Synthesis and Antitumor Activity of Analogues of Ifosfamide Modified in the *N*-(2-Chloroethyl) Group

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A series of 3-(2-chloroethyl)-*N*-(2-X-ethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxides with various X substituents have been prepared by cyclization of racemic ifosfamide or its enantiomers with sodium hydride and subsequent treatment of intermediary products with hydrobromic acid, diethyl hydrogen phosphate, dibenzyl hydrogen phosphate, *p*-toluenesulfonic acid, and acetic acid. All of these compounds were tested in vivo against L 1210 lymphoid leukemia in mice. Only bromo analogue **13** and its enantiomers were effective, exceeding the activity of racemic ifosfamide and cyclophosphamide. The therapeutic index of the racemic **13** and its levorotatory enantiomer was about 1.7 times higher than that for ifosfamide and about 2.7 times higher than that for cyclophosphamide.

Ifosfamide [IF, **1**, *N*,3-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide], like isomeric cyclophosphamide [CP, **2**, *N*,*N*-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide], has a wide range of experimental and clinical antitumor activity.[1-4] It is generally accepted that **1** and **2** require metabolic activation,[5,6] presumably by cytochrome P-450 and mainly in the liver, to exert their cytostatic activity. The principal feature of the metabolic profile[4,5,7] of **1** (Scheme I) involves the initial formation of 4-hydroxyifosfamide (**3**), which equilibrates with the acyclic tautomer, aldoifosfamide (**4**).[8] The resulting **4** undergoes spontaneous decomposition, or enzyme-assisted hydrolysis,[9,10] with release of phosphorodiamidic acid **5**, which is a lethal alkylating agent.[11] Compounds **3** and **4** can undergo further enzymatically mediated oxidation–dehydrogenation to give inactive metabolites 4-ketoifosfamide (**6**) and carboxyifosfamide (**7**).[12,13] In competition with the metabolic reaction **1** → **3**, side-chain hydroxylation occurs, which followed by β-elimination of chloroacetic aldehyde yields inactive N-dechloroethylated metabolites **8** and **9**.[14]

The action mechanism of **5** is thought to resemble that for phosphoramide mustard.[15] The high alkylating reactivity of **5** may be rationalized in terms of an electronically enhanced rate of intramolecular cyclization of conjugate base **5a** to form aziridinium ion **10**.[16] The reaction of **10** with nucleophilic groups of biomolecules (HB) gives a monosubstituted intermediate **11**, which can, in turn, form a second aziridinium ion and, subsequently, afford a disubstituted product **12**.

If the cytotoxic activity of **5** results from alkylation of DNA (biomolecules), any modification of the parent molecule **1**, which does not have a significant effect on the metabolic activation process, but which does change the alkylating activity of **5**, should affect the antitumor activity of ifosfamide congeners. In this context, we have investigated the effect of replacement of the chlorine atom in the *N*-(2-chloroethyl) group of IF by an atom or group undergoing nucleophilic substitution on the antitumor activity of the new analogues.

We now report the synthesis and preliminary biological characteristics of 3-(2-chloroethyl)-*N*-(2-X-ethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide, where X is Br (bromofosfamide, **13**), $(C_2H_5O)_2(O)PO$ (**14**), $(C_6H_5CH_2O)_2(O)PO$ (**15**), p-$CH_3C_6H_4SO_3$ (**16**), $CH_3C(O)O$ (**17**), and F (**18**).

Results and Discussion

Synthesis of Ifosfamide Analogues. Ifosfamide analogues **13**, **14**, **15**, **16**, and **17** have been prepared in two steps (Scheme II) involving intramolecular cyclization of **1** under treatment with sodium hydride,[17] isolation of 2-aziridino-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin 2-oxide (**19**), and subsequent reaction of this last compound with hydrobromic acid, diethyl hydrogen phosphate, dibenzyl hydrogen phosphate, *p*-toluenesulfonic acid, and acetic acid, respectively. Reactions of **19** with the above-mentioned compounds are highly chemoselective and lead to opening of the aziridinyl ring without any observed P–N bond cleavage.[18,19]

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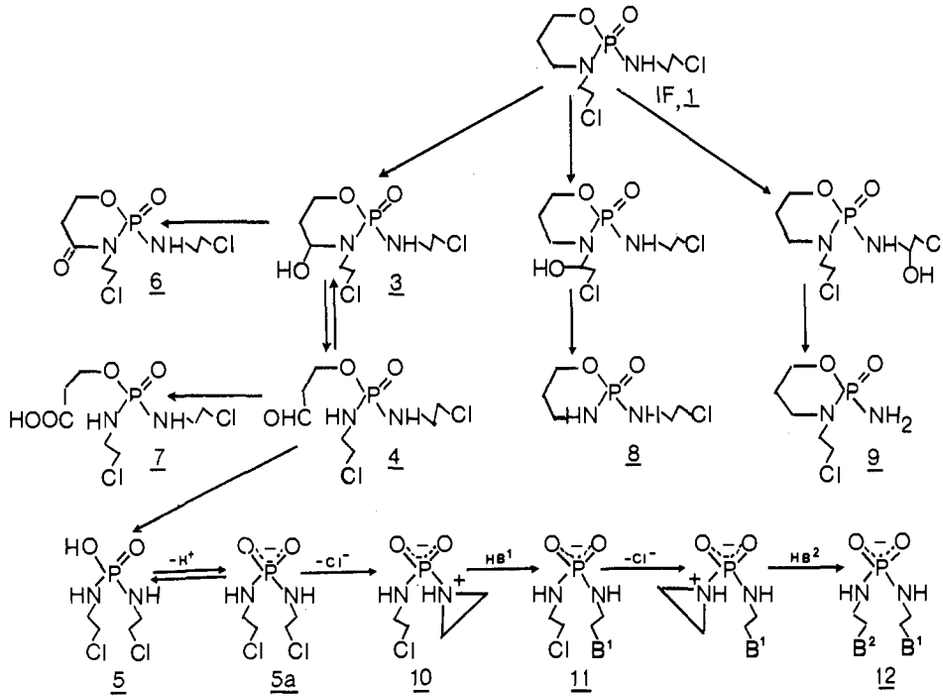
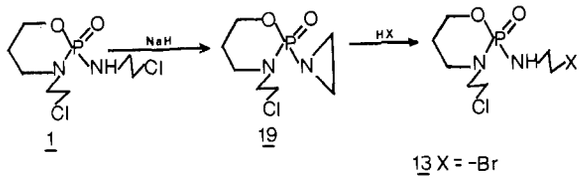
Scheme I



Scheme II



13 X = -Br
14 $-OP(O)(OC_2H_5)_2$
15 $-OP(O)(OCH_2Ph)_2$
16 $-OSO_2C_6H_4CH_3$
17 $-OC(O)CH_3$
20 -I

Attempts to open the aziridinyl ring in **19** by means of hydroiodic acid or an ethereal solution of hydriodide, although successful, led to highly unstable 3-(2-chloroethyl)-*N*-(2-iodoethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide (**20**) [^{31}P NMR (ether) δ 11.0; TLC (chloroform–ethanol, 9:1) R_f 0.42]. Concentration of an ether or chloroform solution of **20** by evaporation of solvent under reduced pressure left dark resinous material. Similar instability of iodo derivatives was observed by Takamizawa[20] for 3-(2-chloroethyl)-4-hydroperoxy-*N*-(2-iodoethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide. Treatment of **19** with poly(hydrogen fluoride)–pyridine complex in THF as a solvent did not afford the desired 3-(2-chloroethyl)-*N*-(2-fluoroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide (**18**). Instead of **18**, 3-(2-chloroethyl)-2-fluorotetrahydro-2*H*-1,3,2-oxazaphosphorin 2-oxide (**21**) was obtained as the sole product. However, the synthesis of **18** has been achieved by the condensation of phosphorus oxychloride with 2-fluoroethylamine hydrochloride (**22**) and subsequent condensation of the resulting *N*-(2-fluoroethyl)phosphoramidic dichloride (**23**) with 3-aziridinopropanol.

Since enantiomeric forms of ifosfamide (**1**) became available[17] due to our long-term interest in stereodifferentiated metabolism and antitumor activity of P-stereogenic oxazaphosphorins,[21,22] we have also prepared the enantiomeric forms of bromofosfamide (**13**). Cyclization of (+)-**1** with sodium hydride and the reaction of a chloroform solution of the resulting **19** with an equimolar amount of 6% aqueous HBr afforded (+)-**13**. In an analogous way, (−)-**1** was converted to (−)-**13**. Bromofosfamides (+)-**13** ($[\alpha]^{25}_D$ = +41.8°) and (−)-**13** ($[\alpha]^{25}_D$ = −41.7°) were enantiomerically pure. The ^{31}P NMR spectra of (+)-**13** and (−)-**13** run in a solution of optically active $Eu(tfc)_3$ in CCl_4 (compound:$Eu(tfc)_3$, 1:1.36, molar ratio) indicated the presence of only one enantiomer with induced chemical shifts of δ_+ −65.35 and δ_- −66.90, respectively, whereas the ^{31}P NMR spectrum of the mixture of (+)-**13** and racemic **13** (85.8:14.2, w/w) indicated the presence of both enantiomers in the ratio 92:8. The assignment of the absolute configuration *S* to the levorotatory and *R* to the dextrorotatory form of **13** was straightforward, because the absolute configuration of ifosfamide was established[17,23] and the conversions **1** → **19** and **19** → **13** both occur without affecting the sense of chirality at the phosphorus atom (that means without cleavage of any bond attached to the phosphorus atom). Albeit enantiomeric forms of compounds **14**–**18** are available, their preparation was abandoned in the light of biological test results obtained with racemic compounds **14**–**18** (vide infra).

Compounds **13**–**18** were isolated as described in the Experimental Section and are characterized by elemental analyses, mass spectrometry, ^{1}H NMR, ^{31}P NMR, ^{13}C NMR, and TLC. Characteristic ^{13}C NMR chemical shifts

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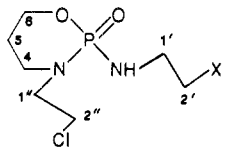
Table I. Selected Carbon-13 Chemical Shifts[a] and Coupling Constants[b] of Analogues 13–18



compd	C-4	C-5	C-6	C-1′	C-2′	C-1″	C-2″
13	42.0 (4.9)	26.3 (4.9)	67.1 (7.3)	43.0 (4.8)	34.1	47.6	50.1
14	42.0 (3.7)	26.3 (4.2)	67.1 (7.0)	41.4 (6.6)	67.7 (t, 5.7)	47.6	50.1 (2.0)
15	42.1 (3.7)	26.3 (4.2)	67.1 (7.3)	41.5 (7.0)	68.2 (t, 5.7)	47.7	50.2 (2.0)
16	42.1 (3.7)	26.3 (4.9)	67.2 (7.3)	40.4	70.5	47.7	50.0
17	42.0 (4.9)	26.4 (4.9)	67.1 (7.3)	40.3	64.6 (4.9)	47.6	50.1
18	42.1 (4.9)	26.4 (4.9)	67.1 (7.3)	41.6 (20)[c]	83.9 (167)[c]	47.7	50.1

[a] Chemical shifts are in parts per million with respect to TMS. [b] J(PC) values are in parentheses. [c] J(FC) values.

Table II. Antitumor Activity against L 1210 Leukemia in Mice[a]

compd	dose, mg/kg	% ILS	survivors on day 60/no. of mice tested (%)
IF (1)	120	74	0/24 (0)
	150	123	4/44 (9)
	180	>250	6/21 (29)
	200	>350	16/38 (42)
	300	>400	17/30 (56)
	400	>500	18/30 (60)
	500	>500	18/30 (60)
	600	>480	9/18 (50)
CP (2)	120	86	1/12 (8)
	150	89	1/19 (5)
	200	>550	8/12 (67)
	300	>550	16/18 (89)
	400	>425	7/18 (39)
13	50	74[b]	0/7[b]
	60	88	4/60 (7)
	80	110	7/25 (28)
	100	>430, >200[b]	24/46, 1/7[b] (52)
	150	>550	16/23 (70)
	200	>550, >600[b]	23/27, 7/7[b] (85)
	300	>550	14/19 (74)
	400	>500	8/12 (67)
(*R*)-(+)-13	120	114	0/6 (0)
	150	>500	4/6 (67)
	200	>550	10/12 (83)
	300	>550	11/12 (92)
	400	>550	11/12 (92)
	500	206	0/6 (0)
(*S*)-(−)-13	60	140	1/12 (8)
	100	>550	8/12 (67)
	150	>550	6/6 (100)
	200	>550	12/12 (100)
	300	>350	7/12 (58)
	400	62	0/6 (0)
14	30–180	0–12	0/6
15	30–180	0–12	0/6
16	30–180	13	0/6
17	30–180	13	0/6
18	15–60	10	0/6
	100	−40	0/6
	200	−40	0/6

[a] Female CD2F$_1$ mice, 10–12 weeks old, were inoculated ip with 10^5 cells of L 1210 leukemia on day 0. Test preparations were dissolved in sterile saline prior to use and were given ip on day 1 in volume of 0.01 mL/g of body weight. Test and control groups consisted of 6–8 and of 18–24 mice, respectively. [b] Data provided by Otsuka Pharmaceutical Co. Preparation was given ip on day 2 to 6-week-old male BDF$_1$ mice bearing L 1210 leukemia.

δ and coupling constants with ^{31}P and ^{19}F (shown in Table I) were in agreement with the assigned structures. The chemical shifts of the ring carbons C-4, C-5, and C-6 as well as the coupling constants ^2J(PNC), ^2J(POC) and ^3J(PC) are almost the same (within experimental error ±1.2 Hz), indicating that the tetrahydro-2*H*-1,3,2-oxazaphosphorin ring adopts the chair conformation.[24] Only δ values for carbons at positions C-1′ and C-2′ were affected by the introduction of the X substituent.

Table III. Toxicity, Effective Doses, and Therapeutic Indices of Bromofosfamides as Compared to Cyclophosphamide and Ifosfamide

compd	ED_{50}:[a] mean ± SD, mg/kg	n[b]	LD_{50},[c] mg/kg	TI[d]
IF (1)	88.6 ± 13.2	7	679 (646–718)	7.7
CP (2)	90.6 ± 14.4	4	445 (427–465)	4.9
rac-13	36.2 ± 5.5	9	470 (453–489)	13.0
(*R*)-(+)-13	66.2 ± 11.6	5	541 (522–562)	8.2
(*S*)-(−)-13	27.7 ± 1.8	5	368 (356–381)	13.3

[a] Effective doses providing 50% increase in life span were determined for each therapeutic experiment separately. Appropriate dose ranges, providing % ILS between 15% and 100%, were given to L 1210 leukemia bearing mice (details described in Table II). Since a linear relationship within these dose ranges were observed, each ED_{50} point was calculated by the method of linear regression analysis of drug dose (four to six levels, six mice/dose) plotted against percentage of ILS. [b] Number of experiments. [c] Lethal doses required to kill 50% of the population were determined after single ip injection of test preparations or reference drugs into healthy female CD2F$_1$ mice. Dose-level groups providing mortality between 0% and 100% (five to six for one evaluation) consisted of 10 or 15 animals. Observation was carried out for 21 days. The LD_{50} values and their 95% confidence limits were calculated by using the probit analysis method. [d] Therapeutic index = LD_{50}/ED_{50}.

Anticancer Screening Data. Cyclophosphamide (**2**), ifosfamide (**1**), and the newly synthesized ifosfamide congeners **13–18** were tested for their in vivo anticancer activity against L 1210 lymphoid leukemia in mice, according to NCI standard screening protocols[25] at the Institute of Immunology and Experimental Therapy. Some tests were performed at the Otsuka Pharmaceutical Co. From the comparative data included in Table II it can be seen that racemic **13** and enantiomers (*R*)-(+)-**13** and (*S*)-(−)-**13** caused higher than 500% increase in lifespan and cured up to 100% of the mice with L 1210 leukemia. Racemic bromofosfamide and its enantiomers were clearly more effective than IF, and to achieve the same maximal curative effects as provided by IF, an approximately two times lower dose of **13** was required. Moreover, the bromofosfamides caused a significant number of cures at a wider dose range than CP. In contrast to the bromofosfamides, compounds **14–18** were inactive in this tumor system.

Effective doses (ED_{50}) of racemic **13**, (*R*)-(+)-**13**, and (*S*)-(−)-**13** were determined as 36.2, 66.2, and 27.2 mg/kg, respectively (Table III), and were lower than those ob-

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tained for CP (90.6 mg/kg) or IF (88.6 mg/kg). Although all three bromofosfamides were more toxic than IF, their cancerotoxic selectivity[26] expressed as a therapeutic index (TI) was higher (about 1.7 times for **13** and (*S*)-(−)-**13**). In comparison to CP, only (*S*)-(−)-**13** was more toxic, but the therapeutic indices of this enantiomer and the racemate were about 2.7 times higher.

Conclusions. The introduction of the bromine substituent instead of chlorine in the *N*-(2-chloroethyl) group of **1** most probably accelerates the rate of aziridinium ion formation[27] from the phosphorodiamidic acid, metabolite of **13**, and leads to an improved therapeutic index. This finding could suggest that cancerotoxic selectivity is connected not only with the primary activation process (**1** → **3**, **4** and reaction of **4** with sulfhydryl compounds with formation of 4-thiooxazaphosphorins) but also with the toxification process (**4** → **5**) and subsequent aziridinium ion formation (**5** → **10**). The increase of the rate of azaridinium ion formation, which is the rate-limiting step of alkylation of biomolecules by **5**, could lead to change of the sites and extent of alkylation of nucleophilic centers of DNA, protein, or DNA-polymerase[6] and, hence, the change of cancerotoxic selectivity. The lack of antitumor activity of compounds **14–18** may be due to their poor tendency, after metabolic transformation, to form aziridinium ion.[27] However, other factors, such as rate of oxidation, formation of the aldehydic metabolites, rate of fragmentation, and lipophilicity, might affect the metabolism of compounds **14–18**, and should have some consequence on anticancer activity.

Detailed studies on the kinetics of decomposition of **5** and its analogues and their interactions with DNA are in progress.

Experimental Section

General Procedures. Melting points were determined in open glass capillary tubes and are uncorrected. Elemental analyses were performed by CMMS Microanalytical Laboratory. Mass spectra were recorded on a LKB 2091 mass spectrometer at 70 eV ionizing energy. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. $^{13}C(^1H)$ NMR and ^1H NMR spectra were recorded at 75.5 and 300 MHz, respectively, with a Bruker MSL-300 instrument. δ values refer to TMS. $^{31}P(^1H)$ NMR spectra were recorded at 24.24 MHz with a JEOL C-60H instrument. Optical purity of enantiomeric bromofosfamides was determined by recording ^{31}P NMR spectra at 80.8 MHz with a Varian XL-200 spectrometer. ^{31}P NMR δ values refer to external 85% H_3PO_4. Silica gel 60 F_{254} plates were used for thin-layer chromatography; spots were visualized with iodine vapor or 5% ethanolic $H_3PO_4 \cdot 12MoO_3$. Silica gel for column chromatography was 230–400 mesh, Merck Art. 9385. Solvents and commercial reagents were distilled and dried by conventional methods before use. Organic extracts were dried over $MgSO_4$. Enantiomeric forms of ifosfamide,[17] diethyl hydrogen phosphate[28] and 3-aziridinopropanol[29] were prepared according to the procedures described previously.

2-Aziridino-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin 2-Oxide (19) and Its Enantiomers (*R*)-(+)-19 and (*S*)-(−)-19. Ethyleneimides **19**, (*R*)-(+)-**19**, and (*S*)-(−)-**19** were prepared in the reaction of racemic, dextrorotatory ($[\alpha]^{25}_D$ = +46.2°), and levorotatory ($[\alpha]^{25}_D$ = −45.8°) ifosfamides, respectively, with sodium hydride in anhydrous THF according to a described procedure.[17] Racemic **19**: mp 25–26 °C; ^{31}P NMR ($CHCl_3$) δ 17.8. (*R*)-(+)-**19**: mp 64–66 °C; $[\alpha]^{25}_D$ = +25.5 °C (*c* 3.0, MeOH); ^{31}P NMR ($CHCl_3$) δ 17.8. (*S*)-(−)-**19**: mp 64–66 °C; $[\alpha]^{25}_D$ = −25.8° (*c* 3.0, MeOH); ^{31}P NMR ($CHCl_3$) δ 17.8.

***N*-(2-Bromoethyl)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (13, Bromofosfamide) and Its Enantiomers (*R*)-(+)-13 and (*S*)-(−)-13.** A water solution of hydrobromic acid (6.6 mL of 6%, 5.0 mmol) was added dropwise to a stirred solution of **19** (1.12 g, 5.0 mmol) in chloroform (10 mL) at room temperature. Stirring was continued for 15 min. The organic layer was separated, and the water layer was extracted twice with chloroform (2 × 5.0 mL). The combined chloroform solutions were dried and concentrated on a rotary evaporator. The solidified material was recrystallized from ether–*n*-pentane to give racemic **13** (1.17 g, 77%): mp 59–60 °C; ^{31}P NMR δ 11.4; ^1H NMR ($CDCl_3$) δ 1.90–2.04 (m, 2), 3.16–3.54 (m, 9), 3.63–3.76 (m, 2), 4.20–4.44 (m, 2); TLC R_f 0.44 ($CHCl_3$–EtOH, 9:1); MS, *m/z* 304 (0.3), 306 (0.3), 225 (30), 83 (84), 31 (100). Anal. ($C_7H_{15}BrClN_2O_2P$) C, H, Br, Cl, N, P. (*R*)-(+)-**13** (1.1 g, 81%) was obtained by starting from (*R*)-(+)-**19** (1.0 g, 4.45 mmol), $[\alpha]^{25}_D$ = +25.5° (*c* 3.0, MeOH), and using an analogous procedure: $[\alpha]^{25}_D$ = +41.8° (*c* 3.0, MeOH); mp 89–90 °C; other parameters (^{31}P NMR, ^1H NMR, ^{13}C NMR, R_f, and MS) as for racemic **13**. Anal. C, H, N, P. In the same way, (*S*)-(−)-**19** [1.0 g, 4.45 mmol; $[\alpha]^{25}_D$ = −25.8° (*c* 3.3, MeOH)] was converted to (*S*)-(−)-**13** (1.1 g, 81%): $[\alpha]^{25}_D$ = −41.7° (*c* 3.0, MeOH); mp 89–90 °C; other parameters (^{31}P NMR, ^1H NMR, ^{13}C NMR, R_f, and MS) as for racemic **13**. Anal. C, H, N, P.

3-(2-Chloroethyl)-*N*-[2-[(diethoxyphosphinyl)oxy]-ethyl]tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (14). A solution of diethyl hydrogen phosphate (0.71 g, 5 mmol) in chloroform (2 mL) was added dropwise to a stirred solution of **19** (0.55 g, 2.5 mmol) in $CHCl_3$ (10 mL) at room temperature. Stirring was continued for 1 h, and then the mixture was diluted with chloroform (8 mL), washed with 10% $KHCO_3$ (10 mL), dried, and concentrated on a rotary evaporator. The residue was chromatographed on silica gel (25 g) with $CHCl_3$–EtOH (18:1) as eluent. Product was isolated as a colorless oil: ^{31}P NMR ($CHCl_3$) δ 11.9 (P-2), 1.3 (P-2′); ^1H NMR ($CDCl_3$) δ 1.34 (t, *J* = 7.1 Hz, 6), 1.89–1.96 (m, 2), 2.15 (s, 1), 3.15–3.29 (m, 5), 3.39–3.46 (m, 1), 3.62–3.68 (m, 2), 4.07–4.37 (m, 8); TLC R_f 0.38 ($CHCl_3$–EtOH, 9:1); MS, *m/z* 381 ($[M + 3]^+$, 1.3), 380 ($[M + 2]^+$, 1.3), 379 ($[M + 1]^+$, 3.4) 378 (M^+, 2.0), 343 ($[M - Cl]^+$, 17), 342 ($[M - HCl]^+$, 14), 329 ($[M - CH_2Cl]^+$, 7.5), 70 (100). Anal. ($C_{11}H_{25}ClN_2O_6P_2$) C, H, N, P.

3-(2-Chloroethyl)-*N*-[2-[[bis(benzyloxy)phosphinyl]-oxy]ethyl]tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (15). By a procedure essentially identical with that applied above to the diethyl phosphate analogue **14**, **19** (0.55 g, 2.5 mmol) and dibenzyl hydrogen phosphate (1.05 g, 3.73 mmol) were converted into **15** (0.9 g, 74%). Product was isolated after column chromatography as colorless oil: ^{31}P NMR ($CHCl_3$) δ 12.0 (P-2), 0.8 (P-2′); ^1H NMR ($CDCl_3$) δ 1.88–1.94 (m, 2), 3.09–3.28 (m, 6), 3.36–3.43 (m, 1), 3.60–3.64 (m, 2), 4.00–4.33 (m, 4), 5.04–5.12 (m, 4), 7.37 (s, 10); TLC R_f 0.33 ($CHCl_3$–EtOH, 9:1); MS, *m/z* 504 ($[M + 2]^+$, 0.3), 502 (M^+, 0.7), 213 ($[M + 2 - CH_2OP(O)(OBz)_2]^+$, 4.6), 211 ($[M - CH_2OP(O)(OBz)_2]^+$, 12.4). Anal. ($C_{21}H_{29}ClN_2O_6P_2$) C, H, N, P.

3-(2-Chloroethyl)-*N*-[2-(tosyloxy)ethyl]tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (16). A solution of *p*-toluenesulfonic acid hydrate (2.0 g, 10.5 mmol) in a chloroform–acetone mixture (3:1, 100 mL) was added dropwise to a stirred solution of **19** (2.24 g, 10.0 mmol) in ether (40 mL) at room temperature. Stirring was continued for 30 min. The mixture was concentrated at reduced pressure, and the residue was dissolved in chloroform (100 mL). The chloroform solution was washed with 5% $NaHCO_3$ (50 mL) and water (50 mL). The aqueous washings were extracted with chloroform (50 mL), and the combined chloroform solutions were dried and concentrated on a rotary evaporator. The residue was chromatographed on silica gel (90 g) with $CHCl_3$–EtOH (18:1) as eluent. Product **16** (2.7 g 68%) was isolated as a colorless oil: ^{31}P NMR ($CHCl_3$) δ 11.5; ^1H NMR ($CDCl_3$) δ 1.88–1.93 (m, 2), 2.34 (s, 1), 2.44 (s, 3),

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(27) Our unpublished ^{31}P NMR kinetic studies (Kinas, R. W.; Pilichowska, S.; Stec, W. J.) of the decomposition of the cyclohexylammonium salt of *N,N′*-bis(2-chloroethyl)phosphorodiamidic acid and *N,N′*-bis(2-bromoethyl)phosphorodiamidic acid have indicated a half-life of ca. 85 and 16 min, respectively (pH 7.4, 37 °C). Under the same experimental conditions,[15] the cyclohexylammonium salt of *N,N′*-bis[2-[(diethoxyphosphinyl)oxy]ethyl]phosphorodiamidic acid does not undergo decomposition.

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***N*-(2-Acetoxyethyl)-3-(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (17).** A solution of **19** (1.1 g, 5.0 mmol) in acetic acid (10 mL) was stirred at room temperature for 1 h and then concentrated under reduced pressure. The solid material was dissolved in chloroform (30 mL), and this solution was washed with 10% $KHCO_3$ (25 mL). The aqueous layer was extracted twice with chloroform (2 × 30 mL). The combined chloroform solutions were dried and concentrated on a rotary evaporator. TLC ($CHCl_3$–acetone, 1:1) revealed two compounds, unreacted **19** (R_f 0.46) and **17** (R_f 0.25). Elution of the mixture from silica gel (30 g) with $CHCl_3$–acetone (1:1) gave unreacted **19** (0.2 g) and **17** (0.6 g, 43%), which was subsequently purified by crystallization from acetone-*n*-pentane to give material (0.5 g) of mp 83–84 °C: ^{31}P NMR ($CHCl_3$) δ 12.4; ^{1}H NMR ($CDCl_3$) δ 1.86–2.18 (m, 2), 2.08 (s, 3), 3.13–3.15 (m, 1), 3.16–3.32 (m, 5), 3.39–3.50 (m, 1), 3.64–3.70 (m, 2), 4.11–4.41 (m, 4); TLC R_f 0.47 ($CHCl_3$–EtOH, 9:1); MS, m/z 287 ([M + 3]$^+$, 0.6), 285 ([M + 1]$^+$, 1.3), 235 ([M – CH_2Cl]$^+$, 43), 226 ([M + 3 – AcO]$^+$, 66), 224 ([M + 1 – AcO]$^+$, 20), 215 ([M + 2 – $AcOCH_2$]$^+$, 60), 213 ([M – $AcOCH_2$]$^+$, 20). Anal. ($C_9H_{18}ClN_2O_4P$) C, H, N, P.

***N*-(2-Fluoroethyl)phosphoramidic Dichloride (23).** A stirred suspension of 2-fluoroethylamine hydrochloride (5.0 g, 50 mmol) in $POCl_3$ (25 mL) was heated under reflux for 3 h. The excess of $POCl_3$ was removed by rotary evaporation, and the residue was distilled under reduced pressure to give **22** (4.6 g, 50%) as a colorless oil: bp 117–118 °C (7 Pa); ^{1}H NMR ($CDCl_3$) δ 3.0–3.9 (m, 2, NCH_2), 4.5 (m, $^2J_{HF}$ = 48 Hz, $^3J_{HH}$ = 6 Hz, $^4J_{HP}$ = 2 Hz, 2, CH_2F), 5.8 (s, 1, NH); ^{31}P NMR ($CHCl_3$) δ 16.6; MS, m/z 179 (M$^+$, 1), 146 ([M – CH_2F]$^+$, 100), 117 ([M – FCH_2CH_2NH]$^+$, 28).

3-(2-Chloroethyl)-*N*-(2-fluoroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-Oxide (18). To a stirred solution of **23** (3.6 g, 20 mmol) in dioxane (50 mL) was added at room temperature a mixture of 3-aziridinopropanol (2.0 g, 20 mmol), triethylamine (2.2 g, 22 mmol), and dioxane (50 mL). The mixture was stirred for 18 h, then filtered, and concentrated to dryness. A solution of the residue in $CHCl_3$ (100 mL) was washed with water (2 × 20 mL), dried, and concentrated under reduced pressure. This material was purified by silica gel (40 g) column chromatography with $CHCl_3$–EtOH (18:1) as eluent and recrystallized from Et_2O. Compound **23** (1.2 g) was obtained in 30% yield: mp 56–57 °C; ^{31}P NMR ($CHCl_3$) δ 12.2; ^{1}H NMR δ 1.89–2.00 (m, 2), 2.96 (s, 1), 3.16–3.36 (m, 5), 3.44–3.56 (m, 1), 3.63–3.70 (m, 2), 4.19–4.29 (m, 1), 4.34–4.44 (m, 1), 4.48 (m, J = 48.4 Hz, J = 4.8 Hz, 2); MS, m/z 195 ([M – CH_2Cl]$^+$, 41), 134 (16), 83 (43), 31 (100). Anal. ($C_7H_{15}ClFN_2O_2P$) C, H, N. P.

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Registry No. **13**, 104149-14-6; (*R*)-(+)-**13**, 104149-16-8; (*S*)-(–)-**13**, 104149-15-7; **14**, 110971-88-5; **15**, 110971-89-6; **16**, 110971-90-9; **17**, 110971-91-0; **18**, 110971-92-1; **19**, 29102-47-4; (*R*)-(+)-**19**, 72578-73-5; (*S*)-(–)-**19**, 72578-74-6; **23**, 110971-93-2; 2-fluoroethylamine hydrochloride, 460-08-2; 3-aziridinopropanol, 31190-87-1.

Spiro Hydantoin Aldose Reductase Inhibitors

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Sorbitol formation from glucose, catalyzed by the enzyme aldose reductase, is believed to play a role in the development of certain chronic complications of diabetes mellitus. Spiro hydantoins derived from five- and six-membered ketones fused to an aromatic ring or ring system inhibit aldose reductase isolated from calf lens. In vivo these compounds are potent inhibitors of sorbitol formation in sciatic nerves of streptozotocinized rats. Optimum in vivo activity is reached in spiro hydantoins derived from 6-halogenated 2,3-dihydro-4*H*-1-benzopyran-4-ones (4-chromanones). In 2,4-dihydro-6-fluorospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione, the activity resides exclusively in the 4*S* isomer, compound **115** (CP-45,634, USAN: sorbinil). This compound is currently being used to test, in humans, the value of aldose reductase inhibitors in the therapy of diabetic complications.

Aldose reductase, the NADPH-linked enzyme that reduces aldehydic substrates, converts glucose to sorbitol; sorbitol can be converted in turn by polyol dehydrogenase to fructose. Because the K_M of aldose reductase for glucose is high, the hypothesis has been put forward[1,2] that significant flux through this "polyol pathway" occurs only at high glucose levels, for instance, in diabetics whose plasma and tissue glucose levels may often exceed the normal range.[2e] Since sorbitol and fructose do not readily diffuse out of cells, it has been postulated that accumulation of sorbitol and fructose in tissues such as lens, nerve, or retina contributes to the development of certain chronic complications of diabetes mellitus, such as cataracts, neuropathies, or retinopathy.[1,2] If this hypothesis were correct, inhibitors of aldose reductase should be of value in preventing or treating these diabetic complications.

The search for aldose reductase inhibitors with which to test this hypothesis has been under way for many years. In 1965 several simple C_6–C_8 fatty acids and their derivatives were disclosed as inhibitors of calf lens aldose reductase.[3] Tetramethyleneglutaric acid (TMG) was reported in 1968 to be an aldose reductase inhibitor which, in contrast to the C_6–C_8 fatty acids, was active and also less toxic in lens cultures,[4a] although TMG failed to dem-

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