WAN, HUIJIE

SYNTHESIS, REACTIVITY, AND STRUCTURAL STUDIES OF N-PHOSPHORYLATED NITROGEN MUSTARDS

DPhil UP 1996

SYNTHESIS, REACTIVITY, AND STRUCTURAL STUDIES OF N-PHOSPHORYLATED NITROGEN MUSTARDS

by

HUIJIE WAN

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SYNTHESIS, REACTIVITY, AND STRUCTURAL STUDIES OF N-PHOSPHORYLATED NITROGEN MUSTARDS

HUIJIE WAN

The results presented in this Thesis have been published as the following articles :

1. H. Wan, T. A. Modro,
   Chemistry of N-phosphorylated Nitrogen Mustards : The Effect of a Second Nitrogen Substituent at Phosphorus on the Stability of the System.
   Phosphorus, Sulfur, and Silicon, **155,** 108(1996).

2. T. A. Modro, C. le Roux, H. Wan, A. M. Modro,
   Reactivity of N-Phosphorylated Mustards,
   Phosphorus, Sulfur, and Silicon, **109-110,** 469(1996)

3. H. Wan, T. A. Modro,
   Preparation of Acyclic and Cyclic Phosphoric Triamides and Diamidoester,
   Synthesis, 1996, in press.

4. H. Wan, A. M. Modro, T. A. Modro, S. Bourne, L. R. Nassimbeni,
   Structural Studies of Phosphoramidates. Conformational Preferences and Hydrogen Bonding,
   J. Phys. Org. Chem., 1996, in press.

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1. H. Wan,
   Reactivity of N-phosphorylated Mustard,
   SACI student symposium at Rand Afrikaanse Universiteit on 8th November 1995.

2. C. le Roux, A. M. Modro, T. A. Modro, H. Wan,
   Chemistry of N-phosphorylated Nitrogen Mustards,

Frank Warren, National Organic Chemistry Conference, Free State, 1995.

3. H. Wan, A. M. Modro, T. A. Modro, S. Bourne, L. R. Nassimbeni,
New Chemistry of Phosphoramidates,
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Abstract

Preliminary research conducted in our laboratory indicated that the degradation behavior of phosphoramidates such as, eg. dimethyl N, N-bis(2-chloroethyl)phosphoramidate is complex. In principle, the substrates can react in three independent directions : 1. 1, 5-cyclization. 2. 1, 3-cyclization. 3. Fragmentation (P-N bond cleavage). In Chapter 1, methyl N, N-diethyl-N', N'-bis(2-chloroethyl)phosphoramidate (diamidate) was prepared. Its reactivity in different media (LiI/ 2-butanone, pyridine-d_5/ D_2O, PhSH/ Et_3N/ acetonitrile-d_3) was studied. Its O-demethylated product led to a highly unstable ion, which underwent spontaneous fragmentation. It is concluded that the electron-rich ionic phosphoramidate substituent, $^-O(R_2N)P(O)$, highly activates the N-(2-chloroethyl) functional group in the alkylation reactions.

In Chapter 2, a series of N-bis(2-chloroethyl)phosphoric triamides (and diamidoesters) was prepared. The suitable bases for the mono- and dicyclization of those compounds were found. Among the prepared compounds, several single crystals of the crystalline products were grown. In Chapter 3, the molecular parameters of those compounds were discussed. The value of the torsion angle of the O=P-N-H function which determines the packing of the molecules was found to determine also the ability of a substrate to form diastereomeric hydrogen-bonded complexes with optically active acids.

Based on the crystal parameters of N-P-N bond angles of those non-, mono-, di-, tricyclic compounds and their specific-range of the ^{31}P NMR chemical shift values, the correlation of ^{31}P NMR chemical shift and N-P-N bond angle was discussed in Chapter 4. The result is consistent with the analogous studies for similar oxygen and sulfur series (O-P-O, S-P-S).

In Chapter 5, the inter and intra molecular nucleophilic competitive reactions of phosphoramidates were studied. The corresponding product of intermolecular reaction showed interesting ^1H NMR spectrum which was correlated with the x-ray structure of that compound.

Opsomming

Voorafgaande navorsing in hierdie laboratorium het daarop gedui dat die degredasie van fosforamiedes soos bv. N,N-bis(2-chloroetiel)fosforamied uiters ingewikkeld is. Hierdie substrate kan drie verskillende reaksies ondergaan: 1. 1,5-siklisasie. 2. 1,3-siklisasie. 3. Fragmentasie (waar die P-N binding gebreek word). Die bereiding van N,N-dietiel-N',N'-bis(2-chloroetiel)fosforamied (diamidaat) word in Hoofstuk 1 bespreek en die reaktiwiteit van hierdie substraat onder verskillende reaksiekondisies (LiI/2-butanoon, pyridine-d_5/D_2O, PhSH/Et_3N/asetonitriel-d_3) is beskryf. Die O-gedemetileerde produk het 'n hoogs onstabiele ioon gelewer wat spontane fragmentasie ondergaan het. Die afleiding kan gemaak word dat die elektronryke ioniese fosforamidaat substituent, $^-O(R_2N)P(O)$ die N-(2-chloroetiel) funksionele groep aktiveer in alkilerings reaksies. In Hoofstuk 2 is die bereiding van 'n hele reeks van N-bis(2-chloroetiel fosforiese triamiede (en diamido esters) uiteengesit. Geskikte basisse vir die selektiewe mono- en disiklisasie van hierdie verbindings is verkry. Verskeie enkel kristalle is van die kristallyne verbindings gegroei. In Hoofstuk 3 word die molekulêre parameters van hierdie verbindings bespreek. Daar is gevind dat die waarde van die wringhoek van die O=P-N-H funksie wat die pakking van die molekules bepaal, ook die vermoë van die substraat om diastereomeriese waterstof gebinde komplekse met opties aktiewe sure sal vorm, beïnvloed. Vanuit die N-P-N bindingshoeke, verkry vanuit die kristalstruktuur data van die asikliese-, mono-, di- en trisikliese verbindings, en die ^{31}P KMR chemiese verskuiwing waardes kon die korrelasie tussen hierdie twee komponente bespreek word in Hoostuk 4. Die resultate wat verkry is stem goed ooreen met analoë studies wat op suurstof- en swawelverbindings (O-P-O, S-P-S) gedoen was.

In Hoofstuk 5 is die inter- en intramolekulêre nukleofiliese kompeterende reaksies van fosforamiede bestudeer. Die produk gevorm in die intermolekulêre reaksie het 'n interessante ^1H KMR spektrum tot gevolg gehad wat met die X-straal struktuur van die stof vergelyk is.

General

Introduction

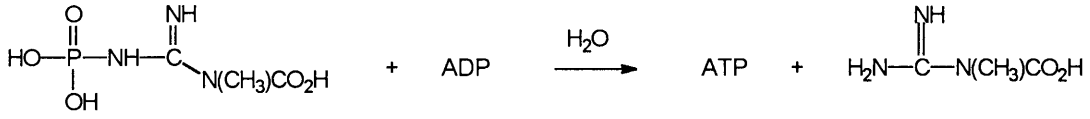
General Introduction

"Without phosphorus, there is no life" -------- Richard Kuhn

Nowadays, phosphorus chemistry has infiltrated into almost all chemical fields[1] : inorganic chemistry, organic chemistry, organometallic chemistry, material science, biochemistry, etc. Especially in the life science, phosphorus plays a central role, it has been found in photosynthesis, metabolisms, saccharride synthesis, nucleic acid helices, it is involved in enzyme system, etc[2]. Some organoposphorus compounds, such as, ATP, ADP, DNA[3], are well-known for their functions in the living organism.

Phosphorus compounds can be classified into many types[4]. Among them, phosphorus-nitrogen compounds are one of the biggest groups. As both phosphorus and nitrogen compounds are essential for some biochemical processes, researches concerning phosphorus-nitrogen compounds are widely reported.

In 1927[5], term phosphagen (phosphocreatine) was introduced to describe an acid-labile, phosphorus-containing compound detectable in frog skeletal muscle. It was isolated and purified by Fiske and Subbarow[6]. Phosphocreatine plays an important role in muscle by 'buffering' the concentration of ATP (Equation 1). During muscular contraction[7, 8], the concentration of ATP remains constant because ADP is phosphorylated by phosphocreatine in a rapid, enzyme-catalyzed reaction (Equation 1)[7, 8, 9].

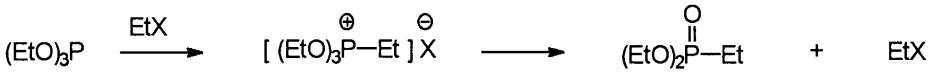


A

Equation 1 *In vivo* phosphorylation of ADP

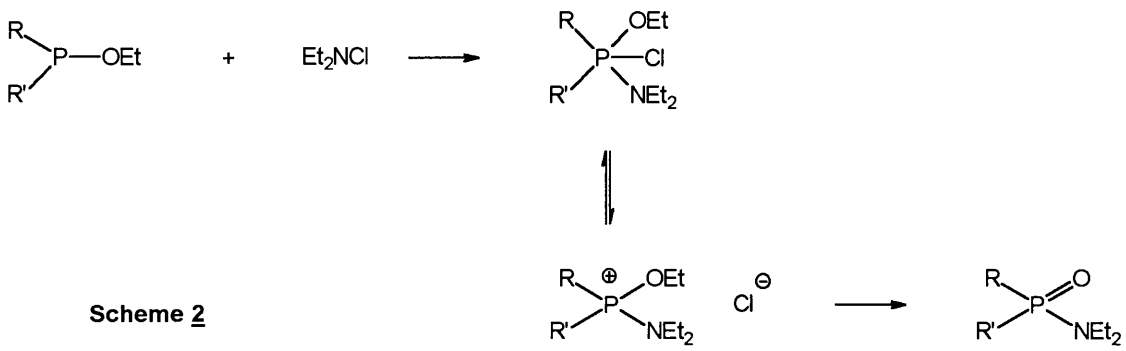
Phosphocreatine A contains a high energy P-N bond which is ruptured in the hydrolysis[10] (Equation 1), and can be seen as an alternative energy store[11]. Its analogues : arginine phosphate, taurocyanine phosphate and guanidinoethyl seryl phosphate also have the same property[4] (high energy P-N bond), but phosphocreatine is the only phosphagen present in detectable quantity in vertebrates.

Synthesis of organophosphorus compounds can be traced back to last century. As early as in 1820, J. L. Lassaigne[12] reacted alcohol with phosphoric acid in a reaction analogous to that with sulfuric acid, and launched the chemistry of organophosphorus compounds[2]. The reaction between tricoordinate phosphorus esters and alkyl halides leading to the tetracoordinate phosphoryl compounds, commonly known as the Arbuzov reaction, was first reported by A. Michaelis in 1883 and explored by A. E. Arbuzov[13, 14].



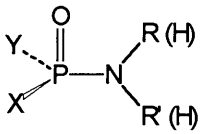
Scheme 1

This reaction can also be used to the synthesis of phosphorus amides[15] (Scheme 2).



Scheme 2

The reactivity (hydrolysis[16-20], alcoholysis[21], alkylating[22], phosphorylating properties[16]) concerning compound B is one of the most extensively studied field of phosphorus chemistry since 1970's.



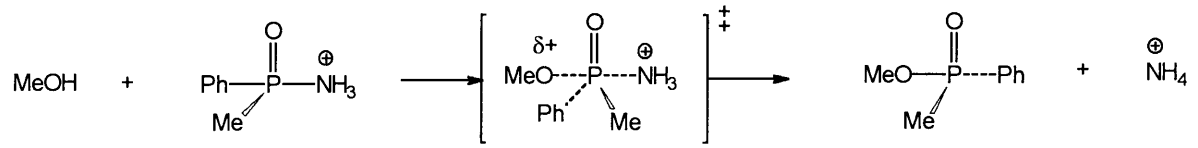
B

Phosphoryl as well as carbonyl group represent functionalities that offer both, the electrophilic (phosphorus or carbon), and nucleophilic (oxygen) centers; the biphilicity being largely responsible for the diverse reactivity of phosphoryl and carbonyl substrates.

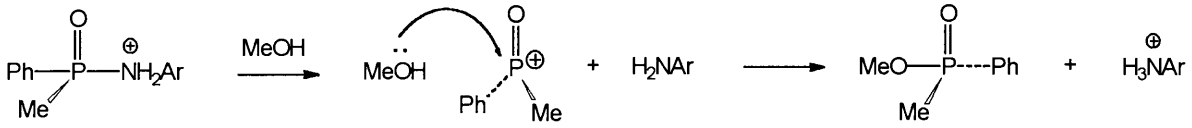
Although phosphorus and nitrogen are in the same group in the Periodic Table, their properties are often very different. When these two elements come together in bond formation they produce one of the most interesting bonding system in chemistry[23]. Single, or double bond can be formed between phosphorus and nitrogen. However, this single σ bond in many cases is not a formal single σ bond, but it is involved in an additional 2p(N)-3d(P) π system. This kind of bond is existing in phosphoric amides[24-26]. This function is similar to that in carboxylic amides (Figure 1). The delocalized π bonding exists in O--C--N and O--P--N groups. This π bonding contribution to this single P-N σ bond is observed in some instances in $R_2P(O)NMe_2$ compounds where the P-N bond shows restricted rotation[27].

Figure 1

Unlike carboxylic amides, phosphoric amides can undergo acid-catalyzed hydrolysis (or methanolysis, P-N bond fission) under much milder condition (relatively to carboxylic amides, which need high temperatures and strong acidic or basic conditions)[20, 28, 29]. Both S_N 1 and S_N 2 mechanisms were expected in the methanolysis of phosphorus amides[30] (Equation 2, 3).



Equation 2



Equation 3

It has been shown that the mechanism is dependent on the nucleophilicity of the departing amine[20, 31-34].

The lability of P-N bonds plays an important role in biochemical systems in phosphorylation of various substrates[28]. It is also widely used in the synthesis of α-methyl α-alkyl phosphonic acid[35] and α-amino α-alkyl phosphonic acid[36, 37].

Another intramolecular P-N bond was recently reported by Yoshifuji[38] (Figure 2).

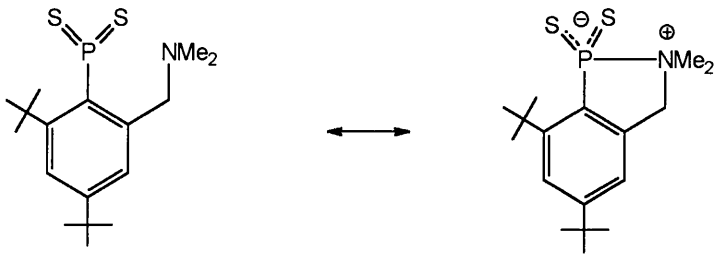


Figure 2

The 2, 4-di-tert-butyl-6-(N, N-dimethylaminomethyl)phenyl group is used to stabilize dithioxophosphorane, the coordination of the nitrogen lone-pair to the P-atom leads to the formation of a five-membered ring, and resonance structures were postulated (Figure 2).

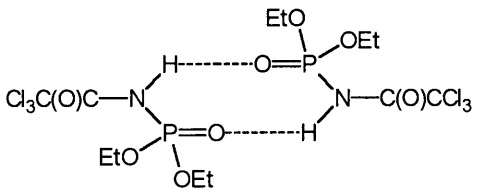
Similar P-N interaction was also described in the cyclization of 1-methyl-1-(N-substituted carbamoyl- or thiocarbamoyl-amino)-alkane-phosphonic acids[39].

Hydrogen bonding is always of interest. In the DNA double helix, the two strands are held together by N-H--O bonds formed between the base pairs. The physicochemical behavior of DNA is also influenced by O-H--O hydrogen bonding between phosphate groups and the surrounding water molecules or directly the phosphate group themselves[4]. Some papers concerning hydrogen bonding of phosphate[40] and phosphorus amides[41] were published.

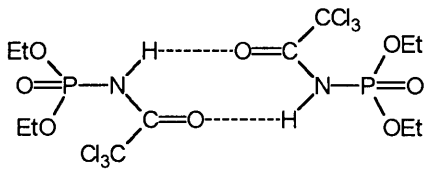
In phosphorus compounds containing >P(O)OH or $P(O)(OH)_2$ groups, the association through H bonding is almost certain to occur[42]. In some non-polar solvent such as benzene, monobasic phosphoric, $(RO)_2POOH$, phosphonic, R(RO)POOH, and phosphinic, R_2POOH, acids form dimers which have stronger hydrogen bonds than the corresponding carboxylic acid dimers[43] (Figure 3).

Figure 3

In phosphoramides, hydrogen bonds involving oxygen and nitrogen are also frequently observed. It is often one of the most important factors in determining geometrical configuration[41, 44] of these compounds. Some reports are interesting, eg. the esters of trichloroacetylamidophosphoric acid exists as C1 rather than C2[45].



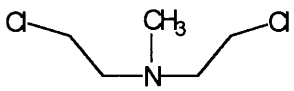
C1



C2

Figure 4

After World War II, investigations concerning nitrogen mustard[46] (D) and phosphoramide mustard[47] started to prosper.



D

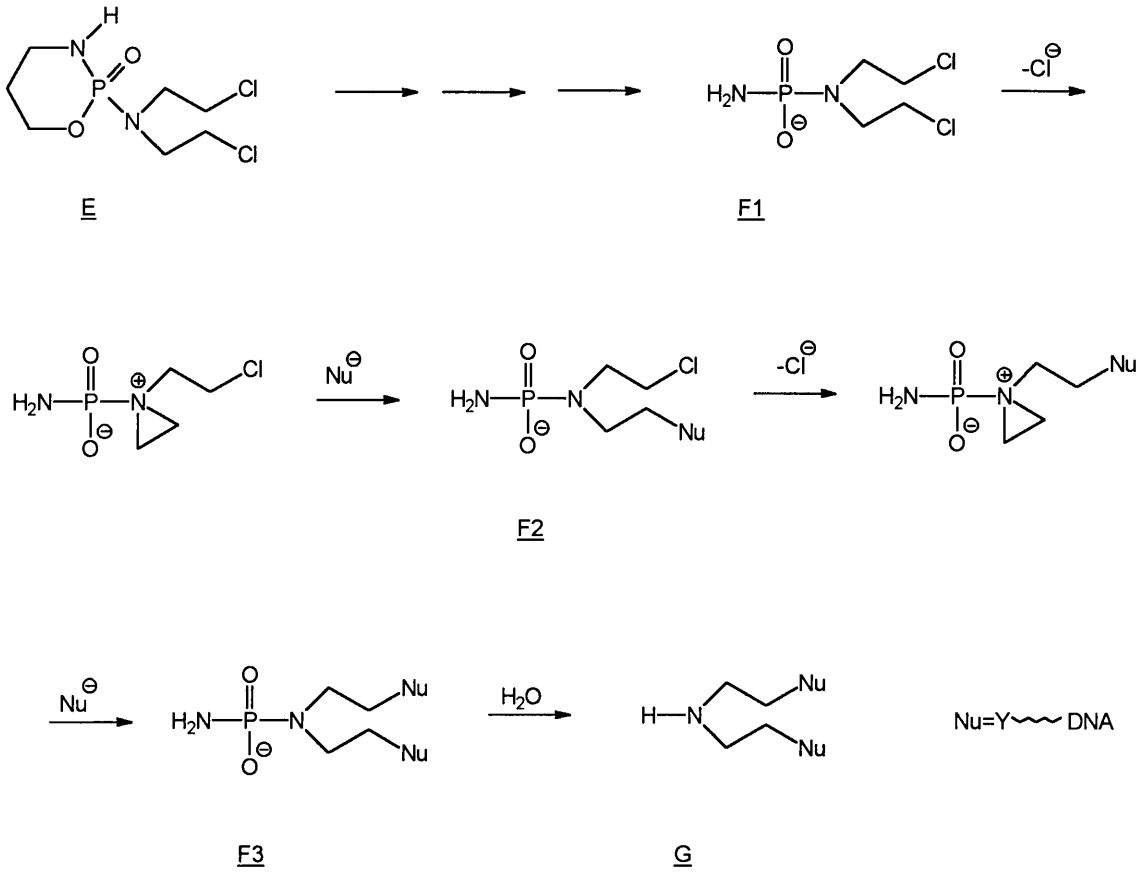
Many phosphoramides[47-49] were synthesized. However, the extensive investigations of this kind of compounds started from the late of 1960's after the cyclophosphamide E[50] was demonstrated to have significant therapeutic activity against a relatively broad spectrum of human cancers[46, 47, 51-56].

The N-phosphorylated mustard F1, which was considered as an intermediate in the *in vivo* degradation of cyclophosphamide[57, 58], is reponsible for the tumor-specific release of N, N-bis(2-chloroethyl)amine[48] (Scheme 3). Scheme 3 shows a postulated mechanism of bisalkylating activity of F1. The first step is the departure of chloride ion, followed by 1,3-cyclization, and an aziridinium ion is formed. Under the external nucleophilic attack (Nu), the ring opening occurs. It gives a mono-alkylated product F2. F2 undergoes two other steps described as above, to give the bis-alkylated product F3. This bisalkylated product F3 then undergoes hydrolysis process, and the P-N bond cleaves. The final bisalkylated product G is formed. This process contains intramolecular cyclizations, intermolecular reactions with biological nucleophiles (Nu) which stop the cellular growth [59].

The importance of F1 in the mechanism of action of cyclophosphamide is related to its high cytotoxicity[60]. The investigation has shown that F1 is a potent alkylating agent at physiological pH : 12 reacts with sulfhydryl groups[61], guanosine[62], guanosine 5-monophosphate[63], and phosphodiester group in DNA[64]; F1 also produces both DNA-protein and intra strand DNA crosslinks[65].

However, the postulated alkylating mechanism of F1 (Scheme 3) is an ideal process. Several questions, such as, whether N-phosphorylated mustard undergoes alkylation reaction as an intact molecule, or the P-N bond cleavage is a prerequisite for the alkylation, remain controversial[57, 59, 66, 67].

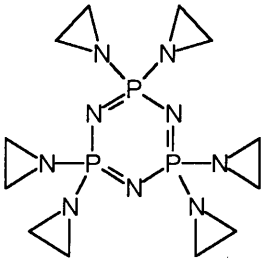
Around this topic, many phosphoramides, containing the bis(β-chloroethyl)amino group, were prepared[48, 49, 66, 68-71] and their reactivities were studied[59, 66, 69, 70].



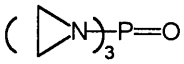
Scheme 3 Postulated mechanism of bisalkylation

Like cyclophosphamides (used as drugs in cancer chemotherapy), many phosphorus amides also found practical applications in various fields[72], such as : fungicides, herbicides, insecticides, chemosterilants, flame retardants, etc.

N-phosphorylated aziridines, such as compounds apholate, tepa, are known inhibitors of cell division. Apholate (hexaaziridinyl cyclotriphosphazene) has drawn attention as potential anticancer agent[73, 74]. Their ability (apholate, tepa), to reduce or eliminate reproductive capacity, can be used for insect control (chemosterilant) [4].

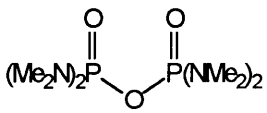


apholate



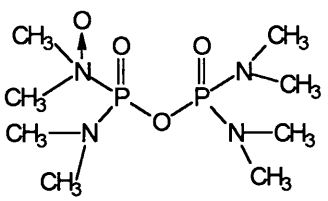
tepa

Since selenium and all it's compounds are toxic in some degree, they are of no use as systemic insecticides, because systemic insecticides must be non-toxic to mammals or broken down at such a rate as to be innocuous when the crop is harvested, if they are used on food crops[75]. Phosphorus amides, octamethyl pyrophosphoramide (O. M. P. A., or Schradan or Pestox III) can overcome this problem.



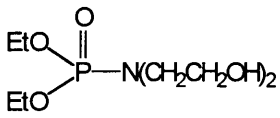
O. M. P. A.

O. M. P. A. was one of the first systemic insecticides, and was made by Schrader. It has been used against aphids on hops and strawberry. Its translocation in the plant has been studied[76] using the compound containing radioactive phosphorus[77]. O. M. P. A. is slowly broken down in the plant by enzymic reactions. Once O. M. P. A. is absorbed into the plant, it is converted into a highly toxic chemical (oxidized O. M. P. A.) (the toxicity is increased 1000-fold).



oxidized O. M. P. A.

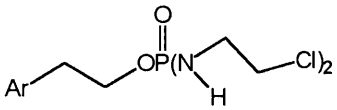
Another example is Fyrol 6[78]. It is a very successful commercial flame retardant for rigid polyurethane foam. Rigid polyurethane foam flame retarded with Fyrol 6 is used extensively for packing and insulation, particularly in household refrigerators, refrigeratered cars and trucks.



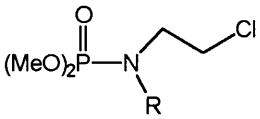
Fyrol 6

Our group has worked on phosphorus amides for many years. Many trisubstituted phosphates belonging to the amidodiester[79], diamidoester[80], and triamidate[71, 81] families were synthesized. Their reactivities[17, 18, 83], such as, 1,3-cyclization and ring-opening reaction[71, 82], alkylation properties[66, 80], were studied.

Especially, several compounds concerning the problem of the alkylating mechanism Scheme 3 were prepared and studied (H, I, 11).



H

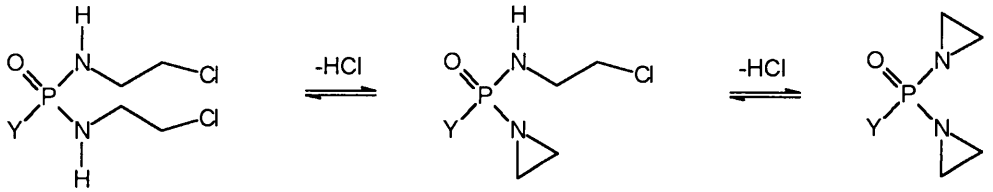


I R=H,

11 CH_2CH_2Cl

The anchimeric assistance of fragmentation[69] (H) and degradations[66] (I, 11) under different conditions were reported.

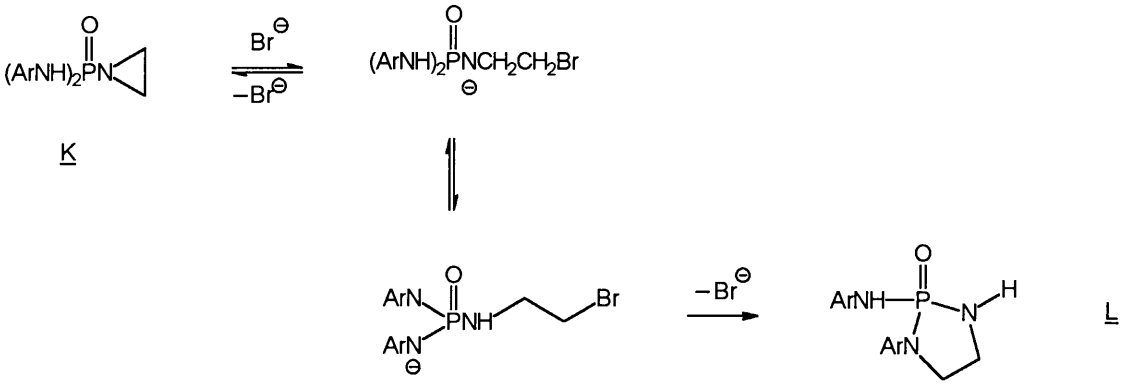
The base promoted mono- and dicyclization of N, N'-di-(2-chloroethyl)-substituted diamides J lead to the N-phosphorylated aziridines. This reaction, however, is complicated by the reversibility of the formation of the aziridynyl ring (Scheme 4).



J

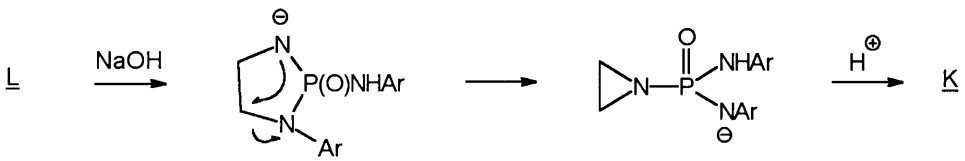
Scheme 4 a: Y=OPh, b: Y=Ph

This kind of interconversion was also observed in another case[71], but this time it involves equilibrium between 1,3-, and 1 ,5-cyclization (Scheme 5, 6).



K L

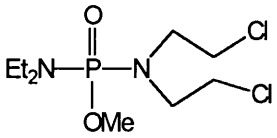
Scheme 5



Scheme 6

Opening of compound K was catalyzed by bromide ion in the convertion into 1, 3, 2-diazaphospholidine derivative L. On the other hand, L was converted into K under the basic condition. All of those researches were related to the alkylating mechanism of F1 (Scheme 3).

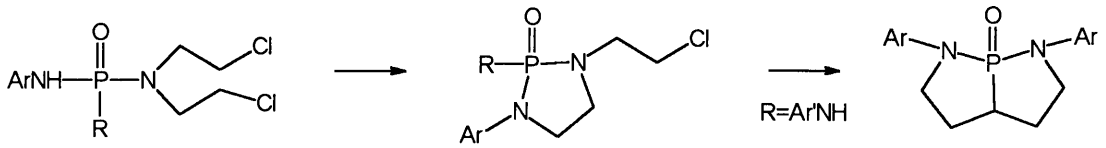
The work described in this Thesis represents the continuation of our study of some aspects of the abovementioned topics. The first major topic (Chapter 1) concerns the reactivity studies of N-phosphorylated nitrogen mustard 1.



1

Methyl N, N-diethyl-N', N'-bis(2-chloroethyl)phosphordiamidate 1 was prepared as a precusor for the corresponding phosphoramidate anion, a model for the phosphoramidate mustard (F1), described in Scheme 3. The demethylation of 1 led to a highly unstable ion, which underwent spontaneous fragmentation. Several media were used for this degradation.

In Chapter 2, the synthesis of a series of N-bis(2-chloroethyl)phosphoric triamides (and diamidoesters) was described. Those acyclic products were cyclized to 1, 3, 2-diazaphospholidine derivatives which could be cyclized again to the phosphotriamidate products of the 1-oxo-2, 8-disubstituted 2, 5, 8-triaza-1-phosphabicyclo[3, 3, 0]octane system (Scheme 7). The suitable base for each cyclization were found and quantitative yields were obtained. This series of compounds (13, 14, 15) are characterized by specific ranges of the ^{31}P NMR chemical shift values. For some of them, several single crystals were grown. Their x-ray molecular structures were determined and the molecular parameters were discussed. The value of the torsion angle of the O=P-N-H function which determines the packing of the molecules was found to determine also the ability of a substrate to form diastereomeric hydrogen-bonded complexes with optically active acids. The Chapter 3 is dedicated to this topic.



13 14 15

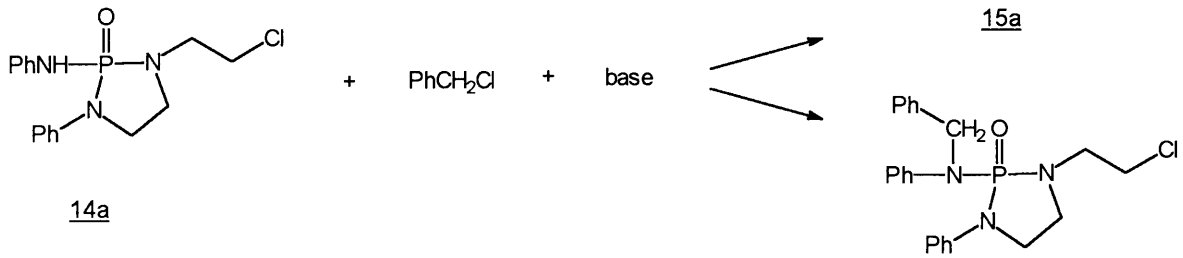
R=Ar'NH

OMe

Scheme 7

In Chapter 4, we discuss the correlation of ^{31}P chemical shift with N-P-N angles based on their crystal parameters.

As we are always interested in the intra, and inter molecular reactivity of phosphorus amides, the compound 14a (when R=Ar=Ph, in 14), was chosen as a substrate to be studied for this topic. Some conclusions were achieved (Scheme 8). The product of the intermolecular nucleophilic reaction 14e showed interesting ^{1}H NMR spectroscopic properties. The single crystal diffraction indicated that the intramolecular hydrogen bonding between one of the benzyl methylene hydrogens and phosphoryl oxygen exists.



14a 15a 14e

Scheme 8

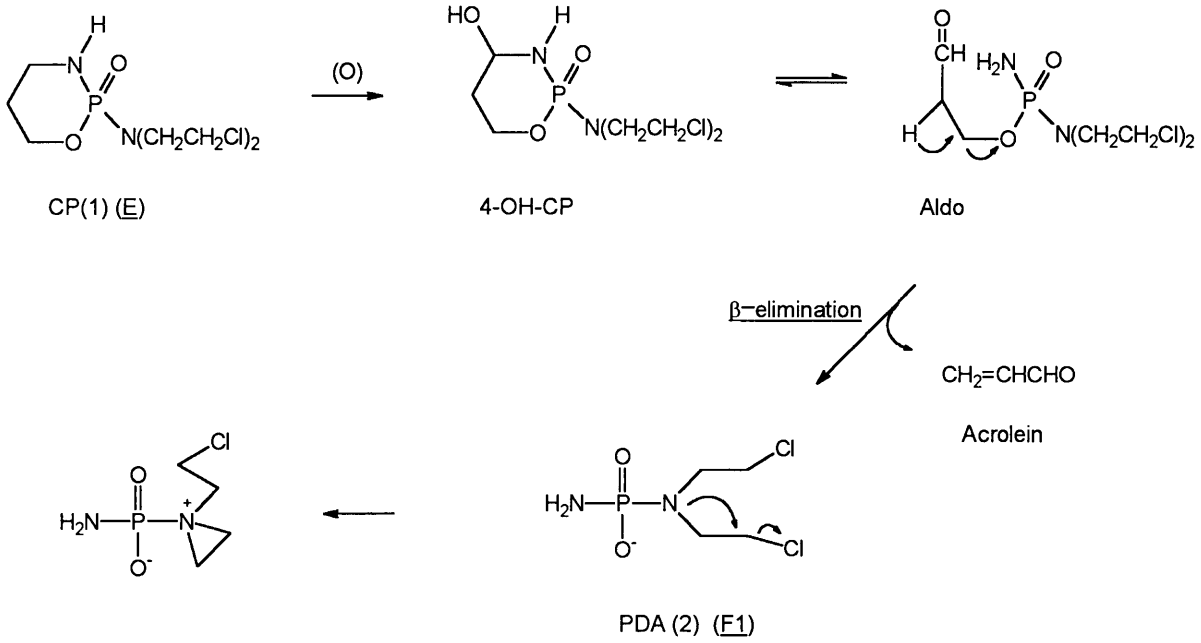
Chapter 1

Chapter 1

Reactivity Studies of N-phosphorylated Nitrogen Mustard

Introduction

The first part of this work is related to the Kwon's mechanism according to which N-phosphorylated nitrogen mustard formed as a biologically active degradation product of cyclophosphoramide drug acts as a biologically alkylating agent[57] and a potent DNA cross-linking agent[61]. The mechanism of the *in vivo* degradation of cyclophosphamide to the reactive N-phosphorylated Nitrogen mustard is represented in Scheme 9.

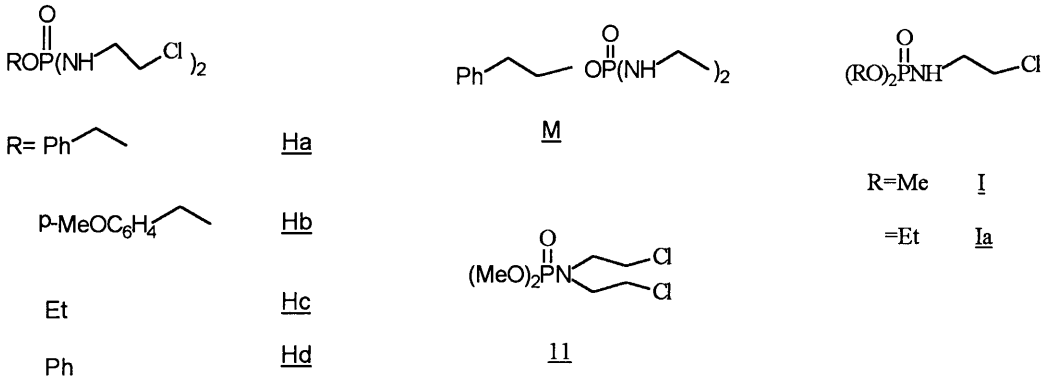


CP(1) (E) 4-OH-CP Aldo

PDA (2) (F1)

Scheme 9

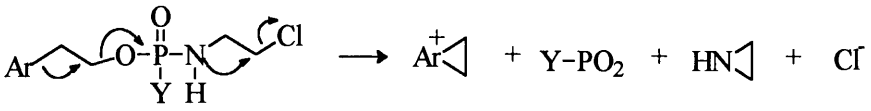
In our laboratory , we have worked with many models of N-phosphorylated mustard to study their reactivities (fragmentation[66, 69, 83, 84], ring forming and opening reactions [82], and synthesis methods[81, 71]). Following are some models which were investigated in our lab (Scheme 10).



Scheme 10

Models H, M and I are phosphoramidate and phosphodiamidate esters. The fragmentation of neutral substrates via anchimeric assistance was studied[69]. It is known from literature[59], that in the N-phosphorylated system, it is necessary to develop a high electronic density at the phosphate group in order to produce the alkylating activity. In other words, the fragmentation will then occur easily. Many studies have also shown that the increment of electronic density can trigger unimolecular fragmentation of substrates[85, 86].

In model H, the system has the ability for anchimeric assistance via the aromatic group (when R=$PhCH_2CH_2$, $MeOC_6H_4CH_2CH_2$) to cleave the C-O bond and it also has a good leaving group (Cl^-) present (Equation 4)



Equation 4

It seems that such system can be a potential precursor of metaphosphate and ethyleneimine products (aziridine derivative). This was confirmed by the fragmentation experiment[69]. But model M is relatively stable (150 ^{0}C, 48 h), because it doesn't have a leaving group at β-position, so 1,3-cyclization won't occur, and no further fragmentation takes place.

On the other hand, model Hd and Ia were also stable under similar conditions, there was no elimination of HCl to yield N-vinylphosphoramidate (1,2-elimination) nor was 1,3,2-diazaphospholidine (1,5-cyclization) produced.

From these experiments, it can be concluded that for the spontaneous fragmentation to occur, N-phosphorylated mustard should meet two basic conditions:

1. It must contain an electron rich anchimeric assistance group (for example, $PhCH_2CH_2$).

2. A good leaving group at β-position of the N-alkyl group is also essential.

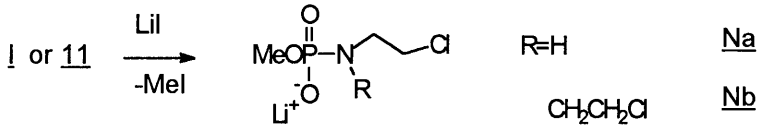
In Kwon's mechanism (Scheme 9), the N-phosphorylated mustard exists as an anion. In order to study the reactivity of ionic substrates, other models were prepared.

The model I and 11 were developed for the following reasons:

1. They can be mono-demethylated to form salts (anions).

2. They are related to phosphoramide mustard [PDA(2)] derived from cyclophosphamide as shown in Scheme 9.

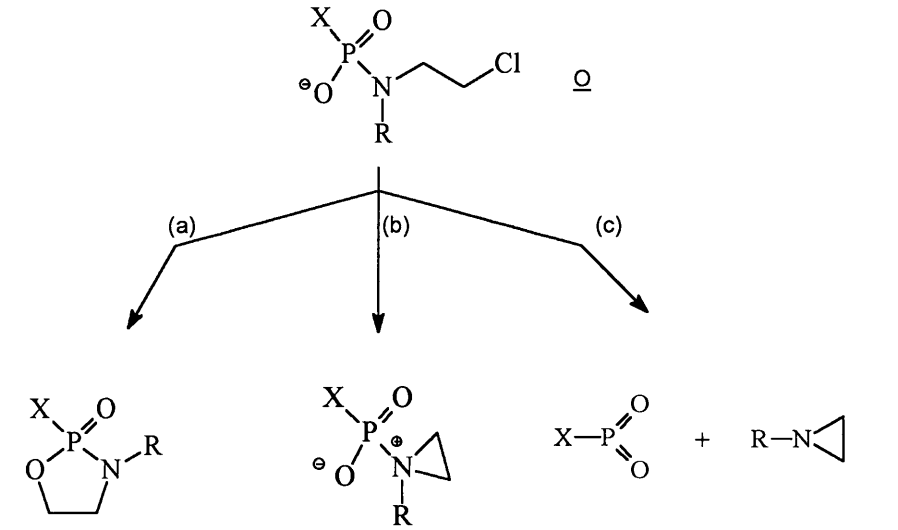
Substrates I, 11 can react with lithium iodide in 2-butanone and give stable Li^+ salts[66] (Na, Nb).



Equation 5

System N (Equation 5) has the potential to fragment as it contains the negatively charged oxygen atom at phosphorus. This type of fragmentation was also observed in the laboratory of L.D. Quin[87]. The fragmentation of anions of phosphorylated mustard is a very complex process. It strongly depends on the following factors : 1. structure of substrate. 2. reaction conditions (temperature, solvent, external nucleophile and reaction time). The fragmentation doesn't simply involve only one pathway, it can proceed via several intramolecular and intermolecular reactions including cyclizations and nucleophilic ring opening steps.

Using a general model O, a possible fragmentation mechanism was proposed[66]. and three pathways have to be considered (Scheme 11).



Scheme 11

The first pathway (a) is a 1,5-cyclization. This intramolecular 1,5-cyclization leads to the formation of 1,3,2-oxazaphospholidine derivative. The pathway (b) is a 1,3-cyclization, N-phosphorylated aziridine derivative is produced. In the pathway (c), P-N bond cleavage occurs directly, and the metaphosphate and aziridine derivatives are generated.

In the previous work, the fragmentation of model I was carried out under the following conditions, and confirmed the above postulated mechanism.

The lithium salt of model I was incubated in water at room temperature (RT), and the reaction was monitored by ^{31}P and ^{1}H NMR spectroscopy. The following results were obtained (Scheme 12):

1. 1,5-cyclization (it has been postulated as the first step in the nonenzymatic hydrolysis of cyclophosphamide[88]). 1,3,2-oxazaphospholidine derivative was observed; this product is considered as to be devoid of alkylating reactivity. The further degradation of this product will follow different pathways which depend on reaction conditions. When the pH of the medium is less than 7 (acidic), the ring opening takes place at the P-N bond, and gives a stable diester salt Ib. When pH > 7, the endocyclic P-O bond cleaves and yield the amidoester salt (Ic).

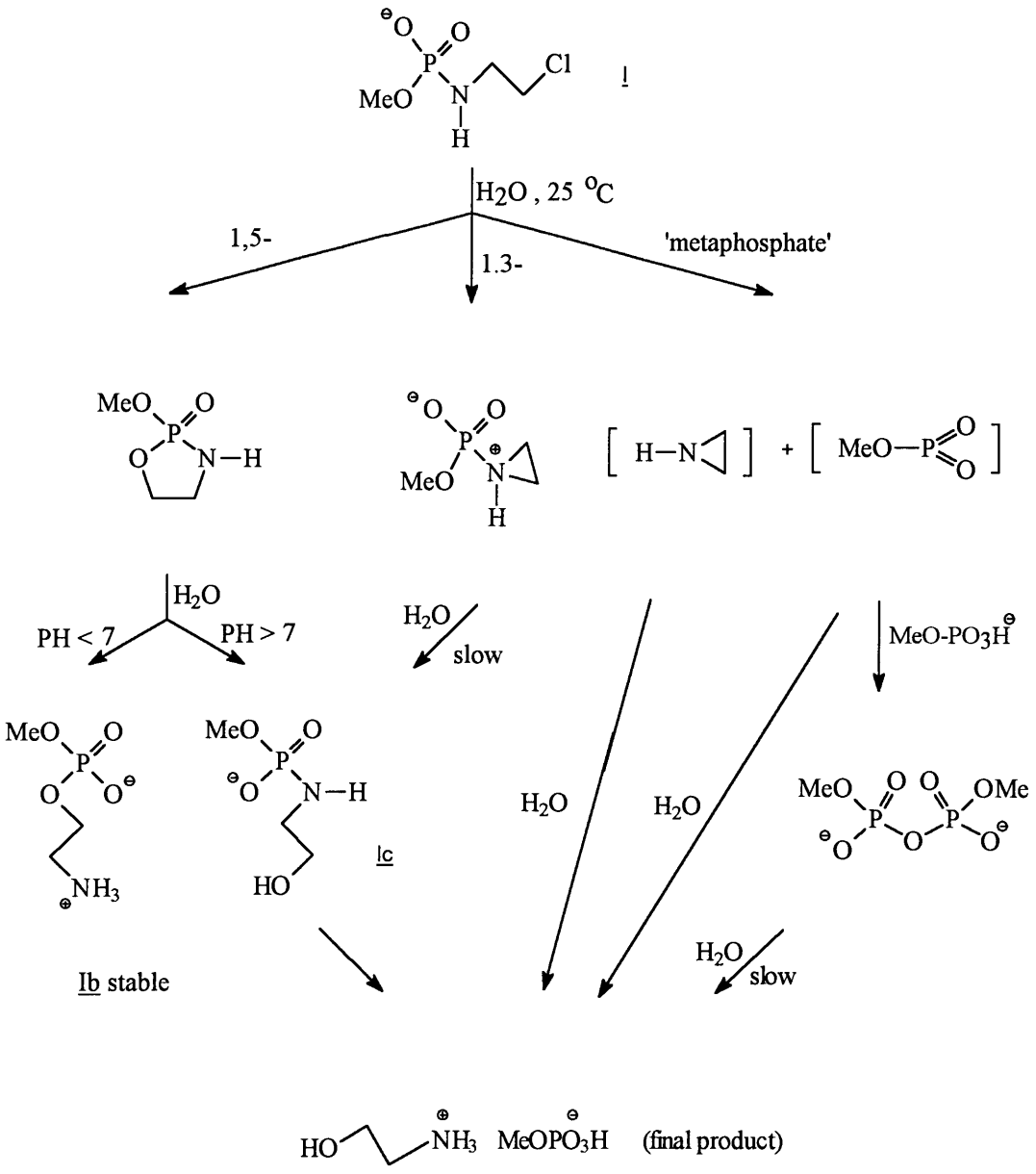
2. 1,3-cyclization. The primary product is, in this case, a N-phosphorylated aziridine derivative, following this step is the nucleophilic ring opening of the aziridine, and final products are generated.

3. The third pathway is the direct formation of metaphosphate and aziridine by a concerted P-N and C-Cl bonds cleavage. As those primary products are highly reactive, they will give further products. Metaphosphate yields methyl ester of phosphoric, diphosphoric and polyphosphoric acids. Aziridine can undergo slow polymerization, especially in acidic condition[66, 69].

This example shows that : this kind of ionic amidoester decomposes easily yielding a variety of primary and secondary products. The fragmentation can follow at least three directions. The 1,3-cyclization and 'metaphosphate' mechanisms lead to the formation of the ethyleneimine derivatives

which can act as biologically critical alkylating agents.

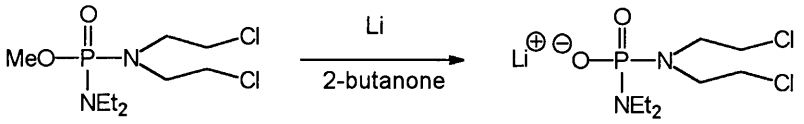
Very similar fragmentation pattern was found for the O-demethylated derivative of 11[66].



Scheme 12

In the biodegradation of cyclophosphamide, the phosphoramidate mustard generated is a diamidate derivative. In our previous work, models studied (model I and 11) were

monoamidoester salts. In the present work, we decided to extend our reactivity studies to the model 1a, the anion of N,N-diethyl-N',N'-bis(2-chloroethyl)phosphodiamidate , using ester 1 as a precusor (Equation 6).



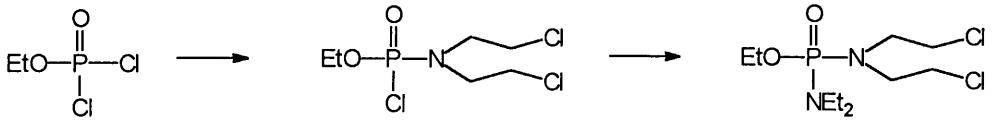
1 1a

Equation 6

Results and discussion

The introduction of second nitrogen to the phosphate moiety had a dramatic effect on the reactivity. Nitrogen is a better electron-releasing atom than oxygen, that means this structural change will increase electronic density of phosphate moiety. In other words, the molecule can be activated by this second N-diethyl group.

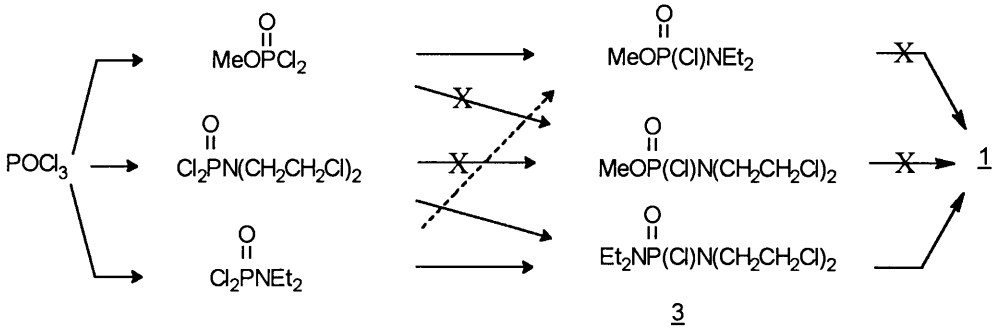
In this case, the first challenge was to make the precursor 1. Similar compounds were prepared by McGuigan[68] . It seemed likely that compound 1 could be generated from methyl phosphorodichloridate sequentially in reactions with $H_2N^+(CH_2CH_2Cl)_2Cl^-$, and Et_2NH. But this approach had failed. After methyl phosphodichloridate was prepared, it was allowed to react with $H_2N^+(CH_2CH_2Cl)_2Cl^-$ at -78 ^{0}C in the presence of 2 mole-equiv. of Et_3N. After 24 h, the ^{31}P NMR spectrum indicated that a mixture of products was found. However, when the same reaction was conducted with ethyl phosphorodichloridate, the product, ethyl N,N-bis(2-chloroethyl)amidophosphorochloridate (2) was obtained successfully (Equation 7).



2

Equation 7

The probable reason for this difference is that methyl group is more reactive (relatively to ethyl group) in nucleophilic displacement. The other possible routes , which could produce compound 1, are shown in Equation 8.



3

Equation 8

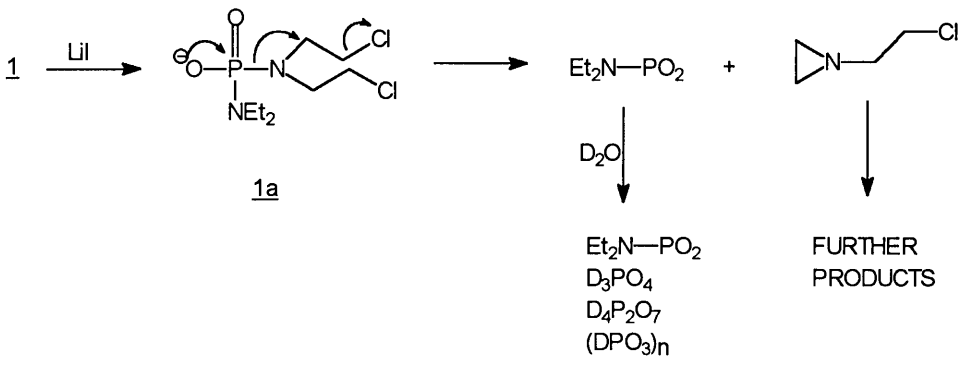
The final way for preparing compound 1 was chosen according to the uncrossed arrows for the following steps (Equation 8). This involved the prior synthesis of the intermediate N,N-diethyl-N',N'-bis(2-chloroethyl)diamidophosphorochloridate 3, followed by its subsequent reaction with sodium methoxide. This route can give a total yield of 1 to be ca. 60%.

After the substrate 1 was obtained, its Li salt was expected to be generated in the same way as were previous models. However, under the "standard" conditions no expected salt was produced. Substrate 1 and lithium iodide were dissolved in 2-butanone and the solution was heated under

reflux for 12 h. A solid that separated was filtered off. ^{1}H and ^{31}P NMR spectroscopy showed that it was not the lithium salt of the demethylated 1. Instead, the ^{31}P NMR spectrum revealed signals at δ_P : 8.5, -0.2, -10.0 and -21.2 ppm. The ^{1}H NMR spectrum indicated that only the Et_3N groups were present. Comparision of those ^{31}P NMR signals with the results reported by Quin and co-workers[89], allowed to identify those signals as N,N-diethyl phosphoramidic acid, phosphoric, diphosphoric, and polyphosphoric acids respectively.

The filtrate (2-butanone solution) was diluted with hexane. The oily material separated out, it was dissolved in $CDCl_3$, and examined with ^{1}H and ^{31}P NMR spectroscopy. ^{31}P NMR spectrum indicated the absence of any phosphorus containing compound, while ^{1}H NMR spectrum showed a mixture giving rise to signals grouped in the ranges of the δ_H values of 1.0-1.2, 2.2-2.4 and 3.0-3.1 ppm. These observed ranges correspond closely to those reported[66] for N-substituted ethylenimine and N,N-bis(2-substituted ethyl)amine type of compounds.

Based on the above results, the following mechamism could be proposed for the reaction (Equation 9):



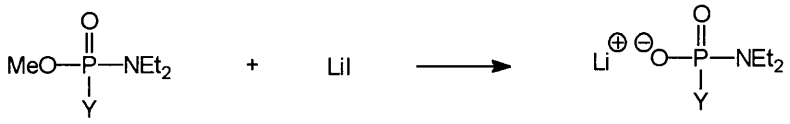
Equation 9

The initial demethylation of 1 was confirmed in the following experiment by the formation of

iodomethane. A solution of compound 1 and LiI in acetone-d_6 was sealed in an NMR tube. The tube was incubated at 40 ^{0}C for 12 h , and the solution was examined by NMR spectroscopy. The single peak was observed (δ_H : 2.16 ppm); by adding the authentic material into the NMR tube, no new peak appeared, only the existing peak increased in intensity, confirming the identity of the product.

After the O-demethylation, the anion 1a formed seems to be so unstable that it fragmented immediately and generated metaphosphate, and aziridine derivates. The metaphosphate underwent immediate condensation reactions to give di or polyphosphoric acid derivates as evidenced by characteristic complex ^{31}P NMR signals in approximate region -10 and -21 ppm. So, the anion of phosphodiamidate 1 is intrinsically unstable and decomposes spontaneously via the unimolecular fragmentation to metaphosphoramidate and N-substituted ethyleneimine as reactive intermediates (primary products).

In order to compare model 1a with other phosphoramidates, four other phosphoramidates---4, 5, 6, 7 were prepared. Compounds 4 and 5 were amidoester and diamidoester, respectively without β-substituted leaving group in the N-substituent (Equation 10).

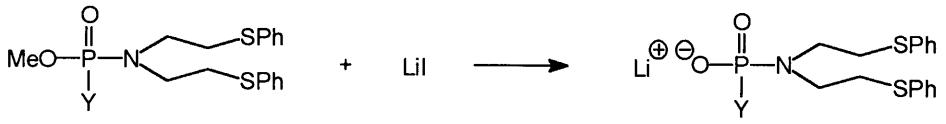


4 Y=OMe

5 Y=NEt_2

Equation 10

Compounds 6 and 7 were amidate and diamidate esters with β-substituted thiophenoxy group which is a poorer leaving group relative to 'Cl' (Equation 11).



6 Y=OMe

7 Y=NEt_2

Equation 11

The Li salts of demethylated 4-7 were also attempted to be prepared under "standard" conditions. Except compound 7, other Li salts were sucessfully obtained, but in different yields : 6 55%, 5 20%, 4 30%. The dimethyl phosphoramidates 4 and 6 gave best results. The important result was that compound 5-- a diamidate ester gave a lithium salt, which was stable both, in solid and in a solution (D_2O) state. The behaviour of 7 was very similar to that observed for substrate 1, i.e., the decay of the substrate was accompanied by the formation of fragmentation products. We can conclude therefore that an electronegative substituent in the β-position is essential for the spontaneous fragmentation of phosphodiamidates such as model 1 and 7.

In the process of preparing those lithium salts, we found that the reaction rates differed significantly. We decided to determine the half-lifes ($t_{1/2}$) of the demethylation of those compounds (4, 5, 6, 7) by LiI. The $t_{1/2}$ was determined by monitoring the decay of the ester by ^{31}P NMR spectroscopy. The results demonstrated the following order :

4 : 22.5h, 5 : >200h, 6 : 5h, 7 : 57h

The demethylation of monoamidate esters (4, 6) is almost ten times faster than that of diamidate esters (5, 7), that is , the diamidate esters are much less reactive in demethylation reaction. On

the other hand, the demethylation of esters with β-substituted thiophenoxy group (6, 7) is about five times faster than those of the corresponding esters wihout β-substituted group (4, 5). The former is more reactive than the latter; in other words, the β-substitution at the NEt_2 function activates the the substrate towards demethylation. We also noticed that the formation of the demethylated products of diamidoesters was a slower process (compared to amidoesters), but if the demethylated products contain a β-leaving group (Cl of model 1, PhS of model 7), they fragment spontaneously as soon as they are formed.

So far, we can arrive at a major conclusion: the structures, which contain a β-substituted leaving group at the NEt_2 function and which belong to the diamidate family, are required if a phosphoramidate ester is expected to act as a precursor to an ionic derivative that would undergo spontaneous fragmentation.

We also tried to get the Li salt of demethylated 7 by changing the solvent to boiling acetone, and keeping the solution under reflux (this reaction didn't work at RT). However, the same result as before was obtained. Finally we decided to change the nucleophile and used thiophenoxide ion (PhS^-), instead of the iodide ion, also used as PhSLi. The reaction (PhSLi, substrate 7, CH_3CN) was initially carried out at RT, but after 280 h, no precipitate formed. The ^{31}P NMR spectrum indicated that no reaction occurred. The reaction temperature was then rised to 60 ^0C and a precipitate was generated. ^{31}P NMR spectrum showed that the product was a complex mixture the same that was obtained under "standard" conditions (LiI, butanone, reflux). Interesting fact is that the same reaction was also performed with substrate 11 at RT. After 120h, the lithium salt was obtained in a high yield (90%). It was identical to the salt obtained under "standard" conditions. These experiments provided further evidence for the lower reactivity of diamidates (relative to monoamidates) in demethylation reaction.

Next we have studied the reactivity of 1a in two other reaction media:

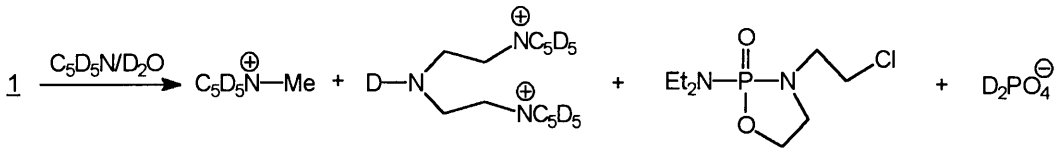
1. Pyridine-d_5/ D_2O 2. PhSH/ Et_3N in CD_3CN

In pyridine-d_5/ D_2O (5/1, v/v) system, pyridine performs two nucleophilic functions:

1. it acts as a demethylating agent to produce anion 1a from precursor 1.

2. it will trap the primary products of fragmentation of anion 1a.

The precursor 1 was placed in D_2O/ pyridine-d_5 solution, and the reaction was carried out at 60 ^{0}C (initailly, the solution was kept at RT for 48 h, but there was no reaction). The course of the reaction was examined by ^{31}P, and ^{1}H NMR spectroscopy at specific time intervals. After about 150 h, the substrate was consumed completely, and the products were identified by the addition of the authentic samples to the examined solution. The results are shown in Equation 12.



8

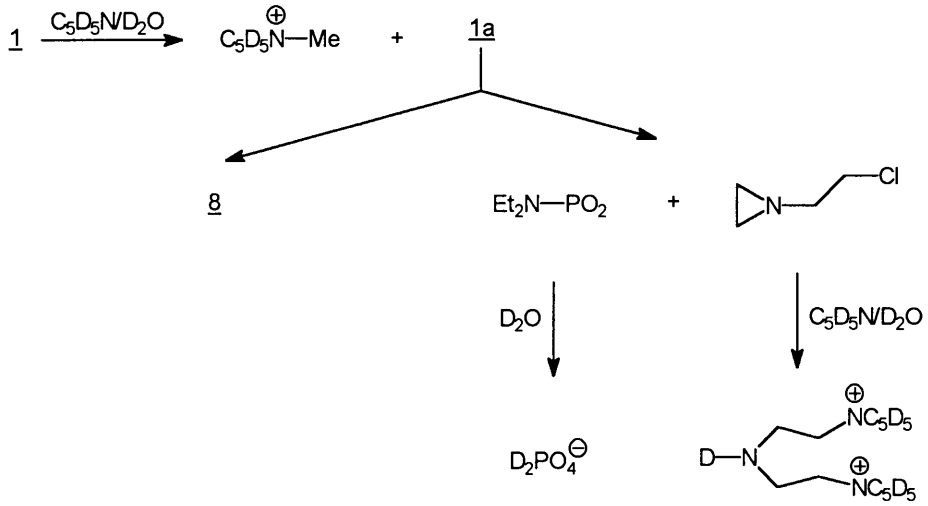
Equation 12

From the ^{1}H NMR spectrum, the signal of the methyl group of $C_5D_5N^+$-Me (singlet, δ_H : 4.5 ppm) increased steadily as the concentration of the substrate decreased. By adding authentic material (C_5D_5N-Me^+ I^-), it was confirmed that the signal of δ_H : 4.5ppm was the signal of the methyl group of $C_5D_5N^+$-Me [the corresponding aromatic signals of the pyridine group are : 7.90 (t),

8.40 (t), 9.20 (d), and were also identified as the pyridine hydrogens of $C_5D_5N^+$-Me cation]. The bis-alkylated product : bis-[2-(N-pyridinio)ethyl]amine was also identified in the mixture. After adding the authentic material to the examined solution, the ethylene group signals at : δ_H 3.30 (t), and 4.90 (t), the corresponding aromatic signals at: 8.06 (t), 8.50 (t), 9.00 (d) of this compound overlaped exactly with existing signals and no new signals appeared.

The ^{31}P NMR spectrum indicated that two products had formed. One was the 1,3,2-oxazaphospholidine derivate 8, the other one was the orthophosphate ion. In the whole course of the reaction, we did not observe any intermediate containing the 2-(N-pyridinio)ethyl substituent with the retained P-N bond.

The postulated mechanism of this reaction is presented in Scheme 13.



Scheme 13

After the demethylation of 1 by pyridine, the anion 1a that is formed decomposes fast. The ethyleneimine derivative is trapped by pyridine and the bis-alkylated product, bis [2-(N-

pyridinio)ethyl]amine, is finally generated. In parallel reactions, the metaphosphoramidate undergoes fast reaction with water and is finally converted to the orthophosphate ion. Another competing reaction, 1,5-cyclization also takes place and product 8 is formed.

In the previous work, substrate 11 was also studied in pyridine/ D_2O system and very similar results were obtained[66].

Before we discuss the behaviour of precusor 1 in the PhSH/ Et_3N/ CD_3CN system, we will first discuss the reactivity studies carried out with substrate 11 in the same system.

The PhSH/ Et_3N/ CD_3CN system contains four mole-equivalents of PhSH and four mole-equivalents of Et_3N with respect to one mole-equivalent of the substrate.

Thiophenoxide ion was used before in the deprotection of methylated oligonucleotides[90]. As it has a high nucleophilicity, it was expected to be a good probe for the alkylating reactivity of the phosphorylated nitrogen mustards.

The substrate 11 , thiophenol and triethylamine were dissolved in acetonitrile-d_3 at RT and the solution was examined by ^{31}P and ^{1}H NMR spectroscopy at specific time intervals. ^{31}P NMR spectrum indicated that the decay of the substrate (11, δ_P 13.3 ppm) was accompanied by the formation of the products with very close ^{31}P NMR chemical shift values (9 : δ_P 13.4, 6 : δ_P 13.5), and other two products of lower δ_P values (11a : δ_P 7.2, 9a : δ_P 7.6). Those products appeared as transient species which were finally converted to a final product (6a : δ_P 8.0). The ^{1}H NMR spectrum showed that the dealkylation product, thioanisole (PhSMe) was formed since a single signal (δ_H : 2.45 ppm) of the methyl group of PhSMe was identified by comparison with authentic material. At the final step, only one product (δ_P : 8.0 ppm) was left in the solution.

The variation of concentration of the substrate and products, measured by the integration of the ^{31}P NMR signals, with reaction time is described in Figure 5.

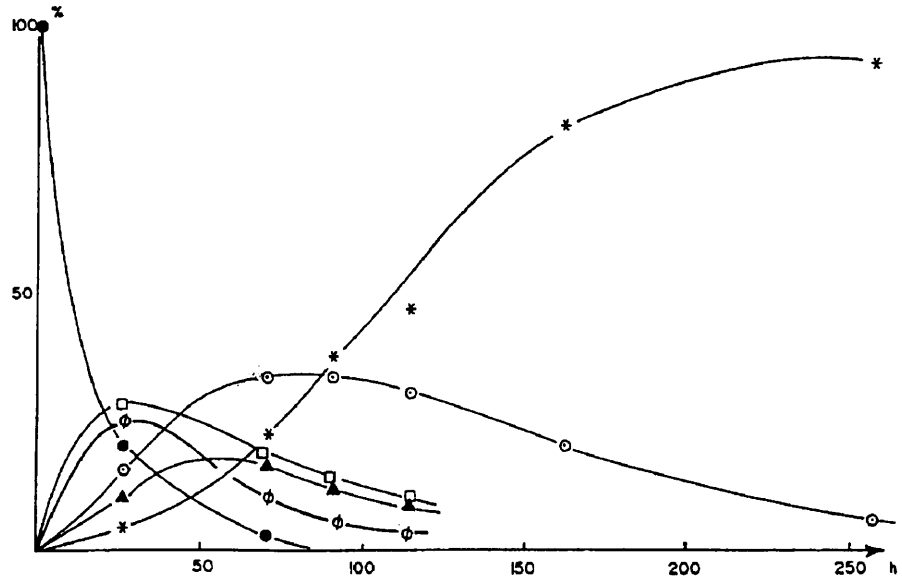
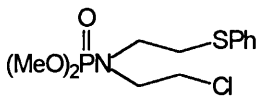


Figure 5 Concentration vs time plot for the reaction of dimethyl N-bis(chloroethyl)-phosphoramidate 11 with PhSH/ Et_3N in acetonitrile. •, substrate 11; *, final product 6a; ⊙, intermediate 9a; □, intermediate 11a; ⌀, intermediate 6; ▲, intermediate 9.

In order to identify the reaction products, compounds 9 and 6 were prepared independently .



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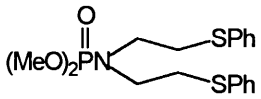

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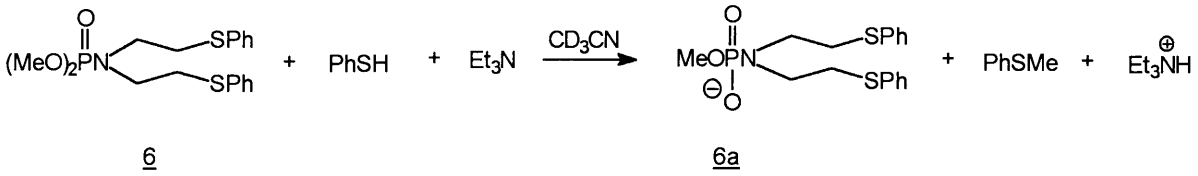
By adding their samples to the reaction mixture, the transient products (9, 6) with δ_P values: 13.4, 13.5 ppm, were identified as dimethyl N-(2-chloroethyl),N-[2-(mercaptophenyl)ethyl] phosphoramidate 9 and dimethyl N,N-bis[2-(mercaptophenyl)ethyl]phosphoramidate 6, respectively.

Other experiments were conducted to identify transient species 11a, 9a, 6a. Compound 6 was placed in the PhSH/ Et_3N/ CD_3CN system, and the reaction was monitored by ^{31}P and ^{1}H NMR

spectroscopy . The ^{31}P NMR spectrum revealed only one product formed, with the signal at δ_P 8.0. After the substrate disappeared, the product with δ_P 8.0 was the only product present. The ^{1}H NMR spectrum indicated the formation of thioanisole and another product which still gave rise to a doublet of the P-OMe group, but integrating for only 3 hydrogens (it means only one methyl group of the substrate was removed by thiophenoxide). The demethylation reaction was suggested (Equation 13)



6 6a

Equation 13

The final product could be identified in this way as the O-demethylated ion of transient product 6. Table 1 presents the ^{31}P and ^{1}H NMR data of both, substrate 6, and the final product 6a. The concentration variation of substrate 6 and product 6a with time is presented in Figure 6.

Table 1. ^{31}P, ^{1}H NMR data of 6 and 6a

	compound 6	product 6a
^{31}P (ppm)	13.5	8.0
^{1}H (ppm)	3.01-3.06 (CH_2N, 2H, m) 3.14-3.20 (CH_2SPh, 2H,m) 3.59 (CH_3O, 6H, d, J_{HP}=11.3)	3.06-3.10 (CH_2N, 2H, m) 3.10-3.21 (CH_2SPh, 2H, m) 3.45 (CH_3O, 3H, d, J_{HP}=10.8)

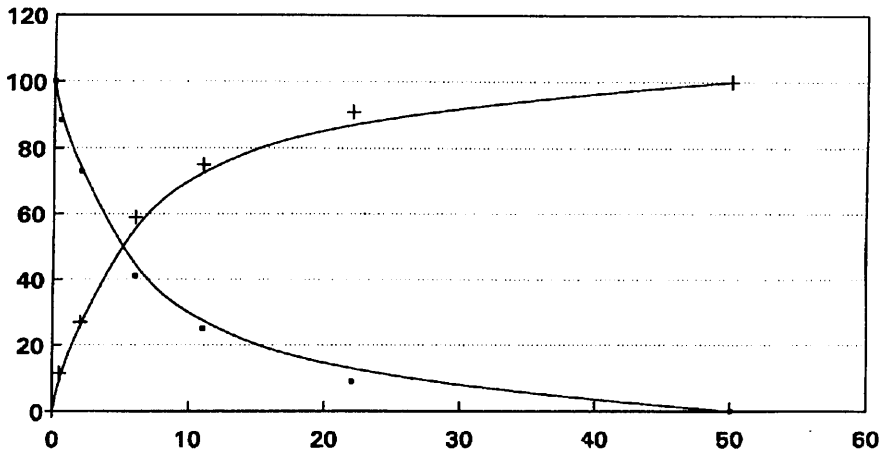
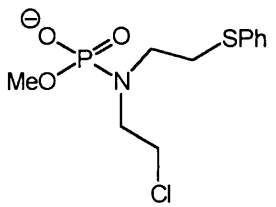
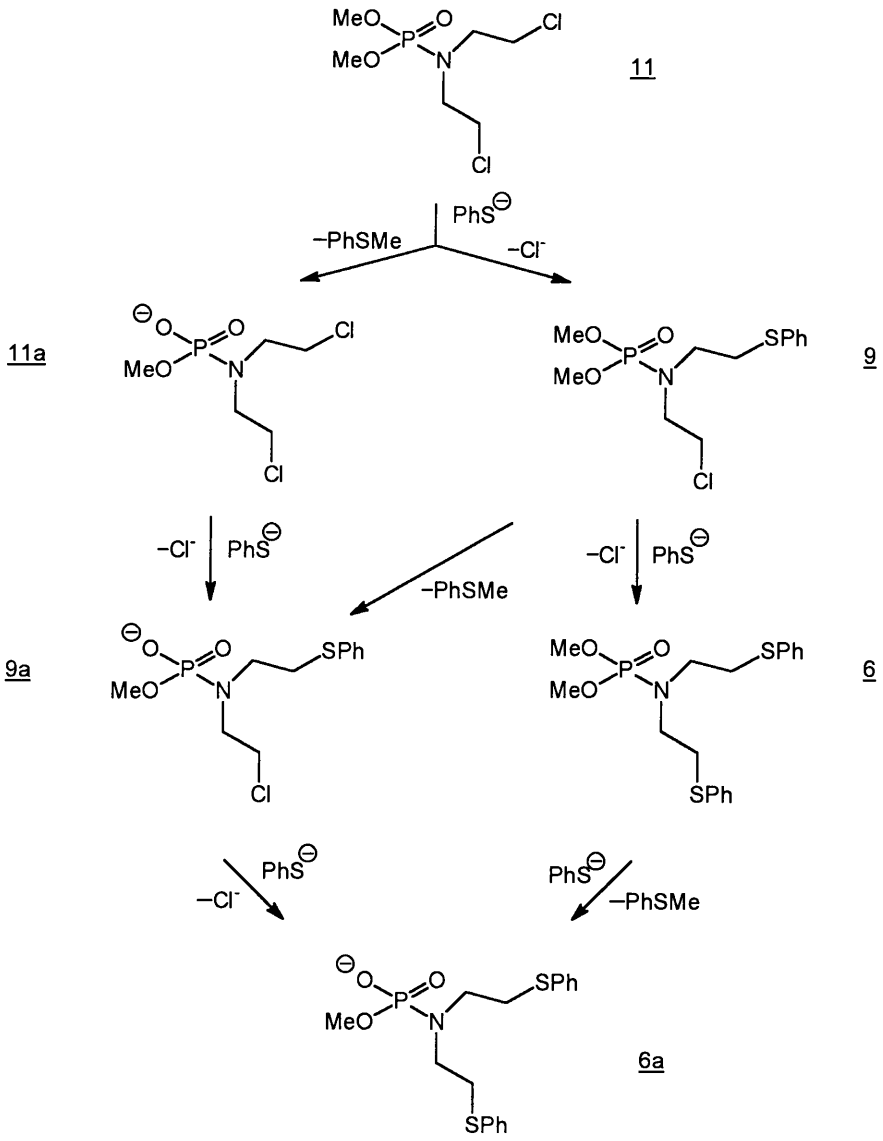


Figure 6 Concentration vs time plot for the reaction of 6 with PhSH/ Et_3N in acetonitrile. ■, substrate 6; +, product 6a.

Another experiment was carried out by placing substrate 9 in the PhSH/ Et_3N/ CD_3CN system. The ^{31}P NMR spectrum showed: that in addition to the appearance of compound 6 (δ_P 13.5) and final product 6a (δ_P=8.0), the product 9a (δ_P=7.6) also formed as a transient species. It was easy to conclude that 9a was the directly O-demethylated product of substrate 9, and that it is from both 11a, and 9.



9a



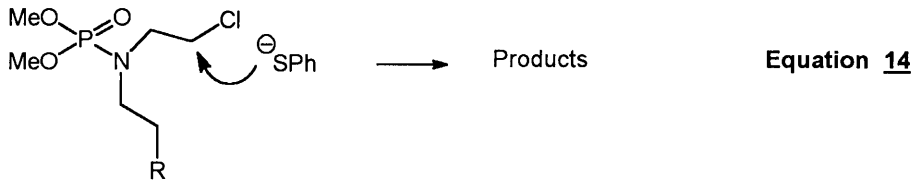
Scheme 14

As for the intermediate 11a (δ_P 7.2) , it was shown to be identical to the previously described ion '1b'[66]. Based on the above facts, a general scheme for the reaction (substrate 11 in PhSH/ Et_3N/ CD_3CN) was developed (Scheme 14).

In Scheme 14, there are obviously two major pathways:

1. O-demethylation reaction.

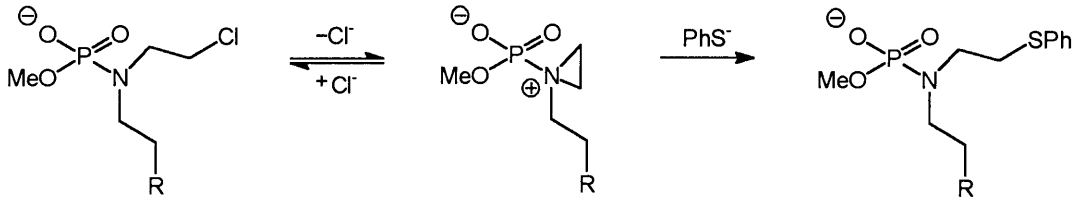
2. Substitution reaction at β-position of N-ethyl group. It can proceed according to two mechanisms: one is the 'classical S_N2 reaction', the alkylation of PhS^- ion by the N-(2-chloroethyl) group, occuring directly for neutral substrates (11, 9) (Equation 14).



Equation 14

9, R=SPh, 11, R=Cl

Another possibility is a pathway involving an aziridinium type intermediate[91] involved for the ionic species (11a, 9a) (Equation 15).



11a R=Cl

9a R=SPh

Equation 15

The following conclusions for the reaction of 11 in the PhSH/ Et_3N/ CD_3CN system can be drawn.

1. The high nucleophilicity of the reagent (PhS^-) led to a non-selective reaction towards the

substrate 11 : in addition to the O-demethylation (to produce 11a, 9a, 6a), a direct substitution of chlorine took place in the nitrogen mustard moiety (to produce 9, 6). On the basis of Figure 5 and Scheme 14, it was possible to assess relative contribution of two competing nucleophilic substitutions, since after 26h only a very small amount (ca. 4%) of final product 6a, which was produced by both pathways, was formed. The total concentration of intermediates 11a and 9a (initial O-demethylation of 11) is 43%, while the combined concentration of 9 and 6 (direct substitution at β-carbon in 11) was 36%. As some intermediate 9a could be generated via the O-demethylation of 9, it seems that thiophenoxide ion attacked at the O-methyl group and at the β-carbon of the mustard function with comparable rates.

2. In this whole process, no P-N bond cleavage was observed. This indicates a strong medium effect on a specific mechanism of the alkylation/ dephosphorylation steps[66].

3. The final product 6a (bisalkylated product) is stable under the reaction condition. It again confirms earlier observation that higher nucleophilicity of the phosphate moiety (second nitrogen atom instead of the MeO function, diamidate), and a better leaving group in the β-position of the N-ethyl function ('Cl' instead of 'SPh') are necessary for a spontaneous fragmentation (dephosphorylation) of the alkylation product.

4. No 1,5-cyclization to a 1,3,2-oxazaphospholidine derivate was observed. It means that in this system the rates of the two pathways discussed above are much bigger than that of the 1,5-intramolecular cyclization. Usually, the 1,5-cyclization is a much slower process than 1,3-cyclization[82, 71, 92] and intermolecular reactions that will be discussed in later chapter.

The same approach was applied to study the reactivity of precursor 1. Substrate 1 was placed in the PhSH/ Et_3N/ CD_3CN system at RT and the reaction course was examined by ^{31}P and ^{1}H NMR spectroscopy. Some neutral compounds were independently prepared and used for the identification of the reaction products. The variation of concentration of all species with time is represented in Figure 7. The general reaction course is developed in Scheme 15.

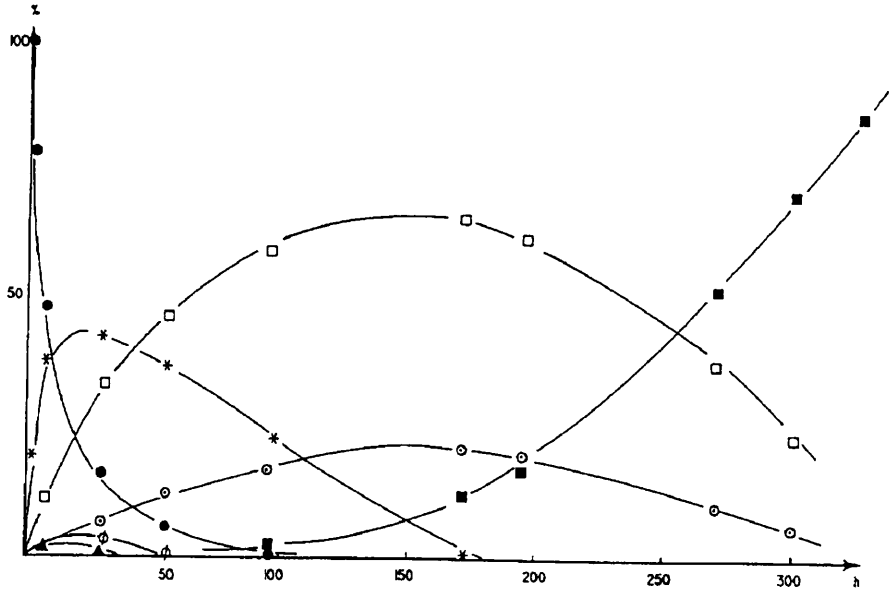
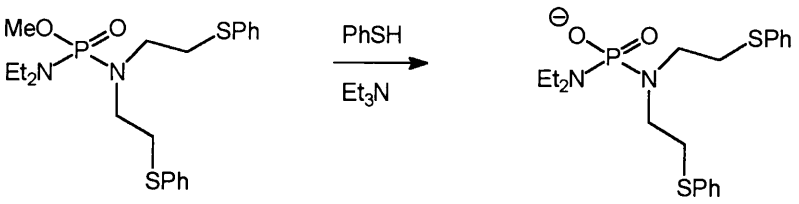


Figure 7 Concentration vs time plot for the reaction of 1 with PhSH/ Et_3N in acetonitrile. •, substrate 1; ■, final product 12; ⊙, product 7a; □, intermediate 7; *, intermediate 10; ⌀, intermediate 10a; ▲, intermediate 1a.

We found that after about 150 h the intermediate corresponding to the δ_P value of 19.7 ppm had formed in high yield (ca. 65%) and it was possible to separate out this intermediate. This compound was obtained on a preparative scale and it was identified as methyl N,N-diethyl-N',N'-bis[2-(mercaptophenyl)ethyl]phosphodiamidate 7.

As for the case of product 6a (Scheme 14), the intermediate 7a (δ_P 10.9) was also identified as the ion resulting from direct O-demethylation of 7 by reacting authentic 7 in the PhSH/ Et_3N/ CD_3CN system (Equation 16).

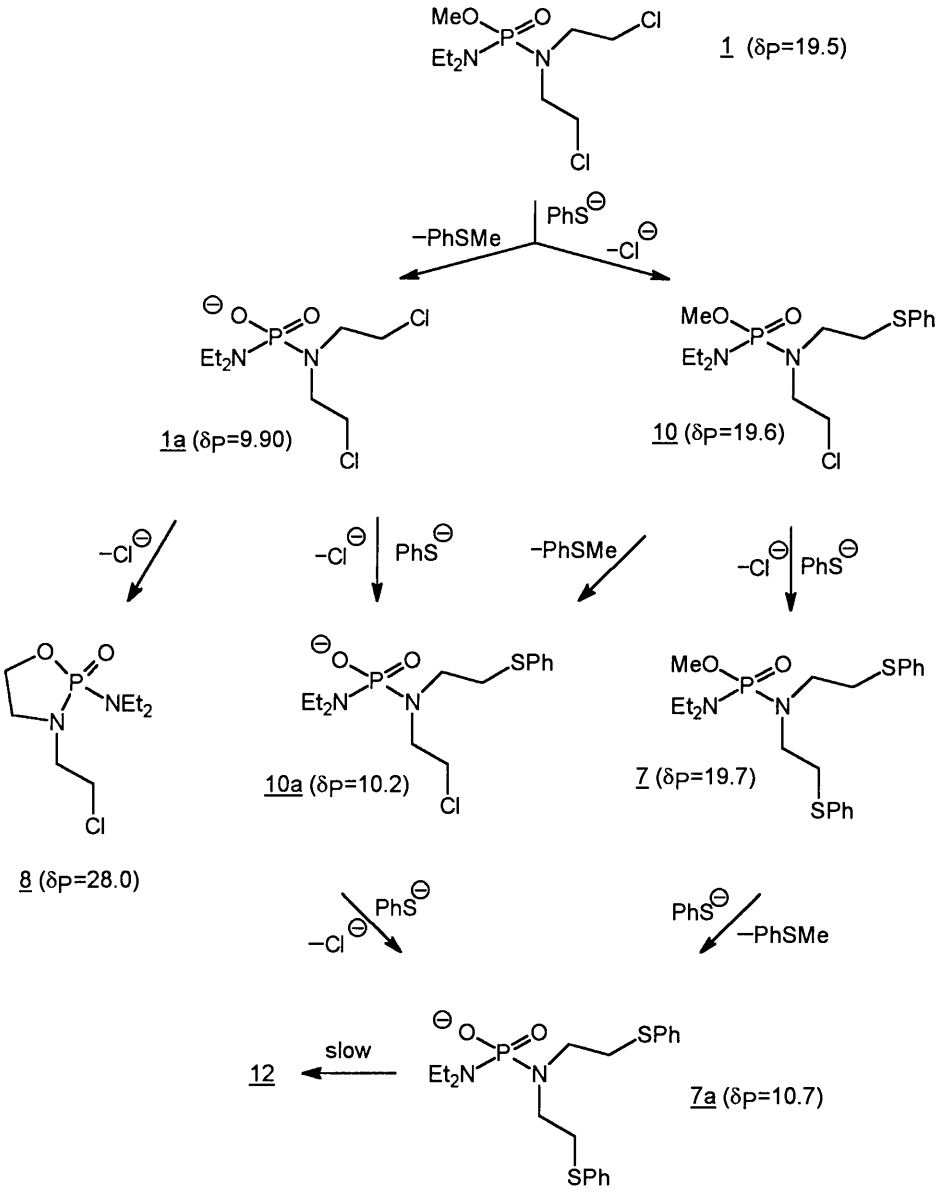


7 7a

Equation 16

Comparison of Figure 7 and Figure 5 led to some important conclusions with respect to the general behavior of the amidate ester and diamidate ester.

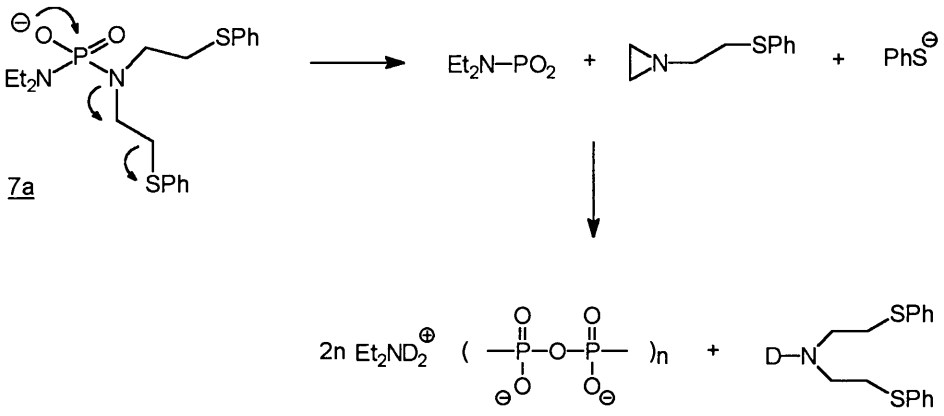
1. It can be seen in Figure 7 that the intermediates 1a and 10a are present at very low contents, while the concentration of the corresponding intermediates 9a and 11a (Figure 5) are much higher. It shows that the reactivity of the ionic intermediates (1a, 10a) is much higher than those of their analogues 9a and 11a. In the first system (Figure 5), the final product 6a was produced mainly from intermediate 9a by the alkylation of PhS^-. In the second system, the final product 7a was formed mainly from the intermediate 7 by the O-demethylation. We can therefore say the O-demethylation reaction of compound 7 is a relatively slow step (Scheme 15), and that the O-demethylaion of compound 6 is a relatively fast reaction compared to alkylation of 9a (Scheme 14). Figure 7 shows that after 52.5 h the total concentration of 10 and 7 (direct substitution at the β-carbon in neutral compounds) is ca. 76%, showing that for substrate 1 the substitution of chlorine is significantly faster than the O-demethylation.



Scheme 15

2. There are two other important differences between the reactions of 11 and 1 in the PhSH/ Et_3N/ CD ÇN system. First, the 1,5-cyclization was observed in the reaction of 1. The

demethylation of 1 was followed by some 1,5-cyclization to 8 (it was also detected before, see Equation 12). However, that direction was not detected in the reaction of 11 under the same conditions. Second, the bis-alkylated ionic product 7a was unstable, while the other final product 6a was stable under those conditions. Product 7a decayed slowly and produced a single phosphorus-containing product 12 (δ_P : -20.3). This final reaction product 12 , after evaporation of the solvent,was treated with D_2O/ benzene-d_6 mixture (1/ 2, v/ v), and both phases were examined by ^{31}P, and ^{1}H NMR spectroscopy. The aqueous solution contained only one phosphorus product (δ_P : -20.3); the ^{1}H NMR spectrum revealed only the presence of the triethylammonium ion. The benzene solution had no phosphorus products and contained only thioanisole and bis[2-(mercaptophenyl)ethyl]amine, which were identified by comparison with the authentic materials. Therefore, the product 12 could be identified as the triethylammonium salt of polyphosphoric acid, which derived from the metaphosphoramidate intermediate (Equation 17). We propose that after the bis-alkylation sequence is completed, dephosphorylation takes place via unimolecular fragmentation of 7a according to the following mechanism.



12

Equation 17

Since the 7a contains a less reactive leaving groups in the β-position of N-ethyl substituent, the fragmentation of 7a is slower than 1a, in which there are two β-chloroethyl groups present. Thiophenoxide generated in the PhSH/ Et_3N/ CD_3CN system was shown to demethylate substrate 1 (Scheme 15). We have mentioned before that PhS as PhSLi in D_3CN failed to demethylate substrate 1. It is obvious that PhS^- can demonstrate different nucleophilicity in different reaction systems.

Experimental

General

1. Solvents and commercially available substrates were purified by conventional methods before use.

2. NMR spectra were recorded on a Bruker AC300 MHz spectrometer, and the chemical shifts are given in δ (ppm), relative to $SiMe_4$ as an internal standard (^1H, ^{13}C), or 85% H_3PO_4 as an external standard (^{31}P), and J values are given in Hz. The following solvents were used for NMR spectroscopy (the organic solvents were dried by molecular sieves) :

Deuterium oxide (D_2O)	(Uvasol, Merck)
Acetone-d_6	(Aldrich, 99.5 atom % D)
Benzene-d_6	(Uvasol, Merck)
Acetonitrile-d_3	(Aldrich, 99.5 atom% D)

3. Mass spectrometry was performed on a Varian MAT-212 double-focusing direct-inlet spectrometer at an ionization potential of 70 ev.

4. Elemental analysis was carried out at the Chemistry Department, University of Cape Town.

5. For column chromatography, Merck silica gel 60 (0.063-0.200 mm) was used as a stationary phase. TLC was performed on 0.25 mm silica gel plates with fluorescent indicator UV254.

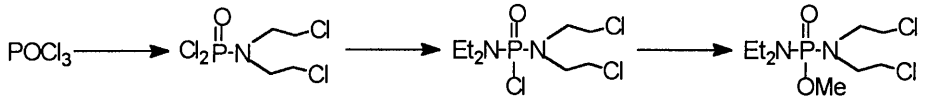
6. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

7. All reported yields are based on pure isolated product unless otherwise stated.

8. Abbreviations used in NMR data, such as : s, d, t, q, m, dd, dt..... , represent singlet, doublet, triplet, quartet, multiplet, double doublet, double triplet...., respectively.

Preparation of Substrates and Standards

Methyl N,N-diethyl-N',N'-bis(2-chloroethyl)phosphodiamidate (1)



3 1

1. Bis(β-chloroethyl)amidophosphoryldichloride

Dry bis(β-chloroethyl)amine hydrochloride (5.00 g, 0.028mol) was mixed with phosphoryl chloride (14 ml), and heated under reflux at 120-140 ^{0}C for 75 h. Excess phosphoryl chloride was evaporated under reduced pressure to give the crude product as off-white solid (100%, 8.44g).

NMR : ($CDCl_3$, ppm)

^{31}P : 18.1

^{1}H : 3.61-3.72 (8H, m, 2 x CH_2CH_2)

^{13}C : 48.9 (CH_2N, J_{HC}=143.0, t)
40.5 (CH_2Cl, J_{HC}=152.8, t)

2. N,N-diethyl-N',N'-bis(β-chloroethyl)diamidophosphorochloride (3)

Triethylamine (0.79 g, 7.82 mmol) in 60 ml dichloromethane was added dropwise with stirring to the mixture of the crude substrate bis(β-chloroethyl)amidophosphoryldichloride (2.00 g, 7.72 mmol) and diethylamine (0.56 g, 7.72 mmol) in 100 ml dichloromethane under an atmosphere of nitrogen at -78 ^{0}C. The mixture was then warmed up to ambient temperature and stirred for 72 h. The solvent was removed under reduced pressure, enough hexane (ca. 200 ml)

was added to the residue, filtered and the solvent was evaporated. The crude product was obtained (75%, 1.71g). Pure product was obtained as a colorless oil by bulb-to-bulb distillation, bp 120-124 ^{0}C/ 0.14 mbar (1.65 g, 72%).

NMR : ($CDCl_3$, ppm)

^{31}P : 26.6

^{1}H :	1.24	(6H, t, J_{HH}=7.12, 2 x CH_3)
	3.26-3.27	(4H, m, 2 x NCH_2 of CH_3CH_2N)
	3.40-3.43	(4H, m, 2 x NCH_2 of NCH_2CH_2Cl)
	3.61	(4H, t, J_{HH}=6.89, 2 x CH_2Cl)
^{13}C :	13.4	(CH_3, dq, J_{HC}=127.0, J_{PC}=2.9)
	40.3	(NCH_2 of CH_3CH_2N, dt, J_{HC}=138.0, J_{PC}=3.1)
	41.5	(CH_2 of CH_2Cl, t, J_{HC}=152.0)
	49.5	(NCH_2 of NCH_2CH_2Cl, dt, J_{HC}=141.0, J_{PC}=4.0)

3. Methyl N,N-diethyl-N',N'-bis(2-chloroethyl)phosphodiamidate (1)

A solution of substrate 3 (0.10 g, 0.34 mmol) in dry methanol (1 ml) was added dropwise to the solution of one mol-equiv. of sodium methoxide (8 mg sodium + 3 ml CH_3OH, 0.35 mmol Na) at RT. After being stirred at ambient temperature for 24 h, the mixture was filtered, and evaporated under reduced pressure. A small volume of chloroform was added to the residue. The suspension was filtered through a layer of anhydrous magnesium sulfate, and then the filtrate was evaporated under reduced pressure, yielding crude product (90%). Pure product was obtained by column chromatography (silica, CH_2Cl_2) as a colorless oil (0.070 g, 70%).

NMR : ($CDCl_3$, ppm)

^{31}P : 18.7

^{1}H :	1.15	(6H, t, J_{HH}=7.04, 3 x CH_3 of CH_3CH_2N)
	3.10	(4H, dq, J_{HH}=7.12, J_{HP}=11.20, 2 x NCH_2 of CH_3CH_2N)
	3.36	(4H, dt, J_{HH}=7.21, J_{HP}=11.70, 2 x NCH_2 of NCH_2CH_2Cl)

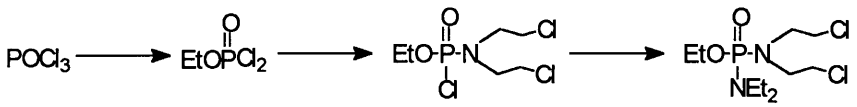
	3.61	(4H, t, J_{HH}=7.04, 2 x CH_2Cl)
	3.73	(3H, d, J_{HP}=11.1, OCH_3)
^{13}C :	14.2	(CH_3 of CH_3CH_2N, q, J_{HC}=125.0)
	39.2	(NCH_2 of CH_3CH_2N, dt, J_{HC}=136.4, J_{PC}=4.1)
	42.4	(CH_2Cl, t, J_{HC}=150.2)
	49.9	(NCH_2 of NCH_2CH_2Cl, dt, J_{HC}=138.8, J_{PC}=4.54)
	51.6	(OCH_3, dq, J_{HC}=147.0, J_{PC}=4.52)

MS : m/z 294, 292, 291, 290 (M^+, 0.5, 2.5, 1.4, 3.8%)
279, 277, 275 (M^+-CH_3, 2.7, 15.6, 23.0%)
243, 241 (M^+-CH_2Cl, 33.0, 100%)
136 (Et_2NHPO_2, 54.0%)
72 (CH_3CH_2NH=$CHCH_3$, 63.0%)

Anal. for $C_9H_{21}C_{l2}N_2O_2P$ (%)

	C	H	N
calcd. :	37.11	7.22	9.62
found :	36.48	7.55	9.38

Ethyl N,N-diethyl-N',N'-bis-(2-chloroethyl)phosphodiamidate (2)



2

1. Ethyl phosphorodichloride

Triethylamine (7.48 g, 0.074 mol) and ethanol (3.15 ml, 0.05 mol) in 100 ml diethyl ether was added to the solution of phosphoryl chloride (8.30 g, 0.05 mol) in 300 ml diethyl ether under nitrogen atomosphere at -78 ^{0}C, with vigorous stirring. The mixture was allowed to warm up to RT, and stirred for 20 h, then filtered, and evaporated under reduced pressure. Pure product was obtained by distillation, bp 60-65 ^{0}C/ 10 mmHg (6.02 g, 75%).

NMR : (CD_3Cl, ppm)

^{31}P : 7.4

^{1}H : 4.28-4.33 (2H, m, CH_2) 1.38-1.43 (3H, m, CH_3)

2. Ethyl N,N-bis-(2-chloroethyl)amidophosphorochloridate

Triethylamine (17.4 g, 0.17 mol) in 350 ml ether was added dropwise with vigorous stirring to the solution of ethyl phosphorodichloridate (14.0 g, 0.086 mol) and bis(β-chloroethyl) amine hydrochloride (15.3 g, 0.086 mol) in 350 ml ether at -78 ^{0}C. After being stirred at ambient temperature for 42 h, the mixture was filtered, and the solvent was removed under reduced pressure. Hexane (900 ml) was added to the residue, filtered, and the solvent was removed under reduced pressure. Crude product was obtained as a colorless oil, 85% yield (18.6g).

NMR : ($CDCl_3$, ppm)

^{31}P : 15.8

^{1}H : 1.41 (CH_3, t, J_{HH}=7.9)
3.40-3.62 (CH_2N x 4, m)
4.31-4.33 (CH_2O, m)

3. Ethyl N,N-diethyl-N',N'-bis-(2-chloroethyl)diamidophosphate (2)

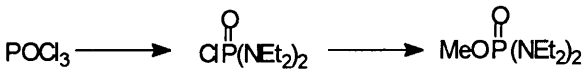
Diethylamine (1.20 g, 16.4 mmol) in 100 ml dichloromethane was added dropwise with vigorous stirring to ethyl N,N-bis(2-chloroethyl)amidophosphorochloridate (2.20 g, 8.22 mmol) in 100 ml dichloromethane at RT under an atomosphere of nitrogen. The mixture was stirred for 72 h, and then the solvent was removed under reduced pressure. The residue was treated with ether (100 ml), filtered, and the solvent was removed under reduced pressure, giving crude product, 2.21 g (97%). Pure product was obtained by column chromatography (Al_2O_3, CH_2Cl_2), as a colorless oil (1.70 g, 75%).

NMR : ($CDCl_3$, ppm)

^1H :	1.10	(6H, t, J_{HH}=7.50, 2 x CH_3 of CH_3CH_2N)
	1.30	(3H, t, J_{HH}=7.20, CH_3 of CH_3CH_2O)
	2.91-3.09	(4H, m, 2 x CH_2N of CH_3CH_2N)
	3.20-3.35	(4H, m, 2 x CH_2N of NCH_2CH_2Cl)
	3.45-3.60	(4H, m, 2 x CH_2Cl)
	3.90-4.10	(2H, m, OCH_2)
^{13}C :	14.2	(CH_3 of CH_3CH_2N, q, J_{HC}=126.4)
	16.3	(CH_3 of CH_3CH_2O, q, J_{HC}=126.9)
	39.6	(CH_2N of CH_3CH_2N, t, J_{HC}=136.4)
	49.6	(CH_2N of NCH_2CH_2Cl, t, J_{HC}=138.8)
	42.5	(CH_2Cl, t, J_{HC}=150.5)
	60.9	(CH_2O, t, J_{HC}=148.8)

MS : m/z 308, 306, 304 (M^+, 2.5, 15.0, 20.9%)
293, 291, 289 (M^+-CH_3, 1.6, 8.8, 12.1%)
257, 255 (M^+-CH_2Cl, 17.6, 58.8%)
136 (Et_2NHPO_2, 100%)
72 ($CH_3CH_2NHCHCH_2$, 76.0%)

Methyl N,N,N',N'-tetraethylphosphodiamidate (5)



5

1. Di(N,N-diethyl)diamidophosphorochloridate

The mixture of diethylamine (4.80 g, 0.066 mol) and triethylamine (16.60 g, 0.16 mol) in dichloromethane (150 ml) was added dropwise at -78 ^{0}C to the solution of phosphoryl chloride (5.05 g, 0.033 mol) in dichloromethane (200ml). The mixture was allowed to warm up to RT, stirred for 72 h, then the solvent was removed under reduced pressure. Enough hexane was

added to residue, filtered, and solvent was evaporated, and the crude product was obtained as a yellow oil (5.10 g, 68%).

NMR : ($CDCl_3$, ppm)

^{31}P :	28.2	
^{1}H :	1.10	(12H, t, J_{HH}=7.12, 4 x CH_3)
	3.01	(8H, dp, J_{HH}=7.14, J_{HP}=14.0, 4 x CH_2)

2. Methyl di-(N,N-diethyl)diamidophosphate (5)

This was preparaed as described for compound 1.

The crude product was purified by the column chromatography (silica, CH_2Cl_2). 1.4 g product was obtained (74%), light yellow oil.

NMR : ($CDCl_3$, ppm)

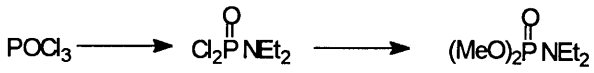
^{31}P :	20.3	
^{1}H :	1.05	(12H, t, J_{HH}=7.00, 4 x CH_3)
	2.99	(8H, dq, J_{HH}=7.19, J_{HP}=11.25, 4 x CH_2N)
	3.56	(3H, d, J_{HP}=11.20, CH_3O)
^{13}C :	13.8	(CH_3, q, J_{HC}=127.4)
	39.1	(CH_2, dt, J_{HC}=136.1, J_{PC}=4.5)
	50.7	(CH_3O, dq, J_{HC}=146.0, J_{PC}=4.71)

MS : m/z 224, 223, 222 (M^+, 0.5, 3.9, 17.0%)
207 (M^+-CH_3, 70.0%)
150 (M^+-NEt_2, 100%)
136 (MH-Et_2NMe, 81.0%)
72 ($CH_3CH_2NHCHCH_3$, 99.0%)

Anal. for $C_9H_{23}N_2O_2P$ (%)

	C	H	N
calcd. :	48.65	10.31	12.61
found :	47.54	9.99	11.45

Dimethyl N,N-diethylphosphoramidate (4)



4

This was prepared as described for compound 5. Pure product was obtained by column chromatography (silica, CH_2Cl_2) (70%).

N,N-diethylamidophosphordichloridate

NMR : ($CDCl_3$, ppm)

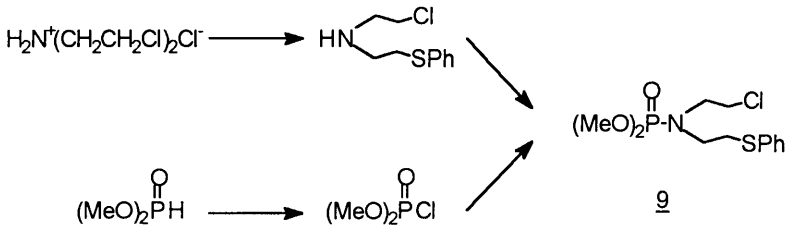
^{31}P :	17.2	
^{1}H :	1.23	(6H, t, J_{HH}=7.14, 2 x CH_3)
	3.33	(4H, dq, J_{HH}=7.13, J_{HP}=16.80, 2 x CH_2)
^{13}C :	13.2	(CH_3, q, J_{HC}=126.8)
	40.8	(CH_2, dt, J_{HC}=138.7, J_{PC}=4.45)

4 NMR : ($CDCl_3$, ppm)

^{31}P :	13.6	(100%)
^{1}H :	1.01	(6H, t, J_{HH}=7.10, 2 x CH_3)
	3.00	(4H, dq, J_{HH}=7.14, J_{HP}=11.4, 2 x CH_2)
	3.58	(6H, d, J_{HP}=11.30, 2 x CH_3O)
^{13}C :	14.0	(CH_3, q, J_{HC}=127.2)
	39.4	(CH_2, dt, J_{HC}=137.0, J_{PC}=4.57)
	52.5	(CH_3O, dq, J_{HC}=147.1, J_{PC}=5.73)

MS : m/z 182, 181 (M^+, 0.9, 6.8%)
167, 166 (M^+-CH_3, 8.8, 100%)
138 (M^+-CH_2CH_2, 10.0%)
109 (M^+-72, 45.5%)
72 ($CH_3CH_2NHCHCH_3$, 19.0%)

Dimethyl N-(2-chloroethyl),N-(2-mercaptophenyl)ethylphosphoramidate (9)



1. N-(2-chloroethyl), N-[2-(mercaptophenyl)ethyl]amine

4.30 g (42.6 ml, 0.042 mol) triethylamine in diethyl ether (40 ml) was added dropwise with vigorous stirring to the solution of 3.00 g (0.017 mol) bis-(2-chloroethyl)amine hydrochloride and 1.85 g (0.017 mol) thiophenol in diethyl ether (400 ml). The mixture was heated under reflux in an atmosphere of nitrogen for 120 h, filtered, and the solvent was removed under reduced pressure. Enough hexane was added, the mixture was filtered and the solvent was evaporated. Crude product was purified by bulb-bulb distillation (oven temperature, 120 ^{0}C/ 2 mbar), 1.26 g (35%).

NMR : ($CDCl_3$, ppm)

^{1}H :	2.81-2.96	(4H, dm, 2 x CH_2N)
	3.04	(2H, t, J_{HH}=6.56, CH_2 of CH_2SPh)
	3.58	(2H, t, J_{HH}=6.01, CH_2Cl)
	7.15-7.35	(5H, m, PhS)

MS : m/z 217, 215 (M^+, 0.2, 0.6%)
166 (M^+-CH_2Cl, 0.3%)
127 ($PhSCH_2$, 5.0%)
109 (PhS, 41.0%)
94 (M^+-CH_2SPh+2, 32.0%)
92 (M^+-CH_2SPh, 100%)

2. O,O-dimethylphosphorochloridate

10.0 g (0.091 mol) dimethylphosphite was dissolved in equal volume of benzene, the solution was added with stirring and protection from moisture to 28.00 g of freshly distilled sulfuryl chloride (0.21 mol) during 20 min at 35-40 ^{0}C. The stirring was continued at 40 ^{0}C for 1 h, then benzene was removed under reduced pressure. The product was obtained as colorless oil, bp 92-95 ^{0}C/ 15 mmHg.

NMR : ($CDCl_3$, ppm)

^{31}P : 8.0

^{1}H : 3.85 (3H, d, J_{HP}=13.40, CH_3)

3. Dimethyl N-(2-chloroethyl),N-(2-mercaptophenyl)ethylphosphoramidate (9)

A solution of triethylamine (0.50 g, 5 ml, 4.95 mol) in diethyl ether (10 ml) was added dropwise at RT to a solution of N-(2-chloroethyl), N-[2-(mercaptophenyl)ethyl]amine (0.90 g, 4.20 mmol) and dimethyl phosphorochloridate (0.61 g, 4.20 mmol) in ether(20 ml). The reaction mixture was stirred at RT for 48 h, and filtered. The solvent was evaporated under reduced pressure. Hexane (50 ml) was added to the residue, filtered, and the solvent was removed from the filtrate. Product was obtained as an orange oil (0.51 g, 34%).

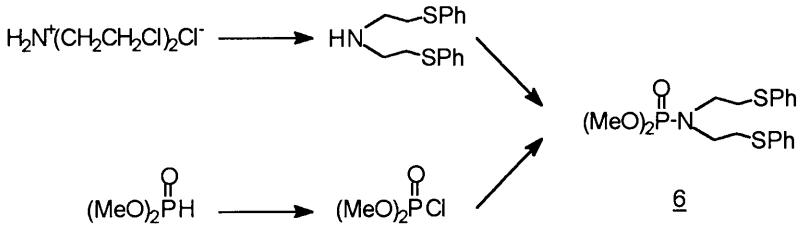
NMR : ($CDCl_3$, ppm)

^{31}P : 13.4

^{1}H :	3.00-3.10	(2H, m, NCH_2 of NCH_2CH_2SPh)
	3.22-3.14	(2H, m, CH_2 of CH_2SPh)
	3.33	(2H, dt, J_{HH}=6.74, J_{HP}=11.67, CH_2N of NCH_2CH_2Cl)
	3.58	(2H, t, J_{HH}=6.30, CH_2Cl)
	3.60	(6H, d, J_{HP}=11.1, 3 x CH_3O)
	7.20-7.40	(5H, m, SPh)
^{13}C :	32.3	(CH_2 of CH_2SPh, t, J_{HC}=141.8)

	43.2	(CH_2Cl, t, J_{HC}=151.1)
	47.5	(NCH_2 of NCH_2CH_2SPh, dt, J_{HC}=141.6, J_{PC}=4.0)
	49.4	(NCH_2 of NCH_2CH_2Cl, dt, J_{HC}=140.7, J_{PC}=4.5)
	53.6	(CH_3O, dq, J_{HC}=147.8, J_{PC}=5.1)
	118.2	(C of Ph, s)
	127.0	(C of Ph, d, J_{HC}=162.1)
	129.2	(C of Ph, d, J_{HC}=161.8)
	130.0	(C of Ph, d, J_{HC}=161.0)
MS : m/z	326, 325, 324,323 (M^+, 0.5, 1.8, 1.1, 5.4%)	
	276, 274 (M^+-CH_2Cl, 0.4, 4.9%)	
	202, 200 (M^+-CH_2SPh, 33.3, 100%)	
	138 [$(MeO)_2PONHCH_2$, 4.7%]	
	123 ($PhSCH_2$, 6.8%)	
	109 (75.0%)	

Dimethyl N,N-bis-[β-(mercaptophenyl)ethyl]phosphoramidate (6)



6

N,N-bis[2-(mercaptophenyl)ethyl]amine was prepared as described for N-(2-chloroethyl) -N-[2-(mercaptophenyl)ethyl]amine by using two mol-equiv. of thiophenol. The product was purified by removing volatile contaminations by keeping the crude product at 50 ^{0}C/ 0.5 mbar for 16 h, yield 75%.

NMR : ($CDCl_3$, ppm)

^{1}H :	2.76	(4H, t, J_{HH}=6.64, 2 x NCH_2)
	3.00	(4H, t, J_{HH}=6.67, 2 x CH_2SPh)

	7.18-7.31	(10H, m, 2 x PhS)
^{13}C :	34.3	(CH_2SPh, t, J_{HC}=138.9)
	48.6	(NCH_2, t, J_{HC}=131.8)
	118.2	(C of Ph, s)
	126.8	(Cof Ph, d, J_{HC}=161.7)
	129.3	(C of Ph, d, J_{HC}=161.3)

MS : m/z 291, 290, 289 (M+, 0.3, 0.7, 2.0%)
166 (M^+-CH_2SPh, 46.8%)
137 ($PhSCH_2CH_2$, 100%)
109 (PhS, 36.0%)

2. Dimethyl N,N-bis-[β-(mercaptophenyl)ethyl]phosphoramidate (6)

A solution of triethylamine (0.50 g, 4.95 mmol) in ether (10 ml) was added dropwise at RT to a solution of dimethyl phosphorochloridate (0.67 g, 4.64 mmol) and N,N-bis-[2-(mercaptophenyl)ethyl]amine (1.33 g, 4.64 mmol) in ether (20 ml). The reaction mixture was stirred at RT for 48 h, filtered and the solvent was removed under reduced pressure. Hexane (50 ml) was added to the residue, filtered , the solvent was removed from the filtrate. The product was obtained as a pale-yellow oil (0.67 g, 36%).

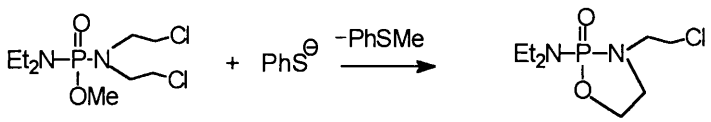
NMR : ($CDCl_3$, ppm)

^{31}P :	12.5	
^{1}H :	2.93-3.04	(4H, m, 2 x CH_2N)
	3.16-3.25	(4H, m, 2 x CH_2 of CH_2SPh)
	3.65	(6H, d, J_{HP}=11.2, 2 x CH_3O)
	7.10-7.30	(10H, m, 2 x PhS)
^{13}C :	32.5	(CH_2SPh, t, J_{HC}=141.2)
	47.2	(NCH_2, dt, J_{HC}=141.1, J_{PC}=4.2)
	53.5	(CH_3O, dq, J_{HC}=147.60, J_{PC}=5.9)
	127.0	(C of Ph, d, J_{HC}=155.0)
	129.6	(C of Ph, d, J_{HC}=161.1)

130.0 (C of Ph, d, J_{HC}=161.1)

MS : m/z 397(M^+, 0.8%)
274(M^+-CH_2SPh, 20.0%)
137($PhSCH_2CH_2$, 100%)
109(PhS, 69.0%)

2-Diethylamino-2-oxo-3-(2-chloroethyl)-1,3,2-oxazaphospholidine (8)



1 8

0.10 g (0.34 mmol) of compound 1, 0.10 g (0.92 mmol) thiophenol, 0.10 g (9.90 mmol,) triethylamine were dissolved in acetonitrile (10 ml). The solution was heated under reflux for 120 h, then the solvent was removed under reduced pressure, 80 ml petroleum ether (40-60 ^{0}C) was added to the residue, and filtered. The filtrate was evaporated under reduced pressure. The pure product was obtained by column chromatography (silica, CH_2Cl_2), 23% yield (0.02g), colorless oil.

NMR : (CD_3CN, ppm)

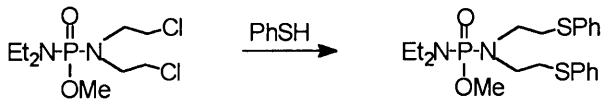
^{31}P : 28.0

^{1}H : 1.10 (6H, t, J_{HH}=7.12, 2 x CH_3)
3.00 (4H, dp, J_{HH}=7.09, J_{HP}=10.90, 2 x CH_2 of CH_3CH_2N)
3.20 (2H, dt, J_{HH}=6.10, J_{HP}=9.01, CH_2N of NCH_2CH_2Cl)
3.36 (2H, dt, J_{HH}=6.80, J_{HP}=10.10, CH_2N of ring)
3.65 (2H, t, J_{HH}=6.71, CH_2Cl)
4.15 (2H, dt, J_{HH}=6.67, J_{HP}=10.50, CH_2O)

^{13}C : 14.8 (CH_3, q, J_{HC}=125.4)
40.2 (CH_2N of NCH_2CH_3, t, J_{HC}=136.7)
43.8 (CH_2Cl, t, J_{HC}=151.2)
47.2 (endo-NCH_2, dt, J_{PC}=5.1, J_{HC}=137.1)
48.0 (exo-NCH_2, dt, J_{PC}=14.8, J_{HC}=138.3)
64.7 (CH_2O, t, J_{HC}=153.0)

MS : m/z 242, 240 (M^+, 2.6, 8.7%)
227, 225 (M^+-CH_3, 27.4, 86.8%)
191 (M^+-CH_2Cl, 40.5%)
170, 168 (M^+-Et_2N, 6.2, 17.8%)
120 (28.7%)
72 ($CH_3CH_2NCH_2CH_3$, 100%)

Methyl N,N-diethyl-N',N'-bis[2-(mercaptophenyl)ethyl]phosphodiamidate (7)



1 7

The solution of 0.10 g (0.34 mmol) compound 1, 0.12 g (1.10 mmol) thiophenol and 0.12 g (1.19 mmol) triethylamine in 10 ml acetonitrile, was kept at room temperature for 140 h. The solvent was removed under reduced pressure, hexane (60 ml) was added to the mixture, then filtered, evaporated, yielding crude product. Pure product was obtained by column chromatography ($CHCl_3$/ hexane, 1:1), as a pale-yellow oil (0.11 g, 62%).

NMR : (CD_3CN, ppm)

^{31}P : 19.7 (100%)

^{1}H : 1.00 (6H, t, J_{HH}=7.07, 2 x CH_3)
2.89-2.99 (4H, m, 2 x NCH_2 of NCH_2CH_2SPh)

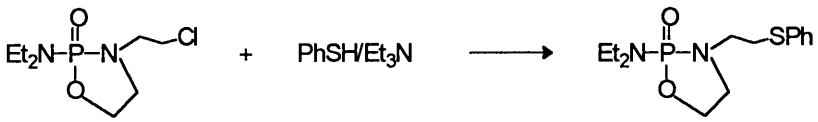
	2.90-2.99	(8H, m, 2 x CH_2 of CH_2SPh and NCH_2CH_3)
	3.50	(3H, d, J_{HP}=11.0, CH_3O)
	7.10-7.40	(10H, m, 2 x PhS)
^{13}C :	14.7	(CH_3 of CH_3CH_2N, q, J_{HC}=126.6)
	32.8	(CH_2 of Ch_2SPh, t, J_{HC}=142.2)
	40.5	(NCH_2 of NCH_2CH_3, t, J_{HC}=136.6)
	47.6	(NCH_2 of NCH_2CH_2SPh, t, J_{HC}=136.5)
	52.0	(CH_3O, q, J_{HC}=147.8)
	118.3	(C of Ph, s)
	127.1	(C of Ph, d, J_{HC}=159.0)
	129.2	(C of Ph, d, J_{HC}=161.0)
	130.0	(C of Ph, d, J_{HC}=161.4)

MS : m/z 439, 438 (M^+, 0.7, 1.8%)
315 (M^+-CH_2SPh, 42.0%)
137 ($PhSCH_2CH_2$, 100%)
109 (PhS, 43.0%)

Anal. for $C_2H_{31}N_2O_2PS_2$ (%)

	C	H	N	S
calcd. :	57.53	7.08	6.39	14.61
found :	56.77	7.21	5.56	13.99

2-Diethylamino-2-oxo-3-[2-(mercaptophenyl)ethyl]-1,3,2-oxazaphospholidine



8

0.10 g (0.42 mmol) of compound 8, 0.14 g (1.28 mmol) thiophenol and 0.13 g (1.29 mmol) triethylamine were dissolved in 15 ml acetonitrile. The mixture was heated under reflux

for 120 h, then the solvent was removed under reduced pressure , 50 ml petroleum ether (40 -60 ^{0}C) was added to the residue, filtered, and the solvent was evaporated. The crude product was obtained in 90% yield. Pure product was obtained by column chromatography (silica, CH_2Cl_2).

NMR : ($CDCl_3$, ppm)

^{31}P : 27.5

^{1}H :	1.02	(6H, t, J_{HH}=7.14, 2 x CH_3)
	2.92-3.30	(8H, m, CH_2CH_2 of NCH_2CH_2SPh and 2 x NCH_2 of Et_2N)
	3.35-3.39	(2H, m, endo-NCH_2)
	4.18-4.22	(2H, m, endo-OCH_2)
	7.15-7.34	(5H, m, PhS)
^{13}C :	14.3	(CH_3, q, J_{HC}=125.3)
	39.5	(CH_2 of CH_3CH_2, dt, J_{HC}=136.5, J_{PC}=4.6)
	32.6	(CH_2 of CH_2SPh, dt, J_{HC}=141.1, J_{PC}=3.6)
	44.0	(endo-CH_2N, dt, J_{HC}=139.6, J_{PC}=4.9)
	47.0	(exo-CH_2N, dt, J_{HC}=142.0, J_{PC}=14.8)
	63.6	(CH_2O, t, J_{HC}=152.4)
	126.3	(C of Ph, d, J_{HC}=161.7)
	129.0	(C of Ph, d, J_{HC}=168.2)
	129.4	(C of Ph, d, J_{HC}=168.2)
	138.0	(C of Ph, s)

MS : m/z 315, 314 (M^+, 0.3, 3.5%)
192, 191 (M^+-CH_2SPh, 9.2, 100%)
242 (M^+-Et_2N, 2.8%)
120 (HM-CH_2SPh-Et_2N, 20.1%)
72 (Et_2N, 19.9%)

Anal. for $C_{14}H_{23}N_2O_2PS$ (%)

	C	H	N	S
calcd. :	53.50	7.32	8.92	10.20
found :	51.78	7.77	8.20	9.42

Lithium methyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidate (6a)

The solution of 0.10 g (0.25 mmol) of compound 6 and 0.04 g (0.29 mmol) of lithium iodide in 2.5 ml dry 2-butanone was heated under reflux for 10 h. The mixture was filtered, the precipitate was washed with 2-butanone and dried under high vacuum. The product was obtained as white solid (0.05 g, 45%), mp 222-224 ^{0}C.

NMR : (D_2O, ppm)

^{31}P :	9.8	
^{1}H :	2.76-2.82	(4H, m, 2 x CH_2N)
	2.92-3.01	(4H, m, 2 x CH_2 of CH_2SPh)
	3.00	(3H, d, J_{HP}=10.9, CH_3O)
	6.87-7.07	(10H, m, 2 x SPh)
^{13}C :	34.8	(CH_2 of CH_2SPh, t, J_{HC}=141.4)
	49.2	(CH_2N, t, J_{HC}=138.6)
	54.4	(CH_3O, dq, J_{HC}=145.5, J_{PC}=5.6)
	128.7	(C of Ph, d, J_{HC}=154.7)
	131.6	(C of Ph, d, J_{HC}=161.3)
	138.9	(C-S of Ph, s)

Anal. for $C_{17}H_{21}PNS_2O_3Li$ (%)

	C	H	N	S
calcd. :	52.44	5.40	3.60	16.45
found :	51.80	5.40	3.48	15.48

Fragmentation Experiments

Demethylation of 4 and 5

Reactions were carried out in 2-butanone using lithium iodide, as described above for the preparation of 6a.

4 gave Lithium methyl N,N-diethylphosphoramidate (30%); mp > 280 ^{0}C; NMR (ppm)

δ_P (D_2O) : 12.0;

δ_H (D_2O) : 0.98 (6H, t, J_{HH}=7.10Hz, 2 x CH_3), 2.87 (4H, dq, J_{HP}=10.90, J_{HH}=7.10Hz, 2 x CH_2), 3.40 (3H, d, J_{HP}=10.70Hz, CH_3O);

δ_C(H-coupled, D_2O) : 16.3 (CH_3, q, J_{HC}=126.4Hz), 42.6 (CH_2, t, J_{HC}=135.8Hz), 54.2 (CH_3O, q, J_{HC}=145.4).

5 gave Lithium N,N,N',N'-tetraethylphosphodiamidate (25%); mp > 260 ^{0}C; NMR (ppm)

δ_P (D_2O) : 17.3;

δ_H (D_2O) : 0.97 (12H, t, J_{HH}=7.1Hz, 4 x CH_3), 2.88 (8H, dq, J_{HP}=9.9, J_{HH}=7.1Hz, 4 x CH_2);

δ_C (H-coupled, D_2O): 16.3 (CH_3, q, J_{HC}=125.2Hz), 41.8 (CH_2, t, J_{HC}=134.7Hz);

Anal. Calcd for $C_8H_{20}LiN_2O_2P$: C, 44.86; H, 9.35; N, 13.08. Found : C, 43.97; H, 8.98; N,11.69.

Attempted preparation of 1a

A solution of 1 (0.20 g, 0.69 mmol) and lithium iodide (0.20 g, 1.46 mmol) in 2-butanone (15 ml) was heated under reflux for 12 h. The precipitate was filtered, washed with 2-butanone and dried under high vacuum. Yield 0.14 g. The precipitate was dissolved in D_2O and the solution was examined by NMR spectroscopy. The presence of the orthophosphate (δ_P -0.2) and diphosphate (δ_P -10.0) ions was confirmed by the addition of the authentic sodium salts. The butanone filtrate was evaporated under reduced pressure, the residue was dissolved in $CDCl_3$, and the solution was examined by NMR spectroscopy.

Fragmentation of 1 in aqueous pyridine

A solution of 1 (0.015 g, 0.05 mmol) in the mixture of C_5D_5N and D_2O (5 : 1, v/ v, 0.5 ml) was incubated at 60 ^{0}C (no reaction was observed at room temperature), and the solution was examined periodically by NMR spectroscopy. The O-demethylation (formation of the N-methylpyridinium ion, confirmed by addition of an authentic sample) was accompanied by the decrease in the intensity of the P-OMe signal of 1. After 150h the disappearance of 1 was

complete and the ^{31}P NMR spectrum showed the presence of two phosphorus-containing products: orthophosphate ion (85%, δ_P 3.5) and 1,3,2-oxaza-phospholidine 8 (15%, δ_P 29.5); both products confirmed by the addition of authentic samples. The ^{1}H NMR spectrum revealed the presence of four products: N-methylpyridinium ion, diethylammonium ion, bis[2-(N-pyridinio) ethyl]amine dication, and 8. All products were confirmed by the addition of samples of authentic species.

Fragmentation of dimethyl N,N-bis(2-chloroethyl)phosphoramidate 11 with PhSH/ Et_3N

The substrate 11 (0.50 g, 2.02 mmol) was added to a solution of thiophenol (0.62 g, 5.74 mmol) and triethylamine (0.58 g, 5.74 mmol) in CD_3CN (4 ml) at room temperature, and the solution was examined periodically by NMR spectroscopy. After 30 min ^{31}P NMR spectrum demonstrated formation of five new phosphorus containing products, and ^{1}H NMR spectrum showed that thioanisole has been formed, as confirmed by the addition of an authentic sample. The changes in the proportions of the individual components of the mixture were then monitored by ^{31}P NMR spectroscopy for a period of 260 h (see Scheme 14 and Figure 5). Intermediates 9 and 6 were identified by the addition of the independently prepared samples. Ionic intermediate 11a was identified by the addition of the authentic material. When the authentic intermediate 6 was treated with PhSH/ Et_3N in CD_3CN under the same conditions, it yielded only one product, identical (^{31}P and ^{1}H NMR spectra) to the final reaction product 6a. In that way 6a was identified as the O-demethylated derivative of 6. Under the same conditions authentic 9 yielded two products, 6 and 9a, which gradually yielded single product 6a. Intermediate 9a (the only component of the mixture not prepared independently) was therefore identified indirectly as the O-demethylated detivative of 9.

Fragmentation of 1 with PhSH/ Et_3N

A solution of 1 (0.015 g, 0.05 mmol), thiophenol (0.028 g, 0.25 mmol) and triethylamine (0.026

g, 0.26 mmol) in CD_3CN (1 ml) was kept at room temperature and examined periodically by NMR spectroscopy. The reaction was followed for 300 h, and the species involved, as well as their proportions are given in Scheme 15 and Figure 7. As before, product 7 (δ_p=19.7) was identified by addition of an authentic sample. The monosubstituted intermediate 10 (precursor of 7) could not be prepared independently, but was identified by its ^{31}P NMR chemical shift (δ_p 19.6), as compared with δ_p=19.5 for its precursor 1, and δ_p=19.7 for its substitution product 7. When authentic 7 was treated with PhSH/ Et_3N it yielded product 7a (δ_p 10.7), thus 7a was identified as the O-demethylated derivative of 7. The two other ionic intermediates, 1a and 10a could not be prepared independently because of their instability. Their structure was assigned on the basis of the analogy with the previous system and a similar sequence of δ_p values (δ_p=9.9 for 1a, δ_p=10.2 for 10a, and δ_p=10.7 for 7a). The concomitant formation of 8 was confirmed by the addition of an authentic sample of 8 to the reaction mixture. It also could be separated out by column chromatography.

The major phosphorus-containing product obtained in the fragmentation of 1, N,N-diethyl-N',N'-bis[2-(mercaptophenyl)ethyl]phosphoramidate anion (7a, δp 10.7) decomposed slowly yielding a new product (δ_p -21.0). The solvent was evaporated under reduced pressure, D_2O (1 ml) and benzene (2 ml) were added and the mixture was stirred for 30 min. The layers were separated and the aqueous solution was examined by NMR spectroscopy. ^{31}P NMR spectrum showed the presence of only one product (δ_p -21.0) and the ^1H NMR spectrum showed the presence of the triethylammonium ion, 1.18 (9H, t, J_{HH} 7.30 Hz), 3.11 (6H, q, J_{HH} 7.30 Hz), and the diethylammonium ion, 1.19 (6H, t, J_{HH} 7.20 Hz), 2.96 (4H, q, J_{HH} 7.20 Hz); both confirmed by the addition of the authentic chloride salts. The benzene layer was evaporated and the residue was dissolved in $CDCl_3$ and examined by NMR spectroscopy. ^{31}P NMR spectrum showed that no phosphorus-containing products were present, while the ^1H NMR spectrum demonstrated the presence of thiophenol, thioanisole, and bis[2-(mercaptophenyl)ethyl]amine; all confirmed by the addition of the authentic samples.

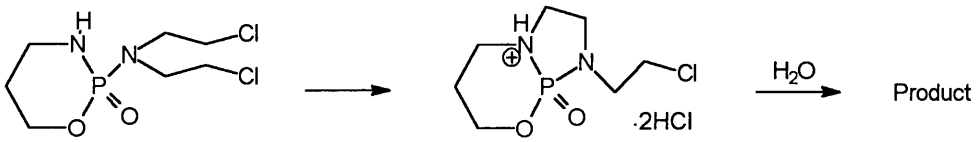
Chapter 2

Chapter 2

Preparation of Acyclic and Cyclic Phosphoric Triamidates and Diamidoesters

Introduction

In the early research, the synthesis of phosphoramide mustards [>P(O)N($CH_2CH_2Cl)_2$] as potential alkylating agents that might be selectively 'activated' in tumors by enzymatic (hydrolytic) release of nornitrogen mustard [HN($CH_2CH_2Cl)_2$] represented one of the design strategies in cancer chemotherapy[48]. Especially 2-[bis(2-chloroethyl)amino-2H-1,3,2-oxazaphosphorinane-2-oxide E emerged as a member which shows a strong antitumor ability. Friedman and coworkers[88, 93, 94] have investigated the enzyme mediated[50] and nonenzymatic[88] hydrolysis of E and postulated that the process is initiated by the intramolecular displacement of chloride ion (Equation 18) and the intermediate P was formed.

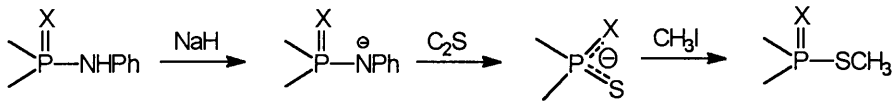


E P

Equation 18

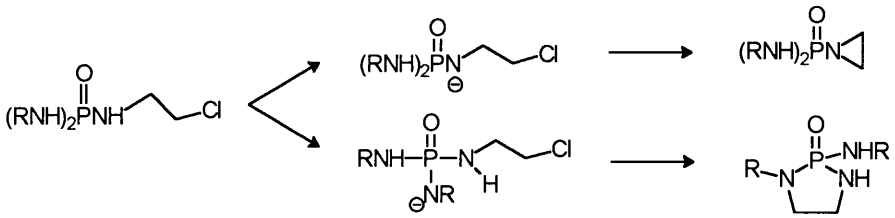
Other investigations[42] showed that P(O)NHR functional group can serve as a simultaneous hydrogen bonding donor and acceptor. The N-aryl phosphoramidate group can be used as an intermediate function in the synthesis of S (Se)-alkylphosphothio (seleno) lates[95] (Equation 19).

This function was also applied to the stereospecific synthesis of cyclic adenosine 3', 5'-(S_P)-[^{18}O] phosphate[96] and other nucleotides[97].



Equation 19 X = O, S, Se

Previously in our lab, N,N'-dialkyl-N''-(2-chloroethyl)phosphotriamidates were synthesized[92, 71] and their reactivities were studied. They can undergo base promoted 1,3- and 1,5- cyclizations, yielding N-phosphorylated aziridines and 1,3,2-diazaphospholidines (Equation 20).



R= Ph, Me, CH_2Ph

Equation 20

In this part of the work, we decided to develop some preparative methods for new phosphoramidate systems. Our aim was to make available some phosphotriamides and diamidoesters contianing the $NHCH_2CH_2Cl$ and the NHAr groups as the phosphoramidate functions. The presence of the relatively acidic N-H hydrogens and the β-chloroethyl groups was expected to use those compounds as substrates for the base-promoted 1,5-cyclization leading to cyclic (or di, as well as tricyclic) phosphoramidate derivatives. We intended to follow the effect

of the structural changes by ^{31}P NMR spectroscopy in order to determine the relationship between the cyclic *vs* noncyclic structure of a phosphoramidate and the corresponding shielding parameters of the phosphorus atom. At the same time it was hoped that some of the new amidates would exhibit interesting hydrogen bonding and other properties, both in solution and in the solid state.

Results and discussion

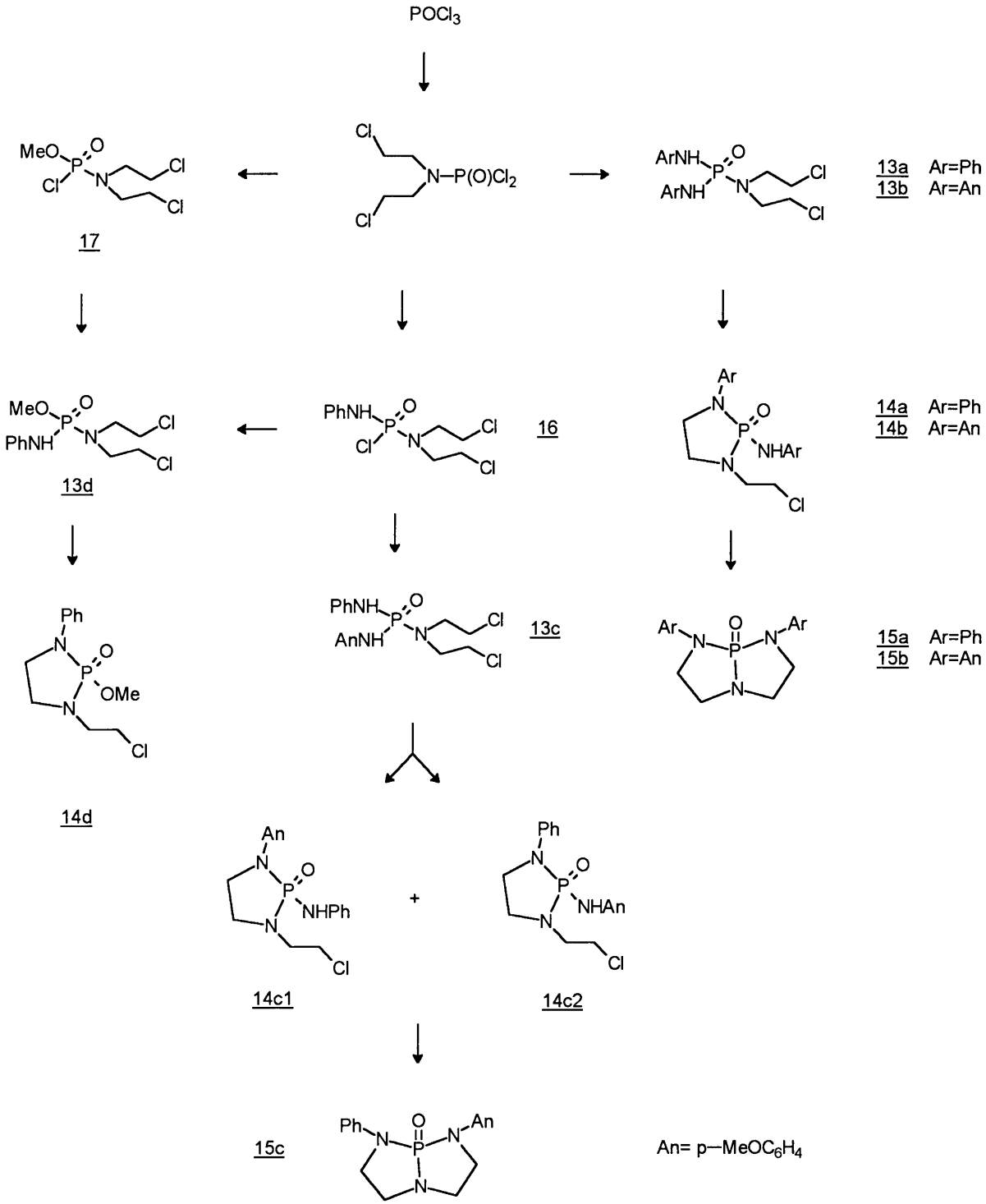
1. Preparation

From Scheme 16, the synthesis of the phosphotriamidates (and diamidoesters) which carry the N-(2-chloroethyl) substituent as an essential structural feature, is based on phosphoryl chloride as a common starting material. We have found that the sequence of the nucleophilic reagents introduced at phosphorus is very important. We found that the N, N-bis(2-chloroethyl) amino group should be introduced at the first step for the following reasons. 1. Phosphoryl chloride reacts with $H_2N^+(CH_2CH_2Cl)_2Cl^-$ to give high yield (100%) of $Cl_2P(O)N(CH_2CH_2Cl)_2$. 2. The low reactivity of $H_2N^+(CH_2CH_2Cl)_2Cl^-$ makes it difficult to replace the chlorine atom from a R_2N-$POCl_2$ or RO-$POCl_2$ (R=Me) intermediates, and such a reaction gives low yields[80] or a decomposed product[98].

The phosphoramidates prepared in this work can be classified into three types:

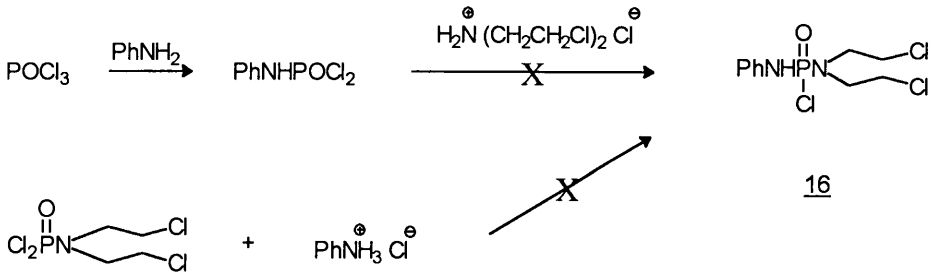
1. Noncyclic phosphoramidates (13a, 13b, 13c, 13d)
2. Monocyclic phosphoramidates (14a, 14b, 14c, 14d)
3. Dicyclic phosphotriamidates (15a, 15b, 15c)

The preparation of 13 involved three subsequent nucleophilic displacements at phosphoryl center. Several ways were tried to make the intermediate, N,N-bis(2-chloroethyl)-N'-phenyldiamido phosphochloridate 16, shown in Equation 21, but these approaches have failed.



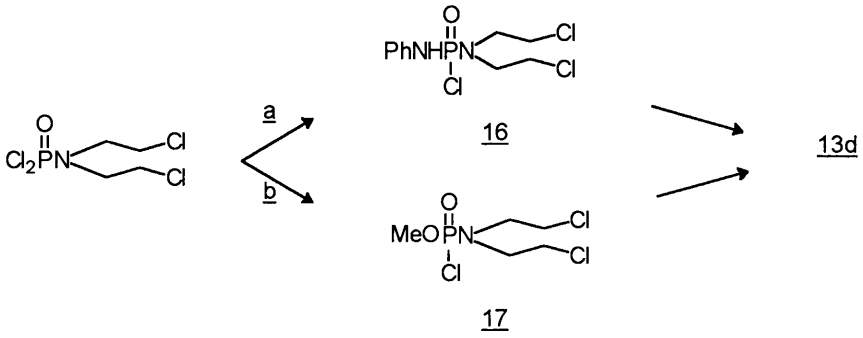
Preparation of Phosphotriamidates and Phosphodiamidoesters

Scheme 16



Equation 21

After the first replacement of chlorine by N-bis(2-chloroethyl) group, the second and third replacements of chlorines are relatively difficult. The reason is that chlorine is a strong electron-withdrawing atom (stronger than nitrogen), so decreasing the number of chlorine atoms will drastically reduce the reactivity of the phosphoryl center.

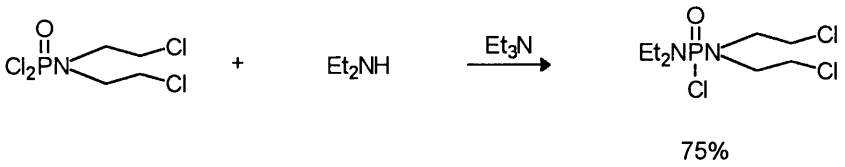


Equation 22

We used two routes to prepare compound 13d (Equation 22); and both of them gave good yields.

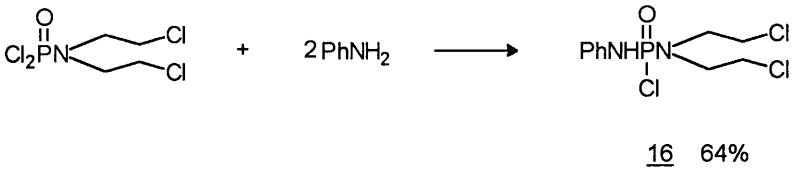
It should be noticed that a route analogous to route a (Equation 22) was once used to synthesize

compound 1 (in Chapter 1), methyl N,N-bis(2-chloroethyl)-N',N'-diehtyl phosphodiamidate. However, there is still a difference between the preparation of these two compounds. In the synthesis of N,N-bis(2-chloroethyl)-N',N'-diethyldiamidophosphorochloridate, triethylamine was used (Equation 23), and 75% yield was obtained.



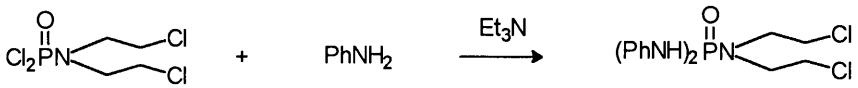
Equation 23

In the case 13d, on the other hand, the intermediate 16 was prepared in the absence of triethylamine. Instead, aniline was used as the base, 64% yield of the product was obtained (Equation 24).



Equation 24

Compound 16 couldn't be obtained when triethylamine was used as a base. Instead, nucleophilic displacement occured twice to give the triamidate product 13a (Equation 25). We can conclude therefore that aniline is also a suitable base to be used to make intermedate 16, as shown in Equation 24.



13a

Equation 25

Route b (Equation 22) was also applied to the synthesis of compound 1 (in Chapter 1)[99]; but the third nucleophilic displacement of chlorine of methyl N,N-bis(2-chloroethyl)amino phosphochloridate by diethylamine gave a very low yield.

A route analogous to route b (Equation 22) was also used by Orji and coworkers to prepare phenyl N,N-bis(2-chloroethyl)-N'-phenyl phosphodiamidate [100].

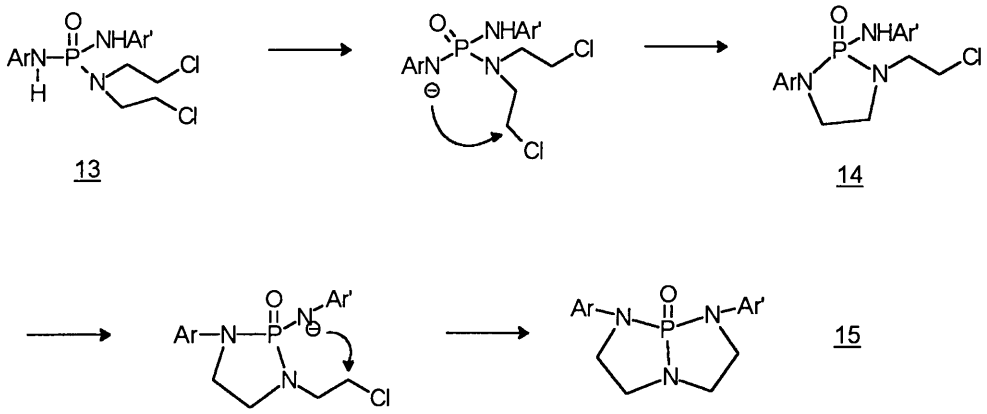
Since anisidine is a stronger base (and nucleophile) than aniline, it was observed that the reaction time necessary for the preparation of 13b (48 h) was shorter than that of 13a (120 h, Scheme 16). At the same time, p-nitroaniline did not react with N,N-bis(2-chloroethyl)amido phosphorodichloridate.

For this reason, anisidine was used at the last step to displace the last chlorine at phosphorus center in the preparation of 13c (Scheme 16). When four equivalents of $ArNH_2$ were used to prepare compounds 13a, 13b (Scheme 16), only one nucleophilic replacement took place giving the phosphochloridate product (Equation 26). Another base (Et_3N) was necessary to complete the reaction.

16

Equation 26

There are two cyclizations involved in the reaction course proceeding from 13 to 14 , and to 15 (Scheme 16). As shown in Scheme 17, the 1,5-cyclization occurs via deprotonation of the amide hydrogen and the intramolecular displacement of the β-chloro atom of the nitrogen mustard function (only this cyclization can occur, while in the reaction described in Equation 20[92, 71], both, 1,3- and 1,5-cyclizations could occur at the same time).



Scheme 17

There could be three factors that affect the cyclization reaction:

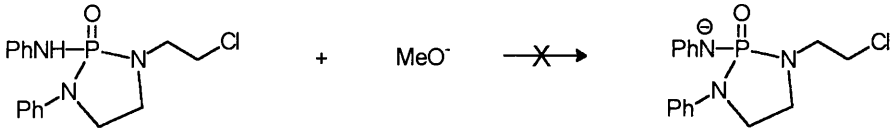
1. NH- group of 14 is less acidic than that of 13, so a stronger base is needed for the second cyclization.

2. The cyclization will lead to the N-P-N bond angles change; the biger the angle changes, the more difficult the reaction should be.

3. The choice of the base is essential for the success of the reaction.

Several experiments were carried out to choose the suitable base. For example, sodium methoxide did not cause the second cyclization of 14a, probably because the corresponding N-

anion was not formed (Equation 27) .



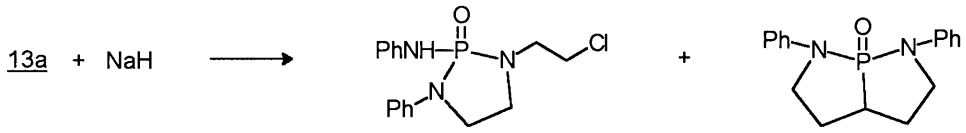
14a

Equation 27

On the other hand, sodium hydride as a strong base, allowed the reaction to go further and generated the final product 15. According to the condition (1), the first cyclization can take place much easier than the second. Indeed, after 18 h, another 6 equivs. of NaH was added to the reaction mixture, the yield of the final product 15a was 100% (see Equation 28).

In conclusion, for the first cyclization (from a amide of type 13 to type 14), sodium methoxide was a suitable base, and quantitative conversion could be obtained without by-products formed. The strong base NaH was however necessary for the second cyclization, and also quantitative conversion could be achieved.

In Equation 28, proportions of three amides involved are given as a function of the amount of NaH used.



14a 15a

	mole ratio of 13a/NaH	time	substrate	14a	15a
1.	1:2	18h	64%	36%	0
2.	1:4	18h	0	10%	90%

Equation 28

2. The NMR and MS characteristics of the prepared compounds.

^{31}P NMR chemical shifts of phosphorus compounds are dependent on intrinsic and extrinsic factors. The intrinsic factors include : bond angle effects [103, 104] and stereoelectronic effects[105]. The extrinsic factors include: solvent[106], and temperature[107]. In our cases, three types of phosphoramidates (13, 14, 15) are characterized by specific ranges of ^{31}P NMR chemical shift values. Their ^{31}P NMR chemical shift values are represented in Table 2. The following conclusions can be drawn :

1. The phosphodiamidoesters (13d, 14d, methyl ester) have higher chemical shift values than the corresponding phosphotriamidates. The strong electron-withdrawing effect of substituents Cl and MeO cause the ^{31}P NMR signal move to low field.

Table 2 Chemical shift (δ, ppm) in the ^{31}P NMR Spectra (in $CDCl_3$) of Phosphoramidates and Diamidoester 13, 14, 15.

Compound	δ
16	14.7
17	17.9
13d	10.4
13a	5.1
13b	6.3
13c	6.0
14d	18.9
14a	13.4
14b	14.7
14c1	14.7
14c2	13.7
15a	33.5
15b	34.1
15c	33.9

2. The average δ_p values for 13a-13c, 14a-14c and 15a-15c are : 5.8 ± 0.6; 14.0 ± 0.7; 33.8 ± 0.3, respectively.

3. It should be noticed, by the ^{31}P NMR chemical shift differences of type 13, 14, 15, the regular deshielding effect can be observed upon each cyclization (first, second). The difference of ^{31}P NMR chemical shift between group 13 and group 14 is average +8.3 ± 0.3 ppm (for the first cyclization), between group 14 and group 15 is +19.7 ± 0.5 ppm (for the second cyclization). Undoubtedly, the differences of chemical shift reflect the changes in N-P-N bond angles, which in turn effect the shielding parameters of the phosphorus nuclei. Similar investigations were carried out for the O-P-O bond angles in phosphates[102] and S-P-S bond angles in cyclic 1, 3, 2-

dithiaphospha compounds[103]. For the latter system, the correlations obtained is shown in Figure 8. The result is the chemical shift of ^{31}P NMR will move to high field when the bond angles decrease.

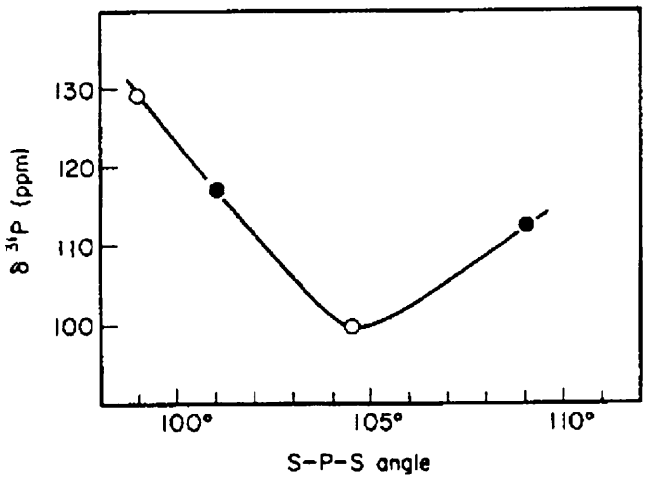
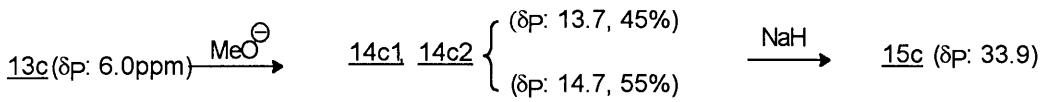


Figure 8 Variation of $\delta\,^{31}P$ (given with positive values downfield from H_3PO_4) as a function of the S-P-S bond angle in 2-thioxo-2-t-butyl-1,3,2-dithiophospha compounds: • = measured S-P-S bond angles; o = estimated values.

A single crystal of compound 15a, suitable for x-ray diffraction was obtained, so its crystal and molecular structure could be determined. In Chapter 3 we discuss the molecular structure of another triamidate (monocyclic), for which we also observe the ^{31}P NMR signal shifting upfield with the decrease of the N-P-N angle. The correlation of the NMR shielding of the ^{31}P nucleus and the molecular parameters will be discussed in more details in Chapter 4.

Phosphotriamidate 13c contains two types of arylamino group (PhNH and p-$CH_3OC_6H_4NH$), what should lead to two competing cyclization reactions at the first cyclization of 13c and should give two isomeric products (14c1, 14c2). Then at the second cyclization, both isomers should converge into one product 15c (Equation 29). The experiment gave the results shown in

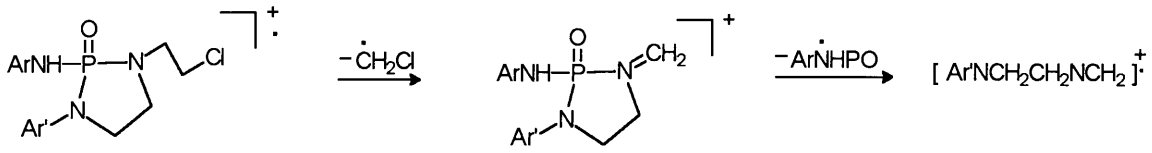
Equation 29.



Equation 29

As far as the intermediate products 14c1 and 14c2 are concerned, only one of them (δ_p=14.7ppm) was isolated as a pure material. The structural assignment of that product was not easy since the spectroscopic (^{31}P, ^{1}H, ^{13}C NMR) characteristics of 14c1, 14c2 compounds should be very similar. At first, we tried to use NMR spectroscopy to identify the pure product by ^{1}H NMR, using NOE and HETCOR experiments. However it didn't work well, and no firm conclusion could be reached. On the other side, the NH proton signal of ArNH was observed at δ_H : 6.29 ppm (d, J_{HP}=8.1 Hz). For 14a (similar exocyclic PhNH group) the corresponding value is δ_H : 6.07 ppm (d, J_{HP}=7.2), while for 14b (exocyclic AnNH group) the value is δ_H : 5.85 ppm (d, J_{HP}=7.9 Hz). So again, it was not possible to identify the isolated product 14c from the signal of the NH proton. The final identification of that pure product was based on its mass spectrum. Like other 1,3,2-diazaphospholidines, the molecular ion signal (M^+, m/z=365, 367; 6.3%, 2.3%) was observed. The typical fragmentation signal (M^+-CH_2Cl) of N-phosphorylated nitrogen mustards [83] at m/z=316 was also detected. The key signal was however the base peak observed at m/z=177. This signal corresponds to the formula $C_{10}H_{13}N_2O$ and can be envisaged as a result of the loss of CH_2Cl, followed by the fission of both endocyclic P-N bonds and yielding a fragment incorporating the N-p-anisyl group in an ethylenediamine system (Scheme 18). The similar fragmentation was also detected in its analogues 14a, 14b, 14d. On the other side, the

corresponding signal at m/z=147 ($C_9H_{11}N_2$), expected from the analogous fragmentation of 14c2, was absent in the mass spectrum of the purified product.

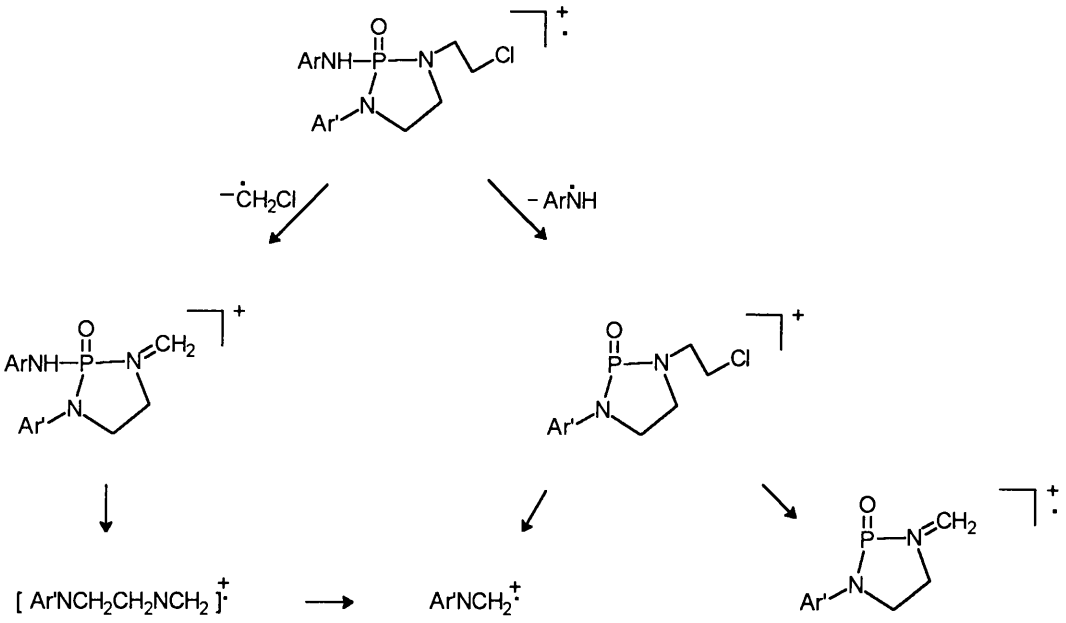


1. Ar=Ph Ar'=p-$MeOC_6H_4$ m/z: 177 14c1

2. Ar=p-$MeOC_6H_4$ Ar=Ph m/z: 147 14c2

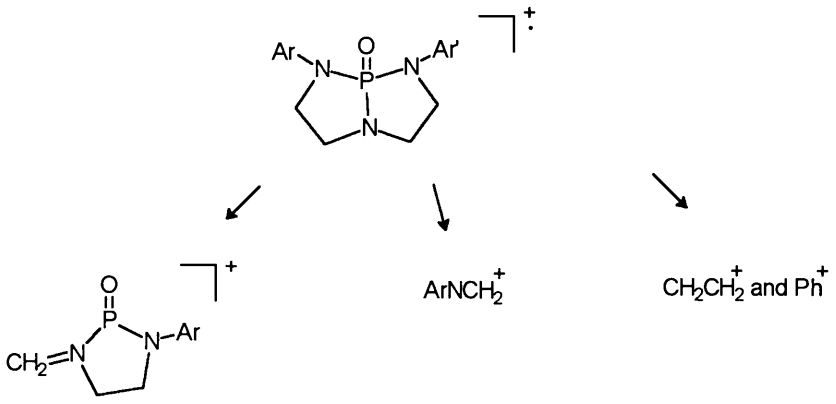
Scheme 18

For the noncyclic compounds 13a, 13b, the mass spectra are complex. Molecular ion peak was not observed, and the attempted resolution of the spectra was difficult. Compounds 13d, 14, 15 give, on the other hand, relatively simple mass spectra. The major electron impact-induced fragmentations of the compounds of type 14 and type 15 are represented in Scheme 19 and 20. The MS of products 14 contain several typical peaks. In addition to the fragment M^+-CH_2Cl ion, such as M^+-ArNH, $ArNHCH_2CH_2NCH_2$, and $ArNCH_2$ can always be observed. In MS of 15, the typical fragments are : M^+-$ArNCH_2$, $ArNCH_2$. For both systems, 14 and 15, the molecular ion signals can be observed and sometimes they represent the base peaks.



Ar, Ar' = Ph, p-MeOC$_6$H$_4$

Scheme 19 Major fragmentations of compounds 14



Ar, Ar' = Ph, p-MeOC$_6$H$_4$

Scheme 20 Major fragmentations of 15

15a is a highly symmetric molecule. The ^1H NMR signals of the endocyclic protons are in agreement with the structure (Figure 9). Figure 9 shows that there are only four kinds of protons in the molecule. By using NOE and HETCOR techniques, they were identified to be H_a, H_b, H_c, H_d, respectively. Normally, this type of ring protons of 5-membered ring is analysed as ABCDX (X=phosphorus) system. Since the group of signals of H_d is symmetric, we tried to resolve it by the first-order rules. The four coupling constants were determined to have the following values (Hz): 14.6, 11.3, 6.4, and 3.1. Following the large body of reported values, the first two constants were assigned as the $^2J_{dc}$ (geminal), and the $^3J_{dp}$ couplings, respectively. The two "small" J values (6.4 and 3.1Hz) result from two vicinal couplings with the methylene hydrogens of the adjacent carbon. The values indicate that the 1,3,7-diazaphospholidine ring is significantly twisted. From Karplus equation it follows that for the torsion angle of ca. 50^0, a $^3J_{HH}$ of ca. 3Hz should be expected. We identified therefore the smallest J value as the $^3J_{da}$ constant, between the two cis hydrogens of the ring. The twist of ca. 50^0 gives the value of the torsion angle between H_d and H_b (trans) of ca. 170^0, for which a J≈8.9Hz should be expected. We believed that the last coupling constant (6.4Hz) corresponds reasonably well to that value, and that it represents the remaining spin-spin coupling in the molecule of 15a (J_{db}, trans). The numbering of protons discussed above is given in Figure 9. This result is very close to the data of 2-oxo-2-R-1,3,6,2-trithiophosphocane[103]. As seen in Figure 9, only the signal for H_d is symmetric, while for H_a, H_b, H_c the corresponding multiplets are not. The possible reason for that is that H_d is far away from the phenyl and the >P=O groups. H_a, H_b, H_c are affected by the deshielding effect of phenyl and >P=O groups, what makes the shapes of their ^1H NMR signals to be distorted and more complex. No other model was tried to resolve analogous signals. Very similar results were also obtained for 15b.

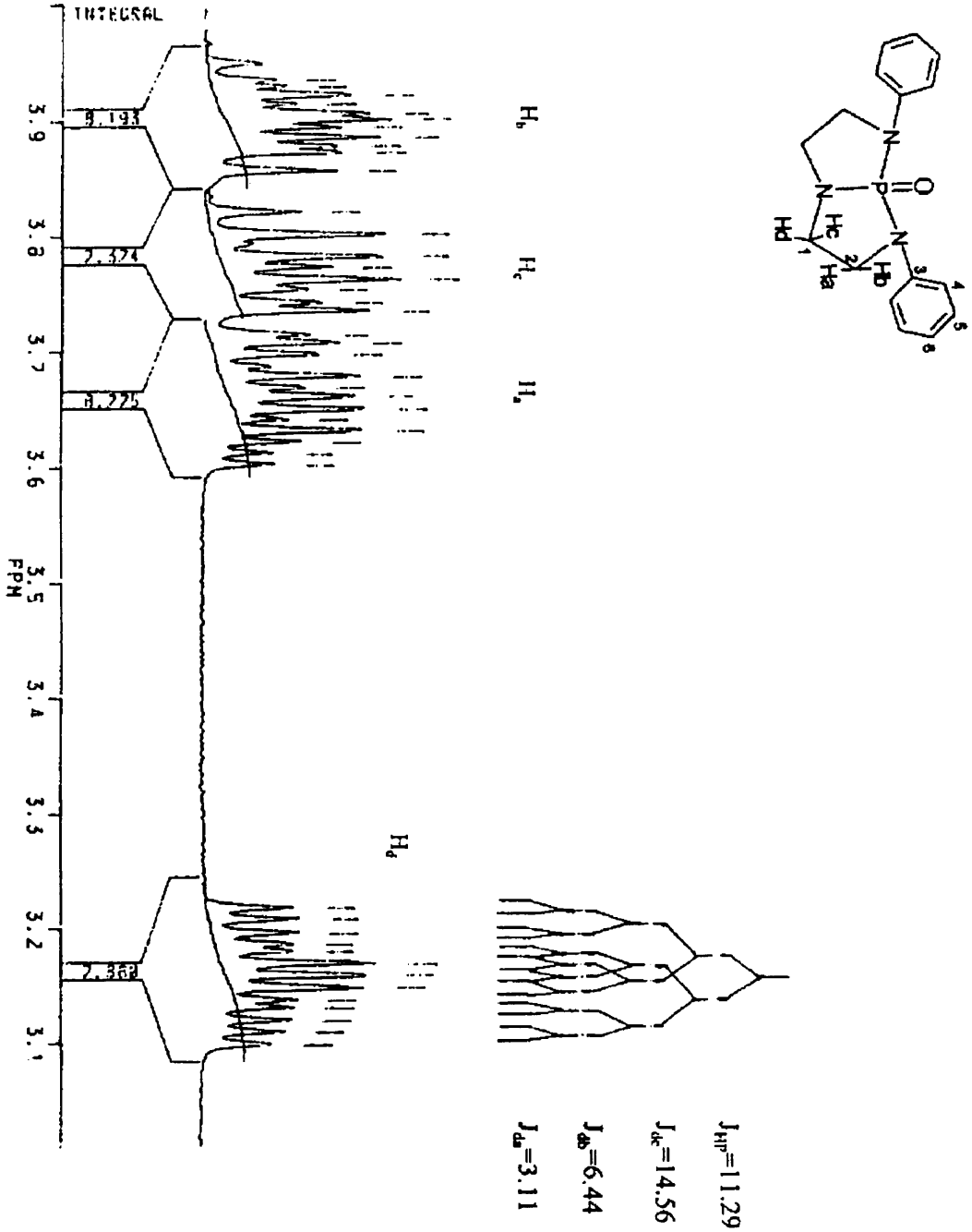


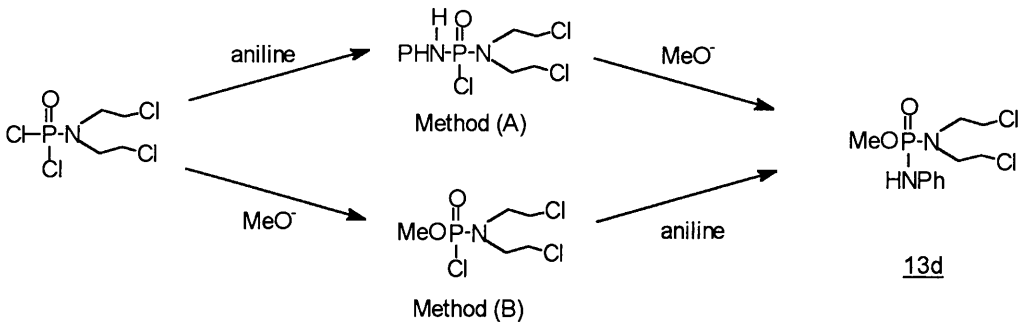
Figure 9 Part of 300MHz ^{1}H NMR spectrum of 15a in $CDCl_3$ solution

Experimental

General see Chapter 1

Preparation

Methyl N-phenyl-N',N'-bis(2-chloroethyl)phosphodiamidate (13d)



1. Method (A)

N-Phenyl-N',N'-bis(2-chloroethyl)chlorophosphodiamidate (16)

N,N-bis(2-chloroethyl)amido phosphoryl dichloride (5.00 g, 0.019 mol) and aniline (3.60g, 0.039mol) were dissolved in 80 ml dichloromethane at room temperature. After 240 h, the mixture was filtered , and the solvent of the filtrate was removed under reduced pressure. The pure product was obtained by recrystallization from benzene/hexane. Colorless solid was obtained, mp 77.5-78.5 ^{0}C, yield 64% (3.89g).

NMR : ($CDCl_3$, ppm)

^{31}P : 14.8

^{1}H : 3.40-3.60 (8H, m, 2 x CH_2CH_2Cl)
6.25 (1H, d, J_{HP}=13.20, N-H)
6.94 (1H, t, J_{HH}=7.26, para-H of Ph)

	6.99	(2H, d, J_{HH}=7.68, 2 x ortho-H of Ph)
	7.14	(2H, t, J_{HH}=7.95, 2 x meta-H of Ph)
^{13}C :	41.4	(CH_2Cl, t, J_{HC}=151.7)
	49.8	(NCH_2, t, J_{HC}=142.1)
	119.8	(meta-C of Ph, dd, J_{HC}=160.3, J_{CP}=7.2)
	123.5	(para-C of Ph, d, J_{HC}=163.5)
	129.5	(ortho-C of Ph, d, J_{HC}=161.8)
	138.1	(N-C of Ph, s)

Ms : m/z 320, 318, 316, 314 (M^+, 0.2, 6.9, 21.2, 22.3 %)
269, 267, 265 (M^+ -CH_2Cl, 8.8, 52.1, 82.3%)
231, 229 (M^+ -CH_2Cl-HCl, 8.2, 28.3%)
92 ($PhNH^+$, 100%)

Anal. for $C_{10}H_{14}N_2Cl_3PO$ (%)

	C	H	N
calcd. :	38.03	4.44	8.87
found :	38.63	4.48	8.83

Methyl N-phenyl-N',N'-bis-(2-chloroethyl)phosphodiamidate (13d)

N-phenyl-N',N'-bis-(2-chloroethyl)phosphodiamidochloridate (3.00 g, 9.54 mmol) was dissolved in methanol (10 ml). The solution of one mol-equivalent of sodium methoxide in methanol (10 ml) was added dropwise with stirring to the first solution at 0-5 ^{0}C. The mixture was then kept at room temperature for 2 h, filtered and evaporated under reduced pressure. The residue was dissolved in chloroform (10 ml), washed with water (2 x 5 ml). Chloroform layer was dried by anhydrous $MgSO_4$. The solvent was then evaporated under reduced pressure. Pure product was obtained by column chromatography (chloroform/ benzene, 2 : 1), colorless crystals (2.22 g, 75%), mp 80-81.5 ^{0}C.

2. Method (B)

Methyl N,N-bis(2-chloroethyl)chlorophosphoramidate (17)

0.27 g (0.012 mol) of sodium was dissolved in 10 ml methanol, the solution was added dropwise to N,N-bis(2-chloroethyl)amido phosphoryl dichloride (3.04 g, 0.012 mol) dissolved in 10 ml methanol at 0-5 ^{0}C; the mixture was kept at the same temperature for 0.5 h, then it was allowed to reach RT. After 2 h, the reaction was stopped, and the mixture was filtered. The solvent was removed under reduced pressure. The product was recovered by adding petroleum ether(40-60 ^{0}C)(100 ml x 4) to the residue and decantation. The combined petroleum ether solution was removed under reduced pressure. Crude product was obtained, 95% yield (2.80g), light yellow oil.

NMR : ($CDCl_3$, ppm)

^{31}P :	17.9	
^{1}H :	3.43	(4H, dq, J_{HH}=7.27, J_{HP}=10.60, 2 x NCH_2)
	3.56	(4H, t, J_{HH}=7.08, 2 x CH_2Cl)
	3.85	(3H, d, J_{HP}=13.70, MeO)
^{13}C :	41.4	(CH_2Cl, t, J_{HC}=151.6)
	49.8	(CH_2N, dt, J_{HC}=142.5, J_{CP}=3.78)
	54.4	(MeO, dq, J_{HC}=149.6, J_{CP}=6.23)

Methyl N-phenyl-N',N'-bis(2-chloroethyl)phosphodiamidate (13d)

1.00 g (3.93 mmol) of the above substrate and 0.73 g (7.93 mmol) of aniline were dissolved in 40 ml acetonitrile, the solution was stirred at 55-60 ^{0}C for 30h. Acetonitrile was removed under reduced pressure, the residue was dissolved in chloroform, and washed with water. The pure product was obtained by column chromatography (silica, benzene/ $CHCl_3$,

1 : 2), white solid, 0.92g, 75% yield. mp 80.0-81.5 ^{0}C.

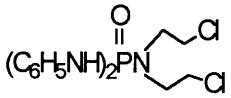
NMR : ($CDCl_3$, ppm)

^{31}P :	10.4	
^{1}H :	3.36-3.45	(4H, m, 2 x CH_2N)
	3.50	(4H, t, J_{HH}=7.06, 2 x CH_2Cl)
	3.76	(3H, d, J_{HP}=11.37, MeO)
	5.43	(1H, d, J_{HP}=8.35, N-H)
	6.96-6.99	(3H, m, para- and ortho-H of Ph)
	7.23	(2H, t, J_{HH}=7.84, meta-H of Ph)
^{13}C :	42.2	(CH_2Cl, t, J_{HC}=151.9)
	49.6	(CH_2N, dt, J_{CP}=4.2, J_{HC}=137.4)
	52.4	(CH_3O, q, J_{HC}=147.8)
	117.8	(ortho-C of Ph, dd, J_{CP}=6.8, J_{HC}=160.6)
	122.2	(para-C of Ph, d, J_{HC}=161.1)
	129.4	(meta-C of Ph, d, J_{HC}=160.8)
	139.4	(N-C of Ph, s)

Ms : m/z
315, 313, 311 (M^+ +1, 0.2, 1.4, 2.0%)
314, 312, 310 (M^+, 1.8, 11.3, 16.8%)
277, 275 (M^+ +1-HCl, 0.93, 2.6%)
276, 274 (M^+ -HCl, 2.4, 6.7%)
264, 262 (M^+ +1-CH_2Cl, 4.2, 13.1%)
263, 261 (M^+, 33.0, 100%)
226, 225 (M^+-HCl-CH_2Cl, 2.3, 19.1%)
170 (M^+-N(CH_2CH_2Cl), 59.3%)
106 ($PHNCH_2$, 21.6%)
92 (93%)

Anal. for $C_{11}H_{17}N_2O_2PCl_2$ (%)

	C	H	N
calcd. :	42.44	5.47	9.00
found :	42.65	5.49	8.92



N,N-bis(2-chloroethyl)-N',N"- diphenylphosphotriamidate (13a)

2.64 g (10.2 mmol) of N,N-bis(2-chloroethyl)amidophosphodichloridate was dissolved in 10 ml dichloromethane. 1.90 g (20.6 mmol) of aniline and 2.09 g (20.7 mmol) of triethylamine were dissolved in 20 ml dichloromethane. The first solution was added dropwise with stirring into the latter at -20 ^{0}C. After 1 h, it was allowed to warm up to RT.

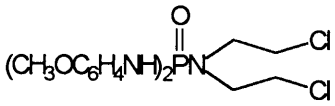
After 120 h, the solvent was removed under reduced pressure. The residue was passed through a chromatographic column (silica, chloroform as eluent). The obtained solid was washed with chloroform. White solid was obtained (2.81 g, 74%), mp 170.0-171.0 ^{0}C.

NMR : ($CDCl_3$, ppm)

^{31}P :	5.1	
^{1}H :	3.53	(4H, dt, J_{HH}=5.68, J_{HP}=11.36, 2 x CH_2N)
	3.67	(4H, t, J_{HH}=5.49, 2 x CH_2Cl)
	5.33	(2H, d, J_{HP}=8.85, 2 x H-N)
	6.93-7.00	(m, 2H of Ph)
	7.08-7.11	(m, 4H of Ph)
	7.20-7.26	(m, 4H of Ph)
^{13}C :	43.3	(CH_2Cl, t, J_{HC}=153.0)
	50.6	(NCH_2, t, J_{HC}=139.8)
	119.0	(ortho-Cof Ph, dd, J_{HC}=159.7, J_{PC}=6.3)
	122.0	(para-C of Ph, d, J_{HC} =158.2)
	130.0	(meta-C of Ph, dd, J_{HC}=158.0, J_{PC}=7.0)
	143.0	(N-C of Ph, s)

Anal. for $C_{16}H_{20}N_3Cl_2OP$ (%)

	C	H	N
calcd. :	51.60	5.38	11.29
found :	51.13	5.42	11.11



N,N'-di(p-methoxyphenyl)-N",N"-bis(2-chloroethyl)phosphotriamidate (13b)

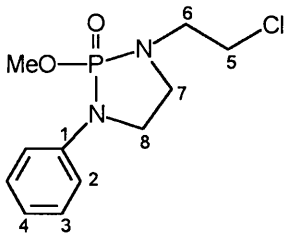
This was prepared as described for compound 13a. Pure product was obtained by column chromatography (silica, chloroform), in 60% yield, mp 156.0-157.0 ^{0}C.

NMR : ($CDCl_3$, ppm)

^{31}P :	6.25	
^{1}H :	3.48	(4H, dt, J_{HH}=5.77, J_{HP}=11.25, 2 x CH_2N)
	3.64	(4H, t, J_{HH}=5.86, 2 x CH_2Cl)
	3.72	(6H, s, 2 x MeO)
	5.15	(2H, d, J_{HP}=8.98, 2 x N-H)
	6.78	(4H, d, J_{HH}=8.94,4 x meta-H of Ph)
	7.16	(4H, d, J_{HH}=8.92, 4 x ortho-H of Ph)
^{13}C :	43.1	(CH_2Cl, t, J_{HC}=153.1)
	50.4	(NCH_2, t, J_{HC}=141.4)
	55.6	(MeO, q, J_{HC}=143.5)
	115.0	(meta-C of Ph, dd, J_{CP}=9.1, J_{HC}=158.2)
	120.6	(ortho-C of Ph, d, J_{HC}=158.9)
	135.5	(N-C of Ph, s)
	155.5	(O-C of Ph, s)

Anal. for $C_{18}H_{24}Cl_2N_3O_3P$ (%)

	C	H	N
calcd. :	50.00	5.56	9.72
found :	49.18	5.53	9.37



1-Phenyl-2-methoxy-2-oxo-3-(2-chloroethyl)-1,3,2-diazaphospholidine (14d)

0.10 g (0.32 mmol) of Methyl N-phenyl-N'N'-bis(2-chloroethyl)phosphodiamidate (13d), 0.016g (0.67 mmol) of sodium hydride, and 0.011 g (0.034 mmol) of tetrabutylammonium bromide (TBAB) were dissolved in 10 ml benzene at room temperature, with vigorous stirring. After 15 minutes, the conversion reached 100% as confirmed by ^{31}P NMR spectroscopy. The mixture was filtered, the benzene solution was washed with water, until aqueous layer was neutral. Benzene solution was dried by anhydrous Na_2SO_4, the solvent was removed under reduced pressure, yielding crude product (0.08 g, 85%). The pure product was obtained by recrystallization from benzene / hexane, mp 114.0-115.0 ^{0}C.

NMR : ($CDCl_3$, ppm)

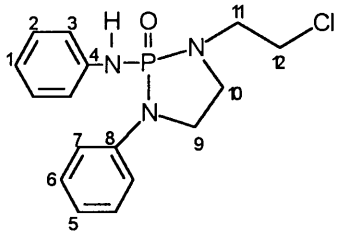
^{31}P :	18.9	
^1H :	3.20-3.40	(2H, m, NCH_2 of NCH_2CH_2Cl)
	3.57	(3H, d, J_{HP}=12.8, CH_3O)
	3.40-3.70	(6H, m, CH_2Cl +2 x endo-CH_2N)
	6.97	(1H, t, J_{HH}=7.40, para-H of phenyl)
	7.13	(2H, d, J_{HH}=8.43, meta-H of phenyl)
	7.29	(2H, t, J_{HH}=8.39, ortho-H of phenyl)
^{13}C :	42.7	(CH_2Cl, t, J_{HC}=150.7)
	43.2	(C_8, dt, J_{PC}=14.3,J_{HC}=142.5)
	44.5	(C_7, dt, J_{PC}=12.0, J_{HC}=146.4)
	47.4	(C_6, dt, J_{PC}=4.75, J_{HC}=139.9)
	58.3	(CH_3O, dq, J_{PC}=7.25, J_{HC}=146.7)

115.7 (C_2, dd, J_{PC}=4.68, J_{HC}=166.2)
121.7 (C_4, d, J_{HC}=159.5)
129.4 (C_3, d, J_{HC}=159.4)
141.0 (C_1, s)

Ms : m/z 277, 275 (M^+ +1, 2.2, 7.0%)
276, 274 (M^+ , 15.7, 45.9%)
226, 225 (M^+ -CH_2Cl, 18.5, 100%)
196, 195 (M^+ -79, 7.4, 57.7%)
170, 169 (M^+ -105, 11.5, 3.2%)
147 ($PhNCH_2CH_2NCH_2$, 4.2%)
106 (17.7%) 105 (50.7%)
104 (19.9%) 77 (ph, 19.8%)

Anal. for $C_{11}H_{16}O_2PN_2Cl$ (%)

	C	H	N
calcd. :	48.09	5.83	10.20
found :	48.58	5.87	10.17



1-Phenyl-2-phenylamino-2-oxo-3-(2-chloroethyl)-1,3,2-diazaphospholidine (14a)

This was prepared as described for compound 14c (base : MeONa). Pure product was obtained by recrystallization from benzene, in 95% yield, mp 190.0-191.5 ^{0}C.

NMR : ($CDCl_3$, ppm)

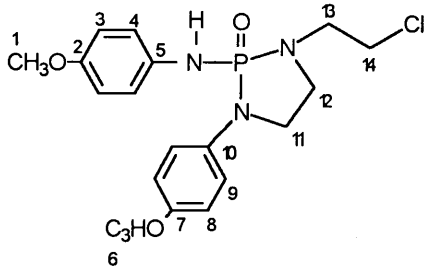
^{31}P : 13.4

^1H :	3.26-3.40	(2H, m, NCH_2 of NCH_2CH_2Cl)
	3.56	(2H, t, J_{HH}=6.70, CH_2Cl)
	3.50-3.65	(4H, m, 2 x H_{10} + CH_2Cl)
	3.68-3.81	(2H, m, 2 x H_9)
	6.07	(1H, d, J_{HP}=7.23, N-H)
	6.83	(2H, d, J_{HH}=7.84, 2 x H_3)
	7.12	(2H, t, J_{HH}=7.79, 2 x H_2)
	7.16-7.23	(6H, m, 2 x H_6 + 2 x H_7 + H_1 +H_5)
^{13}C :	42.5	(CH_2Cl, t, J_{HC}=151.2)
	43.5	(C_9, dt, J_{HC}=140.8, J_{PC}=13.4)
	44.2	(C_{10}, dt, J_{HC}=141.7, J_{PC}=12.3)
	46.5	(NCH_2 of CH_2CH_2Cl, t, J_{HC}=142.7)
	116.1	(C_7, dd, J_{HC}=159.2, Jpc=4.7)
	118.9	(C_3, dd, J_{HC}=157.3, Jpc=6.3)
	122.3	(C_5, d, J_{HC}=156.1)
	121.6	(C_1, d, J_{HC}=159.8)
	129.2-129.1	(C_2+C_6, dd, J_{HC}=159.7)
	135.7	(C_4, s)
	139.7	(C_8, s)

MS : m/z 338, 336 (M^+ +1, 9.4, 28.3%)
337 (M^+, 22.2, 65.3%)
287, 286 (M^+-CH_2Cl, 35.2, 100%)
245, 243 (M^+-$PhNH_2$, 7.5, 21.9%)
181 (29.1%)
147 ($PhNCH_2CH_2NCH_2$, 7.2%)
106 (44.1%)
104 (27.4%)

Anal. for $C_{16}H_{19}N_3OClP$ (%)

	C	H	N
calcd. :	57.23	5.66	12.51
found :	57.71	5.75	12.29



1-(4-methoxyphenyl)-2-[(4-methoxyphenyl)amino]–2–oxo-3-(2-chloroethyl)-1,3,2-phospholidine (14b)

This was prepared as described for compound 14c. Pure product was obtained by recrystallization from benzene, in 95% yield, mp 192.5-193.5 ^{0}C.

NMR : ($CDCl_3$, ppm)

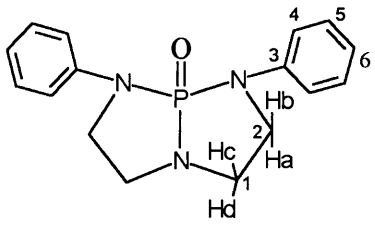
^{31}P :	14.7	
^{1}H :	3.29-3.61	(6H, m)
	3.58	(2H, t, J_{HH}=6.95, CH_2Cl)
	3.70	(3H, s, 3 x H_6)
	3.71	(3H, s, 3 x H_1)
	5.85	(1H, d, J_{HP}=7.85, N-H)
	6.67	(2H, d, J_{HH}=8.97, 2 x H_8)
	6.75	(2H, d, J_{HH}=9.11, 2 x H_3)
	6.76	(2H, d, J_{HH}=8.88, 2 x H_9)
	7.09	(2H, d, 2H, J_{HH}=8.94, 2 x H_4)
^{13}C :	42.5	(CH_2Cl, dt, J_{HC}=147.9, J_{CP}=4.2)
	44.2	(C_{11}, dt, J_{HC}=141.6, J_{CP}=12.6)
	44.5	(C_{12}, dt, J_{HC}=144.4, J_{CP}=11.4)
	46.7	(C_{13}, dt, J_{HC}=143.6, J_{CP}=5.5)
	55.5	(C_1+C_6, dq, J_{HC}=143.1)
	114.3	(C_8, dd, J_{HC}=159.4, J_{CP}=4.8)
	114.6	(C_3, dd, J_{HC}=158.0, J_{CP}=5.0)
	118.1	(C_4, dd, J_{HC}=158.6, J_{CP}=4.3)
	121.7	(C_9, dd, J_{HC}=159.2, J_{CP}=6.0)
	132.6	(C_5, s)

	134.6	(C_{10}, s)
	154.8	(C_1, s)
	155.4	(C_7, s)

MS : m/z 397, 395 (M^+, 38.0, 100%)
382, 380 (M^+ -CH_3, 1.21, 3.65%)
275, 273 (M^+ -$CH_3OC_6H_4NH$, 9.33, 28.3%)
177 ($CH_3OC_6H_4NCH_2CH_2NCH_2$, 4.2%)
136 ($CH_3OC_6H_4NHCH_2$, 19.1%)
56 (28.1%)

Anal. for $C_{18}H_{23}ClN_3O_3P$ (%)

	C	H	N
calcd. :	54.61	5.82	10.62
found :	54.77	5.82	10.51



1-Oxo-2,8-diphenyl-2,5,8-triaza-1-phosphabicyclo(3,3,0)octane (15a)

A solution of 0.015g sodium hydride (0.62 mmol) and 0.010 g tetrabutylammonium bromide (TBAB, 5 mol%) in 4 ml benzene was added dropwise to the solution of 0.1 g (0.30 mmol) of 1-phenyl-2-phenylamino-2-oxo-3-(2-chloroethyl)-1,3,2-diazaphospholidine (14a) in 4 ml benzene at RT. The reaction mixture was stirred vigorously for 6 h, when the conversion reached 100% (checked by ^{31} P NMR spectroscopy), then the mixture was filtered. The filtrate

was washed with water, untill the water was neutral. Benzene solution was dried by anhydrous Na_2SO_4. The crude product was obtained after benzene was removed (0.08 g, 90%) and was recrystallized from benzene/hexane (1:1), mp 148.0-149.5 ^{0}C.

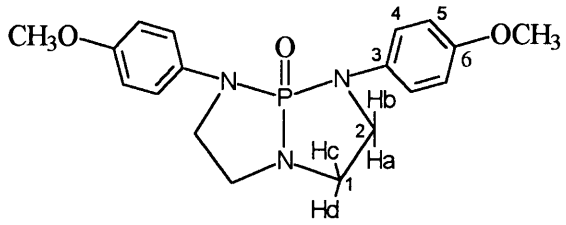
NMR : ($CDCl_3$, ppm)

^{31}P :	33.5	
^{1}H :	3.15	(2H, 13peaks, J_{HP}=11.29, J_{dc}=14.56, J_{db}=6.44, J_{da}=3.11, H_d)
	3.61-3.70	(2H, m, 2 x H_a)
	3.71-3.78	(2H, m, 2 x H_c)
	3.86-3.92	(2H, m, 2 x H_b)
	6.90-6.95	(2H, m, 2 x H_6)
	7.1-7.34	(8H, m, 4 x H_4+ 4 x H_5)
^{13}C :	48.0	(C_1, dt, J_{HC}=145.0, J_{PC}=7.02)
	49.0	(C_2, dt, J_{HC}=142.3, J_{PC}=19.5)
	118.9	(C_4, dd, J_{HC}=159.3, J_{PC}=3.40)
	122.2	(C_6, d, J_{HC}=163.0)
	128.9	(C_5, dd, J_{HC}=162.0, J_{PC}=6.26)
	142.0	(C_3, s)

MS : m/z
300, 299 (M^+, 10.4, 52.2%)
195, 194 (M^+ -104, 13.1, 75.6%)
105 (M^+-194, 75.2%)
104 (M^+-195, 86.3%)
77 (Ph, 99.0%)
28 (CH_2CH_2, 100%)

Anal. for $C_{16}H_{18}N_3OP$ (%)

	C	H	N
calcd. :	62.21	6.02	14.02
found :	62.20	6.04	13.94



1-Oxo-2,8-di-(4-methoxyphenyl)-2,5,8-triaza-1-phosphabicyclo (3,3,0) octane (15b)

This was prepared as described for compound 15a. Pure product was obtained by recrystallization from benzene/ hexane. Needle-like crystals were obtained, mp 168.0-169.5 ^{0}C, yield 92%.

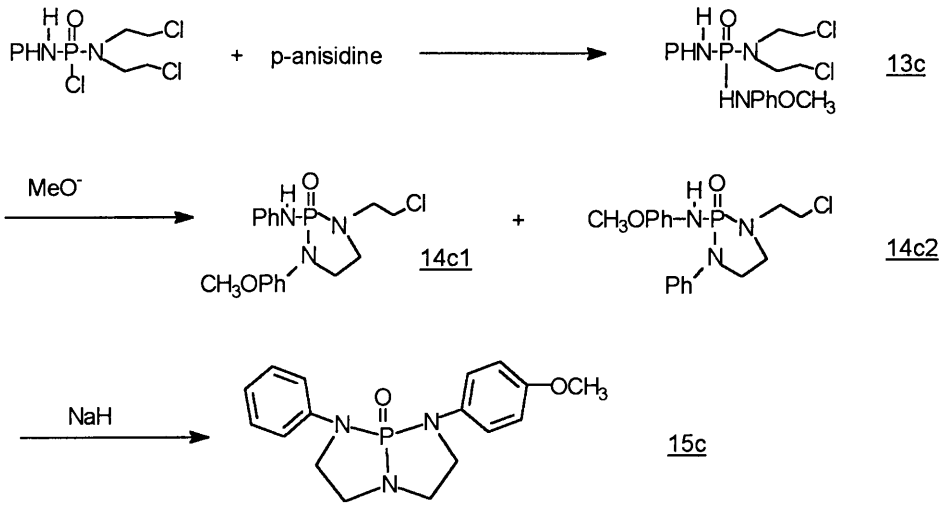
NMR : ($CDCl_3$, ppm)

^{31}P :	34.1	
^{1}H :	3.12	(2H, 13peaks, J_{HP}=11.11, J_{dc}=14.22, J_{da}=6.22, J_{db}=3.31, H_d)
	3.51-3.60	(2H, m, H_a)
	3.68-3.78	(8H, m, H_c+ 2 x CH_3O. CH_3O=3.71ppm, s)
	3.82-3.88	(2H, m, 2 x H_b)
	6.71	(4H, d, J_{HH}=8.96, 4 x H_5)
	7.05	(4H, d, J_{HH}=8.88, 4 x H_4)
^{13}C :	48.0	(C_1, dt, J_{CP}=6.9, J_{HC}=145.3)
	49.5	(C_2, dt, J_{CP}=19.8, J_{HC}=142.2)
	55.5	(MeO, q, J_{HC}=143.2)
	114.4	(C_5, dd, J_{PC}=5.2, J_{HC}=158.2)
	120.9	(C_4, d, J_{HC}=159.7)
	135.1	(C_3, s)
	155.2	(C_6, s)

MS : m/z 360, 359 (M^+, 20.8, 100%)
344 (M^+-CH_3, 12.6%)
224 (M^+-135, 77.1%)
136 (33.0%)
135 ($CH_3OC_6H_4NCH_2$, 11.0%)

Anal. for $C_{18}H_{22}N_3O_3P$ (%)

	C	H	N
calcd. :	60.17	6.13	11.70
found :	60.40	6.19	11.61



1. N-phenyl-N'-(p-methoxyphenyl)-N",N"-bis(2-chloroethyl)phosphotriamidate (13c)

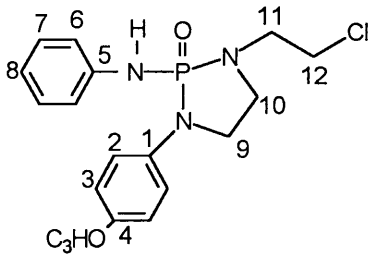
1.00 g (3.18 mmol) of N-phenyl-N',N'-bis(2-chloroethyl)diamidophosphorchloride, 0.39 g (3.20 mmol) of p-anisidine and 0.65 g (6.43 mmol) of triethylamine were dissolved in 30 ml dichloromethane. After 96 h, ^{31}P NMR spectroscopy indicated that all substrate was consumed. The mixture was washed with water (3 x 80 ml), dichloromethane solution was dried by anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was obtained which was used directly for the next step, without further purification. The crude product consisted of a single organophosphorus product (δ_P=6.0 ppm) to which a structure of N-phenyl, N'-(4-methoxyphenyl), N"-bis(2-chloroethyl) phosphotriamidate 13c was assigned.

2. 1-(4-methoxyphenyl)-2-phenylamino-2-oxo-3-(2-chloroethyl)-1,3,2-diazaphospholidine 14c1 and 1-phenyl-2-[(4-methoxyphenyl)amino]-2-oxo-3-(2-chloroethyl)-1,3,2-diazaphospholidine

14c2

1.30 g (3.25 mmol) of N-phenyl-N'-(p-methoxyphenyl)-N",N"-bis(2-chloroethyl)phospho -triamidate 13c was dissolved in 20 ml methanol, then a sodium methoxide solution (0.60 g sodium/ 30 ml methanol, 26 mmol) was added dropwise with stirring at 0-5 ^{0}C. After 1h, the mixture was allowed to warm up to room temperature. After 20 h, ^{31}P NMR analysis showed that compound 13c had disappeared, and two new compounds were formed (according to ^{31}P NMR spectroscopy); δ_P : 14.7 ppm (55%), δ_P : 13.7 ppm (45%). After filtration and evaporation of methanol, chloroform (30 ml) was added to the residue, the solution was washed with water, untill water layer was neutral. The chloroform layer was dried by anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure.

The crude product (14c1, 14c2) was recrystallized from benzene/ hexane. The obtained solid was washed several times with cold benzene untill pure product 14c1 (δ_P : 14.7 ppm, 30% yield, 0.35 g) was obtained, mp 163.5-165.0 ^{0}C.



NMR : ($CDCl_3$, ppm)

^{31}P : 14.8

^{1}H :	3.32-3.49	(3H, m, 3 x CHN)
	3.50-3.60	(5H, m, 3 x CHN + CH_2Cl)
	3.69	(3H, s, CH_3O)
	6.29	(1H, d, J_{HP}=8.12, NH)
	6.66	(2H, d, J_{HH}=8.95, 2 x m-H_{an})
	6.77	(2H, d, J_{HH}=8.64, 2 x o-H_{an})

	6.93	(1H, t, J_{HH}=6.83, H_8)
	7.21	(4H, m)
^{13}C :	42.4	(CH_2Cl, t, J_{HC}=149.0)
	43.5	(C_9, t, J_{HC}=145.0)
	44.2	(C_{10}, t, J_{HC}=144.3)
	46.6	(C_{11}, t, J_{HC}=140.0)
	55.4	(CH_3O, q, J_{HC}=143.2)
	114.3	(C_3, d, J_{HC}=159.5)
	116.0	(C_6, d, J_{HC}=158.9)
	121.4	(C_8, d, J_{HC}=158.9)
	121.9	(C_2, d, J_{HC}=159.5)
	129.1	(C_7, d, J_{HC}=159.0)
	141.2	(C_5, s)
	155.5	(C_4, s)

MS : m/z 367, 365 (M^+, 2.3, 6.3%)
177 ($C_{10}H_{13}N_2O$, 100%)

Anal. for $C_{17}H_{21}ClN_3O_2P$ (%)

	C	H	N
calcd. :	55.81	5.75	11.49
found :	55.95	5.81	11.27

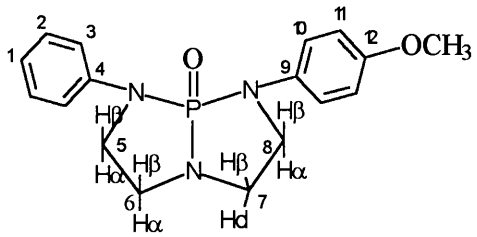
3. 1-Oxo-2-phenyl-8-(4-methoxyphenyl)-2,5,8-triaza-1-phosphabicyclo (3,3,0) octane (15c)

0.56 g of crude product (14c1, 14c2), 0.10 g sodium hydride and 0.06 g tetrabutylammonium bromide (TBAB) were dissolved in 40ml benzene with vigorous stirring at RT. After 5 h, the benzene solution was washed with water (3 x 50 ml), and dried by anhydrous Na_2SO_4. After the solvent was evaporated, the crude product (94%, 0.51 g) was obtained, and was purified by recrystallization from benzene/ hexane. mp 126.5-128.0 ^{0}C.

NMR : ($CDCl_3$, ppm)

^{31}P :	33.9	
^1H :	3.05-3.21	(2H, m, $H_{6\alpha}$+$H_{7\alpha}$)

	3.52–3.79	(7H, m, H_{5a}+H_{8a}+$H_{6\beta}$+$H_{7\beta}$+CH_3)
	3.73	(3H, s, OMe)
	3.81–3.92	(2H, m, $H_{5\beta}$+$H_{8\beta}$)
	6.75	(2H, d, J_{HH}=8.98, H_{11})
	6.89	(1H, t, J_{HH}=7.28, H_1)
	7.05	(2H, d, J_{HH}=9.57, H_3)
	7.11-7.17	(4H, m, H_{10}+H_2)
^{13}C :	47.7	(C_6, dt, J_{PC}=7.1, J_{HC}=143.9)
	48.0	(C_7, dt, J_{PC}=7.7, J_{HC}=143.5)
	48.4	(C_5, dt, J_{PC}=20.2, J_{HC}=142.1)
	50.2	(C_8, dt, J_{PC}=20.0, J_{HC}=143.0)
	55.5	(OCH_3, q, J_{HC}=143.2)
	114.4	(C_{11}, d, J_{HC}=159.0)
	118.8	(C_3, d, J_{HC}=109.5)
	121.7	(C_1, d, J_{HC}=164.1)
	122.2	(C_{10}, d, J_{HC}=159.1)
	128.9	(C2, d, J_{HC}=161.4)
	134.9	(C_9, s)
	141.9	(C_4, s)
	155.6	(C_{12}, s)



MS : m/z
330, 329 (M^+, 20.7, 100%)
315, 314 (M^+-CH_3, 1.6, 9.6%)
224 (M^+-$PhNCH_2$, 42.6%)
194 (M^+-$CH_3OC_6H_4NCH_2$, 46.1%)
135 ($CH_3OC_6H_4NCH_2$, 37.6%)
105 ($PhNCH_2$, 8.9%)

Anal. for $C_{17}H_{20}N_3O_2P$ (%)

	C	H	N
calcd. :	62.01	6.08	12.76
found :	62.03	6.12	12.57

Chapter 3

Chapter 3

Structural Studies of Phosphoramides. Conformational Preferences and Hydrogen Bonding

Introduction

Specific properties of phosphoric amides which contain chiral centers, were widely used and investigated. Many papers concerning these compounds have been published.

As early as in 1960, the oxide of 10-p-dimethylamino phosphane (Figure 10) was resolved into (+)- and (-)- forms by recrystallization of the (+)-camphor-10-sulphonates; the '(-) oxide' formed less soluble salt[108].

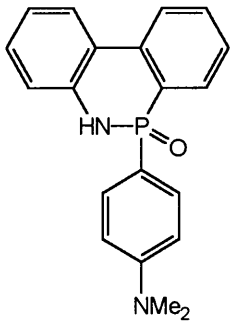


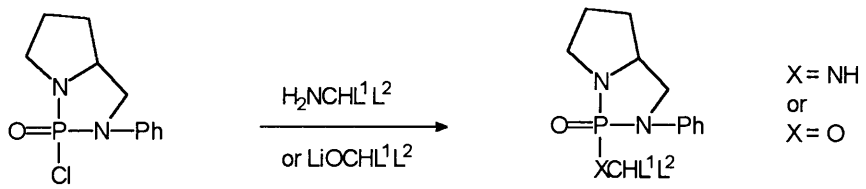
Figure 10

Recently chiral agents were used for the determination of ee of the phosphoric analogues of amino acids [109]. The method was considered as a quick and simple process, the substrate (amine) needs not be enantiomerically pure[110].

Another interesting work was published by T. Oshikama[111]. By converting them into

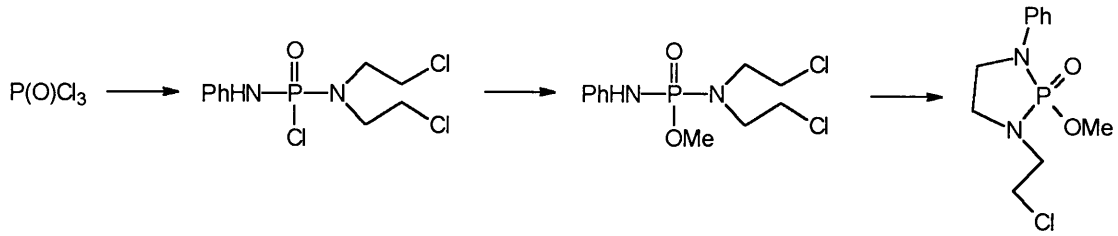
corresponding phosphoramides, the absolute configuration of amines and alcohols could be readily determined by NMR spectroscopy. The experiments also indicated that the stereochemistry at phosphorus was still retained, so no racemization at phosphorus occurred (Equation 30).

According to Equation 30, the amines ($H_2NCHL^1L^2$) or alcohols (in the form of lithiated racemic alcohol) were converted into the phosphoramides.



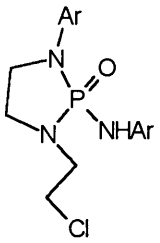
Equation 30

Another example concerning chiral properties of phosphoramides is their use in the Asymmetric Michael Addition Reaction[112]. They have been shown to induce high diastereoselectivity in the Michael Addition to 5-, 6-, 7- member ring enones. In our laboratory, the research on phosphoramidates concerned problems, such as: solid state comparison with carboxyamides [41], hydrogen bonding[40], cyclization[71], and fragmentation of N-phosphorylated nitrogen mustard derivatives[66, 80].



16 13d 14d

Equation 31



14a, Ar=C_6H_5

14b, Ar= 4-$MeOC_6H_4$

Figure 11

In previous chapter, we have already discussed the synthesis of a series of N-bis(2-chloroethyl) phosphoric triamidates and diamidoesters. Among them, there are five chiral compounds that are highly crystalline solids and their single crystals were obtained. Three of them (16, 13d, 14d) are related to each other via the synthetic sequence (Equation 31). Other two (14a, 14b) (Figure 11) represent phosphoramidates of the 1,3,2-diazaphospholidine series.

The molecular structures of those five compounds were determined by x-ray diffraction. The most interesting observation related to their structural parameters are the values of the torsion angles of the O=P-N-H function of these amidates. Those values differed significantly within the series, and the hydrogen bonding properties of individual members were investigated.

Results and discussion

The single crystals of the above compounds were examined by x-ray diffraction. Table 3 represents the selected intramolecular bond distances. The following conclusions can be drawn.

Table 3. Selected bond lengths (Å) of these compounds (with estimated standard deviations in parentheses). For atoms numbering, see Scheme 21.

Compound	16	13d	14d	14a	14b
P=O (1)	1.466 (2)	1.466 (3)	1.453 (2)	1.477 (2)	1.477 (2)
P-N (1)	1.636 (3)	1.637 (3)	1.624 (2)	1.646 (2)	1.645 (2)
P-N (2)	1.629 (3)	1.640 (3)	1.665 (2)	1.656 (2)	1.660 (2)
P-N (3)				1.637 (2)	1.627 (2)
P-Cl	2.034 (1)				
P-O (2)		1.577 (3)	1.577 (2)		

1.The bond lengths of the phosphoryl bond range from 1.453 (2) Å to 1.477 (2) Å. Compound 14d has a shortest 'P=O' bond, since it is the only compound of the series not involved in hydrogen bonding. The values of 'P=O' bond lengths of 16 and 13d are identical. This indicates that 'MeO-' and 'Cl' groups have similar effects on the phosphoryl center. At the same time, compounds 14a and 14b also have the same 'P=O' bonds lengths. According to Emsley[23], the bond between phosphorus and nitrogen in a phosphoramide can be involved in π-bonding based on 2p (N)--3d (P) donor π bonding (Figure 12).

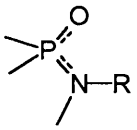


Figure 12

The R group affects the P-O bond by resonance interactions through this 'π-bonding'. But it seems that the phenyl group and the p-anisyl group have the same effect on the 'P=O' moiety; namely, the electronic effects of N-substituents are poorly transmitted to the phosphoryl group. The results suggest a low degree of resonance interactions with the phosphoramidate functionality (as opposed to the carboxyamide function).

2. Comparing triamidates (14a, 14b) and diamidates (16, 13d, 14d), the phosphoryl bond in the former is longer than in the latter, the reason is the net electron-releasing effect of three (in 14a, 14b) (as opposed to two) nitrogen substituents. The similar results can also be obtained: the bond distances around phosphorus atom in thiophosphorylamide[114] (Figure 13) are generally longer than those in phosphoramides. In thiophosphoryl amide, the P=S bond distance is 1.919 (2) Å and P-N bonds are around 1.680 (4) Å ~ 1.694 (4) Å, while P=O bond lengths in these phosphoramidates are around 1.453 (2) Å ~ 1.477 (2) Å and P-N bond are around 1.624 (2) Å ~ 1.665 (2) Å. This is because oxygen has a high electronegativity.

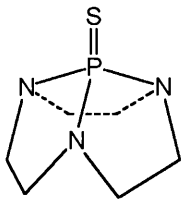


Figure 13

3. The P-N bond lengths ranged from 1.624 (2) Å to 1.665(2)Å are within expected limit[113]. The similarity of the P-N bond lengths for the N-alkyl (av. 1.638 ± 0.009 Å) and for aryl (av. 1.645 ± 0.015 Å) substituents can also be considered as an indication of poor involvement of the phosphoryl group in the resonance interactions with the NHR amide function .

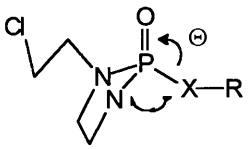
Table 4 gives the bond angles obtained. The geometry of these phosphoramides can be represented by an irregular tetrahedron with the deviations which are typical for phosphate derivatives[115], namely, the angles (O=P-N) involving the 'electron rich' phosphoryl bond are

greater than ideal value of 109.5^0.

Table 4. Selected bonds angles of these substrates (with estimated standard deviations in parentheses). For atoms numbering , see Scheme 21.

Compound	16	13d	14d	14a	14b
O (1)-P-N (1)	111.0 (1)	111.3 (2)	118.3 (1)	117.3 (1)	116.9 (1)
O (1)-P-N (2)	119.2 (2)	115.6 (2)	118.2 (1)	116.5 (1)	117.1 (1)
O (1)-P-Cl	109.5 (1)				
O (1)-P-O (2)		114.7 (2)	106.9 (1)		
O (1)-P-N (3)				106.3 (1)	107.3 (1)
N (1)-P-N (2)	108.0 (1)	107.2 (2)	94.4 (1)	94.3 (1)	93.6 (1)
O (1)-P-N (2)-H(2)	118.0 (1)	130.0 (3)			
O (1)-P-N (3)-H(3)				7 (1)	0 (2)

The O (1)-P-Cl bond in compound 16, where electronic repulsion seems to be counterbalanced by the steric effects of the two remaining substituents, is exactly 109.5^0. In compounds 14d, 14a, and 14b, the O (1)-P-X angles [X=O (2), N (3)] which are not involved in the cyclization, are much smaller than those angles which are involved in the cyclization, because of the stronger steric effects (Figure 14). In substrates 14d, 14a, 14b, the angles of O (1)-P-O(2) or N(3) are around $106.3\ (1)^0 \sim 107.3\ (1)^0$, while the angles of O (1)-P-N (1 or 2) are around $116.5\ (1)^0 \sim 118.2\ (1)^0$.

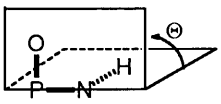


$\Theta = 106.3(1)^0 \sim 107.3(1)^0$
(X=O, NH)

Figure 14

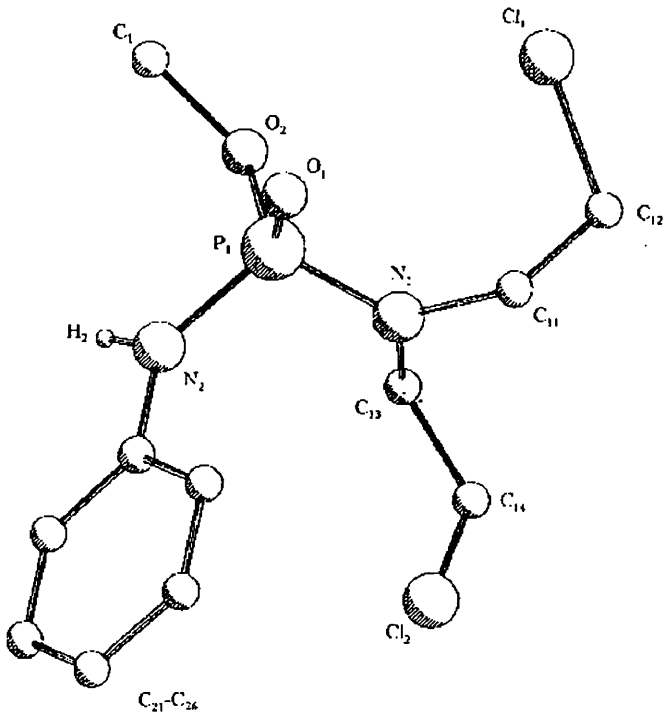
As expected, the N-P-N bonds angles are strongly dependent on the structures of substrates, with all endocyclic values sharply decreased to the value of 94.4 ± 0.40. In the noncyclic compound 13d, the N-P-N bonds angle is 107.2 $(2)^0$, while the value of it's corresponding cyclization compound 14d is 94.4 $(1)^0$; the difference is 12.8^0.

The angles around the phosphorus atom have already been discussed above. They are dependent on the atoms connected to the phosphorus atom and other functional groups, and also provide us with a general picture of those compounds. Below, we shall discuss another important structural feature, that is the torsion angles (O=P-N-H) of compounds 16, 13d, 14a, 14b.



ompound 16 : Θ=118(1) ^{0}

ompound 13d : Θ=130(3)^0

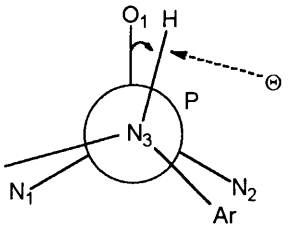


Compound 13d

Figure 15 Structure of 13d with H-N-P=O torsion angle

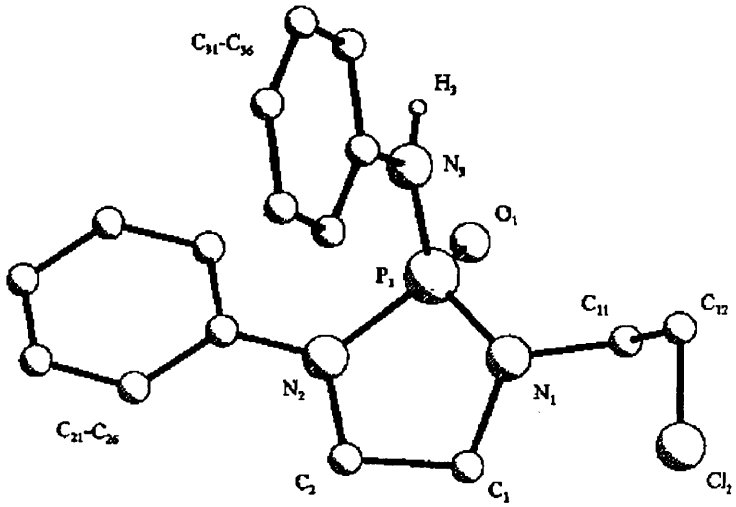
In the acyclic compounds 16 and 13d, the torsion angles O (1)-P-N (2)-H (Figure 15) are large, with values of 118 $(1)^0$ and 130 $(3)^0$, respectively.

However, the O (1)-P-N (3)-H (3) angles in both 1,3,2-diazaphospholidine derivatives 14a, 14b (Figure 16) are 7 $(1)^0$ and 0 $(2)^0$, respectively.



Compound 14a : Θ = 7(1)^0

Compound 14b : Θ = 0(2)^0



Compound 14a

Figure 16 Structure of 14a with H-N-P=O torsion angle

As shown in Figure 16, the N-H in amidates 14a and 14b is placed in an eclipsed position with respect to the 'P=O' bond. The huge difference of the torsion angles between structures shown in Figures 15 and 16 implies possible difference in some properties of those two groups of compounds. First, this torsion angle has a significant effect on the molecular packing of the structures, because compounds 16, 13d and compounds 14a, 14b pack their molecules in different

patterns.

Compounds 16 and 13d pack the molecules in their crystals as a continuous chain system (Figure 17) and the intermolecular hydrogen bonds are formed as a stability factor of the crystals. The results show that the geometry of their torsion angles does not lead to the formations of dimers through hydrogen bonding. Compounds 16 and 13d may be described as a $C_1^1(4)$ pattern[116], as shown in Figure 18.

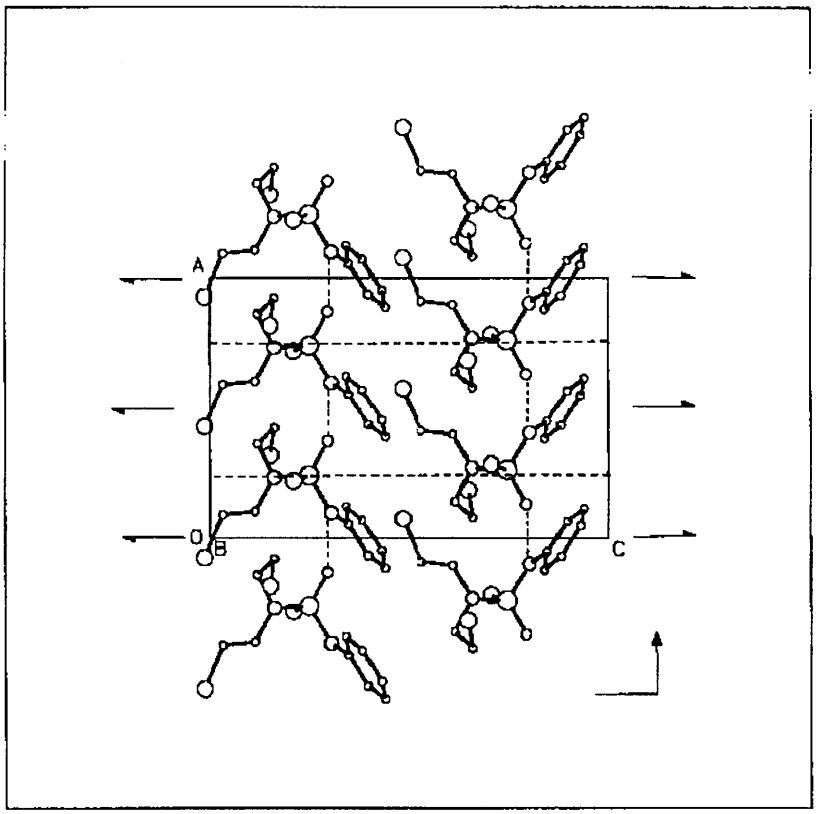


Figure 18 A packing diagram of 16 and 13d, viewed along [0 1 0]. The N-H···O=P hydrogen bonding ribbons run parallel to 'a'. Numbering of the atoms corresponding to that in Scheme 21.

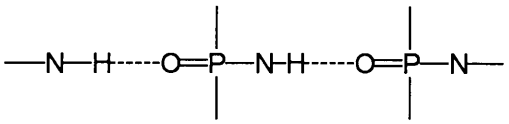


Figure 17

In compound 14a and 14b, the molecules can form hydrogen-bonded dimers (Figure 19).

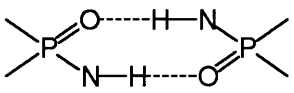


Figure 19

Obviously, the much smaller torsion angles make the 14a and 14b to prefer dimers over 'a continuous chain system' by hydrogen bonding. The pattern can be described as $R_1^2(8)$[116], as shown in Figure 20. This result is in agreement with the literature[120] where the computational and experimental results showed that the parallel orientation of the N-H and the P=O bonds are essential for forming the thermodynamically most stable system of cyclic dimers with N-H←O=P and P=O→H-N hydrogen bridge bonds (Figure 21)[120].

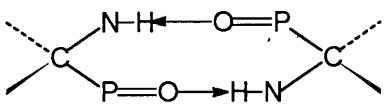


Figure 21

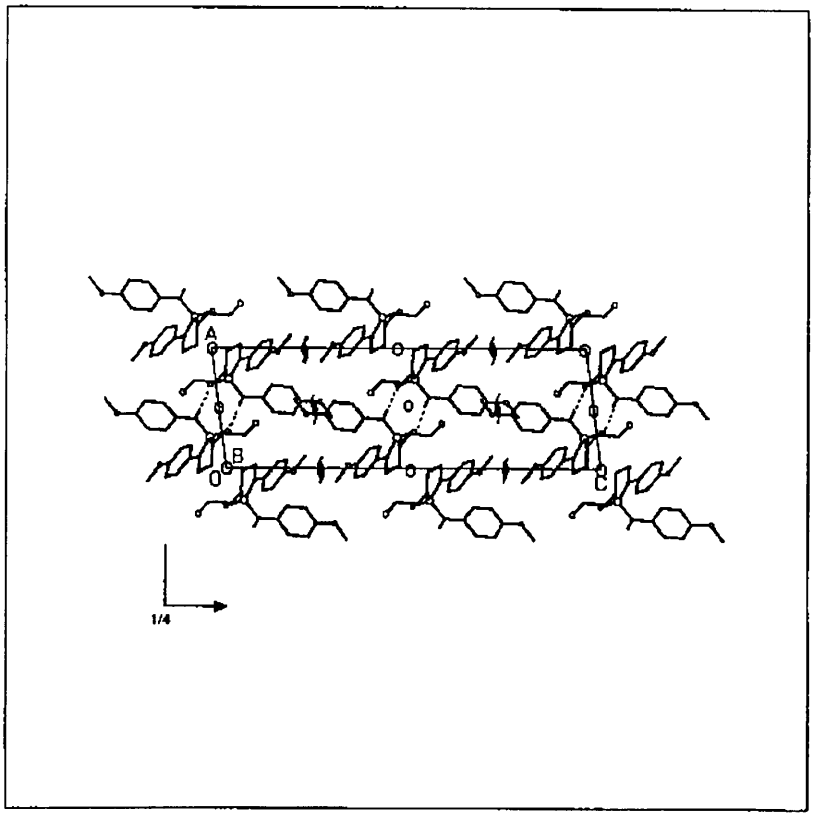


Figure 20 A packing diagram of 14a and 14b, viewed along [0 1 0]. The molecules form hydrogen-bonded dimers across a centre of inversion. Numbering of the atoms corresponding to that in Scheme 21.

Since compound 14d does not have the ability to form hydrogen bonds, the molecules are held in it's crystals only by the usual Van Der Waals forces, as shown in Figure 22. The above facts tell us that the different conformations (around phosphorus atom) between acyclic and cyclic substrates give different packing patterns and also different hydrogen bonding in the solid state. The interesting question is: if the conformational preferences about P-N bonding in the P (O)-NH

moiety can determine the arrangement of the molecules in lattice due to the difference in hydrogen bonding (linear vs. dimeric), can those effects be present in solution to a degree that would force different hydrogen bonding properties upon individual compounds? Two types of experiments were carried out in order to find an indication for the answer : 1. solution studies. 2. solubility studies.

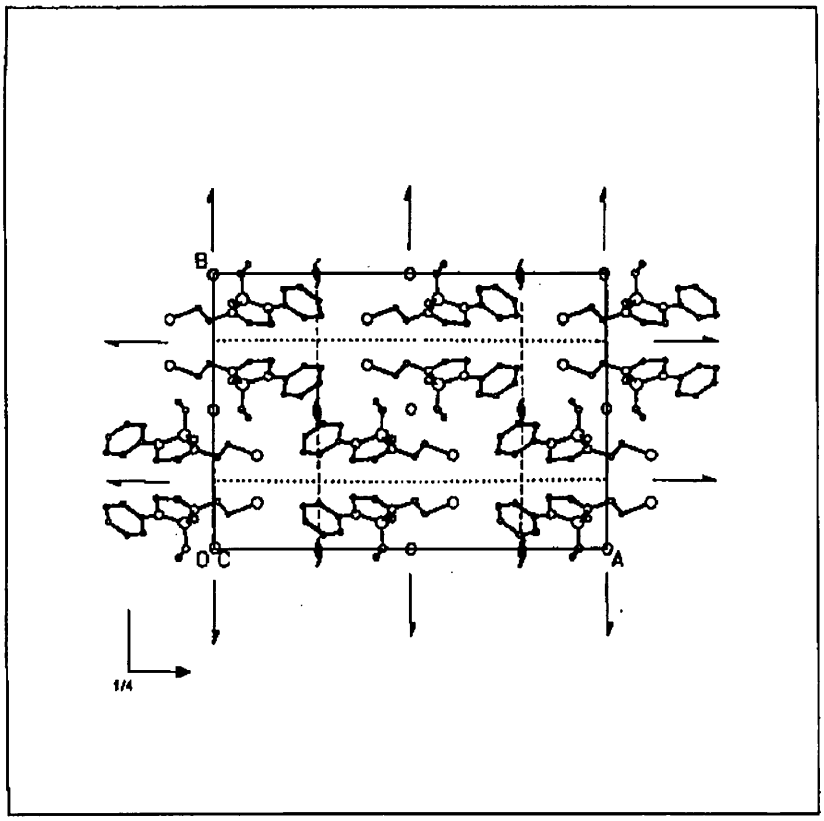


Figure 22 Packing of structure 14d, viewed along [0 0 1] and numbering of the atoms corresponding to that in Scheme 21.

Solution Studies

Early studies have shown that phosphoric acid in solution can be involved in different types of hydrogen bonding[43]. Monobasic phosphoric acids in benzene are dimeric, while dibasic phosphoric acids are polymeric (by hydrogen bonding). The 'N-H----O-P' bonds of phosphoramidates in liquid and solution show complicated effects, they can include free NH, inter-molecularly bonded NH, and intramolecularly bonded NH (Figure 23)[117].

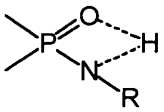


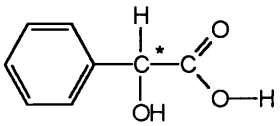
Figure 23

In our case, the functional group P(O)NH of phosphoramidate can act as a hydrogen bonding donor (P=O), and an acceptor center (---N-H), those factors are essential for forming intermolecular hydrogen bonding[42]. However, for forming a dimer, the above condition is not sufficient, the conformation is also a key point. In other words, the ability to form a 1:1 hydrogen-bonded complex with a species also capable of the donor-acceptor interactions should depend on the value of the O=P-N-H torsion angle. This was proved by the following experiments.

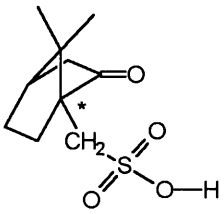
The experiment was carried out in a NMR tube. The phosphoramidate substrate and an optically active acid were mixed (in a 1 : 1 molar ratio) in the NMR tube. Since our substrate is racemic, it should form two diastereomeric hydrogen-bonded complexes with the optically active acid. If the complexation is strong enough to secure significant life time (on the NMR scale) of the complex, the ^{31}P NMR spectrum of the solution should show two signals, corresponding to two

complexes. If, however, the bonding is weak, fast exchange between the complexes should lead to a single (averaged) ^{31}P NMR signal.

The substrates (13d, 14a, 14b) were dissolved in deuterated solvents ($CDCl_3$, CD_3COCD_3, CD_3CN, C_6D_6). The ^{31}P NMR spectra of those solutions of racemic substrates 13d, 14a and 14b gave a single signal indicating that no stable (on the NMR time scale) hydrogen-bonded self-associates were formed. The ^{31}P NMR chemical shifts changed slightly as a function of the polarities of the different solvents. Then, one equimolar ammount of an optically active acid [(+)-mandelic (Q) or (+)-camphor-10-sulfonic (R)] was added to the solution.



L(+)-Mandelic Acid Q



D(+)-Camphor Sulfonic Acid R

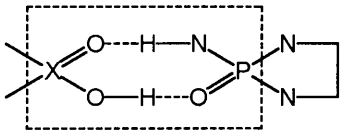
Table 5. Chemical shift differences between the ^{31}P NMR signals of the diastereomeric complexes of phosphoramidates 13d, 14a, 14b with optically active acids Q, R

Substrate	Solvent	$\Delta\delta_P$ (Hz)	
		Acid	
		Q	R
13d	$(CD_3)CO$	0.0	0.0
	C_6D_6	0.0	0.0
14a	$(CD_3)CO$	0.0	0.0
	CD_3CN	0.0	3.8
	$CDCl_3$	3.0	7.8
	C_6D_6	9.2	37.5
14b	$(CD_3)CO$	0.0	0.0
	CD_3CN	0.0	0.0
	$CDCl_3$	3.1	4.6
	C_6D_6	11.7	11.3

The results are given in Table 5; it can be seen that the ^{31}P NMR spectra depended on: 1. the type of the phosphoramide; 2. the solvents; 3. the optically active acids.

1. Substrate 13d yielded only a single ^{31}P NMR signal in the most (acetone-d_6) as well as in the

least (benzene-d_6) polar solvent. This means that any interactions that may develop between 13d and the optically active acid are not strong enough to lead to stable diastereomeric species. On the other hand, substrates 14a and 14b give rise to two signals (in the ratio of 1:1) in the ^{31}P NMR spectra with chemical difference in the range of 3 to 38 Hz. Figure 24 shows the separated signals of substrate 14a in the presence of chiral acid R. This result is taken as an indication of the formation of a relatively stable diastereomeric species via mutual hydrogen bonding interaction between the P(O)NH function of 14a and 14b, and the acidic group of the chiral acid (Figure 25).



Q. X = C
R. X = S

Figure 25 Proposed diastereomeric hydrogen-bonded complex between 14a, 14b and an optically active acid

2. The solvents can also affect the interactions between substrates and chiral acids. In acetone-d_6, no formation of diastereomeric complex was observed. The probably reason is that acetone is itself a sufficiently strong hydrogen bonding acceptor[118] to break any donor-acceptor complexation involving chiral solutes. In other words, in this case, the carbonyl group of acetone acts as an acceptor (>C=O --- H-N-P) masking the effect of chiral acids. In benzene-d_6, for the same substrates, the biggest separation was always observed relative to other solvents. Apparently, it is because benzene is the least polar solvent and does not have the ability to act as an acceptor in hydrogen bonding.

3. The third factor is the optically active acid and it will affect the separation value of ^{31}P NMR signals. The interactions between 14a and R give the biggest difference of ^{31}P NMR signals with $\Delta\delta_P$ (Hz) = 37.5, while for 14b and Q, R almost the same chemical shift differences ($\Delta\delta_P \sim$ 11 Hz) were obtained.

Formation of the complex (Figure 25) requires the syn-periplanar (or nearly syn-periplanar) orientation of the O=P-N-H functionality, that is determined by the conformational preferences of the substrate. In our case, the results (in Table 5) are in accordance with the molecular orientation observed in the solid state (Table 4). Both substrates 14a and 14b show the orientation of the O=P-N-H fragment to be almost ideally syn-periplanar in solid state (Figure 16). That gives them the capability of forming stable diastereomeric complexes with the optically active acids in solutions. Amidate 13d, on the other hand, showing the orientation of the N-H and P=O groups between gauche and anti-periplanar in solid state (Figure 15), displays no tendency towards forming observable diastereomeric complexes in solutions. It seems therefore that those substrates (14a, 14b and 13d) can retain the solid state conformation in the solution state.

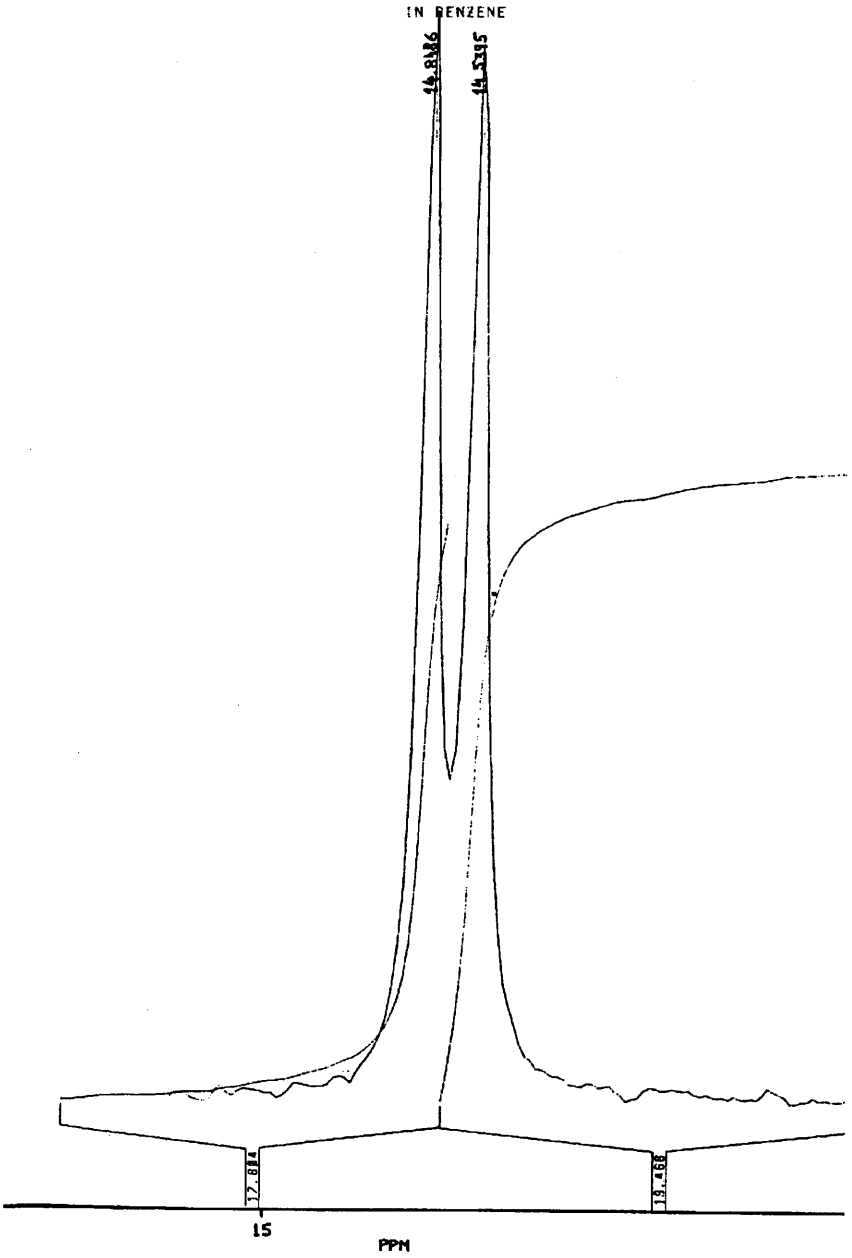


Figure 24 ^{31}P NMR spectrum of 14a with D (+)-camphor sulfonic acid R in Benzene-d_6

Solubility Studies

The strong tendency of substrate 14a and 14b to interact with the acidic species because of the favorable orientation of their P (O)-NH function was also confirmed by solubility experiments. We know that the interaction between solvent and solute or solute and another solute can drastically increase the solubility of the solutes. The interactions can involve hydrogen bonding or Van Der Waals forces, hydrogen bonding being much stronger. Since 14a and R contain strong polar functions, their solubilities in benzene are expectedly to be very low. We determined the solubility of 14a in benzene (25 ^{0}C) as 0.0024 ± 0.0002 g ml $^{-1}$, while the solubility of R (25 ^{0}C) was so low that no reliable value could be determined by using a standard analytical balance. When 14a and R were mixed in an equimolar ratio, the solubility of the mixture in benzene was 0.0110 ± 0.0002 g ml^{-1}. The solubility of 14a was thus increased ca. 5-fold, while the solubility of R was increased by a very larger factor. The ^{31}P NMR spectrum of this solution also showed two signals (ratio 1:1) with the separation of $\Delta\delta_P$=37.5 Hz, the same as in the former experiment shown in Table 5. The experiment indicated the interactions between both components, we take this result as a strong evidence for the postulated diastereomeric complex formed in benzene solution (Figure 25). The complexation engages the most polar fragments of both molecules in mutual hydrogen bonding and leaves the more lipophilic parts exposed to solvation. Thus, the solubility of the whole species was increased in a non-polar solvent (benzene).

Based on the above experiments, the general conclusion is: the geometry of the >P(O)NH function of phosphoramidate, which is modified by the introduction of different functions to the phosphorus moiety, and the examination of their conformational preferences in the solid state can lead to a design of useful reagents capable of chiral recognition via H-bonding interactions.

Experimental

Substrates, solvents and solubility measurements

All substrates were prepared as described in the previous chapter[81]. (+) Mandelic acid (Aldrich) and (+)-10-camphor sulfonic acid (Aldrich) were used as supplied. ^{31}P NMR spectra were recorded on a Bruker AC300 spectrometer at a probe temperature 30 ^{0}C (as in other experiments).

All solvents used for NMR spectroscopy were dried over molecular sieves.

Acetone-d_6	(Aldrich, 99.5 atom% D)
Acetonitrile-d_3	(Aldrich, 99.5 atom% D)
Chloroform-d	(Uvasol, Merck)
Benzene-d_6	(Uvasol, Merck)

Solubility measurements were carried out as follows:

An accurately weighed sample of 14a (or R) was added to dry benzene (5 ml). The concentration of the substrate was 0.20 M. The suspension was stirred at 25 ^{0}C for 48 h. The insoluble material was filtered, dried and it's mass was determined; the filtrate was evaporated to dryness under reduced pressure and the mass of the dissolved material was also determined in the same way. In addition, after the evaporation of benzene, the soluble material was redissolved in C_6D_6 and ^{31}P NMR spectrum of the solution was recorded.

Single crystals and X-ray

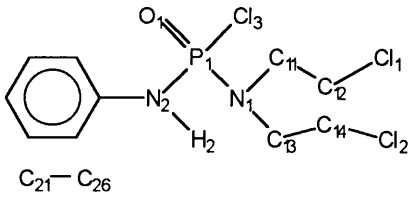
Suitable crystals of all five phosphoramidates were obtained by slow evaporation from benzene / hexane :

Substrates 16, 14a, 14b : Benzene

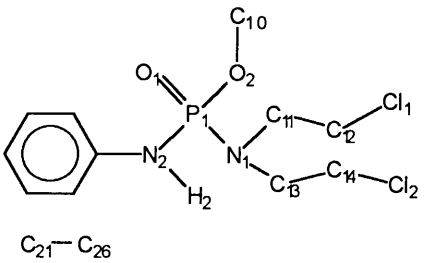
Substrate 14d : Benzene/ hexane--1 : 1

Substrate 13d : Benzene/ hexane--1 : 5

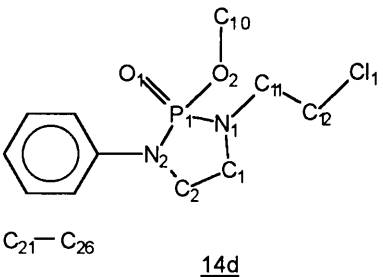
Preliminary cell dimensions and space group symmetry were determined photographically and subsequently refined by standard procedures on a CAD4 diffractometer. The intensities were collected with the ω-2Θ scan mode and crystal stabilities were monitored by periodic reference reflections. The intensities were corrected for Lorentz, polarisation and absorption effects, and the important crystal data and final refinement parameters are given in Table 6. All structures were solved by direct methods using SHELX-S86 and refined by full-matrix least-squares using SHELX-93[119], refining on F^2. The atomic numbering is shown in Scheme 21. In the final models, the non-hydrogen atoms were defined anisotropically, the aromatic, methylene and methyl hydrogens were geometrically constrained [d (C-H)=0.98 Å] and assigned common temperature factors. Special care was taken with the amido hydrogens. These were located in difference electron density maps and either refined independently or with a simple bond length constraint. Crystallographyic data have been deposited under the Cambridge Crystallographic Data Deposition Scheme.



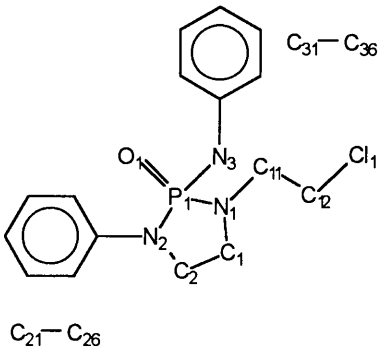
16



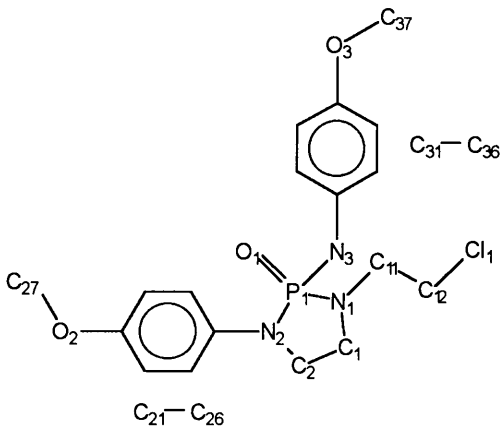
13d



14d



14a



14b

Scheme 21 Structures of 16, 13d, 14d, 14a, 14b with atomic lables

Table 6. Crystal data, experimental and refinement parameters

Compound	**16**	**13d**	**14d**	**14a**	**14b**
Molecular Formula	$C_{10}H_{14}Cl_3N_2OP$	$C_{11}H_{17}Cl_2N_2O_2P$	$C_{11}H_{16}ClN_2O_2P$	$C_{16}H_{19}ClN_3OP$	$C_{18}H_{23}ClN_3O_3P$
Molar mass/g mol^{-1}	315.55	311.15	274.69	335.77	395.81
Space Group	$Pca2_1$	$P2_1/c$	Pbca	$P2_1/c$	$P2_1/c$
a/Å	9.817(2)	9.702(1)	23.766(2)	8.859(1)	8.527(2)
b/Å	9.394(1)	9.871(2)	16.663(3)	15.630(2)	8.813(1)
c/Å	14.977(3)	15.356(2)	6.541(2)	12.821(3)	26.137(3)
β/°	-	91.65(1)	-	109.72(2)	97.22(1)
V/Å^3	1381.2(4)	1470.0(2)	2590.3(9)	1668.3(5)	1948.6(6)
Z	4	4	8	4	4
D_c/g cm^{-3}	1.517	1.406	1.409	1.337	1.349
μ/M_oK_a, mm^{-1}	0.76	0.55	0.41	0.33	0.30
F(000)	648	648	1152	704	832

Crystal dimensions/mm	0.19x0.22x0.25	0.22x0.22x0.25	0.19x0.28x0.38	0.25x0.28x0.38	0.28x0.31x0.31
Θ range scanned/°	2 - 25	2 - 25	2 - 25	2 - 25	2 - 25
Range of indices hkl	11, 11, 17	±11, 11, 18	28, 19, 7	±10, 18, 15	±10, 10, 31
Intensity variation %	2.0	0.2	1.2	0.2	0.1
Scan width (x + 1.05 tanΘ)°	0.85	0.85	0.80	0.85	0.80
No unique reflections	1262	2584	2276	2932	3425
No reflections Irel>2σIrel	1178	1894	1341	2202	2147
R1[(a)]	0.024	0.063	0.043	0.047	0.041
wR2[(b)]	0.062	0.205	0.119	0.145	0.105
Max, Min heights in difference map/eÅ^3	0.14, -0.19	1.09, -0.64	0.21, -0.22	0.60, -0.61	0.17, -0.25

(a) $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; (b) $wR2=[\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2}$

Chapter 4

Chapter 4

Correlation of N-P-N Bond Angle and ^{31}P Chemical Shift in Phosphotriamidates Studied

Introduction

Several monographs on ^{31}P NMR spectroscopy have been published[102, 121]. Attempts to develop a unified theoretical foundation for ^{31}P chemical shifts of phosphorus compounds have been made [122-124]. One of the most successful theoretical approaches was developed by Letcher and Van Wazer [123, 124]. By using approximate quantum-mechanical calculation, they demonstrated that three factors dominate ^{31}P chemical-shift differences $\Delta\delta$, as shown in Equation 32.

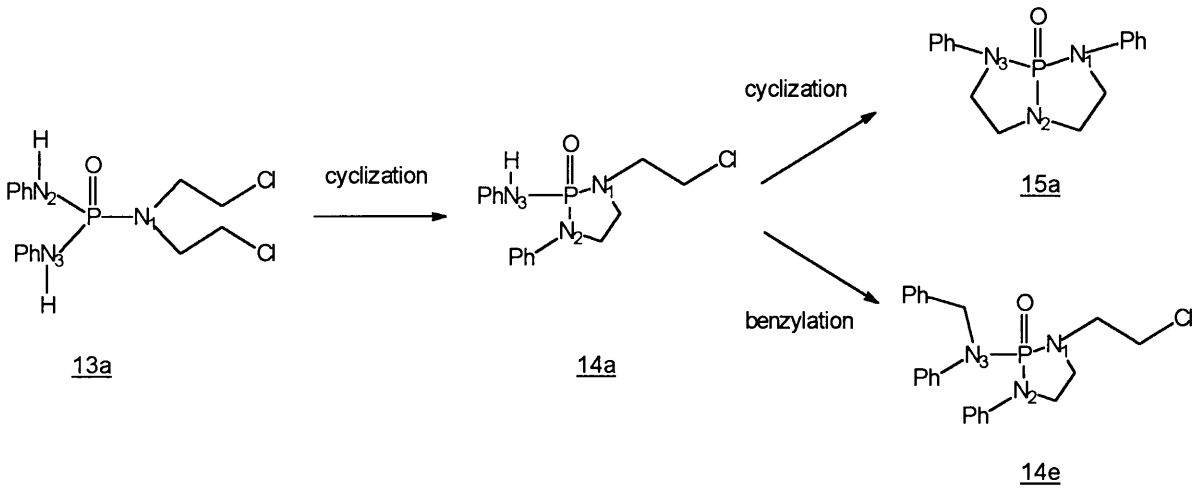
$$\Delta\delta = -C\Delta X_x + k\Delta n_\pi + A\Delta\theta \qquad \textbf{Equation 32}$$

In Equation 32, ΔX is the difference in electronegativity in the P-X bond, Δn_π the change in the π-electron overlap, $\Delta\theta$ the change in the σ-bond angle.

For phosphoryl compounds, however, Letcher and Van Wazer[124] concluded that changes in the σ-bond angles make a negligible contribution ($|A| < 1$) to the ^{31}P chemical shift, with electronegativity effects apparently predominating. Contrary to the theory of Letcher and Van Wazer, Kumamoto[125] and Blackburn[126] have argued on the basis of cyclic phosphate ester shifts that phosphorus bond angles must play some role in determining the values of ^{31}P chemical shifts. The other researches, such as Gorenstein[104], Martin and Robert[103] (mentioned in Chapter 2), have also demonstrated that phosphorus bond angles play an important role for a series of cyclic phosphoryl compounds.

Gorenstein concluded that a decrease in the smallest O-P-O bond angle in the molecule results

in a deshielding (downfield shift) of the phosphorus nucleus. On the other hand, Holms, Gorenstein, and co-workers[127] have also confirmed that the average O-P-O bond angles are likely to be responsible for the ^{31}P chemical shift difference in epimeric esters of the dioxaphosphorinane series. In the course of this work, we have prepared several noncyclic and cyclic phosphotriamidates and performed full NMR spectroscopic characterisation of those compounds. Some of the products were crystalline, and the x-ray diffraction studies provided us with detailed molecular parameters. We decided, therefore, to examine the relationship between the ^{31}P NMR shielding and the bond angle values at phosphorus for a group of related triamidates. To our knowledge, no similar study had been carried out for this particular type of the organophosphorus derivatives.

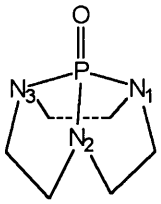


Scheme 22

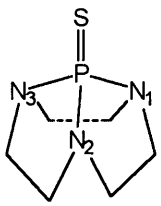
Results and discussion

In Chapter 2 (Table 2), we have reported that the noncyclic, monocyclic and dicyclic phosphotriamidates are characterized by specific ranges of the ^{31}P NMR chemical shift values. Among them, so far, the single crystals of the following compounds : 13a, 14a, 14e, 15a, were grown successfully and the molecular structure of those compounds has been determined from the x-ray diffraction data. The whole series of compounds is derived from compound 13a (precursor) via the transformations shown in Scheme 22.

If all three nitrogen atoms of a phosphotriamidate are fully incorporated in five-membered rings by ethylene bridges, a tricyclic compound, 10-oxo-10-phospha-1, 4, 7-triazatricyclo[5. 2. 1. $0^{4,10}$]decane 18 is obtained.

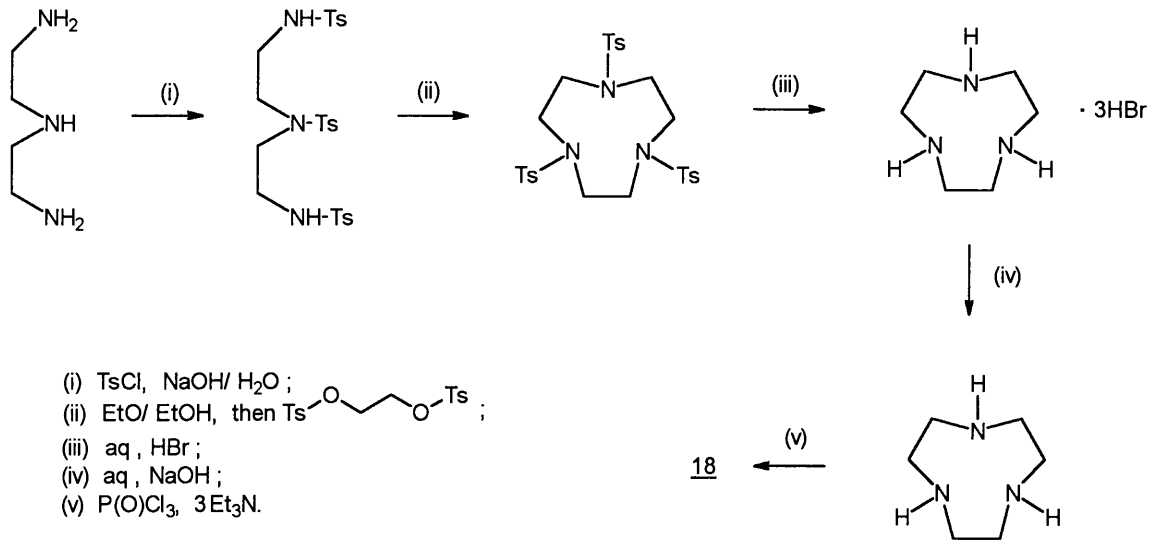


18



18a

The preparation (and the δ_p value) of 18 has been reported[114], but the crystal structure of the compound has not been determined. We repeated the literature synthesis of 18 (Scheme 23) and obtained the product for which we could comfirm the reported $\delta_p$value [lit.[114] δ_p (C_6H_6)=41; this work δ_p ($CDCl_3$)=48.7]. Unfortunately, we were not able to prepare crystals of 18 of the quality suitable for x-ray diffraction. Preparation of larger quantities of 18, required for crystal growth experiments, is currently continued in our laboratory by a new colleague (Dr X. Y. Mbianda). At present, however, for the purpose of the discussed relationship, we accepted for compound 18 the molecular parameters obtained by the same researchers[114] for its close analogue, the corresponding 10-thio derivative, 18a.



Scheme 23

Table 7 lists the N-P-N bond angles and ^{31}P chemical shifts of 13a, 14a, 14e, 15a, 18.

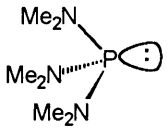
Table 7 N-P-N bond angles and ^{31}P chemical shifts of 13a, 14a, 14e, 15a, 18

Angle (deg.)	13a	14a	14e	15a	18[a]
N(1)-P-N(2)	106.7	94.3	93.3	96.2	100.0
N(1)-P-N(3)	107.0	110.7	108.8	95.7	100.0
N(2)-P-N(3)	103.4	111.6	109.0	114.4	100.1
Average N-P-N	105.7	105.5	103.7	102.1	100.0
^{31}P chemical shift (ppm)[b]	4.4	13.4	17.3	33.3	48.7

a: taken from ref. 114 for 18a; b: in $CDCl_3$.

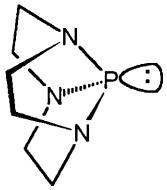
The values of ^{31}P NMR chemical shift give a reasonably good linear correlation (slope = -7.19; $r^2 = 0.9606$) when plotted against the average values of the N-P-N angles. Gorenstein [104] made a similar correlation for a large number of acyclic and cyclic phosphate esters (mono, di, and triesters), and found a similar trend, which led him to the conclusion that the O-P-O bond angle is most important (as opposed to the ionisation of the P-OH group, or the nature of R in the P-OR function) in determining the δ_p value. Similar, but involving much more limited number of compounds, study was carried out for cyclic trithiaphosphocanes, where the δ_p value was correlated with the endocyclic S-P-S bond angle[103]. Those three δ_p vs. X-P-X angle correlations are presented on a common graph in Figure 26.

It is obvious that all three systems follow a similar trend, although it has to be remembered that many additional factors (hydrogen bonding, crystal packing forces, conformational effects, polar effects of R groups, etc) may contribute to the variation in both, the bond angles, as well the chemical shifts. Figure 26 indicates that the correlation is best for our triamidates series. For a pair of non-cyclic and tricyclic phosphortriamidites 19 and 19a, the upfield shifts in the ^{31}P (III) NMR signal was attributed to the enchanced shielding due to increases ***s*** character of the lone pair[129].



19

δ_p : 122



19a

δ_p : 143

We would like to postulate that the relationship observed for series 13a, 14a, 14e, 15a, 18 similarly results from the effect of the molecular geometry (N-P-N angles) on the ***s*** character of

the $\sigma_{P\text{-}O}$ bond, which in turn affects the shielding by the phosphoryl oxygen. More data are however necessary in order to arrive at more uniform picture of the structure-δ_p correlation in organophosphorus compounds.

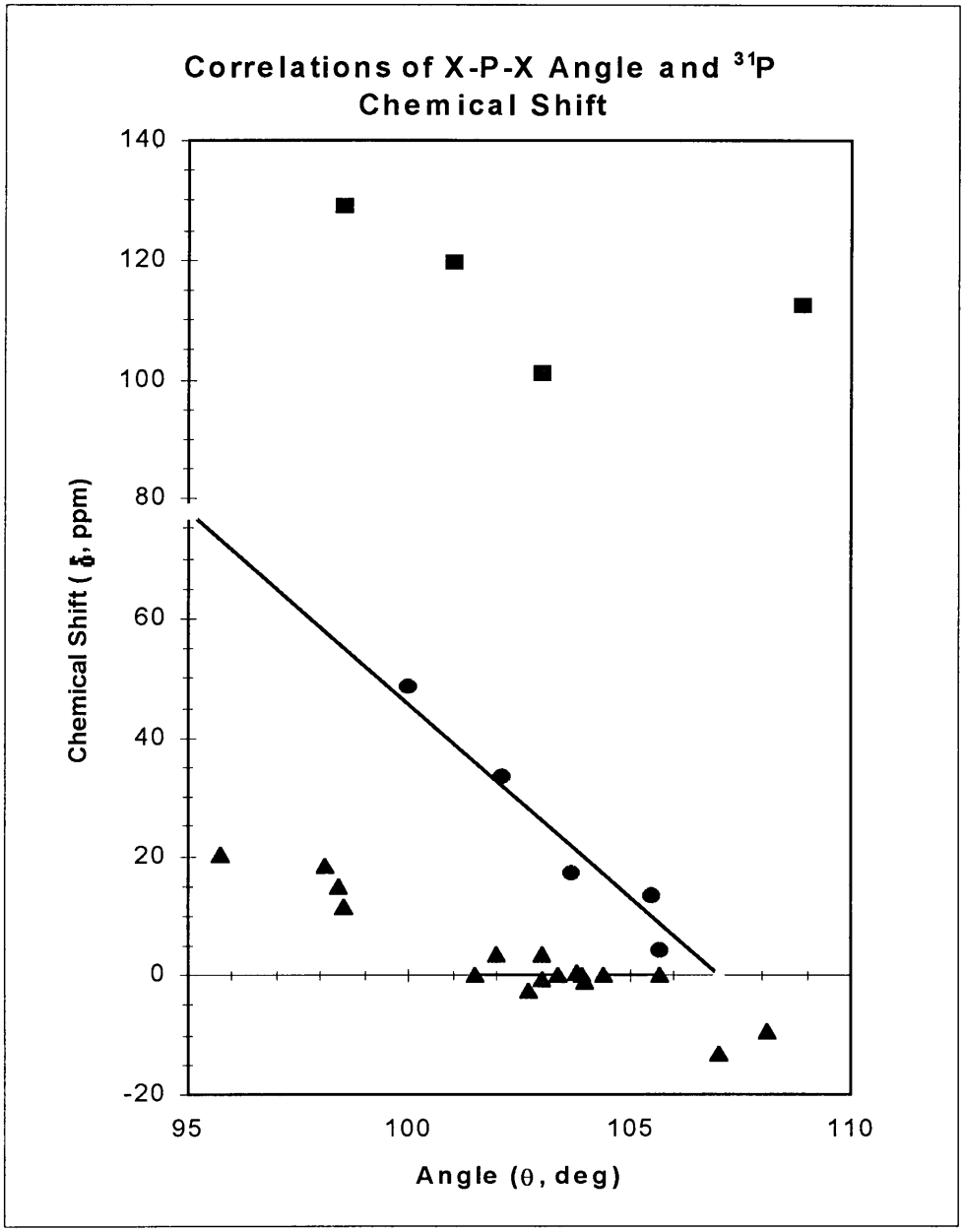


Figure 26 ■ : S-P-S[103] ; ▲ : O-P-O[104] ; ● : N-P-N.

Experimental

Substrates

Compound 13a : the single crystal was grown by recrystallization from ethanol. After recrystallization, its ^{31}P chemical shfit is 4.34ppm.

Compound 14a and 14e : the single crystal was obtained by slow evaporation from benzene.

Compound 15a : the single crystal was grown from our material at the University of Cape Town.

X-ray Diffraction

1. General (see Chapter 3).

2. Compound 13a :

Table 8 and 9 list the conditions and selected bond lengths, angles, respectively. The structure was solved by direct methods and refined on F^2. All non-H atoms were refined anisotropically. One chain (C_{13} - C_{14} - C_{12}) was disordered over two positions (see Figure 27) and was refined with site occupancies of 0.59 (atoms labelled A) and 0.41 (atoms labelled B). Hydrogens attached to N2 and N3 were located in different electron density maps and modelled independently, but with a distance restraint on the N-H bond length. All other hydrogens were placed in calculated positions and modelled with a common isotropic temperature factor.

Table 8. Crystal data and structure refinement for 13a.

Identification code	13a
Empirical formula	C_{16} H_{20} Cl_2 N_3 O P
Formula weight	372.22
Temperature	293(2) K
Wavelength	0.70930 Å
Crystal system	Monoclinic

Space group	P 21/a (14)
Unit cell dimensions	a = 8.7020(10) Å alpha = 90 deg.
	b = 18.564(2) Å beta = 104.520(10) deg.
	c = 11.785(2) Å gamma = 90 deg.
Volume	1843.0(4) A^3
Z	4
Density (calculated)	1.341 Mg/m^3
Absorption coefficient	0.446 mm^-1
F(000)	776
Crystal size	0.20 x 0.20 x 0.20 mm
Theta range for data collection	1.78 to 24.92 deg.
Index ranges	-10<=h<=10, 0<=k<=22, 0<=l<=13
Reflections collected	3234
Independent reflections	3234 [R(int) = 0.0000]
Absorption correction:	
Min, max, ave transmission	0.908, 0.999, 0.967
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3234 / 2 / 237
Goodness-of-fit on F^2	0.859
Final R indices [I>2sigma(I)]	R1 = 0.0679, wR2 = 0.2354
R indices (all data)	R1 = 0.1116, wR2 = 0.2630
Largest diff. peak and hole	0.578 and -0.616 e.A^-3

Table 9. Bond lengths [Å] and angles [deg] for 13a.

P(1)-O(1)	1.464(3)
P(1)-N(2)	1.636(4)
P(1)-N(1)	1.638(4)
P(1)-N(3)	1.644(4)
O(1)-P(1)-N(2)	114.4(2)
O(1)-P(1)-N(1)	111.0(2)
N(2)-P(1)-N(1)	106.7(2)
O(1)-P(1)-N(3)	113.6(2)
N(2)-P(1)-N(3)	103.4(2)
N(1)-P(1)-N(3)	107.0(2)

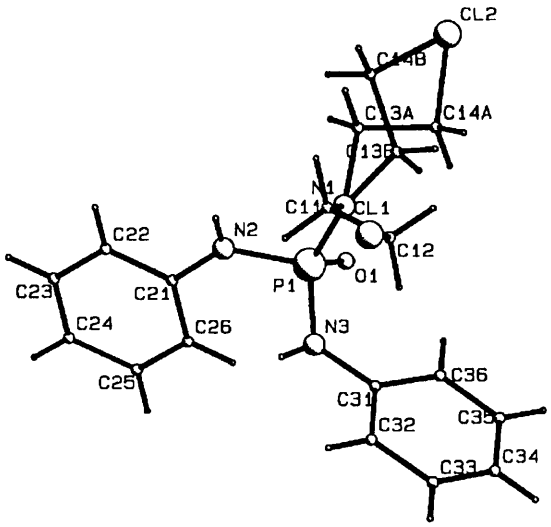


Figure 27 Perspective drawing of the molecule 13a with atomic lables

3. Crystal structure data for compound 14a and 14e are given in Chapter 3 and Chapter 5, respectively.

4. Compound 15a : Table 10 and 11 list the conditions and selected bond lengths, and angles respectively. The structure was solved by direct methods and refined on F^2. All non-H atoms were refined anisotropically. All hydrogens were placed in calculated positions and linked to a common temperature factor. No absorption correction was applied to the data.

Table 10. Crystal data and structure refinement for 15a .

Identification code	15a	
Empirical formula	$C_{16}H_{18}N_3OP$	
Formula weight	299.30	
Temperature	294(2) K	
Wavelength	0.71070 Å	
Crystal system	Orthorhomibic	
Space group	Pbca (61)	
Unit cell dimensions	a = 6.235(3) Å	alpha = 90deg.
	b = 18.758(6) Å	beta = 90 deg.
	c = 25.688 (9) Å	gamma = 90 deg.
Volume	3004(2) A^3	
Z	8	
Density (calculated)	1.323 Mg/m^3	
Absorption coefficient	0.185mm^-1	
F(000)	1264	
Crystal size	0.15 x 0.15 x 0.25 mm	
Theta range for data collection	1.59 to 24.98 deg.	

Index ranges	0<=h<=7, 0<=k<=22, 0<=1<=30
Reflections collected	2645
Independent reflections	2645 [R(int) = 0.0000]
Refinement method	Full-matrix least-squares on F^2
Data / restrains / Parameters	2645 / 0 / 191
Goodness-of-fit on F^2	0.809
Final R indices [I>2sigma(I)]	R1 = 0.0898, wR2 = 0.2264
R indices (all data)	R1 = 0.2228, wR2 =0.2687
Largest diff. peak and hole	0.525 and -0.749 e. A^-3

Table 11 Bond lengths [Å] and angles [deg] for 15a.

P(1)-O(1)	1.437(5)
P(1)-N(1)	1.653(6)
P(1)-N(2)	1.661(6)
P(1)-N(3)	1.676(6)
O(1)-P(1)-N(1)	114.4(3)
O(1)-P(1)-N(2)	121.3(4)
N(1)-P(1)-N(2)	96.2(3)
O(1)-P(1)-N(3)	112.7(3)
N(1)-P(1)-N(3)	114.4(3)
N(2)-P(1)-N(3)	95.7(3)

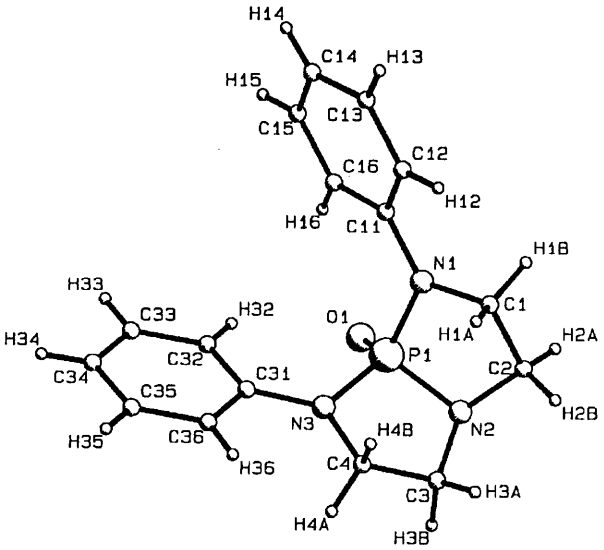


Figure 28 Perspective drawing of the molecule 15a with atomic lables

Preparation of Substrates

13a, 14a, 14e, and 15a is described in other chapters of this Thesis.

Preparation of 18[114] : (Scheme 23)

Step (i) was carried out according to lit.[128] procedure, yield 65%, mp. 174.5-176.0 ^{0}C, lit. mp. 173.0-174.5 ^{0}C.

Step (ii)[114] : yield 60%, mp. 218.0-220.0 ^{0}C.

Step (iii)[114] : yield 65%, mp. 278.0-279.5 ^{0}C.

Step (iv)[114] : a white, deliquescent solid.

Step (v)[114] : a white solid, mp. > 210 ^{0}C, yield 20%, δ_p ($CDCl_3$) : 48.7ppm. Single ^{31}P NMR signal, but ^{1}H NMR spectrum indicates presence of some non-phosphorus impurities.

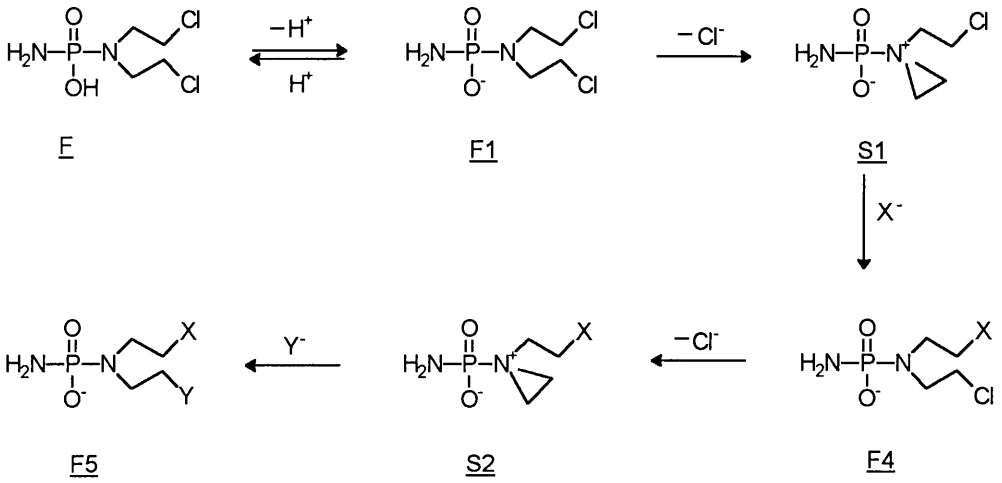
Chapter 5

Chapter 5

Nucleophilicity (intra, inter molecular reactions) and Structure, ^{1}H NMR Spectroscopy Studies

Introduction

Inter and intra molecular reactions of phosphoramide mustard are essential for it's hydrolysis process (degradation) (Scheme 24)[59]



Scheme 24

The phosphorodiamidic acid F forms aziridinium ion S1 via intramolecular cyclization of conjugate base F1. Then, intermolecular reaction of S1 with a biological nucleophile (X^-) gives a monosubstituted intermediate F4, which can in turn form a second aziridinium ion S2 and subsequently afford a bisalkylated product F5.

Inter and intra molecular reactions of organophosphorus compounds are widely studied. The reactions can occur at phosphorus atom[130], and oxygen or nitrogen atom[131] bonded to phosphorus atom. In the previous work carried out in our lab, many organophosphorus compounds were also studied from the point of the intra molecular reactivity.[71]

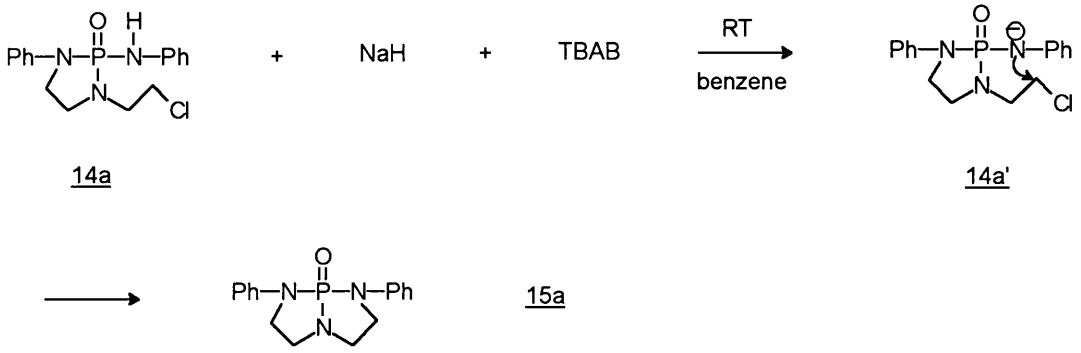
In the introduction of the previous chapter, we have mentioned that the -NHR group can perform some interesting functions[132] and act as an intermediate group to convert phosphoroanilidates [96] into the corresponding phosphorothioates (X=O, Y=S), phosphoroselenoates (X=O, Y=Se), etc.

In this chapter, we shall discuss the behaviour of NHR group in the inter and intra molecular reactions. Some NMR spectroscopic characteristics of the alkylated products 14e, 14f, and the crystal structure of 14e (intermolecular reaction product) will be discussed.

Results and discussion

Inter and intra molecular reaction studies

In the presence of base (NaH), substrate 14a (described in Chapter 2) can form a nitrogen anion. This anion attacks the β-chlorine of N-ethyl group via nucleophilic 1.5-cyclization to form the dicyclic compound 15a. (Scheme 25)



14a 14a' 15a

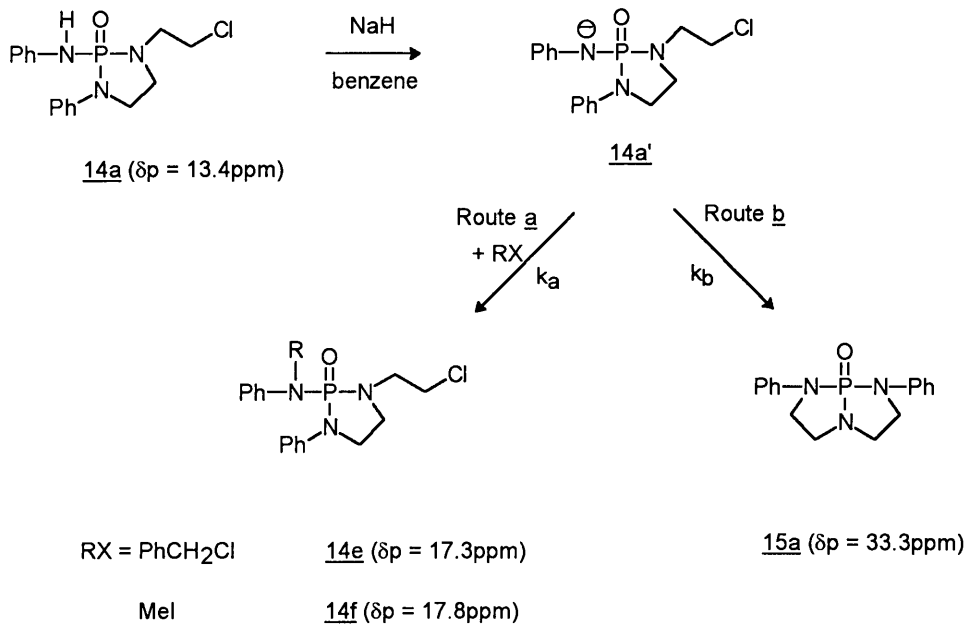
Scheme 25

The acidity of nitrogen hydrogen (14a) is an important factor in this process[92].

If another alkyl halide (RX) is present in the above reaction system, the nitrogen anion (14a') can attack this alkyl halide too. The intermolecular nucleophilic reaction occurs.

In this part of the work, the inter and intra molecular reaction system was developed. The substrates 14a and RX ($PhCH_2Cl$, or MeI) with base (NaH), PTC (TBAB) were put in benzene at RT. A postulated reaction mechanism is represented in Scheme 26.

Obviously, the whole process contains two steps: 1. The formation of intermediate 14a' (deprotonation). 2. Inter and intra molecular competitive reactions (routes a and b).



14a (δp = 13.4ppm)

14a'

RX = $PhCH_2Cl$ 14e (δp = 17.3ppm)

MeI 14f (δp = 17.8ppm)

15a (δp = 33.3ppm)

Scheme 26

The two competitive reactions have the same "substrate"--intermediate 14a'. The intramolecular reaction is a first-order reaction, the reaction rate is determined by the concentration of 14a'. The rate equation is given in Equation 33.

$$-\frac{d[14a']}{dt} = \frac{d[15a]}{dt} = [rate]_b = k_b[14a'] \qquad \textit{Equtation } \underline{33}$$

The intermolecular reaction (route a, from 14a' to 14e) is a second-order reaction. The reaction rate depends on concentrations of both alkyl halide and 14a' (Equation 34).

$$-\frac{d[14a']}{dt} = \frac{d[14e]}{dt} = [rate]_a = k_a[14a'][RX] \qquad \textit{Equation } \underline{34}$$

In order to compare the reaction rates of these two competitive reactions, we decided to find out the ratio of k_a/k_b. From Equation 33 and Equation 34, k_a/k_b can be worked out as follows:

$$\frac{[rate]_a}{[rate]_b} = \frac{k_a[14a'][RX]}{k_b[14a']} = \frac{k_a}{k_b} \times [RX] \qquad \textit{Equation } \underline{35}$$

Therefore:

$$\frac{k_a}{k_b} = \frac{[rate]_a}{[rate]_b} \times \frac{1}{[RX]} = \frac{d[14e]/dt}{d[15a]/dt} \times \frac{1}{[RX]} \qquad \textit{Equation } \underline{36}$$

$$= \frac{d[14e]}{d[15a]} \times \frac{1}{[RX]}$$

$$= \frac{[14e_1] - [14e_0]}{[15a_1] - [15a_0]} \times \frac{1}{[RX]} \qquad \textit{Equation } \underline{37}$$

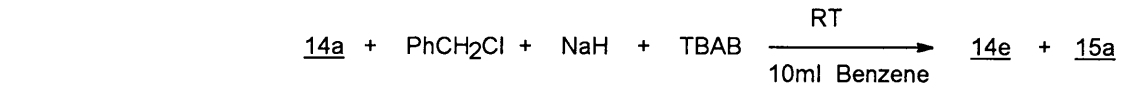
* $[15a_0]$, $[15a_1]$, $[14e_0]$, $[14e_1]$ are the concentrations of compounds 15a and 14e at t_0 and t_1

Because $[15a_0] = [14e_0] = 0$ (t_0=0), therefore

$$\frac{k_a}{k_b} = \frac{[14e_1]}{[15a_1]} \times \frac{1}{[RX]} \qquad \textit{Equation 38}$$

So, the ratio of k_a and k_b can be determined as shown in Equation 38.

Benzyl chloride was chosen as one of the intermolecular reaction substrates. The experiment was carried out according to Equation 39. The whole process was examined by ^{31}P NMR spectroscopy at specific time intervals.



Concentrations (M):	0.03	0.15	0.06	0.003

Equation 39

The results are represented in Table 12.

Table 12: Contents of substrate 14a, and products 14e, 15a (%) based on ^{31}P NMR spectroscopy

Time (h)	14a	14e	15a
1	68	17	15
2	36	35	29
3	4	49	47
4	0	51	49

Since during the first hour concentration of $PhCH_2Cl$ decreased by less than 0.5% of its initial value ($PhCH_2Cl$ is used in 5-fold excess), it can be taken as approximately constant, and the product composition obtained after 1h gives:

$$\frac{k_a}{k_b} = \frac{17}{15} \times \frac{1}{0.15} = 7.6$$

This result shows that in this case the intermolecular reaction rate constant is approximately eight times bigger than that of the intramolecular reaction. However, the reaction rate of intermolecular reaction (route a) strongly depends on the concentration of $PhCH_2Cl$. This was confirmed by the following experiments. These reactions were carried out for different ratio of the substrates (14a and $PhCH_2Cl$). The results are listed in Table 13.

Table 13: Inter and intra molecular competitive reactions in different ratio of substrates

$[14a]_0$ (M)	$[PhCH_2Cl]_0$ (M)	Ratio $[14a]_0$ /$[PhCH_2Cl]_0$	15a (%)	14e (%)
0.02	0.02	1 : 1	89	11
0.02	0.10	1 : 5	57	43
0.018	0.27	1 : 15	36	64

When the substrates 14a and $PhCH_2Cl$ were used in equimolar ratio, the competitive reactions strongly favoured the intramolecular 1.5-cyclization. As the concentration of $PhCH_2Cl$ increased, the intermolecular reaction gradually dominated the process.

Problem:

In Chapter 2, we have already reported that substrate 14a treated with NaH, TBAB in benzene can undergo 1.5-cyclization, and the whole process can be finished in 6 h. When another substrate ($PhCH_2Cl$) takes part in this reaction, the reaction (Scheme 26, RX = $PhCH_2Cl$) can also be completed in 6 h. However, when $PhCH_2Cl$ was replaced by MeI, the reaction gave unexpected results.

Substrate 14a (0.03M) and MeI (0.15M) (with NaH, TBAB) were placed in 10ml of benzene-d_6, and the reaction was examined by ^{31}P NMR spectroscopy at specific time intervals. The results are listed in Table 14.

Table 14: Inter and intra molecular competitive reactions of 14a in the presence of MeI

Time (h)	14a (%)	15a (%)	14f (%)
20	64	0	36
44	46	0	54
120	14	0	86
≥ 240	10	1	89

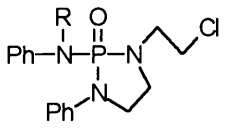
In this case, the intermolecular reaction dominated the whole process, but the reaction time was unexpectedly longer than that of the first case (when, RX = $PhCH_2Cl$). According to the postulated mechanism given in Scheme 26, after the intermediate 14a' is formed, it can react according to route a (inter) or route b (intra). If we accept that the intermolecular reaction between 14a' and MeI is a slow process, the reaction should occur via 1.5-cyclization (route b) and produce product 15a, which is formed in the absence of MeI in 6 h. However, Table 14 shows negligible intramolecular reaction; instead, intermolecular reaction occured very slowly. The identity of the product of the intermolecular alkylation (14f) was demonstrated beyond

resonable doubt. The product was isolated from the reaction mixture, and its structure was determined by NMR (^{31}P, ^{1}H, ^{13}C) spectroscopy, mass spectrometry and elemental analysis. The question is why the formation of 14f is so slow, and the formation of 15a is absent ? Although we are not able at this stage to offer any plausible explanation for the "abnormal" behavior of 14a in the presence of MeI, we think that the observed differences can result from different effects of two haloalkanes on the conformational preferences and ion pairing of the sodium derivatives 14a' in benzene solution.

^{1}H NMR analysis (of 14e and 14f) and X-ray crystallography of 14e.

1. ^{1}H NMR spectroscopy (14e and 14f)

The intermolecular reaction products (Scheme 26, 14e and 14f) were isolated from the reaction mixture and purified by column chromatography. The product 14e is a solid with mp 103.5^{0}C, while 14f is a viscous liquid. The only structural difference of these two compounds is the methyl group (14f) and benzyl group (14e) at the exocyclic-nitrogen (Figure 29).



R= $PhCH_2$ 14e

Me 14f

H 14a

Figure 29

Their ^{1}H NMR spectra (in $CDCl_3$) are of those two compounds, and of the parent amidate are shown in Figures 30 (14e), 31 (14f), 32 (14a). The differences between them are significant. First, the three protons of the N-methy group (Figure 31, 14f) are magnetically equivalent (δ_H

3.10, doublet, $^{3}J_{HP}$=9.20 Hz), while the two protons of benzyl methylene group (Figure 30, 14e) are magnetically non-equivalent (two doublets of doublets). Second, the methylene protons of the CH_2Cl group of 14a (Figure 32, δ_H=3.53 Hz) give rise to a triplet. However, as shown in Figures 30 and 31, the methylene protons of CH_2Cl groups of 14e and 14f are a multiplet and a group of 10 peaks (symmetric), respectively. This part of two spectra is greatly different from other cases discussed in Chapter 1, and Chapter 2.

Compounds 14e and 14f were next dissolved in C_6D_6, and their ^{1}H NMR spectra were recorded [Figures 33 (14e), and 34 (14f)]. Comparison of Figures 31 and 34 shows very strong solvent effects on the spectra, specific to individual groups of signals. For example, the N-Me groups give rise to a signal (doublet) at δ_H 3.10 in $CDCl_3$, and undergo only a very small shift (δ_H 2.94) when transfered to C_6D_6. The hydrogens of the methylene groups (both of the 1,3,2-diazaphospholidine ring, and of the NCH_2CH_2Cl group), on the other hand, undergo dramatic shifts upon the transfer. For the CH_2Cl group, in $CDCl_3$ δ_H=3.66, while in C_6D_6 the signal shifts upfield and merges with the signals of other methylene groups. In $CDCl_3$, the most upfield signal of the methylene protons is δ_H 3.19, but in C_6D_6 it is δ_H 2.48. Obviously, the conformational preferences of the substrate have to be very different in those two solvents.

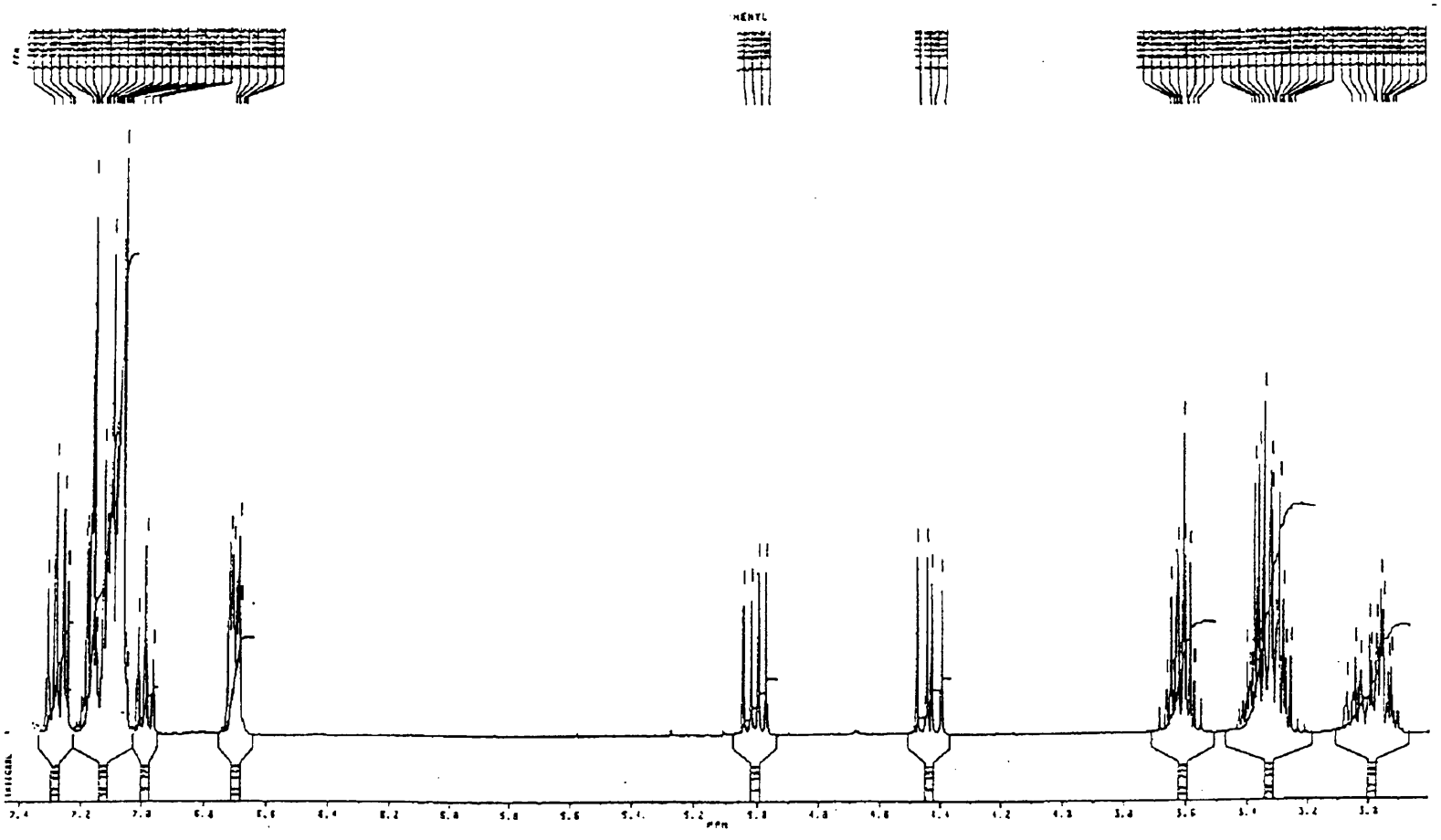


Figure 30 ^{1}H NMR spectrum of 14e in $CDCl_3$

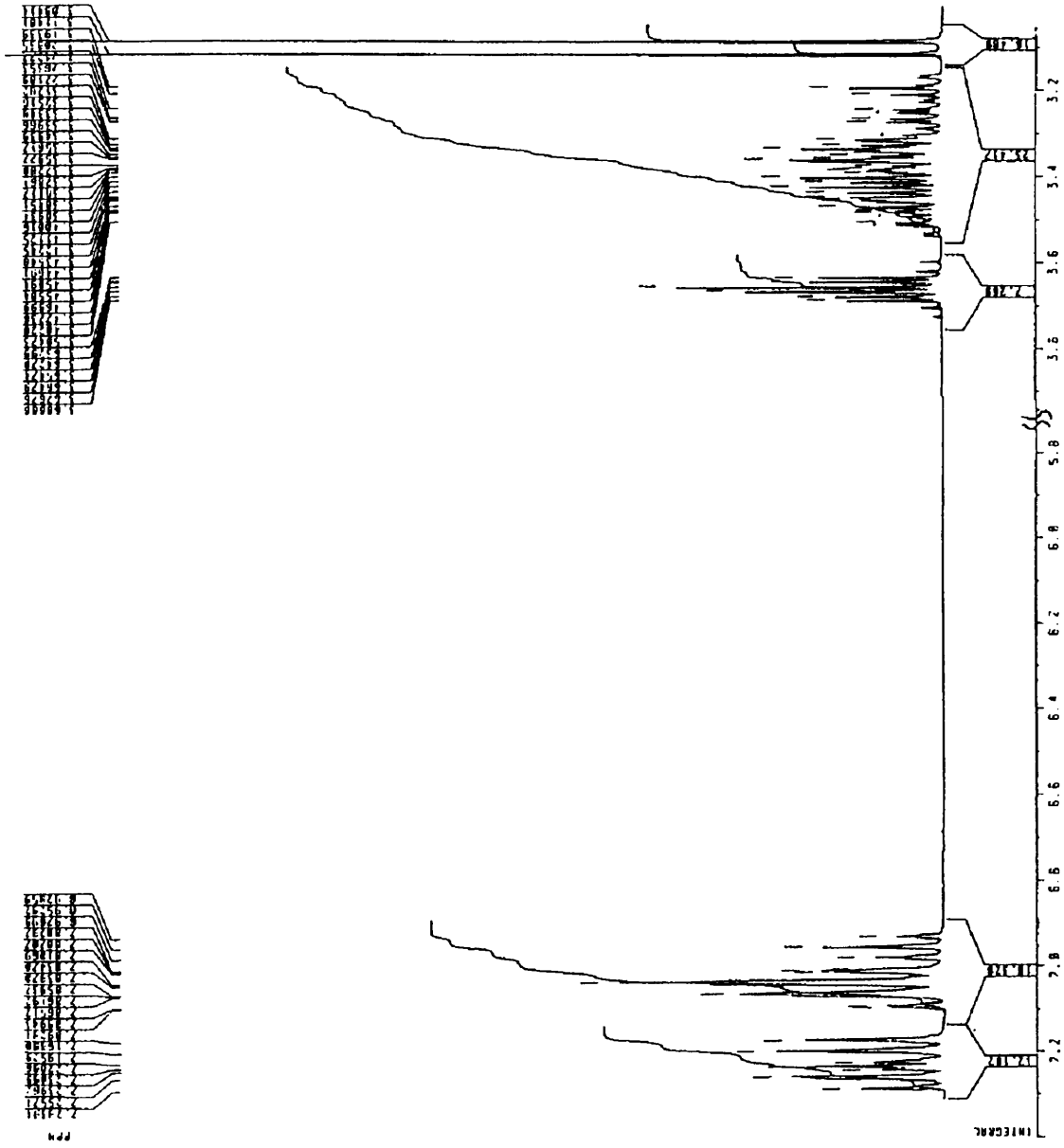


Figure **31** ^{1}H NMR spectrum of 14f in $CDCl_3$

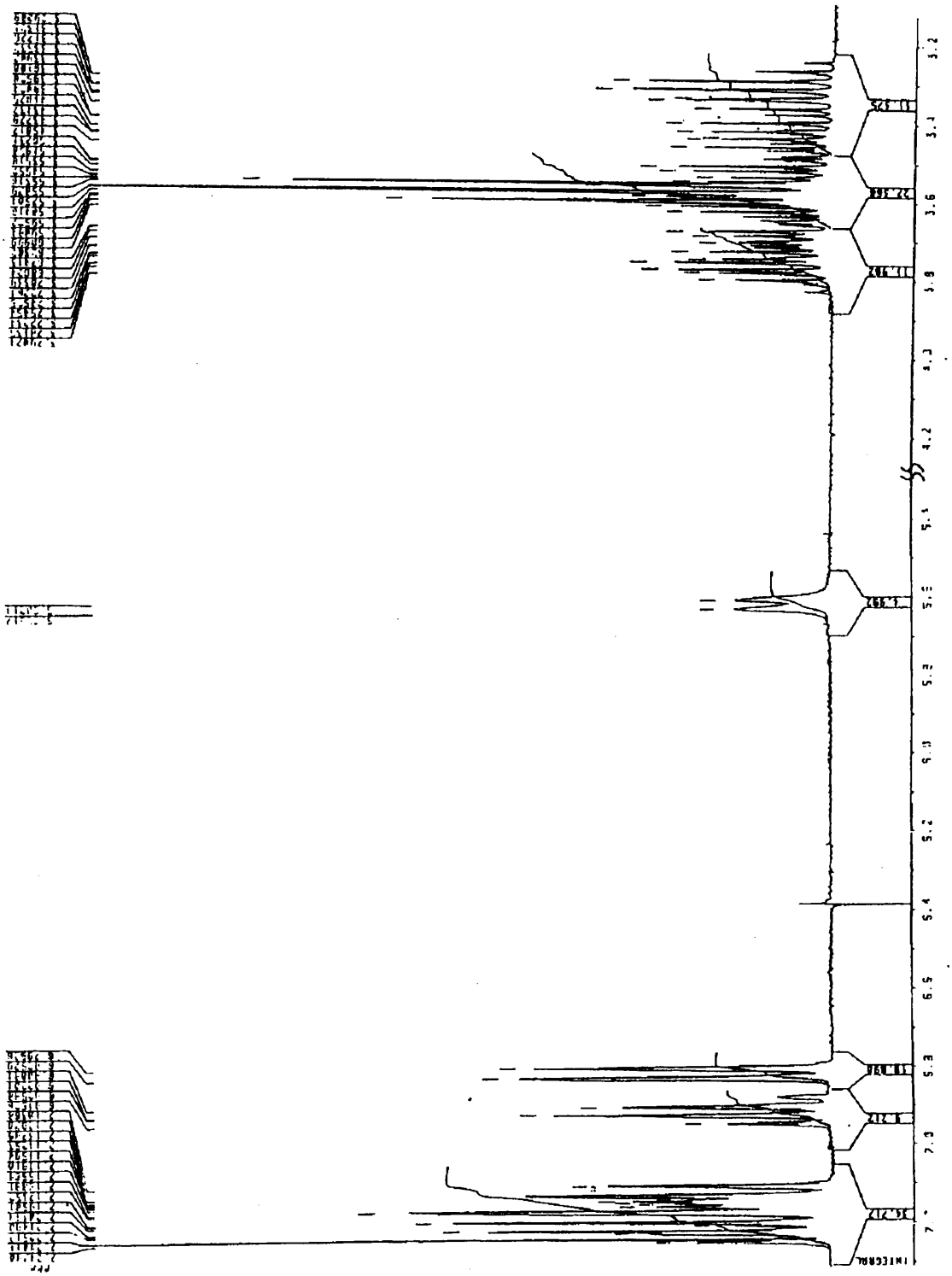


Figure 32 ^{1}H NMR spectrum of 14a in $CDCl_3$

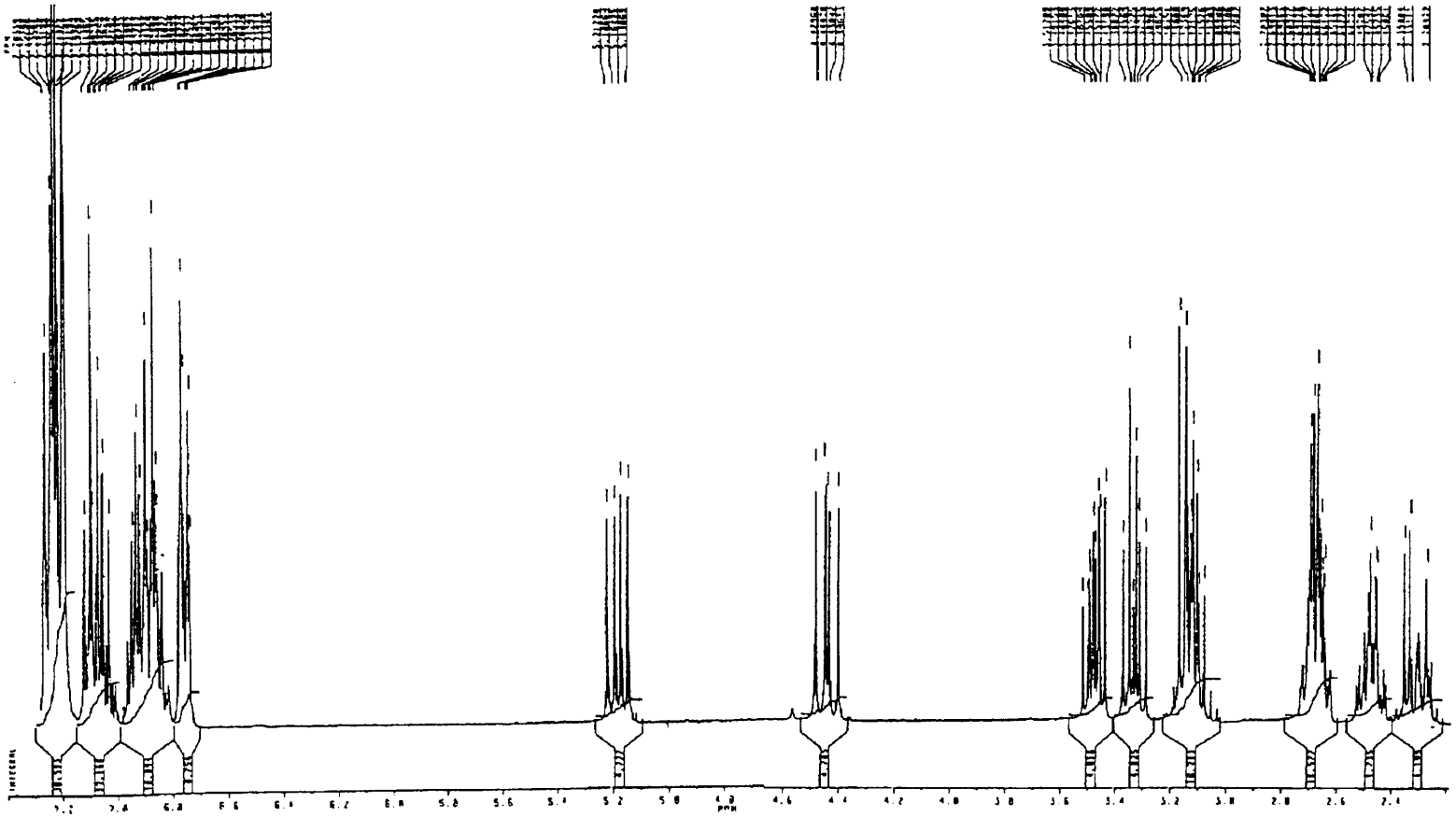


Figure 33 ^{1}H NMR spectrum of 14e in C_6D_6

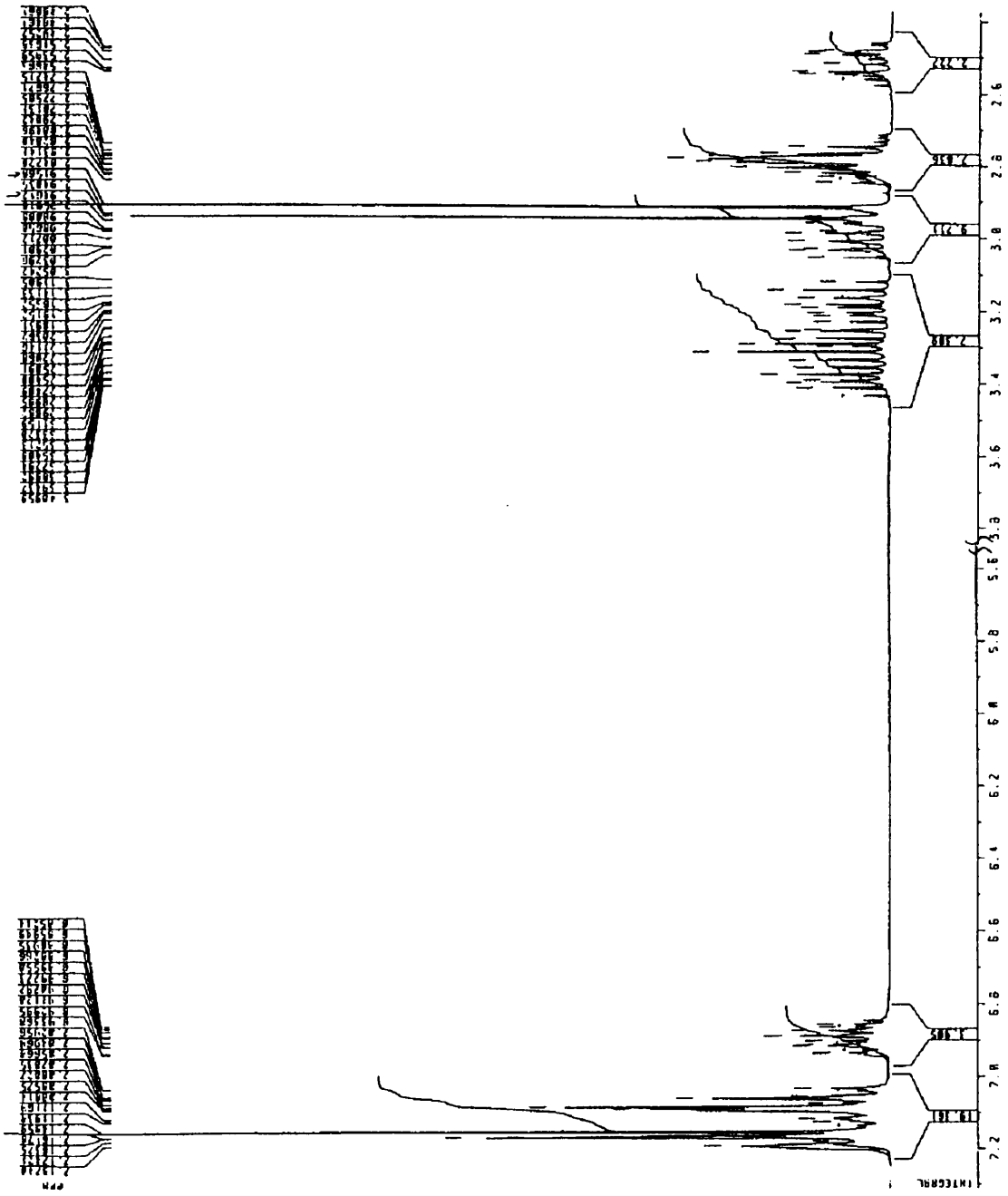


Figure 34 ^{1}H NMR spectrum of 14f in C_6D_6

The effect of solvents on the two benzyl methylene protons of 14e (Figure 30, 33) is shown in Table 15. It seems that the conformational behavior of this part of molecule 14e is similar in both solvents.

The most striking feature of the ^1H NMR spectra of 14e is the enormous difference in the chemical shifts of two geminal hydrogens of the diastereotopic methylene groups of the N-benzyl substituent. The difference is 0.60 and 0.74 ppm in $CDCl_3$ and in C_6D_6, respectively, and it suggests some fixed conformation of the molecule in solution, with very different molecular enviroment of H_A and H_B atoms.

Table 15: Chemical shifts and coupling constants of the diastereotopic hydrogens of the benzyl methylene group of 14e, H_{4A} and H_{4B} in $CDCl_3$ and C_6D_6**

	H_{4A}		H_{4B}	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
δ_H (ppm)	5.00	5.20	4.40	4.46
J_{gem}*	14.50	14.40	14.50	14.40
J_{HP}	7.74	7.67	9.77	10.33

*: The data are similar to the literature data given for nonequivalent geminal protons [133].

**: Similar result was also observed for other systems studied in our lab[134].

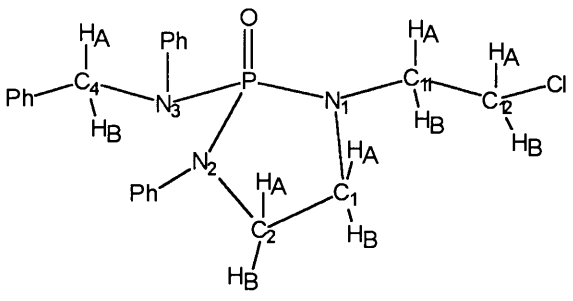


Figure 35

Strong solvent effects were also observed with respect to other methylene signals of 14e (compare Figure 30 with Figure 33). In order to assign the signals observed in both solvents, the corresponding heteronuclear ^{13}C-^{1}H spectra (HETCOR) have been recorded (Figure 36 and 37) and interpreted. The assignments of those signals were made (Table 16).

Table 16: The assignment of methylene protons of 14e in $CDCl_3$ and C_6D_6

(the atomic numbering, see Figure 35)

$CDCl_3$	C_6D_6
H (C_1) 2.95 (m, 2H)	H_{1B} 2.35 (m, 1H) H_{1A} 2.46 (m, 1H)
H (C_2) 3.30 (m)	H_2 2.66 (m, 2H)
H (C_{11}) 3.30 (m)	H_{11} 3.10 (m, 2H)
H (C_{12}) 3.60 (m, 2H)	H_{12B} 3.32 (six signals, J_{gem}=10.8, J_{vic}=7.08, 6.87, 1H)
	H_{12A} 3.47 (eight signals, J_{gem}=10.8, J_{vic}=7.06, 6.18, 1H)

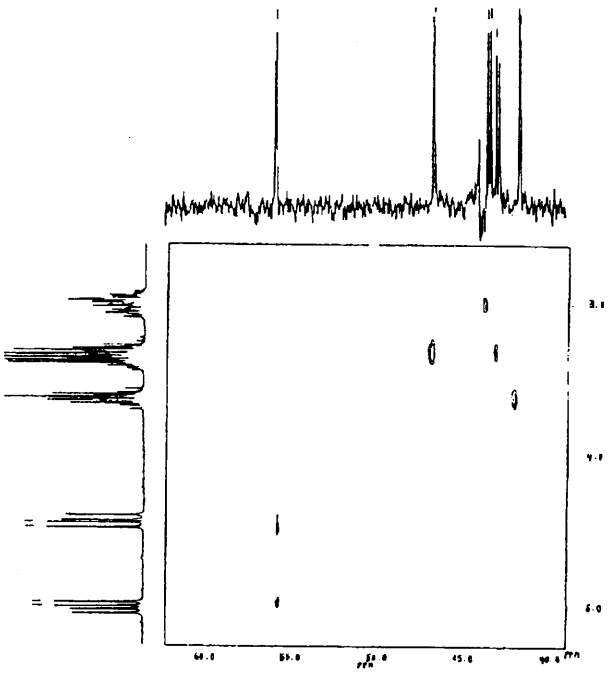


Figure 36 300 MHz HETCOR spectrum of 14e in $CDCl_3$

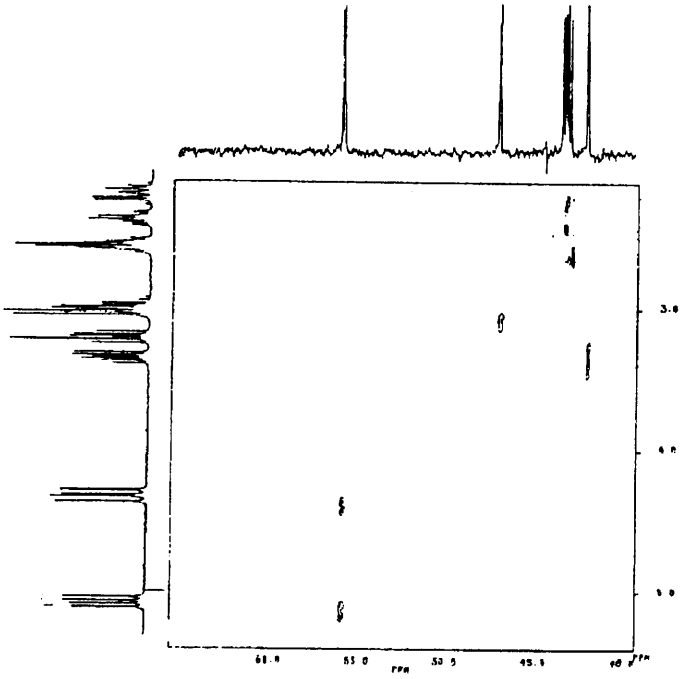


Figure 37 300 MHz HETCOR spectrum of 14e in C_6D_6

The ^{13}C NMR spectra of compounds 14e, 14f, and 14a are very similar (except, of course, for the presence of the N-R group in 14e and 14f). The NMR spectroscopic results indicate therefore that those amidates exist in rigid conformations which impose strong nonequivalence on the hydrogen atoms of individual methylene groups (exo- and endocyclic). The shielding and nonequivalence are also strongly affected by the nature of the solvating molecules. Since a single crystal of 14e, suitable for x-ray diffraction could be prepared, the structure of that compound was also investigated in the solid state.

2. X-ray studies of amidate 14e

The atomic coordinates are listed in Table 17, stereoview of the molecule is represented in Figure 38, and selected bond lengths and valence angles are represented in Table 18.

Table 17. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for 14e. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
P(1)	3080(1)	8922(1)	672(1)	50(1)
O(1)	2452(3)	10299(3)	612(1)	64(1)
C(11)	4638(5)	9252(5)	1613(2)	88(2)
C(12)	5331(6)	10572(6)	1647(2)	101(2)
Cl(1)	5341(2)	11223(2)	2309(1)	128(1)
N(1)	4517(3)	8731(3)	1063(1)	59(1)
C(1)	5841(5)	8403(5)	785(2)	74(1)
C(2)	5356(4)	7717(4)	273(2)	61(1)
N(2)	3893(3)	8261(3)	140(1)	48(1)
C(21)	3136(4)	7829(4)	-326(2)	49(1)
C(22)	1776(4)	8380(4)	-464(2)	56(1)
C(23)	1023(5)	7937(5)	-914(2)	70(1)
C(24)	1618(6)	6984(5)	-1237(2)	80(2)
C(25)	2954(6)	6438(5)	-1113(2)	77(1)
C(26)	3716(5)	6851(4)	-659(2)	63(1)
N(3)	1805(3)	7865(3)	866(1)	45(1)
C(31)	2099(4)	6432(4)	906(2)	43(1)
C(32)	2778(4)	5914(4)	1365(2)	56(1)
C(33)	3019(5)	4516(5)	1411(2)	67(1)
C(34)	2569(5)	3658(4)	1013(2)	70(1)
C(35)	1896(4)	4166(4)	553(2)	63(1)
C(36)	1662(4)	5548(4)	501(2)	50(1)
C(4)	248(5)	8263(5)	895(2)	53(1)
C(41)	-349(4)	8008(4)	1445(2)	46(1)
C(42)	-1011(4)	6793(4)	1560(2)	61(1)
C(43)	-1560(5)	6556(5)	2060(2)	77(1)

C(44)	-1471(5)	7539(6)	2450(2)	86(2)
C(45)	-811(5)	8758(6)	2335(2)	85(2)
C(46)	-244(4)	8989(5)	1835(2)	66(1)

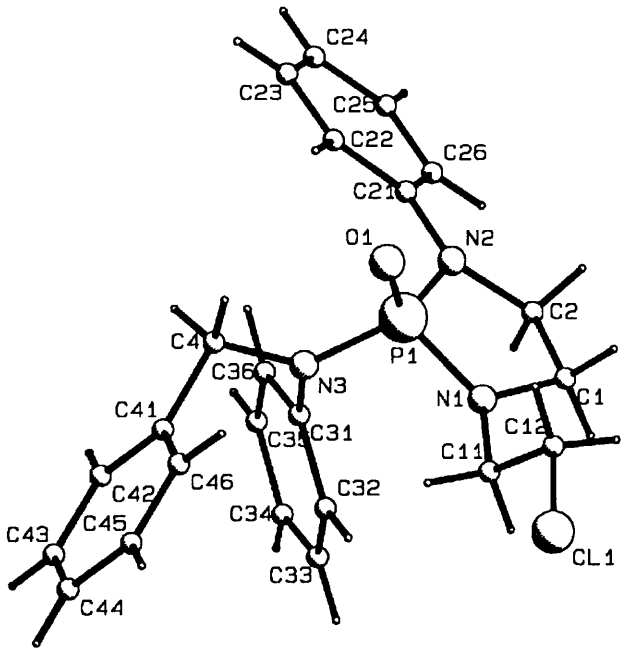


Figure 38 Perspective drawing of the molecule 14e with atomic labels

Table 18. Bond lengths [Å], angles [deg] , and selected torsion angles for compound 14e

bond	length
P(1)-O(1)	1.473(3)
P(1)-N(1)	1.629(3)
P(1)-N(3)	1.644(3)
P(1)-N(2)	1.669(3)
C(11)-C(12)	1.442(6)

C(11)-N(1)	1.462(5)
C(12)-Cl(1)	1.769(5)
N(1)-C(1)	1.451(5)
C(1)-C(2)	1.497(5)
C(2)-N(2)	1.473(4)
N(2)-C(21)	1.403(5)
N(3)-C(31)	1.433(4)
N(3)-C(4)	1.485(5)
C(4)-C(41)	1.510(6)

	angle
O(1)-P(1)-N(1)	118.3(2)
O(1)-P(1)-N(3)	109.1(2)
N(1)-P(1)-N(3)	108.8(2)
O(1)-P(1)-N(2)	117.2(2)
N(1)-P(1)-N(2)	93.3(2)
N(3)-P(1)-N(2)	109.0(2)
C(12)-C(11)-N(1)	113.0(4)
C(11)-C(12)-Cl(1)	111.7(4)
C(1)-N(1)-C(11)	118.8(3)
C(1)-N(1)-P(1)	114.4(3)
C(11)-N(1)-P(1)	124.0(3)
N(1)-C(1)-C(2)	105.8(3)
N(2)-C(2)-C(1)	106.0(3)
C(21)-N(2)-C(2)	120.3(3)
C(21)-N(2)-P(1)	123.5(3)
C(2)-N(2)-P(1)	112.7(3)
C(31)-N(3)-C(4)	115.6(3)
C(31)-N(3)-P(1)	120.3(2)
C(4)-N(3)-P(1)	122.9(3)
N(3)-C(4)-C(41)	112.0(3)

H(11A)-C(11)-H(11B)	107.8(6)
H(12A)-C(12)-H(12B)	108.0(8)
H(4A)-C(4)-H(4B)	111.7(6)

	torsion angle	
H(11A)-C(11)-C(12)-H(12B)	175.53(8)	
H(11A)-C(11)-C(12)-H(12A)	-66.54(8)	
H(11B)-C(11)-C(12)-H(12B)	58.16	0.78
H(11B)-C(11)-C(12)-H(12B)	176.09	0.675

The most important conclusions are the following:

1. The structure revealed the existence of an intramolecular hydrogen bond between H(4) and O(1) , as confirmed by the following data.

H-bonding:	C (4)-H (4)·····O (1) (intramolecular)	
	C-H	0.919 (33) Å
	C···O	2.941 (5) Å
	H···O	2.417 (31) Å
	C-H···O	116.22 (2.41) deg
Torsion angle O(1)-P(1)-C(4)-H(4A)		-0.21 (1.98) deg

O(1)-P(1)-N(3)-C(4)-H(4A) is planar (rms deviation 0.027)

The distance between H(4A) and O(1) is in the normal range of hydrogen bonding[135]. On the other hand, the 'P=O' bond length [1.473 (3)Å, Table 18] is very close to those of 14a and 14b [Chapter 3, Table 3, 1.477 (2)Å] with intermolecular hydrogen bonding, while it is larger than that of 14d [Chapter 3, Table 3, 1.453 (2)Å] without a hydrogen bond.

No intermolecular hydrogen bond was observed. Comparing compound 14e and 14a (Chapter 3), both O(1)-P(1)-N(3)-C(4)-H(4A) and O(1)-P(1)-N(3)-H(3) are planar. Compound 14e forms intramolecular hydrogen bonds.

The intramolecular hydrogen bonding of molecule 14e represents an important structural feature. First, in the solid state, the benzyl methylene hydrogens H_{4A} and H_{4B} are highly non-equivalent [the proton which forms hydrogen bond with O(1) was labeled as H_{4A}]. Hydrogen bond serves therefore as a stabilizing force for the particular conformation of the molecule of 14e. Secondly, this conformation can be retained in the solution by this intramolecular hydrogen bonding (rigid conformation). This can account for the high non-equivalence of H_{4A} and H_{4B} observed in the solution by NMR spectroscopy (Figure 39). It can also explain why the chemical shifts of H_{4A} and H_{4B} are very similar in $CDCl_3$ and C_6D_6 (Table 15), while δ_H values of other protons changed dramatically (Table 16), since the conformation of the H(4A)-C(4)-N(3)-P(1)-O(1) skeleton can be retained by this hydrogen bond (Figure 39).

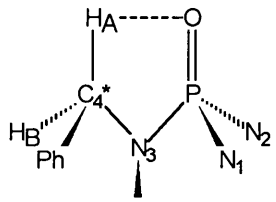


Figure 39

The deshielding effect of the phenyl group are approximately the same for H_{4A} and H_{4B}. Both H_{4A} and H_{4B} are also in the deshielding field of the phosphoryl group, and this anisotropic deshielding effect is now the determining factor responsible for the observed chemical shift differences (Figure 40). The proton H_{4B} is relatively far away from this deshielding field while H_{4A} is locked in a close proximity of the phosphoryl function. The difference in shielding results in the $\Delta\delta_H$

= 220.2 Hz in C_6D_6 (in $CDCl_3$, $\Delta\delta_H$ =170.5 Hz).

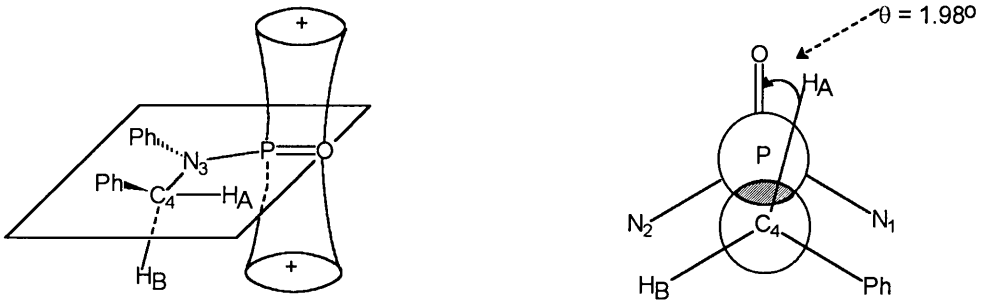
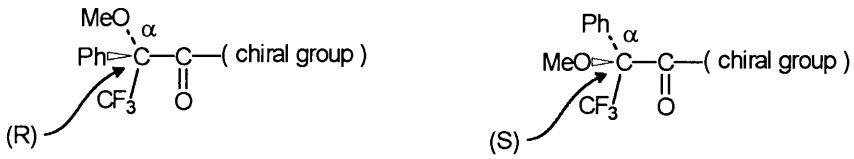


Figure 40

The similar observation was reported by Mosher[136]. They believed that the -CF_3 chemical shift difference observed in the ^{19}F NMR spectra of T1 and T2 (derivatives of Mosher's reagent) results from the anisotropic deshielding of the α- CF_3 substituent by the ester carbonyl group.



T1 T2

2. Some other features of the structure of 14e were helpful in the assignment of signals of H_{1A} and H_{1B} :

(1). All phenyl rings are planar with rms deviations < 0.005 Å.

(2). The P(1)-N(1)-C(1)-C(2)-N(2) ring forms an envelope, with C(1) 0.383 Å above the plane determined by the other atoms (rms deviation =0.031Å) (Figure 41).

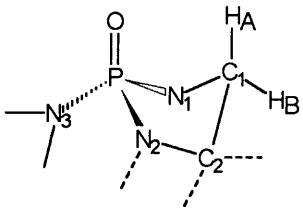


Figure 41

This envelope conformation locates H_{1A} much closer to "P=O" bond than H_{1B} and thus H_{1A} is in more deshielded field and it's chemical shift value is much bigger than that of H_{1B}. On the other hand, the differences of the deshielding effects on H_{2A}, H_{2B} and H_{11A}, H_{11B} are relatively small. Although these methylene hydrogens are too magnetically non-equivalent, the differences of chemical shifts (deshielding effects) are not big enough to allow their separation into two signal groups in ^1H NMR spectrum.

Based on similar reason, H_{12A} and H_{12B} can be assigned, H_{12A} should be closer to "P=O" bond than H_{12B} (Figure 42). From the dihedral angles of the N-chloroethyl group (Table 18), nitrogen and chlorine are in the trans-position.

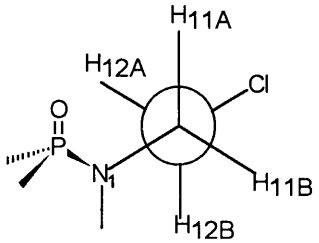


Figure 42

3. Correlation of coupling constants and bond angles

The relationship between coupling constants and molecular parameters is always attractive. In our case, some results can be discussed.

The angle of H_{4A}-C_4-H_{4B} is 111.7 (6) (deg). The geminal coupling constant of H_{4A} and H_{4B} can be obtained from "the geminal Karplus correlation" (Figure 43) [133].

From Figure 43, $J_{gem} \doteq 13$ Hz. This value corresponds reasonably well to the ^1H NMR spectrum of 14e (Table 15), where, J_{gem}=14.5 Hz (C_6D_6) or 14.4 Hz ($CDCl_3$).

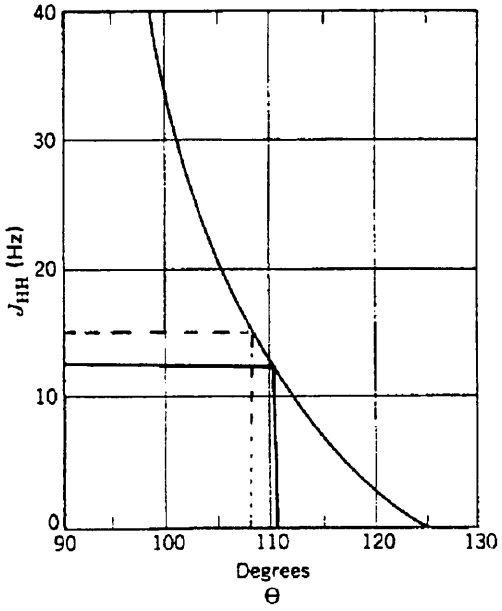


Figure 43 The geminal Karplus correlation. '—' for H_{4A}-C_{14}-H_{4B}, '----' for H_{12A}-C_{12}-H_{12B}

However, the vicinal and geminal Karplus correlation doesn't work well when applied to the N-chloroethyl group (H_{11}-C_{11}-C_{12}-H_{12}). For example, from crystal structure, H_{12A}-C_{12}-H_{12B} angle is

108.0 (8) (deg). From Figure 43, the geminal coupling constant is about 16 Hz. The ^{1}H NMR spectrum of 14e gives J_{gem}=10.8 Hz.

As far as the vicinal relationship of hyrogens is concerned, we used the principle, according to which large values of J are predicted for cis (0^0) and trans (180^0) conformations, but small values of J for gauche (60^0 and 120^0) conformations [137]. In this way, we determined the J_{vic} constants from the values listed in Table 16 for N-chloroethyl group. According to Figure 42, the vicinal coupling constants can be assigned as follows: $J_{H12AH11A}$=6.18, $J_{H12AH11B}$=7.06, $J_{H11BH12B}$=6.87, $J_{H11AH12B}$=7.08 Hz.

Experimental

Inter and intra molecular reaction studies:

1. General (see Chapter 1)
2. General Procedure

Substrate 14a was dissolved in benzene. NaH, TBAB and RX ($PhCH_2Cl$ or MeI) were added into the benzene solution with vigorous stirring at RT. The reaction was monitored by ^{31}P NMR spectroscopy. After the reaction was completed, the mixture was washed by water (50ml x 3). The pure product 14e, or 14f was obtained by column chromatography (silica, ethyl acetate).

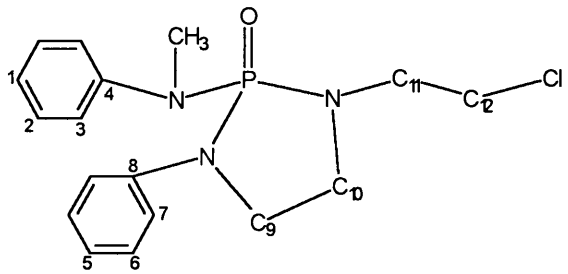
Compound 14f, viscous liquid.

NMR : ($CDCl_3$, ppm)

^{31}P : 17.76

^{1}H :	3.10	(d, 3H, J_{HP}=9.20, CH_3)
	3.18-3.27	(m, 1H, 1H of exo -CH_2N)
	3.19-3.48	(m, 5H, 5H of endo and exo -CH_2N)
	3.63-3.70	(10 signals, 2H, CH_2Cl)
	6.92-7.09	(m, 6H, H of Ph)

	7.17-7.28	(m, 4H, H of Ph)
^{13}C :	38.60	(dq, J_{HC}=138.7, J_{CP}=5.04, CH_3)
	42.40	(dt, J_{HC}=149.6, J_{CP}=4.38, CH_2Cl)
	43.50	(dt, J_{HC}=143.8, J_{CP}=12.75, C_9)
	44.40	(dt, J_{HC}=147.6, J_{CP}=11.77, C_{10})
	46.50	(dt, J_{HC}=138.2, J_{CP}=5.00, C_{11})
	115.6	(dd, J_{HC}=153.1, J_{CP}=4.68, C_7)
	121.3	(d, J_{HC}=159.4, C_5)
	125.1	(d, J_{HC}=161.2, C_1)
	125.5	(dd, J_{HC}=160.1, J_{CP}=2.6, C_3)
	128.8	(d, J_{HC}=158.2, C_6)
	129.1	(d, J_{HC}=160.7, C_2)
	141.7	(s, C_4)
	144.7	(s, C_8)



Ms : m/z 349, 351 (M^+, 100%, 35%)
300 (M^+-CH_2Cl, 86%)
243, 245 (M^+-Ph-NMe, 50%, 19%)
106 (PhNMe, 92%)
77 (Ph, 57%)

Anal. for $C_{17}H_{21}N_3OPCl$ (%)

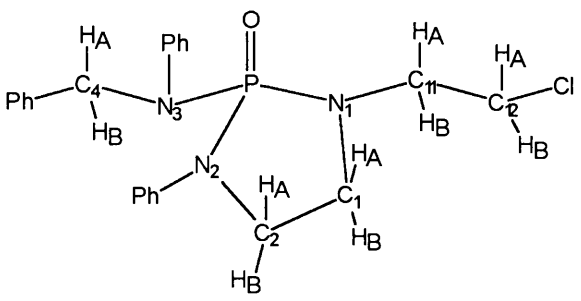
	C	H	N
calcd. :	58.37	6.01	12.02
found :	58.34	6.15	11.89

Compound 14e, solid, mp 103.0-104.5 ^{0}C.

NMR : ($CDCl_3$, ppm)

^{31}P : 17.24

^{1}H :	2.90-3.00	(m, 2H, H_1)
	3.24-3.36	(m, 4H, H_2+H_{11})
	3.52-3.64	(m, 2H, H_{12})
	4.38-4,46	(dd, 1H, J_{gem}=14.5, J_{HP}=9.77, H_{4B})
	4.95-5.02	(dd, 1H, J_{gem}=14.5, J_{HP}=7.74, H_{4A})
	6.66-6.71	(m, 2H, H_{arom})
	6.97	(t, 1H, J_{HH}=7.40, H_{arom})
	7.03-7.13	(m, 7H, H_{arom})
	7.15-7.18	(m, 3H, H_{arom})
	7.25	(t, 2H, J_{HH}=8.47, H_{arom})
^{13}C :	42.16	(dt, J_{HC}=151.4, J_{CP}=4.38, C_{12})
	43.32	(dt, J_{HC}=143.7, J_{CP}=12.2, C_2)
	43.80	(dt, J_{HC}=141.1, J_{CP}=11.47, C_1)
	46.90	(dt, J_{HC}=140.6, J_{CP}=5.13, C_{11})
	55.75	(dt, J_{HC}=139.2, J_{CP}=5.96, C_4)
	115.60	(dd, J_{HC}=159.2, J_{CP}=4.75, C of Ph)
	121.13	(d, J_{HC}=158.4, C of Ph)
	126.16	(d, J_{HC}=137.1, C of Ph)
	127.06-128.97	(m)
	138.70	(s)
	141.60	(s)



NMR : (C_6D_6, ppm)

^{31}P : 16.40

^1H :	2.30-2.40	(m, 1H, H_{1B})
	2.41-2.52	(m, 1H, H_{1A})
	2.60-2.72	(m, 2H, H_2)
	3.04-3.18	(m, 2H, H_{11})
	3.28-3.38	(sixtet, 1H, J_{gem}=10.80, J_{vic}=6.87, J_{vic}=7.08, H_{12B})
	3.43-3.51	(octet, 1H, J_{gem}=10.80, J_{vic}=6.18, J_{vic}=7.14, H_{12A})
	4.40-4.48	(dd, 1H, J_{gem}=14.40, J_{HP}=10.33, H_{4B})
	5.15-5.23	(dd, 1H, J_{gem}=14.40, J_{HP}=7.76, H_{4A})
	6.75-7.27	(m, 15H of Ph)

Ms : m/z
425, 427 (M^+, 16.5%, 6.5%)
376 (M^+-CH_2Cl, 2.1%)
348 (M^+-Ph, 1.0%)
244, 246 (M^+-PhNCHPh, 20.3%, 7.6%)
182 ($PhNCH_2Ph$, 100%)
106 ($PhNCH_3$, 52.1%)
91 ($PhCH_2$, 22.7%)
77 (Ph, 9.2%)

Anal. for $C_{23}H_{25}N_3OPCl$ (%)

	C	H	N
calcd. :	64.86	5.88	9.87
found :	64.83	6.00	9.86

X-ray diffraction:

1. General (see Chapter 3 and Table 19).

Table 19. Crystallographic data and experimental details

Identification code	mod 14e
Empirical formula	C_{23} H_{25} Cl N_3 O P
Formula weight	425.88

Temperature	293(2) K
Wavelength	0.71070 Å
Crystal system	Monoclinic
Space group	P2(1)/c
Unit cell dimensions	a = 9.176(2) Å alpha = 90 deg.
	b = 9.799(2) Å beta = 91.590(10) deg.
	c = 24.913(4) Å gamma = 90 deg.
Volume	2239.2(8) A^3
Z	4
Density (calculated)	1.263 Mg/m^3
Absorption coefficient	0.261 mm^-1
F(000)	896
Crystal size	0.15 x 0.15 x 0.18 mm
Theta range for data collection	1.64 to 24.97 deg.
Index ranges	-8<=h<=10, 0<=k<=11, 0<=l<=29
Reflections collected	3723
Independent reflections	3723 [R(int) = 0.0000]
Absorption correction:	
Min, max, ave transmission	0.952, 0.997, 0.971
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3723 / 0 / 271
Goodness-of-fit on F^2	0.839
Final R indices [I>2sigma(I)]	R1 = 0.0547, wR2 = 0.1117
R indices (all data)	R1 = 0.1698, wR2 = 0.1296
Largest diff. peak and hole	0.215 and -0.243 e.A^-3

2. The single crystal of 14e was grown from benzene by slow evaporation.

Conclusion

And

References

Conclusions

In the first part of this Thesis (Chapter 1), we confirmed that N-phosphorylated mustard system shows diverse reactivity when it is activated by the development of a negative charge at the adjacent phosphate oxygen. The fragmentation experiments indicate that higher nucleophilicity of the phosphate moiety (diamidate), and a better leaving group in the β-position of the N-ethyl function ('Cl') are necessary for a spontaneous fragmentation (dephosphorylation) of the alkylation product. The fragmentation of phosphoramidate strongly depends on the structure of the substrates (eg. compounds 1 and 11) and the media (eg. LiI/ acetone-d_6, pyridine-d_5/ D_2O and PhSH/ Et_3N/ acetonitrile-d_3). The methyl diamidate (1) undergoes a slower demethylation process, but forms a highly reactive anion (O-demethylated derivative). This anion can fragment spontaneously through 1,3-cyclization, P-N bond cleavage, and 1,5-cyclization. The fragmentation mechanism of phosphoramidate shows that 1, 5-cyclization process is a relatively slower process than 1, 3-cyclization and intermolecular reaction. This conclusion is further confirmed by the experiments of the inter- and intramolecular nucleophilic reactions (Chapter 5). However, these competitive reactions depend on the substrates; when MeI is involved, the reaction process shows unexpected results. Further reseach on this topic is necessary.

The structure study of triamidates (and amidoesters) shows that a chiral phosphosphoramidate (eg. 14a) has strong interactions with an optically active acid (eg. R) via H-bonding when the torsion angle O=P-N-H of phosphoramidate is close to zero (syn-periplanar). It is concluded that the geometry of the >P(O)NH function of phosphoramidate, which is modified by the introduction of different functions to the phosphorus moiety, is essential for phosphoramidate in the chiral recognition via H-bonding interactions and the design of useful reagents which are capable of chiral recognition is possible.

The phosphorus NMR chemical shifts of non-, mono-, di-, tricyclic phosphotriamidates are ranged in different areas. The correlations of ^{31}P NMR chemical shifts and N-P-N bond angles show that the decrease of N-P-N bond angles (average) leads to a high field shift of ^{31}P NMR chemical signals. This trend is consistent with the conclusions obtained by other researchers for 'S-P-S' and 'O-P-O' compounds. It once again confirmed that bond angles around phosphorus are important factors to affect the phosphorus chemical shift.

References

(1) E. W. Walsh, E. J. Griffith, R. W. Parry, L. D. Quin, ***Phosphorus Chemistry Development in Science ACS Symposium*** Series 486, Washington D. C. : Am. Chem. Soc., 1992.

(2) C. Fest, K. -J. Schmidt, ***The Chemistry of Organophosphorus Pesticides***, 2nd Ed., Berlin : Springer, 1982.

(3) B. L. Brown, O. R. M. Dobson, ***Advances in Second Messenger and phosphoprotein Research,*** New York : Raven, c1988.

(4) D. E. C. Corbridge, ***Phosphorus : An Outline of Its Chemistry, Biochemistry and Technology,*** 2nd Ed., Amsterdam : Elsevier, 1980.

(5) D. Eggleton, G. P. Eggleton, ***Biochem. J.,*** **21**, 190(1927).

(6) C. H. Fiske, Y. Subbarow, ***J. Biol. Chem.,*** **81**, 629(1929).

(7) H. E. Hurleg, ***Science,*** **164**, 1356(1969).

(8) S. A. Kuby, E. A. Noltanann, ***Enzymes,*** 2nd Ed., **6,** 515(1962).

(9) M. Cohn, J. Reuben, ***Accounts Chem. Res.,*** **4**, 214(1971).

(10) H. Goldwhite, ***Introduction to Phosphorus Chemistry,*** Cambridge University Press, 1981.

(11) H. Dugas, C. Penney, ***Bioorganic Chemistry : A Chemical Approach to Enzyme Action,*** New York : Springer, 1981.

(12) I. -L. Lassaign, ***Chimie Physique,*** **13**(2, série), 294(1820).

(13) A. Michaelis, R. Kaehne, ***Chem. Ber.,*** **31**, 1048(1898).

(14) A. E. Arbuzov, ***J. Russ. Phys. Chem. Soc.,*** **38**, 687(1906).

(15) D. B. Denney, D. Z. Denney, G. DiMiele, ***Phosphorus and Sulfur,*** **4**, 125(1978).

(16) W. J. Stec, ***Phosphorus Chemistry Directed Towards Biology,*** Pergamon press, 1979.

(17) T. A. Modro, B. P. Rijkmans, ***J. Org. Chem.,*** **47**, 3208(1982).

(18) T. A. Modro, M. A. Lawry, E. Murphy, ***J. Org. Chem.,*** **43**, 5000(1978).

(19) P. Haake, P. S. Ossip, ***J. Am. Chem. Soc.,*** **93**, 6924(1971).

(20) T. Koizumi, P. Haake, ***J. Am. Chem. Soc.,*** **95**, 8073(1973).

(21) C. R. Hall, T. D. Inch, ***Tetrahedron Lett.,*** **42**, 3761(1977).

(22) A. Zwierzak, ***Synthesis,*** 332(1982); A. Zwierzak, I. Podstawczyñska, ***Angew. Chem. Int. Ed. Engl.,*** **10**, 16(1977).

(23) J. Emsley, D. Hall, ***The Chemistry of Phosphorus,*** Harper and Row,

London, Chap. 10, 1976.

(24) A. Hung, J. W. Gilje, ***J. Chem. Soc. Chem. Commun.,*** 662(1972).

(25) S. Trippett, P. J. Whittle, ***J. Chem. Soc. Perkin Trans. I,*** 2302(1973).

(26) J. S. Harman, D. W. A. Sharp, ***Inorg. Chem.,*** **10**, 1538(1971).

(27) J. Emsley, J. K. Williams, ***J. Chem. Soc.,*** 1576(1973).

(28) S. J. Benkovic, E. J. Sampson, ***J. Am. Chem. Soc.,*** **93**, 4009(1971).

(29) G. W. Allen, P. Haake, ***ibid,*** **95**, 8080(1973).

(30) M. J. P. Harger, ***J. Chem. Soc. Perkin Trans. I,*** 2057(1977).

(31) P. Haake, T. Koizumi, ***Tetrahedron Lett.,*** **55**, 4845(1970).

(32) P. Haake, D. A. Tyssee, ***Tetrahedron Lett.,*** **40**, 3513(1970).

(33) D. A. Tyssee, L. P. Baushu, P. Haake, ***J. Am. Chem. Soc.,*** **95**, 8066(1970).

(34) M. J. P. Harger, ***J. Chem. Soc. Perkin Trans. II,*** 154(1980).

(35) S. Hanessian, Y. L. Bennani, D. Delorme, ***Tetrahedron Lett.,*** **31**, 6465(1990).

(36) S. Hanessian, Y. L. Bennani, ***Tetrahedron Lett.,*** **31**, 6461(1990).

(37) R. Jacquier, M. Lhassani, C. Petrus, F. Petrus,
Phosphorus, Sulfur, and Silicon, **81**, 83(1993).

(38) M. Yoshifuji, K. Kamijo, K. Toyota, ***Tetrahedron Lett.,*** **35**, 397(1994); M. Yoshifuji, S. Sangu, K. Kamijo, K. Toyota, ***J. Chem. Soc. Chem. Commun.,*** 297(1995).

(39) V. I. Lachkova, G. Petrov, A. H. Hussein, ***Phosphorus, Sulfur, and Silicon,*** 85, 161(1993).

(40) D. R. Bond, T. A. Modro, M. L. Niven, L. R. Nassimbeni, ***S. Afr. J. Chem.,*** **38**, 78(1985).

(41) M. P. du Plessis, T. A. Modro, L. R. Nassimbeni, ***J. Org. Chem.,*** **47**, 2313(1982).

(42) D. E. C. Corbridge, ***The Structure Chemistry of Phosphorus,***
Elsevier Scientific Publishing Company, 1974.

(43) D. F. Peppand, J. R. Ferraro, G. W. Manson, ***Inorg. Nucl. Chem.,***
7, 231(1958); **12**, 60(1959); **16**, 246(1963).

(44) H. Wan, A. M. Modro, T. A. Modro, S. Bourne, L. R. Nassimbeni,
J. Phys. Org. Chem., 1996, 9, in press.

(45) V. I. Shevchenko, A. A. Koval, ***Zh. Obshch. Khim,*** **37**, 1053(1967).

(46) O. M. Friedman, A. Myles, M. Colvin, ***Advances in Cancer Chemotherapy,***
New York, Vol 11, Chap. 4, 1979.

(47) O. M. Friedman, A. M. Seligman, ***J. Am. Med. Assoc.,*** **132**, 126(1946).

(48) O. M. Friedman, A. M. Seligman, ***J. Am. Chem. Soc.,* 76**, 655(1954).

(49) O. M. Friedman, E. Boger, V. Grubliauskas, H. Sommer, ***J. Med. Chem.,* 6**, 50(1963).

(50) H. Anold, F. Bourseaux, ***Angew. Chemie,* 70**, 539(1958).

(51) D. L. Hill, ***A Review of Cyclophosphamide*** (Editor-C. C. Thomas), Springfield Illinois(1975).

(52) G. Gomori, ***Proc. Soc. Exp. Biol. Med.,* 69**, 407(1948).

(53) G. Zon, ***Prog. Med. Chem.,* 19**, 205(1982).

(54) P. J. Cox, P. B. Farmer, M. Jarman, ***Cancer Treat. Rep.,* 60**, 299(1976).

(55) W. J. Stec, ***Organophosphorus Chem.,* 13**, The Royal Society of Chemistry, 145(1982).

(56) N. E. Sladek, ***Pharm. Ther.,* 37**, 301(1988).

(57) C. H. Kwon, K. Y. Moon, N. Baturay, F. N. Shirota, ***J. Med. Chem.,* 34**, 588(1991).

(58) W. J. Stec, ***Specialist Periodical Reports : Organophosphorus Chemistry,*** The Royal Society of Chemistry, London, Vol 13, Chap. 8, 1982.

(59) T. W. Engle, G. Zon, W. Egan, ***J. Med. Chem.,* 25**, 1347(1982).

(60) C. L. Maddock, A. H. Handler, O. M. Friedman, G. E. Foley, S. Farber, ***Cancer Chemother. Rep.,* 50**, 629(1966).

(61) M. Colvin, R. B. Brundrett, M. N. N. Kan, I. Jardine, C. Fenselau, ***Cancer Res.,* 36**, 1121(1976).

(62) J. R. Metha, M. Przybylski, D. B. Ludlum, ***Cancer Res.,* 40**, 4183(1980).

(63) V. T. Vu, C. C. Fenselau, O. M. Colvin, ***J. Am. Chem. Soc.,* 103**, 7362(1981).

(64) H. Lindemann, E. Harbers, ***Arzneim. -Forsch.,* 30**, 2075(1980).

(65) L. C. Erickson, L. M. Ramonas, D. S. Zaharko, K. W. Kohn, ***Cancer Res.,* 40**, 4216(1980).

(66) C. le Roux, A. M. Modro, T. A. Modro, ***J. Org. Chem.,* 60**, 3832(1995).

(67) C. J. O'Connor, W. A. Denny, J. Y. Fan, G. L. Gravalt, B. A. Gregor, D. J. McLennan, ***J. Chem. Soc. Perkin Trans. II,*** 1933(1991).

(68) C. McGuigan, P. Narashiman, ***Synthesis,*** 311(1993).

(69) C. le Roux, A. M. Modro, T. A. Modro, ***J. Chem. Res.(S),*** 38(1995).

(70) P. B. Farmer, P. J. Cox, ***J. Med. Chem.,* 18**, 1106(1975).

(71) S. Bauermeister, A. M. Modro, T. A. Modro, A. Zwierzak, ***Can. J. Chem.***, 69, 811(1991).

(72) A. D. F. Toy, E. N. Walsh, ***Phosphorus Chemistry in Everyday Living,*** 2nd Ed., Washington D. C. : Am. Chem. Soc., 1987.

(73) J. V. Van de Grampel, B. de Ruiter, ***Specialist Periodical Reports : Organophosphorus Chemistry,*** The Royal Society of Chemistry, London, Vol 15, Chap. 9 (1985), and X. W. Allew in the following volumes.

(74) J. -F. Labarre, ***Top. Current Chem.,*** **102**, 1(1982).

(75) B. C. Saunders, ***Phosphorus and Fluorine,*** Cambridge at the University Press, 1957.

(76) W. A. L. David, ***Nature*** London., **166**, 72(1950).

(77) J. E. Gardiner, B. A. Kilby, ***J. Chem. Soc.,*** 1796(1950).

(78) T. M. Beck, E. N. Walsh, ***U. S. Patent, 3,235,517***; Feb., 16, 1966.

(79)C. P. de Jongh, A. Zwierzak, T. A. Modro, ***Phosphorus, Sulfur, and Silicon,*** **61**, 205(1991); M. Froneman, D. L. Cheney, T. A. Modro, ***Phosphorus, Sulfur, and Silicon,*** **47**, 273(1990).

(80) H. Wan, T. A. Modro, ***Phosphorus, Sulfur, and Silicon,*** **108,** 155(1996).

(81) H. Wan, T. A. Modro, ***Synthesis,*** 1996, 6, in press.

(82) C. le Roux, S. Bauermeister, T. A. Modro, ***J. Chem. Res. (S),*** 372(1993).

(83) B. Davidowitz, T. A. Modro, ***Org. Mass. Spectr.,*** **19,** 128(1984).

(84) S. Cocks, and T. A. Modro, ***Tetrahedron Lett.,*** **26,** 945(1985).

(85) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and C. A. Vermoon, ***J. Chem. Soc.,*** 3574(1958).

(86) D. F. Health, ***J. Chem. Soc.,*** 3796(1956).

(87) a) L. D. Quin, S. Jankowski, ***J. Org. Chem.,*** **59,** 4402(1994).

b) R. Bodalski, and L. D. Quin, ***J. Org. Chem.,*** **56,** 2666(1991).

(88) O. M. Friedman, S. Bien, and T. K. Chakrabarti, ***J. Am. Chem. Soc.,*** **87,** 4978(1965).

(89) a) L. D. Quin, J. Szewczyk, K. M. Szewczyk, and T. McPhail, ***J. Org. Chem.,*** **51,** 3341(1986).

b) L. D. Quin, N. D. Sadanani, C. Bourdien, X-P. Wu, G. S. Quin, and R. Bodalski, ***Phosphorus, Sulfur, and Silicon,*** **49/50,** 313(1990).

c) L. D. Quin, C. Bourdieu, and G. S. Quin, ***Phosphorus, Sulfur, and Silicon,*** **63,** 349(1991).

(90) G. W. Daub, and E. E. Van Tamelen, ***J. Am. Chem. Soc.,*** **99,** 3526(1977).

(91) G. W. Klump, ***"Reactivity in Organic Chemistry,"*** Wiley-Interscience, New York, 1982, Chap. 3.2.

(92) S. Bauermeister, A. M. Modro, T. A. Modro, ***Tetrahedron Lett.,*** **30,** 2141(1990).

(93) J. K. Chakrabarti, and O. M. Friedman (2), ***J. Heterocycl. Chem.*, 10,** 55(1973).

(94) G. Zon, S. M. Ludeman, and W. Egan, ***J. Am. Chem. Soc.*, 99,** 5785(1977).

(95) W. J. Stec, A. Okruszek, K. Lesiak, B. Uznanski, J. Michalski,
***J. Org. Chem.*, 41,** 227(1976).

(96) J. Baraniak, K. Lesiak, M. Sochacki, W. J. Stec,
***J. Am. Chem. Soc.*, 102,** 4533(1980).

(97) J. A. Coderre, S. Mehd, J. A. Gerlt, ***J. Am. Chem. Soc.*, 103,** 1872(1981).

(98) C. le Roux, ***Ph.D Thesis,*** University of Pretoria, Chapter 7, 1994.

(99) C. le Roux, ***Ph.D Thesis,*** University of Pretoria, Page 92, 1994.

(100) C. C. Orji, J. H. Reibenspies, E. A. Meyers, and A. G. Pinkus,
***Phosphorus, Sulfur, and Silicon,* 97,** 9(1994).

(101) Ru-Yu Chen, ***Phosphorus, Sulfur, and Silicon,* 89,** 89(1994).

(102) D. G. Gorenstein, ***Phosphorus-31 NMR;*** Academic Press : Orlando, 1984, Chapter 1.

(103) J. Martin, J. B. Robert, ***Org. Magn. Reson.*, 15,** 87(1981).

(104) D. G. Gorenstein, ***J. Am. Chem. Soc.*, 97,** 898(1975).

(105) D. G. Gorenstein, R. P. Meadous, J. T. Metz, E. P. Nikonowicz, C. B. Post, ***Advances in Biophysical Chemistry;*** Bush, C. A., Ed.; JAI Press : Greenswich, 1990, pp47-124.

(106) D. B. Lerner, D. R. Kearns, ***J. Am. Chem. Soc.*, 102,** 7612(1980).

(107) D. G. Gorenstein, ***Annu. Rev. Biophys. Bioengin,* 10,** 355(1981).

(108) I. G. M. Campbell, and J. K. Way, ***J. Chem. Soc.,*** 5034(1960).

(109) Z. Glowacki, M. Hoffman, and J. Rachon,
***Phosphorus, Sulfur, and Silicon,* 104,** 21-32(1995).

(110) Z. Glowacki, M. Hoffman, and J. Rachon,
***Phosphorus, Sulfur, and Silicon,* 82,**39(1993).

(111) T. Oshikawa, M. Yamashita, S. Kumagai, K. Seao, and J. Kobayashi,
J. Chem. Soc. Chem. Commun, 435(1995).

(112) Scott E. Denmark, and Jung-Ho Kim, ***J. Org. Chem.*, 60,** 7335(1995).

(113) F. H. Allen, O. Kennard, D. G. Watson, L. Bramner, A.G. Orpen, and R. Taylor,
J. Chem. Soc. Perkin Trans. II, 2S1-S19(1987).

(114) D. W. White, B. A. Karcher, R. A. Jacobson, and J. G. Verkade,
***J. Am. Chem. Soc.*, 101,** 4921(1979).

(115) D. R. Bond, T. A. Modro, and L. R. Nassimbeni, ***J. Org. Chem.,* 50,** 2281(1985).

(116) J. Bernstein, R. E. Davies, L. Shimoni, and N. L. Chang,
***Angem. Chem. Int. Ed. Engl.,* 34,** 1555(1995).

(117) L. J. Bellamy, and L. Beecher, ***J. Chem. Soc.,*** 1701(1952).

(118) N. S. Isaacs, ***Physical Organic Chemistry,*** Longman Scient., Burnt Hill, p64 (1987).

(119) G. M. Sheldrick, SHELX-93 : A Program for Crystal Structure Determination,
J. Appl. Crystallogr., in preparation.

(120) H. Dronia, S. Failla, P. Finocchiaro, U. Grup, and G. Hägele,
***Phosphorus, Sulfur, and Silicon,* 101,** 149(1995).

(121) J. G. Verkade, L. D. Quin, ***Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis : Organic Compounds and Metal Complexes,*** 1987 VCH Publisher Inc.

(122) H. S. Gutowsky, D. W. McCall, ***J. Chem. Phys.,* 22,** 162(1954).

(123) J. H. Letcher, J. R. Van Wazer, ***J. Chem. Phys.,* 44,** 815(1966).

(124) J. H. Letcher, J. R. Van Wazer, ***Top. Phosphorus Chem.,* 5,** 751(1967).

(125) J. Kumamoto, J. R. Cox, F. H. Westheimer, ***J. Am. Chem. Soc.,* 78,** 4858(1956).

(126) G. M. Blackburn, J. S. Cohen, I. Weatherall, ***Tetrahedron,* 27,** 2903(1970).

(127) R. O. Day, G. D. Gorenstein, R. R. Holmes, ***Inorg. Chem.,* 22,** 2192(1983).

(128) H. Kayama, T. Yoshino, ***Bull, Chem. Soc. Jpn.,* 45,** 481(1972).

(129) M. J. Gallagher in ***Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis,***
Ed. J. G. Verkade and L. D. Quin, VCH, Deerfield Beach, 1987, p.299.

(130) R. Chen, and E. Breuer, ***Phosphorus, Sulfur, and Silicon,* 111,** 165(1996).

(131) Q. J. Yan, Q. Wang, and Y. F. Zhao,
***Phosphorus, Sulfur, and Silicon,* 107,** 181(1995).

(132) I. Tashma, ***Tetrahedron,* 38,** 3745(1982).

(133) R. M. Silverstein, G. C. Bassler, T. C. Morrill, ***Spectrometric Identification of Organic Compounds,*** Fifth Edition, John Wiley & Sons, Inc., New York, 1991.

(134) C. P. de Jongh, ***Ph.D Thesis,*** University of Pretoria, 1992, p68.

(135) A. Irving, ***J. Crystal. and Spectroscopy Res.,* 22,** 533(1992).

(136) G. R. Sullivan, J. A. Dale, H. S. Mosher, ***J. Org. Chem.,* 38,** 2143(1973).

(137) E. D. Becker, ***High Resolution NMR,*** New York : Academic Press, 2nd Ed, 1980.