Table VII[a]

irrad dose, Gy	N	deaths (no.)	p (death)	q (survival)	p·q
9.5	20	0	0	1	0
11.5	20	0	0	1	0
12	20	4	0.2	0.8	0.16
12.5	20	8	0.4	0.6	0.24
13	20	18	0.9	0.1	0.09
13.5	20	20	1	0	0
			$\sum p = 2.5$		$\sum p \cdot q = 0.49$

[a] LD_{50} = 13.5–0.5(2.5–0.5) = 12.5 Gy. 13.5 Gy = lowest irradiation dose giving 100% lethality. 0.5 Gy = difference between two consecutive doses. 2.5 = $\sum p$. $\sigma = (0.5^2 0.49/19)^{1/2} = 0.08$. 0.49 = $\sum p \cdot q$. 19 = $N - 1$. LD_{50} = 12.5 ± 0.16 Gy ≅ 12.5 ± 0.2 Gy ($p < 0.05$). LD_{50} control mice = 8.1 ± 0.2 Gy ($p < 0.05$). DRF = (12.5 ± 0.2)/(8.1 ± 0.2) = 1.54 ± 0.07 ≅ 1.55 ± 0.1 ($p < 0.05$).

doses were tested in order to evaluate the irradiation LD_{50} of protected mice by the Karber method (calculated or graphic).[22]

An example is given for compound **37** in Appendix 1 and Figure 1.

The radiosensitivity of the strain was regularly monitored by the determination of lethality curves of males and females. The LD_{50}/30 days was equal to 7.7 ± 0.3 Gy for males and 8.1 ± 0.2 Gy for females ($P < 0.05$).

Under these conditions significant protection was observed with a DRF value superior to 1.15.

(3) All the compounds were easily dissolved in distilled water. The toxicity was evaluated by a Probit analysis of the LD_{50}, the dose range being determined in a preliminary study. Five groups of 10 mice were then injected with different doses within this range.

(4) Whole-body irradiation were performed with a ^{60}Co γ-ray source (6×10^{13} Bq). The dose rate was equal to 0.65 Gy/min. For the exposure, mice were positioned inside an altuglass box divided into 30 cells in a homogeneous field 28.5 cm × 28.5 cm. The dosimetry was carried out by means of ionization chamber dosimeters and lithium fluoride thermoluminescent dosimeters.

Each irradiation session included a group of 20 mice irradiated with 9 or 9.5 Gy according to their sex after intraperitoneal injection of the solvent alone. A 100% lethality was always observed with a mean survival time equal to 11 ± 1 days. Furthermore, a group of eight unirradiated mice received the test compound with a dose equal to half of its LD_{50}, in order to check for toxic lethality among the injected and irradiated mice. For all the compounds, these animals were alive 30 days after injection.

Remarks

At first sight, injection of equimolecular doses of compounds might seem preferable. However, this would lead to early deaths for the more toxic compounds or to poor protection for the less toxic ones, since radioprotection generally decreases rapidly with decreasing doses.

Acknowledgment. We are grateful to the Direction des Recherches Etudes et Techniques (DRET, Paris, France) Grants 83-1040 and 83-1203 for the support of this work.

Appendix

The determination of irradiation LD_{50}/30 days and DRF in mice receiving an ip injection of 750 mg/kg of **37**, 15 min before irradiation, calculated by the Karber method, is given in Table VII.

Registry No. **4**, 97313-99-0; **5**, 97314-04-0; **6**, 89052-99-3; **7**, 97314-02-8; **8**, 97314-00-6; **9**, 97314-14-2; **10**, 104071-71-8; **11**, 104071-72-9; **12**, 104071-73-0; **13**, 97314-06-2; **14**, 104071-74-1; **15**, 97313-68-3; **15** (free base), 97313-69-4; **16**, 97314-05-1; **17**, 104071-75-2; **17** (free base), 104071-91-2; **18**, 97314-03-9; **18** (free base), 97313-71-8; **19**, 97314-01-7; **19** (free base), 97313-70-7; **20**, 97314-16-4; **21**, 104071-76-3; **21** (free base), 104071-92-3; **22**, 104071-77-4; **22** (free base), 104071-93-4; **23**, 104071-79-6; **23** (free base), 104071-78-5; **24**, 97314-07-3; **24** (free base), 97313-72-9; **25**, 104071-81-0; **25** (free base), 104071-80-9; **26**, 97314-08-4; **27**, 104071-82-1; **28**, 104071-83-2; **29**, 97313-97-8; **30**, 97314-09-5; **31**, 97314-11-9; **32**, 104071-84-3; **33**, 104071-85-4; **34**, 104071-86-5; **35**, 104089-97-6; **36**, 97314-10-8; **36** (free base), 31060-88-5; **37**, 97314-12-0; **38**, 104071-87-6; **38** (free base), 646-08-2; **39**, 104071-88-7; **39** (free base), 73321-59-2; **40**, 97314-13-1; **40** (free base), 97313-79-6; **41**, 97313-95-6; **42**, 104071-89-8; **43**, 104071-90-1; **43** (free base), 72235-32-6; **44**, 97313-83-2; **45**, 97313-84-3; Boc-Ala-OSu, 3392-05-0; Z-β-Ala-OH, 2304-94-1; Z-GABA-OH, 5105-78-2; Z-L-Ala-OH, 1142-20-7; $H_2N(CH_2)_2SCOEt \cdot HCl$, 104071-94-5; $H_2N(CH_2)_2SCOBu\text{-}t \cdot HCl$, 104071-95-6; Boc-Ala-OH, 15761-38-3; $H_2N(CH_2)_2SCOCH_2Cl \cdot HCl$, 90587-64-7; $H_2N(CH_2)_2SCOCHCl_2 \cdot HCl$, 97313-96-7; $H_2N(CH_2)_2SCOCCl_3 \cdot HCl$, 104071-96-7; (benzyloxycarbonyl)glycine, 1138-80-3; *S*-acetylcysteamine hydrochloride, 17612-91-8; (*tert*-butyloxycarbonyl)glycine, 4530-20-5; succinimido (*tert*-butyloxycarbonyl)glycinate, 3392-07-2; cystamine, 51-85-4; cystamine hydrochloride, 1072-22-6; succinimido (benzyloxycarbonyl)glycinate, 2899-60-7.

In the Search for New Anticancer Drugs. 19. A Predictive Design of *N,N:N′,N′:N″,N″*-Tri-1,2-ethanediylphosphoric Triamide (TEPA) Analogues

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The nitroxyl-labeled analogues of *N,N:N′,N′:N″,N″*-tri-1,2-ethanediylphosphoric triamide (TEPA), *N,N:N′,N′*-bis(1,2-ethanediyl)-*N″*-[[(2,2,6,6-tetramethyl-1-oxypiperidin-4-yl)amino]carbonyl]phosphoric triamide (**5a**) and *N,N:N′,N′*-bis(1,2-ethanediyl)-*N″*-[[(2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)amino]carbonyl]phosphoric triamide (**11a**), possess therapeutic indexes that are 8–12 times higher than those of thio-TEPA (**1**) and TEPA (**2**). The introduction of methyl groups into the aziridine ring, or the replacement of the nitroxyl moiety with hydroxylamine or amine derivatives, or with an adamantane moiety, results in compounds of lesser activity. An attempt is made to rationalize these results using a lipophilicity scale. A predictive design pattern is established.

N,N:N′,N′:N″,N″-Tri-1,2-ethanediylphosphorothioic triamide (thio-TEPA) (**1**) was introduced[1] more than 30 years ago into clinical oncology, and it has been applied in Hodgkin's disease,[2] metastatic carcinoma of the breast,[3,4] superficial carcinoma of the bladder,[5–9] carcinomatous meningitis,[10,11] and ovarian cancer.[12] TEPA (**2**), which

(1) Sykes, M.; Karnofsky, D.; Phillips, F.; Burchenal, J. *Cancer* **1953**, *6*, 142.
(2) Silver, R.; Lauper, R. D.; Jarowski, C. *A Synopsis of Cancer Chemotherapy*; Dun-Donnelly: New York, 1977; p 35.
(3) Hart, R. D.; Perloff, M.; Holland, J. F. *Cancer* **1981**, *48*, 1522.
(4) Perloff, M.; Hart, R. D.; Holland, J. F. *Cancer* **1977**, *39*, 1289.
(5) Drew, J. E.; Marshall, V. F. *J. Urol.* **1968**, *99*, 740.
(6) Jones, H. C.; Swinney, J. *Lancet* **1961**, *2*, 615.
(7) National Bladder Cancer Collaborative Group A. *Cancer Res.* **1977**, *37*, 2916.

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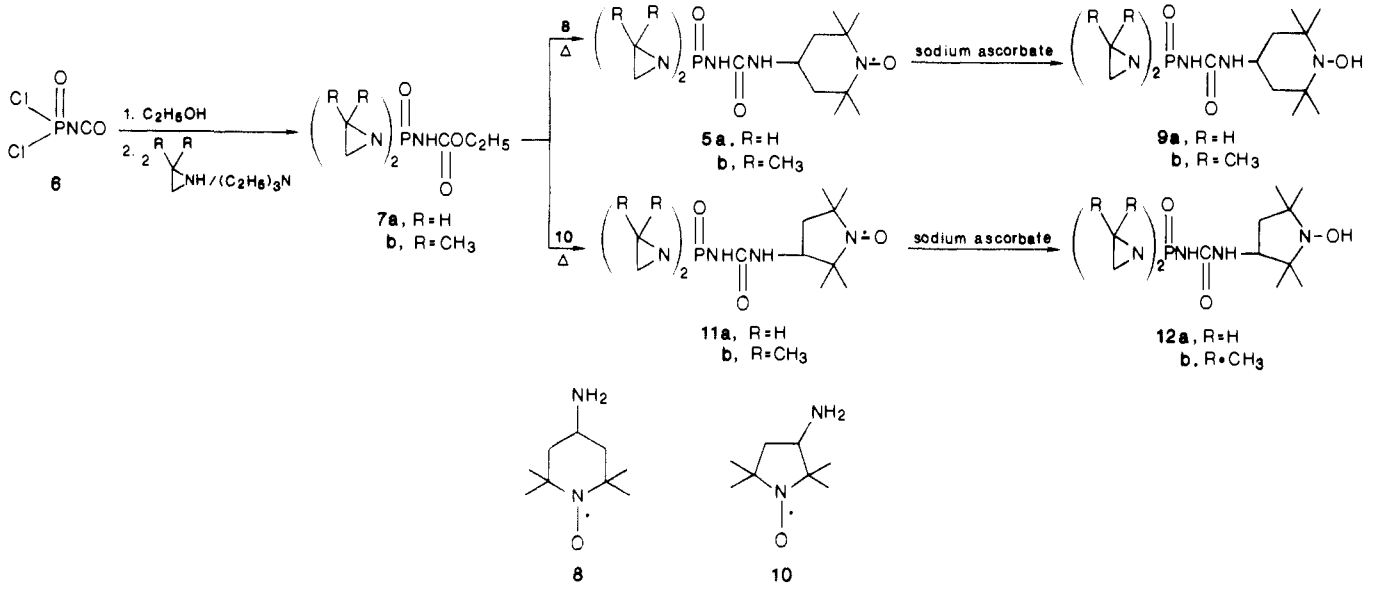
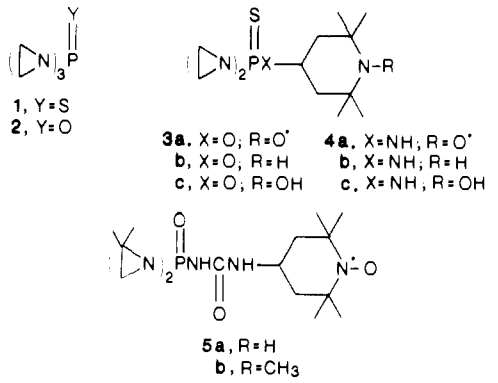
Scheme I



might be the first metabolite of **1**,[13,14] was not applied clinically presumably because of lower stability[13] than that possessed by **1**. Since both compounds (**1** and **2**) are highly toxic, e.g., the LD_{50} of **1** administered iv to rats was found[15,16] to be about 13–18 mg/kg, many attempts have been made over the years to develop more active and/or less toxic analogues of **1** and **2**,[17] however, apparently, without a decisive success. In more recent years, it has been shown[18–22] that the replacement of the very toxic aziridine group with the nontoxic nitroxyl radical results in compounds **3a**, **4a**, and **5a** with promising pharmacological properties. Thus, the most effective compound **5a** has a therapeutic index that is about 9.6 times higher than that of **1**[20,22,23] and 12 times higher than that of **2**. The nitroxyl-labeled compounds have another advantage over the parent compounds, namely they can be followed in pharmacokinetic studies on animal models by the ESR technique,[14] which can be more readily accomplished than radiolabeling of the parent compounds. It was further shown[19,21] that while the nitroxyl moiety has a definite measurable effect on the anticancer activity of derivatives **3a**, **4a**, and **5a**, as compared to the corresponding reduced moieties in **3b**, **3c**, and **4b**, **4c**, and **9a**, **15a** (Scheme I and II), the nitroxyl moiety, per se, has no anticancer activity,[23] is relatively nontoxic,[23] is not carcinogenic or mutagenic,[24]



has little effect on cell growth and cell cycle kinetics,[25] and exhibits no synergistic effect.[21] Hence, the question arises as to what is the function of the nitroxyl moiety, which mediates the anticancer properties of the nitroxyl-labeled drugs. In answer to this question, the following hypothesis is proposed. The nitroxyl moiety is acting as a carrier that facilitates a preferential transport of the drug through membranes of cancerous cells as compared to normal cells, on its way to the cellular DNA. The role of DNA is invoked on the basis of results obtained with flow cytometry[25] that the spin-labeled alkylating agents **3a** and **4a**, similarly to **1** and **2**, cause a retardation of the cell progression through the S phase.

In order to test this hypothesis, an attempt was made to correlate some structural parameters of a series of antineoplastic agents with their hydrophilicity/hydropho-

(8) Veenema, R. J.; Dean, A. L., Jr.; Roberts, M.; Fingerhut, B.; Chowdhury, B. K.; Tarassoly, M. *J. Urol.* **1962**, *88*, 60.
(9) Veenema, R. J.; Dean, A. L., Jr.; Uson, A. C.; Roberts, M.; Longo, F. *J. Urol.* **1969**, *101*, 711.
(10) Gutin, P. H.; Levi, J. A.; Wiernik, P. H.; Walker, M. D. *Cancer Treat. Rep.* **1977**, *61*, 885.
(11) Trump, D. L.; Grassman, S. A.; Thompson, G.; Murray, K.; Wharam, M. *Cancer Treat. Rep.* **1982**, *66*, 1549.
(12) *Chemotherapy of Cancer*, 2nd. ed.; Carter, S. K., Bakowski, M. T., Hellmann, K. Eds.; Wiley: New York, 1981.
(13) Osswald, H. *Maligne Tunmoren*; Schmähl, D., Editio Cantor: Aulendorf, 1981; p 396 and references therein.
(14) Gutierrez, P. L.; Cohen, B. E.; Sosnovsky, G.; Davis, A. T.; Johnson, M. E. *Cancer Chemother. Pharm.* **1985**, *15*, 185.
(15) Scherf, H. R.; Krüger, C.; Karsten, C. *Arzneim.-Forsch.* **1970**, *20*, 1467.
(16) Konovalova, N. P.; Emanuel, N. M. *Current Chemotherapy and Immunotherapy*; Periti, P., Grassi, J. G., Eds.; The American Society of Microbiology: Washington, DC, 1981; Vol. II, p 1543.
(17) MacDiarmid, J. E.; Rose, W. C.; Biddle, W. C.; Perlman, M. E.; Breiner, R. G.; Ambrus, J. L.; Bardos, T. J. *J. Med. Chem.* **1985**, *28*, 1685 and references therein.
(18) Emanuel, N. M.; Konovalova, N. P.; Djachkovskaya, R. F. *Cancer Treat. Rep.* **1976**, *60*, 1605.
(19) Sosnovsky, G.; Paul, B. D. *J. Med. Chem.* **1984**, *27*, 782.
(20) Sosnovsky, G.; Li, S. W. *Life Sci.* **1985**, *36*, 1473.
(21) Sosnovsky, G.; Li, S. W. *Cancer Lett.* **1985**, *25*, 255.
(22) Sosnovsky, G.; Li, S. W. *Drugs Future* **1985**, *10*, 213.
(23) Gutierrez, P. L.; Konieczny, M.; Sosnovsky, G. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1981**, *36B*, 1612.
(24) Afzal, V.; Brasch, R. C.; Nitecki, D. E.; Wolff, S. *Invest. Radiol.* **1984**, *19*, 549.
(25) Ross, D. D.; Gutierrez, P. L.; Sosnovsky, G. *Am. Assoc. Cancer Res., Preclinical Pharmacol. Exp. Ther.* Toronto, Canada, 1984; Abstract 1158.

Table I. Physical Properties of N^3-Substituted Urea Analogues of TEPA and 2,2-Dimethyl-TEPA

compd	yield, %	mp (dec), °C	mol. formula[a,b]	MS,[c] m/e	^{31}P NMR,[d] ppm
5a	63	102–103[e]	$C_{14}H_{27}N_5O_3P$ (344.38)	345 (M^+ + 1, 4), 257 (M^+ - 87, 4), 202 (M^+ - 142, 12), 174 (M^+ - 170, 100)	-32.55[f]
5b	75	142–143[e]	$C_{18}H_{35}N_5O_3P$ (400.48)	401 (M^+ + 1, 100), 400 (M^+, 51), 386 (M^+ - 14, 7)	-30.18[f]
9a	83	158–160	$C_{14}H_{28}N_5O_3P$ (345.38)	346 (M^+ + 1, 3), 345 (M^+, 21), 149 (M^+ - 196, 100)	-32.86
9b	78	192–194	$C_{18}H_{36}N_5O_3P$ (401.48)	402 (M^+ + 1, 100), 401 (M^+, 36), 331 (M^+ - 70, 16)	-30.97
11a	44	122–123[e]	$C_{13}H_{25}N_5O_3P$ (330.35)	331 (M^+ + 1, 100), 288 (M^+ - 42, 14), 174 (M^+ - 156, 32)	-31.97[f]
11b	33	152–154[e]	$C_{17}H_{33}N_5O_3P$ (387.48)	388 (M^+ + 1, 100), 387 (M^+, 50), 317 (M^+ - 70, 22)	-28.96[f]
12a	62	176–178	$C_{13}H_{26}N_5O_3P$ (331.38)	332 (M^+ + 1, 14), 331 (M^+, 34), 135 (M^+ - 196, 100)	-32.12
12b	58	200–205	$C_{17}H_{34}N_5O_3P$ (387.48)	388 (M^+ + 1, 100), 387 (M^+, 37), 317 (M^+ - 70, 36)	-29.98
15a	80	142–143.5	$C_{14}H_{28}N_5O_2P$ (329.38)	330 (M^+ + 1, 3), 314 (M^+ - 15, 4), 174 (M^+ - 155, 20)	-32.86
15b	62	178–180	$C_{18}H_{36}N_5O_2P$ (385.38)	386 (M^+ + 1, 42), 385 (M^+, 24), 315 (M^+ - 70, 100)	-30.46
16a	66	101–104	$C_{15}H_{25}N_4O_2P$ (324.46)	325 (M^+ + 1, 82), 324 (M^+, 100), 282 (M^+ - 42, 65)	-20.97
16b	71	129–131	$C_{19}H_{33}N_4O_2P$ (380.46)	381 (M^+ + 1, 74), 380 (M^+, 12), 379 (M^+ - 1, 40), 205 (M^+ - 175, 100)	-18.97

[a] The microanalyses were in satisfactory agreement with the calculated values (C, H, and N) within ±0.4%. [b] The IR spectra (Nujol) were consistent with the structures. [c] Relative percent intensities of the peaks. [d] All shifts are relative to 85% phosphoric acid, which was used as the external standard, and D_2O as the solvent. [e] ESR: 3 lines, a_N - 15.0 G. [f] The nitroxyl radicals were reduced to the corresponding hydroxylamine derivatives as described in the Experimental Section, and the ^{31}P NMR spectra were recorded as described in ref 27.

Table II. Partition Coefficients and Anticancer Activities of N^3-Substituted Urea Analogues of TEPA and 2,2-Dimethyl-TEPA against P388 Leukemia in CD_2F_1 Male Mice

compd	ILS_{40},[a] mg/kg per day	optimum dose[b]	ILS,[c] %	ILS_{max},[d] %	5-day wt. change,[e] %	cures[f] (survivors/totals)	therapeutic ratio[g]	partn[h] coeff (P) ESR	partn[h] coeff (P) ^{31}P NMR	log P ESR	log P ^{31}P NMR
thio-TEPA (1)	2.18	6	141		-2.1	0/6	2.75[i]		2.54		0.40
TEPA (2)	2.75	6	135		-4.0	0/6	2.20		0.24		-0.62
5-fluorouracil		200[j]	79		-4.0	0/6					
3a		12[k]	103[k]				5.15	60		+1.77	
4a		25[k]	96[k]				2.73	37		+1.57	
5a[l]	3.4	90	170	439	-2.4	6/6	26.5	0.42	0.38	-0.40	-0.43
5b		90	62		+7.3	0/6		2.70	2.50	+0.44	+0.40
9a	16.0	100	153	292	-4.5	2/6	6.2		0.30		-0.52
9b		90	44		+4.8	0/6			3.04		+0.48
11a	4.0	90	178	387	-7.3	3/6	22.5	0.36	0.33	-0.44	-0.48
11b		90	56		+6.2	0/6		2.91	2.81	+0.46	+0.45
12a	19.0	100	142	232	-5.6	2/6	5.3		0.25		-0.60
12b		90	40		+6.1	0/6			3.22		+0.51
15a	17.5	140	157	224	-8.8	2/6	8.0		0.15		-0.80
15b		140	38			0/6			3.52		+0.55
16a		60	35			0/6			65		+1.81
16b		60	32		14.0	0/6			95		+1.98

[a] Daily dose eliciting a 40% increase in life span over the control. [b] Daily dose eliciting the maximum increase in life span. [c] The results obtained on day 30. [d] The results obtained on day 60. [e] The average percentage weight change on day 5 was taken as a measure of drug toxicity. [f] Tumor-free survivors after day 60. [g] Therapeutic ratio = optimum dose/dose ILS_{40}. [h] The partition coefficients, P = [compound in 1-octanol]/[compound in water], were measured either by ^{31}P NMR technique (ref 27) or by ESR spectroscopy (ref 28). [i] Reference 23. [j] 5-Fluorouracil in a single dose of 200 mg/kg per day, administered on day 1, was used as a positive control, according to the NCI protocol (ref 49). [k] Reference 19. [l] LD_{50} = 270 mg/kg; LD_{50} for thio-TEPA is 18 mg/kg (ref 16).

bicity (lipophilicity) properties and the antineoplastic activity.

Results and Discussion

Chemistry. (a) Analytical. There are several analytical techniques for the determination of partition coefficients, namely gas chromatography,[26] Nessler's analysis,[26] visible and ultraviolet spectrophotometry,[26] and NMR[27] and EPR[28] spectroscopies. All methods have their advantages and disadvantages. The EPR method is a general alternative for the determination of partition coefficients of those spin-labeled molecules that cannot be readily analyzed by NMR spectroscopy because of line broadening and which have no suitable absorption maxima in the visible and ultraviolet regions. In the present study we used the EPR as well as ^{31}P NMR techniques as described in the Experimental Section and shown in Table II.

(b) Syntheses. Isocyanotophosphoryl dichloride (**6**) was prepared in 65% yield by a modification of the literature method.[29] The reaction of **6**[30] with ethanol followed by the reaction of either aziridine or 2,2-dimethylaziridine in the presence of triethylamine gave the corresponding urethane **7a** or **7b** (Scheme I). Condensation of either **7a** or **7b** with the nitroxyl-containing amines **8** and **10** in boiling toluene gave the corresponding spin-labeled urea derivatives **5a,b** and **11a,b** (Scheme I). The addition of solid sodium ascorbate to a methanolic solution of compounds **5a,b** and **11a,b** resulted in the reduction of the nitroxyls

(26) Purcell, W. P.; Bass, G. E.; Clayton, J. M. *Strategy of Drug Design: A Guide to Biological Activity*; Wiley: New York, 1973.
(27) Zon, G.; Ludeman, S. M.; Ozkan, G.; Chandrasegaran, S.; Hammer, K. F.; Dickerson, R.; Mizuta, K.; Egan, W. E. *J. Pharm. Sci.* **1983**, *72*, 689.
(28) Keana, J. F.; Lee, T. D.; Bernard, E. M. *J. Am. Chem. Soc.* **1976**, *98*, 3052.
(29) Kirsanov, Z. V. *J. Gen. Chem. USSR Engl. Transl.* **1954**, *24*, 103.
(30) Papanastassiou, Z. B.; Bardos, T. J. *J. Med. Chem.* **1984**, *27*, 782.

Scheme II

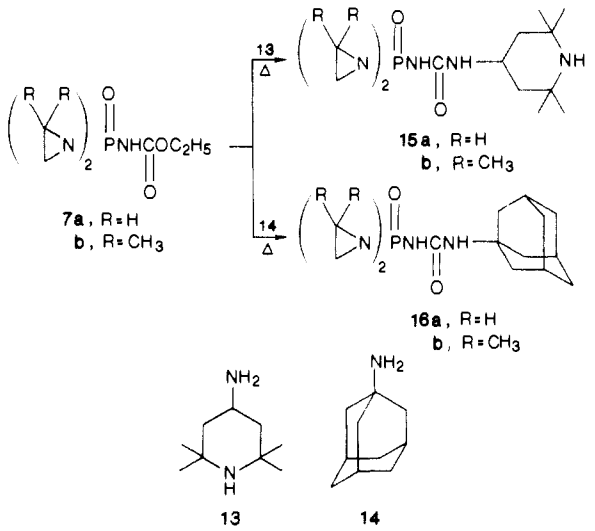


Table III. Anticancer Activities of N^3-Substituted Urea Analogues of TEPA and 2,2-Dimethyl-TEPA against L1210 Leukemia in CD_2F_1 Male Mice

compd	dose, mg/kg per day	test/control survival days	T/C,[a] %	ILS,[a] %	survivors 30 days
thio-TEPA (1)	6.5[b]		155[b]	55[b]	0/6[b]
5a	100	14/6.5	215	115	0/6
	50	12.6/7	181	81	0/6
	25	8.6/7	123	23	0/6
5b	100	11.5/7	164	64	0/6
9a	50	9.3/7	133	33	0/6
9b	50	7.1/7	101	1	0/6
11a	50	12.0/7	172	72	0/6
11b	100	10.4/7	148	48	0/6
12a	50	9.0/7	128	28	0/6
12b	50	7.0/7	100	0	0/6
15a	50	8.6/7	123	23	0/6
	25	8.2/7	117	17	0/6
15b	50	7.4/7	106	6	0/6
16a	25	7.7/7	110	10	0/6
16b	25	6.4/7	92	-8	0/6

[a] Results obtained on day 30. [b] Reference 55.

to the corresponding hydroxylamine derivatives **9a,b** and **12a,b**, respectively. Condensation of **7a** or **7b** with the 4-amino-2,2,6,6-tetramethylpiperidine (**13**) and 1-adamantylamine (**14**) in refluxing toluene afforded the corresponding urea derivatives **15a,b** or **16a,b** (Scheme II). The yields and physical constants of all products are presented in Table I.

(c) Biological. The results of the in vivo antineoplastic activity of compounds **1**, **2**, **3a**, **4a**, **5a,b**, **9a,b**, **11a,b**, **12a,b**, **15a,b**, and **16a,b** are shown in Tables II and III. Compound **5a** was found to be the most active anticancer agent, followed closely by compound **11a**. Other compounds were clearly less active. By use of P388 leukemia, a complete cure of mice was obtained with **5a** as compared to the clinical **1**, in which case all animals succumbed. Agent **5a** was also very much less toxic than **1**. Thus, the LD_{50} that was determined by the administration of **5a** to Swiss male mice was 270 mg/kg as compared to 13–18 mg/kg for **1**.[15,16] The reduced forms of **5a**, i.e., the amine derivative **15a** and the hydroxylamine derivative **9a**, were distinctly less active in both P388 and L1210 leukemia. The replacement of hydrogens in the aziridine ring in **5a** with methyl groups decreased the activity of **5b**. The replacement of the nitroxyl moiety in **5b** with the adamantane moiety caused a further decrease of activity of **16b**. The decrease in activity is coupled to an increase in hydrophobicity from **5a** to **5b** to **16b**.

The choice of the adamantane moiety was based on observations[31] that the adamantane derivatives are excellent perturbers of natural and artificial membranes and tend to make the membranes more fluid. In this connection, results are of interest that were obtained in the partitioning of a nitroxyl-labeled adamantane in a lipid/water system. Thus, when the lipid was fluid, both parts of the molecule, the adamantyl and the nitroxyl moieties, were found in the hydrocarbon region of the lipid. However, when the lipid was less fluid, the nitroxyl moiety entered the aqueous region while the admantyl part remained in the lipid.[31]

On the basis of this result, it might be concluded that, depending on the prevailing environment conditions, the nitroxyl moieties may play a decisive role in ensuring an enhanced permeation of the spin-labeled anticancer drugs through membranes of cancer cells as compared to membranes of normal cells.

Since the transformation of normal to cancer cells is accompanied by extensive structural changes[32,33] and the rate of many metabolic processes such as protein syntheses and DNA replication are greatly enhanced in cancerous over normal cells, it may be that the rates of migration of a drug through cancerous and normal cell membranes are different. The biological effects of a series of alkylating agents may be influenced by a number of factors such as hydrophilicity property, alkylating capability, and metabolism and degradation.

In Table I are shown the correlations of partition coefficients of several selected more active compounds with their anticancer activities. As can be deduced from this table, the replacement of oxygen on phosphorus with sulfur in **2** increases considerably the hydrophobicity of **1**. The replacement of one aziridine moiety with a nitroxyl moiety also increases the hydrophobicity of compounds **3a** and **4a**. The bridge elements between the phosphorous and the nitroxyl moiety also play an important role in this respect. Evidently, the -O- bridge contributes more to the hydrophobic behavior than the NH group. The replacement of one aziridine ring in **2** with the urea-bridged nitroxyl moiety causes only a slight, albeit an important, shift toward the higher hydrophobicity of **5a** and **11a** consistent with a substantial increase in activities. Although in **5a** and **11a** the six- and five-membered nitroxyls have markedly different rates of reduction of the nitroxyl function,[34-36] both compounds possess similar therapeutic indexes and similar hydrophobicities. As a matter of fact, the more readily reducible **5a** containing the six-membered nitroxyl has a somewhat higher therapeutic index than **11a** containing the more stable five-membered nitroxyl. On the basis of this result it appears that the transport of **5a** and **11a** in vivo through cell membranes, mediated by nitroxyl moieties, proceeds in both cases at such a rate that no appreciable enzymatic reduction occurs extracellularly[37]

(31) Morse, P. D., II; Lusczakoski-Nesbit, D. M.; Clarkson, R. B. *Chem. Phys. Lipids* **1982**, *31*, 257.
(32) Oppenheimer, S. B. *Cancer: A Biological and Clinical Introduction*; Allyn and Bacon: New York, 1982.
(33) Oppenheimer, S. B. *Introduction to Embryonic Development*; Allyn and Bacon: New York, 1980.
(34) Couet, W. R.; Brasch, R. C.; Sosnovsky, G.; Lukszo, J.; Prakash, I.; Gnewuch, C. T.; Tozer, T. N. *Tetrahedron* **1985**, *41*, 1165.
(35) Mehlhorn, R. J.; Packer, L. *Can. J. Chem.* **1982**, *60*, 1452.
(36) Mehlhorn, R. J.; Packer, L. *Ann. N. Y. Acad. Sci.* **1983**, *414*, 180.

to the less active amine (**15a**) and hydroxylamine (**9a, 12a**) derivatives. The hypothesis that the nitroxyl moiety is capable of facilitating the transport of an anticancer agent through cell membranes is supported by permeation studies of nitroxyls across cell membranes.[35-37] It was shown that small, neutral or weakly acidic or basic nitroxyls, including amides, equilibrate across human erythrocyte membranes with a half-life of about 100 ms to 1.6 min.[36,37] In the case that the nitroxyl ring contains an amine or a weak acid moiety the most rapid migration occurs at pH 7,[38-40] but when the pH of the environment differs greatly from the pK_a of the acid or base, the permeability decreases.[38-40] Since all derivatives of **1** and **2** are amides of phosphoric or thiophosphoric acid, it can be assumed that these nitroxyl derivatives possess near neutral properties. On the basis of limited pK_a values available in the literature,[41] it appears that the nitroxyl moieties (**5a,b, 11a,b**) should have pK_a values between those of the corresponding amine derivatives (**15a,b**), which will have higher pK_a values, and those of the hydroxylamines (**9a,b, 12a,b**), which will have lower pK_a values.

Furthermore, it can be expected that in the extra- and intracellular environment, the hydrogen bonding will play an important role.[42-47] Thus, while the nitroxyl moiety can be assumed to be a Lewis base and a proton acceptor, the corresponding amine and hydroxylamine derivatives should be both proton donors and acceptors. Hence, it can be anticipated that the amine and hydroxylamine derivatives, as compared to the nitroxyl derivatives, would be retarded during their migration through the extracellular and intracellular aqueous environment. As a consequence of this delayed transport, the reduced drug congeners could undergo undesirable reactions involving the alkylating aziridine moieties and will not be as effective in alkylating[25] the cancerous DNA as the unreduced, nitroxyl-containing drug.

In conclusion, the ideal antineoplastic drug of TEPA or thio-TEPA type should possess a combination of hydrophilic and hydrophobic properties. Thus, in order for the drug to migrate through the aqueous extracellular and intracellular environment of the body, it must possess a certain minimum degree of hydrophilicity. At the same time, in order to permeate the cell membranes, it must also possess a certain degree of compatibility with the hydrophobic portion of the phospholipids and other cell membrane components, although, not to such an extent that it might be "excessively" retarded or even immobilized by binding to the cell membranes. Hence, only a drug with such an "optimum" of lipophilicity characteristics will be able to unfold its full alkylating potential with the ultimate nucleophile, the cellular DNA.

In the present work this postulate was fulfilled. Thus, a graphic correlation of the partition coefficients of all compounds in Table II with their anticancer activities resulted in a distorted "bell-shaped" curve whereby the most active compounds are at or near the apex. This type of correlation can be used to design new anticancer drugs with a predictive level of activity using also carrier moieties other than the nitroxyl moiety, such as the linear and cyclic polyethers,[48] as was recently shown.[48]

Experimental Section

Mice. Male mice CD_2F_1 (for testing; average weight 18–20 g) and DBA/2 (for tumor propagation[49]) 6–7 weeks old were supplied by Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Mice were fed rodent Laboratory Chow 5001 (Ralson Purina Co.) and water ad libitum.

Drugs. Compounds were administered in a 0.85% aqueous sodium chloride solution (Sigma Chemical Company).

Biological Evaluations. Compounds **5a,b**, **9a,b**, **11a,b**, **12a,b**, **15a,b**, and **16a,b** were evaluated in vivo against the lymphocytic leukemia P388 and lymphoid leukemia L1210 in mice following the protocol of the National Cancer Institute.[49] The CD_2F_1 male mice of 18–20 g weight, in groups of six, were inoculated ip either with 10^6 cells of P388 or with 10^5 cells of L1210 tumor at day zero of the experiment. Compounds **5**, **8**, **11**, **12**, **15**, and **16** were injected ip at doses listed in Table II for 9 successive days from day one. The animals were then observed according to the protocol[49] for 30 days, and some of them up to 60 days, keeping a record of deaths and survivors. The anticancer activity was evaluated by comparing the mean survival time of treated with that of the control animals, i.e., by the T/C method where T represents the mean survival time of treated group and C the mean survival time of the tumor-bearing control group. The percent increase in life span (% ILS) parameter was calculated by the formula $[(T - C)/C] \times 100$. The LD_{50} values were determined by Weil's method.[50] Thus, for the determination of LD_{50}, four logarithmically spaced doses were injected ip into four groups of four Swiss male mice. The observation period for the determination of the LD_{50} was 30 days.

Materials. Aziridine[51,52] and 2,2-dimethylaziridine[53] were prepared according to the literature methods. Isocyanatophosphoryl dichloride was prepared by a modification of the literature procedure.[29] The toluene was purified by distillation. A forerun of 15% of the original volume was discarded. The remaining material collected on distillation was stored over molecular sieves, type 4A. The triethylamine was stored over solid potassium hydroxide. *N,N*-bis(aziridinyl)- and *N,N*-bis(2,2-dimethylaziridinyl)phosphorylurethanes (**6a,b**) were prepared according to a literature method.[30]

Analytical Procedures. Melting points were determined on a Thomas-Hoover Apparatus, Model 6406-K with a calibrated thermometer. The IR spectra were recorded either on a Perkin-Elmer spectrophotometer, Model 735B, or on a Nicolet-10MX FTIR spectrophotometer. Mass spectra were recorded on a Hewlett-Packard mass spectrometer, Model 5985 GS, using methane as the reactant gas. Therefore, the $(M^+ + 1)$ values are reported. Microanalyses were performed either on a Perkin-Elmer elemental analyzer, Model 240C, or on a F & M Scientific Corp. carbon, hydrogen, nitrogen analyzer, Model 185. Column chromatography was performed by using either the flash chromatography technique[54] on silica gel 60 (Fluka) finer than 230 mesh

(37) Eriksson, U. G.; Tozor, T. N.; Sosnovsky, G.; Lukszo, J.; Brasch, R. C. *J. Pharm. Sci.* **1986**, *75*, 334.
(38) Mathew, A. E.; Dodd, J. R. *J. Heterocycl. Chem.* **1985**, *22*, 225.
(39) Melhorn, R. J.; Keith, A. D. *Membrane Molecular Biology*; Fox, C. F., Keith, A. D., Eds.; Sinauer Associates: Stanford, 1972; p 192.
(40) Hubbell, W. L.; McConnel, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 12.
(41) Rozantsev, E. G.; Gintsberg, E. G. *Izv. Akad. Nauk SSSR Engl. Transl.* **1966**, *3*, 571.
(42) Cheng, L. Y.; Kun, W. Y. *Acta Chim. Sin.* **1985**, *43*, 155.
(43) Youcheng, L.; Zhongli, L.; Xiuzhi, W.; Yukum, W. *J. Mol. Sci.* **1984**, *2*, 31.
(44) Atherton, N. M.; Manterfield, M. R.; Oral, B.; Zorlu, F. *J. Chem. Soc., Faraday Trans. 1* **1977**, *73*, 430.
(45) Kawamura, T.; Matsunami, S.; Yonezawa, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 111.
(46) Yukum, W.; Youcheng, L. *J. Mol. Sci.* **1984**, *2*, 95.
(47) Lim, Y. Y.; Drago, R. S. *J. Am. Chem. Soc.* **1971**, *93*, 891.
(48) Sosnovsky, G.; Lukszo, J.; Rao, N. U. M. *J. Med. Chem.* **1986**, *28*, 1250.
(49) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A.; Abbott, B. *Cancer Chemother. Rep., Part 3* **1972**, *7*.
(50) Weil, C. S. *Biometrics* **1952**, *8*, 249.
(51) Wenker, H. *J. Am. Chem. Soc.* **1935**, *57*, 2328.
(52) Leighton, P. A.; Perkins, W. A.; Renquist, M. L. *J. Am. Chem. Soc.* **1947**, *69*, 1540.
(53) Campbell, K. N.; Sommers, A. H.; Campbell, B. K. *Org. Synth. Coll. Vol.* **1955**, *3*, 148.
(54) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

or conventional column chromatography on alumina (Basic, Brockmann activity I, 80–200 mesh, Fisher Scientific Co.). TLC analyses were performed on silica gel 60 F_{254} precoated sheets (EM reagents), layer thickness 0.2 mm with visualization using UV light and/or iodine chamber. The purity of compounds **5**, **9**, 11, **12**, **15**, and **16** was checked in a solvent system composed of chloroform and methanol (9:1, v/v). Partition coefficients were obtained by following literature methodologies using either ^{31}P NMR spectroscopy[27] or EPR spectroscopy.[28] Octanol and water layers were presaturated with each other prior to use. Thus, ^{31}P NMR spectra of 10.0 mM solutions (2 mL) of compounds **9**, **12**, **15**, and **16** in water containing 5% (v/v) D_2O were obtained by using a standard set of acquisition parameters, namely 80 pulses ($\pi/2$), no nuclear Overhauser enhancement, 10-s pulse repetition time, and standard set of display parameters.[25] Aliquots (4 mL) of 10.0 mM solutions of compounds were vigorously stirred (25 °C, 10 min) with 1-octanol (2 mL). The ^{31}P NMR spectrum for each of the separated aqueous layers (2 mL) was recorded. A 10.0 mM solution (2 mL) of each of the spin-labeled derivatives **5** and 11 in water containing 5% (v/v) D_2O was reduced with hydrazine hydrate, and for these reduced solutions (2 mL) ^{31}P NMR spectra were obtained as described previously. Aliquots (4 mL) of 10.0 mM solutions of nonreduced spin labels 8 and 11 were vigorously stirred (25 °C, 10 min) with 1-octanol (2 mL). Each of the separated aqueous layers (2 mL) was reduced with hydrazine hydrate, and the ^{31}P NMR spectra of these reduced aqueous layers were recorded. The signal intensities (peak height × width at half-height) were used to compute the concentration of compounds in water and 1-octanol layers. The partition coefficients, P = [compound in 1-octanol]/[compound in water], and their logarithmic values so obtained are shown in Table II. The areas of the double integrals of the first derivatives of the EPR spectra of 5.0 mM solutions of compounds **5** and 11 in octanol were computed at a standard set of display parameters, such as time constant, receiver gain, modulation amplitude, and modulation frequency, using a Varian E-115 spectrometer. Aliquots (3 mL) of 5.0 mM solutions of compounds were vigorously stirred (25 °C, 30 min) with water (60 mL). The octanol layer was separated from the aqueous layer by centrifugation. The ratio of the areas of the initial octanol solution and the separated octanol layer was used to compute the concentrations of the spin-labeled compounds 8 and 11 in octanol and in water layers. The partition coefficients were calculated by using the previous equation for P. These values are presented in Table II.

Preparation of Isocyanatophosphoryl Dichloride (6). The original procedure of Kirsanov[29] was modified by using dichloromethane (bp 40 °C) instead of dichloroethane (bp 83–84 °C). Thus, to a stirred suspension of phosphorus pentachloride (54.5 g, 0.26 mol) in dichloromethane (150 mL) was added in small portions, over a period of 15–20 min, urethane (23.3 g, 0.25 mol) at 25 °C. The reaction mixture was slowly warmed until boiling commenced, and then the mixture was boiled with reflux for 4 h. The reaction mixture was then repeatedly distilled to give 17.0 g (65%) of the pure product **6**: bp 139–140 °C.

Preparation of *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[[(2,2,6,6-tetramethyl-1-oxypiperidin-4-yl)amino]carbonyl]phosphoric Triamide (5a); *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[[(2,2,6,6-tetramethyl-1-oxypiperidin-4-yl)amino]carbonyl]phosphoric Triamide (5b); *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[[(2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)amino]carbonyl]phosphoric Triamide (11a); and *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[[(2,2,5,5-tetramethyl-1-oxypyrrolidin-3-yl)amino]carbonyl]phosphoric Triamide (11b). A solution of either 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (**8**; 0.89 g, 5.0 mmol) or 3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl (**10**; 0.78 g, 5.0 mmol) and either *N*,*N*-bis(aziridinyl)phosphorylurethane (**7a**; 1.28 g, 5.8 mmol) or *N*,*N*-bis(2,2-dimethylaziridinyl)phosphorylurethane (**7b**; 1.60 g, 5.8 mmol) in toluene (15 mL) was heated with reflux for 30 min. The solvent was removed on a rotating evaporator at 40 °C/20 torr, and the resulting residue was purified by flash chromatography on silica gel using dichloromethane and methanol (94:4, v/v) as eluant. The concentration of the combined fractions containing the product on a rotating evaporator at 25 °C/20 torr gave either **5a**, **5b**, 11a, or 11b as pure products. The analytical data are presented in Table I.

Preparation of *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[[(2,2,6,6-tetramethyl-1-hydroxypiperidin-4-yl)amino]carbonyl]phosphoric Triamide (9a); *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[[(2,2,6,6-tetramethyl-1-hydroxypiperidin-4-yl)amino]carbonyl]phosphoric Triamide (9b); *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[[(2,2,5,5-tetramethyl-1-hydroxypyrrolidin-3-yl)amino]carbonyl]phosphoric Triamide (12a); and *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[[(2,2,5,5-tetramethyl-1-hydroxypyrrolidin-3-yl)amino]carbonyl]phosphoric Triamide (12b). A solution of either **5a** (1.0 mmol), **8b** (1.0 mmol), 11a (1.0 mmol), or 11b (1.0 mmol) in methanol (10 mL) was added to solid sodium ascorbate (1.0 mmol) at 24 °C, and the reaction mixture was stirred for 2 h at 24 °C. The solvent was removed on a rotating evaporator at 40 °C/20 torr. The resulting residue was extracted with ethyl acetate (3 × 20 mL), and the combined extracts were dried over anhydrous sodium sulfate. The solid was collected by filtration and washed with anhydrous ether. The combined filtrate and washing were concentrated on a rotating evaporator at 40 °C/20 torr. The resulting residue was recrystallized from methanol and ether (1:5, v/v) to give either **9a**, **9b**, **12a**, or **12b** as pure products. The yields and analytical data are presented in Table I.

Preparation of *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[[(2,2,6,6-tetramethylpiperidin-4-yl)amino]carbonyl]phosphoric Triamide (15a); *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[[(2,2,6,6-tetramethylpiperidin-4-yl)amino]carbonyl]phosphoric Triamide (15b); *N*,*N*:*N*′,*N*′-Bis(1,2-ethanediyl)-*N*″-[(1-adamantylamino)carbonyl]phosphoric Triamide (16a); and *N*,*N*:*N*′,*N*′-Bis(2-methyl-1,2-propanediyl)-*N*″-[(1-adamantylamino)carbonyl]phosphoric Triamide (16b). A solution of either 4-amino-2,2,6,6-tetramethylpiperidine (**13**; 0.7 g, 5.0 mmol) or 1-adamantylamine (**14**; 0.75 g, 5.0 mmol) and either *N*,*N*-bis(aziridinyl)phosphorylurethane (**7a**; 1.28 g, 5.8 mmol) or *N*,*N*-bis(2,2-dimethylaziridinyl)phosphorylurethane (**7b**; 1.60 g, 5.8 mmol) in toluene (15 mL) was heated with reflux for 30 min. The workup procedure was identical to that described for the preparation of either **5a** or 11a. The yields and analytical data for the pure products **15a**, **15b**, **16a**, and **16b** are presented in Table I.

Acknowledgment. These studies were conducted pursuant to a contract with the National Foundation for Cancer Research. The Cancer lines were obtained from the DCT Tumor Repository, NCI—Frederick Cancer Research Facility. We thank Dr. Alan Thompson for the computer program for the EPR spectra and Frank Laib for recording of the ^{31}P NMR spectra.

Registry No. **3a**, 51526-59-1; **4a**, 33683-34-0; **5a**, 96662-64-5; **5b**, 103981-92-6; **6**, 870-30-4; **7a**, 302-49-8; **7b**, 1661-29-6; **8**, 14691-88-4; **9a**, 96662-65-6; **9b**, 103981-94-8; **10**, 34272-83-8; **11a**, 103981-99-3; **11b**, 103981-93-7; **12a**, 103981-95-9; **12b**, 103981-96-0; **13**, 36768-62-4; **14**, 768-94-5; **15a**, 96662-66-7; **15b**, 103981-97-1; **16a**, 65101-39-5; **16b**, 103981-98-2; PCl_5, 10026-13-8; CH_2Cl_2, 75-09-2.

(55) Goldin, A.; Wood, H. B., Jr. *Ann. N. Y. Acad. Sci.* **1969**, *163*, 956.