Other cyclized isomers are recovered from **2** and **3** besides those listed in Table II. Two pathways account for this, ring contraction of cycloalkyl cations and cyclization of secondary cations from reaction 3. Ring contraction of cyclohexyl to 1-methylcyclopentyl and of cycloheptyl to 1-methylcyclohexyl cations occurs rapidly (such that cyclohexyl and cycloheptyl have never been seen by NMR in solution[12]), and we recover the anticipated neutrals[16] in the EBFlow experiments, 1-methylcyclopentene from **3** (0.06 of the C_6H_{10} yield) and 1-methylcyclohexene plus methylenecyclohexane from **4** (0.09 and 0.03 of C_7H_{12}, respectively). We also recover products expected from cyclization of secondary cations: 3- and 4-methylcyclopentene (0.04 or C_6H_{10}; we do not separate these two isomers on our GLC column) from **3**; 3- and 4-methylcyclohexene (0.07 and 0.04 of C_7H_{12}, respectively) from **4**.

Our results not only provide positive evidence of reaction 1 for $n = 2$–4 but also permit us to estimate relative rates of cyclization. In every case, hydride shift (reaction 3) is in competition with cyclization (reaction 1). For the 5-hexenyl case, hydride shift is roughly two times faster; for 4-pentenyl, hydride shift is ten times faster (relative rates are based on the neutral product distributions). If we assume that a 2,1-hydride shift has the same rate constant for all of the cations studied, the implication is that endocyclic electrophilic attack to form a six-member ring is only four to five times faster than to form a five-member ring and that closure to form a seven-member ring is less than twice as fast as to form a five-member ring.

This study quantifies the notion of favoritism for endocyclic closures of the parent cation **1**. In addition, it illustrates how the mechanism of reaction 2 can be used to build a bridge between the realm of mass spectrometry and that of solution chemistry. The analogy between the chemistry of gaseous ion–molecule complexes and solvolysis chemistry seems apt and is the basis of continuing investigations.

Acknowledgments. The authors are grateful to Professor F. H. Westheimer of Harvard University, in whose laboratory much of the synthetic work was done, and to Phillip R. Briggs, who helped record high-resolution mass spectra of Harvard's AEI-MS 9 mass spectrometer. This work was supported by National Institutes of Health Grant NS 14773 to T.H.M.

Supplementary Material Available: Mass spectra of compounds **2**–**4** and their d_2 analogues and product distributions from EBFlow radiolyses of compounds **2**–**4** (7 pages). Ordering information is given on any current masthead page.

(15) Standard control runs (see ref 5) rule out production of the observed products from filament pyrolysis. In an ancillary study, we find that 70-eV electron bombardment of phenoxycyclohexane produces cyclohexene as ≥95% of the C_6H_{10} yield, with methylcyclopentene isomers constituting most of the remaining C_6H_{10}.

(16) Marinelli, W. J.; Morton, T. H. *J. Am. Chem. Soc.* **1978**, *100*, 3536-3539; **1979**, *101*, 1908.

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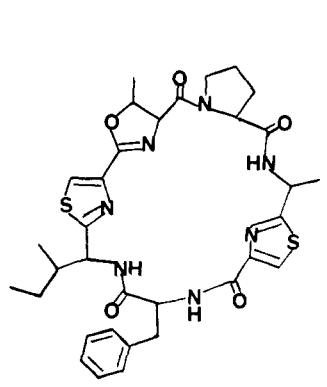
Ulicyclamide and Ulithiacyclamide, Two New Small Peptides from a Marine Tunicate

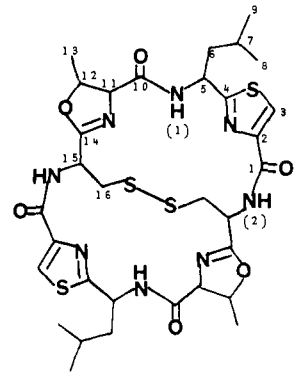
Sir:

Current interest in small peptides, many of which possess antimicrobial or neurophysiological properties,[1] prompts us to report isolation and structure of two new peptides which we encountered in our research into the molecular basis of marine symbiosis.[2]

MeOH extraction of the ascidian *Lissoclinum patella*[3,4] (freeze-dried, 82 g) from Palau, Western Caroline Islands, furnished 0.7 g of residue. Chromatography on Sephadex LH-20 (CH_2Cl_2/hexane, 4:1) and then BisSil A (EtOAc/aqueous NH_3, 95:5) yielded 40 mg of ulicyclamide (**1**) and 35 mg of ulithiacyclamide (**2**) as colorless oils, in addition to several minor constituents.



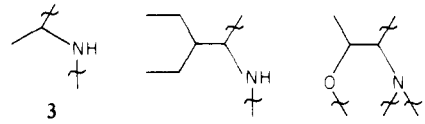
1



2

Ulicyclamide (**1**) has a molecular formula $C_{33}H_{39}N_7O_5S_2$: $[\alpha]^{25}_D$ +35.7° (*c* 2.3, CH_2Cl_2); UV λ_{max} (MeOH) 248 nm (ϵ 7900); high-resolution mass spectroscopy (HRMS), calcd, 677.2439;[7] found, 677.2446. The electron-impact mass spectroscopy (EIMS) exhibited additional peaks at m/z 620 ($M^+ - C_4H_9$) and 586 ($M^+ - C_7H_7$). The IR spectrum was transparent in the OH and COOR regions but showed intense absorptions at 3300, 1670, and 1650 cm^{-1}, indicating peptide linkages. A cyclic peptide was suggested by the lipophilic nature of **1**.

The ^{13}C NMR spectrum of ulicyclamide (Table I) exhibited signals for all 33 carbons. Four singlets between δ 171.9 and δ 170.5 denote a tetrapeptide. Signals for phenylalanine and proline were readily assignable. The olefinic region contained signals for two thiazole rings [161.1 (s), 160.5 (s), 151.4 (s), 148.9 (s), 124.3 (d), and 123.8 (d)]. The 220-MHz ^1H NMR spectrum (Table I), including spin–spin decoupling experiments, confirmed the phenylalanine and proline assignments and exhibited signals at δ 8.08 (1 H, S) and δ 8.03 (1 H, s) for the two thiazole rings, and exhibited signals for three isolated spin systems assignable to part structures **3** [δ 9.06 (1 H, d, J = 5 Hz), 5.38 (1 H, dq, J = 7, 5 Hz), 1.71 (3 H, d, J = 7 Hz)], **4** [δ 7.85 (1 H, d, J = 10 Hz), 5.26 (1 H, dd, J = 10, 7 Hz), 2.60 (1 H, m), 1.20 (2 H, m), 0.85 (3 H, t, J = 7 Hz), 0.75 (3 H, d, J = 7 Hz)], and **5** [δ 4.82 (1 H, dq, J = 4, 7 Hz), 4.26 (1 H, d, J = 4 Hz), 1.44 (3 H, d, J = 7 Hz)].



3 **4** **5**

Hydrolysis of ulicyclamide in refluxing 6 N HCl overnight followed by treatment with C_6H_5COCl and CH_2N_2 yielded *N*-

(1) See, e.g.: *Amino-acids, Pept., Proteins*, **1978**, *9*, 395-416, and previous volumes.

(2) Ireland, C.; Scheuer, P. J. *Science (Washington, D.C)* **1979**, *205*, 922-923.

(3) Eldredge, L. G. *Micronesica* **1966**, *2*, 161-259.

(4) Family Didemnidae, order Enterogona, class Ascidiacaea, subphylum Urochordata (tunicates), phylum Chordata.

(5) The animal was first collected by Mark Yunker in August 1977 and was identified by Dr. Ralph Lewin.

(6) *Uli* in Hawaiian denotes a dark color, as the deep blue of the ocean or the green of vegetation. This ascidian is dark green.

(7) Electron-impact mass spectra were determined on a Varian MAT 311 instrument. The high-resolution mass spectra were measured at the University of Illinois. NMR data (100 MHz ^1H and 25.4 MHz ^{13}C) were determined on a Varian XL 100 spectrometer; proton data at 220 MHz were measured at the facility at the University of California, San Diego.

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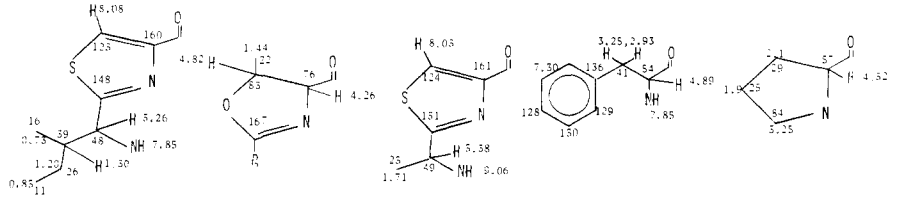
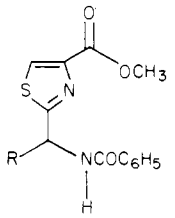
Table I. NMR Data and Assignments for Ulicyclamide (1)



^{13}C ($CDCl_3$) (δ)			
171.9 (3) (s)	136.8 (s)	76.3 (d)	29.8 (t)
170.5 (s)	130.6 (2) (d)	57.5 (d)	26.0 (t)
167.7 (s)	129.5 (2) (d)	54.4 (2) (dt)	25.9 (t)
161.1 (s)	128.2 (d)	49.6 (d)	25.4 (q)
160.5 (s)	124.3 (d)	48.1 (d)	22.9 (q)
151.4 (s)	123.8 (d)	41.8 (t)	16.2 (q)
148.9 (s)	83.3 (d)	39.0 (d)	10.9 (q)
^{1}H ($CDCl_3$, 220 MHz) (δ)			
9.06 (d, J = 5 Hz)	5.38 (dq, J = 5, 7 Hz)	3.25 (m, 3 H)	1.44 (d, 3 H, J = 7 Hz)
8.67 (d, J = 7 Hz)	5.26 (dd, J = 10, 7 Hz)	2.93 (dd, J = 14, 10 Hz)	1.30 (m, 1 H)
8.08 (s)	4.89 (m)	2.60 (m)	1.20 (m, 2 H)
8.03 (s)	4.82 (dq, J = 4, 7 Hz)	2.1 (m, 2 H)	0.85 (t, 3 H, J = 7 Hz)
7.85 (d, J = 10 Hz)	4.52 (t, J = 8 Hz)	1.9 (m, 2 H)	0.73 (d, 3 H, J = 7 Hz)
7.30 (s, 5 H)	4.26 (d, J = 4 Hz)	1.71 (d, 3 H, J = 7 Hz)	

[a] Carbon assignments are based on literature values for amino acids and on values measured for the synthetic thiazole model compound.

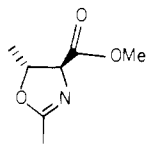
benzamido methyl esters of five amino acids, proline, phenylalanine, threonine, and two thiazoles, 6 and 7. The first three



6, R = Me-
7, R = $CH_3CH_2CH(CH_3)$-
10, R = Me_2CHCH_2-

were identified by comparison with authentic material. Thiazole 6 was synthesized as described.[8] Thiazole **7** was identified by spectral comparison with 6 and analysis of mass spectroscopy (MS) and ^{1}H NMR data.

In contrast, ^{13}C NMR evidence revealed only four peptide linkages. Furthermore, isolation of threonine was surprising since the IR spectrum of ulicyclamide lacks OH absorption. Apparently, the peptide contains an amino acid with a masked threonine that is freed upon hydrolysis, in analogy with acid hydrolysis of thiazoline-containing peptides,[9] which produce cysteine. In our case, the precursor of threonine would have to be an oxazoline. This argument was confirmed by part structure **5** (vide supra), which was secured by spectral comparison with synthetic **8**[10] prepared



8

Table II. NMR Data for Ulithiacyclamide (2)

position	^{13}C ($CDCl_3$), δ	^{1}H (C_6D_6), δ
1[a]	170.5	
2[a]	160.1	
3	124.1	7.72 (s)
4	149.2	
5	48.5 (C-15?)	5.24 (m)
6	46.5	1.35 (m, 2 H)
7	25.3	1.66 (m)
8, 9	22.8, 22.7	0.78 (d, 3 H, J = 7 Hz), 0.90 (d, 3 H, J = 7 Hz)
10	170.0	
11	74.3	4.05 (dd, J = 8, 2 Hz)
12	81.7	4.71 (m)
13	22.1	1.1 (d, 3 H, J = 7 Hz)
14	167.3	
15	48.4 (C-5?)	5.36 (m)
16	46.5	3.22 (dd, J = 14, 6 Hz)
		3.02 (dd, J = 14, 4 Hz)
N-1		7.70 (d, J = 9 Hz)
N-2		8.50 (d, J = 9 Hz)

[a] Assignments agree with the values of a synthetic model compound.

as described.[11] Selective hydrolysis of the oxazoline moiety of ulicyclamide (5% H_2SO_4 in MeOH, followed by acetylation)[12] furnished the acyclic ester **9**. The amino acid sequence of **9** was established by high-resolution electron-impact mass spectroscopy (HREIMS) (Figure 1) and led to structure **1** for ulicyclamide.

The second major component, ulithiacyclamide (**2**), has a molecular formula $C_{32}H_{42}N_8O_6S_4$: $[\alpha]^{25}_D$ +62.4° (*c* 2.9, CH_2Cl_2); HRMS, calcd, 762.2101; found, 762.2105. The IR (CH_2Cl_2) bands at 3300, 1670, and 1650 cm^{-1} and the UV maximum (MeOH) at 247 nm (ϵ 7000) were virtually identical with those of ulicyclamide, thus indicating that ulithiacyclamide was a cyclic peptide with at least one thiazole ring.

The ^{13}C and ^{1}H (C_6D_6) NMR spectra (Table II) exhibited signals for only half of the molecule, which showed that ulithiacyclamide was symmetrical. The NMR data pointed to a thiazole with a leucine side chain, cystine, and an oxazoline ring. Decoupling experiments demonstrated that the oxazoline proton at

(8) Cross, D. F. W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E. *J. Chem. Soc.* **1963**, 2143–2150.

(9) Bycroft, B. W.; Pinchin, R. *J. Chem. Soc., Chem. Commun.* **1975**, 121–122.

(10) **8**: IR (CH_2Cl_2) 1740, 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.62 (1 H, dq, J = 8, 7 Hz), 4.09 (1 H, dq, J = 8, 1 Hz), 3.62 (3 H, s), 1.86 (3 H, d, J = 1 Hz), 1.26 (3 H, d, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 171.4 (s), 167.0 (s), 78.7 (d), 74.7 (d), 52.4 (d), 21.0 (q), 14.2 (q).

(11) Meyers, A. I.; Whitten, C. *Heterocycles* **1976**, *4*, 1687–1692.

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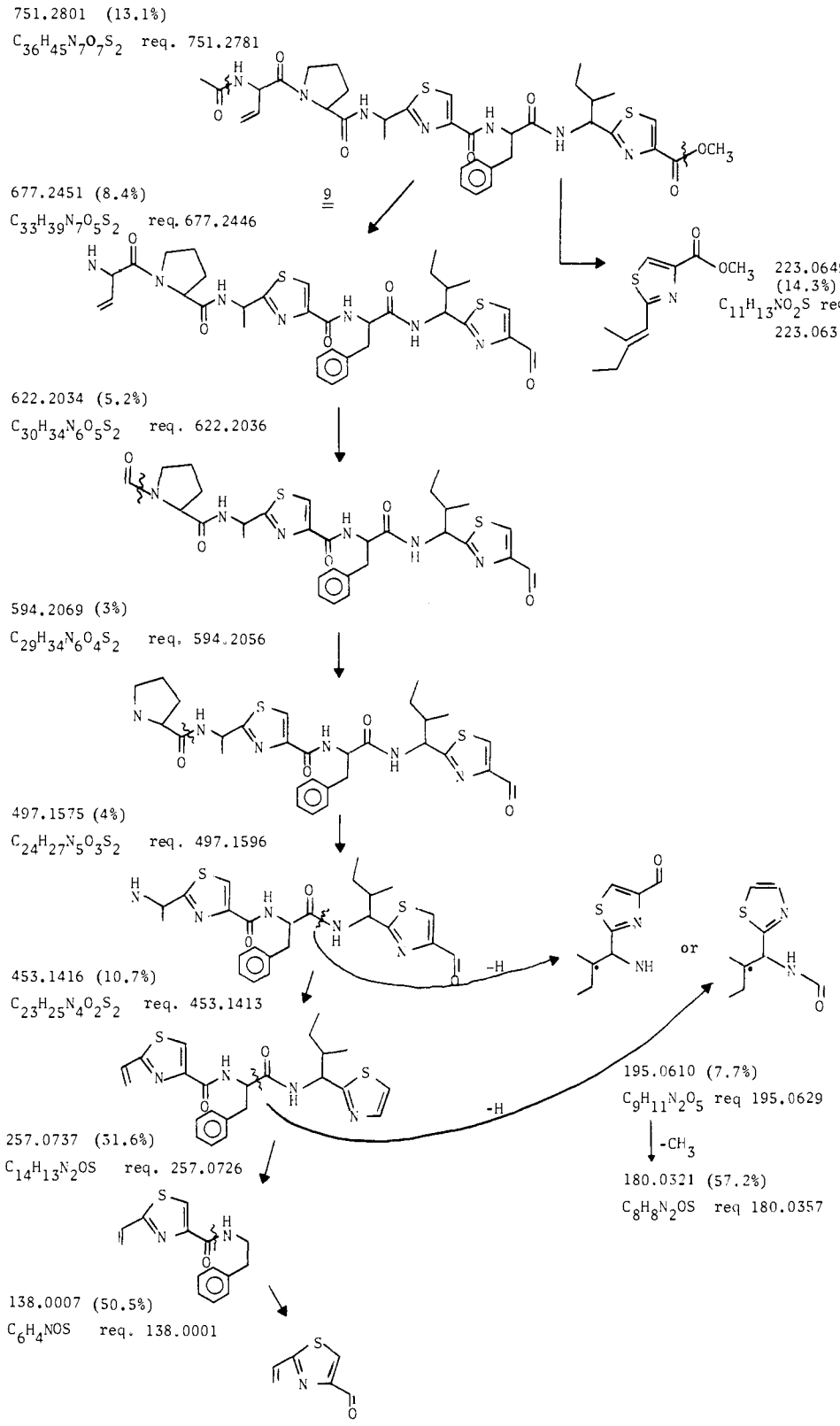

Figure 1. EIMS fragmentation of acyclic ester **9**.

C-11 was homoallylically coupled to the cystine proton at C-15. Therefore, cystine must be condensed with threonine to form the oxazoline. Similar homoallylic coupling is reported for 2-methyl-Δ^2-oxazoline.[13] We also observed this coupling in the oxazoline **8**.

Hydrolysis of ulithiacyclamide with 6 N HCl, followed by the previously described workup, furnished *N*-benzamido methyl esters of cystine, threonine, and thiazole **10** in an approximately 1:2:2 molar ratio.

These data are in full agreement with **2** and with an alternate structure in which the disulfide links two identical cyclic dipeptides. The mass spectrum of ulithiacyclamide readily eliminated the latter structure. Peaks at m/z 729.2304 ($C_{32}H_{41}N_8O_6S_3$ requires 729.2311) correspond to a loss of SH from R–S–S–H$^+$, produced

(13) Weinberger, M. A.; Greenhalgh, R. *Can. J. Chem.* **1963**, *41*, 1038–1041.

by C-S bond fission and H^+ migration, and at 697.2592 ($C_{32}H_{41}N_8O_6S_2$ requires 697.2590) to a loss of S_2H,[14] which is compatible only with **2**.

The biosynthetic origin of ulicyclamide (**1**) and of ulithiacyclamide (**2**) is unknown. Didemnid ascidians are a unique group of invertebrate chordates which harbor symbiotic unicellular algae[15] that possess photosynthetic viability.[16] However, little is known about chemical transport between host and symbiont or about the nitrogen-fixing ability of the algae.

Acknowledgments. We thank Dr. Ralph Lewin for identifying the ascidian, the National Science Foundation for financial support, the University of California, San Diego, NMR facility (NIH Grant RR-00708) for 220-MHz ^1H NMR spectra, and the University of Illinois for HREIMS data.

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(15) Lewin, R. A.; Withers, N. *Nature (London)* **1975**, *256*, 735-737.
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(17) NIH Postdoctoral Fellow 1978-1979; present address: School of Pharmacy, University of Connecticut, Storrs, CT 06268.

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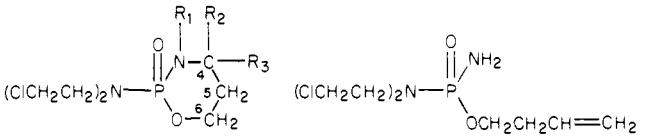
A New Oxidized Derivative of Cyclophosphamide Obtained from Ozonolysis of O-3-Butenyl *N,N*-Bis(2-chloroethyl)phosphorodiamidate

Sir:

The antitumor agent cyclophosphamide (**1**) exerts its cytostatic action after oxidation in vivo by liver microsomes.[1,2] Chemical synthesis of the primary oxidation product in vivo, 4-hydroxycyclophosphamide (**2**), has been described by Takamizawa et al.[3] By this procedure, **2** can be isolated after reduction of 4-hydroperoxycyclophosphamide (**3**), a compound that was synthesized by ozonolysis of O-3-butenyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (**4**). Fenton oxidation of **1** led also to the formation of peroxy compounds[4-6] that were identified as **3** and 4-peroxycyclophosphamide (**5**). In aqueous solution, **3** and **5** are spontaneously converted to **2**.[7] As a consequence, these three compounds exhibit the same cytotoxic properties in in vitro systems. Since **3** and **5** are much more stable than **2** on storage at -20 °C, they are of practical value for in vitro studies on cytostatic effects of **1**.

Routinely, we synthesized **3** by a modified procedure of Takamizawa et al.[3] The modifications led, however, to an unexpected observation, i.e., the occurrence of an as yet unknown oxidized derivative of **1** that could be isolated as a precursor of **3**. Our procedure implied the following:



1, $R_1 = R_2 = R_3 = H$
2, $R_1 = R_2 = H$, $R_3 = OH$
3, $R_1 = R_2 = H$, $R_3 = OOH$
5, ($R_1 = R_2 = H$, $R_3 = O$-)$_2$
6, R_1, $R_2 = O$; $R_3 = H$
7, $R_1 = H$; R_2, $R_3 = O$

4

A solution of 2 g of **4** in 120 mL of acetone/water (2:1), slightly alkalinized with NH_4OH, was ozonized[8] in the presence of 5 mL of 30% H_2O_2 at 0 °C. The reaction was followed by TLC on silica gel, with CH_2Cl_2/*n*-BuOH (9:1) as eluant. After ca. 30 min, the alkylating spot[9] of the starting material ($R_f = 0.4$) had completely disappeared, and almost all alkylating activity was found at $R_f = 0.2$. Acetone was extracted from the reaction mixture with 3 volumes of CH_2Cl_2. The aqueous phase was freeze-dried and the remaining oil dissolved in a small amount of acetone. After the insoluble material was filtered off, ether was added to the filtrate until saturation was reached. From this, a product with rather polar characteristics crystallized at -20 °C (soluble in water, acetone; insoluble in CH_2Cl_2, ether). Isolation of the product had to be carried out as quickly as possible because of its tendency to decompose. The overall yield after crystallization from acetone/ether was ~20%. Crystals of the compound were hygroscopic; when stored dry at -20 °C, its stability is comparable to **3**.

We tentatively identified the compound as 2-bis(2-chloroethyl)aminotetrahydro-2*H*-3,4-epoxy[1,3,2]oxazaphosphorine 2-oxide (**6**). ^1H NMR, ^{13}C NMR, IR, and field desorption (FD) mass spectral analyses[10] were consistent with this structure: ^1H NMR (acetone-*d*, Me_4Si; Varian HA-100) δ 1.75-2.15 (2 H, m, C_5H_2), 3.25-3.85 [8 H, m, $(CH_2CH_2Cl)_2$], 3.90-4.30 (2 H, q, C_6H_2), 5.25 (1 H, t, $J = 6$ Hz, C_4H); ^{13}C NMR (Varian XL-100, solvent and reference acetone-*d*, -40 °C) 33.6 (C_5), 41.8 ($C_{\beta,\beta'}$), 48.5 ($C_{\alpha,\alpha'}$), 61.1 (C_6), 97.5 ppm (C_4).[11] In the ^{13}C NMR "off-resonance" spectrum, the C_4 signal appears as a doublet (while all other signals appear as a triplet), indicating that only one proton is bound at the C_4 atom. IR(KBr): 3410, 3320, 2965, 2945, 2900, 1570, 1455, 1365, 1255, 1205, 1175, 1125, 1090, 1040, 985, 940, 870, 760, and 740 cm^{-1}. FD mass spectrum: *m/z* 274 (2Cl, M^+, relative abundance 75), 141 (2Cl, $[HN(CH_2CH_2Cl)_2]^+$, relative abundance 100), 92 (1Cl, $[CH_2{=}NHCH_2CH_2Cl]^+$, relative abundance 30). Accurate mass measurements of the molecular ion in the FD mode using high resolution and peak matching gave a value of *m/z* 274.0026 (theoretical 274.0041 for $C_7H_{13}O_3N_2Cl_2P$). An isomeric structure of **6**, i.e., the *N*-oxide of the corresponding 3,4-unsaturated compound (Schiff base), is possible from the IR data (absorption at 1570 cm^{-1} is in the >C=N- stretching vibration region) but is unlikely since no peak was detected in the FD mass spectrum at *m/z* 258 $(M - 16)^+$. It has been shown that the loss of oxygen from substances which carry a nitroso group is a characteristic feature in FD mass spectrometry.[12] Also, a possible conversion from **6** to 4-oxocyclophosphamide (**7**) on the probe of the mass spectrometer is unlikely because under the experimental conditions employed **7** is known to give abundant $(M + H)^+$ ions at *m/z* 275 whereas

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(6) Struck, R. F.; Thorpe, M. C.; Coburn, W. C., Jr.; Laster, W. R., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 313-315.
(7) Benckhuysen, C.; van der Steen, J.; Spanjersberg, E. J. *Cancer Treat. Rep.* **1976**, *60*, 369-372.
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(9) Alkylating activity was detected by spraying with 4-pyridinecarboxaldehyde 2-benzothiazolylhydrazone: Norpoth, K. *Cancer Treat. Rep.* **1976**, *60*, 437-443.
(10) Schulten, H.-R. *Int. J. Mass. Spectrom. Ion Phys.* **1979**, *32*, 97-283, and references cited therein.
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