Synthesis and DNA Binding Properties of Bis-9-acridinyl Derivatives Containing Mono-, Di- and Tetra-viologen Units as a Connector of Bis-intercalators

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Bis-9-acridinyl derivatives **1-3** containing mono-, di- and tetra-viologen units as a rigid connector were synthesized. The binding studies of these intercalators for natural and synthetic DNAs showed that these compounds act as bis-intercalators where viologen moieties lie in the minor groove of the DNA duplex. The DNA binding affinity of the intercalators was enhanced with an increase in the number of the viologen unit.

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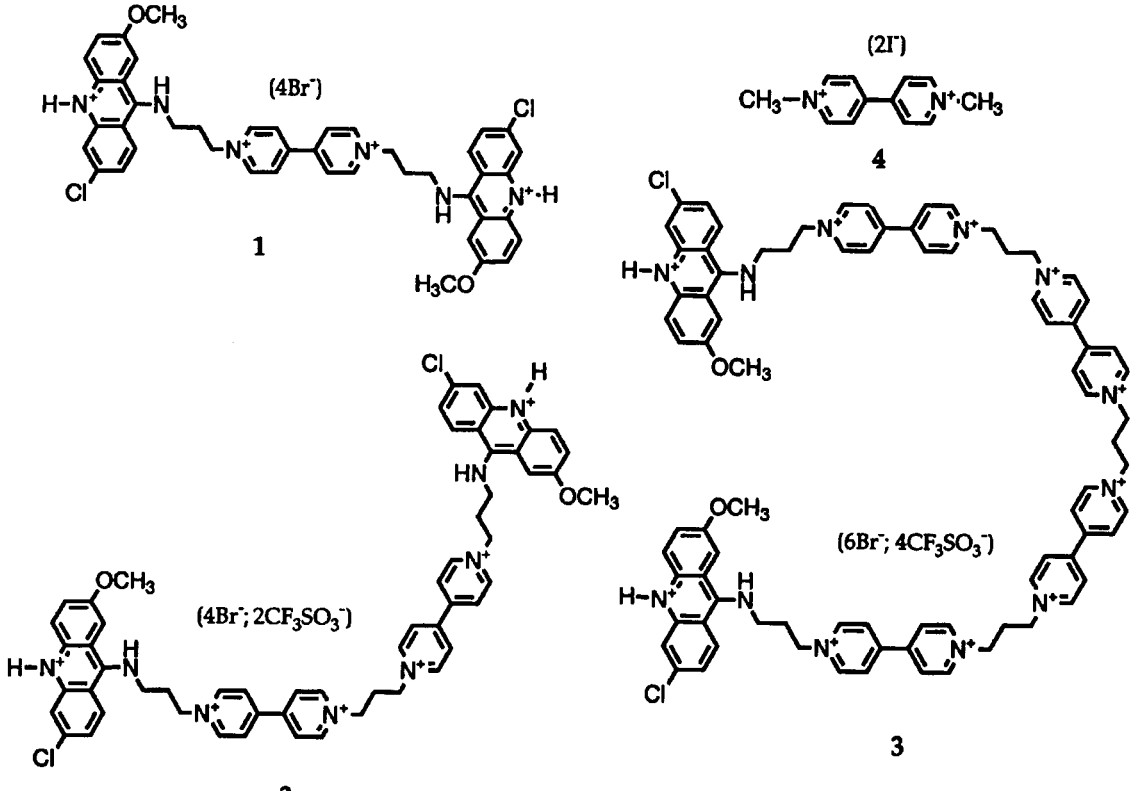
Introduction.

A series of aromatic compounds show a variety of biological activities, some of which are believed to be derived from their interaction with deoxyribonucleic acid (DNA) [1,2]. Bis-intercalators having two aromatic units within the same molecule have been studied with an expectation for increased DNA binding affinity and selectivity for DNA [3]. However, bis-intercalators with two intercalator units bridged by a simple alkyl chain hardly improved DNA sequence specificity [4,5]. This is due in part to a self-stacking within the two intercalator moieties and to a difference in the optimal conformation of the bis-intercalating molecule between the free and DNA-bound states. To overcome these disadvantages, compounds having two intercalator units connected with a rigid linker chain were developed by reference to naturally occurring bis-intercalating antibiotics like echinomycin [6,7]. It was found that these compounds act as molecular staples for duplex DNA. However, the solubility of the compounds in water was rather low compared with ordi-

Scheme 1



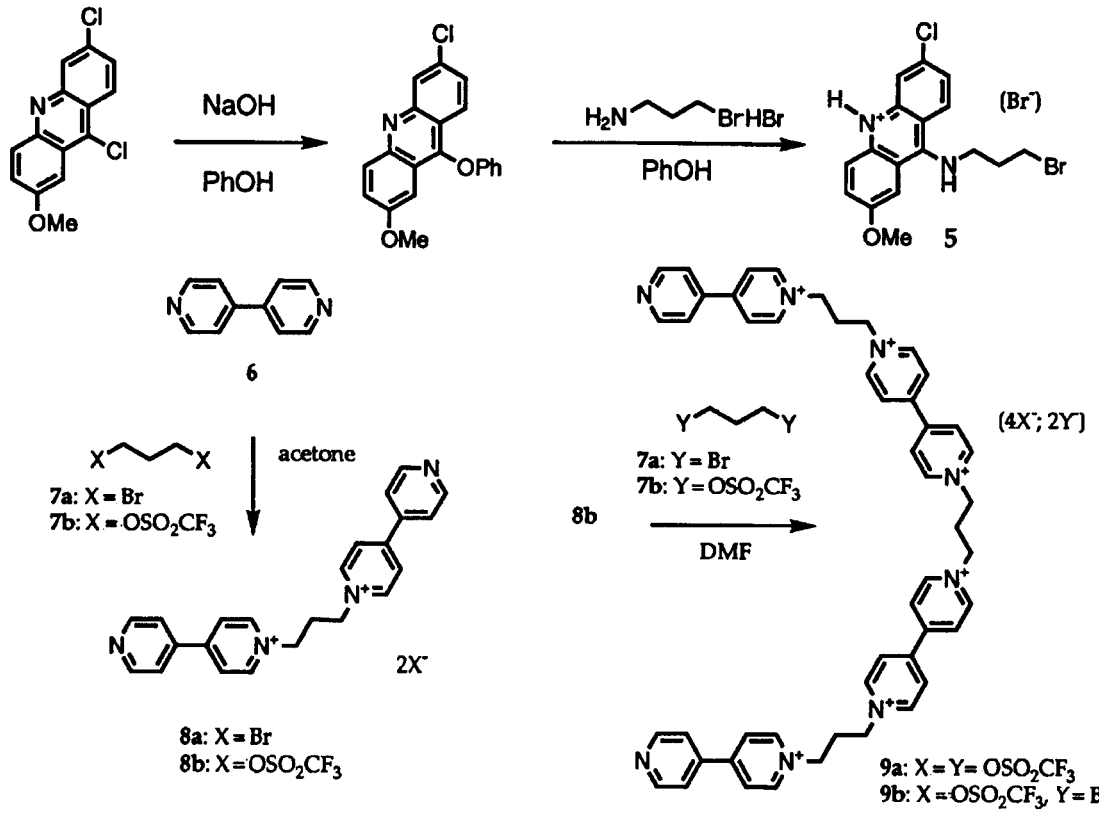
nary bis-intercalators because of the presence of an aromatic linker as the rigid chain [6]. We synthesized bis-9-acridinyl derivative **1** carrying a viologen as a connector of the two intercalator units as shown in Scheme 1 [8,9]. Since compound **1** has good solubility in water in spite of the presence of the rigid aromatic linker, it allowed a detailed analysis of its binding with DNA. Compound **1** is regarded as a molecular staple covering four base pair-region of duplex DNA.

In this paper, we synthesized homologues of **1**, **2** and **3**, carrying di- and tetra-viologen units, respectively, where each viologen is connected by a trimethylene chain (Scheme 1). Compounds **2** and **3** have interchromphore distances of 28 and 48 Å at maximum, respectively, as measured on the Corey-Pauling-Koltun models. Thus, compounds **2** and **3** should be regarded as a molecular staple which can cover six and ten base pairs of DNA duplex, respectively. This property of **2** and **3** may result in an enhanced gene-regulatory activity. Of the bis-intercalators reported to date, compound **3** has the longest inter-chromophore distance.

Results and Discussion.

Bis-acridines **2** and **3** were synthesized essentially by the same route as that for **1**. Briefly, the route consisted of a reaction of 6-chloro-2-methoxy-9-[(3-bromopropyl)-amino]acridine **5** with bipyridine derivatives **8b** and **9b**. The two components were in turn prepared by the routes shown in Scheme 2. However, some of the intermediates for **2** and **3** were insoluble in common solvents and we had to implement a minor modification to the route. For example, to synthesize **2**, **8a** was prepared first by a reaction of **7a** with 1,3-dibromopropane. However, **8a** was insoluble in any solvent tested and its reaction with **5** was impossible. To solve this problem, we next synthesized 1,3-propyl bis(trifluoromethanesulfonate) (**7b**) from 1,3-propanediol and trifluoromethanesulfonate anhydride in 40% yield. A subsequent reaction of **7b** with **6** in acetone yielded diviologen **8b**. Compound **8b** had a good solubility in DMF (dimethylformamide) and after heating with **5** at 80-90° for 19 hours in DMF, **2** precipitated from the reaction solution on cooling. Compound **2** was purified by recrystallization from a mixture of methanol and ethanol in 16% yield. Again, the synthesis of tetraviologen **9a** encountered a problem. Thus, the reaction of **7b** with **8b** gave **9a** containing an impurity which was unable to remove. We then synthesized a mixed counter ion compound **9b** which could be purified and used in the next reaction. After heating **5** and **9b** in DMF at 110° for 12 hours, **3** precipitated from the reaction solution on cooling. Compound **3** was purified by recrystallization from a mixture of methanol and 2-propanol in 1% yield. The ^1H

Scheme 2



nmr spectra of these compounds confirmed their structures. Spectrophotometric *p*H-titration indicated that **1-3** possessed two proton dissociation constants which are pK_{a1} = 4-5 and pK_{a2} = 8.

Spectroscopic properties of compounds **1-3** were studied in 10 m*M* tris(hydroxymethyl)aminomethane hydrochloride buffer (*p*H 8.0) and 100 m*M* sodium chloride at 25° in which one of the acridine moieties was protonated. We used deoxyribonucleic acid from calf thymus (calf thymus DNA) as natural DNA. All of the compounds exhibited hypochromic and bathochromic shifts for the acridine chromophore upon binding to calf thymus DNA, indicative of DNA intercalation. In the case of **1**, isosbestic points were observed for that titration with calf thymus DNA solution. Curve fitting of the Scatchard plot for **1** by the site exclusion model of McGhee and von Hippel [10] afforded the binding constant of K = 1.7 x 10^5 M^{-1}. However, precipitates appeared during the titrations of **2** and **3** with the calf thymus DNA solution, when the charge of DNA phosphates balanced with that of the added compounds. Thus, we evaluated the binding affinity of compounds **1-3** for calf thymus DNA by spectrofluorometric quantitation of the displacement of ethidium bromide from calf thymus DNA. As compounds **1-3** were added increasingly to a solution of 1.26 μ*M* ethidium bromide and 1.0 μ*M* (nucleotide phosphate units) DNA, the enhanced fluorescence intensity of the DNA-bound ethidium gradually decreased. The binding affinity of these compounds was assessed as C_{50} values, which are the intercalator concentration necessary to displace 50% of DNA-bound ethidium [11]. Table 1 shows the C_{50} values of **1-3** with calf thymus DNA, T4 phage DNA and synthetic DNAs which are polydeoxyadenylic-thymidylic acid and polydeoxy-guanylic-deoxycytidylic acid in 1.0 m*M* 2-(*N*-morpho-lino)ethansulfonic acid and 10.0 μ*M* ethylenediamine-tetraacetic acid at *p*H 6.5. The binding affinity of the bis- intercalators is almost 100 times larger than that of methyl viologen **4**, demonstrating that the acridine moieties strongly enhanced the binding affinity of the viologen units. The C_{50} values for calf thymus DNA decreased with an increase in the number of viologen units. For either synthetic DNAs, all compounds exhibited a slightly polydeoxyadenylic-thymidylic acid preference and this tendency increased as the number of viologen units increased (Table 1). These observations demonstrated that the viologen units help enhance DNA-binding affinity and adenine (A)-thymine (T) base pair preference of **1-3** by serving as a groove binder for DNA. To assess the location of the viologens more precisely, *i.e.* the major or minor groove, the C_{50} values for T4 phage DNA were measured under the same conditions. In T4 phage DNA, a glucose residue covalently attached to the hydroxyl group of 5-hydroxymethylcytosine covers the major groove [1]. If the viologen moieties of bis-intercalators lie in the minor groove, C_{50} values of calf thymus DNA and T4 phage DNA may be identical because of almost the same A-T contents. This is indeed the case as shown in Table 1, indicating that the viologen moieties of bis-intercalators lie in the minor groove.

Thermal denaturation profiles for solutions of calf thymus DNA were determined in the presence and absence of **1-3** in 1.0 m*M* 2-(*N*-morpholino)ethansulfonic acid and 10.0 μ*M* ethylenediaminetetraacetic acid at *p*H 6.5. The melting temperature (T_m) of calf thymus DNA was 44.5° under these conditions. The ΔT_m values, defined as the difference in T_m of DNA in the presence and absence of intercalator, are consistent with the above result: the order of the stabilizing effect on DNA helix and DNA-binding affinity was **1** < **2** < **3** (Table 1). Although the same intercalator moieties are present in all of the compounds, the binding affinity of the bis-intercalators for DNA increased with an increase in the number of viologen units. This shows that the viologen units act synergistically in the interaction of these molecules with DNA.

In conclusion, these experiments demonstrated that bis-intercalators **2** and **3** serve as molecular staples for DNA and cover the broader region of DNA than does **1**.

Table 1
DNA Binding Properties of Bis-intercalators **1-3**

Compound	DNA	C_{50} (μM)	ΔT_m (°)	K_{AT}/K_{GC} [a]
1	calf thymus	0.058	4.5	1.75
	T4-phage	0.054		
2	calf thymus	0.040	5.5	1.77
	T4-phage	0.043		
3	calf thymus	0.024	13.4	2.11
	T4-phage	0.024		
4	calf thymus	3.74		

[a] The selectivity (K_{AT}/K_{GC}) was defined as ($1/C_{50}$, polydeoxyadenylic-thymidylic acid)/($1/C_{50}$, polydeoxyguanylic-deoxycytidylic acid).

EXPERIMENTAL

The ^1H nmr spectra were obtained on a Hitachi R-24B and JASCO GSX-400 operating at 60 and 400 MHz, respectively, with TMS as an internal standard. The infrared spectra were recorded with a JASCO IR-800 instrument. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected.

Calf thymus DNA was purchased from Sigma Chemical Co. and purified as described previously [12,13]. Polydeoxyadenylic-thymidylic acid (MW 7.7 x 10^6), polydeoxyguanylic-deoxycytidylic acid, (MW 6.6 x 10^5) and T4 coliphage DNA

were purchased also from Sigma and used without further purification. The DNA concentrations were determined by optical measurement with the following molar absorption coefficients (nucleotide phosphate unit): calf thymus and T4 coliphage DNA, 6600 $M^{-1}cm^{-1}$ at 260 nm; polydeoxyadenylic-thymidylic acid, 6600 $M^{-1}cm^{-1}$ at 260 nm; polydeoxyguanylic-deoxycytidylic acid, 8400 $M^{-1}cm^{-1}$ at 262 nm.

6-Chloro-2-methoxy-9-[(3-bromopropyl)amino]acridine Hydrobromide (**5**).

6-Chloro-2-methoxy-9-phenoxylacridine (13 g, 35 mmoles) [14] and 3-bromopropylamine hydrobromide (7.7 g, 35 mmoles) were dissolved in phenol (40 g) and the solution was heated at 120° for 2 hours. The solution was allowed to cool and poured into ether (500 ml). The solid obtained was dried under reduced pressure to yield 9.8 g (61%) of **5** as a yellow solid, mp 220° dec; tlc R_f = 0.38 (ethyl acetate); ^1H nmr (60 MHz, dimethyl sulfoxide-d_6): δ 3.10 (m, 2H), 3.60 (m, 2H), 3.90 (m, 5H), 4.20 (brs, 1H), 8.10 (m, 6H), 9.60 ppm (brs, 1H).

6,6'-Dichloro-2,2'-dimethoxy-9,9'-[4,4'-bipyridine-1,1'-diibis-(propylamino)]diacridinium Tetrabromide (**1**).

A mixture of **5** (1.8 g, 3.9 mmoles) and 4,4'-bipyridine (0.15 g, 0.96 mmole) was dissolved in DMF (60 ml) and the solution was heated at 95° for 17 hours. The solution was allowed to cool and the precipitate appeared. This was collected and recrystallized from water twice to yield 0.11 g (11%) of **1** as a yellow solid, mp >250°; ^1H nmr (400 MHz, dimethyl sulfoxide-d_6): δ 2.64 (m, 2H), 3.87 (m, 3H), 4.14 (m, 2H), 7.23-7.91 (m, 6H), 8.06 (m, 2H), 8.85 ppm (m, 2H).

Anal. Calcd. for $C_{44}H_{42}N_6O_2Br_4Cl_2 \cdot 2H_2O$: C, 47.46; H, 4.17; N, 7.55. Found: C, 47.39; H, 3.97; N, 7.40.

1,3-Propyl Di(trifluoromethanesulfonate) (**7b**).

To a suspension of anhydrous sodium carbonate (2.0 g) in dichloromethane (10 ml) was added dropwise at 0° trifluoromethanesulfonate anhydride (10.0 g, 35 mmoles) and then 1,3-propanediol (1.2 g, 16 mmoles) under nitrogen. After the mixture has been stirred at 0° for 3 hours, water (20 ml) and dichloromethane (20 ml) were then added dropwise. The dichloromethane layer was separated and dried over magnesium sulfate, filtered, and distilled to yield 2.19 g (40%) of **7b** as a colorless oil, bp 150°/4.5 mm Hg; ir: 1400, 1240-1130 cm^{-1}; ^1H nmr (60 MHz, dimethyl sulfoxide-d_6): δ 2.2-2.6 (m, 2H), 4.5-4.8 ppm (m, 4H).

1,1'-Trimethylenebis-4,4'-bipyridine Ditriflate (**8b**).

A mixture of **7b** (2.2 g, 6.4 mmoles) and 4,4'-bipyridine (2.5 g, 16 mmoles) was dissolved in acetone (40 ml) and the solution was refluxed for 3 hours. The solution was allowed to cool, evaporated to dryness under reduced pressure and the residue was washed with chloroform several times. The viscous oil obtained was dried under reduced pressure to yield 3.7 g (88%) of **8b**; ^1H nmr (60 MHz, dimethyl sulfoxide-d_6): δ 2.50-2.70 (m, 2H), 4.60-5.0 (m, 4H), 8.00-8.30 (m, 4H), 8.60-9.00 (m, 8H), 9.10-9.40 ppm (m, 4H).

6,6'-Dichloro-2,2'-dimethoxy-9,9'-[1,1'-trimethylenebis-4,4'-bipyridine-1",1"'-diibis(propylamino)]diacridinium Tetrabromide Ditriflate (**2**).

A mixture of **5** (1.0 g, 2.2 mmoles) and **8b** (520 mg, 1.0 mmole) was dissolved in DMF (35 ml) and the solution was heated at 80-90° for 19 hours. The solution was allowed to cool and the precipitate appeared. This was collected and recrystallized from a mixture of methanol and ethanol twice to yield 166 mg (16%) of **2** as a yellow solid, mp >250°; ^1H nmr (400 MHz, dimethyl sulfoxide-d_6): δ 2.73 (m, 4H), 2.87 (m, 2H), 3.98 (m, 6H), 4.35 (m, 4H), 4.80-5.00 (m, 8H), 7.54 (dd, 2H, 4.0 and 9.5 Hz), 7.68 (dd, 2H, 4.0 and 9.5 Hz), 7.87 (d, 2H, 9.5 Hz), 7.97 (m, 4H), 8.57 (d, 2H, 9.5 Hz), 8.79 (d, 4H, 6.8 Hz), 8.86 (d, 4H, 6.4 Hz), 9.43 (d, 4H, 6.8 Hz), 9.54 (d, 4H, 6.4 Hz), 9.64 ppm (brs, 2H).

Anal. Calcd. for $C_{59}H_{56}N_8O_8Br_4Cl_2F_6S_2$: C, 45.01; H, 3.56; N, 7.12. Found: C, 45.16; H, 3.38; N, 7.18.

1,1'-[3,3'-(1,1'-trimethylenebis-4,4'-bipyridine-1",1"'-diio)dipropyl]bis-4,4'-dipyridine Dibromide Tetratriflate (**9b**).

A mixture of **8b** (3.69 g, 5.7 mmoles) and dibromopropane (0.54 g, 2.7 mmoles) was dissolved in acetone (30 ml) and the solution was heated at 85° for 6 hours. Upon cooling, the solution was separated into two layers. The lower layer was separated and washed with acetone several times. The viscous oil thus obtained was dried under reduced pressure to yield 1.19 g (30%) of **9b**; ^1H nmr (400 MHz, dimethyl sulfoxide-d_6): δ 2.70-3.90 (m, 6H), 4.80-5.00 (m, 12H), 8.24 (d, 4H, 6.7 Hz), 8.76 (d, 4H, 7.5 Hz), 8.86-8.94 (m, 8H), 9.00 (d, 4H, 6.7 Hz), 9.36 (d, 4H, 7.5 Hz), 9.46-9.54 ppm (m, 8H).

6,6-Dichloro-2,2'-dimethoxy-9,9'-{3,3'-{1,1'-[3,3'-(1,1'-trimethylenebis-4,4'-bipyridine-1",1"'-diio)dipropyl]bis-4,4'-dipyridine-1",1"'-diio}bis(propylamino)}diacridinium Hexabromide Tetratriflate (**3**).

A mixture of **5** (1.30 g, 2.8 mmoles) and **9b** (1.70 g, 1.1 mmole) was dissolved in DMF (5 ml) and the solution was heated at 110° for 12 hours. The solution was allowed to cool and a precipitate appeared. This was collected and recrystallized from a mixture of methanol and 2-propanol twice to yield 25 mg (1%) of **3** as a yellow solid, mp >250°; ^1H nmr (400 MHz, dimethyl sulfoxide-d_6): δ 2.73 (m, 4H), 2.86 (m, 6H), 3.98 (2, 6H), 4.37 (m, 4H), 4.80-5.00 (m, 16H), 7.54 (dd, 2H, 3.5 and 9.5 Hz), 7.68 (d, 2H, 9.5 Hz), 7.86 (d, 2H, 9.5 Hz), 7.95 (m, 4H), 8.57 (d, 2H, 9.5 Hz), 8.81 (d, 4H, 7.2 Hz), 8.84 (d, 4H, 7.2 Hz), 8.89 (m, 8H), 9.45 (d, 4H, 7.2 Hz), 9.59 (m, 12H), 9.69 ppm (brs, 2H)

Anal. Calcd. for $C_{87}H_{84}N_{12}O_{14}Br_6Cl_2F_{12}S_4$: C, 45.02; H, 3.59; N, 7.12. Found: C, 45.16; H, 3.38; N, 7.78.

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