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Photophysics of Lumichrome and Its Analogs

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The spectroscopic and photophysical properties of lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives in acetonitrile and in methanol are presented. In common with the parent molecule, the photophysics of the lumichrome methyl derivatives are dominated by non-radiative transitions in both methanol and acetonitrile. However, fluorescence yields in methanol are higher than in acetonitrile as a result of a reduction in the efficiency of non-radiative deactivation channels. These observations are discussed in terms of the available solvent-solute interactions.

Key words: alloxazine, lumichrome, photophysics, transient absorption, fluorescence lifetime

Lumichrome (7,8-dimethylalloxazine = 7,8-dimethyl-benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) is representative of alloxazines, a class of nitrogen heterocycles related to lumazine and flavins. However, in contrast to lumazine and flavins, lumichrome and other alloxazines have received relatively little attention. Only recently, interest in the alloxazines has become more intense, because it has been shown that these compounds may play an important role in a wide range of biological systems [1]. For example, it has been shown that lumichrome may be used to inhibit flavin reductase in living *Escherichia coli* cells [2]. Said *et al*. [3–5] reported that the mechanism of riboflavin uptake by human-derived liver cells Hep G2, colonic epithelial NCM460 cells and Caco-2 human intestinal epithelial cells is inhibited by lumichrome [4,5]. A number of studies on the photophysics and photochemistry of alloxazines have been performed [6–17]. Photochemical studies of alloxazines have shown that under appropriate circumstances alloxazines, which are unsubstituted at the N(1) position, can undergo an excited-state proton transfer from N(1) to N(10) to give the corresponding isoalloxazinic form. The excited state reaction occurs in the presence of compounds having proton donor and acceptor groups, able to form the *correct* hydrogen bonds with alloxazine molecules [6,15,16,18]. As a class of compounds, alloxazines have proved to be useful models for studying the photophysics of polyatomic molecules in homo and heterogeneous media. For example, it has been shown that alloxazines can act as efficient photosensitisers of singlet oxygen [13,14,19–21]. The efficient polymerization of 2-hydroxyethyl methacrylate photoinitiated by lumichrome in the presence of triethanolamine has also been

reported [22]. The alloxazine nucleosides are potentially of interest as fluorescent probes and have been predicted to exhibit hydrogen-bonding characteristics similar to thymidin [23].

It is rather surprising that model alloxazine compounds, such as lumichrome and a series of its derivatives, have not been systematically studied in aprotic solvents, in which solute-solvent hydrogen bond formation and the possibility of an excited-state proton-transfer reaction with solvent molecules can be excluded. The previous studies of lumichromes are scattered and usually performed in protic solvents, *e.g.* water, alcohols and acetic acid [6,8,10,16,17,24–29]. From the point of view of possible biological roles and also the mechanism of excited-proton transfer reactions it is especially interesting to study lumichromes with and without methyl substituents in the position N(1) and/or N(3) [23]. This paper describes a steady-state and time-resolved study of the singlet and triplet states of lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives. The structures and abbreviations of the lumichromes discussed here are presented in Figure 1. The present investigation was undertaken with the aim of giving a more systematic insight into the photophysics of lumichromes in solution.

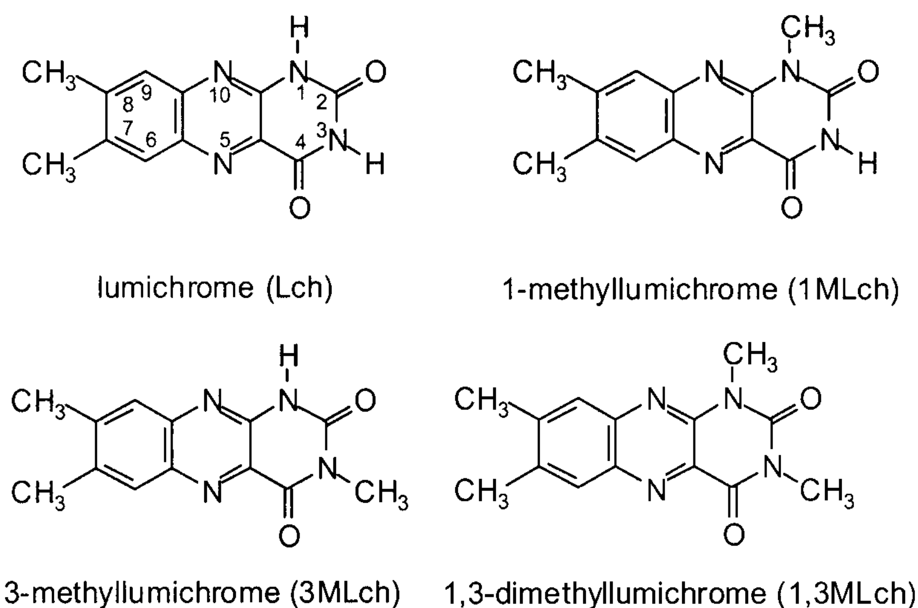

Figure 1. Structures and abbreviations of the lumichromes used in this study.

EXPERIMENTAL

Lumichrome, benzophenone and the solvent methanol (all from Aldrich) were used as received. Acetonitrile (from Aldrich) has been dried by refluxing over calcium hydride just before use. The purity of the solvent was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer. Fluorescence decays were measured by exciting lumichromes in acetonitrile or in

methanol (except lumichrome itself in acetonitrile) at 355 nm using a time-correlated single-photon counting method on a commercially available IBH model 5000U fluorescence lifetime spectrometer. Time-resolved fluorescence measurements of lumichrome in acetonitrile were conducted with a model C-700 fluorometer from Photon Technology International. The system utilizes a nanosecond flash lamp as an excitation source and a stroboscopic detection system [30]. Transient absorption spectra were measured using a computer controlled nanosecond transient spectrophotometer for laser spectroscopy LKS50 instrument (Applied Photophysics). In brief, the spectrophotometer is based on the Q-switched Nd:YAG Laser System (Spectron). The output from the laser was frequency tripled to give 355 nm with typical pulse energies of 8–25 mJ/pulse with a width of *ca.* 8 ns. Steady-state fluorescence spectra were obtained with a Jobin Yvon–Spex Fluoromax3-11 spectrofluorometer. UV-visible absorption spectra were recorded on a Varian Cary 5E spectrophotometer.

RESULTS AND DISCUSSION

Lumichrome and its derivatives exhibit absorption spectra with a few major bands in the UV-visible region, see Figure 2. In the long wavelength region the absorption spectra of lumichrome and its 1- and 3-methyl and 1,3-dimethyl derivatives in acetonitrile and in methanol are essentially identical. However, one can notice some differences in the absorption spectra in the short wavelength region, below 300 nm. The absorption and the corrected fluorescence excitation spectra agree well with each other for all examined compounds in both solvents. The absorption spectrum of lumichrome in the near UV region, with solutions exhibiting a pale yellow colour, shows two well resolved maxima at 335 nm and at about 380 nm, with a shoulder near 400 nm. The molar absorption coefficients and positions of the bands located at lowest energies for the four examined lumichromes are listed in Table 1. The normalised fluorescence emission spectra of lumichrome and its derivatives, excited at 355 nm, are presented in Figure 2. The fluorescence emission spectra of lumichrome and its derivatives in both solvents show a single band with the maximum at about 440 nm and 460 nm in acetonitrile and methanol respectively, the exact position of which depends on the position and number of substituents. All UV-visible absorption and emission bands of alloxazines are assignable to the electric dipole allowed $\pi \rightarrow \pi^*$ transitions [31,32].

Table 1. Spectral and photophysical data for the singlet states of lumichromes in acetonitrile and in methanol [a].

Solvent	Compound	λ^2_{max}/nm	λ^1_{max}/nm	ϕ_F	λ_F/nm	τ_F/ns	$k_r/10^8 s^{-1}$	$\Sigma k_{nr}/10^8 s^{-1}$
ACN	Lch	334	384 (8300)	0.028	437	0.64 0.7 [b]	0.43	15.2
	1MLch	334	379 (7600)	0.027	445	0.63 0.5 [b]	0.43	15.4
	3MLch	335	379 (8100)	0.026	436	0.64	0.41	15.2
	1,3MLch	335	373 (7200)	0.028	444	0.62	0.43	15.2
MeOH	Lch	339	384 (7700)	0.032	453	1.04 [c]	0.30	9.3
	1MLch	340	385 (7500)	0.037	453	0.94	0.35	10.3
	3MLch	340	383 (8000)	0.032	460	1.0 [d]	0.32	9.7
	1,3MLch	340	386 (7500)	0.031	461	1.0 [d]	0.31	9.7

[a] The positions of two long-wavelength bands in the absorption spectra λ^1_{max}, λ^2_{max} with the molar absorption coefficients in parentheses, the fluorescence quantum yield is ϕ_F, the lifetime of fluorescence, τ_F, the radiative rate constant is k_r, and the sum of nonradiative rate constants Σk_{nr};
[b] from [14]; [c] from [22]; [d] from [24].

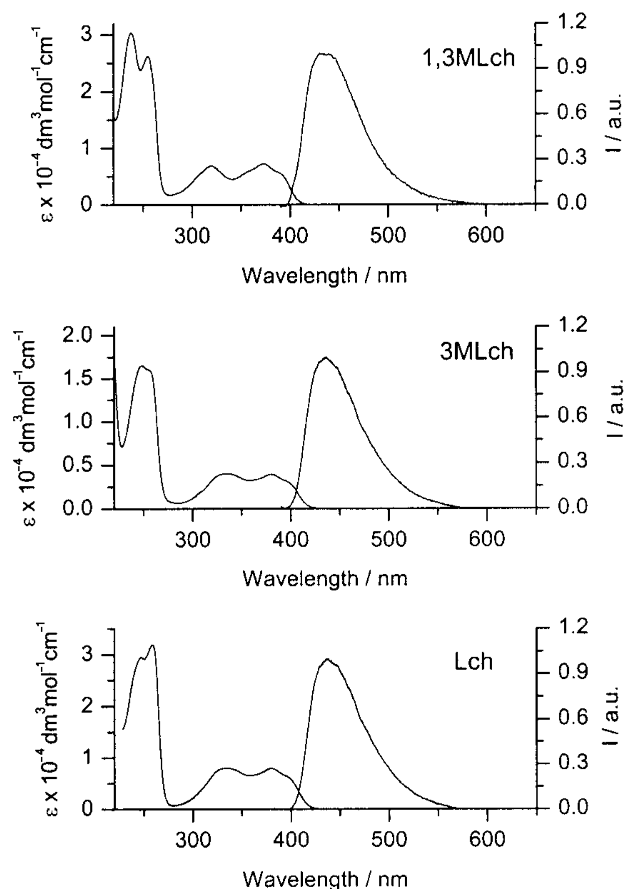

Figure 2. Ground state absorption spectra together with fluorescence spectra of 1,3-dimethyllumichrome, 1,3MLch, 3-methyllumichrome, 3MLch, and lumichrome, Lch, in acetonitrile.

In all cases the fluorescence decays are well modelled by single exponential functions, as shown by the usual statistical measures of "goodness-of-fit". The results of fluorescence lifetime measurements are collected in Table 1. All excited lumichromes exhibit relatively short fluorescence decay times, typical for alloxazines in solution [12,14]. Lumichrome and its 1- and 3-methyl derivatives have very similar lifetimes about of 0.64 ns and 1.0 ns in acetonitrile and methanol, respectively, although there is observed a longer fluorescence lifetime in methanol for all the compounds studied. The recorded lifetimes in acetonitrile, about 0.64 ns, are similar to previously reported fluorescence lifetimes of alloxazines in acetonitrile [14]. The fluorescence lifetimes in methanol and other protic solvents are longer than those measured in acetonitrile. For example, in methanol the reported fluorescence lifetime is about 1.0 ns for all lumichromes (Table 1), in water the reported fluorescence lifetimes are 2.7 ns and 2.2 ns for Lch and 1MLch, respectively [13], and a fluorescence lifetime of 0.88 ns for Lch in ethanol has been reported [12].

The fluorescence quantum yields and fluorescence lifetimes of lumichrome and its derivatives measured in acetonitrile and in methanol are given in Table 1. In methanol, polar protic solvent the absorption and emission bands of all examined lumichromes undergo long wavelength shifts, the fluorescence quantum yields becoming higher and fluorescence lifetimes longer, if compared to lumichromes in acetonitrile. However, in protic solvents the data are much more difficult to interpret because of possible phototautomerisation between the alloxazine and isoalloxazine structures, for Lch and 3MLch. For lumichromes unsubstituted at the N(1) position one may expect the possibility of excited state proton transfer from N(1) to N(10) to form the corresponding isoalloxazine in methanol solution. The similarity of the spectroscopic and photophysical data for Lch and 3MLch unsubstituted at the N(1) position, and those methyl substituted at the N(1) position (1MLch and 1,3MLch) suggests that under the applied conditions no double proton transfer (within a hydrogen bonded complex with the solvent) occurs in the S_1 state of either molecule. It is also reasonable to expect that for lumichromes in methanol a range of hydrogen bonds can be formed between solute (involving N(1), N(3), N(10), and N(5) and both carbonyl oxygens, C(2) and C(4)) and a solvent. The results show that the hydrogen bonding interaction between methanol and the N(1) and N(3) position of lumichrome do not play an important role. Therefore, it is expected that the hydrogen bond interaction of lumichrome involving N(10), and N(5) and both carbonyl oxygens, C(2) and C(4)) and a methanol molecule are important. Indeed, MINDO/3 calculations suggest that both oxygen atoms are more electronegative than any of the nitrogen atoms in the lumichrome structure [32]. Particularly interesting seems the simple hypothesis that the hydrogen bonding interaction between methanol and lumichromes at the N(10) position influences the conjugation such that there is a shift to a more flavine-like structure. For lumichrome and its derivatives in acetonitrile and in methanol the radiative and non-radiative decay constants for the lowest excited singlet states can be calculated from $k_r = \phi_F/\tau_F$ and $\Sigma k_{nr} = (1 - \phi_F)/\tau_F$. Here, k_r is the rate constant for radiative decay of the excited species and Σk_{nr} is the sum of all first order and pseudo-first order rate constants for its non-radiative decay. The sum, Σk_{nr}, could in principle contain contributions from pseudo-first order concentration and oxygen quenching of the excited species. However, the concentrations of both the ground state chromophore ($< 10^{-4}$ M) and oxygen ($< 10^{-3}$ M) are too small to contribute significantly to the rates of decay of the excited singlet state molecules even if the quen- ching were diffusion limited. The values of k_r and Σk_{nr} are also tabulated in Table 1. For all examined compounds the data show that the decay of the singlet state is dominated by the rates of the non-radiative processes, they being almost two orders of magnitude larger those of the radiative processes. The values of rates of both radiative and non-radiative processes differ by no more than a factor of about 1.5 between acetonitrile and methanol, although the rates of both radiative and non-radiative processes are reduced in methanol relative to acetonitrile, with the greatest effect being on the non-radiative components. These small differences between rates of both radiative and non-radiative processes of lumichromes in

methanol if compared to acetonitrile can be explained by the presence of flavine like structure. It is well known that flavines exhibit longer fluorescence lifetimes and a similar order of magnitude for the rates of both radiative and non-radiative processes, and are similar to those of radiative processes for alloxazines. For example, the fluorescence lifetime of lumiflavine has been determined as 7.6 ns [14], due mainly to a remarkable reduction in the rate of non-radiative processes (by more than an order of magnitude) relative to lumichrome. Hence the observation of the relative decrease in the contribution of non-radiative decay to the overall deactivation in methanol may point to hydrogen bonding resulting in changes in charge distribution which result in more flavin-like character.

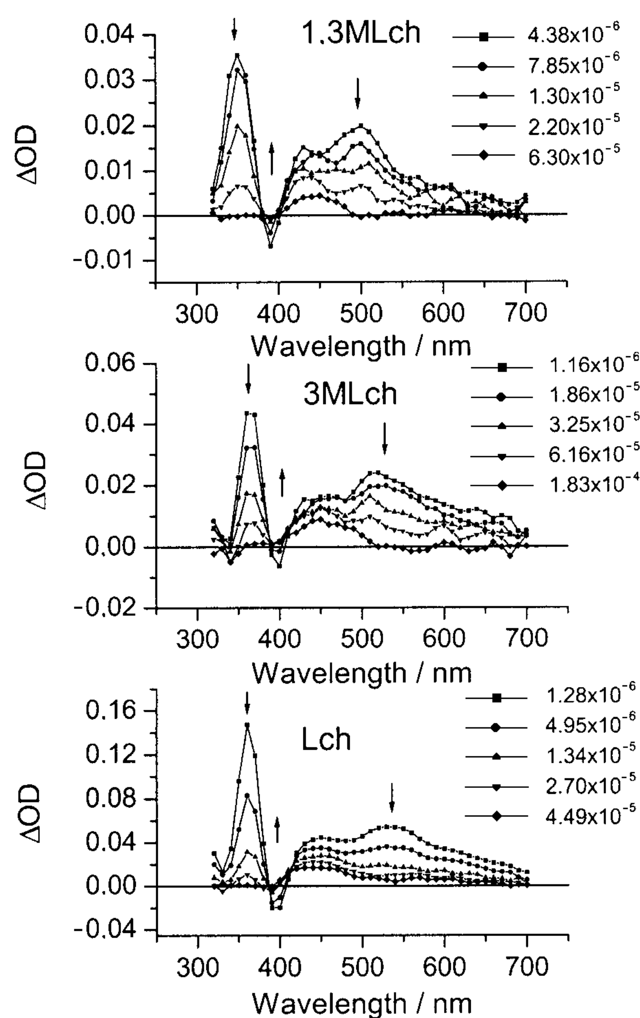

Figure 3. Transient absorption spectra of 1,3-dimethyllumichrome, 1,3MLch, 3-methyllumichrome, 3MLch, and lumichrome, Lch, in deoxygenated acetonitrile at room temperature. Numbers on each panel are the times after laser excitation at 355 nm, (cell pathlength = 1 cm).

Table 2. Spectral and photophysical data for the triplet states of lumichromes in acetonitrile [a].

Compound	λ^3/nm	λ^2/nm	λ^1/nm	$\tau_T/\mu s$	ϕ_T	$k_{ic}/10^9$ s^{-1}	$k_{isc}/10^9$ s^{-1}
Lch	360	450	540	7 11 [b]	0.68	0.46	1.0
1MLch	370	460	560	6.9 6.7 [b]	0.66 [b]	0.62	1.3
3MLch	360	430	520	15			
1,3MLch	350	430	500	15			

[a] The positions of the maxima in the transient absorption spectra of lumichromes are $\lambda^1, \lambda^2, \lambda^3$; the lifetime of triplet states τ_T and the quantum yields of intersystem crossing are ϕ_T. The rate constants for internal conversion is k_{ic} and intersystem crossing is k_{isc};
[b] from [14].

Upon laser excitation at 355 nm, the lumichromes in acetonitrile produce transient species that decay on a microsecond timescale. Transient absorption spectra of lumichrome and 3-methyl- and 1,3-dimethyl derivatives in acetonitrile within different time delays are shown in Figure 3. The spectra exhibit a sharp maximum at about 360 nm, a broader absorption maximum near 450 nm and a broad absorption centred at about 530 nm. The negative absorbance change near 400 nm is attributed to ground-state depletion. Although the transient absorption spectra of all the lumichromes are similar, some differences are apparent especially in the position of the long-wavelength maximum. The position of the long-wavelength maxima vary from 500 nm for 1,3MLch to 560 nm for 1MLch. The decay kinetics of the triplet states have been measured at the long wavelength maximum, and appear to decay by a first-order process. The triplet lifetimes of corresponding lumichromes are reported in Table 2. The triplet state lifetimes of 3-methyllumichrome and 1,3-dimethyllumichrome are a factor of two longer than those for lumichrome and 1-methyllumichrome. At wavelength of 450 nm the decay kinetics is more complicated with a second, more weakly absorbing species decaying with a much longer lifetime. The long-lived band can be ascribed to the radicals derived from lumichrome [8,17]. Possible assignments for this species include for example anion radical of lumichrome with the spectrum presenting main absorption at about 450 nm [10,17,33].

Figure 4 shows the well-known transient absorption spectrum of benzophenone in acetonitrile at different delay times following 355 nm excitation of a nitrogen-saturated solution. The spectrum shows two bands with maxima at 520 nm and 320 nm, respectively, and analysis shows a first-order decay with a triplet lifetime of 12.5 μs. Figure 4 shows the transient absorption spectra of benzophenone containing 1.48×10^{-4} mol dm^{-3} lumichrome. Under conditions of lumichrome concentration necessary to achieve efficient energy transfer, the triplet state of the lumichrome is produced both by direct excitation at 355 nm and by energy transfer from benzophenone. The spectra at subsequent times show a decrease in absorption of benzophenone triplet state at 320 nm with concomitant increase of the band at 360 nm with an isosbestic point at 340 nm. It is clear that in the early stages following excitation, that as the benzophenone triplet decays the lumichrome triplet is formed. The quenching constant associated with this energy transfer is determined as approximately 3×10^9 dm^3 mol^{-1} s^{-1}.

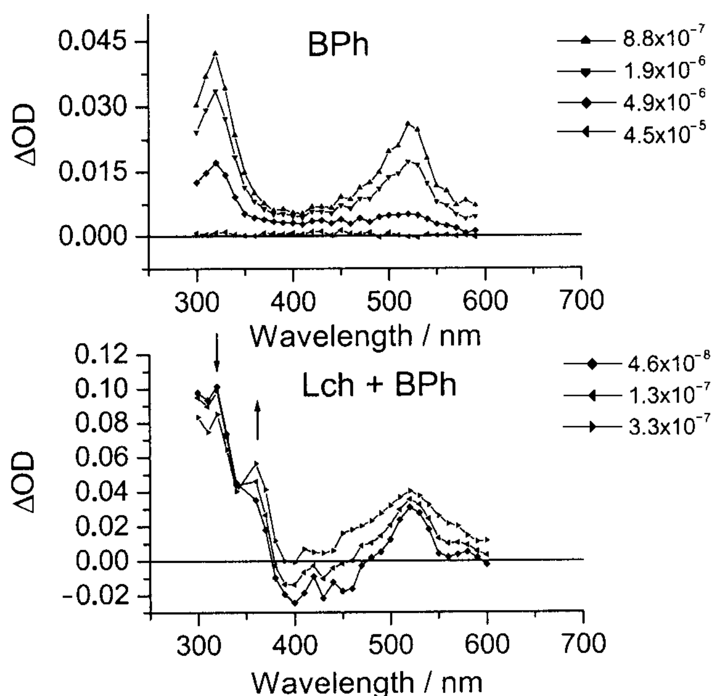

Figure 4. Transient absorption spectra of benzophenone, BPh, and benzophenone in the presence of lumichrome, Lch+BPh, in deoxygenated acetonitrile at room temperature. Numbers on each panel represent the times after laser excitation at 355 nm, (cell pathlength = 1 cm).

The intersystem crossing quantum yield of the lumichrome was calculated by first determining the molar absorption coefficient of the triplet state at the peak of its absorption relative to that of benzophenone in deoxygenated acetonitrile solution in energy transfer experiments. The molar absorption coefficient of the lumichrome at 360 nm is calculated to be 15000 ± 3000 dm^3 mol^{-1} cm^{-1}. The intersystem crossing quantum yield of lumichrome was determined *via* a comparative method using direct excitation of optically matched lumichrome solutions at 355 nm, relative to the benzophenone in acetonitrile. For benzophenone in acetonitrile the quantum yield was taken as 1.00, and the molar absorption coefficient of the benzophenone triplet state as 6500 dm^3 mol^{-1} cm^{-1} at 520 nm [34]. Using the molar absorption coefficient of 15000 dm^3 mol^{-1} cm^{-1} for lumichrome at 360 nm the quantum yield of triplet formation by lumichrome in acetonitrile is calculated to be 0.68 ± 0.1.

The results presented enable a detailed description of all the main deactivation processes for lumichrome. For example, the calculated internal conversion rate constant, k_{ic}, 0.46×10^9 s^{-1} and intersystem crossing rate constant, k_{isc}, 1.0×10^9 s^{-1}, which result in a high intersystem crossing yield, show that non-radiative decay of the excited singlet state of lumichrome is dominated by intersystem crossing to the triplet state.

CONCLUSIONS

Lumichrome and its mono- and di-methyl analogues have been studied in both acetonitrile and methanol solution. In the protic solvent methanol, no evidence for photoinduced proton transfer in the excited state has been observed. Hydrogen bonding interactions with the solvent, primarily at the carbonyl oxygens and the nitrogens at the N(10) and N(5) positions, act to reduce the rates of non-radiative transitions resulting in increased fluorescence lifetimes and quantum yields in methanol as compared to acetonitrile.

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