Synthesis and Biological Properties of Some Spin-Labeled 9-Aminoacridines

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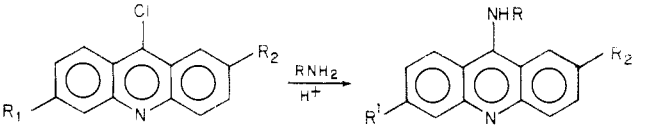
Five spin-labeled 9-aminoacridines, each bearing either a 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) or a 3-(2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety in the 9 position, have been synthesized and assayed for biological activity in three different test systems. Sedimentation velocity measurements indicated that the labels caused unwinding of calf thymus DNA. Those acridines which contained both 6-chloro and 2-methoxy substituents were less toxic to leukemia L1210 in static culture than the corresponding unsubstituted analogues. While the unsubstituted aminoacridines were quite good inhibitors of *Escherichia coli* DNA-primed RNA polymerase, the 6-chloro-2-methoxy-substituted compounds stimulated this enzyme system. In the presence of *E. coli* DNA, the ESR spectrum of 4-[(6-chloro-2-methoxy-9-acridinyl)amino]-2,2,6,6-tetramethyl-1-piperidinyloxyl (12) became broad and highly asymmetric with a maximal hyperfine splitting of 57.5 G. This observation suggests that when 12 intercalates into DNA the piperidinyl moiety that bears the nitroxide group becomes highly immobilized. These results suggest that the spin-labeled 9-aminoacridines will be useful probes for nucleic acids.

The biological properties of the 9-aminoacridines and related analogues are believed to result from the intercalation of these compounds into DNA which prevents DNA from serving as a template in DNA replication and RNA synthesis.[2] Evidence for the intercalation mechanism has come from a wide variety of techniques including viscometry,[3] sedimentation measurements,[3-7] electron microscopy,[8] and x-ray crystallography.[9] In addition to the aminoacridines, a large number of other pharmacologic agents appear to depend upon intercalation into nucleic acids for their therapeutic effects. These include anticancer drugs[10-12] (actinomycin, daunomycin), antimalarial drugs[13,14] (chloroquine, quinacrine), antischistosomasis agents[15] (hycanthone), and trypanocidal drugs[16] (ethidium, propidium).

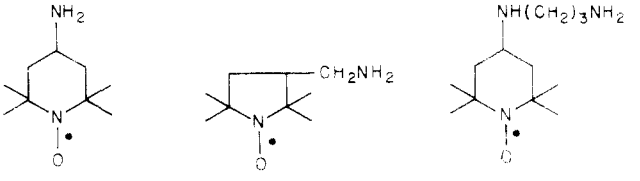
In recent years, spin-labeled drug analogues have become important as probes for studying various drug–macromolecule interactions.[17,18] In previous papers, we have described the synthesis and biological properties of a wide variety of spin-labeled drug molecules containing the nitroxide free radical.[19,20] While some of these compounds were more potent than the parent molecules, others were devoid of biological activity.[20] In particular, the introduction of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) moiety into the amino group of the butyl side chain of primaquine completely abolished the ability of this agent to bind to nucleic acids.[20] We now wish to report on the synthesis and biological properties of some spin-labeled analogues of 9-aminoacridines. In contrast to the aforementioned primaquine spin label, these agents do bind to DNA and RNA and should therefore prove to be of considerable use as probes of nucleic acids and their biologically important complexes such as chromatin. Furthermore, since di-*tert*-butyl nitroxide is toxic to HeLa cells[21] and can sensitize anoxic bacteria to x irradiation,[22] the 9-aminoacridine spin labels are also potential anticancer agents.

Chemistry. Direct condensation of aliphatic amines with 9-chloroacridine or 9-phenoxyacridine has hitherto been the most common procedure used in the synthesis of substituted 9-aminoacridines.[2b,23] Usually such condensations are carried out in the presence of strong acid, e.g., HCl, methanesulfonic acid. Since, however, the nitroxide radical is unstable in strongly acidic solutions,[24] we have carried out the condensation of the appropriate 9-chloroacridine with an excess of spin-labeled amine (1–3) in the presence of a catalytic amount of methanesulfonic acid (Scheme I). While these conditions preserved the nitroxide group, they also resulted in low yields (30–40%) of the spin-labeled aminoacridines. It was often necessary, therefore, to resort to repeated chromatography over neutral alumina in order to obtain a pure product. Many of the spin-labeled 9-aminoacridines gave poor elemental analyses despite repeated recrystallizations. Several of them appeared to undergo limited hydrolysis during recrystallization, possibly as a result of traces of water present in the solvents employed. In addition, the 9-aminoacridines were hygroscopic and often crystallized with traces of solvent which proved difficult to be removed. Solutions of the spin-labeled acridines which were used for biological testing were made up immediately prior to use and any unused solution was discarded.

Scheme I



$R_1 = R_2 = H$ or $R_1 = Cl$, $R_2 = OCH_3$



1 2 3

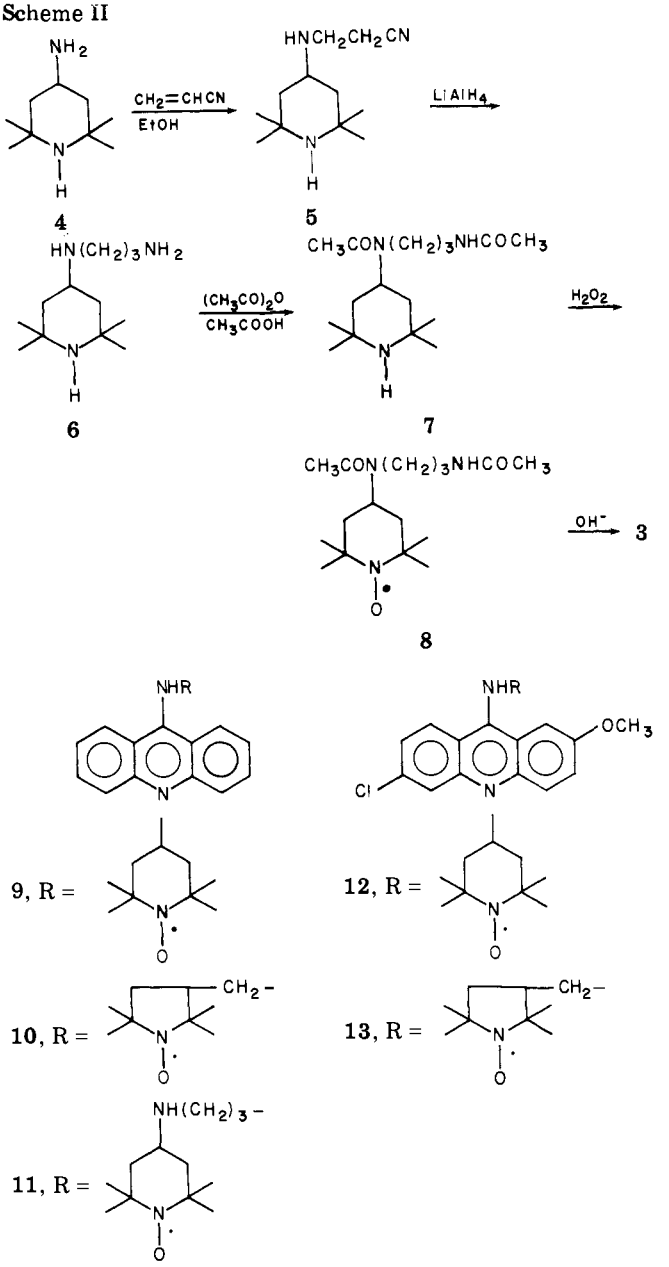
Table I. Sedimentation Coefficients for DNA-Acridine Spin Label Complexes

Drug	Drug concn, M × 10^6	DNA concn, μg/ml	S_{20} DNA	S_{20} DNA + drug	Ratio S_{20} DNA / S_{20} DNA + drug
9	5.2	35	14.3	13.7	1.04
10	5.2	35	14.3	13.2	1.08
12	5.2	35	13.7	13.4	1.02
13	5.2	35	14.4	13.9	1.04
14	5.2	35	14.4	13.7	1.05
Proflavin	20	33	15.4[a]	13.5[a]	1.13[a]

[a] Data taken from Lerman.[3]

The synthesis of spin label **3** (Scheme II) was achieved by reaction of 4-amino-2,2,6,6-tetramethylpiperidine (**4**) with acrylonitrile to give 4-[(2′-cyanoethyl)amino]-2,2,-6,6-tetramethylpiperidine (**5**) in quantitative yield. Reduction of **5** with lithium aluminum hydride afforded the triamine **6** in good yield. Acetylation of **6** with excess acetyl chloride in pyridine under a variety of conditions gave mixtures of the mono- and diacetyl derivatives of **6**. Diacetate **7** was finally obtained by reaction of **6** with excess acetic anhydride in glacial acetic acid at 80–90° for 2 h. Oxidation of **7** with H_2O_2 followed by alkaline hydrolysis gave the desired spin-labeled amine **3**.

Scheme II



Results and Discussion

The biological activity of the spin-labeled 9-aminoacridines was assayed by three different procedures: (a) their ability to cause the unwinding of calf thymus DNA (Table I), (b) their toxicity to leukemia L1210 cells in static culture (Table II), and (c) their effect on *Escherichia coli* RNA polymerase in vitro (Table III). Each of the spin-labeled 9-aminoacridines demonstrated some biological activity in all three test systems.

When a dye molecule intercalates into DNA, the sedimentation coefficient of the nucleic acid will decrease.[3] All of the spin-labeled 9-aminoacridines were found to cause a decrease in the sedimentation coefficient of calf thymus DNA with label **10** producing the most dramatic effect (Table I). While the observed decrease for the spin-labeled aminoacridines was less than that observed by Lerman[3] for the DNA–proflavin system, it should be pointed out that the concentration of proflavin used by Lerman was fourfold higher than those employed in this investigation. It proved impossible to test the spin-labeled acridines at higher concentrations, because the optical densities of the resultant solutions would have been too high for the detection system used in our ultracentrifuge. Nevertheless, the data do suggest that the spin-labeled 9-aminoacridines intercalate into calf thymus DNA.

The cytotoxicity of the 9-aminoacridine spin labels was measured in vitro using leukemia L1210 cells in static culture. The results are shown in Table II together with the cytotoxicity data for **15**, a 9-aminoacridine analogue which has been synthesized by Cain et al.[2b] and which is currently undergoing preclinical trial. While all of the spin labels were found to be toxic after both 24 and 48 h of incubation, labels **12** and **14** were considerably more effective after the 48-h period. Voiculetz and co-workers[25] have studied the toxicity of quinacrine, a structural analogue of labels **12** and **14**, in H1A cells, a subline of

Table II. Cytotoxicity of the Acridine Spin Labels against L1210 Leukemia Cells in Vitro

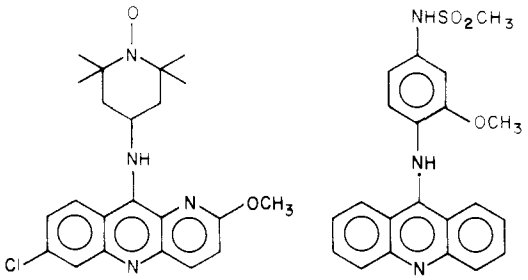
Drug concn, μg/ml	% inhibition of growth[a] 9		10		11		12		13		14		15[b]	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
100	53	87	48	82	47	86	6	78	40	74	0	38	57	92
10	49	84	46	79	56	87	11	63	35	76	0	15	50	87
1	27	56	12	29	10	16	9	24	6	19	0	0	42	82
0.1	1	7	3	2	0	0	12	7	0	5	0	0	29	75
0.01	0	2	0	3	0	0	3	8	10	7	0	0	6	8

[a] Each value is the average of three separate experiments each employing two bottles; two determinations were made on each bottle. The values from the three experiments did not differ from one another by more than 5% with the exception of the data for compound **14** where a variation of 10% was observed. [b] Cysyk, unpublished data.

Table III. Effect of the Acridine Spin Labels on *E. coli* DNA-Primed RNA Polymerase

Drug concn, μg/ml	RNA polymerase activity[a] (% control)						
	9	10	11	12	13	14	9-Amino-acridine
400	9	10	12	116	107	107.5	1.8
200	21	22	20	125	102.5	114	4.0
100	36	45	31	129	106	110	31.0
50	77	70	42	120	106	110	31.0
25	96	87	71	108	112	108	40.0

[a] All assays were run in triplicate. The results represent the average of two separate experiments which did not differ by more than 5%.



14

15

Chinese hamster ovary cells. They found that the toxicity of quinacrine was a function of both time and concentration and have suggested that this drug requires time to reach its cellular receptor sites and express its toxic effect. It is tempting to suggest that the much lower toxicity of labels **12** and **14** at 24 h may be due to slow transport to binding sites on nuclear DNA. The data obtained with the azaacridine spin label **14** showed much greater variability than did the data from the other labels. It is possible that the variability together with the low cytotoxicity of **14** in the L1210 system may be due to the low water solubility of this compound.

Table III shows the in vitro effect of the acridine spin labels on native *E. coli* DNA-primed RNA polymerase. While compounds **9–11** are inhibitors of the RNA polymerase, they are much less active than 9-aminoacridine itself. By contrast, labels **12–14** exhibited a dose-dependent stimulation of RNA polymerase (Table III). This is in contrast to quinacrine, which produces a 50% inhibition of *E. coli* DNA-primed RNA polymerase at a concentration of 2×10^{-5} M (~8 μg/ml).[26] While the stimulation of RNA polymerase may at first appear surprising, it should be pointed out that O'Brien et al.[26] and Krakow[27] have found similar results for spermine and other polyamines. Krakow has suggested[27] that this stimulation may result from binding of the polyamines to RNA which in turn may relieve product inhibition of the polymerase.

In dilute aqueous solution, the ESR spectrum of spin label **12** consists of three sharp lines with a splitting of 17 G between adjacent lines (Figure 1, A). This three-line spectrum results from the anisotropic hyperfine interaction between the unpaired electron and the nuclear spin of the nitrogen atom. In the presence of *E. coli* DNA, the ESR spectrum of spin label **12** became broad and asymmetric with a maximal hyperfine splitting ($2T_{11}$) of 57.5 G (Figure 1, B). This so-called "rigid glass" spectrum is characteristic of a nitroxide radical whose molecular motion is slow on the ESR time scale (i.e., $\tau_c >> 10^{-8}$ s where τ_c is the rotational correlation time of the nitroxide group). Other studies have shown that, in the presence of calf thymus DNA, label **12** has a $2T_{11}$ value of 58.7 G. These results

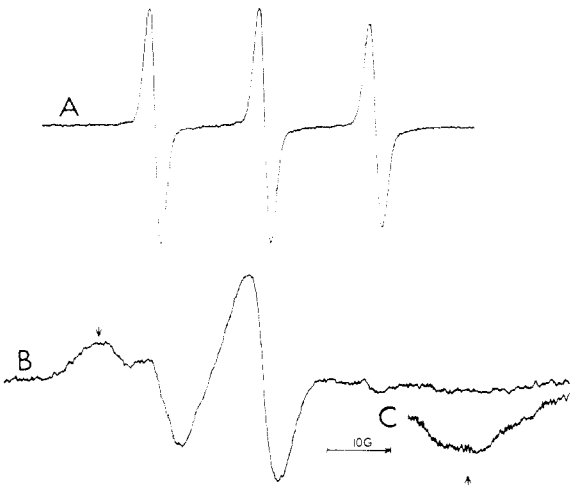


Figure 1. The ESR spectrum of 9-aminoacridine spin label **12** (2×10^{-5} M) in the absence (A) and presence (B, C) of *E. coli* DNA (0.5 mg/ml). All solutions contained 5 mM sodium phosphate buffer, pH 7.4. The gain settings were 1.25×10^3, 5×10^3, and 2×10^4 for spectra A, B, and C, respectively. The arrows show the positions of the low- (left) and high-field (right) hyperfine lines that are characteristic of a highly immobilized nitroxide radical.

demonstrate that when **12** binds to DNA, the motion of the piperidyl moiety is highly restricted. This high degree of immobilization supports the intercalative binding mechanism, since it appears unlikely that the motion of the piperidine ring of label **12** would be reduced so drastically if this compound bound to the phosphate groups on the outside of the DNA helix.

In previous studies, we have reported that the introduction of the 4-(2,2,6,6-tetramethyl-1-piperidinyloxy) moiety into the amino group of the butyl side chain of primaquine abolishes the ability of this drug to bind to nucleic acids.[20] By contrast, the introduction of the same label into 9-aminoacridine produced spin labels that could still intercalate into DNA and that were toxic to tumor cells. Two previous reports have described the use of ESR to monitor the interaction of free-radical ligands with nucleic acids. Ohnishi and McConnell[29] have studied the intercalation of the chlorpromazine cation radical into DNA while Ishizu and co-workers[30] have examined the binding of the 5-methylphenazinium cation radical to nucleic acids. However, both of these radicals have highly complex ESR spectra which result from hyperfine interactions between the unpaired electron and hydrogen and nitrogen atoms in the respective aromatic rings. In addition, both the chlorpromazine cation radical and the 5-methylphenazinium cation radical are unstable in aqueous solution at biological pH values. For these reasons, neither radical has gained acceptance as a noncovalent spin probe for nucleic acids. By contrast, the 9-aminoacridine nitroxide labels described here have relatively simple ESR spectra. They are also very stable in aqueous solution over a wide range of temperatures and pH values. Preliminary ESR studies[28] (Figure 1) strongly suggest that the 9-aminoacridine spin labels will be useful probes for studying nucleic acids both in vitro and in vivo. It is also hoped that these labels can be employed to examine biologically important complexes involving nucleic acids such as chromatin.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. All mass spectra were taken on LKB 9000 with source temperature ~290°. All elemental

analyses were within ±0.4% of the theoretical value, except where indicated.

3-Aminomethyl-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (2) was prepared according to the method of Hsia and Piette[31] in 60% yield.

N^1-[4-(2,2,6,6-Tetramethyl-1-piperidinyloxyl)]-N^1,N^3-diacetyl-1,3-diaminopropane (8) was prepared from **4** by a four-step procedure (Scheme II). In the first step, nitrile **5** was prepared by cyanoethylation of **4** using a procedure described by Tabor et al.[32] To a solution of 15.6 g (0.1 mol) of **4** in 50 ml of 98% EtOH was added 5.83 g (0.11 mol) of acrylonitrile (Aldrich Chemical Co.), and the mixture was heated to reflux for 2 h. The reaction mixture was cooled and allowed to stand overnight at room temperature. Removal of the EtOH and excess acrylonitrile under reduced pressure afforded 20.5 g (98%) of **5** as a colorless liquid: ir (neat) 2230 cm^{-1} (C≡N).

The nitrile **5** (10.5 g, 0.05 mol) in 100 ml of dry Et_2O was added dropwise with stirring to a suspension of $LiAlH_4$ (7.6 g, 0.20 mol) in 250 ml of dry Et_2O. After the addition was complete, the reaction mixture was refluxed for 24 h and the excess $LiAlH_4$ was decomposed with dilute NaOH solution. The Et_2O solution was collected by filtration and the solids were washed well with $CHCl_3$. The combined organic solvents were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give 9.5 g (87%) of pure **6** as a colorless liquid: ir (neat) 3360, 3255, 1580 cm^{-1} (NH).

To the triamine **6** (10.6 g, 0.05 mol) dissolved in 15 ml of glacial acetic acid, 15.3 g (0.15 mol) of acetic anhydride was added with stirring. The reaction mixture was heated at 80–90° on a steam bath for 2 h and then cooled. The acetic acid and excess acetic anhydride were removed under reduced pressure. The semisolid residue was dissolved in a minimum amount of H_2O and neutralized with solid $NaHCO_3$. H_2O was removed and the solids were treated with $CHCl_3$ (4 × 25 ml). The $CHCl_3$ solution was dried (Na_2SO_4) and filtered. Removal of the solvent afforded 12.0 g (60%) of a semisolid which could not be crystallized. Purification was achieved by dissolving the semisolid in a small amount of $CHCl_3$ and precipitating with hexane: ir (neat) 1630, 1660 cm^{-1} (s) (C=O). The mass spectrum (8 eV) was consistent with the desired diacetate **7**: *m/e* (rel intensity) 297 (M^+, 4), 282 (M^+ – 15, 89), 124 (100), 99 (19).

To the crude diacetate **7** (9.0 g, 0.03 mol) suspended in 50 ml of H_2O, 4.0 g of disodium ethylenediaminetetraacetate, 4.0 g of sodium tungstate, and 60 ml of 30% H_2O were added. The pH of the mixture was adjusted to about 8.0, and the mixture was stirred gently for 48 h in a cold room at 4°. The orange solution was extracted with $CHCl_3$ (3 × 50 ml). The combined $CHCl_3$ solutions were washed with dilute HCl and H_2O, dried (Na_2SO_4), and filtered. Removal of the $CHCl_3$ afforded 6.5 g (70%) of a red oil which solidified on standing. Crystallization from $CHCl_3$–Et_2O gave **8** as deep orange crystals, mp 153–154°. The mass spectrum (8 eV) was consistent with **8**: *m/e* (rel intensity) 313 (MH^+, 30), 312 (M^+, 62), 298 (MH^+ – 15, 12), 282 (M^+ – 30, 15), 269 (M^+ – 43, 23), 226 (M^+ – 86, 30), 129 (100). Anal. ($C_{16}H_{30}N_3O_3$).

N-[4-(2,2,6,6-Tetramethyl-1-piperidinyloxyl)]-1,3-diaminopropane (3). A suspension of **8** (6.3 g, 0.02 mol) in 25% aqueous KOH was refluxed for 18 h. The aqueous solution was saturated with NaCl and extracted with Et_2O. The Et_2O solution was washed with H_2O, dried (Na_2SO_4), and filtered. Removal of the solvent afforded 4.0 g (89%) of a red oil. The mass spectrum was consistent with desired amine **3**: *m/e* (rel intensity) 229 (MH^+, 18.5), 228 (M^+, 21.5), 200 (13), 185 (15), 170 (13), 155 (100), 154 (63).

4-(9-Acridinylamino)-2,2,6,6-tetramethyl-1-piperidinyloxy (9). To a solution of 4-amino-2,2,6,6-tetramethyl-1-piperidinyloxy (**1**) (0.75 g, 0.0047 mol) (Aldrich Chemical Co.) in 50 ml of absolute MeOH, 0.50 g (0.0023 mol) of 9-chloroacridine was added. The mixture was heated to reflux and when all the solids had dissolved, 5 drops of methanesulfonic acid was added. The color of the reaction mixture changed from yellow to deep red. The reaction mixture was then refluxed for 24 h and cooled. The yellow solids were filtered and washed with MeOH. Removal of MeOH under reduced pressure afforded a yellow-orange solid which was dissolved in $CHCl_3$ and the solution washed with dilute NH_4OH and H_2O and then dried over Na_2SO_4. Filtration and removal of the $CHCl_3$ under reduced pressure afforded a yellow-orange solid. This material was chromatographed twice on neutral alumina using $CHCl_3$ as eluent. Crystallization from $CHCl_3$–heptane afforded 0.25 g (30%) of pure **9** as an orange-yellow solid, mp 188–190°. The mass spectrum (70 eV) was consistent with structure **9**: *m/e* (rel intensity) 349 (MH^+, 18), 348 (M^+, 10), 333 (M^+ – 15, 15), 318 (M^+ – 30, 5), 292 (15), 275 (43), 262 (100), 194 (54), 178 (27). Anal. ($C_{22}H_{26}N_3O$) H: calcd, 7.52; found, 7.72.

3-(9-Acridinylaminomethyl)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (10) was prepared from 9-chloroacridine and amine **2**. Workup and purification as described for **9** afforded 0.30 g (36%) of pure **10** as an orange-yellow solid, mp 190–191.5°. The mass spectrum (70 eV) was consistent with structure **10**: *m/e* (rel intensity) 348 (M^+, 10), 333 (M^+ – 15, 10), 318 (M^+ – 30, 6), 207 (100), 195 (25), 194 (20). Anal. ($C_{22}H_{26}N_3O$) H, N; C: calcd, 75.83; found, 74.95.

4-[[3′-(9-Acridinylamino)propyl]amino]-2,2,6,6-tetramethyl-1-piperidinyloxyl (11) was prepared from **3** and 9-chloroacridine by a method similar to that described for **9**. Workup and extensive purification gave **11** in 20% yield as a yellow solid, mp 122–124°. The mass spectrum (70 eV) was consistent with **11**: *m/e* (rel intensity), 406 (MH^+, 8), 405 (M^+, 5), 390 (M^+ – 15, 8), 332 (8), 252 (30), 221 (34), 208 (30), 207 (47), 196 (16), 195 (100), 194 (30). Anal. ($C_{25}H_{33}N_4O\cdot CH_3OH$) C, N; H: calcd, 8.15; found, 8.52.

4-[(6-Chloro-2-methoxy-9-acridinyl)amino]-2,2,6,6-tetramethyl-1-piperidinyloxy (12) was prepared from 6,9-dichloro-2-methoxyacridine and amine **1** as described for **9**. Workup and purification by chromatography over neutral alumina followed by crystallization from MeOH–H_2O (95:5) afforded **12** as orange needles, mp 197–198°. The mass spectrum (70 eV) was consistent with **12**: *m/e* (rel intensity) 413 (MH^+, 29), 412 (M^+, 11), 397 (M^+ – 15, 26), 382 (M^+ – 30, 8), 339 (34), 326 (100), 258 (78), 257 (98). Anal. ($C_{23}H_{27}ClN_3O_2\cdot CH_3OH$) C, N; H: calcd, 7.47; found, 7.14.

3-(6-Chloro-2-methoxy-9-acridinylaminomethyl)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (13) was prepared from 6,9-dichloro-2-methoxyacridine and **2** using the same procedure described for **9**. Workup and purification gave pure **13** as a yellow solid, mp 181.5–183°. The mass spectrum (70 eV) was consistent with **13**: *m/e* (rel intensity) 413 (MH^+, 9), 412 (M^+, 9), 397 (M^+ – 15, 23%), 382 (M^+ – 30, 6), 271 (100), 259 (17), 258 (23). Anal. Calcd for $C_{23}H_{27}ClN_3O_2$: C, 66.90; H, 6.60; N, 10.17. Found: C, 67.65; H, 7.01; N, 9.8.

7-Chloro-10-[4-(2,2,6,6-tetramethyl-1-piperidinyloxy)-amino]-2-methoxypyrido[3,2-*b*]quinoline (14) was prepared by reacting amine **1** with 7,10-dichloro-2-methoxypyrido[3,2-*b*]quinoline (Aldrich Chemical Co.) in absolute MeOH according to the method described for the synthesis of **9**. Workup and purification followed by crystallization from aqueous MeOH afforded pure **14** as a yellow solid (20%), mp 215–215.5°. The mass spectrum (70 eV) was consistent with **14**: *m/e* (rel intensity) 414 (MH^+, 18), 398 (M^+ – 15, 10), 259 (24), 43 (100). Anal. ($C_{22}H_{26}ClN_4O_2\cdot H_2O$) C, N; H: calcd, 6.53; found, 6.02.

Biological Testing. RNA Polymerase. Calf thymus DNA, *E. coli* RNA polymerase, and the nucleoside triphosphates were purchased from Sigma Chemical Co. The [8-^{14}C]-ATP (50 mCi/mM) was obtained from New England Nuclear. The activity of RNA polymerase was determined by measuring the amount of [^{14}C]-ATP rendered acid insoluble as determined by the filter paper disk assay of Bollum.[33] The incubation mixture contained in 0.125 ml: Tris–HCl buffer, pH 8.0, 5 μmol; $MgCl_2$, 1.25 μmol; β-mercaptoethanol, 1.25 μmol; CTP, UTP, and GTP each 0.019 μmol; [8-^{14}C]-ATP, 0.019 μmol (65 000 cpm); calf thymus DNA, 3.65 μg. The compounds to be tested were dissolved in dimethyl sulfoxide and 5 μl of the solution was added to the incubation mixture just prior to the addition of enzyme. Controls contained 5 μl of dimethyl sulfoxide. The reaction mixtures were incubated for 10 min at 37°. The acid-insoluble material from an aliquot (100 μl) of each incubation mixture was isolated by the procedure of Bollum[33] and placed in 18 ml of scintillation fluid (6.0 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, 1400 ml of toluene, 600 ml of MeOH) and counted in a Beckman LS-230 liquid scintillation spectrometer. In the absence of added drug, 1.05 nmol of [8-^{14}C]-AMP was incorporated into DNA during the 10-min incubation period.

Cytotoxicity to L1210 Cells. The cytotoxicity of the acridine spin labels was determined in vitro using leukemia L1210 cells

(10^5 cells/ml) in a static culture in RPMI No. 1630 medium supplemented with fetal calf serum according to the method of Moore et al.[34]

Analytical Ultracentrifugation. Sedimentation analysis was carried out with a Beckman Spinco Model E analytical ultracentrifuge equipped with ultraviolet optics and scanner. Mixtures containing calf thymus DNA and the test compounds (5.2×10^{-6} M) were incubated for 30 min at room temperature (~22°), and then an aliquot (0.4 ml) was transferred to one sector of double sector cells maintained at 20°; 0.41 ml of buffer was added to the other sector. Since the optical consequences of drug binding to DNA are complete in much shorter times, it was felt that this incubation was adequate. The sedimentation temperature was maintained at 20°, while the sedimentation data were recorded with the ultraviolet scanning system with the wavelength set at 260 nm and the slit at maximum width. A minimum of eight tracings at 4-min intervals was used to calculate the S_{20} value via a least-squares linear regression on boundary migration. Correlation coefficients were always 0.99 or better. In no case was there any evidence of detectable heterogeneity in either control or drug–DNA mixture. Each run in the four-hole rotor contained a drug-free DNA control and two drug–DNA mixtures. Thus, drug–DNA mixtures and controls sedimented under the same conditions. The sedimentation coefficients of all controls were within 2% of each other. The sedimentation coefficients reported here are not corrected for density or viscosity, although with the buffer employed such corrections would be minimal.

Electron Spin Resonance Spectroscopy. ESR spectra were obtained at 25° with a Varian E-4 spectrometer operating at 9.5 GHz. Power saturation studies were carried out in order to determine the maximum allowable modulation and power settings. All spectra were obtained at 20 mW power with a modulation of 2 G. Samples for ESR spectroscopy were placed in a quartz aqueous sample cell and then introduced into the cavity of the spectrometer.

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References and Notes

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