Binding of dansylamide† derivatives to nucleotides and nucleic acids[1]

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Dansylamide† has been modified by the introduction of several side chains into the sulfonamide substituent. Compounds with no, or with only one positive charge at the side chains were found not to be useful for association studies with nucleotides or nucleic acids. Relatively high binding constants with moderate base selectivities have been observed with a ligand obtained from dansyl chloride and *N*,*N*′-bis(3-aminopropyl)piperazine, which contains *two* dansyl units and two permanently charged peralkylammonium centers. Interactions of this ligand with double-stranded nucleic acids show a biphasic binding, the first at base pair concentrations below 10^{-7} M being only detectable by a decrease of fluorescence intensity. The second phase is characterized by increased fluorescence and by wavelength changes similar to effects observed in lipophilic solvents and by affinities of up to 5×10^5 M. At higher concentrations bathochromic shifts and extinction changes in UV spectra of the dye suggest an intercalation mechanism, in line with preliminary circular dichroism studies.

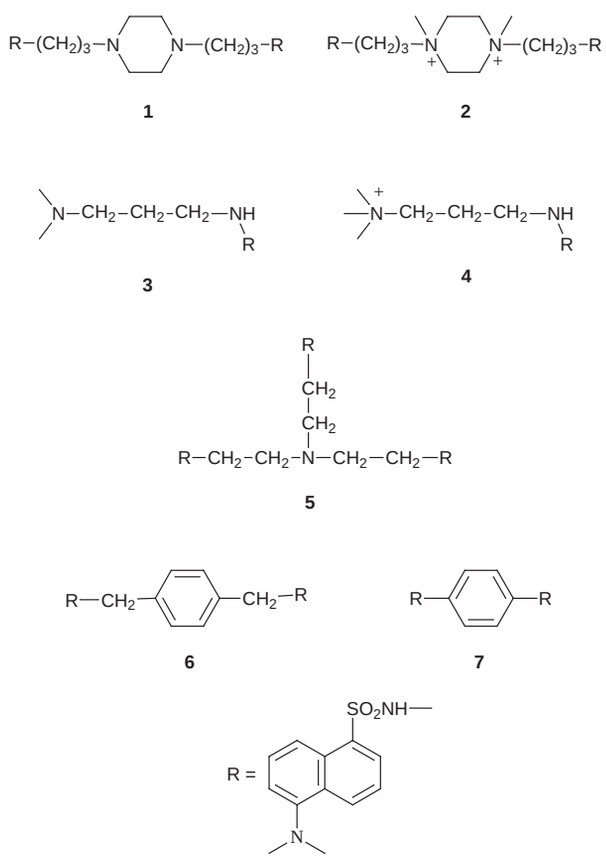
Introduction

The development of new ligands to recognize nucleotides and nucleic acids is of analytical and potentially also of medicinal interest.[2] Considerable effort has been focused on the development of new fluorescent dyes which might have specific effects on nucleic acids. Little attention, however, has been paid to dansylamide (DNSA) derivatives which are well-known fluorescence probes for the study of proteins. We wanted to explore the possibility of increasing the affinity of dansylamide derivatives by the introduction of positive charges in side chains of the dye, and/or to provide two dye residues in one ligand molecule in order to increase stacking interactions with nucleobases.

Results

Compounds such as **1** or **2** were considered to hold particular promise both with respect to affinity constants and selectivity, as they not only contain additional positive charges, but also could allow two dansyl moieties to embrace an aromatic substrate, leading to stacking interactions with potentially some base selectivity. Ligand **2** bears two permanent charges, and thus has the advantage of being usable at all desired pH values. In contrast, the lower water solubility and/or the necessity to use low pH values for complexation with ligand **1** severely limits its application.

For comparison complexation studies were also carried out with several other dansyl derivatives (**3**–**7**), which bear only one positive charge. An additional charge can be introduced at the sulfonamide function in the dansyl moieties by protonation at pH < 4. With 2-naphthylsulfonic acid and 2-naphthylacetic acid as test substrates, fluorescence titrations were carried out in water with 0.5% DMSO, also under acidic conditions. In all these cases the observed fluorescence emission intensity showed a non-linear decrease with increasing substrate concentration. Evaluation by least squares fitting to a 1 : 1 model was found to be satisfactory (error values for association constants were usually < 10%). The association lg *K* values for all these DNSA derivatives as well as for DNSA itself, which bears one positive charge at the applied pH 2, and the naphthalene substrates were all similar with lg $K = 3.0 \pm 0.2$. The dansyl derivatives **6** and **7**, prepared by reaction of dansyl chloride with, *e.g.* α,α-diamino-*p*-xylene or *p*-phenylenediamine, had the disadvantage of very low water solubility of the complex, and showed no regular association behaviour with aromatic substrates or nucleotides. Table 1 summarizes the fluorescence properties of ligands **1** to **7**, and the few data which could be obtained by addition of double-stranded calf thymus DNA to ligands other than **1** and **2** at the accessible low concentrations. The very low solubility of the derivatives **5** to **7** also limited UV and melting point measurements to concentrations where no significant effects could be found. The compounds with only one dansyl unit also showed very low affinity to DNA, with a slight increase of fluorescence intensity ΔI, *i.e.* with ligands **3** and **4**, $\Delta I < 2\%$ at 10^{-3} M DNA. Also, there was no spectral shift



† Dansyl is 5-(dimethylamino)naphthalene-1-sulfonyl.

Table 1 Fluorescence data for dansyl derivatives **1** to **7**[a]

Ligand[a]	Ligand excitation wavelength[b] (λ/nm)	Ligand emission wavelength[c] (λ/nm)	With DNA ($\Delta\lambda$/nm,[d] blue shift)	With DNA ($\Delta I/I_0$[e])
1	326	520	−20	−0.12
2	330	547	−29	(I) −0.23; (II) 0.75
3	330	545	0	0.01
4	330	545	0	0.02
5	350	490	0	−0.74
6	350	493	0	−0.87
7	350	496	0	−0.39

[a] Concentrations of ligands: 3×10^{-6} M in SHE buffer, pH 7.0; ambient temperature. [b] Ligand excitation wavelength at fixed emission (at the λ_{max} of emission); [c] Ligand emission wavelength at fixed excitation (at the λ_{max} of excitation); [d] $\Delta\lambda$ is the wavelength change at the highest DNA concentration used. The concentration range of CT-DNA was varied usually from 10^{-8} to 10^{-5} M, except for ligands **3** and **4** (from 10^{-6} to 10^{-3} M). [e] I_0 is the emission intensity of the ligand in the absence of CT-DNA, ΔI is the maximum emission intensity change upon addition of CT-DNA. For ligand **2**, (I) is the decrease of fluorescence at low ratio of [bp]/[**2**] ($\leqslant$0.01), (II) is the increase of fluorescence at higher DNA concentration (10^{-5} M).

Table 2 Binding constants (K, in M^{-1}) of host compound **2**[a]

Benzenesulfonate	5.2×10^2
2-Naphthalenesulfonate	1.3×10^3
5′-AMP	5.6×10^3
5′-CMP	2.0×10^3
5′-GMP	2.9×10^3
5′-TMP	1.5×10^3
5′-UMP	7.3×10^2

[a] From fluorescence titrations; measurement conditions: 0.02 M phosphate buffer, pH 7.0; the nucleotides at this pH bear two negative charges. Error of binding constants $\leqslant \pm 10\%$.

observed in absorption and fluorescence spectra with these compounds. With ligands such as **5**, **6** and **7**, having two or three dansyl units with and/or without one positive charge, the absorption and fluorescence intensities with added DNA were found to decrease significantly and to reach a final saturation value with increasing nucleic acid concentration, accompanied by negligible spectral shifts. These spectral changes could not, however, be fitted to binding isotherms. After these preliminary experiments only ligand **2** was chosen for further investigation of its interactions with nucleotides and nucleic acids.

Interaction of ligand 2 with nucleotides

Complexation of ligand **2** with aromatic substrates and in particular with nucleotides was studied by UV absorption and by fluorescence spectroscopy. The absence of self-association of the ligand at the concentrations used was ensured by the observation of linear dependencies (linear correlation coefficients $r > 0.9997$) of intensity, or extinction, respectively, *vs.* ligand concentration; these plots began to show curvature by fluorescence only at concentrations above 0.033 mM of **2**. Complex formation of ligand **2** with all aromatic substrates was characterized by the decrease of absorption or fluorescence intensity with almost no shift of the peak positions. Affinities were determined by fluorescence titrations, yielding a satisfactory fit for a 1 : 1 association model (Fig. 1). The values (Table 2) show relatively high equilibrium constants for simple nucleotides, which compare favourably with more complicated synthetic host compounds.[2–5] It should be noted that the relatively high buffer concentration used to maintain a constant pH weakens the ion pairing and thus the affinities considerably. The lg K values can be rationalized by salt bridges between the ammonium centers and the phosphate units, and by moderate stacking interactions between nucleobases and two naphthyl units of the dye, which can be oriented in a cleft-like manner. Computer aided molecular modeling indicates that such a sandwich structure represents a local minimum in the calculated strain energies (Fig. 2). That stacking contributes to the observed binding, although not to a very high degree, is borne out by the smaller affinity observed with benzenesulfonate, as well as by the observation of undetectable (estimated upper value lg $K < 3$) complexation with the simple model compound **4**, which bears only one stacking unit. Ligand **2** also exhibits a

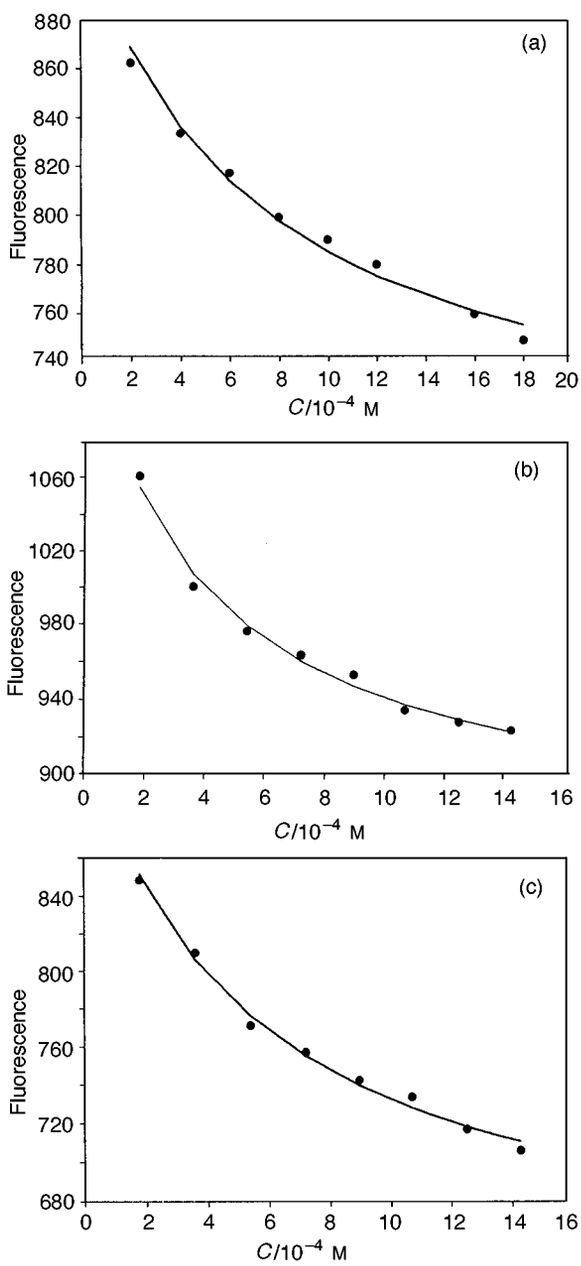


Fig. 1 Fluorescent titration curves of ligand **2** with (a) 5′-TMP, (b) 5′-GMP and (c) 5′-CMP. Excitation wavelength at 330 nm. For Experimental conditions see Table 1.

Fig. 2 CHARMm-simulated structure of the complex between ligand **2** and one nucleotide (5′-AMP)

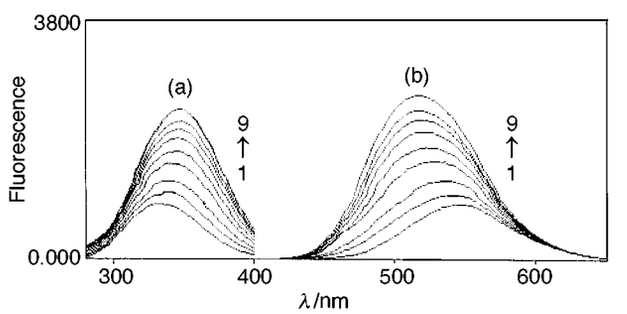


Fig. 3 Fluorescence spectra of ligand **2** (10 μM) with increasing concentration of polyA·polyU. Concentrations of polyA·polyU were as follows: 1, 0.0; 2, 2.1; 3, 4.2; 4, 6.3; 5, 8.4; 6, 10.5; 7, 12.6; 8, 14.6; 9, 17.8 μM. For Experimental conditions see Table 2. (a) Excitation spectra determined with emission at 547 nm; (b) Emission spectra determined with excitation at 330 nm.

moderate binding selectivity to nucleotides, with a preference for purines as in most other cases studied with artificial host compounds.[4]

Interaction of ligand 2 with nucleic acids

Fluorescence studies. The titrations of ligand **2** with nucleic acids show a biphasic binding mode as a function of the biopolymer concentration, as has also been found for other fluorescent antibiotics, such as Hoechst 33 258.[6] At base pair (bp) concentrations of $0.0 < [bp] < 10^{-7}$ M, with a low ratio of $r = [bp]/[\mathbf{2}]$ ($r \leqslant 0.01$), one observes a decrease of fluorescence intensity of **2**, and no change of the peak wavelength with the addition of nucleic acids. At higher ratios of [bp]/[**2**] the fluorescence shows a non-linear increase, with blue shifts of the emission wavelength by up to *ca.* 30 nm, and red shifts of the excitation wavelength by up to *ca.* 20 nm (Fig. 3). The first phase could not be fitted to simple association equilibrium models due to the overlap with the second binding mode; the observed effects, however, indicate lg K_1 values > 6.0. The association at these low concentrations could involve a DNA-induced self-aggregation of the ligand, as has been observed with other groove binders.[7] The association constants for the second binding phase (Table 3) could be obtained in many cases from curve fitting to a 1 : 1 model. Fig. 4 shows an example of the curve fitting for fluorescence titrations of ligand **2** with poly(dG)·poly(dC). In other cases the titration curves showed

Table 3 Binding constants (K, in M^{-1}) of ligand **2** with nucleic acids[a]

	K/M^{-1}[b] (Fluoresc.)	K/M^{-1}[c] (UV–range)
Single-stranded CT-DNA	2.0×10^5[d]	4.0×10^4[d]
Double-stranded CT-DNA	3.0×10^5[d]	3.5×10^4[e]
polyA	5.0×10^5[e]	2.0×10^6[e]
polyA·polyU	1.0×10^5[e]	3.0×10^4[d]
poly(dA)·poly(dT)	1.0×10^5[e]	3.0×10^4[e]
poly(dG)·poly(dC)	1.3×10^5[e]	—
poly(dG-dC)	4.0×10^5[d]	5.0×10^4[e]
poly(dA-dT)	2.0×10^5[d]	4.0×10^4[d]

[a] Measurement conditions: MES buffer (0.01 M MES, 0.001 M EDTA), pH 6.25. [b] Association constants K obtained from fluorescence titrations: [**2**] = 0.010 mM, base pair concentration [bp] was varied usually from 0.0002 to 0.010 mM; [c] Association constants K obtained from UV absorption titrations: [**2**] = 0.020 mM, usually [bp] from 0.003 to 0.030 mM, except with CT-DNA from 0.005 to 0.080 mM. [d] Association constants K estimated from the concentration needed to reach 50% of ΔI, where ΔI is the maximum emission intensity change upon addition of nucleic acids. [e] Association constants K estimated from 1 : 1 curve fitting.

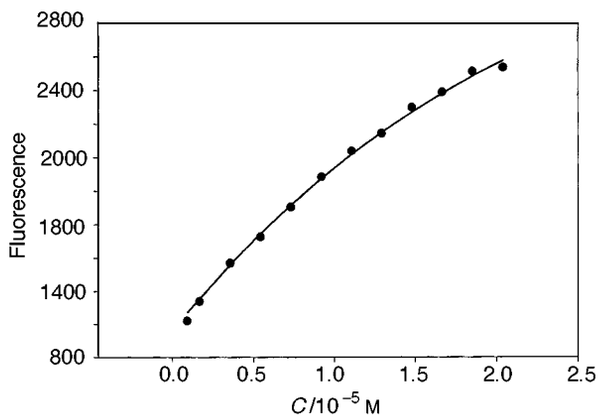


Fig. 4 Fluorescent titration curve of ligand **2** with poly(dG)·poly(dC). Excitation wavelength at 330 nm. For Experimental conditions see Table 2.

systematic deviation from a fit to 1 : 1; in these cases the K value was estimated from the concentration needed to reach 50% of ΔI, where ΔI is the maximum emission intensity change upon addition of nucleic acids.

Dansylamine is also well known as a probe for the microenvironment polarity, for instance in proteins. Therefore, to further reveal the nature of the complexation sites, spectral characteristics of ligand **2** in some organic solvents were investigated. The results (Table 4) indicate that the fluorescence intensity of **2** increases significantly, with a blue shift of emission wavelength and a red shift of the excitation peak with a change in solvent from water to hydrophobic organic solvent. The fluorescence intensity of ligand **2** in alcohol is about ten times stronger than that in aqueous solution. In comparison, the spectral shifts on the second binding step of **2** with nucleic acids show features similar to those in alcohol, which indicates that the dansyl chromophore is involved in a distinctly more hydrophobic environment when bound to nucleic acids.

UV Absorption spectra. Strong interactions of ligand **2** with single- and double-stranded polynucleotides were also visible in UV spectra. One advantage of UV–VIS spectroscopy is that one can measure spectra at higher concentrations of base pairs, until the system becomes insoluble. Typically, ligand **2** upon addition of nucleic acids produced strong decreases of absorption, accompanied by a large red shift of the peak to a position which is similar to that of **2** in organic solvents like methanol or ethanol. In addition, one set of isosbestic points was observed in each case (Table 5). Fig. 5 represents an example of an absorption spectral change of **2** with poly(dA)·poly(dT). The K values (Table 3) obtained with the UV absorption measure-

Table 4 Fluorescence wavelength changes of compound **2** bound either to nucleic acids or in organic solvents (compared to the absorption wavelength in water)

	Excitation wavelength (λ/nm)[a]	Emission wavelength (λ/nm)[b]	$\Delta I/I_0$[c]
Single-stranded CT-DNA	350(+20)	515(−32)	(a) −0.26; (b) 2.52
Double-stranded CT-DNA	350(+20)	518(−29)	(a) −0.23; (b) 0.75
polyA	350(+20)	515(−32)	(a) −0.20; (b) 0.81
polyA·polyU	349(+19)	515(−32)	(a) −0.04; (b) 1.40
poly(dA)·poly(dT)	349(+19)	515(−32)	(a) −0.15; (b) 1.44
poly(dG)·poly(dC)	348(+18)	516(−31)	(a) −0.04; (b) 1.15
poly(dG-dC)	345(+15)	525(−22)	(a) −0.13; (b) 0.36
poly(dA-dT)	350(+20)	515(−32)	(a) −0.27; (b) 1.56
In methanol	347(+17)	518(−29)	—
In ethanol	347(+17)	515(−32)	—
In acetone	349(+19)	509(−38)	—
In THF	340(+10)	490(−57)	—

[a] Excitation spectra with fixed emission wavelength at 547 nm. [b] Emission spectra with fixed excitation wavelength at 330 nm. [c] I_0 is the emission intensity of the ligand in the absence of nucleic acids, ΔI is the maximum emission intensity change upon addition of nucleic acids. (a) is the decrease of fluorescence at low ratio of [bp]/[**2**] (≤0.01), (b) is the increase of fluorescence at higher concentration of nucleic acids. For experimental conditions see Table 3.

Table 5 Spectroscopic properties from UV titrations for ligand **2** bound to nucleic acids[a]

Nucleic acids	H(%)[b]	$\Delta\lambda$/nm (red shift)	Isosbestic points (λ/nm)
ss-CT-DNA	36	8.5	300, 304[c]
ds-CT-DNA	33	6.5	299, 307[d]
polyA	36	8.0	296[e]
polyA·polyU	32	8.5	295
poly(dA)·poly(dT)	31	6.5	298
poly(dG)·poly(dC)	14	8.0	308
poly(dG-dC)	30	6.0	300[f]
poly(dA-dT)	33	7.5	297

[a] Measurement conditions: MES buffer (0.01 M MES, 0.001 M EDTA), pH 6.25. [b] The excitation change [H(%)] was obtained at a ratio of [**2**]/[bp] = 1:1; for CT-DNA: [**2**]/[bp] = 1:2. [c] The isosbestic point shifts from 300 to 304 nm with increasing ss-CT-DNA concentration. [d] The isosbestic point shifts from 299 to 307 nm with increasing ds-CT-DNA concentration. [e] Isosbestic point observed at 296 nm, but blurred at higher concentration ([bp] > 15 μM) of polyA. [f] Isosbestic point observed at 300 nm, but blurred at higher concentration ([bp] > 15 μM) of poly(dG-dC).

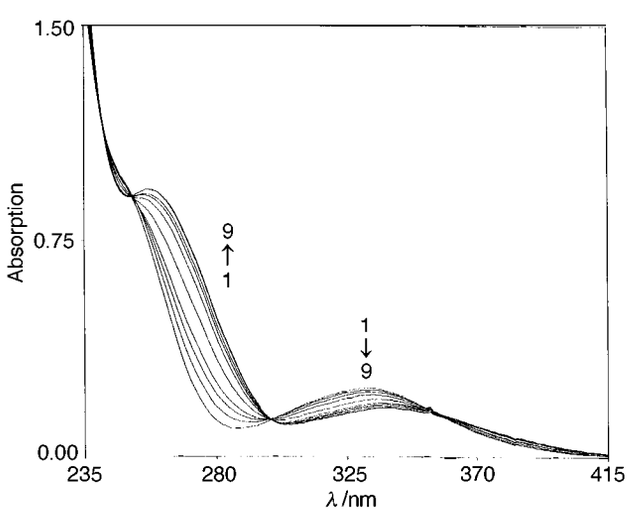


Fig. 5 Absorbance spectra of ligand **2** (20 μM) with increasing concentration of poly(dA)·poly(dT). Concentrations of poly(dA)·poly(dT) were as follows: 1, 0.0; 2, 6.1; 3, 12.2; 4, 18.3; 5, 30.8; 6, 42.9; 7, 49.0; 8, 53.2; 9, 58.0 μM.

ments show deviations compared to those of fluorometric titrations, which indicate another binding mode at the higher concentrations used in the UV measurement range. The K values show an enhanced binding with single-stranded nucleic acids, especially for polyA. One also observed a slight G:C base pair preference. At the higher concentrations of nucleic acids, accessible to UV–VIS measurements, the binding may start to involve intercalation into the double-stranded polymers. NMR analyses with related aminoalkylnaphthalenes indicate intercalation only at such higher concentrations.[8] The magnitude of the excitation changes $\Delta\varepsilon$ and the bathochromic shift is generally believed to indicate intercalation;[9,10] the observed $\Delta\varepsilon$ (usually by *ca.* 30% at the ratio [**2**]/[bp] = 11) and absorption red shift suggest that the intercalation of the dansyl chromophore leads to a close proximity of the dansyl chromophore to the nucleobases. The changes are more pronounced compared to other studies,[9,10] where such large effects were only reported for higher base pair/ligand ratios. The millimolar affinities observed in this work indicate that simple ligand structures may compete with the binding strength of more complicated antibiotics.

Table 6 Effect of ligand **2** on DNA melting point T_m (°C) values[a]

R[b]	CT-DNA (ΔT_m)[c,d]	poly(dA)·poly(dT) (ΔT_m)
0.1	2.3	2.2
0.2	2.7	2.3

[a] Measurement conditions: MES buffer (0.01 M 2-(*N*-morpholino)-ethanesulfonic acid, 0.001 M EDTA and pH 6.25, [NaCl] = 0.01 M). [b] R is a ratio of the concentration of ligand **2** per mol of nucleic acid phosphate of CT-DNA. [c] $\Delta T_m = T_m$ complex − T_m free nucleic acid. The T_m values were obtained from first-derivative plots, dupulicate runs. The error of T_m is ±0.1 °C. [d] For ligand **1** (R = 0.1), ΔT_m with CT-DNA is 0.6 °C; with all other ligands, ΔT_m < 0.1 °C.

Circular dichroism and melting studies. Further evidence for the strong interaction of the ligands with nucleic acids comes from preliminary circular dichroism (CD) spectra and melting studies of nucleic acids. It is found that with the increase of ligand concentration, CD spectra of the nucleic acids at 20 °C changed with intensity decrease (Fig. 6), while the helix melting temperature increased (Table 6).

Fig. 6 shows the CD spectra in the UV range upon addition of ligand **2**; they illustrate a considerable decrease of the peak intensity indicating a distinct conformational change of the DNA duplexes. Even the single-stranded polyA exhibited a decrease of the peak intensity upon addition of ligand **2**, although this change is not as strong as that of the duplexes. It is noteworthy that an isoelliptic point was observed at about 260 nm at the concentrations of ligand **2** below 10 μM; this was blurred above 10 μM of **2**. These observations are consistent with the absorbance changes mentioned above, indicating similar affinities to those obtained from the quantitative

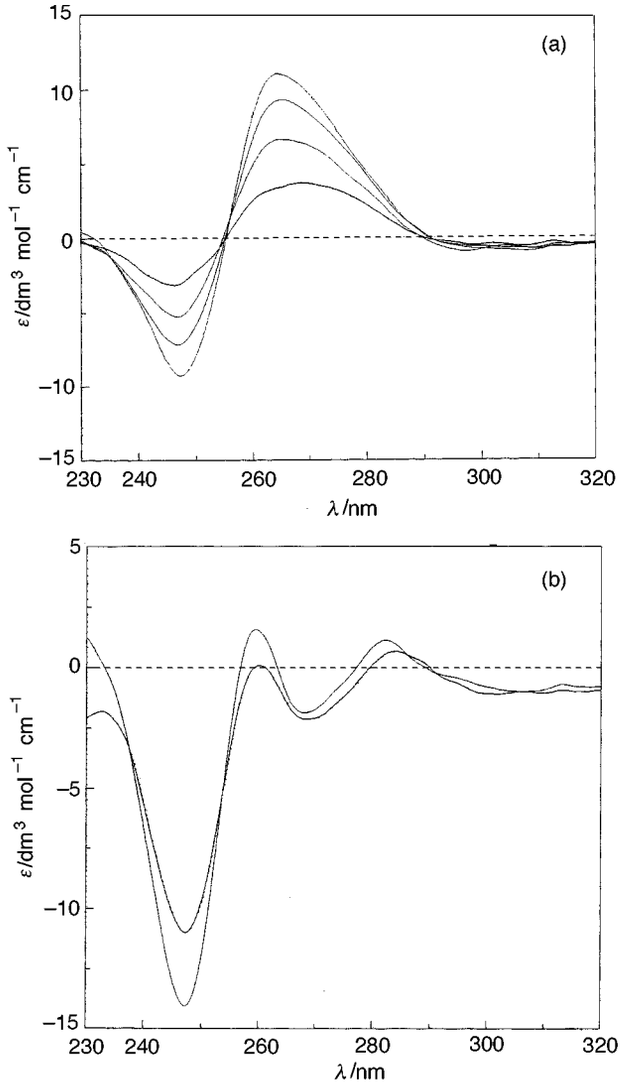


Fig. 6 (a) Circular dichroism (CD) spectra of 6.8 μM polyA in the presence of different concentrations of ligand **2**. Concentrations of **2** were (from top to bottom): 0.0, 3.4, 6.8, 10.2 μM; (b) CD spectra of 24 μM poly(dA)·poly(dT) in the absence (top) and in the presence (bottom) of 12 μM **2**. All CD experiments in MES buffer at pH 7.0, 20 °C.

UV titrations. The lack of solubility of the complexes prevented NMR or viscosimetric studies to further support the intercalating mode.

Conclusions

The results show that a synthetically easily accessible fluorescent dye such as **2** can bind efficiently to aromatic substrates, to nucleotides and to nucleic acids. With double-stranded DNA the association starts at concentrations as low as 10^{-7} M, probably involving binding in the major DNA groove which can induce self-aggregation of the ligand (a similar decrease of emission intensity *I* is observed in the first phase binding to DNA as one finds in curves of *I vs.* ligand concentration, where self-association and quenching start to lead to deviations from linearity). At concentrations between 10^{-7} and 10^{-5} M, accessible to fluorescence titrations with line fitting (second phase), the binding with DNA is characterized by 1:1 association isotherms with affinities as high as 5×10^5 M. The observed changes of fluorescence emission wavelength and intensities are similar to those observed in alcoholic solvents; these data would be in line with a deep groove association of the ligand. At concentrations around 10^{-5} M, accessed by UV titrations, the observed extinction and wavelength changes point to intercalation as the dominant binding mode. This is in line with preliminary circular dichroism data and with earlier results,[8] where naphthyl-shaped moieties were shown to intercalate only at such higher concentrations.

Experimental

Materials

Organic starting material was obtained from Fluka and ACROS Chemical Co. Calf thymus DNA was purchased from Aldrich, polyA·polyU, polyA, poly(dA-dT), poly(dA)·poly(dT) from Sigma Chemical Co., and poly(dG)·poly(dC), poly(dG-dC) from Pharmacia. The nucleic acid concentrations were determined from the molar absorptivity: 9.8 mM^{-1} cm^{-1} at 258 nm for polyA; 6.0 mM^{-1} cm^{-1} at 260 nm for poly(dA)·poly(dT); 7.4 mM^{-1} cm^{-1} at 253 nm for poly(dG)·poly(dC); 6.6 mM^{-1} cm^{-1} at 262 nm for poly(dA-dT); 8.4 mM^{-1} cm^{-1} at 254 nm for poly(dG-dC); 6.0 mM^{-1} cm^{-1} at 260 nm for polyA·polyU. Nucleotide solutions were prepared in 0.02 M phosphate buffer (pH 7), while nucleic acids were dissolved in MES buffers [0.01 M 2-(*N*-morpholino)ethanesulfonic acid, 0.001 M EDTA and pH 6.25, [NaCl] = 0.01 M].

***N,N′*-Bis(3-dansylaminopropyl)piperazine† (1).** *N,N′*-Bis-(3-aminopropyl)piperazine (13.6 mmol) was added to dansyl chloride (34 mmol) in 200 ml dry toluene at 0 °C under stirring; a solution of triethylamine (4 ml) was added dropwise; the mixture was stirred at room temperature and the end of the reaction was checked by TLC. The solid obtained was washed with distilled water several times to obtain the pure compound (45%). δ_H([$^2H_6$]DMSO) 1.37 (m, 4H), 2.00 (m, 12H), 2.80 (t, 4H), 2.84 (s, 12H), 7.28 (d, 2H), 7.63 (m, 4H), 7.91 (s, 2H), 8.11 (d, 2H), 8.30 (d, 2H), 8.48 (d, 2H); δ_C([$^2H_6$]DMSO) 25.90, 44.87, 52.20, 54.65, 114.89, 118.99, 123.17, 127.44, 128.05, 129.06, 136.07, 151.25. (Calc. for $C_{34}H_{46}N_6S_2O_4 \cdot H_2O$: C, 61.07; H, 7.06; N, 12.27. Found: C, 60.80; H, 6.76; N, 12.17%).

Methylated *N,N′*-bis(3-dansylaminopropyl)piperazine (2). Methyl iodide (1 mmol) was added to *N,N′*-bis(3-dansylaminopropyl)piperazine (**1**) (0.5 mmol) in 10 ml dry DMF at 0 °C under stirring, at 50 °C; the end of the reaction was checked by TLC. After evaporation of the solvent and recrystallization from methanol, the pure compound was obtained with a yield of 32%. δ_H([$^2H_6$]DMSO) 1.88 (m, 4H), 2.83 (s, 12H), 2.87 (m, 4H), 3.15 (s, 6H), 3.56 (t, 4H), 3.80 (m, 8H), 7.32 (d, 2H), 7.65 (m, 4H), 8.06 (s, 2H), 8.12 (d, 2H), 8.30 (d, 2H), 8.48 (d, 2H); δ_C([$^2H_6$]DMSO) 22.17, 45.30, 53.42, 115.64, 119.37, 123.90, 128.18, 128.59, 129.10, 129.62, 135.52, 151.00. (Calc. for $C_{36}H_{52}N_6S_2O_4I_2$: C, 45.44; H, 5.47; N, 8.83. Found: C, 43.75; H, 5.20; N, 8.22%).

***N*-Dansyl-3-(dimethylamino)propylamine (3).** 3-(Dimethylamino)propylamine (10.5 mmol) was added to dansyl chloride (7 mmol) in 90 ml dry CH_3CN at 0 °C under stirring. Then a solution of triethylamine (2 ml) was added dropwise. The mixture was stirred at room temperature overnight; after acidification (pH 4), the mixture was extracted with diethyl ether. The aqueous layer was made basic with aqueous NaOH, then extracted with $CHCl_3$ and dried over Na_2SO_4. A solid was obtained after evaporation of solvent. Recrystallization from ethanol yielded a pure compound (48%). δ_H([$^2H_6$]DMSO) 1.40 (m, 2H), 1.92 (s, 6H), 2.02 (t, 2H), 2.85 (m, 8H), 7.29 (d, 1H), 7.64 (m, 2H), 7.90 (s, 1H), 8.13 (d, 1H), 8.31 (d, 1H), 8.49 (d, 1H); δ_C([$^2H_6$]DMSO) 27.02, 40.10, 44.96, 45.13, 56.31, 115.18, 119.28, 123.62, 127.86, 128.44, 129.27, 129.42, 136.24, 151.49.

***N*-Dansyl-3-(trimethylamino)propylamine iodide (4).** Methyl iodide (4 mmol) was added to dansyl-3-(dimethylamino)-propylamine (**3**) (2 mmol) in 40 ml dry methanol at 0 °C under stirring. The mixture was stirred at room temperature for 18 h. After evaporation, the pure compound was obtained by recrystallization from methanol (55%). δ_H([$^2H_6$]DMSO) 1.79 (m, 2H), 2.85 (m, 8H), 2.97 (s, 9H), 3.23 (t, 2H), 7.29 (d, 1H), 7.64 (m, 2H), 8.07 (s, 1H), 8.13 (d, 1H), 8.29 (d, 1H), 8.49 (d, 1H); δ_C([$^2H_6$]DMSO) 23.01, 45.16, 52.40, 63.34, 115.26, 118.90,

123.69, 128.05, 128.48, 129.00, 129.61, 135.47, 151.49. (Calc. for $C_{18}H_{28}N_3SO_2I$: C, 45.31; H, 5.92; N, 8.81. Found: C, 45.49; H, 5.84; N, 8.71%).

Tris(2-dansylethyl)amine (5). Tris(2-aminoethyl)amine (1 mmol) was added to dansyl chloride (3.5 mmol) in 30 ml dry toluene at 0 °C under stirring. Then a solution of triethylamine (1 ml) was added dropwise into the clear solution. The mixture was allowed to stir at room temperature and the end of the reaction was checked by TLC. The precipitate formed was filtered and the solution was evaporated. The solid obtained was washed several times with distilled water to obtain the pure compound (38%). $\delta_H([^2H_6]DMSO)$ 2.11 (t, 6H), 2.64 (m, 6H), 2.84 (s, 18H), 7.18 (d, 3H), 7.54 (m, 6H), 7.76 (s, 3H), 8.08 (d, 3H), 8.28 (d, 3H), 8.44 (d, 3H); $\delta_C([^2H_6]DMSO)$ 45.13, 53.55, 115.09, 119.05, 123.52, 127.79, 128.11, 129.09, 129.35, 136.16, 151.38. (Calc. for $C_{41}H_{51}N_7S_3O_6 \cdot H_2O$: C, 58.38; H, 6.187; N, 11.35. Found: C, 58.25; H, 6.224; N, 11.31%).

α,α′-Bis(dansylamino)-*p*-xylene (6). α,α′-Diamino-*p*-xylene (2 mmol) was added to dansyl chloride (5 mmol) in 20 ml dry toluene at 0 °C under stirring; a solution of triethylamine (2 ml) was added dropwise; the mixture was stirred at room temperature and the end of the reaction was checked by TLC. The precipitate obtained was washed with distilled water several times to obtain the pure compound (60%). $\delta_H([^2H_6]DMSO)$ 2.85 (s, 12H), 3.95 (s, 4H), 6.97 (s, 4H), 7.27 (d, 2H), 7.60 (m, 4H), 8.08 (d, 2H), 8.32 (d, 2H), 8.43 (s, 2H), 8.46 (d, 2H); $\delta_C([^2H_6]DMSO)$ 45.16, 45.74, 115.15, 119.28, 123.56, 127.20, 127.86, 128.30, 129.16, 129.42, 136.33, 136.64, 151.43. (Calc. for $C_{32}H_{34}N_4S_2O_4$: C, 63.76; H, 5.69; N, 9.29. Found: C, 63.16; H, 5.74; N, 9.11%).

1,4-Bis(dansylamino)phenylene (7). Phenylene-1,4-diamine (2 mmol) was added to dansyl chloride (5 mmol) in 20 ml dry toluene at 0 °C under stirring; a solution of triethylamine (2 ml) was added dropwise; the mixture was stirred at room temperature and the end of the reaction was checked by TLC. The precipitate obtained was washed with distilled water several times to obtain the pure compound (48%). $\delta_H([^2H_6]DMSO)$ 2.82 (s, 12H), 6.80 (s, 4H), 7.21 (d, 2H), 7.52 (m, 4H), 8.07 (d, 2H), 8.24 (d, 2H), 8.42 (d, 2H), 10.40 (s, 2H); $\delta_C([^2H_6]DMSO)$ 45.24, 115.37, 118.87, 120.78, 123.61, 128.17, 129.15, 129.70, 130.19, 133.72, 135.10, 151.60. (Calc. for $C_{30}H_{30}N_4S_2O_4$: C, 62.70; H, 5.26; N, 9.75. Found: C, 62.10; H, 5.10; N, 9.66%).

Spectroscopic methods

Fluorescence spectra were obtained with a Hitachi Fluorescence Spectrophotometer F-2000 at 25 °C. The excitation and emission slit width was 5 mm. UV spectra were recorded with a Kontron Uvikon 860 instrument at 25 °C. Denaturation experiments were performed in MES buffers with 6.0×10^{-5} M poly(dA)·poly(dT) or 7.6×10^{-5} M CT-DNA phosphate. $\Delta T_m = T_m$ complex $- T_m$ free nucleic acid. The T_m values were obtained from first-derivative plots. CD spectra were obtained with a JASCO J-715 spectrophotometer interfaced to a PC. All CD experiments were carried out at 20 °C in 1 cm path length cuvettes. Curves presented are the average of three scans.

Spectroscopic titrations were performed as described earlier,[8] using non-linear least squares fit for association constants. With the first part of the biphasic behavior of the fluorescence titrations, no satisfactory fit could be obtained; in these cases lg *K* values were approximated from the concentrations necessary to reach 50% of the total observed change in fluorescence emission. The presence of 0.5% DMSO in the measured solution does not lead to any significant lg *K* changes, as was established in a test with compound **1** and 2-naphthylacetic acid with 15% DMSO.

Acknowledgements

An Alexander von Humboldt fellowship for X. W. and financial support by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie are gratefully acknowledged.

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Paper 8/01609G
Received 25*th February* 1998
Accepted 16*th March* 1998