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Dual Fluorescence of Ellipticine: Excited State Proton Transfer from Solvent versus Solvent Mediated Intramolecular Proton Transfer

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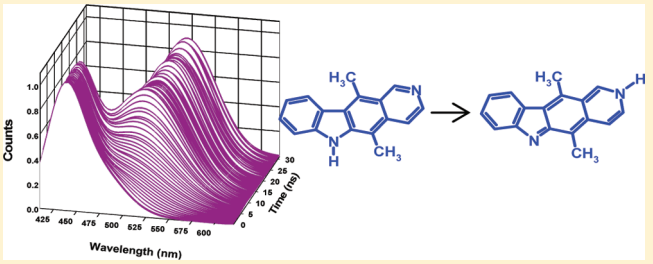
Supporting Information

ABSTRACT: Photophysical properties of a natural plant alkaloid, ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole), which comprises both proton donating and accepting sites, have been studied in different solvents using steady state and time-resolved fluorescence techniques primarily to understand the origin of dual fluorescence that this molecule exhibits in some specific alcoholic solvents. Ground and excited state calculations based on density functional theory have also been carried out to help interpretation of the experimental data. It is shown that the long-wavelength emission of the molecule is dependent on the hydrogen bond donating ability of the solvent, and in methanol, this emission band arises solely from an excited state reaction. However, in ethylene glycol, both ground and excited state reactions contribute to the long wavelength emission. The time-resolved fluorescence data of the system in methanol and ethylene glycol indicates the presence of two different hydrogen bonded species of ellipticine of which only one participates in the excited state reaction. The rate constant of the excited state reaction in these solvents is estimated to be around $4.2-8.0 \times 10^8\ s^{-1}$. It appears that the present results are better understood in terms of solvent-mediated excited state intramolecular proton transfer reaction from the pyrrole nitrogen to the pyridine nitrogen leading to the formation of the tautomeric form of the molecule rather than excited state proton transfer from the solvents leading to the formation of the protonated form of ellipticine.



1. INTRODUCTION

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, Chart 1), a natural plant alkaloid, has long been known for its anticancer activity with brain tumor specificity.[1−5] In recent years, ellipticine and structurally related compounds have found use in the treatment of obesity and tested for human pre-AIDS treatment in association with other drugs.[6,7] Although the potential of ellipticine as an antitumor drug was discovered in the early sixties, its use is limited for its low solubility in aqueous media and in many organic solvents.[8−11] This problem, however, has been circumvented in recent years using modern drug delivery technologies by attaching polymers, peptides, or micelles.[12−17] Ellipticine and its derivatives, which belong to the family of azacarbazoles, comprise both proton donating and accepting sites, and can exist in different prototropic forms. Studies in living cells have revealed that ellipticine exists both in neutral and in protonated forms in aqueous cytoplasm, but only in its protonated form in the nucleus.[18,19]

Much of research work on ellipticine is focused on its biological activity, sequence selectivity, and metabolism.[20−30] Very few photophysical studies have so far been performed on this system.[2,18,31−33] As ellipticine and its derivatives can exist in different prototropic forms, understanding the influence of environment on the prototropic equilibria is extremely important from the point of view of understanding the photophysical behavior of the system, which, in turn, can help monitor the transport of ellipticine to its target and its uptake and release by/from the carrier, Chen and co-workers recently studied the solvent effect on the photophysical properties of ellipticine.[31] In this work, which involved several media of a wide polarity range, solvent dependence of the absorption and fluorescence spectral shift were studied for the estimation of change of dipole moment on electronic excitation of the molecule. In addition to establishing the polar nature of the emitting state of the molecule, it was

Chart 1

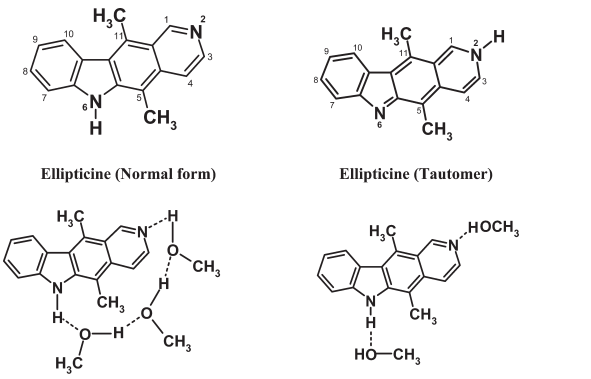


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Table 1. Spectral and Temporal Parameters of Ellipticine in Selected Solvents of Different Polarity (Measured in E_T^N Scale) and Hydrogen Bond Donor Acidity (Measured in α Scale)

solvents	E_T^N [a]	α [b]	λ_{max}^{abs} (nm)	λ_{max}^{flu} (nm)	decay parameters[c] τ_i (ns) [a_i]
hexane	0.009	0.00	382	401	15.3
acetonitrile	0.46	0.19	392	432	11.2
2-propanol	0.546	0.76	399	442	29.8
1-butanol	0.586	0.79	399	444	28.2
ethanol	0.654	0.83	400	444	29.5
methanol	0.762	0.98	398	443, 521	[d]
ethylene glycol	0.79	0.9	402,424	444, 529	[d]
trifluoroethanol	0.89	1.51	424	530	3.6 [0.009], 7.6 [0.99]
HFP	1.068	1.96	428[e]	525[e]	6.4[e]

[a] From ref 46. [b] From ref 47. [c] monitored at 435 nm. [d] See Table 2. [e] From ref 32.

found that specific hydrogen bonding interaction between ellipticine and protic solvents led to a more pronounced shift of the emission maxima in these media and contributed to a longer fluorescence lifetime compared to nonalcoholic solvents. It was also found that ellipticine exhibits a second fluorescence band in methanol. Even though the dual fluorescence was attributed to two different forms of ellipticine, the identity of the two forms was not determined or speculated and no attempt was made to find out whether the two forms arose from a ground or excited state reaction. The issue of why methanol behaved differently from the other alcohols was also not explored. Earlier Cabo et al. studied the photophysical properties of olivacine (1,5-dimethyl-6H-pyrido[4,3-b]carbazole), a molecule differing from ellipticine only in the position of a methyl group, and observed dual emission of this system as well in methanol.[34] The second emission was attributed to the tautomeric form of the molecule (Chart 1), with the latter formed via solvent-assisted excited state intramolecular proton transfer reaction from the pyrrole to the pyridine nitrogen. Miskolczy et al., who later studied the photophysical behavior of ellipticine and 6-methylellipticine, observed dual emission for both the systems in methanol.[32] They argued against the long distance, solvent-assisted excited-state proton transfer reaction proposed by Cabo et al. for structurally related molecule, olivacine, and instead, based on the observation of dual emission in 6-methylellipticine, which lacks the transferable hydrogen atom, attributed the dual fluorescence of ellipticine and its 6-methyl derivative to excited-state protonation of the pyridine moiety of the molecule by the solvent.[34] The spectral behavior of ellipticine in different solvents was correlated with the pK_a values of the respective solvents in dimethyl sulfoxide (DMSO).[32] Even though this work proposed a new mechanism for dual fluorescence of ellipticine observed only in some select alcoholic solvents, a number of important issues concerning the excited state reaction were not addressed in this work, which necessitates further investigations. For example, neither the spectral data, nor the temporal data presented in this work provide evidence of the excited state reaction in neat alcoholic solvents. The dual fluorescence of ellipticine in methanol can very well be due to excitation of the normal form and a second species (assigned as the protonated form of ellipticine produced by reaction with the solvent), both of which are present in the ground state.[35] The time-resolved fluorescence data of the system also does not provide unambiguous evidence for the excited state reaction, as no lifetime component with a negative pre-exponential factor (rise component) is reported. Second, the kinetics of the excited state reaction, which is so crucial to the understanding of the mechanism of dual fluorescence in methanol was not investigated in neutral solvents using time-resolved fluorescence technique. As the rate parameters were estimated in the presence of external acid and base, they do not represent the reaction rates in neutral solvents. There are additional issues discussed at later stages of the manuscript, which require further investigation. The objective of this work is to obtain a deeper insight and provide a more comprehensive picture of the origin of dual fluorescence of ellipticine in methanol taking into consideration additional results and theoretical findings. In this work, we have studied in detail the fluorescence decay behavior of the molecule in several carefully chosen solvents, which include some mixed solvents as well. It appears that solvent-assisted excited state intramolecular proton transfer (from the pyrrole nitrogen to the pyridine nitrogen), which is similar to that observed in other systems such as 7-hydroxyquinoline, where the proton donor and acceptor sites are separated from each other by a large distance,[36−38] better explains the dual fluorescence of ellipticine.

2. EXPERIMENTAL SECTION

2.1. Materials. Ellipticine (for fluorescence, ≥98% HPLC) was purchased from Sigma-Aldrich and used without further purification. The compound was stored in a glovebox below −20 °C. The solvents used in this work were of spectroscopic grade but were dried by following standard procedures.[39]

2.2. Instrumentation. Steady state absorption and fluorescence spectra were measured by using Cary 100 UV−vis spectrophotometer (Varian) and Fluorolog-3 spectrofluorimeter (Horiba Jobin Yvon), respectively. Fluorescence lifetime measurements were carried out using a time- correlated single-photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH).[40−42] Laser diodes (λ_{exc} = 405 or 439 nm) were used as the excitation source and an MCP photomultiplier (Hamamatsu R3809U-50) as the detector. The instrument response function, which was limited by the fwhm of the exciting pulse, were 118 and 117 ps for the 405 and 439 nm lasers, respectively. The lamp profile was recorded by placing a scatterer (dilute solution of Ludox in water) in place of the sample. The decay curves were analyzed by nonlinear least-squares iteration procedure using IBH DAS6 (Version 2.2) decay analysis software. The quality of the fit was assessed by the χ^2 values and the distribution of the residuals.

2.3. Method of Theoretical Calculation. All calculations were performed with the Gaussian 03 software package. The ground state geometries of the different forms of the system were

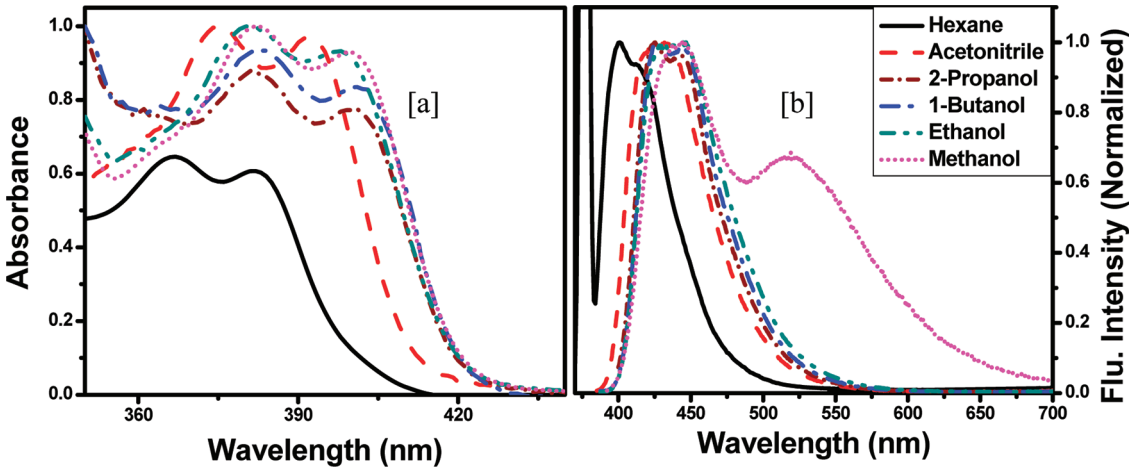

Figure 1. Absorption [a] and fluorescence spectra [b] (λ_{exc} = 375 nm) of ellipticine in hexane (solid line), acetonitrile (dash), 2-propanol (short dash dot), 1-butanol (dash dot), ethanol (dash dot dots), and methanol (short dot). All spectra normalized at their peak maximum.

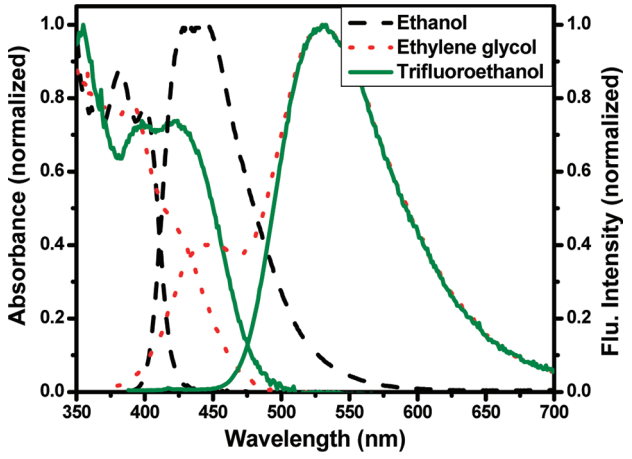

Figure 2. Absorption and fluorescence emission spectra (λ_{exc} = 405 nm) of ellipticine in ethanol (dash), ethylene glycol (dot), and trifluoroethanol (solid line). All spectra normalized at their peak maximum.

optimized in the gas phase using the hybrid DFT functional B3LYP at 6-31G* level. Excited-state calculations were performed within the time-dependent (TD-DFT) framework at the B3LYP/6-31G* level.[43,44] Both ground and excited state calculations were also carried out in different solvents by self-consistent reaction field (SCRF) method using polarized continuum (PCM) model.[45]

3. RESULTS

3.1. Spectral Studies in Neat Solvents. The spectral behavior of ellipticine has been studied in nonpolar solvent, hexane, polar aprotic solvent, acetonitrile, and polar protic solvents, 2-propanol, 1-butanol, ethanol, methanol, ethylene glycol (EG), and 2,2,2-trifluoroethanol (TFE). The polarity of these solvents, as indicated by their microscopic solvent polarity parameter, E_T^N values[46,47] and the hydrogen bond donating ability of the solvents, as characterized by the Kamlet−Taft's hydrogen bond acidity parameter (α),[46,47] are collected in Table 1. Representative absorption and fluorescence spectra of ellipticine in these solvents are shown in Figures 1 and 2. As can be seen, the first absorption maximum of ellipticine appears at ∼382 nm in *n*-hexane and the band shows small bathochromic shift with increase in polarity of the medium. In protic solvents such as 2-propanol, 1-butanol, ethanol, and methanol, the spectral shift is more pronounced compared to polar aprotic solvent, such as acetonitrile. This behavior is consistent with the literature and in accordance with specific hydrogen bonding interaction with the protic solvents.[31] It is interesting to note the appearance of a new absorption band above 420 nm in EG (and also in TFE), which has resulted from the interaction of ellipticine with the solvent in the ground state. Miskolczy et al. attributed the new absorption band to the protonated form of ellipticine as a similar absorption band was observed in more acidic solvent, 1,1,1,3,3,3-hexafluoro-2-propanol (HFP).[32] We are, however, not so sure about this assignment as methanol and EG may not be acidic enough to protonate ellipticine in the ground state. The 420 nm species can very well be due to the hydrogen-bonded complex of ellipticine, as hydrogen bonding also leads to the formation of long-wavelength absorption band.[48,49] It should be noted in this context, contrary to the findings of Miskolczy et al., who observed a similar absorption band in methanol as well,[32] we did not observe this new absorption band in methanol.

The steady state fluorescence spectrum of ellipticine is characterized by a broad band in all the solvents. The fluorescence displays a more pronounced red shift compared to the absorption spectrum with increase in polarity of the medium. This is due to the more polar nature of the emitting state of ellipticine compared to the ground state.[31] In *n*-hexane, the emission maximum is observed at 401 nm and it shifts to 432 nm in acetonitrile. A further red shift due to hydrogen bonding interaction is observed in alcoholic solvents (Table 1). The most interesting aspect of the fluorescence behavior of ellipticine is that the molecule exhibits a second emission band at a longer wavelength ($\lambda \geq 520$ nm) in methanol and EG. However, in 2-propanol, 1-butanol, and ethanol, this long wavelength emission band could not be observed. In TFE, the molecule exhibits only the long wavelength emission band (Figure 2). Miskolczy et al., who found that in highly acidic HFP, ellipticine shows only the long wavelength fluorescence band similar to that observed in TFE, suggested that this emission band arises from

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the protonated form of ellipticine.[32] We find the fluorescence excitation spectra of ellipticine corresponding to the long wavelength emission in methanol and EG (Figure 3), which is such an important experimental data, but was not recorded earlier, to be quite instructive. These spectra clearly indicate that, in methanol, the species responsible for the second emission band are formed only in the excited state. This finding is contrary to the earlier report.[32] In EG, the species is, however, formed in the ground state.

3.2. Time-Resolved Fluorescence Studies in Neat Solvents. In hexane, acetonitrile, 2-propanol, 1-butanol, and ethanol, where only one emission band is observed, the fluorescence decay behavior is found to be a single exponential with a lifetime ranging from 11 to 30 ns (Table 1). However, in methanol and EG, where dual fluorescence is observed, the fluorescence decay profiles corresponding to both the short and the long wavelength emission are best described by a sum of two exponentials. As can be seen from the decay parameters collected in Table 2, the two measured lifetime components in methanol in the short wavelength region (420−470 nm) are 2.2 (20%) and 3.5 ns (80%). On the other hand, the fluorescence time profiles of the long wavelength emission are characterized by a rise component (which is associated with a negative pre-exponential factor) of 2.2 ns, along with a decay component of 8.2 ns. Representative decay profiles for the two emission bands along with the biexponenial fits to the decay profiles are shown in Figure 4.

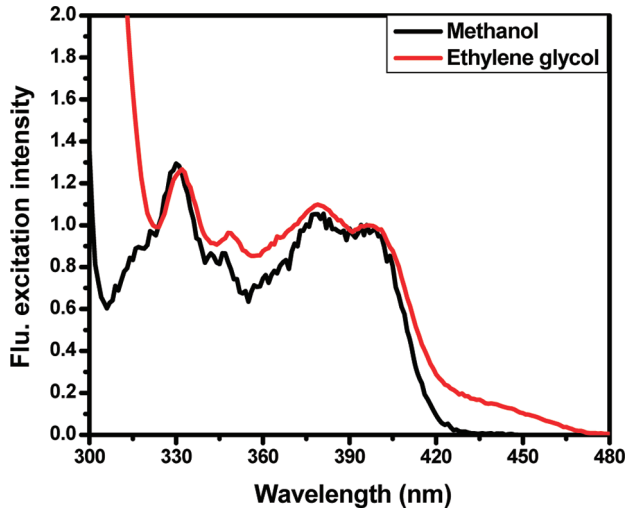

Figure 3. Fluorescence excitation spectra of ellipticine in methanol and ethylene glycol. The monitoring wavelength was 575 nm (normalized at 396 nm).

The time-resolved emission spectra (TRES) show time-evolution of the two different components of ellipticine in methanol (Figure 5), in particular, it clearly captures the evolution of the long wavelength component, thus, providing the first direct evidence of the formation of this species as a result of an excited state reaction. In EG, where dual emission is also observed, the fluorescence decay parameters (λ_{exc} = 405 nm) shown in Table 2 suggest that the time-dependence of the two emission bands is very similar to that in methanol. However, when EG solution of ellipticine is excited at 439 nm, which corresponds to the long wavelength absorption band arising from ground state interaction, no rise component is observed (Figure S1, Supporting Information).

In TFE, where only the long wavelength emission is observed, the decay profile is best represented by a biexponential decay function with the characteristic decay times of 3.6 and 7.6 ns (Figure 6) and no rise component is observed.

3.3. Spectral and Temporal Studies in Mixed Solvents. *3.3.1. Addition of Methanol.* To understand the origin of the second fluorescence band of ellipticine observed only in some alcohols and the nature of the interaction responsible for the formation of this species, the study has been extended to mixed solvents containing a controlled amount of protic solvent in a polar aprotic solvent (such as acetonitrile and tetrahydrofuran (THF)). Figure 7 displays characteristic changes of the absorption spectrum of an acetonitrile solution of ellipticine in the presence of various quantities of methanol. As can be seen, addition of methanol leads to small changes in the absorbance value with a red shift of the spectrum, but no isosbestic point is observed. Lack of isosbestic point is suggestive of the presence of more than two absorbing components in the solution. As far as the emission is concerned, a small increase in intensity is observed initially. However, subsequent addition leads to quenching of the short wavelength fluorescence. It is important to note here that neither the second emission band is observed, nor did we observe a noticeable increase in the fluorescence intensity in the long wavelength emission region (510−550 nm) even when a large quantity (∼50% by volume) of methanol was added. When THF is used in place of acetonitrile, a similar result is observed. The time-resolved fluorescence data in mixed solvents (Table S1, Supporting Information) did not show any rise component associated with the long wavelength emission component.

3.3.2. Addition of EG. Addition of EG to an acetonitrile solution of ellipticine leads to the appearance of a long wavelength absorption band around 420 nm and, also, the long wavelength emission band (Figure 8). No isosbestic point is observed in this case also. The time-resolved fluorescence decay parameters in

Table 2. Fluorescence Decay Parameters of Ellipticine (λ_{exc} = 405 nm) in Methanol and Ethylene Glycol (EG)

	decay parameters: τ_i (ns) [a_i]		χ^2	
monitoring wavelength (nm)	in methanol	in EG	MeOH	EG
420	2.2 [0.185], 3.55 [0.815]	1.1 [0.632], 3.4 [0.367]	1.05	1.1
430	2.17 [0.184], 3.57 [0.815]	1.2 [0.605], 3.4 [0.394]	1.08	0.9
440	2.21 [0.205], 3.58 [0.794]	1.1 [0.594], 3.4 [0.405]	1.01	1.1
520	2.0 [−0.302], 8.1 [0.070]	1.1 [−0.051], 8.2 [0.949]	1.12	1.01
530	2.2 [−0.36], 8.2 [0.638]	1.2 [−0.126], 8.2 [0.873]	1.1	1.05
540	2.2 [−0.39], 8.2 [0.601]	1.3 [−0.157], 8.2 [0.843]	1.13	1.07
550	2.2 [−0.421], 8.2 [0.578]	1.2 [−0.184], 8.3 [0.815]	1.18	1.04

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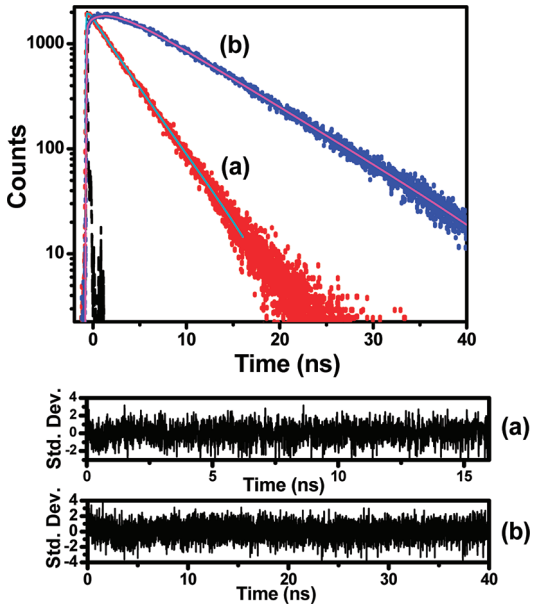

Figure 4. Fluorescence decay profiles (λ_{exc} = 405 nm) of ellipticine in methanol at 435 (a) and 535 nm (b). The experimental decay profiles are indicated by the dots and the instrument profile as dash. The solid lines represent the best-fit to the decay curves. The residuals are indicated below for the respective decay profiles.

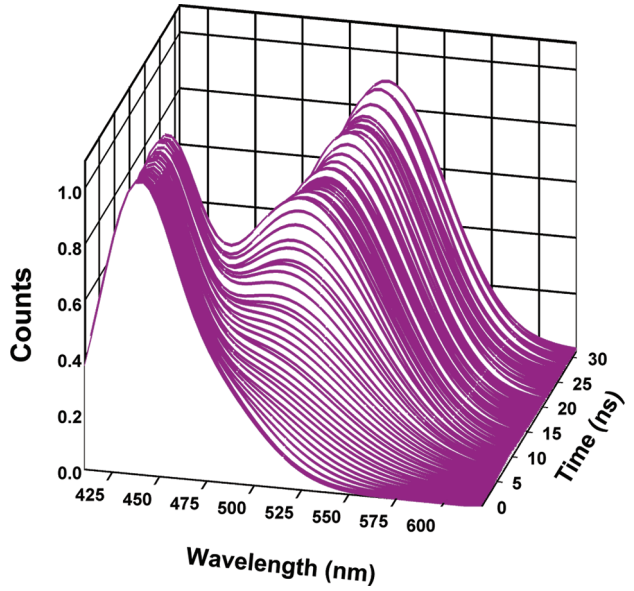

Figure 5. Time-resolved fluorescence emission spectra of ellipticine in methanol (λ_{exc} = 405 nm).

mixed solvents, which are presented as Supporting Information (Table S2, Supporting Information), are in accordance with expectation. Similar results were obtained on addition of EG to ellipticine dissolved in hexane, toluene, *N*,*N*-dimethylformamide, and so on.

3.4. Theoretical Calculations. The calculated energies corresponding to the optimized (B3LYP/6-31G*) geometries of the normal and tautomeric forms indicate that in the ground state the normal form of ellipticine more stable than its tautomer by ~0.927 eV (Figure 9) in the gas phase. Even though this

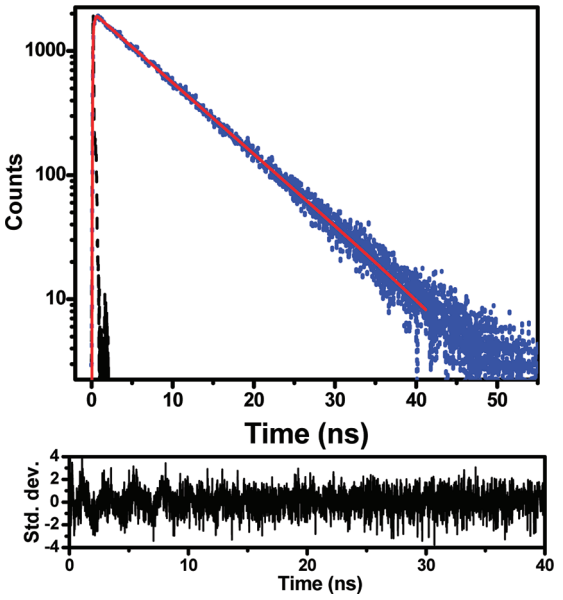

Figure 6. Fluorescence decay profiles (λ_{exc} = 405 nm) of ellipticine in TFE at 520 nm. The experimental decay profile is indicated by the dots and the instrument profile is indicated by a dash. The solid lines represent the best-fit to the decay curves. The residuals for the fit to the decay profile are indicated below.

energy gap between the two forms decreases substantially in acetonitrile and methanol, it still remains much higher than the thermal energy available at room temperature, indicating that ellipticine exists in its normal form in the ground state in these solvents as well. The excited state calculations (TD-DFT, B3LYP/6-31G* level), however, show that the first excited singlet state of the tautomer, whose energy is estimated by adding the calculated excitation energy for the lowest energy transition with the ground state energy, is energetically lower than that of the normal form of ellipticine by about 0.324 eV in vacuo. In acetonitrile and methanol, this energy gap is slightly higher (Figure 9). Thus, the theoretical calculations indicate that the transformation of the normal form to its tautomer is energetically feasible in the excited state. Hence, the long wavelength emission can possibly arise from the tautomer of ellipticine.

The calculated lowest energy transition for the normal form of ellipticine is found to be 3.247 eV in acetonitrile, which is comparable to the experimental energy (3.166 eV) estimated from the λ_{max}^{abs} value in acetonitrile (Table 3). The molecular orbitals associated with the lowest energy transition are also studied and careful examination of the shapes of the molecular orbitals reveals the nature of the transition. Three different excitations, HOMO−LUMO, (HOMO − 1)−(LUMO + 1), and (HOMO − 1)−LUMO contribute to the first excited state of the normal form in vacuum. In all cases, the excitation mainly arises from the HOMO−LUMO transition indicated by the transition coefficients, having $\pi-\pi^*$ character (Table 3).

4. DISCUSSION

Let us concentrate on the findings in methanol and EG, the two solvents in which dual fluorescence is observed. In all other solvents, where only one emission band is observed, it is not difficult to figure out its origin. As the theoretical calculations suggest that ellipticine exists in its normal form in the ground

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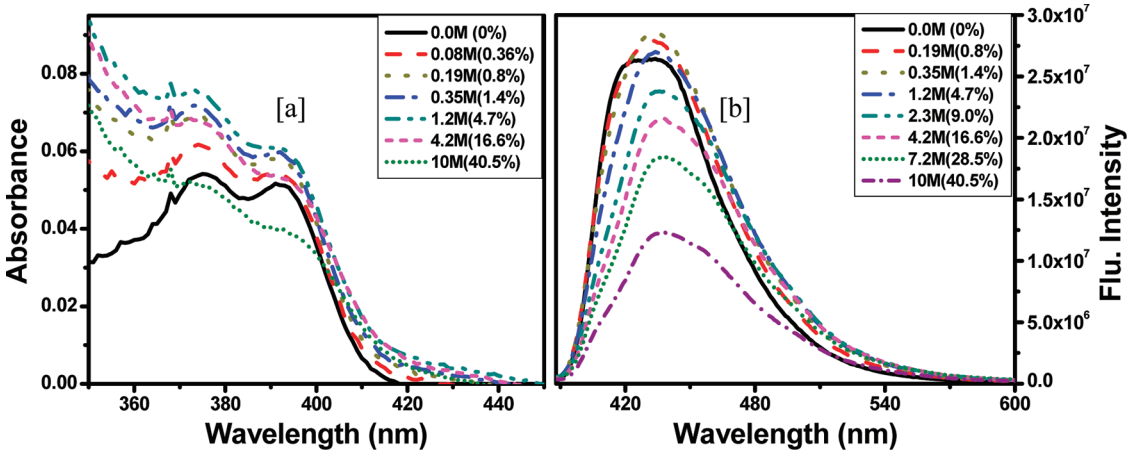

Figure 7. Absorption [a] and fluorescence emission [b] (λ_{exc}= 405) spectra of ellipticine in acetonitrile for different concentrations of added methanol (0−10 M).

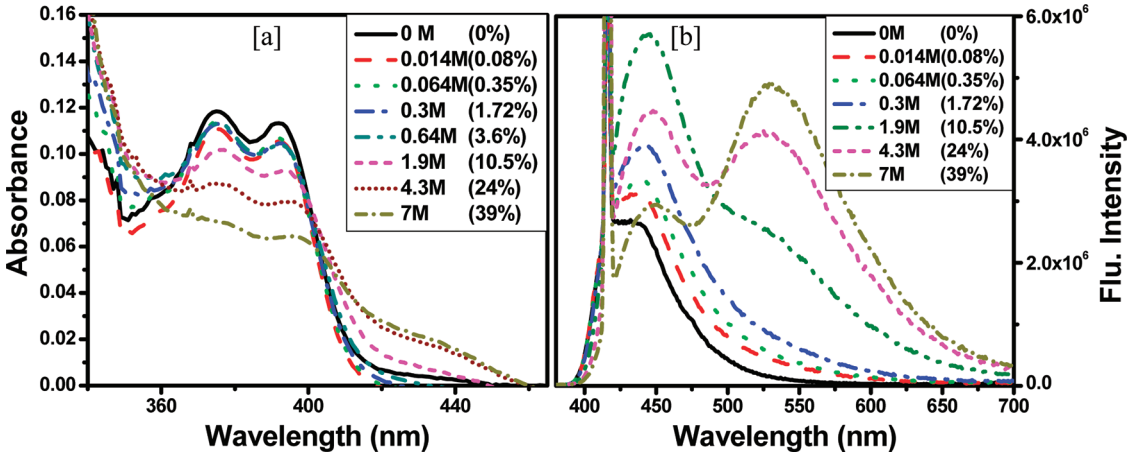

Figure 8. Absorption [a] and fluorescence emission [b] (λ_{exc} = 415 nm) spectra of ellipticine in acetonitrile for different concentration of added ethylene glycol (0−7 M).

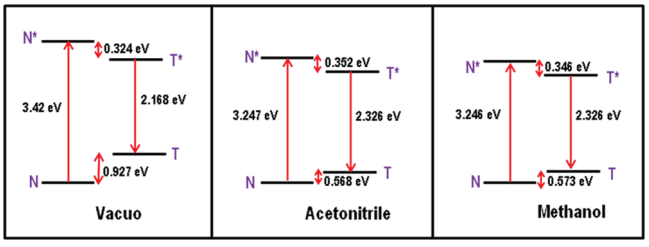

Figure 9. Schematic representation of the potential energies of ellipticine for both normal (N) and tautomeric form (T), as obtained by theoretical calculations.

state, it is evident that, in not too acidic protic solvents ($\alpha \leq 0.83$),[46,47] the normal form and its various hydrogen bonded forms are present in the ground state. In highly acidic solvents, such as in TFE and HFP, whose α values are greater than 1.5,[46,47] the basic pyridine nitrogen of the molecule is expected to be protonated, and hence, ellipticine is present in its protonated form in the ground state.

Among all the alcohols, dual emission is observed only in neat methanol and EG, whose α values (0.98 and 0.90, respectively) are in between these two group of protic solvents. As the long wavelength absorption and emission bands are related to the hydrogen bond donating ability of the solvent, it is quite reasonable to assume that these bands arise from the protonated form of ellipticine, as Miskolczy et al. suggested.[32] However, this picture presents the following difficulties.

(i) Even though methanol has a higher hydrogen bond donating ability compared to EG,[46,47] the long wavelength absorption band ($\lambda_{max} \sim 424$ nm) is observed in EG and in mixed solvents containing EG, but not in methanol. (ii) A higher intensity of the long wavelength emission relative to the short wavelength emission (I_{LW}/I_{SW}) is also not expected for the same reason. (iii) If solvent acidity is the only parameter responsible for the formation of the long wavelength emission band, one would have expected this emission in methanol containing mixed solvents as well. However, the absence of this band in acetonitrile−methanol mixture suggests that other factors also contribute to this emission. (iv) A biexponential fluorescence decay behavior of the short wavelength emission band with the kind of decay parameters observed cannot

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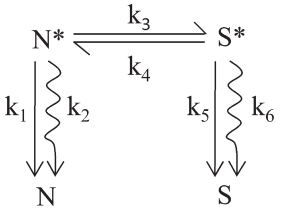
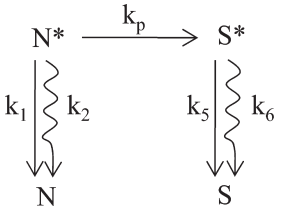
Table 3. Lowest Energy Transition (B3LYP/6-31G*), Oscillator Strength, And Nature of Transition in the Normal Form of Ellipticine in Vacuo and in Other Solvents

	transition energy (eV)				
medium	theory	expt	osc. strength	MOs involved (tr. coeff.)	nature
vacuo	3.420		0.0568	HOMO → LUMO (0.64414)	$\pi \rightarrow \pi^*$
				HOMO − 1 → LUMO + 1 (0.12850)	
				HOMO − 1 → LUMO (0.12109)	
ACN	3.247	3.166	0.0661	HOMO → LUMO (0.65799)	$\pi \rightarrow \pi^*$
				HOMO − 1 → LUMO + 1 (−0.10693)	
MeOH	3.246	3.118	0.0654	HOMO → LUMO (0.65779)	$\pi \rightarrow \pi^*$
				HOMO − 1 → LUMO + 1 (−0.10735)	

Scheme 1



Scheme 2



be explained if the excited state reaction leads to the formation of the protonated form of ellipticine. This is because if ellipticine undergoes protonation in the excited state and the two species are in equilibrium, one would expect biexponential fluorescence time profile for both the species with two identical lifetimes. On the other hand, if the two species are not in equilibrium, one would expect single exponential decay for ellipticine and biexponential decay (with one lifetime component same as that observed for ellipticine) for the protonated species. However, as can be seen, the experimental decay parameters (Table 2) do not correspond to either of these two possible scenarios.

To understand the nature of the excited state reaction in methanol and to determine the kinetics of the reaction, we begin by considering the presence of the normal form of ellipticine and its hydrogen bonded forms, which are represented as N in the following schemes, in the ground state. When excited, these species give rise to the short wavelength emission and undergo reaction to form another species (S*), which is responsible for the long wavelength emission. S* can be the protonated form of ellipticine, or its tautomer (Chart 1).[50] If N* is in equilibrium with S*, as shown in Scheme 1, then the time dependence of the two species is given by eqs 1 and 2, which imply that both the species would exhibit a biexponential fluorescence time profile with the two decay times associated with N * and S* are identical. However, the kinetic data (Table 2) for the present system is not consistent with this scheme.

$$[N^*] = \frac{[N^*]_0}{\lambda_2 - \lambda_1}\left[(\lambda_2 - X)e^{-\lambda_1 t} + (X - \lambda_1)e^{-\lambda_2 t}\right] \quad (1)$$

$$[S^*] = \frac{k_3[N^*]_0}{\lambda_2 - \lambda_1}\left[e^{-\lambda_1 t} - e^{-\lambda_2 t}\right] \quad (2)$$

where $X = k_1 + k_2 + k_3$ and $Y = k_4 + k_5 + k_6$.

$$\lambda_1 = \frac{1}{2}\left\{(X + Y) - [(X - Y)^2 + 4k_3k_4]^{1/2}\right\}$$

$$\lambda_2 = \frac{1}{2}\left\{(X + Y) + [(X - Y)^2 + 4k_3k_4]^{1/2}\right\}$$

However, if the equilibrium is not established in the excited state and the formation of S* from N* is represented by Scheme 2, then the time-dependence of N* and S* is given by eqs 3 and 4. In this case, N* should show a single exponential decay and S* should exhibit a rise time identical with the decay time of N* and a decay time that is characteristics of the lifetime of S* under the experimental condition.

$$[N^*] = [N^*]_0 e^{-(k_N + k_p)t} \quad (3)$$

$$[S^*] = \left(\frac{[N^*]_0 k_p}{k_N + k_p - k_S}\right)\left[e^{-k_S t} - e^{-(k_N + k_p)t}\right] \quad (4)$$

The kinetic data presented in Table 2 is, however, not consistent with this mechanism as well. An inspection of the decay characteristics reveals that while the 1.1−2.2 ns lifetime component is connected with the excited state reaction (as it is associated with both *N** and *S**), the 3.4−3.6 ns decay component of *N**, whose origin needs to be found, is not connected with the excited state reaction.

Marks et al. studied the photophysical behavior of pyridocarbazole derivatives, and observed dual emission of dipyrido [2,3-*a*:3′,2′-*i*]carbazole (DPC) and 1*H*-pyrrolo[3,2-*h*]quinoline (PQ) in alcoholic solvents.[51] The short wavelength fluorescence emission was attributed to the normal solute−solvent complex and long wavelength band was ascribed to the tautomeric form of the cyclic solute−solvent complex.[51] The long wavelength emission displayed a rise component that matched with one of the decay components of the first emission. The decay characteristics of the two emission bands indicated solvent mediated excited state intermolecular proton transfer from a cyclic

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solute−solvent complex. Interestingly, the presence of another species, which does not undergo excited state proton transfer, was found and the same was attributed to the "blocked" solute−solvent complex.[51] In fact, long distance solvent mediated excited state intramolecular proton transfer has been proposed also for other amphoteric molecule such as 7-hydroxyquinoline in methanol.[36−38]

Taking into consideration the above literature, the kinetic data of ellipticine can be explained by suitably modifying Scheme 2 and proposing two different types of hydrogen bonded ellipticine molecules. The first set of solvated molecules of ellipticine, which does not undergo excited state reaction, is responsible for the long-lived (3.4−3.6 ns) component of N^*. The "blocked" solvated species shown in Chart 1 is one of these species. The other set of solvated species, which can form the "cyclic" hydrogen-bonded species (Chart 1) during the excited state lifetime of ellipticine and facilitate the excited state reaction across the hydrogen bonded chain, contributes to the short lifetime component of N^*. Essentially, the excited state reaction involves two steps: (i) solvent reorganization around ellipticine to form the "cyclic" solvated species and (ii) rapid proton transfer (relay) along the chain. The rate constant of the excited state reaction, estimated from the rise time associated with S^* emission (8 and 4.2×10^8 s^{-1} in EG and methanol, respectively), obviously represents the kinetics of the slowest of the two steps, which is clearly the formation of the "cyclic" complex. The very fact that only one particular type of solvated N^* (of the two types present) undergoes the excited state reaction, as indicated by the time-resolved study, implies that S^* is the tautomer of ellipticine. Had the excited state process been a bimolecular reaction between ellipticine and solvent molecules, it would not have been possible to think of two different types of ellipticine of which only one undergoes protonation.

The excited state reaction mechanism proposed here is not only consistent with the theoretical calculations, but can also account for the observations, which cannot be interpreted in terms of proton transfer from the solvent. For example, a higher I_{LW}/I_{SW} in EG compared to methanol can be explained as follows. The pyrrole hydrogen atom is far off is (∼7 Å) from the pyridine nitrogen atoms. This implies that at least three molecules of methanol are needed to form the cyclic solute−solvent complex through which this proton can be transferred. However, in the case of EG, only two molecules are sufficient. A higher I_{LW}/I_{SW} in EG compared to methanol is a reflection of the relative ease of formation of the cyclic solute−solvent complex involving two molecules rather with three molecules. This also explains why the rate constant of the excited state reaction is higher in EG compared to methanol despite better hydrogen bond donating ability of the latter.

Both spectral and time-resolved data has shown that the excited state reaction of ellipticine does not occur in mixed solvents (methanol + acetnitrile/THF). This is another observation that cannot be explained in terms of excited state protonation of the molecule. As cyclic solute−solvent complex formation involving three methanol molecules is prevented by a large number of nonalcoholic solvent molecules present in the medium, one can account for this observation easily with the help of this new mechanism.

5. CONCLUSION

The origin of dual fluorescence of ellipticine, as observed in some alcoholic solvents, is reinvestigated. The steady state and time-resolved experiments presented here provide the first direct evidence of the excited state reaction in ellipticine and the rate constant of this process in neat methanol and ethylene glycol. Contrary to the earlier studies, which suggested the dual emission of ellipticine in methanol to originate from the photoexcited normal and protonated forms of the molecule with the latter produced as a result of proton transfer from the solvent, the present results seem to indicate that the excited state reaction involves solvent reorganization around ellipticine to form the "cyclic" solvated species followed by rapid proton transfer (relay) along the chain and the two emission bands arise from the normal and tautomeric forms of ellipticine. The measured rate constant of the reaction perhaps represents the kinetics of the formation of the "cyclic" complex, which is the slowest of the two-step process. The ease of formation of the cyclic complex involving two molecules of EG compared to that requiring three molecules of methanol explains why the excited state reaction is faster in EG compared to methanol, even though the hydrogen bond donating ability of the latter is more than the former. The time-resolved experiments also reveal the existence of a second set of hydrogen bonded ellipticine molecules, which do not contribute to the excited-state proton-transfer process.

■ ASSOCIATED CONTENT

Supporting Information. Fluorescence decay profiles (λ_{exc} = 439 nm) of ellipticine in ethylene glycol, MO picture associated with the electronic transition in the normal form of ellipticine, time-resolved fluorescence data of ellipticine (λ_{exc} = 405 nm) in THF and ACN with various amounts of methanol and EG. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Garbett, N. C.; Graves, D. E. *Curr. Med. Chem.* **2004**, *4*, 149.
(2) Smith, A. F.; Horning, E. C.; Goodwin, S. *J. Am. Chem. Soc.* **1959**, *81*, 1903.
(3) Le Pecq, J.-B.; Xuong, N.-D.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(4) Ohashi, M.; Oki, T. *Expert Opin. Ther. Pat.* **1996**, *6*, 1285.
(5) Woodward., R. B.; Iacobucci., G. A.; Hochstein., F. A. *J. Am. Chem. Soc.* **1959**, *81*, 4434.
(6) Ellies, D.; Rosenberg, W. Ellipticine compounds for treating obesity. PCT Int. Appl. WO 2010107704; 2010, p 1.
(7) Hallard, M.; Pontiggia, P.; Mathe, G. *Biomed. Pharmacother.* **1996**, *50*, 510.
(8) Clarysse, A.; Brugarolas, A.; Siegenthaler, P.; Abele, R.; Cavalli, F.; De Jager, R.; Renard, G.; Rozencweig, M.; Hansen, H. H. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 243.

(9) Dodion, P.; Rozencweig, M.; Nicaise, C.; Piccart, M.; Cumps, E.; Crespeigne, N.; Kisner, D.; Kenis, Y. *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 519.
(10) Gouyette, A.; Huertas, D.; Droz, J.-P.; Rouesse, J.; Amiel, J.-L. *Eur. J. Cancer Clin. Oncol.* **1982**, *18*, 1285.
(11) Paoletti, C.; Le Pecq, J. B.; Dat-Xuong, N.; Juret, P.; Garnier, H.; Amiel, J.-L.; Rouesse, J. *Recent Res. Cancer Res.* **1980**, *40*, 107.
(12) Liu, J.; Xiao, Y.; Allen, C. *J. Pharm. Sci.* **2004**, *93*, 132.
(13) Searle, F.; Gac-Breton, S.; Keane, R.; Dimitrijevic, S.; Brocchini, S.; Sauville, E. A.; Duncan, R. *Bioconjugate Chem.* **2001**, *12*, 711.
(14) Trubetskoy, V. S.; Torchilin, V. P. *Adv. Drug Delivery Rev.* **1995**, *16*, 311.
(15) Czerwinski, G.; Tarasova, N. I.; Michejda, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11520.
(16) Moody, T. W.; Czerwinski, G.; Tarasova, N. I.; Moody, D. L.; Michejda, C. J. *Regul. Pept.* **2004**, *123*, 187.
(17) Moody, T. W.; Czerwinski, G.; Tarasova, N. I.; Michejda, C. *J. Life Sci.* **2002**, *71*, 1005.
(18) Sbai, M.; Lyazidi, S. A.; Lerner, D. A.; del Castillo, B.; Martin, M. A. *Anal. Chim. Acta* **1995**, *303*, 47.
(19) Schwaller, M.-A.; Allard, B. *J. Phys. Chem.* **1994**, *98*, 4209.
(20) Reha, D.; Kabelac, M.; Ryjacek, F.; Sponer, J.; Sponer, J. E.; Elstner, M.; Suhai, S.; Hobza, P. *J. Am. Chem. Soc.* **2002**, *124*, 3366.
(21) Froelich-Ammon, S. J.; Patchan, M. W.; Osheroff, N.; Thompson, R. B. *J. Biol. Chem.* **1995**, *270*, 14998.
(22) El Hage Chahine, J. M.; Bertigny, J.-P.; Schwaller, M.-A. *J. Chem. Soc., Perkin Trans. 2* **1989**, 629.
(23) Auclair, C. *Arch. Biochem. Biophys.* **1987**, *259*, 1.
(24) Bailly, C.; Ohuigin, C.; Rivalle, C.; Bisagni, E.; Waring, M. *J. Nucleic Acids Res* **1990**, *18*, 6283.
(25) Behravan, G.; Leijon, M.; Selhlstedt, U.; Vallberg, H.; Bergamn, J.; Gra slund, A. *Biopolymers* **1994**, *34*, 599.
(26) Canals, A.; Purciolas, M.; Aymami, J.; Coll, M. *Acta Crystallogr.* **2005**, *61*, 1009.
(27) Dodin, G.; Schwaller, M. A.; Aubard, J.; Paoletti, C. *Eur. J. Biochem.* **1988**, *176*, 371.
(28) Elcock, A. H.; Rodger, A.; Richards, W. G. *Biopolymers* **1996**, *39*, 309.
(29) Jain, S. C.; Bhandary, K. K.; Sobell, H. M. *J. Mol. Biol.* **1979**, *135*, 813.
(30) Kohn, K. W.; Waring, M. J.; Glaubiger, D.; Friedman, A. *Cancer Res.* **1975**, *35*, 71.
(31) Fung, S. Y.; Duhamel, J.; Chen, P. *J. Phys. Chem. A* **2006**, *110*, 11446.
(32) Miskolczy, Z.; Biczok, L.; Jablonkai, I. *Chem. Phys. Lett.* **2006**, *427*, 76.
(33) Sbai, M.; Ait Lyazidi, S.; Lerner, D. A.; del Castillo, B.; Martin, M. A. *J. Pharm. Biomed. Anal.* **1996**, *14*, 959.
(34) Cabo, J. L.; Faria, H. B.; Portuga, S. G. M.; Silva, M. A. A.; Brinn, I. M. *Photochem. Photobiol.* **1999**, *69*, 664.
(35) In fact, one obtains this picture from the spectral data of ellipticine in methanol, as presented in Table 1 of ref 32, indicating the formation of a new species in the ground state.
(36) Itoh, M.; Adachi, T.; Tokumura, K. *J. Am. Chem. Soc.* **1984**, *106*, 850.
(37) Bhattacharya, B.; Samanta, A. *J. Phys. Chem. B* **2008**, *112*, 10101.
(38) Kang, B.; Ko, K. C.; Park, S.-Y.; Jang, D.-J.; Lee, J. Y. *Phys. Chem. Chem. Phys.* **2011**, *13*, 6332.
(39) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon Press: New York, 1980.
(40) Paul, A.; Samanta, A. *J. Phys. Chem. B* **2008**, *112*, 16626.
(41) Santhosh, K.; Banerjee, S.; Rangaraj, N.; Samanta, A. *J. Phys. Chem. B* **2010**, 1967.
(42) Santhosh, K.; Samanta, A. *J. Phys. Chem. B* **2010**, *114*, 9195.
(43) Bauernschmitt, R.; Ahlrichs, R. *Chem. Phys. Lett.* **1996**, *256*, 454.
(44) Casida, M. E.; Jamorski, C.; Casida, K. C.; Salahub, D. R. *J. Chem. Phys. Rev. B* **1998**, *108*, 4439.
(45) Cossi, M.; Barone, V. *J. Chem. Phys.* **2000**, 2427.
(46) Reichardt, C. *Chem. Rev* **1994**, *94*, 2319.
(47) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*; Wiley-VCH: New York, 2004; p 433.
(48) Diverdi, L. A.; Topp, M. R. *J. Phys. Chem.* **1984**, *88*, 3447.
(49) Mataga, N.; Tsuno, S. *Bull. Chem. Soc. Jpn.* **1957**, *30*, 368.
(50) We take note of the argument used by Miskolczy et al. (ref 32) in support of their mechanism. However, we are left with no choice but to reconsider an alternative mechanism to account for the new results presented in this work. At this moment, we have no idea how to explain the dual fluorescence of 6-methylellipticine (ref 32) using our mechanism. This is an issue that needs to be looked into.
(51) Marks, D.; Zhang, H.; Borowicz, P.; Waluk, J.; Glasbeek, M. *J. Phys. Chem. A* **2000**, *104*, 7167.