In the Search for New Anticancer Drugs, VI⁺ Structural Modifications of Cyclophosphamide

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Taking into consideration the known mechanism of the drug action of cyclophosphamide (**1**), several analogs and related compounds **5**, **6**, **7**, **9**, **11**, **12** and **27** were synthesized, and tested against lymphocytic leukemia P 388 in mice.

Moderate to high activity was found with **5** ($T/C \times 100 = 243$ at a dose of 300 mg/kg), **7** ($T/C \times 100 = 173$ at a dose of 10 mg/kg), **9** ($T/C \times 100 = 248$ at a dose of 100 mg/kg) and **11** ($T/C \times 100 = 155$ at a dose of 55 mg/kg). None of these compounds was found to be more active than **1** ($T/C \times 100 = 339$ at a dose of 65 mg/kg). Although the essential position C4 for the enzymatic activation is completely blocked in **5**, the compound possesses a high degree of activity. This result justifies a reevaluation of the proposed mechanism of drug action of **1** and its analogs.

Introduction

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]-tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (**1**), first synthesized in 1958 [1], is a very effective drug in the chemotherapy of various human cancers [2–4].

In 1948 [5], it was demonstrated that certain human neoplasms contain high levels of phosphamid-

ase. On the basis of this result, it was hypothesized [6, 7] that some nontoxic phosphamide derivatives of nitrogen mustard could be synthesized which would be enzyme-activated, preferentially in the neoplastic cells, to give the cytotoxins. Several groups of scientists [1, 6–11] pioneered this research, and cyclophosphamide (**1**) was assumed [1] to be such a drug. After **1** was introduced into the clinical use [2, 3] a considerable number of structural modifications [12–17], based on the mechanism of drug action, have been devised but no modified analog of **1** was found to be more active than the parent compound. In studying the action of **1**, Foley *et al.* found [18] that the activation is primarily accomplished by the liver. Brock and Hohorst found [19–21] that an enzyme-mediated oxidation rather than a phosphamidase hydrolysis plays the major role in the activation of the drug. A simplified

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presentation of the metabolic transformations and drug action, originally suggested by Hill *et al.* [22] and later modified by Colvin *et al.* [23], is shown in Scheme 1. Further extensive work [24–30] justifies these viewpoints. The initial activation of **1** is considered [22–27] to involve an enzymatic hydroxylation at the position C4 to form the active metabolite, 2-[bis(2-chloroethyl)amino]-4-hydroxytetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (**2**). The selective cytotoxic effect of **2** towards neoplastic cells, as compared to the vital organs, was suggested [26–30] to be derived from the differences in the toxification and detoxification processes (Scheme 1). Such detoxification is considerably higher in liver, kidney and intestinal mucosa than in tumor cells [28]. Although detoxification of **2** could either be enzymatic or nonenzymatic, toxification was suggested [27] to proceed through a chemical β -elimination which necessitates the presence of at least one hydrogen at the C5 position in **1**. The proposed mechanism (Scheme 1) of drug action of **1** seems to support the results obtained with 2-bis(2-chloroethyl)amino-4,4,6-trimethyltetrahydro-2H-1,3,2-

oxazaphosphorine 2-oxide (**3**), containing no hydrogens at the position C4, and 2-bis(2-chloroethyl)amino-5,5-dimethyltetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (**4**), containing no hydrogens at the position C5 found to be inactive [9, 24].

Since these results were obtained at a time when no well-developed procedure for *in vivo* testing of compounds was available, it was now decided to reinvestigate the role of hydrogens at the position C4 (adjacent to N3) in **1** and related compounds following an improved standardized procedure [31]. For this purpose 2-[bis(2-chloroethyl)amino]-4,4-dimethyltetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (**5**) was synthesized and evaluated against the lymphocytic leukemia P 388 in mice.

In order to determine the effect of the ring size on the anticancer activity, the seven membered com-

Scheme 1

pounds, 2-[bis(2-chloroethyl)amino]-4,4-dimethylhexahydro-1,3,2-oxazaphosphepine 2-oxide (**6**) and 2-[bis(2-chloroethyl)amino]-4,4,7-trimethylhexahydro-1,3,2-oxazaphosphopine 2-oxide (**7**) were prepared.

On the basis of the structure-activity studies [9, 24], it appeared that a decrease in available hydrogens at the position C4 in **1** resulted in a decrease in the therapeutic effectiveness (Table I).

Table I. Influence of methyl substitution at C4 position in **1** on the therapeutic effectiveness [9, 24].

Compound	LD ₅₀ /CD ₅₀ ^a
1	20
12.5
3	0.5

^a LD₅₀ = lethal dose at 50% survival;
CD₅₀ = curative dose at 50% cured.

We have, therefore, hypothesized that an increase in active sites adjacent to the endocyclic nitrogen (N3) of compound **1** may lead to compounds with a higher degree of activity. Consequently, 1-[bis(2-chloroethyl)amino]hexahydro-1H,3H-pyrido[1,2-c][1,3,2]oxazaphosphorine 1-oxide (**9**) was prepared.

The compound presumably has two active sites (C6 and C10) for oxidative attack adjacent to the endocyclic nitrogen (N1). An attempt to prepare another compound with two active sites (C6 and C8) adjacent to the endocyclic nitrogen (N1), 2-[bis(2-chloroethyl)amino]-3-oxa-1-aza-2-phosphabicyclo[2,2,2]octane 2-oxide (**10**) failed. The only product isolated in this case was the intermediate, N,N-bis(2-chloroethyl)-N',N'-(3-hydroxy-1,5-pentanediy)phosphordiamidic chloride (**11**). Nevertheless, this compound **11**, although not a oxazaphosphorine derivative, has presumably two active sites (C2 and C6) adjacent to the endocyclic nitrogen (N1) for an oxidative enzyme attack.

The compound 9-(2-chloroethyl)5,5-dimethyl-2-oxa-6,9-diaza-1-phosphabicyclo[4,3,0]nonane 1-oxide (**12**) which contains two possible active sites (C7 and C8) adjacent to N6 and N9 but only one alkylating 2-chloroethyl moiety was also synthesized.

Results and Discussion

The synthetic approach for the preparation of **5**, **6**, **7**, **9** and **11** involved the condensation of bis(2-chloroethyl)phosphoramidic dichloride (**13**) with an appropriate amino alcohol in the presence of triethylamine in analogy to the literature procedure [10].

The dichloride **13** [12] was prepared by the reaction of phosphoryl chloride with the commercially available bis(2-chloroethyl)amine hydrochloride in the presence of triethylamine and was used without isolation. The synthesis of 3-amino-3-methylbutanol (**14**) required the initial preparation of 3-amino-3-methylbutyric acid (**17**) from 3,3-dimethylacrylic acid (**18**) and concentrated aqueous ammonia [32]. Reduction of **17** with lithium aluminium hydride afforded **14** in 86% yield.

Although synthesis of **14** was reported earlier [33, 34], involving the reaction of isoprene dihydrochloride with aqueous ammonia [33], and the Ritter reaction using either 3-methyl-3-butene-1-ol or 3-methyl-2-butene-1-ol with hydrocyanic acid and sulfuric acid [34], the present procedure offers a distinct experimental advantage for a laboratory scale preparation resulting in a product of high purity. The synthesis of 4-amino-4-methylpentanol (**15**) and of 5-amino-5-methyl-2-hexanol (**16**) was achieved by the following route. Michael condensation of 2-nitropropane (**19**) with either ethyl acrylate (**20**) or methyl vinyl ketone (**21**) yielded ethyl 4-methyl-4-nitropentanoate (**22**) and 5-methyl-5-nitro-2-hexanone (**23**), respectively. Since only the carbonyl group in **22** and **23** could be reduced by lithium aluminium hydride to give 4-methyl-4-nitropentanol (**24**) and 5-methyl-5-nitro-2-hexanol (**25**), respectively, the nitroalcohols thus obtained were further reduced with hydrogen in the presence of platinum dioxide to yield **15** and **16**, respectively.

Compound **9** was prepared by the reaction of 2-(2-hydroxyethyl)-piperidine (**26**) with **13** in the presence of triethylamine.

Compound **9** was previously prepared and tested [10] as an impure material. It was later synthesized [35] in a pure form, however, no test results are available. Compound **11** was prepared by the reaction of 4-hydroxypiperidine with **13** in the presence

of triethylamine. All attempts to achieve an intramolecular cyclization of **11** using either triethylamine or sodium hydride failed to yield the desired product **10**. Prolonged heating at 80 °C resulted in a decomposition of **11**.

The reaction of **11** with propylamine proceeded readily to give N,N-bis[2-chloroethyl]-N',N'-(3-hydroxy-1,5-pentanediy)-N''-propylphosphoric triamide (**27**) in 74% yield.

The intramolecular cyclization of the 2-chloroethyl group in compound **5** using sodium hydride proceeded, similarly to the reported [36] cyclization of the 2-chloroethyl group in **1**, to give the fused bicyclic product **12**. The physical properties of compounds **5**, **6**, **7**, **9**, **11**, **12** and **27** are shown in Table II.

Tab. II. Physical properties of selected antitumor agents.

Com-pound	Yield [%]	Formula ^a	m.p. [°C]	n_D^{23}	IR ^b [cm ⁻¹]	MS [M ⁺ + 1]	¹ H NMR [δ, ppm]
5	82	C ₉ H ₁₉ Cl ₂ N ₂ O ₂ P	73–74		3100(NH), 1480, 1390, 1240, 1080, 1000, 770	289(100), 291(65), 293(11)	1.25 (s, 3H, CH ₃), 1.4 (s, 3H, CH ₃), 1.6–1.8 (m, 2H, CH ₂), 3.2–3.8 (m, 9H, NCH ₂ CH ₂ Cl, NH), 4.2–4.6 (m, 2H, CH ₂ O).
6	22	C ₁₀ H ₂₁ Cl ₂ N ₂ O ₂ P	52–53		3150(NH), 1430, 1370, 1220, 1020, 740	303(73), 305(46), 307(10)	1.5 (s, 6H, 2CH ₃), 2.0–2.2 (m, 4H, 2CH ₂), 3.5–4.3 (m, 9H, NCH ₂ CH ₂ Cl, NH), 4.3–4.8 (m, 2H, CH ₂ O).
7	25	C ₁₁ H ₂₃ Cl ₂ N ₂ O ₂ P	77–79		3150(NH), 1450, 1370, 1210, 1000, 980, 740	317(78), 319(51), 321(9)	1.3 (s, 6H, 2CH ₃), 1.4 (s, 3H, CH ₃), 1.7–1.9 (m, 4H, 2CH ₂), 3.0–4.1 (m, 9H, NCH ₂ CH ₂ Cl, NH), 4.3–4.8 (m, 1H, CHO).
9	89	C ₁₁ H ₂₁ Cl ₂ N ₂ O ₂ P	oil	1.5122	1480, 1360, 1240, 1110, 970, 920, 780	315(100), 317(63), 319(12)	1.4–1.9 (m, 8H, 4CH ₂), 3.2–3.7 (m, 11H, NCH ₂ CH ₂ Cl, CH ₂ N, CHN), 4.2–4.5 (m, 2H, CH ₂ O).
11	84	C ₉ H ₁₈ Cl ₃ N ₂ O ₂ P	oil	1.5250	3500–3200(OH), 1450, 1340–1360, 1230, 1050, 960, 770	323(100), 325(95), 327(34), 329(5)	1.5–2.3 (m, 4H, 2CH ₂), 3.2–4.5 (m, 14H, NCH ₂ CH ₂ Cl, 2CH ₂ N, CHO, OH).
12	62	C ₉ H ₁₈ ClN ₂ O ₂ P	86–88		1440, 1360, 1220, 1030, 870, 780	253(100), 255(33)	1.25 (s, 3H, CH ₃), 1.5 (s, 3H, CH ₃), 1.8–2.1 (m, 2H, CH ₂), 3.0–3.7 (m, 8H, NCH ₂ CH ₂ Cl, 2CH ₂ N), 4.2–4.6 (m, 2H, CH ₂ O).
27	74	C ₁₂ H ₂₆ Cl ₂ N ₃ O ₂ P	oil	1.5335	3550–3100(NH, OH), 1430, 1340, 1180, 1030, 720	310(100) ^c , 312(32) ^c	1.0 (t, 3H, CH ₃), 1.4–2.2 (m, 6H, 3CH ₂), 2.8–4.2 (m, 15H, NCH ₂ CH ₂ Cl, 3CH ₂ N, NH).

^a The microanalysis were in satisfactory agreement with the calculated values: C $\pm$ 0.34, H $\pm$ 0.34, N $\pm$ 0.43, except for **12** and **27** which are unstable: C $\pm$ 0.34, H $\pm$ 0.34, N $\pm$ 0.57; ^b liquids were analysed neat, solid samples as Nujol mull; ^c MS reported as [(M⁺ + 1) — HCl]. M⁺ + 1 not observed.

The *in vivo* anticancer activity of **5**, **6**, **7**, **9**, **11**, **12** and **27**, as well as that of cyclophosphamide (**1**) was evaluated against lymphocytic murine leukemia P 388 according to the protocol of the National Cancer Institute [31]. The mean survival times of the test animals (T), *i.e.*, animals treated with the tumor and drug, were compared with those of controls (C), *i.e.*, animals treated with the tumor only. Compounds exhibiting a test/control (T/C) percentage greater than 125 are considered [31] to be active in this preliminary evaluation. The results are shown in Table III. Since enzyme-mediated C4 oxidation is not expected in **5**, it is reasonable to assume that this compound would have either no activity or a low antitumor activity. Contrary to this expectation, our screening results indicate that compound **5** is considerably active (T/C $\times$ 100 = 243 at a dose of 300 mg/kg). Compound **9** in an earlier test [10] was found to cure all noninbred rats implanted with Walker 256 tumor, however, the therapeutic index, LD₁₀/ED₉₀ was found to be approxi-

mately 1. Present test results with a pure sample against lymphocytic leukemia P 388 indicate (Table III) a high degree of activity (T/C $\times$ 100 = 248 at a dose of 100 mg/kg). Of the two seven-membered ring compounds, **6** and **7**, only **7** was found to be active (T/C $\times$ 100 = 173 at a dose of 10 mg/kg). Compound **11**, although not a oxazaphosphorine derivative, was also found to be active (T/C $\times$ 100 = 155 at a dose of 55 mg/kg). Conversion of compound **11** to the triamide **27** negated the activity. In the present tests (Table III) none of the compounds was found to be more active than cyclophosphamide (**1**). Nevertheless, promising activities of **5**, **7**, **9** and **11** warrant a further study of these compounds against other types of tumors.

In conclusion, the considerable activity of **5** where both C4 positions are blocked by two methyl groups appears to indicate that either the enzymatic oxidation at C4 may not be an exclusively determining factor in the activation of **1**, or the activation of **5** may proceed by a different metabolic path than that

Table III. Anticancer activity of some phosphoramidate nitrogen mustards against lymphocytic Leukemia P 388 in mice^a.

Com-pound	Daily dose ^b (mg/kg)	Test/Control (T/C) Survival (days)	Percent	30 day Survival/ Total	ILS ^c percent
1	32.5	23.7/8.9	266	0/6	166
	65	30.1/8.9	339	5/6	239
	130	6.8/8.9	77	0/6	-23
5	37.5	13.2/9.6	137	0/6	37
	75	15.5/9.6	161	0/6	61
	150	18.0/9.6	187	0/6	87
	300	23.3/9.6	243	1/6	143
6	25	9.3/9.1	103	0/6	3
	50	7.0/9.1	75	0/6	-25
7	10	14.5/8.4	173	0/6	73
	20	9.7/8.4	115	0/6	15
	30	7.5/8.4	89	0/6	-11
	40	6.5/8.4	77	0/6	-23
9	25	13.2/9.6	137	0/6	37
	50	17.5/9.6	182	0/6	82
	100	23.8/9.6	248	1/6	148
	200	10.0/9.6	104	0/6	4
11	50	13.0/8.4	155	0/6	55
	100	14.7/8.4	105	0/6	5
12	100	8.8/8.4	105	0/6	5
	200	8.5/8.4	101	0/6	1
27	25	9.0/9.1	99	0/6	-1
	50	9.2/9.1	101	0/6	1
	100	9.3/9.1	103	0/6	3
	200	10.5/9.1	115	0/6	15

^a CD₂F₁(CDF₁) male mice of average weight 18–21 g were used;

^b compounds injected intraperitoneally daily for 9 days after the day of transplantation of tumor, except compounds 6 and 7 which were injected for only 5 days;

^c ILS % = increase life span calculated from [(T-C)/C] × 100.

postulated for 1. Furthermore, the introduction of additional sites for a possible oxidative activation, as in compound 9, seems to have no noticeable effect on the increase of activity in analogs of 1. Removal of one of the 2-chloroethyl groups in 5 resulted in the product 12 which was devoid of activity, in spite of the availability of active sites. This result can be attributed to the loss of cross linking ability which is believed to be essential for alkylating drugs of type 1.

Experimental

Materials: All reagents were of the best quality commercially available, and were used without further purification. The cyclophosphamide (1) was prepared according to the literature procedure [11]. Solvents were dried using standard procedures [37].

For bioassay, the CD₂F₁ (CDF₁) mice were procured either from Charles River Breeding Laboratories, Inc., Wilmington, MA, or from Harlan Sprague Dawley, Inc., Indianapolis, IN.

Analytical procedures: The melting points were obtained with a Thomas Hoover Capillary Melting Point apparatus using a calibrated thermometer. The IR and NMR spectra were recorded on a Perkin-Elmer, 735 B, and on a Varian EM-360 L, 60 MHz spectrometer, respectively. Mass spectra were obtained on a Hewlett Packard Mass Spectrometer, model 5985 GS using a direct insertion probe, a source pressure of 2×10^{-7} torr, and methane as reactant gas for chemical ionization. Therefore, MS data are reported as (M⁺ + 1). In case of chloro compounds, all (M⁺ + 1) corresponding to isotopic Cl³⁵ and Cl³⁷ are reported in Table II. Their ratios of relative abundance is in agreement with the theory [38]. Microanalysis were performed either on an F & M Scientific Corporation Carbon, Hydrogen, Nitrogen Analyzer, model 185, or by the Atlantic Microlab, Inc., Atlanta, GA. Homogeneity of compounds was checked on pre-coated T.L.C. plastic sheets of either silica gel 60 F₂₅₄ or aluminium oxide 60 F₂₅₄, neutral (type E), layer thickness 0.2 mm, E. Merck Laboratories, Inc. The compounds were visualized by exposure of the T.L.C. plates to iodine vapor. For column chromatography either silica gel or neutral alumina, dry-column grade (activity III/20 mm), M. Woelm, Germany were used. Solvents were always removed in work-up procedures on a rotating evaporator at 30–35 °C/15–20 torr.

Bioassays: The compounds (1, 5, 6, 7, 9, 11, 12 and 27) were dispersed in tween "80" (0.1 ml) and diluted with water to the proper concentration. Male mice, CD₂F₁(CDF₁) of average weight of 18–21 g were injected intraperitoneally (i.p.) with diluted ascitic fluid (0.1 ml) containing lymphocytic leukemia P 388 cells (10⁶ cells). On the day following the tumor transplantation, the compound was injected i.p. in solution for 9 days. The mean survival time of the test animals (T) was compared with that of the control (C) [31] and the percentage of T/C was recorded (Table III).

Preparation of 3-amino-3-methylbutanoic acid (17)

A mixture of 3,3-dimethylacrylic acid, (18, 25 g, 0.25 mole) and concentrated aqueous ammonia (250 ml, 28–30% ammonia) was heated in a parr pressure apparatus at 150 °C and 700 psi for 16 h. After the reaction, the solution was filtered and concentrated to 100 ml on a rotating evaporator. After addition of barium hydroxide (8 g), the solution was heated on a steam bath for about 2 h, until no more ammonia was evolved. Sufficient sulfuric acid (1 N) was added to the mixture to completely precipitate all barium cations. The mixture was then filtered and the filtrate was concentrated on a rotating evaporator. The remain-

ing thick oil was triturated with ether, and the ether decanted. The semi-solid mass was recrystallized from anhydrous alcohol to give product **17** (12 g, 42%), m.p. 228–229 °C, lit. [32] m.p. 218 °C.

MS: $m/e = 118$ ($M^+ + 1$, 100).

$^1\text{H NMR}$: ($\text{D}_2\text{O}/\text{TSPa}$): $\delta = 1.35$ (s, 6H, 2 CH_3), 2.40 ppm (s, 2 H, CH_2).

^a 3-(Trimethylsilyl)propionic acid sodium salt

Preparation of 3-amino-3-methylbutanol (**14**)

To a stirred suspension of lithium aluminium hydride (3.8 g, 0.1 mole) in tetrahydrofuran (300 ml) was added **17** (8.2 g, 0.7 mole) portionwise over a period of 1 h. The reaction mixture was boiled for 5 h, and stirred for 36 h at room temperature. Ethyl acetate (10 ml) was then added to decompose the excess of hydride, and the intermediate complex was hydrolyzed with a solution of aqueous sodium hydroxide (6.5 ml, 15% w/v). The mixture was stirred with anhydrous sodium sulfate (10 g) for 15 min, filtered, and the filtrate was concentrated on a rotating evaporator. The remaining brownish thick oil was distilled to give **14** (5 g, 86%), b.p. 115–118 °C/24 torr, n_D^{23} 1.4502, lit. [33], b.p. 71 °C/9 torr, n_D 1.4496.

$\text{C}_5\text{H}_{13}\text{NO}$ (103.20)

Calcd	C 58.21	H 12.70	N 13.58,
Found	C 58.02	H 12.71	N 13.37.

IR (film): $\nu = 3500\text{--}3000\text{ cm}^{-1}$ (OH and NH).

MS: $m/e = 104$ ($M^+ + 1$, 100), 88(19), 58(24).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.25$ (s, 6H, 2 CH_3), 1.6 (t, 2 H, CH_2), 2.95 (broad s, 3 H, NH_2 , OH), 3.9 ppm (t, 2 H, CH_2O).

Preparation of ethyl 4-methyl-4-nitropentanoate (**22**)

Powdered potassium hydroxide (0.250 g) was added to a vigorously stirred solution of 2-nitropropane (**19**, 44.5 g, 0.5 mole) in anhydrous ethanol (100 ml). To this mixture was added dropwise at 60 °C a solution of ethyl acrylate (**20**, 50 g, 0.5 mole) in anhydrous ethanol (100 ml). The exothermic reaction was kept at 60–65 °C by an external cooling of the reaction flask. After the addition, the solution was boiled for 2 h, stirred for 16 h at room temperature, and filtered. The filtrate was concentrated on a rotating evaporator. The remaining oil was dissolved in ether (150 ml), and the solution first washed with aqueous hydrochloric acid (0.1 N, 2×40 ml) and then with water until the ether layer was free of acid. The ether layer was dried over anhydrous sodium sulfate (20 g), filtered, and the filtrate concentrated on a rotating evaporator. The remaining oil was distilled twice to give product **22** (81.8 g, 87%), b.p. 113.5–144.5 °C/9 torr, n_D^{23} 1.4369.

$\text{C}_8\text{H}_{15}\text{NO}_4$ (189.21)

Calcd	C 50.78	H 7.99	N 7.40,
Found	C 50.13	H 8.27	N 7.38.

IR (film): $\nu = 1723\text{ cm}^{-1}$ (C=O).

MS: $m/e = 190$ ($M^+ + 1$, 3), 143(100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.3$ (t, 3 H, CH_3), 1.7 (s, 6 H, 2 CH_3), 2.5 (s, 4 H, CH_2CH_2), 4.5 ppm (q, 2 H, CH_2O).

Preparation of 4-methyl-4-nitropentanol (**24**)

A solution of **22** (19 g, 0.1 mole) in tetrahydrofuran (150 ml) was added to a well stirred suspension of lithium aluminium hydride (3 g, 79 mmole) in tetrahydrofuran (200 ml) over a period of 20 min. After the addition, stirring was continued for 20 h at room temperature. The mixture was diluted with tetrahydrofuran (100 ml). The excess lithium aluminium hydride and the intermediate complex were carefully decomposed by successive additions of water (12 ml), and an aqueous solution of sodium hydroxide (3 ml, 15% w/v). The mixture was stirred with anhydrous sodium sulfate (25 g) for 15 min, filtered, and the filtrate concentrated on a rotating evaporator. The remaining crude pale yellow oil was distilled to give a colorless product, **24** (12.4 g, 84%), b.p. 84–86 °C/0.48 torr, n_D^{23} 1.4484.

$\text{C}_6\text{H}_{13}\text{NO}_3$ (147.17)

Calcd	C 48.96	H 8.90	N 9.52,
Found	C 48.52	H 9.36	N 9.45.

IR (film): $\nu = 3100\text{--}3600\text{ cm}^{-1}$ (OH).

MS: $m/e = 101$ ($M^+ - \text{NO}_2$, 78), 83(100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.7$ (s, 6 H, 2 CH_3), 2.0–2.4 (m, 4 H, CH_2CH_2), 3.9 ppm (t, 2 H, CH_2O).

Preparation of 4-amino-4-methylpentanol (**15**)

A solution of **24** (10.7 g, 72.8 mmole) in ethanol (95%, 150 ml) was hydrogenated in the presence of platinum dioxide (100 mg) at 110 °C and a hydrogen pressure of 200 psi for 16 h. The solution was filtered, and the filtrate concentrated on a rotating evaporator. The remaining oil was dissolved in hexane (15 ml). Cooling of the solution to 4–6 °C gave the crystalline product, **15** (7.1 g, 83%), m.p. 42–43 °C.

$\text{C}_6\text{H}_{15}\text{NO}$ (117.19)

Calcd	C 61.49	H 12.90	N 11.95,
Found	C 61.29	H 12.70	N 12.01.

IR (Nujol): $\nu = 3300$ (NH), 3250–3000 cm^{-1} (OH).

MS: $m/e = 118$ ($M^+ + 1$, 100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.25$ (s, 6 H, 2 CH_3), 1.5–1.8 (m, 4 H, CH_2CH_2), 3.0 (broad s, NH_2 , OH), 4.0 ppm (t, 2 H, CH_2O).

Preparation of 5-methyl-5-nitro-2-hexanone (**23**)

To a solution of 2-nitropropane (**19**, 22.3 g, 0.25 mole) and methyl vinyl ketone (**21**, 26.3 g,

0.37 mole) in methanol (125 ml) was added powdered potassium hydroxide (0.12 g). The solution was boiled for 3 h, and then concentrated on a rotating evaporator. The remaining oil was dissolved in ether (150 ml), and the solution first washed with aqueous hydrochloric acid (0.1 N, 3 × 40 ml), and then with water until the ether layer was free from acid. The ether layer was dried over anhydrous sodium sulfate (30 g), filtered, and the filtrate concentrated on a rotating evaporator. The remaining oil was distilled twice to give product **23** (29.4 g, 65%), b.p. 69–72 °C/0.11 torr, n_D^{25} 1.4451, lit. [39] b.p. 119 °C/11 torr, n_D^{25} 1.4447.

IR (film): $\nu = 1710 \text{ cm}^{-1}$ (C=O).

MS: $m/e = 113$ ($M^+ - \text{NO}_2$, 100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.6$ (s, 6 H, 2 CH_3), 2.1 (s, 3 H, CH_3), 2.2–2.6 ppm (m, 4 H, CH_2CH_2).

Preparation of 5-methyl-5-nitro-2-hexanol (**25**)

The procedure was essentially the same as that used for preparation of **24**. Compound **23** (7.8 g, 50 mmole) was reduced with lithium aluminum hydride (1.9 g, 50 mmole) in tetrahydrofuran (200 ml). After the reaction, the mixture was worked up as described for **24**. Analysis of the crude oil by T.L.C. on alumina (benzene:ether, 1:1, v/v) indicated the presence of three compounds. Therefore, the oil was dissolved in benzene (10 ml) and chromatographed through a column of neutral alumina (30 × 25 cm) using petroleum ether (100 ml), a mixture of benzene and petroleum ether (1:1, v/v, 100 ml), and finally ether (150 ml) as eluants. Concentration of the ether fraction on a rotating evaporator yielded pure product (**25**, 4.8 g, 61%), b.p. 79–81 °C/0.23 torr, n_D^{25} 1.4542.

$\text{C}_7\text{H}_{15}\text{NO}_3$ (161.20)

Calcd C 52.15 H 9.38 N 8.69,

Found C 51.78 H 9.94 N 8.59.

IR (film): $\nu = 3500\text{--}3000 \text{ cm}^{-1}$ (OH).

MS: $m/e = 115$ ($M^+ - \text{NO}_2$, 65), 97(100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.2$ (d, 3 H, CH_3), 1.4–1.6 (m, 2 H, CH_2), 1.7 (s, 6 H, 2 CH_3), 1.9–2.3 (m, 2 H, CH_2), 3.8–4.2 ppm (m, 1 H, CHO).

Preparation of 5-amino-5-methyl-2-hexanol (**16**)

The procedure was essentially the same as that for **15**. Thus, a solution of **25** (16.1 g, 0.1 mole) in ethanol (95%, 100 ml) was hydrogenated in the presence of platinum dioxide (200 mg) at 100 °C and a hydrogen pressure of 200 psi for 24 h. The solution was filtered, and the filtrate concentrated on a rotating evaporator. Distillation of the remaining crude oil gave **16** (12.6 g, 96%), b.p. 89–92 °C/9 torr, n_D^{25} 1.4544, lit. [39] b.p. 100 °C/12 torr, n_D^{25} 1.4512.

IR (film): $\nu = 3550\text{--}3000 \text{ cm}^{-1}$ (NH and OH).

MS: $m/e = 132$ ($M^+ + 1$, 35), 114(30), 97(100).

$^1\text{H NMR}$ (CDCl_3/TMS): $\delta = 1.1$ (s, 6 H, 2 CH_3), 1.15 (d, 3 H, CH_3), 1.3–1.6 (m, 4 H, CH_2CH_2), 2.65 (broad s, 3 H, NH_2 , OH), 3.5–3.9 ppm (m, 1 H, CHO).

Preparation of 2-[bis(2-chloroethyl)amino]-4,4-dimethyltetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (5), 2-[bis(2-chloroethyl)amino]-4,4-dimethylhexahydro-1,3,2-oxazaphosphepine 2-oxide (6), 2-[bis(2-chloroethyl)amino]-4,4,7-trimethyl-hexahydro-1,3,2-oxazaphosphepine 2-oxide (7) and 1-[bis(2-chloroethyl)amino]-hexahydro-1H, 3H-pyrido[1,2-c][1,3,2]oxazaphosphorine 1-oxide (9)

General Procedure: To a stirred solution of phosphoryl chloride (0.02 mol) in methylene chloride (50 ml) at 0–4 °C was added dropwise a solution of bis(2-chloroethyl)amine hydrochloride (0.02 mole) and triethylamine (0.04 mole) in methylene chloride (150 ml) over a period of 30 min. After the addition the reaction mixture was stirred for 2 h at room temperature, concentrated to 25 ml on a rotating evaporator, and then mixed with benzene (100 ml). The precipitated triethylamine hydrochloride (98%) was filtered off, and the filtrate containing the bis-(2-chloroethyl)phosphoramidic dichloride **13** [12] was used in the subsequent step without further purification.

A solution of the corresponding amino alcohol **14**, **15**, **16**, or **26** (0.02 mole) and triethylamine (0.04 mole) in tetrahydrofuran (100 ml) was added dropwise to the above solution of **13** at 0–4 °C. The reaction mixture was stirred for 20 h at room temperature. The precipitated triethylamine hydrochloride was filtered off, and the filtrate concentrated on a rotating evaporator. All compounds were then purified by column chromatography on silica gel (25 × 2.5 cm) using methylene chloride as eluant. Purity control of the products **5**, **6**, **7**, or **9** was performed by T.L.C. on silica gel (methylene chloride:methanol, 9:1, v/v). The yields of the products, and their physical properties are shown in Table II.

Preparation of N,N-bis(2-chloroethyl)-N',N'-(3-hydroxy-1,5-pentanediy)-phosphorodiamidic chloride (11)

To a stirred solution of **13**, obtained as described in the preceding experiment, was added at 0–4 °C a solution of 4-hydroxypiperidine (2.02 g, 0.02 mole) and triethylamine (2.02 g, 0.02 mole) in tetrahydrofuran (100 ml) over a period of 30 min. The reaction mixture was stirred for 20 h at room temperature. The precipitated triethylamine hydrochloride was filtered off, and the filtrate concentrated on a rotating evaporator. The remaining oil was dissolved

in methylene chloride (8 ml), and chromatographed through a silica gel column (25 × 2.5 cm) using methylene chloride as eluant. The eluate was concentrated on a rotating evaporator. Analysis of the remaining oil by T. L. C. on silica gel (methylene chloride:acetone, 7:3, v/v) indicated one component. The yield and physical properties of the product **11** are shown in Table II.

Preparation of 9-(2-chloroethyl)-5,5-dimethyl-2-oxa-6,9-diaza-1-phosphabicyclo[4.3.0]nonane 1-oxide (12)

A 57% suspension of sodium hydride in oil (500 mg) was freed from oil by mixing it with benzene (3 × 5 ml), and decanting the supernatant liquid. The sodium hydride was then mixed with **5** (1.44 g, 5 mmole) in benzene (50 ml) and the reaction mixture stirred for 24 h at room temperature. The mixture was filtered, and the filtrate concentrated on a rotating evaporator to a semi-solid mass. The product was dissolved in methylene chloride (10 ml) and chromatographed through a silica gel column (20 × 2.5 cm) using methylene chloride as eluant. Removal of solvent on a rotating evaporator gave **12**. The yield and physical properties of **12** are shown in Table II.

Preparation of N,N-bis[2-chloroethyl]-N',N'-(3-hydroxy-1,5-pentanediy)-N''-propylphosphoric triamide (27)

A solution of **11** (9.69 g, 0.03 mole) and *n*-propylamine (5.31 g, 0.09 mole) in methylene chloride (60 ml) was boiled for 20 h. The solution was concentrated on a rotating evaporator to an oil. The oil was mixed with tetrahydrofuran (60 ml), and the precipitated *n*-propylamine hydrochloride was filtered off. The filtrate was concentrated on a rotating evaporator to a volume of 25 ml, and then chromatographed through a silica gel column (28 × 2.5 cm) using methylene chloride as eluant. Subsequent evaporation of the solvent on a rotating evaporator gave the oily product. The yield and physical properties of **27** are shown in Table II.

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