High-Resolution Nuclear Magnetic Resonance Investigations of the Chemical Stability of Cyclophosphamide and Related Phosphoramidic Compounds

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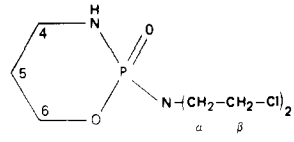
Abstract: High-resolution ^1H (220 MHz) and ^{13}C (68 MHz) nuclear magnetic resonance (NMR) spectroscopy was used to investigate the chemical stability of the anticancer drug cyclophosphamide [2-[bis(2-chloroethyl)amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide, **1**], *N*-methylcyclophosphamide (**6**), isophosphamide [2-(2-chloroethylamino)-3-(2-chloroethyl)-2*H*-1,3,2-oxazaphosphorinane 2-oxide, **9**], *N*-α-methylbenzylcyclophosphamide (**12**), and related phosphoramidic compounds. Hydrolysis (100 °C) of **6** led to isolation of *N*-(2-chloroethyl)-*N'*-methyl-*N'*-(3-phosphatopropyl)ethylenediammonium ion as an oxalate derivative (**7**·oxalate), while similar reaction of β,β'-**6**-d_4 [2-[bis(2-chloro-2,2-dideuterioethyl)amino]-3-methyl-2*H*-1,3,2-oxazaphosphorinane 2-oxide] was used to demonstrate via NMR that **7**-d_4 was formed *without* positional scrambling of adjacent α and β carbons in the mustard functionality [$N(C^{\alpha}H_2C^{\beta}D_2Cl)_2$] of the starting material. Product NMR data from analogous deuterium-labeling experiments with β,β'-**1**-d_4 were consistent with a similar stereospecific process for conversion of **1** into *N*-(2-chloroethyl)-*N'*-(3-phosphatopropyl)ethylenediammonium ion (**3**). ^1H NMR derived pseudo-first-order rate constants (k) for the hydrolysis (37 °C) of **1**, β,β'-**1**-d_4, **9**, and **12** indicated that $k_H/k_D = 1.14 \pm 0.07$ (per deuterium) for **1**, and that increasing steric bulk about the N-3 position in these 1,3,2-oxazaphosphorinane derivatives leads to decreasing hydrolysis rates: $k_9/k_1 = 4.8 \times 10^{-2}$ and $k_{12}/k_1 < 1.1 \times 10^{-2}$. These observations, in concert with other kinetic data, implicate an initial hydrolysis mechanism for **1** wherein rate-limiting carbon–chlorine bond cleavage is accompanied by participation of the endocyclic nitrogen lone pair to yield a labile intermediate [1-(2-chloroethyl)tetrahydro-1*H*,5*H*-[1,3,2]diazaphospholo[2,1-*b*][1,3,2]oxazaphosphorine 9-oxide (**2**)] whose existence was suggested some years ago by Friedman and coworkers [O. M. Friedman, S. Bien, and J. K. Chakrabarti, *J. Am. Chem. Soc.*, **87**, 4978 (1965)]. Additional deuterium-labeling studies by ^1H NMR using β,β'-**1**-d_4 revealed that secondary hydrolytic conversion of **3** into *N*-(2-hydroxyethyl)-*N'*-(3-phosphatopropyl)ethylenediammonium ion (**22**) occurs with label scrambling, which is consistent with the intermediacy of a symmetrical aziridinium ion (**24**). Compound **2** was synthesized by reaction of **1** with sodium hydride and proton NMR was also used to both study the kinetics for hydrolysis of **1**, **2**, **6**, **9**, and **12** under acidic conditions and to identify their respective products.

The synthesis of phosphoramide mustards [>P(O)-$N(CH_2CH_2Cl)_2$] as latentiated alkylating agents that might be selectively "activated" in tumors by enzymatic (hydrolytic) release of nornitrogen mustard [$HN(CH_2CH_2Cl)_2$; nor-HN_2] represents one of the earliest design strategies in cancer chemotherapy.[2a] Hundreds of candidate compounds belonging to this structural class have been screened[2b,c] and cyclophosphamide,[2c] 2-[bis(2-chloroethyl)amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide (**1**), has uniquely emerged as that



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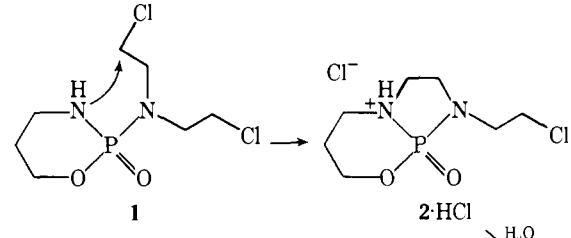
member which exhibits clinical effectiveness against a relatively wide spectrum of human cancers.[3] A host of studies attempting to determine the causative factors which underlie the superior anticancer activity of **1** have attenuated the interest in the in vivo enzymatic hydrolysis of **1** and have instead concentrated on an oxidative metabolic pathway leading to activation.[4] Among those investigators concerned with enzyme-mediated[5] and nonenzymatic[6-9] hydrolyses of **1**, Friedman and coworkers[7] have postulated that the latter process is initiated by intramolecular displacement of chloride ion (eq 1), which is then followed by sequential P–N, C–Cl, and P–O bond hydrolyses to give the various products isolated; however, direct evidence for the intermediacy of **2** was not obtainable. In view of the fact that this mechanism has been widely incorporated into the chemical[4b,10] and clinical[11] literature regarding **1**



(1)

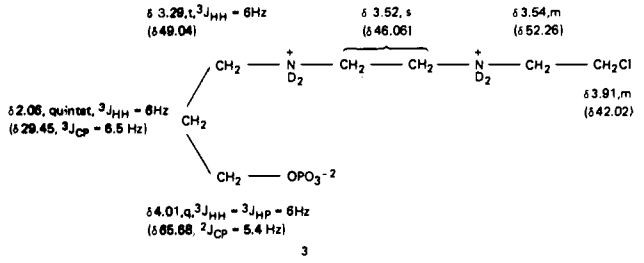
without further testing, we have reinvestigated the nonenzymatic hydrolysis of **1** using high-resolution nuclear magnetic resonance (NMR) spectroscopy. Such NMR methods have proven useful in our ongoing stereochemical[12,13] and dynamical[14] studies of **1** and it was therefore believed that NMR could be further developed as a reliable and convenient analytical tool for hydrolytic studies of **1** and related molecules, in contrast to indirect and less convenient colorimetric[8,15] or chromatographic[9,11] techniques currently used.

We now report persuasive ^1H and ^{13}C NMR evidence which supports and elaborates the original "Friedman mechanism" (eq 1). Data that reveal structural and media factors influencing the hydrolytic behavior of the 1,3,2-oxazaphosphorinane 2-oxide skeleton are also reported, together with a synthetic method which allowed for the preparation of **2**.

Results and Discussion

Identification of Cyclophosphamide Hydrolysis Products. Hydrolysis of an unbuffered aqueous solution of **1** (0.1 M) at 100 °C was reported by Friedman and coworkers[7] to afford

a product array that is a function of the heating period, with 30 min of reflux yielding hygroscopic material that was characterized as *N*-(2-chloroethyl)-*N'*-(3-phosphatopropyl)ethylenediamine hydrochloride (**3**·HCl) on the basis of elemental analysis and 60-MHz ^{1}H NMR data. Our careful repetition of this experiment gave material which exhibited a 60-MHz ^{1}H NMR spectrum that differed significantly from the original literature data;[16] consequently, the sample in question was treated with oxalic acid and gave an oxalate derivative (**3**·oxalate) having essentially the same melting point (192–193 °C dec) as that reported[7c] earlier (190–191 °C dec). High-resolution ^{1}H (220 MHz) and ^{13}C (68 MHz) NMR data for a D_2O–TSP[17] solution of **3**·oxalate, as summarized below (^{13}C data in parentheses), were consistent with the originally proposed structure for **3**; however, our more clearly resolved ^{1}H spectrum and other comparative information (vide infra) allowed for correction of previous spectral assignments.[7c] In particular, the CH_2Cl protons appear as the low-field portion



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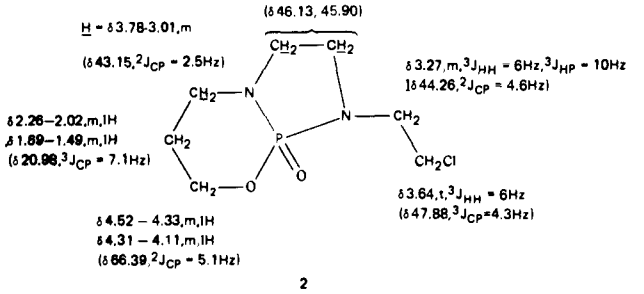
of an AA'XX' multiplet at δ 3.91 rather than the higher field triplet absorption at δ 3.29 which is instead due to the CH_2N^+ protons of the phosphatopropyl group.

Past attempts to obtain either indirect chromatographic evidence for the intermediacy of **2** or actually isolate **2** by conducting the hydrolysis of **1** in the presence of an equivalent amount of sodium bicarbonate were reported as being unsuccessful.[7c] Our studies regarding this putative intermediate therefore focused on direct synthesis of **2** under anhydrous conditions in order to avoid hydrolysis of the strained P-N bridge bond. As reported in the Experimental Section, cyclization of **1** with sodium hydride gave material having ^{1}H and



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^{13}C spectral properties wholly consistent with that expected for **2**.[18]

The anticipated greater lability of **2** under hydrolytic conditions used for **1** (100 °C) was supported by the observation that treatment of a D_2O solution of **2** at 20 °C with 1 equiv of DCl afforded after 2 h a ^{1}H NMR spectrum which was identical with that of **3**·HCl and led to a 100% isolated yield of an oxalate derivative that was identical (melting point, ^{1}H NMR) to **3**·oxalate. Conversion of **2** → **3** also provides an independent check on the spectrally based structure assigned to the latter material. In this connection it is worthwhile to note here that column chromatographed *anhydrous* samples of **1** gradually changed from their initial oily state to a semisolid mass, upon storage at room temperature in a desiccator. The ^{1}H NMR spectrum in D_2O solution of the semisolid formed after ~5 months indicated that ~50% conversion of **1** → **2**·HCl had taken place. In contradistinction to anhydrous **1**, samples of

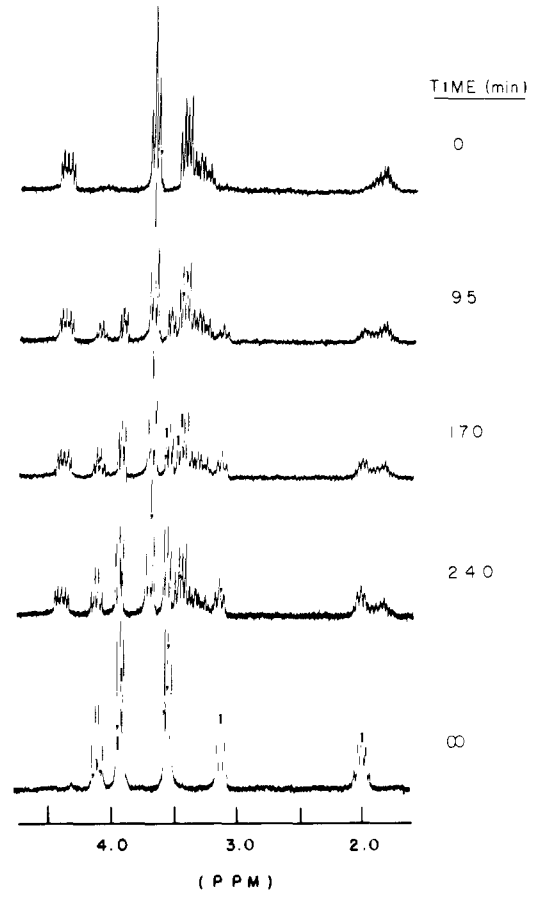


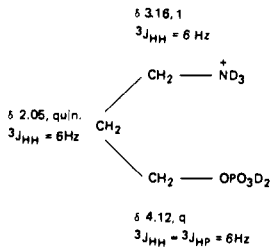
Figure 1. Continuous-wave 220-MHz ^{1}H NMR spectrum of **1** (T = 20–25 °C; initial pH 0.25) as a function of time.

crystalline **1**·H_2O and commercially available Cytoxan[19] (**1**·H_2O plus NaCl) were found by periodic ^{1}H NMR analyses to be essentially inert over equally prolonged periods of storage. These observations illustrate, among other things, the practical utility of NMR as an alternative to gas chromatography for pharmaceutical assay[11] of Cytoxan.

Monitoring the ambient pH during hydrolysis of **1** (0.02 M) at 85 °C showed an initially rapid decrease in pH that was followed by a gradual leveling off upon approach to complete disappearance of starting material: (min, pH) 0, 4.30; 5, 2.90; 20, 2.60; 50, 2.45; and 80, 2.35. NMR kinetic measurements (vide infra) revealed that despite this substantial acidity increase the reaction obeyed a pseudo-first-order rate law. A similar independence of hydrolysis rate on pH has been reported in connection with various studies[8,9a] of **1**; however, Hirata et al.[8] also found that the observed disappearance rate constant (k) for **1** was subject to acidic and basic catalysis at pH values outside the range of ~2–10: $k(75\ °C) = k_H[H^+] + k_{OH}[^-OH] + k_0$, with $k_H = 4.0 \times 10^{-3}$, $k_{OH} = 3.3 \times 10^{-3}$, and $k_0 = 1.2 \times 10^{-4}\ s^{-1}$. Additional measurements carried out by these investigators, which compared k with the formation rate of chloride ion (k_{Cl}), showed that whereas $k = k_{Cl}$ at pH ~2–14, $k = 1000k_{Cl}$ at pH ~0. Attempts by Hirata et al.[8] to characterize the hydrolysate by chromatographic methods were unsuccessful and no mechanistic rationale for the aforementioned kinetic observations was offered. We therefore investigated the use of ^{1}H NMR to directly analyze solutions obtained from hydrolysis of **1** under conditions of strong acidity and basicity.

A solution of **1** (0.1 M) in unbuffered DCl-D_2O having an initial pH[20] of 0.25 ($[D^+]/[1] = 10$) and kept at 20–25 °C was monitored by ^{1}H NMR. After ~24 h signals for **1** were absent, and the resultant spectrum (see Figure 1) was consistent with

the presence of an equimolar mixture of O-phosphorylated ammonium ion **4** and bis(2-chloroethyl)ammonium chloride (**5**); the pH was essentially unchanged (0.30) and absorptions due to other products were not detectable (<~3%). As in the case of **3**, the phosphonate moiety in **4** was clearly evident from



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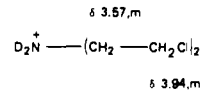

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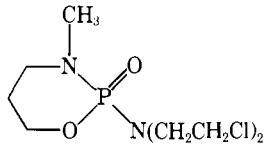
the CH_2OP proton signals, which appeared as a 1:3:3:1 quartet due to the equivalence of three-bond spin-spin coupling (6 Hz) to adjacent ^1H and ^{31}P nuclei. The spectral pattern associated with **5** has the gross appearance of two triplets for an AA'XX' spin system with $J_{HH} \sim 6$ Hz. Additional support for the identification of these two acid hydrolysis products was obtained by NMR spectral comparisons with an equimolar sample of authentic 3-aminopropanol and nor-HN_2·HCl in DCl-D_2O at pH ~0. Conversion of **1** into **4** and **5** under these strongly acidic conditions thus accounts for the previously unexplained disappearance of **1** *without* chloride ion production that was reported by Hirata and coworkers.[8]

Continual ^1H NMR monitoring of the alkaline hydrolysis of **1** (0.1 M) in NaOD-D_2O at pH ~11-14 and a probe temperature of 20 °C was foiled by crystallization of **1** as its hydrate due to a "salting-out" effect. Such samples, which remained homogeneous at 20 °C following heating at 37 °C (15 h), afforded a complex spectrum that was not interpretable with regard to product identification, except for a low-field doubled triplet indicative of CH_2CH_2OP ($^3J_{HH}$ = 6 Hz, $^3J_{HP}$ = 14 Hz) and a high-field AA'XX' aziridinyl ring proton pattern.[21] It was also found that basification (pH ~14) of control solutions of either **3**·DCl (0.1 M) or 3-aminopropanol and nor-HN_2·HCl (1:1, 0.1 M) did *not* produce spectra which simulated the alkaline reaction mixture derived from **1**; however, the liberated nor-HN_2 did lead to aziridinyl proton absorptions. These observations militate against the intermediacy of **3** and indicate that the chloride ion formation reported earlier[8] for **1** under basic conditions results from facile[21] 2-chloroethylamino group cyclization.

Identification of *N*-Alkylcyclophosphamide and Isophosphamide Hydrolysis Products. The effect of structural changes on the hydrolytic behavior of cyclophosphamide (**1**) derivatives has received surprisingly little attention. Our success in analyzing **1** therefore led to the investigation of its unstudied derivatives using NMR. Refluxing an aqueous solution of *N*-methylcyclophosphamide (**6**) under the same conditions used for **1** was followed by treatment with oxalic acid and gave a solid oxalate derivative (**7**·oxalate), which required careful repetitive recrystallization from methanol-water in order to remove minor impurities (as detected in the ^{13}C NMR spectrum). Comparison of the ^1H and ^{13}C spectra of **7**·oxalate with those of **3**·oxalate provided a firm basis for the structural as-



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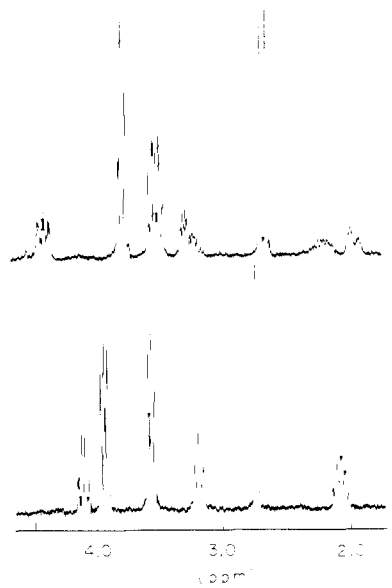
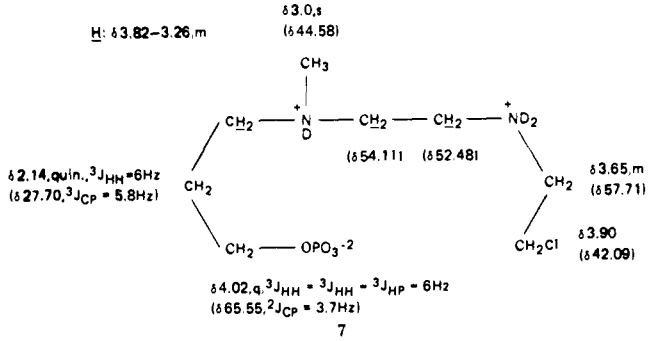


Figure 2. Continuous-wave 220-MHz ^1H NMR spectra of **6** (top) and its hydrolysis (T = 20 °C; initial pH 0.2) products, **5** and **8**.

signment shown below; deuterium-labeling experiments which are discussed in a following section allowed for identification



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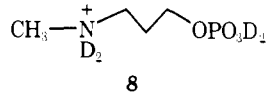
of the carbon resonances at δ 42.09, 52.48, 54.11, and 57.71.

Hydrolysis of **6** (0.1 M) under strongly acidic conditions (pH 0.20; $[D^+]/[6] = 10$) was directly monitored by ^1H NMR at 20 °C and signals from the starting material were no longer evident after ~1 h. The composition of the hydrolysate (pH 0.40) was found to be an equimolar mixture of **8** and **5**, based



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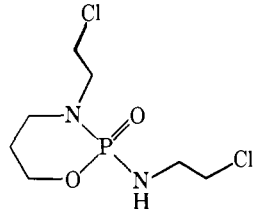
on the close spectral similarity to that of **4** and **5** from **1**, with the presence of an additional singlet at δ 2.74 for CH_3N^+ (see Figure 2).

Isophosphamide (Ifosfamide, **9**) is an important isomer of **1** by virtue of its potential utility as an anticancer drug.[22] In unbuffered D_2O solution the disappearance rate of **9** was found by ^1H NMR to be considerably slower than either **1** or **6** (vide infra), with only ~50% reaction occurring after 119 days at



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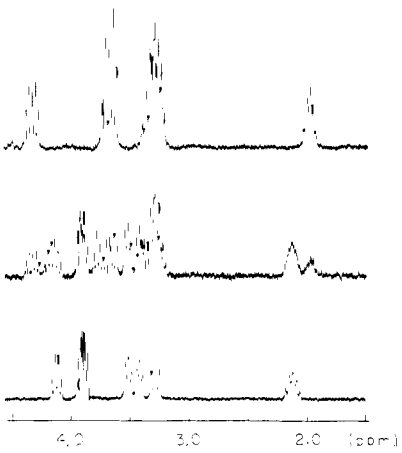


Figure 3. Continuous-wave 220-MHz ^{1}H NMR spectra of **9** (top) and its hydrolysis (T = 20 °C; pH ~0) products, an equimolor mixture of 2-chloroethylammonium ion and **11** (bottom). The middle trace shows the hydrolysis mixture at an intermediate time.

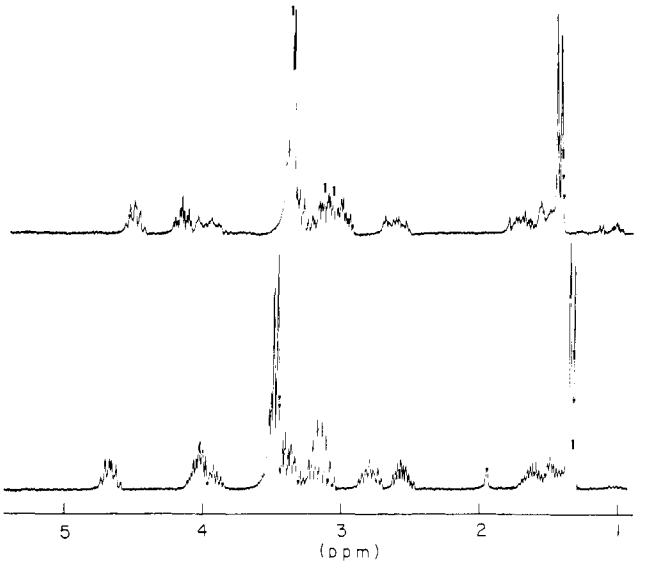
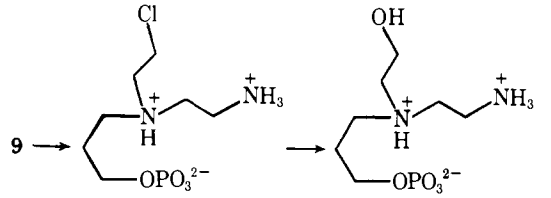


Figure 4. Continuous-wave 220-MHz ^{1}H NMR spectra of the diastereomerically homogeneous synthetic precursors **12b** (top) and **12a** (bottom) to optically pure cyclophosphamides (see ref 13).

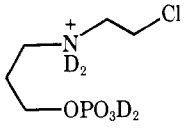
37 °C. An aqueous solution of **9** was therefore refluxed for 12 days and paper chromatography was used to isolate the major reaction product, after initial unsuccessful attempts to obtain a crystalline oxalate derivative. ^{1}H NMR spectral comparisons between this product, **3**·oxalate, and **7**·oxalate revealed the presence of two low-field multiplets characteristic of nonequivalent CH_2O groups and indicative of structure **10.** The absence of covalently bound chlorine in **10** was confirmed by elemental analysis and formation of **10** from **9** may be accommodated by the reaction sequence shown in Scheme I,

Scheme I



which is akin to that exhibited by **1** under extended hydrolysis conditions (vide infra).

Strongly acidic (pH ~0) hydrolysis of **9** at 20 °C in D_2O solution likewise afforded a product mixture similar to that derived from either **1** or **6,** with an equimolar mixture of **11** and



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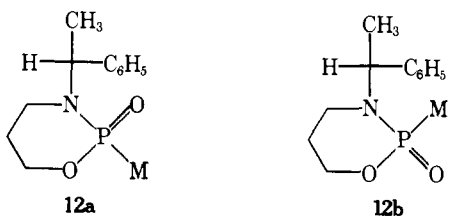
2-chloroethylammonium ion being readily evidenced by the typical 3-phosphatopropyl proton resonances plus two sets of partially overlapped AA′XX′ spin multiplets centered at δ 3.45 and 3.90 that are characteristic of nonequivalent $N^+CH_2CH_2Cl$ groups (see Figure 3).

Diastereomers **12a** and **12b,** which were obtained by a previously reported[12] synthetic route starting from optically pure (+)-(*R*)-α-methylbenzylamine, exhibit ^{1}H NMR spectra (see Figure 4) in which the methyl group proton signals appear at δ 1.55 and 1.68, respectively. These two compounds thus provided an opportunity to study the effect of a "bulky" *N*-alkyl substituent on the hydrolytic behavior of **1** while simultaneously monitoring the relative rate of possible stereomutation (**12a** ⇄ **12b**) at the chiral phosphorus center. The hydrophobicity of **12** required the use of 1:3 (v/v) D_2O–dimethyl-d_6 sulfoxide (Me_2SO-d_6) as the hydrolysis medium for studies carried out at 37 °C with a concentration of 0.08 M. The fact that neither diastereomer underwent detectable reaction (<5%) after 52 days of heating demonstrated that the α-methylbenzyl group exerts a dramatic rate-retarding effect on hydrolysis. The NMR spectra also demonstrated that no stereomutation (<5%) took place during this period of time.[23] In our earlier studies[12] with **12** we have shown by use of ^{18}O-enriched water that stereospecific oxygen exchange with the phosphoryl group does not occur after 15 h at 50 °C.



The unique stability of **12** toward hydrolysis likewise obtains under strongly acidic conditions, as indicated by rate data (vide infra) pertaining to 37 °C solutions in 2:3 (v/v) D_2O–CD_3OD at pH ~0. The reaction products from **12,** which were identified by ^{1}H NMR, are analogous to those obtained from acid hydrolysis of **1, 6,** and **9,** with $D_2N^+(CH_2CH_2Cl)_2$ being accompanied by the *N*-α-methylbenzyl counterpart of **4.** During the course of this relatively slow transformation, no stereomutation (<5%) of **12** was in evidence via the methyl group proton region.

Hydrolysis Kinetic Measurements by NMR. Analytical methods which have been employed for determining the concentration of **1** in aqueous solution generally involve selective extraction of **1** with an organic solvent followed by either colorimetric assay [4-(*p*-nitrobenzyl)pyridine[15a] or cobalt thiocyanate reagents[8]], IR analysis,[25] or conversion to an *N*-trifluoroacetyl derivative for gas chromatography.[11] Stable isotope dilution using deuterium-labeled **1** and mass spectroscopy is a newer method which has shown considerable utility.[26] Our interest in developing NMR as an alternative analytical tool stemmed from the desire to have a more general procedure that could be easily applied to **1** and various compounds of a related structural type. Moreover, an NMR method allows for direct observation and possible identification of products, as well as metastable intermediates leading to the final products. The

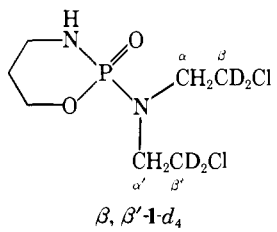
Table I. Kinetic Data for Hydrolysis of Cyclophosphamide (1) and Related Phosphoramidic Compounds

Compd	Solvent	Concn, M	Hydrolysis conditions[a]	k,[b] s^{-1}	$\tau_{1/2}$, days
1	D_2O	0.12	S + NaCl	$1.38 \pm 0.06 \times 10^{-6}$ [c]	5.7
β,β'-**1**-d_4	D_2O	0.12	S + NaCl	$1.07 \pm 0.08 \times 10^{-6}$ [d]	7.5
1	D_2O	0.08	S	$1.43 \pm 0.12 \times 10^{-6}$	5.6
1	D_2O-Me_2SO-d_6 (1:2)	0.05	S	$1.07 \pm 0.05 \times 10^{-6}$	7.5
1	D_2O-Me_2SO-d_6 (1:3)	0.12	S	$1.02 \pm 0.03 \times 10^{-6}$	7.9
1	D_2O	0.10	A	$4.35 \pm 0.06 \times 10^{-5}$	1.8×10^{-1}
2	D_2O	0.13	A	$5.79 \pm 0.05 \times 10^{-4}$	1.4×10^{-2}
6	D_2O	0.10	S	$1.78 \pm 0.05 \times 10^{-6}$	4.5
6	D_2O	0.10	A	$3.45 \pm 0.44 \times 10^{-5}$	2.3×10^{-1}
9	D_2O	0.26	S + NaCl	$6.70 \pm 0.37 \times 10^{-8}$	1.2×10^{2}
9	D_2O	0.10	A	$1.72 \pm 0.24 \times 10^{-3}$	4.7×10^{-3}
12a	D_2O-Me_2SO-d_6 (1:3)	0.08	S	$<1.1 \times 10^{-8}$ [e]	$>7.0 \times 10^{2}$
12a	D_2O-CD_3OD (2:3)	0.10	A[f]	4.47×10^{-7}	1.8×10^{1}
13[g]	D_2O-Me_2SO-d_6 (1:2)	0.05	S	$<4.8 \times 10^{-8}$ [e]	$>1.6 \times 10^{2}$
14	D_2O-Me_2SO-d_6 (1:1)	0.12	S	$<1.3 \times 10^{-8}$ [e]	$>6.0 \times 10^{2}$

[a] S = standard reaction conditions, viz. 37.4 ± 0.1 °C and ambient pD; S + NaCl = standard reaction conditions plus 2 equiv of NaCl to simulate the **1**:NaCl ratio in clinical samples of Cytoxan (see ref 19); A = acidic reaction conditions, viz. 20 ± 1 °C and pD ~ 0 (see ref 20), except as noted. [b] Pseudo-first-order rate constants (k) from linear least-squares treatment of relative substrate concentration calculated from 220-MHz ^1H NMR integrated signal intensities, except as noted. [c] Average value for three runs. [d] Average value for two runs. [e] Value obtained by assuming that <5% reaction occurred after 52 days. [f] Refers to 37.4 ± 0.1 °C. [g] Synthesized according to ref 46.

need for aliquot removal may not be necessary and with such reactions pulse techniques can be used to measure moderately fast reaction rates ($\tau_{1/2} \sim 30$ s). Further advantages of NMR over earlier methods used for the study of **1** relate to the use of ^2H and/or ^{13}C labeling to probe mechanisms. Our application of ^1H NMR to the hydrolysis of **1**, which is described below, illustrates a number of these points.

Comparison of the high-resolution ^1H NMR spectrum of a sample **1**·H_2O in D_2O with spectra obtained after heating at 37 °C clearly showed the gradual appearance of new signals centered at δ 4.0. Relative integrated signal intensities for the CH_2O protons of residual **1** vs. the signals at δ 4.0 vs. the high-field $CH_2CH_2CH_2$ absorption region in these spectra, which were internally consistent with the δ 4.0 signals being due to 4 protons in **3**, thus allowed for direct determination of the percentage of unreacted **1** as a function of time; resonances associated with **2** were not discernible. Using such data to generate standard plots of ln $([\mathbf{1}]_0/[\mathbf{1}]_t)$ vs. time gave acceptably linear least-squares fits (±5–10% slope error) over reaction periods of ~50–75%. An analogous procedure with β,β'-**1**-d_4 likewise allowed for determination of a pseudo-first-order hydrolysis rate constant (k) from which k_H/k_D (per



β,β'-**1**-d_4

deuterium) was found to be 1.14 ± 0.07 (see Table I). Hydrolysis of **1** in D_2O-Me_2SO-d_6 could be monitored by simply comparing integrals for the $CH_2CH_2CH_2$ absorptions of **1** and **3**, which fortuitously exhibit a substantial chemical-shift difference in this mixed solvent system.

For acidic (pH ~0) hydrolysis of **1** in DCl-D_2O, which proceeds at a convenient rate with a probe temperature of 20

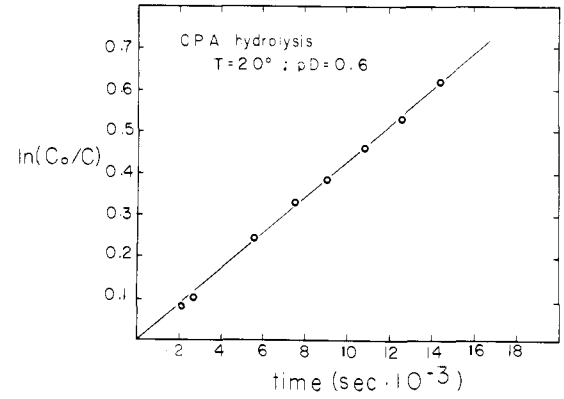
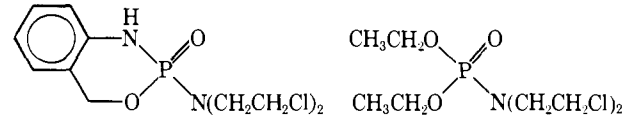


Figure 5. Plot of the time dependence of the change in cyclophosphamide concentration [ln (C_0/C)] under hydrolytic conditions (T = 20 °C; pD = 0.6).

°C, integrated signal comparisons as a function of time could be made between the CH_2O protons of **1** and either the CH_2O protons of **3** or the CH_2Cl protons of fragment **5** (see Figure 1). A typical kinetic plot which was derived from such NMR spectral data is shown in Figure 5.

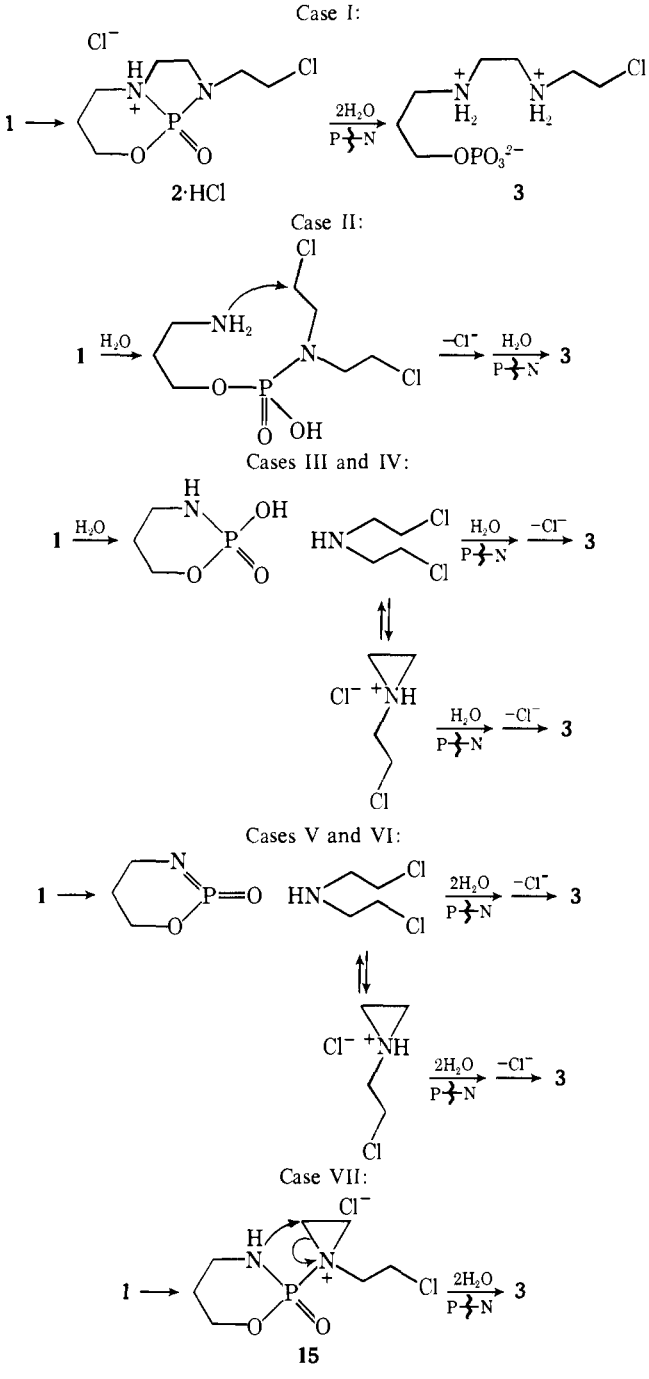
Relative signal integrations for starting material vs. product analogous to those described above for conversion of **1** into **3** were subsequently used to obtain the hydrolytic rate data summarized in Table I for **2, 6, 9,** and **12a–14.**



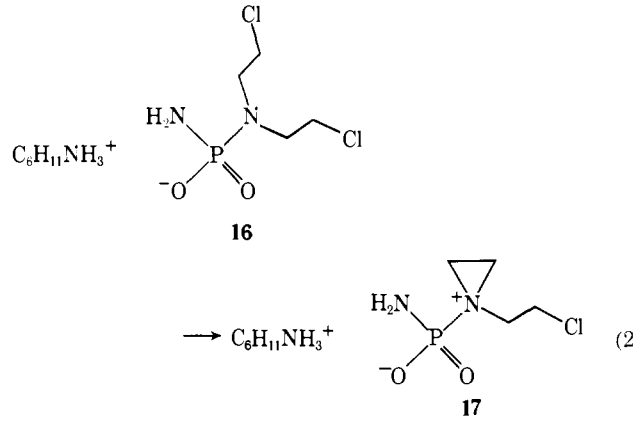
13 **14**

Mechanism of Initial Cyclophosphamide Hydrolysis: 1 → 3. From their assigned structure for **3**, and the absence of detectable quantities of $HN(CH_2CH_2Cl)_2$ (nor-HN_2) during reaction of **1** in unbuffered water at reflux, Friedman et al.[7] suggested a hydrolysis mechanism involving intramolecular

Chart I



N-alkylation to give **2**·HCl, which then undergoes sequential P-N bond cleavage to afford **3** (see Chart I, case I). While case I neatly accommodates production of **3**, the reported[27] inability to effect intermolecular N-alkylation of phosphoramidates, and the known[28,29] susceptibility of P-N bonds toward reaction with water suggested to us that an alternative mechanism for **1** → **3** might be operative. Such possibilities are outlined in Chart I and include: endocyclic P-N cleavage followed by cyclization (case II); exocyclic P-N cleavage with subsequent ring opening and then N-alkylation (case III) or cyclization to an aziridinium ion followed by an analogous sequence (case IV);[30] elimination of nor-HN_2 followed by N-alkylation and P-N bond hydrolyses (cases V and VI); and variants of an intramolecular isomerization mechanism (case VII).[31] On the other hand, some analogy for case I can be found in the relatively high alkylating activity of the cyclohexylammonium salt of phosphoramide mustard (**16**), which was rationalized by Friedman[32] in terms of an enhanced rate of **16** → **17** (eq 2)

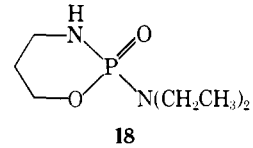


resulting from increased nucleophilicity of the mustard nitrogen, relative to O-alkylated derivatives of **16.**

The potential for case VII to result in a virtual loss of identity between the α- and β-CH_2 mustard positions in **1** (cf. diastereotopic aziridinyl carbons in **15**) and the possibility for cases IV and VI to effect analogous positional equivalence via cyclization of nor-HN_2 prior to N-alkylation suggest a labeling experiment. A simple method for carrying out such a mechanistic test involves the use of β,β'-**1**-d_4 and subsequent NMR analysis of **3** for deuterium content at the positions of interest, viz. the two ethylenediammonium carbons.[33] However, the "locally symmetric" environment about this molecular fragment leads to ^1H and ^{13}C signal isochrony (vide supra) in D_2O solvent, even at the superconducting magnetic field strengths available, and in the presence of the water-soluble shift reagent praseodymium perchlorate [$Pr(ClO_4)_3$]. In facing this problem, we initially resorted to the study of β,β'-**6**-d_4 since, as was seen above, the methyl group in **6** induces a measurable ^{13}C chemical-shift difference between the $N^+CH_2CH_2N^+$ carbons in its corresponding hydrolysis product, **7.** The observation that hydrolyses of **6** and **1** (D_2O or DCl-D_2O) proceed at roughly the same rates (see Table I) and afford analogous products supported the assumption that the relatively small methyl substituent effect does not cause fundamental mechanistic differences between **6** and **1.**

Hydrolysis of β,β'-**6**-d_4 in refluxing water for 30 min led to isolation of **7**-d_4·oxalate wherein the deuterium labels were found by a combination of ^1H and ^{13}C NMR to reside on the chlorine-bearing carbon and *one* of the ethylenediamine carbons. This conclusion is evidenced in the proton spectrum by the absence of a CH_2Cl triplet and the decrease by two protons in the relative intensity of the δ 3.82-3.26 CH_2N^+ multiplet, while the carbon spectrum (see Figure 6) reveals the virtual absence of absorption at chemical shifts corresponding to CH_2Cl and one of the CH_2N^+ groups, due to expected[34] relaxation and multiplicity effects of directly bonded deuterons. These results, which exclude β-/α-CH_2 positional scrambling within the mustard group during transformation of **6** into **7,** argue against cases IV, VI, and VII (or any other mechanisms) that involve symmetrization of a NCH_2CH_2Cl unit. Corroborative (albeit equivocal) evidence for extension of this mechanistic conclusion to cyclophosphamide (**1**) was obtained by a similar hydrolysis experiment with β,β'-**1**-d_4. The ^{13}C spectra for the **3**-d_4 product and **3** are shown in Figure 7 and feature an ethylenediammonium absorption for **3**-d_4 which is approximately half the intensity as that in **3.**

Various kinetic data were next obtained in order to select



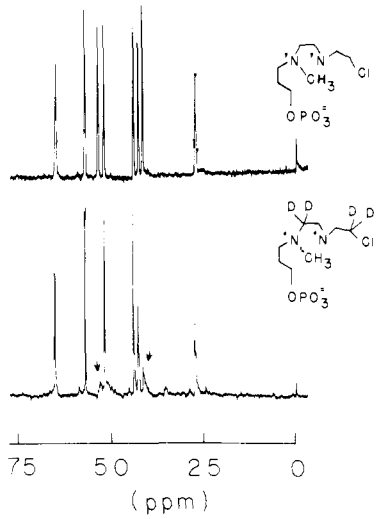
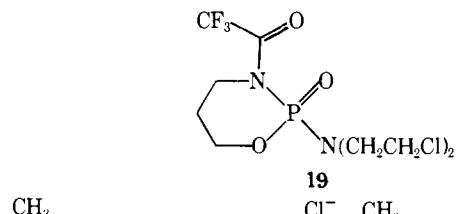


Figure 6. Aliphatic portion of the 67.8-MHz ^{13}C NMR spectra (Fourier transform mode) of the 7-oxalate resulting from the hydrolysis (30 min; 100 °C; pH ~7) of **6** (top) and the 7-d_4-oxalate resulting from the hydrolysis (30 min; 100 °C; pH ~7) of β,β'-**6**-d_4 (bottom).

the most likely candidate among the remaining mechanisms for **1** → **3**. Thus, the hydrolytic stability found for **18** (no detectable reaction after 15 h in D_2O-Me_2SO-d_6 at 85 °C) vs. **1** ($\tau_{1/2} \simeq 0.5$ h, same conditions) is contrary to expectations based on cases II-VI, as this model compound is capable of undergoing initial P-N hydrolysis at a rate comparable to **1**. By the same token, the relatively inert hydrolytic character found for either **12** or **14** (see Table I), which both contain a phosphoramidic mustard functionality, is incompatible with their common reaction via case VII. Finally, the likelihood of cases V and VI for reaction of **1** is further discounted by the insensitivity of the hydrolysis rate for **1** in the pH 8-10 range.

Friedman's original hypothesis[7] (case I) remains as that mechanism which is consistent with both the labeling data and the relative ratio of hydrolytic rate constants (see Table I, ambient pH) for **9**/**1** (4.8×10^{-2}) and **12a**/**1** ($<1.1 \times 10^{-2}$) that reflect increasing steric hindrance about the endocyclic nitrogen position. Electronic factors may also exert a measurable effect on the initial intramolecular chloride ion displacement step. Thus, decreased nucleophilicity of the π-conjugated ring nitrogen in **13** explains the hydrolytic stability found for this benzoannulated derivative of **1**, while a combination of electron-withdrawing and steric factors accommodates the extreme thermal stability of *N*-trifluoroacetylcyclophosphamide (**19**), which survives vapor-phase chromatography at 200-250 °C.[11a] In contradistinction to such stabilizing effects, it is now possible to reasonably propose the existence of electronic situations that *accelerate* intramolecular alkylation of the type which takes place in **1**. An example of this phenomenon is compound **20**, which has been



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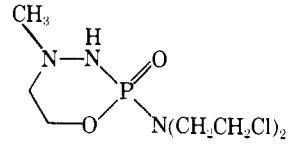

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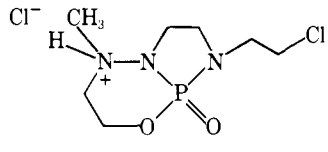

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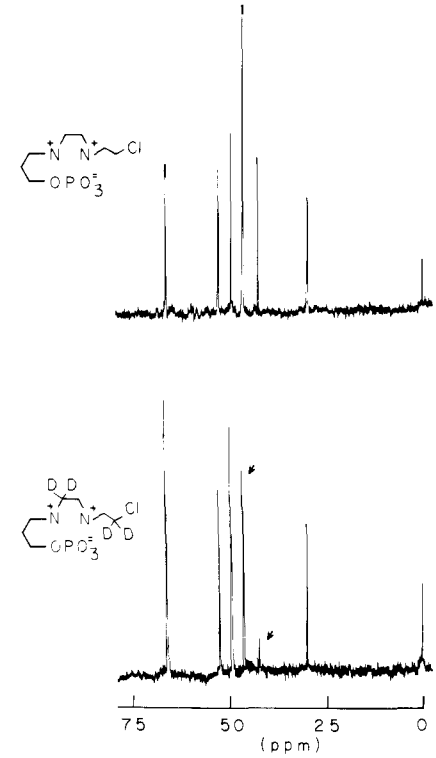


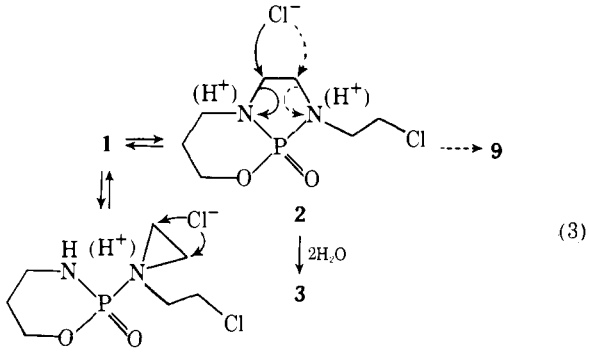
Figure 7. Aliphatic portion of the 67.8-MHz ^{13}C NMR spectra (Fourier transform mode) of the oxalates, **3** (top) and **3**-d_4 (bottom) obtained from the hydrolyses (30 min; 100 °C; pH ~7) of **1** and β,β'-**1**-d_4.

found[35] to generate chloride ion at a rate estimated[36] by us to be approximately 100 times faster than anhydrous **1** in their neat liquid state. The product from **20** has been assigned[35] structure **21** on the basis of infrared data and we suggest that its relatively rapid formation rate results from relief of adjacent nitrogen lone-pair repulsive forces,[37] which is similar to the α effect.[38]

Implicit in the foregoing discussions is the assumption of rate-limiting intramolecular nucleophilic substitution. Granted this condition, it follows that further mechanistic details for **1** → **2** can be deduced from the relative ratio of hydrolytic rate constants for **1** and β,β'-**1**-d_4 which is equal to 1.29 (see Table I). Since only one of the CD_2Cl groups in the latter compound serves as the reaction center, the square root of 1.29 affords a value of $k_H/k_D = 1.14 \pm 0.07$ per deuterium, which is somewhat greater than the α-deuterium isotope effect for S_N2 reactions (~0.95-1.05[39]) and is more in line with an S_N1-type process (~1.15 for substitution of chloride[40]). However, a limiting dissociative mechanism for **1** → **2** that excludes involvement of the endocyclic nitrogen is contrary to general conceptions[41] regarding solvolytic reactivity of primary systems and would, furthermore, not be in keeping with the aforementioned steric and electronic effects of substituents bonded to this nitrogen position. In the absence of more refined k_H/k_D data and measurement of carbon or chlorine kinetic isotope effects, a compromised rate-determining transition state is suggested, wherein extensive C-Cl bond breaking is assisted by endocyclic nitrogen lone-pair participation. Increased conformational restrictions placed on this transition state, relative to ground-state dynamics for **1**, are consistent with the negative and moderately large value of $\Delta S^{\ddagger} = -13$ eu for hydrolysis of **1**, which we have calculated[42] using literature rate data.[43]

It is noteworthy that the hydrolysis rate for **1** was not significantly affected by the presence of added silver nitrate, as evidenced by an only twofold rate acceleration factor found for ~50% conversion of **1** (0.1 M) → **3** in water containing 1

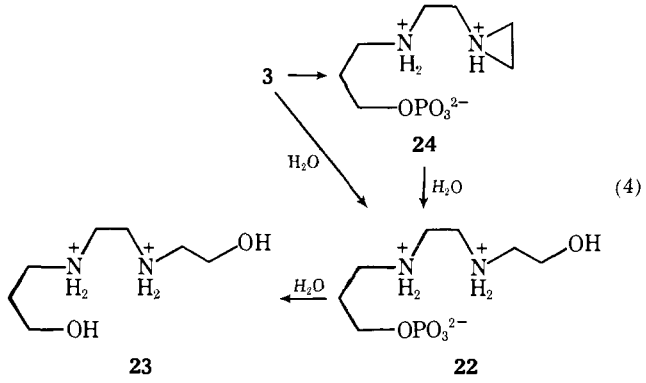
equiv of silver nitrate. While the absence of substantial Ag(I) ion-assisted chloride ionization could be due to preferential complexation of the metal to the phosphoryl oxygen,[44] our interest in testing for the possibility of such assistance derived from its implications within the in vivo metabolism of **1**. Thus, whereas the inherent rate for **1** → **2** is extremely slow relative to the metabolic timescale for **1**,[4b] biological metal ions and/or metalloenzymes could conceivably accelerate the transformation of **1** into **2** to an extent such that in vivo production of **2** would no longer be negligible. Moreover, attack of chloride ion on approximately protonated **2** (internal return) could either regenerate **1** or produce isophosphamide (**9**), as represented in eq 3. The observation of linear pseudo-first-order



(3)

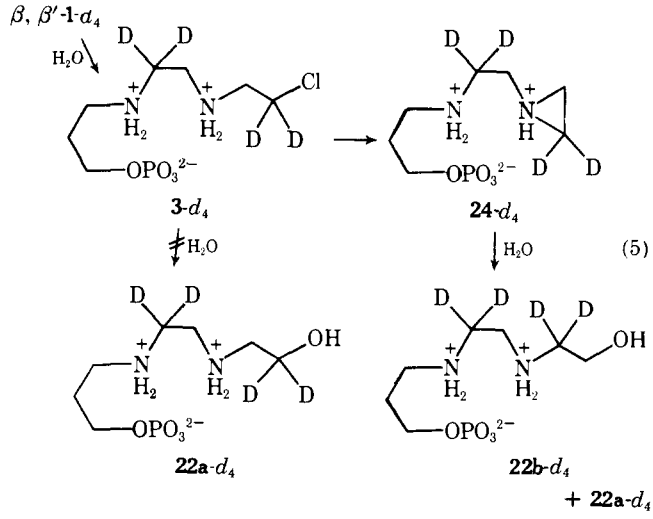
kinetics for disappearance of **1**, even in the presence of added NaCl (see Table I), indicates that leakage to slower reacting **9** ($k_9/k_1 = 0.05$) cannot be substantial. Formation of aziridinium ion **15** in eq 3 and regeneration of **1** is likewise ineffective, relative to **1** → **2** → **3**, since the resultant scrambling of deuterium between the α and β carbons starting with β,β'-**1**-d_4 was not evidenced in the ^{1}H NMR spectra of unhydrolyzed **1** during the monitored reaction period (~3 half-lives) and is inconsistent with the stereospecific location of deuterium labels in the final **3**-d_4 hydrolysis product. It should be pointed out, however, that these in vitro data, as well as those for reaction of **1** in the presence of a strong electrophile such as Ag(I), cannot be extrapolated to in vivo systems without the usual caveats applicable to enzymatic processes that are difficult, if not impossible, to adequately mimic in the laboratory.

Mechanism for Secondary Hydrolysis of Cyclophosphamide: Hydrolytic Reaction of 3. Prolonged reflux of an aqueous solution of **1** reportedly[7] leads to secondary hydrolytic conversion of **3** into *N*-(2-hydroxyethyl)-*N'*-(3-phosphatopropyl)ethylenediammonium ion (**22**) and, ultimately, dephosphorylation of **22** to give **23**. Starting with ca. 0.1 M **1** in unbuffered water requires that formation of **22** from **3** take place at relatively low pH (~2); consequently, it is not obvious whether this hydrolytic step occurs by direct displacement of chloride ion by water in protonated **3** or by prior cyclization of a free amine form of **3** to give intermediate aziridinium ion **24**, which then



(4)

reacts with solvent (eq 4). In order to resolve this mechanistic ambiguity, β,β'-**1**-d_4 was hydrolyzed under conditions leading to **22**-d_4 (via **3**-d_4). The positions of the deuterium labels, which were deduced by ^{1}H NMR signal integrations, were found to be consistent with an approximately equimolar mixture of **22a**-d_4 and **22b**-d_4, thereby excluding the direct displacement pathway that would lead exclusively to **22a**-d_4 (cf. eq 5). The generality of these results in favor of intermediate



(5)

24, as applied to other β-chloroethylammonium systems and various nucleophiles, remains to be established.

Concluding Remarks

The present studies, which have clarified mechanistic details regarding the hydrolytic chemistry of **1**, serve to demonstrate that ^{1}H and ^{13}C NMR spectroscopy offer themselves as useful and complementary analytical tools for investigating phosphoramidic mustard compounds. Our extensions of this approach to enzymatic and nonenzymatic aspects of cyclophosphamide, isophosphamide, and their metabolites will be reported in future papers.

Experimental Section

Melting points were obtained with a Thomas-Hoover capillary melting point apparatus and are uncorrected, as are reported boiling points. Elemental analyses were performed by Chemalytics, Inc. IR measurements were obtained with a Perkin-Elmer Model 337 spectrophotometer. ^{1}H NMR spectra at 60 MHz were recorded on a Varian A 60 instrument at ambient probe temperature, using ~10% v/v solutions in either $CDCl_3$ or D_2O with tetramethylsilane or TSP[17] as an internal reference, except as noted. ^{1}H NMR spectra at 220 MHz were obtained in either the continuous wave or Fourier transform mode on a Varian HR 220 spectrometer equipped with a Fourier transform accessory and 620 L computer. The accumulated free induction decay signal (8K data points) was transformed to give spectra with either 2500- or 1000-Hz sweep widths; ambient probe temperature was 20 ± 1 °C.

^{13}C NMR spectra were obtained in the pulse Fourier transform mode utilizing a Bruker superconducting magnet with a "homebuilt" spectrometer and probe. A Nicolet 1080 computer system, modified for quadrature phase detection, was used for data collection and transformation. The accumulated free induction decay signal (32K data points) was transformed to give a spectrum with 15151.5-Hz sweep width. A 90° ^{13}C pulse was approximately 27 μs. Spectra were recorded under broad-band pseudo-random-noise proton decoupling conditions at a probe temperature of 28 ± 2 °C unless otherwise noted. Precooled nitrogen gas was used to obtain the low-temperature ^{13}C spectra. A chloroform solution containing a copper-constantan thermocouple connected to a precalibrated Doric Trendicator 400 Type T/C digital readout temperature meter was used to establish probe temperature before and after sample runs.

Except as noted, analytical thin-layer chromatography utilized either 2.5 × 10 cm or 5 × 20 cm Analtech plates with a 250-μm layer

of silica gel GF, while analogous preparative separations were performed with 20 × 20 cm plates having a 1000-μm coating; component visualization as brown colored spots was achieved by exposure to iodine vapor. Column chromatography employed Baker 60–200 mesh silica gel. Analytical and preparative descending paper chromatography with Whatman No. 3MM chromatographic paper used 1% ninhydrin in acetone spray followed by heating for color visualization. All of the reported R_f values are approximate.

All reactions performed in nonaqueous media were conducted with protection from atmospheric moisture.

2-[Bis(2-chloroethyl)amino] - 2*H*-1,3,2 - oxazaphosphorinane 2-Oxide (Cyclophosphamide, 1). A solution of bis(2-chloroethyl)phosphoramidic dichloride (25.9 g, 0.1 mol) in ethyl acetate (150 mL) was added (1.5 h) to a magnetically stirred solution of 3-amino-1-propanol (7.6 mL, 0.1 mol) and triethylamine (27.8 mL, 0.2 mol) in ethyl acetate. After 48 h at ambient temperature, triethylamine hydrochloride was removed by suction filtration and the filtrate was concentrated on a rotary evaporator. The residual oil was column chromatographed using chloroform–methanol (9:1) as eluent. Anhydrous **1** (R_f 0.65) was isolated (50%) as a colorless viscous oil, which was found to undergo relatively slow decomposition when stored at room temperature; however, rechromatography of such samples led to recovery of unreacted **1**. A ^{1}H NMR spectrum of **1** is shown in Figure 1.

2-[Bis(2-chloro - 2,2-dideuterioethyl)amino]-2*H* - 1,3,2-oxazaphosphorinane 1-Oxide (β,β′-1-d_4). Use of bis(2-chloro-2,2-dideuterioethyl)phosphoramidic dichloride[33] in the above procedure for **1** led to isolation (71%) of pure β,β′-**1**-d_4, which was readily characterized by comparison of its ^{1}H NMR spectrum (D_2O) with that of **1**.

2-[Bis(2-chloroethyl)amino] - 3-methyl-2*H* - 1,3,2-oxazaphosphorinane 1-Oxide (*N*-Methylcyclophosphamide, 6). A solution of bis(2-chloroethyl)phosphoramidic dichloride (1.3 g, 5 mmol) in ethyl acetate (7 mL) was reacted with *N*-methyl-3-amino-1-propanol (0.45 g, 5 mmol) and triethylamine (1.4 mL, 10 mmol) in ethyl acetate (5 mL) according to the method described for **1**. Column chromatography with ether–chloroform (1:1) afforded **6** (0.6 g, 43%) as beige crystals: mp 50–55 °C; ^{1}H NMR (220 MHz, D_2O) δ 4.45–4.29 (m, CH_2O), 3.73 (t, $^3J_{HH}$ = 6 Hz, CH_2Cl), 3.47 (d of t, $^3J_{HH}$ = 6 Hz, $^3J_{HP}$ = 12 Hz, NCH_2CH_2Cl), 3.31–3.09 (m, CH_2N), 2.62 (d, $^3J_{HP}$ = 11 Hz, CH_3), 2.27–2.04 (m, 1 H), and 2.00–1.82 (m, 1 H). Anal. Calcd for $C_8H_{17}N_2O_2PCl_2 \cdot 0.5\,H_2O$: C, 33.82; H, 6.39. Found: C, 33.75 (34.15); H, 6.20 (6.19).

2-[Bis(2-chloro-2,2-dideuterioethyl)amino]-3-methyl-2*H*-1,3,2-oxazaphosphorinane 1-Oxide (β,β′-6-d_4). Use of bis(2-chloro-2,2-dideuterioethyl)phosphoramidic dichloride in the above procedure for **6** led to isolation (65%) of β,β′-**6**-d_4, which exhibited ^{1}H NMR spectra similar to that of **6** except for the expected absence of β-CH_2 absorptions and the appearance of α-CH_2 protons as a doublet ($^3J_{HP}$ = 12 Hz). Signal integration and TLC indicated the presence of a relatively small amount of slower eluting contaminant(s) that was removed by careful rechromatography.

1-(2-Chloroethyl)tetrahydro - 1*H*,5*H* - [1,3,2]diazaphospholo[2,1-*b*][1,3,2]oxazaphosphorine 9-Oxide (2). A solution of anhydrous **1** (1.04 g, 4 mmol) in ether (40 mL) was added (1 h) to a magnetically stirred suspension of sodium hydride (0.58 g, 24 mmol) in ether (8 mL), and after 18 h at ambient temperature the reaction mixture was suction filtered and the filtrate was then concentrated on a rotary evaporator. The residual oil was column chromatographed using chloroform–methanol (9:1) as eluent and **2**, which elutes slightly ahead of **1** and gives a more persistent color upon exposure to iodine vapors, was isolated (60%) as a colorless oil that turned into a waxy solid, mp 142–143 °C dec, upon storage at 10 °C. Conclusive ^{1}H and ^{13}C NMR data for the structure assigned to **2** are pictured in the Results and Discussion section; however, repeated attempts to obtain a satisfactory elemental analysis were unsuccessful. It should be noted that NMR samples of **2** in $CDCl_3$ are subject to relatively rapid decomposition (precipitate formation) and should therefore be kept at low temperature whenever possible.

***O,O*-Diethyl Bis(2-chloroethyl)aminophosphoramidate (14).** Bis(2-chloroethyl)phosphoramidic dichloride and absolute ethanol were reacted according to the general procedures reported by Friedman et al.[45] Column chromatography of the crude reaction product using chloroform solvent led to elution of *O*-ethyl bis(2-chloroethyl)aminophosphorochloridate (R_f 0.7) and then **14** (R_f 0.2), which were readily identified from their characteristic ^{1}H NMR (60 MHz) spectra.

2-(Diethylamino) - 2*H*-1,3,2-oxazaphosphorinane 2-Oxide (18). A solution of phosphorus oxychloride (60 mL, 0.65 mol) in recently dried methylene chloride (400 mL) was added (4 h) to a mechanically stirred and chilled solution of 3-amino-1-propanol (55 mL, 0.72 mol) and triethylamine (165 mL, 1.18 mol) in methylene chloride (700 mL) at such a rate as to keep the temperature below 10 °C. After 24 h at ambient tempertture the reaction mixture was suction filtered and the filtrate was then cooled to −10 °C before addition of cold water (120 mL), shaking, and then drying of the separated organic layer over anhydrous magnesium sulfate. Removal of volatiles at reduced pressure afforded (52%, corrected) crude 2-chloro-2*H*-1,3,2-oxazaphosphorinane 2-oxide, which was found by ^{1}H NMR to contain a substantial amount (37%) of triethylamine hydrochloride and used without further purification. A magnetically stirred solution of the crude chloro compound (~0.3 mol) in methylene chloride (170 mL) was treated with 1.5 equiv of triethylamine and then with 1.5 equiv of diethylamine, which led to reflux. After stirring for 12 h the reaction mixture was filtered and the filtrate was then concentrated on a rotary evaporator. Kugelrohr distillation of the residue yielded (12%) **18** (bp 130 °C, 0.015 mm) as a colorless hygroscopic oil, which exhibited a ^{1}H NMR spectrum that was a simple variant of that for **1**. Anal. Calcd for $C_7H_{17}N_2O_2P \cdot 0.5H_2O$: C, 41.79; H, 9.02. Found: C, 41.43; H, 8.71.

Hydrolytic Conversion of 1 into 3·HCl and 3·Oxalate. Freshly chromatographed **1** (0.5 g, 1.9 mmol) in water (25 mL) was refluxed for 0.5 h and the reaction mixture was then concentrated in vacuo at room temperature. The residue was dissolved in methanol (25 mL), reconcentrated, and then taken up in a minimal amount of warm methanol (5 mL). Addition of absolute ethanol followed by cooling repeatedly gave an oil; however, drying this material over P_2O_5 led to formation of an extremely hygroscopic microcrystalline solid (0.11 g) which exhibited a ^{1}H NMR spectrum[16] roughly compatible with that reported[7c] for **3**·HCl. The recovered sample was dissolved in water (2 mL) and then treated with a solution of oxalic acid (0.13 g) in water (1.5 mL). A warm aqueous solution (10 mL) of the resultant precipitate was diluted with methanol (0.5 mL) and the white powder which formed upon cooling to room temperature was collected and dried in vacuo over P_2O_5. ^{1}H and ^{13}C NMR data (see Results and Discussion section) for this material [50 mg, mp 192–193 °C dec; lit.[7c] 190–191 °C dec] were consistent with its identification as **3**·oxalate.

Ambient solution acidity during the unbuffered hydrolysis of **1** (0.02 M) was monitored by periodically removing the reaction vial from an oil bath maintained at 85 °C, cooling to 5 °C, and then measuring pH with a precalibrated standard glass electrode system. Reaction times (minutes) and corresponding pH values were as follows: 0, 4.30; 5, 2.90; 20, 2.60; 50, 2.45; and 80, 2.35. Complete conversion of **1** → **3**·HCl with production of 1 equiv of "free" H^+ would give a pH of 1.7.

Prolonged Hydrolyses of 1 and β,β′-1-d_4. A solution of **1** (170 mg, 0.65 mmol) in water (8.5 mL) was refluxed for 6 h. Concentration of the reaction mixture at reduced pressure gave an oil which was dissolved in a minimal volume of methanol and then applied to eight sheets (18 × 35 cm) of chromatographic paper for descending elution (20 h, room temperature) with propanol–water (8:1). Visualization of sample end strips revealed the presence of at least nine components; however, only the slowest eluting band (R_f 0.01) was removed by extraction with water. The small amount of material (~10 mg) which remained after freeze drying was spectroscopically identified as partially contaminated **22**: ^{1}H NMR (220 MHz, D_2O) δ 4.09–3.93 (m, 2 H, CH_2OP), 3.93–3.82 (m, 2 H, CH_2O), 3.47 (br s, 4 H, two $CH_2CH_2N^+$ type), 3.25 (br s, 4 H, $N^+CH_2CH_2N^+$), and 2.14–1.86 (m, 2 H, $CH_2CH_2CH_2$).

Repetition of the above procedure with β,β′-**1**-d_4 gave material which was similarly analyzed by ^{1}H NMR. Relative signal intensities obtained by the cut-and-weigh technique for the five anisochronous sets of protons listed above were 2.3, 0.8, 1.3, 3.4, and 2.2 ± 0.1, respectively. For an equimolar mixture of products **22a**-d_4 and **22b**-d_4, these proton sets are expected in a relative ratio of 2:1:2:3:2. Differences between the found and expected ratios may be due to inter alia, H-for-D exchange and/or NMR sample contamination that was evident from the appearance of extraneous peaks.

Hydrolytic Conversion of 6 into 7·Oxalate. A solution of **6** (0.25 g, 0.9 mmol) in water (10 mL) was refluxed for 0.5 h and then concentrated in vacuo at room temperature. The gummy residue was dissolved in hot methanol (4 mL) and triturated to a persistent cloudiness with absolute ethanol. Gradual cooling to −5 °C resulted in formation

of an oil, which was isolated and crystallized by drying in vacuo. The ^1H NMR spectrum of this material (0.16 g) in D_2O was consistent with that expected for **7**·HCl; however, ^{13}C analysis revealed the presence of 12 rather than 8 carbon signals. The recovered crude product was therefore dissolved in water (1.5 mL) and then treated with an aqueous solution (0.75 mL) of oxalic acid (0.15 g), followed by addition of methanol (2.25 mL) and cooling at −5 °C. The resultant material (0.12 g, mp 158–158.5 °C dec) was recrystallized once more and thus afforded pure **3**·oxalate, as judged from its characteristic ^{13}C NMR spectrum (see Results and Discussion section).

Hydrolytic Conversion of 9 into 10. A solution of **9** (600 mg, 2.3 mmol) in water (25 mL) was refluxed for 12 days and was then concentrated on a rotary evaporator. The residual colorless oil was chromatographically purified as described for **1** → **22** and the relatively small amount (45 mg) of product **10** (R_f 0.01) was spectroscopically identified: ^1H NMR (220 MHz, D_2O, external TSP) δ 4.47 (apparent q, $J \simeq 6$ Hz, 2 H, CH_2OP), 4.43 (apparent q, $J \simeq 6$ Hz, CH_2O), 4.35 (apparent t, $J \simeq 6$ Hz, 2 H), 4.08–4.00 (m, 6 H), and 3.49–3.42 (quintet, 2 H, $CH_2CH_2CH_2$). To obtain a sample of **10** that was free of any chloride ion, the NMR sample was chromatographed on Dowex 50W-X8 exchange resin (1.5 mL) using 0.1 M acetic acid eluent until a negative silver chloride test was obtained upon addition of silver nitrate. The column was next washed with water until neutral to litmus paper, and the product was finally eluted with 6 M ammonium hydroxide. Ninhydrin spray was used to identify those fractions containing **10** and freeze drying of the combined fractions yielded material (26 mg) which was found by combustion analysis to be free of detectable amounts of chlorine. Anal. Calcd for $C_7H_{19}N_2O_5P$: C, 34.70; H, 7.90. Found: C, 34.15; H, 6.65.

Hydrolysis Kinetics by NMR. Compounds to be hydrolyzed were weighed in small glass vials, dissolved in volumetrically delivered amounts of the appropriate deuterated solvent(s), and the solutions were then transferred to NMR sample tubes. After recording initial "zero time" spectra, the samples were submerged in a circulating water bath maintained at 37.4 ± 0.1 °C and then periodically removed for ^1H NMR analysis at 220 MHz. In such cases, the amount of reaction which took place during NMR analysis was negligibly small. Samples for acidic or basic hydrolysis were prepared as above and then rapidly mixed with the appropriate amount of commercially available (Merck, Sharp, and Dohme) 12 N DCl–D_2O or 40% NaOD–D_2O. Measurements of pH[20] were made on a precalibrated Radiometer Model 26 instrument equipped with an Ingold combination electrode which could be inserted directly into a 5 mm NMR tube.

Normalized integrated signal intensities used to evaluate the relative concentrations of starting material and product(s) were averaged over five scans. Linear least-squares fits of pseudo-first-order plots of ln ([starting material]$_0$/[starting material]$_t$) vs. t gave the values of k listed in Table I.

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Communications to the Editor

Synthesis and Reactions of Molybdenum Vinyl Complexes. Evidence for Formation of Carbyne Complexes

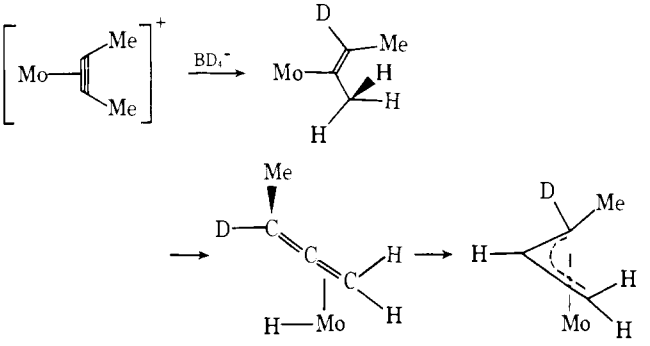
Sir:

The chemistry of σ-bonded transition metal vinyl complexes is a relatively unexplored area because of the difficulties associated with their synthesis. Our discovery[1] of a simple route to the formally 16-electron acetylene substituted molybdenum cations (**1** and **2**) provided an opportunity to study their reactions with, for example, the borohydride anion. The resulting vinyl species undergo novel rearrangements to give η^3-allylic and carbyne complexes.

Reaction (−78 °C, THF) of sodium borohydride with the violet crystalline cation (**1**, R = H, R^1 = Bu-*t*) in the presence of an excess of $P(OMe)_3$ affords the yellow crystalline vinyl complex[3] (**3**): ^1H NMR resonances (C_6D_6) at τ 3.3 (1 H, m, H^1, J_{12} = 17.0 Hz, J_{H^1Pa} = 14.0 Hz, J_{H^1Pb} = 7.0 Hz), 4.6 (1 H, dt, H^2, J_{H^2Pa} = 2.0 Hz), 5.1 (5 H, q, C_5H_5, J_{HP} = 1.0 Hz), 6.6 (27 H, m, POMe), and 8.8 (9 H, s, Bu); ^{13}C NMR resonances (C_6D_6) at −151.3 (t, Mo**C**=C, $^2J_{CP}$ = 4.5 Hz.), 128.1 (s, MoC=**C**), −87.4 (s, C_5H_5), −51.4 (s, POMe), −37.3 (s, **C**Me$_3$), −30.6 ppm (s, C**Me**$_3$). Treatment with $NaBD_4$ gives the corresponding deuterio complex of **3**, where H^2 is replaced by deuterium, indicating that the formation of **3** involves the intermediacy of a hydride, $MoH[P(OMe)_3]_2(\eta^2$-Bu-*t*-$C_2H)(\eta^5$-$C_5H_5)$, which then undergoes a cis-insertion reaction. This kind of reaction has not been observed previously, although nucleophilic attack by methoxide anion on a cationic platinum(II)-acetylene complex has been shown[4] by Clark and Chisholm to lead to the formation of carbene complexes. However, it is likely in the Pt system initial nucleophilic attack occurs at carbon rather than at the metal center as suggested for the molybdenum cation.

In solution (C_6D_6) and also in the solid state (room temperature) the vinyl complex **3** undergoes a very unusual rearrangement (quantitative yield) to form the crystalline *carbyne* complex **4**: ^1H NMR resonances (C_6D_6) at τ 4.8 (5 H, t, C_5H_5, J_{HP} = 1.0 Hz), 6.5 (18 H, apparent *t*, POMe, $|J|_{POCH}$ = 12 Hz), 7.8 (2 H, t, C**H**$_2$Bu-*t*, $^4J_{HP}$ = 4.0 Hz), and 8.9 (9 H, s, Bu-*t*); ^{13}C NMR resonances (C_6D_6) at −299.8[5] (t, MoCCH$_2$, $^2J_{CP}$ = 27.0 Hz, t in off-resonance spectrum), −89.3 (s, C_5H_5), −62.5 (s, CH_2, t in off-resonance spectrum), −50.9 (s, POMe), −33.0 (s, **C**Me$_3$), and −29.9 ppm (s, C**Me**$_3$). The rearrangement reaction is suppressed by the presence of free trimethyl phosphite suggesting the requirement of a vacant coordination site for the H shift to occur. The rearrangement of a vinyl complex to a carbyne complex has not been previously observed, carbyne metal complexes normally being prepared via carbene complexes,[5] and appropriate labeling experiments are in hand, which will, it is hoped, elucidate the mechanistic details of this new reaction.

Scheme I[a]



[a] Ligands omitted for clarity.

An indication of the potential chemistry of **4** is provided by the observation that protonation (HBF_4/Ac_2O, −78 °C) proceeds in high yield to give the cationic hydride species **5**: ν_{MoH} (Nujol) 1760 cm^{-1} (s); ^1H NMR resonances ($CDCl_3$) at τ 4.2 (5 H, t, C_5H_5, J_{HP} = 1.0 Hz.), 6.2 (18 H, d, POMe, $|J|_{POCH}$ = 12 Hz), 7.6 (2 H, t, C**H**$_2$Bu-*t*, J_{HP} = 4.0 Hz), 9.0 (9 H, s, Bu-*t*), and 12.5 (1 H, t, MoH, $^2J_{HP}$ = 67.0 Hz.); ^{13}C NMR resonances ($CDCl_3$) at −346.7 (t, Mo≡**C**CH$_2$, J_{CP} = 24.0 Hz, t in off-resonance spectrum), −96.2 (s, C_5H_5), −64.2 (s, CH_2, t in off-resonance spectrum), −53.5 (s, POMe), −33.6 (s, **C**Me$_3$), and −29.2 ppm (s, C**Me**$_3$). The cation **5** is stable and shows no tendency to rearrange.

It is interesting that the but-2-yne 16-electron cation **2** (R = R^1 = Me) undergoes a different type of reaction on treatment with $NaBH_4$ (room temperature, THF) forming selectively the dark red crystalline *anti*-crotyl complex **6**, which at room temperature exists in solution as equilibrating exo and endo isomers.[6] At −40 °C (toluene-d_8) the ^1H spectrum (^{31}P decoupled) showed resonances corresponding to the two anti conformers. It is suggested that an initially formed vinyl complex undergoes a β-elimination reaction as illustrated in Scheme I giving a molybdenum hydride(allene) complex,