5, 9.3, 12), 3.77 (ddd, 1 H, C-2 exo H, J = 3.5, 5, 9.3), 5.11 (d, 1 H, C-1 methine, J = 5), 5.30 (d, 1 H, C-4 methine, J = 5), 7.07–7.50 (m, 4 H, Ar H); MS, m/e 161 (M^+), 118 (base). The oxalate salt was prepared and recrystallized from absolute EtOH as white crystals: mp 189–190 °C. Anal. ($C_{10}H_{11}NO \cdot 0.5C_2H_2O_4$) C, H, N.

***endo*-2-Amino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene (4b).** A solution of 1.77 g (7.5 mmol) of **11b** in 100 mL of Et_2O was treated with $LiAlH_4$ (0.63 g, 17 mmol) and heated at reflux for 4 h. The reaction was worked up as for **4a** and flash chromatographed on neutral alumina (0.5% MeOH/$CHCl_3$) to give 0.27 g of **4b**: R_f 0.58; IR (neat) 3290 and 3370 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 0.90 (dd, 1 H, C-3 endo H, J = 3.3, 12), 1.59 (br s, 2 H, NH_2), 2.50 (ddd, 1 H, C-3 exo H, J = 5, 9, 12), 3.60–3.85 (br s, 7 H, C-2 exo H and OCH_3), 5.36 (d, 1 H, C-1 methine, J = 5), 5.46 (d, 1 H, C-4 methine, J = 5), 6.71 (s, 2 H, Ar H); MS, m/e 221 (M^+), 178 (base). The oxalate was prepared and recrystallized from absolute EtOH to give a white powder: mp 202.5–203.5 °C dec. Anal. ($C_{12}H_{15}NO_3 \cdot C_2H_2O_4$) C, H, N.

***exo*-2-Guanidino-5,8-dimethoxy-1,2,3,4-tetrahydro-1,4-epoxynaphthalene Nitrate (5).** A solution of **3b** (2.21 g, 10 mmol) and 3,5-dimethylpyrazolecarboxamidine nitrate (2.01 g, 10 mmol) in 10 mL of absolute EtOH was heated at reflux for 22 h. HPLC analysis showed the reaction to essentially stop at approximately 50% conversion. Upon cooling, EtOH was removed in vacuo and the resultant oil partitioned between Et_2O and H_2O. The aqueous portion was concentrated in vacuo and flash chromatographed on a column of neutral alumina eluting with EtOAc/MeOH/NH_4OH (85:10:5) to give 0.95 g of **5** (36%): R_f 0.14. Recrystallization from EtOH/Et_2O gave cream crystals: mp 183–184 °C; IR (KBr) 3180–3440 (br, guanidinium), 1620, and 1675 cm^{-1} (guanidinium I and II bands); ^1H NMR (CD_3OD) δ 1.91 (m, 2 H, C-3 methylene), 3.60–3.90 (m, 7 H, C-2 endo and OCH_3), 5.36 (s, 1 H, C-1 methine), 5.58 (d, 1 H, C-4 methine), 6.75 (s, 2 H, Ar H); MS, m/e 264 (M + 1), 163 (base). Anal. ($C_{13}H_{17}N_3O_3 \cdot HNO_3$) C, H, N.

Pharmacological Methodology. Field Stimulated Rat Vas Deferens. Vas deferentia were extirpated from young adult (2–4 months old) Sprague–Dawley rats (Charles River Crl:CD (SD)BR) which had been sacrificed by a sharp blow to the head. Tissues were dissected free of connective tissue and blood vessels and cut to a length of 25 mm by removal of the epididimal end. The tissues were suspended between two parallel platinum electrodes 4–7 mm apart in a 25-mL organ bath containing Krebs–bicarbonate solution[25] at 37 °C gassed with 95% O_2 and 5% CO_2. Tissues were placed under 500 mg of resting tension, and responses to single stimuli 0.1 Hz, 60 V, 0.2-ms duration measured via a Narco Biosystems F-60 transducer linked to a Narco Biosystems Model DMP-4B physiograph with Type 7070 amplifier. Tissues were allowed a minimum of 30 min to develop consistent control responses before the effects of test compounds were assessed on the nerve-stimulation-evoked responses. Test compounds were dissolved in normal saline and added cumulatively to the bath in volumes less than 250 μL. Compounds **3a,b** and **4a,b** were evaluated as their oxalates while **5** was tested as its nitrate. Oxalic acid was shown to have no effect on the preparation in the concentration range at which the compounds were tested.

α_1-Adrenergic agonist activity was determined from linear regression analyses of cumulative log dose–response curves or by extrapolation of double-reciprocal plots. A period of 10 min was allowed to elapse between successive dosage increases of agonist. Direct adrenergic activity was substantiated by pretreatment with reserpine (3 mg/kg) 16 h prior to sacrifice.[26] α_1-Adrenergic activity was verified by the ability of prazosin (Pfizer) (78 nM) to cause a parallel shift to the right in the log dose–response curve of agonist. (±)-Methoxamine hydrochloride (Burroughs-Wellcome) was employed as the standard to which test compounds were compared.

α_2-Adrenergic activity was assessed by pretreating tissues with prazosin (78 nM) 30 min prior to addition of agonist to block α_1-mediated effects. α_2-Agonists were added cumulatively to the bath at 10-min intervals to allow their full inhibitory effects to develop. Responses to electrical stimuli were measured as a percent of predrug control levels and expressed as the negative log of the molar concentration required to produce a 50% inhibition of the predrug control response plus or minus the standard error of the mean by using linear regression analysis of 16–84% values. α_2-Adrenergic activity was verified by the ability of yohimbine (Sigma) (8 nM) to cause a parallel shift to the right in the log dose–response curve of the agonist. Guanabenz acetate (Wyeth) was used as the standard α_2-agonist to which test compounds were compared.

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Spectroscopic Detection of Iminocyclophosphamide and Its Possible Role in Cyclophosphamide Metabolism

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Iminocyclophosphamide (**4**) has been identified by ^1H NMR as a product from base treatment of 4-alkylthio-substituted cyclophosphamide derivatives, viz., *cis*-4-(propylthio)cyclophosphamide (*cis*-**7**). A maximum concentration of approximately 12% of total product was observed by treating *cis*-**7** with ethyl propiolate and NaH or deuteriated dimsyl anion in anhydrous Me_2SO-d_6. Treatment of *cis*-**7** with base alone established a rapid *cis*-/*trans*-**7** equilibrium via the imine intermediate **4**. Base-catalyzed expulsion of 1-propanethiol (**8**) from *cis*-**7** and thiol trapping afforded formation of **4**, which subsequently underwent elimination to the relatively more stable conjugated (vinylimino)-phosphamide (**9**). Iminocyclophosphamide (**4**) was also identified by fast atom bombardment mass spectrometry as a product generated upon analysis of cyclophosphamide derivatives substituted in the 4-position of the oxazaphosphorine ring with various leaving groups.

The metabolic activation of cyclophosphamide (**1**) to 4-hydroxycyclophosphamide (**2**) initiates a complex but interesting array of nonenzymatic processes. It is generally agreed that **2** undergoes ring opening to aldophosphamide (**3**) followed by generation of the ultimately cytotoxic phosphoramide mustard by base-catalyzed β-elimination. *cis*-**2** in aqueous buffer establishes a pseudoequilibrium consisting of *cis*-**2**, *trans*-**2**, the aldehyde **3**, and its hydrate.[1]

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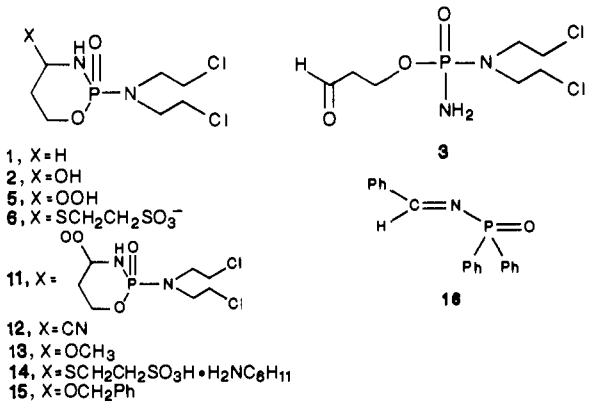
Chart I



The *cis*-/*trans*-**2** equilibration occurs by an acid-catalyzed ring opening of *cis*-**2** to **3** followed by its reclosure to both isomers of **2** (Chart I).[2]

It is well-known that reactions of aldehydes with amines involve the carbinolamine as an intermediate, which in turn is in equilibrium with imine.[3] By structural analogy, 4-substituted cyclophosphamides may also undergo exchange reactions via iminocyclophosphamide (**4**), which is generated by expulsion of the 4-substituent. Fenselau et al.[4] have provided evidence for the existence of imine **4** from the decomposition of 4-hydroperoxycyclophosphamide (**5**) as well as from the enzymatic activation of **1**. More recently, convincing kinetic evidence was presented for the involvement of **4** as a transient intermediate in the hydrolysis of 4-hydroperoxycyclophosphamide (**5**)[5] and mafosfamide (**6**).[6] There is currently no evidence to support the intermediacy of iminocyclophosphamide in the direct activation of **1**,[1-2,7] and the basic conditions required for the elimination of hydroperoxide from **5** suggests that elimination of water from **2** will be very slow under physiologic conditions.[5] In contrast, exchange and hydrolysis of thiol substituents via **4** occurs rapidly at neutral pH, indicating that **4** is the critical intermediate in the activation of mafosfamide and other 4-thio-substituted intermediates.[6]

All evidence to date in support of imine **4** is indirect. Attempts to synthesize **4** and its related heterocyclic structures as well as acyclic model compounds have been unsuccessful.[7,8] Nuclear magnetic resonance spectroscopic (^{1}H, ^{2}H, ^{13}C, and ^{31}P) methods[7] that employ chemical reactions of 4-hydroxycyclophosphamide (**2**) in aqueous solution (pH 5.5–7.8, 37 °C) also have not been successful in detecting **4**. We report here the first direct observation of **4** produced by base-catalyzed elimination of a 4-substituted cyclophosphamide derivative and observed by ^{1}H NMR. In addition, we report mass spectrometric data for **4** that substantiate its identity as a species detectable by another physical method, thus lending support to the conclusions derived from the NMR studies.

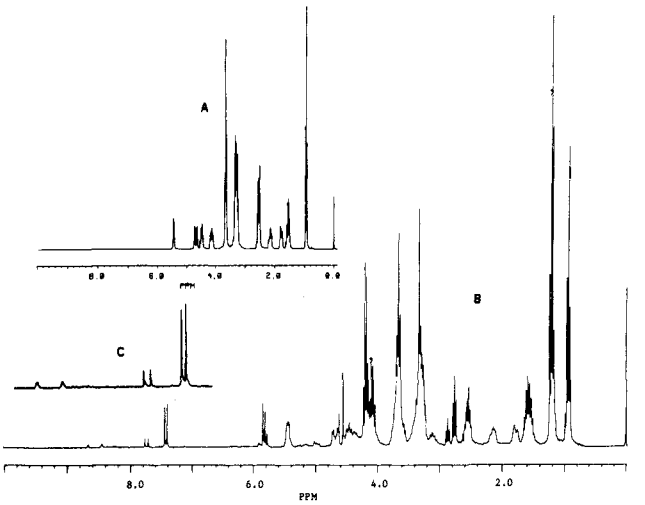


Figure 1. ^{1}H NMR spectra of (A) *cis*-4-(propylthio)cyclophosphamide (*cis*-**7**) in anhydrous Me_2SO-d_6, (B) *cis*-**7** treated with NaH and ethyl propiolate (1 equiv of each), (C) partial expanded spectrum of (B).

Results and Discussion

^{1}H NMR Studies. Generation and detection of the imine **4** was monitored by ^{1}H NMR, and the intermediates were quantitated by peak intensity. *cis*-4-(Propylthio)-cyclophosphamide (*cis*-**7**) was selected as the precursor to **4** because the thiolate group should undergo facile base-catalyzed elimination. It was assumed that readdition of thiol to the imine could be prevented by trapping the thiol liberated in the elimination reaction. A nonnucleophilic solvent was used since the imine, once generated, would be readily intercepted by solvent to form the more stable 4-substituted product. Among the nonnucleophilic bases used to effect elimination of 1-propanethiol (**8**) from *cis*-**7**, NaH was most suitable since even sterically hindered organic bases, i.e., DBN[9] or 2,6-lutidine, underwent addition to the thiol trapping agents used.

Treatment of *cis*-**7** with NaH (1 equiv) alone in anhydrous Me_2SO-d_6 resulted in complete *cis*/*trans*-**7** isomerization within 5 min. Isomerization was confirmed by the following: the C_4 proton at 4.75 ppm (ddd) became a complex multiplet, which reflected the difference in $J_{H,P}$ for $C_{4\text{-}H}$ of *cis*- and *trans*-**7**, and the ^{31}P NMR spectrum changed from a single peak for *cis*-**7** at −16.9 ppm to two peaks at −16.9 ppm and −15.1 ppm of approximately equal intensity. We assume that the *cis*-/*trans*-**7** isomerization proceeds via the imine **4** by base-catalyzed expulsion of the thiol **8** from *cis*-**7** and facile readdition of **8** to give both isomers of **7**.[6]

We then attempted to prepare the imine by reaction of **7** with base and the Michael acceptors ethyl propiolate or *N*-ethylmaleimide. Thus, *cis*-4-(propylthio)cyclophosphamide (*cis*-**7**) was treated with NaH and ethyl propiolate (1 equiv of each) in anhydrous Me_2SO-d_6. A 2:1 mixture of *cis*- and *trans*-2-(propylthio)acrylate (**10**) was rapidly generated; the presence of both isomers was evident from the two sets of vinyl protons (J = 9.1 and 14.6 Hz) and by comparison with an authentic sample of the addition product. Careful examination of the spectrum revealed the presence of the imine **4**, with the C_4 proton at 8.55 ppm as a broad doublet, $J_{H,P}$ = 54 Hz (Figure 1C). Although the magnitude of this proton–phosphorus coupling appears large, there is no precedent for an iminophosphamide

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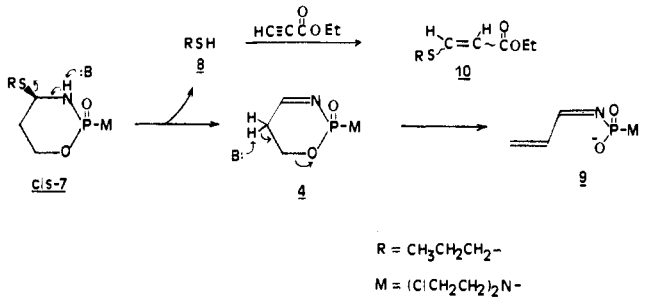
(9) 1,5-Diazabicyclo[4.3.0]non-5-ene.

Scheme I



moiety with trans proton–phosphorus stereochemistry. We have prepared a related phosphinamide **16** with cis stereochemistry;[23] the imine proton resonance of **16** occurs at 9.27 ppm with $J_{H,Pcis}$ = 33 Hz. The trans proton–phosphorus coupling constant should be significantly larger than the cis value.[24]

This resonance gradually disappeared over 20 min and was replaced by another signal at 8.31 ppm that appeared as a doublet of doublets, with $J_{H,P}$ = 28 Hz and $J_{H,H}$ = 9 Hz. This resonance was accompanied by the appearance of signals at 6.36, 5.93, and 5.82 ppm that are characteristic of a conjugated vinyl group. Homonuclear decoupling experiments demonstrated coupling between the imine proton at 8.31 ppm and the vinyl proton at 6.36 ppm and also between the latter proton and the terminal vinyl protons at 5.93 and 5.82 ppm. This product was tentatively assigned the structure **9** and is presumed to arise as shown in Scheme I. Its structure was further supported by comparing the ^{1}H NMR spectrum of the structurally analogous acrolein 2,4-dinitrophenylhydrazone. The imine proton of this hydrazone appeared as a doublet centered at 8.38 ppm in Me_2SO-d_6, with $J_{H,H}$ = 9.3 Hz in THF-d_8.[10] This coupling constant is essentially identical with that of the imine proton of **9**. This vinyl imine was also unstable and gradually disappeared over 1 h.

Several attempts were made to increase the quantity of imine **4** produced in this reaction. Addition of catalytic (0.07 equiv) deuteriated dimsyl anion to a solution of **7** and ethyl propiolate generated approximately 10% of imine **4** and 7% of vinyl imine **9** (Figure 2). Subsequent additions of dimsyl anion led to disappearance of **7** and increased formation of trapped thiol adduct **10**. However, a concomitant increase in imine **4** was not observed. After addition of ca. 1.3 equiv of base, **7** and **4** had completely disappeared, but vinyl imine **9** accounted for only 22% of the product. It is apparent that, under these conditions, imine **4** is unstable and undergoes a variety of subsequent reactions. This conclusion is supported by the following ^{31}P experiments.

^{31}P NMR Studies. Admixture of equimolar quantities of *cis*-**7** and ethyl propiolate in Me_2SO-d_6 exhibited an NMR spectrum showing a single resonance at −16.9 ppm, confirming the stability of this mixture in the absence of base. Addition of NaH to the mixture resulted in immediate (within 3 min) conversion of **7** into a number of unidentified products. *cis*- and *trans*-**7** together constituted only 50% of the reaction mixture, and the presence of 14% of *trans*-**7** suggests that imine **4** competes effectively with ethyl propiolate for the thiolate released in the elimination reaction.[6] The phosphorus chemical shift for the carbinolamine of **16** is ca. 3.4 ppm downfield compared to that of imine **16**; a comparable chemical shift difference

(10) The imine resonance overlapped with an aromatic proton in Me_2SO-d_6; therefore the coupling constant was measured in THF-d_8.

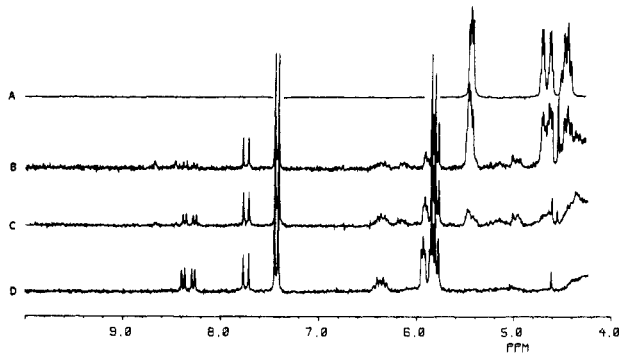


Figure 2. Partial ^{1}H NMR spectra (4.3–10.0) of (A) *cis*-4-(propylthio)cyclophosphamide (*cis*-**7**) in Me_2SO-d_6; *cis*-**7** treated with ethyl propiolate (1 equiv) and (B) 0.07 equiv, (C) 0.37 equiv, (D) 1.33 equiv of deuteriated dimsyl anion.

for **4** vs. **2** would place the signal for **4** at ca. −13 ppm. Given the number of transient peaks appearing in the phosphorus spectrum, however, it was impossible to assign the phosphorus resonance of **4**. Addition of excess NaH led to the appearance of a resonance at −18.1 ppm (12% of the total phosphorus intensity) that gradually disappeared over 1 h. A ^{1}H spectrum of this mixture taken with the decoupler coil in the multinuclear probe showed the presence of the vinylimine proton at 8.31 ppm. We tentatively assign the phosphorus resonance at −18.1 ppm to the vinyl imine **9**. This resonance was also the major identifiable product resonance observed when ethyl propiolate was added to a mixture of **7** and NaH.

Further evidence in support of the formation and rapid decomposition of imine **4** was obtained by reacting **7** with NaH and ethyl propiolate in the presence of excess benzyl alcohol. Under these conditions the major product observed by ^{1}H and ^{31}P NMR was 4-(benzyloxy)cyclophosphamide (^{31}P = −16.2 ppm); the plethora of other signals noted in previous experiments was totally absent.

Mass Spectrometric Studies. Fast atom bombardment mass spectrometric (FABMS) analysis of **2**, **5**, *cis*-**7**, 4-peroxycyclophosphamide (**11**), 4-cyanocyclophosphamide (**12**), 4-methoxycyclophosphamide (**13**), and mafosfamide (ASTA Z 7557, **14**) all yielded evidence of the production of **4** during analysis. In addition to peaks for the appropriate parent compound, spectra included m/z 259 (2 Cl) in the positive mode, which corresponds to $(M + 1)^+$ for **4**, and m/z 257 (2 Cl) in the negative mode, which corresponds to $(M - 1)^-$ for **4**. The FAB mass spectrum of **2** in the negative mode also gave m/z 349 (2 Cl) corresponding to $(M - 1 + \text{glycerol})^-$ for **4**. Additional support for the production of **4** during analysis of **2**, after treatment with ammonium hydroxide (pH 8) and lyophilization, was provided by the presence of a peak of m/z 351 (2 Cl) in the positive mode, corresponding to $(4 + \text{glycerol} + 1)^+$, and m/z 349 (2 Cl) and m/z 385 (3 Cl) in the negative mode, the latter corresponding to $(4\text{-}1 + \text{glycerol} + \text{Cl})^-$. In addition, recording the spectrum of this preparation in the high mass range in the positive mode yielded m/z 517 (4 Cl), which corresponds to $(2\ M + 1)^+$ for **4**. The FAB mass spectrum of **13** in the positive mode is shown in Figure 3, where m/z 291 (2 Cl) $(M + 1)^+$ and m/z 581 (4 Cl) $(2\ M + 1)^+$ for **13** are observed along with m/z 259, 351, and 517 for **4**; also present is m/z 549 (4 Cl), corresponding to a cluster of $(M258 + M290 + 1)^+$, i.e., $(4 + 13 + 1)^+$. The FAB mass spectrum of *cis*-**7**, the intermediate from which **4** was generated in the NMR studies, similarly contained a cluster ion of m/z 593 (4 Cl) in the positive mode, corresponding to $(M258 + M334 + 1)^+$, i.e., $(4 + \textit{cis}\text{-}7 + 1)^+$, and a cluster of m/z 379 (2 Cl), corresponding to (M258

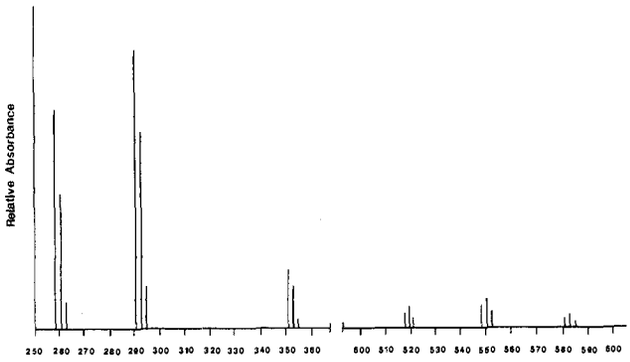


Figure 3. Partial FAB mass spectrum of 4-methoxycyclophosphamide (**13**) in the positive mode.

+ sulfolane + 1)$^+$; also observed were m/z 517 (4 Cl) and m/z 669 (4 Cl), the latter corresponding to (2 M + 1)$^+$ for *cis*-**7**. The FAB mass spectrum of *cis*-**7** in the negative mode gave m/z 369 (3 Cl), corresponding to (M + Cl)$^-$ for *cis*-**7**, in addition to m/z 257 (2 Cl).

Conclusion

The electrophilic reactivity of imines is well recognized.[11,12] Imine **4** is a phosphoryl analogue of *N*-acyl imines, which are known to be more reactive than simple imines and spontaneously add water to form carbinolamines.[13] The *N*-phosphoryl imine moiety appears to be even more reactive, and its lifetime in the presence of nucleophiles is extremely short. We have shown that, given a suitable leaving group at C_4 in the absence of nucleophiles and under conditions that prevent readdition of the leaving group, imine **4** can be generated via base-catalyzed elimination and detected spectroscopically. Iminocyclophosphamide (**4**) appears to be rather unstable, however; even in the absence of nucleophiles, subsequent ring-opening with elimination of the phosphoramidate anion occurs to generate the acrolein imine of phosphoramide mustard (**9**). It should be emphasized that the rate of addition of nucleophiles far exceeds that of elimination, so it is unlikely that **9** would be generated under in vivo conditions.

We have also shown that **4** is readily produced upon FABMS analysis of derivatives of **1** substituted in the 4-position of the oxazaphosphorine ring with various leaving groups. It is possible that previously reported mass spectral studies on various 4-substituted-thio derivatives of cyclophosphamide and on compounds **5** and **12** also detected **4**. Particularly abundant peaks corresponding to (M + 1)$^+$ for **4** were observed upon chemical-ionization mass spectral analysis of **12**[4] and upon field desorption analysis of **5** and several 4-alkylthio derivatives of cyclophosphamide,[14] suggesting that **4** might have been the species accounting for these peaks, although it is also possible that the peaks were indeed fragment ions as intimated by the investigators.[4,14]

Thus we have confirmed the existence of iminocyclophosphamide, which has been postulated as an intermediate in the reactions of 4-substituted cyclophosphamides.[1,4,5] The unusual electrophilicity of the imine moiety in **4**, however, suggests that its lifetime will be extremely short in aqueous solution. Recent kinetic studies indicate that, under physiologic conditions, iminocyclophosphamide is an important intermediate only for those analogues and metabolites that posses a good leaving group at the C-4 position.[1,2,5,6] Although iminocyclophosphamide is unlikely to be important in the direct metabolism of cyclophosphamide, it is probably the critical intermediate in the activation of mafosfamide and the further metabolism of other potential 4-thio-substituted cyclophosphamide intermediates.

Experimental Section

All organic reagents and solvents were reagent grade and purchased from Aldrich Chemical Co. ^{1}H NMR spectra were recorded on an IBM WP-270-SY instrument using 5-mm sample tubes, a 4000-Hz spectral width, 90° pulse, 2-s repetition time, and 32 scans. Chemical shifts are reported in parts per million from internal Me_4Si reference. ^{31}P NMR spectra were recorded on the same instrument equipped with an IBM-VSP multinuclear probe set for 109.368 MHz using 10-mm sample tubes, 500-Hz spectral width, 90° pulse, 0.8-s pulse repetition time, and 64 scans. Broad-band gated proton decoupling was used. Chemical shifts are reported in parts per million from 5% triphenylphosphine oxide in toluene-d_8 as a coaxial reference.

Mass spectra were determined with a Varian MAT 311A spectrometer equipped for fast atom bombardment (FAB) capabilities. Glycerol or sulfolane was used as the solvent.

Mafosfamide (**14**) was obtained as a generous gift from Professor Norbert Brock and Dr. Peter Hilgard of Asta-Werke, Degussa Pharma Gruppe, Bielefeld, West Germany. 4-Hydroxycyclophosphamide (**2**) and 4-peroxycyclophosphamide (**11**) were prepared as described previously.[15,16]

***cis*-4-Hydroperoxycyclophosphamide (5)** was prepared in 33% yield as described elsewhere:[2] mp 106–107 °C dec (lit.[17] mp 107–108 °C); ^{1}H NMR (Me_2SO-d_6) δ 11.56 (1 H, s, OOH), 5.90 (1 H, m, NH), 4.92 (1 H, ddd, J_{HP} = 25 Hz, H_4), 4.25–4.40 (1 H, m, H_{6a}), 4.00–4.20 (1 H, m, H_{6e}), 3.20–3.70 (8 H, m, NCH_2CH_2Cl), 1.87–1.93 (2 H, m, H_5).

***cis*-4-(Propylthio)cyclophosphamide (*cis*-7)** was prepared by a modification of the published procedure.[18,19] *cis*-4-Hydroperoxycyclophosphamide (478 mg, 1.43 mmol) was deoxygenated with triethyl phosphite (237 mg, 0.245 mL, 1.43 mmol) in 10 mL of methylene chloride at 0 °C for 10 min. 1-Propanethiol (217 mg, 0.259 mL, 2.85 mmol) was added to the reaction mixture and followed by 0.1 mL of trifluoroacetic acid. The reaction was allowed to proceed for 2 h at 0 °C, and the mixture was brought to room temperature. The solution was washed with 0.1 N $NaHCO_3$ (5 mL) and H_2O (5 mL), the separated organic layer dried over sodium sulfate, and the filtrate evaporated in vacuo. The resulting oil was dissolved in anhydrous ether and precipitated by addition of hexane. The precipitate was crystallized from ether/hexane to give 256 mg (53% yield) of crystalline *cis*-**7**: mp 71–72 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.50 (1 H, fused t, $J_{NH,C4\text{-}H}$ = 4.5 Hz, NH), 4.75 (ddd, $J_{C4\text{-}H,NH}$ = 4.5 Hz, $J_{C4\text{-}H,P}$ = 21.5 Hz, H_4), 4.60–4.45 (1 H, m, H_{6a}), 4.25–4.10 (1 H, m, H_{6e}), 3.80–3.60 (4 H, m, CH_2Cl), 3.45–3.20 (4 H, m, CH_2N), 2.70–2.50 (2 H, m, CH_2S), 2.30–2.10 and 1.90–1.75 (2 H, m, H_5), 1.70–1.50 (2 H, m, CH_2CH_2S), 0.95 (3 H, t, $J_{H,H}$ = 7.3, CH_2CH_3); ^{31}P NMR (Me_2SO-d_6) −16.9 ppm.

Acrolein 2,4-dinitrophenylhydrazone was prepared as described:[20] mp 166 °C (lit.[21] mp 165 °C); ^{1}H NMR (THF-d_8) δ 11.25

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(1 H, br, NH), 9.00 (1 H, fused d, $J_{H,H}$ = 2.6 Hz, aromatic H), 8.35 (1 H, m, aromatic H), 8.10 (1 H, d, $J_{H,H}$ = 9.3 Hz, N=CH), 7.96 (1 H, d, $J_{H,H}$ = 9.6 Hz, aromatic H), 6.65 (1 H, m, CH=CH_2), 5.75 (2 H, m, CH=CH_2).

Deuteriated methylsulfinyl carbanion (dimsyl anion) was prepared essentially as described.[22] The prepared solution contained 0.01 mmol of deuteriated methylsulfinyl carbanion sodium salt in 15 μL of Me_2SO-d_6.

4-Cyanocyclophosphamide (12)[4] was prepared from **14** by dissolving 10 mg in 0.5 mL 0.5 N sodium cyanide, allowing the solution to stand 15 min at room temperature, and lyophilizing. TLC of the residue on silica gel in acetone/chloroform (3:1, v/v) gave the two isomers[4] at R_f 0.9 and 0.7, which were isolated by elution of appropriate TLC bands with acetone and evaporation. FABMS identified **12** and **4** in both isolates: positive mode [relative abundance] – m/z 259 (2 Cl), $(M + 1)^+$ for **4** [40]; m/z 286 (2 Cl), $(M + 1)^+$ for **12** [100]; m/z 378 (2 Cl), (M + 1 + glycerol)$^+$ for **12** [10]; negative mode – m/z 257 (2 Cl), $(M - 1)^-$ for **4**; m/z 284 (2 Cl), $(M - 1)^-$ for **12**; m/z 320 (3 Cl), $(M + Cl)^-$ for **12**; m/z 376 (2 Cl), (M – 1 + glycerol)$^-$ for **12**.

4-Methoxycyclophosphamide (13) was prepared from **14** by dissolving 20 mg in 1 mL of methanol, adding one drop of 0.1 N ammonium hydroxide, allowing to stand 24 h at room temperature, and evaporating in a stream of nitrogen. The residue was triturated with 0.5 mL of cold acetone and filtered to remove **14**. The filtrate was fractionated by preparative TLC on a scored (2-in. sections) 8-in. 500 silica gel plate in acetone/chloroform (3:1, v/v), and the mobile band (R_f 0.7) was collected and eluted with acetone. Silica gel was removed by centrifugation, and separation and evaporation of the eluate in a stream of nitrogen yielded **13**. FABMS analysis of **13** gave the peaks identified above in the Results and Discussion section for **13** in the positive mode. The following peaks were observed in the negative mode: m/z 257 (2 Cl), $(M - 1)^-$ for **4**; m/z 289 (2 Cl), $(M - 1)^-$ for **13**; m/z 325 (3 Cl), $(M + Cl)^-$ for **13**.

4-(Benzyloxy)cyclophosphamide (15) was prepared by dissolving **5** (30 mg, 0.09 mmol) in methylene chloride (10 mL) and adding triphenylphosphine (26 mg, 0.1 mmol), benzyl alcohol (22 mg, 0.2 mmol), and a catalytic amount of *p*-toluenesulfonic acid. After being stirred at room temperature for 2 h, the mixture was evaporated in vacuo and chromatographed by preparative TLC (1000-μm silica gel H), eluting with 3:1 v/v ethyl acetate/hexanes. The UV-absorbing band at R_f 0.3 was removed, extracted with acetone, filtered, and evaporated in vacuo to give **15** (14 mg) contaminated with triphenylphosphine: ^{31}P NMR –16.2 ppm (Me_2SO-d_6).

***N*-Benzylidenediphenylphosphinamide (16)** was prepared from benzaldoxime and chlorodiphenylphosphine as described:[23] mp 125–127 °C (lit. mp 141–142 °C); ^1H NMR (Me_2SO-d_6) δ 9.27 (1 H, d, $J_{H,P}$ = 33 Hz, HC=), 8.2–7.4 (15 H, m, aromatic H); ^{31}P NMR (Me_2SO-d_6) –1.52 ppm.

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Registry No. 4, 84489-09-8; **5**, 56922-83-9; *cis*-**7**, 107961-74-0; **12**, 84489-10-1; **13**, 81733-42-8; **14**, 99280-05-4; **15**, 107961-75-1; **16**, 67764-52-7; acrolein 2,4-dinitrophenylhydrazone, 888-54-0; 1-propanethiol, 107-03-9; benzyl alcohol, 100-51-6.

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