Photophysics of aromatic thiourea derivatives and their complexes with anions. Fast and ultrafast spectroscopic investigations

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In order to collect detailed information on the interaction mechanism between fluorescent thiourea derivatives and anions, 9-[4-(trifluoromethyl)phenylthioureidomethyl]anthracene (1) and the corresponding 10-cyanoanthracene derivative (2) were synthesized and investigated in DMSO and MeCN by using absorption and emission steady state techniques, both in the absence and in the presence of different anions (AcO⁻, H₂PO₄⁻, HSO₄⁻, and Br⁻). A wide examination of the mechanism of anion recognition was also performed by time-resolved transient absorption spectroscopy, with nano- and femto-second time resolution. A complete picture of the excited state deactivation pathways of 1 and 2, where the main operative processes were fluorescence, intersystem crossing and internal conversion, was obtained. Even if steady-state measurements suggest that 1 and 2 selectively interact in the ground state with the anions $H_2PO_4^-$ and AcO⁻, time-resolved investigations demonstrate that the substrates are able to complex all the four anions. The photophysics of such complexes was fully characterized. The anions mainly modify the lifetime of the lowest excited singlet state and, especially in the cases of $H_2PO_4^-$ and AcO⁻, the efficiencies of fluorescence emission and triplet formation. In particular, no evidence was found of further deactivation processes such as photoinduced electron transfer, photodissociation. and photoionization.

Introduction

Over the last years various fluorescent sensors for anions have been synthesized,¹⁻⁴ but many of them are based on charged receptors⁵⁻¹¹ that present the limit to make easier the competition between the anion and the solvent for binding sites. More recently simple and efficient sensors have been developed by using charge neutral receptors.

Among the reported uncharged receptors of anions, the aliphatic and aromatic urea receptors have been widely investigated.¹²⁻²¹ Anion recognition occurs through hydrogen bonding interactions between the urea protons and the anion forming anionreceptor complexes. Gunnlaugsson et al. proposed fluorescent anion sensors, based on the "fluorophore-spacer-anion receptor" system, employing anthracene as fluorophore and substituted thioureas as receptor, bonded to the 9-position of the anthracene via a methylene spacer. Different isothiocyanates were used in order to monitor the acidity of the thiourea-based receptors, which influences the stability of the anion-receptor complexes, and the resulting affinity and selectivity. This class of sensors demonstrated an ideal recognition of the anion: only the fluorescence lifetime and quantum yield would be influenced upon ion detection. Therefore any further spectral changes, such as exciplex or excimer emission,²² were not observed.

The operating mechanism in these sensors, in competition with the fluorescent emission, has been suggested to be photoinduced electron transfer (PET).^{23,24} This hypothesis is supported considering that, upon anion recognition through hydrogen bonding, the oxidation potential of the thiourea receptor decreases, thus inducing a more efficient PET from the anion–receptor unit to the excited anthracene moiety. Anyway, no direct investigations have been yet performed to demonstrate the effective mechanism of action.

In this work we examined 9-[4-(trifluoromethyl)phenylthioureidomethyl]anthracene (1) and the corresponding 10-cyanoanthracene derivative (2) (Scheme 1). Compound 2 was simply prepared following the synthesis already reported for 1,¹⁴ mixing 9-(aminomethyl)anthracenes with the commercial 4-(trifluoromethyl)phenyl isothiocyanate in opportune experimental conditions. A first part of the study regarded the investigation of ground state and excited state, by absorption and emission measurements, both in the absence and the presence of different anions (AcO⁻, H₂PO₄⁻, HSO₄⁻, and Br⁻) used as tetrabuthylammonium salts, in DMSO and MeCN. An extensive photophysical study has already been reported on 1 in DMSO,^{12,14} so in this solvent only some measurements were carried out in order to compare the results with those obtained with 2 in the same experimental conditions. A wide examination of the mechanism of anion recognition, responsible for the fluorescence quenching, was performed by timeresolved spectroscopy, with nano- and femto-second time resolution. This study represents, to the best of our knowledge, one of the first attempts of an exhaustive investigation of

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Scheme 1 Molecular structures of 1 (X = H) and 2 (X = CN).

the mechanism that is at the base of fluorescent sensor working.

Experimental

Synthesis of 1 and 2

Compound **1** was synthesized in three steps, starting from 9-methylanthracene which is commercially available. Firstly, 9-(bromomethyl)anthracene was prepared by bromination of 9-methylanthracene with *N*-bromosuccinimide in carbon tetrachloride.²⁵ This product was refluxed with hexamethylenetetramine in anhydrous chloroform and the resulting product (9-(aminomethyl)antracene) was dissolved in anhydrous dichloromethane with 4-(trifluoromethyl)phenyl isothiocyanate to give **1**, 9-[4-(trifluoromethyl)phenyl thioureidomethyl]anthracene.¹⁴

The synthesis of 2 was carried out following the same procedure reported for 1. In this case the starting reactant, 10-methyl-9-cyanoanthracene, was prepared from 10-methyl-anthracene-9-carboxaldehyde as previously described.²⁶

10-(Aminomethyl)-9-cyanoanthacene: ¹H-NMR δ (200 MHz, CDCl₃): 8.51–8.43 (m, Ar–1H, Ar–8H, Ar–4H, Ar–5H), 7.78–7.64 (m, Ar–2H, Ar–3H, Ar–6H, Ar–7H), 4.89 (s, CH₂NH₂); **2** ¹H-NMR δ (400 MHz, CDCl₃): 9.78 (s, NH), 8.69 (d, Ar–4H, Ar–5H), 8.63 (s, NH), 8.43 (d, Ar–1H, Ar–8H), 7.97 (t, Ar–3H, Ar–6H), 7.86 (t, Ar–2H, Ar–7H), 7.79 (d, Ar–19H, Ar–20H), 7.65 (d, Ar–18H, Ar–21H), 5.84 (s, CH₂).

Photophysical measurements

Absorption spectra were recorded with a Perkin-Elmer Lambda 800 spectrophotometer. Fluorescence emission spectra were measured with a Fluorolog-2 (Spex, F112AI) spectrophoto-fluorimeter. Fluorescence quantum yields (experimental error of *ca.* 7%, from three independent experiments) were determined from the emission spectra of the samples recorded at an absorbance smaller than 0.1 at the excitation wavelength, to avoid self-absorption effects, by use of anthracene in ethanol as reference ($\phi_F = 0.22$).²⁷

Triplet formation quantum yields and lifetimes were measured by a nanosecond laser flash photolysis setup previously described (Nd:YAG Continuum, Surelite II, third harmonics, $\lambda_{exc} = 355$ nm, pulse width *ca*. 7 ns and energy \leq 1 mJ pulse⁻¹).^{28,29} First-order kinetics were observed for the decay of the lowest triplet state (T–T annihilation was prevented by the low excitation energy). The triplet lifetimes were measured at an absorbance of *ca*. 0.2; the concentration effect on triplet lifetime was not investigated. The transient spectra were obtained by monitoring the change of absorbance over the 300–900 nm range and averaging at least 10 decays at each wavelength. The experimental setup was calibrated by an optically matched solution of benzophenone ($\phi_T = 1$ and $\Delta \varepsilon_T = 6500 \text{ M}^{-1} \text{ cm}^{-1}$ at the corresponding absorption maximum) in MeCN.³⁰ Triplet–triplet molar absorption coefficients (ε_T), and then ϕ_T values, were determined by energy transfer from benzophenone in MeCN. The experimental errors on τ_T were estimated to be about $\pm 10\%$ and on ε_T and ϕ_T of about $\pm 15\%$. The quenching constants of the triplets by molecular oxygen were evaluated by the slope of the linear plots of triplet lifetime *vs.* different oxygen concentrations. All measurements were carried out at room temperature; the solutions were saturated by bubbling with nitrogen.

The transient behaviour on the ultrafast time scale was investigated using femtosecond pulses generated by an amplified titanium-sapphire laser system (Spectra Physics, Mountain View, CA), that produces 1 W pulses centred at 800 nm at a repetition rate of 1 kHz. Pump pulses centred at 400 nm were obtained by second harmonic generation in a 500 μm β-barium-borate crystal. In the transient absorption set up (Helios, Ultrafast Systems) the pump pulses were passed through a chopper which cut out every second pulse and collimated to the sample in a 2 mm quartz cuvette. Probe pulses for optical measurements were produced by passing a small portion of 800-nm light through an optical delay line (with a time window of 1600 ps) and focusing into a 2 mm thick Ti:Sapphire window to generate a white-light continuum in the 430-800 nm window. The white light was focused onto the sample and the chirp inside the sample cell was determined by measuring the laser-induced Kerr signal of the solvent. The temporal resolution of our time resolved spectroscopic technique is ~ 150 fs while the spectral resolution is 1.5 nm. All the measurements were carried out under magic angle in order to avoid the rotational decay processes, in a 2 mm cell and at an absorbance of about 0.5 at 400 nm. The solution was stirred during the experiments to avoid photodegradation processes. Transient absorption data were analyzed using the Surface Xplorer PRO (Ultrafast Systems) software. It was possible to visualize the 3D and 2D data surface, to simultaneously display and save multiple spectra and kinetics, to perform temporal chirp corrections, to make time zero adjustments, to fit the kinetics by exponential(s) including instrumental response function deconvolution, to perform Singular Value Decomposition^{31,32} of the 3D surface into principal components (spectra and kinetics) and to perform Global Analysis.

Results and discussion

Absorption and emission spectra

The $S_0 \rightarrow S_n$ absorption spectra of **1** and **2** in MeCN and DMSO are shown in Fig. 1. Both compounds present a broad vibrationally structured band in the 320–450 nm range. The relatively high values of the absorption coefficients (7700–11 500 M⁻¹ cm⁻¹) indicate the allowed transitions (Table 1). These data are in good agreement with the absorption maxima and oscillator strengths obtained by quantummechanical calculations by using the INDO/1-CI method (data not shown).



Fig. 1 Absorption and emission spectra of **1** (a) and **2** (b) in MeCN (full lines) and dimethylsulfoxide (dashed lines).

 $\label{eq:table_$

	Solvent	$\lambda_{max,abs}/nm$	$\epsilon/M^{-1}cm^{-1a}$	$\lambda_{max,em}/nm$	$\phi_{ m F}$			
1	MeCN	333,349, 367 ,386	11 500	390,413,437,463	0.035			
	DMSO	336,353, 370 ,390	8400	396,418,443,471	0.096			
2	MeCN	353,369,388,409	8600	423,442	0.0013			
	DMSO	353,371, 394 ,416	7700	430,454	0.0016			
a	⁴ Absorption coefficient at the maximum (in bold type).							

With respect to the spectrum of anthracene in apolar solvent,³³ the absorption spectrum of **1** is red shifted and exhibits larger bands compared to those of the fluorophore itself. The introduction of the cyano group in the anthracenic 10 position (compound **2**) shifts bathcoromically the absorption spectrum while the vibrational structure is less evident (larger bands and less profound minima). A small blue shift was observed on going from DMSO to MeCN.

The fluorescence emission spectrum of **1** is characterized by maxima centred at 396, 418, 443 and 471 nm in DMSO which undergo a ~5 nm blue shift in MeCN, similarly to what already observed for the absorption spectra (Fig. 1). However, a significant dependence of the fluorescence quantum yield on the solvent was observed (Table 1). In fact, the fluorescence quantum yield measured for **1** was drastically reduced switching from DMSO to MeCN ($\phi_F = 0.096$ and 0.035 in DMSO and MeCN, respectively). Compared to the emission spectrum of anthracene in non polar solvents,³³ the spectra of **1** are red shifted and less structured. The emission spectrum of **2** (Fig. 1) shows a less structured band centred at ~440 and 450 nm in MeCN and DMSO, respectively. The fluorescence quantum yields measured for **2** were very low ($\phi_F \sim 10^{-3}$, Table 1).

Upon addition of the tetrabutyl ammonium salts of four different anions (Br⁻, HSO₄⁻, H₂PO₄⁻, AcO⁻) the vibrational structure of the absorption spectra is still present even at high anion concentration (Table 2). The absorption spectra show significant changes in shape in the region below 340 nm where



Fig. 2 Absorption spectra of **2** (left) and emission spectra of **1** (right) in MeCN in the absence and in the presence of increasing amounts of TBAH₂PO₄ (up to 1.6×10^{-3} M and 4×10^{-2} M respectively). Inset: concentration effect of the salt on the absorbance at 400 nm (left) and on the fluorescence intensity at 417 nm (right); the full line represents the best titration fitting (see text).

the receptor chromophore C=S absorbs. This experimental evidence indicates that there is an interaction anion-substrate which does not involve the anthracenic fluorophore. It is feasible that the interaction takes place through hydrogen bonding of the anion with the thiourea protons.³⁴ This effect was the only spectral change observed upon addition of the anions Br⁻ and HSO₄⁻ to solutions of **1** and **2** in both MeCN and DMSO. When H₂PO₄⁻ and AcO⁻ were added to the substrate **2**, a bathochromic shift of the absorption bands was observed increasing the anion concentration and several isosbestic points appeared (Fig. 2A). This may be due to a partial interaction of the two anions with the anthracenic portion of **2** and it is more important in MeCN, a less polar solvent with respect to DMSO and so less competitive for the anion in binding the substrate.

Anion addition to 1 induced a fluorescence quenching: in general the spectral shape was not modified by the presence of the salts but there was an important variation in the fluorescence intensity especially when $H_2PO_4^-$ and AcO^- were used (Fig. 2B and 3). The values of the fluorescence quantum yields of 1 and 2 measured in the presence of a 0.04 M concentration of the anions are reported in Table 2. At this anion concentration the fluorescence intensity should have reached a plateau limit value as Fig. 3 shows. The quantum yield values confirmed the little effect of HSO_4^- e Br⁻ on the efficiency of the fluorescence process of the substrates 1 and 2. A variation of both spectral shape and fluorescence quantum yields was observed upon complexation of 2 with $H_2PO_4^-$ and AcO⁻. In general, the effect on the fluorescence intensity significantly depends on the nature of the anion according to the following trend: $HSO_4^- \le Br^- \ll H_2PO_4^- < AcO^-$, in agreement with the ability of the anion to form hydrogen bonds with the thiourea protons. The stronger the bases the more efficiently these anions interact with the two substrates ($K_a = 1.8 \times 10^{-5}$ for AcOH, $K_a = 7.1 \times 10^{-3}$ for H₃PO₄, $K_a = 1 \times 10^3$ for H₂SO₄, and $K_a = 1 \times 10^9$ for HBr; where K_a is the acid constant).

The absorbance of **2** recorded in the presence of an increasing amount of $H_2PO_4^-$ and AcO⁻ was analyzed by use of eqn (1)³⁵

Table 2 Spectral properties of 1 and 2 in the presence of 0.04 M additives together with the fluorescence quantum yields

	Solvent	Additive (0.04 M)	$\lambda_{ m max,abs}/ m nm$	$\lambda_{ m max,em}/ m nm$	$\phi_{ m F}$
1	MeCN	TBABr	333,349,367,387	390,413,437,463	0.032
		TBAHSO ₄	333,349,367,387	390,413,437,463	0.032
		TBAH ₂ PO ₄	333,349,367,387	390,413,437,463	0.007
		TBAAcO	333,349,367,387	390,413,437,463	0.006
	DMSO	TBABr	335,352,370,391	396,418,443,471	0.095
		TBAHSO ₄	336,352,371,391	396,418,443,471	0.092
		TBAH ₂ PO ₄	336,352,371,391	396,418,443,471	0.027
		TBAACO	336,352,371,391	396,418,443,471	0.016
2	MeCN	TBABr	352,369,389,410	423,442	0.0011
		TBAHSO ₄	351,369,388,410	423,442	0.0013
		TBAH ₂ PO ₄	352,369,391,412	417, 438, 451	0.0005
		TBAAcO	352,369,391,412	416, 438	0.0013
	DMSO	TBABr	352,371,394,416	430,454	0.0017
		TBAHSO ₄	352,371,395,416	430,454	0.0017
		TBAH ₂ PO ₄	352,371,396,417	430, 444, 456	0.0009
		TBAAcO	352,371,395,416	416, 443	0.0031



Fig. 3 Changes in the relative fluorescence intensity at 413 nm of 1 upon addition of TBABr ($\mathbf{\nabla}$), TBAHSO₄ ($\mathbf{\Delta}$), TBAH₂PO₄ ($\mathbf{\Box}$), TBAOAc ($\mathbf{\Theta}$) in MeCN ($\lambda_{exc} = 367$ nm).

to determine the values of the association constants for the substrate-anion interaction (Fig. 2A, inset):

$$A = \frac{A_{\rm S} + A_{\rm C} \times K_{\rm ass} \times [\rm{anion}]}{1 + K_{\rm ass} \times [\rm{anion}]} \tag{1}$$

where A is the absorbance at a wavelength were a great variation is observed upon anion addition, [anion] is the anion concentration in solution, $A_{\rm S}$ and $A_{\rm C}$ are the limit values of absorbance of the free substrate and of the fully complexed substrate respectively, $K_{\rm ass}$ is the association constant of the complex. Instead, in case of the interaction of 1 with H₂PO₄⁻ and AcO⁻, where the absorption changes are rather small, the better information on the complexation was obtained from the fluorescence titrations. The emission intensities were analyzed by use of eqn (2)³⁶ to determine the K_{ass} values (Fig. 2B, inset):

$$\frac{I_{\rm F}}{I_{\rm F}^{\circ}} = \frac{1 + \Delta F \times K_{\rm ass} \times [\rm{anion}]}{1 + K_{\rm ass} \times [\rm{anion}]}$$
(2)

where $I_{\rm F}$ is the fluorescence intensity upon anion addition, $I^{\circ}_{\rm F}$ is the fluorescence intensity of the free substrate, [anion] is the anion concentration in solution, and ΔF is the fluorescence intensity variation.

It has to be noted that the anion concentrations used in the above experiments were always significantly larger than those of **1** and **2** and, therefore, the concentration of the complex in solution was much smaller than that of the free anion.

The measured association constants (Table 3) are in the 300–46 000 M⁻¹ range. They are larger for the complexes with acetate with respect to those with dihydrogen phosphate, in agreement with the relative basicity. Furthermore, in the case of 1, the solvent effect on the association constant ($K_{ass,DMSO} < K_{ass,MeCN}$) reflects the ability of DMSO to compete with the anion in the complex formation, as also suggested by the β -scale of hydrogen-bond acceptor basicities.³⁷ Instead, higher K_{ass} values were generally obtained in the case of the more polar substrate **2**.

Triplet properties

The study of the triplet state of 1 and 2 was performed in MeCN and DMSO by laser flash photolysis. For both compounds a single transient was observed in all cases. It was assigned to the $T_1 \rightarrow T_n$ absorption on the basis of the following considerations: (1) it is formed during the laser pulse (ca. 7 ns); (2) it decays by a first-order kinetics; (3) its lifetime is quenched by molecular oxygen at an almost diffusional rate; and (4) it is sensitized by higher-energy triplet donors, such as benzophenone. The time resolved absorption spectra obtained for 2 in MeCN are shown in Fig. 4 as an example, together with the decay kinetics recorded at the absorption maximum of ca. 430 nm (Fig. 4, inset); similar results were obtained in the other experiments. In our experimental conditions at very low laser intensity, the time-resolved absorption spectra and the decay curves of 1 and 2 did not show any sign of absorptions of cation radicals and free radicals produced by photoionization and photodissociation, respectively.

Table 3 Association equilibrium constants (K_{ass}) of 1 and 2

Compd.	Solvent	Additive	$K_{\rm ass}/{ m M}^{-1}$
1	MeCN	$TBAH_2PO_4$ TBAACO	3000 45,000
	DMSO	TBAH ₂ PO ₄	300
2	MeCN	TBAACO TBAH ₂ PO ₄	670
	DMSO	TBAACO TBAH ₂ PO ₄ TBAACO	46 000 6200 32 000



Fig. 4 Triplet–triplet absorption spectra of **2** in MeCN recorded at 3 (\Box), 18 (\bigcirc), 50 (\triangle), 100 (\bigtriangledown) and 150 (\lhd) µs after the laser pulse ($\lambda_{\text{exc}} = 355$ nm). Inset: decay kinetics recorded at 430 nm.

The triplet yields (Table 4) are rather high (*ca.* 0.4 and 0.7 for **1** and **2**, respectively), indicating that the $S_1 \rightarrow T_1$ intersystem crossing is an efficient decay channel of the lowest excited singlet state of these compounds, as already reported for anthracene itself ($\phi_T = 0.66-0.71$).²⁷ It has to be noted that the quantum yields were obtained by using in DMSO the triplet-triplet absorption coefficients measured in MeCN.

The triplet lifetimes of 1 and 2, longer than 150 μ s (Table 4), represent lower limits for τ_T which is strongly affected by molecular oxygen and excitation energy. Thus they were measured at an excitation energy smaller than 1 mJ pulse⁻¹ after a vigorous bubbling with nitrogen.

The transient absorption spectra in the presence of the salts (Table 5) showed a sharp maximum at \sim 430 nm which decays with a time constant of hundreds of microseconds as in the case of the free substrates. Thus, the spectral and kinetic properties of the triplet state depend only on the fluorophore nature and are not affected by the anion complexation. The intersystem crossing quantum yields ($\phi_{\rm T}$) were calculated by assuming the absorption coefficients of the triplet state of the complexes to be equal to the correspondent one measured for the uncomplexed substrate in MeCN and are shown in Table 5. In general, the efficiency of production of the triplet state resulted slightly influenced by the interaction with the anion, being $\phi_{\rm T} = 0.36-0.50$ and 0.55-0.80 for complexes with 1 and 2, respectively. Only in the presence of the acetate anion the triplet quantum yield was decreased to values of 0.26-0.28 for both the substrates in both the solvents.

Femtosecond absorption

The primary decay processes of **1** and **2** were investigated upon ultrafast excitation at 400 nm. Taking into account the absorption spectra of the substrates and the spectral broadening of the

Table 4 Properties of the lowest triplet state of 1 and 2 ($\lambda_{exc} = 355 \text{ nm}$)

	Solvent	λ _{max}	$\tau_T/\mu s$	${\epsilon_{T} \over M^{-1}} \ cm^{-1}$	ϕ_{T}	$rac{k_{ m ox}}{10^9}{ m M}^{-1}~{ m s}^{-1}$
1	MeCN DMSO	425 430	160 150	23 000	0.37	2.2
2	MeCN DMSO	430 435	150 150 250	11 000	0.74 0.71	2.0 1.9

Table 5 Triplet properties of 1 and 2 in the presence of 0.04 M additives ($\lambda_{exc} = 355$ nm)

	Solvent	Additive (0.04 M)	$\lambda_{\rm max}/{\rm nm}$	$\tau_T/\mu s$	ϕ_{T}	$rac{k_{ m ox}}{10^9}{ m M}^{-1}~{ m s}^{-1}$
1	MeCN	TBABr	425	160	0.50	2.2
		TBAHSO ₄	425	180	0.38	2.1
		$TBAH_2PO_4$	425	140	0.37	2.0
		TBAAcO	425	170	0.28	1.5
	DMSO	TBABr	430	170	0.50	1.8
		TBAHSO ₄	430	160	0.47	2.2
		$TBAH_2PO_4$	430	180	0.43	1.4
		TBAAcO	430	190	0.36	1.5
2	MeCN	TBABr	430	220	0.62	1.9
		TBAHSO ₄	430	190	0.55	2.0
		$TBAH_2PO_4$	430	200	0.27	1.6
		TBAAcO	430	250	0.26	2.9
	DMSO	TBABr	435	220	0.74	1.6
		TBAHSO ₄	435	200	0.80	1.8
		$TBAH_2PO_4$	435	170	0.67	1.6
		TBAAcO	435	300	0.26	1.9

ultrafast excitation pulses,^{38–41} the lowest vibrational levels of the π,π^* excited singlet state of anthracenic character were populated upon excitation.

The time-resolved absorption spectra of the two substrates in MeCN are shown in Fig. 5, together with the decay kinetics recorded at two noteworthy wavelengths; similar spectra were recorded in DMSO. A broad absorption band centred at 575–595 nm was detected just after the laser pulse. At longer time delays this absorption was replaced by a rest absorption with a maximum ($\lambda_{max} < 470$ nm) located outside the investigated spectral interval.

The decay traces were analyzed at different wavelengths: the best fittings were obtained by triexponential functions for 1 and biexponential functions for 2 (Table 6) convoluted with the instrumental response profile with Gaussian shape.



Fig. 5 Pump–probe time resolved absorption spectra of **1** (A) recorded 0.2 (a), 5 (b), 20 (c), 60 (d), 200 (e), 500 (f) and 1020 (g) ps and **2** (B) recorded 0.3 (a), 1.5 (b), 7 (c), 18 (d), 25 (e), 50 (f) and 1400 (g) ps after the laser pulse ($\lambda_{exc} = 400$ nm) in MeCN. Insets: decay kinetics recorded at 595 (full lines) and 473 (dashed lines) nm for **1** (A) and at 575 (full line) and 475 (dashed line) nm for **2** (B) in MeCN.



Fig. 6 Amplitudes of the decay components of **1** (A) and **2** (B) in MeCN obtained by global analysis.

The global analysis of the decay kinetics also revealed the presence of three and two exponential components (a shortand a longer-lived one, S and L, respectively and a rest absorption formed while L disappeared and that did not decay during the investigated time interval, Fig. 6A).

In the case of **2** the data analysis revealed the presence of two components: a transient characterized by a lifetime of 30 ps in MeCN and 27 ps in DMSO (L) and a rest absorption (Fig. 6B). The L component shows a negative signal at wavelengths shorter than 500 nm and a positive absorption with a maximum at 575 nm in both solvents; instead, the rest transient shows a positive signal at wavelengths shorter than 460 nm. This spectral properties are in agreement with a precursor–successor scheme which involves the L \rightarrow rest step. Moreover the spectral shape obtained for L exhibits a negative absorption signal in the region of the steady-state fluorescence spectrum.

The L transient was assigned to the lowest excited singlet state S_1 , while the rest component was attributed to the lowest excited triplet state T_1 , formed upon the disappearance of S_1 (Fig. 4).

For 1, the data analysis revealed the presence of an additional short-lived component, S, characterized by a lifetime of 11 ps in both MeCN and DMSO while the lifetimes associated with the component L were 330 and 300 ps in MeCN and DMSO, respectively. For the same reasons already discussed for 2, L was assigned to the lowest excited singlet state S_1 and rest to the lowest excited triplet state T_1 . The S transient, associated with a positive absorption band centered at 590 nm, was due to the vibrational relaxation of the vibrationally hot

Table 6 Time constants of **1** and **2** ($\lambda_{exc} = 400 \text{ nm}$)

	Solvent	$\lambda_{max,1}/nm$	τ_1/ps	$\lambda_{max,2}/nm$	τ_2/ps	$\lambda_{\rm max,3}/\rm nm$	$ au_3/ps$
1 2	MeCN DMSO MeCN	590 450, 590	11 11	595 590 575	330 300 30	<470 <435 <460	Rest Rest Rest
	DMSO			575	27	<460	Rest

 S_1 state which takes place in about 11 ps (Fig. 6A). The similar results obtained in MeCN and DMSO indicate that the solvent does not play an important role in determining the nature of the excited states formed upon laser irradiation and their lifetimes.

The short lifetimes of the S₁ states, coupled with the Stern–Volmer constants (K_{SV}) obtained by the steady-state fluorescence titrations of the substrates in both MeCN and DMSO with the different anions (Br⁻, HSO₄⁻, H₂PO₄⁻, AcO⁻), allowed to obtain fluorescence quenching rate constants ($k_q = K_{SV}/\tau_F$) much larger than the diffusion limit of 10^{10} M⁻¹ s^{-1.42} This result is in agreement with an interaction between the substrates and the ions which takes place in the ground state and is not diffusion controlled.

The effect of the anions Br^- , HSO_4^- , $H_2PO_4^-$, AcO^- as tetrabutylammonium salts on the primary deactivation processes of **1** and **2** was investigated both in MeCN and DMSO. In particular, femtosecond transient absorption measurements were carried out for the two substrates in the presence of a 0.04 M concentration of the salts, value at which all the substrate receptors in the sample are complexed with the anions. Therefore, the deactivation pathways found to be operative with this investigation could be considered characteristic of the complex substrate–anion formed in the ground state before laser excitation.

The time resolved absorption spectra and the decay kinetics recorded for the two substrates in the presence of the anions were very similar to those recorded for the free molecules (Fig. 7). The data analysis revealed also in these cases the presence of two or three exponential components characterized by spectral shapes analogous to the ones observed for the substrates itself (Fig. 8 and Table 7).



Fig. 7 Time resolved absorption spectra of **1** in MeCN (A) in the presence of 0.04 M TBABr recorded 0.45 (a), 3 (b), 8 (c), 30 (d), 100 (e), 400 (f) and 1400 (g) ps after the laser pulse and (B) in the presence of 0.04 M TBAAcO recorded 0.2 (a), 1.5 (b), 4 (c), 7 (d), 11 (e), 30 (f), 150 (g) and 500 (h) ps after the laser pulse ($\lambda_{exc} = 400$ nm). Insets: decay kinetics recorded at 593 (full lines) and 435 (dashed lines) nm for **1** in MeCN in the presence of TBAAcO (B).



Even if the shape of the time-resolved absorption spectra was only slightly affected by the presence of the anions, the lifetimes of the lowest excited singlet states of 1 and 2 were significantly changed by the interaction with the anions (Table 7). In general, after a fast vibrational relaxation which takes place in less than 15 ps, the longer lived S_1 state was detected and characterized. The results of this analysis show that the interaction between the substrates and the anions cause effects much different than those recorded by steadystate experiments. In particular, the effect on the lifetimes is not parallel to that on the fluorescence quantum yields, thus indicating that the photophysical behaviour of the systems cannot be understood just taking into account of a further decay pathway, such as the photoinduced electron transfer, which takes place in the complexes. In particular, Br⁻ and HSO₄⁻, which change the fluorescence quantum yields only slightly, are able to reduce significantly the S_1 lifetimes of 1 in MeCN (from 330 ps to 190 and 120 ps respectively). Instead, in some cases where the fluorescence quantum yield is quenched, the S₁ lifetimes become even longer than those of the substrate alone (e.g. $1/AcO^{-}$ in MeCN and $2/H_2PO_4^{-}$ in both the solvents). In other cases, parallel behaviours were found.

Interaction mechanism

The steady-state and the time-resolved measurements provided a complete characterization of the lowest excited states deactivation pathways of 1 and 2 in polar solvents. In particular combining the data of the measured quantum yields $(\phi_{\rm F}, \phi_{\rm T})$ it was possible to estimate also the efficiency of internal conversion ($\phi_{IC} = 1 - \phi_F - \phi_T$), since in our experimental conditions there was no evidence of photoreactions, photodissociations or photoionization. Using the values of the lowest excited state lifetimes, the rate constants for three deactivation processes observed (fluorescence, intersystem crossing and internal conversion) were calculated for the free substrates (Table 8).

It is worthy to note that the fluorescence rate constant $(k_{\rm F})$ assumes values of 1.1×10^8 and 3.2×10^8 s⁻¹ for 1 in MeCN and DMSO, respectively, confirming that the radiative transition $S_1 \rightarrow S_0$ is allowed, while it is 4.3×10^7 and 5.9×10^7 s⁻¹ for 2 in MeCN and DMSO, thus indicating a slightly less allowed transition. On the other hand, the high intersystem crossing (ISC) rate constants of 1 and 2 ($k_{\rm ISC} \approx 10^9 \, {\rm s}^{-1}$ and 10^{10} s⁻¹, respectively) suggest the occurrence of an efficient spin-orbit coupling between the lowest excited singlet and triplet states which allows a very fast ISC process to be operative. It has to be noted that in the case of 1, the solvent effect on $k_{\rm F}$ could reflect the influence of the different solvent polarizability (0.212 and 0.284 for MeCN and DMSO, respectively) on the relative position of the lowest excited singlet states S1 and S2. In fact, for this molecule quantum mechanical INDO1/CI calculations show the presence of close lying states of π,π^* anthracenic character with different oscillator strengths (S₁ forbidden and S₂ allowed). The solvent could affect the mixing between the two states and then the experimental $k_{\rm F}$ values.^{43,44}

Irradiation at 400 nm generates vibrationally hot levels of the lowest excited singlet state of the substrates 1 and 2 which reach within about ten picoseconds the relaxed S1 state. This state decays in about 300 ps and 30 ps for 1 and 2, respectively.

Table 7 Time constants of 1 and 2 in the presence of 0.04 M additives ($\lambda_{exc} = 400 \text{ nm}$)

6ps

rest

8ps

rest

----- 400ps

700

750

650

----- 190ps

	Solvent	Additive (0.04 M)	$\lambda_{\max,1}/nm$	τ_1/ps	$\lambda_{\max,2}/nm$	τ_2/ps	$\lambda_{\rm max,3}/\rm nm$	τ_3/ps
1	MeCN	TBABr	590	6	590	190	<440	Rest
		TBAHSO ₄	590	4	595	120	<470	Rest
		TBAH ₂ PO ₄	585	4	590	35	<470	Rest
		TBAAcO	590	8	600	400	<440	Rest
	DMSO	TBABr	600	10	595	250	<450	Rest
		TBAHSO ₄	600	14	595	250	<470	Rest
		TBAH ₂ PO ₄	600	11	590	80	<460	Rest
		TBAAcO			585	25	<470	Rest
2	MeCN	TBABr	570	4	570	36	<470	Rest
		TBAHSO ₄			570	26	<460	Rest
		TBAH ₂ PO ₄	570	4	565	100	<470	Rest
		TBAAcO	570	3.5	580	400	<470	Rest
	DMSO	TBABr			565	19	<470	Rest
		TBAHSO ₄			570	24	<470	Rest
		TBAH ₂ PO ₄	570	6.6	575	280	<460	Rest
		TBAAcO	565	4	650	130	<440	Rest

Table 8 Fluorescence, intersystem crossing and internal conversion rate constants of 1 and 2

	Solvent	$k_{\rm F}/10^9~{\rm s}^{-1}$	$k_{\rm ISC}/10^9~{\rm s}^{-1}$	$k_{\rm IC}/10^9~{\rm s}^-$
1	MeCN	0.11	1.1	1.8
	DMSO	0.32	1.6	1.4
2	MeCN	0.043	25	8.6
	DMSO	0.059	26	11

 S_1 is the fluorescent state and the precursor of the lowest excited triplet state T_1 whose formation could be followed by the femtosecond resolved measurements and whose decay was observed during the nanosecond laser flash photolysis experiments (Scheme 2).

Upon addition of the tetrabutylammonium salts of the four anions (Br⁻, HSO₄⁻, H₂PO₄⁻, AcO⁻) the singlet-state lifetimes were modified, together with the efficiency of the fluorescence process and formation of the triplet. The steady-state and time-resolved measurements carried out on the anionsubstrate complexes allowed us to get a complete description of the rate constants (Table 9) of the three main relaxation pathways observed for the singlet state. In particular, the complexes with the anions Br⁻ and HSO₄⁻ show rate constants $k_{\rm F}$ and $k_{\rm ISC}$ quite close of 1 and 2 (the difference is smaller than the factor of 2) while those with the anions $H_2PO_4^-$ and AcO⁻ has k_F and k_{ISC} values of about one order of magnitude smaller than those of the substrates alone. The small effect of the heavy atom (Br⁻) effect on the intersystem crossing rate constant can be due to the high spin-orbit coupling effect which is responsible of particularly high $k_{\rm ISC}$ already in the substrates alone.

Moreover the time resolved measurements did not show any evidence of further deactivation pathways induced by the presence of anions, such as photoinduced electron transfer (PET). In fact, both the nanosecond and the femtosecond measurements did not detect transients which could be formed as a consequence of this process (for example the anion radicals of the anthracene and cyano-anthracene fluorophores).⁴⁵ The excited state dynamics of the free (S) and complexed (C) substrates are summarized in Scheme 2.

The nature of the anion has an impact on the effect observed both on the lifetimes and the quantum yields of the two substrates. In fact ions like Br^- and HSO_4^- , which interact weakly with the thiourea protons because of their low basicity,



Scheme 2 Deactivation pathways of the excited states of 1 and 2 (S) and of their complexes with anions (C).

	Solvent	Additive (0.04 M)	$k_{\rm F}/10^9~{\rm s}^{-1}$	$k_{\rm ISC}/10^9~{\rm s}^{-1}$	$k_{\rm IC}/10^9~{\rm s}^{-1}$
1	MeCN	TBABr	0.17	2.6	2.5
		TBAHSO ₄	0.27	3.2	4.9
		TBAH ₂ PO ₄	0.20	11	18
		TBAAcO	0.015	0.70	1.8
	DMSO	TBABr	0.38	2.0	1.6
		TBAHSO ₄	0.37	1.9	1.8
		TBAH ₂ PO ₄	0.34	5.4	6.8
		TBAAcO	0.48	14	25
2	MeCN	TBABr	0.031	21	6.9
		TBAHSO ₄	0.050	21	17
		TBAH ₂ PO ₄	0.005	2.7	7.3
		TBAAcO	0.003	0.70	1.8
	DMSO	TBABr	0.089	39	14
		TBAHSO ₄	0.071	33	8.3
		TBAH ₂ PO ₄	0.003	2.4	1.2
		TBAAcO	0.024	2.0	5.7

 Table 9 Decay rate constants of 1 and 2 in the presence of 0.04 M additives

induced only a small variation in the properties of the fluorescent lowest excited singlet state. The changes induced by the two very basic $H_2PO_4^-$ and AcO^- species are remarkable, in agreement with the values of the association constants between the ground state of the substrates and these salts. This experimental evidence confirmed the possibility of using the substrates 1 and 2 as selective sensors of dihydrogen phosphate and acetate even in small amounts.

Conclusions

In this work compounds 1 and 2 were synthesized and characterized by steady-state absorption and emission techniques, both in the case of the free substrates and of their complexes with different anions (Br⁻, HSO₄⁻, H₂PO₄⁻ and AcO⁻) in MeCN and DMSO. A complete investigation of the excited state dynamics of 1 and 2 and of their complexes with the salts was carried out by using time-resolved transient absorption spectroscopy, with nano- and femto-second time resolution. The observed singlet and triplet excited states were spectrally and kinetically characterized and three main deactivation pathways found to be operative (fluorescence, intersystem crossing and internal conversion) were quantitatively described in terms of their efficiencies and rate constants. In particular, the substrate 1 is more fluorescent ($\phi_{\rm F} = 0.035$ in MeCN) and shows a longer S_1 state ($\tau_F = 330$ ps in MeCN) than the compound 2 ($\phi_{\rm F} = 0.0013$ and $\tau_{\rm F} = 30$ ps in MeCN). Instead, the intersystem crossing is more efficient for 2 ($\phi_{\rm T} = 0.74$ in MeCN) than for 1 ($\phi_{\rm F} = 0.37$ in MeCN).

The impact of Br⁻ and HSO₄⁻ on the photophysical properties of **1** and **2** was rather small; in fact the decay rate constants of the lowest singlet state were changed by a factor of ≤ 2 . Instead, the substrates strongly bound the anions H₂PO₄⁻ and AcO⁻ in the ground state with association constants of 10^2-10^5 M⁻¹, depending on the substrate and the solvent. The main effect of acetate and dihydrogen phosphate was on the lifetime of the lowest excited singlet state and on the fluorescence and triplet formation quantum yields.

The experiments carried out with nano- and femto-second time resolution did not give evidence of further transients ascribable to radical ions and radicals, which would be expected if a photoreaction process, such as photoinduced electron 20 R. transfer (PET), was operative both in the absence and presence

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of anions.

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