In the Search for New Anticancer Drugs. 17. Linear and Cyclic Polyether 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

George Sosnovsky,* Jan Lukszo, and N. Uma Maheswara Rao

Department of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201. *Received September 6, 1985*

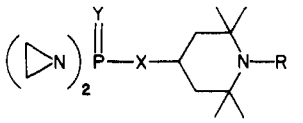
Linear and cyclic polyether derivatives of *N,N:N',N':N'',N''*-tri-1,2-ethanediylphosphoric triamide (TEPA) and *N,N:N',N':N'',N''*-tri-1,2-ethanediylphosphorothioic triamide (thio-TEPA) are synthesized and evaluated for their antineoplastic activity against the murine lymphocytic leukemia P388. All compounds, except for **7d**, were active ranging from 42% to 287% increase in life span (% ILS). All CD_2F_1 male mice treated with the most active compound (**7a**) at 90 mg/kg per day for 9 days were alive after 30 days, whereas all mice treated with the clinical drug thio-TEPA were dead. The % ILS for compound **7a** on day 60 was 525. A correlation is presented between the structural features of compounds and their lipophilicities and antineoplastic activities.

The substituted and unsubstituted 1,2-ethanediylimine (aziridine, ethylenimine) moieties are found in a number of synthetic and naturally occurring antineoplastic agents.[1,2] In particular, the small synthetic compounds, such as, *N,N:N',N':N'',N''*-tri-1,2-ethanediylphosphoric triamide (TEPA, **1**), *N,N:N',N':N'',N''*-tri-1,2-ethanediylphosphorothioic triamide (thio-TEPA, **2**), and

1 **2**

their derivatives have received considerable attention[2-4] over the past 3 decades. Thio-TEPA (**2**) was introduced into the clinical use more than 30 years ago.[5] The drug has been applied in the chemotherapy of Hodgkins' disease,[2] metastatic carcinoma of the breast,[6,7] superficial carcinoma of the bladder,[8-12] carcinomatous meningitis,[13,14] and ovarian cancer.[15]

In contrast, TEPA (**1**), which could be the first metabolite[16,17] of **2**, has not been applied in oncology, presumably, because of a lower stability[16] than that possessed by **2**. TEPA (**1**) and thio-TEPA (**2**) are triamides of the phosphoric and thiophosphoric acids, and, hence, the presence of three 1,2-ethanediylimine groups seems to be more than adequate to enable these compounds (**1** and **2**) to act not only as alkylating but also as cross-linking agents of the cellular DNA strands.[18] Therefore, since, theoretically, only two aziridine groups are required for a drug to display its full activity, many attempts have been made over the years to develop more active and/or less toxic derivatives of **1** and **2** by replacing one of the aziridine groups with different moieties, including the nitroxyl radical and its reduced forms (**3a–f**).[4,19-24]



3a: X = O; Y = O; R = O·, OH
b: X = O; Y = S; R = O·, OH, H
c: X = NH; Y = S; R = O·, OH, H
d: X = NCH_3; Y = S; R = O·, OH, H
e: X = NHC(O)O; Y = O; R = O·, OH
f: X = NHC(O)NH; Y = O; R = O·, OH, H

In particular, the TEPA derivative **3f**, R = O·, was shown[19-21] to have a much higher therapeutic index (26.5) than that (2.75) of the clinically used drug **2**. It was also shown[21] that while the nitroxyl moiety imparts a beneficial influence on the antineoplastic properties of a drug, by itself it is not active[17] and has no synergistic effect.[19]. This phenomenon can be explained by our hypothesis[25] that the nitroxyl moiety is facilitating the transport of the drug through the biological membranes on its way to the cellular DNA. As an extension of this idea, it was compelling to test the usefulness of other carrier moieties in designing of new antineoplastic agents.

The ability of synthetic and naturally occurring ionophores (siderophores) to complex various metal ions, such as, alkali, alkaline earth and iron, and to facilitate their transport through biological membranes is well documented.[26-31] In the present study, it was hypothesized that

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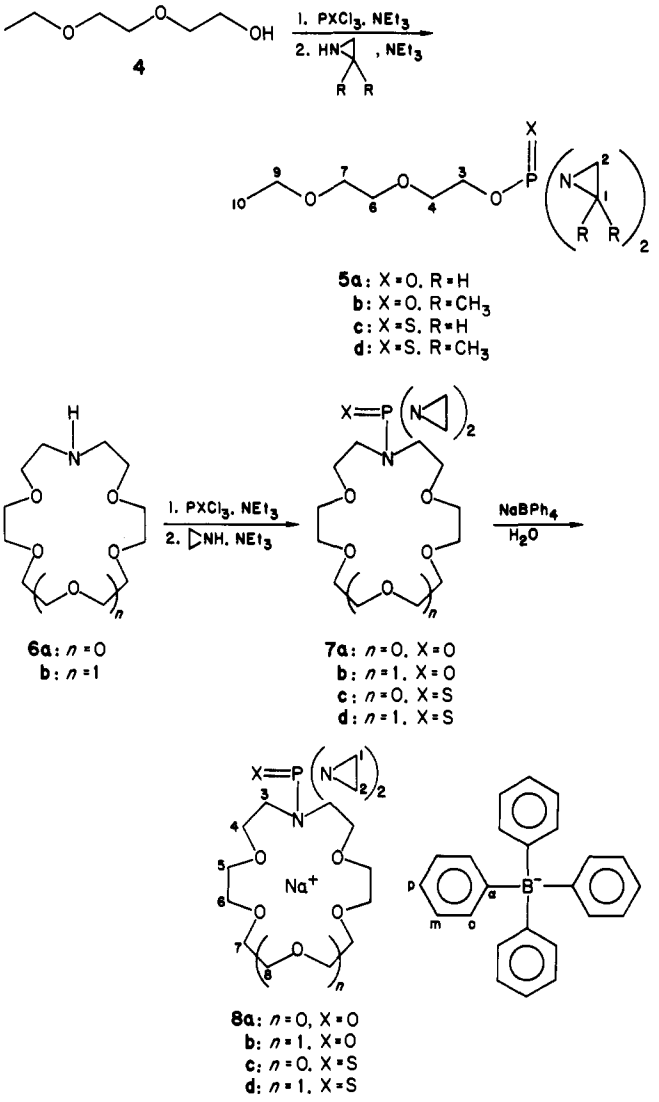
a replacement of one aziridine group in **1** and **2** with such a complexon, or with structural components that mimic the ionophore properties, could result in an enhancement of the rate of transport of the drug through the cell membranes. As a consequence of this enhanced membrane penetration by the modified drug, it was anticipated that a higher level of antineoplastic activity than that possessed by the parent compounds **1** and **2** would be achieved. Furthermore, it was speculated that by the use of a complexon of suitable dimensions it would be possible to upset the sodium/potassium balance of the cells. Assuming further that the transport through the membranes of cancerous cells is different to that of the noncancerous cells, the effect of the ion imbalance in conjunction with the increased alkylating potential of the aziridine moieties, facilitated by the enhanced rate of transport, could contribute to a preferential destruction of cancer cells. Because of structural complexities of naturally occurring ionophores (siderophores), such as, nigericin, monensin, valinomycin, nonactin, and others,[26] their often exhibited toxicities,[26] high cost, and difficulties in obtaining these components in quantity, it was decided to use model substances that have analogous structural components to the natural compounds. For example, the ionophores of the nigericin/monensin group possess a linear polyether chain as a common structural feature. The oxygen atoms that participate in the complexing of cations are spaced by two methylene units and bear close resemblance to oligomers of ethylene glycol, such as, 3,6-dioxaoctan-1-ol (carbitol, **4**). Since some natural ionophores, e.g., nonactin, possess cyclic structures, it was decided to use also synthetic azacrown complexons, in order to mimic the properties of this type of carriers. Recently we published a preliminary communication[32] on this topic. Now, we report the details of this study.

Results and Discussion

Chemistry. Compounds **5a–d** were prepared in 75–85% yields by the reaction of 3,6-dioxaoctan-1-ol (carbitol, **4**) with either phosphoryl or thiophosphoryl chloride in the presence of triethylamine, followed by the reaction of the intermediates with either aziridine or 1,1-dimethylaziridine in the presence of triethylamine (Scheme I). The azacrown derivatives **7a–d** were prepared in 18–64% yields by a similar methodology (Scheme I). The physical constants of the compounds **5a–d** and **7a–d** are shown in Table I.

In order to establish the complexing capabilities of compounds **5** and **7**, aqueous solutions of these compounds were mixed with equimolar amounts of aqueous solutions of sodium tetraphenylborate reagent.

Scheme I



While no visible signs of a reaction were observed with linear polyether derivatives **5a–d**, the reactions of the crown compounds **7a–d** resulted instantly in a turbidity, which slowly changed to a precipitate of white crystalline products **8a–d** in 18–69% yields. The composition of the complexes **8a–d** was confirmed by microanalysis and IR, ^{13}C NMR, and ^{31}P NMR spectroscopies (Table II). In the ^{31}P NMR spectra a single signal for the phosphorus atom and in ^{13}C NMR spectra signals for the crown and aziridine ring carbon atoms, as well as three out of four signals for the aromatic atoms, were observed. The signal for the quaternary aromatic carbon atom Cα (Scheme I) expected as a low-intensity quartet could not be detected because of a low signal to noise ratio.

The ^{23}Na NMR spectroscopy of the complex **8a** revealed a broad single line that was shifted nearly 0.53-ppm downfield from the signal recorded for the sodium tetraphenylborate reagent. A similar result was previously observed[33] for a complex of dibenzo-18-crown-6 with the sodium cation. The broadening of the ^{23}Na signal was attributed[33] to a lack of cubic symmetry around the sodium in the complex as compared to the solvated sodium cation. Therefore, this observation of a broad line in the ^{23}Na NMR spectrum of **8a** provides an additional evidence for a complex formation.

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Table I. Physical Properties of the Linear and Cyclic Polyether Analogues of TEPA and Thio-TEPA

no.	yield, %	mp, °C	molecular formula	MS, m/e[a]	IR:[b] ν_{max}, cm^{-1}	^1H NMR,[c] δ	^{13}C NMR,[c] δ	^{31}P NMR,[d] δ
5a	84	oil	$C_{10}H_{21}N_2O_4P$[e] (264.26)	265 ($M^+ + 1$, 100), 222 ($M^+ - 42$, 6), 117 ($M^+ - 147$, 21)	799, 818, 840, 935, 1053, 1114, 1260, 2870, 2931, 2975	1.15 (t, 3 H), 2.05 (d, 8 H), 3.32–3.40 (m, 2 H), 3.46–3.54 (m, 6 H), 4.01–4.07 (m, 2 H)	15.23 (s, C10), 23.84 + 24.18 (d, C1, C2), 66.03 + 66.30 (d, C3), 66.37 (s, C9), 69.91 (s, C7), 70.23 + 70.37 (d, C4), 70.60 (s, C6)	48.64
5b	82	oil	$C_{14}H_{29}N_2O_4P$[e] (320.37)	321 ($M^+ + 1$, 100), 250 ($M^+ - 70$, 5), 117 ($M^+ - 203$, 50)	967, 1056, 1114, 1142, 1299, 1344, 1377, 2872, 2909, 2975	1.10 (t, 3 H), 1.45 (s, 12 H), 2.05 (d, 4 H), 3.22–3.29 (m, 2 H), 3.38–3.47 (m, 6 H), 4.02–4.08 (m, 2 H)	15.32 (s, C10), 22.93 + 23.17 (d, CH_3-C1), 23.63 + 23.86 (d, CH_3-C1), 37.99 + 38.34 (d, C1), 40.03 + 40.39 (d, C2), 65.28 + 65.64 (d, C3), 66.07 + 66.17 (d, C4), 70.02 (s, C9), 70.41 (s, C6), 70.59 (s, C7)	50.32
5c	78	oil	$C_{10}H_{21}N_2O_3PS$[e] (280.32)	281 ($M^+ + 1$, 100), 238 ($M^+ - 42$, 24), 117 ($M^+ - 163$, 18)	967, 1057, 1114, 1142, 1299, 1377, 2872, 2909, 2931, 2976	1.05 (t, 3 H), 2.00 (d, 8 H), 3.17–3.23 (m, 2 H), 3.30–3.39 (m, 6 H), 3.77–3.84 (m, 2 H)	15.23 (s, C10), 23.85 + 24.19 (d, C1, C2), 66.06 + 66.40 (d, C3), 69.84 (s, C9), 69.94 (s, C7), 70.27 + 70.45 (d, C4), 70.63 (s, C6)	80.24
5d	75	oil	$C_{14}H_{29}N_2O_3PS$[e] (336.43)	337 ($M^+ + 1$, 100), 266 ($M^+ - 70$, 34), 117 ($M^+ - 219$, 27)	1047, 1052, 1113, 1116, 1104, 1141, 2871, 2929, 2939, 2974	1.05 (t, 3 H), 1.30 (s, 12 H), 1.95 (d, 4 H), 3.14–3.20 (m, 2 H), 3.26–3.35 (m, 6 H), 3.85–3.91 (m, 2 H)	15.33 (s, C10), 22.94 + 23.18 (d, CH_3-C1), 23.65 + 23.87 (d, CH_3-C1), 38.02 + 38.37 (d, C1), 40.07 + 40.42 (d, C2), 65.30 + 65.67 (d, C3), 66.09 + 66.19 (d, C4), 70.04 (s, C9), 70.61 (s, C6), 71.22 (s, C7)	81.31
7a	44	oil	$C_{14}H_{28}N_3O_5P$[f] (349.35)	350 ($M^+ + 1$, 100), 307 ($M^+ - 42$, 24)	931, 947, 1018, 1127, 1165, 1196, 1260, 1359, 1451, 2866	1.97–2.06 (m, 8 H), 3.33–3.37 (m, 4 H), 3.55–3.66 (m, 16 H)	23.33 + 23.42 (d, C1, C2), 48.27 + 48.33 (d, C3), 70.21 + 70.44 + 71.12 + 71.62 (C4-C7)	42.44
7b	63	oil	$C_{16}H_{32}N_3O_6P$[g] (393.42)	394 ($M^+ + 1$, 100), 351 ($M^+ - 42$, 21)	934, 947, 1020, 1118, 1165, 1195, 1260, 1354, 1456, 2865	1.97–2.06 (m, 8 H), 2.38–3.45 (m, 4 H), 3.55–3.62 (m, 20 H)	23.39 + 23.48 (d, C1, C2), 46.56 + 46.62 (d, C3), 70.39 + 70.68 + 70.77 + 71.44 (C4-C8)	36.73
7c	41	47-8	$C_{14}H_{28}N_3O_4PS$[e] (365.42)	366 ($M^+ + 1$, 100), 323 ($M^+ - 42$, 85)	927, 1011, 1128, 1257, 1297, 1449, 2864, 2965	1.85–2.00 (m, 8 H), 3.54–3.69 (m, 20 H)	23.83 + 23.91 (d, C1, C2), 48.67 + 48.72 (d, C3), 70.30 + 70.54 + 71.17 + 71.83 (C4-C7)	71.72
7d	18	oil	$C_{16}H_{32}N_3O_5PS$[e] (409.49)	410 ($M^+ + 1$, 100), 367 ($M^+ - 42$, 84)	926, 947, 1012, 1118, 1122, 1256, 1353, 2869, 2982	1.81–2.01 (m, 8 H), 3.55–3.65 (m, 24 H)	23.85 + 23.94 (d, C1, C2), 47.02 + 47.08 (d, C3), 70.51 + 70.76 + 70.88 + 71.66 (C4-C8)	72.14

[a] Relative percent intensities of the peaks. [b] With the exception of the compound **7c** which was analyzed as a KBr pellet, all other spectra were recorded by using thin film on silver chloride disk. [c] All spectra were recorded with use of deuteriochloroform($CDCl_3$) solutions with tetramethylsilane (Me_4Si) as the internal standard. [d] With the exception of the spectrum of compound **7a** which was recorded with use of a hexadeuterioacetone (CD_3COCD_3) solution, all other spectra were obtained with use of deuteriochloroform ($CDCl_3$) solutions. All shifts are relative to 85% phosphoric acid, which was used as the external standard. [e] The microanalyses were in satisfactory agreement with the calculated values within ±0.4%. [f] Microanalysis **7a**, $C_{14}H_{28}N_3O_5P \cdot {}^1/_2H_2O$ (367.37). Calcd: C, 46.92; H, 8.15; N, 11.72. Found: C, 46.64; H, 7.74; N, 11.73. [g] Microanalysis **7b**, $C_{16}H_{32}N_3O_6P \cdot H_2O$ (411.44). Calcd: C, 46.70; H, 8.33; N, 10.21. Found: C, 46.32; H, 8.16; N, 9.85.

Table II. Physical Properties of Compounds **8a–d**

compd	yield, %	mp, °C	molecular formula	IR:[a] ν_{max}, cm^{-1}	^{13}C NMR,[b] δ	^{31}P NMR,[c] δ
8a[d]	45	208–220, sinters at 200	$C_{33}H_{48}BNaN_3O_5P$[e]	928, 961, 1115, 1223, 1257, 3056	23.38 + 23.47 (d, C1, C2), 48.97 (C3), 70.84 + 71.68 + 72.04 (C4-C7), 122.17 (Cp), 125.93 (Cm), 137.02 (Co)	42.23
8b	64	206–210	$C_{40}H_{52}BNaN_3O_6P$[f]	936, 951, 1101, 1196, 1262, 2875, 2916, 3054	24.59 + 24.71 (d, C1, C2), 48.56 (C3), 68.29 + 68.47 + 69.36 + 70.30 (C4-C8), 121.65 (Cp), 125.48 (Cm), 136.35 (Co)	32.57
8c	18	150–153	$C_{38}H_{48}BNaN_3O_4PS$[g]	928, 944, 1084, 1106, 1125, 1253, 1480, 2877, 2916, 3055	25.26 (C1, C2), 50.11 (C3), 67.99 + 68.72 + 69.45 (C4-C7), 121.88 (Cp), 125.69 (Cm), 136.38 (Co)	109.25
8d	69	124–127	$C_{42}H_{52}BNaN_3O_5PS$[h]	930, 949, 1097, 1114, 1255, 2872, 2919, 3053	24.93 (C1, C2), 48.70 (C3), 68.23 + 69.00 + 69.35 + 71.24 (C4-C8), 121.68 (Cp), 125.50 (Cm), 136.31 (Co)	106.35

[a] Recorded as KBr disk. [b] In $CDCl_3$ with tetramethylsilane (Me_4Si) as internal standard. [c] Compounds **8a** and **8b** in acetone-d_6, compounds **8c** and **8d** in $CDCl_3$; all shifts are relative to 85% phosphoric acid as external standard. [d] Na NMR of **8a** in acetone-d_6: δ 0.53 (br s) relative to a 15 mM solution of $NaBPh_4$ in acetone-d_6; a 1:1 mixture of **8a** and $NaBPh_4$: δ 0.33 (br s). [e] Microanalysis **8a**. Calcd: C, 65.99; H, 7.00; N, 6.08. Found: C, 65.31; H, 6.85; N, 5.95. [f] Microanalysis **8b**. Calcd: C, 65.30; H, 7.12; N, 5.71. Found: C, 64.61; H, 7.03; N, 5.63. [g] Microanalysis **8c**. Calcd: C, 64.49; H, 6.84; N, 5.94. Found: C, 63.24; H, 6.65; N, 5.80. [h] Microanalysis **8c**. Calcd: C, 67.10; H, 6.97; N, 5.59. Found: C, 66.71; H, 7.03; N, 5.45.

The ^{23}Na NMR spectrum of a mixture of acetone solutions of the **8a** complex and sodium tetraphenylborate contained again a broad line at 0.33 ppm, implying a fast exchange of the sodium cation.

Biology. Compounds **5a–d** and **7a–d** were first tested in vivo, using Swiss mice for the acute toxicity at single doses of 50, 100, 150, and 200 mg/kg. No toxicity was observed at 50, 100, and 150 mg/kg. The compounds were then tested in vivo with use of the lymphocytic leukemia P388 at doses of 20 and 60 mg/kg per day. Compound **7a**, because of its outstanding activity, was retested with use of an expanded range of doses from 5 to 125 mg/kg per day. The unsubstituted azacrown **6a**, representing the carrier component of the very active compound **7a**, was tested in order to establish whether the assumed disturbance of the sodium/potassium balance in murine cells would manifest itself in an antineoplastic effect. However, no activity was observed for this compound (**6a**). Since a rapid sodium exchange was demonstrated for compound **8a**, it was assumed that the uncomplexed compounds would, in any case, rapidly equilibrate with the sodium ions in cells. Therefore, the sodio derivatives **8a–d** were not tested for anticancer activity.

Compounds **5a–d** and **7b–d** exhibited at the optimum dose of 60 mg/kg per day distinct activity ranging from low for **5d** (borderline case) to very high for compound **5a** as evidenced by the percent increase in life span values (% ILS, Table III). Compound **7a** was exceptional, possessing a high activity over the whole tested range from 5 to 90 mg/kg per day. At these doses no chronic toxicity was observed with **7a**. However, chronic toxicity was observed at the highest tested dose of 125 mg/kg per day. The therapeutic index (16) for this compound (**7a**), which was obtained by dividing the dose eliciting the optimum % ILS (90 mg/kg per day) by the dose eliciting a 40% ILS (5 mg/kg per day), exceeded by far the value of 2.75[34] for the clinical drug **2**. All mice treated with compound **7a** at the optimum dose of 90 mg/kg per day were alive after 30 days (six out of six). The % ILS at this stage was 287. After 60 days five mice out of six were alive. The % ILS after 60 days was 525. In contrast, all mice treated with **2** at the optimum dose of 6 mg/kg per day were dead on day 30.

In order to establish a measurable and predictable parameter for the correlation of activities of drugs **5a–d** and **7a–d** with the presumed migratory amplitudes through cell membranes, an attempt was made to relate the lipophilicities of drugs **5a–d** and **7a–d** to the structural features and to the levels of activities. For this purpose, the partition coefficients for these drugs were determined with use of 1-octanol/water solvent system and ^{31}P NMR spectroscopy.[35] The results are shown in Table III. The higher the P (or log P) value, the higher is the lipophilicity of a compound. Although all compounds are water soluble, they possessed a wide range of partition coefficients. In the linear polyether series, the introduction of either methyl groups into the aziridine ring or the replacement of the oxygen on phosphorus with sulfur caused (predictably) an increase in lipophilicity with a concomitant decrease in activity. The most active compound (**5a**) possessed the lowest lipophilicity (log P = 0.10), except for **1**, at the low end of the lipophilicity scale, and for **2**, at the high end of the lipophilicity scale.

In the azacrown series, the least active compound at the low end of the lipophilicity scale was **7b** with a log P = −1.36. This values is even lower than that of **1** (log P = −0.62). An increase in lipophilicity was achieved by eliminating one $-OCH_2CH_2-$ group in the azacrown ring to give the compound **7a** with a log P = 0.12. A replacement of the oxygen on phosphorus in **7a** with sulfur resulted (predictably) in a more lipophilic (log P = 0.41) and less active compound **7c**. A replacement of the oxygen with sulfur in **7b** resulted (predictably) in a further increase in lipophilicity (log P = 0.75) and a further decrease in ac-

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Table III. Partition Coefficients and Anticancer Activities of Compounds **5a–d**, **6a**, and **7a–d** against P388 Leukemia in CD_2F_1 Male Mice

compound	dose,[a] mg/kg per day	T/C,[b] %	ILS,[b] %	day 5[c] wt change, %	survivors 30 days	survivors 60 days	partition[d] coeff P	log P
5-fluorouracil	200[e]	179	79	−4.0	0/6			
TEPA (1)	6	236	136	−4.0	1/6	0/6	0.24	−0.62
thio-TEPA (2)	6	241	141		0/6		2.54	0.40
				Linear Polyethers				
5a	20	140	40	+2.8	0/6		1.25	0.10
	60	231	131	+4.5	0/6			
5b	20	128	28	+1.5	0/6		1.65	0.22
	60	188	88	+5.5	0/6			
5c	20	124	24	+2.6	0/6		2.40	0.38
	60	177	77	+1.4	0/6			
5d	20	119	19	+1.6	0/6		2.95	0.47
	60	142	42	+1.4	0/6			
				Azacrowns				
6a	20	102	2	+8.3	0/6			
	60	100	0	+7.9	0/6			
7a	5	141	41	+3.3	0/6		1.32	0.12
	10	166	66	+6.2	0/6			
	20	199	99	−2.0	0/6			
	30	222	122	0	0/6			
	40	237	137	−6.8	0/6			
	60	329 (393)	229 (293)	−4.4	3/6	1/6		
	90	387 (625)	287 (525)	−1.8	6/6	5/6		
	125	87	−13	−9.0	0/6			
7b	20	147	47	+7.6	0/6		0.044	−1.36
	60	188	88	+0.5	0/6			
7c	20	124	24	+7.9	0/6		2.60	0.41
	60	180	80	−6.4	0/6			
7d	20	102	2	+10.6	0/6		5.60	0.75
	60	120	20	+12.3	0/6			

[a] Compounds **5** were administered ip as aqueous solutions containing 2% Tween 80, and compounds **6** and **7** were administered ip as saline solutions. [b] The results obtained within 30 days are reported. Numbers in the parentheses represent results obtained on extension of the experiments to 60 days. [c] The average percentage weight change on day 5 was taken as a measure of drug toxicity. [d] The partition coefficients p = [compound in 1-octanol]/[compound in water] were measured by ^{31}P NMR technique, according to a literature method.[35] [e] 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.[38]

tivity to give the least active compound **7d** at the high end of the lipophilicity scale.

Thus, it appears that in the azacrown ether series a better distribution of lipophilicity and a better correlation with activities were achieved than in the linear polyether series. Interestingly, the most active compounds from both series **5a** and **7a**, respectively, possessed almost identical lipophilicity properties as evidenced by their partition coefficients.

A graphic correlation of the partition coefficients of **1**, **2**, and **7a–d** with their anticancer activities result in a bell-shaped curve, whereby the most active compound is at the apex of the bell. A similar type of relationship was previously described[36] for the nitrosoureas.

In conclusion, although the system for obtaining indirect information on the presumed relative partitioning of a particular class of drugs in cell membranes, and the correlation of these results with activities is somewhat simplistic, nevertheless, it is felt, that an astoundingly good correlation was obtained in the present study with a potential for future planning in the design of anticancer drugs of TEPA and thio-TEPA type.

Experimental Section

Mice. Male CD_2F_1 mice (for testing; average weight 19–22 g) and male DBA/2 mice (for tumor propagation[37]), 6–7 weeks old, were supplied by Harlan Sprague–Dawley (Indianapolis, IN). Mice were fed rodent Laboratory Chow 5001 (Ralston Purina Co.) with a hopper and water ad libitum.

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

Biological Evaluations. Compounds **5a–d**, **6a**, and **7a–d** were evaluated in vivo against the lymphocytic leukemia P388 in mice following the protocol of the National Cancer Institute.[37] The CD_2F_1 male mice of 19–22 g weight, in groups of six, were inoculated ip with 10^6 cells of P388 tumor at day zero of the experiment. Compounds **5** were injected ip as aqueous solutions containing 2% Tween 80 and compounds **6** and **7** were injected ip as saline solutions at doses listed in Table III for 9 successive days from day one. The animals were then observed according to the protocol[37] for 30 days and some of them up to 60 days, and a record of deaths and survivors was kept. 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 the treated group and the C the mean survival time of the tumor bearing group. The percent of increase in life span (% ILS) parameter was calculated by the formula $[(T - C)/C] \times 100\%$. The results are summarized in Table III.

Materials. Aziridine,[38,39] 2,2-dimethylaziridine,[40] 1,4,7,10-tetraoxa-13-azacyclopentadecane (**6a**),[41] and 1,4,7,10,13-pentaoxa-16-azacyclooctadecane (**6b**)[41] were prepared according to the literature methods. 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. Sodium tetraphenylborate was purchased from MCB Reagents. All other chemicals were reagent grade.

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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-10 MX FTIR spectrophotometer. ^{1}H, ^{13}C, ^{23}Na, and ^{31}P NMR spectra were recorded on a 250-MHz Bruker NMR spectrometer (Model WM-250). Because of the low concentration of the solutions of the complexes **8a–d**, their ^{13}C NMR spectra showed, with two exceptions, single peaks for C1, C2, and C3 carbon atoms instead of the expected doublets. The relatively low peak to noise ratio in these spectra rendered also indistinguishable the shifts related to the quaternary atom Cα, expected to appear as a low-intensity quartet due to a boron–carbon coupling.[42] The ^{23}Na shift in the ^{23}Na NMR spectrum of **8a** was recorded relative to a 15 mM solution of $NaBPh_4$ in acetone-d_6. 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 by the Atlantic Microlab, Inc., Atlanta, GA. Column chromatography was performed either using the flash chromatography technique[43] on silica gel 60 (Fluka) finer than 230 mesh or by 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. Purity of the compounds **5a–d** and **7a–d** was checked in a solvent system composed of chloroform and methanol (9:1, v/v). Partition coefficients were obtained by following a literature methodology[35] using ^{31}P NMR spectroscopy. Thus, ^{31}P NMR spectra of 10.0 mM solutions of compounds **5a–d** and **7a–d** in water containing 5% (v/v) D_2O were obtained with use of a standard set of acquisition parameters, namely, 80 pulses ($\pi/2$), no nuclear Overhauser enhancement, 10-s pulse–repetition time, and a standard set of display parameters. Aliquots (4 mL) of 10 mM solutions of compounds were vigorously stirred (25 °C, 5 min) with 2 mL of 1-octanol. The ^{31}P NMR spectrum for each of the separated aqueous layers (2 mL) was recorded as described above, and 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 III.

Preparation of 3,6-Dioxaoctyl *N*,*N*:*N*′,*N*′-Di-1,2-ethanediylphosphorodiamidate (5a) and 3,6-Dioxaoctyl *N*,*N*:*N*′,*N*′-Bis[2-methyl-1,2-propanediyl]phosphorodiamidate (5b). A solution of 3,6-dioxaoctanol (1.34 g, 10.0 mmol) and triethylamine (1.31 g, 13.0 mmol) in toluene (10 mL) was added dropwise, over a period of 10–15 min, with stirring to a solution of phosphoryl chloride (1.53 g, 10.0 mmol) in toluene (10 mL) at −15 °C. The reaction mixture was cooled to −15 °C and a solution of triethylamine (2.52 g, 25.0 mmol) in toluene (10 mL) was added rapidly followed by a dropwise addition of a toluene (10 mL) solution of either aziridine (0.86 g, 20.0 mmol) or 2,2-dimethylaziridine (1.42 g, 20.0 mmol) over a period of 10–15 min while the temperature was maintained at −15 °C. The reaction mixture was subsequently stirred for 4 h at −15 °C and then kept overnight in a refrigerator at 5 °C. The solid material was collected by filtration and washed successively with toluene and anhydrous ether. The combined filtrate and washings were concentrated on a rotating evaporator at 40 °C (20 torr). The crude oil thus obtained was purified by flash chromatography on silica gel with chloroform/methanol (12:1, v/v) as eluant. The concentation of the combined fractions containing the product on a rotating evaporator at 25 °C (20 torr) gave either 2.22 g (84%) of the pure product **5a** or 2.36 g (82%) of the pure product **5b** as colorless viscous oils. The yields and analytical data are presented in Table I.

Preparation of 3,6-Dioxaoctyl *N*,*N*:*N*′,*N*′-Di-1,2-ethanediylphosphorodiamidothioate (5c) and 3,6-Dioxaoctyl *N*,*N*:*N*′,*N*′-Bis[2-methyl-1,2-propanediyl]phosphorodiamidothioate (5d). A solution of 3,6-dioxaoctanol (1.34 g, 10.0 mmol) and triethylamine (1.31 g, 13.0 mmol) in toluene (10 mL) was added dropwise, over a period of 10–15 min, with stirring to a solution of thiophosphoryl chloride (1.69 g, 10.0 mmol) in toluene at −15 °C. The reaction mixture was stirred for 4 h at −15 °C and for 6 h at 24 °C. The reaction mixture was cooled to −15 °C and a solution of triethylamine (2.52 g, 25.0 mmol) in toluene (10 mL) was added rapidly, followed by a dropwise addition of a toluene (10 mL) solution of either aziridine (0.86 g, 20.0 mmol) or 2,2-dimethylaziridine (1.42 g, 20.0 mmol) over a period of 10–15 min while the reaction temperature was maintained at −15 °C. The reaction mixture was stirred for 8 h at −15 °C and for 15 h at 5 °C. The solid material was collected by filtration and successively washed with toluene and anhydrous ether. The combined filtrate and washings were concentrated on a rotating evaporator at 40 °C (20 torr). The crude oil thus obtained was purified by flash chromatography on silica gel with chloroform/methanol (12:1, v/v) as eluant. The concentration of the combined fractions containing the product, on a rotating evaporator at 25 °C (20 torr), gave either 2.18 g (78%) of the pure product **5c** or 2.52 g (75%) of the pure product **5d** as colorless viscous oils. The yields and analytical data are presented in Table I.

Preparation of 1,4,7,10-Tetraoxa-13-(di-*N*-aziridinylphosphoryl)azacyclopentadecane (7a) and 1,4,7,10,13-Pentaoxa-16-(di-*N*-aziridinylphosphoryl)azacyclooctadecane (7b). To a stirred solution of phosphoryl chloride (0.614 g, 4.0 mmol) in toluene (10 mL) was added over a period of 10 min either a solution of **6a** (0.876 g, 4.0 mmol) or **6b** (1.052 g, 4.0 mmol) and triethylamine (0.505 g, 4.0 mmol) in toluene (10 mL), at 0–5 °C. The reaction mixture was stirred for 2 h at 20 °C and then cooled to −15 °C. A solution of aziridine (0.387 g, 9.0 mmol) and triethylamine (0.909 g, 9.0 mmol) in toluene (10 mL) was added dropwise to the reaction mixture, in 10 min at −15 to −20 °C. After addition the reaction mixture was stirred for 30 min at −10 °C and for 12 h at 20 °C to 25 °C and then filtered. The solid material was washed with anhydrous ethyl ether (3 × 20 mL), and the combined filtrate and ether washings were concentrated on a rotating evaporator at 30 °C (20 torr). The crude, oily products **7a** and **7b** were further purified as follows. Compound **7a** was repeatedly flash chromatographed on silica gel with acetone/methanol (9:1, v/v) as eluant. Compound **7b** was first chromatographed on basic alumina with methanol as eluant, followed by flash chromatography on silica gel with acetone/methanol (92:8, v/v) as eluant. The yields and analytical data are presented in Table I.

Preparation of 1,4,7,10-Tetraoxa-13-(di-*N*-aziridinylthiophosphoryl)azacyclopentadecane (7c) and 1,4,7,10,13-Pentaoxa-16-(di-*N*-aziridinylthiophosphoryl)azacyclooctadecane (7d). To a stirred solution of freshly distilled thiophosphoryl chloride (0.847 g, 5.0 mmol) in toluene (10 mL) was added over a period of 5 min either a solution of **6a** (1.095 g, 5.0 mmol) or **6b** (1.315 g, 5.0 mmol) and triethylamine (0.606 g, 6.0 mmol) in toluene (10 mL), at −5 to −10 °C. The reaction mixture was stirred for 15 h at 25 °C and then cooled to −15 °C. A solution of aziridine (0.430 g, 10.0 mmol) and triethylamine (1.212 g, 12.0 mmol) in toluene (10 mL) was then added dropwise during a period of 6 min at −15 to −10 °C. The reaction mixture was stirred for 20 h at 25 °C and then filtered. The solid was washed with anhydrous ethyl ether (3 × 20 mL), and the combined filtrate and ether washings were concentrated on a rotating evaporator at 30 °C (20 torr). The crude, oily products **7c** and **7d** were purified as follows. Compound **7d** was first purified by column chromatography on basic alumina with *tert*-butyl methyl ether/methanol (96:4, v/v), followed by flash chromatography on silica gel with a 1% (v/v) solution of methanol in chloroform as eluant. Compound **7c** was purified by flash chromatography on silica gel with *tert*-butyl methyl ether/acetone (92:8, v/v) as eluant, followed by recrystallization of the solid product from *n*-hexane. The yields and analytical data are presented in Table I.

Preparation of Complexes 8a–d by the Reaction of Sodium Tetraphenylborate with the Crown Derivatives 7a–d. A solution of sodium tetraphenylborate (0.034 g, 0.10 mmol) in water (1.0 mL) was added at once to a stirred solution of the appropriate crown derivative **7** (0.10 mmol) in water (0.5 mL). The instantly formed white emulsions were stirred at 25 °C until precipitates

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were formed (1–6 h). The solid products **8a–d** were collected either by filtration (**8b–d**) or centrifugation (**8a**) and then successively washed with water (2 × 1.0 mL) and ethyl ether (1.0 mL) and dried at 40 °C (0.01 torr). Difficulties were experienced in combustion of **8a–c**. As a consequence, the microanalyses are not within 0.4% for **8a–c**. The yields and analytical data are presented in Table II.

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Registry No. 4, 111-90-0; **5a**, 101347-40-4; **5b**, 101347-41-5; **5c**, 101347-42-6; **5d**, 101347-43-7; **6a**, 66943-05-3; **6b**, 33941-15-0; **7a**, 101347-44-8; **7b**, 101347-45-9; **7c**, 101347-46-0; **7d**, 101347-47-1; **8a**, 101248-21-9; **8b**, 101248-23-1; **8c**, 101248-25-3; **8d**, 101315-82-6; aziridine, 151-56-4; 2,2-dimethylaziridine, 2658-24-4.

Conformations of Complexes between Mitomycin and Decanucleotides. 2. Application of the Model to Mitomycin C Derivatives. Extension to Covalent Binding with Adenine[1]

William A. Remers,*[2] Shashidhar N. Rao, U. Chandra Singh, and Peter A. Kollman

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.
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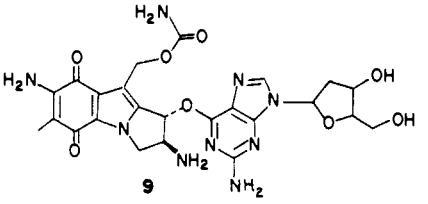
Molecular mechanics simulation of the interactions of important mitomycin C analogues monocovalently bound to DNA models are presented. These analogues included substituents such as *p*-hydroxyphenyl, 2-mercaptoethyl, and dimethylamidinium on N7 of mitomycin C and the DNA models consisted of $d(GCGCGCGCGC)_2$ and $d(GCGCATGCGC)_2$. The excellent fits and strong binding affinities of these highly potent analogues support the usefulness of the model. The binding of a mitomycin-related *N*-phenylpyrrole with a carbamoyloxy substituent to O6 of guanine was studied. Finally, a reactive mitomycin intermediate proposed by Moore was shown to interact with DNA in a way consistent with the formation of a covalent adduct.

The mode of binding of mitomycins to DNA has challenged investigators for over 20 years. An early proposal by Iyer and Szybalski that mitomycin C (**1**) is activated by reduction to a hydroquinone **2** followed by spontaneous loss of methanol (Scheme I) and that the resulting intermediate **3** alkylates DNA probably at O6 of guanine[3] has generally stood the test of time. However, a number of modifications have been proposed. Lown suggested that the initial alkylation probably involves aziridine ring opening with C1 of the mitomycin becoming the electrophilic center (**6** → **8**).[4] The second alkylation, involving DNA cross-linking, is a separate step (**8** → **7**).

More recently, an alternative reactive intermediate derived from the hydroquinone by aziridine ring opening and protropic rearrangement (Scheme I) was proposed by Moore.[5] This intermediate (**5**) has received indirect support from experiments that showed that reduction of mitomycin C in acidic medium gave mostly the 1-unsubstituted mitosene **4**.[6,7] Bachur has demonstrated that in biological systems the first intermediate formed from mitomycin C is a radical anion.[8] It would be surprising if this negatively charged species alkylates DNA directly. Whether it undergoes disproportionation to hydroquinone **3** is unknown.

Although the status of various reactive intermediates remains unclear, progress has been made in establishing the alkylation sites on DNA. By digestion of mitomycin-alkylated DNA with phosphatases, Hashimoto's group isolated mononucleotides covalently bound to the 1-position of the resulting 2-aminomitosene (**8**). The nucleotide bonds were O6 of guanine, *N*6 of adenine, and *N*2 of guanine, in decreasing order of abundance.[9] Tomasz confirmed the fragment **9** involving O6 of guanine.[10] Hashimoto further showed that the N^7-(*p*-hydroxyphenyl)



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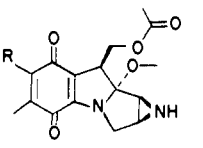
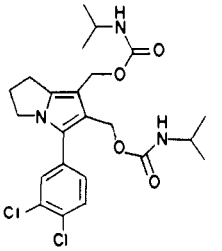


10 R = NHC_6H_5OH-P
11 R = NHC_6H_5I-P
12 R = $NHCH_2CH_2SH$
13 R = $N{=}CHN(CH_3)_2$
14 R = $NHCH{=}\overset{+}{N}(CH_3)_2$



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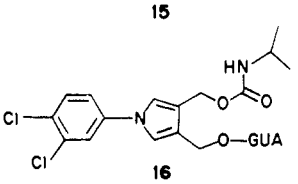

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