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Synthesis of pyrene–acridine bis-intercalators and effects of binding to DNA

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Abstract

A bis-intercalating compound containing pyrene and 9-aminoacridine chromophores (*N*-(5-(1-pyrenyl)-pentyl)-6-(9-acridinylamino)hexylamide, **I**), was prepared and its interaction with double-stranded DNA was investigated. Homologous compounds in which the two chromophores were connected by a linear carbon chain (pentamethylene (**II**), tetramethylene (**III**) and methylene (**IV**)) were also prepared. In acetonitrile solutions of the free ligands, the presence of the proximal pyrene results in reduced acridine fluorescence relative to 9-methylaminoacridine (9-MAA), and the degree of quenching increases with decreasing chain length. The quenching process is assigned to exothermic electron transfer from pyrene to the excited 9-aminoacridine (9-AA) chromophore. In the presence of DNA, the relative quenching order is reversed, and **I** and **IV** are quenched more strongly than **II** and **III**. From linear dichroism experiments, it is concluded that **I** binds by bis-intercalation of the pyrene and acridine moieties, **III** and **IV** undergo intercalation of the acridine chromophore and **II** binds by partial bis-intercalation at two contiguous sites.

Keywords: Bis-intercalators; DNA intercalation; Fluorescence quenching; Linear dichroism

1. Introduction

The discovery that electron transfer can proceed over long distances in biological polymers, such as proteins [1] and DNA [2], has received much attention in recent years. In particular, the effect of these environments on the rate and mechanism of photoinduced electron transfer is a topic of debate. Although it is well established that electron transfer can proceed over long distances in protein matrices via tunnelling [3], the relative importance of the factors which govern electron transfer between donor–acceptor pairs bound to DNA is difficult to evaluate. Enhancement of the electron transfer rate may be expected as a result of an increased local concentration of bound reactants, an increase in the lifetime of the excited donor or acceptor, variations in the redox potential of the reactants, more favourable relative orientation of the donor and acceptor and modulation of electron transfer by the π-stacked nucleotide bases. The latter possibility, that electron transfer may be mediated by the central core of the DNA helix acting as a molecular wire, is by far the most intriguing.

A number of donor–acceptor systems have been proposed to undergo photoinduced electron transfer mediated by the DNA core. Baguely et al. [4] observed efficient quenching of intercalated ethidium fluorescence on addition of substituted 9-anilinoacridines under conditions in which less than 1% of the ethidium was displaced from the DNA. In their investigations of intermolecular electron transfer between various intercalators, Brun and Harriman [5] found that efficient electron transfer occurred between excited ethidium or acridine orange and *N,N'*-dimethyl-2,7-diazapyrenium when both reagents were intercalated in double-stranded DNA. However, the same workers reported that singlet–singlet energy transfer via an electron exchange mechanism was uncompetitive in systems composed of palladium porphyrins bound to DNA by intercalation [6].

Electron transfer between donor–acceptor pairs in which one or both partners are bound to DNA by intercalation has been a topic of several recent studies. However, this approach suffers from a lack of information concerning the distance separating the bound species, and the data must be interpreted assuming a statistical distribution of reactants. This compli-

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cation has been avoided by the preparation of synthetic double-stranded oligonucleotides containing covalently bound donor and acceptor complexes. One such system, possessing a ruthenium donor complex and a rhenium acceptor complex tethered to opposite ends of a synthetic DNA strand composed of 15 base pairs, has been described [7]. The observation of efficient electron transfer over such a long distance ($d > 40$ Å) between the intercalated species supports the occurrence of electron transfer through DNA. Though rigorous, the latter approach is rendered difficult by the need to synthesize substantial quantities of derivatized synthetic polynucleotides. Moreover, efficient photoinduced electron transfer has also been observed in another covalent assembly ($d \approx 21$ Å) in which neither the donor nor the acceptor undergoes intercalative binding to DNA [8]. Hence, covalent modification of the DNA duplex may influence the observed electron transfer rates. An alternative approach may involve the preparation of tethered donor–acceptor pairs, capable of intercalative binding to DNA and of undergoing photoinduced electron transfer. Although many bis-intercalating compounds have been synthesized and their interaction with DNA studied [9], none possess chromophores suited to the occurrence of photoinduced electron transfer.

In this paper, we report the synthesis of several linked pyrene–aminoacridine bis-intercalators and their binding to double-stranded DNA. Both pyrene [10] and 9-aminoacridine (9-AA) [11] are known DNA intercalators possessing well-separated absorption and emission spectra, and electron transfer from pyrene to the excited acridinium ion is moderately exothermic in polar solvents.

2. Experimental details

2.1. General methods

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian VX300 instrument with tetramethylsilane (TMS) as internal standard. UV absorption spectra were obtained using a Hewlett-Packard 8452 diode-array spectrophotometer or a Perkin–Elmer Lambda 9 instrument. Steady state fluorescence measurements were recorded on a Spex Fluorolog 2 (model 111 C) fitted with an R928 photomultiplier and are uncorrected. Mass spectral analysis was performed on a 5971 Hewlett-Packard mass spectrometer coupled to a Hewlett-Packard 5890 gas chromatograph equipped with a 20 m OV1 capillary column. Fluorescence quantum yields were determined using solutions of matched absorbance (approximately 0.07 at the excitation wavelength) degassed by three freeze–pump–thaw cycles. 9-AA in ethanol ($\Phi_f = 0.99$) [12] was used as a secondary standard.

2.2. DNA binding studies

Solutions used in the competitive binding assays contained 1.82×10^{-6} M base pairs, 2.35×10^{-6} M ethidium bromide and 0.04 M phosphate buffer. The fluorescence yield of the bound ethidium on excitation at 520 nm was monitored at 610 nm as a function of added ligand. No correction was made for the emission of displaced ethidium. Linear dichroism spectra were obtained on a modified Cary 61 spectrometer using a cylindrical quartz Couette cuvette with a spinning inner cylinder ($G \approx 6000\ s^{-1}$) and a 1 mm path length [13]. Solutions contained 1 mM DNA base pairs, approximately 10 μM ligand, 40 mM phosphate buffer and 0.1 M sodium chloride in water.

2.3. Kinetic and spectrographic flash photolysis

Kinetic flash photolysis on the millisecond time scale was performed on a conventional system with an electrical discharge of 1000 J or less through partially evacuated quartz tubes which produced a flash of approximately 20 μs duration. Nanosecond laser flash photolysis used pulses from a Lambda Physics EMG 101 MSC excimer laser filled with either XeF or XeCl (351 or 308 nm respectively; 25 ns pulse width; approximately 100 mJ per pulse) and a pulsed xenon arc lamp as probe light. The signal was measured with an EMI 978QA photomultiplier, fed into a digitizer (Tektronix TDS 540) and analysed by least-squares fitting. The set-up employed to obtain the transient absorption spectra has been described elsewhere [14].

2.4. Materials

Pyrene (Fluka), 9-aminoacridine hydrochloride (Fluka), acridone (Fluka) and ethidium bromide (Merck) were used as received. Acetonitrile (Merck) was of spectrophotometric grade. Buffers were 50 mM in phosphate (pH 7) and 100 mM in sodium chloride dissolved in water previously purified on a Millipore Q filtration unit, and were stored at 4 °C. Purified sonicated calf thymus-DNA (ct-DNA) (average chain length, 300 bases) was employed in the binding assays. For spectral measurements and circular dichroism experiments, highly polymerized lyophilized ct-DNA (Fluka) was dissolved in phosphate buffer and was used without further purification. Compounds **II**, **III**, and **IV** were prepared by reaction of the corresponding ω-aminoalkylpyrene with 9-chloroacridine (prepared from 9-acridone according to a literature procedure [15]) and do not have well-defined melting points. Their purity was controlled by thin layer chromatography (TLC) using alumina (2% methanol in chloroform, $R_f = 0.5$–0.7) and silica (15% methanol in chloroform, $R_f = 0.2$–0.3) supports and by elemental analysis.

2.4.1. 4-(1-Pyrenyl)-4-oxo-pentanoic acid

Glutaric anhydride (22.8 g, 0.20 mol) was added to a solution of anhydrous aluminium chloride (53.2 g, 0.40 mol) dissolved in 200 ml of nitrobenzene in a 1 l round-bottomed flask equipped with a mechanical stirrer and cooled in an ice–water bath. Powdered pyrene (40.2 g, 0.20 mol) was then added in small portions over a 15 min period, and the mixture

was stirred at room temperature for 8 h. The reaction mixture was then carefully quenched with aqueous hydrochloric acid and the nitrobenzene was removed by steam distillation. The resulting slurry was made basic with potassium carbonate, steam distilled for an additional 15 min and filtered from suspended aluminium hydroxide using celite as a filtration aid. The solution was then neutralized with hydrochloric acid and the fluffy white precipitate was collected by filtration and air dried to yield 12 g (0.038 mol) of 4-(1-pyrenyl)-4-oxo-pentanoic acid in 19% yield. ^{1}H NMR ($CDCl_3$) δ: 2.24 (m, 2H); 2.60 (t, 2H, J=7.2 Hz); 3.33 (t, 2H, J=7.1 Hz); 8.0–8.2 (m, 8H); 8.91 (d, 1H, J=12.4 Hz). Mass spectrum (MS) (m/z): 298 ($M^+ - H_2O$, 100%), 256 (10), 242 (14), 229 (20), 201 (28).

2.4.2. 5-(1-Pyrenyl)-pentylamine

4-(1-Pyrenyl)-4-oxo-pentanoic acid (3.4 g, 0.01 mol) was dissolved in a solution of 2.0 g of potassium hydroxide in 30 ml of ethylene glycol contained in a round-bottomed flask. Hydrazine (95%, 2.2 g) was added and the solution was refluxed at 140 °C for 1 h. The condenser was then removed and the temperature was allowed to increase to 200 °C; the condenser was then replaced and reflux was continued for 5 h. The solution was cooled, diluted with three times its volume of water and poured into 2 N hydrochloric acid. The white precipitate was collected by filtration and dried to yield 2.45 g (8.1 mmol, 75%) of 5-(1-pyrenyl)-pentanoic acid. The acid (1 g, 3.3 mmol) was suspended in 15 ml of chloroform to which was added one drop of *N,N*-dimethylformamide (DMF) and 2 ml of oxalyl chloride. The mixture was stirred at room temperature for 4 h until all the compound had dissolved. The solvent and excess oxalyl chloride were removed on a rotary evaporator and the solid residue was taken up in dry benzene and added dropwise to a stirred suspension of 20 ml of ammonium hydroxide and 10 ml of benzene. Stirring was continued for 2 h and the mixture was extracted with three 20 ml portions of benzene. The organic portions were combined and dried over anhydrous magnesium sulphate; the solvent was removed on a rotary evaporator to yield 0.75 g of an off-white solid. The amide thus obtained was dissolved in 1,2-dimethoxyethane and added dropwise to a stirred suspension of 3.0 g of lithium aluminium hydride in 60 ml of 1,2-dimethoxyethane under nitrogen. The solution was then refluxed overnight and the excess reagent was decomposed by careful successive addition of 3 ml of water, 3 ml of aqueous sodium hydroxide and 9 ml of water. The solution was filtered from the granular precipitate and the latter was washed with several portions of tetrahydrofuran (THF). The ether fractions were combined, washed several times with water and dried over anhydrous magnesium sulphate; the solvent was removed on a rotary evaporator to yield 0.65 g (2.3 mmol, 69%) of 5-(1-pyrenyl)-pentylamine as a viscous oil which solidified on standing. ^{1}H NMR ($CDCl_3$) δ: 1.63 (m, 4H); 1.88 (m, 2H); 3.34 (t, 2H, J=7.1 Hz); 3.62 (t, 2H, J=6.8 Hz); 7.8–8.3 (m, 9H). MS (m/z): 287 (M^+, 35%), 215 (100), 202 (30), 189 (10).

2.4.3. 9-(6-Carboxyhexylamino)acridine

In a 25 ml round-bottomed flask were placed 0.50 g (2.3 mmol) of 9-chloroacridine, 0.31 g (2.4 mmol) of 6-amino-caproic acid and 2 g of phenol. The mixture was heated with stirring at 120 °C for 1 h and the phenol was removed by distillation. The remaining solid was dissolved in acetone and poured into 50 ml of 2 N sodium hydroxide solution. The latter was extracted with chloroform and the organic fractions were collected, washed twice with aqueous sodium hydroxide and several times with water. The chloroform was removed on a rotary evaporator and the yellow solid was recrystallized from acetone to yield 0.29 g (1.0 mmol, 44%) of 9-(6-carboxyhexylamino)acridine as a yellow crystalline powder. ^{1}H NMR ($CDCl_3$ + dimethylsulphoxide-d_6 (DMSO-d_6)) δ: 1.30 (m, 2H); 1.45 (m, 2H); 1.82 (m, 2H); 2.18 (t, 2H, J=7.3 Hz); 3.86 (t, 2H, J=8.0 Hz); 7.20 (t, 2H, J=7.7 Hz); 7.52 (t, 2H, J=7.7 Hz); 7.91 (d, 2H, J=8.6 Hz); 8.23 (t, 2H, J=9.5 Hz). Analysis: calculated for $C_{19}H_{20}N_2O_2 \cdot 2.5H_2O$: C, 64.54; H, 7.13; N, 7.92; O, 20.41; found: C, 65.11; H, 6.10; N, 7.92.

2.4.4. N-(5-(1-Pyrenyl)-pentyl)-6-(9-acridinylamino)hexylamide (I)

In a round-bottomed flask containing 30 ml of acetone (previously dried over molecular sieves) and 0.75 ml of triethylamine was dissolved 0.30 g (1.0 mmol) of 9-(6-carboxyhexylamino)acridine. The solution was stirred under nitrogen while cooling in an ice–water bath for 15 min, and 85 μl of ethyl chloroformate in 1 ml of dry acetone was then added dropwise. The solution was stirred at 0 °C for 1 h and 0.25 g (0.88 mmol) of 5-(1-pyrenyl)-pentylamine in 10 ml of dry acetone was added. Stirring was continued at 0 °C for 2 h and the acetone was then removed on a rotary evaporator. The residue was dissolved in chloroform and washed once with 30 ml of 2 N sodium hydroxide solution and twice with water. The organic phase was then reduced on a rotary evaporator and purified by liquid chromatography on an acid alumina (pH 5.5) column (eluent, 5% CH_3OH in chloroform) to yield 200 mg (0.35 mmol, 40%) of **I** as a yellow powder. ^{1}H NMR ($CDCl_3$) δ: 1.2–1.7 (m, 10H); 1.91 (m, 2H); 2.12 (m, 2H); 3.22 (m, 2H); 3.3 (t, 2H, J=7.5 Hz); 3.78 (t, 2H, J=7.5 Hz); 5.1 (br s, 1H); 5.91 (br t, 1H); 7.23 (t, 2H, J=7.7 Hz); 7.50 (t, 2H, J=7.7 Hz); 7.7–8.3 (m, 13H). Analysis: calculated for $C_{40}H_{39}N_3O \cdot 2.5H_2O$: C, 77.12; H, 7.12; N, 6.75; O, 9.01; found: C, 77.33; H, 6.78; N, 6.72.

2.4.5. 9-(5-(1-Pyrenyl)-pentylamino)-acridine (II)

This compound was prepared in 35% isolated yield from 5-(1-pyrenyl)-pentylamine and 9-chloroacridine in phenol at 120 °C, and was purified as described above. ^{1}H NMR ($CDCl_3$) δ: 1.52 (m, 2H); 1.80 (m, 2H); 1.88 (m, 2H); 3.32 (t, 2H J=7.2 Hz); 3.77 (t, 2H, J=7.2 Hz); 5.0 (br s, 1H); 7.29 (t, 2H, J=7.7 Hz); 7.65 (t, 2H, J=7.7 Hz); 7.7–8.2 (m, 13H). Analysis: calculated for $C_{34}H_{28}N_2 \cdot 1.6H_2O$: C, 82.75; H, 6.37; N, 5.68; O, 5.20 found: C, 82.71; H, 6.53; N, 5.40.

2.4.6. 9-(4-(1-Pyrenyl)-butylamino)-acridine (III)

4-(1-Pyrenyl)-butyric acid (Fluka, 0.50 g, 1.7 mmol) was converted to 4-(1-pyrenyl)-butylamine via the primary amide as described above to yield 0.20 g (0.7 mmol, 42%) of a clear yellow oil. The primary amine was then reacted with 9-chloroacridine in the presence of phenol at 120 °C and the product was purified by preparative TLC (neutral alumina, eluted with 2% CH_3OH in chloroform) to yield 90 mg (12% overall yield) of **III** as a yellow powder. ^1H NMR ($CDCl_3$) δ: 2.15 (m, 4H); 3.40 (t, 2H $J=7.4$ Hz); 3.88 (t, 2H, $J=7.2$ Hz); 7.05 (t, 2H, $J=7.7$ Hz); 7.42 (t, 2H, $J=7.7$ Hz); 7.7–8.2 (m, 13H). Analysis: calculated for $C_{33}H_{26}N_2 \cdot 2.9H_2O$: C, 78.78; H, 6.38; N, 5.57; O, 9.28; found: C, 79.08; H, 5.98; N, 5.31.

2.4.7. 9-(1-Pyrenylmethylamino)-acridine (IV)

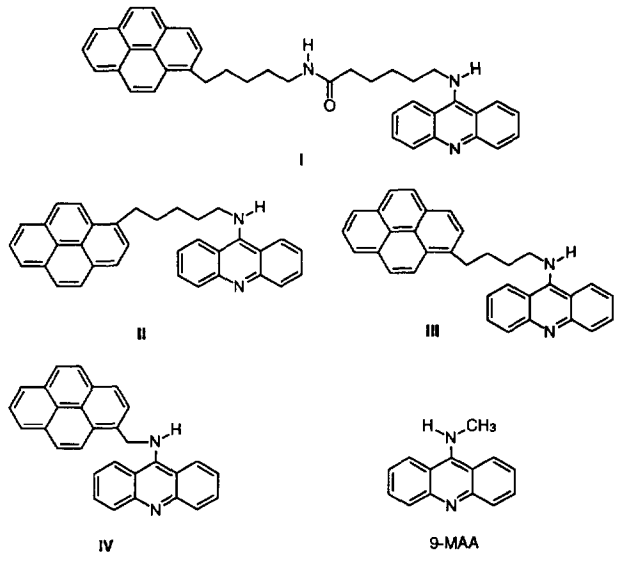
1-Pyrenylmethylamine hydrochloride (Aldrich, 0.46 g, 1.7 mmol) was reacted with 9-chloroacridine (0.40 g, 1.9 mmol) in the presence of phenol (3.0 g) at 140 °C for 1.5 h. The phenol was then removed by distillation under reduced pressure and the remaining solid, dissolved in a minimal quantity of methanol, was poured into 100 ml of acetone and filtered to yield 0.25 g of yellow powder. The product thus obtained was liberated from the hydrochloride salt by shaking with 100 ml of 2 N sodium hydroxide solution and extracted with chloroform. The organic fractions were combined and dried over anhydrous magnesium sulphate; the solvent was removed on a rotary evaporator to yield, after purification by column chromatography, 0.20 g (30% yield) of **IV** as a yellow powder. ^1H NMR ($CDCl_3$) δ: 5.69 (s, 1H); 7.28 (t, 2H, $J=7.5$ Hz); 7.68 (t, 2H, $J=7.5$ Hz); 8.0–8.3 (m, 13H). Analysis: calculated for $C_{30}H_{20}N_2 \cdot 2.9H_2O$: C, 78.78; H, 6.38; N, 5.57; O, 9.28; found: C, 79.08; H, 5.98; N, 5.31.

3. Results

A bis-intercalator composed of pyrene and 9-AA linked by a tether of sufficient length to span two base pairs (compound **I**, Scheme 1) was prepared from 5-(1-pyrenyl)-pentylamine and 9-(6-carboxyhexylamino)acridine. To compare fluorescence quenching, electron transfer rates and binding to DNA, homologous compounds in which the pyrene and 9-AA chromophores were separated by a chain of five, four or one carbon atoms (compounds **II**, **III** and **IV** respectively, Scheme 1) were prepared from the corresponding ω-aminopyrenylalkanes and 9-chloroacridine. 9-Methylaminoacridine (9-MAA) was used as a reference compound to account for the effects of alkyl substitution on 9-AA. The compounds were purified by column chromatography (weakly acidic alumina) or by recrystallization from acetone.

3.1. Photochemical properties

The long-wavelength region of the absorption spectra of compounds **I**, **IV** and 9-MAA in acetonitrile containing 0.005 M $HClO_4$ (to ensure complete protonation of the acridine chromophore) are shown in Fig. 1. Although compounds **I**, **II** and **III** are stable under the acidic conditions employed, the amide function of compound **IV** hydrolyses with a first-order rate constant of approximately 0.03 s^{-1}, determined from kinetic analysis of the absorbance changes. The hydrolysis of **I** eventually leads to the appearance of an absorption spectrum identical with that of 9-AA under the same conditions, and the concomitant appearance of strong fluorescence, presumably due to 1-hydroxymethylpyrene.



Scheme 1.

Excitation of the pyrene chromophore at 341 nm in compounds **I–IV** in aerated acetonitrile (0.005 M $HClO_4$) solutions results in a weak fluorescence emission from the acridine chromophore and some residual pyrene emission. Excitation at longer wavelengths ($\lambda_{ex}=396$ nm), where only the acridine chromophore absorbs, results in the observation of weak acridine emission (Fig. 2). Compared with 9-AA, 9-MAA and compounds **I–IV** possess considerably lower fluorescence quantum yields (Table 1) which, unlike that of 9-AA, are unaffected by the presence of air. The fluorescence of **IV** can only be measured in neutral acetonitrile because of the occurrence of hydrolysis under acidic conditions. The absorption and emission maxima of **I–IV** in acidic acetonitrile are given in Table 1.

The fluorescence of 9-AA in acetonitrile (0.005 M $HClO_4$) solution is quenched by the addition of pyrene with a Stern–Volmer constant $K_{SV}=k_q\tau=385\ M^{-1}$. Assuming an excited state lifetime for the 9-aminoacridinium cation of $\tau=18$ ns [16], a quenching rate constant of $k_q=2.1\times10^{10}\ M^{-1}\ s^{-1}$ is calculated. The quenching may be attributed to exothermic electron transfer from pyrene to excited acridine based on the direct observation of the resulting radical ions by transient absorption spectroscopy (see below). A value for the free energy of electron transfer ($\Delta_{et}G^\circ=-0.53$ eV) can be calculated from the pyrene oxidation potential ($E_D^{ox}=1.16$ V vs. saturated calomel electrode (SCE) [17]), the reduction

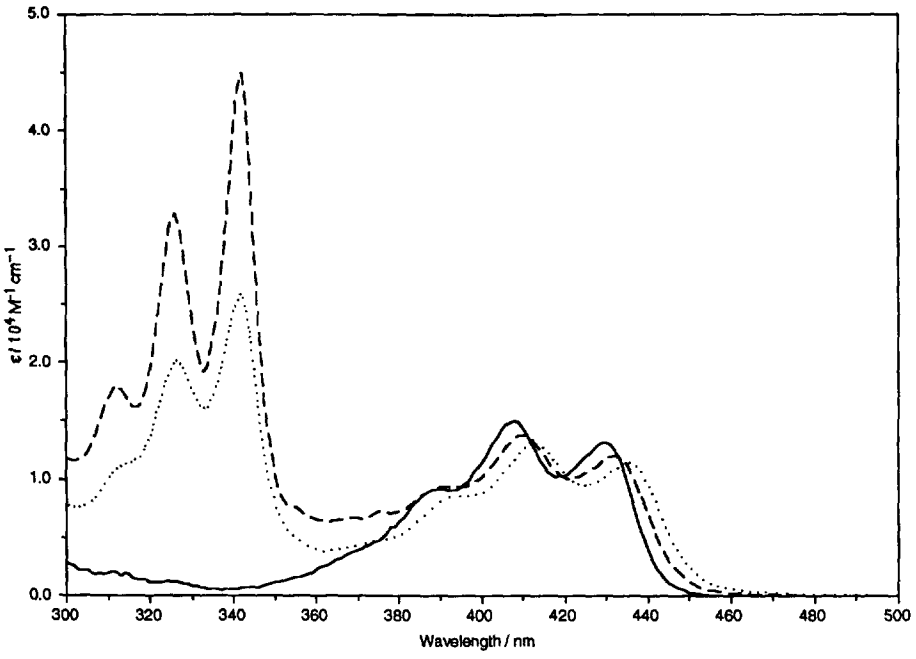


Fig. 1. Absorption spectra of 9-MAA (———), **I** (– – –) and **IV** (· · ·) in acetonitrile–$HClO_4$ (0.005 M) solutions.

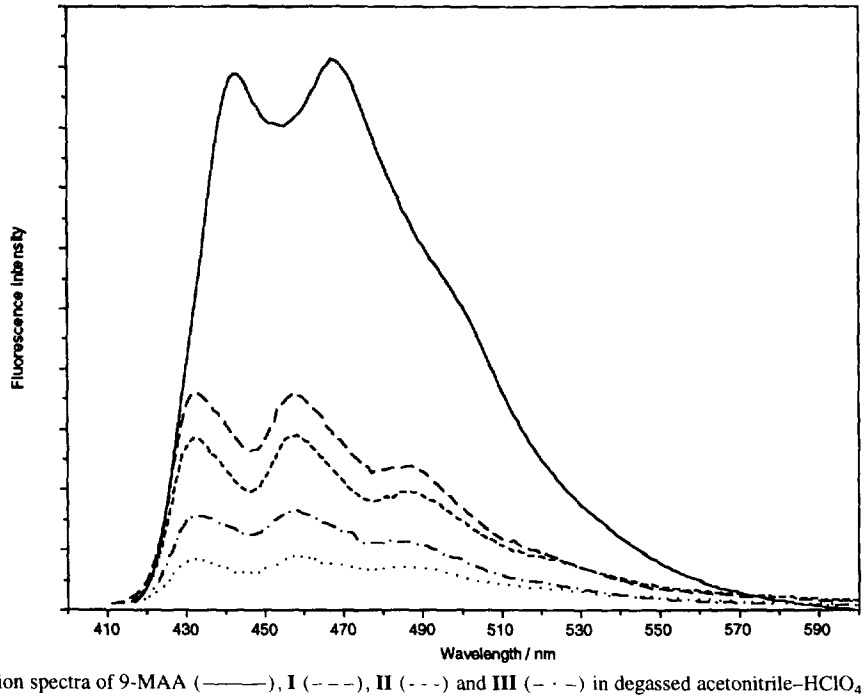


Fig. 2. Fluorescence emission spectra of 9-MAA (———), **I** (– – –), **II** (- - -) and **III** (– · –) in degassed acetonitrile–$HClO_4$ (0.005 M) solutions and **IV** (· · ·) in acetonitrile. Excitation wavelength, 396 nm.

potential of 9-AAH^+ ($E_A^{red} = -1.21$ V vs. SCE [18]) and the singlet energy of the latter ($^1E^* = 2.92$ eV) using Eq. (1) proposed by Rehm and Weller [19]

$$\Delta_{et}G^\circ = E_D^{ox} - E_A^{red} - {}^1E^* - 0.06 \quad (1)$$

The fluorescence emission arising from excitation of the acridine chromophore in the linked compounds is substantially quenched compared with that of 9-MAA as a result of the proximal pyrene. Intramolecular electron transfer quenching has been observed in numerous systems composed of donor–acceptor molecules joined by flexible linkers [20–22], and the rates of electron transfer are generally found to decrease with increasing chain length. The degree of acridine fluorescence quenching observed in compounds **I**, **II** and **III** (Table 1) is consistent with intramolecular electron transfer quenching, being greater for those compounds possessing the shortest connecting chains. Both electron and energy transfer are thermodynamically feasible from the pyrene S_1 excited state. The occurrence of energy transfer is indicated by the observation of acridine-like fluorescence in the emission

Table 1
Spectroscopic data of 9-MAA and **I–IV** in acetonitrile and bound to DNA

Compound	Acetonitrile[a]		Φ_f^b	DNA[c]		C_{50}^d (μM)
	λ_{max}(abs) (nm)	λ_{max}(fluo) (nm)		λ_{max}(abs) (nm)	λ_{max}(fluo) (nm)	
9-MAA	406, 430	441, 467	0.041	412, 437	453, 469	10
I	326, 342	379, 399	0.016	334, 349	455, 478	2
	410, 424	432, 458		415, 439		
II	326, 342	379, 399	0.013	334, 350	452, 475	2
	411, 423	432, 458		416, 440		
III	327, 343	379, 399	0.007	333, 350	452, 478	2
	411, 433	432, 458		416, 438		
IV	327, 342	389, 408	(0.004)[e]	331, 345	455, 480	2.5
	412, 436	432, 458[e]			418, 439	

[a]Solution containing 0.005 M $HClO_4$. The spectrum of **IV** was measured using a diode-array spectrometer within 2 s after mixing with acid.
[b]In degassed acetonitrile containing 0.005 M $HClO_4$.
[c]In the presence of 0.5 mM ct-DNA.
[d]The concentration of added compound necessary to cause a 50% reduction in the concentration of ethidium bound to DNA.
[e]In acetonitrile.

spectra recorded on excitation of **I**, **II** and **III** at 341 nm, where more than 98% of the light is absorbed by the pyrene chromophore.

3.2. Binding to DNA

In the presence of ct-DNA, the aqueous solubility of compounds **I–IV** is enhanced, and the absorption spectra of both the pyrene and acridine chromophores shift bathochromically and exhibit strong hypochromism. The absorption and emission spectra of compounds **I**, **II**, **III** and **IV** in the presence of ct-DNA are shown in Figs. 3(a) and 4 respectively.

Efforts to determine the binding constants to DNA of compounds **I–IV** via direct UV titration were impeded by their low aqueous solubility. Indirect methods employing competitive binding assays only yield relative binding affinities, as in the case of ethidium bromide displacement [23] (Fig. 5). The C_{50} values (i.e. the concentration of ligand necessary to cause a 50% decrease in the fluorescence intensity of ethidium bound to DNA) are reported in Table 1. In reverse addition of ethidium to solutions of DNA and compounds **I–IV**, ethidium could not displace the DNA-bound species within the experimentally accessible conditions.

To characterize the mode of binding to DNA, linear dichroism spectra of compounds **I–IV** were measured in the presence of ct-DNA. It is well known that the binding of small molecules to DNA results in the observation of dichroism in the region of absorption of the bound molecule when the DNA is oriented by flow or other techniques. The application of linear dichroism to the elucidation of drug–DNA interactions has been reviewed recently [24]. Provided that the bound species absorbs in a region in which the DNA is transparent, the orientation of its electronic transition vector relative to the long axis of DNA can be determined. The compounds studied herein are well suited to analysis by linear dichroism as they contain planar aromatic chromophores which absorb in two separate regions of the spectrum beyond

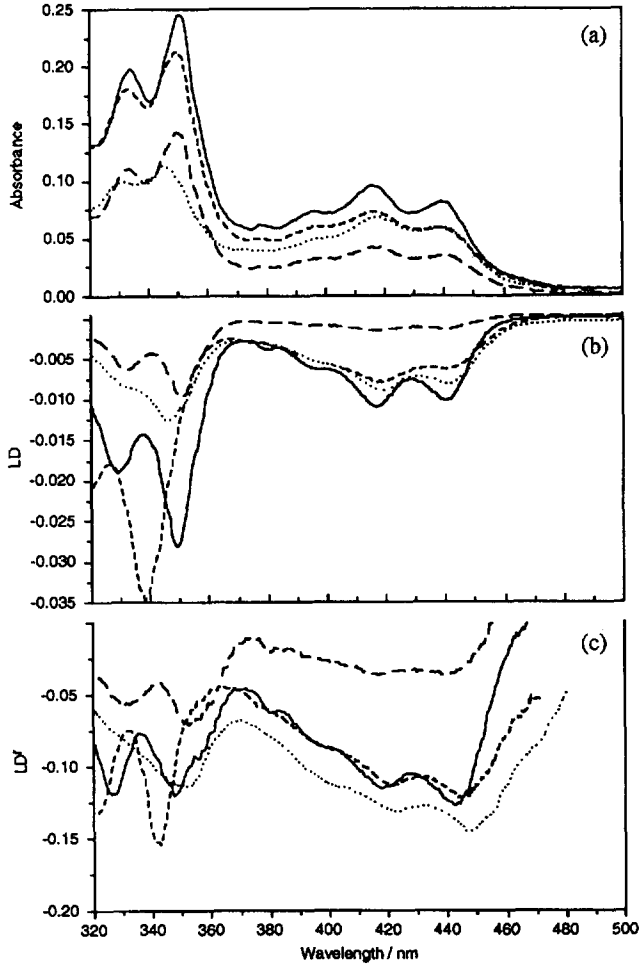


Fig. 3. Absorption (a), linear dichroism (b) and reduced linear dichroism (c) spectra of **I** (———), **II** (– – –), **III** (- - -) and **IV** (· · ·) in the presence of 1 mM ct-DNA.

the absorption envelope of DNA. To ensure complete binding to DNA, and that the bound molecules are sufficiently separated along the DNA chain to avoid complications resulting

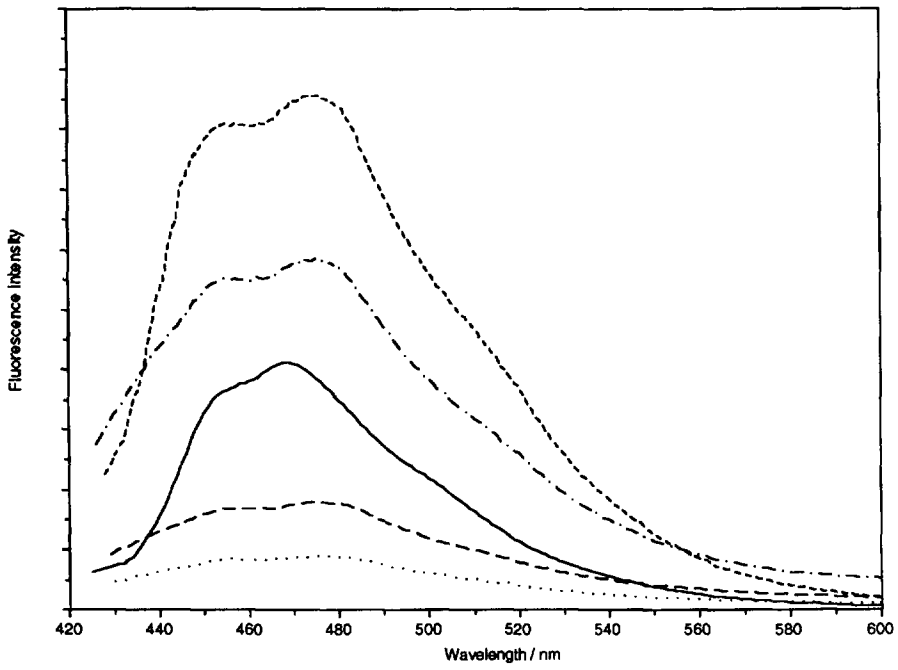


Fig. 4. Fluorescence emission spectra of 9-MAA (———), **I** (– – –), **II** (- - -), **III** (– · –) and **IV** (· · ·) in the presence of ct-DNA. The spectra were measured under conditions of matched absorbance ($A = 0.07$) at the excitation wavelength ($\lambda_{ex} = 400$ nm), and solutions contained 0.5 mM DNA base pairs in aqueous phosphate buffer (pH 7) containing 0.1 M sodium chloride.

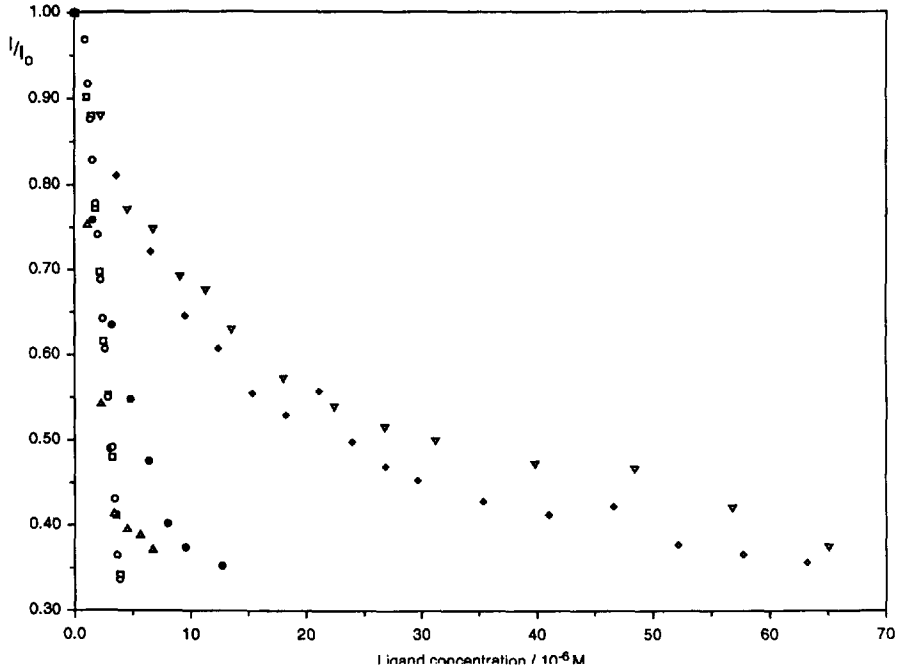


Fig. 5. Reduction in the fluorescence of DNA-bound ethidium on addition of 9-AA (▽), 9-MAA (◇), **I** (□), **II** (○), **III** (△) and **IV** (●). [Ethidium] = 2.35 μM. [DNA] = 1.82 μM base pairs in 40 mM phosphate buffer.

from co-operative-type binding, loadings of 0.01 ligand to one DNA base residue or less were employed. The linear dichroism spectra and calculated reduced linear dichroism ($LD^r = LD/A_{iso}$, where A_{iso} is the isotropic absorbance of the sample) are presented in Figs. 3(b) and 3(c) respectively.

3.3. Transient absorption spectroscopy

To determine the spectra of the radical ions which may be formed via intramolecular electron transfer quenching of the excited acridine chromophore by pyrene, 9-AA and pyrene were investigated separately under conditions in which the generation of the corresponding radical ions is expected. Laser excitation of acetonitrile solutions of naphthalene or anthracene has been reported to result in the photoinduced ionization of the aromatic hydrocarbon and generation of the corresponding radical cation [25]. On laser excitation of pyrene (6.5×10^{-5} M) at 308 nm in aerated acetonitrile solution, the transient absorption spectrum shown in Fig. 6 is

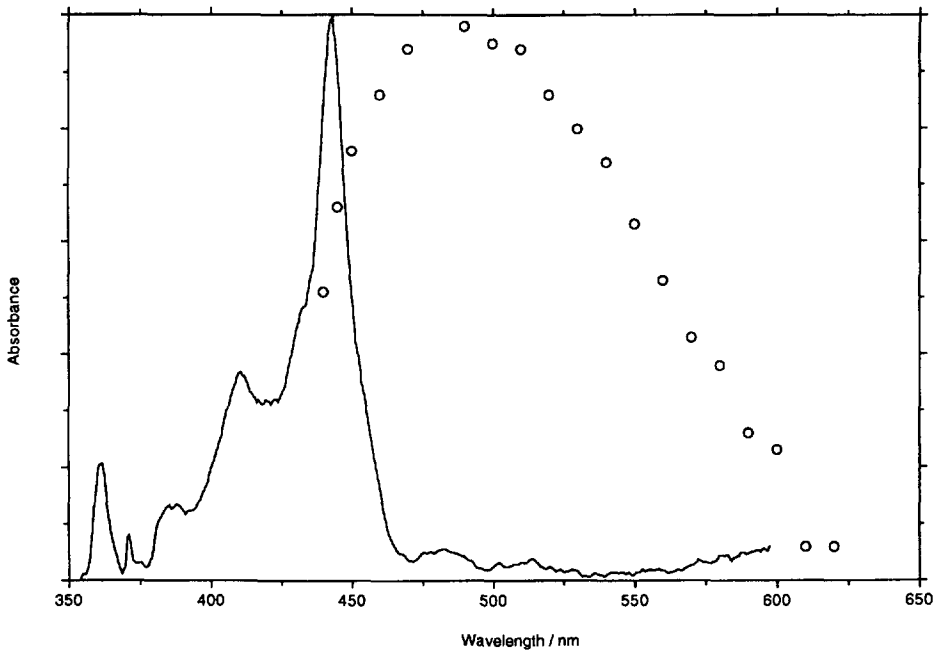


Fig. 6. Transient absorption spectra of pyrene$^{\cdot+}$ (full line) in acetonitrile and 9-AAH (open circles) in acetonitrile–$HClO_4$ (0.005 M).

formed within the duration of the excitation flash (approximately 20 ns). The spectrum agrees well with that of the pyrene radical cation obtained in a freon matrix by Shida [26]. The same spectrum is obtained on excitation of 5×10^{-5} M pyrene solutions containing 0.01 M *p*-dicyanobenzene, a strong electron acceptor. Two components to its formation may be identified: one formed within the laser flash, and one that is time resolved. The latter component is attributed to diffusional electron transfer quenching of the pyrene excited state by *p*-dicyanobenzene.

The reduced form of protonated 9-AA was obtained by excitation of 9-AA hydrochloride in the presence of potassium thiocyanate (0.1 M) in aerated acetonitrile solution. The quenching of aromatic molecules by inorganic anions was recently investigated by Mac et al. [27], who reported the detection of the corresponding aryl radical anions at quencher concentrations sufficient to promote charge separation in the initially formed charge transfer exciplex. The transient absorption spectrum (Fig. 6, $\lambda_{max}=490$ nm), obtained on conventional flash photolysis (using microsecond discharge flash lamps fitted with filters to exclude light below 400 nm) of 9-AAH$^+$ in aerated acetonitrile (0.005 M $HClO_4$, 0.1 M KSCN) solution, is therefore assigned to the 9-AA radical. Selective excitation of 9-AA in the presence of pyrene (2.5×10^{-5} M and 0.01 M respectively in aerated acetonitrile solution) by conventional flash photolysis results in the observation of strong transient absorption signals at 445 nm and 500 nm which are assigned to the decay of the pyrene radical cation and 9-AA radical respectively. Although the signal at 500 nm is non-exponential, the signal at 445 nm decays with a first-order rate constant $k=1.4\times10^3$ s^{-1}.

On excitation of 9-AAH$^+$ in the absence of potassium thiocyanate using a conventional or laser flash photolysis apparatus, a weak transient species possessing an absorption maximum at 470 nm is detected. The latter is identical with that obtained on 308 nm laser excitation of degassed acetonitrile (0.005 M $HClO_4$) solution containing 0.01 M benzophenone and 1.0×10^{-4} M 9-AA. Kinetic analysis resolves a grow-in of the 470 nm transient equal to the decay rate of the benzophenone triplet. Since the triplet energy of benzophenone (288 kJ mol^{-1} [28]) is higher than that of 9-AA (191 kJ mol^{-1} [16]), energy transfer from triplet benzophenone to 9-AA is expected to be efficient and the observed transient spectrum is therefore assigned to triplet 9-AAH$^+$. The absorption of triplet 9-AAH$^+$ is observed with a lifetime of 150 ns in aerated acetonitrile solution, from which a value of $k_q=3.6\times10^9$ M^{-1} s^{-1} is calculated for the rate of quenching by oxygen assuming $[O_2]=1.9\times10^{-3}$ M [29].

Direct excitation of compounds **I–IV** in degassed acetonitrile solution (0.005 M $HClO_4$), using pulsed flash lamps fitted with cut-off filters to exclude light below 395 nm, leads to the observation of a weak transient absorption with a maximum at approximately 460–470 nm, which is not observed in the presence of air. The weak transient absorption spectrum is assigned to the triplet state of acridine based on its similarity with the spectrum of triplet 9-AAH$^+$ and the occurrence of oxygen quenching. Because the acridine triplet state (191 kJ mol^{-1} [16]) is lower in energy than the pyrene triplet state (202 kJ mol^{-1} [30]), its formation may be expected on return electron transfer. Fast return electron transfer has been observed in many intramolecular donor–acceptor systems, including linked pyrene–amines [20].

In the presence of DNA, no transient absorption can be detected on the nanosecond or millisecond time scale in degassed solutions. The absence of signals, under conditions in which they would normally have been observed, suggests

the occurrence of a deactivation mechanism faster than the time resolution of the instrumentation.

4. Discussion

4.1. Photophysics

The absorption spectra of 9-AA [31] and pyrene [32] have been the subject of numerous investigations. Pyrene possesses a highly structured absorption spectrum with a parity-forbidden $S_1 \leftarrow S_0$ electronic transition beginning at 372 nm. The second $\pi\pi^*$ transition ($\lambda_{max} = 338$ nm) is allowed and exhibits prominent 1400 cm^{-1} progressions. A consequence of the weak oscillator strength of the $S_1 \leftarrow S_0$ transition is the uncommonly long lifetime of singlet excited pyrene in degassed solutions (650 ns [33]) rendering it particularly susceptible to quenching. The electronic transitions to S_3 and S_4 occur at 274 nm and 241 nm respectively, and partially overlap the DNA absorption. Alkyl substitution on the C1 position of pyrene has only a modest effect on the absorption spectrum, resulting in a bathochromic shift of 4 nm for the $S_2 \leftarrow S_0$ transition and an intensification of the $S_1 \leftarrow S_0$ transition.

The basicity of acridine is enhanced by the presence of the 9-amino substituent and, in aqueous solutions at pH 7, the ring nitrogen of 9-AA ($pK_a = 9.6$) [31] is quantitatively protonated to form the 9-aminoacridinium cation, 9-AAH^+. Although acridine and 9-AA possess close-lying excited states of $\pi\pi^*$ and $n\pi^*$ nature, the lowest excited state has been proposed to be of $\pi\pi^*$ character in all solvents [16,34]. Alkyl substitution on the exocyclic amino group in 9-AA results in a small shift of the first band to longer wavelengths due to the electron-releasing inductive effect of the alkyl group. A more important effect of substitution is the large reduction in fluorescence intensity observed in 9-alkylaminoacridines compared with 9-AA [31].

Linkage of the 9-AA and pyrene chromophores via a tether of more than one carbon atom as in **I**, **II** or **III** does not result in any additional changes in the absorption or emission spectra, other than those expected for alkyl substitution at the C1 position of pyrene and at the 9-amino group of 9-AA. The larger inductive effect of the longer chain is reflected in an increased bathochromic shift of the acridine absorption band in **I**, **II** and **III** compared with 9-MAA and 9-AA. Although acridine is known to have a high propensity towards ground state aggregation [31], no additional absorption bands at longer wavelengths, assignable to intramolecular electron donor–acceptor (EDA) complex formation, are detected in the linked compounds. In the case of **IV**, the electronic interactions through the methylene bridge are sufficiently strong to cause more substantial changes in the absorption and emission spectra of the two chromophores. The acridine absorption in **IV** is further shifted to longer wavelengths, and a substantial reduction in the oscillator strength of the pyrene S_2 transition is observed (Fig. 1).

4.2. Binding to DNA

Pyrene and other planar aromatic hydrocarbons have been found to bind to B-form DNA by intercalation between adjacent nucleotide base pairs [35]. In the case of pyrene, both intercalative (type I) and groove or exterior (type II) binding are observed [10,36,37]. Type I binding is characterized by strong hypochromism in the absorption spectrum, which is shifted bathochromically by 10 nm, and strong quenching of the fluorescence emission, whereas type II binding is much weaker and produces no changes in the absorption or emission spectra. Comparison of the relative solubilization of pyrene by synthetic polynucleotides reveals a preference for type I binding to AT sequences in DNA [36]. Recently, investigation of the electron transfer quenching of excited state pyrene by nucleosides has been reported [38].

Because of the anti-tumour activity of acridine and many of its derivatives, such as *m*-amsacrine, the interaction of acridines and, in particular 9-AA, with DNA has been the subject of numerous investigations and is well documented [4,35]. As in the case of pyrene, both intercalative and surface binding are possible. The latter is much weaker and suppressed at moderate ionic strengths, and has been attributed to stacking on the surface of DNA due to electrostatic interactions [31]. Intercalative binding is accompanied by a reduction in the extinction coefficient (hypochromism) of the long-wavelength band, which is shifted to longer wavelengths by 6 nm [39]. The fluorescence emission is similarly shifted and the fluorescence quantum yield is reduced. Studies by Weisblum and de Haseth [40] have shown that the fluorescence of DNA-bound acridine is quenched by the presence of nearby GC base pairs.

Joining of two acridine molecules by a flexible chain may result in the formation of compounds capable of bis-intercalative binding to DNA [41–43]. The mode of binding, mono- vs. bis-intercalative, depends on the length of the chain, its point of attachment and the presence of substituents on the chain or chromophores. Bis-intercalation is observed only in cases in which the chain is of sufficient length to span two base pairs (10 Å or more). This was interpreted as an extension of the neighbour exclusion principle, where binding of one intercalator effectively excludes binding at adjacent sites [42]. Exceptions to this rule have, however, been observed. Atwell et al. [44] reported the bis-intercalative binding of a rigid diacridine derivative at two contiguous DNA intercalation sites. Binding in violation of the neighbour exclusion principle has also been proposed in a bis-acridine linked by a pentamethylene chain [43].

Because the first electronic transitions of the pyrene and acridine moieties in compounds **I–IV** are well separated, it is possible to investigate the binding of each chromophore separately. This has not been the case in the other bis- and multi-intercalating compounds studied previously. All of the compounds synthesized and studied in this work undergo the characteristic spectral changes in their UV–visible absorption spectra assigned to type I binding in the presence of DNA,

namely strong hypochromism and a shift to longer wavelengths. As a result of the low water solubility of these compounds, conventional methods of obtaining binding constants, such as Scatchard plots, do not give meaningful results. Relative binding strengths were determined by comparing the capability of each ligand to displace intercalated ethidium from double-stranded DNA. Fig. 5 charts the decrease in ethidium fluorescence resulting from the displacement of bound ethidium by 9-AA, 9-MAA and compounds **I–IV**. The introduction of a methyl group on the exocyclic nitrogen of 9-AA has already been reported to result in little change in its binding properties to DNA [41], and this is reflected in the similar displacement curves obtained for 9-AA and 9-MAA in Fig. 5. Conversely, the presence of the pyrene group leads to a dramatic increase in the DNA binding affinity of **I–IV**. The similarity between the absorption spectra of the bound species (Fig. 3(a)) observed for **I, II** and **III** suggests similar binding modes for the three molecules. However, the observation of changes in the absorption spectra of the molecules on binding to DNA cannot be used as clear-cut evidence for intercalative-type binding, a fact that has already been pointed out by Long and Barton [45], and additional confirmation of intercalation must be sought.

More precise information concerning the difference in binding modes can be obtained from an inspection of the linear dichroism and reduced linear dichroism spectra shown in Figs. 3(b) and 3(c). Intercalation of a planar aromatic chromophore is expected to lead to a reduced linear dichroism signal of similar magnitude and direction as that observed for the base pairs if the electronic transition moment is perpendicular to the long axis of the DNA strand. The wavelength dependence of the vibronic bands in the reduced linear dichroism spectrum has been associated with a deviation from coplanarity of the chromophore with respect to the base pairs [24]. In the case of pyrene, the $S_2 \leftarrow S_0$ transition at 350 nm is aligned with the long axis of the chromophore, whereas 9-AA possesses an electronic transition at 401 nm aligned with the short axis of the molecule [24]. To compensate for the modest oscillator strength of the pyrene $S_2 \leftarrow S_0$ and acridine $S_1 \leftarrow S_0$ transitions, ligand concentrations of approximately 1×10^{-5} M were employed. Because of the high DNA concentration used (1 mM in base pairs), direct comparison of the signal from the DNA bases and the intercalated compounds is not possible, and exact ligand–DNA orientations cannot be determined. However, approximate relative orientations of the chromophores can be inferred by assuming that the strongest undistorted signal is due to intercalation with a planar or near-planar orientation with respect to the base pairs. Inspection of the LD^r signals of compounds **I–IV** reveals important differences, and only compound **I** meets all the criteria expected for near-planar intercalation of both the pyrene and the acridine chromophores. It can be observed that both chromophores in **I** possess LD^r signals of equal magnitude and sign, and that each of the vibronic bands in pyrene is of equal intensity. In contrast, compound **II** yields much smaller LD^r signals which are severely distorted. Such signals are consistent with either a mixture of pyrene and acridine mono-intercalated molecules or partial bis-intercalation at adjacent base pairs. Large deviations from coplanarity of the chromophores would be expected in the latter case, as the tether in **II** is of insufficient length to allow full intercalation of both acridine and pyrene moieties. To elucidate the binding geometry of **II**, it is useful to examine the homologous compound possessing one methylene group less in the connecting chain. Because the chain in **III** is too short to allow bis-intercalation even at adjacent binding sites, a similar behaviour to **II** would indicate that both ligands bind as a mixture of pyrene and acridine mono-intercalated compounds. However, this is not the case, as only the acridine signal in **III** corresponds to that expected for intercalation; the pyrene signal, on the other hand, is displaced to shorter wavelengths (where the free pyrene absorbs) and is assigned to exterior-bound pyrene. Compound **IV** behaves similarly to **III**, intercalating only its acridine moiety and producing a broad distorted signal in the region of pyrene absorption. The presence of LD signals for pyrene in **II** and **IV**, even in the absence of intercalation, may reflect the restricted conformational mobility induced by the intercalation of the acridine moiety or the occurrence of external binding.

Binding of compounds **I–IV** to DNA is accompanied by a reduction in the fluorescence quantum yield of the acridine chromophore, as expected from the additive effects of quenching by pyrene and the DNA bases (Fig. 4). Guanine is known to quench acridine excited states and will therefore compete with pyrene for electron transfer to the acridine chromophore. Unfortunately, precise data concerning the oxidation potentials of nucleosides are not available owing to irreversible oxidation in aqueous and aprotic media, and it is not currently possible to assign conclusively the quenching to electron transfer from pyrene or from the DNA bases. Lecomte et al. [46] have estimated the oxidation potential of 2′-deoxyguanosine-5′-monophosphate (GMP) in aqueous solution to be $E_{ox}(GMP^{\cdot +}/GMP) = 1.29$ V vs. SCE, slightly above that of pyrene (1.16 V) [17]. However, a lower value (1.00 V vs. normal hydrogen electrode (NHE)) was estimated by Faraggi and Klapper [47] from a combination of cyclic voltammetry and differential pulse polarography.

Compared with 9-MAA bound to DNA, compounds **II** and **III** possess increased fluorescence yields, probably indicative of a reduced quenching efficiency by the DNA bases and/or a preference for AT regions in the DNA strand. The strongest emission is detected from **II**, which is proposed to be only partially intercalated on the basis of the linear dichroism results. In contrast, the fluorescence emissions of **I** and **IV** are strongly quenched with respect to that of 9-MAA (Fig. 4). In the case of **I**, the pyrene moiety is proposed to lie coplanar to the acridine chromophore, separated by two base pairs (approximately 10 Å), assuming conventional bis-intercalation. Pyrene and 9-AA preferentially bind to GC and AT regions in DNA respectively. Hence, the observed reduction in the fluorescence quantum yield may result from preferential binding of **I** to CG-rich regions in the DNA rather

than from electron transfer from pyrene to acridine through the DNA core. Further investigations by picosecond time-resolved fluorescence emission and pump-probe absorption spectroscopy may serve to identify the process responsible for fluorescence quenching and to determine the rates for intramolecular electron transfer in **I** in the presence and absence of DNA. Finally, a detailed characterization of the binding sites of **I** at synthetic oligonucleotides in solution may be attained by NMR methods [48].

5. Conclusions

In solution, the degree of fluorescence quenching of acridines linked to a pyrene quencher was found to decrease with increasing chain length, as expected for intramolecular quenching. This trend was reversed in the presence of DNA where **I** was more efficiently quenched than **II** or **III**. Linear dichroism experiments indicated that compounds **III** and **IV**, possessing tethers of four and one methylene units respectively, underwent binding to DNA by mono-intercalation of the acridine chromophores. Compound **II**, possessing a pentamethylene linker, appeared to undergo partial bis-intercalative binding, in violation of the neighbour exclusion principle, where the two chromophores were partially inserted in the DNA and were tilted with respect to the base pairs. In compound **I**, the two chromophores were linked by a chain of sufficient length to span two base pairs and this compound was bound to DNA in accordance with the neighbour exclusion principle. The experimental data were fully consistent with bis-intercalation and an essentially coplanar orientation of the two chromophores in the DNA complexes of compound **I**.

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References

[1] D.N. Beratan and J.N. Onuchic, *Photosynth. Res., 22* (1989) 173. S.A. Wallin, E.D.A. Sterp, A.M. Everest, J.M. Nocek, T.L. Netzel and B.M. Hoffman, *J. Am. Chem. Soc., 113* (1991) 1842. A.W. Axup, M. Albin, S.L. Mayo, R.J. Crutchley and H.B. Gray, *J. Am. Chem. Soc., 110* (1988) 435.

[2] J.K. Barton, V.K. Challa and N.J. Turro, *J. Am. Chem. Soc., 108* (1986) 6391. M.D. Purugganan, C.V. Kumar, N.J. Turro and J.K. Barton, *Science, 241* (1988) 1645. P. Fromherz and B. Rieger, *J. Am. Chem. Soc., 108* (1986) 5361. N.J. Turro, J.K. Barton and D.A. Tomalia, *Acc. Chem. Res., 24* (1991) 332. C.J. Murphy, M.M. Arkin, N.D. Ghatlia, S. Bossman, N.J. Turro and J.K. Barton, *Proc. Natl. Acad. Sci. USA, 91* (1991) 5315. E.D.A. Stemp, M.R. Arkin and J.K. Barton, *J. Am. Chem. Soc., 117* (1995) 2375.

[3] D.N. Beratan, J.N. Betts and J.N. Onuchic, *Science, 252* (1991) 1285. B.A. Jacobs, M.R. Mauk, W.D. Funk, R.T.A. MacGillivray, A.G. Mauk and H.B. Gray, *J. Am. Chem. Soc., 113* (1991) 4390.

[4] B.C. Baguely, W.A. Denny, G.J. Atwell and B.F. Cain, *J. Med. Chem., 24* (1981) 170.

[5] A.M. Brun and A. Harriman, *J. Am. Chem. Soc., 114* (1992) 3656.

[6] A.M. Brun and A. Harriman, *J. Am. Chem. Soc., 116* (1994) 10 383.

[7] C.J. Murphy, M.M. Arkin, N.D. Jenkins, N.D. Ghatlia, S. Bossman, N.J. Turro and J.K. Barton, *Science, 262* (1993) 1025.

[8] T.J. Meade and J.F. Kayyem, *Angew. Chem., 107* (1995) 358.

[9] L.P.G. Wakelin, *Med. Res. Rev., 6* (1986) 275. J. Markovits, J. Ramstein, P. Roques and J.-B. Le Pecq, *Biochemistry, 22* (1983) 3231. B. Gaugain, J. Barbet, R. Oberlin, B.P. Roques and J.-B. Le Pecq, *Biochemistry, 17* (1978) 5071. B. Gaugain, J. Barbet, N. Capelle, B.P. Roques and J.-B. Le Pecq, *Biochemistry, 17* (1978) 5078. S.C. Zimmerman, C.R. Lamberson, M. Cory and T.A. Fairly, *J. Am. Chem. Soc., 111* (1989) 6805. B.F. Cain, B.C. Baguely and W.A. Denny, *J. Med. Chem., 21* (1978) 658.

[10] M. Kodama, Y. Tagashira, A. Inamura and C. Nagata, *J. Biochem., 59* (1966) 257. F.M. Chen, *Anal. Biochem., 130* (1983) 346.

[11] A.R. Peacocke and N.J.H. Sherrett, *Trans. Faraday Soc., 52* (1956) 261. L.S. Lerman, *J. Mol. Biol., 3* (1961) 18.

[12] W.R. Ware and B.A. Baldwin, *J. Chem. Phys., 40* (1964) 1703.

[13] H. Vogt, G. Hänisch and R.A. Hochstrasser, *Rev. Sci. Instrum., 66* (1995) 4385.

[14] J. Wirz, *Pure Appl. Chem., 56* (1984) 1289.

[15] R.M. Acheson, *Acridines; The Chemistry of Heterocyclic Compounds*, Vol. 9, Wiley, London, 1956, p. 37.

[16] K. Kasama, K. Kikuchi, Y. Nishida and H. Kokobun, *J. Phys. Chem., 85* (1981) 4148.

[17] N.C. Yang, *J. Am. Chem. Soc., 85* (1963) 2124.

[18] R.C. Kaye and H.I. Stonehill, *J. Chem. Soc.*, (1951) 2638.

[19] D. Rehm and A. Weller, *Isr. J. Chem., 8* (1970) 259.

[20] T. Okada, I. Karaki, E. Matsuzawa, N. Mataga, Y. Sakata and S. Misumi, *J. Phys. Chem., 85* (1981) 3957. F.C. De Schryver, D. Declercq, S. Depaemelaere, E. Hermans, A. Onkelinx, J.W. Verhoeven and J. Gelan, *J. Photochem. Photobiol. A: Chem., 82* (1994) 171.

[21] F.D. Lewis, D.M. Bassani, E.L. Burch, B.E. Cohen, J.A. Englemann, D.G. Reddy, S. Schneider, W. Jaeger, P. Gedeck and M. Gahr, *J. Am. Chem. Soc., 117* (1995) 660. F.D. Lewis, D.G. Reddy, D.M. Bassani, S. Schneider and M. Gahr, *J. Am. Chem. Soc., 116* (1994) 597. F.D. Lewis, D.M. Bassani and D.G. Reddy, *Pure Appl. Chem., 9* (1992) 1271.

[22] M. Van der Auweraer, A.M. Swinnen and F.C. De Schryver, *J. Chem. Phys., 77* (1982) 4110. A.M. Swinnen, M. Van der Auweraer and F.C. De Schryver, *J. Photochem., 28* (1985) 315. M. Van der Auweraer, *Acad. Anal., 48* (1986) 27. M. Van der Auweraer, Z.R. Grabowski and W. Rettig, *J. Chem. Phys., 95* (1991) 2083. Ph. Van Haver, N. Helsen, S. Depaemelaere, M. Van der Auweraer and F.C. De Schryver, *J. Am. Chem. Soc., 113* (1991) 6849.

[23] B.C. Baguely and E.-M. Falkenhaug, *Nucleic Acid Res., 5* (1978) 161. B.F. Cain, B.C. Baguely and W.A. Denny, *J. Med. Chem., 21* (1978) 658.

[24] B. Nordén and T. Kurucsev, *J. Mol. Rec., 7* (1994) 141. B. Nordén, M. Kubista and T. Kurucsev, *Q. Rev. Biophys., 25* (1992) 51.

[25] E. Vauthey, E. Haselbach and P. Suppan, *Helv. Chim. Acta, 70* (1987) 347. S. Steenken, C.J. Warren and B.C. Gilbert, *J. Chem. Soc., Perkin Trans. 2*, (1990) 335.

[26] T. Shida, *Electronic Absorption Spectra of Radical Ions; Physical Science Data*, Vol. 34, Elsevier, Amsterdam, 1988, p. 86.

[27] M. Mac, J. Wirz and J. Najbar, *Helv. Chim. Acta, 76* (1993) 1319.

[28] W.J. Leigh and D.R. Arnold, *J. Chem. Soc., Chem. Commun.*, (1980) 406.

[29] R. Battino, *Solubility Data Series: Vol. 7, Oxygen and Ozone*, Pergamon, Oxford, 1981, p. 19.

[30] M.Z. Zander, *Z. Naturforsch., Teil A, 33* (1978) 998.

[31] M. Bailey, in R.M. Acheson (ed.), *Acridines; The Chemistry of Heterocyclic Compounds*, Vol. 9, Wiley, London, 2nd edn., 1973.
[32] J.B. Birks, *Photophysics of Aromatic Compounds*, Wiley, London, 1970.
[33] J.F. Deluis, J.A. Delaire and N. Ivanoff, *Chem. Phys. Lett., 61* (1979) 343.
[34] K. Kasama, K. Kikuchi, K. Yamamoto, Y. Uji-ie, Y. Nishida and H. Kokobun, *J. Phys. Chem., 85* (1981) 1291.
[35] N.E. Geacintov, in R.G. Harvey (ed.), *Polycyclic Aromatic Hydrocarbons and Carcinogenesis*, ACS Symposium Series 283, American Chemical Society, Washington DC, 1985.
[36] F.M. Chen, *Nucleic Acid Res., 11* (1983) 7231.
[37] N. Cho and S.A. Asher, *J. Am. Chem. Soc., 115* (1993) 6349.
[38] M. Manoharan, K.L. Tivel, M. Zhao, K. Nafisi and T.L. Netzel, *J. Phys. Chem., 99* (1995) 17 461. V.Y. Shafirovich, P.P. Levin, V.A. Kuzmin, T.E. Thorgeirsson, D.S. Kliger and N.E. Geacintov, *J. Am. Chem. Soc., 116* (1995) 63.
[39] D.S. Drummond, V.F.W. Simpson-Gildemeister and A.R. Peacocke, *Biopolymers, 3* (1965) 135.
[40] B. Weisblum and P.L. de Haseth, *Proc. Natl. Acad. Sci. USA, 69* (1972) 629.
[41] R.G.McR. Wright, L.P.G. Wakelin, A. Fieldes, R.M. Acheson and M.J. Waring, *Biochemistry, 19* (1980) 5825. N. Assa-Munt, W. Leupin, W.A. Denny and D.R. Kearns, *Biochemistry, 24* (1985) 1449.
[42] N. Assa-Munt, W.A. Denny, W. Leupin and D.R. Kearns, *Biochemistry, 24* (1985) 1441.
[43] L.P.G. Wakelin, M. Romanos, D. Chen, E.S. Glaubiger, E.S. Canellakis and M.J. Waring, *Biochemistry, 17* (1978) 5057.
[44] G.J. Atwell, G.M. Stewart, W. Leupin and W.A. Denny, *J. Am. Chem. Soc., 107* (1985) 4335.
[45] E.C. Long and J.K. Barton, *Acc. Chem. Res., 23* (1990) 271.
[46] J.-P. Lecomte, A. Kirsch-De Mesmaeker, J.M. Kelly, A.B. Tossy and H. Görner, *Photochem. Photobiol., 55* (1992) 681.
[47] M. Faraggi and M.H. Klapper, *J. Chim. Phys., 91* (1994) 1054.
[48] A. Fede, M. Billeter, W. Leupin and K. Wüthrich, *Structure, 1* (1994) 177.