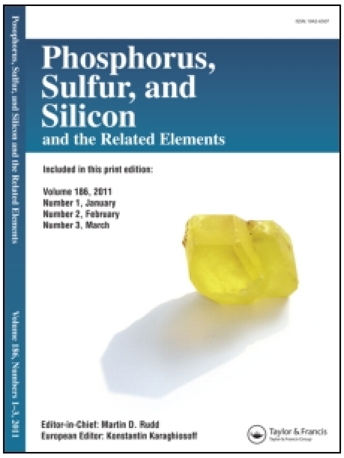

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CHEMISTRY OF N-PHOSPHORYLATED NITROGEN MUSTARDS: THE EFFECT OF A SECOND NITROGEN SUBSTITUENT AT PHOSPHORUS ON THE STABILITY OF THE SYSTEM

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CHEMISTRY OF N-PHOSPHORYLATED NITROGEN MUSTARDS: THE EFFECT OF A SECOND NITROGEN SUBSTITUENT AT PHOSPHORUS ON THE STABILITY OF THE SYSTEM

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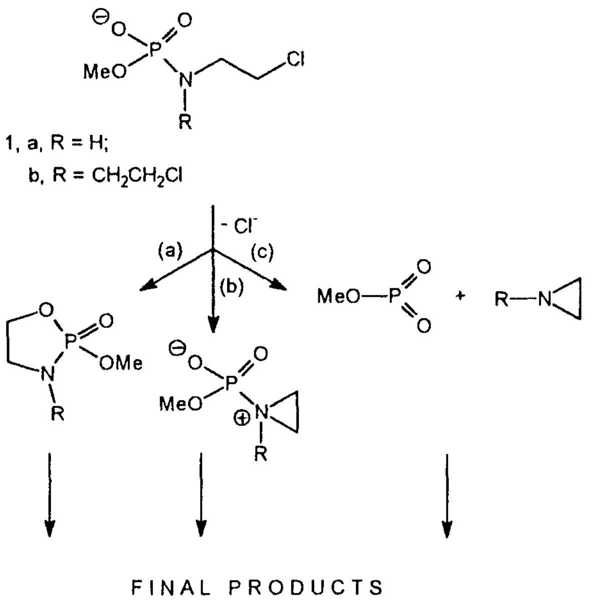
Methyl N,N-diethyl-N'N'-bis(2-chloroethyl)phosphoramidate was prepared as a precursor for the corresponding phosphordiamidate anion, a model for the phosphoramidate mustard, biologically active degradation product of cyclophosphamide drug. Demethylation of the precursor led to a highly unstable ion which underwent spontaneous fragmentation. In the absence of an external nucleophile, the ion decomposed yielding metaphosphoramidate and N-substituted ethyleneimine as primary intermediates. In the presence of pyridine, bis-alkylation of two pyridines took place yielding bis [2-(N-pyridinio)ethyl]amine dication, in addition to some 1,3,2-oxazaphospholidine derivative, formed via the competitive 1,5-cyclization of the demethylated anion. Incubation of the precursor in the presence of thiophenol/triethylamine resulted in two parallel nucleophilic displacements: (i) the O-demethylation followed by bis-alkylation of two molecules of thiophenol, together with some 1,5-cyclization; (ii) initial direct displacement of the chlorine at the β-carbon of the precursor, followed by the fragmentation of the system. It is concluded that the electron-rich ionic phosphoramidate substituent, $^{-}O(R_2N)P(O)$, highly activates the N-(2-chloroethyl) functional group in alkylation reactions.

Key words: Methyl N,N-diethyl-N',N'-bis(2-chloroethyl)phosphordiamidate, dimethyl N,N-diethylphosphoramidate, methyl N,N,N',N'-tetraethylphosphordiamidate, methyl N,N-diethyl-N',N'-bis[2-(mercaptophenyl)ethyl]phosphordiamidate, dimethyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidate, stability.

INTRODUCTION

In the first part of this investigation[1] we have demonstrated that the anions of methyl N-(2-chloroethyl) [or N,N-bis(2-chloroethyl)] phosphoramidates (**1**), prepared as models of N-phosphorylated mustards, decompose easily yielding a variety of primary and secondary products. The fragmentation, triggered by the accumulation of the negative charge at the phosphate group, can follow the 1,5- and the 1,3-cyclization mechanism, the latter proceeding with or without retention of the P—N bond (Scheme I). Contribution of the individual pathways is important, since routes (b) and (c) result in the formation of the ethyleneimine derivatives that can act as biologically critical alkylating (or bis-alkylating) agents, while the 1,3,2-oxazaphospholidine product (pathway a) is devoid of further alkylating reactivity. Since the phosphoramidate mustard, $HO(H_2N)P(O)N(CH_2CH_2Cl)_2$, responsible for the alkylating reactivity of the anticancer drug cyclophosphamide[2] belongs to the family of phosphor*diamidates*, we have extended our reactivity studies on the model substrate belonging to that family, namely the N-N-diethyl-N',N'-bis(2-chloroethyl)phosphordiamidate anion (**2**). It seemed of interest to determine the effect of the increase in the nucleophilicity of the phosphate moiety (second nitrogen substituent at phos-

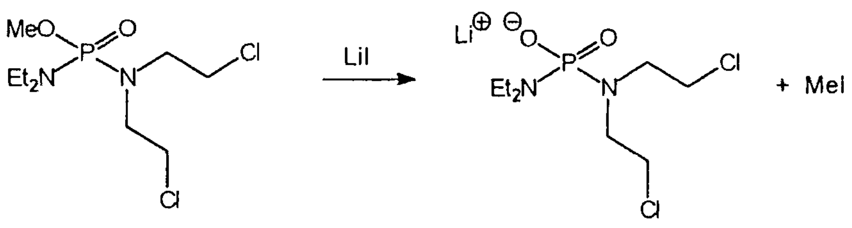


SCHEME I

phorus) on: (i) the intrinsic stability of the system; (ii) the mechanism of the fragmentation.

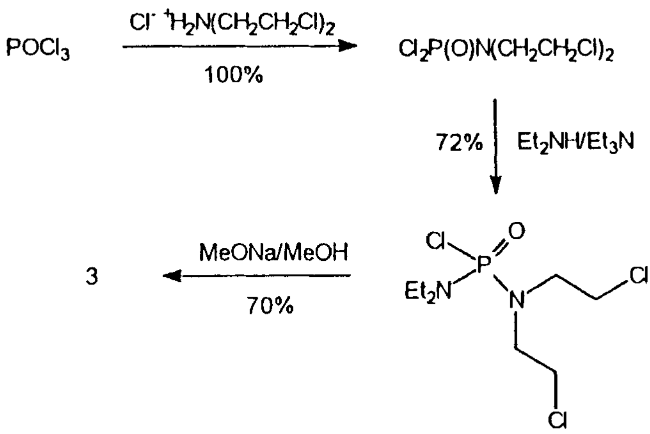
RESULTS AND DISCUSSION

As before,[1] we attempted to prepare **2** as a lithium salt by the nucleophilic O-demethylation of the corresponding diamidoester precursor **3** (Equation 1). Preparation of substrate **3** presented some problem in itself, as the sequence of the introduction of substituents at phosphorus in $POCl_3$, as well as the reaction conditions, determine the final outcome of the synthesis.[3] Finally **3** was prepared in good yield according to Equation 2. Contrary to the behavior of the lithium salt of the ester analogue, **1b**, which could be stored in the solid state in a refrigerator, salt **2** proved so unstable that we were never able to isolate it. Under standard conditions (LiI in refluxing 2-butanone) **3** reacted easily, but no precipitation of the lithium phosphordiamidate salt



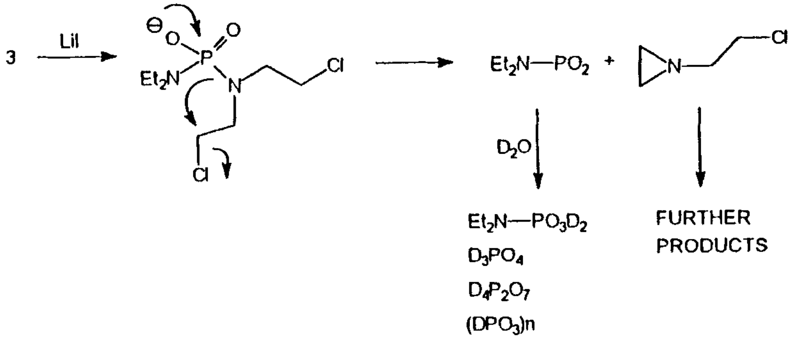
3 2

(1)



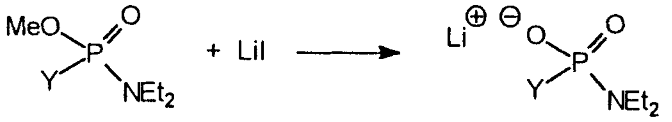
(2)

was observed. Instead, a polymeric material separated out indicating fast and spontaneous decomposition of the initially formed anion **2**. The initial demethylation of **3** was demonstrated in a separate experiment by the formation of iodomethane, identified by ^1H NMR spectroscopy. The decomposition product was filtered off and the soluble and insoluble products were examined by NMR spectroscopy. The insoluble material was dissolved in D_2O and the ^{31}P NMR spectrum revealed signals at δ_P 8.5, -0.2, -10.0, and -21.2; the ^1H NMR spectrum showed only the presence of the Et_2N function. Taking advantage of the extensive NMR spectroscopic data reported by Quin *et al.* on the secondary products formed from the free metaphosphoramidates generated in solution,[4] we identified the four phosphorus-containing products as triethyl ammonium salts of N,N-diethylphosphoramidic acid, and phosphoric, diphosphoric, and polyphosphoric acids, respectively. The 2-butanone solution was diluted with hexane, the oily material that separated out was dissolved in $CDCl_3$ and examined by NMR spectroscopy. ^{31}P NMR spectrum demonstrated absence of any P-containing products, while ^1H NMR spectrum indicated 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. The observed ranges correspond closely to those reported[1] for the N-substituted ethyleneimine and N,N-bis-(2-substituted ethyl)amine type of compounds. We conclude therefore that anion **2** is intrinsically unstable and decomposes spontaneously via the unimolecular fragmentation to the metaphosphoramidate and N-substituted ethyleneimine as reactive intermediates (Equation 3).



(3)

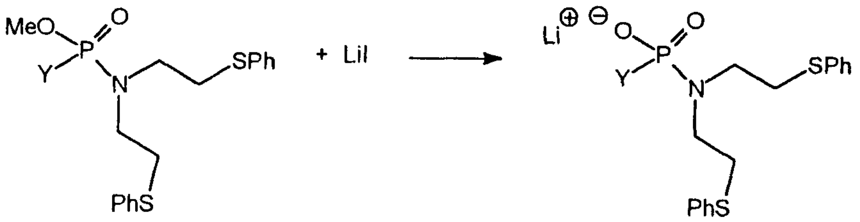
In order to compare the stability of **2** with that of other phosphoramidate anions, dimethyl N,N-diethylphosphoramidate (**3a**) and methyl N,N,N′,N′-tetraethylphosphordiamidate (**3b**) were demethylated with LiI yielding the corresponding salts that were found to be indefinitely stable, both in solution, and in the solid state (Equation 4). Demethylation of **3a** and **3b** was then compared with that of their analogues (**3c** and **3d**) containing the N-bis[2-(mercaptophenyl)ethyl] substituent in place of the NEt$_2$ group (Equation 5). Only **3c** gave a stable Li salt; **3d** behaved similarly to **3**,



(4)

3a, Y = OMe

3b, Y = NEt$_2$



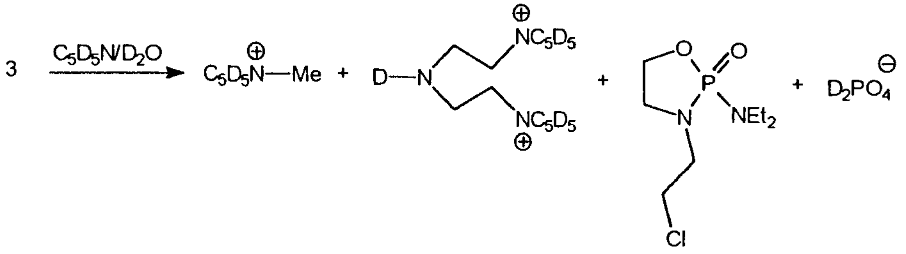
(5)

3c, Y = OMe

3d, Y = NEt$_2$

i.e. the disappearance of the substrate was accompanied by the formation of fragmentation products (*vide infra*). The methyl esters **3** differ significantly in the rates of their O-demethylation by LiI. The half-lives of the demethylation by LiI in refluxing 2-butanone were determined by monitoring the decay of the ester by ^{31}P NMR spectroscopy, and the following order of $t_{1/2}$ (h) was obtained: **3a**, 22.5; **3b**, >200; **3c**, 5; **3d**, 57. It is clear that the ***diamidate*** esters are much less reactive in the demethylation reaction (the leaving group representing a conjugate base of a much weaker acid), and that the β-substitution at the NEt$_2$ function activates the substrate toward dealkylation. On the other hand, the products of the demethylation of the diamidates, although produced in a slow reaction, if containing a potential leaving group in the β-position of the N-ethyl group (**2** and the demethylation product of **3d**) are highly unstable and decompose spontaneously as soon as they are formed. Those structural conditions are essential for a phosphoramidate precursor to serve as an alkylating (hence, biologically active) agent.

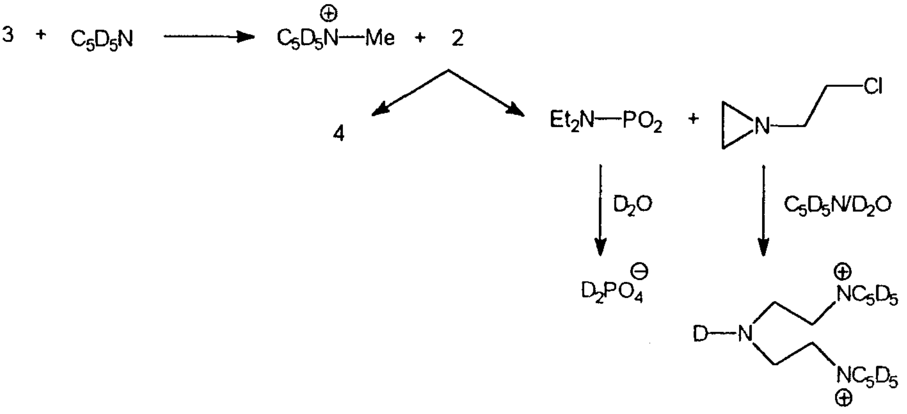
Alkylating reactivity of the ion **2** was then tested by generating **2** from **3** in the D_2O/pyridine-d_5 mixture.[1] It was assumed that pyridine will serve two nucleophilic functions: (i) it will displace the O-methyl group in **3**[5] yielding **2**, and (ii) it will act as a nucleophilic trapping agent toward the primary products of the fragmentation of **2**. The reaction was carried out in a 5:1 (v/v) pyridine-d_5/D_2O solution at 60°C, and the course of the reaction was monitored by NMR (^1H and ^{31}P) spectroscopy. The products formed were identified by the addition of the authentic samples to the examined solution. After ca 150 h **3** disappeared completely, and the product had a composition as shown in Equation 6. The results indicate clearly that **3** undergoes



4

(6)

demethylation to **2** (formation of the N-methylpyridinium ion), and that that intermediate decomposes under the reaction conditions. The ethyleneimine derivative formed is trapped by pyridine to yield the bis-alkylated product, bis-[2-(N-pyridinio)ethyl]amine dication. The metaphosphoramidate intermediate is finally converted to orthophosphate; the 1,3,2-oxazaphospholidine derivative **4** results from the 1,5-cyclization occurring parallel to the fragmentation of **2** (Scheme I, pathway a). At no stage of the reaction were we able to detect an intermediate containing the 2-(N-pyridinio)ethyl substituent with the *retained* P—N bond; we conclude therefore that the cleavage of the P—N linkage occurs *before* the nucleophilic opening of the ethyleneimine function (Scheme I, pathway c). Reaction of **3** in D_2O/C_5D_5N mixture can be therefore presented by Equation 7.



(7)

Next experiment involved application of the thiophenoxide ion as a nucleophilic reagent. The PhS^- ion was chosen because of its use in the deprotection of methylated oligonucleotides,[6] and because of its high nucleophilicity,[7] expected to make that ion a good probe for the alkylating reactivity of the phosphorylated nitrogen mustards. The reaction system consisted of the solution of four mole-equivalents of

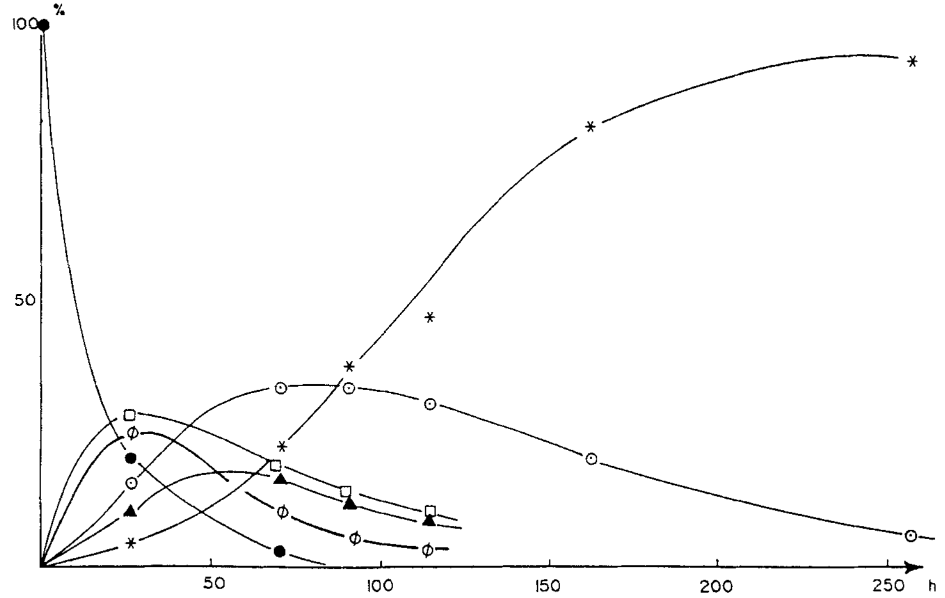
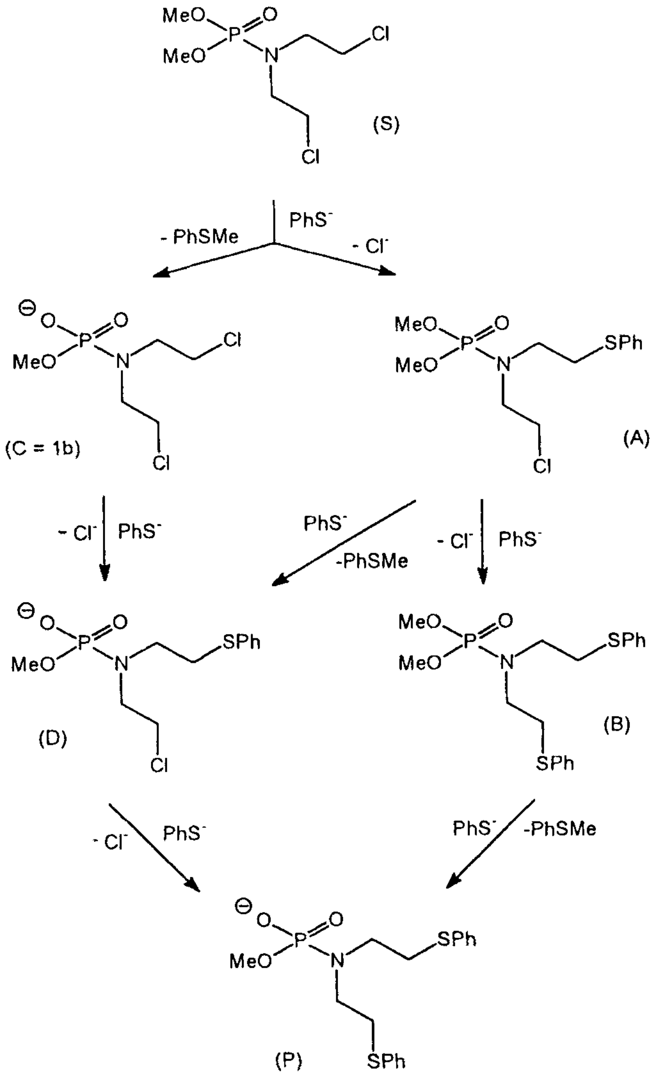


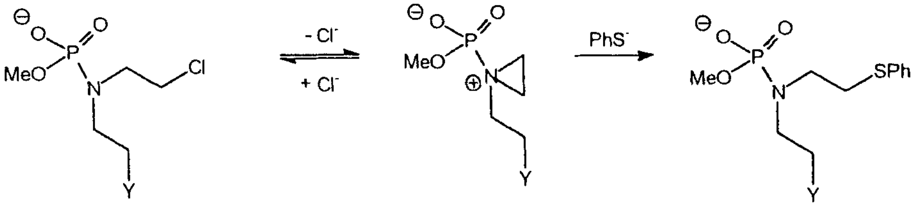
FIGURE 1 Concentration vs time plot for the reaction of dimethyl N-bis(2-chloroethyl)phosphoramidate (**S**) with PhSH/Et$_3$N in acetonitrile. ●, substrate **S**; *, final product **P**; ⊙, intermediate **D**; □, intermediate **C** (**1b**); ϕ, intermediate **B**; ▲, intermediate **A**.

thiophenol and triethylamine in acetonitrile-d$_3$, and the first substrate tested was the previously studied[1] dimethyl N-bis(2-chloroethyl)phosphoramidate, the precursor of ion **1b**. The reaction was carried out at room temperature and its course was monitored by ^{31}P NMR spectroscopy. The decay of the substrate (**S**, δ_P 13.3) was accompanied by the formation of two products of similar ^{31}P NMR chemical shift values (**A**, δ_P 13.4; **B**, δ_P 13.5), and two products of lower δ_P values (**C**, δ_P 7.2; **D**, δ_P 7.6). All those products appeared as transient species which eventually gave way to a final, single product (**P**, δ_P 8.0). In addition, ^{1}H NMR spectrum of the reaction product revealed the formation of thioanisole (δ_H 2.45, s, SMe). The variation of the concentration of the individual species, determined by the integration of the ^{31}P NMR signals, with reaction time is shown in Figure 1. The intermediates **A** and **B** were identified as dimethyl N-(2-chloroethyl),N-[2-(mercaptophenyl)ethyl]phosphoramidate, and dimethyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidate (**3c**) by independent synthesis of those compounds and by adding their samples to the reaction mixture. The final product **P** was identified by NMR spectroscopy as the ion of methyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidic acid, and its structure was confirmed by its independent formation in the O-demethylation of **B** by PhSH/Et$_3$ N. Intermediate **C** was shown to be identical to the previously described[1] ion **1b**. Products identity together with the concentration plots allowed us to develop a general scheme for the reaction (Scheme II). The results presented in Scheme II led to the following conclusions. (i) High nucleophilicity of the reagent resulted in a non-selective reaction of the substrate: in addition to the O-demethylation (**S** → **1b**) a



SCHEME II

direct substitution of Cl took place in the nitrogen mustard moiety (**S** → **A**; **A** → **B**). Since after 26 h only a very small quantity (ca. 4%) of **P**, the final product furnished by both pathways is formed, it was possible to assess relative contribution of two competing nucleophilic substitutions. The combined concentration of intermediates **C** and **D** (initial O-demethylation of **S**) is 43%, while the combined concentration of **A** and **B** (substitution at β-carbon in **S**) is 36%. Considering that some **D** can be also produced via the O-demethylation of **A**, it seems that the attack of thiophenoxide at the methyl ester group and at the β-carbon of the mustard function proceeds with comparable rates. We believe that the alkylation of the PhS^- ion by the N-(2-chloroethyl) group occurs directly (a "classical" S_N2 reaction) for neutral substrates (**S**, **A**), and via an aziridinium type intermediate[8] for the ionic species



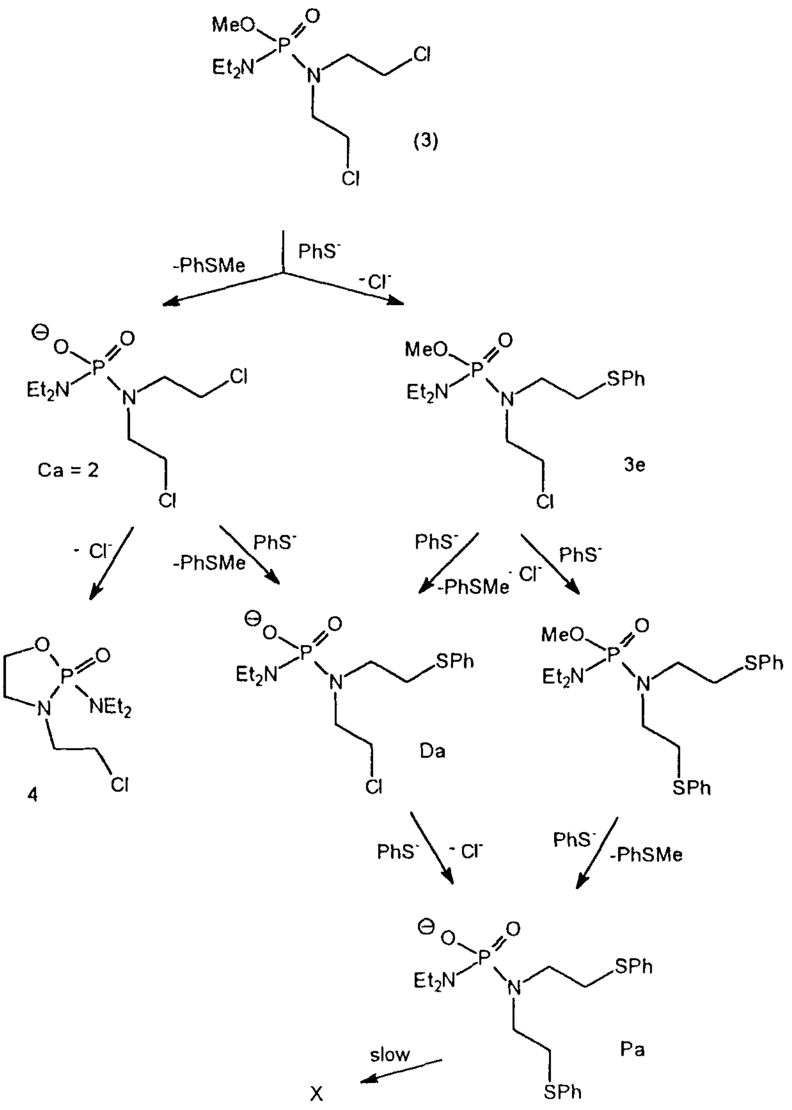
1b, Y = Cl;
D, Y = SPh

D, Y = Cl;
P, Y = SPh

(8)

(**1b**, **D**) (Equation 8). (ii) The P—N bond is retained for the whole course of the reaction indicating strong medium effect on the specific mechanism of the alkylation/dephosphorylation steps.[1] (iii) The bis-alkylated product **P** is stable under the reaction conditions demonstrating that higher nucleophilicity of the phosphate moiety (second nitrogen atom instead of the OMe function), and 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. (iv) No 1,5-cyclization (formation of 1,3,2-oxazaphospholidine derivative) was observed.

When substrate **3** was caused to react with the PhSH/Et_3N (excess) system in CD_3CN and the reaction course examined as above, a similar, albeit even more complex, picture was obtained. Again, neutral products (final and transient) were independently prepared and used for products identification. The total reaction scheme could be then constructed and is given below (Scheme III). Figure 2 shows the variation of the proportions of all species with time. Although the general reaction profile resembles that observed for **S** (Figure 1), it is clear that the reactivity of the ionic intermediates (**2** and **Da**) is much higher than that of the corresponding **1b** and **D** analogues. Both, **2** and **Da**, appeared as very transient species and at very low concentrations, as opposed to a relatively high accumulation of **1b** and, in particular, of **D** (Figure 1). For the reaction of **3** the most transient signal at δ_P 9.9 was assigned to the first ionic species **2**, and its fast disappearance confirmed our earlier failure in preparing a sample of the lithium salt of **2** (*vide supra*). After 52.5 h the combined concentration of **3e** and **3d** (direct substitution at the β-carbon in neutral compounds) is ca. 76%, showing that for **3** the substitution of Cl is significantly faster than the O-demethylation, presumably because of the poorer leaving group (phosphoramidate anion) involved in the latter reaction. There are two important features of the reaction that were not observed in the reaction presented in Scheme II. First, as in the reaction in C_5D_5N/D_2O (Equation 6), demethylation of **3** was followed by some 1,5-cyclization to **4**, not observed for the reaction of **1b** under the same conditions. Second, the important difference is that the bis-alkylation product **Pa** was, contrary to **P** (Scheme II) not stable, but decayed slowly giving rise to a single phosphorus-containing product **X** (δ_P −20.3). The final reaction product **X**, after evaporation of the solvent, was treated with D_2O/benzene (1:2, v/v) mixture, and both phases were examined independently. The ^{31}P NMR spectrum of the aqueous solution revealed the presence of a single product (δ_P −21.0), while the ^1H NMR spectrum showed only the presence of the triethylammonium ion. The benzene solution was shown to



SCHEME III

contain no phosphorus products, but only thioanisole and bis[2-(mercaptophenyl)-ethyl]amine. We identify therefore the product **X** as the triethylammonium salt of polyphosphoric acid derived from the metaphosphoramidate intermediate, and we propose that after the dialkylation sequence is completed, dephosphorylation takes place via unimolecular fragmentation of **Pa** according to the previously proposed mechanism (Equation 9). The fragmentation is in this case much slower than of **2** since the substrate contains a less reactive leaving group in the *β*-position of the N-ethyl substituent.

In summary, reactivity studies confirm that a N-phosphorylated nitrogen mustard

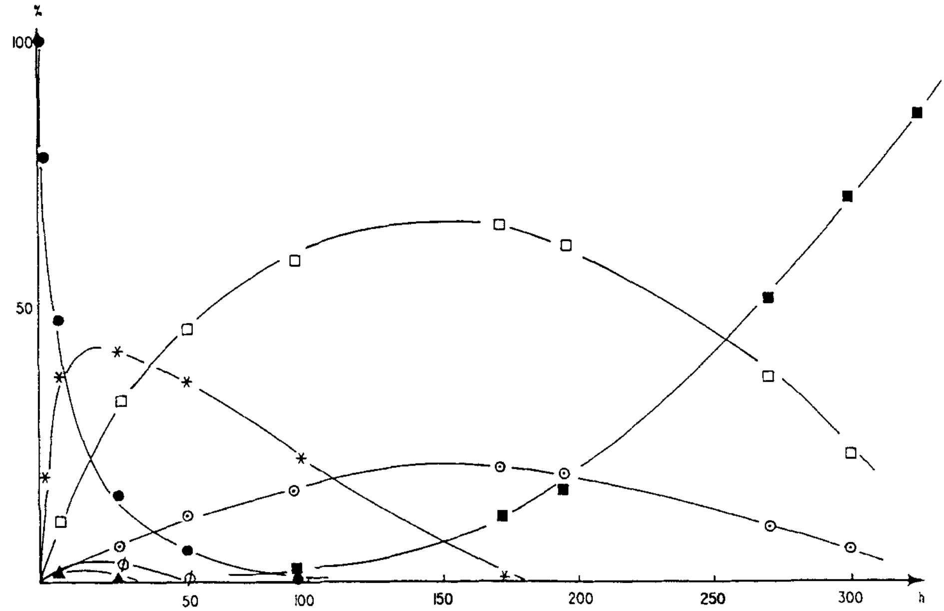
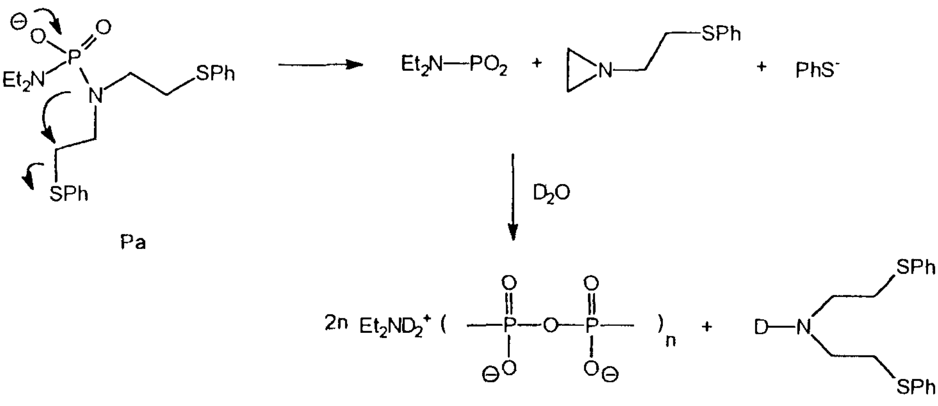


FIGURE 2 Concentration vs time plot for the reaction of **3** with PhSH/Et_3N in acetonitrile. ●, substrate **3**; ■, final product **X** (δ_P −20.3); ⊙, product **Pa**; □, intermediate **3d**; *, intermediate **3e**; ϕ, intermediate **Da**; ▲, intermediate **Ca** (**2**).

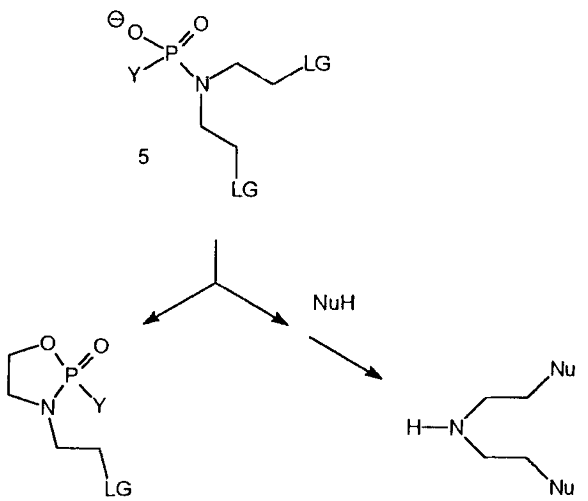
system of a general structure **5** is capable of spontaneous bis-alkylation of an external nucleophile NuH,[9] provided that it contains a good leaving group LG, and that the substituent Y increases the net electronic charge of the phosphate group. The concomitant 1,5-cyclization is expected to compete with the alkylation sequence (Scheme IV).



(9)

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC300 spectrometer with TMS as internal standard for ^1H and ^{13}C NMR and 85% H_3PO_4 as external standard for ^{31}P NMR. Mass spectrometry was performed on a Varian MAT-12 double



SCHEME IV

focusing direct inlet spectrometer at an ionization potential of 70 eV. Elemental analyses were performed at the Chemistry Department, University of Cape Town. Merck Kieselgel 60 (0.063–0.200 mm) was used for column chromatography. All solvents and reagents were dried and purified by conventional methods immediately before use.

Preparation of Substrates and Standards

Dimethyl N,N-bis(2-chloroethyl)phosphoramidate and lithium methyl N,N-bis(2-chloroethyl)phosphoramidate (**1b** = **C**) were prepared as described before.[1] N,N-bis(2-chloroethyl)phosphoramidodichloridate was prepared according to the literature procedure.[11] N,N-Diethyl-N′,N′-bis-(2-chloroethyl)phosphoramidochloridate was prepared by adding triethylamine (one mol-equiv.) in CH_2Cl_2 to a solution of the above dichloridate (one mol-equiv.) and diethylamine (one mol-equiv.) in CH_2Cl_2 at −78°C. The mixture was allowed to warm to ambient temperature, stirred for 72 h, and the solvent was removed under reduced pressure. Large volume of hexane (ca. 30 mL per mmol) was added, the amine salt was filtered off, the solvent was evaporated, and the crude product (75%) was purified by bulb-to-bulb distillation (oven temp. 120–124°C/0.14 mbar) (72%); δ_H ($CDCl_3$) 1.24 (6H, t, J_{HH} 7.1 Hz), 3.26 (4H, m), 3.61 (4H, t, J_{HH} 6.9 Hz); δ_C (H-coupled, $CDCl_3$) 13.4 (q, J_{CH} 127.0 Hz), 40.3 (t, J_{CH} 138.0 Hz), 41.5 (t, J_{CH} 152 Hz), 49.5 (t, J_{CH} 141.0 Hz); δ_P ($CDCl_3$) 26.6.

Methyl N,N-diethyl-N′,N′-bis(2-chloroethyl)phosphordiamidate (**3**): A solution of the above phosphordiamidochloridate in dry methanol (3 mL per mmol of substrate) was added dropwise at room temperature to the solution of one mol-equiv. of sodium methoxide in methanol (9 mL per mmol). The mixture was stirred at ambient temperature for 24 h, filtered and evaporated under reduced pressure. A small volume of chloroform was added, the suspension was filtered through a layer of anh. $MgSO_4$, and the filtrate was evaporated under reduced pressure. Crude product (>90%) was purified by column chromatography (CH_2Cl_2) yielding a colorless oil (70%); δ_H ($CDCl_3$) 1.15 (6H, t, J_{HH} 7.0 Hz), 3.10 (4H, dq, J_{HP}, J_{HH} 11.2, 7.1 Hz), 3.36 (4H, dt, J_{HP}, J_{HH} 11.7, 7.2 Hz), 3.61 (4H, t, J_{HH} 7.0 Hz), 3.73 (3H, d, J_{HP} 11.1 Hz); δ_C (H-coupled, $CDCl_3$) 14.2 (q, J_{CH} 125.0 Hz), 39.2 (t, J_{CH} 136.4 Hz), 42.4 (t, J_{CH} 150.2 Hz), 49.9 (t, J_{CH} 138.8 Hz), 51.6 (q, J_{CH} 147.0 Hz); δ_P ($CDCl_3$) 18.7 MS m/z 294 (M+4, 0.5%), 292 (M+2, 2.5), 291 (M+1, 1.4), 290 (M, 4), 275 (M-CH_3, 23), 241 (M-CH_2Cl, 100), 136 (54), 72 (63). Anal. Calcd for $C_9H_{21}Cl_2N_2O_2P$: C, 37.11; H, 7.22; N, 9.62. Found: C, 36.48; H, 7.55; N, 9.38.

Dimethyl N,N-diethylphosphoramidate (**3a**) and methyl N,N,N′,N′-tetraethylphosphordiamidate (**3b**) were prepared from the corresponding phosphoramidodichloridate and phosphordiamidochloridate and sodium methoxide as described above. **3a**, purified by column chromatography (CH_2Cl_2) (70%); δ_H 1.02 (6H, t, J_{HH} 7.1 Hz), 2.98 (4H, dq, J_{HP}, J_{HH} 11.4, 7.1 Hz), 3.58 (6H, d, J_{HP} 11.3 Hz); δ_C (H-coupled, $CDCl_3$) 14.0 (q, J_{CH} 127.2 Hz), 39.4 (t, J_{CH} 137.0 Hz), 52.5 (q, J_{CH} 147.1 Hz); δ_P ($CDCl_3$) 13.6. MS m/z 182 (M+1, 0.9%), 181 (M, 7), 166 (M-CH_3, 100), 138 (10), 109 (46), 72 (19). **3b**, purified by column chromatography (hexane/$CHCl_3$, 2:1) (74%); δ_H ($CDCl_3$) 1.05 (12H, t, J_{HH} 7.0 Hz), 2.99 (8H, dq, J_{HP}, J_{HH} 11.3, 7.2 Hz), 3.56 (3H, d, J_{HP} 11.2 Hz): δ_C (H-coupled, $CDCl_3$) 13.8 (q, J_{CH} 127.4 Hz), 39.1 (t, J_{CH} 136.1 Hz), 50.7 (q, J_{CH} 146.0 Hz); δ_P ($CDCl_3$) 20.3 MS m/z 224 (M+2, 0,5%), 223 (M+1, 3.9), 222 (M, 18), 207 (M-CH_3, 71), 150 (M-NEt_2, 100), 136 (81), 72 (99).

N-(2-chloroethyl),N-[2-(mercaptophenyl)ethyl]amine: A solution of triethylamine (4.30 g, 42.6 mmol) in ether (400 mL) was added dropwise with vigorous stirring to a suspension of bis (2-chloroethyl)ammonium chloride (3.0 g, 16.8 mmol) in ether (400 mL) containing thiophenol (1.85 g, 16.8 mmol). The mixture was heated under reflux in an atmosphere of dry nitrogen for 120 h, filtered, and the solvent was removed under reduced pressure. After addition of some hexane, filtration and evaporation of the solvent, the crude product was purified by bulb-to-bulb distillation (oven temp. 120°C/2 mbar), 1.26 g (35%); δ_H ($CDCl_3$) 2.81–2.96 (4H, two m), 3.05 (2H, t, J_{HH} 6.6 Hz), 3.59 (2H, t, J_{HH} 6.0 Hz), 7.15–7.36 (5H, m). MS m/z 217 (M+2, 0.2%), 215 (M, 0.6), 123 (5), 109 (41), 94 (32), 92 (M-CH_2SPh, 100).

N,N-Bis[2-(mercaptophenyl)ethyl]amine was prepared as described above using two mol-equiv. of thiophenol. Purified by removing volatile contaminations by keeping the crude product at 50°C/0.5 mbar for 16 h. Yield 74%; δ_H ($CDCl_3$) 2.76 (4H, t, J_{HH} 6.6 Hz), 3.01 (4H, t, J_{HH} 6.7 Hz), 7.18–7.36 (10 H, m); δ_C (H-coupled, $CDCl_3$) 34.3 (t, J_{CH} 138.9 Hz), 48.6 (t, J_{CH} 131.8 Hz), 118.2 (s), 125.8, 127.9 (two d, J_{CH} 161.7 Hz), 129.0 (d, J_{CH} 161.3 Hz). MS m/z 291 (M+2, 0.3%), 290 (M+1, 0.7), 289 (M, 2), 166 (47), 137 (100), 109 (36).

2-Diethylamino-2-oxo-3-(2-chloroethyl)-1,3,2-oxazaphospholidine (**4**): Substrate **3** (0.10 g, 0.42 mmol) was dissolved in acetonitrile (10 mL), thiophenol (0.10 g, 0.91 mmol) and triethylamine (0.10 g, 1.0 mmol) were added and the solution was heated under reflux for 120 h. The solvent was removed under reduced pressure, pet. ether (80 mL) was added and the mixture was filtered. The filtrate was evaporated under reduced pressure and **4** was purified by column chromatography (CH_2Cl_2). Colorless oil, 0.023 g (23%); δ_H ($CDCl_3$) 1.12 (6H, t, J_{HH} 7.1 Hz), 2.99 (4H, dq, J_{HP}, J_{HH} 10.9, 7.1 Hz), 3.18 (2H, dt, J_{HP}, J_{HH} 9.0, 6.1 Hz), 3.36 (2H, dt, J_{HP}, J_{HH} 10.1, 6.8 Hz), 3.65 (2H, t, J_{HH} 6.7 Hz), 4.16 (2H, dt, J_{HP}, J_{HH} 10.5, 6.8 Hz); δ_C (H-coupled, $CDCl_3$) 14.8 (q, J_{CH} 125.4 Hz), 40.2 (t, J_{CH} 136.7 Hz), 43.8 (t, J_{CH} 151.2 Hz), 47.2 (t, J_{CH} 137.1 Hz), 48.0 (t, J_{CH} 138.3 Hz), 64.7 (t, J_{CH} 153.0 Hz); δ_P ($CDCl_3$) 28.0 MS m/z 242 (M+2, 2.6%), 240 (M, 9), 225 (M-CH_3, 87), 191 (M-CH_2Cl, 41), 168 (18), 120 (29), 72 (100).

Methyl N,N-diethyl-N′,N′-bis[2-(mercaptophenyl)ethyl]phosphordiamidate (**3d**): Substrate **3** (0.10 g, 0.42 mmol) was dissolved in acetonitrile (10 mL), thiophenol (0.12 g, 1.1 mmol) and triethylamine (0.12 g, 1.2 mmol) were added and the solution was kept at room temperature for 140 h. Solvent was removed under reduced pressure, hexane (60 mL) was added, the mixture was filtered, and the filtrate was evaporated yielding crude product which was purified by column chromatography ($CHCl_3$/hexane, 1:1). Pure **3d** was obtained as a pale-yellow oil, 0.11 g (62%); δ_H (CD_3CN) 1.05 (3H, t, J_{HH} 7.1 Hz), 2.94 (4H, m), 2.96 (8H, m), 3.55 (3H, d, J_{HP} 11.0 Hz), 7.10–7.40 (10H, m); δ_C (H-coupled, CD_3CN) 14.7 (q, J_{CH} 126.6 Hz), 32.8 (t, J_{CH} 142.2 Hz), 40.5 (t, J_{CH} 136.6 Hz), 47.6 (t, J_{CH} 136.5 Hz), 52.0 (q, J_{CH} 147.8 Hz), 118.3 (s), 127.1 (d, J_{CH} 159.0 Hz), 129.2 (d, J_{CH} 161.0 Hz), 130.0 (d, J_{CH} 161.4 Hz); δ_P (CD_3CN) 19.7. MS m/z 439 (M+1, 0.7%), 438 (M, 1.8), 315 (M-SPh, 42), 137 (100), 109 (43). Anal. Calcd for $C_{21}H_{31}N_2O_2PS_2$: C, 57.53; H, 7.08; N, 6.39; S, 14.61. Found: C, 56.77; H, 7.21; N, 5.56; S, 13.99.

Dimethyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidate (**3c**): A solution of triethylamine (0.50 g, 5.0 mmol) in either (10 mL) was added dropwise at room temperature to a solution of dimethylphosphorchloridate (0.67 g, 4.7 mmol) and bis[2-(mercapto-phenyl)ethyl]amine (1.36 g, 4.7 mmol) in ether (20 mL). The reaction mixture was stirred at room temperature for 48 h, filtered, the solvent was removed under reduced pressure, hexane (5 mL) was added to the residue, the mixture was filtered and the solvent was removed from the filtrate. Product **3c** was obtained as a pale-yellow oil, 0.67 g (36%); δ_H ($CDCl_3$) 2.98 (4H, m), 3.20 (4H, m), 3.65 (6H, d, J_{HP} 11.0 Hz), 7.10–7.30 (10H, m); δ_C (H-coupled, $CDCl_3$) 32.5 (t, J_{CH} 141.2 Hz), 47.2 (t, J_{CH} 141.1 Hz), 53.5 (q, J_{CH} 147.6 Hz), 118.2 (s), 127.0 (d, J_{CH} 155.0 Hz), 129.6 (d, J_{CH} 161.1 Hz), 130.0 (d, J_{CH} 161.1 Hz); δ_P ($CDCl_3$) 12.5. MS m/z 397 (M, 1%), 274 (M-CH_2Ph, 20), 137 (100), 109 (69).

Dimethyl N-(2-chloroethyl),N-[2-(mercaptophenyl)ethyl]phosphoramidate (**A**): Prepared from dimethyl phosphorochloridate and N-(2-chloroethyl),N-[2-(mercaptophenyl)ethyl]amine as described for **3c**. Yield 34%; δ_H ($CDCl_3$) 3.05 (2H, m), 3.18 (2H, m), 3.32 (2H, dt, J_{HP}, J_{HH} 11.6, 6.7 Hz), 3.58 (2H, t, J_{HH} 6.3 Hz), 3.60 (6H, d, J_{HP} 11.1 Hz), 7.20–7.40 (5H, m); δ_C (H-coupled, $CDCl_3$) 32.3 (t, J_{CH} 141.8 Hz), 43.2 (t, J_{CH} 151.1 Hz), 47.5 (t, J_{CH} 141.6 Hz), 49.4 (t, J_{CH} 140.7 Hz), 53.6 (q, J_{CH} 147.8 Hz), 118.2 (s), 127.0 (d, J_{CH} 162.1 Hz), 129.2 (d, J_{CH} 161.8 Hz), 130.0 (d, J_{CH} 161.0 Hz). MS m/z 326 (M+3, 0.5%), 325 (M+2, 1.8), 324 (M+1, 1.1), 323 (M, 5), 274 (M-CH_2Cl, 5), 202 (M-CH_2SPh, 33), 200 (M-CH_2SPh, 100), 138 (47), 123 (7), 109 (75).

Bis[2-(N-pyridinio)ethyl]amine dichloride was prepared as described before.[1]

N-Methylpyridinium ion was prepared as an iodide salt by methylation of pyridine with iodomethane in hexane. Mp 117–118°C; δ_H (C_5D_5N/D_2O, 5:1, v/v) 4.57 (3H, s), 8.00 2H, t, J_{HH} 6.6 Hz), 8.42 (1H, t, J_{HH} 6.9 Hz), 9.20 (2H, d, J_{HH} 6.2 Hz); δ_C (H-coupled, C_5D_5N/D_2O, 5:1) 49.0 (q, J_{CH} 145.0 Hz), 127.9 (d, J_{CH} 171.8 Hz), 145.1 (d, J_{CH} 165.6 Hz), 145.4 (d, J_{CH} 192.6 Hz).

Lithium methyl N-bis[2-(mercaptophenyl)ethyl]phosphoramidate (**P**): A solution of **3c** (0.10 g, 0.25 mmol) and lithium iodide (0.040 g, 0.30 mmol) in dry 2-butanone (2.5 mL) was heated under reflux for 10 h. The precipitate was filtered, washed with 2-butanone and dried under high vacuum. White solid, 0.044 g (45%); mp 222–224°C; δ_H (D_2O) 2.79 (4H, m), 2.96 (4H, m), 3.30 (3H, d, J_{HP} 10.9 Hz), 6.87–7.07 (10H, m); δ_C (H-coupled, D_2O) 34.8 (t, J_{CH} 141.4 Hz), 49.8 (t, J_{CH} 138.6 Hz), 54.5 (q, J_{CH} 145.5 Hz), 128.2 (d, J_{CH} 154.7 Hz), 131.4, 131.7 (two d, J_{CH} 161.3 Hz), 138.1 (s); δ_P (D_2O) 9.8. Anal. Calcd for $C_{17}H_{21}LiNO_3PS_2$: C, 52.44; H, 5.40; N, 3.60; S, 16.45. Found: C, 51.80; H, 5.40; N, 3.48; S, 15.48.

Fragmentation Experiments

Demethylation of **3a** *and* **3b**: Reactions were carried out in 2-butanone using LiI, as described above for the preparation of **P**. **3a** gave lithium methyl N,N-diethylphosphoramidate (30%); mp > 280°C; δ_H (D_2O) 0.98 (6H, t, J_{HH} 7.1 Hz), 2.87 (4H, dq, J_{HP}, J_{HH} 10.9. 7.1 Hz), 3.40 (3H, d, J_{HP} 10.7 Hz); δ_C (H-coupled, D_2O) 16.3 (q, J_{CH} 126.4 Hz), 42.6 (t, J_{CH} 135.8 Hz), 54.2 (q, J_{CH} 145.4 Hz); δ_P (D_2O) 12.0. **3b** gave lithium N,N,N',N'-tetraethylphosphordiamidate (25%); mp > 260°C; δ_H (D_2O) 0.97 (12H, t, J_{HH} 7.1 Hz), 2.88 (8H, dq, J_{HP}, J_{HH} 9.9, 7.1 Hz); δ_C (H-coupled, D_2O) 16.3 (q, J_{CH} 125.2 Hz), 41.8 (t, J_{CH} 134.7 Hz); δ_P (D_2O) 17.3. 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 **2**: A solution of **3** (0.20 g, 0.69 mmol) and LiI (0.20 g, 1.49 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 (see Discussion). 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 (see Discussion).

Fragmentation of **3** *in aqueous pyridine*: A solution of **3** (0.015 g, 0.052 mmol) in the mixture of C_5D_5N and D_2O (5:1, v/v, 0.5 mL) was incubated at 60°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 **3**. After 150 h the disappearance of **3** 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 **4** (15%, δ_P 29.5); both products confirmed by the addition of authentic samples. The ^1H NMR spectrum revealed the presence of four products: N-methylpyridinium ion, diethylammonium ion, bis[2-(N-pyridinio)ethyl]amine dication, and **4**. All products were confirmed by the addition of samples of authentic species.

Fragmentation of dimethyl N,N-bis(2-chloroethyl)phosphoramidate with PhSH/Et_3N: The substrate **S** (0.500 g, 2.0 mmol) was added to a solution of thiophenol (0.630 g, 5.7 mmol) and triethylamine (0.577 g, 5.7 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 ^1H 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 II and Figure 1). Intermediates **A** and **B** (**3c**) were identified by the addition of the independently prepared samples. Ionic intermediate **C** (**1b**) was identified by the addition of the authentic material.[1] When the authentic intermediate **B** (**3c**) was treated with PhSH/Et_3N in CD_3CN under the same conditions, it yielded only one product, identical (^{31}P and ^1H NMR spectra) to the final reaction product **P**. In that way **P** was identified as the O-demethylated derivative of **B** (**3c**). Under the same conditions authentic **A** yielded two products, **B** (**3c**) and **D**, which gradually yielded single product **P**. Intermediate **D** (the only component of the mixture not prepared independently) was therefore identified indirectly as the O-demethylated derivative of **A**.

Fragmentation of **3** *with PhSH/Et_3N*: A solution of **3** (0.015 g, 0.052 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 III and Figure 2. As before, product **3d** (δ_P 19.7) was identified by addition of an authentic sample. The monosubstituted intermediate **3e** (precursor of **3d**) could not be prepared independently, but was identified by its ^{31}P NMR chemical shift (δ_P 19.6), as compared with $\delta_P = 19.5$ for its precursor **3**, and $\delta_P = 19.7$ for its substitution product **3d**. When authentic **3d** was treated with PhSH/Et_3N it yielded product **Pa** (δ_P 10.7), thus **Pa** was identified as the O-demethylated derivative of **3d**. The two other ionic intermediates, **Ca** (**2**) and **Da** 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 ($\delta_P = 9.9$ for **Ca**, $\delta_P = 10.2$ for **Da**, and $\delta_P = 10.7$ for **Pa**). The concomitant formation of **4** was confirmed by the addition of an authentic sample of **4** to the reaction mixture.

In addition, **4** was isolated from the reaction product in the following experiment. A solution of **3** (0.50 g, 1.72 mmol), thiophenol (0.628 g, 5.7 mmol) and triethylamine (0.577 g, 5.7 mmol) in CH_3CN (40 mL) was heated under reflux for 280 h. After removal of the solvent, hexane (50 mL) was added and the insoluble material was filtered off. The hexane solution was evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/acetone, 5:1) yielding 0.090 g (22%) of pure **4**. The NMR (^{31}P, ^1H, ^{13}C) spectra and the mass spectrum of this product were identical to those obtained for the independently prepared **4** (*vide supra*).

The major phosphorus-containing product obtained in the fragmentation of **3**, N,N-diethyl-N′,N′-bis[2-(mercaptophenyl)ethyl]phosphoramidate anion (**Pa**, δ_P 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} 7.3 Hz), 3.11 (6H, q, J_{HH} 7.3 Hz), and the diethylammonium ion, 1.19 (6H, t, J_{HH} 7.2 Hz), 2.96 (4H, q, J_{HH} 7.2 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.

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