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Synthesis and Antitumor Activity of Preactivated Isophosphamide Analogues Bearing Modified Alkylating Functionalities¹

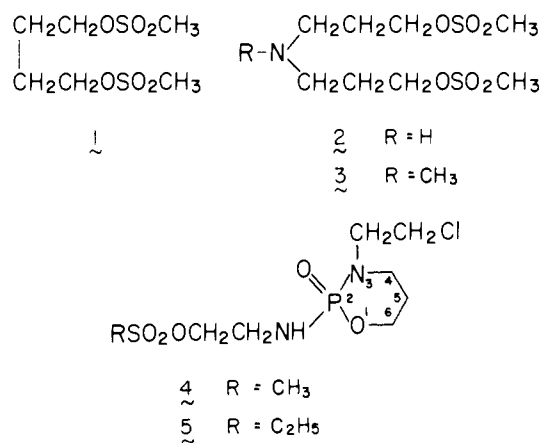
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In search of cancer chemotherapeutic agents with greater efficacy than cyclophosphamide, 4-hydroperoxyisophosphamide analogues bearing modified alkylating functionalities such as 2-bromoethyl, 2-iodoethyl, 2-methylsulfonyloxyethyl, and 2-ethylsulfonyloxyethyl groups were prepared by ozonolytic cyclization reaction of N,N'-substituted 3-butenyl phosphorodiamidates. Comparative cytotoxicity against L1210 cells and antileukemic life-span activity against L1210 implanted BDF₁ mice of the newly synthesized compounds were tabulated. The 4-hydroperoxyisophosphamide analogues which have different alkylating groups in a molecule showed slightly greater cytotoxicity in vitro than those with the same alkylating groups. Most of the compounds having different alkylating groups also showed high antileukemic activity in vivo. Among them, the highest efficacy was found for 2-[N-methyl-N-(2-chloroethyl)]amino-3-(2-methylsulfonyloxyethyl)-4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxide (NSC 280122D) whose life-span activity was also greater than that of 4-hydroperoxyisophosphamide, cyclophosphamide, and isophosphamide. The superiority of this compound was especially apparent by oral administration.

Busulfan (1)² is a representative antitumor alkyl-sulfonate having potential alkylating activity. However, in comparison with nitrogen mustard, which is representative of antitumor alkylating agents bearing 2-chloroethylamino groups and is effective against various kinds of experimental tumors, busulfan exerts only limited activity against Walker-256 carcinosarcoma and is practically ineffective against L1210 leukemia and other animal tumors. In 1964, Sakurai and El-Merzabani³ synthesized nitrogen-containing methanesulfonates such as 2 and 3 which are structurally related to nitrogen mustard and found that these compounds showed increased activity against some tumors which were unaffected by busulfan.^{4,5} In 1974, Brock and his co-workers^{6,7} reported on the antitumor activities of a number of cyclophosphamide and isophosphamide analogues bearing alkylsulfonyloxyalkyl and chloroethyl groups. Most of the compounds reported by Brock et al. showed considerable activities against Yoshida ascitic sarcoma in rats, Walker-256 carcinosarcoma in rats, and L1210 leukemia in mice, and they concluded that isophosphamide analogues bearing mixed alkylating functions such as 4 and 5 were especially effective. Recently, we^{8,9} synthesized C₄-oxidized cyclophosphamide and isophosphamide derivatives and found that C₄-hydroperoxylation was as effective as C₄-hydroxylation for activating these drugs and that the C₄-hydroperoxides showed greater stability than the corresponding C₄-hydroxy derivatives. The action mechanisms of the compounds reported by Brock et al. are thought to resemble those for isophosphamide (and cyclophosphamide), i.e., the antitumor effect might be exerted after in vivo C₄-oxidation of the 1,3,2-oxazaphosphorinane ring.¹⁰ Therefore, the C₄-hydroperoxy derivative of 4 and 5 might also exert increased activity. We now wish

Chart I



to report on the synthesis and antileukemic activity of C₄-hydroperoxyisophosphamide analogues bearing mixed alkylating functions related to compounds 4 and 5 (Chart I).

The ozonolysis reaction of 3-butenyl phosphorodiamidate, which is a general synthetic method for preparing C₄-functionalized 1,3,2-oxazaphosphorinane 2-oxides and related phosphorus-containing heterocyclics,^{8,9,11,12} was also used for the present syntheses. A variety of 3-butenyl phosphorodiamidates bearing different alkylating substituents at the phosphorodiamidic nitrogen atoms were prepared via three routes (see Scheme I). Route a consisted of reaction of phosphoryl halide 6 (POX₃, X = Cl or Br) with 3-buten-1-ol followed by treatment with the corresponding N-substituted 2-haloethylamine salt (XCH₂CH₂NHR·HX, X = Cl or Br) in the presence of

Table I. N,N'-Substituted 3-Butenyl Phosphorodiamidates (9a-m)

Compd	X	Y	R	Formula	Analyses	Route	Overall yield, %
9a	Cl	OSO ₂ CH ₃	CH ₃	C ₁₀ H ₂₂ N ₂ O ₅ PSCl	C, H, N, P, Cl	a	70
9b	Cl	OSO ₂ C ₂ H ₅	CH ₃	C ₁₁ H ₂₄ N ₂ O ₅ PSCl	C, H, N, P, Cl	b	35 ^f
9c	Cl	OSO ₂ CH ₃	C ₂ H ₅	C ₁₁ H ₂₄ N ₂ O ₅ PSCl	C, H, N, Cl; P ^a	a	65
9d	Cl	OSO ₂ CH ₃	CH ₂ CH ₂ Cl	C ₁₁ H ₂₃ N ₂ O ₅ PSCl ₂	C, H, N, P, Cl	a	67
9e	OSO ₂ CH ₃	Cl	CH ₃	C ₁₀ H ₂₂ N ₂ O ₅ PSCl	C, H; N ^b	b	40 ^f
9f	OSO ₂ CH ₃	Cl	C ₂ H ₅	C ₁₁ H ₂₄ N ₂ O ₅ PSCl	C, H, N	b	43 ^f
9g	Cl	Br	CH ₃	C ₉ H ₁₉ N ₂ O ₂ PClBr	C, H, N, Cl, Br; P ^c	a	65
9h	Cl	I	CH ₃	C ₉ H ₁₉ N ₂ O ₂ PClI	C, H, N	a	64
9i	Br	Cl	CH ₃	C ₉ H ₁₉ N ₂ O ₂ PClBr	C, H, N, P, Cl, Br	a	65
9j	Br	OSO ₂ CH ₃	CH ₃	C ₁₀ H ₂₂ N ₂ O ₅ PSBr	C, H, N; P, ^d Br ^e	a	40 ^f
9k	Cl	OSO ₂ CH ₃	H	C ₉ H ₂₀ N ₂ O ₅ PSCl	C, H, N, P, Cl	a	30 ^f
9l	OSO ₂ CH ₃	OSO ₂ CH ₃	H	C ₁₀ H ₂₃ N ₂ O ₈ PS ₂	C, H, N, P	c	45 ^f
9m	Br	Br	H	C ₈ H ₁₇ N ₂ O ₂ PBr ₂	C, H, N, P, Br	c	55 ^f

^a P: calcd, 8.54; found, 8.03. ^b N: calcd, 8.03; found, 8.48. ^c P: calcd, 9.28; found, 10.01. ^d P: calcd, 7.88; found, 7.38. ^e Br: calcd, 20.32; found, 19.87. ^f Yield of the product purified by column chromatography of the crude product with silica gel and acetone-chloroform (1:1). Other products were used for the ozonolysis reaction without chromatographic purification.

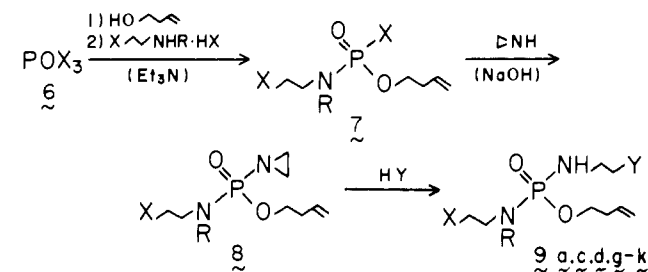
triethylamine; then the resulting 3-butenyl phosphoramidoalate 7 was treated with ethylenimine in the presence of aqueous sodium hydroxide to give 3-butenyl N-2-haloethyl-N',N'- ethylenephosphorodiamidate 8 which was finally treated with acid (HY: HCl, HBr, HI, or methanesulfonic acid), yielding the corresponding N,N'-substituted 3-butenyl phosphorodiamidates. By this route, 9a,c,d,g-k were prepared with satisfactory purity in overall yields as shown in Table I, and most of these products could be used for the ozonolysis reaction without chromatographic purification. Route b consisted of reaction of 3-butenyl phosphoramidochloridate 10, which was produced by a procedure similar to that in route a, with N-substituted 2-ethanolamine (HOCH₂CH₂NHR') in the presence of triethylamine followed by sulfonylation of the resulting alcohol 11 with sulfonyl chloride and pyridine, giving 9b,e,f in reasonable yields after chromatographic purification (Table I). Route c, which was used to prepare 9l and 9m, consisted of reaction of phosphoryl chloride with 3-buten-1-ol followed by treatment with ethylenimine in the presence of sodium hydroxide. The resulting bis(aziridinyl) phosphorodiamidate 12 was cleaved by acid (HY: methanesulfonic acid or HBr) to give the corresponding 3-butenyl phosphorodiamidates in the overall yields shown in Table I.

In the previous synthesis of 4-hydroperoxyisophosphamide, we obtained a small amount of a by-product into which a molecule of acetone was incorporated when the ozonolysis of an appropriate 3-butenyl phosphorodiamidate was carried out in aqueous acetone.^{9b,13} Ozonolysis of 9a-m was therefore carried out in 1:1 aqueous tetrahydrofuran to avoid formation of such a by-product; then the ozonized solutions were treated with 30% hydrogen peroxide. After the reaction mixture had stood for 72 h at 3 °C, tetrahydrofuran was removed by evaporation in vacuo and the remaining aqueous layer was extracted with chloroform. The crystalline cyclic hydroperoxides listed in Table II were isolated from the chloroform extract after concentration in vacuo and crystallization of the resulting oily residue by trituration in ether-acetone. The oily products shown in Table II were purified by column chromatography of the crude oil obtained from the chloroform extract.

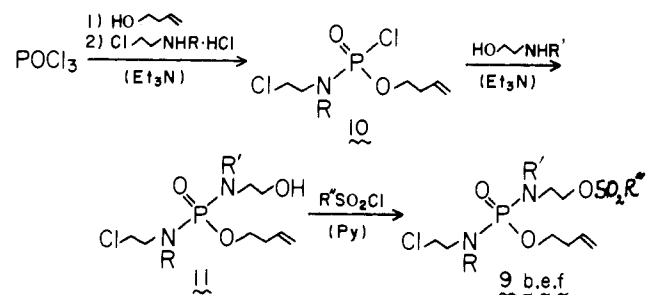
In the case of the ozonolytic cyclization reaction producing 4-hydroperoxyisophosphamide, we^{9b,13} obtained

Scheme I

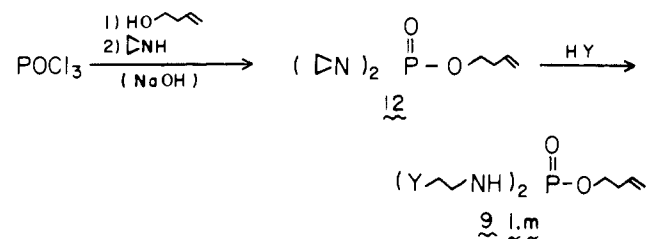
Route a



Route b



Route c



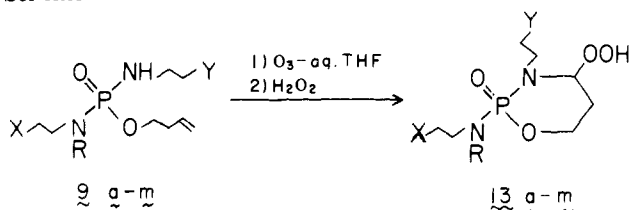
two stereoisomers having cis and trans configurations of C₄-OOH and P=O groups,¹⁴ whereas only a cis isomer has been isolated in a similar synthesis of 4-hydroperoxy-cyclophosphamide.^{8b,15} Although all of the products listed in Table II were homogeneous according to thin-layer chromatography (TLC) with silica gel in ethyl acetate or in a mixture of various ratios of acetone and chloroform, the possibility that they were a mixture of stereoisomers could not be ruled out at least for the oily products. The following experiments indeed confirmed the formation of

Table II. 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m)

Compd	X	Y	R	Formula	Analyses	Yield, %	Mp, °C
13a	Cl	OSO ₂ CH ₃	CH ₃	C ₉ H ₂₀ N ₂ O ₇ PSCl	C, H, N, P, Cl	42	119-121 dec ^g
13b	Cl	OSO ₂ C ₂ H ₅	CH ₃	C ₁₀ H ₂₂ N ₂ O ₇ PSCl	C, H, N, P, Cl	30	103-105 dec ^g
13c	Cl	OSO ₂ CH ₃	C ₂ H ₅	C ₁₀ H ₂₂ N ₂ O ₇ PSCl	C, H, N; P, ^a Cl ^b	30 ^f	Oil
13d	Cl	OSO ₂ CH ₃	CH ₂ CH ₂ Cl	C ₁₀ H ₂₁ N ₂ O ₇ PSCl ₂	C, H, N	25 ^f	Oil
13e	OSO ₂ CH ₃	Cl	CH ₃	C ₉ H ₂₀ N ₂ O ₇ PSCl	C, H, N	32 ^f	Oil
13f	OSO ₂ CH ₃	Cl	C ₂ H ₅	C ₁₀ H ₂₂ N ₂ O ₇ PSCl	C, H; N ^c	28 ^f	Oil
13g	Cl	Br	CH ₃	C ₈ H ₁₇ N ₂ O ₄ PCLBr	C, H, N, P, Cl, Br	39	101-102 dec ^g
13h	Cl	I	CH ₃	C ₈ H ₁₇ N ₂ O ₄ PClI	C, H, N, P, Cl, I	5 ^f	96-97 dec ^g
13i	Br	Cl	CH ₃	C ₈ H ₁₇ N ₂ O ₄ PCLBr	C, H, N, P, Cl, Br	34	97-98 dec ^g
13j	Br	OSO ₂ CH ₃	CH ₃	C ₉ H ₂₀ N ₂ O ₇ PSBr	H, N, Br; C ^d	27 ^f	108-109 dec ^g
13k	Cl	OSO ₂ CH ₃	H	C ₈ H ₁₈ N ₂ O ₇ PSCl	C, H, N, P, Cl	18 ^f	Oil
13l	OSO ₂ CH ₃	OSO ₂ CH ₃	H	C ₉ H ₂₁ N ₂ O ₁₀ PS ₂	C, H; N ^e	29 ^f	Oil
13m	Br	Br	H	C ₇ H ₁₅ N ₂ O ₄ PBr ₂	C, H, N, P, Br	37	98-99 dec ^g

^a P: calcd, 8.13; found, 8.60. ^b Cl: calcd, 9.31; found, 8.85. ^c N: calcd, 7.36; found, 6.89. ^d C: calcd, 26.29; found, 26.77. ^e N: calcd, 6.79; found, 6.31. ^f After purification by column chromatography with silica gel and acetone-chloroform (1:1). ^g Recrystallized from acetone-ether.

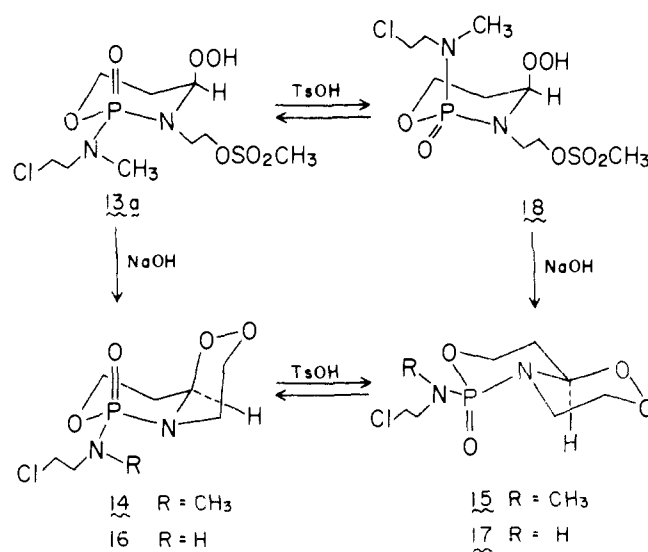
Scheme II



a small amount of a possible stereoisomer in the ozonolysis of **9a** (Scheme II). When the isolated crystalline product **13a** was treated with 5% aqueous sodium hydroxide in chloroform, a bicyclic peroxide **14** was obtained quantitatively, whereas alkali treatment of the crude ozonolysis product gave **14** and an isomeric product **15** which were separated in the 14:15 ratio of approximately 5:1 after column chromatography with silica gel in acetone-chloroform (1:1). As found for the corresponding bicyclic isomers **16** and **17**, which were produced from 4-hydroperoxyisophosphamide and its stereoisomer 2-*epi*-4-hydroperoxyisophosphamide, respectively,¹⁶ these products **14** and **15** were also interconvertible by the action of *p*-toluenesulfonic acid (TsOH) at room temperature, giving an equilibrium mixture with the 14:15 ratio of 3:4. The 60-MHz nuclear magnetic resonance (NMR) spectra in CDCl₃ solution of these isomers showed signals corresponding to an angular methine proton (C₄-H) at δ 5.40 ppm [J (P, H) = 21.8 Hz] for **14** and δ 5.48 ppm [J (P, H) = 8.9 Hz] for **15**, which are almost comparable to those for **16** and **17**, respectively.^{13,16} These results indicate that the major ozonolysis product **13a** has the same stereochemistry as 4-hydroperoxyisophosphamide and that the possible stereoisomer **18** has an inverted configuration at the phosphorus atom as proposed for 2-*epi*-4-hydroperoxyisophosphamide.¹⁴ The stereoisomer **18** was also produced by the TsOH-catalyzed isomerization reaction of **13a** in almost the same ratio as that produced by the ozonolysis of **9a**, which was also confirmed by being converted into **15** by alkali treatment (Scheme III).

In the case of the ozonolysis of **9k**, **13k** was isolated as a 1:1 stereoisomeric mixture after repeated column chromatography of the crude ozonolysis mixture. This product, **13k**, quantitatively afforded a crystalline lactam **19** on treatment with ferrous sulfate, while alkali treatment

Scheme III



converted it into an approximately 1:1 mixture of **16** and **17**, which was separated and identified by comparison of their NMR spectra with those of authentic specimens.^{9b} Another cyclization product, **20**, and its stereoisomer could not be separated from **13k**, but their formation was suggested because an isomeric lactam **21** was obtained besides **19** when the crude ozonolysis mixture was treated with ferrous sulfate.

The ozonolysis of **9h** gave an iodo-substituted cyclic hydroperoxide **13h** in a crystalline state (Scheme IV). Although satisfactory NMR and analytical data were obtained for **13h**, this compound was more unstable than the other cyclic hydroperoxides, rapidly turning into a brown resinous material on standing at room temperature; therefore, bioassay experiments were omitted. Table III gives characteristic NMR data for the synthesized 4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxide derivatives.

Comparative *in vitro* cytotoxicities of the 4-hydroperoxyisophosphamide analogues **13a-m** against L1210 cells are listed in Table IV. As apparent from Table IV, **13d** was the most toxic and all the compounds having mixed alkylating groups (**13a-k**) were more toxic than 4-hydroperoxyisophosphamide and those having the same

Table III. 60-MHz Proton NMR Data for 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m)^a

Compd	Solvent	δ (C ₄ -H), ppm	J (P, C ₄ -H), Hz	δ (NCH ₃), ppm	J (P, NCH ₃), Hz	δ (OSO ₂ CH ₃), ppm
13a	Me ₂ SO- <i>d</i> ₆	4.97	20.2	2.58	9.9	3.17
13b	CDCl ₃	5.08	21.8	2.75	10.0	
13c	CDCl ₃	5.05	21.7			3.07
13d	CDCl ₃	5.02	18.0			3.07
13e	CDCl ₃	5.03	22.0	2.70	10.0	3.08
13f	CDCl ₃	5.01	21.8			3.07
13g	Me ₂ SO- <i>d</i> ₆	4.99	19.9	2.58	10.0	
13h	CDCl ₃	5.00	18.6	2.75	10.0	
13i	Me ₂ SO- <i>d</i> ₆	4.98	19.9	2.60	10.0	
13j	Me ₂ SO- <i>d</i> ₆	4.99	20.0	2.56	10.0	3.19
13k	CDCl ₃	5.04	19.6			3.11
13l	CDCl ₃	4.97	20.0			3.29
13m	Me ₂ SO- <i>d</i> ₆	5.04	20.0			

^a Determined at 28 °C with the probe concentration at approximately 10% (v/w) for each sample, and tetramethylsilane was used as an internal standard.

Table IV. Comparative in Vitro Cytotoxicity of 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m) and 4-Hydroperoxyisophosphamide against L1210 Cells

	13a	13b	13c	13d	13e	13f	13g	13i	13j	13k	13l	13m	4-Hydroperoxyisophosphamide
ED ₅₀ , ^a μg/mL	0.8	1.0	0.5	0.06	0.5	0.8	0.5	0.3	1.0	0.8	3.9	2.0	1.8

^a Cell number was counted 72 h after addition of the drug to the cell culture.

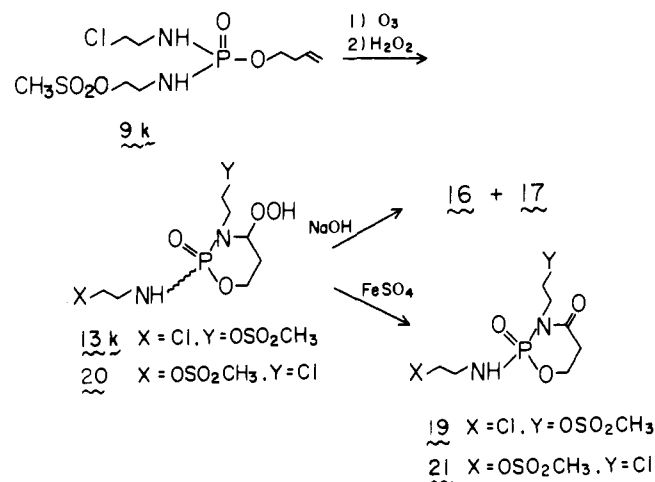
Table V. Comparative Antileukemic Life-Span Activity of 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m) and 4-Hydroperoxyisophosphamide against L1210 BDF₁ Mice^a

	13a	13b	13c	13d	13e	13f	13g	13i	13j	13k	13l	13m	4-Hydroperoxyisophosphamide
ILS ₃₀ , ^b mg/kg	2	5	6	10	3	10	7	6	3	2	25	6	15
ILS _{max} , ^c mg/kg	80	70	70	80 ^d	60	80	35	70	60	75	50	70	100
ILS _{max} /ILS ₃₀	40	14	12	8	20	8	5	12	20	38	2	12	7

^a BDF₁ mice were inoculated (ip) with 10⁵ L1210 cells and the drugs were administered (iv) 24 h after cell inoculation. Eight mice were used for each experiment. ^b Dose required for 30% increase of life span over control. ^c Maximum dose required for survival over 30 days of all the tested mice. ^d One of the eight mice died within 30 days.

alkylating groups such as 13l and 13m. Comparative in vivo antileukemic life-span activities against L1210 leukemia in BDF₁ mice inoculated with 10⁵ cells are given in Table V in which the minimum effective dose for a 30% increase of life span over the control (ILS₃₀), the maximum tolerant dose for the maximum increase of life span (ILS_{max}), and their ratio (ILS_{max}/ILS₃₀) are given. The ILS₃₀ and ILS_{max} values were estimated from the dose-response curve, as described in an earlier paper,¹³ by plotting the ILS value vs. the logarithmic scale of the dosages at 5, 10, 25, 50, 100, and 200 mg/kg iv administration of the drugs using eight mice for each experiment. At the ILS_{max} dose, all compounds except 13d allowed survival over 30 days of all the tested mice. In the case of 13d, as suggested by its high cytotoxicity (Table IV), toxic symptom was observed with the ILS_{max} dose (80 mg/kg) at which one of the eight mice died within 30 days. Note that 13l was the least effective according to the ILS_{max}/ILS₃₀ ratio, but the compounds having mixed alkylating groups were superior to 13l and some of them were also superior to 4-hydroperoxyisophosphamide. Comparison of the ILS₃₀ and ILS_{max} values of 13a and 13b suggests that an *N*-methyl substituent at the exocyclic nitrogen atom causes essentially no effect. This result is interesting in contrast to the case of 4-hydroperoxyisophosphamide whose antileukemic activity slightly decreased on *N*-methylation.^{9b} On the contrary, the biologic activity of 4-hydroperoxycyclophosphamide markedly decreased on *N*-methylation of its ring nitrogen atom.^{17,18} Among the compounds listed in Table V, 2-[*N*-methyl-*N*-(2-chloroethyl)]amino-3-(2-methylsulfonyloxyethyl)-

Scheme IV



4-hydroperoxy-1,3,2-oxazaphosphorinane 2-oxide (NSC 280122D) (13a) was especially effective as suggested by its having the greatest ILS_{max}/ILS₃₀ ratio. Although 13a showed somewhat increased host toxicity compared with 4-hydroperoxyisophosphamide (Table VI), it also showed high life-span activity against a larger inoculum of L1210 cells (10⁷) in BDF₁ mice (Table VII). As shown in Table VII, the number of mice which survived over 30 days was largest for 13a among the listed compounds at an optimal dose (50 mg/kg) by ip administration. Greater efficacy of 13a is further revealed in Table VIII in which com-

Table VI. Comparative Acute Toxicity of 13a and 4-Hydroperoxyisophosphamide

	Route	13a	4-Hydroperoxyisophosphamide
Mouse ^a	iv	120	220
LD ₅₀ , mg/kg	ip	134	220
	po	284	1000
Rat ^b	iv	66	250
LD ₅₀ , mg/kg	ip	41	250
	po	238	800

^a Ten BDF₁ mice (male) were used for each experiment.^b Ten Wistar rats (female) were used for each experiment.

parative complete cure-dose indexes of 13a are compared with those of 4-hydroperoxyisophosphamide, cyclophosphamide, and isophosphamide for different administration routes. The C_{\min} and C_{\max} values, respectively, indicate the minimum effective and maximum tolerant doses for 60-day survival (complete cure) of all the tested mice; they were similarly estimated by the dose-response curve cited above. As apparent in Table VIII, the cure-dose range ($C_{\max} - C_{\min}$) of 13a is wider than that of 4-hydroperoxyisophosphamide for every route (iv, ip, and po), and particularly interesting is the superiority of 13a by oral administration as clearly demonstrated by its having the largest $C_{\max} - C_{\min}$ and C_{\max}/C_{\min} values among the listed compounds. Further evaluations of the anti-tumor effects of this compound against various kinds of

experimental tumors are now in progress.

Experimental Section

Melting points were determined in open glass capillary tubes in a silicone oil bath using a Yamato MP-1 apparatus and are uncorrected. Proton NMR data were determined with a Varian Model A-60 spectrometer with tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on precoated silica gel plates (Merck, Kieselgel F-254, 0.25 mm). Column chromatography was carried out using silica gel (Merck, Kieselgel 60). Where analyses are indicated only by element symbols, the analytical results were within $\pm 0.4\%$ of the theoretical values. 3-Buten-1-ol and 2-chloroethylamine hydrochloride were purchased from Chemical Samples Co., Ltd., and Aldrich Chemical Co., Inc., respectively. Phosphorus oxybromide and *N,N*-bis(2-chloroethyl)amine hydrochloride were purchased from Tokyo Kasei Chemical Industries, Ltd. *N*-Methyl-*N*-(2-chloroethyl)amine and *N*-ethyl-*N*-(2-chloroethyl)amine hydrochlorides were prepared by chlorination of the corresponding 2-ethanolamines with SOCl_2 according to the usual procedure. 2-Bromoethylamine and *N*-methyl-*N*-(2-bromoethyl)amine hydrobromides were obtained from the corresponding 2-ethanolamines by heating in concentrated hydrobromic acid. Satisfactory NMR data were obtained for all the 3-butenyl phosphorodiamidates 9a-m.

General Procedures for the Preparation of 3-Butenyl Phosphorodiamidates (9a-m). **Route a.** To a mechanically stirred solution of POX_3 ($X = \text{Cl}$ or Br) (100 mmol) in CH_2Cl_2 (100 mL) was added dropwise a solution of 3-buten-1-ol (7.2 g, 100 mmol) for 30 min at $-10 \pm 1^\circ\text{C}$; then the mixture was stirred for 3 h at $-5 \pm 1^\circ\text{C}$. Next, the reaction mixture was cooled to

Table VII. Comparative Antileukemic Life-Span Activity of 13a, 4-Hydroperoxyisophosphamide, Cyclophosphamide, and Isophosphamide against BDF₁ Mice with 10^7 L1210 Cell Inoculum (ip)

Compd	Dose, ^a mg/kg	No. of mice	Mean survival, days	ILS, ^b %	Survivors over 30 days
13a	0	10	4.6 \pm 0.16	0	0
	2	8	6.6 \pm 0.18	44	0
	10	8	11.4 \pm 0.80	147	0
	50	8	> 26.1 \pm 2.54	> 468	6
	100	8	> 15.8 \pm 3.62	> 242	2
4-Hydroperoxyisophosphamide	0	10	4.6 \pm 0.16	0	0
	20	8	8.9 \pm 0.23	93	0
	50	8	12.5 \pm 0.38	172	0
	100	8	> 23.8 \pm 2.87	> 416	4
	200	8	> 26.5 \pm 1.51	> 476	4
Cyclophosphamide	0	10	4.6 \pm 0.16	0	0
	50	8	7.8 \pm 0.25	68	0
	100	8	12.4 \pm 0.32	169	0
	200	8	> 18.9 \pm 1.91	> 310	1
	400	8	> 17.6 \pm 2.80	> 283	1
Isophosphamide	0	10	4.6 \pm 0.16	0	0
	70	8	8.8 \pm 0.45	90	0
	130	8	11.9 \pm 0.35	158	0
	330	8	> 24.3 \pm 1.98	> 427	3
	530	8	> 16.5 \pm 3.88	> 251	1

^a The drugs were administered (ip) 24 h after the cell inoculation. ^b Increase of life span in dying animals over control.Table VIII. Comparative Complete Cure-Dose Indexes of 13a, 4-Hydroperoxyisophosphamide, Cyclophosphamide, and Isophosphamide against L1210 BDF₁ Mice^a

Compd	Route	C_{\min} , mg/kg ^b	C_{\max} , mg/kg ^c	$C_{\max} - C_{\min}$	C_{\max}/C_{\min}
13a	iv	15	100	85	6.6
	ip	10	100	90	10.0
	po	50	200	150	4.0
4-Hydroperoxyisophosphamide	iv	90	160	70	1.8
	ip	30	70	40	2.3
	po	400 ^d	400 ^d	0	1.0
Cyclophosphamide	iv	200	200	0	1.0
	ip	100	200	100	2.0
	po	200	200	0	1.0
Isophosphamide	iv	280	320	40	1.1
	po	250	250	0	1.0

^a BDF₁ mice were inoculated (ip) with 10^5 L1210 cells and the drugs were administered 24 h after cell inoculation. Eight mice were used for each experiment. ^b Minimum effective dose required for 60-day survival of all the tested mice. ^c Maximum tolerant dose required for 60-day survival of all the tested mice. ^d One of the eight mice died within 60 days.

-35 to -40 °C, CH₂Cl₂ (100 mL) and N-substituted 2-haloethylamine halide (XCH₂CH₂NHR·HX, X = Cl or Br) (100 mmol) were added, and then triethylamine (Et₃N) (50.5 g, 500 mmol) was added dropwise to the stirred reaction mixture, which was maintained at -35 to -40 °C. After addition of Et₃N was completed, stirring was continued for 2 h at -35 ± 5 °C. Then the precipitated triethylammonium halide was removed by filtration and the filtrate was washed with cold H₂O (100 mL × 2) and added dropwise to a vigorously stirred solution of an excess amount of ethylenimine (10 g) in 5% aqueous NaOH (100 mL) for 1 h in an ice-water bath. After additional stirring for 1 h at 3 ± 1 °C, the CH₂Cl₂ layer was separated from the alkali layer, then washed with water (100 mL × 2), dried over anhydrous Na₂SO₄, and finally concentrated in vacuo to give the corresponding crude 3-butenyl phosphoramidoylaziridine 8 (X = Cl or Br) as an oily residue which gave satisfactory NMR data. [This product, 8, generally showed a typical doublet due to the aziridine ring protons in CDCl₃ solution: R = CH₃, X = Cl, δ 2.13 ppm [*J* (P, H) = 15.6 Hz]; R = CH₃, X = Br, δ 2.13 ppm [*J* (P, H) = 16.0 Hz]; R = C₂H₅, X = Cl, δ 2.15 ppm [*J* (P, H) = 15.8 Hz]; R = H, X = Cl, δ 2.15 ppm [*J* (P, H) = 16.0 Hz].] Product yields were 85–90% of the theoretical amount. To a magnetically stirred solution (CH₂Cl₂, 100 mL) of 8 was added dropwise an aqueous solution of 1% HX (X = Cl or I) or a solution of 5% CH₃SO₃H in CH₂Cl₂ at -20 ± 5 °C, while the reaction mixture was monitored by TLC [CHCl₃-Me₂CO (1:1)]. When the spot of 8 (*R_f* 0.35–0.37) disappeared, addition of the acid was stopped and the mixture was washed with H₂O (100 mL × 2). The CH₂Cl₂ layer was dried over anhydrous Na₂SO₄ and then concentrated in vacuo to give the corresponding 3-butenyl phosphorodiamidates 9a,c,d,g-k as an oily residue. Their overall yields starting from POX₃ are given in Table I. Before the ozonolysis reaction, 9j,k were purified by column chromatography in CHCl₃-Me₂CO (1:1), but all other products were ozonized without further purification. All the analytical samples were purified by column chromatography [CHCl₃-Me₂CO (1:1)].

Route b. POCl₃ (15.3 g, 100 mmol), 3-buten-1-ol (7.2 g, 100 mmol), and N-substituted 2-chloroethylamine hydrochloride (ClCH₂CH₂NHR·HCl, R = H or CH₃) (100 mmol) were allowed to react in CH₂Cl₂ (200 mL) as in route a. Next, a mixture of 2-ethanolamine (HOCH₂CH₂NHR', R' = H, CH₃, or C₂H₅) (100 mmol) and Et₃N (10.1 g, 100 mmol) in CH₂Cl₂ (50 mL) was added dropwise with mechanical stirring over 30 min at -5 ± 1 °C. Next, the reaction mixture was stirred for 1 h at room temperature and then filtered. The filtrate was washed with cold H₂O (100 mL × 1), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give the corresponding crude product 11 as an oil. This product, 11, was dissolved in a mixture of pyridine (20 mL) and CHCl₃ (50 mL); then a solution of sulfonyl chloride (R''SO₂Cl, R'' = CH₃ or C₂H₅) (100 mmol) in CHCl₃ (20 mL) was added dropwise to the mechanically stirred solution over 30 min at -20 ± 5 °C. After being stirred for 2 h at -10 ± 5 °C, the reaction mixture was allowed to stand overnight at room temperature; then the mixture was concentrated in vacuo and the resulting residue was dissolved in CHCl₃ (100 mL) and washed with H₂O (100 mL × 2), dried over anhydrous Na₂SO₄, and concentrated in vacuo, giving the corresponding crude sulfonates 9b,e,f as an oily brown residue. The crude products were purified by column chromatography [CHCl₃-Me₂CO (1:1)] to give the pure products in the overall yields shown in Table I.

Route c. POCl₃ (15.3 g, 100 mmol) and 3-buten-1-ol (7.2 g, 100 mmol) were allowed to react in CH₂Cl₂ (100 mL) as in route a. The reaction mixture was then added dropwise to a vigorously stirred solution of ethylenimine (20 mL) in 5% aqueous NaOH (100 mL) with cooling in an ice-water bath. After the addition was completed (40 min), the reaction mixture was stirred for 1 h at room temperature; then the CH₂Cl₂ layer was separated, washed with water (100 mL × 2), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give bis(aziridiny)-3-butenyl phosphorodiamidate (12) as an oily residue which was purified by column chromatography (acetone) to obtain a pure product (14 g, 69%) [NMR (CDCl₃) δ 2.20 ppm [*J* (P, H) = 15.2 Hz]]. The pure product 12 (14 g) was dissolved in CH₂Cl₂ (100 mL); then a solution of 5% CH₃SO₃H in CH₂Cl₂ or 1% aqueous HBr was added dropwise to the magnetically stirred solution of 12 with cooling in an ice-water bath. The reaction mixture was monitored

by TLC [CHCl₃-Me₂CO (1:1)] and the addition of acid was stopped when the spot of 12 (*R_f* 0.40) disappeared. The reaction mixture was then washed with H₂O (100 mL × 2), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give the corresponding products, 9l,m, as a crude oil which was purified by column chromatography (Me₂CO) to yield the pure product in the overall yields shown in Table I.

4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m). General Procedure. Into a magnetically stirred solution of 3-butenyl phosphorodiamidates (9a-m) (10 mmol) in aqueous THF [H₂O-THF (1:1)] (40 mL), O₃ (720 mg, 15 mmol) was bubbled at a rate of approximately 50 mg/min for 14–15 min with cooling in an ice-water bath. Next, 30% H₂O₂ (3 mL) was added to the ozonized solution. After the solution had stood for 72 h at 3 °C, THF was evaporated in vacuo below 40 °C and the remaining aqueous layer was extracted with CHCl₃ (20 mL × 3). The combined CHCl₃ extract was washed with H₂O (50 mL × 1), dried over anhydrous Na₂SO₄, and concentrated in vacuo below 30 °C to give a colorless oily residue. In the cases of the cyclic hydroperoxides 13a,b,g-i,m, they were crystallizable by triturating the crude oil in Et₂O-Me₂CO (10:1) and then recrystallized from Me₂CO-Et₂O (1:1). Other products were purified by column chromatography [CHCl₃-Me₂CO (1:1)]. Table II gives the yields of the purified products.

Isomeric Bicyclic Peroxides, 6-[N-Methyl-N-(2-chloroethyl)aminoperhydro[1,2,4]dioxazino[4,3-c][1,3,2]oxazaphosphorine 6-Oxide (14) and Its Isomer 15. 9a (3.49 g, 10 mmol) was ozonized and the ozonized solution was treated with 30% H₂O₂ according to the general procedure described above. The CHCl₃ layer (60 mL) extracted from the concentrated aqueous ozonized solution was added to 5% aqueous NaOH solution (30 mL) and the mixture was vigorously stirred for 30 min in an ice-water bath. Next, the CHCl₃ layer was separated, washed with water (30 mL × 2), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give an oily residue, which according to TLC [CHCl₃-Me₂CO (1:1)] contained two components [*R_f* 0.35 (major) and *R_f* 0.31 (minor)] in the ratio of approximately 5:1. Column chromatography [CHCl₃-Me₂CO (3:2)] of the oily mixture was used to isolate the major product 14 as the faster migrating component in the form of a crystalline solid (1.22 g, 45%) which was recrystallized from Et₂O-Me₂CO (2:1) giving colorless prisms: mp 71–72 °C; NMR (CDCl₃) δ 2.72 [d, 3 H, *J* (P, H) = 9.7 Hz, NCH₃], 5.40 [d of dd, 1 H, *J* (P, H) = 21.8 Hz, *J* (H, H) = 4.1 Hz, *J'* (H, H) = 2.9 Hz, >CH_{ax}-O-O]. Anal. (C₈H₁₆N₂O₄PCl) C, H, N, P, Cl. The minor stereoisomer 15 was eluted as the slower migrating component in the form of a colorless oil (0.27 g, 10%): NMR (CDCl₃) δ 2.79 [d, 3 H, *J* (P, H) = 10.0 Hz, NCH₃], 5.48 [d of t, 1 H, *J* (P, H) = 8.9 Hz, *J* (H, H) = 5.6 Hz, >CH_{ax}-O-O]. Anal. (C₈H₁₆N₂O₄PCl) C, H, N, P, Cl. When the crystalline product of 13a (367 mg, 10 mmol) was similarly treated with 5% aqueous NaOH solution (10 mL) in CHCl₃ (20 mL), 14 was quantitatively obtained (257 mg, 95%) from the CHCl₃ layer after evaporation in vacuo.

Experiments on the TsOH-Catalyzed Isomerization of 13a, 14, and 15. (a) To a magnetically stirred solution of 13a (367 mg, 1 mmol) in CHCl₃ (20 mL) was added *p*-toluenesulfonic acid (TsOH) monohydrate (19 mg, 0.1 mmol) and the mixture was stirred overnight at room temperature. Next, an aqueous 5% NaOH solution (10 mL) was added to the solution and the mixture was vigorously stirred for 30 min in an ice-water bath. The CHCl₃ layer was separated, washed with H₂O, dried over anhydrous Na₂SO₄, and concentrated in vacuo, and the resulting oily residue was chromatographed [CHCl₃-Me₂CO (3:2)] to give 14 (176 mg, 65%) and 15 (41 mg, 15%) which were identified with authentic specimens^{9b} by comparison of their NMR spectra in CDCl₃ solution.

(b) To a solution of 14 (27 mg, 0.1 mmol) in CHCl₃ (5 mL) was added a small amount of TsOH·H₂O (ca. 2 mg, 0.01 mmol), and the mixture was allowed to stand overnight at room temperature. A mixture of 15 (27 mg, 0.1 mmol) and TsOH·H₂O (ca. 2 mg, 0.01 mmol) in CHCl₃ (5 mL) was also allowed to stand overnight at room temperature. The TLC patterns of these mixtures in CHCl₃-Me₂CO (1:1) were identical, giving two spots of 14 (*R_f* 0.35) and 15 (*R_f* 0.31) in the ratio of approximately 5:1.

Isolation of the Stereoisomeric Mixture 13k Produced from the Ozonolysis of 9k. 9k (3.35 g, 10 mmol) was ozonized

and the ozonized solution was treated with 30% H₂O₂ (3 mL) according to the general procedure. After evaporation of THF in vacuo, the remaining aqueous layer was extracted with CHCl₃ (20 mL × 3). The combined CHCl₃ extract was washed with H₂O (40 mL × 1), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give an oily residue (2.5 g). The residue was chromatographed on a column (4.5 × 10 cm) eluted with CHCl₃-Me₂CO (1:1). TLC [CHCl₃-Me₂CO (1:1)] was used to monitor and separate the eluted fractions into three parts. The first part contained unidentified peroxidic oily material (250 mg) which gave a negative Epstein test.¹⁹ The second part gave a colorless oil (480 mg) whose TLC [CHCl₃-Me₂CO (1:1)] showed two partly overlapped spots giving a positive Epstein test at approximately R_f 0.24 and 0.22 with almost equal intensity. The third part gave a colorless oil (1.3 g) which contained mainly the R_f 0.22 component and a small amount of the R_f 0.24 component. The second part was further chromatographed on a column (2 × 6 cm) eluted with CHCl₃-Me₂CO (2:1), but the two components could not be completely separated. The NMR spectrum (CDCl₃ solution) of this mixture showed an unresolved broad peak at δ 3.10 ppm due to the protons of the methanesulfonyl group and complex multiplets corresponding to the C₄ proton at δ 4.90–5.35 ppm. The third part was chromatographed twice on columns (2.5 × 7 cm) eluted with CHCl₃-Me₂CO (1:1) and the major component of R_f 0.22 (**13k**) was completely separated from the minor component as a colorless oil (600 mg, 18%) whose NMR spectrum showed a sharp singlet at δ 3.11 ppm due to the SO₂CH₃ protons (see Table III).

Formation of Bicyclic Peroxides 16 and 17 from 13k. To a magnetically stirred solution of **13k** (350 mg, 1 mmol) in CHCl₃ (20 mL) was added a 5% aqueous NaOH solution (10 mL), and the mixture was vigorously stirred for 30 min at room temperature. The CHCl₃ layer was separated, washed with H₂O (10 mL × 2), dried over anhydrous Na₂SO₄, and finally concentrated in vacuo to give an oily residue. The oil was chromatographed on a column (2.5 × 8 cm) eluted with CHCl₃-Me₂CO (1:1) to give **16** [mp 127–129 °C. Anal. (C₇H₁₄N₂O₄PCl) C, H, N, P, Cl (80 mg)] as a faster migrating product and **17** [mp 103–105 °C. Anal. (C₇H₁₄N₂O₄PCl) C, H, N, P, Cl (90 mg)] as a slower migrating isomer. The NMR spectra of these products in CDCl₃ solution were identical with those of the authentic specimens.^{9b}

Formation of Lactams 19 and 21 from the Ozonolysis Products of 9k. (a) To a stirred solution of **13k** (175 mg, 0.5 mmol) in CHCl₃ (20 mL) was added an aqueous solution of FeSO₄·7H₂O (280 mg, 1 mmol) (10 mL). After the mixture had been vigorously stirred for 30 min at room temperature, the CHCl₃ layer was washed with H₂O (10 mL × 1), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give **19** as a colorless oil (116 mg, 70%) which solidified on standing overnight at -20 °C. Recrystallization of the solid from EtOAc afforded colorless prisms: mp 112–113 °C; NMR (CDCl₃) δ 2.63–2.92 (m, 2 H, -COCH₂-), 3.08 (s, 3 H, SO₂CH₃), 3.25–4.00 (m, 7 H, ClCH₂C-H₂NH, NCH₂), 4.18–4.53 (m, 4 H, -CH₂OSO₂, -CH₂OPO); IR ν_{max} (Nujol) 1687 (CO), 3220 cm⁻¹ (NH). Anal. (C₈H₁₆N₂O₆PSCl) C, H, N, P, Cl.

(b) An oil (200 mg) obtained from the second part of the fractions eluted by column chromatography [CHCl₃-Me₂CO (1:1)] of the crude ozonolysis products of **9k** was dissolved in CHCl₃ (10 mL). Next, an aqueous solution of FeSO₄·7H₂O (280 mg, 1 mmol) (10 mL) was added to the solution. After the mixture had

been vigorously stirred for 30 min at room temperature, the CHCl₃ layer was washed with H₂O (10 mL × 1), dried over anhydrous Na₂SO₄, and concentrated in vacuo, giving an oily residue (160 mg) whose TLC [CHCl₃-Me₂CO (3:2)] indicated the presence of two major products with R_f 0.31 and 0.28. The R_f of the slower migrating product was identical with that of the lactam **19**. The oily residue was chromatographed twice on columns (1.5 × 5 cm) eluted with CHCl₃-Me₂CO (3:2), giving the lactam **21** as a colorless oil (45 mg): NMR (CDCl₃) δ 2.61–2.91 (m, 2 H, -COCH₂-), 3.10 (s, 3 H, SO₂CH₃), 3.25–4.10 (m, 7 H, ClCH₂CH₂NH, NCH₂), 4.16–4.52 (m, 4 H, -CH₂OSO₂, CH₂OPO); IR ν_{max} (film) 1689 (CO), 3220 cm⁻¹ (NH). Anal. (C₈H₁₆N₂O₆PSCl) C, H, N, P, Cl.

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