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# Effect of N-substituent in 4-styrylpyridinium dyes on their binding to DNA

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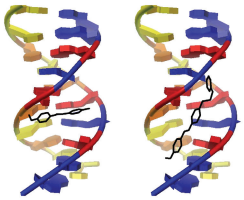
**4-Styrylpyridinium dyes containing *N*-methyl and *N*-(3-pyridiniopropyl) substituents have been prepared and characterized by optical and CD spectroscopy. These compounds demonstrate affinity to calf thymus DNA, with dye–DNA binding mode being different for various N-substituents.**

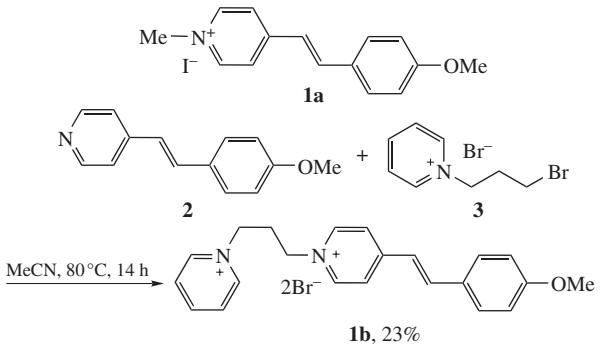


*Keywords*: styryl dye, DNA, intercalation, groove binding, fluorescent probes.

Polymethine dyes represent a well-established class of organic compounds and are used in biology, medicine and drug discovery as fluorescent light-up probes as well as labels for cells, micelles and organelles.[1–3] In this context, styryl dyes have found an application as fluorescent probes for DNA, RNA, β-amyloid peptides, chymotrypsin, heparin and other bioanalytes.[4–9] The design and synthesis of new fluorescent dyes as probes for DNA still represent an urgent task in biological and medical areas.

A pivotal point in the characterization of a dye–DNA complex is the determination of its stoichiometry and mode of binding, such interactions being known to have different mechanisms, namely intercalation, groove binding or external stacking.[10] It was demonstrated, that cationic styryl dyes were capable of forming helical aggregates assembled along the DNA molecule.[11–13] This notion is important to understand the structure–property relationship and to design promising structural types of fluorescent probes for DNA, starting from styryl dyes.

In this work, we present the synthesis of 4-styrylpyridinium dyes with methoxy group in benzene moiety and *N*-methyl- or *N*-(3-pyridiniopropyl) substituent in pyridine ring, along with the investigation of their optical and DNA binding properties. The styrylpyridinium dye scaffold was chosen because of its potential light-up effect upon the interaction with calf thymus DNA, which consisted in the increase in intensity of the dye fluorescence signal in the DNA complex.[14,15]




**Scheme 1** Structure of dye **1a**[16] and synthesis of dye **1b**.

Styryl dye **1a** was prepared according to known procedure,[16] dye **1b** was obtained as shown in Scheme 1. The Knoevenagel condensation of 4-methoxybenzaldehyde with 4-methylpyridine[17] resulted in compound **2**. In the next step, this product reacted with *N*-(3-bromopropyl)pyridiniun bromide affording dye **1b** in 23% yield. The new compound **1b** was identified and characterized by NMR spectroscopy, mass spectrometry and elemental analysis (see Online Supplementary materials).†

The absorption spectra of dyes **1a** and **1b** demonstrate intensive bands at 373 and 382 nm, respectively, which originate from an intramolecular charge transfer from the electron donating methoxyphenyl group to the positively charged pyridinium rings [Figure 1, Figure S1(*a*) and Table 1].‡ The interaction of dyes **1a**,**b** with calf thymus DNA was investigated using optical spectroscopy in aqueous BPE buffer at pH 7. The addition of increasing amounts of DNA resulted in a decrease in the absorption intensity of dyes along with moderate red shifts, namely 21 nm for **1a** and 22 nm for **1b**, up to the saturation of binding was reached [see Figure 1, Figure S1(*a*) and Table 1]. For both compounds,

† *(E)-4-(4′-methoxystyryl)-1-(3″-pyridiniopropyl)pyridinium dibromide* **1b**. A mixture of compounds **2** (0.08 g, 0.4 mmol) and **3** (0.11 g, 0.4 mmol) in MeCN (4 ml) was stirred at 80 °C for 14 h. Then the solvent was evaporated and the residue was purified by column chromatography on neutral $Al_2O_3$ using $CH_2Cl_2$–MeOH as an eluent to afford product **1b** as a yellow oil. Yield 23%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.64 (m, 2H, 2″-H), 3.83 (s, 3H, OMe), 4.63 (t, 2H, 1″-H, *J* 7.0 Hz), 4.75 (t, 2H, 3″-H, *J* 7.0 Hz), 7.07 (d, 2H, 3′-H, *J* 8.5 Hz), 7.41 (d, 1H, α-styryl-H, *J* 16.4 Hz), 7.73 (d, 2H, 2′-H, *J* 8.5 Hz), 8.04 (d, 1H, β-styryl-H, *J* 16.0 Hz), 8.21 (t, 2H, 3‴-H, *J* 7.0 Hz), 8.22 (d, 2H, 3-H, *J* 6.6 Hz), 8.65 (t, 1H, 4‴-H, *J* 7.4 Hz), 8.96 (d, 2H, 2-H, *J* 6.2 Hz), 9.17 (d, 2H, 2‴-H, *J* 5.8 Hz). $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 31.8, 55.5, 56.3, 57.6, 114.74, 120.83, 123.41, 128.2, 130.09, 141.20, 141.23, 144.06, 145.01, 145.8, 153.61, 161.36. MS (ESI in $H_2O$), *m/z*: 166.1 $[M - 2Br]^{2+}$ (calc. for $C_{22}H_{24}N_2O_2^+$, *m/z*: 166.09). Found (%): C, 54.02; H, 4.52; N, 5.53. Calc. for $C_{22}H_{24}Br_2N_2O$ (%): C, 53.68; H, 4.91; N, 5.69.

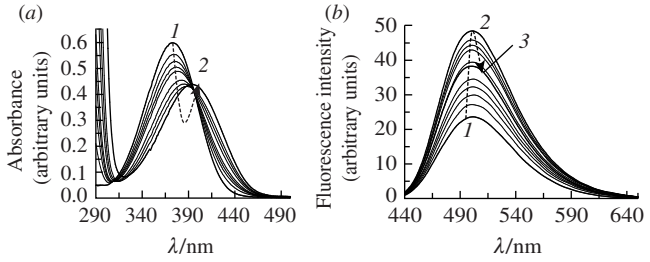



**Figure 1** Shift of spectra upon titration procedure for dye **1a**: (*a*) absorption spectra for $7\times10^{-5}$ M dye with (*1*) 0 and (*2*) $3.5\times10^{-3}$ M calf thymus DNA; (*b*) fluorescence emission spectra at $\lambda_{ex}$ = 394 nm for $2.3\times10^{-5}$ M dye with (*1*) 0, (*2*) $1.76\times10^{-3}$ and (*3*) $2.81\times10^{-3}$ M calf thymus DNA, all in aqueous BPE buffer.

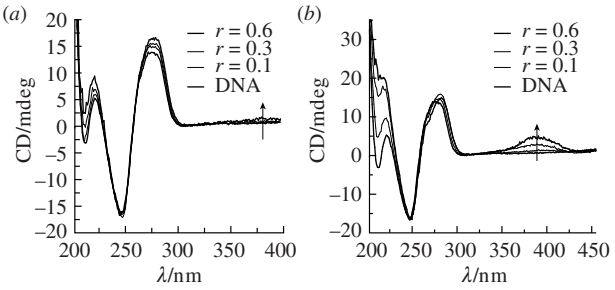



**Figure 2** CD spectra of $1\times10^{-4}$ M calf thymus DNA mixtures with dye ligands (*a*) **1a** and (*b*) **1b** in aqueous buffer at different ligand-to-DNA ratios (*r*).

isosbestic points were observed until almost the end of titration, *i.e.* predominantly single binding mode was revealed.

The analysis of fluorimetric titration data [Figure 1 and Figure S1(*b*)] following the neighbor exclusion model developed by McGhee and von Hippel[18–20] allowed us to determine the binding-site sizes *n*, *i.e.* the number of DNA base pairs per one bound molecule of a dye (Table 1), as well as the binding constants $K_b$, which proved to be equal to $2.4\times10^3$ mol$^{-1}$ dm$^3$ for compound **1a**, $2.5\times10^4$ mol$^{-1}$ dm$^3$ for compound **1b** (Table 1) and were close to the one obtained for styryl dye of similar structure.[15]

The association of the dyes with calf thymus DNA is accompanied by an increase in fluorescence intensity. A moderate enhancement by 2.1 times is observed for compound **1a**, and further elevation of DNA concentration leads to a decrease in the fluorescence intensity by 1.3 times related to its maximum. The fluorimetric response for compound **1b** consists in an intensity increase by 2.3 times with elevation of DNA concentration and no further intensity reduction up to the end of titration. The maximum of fluorescence emission slightly moves up to 3 nm towards red region for dye **1b** and does not change for dye **1a** upon the addition of DNA. The numbers of DNA base pairs occupied by one bound dye molecule are equal to 3.74 and 7.46 for compounds **1a** and **1b**, respectively.

**Table 1** Absorption and fluorescence data for free and DNA bound dyes.[a]

| Dye | $\lambda_{abs}$/nm | | $\lambda_{fl}$/nm | | $I_{max}/I_0$ | $\varphi_{dye}^{free}$ / $\varphi_{dye}^{bound}$ | $K_b$/ mol$^{-1}$ dm$^3$ | $n$ (bp) |
|---|---|---|---|---|---|---|---|---|
| | free | bound | free | bound | | | | |
| | | | | | calf thymus DNA | | | |
| **1a** | 373 | 395 | 501 | 501 | 2.1 | 1.20/2.33 | $2.4\times10^{3\,b}$ | 3.74 |
| **1b** | 382 | 404 | 511 | 506 | 2.3 | 3.36/6.49 | $2.5\times10^{4\,b}$ | 7.46 |
| | | | | | poly(dG–dC)$_2$ | | | |
| **1b** | 382 | 396 | 515 | 512 | 4.7/10 (0.47) | 3.36/1.0 | 4.10$^c$ | – |
| | | | | | poly(dA–dT)$_2$ | | | |
| **1b** | 382 | 393 | 515 | 514 | 46.2/9.5 (4.9) | 3.36/14.4 | 4.10$^c$ | – |

[a] $\lambda_{abs}$ and $\lambda_{fl}$ are absorption and fluorescence emission maxima, respectively; $I_{max}/I_0$ is the change in fluorescence intensity; $\varphi_{dye}^{free}$ and $\varphi_{dye}^{bound}$ are fluorescence quantum yields for the free and bound dyes, respectively; $K_b$ is stability constant and $n$ is the size of binding site, both in aqueous BPE buffer. [b] Calculated using McGhee and von Hippel model.[18] [c] Calculated using SPECFIT program.[21]

The additional support for dye–DNA mode of interaction was found using circular dichroism (CD) tests. Complementary CD investigation demonstrated the development of a positive induced CD (ICD) signal for compounds **1a**,**b** in their range of absorption [Figures 2(*a*),(*b*)].

The CD titration spectra of calf thymus DNA by compound **1a** as well as by compound **1b** with constant DNA concentration of $1\times10^{-4}$ M, varying ligand-to-DNA ratios from 0.1 to 0.6, are shown in Figure 2. Due to achirality of the dye molecules, *i.e.* identity with their mirror images, they do not give themselves any signals in CD spectra, but when DNA is added, an induced circular dichroism signal appears.[23] The spectrum of dye **1a** demonstrates a CD signal with low intensity at ~381 nm [Figure 2(*a*)]. We propose, that the observed spectral changes originate from the interaction of compound **1a** through intercalation between DNA base pairs. The same mode of binding has been observed for 3-bromopropoxy substituted 4-styrylpyridinium dye.[15]

For compound **1b**, the addition of DNA led to the formation of a gradually increasing positive signal in the dye absorption region at 388 nm. The preference for the minor groove binding by compound **1b** is confirmed by its larger ICD signal compared with dye **1a**. In contrast to the intercalation site between base pairs, the minor groove of DNA provides more chirality to the environment, thus affording a larger ICD signal.

In summary, we have demonstrated, that the change of substituent at N-atom of pyridine in 4-styrylpyridinium dyes from *N*-methyl to *N*-(3-pyridiniopropyl) results in a switch of the binding mode for dye–DNA interactions from intercalation to minor groove binding. This may originate from interaction of the positively charged

‡ UV-VIS spectra were measured using a Cary 300 two-channel spectrophotometer (Varian, USA). Fluorescence spectra were measured at 20±1 °C on a Cary Eclipse spectrophotometer (Agilent, USA). Coumarin 6 dye in ethanol (quantum yield of fluorescence $\varphi_{fl}$ = 78.0%) and quinine in 1.0 N sulfuric acid ($\varphi_{fl}$ = 54.6%) were used as references for the quantum yield measurements.

CD spectra were obtained using a J-500C spectropolarimeter (JASCO, Japan). The solutions investigated were placed in an 1-cm rectangular cuvette and recording was carried out at 50 nm min$^{-1}$ with conventional sensitivity for different wavelength ranges.

3-(pyridiniopropyl) substituent and the 4-styrylpyridinium moiety in dye **1b** with suitable groups in DNA, whereas dye **1a** has only one pyridinium moiety for coordination with DNA. The results obtained can help in prediction of the binding mode as well as in a rational design of fluorescent ligands corresponding to the definite types of their interactions with DNA.

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*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.03.029.

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