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Solvent effect on the singlet excited state dynamics of 5-fluorouracil in acetonitrile as compared to water

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Title running head : Femtosecond fluorescence and TDDFT study of 5-fluorouracil in acetonitrile

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Abstract. The excited state dynamics of 5-fluorouracil in acetonitrile has been investigated by femtosecond fluorescence upconversion spectroscopy in combination with quantum chemistry TD-DFT calculations ((PCM/TD-PBE0). Experimentally it was found that when going from water to acetonitrile solution the fluorescence decay of 5FU becomes much faster. The calculations show that this is related to the opening of an additional decay channel in acetonitrile solution since the dark n/π^* excited state becomes near degenerate with the bright π/π^* state, forming a conical intersection close to the Franck-Condon region. In both solvents a $S_1 - S_0$ conical intersection, governed by the out-of-plane motion of the fluorine atom is active, allowing an ultrafast internal conversion to the ground state.

Key Words.

DNA bases, pyrimidine bases, time-resolved fluorescence, fluorescence upconversion, TDDFT density functional calculations, conical intersections,

Introduction. There is currently a keen interest in characterizing the electronically excited states of the DNA bases. These are known to undergo extremely fast non-radiative deactivation, but the underlying mechanisms remained largely unknown up to very recently. Rapid advances in time-resolved spectroscopic techniques and excited state quantum mechanical calculations have now made it possible to get fundamental insights in their excited state dynamics, both in the gas phase and in aqueous solution.¹⁻⁵ However, most recent studies explain the ultrafast decay on purely intramolecular grounds, only marginally affected by solvent effects. The role played by environmental effects in the excited state dynamics is instead far from being completely assessed. The large majority of the ultrafast studies have been performed in aqueous solution, whereas the number of comparative studies in different solvents is still very limited.⁶⁻⁸ In these investigations, the excited state deactivation of DNA bases is still found to be ultrafast, but modulated by $\pm 50\%$ with respect to aqueous solution, depending on the base and the solvent. Crespo-Hernandez et al. summarized solvent effects on the singlet excited states of DNA bases as modest.⁹ It should be noted though, that in none of these studies were the experimental results accompanied by any theoretical calculations.

In this communication we show how the nature of the solvent significantly modulates, by a factor of four, the excited state lifetime of a pyrimidine nucleobase. More precisely, 5-fluorouracil (5FU, inset in Figure 2) has been investigated in acetonitrile, a polar but aprotic solvent, by femtosecond fluorescence upconversion. The experimental observations are compared with recent results concerning different uracils in aqueous solution¹⁰ and interpreted with the aid of quantum chemistry TD-DFT calculations ((PCM/TD-PBE0). From a purely theoretical point of view, obtaining a reliable description of the relaxed excited state geometry of a large organic molecule by TD-DFT calculations including solvent effects is today only at its beginning.^{1,10-12}

Experimental details. 5FU was purchased from Sigma Aldrich. Acetonitrile (Merck UV spectroscopic grade) was used without further purification. Absorption and fluorescence spectra were recorded with a Perkin Lambda 900 spectrophotometer and a SPEX Fluorolog-2 spectrofluorometer

respectively. The femtosecond fluorescence upconversion setup used has been described earlier.¹³ It uses the frequency-tripled output at 267 nm from a Ti:S laser for excitation. The Gaussian apparatus function (fwhm \approx 310 fs) allows a time resolution of about 100 fs after deconvolution. Total fluorescence decays shown below were constructed from the parallel and perpendicular signals ($I_{par}(t)$ and $I_{perp}(t)$) according to $I(t) = I_{par}(t) + 2I_{perp}(t)$. All upconversion measurements were performed at room temperature (20 ± 1 °C) under aerated conditions. Solutions ($\approx 2.5 \times 10^{-3}$ mol/dm⁻³, 25 ml) were kept flowing through a 0.4 mm quartz cell, which itself was kept in continuous motion perpendicular to the excitation beam.

Computational Details. Ground state and excited state geometry optimizations on 5FU in acetonitrile were performed by DFT (PCM/PBE0) and TD-DFT (PCM/TD-PBE0) calculations. These allowed the determination of the relative energy in the Franck-Condon region and the calculation of the minima of the two lowest energy excited states deriving from the bright HOMO/LUMO π/π^* and the dark HOMO-1/LUMO n/π^* transitions (hereafter S_π and S_n). Conical Intersections (CI) between the ground and the π/π^* excited state were located at the CASSCF(8/8)/6-31G(d) level, by using the method of Bearpark et al.,¹⁴ including 6 π molecular orbitals and the two n_O valence orbitals. The PCM/TD-PBE0 calculations allowed ground- and excited- state geometry optimization including solvent effects. All the calculations have been performed by using a development version of the Gaussian package.¹⁵ Further details regarding the calculations are given elsewhere.¹⁰ Bulk solvent effect on the excited state has been calculated by using the PCM/TD-DFT implementation, based on the linear response (LR) theory, described in ref. 16. In some cases we have also applied the new State Specific (SS) implementation of TD-DFT methods, that should allow for a more balanced treatment of the n/π^* and π/π^* transitions.¹⁷ In brief, LR methods avoid the calculation of the exact excited state electron density in favor of a direct determination of excitation energies. On the other hand, in the SS procedure the excited state electron

density and the corresponding response surface charges (characterizing the PCM method) are self-consistently optimized by means of an iterative procedure.

Several papers show that PCM alone is fully adequate to model polar solvents as CH₃CN without including explicit solvent molecules.¹⁸⁻²¹ The proper description of solvent shifts in aqueous solutions requires instead also the explicit inclusion of water molecules belonging to the first solvation shell.¹ Taking into account experimental suggestions²², all the PCM calculations of 5FU in water solution included four explicit water molecules in the first solvation shell.¹⁰ Our model is confirmed by the results of a recent MD study of 5-fluorouracil, where no indication of hydrogen bonding between the fluorine atom and water hydrogens was found.²³ Here we only treat the diketo form of 5FU. This is the most stable form in the electronic ground state,^{24,25} and the only tautomer identified in solution and in the gas phase.^{4,26}

Results and discussion. Steady-state absorption and fluorescence spectra of 5FU in acetonitrile and aqueous solution are shown in Figure 1. Both absorption and fluorescence spectra are shifted toward shorter wavelengths in acetonitrile as compared to water, indicating a weaker solute-solvent interaction in the excited state. Corresponding fitted peak frequencies are given in Table 1 (see SI for details). The fluorescence quantum yield of 5FU in acetonitrile is about four times lower than in water. The most striking difference is that the fluorescence spectrum of 5FU in acetonitrile is much broader than in water, with a short wavelength flank that is displaced by 25 nm towards the blue while the red side is nearly superposable with that observed in water.

(Figure 1)

Fluorescence decays at 330 nm of 5-fluorouracil in H₂O and CH₃CN are shown in Figure 2. It is quite apparent that the decay in acetonitrile is much faster than that in water. We performed merged nonlinear fitting/deconvolution processes using mono- or bi-exponential impulse response model functions $i(t)$ convoluted by the Gaussian instrument response function, $I(t) \propto i(t) \otimes G(t)$. Fitted values are given in Table 2. Although mono-exponential fits are not perfect, such fits give easily comparable characteristic

times, having the values of 1.4 and 0.4 ps in H₂O and CH₃CN, respectively. These values are in line with the relative fluorescence intensities mentioned above.

(Figure 2)

The Vertical Excitation Energy (VEE) of the S_π state (mainly arising from the HOMO-LUMO excitation, with π/π* character)^{1,12} provided by PCM/TD-PBE0/6-311+G(2d,2p) calculations on a PCM/PBE0/6-31G(d) optimized geometry in acetonitrile solution is 39.700 cm⁻¹, to be compared with a value of 39.400 cm⁻¹ obtained in aqueous solution. Computed VEE's are thus in good agreement with experimental data. Even if the π/π* VEE is slightly overestimated (by ~ 2000 cm⁻¹), it is noteworthy that our calculations provide a very good estimate of the water→acetonitrile solvent blue-shift. The experimental value (300 cm⁻¹) is indeed very close to its PCM/TD-PBE0 counterpart.

In agreement with previous computational studies on uracils in gas phase and in aqueous solution,^{1,9,10} both LR and SS PCM/TD-PBE0 calculations predict that in the FC region a dark state (S_n) has a similar stability to S_π. The S_n electronic transition has a predominant HOMO-1 → LUMO character (n/π*), mainly involving the C4-O8 carbonyl group.

Figure 3

The structure of the S_π state optimized in acetonitrile (see Figure 3) is very similar to that obtained in aqueous solution and already described in ref. 10. The pyrimidine ring adopts a "boat-like" conformation, with N3 and C6 atoms out of the average plane defined by N1, C2, C4, and C5 atoms. The largest variations of the bond lengths involve the lengthening of C4O8 and, especially, C5C6 bond distances in line with the bonding/antibonding character of HOMO and LUMO with respect to those bonds.^{1,10}

The computed fluorescence energy is 28.500 cm⁻¹ to be compared to a value of 30.100 cm⁻¹ in aqueous solution. The comparison with the experimental results is not straightforward, since the observed fluorescence spectrum of 5FU in acetonitrile is abnormally broad and the lifetime is much shorter than in water. For acetonitrile the main part of the fluorescence could thus come from the

Franck-Condon region and the excited state population be quenched before it reaches the S_π local minimum.

The stabilities of the S_π and the "dark" S_n excited states are comparable: in particular, state specific PCM/TD-PBE0/6-311+G(2d,2p) calculations provide 0-0 transition energies of 37000 and 36700 cm^{-1} for the S_π and the S_n state, respectively. PCM/TD-PBE0 geometry optimizations predict that the equilibrium geometry of S_n is planar, as the ground state. The most relevant geometry shifts with respect to the ground state geometry involves instead the C4O8 and C5C6 bond lengths that increase by 0.1 Å and 0.04 Å, respectively. The n/π^* transition involves indeed the transfer of an electron from the orbital corresponding mainly to the O8 lone pair to a π^* orbital localized mainly on the C5C6 and C4O8 bonds. Except for the loss of planarity, the geometry shifts of the S_n minimum are thus quite similar to that of the S_π minimum. The latter exhibits a longer C5C6 bond distance, since the π HOMO has a bonding C5C6 character; the former has a longer C4O8 bond distance, due to the involvement of the O8 lone pair. On the balance, however, it is likely that, at least in the first instants after excitation to the S_π state, the geometry shifts induced by the absorption also lead to a stabilization of the close lying S_n state. In fact, preliminary PCM/TD-PBE0 calculations along a one-dimensional path leading from the FC region to the S_π minimum indicates the presence of crossing between S_π and S_n states.

These results suggest that the dynamical behavior of the S_π state could be influenced by the S_n state, especially in the proximity of the Franck-Condon region corresponding to a planar geometry. In this respect, it is worth noticing that resonant Raman experiments on uracil show that all but one of the vibrational modes that are more strongly affected by the electronic transition involve in-plane stretching and bending^{27,28}, suggesting that, soon after excitation to the S_π state, uracil-like molecules keep the planar geometry characteristic of the Franck-Condon region.

In order to better investigate this point, we have optimized the geometry of the S_π state under the constrain of planarity: it is noteworthy that now the S_π state is just 0.3 eV more stable than S_n (solvent fully equilibrated with the S_π state) and that the difference between the energy of the two states is just

0.1 eV, when considering the equilibrium solvation energy of S_n . The presence of a conical intersection between these two states in this region is thus likely, and solvent fluctuations could act as coupling modes between S_π and S_n . As a matter of fact, CASSCF calculations predicts that a Conical Intersection between S_n and S_π states (hereafter $CI^{n/\pi}$) does exist in vacuo for a geometry close to planarity. The structure of $CI^{n/\pi}$ is very similar to that found for uracil in the gas phase by Matsika at the CASSCF level,^{5,29} and PCM/TDPBE0 calculations confirm that this CI is present also in CH_3CN solution, since the energy difference between the two states is only 0.09 eV (PCM/TD-PBE0 6-31G(d) calculations).

The main process governing the ultrafast internal conversion from S_π to the S_0 ground state is the conical intersection $CI^{S1/S0}$. Confirming our previous results concerning aqueous solution, also in acetonitrile the reaction path on S_π is dominated by a pyramidalisation of C5 and an out-of-plane motion of the 5-substituent (ϕ dihedral), leading to the $CI^{S1/S0}$. In order to ascertain if solvent affects the energy barrier possibly present on the S_π potential energy surface (PES) when moving from the FC region towards $CI^{S1/S0}$, we have performed a fully relaxed energy scan of the S_π state as a function of the out of plane motion of the Fluoro substituent (ϕ dihedral) in CH_3CN (5F) and in water (5F•4H₂O), by performing excited state PCM/TD-PBE0 geometry optimizations for fixed values of the ϕ dihedral. The resulting curves are shown in Figure 4.

(Figure 4)

The picture obtained in the two solvents is very similar. The curves exhibit a very shallow minimum for $\phi = 170^\circ$, i.e. the value corresponding to the S_π energy minimum and rise slowly up to $\phi=140^\circ$. After this point, the energy of the S_π state drops and the geometry starts approaching that of the CI, where TD-PBE0 geometry optimizations suffer from severe convergence problems. Nevertheless, a partially relaxed single-point calculation for $\phi = 135^\circ$ confirms that the energy of S_π decreases with respect to $\phi = 140$ suggesting that this point is a saddle point on the isomerization path.

The computed energy barriers on the S_π state surface, separating the Franck-Condon region from the $CI^{S1/S0}$ conical intersection, are very similar: 0.13 eV in CH_3CN and 0.15 eV in H_2O (PCM/TD-PBE0/6-

31G(d) calculations). At the 6-311+G(2d,2p) level the energy barrier increases to 0.15 eV in CH₃CN and to 0.165 eV in H₂O. The stabilization with respect to the Franck-Condon region is ca. 0.4 eV in both solvents.

Even if the present computational analysis does not allow excluding the possibility that the solvent can modulate also the barrier heights on the path towards the CI^{S₁/S₀} conical intersection, the comparison of the computational results obtained for 5FU in acetonitrile with those obtained for water solution¹⁰ strongly suggests that it affects the S_π lifetime mainly by tuning the relative energy of the S_π state and the close lying S_n dark state. The stability of π/π* states increases both with the polarity and, especially, the hydrogen bonding ability of the solvent, implying that in water the dynamics on S_π is not influenced by S_n. In acetonitrile, instead, the S_n and the S_π states are very close (their relative energy being within 0.1 eV) in the region of the configuration space close to the Franck-Condon region and the S₁ local minimum.

According to the picture that emerges from this study (schematically illustrated in Figure 5, an additional decay channel, very likely a conical intersection between S_π and S_n, opens up for 5FU in acetonitrile, leading to a decrease of its lifetime, in full agreement with experiments. The broad fluorescence spectrum of 5FU in acetonitrile is another hint of the possible involvement of an additional electronic state.

(Figure 5)

The efficiency of non-radiative decay through CI^{n/π} could be even more important in the gas phase or in nonpolar solvents, where the relative stability of the S_n state is larger than in CH₃CN. This is supported by experimental results on thymine in the gas phase indicating that following photoexcitation to the S_π state, the system is trapped in a dark state.²²

Further experimental and theoretical (for example, dynamical computational studies) work is obviously necessary to fully assess the precise role of the solvent in these processes. We are currently extending our ultrafast fluorescence studies to other bases and other solvent environments.

On the balance, the present study represents a very promising step towards a deeper understanding of the microscopic mechanisms underlying the photophysical behavior of nucleic acids and their constituents, showing the potentialities of the combined application of experimental and computational methods in comparative studies of different nucleobases in different solvents.

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Supporting Information. Steady-state absorption and fluorescence spectra on a wavenumber scale with fits, fluorescence decays on a linear scale with fits and PCM/PBE0/6-31G(d) calculated cartesian coordinates of the S_0 , S_π and S_1 minima. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Table 1. Characteristic parameters of the first absorption and fluorescence bands of 5-fluorouracil in acetonitrile and water. The peak frequencies ν_{\max} and the Stokes shift $\Delta\nu$ (peak absorption minus peak fluorescence). Also given are π/π^* vertical transition energies and fluorescence transitions. All values are in 1000 cm^{-1} .

		ν_{\max} (abs)	ν_{\max} (fluo)	$\Delta\nu$ (cm^{-1}) $\times 10^3$
CH ₃ CN	Exp	37.9	29.9	8.0
	Th	39.7	28.5	8.0
H ₂ O	Exp	37.6	29.5	8.1
	Th	39.4	30.1	8.1

Table 2. Characteristic times of the fluorescence decays of 5-fluorouracil in room-temperature acetonitrile and aqueous solutions ($\sim 2.5 \times 10^{-3} \text{ M}$). Results are from bi-exponential (τ_1 , τ_2) and mono-exponential fits (τ_0). Also given is the mean time ($\langle \tau \rangle = \alpha\tau_1 + (1-\alpha)\tau_2$) of the bi-exponential fit.

	α	τ_1 (ps)	τ_2 (ps)	$\langle \tau \rangle$ (ps)	τ_0 (ps)
CH ₃ CN	0.81 ± 0.11	0.26 ± 0.04	0.74 ± 0.18	0.36 ± 0.06	0.39 ± 0.07
H ₂ O	0.39 ± 0.04	0.69 ± 0.06	1.74 ± 0.05	1.32 ± 0.05	1.38 ± 0.01

Figure Captions

Figure 1. Steady-state absorption and fluorescence spectra of 5-fluorouracil in room-temperature H₂O and CH₃CN.

Figure 2. Fluorescence decays at 330 nm of 5-fluorouracil in CH₃CN and H₂O ($\approx 2.5 \times 10^{-3}$ M) after excitation at 267 nm. Also shown is the Gaussian apparatus function (fwhm ≈ 310 fs).

Figure 3: Minimum of the S_n (a) and S_π (b) states, according PCM/TD-PBE0-631G(d) excited state geometry optimizations in acetonitrile solution. Some selected bond distances (in Å) are also reported. In the ground state they are, respectively: C4O8=1.22 Å, C4C5=1.45 Å, C5C6 1.34 Å.

Figure 4. Energies of the S₀ and the S_π states as a function of the out of plane motion of the 5-substituent according to PCM/TD-PBE0/6-31G(d) excited state geometry optimization in acetonitrile. For comparison, the corresponding curves in water are also shown.

Figure 5. Schematic picture of the most important processes involving the electronically excited states of 5FU in acetonitrile solution. The destabilization of the S_n state in water solution is also schematically depicted.

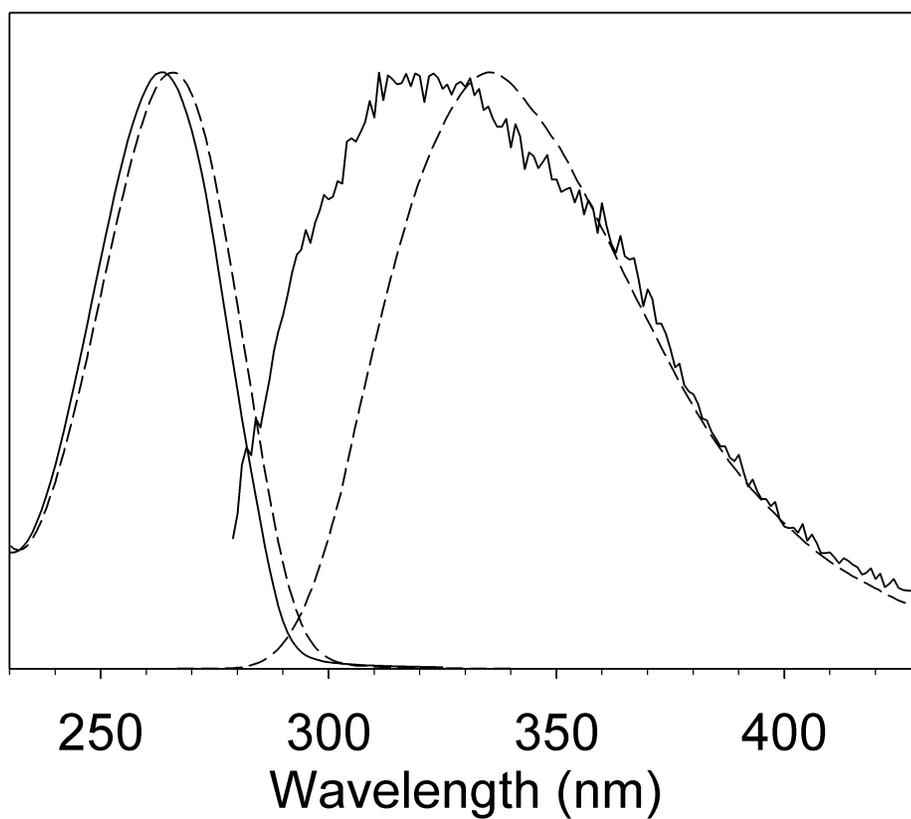


Figure 1. Steady-state absorption and fluorescence spectra of 5-fluorouracil in room-temperature H₂O (dashed lines) and CH₃CN (solid lines).

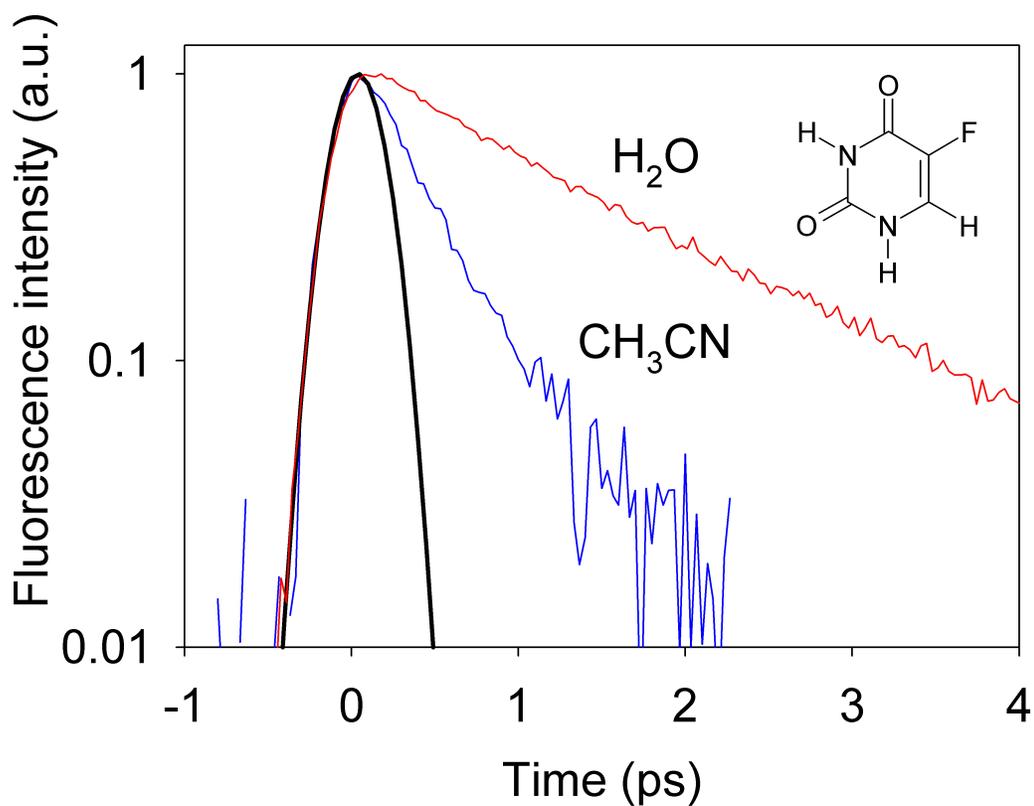


Figure 2. Fluorescence decays at 330 nm of 5-fluorouracil in CH₃CN and H₂O ($\approx 2.5 \times 10^{-3}$ M) after excitation at 267 nm. Note that a semi-log scale is used. Also shown is the Gaussian apparatus function (fwhm ≈ 310 fs).

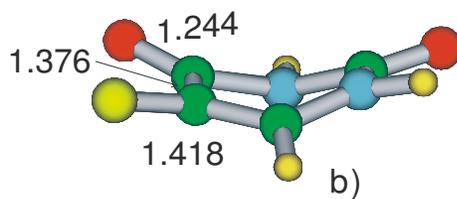
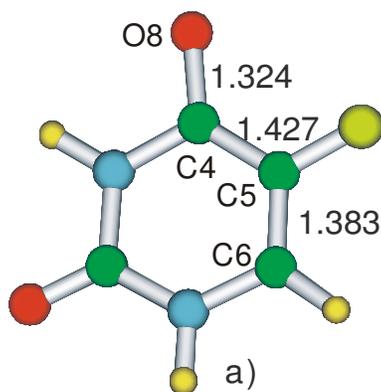


Figure 3

Figure 3: Minimum of the S_n (a) and S_π (b) states, according to PCM/TD-PBE0-631G(d) excited state geometry optimizations in acetonitrile solution. Some selected bond distances (in Å) are also reported. In the ground state they are, respectively: C4O8=1.22 Å, C4C5=1.45 Å, C5C6 1.34 Å.

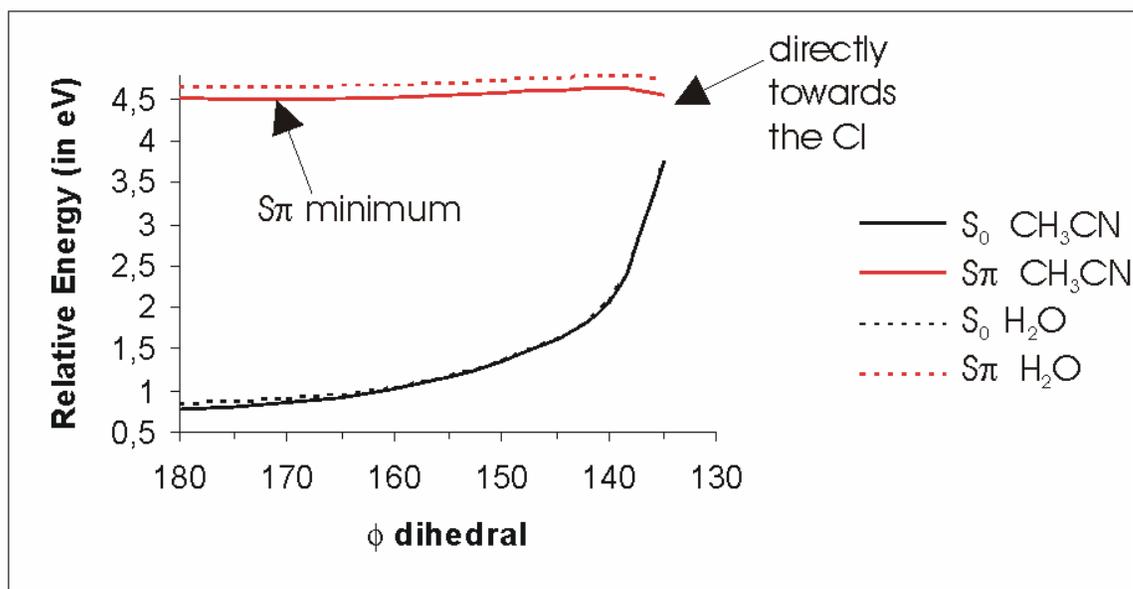


Figure 4. Energies of the S_0 and the S_π states as a function of the out-of-plane motion of the 5-substituent according to PCM/TD-PBE0/6-31G(d) excited state geometry optimization in acetonitrile. For comparison, the corresponding curves in water are also shown (dotted curves).

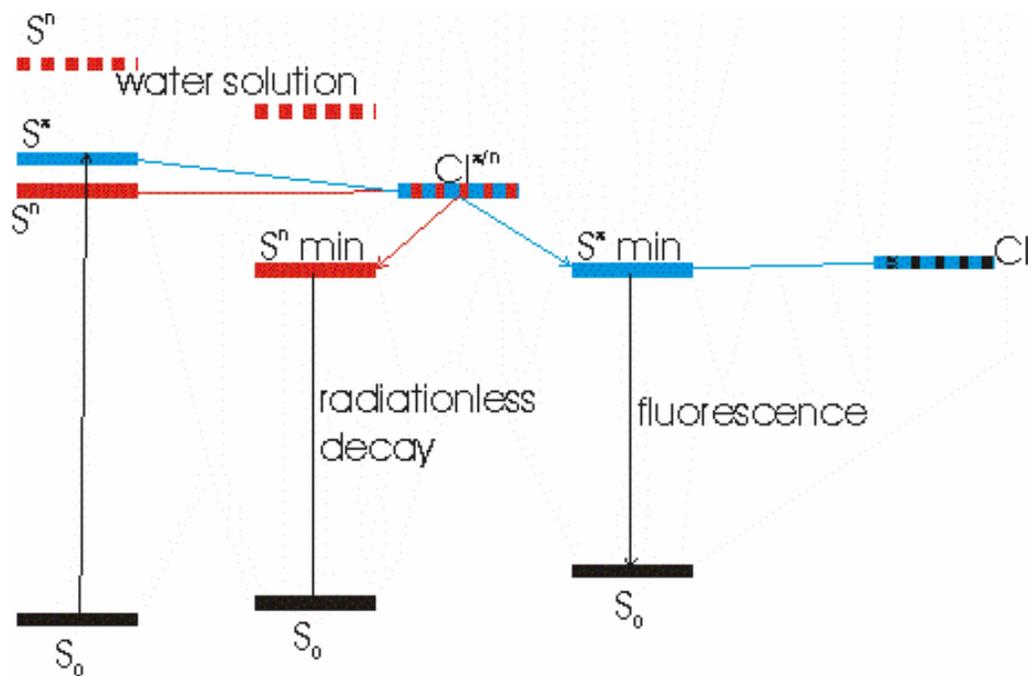


Figure 5. Schematic picture of the most important processes involving the electronically excited states of 5FU in acetonitrile solution. The destabilization of the S_1^n state in water solution is also schematically depicted (dashed red lines).

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