Topologically Constrained Bifunctional Intercalators: DNA Intercalation by a Macrocyclic Bisacridine

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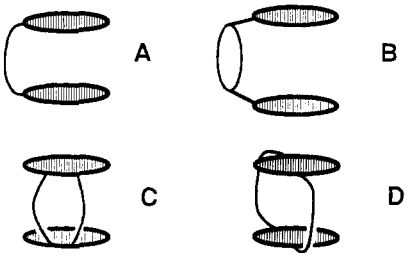
Abstract: A topologically novel DNA bifunctional intercalator, **4**, has been synthesized and its DNA binding compared with the binding of the monointercalator 9-aminoacridine (**6**) and spermine bisacridine (**5**), a known bisintercalator. Water-soluble macrocyclic bisacridine **4** was synthesized by reaction of 4-(bromomethyl)-9-chloroacridine with *N,N'*-bis(2-mercaptoethyl)succinamide and base. The resulting dichloride was converted to the corresponding bis(9-phenoxyacridine), which reacted with spermine tetrahydrochloride to form macrocyclic bisacridine **4**. At a 1:10 ratio of **4** to calf thymus DNA phosphate, a ΔT_m of >40 °C was measured, which indicates a high binding affinity. Viscometric analysis of helix extension using sonicated calf thymus DNA gave slopes for compounds **4** and **5** that were much greater than that for **6**. Metachromic shifts were observed in the absorption spectra of **4** upon addition of DNA. These metachromic shifts were similar to those shown by **5** and **6** under the same conditions. Additions of **4** to closed circular supercoiled plasmid DNA (pOP1Δ6) removed and reversed the supercoiling as measured by the changes in the viscometric properties of the plasmid. The similarities in binding data obtained for macrocycle **4** and the known bisintercalator spermine bisacridine (**5**) indicate that the former also binds to DNA as a bifunctional intercalator.

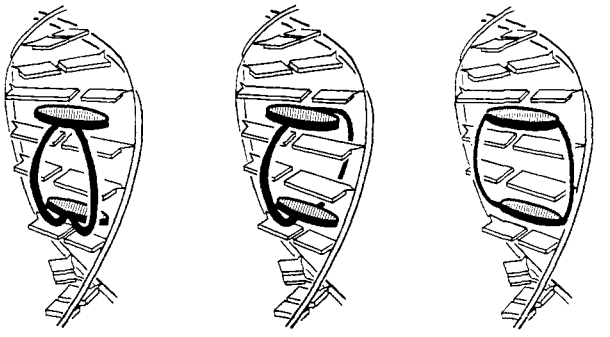
For more than a decade extensive effort has been directed toward the synthesis and study of DNA polyintercalators, primarily based on their potential as effective antitumor agents.[1] Additionally, these compounds can serve as experimental tools to probe DNA structure. Studies of more than 100 DNA bisintercalators have been published, their structures differing in length, rigidity, and charge of the linking chain and by the intercalating chromophore itself.[2] What is invariant in these bisintercalators is their *topology*. These synthetic "receptors" for DNA have been exclusively of the nonmacrocyclic structure represented by A.



Two classes of naturally occurring DNA bisintercalators are known: Members of both have aromatic chromophores attached to a macrocyclic peptide as represented by structure B. In the naturally occurring bisquinoxaline antitumor antibiotics[2,3] (e.g., triostin A and echinomycin), the intercalating chromophores are linked by a bicyclic octadepsipeptide, while in luzopeptin quinoline chromophores are attached to a somewhat more flexible macroring containing ten amino acids and two tetrahydropyrazine rings.[2,4] The crystal structure of the triostin A–DNA complex shows that the peptide backbone lies in the minor groove, with the quinoxaline rings intercalated into the helix and spanning two base pairs.[5]

Bisintercalators with aromatic rings linked by *two* chains, as represented by C and D, have not been investigated, probably due to the *topological* constraint imposed by the DNA on the bifunctional intercalation process. With acridine chromophores, if the two linking chains are closely attached on the major axis (i.e., C), normal bifunctional intercalation might occur with both chains in a single (presumably minor) groove, thus forming complex **1**.[6]

A bisintercalator with linking chains attached on the major axis of the acridine ring, but on *opposing sides* (i.e., D), could (1) bind



by perpendicular insertion of a small portion of the acridine rings, forming complex **3**, (2) bind as a "normal" bifunctional intercalator by forming *catenated complex* **2**, or (3) not bind as a bisintercalator at all. Normal implies alignment of the long axes of the intercalator and DNA base pairs as invariably seen in the X-ray analysis of intercalator miniduplex complexes.[7] An exception

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(6) Schematics A-D and **1-3** are not meant to convey information regarding major vs minor axis attachment of the bisintercalator side chains.

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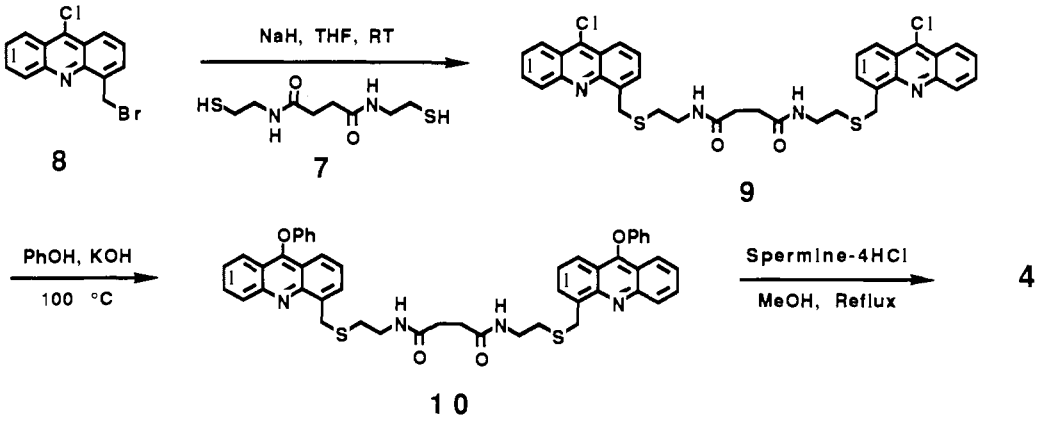
Scheme I



is daunomycin, which intercalates perpendicularly.[8] However, the oxygen-containing functionality along the major axis of daunomycin allows the intercalation site to be more fully filled. Partial intercalation models involving small aromatic compounds have been proposed, but they are controversial and undocumented.[9] Thus, the partially intercalated complex **3** is without precedent and might be predicted to be weak, because only a small portion of the acridine ring can overlap with the base pairs.[9]

The alternative catenated complex **2** would have the acridine chromophores oriented in the normal fashion (vide supra). However, **2** could be formed only if the bisintercalator located transient openings in Watson–Crick base pairs, allowing one linking chain in the minor groove and one in the major groove, followed by re-formation of the hydrogen-bonded base pairs. This type of complex is also without precedent. Several monointercalators—most notably nogalamycin—have been proposed to require breakage of at least one hydrogen-bonded base pair in order to bind.[10,11] Additionally, the crystal structure of the triostin A complex with an octanucleotide shows the presence of four Hoogsteen base pairs, two located at internal positions on the helix and two located at the end of the octanucleotide, suggesting that the formation of an intercalation complex can disrupt, at least in a transient manner, the normal Watson–Crick base pairing.[5]

As a result of these considerations, we have initiated a program directed toward the synthesis and study of macrocyclic DNA bisintercalators. We report a macrocyclic bisacridine of structure type D (**4**) that binds to DNA in a fashion strikingly similar to that of spermine bisacridine (**5**), a nonmacrocyclic analogue and a known bifunctional intercalator. Although assignment of the bisintercalation complex as either structure **2** or **3** cannot be made, the result indicates that DNA can exhibit high plasticity in order to accommodate the bisintercalation process.

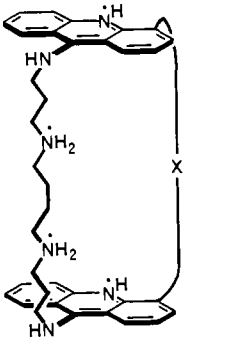
Table I. Thermal Denaturation Data (ΔT_m, °C) for Acridines at Various Compound to DNA Phosphate Ratios[a,b]

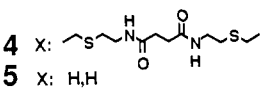
	calf thymus DNA compd to DNA-P ratio				poly(dA)·poly(dT)	poly[d(GC)]
no.	1:10	1:20	1:40	1:80	1:10	1:10
4	>40	>40[c]	7[c]	4	51	>10
5	>40	40[c]	7[c]	3	52	>10
6	9	6	4	3	7	4

[a] Experiments were conducted at pH 7.55 in 5 mM Tris buffer containing 1.25–5.0 μM compound, 50 μM DNA-P, 50 μM EDTA, and 5% DMSO. [b] Greater than symbol indicates DNA not fully denatured up to the maximum temperature (100 °C) obtainable under the experimental conditions. [c] The thermal denaturation profile of the compound in these experiments was biphasic.

Results and Discussion

The linking chains in **4** were designed to increase water solubility and are long enough to allow a favorable conformation for binding with a neighbor-excluded geometry. Additionally, 9-aminoacridine (**6**) and spermine bisacridine (**5**) are well-studied mono- and bifunctional DNA intercalators.[12] The synthesis of **4** is outlined





4 X:

5 X: H,H

in Scheme I. The bisamide linker was synthesized by reaction of succinyl chloride and 2-aminoethanethiol, producing dithiol **7** in 33% yield (based on succinyl chloride). (Bromomethyl)acridine **8** was produced in 67% yield by NBS bromination of 9-chloro-4-methylacridine.[13] The dithiolate generated from **7** was reacted with bromide **8** to produce dichloride **9**. Dichloride **9** was unstable to purification and was reacted directly with phenol and potassium hydroxide to afford its 9-phenoxy derivative, **10**, in 31% overall yield from **8**. High-dilution cyclization of **10** with spermine

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(13) Acheson, R. M.; Bolton, R. G. *J. Chem. Soc., Perkin Trans. 1* **1975**, 650. Acheson, R. M.; Constable, E. C.; Wright, R. G. M.; Taylor, G. N. *J. Chem. Res., Synop.* **1983**, (1), 2–3.

Table II. Spectroscopic Data for Ligand Free and Bound to DNA[a]

compd	λ_{max_f}	ϵ_f	λ_{max_b}	ϵ_b	H^b
4	396	13000	399	7900	39
	413	18000	417	12000	33
	435	15000	441	11000	27
5	393	12000	397	9600	20
	412	18000	416	13000	28
	434	13000	440	11000	15
6	380	6400	386	3200	50
	400	9800	406	4800	51
	423	7800	428	4000	49

[a] Experiments were conducted at pH 7.55 in 5 mM Tris buffer containing 10 μM EDTA and 5% DMSO. Addition of aliquots of a 18 mM DNA-P solution was made to a solution 2–5 mM in compound. [b] H is percent hypochromicity [% $H = (1 - \epsilon_b/\epsilon_f) \times 100$].

tetrahydrochloride in methanol produced macrocyclic bisacridine **4** in 42% yield.

Intercalators, in binding selectively to double-stranded DNA, increase the helix–coil transition temperature (T_m). The usual measure of this effect is the difference in the transition temperature for the DNA with and without the intercalator (ΔT_m), which gives an indication of the binding affinity. Thus, DNA ΔT_m values were determined on sonicated calf thymus DNA or commercially available homopolynucleotides in low ionic strength buffer by the method of Cory.[14] At a 1:10 compound/DNA phosphate ratio, the ΔT_m values were too high to measure for compounds **4** and **5** (Table I). The 1:10 ratio was chosen because it is below saturation of the DNA if a neighbor-exluded monofunctional intercalation mechanism is assumed. The ΔT_m values in Table I indicate that **4** and **5** have a much higher affinity for DNA than 9-aminoacridine (**6**), a result in part reflecting the three additional cationic charges in the diacridines. Demonstrating that the second acridine ring in **4** and **5** contributes to the increase in binding affinity is difficult since the high ionic strength conditions ([NaCl] > 1 M) needed to negate the ionic contributions from the side chain[15] would result in a very high DNA T_m (ca. 100 °C) making ΔT_m measurements difficult.

The binding of **4–6** to DNA was studied spectrophotometrically, since intercalation of the acridine chromophore leads to metachromic shifts in its absorption spectra.[16] Relative to the free-compound absorption spectra, the three major ultraviolet bands in **4–6** bound to DNA are red-shifted and display hypochromic effects (Table II). The hypochromicity seen in the bisintercalators is less than that seen with 9-aminoacridine. This could result from a number of factors including (1) incomplete insertion of both chromophores of **4** and **5**, (2) tilting of one or both of the acridine rings in **4** and **5** relative to the DNA base pairs, (3) the difference in substitution of the chromophores, and (4) intramolecular stacking of the acridines in unbound **4** and **5**. Comparison of the ϵ_f values for **4–6** indicates the latter to be a contributor to the lowered hypochromicity seen with **4** and **5**.

The most crucial test of intercalative binding is a compound's ability to unwind closed circular supercoiled DNA, as detected by changes in hydrodynamic properties.[17] Addition of increasing amounts of compounds **4–6** to pOP1Δ6 DNA caused removal and then reversal of the supercoils (Figure 1). Ordinarily a bifunctional intercalator should exhibit an unwinding angle twice that of an analogous monointercalator. However, it is known that bifunctional diacridines containing two basic nitrogens in the linking chain have unwinding angles not greatly different from that of 9-aminoacridine.[2] While the origin of this effect is not known, the results with **4–6** are consistent with the literature data.

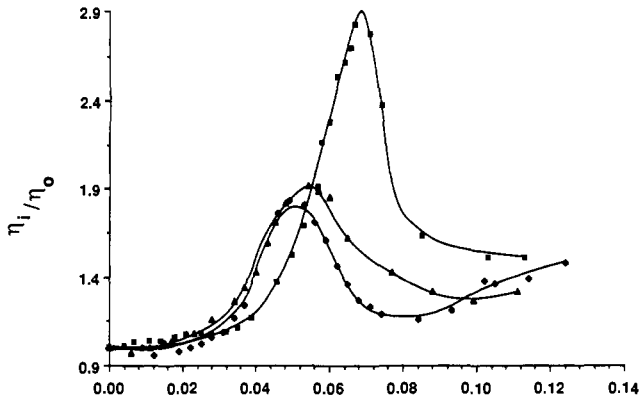

Figure 1. Representative experiment showing unwinding of closed circular supercoiled plasmid pOP1Δ6 DNA by compounds **4** (♦), **5** (▲), and **6** (■). Experiments were conducted at pH 6.3 in 2 mM MES containing 1 mM EDTA, 1 mM NH_4F, and 5% DMSO. Data analysis was done by the methods in ref 25.

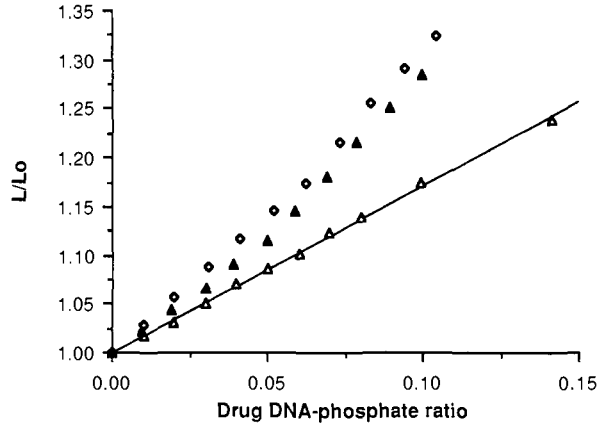

Figure 2. Representative experiment showing the increase in viscosity of sonicated calf thymus DNA by intercalating compounds **4** (▲), **5** (◊), and **6** (△). Experiments were conducted at pH 7.0 in 2 mM HEPES containing 10 μM EDTA and 9.4 mM NaCl. Data analysis was done by the methods in ref 25.

Thus, the unwinding angles obtained for macrocycle **4** (20°) and spermine bisacridine **5** (18°) were greater than that of 9-aminoacridine (**6**) (15.8°) by only 1.3- and 1.1-fold, respectively.

Definitive evidence that both acridine rings of **4** are intercalated upon binding to DNA comes from comparison of its helix extension parameter with that of **5** and **6**.[2] The helix extension parameters were measured under the ionic strength conditions of Wakelin.[19] The slope obtained from the L/L_0 vs drug/DNA phosphate ratio plot for **6** (slope 1.59 ± 0.19; lit.[19] slope 1.79) was much smaller than that seen for both **4** and **5**. As seen in Figure 2 spermine bisacridine (**5**) and macrocycle **4** afforded helix extension plots with upward curvature that analyzed best as two lines with a break point at $R = 0.06$. While the origin of this frequently observed behavior is not known, the higher slopes in the helix extension plot for **4** (3.28 ± 0.13) and **5** (3.40 ± 0.12) are over twice that seen with 9-AA (**6**) (9-AA = 9-aminoacridine). The slopes at the lower saturation values for **4** (2.23 ± 0.05) and **5** (2.63 ± 0.16) are 1.4 and 1.65 times greater, respectively, than the value for 9-AA. These data are fully consistent with intercalation of both acridine chromophores in **4** and **5**.[20]

The strikingly similarity in binding data obtained for macrocycle **4** and spermine bisacridine (**5**) support the conclusion that **4** also

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(20) Flow dichroism studies indicate that both chromophores in **4** and **5** are oriented perpendicular to the helix axis also consistent with bisintercalation. Wilson, W. D. Personal communication.

binds to DNA as a bifunctional intercalator. They do not, however, distinguish between complexes **2** and **3**. Modeling studies suggest that the best fit between DNA and **4** occurs in catenated complex **2**, in which the long axis of the acridines and the DNA base pairs are aligned in the normal fashion. It would be remarkable, however, if a complex such as **2** was formed so readily. The probability of base-pair opening is only about 10^{-5}, although this number has been controversial.[21] Additionally, it has been argued that DNA breathing occurs by the opening of a single base pair,[21a,c] while formation of complex **2** (neighbor-exclusion model) requires a minimum of two base pairs to open.

The alternative complex, **3**, in which both chains lie in a single groove and a small portion of the acridine chromophores is inserted perpendicularly between base pairs is without precedent but could be stabilized by the strong ionic interactions between the charged linking chain and the phosphate backbone. In either case, this work demonstrates that macrocyclic structures are fully compatible with the bisintercalation process. Further study of this new class of DNA bisintercalators is under way and will elucidate the structure and dynamics[22] of complex formation as well as potential sequence selectivity and biological activity.

Experimental Section

General Procedures. All reactions were carried out under 1 atm of dry nitrogen. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl prior to use. Methanol was distilled from magnesium methoxide and stored over 3-Å molecular sieves. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride, and carbon tetrachloride was passed through a column of neutral alumina. Phenol and succinyl chloride were fractionally distilled under reduced pressure prior to use. *N*-Bromosuccinimide was recrystallized from water and dried in a vacuum desiccator over phosphorus pentoxide. All other solvents or reagents were of reagent-grade quality and were used without further purification. Spermine bisacridine (**5**) was prepared by a higher yielding modification of the literature procedure (see below).[12] Analytical TLC was performed on 0.2-mm silica 60 coated plastic plates (EM Science) with F-254 indicator. Flash chromatography was performed on Merck 40–63-μm silica gel according to the procedure of Still.[23] Melting points were measured on a Thomas Hoover melting point apparatus and are uncorrected.

^{1}H NMR spectra were performed in $CDCl_3$ and recorded on a General Electric QE-300 spectrometer unless otherwise stated. ^{13}C NMR spectra were performed in $CDCl_3$ and recorded on a General Electric QE-300 spectrometer at 75 MHz unless otherwise stated. Spectra were referenced to internal TMS in deuterochloroform, to the residual protio solvent peak in methanol-d_4, or to sodium 3-(trimethylsilyl)propionate (TSP) in deuterium oxide. Coupling constants are reported in hertz (Hz). A presaturation pulse sequence was used for spectra obtained in deuterium oxide. The ^{1}H NMR assignments of **4** were made from its COSY spectrum obtained on a GN-500 spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1320 spectrometer. Mass spectra were obtained on a Finnigan-MAT CH-5 (EI) or on a Finnigan-MAT 731 (FD and FI) spectrometer. Elemental analyses were performed at the University of Illinois School of Chemical Sciences.

DNA. Calf thymus DNA for the thermal denaturation and linear viscosity experiments was prepared by the method of Cory.[14] The high copy number, 6.9 kilobase, closed circular supercoiled plasmid pOP1Δ6 was used for the unwinding angle determinations. It was isolated by the method of Garger.[24]

Thermal Denaturation. Thermal denaturation studies were performed by the procedure of Cory[14] on a Varian 2290 UV–visible spectrophotometer with a heating rate of 18 °C/h. A cuvette dwell time of 5 s and a five-cuvette cycle time of 2 min were used. T_m results were determined graphically for each cuvette from the spectrophotometer printout. The ΔT_m values for each compound were determined as the difference between the compound T_m and the DNA or homopolymer T_m. Under these conditions T_m for calf thymus DNA was 57 °C, for poly(dA)·poly(dT) was 42 °C, and for poly[d(GC)] was 84 °C. Poly(dA)·poly(dT) was obtained from Boehringer-Mannheim and poly[d(GC)] from Pharmacia Molecular Biology Division.

Viscometric Titrations. The viscometric titrations were done by the method of Cohen and Eisenberg[18] as modified in ref 14. Time readings were recorded automatically by use of a Wescan (Wescan Instruments, Santa Clara, CA) viscosity timer attached to the Cannon-Ubblehode viscometers. Reduced specific viscosity was calculated by the method of Cohen and Eisenberg.[18] For compounds **4** and **5** data at a compound to DNA phosphate ratio between 0.0 and 0.1 were fit to a straight line. For compound **6** data from 0.0 to 0.2 were used. The results of the titrations for compounds **4–6** are shown in Figure 2. Compounds **4** and **5** gave precipitates at ratios higher than 0.15 so the stoichiometry of saturation could not be determined.

Unwinding Angle Determinations. Unwinding angle determinations were done by the method of Revet.[17] Some early experiments were done with the plasmid pBR322, but the majority of the experiments were done with pOP1Δ6. Comparison of the unwinding angle measurements for ethidium bromide using both plasmids showed that the data could be merged. The slope of the Vinograd plot[17] for ethidium bromide using pOP1Δ6 was considered to represent an unwinding angle of 26°. At least five titrations with compounds **4–6** were done at various DNA concentrations, and the point of highest viscosity, representing fully unwound plasmid equilibrium points, was fit to a line whose slope was compared with that of ethidium bromide.[25] Figure 1 shows one titration from this series of experiments with each of compounds **4–6**.

UV Spectrophotometric Titrations. UV spectral data were recorded on a Cary 118 spectrophotometer by titrating a solution of DNA into a solution of the compound in 5 mM Tris, 10 mM EDTA, pH 7.55, buffer containing 5% DMSO. A DNA stock solution of 18.3 mM was added in aliquots to a solution of the compound (generally between 2 and 5 mM) dissolved in buffer. The UV spectrum was scanned after each addition. Titrations were stopped when no change in the spectrum was detected between additions. In low ionic strength buffer an isosbestic point was detected with **6** but not with **4** or **5**. The data are summarized in Table II.

***N*,*N*′-Bis(2-mercaptoethyl)butane-1,4-diamide (7).** To a suspension of 2.5 g (32.4 mmol) of 2-aminoethanethiol in 130 mL of CH_2Cl_2 at 0 °C was added 8.92 mL (8.1 mmol) of succinyl chloride. The reaction was warmed to room temperature, and the heterogeneous mixture was stirred at 25 °C for 4 h. The solvent was evaporated, and the residue was dissolved in 25 mL of saturated aqueous ammonium chloride solution and washed four times with 10% 2-propanol–chloroform. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford 1.38 g of a white solid. Recrystallization from ethyl acetate yielded 0.71 g (37%) of **7** as a white powder: mp 163–166 °C; TLC R_f = 0.3, 10% methanol–CH_2Cl_2; IR (KBr) 3290, 2540, 1634, 1550 cm^{-1}; ^{1}H NMR δ 1.44 (t, 2 H, J = 8.5, SH), 2.60 (s, 4 H, $COCH_2$), 2.69 (dt, 4 H, J_1 = 8.5, J_2 = 6.5, CH_2S), 3.46 (q, 4 H, J = 6.5, CH_2N), 6.40 (br s, 2 H, NH); ^{13}C NMR (CD_3OD) δ 24.4, 32.4, 43.5, 174.4; MS (FI, 150 °C) m/z (relative intensity) 236 (M^+, 100). Anal. Calcd for $C_8H_{16}N_2O_2S_2$: C, 40.67; H, 6.83; N, 11.86; S, 27.09. Found: C, 40.57; H, 6.72; N, 11.72; S, 27.11.

4-(Bromomethyl)-9-chloroacridine (8). To a mixture of 6.57 g (36.9 mmol) of *N*-bromosuccinimide (NBS) in 300 mL of carbon tetrachloride was added 8.0 (35 mmol) of 4-methyl-9-chloroacridine. A 100-W light bulb was placed about 2 in. below the reaction flask, and the setup was loosely covered with aluminum foil. The reaction was photolyzed at reflux until the starting material was consumed (TLC). The mixture was diluted with 200 mL of carbon tetrachloride, filtered, and concentrated under reduced pressure. The dark yellow solid was recrystallized from hexane–ethyl acetate to yield 7.22 g (67%) of **8** as light yellow needles: mp 187–189 °C dec; TLC R_f = 0.3, 35% CH_2Cl_2–hexane; ^{1}H NMR δ 5.39 (s, 2 H, $ArCH_2$), 7.60 (dd, 1 H, $J_{1,2}$ = 8.7, $J_{2,3}$ = 7.0, H-2), 7.66 (m, 1 H, H-7), 7.83 (dd, 1 H, $J_{5,6}$ = 8.7, $J_{6,7}$ = 7.0, H-6), 7.95 (d, 1 H, $J_{2,3}$ = 7.0, H-3), 8.30 (d, 1 H, $J_{5,6}$ = 8.7, H-5), 8.42 (d, 2 H, $J_{7,8}$ = 8.7, H-1), 8.42 (d, 2 H, $J_{1,2}$ = 8.7, H-1); MS (EI, 70 eV) m/z (relative intensity) 307 (M^+ for ^{81}Br, 21), 305 (M^+ for ^{79}Br, 15), 226 (M^+ − Br, 100). Anal. Calcd for $C_{14}H_9NClBr$: C, 54.85; H, 2.96; N, 4.57. Found: C, 54.68; H, 3.12; N, 4.83.

***N*,*N*′-Bis[2-[[(9-chloroacridin-4-yl)methyl]thio]ethyl]butane-1,4-diamide (9).** A slurry of 1.06 g (4.5 mmol) of powdered dithiol **7** and 226 mg (9.4 mmol) of NaH (97%) in 70 mL of dry THF was sonicated until the

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(22) Complex **2** might be expected to be kinetically more stable than complex **3**. Preliminary studies indicate that **4** and **5** dissociate with similar rates from poly[d(AT)] but that **4** dissociates ca. 20-fold slower from poly[d(GC)] than does **5**. However, **4** dissociates from poly[d(GC)] >100 times faster than does nogalamycin. Wilson, W. D. Personal communication.

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evolution of hydrogen gas ceased. The slurry was diluted with 10 mL of THF, and 2.75 g (9.0 mmol) of **8** was added. The heterogeneous light yellow mixture changed to a dark yellow viscous solution. The mixture was stirred at ambient temperature for 3 h, and the solvent was evaporated under reduced pressure to yield 4.18 g of crude **9** (68% yield). This material was about 80% pure by ^{1}H NMR. Purification by silica gel chromatography caused decomposition, so the product was used without further purification: mp 204-210 °C dec; TLC R_f = 0.4, 6% methanol-CH_2Cl_2; ^{1}H NMR δ 2.45 (s, 4 H, $COCH_2$), 2.68 (t, 4 H, J = 6.0, CH_2S), 3.58 (dd, 4 H, J_1 = 6.0, J_2 = 6.0, CH_2N), 4.52 (s, 4 H, $ArCH_2$), 6.50 (br s, NH), 7.56 (dd, 2 H, $J_{1,2}$ = 9.0, $J_{2,3}$ = 7.0, H-2), 7.62 (dd, 2 H, $J_{6,7}$ = 7.0, $J_{7,8}$ = 9.0, H-7), 7.77 (m, 4 H, H-3, H-6), 8.23 (d, 2 H, $J_{5,6}$ = 9.0, H-5), 8.35 (dd, 2 H, $J_{1,2}$ = 9.0, $J_{1,3}$ = 1.0, H-1), 8.41 (d, 2 H, $J_{7,8}$ = 9.0, H-8); MS (FD, 22 mA) m/z (relative intensity) 686 (M^+, 100).

***N,N'*-Bis[2-[[(9-phenoxyacridin-4-yl)methyl]thio]ethyl]butane-1,4-diamide (10).** A mixture of 3.0 g (4.36 mmol) of crude **9** and 56 g (596 mmol) of phenol was immersed in a 95 °C oil bath until the phenol melted. One 980-mg (17.4-mmol) portion was added of powdered KOH. The solution was stirred at 100 °C for 1 h and cooled slightly and the viscous solution poured into 650 mL of 1 M NaOH. This solution was washed four times with 50 mL of 10% 2-propanol-chloroform, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The solid residue was purified by flash chromatography (10% CH_2Cl_2-ethyl acetate) and then recrystallized from acetonitrile to yield 1.1 g (31%) of **10** as a light yellow powder: mp 167-169 °C; TLC R_f = 0.3, 10% CH_2Cl_2-ethyl acetate; IR (KBr) 3300, 1634 cm^{-1}; ^{1}H NMR δ 2.50 (s, 4 H, $COCH_2$), 2.74 (t, 4 H, J = 6.0, CH_2S), 3.65 (dd, 4 H, J_1 = 6.0, J_2 = 6.0, CH_2N), 4.56 (s, 4 H, Ar-CH_2), 6.73 (t, 2 H, J = 6.0, NH), 6.83 (d, 4 H, $J_{10,11}$ = 8.0, H-10), 7.03 (t, 2 H, $J_{11,12}$ = 7.0, H-12), 7.26 (m, 4 H, H-11), 7.41 (m, 4 H, H-2, H-7), 7.75 (m, 4 H, H-3, H-6), 8.02 (dd, 2 H, $J_{5,6}$ = 8.8, $J_{5,7}$ = 1.0, H-5), 8.05 (d, 2 H, $J_{1,2}$ = 8.5, H-1), 8.26 (d, 2 H, $J_{7,8}$ = 8.8, H-8); ^{13}C NMR (500 MHz) δ 31.20, 31.73, 32.06, 38.67, 115.53, 120.25, 120.25, 122.19, 122.54, 122.63, 125.52, 126.04, 129.92, 130.25, 130.56, 137.23, 148.65, 149.83, 155.36, 159.54, 172.16; MS (FD, 18 mA) m/z (relative intensity) 802 (M^+, 100), 401 (M^{2+}, 10); Anal. Calcd for $C_{48}H_{42}N_4S_2O_4$: C, 71.80; H, 5.27; N, 6.98; S, 7.99. Found: C, 71.71; H, 5.30; N, 7.10; S, 7.93.

1,5,10,14,33,38-Hexaaza-30,41-dithia[14.14](4,9)acridinophane-34,37-dione (4). To a solution of 365 mg (1.05 mmol) of spermine tetrahydrochloride in 1 L of dry methanol was added 825 mg (1.03 mmol) of **10** followed by 30 mL of methanol (1 mM in bisacridine). The reaction was stirred at reflux for 24 h and then concentrated at reduced pressure to yield 1.07 g of crude product. The solid was slurried in ethanol, absorbed onto silica gel, and flash chromatographed, eluting with 100% ethanol until the first yellow band came off. The column was eluted with 4% diethylamine-ethanol to produce 360 mg (42%) of **4** as a dark yellow oily solid. The tetrahydrochloride of **4** was formed by dissolving the free base in methanol and adding methanol saturated with gaseous HCl at 0 °C. The methanol was removed under reduced pressure to yield a light yellow powder. The solid was repeatedly dissolved in water and lyophilized to yield a fluffy canary yellow solid: mp 189-191 °C dec; TLC R_f = 0.30, 4% diethylamine-ethanol; IR (KBr) 1625 cm^{-1}; ^{1}H NMR (500 MHz, D_2O, 16 mM, 20 °C) δ 1.91 (br s, 4 H, H-7), 2.32 (br s, 4 H, H-35), 2.38 (t, 4 H, $J_{2,3}$ = 6.8, $J_{3,4}$ = 6.8, H-3), 2.54 (t, 4 H, $J_{31,32}$ = 5.7, H-31), 3.21 (br s, 4 H, H-6), 3.29 (br s, 8 H, H-4, H-32), 3.93 (br s, 4 H, H-29), 4.02 (t, 4 H, $J_{2,3}$ = 6.8, H-2), 6.80 (br s, 2 H, H-26), 7.32 (m, 4 H, H-18, H-25), 7.52 (d, 2 H, $J_{18,19}$ = 6.2, H-19), 7.59 (d, 2 H, $J_{24,25}$ = 8.3, H-24), 7.77 (d, 2 H, $J_{26,27}$ = 7.8, H-27), 8.00 (d, 2 H, $J_{17,18}$ = 8.6, H-17); ^{13}C NMR (500 MHz, D_2O) δ 24.92, 28.59, 33.06, 33.78, 34.03, 40.85, 47.07, 48.53, 48.87, 120.73, 126.07, 126.43, 126.47, 128.00, 137.82, 139.01, 160.55, 177.05 (missing resonances due to low S/N); MS (FAB) m/z (relative intensity) 817 (M^+ + 1, 100 for peaks m/z > 650). Anal. Calcd for $C_{46}H_{56}N_8S_2O_2 \cdot 4HCl \cdot 6H_2O$: C, 51.57; H, 6.78; N, 10.47; S, 5.97; Cl, 13.25. Found: C, 51.29; H, 6.19; N, 10.07; S, 5.64; Cl, 13.48.

***N,N'*-Bis[3-(9-acridinylamino)propyl]butane-1,4-diamine (Spermine Bisacridine) (5).** By the method outlined for **4** (1 mM in 9-phenoxyacridine), 356 mg (56%) of **5** was obtained as a yellow solid: mp >235 °C; TLC R_f = 0.3, 6% diethylamine-ethanol; ^{1}H NMR (D_2O) δ 1.87 (br s, 4 H, H-5'), 2.40 (m, 4 H, H-2'), 3.18 (br s, 4 H, H-4'), 3.29 (t, 4 H, $J_{2',3'}$ = 7.8, H-3'), 4.20 (t, 4 H, $J_{1',2'}$ = 7.2, H-1'), 7.55 (dd, 4 H, $J_{1,2}$ = 8.7, $J_{2,3}$ = 7.7, H-2), 7.66 (d, 4 H, $J_{3,4}$ = 8.5, H-4), 7.94 (dd, 4 H, $J_{3,4}$ = 8.5, $J_{2,3}$ = 7.7, H-3), 8.25 (d, 4 H, J = 8.7, H-1); MS (FD, 8 mA) m/z (relative intensity) 556 (M^+, 100). Anal. Calcd for $C_{36}H_{40}N_6 \cdot 4HCl \cdot 1.5H_2O$: C, 59.26; H, 6.49; N, 11.52. Found: C, 59.40, H, 6.59; N, 11.33.

Acknowledgment. We thank Heidi Guzalo for preliminary synthetic efforts, Jack Starmer and Lee Chenier for assistance with the DNA binding studies, and Dr. Lynn Elwell for assistance in preparing the plasmid used in these experiments. Funding from the donors of the Petroleum Research Fund, administered by the American Chemical Society, the American Cancer Society (Junior Faculty Award to S.C.Z.), and the National Science Foundation PYI Program (Grant CHE-8858202) is gratefully acknowledged.

Mutual Recognition between Polymerized Liposomes: Macrophage Model System by Polymerized Liposomes†

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Received June 30, 1988

Abstract: A macrophage model system using polymerized liposomes was examined. Recognition and subsequent attack of an avidin-carrying *partly* polymerized liposome by a biotin-carrying and phospholipase A_2 (PLA_2) carrying polymerized liposome were studied by the turbidimetry and fluorescence methods. While the hydrolysis of dimyristoylphosphatidylcholine (DMPC) molecules was lowered, the introduction of avidin and biotin onto the surface of liposomes promoted the concentrated attack of PLA_2 to DMPC molecules in a narrow region on the avidin-carrying liposome; an effective "uncorking" of the avidin-carrying liposome is thus realized. Effects of the surface morphology and the surface density of the complementary ligands on the recognition phenomena were examined.

Recognition of invading cells and subsequent attack on cells by other cells having immunological or phagocytic roles are essential processes in the protective system of living bodies. For example, activated macrophage cells are reported to attack tumor cells and make a lysis of the wall of the tumor cells.[1] To mimic such phenomena, we examined here the recognition and subsequent attack (lysis) of a model cell by another model cell using a polymerized liposome system.

† Presented at the 37th Annual Meeting of the Society of Polymer Science, Japan, at Nagoya, May 1988.

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