The extracts were combined, dried over magnesium sulfate, and evaporated. The resulting residues were purified by recrystallization from a petroleum ether–benzene mixture or by column chromatography on silica gel (40 mesh) with a stepwise solvent gradient of petroleum ether and diethyl ether.

4-Aminobenzamides. A solution of 5.0 g of the appropriate *p*-nitrobenzamide in absolute ethanol (150 mL) was added to a Paar hydrogenation bottle along with 250 mg of 5% palladium on carbon and subjected to low-pressure hydrogenation (45 psi) for 3 h. The bottle was then removed, and the contents were filtered through Celite. The filtrate was evaporated, and the resulting residue was purified by recrystallization from benzene–petroleum ether mixtures or by column chromatography on silica gel (40 mesh) with a stepwise solvent gradient of petroleum ether (boiling range 30–60 °C) and diethyl ether.

Pharmacology.[19] Initial anticonvulsant evaluation of these compounds was conducted with at least three dose levels (30, 100, and 300 mg/kg) and in some cases a fourth dose of 600 mg/kg. All tests were performed with male Carworth Farms number-one mice. Test solutions of all compounds were prepared in 30% polyethylene glycol 400, and animals were dosed intraperitoneally at 30 min prior to testing.

Maximal electroshock seizures (MES) were elicited with a 60 cycle alternating current of 50-mA intensity delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of electrodes. Abolition of the hindlimb tonic extension component of the seizure was defined as protection in the MES test.

The subcutaneous pentylenetetrazole (metrazol) seizure threshold test (scMet) was conducted by administering 85 mg/kg of pentylenetetrazole as a 0.5% solution in the posterior midline. Protection in this test was defined as a failure to observe a single episode of clonic spasms of at least 5-s duration during a 30-min period following administration of the test compound.

Neurological deficit was measured in mice by the rotorod test. The dosed animal was placed on a 1-in. diameter knurled plastic rod rotating at 6 rpm. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min. The median anticonvulsant potency (ED50) and toxicity (TD50) were determined by the graphical method.

(19) The pharmacological evaluation of these compounds was conducted in the laboratories of the Anticonvulsant Drug Development Program, Epilepsy Branch, NINCDS, Bethesda, MD.

Acknowledgment. The authors deeply appreciate the assistance of the Anticonvulsant Drug Development Program, Epilepsy Branch, NINCDS, in the pharmacological testing of these compounds. The help of Gill D. Gladding of the ADD Program is gratefully acknowledged.

Registry No. 1, 2835-68-9; **2**, 6274-22-2; **3**, 89399-17-7; **4**, 38681-78-6; **5**, 51207-84-2; **6**, 85592-76-3; **7**, 85592-77-4; **8**, 17675-42-2; **9**, 79868-19-2; **10**, 782-45-6; **11**, 54977-92-3; **12**, 85592-75-2; **13**, 61251-99-8; **14**, 13004-65-4; **15**, 89399-18-8; **16**, 85592-78-5; **17**, 89399-19-9; *p*-nitrobenzamide, 619-80-7; *p*-nitrobenzoyl chloride, 122-04-3; *N*-methyl-4-nitrobenzamide, 2585-23-1; *N*-ethyl-4-nitrobenzamide, 50445-50-6; 4-nitro-*N*-propylbenzamide, 2585-24-2; *N*-butyl-4-nitrobenzamide, 51207-98-8; 4-nitro-*N*-pentylbenzamide, 89399-20-2; *N*-hexyl-4-nitrobenzamide, 89399-21-3; *N*-cyclohexyl-4-nitrobenzamide, 7506-46-9; *N,N*-dipropyl-4-nitrobenzamide, 79868-22-7; 4-nitro-*N*-phenylbenzamide, 3460-11-5; *N*-benzyl-4-nitrobenzamide, 2585-26-4; *d,l*-4-nitro-*N*-(α-methylbenzyl)benzamide, 85592-74-1; 4-nitro-*N*-phenethylbenzamide, 62497-65-8; 4-nitro-*N*-(β-methylphenethyl)-benzamide, 15269-43-9; 4-nitro-*N*-(α-methylphenethyl)benzamide, 89399-22-4; *N*-benzyl-*N*-methyl-4-nitrobenzamide, 89399-23-5; 4-nitro-*N*-(α-phenylbenzyl)benzamide, 88229-34-9.

In the Search for New Anticancer Drugs. 9. Synthesis and Anticancer Activity of Spin-Labeled Analogues of *N,N*:*N′,N′*:*N″,N″*-Tri-1,2-ethanediylphosphoric Triamide and *N,N*:*N′,N′*:*N″,N″*-Tri-1,2-ethanediylphosphorothioic Triamide

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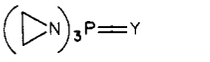
Department of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201. *Received September 2, 1983*

A number of *N,N*:*N′,N′*:*N″,N″*-tri-1,2-ethanediylphosphoric triamide (TEPA) and *N,N*:*N′,N′*:*N″,N″*-tri-1,2-ethanediylphosphorothioic triamide (thio-TEPA) derivatives containing either two aziridine moieties (**1a**) or two (2-chloroethyl)amino functions (**1b**) and either a 2,2,6,6-tetramethylpiperidine, 1-oxy-2,2,6,6-tetramethylpiperidine or 1-hydroxy-2,2,6,6-tetramethylpiperidine component were synthesized and tested against lymphocytic leukemia P388 in mice. In a structure–activity comparison it was found that at optimum dose all compounds containing the nitroxyl radical were more active than the corresponding hydroxylamine derivatives. The open-chain compounds (**1b**) were less active than the corresponding aziridine ring compounds (**1a**). The replacement of the X = bridge in **1a** with the X = $N(CH_3)$ group resulted in lowering of the anticancer activity.

Thio-TEPA [*N,N*:*N′,N′*:*N″,N″*-tri-1,2-ethanediylphosphorothioic triamide (1, Y = S)] is a clinically used



1, Y = S, O

anticancer agent that is effective against the Hodgkin's disease and carcinoma of the breast, bladder, and ovary.[1,2] TEPA [*N,N*:*N′,N′*:*N″,N″*-tri-1,2-ethanediylphospohoric triamide (1, Y = O)] is not used clinically. The synthesis of 1 and of a number of thio-TEPA derivatives containing either two or three substituted or unsubstituted di-1,2-ethanediylimine (aziridine, ethylenimine) groups at the pentavalent phosphorus has been reported.[3-12] Although only 1, Y = S, is clinically used, several other com-

†Naval Hospital, Toxicology Branch, S-33, NAS, Norfolk, VA.

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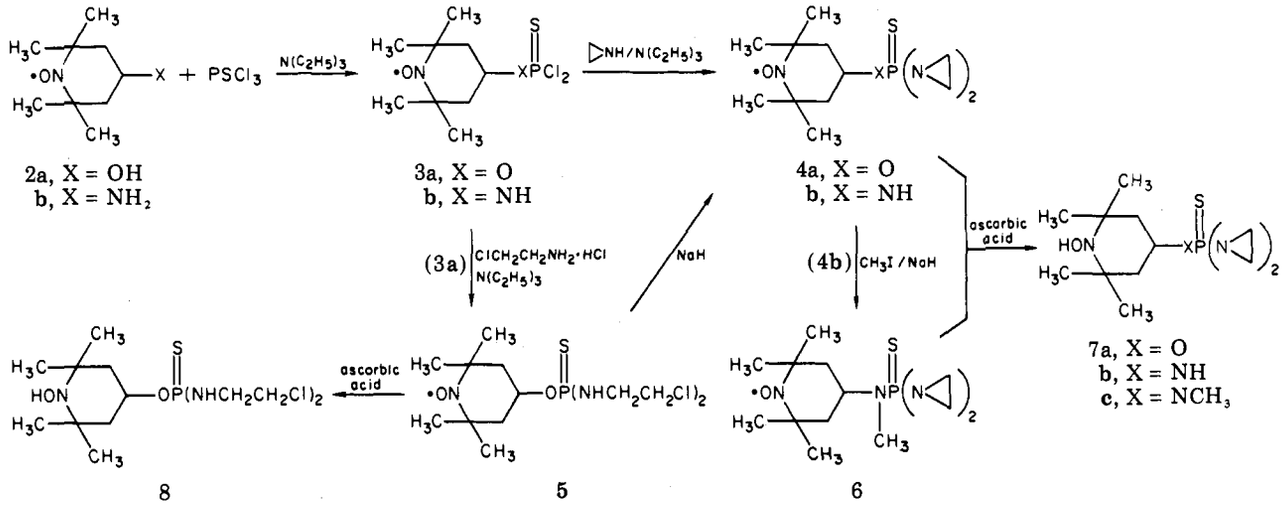
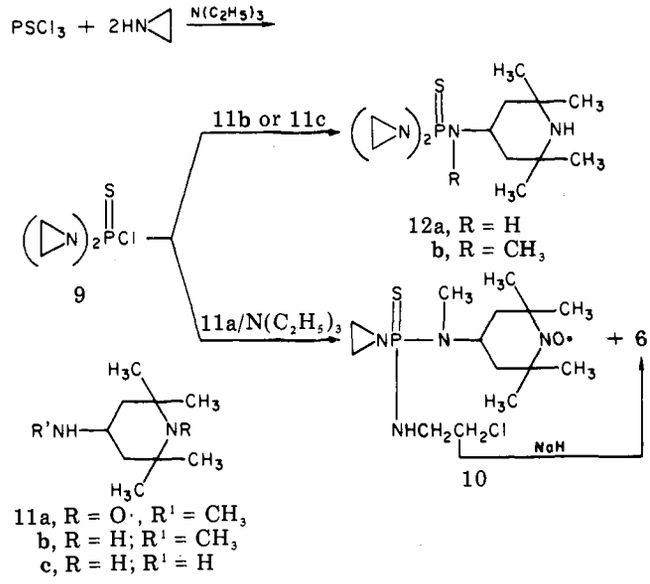
Scheme I

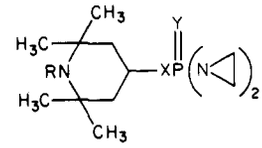


pounds[5,6,10,11] in this series were found, in animal models, to have a comparable activity to that of the parent compound 1, Y = S. Studies[13] on the interaction of nitrogen mustard with DNA indicated that a cross-linking between strands essentially requires only two alkylating groups to prevent strand separation. If such an effect is anticipated in TEPA and thio-TEPA, actually only two aziridine groups would be sufficient for the cross-linking of the DNA strands. Hence, it should be possible by substituting the third alkylating aziridine moiety by a suitable group to produce compounds that might be less toxic and equally as active as 1, Y = S. It is believed[14-23] that a nitroxyl radical could be such a group. In addition, the incorporation of a spin-label into 1, Y = S, as suggested in a number of publications,[14-23] may not only produce compounds with comparable activity and/or lower toxicity than 1, Y = S, but also could lead to a new approach in the pharmacology of drugs using the ESR technique.[21]

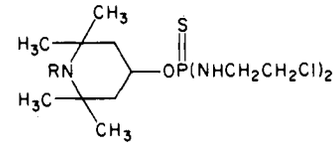
Scheme II



Although several compounds in this series were found[19-23] to have anticancer activity comparable to that of 1, Y = S, the role of the nitroxyl radicals in the activity has not been convincingly demonstrated. In view of this fact, it became imperative to us to investigate the role of nitroxyl radicals in the anticancer activity of spin-labeled compounds. Therefore, we synthesized several di-1,2-ethanediylphosphoramides of structure **1a** and evaluated



1a, R = H, O·, OH; X = O, NH, N(CH3), O(O)CNH; Y = O, S



1b, R = O·, OH

their anticancer activities. Assuming that in biological systems the cross-linking of the DNA strands by the nitrogen mustards may be caused either "directly" by the

(8) I. A. Nuretdinov and N. P. Grech, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1883 (1964).
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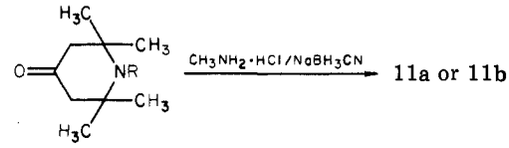
bis[(2-chloroethyl)amino] moieties or via the intermediate formation of the bis(aziridinyl) moieties, compounds of type **1b** were prepared for a comparison of the anticancer activity of these open-chain compounds (**1b**) with the corresponding ring structures (**1a**). Judiciously chosen bridge elements X in the design of compounds **1a** were expected to result in changes of anticancer activity that might give a clue to the significance of steric, inductive, and hydrogen bonding effects of bridge elements X in the alkylating drugs of type **1a**.

Results and Discussion

Chemistry. The synthetic methodology for the preparation of spin-labeled thio-TEPA compounds is depicted in Schemes I and II. Either 4-hydroxy-1-oxy-2,2,6,6-tetramethylpiperidine[24] (**2a**) or 4-amino-1-oxy-2,2,6,6-tetramethylpiperidine[24,25] (**2b**) was reacted with thiophosphoryl chloride in the presence of triethylamine to produce the intermediates, 1-oxy-2,2,6,6-tetramethyl-4-piperidyl thiophosphorodichloridate[15-17] (**3a**) or 1-oxy-2,2,6,6-tetramethyl-4-piperidyl thiophosphoramidodichloridate[14,21] (**3b**) (Scheme I). These intermediates (**3a,b**), without isolation, were further reacted either with aziridine or 2-chloroethylamine hydrochloride in the presence of triethylamine to give **4a**,[15-17] **4b**,[14,21] or **5**, respectively. Compound **4b** was N-methylated with methyl iodide in the presence of sodium hydride to give **6**. The 2-chloroethylamine group in **5** was cyclized with sodium hydride to the aziridine group to produce **4a**. All nitroxyl compounds,**4a,b**, **5**, and **6**, were reduced by ascorbic acid to the corresonding hydroxylamine derivatives, **7a,b**, **8**, and **7c**, respectively, by a procedure described earlier[26] (Table I). The intermediate *N,N:N'N'*-di-1,2-ethanediylphosphorodiamidothioic chloride (**9**) (Scheme II) was synthesized from thiophosphoryl chloride and aziridine in the presence of triethylamine in accordance with the procedure described earlier.[7,17] This intermediate (**9**) was then utilized in the reactions with 4-(methylamino)-1-oxy-2,2,6,6-tetramethylpiperidine[25] (**11a**), 4-(methylamino)-2,2,6,6-tetramethylpiperidine (**11b**), and 4-amimo-2,2,6,6-tetramethylpiperidine (**11c**) to give products **6**, **12a**, and **12b**, respectively. Since 2,2,6,6-tetramethylpiperidine compounds are considered[27] to be excellent scavangers of hydrogen chloride,[27] 2 equiv of starting base **11b** or **11c** were used for the synthesis of **12a** or **12b**, respectively. The synthesis of **6**, following Scheme II, proceeded with the formation of the byproduct **10**. The ring-opening reaction of **6** is presumed to be caused by triethylamine hydrochloride, present in the reaction mixture. Nevertheless, the (2-chloroethyl)amino group in **10** was readily cyclized by sodium hydride to give compound **6**. The starting compounds **11a** and **11b** were prepared from 4-oxo-1-oxy-2,2,6,6-tetramethylpiperidine[24] (**13a**) and 4-oxo-



13a, R = O·
b, R = H

2,2,6,6-tetramethylpiperidine[24] (**13b**), respectively, by the

Table I. Physical Properties of 4-Phosphorylated *N*-Hydroxy-2,2,6,6-tetramethylpiperidines

no.	yield, %	formula[a]	M_r	mp, °C	IR,[b] cm^{-1}	MS,[c] *m/e*	^1H NMR, δ
7a	86	$C_{13}H_{26}N_3O_2PS$	319.40	128–129	3325 (OH), 1440, 1240, 980, 920	320 (100), 165 (47), 156 (47)	1.2 (s, 12 H, 4 CH_3), 1.9–2.5 (m, 12 H, 2 CH_2, 4 CH_2N), 4.4 (broad, 1 H, CHO)
7b	92	$C_{13}H_{27}N_4OPS$	318.42	167–168	3250 (NH), 3350 (OH), 1420, 1240, 910	319 (100), 301 (31)	1.2 (s, 12 H, 4 CH_3), 1.8–2.4 (m, 13 H, 2 CH_2, 4 CH_2N, NH), 3.6 (broad, 1 H, CHN)
7c	84	$C_{14}H_{29}N_4OPS$	332.45	156–157	3350 (OH), 1440, 1250, 920	333 (100), 315 (23)	1.3 (s, 12 H, 4 CH_3), 1.9–2.6 (m, 12 H, 2 CH_2, 4 CH_2N), 2.9–3.3 (m, 4 H, CH_3N, CHN)
8	93	$C_{13}H_{28}Cl_2N_3O_2PS$	392.33	87–88	3250 (broad, NH, OH), 1230, 990, 910	356 (100),[d] 358 (35)[d]	1.2 (s, 12 H, 4 CH_3), 1.9–2.2 (m, 10 H, 2 CH_2, 2 CH_2N, 2 NH), 3.5 (m, 4 H, 2 CH_2Cl), 4.3 (broad, 1 H, CHO)
15	88	$C_{14}H_{27}N_4O_4P$	346.37	150–151	3250 (broad, NH, OH), 1450, 1230, 950	347 (3), 174 (100), 156 (31)	1.1 (d, 12 H, 4 CH_3), 1.8–2.0 (m, 5 H, 2 CH_2, NH), 2.2 (d, 8 H, 4 CH_2N), 4.9 (broad, 1 H, CHO)

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%. [b] Dispersed in Nujol mull. [c] Chemical ionization with methane as the reactant gas. Relative abundance is shown in parentheses. [d] Since $M^+ + 1$ was weak, the two isotope peaks for ^{35}Cl and ^{37}Cl of $(M + 1) - HCl$ are reported.

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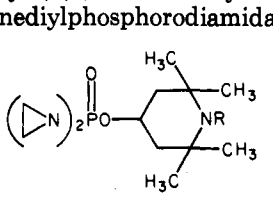
Table II. Anticancer Activity of Some TEPA and Thio-TEPA Analogues against P388 Leukemia in CD_2F_1 Mice[a]

no.	daily dose,[b] mg/kg	test/control survival, days	T/C × 100, %	30 day survival/total	ILS,[c] %
1 (Y = S)	6	22.2/9.2	241	1/6	141
	3	16.8/9.2	183	0/6	83
4a	15	8.5/9.2	92	0/6	-8
	12	18.7/9.2	203	1/6	103
	6	13.3/9.2	145	0/6	45
4b	25	18/9.2	196	0/6	96
	20	14/9.2	152	0/6	52
	10	12.3/9.2	134	0/6	34
5	160	13.8/9.6	144	0/6	44
	80	12.5/9.6	130	0/6	30
	40	11.2/9.6	117	0/6	17
	20	10.9/9.6	113	0/6	13
6	20	11.8/9.3	127	0/6	27
	15	10.8/9.3	116	0/6	16
7a	20	9.3/9.2	102	0/6	2
	15	11.8/9.2	129	0/6	29
	7.5	13/9.2	141	0/6	41
7b	15	12.5/9.3	134	0/6	34
	10	11.6/9.3	125	0/6	25
7c	20	10.8/9.3	116	0/6	16
	15	9.7/9.3	104	0/6	4
8	160	10.6/8	133	0/6	33
	80	9.2/8	115	0/6	15
10	20	11.2/9.3	120	0/6	20
	15	10.5/9.3	113	0/6	13
12a	40	17.3/9.2	188	0/6	88
	30	13/9.2	141	0/6	41
12b	20	12.3/9.3	133	0/6	33
	15	11.2/9.3	120	0/6	20
14	30	16.8/9.3	181	0/6	81
	15	13.2/9.3	142	0/6	42
15	30	11.8/8	148	0/6	48
	15	10/8	125	0/6	25
16a	15	9.7/9.2	105	0/6	5
	10	11.8/9.2	129	0/6	29
16b	15	7.8/8	98	0/6	-2
	10	9.8/8	123	0/6	23

[a] CD_2F_1 male mice of average weight 18-20 g were used. [b] Compounds injected intraperitoneally daily for 9 days after the day of transplantation of tumor (10^6 cells). [c] % ILS = percent increase in life span calculated from $[(T - C)/C]100$.

reductive methylamination with methylamine hydrochloride and sodium cyanoborohydride.[25,28] Although the synthesis of **11a** was described[25] to give an oil, in the present work, a crystalline compound was obtained by a chromatographic purification procedure. The TEPA-type compound **14**,[18] prepared in accordance with the Scheme III, was reduced by the ascorbic acid procedure[26] to the corresponding hydroxylamine derivative **15**. The attempted synthesis of the *N*-deoxy analogues of **4a** and **5** from 4-hydroxy-2,2,6,6-tetramethylpiperidine[24] following either Scheme I or II were unsuccessful. The TEPA-type compounds, 1-oxy-2,2,6,6-tetramethyl-4-piperidyl *N,N:N',N'*-di-1,2-ethanediylphosphorodiamidate[16,17] (**16a**) and

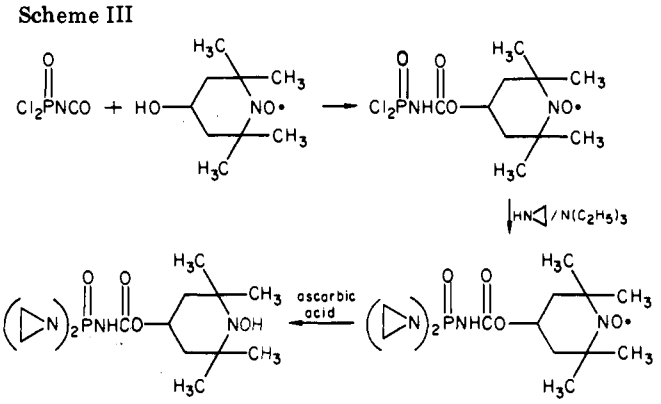


16a, R = O·
b, R = OH

1-hydroxy-2,2,6,6-tetramethyl-4-piperidyl *N,N:N',N'*-di-1,2-ethanediylphosphorodiamidate[26] (**16b**), were prepared by procedures described earlier.[16,17,26]

Biology. Compounds 1 (Y = S), 4-8, **10**, **12a**, **b**, and **14-16** were tested in vivo against the P388 lymphocytic leukemia in CD_2F_1 male mice by the National Cancer Institute protocol,[29] and the results are reported in Table II. Compounds with a test/control (T/C) percentage greater than 125 or with a percent increase in life span (% ILS) greater than 25 are considered to be active.[29] According to the protocol,[29] compounds **7c**, **10**, and **16b** are considered to be inactive. All other compounds were found to be active. Although thio-TEPA (1), Y = S, with a percent

Scheme III



15 14

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Table III. Comparison of the Percent ILS of the Corresponding Nitroxyl (>NO·) Compounds and Hydroxylamine (>NOH) Derivatives

>NO· % ILS (dose, mg/kg)	>NOH % ILS (dose, mg/kg)
4a 103 (12)	7a 41 (7.5)
4b 96 (25)	7b 34 (15)
5 44 (160)	8 33 (160)
6 27 (20)	7c 16 (20)
14 81 (30)	15 48 (30)
16a 29 (10)	16b 23 (10)

ILS of 141 at a dose of 6 mg/kg was found to be the most active compound in this group, it is also probably the most toxic.[21] Promising activity was confirmed[21] for compounds **4a** (% ILS = 103 at a dose of 12 mg/kg) and **4b** (% ILS = 96 at a dose of 25 mg/kg). Although the TEPA-type compound **14** was reported to be inactive against the P388 lymphocytic leukemia,[19] we found it to be considerable active with a % ILS of 81 at a dose of 30 mg/kg.

A comparison of the anticancer activities of the nitroxyl compounds of various structures, **4a**, **4b**, **5**, **6**, **14**, **16a**, with the corresponding hydroxylamine derivatives, **7a**, **7b**, **8**, **7c**, **15**, and **16b**, respectively (Table III), leaves no doubt about the superiority of the nitroxyl compounds. In our in vitro metabolism studies,[30] we found that the nitroxyl radicals are transformed to the hydroxylamine derivatives in mice and rat liver homogenates. Such nitroxyl reduction processes are considered to be reversible in vivo.[23] Similar biological reductions have been also observed by several other investigators.[31-33] In biological systems in vivo there probably exists an equilibrium between the nitroxyl compound and the corresponding nitroxylamine derivatives.[34,35] On the basis of our results, it appears that the reductive process involving the nitroxyl group constitutes a deactivation process of that drug. A comparison of activities of compound **4a** (% ILS = 103 at a dose of 12 mg/kg) and **16a** (% ILS = 29 at a dose of 10 mg/kg) clearly points to the contribution of the P=S group to the anticancer activity as compared to the P=O group in this type of compounds.

In one experiment, the nitroxyl compound **4b** with a % ILs of 96 at a dose of 25 mg/kg is slightly superior to the *N*-deoxy congener **12a**, with a ILS of 88 at a dose of 40 mg/kg, whereas in another example, the nitroxyl compound **6** with a % ILS of 27 at a dose of 20 mg/kg is about equal to the *N*-deoxy congener **12b**, with a % ILS of 33 at a dose of 20 mg/kg. Thus, within the experimental error of the in vivo experimentation, it can be assumed, at this point, that the nitroxyl compounds possess either about equal or higher activity than the deoxy congeners. It is conceivable that some of the *N*-deoxy congener is biologically converted in vivo into the nitroxyl radical.[34,35]

The bridge element between the phosphorus moiety and the tetramethylpiperidine ring seems to play a decisive part in the structure–activity relationship, whereby inductive, steric, and hydrogen-bonding effects could be involved. Thus, in the P=O series, the NHC(O)O bridge, as in compound **14** with a % ILS of 42 at a dose of 15 mg/kg, is superior to the O bridge, as in compound **16a** with a % ILS of 5 at a dose of 15 mg/kg. In the P=S series, the compound (**4a**) containing the O bridge seemes to be somewhat superior to the compound (**4b**) containing the NH bridge, since a 103% ILS is obtained for **4a** at a dose of 12 mg/kg, whereas a 96% ILS is attained for **4b** at a double dose of 25 mg/kg. Analogous results were obtained[21] in a study of **4a**,**b** under slightly different conditions. The introduction of a methyl group into the NH bridge results in a lowering of the activity, as evidenced by comparison of the % ILS of the nitroxyls **4b** and **6** and the corresponding hydroxylamine derivatives **7b**,**c**, respectively.

The opening of the aziridine ring as in **5** (% ILS = 44 at a dose of 160 mg/kg) results in a considerable lowering of the anticancer activity as compared to the ring compound **4a** (% ILS = 103 at a dose of 12 mg/kg), although it seems that the components for cross-linking DNA exist in both **4a** and **5**.

In conclusion, it was shown that the nitroxyl moiety has a decisive effect on the anticancer activity of the spin-labeled TEPA and thio-TEPA derivatives, since the corresponding hydroxylamine derivatives are less active (Table III). The activity of the deoxy congeners (**12a**,**b**) seems to be about equal (**12a**) or lower (**12b**) than the activity of the corresponding nitroxyl compounds **4b** and **6**, respectively. Since the nitroxyl derivatives in the TEPA and thio-TEPA series are usually easier to synthesize than the *N*-deoxy congeners and since the nitroxyl derivatives may also provide additional diagnostic features,[21] the nitroxyl derivatives would be preferred in clinical applications and research. The bridge between the phosphorus and the tetramethylpiperidine moieties has a definite contributing effect, whereby inductive, steric, and hydrogen bonding may play a role. In some cases, the P=S moiety is increasing the anticancer activity, as compared to the P=O moiety.

Experimental Section

Materials. All reagents were of the best quality commercially available and were used without further purification. Solvents were dried by standard procedures.[36] For bioassay, the CD_2F_1 (CDF_1) male mice were procured from Harlan Sprague–Dawley, Inc., Indianapolis, IN.

Analytical Procedures. All melting points were obtained with a Thomas-Hoover capillary melting point apparatus using a calibrated thermometer. The IR and ^1H NMR spectra were recorded on a Perkin-Elmer 735B and a Varian EM-360L (60 MHz) spectrometer, respectively. Mass spectra were obtained on a Hewlett Packard mass spectrometer, Model 5985 GS, using a direct insertion probe, a source pressure of 2×10^{-7} torr, and methane as reactant gas for chemical ionization. Therefore, the MS data are reported as ($M^+ + 1$). The EPR spectra of approximately 4.0×10^{-5} M solutions of nitroxyl radical in benzene were obtained on a Varian E-115 EPR spectrometer. Gas chromatography was performed on a Varian Aerograph, Model 90-P3, using a column packed with 20% SE-30. Microanalyses were performed either on a F&M Scientific Corp. carbon, hydrogen, nitrogen analyzer, Model 185, or by the Atlantic Microlab, Inc., Atlanta, GA. Homogeneity of the compounds was checked on precoated TLC plastic sheets of either silica gel 60 F_{254} or aluminum oxide 60 F_{254}, neutral (type E), layer thickness 0.2 mm, E. Merck Inc. The compounds were visualized either by exposure of the TLC plates to iodine vapor or by UV light. For column chromatography, either silica gel or neutral alumina, dry-column grade (activity III/20 mm) M Woelm, Germany, were used.

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Solvents were always removed in the workup procedures on a rotating evaporator at 30–35 °C (15-20 mm).

2,2,6,6-Tetramethyl-1-oxy-4-piperidyl *N,N'*-Bis(2-chloroethyl)phosphorodiamidothioate (5). A solution of **2a**[24] (3.44 g, 20 mmol) and triethylamine (2.02 g, 20 mmol) in methylene chloride (100 mL) was added dropwise to a stirred solution of thiophosphoryl chloride (3.38 g, 20 mmol) in methylene chloride (100 mL) at 0–4 °C. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 72 h. The solution was concentrated under vacuum to a semisolid mass, which was mixed with benzene (50 mL), and the crystalline triethylamine hydrochloride was filtered. The filtrate containing **3a** was diluted with methylene chloride (100 mL), and the solution was cooled to 0–4 °C. To this solution was added dropwise a solution of 2-chloroethylamine hydrochloride (4.64 g, 40 mmol) and triethylamine (8.08 g, 80 mmol) in methylene chloride (200 mL). The reaction mixture was allowed to warm to room temperature, and stirring was continued for 72 h. The mixture was concentrated under vacuum, and the concentrate was diluted with benzene (150 mL) and filtered. Concentration of the filtrate under vacuum gave crude product **5**, which, on the basis of TLC analysis (silica gel; benzene/acetone, 8:2, v/v), contained one major compound (R_f 0.55) and two minor impurities. The compound was purified by column chromatography on silica gel (1 × 14 in.) with benzene/acetone (8:2, v/v) as eluant. The fractions were monitored by TLC. After removal of the solvents under vacuum, recrystallization of the residual oil from hexane gave 4.1 g (52%) of pure **5**. After drying at 40 °C (0.1 mm) for 20 h, the compound melted at 120–121 °C. The mass spectrum (CI) was typical of a compound containing two chlorine atoms: MS (CI), *m/e* 392 ($M^+ + 1$), 394 ($M^+ + 3$), 396, ($M^+ + 5$), at a ratio of 100:60:15; IR (Nujol) 3200 (NH), 1450, 1010 cm^{-1}; ESR (C_6H_6) 3 lines, a_N = 15.5 G. Anal. ($C_{13}H_{27}Cl_2N_3O_2PS$) C, H, N (±0.35%).

***N,N':N',N'*-Di-1,2-ethanediyl-*N''*-methyl-*N''*-(2,2,6,6-tetramethyl-1-oxy-4-piperidiyl)phosphorothioic Triamide (6).** Commercial sodium hydride (0.63 g, 57% NaH in oil, 15 mmol) was freed from oil by rinsing it with benzene (3 × 10 mL). It was then added to a solutin of **4b**[14,21] (3.2 g, 10 mmol) and CH_3I (2.13 g, 15 mmol) in benzene (40 mL). The mixture was boiled for 20 h and then filtered. The filtrate was concentrated under vacuum to an oil, which, on the basis of TLC analysis (silica gel; benzene/acetone, 8:2, v/v), was impure **6**. The compound was purified by column chromatography on silica gel (1 × 14 in.) with methylene chloride as eluant. The fractions were monitored by TLC (silica gel; benzene/acetone, 8:2, v/v), and the fractions containing compound **6** were combined. Removal of the solvents under vacuum resulted in 2.2 g (67%) of crystalline **6**: mp 115–116 °C; MS, *m/e* 332 ($M^+ + 1$); ESR (C_6H_6) 3 lines, a_N = 15.5 G. Anal. ($C_{14}H_{28}N_4OPS$) C, H, N (±0.35%).

Preparation of 1-Hydroxy-2,2,6,6-tetramethyl-4-piperidyl *N,N':N',N'*-phosphorodiamidothioate (7a), *N,N':N',N'*-Di-1,2-ethanediyl-*N''*-(1-hydroxy-2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic Triamide (7b), *N,N':N',N'*-Di-1,2-ethanediyl-*N''*-methyl-*N''*-(1-hydroxy-2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic Triamide (7c), 1-Hydroxy-2,2,6,6-tetramethyl-4-piperidyl *N,N'*-Bis(2-chloroethyl)phosphorodiamidothioate (8), and *N,N':N',N'*-Di-1,2-ethanediyl-*N''*-[(1-hydroxy-2,2,6,6-tetramethyl-4-piperidyloxy)carbonyl]phosphoric Triamide (15). General Procedure. To a stirred solution of nitroxyl radical [1-oxy-2,2,6,6-tetramethyl-4-piperidyl *N,N:N',N'*-di-1,2-ethanediylphosphorodiamidothioate (**4a**), *N,N:N',N'*-di-1,2-ethanediyl-*N''*-(1-oxy-2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic triamide (**4b**), 1-oxy-2,2,6,6-tetramethyl-4-piperidyl *N,N'*-bis(2-chloroethyl)-phosphorodiamidothioate (**5**), *N,N:N',N'*-di-1,2-ethanediyl-*N''*-methyl-*N''*-(1-oxy-2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic triamide (**6**), or *N,N:N',N'*-di-1,2-ethanediyl-*N''*-[(1-oxy-2,2,6,6-tetramethyl-4-piperidyloxy)carbonyl]phosphoric triamide, (**14**), (10 mmol) in anhydrous methanol (20 mL) was added a solution of ascorbic acid (1.94, 1.1 mmol) in anhydrous methanol (25 mL). The completion of the reaction was indicated by the disappearance of color within 5 min. The solution was evaporated under vacuum to dryness. The remaining solid was extracted with methylene chloride (3 × 15 mL), and the combined extracts were filtered. The filtrate was concentrated to 15 mL under vacuum, and the concentrate was chromatographed on a silica gel column (1 × 14 in.). The column was first eluted with methylene chloride until no colored impurities remained, and then it was eluted with methylene chloride/ethyl acetate (1:1, v/v). The fractions were monitored by TLC (silica gel; ethyl acetate/methanol, 9:1, v/v). Removal of the solvents from the methylene chloride/ethyl acetate fractions under vacuum gave the pure hydroxylamines listed in the Table I.

4-(Methylamino)-1-oxy-2,2,6,6-tetramethylpiperidine (11a). The procedure, which is described in literature,[25] was modified as follows. A solution of methylamine hydrochlride (23.8 g, 353 mmol) in methanol (500 mL) was adjusted to pH 7–8 by dissolving solid sodium hydroxide in it. To this solution was added a solution of **13a**[24] (10 g, 58.8 mmol) in methanol (100 mL). After the solution was stirred for 10 min, solid sodium cyanoborohydride (2.5 g, 39.7 mmol) was added to the solution all at once. The reaction mixture was stirred for 48 h at room temperature and filtered, and the filtrate was concentrated under vacuum. The remaining solid was dissolved in water (80 mL), and the solution was adjusted to pH 13 with aqueous 12 N potassium hydroxide solution. The solution was then saturated with solid sodium chloride and extracted with ethyl ether (5 × 60 mL). The combined ether layers were dried (Na_2SO_4) and filtered, and the filtrate was concentrated under vacuum. TLC analysis (neutral alumina; methylene chloride/methanol, 10:0.4, v/v) of the remaining red oil (10.9 g) indicated a mixture of **11a** (R_f 0.5) and a less polar impurity (R_f 0.8). Therefore, the oil was mixed with benzene (10 mL) and chromotographed on a neutral alumina column (1 × 14 in.) with methylene chloride/ethyl acetate (9:1, v/v) as eluant. The fractions were monitored by TLC. Concentration of the fractions containing the pure product **11a** (R_f 0.5) under vacuum gave 7.9 g (73%) of **11a**: bp 73–75 °C (0.11 mm) [lit.[25] bp 56–59 °C (0.07 mm)]. The compound was crystallized from petroleum ether at −10 °C: mp 52–53 °C; IR (neat) 3260 (NH) cm^{-1}; MS, *m/e* 186 ($M^+ + 1$, 100), 171 (44), 169 (45); ESR (C_6H_6) 3 lines, a_N = 15.5 G.

4-(Methylamino)-2,2,6,6-tetramethylpiperidine (11b). A solution of methylamine hydrochloride (23.8 g, 352.6 mmol) and **13b**[24] (10 g, 64.5 mmol) in methanol (400 mL) was adjusted to pH 7–8 with concentrated hydrochloric acid. To this solution was added sodium cyanoborohydride (2.4 g, 38.1 mmol) in methanol (100 mL), and the solution was stirred for 6 days at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under vacuum. The remaining oil was dissolved in water (40 mL), and the solution was adjusted to pH 13 by dissolving solid sodium hydroxide in it. The solution was then saturated with solid sodium chloride and extracted with ethyl ether (3 × 60 mL). The combined ether extracts were dried (Na_2SO_4) and filtered, and the filtrate was concentrated under vacuum. Distillation of the remaining oil (6.4 g) gave 5.4 g (50%) of **11b**: bp 82–84 °C (15 mm); n^{25}_D 1.4656. The compound was found to be pure by TLC, R_f 0.5 (neutral alumina; benzene/methylene chloride/methanol, 4:4:0.5, v/v) and GLC (injection 190 °C, column 185 °C, detector 215 °C); IR (neat) 3250 (NH) cm^{-1}; MS, *m/e* 171 ($M^+ + 1$, 100), 140 (49), 83 (21); ^1H NMR ($CDCl_3/Me_3Si$) δ 1.0 (d, 6 H, 2 CH_3), 1.3 (d, 6 H, 2 CH_3), 1.7–2.2 (m, 6 H, 2 CH_2, 2 NH), 2.6 (d, 3 H, CH_3N), 3.2 (m, 1 H, CHO). Anal. ($C_{10}H_{22}N_2$) C, H, N (±0.35%).

***N*-(2-Chloroethyl)-*N'*-1,2-ethanediyl-*N''*-methyl-*N''*-(1-oxy-2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic Triamide (10).** To a stirred solution of **9**[7,17] (1.82 g, 10 mmol) in benzene (100 mL) at 0–4 °C was added dropwise a solution of **11a** (1.86 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) in methylene chloride (50 mL). The reaction mixture was allowed to warm to room temperature, and the stirring was continued for 4 days. The mixture was concentrated under vacuum, and the concentrate was mixed with benzene (100 mL) and filtered. The filtrate was concentrated to 10 mL, and the concentrate was chromatographed on a silica gel column (1 × 14 in.) with benzene/acetone (9:1, v/v) as eluant. Concentration of the fraction containing the first red band gave 0.7 g (21%) of **10** as oil. The oil was found by TLC analysis (silica gel; benzene/acetone, 8:2, v/v) to be pure **10** (R_f 0.7), n^{25}_D 1.5355; IR (neat) 3225 (NH), 1440, 1235, 910 cm^{-1}; MS, *m/e* 368 ($M^+ + 1$, 100), 353 (42), 333 (29), 332 (27); ESR (C_6H_6) 3 lines, a_N = 15.5 G. Anal. ($C_{14}H_{29}ClN_4OPS$) C, H, N (±0.35%). Further elution of the column with benzene/acetone (8:2, v/v) and subsequent removal of the solvents

yielded 0.6 g (18%) of the crystalline product **6**, found to be pure by TLC (silica gel; benzene/acetone, 8:2, v/v): R_f 0.6; mp 115–116 °C.

Preparation of 6 from Compound 10. Commercial sodium hydride (0.063 g, 57% NaH in oil, 1.5 mmol) was freed from oil by rinsing it with benzene (3 × 5 mL). It was then added to a solution of **10** (0.331 g, 1 mmol) in benzene (20 mL). The reaction mixture was stirred for 48 h and filtered, and the filtrate was concentrated under vacuum. Recrystallization of the remaining solid from ethyl ether/petroleum ether at −10 °C gave 0.250 g (86%) of pure **6**, mp 115–116 °C.

***N,N*:*N'*,*N'*-Di-1,2-ethanediyl-*N''*-(2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic Triamide (12a).** To a stirred solution of **9**[7,17] (1.82 g, 10 mmol) in benzene/methylene chloride (100 mL, 2:1, v/v) at 0–4 °C was added a solution of 4-amino-2,2,6,6-tetramethylpiperidine (**11c**; 3.13 g, 20 mmol) in methylene chloride (50 mL). The reaction mixture was allowed to warm to room temperature, and the stirring was continued for 16 h. The mixture was filtered, and the filtrate was concentrated under vacuum. The remaining solid was dispersed in benzene (30 mL), and the dispersion was filtered. The filtrate was concentrated. The remaining solid was mixed with ethyl ether (30 mL), and the solution was filtered. Concentration of the filtrate gave a solid, which on recrystallization from petroleum ether yielded 2.2 g (70%) of crystalline **12a**. TLC analysis (neutral alumina; benzene/methanol, 9:1, v/v) indicated a pure product **12a**: R_f 0.7; mp 121–122 °C; IR (Nujol mull) 3350 (NH), 1430, 1240, 920 cm^{-1}; MS 303 ($M^+ + 1$, 100), 287 (16), 260 (21); ^1H NMR ($CDCl_3/Me_3Si$), δ 1.1 (d, 12 H, 4 CH_3), 1.7–2.2 (m, 13 H, 2 CH_2, 4 CH_2N, NH), 2.5 (t, 1 H, CHN), 3.7 (broad, 1 H, NH). Anal. ($C_{13}H_{27}N_4PS$) C, H, N (±0.35).

***N,N*:*N'*,*N'*-Di-1,2-ethanediyl-*N''*-methyl-*N'''*-(2,2,6,6-tetramethyl-4-piperidyl)phosphorothioic Triamide (12b).** To a stirred solution of **9**[7,17] (1.82 g, 10 mmol) in benzene/methylene chloride (200 mL, 1:1, v/v) at 0–4 °C was added all at once a solution of **11b** (3.4 g, 20 mmol) in methylene chloride (50 mL). The reaction mixture was allowed to warm to room temperature, and the stirring was continued for 72 h. The solution was then concentrated under vacuum, and the remaining solid was mixed with benzene (80 mL) and filtered, and the filtrate was concentrated to 10 mL. The concentrate was chromatographed on a neutral alumina column (1 × 14 in.) with benzene/ethyl acetate (8:2, v/v) as eluant. The fractions were monitored by TLC (neutral alumina; benzene/methanol, 9:0.8, v/v). Concentration of the fractions containing the pure compound, R_f 0.7, under vacuum gave 1.2 g (36%) of the crystalline **12b**: mp 94–95 °C; IR (Nujol mull) 1450, 1370, 1250, 920 cm^{-1}; MS, *m/e* 317 ($M^+ + 1$, 100), 301 (24); ^1H NMR ($CDCl_3/Me_4Si$) δ 1.3 (d, 12 H, 4 CH_3), 1.8–2.7 (m, 12 H, 2 CH_2, 4 CH_2N), 2.9–3.3(m, 4 H, CH_3N, CHN), 5 (broad, 1 H, NH). Anal. ($C_{14}H_{29}N_4PS$) C, H, N (±0.35%).

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Registry No. **1**, 52-24-4; **2a**, 2226-96-2; **2b**, 14691-88-4; **3a**, 51585-38-7; **3b**, 89486-95-3; **4a**, 51526-59-1; **4b**, 33683-34-0; **5**, 89486-96-4; **6**, 89486-97-5; **7a**, 78996-69-7; **7b**, 89486-98-6; **7c**, 89486-99-7; **8**, 89487-00-3; **9**, 62679-38-3; **10**, 89487-01-4; **11a**, 42585-33-1; **11b**, 40327-96-6; **11c**, 36768-62-4; **12a**, 89487-02-5; **12b**, 89487-03-6; **13a**, 2896-70-0; **13b**, 826-36-8; **14**, 70484-16-1; **15**, 89487-04-7; **16a**, 51526-57-9; **16b**, 64566-76-3; $PSCl_3$, 3982-91-0; $ClCH_2CH_2NH_2$, 689-98-5; aziridine, 151-56-4; phosphorisocyanatidic dichloride, 870-30-4; 4-(dichlorophosphinoaminocarbonyl)-1-oxy-2,2,6,6-tetramethylpiperidine, 89487-05-8.

Substituent Branching in Phenethylamine-Type Hallucinogens: A Comparison of 1-[2,5-Dimethoxy-4-(2-butyl)phenyl]-2-aminopropane and 1-[2,5-Dimethoxy-4-(2-methylpropyl)phenyl]-2-aminopropane

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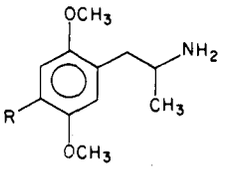
Two novel hallucinogen analogues related to 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, STP) were synthesized and evaluated in the two-lever drug discrimination paradigm by using 0.08 mg/kg of LSD as the training drug stimulus. The two compounds differ from each other only with respect to the point of branching in the 4-alkyl group. However, pharmacological evaluation revealed a clear difference in potency and degree of LSD generalization for the two isomers. Branching adjacent to the ring, as in the 4-(2-butyl) analogue, may provide steric interference to the formation of the drug–receptor complex, while branching one methylene unit removed from the ring, as in the 4-(2-methylpropyl) analogue, poses less of a steric problem for the drug–receptor interaction. This is consistent with the idea that formation of a charge-transfer complex between the hallucinogen molecule and the receptor may be one of the features of this drug–receptor interaction.

In the 1-phenylisopropylamine hallucinogens, "substituted amphetamines", maximum activity resides in compounds with the 2,4,5 aromatic trisubstitution pattern.[1] Greatest potency results when the substituent at the 4-position is an alkyl or halogen (**1**).[2] Variation of the 4-alkyl group in 1-(2,5-dimethoxy-4-alkylphenyl)isopropylamine has shown that optimum activity resides in analogues containing a straight chain, while branching adjacent to the ring drastically attenuates activity.[3,4]

It has been suggested that a planar surface may serve as a model for the interaction of hallucinogenic phenethylamines with the receptor.[5,6] Furthermore, present evidence suggests that binding to the receptor may involve



1, R = alkyl; halogen
2, R = $CH_2CH(CH_3)_2$
3, R = $CH(CH_3)CH_2CH_3$

the formation of a charge-transfer complex.[7-9] The steric bulk of the 4-alkyl group may therefore have an important

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