Nucleophilic Reactivity of *N*-Phosphorylated Ethyleneimine†

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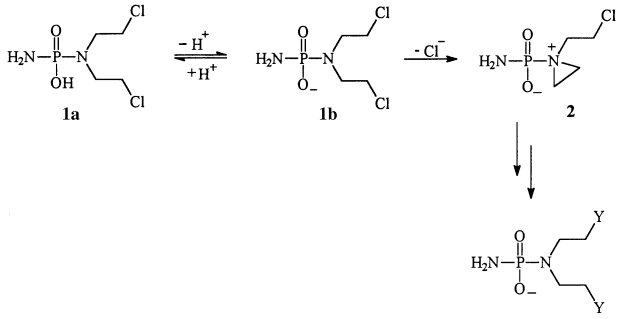
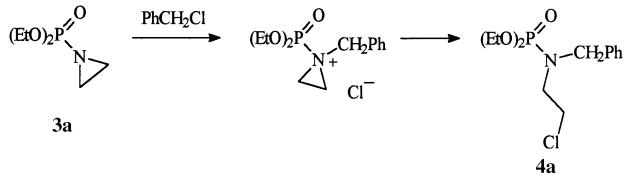
The diesters of *N*-phosphorylated ethyleneimine (aziridine) are unreactive towards alkylating agents, but after being converted into the ionic monoesters, they undergo facile *N*-methylation with MeI, followed by fast opening at the aziridinium ring by the iodide ion; the results can be related to the bis-alkylating reactivity of *N*-phosphorylated nitrogen mustards.

The bisalkylating (cross-linking) reactivity of phosphoramide mustard **1a**, released in the *in vivo* degradation of an anti-tumor drug cyclophosphamide, is attributed to the intramolecular cyclization of its conjugate base **1b** to an aziridinium ion **2**, the reactive intermediate in the sequence of two ring-opening reactions with an external nucleophile Y (Scheme 1).[1] We have demonstrated the participation of



Scheme 1

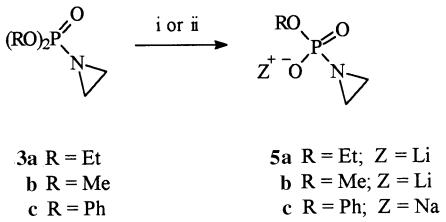
aziridinium ions of the type **2** in the degradation of *N*-(2-chloroethyl)phosphoramidates (*N*-phosphorylated nitrogen mustards).[2] The results obtained so far indicate that the accumulation of the negative charge at the phosphate group is a prerequisite for a facile 1,3-cyclization, while the positively charged nitrogen atom in **2** is responsible for the high electrophilicity of the aziridine carbon atoms. There is, however, a single literature report[3] according to which *N*-(diethylphosphoryl)ethyleneimine **3a** reacts with benzyl chloride yielding the *N*-benzylated, ring-opened product **4a**; the reaction can be explained by the mechanism given in Scheme 2



Scheme 2

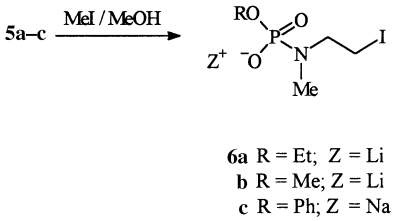
involving the initial *N*-benzylation of the substrate. In view of the available reports on the alkylation of phosphoramidates,[4] it seems unlikely that the nitrogen atom in a *neutral* substrate like **3a** would be nucleophilic enough to be alkylated to a quaternary derivative, susceptible to the ring-opening reaction. We have prepared the *N*-phosphorylated ethyleneimine **3a** and its *O*,*O*-dimethyl (**3b**) and *O*,*O*-diphenyl (**3c**) analogues and tested those three substrates towards alkylation (using benzyl chloride or iodomethane) under various conditions. No reaction with $PhCH_2Cl$ was observed for **3a** or **3b** upon boiling in chlorobenzene for 30 h or on heating the substrates neat at 100 °C for 24 h; **3b** underwent under those conditions partial degradation (most likely resulting from facile *O*-demethylation[5]) to a complex mixture of phosphorus-containing products. All three substrates proved unreactive towards iodomethane in $CDCl_3$ solutions at 40 °C for 24 h. We did not, therefore, confirm the earlier reports[3] on the *N*-alkylation of **3a**, and we demonstrate in this work that the ionization of the phosphate function has a dramatic effect on the nucleophilic reactivity of the *N*-phosphorylated ethyleneimine.

Phosphoramidates **3** were converted into the corresponding monoanionic salts **5** either by the nucleophilic *O*-dealkylation (**3a**, **3b**) or by alkaline hydrolysis (**3c**) (Scheme 3).



Scheme 3 *Reagents:* i, LiI, butan-2-one; ii, aq. NaOH

The salts **5** were then subjected to the alkylation reactions in methanolic solutions at room temperature. With benzyl chloride the reactivity of **5** was very high, but non-selective, leading to a complex mixture of phosphorus-containing products, all giving rise to ^{31}P NMR signals at about 11 ppm (no P—N bond cleavage). The reaction with MeI, on the other hand, yielded in each case the expected products **6** (scheme 4) as a single product, with the reactivity of the salts



Scheme 4

decreasing in the order **5b** > **5a** > **5c**. Presumably the products **6** are formed *via* the attack of I^- ion at the *N*-methylated zwitterionic derivative of **5** (see Scheme 2); the formation of the latter intermediate confirms the structural condition of a substrate (the anionic phosphate group) necessary for the *N*-alkylation to occur. The *N*-methylated derivative of **5** can be considered a model for an intermediate **2** (Scheme 1), postulated as a key intermediate in the bis-alkylation sequence of phosphoramide mustard.[6]

The enhanced basicity (and nucleophilicity) of the aziridine nitrogen in the ionic substrates **5**, allowing the ring-

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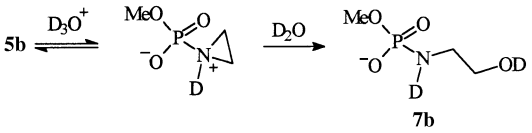
opening *via* the *N*-protonated (or *N*-alkylated) intermediate, was confirmed in the following experiment. When **5b** was incubated in a weakly acidic solution in D_2O (pD ≈ 4) at 40 °C for 1 h, it was quantitatively converted into the *N*-(2-hydroxyethyl)phosphoramidate **7b**, presumably *via* the attack of water at the *N*-protonated form of the substrate (Scheme 5).



Scheme 5

It is interesting to note that in the conjugate acid of **5b**, it is the aziridine carbon, not the phosphorus atom, that represents the electrophilic reaction centre, and that the reaction leads to a phosphoramidate product, not to P—N bond cleavage, as is usually observed for a phosphoric amide system under acidic conditions.[7] As expected, all ions **5** were found to be perfectly stable when incubated in the D_2O–[$^2H_5$]-pyridine (4:1, v/v) at 40 °C for 48 h. Since in that medium the *N*-protonation of **5** should be negligible, no reaction takes place, notwithstanding the presence of highly nucleophilic species.

In conclusion, our results indicate that the biologically important bis-alkylating reactivity of *N*-phosphorylated mustards is closely related to a local pH of the medium, which should control the deprotonation equilibria (**1a**⇄**1b**, Scheme 1), critical for the 1,3-cyclization and the nucleophilic ring-opening steps.

Experimental

NMR spectra were recorded on a Bruker AC 300 spectrometer in $CDCl_3$ (unless otherwise stated) and δ values are given relative to $SiMe_4$ (^{1}H, ^{13}C) or 85% H_3PO_4 (^{31}P). ^{13}C NMR spectra are given as proton-decoupled, but the proton-coupled spectra gave the expected patterns of signals. Mass spectra were recorded on a Varian MAT-212 double-focusing direct-inlet spectrometer at an ionization potential of 70 eV. Elemental analyses (C, H, N) were performed at the Chemistry Department, University of Cape Town.

N-Phosphorylated aziridines **3** were prepared from the corresponding phosphorochloridates and ethyleneimine (prepared according to the literature procedure[8]) in the presence of triethylamine in diethyl ether at 10 °C. After filtration through a layer of $MgSO_4$–Celite, the solution was washed with 10% aqueous K_2CO_3, dried ($MgSO_4$) and concentrated under reduced pressure. **3a**, oil (59%); bp 139–141 °C) at 1 mmHg; δ_H 1.29 (6 H, t, *J* 6.8 Hz), 2.10 (4 H, d, *J* 15.5 Hz), 4.10 (4 H, m); δ_C 16.2 (s), 24.2 (d, *J* 6.3 Hz), 63.3 (d, *J* 4.2 Hz); δ_P 15.9; *m*/*z* 179 (6%, M^+), 166 (100, $M^+ - CH$), 138 (39, $M^+ + 1 - C_2H_4N$), 110 (99, $M^+ + 1 - C_2H_4N - C_2H_4$). **3b**,[9] oil (62%); bp 83–84 °C at 0.7 mmHg; δ_H 2.14 (4 H, d, *J* 15.5 Hz), 3.77 (6 H, d, *J* 10.8 Hz); δ_C 23.4 (d, *J* 6.0 Hz), 52.9 (d, *J* 5.5 Hz); δ_P 18.4. **3c**, oil (51%); bp 187–189 °C at 0.7 mmHg; δ_H 2.31 (4 H, d, *J* 16.4 Hz), 7.22 (10 H, m); δ_C 25.5 (d, *J* 6.5 Hz), 120.2 (s), 125.2 (s), 129.8 (d, *J* 8.6 Hz), 149.2 (s); δ_P 6.8; *m*/*z* 276 (100%, $M^+ + 1$), 262 (22, $M^+ - CH$), 166 (20, $M^+ + 1 - CH - 2 \times Ph$).

Salts **5a** and **5b** were prepared from **3a** and **3b** by heating the solutions of substrates with 1 mol equiv. of LiI in butan-2-one under reflux for 3 h. The precipitate was filtered off, washed several times with anhydrous $CHCl_3$ and dried under high vacuum. **5a**, white powder (81%); δ_H (D_2O) 1.18 (3 H, t, *J* 7.2 Hz), 1.89 (4 H, d, *J* 14.3 Hz), 3.91 (2 H, m); δ_P 13.9 (Found: C, 30.20; H, 6.05; N, 8.85. $C_4H_9LiNO_3P$ requires C, 30.59; H, 5.78; N, 8.92%). **5b**, white powder (81%); δ_H (D_2O) 1.91 (4 H, d, *J* 14.2 Hz), 3.57 (3 H, d, *J* 10.5 Hz); δ_P 15.1 (Found: C, 24.95; H, 5.08; N, 9.55. $C_3H_7LiNO_3P$ requires C, 25.20; H, 4.93; N, 9.79%). **5c** was prepared from **3c** by heating a suspension of **3c** in 0.15 M aqueous NaOH containing 1 mol equiv. of NaOH until a homogeneous solution was obtained (*ca*. 17 h). Most of the water was removed under reduced pressure and complete drying was achieved by means of the Dean–Stark method. **5c**, white solid (64%); δ_H (D_2O) 1.98 (4 H, d, *J* 14.6 Hz), 7.13 (5 H, m); δ_P 10.5 (Found: C, 43.00; H, 4.25; N, 6.30. $C_8H_9NaNO_3P$ requires C, 43.45; H, 4.10; N, 6.33%).

Methylation reactions of salts **5** were carried out by incubating a solution of **5** in CD_3OD (1.4 mL per mmol of **5**) in the presence of MeI (2.8 mol equiv.) at room temperature and monitoring the reaction progress by ^{31}P NMR spectroscopy. After a certain period of time (24, 52 and 72 h for **5b**, **5a**, and **5c**, respectively) the spectrum showed complete disappearance of substrate and the formation of a single phosphorus-containing product. The solvent and the excess of MeI were evaporated under reduced pressure, and the residue was washed with anhydrous ether and dried under high vacuum. **6a**, white powder (98%); δ_H ($CD_3OD + NaOD$; pD ≈ 11) 1.18 (3 H, t, *J* 7.0 Hz), 2.50 (3 H, d, *J* 9.5 Hz), 3.00 (2 H, m), 3.23 (2 H, t, *J* 6.4 Hz), 3.30 (2 H, m); δ_P 10.9 (Found: C, 19.85; H, 4.20; N, 4.50. $C_5H_{12}ILiNO_3P$ requires C, 20.09; H, 4.05; N, 4.69%). **6b**, white hygroscopic solid (100%); δ_H ($CD_3OD + NaOD$; pD ≈ 11) 2.50 (3 H, d, *J* 9.1 Hz), 3.01 (2 H, m), 3.25 (2 H, t, *J* 6.6 Hz), 3.36 (3 H, d, *J* 11.0 Hz); δ_P 12.3 (Found: C, 16.44; H, 3.85; N, 4.77. $C_4H_{10}ILiNO_3P$ requires C, 16.86; H, 3.54; N, 4.92%). **6c**, white powder (88%); δ_H ($CD_3OD + NaOD$; pD ≈ 11) 2.51 (3 H, d, *J* 9.6 Hz), 3.02 (2 H, m), 3.21 (2 H, t, *J* 6.4 Hz), 7.05 (5 H, m); δ_P 8.1 (Found: C, 29.60; H, 3.50; N, 3.70 ($C_9H_{12}INaNO_3P$ requires C, 29.78; H, 3.33; N, 3.86%).

Hydrolysis of Lithium Methyl N,N-*Ethylenephosphoramidate* (**5b**).—Salt **5b** was dissolved in D_2O (0.050 g in 0.4 mL), the pD of the solution was adjusted to *ca*. 4 with CF_3CO_2D and the solution was incubated at 40 °C for 1 h. NMR (^{1}H and ^{31}P) spectra of the solution demonstrated complete disappearance of **5b** and the formation of a single product, identical with that obtained previously in the alkaline hydrolysis of 2-methoxy-2-oxo-1,3,2-oxazaphospholidine.[2] The reaction was repeated on a larger scale in the H_2O–CF_3CO_2H solution and the product **7b** was isolated as an amorphous, hygroscopic solid (95%); δ_H (D_2O) 2.79 (2 H, m), 3.38 (3 H, d, *J* 10.9 Hz), 3.45 (2 H, t, *J* 5.6 Hz); δ_P 10.2 (Found: C, 22.10; H, 5.95; N, 8.50. $C_3H_9LiNO_4P$ requires C, 22.38; H, 5.63; N, 8.70%).

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