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DNA Effect on the Photoisomerization of Naphthalenevinylpyridinium Derivatives

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The binding study, photoisomerization and spectral behavior of novel DNA interacting dyes, 1-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (**1**) and 2-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (**2**), are reported. Ligand-DNA interactions were investigated by UV-Vis absorption and circular dichroism measurements. The ligands have different binding characteristics, depending on the structure of the isomers. The nonplanar *cis* isomers have lower affinity to DNA. Photoisomerization experiments in the absence and the presence of DNA showed significant differences in the composition of resulting photostationary states (pss). The lower values of pss in the presence of DNA indicate that *trans* → *cis* isomerization of DNA-bound ligands is suppressed, which leads finally to *trans* isomer-rich pss. Moreover, the quantum yield of *trans* → *cis* photoisomerization (φ_{TC}) decreased dramatically.

Key words: DNA, naphthalenevinylpyridinium ligands, photoisomerization

The photophysical and photochemical properties of aza-derivatives of 1,2 diarylethylenes have received much attention, because (n, π^*) states introduced by nitrogen atom significantly affect the photochemical and photophysical properties of the compounds [1]. The fluorescent *trans* isomers have been studied more extensively and it was found that they generally follow the same photoisomerization mechanism, widely accepted for stilbenes. A singlet or triplet reaction mechanism depends strongly on the size and the nature of the aryl groups linked to the ethenic central bond [2,3]. For example, direct excitation of 2-styrylnaphthalene and naphthalenevinylpyridines does not produce triplet state, but isomerization proceeds through the singlet manifold [1]. On the contrary, *trans* 1-styrylnaphthalene exhibits a co-mixed singlet and triplet mechanism of photoisomerization. In nonpolar solvents the triplet mechanism prevails below 275 K and in polar solvents below 240 K. Above these temperatures the photoisomerization proceeds through the singlet mechanism [4]. Photoisomerization mechanism of *cis* 1,2-diarylethenes was extensively studied by Mazzucato and co-workers [2–5]. They found adiabatic *cis-trans* isomerization mechanism instead of the well-know classical pathway, which implies internal conversion to ground state from the perpendicular configuration. The efficiency of the adiabatic process depended on the nature of the aryl groups and the solvent polarity [5].

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The specific interactions of diarylethylenes have played important role in biological activities of enzymes. These compounds were successfully applied as inhibitors of choline acetyltransferase and photoisomerization was used to photoswitch enzyme activity [6–9]. On the other hand, many simple organic ligands have been used in anticancer and antiviral therapy. The most important feature of these ligands is their DNA binding affinity. One can assume that geometrical isomers should exhibit different DNA binding affinity. Therefore, photoisomerization could be exploited to tune binding affinity of the ligand to DNA. Bisvinylpiridiniumbenzene dyes have been reported to interact with DNA by both groove binding and intercalation, depending of the base-pair composition of DNA [10]. Interestingly, these compounds undergo *cis-trans* photoisomerization but the structural differences between isomers do not affect DNA-binding affinity significantly. These peculiarities have been explained in terms of the partial interaction of the ligand with DNA, thus, allowing the remaining part of the molecule to undergo photoisomerization [10].

To explore further the effect of photoisomerization on DNA binding properties of ligands, we report here the synthesis, DNA-binding studies and photoisomerization behavior of novel naphthalene derivatives, possessing only one isomerizable arm, 1-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (**1**) and 2-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (**2**).

EXPERIMENTAL

Materials: Preparation of 1-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (1): A mixture of 1.881 g (8 mmol) of N-methyl-4-picolinium iodide and 1.09 g ml (8 mmol) of 1-naphthaldehyde in 50 ml of ethanol was heated under reflux for 2 h with 1 ml of piperidine as a catalyst. After being cooled to room temperature, the precipitate was collected by filtration, washed with hot ethanol, and dried under reduced pressure. The crystalline product was dissolved in methanol and recrystallized from methanol and ethanol. Yield: 12.25%; m. p. 285°C. Found C, 58.04; H, 4.21; N, 3.76; I, 33.4%. Calc. for $C_{18}H_{16}NI$: C, 57.87; H, 4.29; N, 3.75; I, 34%. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 4.38 (3H, s, N^+Me), 7.62 (1H, d, naphthyl H-5), 7.66 (2H, dd, naphthyl H-6, 7), 7.69 (1H, m, naphthyl H-3), 8.04 (1H, d, naphthyl H-4), 8.075 (1H, d, J = 15.2, Hz, H-vinylic), 8.11 (1H, naphthyl H-8), 8.46 (2H, d, J = 7.14 Hz, pyridyl H-3, 5), 8.61 (1H, d, naphthyl H-2), 8.83 (1H, d, J = 16 Hz, H-vinylic), 8.94 (2H, d, J = 6.9 Hz, pyridyl H-2, 6).

Preparation of 2-[2-(N-methylpyridinium-4-yl)vinyl]naphthalene iodide (2): A mixture of 1.881 g (8 mmol) of N-methyl-4-picolinium iodide and 1.09 g ml (8 mmol) of 2- naphthaldehyde in 50 ml of ethanol was heated under reflux for 4 h with 1 ml of piperidine as a catalyst. After being cooled to room temperature, the crystalline was collected by filtration, washed with hot ethanol, and finally recrystallized from methanol. Yield: 23.74%; m.p. 169°C. Found C, 57.95; H, 4.25; N, 3.65; I, 33.7%. Calc. for $C_{18}H_{16}NI$: C, 57.87; H, 4.29; N, 3.75; I, 34%. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 4.28 (3H, s, N^+Me), 7.61 (2H, dd, naphthyl H-6, 7), 7.68 (2H, d, J = 16.48 Hz, H-vinylic), 7.99 (1H, d, naphthyl H-5), 8.0 (1H, d, naphthyl H-8), 8.02 (1H, d, naphthyl H-4), 8.05 (1H, naphthyl H-3), 8.2 (1H, d, J = 16.48 Hz, H-vinylic), 8.23 (1H, d, naphthyl H-1), 8.28 (2H, d, J = 6.9 Hz, pyridyl H-3, 5), 8.9 (2H, d, J = 7.14 Hz, pyridyl H-2, 6).

Calf thymus DNA (CT-DNA), $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ were purchased from Sigma, and were used without further purification. All the experiments were carried out in HEPES buffer (10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, pH = 7.2). Sodium chloride was added to obtain the desired salt concentration. A Milli-Q filtered water (Millipore Co.) was used throughout.

Measurements: ^{1}H NMR spectra were recorded on a Varian spectrometer operating at 300 MHz, using tetramethylsilane (TMS) as an internal standard. Absorption spectra were recorded with a Specord M40 (Jena Germany). The fluorescence measurements were carried out using Perkin–Elmer MPF-3 spectrofluorimeter with excitation and emission bandwidths of 12 and 16 nm, respectively. Cell compartments were thermostated at 25°C. All measurements were carried out using a 10 mm quartz cell. The circular dichroizm spectra were recorded in the 220–700 nm spectral range on a Jasco J-810 spectropolarimeter.

Spectrophotometric titration. A titration consisted of successive replacements of small amounts (2–4 μl) of a 10 μM solution of dye in a buffer (containing 25 mM NaCl) by the same volume of concentrated DNA solution containing a buffer and a dye, followed by stirring and after achieved an equilibrium, recording of the spectrum. The binding constants were calculated on the basis of spectrophotometric titration's results.

Photoisomerization experiments. A dye solution (10 μM) in HEPES buffer was irradiated in the thermostated (25°C) cell compartment of a Shimadzu RF5000 spectrofluorimeter equipped with a 150 W Xe lamp at a selected wavelength. Nitrogen was purged through solution during irradiation and absorption spectra were recorded at desired time intervals. *Trans* isomers were irradiated at 410 nm and *cis* isomers at 320 nm. Photoisomerization experiments were carried out in the absence and the presence of CT-DNA (2 mM).

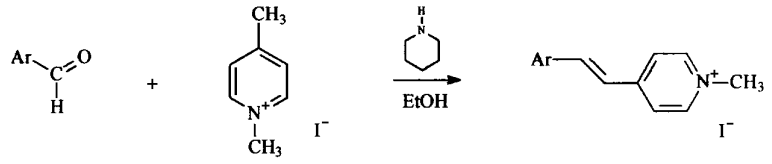
Viscosity titration. Titrations were carried out with a PC-controlled automatic system equipped with a capillary Ubbelohde-type viscometer, automatic pump/stop watch unit, and thermostated water bath at 30± 0.1°C. Small amounts (5 μl) of dye solution (5 mM) were added to a 3 ml DNA sample (about 0.2 mM) by means of a microsyrine without removing the solution from the viscometer. Measurements were repeated after each dye addition until reproducible flow times were observed. The relative viscosity ratios of the DNA-dye complex and the DNA were calculated using the expression $(\eta/\eta_0) = (t - t_0)/(t_{DNA} - t_0)$, where t_0 is the flow time of the buffer; t and t_{DNA} are the flow times of the DNA sample in the presence and in the absence of the dye, respectively.

RESULTS AND DISCUSSION

Synthesis and spectral properties: The *trans* isomers of naphthalene derivatives **1** and **2** were obtained using the aldol condensation, as shown in Figure 1. The ligand identity was evidenced by UV-Vis and ^{1}H NMR spectroscopies (the coupling constants of vinyl protons were 15.6 Hz for **1** and 16.5 Hz for **2**, characteristic for derivatives of *trans*-stilbene).

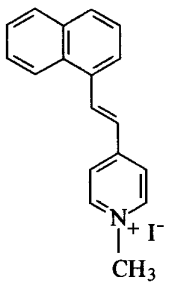
The corresponding *cis* isomers were isolated from a mixture of isomers. The mixture, which was obtained upon an exposure of *trans* isomer to visible light, was separated on HPLC ODS column using acetonitrile/water mixture (1:1) with 100 mM ammonium acetate (pH = 6) as a mobile phase. The irradiation of *trans* isomer gave for both ligands only two components: *cis* and *trans* isomers. Their spectral characteristics are collected in Table 1. All ligands show strong absorption band between 220–260 nm and a broad long wavelength band in the visible region, corresponding to a charge transfer (CT) transition from naphthalene unit to the pyridinium ring.

Absorption spectra of naphthalenevinylpyridinium derivatives are generally similar to those reported for stilbenes. However, absorption bands for naphthalenevinylpyridinium ligands are shifted to longer wavelengths. For example, the second band for *trans* stilbene occurs at 294 nm [11], that for **1** is observed at 380 nm. *Cis* and *trans* isomers show isosbestic points (λ_{iso}) at 294 and 279 nm for **1** and **2**, respectively. The *trans* isomers exhibit an emission at 520 nm for **1** and 510 nm for **2**, while *cis* conformers are not fluorescent.

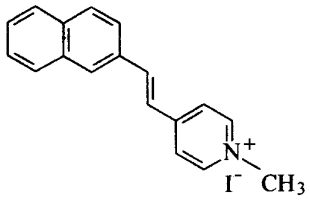


where Ar is:

1-naphthyl



2-naphthyl



1 **2**

Figure 1.

Table 1. Spectral properties of naphthalenevinylpyridinium ligands.

Ligand	λ_{max} [nm] ($\varepsilon \times 10^{-4}$/M^{-1} dm^3 cm^{-1})	λ_{iso} [nm] ($\varepsilon \times 10^{-4}$/M^{-1} dm^3 cm^{-1})	Emission λ_{max} [nm]
trans **1**	380 (2.44)	294 (1.27)	520
cis **1**	360 (0.69)	294 (1.27)	–
trans **2**	354 (3.90)	279 (1.46)	510
cis **2**	340 (1.19)	279 (1.46)	–

Using semiempirical PM3 method (CAChe, Fujitsu), the optimized equilibrium geometries of all isomers were calculated, and shown in Figure 2. There are no significant differences in the geometries of particular conformations between ligands **1** and **2**.

The *trans* geometries are in good agreement with the earlier published structures [6]. The computed values of dihedral angles between naphthalene-ethylene planes and pyridine-ethylene planes are –0.62 and –179.72° for *trans* **1** (0.06 and –179.9° for *trans* **2**). These results indicated that the *trans* structures at global minima are planar.

In *cis* conformations the pyridinium ring and naphthalene moiety are not coplanar with the ethylene bond (Fig. 2, Tab. 2). The dihedral angles between naphthalene-ethylene and pyridine-ethylene are 101.77 and –32.64° for cis **1** (112 and –26.2° for *cis* **2**). In all coplanar conformations the *trans*-ethylene bridge allows an extended conju-

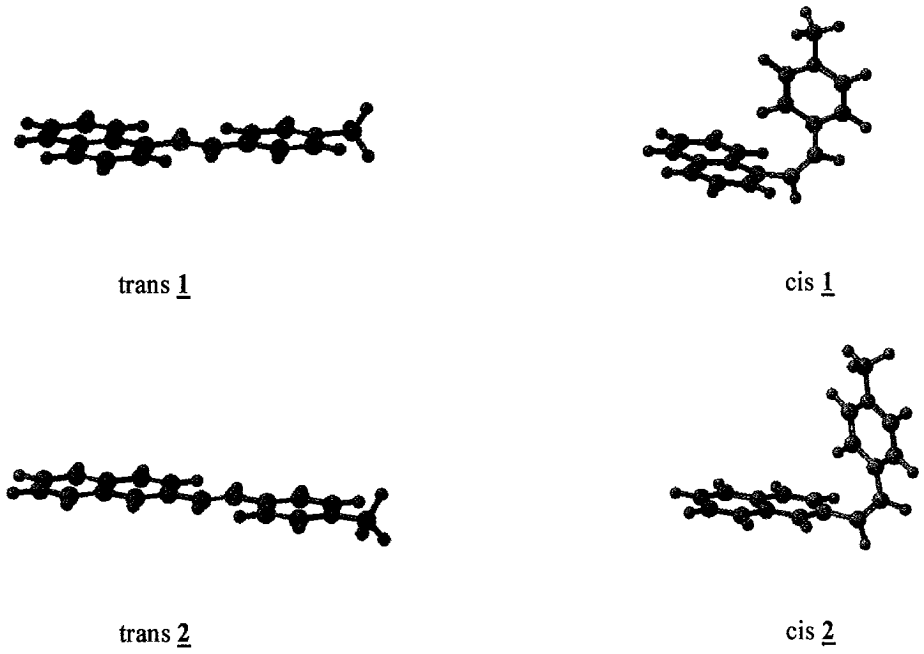
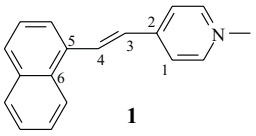
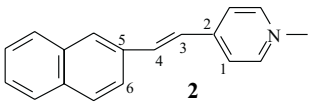


Figure 2. CAChe-generated optimized structures of *trans* and *cis* isomers of ligands **1** and **2**.

gation of π-system. This explains the stability of these structures in comparison with the no planar ones. *Trans* configurations are thermodynamically more stable than *cis* geometries; the energy differences are 5.31 and 4.94 kcal/mol for **1** and **2**, respectively.

Table 2. Geometries of the naphthalenevinylpyridinium derivatives in *trans* and *cis* conformations optimized by the semiempirical PM3 calculations (CAChe).





	R[1]/[Å]			v[2] [°]			E[3]
	2-3	3-4	4-5	1-2-3-4	2-3-4-5	3-4-5-6	[kcal mol^{-1}]
trans **1**	1.43	1.36	1.44	–0.62	180	–179.72	231.74
cis **1**	1.45	1.34	1.46	–32.64	0	101.77	237.05
trans **2**	1.43	1.35	1.44	0.06	180	–179.9	231.09
cis **2**	1.45	1.34	1.46	–26.2	–3.6	112	236.03

[1]interatomic distance; [2]dihedral angle; [3]optimized potential energy.

DNA binding properties. Ligand DNA interactions were investigated by spectrophotometric titration (Fig. 3). The *trans* isomers exhibited a similar tendency in spectral changes upon binding to CT-DNA. Red shifts of visible absorption bands and

quite uniform absorbance changes with large hypochromicity are observed for both ligands (Fig. 3a, c). These changes indicate a strong stacking between dyes and DNA base planes, thus, suggesting an intercalating binding mode. In the case of *cis* isomers the observed spectral changes are distinctly different. The hypochromicity is less pronounced, what can indicate a lower binding affinity to DNA.

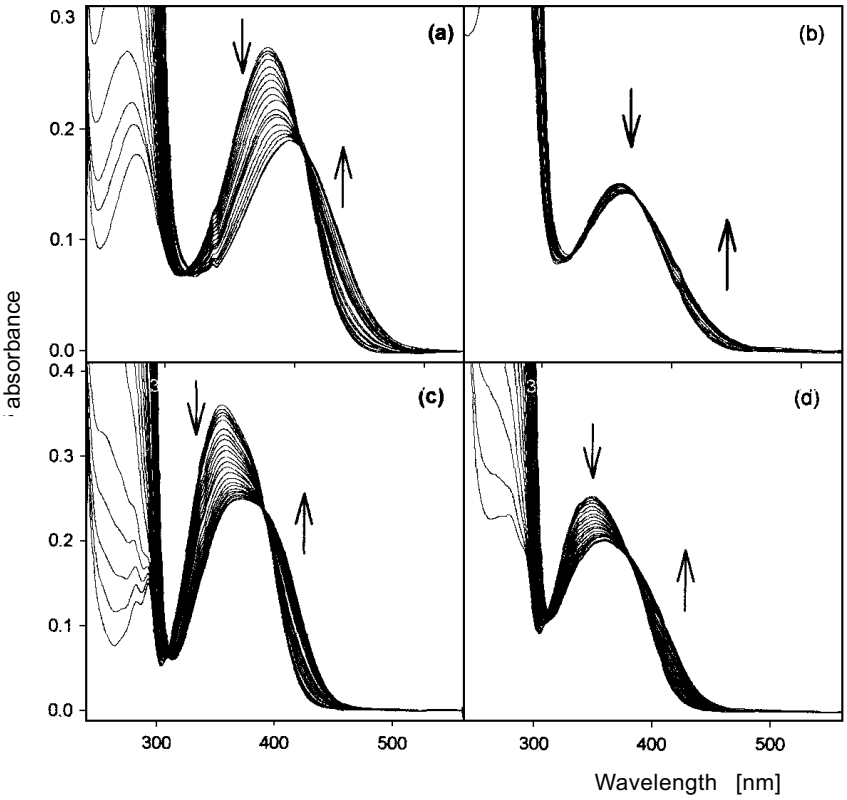


Figure 3. Spectrophotometric titration of naphthalenevinylpyridinium derivatives with CT-DNA. Conditions: [*trans*] = 10 μM, [*cis*] = 20 μM, [CT-DNA] = 0–1250 μM, [NaCl] = 25 mM, HEPES buffer (10 mM, pH 7.2); (a) *trans* **1**/CT-DNA, (b) *cis* **1**/CT-DNA, (c) *trans* **2**/CT-DNA, (d) *cis* **2**/CT-DNA.

Binding isotherm were constructed and analyzed according to the Scatchard model [12] characterized by:

$$K = \frac{r}{(n-r)[L]}$$

where: K – the binding constant, n – the number of binding sites per nucleotide, $[L]$ – concentration of free ligand, r = C/N, C – concentration of the complex, N – concentration of DNA (in base). The calculated binding parameters obtained in the presence

of 25 mM NaCl are given in Table 3. Both *cis* and *trans* conformations of **1** exhibit a greater binding affinity to DNA, compared to the complexes of **2**/CT-DNA. Significant differences in the binding constants are observed for *cis* conformations. The *cis* isomers exhibit 2-times lower binding constants: 7.8×10^4 for *trans* **1** and 4.5×10^4 for *cis* **1** (3.8×10^4 for *trans* **2** and 2.2×10^4 for *cis* **2**). Nonplanar structures of *cis* conformations disturb the effective insertion of naphthyl planes between DNA base pairs and ligands show a lower binding affinity to DNA.

Table 3. Parameters for the binding of ligands to CT-DNA calculated according to Scatchard model (K – binding constant, n – the number of binding sites per DNA base). Conditions: 10 mM HEPES buffer, 25 mM NaCl.

Ligand	λ_{max} [nm] free	λ_{max} [nm] bound	$K\times10^4$ [M^{-1}]	n
trans **1**	380	398	7.8	0.2
cis **1**	360	367	4.5	0.2
trans **2**	354	373	3.8	0.2
cis **2**	340	358	2.2	0.2

The binding mode was characterized by recording circular dichroism spectra (CD). Free ligands exhibit no CD activity. However, upon binding to DNA, the induced CD spectrum (ICD) of the ligand may appear, and its characteristics reflect the binding mode. Figure 4 shows the CD spectra of *trans* **2** complexes with synthetic polynucleotides, $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ in the presence of 5 mM NaCl. The spectral range below 310 nm contains CD bands of DNA, and is not discussed here. The dramatic difference between complexes with AT and GC polymers is evident in the diagnostic region, where the ligands absorb (310–450 nm). The strong positive ICD signal observed in the presence of $[poly(dA\text{-}dT)]_2$ was attributed to a groove-binding process. The ICD signal at 380 nm increases along with the concentration of DNA, and the shape of the spectra remains the same at low and high P/D ratios (DNA to ligand concentration).

Interestingly, in the presence of higher concentration of NaCl, the ICD signal disappeared for *trans* **2**/$[poly(dA\text{-}dT)]_2$. This indicates that groove binding mode becomes less important at higher NaCl and ligand **2** probably interacts with DNA by intercalation.

The complexes of *trans* **1** with $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ did not show positive signal in CD spectra. The lack of ICD in the case of ligand **1**/DNA complexes suggests intercalation binding mode for both AT and GC sequences.

To prove further the validity of the assigned type of binding mode, viscometric titrations were carried out using AT and GC polymers. The increase in the viscosity of the solution reflects the increase in the DNA contour length upon ligand binding, and is regarded as evidence of intercalation [13]. Titration of $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ with both ligands produced a substantial increase in the specific vi-

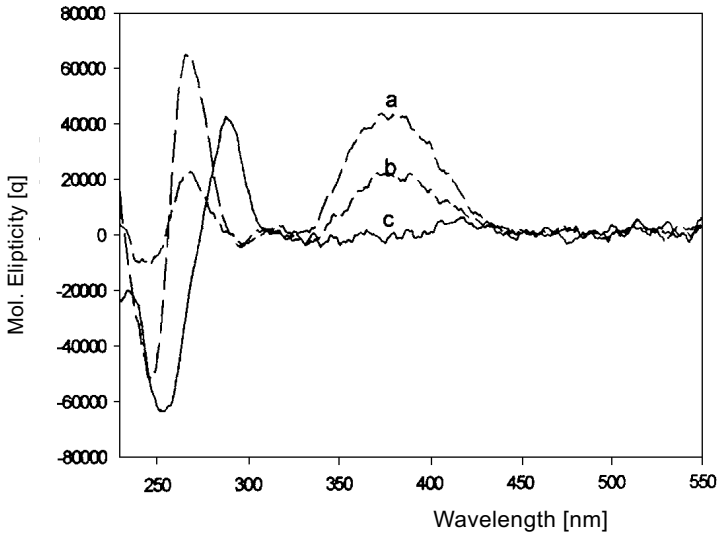


Figure 4. Circular dichroism spectra of **2**/[poly(dA-dT)]$_2$ complex (broken line: a, b), and **2**/[poly(dG-dC)]2 complex (solid line: c). Conditions: [DNA] = 25 μM (b), 60 μM (c), 95 μM (a), [dye] = 25 μM, 10 mM HEPES buffer, 5 mM NaCl.

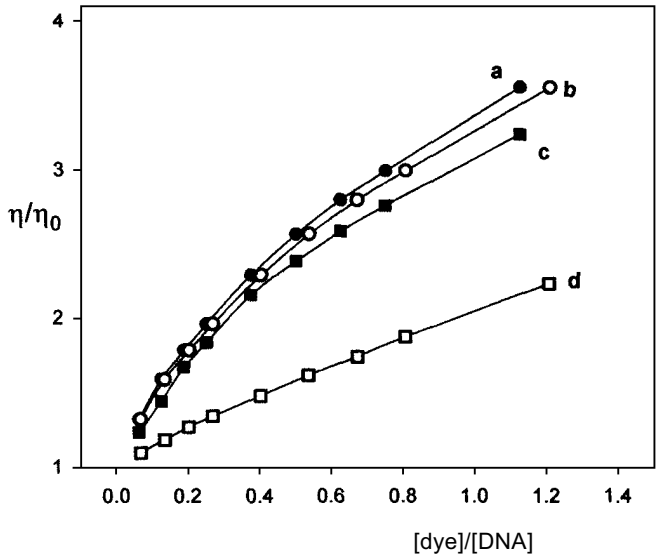


Figure 5. Viscometric titration of [poly(dA-dT)]$_2$ with **1** (a) and **2** (c), and [poly(dG-dC)]$_2$ with **1** (b) and **2** (d). Conditions: [DNA] = 0.2 mM, 10 mM HEPES (pH 7.2), temp = 30°C.

scosity, as illustrated in Figure 5. The gradual increase in the specific viscosity confirms the ability of the ligands to bind by intercalation.

Photoisomerization. The process of photoisomerization was monitored by recording absorbance changes in UV-VIS spectra. A sample solution of ligand in the absence and the presence of CT-DNA were irradiated at selected wavelength: *trans* isomers at 410 nm and *cis* isomers at 320 nm. Figure 6 shows the *trans* → *cis* isomerization of free and DNA-bound ligands. Upon exposure to visible light, absorption spectra of aqueous solution of dyes (Fig. 6a, c) gradually change, showing a blue shift of the long-wavelength absorption band and a significant hypochromicity. During *cis* → *trans* isomerization, the spectral changes occurred in opposite direction (data not shown). In both cases, the clear isosbestic points suggest only a two-component system. Remarkable hypochromicity, observed during irradiation, suggests an efficient conversion of *trans* isomer into *cis* conformer, possessing a much lower absorptivity.

On the other hand, in the presence of DNA, the longer exposure time of irradiation and modest absorbance changes (Fig, 6b, d) suggest a less efficient isomerization and a lower content of *cis* isomer at the photostationary state (pss).

The mechanism of photoisomerization has been extensively investigated for many stilbenes, in particular for the *trans* isomers. The isomerization proceeds in the singlet manifold by an excited perpendicular state (p^{1*}), from which an internal conversion to the ground state takes place. The deactivation of p^{1*} involving completion of 180° rotation leads to *cis* isomer, but rotation in opposite direction produces the *trans* isomer [14].

The studies of solvent effect on the quantum yields showed, that φ_{TC} depends on solvent polarity [14,15]. The pyridine derivatives possess a lower φ_{TC} in polar solvent, for example: 1-pyridinevinylnaphthalene the quantum yield amounts 0.58 [14] in nonpolar solvent, but in polar solvent decreases to 0.51 [15] (we obtained comparable values).

Quantum yields of isomerization were calculated using the method of Gauglitz [16] and the results of calculations are collected in Table 4. The different values of pss obtained for systems, where isomerization started from *cis* or *trans* isomers, reflect different wavelengths applied for irradiation. Isomeric composition of photostationary state (pss) depends on irradiation wavelength according to:

$$\varphi_{TC} \times \varepsilon_T \times c_T = \varphi_{TC} \times \varepsilon_C \times c_C; \qquad \text{pss} = \frac{c_C}{c_T} = \frac{\varphi_{TC} \times \varepsilon_T}{\varphi_{CT} \times \varepsilon_C}$$

Molar absorption coefficients (Tab. 4) depend on wavelength and pss values obtained reflect these differences. However, the ratio of $\varphi_{TC}/\varphi_{CT}$ does not depend on irradiation wavelength and the obtained values of $\varphi_{TC}/\varphi_{CT}$ should be the same, regardless which isomer has been irradiated. Indeed, the experimental values of $\varphi_{TC}/\varphi_{CT}$ are comparable (within experimental error) for *cis* and *trans* isomers. Photoisomerization experiments in the absence and the presence of DNA showed significant differ-

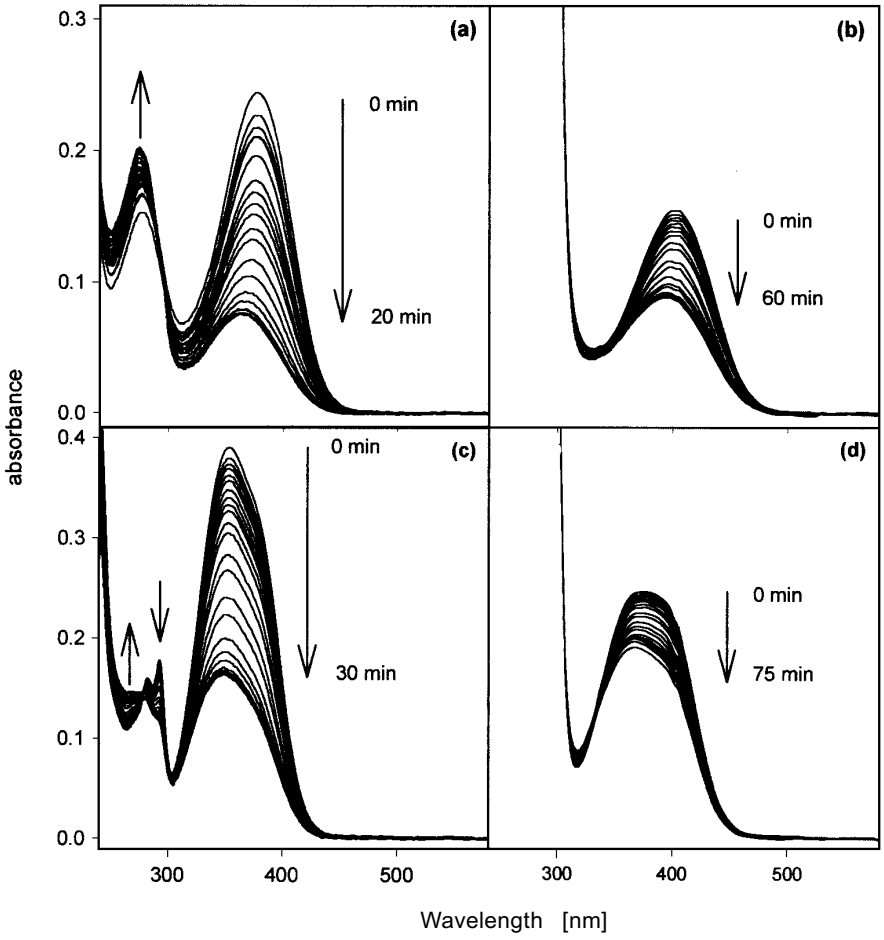


Figure 6. Photoisomerization of *trans* **1** and *trans* **2** in the absence and the presence of CT-DNA: (a) free **1**, (b) bound **1**, (c) free **2**, (d) bound **2**. Ligands irradiated at 410 nm. Conditions: [dye] = 10 μM, [NaCl] = 17 mM, [HEPES] = 10 mM, pH 7.2, [CT-DNA] = 2 mM.

ences in the resulting photostationary states. The lower values of pss in the presence of DNA indicate that *trans* → *cis* isomerization of DNA-bound ligands leads finally to *trans* isomer-rich pss. Moreover, the quantum yields of *trans* → *cis* photoisomerization decrease significantly. The *trans* isomers, which have planar structure, are stronger bound to DNA and photoisomerization occurs less efficiently.

Table 4. Photoisomerization data of naphthalenevinylpyridinium derivatives: pss (photostationary state composition), φ_{TC}, φ_{CT} (quantum yield of *trans-cis* and *cis-trans* photoisomerization).

Irradiated system	λ_{irr} [nm]	$\varepsilon \times 10^{-4}$ $M^{-1}\,dm^3\,cm^{-1}$	$pss\left(\frac{c_C}{c_T}\right)$	$\frac{\varphi_{TC}}{\varphi_{CT}}$	φ_{TC}	φ_{CT}
free *trans* **1**	410	1.30	7.30	1.93	0.60	0.31
free *cis* **1**	320	0.40	3.50	2.15	0.58	0.27
trans **1** bound to CT-DNA	410	2.17	1.45	0.30	0.26	0.87
cis **1** bound to CT-DNA	320	0.37	0.90	0.28	0.24	0.89
free *trans* **2**	410	0.78	4.55	1.14	0.49	0.43
free *cis* **2**	320	0.98	1.71	1.21	0.58	0.48
trans **2** bound to CT-DNA	410	2.23	0.70	0.21	0.12	0.58
cis **2** bound to CT-DNA	320	0.79	0.60	0.21	0.15	0.70

The presented results showed that the ligands have different binding characteristics, depending on the structure of the isomers. The nonplanar *cis* isomers have a lower affinity to DNA. Moreover, DNA influences the photoisomerization of naphthalenevinylpyridine derivatives, giving different parameters of isomerization.

Further investigations, including identification of possible products of photoisomerization in the presence of DNA and upon prolonged irradiation are in progress.

Acknowledgments

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