

**STRUCTURE AND SYNTHESIS  
OF SOME PHOSPHORUS COMPOUNDS OF POTENTIAL BIOLOGICAL ACTIVITY.**

A thesis submitted to

The University of Cape Town

in the fulfilment of the requirements of the degree of

**Master of Science**

by

**Panos Kyriacou**

**B.Sc. (HONS.) (U.C.T.)**

Department of Organic Chemistry

University of Cape Town

February 1988.

The University of Cape Town has been given  
the right to reproduce this thesis in whole  
or in part. Copyright is held by the author.

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

<u>CONTENTS</u>	Page.
Acknowledgements	(iii)
<u>PART I</u>	
Structural study of the copper complex of the N-thiophosphorylated thiourea.	1
Abstract	2
Chapter 1: Introduction.	3
1.1 Structures of Cu(I) compounds.	4
Chapter 2: Cu(I) Complex of [N-(Diethylthiophosphoryl)- N'-Phenyl Thiourea].	14
2.1 Results and discussion.	17
Chapter 3: Experimental.	30
3.1 General.	31
3.2 Reagents.	31
3.3 Preparation of the ligand (EtO) <sub>2</sub> P(S)-NH-C(S)-NHPh.	32
3.4 Crystals of [Cu <sub>10</sub> (C <sub>11</sub> H <sub>16</sub> O <sub>2</sub> N <sub>2</sub> S <sub>2</sub> P) <sub>9</sub> ]ClO <sub>4</sub> .	34
3.5 X-Ray Data collection and solution of Structure.	35
Appendix.	38
References.	42

PART II

<b>A novel synthesis of phospholipids</b>	45
<b>Abstract</b>	46
<b>Chapter 1: Introduction.</b>	47
<b>Chapter 2: Outline of proposed synthetic approach.</b>	52
<b>Chapter 3: Synthesis of aminoalcohol.</b>	56
<b>Chapter 4: Synthesis of cetyl methyl chlorophosphate.</b>	61
<b>Chapter 5: Reaction of aminoalcohol with cetyl methyl chlorophosphate.</b>	73
<b>Chapter 6: N-methylation and O-Demethylation of the neutral triester.</b>	77
<b>Chapter 7: Experimental.</b>	96
<b>References.</b>	109

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my two supervisors, Professor Tomasz A Modro and Dr Colin J Paterson-Jones, for their invaluable guidance and assistance throughout the course of this project. My thanks also to Dr Sunil Vather, for his help and encouragement, and to my friends and colleagues who contributed in any way to this thesis.

Special thanks to Dr Margaret Niven of the crystallography unit, for the determination of the crystal structure and in her assistance in helping me with the interpretation of the structure.

My sincere thanks also to Mrs Annatjie Kok for the typing of this thesis.

Lastly, I would like to thank my parents, for their tremendous support throughout my years of study.

PART I

STRUCTURAL STUDY OF THE COPPER COMPLEX OF THE N-THIOPHOSPHORYLATED

THIOUREA

**ABSTRACT**

The pale-yellow complex formed by the reaction of copper(II) and N-(O,O-diethylthiophosphoryl)-N'-phenylthiourea is shown to be a cationic Cu(I) aggregate,  $[\text{Cu}_{10}(\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2\text{P})_9]^+$ . Crystals were obtained with  $\text{ClO}_4^-$  as a counterion and the structure of the aggregate was determined by X-ray diffraction. The unit cell is triclinic, space group  $\text{P}\bar{1}$ , with  $a = 16.06(1)$ ,  $b = 22.19(1)$ ,  $c = 22.455(6)$  Å,  $\alpha = 90.16(3)$ ,  $\beta = 108.63(4)$ ,  $\gamma = 91.37(5)^\circ$ ,  $V = 7580$  Å<sup>3</sup>,  $D_m = 1.52$ ,  $D_c = 1.51$  g cm<sup>-3</sup> for  $Z = 2$ . The intensities of 8903 reflections were collected ( $\theta_{\text{max}} = 18^\circ$ ). The final weighted residual for 7249 reflections with  $I > 2\sigma I$  is 0.100. Each aggregate consists of (1) ten Cu(I) atoms arranged in an approximate tetrahedron whose sides are delineated by three copper atoms, (2) nine ligand molecules, each chelating one copper atom via two sulfur atoms, (3) one perchlorate anion neutralising the positive charge carried by a single, non-chelated copper. This  $\text{Cu(I)}_{10}\text{S}_{18}$  core represents the definitive member of a new family of copper-sulfur aggregates. Cu-Cu distances observed (3.11 - 4.06 Å) indicate the absence of any significant metal-metal bonding. Sulfur coordination varies from 1 and 2 for P=S centres to 2 and 3 for C-S<sup>-</sup> groups.

CHAPTER 1

INTRODUCTION



## 1 INTRODUCTION

### 1.1 Structures of Cu(I) compounds

Copper, in its normal chemistry, forms compounds in the oxidation states of +1 and +2, although under special circumstances some compounds of trivalent copper can be prepared.

Copper has a single s electron outside the filled 4d shell and is expected to form ions  $M^+$  by loss of the single electron in the outermost shell or to use the s and p orbitals of that shell to form collinear sp or tetrahedral  $sp^3$  bonds. The more stable ion of copper is the  $Cu^{2+}$ , although the cuprous state may be stabilised by coordination.

Copper(I) complexes are usually obtained by:

- i) direct interaction of ligands with copper(I) halides.
- ii) Reduction of corresponding copper(II) compounds.
- iii) Reduction of  $Cu^{2+}$  in the presence of or by the ligand.

Copper(I) forms numerous types of complexes which include mononuclear, binuclear and polynuclear structures, with copper being two-, three-, or four-coordinate, or forming infinite chains of atoms<sup>1</sup>.

Mononuclear species can belong to the structural types as indicated in figure 1.<sup>2</sup>

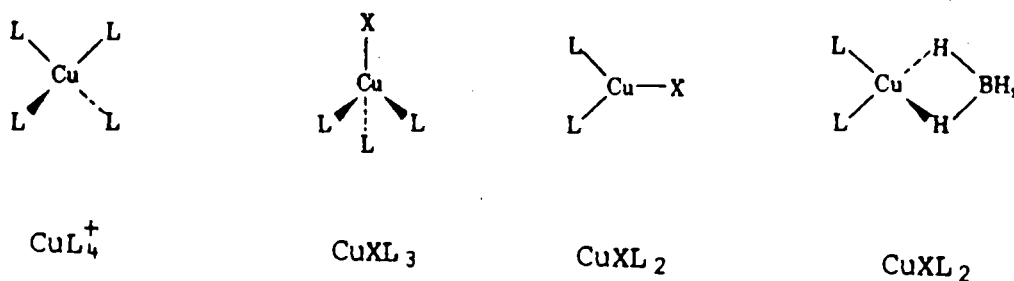


Figure 1

Examples of mononuclear<sup>3</sup> species include,  $[\text{Cu}(\text{PPh}_3)_3]\text{BF}_4$  and the tris-trimethyl phosphine sulfide Cu(I) perchlorate;  $[\text{Cu}(\text{SPMe}_3)_3]\text{ClO}_4$ .

Binuclear species are of the structure as indicated in figure 2.<sup>2</sup>

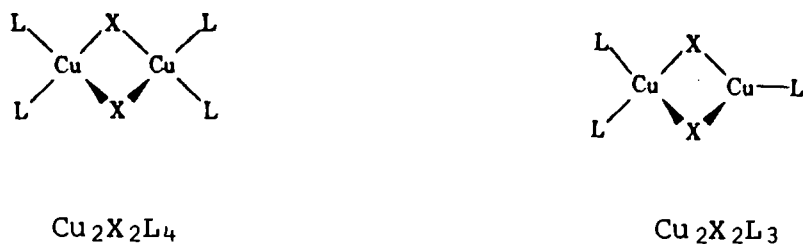


Figure 2

An example of the  $\text{Cu}_2\text{X}_2\text{L}_3$  complex which has both three- and four-coordinate copper(I) is  $\text{Cu}_2\text{Cl}_2(\text{PPh}_3)_3$ .

The tris-thiourea Cu(I) chloride complex,  $[\text{Cu}\{\text{SC}(\text{NH}_2)_2\}_3]\text{Cl}$ ,

represents an example of a chain structure, in which one of the three thiourea molecules forms a bridge between two copper atoms<sup>4</sup>. (figure 3).

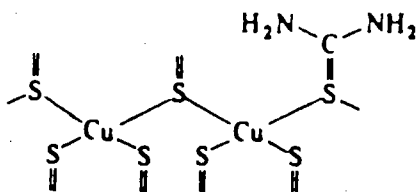


Figure 3

Another type of chain is that found in  $\text{NaCu}(\text{CN})_2 \cdot 2\text{H}_2\text{O}$ , where there is a spiral of almost planar trigonal  $\text{Cu}(\text{I})$  atoms, linked by CN bridge<sup>2</sup>. (figure 4).

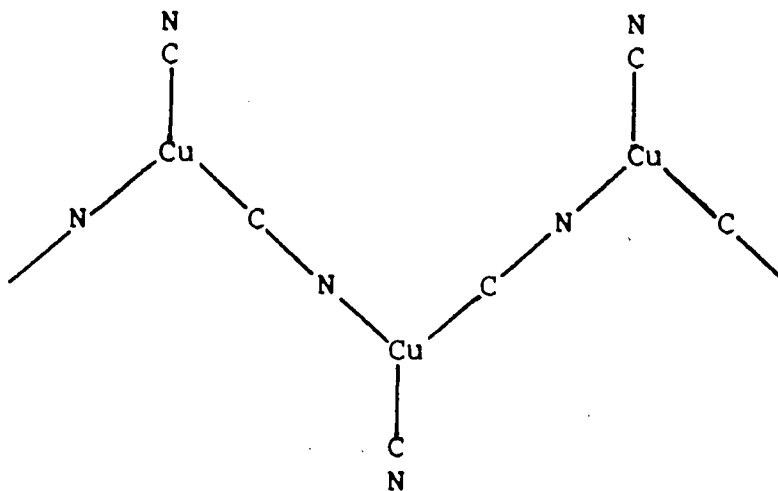


Figure 4

Tetrameric  $\text{Cu}_4(\text{I})$  complexes may have the structures in which the four copper atoms are:

- i) in a parallelogram, rectangle or a square.

- ii) most commonly at the vertices of a tetrahedron, regular or slightly distorted.
- iii) in a halogen bridged step structure.

The first tetrameric structure of importance is the  $(\text{CuXL})_4$  complex, where X is a halogen and L is usually a tertiary phosphine. For these complexes there are two limiting structures, each of which may be distorted. The first is the so called cubane structure<sup>2</sup> (figure 5), in which there is a  $\text{Cu}_4$  tetrahedron with a triply bridging halide and a ligand on each copper atom, which is four-coordinate. The second<sup>2</sup> (figure 6), has the step form with double and triple halide bridges and two four-coordinate tetrahedral and two three-coordinate trigonal copper atoms.

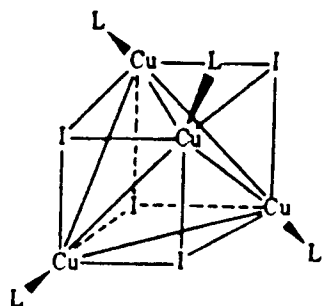


Figure 5

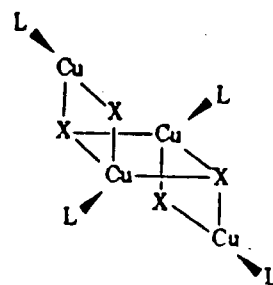


Figure 6

A specific structure of a complex depends on the sizes of the metal and halide atoms and on the steric bulk of the ligand. Copper cubanes therefore, are formed with iodide and non-bulky phosphines, and with chloride and a bulky phosphine (or arsine): eg.  $(\text{CuIAsEt}_3)_4$  and  $(\text{CuClPPh}_3)_4$ .

Step structures are found for  $(\text{CuBrPPh}_3)_4$  and  $\text{Cu}_4\text{I}_4(\text{Ph}_2\text{PCH}_2\text{CH}_2\text{PPh}_2)_2$ .

Other compounds<sup>2</sup> that have a tetrahedral or distorted tetrahedral  $\text{Cu}_4$  core are a number of sulfur complexes such as  $\text{Cu}_4(\text{S}_2\text{PPR}_2^i)_4$ ;  $[\text{Cu}_4(\mu_2\text{-SPh})_6]^{2-}$ ; and  $\{\text{Cu}_4[\text{SC}(\text{NH}_2)_2]_6\}^{4+}$ . The last two show a characteristic structure for such sulfur complexes. There is a  $\text{Cu}_4$  tetrahedron, but the atoms are bridged by sulfur to give a  $\text{Cu}_4\text{S}_6$  core that has an adamantane type structure with linked six membered  $\text{CuSCuSCuS}$  rings<sup>5</sup>.

Pentanuclear complexes are uncommon, but one example is  $[\text{Cu}_5(\mu_2\text{-SBut})_6]^-$ , which has trigonal bipyramidal  $\text{Cu}(\text{I})$  with bridging SR groups<sup>2</sup>.

Hexanuclear complexes are confined to the hydride  $[\text{CuHPPH}_3]_6 \cdot \text{Me}_2\text{NCHO}$ . It has the structure represented in figure 7. The hydrogen atoms probably lie as bridges along the six longer Cu-Cu edges<sup>2</sup>.

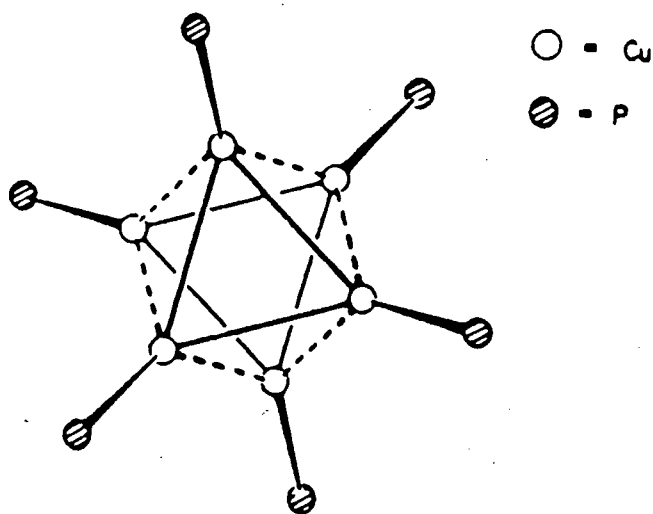


Figure 7

A family of structures attracting increasing attention is the type characterised by a  $\text{Cu(I)}_8\text{S}_{12}$  core, consisting of a cube of copper(I) atoms inside an icosahedron of sulfur atoms and found in copper(I) complexes with 1,1- and 1,2-dithiolate ligands such as 1,1-dicyanoethylene-2,2-dithiolate<sup>6</sup> or 1,2-dithiosquarate<sup>7</sup> anions.

The 1,1-dicyanoethylene-2,2-dithiolate complex,  $(\text{Cu}_8(\text{i-MNT})_6]^{4-})$ , consists of a slightly distorted cube of eight copper atoms embedded within the skeleton of 12 sulfur atoms, which lie on the vertices of a systematically distorted icosahedron. This group of 20 atoms is enclosed by six  $\text{CS}_2$  carbon atoms located at the vertices of an icosahedron.

In the 1,2-dithiosquarate complex,  $[(\text{Cu}_8(\text{DTS})_6)]^{4-}$ , the ligands bridge the six faces of the cube in a manner similar to the bonding observed for the dithiolate copper clusters. Each copper is coordinated by three sulfur atoms from different ligands with each sulfur atom coordinating two copper atoms on an edge of the cube. (figure 8).

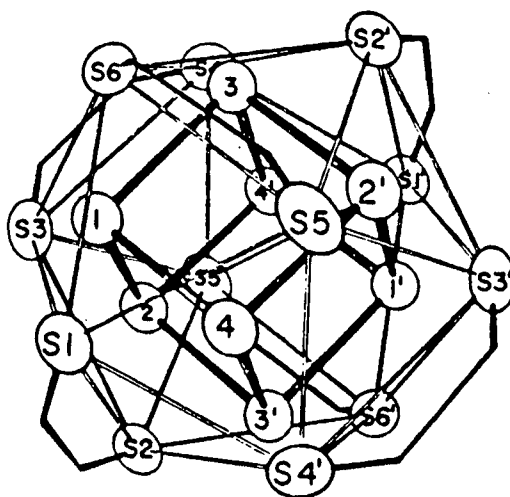


Figure 8

The same  $\text{Cu(I)}_8 \cdot \text{S}_{12}$  core has been recognised<sup>8</sup> in the structure of a  $\text{Cu(I)} \text{ Cu(II)}$  mixed valence cluster complex formed by the reaction of  $\text{Cu(II)}$  and D-penicillamine and studied in relation to the use of this ligand in the treatment of Wilsons disease. The complex crystallised as a highly hydrated thallium(I) salt and was found to consist of mixed valence

clusters with the composition  $[\text{Cu}_8^{\text{I}}\text{Cu}_6^{\text{II}}\text{L}_{12}\text{Cl}]^{5-}$  surrounded by  $\text{Tl}^+$  ions and water molecules. In each cluster there are eight copper atoms which are coordinated by three penicillamine S(thiol) atoms in an approximately trigonal planar geometry. These eight metal atoms were therefore identified as Cu(I). Two of the Cu(I) sites, as well as the  $\text{Cl}^-$  ion at the centre of the cluster, lie on a three-fold rotation axis. The remaining six copper atoms are identified as Cu(II) by their square planar coordination geometries. Every S(thiol) atom in the structure is thus bonded to one Cu(II) and two Cu(I) atoms. Figure 9 represents the Cu-Cl-N-S framework of the copper-penicillamate cluster.<sup>8</sup>

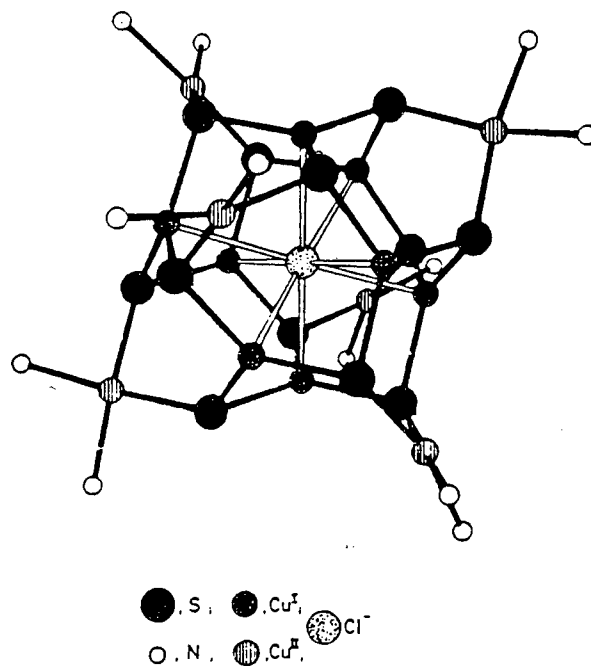
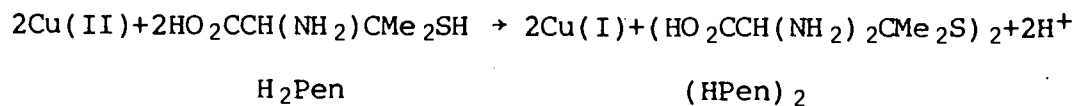


Figure 9

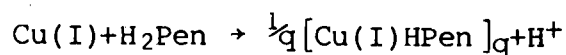


The cluster is formed in a sequence of reactions, the first of which involves reduction of Cu(II) to Cu(I) by penicillamine, H<sub>2</sub>Pen. (Equation 1).



Equation 1

The Cu(I) produced can then react with the excess of the ligand to form a yellow, polymeric 1:1 Cu(I)-HPen complex. (Equation 2).



Equation 2

In this complex, spectroscopically characterised by Sugiura and Tanaka<sup>9</sup>, the metal atoms are coordinated by the thiolate groups of the ligands and not by the amino groups, which are still in their protonated NH<sub>3</sub><sup>+</sup> form. It should be noted that the pale-yellow complex obtained in equation 2 occurs only when the penicillamine is in excess with respect to the cupric ion. However, if the cupric ion is present in equivalence or in a little excess, the mixed valence cluster is obtained.

Cu-Cu interaction in Cu(I) aggregate compounds.

It is important to note whether significant Cu(I)-Cu(I) bonding occurs in the complexes discussed.

Table I represents a comparison of the Cu(I)-Cu(I) distances in the various aggregates mentioned

Cu-complexes	Cu-Cu (Å)
metallic Cu; <sup>10</sup>	2.56
Cu <sub>4</sub> I <sub>4</sub> (AsEt <sub>3</sub> ) <sub>4</sub> ; <sup>11</sup>	2.78
[(C <sub>3</sub> H <sub>7</sub> ) <sub>2</sub> NCOSCu] <sub>6</sub> ; <sup>4</sup>	2.70-3.06
[Cu <sub>8</sub> (i-MNT) <sub>6</sub> ] <sup>4-</sup> ; <sup>6</sup>	2.83
[Cu <sub>8</sub> (DTS) <sub>6</sub> ] <sup>4-</sup> ; <sup>7</sup>	2.84
[Cu <sub>8</sub> I <sub>4</sub> Cu <sub>6</sub> II <sub>12</sub> Cl] <sup>5-</sup> ; <sup>8</sup>	3.24-3.37

TABLE I

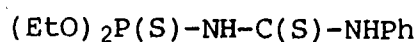
Comparing the Cu-Cu distances found in the five complexes above with that of metallic copper, it would appear that direct Cu-Cu bonding in the complexes is at best weak and possibly negligible.

Certainly, as far as the mixed valence cluster ([Cu<sub>8</sub>I<sub>4</sub>Cu<sub>6</sub>II<sub>12</sub>Cl]<sup>5-</sup>) is concerned, the Cu(I)-Cu(I) distances are so large that metal-metal bonding interactions are clearly absent.

CHAPTER 2Cu(I) COMPLEX OF [N-(DIETHYLTHIOPHOSPHORYL)-N'-PHENYL THIOUREA]

2 Cu(I) COMPLEX OF [N-(DIETHYLTHIOPHOSPHORYL)-N'-PHENYL THIOUREA]

In view of the great diversity found in the copper(I) sulfur complexes, the stoichiometry of which give little clue to their structure<sup>1,2</sup>, it was decided to investigate the reaction between cupric salts and the bifunctional neutral sulfur substrate, N-(O,O-diethyl thiophosphoryl)-N'-phenylthiourea, (1)

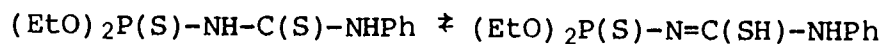


(1)

At least three reasons were seen for choosing this compound as a ligand for structural study.

Firstly, (1) contains two different sulfur centres:

- (i) the thiophosphoryl group P=S, capable of forming with Cu(I) mononuclear species<sup>2</sup> and
- (ii) the thiocarbonyl group which can act as a neutral donor centre, or due to the tautomeric equilibrium (equation 3), can produce the sulfhydryl type ligand, (1a).



(1)

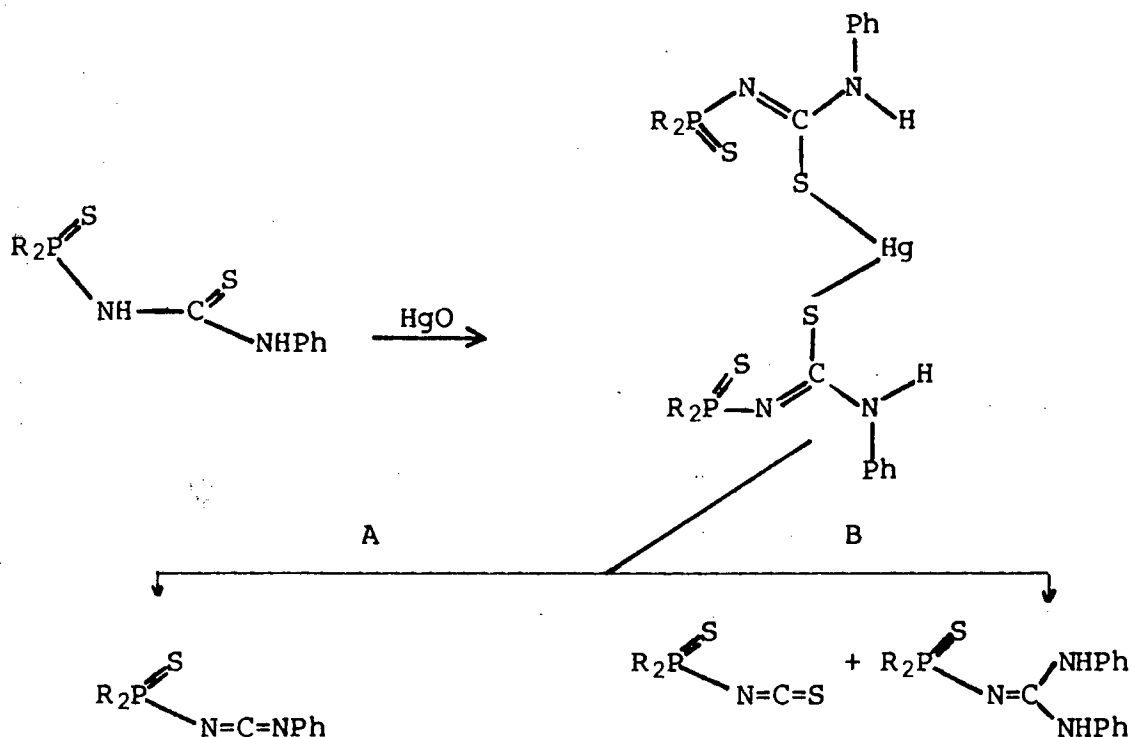
(1a)

Equation 3

Compound (1a) contains a sulfhydryl group and would be expected to reduce Cu(II) ions in a manner similar to other thiols and thus give rise to a Cu(I) - (1a) complex.

Secondly, (1) represents the 1,5-dithio system and such a geometry could result in the copper complex possessing a structure quite different from those obtained for monothio, or 1,1- and 1,2-dithio ligands.

Thirdly, it was reported by Zontova, Grapov and Mel'nikov<sup>12</sup> that (1) forms the S-mercury derivative, which easily undergoes fragmentation to N-thiophosphinyl-carbodiimides, or mixtures of N-thiophosphinyl-substituted isothiocyanates and guanidines. (Scheme 1).



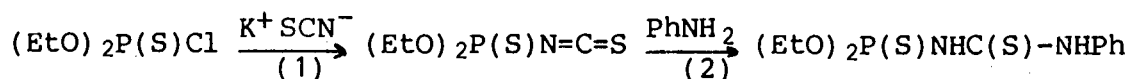
**Scheme 1**

It was therefore of interest to see what the effect of the Cu(I) complexation on the stability of the thiourea skeleton in (1) would be.

## 2.1 Results and discussion

### 2.1.1 Synthesis of (EtO)<sub>2</sub>P(S)NHC(S)-NHPh (1).

The synthesis of (1) was carried out according to the following scheme. (Scheme 2).



#### Scheme 2

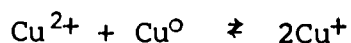
In what is representative of a substitution reaction at phosphorus, (EtO)<sub>2</sub>P(S)Cl is added to K<sup>+</sup>SCN<sup>-</sup> to form the substitution product (EtO)<sub>2</sub>P(S)N=C=S. (EtO)<sub>2</sub>P(S)NHC(S)-NHPh is then formed by an addition reaction to the N=C bond, in which aniline and (EtO)<sub>2</sub>P(S)N=C=S are heated in toluene for three hours.<sup>13</sup> The product, (EtO)<sub>2</sub>P(S)NHC(S)-NHPh, is then precipitated with cold petroleum ether. <sup>1</sup>H NMR spectra and elemental analysis were in agreement with the expected structure of (1) for the product.

### 2.1.2 Reaction of (1) with copper.

When an ethanolic solution of Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O is added at -60°C to an ethanolic solution of (1) (ligand : copper ratio > 2:1), an intensely purple solution is obtained immediately. The colour disappears after the addition is com-

plete and a pale-yellow precipitate forms. The same precipitate is directly obtained when the mixing is carried out at room or high (60°) temperature. Elemental analysis of the precipitate indicated the presence of one atom of copper in the molecule and the  $^1\text{H}$  NMR spectroscopy showed that the structure of the ligand remained intact. Evidence that a Cu(I) and not a Cu(II) derivative had been obtained, was clear from the  $^1\text{H}$  NMR spectrum, which showed well resolved peaks. Cu(II), as a paramagnetic ion, would give broad and unresolved peaks in the  $^1\text{H}$  NMR spectrum.

The observed behaviour corresponds closely to that reported<sup>8</sup> for the reaction between Cu(II) and penicillamine. i.e., fast reduction of Cu(II) to Cu(I) by the SH group of the ligand, followed by initial formation of a Cu(I)/Cu(II) mixed valence complex, (the purple colour observed), and finally precipitation of an almost colourless, polymeric Cu(I) complex. Although the conditions for complex formation were varied, (reactions were carried out at high and low temperatures, under nitrogen, varying the rates of mixing and reagent concentrations) the same product was always obtained. Unfortunately the precipitate was a powder and was therefore unsuitable for X-ray diffraction experiments. It was found however, that crystals suitable for X-ray analysis could be conveniently obtained if the reaction between Cu(II) and (1) is carried out in the presence of copper powder, which assists the formation of Cu(I) ions<sup>2</sup>. (Equation 4).



Equation 4

The crystals were prepared by dissolving the ligand (1) in ethanol and adding copper perchlorate as a solid and copper powder to the ligand-ethanol solution. The copper powder was then filtered off and the solution allowed to stand overnight. Since the crystals were found to be unstable in air, a single crystal was selected and placed rapidly in a Lindermann tube, together with the mother liquor. After sealing the tube at both ends, the crystal was mounted on a diffractometer and irradiated with  $\text{MoK}\alpha$  radiation. The copper atoms were located by direct methods using SHELXS-86<sup>14</sup> and the remaining non-hydrogen atoms of the aggregate were revealed in subsequent difference Fourier using SHELX 76<sup>15</sup>.

Figure 10, represents the structure of the Cu(I) complex obtained.

Each molecule has ten copper atoms which are coordinated to nine ligands, each one acting as a bidentate ligand, using its two sulfur atoms for chelating copper atoms. The ligand molecules have all lost one proton at NS1 (for the ligand structure and numbering of atoms, see figure 11). Electrical neutrality is then achieved by the co-crystallisation of one perchlorate anion per aggregate.

#### The arrangement of the Cu and S atoms.

Within an aggregate, Cu-Cu distances are all in the range 3.11 - 4.06 Å. As with the complexes in Table 1, there appeared to be no significant direct Cu-Cu bonding in the



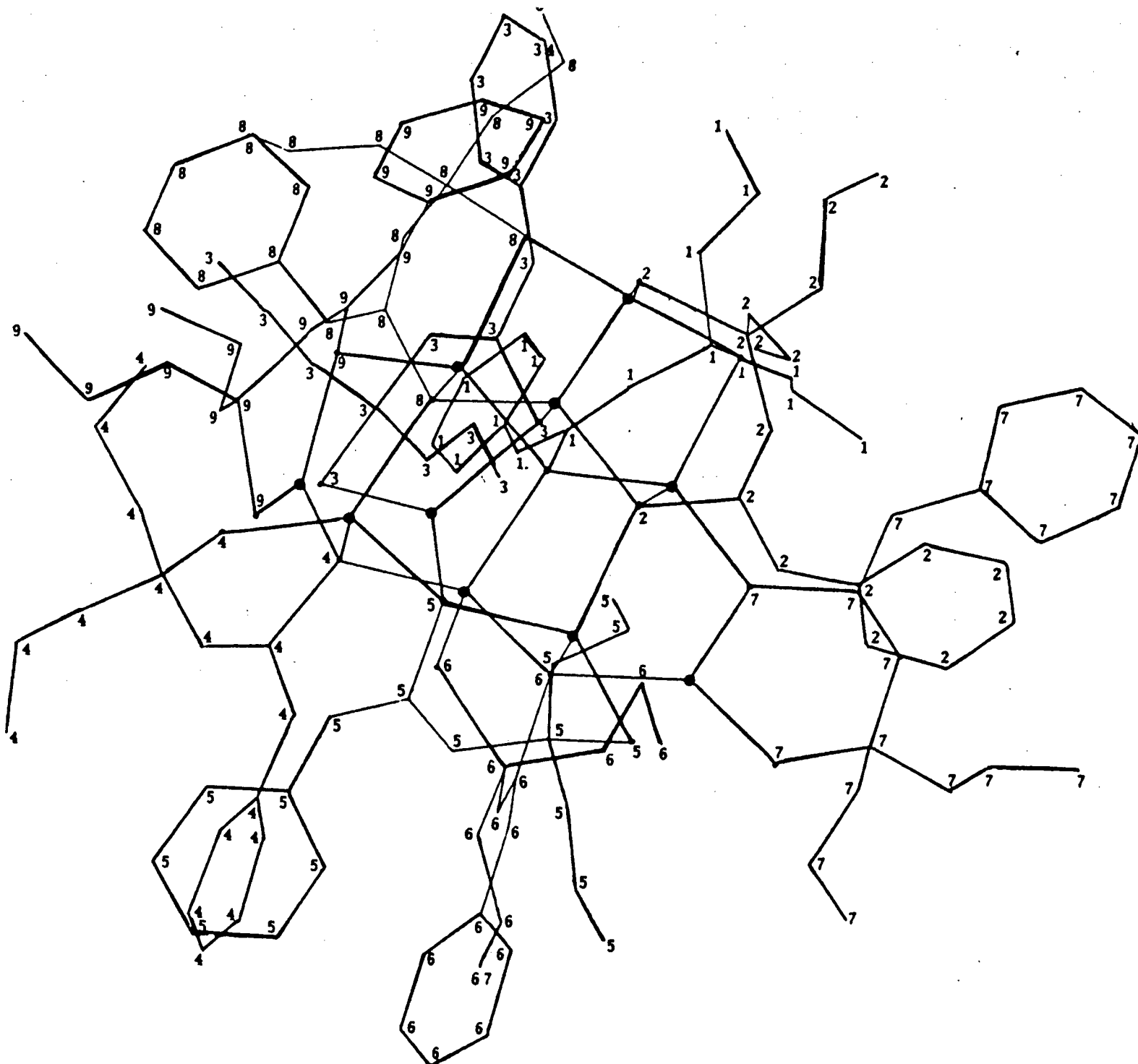
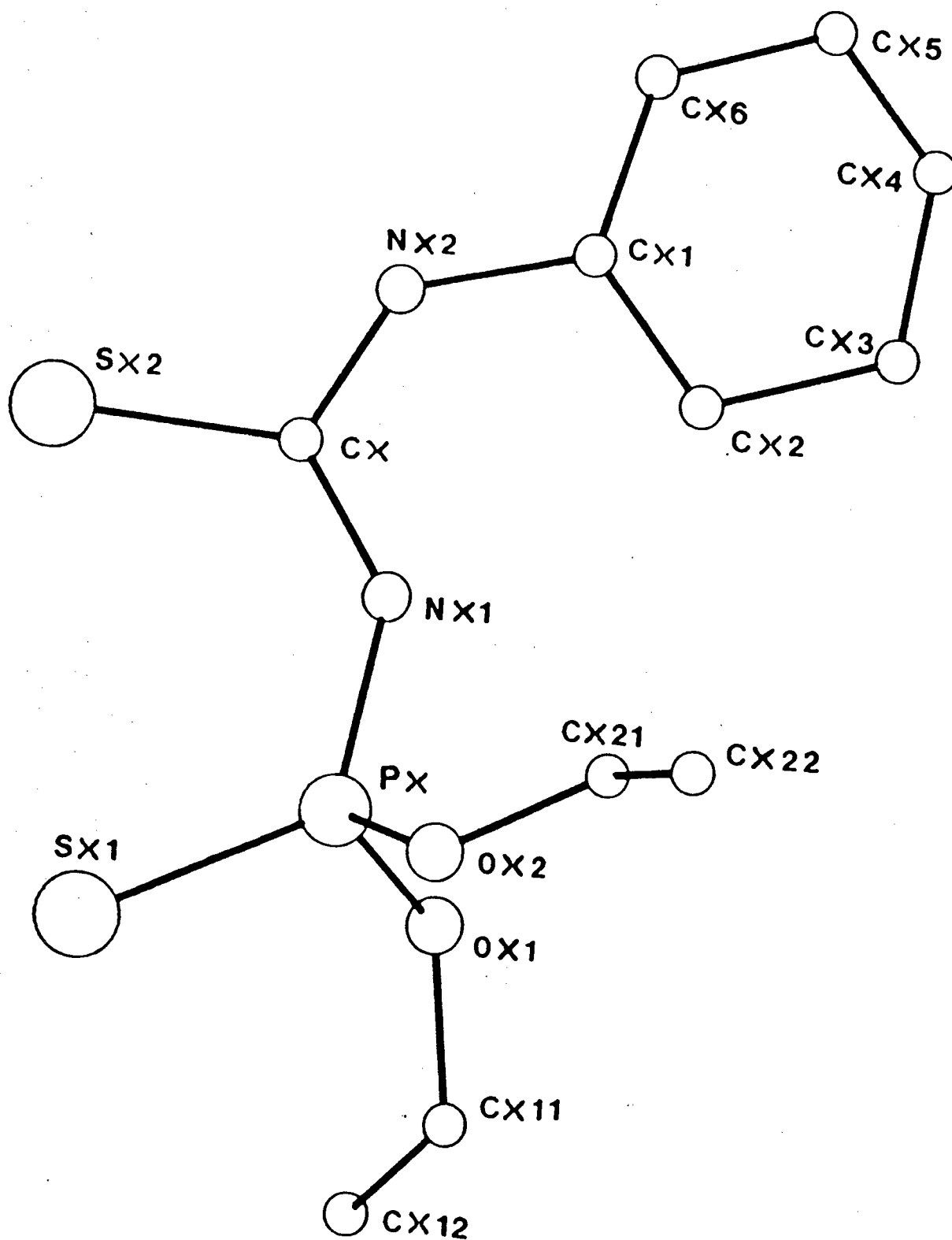


Figure 10. The  $[Cu_{10}(C_{11}H_{16}O_2N_2S_2P)_9]$  aggregate ( $ClO_4^-$  anion has been omitted for clarity). copper atoms are represented by black dots; numbers 1-9 refer to the nine ligands.

Figure 11. Structure of the ligand (1) showing the atomic numbering:



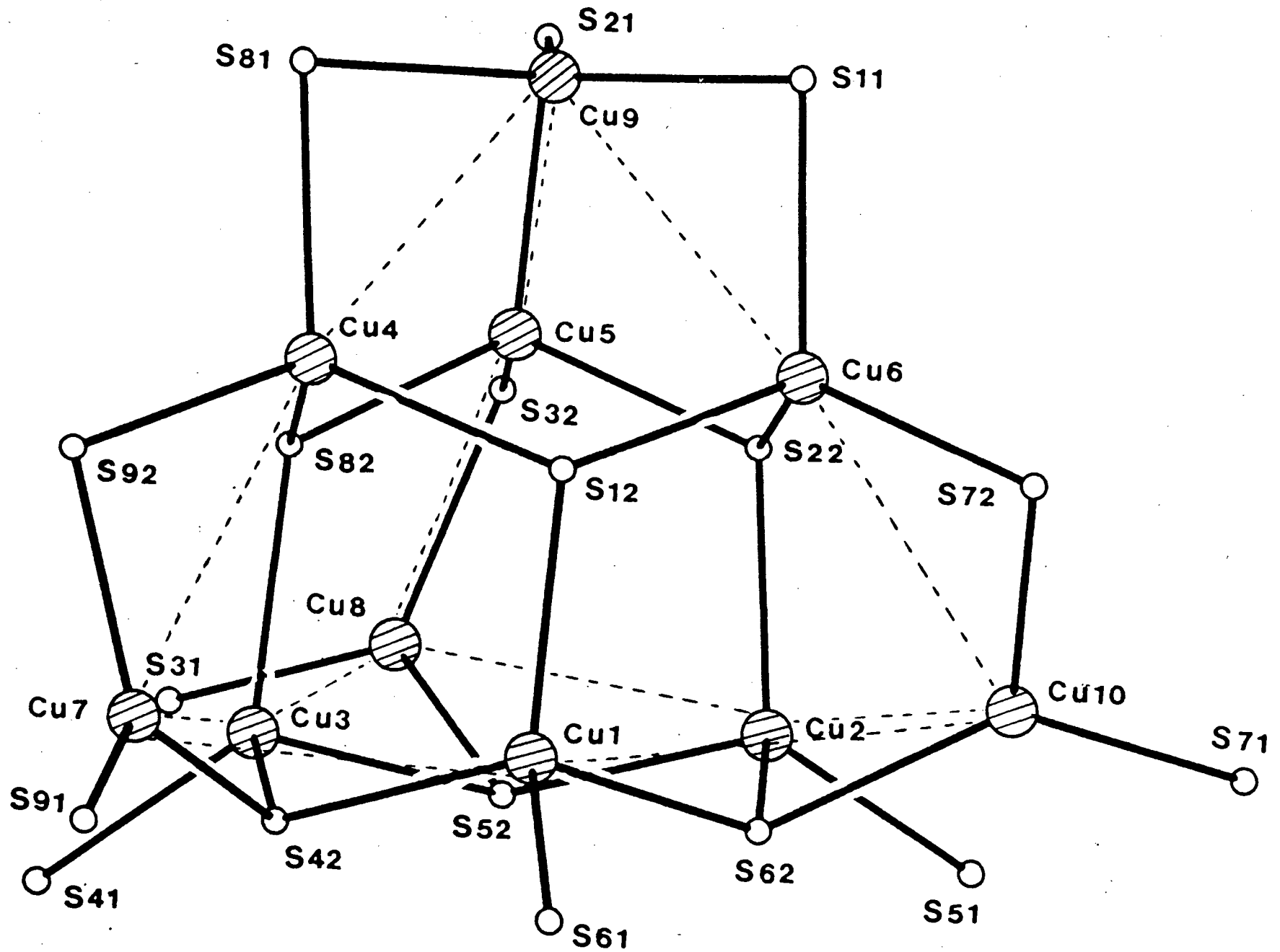
clusters. Molecular orbital calculations<sup>16</sup> have in fact indicated that in such systems, there are no real significant metal-metal interactions. Since the ligand possesses a 1,5-dithio geometry, which allows for greater intermetallic distances, there are no significant metal-metal bonds and consequently<sup>2</sup> the complex is referred to as an aggregate and not a cluster. The Cu(I) atoms are therefore four- and three-coordinate to the sulfur atoms of the ligand.

As shown in figure 12a, the ten copper atoms are arranged in an approximate tetrahedron whose sides are delineated by Cu9...Cu5...Cu8; Cu9...Cu6...Cu10; Cu9...Cu4...Cu7; Cu8...Cu3...Cu7; Cu7...Cu1...Cu10; and Cu10...Cu2...Cu8 atoms. In each case, the three copper atoms forming a side are not in fact linear (the angle subtended at the centre copper atom by the other two is in the range of 162° - 167°); hence the description of the shape as a tetrahedron is a simplification. Figure 12b represents the stereo drawing of the aggregate.

Figure 13 represents the framework of the copper atoms showing clearly the novel arrangement in the Cu<sub>10</sub>S<sub>18</sub> aggregate, different from the more common tetrahedral structure of the Cu<sub>4</sub>S<sub>6</sub><sup>5</sup> core or the cube structure of the C<sub>8</sub>S<sub>12</sub><sup>6,7</sup> systems.

As far as the copper coordination is concerned, Cu1', Cu2, Cu3, Cu4, Cu5 and Cu6 are four-coordinate to the sulfur atoms, (with the S-Cu-S angles in the range of 98.5° - 131.0°), while Cu7, Cu8, Cu9 and Cu10 are three-coordinate

Figure 12. a) The Cu-S-Cu framework of the copper-ligand (1) aggregate.



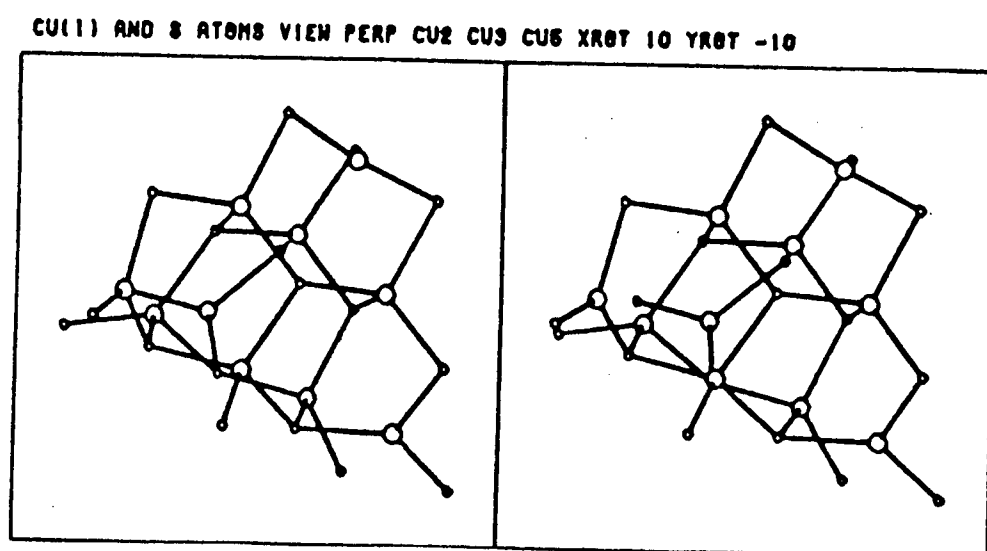


Figure 12b: Stereo drawing of aggregate

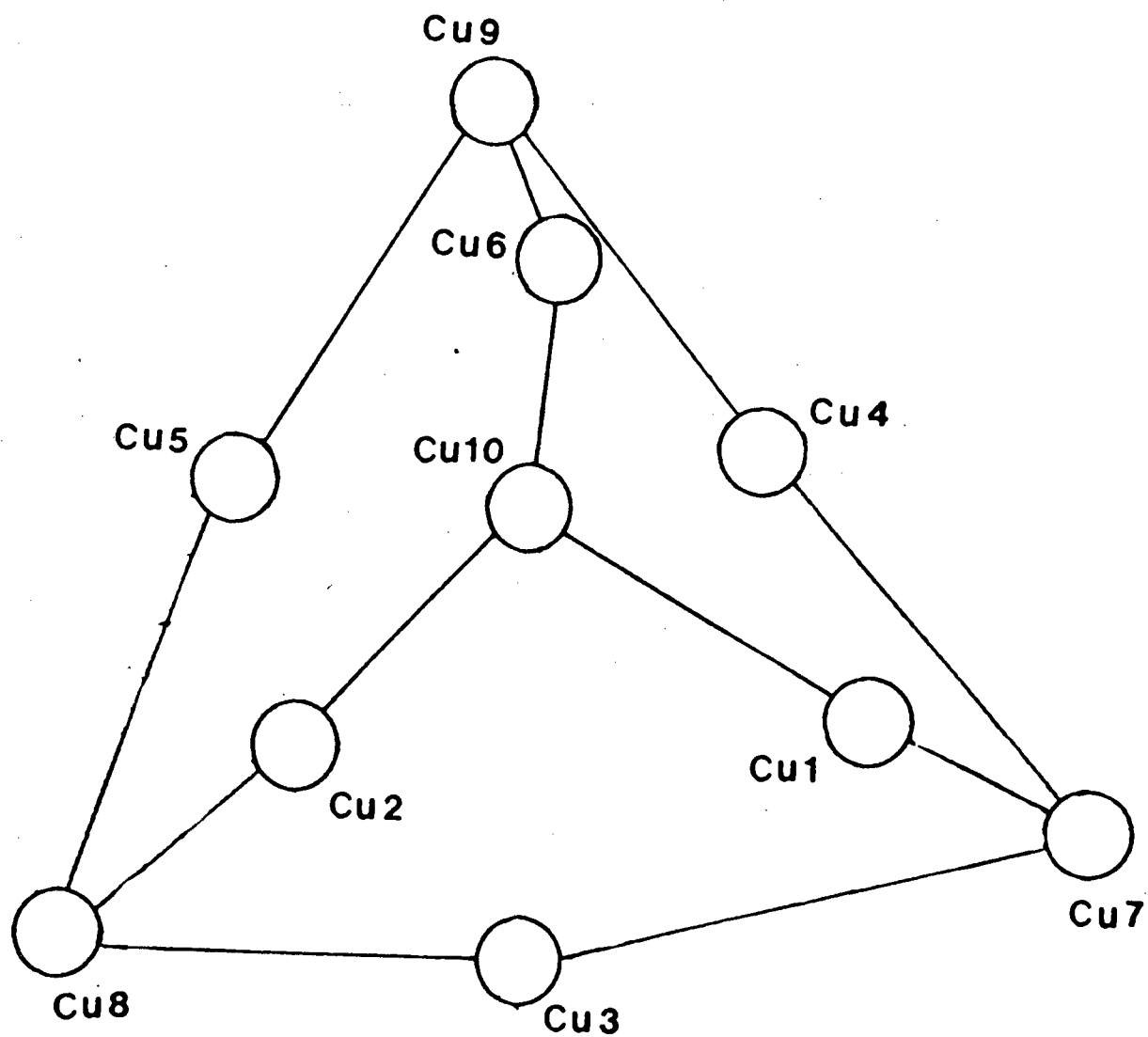


Figure 13: "Tent-like" arrangement of copper atoms in the aggregate.

to the sulfur atoms, (With the S-Cu-S angles in the range of  $111.0 - 131.9^\circ$ ). Nine out of ten copper atoms in the aggregate (Cu1 - Cu8, Cu10) are each chelated by the sulfur atoms of the C-S<sup>-</sup> and P=S of one ligand molecule, forming nine six membered CuSCNPS rings. One (Cu7, Cu8 and Cu10) or two (Cu1 - Cu6) additional coordinations take place with the C-S<sup>-</sup> centres of the adjacent ligand molecules. A single copper atom (Cu9) is unique within the aggregate; - it is not chelated but coordinates trigonally to three sulfur atoms of the neutral thiophosphoryl groups of the three adjacent molecules of (1). These are ligands 1, 2 and 8. Consequently, Cu9 carries a full positive charge counter-balanced by the perchlorate anion present in the aggregate.

The coordination numbers of the eighteen sulfur atoms in the aggregate show a marked difference between the thiophosphoryl and the (ionised) isothioureate sulfur atoms. Six of the P=S sulfur atoms (S31, S41, S51, S61, S71 and S91) are mono-coordinate (Cu-S in the range of 2.20 - 2.31 Å), involved only in the chelation of six copper atoms. Three remaining thiophosphoryl centres (S11, S21 and S81) are di-coordinate because of the additional coordination to the positively charged "unique" Cu9 atom. Three of the C-S<sup>-</sup> sulfur atoms (S32, S72 and S92) are also di-coordinate; the Cu-S distance of all di-coordinate sulfur atoms is in the range of 2.20 - 2.38 Å. Six other C-S<sup>-</sup> sulfur atoms each form a bridge among three copper atoms (Cu-S distance in the range 2.23 - 2.38 Å).

The bonding network between the copper and sulfur atoms gives rise to a system of fourteen six membered Cu-S-Cu-S-Cu-S rings. A conformational analysis<sup>17</sup> of these rings was carried out. The three rings involving Cu9 are each in the chair conformation; the two rings involving Cu7, Cu8 and Cu10 respectively (six in all) have at least some boat character. The rings involving copper atoms 2,3,5; 1,3,4; and 1,2,6 are boats or screw boat/half chair, while the remaining two rings (copper atoms 1,2,3 and 4,5,6) are chairs. Figure 14 represents the conformation used in the Cu<sub>10</sub>S<sub>18</sub> aggregate.

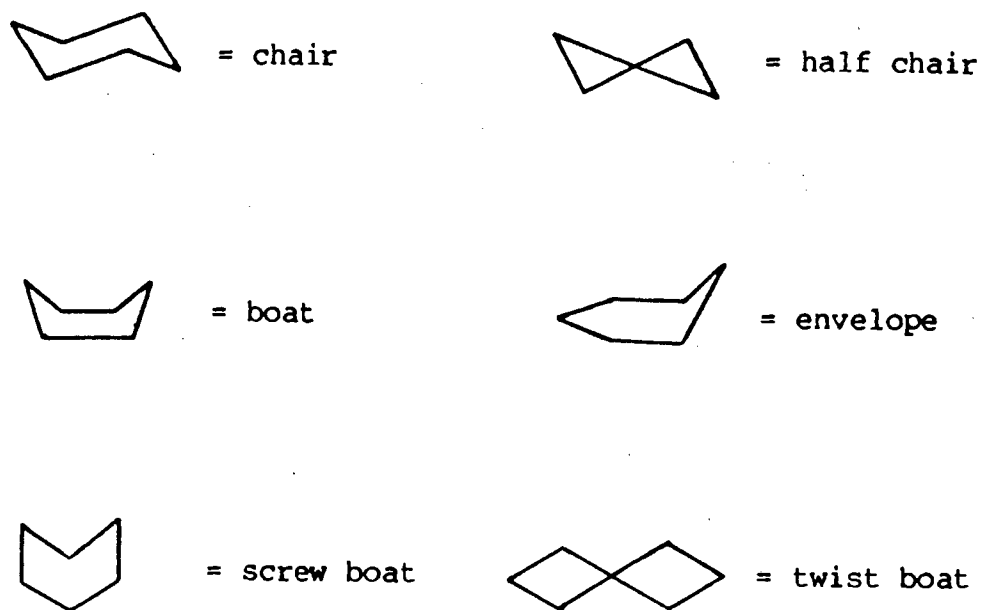
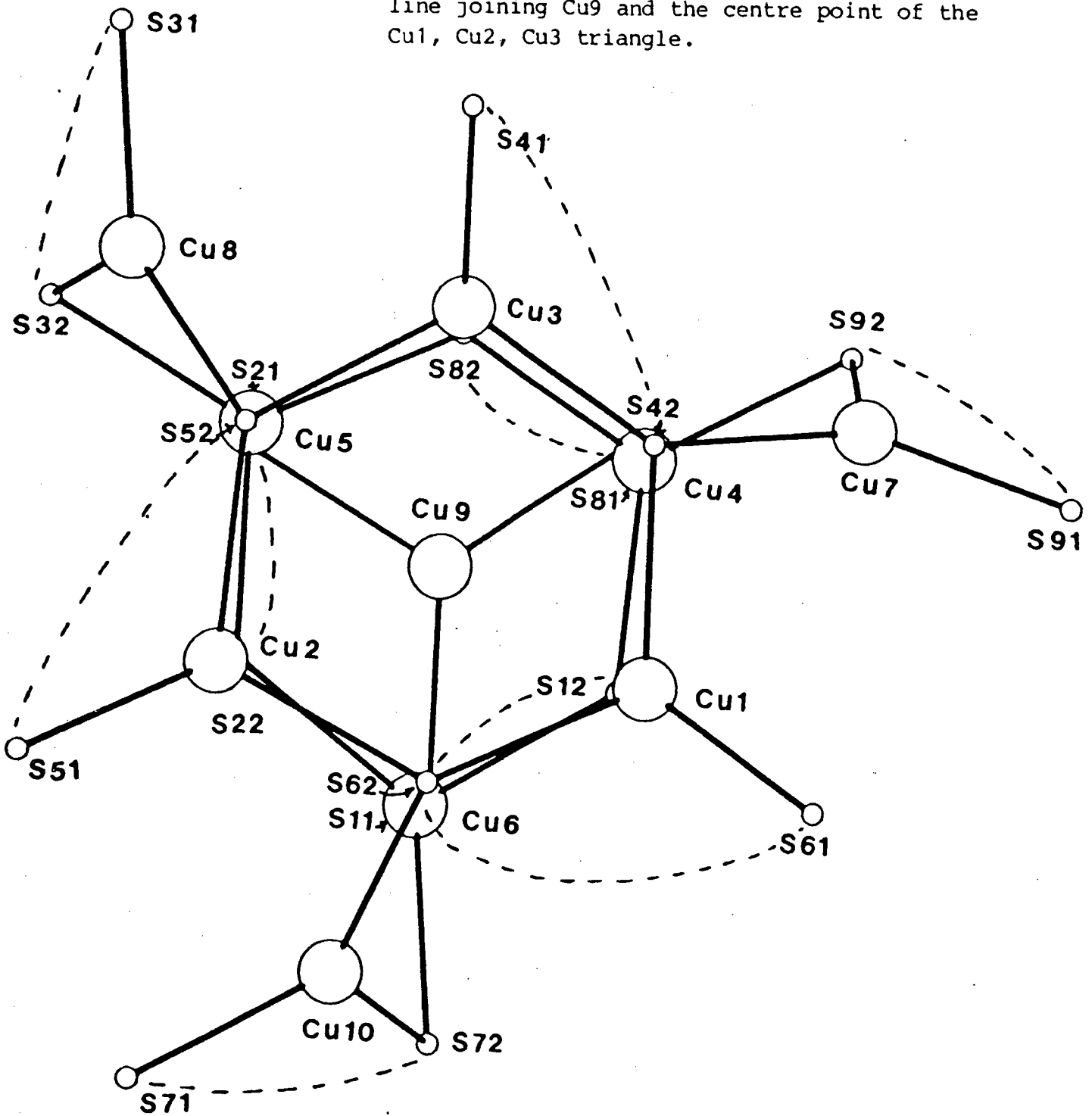


Figure 14

A view of the aggregate projected down the line joining Cu9 and the centre point of the Cu1, Cu2, Cu3 triangle (figure 15), reveals clearly that the aggregate has approximate C<sub>3</sub> symmetry.



Figure 15: A view of the aggregate projected down the line joining Cu9 and the centre point of the Cu1, Cu2, Cu3 triangle.



In conclusion therefore, a new type of Cu(I)-S aggregate has been described, different in structure from the  $\text{Cu(I)}_8\text{S}_{12}$  core, which consists of a cube of copper atoms and found in Cu(I) complexes with 1,1- and 1,2-dithiolate ligands. The new structure has been directly attributed to the fact that there is a ligand consisting of a 1,5-dithiolate system. It would therefore be interesting to see whether the new aggregate obtained, is an isolated case for this ligand or whether this structure is general for all complexes prepared from 1,5-dithiolate systems.

CHAPTER 3

EXPERIMENTAL

3 EXPERIMENTAL PROCEDURES FOR THE SYNTHESIS OF THE LIGAND, (EtO)<sub>2</sub>P(S)-NH-C(S)-NHPh, THE PREPARATION OF THE Cu(I)-LIGAND AGGREGATE AND THE SOLUTION OF THE STRUCTURE.

3.1 General

<sup>1</sup>H NMR spectra were recorded in deuterated chloroform (with trimethylsilane as internal standard) or in deuterated DMSO (with trimethylsilane as internal standard) on a Brüker 90 MHz spectrometer.

Melting points were determined on a Fischer-Johns m.p. apparatus.

Analysis for C, H, N were performed on a Heraeus Universal combustion analyser by Mr W R T Hemsted, in the Organic Chemistry Department, University of Cape Town.

3.2 Reagents

The following reagents were used, and were purified by drying and/or distillation, according to standard procedures: thiophosphoryl chloride, benzene, diethyl ether, potassium thiocyanate, acetonitrile, aniline, toluene, petroleum ether (30 - 40°C), ethanol.

The following compounds were used as supplied: copper perchlorate, copper powder, sodium metal.

### 3.3 Preparation of the ligand (EtO)<sub>2</sub>P(S)-NH-C(S)-NHPh

#### 3.3.1 Synthesis of (EtO)<sub>2</sub>P(S)Cl<sup>18</sup>

A solution of sodium ethoxide was prepared by adding during one hour sodium (22 g; 0.96 mole) to dry ethanol (520 ml) contained in a one litre flask fitted with a reflux condenser and drying tube. This solution was added dropwise to a solution of freshly distilled thiophosphoryl chloride (80.1 g; 0.473 mole) in dry benzene (225 ml) contained in a two litre flask, while maintaining the temperature between 5° and 10°C. The solution turned to a white suspension, as NaCl formed. The mixture was kept cool to avoid dealkylation of the product by chloride ions. The excess ethanol and benzene were removed on a rotary evaporator until a thick white suspension was obtained. The suspension was transferred to a large separating funnel and benzene (100 ml) and water (250 ml) were the added.

The benzene layer (which contained the product) separated and was removed.

The aqueous layer was extracted with benzene (3 x 50 ml) and water (75 ml) was then added to the combined benzene solutions. Separation however, would not occur, as the benzene and product density equalled the water density. Absolute diethyl ether (20 ml) was then added to the solution, which then readily separated. The benzene-ether layer was removed and dried over magnesium sulphate. The

solution was filtered and the solvent removed. The crude product was then distilled under reduced pressure.

Yield: 67.6%    b.p.: 54 - 56°C/1.5 mmHg .

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$ 1.4 (6H, t,  $J_{\text{H,H}}$  7 Hz, 2 x  $\text{CH}_3$ )  
 $\delta$ 4.3 (4H, d of q,  $J_{\text{H,H}}$  7 Hz,  $J_{\text{H,P}}$  11 Hz,  
 2 x  $\text{CH}_2$ )

### 3.3.2 Synthesis of $(\text{EtO})_2\text{P}(\text{S})-\text{NCS}$ <sup>19</sup>

$(\text{EtO})_2\text{P}(\text{S})\text{Cl}$  (16.0 g; 0.085 mole) was added to a solution of potassium thiocyanate (12.4 g; 0.127 mole) in dry acetonitrile (160 ml) and stirred for four hours at 50°C and at room temperature overnight. The acetonitrile was removed under reduced pressure and benzene (50 ml) was added to the residue. The KCl which had formed was removed by filtration and the benzene then removed, leaving behind the crude product. This was then distilled under reduced pressure to produce a clear liquid.

Yield: 51.6%    b.p.: 62° - 66°C/0.33 mmHg

Anal. Calcd for  $\text{C}_5\text{H}_{10}\text{NO}_2\text{PS}_2$ :    C 28.4;    H 4.8;    N 6.6 %

Found :    C 28.6;    H 4.8;    N 6.5 %

### 3.3.3 Synthesis of (EtO)<sub>2</sub>P(S)NHC(S)-NHPh<sup>20</sup>

Aniline (2.70 ml) was added to a solution of (EtO)<sub>2</sub>P(S)NCS (6.09 g; 0.029 mole) in toluene (10 ml), the mixture stirred at 80°C (water bath) for four hours and cooled when some crystals of the product separated. An equal volume of petroleum ether (30° - 40°C) was added and more crystals precipitated. These were removed by filtration through a sintered glass funnel and washed with cold petroleum ether.

Yield: 71.4% m.p.: 94° - 96°C (lit. <sup>20</sup>mp 96°C).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ1.38 (6H, t, J<sub>H,H</sub> 7 Hz, 2 x CH<sub>3</sub>).

δ4.20 (4H, d of q, J<sub>H,H</sub> 8 Hz, J<sub>H,P</sub> 11 Hz, 2 x CH<sub>2</sub>).

δ7.0 - 8.0 (7H, m, upon D<sub>2</sub>O wash reduced to 5H, M, 2 x NH, C<sub>6</sub>H<sub>5</sub>).

Anal. Calcd for C<sub>11</sub>H<sub>17</sub>O<sub>2</sub>PN<sub>2</sub>S<sub>2</sub>: C 43.4; H 5.6; N 9.2%

Found : C 44.2; H 5.6; N 9.3%

### 3.4 Crystals of [Cu<sub>10</sub>(C<sub>11</sub>H<sub>16</sub>O<sub>2</sub>N<sub>2</sub>S<sub>2</sub>P)<sub>9</sub>]ClO<sub>4</sub>

Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (0.37 g; 1 mmole) was added to a stirred solution of (1) (0.75 g; 2.47 mmole) in ethanol (12 ml). An excess of copper powder was then added and the mixture was stirred at room temperature for 10 minutes. The copper powder

was then filtered off and the pale-yellow solution was left in an opened flask at room temperature overnight. The crystalline precipitate formed was filtered off, washed rapidly with cold ethanol and subjected to spectroscopic ( $^1\text{H}$  NMR) and elemental analysis. The  $^1\text{H}$  NMR spectrum (in deuterated DMSO) was practically identical to that of (1) (except some down-field shift of signals), indicating that no change in the ligand structure had taken place.

Anal. Calcd. for  $\text{Cu}_{10}\text{C}_{99}\text{H}_{144}\text{ClN}_{18}\text{O}_{22}\text{P}_9\text{S}_{18}$ :

C 34.32; H 4.19; N 7.28 %

Found : C 34.35; H 4.10; N 7.20 %

### 3.5 X-ray Data collection and Solution of Structure

The compound was found to be unstable to air. Hence on recrystallization from an ethanol solution, a single crystal was selected and placed rapidly in a Lindemann tube together with mother liquor. After sealing of the tube at both ends, the crystal was mounted on an Enraf-Nonius CAD4 diffractometer and irradiated with  $\text{MoK}\alpha$  radiation ( $\lambda = 0.711\text{\AA}$ ).

Least squares analysis of the setting angles of 24 reflections with  $10^\circ < \theta < 11^\circ$  yielded the triclinic cell reported. During the data collection reorientation and intensity checks were carried out periodically to monitor crystal and machine stability. The data were LP processed and an empirical absorption correction<sup>21</sup> was applied.



The ten copper atoms were located by direct methods using SHELXS-86<sup>14</sup> and the remaining non-hydrogen atoms of the aggregate were revealed in subsequent difference Fourier's using SHELX 76.<sup>15</sup>

Methylene hydrogens were placed in calculated positions at 1.00Å from their parent carbons as were the single amino hydrogens on NX2 and the phenyl hydrogens. The methyl hydrogens were treated as rigid groups, again with a single temperature factor. The Cl of the perchlorate anion was easily located at this stage. However, due to the considerable disorder of the four oxygen atoms in the ClO<sub>4</sub>, the following approach was adopted. The spherically averaged molecular scattering factor for ClO<sub>4</sub> were calculated using dummy coordinate input to NORMAL of MULTAN.<sup>22</sup> An approximation of the scattering with  $\sin\theta/\lambda$  was then obtained in the form suitable for SHELX 76<sup>15</sup> by the addition of normal distribution functions viz:

$$f(x) = 38.8 \exp(43.4x^2) + 4.0 (\exp 1.79x^2) + 1.5 (\exp 0.63x^2) + 5.69, \text{ where } x = \sin\theta/\lambda.$$

The molecular scatterer was then placed at the location of the Cl with  $U_{iso}$  fixed at 0.35 to model disorder. In the final refinements, the copper atoms were treated anisotropically and all other atoms isotropically. A weighting scheme,  $(\sigma^2F)^{-1}$ , was employed. In the final difference map, max/min residual electron densities were 1.3/-1.6 eÅ<sup>-3</sup>. Complex neutral atom scattering factors were taken from Cromer and Mann<sup>23</sup> for non-hydrogen atoms (excluding ClO<sub>4</sub>) and from

Stewart et al.<sup>24</sup> for H, with dispersion corrections from Cromer and Liberman.<sup>25</sup> Molecular parameters were calculated using PARST<sup>26</sup> and drawings obtained using PLUTO.<sup>27</sup> All computations were performed at the Computer Centre of the University of Cape Town on a Sperry 1100/81. Full details of the data collection, structure solution and refinement are given in Table 2.

**APPENDIX**

**TABLE 2:** Crystal data, details of the data collection and final refinements for  $[\text{Cu}_{10}(\text{C}_{11}\text{H}_{16}\text{O}_2\text{N}_2\text{S}_2\text{P})_9] \text{ClO}_4$

Molecular formula	$\text{C}_{99}\text{H}_{144}\text{O}_{22}\text{N}_{18}\text{S}_{18}\text{P}_9\text{Cu}_{10}\text{Cl}$
Mr	3465.04
Space group	$\bar{P}1$
$\underline{a}$ (Å)	16.06(1)
$\underline{b}$ (Å)	22.19(1)
$\underline{c}$ (Å)	22.455(6)
$\alpha$ (°)	90.16(3)
$\beta$ (°)	108.63(4)
$\gamma$ (°)	91.37(5)
$V$ (Å <sup>3</sup> )	7580
$D_m$ (bromobenzene/ $\text{CCl}_4$ ) ( $\text{gcm}^{-3}$ )	1.52
$D_c$ (for $Z = 2$ ) ( $\text{gcm}^{-3}$ )	1.51
$F(000)$	3506
$\mu$ (MoK $\alpha$ ) ( $\text{cm}^{-1}$ )	17.14
Crystal dimensions (mm)	0.25 x 0.37 x 0.43
Scan mode	$\omega - 2\theta$
Scan width (°)	$(0.85 + 0.35\tan\theta)$
Aperture width (mm)	$(2.40 + 1.05\tan\theta)$
Range scanned ( $\theta^\circ$ )	1 - 18
Range of Miller Indices (h;k;l;)	$\pm 13; \pm 19; 0, 19;$
Number of unique reflections collected	8903
R merge	0.044
Number of reflections observed, N	7249
(with $I \text{ rel} > 2\sigma I \text{ rel}$ )	
Number of parameters, NP	799
$R = \sum   F_o  -  F_c   / \sum  F_o  = \sum \Delta / \sum  F_o $	0.101
$R_w = \sum w^1_2  \Delta  / \sum w^1_2  F_o $	0.100
$w =$	$(\sigma^2 F)^{-1}$
$S = (\sum w  \Delta ^2 / N - NP)$	12.6
Crystal stability (%)	9.7

Figure 16:  $^1\text{H}$  NMR spectrum of  $(\text{EtO})_2\text{P}(\text{S})\text{NHC}(\text{S})\text{-NHPh}$  in  $\text{CDCl}_3$  at 90 MHz

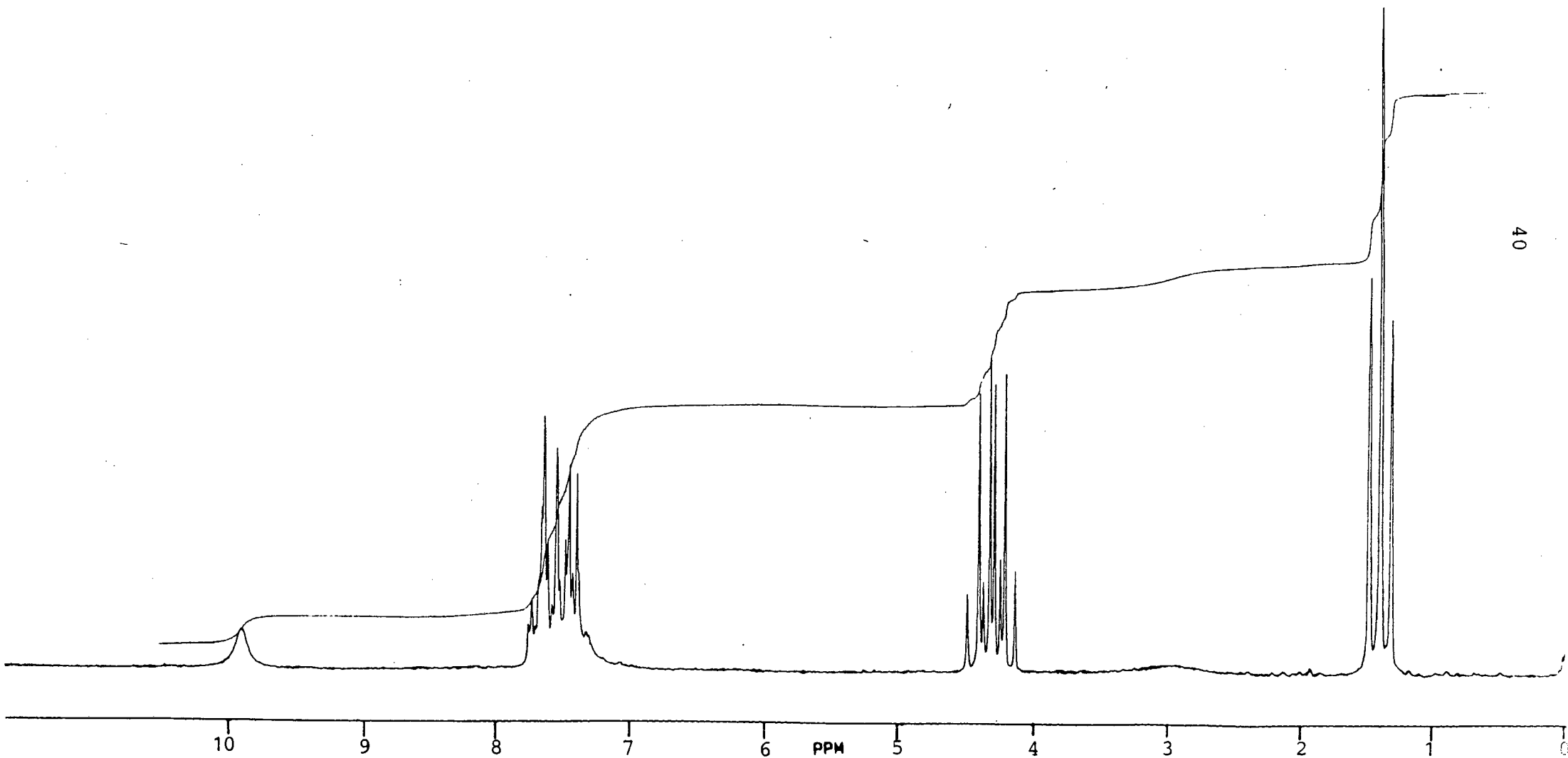
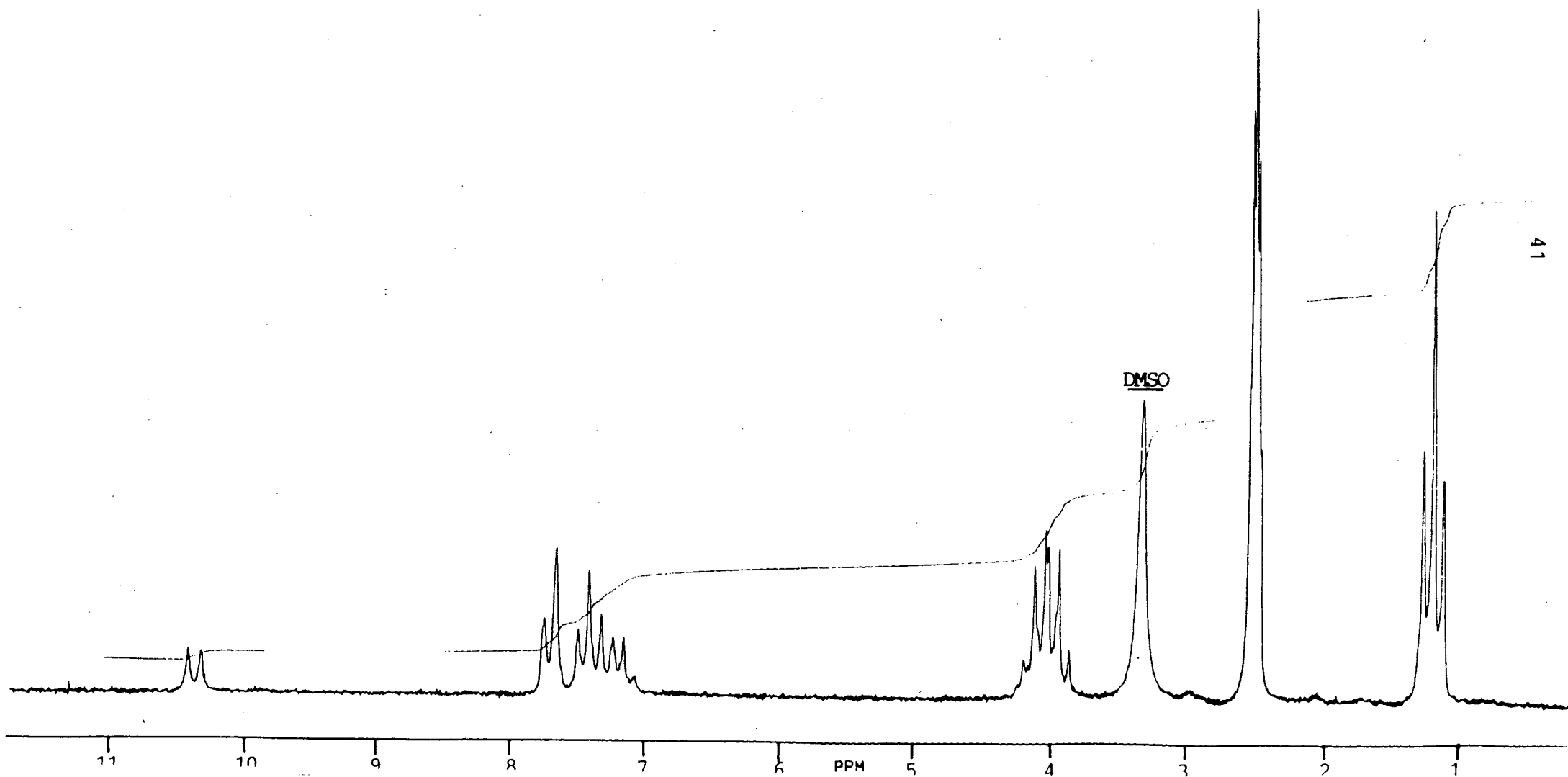


Figure 17:  $^1\text{H}$  NMR spectrum of  $\text{Cu}^{2+}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  complex of (1) in DMSO at 90 MHz



REFERENCES

1. F.J. Jardine, *Adv. Inorg. Chem. Radiochem.*, 1975, 17, 116.
2. F.A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, 4th ed., Wiley-Interscience, 1980, Ch. 21-H-2.
3. P.G. Eller and P.W.R. Corfield, *J. Chem. Soc. Chem. Comm.*, 1971, 105.
4. A.F. Wells, *Structural Inorganic Chemistry*, 5th ed., Clarendon Press, Oxford, 1984, p.1108.
5. J.G. Dance and J.C. Calabrese, *Inorg. Chim. Acta*, 1976, 19, L41.
6. L.E. McCandlish, E.C. Bissell, D. Coucouvanis, J.P. Fackler and K. Knox, *J. Am. Chem. Soc.*, 1968, 90, 7357.
7. F.J. Hollander, D. Coucouvanis, *J. Am. Chem. Soc.*, 1974, 96, 5646.
8. P.J.M.W.L. Birker and H.C. Freeman, *J. Am. Chem. Soc.*, 1977, 99, 6890.
9. Y. Sugiura and H. Tanaka, *Chem. Pharm. Bull.*, 1970, 18, 368.
10. A.F. Wells, *Structural Inorganic Chemistry*, 5th ed., Clarendon Press, Oxford, 1984, p.1104.

11. M.R. Churchill and K.L. Kalra, *Inorg. Chem.*, 1974, 13, 1899;
12. V.N. Zontova, A.F. Grapov and N.N. Melnikov, *Dokl. Akad. Nauk SSSR*, 1980, 251, 882; *Zh. Obshch. Khim.*, 1982, 52, 64.
13. J. Michalski and J. Wieczorkowski, *Roczniki Chem.*, 1957, 31, 585.
14. G.M. Sheldrick, SHELX-86 in "Crystallographic Computing 3", eds. G.M. Sheldrick, C. Kruger and R. Goddard, Oxford University Press, 1985, p.175.
15. G.M. Sheldrick, SHELX-76 in "Computing in Crystallography", eds. H. Schenk, R. Olthof-Hazekamp, H. von Koningsveld and G.C. Bassi, Delft University Press, 1978, p.34.
16. A. Avdeef and J.P. Fackler, *Inorg. Chem.*, 1978, 17, 2182; P.K. Mehrota and R. Hoffmann, *Inorg. Chem.*, 1978, 17, 2187.
- 17a. D. Cremer and J.A. Pople, *J. Am. Chem. Soc.*, 1975, 97, 1354;  
b. J.C.A. Boeyens, *J. Cryst. Mol. Struct.*, 1978, 8(6), 317.
18. J.H. Fletcher, *J. Am. Chem. Soc.*, 1950, 72, 2761.
19. J. Michalski, J. Wieczorkowski, *ROCZ. Chem.*, 1957, 31, 585.
20. G. Schrader, DBP, 952712, Farb. Bayer, Erf., 1955, *Chem. Zentr.*, 1957, 3651.



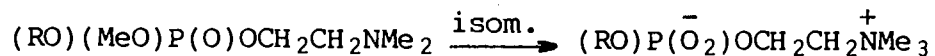
21. A.C.T. North, D.C. Phillips and F. Scott Mathews, *Acta Cryst.*, 1968, A24, 351.
22. P. Main, S.E. Hull, L. Lessinger, G. Germain, J-P. Declercq and M.M. Woolfson, MULTAN 78, Universities of York, England and Louvain, Belgium, 1978.
23. D.T. Cromer and J.B. Mann, *Acta Cryst.*, 1968, A24, 321.
24. R.F. Stewart, E.R. Davidson and W.T. Simpson, *J. Chem. Phys.*, 1965, 42, 3157.
25. D.T. Cromer and D. Liberman, *J. Chem. Phys.*, 1970, 53, 1891.
26. M. Nardelli, *Comput. Chem.*, 1983, 7, 95.
27. W.D.S. Motherwell, PLUTO Plotting program, 1974, private communication.

PART II

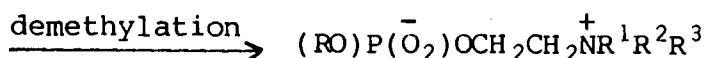
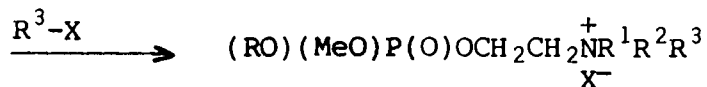
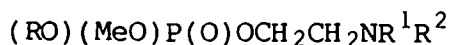
A NOVEL APPROACH TO THE SYNTHESIS OF PHOSPHOLIPIDS

ABSTRACT

Lacey and Loew<sup>11</sup>, prepared phospholipids by the following route:



Unfortunately, during the isomerization period, the unwanted aziridinium ion is also formed. A novel approach for the preparation of phospholipids is described, in which, in principle, the substituents at nitrogen can be varied and possible fragmentation of the phosphate triester to the unwanted aziridinium ion is eliminated.



**CHAPTER 1**

**INTRODUCTION**

## 1 INTRODUCTION

Commercially, natural rubber is obtained almost exclusively from *hevea brasiliensis*, a tree indigenous to South America, where it grows wild to 120 feet. *Hevea*, is a latex-producing tree (latex, being the milky white liquid emulsion found in the cells of trees, such as *Hevea*), and since other latex-producing trees do not compare with *Hevea* for efficiency, industrial botanists have concentrated their efforts almost exclusively upon this species.

The regulation of rubber biosynthesis has been studied primarily in *Hevea brasiliensis* and guayule (*Parthenium argentatum*), a shrub native to the Northern deserts of Mexico, and known for a long time to be a source of high quality natural rubber, cis-polyisoprene.

In *Hevea*, ethylene stimulates the yield of latex. It was suggested<sup>1</sup> that the hormone may affect the genes responsible for the latex clotting mechanism, and that turning off these genes, leads to a doubling of rubber production. No such mechanism is operable in guayule, because it is not a laticifer. However, rubber production in guayule is cyclic, accumulating only in the fall and winter, so that a control mechanism must be operable in rubber synthesis. It was shown by Bonner,<sup>2,3</sup> that a low night temperature of 7°C stimulates rubber production in guayule and suggests that the low temperature stimulates transcription of the genes, coding for the enzymes involved in rubber synthesis. A notable discovery in the regulation of rubber biosynthesis in

guayule, was the finding<sup>4</sup> that treatment of young plants with 2-(3,4-dichlorophenoxy)triethylamine (DCPTA) (figure 1), resulted in the stimulation of cis-polyisoprenoid rubber production,<sup>5,6</sup> and also caused shoot formation in callus cultures of guayule tissue<sup>7</sup>.

By applying DCPTA to the foilage of guayule plants, rubber synthesis over an extended period is increased, and this may prove to be important in the economic production of rubber in guayule, since natural stands of guayule do not synthesize rubber during the summer months of most active growth. Rubber is synthesized only during the winter months, as the plant experiences the low night temperatures necessary for rubber accumulation.

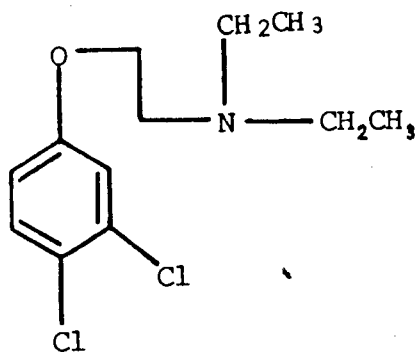


Figure 1: DCPTA.

By applying DCPTA to the guayule plant, it is therefore possible to obtain an increase in rubber synthesis during the summer growth period.

The expanding field of membrane research has fostered the need for simple synthetic approaches to phospholipid and phospholipid

analogues. The aim of the project was therefore to incorporate this DCPTA structure or its methyl analogue into a phospholipid structure, to form a phosphatidyl choline (figure 2), using a novel synthetic approach.

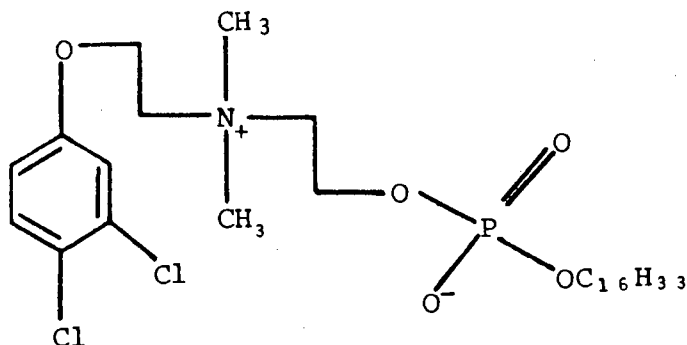


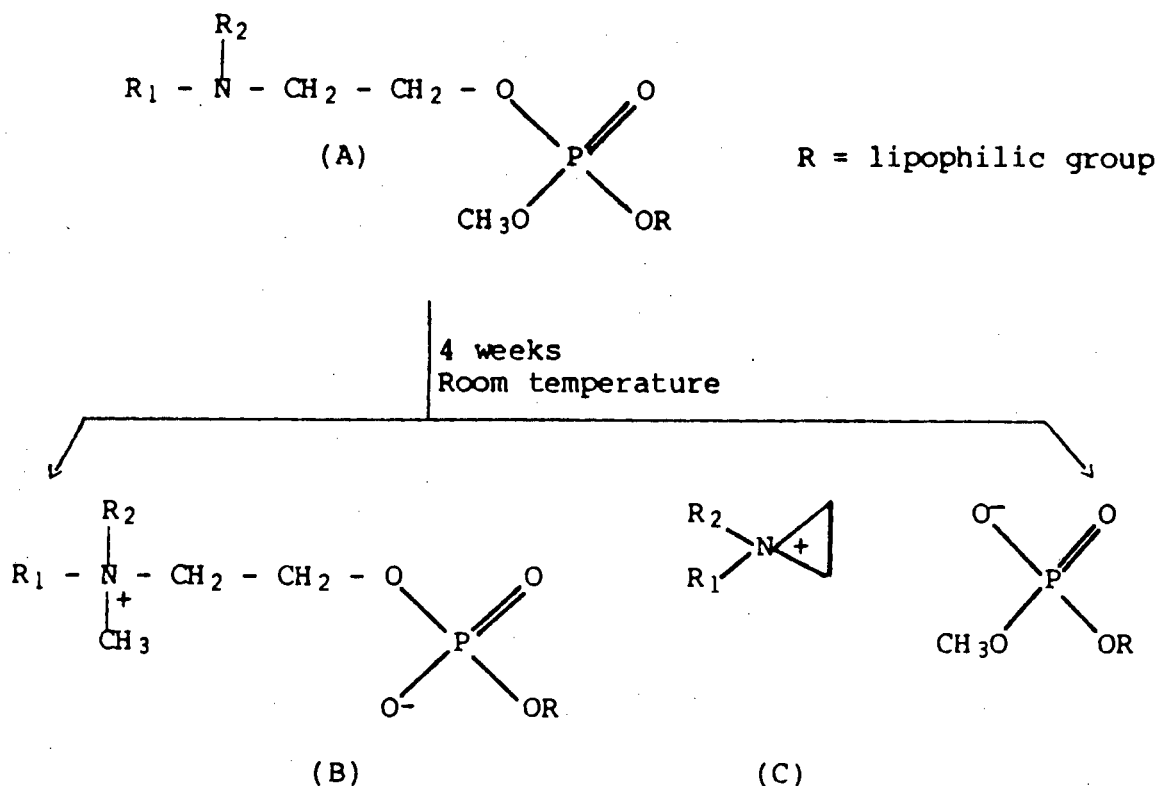
Figure 2.

As far as the preparation of phosphatidylcholines is concerned, the available methods can be divided into three groups:

- 1) The formation of phosphate ester linkages to a diglyceride and choline as the last steps<sup>8</sup>.
- 2) The introduction of both fatty acid ester linkages simultaneously into glycerophosphorylcholine to provide symmetrical phosphatidyl cholines<sup>9</sup>.
- 3) The preparation of mixed carboxy ester of phosphatidylcholines by acylation of lysophosphatidylcholine<sup>10</sup>.

The approach described by Lacey and Loew<sup>11</sup> did not fit well into any of the above groups. It allowed the preparation of mixed carboxy ester of phosphatidylcholine from a phosphate diester of choline. Scheme 1 represents the method described by Lacey and Loew. Here, a triester (A), is converted via an isomerization

to the desired ionic diester (B). The mechanism of the isomerization appears to be intermolecular, as based on a kinetic study<sup>12</sup>, and competes with an intramolecular extrusion of aziridinium phosphate (C).



Scheme 1

As can be seen, the method is ambiguous, for when (A) is allowed to isomerize to form the phosphatidyl choline (B), the unwanted aziridinium ion (C) is also formed.

The aim of this project was therefore to develop a new synthesis, which would allow the formation of the phosphatidyl choline to take place, while at the same time eliminating any possibility of the aziridinium ion being formed.

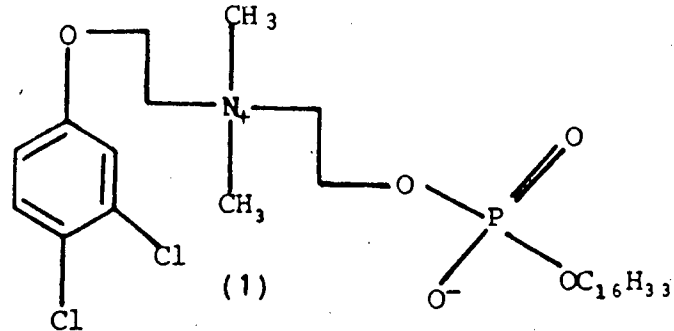


CHAPTER 2

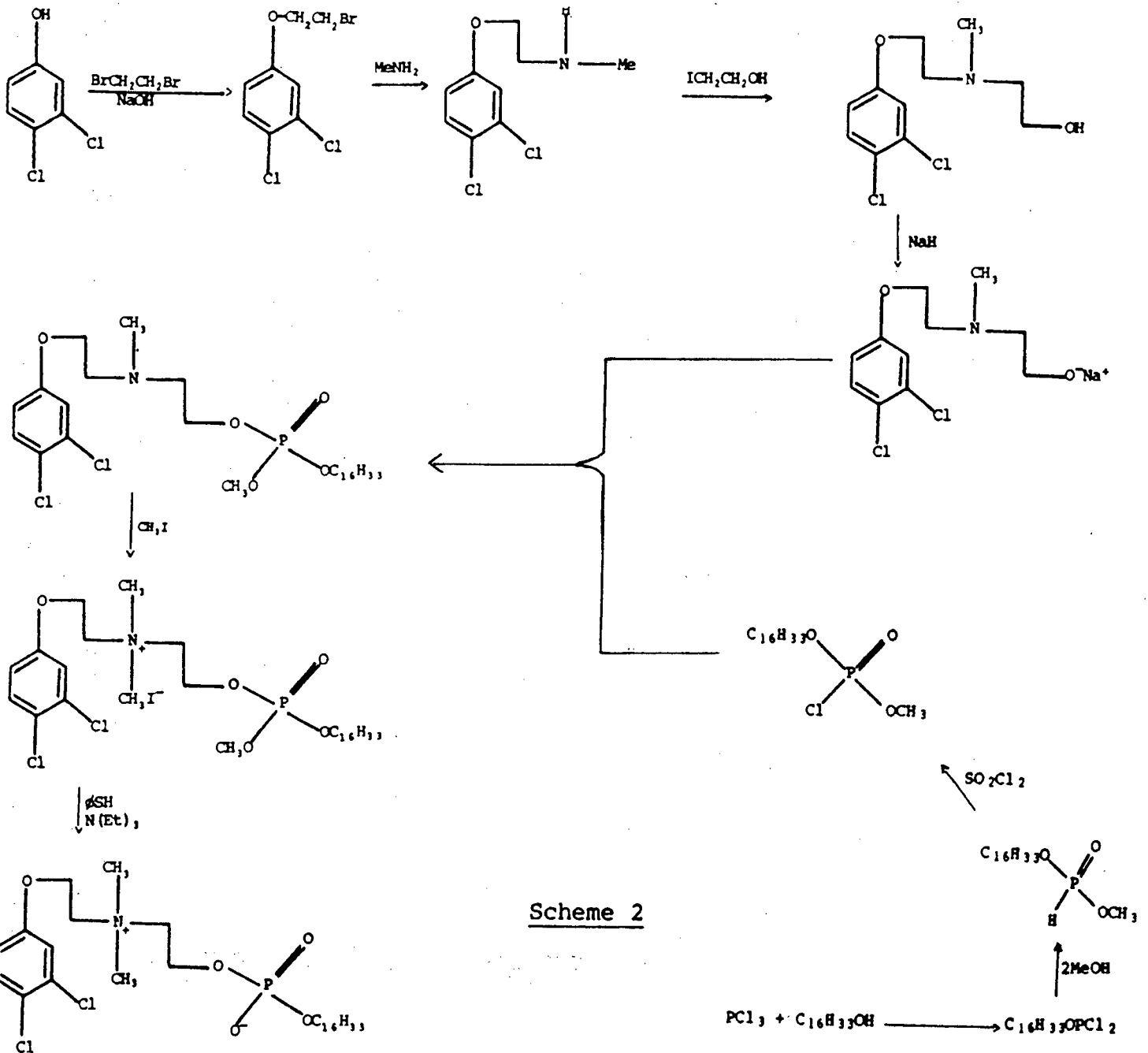
OUTLINE OF PROPOSED SYNTHETIC APPROACH

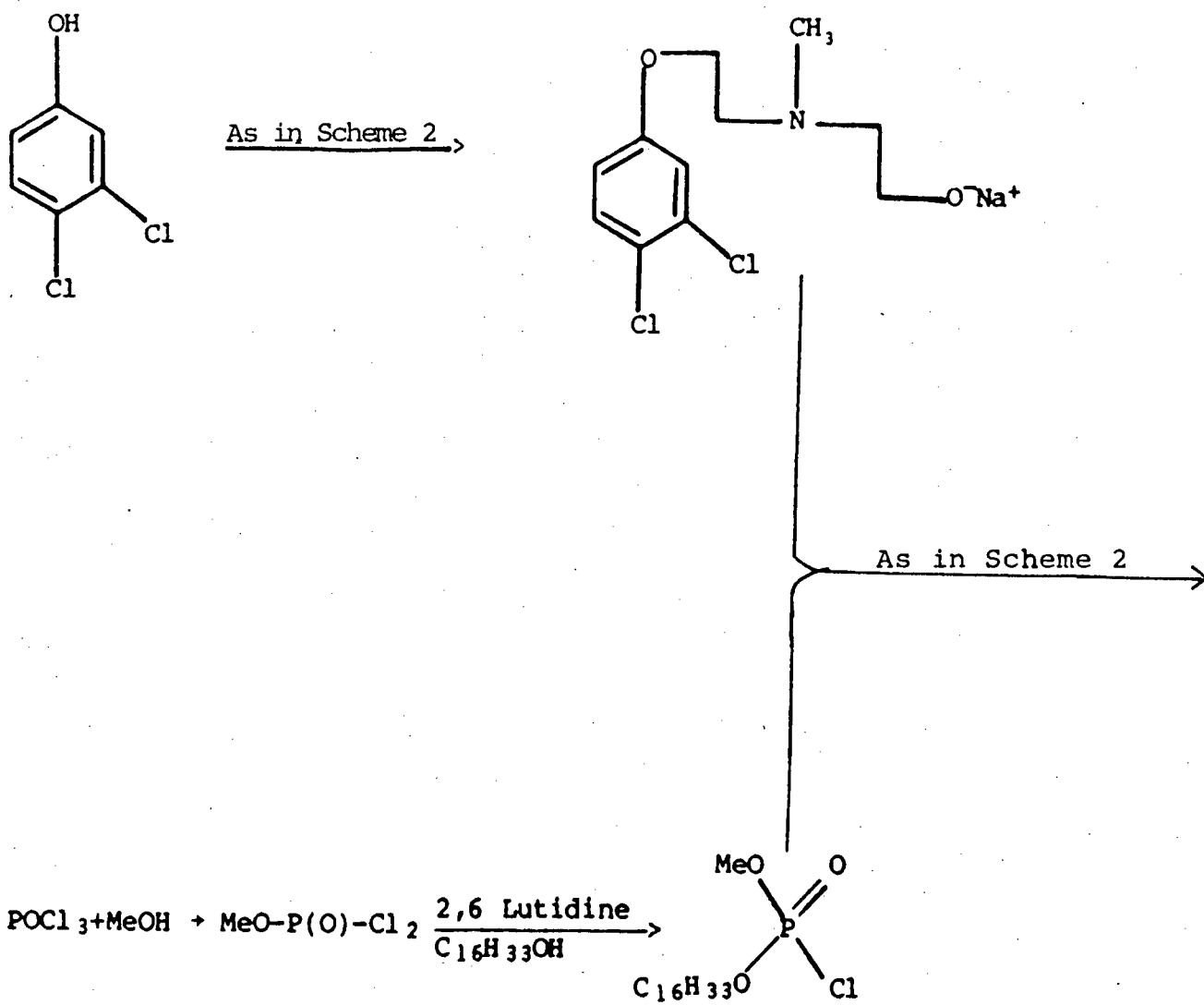
## 2. OUTLINE OF PROPOSED SYNTHETIC APPROACH

Two routes were considered for the synthesis of the phosphatidyl choline (1), and are shown below.



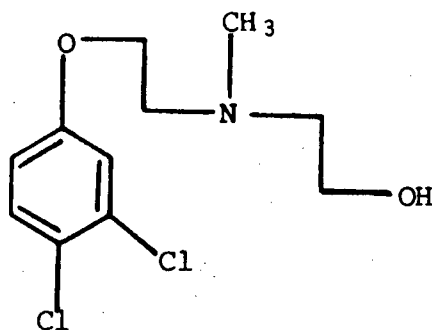
### Route 1



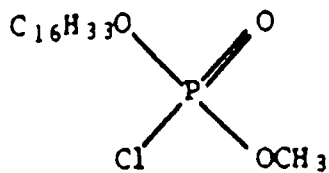
Route 2Scheme 3

The phosphatidyl choline (1) was synthesized in four steps.

(i) Synthesis of the aminoalcohol (2).

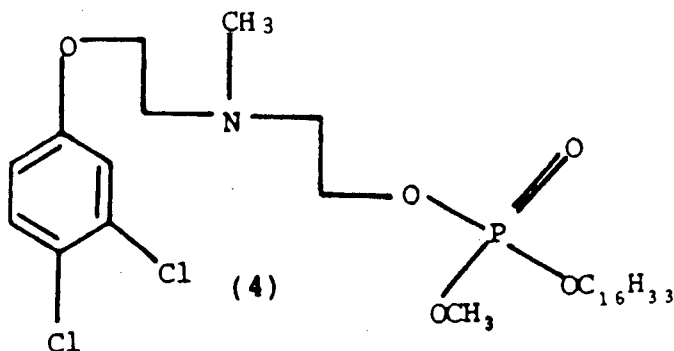


(ii) Synthesis of cetyl methyl chlorophosphate (3).



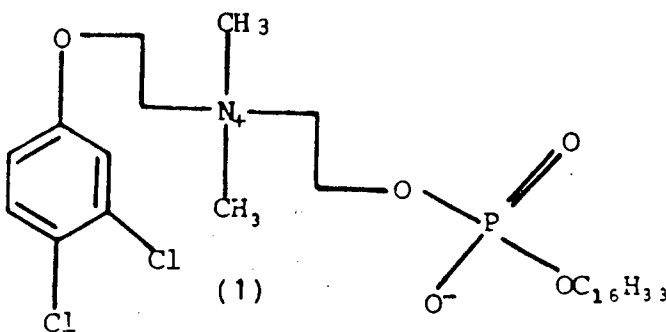
(3)

(iii) Reaction of (2) with (3) to form the neutral phosphate triester (4).



(4)

(iv) The methylation of the nitrogen and the demethylation of the phosphate  $\text{OCH}_3$  group to produce the final compound (1).



(1)

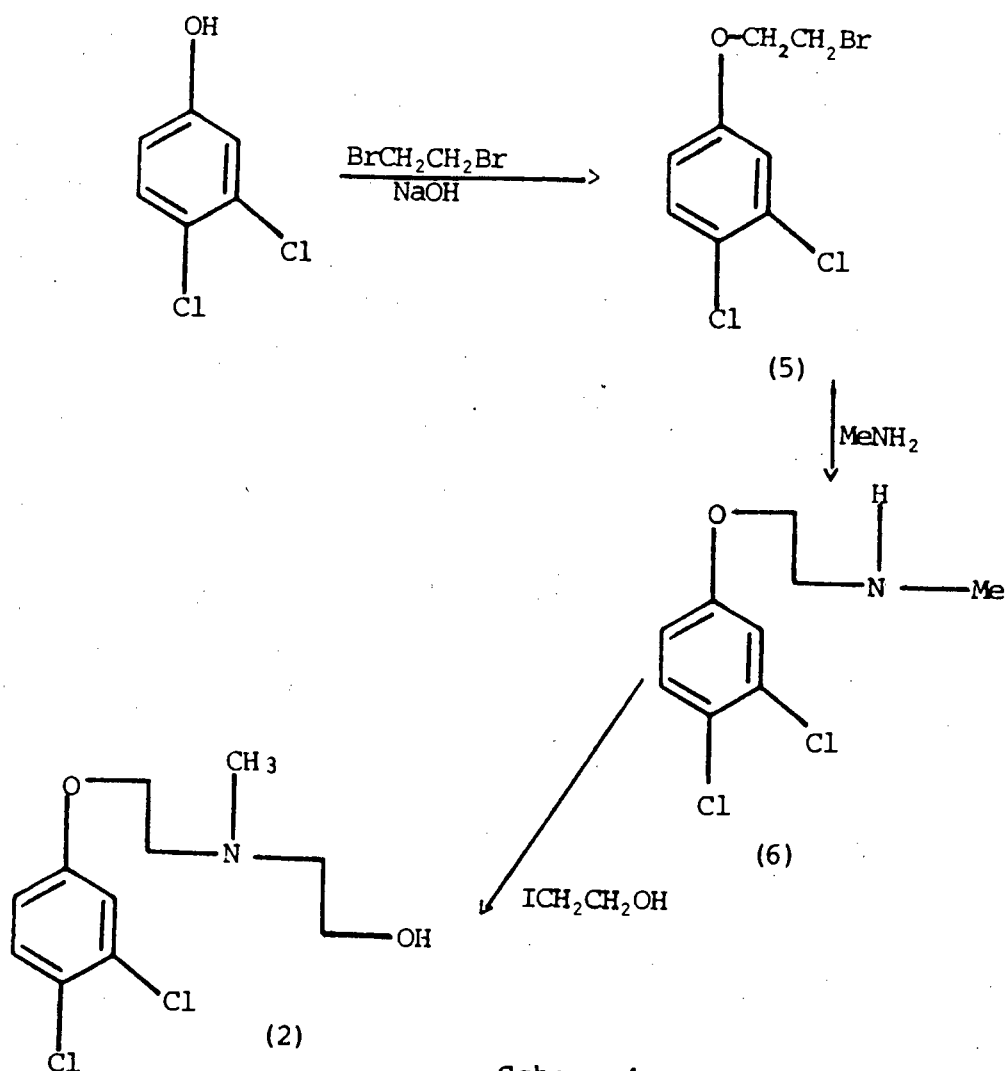
The four steps of the synthesis, will be discussed in the ensuing chapters.

CHAPTER 3

SYNTHESIS OF AMINOALCOHOL

### 3. SYNTHESIS OF AMINOALCOHOL (2)

The synthesis of methyl(2-hydroxyethyl)-[2-(3,4-dichlorophenoxy)ethyl]amine (2), which will be referred to as the aminoalcohol, was prepared by the route shown in scheme 4.



Scheme 4

1-bromo-2-(3,4-dichlorophenoxy)ethane (5) can be prepared<sup>13</sup> from 3,4-dichlorophenol and 1,2-dibromoethane in about 85% under phase transfer catalysis conditions, using a large excess of 1,2-dibromoethane in the presence of tetrabutylammonium bromide (5 mol %). Addition of the phase transfer

catalyst, tetrabutylammonium bromide, to the reaction mixture, facilitates the transfer of the phenoxide ion into the organic phase. In this case, the excess of 1,2-dibromoethane serves as the organic phase for phase transfer as well as a solvent for the product. The high molar ratio of 1,2-dibromoethane: phenol used, almost completely eliminates the formation of the disubstituted product, 1,2-bis-(3,4-dichlorophenoxy)ethane. During the work up of the reaction mixture, more than 80% of unreacted 1,2-dibromoethane can be recovered by distillation and used for further synthesis.

A small degree of dehydrobromination can result in some decrease in the basicity of the aqueous phase and reduce the yield of (5). It is therefore necessary to adjust the pH of the reaction mixture to 11, safely above the pKa of 3,4-dichlorophenol.

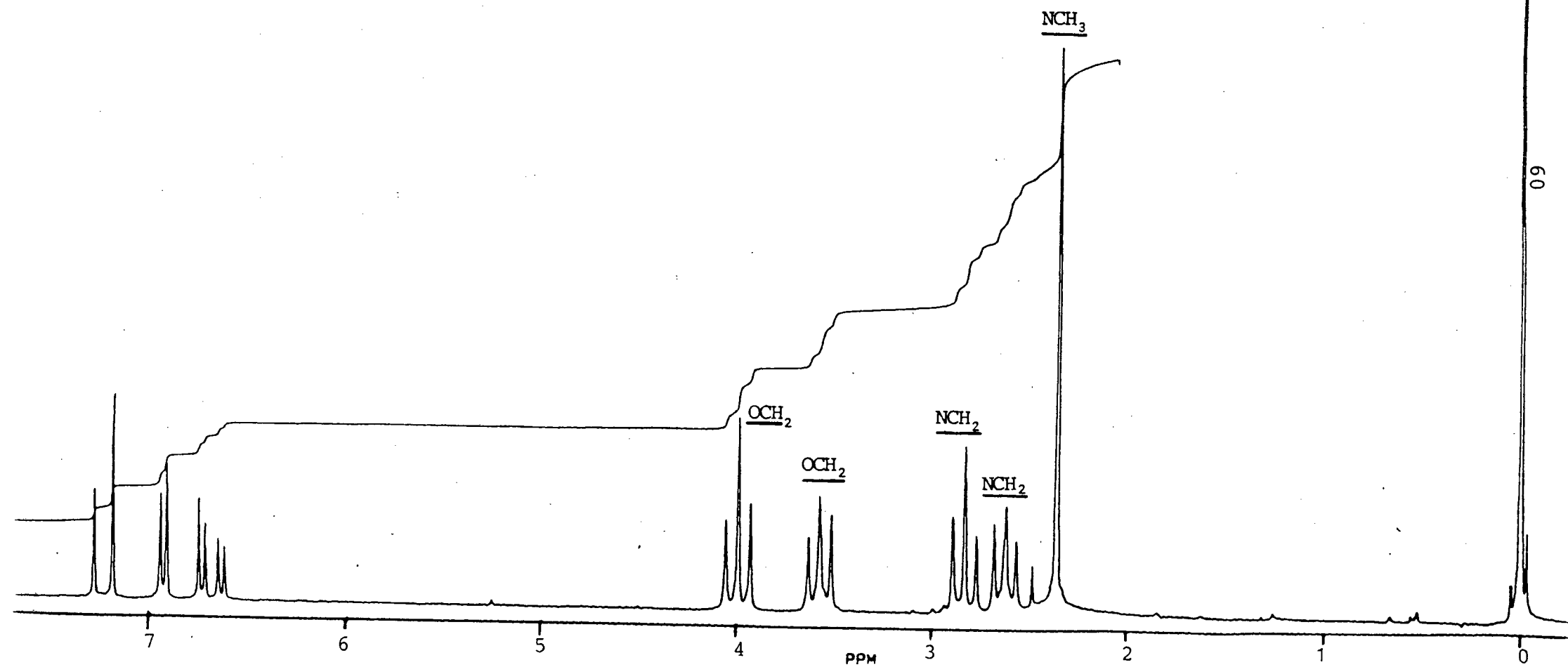
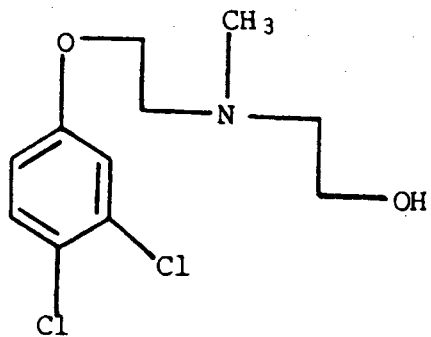
The amination of 1-bromo-2-(3,4-dichlorophenoxy)ethane to form 2-(3,4-dichlorophenoxy)ethylmethanamine (6), can be easily carried out by treating (5) with an excess of methanamine in ethanol at 80°C for 48 hours. The high molar ratio of methanamine: (5) almost completely eliminates the formation of the disubstituted product, methyl-bis-[2-(3,4-dichlorophenoxy)ethyl]amine, and the yields obtained are in the region of 80%.

Synthesis of the aminoalcohol (2) is then achieved by the reaction of iodo-ethanol with (6).

Figure 3, shows the  $^1\text{H}$  NMR spectrum of the aminoalcohol obtained.



Figure 3.  $^1\text{H}$  NMR spectrum of aminoalcohol in  $\text{CDCl}_3$  at 90 MHz



CHAPTER 4

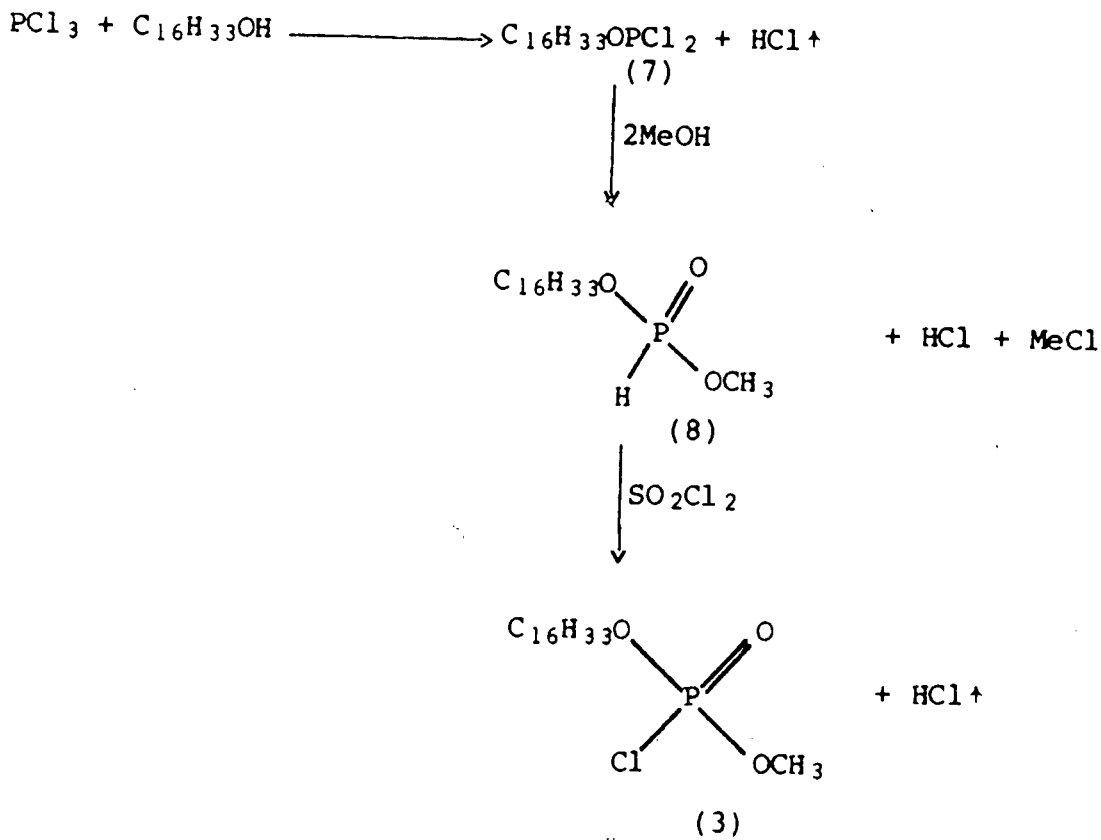
SYNTHESIS OF CETYL METHYL CHLOROPHOSPHATE

#### 4. SYNTHESIS OF CETYL METHYL CHLOROPHOSPHATE

Two routes were used in the synthesis of the cetyl methyl chlorophosphate, of which one proved successful and the other unsuccessful. The two routes are discussed below.

##### 4.1 Attempted synthesis starting with $\text{PCl}_3$

The route for the attempted synthesis is outlined below in scheme 5.



Scheme 5

Since alkyl dichlorophosphites (such as (7)) are extremely sensitive to hydrolysis, the first two reactions in scheme 5 were carried out as a "one pot" procedure, without any attempts to isolate intermediate product (7).

Cetyl alcohol and phosphorus trichloride are reacted in a 1:1 molar ratio to form the cetyl dichlorophosphite (7). The cetyl dichlorophosphite is then reacted with two moles of methanol, to form the cetyl methyl phosphite (8), with the release of HCl and MeCl. After purification, the cetyl methyl phosphite is reacted with sulfuryl chloride to produce the cetyl methyl chlorophosphate (3).

The synthesis however, proved to be problematic. The  $^{31}\text{P}$  NMR spectrum of the crude cetyl methyl phosphite (8), showed the existence of two major species. After purification of this product by column chromatography (dichloromethane: acetone; 95:5), three species were obtained in the following yields:

- (i) cetyl chloride ( $\text{C}_{16}\text{H}_{33}\text{Cl}$ ); 16%.
- (ii) dicetyl phosphite ( $(\text{C}_{16}\text{H}_{33}\text{O})_2\text{POH}$ ); 40%.
- (iii) cetyl methyl phosphite; 17%.

Although it is not known how dicetyl phosphite is formed in this reaction, evidence of its formation can be seen from the  $^1\text{H}$  NMR spectrum (figure 4), where there is an absence of a doublet caused by the P-OCH<sub>3</sub> signal being split by the  $^{31}\text{P}$  nucleus. It is seen that the proton attached to the phosphorus is also split to a doublet ( $J_{\text{H,P}} = 693 \text{ Hz}$ ).

Integration of the P-H peaks, and the two overlapping triplets at  $\delta 4.1$  ( $J_{\text{H,P}} = 9 \text{ Hz}$ ), due to the P-OCH<sub>2</sub> signal also being split by the  $^{31}\text{P}$  nucleus, as well as the high

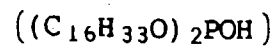
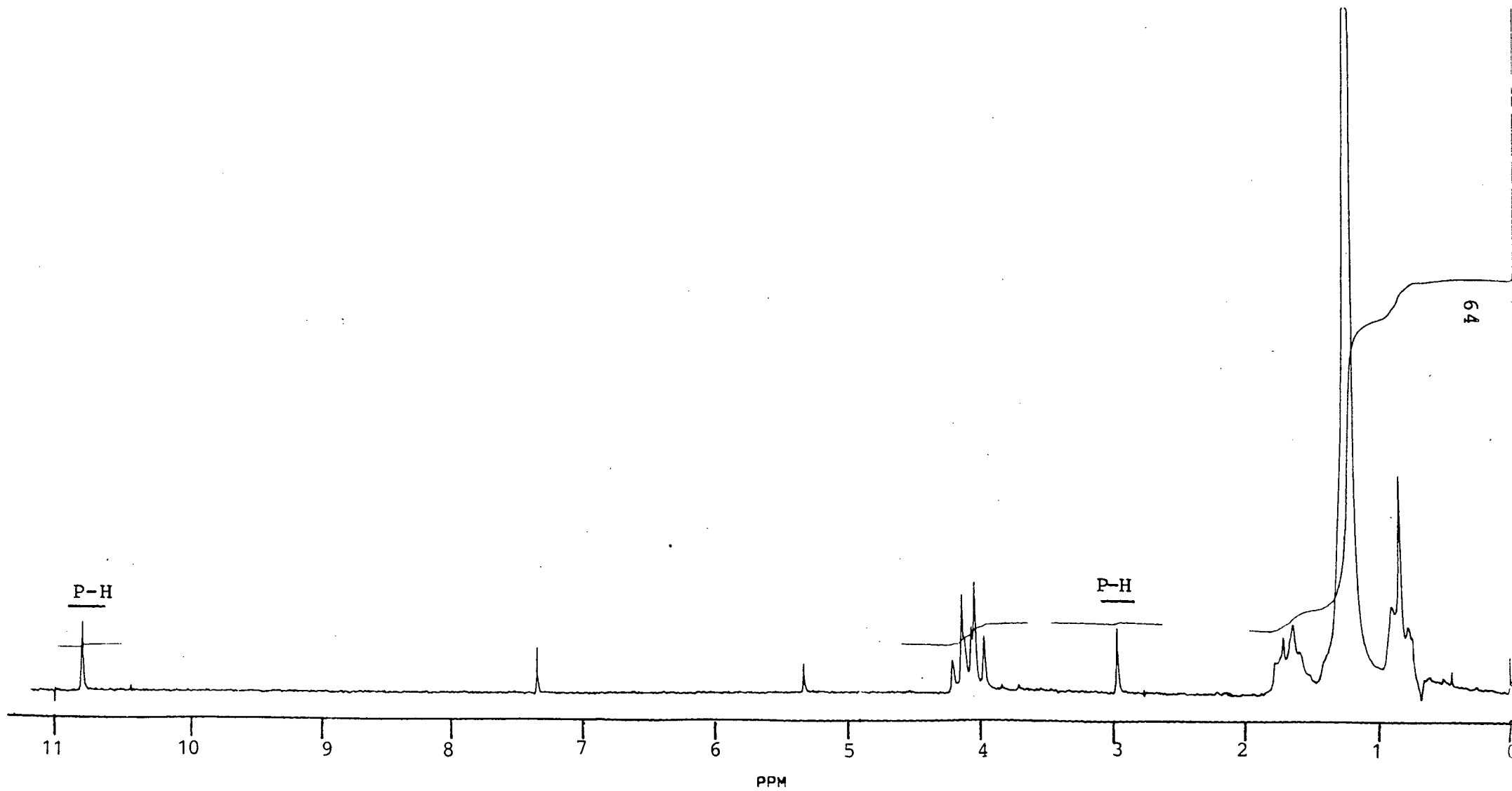


Figure 4.  $^1H$  NMR spectrum of dicetyl phosphite in  $CDCl_3$  at 90 MHz



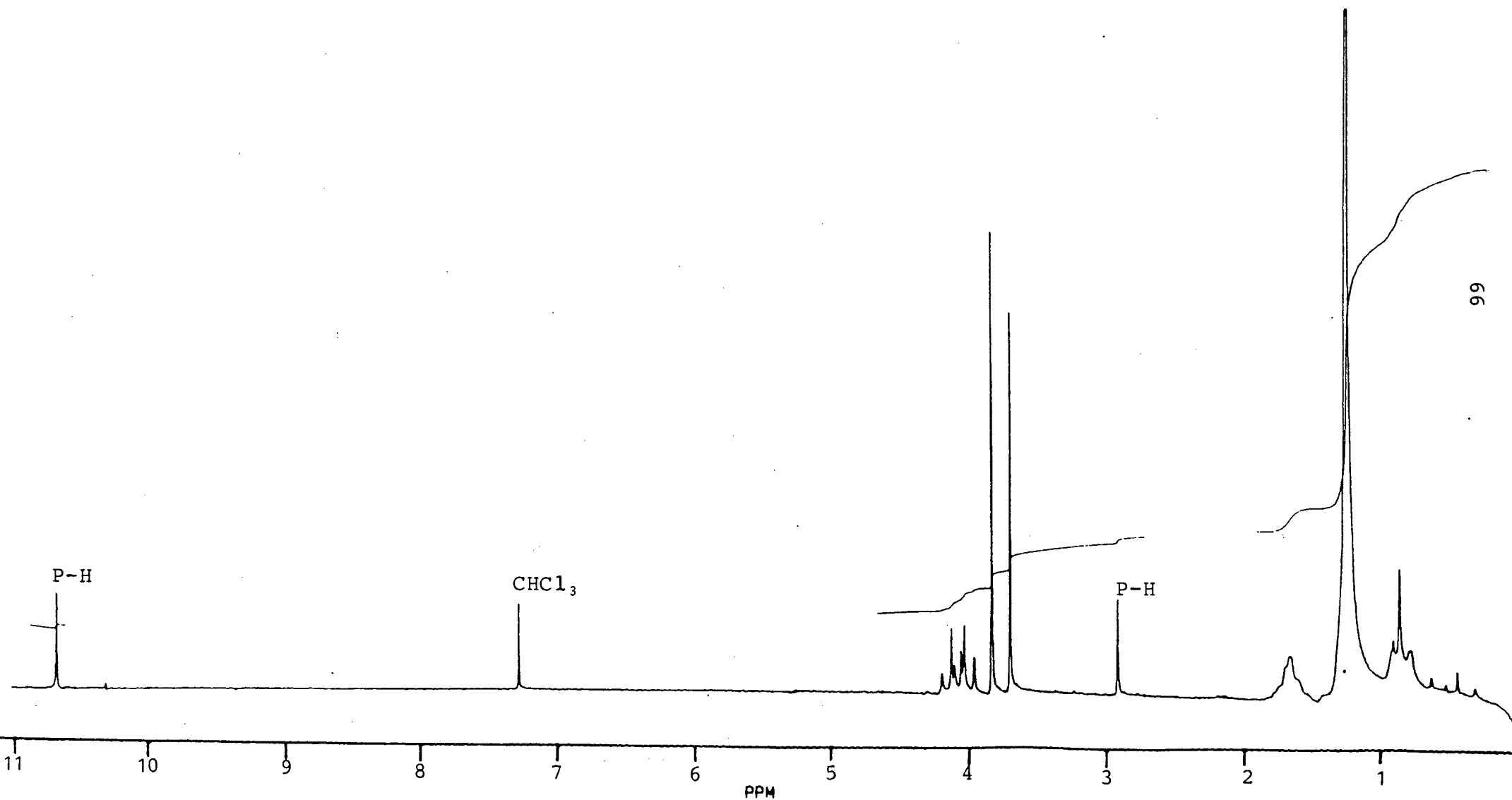
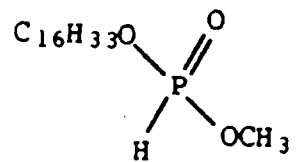
field signal of the cetyl chain, show them to be in the ratio of 1:4:62. Further evidence of its formation is supported by elemental analysis (see Experimental part).

Figure 5 shows the  $^1\text{H}$  NMR spectrum of the cetyl methyl phosphite. The doublet at  $\delta 3.78$  ( $J_{\text{H,P}} = 11.7$  Hz) is identified as the P-OCH<sub>3</sub> signal, split by the  $^{31}\text{P}$  nucleus. The two overlapping triplets at  $\delta 4.1$  are assigned to the P-OCH<sub>2</sub> signal which is also split by the  $^{31}\text{P}$  nucleus. The two P-H peaks ( $J_{\text{H,P}} = 693$  Hz) are also clearly visible. The integrals of the P-H, P-OCH<sub>2</sub> and P-OCH<sub>3</sub> signals are in the ratio of 1:2:3.

From the yield of 17% obtained for the desired cetyl methyl phosphite, it became apparent that the reaction had not been as successful as initially thought and the following attempts were made to optimize the reaction and improve the yield.

- (i) Different solvents were used to carry out the reaction, including benzene, carbon tetrachloride, petroleum ether and ether.
- (ii) The reaction was carried out at different temperatures, ranging from room temperature to  $-70^\circ\text{C}$ .
- (iii) Cetyl alcohol was added dropwise to phosphorus trichloride at different temperatures in different solvents.

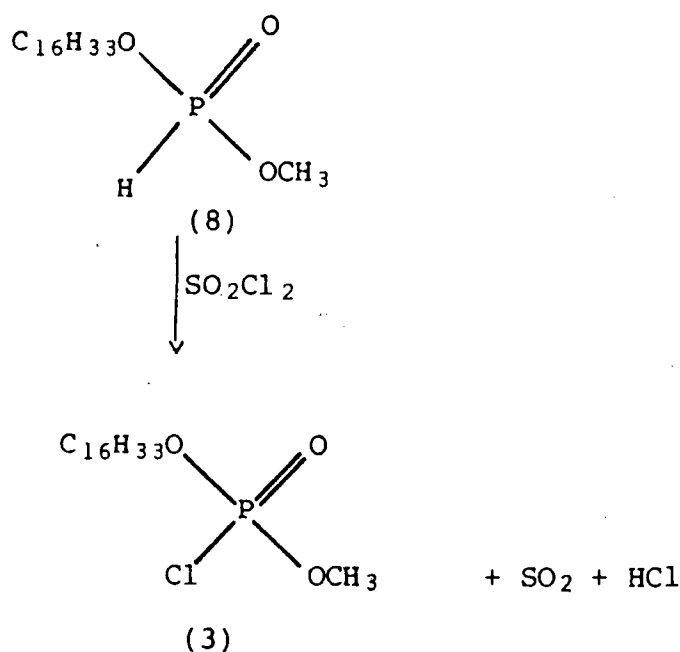
Figure 5.  $^1\text{H}$  NMR spectrum of cetyl methyl phosphite in  $\text{CDCl}_3$  at 90 MHz



- (iv) Cetyl alcohol was added all at once to phosphorus trichloride at different temperatures in different solvents.
- (v) The reaction was carried out under nitrogen.
- (vi) The reaction was allowed to stir for differing lengths of time.

All the above efforts however, proved to be unsuccessful, as the yield of the cetyl methyl phosphite remained poor, with dicetyl phosphite being formed in over twice as much yield.

Efforts to understand why the cetyl methyl phosphite was produced in such low yields were unsuccessful and it was decided to accept the low yield and proceed to the chlorination of (8) to produce the cetyl methyl chlorophosphate (3). (Scheme 6).



Scheme 6.



Following the directions of F.R. Atherton et al,<sup>14</sup> sulfonyl chloride was used to chlorinate (8). A ten-fold excess of sulfonyl chloride was added to a solution of (8) and the mixture stirred overnight. The solvent and excess sulfonyl chloride were removed using a rotary evaporator and a <sup>1</sup>H NMR spectrum of the residue taken. Figure 6 clearly shows the disappearance of the P-H peaks and the slight downfield shift of the P-OCH<sub>3</sub> doublet from δ3.77 to δ3.89, due to the electron withdrawing effect of the chloride atom.

However, attempts to react the cetyl methyl chlorophosphate (3) formed in this way, with the aminoalcohol (2) proved unsuccessful. The reaction was carried out in the same way as described in the experimental part and gave an unidentified mixture of products, in which neither the starting aminoalcohol, nor any significant quantities of expected product could be detected. Although the mass spectrum of the chlorophosphate used, showed the presence of the expected molecular ion (M<sup>+</sup>, m/e = 355), it was possible that the substrate contained some other components. It was decided therefore, to abandon the route involving synthesis of the chlorophosphate from phosphorus trichloride because of the following reasons.

- (i) There was no conclusive evidence to prove beyond doubt that the species was in fact cetyl methyl chlorophosphate.
- (ii) In forming the chlorophosphate, the yields were very poor and could not be improved.

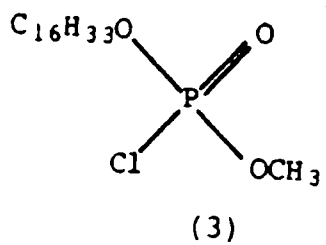
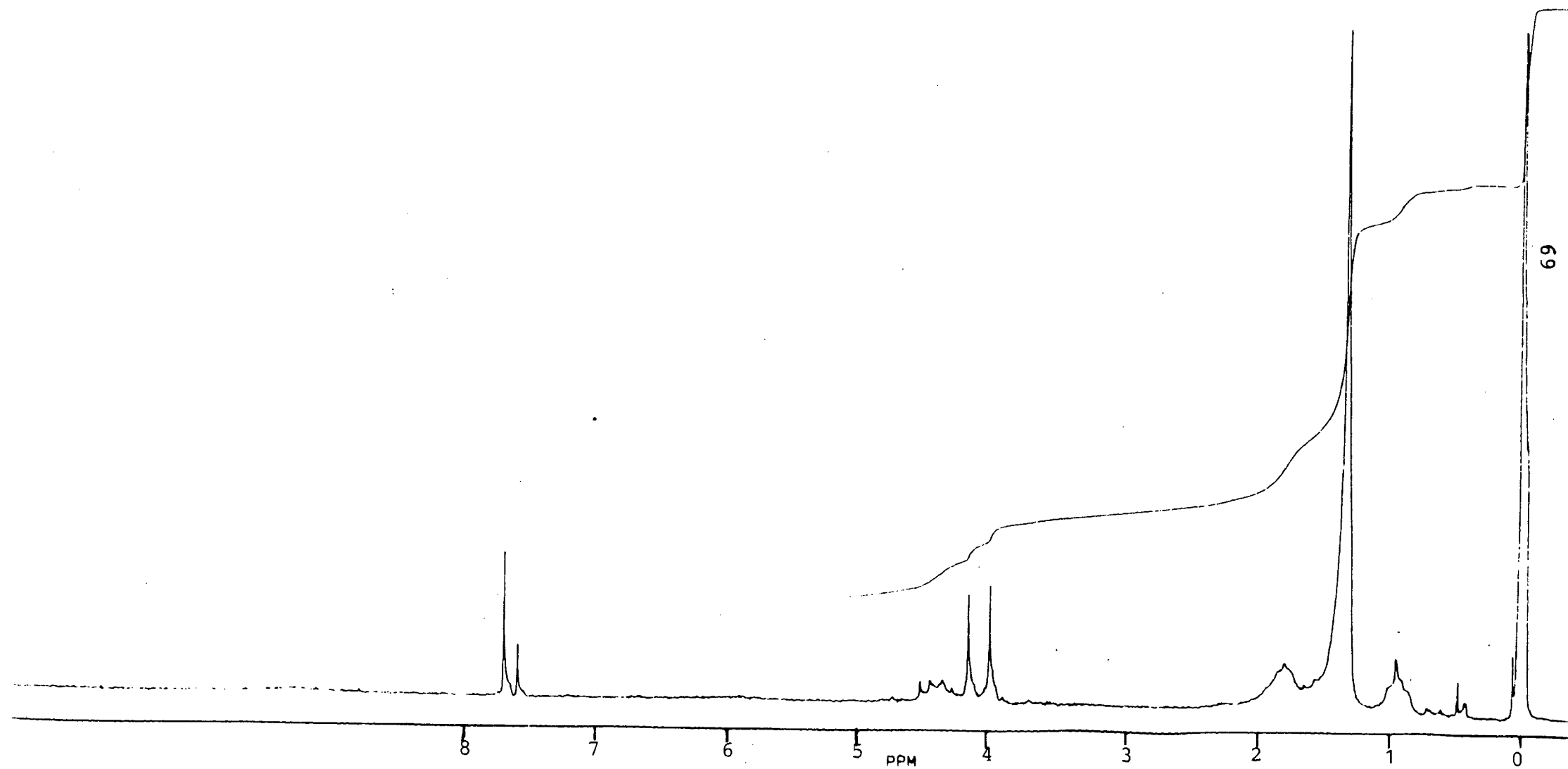


Figure 6.  $^1\text{H}$  NMR spectrum of cetyl methyl chlorophosphate in  $\text{CDCl}_3$  at 90 MHz

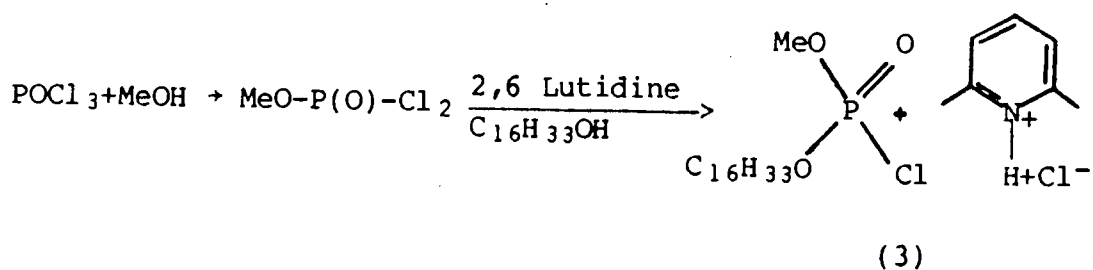


(iii) The reaction between the chlorophosphate and the aminoalcohol proved to be unsuccessful.

It was therefore decided to use the route described below to prepare the cetyl methyl chlorophosphate.

#### 4.2 Synthesis starting with POCl<sub>3</sub>

The route for the synthesis is outlined below in scheme 7.



Scheme 7.

It was previously reported<sup>11</sup> that by reacting methyl dichlorophosphate, formed by the reaction of methanol and phosphorus oxychloride, one could phosphorylate alcohols in a straightforward manner.

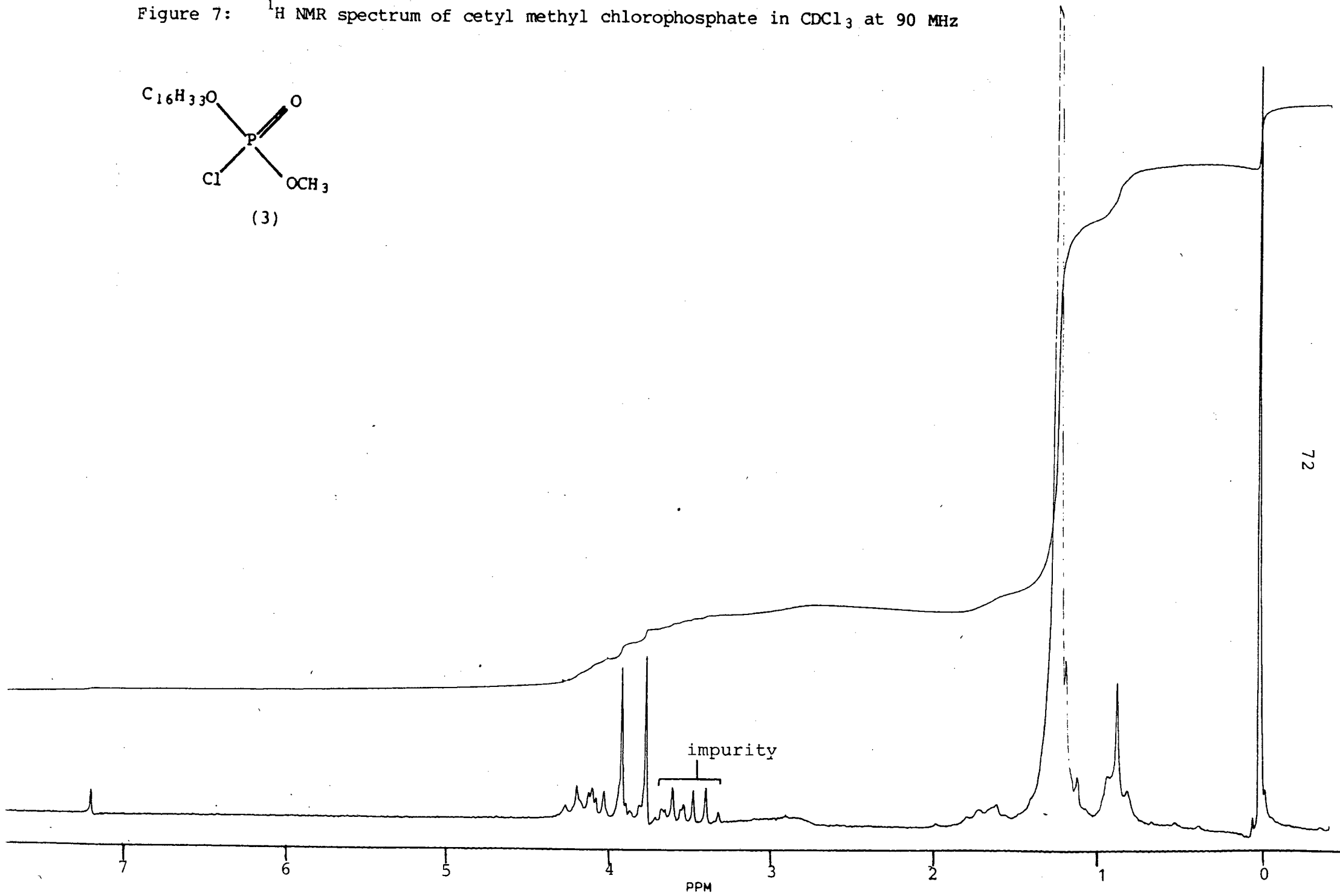
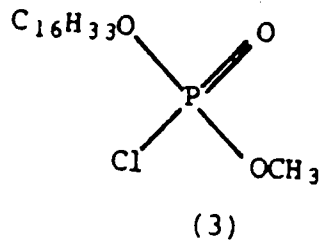
A solution of cetyl alcohol and 2,6-lutidine was reacted with a solution of methyl dichlorophosphate with the almost immediate precipitation of white 2,6-lutidine hydrochloride.

Recovery of the hydrochloride salt is essentially quantitative and the yield of the reaction is typically 85%. From the  $^1\text{H}$  NMR spectrum (figure 7), it was seen that the product (3) was essentially pure (~80%) and it was decided to react the crude product directly with the aminoalcohol. Since the resulting neutral phosphate triester would need to be purified by column chromatography, the slight impurity present in the cetyl methyl chlorophosphate could then be eliminated at that stage.

Problems initially experienced in the reaction included:

- (i) Use of petroleum ether as solvent in the reaction caused the lutidine hydrochloride salt to become sticky and filtration with suction was difficult. The problem was overcome using carbon tetrachloride as solvent.
- (ii) Slight demethylation of the  $-\text{OCH}_3$  group was observed (decrease of the OMe doublet in  $^1\text{H}$  NMR spectrum) when heat was applied to remove the solvent using a rotary evaporator. Demethylation does not occur however, when the solvent is removed at room temperature.

Figure 7:  $^1\text{H}$  NMR spectrum of cetyl methyl chlorophosphate in  $\text{CDCl}_3$  at 90 MHz

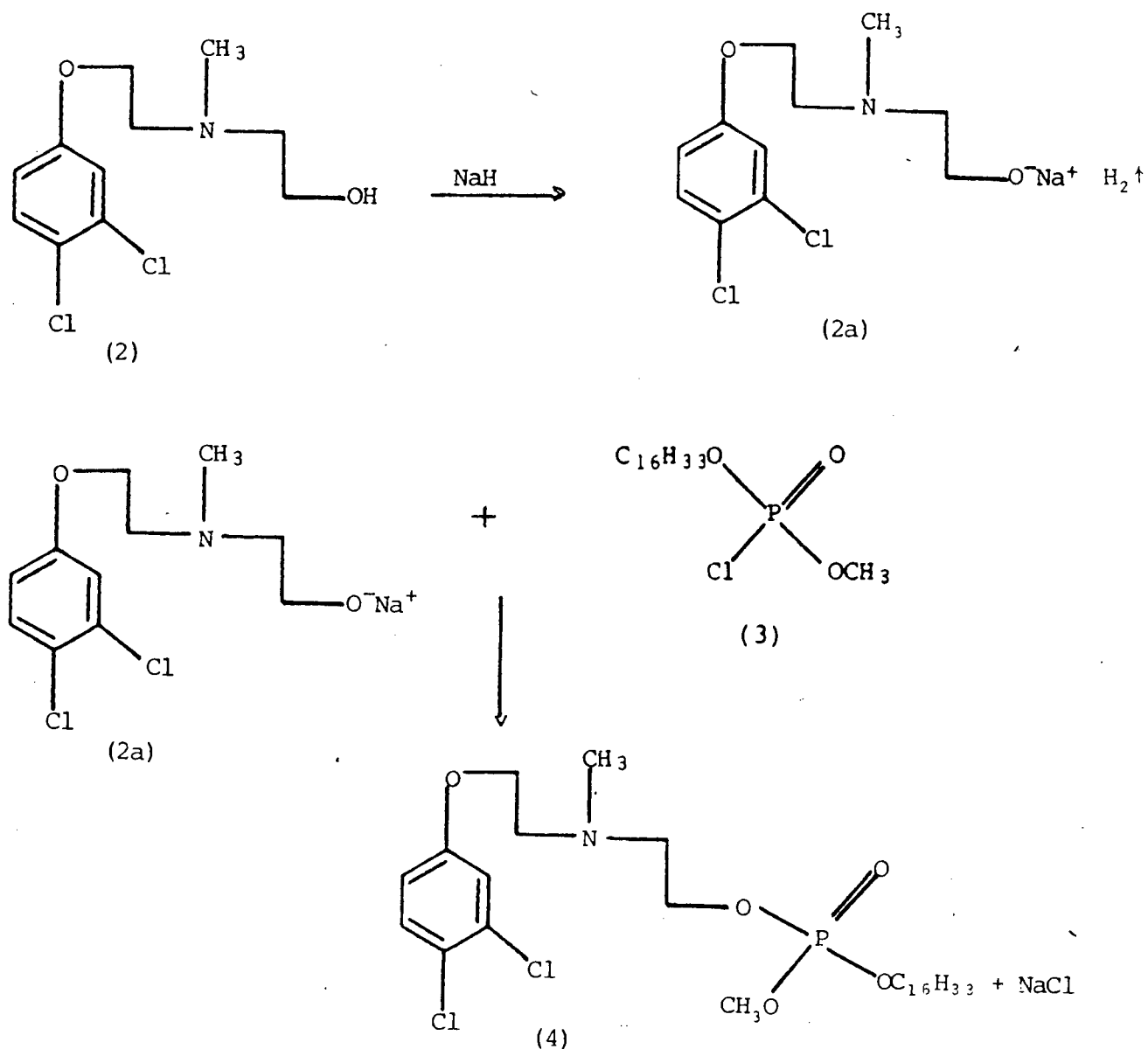


CHAPTER 5

REACTION OF AMINOALCOHOL WITH CETYL METHYL CHLOROPHOSPHATE

## 5. REACTION OF AMINOALCOHOL WITH CETYL METHYL CHLOROPHOSPHATE

The third part of the synthesis involved the formation of a phosphate ester linkage between the aminoalcohol (2) and the cetyl methyl chlorophosphate (3).



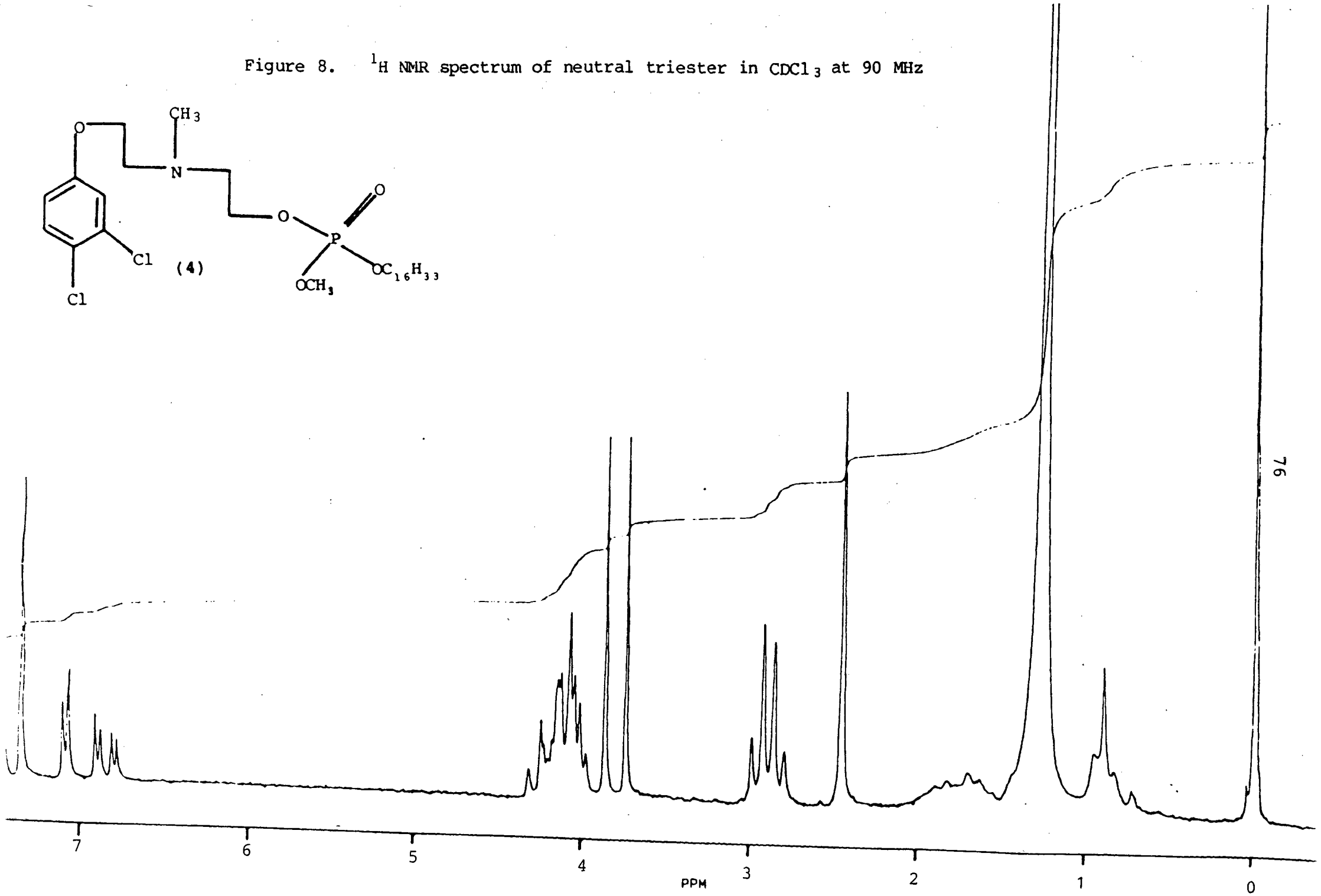
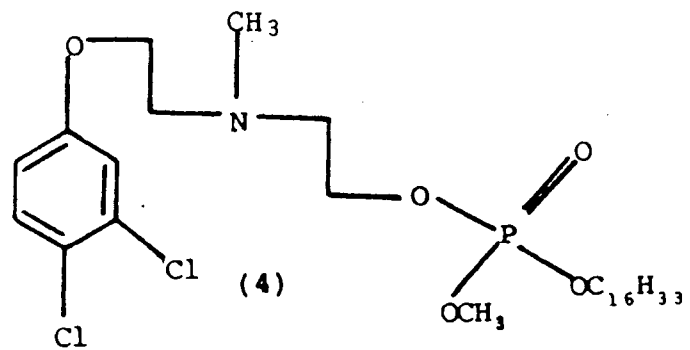
Scheme 8.

The aminoalcohol (2) is reacted with sodium hydride to form the sodium salt (2a), which is in turn reacted with (3) to form the neutral phosphate triester (4).

Figure 8, represents the  $^1\text{H}$  NMR spectrum of (4) obtained after purification. The P-OCH<sub>3</sub> doublet has shifted from  $\delta 3.92$  in the cetyl methyl chlorophosphate to  $\delta 3.78$  in the neutral triester (4), with the coupling constant changing from 14.4 Hz to 11.7 Hz. The multiplet at  $\delta 2.86$  is due to the N-CH<sub>2</sub> protons, whilst the multiplet at  $\delta 4.0$  is due to the O-CH<sub>2</sub> protons. The singlet at  $\delta 2.4$  is assigned to the N-CH<sub>3</sub> protons.



Figure 8.  $^1\text{H}$  NMR spectrum of neutral triester in  $\text{CDCl}_3$  at 90 MHz

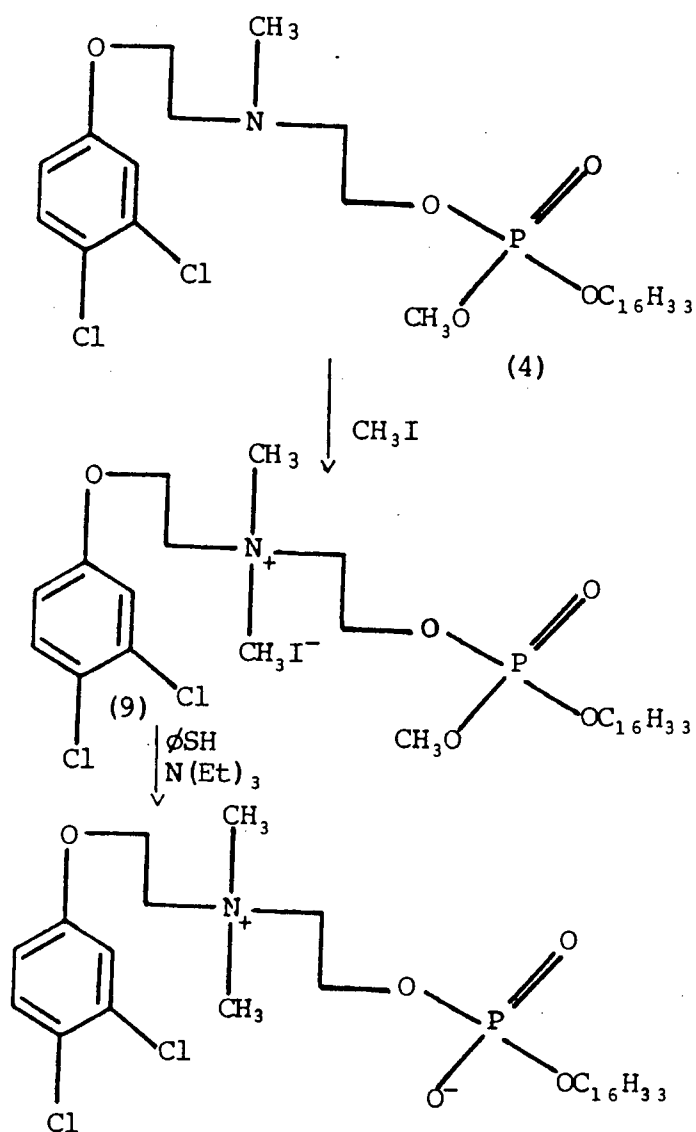


CHAPTER 6

N-METHYLATION AND O-DEMETHYLATION OF THE NEUTRAL TRIESTER

6. N-METHYLATION AND O-DEMETHYLATION OF THE NEUTRAL TRIESTER (4).

The final two steps of the synthesis involved the quaternization of the nitrogen by a methyl group and the demethylation of the P-OCH<sub>3</sub> group to produce the zwitterionic product. (Scheme 9).



Scheme 9.

In a method reported by Lacey and Loew,<sup>11</sup> the neutral triester is allowed to isomerize during a period of 4 weeks to produce the zwitterionic compound. However, during the isomerization period, the unwanted aziridinium ion is also formed. (Scheme 10).



iodide and a comparison of portions of the  $^1\text{H}$  NMR spectra of the ionic species obtained from the above reaction with that of the neutral triester, showed a number of differences: (figure 9).

Compare:

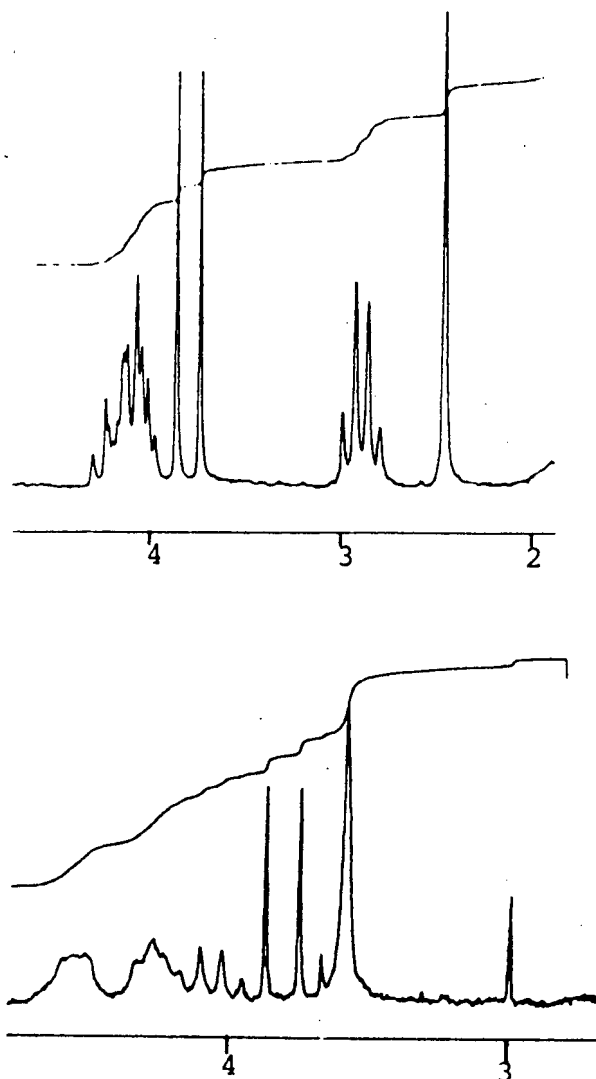


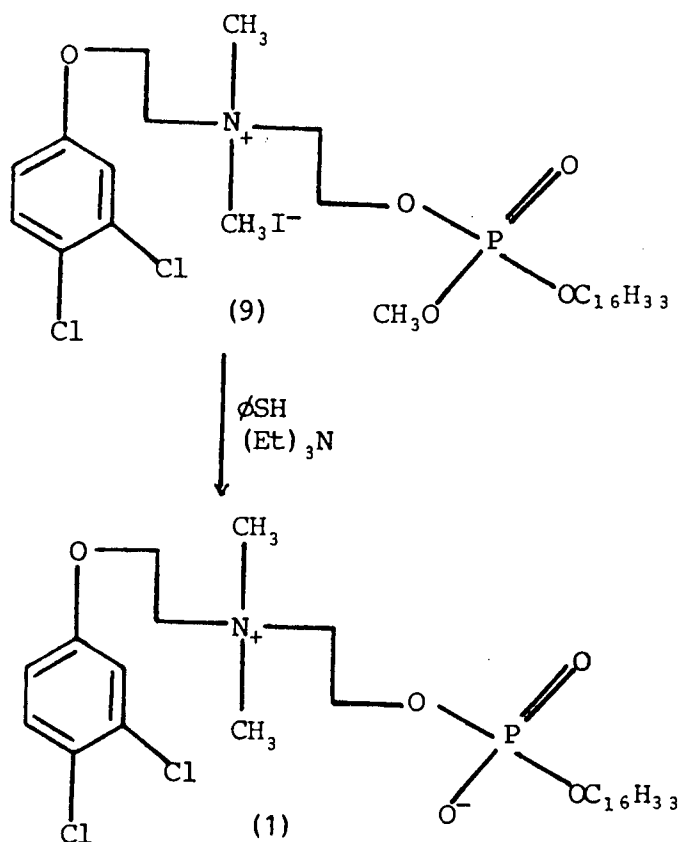
Figure 9.

The singlet at  $\delta 2.4$  in the neutral triester, due to the  $\text{N-CH}_3$  protons, has disappeared, with the formation of a new singlet at  $\delta 3.58$  due to the  $\text{N}^+\text{-CH}_3$  protons. Loss of the multiplet at  $\delta 2.86$  due to the  $\text{N-CH}_2$  protons is also observed, with the multiplet now being found downfield at  $\delta 4.08$  due to the positive charge on the nitrogen. Retention of the doublet is

also observed (although shifted slightly downfield from  $\delta 3.76$  to  $\delta 3.82$ ), indicating that no demethylation of the P-OCH<sub>3</sub> group had taken place.

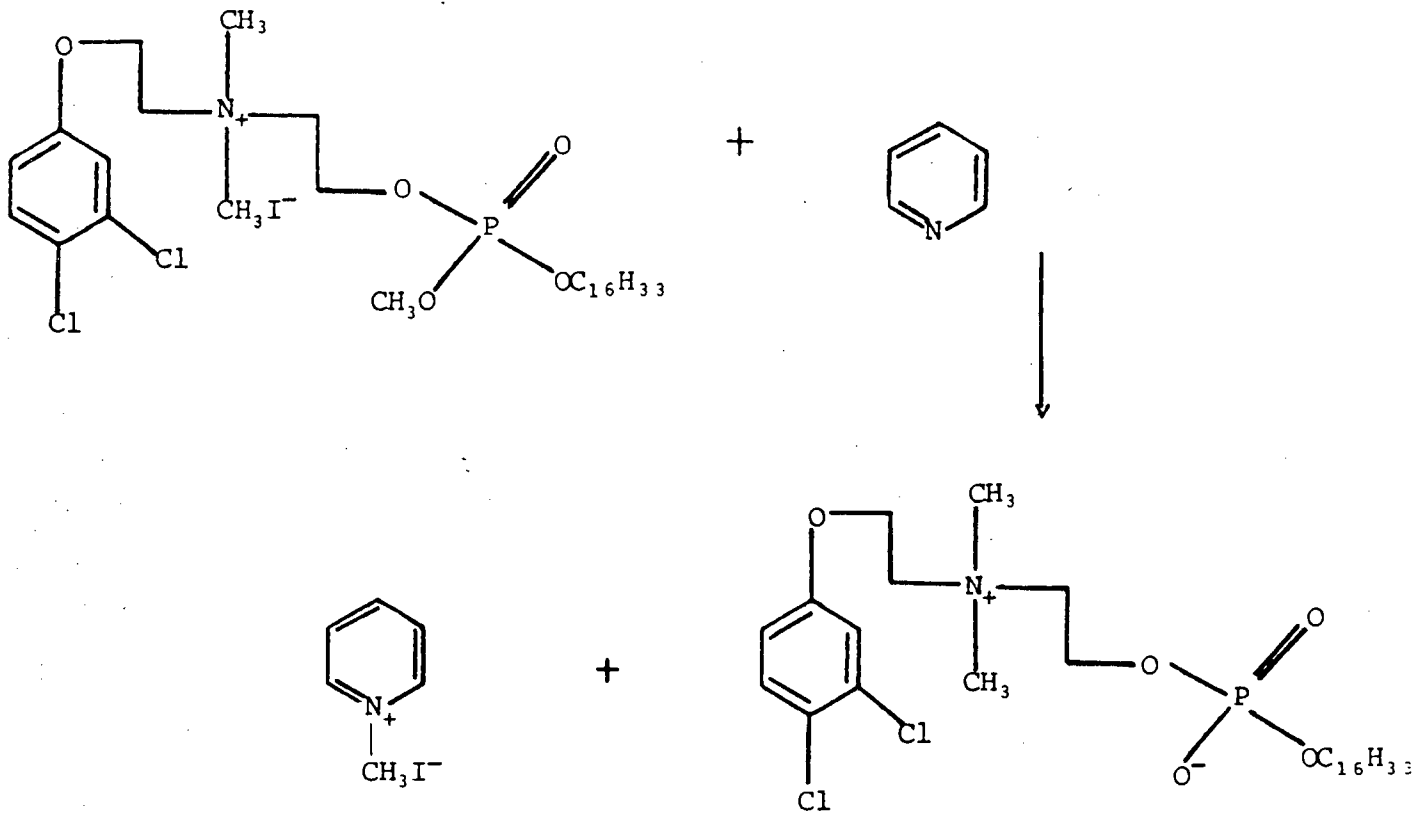
## 6.2 O-Demethylation of (9).

Scheme 11, shows the final step of the synthesis.



Scheme 11.

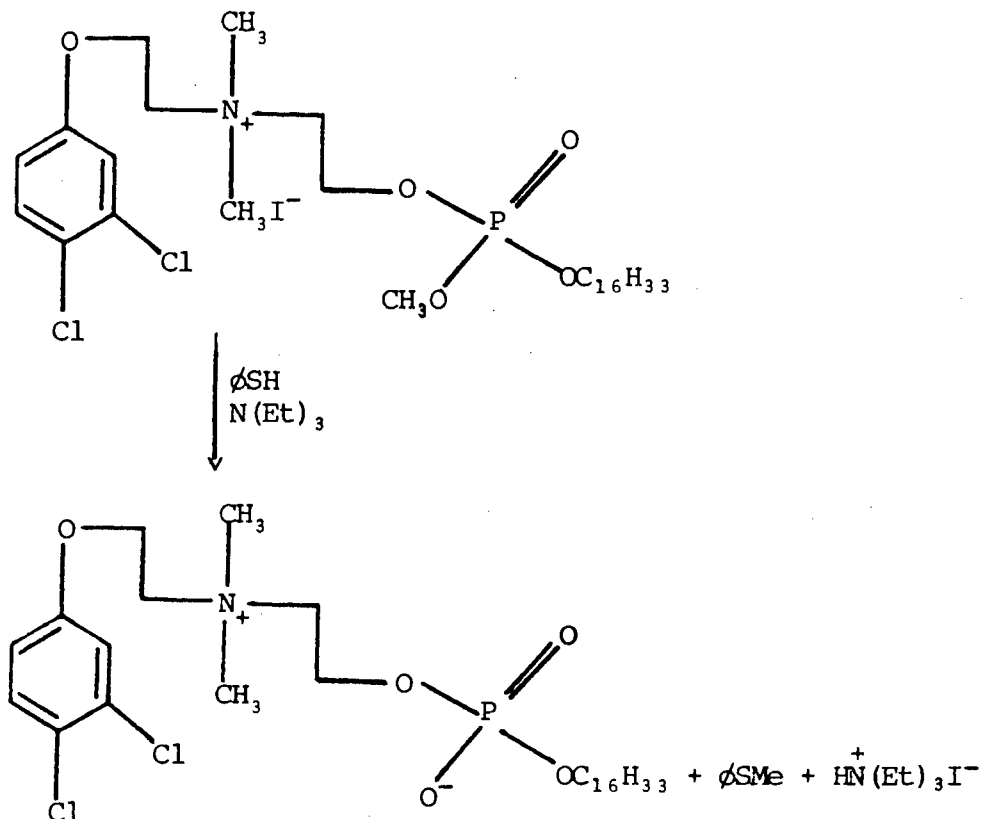
Initial attempts at demethylation using pyridine proved unsuccessful. Compound (9) was dissolved in chloroform, and a 1.1 molar excess of pyridine added, and the solution placed in a water bath at 60°C for four days, in the hope that the reaction illustrated in scheme 12 would take place.



Scheme 12.

The <sup>1</sup>H NMR spectrum showed that complete demethylation had occurred, as proved by the absence of the P-OCH<sub>3</sub> doublet. However, separation of the phosphatidyl choline from the N-methylpyridinium salt proved difficult. An extraction was attempted using chloroform/water in the hope that the phosphatidyl choline would remain in the chloroform and the N-methylpyridinium salt would dissolve in water. This however, was unsuccessful, as the phosphatidyl choline seemed to hydrolyse. Separation by column chromatography was also attempted (silica gel, CHCl<sub>3</sub>). This also proved unsuccessful, as a mixture of products was obtained. It was therefore decided to abandon this method of demethylation in favour of the one described below.

Thiophenol was used as a demethylating agent (scheme 13), according to the method reported by Daub and Tamelen,<sup>15</sup> in which the methyl group was removed under mild conditions, after serving as a protecting group in phosphodiester synthesis:



Scheme 13.

In the reported<sup>15</sup> method, a hundred-fold excess of thiophenol and triethylamine was used and the reaction carried out at room temperature. It was decided however to use only a three-fold excess of thiophenol and triethylamine (for practical purposes of separation), and to carry out the reaction at 60°C.

By dissolving (9) in deuterated chloroform, the rate of demethylation could be followed in the  $^1\text{H}$  NMR spectrum by observing the rate of disappearance of the P-OCH<sub>3</sub> doublet.



Figure 10, illustrates the progress of demethylation over a period of two days.

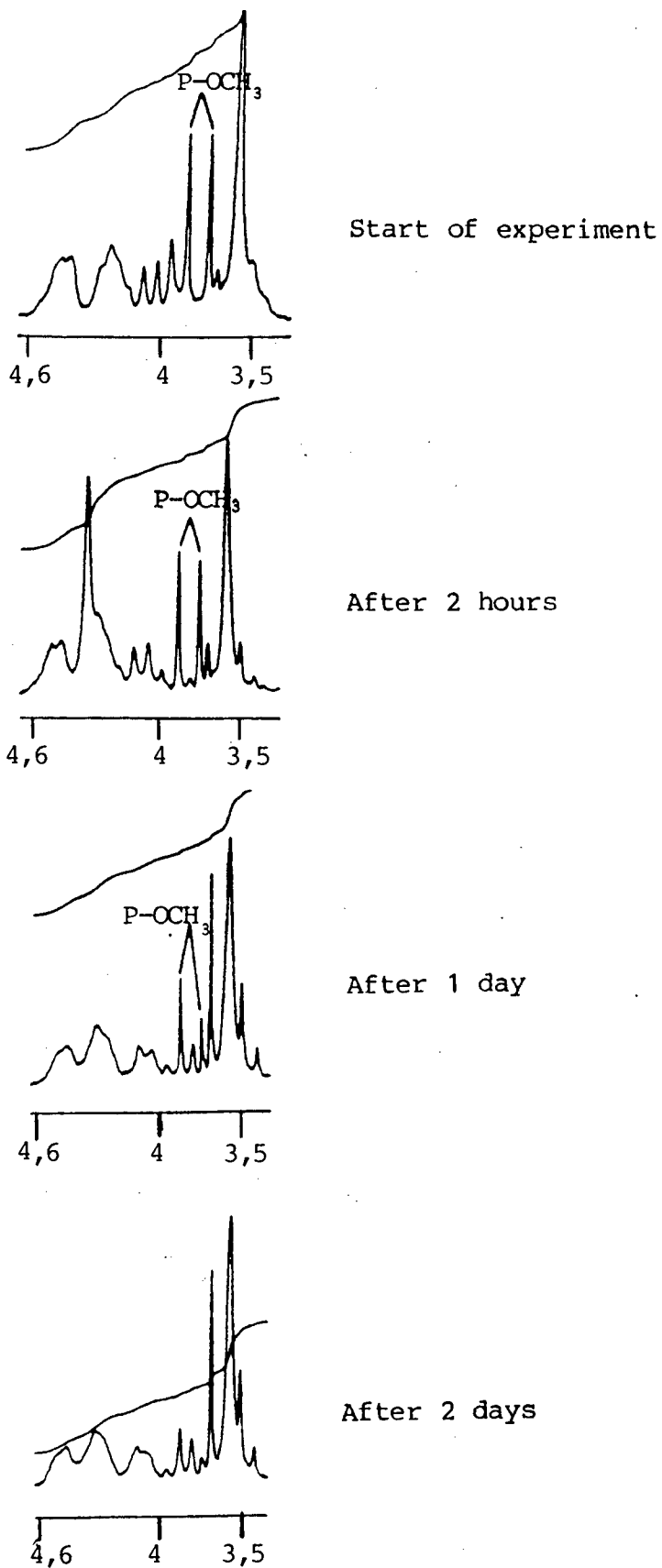


Figure 10.

Demethylation is complete after two days, since the doublet has disappeared. The rest of the  $^1\text{H}$  NMR spectrum has remained essentially the same.

The next problem however, was the separation of the phosphatidyl choline (1) from the reaction mixture. Owing to the experiences of previous demethylation experiments with pyridine, it was decided not to use extraction procedures but to attempt to isolate (1) from the reaction mixture using column chromatography. The purification procedure involved separation of the following components of the mixture

- (i) the phosphatidyl choline (1).
- (ii) Excess of thiophenol.
- (iii) Excess of triethylamine.
- (iv) Methyl phenyl sulfide.
- (v) The triethylammonium iodide.

From thin layer chromatography (silica gel; dichloromethane: methanol; 4:1) it was found that the thiophenol moved with the solvent front and the triethylamine was found at a  $R_f$  value of 0.61. Since the triethylammonium salt was expected to remain on the column and not be eluted, the only two species whose elution pattern was not known, was that of (1) and the

methyl phenyl sulfide.

The reaction mixture was then eluted through a silica gel column using an initial solvent system of dichloromethane: methanol (4:1) and it was found that the first fraction consisted of a mixture of thiophenol and methyl phenyl sulfide. It would appear that the two species coeluted and this could be concluded from the singlet which appeared at  $\delta 2.48$  in the  $^1\text{H}$  NMR spectrum (figure 11) of this fraction, due to the S-Me protons. The second fraction eluted, contained the excess triethylamine.

It was then found that after the triethylamine fraction, nothing else appeared to be eluted from the column. The solvent system was then changed to a dichloromethane : methanol ratio of 1:1. However, even with the increased polarity of the solvent system, nothing more was eluted from the column.

The dichloromethane was then abandoned and pure methanol was used in the elution of the column. The methanolic fractions contained the product (1), of which the  $^1\text{H}$  NMR spectrum is shown in figure 12. The singlet of the  $\text{N}^+\text{-Me}$  is clearly seen at  $\delta 3.4$ , as are the aromatic hydrogens found downfield at  $\delta 6.8\text{-}7.4$ .

In theory, one would expect to see five distinct triplets, due to the O-CH<sub>2</sub> and N-CH<sub>2</sub> protons. In practice however, this is not the case, as the signals overlap and therefore appear as broad singlets. There also appears to be a broad singlet at  $\delta 3.85$  (marked X) not associated with the zwitterionic

phen  
øSMe

Figure 11. A NMR spectrum (90 MHz) of the first fraction obtained in column chromatography of the crude demethylation product. The fraction contains thiophenol and phenyl methyl sulfide.

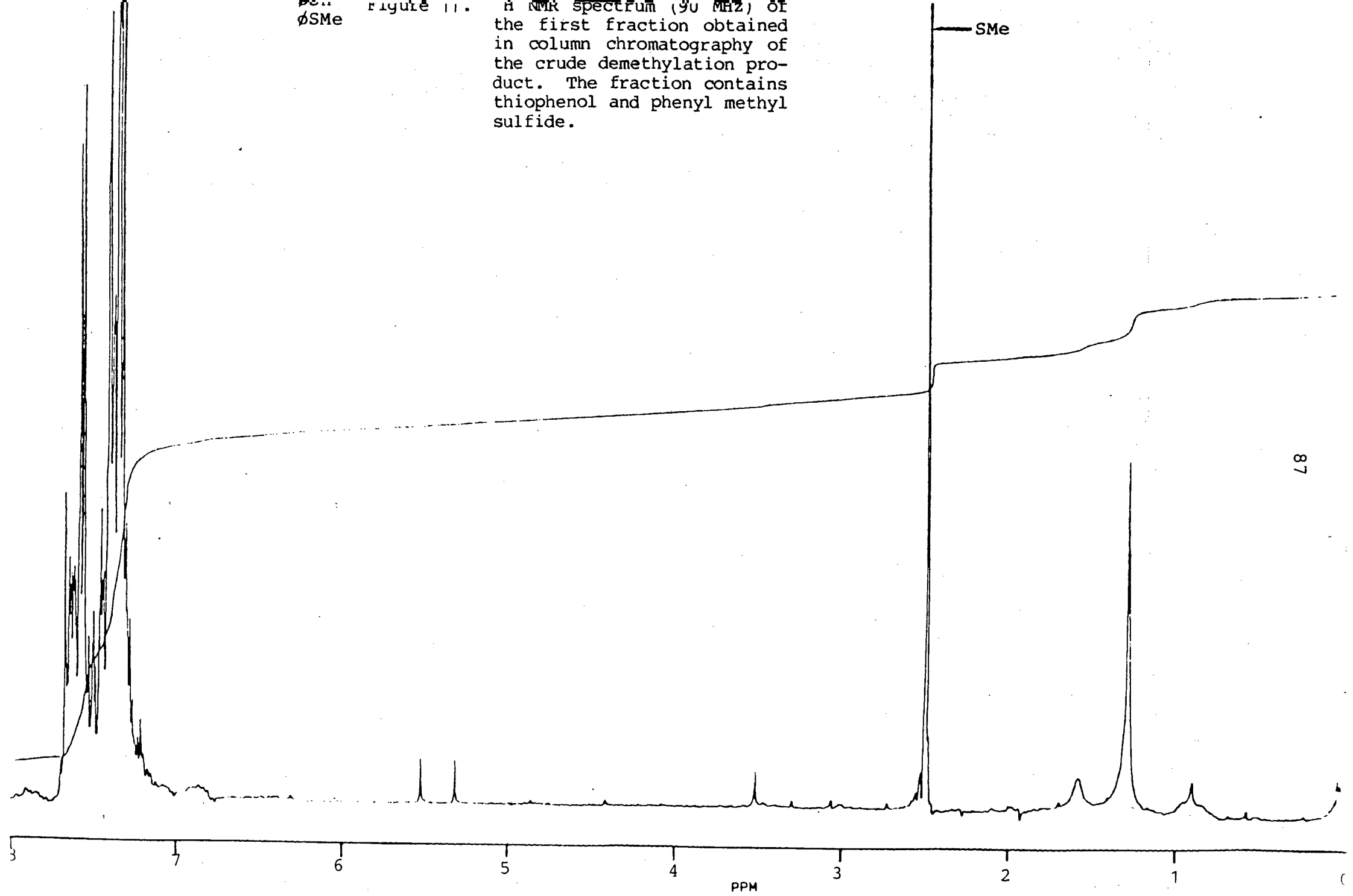
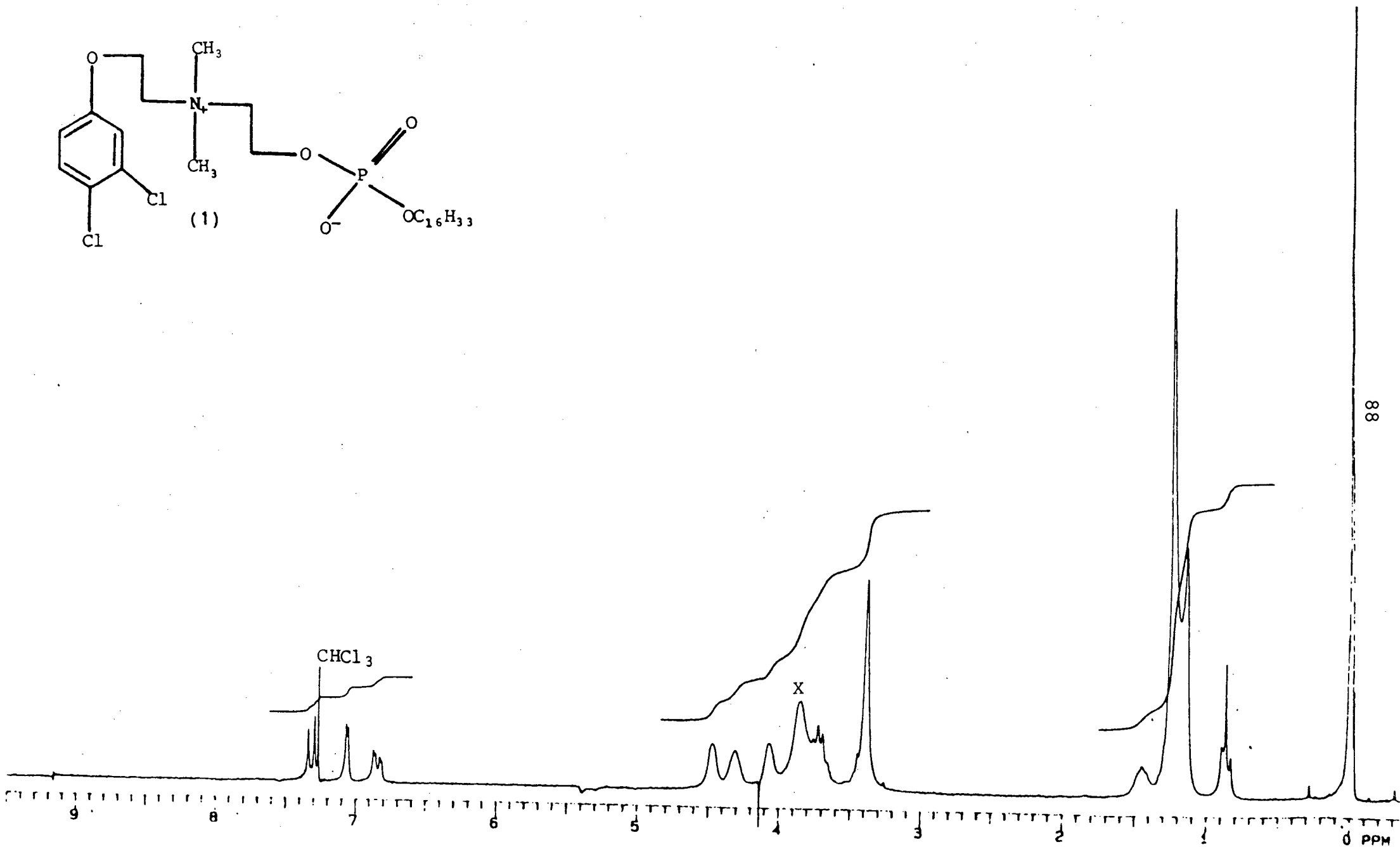
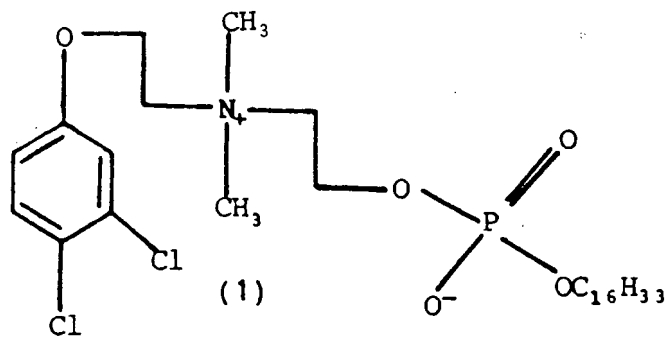


Figure 12.  $^1\text{H}$  NMR spectrum of (1) in  $\text{CDCl}_3$  at 200 MHz

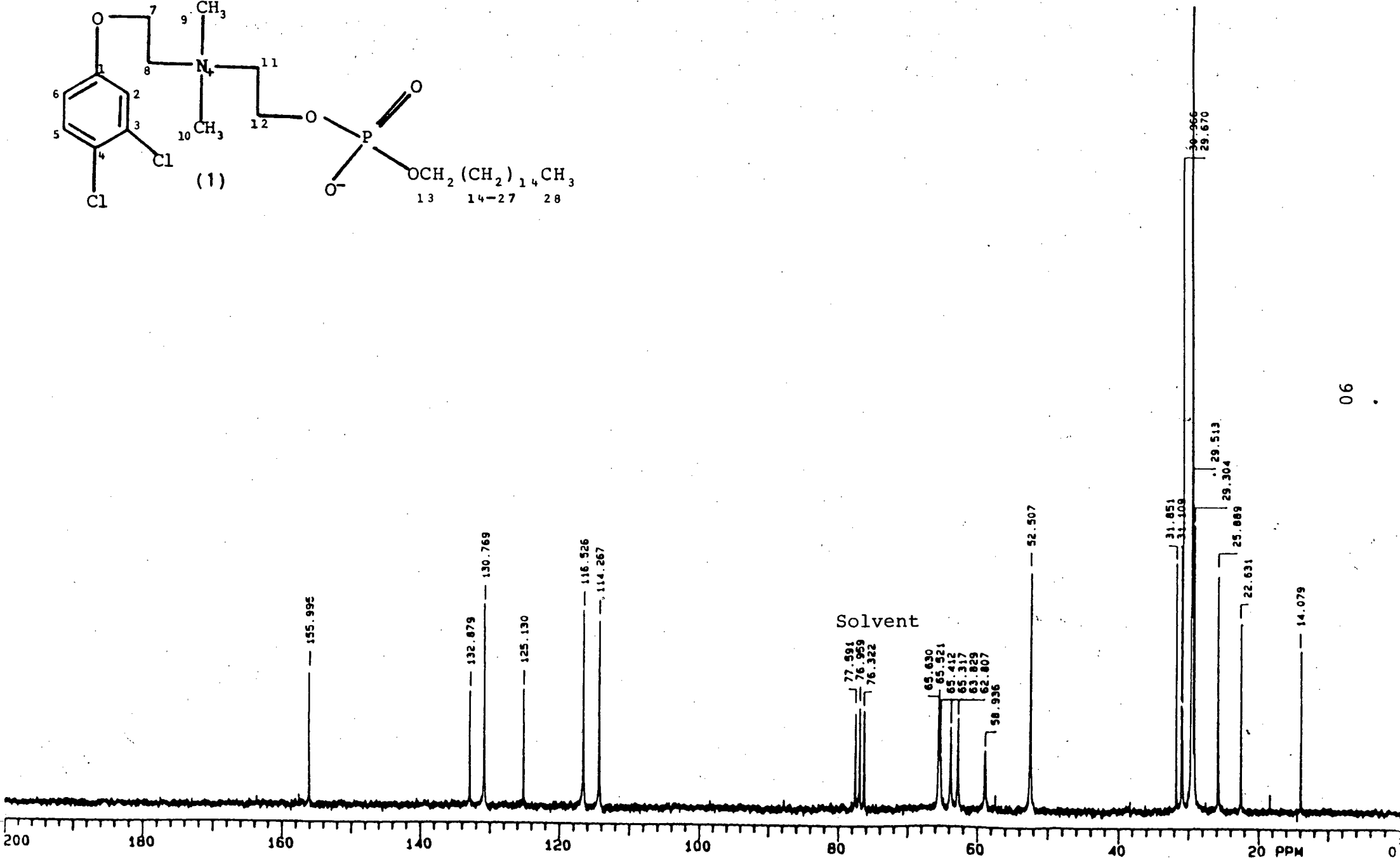
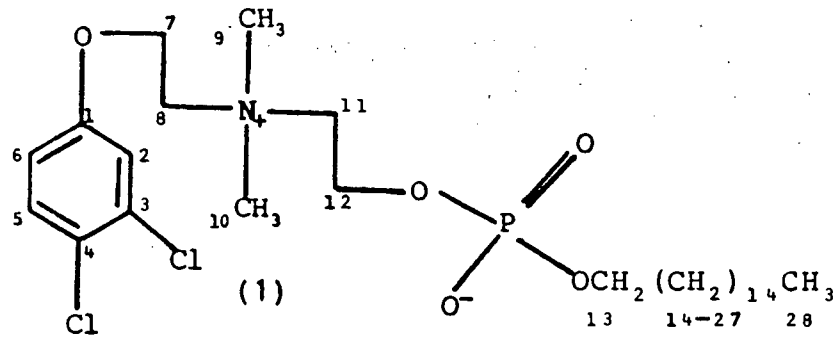


compound (1). Upon D<sub>2</sub>O wash of (1) it was found that the singlet disappeared. It was therefore concluded that this singlet could only be due to water molecules being associated with the product. When two molecules of water per molecule of product were included in the calculations, elemental analysis obtained for this product, agreed well with calculated values.

The <sup>31</sup>P NMR spectrum of the phosphatidyl choline (1), showed a single peak at δ-2.6, indicating that no other phosphorus species was present and that the compound was pure.

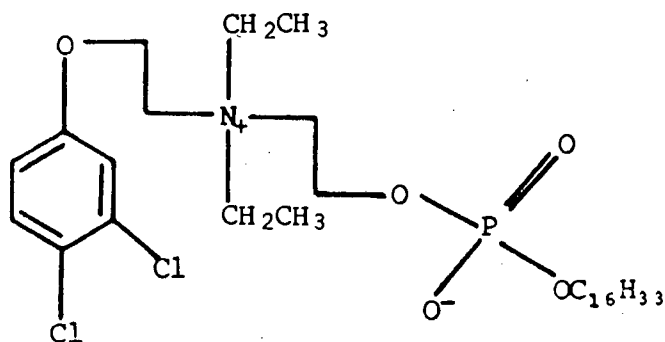
Figure 13, shows the <sup>13</sup>C NMR spectrum of (1). The singlet at 14.1 ppm represents the terminal CH<sub>3</sub> carbon (C 28) of the cetyl chain. At 22-32 ppm are found the CH<sub>2</sub> carbons (C 14 - C 27) of the cetyl chain. The singlet at 52.5 ppm is representative of the two methyl group carbons (C 9, C 10) of the quaternary ammonium group. The signal at 58.9 ppm is assigned to the OCH<sub>2</sub> carbon (C 7), whilst the signals at 62.8 ppm and 63.8 ppm are assigned to the N-CH<sub>2</sub>(C 11) and N-CH<sub>2</sub>(C 8) carbons respectively. The next signal at approximately 65.4 ppm and displaying splitting, is due to the CH<sub>2</sub>-OP carbons (C 12, C 13). Each carbon is split by the <sup>31</sup>P nucleus to produce a doublet. The two doublets overlap however, and it is not possible to distinguish them. The signals found low field at 114.2 ppm, 116.5 ppm and 130.7 ppm represent the C-H carbons of the aromatic ring (C 6, C 2 and C 5 respectively). The signals at 125.1 ppm (C 4), 132.8 ppm (C 3) and 155.9 ppm (C 1) represent the quaternary carbon atoms.

Figure 13.  $^{13}\text{C}$  NMR spectrum of (1) in  $\text{CDCl}_3$



In  $^{13}\text{C}$  - NMR spectroscopy it is possible to distinguish between signals of quaternary and methylene carbons on the one hand, and those of methine and methyl carbons on the other. Figure 14 shows the result of an attached proton test (APT) performed on (1), in order to distinguish between signals of quaternary C atoms and  $\text{CH}$ ,  $\text{CH}_2$  and  $\text{CH}_3$  groups. The signals of quaternary C atoms and those of the  $\text{CH}_2$  groups, have a positive phase, and those of  $\text{CH}$  and  $\text{CH}_3$  groups a negative phase.

Although the phosphatidyl choline (1) has been successfully synthesized and appears to show biological activity, it is the diethyl analogue (10) that is expected to show even greater biological activity.



(10)

The synthesis of (10) has been successfully completed up to the neutral phosphate triester (11) stage in the same manner as described for (4), and with similar yields, with the only difference being that a solution of ethylamine in water was used instead of methylamine solution. Figure 15 represents the  $^1\text{H}$  NMR spectrum of the neutral phosphate triester (11). In an attempt to quaternize the nitrogen atom in (11), the



Figure 14.

ATTACHED PROTON TEST (APT) FOR (1):

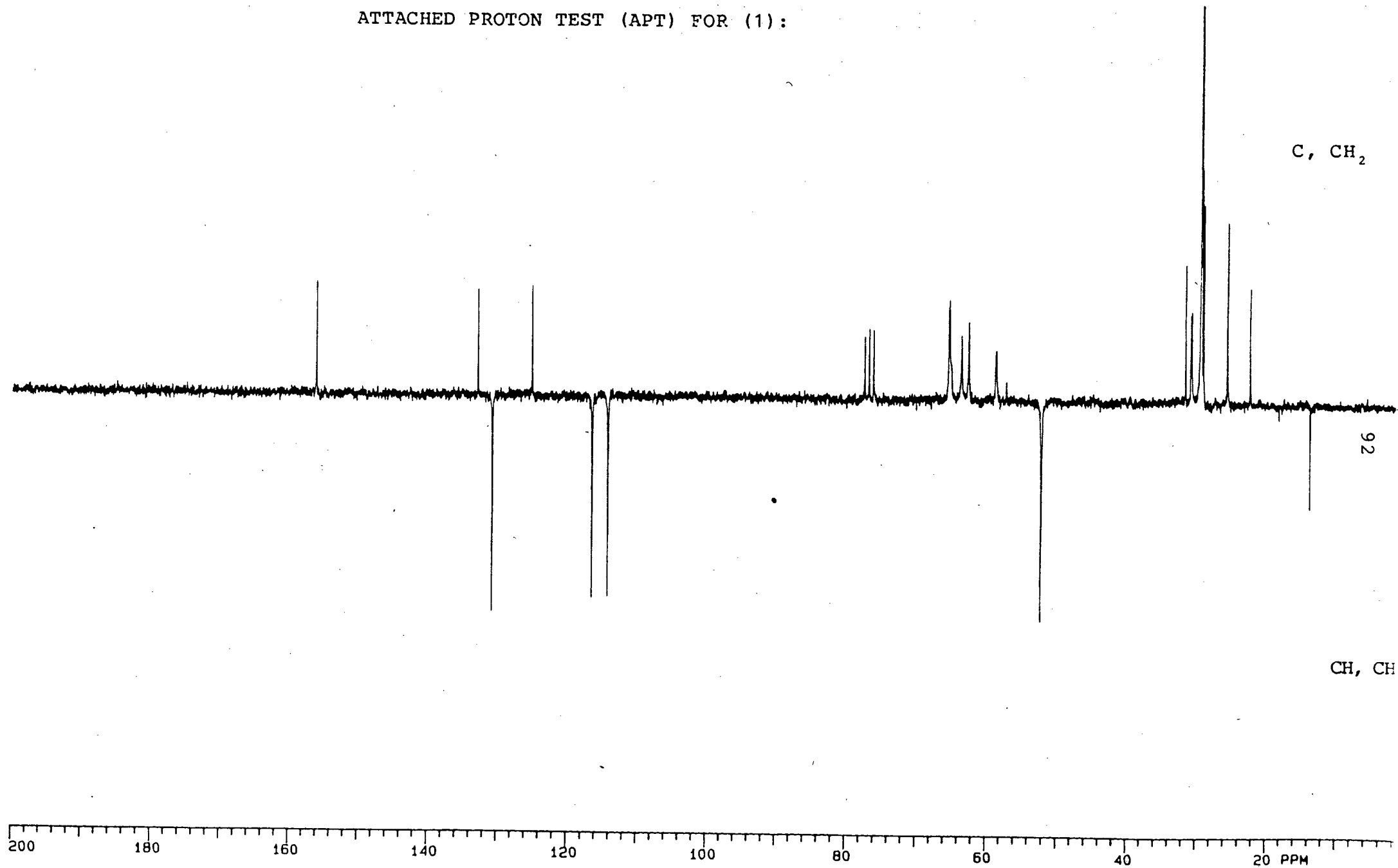
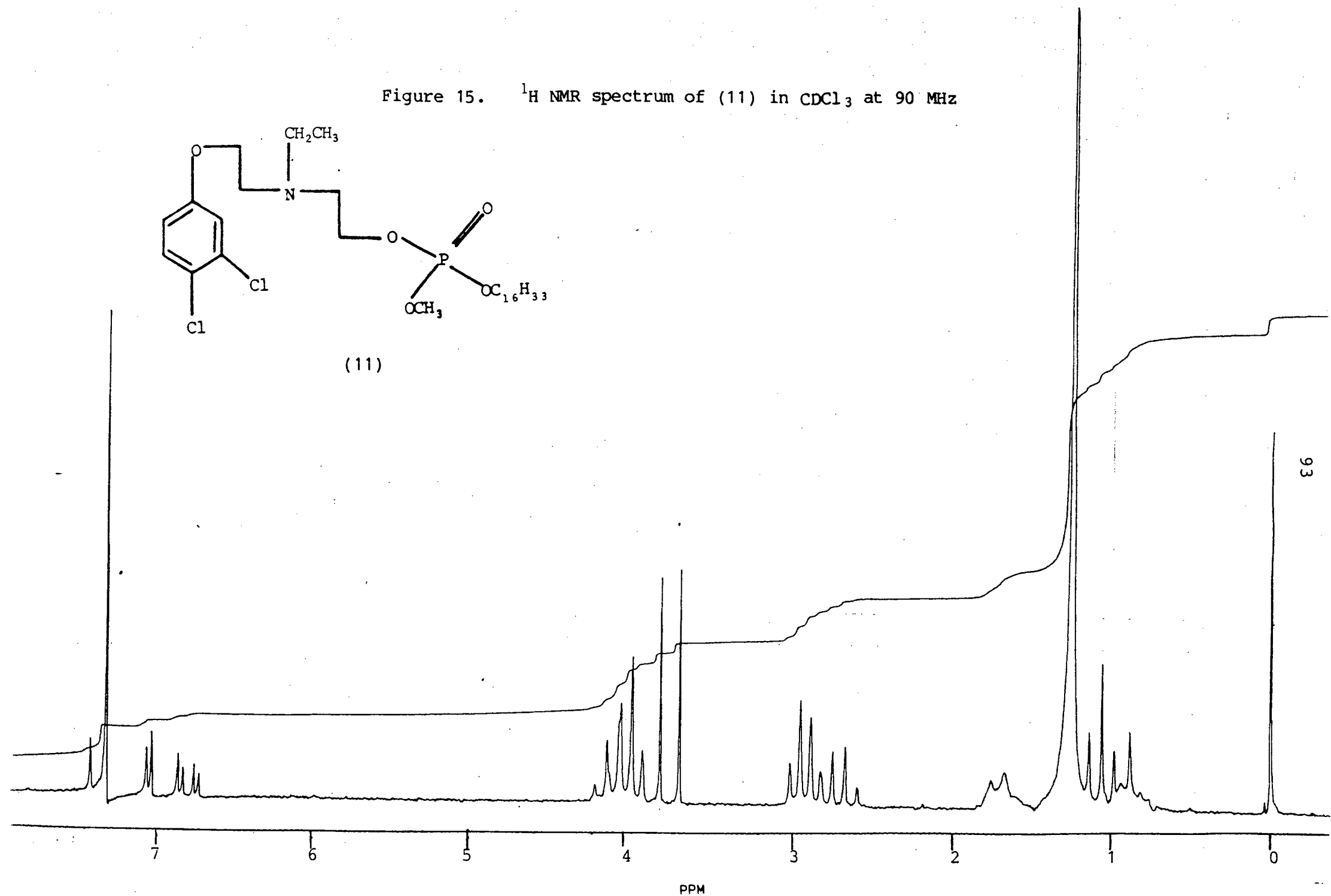
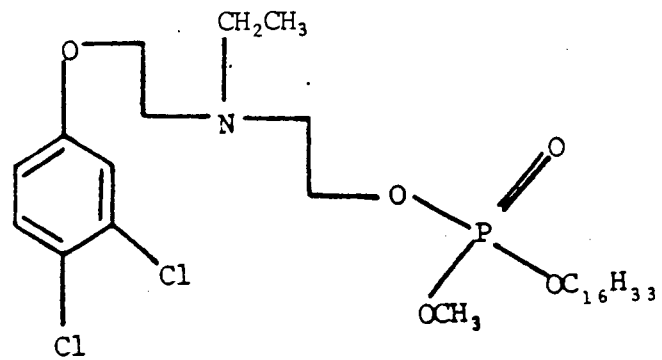
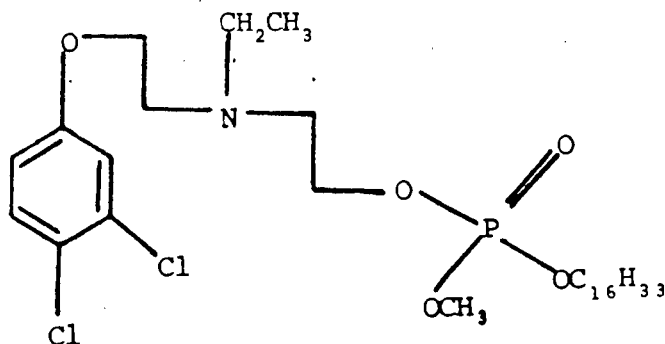


Figure 15.  $^1\text{H}$  NMR spectrum of (11) in  $\text{CDCl}_3$  at 90 MHz





(11)

compound was dissolved in carbon tetrachloride and a thirty-fold excess of ethyl iodide was added. The reaction mixture was stirred at room temperature for one week, after which the excess ethyl iodide and carbon tetrachloride were removed using a rotary evaporator. The  $^1\text{H}$  NMR spectrum showed that no reaction had taken place. It was therefore decided to heat the reaction mixture at reflux temperature for four days. Analysis of the  $^1\text{H}$  NMR spectrum for the above reaction, reveals that N-ethylation of (11) with ethyl iodide yields a mixture of the N,N-diethylammonium salt (desired product) and the N-ethyl-N-methylammonium salt. The latter is formed by N-methylation of (11) with methyl iodide, formed by the O-demethylation of the phosphate group by iodide ion. This ion is, in turn, formed as a leaving group during the initial N-ethylation reaction. Due to the fact that these two quaternary salts have almost identical masses (differing only by a single  $\text{CH}_2$  group), it was felt that their properties would be too similar for effective separation to take place.

Ethylation was then attempted using an excess of diethyl sulphate instead of ethyl iodide. The reaction again did not proceed at room temperature so the mixture was heated at 60°C for four days. The heating caused a precipitate to form, which was filtered and a <sup>1</sup>H NMR spectrum of the filtrate and precipitate obtained. The filtrate was shown to contain excess diethyl sulphate, whilst that of the precipitate showed the quaternized product.

With the quaternized product dissolved in DMSO (as this appeared to be the only solvent in which the product would dissolve in), demethylation was attempted using a three-fold excess of thiophenol and triethylamine. The reaction mixture was heated at 60°C for three days, after which a <sup>1</sup>H NMR spectrum was recorded. This showed that demethylation had indeed occurred. Chromatographic purification of this product was initially complicated by solubility problems. Due to the time limitations, the synthesis of the diethyl analogue (10) was not completed, but left at the neutral triester (11)/N,N-diethyl quaternary ammonium salt stage. The quaternization and demethylation steps require still some refining. When Et<sub>2</sub>SO<sub>4</sub> is used for N-ethylation, it is difficult to remove the excess of the reagent from the product. Ethylation using a reagent such as triethyloxonium fluoroborate should be attempted. It will be also necessary to find suitable conditions for the chromatographic purification of the final zwitterionic product.

CHAPTER 7

EXPERIMENTAL

## 7. EXPERIMENTAL

### 7.1 General

$^1\text{H}$  NMR spectra were recorded using the following instruments: 90 MHz, Brüker WH-90, and 200 MHz, Varian VXR-200. All  $^1\text{H}$  NMR spectra were recorded in deuterated chloroform using tetramethylsilane as an internal standard.

Column chromatography was carried out, using Merck Kieselgel 60 (70-230 mesh) as adsorbent. Aluminium-backed silica gel plates (Merck, Kieselgel 60 F<sub>254</sub>) were used for thin layer chromatography (t.l.c.).

Analyses for C, H, N were performed on a Heraeus Universal combustion analyser by Mr W R T Hemsted in the Organic Chemistry Department, University of Cape Town.

### 7.2 Reagents

The following reagents were purified by drying and/or distillation: 1,2 dibromoethane, sodium iodide, 2-chloroethanol, acetone, toluene, methanol, phosphorus oxychloride, cetyl alcohol, carbon tetrachloride, ether, petroleum ether.

The following reagents were used as supplied: 3,4-dichlorophenol, tetrabutylammonium bromide, sodium hydroxide, dichloromethane, chloroform, methylamine (33% in ethanol), hydrochloric acid (conc), magnesium sulphate, 2,6-lutidine, methyl

iodide, triethylamine, thiophenol. Sodium hydride was supplied as a dispersion in paraffin oil.

### 7.3 Synthesis of 1-bromo-2-(3,4-dichlorophenoxy)ethane<sup>33</sup> (5)

1,2-dibromoethane (555 ml; 6.406 mole) was added to a solution of 3,4-dichlorophenol (40 g; 0.245 mole) and tetrabutylammonium bromide (4 g; 0.012 mole) in aqueous sodium hydroxide (5%; 600 ml) and the mixture heated at 80°C with vigorous stirring for twelve hours. The pH of the aqueous phase was kept at 11 by occasional addition of aqueous sodium hydroxide. The mixture was then cooled, the organic layer separated and the aqueous layer extracted with dichloromethane (3 x 50 ml). The combined organic solutions were washed with aqueous sodium hydroxide (2%; 30 ml), then with water (2 x 50 ml), and dried (MgSO<sub>4</sub>). After removal of dichloromethane on a rotary evaporator, excess 1,2-dibromoethane was distilled off under reduced pressure (b.p. 40 - 50°C/20 mmHg), and was shown by <sup>1</sup>H NMR spectroscopy to be essentially pure. The product, 1-bromo-2-(3,4-dichlorophenoxy)ethane was then distilled under reduced pressure

Yield: 85%                      b.p. 129 - 131°C/0.5 mmHg.

<sup>1</sup>H NMR (CDCl<sub>3</sub>)

83.50	(2H, t, J <sub>H,H</sub> 6 Hz, CH <sub>2</sub> Br)
84.14	(2H, t, J <sub>H,H</sub> 6 Hz, CH <sub>2</sub> O)
86.83	(1H, dd, J <sub>H,H</sub> 9 Hz and 2.6 Hz, 6-H)
86.92	(1H, d, J <sub>H,H</sub> 2.6 Hz, 2-H)
87.27	(1H, d, J <sub>H,H</sub> 9 Hz, 5-H)

Anal. Calcd for  $C_8H_7OBrCl_2$ : C 35.57; H 2.59%

Found : C 35.60; H 2.60%

#### 7.4 Synthesis of 2-(3,4-dichlorophenoxy)ethylmethanamine. (6)

A 33% solution (140 ml; 1.48 mole) of methanamine in ethanol was added to a solution of (5) (40 g; 0.148 mole) in 96% ethanol (120 ml). The mixture was sealed in three glass tubes and placed in a water bath at 80°C for sixty six hours. The tubes were then cooled, opened and after removing the solvent on a rotary evaporator, aqueous sodium hydroxide (32 g NaOH pellets/400 ml H<sub>2</sub>O) was added and the mixture extracted with ether (4 x 50 ml). The combined ethereal solutions were extracted with 3.3 M hydrochloric acid (3 x 100 ml). The acid solution was made alkaline with sodium hydroxide (100 g NaOH pellets/200 ml H<sub>2</sub>O) and extracted with ether (3 x 200 ml). The ethereal solutions were dried (MgSO<sub>4</sub>) and the ether removed on a rotary evaporator. The product was then distilled under reduced pressure.

Yield: 76%.      b.p. 126 - 128°C/0.5 mmHg.

<sup>1</sup>H NMR (CDCl<sub>3</sub>)

	δ1.58 (1H, s, NH)
	δ2.48 (3H, s, NCH <sub>3</sub> )
	δ2.94 (2H, t, J <sub>H,H</sub> 6 Hz, NCH <sub>2</sub> )
	δ4.00 (2H, t, J <sub>H,H</sub> 6 Hz, OCH <sub>2</sub> )
	δ6.82 (1H, dd, J <sub>H,H</sub> 9 Hz and 2.6 Hz, 6-H)
	δ7.02 (1H, d, J <sub>H,H</sub> 2.6 Hz, 2-H)



$\delta$ 7.38 (1H, d,  $J_{H,H}$  9 Hz, 5-H)

Anal. calcd. for  $C_9H_{11}ONCl_2$ : C 49.09; H 5.0; N 6.36%.

Found: C 48.9; H 5.0; N 6.3%.

### 7.5 Synthesis of 2-Iodo-Ethanol.

A solution of sodium iodide (71.5 g; 0.477 mole) and 2-chloroethanol (26.7 ml; 0.398 mole) in dry acetone (300 ml) was refluxed for seventeen hours. The NaCl which had formed was filtered off and the acetone removed using a rotary evaporator. Water (100 ml) was added and the solution extracted with ether (3 x 50 ml). The ethereal solutions were dried ( $MgSO_4$ ) and the ether removed on a rotary evaporator. The product was then distilled under reduced pressure.

Yield: 60.35%      b.p. 78 - 79°C/20 mmHg

$^1H$  NMR ( $CDCl_3$ )       $\delta$ 3.25 (2H, t,  $J_{H,H}$  6 Hz,  $ICH_2$ )  
                                  $\delta$ 3.80 (2H, t,  $J_{H,H}$  6 Hz,  $OCH_2$ )  
                                  $\delta$ 4.20 (1H, s, OH)

### 7.6 Synthesis of aminoalcohol. (2)

Iodo-ethanol (9.2 g; 0.054 mole) in toluene was added dropwise to (6) also in toluene at reflux temperature. During the two hour reflux, an oil settled at the bottom. The toluene was decanted off and the oil treated with sodium

hydroxide solution (2.1 g NaOH/20 ml H<sub>2</sub>O) and stirred well. The mixture was extracted with ether (3 x 50 ml). The combined ethereal solutions were washed with water (2 x 30 ml) and dried (MgSO<sub>4</sub>). The ether was removed on a rotary evaporator and the product distilled under reduced pressure.

Yield: 41.6%      b.p. 172 - 174°C/0.1 mmHg

<sup>1</sup>H NMR (CDCl<sub>3</sub>)

δ2.36	(1H, s, NCH <sub>3</sub> )
δ2.64	(2H, t, J <sub>H,H</sub> 5.4 Hz, NCH <sub>2</sub> )
δ2.86	(2H, t, J <sub>H,H</sub> 5.4 Hz, NCH <sub>2</sub> )
δ3.6	(2H, t, J <sub>H,H</sub> 5.4 Hz, OCH <sub>2</sub> )
δ4.0	(2H, t, J <sub>H,H</sub> 5.4 Hz, OCH <sub>2</sub> )
δ6.82	(1H, dd, J <sub>H,H</sub> 9 Hz and 2.6 Hz, 6-H)
δ7.08	(1H, d, J <sub>H,H</sub> 2.6 Hz, 2-H)
δ7.38	(1H, d, J <sub>H,H</sub> 9 Hz, 5-H)

Anal. calcd. for C<sub>11</sub>H<sub>15</sub>O<sub>2</sub>NCl<sub>2</sub>: C 50.00; H 5.68; N 5.30%

Found: C 50.0; H 5.8; N 5.3%

### 7.7 Synthesis of methyl dichlorophosphate.

Dry methanol (20.5 ml; 0.5 mole) was added with stirring at 0°C to a solution of phosphorus oxychloride (45.7 ml; 0.5 mole) in ether. The reaction mixture was allowed to warm up to room temperature and stirred for a further two hours. The solvent was then removed on a rotary evaporator and the product distilled under reduced pressure.

Yield: 81%                      b.p. 64 - 66°C/20 mmHg

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )                       $\delta$ 4.0 (3H, d,  $J_{\text{H,P}}$  17.1 Hz,  $\text{OCH}_3$ )

## 7.8 Synthesis of cetyl methyl chlorophosphate

### 7.8.1 Attempted synthesis starting with $\text{PCl}_3$

A solution of cetyl alcohol (8 g;  $3.30 \times 10^{-2}$  mole) in petroleum ether (70 ml) was added dropwise with stirring at 0°C to a solution of phosphorus trichloride (2.88 ml;  $3.30 \times 10^{-2}$  mole) in petroleum ether. The reaction mixture was stirred for one hour, after which, without the intermediate cetyl dichlorophosphite being isolated and analysed, was added a solution of methanol (2.67 ml;  $6.60 \times 10^{-2}$  mole) in petroleum ether (10 ml). The mixture was left to equilibrate to room temperature and the solvent then removed using a rotary evaporator. The crude product revealed the existence of three products which were separated by column chromatography (silica gel; dichloromethane: acetone; 95:5).

(i) cetyl chloride : yield 16%

(ii) dicetyl phosphite : yield 40%

Anal. calcd. for  $\text{C}_{32}\text{H}_{67}\text{O}_3\text{P}$ : C 72.5; H 12.6%

Found : C 72.7; H 12.3%

(iii) cetyl methyl phosphite: yield 17%

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )       $\delta$ 0.8 (3H, t,  $J_{\text{H,H}}$  5.4 Hz,  $\text{CH}_3$ )  
                                   $\delta$ 1.24 (28H, s, 14 x  $\text{CH}_2$ )  
                                   $\delta$ 3.77 (3H, d,  $J_{\text{H,P}}$  11 Hz,  $\text{OCH}_3$ )  
                                   $\delta$ 3.80-4.20 (2H, dt,  $\text{CH}_2$ )  
                                   $\delta$ 6.81 (1H, d,  $J_{\text{H,P}}$  700 Hz, PH)

Anal. calcd. for  $\text{C}_{17}\text{H}_{37}\text{O}_3\text{P}$ : C 63.8; H 11.6%

Found : C 64.0; H 11.7%

After purification a ten fold excess of sulphuryl chloride<sup>14</sup> (1.1 ml;  $1.28 \times 10^{-2}$  mole) was added at room temperature to a solution of cetyl methyl phosphite (0.41 g;  $1.28 \times 10^{-3}$  mole) in benzene (5 ml). The reaction mixture was stirred overnight and the excess sulphuryl chloride then removed using a rotary evaporator.

Yield : 80%

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )       $\delta$ 0.8 (3H, t,  $J_{\text{H,H}}$  5.4 Hz,  $\text{CH}_3$ )  
                                   $\delta$ 1.24 (28H, s, 14 x  $\text{CH}_2$ )  
                                   $\delta$ 3.90 (3H, d,  $J_{\text{H,P}}$  11 Hz,  $\text{OCH}_3$ )  
                                   $\delta$ 3.95-4.30 (2H, dt,  $\text{CH}_2$ )

### 7.8.2 Synthesis starting with POCl<sub>3</sub>

A solution of 2,6-lutidine (0.96 ml;  $8.26 \times 10^{-3}$  mole) and cetyl alcohol (2.0 g;  $8.26 \times 10^{-3}$  mole) in carbon tetrachloride was added dropwise with stirring at 0°C to methyl dichlorophosphate (1.2 g). The reaction mixture was stirred for a further two hours, after which the precipitate which had formed (2,6-lutidine hydrochloride) was filtered and the solvent removed on a rotary evaporator. The crude product was used directly in the next step.

Yield: 85%

<sup>1</sup> H NMR (CDCl <sub>3</sub> )	δ0.88 (3H, t, J <sub>H,H</sub> 5.4 Hz, CH <sub>3</sub> )
	δ1.24 (28H, s, 14 x CH <sub>2</sub> )
	δ3.86 (3H, d, J <sub>H,P</sub> 14.4 Hz, OCH <sub>3</sub> )
	δ4.16 (2H, m, OCH <sub>2</sub> )

### 7.9 Reaction of aminoalcohol with cetyl methyl chlorophosphate.

Sodium hydride (0.3 g;  $1.29 \times 10^{-2}$  mole) was washed twice with petroleum ether and once with ether before being used.

A solution of (2) 2.0 g;  $7.58 \times 10^{-3}$  mole) in ether was added with stirring to the suspension of sodium hydride (1.7 fold excess) in ether in an atmosphere of nitrogen which had

been dried over concentrated sulfuric acid and phosphorus pentoxide. After addition, the mixture was stirred for a further two hours at room temperature. The contents of the flask were then transferred to a dropping funnel (under nitrogen) connected to a three-necked flask containing cetyl methyl chlorophosphate in ether and a  $P_2O_5$  drying tube. The sodium salt in the dropping funnel was then added dropwise with stirring at  $0^\circ C$  to the cetyl methyl chlorophosphate solution. Stirring was continued for a further two hours, after which the solvent was removed on a rotary evaporator and the crude product purified by column chromatography (silica gel), using chloroform as the eluent, to produce the neutral phosphate triester (4).

Yield: 33%

$^1H$ NMR ( $CDCl_3$ )	$\delta$ 0.88 (3H, t, $J_{H,H}$ 5.4 Hz, $CH_3$ )
	$\delta$ 1.24 (28H, s, 14 x $CH_2$ )
	$\delta$ 2.40 (3H, s, $NCH_3$ )
	$\delta$ 2.86 (4H, m, 2 x $NCH_2$ )
	$\delta$ 3.76 (3H, d, $J_{H,P}$ 11 Hz, $OCH_3$ )
	$\delta$ 4.06 (6H, m, 3 x $OCH_2$ )
	$\delta$ 6.82 (1H, dd, $J_{H,H}$ 9 Hz and 2.6 Hz, 6-H)
	$\delta$ 7.08 (1H, d, $J_{H,H}$ 2.6 Hz, 2-H)
	$\delta$ 7.38 (1H, d, $J_{H,H}$ 9 Hz, 5-H)

Anal. calcd. for  $C_{28}H_{50}NO_5PCl_2$ : C 57.73; H 8.59; N 2.41%

Found : C 57.6; H 8.5; N 2.4%

### 7.10 N-methylation of the neutral triester (4).

A thirty-fold excess of methyl iodide (1.68 ml;  $2.7 \times 10^{-2}$  mole) was added to a solution of (4) (0.5 g;  $9.0 \times 10^{-4}$  mole) in carbon tetrachloride (10 ml). The reaction mixture was stirred at room temperature for four days, after which the excess methyl iodide and carbon tetrachloride were removed using a rotary evaporator, leaving behind the ionic product (9).

Yield: 85%

$^1\text{H NMR (CDCl}_3)$	$\delta 0.88$ (3H, t, $J_{\text{H,H}}$ 5.4 Hz, $\text{CH}_3$ )
	$\delta 1.24$ (28H, s, 14 x $\text{CH}_2$ )
	$\delta 3.56$ (6H, s, 2 x $\text{NCH}_3^+$ )
	$\delta 3.80$ (3H, d, $J_{\text{H,P}}$ 11 Hz, $\text{OCH}_3$ )
	$\delta 3.90 - 4.70$ (10H, m, 2 x $\text{NCH}_2$ , 3 x $\text{OCH}_2$ )
	$\delta 6.94$ (1H, dd, $J_{\text{H,H}}$ 9 Hz and 2.6 Hz, 6-H)
	$\delta 7.08$ (1H, d, $J_{\text{H,H}}$ 2.6 Hz, 2-H)
	$\delta 7.40$ (1H, d, $J_{\text{H,H}}$ 9 Hz, 5-H)

Anal. calcd. for  $\text{C}_{29}\text{H}_{53}\text{O}_5\text{NPCl}_2\text{I}$ : C 48.07; H 7.32; N 1.93%

Found : C 47.6; H 7.0; N 1.8%

### 7.11 O-Demethylation of (9).

To a solution of compound (9) (0.1 g;  $1.38 \times 10^{-4}$  mole) in chloroform was added a three-fold excess of thiophenol

(0.04 ml;  $4.14 \times 10^{-4}$  mole) and triethylamine (0.06 ml;  $4.14 \times 10^{-4}$  mole). The reaction mixture was then placed in a water bath at  $60^\circ\text{C}$  for four days until demethylation of the P-OCH<sub>3</sub> group was complete. The components of the mixture were then separated by column chromatography, using an initial solvent system of dichloromethane:methanol; 4:1. Once the excess thiophenol, triethylamine and methyl phenyl sulfide had been eluted, the dichloromethane was abandoned and the column then eluted with pure methanol. The methanolic fractions, which contained the product (1), were combined and the solvent removed on a rotary evaporator.

Yield: 65%

<sup>1</sup> H NMR (CDCl <sub>3</sub> )	δ0.88 (3H, t, J <sub>H,H</sub> 5.4 Hz, CH <sub>3</sub> )
	δ1.24 (28H, s, 14 x CH <sub>2</sub> )
	δ3.40 (6H, s, 2 x NCH <sub>3</sub> <sup>+</sup> )
	δ3.72 (4H, m, 2 x OCH <sub>2</sub> )
	δ4.08 (2H, br s, NCH <sub>2</sub> )
	δ4.30 (2H, br s, NCH <sub>2</sub> )
	δ4.50 (2H, br s, OCH <sub>2</sub> )
	δ6.82 (1H, dd, J <sub>H,H</sub> 9 Hz and 2.6 Hz, 6-H)
	δ7.08 (1H, d, J <sub>H,H</sub> 2.6 Hz, 2-H)
	δ7.38 (1H, d, J <sub>H,H</sub> 9 Hz, 5-H)

<sup>13</sup> C NMR (CDCl <sub>3</sub> )	δ14-32 (m, carbons of cetyl chain)
	δ52.5 (s, N <sup>+</sup> CH <sub>3</sub> )
	δ58.9 (s, ArOCH <sub>2</sub> CH <sub>2</sub> N)
	δ62.8 (s, NCH <sub>2</sub> CH <sub>2</sub> OPOR)



- δ63.8 (s, ArOCH<sub>2</sub>CH<sub>2</sub>N)  
δ65.4 (dd, J<sub>P,C</sub> 21 Hz, 19 Hz, CH<sub>2</sub>O-P-OCH<sub>2</sub>CH-)  
δ114.2 (s, aromatic C-6)  
δ116.5 (s, aromatic C-2)  
δ130.7 (s, aromatic C-5)  
δ125.1 (s, C-Cl)  
δ132.8 (s, C-Cl)  
δ155.9 (s, C-O)

Anal. calcd. for C<sub>28</sub>H<sub>50</sub>O<sub>5</sub>NPCl<sub>2</sub>·2H<sub>2</sub>O: C 54.37; H 8.74; N 2.26%

Found : C 53.9; H 8.9; N 2.2%

**REFERENCES**

1. J. Bonner, Physiology and chemistry of guayule. In NG McGinnes, EF HAAS, eds, An international conference on the utilization of guayule. Office of land studies, University of Arizona, Tuscon, AZ, 1975, 78-83.
2. J. Bonner. Effects of temperature on rubber accumulation by the guayule plant. *Bot Gaz*, 1943, 105, 233-243.
3. J. Bonner. Rubber biosynthesis In. P Bernfield, ed, Biogenesis of natural compounds, Ed 2, Pergamon, Oxford, 1967, 941-952.
4. H. Yokoyama, E.P. Hayman, W.J. Han, S.H. Poling and A.J. Bauman, *Science*, 1977, 197, 1076.
5. A.J. Bauman, US Pat. 4159 903, July 3 1979.
6. C.R. Benedict, P.H. Reibach, S. Madhavan, R.P. Stipanovic, J.H. Keithly and H. Yokoyama, *Plant Physiol.*, 1983, 72, 897.
7. The chemistry of carbon compounds (E.H. Rodd, ed.), Vol. 4A, Elsevier, Amsterdam, 1957, p15.
- 8a. E. Baer, M. Kates, *J. Am. Chem. Soc.*, 1950, 72, 942.
- b. E. Baer, J. Maurukus, *J. Am. Chem. Soc.*, 1952, 74, 158.

- 9a. E. Baer, D. Buchnea, A.G. Newcombe, *J. Am. Chem. Soc.*, 1956, 78, 232.
- b. N.H. Tattrie, C.S. McArthur, *Can. J. Biochem. Physiol.*, 1957, 35, 1165.
10. G.H. de Haas, L.L.M. van Deenen, *Tetrahedron Lett.*, 1960, 7.
11. C.J. Lacey, L.M. Loew, *J. Org. Chem.*, 1983, 48, 5214.
12. P.A. Manninen, *Acta Chem. Scand.*, Ser. B, B32, 1978, 269.
13. J.C. Paterson-Jones, A. Le Roux, T.A. Modro, *S. Afr. J. Chem.*, 1984, 37, 161-163.
14. F.R. Atherton et al, *J. Chem. Soc.*, 1948, 1106.
15. G.W. Daub, E.E. van Tamelen, *J. Am. Chem. Soc.*, 1977, 99, 3526.