety of head groups and hydrocarbon chains.

Three series of phospholipids consisting of the N-methyl, *t*-butyl and free amino head groups were prepared by a rapid three-step procedure involving phosphoramidite intermediates.

The corresponding phosphitylating agents containing the amino group in a protected cyclic form were condensed with a variety of alcohols to yield cyclic phosphoramidites. The alcohols ranged from short to long alkyl chain primary, secondary and tertiary forms, including those containing a double bond and ester functionalities. The phosphites were then oxidised to the corresponding phosphates in high yield. Finally, P-N cleavage by simple hydrolyses with water gave the phospholipid analogues.

A series of analogues with a serinol head group were prepared *via* phosphate methodology. Thus reaction of 2-amino-2-methyl-1,3-propanediol with alkyl phosphorodichloridates gave the corresponding cyclic phosphates. Subsequent hydrolyses yielded the desired serinol phospholipid analogues.

An alternative route to the phospholipids *via* H-phosphonate methodology was pursued. Hydrolyses of cyclic phosphoramidites gave the H-phosphonate intermediates. However, subsequent attempts to oxidise and sulphurise the intermediates failed to yield the desired phospholipid analogues.

The initial synthesis of a phosphitylating agent, where the oxygen had been replaced by a methylene was also pursued with some success.

The biological evaluation of several of the novel phospholipid analogues as potential anti-HIV agents is reported.

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CHAPTER 1

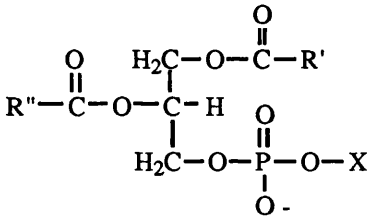
A Review of Phospholipids.

A Review of Phospholipids.

1.1. The Structure of Phospholipids.

Phospholipids are phosphorus-containing lipids. They possess a hydrophilic head group and a hydrophobic chain linked by a phosphodiester bridge.[1,2,3,4]

There are two major types of phospholipids, the glycerophospholipids **(1)**, which have a glycerol backbone and the sphingolipids **(2)**, whose structure is based on sphingosine.[4,5]



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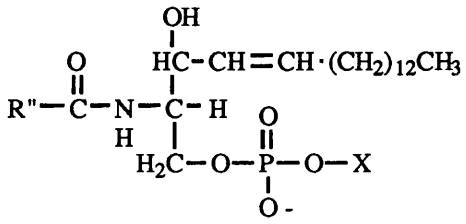
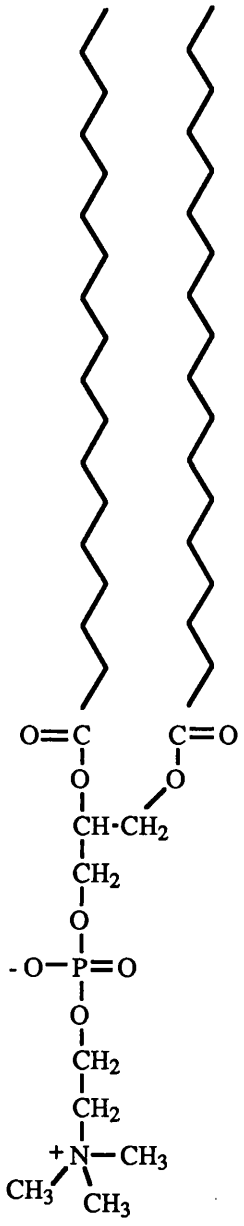


(2)

The glycerophospholipids can be further subdivided according to the nature of the head group X,[5,6] which may be choline in phosphatidylcholine **(3)**, ethanolamine in phosphatidylethanolamine **(4)**, serine in phosphatidylserine **(5)**, *myo*-inositol in phosphatidylinositol **(6)** or glycerol in diphosphatidylglycerol e.g. cardiolipin **(7)**.



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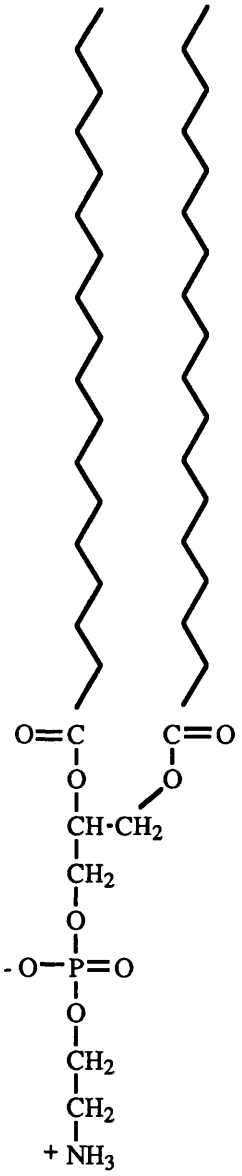

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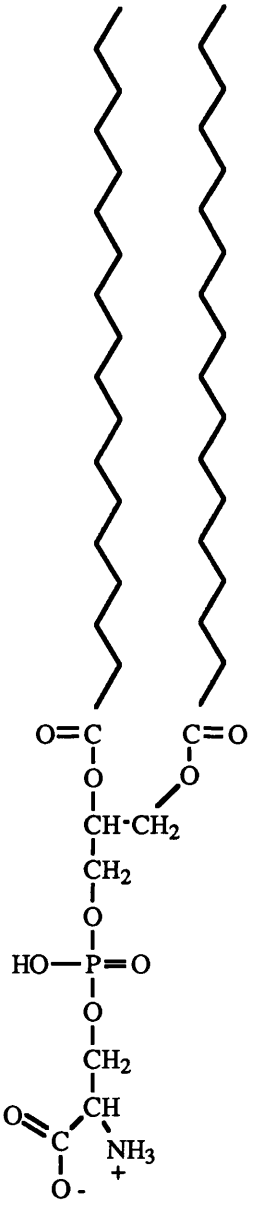

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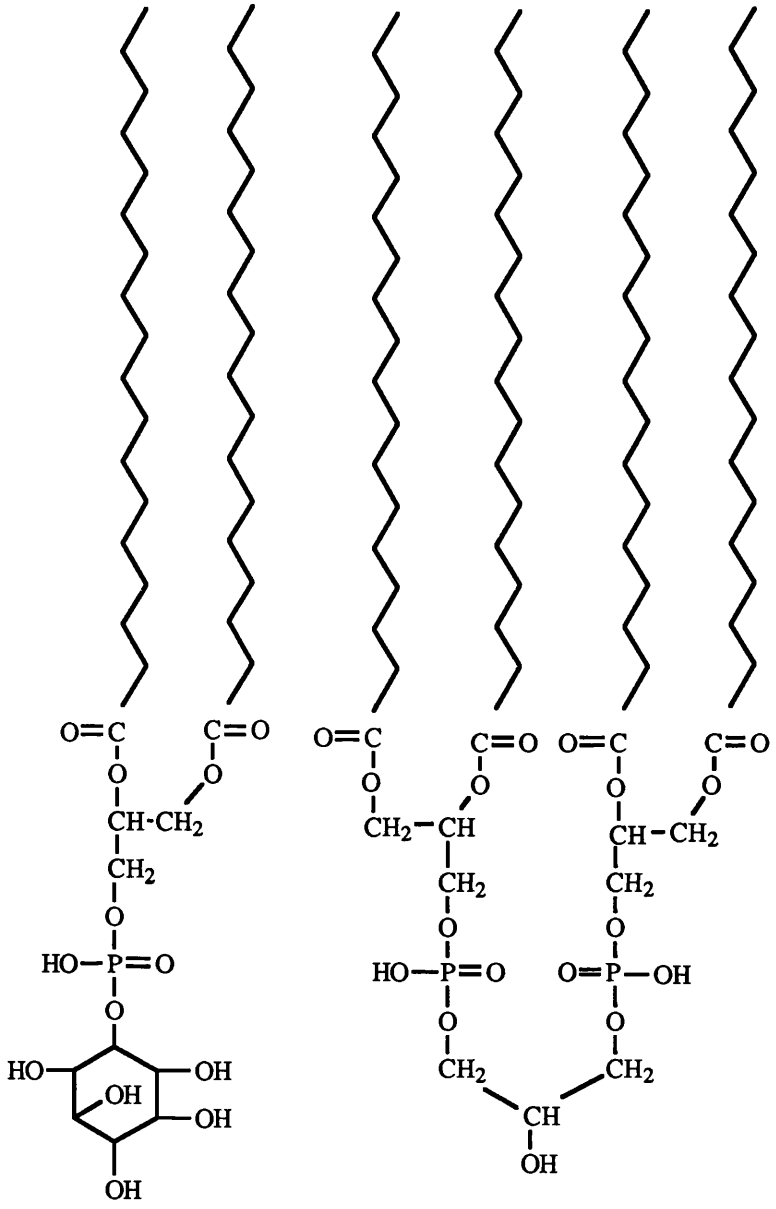


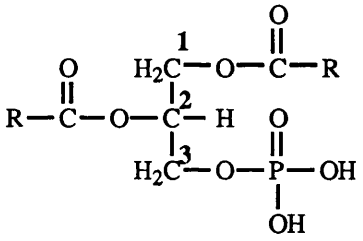
(6) (7)

The nature and the length of the acyl chain also varies. Animal phospholipids contain fatty acids between chain length 14 and 24, with the 16 and 18 carbon chains predominant. The chains can be saturated or unsaturated e.g. oleic (18:1) and linoleic (18:2). The configuration of these alkenes is nearly always *cis*.[2] Plant phospholipids have a more limited range with few fatty acids of

greater than 18 carbons, whilst bacteria have a more complex range of short saturated chains that are often branched and esterified.[4] Natural phospholipids also contain a chiral carbon at position C-2 of the glycerol backbone and in higher plants and animals only the *R*-enantiomer exists.[7]

1.2. Nomenclature.

The nomenclature of phospholipids is based on a proposal by Hirschmann.[8] The carbons of the glycerol backbone are numbered from 1 to 3, thus, phosphatidic acid **(8)** is 1,2-diacyl-*sn*-glycero-3-phosphate, where *sn* refers to stereospecific numbering.



(8)

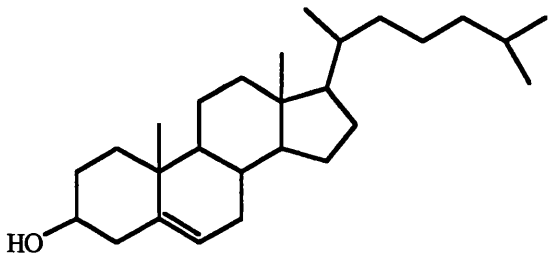
The specific distribution over positions 1, 2 and 3 of the glycerol molecule introduces chirality. The respective *S*-enantiomer (i.e the unnatural isomer) is 2,3-diacyl-*sn*-glycero-1-phosphate.

1.3. The Functions of Phospholipids.

Phospholipids play a major role as components of both cell surface membranes and subcellular organelles and are also implicated in a wide range of physiological processes.[4]

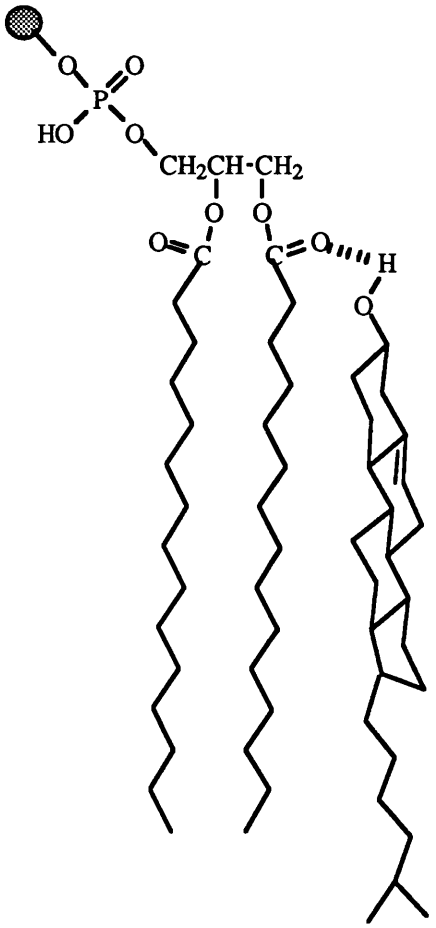
1.3.1. Cell Membranes.

It is the amphipathic character of phospholipids which result in their ability to self-associate in aqueous solutions forming bilayers enclosing aqueous compartments. Aggregation organises the head groups to oppose the water and allows the adjacent hydrocarbon tails to interact, *via* Van der Waals forces, protected from the water.[9] Other lipids, e.g. cholesterol **(9)**, which also has amphipathic character by virtue of its polar hydroxyl group bonded to the steroid ring system, orientate themselves similarly to the phospholipids.[10](Refer to figure 1).



(9)

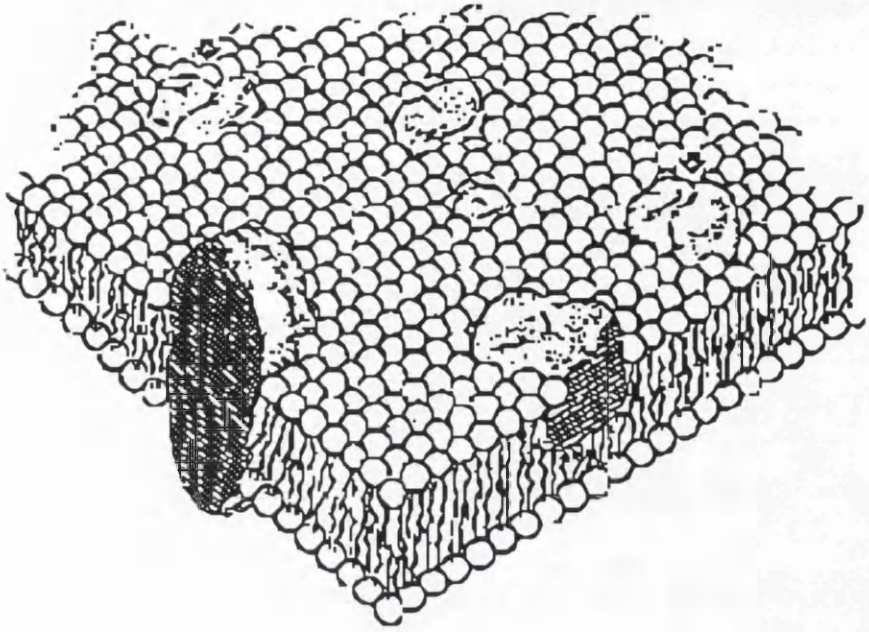
Figure 1. Possible interaction between cholesterol and a phospholipid molecule.



Cholesterol acts as a membrane stabiliser, maintaining the membrane integrity by reducing the effective area of the phospholipids and adjusting their flexibility and permeability.[11]

The other main component of cell membranes, proteins, are embedded in the membrane and may even completely span it. (Refer to figure 2). The proteins contact both hydrophilic and hydrophobic portions of the lipids surrounding them.[10]

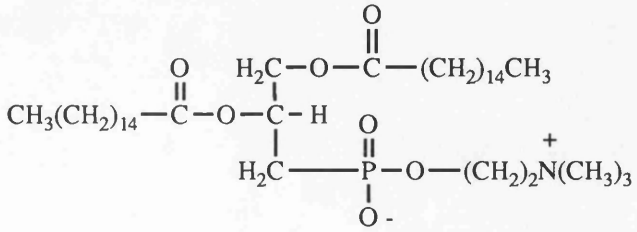
Figure 2. The Cell Membrane.[12]



The ratio of lipid to protein depends on the role of the cell.[13] At one extreme the inner mitochondrial membrane contains about 25% lipid to 75% protein, while at the other extreme the myelin membrane contains as much as 75% lipid.

1.3.2. Lung Function.[4]

Normal lung function depends on a constant supply of the surfactant dipalmitoylcholine **(10)**.



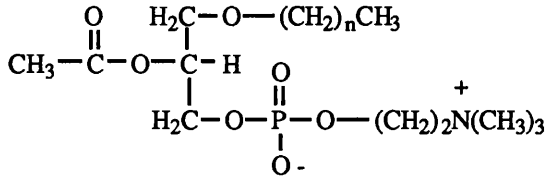
(10)

This phospholipid is produced by type II epithelial cells and contributes more than 80% of all the phospholipid in the

extracellular liquid layer lining the alveoli. It acts by decreasing surface tension of the aqueous surface of the alveoli, preventing complete collapse of the alveoli at the end of expiration. A deficiency of pulmonary surfactant at birth is the underlying cause of respiratory distress syndrome of premature infants, which is a major source of neonatal mortality.[4] In adults, pulmonary surfactant dysfunction has been implicated in a variety of lung diseases, leading to adult respiratory distress syndrome. This has le d to the interest in the synthesis of exogenous surfactants, i.e. analogues of dipalmitoylcholine to replace the deficient or inactivated natural lung surfactant.[14,15]

1.3.3. Hypersensitivity and Allergic Response.

Platelet activating factor (PAF) is a major mediator of hypersensitivity, acute anti-inflammatory reactions and anaphylactic shock.[13] It was the first bioactive phospholipid to be discovered,[16,17]although its chemical structure was not properly elucidated until 1979 [18,19,20] when it was characterised as 1-*O*-hexadecyl/octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine **(11)**.



(11) n=14,16

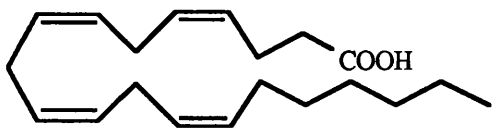
It is produced by many types of cells, including basophils, neutrophils, eosinophils, macrophages, platelets and endothelial cells. When stimulated they produce PAF which accumulates in the parent cell membrane and further activates adjacent cells.[21] The

actual liberation of PAF from the cells appears to be related to several factors including the type of cell involved.[22] PAF has the effect of platelet aggregation and is a chemoattractant for the granulocytes. The latter is accompanied by superoxide generation and degranulation leading to the release of lysosomal enzymes and cationic proteins.[23]

Injected intravenous PAF elicits systemic hypotension, bronchoconstriction, increased microvascular permeability, thrombocytopenia and neutropenia. Inhalation of PAF triggers an inflammatory response involving oedema, cell accumulation, mucus secretion and bronchoconstriction.[24] Interest in PAF heightened following the observation that it induced bronchial hyperresponsiveness in normal human subjects[25] and therefore could be of use in asthma therapy. However, subsequent studies of this effect have produced conflicting results. Some research supports the hyperresponsive effect,[26] while others failed to show this.[27] The former studies suggest that PAF acts by causing the secondary release of other mediators e.g. leukotrienes.

1.3.4. Precursors of Biologically Active Metabolites.

Membrane phospholipids are precursors of arachidonic acid **(12)**, which, once released, is rapidly metabolised to biologically active metabolites (eicosanoids).[28]

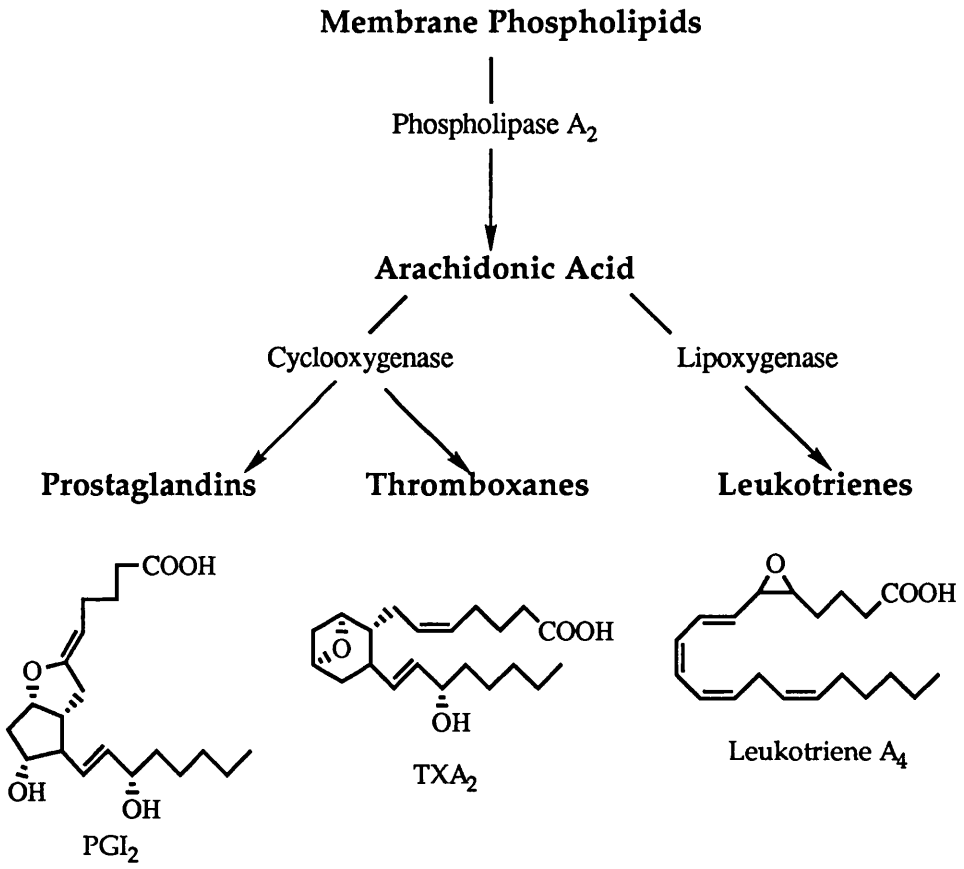


(12)

The release of arachidonic acid is catalysed mainly by phospholipase A_2, which is thought to be regulated by several factors including Ca^{2+}, protein kinase C and PLA_2 regulating proteins.[29]

Figure 3 shows the enzymes responsible for and the products of the biosynthesis of arachidonic acid.

Figure 3. Arachidonic Acid Cascade.[30,31]

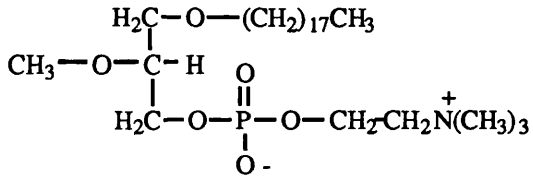


These eicosanoids are formed from C_{20} polyunsaturated fatty acids and have a variety of biological activities.[32] They are generally considered to be hormones that exert their effect locally. This is because they are generated *in situ* and are rapidly metabolised. The group known as the prostaglandins are natural mediators of

inflammation and have the effect of increasing local blood flow by arterial vasodilation which in turn, lowers the systemic arterial pressure. They also cause smooth muscle contraction and inhibit platelet aggregation and adhesion. Thus they act as antagonists to the thromboxanes which cause vasoconstriction and platelet aggregation. The leukotrienes have the ability to contract respiratory, vascular and intestinal smooth muscle, although the exact mechanism of their action is unknown.[33]

1.3.5. Anti-Cancer Agents.

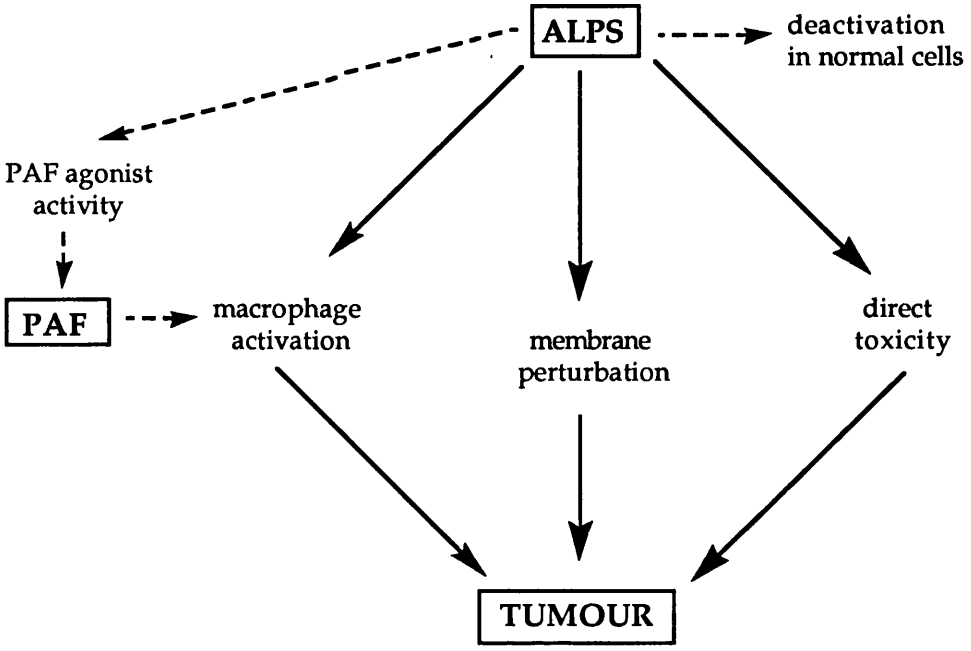
The ether phospholipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine **(13)** is known to be a potent inhibitor of tumour cell growth.[34]



(13)

Its specificity for tumour cells was originally explained by the lack of the degradation 1-*O*-alkyl cleavage enzyme in tumour cells leading to membrane perturbation.[35,36,37,38] However, more recent work supports that several factors are involved in anti-tumour action, including its inhibition of a phospholipid-sensitive Ca^{2+}-dependent protein phosphorylation system[39,40] and stimulation of macrophages to destroy tumour cells.[41]

Figure 4. Proposed mode of anti-tumour action.[41]



Much research has been carried out into the synthesis and evaluation of a range of ALP analogues.[42,43,44] In general, phase 1 clinical trials performed on patients with advanced malignancies have shown some success in terms of tumour shrinkage. Noted side effects of these ALP analogues were related to PAF activity, e.g. pulmonary oedema. Encouragingly however, the usual side effects of other anti-tumour cytotoxins, e.g. myelosuppression and alopecia were not evident.[45,46] Thus ALP analogues show promise as anti-tumour agents if their PAF activity can be minimised or eliminated.

1.4. Phospholipid Turnover.

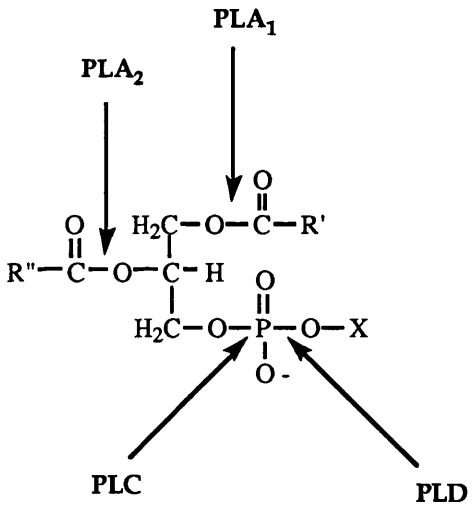
For a long time it was thought that phospholipids were metabolically inert and it was not until radioactive isotopes of phosphorus (^{32}P) became available that this myth was disproved. In a pulse experiment it was found that all of the phospholipids in the

sample became labelled with radio-labelled phosphate. Further, the incorporated radioactivity disappeared rapidly from the phospholipid fraction.[47] These observations led to the concept of phospholipid turnover in which a constant supply of newly synthesised phospholipids is balanced by the removal of existing phospholipids, thus maintaining the structural integrity and function of the membrane.

1.5. Phospholipases.[33,48,49]

The enzymes responsible for phospholipid turnover are the phospholipases. Their sites of action are shown in figure 5.

Figure 5.



The activity of the phospholipases depends on the physiochemical nature of the phospholipid substrate. Since phospholipids form micelles above the critical micelle concentration (cmc), Michaelis-Menton kinetics cannot be used to study these enzymes.[48] The value of the cmc is related to the structure of the

phospholipid and physical parameters such as surface charge, temperature and ionic environment. [50]

1.5.1. Phospholipase A_1.

Phospholipase A_1 specifically removes the fatty acid from the C-1 position of the glycerol backbone to give the corresponding lysophospholipid. This enzyme also has some lysophospholipase and lipase activity.[51]

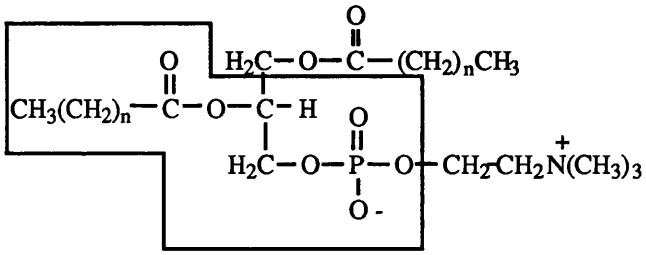
1.5.2. Phospholipase A_2.

PLA_2 is by far the most intensely studied of the phospholipases, mainly because it produces the substrates for the generation of arachidonic acid and PAF. It catalyses the ester cleavage at the C-2 position of the glycerol backbone to give the corresponding fatty acid.[52] Because of its role in generating arachidonic acid, a good way of regulating the activity of the eicosanoids, thus controlling inflammation and response to tissue injury, would be by PLA_2 inhibition.

Many PLA_2 inhibitors have been found.[53] Some of these compounds are inhibitors by virtue of their ability to interfere with the substrate enzyme interface, e.g. halothane which makes the membrane more fluid or cholesterol which makes the membrane more rigid.[54] Some anti-inflammatory agents are thought to have therapeutic effects because they inhibit cyclooxygenase, however, the anti-inflammatory agent indomethacin also inhibits PLA_2 at higher concentrations.[55]

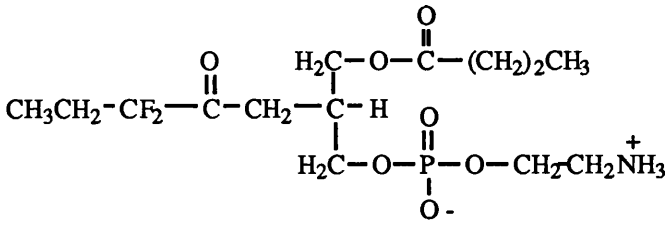
The design of active site directed inhibitors has been explored. Van Deenen and de Haas[56] observed that the minimal requirements for a compound to be a PLA_2 substrate were a fatty acid ester bonded vicinally to a phosphate diester. (Refer to figure 6).

Figure 6. Minimal Requirements for a PLA_2 Substrate.



By systematic modification of the phosphatidylcholine molecule, van Deenen and de Haas went on to produce a series of active site directed PLA_2 inhibitors. These included analogues with unnatural stereochemical configuration, C-2 position ester bond modifications e.g. branched chains, and an amide linkage in place of an ester bond.[56] This work supported the earlier minimal requirements theory and highlighted the stereospecificity factor since only the *R* form could be hydrolysed.

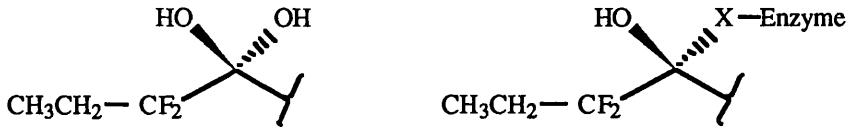
Transition state-like inhibitors of PLA_2 have also been synthesised.[57,58] Gelb and co-workers synthesised a difluoromethylene ketone analogue **(14)** and found it was a potent inhibitor of PLA_2.[57]



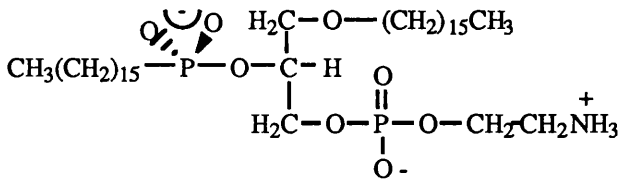
(14)

The proposed reason for activity was that in aqueous solution the difluoromethylene ketone moiety would be hydrated and might mimic the high energy tetrahedral intermediate that is presumed to form during hydrolysis. Alternatively the analogue could bind to the enzyme *via* a nucleophile present at the active site. (Refer to figure 7).

Figure 7. Possible Modes of Enzyme Interaction.



Gelb went on to extend these PLA_2 inhibitory studies by synthesising phosphonate analogues of phospholipids.[58] In this work a phosphonate group was employed to mimic the high energy tetrahedral intermediate in hydrolysis. A two chain phosphonate analogue **(15)** was found to be a more potent inhibitor than the difluoromethylene ketone analogue.



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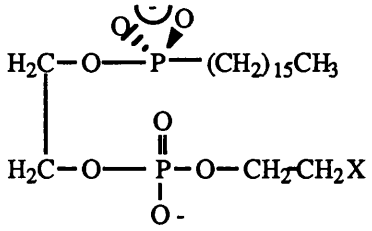
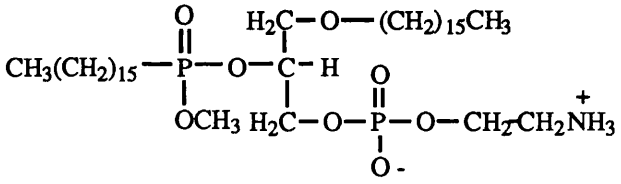
Further a single chain analogue **(16)** was found to be much less inhibitory than the two chain analogue, implying some C-1 alkyl chain interaction with the enzyme is involved.



(16)

X
$^{+}NH_3$
$^{+}N(CH_3)_3$

Also a phosphonate triester **(17)** was found to be much less active than the phosphonate diester implying that the negative charge on the phosphonate is essential for interaction with the enzyme.



(17)

1.5.3. Phospholipase C.

PLC is mainly found in bacteria,[59,60] but there is also a mammalian PLC which is specific for phosphatidylinositol **(6)**.[61] It catalyses the hydrolysis of the glycerophosphate ester bond with the formation of 1,2-diacylglycerol and a phosphate monoester.[49] PLC action is linked to the release of arachidonic acid, since the diacylglycerol released may then be acted on by a lipase to release arachidonic acid and monoacylglycerol.[62]

1.5.4 Phospholipase D.

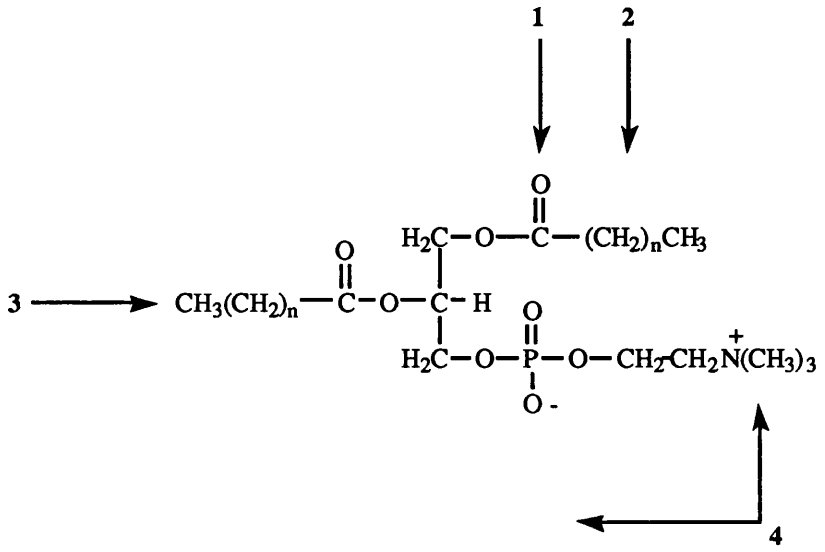
There are few reports of mammalian PLD, although, brain and lung tissue have been shown to have some activity.[63] Most studies of PLD activity involve enzymes isolated from plant sources.[64,65] There is still uncertainty about the physiological role of this enzyme in cellular metabolism or phospholipid turnover, however, it is unique among the phospholipases in its ability to transfer a phosphatidyl moiety between two nucleophilic compounds containing a primary hydroxyl group.[66] It may be involved in converting 1-alkyl lysophosphatidylethanolamine to the 1-alkyl lysophosphatidylcholine.[67]

1.6. The Synthesis of Phospholipids.

The increasing significance of phospholipids as potential chemotherapeutic agents has resulted in the development of synthetic routes to both natural and unnatural phospholipids.

Some potential modifications of the phospholipid structure to give novel phospholipid analogues are shown in figure 8.

Figure 8.



1. The ester linkage can be replaced with an ether[35] or thioether[68] linkage to give analogues of ALP.

2. The number of carbon atoms, n, in the side chains and degree of unsaturation can be varied to give analogues of PAF[69] and ALP.

3. The chain at the C-2 position can vary in terms of points 1 and 2 above, as well as in functionality, e.g. carbamyl[70] and acylamino[71,72] derivatives. Substitution with short ether linked groups e.g. OCH_3 and OCH_2CH_3 gives ALP analogues, while short ester linked groups give PAF analogues.

4. Modifications of the polar head group alters the charge pattern of the molecule.[73,74] While substitution of oxygen with sulphur increases lipophilicity and substitution with methylene prevents PLC catalysed hydrolyses of the head group.[75]

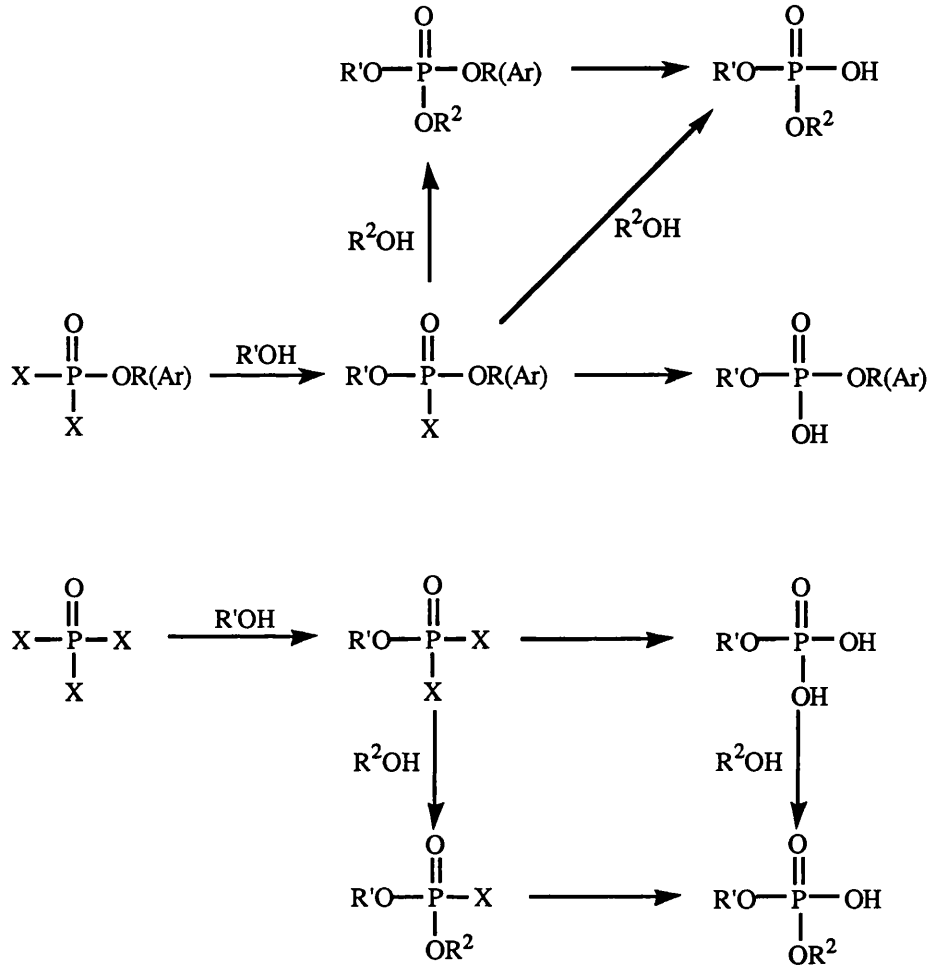
Generally, the synthesis involves the preparation of a symmetric or asymmetric diacylglycerol[76,77] followed by its phosphorylation to introduce the polar head group moiety. The phosphorylation technique used can involve either a phosphate or a phosphite species.

1.6.1. Phosphate Methodology.

There are three major phosphorus (V) phosphorylation techniques used in phospholipid synthesis. These are phosphodiester, phosphotriester and phosphorochloridate methodologies.

Scheme 1 shows both the phosphodiester and phosphotriester routes. In a given phosphorylating agent the atom or group X represents a good leaving group. The alkoxy or aryloxy group represented by OR or OAr serves as a protecting group at phosphorus.

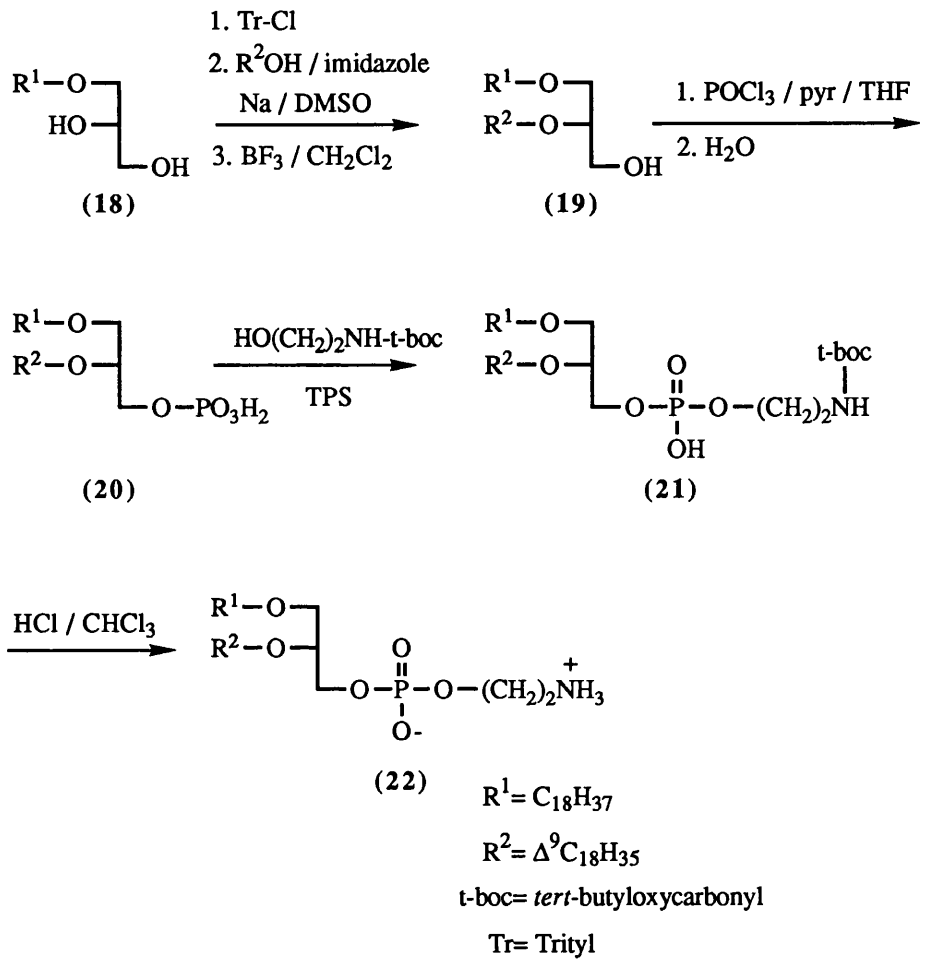
Scheme 1.



Typical condensing agents used to bring about the condensation reaction between an alcohol ROH and the POH function are dicyclohexylcarbodiimide (DCC),[78] 2,4,6-triisopropylbenzenesulphonylchloride (TPS)[79] and similar derivatives e.g. 2,4,6-triisopropylbenzenesulphonyl-(3-nitro-1,2,4-triazole) (TPS-nitrotriazole).[80]

A diester approach to phospholipid synthesis usually entails activated condensation of phosphatidic acid with an alcohol. An example of this is shown in scheme 2.

Scheme 2.

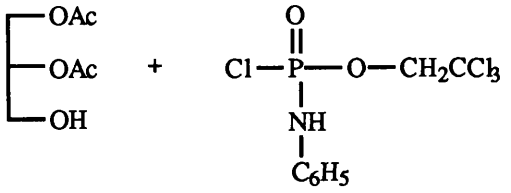


This route was developed by Paltauf[81,82] from earlier work on diacyl analogues, involving the use of TPS as a condensing reagent, by Barzilay and Lapidot[83] and Aneja and co-workers.[84] The condensation of the phosphatidic acid **(20)** with the tertiary-butyloxycarbonyl protected ethanolamine was brought about in the presence of TPS. The phospholipid product **(22)** was obtained by removal of the t-boc protecting group under acidic conditions.

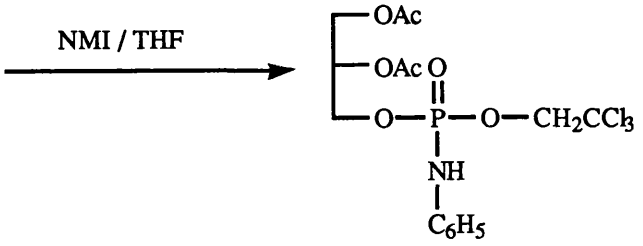
Triester routes were developed out of the desire to reduce the possibility of by-product formation that often occurs when

phosphodiester methodology is employed. Lammers and van Boom[85] criticised the phosphorylation of diacylglycerols with alkyl phosphoric acid dichlorides on the grounds that by-product formation occured due to the bifunctionality of the phosphorylating agent. They instead favoured the phosphate triester approach outlined in scheme 3, which was influenced by similar trends in oligonucleotide synthesis.[86]

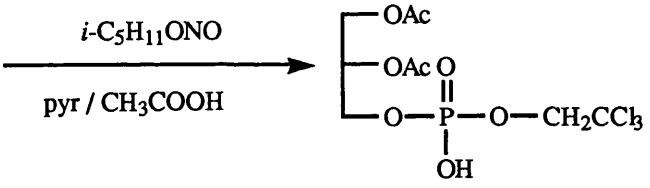
Scheme 3.



(23) **(24)**



(25)



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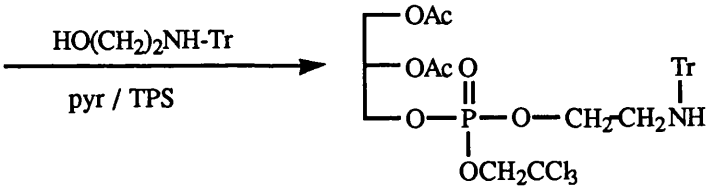

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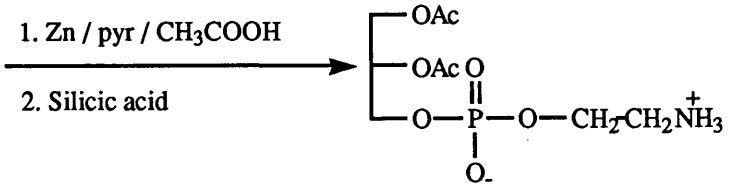
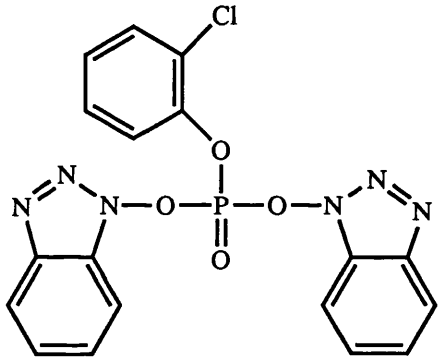


(28)

Ac = $CH_3(CH_2)_{16}C(O)$

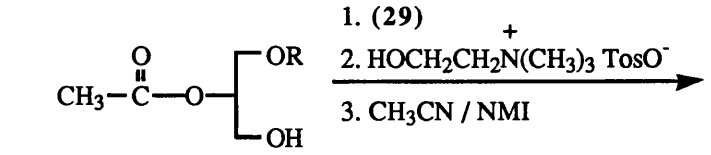
Here the protecting group is trichloroethyl which is subsequently removed using a zinc/acetic acid mixture in pyridine. The condensing agent used is TPS.

Van Boom also presented a phosphotriester route to phospholiponucleotides,[86] phosphate polysaccharides[87] and a PAF enantiomer [88] using the phosphorylating agent 2-chlorophenyl-bis-(1-hydroxybenzotriazole) phosphate **(29)**. (Refer to scheme 4).



(29)

Scheme 4.



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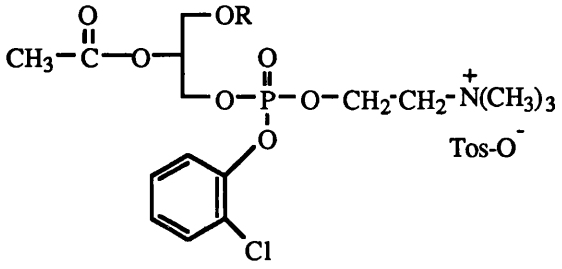
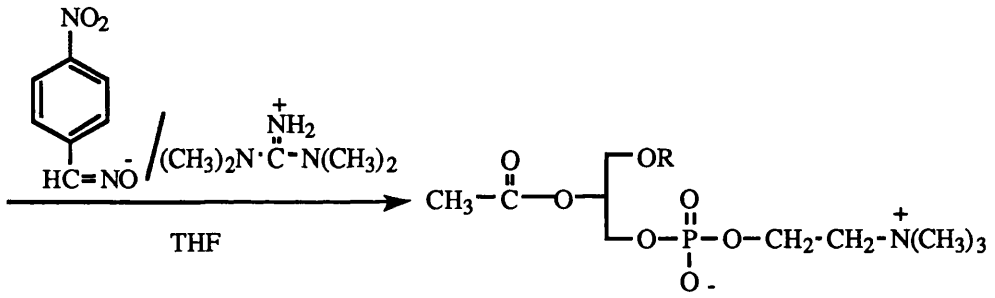


(31)



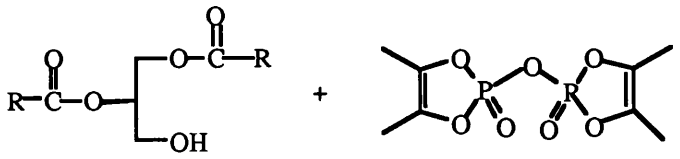
(32)

R= $C_{16}H_{33}$

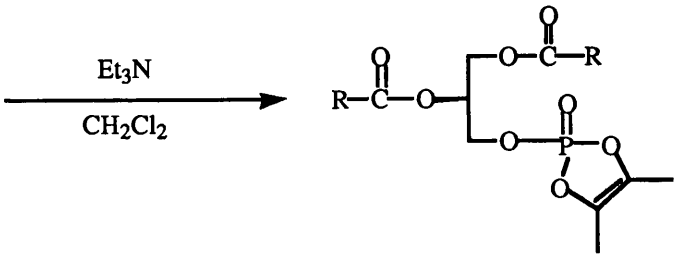
The PAF enantiomer **(32)** was obtained in an overall yield of 75% based on the starting alcohol, with no reported acyl migration.

Ramirez *et al* synthesised a variety of phospholipids including a cardiolipin analogue **(37)** using a novel phosphotriester route.[89,90] This involves a cyclic enediolphosphoryl reagent di(1,2-dimethylethylene) pyrophosphate **(34)**. (Refer to scheme 5).

Scheme 5.



(33) (34)



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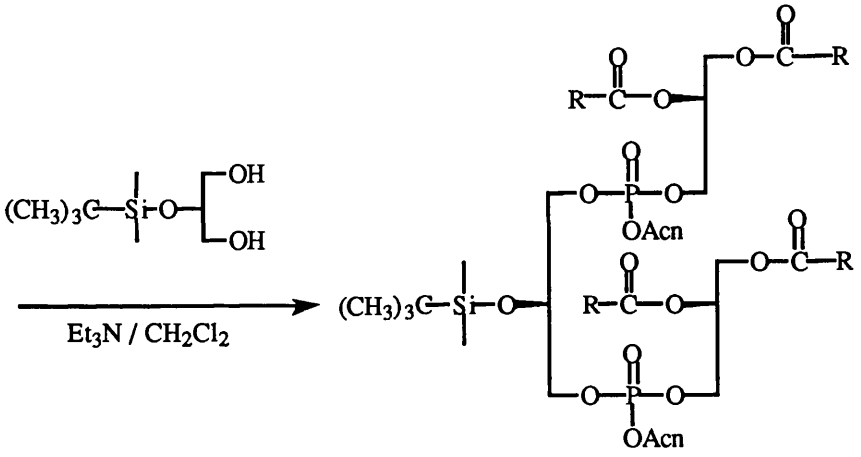
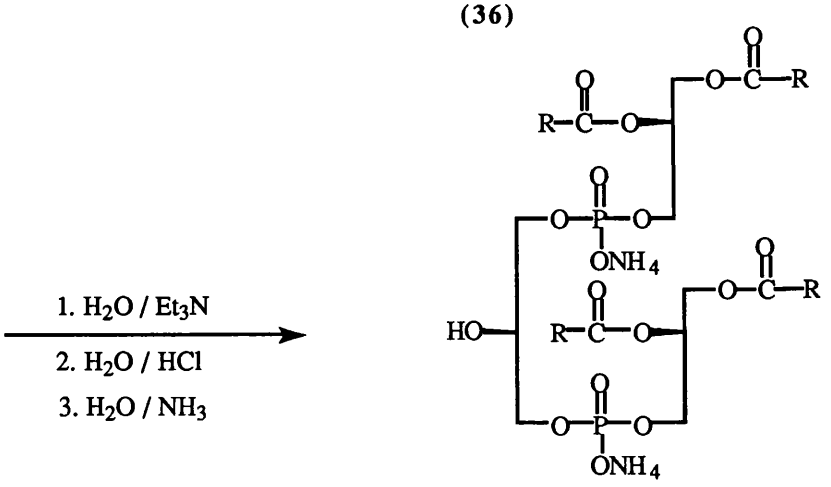


(36)

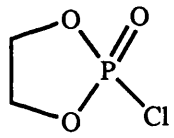


(37) R= $C_{17}H_{35}$

The reagent **(34)** is condensed with the alcohol **(33)** in the presence of triethylamine base and the resulting cyclic compound **(35)** then ring opened with 2-(*t*-butyldimethylsilyl)glycerol. The protecting group methylacetonyl (Acn) arises from the ring opening step of the synthesis and is subsequently removed using triethylamine, pyridine and water. The end product **(37)** was isolated in only 29% overall yield.

The major criticism of these methods is that many reaction steps are involved and the overall yield based on the starting alcohol is often low. Thus the improvements on earlier methods are only marginal in most cases.

The phosphorochloridates used in phospholipid synthesis can be divided into two categories, cyclic or acyclic. The most frequently used cyclic phosphorochloridate is 2-chloro-1,3,2-dioxaphosphacyclopentane-2-oxide **(38)**.

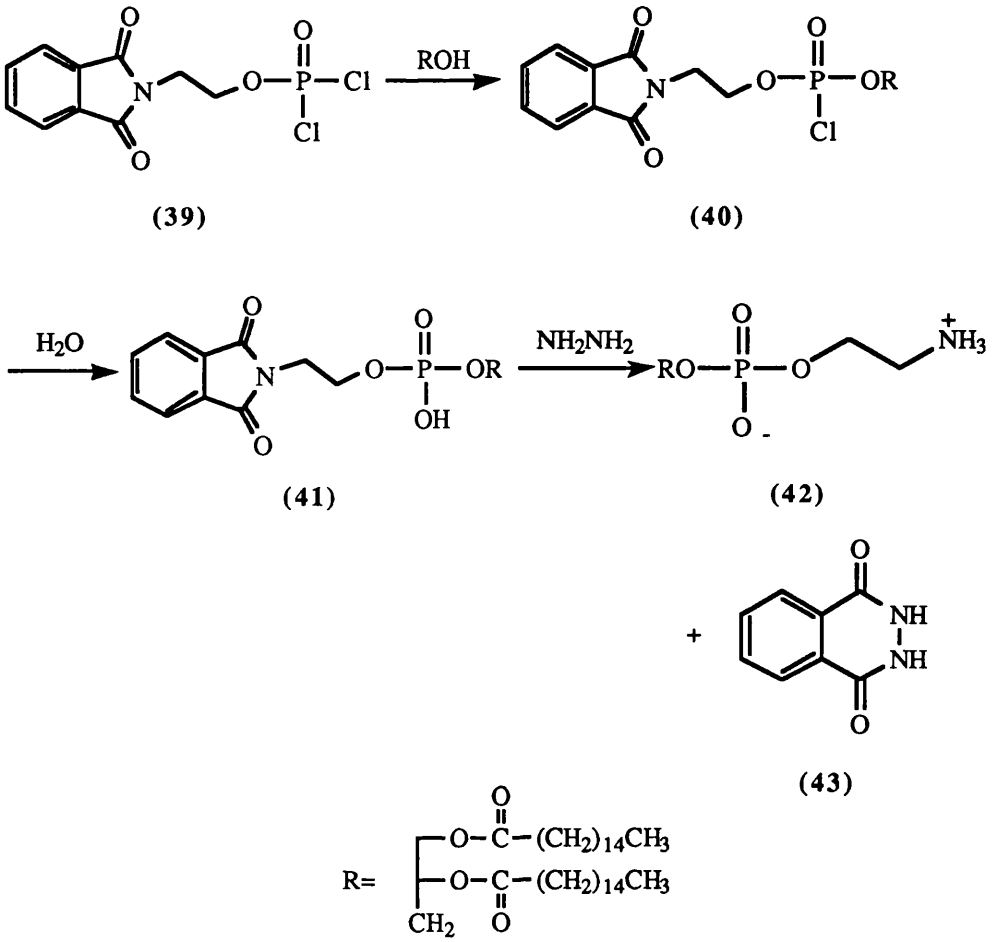


(38)

Whilst the most commonly used acyclic phosphorochloridates are phosphorus oxychloride and alkyl phosphorodichloridates.

The classical route to phosphatidylethanolamines was described by Rose.[91] He introduced the phthalimido protecting group which was removed in the last step of the synthesis, by hydrazinolysis. (Refer to scheme 6).

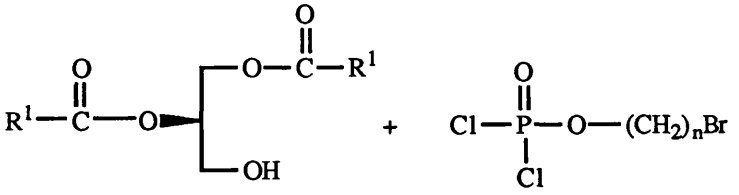
Scheme 6.



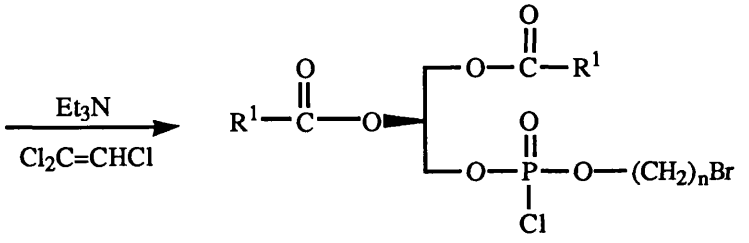
(39) (40) (41) (42) (43)

Bromoalkyl phosporodichloridates were first used for phospholipid synthesis by Hirt and Berchtold[92] in 1957 when they introduced bromoethyl phosphorodichloridate as a phosphorylating agent. Eibl and Nicksch[93] and Eibl and Diembeck[94] further developed this route to phosphatidylethanolamines and their analogues. (Refer to scheme 7).

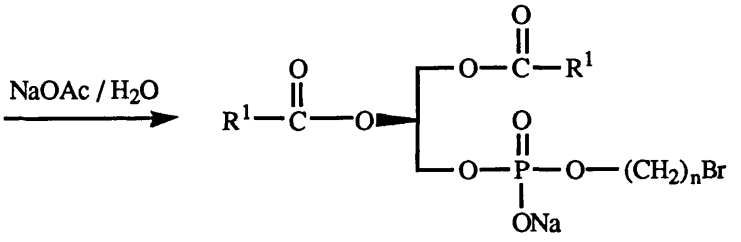
Scheme 7.



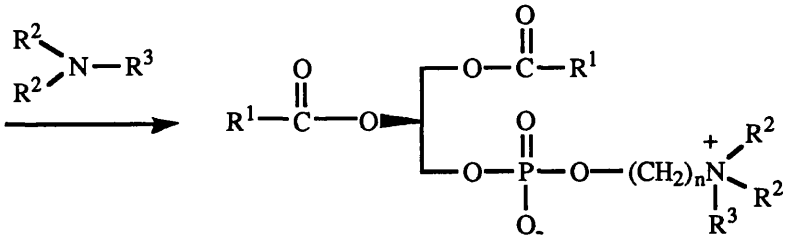
(44) **(45)**



(46)



(47)



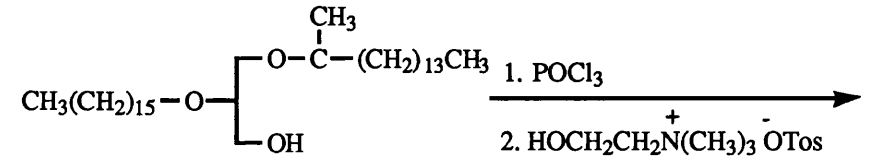
(48)

$R^1 = C_{15}H_{35}$
$R^2 = CH_3, R^3 = H$
$R^2 = H, R^3 = CH_3$
n= 3-11

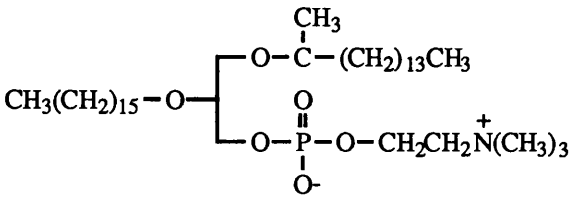
The final stage of the reaction involved direct amination with ammonia, methylamine, dimethylamine or trimethylamine respectively. Overall yields of between 75 and 85% of phospholipid were obtained using this method. This route appears to be particularly good for synthesising novel analogues of differing polar head group type as well as variations in head group chain length. However, many other workers[95,96,97] have utilised this technique and report lower overall yields. Most of these cases involve the syntheses of PAF and PAF analogues and the reported lower yields are most likely because of side reactions.

Phosphorus oxychloride is often used in conjunction with choline tosylate to introduce the phosphorylcholine moiety into a diacylglycerol. Bittman and Witzke[98] synthesised racemic mixed chain ether glycerophosphorylcholine in 51% yield using just such a system. (Refer to scheme 8).

Scheme 8.



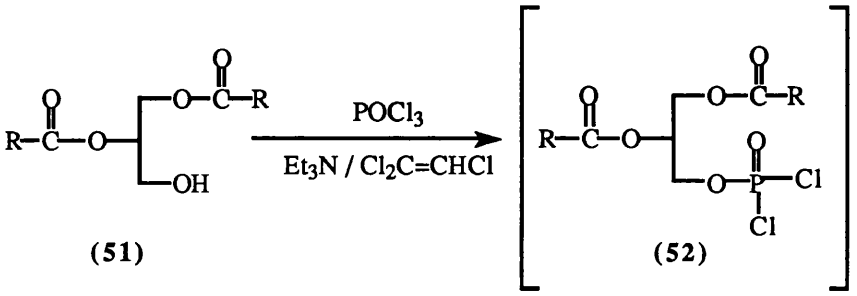
(49)



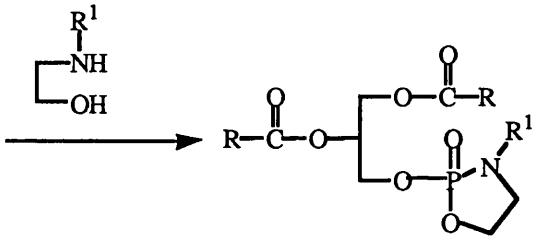
(50)

An efficient synthesis of phosphatidylethanolamines was described in 1978 by Eibl[93] in which phosphorus oxychloride was employed. (Refer to schemes 9a and 9b).

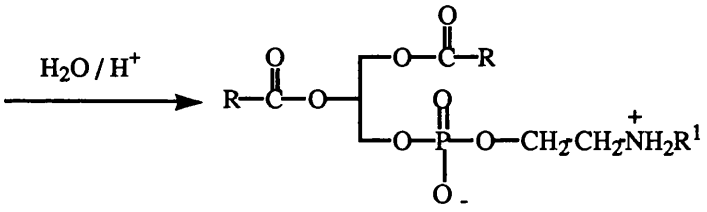
Scheme 9a.



(51) (52)



(53)

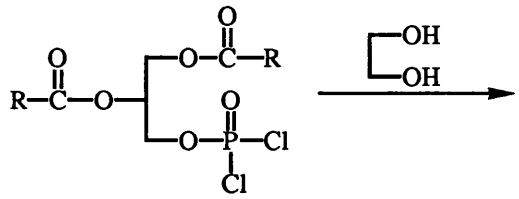


(54)

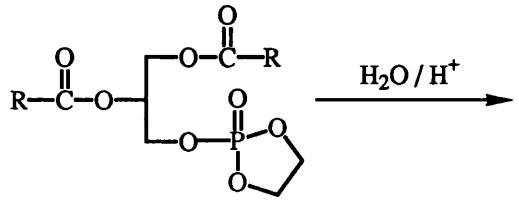
$R = C_{15}H_{31}$

$R^1 = H, CH_3$

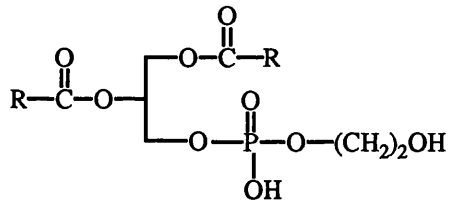
Scheme 9b.



(52)



(55)



(56)

$R = C_{15}H_{31}$

$R^1 = H, CH_3$

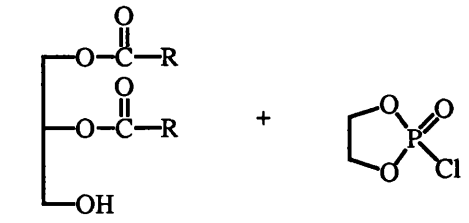
Eibl reported a quantitative yield of the initial alkyl phosphorodichloridate **(52)** with no by-product formation. This intermediate was then reacted with ethanolamine, N-methylethanolamine, ethyleneglycol or serine benzyl ester to give derivatives of the 1,3,2-dioxaphosphacyclopentane-2-oxide **(55)** and 1,3,2-oxazaphosphacyclopentane-2-oxide **(53)** ring systems in reported yields of 95-100%. The cyclic intermediates were then

hydrolysed using 20% acetic acid to give the corresponding phosphatidylethanolamine, phosphatidyl-N-methylethanolamine, phosphatidylethyleneglycol and phosphatidylserine benzyl ester in approximately 90% overall yields.

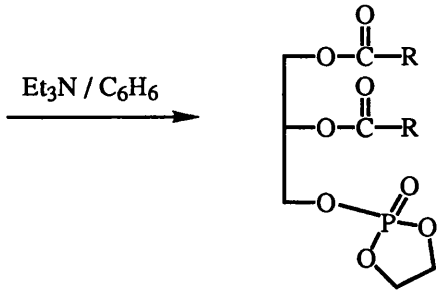
A major drawback with this otherwise excellent synthetic route is if the diacylglycerol is very sterically hindered, in which case the phosphorylation reaction might prove less efficient. Another problem occurs in the aqueous extraction and precipitation procedures used by Eibl to isolate the products, since emulsion formation and solubility problems are more likely to occur if alcohols with longer side chains (i.e. longer than sixteen carbons) are involved.

The use of a cyclic phosphorochloridate in phospholipid synthesis was first reported in 1976 by Phnong *et al.*[99] (Refer to scheme 10).

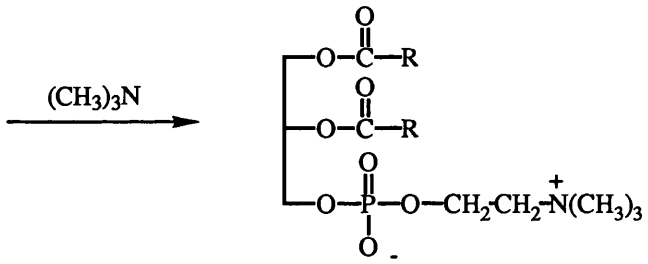
Scheme 10.



(57) **(38)**



(58)



(59) R= $C_{15}H_{31}$

The phosphorylating reagent **(38)** was reacted with diacylglycerol in the presence of base followed by ring opening of the cyclic phosphate triester **(58)** *via* endocyclic C-O bond cleavage using trimethylamine.

Chandrakumar and Hajdu[100,101,102] used this reagent extensively to synthesise a variety of phospholipids including 2-acylamido analogues using L-serine as the starting material.

Similarly, Bhatia and Hajdu[68] have synthesised ether and thioether phospholipid analogues.

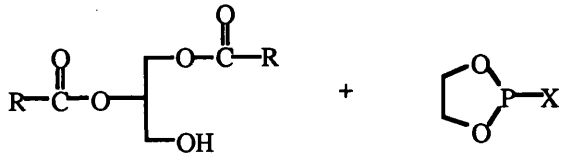
The major drawback with this route is that forcing conditions are required to bring the reaction to completion, i.e. overnight heating to 65 °C in a pressure bottle, which can lead to exocyclic P-O cleavage and diacylglycerol hydrolysis.

1.6.2. Phosphite Methodology.

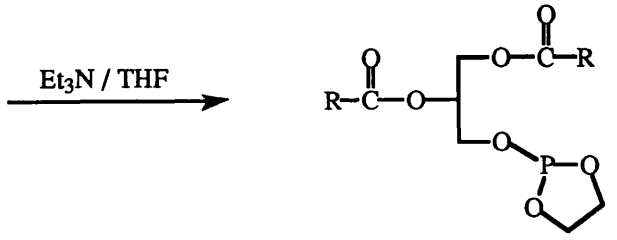
Phosphite phosphorylating procedures require an extra oxidation step to convert the phosphite to the corresponding phosphate. However, this is not viewed as a disadvantage because a variety of oxidising agents are available including iodine/water,[103] tertiary butylhydroperoxide[104] and dinitrogen tetroxide[105] which bring about the conversion in quantitative yields, under mild conditions. It also gives rise to possible variations of the phosphate group e.g. sulphurisation and selenisation.

Nifantev *et al*[106] in 1978 first applied phosphite techniques to the synthesis of phospholipids. (Refer to schemes 11 and 12).

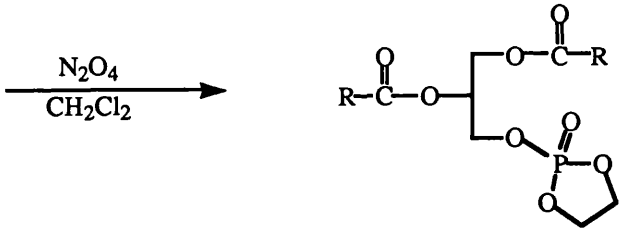
Scheme 11.



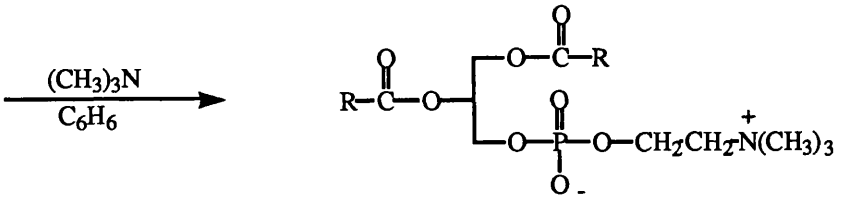
(60) **(61)**



(62)



(63)

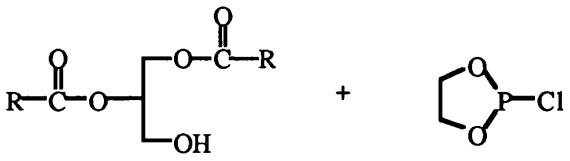


(64)

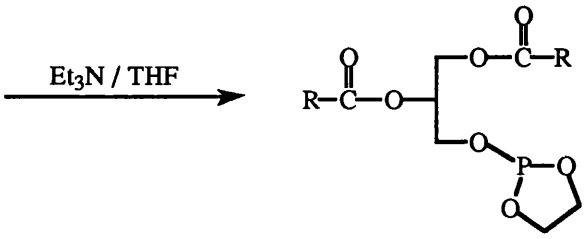
X= Cl, Et_2N

R= $C_{15}H_{31}$, $C_{17}H_{35}$

Scheme 12.



(60) (65)



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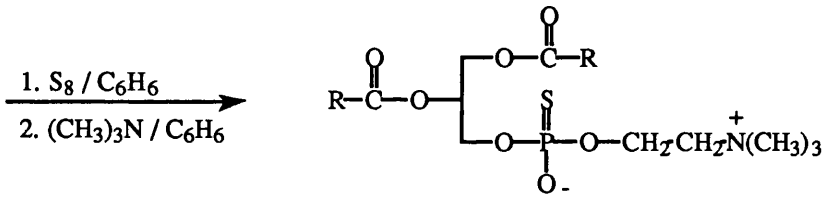


(66)

X= Cl, Et_2N

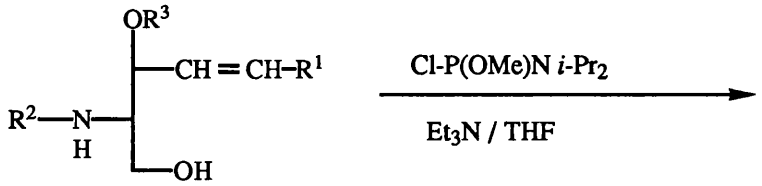
R= $C_{15}H_{31}$, $C_{17}H_{35}$

The phosphite triester **(62)** obtained in 83-93% yield was either oxidised or sulphurised. The former oxidation with N_2O_4 gave a quantitative yield of cyclic phosphate **(63)**. While the sulphurisation with elemental sulphur gave the corresponding phosphorothioate in a 78% yield.

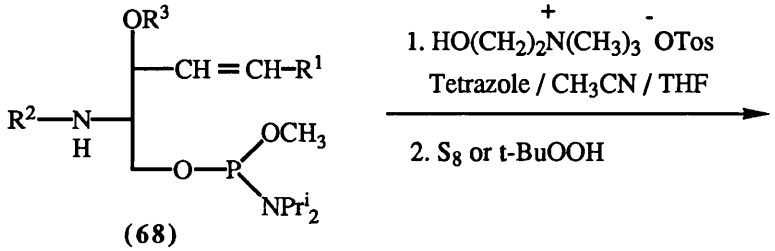
It was not until 1986 that phosphite methodology was again used to synthesise phospholipids. Bruzik[107] presented the synthesis of

thiosphingomyelins *via* a phosphoramidite procedure designed for the synthesis of oligonucleotides. (Refer to scheme 13).

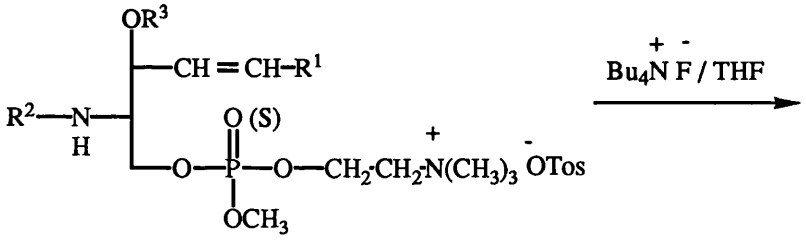
Scheme 13.



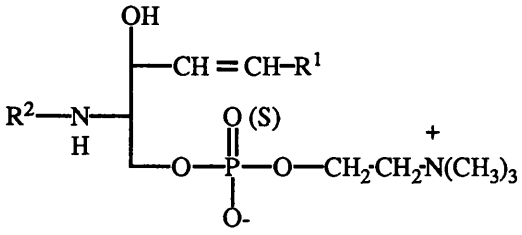
(67)



(68)



(69)



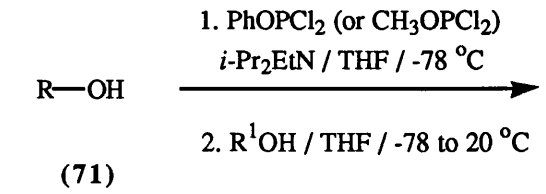
(70) $R^1 = C_{13}H_{27}$
$R^2 = C_{17}H_{35}CO$
$R^3 = Ph_2Bu\ t\text{-}Si$

The alcohol **(67)** was phosphitylated with chloro-(N,N-diisopropylamino)-methoxyphosphite in the presence of base. Subsequent nucleophilic displacement of the diisopropylamino group with choline tosylate in the presence of tetrazole, followed by either oxidation or sulphurisation gave the triester **(69)**. Deprotection gave the final products as four diastereoisomers in 56% overall yields.

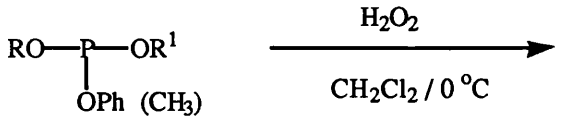
Stec and Bruzik[107] applied the same phosphoramidite methodology to the synthesis of glycerophospholipids and a variety of phosphorothioyl analogues with differing polar head groups, in overall yields of 60-70%.

In 1988, Josey and Martin[108] presented a novel, high yielding synthetic route to phosphatidylcholine and phosphatidylserine analogues using the phosphite methodology outlined in scheme 14.

Scheme 14.



(71)



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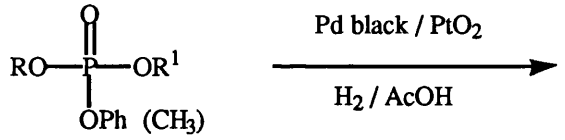
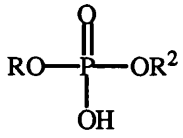


(73)



(74)

$R = C_{18}H_{37}$

$R^1 = (CH_2)_2NHBoc$

$= CH_2CH(NHCbz)CO_2Bn$

$R^2 = (CH_2)_2\overset{+}{N}H_3\ \overset{-}{O}Ac$

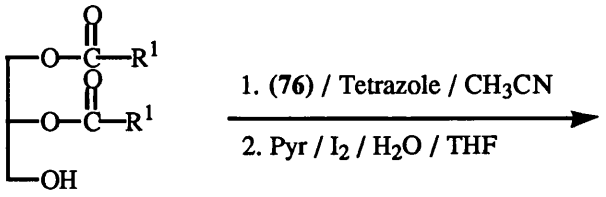
$= CH_2CH(\overset{+}{N}H_3\ \overset{-}{O}Ac)CO_2H$

The alcohol **(71)** was coupled with carbobenzyloxyethanolamine and benzylcarbobenzyloxyserine using phenyl dichlorophosphite (or methyl dichlorophosphite) in the presence of N,N-diisopropylethylamine. Subsequent oxidation using hydrogen peroxide gave the corresponding phosphate triesters **(73)** in overall yields of 89-92%. The phenyl, benzyl and carbobenzyloxy protecting

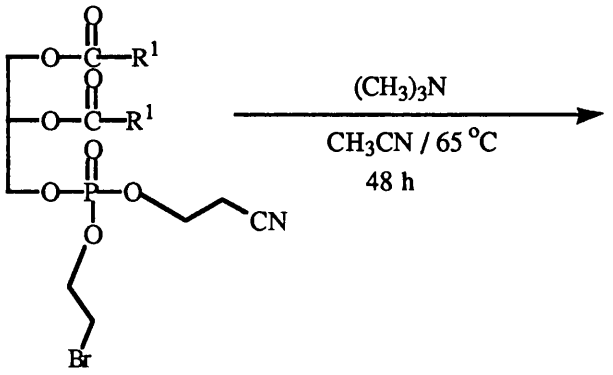
groups were then readily removed by hydrogenolysis under standard conditions to yield the corresponding phosphatidylethanolamine and phosphatidylserine analogues.

Hebert and Just[109] presented a novel synthetic route to glycerophosphocholines *via* an inverse phosphite triester approach shown in scheme 15.

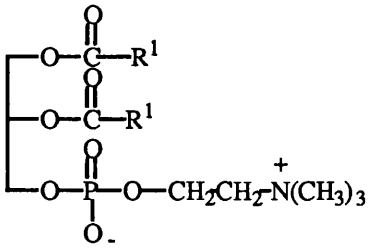
Scheme 15.



(75)

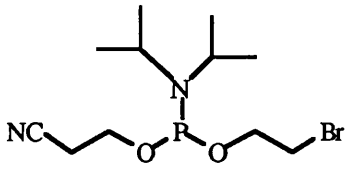


(77)



(78)

$R^1 = C_{15}H_{37}$



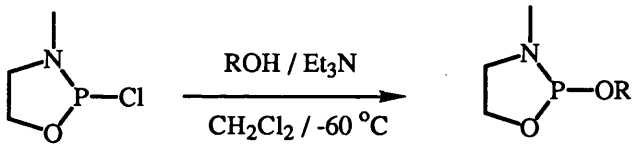
(76)

The phosphoramidite **(76)** was prepared by condensing 2-cyanoethyl(N,N-diisopropylamino)chlorophosphite with bromoethanol in dichloromethane in the presence of triethylamine base. This was then coupled to the diacylglycerol **(75)** using an excess of tetrazole. The intermediate triester was oxidised *in situ* by the

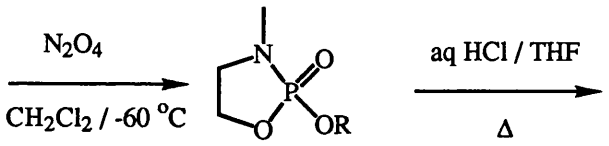
addition of pyridine and a solution of iodine in aqueous THF. The final step involved the simultaneous removal of the cyanoethyl group and the displacement of the bromide with trimethylamine. The reaction conditions for this final step were fairly harsh; heating to 65 °C for forty-eight hours in a pressure bottle, however, the final product was obtained in a 92% yield.

McGuigan[110] developed a synthetic route to novel phospholipids employing the 2-chloro-1,3,2-oxazaphosphacyclopentane **(79)** phosphitylating agent. (Refer to scheme 16).

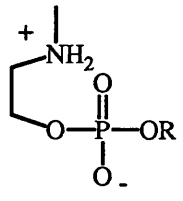
Scheme 16.



(79) **(80)**



(81)

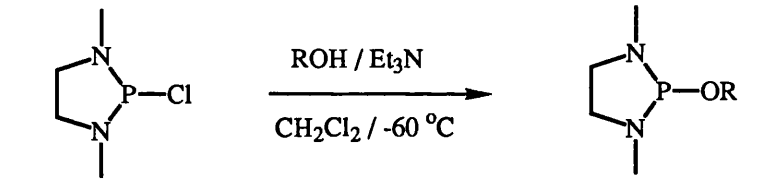


R= C_6H_{13}
C_8H_{17}
$C_{18}H_{37}$
$\Delta^9C_{18}H_{35}$

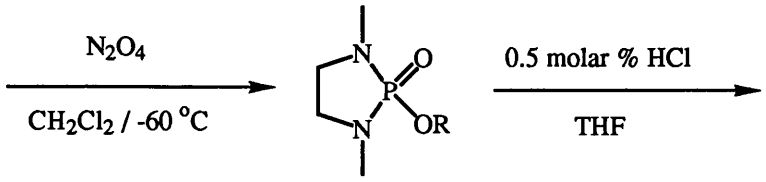
(82)

This route offers a high yielding three step procedure in which the amino group is present, throughout, in a protected (cyclic) form and is easily freed at the end of the synthesis. This route can be seen to be a hybrid of the procedures used by Nifantev[106] and Eibl.[7] Thus, the alcohol is firstly phosphitylated and the resulting phosphite is then oxidised, as for Nifantev's route, and then the phospholipid product is obtained by endocyclic P-N bond cleavage under mild acid conditions, as for Eibl's route. This route was applied to the synthesis of novel phospholipid analogues based on ethylene diamine[111,112] as shown in scheme 17.

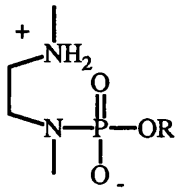
Scheme 17.



(83) **(84)**



(85)



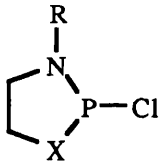
(86)

R= C_6H_{13}
$C_{12}H_{25}$
$C_{18}H_{37}$
$\Delta^9C_{18}H_{35}$

This work showed that the P-N bond could be cleaved, in the final stage, under the milder conditions of catalytic amounts of hydrochloric acid and ambient temperature.

This particular phosphite route offers many possibilities for the construction of novel phospholipids with various modifications of the head group and hydrocarbon chain. Favourably, the overall yield in this three step synthesis is high, thus, the main limiting factor is whether the heterocycles of the structure shown in figure 9 can be synthesised in sufficient purity.

Figure 9.



X= O, CH_2

R= CH_3, t-butyl, Bzl, t-boc

Also, since this particular method has not been applied extensively to the phosphorylation of diacylglycerols, it would be interesting to see how successful it would be.

The following report outlines attempts to synthesise a range of novel phospholipids using phosphoramidite procedures.

CHAPTER 2

The Synthesis of Some Novel N-methylethanolamine Phospholipid Analogues.

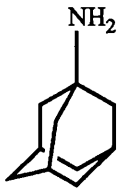
The Synthesis of Some Novel N-methylethanolamine Phospholipid Analogues.

2.1. Introduction.

This chapter is concerned with the synthesis of a series of novel phospholipid analogues with an N-methylethanolamine head group. The hydrocarbon chain was varied in terms of length and functionality, to give a range of analogues. Primary, secondary and tertiary alcohols were successfully reacted with the N-methylethanolamine phosphitylating agent **(79)**. The primary alcohols were chosen to mimic the long chain moieties of phospholipids. Synthesis of an ethyl glycolate analogue showed that the methodology could be applied to an hydrolysis susceptible ester function, whilst the oleyloxy analogue showed that it could also be applied to easily oxidisable unsaturated moieties. Dipalmitoyl analogues were successfully synthesised using the same methodology showing that the route was applicable to natural alcohols. Attempts were also made to synthesise an ALP analogue from 3-*O*-hexadecyl-2-*O*-ethylglycerol.

The secondary and tertiary alcohols were chosen to test the application of the methodology to more hindered alcohols. Furthermore, an adamantanyloxy analogue was synthesised to investigate the effect of incorporating a structure related to a known antiviral agent, i.e. amantidine **(87)**[113] into a phospholipid. Amantidine is active against the influenza A virus and is thought to

be effective by preventing the adsorption of the virus onto membranes and thereby preventing penetration into the cell.[114]



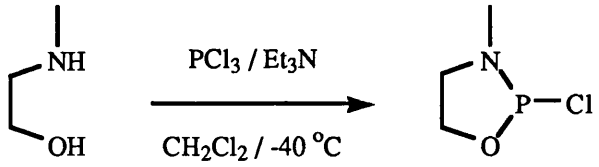
(87)

The cholesteryloxy analogue was synthesised to investigate the effect of incorporating a cholesterol moiety into a phospholipid structure, since cholesterol **(9)** is itself a structural component of the cell membrane and is known to affect its fluidity.[115]

2.2. Results and Discussion.

2.2.1. Cyclisation Reaction.

The phosphitylating agent 2-chloro-3-methyl-1,3,2-oxazaphosphacyclopentane **(79)** was prepared by the low temperature reaction of phosphorus trichloride with N-methylethanolamine in the presence of triethylamine base.[116]



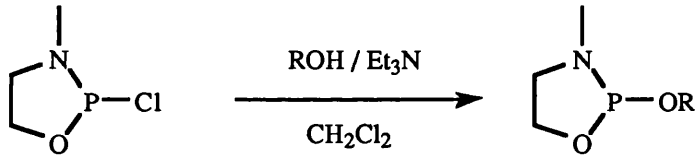
(79)

Polymeric by-product formation was minimised by employing fairly dilute conditions. The product, obtained in a 49% yield following high vacuum distillation, was characterised by nuclear magnetic resonance, infra-red and electron impact mass

spectroscopy. Both ^{1}H and ^{13}C nmr spectra show two bond phosphorus coupling and of particular interest in the ^{13}C nmr spectrum is the OCH_2 resonance signal which is shifted *ca* 10ppm downfield, relative to N-methylethanolamine.[117] The ^{31}P nmr shift at δ167.3 is consistent with similar phosphite species.[118]

2.2.2. Condensation Reactions.

Compound **(79)** was reacted with a series of primary, secondary and tertiary alcohols. The alcohols, reaction temperatures, yields and ^{31}P nmr shifts of the corresponding phosphite triester products are shown in tables 1 and 2.



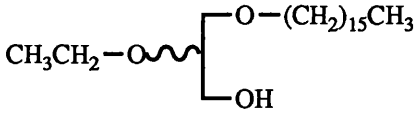
(79) **(80a-o)**

Table 1. Condensations with primary alcohols.

ROH (R=)	Temp. °C	Product	Yield%	δ ^{31}P nmr
n-C_6H_{13}	-60	**(80a)**	83	135.0
n-C_8H_{17}	-60	**(80b)**	96	136.4 *
n-$C_{12}H_{25}$	-40	**(80c)**	100	135.8
n-$C_{18}H_{37}$	-40	**(80d)**	100	135.7
$\Delta^9C_{18}H_{35}$	-60	**(80e)**	100	136.2
Ethyl glycol-ate	-60	**(80f)**	100	138.7
Et-O-16	0	**(80g)**	>100 impure	140.4, 137.1, 10.19 (2:1:7)
rac dipalm.	0	**(80h)**	95	138.7, 137.8 (4:3)
sn dipalm.	0	**(80i)**	100	138.7, 137.8 (2:3)

* nmr recorded in CH_2Cl_2 with D_2O centre lock, otherwise recorded in $CDCl_3$.

Ethyl glycolate= $CH_3CH_2O-\overset{\overset{O}{\|}}{C}-CH_2OH$

Et-O-16= 

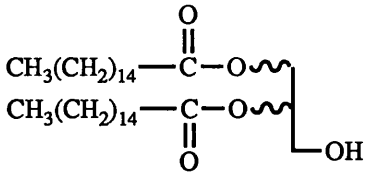
dipalm.= 

Table 2. Condensation reactions with secondary and tertiary alcohols.

Alcohol	Temp. °C	Product	Yield%	δ ^{31}P nmr ($CDCl_3$)
Octan-2-ol	-60	**(80j)**	86	137.8, 137.3 (3:2)
4-Methyl-heptan-3-ol	-60	**(80k)**	99	140.0, 139.8, 138.7, 138.6 (1.5:1.0:1.4:1.2)
5-Methyl-heptan-3-ol	-60	**(80l)**	99	139.5, 139.2, 139.1, 138.8 (1.5:1.2:1.1:1.0)
Cholesterol	-20	**(80m)**	96	137.4
1,1-Dimethyl-octadecanol	-60	**(80n)**	99	132.8
Adamantanol	-60	**(80o)**	100	132.1

Compound **(79)** reacted readily with hexan-1-ol in triethylamine and dichloromethane at low temperature. The phosphite triester **(80a)** was isolated, by saturated aqueous sodium hydrogen carbonate extraction in high yield, and characterised by ^{1}H and ^{31}P nmr spectroscopy. The former shows that although the chemical shift of the N-methyl moiety is unchanged relative to **(79)**, the magnitude of the phosphorus coupling is reduced from 13.6 to 11.8 Hz. The ^{31}P nmr shift is greatly altered from δ167.3 for **(79)** to δ135.0 for **(80a)**

which is consistent with previous reports on similar species (e.g. δ143 for $PNMe_2(OEt)_2$).[119]

The octanyloxy, dodecyloxy and octadecyloxy phosphite triesters **(80b-d)** were prepared in a similar manner, although a slightly higher temperature was required for the preparation of **(80c)** and **(80d)** on account of the poorer solubility of the alcohol in dichloromethane. Phosphite triester **(80b)** was isolated in high yield following hexane extraction and characterised by ^{31}P nmr spectroscopy. Phosphite triesters **(80c)** and **(80d)** were isolated in high yield following aqueous sodium hydrogen carbonate extraction. Significantly, no real problems were encountered during aqueous extraction e.g. emulsion formation. The products were characterised by ^{31}P and ^{13}C nmr spectroscopy and data found to be similar to the other phosphites. The ^{13}C nmr spectra of compounds **(80c)** and **(80d)** showed two and three bond phosphorus coupling and a significant reduction of the N-methyl coupling constant, from 13.6 in **(79)** to *ca* 7.0 Hz in the phosphite triesters **(80c)** and **(80d)**. The signals were assigned with reference to compound **(79)** and model compounds such as decan-1-ol.[117]

Condensation of **(79)** with oleyl alcohol proceeded in quantitative yield, and again aqueous extraction was not detrimental. The phosphite triester **(80e)** was characterised by ^{31}P nmr spectroscopy, the chemical shift being consistent with the previous phosphite triesters. However, it was noted that despite the similarities between **(80d)** and **(80e)** in terms of spectroscopic properties their physical properties were very different. Compound

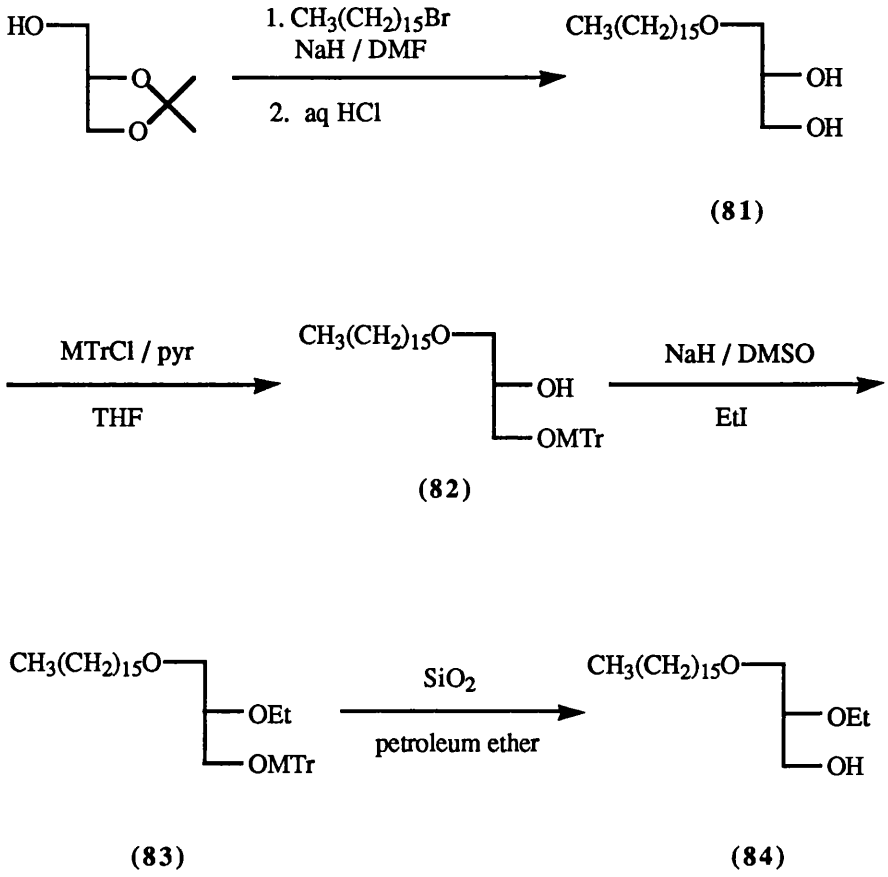
(80d) being a white solid whilst compound **(80e)** a colourless oil which compares with the physical states of the corresponding alcohols. Octadecan-1-ol being a white solid, whilst the unsaturated oleyl alcohol is a liquid.

Condensation of **(79)** with ethyl glycolate, which was prepared by standard methods,[120] gave the corresponding phosphite triester **(80f)** in quantitative yield. The product was isolated by hexane extraction rather than aqueous extraction because of its high water solubility. ^{31}P nmr spectroscopy revealed a chemical shift *ca* 2 ppm further downfield than that of **(80a-e)**, presumably due to the electron withdrawing nature of the glycolyl moiety.

Synthesis of an ALP analogue was pursued since such a phospholipid might show interesting biology in terms of anti-viral activity, although the ALP phospholipids are known more as anti-cancer agents.

The alcohol 3-*O*-hexadecyl-2-*O*-ethyl-*rac*-glycerol **(84)** was not readily available and therefore was synthesised using a combination of the methods of Van Boom *et al*[88] and Bittman *et al*[121] used to prepare alcohols of this type. Scheme 18 outlines the route used.

Scheme 18.



Thus, 1,2-*O*-isopropylidene-*rac*-glycerol was reacted with 1-bromohexadecane, followed by the removal of the isopropylidene group in aqueous hydrochloric acid to give the 1-*O*-hexadecyl-*rac*-glycerol **(81)** in *ca* 53% yield. Subsequent treatment of **(81)** with methoxytrityl chloride gave the protected product **(82)** in *ca* 70% yield. The free hydroxy group of **(82)** was ethylated using ethyl iodide in the presence of sodium hydride and the protected product **(83)** was obtained in 99% yield. Deprotection of **(83)** was carried out using silicic acid in petroleum ether.[88] The alcohol **(84)** was isolated in 69% yield after purification by chromatography and characterised by ^1H and ^{13}C nmr spectroscopy. Carbon signals were assigned by

comparison with hexadecane,[122] dihexyl ether,[117] 1-ethoxy-3-methyl-1,3-diphenylbutan-2-one[123] and 18-nonadecyl-1-ol.[124]

Condensation of **(79)** with **(84)** was attempted using modified conditions, relative to those used in the previous synthesis of the phosphite triesters **(80a-f)**. Anson[125] had found previously that condensation reactions of alcohols like **(84)** with an N,N'-dimethyl phosphitylating agent required higher temperatures i.e. 0 °C, an equimolar ratio of alcohol to phosphitylating agent and a solvent that facilitates the precipitation of the base hydrochloride by-product. This latter factor is based on literature precedent[72,100,101,106,126] that suggests the use of such solvents provide a driving force for completion of the reaction. Hence, benzene was employed as the solvent and the reaction was carried out at 0 °C. In the initial attempt, the reaction was left for 1 hour at ambient temperature before aqueous extraction. A crude product in excess of 100% yield was obtained and characterised by ^{31}P nmr spectroscopy. Three signals were observed at δ140.4, 137.1 and 10.1 in a ratio of 2:1:7. The two former shifts correspond to the phosphite product, whilst the latter is apparently a by-product resulting from the decomposition of **(79)** which is thought to have arisen during the product isolation procedure.

The condensation reaction was repeated, but this time the reaction mixture was stirred at ambient temperature for 2 hours. Also a non-aqueous extraction procedure was employed in an attempt to avoid undesired decomposition of **(79)**. Unfortunately, ^{31}P nmr spectroscopy of the product of this reaction revealed a

complete absence of the desired phosphite triester product **(80g)** and only signals for decomposition by-products. It was thought that problems with efficient drying of the synthesised alcohol (which are detailed in the appendix) were responsible for this. Due to the constraints of time, further work on the synthesis of such analogues was not possible.

1,2-dipalmitoyl-*sn*-glycerol or 1,2-dipalmitoyl-*rac*-glycerol are often used as starting alcohols in phospholipid synthesis[90] since they are readily available and their synthesis is straightforward.[76] Again previous work by Anson[125] showed that reaction conditions used for the alcohols **(80a-f)** were unsuitable for the condensation reaction with the dipalmitoyl glycerols. Alcohol insolubility at low temperature necessitated increasing the reaction temperature to 0 °C, whilst the strong possibility of emulsion formation necessitated use of a non-aqueous extraction.

Thus, 1,2-dipalmitoyl-*rac*-glycerol was condensed with compound **(79)** at 0 °C. Hexane extraction gave a high yield of the phosphite triester product **(80h)** which was characterised by ^{31}P nmr spectroscopy. Two signals were observed at δ138.7 and 137.8 in a ratio of 4:3, corresponding to diastereoisomeric products, due to the presence of a chiral centre at the C-2 position of the glycerol backbone.

The same procedure was repeated using 1,2-dipalmitoyl-*sn*-glycerol, to give the phosphite triester **(80i)** in a quantitative yield. Again, ^{31}P nmr spectroscopy of the product revealed two signals at

δ138.7 and 137.8 in a ratio of 2:3, corresponding to diastereoisomeric products.

Until now the condensation reaction had been applied to primary alcohols only. It was decided to investigate the reaction of more hindered secondary and tertiary alcohols with the phosphitylating agent **(79)**. In general, the reaction was found to be no slower than when primary alcohols were involved, indicating that the condensation is rapid and probably complete in less than 30-60 minutes.

Firstly a series of structural isomers of formula $C_8H_{18}O$ were used in the condensation reaction. Compound **(79)** was reacted with octan-2-ol at low temperature. Non-aqueous extraction gave an 86% yield of the phosphite triester **(80j)**, which was characterised by nmr spectroscopy. Since the starting alcohol has a chiral centre, the product **(80j)** contains two chiral centres due to the chiral carbon and the phosphorus. This gives rise to diastereoisomerism, evidence of which is seen in all the nmr spectra. The ^1H nmr spectrum shows the effects of chirality and phosphorus coupling, in particular, the methyl group of the chain closest to phosphorus appears as two doublets (δ1.15). As does the N-methyl signal (δ2.70) compared with a doublet for similar products without a chiral centre. The signals observed in the ^{13}C nmr spectrum were assigned with reference to data for octan-2-ol.[117] These signals also show a doubling due to both two bond phosphorus coupling and chirality. The ratio of signal intensities for the diastereoisomers ranged from 1:1 to 3:2. Two

signals were observed in the ^{31}P nmr spectrum corresponding to diastereoisomers in a 3:2 ratio.

Similarly, 4-methyl-heptan-3-ol and 5-methyl-heptan-3-ol were condensed with compound **(79)** to give the corresponding phosphite triesters **(80k)** and **(80l)** in high yields. The products were characterised by nmr spectroscopy, all of which showed the effects of phosphorus coupling and diastereoisomerism. However, in these cases there are two chiral centres in the starting alcohols and therefore three in the corresponding products. This explains the multiplicity of signals in the ^{1}H and ^{13}C nmr spectra and the four signals observed in the ^{31}P nmr spectra.

Compound **(79)** was condensed with cholesterol **(9)**, but a higher temperature of -20 ^{o}C was necessary because of the poor solubility of the alcohol in dichloromethane. The phosphite triester **(80m)** was isolated after non-aqueous extraction in 96% yield and characterised by ^{31}P nmr spectroscopy. The signal observed was consistent with similar phosphite species.

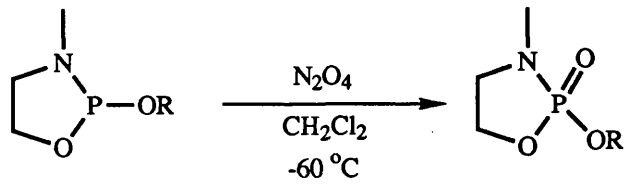
The tertiary alcohol 1,1-dimethyloctadecan-1-ol was also condensed with compound **(79)** at low temperature. The phosphite triester **(80n)** was isolated in a 99% yield, following non-aqueous extraction, and characterised by nmr spectroscopy. The absence of chiral centres somewhat simplified the spectra which were not dissimilar to those of the octadecyloxy compound **(80d)**. Interestingly, the reaction time for the condensation of this more hindered alcohol was not significantly longer than that for the primary octadecan-1-ol. Infact the condensation reaction with the

tertiary alcohol was complete within 1 hour although the condensation reaction with the primary alcohol was left for 2 hours. Predictably, this appears to indicate that the general condensation reaction is very rapid.

Condensation of **(79)** with adamantanol proceeded in quantitative yield. A non-aqueous extraction procedure was employed and the phosphite triester **(80o)** was characterised by nmr spectroscopy. As expected, two and three bond phosphorus coupling were observed in the ^{1}H and ^{13}C nmr spectra, carbon signals in the latter being assigned with reference to ^{13}C nmr data of dichloroaminoadamantane.[127]

2.2.3. Oxidation Reactions.

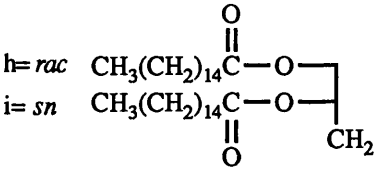
The next stage of the synthetic strategy was the oxidation of the cyclic phosphite triesters **(80a-f)** and **(80h-o)** to their corresponding phosphates **(85a-f)** and **(85h-o)**. Dinitrogen tetroxide[128] had been found to be the best oxidant for this conversion.[111] However, the one major concern with this oxidant was its application to the oxidation of the unsaturated oleyloxy compound **(80e)** and the possibility of undesired addition across its double bond.[129] Schechter[130] reports optimum temperatures in the range of -10 to 25 ^{o}C for such additions, along with the use of oxygen containing solvents to facilitate the addition.[131] Thus the oxidations of the phosphite triesters **(80a-f)** and **(80h-o)** were conducted at -60 ^{o}C in dichloromethane, using a standard solution of dinitrogen tetroxide.[132] Thus by assuming 1:4 phosphite:oxidant stoichiometry it was possible to use only the required quantity of reagent.



(80a-o) **(85a-o)**

R

a= n-C_6H_{13}
b= n-C_8H_{17}
c= n-$C_{12}H_{25}$
d= n-$C_{18}H_{37}$
e= $\Delta^9C_{18}H_{35}$
f= $CH_3CH_2OC(O)CH_2$



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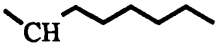
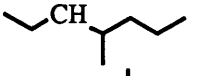
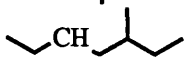
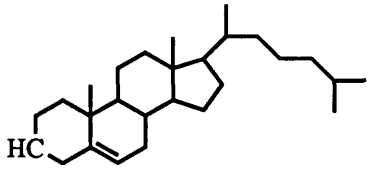
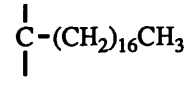
j= 

k= 

l= 

m= 

n= 

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The reaction times, yields and ^{31}P nmr shifts of the phosphates **(85a-f)** and **(85h-o)** are shown in table 3.

Table 3. Oxidations.

Phosphate	Reaction time (min)	Yield%	δ ^{31}P nmr ($CDCl_3$)
(85a)	30	96	18.9
(85b)	30	100	19.8
(85c)	30	100	18.7
(85d)	60	100	18.7
(85e)	a	100	19.6
(85f)	a	100	20.1
(85h)	10	98 (impure)	19.9, -1.3 (1:5)
(85i)	10	100 (impure)	19.8, -2.3 (1:3)
(85j)	10	100	20.9
(85k)	10	100	19.4
(85l)	10	100	19.2
(85m)	10	99	18.9
(85n)	10	100	18.5
(85o)	10	100	16.2

[a] solvent removed under reduced pressure immediately on reaction mixture reaching ambient temperature.

It is particularly noticeable here that there is a dramatic shift in the ^{31}P nmr signals, *ca* δ18-20 as compared to *ca* δ132-140 for the phosphite species **(80a-o)**. This compares closely to data recorded for analogous phosphates[133] e.g. δ11 for $OPNMe_2(OEt)_2$.

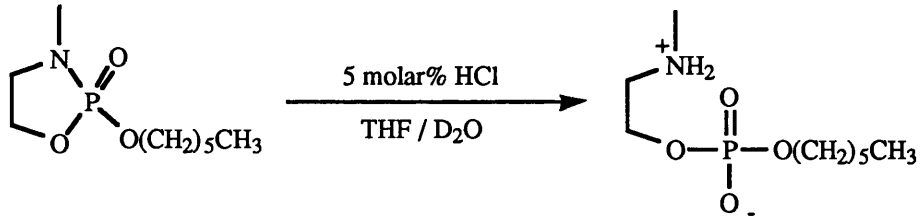
The table shows that the dipalmitoyloxy phosphate analogues **(85h)** and **(85i)** were obtained in an impure state as shown by ^{31}P nmr spectroscopy. However, the most intense peaks were assumed to be the product of P-N cleavage on the basis of ^{31}P nmr chemical shift values and therefore the impure phosphates were subjected to hydrolysis conditions to complete the transformation.

Interestingly, the phosphates containing chiral centres that had previously given rise to diastereoisomeric signals in the ^{31}P nmr spectra now appeared as single signals, presumably due to overlapping of signals or the preferential formation of one of the diastereoisomers. The former seems to be the most likely explanation since there are still diastereoisomeric signals present in the ^{13}C nmr spectra. (Compare for example the ^{13}C nmr spectra, the data of which is recorded in the experimental section, of the 2-octanyloxy intermediates **(80j)** and **(85j)** both of which show diastereoisomeric effects). ^{1}H nmr spectroscopy was also used to characterise some of the phosphates i.e. **(85a, 85c, 85j and 85m)**. The most noticeable change being that of the P-NCH_3 coupling constant which decreases by *ca* 2.0 Hz. This parameter seems particularly receptive to the precise environment of the phosphorus atom since it has altered at each stage of the reaction so far.

2.2.4. Hydrolysis Reactions.

A major advantage of this route to phospholipids had been viewed as the final step, i.e. the mild acid-catalysed hydrolysis of the P-N bond to yield acyclic phosphate diesters. Although the phosphoramidate bond is known to be acid labile[134] the precise acid lability of this particular heterocycle was unclear. Previous work had reported the use of rather forcing conditions i.e refluxing THF/2M HCl[110] or acetic acid.[93] In order to investigate the hydrolysis, it was decided to carry out kinetic studies, 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.[135]

Compound **(85a)** was mixed with 5 molar% hydrochloric acid in THF/D_2O at ambient temperature with the intention of recording ^{31}P nmr spectra at various times. Suprisingly however, the reaction was complete within 10 minutes, giving a single product with a chemical shift of *ca* 0.39 ppm.



(85a) **(86a)**

Neutralisation and lyophilisation gave the phospholipid product **(86a)** in quantitative yield. Characterisation by spectroscopy gave firm evidence of P-N cleavage. The ^1H nmr spectrum is particularly revealing, since the N-methyl signal at δ2.67 now appears as a

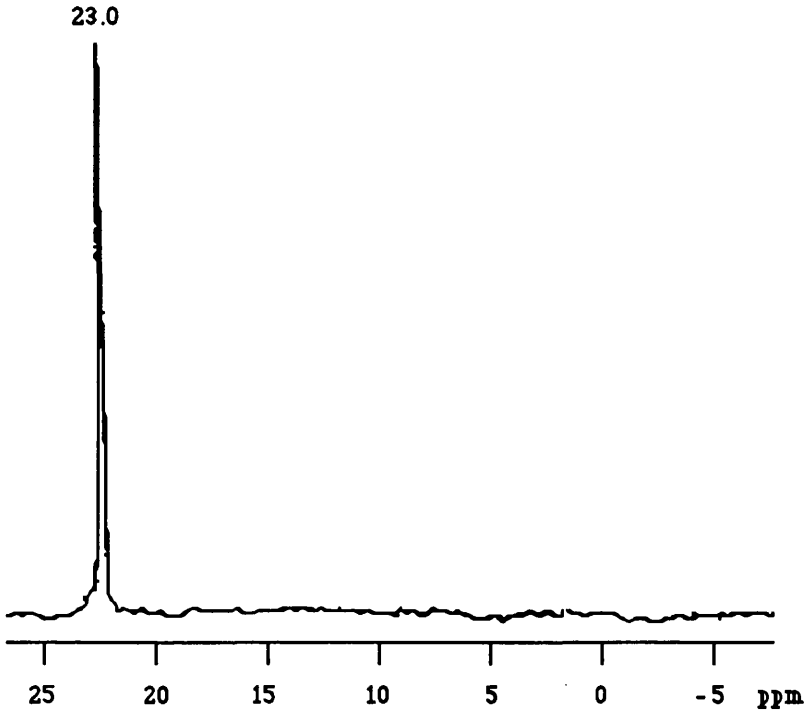
singlet, having lost phosphorus coupling. The cleavage is further confirmed by ^{31}P nmr spectroscopy which reveals a (solvent dependent) signal close to 0 ppm, as anticipated for a phosphate diester.[135] The ^{13}C nmr spectrum (refer to table 5) also supports P-N cleavage, particularly the N-CH_3 signal at δ31.5 which is now clearly a singlet having lost phosphorus coupling.

Having noted the marked acid lability of **(85a)** 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 diacyl glycerols, but also the potentially problematic neutralisation step would be obviated, since undesired P-O cleavage[136,137] can occur if over-neutralisation takes place.

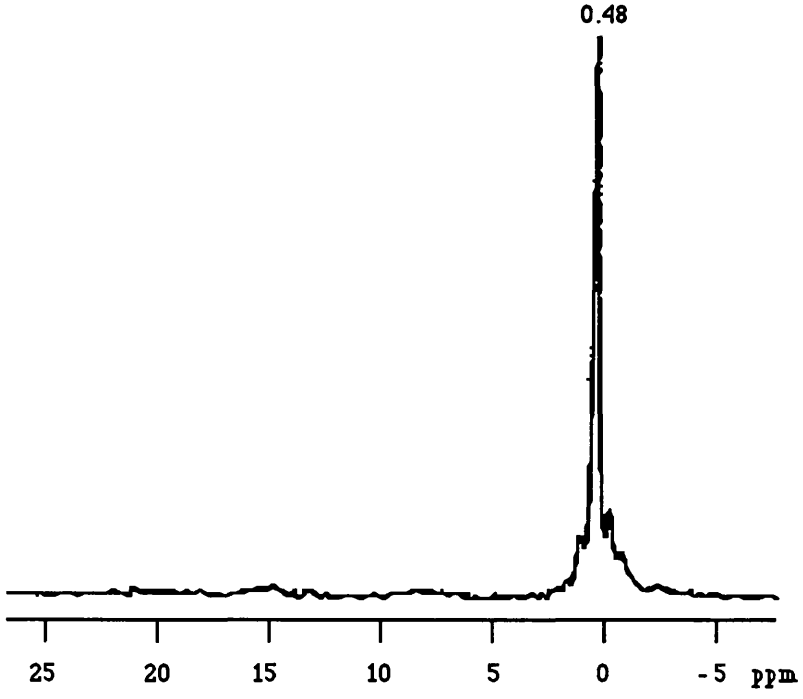
Thus, compound **(85a)** was dissolved in THF/D_2O and ^{31}P nmr spectra recorded at appropriate intervals. Remarkably, hydrolysis proceeded as before in the absence of added acid, although at a slower rate. Hydrolysis was complete in under 75 minutes, (refer to figure 10) and simple lyophilisation of the reaction mixture gave the product **(86a)** in quantitative yield. The product was fully characterised and all analytical data supported the structure of the product.

Figure 10. ^{31}P nmr kinetic study of the hydrolysis of **(85a)**.

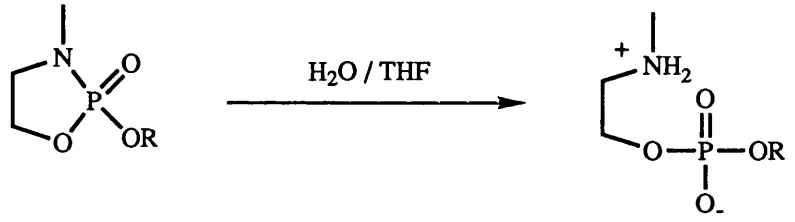
Time = 0 minutes.



Time = 75 minutes



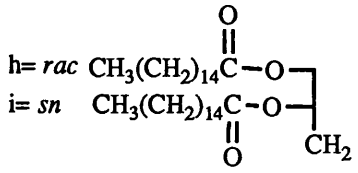
The exact mechanism of this 'acid free' hydrolysis is unclear, but it may be catalysed by traces of acid present from the oxidation stage. However, the method does appear to be generally applicable since the phosphates **(85b-f)** and **(85h-o)** were hydrolysed in the same way, although the reaction time varied. The longer chained phosphates and the more hindered phosphates appeared to take longer to hydrolyse. (Refer to table 4).



(85a-o) **(86a-o)**

R

a= n-C_6H_{13}
b= n-C_8H_{17}
c= n-$C_{12}H_{25}$
d= n-$C_{18}H_{37}$
e= $\Delta^9C_{18}H_{35}$
f= $CH_3CH_2OC(O)CH_2$



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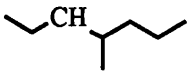
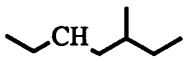
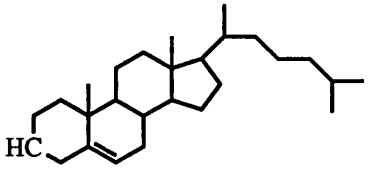
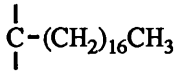
k= 

l= 

m= 

n= 

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The hydrolysis of the phosphate triester **(85j)** was investigated by a kinetic study, again following the reaction by ^{31}P nmr spectroscopy. The hydrolysis was found to be complete within 180 minutes,

compared with 75 minutes for the hexyloxy analogue **(85a)**. The product **(86j)** was isolated in a quantitative yield following lyophilisation.

The phosphate triesters **(85n)** and **(85o)** took longest to hydrolyse. After 8 hours stirring in water at ambient temperature the ratio of cyclic phosphate **(85n)** to cleaved product **(86n)** was 1.3:1.0. To ensure completion of hydrolysis the reaction mixture was left stirring in water at ambient temperature overnight. A quantitative yield of the product was isolated after lyophilisation and fully characterised. The phosphate triester **(85o)** was left stirring in water for 10 hours to ensure complete hydrolysis and the reaction was found to be complete within this time.

The phospholipid analogues **(86e)**, **(86f)**, **(86k)** and **(86o)** required chromatographic purification, because of phosphorus containing minor impurities observed in their ^{31}P nmr spectra with shifts *ca* δ6.0-8.0. This accounts for the lower yields of these products which correlates with the well known difficulties encountered in the chromatography of phospholipids.[102,138]

All of the products were fully characterised spectroscopically. The ^{1}H nmr spectra of the phospholipid products show the N-CH_3 signal as a singlet supporting P-N cleavage and the ^{31}P nmr spectra signals at *ca* 0 ppm are consistent with phosphates of this type. (Refer to table 4).

Table 4.

Product	Yield%	Reaction Time (min)	δ ^{1}H nmr (NCH_3)	δ ^{31}P nmr
(86a)	100	75	2.63, s	-1.4
(86b)	99	180	2.62, s	-1.5
(86c)	100	210	2.61, s	-1.2
(86d)	97	180	2.70, s	-1.7
(86e)	65	120	2.68, s	-1.9
(86f)	82	60	2.72, s	-2.5
(86h)	100	180	2.71, s	0.3
(86i)	100	120	2.72, s	-0.8*
(86j)	100	180	2.59, s	-0.2
(86k)	69	180	2.65, s	-1.0
(86l)	100	240	2.63, s	-1.7
(86m)	98	360	2.64, s	0.7
(86n)	100	1920	2.62, s	-4.9
(86o)	77	600	2.68, s	-5.3

* nmr recorded in CH_2Cl_2 with D_2O centre lock, otherwise recorded in $CDCl_3$.

The ^{13}C nmr spectral data for the phospholipid products is shown in tables 5, 6, and 7. The ^{13}C nmr spectrum of the oleyloxy analogue **(86e)** is particularly interesting because the two alkenyl carbon atoms are non-equivalent and their chemical shifts

(δ130.0/129.9) are very similar to that recorded for *cis*-dec-5-ene (δ130.2) and different to that recorded for *trans*-dec-5-ene (δ130.8).[139] Also the resonances of the adjacent methylene groups for these three compounds (27.2, 27.5 and 32.9 respectively) show a similar trend, which supports the *cis* geometry of the alkenyl moiety in **(86e)**.

The signals in the ^{13}C nmr spectra of the dipalmitoyloxy analogues **(86h)** and **(86i)** were assigned with reference to ^{13}C nmr data for the methyl ester of hexadecanoic acid[140] and tributyrylglycerol.[117]

Table 5. ^{13}C nmr data for (86a-f) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.

$CH_3CH_2CH_2(CH_2)_nCH_2CH_2CH_2OP(O)(OH)OCH_2CH_2NHCH_3$

A B C D E F G H I

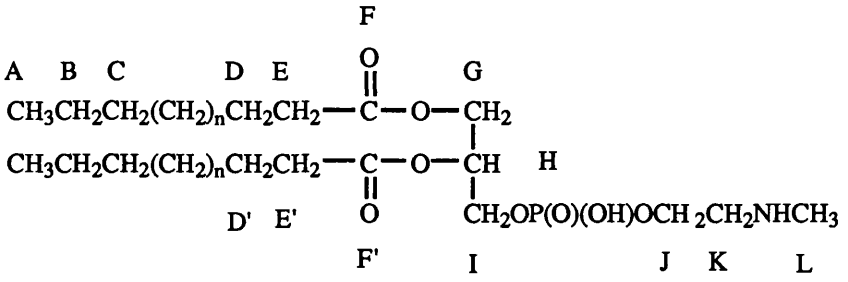
Signal	(86a) n=0	(86b) n=2	(86c) n=6	(86d) n=12	(86e)[a] n=12	(86f)[b]
A	13.9	14.1	14.1	14.2	14.1	-
B	22.4	22.7	22.6	22.7	22.7	-
C	31.4	31.8	31.8	32.0	31.9	14.2
D	25.3	25.9	25.8	25.7	25.9	60.9
E	30.5 (7.5)	30.8 (6.9)	30.7 (7.4)	30.7 (7.4)	30.8 (7.5)	169.8 (7.7)
F	60.6 (3.8)	60.9 (2.7)	60.7 (4.9)	61.0 (5.2)	60.9 (3.3)	62.7 (3.9)
G	65.8 (5.8)	62.9 (5.4)	66.0 (5.9)	66.4 (5.6)	66.0 (5.8)	61.2 (5.1)
H	49.8 (6.3)	50.0 (5.0)	50.0 (6.0)	50.0 (6.7)	50.2 (5.5)	49.8 (5.7)
I	33.5	33.7	33.6	33.7	33.6	33.6

[a] Includes: CH=CH δ130.0 and 129.9 and $\underline{C}H_2CH=CH\underline{C}H_2$ δ27.23.

[b] $CH_3CH_2OC(O)CH_2OP$-

C D E F

Table 6. ^{13}C nmr data for (86h) and (86i) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.



Signal	(86h)	(86i)
A	14.1	14.1
B	22.6	22.6
C	31.8	31.9
D	24.80	24.80
D'	24.83	24.83
E	33.97	33.98
E'	34.04	34.04
F	173.3	173.3
F'	172.9	172.9
G	62.1	62.1
H	64.5	64.5
I	61.6 (1.5)	61.6 (1.3)
J	69.7 (4.5)	69.7 (4.6)
K	49.7 (7.2)	49.7 (7.5)
L	34.1	34.1

Table 7. ^{13}C nmr data for (86j-m) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.

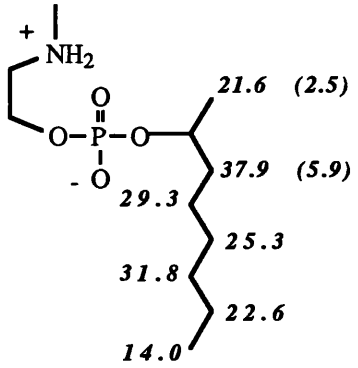
$RCH_nOP(O)(OH)OCH_2CH_2NHCH_3$

F G H I

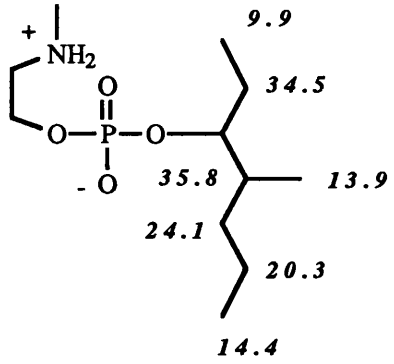
Signal	(86j) n=1	(86k) n=1	(86l) n=1	(86m) n=1	(86n) n=0	(86o) n=0
F	60.7 (3.9)	61.1 (3.2)	60.8 (2.5)	60.2[a]	50.0 (3.9)	61.0 (2.0)
G	73.1 (6.1)	83.3 (4.4/ 3.3)	76.2 (6.1/ 6.3)	73.8[a]	80.4 (7.4)	77.3 (3.1)
H	50.1 (6.0)	49.8 (4.4)	50.3 (5.5)	49.9[a]	43.4 (5.1)	50.5
I	33.6	33.6	33.6	31.7	31.9	33.7

[a] Coupling not resolved, signal appears as a broad singlet.

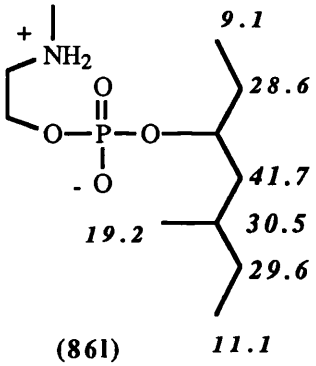
Figure 11. ^{13}C nmr data for compounds **(86j-86l)** and **(86n)** recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.



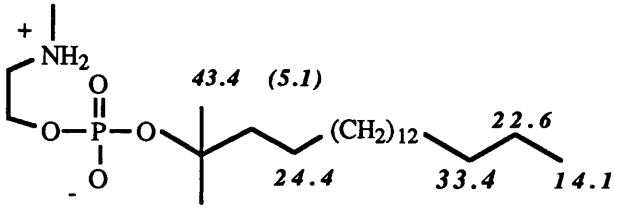
(86j)



(86k)

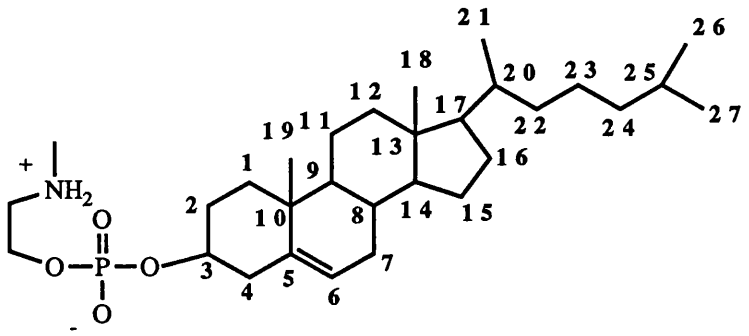


(86l)

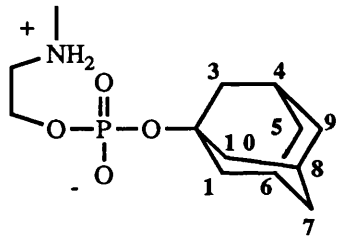


(86n)

Figure 12. Numbering used for assignment of ^{13}C nmr signals in compounds **(86m)** and **(86o)**.



(86m)



(86o)

Electron Impact Mass Spectroscopy was used to characterise the product **(86a)**, whilst, mass spectroscopy by Fast Atom Bombardment technique was used to characterise the products **(86 b-f)** and **(80h-o)**. (EIMS could not be applied to the less volatile products with higher mass). Observation of protonated molecular ions along with fragmentation by successive loss of alkyl groups helped confirm the proposed structures of these phospholipid products.

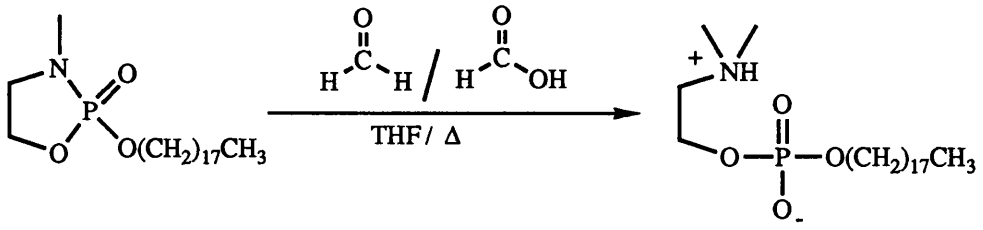
Microanalysis of the products showed deviations commonly encountered with phospholipid derivatives[141] i.e. associated water.

The infra-red spectra of the products showed the presence of phosphate functionality in the range of *ca* 1248-1218 cm^{-1} and those products containing a carbonyl functionality i.e. **(86f)**, **(86h)** and **(86i)** showed a strong signal in the range of *ca* 1753-1735 cm^{-1}.

2.2.5. N-Methylation.

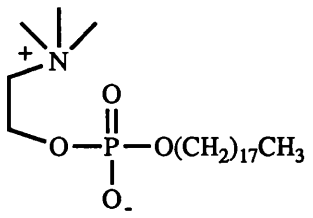
In an attempt to synthesise a choline phospholipid analogue standard N-methylation[142,143] of the octadecyloxy phosphate **(85d)** was pursued.

Thus compound **(85d)** was treated with 90% aqueous formic acid and 37% aqueous formaldehyde and refluxed overnight. The reaction mixture was neutralised by addition of aqueous sodium hydroxide solution and then lyophilised. Chloroform extraction of the residue gave a quantitative yield of a dimethylethanolamine octadecyloxy analogue **(87d)**.



(85d) **(87d)**

The product was fully characterised and indicated that none of the desired choline analogue **(88d)** had been formed.



(88d)

Most noticeable in the ^1H nmr spectrum is the signal at δ2.78 which corresponds to N-methyl integrating to *ca* six protons indicative of a dimethyl product. If the trimethyl product had been formed this N-methyl signal would be shifted to *ca* δ3.20. FAB mass spectroscopy revealed a protonated molecular ion of m/e 422 corresponding to the proposed dimethyl product **(87d)**. There was no evidence of the choline analogue **(88d)** which would have a protonated molecular ion of m/e 436.

2.3. Experimental.

2-Chloro-3-methyl-1,3,2-oxazaphosphacyclopentane (79).

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. Seperately 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 x 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 (12.0 g, 49%); b.p. 30-35 °C, 0.1 mmHg.

^{1}H nmr δ($CDCl_3$) 2.66 (3H, d, CH_3N, J=15 Hz), 3.08 (2H, m, NCH_2), 4.34 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 167.3.

^{13}C nmr δ($CDCl_3$) 30.9 (d, CH_3N, $^2J_{C-P}$ =13.6 Hz), 48.7 (d, NCH_2, $^2J_{C-P}$ =7.2 Hz), 70.9 (d, CH_2O, $^2J_{C-P}$ =9.5 Hz).

EIMS m/e 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).

$\upsilon_{max}(CH_2Cl_2)$ 2960, 2420, 1640, 1245, 1000, 700 cm^{-1}.

2-Hexyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane (80a).

Anhydrous hexan-1-ol (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 **(79)** (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%).

^{1}H nmr δ($CDCl_3$) 0.83 (3H, t, $C\underline{H}_3CH_2$), 1.36 (8H, m, CH_2 x 4), 2.67 (3H, d, CH_3N, J=11.8 Hz), 2.95 (2H, m, NCH_2), 3.66 (2H, m, RCH_2O), 4.23 (2H, m, OCH_2).

^{31}P nmr δ($CDCl_3$) 135.0.

3-Methyl-2-octanyloxy-1,3,2-oxazaphosphacyclopentane (80b).

Compound **(79)** (0.20 g, 1.43 mmol) in dichloromethane (12 mL) was added dropwise with vigorous stirring to anhydrous octan-1-ol (0.19 g, 1.43 mmol) and triethylamine (0.20 mL, 1.45 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 the solvent evaporated under reduced pressure. The residue was then extracted with dry hexane (80 mL) and the extract

evaporated under reduced pressure to give the product as a colourless oil (0.32 g, 96%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 136.4.

2-Dodecyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane (80c).

Compound **(79)** (0.50 g, 3.58 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous dodecan-1-ol (0.67 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%).

^{31}P nmr δ($CDCl_3$) 135.8.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.9 (C-10), 29.4 (d, C-11, $^3J_{C-P}$ =2.7 Hz), 29.6-31.6 (m, C-4 to C-9), 31.6 (d, CH_3N, $^2J_{C-P}$ =7.1 Hz), 32.0 (C-3), 49.6 (d, NCH_2, $^2J_{C-P}$ =5.5 Hz), 63.3 (d, C-12, $^2J_{C-P}$ =11.7 Hz), 69.0 (d, CH_2O, $^2J_{C-P}$ =10.6 Hz).

3-Methyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane (80d).

Compound **(79)** (0.50 g, 3.58 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous octadecan-1-ol (0.97 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 dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as a white solid (1.34 g, 100%).

^{31}P nmr δ($CDCl_3$) 135.7.

^{13}C nmr δ($CDCl_3$) 14.2 (C-1), 22.8 (C-2), 25.9 (C-16), 29.4 (d, C-17, $^3J_{C-P}$ =4.0 Hz), 29.2-32.0 (m, C-4 to C-15), 31.5 (d, CH_3N, $^2J_{C-P}$ =7.3 Hz), 31.9 (C-3), 49.6 (d, NCH_2, $^2J_{C-P}$ =5.7Hz), 63.2 (d, C-18, $^2J_{C-P}$ =11.7 Hz), 68.9 (d, CH_2O, $^2J_{C-P}$ =10.5 Hz).

2-Methyl-2-oleyloxy-1,3,2-oxazaphosphacyclopentane (80e).

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 **(79)** (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%).

^{31}P nmr δ($CDCl_3$) 136.2.

2-(Ethoxycarbonyl)-methoxy-3-methyl-1,3,2-oxazaphosphacyclo pentane (80f).

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 **(79)** (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%).

^{31}P nmr δ($CDCl_3$) 138.7.

3-*O*-Hexadecyl-*sn*-glycerol (81).

1,2-*O*-isopropylidene glycerol (2.64 g, 0.02 mol) in DMF (5 mL) was added dropwise with vigorous stirring to 80% sodium hydride (0.91 g, 0.03 mol) in DMF (40 mL). 1-Bromohexadecane (9.15 g, 0.03 mol) was then added dropwise and the solution stirred at ambient temperature for 24 h. Methanol (1 mL) followed by H_2O (100 mL) were then added and the solution extracted with diethyl ether (2 x 50 mL). The combined organic portions were dried ($MgSO_4$) and evaporated under reduced pressure to give a brown oil. The crude product was chromatographed on silica (80 g) eluting with 20% diethyl ether in 60-80 °C petroleum ether. Appropriate fractions were pooled and evaporated under reduced pressure to yield the protected product as a pale yellow oil (3.80 g, 53%).

The protected product (3.80 g, 0.01 mol) in 1M aqueous hydrochloric acid (15 mL) and methanol (135 mL) was refluxed for 2 h. The solvent was then removed under reduced pressure to yield the product **(81)** as a white solid (3.16 g, 100%).

3-*O*-Hexadecyl-1-*O*-(4-methoxytrityl)-*rac*-glycerol (82).

4-Methoxytrityl chloride (4.1 g, 15.3 mmol) in THF (5 mL) was added to compound **(81)** (3.0 g, 9.50 mmol) in pyridine (10 mL) at 0 °C. The solution was warmed to ambient temperature with stirring for 2 h and the solvent then removed under reduced pressure. The residue was dissolved in chloroform (80 mL) then extracted with 10% aqueous sodium hydrogen carbonate (20 mL), followed by water (20 mL). The organic portion was dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil. The crude product was chromatographed on silica (70 g) eluting with 33% diethyl ether in 60-80 °C petroleum ether. Appropriate fractions were pooled and evaporated under reduced pressure to yield the product **(82)** as a pale yellow oil (3.91 g, 70%).

1-*O*-Hexadecyl-2-*O*-ethyl-3-*O*-(4-methoxytrityl)-*rac*-glycerol (83).

80% Sodium hydride (0.29 g, 12.30 mmol) in DMSO (10.4 mL) was stirred under an atmosphere of nitrogen for 30 min. Compound **(82)** (1.5 g, 2.55 mmol) in toluene (6 mL) was added and the solution stirred for 15 min. Iodo ethane (0.80 g, 5.1 mmol) was then added and the solution stirred for 4 h. Hexane (35 mL) and water (35 mL) were added and the organic layer separated and washed with water (3 x 30 mL). The combined aqueous portions were extracted with hexane (3 x 30 mL) and the organic portions combined, dried ($MgSO_4$) and evaporated under reduced pressure to yield the product **(83)** as a pale yellow oil (1.56 g, 99%).

3-*O*-Hexadecyl-2-*O*-ethyl-*rac*-glycerol (84).

Compound **(83)** (1.0 g, 1.62 mmol) and silicic acid (50 g, Mallinckrodt) in 60-80 °C petroleum ether were stirred at ambient temperature for 70 h. The mixture was then filtered, washing with 50% diethyl ether in 60-80 °C petroleum ether (1000 mL). The solvent was removed under reduced pressure to give a yellow oil. The crude product was chromatographed on silica (60 g) eluting with 50% diethyl ether in 60-80 °C petroleum ether. Appropriate fractions were pooled and evaporated under reduced pressure to yield the product **(84)** as a pale yellow oil (0.37 g, 69%).

^{1}H nmr δ(CDCl$_3$) 0.85 (3H, m, C$\underline{H}_3$CH$_2$), 1.19 (29H, m, CH$_2$ x 13 and C$\underline{H}_3$CH$_2$O), 1.50 (2H, m, C$\underline{H}_2$CH$_2$O), 2.30 (1H, s_b, OH), 3.45 (9H, m, C$\underline{H}_2$OH, CH$_2$OCH$_2$ and CH$_2$OCH).

^{13}C nmr δ(CDCl$_3$) 14.1 (C$\underline{H}_3$CH$_2$CH$_2$), 15.5 (C$\underline{H}_3$CH$_2$O), 22.7 (CH$_3$C$\underline{H}_2$CH$_2$), 26.0 (C$\underline{H}_2$CH$_2$CH$_2$O), 29.3 - 29.6 (CH$_2$ x 11), 31.9 (C$\underline{H}_2$CH$_2$O), 62.9 (C$\underline{H}_2$OH), 65.5 (CH$_3$C$\underline{H}_2$O), 70.8 (CH$_2$C$\underline{H}_2$O), 71.8 (C$\underline{H}_2$O), 78.2 (C$\underline{H}$OCH$_2$CH$_3$).

3-Methyl-2-(1-*O*-hexadecyl-2-*O*-ethyl-glycerol-*rac*-3-yloxy)-1,3,2-oxazaphosphacyclopentane (80g). 1st Attempt.

Compound **(79)** (0.08 g, 0.61 mmol) in benzene (10 mL) was added dropwise with vigorous stirring to anhydrous compound **(84)** (0.21 g, 0.61 mmol) and triethylamine (0.05 mL, 0.61 mmol) in benzene (15 mL) at 0 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h

and the solvent removed under reduced pressure. The residue was then dissolved in dichloromethane (80 mL) and extracted with saturated aqueous sodium hydrogen carbonate (3 x 15 mL), followed by saturated brine (15 mL). The organic portion was dried ($MgSO_4$) and the solvent evaporated under reduced pressure to give a pale yellow gum (0.30 g, >100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 140.4, 137.1 and 10.1 (2:1:7).

3-Methyl-2-(1-*O*-hexadecyl-2-*O*-ethyl-glycerol-*rac*-3-yloxy)-1,3,2-oxazaophosphacyclopentane (80g). 2nd Attempt.

Compound **(79)** (0.04 g, 0.29 mmol) in benzene (10 mL) was added dropwise with vigorous stirring to anhydrous compound **(84)** (0.10 g, 0.29 mmol) and triethylamine (0.04 mL, 0.30 mmol) in benzene (15 mL) at 0 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 2 h and the solvent then removed under pressure. The residue was extracted with hexane (60 mL) and the extract evaporated under reduced pressure to give a cloudy white oil (0.20 g, >100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 10.0 and 4.7 (2:3).

3-Methyl-2-(1-2-dipalmitoylglycerol-*rac*-3-yloxy)-1,3,2-oxazaophospha cyclopentane (80h).

Compound **(79)** (0.043 g, 0.305 mmol) in dichloromethane (8 mL) was added dropwise with vigorous stirring to anhydrous 1,2-dipalmitoyl-*rac*-glycerol (0.170 g, 0.299 mmol) and triethylamine (0.042 mL, 0.305 mmol) in dichloromethane (10 mL) at 0 °C under an

atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 2 h and the solvent removed under reduced pressure. The residue was extracted with dry hexane (60 mL) and the extract evaporated under reduced pressure to yield the product **(80h)** as a white solid (0.19 g, 95%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 138.7 and 137.8 (diastereoisomers 4:3).

3-Methyl-2-(1-2-dipalmitoylglycerol-*sn*-3-yloxy)-1,3,2-oxazaophospha cyclopentane (80i).

This was prepared in an analogous manner to compound **(80h)** above. Thus from the condensation of compound **(79)** (0.038g, 0.269 mmol) and anhydrous 1,2-dipalmitoyl-*sn*-glycerol (0.150 g, 0.264 mmol) was isolated the title compound **(80i)** (0.18 g, 100%).

^{31}Pnmr δ(CH_2Cl_2/D_2O centre lock) 138.7 and 137.8 (diastereoisomers 2:3).

3-Methyl-2-(2-octanyloxy)-1,3,2-oxazaphosphacyclopentane (80j)

Compound **(79)** (0.22 g, 1.54 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous octan-2-ol (0.20 g, 1.54 mmol) and triethylamine (0.22 mL, 1.56 mmol) in dichloromethane (15 mL) at -60 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 30 mins and the solvent evaporated under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract

evaporated under reduced pressure to give the product as a colourless oil (0.31 g, 86%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.15 (3H, 2d, $C\underline{H}_3CHO$), 1.31 (10H, m, CH_2 x 5), 2.70 (3H, 2d, CH_3N), 3.0 (2H, m, NCH_2), 4.26 (3H, m, $CH_3C\underline{H}O$ and CH_2O).

^{31}P nmr δ($CDCl_3$) 137.8 and 137.3 (diastereoisomers, 3:2).

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 22.9, 23.0 (2d, $\underline{C}H_3CH$, diast., 2:3, $^3J_{C-P}$ =2.3 and 2.7 Hz) 25.5 (d, $R\underline{C}H_2CH_2CHO$, $^4J_{C-P}$ =3.5 Hz), 29.1 (C-4), 31.7 (m, CH_3N, diast.) 32.2 (C-3) 38.3, 38.5 (2d, C-6, diast., 3:2, $^3J_{C-P}$ =2.0 and 5.2 Hz), 49.3, 49.4 (2d, NCH_2, diast., 1:1, $^3J_{C-P}$ =5.0 and 4.7 Hz), 68.4, 68.5 (2d, RCHO, diast., 1:1, $^2J_{C-P}$ =9.9 and 10.0 Hz), 70.0, 70.2 (2d, CH_2O, diast.,1:1, $^2J_{C-P}$ =15.5 and 15.3 Hz).

3-Methyl-2-(4-methyl-3-heptanyloxy)-1,3,2-oxazaphosphacyclo pentane (80k).

Compound **(79)** (0.15 g, 1.08 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous 4-methyl-heptan-3-ol (0.13 g, 1.08 mmol) and triethylamine (0.15 mL, 1.09 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 the solvent evaporated under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.25 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.85 (9H, m, $C\underline{H}_3CH_2$ x 2 and $C\underline{H}_3CH$), 1.29 (7H, m, CH_2 x 3 and $CH_3C\underline{H}$), 2.71 (m, CH_3N), 3.01 (2H, m, NCH_2), 3.70 (1H, m, CHO), 4.25 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 140.0, 139.8 138.7 and 138.6 (diastereoisomers, 1.5:1.0:1.4:1.2).

^{13}C nmr δ($CDCl_3$) 10.3 (m, $\underline{C}H_3CH_2CH$), 14.4 (m, $\underline{C}H_3CH$), 15.0 (m, $\underline{C}H_3CH_2CH_2$), 20.4 ($CH_3\underline{C}H_2CH_2$), 25.5 (m, $CH_3CH_2\underline{C}H_2$), 32.0, 32.4 (2d, CH_3N, diast., 3:2, $^2J_{C\text{-}P}$ =3.1 and 5.4 Hz), 34.8 (m, $CH_3\underline{C}H_2CH$), 37.4 (m, $CH_3\underline{C}H$), 49.2, 49.5 (2d, NCH_2, diast., 2:3, $^2J_{C\text{-}P}$ =4.4 and 5.2 Hz), 68.2, 68.4 (2d, OCH, diast., 1:1, $^2J_{C\text{-}P}$ =3.6 and 3.0 Hz), 78.9, 79.5 (2d, CH_2O, diast., 1:1, $^2J_{C\text{-}P}$ =14.3 and 13.7 Hz).

3-Methyl-2-(5-methyl-3-heptanyloxy)-1,3,2-oxazaphosphacyclo pentane (80l).

Compound **(79)** (0.15 g, 1.08 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous 5-methyl-heptan-3-ol (0.13 g, 1.08 mmol) and triethylamine (0.15 mL, 1.09 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 the solvent evaporated under reduced pressure.The residue was extracted with dry hexane (60 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.25 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.89 (9H, m, $C\underline{H}_3CH_2$ x 2 and $C\underline{H}_3CH$), 1.32 (7H, m, CH_2 x 3 and $CH_3C\underline{H}$), 2.72 (3H, d, CH_3N, J=12.0 Hz), 3.00 (2H, m, NCH_2), 3.93 (1H, m, CHO), 4.25 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 139.5, 139.2, 139.1 and 138.8 (diastereoisomers, 1.5:1.2:1.1:1.0).

^{13}C nmr δ($CDCl_3$) 9.8 (m, $\underline{C}H_3CH_2CHO$), 11.2 (m, $\underline{C}H_3CH_2CH$), 19.2 (m, $\underline{C}H_3CH$), 29.0 (m, $CH_3\underline{C}H_2CHO$), 29.5 ($CH_3\underline{C}H_2CH$), 30.9 (m, $CH_3\underline{C}H$), 32.3 (d, CH_3N, $^2J_{C\text{-}P}$ =2.6 Hz), 43.0 (m, $CH_3CH_2CH\underline{C}H_2$), 49.4 (d, NCH_2, $^2J_{C\text{-}P}$ =4.2 Hz), 68.3, 68.4 (2d, CHO, diast., 3:2, $^2J_{C\text{-}P}$ =2.3 and 4.0 Hz), 73.3, 73.5 (2d, CH_2O, diast., 2:3, $^2J_{C\text{-}P}$ =7.0 and 7.5 Hz).

2-Cholesteryloxy-3-methyl-1,3,2-oxazaphosphacyclopentane 80m).

Compound **(79)** (0.15 g, 1.08 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous cholesterol (0.42 g, 1.08 mmol) and triethylamine (0.15 mL, 1.09 mmol) in dichloromethane (20 mL) at -20 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h and the solvent evaporated under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white solid (0.51 g, 96%).

^{31}P nmr δ($CDCl_3$) 137.4.

2-(1,1-Dimethyloctadecyloxy)-3-methyl-1,3,2-oxazaphosphacyclo pentane (80m).

Compound **(79)** (0.20 g, 1.43 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous 1,1-dimethyloctadecan-1-ol (0.43 g, 1.43 mmol) and triethylamine (0.20 mL, 1.45 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 the solvent evaporated under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.57 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.29 (6H, s, CH_3 x 2), 1.35 (32H, m, CH_2 x 16), 2.61 (3H, d, CH_3N, J=13 Hz), 3.0 (2H, m, NCH_2), 4.2 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 132.75.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 24.0 (C-16), 28.9 - 30.2 (m, C-4 to C-15 and C-17), 31.9 (d, CH_3N, $^2J_{C-P}$ =4.1 Hz), 32.2 (C-3), 43.9 (d, CH_3 x 2, $^3J_{C-P}$ =5.7 Hz), 49.0 (d, NCH_2, $^2J_{C-P}$ =5.7 Hz), 68.2 (d, RCO, $^2J_{C-P}$ =9.6 Hz), 76.5 (d, CH_2O, $^2J_{C-P}$ =6.8 Hz).

2-Adamantanyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane (80n).

Compound **(79)** (0.15 g, 1.08 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous adamantanol (0.16 g, 1.08 mmol) and triethylamine (0.15 mL, 1.09 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 the solvent evaporated under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.28 g, 100%).

^{1}H nmr δ($CDCl_3$) 1.63 (6H, m, CH_2 x 3), 1.89 (6H, m, CH_2 x 3), 2.09 (3H, m, CH x 3), 2.62 (3H, d, CH_3N, J=6.6 Hz), 2.94. (2H, m, NCH_2), 4.22 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 132.13.

^{13}C nmr δ($CDCl_3$) 30.8 (C-4, C-6 and C-8), 32.1 (d, CH_3N, $^2J_{C-P}$ =19.8 Hz), 36.1 (C-5, C-7 and C-9), 45.1 (d, C-1, C-3 and C-10, $^3J_{C-P}$ =12.5 Hz), 49.1 (d, NCH_2, $^2J_{C-P}$ =5.5 Hz), 68.2 (d, RCO, $^2J_{C-P}$ =9.6 Hz), 73.8 (d, CH_2O, $^2J_{C-P}$ =5.5 Hz).

<u>Preparation of standard dinitrogen tetroxide solutions</u>

Dinitrogen tetroxide was condensed at 0 °C and treated with dry oxygen gas for 15 mins. Phosphorus pentoxide was added and the oxidant distilled by gentle warming. The distillate was collected by cooling the receiver in an acetone/cardice bath. Purified dinitrogen tetroxide could be stored for prolonged periods under deep freeze conditions. The standard solutions were prepared by allowing the distillate to warm to ambient temperature, withdrawing aliquots and adding these to an accurately weighed volume of dry dichloromethane (20 mL). The standard solutions could be stored at low temperature for several weeks without appreciable deterioration.

3-Hexyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane 2-oxide (85a).

A portion of standard dinitrogen tetroxide solution (11 mL, containing 1.30 mmol oxidant, sufficient to oxidise 5.20 mmol of phosphite) was added dropwise with vigorous stirring to compound **(80a)** (1.0 g, 4.90 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 pale yellow oil (1.04 g, 96%).

^{1}H nmr δ($CDCl_3$) 0.82 (3H, t, $C\underline{H}_3CH_2$), 1.40 (8H, m, CH_2 x 4), 2.62 (3H, d, CH_3N, J=10.0 Hz), 3.25 (2H, m, NCH_2), 3.93 (2H, m, RCH_2O), 4.21 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 18.9.

3-Methyl-2-octanyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (85b).

This was prepared by an analogous manner to **(85a)** above. Thus from compound **(80b)** (0.34 g, 1.36 mmol) was isolated the title compound **(85b)** (0.34 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.8.

2-Dodecyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane 2-oxide (85c).

This was prepared by an analogous manner to **(85a)** above. Thus from compound **(80c)** (1.90 g, 6.57 mmol) was isolated the title compound **(85c)** (2.0 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.87 (3H, t, $C\underline{H}_3CH_2$), 1.43 (20H, m, CH_2 x 10), 2.70 (3H, d, CH_3N, J=10.0 Hz), 3.40 (2H, m, NCH_2), 4.02 (2H, m, RCH_2O), 4.20 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 18.7.

3-Methyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (85d).

This was prepared by an analogous manner to **(85a)** above except that stirring at ambient temperature was continued for 1 h. Thus from compound **(80d)** (1.34 g, 3.59 mmol) was isolated the title compound **(85d)** as a white solid (1.39 g, 100%).

^{31}P nmr δ($CDCl_3$) 18.7.

3-Methyl-2-oleyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (85e).

This was prepared by an analogous manner to **(85d)** above except that the solution was concentrated immediately upon reaching ambient temperature. Thus from compound **(80e)** (1.27 g, 3.42 mmol) was isolated the title compound **(85e)** as a colourless oil (1.30 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.6.

2-(Ethoxycarbonyl)-methoxy-3-methyl-1,3,2-oxazaphosphacyclo -pentane 2-oxide (85f).

This was prepared by an analogous manner to **(85e)** above. Thus from compound **(80f)** (0.67 g, 3.25 mmol) was isolated the title compound **(85f)** as a colourless oil (0.73 g, 100%).

^{31}P nmr $\delta(CDCl_3)$ 20.1.

3-Methyl-2-(1,2-dipalmitoylglycerol-*rac*-3-yloxy)-1,3,2-oxazaphospha cyclopentane 2-oxide (85h).

Attempts were made to prepare this by an analogous manner to **(85a)** above except that stirring at ambient temperature was continued for 10 mins upon reaching ambient temperature. Thus from compound **(80h)** (0.19 g, 0.28 mmol) was isolated a white solid (0.19 g, 98%).

^{31}P nmr $\delta(CDCl_3)$ 19.9 and -1.3 (1:5).

3-Methyl-2-(1,2-dipalmitoylglycerol-*sn*-3-yloxy)-1,3,2-oxazaphospha cyclopentane 2-oxide (85i).

Attempts were made to prepare this by an analogous manner to **(85h)** above. Thus from compound **(80i)** (0.18 g, 0.27 mmol) was isolated a white solid (0.18 g, 100%).

^{31}P nmr $\delta(CDCl_3)$ 19.8 and -3.0 (1:3).

3-Methyl-2-(2-octanyloxy)-1,3,2-oxazaphosphacyclopentane 2-oxide (85j).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80j)** (0.31 g, 1.33 mmol) was isolated the title compound **(85j)** as a pale yellow oil (0.33 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.82 (3H, t, $C\underline{H}_3CH_2$), 1.24 (3H, d, $C\underline{H}_3CHO$, J=3.1 Hz), 1.44 (10H, m, CH_2 x 5), 2.64 (3H, m, CH_3N,), 3.27 (2H, m, NCH_2), 4.30 (3H, m, $CH_3C\underline{H}O$ and CH_2O).

^{31}P nmr δ($CDCl_3$) 20.9.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1). 21.8 (d, $\underline{C}H_3CH$, $^3J_{C\text{-}P}$ =6.0 Hz), 22.5 (C-2), 25.2 (d, $R\underline{C}H_2CH_2CHO$, $^4J_{C\text{-}P}$ =2.1 Hz), 29.0 (C-4), 31.3 (2d, CH_3N, diast., 1:1, $^2J_{C\text{-}P}$ =4.8 and 6.5 Hz), 31.7 (C-3), 37.4 (2d, $R\underline{C}H_2CHO$, diast., 1:1, $^3J_{C\text{-}P}$ =4.5 and 6.8 Hz), 49.2 (2d, NCH_2, diast., 1:1, $^2J_{C\text{-}P}$ =4.4 and 4.5 Hz), 63.7 (RCHO), 75.7 (2d, CH_2O, diast., 3:2, $^2J_{C\text{-}P}$ =2.6 and 2.6 Hz).

3-Methyl-2-(4-methyl-3-heptanyloxy)-1,3,2-oxazaphosphacyclo pentane 2-oxide (85k).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80k)** (0.25 g, 1.07 mmol) was isolated the title compound **(85k)** as a pale yellow oil (0.27 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.4.

3-Methyl-2-(5-methyl-3-heptanyloxy)-1,3,2-oxazaphosphacyclo pentane 2-oxide (85l).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80l)** (0.33 g, 1.42 mmol) was isolated the title compound **(85l)** as a pale yellow oil (0.35 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.2.

2-Cholesteryloxy-3-methyl-1,3,2-oxazaphosphacyclopentane 2-oxide (85m).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80m)** (0.51 g, 1.04 mmol) was isolated the title compound **(85m)** as a white solid (0.52 g, 99%).

^{1}H nmr δ($CDCl_3$) 1.54 (42H, m, cholesteryl protons), 2.68 (3H, d, CH_3N, J=10.2 Hz), 3.30 (2H, m, NCH_2), 4.23 (3H, m, CH_2O and CH of cholesteryl), 5.37 (1H, m, CH=C).

^{31}P nmr δ($CDCl_3$) 18.9.

^{13}C nmr δ($CDCl_3$) 11.8 (C-18), 18.7 (C-21), 19.2 (C-19), 21.0 (C-11), 22.5 (C-27), 22.8 (C-26), 23.8 (C-23), 24.2 (C-15), 28.0 (C-25), 28.2 (C-12), 29.7 (d, C-2, $^{3}J_{C\text{-}P}$ =2.7 Hz), 31.3 (d, CH_3N, $^{2}J_{C\text{-}P}$ =4.6 Hz), 31.8 (C-8), 36.1 (C-7), 36.3 (C-20), 36.9 (C-10/C-22), 39.5 (C-1), 39.6 (C-24), 40.0 (d, C-4, $^{3}J_{C\text{-}P}$ =2.6 Hz), 42.2 (C-9/C-16), 49.2 (d, NCH_2, $^{2}J_{C\text{-}P}$ =15.6 Hz), 49.9 (C-13), 56.1 (C-17), 56.6 (C-14), 63.8 (C-3, unresolved), 76.5 (d, CH_2O, $^{2}J_{C\text{-}P}$ =9.4 Hz), 122.8 (C-6), 139.4 (C-5).

υ_{max}(CH_2Cl_2) 3940, 3052, 2985, 1425, 1264 (P=O) cm^{-1}.

2-(1,1-Dimethyloctadecyloxy)-3-methyl-1,3,2-oxazaphosphacyclo pentane 2-oxide (85n).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80n)** (0.58 g, 1.45 mmol) was isolated the title compound **(85n)** as a pale yellow oil (0.60 g, 100%).

^{31}P nmr δ($CDCl_3$) 18.5.

2-Adamantanyloxy-3-methyl-1,3,2-oxazaphosphacyclopentane 2-oxide (85o).

This was prepared by an analogous manner to **(85h)** above. Thus from compound **(80o)** (0.28 g, 1.09 mmol) was isolated the title compound **(85o)** as a pale yellow oil (0.29 g, 100%).

^{31}P nmr δ($CDCl_3$) 16.2.

Attempted Kinetic Study of Acid-catalysed Hydrolysis of (85a).

Compound **(85a)** (0.40 g, 1.81 mmol) was dissolved in THF (1 mL) and D_2O (1 mL) and treated with 0.2 M hydrochloric acid (0.53 mL, 5 molar %). ^{31}P nmr spectra were recorded after 10 min at ambient temperature; δ(THF/D_2O) 0.39. The reaction mixture was then neutralised by careful addition of 0.2 M 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%).

^{1}H nmr δ($CDCl_3$) 0.81 (3H, t, $C\underline{H}_3CH_2$), 1.39 (8H, m, CH_2 x 4), 2.63 (3H, s, CH_3N), 3.08 (2H, m, NCH_2), 3.78 (2H, m, RCH_2O), 4.12 (2H, m, CH_2O), 10.0 (2H, s_b, NH_2).

^{31}P nmr δ($CDCl_3$) -1.4.

^{13}C nmr δ($CDCl_3$) 13.9 (C-1), 22.4 (C-2), 25.3 (C-4), 30.5 (d, C-5, $^3J_{C-P}$ =7.5 Hz), 31.4 (C-3), 33.5 (CH_3N), 49.8 (d, NCH_2, $^3J_{C-P}$ =6.3 Hz), 60.6 (d, RCH_2O, $^2J_{C-P}$ =5.8 Hz), 65.8 (d, CH_2O, $^2J_{C-P}$ =3.8 Hz).

υ_{max}(CH_2Cl_2) 3679, 3597, 3381, 2949, 2925, 2855, 2721, 1604, 1461, 1224 (P=O), 1078 cm^{-1}.

<u>Kinetic Study of Water Mediated Hydrolysis of (85a).</u>

Compound **(85a)** (0.45 g, 2.04 mmol) was dissolved in THF (1 mL) and D_2O (1 mL) at ambient temperature and ^{31}P nmr spectra recorded at appropriate intervals over a period of 75 min; t=0, δ(D_2O/THF) 23.0; t=75 min δ(D_2O/THF) 0.48. The reaction mixture was lyophilised to yield **(86a)** as a white solid (0.49 g, 100%).

EIMS m/e 240.1371 (MH^+, calc. for $C_9H_{23}NO_4P$ 240.1359, 3%), 224 (M^+- CH_3), 210 (M^+- C_2H_5), 196 (M^+- C_3H_7), 183 (MH^+- C_4H_9), 138 (M^+- $C_6H_{13}O$), 57 ($CH_3NCH_2CH_2^+$, base peak).

Analysis Found: C, 44.60; H, 9.23; N, 5.81%; $C_9H_{22}NO_4P$ requires C, 45.18; H, 9.27; N, 5.85%.

Water Mediated Hydrolysis of (85b).

Compound **(85b)** (0.34 g, 1.36 mmol) was dissolved in water (15 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised yielding **(86b)** as a cream coloured solid (0.36 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.38 (12H, m, CH_2 x 6), 2.62 (3H, s, CH_3N), 3.06 (2H, m, NCH_2), 3.78 (2H, m, RCH_2O), 4.10 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) -1.5.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.65 (C-2), 25.86 (C-6), 29.29 (C-5 and C-6), 30.8 (d, C-7, $^3J_{C\text{-}P}$ =6.9 Hz), 31.8 (C-3), 33.7 (CH_3N), 50.0 (d, NCH_2, $^3J_{C\text{-}P}$ =5.0 Hz), 60.9 (d, RCH_2O, $^2J_{C\text{-}P}$ =2.7 Hz), 62.9 (d, CH_2O, $^2J_{C\text{-}P}$ =5.4 Hz).

FAB MS m/e 268 (MH^+, 31%), 156 (3), 154 (M^+- C_8H_{17}, 3), 58 ($CH_3NHCH_2CH_2^+$, <1), 53 (base peak).

Analysis Found: C, 48.54; H, 9.92; N, 5.08; P, 11.16%; $C_{11}H_{26}NO_4P$ requires C, 49.43; H, 9.80; N, 5.24; P, 11.59%.

υ_{max}(CH_2Cl_2) 3037, 2924, 2852, 1463, 1415, 1224 (P=O), 1074, 1044 cm^{-1}.

Water Mediated Hydrolysis of (85c).

Compound **(85c)** (0.50 g, 1.64 mmol) was dissolved in D_2O (1.2 mL) at ambient temperature and ^{31}P nmr spectra recorded at appropriate intervals over 30 min at which point an emulsion

formed precluding further spectroscopic investigation. The reaction mixture was diluted with water (8 mL) and stirred for a further 3 h and then lyophilised yielding **(86c)** as a white solid (0.53 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.81 (3H, t, $C\underline{H}_3CH_2$), 1.33 (20H, m, CH_2 x 10), 2.61 (3H, s, CH_3N), 3.06 (2H, m, NCH_2), 3.79 (2H, m, RCH_2O), 4.10 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) -1.2.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.8 (C-10), 29.3-30.3 (C-4 to C-9), 30.7 (d, C-11, $^3J_{C\text{-}P}$ =7.4 Hz), 31.8 (C-3), 33.6 (CH_3N), 50.0 (d, NCH_2, $^3J_{C\text{-}P}$ =6.0 Hz), 60.7 (d, RCH_2O, $^2J_{C\text{-}P}$ =4.9 Hz), 66.0 (d, CH_2O, $^2J_{C\text{-}P}$ =5.9 Hz).

EIMS m/e 183 (M^+- $C_{10}H_{20}$, 2%), 138 (M^+- $C_{12}H_{25}O$, 21), 43 ($CH_3NCH_2^+$, 70).

FAB MS m/e 324 (MH^+, 41%), 138 (M^+- $C_{12}H_{25}O$, 40), 58 ($CH_3NCH_2CH_2^+$, base peak).

Analysis Found: C, 55.45; H, 10.70; N, 4.05; P, 9.37%; $C_{15}H_{34}NO_4P$ requires C, 55.70; H, 10.60; N, 4.33; P, 9.58%.

υ_{max}(CH_2Cl_2) 3679, 3587, 2925, 2849, 1604, 1461, 1218 (P=O), 1075, 1043 cm^{-1}.

<u>Water Mediated Hydrolysis of (85d).</u>

Compound **(85d)** (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 **(86d)** as a white solid (1.40 g, 97%).

^{1}H nmr δ($CDCl_3$) 0.88 (3H, t, $C\underline{H}_3CH_2$), 1.42 (32H, m, CH_2 x 16), 2.70 (3H, s, CH_3N), 3.13 (2H, m, NCH_2), 3.87 (2H, m, RCH_2O), 4.18 (2H, m, CH_2O), 9.98 (2H, s_b, NH_2).

^{31}P nmr δ($CDCl_3$) -1.7.

^{13}C nmr δ($CDCl_3$) 14.2 (C-1), 22.7 (C-2), 25.7 (C-16), 29.4-30.8 (C-4 to C-15), 30.7 (d, C-17, $^3J_{C-P}$ =7.4 Hz), 31.9 (C-3), 33.7 (CH_3N), 50.0 (d, NCH_2, $^3J_{C-P}$ =6.7 Hz), 61.0 (d, RCH_2O, $^2J_{C-P}$ =5.2 Hz), 66.4 (d, CH_2O, $^2J_{C-P}$ =5.6 Hz).

EIMS m/e 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 ($CH_3NCH_2CH_2^+$, 70).

FAB MS m/e 408 (MH^+, 42%), 138 (M^+- $C_{18}H_{37}O$, 9), 58 ($CH_3NCH_2CH_2^+$, base peak).

Analysis Found: C, 58.82; H, 11.36; N, 3.61%; $C_{21}H_{46}NO_4P$ requires C, 61.89; H, 11.38; N, 3.44%; $C_{21}H_{46}NO_4PH_2O$ requires C, 59.27; H, 11.37; N, 3.29%.

υ_{max}(CH_2Cl_2) 3042, 2925, 2849, 1464, 1221 (P=O), 1072, 1043 cm^{-1}.

Water Mediated Hydrolysis of (85e).

Compound **(85e)** (1.30 g, 3.36 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 2 h. The reaction mixture was then lyophilised and the residue was extracted with

chloroform (100 mL). The extract was 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 **(86e)** as a white solid (0.88 g, 65%).

^{1}H nmr δ($CDCl_3$) 0.88 (3H, t, $C\underline{H}_3CH_2$), 1.26 (22H, m, CH_2 x 11), 1.59 (2H, m, $RC\underline{H}_2CH_2O$), 2.01 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 2.68 (3H, s, CH_3N), 3.10 (2H, m, NCH_2), 3.84 (2H, m, RCH_2O), 4.17 (2H, m, CH_2O), 5.34 (2H, m, CH=CH).

^{31}P nmr δ($CDCl_3$) -1.9.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.8 (C-16), 29.3-29.8 (C-4 to C-7 and C-12 to C-15), 30.8 (d, C-17, $^3J_{C\text{-}P}$ =7.5 Hz), 31.9 (C-3), 43.6 (CH_3N), 50.2 (d, NCH_2, $^3J_{C\text{-}P}$ =5.5 Hz), 60.9 (d, RCH_2O, $^2J_{C\text{-}P}$ =3.3 Hz), 66.0 (d, CH_2O, $^2J_{C\text{-}P}$ =5.8 Hz), 129.8 (C-9 or C-10), 130.0 (C-9 or C-10).

FAB MS m/e 406 (MH^+, 5%), 321 (6), 138 (M^+- $C_{18}H_{35}O$, 2), 107 (3), 58 ($CH_3NCH_2CH_2^+$, base peak).

Analysis Found: C, 55.91; H, 10.30; N, 4.05%; $C_{21}H_{44}NO_4P$ requires C, 62.19; H, 10.94; N, 3.45%; $C_{21}H_{44}NO_4P[H_2O]_{2.5}$ requires C, 55.98; H, 10.96; N, 3.11%.

υ_{max}(CH_2Cl_2) 3679, 3597, 2925, 2849, 1604, 1464, 1271, 1248 (P=O), 1154, 1072 cm^{-1}.

Water Mediated Hydrolysis of (85f).

Compound **(85f)** (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 50-70% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(86f)** as a white solid (0.90 g, 82%).

^{1}H nmr δ($CDCl_3$) 1.26 (3H, t, $C\underline{H}_3CH_2$, J=7.1 Hz), 2.72 (3H, s, CH_3N), 3.17 (2H, m, NCH_2), 4.18 (4H, m, CH_2O and $CH_3C\underline{H}_2$), 4.45 (2H, d, RCH_2O, J=9.2 Hz), 8.6 (2H, s_b, NH_2).

^{31}P nmr δ($CDCl_3$) -2.5.

^{13}C nmr δ($CDCl_3$) 14.2 (C-1), 33.6 (CH_3N), 49.8 (d, NCH_2, $^3J_{C\text{-}P}$ =5.7 Hz), 60.9 (d, $CH_3\underline{C}H_2O$, $^2J_{C\text{-}P}$ =3.3 Hz), 61.2 (d, CH_2O, $^2J_{C\text{-}P}$ =3.9 Hz), 62.7 (d, $O\underline{C}H_2C(O)R$, $^2J_{C\text{-}P}$ =5.1 Hz), 169.0 (d, C=O, $^3J_{C\text{-}P}$ =7.7 Hz).

FAB MS m/e 242 (MH^+, base peak), 169 (MH^+- $CO_2CH_2CH_3$, 2%), 58 ($CH_3NCH_2CH_2^+$, 93).

Analysis Found: C, 33.75; H, 6.45; N, 5.55; P, 12.70%; $C_7H_{16}NO_6P$ requires C, 34.86; H, 6.69; N, 5.81; P, 12.84%; $C_7H_{16}NO_6P[H_2O]_{0.5}$ requires C, 33.61; H, 6.85; N, 5.60; P, 12.38%.

υ_{max}(CH_2Cl_2) 3679, 3597, 2960, 2727, 2464, 1753 (C=O), 1604, 1464, 1227 (P=O), 1119, 1084, 1040 cm^{-1}.

Water Mediated Hydrolysis of (85h).

The crude compound **(85h)** (0.19 g, 0.28 mmol) was suspended in THF/H_2O (1:1, 16 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised to yield **(86h)** as a white solid (0.19 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.85 (6H, t, $C\underline{H}_3CH_2$ x 2), 1.26 (48H, m, CH_2 x 24), 1.55 (4H, m, $OC(O)CH_2C\underline{H}_2$ x 2), 2.27 (7H, m, $OC(O)C\underline{H}_2$ x 2 and CH_3N), 3.10 (2H, m, NCH_2), 4.12 (6H, m, CH_2O x 2 and $C\underline{H}_2OC(O)CH_2$), 5.20 (1H, m, $OCH_2C\underline{H}CH_2O$).

^{31}P nmr δ($CDCl_3$) 0.3.

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2$ x 2), 22.6 ($CH_3\underline{C}H_2$ x 2), 24.80 ($CH_2OC(O)CH_2\underline{C}H_2$), 24.83 ($CHOC(O)CH_2\underline{C}H_2$), 29.1 - 29.7 (m, C-4 to C-13), 31.9 ($CH_3CH_2\underline{C}H_2$ x 2), 33.97 ($CH_2OC(O)\underline{C}H_2$), 34.04 ($CHOC(O)\underline{C}H_2$), 34.1 ($CH_3N$), 49.7 (d, NCH_2, $^3J_{C\text{-}P}$ =7.2 Hz), 61.6 (d, RCH_2O, $^2J_{C\text{-}P}$ =1.5 Hz), 62.1 ($\underline{C}H_2OC(O)$), 64.5 ($\underline{C}HOC(O)$), 69.7 (d, CH_2O, $^2J_{C\text{-}P}$ =4.5 Hz), 172.9 ($CHO\underline{C}(O)$), 173.3 ($CH_2O\underline{C}(O)$).

FAB MS m/e 729 (MNa^+, 19%), 707 (MH^+, 13), 552 (MH^+- $C_{11}H_{23}$, 91), 415 (6), 361 (15), 313 (31), 223 (9), 156 (62), 121 (59).

Analysis Found: C, 63.79; H, 10.93; N, 1.98; P, 4.29%; $C_{38}H_{76}NO_8P$ requires C, 64.65; H, 10.85; N, 1.98; P, 4.39%; $C_{38}H_{76}NO_8P[H_2O]_{0.5}$ requires C, 63.83; H, 10.86; N, 1.96; P, 4.33%.

υ_{max}(CH_2Cl_2) 2924, 2852, 1735 (C=O), 1639, 1511, 1460, 1227 (P=O), 1167, 1092, 1065 cm^{-1}.

Water Mediated Hydrolysis of (85i).

The crude compound **(85i)** (0.18 g, 0.26 mmol) was suspended in THF/H_2O (1:1, 12 mL) and stirred at ambient temperature for 2 h. The reaction mixture was then lyophilised to yield **(86i)** as a white solid (0.18 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.86 (6H, t, $C\underline{H}_3CH_2$ x 2), 1.27 (48H, m, CH_2 x 24), 1.57 (4H, m, $OC(O)CH_2C\underline{H}_2$ x 2), 2.28 (7H, m, $OC(O)CH_2$ x 2), 2.72 (3H, s_b, CH_3N), 3.14 (2H, m, NCH_2), 4.05 (2H, m, $CH_2OC(O)$), 4.23 (4H, m, CH_2O x 2), 5.20 (1H, m, $OCH_2C\underline{H}CH_2O$), 9.33 (2H, s_b, NH_2).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) -0.81.

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2$ x 2), 22.6 ($CH_3\underline{C}H_2$ x 2), 24.80 ($CH_2OC(O)CH_2\underline{C}H_2$), 24.83 ($CHOC(O)CH_2\underline{C}H_2$), 29.1 - 29.7 (m, C-4 to C-13), 31.88 ($CH_3CH_2\underline{C}H_2$ x 2), 33.98 ($CH_2OC(O)\underline{C}H_2$), 34.04 ($CHOC(O)\underline{C}H_2$), 34.1 ($CH_3N$), 49.7 (d, NCH_2, $^3J_{C\text{-}P}$ =7.5 Hz), 61.6 (d, RCH_2O, $^2J_{C\text{-}P}$ =1.3 Hz), 62.1 ($\underline{C}H_2OC(O)$), 64.5 ($\underline{C}HOC(O)$), 69.7 (d, CH_2O, $^2J_{C\text{-}P}$ =4.6 Hz), 172.9 ($CHO\underline{C}(O)$), 173.3 ($CH_2O\underline{C}(O)$).

FAB MS m/e 1411 ($2M^+$, 3%), 707 (MH^+, 26), 552 (MH^+- $C_{11}H_{23}$, 54), 468 (MH^+- $C_{16}H_{31}O$, 1), 367 (7), 313 (15), 154 (base peak).

Analysis Found: C, 62.96; H, 10.90; N, 1.98; P, 4.30%; $C_{38}H_{76}NO_8P$ requires C, 64.65; H, 10.85; N, 1.98; P, 4.39%; $C_{38}H_{76}NO_8PH_2O$ requires C, 63.04; H, 10.86; N. 1.93; P, 4.28%.

υ_{max}(CH_2Cl_2) 2918, 2852, 1735 (C=O), 1631, 1511, 1457, 1157, 1230 (P=O), 1167, 1065, 1041 cm^{-1}.

Kinetic Study of Water Mediated Hydrolysis of (85j).

Compound **(85j)** (0.29 g, 1.16 mmol) was dissolved in D_2O (2 mL) at ambient temperature and ^{31}P nmr spectra recorded at appropriate intervals over a period of 3 h; t=0, $\delta(D_2O)$ 25.11; t=180 min, $\delta(D_2O)$ 2.74. The reaction mixture was further diluted with water (10 mL) and then lyophilised to yield **(86j)** as a white solid (0.31 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.78 (3H, t, $C\underline{H}_3CH_2$), 1.33 (13H, m, $C\underline{H}_3CH$ and CH_2 x 5), 2.59 (3H, s, CH_3N), 3.04 (2H, m, NCH_2), 4.07 (3H, m, CH_2O and CHO), 10.02 (2H, s_b, NH_2).

^{31}P nmr $\delta(CDCl_3)$ -0.17.

^{13}C nmr $\delta(CDCl_3)$ 14.0 (C-1), 21.6 (d, $\underline{C}H_3CH$, $^3J_{C\text{-}P}$ =2.5 Hz), 22.6 (C-2), 25.3 (C-4), 29.3 (C-5), 31.8 (C-3), 33.6 (CH_3N), 37.9 (d, $R\underline{C}H_2CHO$, $^3J_{C\text{-}P}$ =5.9 Hz), 50.1 (d, NCH_2, $^3J_{C\text{-}P}$ =6.0 Hz), 60.7 (d, RCHO, $^2J_{C\text{-}P}$ =3.9 Hz), 73.1 (d, CH_2O, $^2J_{C\text{-}P}$ =6.1 Hz).

FAB MS m/e 268 (MH^+, 48%), 156 (43), 154 (M^+- C_8H_{17}, 5), 138 (M^+ - $C_8H_{17}O$, 10), 58 ($CH_3NCH_2CH_2^+$,<1), 53 (base peak).

Analysis Found: C, 49.47; H, 9.85; N, 5.19; P, 11.50%; $C_{11}H_{26}NO_4P$ requires C, 49.43; H, 9.80; N, 5.24; P, 11.59%.

$\upsilon_{max}(CH_2Cl_2)$ 2930, 2852, 2720, 2433, 1623, 1463, 1376, 1218 (P=O), 1074, 1044 cm^{-1}.

Water Mediated Hydrolysis of (85k).

Compound **(85k)** (0.27 g, 1.07 mmol) was dissolved in water (12 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised to yield the crude product as a yellow gum (0.29 g, 100%).

^{31}P nmr δ($CDCl_3$) 5.65 (minor impurity) and -3.02.

The crude product was purified by column chromatography on silica (20 g), eluting with 10-20% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(86k)** as a pale yellow gum (0.20 g, 69%).

^{1}H nmr δ($CDCl_3$) 0.88 (9H, t, $C\underline{H}_3CH_2$, $C\underline{H}_3CH$ and $C\underline{H}_3CH_2$), 1.33 (7H, m, $C\underline{H}$ and CH_2 x 3), 2.65 (3H, s, CH_3N), 3.04 (2H, m, NCH_2), 4.20 (3H, m, CH_2O and CHO).

^{31}P nmr δ($CDCl_3$) -0.95.

^{13}C nmr δ($CDCl_3$) 9.9 (m, $\underline{C}H_3CH_2CHO$),13.9 (m, $\underline{C}H_3CH$), 14.4 (m, $\underline{C}H_3CH_2CH_2$), 20.3 (m, $CH_3\underline{C}H_2CH_2$), 24.1 (m, $CH_3CH_2\underline{C}H_2$), 33.6 (CH_3N), 34.5 ($CH_3\underline{C}H_2CH$), 35.8 (m, $CH_3\underline{C}H$), 49.8 (d, NCH_2, $^{3}J_{C\text{-}P}$ =4.4 Hz), 61.1 (d, CHO, $^{2}J_{C\text{-}P}$ =3.2 Hz), 83.0, 83.3 (2d, CH_2O, diast.,1:1, $^{2}J_{C\text{-}P}$ =4.4 and 3.3 Hz).

FAB MS m/e 268 (MH^+, 25%), 156 (39), 138 (M^+- $C_8H_{17}O$, 4), 53 (base peak).

Analysis Found: C, 49.50; H, 9.87; N, 5.25; P, 11.55%; $C_{11}H_{26}NO_4P$ requires C, 49.43; H, 9.80; N, 5.24; P, 11.59%.

$\upsilon_{max}(CH_2Cl_2)$ 2947, 2708, 2433, 1604, 1460, 1376, 1218 (P=O), 1077 cm^{-1}.

Water Mediated Hydrolysis of (85l).

Compound **(85l)** (0.35 g, 1.40 mmol) was dissolved in water (15 mL) and stirred at ambient temperature for 4 h. The reaction mixture was then lyophilised yielding **(86l)** as a pale yellow gum (0.37 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.87 (9H, m, $C\underline{H}_3CH_2$ x 2 and CH_3CH), 1.36 (7H, m, CH_2 x 3 and $CH_3C\underline{H}$), 2.63 (3H, s, CH_3N), 3.09 (2H, m, NCH_2), 4.13 (3H, m, CH_2O and CHO), 10.09 (2H, s_b, NH_2).

^{31}P nmr $\delta(CDCl_3)$ -1.74.

^{13}C nmr $\delta(CDCl_3)$ 9.1 (m, $\underline{C}H_3CH_2CHO$), 11.1 (m, $\underline{C}H_3CH_2CH$), 19.2 (m, $\underline{C}H_3CH$), 28.6 ($CH_3\underline{C}H_2CHO$), 29.6 (m, $CH_3\underline{C}H_2CH$), 30.6 (m, $CH_3\underline{C}H$), 33.6 (CH_3N), 41.7 (m, $CH_3CH_2CH\underline{C}H_2$), 50.3 (d, NCH_2, $^3J_{C\text{-}P}$ =5.5 Hz), 60.8 (d, CHO, $^2J_{C\text{-}P}$ =2.5 Hz), 76.1, 76.2 (2d, CH_2O, diast., 3:2, $^2J_{C\text{-}P}$ =6.1 and 6.3 Hz).

FAB MS m/e 268 (MH^+, 10%), 156 (46), 138 (M^+- $C_8H_{17}O$, 4), 77 (11), 53 (base peak).

Analysis Found: C, 48.75; H, 9.88; N, 5.13; P, 11.40%; $C_{11}H_{26}NO_4P$ requires C, 49.43; H, 9.80; N, 5.24; P, 11.59%; $C_{11}H_{26}NO_4P[H_2O]_{0.2}$ requires C, 48.77; H, 9.82; N, 5.17; P, 11.43%.

$\upsilon_{max}(CH_2Cl_2)$ 2959, 2930, 2732, 2445, 1604, 1460, 1215 (P=O), 1074 cm^{-1}.

Water Mediated Hydrolysis of (85m).

Compound **(85m)** (0.50 g, 0.99 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 6 h. The reaction mixture was then lyophilised yielding **(86m)** as a cream coloured solid (0.51 g, 98%).

^{1}H nmr δ($CDCl_3$) 1.50 (42H, m, cholesteryl protons), 2.64 (3H, s, CH_3N), 3.10 (2H, m, NCH_2), 4.05 (3H, m, CH_2O and RCHO), 5.29 (1H, m, $C\underline{H}=CH_2$).

^{31}P nmr δ($CDCl_3$) 0.72.

^{13}C nmr δ($CDCl_3$) 11.8 (C-18), 18.7 (C-21), 19.3 (C-19), 21.0 (C-11), 22.6 (C-27), 22.8 (C-26), 23.8 (C-23), 24.3 (C-15), 28.0 (C-25), 28.2 (C-12), 29.7 (C-2), 31.7 (CH_3N), 31.8 (C-8), 35.8 (C-7), 36.2 (C-20), 36.8 (C-20), 39.5 (C-22/C-10), 39.7 (C-1/C-24), 39.9 (C-4), 42.3 (C-9/C-16), 49.9 (NCH_2), 50.0 (C-13), 56.1 (C-17), 56.6 (C-14), 60.2 (C-3, unresolved), 73.8 (CH_2O, unresolved), 122.8 (C-6), 140.0 (C-5).

FAB MS m/e 524 (MH^+, 6%), 369 (12), 213 (3), 178 (4), 173 (2), 172(2), 167 (3), 161 (5), 156 (base peak), 154 (23), 149 (11), 138 (11), 137 (12), 136 (21), 121 (14), 119 (10), 109 (12), 107 (22), 105 (22), 95 (26), 91 (29), 59 ($CH_3NH_2CH_2CH_2$, 9), 57 ($CH_3NCH_2CH_2^+$, 7).

Analysis Found: C, 65.53; H, 10.30; N, 2.84; P, 6.13%; $C_{30}H_{54}NO_4P$ requires C, 68.80; H, 10.39; N, 2.67; P, 5.91%; $C_{30}H_{54}NO_4P[H_2O]_{1.5}$ requires C, 65.42; H, 10.43; N, 2.54; P, 5.64%.

$\upsilon_{max}(CH_2Cl_2)$ 2936, 2864, 2720, 2445, 1619, 1463, 1379, 1218 (P=O), 1074, 1026 cm^{-1}.

Water Mediated Hydrolysis of (85n).

Compound **(85n)** (0.60 g, 1.44 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 8 h. The reaction mixture was then lyophilised yielding a white solid.

^{31}P nmr $\delta(CDCl_3)$ 16.21 and -5.09 (1.3 : 1.0).

The reaction mixture was resuspended in water (15 mL) and stirred at ambient temperature for 24 h. The reaction mixture was then lyophilised to yield **(86n)** as a white solid (0.62 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.25 (30H, m, CH_2 x 15), 1.37 (6H, s, CH_3 x 2), 1.58 (2H, $RC\underline{H}_2C(CH_3)_2$), 2.62 (3H, s, CH_3N), 3.07 (2H, m, NCH_2), 4.13 (2H, m, CH_2O).

^{31}P nmr $\delta(CDCl_3)$ -4.96.

^{13}C nmr $\delta(CDCl_3)$ 14.1 (C-1), 22.6 (C-2), 24.4 (C-16), 27.3 - 30.2 (m, C-4 to C-15 and C-17), 31.9 (CH_3N), 33.4 (C-3), 43.4 (d, CH_2 x 3, $^{3}J_{C-P}$ =5.1 Hz), 50.0 (d, NCH_2, $^{2}J_{C-P}$ =3.9 Hz), 70.7 (RCO, unresolved), 80.4 (d, CH_2O, $^{2}J_{C-P}$ =7.4 Hz).

FAB MS m/e 436 (MH^+, <1%), 349 (3), 291 (2), 236 (1), 156 (base peak), 154 (M^+-1,1-dimethyloctadecyl, 4), 138 (M^+- 1,1- dimethyloctadecylO, 4), 58 ($CH_3NHCH_2CH_2^+$, 58).

Analysis Found: C, 58.83; H, 11.48; N, 3.06; P, 6.85%; $C_{23}H_{50}NO_4P$ requires C, 63.41; H, 11.57; N, 3.22; P, 7.11%; $C_{23}H_{50}NO_4P[H_2O]_{2.0}$ requires C, 58.50; H, 11.54; N, 2.97; P, 6.57%.

$\upsilon_{max}(CH_2Cl_2)$ 2983, 2924, 2852, 2732, 2445, 1610, 1463, 1215 (P=O), 1068 cm^{-1}.

<u>Water Mediated Hydrolysis of (85o).</u>

Compound **(85o)** (0.29 g, 1.07 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 10 h. The reaction mixture was then lyophilised to yield a pale yellow solid.

^{31}P nmr $\delta(CDCl_3)$ 0.2 (minor impurity) and -5.6.

The crude product was purified by column chromatography on silica (15 g), eluting with 10-50% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(86o)** as a cream coloured solid (0.24 g, 77%).

^{1}H nmr $\delta(CD_3OD)$ 1.63 (6H, m, C-1, C-3, C-10), 2.01 (6H, m, C-5, C-7, C-9), 2.02 (3H, m, C-4, C-6, C-10), 2.68 (3H, s, CH_3N), 3.16 (2H, m, NCH_2), 4.02 (2H, m, CH_2O).

^{31}P nmr $\delta(CDCl_3)$ -5.25.

^{13}C nmr $\delta(CDCl_3)$ 30.9 (C-4, C-6 and C-8), 33.7 (CH_3N), 36.0 (C-5, C-7 and C-9), 43.5 (d, C-1, C-3 and C-10, $^{3}J_{C-P}$ =3.7 Hz), 50.5 (NCH_2), 61.0 (d, RCOP, $^{2}J_{C-P}$ =2.0 Hz), 77.3 (d, CH_2O, $^{2}J_{C-P}$ =3.1 Hz).

FAB MS m/e 312 (MNa^+, 2%), 290 (MH^+, 40), 154 (M^+- $C_{10}H_{15}$, 21), 149 (33), 138 (10), 137 (15), 136 (31), 135 (base peak), 58 ($CH_3NHCH_2CH_2^+$, 43).

Analysis Found: C, 53.90; H, 8.28; N, 4.71; P, 10.68%; $C_{13}H_{24}NO_4P$ requires C, 53.97; H, 8.36; N, 4.84; P, 10.71%.

$\upsilon_{max}(CH_2Cl_2)$ 2912, 2852, 2660, 2481, 1625, 1604, 1514, 1448, 1352, 1224 (P=O), 1053 cm^{-1}.

N-methylation of 3-methyl-2-octadecyloxy-1,3,2-oxazaphosphacyclo pentane 2-oxide (85d).

90% aqueous formic acid (0.20 mL, 4.5 mmol), followed by 37% aqueous formaldehyde (0.31 mL, 4.2 mmol) were added to compound **(85d)** (0.50 g, 1.29 mmol) in THF (10 mL) at 0 °C. The solution was heated to 80 °C for 30 h, and then neutralised by the addition of 0.5 M aqueous sodium hydroxide solution. The reaction mixture was then lyophilised and the resulting residue extracted with chloroform (100 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a yellow solid which was recrystallised from dichloromethane/acetone. The product was isolated as a cream coloured solid (0.5 g, 92%).

^1H nmr $\delta(CDCl_3)$ 0.81 (3H, t, $C\underline{H}_3CH_2$), 1.35 (20H, m, CH_2 x 10), 2.78 (6H, s, CH_3 x 2), 3.21 (2H, m, NCH_2), 3.93 (4H, m, CH_2O x 2).

^{31}P nmr $\delta(CDCl_3)$ -1.10.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 25.8 (C-16), 29.3-29.7 (C-4 to C-15), 30.8 (d, C-17, $^3J_{C-P}$ =7.9 Hz), 31.8 (C-3), 43.5 (NCH_2, unresolved), 57.5 (CH_3N), 59.7 (C-18, unresolved), 65.8 (d, CH_2O, $^2J_{C-P}$ =3.7 Hz).

FAB MS m/e 444 (MNa^+, 5%), 422 (MH^+, 7), 154 (7), 152 (M^+-$C_{18}H_{37}O$, 4), 68 (base peak).

Analysis Found: C, 55.93; H, 10.58; N, 2.46; P, 6.37%; $C_{22}H_{48}NO_4P$ requires C, 62.68; H, 11.48; N, 3.32; P, 7.35%; $C_{22}H_{48}NO_4P[H_2O]_{3.0}$ requires C, 55.55; H, 11.44; N, 2.94; P, 6.51%.

υ_{max}(CH_2Cl_2) 3673, 3591, 2919, 2843, 1668, 1604, 1464, 1227 (P=O), 1178, 1084, 1061 cm^{-1}.

CHAPTER 3

The Synthesis of Some Novel *t*-Butylethanolamine Phospholipid Analogues.

The Synthesis of Some Novel *t*-Butylethanolamine Phospholipid Analogues.

3.1. Introduction.

This chapter is concerned with the synthesis of novel phospholipid analogues with a *t*-butylethanolamine head group.

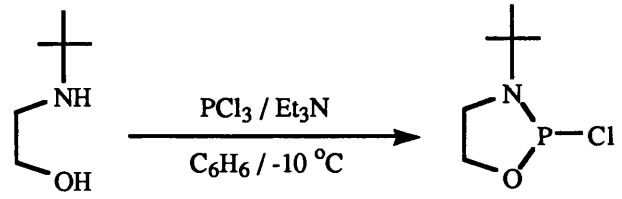
At the end of the previous chapter the N-methylation of an ethanolamine analogue was discussed. It was found that a choline analogue could not be obtained using the method described[142,143] and since the phosphoramidite methodology employed in our synthetic route can not be applied directly to the synthesis of choline analogues, it was decided to attempt to sterically mimic a choline functionality with a *t*-butyl group.

Primary, secondary and tertiary alcohols were successfully reacted with the *t*-butylethanolamine phosphitylating agent **(89)** to give the corresponding phosphite intermediates. These in turn were oxidised and then hydrolysed to the phospholipid analogues.

3.2. Results and Discussion.

3.2.1. Cyclisation Reaction.

The phosphitylating agent 3-*t*-butyl-2-chloro-1,3,2-oxazaphosphacyclopentane **(89)** was prepared by the low temperature reaction of phosphorus trichloride with 2-*t*-butylaminoethanol in the presence of triethylamine base.

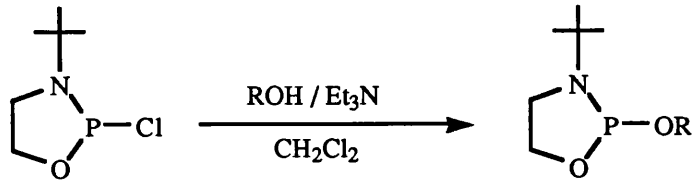


(89)

The product was obtained in a 63% yield following high vacuum distillation and characterised by nmr and FAB mass spectroscopy, the data being similar to that of the N-methyl phosphitylating agent **(79)**, with the exception of boiling point. That of the N-methyl phosphitylating agent **(79)** being 30-35 °C at 0.1 mmHg, compared with 64-68 °C at 0.08 mmHg for the *t*-butyl phosphitylating agent **(89)**. The signals in the ^{13}C nmr spectrum were assigned with reference to data on 2-methyl-1-heptylaminoethanol.[117] One of the most striking features seen in the ^{13}C nmr spectrum is the large magnitude of the three bond phosphorus coupling to the *t*-butyl group i.e. *ca* 12 Hz. The ^{31}P nmr shift at *ca* δ166 is very similar to that recorded for the N-methyl analogue **(79)**.

3.2.2. Condensation Reactions.

Compound **(89)** was reacted with a range of primary, secondary and tertiary alcohols. The alcohols, reaction temperatures, yields and ^{31}P nmr shifts of the corresponding phosphite triester products are shown in tables 8 and 9.



(89) **(90a-m)**

Table 8. Condensations with primary alcohols.

ROH (R=)	Temp. °C	Product	Yield%	δ ^{31}P nmr
n-C_6H_{13}	-60	**(90a)**	98	134.2
n-$C_{12}H_{25}$	-60	**(90b)**	100	134.1
n-$C_{18}H_{37}$	-40	**(90c)**	98	134.2
$\Delta^9C_{18}H_{35}$	-60	**(90d)**	99	134.3
Ethyl glycolate	-60	**(90e)**	99	136.8
Et-O-16	0	**(90f)**	100 impure	134.6, 10.3 (5:1)*
rac dipalm.	0	**(90g)**	100	136.2, 135.4 (4:3)*
sn dipalm.	0	**(90h)**	100	136.3, 135.5 (4:5)*

* nmr recorded in CH_2Cl_2 with D_2O centre lock, otherwise recorded in $CDCl_3$.

Table 9. Condensation with secondary and tertiary alcohols.

Alcohol	Temp. °C	Product	Yield%	δ ^{31}P nmr
d/l octan-2-ol	-60	**(90i)**	99	137.6, 137.1 (3:2)
l octan-2-ol	-60	**(90j)**	100	137.6, 137.1 (2:3)
cholesterol	-20	**(90k)**	98	136.3, 136.0 (2:3)*
1,1-dimethyl-octadecan-1-ol	-60	**(90l)**	100	130.2
adamantanol	-60	**(90m)**	100	131.1*

* nmr recorded in CH_2Cl_2 with D_2O centre lock, otherwise recorded in $CDCl_3$.

Compound **(89)** readily reacted with hexan-1-ol at low temperature to give the corresponding phosphite triester **(90a)** in 98% yield. The product was characterised by nmr spectroscopy, which was not dissimilar to the spectra recorded for the N-methyl analogue **(80a)**. Once again, most noticeable is the signal in the ^{31}P nmr spectrum which is much further upfield, i.e. δ134 than that noted for the phosphitylating agent **(89)**, i.e. δ166. The ^{13}C nmr spectrum contains six doublets due to either two or three bond phosphorus coupling. Noticeably, there is a reduction in the coupling constant for the methyl carbons of the *t*-butyl group, i.e. from *ca* 12 Hz in **(89)** to *ca* 9.5 Hz in **(90a)**. Whilst, the coupling constant for $Me_3\underline{C}$ has increased from 6.6 Hz in **(89)** to *ca* 13 Hz in **(90a)**.

In a similar manner, compound **(89)** was treated with dodecan-1-ol, octadecan-1-ol and oleyl alcohol to give the corresponding phosphite triesters **(90b-d)**, in high yields. The reaction with octadecan-1-ol was carried out at a slightly higher temperature of -40 ^{o}C on account of its poor solubility in dichloromethane at very low temperatures. Compounds **(90b)** and **(90c)**, like **(90a)**, were isolated using an aqueous partition procedure. However, compound **(90d)** was isolated by hexane extraction, to avoid any possible difficulties with emulsion formation.

To obtain the model ester analogue, ethyl glycolate was reacted with compound **(89)** at low temperature. The phosphite triester product **(90e)** was obtained in quantitative yield, using a non-aqueous isolation procedure. The ^{31}P nmr spectrum of the product confirmed its structure. A shift at δ136.8 was recorded, which not only corresponds to the shift recorded for the N-methyl analogue **(80f)**, but also supports the trend of downfield shift (*ca* 3 ppm) due to the electron withdrawing nature of the glycolyl moiety.

Application of the route to the synthesis of ALP analogues was again pursued by reacting compound **(89)** with alcohol **(84)** at 0 ^{o}C. A product isolated in high yield, following hexane extraction, was characterised by ^{31}P nmr spectroscopy. Two signals were observed at δ134.6 and 10.3 in a 5:1 ratio. The former shift represents the desired phosphite triester **(90f)**, whilst the latter is presumably a by-product arising from the hydrolysis of either the phosphitylating agent **(89)** or the product **(90f)**. A similar spectrum was observed for the corresponding N-methyl analogue **(80g)**, although there was a

greater amount of the undesired by-product formed in that reaction. Since this particular condensation reaction of the phosphitylating agent with this alcohol **(84)** was so problematic and since a reasonable amount of the desired product **(90f)** was present it was decided to continue the synthesis of the Et-O-16 phospholipid analogue in its crude form and then purify at a later stage.

The 1,2-dipalmitoyl-*sn*-glyceroloxy and 1,2-dipalmitoyl-*rac*-glyceroloxy phosphite triesters **(90g)** and **(90h)** were obtained by condensing compound **(89)** with the corresponding dipalmitoyl glycerols. Both phosphite triesters were isolated in quantitative yields, following hexane extraction, and characterised by ^{31}P nmr spectroscopy. Two signals, of almost identical shifts, due to diastereoisomerism were observed in the spectra of the phosphite triesters **(90g)** and **(90h)**, although the ratio of diastereoisomers varied from 4:3 in **(90g)** to 4:5 in **(90h)**.

The condensation reaction was applied successfully to both secondary and tertiary alcohols also. Thus, compound **(89)** was reacted with(+)/(-)octan-2-ol at low temperature to give the phosphite triester **(90i)**. The product was characterised by nmr spectroscopy and, as previously discussed in chapter 2, the presence of two chiral centres gave rise to diastereoisomerism, the effects of which were clearly visible in all of the nmr spectra recorded. Signals in the ^{13}C nmr spectrum were assigned with reference to ^{13}C nmr data for octan-2-ol.[117]

It was felt at this stage that it might be interesting to synthesise a similar phosphite product from(-) octan-2-ol and compare the nmr

spectra of the intermediates and later the possible biological activity of the two octanyloxy phospholipid analogues **(92i)** and **(92j)**. Thus in a similar manner to that above, the phosphite triester **(90j)** was prepared by the low temperature condensation of compound **(89)** with (-) octan-2-ol. The product was characterised by nmr spectroscopy. All signals were virtually identical to those recorded for **(90i)**, although the ratios of the diastereoisomers varied in both the ^{31}P and ^{13}C nmr spectra.

Compound **(89)** was reacted with cholesterol **(9)** at the slightly higher temperature of -20 ^{o}C, due to the alcohols poorer solubility in dichloromethane at very low temperatures. The phosphite triester **(90k)** was isolated in high yield following hexane extraction and characterised by ^{31}P nmr spectroscopy. Two signals, due to diastereoisomerism, were observed in a ratio of 2:3.

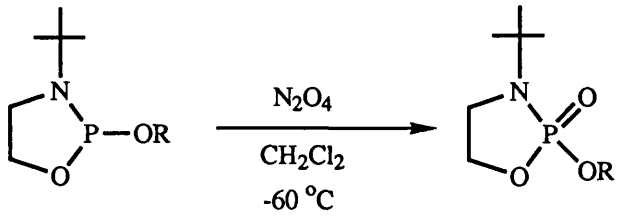
The dimethyloctadecyloxy analogue was obtained following the condensation of **(89)** with 1,1-dimethyloctadecan-1-ol at low temperature. A quantitative yield of the phosphite triester **(90l)** was obtained and characterised by nmr spectroscopy. The spectra recorded were similar to those recorded for the octadecyloxy phosphite triester **(90c)**, except that the carbon atoms adjacent to the phosphorus atom i.e. RCOP and cyclic CH_2OP were seen to be further downfield by *ca* 4.5 and 8.5 ppm respectively compared with that of RCH_2OP and cyclic CH_2OP in the octadecyloxy phosphite **(90c)**.

Compound **(89)** was condensed with adamantanol at low temperature. The phosphite triester **(90m)** was obtained in a

quantitative yield following hexane extraction and characterised by ^{31}P nmr spectroscopy. The shift observed at δ131.1 is consistent with that of the N-methyl analogue **(80o)**.

3.2.3. Oxidation Reactions.

The next stage of the synthetic route involved the dinitrogen tetroxide oxidation of the phosphite triesters **(90a-m)** to the corresponding phosphate triesters **(91a-m)**.



(90a-m) **(91a-m)**

Yields and ^{31}P nmr shifts for the phosphate triesters **(91a-m)** are shown in table 10.

All of the phosphate triesters were entirely pure by spectroscopy, with the exception of **(91f)** which was obtained from the oxidation of crude **(90f)**. Four signals were observed in the ^{31}P nmr spectrum of the crude phosphate triester **(91f)**. The signal at δ18.9 corresponds to the desired phosphate triester product, whilst the signals at δ9.7 and 7.4 are again presumably due to the presence of hydrolysed phosphitylating agent that arose during the condensation reaction. The signal at δ-1.0 is presumably the result of P-N cleavage of the phosphate triester **(91f)**.

Table 10. Oxidations.

Phosphate	Yield%	δ ^{31}P nmr ($CDCl_3$)
(91a)	98	16.1
(91b)	100	17.0
(91c)	100	16.3
(91d)	100	17.1
(91e)	100	17.4
(91f)	100 impure	18.9, 9.7, 7.4, -1.0 (4:1:1.5:5)
(91g)	100	17.2
(91h)	100	17.1
(91i)	100	16.9, 16.7 (3:2)
(91j)	100	16.9, 16.7 (4:5)
(91k)	100	16.4
(91l)	100	13.5
(91m)	100	13.5

The N-methyl phosphite intermediates containing a chiral centre had shown two signals in the ^{31}P nmr spectra, however, when oxidised to their corresponding phosphate triesters only one signal was observed in the ^{31}P nmr spectra, presumably due to overlapping of the signals. Here, the same effect is observed for the phosphates **(91g)**, **(91h)** and **(91k)**, whilst the octanyloxy phosphate

intermediates **(91i)** and **(91j)** continue to show two signals due to diastereoisomerism.

The structures of the phosphates **(91g)**, **(91h)**, **(91j)**, **(91l)** and **(91m)** were further supported by ^{1}H and ^{13}C nmr spectroscopy. The signals in the ^{13}C nmr spectra of **(91g)** and **(91h)** were assigned with reference to data for the methyl ester of hexadecanoic acid[140] and tributyrylglycerol.[117]

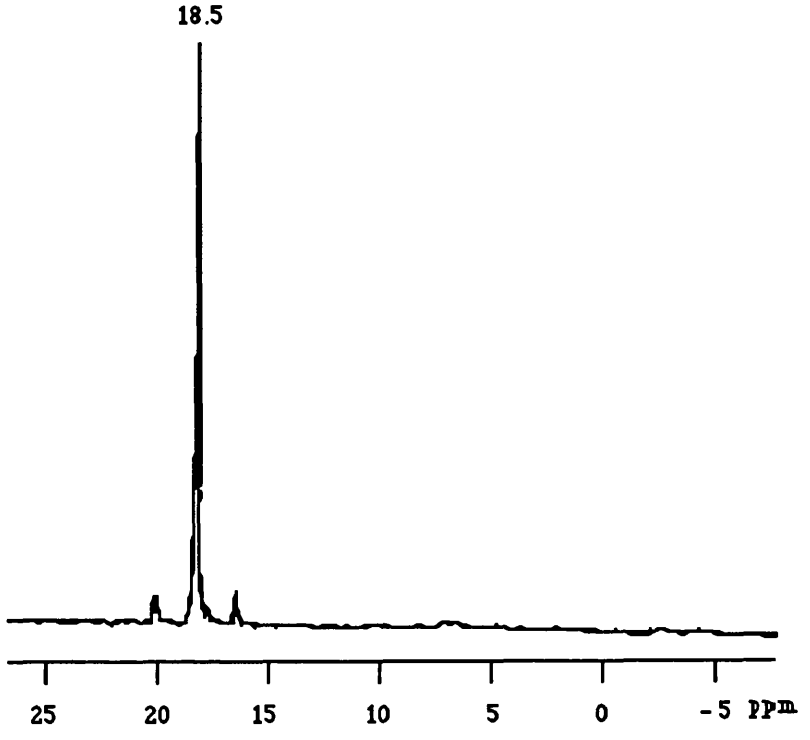
3.2.4. Hydrolysis Reactions.

In the previous chapter, it was revealed that the hydrolysis of the N-methyl phosphate compounds could be carried out successfully in the absence of acid at ambient temperature. However, it seemed likely that the *t*-butyl phosphates would hydrolyse less readily on steric grounds at least.

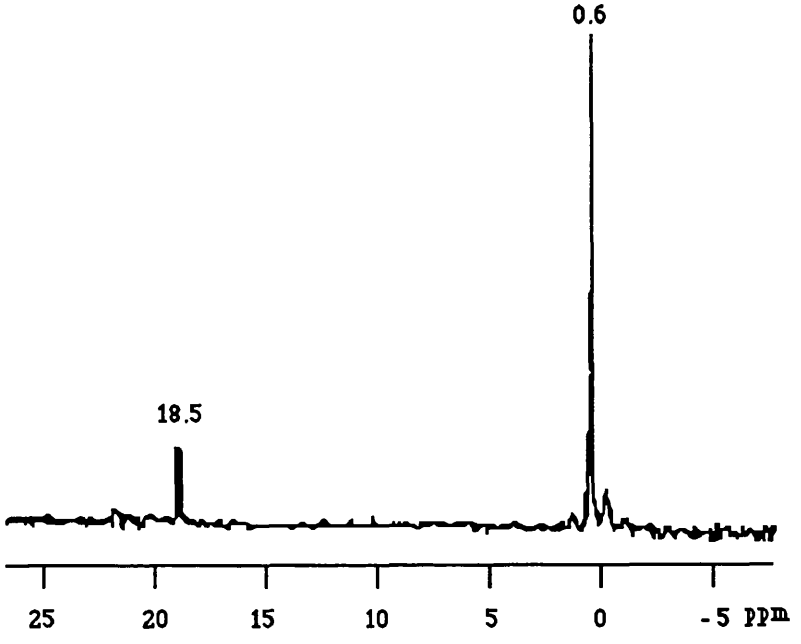
Thus the hydrolysis of **(91a)** was followed by ^{31}P nmr in D_2O at ambient temperature. The presence of cleaved product **(92a)** was noted after only 30 minutes, but the reaction was not complete until 8 hours had elapsed (refer to figure 13). This compares with complete hydrolysis of the corresponding N-methyl phosphate **(86a)** within 75 minutes.

Figure 13. ^{31}P nmr kinetic study of the hydrolysis of **(91a)**.

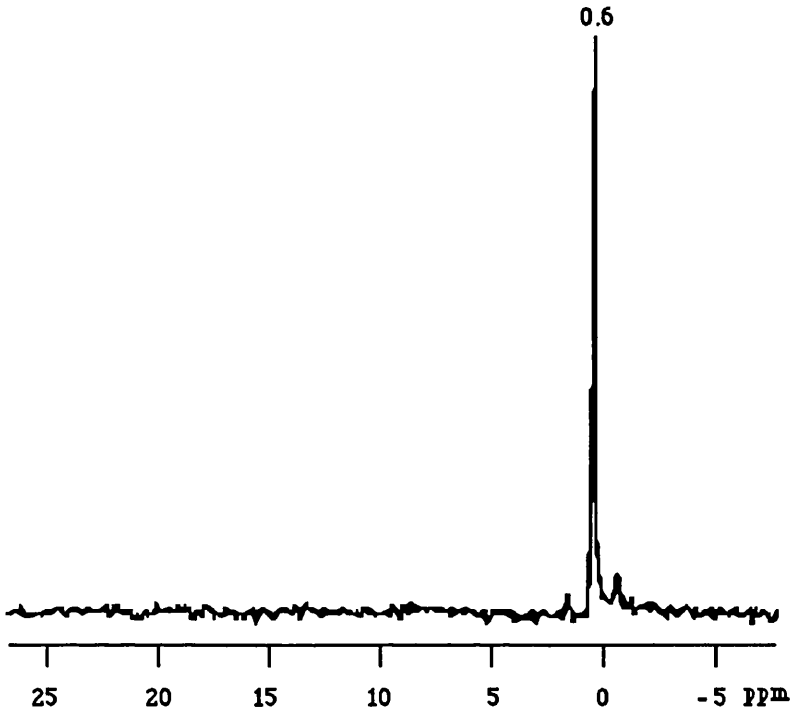
Time = 0 hours



Time = 7.5 hours



Time = 8 hours



Simple lyophilisation gave **(92a)** in a near quantitative yield, the product being pure by both spectroscopy and microanalysis. Thus **(92a)** displayed one signal in the ^{31}P nmr at *ca* δ-0.4, which is consistent with the cleavage of the P-N bond to yield a phosphate diester. The ^{1}H and ^{13}C nmr spectra (for details of the latter see table 11) are also informative. Phosphorus coupling to the carbon atoms of the *t*-butyl moiety is now entirely removed, whilst that to other neighbouring carbon atoms is retained. This is strong evidence of P-N cleavage rather than P-O cleavage. FAB mass spectrum also confirmed the structure of **(92a)** with peaks noted for the protonated molecular ion and its dimer.

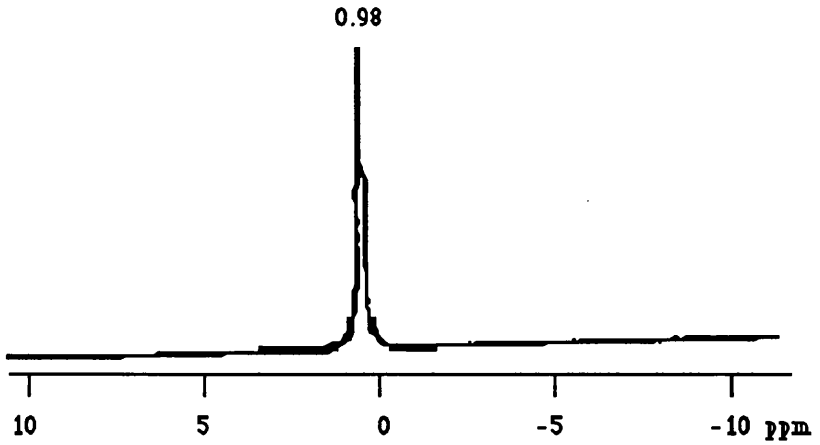
The dodecyloxy phosphate **(91b)** was similarly hydrolysed in water at ambient temperature for 8 hours. Lyophilisation gave **(92b)**

which was pure by spectroscopy but not by analysis. Pure **(92b)** was obtained following chromatography on silica, although the yield was somewhat reduced as a result of the chromatography.[68,102] The product was fully characterised and showed similar spectroscopic data to **(92a)**.

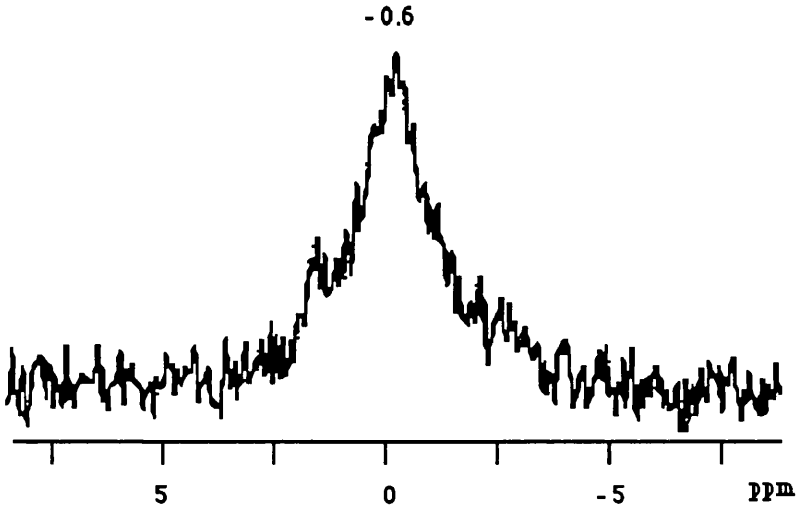
Similarly prepared were the octadecyloxy and oleyloxy phospholipid analogues **(92c)** and **(92d)**. The former hydrolysis was conducted for 8 hours, as above, whilst the latter was noted to be slower and was extended to 48 hours to ensure complete hydrolysis. Interestingly, both of these long chain compounds displayed very broad ^{31}P nmr resonances, suggestive of the formation of micelles or other organised structures[144] (refer to figure 14).

Figure 14. ^{31}P nmr spectra of compounds **(92b)** and **(92c)**, the latter showing the broad signal associated with micelle formation.

Compound **(92b)** δP ($CDCl_3$).



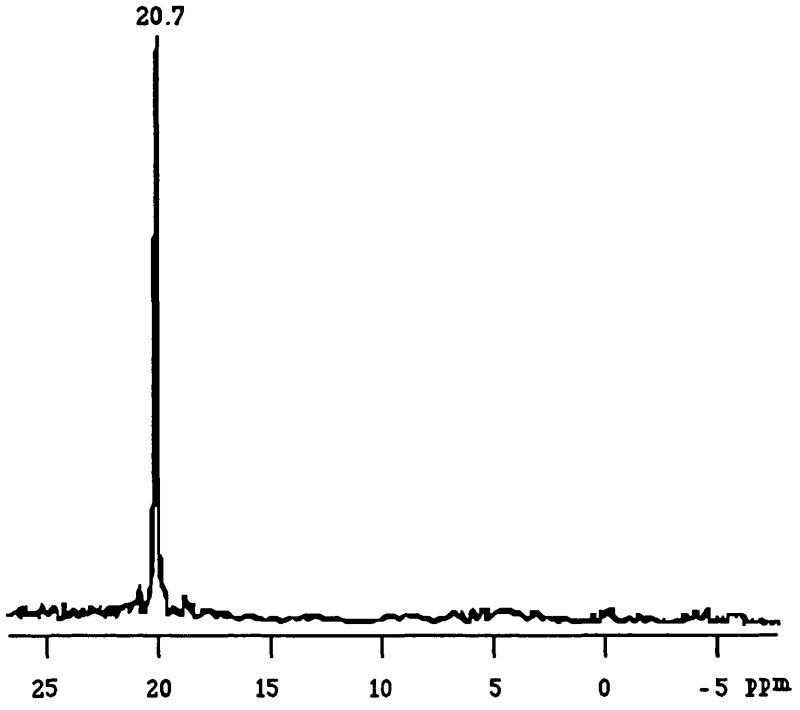
Compound **(92c)** δP ($CDCl_3$).



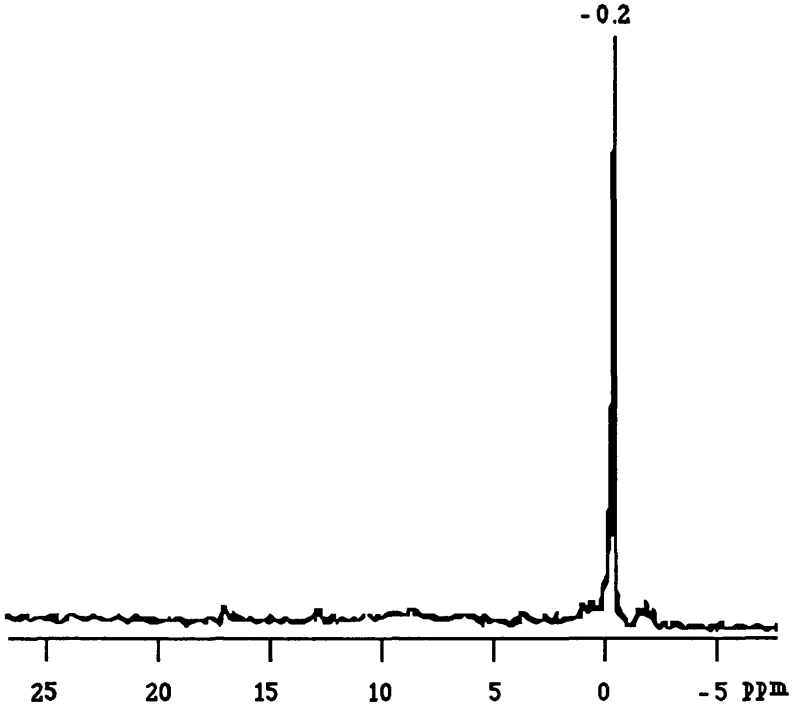
The ethyl glycolyl phosphate **(91e)** was hydrolysed to yield the product **(92e)**. The reaction was rapid, being followed by ^{31}P nmr spectroscopy, and found to be complete in under 1 hour (refer to figure 15).

Figure 15. ^{31}P nmr kinetic study of the hydrolysis of **(91e)**.

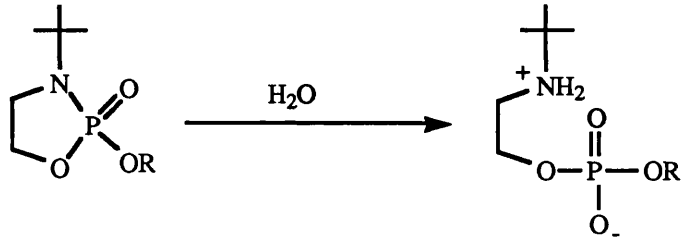
Time = 0 minutes



Time = 105 minutes



The product was fully characterised and displayed similar spectra to **(92a-d)**, although not suprisingly, apparent micelle formation was not noted for this short chain analogue.



(91a-e) **(92a-e)**

R

a= n-C_6H_{13}
b= n-$C_{12}H_{25}$
c= n-$C_{18}H_{37}$
d= $\Delta^9C_{18}H_{35}$
e= $CH_3CH_2OC(O)CH_2$

Table 11. ^{13}C nmr data for compounds (92a-e) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.

$CH_3CH_2CH_2(CH_2)_nCH_2CH_2CH_2OP(O)(OH)OCH_2CH_2NHCMe_3$

A B C D E F G H I J

Signal	(92a) n=0	(92b) n=6	(92c) n=12	(92d) n=12[a]	(92e)[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	14.1
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)	61.5 (4.0)
G	66.3 (5.9)	66.2 (6.2)	66.2 (5.2)	66.1 (4.4)	62.6 (4.6)
H	42.6 (1.5)	42.9[d]	42.5[d]	42.3 (4.9)	42.2 (4.2)
I	55.5	55.1	55.7	55.8	55.9
J	25.5	25.7	25.7	25.7	25.6

[a] Includes CH=CH δ129.9 and 129.7 and $CH_2CH{=}CHCH_2$ δ27.2

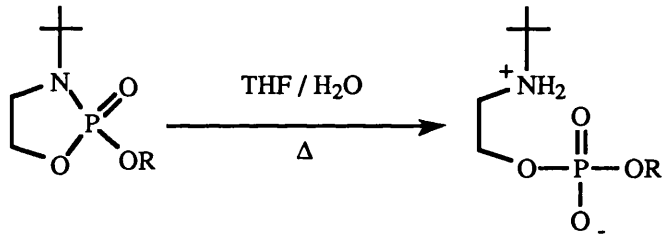
[b] $CH_3CH_2OC(O)CH_2OP$

C D E F

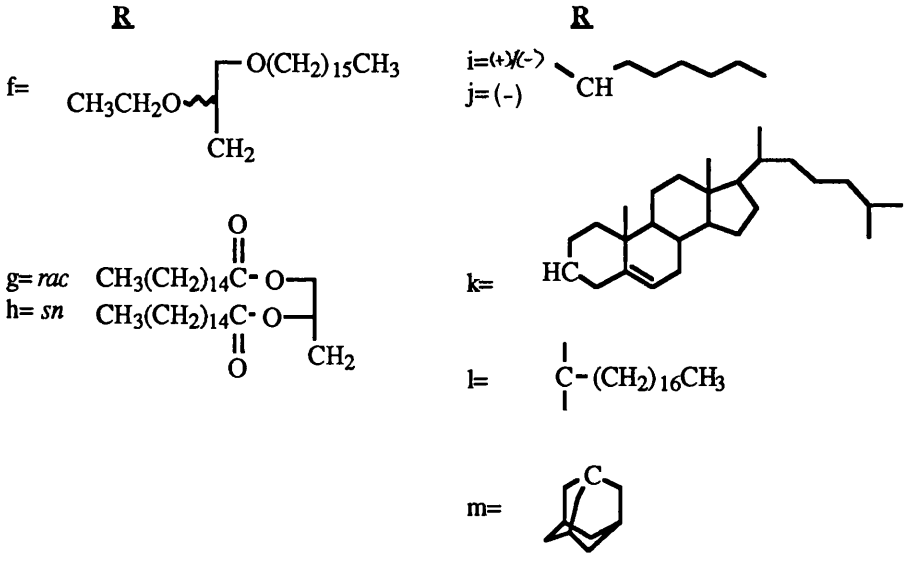
[c] Signal coincident with Me_3 δ25.7.

[d] Coupling not resolved, signal appears as a broad singlet.

Hydrolysis of the phosphate **(91f)** required the use of a co-solvent: THF, due to its poor solubility in water alone. Thus the crude phosphate was refluxed in a 1:1 mixture of THF and water for 10 hours, to ensure complete hydrolysis. The reaction mixture was then lyophilised and the crude product purified by chromatography, the yield of the product being 62%, predictably diminished by this purification step. The product was fully characterised with spectra supporting the proposed structure of the product. Signals in the ^{13}C nmr spectra of **(92f)** were assigned with reference to data for similar compounds and details of the former are shown later in table 13.

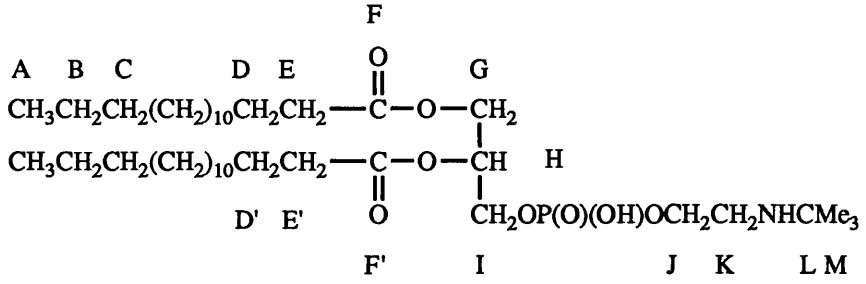


(91f-m) **(92f-m)**



Similarly, the dipalmitoyloxy phosphate intermediates **(91g)** and **(91h)** were hydrolysed by refluxing in a 1:1 mixture of THF and water over a period of 48 hours. The corresponding products **(92g)** and **(92h)** were obtained in quantitative yields following lyophilisation and characterised by spectroscopic and analytical methods. The data recorded was not dissimilar to that of the N-methyl dipalmitoyloxy analogues **(86h)** and **(86i)**. Table 12 shows some of the ^{13}C nmr data recorded for the two dipalmitoyloxy analogues. Signals were assigned with reference to ^{13}C nmr data for the methyl ester of hexadecanoic acid[140] and tributyrylglycerol.[117]

Table 12. ^{13}C nmr data for (92g) and (92h) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.



Signal	(92g)	(92h)
A	14.1	14.0
B	22.7	22.6
C	31.9	31.8
D	24.85	24.80
D'	24.90	24.90
E	34.05	34.00
E'	34.22	34.20
F	173.3	173.3
F'	173.0	172.9
G	62.4	62.4
H	64.2[a]	64.0[a]
I	61.2 (1.9)	62.1 (2.0)
J	70.0 (6.5)	70.0 (6.2)
K	45.6[a]	45.9[a]
L	55.9	55.3
M	25.7	25.6

[a] Coupling not resolved, signal appears as a broad singlet.

The octanyloxy phosphate intermediates **(91i)** and **(91j)** required much longer reaction times to complete their hydrolyses than the short chain primary analogues **(91a)** and **(91b)** which is presumably related to steric factors. The hydrolysis of **(91j)** was found to be incomplete after 18 hours at ambient temperature, so the reaction mixture was subjected to refluxing conditions to ensure complete hydrolysis. Compound **(91i)** required stirring at ambient temperature for 48 hours before the hydrolysis reaction was complete. Both products were characterised by nmr, infra-red, FAB mass spectroscopy and microanalysis. The data collected supports P-N bond cleavage under both sets of hydrolysis conditions. Some of the ^{13}C nmr spectral data is shown in table 13. Signals were assigned with reference to ^{13}C nmr data for n-hexadecane,[122] dihexyl ether,[117] 1-ethoxy-3-methyl-1,3-diphenylbutan-2-one[123] and 18-nonadecyl-1-ol[124] for compound **(92f)**, octan-2-ol,[117] for compounds **(92i)** and **(92j)**, cholestan-3-one[145] and cholest-5-en-3-ol,[117] for compound **(92k)**, decan-1-ol,[117] and octan-2-ol,[117] for compound **(92l)** and dichloroaminoadamantane[127] for compound **(92m)**.

The somewhat hindered phosphate intermediates **(91k)**, **(91l)** and **(91m)** were subjected to the more forcing hydrolysis conditions of refluxing in THF and water for long periods of time to ensure complete hydrolysis.

The dimethyloctadecyloxy phosphate **(91l)** seemed particularly resistant to hydrolysis taking *ca* 9 days to fully hydrolyse. Each of the phospholipid analogues **(92k)**, **(92l)** and **(92m)** were fully

characterised by nmr, infra-red, FAB mass spectroscopy and microanalysis.

Table 13. ^{13}C nmr data for compounds (92f), and (92i-m) in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are shown in parentheses.

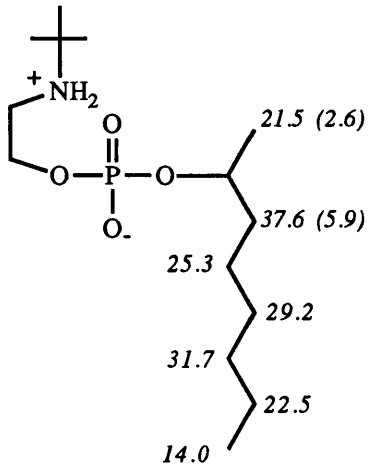
$RCH_nOP(O)(OH)OCH_2CH_2NHCMe_3$

F G H I J

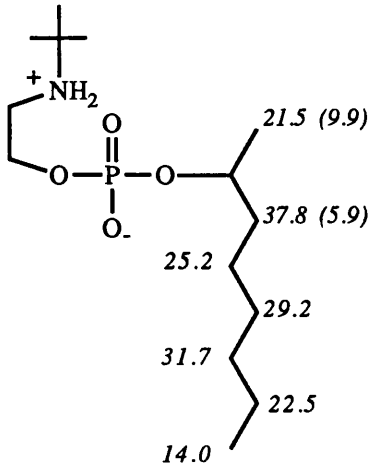
Signal	(92f) n=2	(92i) n=1	(92j) n=1	(92k) n=1	(92l) n=0	(92m) n=0
F	61.3 (4.3)	60.9[a]	61.2 (4.8)	62.6[a]	61.5[a]	62.2 (5.2)
G	74.2 (5.1)	73.2[a]	65.5 (6.0)	84.1 (8.6)	76.2 (5.2)	78.5 (7.9)
H	42.2 (6.0)	42.5[a]	42.3 (5.3)	43.2 (6.7)	49.9[a]	44.7 (3.5)
I	56.3	55.4	56.1	52.1	55.2	57.5
J	25.5	25.7	25.7	25.7	25.9	25.8

[a] Coupling not resolved, signal appears as a broad singlet.

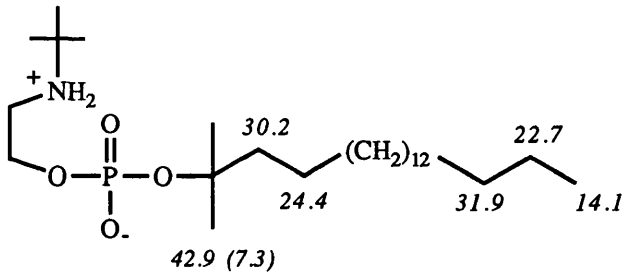
Figure 13. ^{13}C nmr data for compounds **(92i-j)** and **(92l)** in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are shown in parentheses.



(92i)



(92j)



(92l)

All of the phospholipid analogues were analysed by microanalysis and just like the N-methyl analogues the *t*-butyl phospholipids regularly showed deviations of associated water, despite thorough drying. This is a strong indication of their affinity for water.

Infra-red spectra of the analogues revealed the phosphate functionality in the range of *ca* 1230-1200 cm^{-1} and those containing ester linkages showed a strong signal for the carbonyl functionality in the range of *ca* 1750-1730 cm^{-1}.

3.3. Experimental.

3-*t*-Butyl-2-chloro-1,3,2-oxazaphosphacyclopentane (89).

Dry 2-*t*-butylaminoethanol (10.0 g, 0.085 mol) and triethylamine (30 mL, 0.21 mol) 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, 0.094 mol) in benzene (60 mL) was added dropwise. The reaction mixture was allowed to warm to ambient temperature with stirring for 2 h. The mixture was filtered, washing the residue with benzene (50 mL) and the filtrate evaporated under reduced pressure to give a yellow oil, which was vacuum distilled. The product was collected as a clear colourless oil (9.69 g, 63%), b.p. 64-68 °C, 0.08 mmHg.

^{1}H nmr δ($CDCl_3$) 1.33 (9H, m, CH_3 x 3), 3.12 (2H, m, NCH_2), 4.45 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 165.6.

^{13}C nmr δ($CDCl_3$) 29.0 (d, CH_3 x 3, $^3J_{C-P}$ =11.9 Hz), 41.8 (d, NCH_2, $^2J_{C-P}$ =8.1 Hz), 53.3 (d, $Me_3\underline{C}$, $^2J_{C-P}$ =6.6 Hz), 70.3 (d, CH_2O, $^2J_{C-P}$ =8.1 Hz).

FAB MS m/e 544 ($3MH^+$, 1.0%), 363 ($2MH^+$, 17), 182 (MH^+, 93), 136 (10), 126 (15), 118 (57), 100 (21), 89 (7), 62 (26), 58 (22), 57 (38), 44 (base peak).

3-*t*-Butyl-2-hexyloxy-1,3,2-oxazaphosphacyclopentane (90a).

Compound **(89)** (0.30 g, 1.65 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous 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 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 (2 x 50 mL). The solution was dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as an oil (0.40 g, 98%).

^{1}H nmr δ($CDCl_3$) 0.83 (3H, t, $C\underline{H}_3CH_2$), 1.29 (15H, m, CH_3 x 3 and CH_2 x 3), 1.51 (2H m, $C\underline{H}_2CH_2O$), 3.00 (2H, m, NCH_2), 3.66 (2H, m, RCH_2O), 4.29 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 134.2.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 25.5 (C-4), 29.9 (d, CH_3 x 3, $^3J_{C-P}$ =9.7 Hz), 31.4 (d, C-5, $^3J_{C-P}$ =4.2 Hz), 31.6 (C-3), 42.4 (d, NCH_2, $^2J_{C-P}$ =5.3 Hz), 51.7 (d, $Me_3\underline{C}$, $^2J_{C-P}$ =13.1 Hz), 62.7 (d, C-6, $^2J_{C-P}$ =12.2 Hz), 68.2 (d, CH_2O, $^2J_{C-P}$ =9.3 Hz).

3-*t*-Butyl-2-dodecyloxy-1,3,2-oxazaphosphacyclopentane (90b).

Compound **(89)** (0.30 g, 1.65 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous dodecan-1-ol (0.31 g, 1.65 mmol) and triethylamine (0.23 mL, 1.67 mmol) in dichloromethane (20 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 (30 mL), followed by saturated brine (2 x 30 mL). The solution was dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as an oil (0.55 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.24 (27H, m, CH_3 x 3 and CH_2 x 9), 1.50 (2H m, $C\underline{H}_2CH_2O$), 3.00 (2H, m, NCH_2), 3.67 (2H, m, RCH_2O), 4.25 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 134.1.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 25.9 (C-10), 28.9 - 29.6 (m, C-4 to C-9) 29.6 (d, CH_3 x 3, $^3J_{C\text{-}P}$ =9.6 Hz), 31.5 (d, C-11, $^3J_{C\text{-}P}$ =4.6 Hz), 31.9 (C-3), 42.4 (d, NCH_2, $^2J_{C\text{-}P}$ =5.4 Hz), 51.7 (d, $Me_3\underline{C}$, $^2J_{C\text{-}P}$ =13.5 Hz), 62.7 (d, C-12, $^2J_{C\text{-}P}$ =12.3 Hz), 68.2 (d, CH_2O, $^2J_{C\text{-}P}$ =9.1 Hz).

3-*t*-Butyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane (90c).

Compound (89) (0.20 g, 1.10 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous octadecan-1-ol (0.30 g, 1.10 mmol) and triethylamine (0.15 mL, 1.11 mmol) in dichloromethane (20 mL) at -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 (30 mL), followed by saturated brine (2 x 30 mL). The solution was dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as an oil (0.45 g, 98%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.26 (39H, m, CH_3 x 3 and CH_2 x 15), 1.55 (2H m, $C\underline{H}_2CH_2O$), 3.00 (2H, m, NCH_2), 4.10 (4H, m, RCH_2O and CH_2O).

^{31}P nmr δ($CDCl_3$) 134.2.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.8 (C-16), 28.9 - 29.6 (m, C-4 to C-15), 29.9 (d, CH_3 x 3, $^3J_{C\text{-}P}$ =9.6 Hz), 31.5 (d, C-17, $^3J_{C\text{-}P}$ =4.2 Hz), 31.9 (C-3), 42.4 (d, NCH_2, $^2J_{C\text{-}P}$ =5.3 Hz), 51.6 (d, $Me_3\underline{C}$, $^2J_{C\text{-}P}$ =13.0 Hz), 62.7 (d, C-18, $^2J_{C\text{-}P}$ =11.9 Hz), 68.2 (d, CH_2O, $^2J_{C\text{-}P}$ =9.1 Hz).

3-*t*-Butyl-2-oleyloxy-1,3,2-oxazaphosphacyclopentane (90d).

Compound **(89)** (0.20 g, 1.10 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous 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 solution was warmed to ambient temperature with stirring for 1 h and then the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a pale yellow oil (0.45 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.23 (33H, m, CH_3 x 3 and CH_2 x 12), 2.01 (4H m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.00 (2H, m, NCH_2), 3.80 (2H, m, RCH_2O), 4.20 (2H, m, CH_2O), 5.32 (2H, m, CH=CH).

^{31}P nmr δ($CDCl_3$) 134.3.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.9 (C-16), 27.2 (C-8 and C-11), 28.8 - 29.7 (m, C-4 to C-7 and C-12 to C-15), 29.9 (d, CH_3 x 3, $^3J_{C-P}$ =10.1 Hz), 31.5 (d, C-17, $^3J_{C-P}$ =4.3 Hz), 31.9 (C-3), 42.4 (d, NCH_2, $^2J_{C-P}$ =5.1 Hz), 51.5 (d, $Me_3\underline{C}$, $^2J_{C-P}$ =13.0 Hz), 62.7 (d, C-18, $^2J_{C-P}$ =12.1 Hz), 68.2 (d, CH_2O, $^2J_{C-P}$ =9.3 Hz), 129.77 (C-9 or C-10), 129.84 (C-9 or C-10).

3-*t*-Butyl-2-(ethoxycarbonyl)methoxy-1,3,2-oxazaphosphacyclopentane (90e).

Compound **(89)** (0.40 g, 2.20 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous ethyl glycolate (0.22 g, 2.16 mmol) and triethylamine (0.31 mL, 2.20 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.53 g, 99%).

^{31}P nmr δ($CDCl_3$) 136.8.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 29.8 (d, CH_3 x 3, $^3J_{C-P}$ =9.7 Hz), 42.1(d, NCH_2, $^2J_{C-P}$ =5.4 Hz), 51.8 (d, $Me_3\underline{C}$, $^2J_{C-P}$ =12.1 Hz), 60.1(d, $PO\underline{C}H_2C(O)$, $^2J_{C-P}$ =13.7 Hz), 60.9 (C-2), 68.4(d, CH_2O, $^2J_{C-P}$ =8.7 Hz), 170.4 (d, C=O, $^3J_{C-P}$ =3.9 Hz).

3-*t*-Butyl-2-(1-*O*-hexadecyl-2-*O*-ethyl-glycerol-*rac*-3-yloxy)-1,3,2-oxazaphosphacyclopentane (90f).

Compound **(89)** (0.08 g, 0.44 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous compound **(84)** (0.15 g, 0.44 mmol) and triethylamine (0.06 mL, 0.44 mmol) in dichloromethane (10 mL) at 0 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 2 h and the solvent then removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to yield a white solid (0.21 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 134.6 and 10.3 (5:1).

3-*t*-Butyl-2-(1,2-dipalmitoyl-*rac*-3-yloxy)-1,3,2-oxazaphospha cyclopentane (90g).

Compound **(89)** (0.05g, 0.27 mmol) in dichloromethane (6 mL) was added dropwise with vigorous stirring to anhydrous 1,2-dipalmitoyl-*rac*-glycerol (0.15 g, 0.26 mmol) and triethylamine (0.04 mL, 0.27 mmol) in dichloromethane (10 mL) at 0 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 2 h and the solvent then removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to yield the product **(90g)** as a white solid (0.19 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 136.2 and 135.4 (diastereoisomers 4:3).

3-*t*-Butyl-2-(1,2-dipalmitoyl-*sn*-3-yloxy)-1,3,2-oxazaphospha cyclopentane (90h).

This was prepared in an analogous manner to compound **(90g)** above. Thus from the condensation of compound **(89)** (0.05 g, 0.27 mmol) and anhydrous 1,2-dipalmitoyl-*sn*-glycerol (0.15 g, 0.26 mmol) was isolated the title compound **(90h)** (0.19 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 136.3 and 135.5 (diastereoisomers 4:5).

3-*t*-Butyl-2-(2-octanyloxy)-1,3,2-oxazaphosphacyclopentane (90i).

Compound **(89)** (0.20 g, 1.10 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous racemic octan-2-ol (0.14 g, 1.10 mmol) and triethylamine (0.15 mL, 1.11 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.30 g, 99%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.24 (22H, m, CH_2 x 5, $C\underline{H}_3CHO$ and CH_3 x 3), 3.00 (2H, m, NCH_2), 4.25 (3H, m, CH_2O and $CH_3C\underline{H}O$).

^{31}P nmr δ($CDCl_3$) 137.6 and 137.1 (diastereoisomers, 3:2).

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2CH_2$), 22.6 ($CH_3\underline{C}H_2CH_2$), 22.87, 22.92 (2d, $\underline{C}H_3CH$, diast., 1:1, $^3J_{C-P}$ =12.7 and 10.0 Hz), 25.6 (m, $CH_3CH_2CH_2CH_2\underline{C}H_2$), 29.2 (m, $CH_3CH_2CH_2\underline{C}H_2$), 29.8, 29.9 (2d, CH_3 x 3, diast., 1:1, $^3J_{C-P}$ =3.3 and 3.3 Hz), 31.8 ($CH_3CH_2\underline{C}H_2$), 38.5 (d, $R\underline{C}H_2CHO$ $^3J_{C-P}$ =5.2 Hz), 42.3, 42.4 (2d, NCH_2, diast., 1:1, $^3J_{C-P}$ =4.8 and 4.7 Hz), 51.7, 51.9 (2d, $Me_3\underline{C}$, diast., 4:5, $^2J_{C-P}$ =6.0 and 5.8 Hz), 67.5, 67.6 (2d, RCHO, diast., 4:5, $^2J_{C-P}$ =8.2 and 8.6 Hz), 69.8, 70.3 (2d, CH_2O, diast., 4:5, $^2J_{C-P}$ =16.9 and 18.3 Hz).

3-*t*-Butyl-2-(2-octanyloxy)-1,3,2-oxazaphosphacyclopentane (90j).

Compound **(89)** (0.15 g, 0.83 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous *l*-octan-2-ol (0.11 g, 0.83 mmol) and triethylamine (0.12 mL, 0.84 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.23 g, 100%).

^{31}P nmr δ($CDCl_3$) 137.6 and 137.1 (diastereoisomers, 2:3).

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2CH_2$), 22.6 ($CH_3\underline{C}H_2CH_2$), 22.8, 22.9 (2d, $\underline{C}H_3CH$, diast., 4:5, $^3J_{C-P}$ =12.8 and 10.1 Hz), 25.6 (m, $CH_3CH_2CH_2CH_2\underline{C}H_2$), 29.1 (m, $CH_3CH_2CH_2\underline{C}H_2$), 29.8, 29.9 (2d, CH_3 x 3, diast., 1:1, $^3J_{C-P}$ =3.5 and 3.2 Hz), 31.8 ($CH_3CH_2\underline{C}H_2$), 38.5 (d,

R$\underline{C}H_2CHO$, $^3J_{C\text{-}P}$ =5.4 Hz), 42.3, 42.4 (2d, NCH_2, diast., 1:1, $^3J_{C\text{-}P}$ =4.5 and 4.9 Hz), 51.7, 51.9 (2d, $Me_3\underline{C}$, diast., 2:3, $^2J_{C\text{-}P}$ =6.4 and 6.0 Hz), 67.5, 67.6 (2d, RCHO, diast., 4:5, $^2J_{C\text{-}P}$ =8.3 and 8.7 Hz), 69.8, 70.3 (2d, CH_2O, diast., 4:5, $^2J_{C\text{-}P}$ =16.6 and 18.2 Hz).

3-*t*-Butyl-2-cholesteryloxy-1,3,2-oxazaphosphacyclopentane (90k).

Compound **(89)** (0.15 g, 0.83 mmol) in dichloromethane (15 mL) was added dropwise with vigorous stirring to anhydrous cholesterol (0.32 g, 0.83 mmol) and triethylamine (0.12 mL, 0.84 mmol) in dichloromethane (15 mL) at -20 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h and then the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white solid (0.43 g, 98%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 136.3 and 136.0 (diastereoisomers, 2:3).

3-*t*-Butyl-2-(1,1-dimethyloctadecyloxy)-1,3,2-oxazaphosphacyclopentane (90l).

Compound **(89)** (0.15 g, 0.83 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous 1,1-dimethyloctadecan-1-ol (0.25 g, 0.83 mmol) and triethylamine (0.12 mL, 0.83 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane

(80 mL) and the extract evaporated under reduced pressure to give a colourless oil (0.37 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.32 (47H, m, CH_3 x 2, CH_2 x 16 and CH_3 x 3), 3.00 (2H, m, NCH_2), 4.21 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 130.2.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 24.1 (C-16), 28.7-29.7 (m, C-4 to C-15), 29.8 (d, CH_3 x 3, $^3J_{C\text{-}P}$ =7.8 Hz), 30.1 (C-17), 31.9 (C-3), 41.7 (d, CH_3 x 2, $^2J_{C\text{-}P}$ =6.2 Hz), 44.2 (d, NCH_2, $^2J_{C\text{-}P}$ =5.3 Hz), 51.8 (d, $Me_3\underline{C}$, $^2J_{C\text{-}P}$ =9.4 Hz), 67.2 (d, COP, $^2J_{C\text{-}P}$ =7.5 Hz), 76.6 (d, CH_2O, $^2J_{C\text{-}P}$ =4.8 Hz).

2-Adamantanyloxy-3-*t*-butyl-1,3,2-oxazaphosphacyclopentane (90m).

Compound **(89)** (0.20 g, 1.10 mmol) in dichloromethane (12 mL) was added dropwise with vigorous stirring to anhydrous adamantan-1-ol (0.17 g, 1.10 mmol) and triethylamine (0.15 mL, 1.11 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give a colourless oil (0.33 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 131.1.

3-*t*-Butyl-hexyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91a).

A portion of standard dinitrogen tetroxide solution (3.0 mL, containing 0.48 mmol of oxidant, sufficient to oxidise 1.90 mmol of phosphite) was added dropwise with vigorous stirring to compound **(90a)** (0.40 g, 1.62 mmol) in dichloromethane (10 mL) at -60 °C. The solution was warmed to ambient temperature with stirring for 30 mins and the solvent removed under reduced pressure to yield the product as a pale yellow oil (0.42 g, 98%).

^{31}P nmr $\delta(CDCl_3)$ 16.1.

3-*t*-Butyl-dodecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91b).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90b)** (0.55 g, 1.65 mmol) was isolated the title compound **(91b)** (0.57 g, 100%).

^{31}P nmr $\delta(CDCl_3)$ 17.0.

3-*t*-Butyl-octadecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91c).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90c)** (0.50 g, 1.20 mmol) was isolated the title compound **(91c)** (0.52 g, 100%).

^{31}P nmr $\delta(CDCl_3)$ 16.3.

3-*t*-Butyl-oleyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91d).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90d)** (0.45 g, 1.09 mmol) was isolated the title compound **(91d)** (0.47 g, 100%).

^{31}P nmr δ($CDCl_3$) 17.1.

3-*t*-Butyl-(ethoxycarbonyl)-methoxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91e).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90e)** (0.28 g, 1.12 mmol) was isolated the title compound **(91e)** (0.29 g, 100%).

^{31}P nmr δ($CDCl_3$) 17.4.

3-*t*-Butyl-2-(1-*O*-hexadecyl-2-*O*-ethyl-glycerol-*rac*-3-yloxy)-1,3,2-oxazaphosphacyclopentane 2-oxide (91f).

This was prepared by an analogous manner to **(91a)** above. Thus from crude compound **(90f)** (0.21 g, 0.43 mmol) was isolated the title compound **(91f)** in a crude form (0.22 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 18.9, 9.7, 7.4 and -1.0 (4:1:1.5:5).

3-*t*-Butyl-2-(1,2-dipalmitoyl-*rac*-3-yloxy)-1,3,2-oxazaphospha cyclopentane 2-oxide (91g).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90g)** (0.19 g, 0.27 mmol) was isolated the title compound **(91g)** (0.19 g, 100%).

^{1}H nmr δ(CDCl$_3$) 0.87 (6H, t, C$\underline{H}_3$CH$_2$ x 2), 1.29 (48H, m, CH$_2$ x 24), 1.42 (9H, s, CH$_3$ x 3), 1.59 (4H, m, OC(O)CH$_2$C$\underline{H}_2$ x 2), 2.29 (4H, m, OC(O)C$\underline{H}_2$ x 2), 3.13 (2H, m, NCH$_2$), 4.04 (2H, m, CH$_2$OC(O)), 4.25 (4H, m, CH$_2$O x 2 cyclic and chain), 5.25 (1H, m, OCH$_2$C$\underline{H}$CH$_2$O).

^{31}P nmr δ(CDCl$_3$) 17.2.

^{13}C nmr δ(CDCl$_3$) 14.1 (C$\underline{H}_3$CH$_2$ x 2), 22.7 (CH$_3$C$\underline{H}_2$ x 2), 24.8 (CH$_2$OC(O)CH$_2$C$\underline{H}_2$), 25.5 (CHOC(O)CH$_2$C$\underline{H}_2$), 29.0-29.7 (m, CH$_2$ x 20 and CH$_3$ x 3), 31.9 (CH$_3$CH$_2$C$\underline{H}_2$ x 2), 34.01 (CH$_2$OC(O)C$\underline{H}_2$), 34.03 (CHOC(O)C$\underline{H}_2$), 43.1 (d, NCH$_2$, $^2J_{C\text{-}P}$ =17.0 Hz), 57.1 (Me$_3$C), 61.9 (d, RCH$_2$O, $^2J_{C\text{-}P}$ =2.5 Hz), 62.07 (C$\underline{H}_2$OC(O)), 62.12 (C$\underline{H}$OC(O)), 69.7 (d, CH$_2$O, $^2J_{C\text{-}P}$ =8.1 Hz), 173.0 (CHOC(O)), 173.3 (CH$_2$OC(O)).

FAB MS m/e 652 (1%), 551 (M$^+$- $C_{35}H_{67}O_4$, 9), 198 (Me$_3$CNH$_2$CH$_2$CH$_2$OP(OH)$_2$O, 28), 154 (10), 136 (14), 121 (27), 109 (13), 107 (CH$_2$CH$_2$OPO$_2$, 22), 105 (10), 100 (base peak).

3-*t*-Butyl-2-(1,2-dipalmitoyl-*sn*-3-yloxy)-1,3,2-oxazaphospha-cyclopentane 2-oxide (91h).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90h)** (0.19 g, 0.27 mmol) was isolated the title compound **(91h)** (0.19 g, 100%).

^{1}H nmr δ(CDCl$_3$) 0.86 (6H, t, C$\underline{H}_3$CH$_2$ x 2), 1.28 (57H, m, CH$_2$ x 24 and CH$_3$ x 3), 1.56 (4H, m, OC(O)CH$_2$C$\underline{H}_2$ x 2), 2.29 (4H, m, OC(O)C$\underline{H}_2$ x 2), 3.27 (2H, m, NCH$_2$), 4.19 (6H, m, CH$_2$OC(O) and CH$_2$O x 2 cyclic and chain), 5.24 (1H, m, OCH$_2$C$\underline{H}$CH$_2$O).

^{31}P nmr δ($CDCl_3$) 17.1.

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2$ x 2), 22.7 ($CH_3\underline{C}H_2$ x 2), 24.8 ($CH_2OC(O)CH_2\underline{C}H_2$), 25.5 ($CHOC(O)CH_2\underline{C}H_2$), 29.0-29.7 (m, CH_2 x 20 and CH_3 x 3), 31.9 ($CH_3CH_2\underline{C}H_2$ x 2), 34.0 ($CH_2OC(O)\underline{C}H_2$), 34.2 ($CHOC(O)\underline{C}H_2$), 43.1(d, NCH_2, $^2J_{C\text{-}P}$ =17.2 Hz), 56.8 ($Me_3\underline{C}$), 61.8 (d, RCH_2O, $^2J_{C\text{-}P}$ =3.0 Hz), 62.1 ($\underline{C}H_2OC(O)$), 63.2 ($\underline{C}HOC(O)$), 69.6 (d, CH_2O, $^2J_{C\text{-}P}$ =8.0 Hz), 172.9 ($CHO\underline{C}(O)$), 173.3 ($CH_2O\underline{C}(O)$).

3-*t*-Butyl-2-(2-octanyloxy)-1,3,2-oxazaphosphacyclopentane 2-oxide (91i).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90i)** (0.23 g, 0.84 mmol) was isolated the title compound **(91i)** (0.24 g, 100%).

^{31}P nmr δ($CDCl_3$) 16.9 and 16.7 (diastereoisomers, 3:2).

3-*t*-Butyl-2-(2-⊖-octanyloxy) -1,3,2-oxazaphosphacyclopentane 2-oxide (91j).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90j)** (0.25 g, 0.91 mmol) was isolated the title compound **(91j)** (0.26 g, 100%).

^{31}P nmr δ($CDCl_3$) 16.9 and 16.7 (diastereoisomers, 4:5).

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 21.6 (d, $\underline{C}H_3CHO$, $^3J_{C\text{-}P}$ =4.5 Hz), 22.5 (C-2), 25.2 (m, C-5), 28.2 (C-4), 29.2 (CH_3 x 3), 31.7 (C-3), 37.4, 37.5 (2d, C-6, diast., 2:3, $^3J_{C\text{-}P}$ =4.5 and 3.7 Hz), 43.0, 43.3 (2d, NCH_2, diast., 4:5, $^2J_{C\text{-}P}$ =2.2 and 1.9 Hz), 52.1, 52.2 (2d, $Me_3\underline{C}$, diast.,1:1, $^2J_{C\text{-}P}$ =5.0 and 5.0 Hz),

62.7 (CHOP, unresolved), 75.4, 75.5 (2d, CH_2O, diast., 4:5, $^2J_{C-P}$ =7.1 and 7.3 Hz).

3-*t*-Butyl-2-cholesteryloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (91k).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90k)** (0.28 g, 0.53 mmol) was isolated the title compound **(91k)** (0.29 g, 100%).

^{31}P nmr δ($CDCl_3$) 16.4.

^{13}C nmr δ($CDCl_3$) 11.8 (C-18), 18.7 (C-21), 19.3 (C-19), 21.0 (C-11), 22.6 (C-27), 22.8 (C-26), 23.8 (C-23), 24.2 (C-15), 25.7 (C-25), 28.0 (C-12), 28.2 (d, CH_3 x 3, $^3J_{C-P}$ =2.8 Hz), 29.6 (C-7), 29.7 (C-2), 31.8 (C-8), 35.8 (C-20), 36.1 (C-22), 36.4 (C-10), 36.9 (C-1), 39.4 (C-24), 39.5 (C-16), 39.7 (C-9), 42.3 (C-13), 43.1 (2d, NCH_2, $^2J_{C-P}$ =17.0 Hz), 49.9 (C-4), 52.2 (d, $Me_3\underline{C}$, $^2J_{C-P}$ =4.5 Hz), 56.1 (C-17), 56.6 (C-14), 62.9 (C-3, unresolved), 77.1 (d, CH_2O, $^3J_{C-P}$ =9.2 Hz), 122.7 (C-6), 139.6 (C-5).

3-*t*-Butyl-2-(1,1-dimethyloctadecyloxy)-1,3,2-oxazaphosphacyclopentane 2-oxide (91l).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90l)** (0.36 g, 0.81 mmol) was isolated the title compound **(91l)** (0.37 g, 100%).

^{31}P nmr δ($CDCl_3$) 13.5.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 24.2 (C-16), 27.3 - 29.7 (m, C-4 to C-15), 29.1 (d, CH_3 x 3, $^3J_{C-P}$ =3.1 Hz), 29.9 (C-17), 31.9 (C-3), 43.1 (d,

CH_3 x 2, $^2J_{C\text{-}P}$ =5.4 Hz), 43.2 (d, NCH_2, $^2J_{C\text{-}P}$ =6.0 Hz), 52.1 (d, $Me_3\underline{C}$, $^2J_{C\text{-}P}$ =4.9 Hz), 62.6 (COP, unresolved), 84.1 (d, CH_2O, $^2J_{C\text{-}P}$ =8.8 Hz).

FAB MS m/e 482 (MNa^+, 6%), 202 (75), 186 (15), 180 (base peak), 164 (20), 146 (14), 124 (33), 121 (11), 105 (11), 100 ($Me_3CNHCH_2CH_2$, 1).

υ_{max}**(CH_2Cl_2)** 3054, 2984, 2925, 2849, 1259 (P=O), 1075 cm^{-1}.

2-Adamantanyloxy-3-*t*-Butyl-1,3,2-oxazaphosphacyclopentane 2-oxide (91m).

This was prepared by an analogous manner to **(91a)** above. Thus from compound **(90m)** (0.33 g, 1.11 mmol) was isolated the title compound **(91m)** (0.35 g, 100%).

^{31}P nmr δ($CDCl_3$) 13.5.

^{13}C nmr δ($CDCl_3$) 28.1 (d, CH_3 x 3, $^3J_{C\text{-}P}$ =3.0 Hz), 31.0 (C-4, C-6 and C-8), 35.8 (C-5, C-7 and C-9), 42.9 (d, C-1, C-3 and C-10, $^3J_{C\text{-}P}$ =16.2 Hz), 43.4 (d, NCH_2, $^2J_{C\text{-}P}$ =4.0 Hz), 52.1 (d, $Me_3\underline{C}$, $^2J_{C\text{-}P}$ =4.3 Hz), 62.6 (RCO), 80.9 (d, CH_2O, $^2J_{C\text{-}P}$ =8.7 Hz).

FAB MS m/e 314.1917 (MH^+ calc. for $C_{16}H_{29}NO_3P$, 314.1885, 0.9%), 299 (62), 178 (M^+- $C_{10}H_{15}$, 3), 164 (base peak), 162 (M^+- $C_{10}H_{15}O$, 4), 99 ($Me_3CNCH_2CH_2$, 3).

Water Mediated Hydrolysis of (91a).

Compound **(91a)** (0.69 g, 2.62 mmol) was dissolved in D_2O (2 mL) at ambient temperature and ^{31}P nmr spectra were recorded at appropriate intervals over a period of 8 h; t=0 δ(D_2O) 18.5; t=8 h

$\delta(D_2O)$ 0.6. The reaction mixture was then lyophilised to give a white solid (0.72 g, 98%).

^{1}H nmr $\delta(CDCl_3)$ 0.81 (3H, t, $C\underline{H}_3CH_2$), 1.41 (17H, m, CH_2 x 4 and CH_3 x 3), 3.05 (2H, m, NCH_2), 3.85 (2H, m, RCH_2O), 4.19 (2H, m, CH_2O).

^{31}P nmr $\delta(CDCl_3)$ -0.4.

^{13}C nmr $\delta(CDCl_3)$ 14.0 (C-1), 22.5 (C-2), 25.2 (C-4), 25.5 (CH_3 x 3), 30.5 (d, C-5, $^3J_{C\text{-}P}$ =7.0 Hz), 31.4 (C-3), 42.6 (d, NCH_2, $^3J_{C\text{-}P}$ =1.5 Hz), 55.5 ($Me_3\underline{C}$), 61.0 (d, C-6, $^2J_{C\text{-}P}$ =4.6 Hz), 66.3 (d, CH_2O, $^2J_{C\text{-}P}$ =5.9 Hz).

FAB MS m/e 563 ($2MH^+$, 3%), 282 (MH^+, 44), 266 (M^+- CH_3, 1), 118 (MH^+- $C_6H_{13}OPO_2$, 1), 101 (M^+- $C_6H_{13}OPO_3$, 2), 99 ($Me_3CNCH_2CH_2$, 20), 57 (Me_3C^+, 15), 44 ($NH_2CH_2CH_2$, base peak).

Analysis Found: C, 49.17; H, 9.83; N, 4.94%; $C_{12}H_{28}NO_4P$ requires C, 51.23; H, 10.03; N, 4.98%; $C_{12}H_{28}NO_4P$ $[H_2O]_{0.5}$ requires C, 49.64; H, 10.07; N, 4.82%.

$\upsilon_{max}(CH_2Cl_2)$ 3037, 2947, 2930, 2858, 2642, 1613, 1463, 1376, 1200 (P=O), 1065, 1020 cm^{-1}.

<u>Water Mediated Hydrolysis of (91b).</u>

Compound **(91b)** (0.57 g, 1.64 mmol) was suspended in water (12 mL) and stirred at ambient temperature for 8 h. The reaction mixture was then lyophilised to give a cream coloured solid.

^{31}P nmr $\delta(CDCl_3)$ 1.40 (minor impurity) and -0.03.

This was further purified by column chromatography on silica (50 g) eluting with 20% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(92b)** as a white solid (0.42 g, 71%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.39 (29H, m, CH_2 x 10 and CH_3 x 3), 3.08 (2H, m, NCH_2), 3.88 (2H, m, RCH_2O), 4.25 (2H, m, CH_2O).

^{31}P nmr δ($CDCl_3$) 0.98.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.7 (CH_3 x 3 and C-10), 29.3 - 29.6 (m, C-4 to C-9), 30.6 (d, C-11, $^3J_{C-P}$ =7.5 Hz), 31.8 (C-3), 42.9 (NCH_2), 55.1 ($Me_3\underline{C}$), 60.8 (d, C-12, $^2J_{C-P}$ =4.1 Hz), 66.2 (d, CH_2O, $^2J_{C-P}$ =6.2 Hz).

FAB MS m/e 731 ($2MH^+$,1%), 366 (MH^+, 56), 350 (M^+- CH_3, 1), 101 (M^+- $C_{12}H_{25}OPO_3$, 0.5), 99 ($Me_3CNCH_2CH_2$, 2), 57 (Me_3C^+, 14), 44 ($NH_2CH_2CH_2$, base peak).

Analysis Found: C, 58.54; H, 10.60; N, 3.67; $C_{18}H_{40}NO_4P$ requires C, 59.15; H, 11.03; N, 3.83%; $C_{18}H_{40}NO_4P$ $[H_2O]_{0.3}$ requires C, 58.29; H, 11.03; N, 3.78%.

υ_{max}(CH_2Cl_2) 2924, 2846, 2642, 1463, 1376, 1200 (P=O), 1065, 1026 cm^{-1}.

<u>Water Mediated Hydrolysis of (91c).</u>

Compound **(91c)** (0.52 g, 1.21 mmol) was suspended in water (20 mL) and stirred at ambient temperature for 8 h. The reaction mixture was then lyophilised to give a white solid.

^{31}P nmr δ($CDCl_3$) -0.82 (major) and -4.20 (minor impurity).

This was further purified by column chromatography on silica (50 g) eluting with 15% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(92c)** as a white solid (0.35 g, 64%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.37 (41H, m, CH_2 x 16 and CH_3 x 3), 3.10 (2H, m, NCH_2), 3.87 (2H, m, RCH_2O), 4.20 (2H, m, CH_2O), 10.0 (2H, s_b, NH_2).

^{31}P nmr δ($CDCl_3$) -0.6.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.7 (CH_3 x 3), 25.8 (C-16), 29.1-29.7 (m, C-4 to C-15), 30.7 (d, C-17, $^{3}J_{C-P}$ =7.4 Hz), 31.9 (C-3), 42.5 (NCH_2), 55.7 ($Me_3\underline{C}$), 61.0 (d, C-18, $^{2}J_{C-P}$ =4.1 Hz), 66.2 (d, CH_2O, $^{2}J_{C-P}$ =5.2 Hz).

FAB MS m/e 450 (MH^+, 10%), 101 (M^+- $C_{18}H_{37}OPO_3$, 1), 99 ($Me_3CNCH_2CH_2$, 1), 57 (Me_3C^+, 37), 44 ($NH_2CH_2CH_2$, base peak).

Analysis Found: C, 62.18; H, 11.25; N, 2.92%; $C_{24}H_{52}NO_4P$ requires C, 64.11; H, 11.66; N, 3.11%; $C_{24}H_{52}NO_4PH_2O$ requires C, 61.64; H, 11.64; N, 2.99%.

υ_{max}(CH_2Cl_2) 2917, 2849, 1464, 1219 (P=O), 1063, 1026 cm^{-1}.

<u>Water Mediated Hydrolysis of (91d).</u>

Compound **(91d)** (0.47 g, 1.09 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 48 h. The reaction mixture was then lyophilised to give a cream coloured solid.

^{31}P nmr δ($CDCl_3$) 7.60, 1.60 (minor impurities) and 0.50 (major product).

This was further purified by column chromatography on silica (40 g) eluting with 10% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(92d)** as a waxy solid (0.34 g, 70%).

^{1}H nmr δ($CDCl_3$) 0.84 (3H, t, $C\underline{H}_3CH_2$), 1.33 (33H, m, CH_2 x 12 and CH_3 x 3), 1.97 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.07 (2H, m, NCH_2), 3.85 (2H, m, RCH_2O), 4.20 (2H, m, CH_2O), 5.31 (2H, m, CH=CH).

^{31}P nmr δ($CDCl_3$) 0.5.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.7 (CH_3 x 3), 25.8 (C-16), 27.2 (C-8 and C-11), 28.9-29.8 (m, C-4 to C-7, C-12 to C-15), 30.7 (d, C-17, $^{3}J_{C-P}$ =7.4 Hz), 31.8 (C-3), 42.3 (d, NCH_2, $^{3}J_{C-P}$ =4.9 Hz), 55.8 ($Me_3\underline{C}$), 61.0 (d, C-18, $^{2}J_{C-P}$ =4.4 Hz), 66.1 (d, CH_2O, $^{2}J_{C-P}$ =4.4 Hz), 129.7 (C-9 or C-10), 129.9 (C-9 or C-10).

FAB MS m/e 448 (MH^+, 1%), 57 (Me_3C^+, 1), 56 (base peak), 44 ($NH_2CH_2CH_2$, 53).

Analysis Found: C, 60.23; H, 10.62; N, 3.15; P, 6.70%; $C_{24}H_{50}NO_4P$ requires C, 64.40; H, 11.26; N, 3.13; P, 6.92%; $C_{24}H_{50}NO_4P[H_2O]_{1.5}$ requires C, 60.73; H, 11.26; N, 2.95; P, 6.53%.

υ_{max}(CH_2Cl_2) 2925, 2849, 1613, 1461, 1373, 1221, 1201 (P=O), 1081, 1067, 1026 cm^{-1}.

Kinetic Study of Water Mediated Hydrolysis of (91e).

Compound **(91e)** (0.56 g, 2.11 mmol) was dissolved in D_2O (2 mL) at ambient temperature and ^{31}P nmr spectra were recorded at appropriate intervals over a period of 105 min; t=0 min $\delta P(D_2O)$ 20.7; t=105 min; $\delta P(D_2O)$ -0.22. The reaction mixture was diluted with water (5 mL) and then lyophilised to yield **(92e)** as a white solid (0.58 g, 97%).

^{1}H nmr $\delta(CDCl_3)$ 1.23 (3H, t, $C\underline{H}_3CH_2$), 1.35 (9H, s, CH_3 x 3), 3.13 (2H, m, NCH_2), 4.20 (4H, m, RCH_2O and $CH_3C\underline{H}_2O$), 4.45 (2H, m, CH_2O).

^{31}P nmr $\delta(CDCl_3)$ -3.1.

^{13}C nmr $\delta(CDCl_3)$ 14.0 (C-1), 25.6 (CH_3 x 3), 42.2 (d, NCH_2, $^3J_{C-P}$ =4.2 Hz), 55.9 ($Me_3\underline{C}$), 60.8 (C-2), 61.5 (d, RCH_2O, $^2J_{C-P}$ =4.0 Hz), 62.6 (d, CH_2O, $^2J_{C-P}$ =4.6 Hz), 169.8 (d, C=O, $^3J_{C-P}$ =7.9 Hz).

FAB MS m/e 284 (MH^+, 25%), 270 (2), 136 (8), 118 (MH^+-$CH_3CH_2CO_2PO_2$, 1), 100 (44), 99 ($Me_3CNCH_2CH_2$, 1), 57 (Me_3C^+, 1), 45 (base peak).

Analysis Found: C, 39.66; H, 7.68; N, 4.73; P, 10.23%; $C_{10}H_{22}NO_6P$ requires C, 42.40; H, 7.83; N, 4.94; P, 10.93%; $C_{10}H_{22}NO_6PH_2O$ requires C, 39.87; H, 8.03; N, 4.65; P, 10.28%.

$\upsilon_{max}(CH_2Cl_2)$ 2966, 2931, 2651, 1753 (C=O), 1613, 1379, 1215 (P=O), 1078, 1026 cm^{-1}.

Water Mediated Hydrolysis of (91f).

Compound **(91f)** (0.22 g, 0.44 mmol) was dissolved in THF/water (1:1; 12 mL) and refluxed for 10 h. The reaction mixture was then lyophilised to yield a cream-white solid. This was further purified by column chromatography on silica (15 g) eluting with chloroform followed by 10-30% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(92f)** as a white solid (0.139g, 62%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3(CH_2)_{15}$), 1.18 (29H, m, CH_2 x 13 and $C\underline{H}_3CH_2O$), 1.39 (9H, s, CH_3 x 3), 1.60 (2H, m, $CH_3(CH_2)_{13}C\underline{H}_2CH_2$), 3.15 (2H, m, NCH_2), 3.85 (9H, m, CH_2O x 2, CH_2OR and $C\underline{H}OC\underline{H}_2CH_3$).

^{31}P nmr δ($CDCl_3$) -3.1.

^{13}C nmr δ($CDCl_3$) 14.1 ($\underline{C}H_3CH_2CH_2$), 15.6 ($\underline{C}H_3CH_2O$), 22.7 ($CH_3\underline{C}H_2CH_2$), 25.6 ($CH_2OCH_2\underline{C}H_2$), 25.7 ($CH_3$ x 3), 26.1-29.7 (m, C-4 to C-14), 31.9 (C-3), 42.3 (d, NCH_2, $^3J_{C\text{-}P}$ =5.5 Hz), 56.1 ($Me_3\underline{C}$), 61.2 (d, $POCH_2$, $^2J_{C\text{-}P}$ =4.8 Hz), 65.5 (d, CH_2O, $^2J_{C\text{-}P}$ =6.0 Hz), 65.5 (d, $CH_3\underline{C}H_2O$, $^2J_{C\text{-}P}$ =4.6 Hz), 70.5 ($CH_2O\underline{C}H_2$), 71.7 ($\underline{C}H_2OCH_2$), 77.6 (d, $\underline{C}HOCH_2CH_3$, $^3J_{C\text{-}P}$ =3.6 Hz).

FAB MS m/e 524 (MH^+, 12%), 313 (MH^+- $C_{15}H_{31}$, 7), 122 (2), 100 (base peak).

Analysis Found: C, 60.90; H, 11.08; N, 2.60; P, 5.85%; $C_{27}H_{58}NO_6P$ requires C, 61.92; H, 11.16; N, 2.67; P, 5.91%; $C_{27}H_{58}NO_6P[H_2O]_{0.5}$ requires C, 60.87; H, 11.16; N, 2.63; P, 5.81%.

$\upsilon_{max}(CH_2Cl_2)$ 2924, 2852, 1619, 1457, 1376, 1320, 1224, 1203 (P=O), 1083 cm^{-1}.

Water Mediated Hydrolysis of (91g).

Compound **(91g)** (0.19 g, 0.26 mmol) was dissolved in THF/water (1:1; 16 mL) and refluxed for 48 h. The reaction mixture was then lyophilised to yield a white solid (0.19 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.86 (6H, t, $C\underline{H}_3CH_2$ x 2), 1.24 (48H, m, CH_2 x 24), 1.38 (9H, s, CH_3 x 3), 1.57 (4H, m, $OC(O)CH_2C\underline{H}_2$ x 2), 2.28 (4H, m, $OC(O)C\underline{H}_2$ x 2), 3.10 (2H, m, NCH_2), 4.10 (2H, m, $CH_2OC(O)$), 4.30 (4H, m, $CH\underline{2}O$ x 2), 5.21 (1H, m, $CH_2C\underline{H}CH_2$), 9.76 (2H, s_b, NH_2).

^{31}P nmr $\delta(CDCl_3)$ 2.3.

^{13}C nmr $\delta(CDCl_3)$ 14.1 ($\underline{C}H_3CH_2$ x 2), 22.7 ($CH_3\underline{C}H_2$ x 2), 24.8 ($CH_2OC(O)CH_2\underline{C}H_2$), 24.9 ($CHOC(O)CH_2\underline{C}H_2$), 25.7 ($CH_3$ x 3), 29.1-29.7 (m, CH_2 x 20), 31.9 ($CH_3CH_2\underline{C}H_2$ x 2), 34.1 ($CH_2OC(O)\underline{C}H_2$), 34.2 ($CHOC(O)\underline{C}H_2$), 42.6 ($NCH_2$), 55.9 ($Me_3\underline{C}$), 61.2 (d, RCH_2O, $^2J_{C\text{-}P}$ =1.9 Hz), 62.4 ($\underline{C}H_2OC(O)$), 64.2 ($C\underline{H}OC(O)$), 70.0 (d, CH_2O, $^2J_{C\text{-}P}$ =6.5 Hz), 173.0 ($CHO\underline{C}(O)$), 173.3 ($CH_2O\underline{C}(O)$).

FAB MS m/e 749 (MH^+, 19%), 552 (MH^+- $C_{14}H_{29}$, 26), 367 (5), 313 (6), 238 (4), 198 (29), 154 (16).

Analysis Found: C, 64.18; H, 11.06; N, 1.90; P, 4.00%; $C_{41}H_{82}NO_8P$ requires C, 65.83; H, 11.05; N, 1.87; P, 4.14%; $C_{41}H_{82}NO_8PH_2O$ requires C, 64.28; H, 11.05; N, 1.83; P, 4.04%.

$\upsilon_{max}(CH_2Cl_2)$ 2924, 2852, 1735, 1613, 1460, 1376, 1230 (P=O), 1197, 1065 cm^{-1}.

Water Mediated Hydrolysis of (91h).

Compound **(91h)** (0.19 g, 0.26 mmol) was dissolved in THF/water (1:1; 12 mL) and refluxed for 90 h. The reaction mixture was then lyophilised to yield a white solid (0.19 g, 100%).

^{1}H nmr δ(CDCl$_3$) 0.81 (6H, t, C$\underline{H}_3$CH$_2$ x 2), 1.19 (48H, m, CH$_2$ x 24), 1.33 (9H, s, CH$_3$ x 3), 1.52 (4H, m, OC(O)CH$_2$C$\underline{H}_2$ x 2), 2.22 (4H, m, OC(O)C$\underline{H}_2$ x 2), 3.07 (2H, m, NCH$_2$), 3.95 (2H, m, CH$_2$OC(O)), 4.20 (4H, m, C$\underline{H}_2$O x 2), 5.16 (1H, m, CH$_2$C$\underline{H}$CH$_2$), 9.78 (2H, s$_b$, NH$_2$).

^{31}P nmr δ(CDCl$_3$) 2.0.

^{13}C nmr δ(CDCl$_3$) 14.0 (C$\underline{H}_3$CH$_2$ x 2), 22.6 (CH$_3$C$\underline{H}_2$ x 2), 24.8 (CH$_2$OC(O)CH$_2$C$\underline{H}_2$), 24.9 (CHOC(O)CH$_2$C$\underline{H}_2$), 25.6 (CH$_3$ x 3), 29.1-29.6 (m, CH$_2$ x 20), 31.8 (CH$_3$CH$_2$C$\underline{H}_2$ x 2), 34.0 (CH$_2$OC(O)C$\underline{H}_2$), 34.2 (CHOC(O)C$\underline{H}_2$), 45.9 (NCH$_2$), 55.3 (Me$_3$C), 61.1 (d, CH$_2$OP, $^2J_{C\text{-}P}$ =2.0 Hz), 62.4 (C$\underline{H}_2$OC(O)), 64.0 (C$\underline{H}$OC(O)), 70.0 (d, CH$_2$O, $^2J_{C\text{-}P}$ =6.2 Hz), 172.9 (CHOC(O)), 173.2 (CH$_2$OC(O)).

FAB MS m/e 749 (MH$^+$, 8%), 552 (MH$^+$- C$_{14}$H$_{29}$, 21), 313 (5), 198 (22), 124 (6), 100 (base peak).

Analysis Found: C, 64,12; H, 11.18; N, 2.05; P, 4.30%; $C_{41}H_{82}NO_8P$ requires C, 65.83; H, 11.05; N, 1.87; P, 4.14%; $C_{41}H_{82}NO_8P[H_2O]_{3.0}$ requires C, 63.86; H, 11.50; N, 1.82; P, 4.02%.

$\upsilon_{max}(CH_2Cl_2)$ 3049, 2918, 2852, 1732, 1607, 1460, 1293, 1233, 1200 (P=O), 1173, 1065 cm^{-1}.

Water Mediated Hydrolysis of (91i).

Compound **(91i)** (0.24 g, 0.82 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 48 h. The reaction mixture was then lyophilised to yield **(92i)** as a white solid (0.25 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.84 (3H, t, $C\underline{H}_3CH_2$), 1.42 (22H, m, CH_2 x 5, $C\underline{H}_3CHO$ and CH_3 x 3), 3.10 (2H, m, NCH_2), 4.26 (3H, m, CH_2O and $CH_3C\underline{H}O$), 9.59 (2H, s_b, NH_2).

^{31}P nmr $\delta(CDCl_3)$ -2.1.

^{13}C nmr $\delta(CDCl_3)$ 14.0 (C-1), 21.5 (d, C-8, $^3J_{C\text{-}P}$ =2.6 Hz), 22.5 (C-2), 25.3 (C-5), 25.5 (CH_3 x 3), 29.2 (C-4), 31.7 (C-3), 37.6 (d, C-6, $^3J_{C\text{-}P}$ =5.9 Hz), 42.2 (d, NCH_2, $^3J_{C\text{-}P}$ =6.0 Hz), 56.3 ($Me_3\underline{C}$), 61.3 (d, RCHO, $^2J_{C\text{-}P}$ =4.3 Hz), 74.2 (d, CH_2O, $^2J_{C\text{-}P}$ =5.1 Hz).

FAB MS m/e 332 (MNa^+, 2%), 310 (MH^+, 4), 239 (MH^+- C_5H_{11}, 2), 198 (4), 121 (7), 107 (3), 100 (51), 77 (9), 73 (Me_3CNH_2, 2), 45 (base peak).

Analysis Found: C, 48.96; H, 10.08; N, 4.30; P, 9.78%; $C_{14}H_{32}NO_4P$ requires C, 54.35; H, 10.43; N, 4.53; P, 10.01%; $C_{14}H_{32}NO_4P[H_2O]_{2.0}$ requires C, 48.68; H, 10.51; N, 4.05; P, 8.97%.

$\upsilon_{max}(CH_2Cl_2)$ 3623, 3378, 2930, 2774, 1616, 1463, 1376, 1227, 1203 (P=O), 1083, 1012 cm^{-1}.

Water Mediated Hydrolysis of (91j).

Compound **(91j)** (0.18 g, 0.62 mmol) was suspended in water (15 mL) and stirred at ambient temperature for 18 h.

^{31}P nmr δ(H_2O/D_2O centre lock) 16.9, 16.2 and 0.78 (product).

The reaction mixture was diluted with THF (2 mL) and refluxed for 24 h. The reaction mixture was then lyophilised to yield **(92j)** as a white solid (0.14 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.79 (3H, t, $C\underline{H}_3CH_2$), 1.34 (22H, m, CH_2 x 5, $C\underline{H}_3CHO$ and CH_3 x 3), 3.02 (2H, m, NCH_2), 4.18 (3H, m, CH_2O and $CH_3C\underline{H}O$), 10.0 (2H, s_b, NH_2).

^{31}P nmr δ(H_2O/THF/D_2O centre lock) 0.4.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 21.5 (d, C-8, $^{3}J_{C\text{-}P}$ =9.9 Hz), 22.5 (C-2), 25.2 (C-5), 25.7 (CH_3 x 3), 29.2 (C-4), 31.7 (C-3), 37.8 (d, C-6, $^{3}J_{C\text{-}P}$ =5.9 Hz), 42.5 (s_b, NCH_2), 55.4 ($Me_3\underline{C}$), 60.9 (s_b, CHOP), 73.2 (s_b, CH_2O).

FAB MS m/e 310 (MH^+, 8%), 239 (MH^+- C_5H_{11}, 5), 121 (10), 99 ($Me_3CNCH_2CH_2$, 4), 73 (Me_3CNH_2, 1), 45 (base peak).

Analysis Found: C, 50.56; H, 9.89; N, 4.24; P, 9.57%; $C_{14}H_{32}NO_4P$ requires C, 54.35; H, 10.43; N, 4.53; P, 10.01%; $C_{14}H_{32}NO_4P[H_2O]_{1.3}$ requires C, 50.53; H, 10.48; N, 4.21; P, 9.31%.

υ_{max}(CH_2Cl_2) 3052, 2978, 2305, 1614, 1421, 1274, 1250, 1201 (P=O), 1078, 1033 cm^{-1}.

Water Mediated Hydrolysis of (91k).

Compound **(91k)** (0.20 g, 0.36 mmol) was suspended in THF/water (1:2; 6 mL) and refluxed for 96 h. The reaction mixture was then lyophilised to yield **(92k)** as a cream coloured solid (0.2 g, 100%).

^{1}H nmr δ($CDCl_3$) 1.52 (51H, m, cholesteryl protons and CH_3 x 3), 3.07 (2H, m, NCH_2), 4.10 (3H, m, RCHO and CH_2O), 5.30 (1H, m, C=CH), 10.15 (2H, s_b, NH_2).

^{31}P nmr δ($CDCl_3$).1.37.

^{13}C nmr δ($CDCl_3$) 11.8 (C-18), 18.7 (C-21), 19.3 (C-19), 21.0 (C-11), 22.5 (C-27), 22.8 (C-26), 23.8 (C-23), 24.3 (C-15), 25.9 (CH_3 x 3)), 28.0 (C-25), 28.2 (C-12), 29.8 (d, C-2, $^3J_{C\text{-}P}$ =3.5 Hz), 31.8 (C-8), 35.8 (C-7), 36.2 (C-20), 36.5 (C-10/C-22), 39.5 (C-1), 39.7 (C-24), 40.3 (d, C-4, $^3J_{C\text{-}P}$ =4.0 Hz), 42.3 (C-9/C-16), 49.9 (NCH_2, unresolved), 50.0 (C-13), 55.2 ($Me_3\underline{C}$), 56.1 (C-17), 56.7 (C-14), 61.5 (C-3, unresolved), 76.2 (d, CH_2O, $^2J_{C\text{-}P}$ =5.2 Hz), 122.0 (C-6), 140.5 (C-5).

FAB MS m/e 602 (MH^+, 0.4%), 586 (2), 565 (2), 278 (2), 242 (2), 220 (13), 204 (3), 201 (3), 199 (5), 198 (62), 182 (10), 165 (3), 164 (3), 161 (3), 159 (6), 157 (3), 147 (7), 146 (3), 145 (9), 143 (14), 142 (17), 141 (5), 136 (6), 131 (9),129 (9), 121 (50), 119 (11), 107 (17), 105 (25), 100 (29), 99 ($Me_3CNCH_2CH_2$, 3), 42 (base peak).

Analysis Found: C, 64.43; H, 10.49; N, 2.51; P, 5.24%; $C_{33}H_{60}NO_4P$ requires C, 70.05; H, 10.69; N, 2.48; P, 5.47%; $C_{33}H_{60}NO_4P[H_2O]_{2.5}$ requires C, 64.89; H, 10.73; N, 2.29 and P, 5.07%.

$\upsilon_{max}(CH_2Cl_2)$ 2941, 2631, 2439, 1616, 1466, 1376, 1212, 1200 (P=O), 1083, 1018 cm^{-1}.

<u>Water Mediated Hydrolysis of (91l).</u>

Compound **(91l)** (0.34 g, 0.74 mmol) was suspended in THF/water (1:1; 8 mL) and refluxed for 120 h.

^{31}P nmr $\delta(H_2O/D_2O$ centre lock/THF) 15.54 and 0.99 (product).

The reaction mixture was refluxed for a further 96 h and was then lyophilised to yield **(92l)** as a white solid (0.35 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.17 (6H, s, CH_3 x 2), 1.45 (41H, m, CH_2 x 16 and CH_3 x 3), 3.20 (2H, m, NCH_2), 4.10 (2H, m, CH_2O).

^{31}P nmr $\delta(D_2O)$ 0.97.

^{13}C nmr $\delta(CDCl_3)$ 14.1 (C-1), 22.7 (C-2), 24.4 (C-16), 25.7 (CH_3 x 3), 27.6-29.9 (m, C-4 to C-15), 30.2 (C-17), 31.9 (C-3), 42.9 (d, CH_3 x 2, $^{2}J_{C-P}$ =7.3 Hz), 43.2 (d, NCH_2, $^{2}J_{C-P}$ =6.7 Hz), 52.1 ($Me_3\underline{C}$), 62.6 (s_b, COP), 84.1 (d, CH_2O, $^{2}J_{C-P}$ =8.6 Hz).

FAB MS m/e 478 (MH^+, 38%), 391 (5), 99 ($Me_3CNCH_2CH_2$, 9), 57 (Me_3C^+, 15), 44 ($NH_2CH_2CH_2$, base peak)

Analysis Found: C, 64.12; H, 11.77; N, 2.90; P, 6.45%; $C_{26}H_{56}NO_4P$ requires C, 65.37; H, 11.82; N, 2.93; P, 6.48%; $C_{26}H_{56}NO_4P[H_2O]_{0.5}$ requires C, 64.16 H, 11.80; N, 2.88; P, 6.36%.

$\upsilon_{max}(CH_2Cl_2)$ 3408, 2924, 2852, 1768, 1601, 1463, 1373, 1224 (P=O), 1074 cm^{-1}.

Water Mediated Hydrolysis of (91m).

Compound **(91m)** (0.33 g, 1.05 mmol) was dissolved in THF/water (1:1; 8 mL) and refluxed for 72 h. The reaction mixture was then lyophilised to yield **(92m)** as a white solid (0.35 g, 100%).

^{1}H nmr δ(CD_3OD) 1.37 (9H, s, CH_3 x 3), 1.58 (6H, m, C-1, C-3, C-10), 2.03 (6H, m, C-5, C-7, C-9), 2.09 (3H, m, C-4, C-6, C-8), 3.18 (2H, m, NCH_2), 4.30 (2H, m, CH_2O).

^{31}P nmr δ(THF/D_2O) -2.8.

^{13}C nmr δ($CDCl_3$) 25.8 (CH_3 x 3), 32.5 (C-4, C-6 and C-8), 37.1 (C-5, C-7 and C-9), 44.10 (d, C-1, C-3 and C-10, $^3J_{C-P}$ =3.4 Hz), 44.7 (d, NCH_2, $^3J_{C-P}$ =3.5 Hz), 57.5 ($Me_3\underline{C}$), 62.2 (d, COP, $^2J_{C-P}$ =5.2 Hz), 78.5 (d, CH_2O, $^2J_{C-P}$ =7.9 Hz).

FAB MS m/e 332 (MH^+, 49%), 198 (3), 136 (19), 135 (adamantyl, base peak), 100 ($Me_3CNHCH_2CH_2$, 46), 73 (Me_3CNH_2, 11).

Analysis Found: C, 55.23; H, 9.31; N, 4.08; P, 9.07%; $C_{16}H_{30}NO_4P$ requires C, 57.99; H, 9.13; N, 4.23; P, 9.35%; $C_{16}H_{30}NO_4P[H_2O]_{1.0}$ requires C, 55.00; H, 9.23; N, 4.01, P, 8.06%.

υ_{max}(CH_2Cl_2) 3043, 2912, 2636, 2433, 1609, 1526, 1355, 1197, (P=O), 1086, 1044 cm^{-1}.

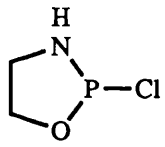
CHAPTER 4

The Synthesis of Some Novel Ethanolamine Phospholipid Analogues.

The Synthesis of Some Novel Ethanolamine Phospholipid Analogues.

4.1. Introduction.

This chapter deals with the attempt to adapt the cyclic phosphoramidite synthetic route to give a range of phospholipid analogues with a natural ethanolamine headgroup. A 2-chloro-1,3,2-oxazaphosphacyclopentane phosphitylating agent **(93)** would be far too unstable to be used in such a synthesis, by virtue of its reactivity at the amine centre and therefore its tendency to self condense and polymerise.

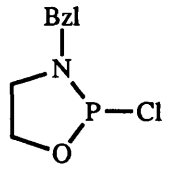


(93)

Thus a suitable N-H protecting group would have to be found that could be removed under mild conditions which would not be deleterious to the rest of the phospholipid molecule, e.g. the base labile P-O bonds[136,137] and the acid and base labile ester functionalities.[146]

Benzyl protection has been used widely for alcohols[95,96,147] and amines[148] and its removal involves facile catalytic hydrogenation under neutral conditions.[149] Another advantage of benzyl protection was that the N-benzylethanolamine required for the synthesis of the phosphitylating agent 3-benzyl-2-chloro-1,3,2-oxazaphosphacyclopentane **(94)** is readily available.

Thus, the synthesis of the benzyl protected phosphitylating agent **(94)** was pursued, followed by its condensation with alcohol to give a phosphite intermediate, which was readily oxidised to a phosphate triester.

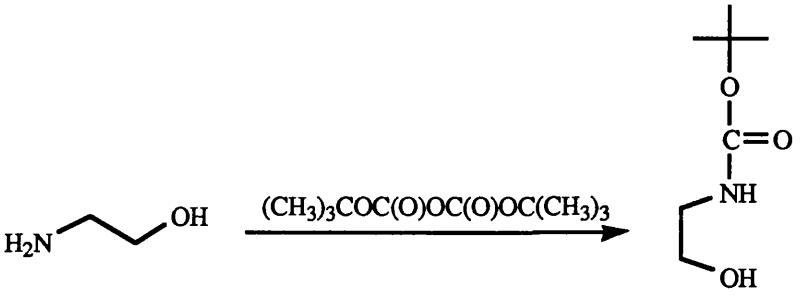


(94)

However, deprotection of the phosphate triester proved problematic and the ethanolamine phospholipid analogues could not be obtained.

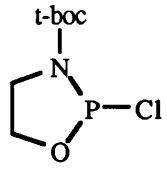
As an alternative, the *t*-butoxycarbonyl (*t*-boc) protecting group was utilised. The N-*t*-boc-ethanolamine starting material was not available and so had to be synthesised, (refer to scheme 19), before the N-*t*-boc phosphitylating agent **(96)** could be prepared.

Scheme 19.



(95)

From this it was possible to synthesise 3-*t*-boc-2-chloro-1,3,2-oxazaphosphacyclopentane phosphitylating agent **(96)**.

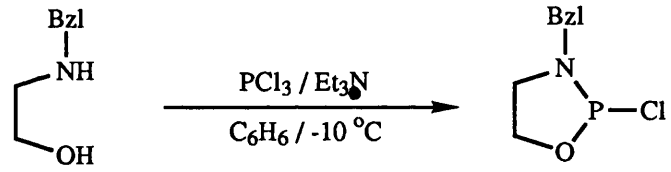


(96)

4.2. Results and Discussion.

4.2.1. Cyclisation Reactions.

The phosphitylating agent **(94)** was prepared by the low temperature reaction of phosphorus trichloride with N-benzylethanolamine in the presence of triethylamine base.

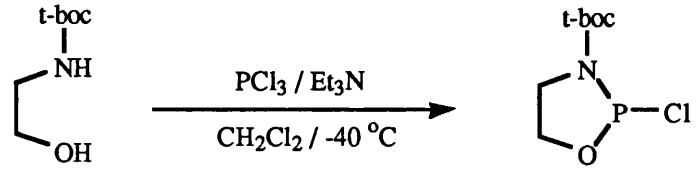


(94)

The product was isolated in only a 17% yield following high vacuum distillation. This low yield was associated with the physical nature of the phosphitylating agent i.e. it is a viscous gum that is difficult to distil even under short path conditions. The product obtained was characterised by nmr and FAB mass spectroscopy. Both the ^{1}H and ^{13}C nmr spectra show two bond phosphorus coupling. The signals in the ^{13}C nmr spectrum were assigned with reference to data for N-methylbenzenemethanamine.[117] The ^{31}P nmr shift at *ca* δ167 is consistent with that reported for the other phosphitylating agents. The FAB mass spectrum revealed a protonated molecular ion peak of m/e 216, along with a significant percentage of an unknown species of m/e 351. The nature of this contaminant was

not elucidated by the nmr spectra recorded and no further information on this was collected.

The phosphitylating agent **(96)** was prepared by the reaction of phosphorus trichloride with N-*t*-boc-ethanolamine **(95)** in the presence of triethylamine base.

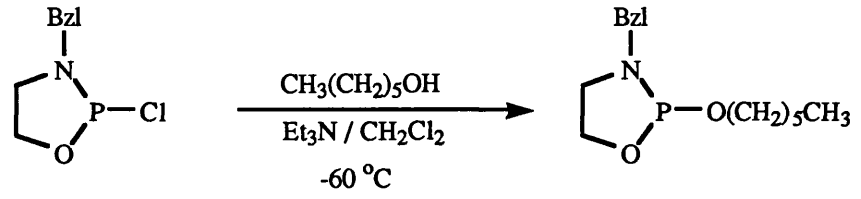


(95) **(96)**

The product was obtained in a 53% yield following high vacuum distillation and characterised by nmr and FAB mass spectroscopy. The ^{1}H and ^{13}C nmr spectra show two bond phosphorus coupling and the ^{31}P nmr shift at δ151.2 is consistent with similar phosphite species. Noticeably the shift is *ca* 15 ppm upfield compared with the shifts observed for the phosphitylating agents **(79)**, **(89)** and **(94)**, presumably due to the presence of the carbonyl functionality in the protecting group. Signals in the ^{13}C nmr spectrum were assigned with reference to the signals observed for the N-methyl phosphitylating agent **(79)**. The FAB mass spectrum revealed a protonated molecular ion of m/e 226. The boiling point of the phosphitylating agent **(96)** was observed as 130-135 °C at 0.02 mmHg compared to that of the N-methyl and *t*-butyl phosphitylating agents **(79)** and **(89)** which boiled at 30-35 °C at 0.1 mmHg and 64-68 °C at 0.08 mmHg respectively.

4.2.2. Condensation Reactions.

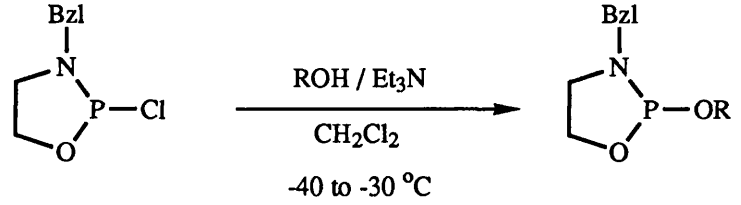
Compound **(94)** was reacted with hexan-1-ol at low temperature to give a product in 90% yield.



(94) **(97a)**

The product was characterised by nmr spectroscopy. The ^{1}H nmr spectrum indicated the desired product **(97a)** in a pure state. However, the ^{31}P nmr spectrum revealed three signals at δ142.1, 138.5 and 134.3 in a ratio of 1:1:16. The former two signals were minor impurities that possibly arose from by-product formation in the cyclisation reaction. It was hoped that they would not be detrimental to the next stages of the synthesis.

Similarly compound **(94)** was treated with dodecan-1-ol and octadecan-1-ol to give the corresponding phosphite triesters **(97b)** and **(97c)** in high yields.



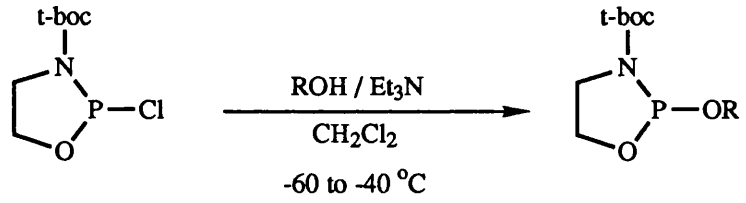
(94) **(97b-c)**

R

b= n-$C_{12}H_{25}$
c= n-$C_{18}H_{37}$

The products were characterised by ^{31}P nmr spectroscopy. The spectrum for **(97b)** revealed two signals at δ137.6 and 134.3 in a ratio of 6:5, whilst that for compound **(97c)** revealed a single shift at δ135.2. Product **(97b)** was considered to be too impure for further reaction. The appearance of two signals in the ^{31}P nmr, in this case, would appear to indicate the formation of desired phosphite triester **(97b)** along with some undesired phosphite by-product. This latter product possibly arising from the presence of catalytic amounts of water during the condensation reaction, presumably due to insufficiently dried alcohol.

Compound **(96)** was reacted with hexan-1-ol, dodecan-1-ol, octadecan-1-ol and oleyl alcohol at low temperature to give the corresponding phosphite triesters **(98a-d)**.



(96) **(98a-d)**

R

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

The products were isolated by hexane extraction and characterised by ^{31}P nmr spectroscopy. The shifts observed are shown in table 14.

Table 14. Condensation of compound (96) with primary alcohols.

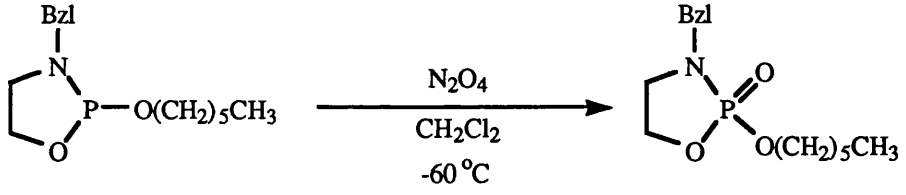
ROH (R=)	Temp. °C	Product	Yield%	δ ^{31}P nmr*
n-C_6H_{13}	-60	**(98a)**	100	124.9
n-$C_{12}H_{25}$	-60	**(98b)**	100	124.9
n-$C_{18}H_{37}$	-40	**(98c)**	100	124.5
Δ^9 $C_{18}H_{35}$	-60	**(98d)**	99	124.6

*nmr recorded in CH_2Cl_2 with D_2O centre lock.

4.2.3. Oxidation Reactions.

The next stage of the route involved the oxidation of the phosphite triesters to their corresponding phosphate triesters.

The hexyloxy phosphite **(97a)** was oxidised using a standard solution of dinitrogen tetroxide at low temperature.

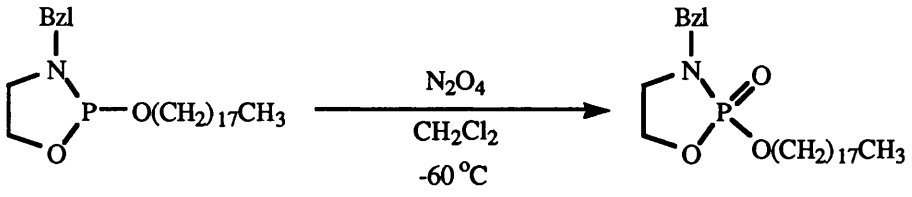


(97a) **(99a)**

The phosphate product **(99a)** was isolated in quantitative yield and characterised by ^{31}P nmr spectroscopy. Two signals were observed at δ19.0 and 6.8, the former corresponding to the desired product whilst the latter represented a minor impurity i.e. possibly as a result of hydrolysis of the phosphite **(97a)** or of the material that

showed up as minor impurities in the ^{31}P nmr spectrum of the hexyloxy phosphite **(97a)**.

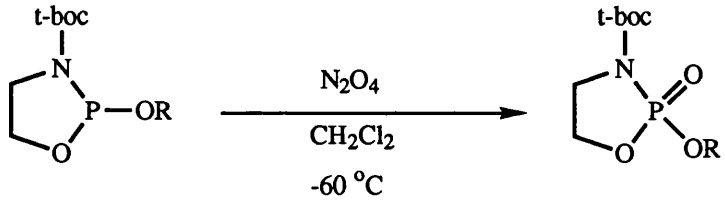
Similarly, the octadecyloxy phosphite **(97c)** was oxidised with dinitrogen tetroxide at low temperature.



(97c) **(99c)**

The phosphate product **(99c)** was obtained in a quantitative yield and characterised by ^{13}C, ^{31}P nmr and infra-red spectroscopy. The ^{31}P nmr chemical shift observed at δ19.1 is consistent with similar phosphate intermediates previously reported.

The *t*-boc protected phosphite intermediates **(98a-d)** were similarly oxidised to give the corresponding phosphate intermediates **(100a-d)**. Details of yields and ^{31}P nmr chemical shifts for these are shown in table 15.



(98a-d) **(100a-d)**

R

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

Table 15. Oxidations.

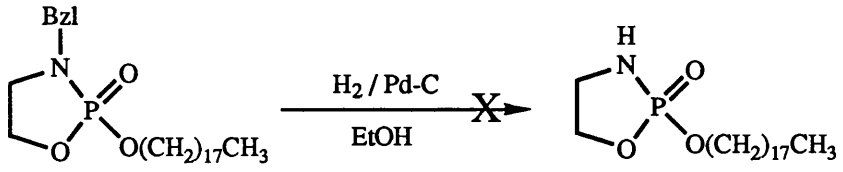
Phosphate	Yield%	δ ^{31}P nmr*
(100a)	100	8.1
(100b)	98	8.2
(100c)	99	8.1
(100d)	100	8.0

* nmr recorded in $CDCl_3$

These phosphates were also characterised by ^{1}H, ^{13}C nmr and infra-red spectroscopy, which fully supported the proposed structure of the phosphate intermediates. The signals were assigned with reference to the spectral data collected for the corresponding N-methyl analogues, which were disscussed in chapter 2. The change in ^{31}P nmr shift from *ca* δ16-20 observed for the N-methyl, *t*-butyl and N-benzyl phosphate analogues to *ca* δ8 in the corresponding *t*-boc phosphates is presumably due to effects from the carbonyl group.

4.2.4. Hydrogenation.

The attempted hydrogenation of compound **(99c)** was carried out under standard conditions at atmospheric pressure. Previous work by Anson[125] on the hydrogenation of N,N'-dibenzylethylenediamine analogues had revealed their resistance to deprotection under a variety of conditions, including Parr hydrogenation and catalytic transfer hydrogenation, as well as the conditions described below. It seemed reasonable to assume that this hydrogenation reaction would also prove problematic.



(99c) **(101c)**

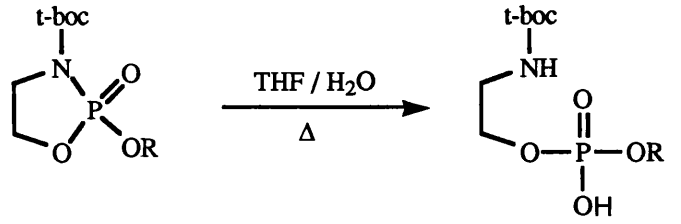
There is some literature[149] precedent suggesting that the hydrogenation of phosphorus containing species can prove problematic due to poisoning of the catalyst.

The reaction was followed by TLC. After *ca* 16 hours and the addition of three portions of 10% palladium on charcoal the TLC appeared to show the loss of starting material **(99c)**, with the appearance of base line material. However, ^{1}H and ^{13}C nmr spectroscopy of the isolated product showed the presence of aromatic signals at δ4.12 and 7.36 in the ^{1}H nmr spectrum and δ51.0, 129.1 and 130.5 in the ^{13}C nmr spectrum. The low yield (42%) and spectral data would appear to indicate loss of the deprotected product **(101c)** during the isolation procedure.

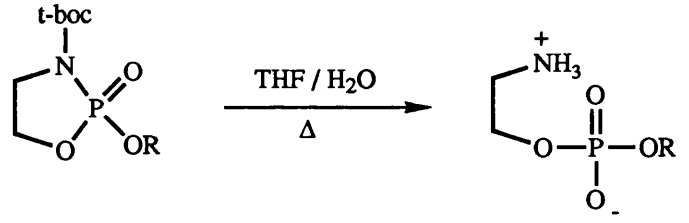
Since this deprotection appeared to have many drawbacks in terms of rate of reaction, the need for excessive amounts rather than catalytic amounts of palladium on charcoal and isolation of the deprotected product it seemed advisable to abandon this route.

4.2.5. Hydrolysis Reactions.

It was decided to pursue the hydrolysis of the *t*-boc protected phosphate analogues **(100a-d)** to give the cleaved *t*-boc protected intermediates **(102a-d)** and then deprotect under acidic conditions.



(100a-d) **(102a-d)**



(100a-d) **(103a-d)**

R

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

Thus, compound **(100a)** was dissolved in water and stirred at ambient temperature for 4 hours, ^{31}P nmr spectroscopy of the reaction mixture, after this time, showed no appreciable hydrolysis had occured. The reaction mixture was further diluted with THF to give a 1:1 ratio of THF to water and then refluxed for 32 hours. The product, isolated by lyophilisation, was characterised by nmr and infra-red spectroscopy. Spectral analysis showed that none of the *t*-boc protected compound **(102a)** had been isolated since there was an absence *t*-boc signals in the ^1H and ^{13}C nmr spectra and an absence of a carbonyl signal at *ca* 1750 cm^{-1} in the infra-red spectrum. In fact it was the hexyloxy ethanolamine **(103a)** that had been isolated in 94%

yield. Further characterisation by FAB mass spectroscopy and microanalysis supported the presence of the deprotected product, the former revealing a protonated molecular ion of m/e 226. Whilst, P-N cleavage to give the phospholipid analogue was supported by the observed chemical shift at δ1.0 in the ^{31}P nmr spectrum.

Thus it seemed that the hydrolysis conditions used were sufficiently acidic to bring about the desired deprotection as well as the P-N bond cleavage. With this in mind, the dodecyloxy, octadecyloxy and oleyloxy phosphate intermediates **(100b-d)** were refluxed in THF and water.

Hydrolysis and deprotection of the dodecyloxy phosphate **(100b)** was accomplished within 72 hours. Characterisation by nmr, infra-red, FAB mass spectroscopy and microanalysis supported the proposed structure of the product **(103b)**.

The octadecyloxy phosphate **(100c)** was similarly refluxed for 90 hours, at this time ^{31}P nmr spectroscopy of the reaction mixture revealed two signals at δ-2.28 and -2.23 in a ratio of 2:3, signifying complete hydrolysis, but incomplete deprotection. Thus the reaction mixture was further refluxed for 72 hours to ensure complete deprotection. The product **(103c)** was isolated in a 91% yield following lyophilisation and fully characterised. This time the ^{31}P nmr spectrum revealed a single, solvent dependent, signal at δ-0.3. The ^{1}H and ^{13}C nmr spectra show the absence of signals corresponding to the *t*-boc group, but the presence of signals to support the proposed structure of the ethanolamine product **(103c)**. (Details of the ^{13}C nmr spectrum are shown in table 16). The infra-

red spectrum supports the loss of the *t*-boc group since there is no carbonyl signal present and in the FAB mass spectrum a protonated molecular ion of m/e 394 was observed.

Similarly the oleyloxy phosphate intermediate **(100d)** required much longer reaction time to ensure full deprotection, i.e. 180 hours. After 90 hours ^{31}P nmr spectroscopy of the reaction mixture showed a single signal at δ-2.3, however, infra-red of this product revealed that the *t*-boc group was still present since a carbonyl signal was clearly visible at 1708 cm^{-1}. Presumably, this product is the cleaved *t*-boc protected compound **(102d)**. The reaction was found to be complete after refluxing the reaction mixture for a further 90 hours and isolating the product by lyophilisation. The product **(103d)** was fully characterised and the data collected supported its proposed structure.

Table 16. ^{13}C nmr data for compounds (103a-d) recorded at 100 MHz in $CDCl_3$. Phosphorus-carbon coupling constants (Hz) are given in parentheses.

$CH_3CH_2CH_2(CH_2)_nCH_2CH_2CH_2OP(O)(OH)OCH_2CH_2NH_2$

A B C D E F G H

Signal	(103a) n=0	(103b) n=6	(103c) n=12	(103d) n=12[a]
A	14.0	14.1	14.1	14.1
B	22.6	22.7	22.7	22.6
C	31.6	31.9	32.0	31.8
D	25.4	25.7	26.9	25.8
E	30.6[b]	31.8[b]	31.6[b]	30.7 (7.4)
F	62.9[b]	63.0[b]	63.0[b]	62.8[b]
G	66.3 (2.8)	66.5[b]	67.8[b]	66.0 (4.6)
H	40.6 (6.5)	40.5[b]	41.4[b]	40.4[b]

[a] Includes CH=CH δ130.3 and 130.1 and $\underline{C}H_2CH=CH\underline{C}H_2$ δ27.2

[b] Coupling not resolved, signal appears as a broad singlet.

The products were further characterised by microanalysis which showed the familiar phenomena of associated water despite extensive drying under high vacuum conditions. This fully supports the theory of their affinity for water and the percentages are quoted in the experimental both with and without the associated water.

4.3. Experimental.

3-Benzyl-2-chloro-1,3,2-oxazaphosphacyclopentane (94).

Dry N-benzylethanolamine (10.0 g, 0.07 mol) and triethylamine (17.8 g, 0.18 mol) in benzene (50 mL) were added dropwise with vigorous stirring to benzene (150 mL) at -10 °C under an atmosphere of nitrogen. Separately but simultaneously, phosphorus trichloride (11.5 g, 0.08 mol) in benzene (50 mL) was added dropwise. The reaction mixture was allowed to warm to ambient temperature with stirring for 2 h. The mixture was filtered, washing the residue with benzene (2 x 30 mL) and the filtrate concentrated under reduced pressure to give a pale yellow oil, which was vacuum distilled. The product was collected as a white gum (1.80 g, 17%), b.p. 155-160 °C, 0.08 mmHg.

^{1}H nmr δ($CDCl_3$) 3.07 (2H, m, NCH_2), 4.15 (2H, d, CH_2O, J=8.1 Hz), 4.43 (2H, m, Ph$C\underline{H}_2$), 7.31 (5H, m, Ph).

^{31}P nmr δ($CDCl_3$) 166.9.

^{13}C nmr δ($CDCl_3$) 46.1 (d, NCH_2, $^2J_{C\text{-}P}$ =7.2 Hz), 49.0 (d, Ph$\underline{C}H_2$, $^2J_{C\text{-}P}$ =14.9 Hz), 70.8 (d, CH_2O, $^2J_{C\text{-}P}$ =9.3 Hz), 129.0 (Ph), 136.4 (*ipso* C).

FAB MS m/e 351 (38%), 216 (MH^+, 59), 152 (54), 134 (51), 107 (18), 91 (base peak), 77 (21).

N-(*t*-Butoxycarbonyl)ethanolamine (95).

Di-*t*-butylpyrocarbonate (5.0 g, 0.023 mol) and 4-dimethylaminopyridine (0.075 g) in acetonitrile (30 mL) were added

dropwise to ethanolamine (2.80 g, 0.046 mol) and triethylamine (6.40 mL, 0.046 mol) in acetonitrile (30 mL). The solution was stirred for 1 h, then methanol (150 mL) was added and the solution stirred for a further 20 min. The solvent was removed under reduced pressure, the residue dissolved in chloroform (150 mL) and extracted with saturated aqueous sodium hydrogen carbonate (30 mL), followed by saturated brine (2 x 30 mL). The organic solution was dried ($MgSO_4$) and evaporated under reduced pressure to yield the product as a pale yellow oil (3.29 g, 89%).

^{1}H nmr δ($CDCl_3$) 1.33 (9H, s, CH_3 x 3), 3.15 (2H, m, NCH_2), 3.55 (2H, m, CH_2O), 3.90 (1H, s_b, OH), 5.20 (1H, s_b, NH).

^{13}C nmr δ($CDCl_3$) 28.2 (CH_3 x 3), 42.8 (NCH_2), 61.3 (CH_2O), 79.1 ($Me_3\underline{C}$), 156.6 (C=O).

3-*t*-Boc-2-chloro-1,3,2-oxazaphosphacyclopentane (96).

Compound **(95)** (2.29 g, 0.014 mol) and triethylamine (4.40 mL, 0.031 mol) in dichloromethane (60 mL) were added dropwise with vigorous stirring to dichloromethane (100 mL) at -40 °C under an atmosphere of nitrogen. Seperately but simultaneously, phosphorus trichloride (1.50 mL, 0.017 mol) in dichloromethane (50 mL) was added dropwise. The solution was warmed to ambient temperature with stirring for 2 h and then the solvent removed under reduced pressure. The residue was extracted with dry diethyl ether (3 x 80 mL) and the extract evaporated under reduced pressure to give a pale yellow oil, which was vacuum distilled. The product was

collected as a clear colourless oil (1.67 g, 53%), b.p. 130-135 °C, 0.02 mmHg.

^{31}P nmr δ($CDCl_3$) 151.2.

^{13}C nmr δ($CDCl_3$) 28.1 (CH_3 x 3), 42.3 (d, NCH_2, $^2J_{C-P}$ =5.2 Hz), 70.7 (d, CH_2O, $^2J_{C-P}$ =6.6 Hz), 83.1 ($Me_3\underline{C}$), 152.3 (d, C=O, $^2J_{C-P}$ =9.1 Hz).

FAB MS m/e 248 (MNa^+, 15%), 226 (MH^+, 1), 170 (9), 126 (14), 88 (13), 57 (base peak).

3-Benzyl-2-hexyloxy-1,3,2-oxazaphosphacyclopentane (97a).

Compound **(94)** (0.50 g, 2.32 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous hexan-1-ol (0.24 g, 2.32 mmol) and triethylamine (0.36 mL, 2.55 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.59 g, 90%).

^{1}H nmr δ($CDCl_3$) 0.89 (3H, t, $C\underline{H_3}CH_2$), 1.42 (8H, m, CH_2 x 4), 2.89 (2H, m, NCH_2), 3.75 (2H, m, RCH_2O), 4.25 (2H, m, CH_2O and $PhC\underline{H_2}$), 7.31 (5H, m, Ph).

^{31}P nmr δ($CDCl_3$) 142.1, 138.5 and 134.3 (1:1:16).

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.6 (C-4), 31.5 (C-5), 31.6 (C-3), 46.5 (d, NCH_2, $^2J_{C-P}$ =5.1 Hz), 49.3 (d, $Ph\underline{C}H_2$, $^2J_{C-P}$ =23.1 Hz), 63.3 (d,

C-6, $^2J_{C-P}$ =10.8 Hz), 69.0 (d, CH_2O, $^2J_{C-P}$ =10.7 Hz), 127.8 (m, Ph), 139.2 (m, *ipso* C).

3-Benzyl-2-dodecyloxy-1,3,2-oxazaphosphacyclopentane (97b).

Compound **(94)** (0.25 g, 1.16 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous dodecan-1-ol (0.22 g, 1.16 mmol) and triethylamine (0.20 mL, 1.17 mmol) in dichloromethane (10 mL) at -30 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h and then the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a colourless oil (0.41 g, 97%).

^{31}P nmr δ($CDCl_3$) 137.6 and 134.3 (6:5).

3-Benzyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane (97c).

Compound **(94)** (0.10 g, 0.46 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous octadecan-1-ol (0.13 g, 0.46 mmol) and triethylamine (0.06 mL, 0.47 mmol) in dichloromethane (10 mL) at -40 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h and then the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.21 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 135.2.

3-*t*-Boc-2-hexyloxy-1,3,2-oxazaphosphacyclopentane (98a).

Compound **(96)** (0.20 g, 0.89 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous hexan-1-ol (0.09 g, 0.89 mmol) and triethylamine (0.12 mL, 0.90 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (60 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.26 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 124.9.

3-*t*-Boc-2-dodecyloxy-1,3,2-oxazaphosphacyclopentane (98b).

Compound **(96)** (0.30 g, 1.33 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous hexan-1-ol (0.25 g, 1.33 mmol) and triethylamine (0.19 mL, 1.34 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (60 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.50 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 124.9.

3-*t*-Boc-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane (98c).

Compound **(96)** (0.15 g, 0.66 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous octadecan-1-ol (0.18 g, 0.66 mmol) and triethylamine (0.09 mL, 0.67 mmol) in dichloromethane (15 mL) at -40 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 1 h and then the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.31 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 124.5.

3-*t*-Boc-2-oleyloxy-1,3,2-oxazaphosphacyclopentane (98d).

Compound **(96)** (0.15 g, 0.66 mmol) in dichloromethane (10 mL) was added dropwise with vigorous stirring to anhydrous oleyl alcohol (0.18 g, 0.66 mmol) and triethylamine (0.09 mL, 0.67 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 the solvent removed under reduced pressure. The residue was extracted with dry hexane (80 mL) and the extract evaporated under reduced pressure to give the product as a white oil (0.30 g, 99%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 124.6.

3-Benzyl-2-hexyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (99a).

A portion of standard dinitrogen tetroxide solution (2.0 mL, containing 0.54 mmol of oxidant, sufficient to oxidise 2.20 mmol of phosphite) was added dropwise with vigorous stirring to compound **(97a)** (0.59 g, 2.10 mmol) in dichloromethane (10 mL) at -60 °C. The solution was warmed to ambient temperature and the solvent removed under reduced pressure to yield the product as a pale yellow oil (0.62 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.0 and 6.8 (minor impurity).

3-Benzyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (99c).

This was prepared by an analogous manner to **(99a)** above. Thus from compound **(97c)** (0.25 g, 0.56 mmol) was isolated the title compound **(99c)** (0.26 g, 100%).

^{31}P nmr δ($CDCl_3$) 19.1.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.5 (C-16), 29.2 - 30.2 (m, C-4 to C-15), 30.5 (d, C-17, $^3J_{C\text{-}P}$ =5.4 Hz), 31.8 (C-3), 46.4 (d, NCH_2, $^2J_{C\text{-}P}$ =4.5 Hz), 51.2 ($PhCH_2$), 64.0 (RCH_2O), 68.0 (d, CH_2O, $^2J_{C\text{-}P}$ =6.7 Hz), 128.5 (m, Ph), 130.4 (m, *ipso* C).

υ_{max}(CH_2Cl_2) 3679, 3597, 3340, 2919, 2849, 1604, 1458, 1291 (P=O), 1064, 1026 cm^{-1}.

3-*t*-Boc-2-hexyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (100a).

A portion of standard dinitrogen tetroxide solution (0.37 mL, containing 0.23 mmol of oxidant, sufficient to oxidise 0.90 mmol of phosphite) was added dropwise with vigorous stirring to compound **(98a)** (0.26 g, 0.89 mmol) in dichloromethane (15 mL) at -60 °C. The solution was warmed to ambient temperature and the solvent removed under reduced pressure to yield the product as a pale yellow oil (0.27 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.87 (3H, t, $C\underline{H}_3CH_2$), 1.35 (6H, m, CH_2 x 3), 1.50 (9H, s, CH_3 x 3), 1.68 (2H, m, $RC\underline{H}_2CH_2O$), 3.62 (2H, m, NCH_2), 4.20 (4H, m, CH_2O and RCH_2O).

^{31}P nmr δ($CDCl_3$) 8.1.

^{13}C nmr δ($CDCl_3$) 13.9 (C-1), 22.5 (C-2), 25.1 (C-4), 28.0 (CH_3 x 3), 30.3 (d, C-5, $^3J_{C\text{-}P}$ =6.9 Hz), 31.3 (C-3), 44.3 (d, NCH_2, $^2J_{C\text{-}P}$ =16 Hz), 63.2 (C-6), 69.7 (d, CH_2O, $^2J_{C\text{-}P}$ =6.6 Hz), 83.2 ($Me_3\underline{C}$), 151.2 (d, C=O, $^2J_{C\text{-}P}$ =8.5 Hz).

υ_{max}(CH_2Cl_2) 3673, 3439, 2949, 2931, 2861, 1718 (C=O), 1338, 1268 (P=O), 1154, 1026 cm^{-1}.

3-*t*-Boc-2-dodecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (100b).

This was prepared by an analogous manner to **(100a)** above. Thus from compound **(98b)** (0.50 g, 1.33 mmol) was isolated the title compound **(100b)** (0.52 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.84 (3H, t, $C\underline{H}_3CH_2$), 1.29 (18H, m, CH_2 x 9), 1.49 (9H, s, CH_3 x 3), 1.60 (2H, m, $RC\underline{H}_2CH_2O$), 3.76 (2H, m, NCH_2), 4.25 (4H, m, CH_2O and RCH_2O).

^{31}P nmr δ($CDCl_3$) 8.2.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 25.4 (C-10), 28.0 (CH_3 x 3), 29.1 - 29.6 (m, C-4 to C-9), 30.3 (d, C-11, $^3J_{C-P}$ =6.6 Hz), 31.8 (C-3), 44.3 (d, NCH_2, $^2J_{C-P}$ =16.1 Hz), 63.2 (C-12), 69.7 (d, CH_2O, $^2J_{C-P}$ =6.8 Hz), 83.4 ($Me_3\underline{C}$), 151.5 (d, C=O, $^2J_{C-P}$ =8.4 Hz).

υ_{max}(CH_2Cl_2) 3676, 3443, 2923, 2850, 1718 (C=O), 1369, 1348, 1268 (P=O), 1152, 1026 cm^{-1}.

3-*t*-Boc-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (100c).

This was prepared by an analogous manner to **(100a)** above. Thus from compound **(98c)** (0.31 g, 0.67 mmol) was isolated the title compound **(100c)** (0.31 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.82 (3H, t, $C\underline{H}_3CH_2$), 1.21 (30H, m, CH_2 x 15), 1.48 (9H, s, CH_3 x 3), 1.61 (2H, m, $RC\underline{H}_2CH_2O$), 3.72 (2H, m, NCH_2), 4.18 (4H, m, CH_2O and RCH_2O).

^{31}P nmr δ($CDCl_3$) 8.1.

υ_{max}(CH_2Cl_2) 3671, 3599, 3444, 2924, 2852, 1720 (C=O), 1601, 1463, 1367, 1334, 1263 (P=O), 1152, 1026 cm^{-1}.

3-*t*-Boc-2-oleyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (100d).

This was prepared by an analogous manner to **(100a)** above. Thus from compound **(98d)** (0.30 g, 0.66 mmol) was isolated the title compound **(100d)** (0.31 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.82 (3H, t, $C\underline{H}_3CH_2$), 1.20 (22H, m, CH_2 x 11), 1.47 (9H, s, CH_3 x 3), 1.60 (2H, m, $RC\underline{H}_2CH_2O$), 1.93 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.66 (2H, m, NCH_2), 4.19 (4H, m, CH_2O and RCH_2O), 5.29 (2H, m, CH=CH).

^{31}P nmr δ($CDCl_3$) 8.0.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.5 (C-2), 25.3 (C-16), 27.8 (C-8 and C-11), 27.9 (CH_3 x 3), 28.9 - 32.5 (m, C-4 to C-7, C-12 to C-15 and C-17), 31.8 (C-3), 44.2 (d, NCH_2, $^2J_{C\text{-}P}$ =15.8 Hz), 63.0 (C-18), 63.4 (d, CH_2O, $^2J_{C\text{-}P}$ =3.7 Hz), 82.9 ($Me_3\underline{C}$), 130.1 (C-9 or C-10), 130.3 (C-9 or C-10), 151.6 (d, C=O, $^2J_{C\text{-}P}$ =8.0 Hz).

Attempted Catalytic Hydrogenation of 3-Benzyl-2-octadecyloxy-1,3,2-oxazaphosphacyclopentane 2-oxide (99c).

Compound **(99c)** (0.19 g, 0.41 mmol) in ethanol (12 mL) was treated with 10% palladium on charcoal (0.19 g) and stirred at ambient temperature under an atmosphere of hydrogen. The reaction was monitored by TLC and found to have not occured to any appreciable extent after 6 h. A further portion of 10% palladium on charcoal (0.19 g) was added and the reaction mixture stirred under an atmosphere of hydrogen for a further 6 h. TLC showed approximately 50% reduction in the intensity of the starting

material. A further portion of 10% palladium on charcoal (0.19 g) was added and the reaction continued as before for 4 h. TLC after this time appeared to show no unreacted starting material and the reaction mixture was filtered through celite, washing with ethanol (50 mL). The filtrate was evaporated under reduced pressure to give a cream coloured solid (0.07 g, 42%).

^{1}H nmr δ($CDCl_3$) 0.84 (3H, t, $C\underline{H}_3CH_2$), 1.40 (32H, m, CH_2 x 16), 3.00 (2H, m, NCH_2), 3.79 (2H, m, RCH_2O), 4.12 (4H, m, CH_2O and $PhC\underline{H}_2$), 7.36 (5H, m, Ph).

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.7 (C-16), 29.3 - 29.7 (m, C-4 to C-15), 30.5 (d, C-17, $^3J_{C\text{-}P}$ =7.1 Hz), 31.9 (C-3), 46.6 (d, NCH_2, $^2J_{C\text{-}P}$ =4.4 Hz), 51.0 ($Ph\underline{C}H_2$), 61.0 (d, RCH_2O, $^2J_{C\text{-}P}$ =3.6 Hz), 66.6 (CH_2O), 129.1 (m, Ph), 130.5 (m, *ipso* C).

Water Mediated Hydrolysis of (100a).

Compound **(100a)** (0.26 g, 0.85 mmol) was dissolved in water (8 mL) and stirred at ambient temperature for 4 h. The reaction mixture was then lyophilised to give a pale yellow gum.

^{31}P nmr δ($CDCl_3$) 8.3.

The unreacted compound **(100a)** was then dissolved in THF/H_2O (1:1, 5 mL) and refluxed for 32 h. The reaction mixture was then lyophilised to yield the product as a white solid (0.18 g, 94%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.30 (6H, m, CH_2 x 3), 1.50 (2H, m, $C\underline{H}_2CH_2O$), 3.22 (2H, m, NCH_2), 3.97 (4H, m, CH_2O and RCH_2O), 8.10 (3H, s_b, NH_3).

^{31}P nmr δ(THF/D_2O centre lock) 1.0.

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 25.4 (C-4), 30.6 (C-5). 31.6 (C-3), 40.6 (d, NCH_2, $^2J_{C-P}$ =2.8 Hz), 66.3 (d, CH_2O, $^2J_{C-P}$ =2.8 Hz).

FAB MS m/e 226 (MH^+, 1%), 154 (M^+- C_5H_{11}, 3), 136 (4), 124 (M^+-$C_6H_{13}O$, 3), 107 (4), 99 (8), 89 (8), 77 (12), 57 (59), 55 (26), 45 ($NH_3CH_2CH_2$, 2), 27 (base peak).

Analysis Found: C, 37.98; H, 9.15; N, 5.70; P, 12.16%; $C_8H_{20}NO_4P$ requires C, 42.66; H, 8.95; N, 6.22; P, 13.75%; $C_8H_{20}NO_4P[H_2O]_{1.5}$ requires C, 38.09; H, 9.19; N, 5.55; P, 12.28%.

υ_{max}(CH_2Cl_2) 3671, 3593, 3402, 2924, 1601, 1457, 1215 (P=O), 1074, 1020 cm^{-1}.

<u>Water Mediated Hydrolysis of (100b).</u>

Compound **(100b)** (0.40 g, 1.02 mmol) was dissolved in THF/H_2O (1:3, 12 mL) and refluxed for 72 h. The reaction mixture was then lyophilised to yield the product as a white solid (0.29 g, 92%).

^{1}H nmr δ($CDCl_3$) 0.87 (3H, t, $C\underline{H}_3CH_2$), 1.25 (20H, m, CH_2 x 10), 3.20 (2H, m, NCH_2), 4.00 (4H, m, CH_2O and RCH_2O), 8.10 (3H, s_b, NH_3).

^{31}P nmr δ($CDCl_3$) -1.9.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 25.7 (C-10), 28.4-30.3 (C-4 to C-9), 31.8 (s_b, C-11), 31.9 (C-3), 40.5 (s_b, NCH_2), 63.0 (s_b, C-12), 66.5 (s_b, CH_2O).

FAB MS m/e 332 (MNa^+, 1%), 310 (MH^+, 0.5), 154 (M^+- $C_{11}H_{23}$, 1), 125 (MH^+- $C_{12}H_{25}O$, 2), 107 (3), 106 (3), 100 (7), 92 (4), 90 (4), 82 (5), 78 (9), 70 (16), 68 (9), 59 (33), 57 (42), 46 (42), 45 ($NH_3CH_2CH_2$, 70), 43 (base peak).

Analysis Found: C, 51.14; H, 9.76; N, 4.45; P, 9.13%; $C_{14}H_{32}NO_4P$ requires C, 54.35; H, 10.43; N, 4.53; P, 10.01% $C_{14}H_{32}NO_4PH_2O$ requires C, 51.36; H, 10.47; N, 4.28; P, 9.46%.

$\upsilon_{max}(CH_2Cl_2)$ 3615, 3451, 2937, 2914, 2832, 1461, 1385, 1335, 1221 (P=O), 1078, 1031, 1014 cm^{-1}.

<u>Water Mediated Hydrolysis of (100c).</u>

Compound **(100c)** (0.32 g, 0.67 mmol) was dissolved in THF/H_2O (1:4, 10 mL) and refluxed for 90 h. The reaction mixture was then lyophilised to yield the product as a white solid.

^{31}P nmr $\delta(CDCl_3)$ -2.28 (major) and -2.63.

The crude product was redissolved in THF/H_2O (1:4, 15 mL) and refluxed for a further 72 h. The reaction mixture was then lyophilised to yield the product as a white solid (0.24 g, 91%).

^1H nmr $\delta(CDCl_3)$ 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.47 (32H, m, CH_2 x 16), 3.64 (2H, m, NCH_2), 4.03 (4H, m, CH_2O and RCH_2O).

^{31}P nmr $\delta(CH_2Cl_2/D_2O$ centre lock) -0.3.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 26.9 (C-16), 29.1-29.7 (m, C-4 to C-15), 31.6 (s_b, C-17), 32.0 (C-3), 41.4 (s_b, NCH_2), 63.0 (s_b, C-18), 67.8 (s_b, CH_2O).

FAB MS m/e 394 (MH^+, 4%), 155 (MH^+- $C_{17}H_{35}$, 7), 154 (M^+- $C_{17}H_{35}$, 56), 142 (3), 139 (3), 138 (14), 137 (31), 136 (43), 125 (MH^+- $C_{18}H_{35}O$, 1), 107 (18), 102 (21), 99 (21), 89 (21), 83 (17), 81 (12), 77 (28), 67 (19), 44 (base peak).

Analysis Found: C, 55.75; H, 10.98; N, 3.40; P, 7.15%; $C_{20}H_{44}NO_4P$ requires C, 61.04; H, 11.27; N, 3.56; P, 7.87%; $C_{20}H_{44}NO_4P[H_2O]_{2.0}$ requires C, 55.92; H, 11.26; N, 3.26; P, 7.21%.

υ_{max}(CH_2Cl_2) 3611, 3468, 2924, 2846, 1628, 1550, 1457, 1334, 1218 (P=O), 1074, 1020 cm^{-1}.

<u>Water Mediated Hydrolysis of (100d).</u>

Compound **(100d)** (0.42 g, 0.89 mmol) was dissolved in THF/H_2O (1:4, 10 mL) and refluxed for 90 h. The reaction mixture was then lyophilised to yield a yellow gum(0.34 g, 98%).

^{31}P nmr δ($CDCl_3$) -2.3.

υ_{max}(CH_2Cl_2) 2924, 2852, 1708 (C=O),1625, 1218 (P=O), 1074, 1032 cm^{-1}.

The crude product was redissolved in THF/H_2O (1:4, 10 mL) and refluxed for a further 90 h. The reaction mixture was then lyophilised to yield a pale yellow foam (0.25 g, 72%).

^{1}H nmr δ($CDCl_3$) 0.85 (3H, t, $C\underline{H}_3CH_2$), 1.30 (22H, m, CH_2 x 11), 1.54 (2H, m, $RC\underline{H}_2CH_2O$), 1.96 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.10 (2H, m, NCH_2), 3.83 (4H, m, CH_2O and RCH_2O), 5.33 (2H, m, CH=CH), 8.31 (2H, s_b, NH_2).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) -0.8.

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.6 (C-2), 25.8 (C-16), 27.2 (C-8 and C-11), 29.0-29.7 (m, C-4 to C-7 and C-12 to C-15), 30.7 (d, C-17, $^3J_{C\text{-}P}$ =7.4 Hz), 31.8 (C-3), 40.4 (s_b, NCH_2), 62.8 (s_b, C-18), 66.0 (d, CH_2O, $^2J_{C\text{-}P}$ =4.6 Hz), 130.1 (C-9 or C-10), 130.3 (C-9 or C-10).

FAB MS m/e 392 (MH^+, 10%), 142 (2), 138 (8), 137 (1), 136 (25), 107 (12), 102 (5), 99 (28), 77 (10), 67 (5), 44 (base peak).

Analysis Found: C, 54.12; H, 10.57; N, 3.35; P, 6.53%; $C_{20}H_{42}NO_4P$ requires C, 61.35; H, 10.81; N, 3.58; P, 7.91%; $C_{20}H_{42}NO_4P[H_2O]_{3.0}$ requires C, 53.91; H, 10.86; N, 3.14; P, 6.95%.

υ_{max}(CH_2Cl_2) 3677, 3593, 2918, 2846, 1604, 1547, 1463, 1421, 1218 (P=O), 1074, 1026 cm^{-1}.

CHAPTER 5

The Synthesis of Some Novel Serinol Phospholipid Analogues.

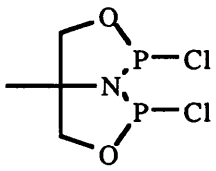
The Synthesis of Some Novel Serinol Phospholipid Analogues.

5.1. Introduction.

This chapter deals with the attempts to synthesise some cardiolipin analogues and the synthesis of a range of phospholipid analogues with a serinol head group.

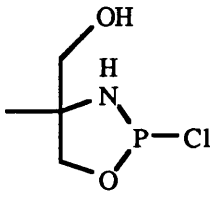
The cardiolipins or diphosphatidylglycerols constitute a class of complex phospholipids which occur mainly in heart and skeletal muscle cells[150] and are usually associated with membranes of subcellular fractions showing high metabolic activity, e.g. mitochondria.[151] The full biological functions of cardiolipins are not fully understood, however, it is believed that an increased presence has a profound effect on the transition of normal healthy cells to a cancerous state.[152]

Unpublished results[153] involving the synthesis of cardiolipin analogues using our phosphoramidite methodology revealed that the bis-phosphitylating agent **(104)** could not be synthesised by the reaction of 2-amino-2-methyl-1,3-propanediol with phosphorus trichloride.



(104)

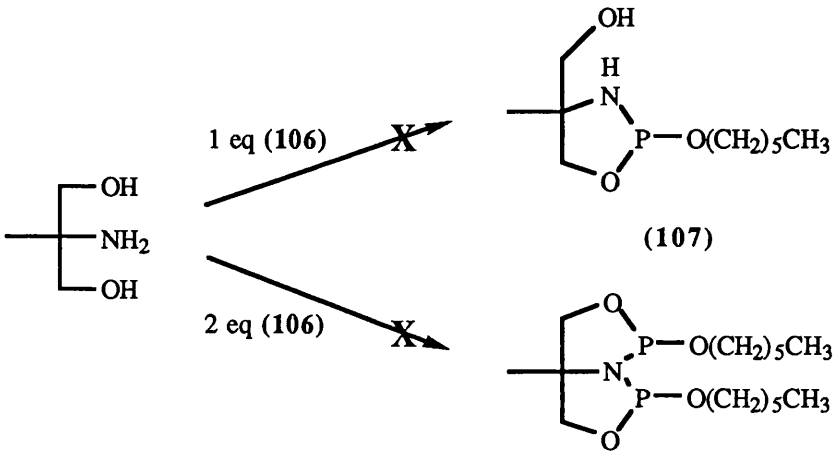
Similarly, the phosphitylating agent **(105)** could not be synthesised by reacting 2-amino-2-methyl-1,3-propanediol with a molar equivalent of phosphorus trichloride.



(105)

In an effort to utilise phosphite methodology, but abandon the original quest for the phosphitylating agent **(104)**, 2-amino-2-methyl-1,3-propanediol was reacted with both 1 and 2 molar equivalents of hexyl phosphorodichloridite **(106)** as shown in scheme 20.

Scheme 20.



In neither circumstances was any of the desired product isolated.

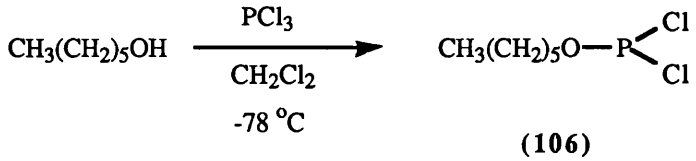
Finally, it was decided to attempt to synthesise a phosphate analogue by reacting 2-amino-2-methyl-1,3-propanediol with an alkyl phosphorodichloridate and if successful, then react the cyclic

phosphate intermediate with a phosphite e.g. phosphorus trichloride or hexyl phosphorodichloridite to form a bis-compound.

5.2. Results and Discussion.

5.2.1. Hexyl phosphorodichloridite.

Hexan-1-ol was reacted with an excess of phosphorus trichloride at low temperature.

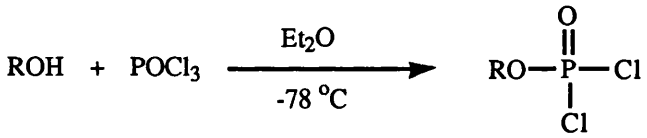


(106)

The product **(106)** was isolated in a quantitative yield and characterised by ^{31}P nmr spectroscopy. The shift observed at δ175.9 is consistent with similar phosphites.

5.2.2. Alkyl phosphorodichloridates.

Hexan-1-ol, octadecan-1-ol and oleyl alcohol were in turn reacted with a molar equivalent of phosphoryl chloride at low temperature.



(109a-c)

R

a= C_6H_{13}

b= $C_{18}H_{37}$

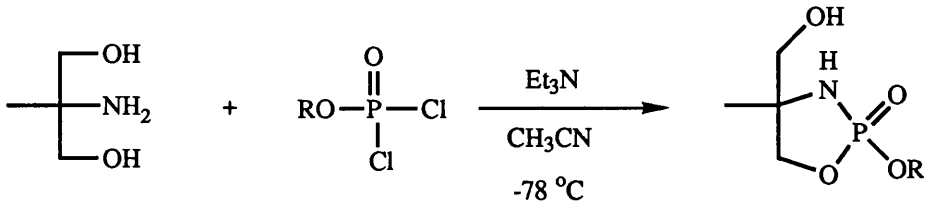
c= $\Delta^9C_{18}H_{35}$

The phosphate products **(109a-c)** were isolated in quantitative yields after 15-18 hours reaction time and characterised by ^{31}P nmr

spectroscopy. The ^{31}P nmr chemical shifts observed at *ca* δ5-6 are entirely consistent with phosphates of this type.[133]

5.2.3. Condensation Reactions.

2-Amino-2-methyl-1,3-propanediol was in turn reacted with 1 molar equivalent of the alkyl phosphorodichloridates **(109a-c)** at low temperature in the presence of triethylamine.



(109a-c) **(110a-c)**

R
a=n-C_6H_{13}
b=n-$C_{18}H_{37}$
c=$\Delta^9C_{18}H_{35}$

The reaction was carried out in acetonitrile on account of the poor solubility of the 2-amino-2-methyl-1,3-propanediol starting material. The hexyloxy and octadecyloxy intermediates **(110a)** and **(110b)** were isolated after 18-20 hours by extraction using diethyl ether, whilst the oleyloxy intermediate **(110c)** was isolated after 18 hours by hexane extraction. Compounds **(110a)** and **(110c)** were obtained in quantitative yields, whilst **(110b)** was obtained in only 67% yield presumably because of its poor solubility in diethyl ether.

Noticeably, the reaction time needed to be increased markedly by 17-19 hours because of the poorer reactivity of phosphate species compared with phosphites. (Refer to the rapid rates of the

condensation reactions of the phosphitylating agents and alcohols, discussed in the previous chapters).

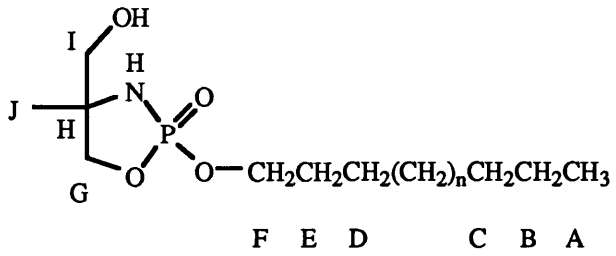
All of the phosphate intermediates **(110a-c)** were characterised by nmr spectroscopy, details of which are shown in tables 17 and 18.

Table 17. ^{31}P nmr data for compounds (110a-c) recorded at 80MHz in $CDCl_3$. The ratio of diastereoisomers are shown in parentheses.

Phosphate	δ ^{31}P nmr
(110a)	23.4, 22.9 (3:2)
(110b)	23.8, 23.3 (1:2)
(110c)	23.5, 22.7 (3:2)

The presence of 2 chiral centres in the phosphate intermediates **(110a-c)** results in diastereoisomerism as noted in the ^{31}P nmr spectra. The chemical shifts observed are consistent with phosphate compounds of this type.[133]

Table 18. ^{13}C nmr data for compounds (110a-c) recorded at 50 MHz and in $CDCl_3$. Diastereoisomeric ratios and coupling constants (Hz) are given in parentheses.



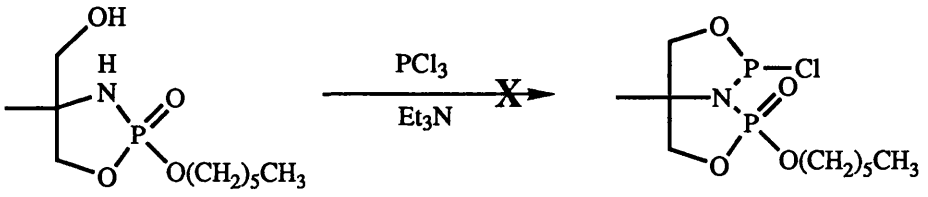
Signal	(110a) n=0	(110b) n=12[a]	(110c) n=12[b]
A	14.0	14.1	14.0
B	22.6	22.7	22.5
C	31.4	31.9	31.8
D	25.2	25.5	25.4
E	30.27, 30.3 (3:2), (J=7.2, 6.8 Hz)	30.3, 30.4 (2:3), (J=6.5, 6.7 Hz)	30.0, 30.2 (2:3), (J=4.9, 6.0 Hz)
F	60.1, 60.2 (3:2), (J=9.5, 9.5 Hz)	60.2, 60.4 (3:2), (J=9.3, 9.3 Hz)	59.9, 60.1 (2:3), (J=6.7, 6.0 Hz)
G	67.7, 67.9 (2:3), (J=6.3, 7.1 Hz)	67.8, 67.9 (1:1), (J=3.9, 6.7 Hz)	67.3, 67.5 (1:1), (J=6.6, 6.9 Hz)
H	53.6	54.6	53.4
I	73.7 (J=2.1 Hz)	73.1 (J=2.1 Hz)	73.4 (J=2.2 Hz)
J	23.8, 23.9 (2:3), (J=6.3, 6.3 Hz)	23.8, 23.9 (1:1), (J=2.3, 4.4 Hz)	23.8, 23.9 (1:1), (J=2.3, 1.7 Hz)

[a] Includes signals δ 29.2-30.0 for CH_2 x 12.

[b] Includes CH=CH at δ129.5 and 129.7, CH_2 x 8 at δ28.7-29.9, and $\underline{C}H_2CH{=}CH\underline{C}H_2$ at δ27.1.

5.2.4. Bicyclic Systems.

Compound **(110a)** was reacted with phosphorus trichloride at low temperature.

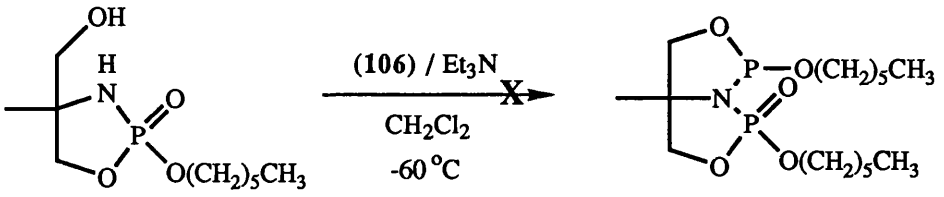


(110a) **(111a)**

The first attempt was carried out using dichloromethane as the solvent. A product was isolated in a greater than 100% yield based on the moles of starting compound **(110a)** and ^{31}P nmr spectroscopy revealed several signals at δ22.1, 3.8, -0.6, -0.7 and -3.7 in a ratio of 1:1:2:2:5. None of these correspond to a phosphite species, whilst the signal at δ22.1 is presumably of cyclic phosphate origin. Those signals observed between δ-0.6 and -3.7 presumably arose as a result of phosphite and cyclic phosphate hydrolysis.

In the second and third attempts benzene was employed as the solvent. Both reactions gave products that were characterised by ^{31}P nmr spectroscopy. Again signals were observed in the regions corresponding to phosphite, phosphate and hydrolysed species. In both cases high proportions of the undesired hydrolysed material were present presumably as a result of side reactions of the highly reactive phosphite **(111a)**.

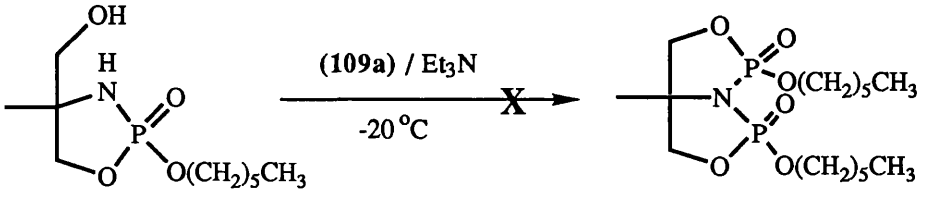
Compound **(110a)** was reacted with the hexyl phosphorodichloridite **(106)** at low temperature in the presence of triethylamine.



(110a) **(112a)**

The product was characterised by ^{31}P nmr spectroscopy and signals were observed in the regions corresponding to phosphite, i.e. δ139.3 and 138.8, phosphate i.e. δ23.9, 22.2, 22.1, 21.9, 21.8, 21.0 and 17.5, and hydrolysed material i.e. δ6.9 to -12.0. However, it was the hydrolysed material that was present in the highest proportions. Attempts to purify this product by column chromatography failed to significantly improve the quality of the product in terms of elimination of the undesired hydrolysed material. This is presumably because of the high reactivity of the phosphite portion of the product **(112a)** and the risk of its hydrolysis during chromatography.

Compound **(110a)** was reacted with hexyl phosphorodichloridate **(109a)** at low temperature in the presence of triethylamine.



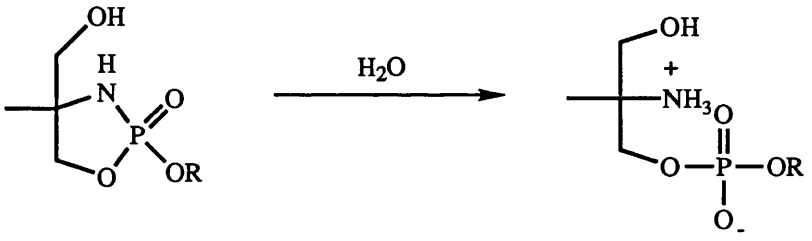
(110a) **(113a)**

This reaction was carried out firstly using dichloromethane as the solvent and then using acetonitrile. In both cases the products of the reaction were characterised by ^{31}P nmr spectroscopy, and showed signals in the region corresponding to phosphate species i.e. *ca* δ20, but also numerous signals in the region corresponding to hydrolysed material i.e. *ca* δ-3.2 to -26.9. The change of solvent did not appear to improve the outcome of this reaction.

5.2.5. Hydrolysis Reactions.

It seemed reasonable to predict that the hydrolysis of the phosphates **(110a-c)** to the corresponding serinol phospholipid products **(114a-c)** would be rapid compared with that of the more sterically hindered phosphates discussed in previous chapters.

The hydrolysis of the hexyloxy, octadecyloxy and oleyloxy phosphates **(110a-c)** were caried out by stirring at ambient temperature for between 1 and 4 hours.



(110a-c) **(114a-c)**

R

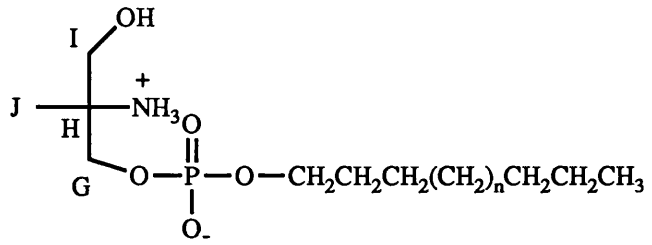
a= C_6H_{13}

b= $C_{18}H_{37}$

c= $\Delta^9C_{18}H_{35}$

The products **(114a-c)** were isolated in quantitative yields following lyophilisation and were fully characterised. All spectroscopic data supported the proposed structure of the serinol phospholipid products. (^{13}C nmr data is shown in table 19).

Table 19. ^{13}C nmr data for compounds (114a-c) recorded at 100 MHz. Phosphorus-carbon coupling constants (Hz) are given in parentheses.



Signal	(114a) n=0[a]	(114b) n=12[b]	(114c) n=12[b c]
A	13.6	14.1	13.9
B	22.9	22.7	22.4
C	31.9	31.9	31.6
D	25.7	25.8	25.6
E	30.9 (7.5)	29.7 (7.6)	30.4[d]
F	63.6[d]	62.2[d]	62.1[d]
G	66.2 (6.0)	66.2[d]	66.1 (5.3)
H	58.0 (7.7)	57.8[d]	57.2[d]
I	66.9	66.9	66.8
J	17.4	18.0	17.7

[a] Recorded in CH_3OD.

[b] Recorded in $CDCl_3$.

[c] Includes: CH=CH at δ129.6 and 129.5 and $\underline{C}H_2CH=CH\underline{C}H_2$ at δ26.9.

[d] Coupling not resolved, signal appears as a broad singlet.

Most noticeable is the shift of the signal corresponding to the methyl of the serinol head group from *ca* δ24 in the cyclic phosphate intermediates **(110a-c)** to *ca* δ18 in the cleaved products **(114a-c)**. P-N bond cleavage is also confirmed since the methyl signal now appears as a singlet having lost phosphorus coupling.

The solvent dependent chemical shifts observed in the ^{31}P nmr spectra for these compounds at *ca* δ1.0 to -2.3 also confirms P-N cleavage, since the chemical shift has altered from *ca* δ23 observed for the cyclic phosphate intermediates.

The FAB mass spectra of the products **(114a-c)** reveal protonated molecular ions of m/e 270, 438 and 436 respectively along with predictable fragmentation of the phospholipid molecules.

The products were also characterised by infra-red spectroscopy and microanalysis, which both supported the proposed structures of these serinol phospholipid analogues. Microanalysis of the products showed deviations commonly encountered with phospholipid derivatives i.e. associated water.[141]

5.3. Experimental.

Hexyl phosphorodichloridite (106).

Anhydrous hexan-1-ol (2.0 g, 0.02 mmol) in dichloromethane (50 mL) was added dropwise with vigorous stirring to phosphorus trichloride (27.0 g, 0.20 mol) in dichloromethane (50 mL) at -78 °C under an atmosphere of nitrogen. The solution was stirred for 2 h, maintaining the temperature at -78 °C, then warmed to ambient temperature with stirring for 1 h. The solvent was removed under reduced pressure to yield the product as a colourless oil (4.00 g, 99%).

^{31}P nmr $\delta(CDCl_3)$ 175.9.

Hexyl phosphorodichloridate (109a).

Anhydrous hexan-1-ol (1.0 g, 9.80 mmol) and triethylamine (1.37 mL, 9.80 mmol) in diethyl ether (20 mL) were added dropwise with vigorous stirring to phosphoryl chloride (1.50 g, 9.80 mmol) in diethyl ether (25 mL) at -78 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 15 h. The insoluble triethylamine hydrochloride was filtered, washing with diethyl ether (20 mL) and the filtrate evaporated under reduced pressure to yield the product as a colourless oil (2.10 g, 98%).

^{31}P nmr $\delta(CDCl_3)$ 4.9.

Octadecyl phosphorodichloridate (109b).

Anhydrous octadecan-1-ol (3.0 g, 0.011 mol) and triethylamine (1.60 mL, 0.011 mol) in diethyl ether (100 mL) were slowly added

dropwise, over a period of 2 h, to phosphoryl chloride (1.04 mL, 0.011 mol) in diethyl ether (100 mL) at -78 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring for 18 h. The insoluble triethylamine hydrochloride was filtered, washing with diethyl ether (50 mL) and the filtrate evaporated under reduced pressure to yield the product as a colourless oil (4.20 g, 98%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 6.5.

<u>Oleyl phosphorodichloridate (109c).</u>

This was prepared in an analogous manner to compound **(109b)** above, thus anhydrous oleyl alcohol (3.00 g, 0.011 mmol) was reacted with phosphoryl chloride (1.04 mL, 0.011 mol). The product **(109c)** was isolated as a pale yellow oil (4.25 g, 100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 6.1.

<u>3-Amino-2-hexyloxy-4-methyl-4-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (110a).</u>

Anhydrous 2-amino-2-methyl-1,3-propanediol (1.0 g, 9.52 mmol) and triethylamine (2.64 mL, 19.04 mmol) in acetonitrile (60 mL) were added dropwise with vigorous stirring to acetonitrile (100 mL) at -70 °C under an atmosphere of nitrogen. Separately but simultaneously, compound **(109a)** (2.08 g, 9.52 mmol) in acetonitrile (40 mL) was added dropwise. The additions were carried out over 1 h, maintaining the temperature at -70 °C. The solution was warmed to ambient temperature with stirring for 20 h and the solvent then

removed under reduced pressure. The residue was extracted with diethyl ether (2 x 100 mL) and the extract evaporated under reduced pressure to yield the product as a colourless oil (2.40 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.89 (3H, t, $C\underline{H}_3CH_2$), 1.26 (3H, s, CH_3C), 1.36 (6H, m, CH_2 x 3), 1.67 (2H, m, $RC\underline{H}_2CH_2O$), 3.49 (2H, m, $C\underline{H}_2OH$), 4.07 (6H, m, RCH_2O, CH_2O, NH and OH).

^{31}P nmr δ($CDCl_3$) 23.4 and 22.9 diastereoisomers (3:2).

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.6 (C-2), 23.8, 23.9 (d, $\underline{C}H_3C$, diast., 2:3, $^3J_{C\text{-}P}$ =6.3 Hz), 25.2 (C-4), 30.27, 30.34 (2d, C-5, diast., 3:2, $^3J_{C\text{-}P}$ =7.2 and 6.8 Hz), 31.4 (C-3), 53.6 ($CH_3\underline{C}$), 60.1, 60.2 (2d, C-6, diast., 3:2, $^2J_{C\text{-}P}$ =9.5 and 9.5 Hz), 67.7, 67.9 (2d, CH_2O, diast., 2:1, $^2J_{C\text{-}P}$ 6.3 and 7.1 Hz), 73.7 (d, CH_2OH, $^3J_{C\text{-}P}$ =2.1 Hz).

3-Amino-2-octadecyloxy-4-methyl-4-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (110b).

Anhydrous 2-amino-2-methyl-1,3-propanediol (0.30 g, 2.86 mmol) and triethylamine (0.79 mL, 5.72 mmol) in acetonitrile (30 mL) were added dropwise with vigorous stirring to acetonitrile (50 mL) at -30 °C under an atmosphere of nitrogen. Separately but simultaneously, compound **(109b)** (1.11 g, 2.86 mmol) in acetonitrile (30 mL) was added dropwise. The additions were carried out over 1 h, maintaining the temperature at -30 °C. The solution was warmed to ambient temperature with stirring for 18 h and the solvent then removed under reduced pressure. The residue was extracted with diethyl ether (3 x 50 mL) and the extract evaporated under reduced pressure to yield the product as a white solid (0.80 g, 67%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.35 (35H, m, CH_3C and CH_2 x 16), 3.86 (6H, m, CH_2O x 2 and $C\underline{H}_2OH$).

^{31}P nmr δ($CDCl_3$) 23.8 and 23.3 diastereoisomers (1:2).

^{13}C nmr δ($CDCl_3$) 14.1 (C-1), 22.7 (C-2), 23.8, 23.9 (2d, $\underline{C}H_3C$, diast. 1:1, $^3J_{C\text{-}P}$ =2.3 and 4.4 Hz), 25.5 (C-16), 29.2-30.0 (m, C-5 to C-15), 30.3, 30.4 (2d, C-17, diast., 2:3, $^3J_{C\text{-}P}$ =6.5 and 6.7 Hz), 31.9 (C-3), 54.6 ($CH_3\underline{C}$), 60.2, 60.4 (2d, C-18, diast., 3:2, $^2J_{C\text{-}P}$ =9.3 and 9.3 Hz), 67.8, 67.9 (2d, CH_2O, diast., 1:1, $^2J_{C\text{-}P}$ =3.9 and 6.7 Hz), 73.1 (d, CH_2OH, $^3J_{C\text{-}P}$ =2.1 Hz).

FAB MS m/e 420 (MH^+, 11%), 248 (2), 179 (7), 169 (4), 168 (base peak), 167 (MH^+- $C_{18}H_{35}$, 0.4), 166 (M^+- $C_{18}H_{35}$, 4), 154 (12), 152 (6), 151 (MH^+- $C_{18}H_{35}O$, 3), 150 (M^+- $C_{18}H_{35}O$, 33), 149 (4), 138 (14), 137 (11), 136 (49), 120 (5), 107 (8), 106 (6), 105 (4), 99 (19), 98 (20), 88 ($CH_3C(CH_2OH)CH_2NH_2$, 82).

3-Amino-2-oleyloxy-4-methyl-4-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (110c).

This was prepared in an analogous manner to compound **(110b)** above except that the product was isolated by hexane trituration. Thus 2-amino-2-methyl-1,3,2-propanediol (0.41 g, 3.90 mmol) was reacted with compound **(109c)** (1.50 g, 3.90 mmol) gave the product **(110c)** as a pale yellow oil (1.60 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.81 (3H, t, $C\underline{H}_3CH_2$), 1.26 (25H, m, CH_3C and CH_2 x 11), 1.58 (2H, m, $RC\underline{H}_2CH_2O$), 1.94 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.07 (1H, s_b, OH), 3.44 (2H, m, $C\underline{H}_2OH$), 3.99 (4H, m, RCH_2O and CH_2O), 5.26 (2H, m, CH=CH).

^{31}P nmr δ($CDCl_3$) 23.5 and 22.7 diastereoisomers (3:2).

^{13}C nmr δ($CDCl_3$) 14.0 (C-1), 22.5 (C-2), 23.8, 23.9 (2d, $\underline{C}H_3C$, diast. 1:1, $^3J_{C-P}$ =2.3 and 1.7 Hz), 25.4 (C-16), 27.1 (C-8 and C-11), 28.7 - 29.9 (C-4 to C-7 and C-12 to C-15), 30.0, 30.2 (2d, C-17, diast., 2:3, $^3J_{C-P}$ =4.9 and 6.0 Hz), 31.8 (C-3), 53.4 ($CH_3\underline{C}$), 59.9, 60.1 (2d, C-18, diast., 2:3, $^2J_{C-P}$ =6.7 and 6.0 Hz), 67.3, 67.5 (2d, CH_2O, diast., 1:1, $^2J_{C-P}$ =6.6 and 6.9 Hz), 73.39, 73.43 (2d, CH_2OH, diast., 2:3, $^3J_{C-P}$ =2.2 and 5.8 Hz), 129.5 (C-9 or C-10), 129.7 (C-9 or C-10).

Reaction of Compound (110a) with Phosphorus Trichloride. 1st Attempt.

Compound **(110a)** (0.50 g, 1.99 mmol) and triethylamine (0.58 mL, 4.18 mmol) in dichloromethane (50 mL) were added dropwise with vigorous stirring to dichloromethane (50 mL) at -40 °C under an atmosphere of nitrogen. Separately but simultaneously, phosphorus trichloride (0.27 g, 1.99 mmol) in dichloromethane (30 mL) was added dropwise. The solution was warmed to ambient temperature with stirring for 3 h and the solvent then removed under reduced pressure. The residue was extracted with diethyl ether (3 x 50 mL) and the extract evaporated under reduced pressure to yield a colourless oil (1.20 g, >100%).

^{31}P nmr δ($CDCl_3$) 22.1, 3.8, -0.6, -0.7 and -3.7 (1:1:2:2:5).

Reaction of Compound (110a) with Phosphorus Trichloride. 2nd Attempt.

Compound **(110a)** (0.50 g, 1.99 mmol) and triethylamine (0.58 mL, 4.18 mmol) in benzene (30 mL) were added dropwise with vigorous stirring to benzene (50 mL) at -10 °C under an atmosphere of nitrogen. Separately but simultaneously, phosphorus trichloride (0.27 g, 1.99 mmol) in benzene (30 mL) was added dropwise. The reaction mixture was warmed to ambient temperature with stirring for 2 h and the insoluble triethylamine hydrochloride filtered, washing with benzene (30 mL). The filtrate was evaporated under reduced pressure to yield a white oil (0.58 g, 92%).

^{31}P nmr δ($CDCl_3$) 162.5, 161.9, 159.3, 158.8, 21.8, 21.3, 14.2, -3.9, -4.4 and -15.5 (2:2:2:2:2:2:2:4:1:1).

Reaction of Compound (110a) with Phosphorus Trichloride. 3rd Attempt.

Compound **(110a)** (0.25 g, 1.00 mmol) was azeotroped with toluene and then added dropwise with triethylamine (0.30 mL, 2.09 mmol) in benzene (20 mL) were added dropwise with vigorous stirring to benzene (30 mL) at -30 °C under an atmosphere of nitrogen. Separately but simultaneously, phosphorus trichloride (0.09 mL, 1.00 mmol) in benzene (20 mL) was added dropwise. The reaction mixture was warmed to ambient temperature with stirring for 2 h and the insoluble triethylamine hydrochloride filtered, washing with benzene (30 mL). The filtrate was evaporated under reduced pressure to yield a white oil (0.30 g, 95%).

^{31}P nmr δ($CDCl_3$) 162.5, 161.9, 159.3, 158.8, 14.3, 13.8, -4.5, -6.9 and -14.7 (1:1:1:1;3:2:1:1:1).

Reaction of Compound (110a) with Hexyl phosphorodichloridite (105).

Compound **(110a)** (0.25 g, 0.99 mmol) and triethylamine (0.30 mL, 2.10 mmol) in dichloromethane (20 mL) were added dropwise with vigorous stirring to dichloromethane (20 mL) at -60 °C under an atmosphere of nitrogen. Separately but simultaneously, compound **(106)** (0.20 g, 1.01 mmol) in dichloromethane (20 mL) was added dropwise. The solution was warmed to ambient temperature with stirring for 2 h and the solvent then removed under reduced pressure. The residue was extracted with diethyl ether (3 x 50 mL) and the extract evaporated under reduced pressure to yield a pale yellow oil (0.49 g, >100%).

^{31}P nmr δ($CDCl_3$) 139.3, 138.8, 23.9, 22.2, 22.1, 21.9, 21.8, 21.0, 17.5, 6.9, 6.1, 3.0, -3.4 and -12.2 (2:2:1:2:2:4:2:4:4:2.5:2:4:5:5:1:1).

The crude product was chromatographed on silica (20 g), eluting with 5% methanol in chloroform. Appropriate fractions were pooled and evaporated under reduced pressure to yield a pale yellow oil.

^{31}P nmr δ($CDCl_3$) 142.3, 141.1, 19.3, 18.6, 7.7 and 5.7 (2:2:3:3:1:3).

Reaction of Compound (110a) with Hexyl phosphorodichloridate (109a). 1st attempt.

Compound **(110a)** (0.29 g, 1.16 mmol) and triethylamine (0.40 mL, 2.44 mmol) in dichloromethane (15 mL) were added dropwise with vigorous stirring to dichloromethane (20 mL) at -20 °C under an atmosphere of nitrogen. Separately but simultaneously, compound **(109a)** (0.25 g, 1.16 mmol) in dichloromethane (15 mL) was added dropwise. The solution was warmed to ambient temperature with stirring for 23 h and the solvent then removed under reduced pressure. The residue was extracted with carbon tetrachloride (2 x 50 mL) and the extract evaporated under reduced pressure to yield a pale yellow oil (0.65 g, >100%).

^{31}P nmr δ($CDCl_3$) 22.3, 22.2, 21.6, 21.3, 21.0, 9.9, 9.5, 9.3, -2.8, -3.2, -7.7, -14.0, -14.5, -14.8, -26.9 (1:1:3:1:1:5:2:2:1:3:2.5:2:2:1).

Reaction of Compound (110a) with Hexyl phosphorodichloridate (109a). 2nd attempt.

Compound **(110a)** (0.50 g, 4.76 mmol) and triethylamine (2.60 mL, 19.04 mmol) in acetonitrile (50 mL) were added dropwise with vigorous stirring to acetonitrile (50 mL) at -70 °C under an atmosphere of nitrogen. Separately but simultaneously, compound **(109a)** (2.08 g, 9.52 mmol) in acetonitrile (20 mL) was added dropwise. The solution was warmed to ambient temperature with stirring for 24 h and the solvent then removed under reduced pressure. The residue was extracted with diethyl ether (3 x 100 mL) and the extract

evaporated under reduced pressure to yield a white oil (1.97 g, >100%).

^{31}P nmr δ($CDCl_3$) 23.5, 21.8, -7.8, -8.9, -10.7, -13.4 (1.5:1:1.5:2:8:3).

Water Mediated Hydrolysis of (110a).

Compound **(110a)** (0.50 g, 1.99 mmol) was dissolved in water (10 mL) and stirred at ambient temperature for 1 h. The reaction mixture was then lyophilised to yield the product as a white solid (0.53 g, 100%).

^{1}H nmr δ(d_6-DMSO) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.13 (3H, m, CH_3C), 1.24 (6H, m, CH_2 x 3), 1.51 (2H, m, $RC\underline{H}_2CH_2O$), 3.36 (2H, m, $C\underline{H}_2OH$), 3.66 (4H, m, RCH_2O and CH_2O), 8.25 (1H, s_b, NH/OH).

^{31}P nmr δ(D_2O) 0.9.

^{13}C nmr δ(CH_3OD) 13.6 (C-1), 17.4 ($\underline{C}H_3C$), 22.9 (C-2), 25.7 (C-4), 30.9 (d, C-5, $^{3}J_{C\text{-}P}$ =7.5 Hz), 31.9 (C-3), 58.0 (d, $CH_3\underline{C}$, $^{3}J_{C\text{-}P}$ =7.7 Hz), 63.6 (s_b, C-6), 66.2 (d, CH_2O, $^{2}J_{C\text{-}P}$ =6.0 Hz), 66.9 (CH_2OH).

FAB MS m/e 270 (MH^+, 3%), 252 (M^+- OH, 8), 168 (M^+- $C_6H_{13}O$, 10), 154 (5), 150 (4), 137 (2), 107 (4), 106 (MH^+- $C_6H_{13}O_3P$, 2), 105 (M^+- $C_6H_{13}O_3P$, 2), 99 (14), 89 (12), 88 (45), 57 (CH_3CNHCH_2, 16), 56 (16), 55 (22), 27 (base peak).

Analysis Found: C, 42.29; H, 8.90; N, 4.98; P, 11.00%; $C_{10}H_{24}NO_5P$ requires C, 44.60; H, 8.98; N, 5.20; P, 11.50%; $C_{10}H_{24}NO_5PH_2O$ requires C, 41.81; H, 9.12; N, 4.88; P, 10.78%.

$\upsilon_{max}(CH_2Cl_2)$ 3611, 3270, 3013, 2924, 2894, 2433, 2397, 1636, 1520, 1475, 1209 (P=O), 1047 cm^{-1}.

Water Mediated Hydrolysis of (110b).

Compound **(110b)** (0.15 g, 0.04 mmol) was dissolved in water (10 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised to yield the product as a white solid (0.16 g, 100%).

^{1}H nmr $\delta(CDCl_3)$ 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.52 (35H, m, CH_2 x 16 and CH_3C), 3.10 (2H, m, $C\underline{H}_2OH$), 3.95 (4H, m, RCH_2O and CH_2O).

^{31}P nmr $\delta(CDCl_3)$ -2.3.

^{13}C nmr $\delta(CDCl_3)$ 14.1 (C-1), 18.0 ($\underline{C}H_3C$), 22.7 (C-2), 25.8 (C-16), 29.4-29.5 (m, C-4 to C-16), 29.7 (d, C-17, $^3J_{C-P}$ =7.6 Hz), 31.9 (C-3), 57.8 ($CH_3\underline{C}$), 62.2 (s_b, C-18), 66.2 (s_b, CH_2O), 66.9 (CH_2OH).

FAB MS m/e 460 (MNa^+, 3%), 438 (MH^+, 2), 373 (3), 176 (16), 168 (M^+-$C_{18}H_{37}O$, 2), 154 (35), 152 (4), 150 (3), 149 (4), 138 (9), 137 (19), 136 (41), 121 (13), 107 (20), 91 (17), 90 (15), 89 (M^+- $C_{18}H_{37}OPO_3$, 31), 88 ($CH_3C(CH_2OH)CH_2NH_2$, base peak).

Analysis Found: C, 55.54; H, 10.84; N, 2.58; P, 6.22%; $C_{22}H_{48}NO_5P$ requires C, 64.98; H, 11.90; N, 3.44; P, 7.62%; $C_{22}H_{48}NO_5P[H_2O]_{2.0}$ requires C, 55.79; H, 11.07; N, 2.96; P, 6.54%.

$\upsilon_{max}(CH_2Cl_2)$ 3258, 3055, 2924, 2852, 1622, 1550, 1463, 1418, 1200 (P=O), 1068, 1047 cm^{-1}.

Water Mediated Hydrolysis of (110c).

Compound **(110c)** (1.60 g,3.84 mmol) was dissolved in THF/H_2O (1:3, 12 mL) and stirred at ambient temperature for 4 h. The reaction mixture was then lyophilised to yield the product as a white solid (1.60 g, 100%).

^{1}H nmr δ($CDCl_3$) 0.86 (3H, t, $C\underline{H}_3CH_2$), 1.31 (27H, m, CH_2 x 12 and CH_3C), 1.98 (4H, m, $C\underline{H}_2CH{=}CHC\underline{H}_2$), 3.05 (2H, m, $C\underline{H}_2OH$), 3.95 (4H, m, RCH_2O and CH_2O), 5.30 (2H, m, CH=CH), 8.30 (2H, s_b, NH/OH).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) -0.1.

^{13}C nmr δ($CDCl_3$) 13.9 (C-1), 17.7 ($\underline{C}H_3C$), 22.4 (C-2), 25.6 (C-16), 26.9 (C-8 and C-11), 28.7-30.3 (m, C-4 to C-7 and C-12 to C-15), 30.4 (C-17), 31.6 (C-3), 57.2 ($CH_3\underline{C}$), 62.1 (s_b, C-18), 66.1 (d, CH_2O, $^2J_{C\text{-}P}$ =5.3 Hz), 66.8 (CH_2OH), 129.5 (C-9 or C-10), 129.6 (C-9 or C-10).

FAB MS m/e 436 (MH^+, 3%), 179 (1), 168 (M^+- $C_{18}H_{35}O$, 4), 154 (12), 150 (3), 149 (4), 136 (12), 123 (3), 109 (5), 108 (2), 107 (8), 106 (MH^+- $C_{18}H_{35}PO_3$, 6), 105 (M^+- $C_{18}H_{35}PO_3$, 5), 102 (base peak), 99 (13), 88 ($CH_3C(CH_2OH)CH_2NH_2$, 72).

Analysis Found: C, 58.63; H, 10.66; N, 2.89; P, 7.01%; $C_{22}H_{46}NO_5P$ requires C, 64.98; H, 10.64; N, 3.22; P, 7.11%; $C_{22}H_{46}NO_5PH_2O$ requires C, 58.25; H, 10.67; N, 3.09; P, 6.83%.

υ_{max}(CH_2Cl_2) 3252, 2924, 2852, 2589, 2439, 1625, 1538, 1460, 1218 (P=O), 1056 cm^{-1}.

CHAPTER 6

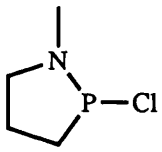
The Synthesis of Phosphonates as Phospholipid Analogues.

The Synthesis of Phosphonates as Phospholipid Analogues.

6.1. Introduction.

Replacement of an oxygen-phosphorus bond with a carbon-phosphorus bond is a common strategy used in attempts to overcome hydrolytic lability. In phospholipid analogues, it would presumably result in their non-recognition by the phospholipase enzymes PLC and PLD.[154,155]

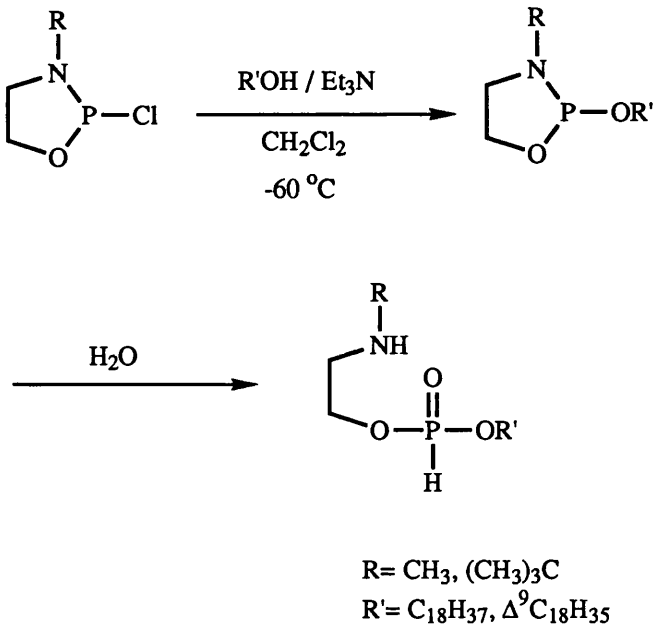
In an effort to synthesise phospholipid analogues in which the oxygen of the head group is replaced with a methylene group, attempts were made to prepare a suitable phosphitylating agent **(120)**, which could be used in the established phosphoramidite route.



(120)

It was also decided to investigate the possibilities of adapting the current phosphoramidite route to produce H-phosphonate derivatives which might have interesting biological activity since P-H centres are known to be particularly active.[156] This adaptation would involve hydrolysis of the phosphite intermediate and is outlined in scheme 21.

Scheme 21.



R= CH_3, $(CH_3)_3C$
R'= $C_{18}H_{37}$, $\Delta^9C_{18}H_{35}$

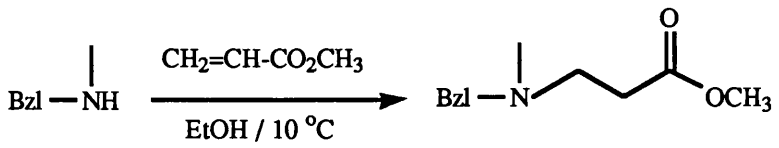
Once formed, the H-phosphonate intermediate could be converted to a more natural phospholipid *via* oxidation with iodine in water[103,156] or into a thio or seleno analogue using sulphur or selenium as the oxidant, respectively. These latter conversions have been successfully executed by Lindh and Stawinski,[156] so it would be interesting to compare this alternative route to the phosphoramidite route described in previous chapters.

6.2. Results and Discussion.

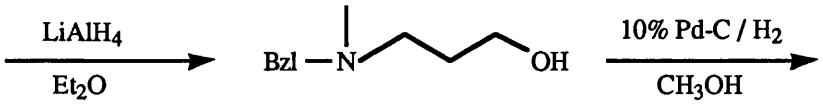
6.2.1. Cyclisation Reaction.

The starting material required for the cyclisation reaction i.e. N-methyl-3-bromopropylamine hydrobromide **(118)** was not readily available and was synthesised using the method described by Parcell and Hauck,[157] which is outlined in scheme 22.

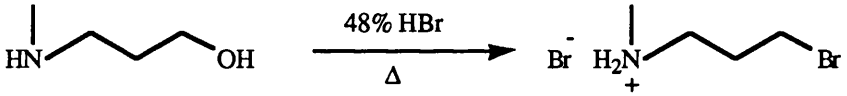
Scheme 22.



(115)

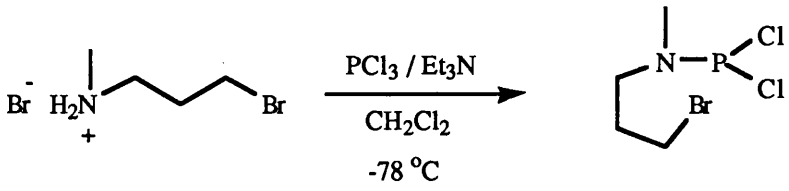


(116)



(117) **(118)**

The hydrobromide salt **(118)** was then reacted with a molar equivalent of phosphorus trichloride, at low temperature, in the presence of a slight excess of two molar equivalents of triethylamine base, i.e. one to free the base and the second to facilitate proton abstraction from the amine.



(118) **(119)**

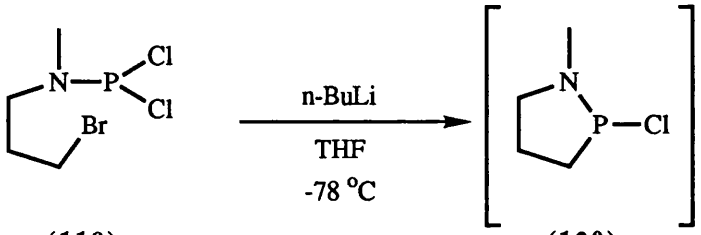
The product was obtained in an 89% yield following benzene extraction and characterised by ^{31}P nmr spectroscopy. The latter

revealed a chemical shift at δ164.5 which is consistent with phosphite species of this type.

In order to bring about the cyclisation of compound **(119)**, generation of a carbanion at the methylene adjacent to the bromine, by proton abstraction, would be necessary or formation of a Grignard reagent. Then, subsequent cyclisation by coupling between the phosphorus and the carbanion or the negatively polarised carbon, in the case of the Grignard reagent, followed by elimination of bromine and chlorine could be possible.

It was envisaged that this reaction could be brought about by using n-butyl lithium or by generating a Grignard reagent. However, the reactivity of the phosphite product would not permit the general isolation procedures of aqueous extraction to remove the metal salt by-products. Instead, it was hoped that the product could be isolated by distillation.

In the first attempt at the cyclisation, n-butyl lithium was added to compound **(119)** at low temperature.

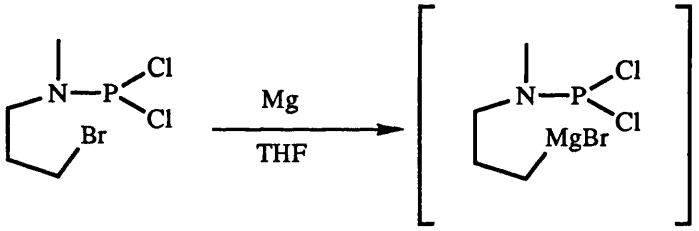


(119) **(120)**

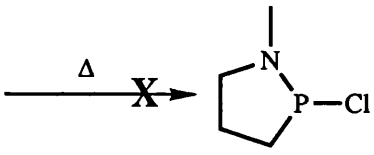
The product was isolated by diethyl ether extraction and characterised in its crude form by ^{31}P nmr spectroscopy. Three signals were observed at δ164.3, 55.4 and 23.6, in a ratio of 1:10:3. The former signal represents unreacted **(119)**, whilst the next is

presumably the desired product **(120)**. The signal at δ23.6 is presumably the result of oxidation of the product **(120)** or the starting material **(119)**, since this region is characteristic of phosphate species. Attempted purification by short path distillation under high vacuum failed to give the desired product even after heating to 250 °C at pressures of 0.05 mmHg.

As an alternative, the cyclisation reaction was attempted *via* a Grignard intermediate. It was hoped that this might yield the desired product in a form that would not require further purification other than the removal of the magnesium salt by-products. Thus, compound **(119)** was reacted with magnesium turnings and refluxed for 6 hours.



(119)



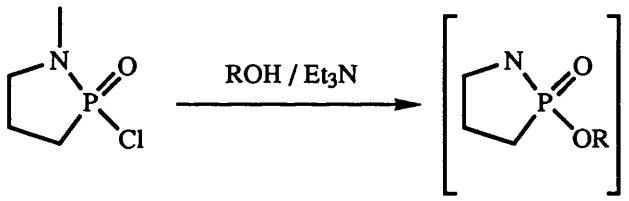
(120)

The product was isolated by dichloromethane extraction and characterised by ^{31}P nmr spectroscopy. Five signals were observed at δ80.2, 78.7, 67.6, 24.9 and 21.1 in a ratio of 10:3:8:1:3. The signals between δ80.2 and 67.6 would appear to represent phosphite species, whilst those at δ24.9 and 21.1 are presumably of phosphate nature.

Again attempted purification of the crude product by short path distillation under high vacuum failed to give the desired product **(120)** even after heating to 250 °C at pressures of 0.02 mmHg.

Thus it appeared that the former reaction involving n-butyl lithium was cleaner but isolation of the phosphitylating agent **(120)** in a pure form from either reaction was not possible by high vacuum distillation. Future work might entail repeating the n-butyl lithium method, ensuring complete reaction of the starting material **(119)**, and then oxidising the reaction mixture to give the phosphate **(121)**. This could then be condensed with alcohol to give a phosphate triester, as shown in scheme 23.

Scheme 23.

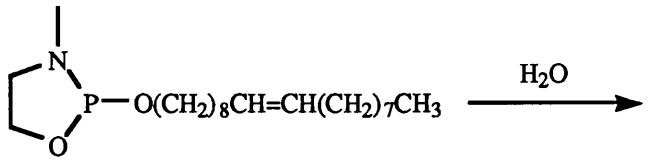


(121)

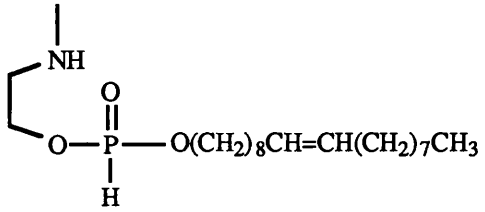
6.2.2. H-Phosphonates.

The synthesis of the phosphite intermediates **(80e)** and **(90c)** were discussed in previous chapters.

Compound **(80e)** was hydrolysed by stirring in water at ambient temperature for 3 hours.



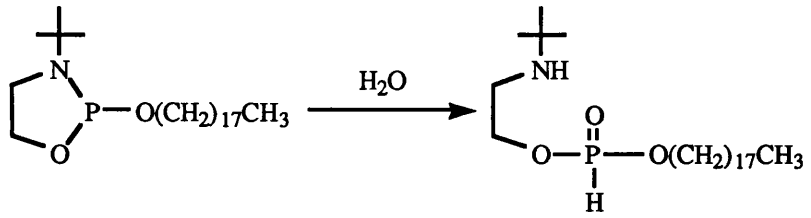
(80e)



(122e)

The product was isolated by lyophilisation and characterised by ^{31}P nmr spectroscopy. Two signals were observed at δ8.0 and 4.9 in a ratio of 1:10. The former is a minor product of unknown origin, most probably a phosphate on account of its ^{31}P nmr shift, whilst the latter signal represents the desired H-phosphonate product **(122e)**. The product was purified by column chromatography and isolated in a 54% yield. The decoupled ^{31}P nmr spectrum of the product revealed a single signal at δ4.9, which is consistent with H-phosphonate species e.g. $(CH_3CH_2CH_2O)_2P(O)H$ δ7.6. [158] Whilst in the coupled spectrum this signal was split into a quartet at δ15.0 and a quartet at δ-4.1. A coupling constant of 578 Hz was observed which is consistent with H-phosphonates[133] and supports the proposed structure of the product.

Compound **(90c)** was similarly hydrolysed.

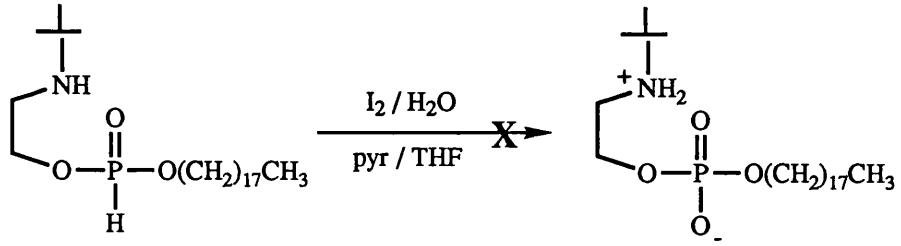


(90c) (123c)

The product was isolated by lyophilisation and characterised by ^{31}P nmr spectroscopy. The latter showed the presence of phosphorus impurities and so the product was purified by column chromatography. The product **(123c)** was obtained in 64% yield and was characterised by ^{31}P nmr spectroscopy, which revealed a signal of chemical shift δ5.4.

6.2.3. Oxidation and Sulphurisation.

The attempted oxidation of compound **(123c)** was carried out using iodine and water.[103] Thus compound **(123c)** in pyridine and THF was treated with iodine in pyridine and water.

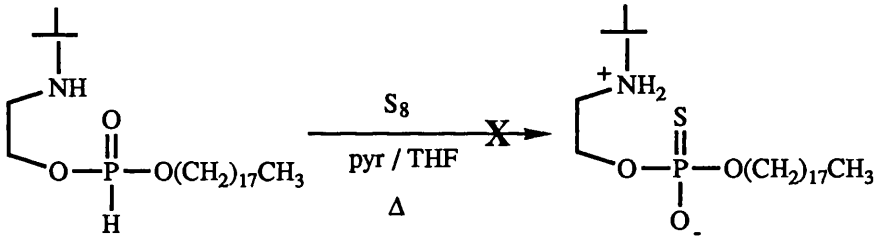


(123c) (124c)

The product was isolated by aqueous extraction after 4 hours, although Lindh and Stawinski[156] found their oxidation was complete in 5 minutes. The product was characterised by ^{31}P nmr spectroscopy which revealed a signal at δ4.9 only. This would appear

to indicate that no oxidation had occured, unless the oxidised product had been lost during the aqueous isolation procedure.

Similarly, sulphurisation of compound **(123c)** was pursued using the method reported by Lindh and Stawinski,[156] in which sulphurisation of an H-phosphonate intermediate was complete after 2 hours at ambient temperature. Thus compound **(123c)** was treated with elemental sulphur and the reaction followed by TLC. After 6 hours no appreciable reaction had occured so a further portion of sulphur was added and the reaction mixture refluxed for 20 hours.[159]



(123c) **(125c)**

^{31}P nmr spectroscopy of the product revealed a single signal at δ2.6, which indicated the reaction had failed since the chemical shift for a sulphur analogue such as **(125c)** would occur at *ca* δ55.

Thus it would seem from preliminary investigations, that a route involving H-phosphonates as an alternative to the phosphoramidite route gives poorer results in terms of ease of isolation and yields of intermediates. It also would appear that the phosphite intermediates are more receptive to oxidation than the H-phosphonate intermediates.

6.3. Experimental.

3-(N-benzyl-N-methylamino) propanol (116).

Methylacrylate (17.8 g, 0.26 mol) was added to N-benzylmethylamine (25.0 g, 0.21 mol) in ethanol (40 mL) at 10 °C. The solution was stirred at 0 °C for 2 h and then at ambient temperature for 24 h. The solvent was removed under reduced pressure and the residue dissolved in benzene (100 mL). The solvent was then evaporated under reduced pressure and the residue dissolved in diethyl ether (30 mL) and added dropwise over 3 h to a stirred slurry of lithium aluminium hydride (6.57 g, 0.17 moles) in diethyl ether (300 mL). The reaction mixture was then left to stand for 18 h. Water (7 mL), 50% sodium hydroxide solution (5 mL), followed by water (23 mL) were each added dropwise and the resulting precipitate filtered, washing with diethyl ether (2 x 25 mL). The filtrate was evaporated under reduced pressure to give a colouless oil which was vacuum distilled. The product was collected as a colourless oil (32.7 g, 89%); b.p. 98-100 °C, 0.25 mmHg.

3-N-methylamino propanol (117).

Compound **(116)** (32.7 g, 0.18 mol) in methanol (100 mL) was treated with 10% palladium on charcoal (6.54 g) and stirred for 8 h under an atmosphere of hydrogen. The reaction mixture was then filtered through kieselguhr and the filtrate evaporated under reduced pressure to give a yellow oil. The product was collected as a colourless oil (15.43 g, 95%); b.p. 120-124 °C, 12-15 mmHg.

N-methyl-3-bromopropylamine hydrobromide (118).

Compound **(117)** (15.43 g, 0.17 mol) was mixed carefully with 48% hydrobromic acid (59 mL) and the solution refluxed, using Dean and Stark apparatus, for 7 h. Water (30 mL) was removed as formed below 105 °C. The residual solution was concentrated and the residue triturated with acetone/diethyl ether (1:1, 50 mL). The product was obtained as a pale yellow crystalline solid (22.6 g, 96%).

N-methyl-(3-bromopropyl) aminophosphorodichloridite (119).

Triethylamine (2.32 mL, 0.017 mol) was added dropwise with vigorous stirring, over a period of 3 h, to compound **(118)** (1.92 g, 8.24 mmol) and phosphorus trichloride (0.72 mL, 8.32 mmol) in dichloromethane (50 mL) at -78 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature over 2.5 h and then concentrated under reduced pressure. The residue was extracted with benzene (2 x 30 mL) and the extract evaporated under reduced pressure to give a white oil (1.86 g, 89%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 164.5.

1-Chloro-2-methyl-2-azaphosphacyclopentane (120). 1st Attempt.

n-Butyl lithium (3.26 mL, 8.09 mmol of a 2.5 M solution) was added dropwise with vigorous stirring to compound **(119)** (1.86 g, 7.35 mmol) in THF (50 mL) at -78 °C under an atmosphere of nitrogen. The solution was warmed to ambient temperature with stirring over a period of 2 h and the solvent then removed under reduced pressure. The residue was extracted with diethyl ether (3 x

50 mL) and then evaporated under reduced pressure to yield a colourless oil (1.05 g, >100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 164.3, 55.4 and 23.6 (1:10:3).

Attempted short path distillation of the crude product failed to yield the desired product even after heating to 250 °C, 0.05 mmHg.

1-Chloro-2-methyl-2-azaphosphacyclopentane (120). 2nd Attempt.

Compound **(119)** (6.90 g, 0.030 mol) in THF (15 mL) was added dropwise with vigorous stirring to magnesium turnings (0.79 g, 0.033 mol) in THF (50 mL) under an atmosphere of nitrogen. The reaction mixture was refluxed for 6 h and the solvent then removed under reduced pressure. The residue was extracted with dichloromethane (3 x 50 mL) and the extract evaporated under reduced pressure to give a yellow oil (4.2 g, >100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 80.24, 78.72, 67.58, 24.88 and 21.14 (10:3:8:1:3).

Attempted short path distillation of the crude product failed to yield the desired product even after heating to 250 °C, 0.02 mmHg.

Hydrolysis of (80e).

Compound **(80e)** (0.53 g, 1.43 mmol) was suspended in water (10 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised to give a colourless gum (0.55 g, 100%).

^{31}P nmr δ($CDCl_3$) 8.0 and 4.9 (1:10).

This was further purified by column chromatography on silica (20 g) eluting with 10-20% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(122e)** as a sticky white crystalline solid (0.30 g, 54%).

^{31}P nmr δ($CDCl_3$) 4.9.

^{31}P nmr δ($CDCl_3$) coupled 15.0 (quartet) and -4.1 (quartet), J_{P-H} =578 Hz.

Hydrolysis of (90c).

Compound **(90c)** (1.14 g, 2.75 mmol) was suspended in THF/water (1:3, 12 mL) and stirred at ambient temperature for 3 h. The reaction mixture was then lyophilised to give a white gum (1.20 g, >100%).

^{31}P nmr δ(CH_2Cl_2/D_2O centre lock) 8.9, 5.6 and 2.9 (2:1:6).

This was further purified by column chromatography on silica (30 g) eluting with 5-30% methanol in chloroform. Pooling and evaporation of appropriate fractions gave **(123c)** as a sticky white crystalline solid (0.76 g, 64%).

^{31}P nmr δ(CH_3OH/D_2O centre lock) 5.4.

Attempted Iodine/water oxidation of (123c).

Compound **(123c)** (0.25 g, 0.58 mmol) in pyridine/THF (2:1, 4.5 mL) was treated with iodine (0.29 g, 1.15 mmol) in pyridine/water (4.9:0.1, 5 mL) and stirred at ambient temperature for 4 h. The

solution was diluted with chloroform (100 mL), washed with 5% sodium bisulphite solution (25 mL) and the aqueous portion extracted with chloroform (20 mL). The combined organic portion was dried ($MgSO_4$) and evaporated under reduced pressure. The pyridine was removed by co-evaporation with toluene, yielding a yellow gum (0.20 g).

^{31}P nmr δ(pyridine/D_2O centre lock) 4.9.

Attempted Sulphurisation of (123c).

Compound **(123c)** (0.27 g, 0.62 mmol) in toluene (5 mL) was treated with elemental sulphur (0.04 g, 1.25 mmol) in pyridine/toluene (1:1, 2 mL) and stirred at ambient temperature. TLC showed no reaction had occured after 6 h and a further portion of sulphur (0.06 g, 1.87 mmol) was added and the reaction mixture refluxed for 20 h. The solvent was removed under reduced pressure and the residue extracted with dichloromethane (50 mL). The extract was evaporated under reduced pressure to give a yellow oil (0.20 g).

^{31}P nmr δ(toluene/D_2O centre lock) 2.6.

CHAPTER 7

Biological Testing.

Biological Testing.

7.1. Introduction.

The implication of phospholipids in a wide range of physiological processes has lead to the interest in their potential as chemotherapeutic agents.

There is mounting evidence that changes in the lipid bi-layer of cells is intimately involved in viral infection and this has resulted in the recent evaluation of lipids[160] and phospholipid analogues[73] as a possible non-genotoxic therapy for Acquired Immunodeficiency Syndrome (AIDS).

7.2. Mode of Action as Anti-viral Agents.

The exact mode of action of phospholipid analogues against HIV is unknown. However, it is evident that viruses must perturb the membrane structure in order to penetrate (and exit from) host cells.

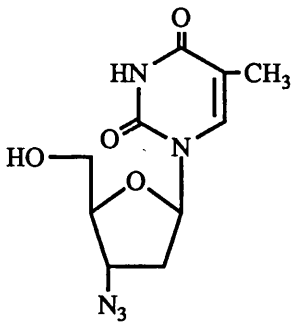
Kinchington and co-workers[160] demonstrated that infection of H9 cells with HIV 1 lead to reproducible changes in the ratio of saturated to unsaturated fatty acids in the host cell membrane, probably leading to a more fluid membrane and thus facilitating viral penetration. Moreover, they reported that 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.

Lewis *et al*[161] noted anti-viral activity at *ca* 15 μM for several phosphatidyl cholines, ethanolamines and inositols. Interestingly this group also noted that polyunsaturation of the 2-acyl chain is preferable, with the saturated phospholipids being inactive.

More recently, Kucera and co-workers reported the evaluation of novel ether lipids[73] and ether lipid nucleoside conjugates[162] against HIV 1. In both cases they noted anti-HIV activity and postulated that the phospholipids inhibit a late step in HIV replication involving virus assembly and infectious virus production.

7.3. Testing Procedures.

A number of the synthesised phospholipid analogues were evaluated against HIV at three test centres. In these studies, AZT **(126)** was used as a reference compound based on the fact that it is currently the major approved therapeutic agent for the treatment of AIDS. However, it should be noted that AZT does not represent an ideal standard for phospholipid analogues.



(126)

The following is a brief description of the test methods used at the three centres.

7.3.1. Test Centre 1, St. Marys.

Ten $TCID_{50}$ of HIV 1_{RF}/C8166 are adsorbed to 2×10^5 cells for 90 minutes at 37 °C. The cells are washed, resuspended in growth medium and cultured in 6 mL tubes with three concentrations of compound i.e. 200, 20 and 2 μM for 72 hours. The culture fluid is then assayed for the presence of the antigen p24. Dilutions ranging from 200-0.00002 μM are selected for subsequent tests. Cytotoxicity assays are carried out simultaneously with secondary evaluation of the compound i.e. cells 2×10^5 are cultured in 6 mL tubes with three concentrations of compound (200, 20 and 2 μM) for 72 hours. The cells are resuspended with ^{14}C-protein hydrolysate and after overnight incubation the incorporated ^{14}C is measured. (Value for AZT **(126)**: EC_{50}= 0.03 μM).

7.3.2. Test Centre 2, Mill Hill.

Cells are infected at room temperature with 10 $TCID_{50}$ of HIV 1_{RF}/C8166. After 90 minutes the cells are washed, resuspended in fresh medium and 100 μL volumes placed in wells of a microtitre plate containing 10 fold dilutions of the compound. After 5 days at 37 °C, syncitia are examined microscopically and p24 release measured by ELISA and virus infectivity assayed. (Value for AZT **(126)**: EC_{50}= 0.016μM).

7.3.3. Test Centre 3, Cambridge.

10^4 cells in 200 µL medium are added to each well of a microtitre plate. After 4 hours the medium is replaced with medium containing 10 fold dilutions of the compound. After overnight incubation the medium is aspirated and 10^4 $TCID_{50}$ of HIV 1_{NKR}/MOLT 4 is added. After 1 hour at 37 °C the cells are fed with medium containing dilutions of the compound. On the fourth and seventh days each well is subcultured to two new cells along with the addition of 200 µL of fresh medium plus compound. The cells are examined microscopically each day for a total of 10 days and the induction of ballooned pycnotic cells scored. Parallel control cultures are also used. (Value for AZT **(126)**: EC_{95}= 0.004µM).

7.4. Results.

The results from the three test centres (TC) are shown in the tables below as either EC_{95} or EC_{50} values (µM). The compounds were dissolved in ethanol, whilst the AZT reference was dissolved in DMSO.

KEY:

EC_x	concentration which reduces Ag gp120 by x% in infected cell cultures, where x= 50 or 95%.
TC	Test centre.
Tx	Toxic.
inact.	Inactive.
NT	Not tested.

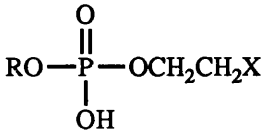


Table 20.

Compound	Alkyl (R)	Head Group (X)	TC1 (EC_{50})	TC2 (EC_{50})	TC3 (EC_{95})*
(86a)	n-C_6H_{13}	N-methyl	inact.	inact.	inact.
(92a)		*t*-butyl	40	inact.	>200
(103a)		amino	NT	inact.	
(114a)		serinol	inact.	NT	NT
(86b)	n-C_8H_{17}	N-methyl	NT	100	>200 (tx)
(86c)	n-$C_{12}H_{25}$	N-methyl	inact.	100	NT
(92b)		*t*-butyl	10	inact.	toxic
(103b)		amino	NT	NT	NT
(86d)	n-$C_{18}H_{37}$	N-methyl	25	>100	inact.
(92c)		*t*-butyl	insol.	40 (tx40)	50 (tx200)
(103c)		amino	NT	inact.	NT
(114b)		serinol	NT	16 (tx16)	30% @ 0.003(tx0.2)
(86e)	Δ^9 $C_{18}H_{35}$	N-methyl	10	10	20
(92d)		*t*-butyl	3	inact.	>200 (tx)
(103d)		amino	NT	inact.	NT
(114c)		serinol	NT	NT	NT

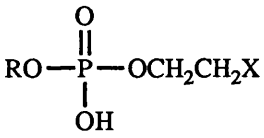


Table 20 (continued)

Compound	Alkyl (R)	Head Group (X)	TC1 (EC_{50})	TC2 (EC_{50})	TC3 (EC_{95})*
(86f)	$CH_3CH_2OC(O)CH_2$	N-methyl	inact.	NT	NT
(92e)		*t*-butyl	>200	inact.	200 (tx)
(86h)	*rac* dipalm.	N-methyl	NT	inact.	NT
(92g)		*t*-butyl	NT	inact.	NT
(86i)	*sn* dipalm.	N-methyl	NT	inact.	NT
(92h)		*t*-butyl	NT	inact.	NT
(92f)	Et-O-16	*t*-butyl	NT	inact.	NT
(86j)	*d/l* 2° octyl	N-methyl	NT	inact.	20% @ 0.04 (tx 0.1)
(92i)	*d/l* 2° octyl	*t*-butyl	NT	inact.	>200 (tx)
(92j)	*l* 2° octyl	*t*-butyl	NT	inact.	>200 (tx)
(86m)	cholesteryl	N-methyl	NT	inact.	<2 (tx 10)
(92k)		*t*-butyl	NT	inact.	>200 (tx)
(86n)	1,1 dimethyl-octadecyl	N-methyl	NT	8 (tx10)	10 (tx10)
(92l)		*t*-butyl	NT	inact.	>200 (tx)
(86o)	adamantyl	N-methyl	NT	500 (tx 1000)	>200 (tx)
(92m)		*t*-butyl	NT	inact.	>200 (tx)

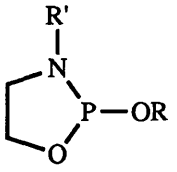


Table 21. Cyclic Phosphite intermediates.

Compound	Alkyl (R)	(R')	TC1 (EC_{50})	TC2 (EC_{50})	TC3 (EC_{95})
(80e)	Δ^9 $C_{18}H_{35}$	CH_3	NT	inact.	toxic
(80m)	cholesteryl	CH_3	NT	100 (tx 200)	20% @ 0.2
(90l)	1,1-dimethyl-octadecyl	$C(CH_3)_3$	NT	inact.	150 (tx 150)

Table 22. Cyclic Phosphate intermediates.

Compound	Alkyl (R)	(R')	TC1 (EC_{50})	TC2 (EC_{50})	TC3 (EC_{95})
(85m)	cholesteryl	CH_3	NT	inact.	inact.
(91k)		$C(CH_3)_3$	NT	inact.	150 (tx 150)
(91m)	adamantyl	$C(CH_3)_3$	NT	inact.	>200 (tx)
(110b)	n-$C_{18}H_{37}$	serinol	NT	16 (tx 16)	toxic

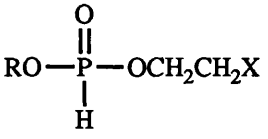


Table 23. H-Phosphonates.

Compound	Alkyl (R)	Head Group (X)	TC1 (EC_{50})	TC2 (EC_{50})	TC3 (EC_{95})
(122e)	Δ^9 $C_{18}H_{35}$	N-methyl	NT	inact.	30% @ 0.2
(123c)	Δ^9 $C_{18}H_{35}$	*t*-butyl	NT	inact.	>200

7.5. Conclusions.

Generally it was found that of the primary alkyl phospholipid analogues, there was increased activity with increasing chain length and that the presence of a double bond, i.e. the oleyl analogues, enhanced activity.

The head group also seemed to play a role in activity since even the shorter chain primary alkyl phospholipid analogues showed some activity in the *t*-butyl series compared to inactivity of the shorter chained compounds, e.g. the hexyl analogue, of the N-methyl series.

The results for the more hindered alkyl phospholipid analogues e.g. the cholesteryl, adamantyl and dimethyloctadecyl analogues varied, but generally showed toxicity along with activity. This was also noted when the cyclic phosphite and phosphate intermediates were tested. Since the phosphite intermediates and to an extent the phosphates are very reactive it is likely that the compounds hydrolysed once they were dissolved in the solvent (ethanol), thus forming either the corresponding H-phosphonates or the phospholipids themselves or a mixture of these.

Most of the results varied markedly from each test centre, although it should be stressed that these tests are designed for nucleoside analogues. (The results given are relative to the results for AZT and none of the analogues showed better activity than this compound). However, the oleyl phospholipid analogues, in particular the N-methyl phospholipid analogue **(86e)** which was

active in all three tests (refer to table 20), does show some potential as a non-toxic anti-HIV treatment.

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Appendix I.

Abbreviations.

A	Angstrom
ALP	Alkyl lysophospholipid
t-boc	Tertiary butyloxycarbonyl
Bzl	Benzyl
b.p.	Boiling point
d	Doublet
d	*Dextro*
DCC	Dicyclohexylcarbodiimide
diast.	Diastereoisomers
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
ed.	Edition
E.I.	Electron Impact
eq	Equivalents
EtI	Iodoethane
EtOH	Ethanol
FAB	Fast Atom Bombardment
g	Gramme
h	Hour(s)
Hz	Hertz
I.R.	Infra-red
J	Coupling constant
l	*Laevo*
M	Molar
m	Multiplet

mm	Millimetre
MHz	Megahertz
min.	Minutes
mL	Millilitre
mmol	Millimole
M.S.	Mass spectrometry
MTr	Methoxytrityl
nmr	Nuclear magnetic resonance
PAF	Platelet activating factor
PLA_1	Phospholipase A_1
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospholipase D
ppm	Parts per million
pyr	Pyridine
q	Quartet
R	*Rectus*
rac	Racemic
s	Singlet
s_b	Broad singlet
S	*Sinister*
sn	Stereospecific numbering
t	Triplet
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Tos	Tosyl

TPS	2,4,6-triisopropylbenzenesulphonyl chloride
TrCl	Trityl chloride

Appendix II.

Apparatus and Reagents.

All reactions, excluding hydrolyses, were carried out under scrupulously dry conditions. Benzene, dichloromethane, diethyl ether, hexane and triethylamine were dried by distillation from calcium hydride at atmospheric pressure. All but triethylamine were further dried over 4A molecular sieves. Ethanolamine, N-benzylethanolamine and N-methylethanolamine were distilled and dried over 4A molecular sieves. 2-Amino-2-methyl-1,3-propanediol was dried under high vacuum conditions. Appropriate alcohols were distilled and further dried over 4A molecular sieves. The alcohols that are solid at room temperature, including the dipalmitoyl glycerols, were dried for several days under high vacuum conditions. The alcohol 3-*O*-Hexadecyl-2-*O*-ethyl-*rac*-glycerol was repeatedly azeotroped with toluene and subjected to high vacuum for 5 days before use. 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 the unbranched alkyl chains and 1,1-dimethyloctadecyl are numbered from the terminus. The

cholesteryl and adamantanyl analogues were numbered according to the system shown in chapter 2, figure 12. The 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 a matrix, courtesy of the University of London Mass Spectrometry Service (FAB MS). Microanalyses were performed at UCL courtesy of Mr. A.T.T. Stones. Infra-red were recorded using a Perkin Elmer 983. ^{13}C NMR SPECTRA WERE ALSO OBTAINED AT 100 MHz USING A VXR400 SPECTROMETER