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Photobehaviour of some 1-heteroaryl-2-(1-methylpyridinium-2-yl)ethene iodides (free and complexed with DNA)

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Abstract

The fluorescence emission and the photoreactivity of three *trans*-1-heteroaryl-2-(1-methylpyridinium-2-yl)ethene iodides, where the heteroaryl group is thiophen-2-yl, 5-bromothiophen-2-yl or 1-methylpyrrol-2-yl, have been investigated in water and acetonitrile (MeCN). The main relaxation pathway of the lowest excited singlet states of these compounds leads to *trans* → *cis* photoisomerization with high quantum yields, particularly for the two thiophen derivatives. The intervention of a thermal isomerization, important only above room temperature, was observed in acetonitrile. The yield of the radiative deactivation is very small and becomes substantial in rigid matrices at low temperature only. Preliminary experiments on the binding of these pyridinium salts to DNA and on its effect on the photoreactivity showed a modest binding affinity to DNA with formation of complexes which did not affect significantly the photoisomerization process.

Keywords: Diarylethenes; Photoisomerization; Push–pull compounds; Interactions with DNA

1. Introduction

Diarylethenes, where the aryl groups have different donor/acceptor electronic properties, may be of interest for potential applications in optoelectronics. The intramolecular charge transfer (CT) character of compounds, where a π-excessive pentatomic group acts as a donor and a π-deficient pyridinium group acts as acceptor, has been described and found to increase on passing from thiophen to pyrrole derivatives [1]. Moreover, these compounds can interact with DNA [2] leading to complexes which can affect the cell metabolism being, thus of interest as candidates for the therapy of human pathologies [3,4].

The three *trans*-1-heteroaryl-2-(1-methylpyridinium-2-yl)ethene iodides here investigated (Scheme 1), where the heteroaryl group is thiophen-2-yl, 5-bromothiophen-2-yl or 1-methylpyrrol-2-yl, were previously prepared and the synthesis and spectroscopic characterization as well as preliminary in vitro antitumor tests (the latter for compound **3** only) have been reported [4].

In the present work, the results of a photophysical and photochemical investigation of the three compounds in two solvents are reported. It was found that under UV irradiation, they undergo *trans* → *cis* photoisomerization with high quantum yields, particularly for the two thiophen derivatives, while the yield of the radiative deactivation is very small and becomes substantial in rigid low temperature matrices only.

When styrylpyridinium ligands are organized on the DNA, their photoisomerization quantum yield and photostationary state (pss) composition could be significantly modified because of the different binding affinity of DNA towards the *trans* or *cis* isomer [2]. A preliminary study of the interaction between DNA and the three pyridinium salts investigated here, showed a modest binding affinity with formation of complexes which did not affect significantly the photoreactivity.

2. Experimental

The three compounds investigated have been synthesized at the Catania laboratory for a previous work [4]. Their photo-

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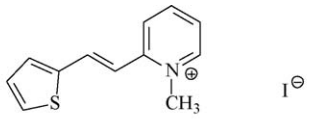
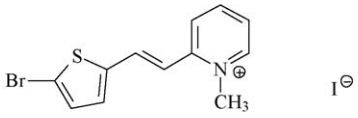
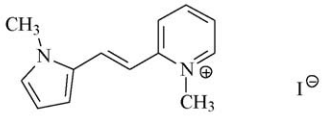


trans-1-(thiophen-2-yl)-2-(1-methylpyridinium-2-yl)ethene iodide (**1**)



trans-1-(5-bromothiophen-2-yl)-2-(1-methylpyridinium-2-yl)ethene iodide (**2**)



trans-1-(1-methylpyrrol-2-yl)-2-(1-methylpyridinium-2-yl)ethene iodide (**3**)

Scheme 1.

physical and photochemical behaviour was studied in water and acetonitrile (MeCN). Some optical measurements at liquid nitrogen temperature were also carried out in 5/5/2 (v/v/v) diethyl ether/isopentane/ethanol (EPA).

A Perkin-Elmer Lambda 800 spectrophotometer was used for the absorption measurements. The fluorescence spectra were measured by a Spex Fluorolog-2 F112AI spectrofluorimeter. Dilute solutions (absorbance < 0.1 at the excitation wavelength, λ_{exc}) were used for fluorimetric measurements. The emission quantum yields were determined at λ_{exc} corresponding to the maximum of the first absorption band (λ_{max}). 9,10-Diphenylanthracene in cyclohexane was used as fluorimetric standard ($\phi_F = 0.90$ in de-aerated solvent [5]). For photochemical measurements (potassium ferrioxalate in water as actinometer), a 150 W high pressure xenon lamp coupled with a monochromator was used. The photoreaction (solute concentrations $\sim 10^{-4}$ M) was monitored by HPLC using a Waters apparatus equipped with analytical Simmetry C18 (4.6 mm × 250 mm; 5 μm) or Prontosil 200-3-C30 (4.6 mm × 250 mm; 3 μm) columns and UV detector. Water/MeCN mixtures were used as eluents. The monitoring wavelength was at the isosbestic point. The conversion percentage was held at below 8% to avoid the competition from the back photoreaction. Sensitized experiments were carried out using biacetyl in benzene as triplet donor.

The compounds were characterized by ^{1}H NMR spectra in deuterated methanol using a Bruker AC 400 spectrometer and TMS as reference.

Fluorescence lifetimes of these low-emitting compounds were only measured at low temperature by an Edinburgh Instrument 199S spectrofluorimeter, using the single photon counting method and a Spex Fluorolog-τ2 apparatus, based on the phase modulation technique.

The triplet state was investigated by nanosecond laser flash photolysis. For direct and benzophenone-sensitized measurements ($\lambda_{exc} = 355$ nm), a Continuum Surelite II Nd:YAG laser was used.

All measurements were carried out in de-aerated solutions by purging with nitrogen. The parameters reported in the Tables are averages of at least three independent experiments with mean deviation of ca. 15% for fluorescence quantum yields and ca. 10% for the fluorescence lifetimes and photoisomerization quantum yields. The uncertainty increases to 20% for the sensitized yield of **3** ($\lambda_{exc} = 280$ nm) because the compound absorbs in the same region of the sensitizer, thus requiring a correction to the measured yield. Further details on spectrophotometric, fluorimetric and laser flash photolysis measurements are reported elsewhere [6].

Some theoretical calculations of the electronic spectra, conformational equilibria and heats of formation for the **1** and **3** cations (excluding the Br-substituted compound **2**) were carried out using the HyperChem computational package (Version 6.1). The calculated electronic spectra (transition energy and oscillator strength) were obtained by ZINDO/S using optimized geometries (according to PM3 method). Calculations of the configuration interaction included 81 (9 × 9) single excited configurations. The overlap weighting factor was set $f_{p\pi} = 0.73$ to obtain the best correlation with the experimental electronic spectra.

The tests on the binding of the ethenic compounds to DNA were carried out by successive additions of an aqueous buffered (ETN, 0.01 M NaCl, pH 7.0) solution of salmon DNA to an initial 2×10^{-5} M concentration of ligand in the same buffer. The spectrum of the Z-DNA complex was obtained after addition of a suitable DNA concentration to a Z-rich photostationary (pss) mixture of the free isomers and subtraction of the E-DNA contribution.

3. Results and discussion

3.1. Spectral behaviour

Fig. 1 and Table 1 show the absorption and emission spectra of compounds **1**–**3** in MeCN. The bathochromic electronic transition of the E isomer is a bell-shaped and intense absorption band centred at 368 nm, 373 nm and 422 nm for **1**, **2** and **3**,

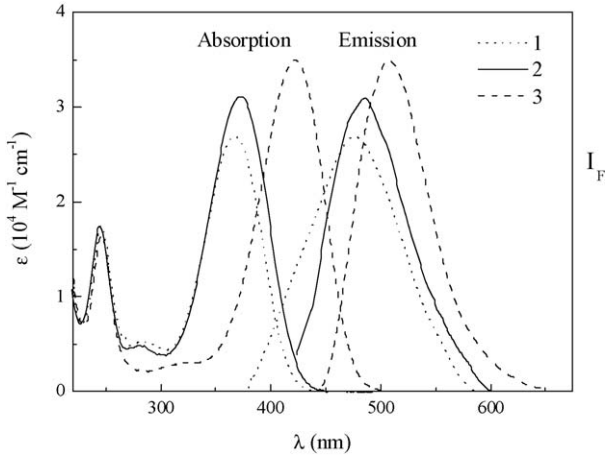


Fig. 1. Absorption and emission spectra of the E isomers of **1**–**3** in MeCN at room temperature.

Table 1
Spectral and photophysical parameters of the E isomers of **1**–**3** in MeCN and water at room temperature

Compound	Solvent	λ_{abs}^{max}(nm)	ε_{max} ($M^{-1}cm^{-1}$)	λ_{F}^{max}(nm)	ϕ_F ($\times10^4$)
1	MeCN	368	26900	480	1.5
	H_2O	365	26000	480	1.2
2	MeCN	373	31000	485	1.5
	H_2O	373	27000	485	1.3
3	MeCN	422	35000	510	7.0
	H_2O	412	27700	510	4.0

respectively. A much less intense absorption around 270–320 nm was also observed. The narrow band at 245 nm (that shifts to 225 nm in water) corresponds to the characteristic iodide absorption. The shapes of the emission spectra is quite similar to those of the absorption spectra and show large Stokes shifts between the two maxima indicating intramolecular charge separation.

Irradiation of **1**–**3** caused a decrease of the bathochromic band and an increase of a new band (overlapped to the second band of the E compounds and assigned to the Z isomers) in the 260–300 nm region, as shown in Fig. 2. The isosbestic points confirmed the occurrence of a clean photoreaction of a two-component system. Reversibility of the E–Z photoisomerization was checked by HPLC measurements. The qualitative spectra of the Z stereo-isomers in the eluent mixture (water containing ~5% MeCN), normalized at the isosbestic points, are shown in Fig. 2 (dotted lines).

In the case of **1**, the structure was also confirmed by NMR measurements. The ^{1}H NMR spectrum of the starting E-**1** isomer in deuterated methanol (in agreement with that in deuterated DMSO previously reported [3]) showed two doublets at 7.30 ppm and 8.12 ppm with a coupling constant of 15.7 Hz, characteristic of ethenic hydrogens in E configuration. The spectrum carried out after prolonged irradiation of E-**1** showed the appearance of NMR peaks in agreement with a Z structure. In particular, the doublets at 6.66 ppm and 7.47 ppm, with a coupling constant of 12.5 Hz, indicated the presence of ethenic hydrogens in Z configuration. The residual E isomer produced weak peaks that allowed to estimate the photostationary state composition (see later).

The calculated spectra of the most stable conformers [7], showed as vertical bars in Fig. 2, are in reasonable agreement with the experimental data. The calculated orbitals of **3** showed that the nitrogen atom of the pyrrole group contributes more to LUMO than to HOMO. Therefore, a major stabilization of LUMO with respect to HOMO is induced in **3**, in agreement with the large bathochromic shift observed in the experimental absorption spectra.

Due to the rather strong electron withdrawing character of the pyridinium group with respect to the five-member heteroaromatic ring (electron donor) linked by the ethenyl group, these compounds behave as push–pull (donor–acceptor) systems [1,8], as confirmed by the solvatochromic spectral shift previously reported [4].

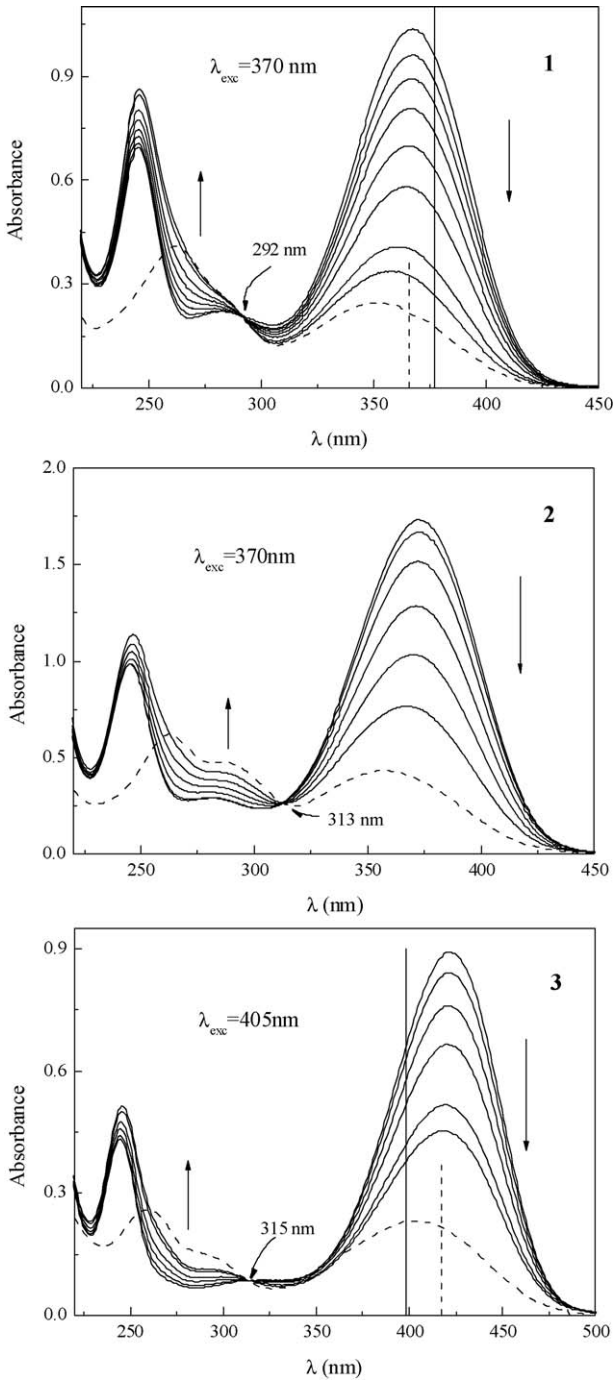


Fig. 2. Absorption spectra of **1**–**3** in MeCN as a function of the irradiation time. Dotted lines show the spectrum of the Z isomers in the HPLC eluent mixture (~5% MeCN in water) normalized at the isosbestic point. Bars indicate the calculated transitions for the first band of E (—) and Z (- - -) isomers of **1** and **3**.

The results of the theoretical calculations performed for **1** and **3** showed that the CT character of the first transition increases in the Z derivatives where the conformations are more distorted. This is, particularly evident for **3** where the computed spectrum of the Z isomer is shifted to the red with respect to the E isomer. The calculations probably overestimate the CT contribution considering that the experimental spectrum is slightly shifted in the opposite direction.

Table 2
Excitation and emission fluorescence spectra and photophysical parameters of the E isomers of **1**–**3** in EPA at 77 K

Compound	λ_{max}^{exc} (nm)	λ_{max}^{fluo} (nm)	ϕ_F	τ_F (ns)	k_F ($\times 10^8$ s^{-1})
1	385	450	0.24	2.3	1.0
2	388	456	0.23	2.1	1.1
3	450	468	0.32	2.1	1.5

The conformers of the E isomers shown in Scheme 1 are the most stable ones, having the smallest (calculated) formation enthalphy difference (ΔH_f^0). Rotation of the methylpyridinium group around the quasi-single bond implies an increase in ΔH_f^0 of 3–4 kcal mol^{-1}, due to the increased steric hindrance between the methyl group and the ethenic hydrogen, whereas rotation of the other heteroaromatic groups produces conformers with more similar ΔH_f^0 values (only 0.6–1 kcal mol^{-1} higher than the stable ones).

3.2. Photophysical and photochemical behaviour

The fluorescence parameters measured for the three compounds are shown in Table 1. Given the weak fluorescence yields measured in both water and MeCN, which prevented us from measuring the fluorescence lifetimes at room temperature, the emission parameters were measured in a rigid matrix of EPA at 77 K (Table 2). Even in these conditions, where the reactive relaxation is inhibited, the yields of the radiative relaxation remain rather lower than the unitary value indicating the intervention of substantial non-radiative deactivation (internal conversion to S_0 or intersystem crossing to T_1). The high value of the radiative rate constant indicates that fluorescence originates from an allowed state in agreement with the calculated high oscillator strenght for the first transition ($S_0 \rightarrow S_1$).

The E → Z photoisomerization quantum yields in MeCN are reported in Table 3. If one assumes a diabatic mechanism for the photoreaction (twisting from the E to the perpendicular configuration, P*, and then internal conversion to the P ground state and partitioning to E and Z isomers), as assumed for stilbene and several stilbene-like molecules [9], the high values measured for the three compounds, particularly for the thienyl derivatives, indicate that the main deactivation pathway of S_1 is the torsion around the double bond. The pss compositions ([Z]/[E]) in Table 3 correspond to isomer mixtures enriched in the Z isomer, as generally found for diarylethenes, due to the higher molar absorption coefficient of the E isomers.

Table 3
Photostationary state composition and direct and sensitized photoisomerization quantum yields of **1**–**3** in MeCN at room temperature

Compound	pss (λ_{exc} (nm))	$\phi_{E\rightarrow Z}$	$\phi_{Z\rightarrow E}$[a]	$\phi_{E\rightarrow Z}^{sens}$
1	4.4 (370)	0.58	0.66	0.15
2	2.8 (370)	0.50	0.77	0.21
3	2.4 (405)	0.37	0.47	0.14

[a] Calculated by the equilibrium composition.

The pss at a given λ_{exc} allowed the quantum yields of the back Z → E photoreaction to be derived from $[Z]/[E] = (\varepsilon_T/\varepsilon_C) \times (\phi_{E\rightarrow Z}/\phi_{Z\rightarrow E})$. The high values, thus obtained showed an high photoreactivity of the Z isomers, even higher than that of the E isomers.

Measurements by nanosecond laser flash photolysis did not show any sign of $T_1 \rightarrow T_n$ absorption at room temperature. The triplet state populated by sensitization from biacetyl in MeCN underwent isomerization to Z with reduced quantum yields probably because of a fast back (Z → E) isomerization. This finding points to the singlet state as the main responsible for the direct photoreaction.

In order to compare the photoreactivity of these compounds with that in the presence of DNA, the photoisomerization quantum yields were also measured in aqueous buffered (pH 7) medium (see later).

3.3. Thermal isomerization

Interestingly, the [Z]/[E] ratio at the pss in MeCN did not remain constant after removal of the irradiation source but decreased due to a thermal back reaction which enriched the isomer mixture in the E component. This behaviour was investigated in greater detail for **1** and **3**. The results obtained are summarized in Table 4.

A solution of the E isomer left in the dark for 2 days led to a stationary state (ss) composition ($[Z]/[E] \cong 0.04$ and 0.09 for **1** and **3**, respectively) that confirmed the presence of a thermal equilibrium between the two geometrical isomers, largely shifted towards the more stable E component with respect to the pss composition ($\Delta H_{Z\text{–}E} \cong 2$ kcal mol^{-1}). The temperature effect on the thermal steps was followed for **1** in the range 298–308 K leading to activation energies of 22 kcal mol^{-1} and 20 kcal mol^{-1} for the E → Z and Z → E reaction, respectively.

Similar experiments carried out in water did not show any sign of thermal equilibration probably because of higher activation energies in both directions, due to a further stabilisation of the ionic species in water.

3.4. Interaction with DNA

Some spectrophotometric measurements were carried out to test the formation of *E*-diarylethene–DNA complexes by adding DNA to a constant olefin concentration ($\sim 2 \times 10^{-5}$ M). All three

Table 4
Kinetic constants ($k(10^{-4}$ s^{-1})), stationary state ratios and enthalpy differences (ΔH(kcal mol^{-1})) for the thermal isomerization of **1** and **3** in MeCN at room temperature

Compound	T (K)	$k_{E\rightarrow Z}$	$k_{Z\rightarrow E}$	[Z]/[E]	ΔH[a]
1	298	1.5	0.3	0.037	2.0
	303	2.6	0.8		
	308	2.3	1.0		
3	313	0.6	0.021	0.087	1.5

[a] Calculated by the equilibrium composition.

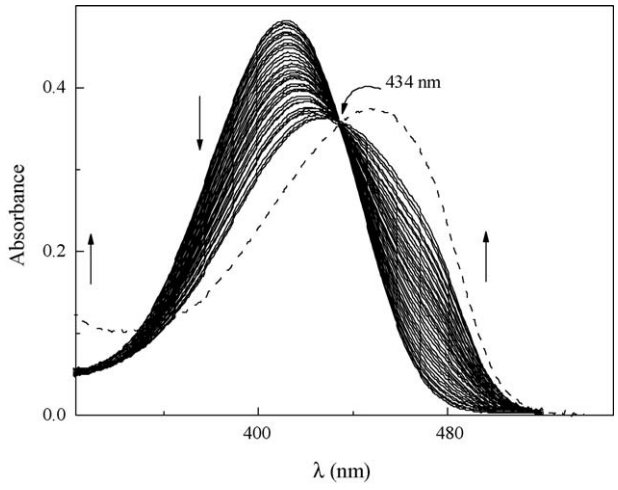


Fig. 3. Absorption spectra of E-**3** (corrected for dilution) recorded after successive addition of a DNA solution (in the range 2.4×10^{-6} to 3.7×10^{-4} M) to an initial olefin concentration of 2.5×10^{-5} M. The dashed curve is to the limit spectrum obtained at high DNA concentrations and assigned to the **3**–DNA complex.

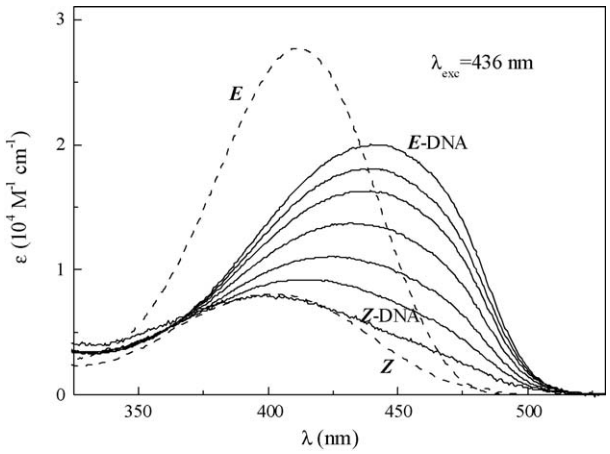


Fig. 4. Spectral evolution under irradiation of the E-**3**–DNA complex in buffered water. The figure shows also the spectrum of Z-**3**–DNA and those of the free E and Z isomers (dashed lines).

compounds showed sign of interactions with DNA as evidenced by the red-shift and the hypochromicity of the absorption maximum of the olefin.

For compounds **1** and **2**, the spectral shift was very small indicating formation of complexes with similar absorption spectrum. Much more evident spectral changes were found for **3**, as shown in Fig. 3, where the new band of the complex appears as a shoulder of the olefin spectrum. By increasing substantially the DNA concentrations, a final spectrum was obtained which probably corresponds to an equilibrium completely shifted towards the complexed form. It has to be noted that in all cases, total complexation required the addition of rather high DNA concentrations pointing to weak interactions.

A treatment of the experimental data using the equation of McGhee and von Hippel [10] allowed the complexation constants (K) to be estimated for the three compounds. The uncertainty in the fitting procedure of the present experiments (repeated at least three times, also varying the relative ligand–DNA ratio) is rather high, due to the modest changes in absorbance and additional errors associated to corrections for dilution. In any case, the K values, thus obtained (of the order of $(2–5) \times 10^3\ M^{-1}$) evidence the formation of ligand–DNA complexes but are probably too low for applications of these compounds in phototherapy (see, e.g. the K values recently obtained for analogous compounds, which are higher by one order of magnitude or more [2]).

The anti-proliferative activity of these compounds, tested against two tumor cell lines (breast and prostate carcinomas) following the method described elsewhere [3], has been reported to be rather weak but more important for **3**, in agreement with the present measurements.

It was interesting to investigate if and how the binding equilibria might affect the relaxation properties of the compounds investigated. The radiative deactivation was found practically unchanged for the complexes **1** and **2**, whereas the value for **3**–DNA increased significantly ($\phi_F = 3 \times 10^{-3}$). The irradiation of the pyridinium salts in the presence of DNA showed that photoisomerization takes place at a rate which is not much different from that of the free molecule. Fig. 4 shows a sequence of spectra of **3**–DNA recorded at increasing irradiation time. Even if the less planar Z isomer is expected to have a smaller binding affinity to DNA [2], the spectrum of the E–DNA complex moved towards that of a complexed Z isomer, with a final spectrum rather similar to that of the free molecule (slightly broadened towards the red). Table 5 collects the pss compositions and E → Z quantum yields of the complexed and free molecules in the aqueous medium. The pss composition at λ_{exc}

Table 5
Molar absorption coefficients ($M^{-1}\ cm^{-1}$) at λ_{max} (nm), photostationary state composition and photoisomerization quantum yields of the free and complexed **1**–**3** in buffered water at room temperature

Compound	$\varepsilon_E^{\lambda max}(\lambda_{max})$	$\varepsilon_Z^{\lambda max}(\lambda_{max})$	pss (λ_{exc})	$\phi_{E\rightarrow Z}$	$\phi_{Z\rightarrow E}$[a]
1	26000 (365)	7330 (351)	11.2 (366)	0.60	0.22
1–DNA	21750 (375)	5850 (351)	4.5 (372)	0.38	0.31
2	27000 (374)	7590 (357)	6.6 (366)	0.55	0.30
2–DNA	22300 (390)	6930 (359)	2.0 (372)	0.28	0.46
3	27720 (412)	8060 (402)	1.6 (366)	0.37	0.46
3–DNA	20500 (449)	7880 (400)	3.9 (436)	0.35	0.31

[a] Calculated by the equilibrium composition.

in the presence of DNA is again richer in the Z isomer, as found for the free compounds. The quantum yields are not much different from those of the free molecules: practically unchanged for **3** and slightly smaller for **1** and **2**. Even in the presence of DNA, the quantum yields calculated for the back Z → E photoreation were found rather high indicating an efficient two-ways photoisomerization.

In the diluted ligand solutions used in the present case ($\sim 10^{-5}$ mol dm^{-3}), irradiation gave only a mixture of Z and E isomers without formation of cyclobutane photodimers, which were often detected in styrylpyridinium salt photochemistry [2,11]. This suggests that groove binding is not implied in the interaction in our experimental conditions and that the photoisomerization occurs in intercalated molecules.

A more detailed study of the photochemistry of these DNA–pyridinium complexes, extended to a larger series of compounds, is planned.

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