Decomposition of N-Phosphorylated Nitrogen Mustards: A Mechanistic Investigation

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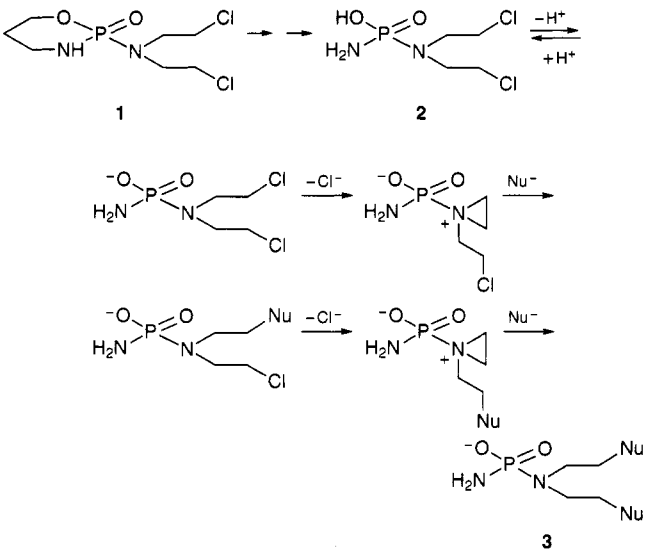
Lithium methyl *N*-(2-chloroethyl)phosphoramidate (**2b**) and lithium methyl *N,N*-bis(2-chloroethyl)-phosphoramidate (**2c**) were prepared as models of N-phosphorylated mustards used in cancer chemotherapy. The decomposition of those substrates in D_2O and in D_2O–pyridine-d_5 was studied to elucidate the mechanism of their alkylating reactivity. The products of the decomposition and the variation of the proportions of the products with time were determined, and the results led to the following conclusions. Decomposition of substrates of the type **2** can follow three independent pathways: (i) 1,5-cyclization to a 1,3,2-oxazaphospholidine derivative, followed by fast ring opening via the pH-dependent P–O or P–N bond cleavage; (ii) 1,3-cyclization to a N-phosphorylated aziridinium derivative, followed by the nucleophilic opening of the aziridine ring; (iii) fragmentation to metaphosphate and aziridine species, followed by rapid reactions of those intermediates with nucleophiles. The first pathway deactivates the substrate with respect to the alkylating reactivity. Relative contributions of individual pathways to the decomposition are highly sensitive to the detailed structure of the substrate and to the nucleophilic composition of the reaction medium.

Introduction

The discovery that nitrogen mustard [methyl bis(2-chloroethyl)amine] produces remissions in various forms of lymphoma[1] stimulated intensive research in the synthesis of N-phosphorylated mustards, which led to the preparation of cyclophosphamide **1**,[2] a compound demonstrating significant activity against a broad spectrum of human cancers.[3] The metabolism of the prodrug **1** is well understood and results in the release of the phosphoramidate mustard **2**, the biologically active alkylating agent.[4] The alkylating reactivity of **2** was attributed to the intramolecular cyclization of its conjugate base, followed by a sequence of nucleophilic ring opening steps, yielding the final bisalkylated ("cross-linked") product **3** (Scheme 1).[5] The exact mechanism of the alkylation chemistry of **2** is, however, still a relatively poorly understood subject. The stepwise pathway involving the intermediate aziridine derivatives has been demonstrated by deuteration experiments,[6] but whether **2** undergoes alkylation reactions as an intact molecule, or the P–N bond cleavage is a prerequisite for the alkylation, remains controversial. The claim that the cleavage of **2** to nornitrogen mustard ($[HN(CH_2CH_2Cl)_2]$ *does not* take place in its reaction with model nucleophiles was made by Colvin *et al.*[6] on the basis of the colorimetric assay of the alkylation of nitrobenzylpyridine (NBP) by **2** and by nornitrogen mustard, and by the detection of the $m/z = 315$ (M + 1) signal in the CI mass spectrum of the trimethyl derivative of the product of the alkylation of two molecules of etanethiol by **2**, identified as the trimethyl derivative of **3** (Nu = Nu′ = SEt). The results

Scheme 1



of the alkylation of NBP seem ambiguous in view of the higher reactivity observed at lower pH, when both the cyclization of **2** and the nucleophilicity of NBP should be retarded. The reported MS can also be (even better) explained by a different structural assignment, based on the initial cyclization of **2** to the 1,3,2-oxazaphospholidine derivative, followed by the ring opening by EtSH (*vide infra*). A more convincing evidence of at least partial involvement of the intact **2** in alkylation was produced by FAB mass spectra of the products of the reaction of guanosine 5′-monophosphate with **2**.[7] A thorough ^{31}P NMR kinetic study of the reactivity of **2** and cognate substrates[8] demonstrated the participation of a 1,3,2-oxazaphospholidine compound as one of the intermediates, but the assignment of some signals to the transient N-phosphorylated aziridinium ions still needs to be

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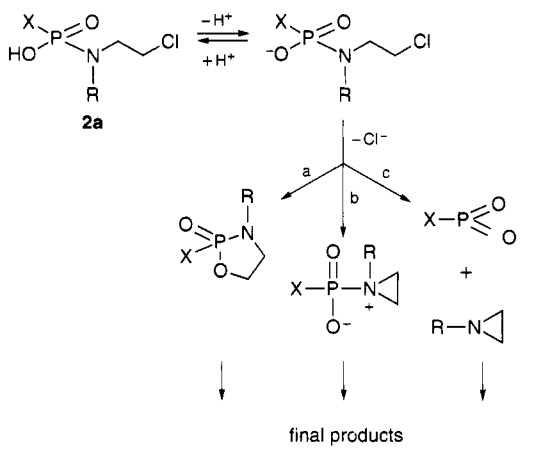
(1) Friedman, O. M.; Myles, A.; Colvin, M. In *Advances in Cancer Chemotherapy*; Rosowsky, A., Ed.; Marcel Dekker: New York, 1979; Vol. 1, Chapter 1.
(2) Arnold, H.; Bourseaux, F. *Angew. Chem.* **1958**, *70*, 539.
(3) Hill, D. L. *A Review of Cyclophosphamide*; Thomas: Springfield, IL, 1975.
(4) Stec, W. J. In *Organophosphorus Chemistry*; The Royal Society of Chemistry: London, 1982; Vol. 13, Chapter 8.
(5) Friedman, O. M. *Cancer Treat. Rep.* **1967**, *51*, 347.
(6) Colvin, M.; Brundrett, M. N.; Kan, N.; Jardine, I.; Fenselau, C. *Cancer Res.* **1976**, *36*, 1121.
(7) Vu, V. T.; Fenselau, C.; Colvin, M. *J. Am. Chem. Soc.* **1981**, *103*, 7362.
(8) Engle, T. W.; Zon, G.; Egan, W. *J. Med. Chem.* **1982**, *25*, 1347.

0022-3263/95/1960-3832$09.00/0

confirmed. Hemminki studied binding of metabolites of **1** to DNA and concluded that **2** is the primary alkylating agent and that the phosphorus-free alkylated guanidine products are formed after dephosphoramidation.[9] The benzyl ester of **2** has been shown to gain alkylating reactivity via the oxidation by microsomal enzymes,[10] but the exact nature of the reactive species produced by the oxidative removal of the benzyl group has not been established.

Close examination of the conjugate base of **2** (Scheme 1) leads to a conclusion that its intramolecular reactivity can be expected to be much more complex than suggested in the scheme. First, although the 1,3-cyclization to the aziridinium ion is feasible, the reaction should be reversible, as has been demonstrated for neutral N-phosphorylated aziridines.[11] Second, the formation of a neutral 1,3,2-oxazaphospholidine intermediate has to be expected, as the 1,5-cyclization can effectively compete with the formation of the aziridine ring in those[8] and related[12] phosphoramidate systems. Finally, the deprotonated **2** represents an organophosphorus system containing a leaving group (Cl) and an accumulation of negative charge on an atom directly bonded to phosphorus. Such structural features are usually a prerequisite for the fragmentation of a substrate with the extrusion of a metaphosphate derivative, $X-PO_2$, as a reactive intermediate.[13] In a most closely related example, we have recently demonstrated that a 2-aryl group in 2-arylethyl esters of *N*-bis(2-chloroethyl)phosphoramidates is responsible for such reactivity via the anchimerically assisted fragmentation of the substrate to arenium and chloride ions and the metaphosphate and aziridine species.[14] Taking into consideration the available options, the expected intramolecular reactivity of the systems of the type **2a** can be illustrated in general terms by Scheme 2. In the present work, we describe first results[15] of an investigation of the decomposition of some model substrates **2a** from the point of view of the contribution of the individual pathways shown in Scheme 2.

Scheme 2



Experimental Section

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 focusing direct inlet spectrometer at an ionization potential of 70 eV. pH measurements were conducted with a Metrohom 691 pH meter using a platinum electrode. Elemental analyses were performed at the Chemistry Department, University of Cape Town. Kieselgel 60 was used for column chromatography. All solvents and reagents were dried and purified by conventional methods immediately before use.

Dimethyl *N*-(2-Chloroethyl)phosphoramidate. Dry and powdered (2-chloroethyl)ammonium chloride (12.06 g, 104 mmol) was added to a solution of dimethyl phosphorochloridate (15.0 g, 104 mmol) in dry dichloromethane (50 mL), and the suspension was cooled to 0 °C with vigorous stirring. A solution of triethylamine (28.8 mL, 208 mmol) in dichloromethane (30 mL) was added dropwise, the cooling bath was removed, and the stirring was continued for 20 h. After filtration through $MgSO_4$/Celite and evaporation of the solvent, the product was purified by distillation, yielding 12.4 g (66.6 mmol, 64%): bp 125–126 °C (0.7 mm) (lit.[16] bp 124–126 °C (0.5 mm)); ^1H NMR ($CDCl_3$) δ 3.18 (2H, m), 3.51 (2H, t, J = 5.9 Hz), 3.65 (6H, d, J = 11.2 Hz); proton-coupled ^{13}C NMR ($CDCl_3$) δ 42.8 (t, J = 142.5 Hz), 44.7 (dt, J = 156.5, 4.5 Hz), 52.7 (dq, J = 150.4, 5.6 Hz); ^{31}P NMR ($CDCl_3$) δ 11.7; MS m/z 187 (M^+, 2), 152 (24), 124 (8), 109 (100), 95 (46).

Lithium Methyl *N*-(2-Chloroethyl)phosphoramidate (2b). A solution of dimethyl *N*-(2-chloroethyl)phosphoramidate (0.30 g, 1.6 mmol) and lithium iodide (0.22 g, 1.9 mmol) in dry 2-butanone (15 mL) was heated under reflux for 5 h. The precipitate was filtered, washed with chloroform (25 mL), and dried under high vacuum: white solid, 0.20 g (69%); ^1H NMR (D_2O) δ 3.07 (2H, dt, J = 10.8, 6.1 Hz), 3.45 (3H, d, J = 10.8 Hz), 3.55 (2H, t, J = 6.1 Hz); ^{31}P NMR (D_2O) δ 10.3. Anal. Calcd for $C_3H_8ClLiNO_3P$: C, 20.1; H, 4.5; N, 7.8. Found: C, 19.9; H, 4.6; N, 7.7.

Dimethyl *N*-(2-Bromoethyl)phosphoramidate.[16] To a stirred and refluxing suspension of dimethyl phosphorochloridate (13.4 g, 93 mmol) and dry (2-bromoethyl)ammonium bromide (23.8 g, 110 mmol) in dry ether (116 mL) was added a solution of triethylamine (31.1 mL, 250 mmol) in ether (35 mL),, and the mixture was kept under reflux for 7 h, followed by stirring overnight at room temperature. After filtration ($MgSO_4$/Celite) and evaporation of the solvent, the product was purified by distillation, yielding 12.1 g (52 mmol, 56%): bp 190 °C (0.3 mm); ^1H NMR ($CDCl_3$) δ 3.19 (2H, m), 3.34 (2H, t, J = 6.2 Hz), 3.63 (6H, d, J = 11.2 Hz); proton-coupled ^{13}C NMR ($CDCl_3$) δ 33.6 (t, J = 155.5 Hz), 43.0 (dt, J = 144.4, 4.6 Hz), 53.1 (dq, J = 150.7, 5.5 Hz); ^{31}P NMR ($CDCl_3$) δ 11.5.

Lithium methyl *N*-(2-bromoethyl)phosphoramidate (2b') was prepared by following the method used for **2b** from the above substrate (3.59 g, 15 mmol) and lithium bromide (1.34 g, 15 mmol) in 2-butanone (80 mL): white solid, 2.32 g (69%); ^1H NMR (D_2O) δ 3.13 (2H, m), 3.41 (3H, d, J = 10.8 Hz), 3.44 (2H, t, J = 6.5 Hz); ^{31}P NMR (D_2O) δ 10.1. Anal. Calcd for $C_3H_8BrLiNO_3P$: C, 16.1; H, 3.6; N, 6.25. Found: C, 15.8; H, 3.5; N, 6.1.

Ethyleneimine.[17] A solution of (2-chloroethyl)ammonium chloride (15.0 g, 130 mmol) and NaOH (15.6 g, 390 mmol) in water (30 mL) was heated at 50 °C for 2 h under reduced pressure (25 mm), and the distillate (bp 30–35 °C) was collected in an ice-cooled receiver. A second distillation of the crude product was carried out at atmospheric pressure, with rigorous exclusion of moisture, and the distillate was collected

(9) Hemminki, K. *Cancer Res.* **1985**, *45*, 4237.
(10) Kwon, C.-H.; Moon, K.-Y.; Baturay, N.; Shirota, F. N. *J. Med. Chem.* **1991**, *34*, 588.
(11) Le Roux, C.; Bauermeister, S.; Modro, T. A. *J. Chem. Res., Synop.* **1993**, 372.
(12) Bauermeister, S.; Modro, A. M.; Modro, T. A.; Zwierzak, A. *Can. J. Chem.* **1991**, *69*, 811.
(13) Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313.
(14) Le Roux, C.; Modro, A. M.; Modro, T. A. *J. Chem. Res., Synop.* **1995**, 38.
(15) Reactivity of the corresponding diamides (**2a**, X = NR_2) will be the subject of a future publication. The diamidate system (more closely related to **2**) is, however, much more reactive, so the preliminary studies were more conventiently carried out using monoamidates as substrates.
(16) Davidowitz, B.; Modro, T. A. *S. Afr. J. Chem.* **1982**, *35*, 63.
(17) Wystrach, V. P.; Kaiser, D. W.; Schaefer, F. C. *J. Am. Chem. Soc.* **1955**, *77*, 5915.

over solid NaOH: colorless liquid, 3.8 g (91 mmol, 70%): bp 55–57 °C (lit.[24] bp 56–58 °C); ^{1}H NMR (D_2O) δ 1.56 (s); lit.[18] δ 1.60.

Dimethyl *N*-Ethylenephosphoramidate. A solution of dimethyl phosphorochloridate (5.0 g, 35 mmol) in dry ether (10 mL) was added dropwise to a stirred and cooled solution of ethyleneimine (1.5 g, 42 mmol) and triethylamine (3.5 g, 35 mmol) in ether (20 mL) at 10 °C. The cooling was removed, and the stirring was continued for 2 h. After filtration of triethylammonium chloride and removal of the solvent, the crude product was dissolved in ether (30 mL), washed with 10% aqueous K_2CO_3 (20 mL), dried, and evaporated. Distillation of the residue yielded 3.2 g (21 mmol, 60%) of the pure product: bp 83–84 °C (0.7 mm) (lit.[19] bp 99–100 °C (10 mm)); ^{1}H NMR ($CDCl_3$) δ 2.14 (4H, d, J = 15.5 Hz), 3.70 (6H, d, J = 10.8 Hz); proton-coupled ^{13}C NMR ($CDCl_3$) δ 23.4 (dt, J = 174.2, 6.0 Hz), 52.9 (dq, J = 150.9, 5.5 Hz); ^{31}P NMR ($CDCl_3$) δ 18.4.

Lithium methyl *N*-ethylenephosphoramidate (9) was prepared by following the method used for **2b** from the above substrate (0.5 g, 3.3 mmol) and lithium iodide (0.44 g, 3.3 mmol) in 2-butanone (10 mL): white solid, 0.38 g (2.7 mmol, 81%); ^{1}H NMR (D_2O) δ 1.91 (4H, d, J = 14.2 Hz), 3.57 (3H, d, J = 10.5 Hz); ^{31}P NMR (D_2O) δ 15.5. Anal. Calcd for $C_3H_7LiNO_3P$: C, 25.2; H, 4.9; N, 9.8. Found: C, 24.9; H, 4.7; N, 9.6.

2-Methoxy-2-oxo-1,3,2-oxazaphospholidine (4). A solution of triethylamine (4.05 g, 40 mmol) in dry chloroform (20 mL) was placed in a three-necked flask equipped with a magnetic stirrer, two dropping funnels, and a drying tube. To this stirred solution were added a solution of methyl phosphorodichloridate (3.0 g, 20 mmol) in chloroform (15 mL) and a solution of 2-aminoethanol (1.22 g, 20 mmol) in chloroform (15 mL) dropwise simultaneously from two dropping funnels at 5 °C. The cooling was removed, and the stirring was continued for 1 h. The precipitate was filtered off, the solvent was removed under reduced pressure, the viscous oil was treated with dry ether (20 mL), and the residual triethylammonium chloride was filtered off. The precipitation process was repeated several times until no more salt precipitated. Evaporation of the solvent afforded pure product as colorless crystals, 0.90 g (6.6 mmol, 33%): mp 197.2–199.1 °C; ^{1}H NMR ($CDCl_3$) δ 3.35 (1H, m), 3.50 (1H, m), 3.67 (3H, d, J = 11.8 Hz), 4.21 (1H, m), 4.30 (1H, m); proton-coupled ^{13}C NMR ($CDCl_3$) δ 48.1 (dt, J = 147.2, 15.9 Hz), 53.7 (dq, J = 149.8, 6.8 Hz), 62.8 (t, J = 157.0 Hz); ^{31}P NMR ($CDCl_3$) δ 27.4. Anal. Calcd for $C_3H_8NO_3P$: C, 26.5; H, 5.9; N, 10.3. Found: C, 26.2; H, 6.0; N, 10.0.

Methyl *N*-(2-Hydroxyethyl)phosphoramidate (5). Substrate **4** (0.040 g, 0.3 mmol) was dissolved in D_2O (0.4 mL), the pD of which was adjusted to ~11 by the addition of a 40% solution of NaOD in D_2O. The NMR spectrum was recorded immediately and demonstrated complete disappearance of **4** and the formation of a single product which was identified in solution as methyl *N*-(2-hydroxyethyl) phosphoramidate: ^{1}H NMR (D_2O) δ 2.79 (2H, m), 3.38 (3H, d, J = 10.9 Hz), 3.45 (2H, t, J = 5.6 Hz); ^{31}P NMR (D_2O) δ 10.1. The experiment was repeated on a larger scale (0.20 g of **4**, 1.5 mmol) in H_2O containing NaOH, and the sodium salt of **5** was obtained after evaporation and recrystallization from ethanol: glassy, hygroscopic substance, 0.21 g (1.12 mmol, 78%); ^{1}H and ^{31}P NMR spectra identical to those given above. Anal. Calcd for $C_3H_9NaNO_4P$: C, 20.3; H, 5.1; N, 7.9. Found: C, 19.9; H, 5.3; N, 7.6.

2-Aminoethyl Methyl Phosphate (6). As above, **4** was dissolved in D_2O (neat), or in D_2O, the pD of which was adjusted to ~4 by the addition of trifluoroacetic acid-*d*. NMR spectra demonstrated complete disappearance of **4** and the formation of a single product, identified as methyl (2-aminoethyl) phosphate: ^{1}H NMR (D_2O) δ 3.18 (2H, t, J = 5.3 Hz), 3.52 (3H, d, J = 10.8 Hz), 4.01 (2H, m); ^{31}P NMR (D_2O) δ 2.4. After evaporation and drying under high vacuum, crude **6** was obtained as a semisolid material (100%). Anal. Calcd for $C_3H_{10}NO_4P$: C, 23.2; H, 6.5; N, 9.0. Found: C, 23.0; H, 6.8; N, 8.8.

(2-Hydroxyethyl)ammonium Methyl Phosphate (7). A solution of 2-aminoethanol (0.54 g, 8.8 mmol) in dry ethanol (5 mL) was added slowly to a solution of methyl phosphate (0.99 g, 8.8 mmol) in ethanol (5 mL). The solution was stirred for 1 h, ether (10 mL) was added, and the mixture was left overnight in a refrigerator. The precipitate was centrifugated, washed with dry ether, and dried under high vacuum: white, very hygroscopic solid, 1.39 g (8.0 mmol, 91%); ^{1}H NMR (D_2O) δ 3.03 (2H, t, J = 5.3 Hz), 3.46 (3H, d, J = 10.8 Hz), 3.71 (2H, t, J = 5.3 Hz); ^{31}P NMR (D_2O) δ 2.2. Anal. Calcd for $C_3H_{12}NO_5P$: C, 20.8; H, 6.9; N, 8.1. Found: C, 20.5; H, 7.1; N, 8.0.

(2-Hydroxyethyl)ammonium Picrate was prepared from equimolar quantities of 2-aminoethanol and picric acid in benzene/ethanol (30:1, v/v). After filtration, the product was washed several times with dry benzene and dried under high vacuum: yellow crystals (79%); mp 157.5–158.5 °C; ^{1}H NMR (D_2O) δ 3.07 (2H, t, J = 5.4 Hz), 3.75 (2H, t, J = 5.3 Hz), 8.83 (2H, s). Anal. Calcd for $C_8H_{10}N_4O_8$: C, 33.1; H, 3.4; N, 19.3. Found: C, 33.1; H, 3.5; N, 19.2.

***N*-(2-Aminoethyl)pyridinium Chloride (Cation of 11).** (2-Hydroxyethyl)ammonium chloride (3.0 g, 25.8 mmol) was dissolved in water (2 mL), pyridine (8 mL) was added, and the solution was heated under reflux for 18 h. The pyridine and water were distilled off, and the residual water was removed by means of the Dean–Stark method, yielding the product as colorless crystals, 3.46 g (22 mmol, 85%): mp 210.1–211.8 °C; ^{1}H NMR (D_2O) δ 3.46 (2H, t, J = 6.4 Hz), 3.78 (2H, t, J = 6.4 Hz), 8.85 (5H, m). Anal. Calcd for $C_7H_{11}ClN_2$: C, 53.0; H, 7.0; N, 17.7. Found: C, 52.9; H, 7.1; N, 17.5.

***N,N*-Bis(2-chloroethyl)phosphoramidodichloridate** was prepared from bis(2-chloroethyl)ammonium chloride and phosphoryl chloride:[20] 71%; bp 125 °C (0.7 mm); mp 55–56 °C (lit.[20] mp 54–56 °C); ^{1}H NMR ($CDCl_3$) δ 3.35 (4H, m), 3.65 (4H, t, J = 6.8 Hz); ^{31}P NMR ($CDCl_3$) δ 18.0.

Dimethyl *N,N*-Bis(2-chloroethyl)phosphoramidate. The above dichloridate (5.0 g, 20 mmol) was dissolved in dry methanol (20 mL), and a solution of sodium methoxide (0.92 g of Na, 40 mmol) in methanol (20 mL) was added dropwise at 5 °C. The cooling was removed, and the mixture was stirred for 20 h. After evaporation of the solvent, chloroform (30 mL) was added, NaCl was filtered off, and the solvent was removed under reduced pressure. Crude product was purified by distillation, yielding pale yellow oil, 3.6 g (14 mmol, 73%): bp 111–113 °C (0.7 mm); ^{1}H NMR ($CDCl_3$) δ 3.38 (4H, m), 3.59 (4H, t, J = 6.7 Hz), 3.70 (6H, d, J = 11.2 Hz); proton-coupled ^{13}C NMR ($CDCl_3$) δ 42.3 (t, J = 153.5 Hz), 49.5 (dt, J = 1441.1, 5.6 Hz), 53.3 (dq, J = 150.1, 5.6 Hz); ^{31}P NMR ($CDCl_3$) δ 12.0; MS m/z 250 (M^+, 3), 215 (25), 200 (58), 150 (53), 138 (100). Anal. Calcd for $C_6H_{14}Cl_2NO_3P$: C, 28.8; H, 5.6; N, 5.6. Found: C, 28.6; H, 5.7; N, 5.5.

Lithium methyl *N,N*-Bis(2-chloroethyl)phosphoramidate (2c) was prepared from the above substrate (0.5 g, 2 mmol) and LiI (0.27 g, 2 mmol) in 2-butanone, as described for **2b**: colorless solid, 0.27 g (1.1 mmol, 55%); ^{1}H NMR (D_2O) δ 3.26 (4H, m), 3.43 (3H, d, J = 11.0 Hz), 3.58 (4H, t, J = 7.1 Hz); ^{31}P NMR (D_2O) δ 10.1. Anal. Calcd for $C_5H_{11}Cl_2LiNO_3P$: C, 24.8; H, 4.5; N, 5.8. Found: C, 24.5; H, 4.5; N, 5,6.

Dimethyl *N,N*-bis(2-hydroxyethyl)phosphoramidate was prepared from dimethyl phosphite and diethanolamine in CCl_4, following the geneal procedure,[21] and then purified by bulb-to-bulb distillation (ot 160 °C (0.15 mm)), 68%: ^{1}H NMR ($CDCl_3$) δ 3.12 (4H, m), 3.65 (4H, t, J = 6.2 Hz), 3.66 (6H, d, J = 11.1 Hz), 4.90 (2H, br s); proton-coupled ^{13}C NMR ($CDCl_3$) δ 40.3 (t, J = 146.5 Hz), 47.9 (dt, J = 146.1, 5.8 Hz), 54.3 (dq, J = 151.1, 5.8 Hz); ^{31}P NMR ($CDCl_3$) δ 14.3; MS m/z 213 (M^+, 0.9), 182 (100), 152 (51) 138 (52).

Lithium methyl *N,N*-bis(2-hydroxyethyl)phosphoramidate was prepared from the above substrate (2.0 g, 9.3

(18) Silverstein, R. M.; Bassler, G. C.; Morril, T. C. *Spectrometric Identification of Organic Compounds*; J. Wiley: New York, 1981; p 226.
(19) Grechkin, N. P. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1956**, 538.
(20) Friedman, O. M.; Seligman, A. M. *J. Am. Chem. Soc.* **1954**, *76*, 655.
(21) Zwierzak, A.; Osowska, K. *Synthesis* **1984**, 223.

mmol) and LiI (1.5 g, 11.0 mmol) in 2-butanone, as described for **2b**: pale yellow solid, 0.63 g (3.1 mmol, 33%); ^{1}H NMR (D_2O) δ 3.10 (4H, m), 3.42 (3H, d, J = 10.8 Hz), 3.60 (4H, t, J = 6.4 Hz); ^{31}P NMR (D_2O) δ 11.8. Anal. Calcd for $C_5H_{13}LiNO_5P$: C, 29.3; H, 6.4; N, 6.8. Found: C, 28.9; H, 6.5; N, 6.5.

2-Methoxy-2-oxo-3-(2-chloroethyl)-1,3,2-oxazaphospholidine (4a). Dimethyl *N,N*-bis(2-chloroethyl)phosphoramidate (0.50 g, 2 mmol) was dissolved in dry pyridine (20 mL), and the solution was heated at 80 °C until ^{31}P NMR spectra showed complete disappearance of the substrate and the formation of a single phosphorus-containing product. After removal of the solvent under reduced pressure, ether (20 mL) was added, the precipitate was filtered off, and the filtrate was evaporated under reduced pressure and finally dried under high vacuum to give a pale yellow, viscous oil, 0.32 g (1.6 mmol, 80%) which could not be purified by distillation without decomposition: ^{1}H NMR (pyridine-d_5) δ 3.32 (2H, m), 3.35 (2H, m), 3.70 (2H, t, J = 6.5 Hz), 3.71 (3H, d, J = 11.4 Hz), 4.21 (2H, m); proton-coupled ^{13}C NMR ($CDCl_3$) δ 42.1 (t, J = 142.2 Hz), 45.2 (dt, J = 155.9, 4.2 Hz), 48.5 (dt, J = 146.8, 14.9 Hz), 54.2 (dq, J = 150.0, 6.9 Hz), 61.9 (t, J = 156.8 Hz); ^{31}P NMR (pyridine-d_5) δ 22.4. Anal. Calcd for $C_5H_{11}ClNO_3P$: C, 30.2; H, 5.5; N, 7.0. Found: C, 29.4; H, 5.9; N, 6.5.

***N*-(2-Chloroethyl)-2-aminoethyl methyl phosphate (13)** was prepared by incubation of **4a** in D_2O with pD adjusted to ~4 (CF_3CO_2D) at 60 °C until the ^{31}P NMR spectrum showed complete disappearance of **4a** and the formation of a single product: ^{1}H NMR (D_2O) δ 3.13 (2H, t, J = 5.8 Hz), 3.21 (2H, m), 3.57 (3H, d, J = 10.9 Hz), 3.89 (2H, t, J = 5.7 Hz), 4.01 (2H, m); ^{31}P NMR (D_2O) δ 2.2. After evaporation and drying, crude **13** was obtained as a glassy substance (98%). Anal. Calcd for $C_5H_{13}ClNO_3P$: C, 29.9; H, 6.5; N, 7.0. Found: C, 29.5; H, 6.2; N, 6.9.

Bis[2-(*N*-pyridinio)ethyl]amine Dichloride (14). Bis-(2-chloroethyl)ammonium chloride (3.0 g, 16.8 mmol) was dissolved in a pyridine/water mixture (20 mL, 4:1 v/v), and the solution was heated under reflux for 24 h. The solvent was removed under reduced pressure, traces of water were removed by the Dean–Stark method, and the product was dried under high vacuum to give colorless crystals, 3.8 g (14.1 mmol, 84%): mp 199.2–201.1 °C; ^{1}H NMR (D_2O) δ 3.42 (4H, t, J = 6.4 Hz), 3.78 (4H, t, J = 6.3 Hz), 8.51 (10H, m). Anal. Calcd for $C_{14}H_{19}N_3Cl_2$: C,56.0; H, 6.4; N, 14.0. Found: C, 55.7; H, 6.2; N, 14.2.

***N,N*-Bis(2-hydroxyethyl)ammonium picrate** was prepared from equimolar quantities of *N,N*-bis(2-hydroxyethyl)-amine and picric acid in benzene/ethanol (15:1, v/v). After filtration, the product was washed several times with cold benzene and dried under high vacuum to give pale yellow crystals (60%): mp 110–111 °C; ^{1}H NMR (D_2O) δ 3.18 (4H, t, J = 5.2 Hz), 3.80 (4H, t, J =5.2 Hz), 8.74 (2H, s). Anal. Calcd for $C_{10}H_{14}N_4O_9$: C, 35.9; H, 4.2; N, 16.8. Found: C, 35.8; H, 4.0; N, 16.6.

Degradation of 2b. (i) The substrate was dissolved in D_2O (40 mg in 0.4 mL) in an NMR tube, and the tube was sealed and kept in a water bath at constant temperature. Reaction progress was monitored by NMR (^{1}H and ^{31}P) spectroscopy, and the proportion of individual products was determined by the integration of the ^{31}P NMR signals (see Table 1). Whenever possible, authentic samples of the products were added separately to the reaction mixture to assure unambigous identification. The following products were observed.

2-(Hydroxyethyl)ammonium methyl phosphate (7) was identified by the addition of an authentic sample. Additionally, the cation was identified as a picrate salt (*vide supra*) and the anion as an anilinium salt; mp 166–168 °C (lit.[22] mp 166–169.5 °C).

Methyl (2-aminoethyl) phosphate (6) was identified by the addition of the product of acidic hydrolysis of **4** (*vide supra*).

Methyl *N*-(2-hydroxyethyl)phosphoramidate (5) was identified by the addition of the product of the alkaline hydrolysis of **4** (*vide supra*).

(22) Modro, A. M.; Modro, T. A. *O.P.P.I. Briefs* **1992**, *24*, 57.

***P*^{1},*P*^{2}-Dimethyl diphosphate (8)** was identified by NMR spectroscopy: ^{1}H NMR (D_2O) δ 3.46 (d, J = 11.2 Hz); ^{31}P NMR (D_2O) δ −9.3 (lit.[23] δ −9.3).

Degradation of **2b'** was carried out in the same way, and the products were identified as described above.

(ii) Substrate **2b** was dissolved in a pyridine-d_5/D_2O mixture (4:1, v/v) in an NMR tube, and the reaction was studied as described above. The following products were observed.

Methyl *N*-[2-(*N'*-pyridinioethyl)]phosphoramidate (10) was identified in solution by NMR spectroscopy: ^{1}H NMR (pyridine-d_5/D_2O, 4:1, v/v) δ 3.56 (3H, d, J = 11.0 Hz), 3.64 (2H, m), 4.78 (2H, t, J = 5.5 Hz), 7.6 (residual absorption); ^{31}P NMR δ 9.5. The product could not be isolated by evaporation as it hydrolyzed partially to **11**.

***N*-(2-Aminoethyl)pyridinium Methyl Phosphate (11).** The cation was identified by the addition of the authentic chloride salt (*vide supra*); the anion (^{1}H NMR δ 3.81, d, J = 10.3 Hz; ^{31}P NMR δ 5.0[24]) was identified as the anilinium salt: mp 165–167 °C (lit.[22] mp 166–169.5 °C).

Degradation of Lithium Methyl *N*-Ethylenephosphoramidate (9). Salt **9** was dissolved in D_2O (50 mg in 0.4 mL), and the pD of the solution was adjusted to ~4 (CF_3CO_2D). The solution was transferred to an NMR tube and sealed, the tube was incubated in a water bath at 40 °C, and the NMR spectra of the solution were recorded periodically. After 1 h, all **5** has reacted and yielded a single product which was identified as methyl *N*-(2-hydroxyethyl)phosphoramidate (**5**), whose ^{31}P and ^{1}H NMR spectra were identical to those of the same product obtained in the degradation of **2b** and in the alkaline hydrolysis of **4** (*vide supra*). The solution was then incubated at 40 °C for a further period of time. Product **5** was stable for approximately 72 h, after which slow formation of the P–N bond cleavage product **7** could be observed.

Reactivity of (2-Chloroethyl)ammonium Chloride. The salt was dissolved in D_2O (50 mg in 0.4 mL), the solution was transferred to an NMR tube, and the solution was kept at 40 °C for 96 h. NMR (^{1}H) analysis showed that the compound was perfectly stable. The same experiment was repeated using pyridine-d_5/D_2O (4:1, v/v) as a medium. After 96 h a complete conversion to *N*-(2-aminoethyl)pyridinium ion (cation of **11**) was observed: ^{1}H NMR δ 3.48 (2H, t, J = 6.3 Hz), 3.80 (2H, t, J = 6.3 Hz,), 8.90 (residual absorption).

Degradation of 2c. (i) A solution of **2c** in D_2O (50 mg in 0.4 mL) was incubated at 40 °C, and the reaction progress was monitored by NMR spectroscopy. A spectrum recorded after 24 h showed the disappearance of **2c** and formation of two products. No further change was observed for a total period of 96 h. The reaction products were identified as follows.

***N*-(2-Chloroethyl)-*N*-(2-hydroxyethyl)ammonium methyl phosphate (12):** 95%; ^{1}H NMR (D_2O) δ 3.19 (2H, m), 3.45 (2H, m), 3.50 (3H, d, J = 10.7 Hz), 3.79 (2H, t, J = 6.4 Hz), 3.84 (2H, t, J = 6.8 Hz); ^{31}P NMR δ 2.2. The cation of **12** was isolated as a picrate, mp 124–125 °C. Anal. Calcd for $C_{10}H_{13}ClN_4O_8$: C, 34.1; H, 3.7; N, 15.9. Found: C, 34.0; H, 3.3; N, 15.7. The anion of **12** was isolated as an anilinium salt, mp 165–168 °C (lit.[22] mp 166–169.5 °C).

Methyl 2-[*N*-(2-chloroethyl)amino]ethyl phosphate (13): 5%; ^{1}H NMR (D_2O) δ 3.21 (2H, m), 3.35 (2H, t, J = 5.0 Hz), 3.53 (3H, d, J = 10.9 Hz), 3.78 (2H, m), 4.02 (2H, m); ^{31}P NMR δ 2.1. **13** was additionally identified by addition of the product of acidic hydrolysis of **4a**.

(ii) The experiment was repeated using D_2O containing NaOD (pD ~11) as a reaction medium. The reaction was completed after 5 h, and the ^{31}P NMR spectrum showed formation of the methyl phosphate anion. ^{1}H NMR spectra showed the formation of three amine-derived products which were identified as follows.

***N,N*-Bis(2-hydroxyethyl)amine:** ^{1}H NMR δ 2.45 (4H, t, J = 5.8 Hz), 3.39 (4H, t, J = 5.6 Hz), confirmed by the addition

(23) Satterthwait, A. C.; Westheimer, F. H. *J. Chem. Soc.* **1978**, 3197; *J. Am. Chem. Soc.* **1980**, *102*, 4464.

(24) ^{31}P NMR chemical shift of methyl phosphate salts in D_2O is 2.2. In pyridine-d_5/D_2O (4:1, v/v) the δ_P value of this and other (*e.g.* anilinium) salts of that acid is 5.0.

of the authentic material, and isolated as a picrate salt; mp 109 - 110°C (mixed mp 109–110 °C).

***N*-(2-Chloroethyl)ethyleneimine:** ^{1}H NMR δ 1.06 (2H, m), 1.42 (2H, m), 2.25 (2H, t, J = 5.6 Hz), 3.37 (2H, t, J = 5.5 Hz)(lit.[25b] δ 1.21, m, 1.41, m, 2.41, t, 3.55, t).

***N*-(2-Hydroxyethyl)ethyleneimine:** ^{1}H NMR δ 0.97 (2H, m), 1.37 (2H, m), 2.02 (2H, t, J = 6.0 Hz), 3.43 (2H, t, J = 5.9 Hz) (lit.[26] δ (DMSO-d_6) 1.08, m, 1.54, m, 2.20, t, 3.50, t). Further incubation resulted in the disappearance of both ethyleneimine derivatives and in the exclusive formation of *N*,*N*-bis(2-hydroxyethyl)amine.

(iii) Degradation was carried out in a pyridine-d_5/D_2O mixture (4:1, v/v) as described for **2b**. The following products were obtained.

Methyl Phosphate Anion. A sole phosphorus-containing product: ^{1}H NMR δ 3.84 (3H, d, J = 10.9 Hz); ^{31}P NMR δ 3.4; isolated as anilinium salt, mp 166–167 °C.

N-{2-[*N*'-(2-Chloroethyl)ethyl]}pyridinium ion (tentative identification, as the ion could not be isolated as a stable salt): ^{1}H NMR δ 3.01 (2H, t, J = 6.1 Hz), 3.40 (2H, t, J = 6.0 Hz), 3.64 (2H, t, J = 6.1 Hz), 5.01 (2H, t, J = 6.0 Hz), 7.80 (residual absorption).

Bis[2-(*N*-pyridinio)ethyl]amine ion: ^{1}H NMR δ 3.27 (4H, t, J = 6.3 Hz), 3.87 (4H, t, J = 6.3 Hz), 8.45 (residual absorption), confirmed by the addition of the authentic material (**14**). Further incubation resulted in the exclusive formation of the latter (symmetrical) amine product.

Alkylating Behavior of Bis(2-chloroethyl)ammonium Ion. The chloride salt was dissolved in a required medium (35 mg in 0.4 mL), incubated at 40 °C, and the reaction progress was monitored by ^{1}H NMR spectroscopy.

(i) In D_2O, the salt is stable for at least 96 h.

(ii) In D_2O containing NaOD (pD ~11), formation of two products was observed.

***N*-(2-Chloroethyl)ethyleneimine** (transient): ^{1}H NMR δ 1.15 (2H, m), 1.45 (2H, m), 2.02 (2H, t, J = 5.9 Hz), 3.22 (2H, t, J = 5.8 Hz).

***N*-(2-Hydroxyethyl)ethyleneimine** (final product): ^{1}H NMR δ 1.00 (2H, m), 1.37 (2H, m), 2.02 (2H, t, J = 5.9 Hz), 3.32 (2H, t, J = 5.9 Hz).

(iii) In pyridine-d_5/D_2O (4:1, v/v), two products were observed.

N-{2-[*N*'-(2-Chloroethyl)ethyl]}pyridinium ion (transient, tentative identification): ^{1}H NMR δ 3.02 (2H, t, J = 6.1 Hz), 3.41 (2H, t, J = 6.1 Hz), 3.65 (2H, t, J = 6.2 Hz), 5.01 (2H, t, J = 6.0 Hz), 7.80 (residual absorption).

Bis[2-(*N*-pyridinio)ethyl]amine ion (final product): ^{1}H NMR δ 3.26 (4H, t, J = 6.2 Hz), 3.89 (4H, t, J = 6.2 Hz), 8.20 (residual absorption), confirmed by the addition of the authentic material (**14**).

Results and Discussion

The decomposition of two salts, lithium methyl *N*-(2-chloroethyl)phosphoramidate, **2b** (**2a**, X = OMe; R = H), and lithium methyl *N*,*N*-bis(2-chloroethyl)phosphoramidate, **2c** (**2a**, X = OMe; R = CH_2CH_2Cl), was studied in aqueous solutions,[27] in the absence and in the presence of additional nucleophilic reagents.

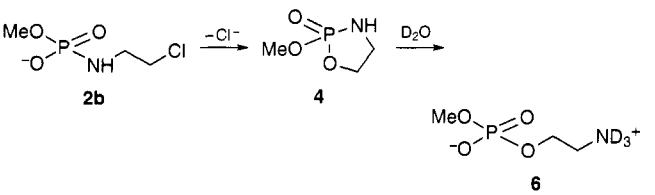
Degradation of 2b. A solution of **2b** in unbuffered D_2O was kept at room temperature and at 80 °C, and the change in the composition of the reaction system was monitored by ^{31}P NMR spectroscopy. Four phosphorus-containing products were formed, their proportions changing with time (Table 1). The major product (**7**) was easily identified as the (2-hydroxyethyl)ammonium salt of methyl phosphate ($DOCH_2CH_2ND_3^+$ $MeOPO_3D^-$), the ultimate product of the alkylation of the D_2O molecule by **2b**, and the hydrolysis of the P–N bond. The minor product (**6**) was identified as a diester, methyl 2-aminoethyl phosphate, $^+D_3NCH_2CH_2OP(OMe)O_2^-$. The first transient species (**5**) corresponds to methyl *N*-(2-hydroxyethyl)phosphoramidate, the product of the substitution of Cl in **2b** by the hydroxyl group. A second intermediate product (**8**), observed only at high conversion and disappearing at the end of the reaction, was identified as P^1,P^2-dimethyl diphosphate.

Table 1. Products and Yields[a] from the Hydrolysis of 2b in D_2O

temp (°C)	time (h)	**2b** (δ_P 10.3)	**5** (δ_P 10.1)	**6** (δ_P 2.4)	**7** (δ_P 2.2)	**8** (δ_P −9.3)
25	20	93	4	1	2	
	48	87	7	2	4	
	72	85	8	2	5	
	168	71	16	3	10	
	240	64	17	3	16	
80	20		15	4	71	10
	48		10	2	79	9
	67		9	3	80	8
	96			3	97	

[a] % obtained from the integration of the ^{31}P NMR signals.

Scheme 3



The presence of the stable, minor product **6** gives evidence for the intermediate formation of the 1,3,2-oxazaphospholidine product **4** (Scheme 2, pathway a), followed by its hydrolysis involving cleavage of the P–N bond (Scheme 3). The formation of transient product **5** can, in principle, be explained by three different routes, as shown in Scheme 4. The direct displacement (pathway f) was easily ruled out by showing the hydrolytic stability of the CH_2Cl group in the *N*-(2-chloroethyl)phosphoramidate function of other substrates. For example, the neutral precursor of **2b**, dimethyl *N*-(2-chloroethyl)-phosphoramidate, $(MeO)_2P(O)NHCH_2CH_2Cl$, when heated at 80 °C in D_2 O, underwent the hydrolysis of the P–N bond without any release of the Cl^- ion. The P–O bond hydrolysis in the cyclic intermediate **4** represents another option of the hydrolytic ring opening in a 1,3,2-oxazaphospholidine system, as opposed to the reaction shown in Scheme 3. We have prepared independently compound **4** and studied the products of its hydrolysis. The reaction was found to be strictly pH dependent: at pD ≤7 the hydrolysis yielded exclusively **6** (P–N cleavage), while at pD ≥9, only P–O (endocyclic) cleavage was observed (formation of **5**). The pD of the reaction mixture for the hydrolysis of **2b** in D_2O was then monitored over the whole duration of the experiment (336 h) and was found to decrease from the initial value of pD ~7 to the final value of pD = 4.[28] It is obvious, therefore, that, under the conditions of the experiment, **6** is formed from

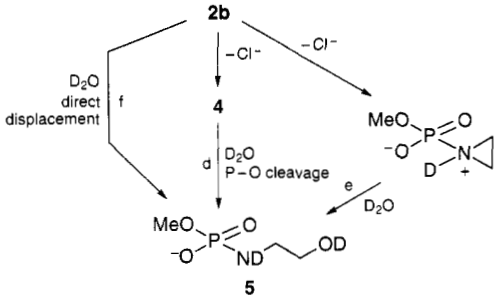
(25) (a) Pettit, G. R.; Settepani, J. A.; Hill, R. A. *Can. J. Chem.* **1965**, *43*, 1792. (b) Levins, P. L.; Papanastassiou, Z. B. *J. Am. Chem. Soc.* **1965**, *87*, 826.

(26) Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*; Aldrich Chemical Co.: Milwaukee, 1993, Vol. I, p 557.

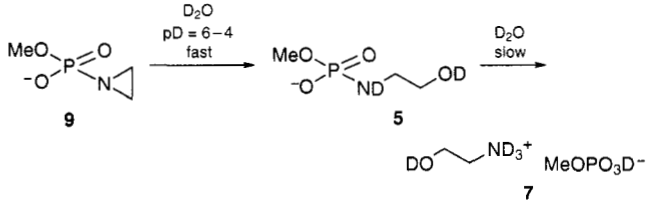
(27) The reactions were followed by NMR (^{31}P, ^{1}H) spectroscopy using D_2O as the reaction medium. Consequently, in all chemical equations the exchangable hydrogen atoms will be represented by D not by H atoms.

(28) The pH of the solution of the independently prepared salt, (2-hydroxyethyl)ammonium methyl phosphate at a concentration corresponding to that used in our experiments has a value of ~4.

Scheme 4



Scheme 5



the 1,3,2-oxazaphospholidine precursor **4** and that **5** *cannot* be produced via the same route. Product **5** is therefore produced via the hydrolytic ring opening of the aziridinium intermediate resulting from the 1,3-cyclization of **2b** (Scheme 4, pathway e). To confirm that mechanism, we have prepared the conjugate base of that intermediate (**9**) and studied its hydrolytic behavior. At the pD values in the range of 6–4, the ^{31}P NMR spectroscopy demonstrated fast hydrolytic opening of the aziridinium ring (formation of **5**), followed by very slow hydrolysis to the salt **7** (Scheme 5). It is interesting to note that at pD = 2, **9** undergoes fast hydrolysis of the P–N bond, *without* opening of the aziridine ring, yielding a stable aziridinium salt of methyl phosphate. This dependence of the regioselectivity of the hydrolysis of **9** on pH opens a possibility of directing the nucleophilic substitution of N-phosphorylated aziridines. Hydrolytic behavior of **9** led to two important conclusions. First, it explained the formation of $\mathbf{P_i}$ in the decomposition of **2b**; thus it confirmed the operation of pathway b (Scheme 2) in addition to the previously confirmed pathway a. Second, very slow hydrolysis of **5** in weakly acidic solutions shows that pathway b alone cannot be responsible for the formation of salt **7** as the major reaction product. We conclude therefore that **7** is formed primarily via pathway c, that is, substrate **2b** is capable of undergoing fragmentation to methyl metaphosphate and aziridine; the reaction is driven by the electronic charge at the phosphate group and the presence of a good leaving group. To confirm that mechanism, we have prepared a solution of equimolar quantities of methyl phosphate and aziridine in D_2O, heated the solution at 70–80 °C, and monitored the reaction progress by NMR spectroscopy. The reaction was completed after 24 h, and the NMR (^{31}P, ^{1}H) spectra showed almost exclusive formation of salt **7**, with only minor side products corresponding to self-condensation of aziridine.[29] Additional support for the fragmentation according to pathway c is taken from the presence of the symmetrical diphosphate **8**. This product, observed when methyl metaphosphate was generated in aqueous media,[23] results from the trapping of that intermediate by the already present hydrolysis product—methyl phosphate (Scheme 6). At the concentrations used, the molar ratio of the two available nucleophiles (D_2O and $MeOPO_3D^-$) is between 100 and 200. The nucleophilicity of the latter (for $H_2PO_4^-$, $n = 2.2$[30]) is, however, approximately 160 times higher than that of the solvent; thus comparable yields of both trapped products could be expected. The final conclusions can be formulated as follows. (i) Decomposition of **2b** in water follows all three pathways indicated in Scheme 2, with pathway a representing a minor route (*ca.* 3%). (ii) Pathway b, generally regarded as the mechanism of alkylation by *N*-(2-chloroethyl)phosphoramidate ions, does operate, but it is the "metaphosphate" route (c) that seems to be a predominant direction.

Scheme 6

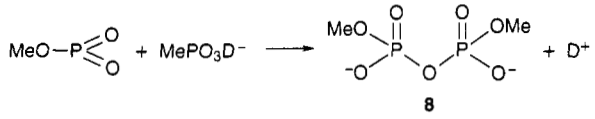


Table 2. Products and Yields[a] from the Hydrolysis of 2b′ in D_2O at 25 °C

time (h)	2b′	5	6	7	8
24	45	30		25	
36	40	31		29	
96	23	36		41	
168	11	36		48	5
216	8	34		54	4
480		29		65	6

[a] % obtained from the integration of the ^{31}P NMR signals.

Table 3. Products and Yields[a] for the Degradation of 2b in Pyridine-d_5/D_2O (4:1, v/v) at 60 °C

time (h)	2b	10	11
1	18	65	17
2	4	74	22
5		65	35
48		47	53
72		40	60
96		6	94
120		3	97

[a] % obtained from the integration of the ^{31}P NMR signals.

The effect of the leaving group in the 2-position of the N-substituent can be illustrated by the degradation of the bromo analogue of **2b**, lithium methyl *N*-(2-bromoethyl)phosphoramidate, **2b′** (Table 2). Substrate **2b′**, containing a better leaving group,[31] was found to be about three times more reactive than **2b**, indicating the involvement of of the C–X bond cleavage in the rate-determining transition state. The absence of the product **6** implies that the 1,5-cyclization of **2b′** to **4** is negligible. Apart from these differences, the general pattern of the reactivity of both substrates is the same.

In the next experiment, **2b** was incubated in a D_2O/pyridine-d_5 (1:4, v/v) mixture at 60 °C, and the reaction progress was monitored as before. Two phosphorus-containing products, **10** (δ_P 9.5) and **11** (δ_P 5.0), were formed; Table 3 gives the composition of the reaction mixture as a function of time. The change in the product composition is typical for a two-step conversion involving a relatively stable intermediate, *i.e.* **2b** first changes to **10**, which is in turn converted to the final product **11**.

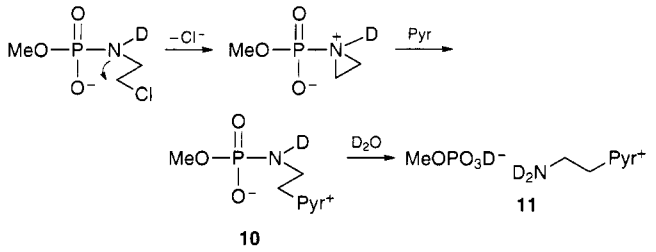
(29) Dermer, O. C.; Ham, G. E. *Ethylenimine and other Aziridines*; Academic Press: New York, 1969.

(30) Isaacs, N. S. *Physical Organic Chemistry*; Longman: New York, 1987, p 134.

(31) With respect to the azide ion in MeOH, $k_{MeBr}/k_{MeCl} = 63$.[32] In case of **2b**, the observed lower value of k_{rel} suggests lower advancement of the C–X bond cleavage in the transition state of the reaction.

(32) Parker, A. J. *Chem. Rev.* **1969**, *69*, 1.

Scheme 7



[a] Pyr = C_5D_5N.

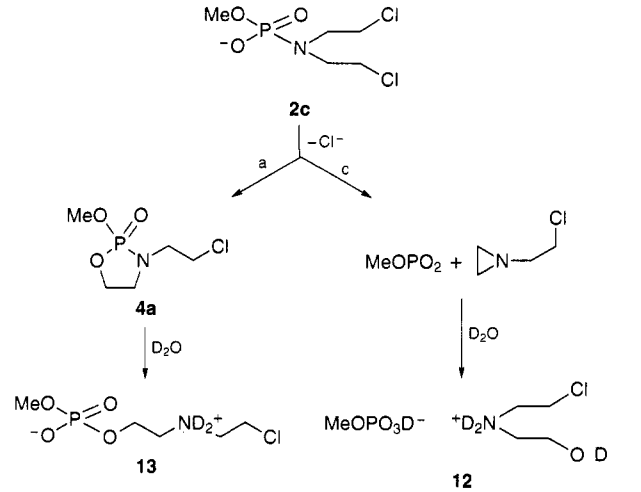
11 was identified as an analogue of salt **7**, that is, *N*-(2-aminoethyl)pyridinium methyl phosphate.[33] Its precursor, **10**, was identified as the zwitterionic methyl *N*-[2-(*N'*-pyridinioethyl)]phosphoramidate. A proposed mechanism for the reaction is outlined in Scheme 7. The same *N*-(2-aminoethyl)pyridinium cation was found as an exclusive product when (2-chloroethyl)ammonium chloride was incubated for several days at 40 °C in the same medium. Under these conditions, the medium provides enough base to cause the closure to the aziridinium ring and enough of a good nucleophile to make its alkylation fully selective. The similarity of the behavior of the chloride salt and of **2b** is obvious. In conclusion, in the water–pyridine system, we did not obtain any evidence for the operation of pathways a and c (Scheme 2). It seems that the nature of the reaction medium and the available nucleophile have a profound effect on the reaction mechanism; in the latter case only the initial cyclization to the aziridinium derivative is responsible for degradation.

Degradation of 2c. In a first experiment, **2c** was incubated in D_2O at 40 °C for 96 h. The complete disappearance of the substrate was achieved after 24 h, and the subsequent analysis indicated no change in the composition of the reaction product. Two phosphorus-containing products, **12** (95%, δ_P 2.2) and **13** (5%, δ_P 2.1), were formed. The former was identified as *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)ammonium methyl phosphate (an analogue of **7** formed from **2b**), while the minor product was identified as methyl 2-[*N*-(2-chloroethyl)-amino]ethyl phosphate (an analogue of **6**). The formation of these products was ascribed to the operation of two independent pathways of the degradation of **2c** (Scheme 8). As before, the intermediate 1,3,2-oxazaphospholidine product **4a** was prepared independently, and its hydrolysis at pD ≤7 yielded **13** as the exclusive product. The pD of the reaction mixture of **2c** decreased during the first hour from ~7 to ~4 and remained constant for the further duration of the experiment. The formation of the major product (**12**) with the unsymmetrically substituted ammonium cation (alkylation of only one molecule of D_2O) is the consequence of the acidity of the reaction medium.

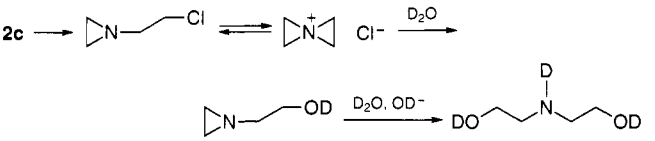
1-(2-Chloroethyl)ethyleneimine released in the fragmentation (Scheme 8) undergoes protonation, followed by the nucleophilic ring opening by water. The *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)ammonium ion formed is under those conditions stable, as the acidity of the medium is too low to allow for the next cyclization,

(33) The observed ^{31}P NMR chemical shift difference between **7** and **11** results from medium effects (D_2O *vs* D_2O/pyridine-d_5). Both salts were unambiguously identified by comparison with authentic samples, and their NMR spectra recorded in the respective media matched perfectly those of original reaction mixtures.

Scheme 8



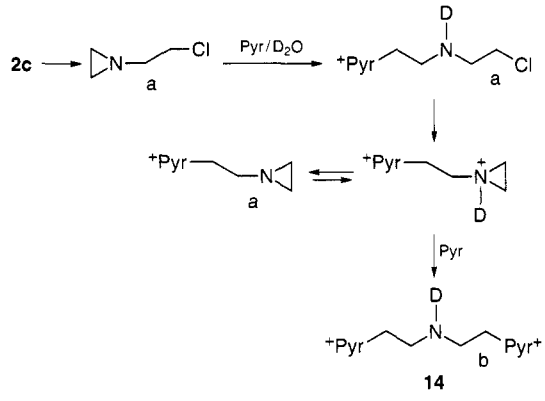
Scheme 9



necessary for the substitution of Cl by another molecule of D_2O. When **2c** was incubated at 40 °C in D_2O with pD adjusted to ~11, the NMR analysis demonstrated that the degradation was complete after 5 h, with the formation of only one, stable phosphorus product—the anion of methyl phosphate. ^1H NMR analysis showed that after 5 h three amine products were initially present, and they were identified as 1-(2-chloroethyl)ethylenimine, 1-(2-hydroxyethyl)ethylenimine, and *N,N*-bis(2-hydroxyethyl)-amine. The former two gradually disappeared, yielding the third amine as the final, stable product. It is obvious that in this case, due to the basicity of the medium, the initially formed 1-(2-chloroethyl)ethyleneimine undergoes further reactions leading finally to the bis(hydroxyethyl) product (Scheme 9). We conclude that, as in the previous experiment, no pathway b (Scheme 2) operated for substrate **2c**, that the 1,5-cyclization is supressed at high pD, and that alkylation of water takes place after the P–N bond cleavage. Support for this claim comes from two sources. First, the mere fact that we observe two intermediate aziridine derivatives without two corresponding signals in the ^{31}P NMR spectra demonstrated that the intermediates are two separate, phosphorus-free entities. Second, we have prepared the lithium salt of methyl *N,N*-bis(2-hydroxyethyl)phosphoramidate, the expected product of the bis-alkylation of D_2O by **2c** with the P–N bond intact (bis-alkylation via pathway b). The compound was then incubated in neutral, weakly acidic (pD ~4), and alkaline (pD ~11) D_2O at 40 °C for 48 h, and the NMR analysis demonstrated that the substrate was perfectly stable under these conditions. We conclude, therefore, that the bis(2-hydroxyethyl)phosphoramidate was never formed during the aqueous degradation of **2c**.

In aqueous pyridine (D_2O/C_5D_5N, 1:4, v/v), the course of the degradation of **2c** was different from that observed for **2b**. Incubation of the substrate at 40 °C for 48 h and monitoring the composition changes by NMR spectroscopy did not reveal the formation of any intermediate phosphoramidate product, the methyl phosphate anion being identified as a sole phosphorus-containing product.

Scheme 10



[a] (a) Observed as transient species. (b) Final product.

Similar to the reaction of **2c** in pD ~11, we observed the formation, and the subsequent disappearance of all phosphorus-free amine species expected for the release of the initial aziridine derivative, followed by the reaction with the nucleophilic medium, yielding finally the bis-alkylated product bis[2-(*N*-pyridinio)ethyl]amine dication (**14**) (Scheme 10). The important difference is that for **2c** alkylation of pyridine took place exclusively *after* the P–N bond cleavage, that is only the fragmentation to the neutral aziridine derivative ("metaphosphate" mechanism) was operating. As a final remark, it is worth mentioning that a literature report claims that bis(2-chloroethyl)amine has no alkylating activity at high pH.[1] We have found that in the D_2O/C_5D_5N (1:4, v/v) system substrate is capable of bis-alkylating the nucleophilic component (two molecules of pyridine). In purely aqueous medium, at higher pH, only the monoalkylating behavior was observed, yielding 1-(2-hydroxyethyl)ethyleneimine.

The results of this study show that a nitrogen mustard derivative, phosphorylated at nitrogen by an ionic phosphate group, represents a highly reactive system capable of reacting according to different mechanisms. The major directions (initial 1,5-cyclization, 1,3-cyclization, and the "metaphosphate" fragmentation) differ in that the second and the third (pathways b and c, Scheme 2) can be considered as "active", as they lead to the alkylation of an external nucleophile by an aziridinyl function. The first route (pathway a, Scheme 2) represents a "deactivation" direction, as the 1,3,2-oxazaphospholidine derivative formed, although reactive, is devoid of alkylating properties. From the point of view of the role of metaphosphate intermediates in organophosphorus chemistry, pathway c shows that the N-phosphorylated mustards of the type **2a** represent yet another example of a system in which a "metaphosphate" species is incorporated into a molecular framework, from which it can be easily released, together with the departure of a leaving group and the simultaneous formation of another reactive species (*e.g.*, aziridine derivative).

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