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Synthesis and Antitumor Activity of Preactivated Isophosphamide Analogues Bearing Modified Alkylating Functionalities[1]

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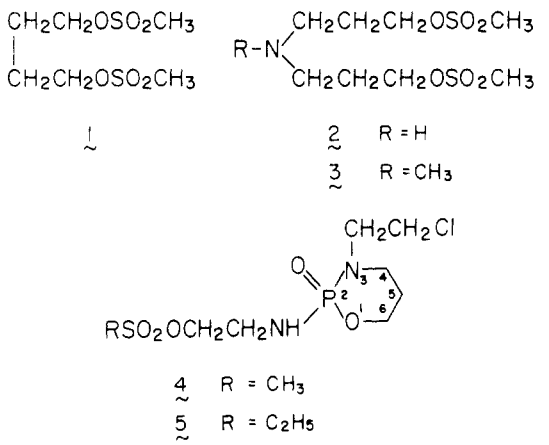
In search of cancer chemotherapeutic agents with greater efficacy than cyclophosphamide, 4-hydroperoxyisophosphamide analogues bearing modified alkylating functionalities such as 2-bromoethyl, 2-iodoethyl, 2-methylsulfonyloxyethyl, and 2-ethylsulfonyloxyethyl groups were prepared by ozonolytic cyclization reaction of N,N′-substituted 3-butenyl phosphorodiamidates. Comparative cytotoxicity against L1210 cells and antileukemic life-span activity against L1210 implanted BDF_1 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 alkylsulfonate 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-Merzabani[3] 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 C_4-oxidized cyclophosphamide and isophosphamide derivatives and found that C_4-hydroperoxylation was as effective as C_4-hydroxylation for activating these drugs and that the C_4-hydroperoxides showed greater stability than the corresponding C_4-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 C_4-oxidation of the 1,3,2-oxazaphosphorinane ring.[10] Therefore, the C_4-hydroperoxy derivative of **4** and **5** might also exert increased activity. We now wish to report on the synthesis and antileukemic activity of C_4-hydroperoxyisophosphamide analogues bearing mixed alkylating functions related to compounds **4** and **5** (Chart I).

Chart I



The ozonolysis reaction of 3-butenyl phosphorodiamidate, which is a general synthetic method for preparing C_4-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** (POX_3, X = Cl or Br) with 3-buten-1-ol followed by treatment with the corresponding N-substituted 2-haloethylamine salt ($XCH_2CH_2NHR \cdot HX$, X = Cl or Br) in the presence of

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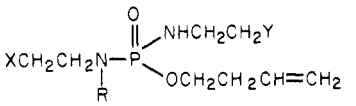
Table I. N,N′-Substituted 3-Butenyl Phosphorodiamidates (9a–m)



Compd	X	Y	R	Formula	Analyses	Route	Overall yield, %
9a	Cl	OSO_2CH_3	CH_3	$C_{10}H_{22}N_2O_5PSCl$	C, H, N, P, Cl	a	70
9b	Cl	$OSO_2C_2H_5$	CH_3	$C_{11}H_{24}N_2O_5PSCl$	C, H, N, P, Cl	b	35[f]
9c	Cl	OSO_2CH_3	C_2H_5	$C_{11}H_{24}N_2O_5PSCl$	C, H, N, Cl; P[a]	a	65
9d	Cl	OSO_2CH_3	CH_2CH_2Cl	$C_{11}H_{23}N_2O_5PSCl_2$	C, H, N, P, Cl	a	67
9e	OSO_2CH_3	Cl	CH_3	$C_{10}H_{22}N_2O_5PSCl$	C, H; N[b]	b	40[f]
9f	OSO_2CH_3	Cl	C_2H_5	$C_{11}H_{24}N_2O_5PSCl$	C, H, N	b	43[f]
9g	Cl	Br	CH_3	$C_9H_{19}N_2O_2PClBr$	C, H, N, Cl, Br; P[c]	a	65
9h	Cl	I	CH_3	$C_9H_{19}N_2O_2PClI$	C, H, N	a	64
9i	Br	Cl	CH_3	$C_9H_{19}N_2O_2PClBr$	C, H, N, P, Cl, Br	a	65
9j	Br	OSO_2CH_3	CH_3	$C_{10}H_{22}N_2O_5PSBr$	C, H, N; P,[d] Br[e]	a	40[f]
9k	Cl	OSO_2CH_3	H	$C_9H_{20}N_2O_5PSCl$	C, H, N, P, Cl	a	30[f]
9l	OSO_2CH_3	OSO_2CH_3	H	$C_{10}H_{23}N_2O_8PS_2$	C, H, N, P	c	45[f]
9m	Br	Br	H	$C_8H_{17}N_2O_2PBr_2$	C, H, N, P, Br	c	55[f]

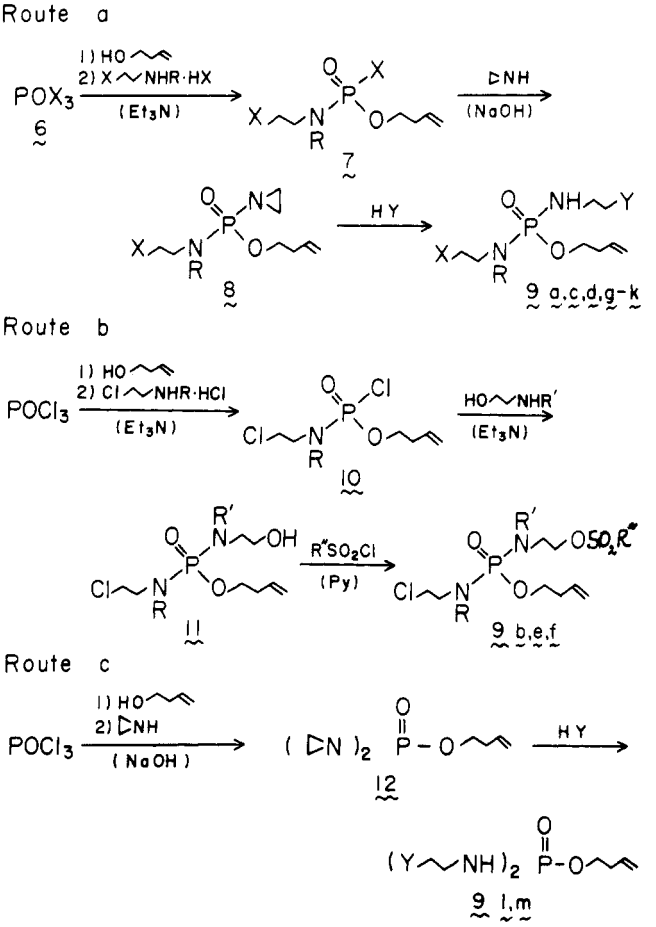
[a] P: calcd, 8.54; found, 8.03. [b] N: calcd, 8.03; found, 8.48. [c] P: calcd, 9.28; found, 10.01. [d] P: calcd, 7.88; found, 7.38. [e] Br: calcd, 20.32; found, 19.87. [f] Yield of the product purified by column chromatography of the crude product with silica gel and acetone–chloroform (1:1). Other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

triethylamine; then the resulting 3-butenyl phosphoramidohaloate **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 ($HOCH_2CH_2NHR'$) 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.

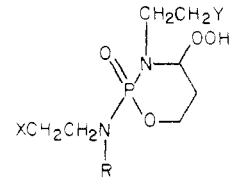
In the case of the ozonolytic cyclization reaction producing 4-hydroperoxyisophosphamide, we [9b,13] obtained

Scheme I



two stereoisomers having cis and trans configurations of C_4-OOH and P=O groups,[14] whereas only a cis isomer has been isolated in a similar synthesis of 4-hydroperoxycyclophosphamide.[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

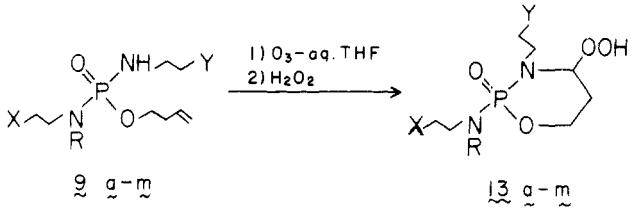
Table II. 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m)



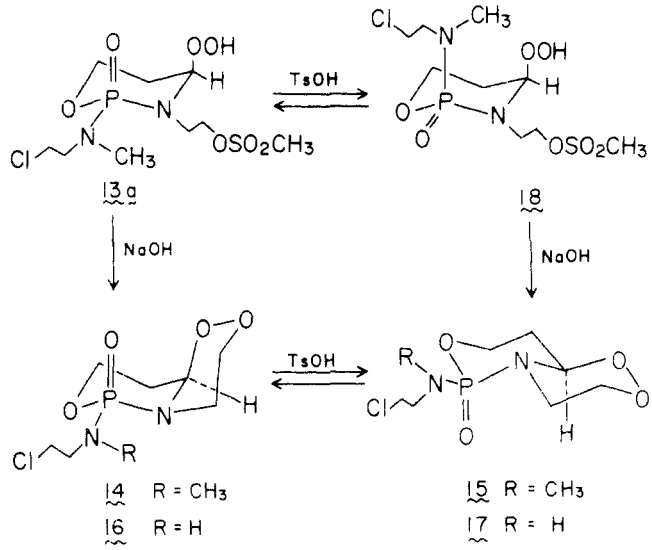
Compd	X	Y	R	Formula	Analyses	Yield, %	Mp, °C
13a	Cl	OSO_2CH_3	CH_3	$C_9H_{20}N_2O_7PSCl$	C, H, N, P, Cl	42	119-121 dec[g]
13b	Cl	$OSO_2C_2H_5$	CH_3	$C_{10}H_{22}N_2O_7PSCl$	C, H, N, P, Cl	30	103-105 dec[g]
13c	Cl	OSO_2CH_3	C_2H_5	$C_{10}H_{22}N_2O_7PSCl$	C, H, N; P,[a] Cl[b]	30[f]	Oil
13d	Cl	OSO_2CH_3	CH_2CH_2Cl	$C_{10}H_{21}N_2O_7PSCl_2$	C, H, N	25[f]	Oil
13e	OSO_2CH_3	Cl	CH_3	$C_9H_{20}N_2O_7PSCl$	C, H, N	32[f]	Oil
13f	OSO_2CH_3	Cl	C_2H_5	$C_{10}H_{22}N_2O_7PSCl$	C, H; N[c]	28[f]	Oil
13g	Cl	Br	CH_3	$C_8H_{17}N_2O_4PClBr$	C, H, N, P, Cl, Br	39	101-102 dec[g]
13h	Cl	I	CH_3	$C_8H_{17}N_2O_4PClI$	C, H, N, P, Cl, I	5[f]	96-97 dec[g]
13i	Br	Cl	CH_3	$C_8H_{17}N_2O_4PClBr$	C, H, N, P, Cl, Br	34	97-98 dec[g]
13j	Br	OSO_2CH_3	CH_3	$C_9H_{20}N_2O_7PSBr$	H, N, Br; C[d]	27[f]	108-109 dec[g]
13k	Cl	OSO_2CH_3	H	$C_8H_{18}N_2O_7PSCl$	C, H, N, P, Cl	18[f]	Oil
13l	OSO_2CH_3	OSO_2CH_3	H	$C_9H_{21}N_2O_{10}PS_2$	C, H; N[e]	29[f]	Oil
13m	Br	Br	H	$C_7H_{15}N_2O_4PBr_2$	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



Scheme III



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 $CDCl_3$ solution of these isomers showed signals corresponding to an angular methine proton (C_4-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 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_2CH_3), ppm
13a	Me_2SO-d_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_2SO-d_6	4.99	19.9	2.58	10.0	
13h	$CDCl_3$	5.00	18.6	2.75	10.0	
13i	Me_2SO-d_6	4.98	19.9	2.60	10.0	
13j	Me_2SO-d_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_2SO-d_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-Hydroperoxy-isophosphamide
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.

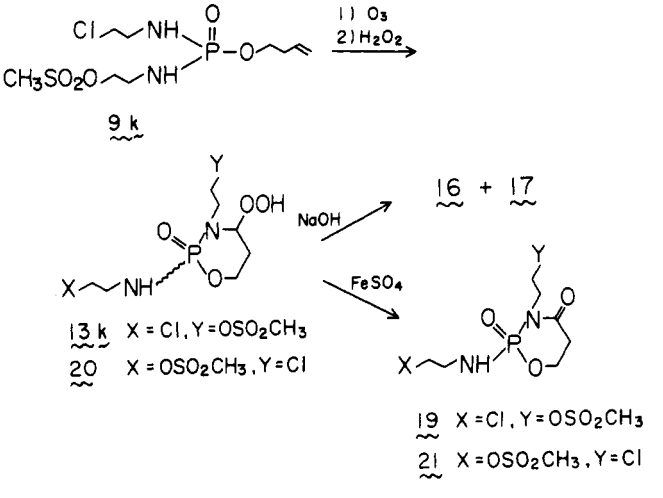
Table V. Comparative Antileukemic Life-Span Activity of 4-Hydroperoxy-1,3,2-oxazaphosphorinane 2-Oxides (13a-m) and 4-Hydroperoxyisophosphamide against L1210 BDF_1 Mice[a]

	13a	**13b**	**13c**	**13d**	**13e**	**13f**	**13g**	**13i**	**13j**	**13k**	**13l**	**13m**	4-Hydroperoxy-isophosphamide
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] BDF_1 mice were inoculated (ip) with 10^5 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 BDF_1 mice inoculated with 10^5 cells are given in Table V in which the minimum effective dose for a 30% increase of life span over the control (ILS_{30}), the maximum tolerant dose for the maximum increase of life span (ILS_{max}), and their ratio (ILS_{max}/ILS_{30}) are given. The ILS_{30} 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}/ILS_{30} 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 ILS_{30} 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}/ILS_{30} 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 (10^7) in BDF_1 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-Hydroperoxy-isophosphamide
Mouse[a]	iv	120	220
LD_{100},	ip	134	220
mg/kg	po	284	1000
Rat[b]	iv	66	250
LD_{100},	ip	41	250
mg/kg	po	238	800

[a] Ten BDF_1 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 antitumor 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 ±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 = 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 ± 1 °C; then the mixture was stirred for 3 h at −5 ± 1 °C. Next, the reaction mixture was cooled to

Table VII. Comparative Antileukemic Life-Span Activity of **13a**, 4-Hydroperoxyisophosphamide, Cyclophosphamide, and Isophosphamide against BDF_1 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 ± 0.16	0	0
	2	8	6.6 ± 0.18	44	0
	10	8	11.4 ± 0.80	147	0
	50	8	>26.1 ± 2.54	>468	6
	100	8	>15.8 ± 3.62	>242	2
4-Hydroperoxy-isophosphamide	0	10	4.6 ± 0.16	0	0
	20	8	8.9 ± 0.23	93	0
	50	8	12.5 ± 0.38	172	0
	100	8	>23.8 ± 2.87	>416	4
	200	8	>26.5 ± 1.51	>476	4
Cyclophosphamide	0	10	4.6 ± 0.16	0	0
	50	8	7.8 ± 0.25	68	0
	100	8	12.4 ± 0.32	169	0
	200	8	>18.9 ± 1.91	>310	1
	400	8	>17.6 ± 2.80	>283	1
Isophosphamide	0	10	4.6 ± 0.16	0	0
	70	8	8.8 ± 0.45	90	0
	130	8	11.9 ± 0.35	158	0
	330	8	>24.3 ± 1.98	>427	3
	530	8	>16.5 ± 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 BDF_1 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-Hydroperoxy-isophosphamide	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] BDF_1 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, CH_2Cl_2 (100 mL) and N-substituted 2-haloethylamine halide ($XCH_2CH_2NHR \cdot HX$, X = Cl or Br) (100 mmol) were added, and then triethylamine (Et_3N) (50.5 g, 500 mmol) was added dropwise to the stirred reaction mixture, which was maintained at −35 to −40 °C. After addition of Et_3N 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 H_2O (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 CH_2Cl_2 layer was separated from the alkali layer, then washed with water (100 mL × 2), dried over anhydrous Na_2SO_4, 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 $CDCl_3$ solution: R = CH_3, X = Cl, δ 2.13 ppm [J (P, H) = 15.6 Hz]; R = CH_3, X = Br, δ 2.13 ppm [J (P, H) = 16.0 Hz]; R = C_2H_5, 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 (CH_2Cl_2, 100 mL) of **8** was added dropwise an aqueous solution of 1% HX (X = Cl or I) or a solution of 5% CH_3SO_3H in CH_2Cl_2 at −20 ± 5 °C, while the reaction mixture was monitored by TLC [$CHCl_3$–Me_2CO (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 H_2O (100 mL × 2). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 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 POX_3 are given in Table I. Before the ozonolysis reaction, **9j,k** were purified by column chromatography in $CHCl_3$–Me_2CO (1:1), but all other products were ozonized without further purification. All the analytical samples were purified by column chromatography [$CHCl_3$–Me_2CO (1:1)].

Route b. $POCl_3$ (15.3 g, 100 mmol), 3-buten-1-ol (7.2 g, 100 mmol), and N-substituted 2-chloroethylamine hydrochloride ($ClCH_2CH_2NHR \cdot HCl$, R = H or CH_3) (100 mmol) were allowed to react in CH_2Cl_2 (200 mL) as in route a. Next, a mixture of 2-ethanolamine ($HOCH_2CH_2NHR'$, R′ = H, CH_3, or C_2H_5) (100 mmol) and Et_3N (10.1 g, 100 mmol) in CH_2Cl_2 (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 H_2O (100 mL × 1), dried over anhydrous Na_2SO_4, 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 $CHCl_3$ (50 mL); then a solution of sulfonyl chloride ($R''SO_2Cl$, R″ = CH_3 or C_2H_5) (100 mmol) in $CHCl_3$ (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 $CHCl_3$ (100 mL) and washed with H_2O (100 mL × 2), dried over anhydrous Na_2SO_4, 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 [$CHCl_3$–Me_2CO (1:1)] to give the pure products in the overall yields shown in Table I.

Route c. $POCl_3$ (15.3 g, 100 mmol) and 3-buten-1-ol (7.2 g, 100 mmol) were allowed to react in CH_2Cl_2 (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 CH_2Cl_2 layer was separated, washed with water (100 mL × 2), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give bis(aziridinyl)-3-butenyl phosphorodiamidate (**12**) as an oily residue which was purified by column chromatography (acetone) to obtain a pure product (14 g, 69%) [NMR ($CDCl_3$) δ 2.20 ppm [J (P, H) = 15.2 Hz]]. The pure product **12** (14 g) was dissolved in CH_2Cl_2 (100 mL); then a solution of 5% CH_3SO_3H in CH_2Cl_2 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 [$CHCl_3$–Me_2CO (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 H_2O (100 mL × 2), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the corresponding products, **9l,m**, as a crude oil which was purified by column chromatography (Me_2CO) 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 [H_2O–THF (1:1)] (40 mL), O_3 (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% H_2O_2 (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 $CHCl_3$ (20 mL × 3). The combined $CHCl_3$ extract was washed with H_2O (50 mL × 1), dried over anhydrous Na_2SO_4, 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 Et_2O–Me_2CO (10:1) and then recrystallized from Me_2CO–Et_2O (1:1). Other products were purified by column chromatography [$CHCl_3$–Me_2CO (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% H_2O_2 according to the general procedure described above. The $CHCl_3$ 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 $CHCl_3$ layer was separated, washed with water (30 mL × 2), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give an oily residue, which according to TLC [$CHCl_3$–Me_2CO (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 [$CHCl_3$–Me_2CO (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 Et_2O–Me_2CO (2:1) giving colorless prisms: mp 71–72 °C; NMR ($CDCl_3$) δ 2.72 [d, 3 H, J (P, H) = 9.7 Hz, NCH_3], 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, $>CH_{eq}$–O–O]. Anal. ($C_8H_{16}N_2O_4PCl$) 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 ($CDCl_3$) δ 2.79 [d, 3 H, J (P, H) = 10.0 Hz, NCH_3], 5.48 [d of t, 1 H, J (P, H) = 8.9 Hz, J (H, H) = 5.6 Hz, $>CH_{ax}$–O–O]. Anal. ($C_8H_{16}N_2O_4PCl$) 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 $CHCl_3$ (20 mL), **14** was quantitatively obtained (257 mg, 95%) from the $CHCl_3$ 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 $CHCl_3$ (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 $CHCl_3$ layer was separated, washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated in vacuo, and the resulting oily residue was chromatographed [$CHCl_3$–Me_2CO (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 $CDCl_3$ solution.

(b) To a solution of **14** (27 mg, 0.1 mmol) in $CHCl_3$ (5 mL) was added a small amount of $TsOH \cdot H_2O$ (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 $TsOH \cdot H_2O$ (ca. 2 mg, 0.01 mmol) in $CHCl_3$ (5 mL) was also allowed to stand overnight at room temperature. The TLC patterns of these mixtures in $CHCl_3$–Me_2CO (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% H_2O_2 (3 mL) according to the general procedure. After evaporation of THF in vacuo, the remaining aqueous layer was extracted with $CHCl_3$ (20 mL × 3). The combined $CHCl_3$ extract was washed with H_2O (40 mL × 1), dried over anhydrous Na_2SO_4, 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 $CHCl_3$–Me_2CO (1:1). TLC [$CHCl_3$–Me_2CO (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 [$CHCl_3$–Me_2CO (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 $CHCl_3$–Me_2CO (2:1), but the two components could not be completely separated. The NMR spectrum ($CDCl_3$ 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 C_4 proton at δ 4.90–5.35 ppm. The third part was chromatographed twice on columns (2.5 × 7 cm) eluted with $CHCl_3$–Me_2CO (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 SO_2CH_3 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 $CHCl_3$ (20 mL) was added a 5% aqueous NaOH solution (10 mL), and the mixture was vigorously stirred for 30 min a room temperature. The $CHCl_3$ layer was separated, washed with H_2O (10 mL × 2), dried over anhydrous Na_2SO_4, and finally concentrated in vacuo to give an oily residue. The oil was chromatographed on a column (2.5 × 8 cm) eluted with $CHCl_3$–Me_2CO (1:1) to give **16** [mp 127–129 °C. Anal. ($C_7H_{14}N_2O_4PCl$) C, H, N, P, Cl (80 mg)] as a faster migrating product and **17** [mp 103–105 °C. Anal. ($C_7H_{14}N_2O_4PCl$) C, H, N, P, Cl (90 mg)] as a slower migrating isomer. The NMR spectra of these products in $CDCl_3$ 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 $CHCl_3$ (20 mL) was added an aqueous solution of $FeSO_4·7H_2O$ (280 mg, 1 mmol) (10 mL). After the mixture had been vigorously stirred for 30 min at room temperature, the $CHCl_3$ layer was washed with H_2O (10 mL × 1), dried over anhydrous Na_2SO_4, 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 ($CDCl_3$) δ 2.63–2.92 (m, 2 H, $-COCH_2-$), 3.08 (s, 3 H, SO_2CH_3), 3.25–4.00 (m, 7 H, $ClCH_2CH_2NH$, NCH_2), 4.18–4.53 (m, 4 H, $-CH_2OSO_2$, $-CH_2OPO$); IR ν_{max} (Nujol) 1687 (CO), 3220 cm^{-1} (NH). Anal. ($C_8H_{16}N_2O_6PSCl$) C, H, N, P, Cl.

(b) An oil (200 mg) obtained from the second part of the fractions eluted by column chromatography [$CHCl_3$–Me_2CO (1:1)] of the crude ozonolysis products of **9k** was dissolved in $CHCl_3$ (10 mL). Next, an aqueous solution of $FeSO_4·7H_2O$ (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 $CHCl_3$ layer was washed with H_2O (10 mL × 1), dried over anhydrous Na_2SO_4, and concentrated in vacuo, giving an oily residue (160 mg) whose TLC [$CHCl_3$–Me_2CO (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 $CHCl_3$–Me_2CO (3:2), giving the lactam **21** as a colorless oil (45 mg): NMR ($CDCl_3$) δ 2.61–2.91 (m, 2 H, $-COCH_2-$), 3.10 (s, 3 H, SO_2CH_3), 3.25–4.10 (m, 7 H, $ClCH_2CH_2NH$, NCH_2), 4.16–4.52 (m, 4 H, $-CH_2OSO_2$, CH_2OPO); IR ν_{max} (film) 1689 (CO), 3220 cm^{-1} (NH). Anal. ($C_8H_{16}N_2O_6PSCl$) C, H, N, P, Cl.

Acknowledgment. The authors are greatly indebted to Dr. K. Tori and his co-workers for discussions and measurements of NMR spectra.

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