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**Assessing solvent effects on the singlet excited state
lifetime of uracil derivatives: a femtosecond fluorescence
upconversion study in alcohols and D₂O**

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Abstract

The excited state lifetimes of uracil, thymine and 5-fluorouracil have been measured using femtosecond UV fluorescence upconversion in various protic and aprotic polar solvents. The fastest decays are observed in acetonitrile and the slowest in aqueous solution while those observed in alcohols are intermediate. No direct correlation with macroscopic solvent parameters such as polarity or viscosity is found, but hydrogen bonding is one key factor affecting the fluorescence decay. It is proposed that the solvent modulates the relative energy of two close-lying electronically excited states, the bright $\pi\pi^*$ and the dark $n\pi^*$ states. This relative energy gap controls the non-radiative relaxation of the $\pi\pi^*$ state through a conical intersection close to the Frank-Condon region competing with the ultrafast internal conversion to the ground state. In addition, an inverse isotope effect is observed in D_2O where the decays are faster than in H_2O .

Introduction

The study of the photophysics of single DNA bases is currently undergoing a revival triggered by the availability of new improved experimental techniques and theoretical methods [1]. Various ultrafast spectroscopic techniques have been applied with success to the different nucleobases, nucleosides and nucleotides, showing that the excited states decay mainly on the sub-picosecond time scale, but in a complex manner and with large variations from one molecule to another [2]. In parallel, theoretical calculations have explained the ultrafast decay by revealing the existence of efficient conical intersections between the first singlet excited state and the ground states in uracil [3-8], cytosine [7,9-16] and adenine [4,17-28].

For uracil and its derivatives there is general agreement that the conical intersection (CI) between the bright $\pi\pi^*$ excited state (hereafter **S π**) and the ground state (S_0) is reached by pyramidalization around C5 while an out of plane motion leads the C5 substituent (see Chart I) to a pseudo-perpendicular arrangement with respect to the molecular plane [3-8,16,29]. In aqueous solution, this motion is characterized by a very low barrier (almost vanishing for uracil) which accounts for the very short **S π** lifetimes. The presence of a fluorine atom in at the 5-position increases the energy barrier separating the minimum of **S π** and the CI with S_0 , explaining the relatively longer excited state lifetime of 5-fluorouracil (5FU) in water [29]. If the role of the 5-substituent motion is rather well established, the evolution of the wavepacket in the Franck-Condon region is still a matter of debate [8].

According to the above picture, the ultrafast decay would thus be due to a purely intramolecular mechanism, little or not affected by the solvent. However, very recent experimental [30-32] and computational [33] studies indicated a more complex mechanism. Indeed, time-resolved fluorescence upconversion experiments show that the excited state

lifetimes of thymine and 5-fluorouracil are significantly shorter in acetonitrile than in water. It was proposed that a dark state of $n\pi^*$ character (hereafter **S_n**) almost isoenergetic with **S π** , provides an extra decay channel for **S π** in acetonitrile. The existence of **S_n** in uracil was reported by previous theoretical studies but these did not take into account the solvent effect [3,4,6-8,16].

In parallel, transient absorption experiments on 1-cyclohexyluracil indicated that the sub-picosecond lifetime of the photoexcited bright state exhibits a slight solvent dependence [34]. These experiments also revealed the existence of a longer living dark state, whose lifetime depends strongly on the solvent and was attributed to the **S_n** state. Even though the decay mechanism of **S_n** was not elucidated it was recognized that hydrogen bonds play an important role.

On the basis of the above mentioned calculations it is possible to explain the solvent dependence of the **S π** lifetime of uracils, as due to a modulation of the relative energies of the **S π** and **S_n** states, and thus, the availability of this additional quenching route of the fluorescent state. Quantum mechanical calculations in aqueous solution show indeed that **S_n** is destabilized by an increase of the solvent polarity, and, especially, by the presence of hydrogen bonds with solvent molecules. Consequently, the **S π** state of 5FU in aqueous solution lies always below the **S_n** state along the path connecting the Franck-Condon (FC) region with the conical intersection with S_0 . In contrast, in acetonitrile, **S_n** is more stable than **S π** in the FC region and the predicted crossing between these two states provides another effective decay route for the **S π** [33]. This was attested by the shorter measured fluorescence lifetime of 5FU in acetonitrile.

There is thus much evidence that the solvent can affect the non-radiative decay of excited states of uracil derivatives, making a more in-depth knowledge of the underlying microscopic mechanisms highly desirable. Assessing the role played by environmental effects is important

not only for the study of the monomeric nucleobases, but would also make it easier to understand the excited state decay mechanism of more complex systems such as single and double stranded nucleic acids, where the photophysical behavior can be modulated by different interrelated intramolecular effects (base stacking, base pairing) [27,35-40].

In the present work we focus our interest on uracil (U), thymine (5-methyluracil, T) and 5-fluorouracil (Chart 1) in alcohols (methanol, ethanol and 1-propanol) and in D₂O. We study them by fluorescence upconversion and compare the results with those obtained earlier for the same compounds in aqueous solution [29] and acetonitrile [30,31]. This approach may provide useful information about the role of the solvent in terms of macroscopic parameters such as polarity and viscosity as well as hydrogen bonding ability and deuterium isotope effect.

Materials and methods

Uracil, thymine and 5-fluorouracil were purchased from Sigma Aldrich. Acetonitrile (Merck UV spectroscopic grade), methanol, ethanol, 1-propanol (Fluka for UV spectroscopy) and D₂O (Euroisotope) were used without further purification.

(Chart 1)

The femtosecond fluorescence upconversion setup has been described earlier in detail [41]. Briefly, fluorescence decays were recorded at 330 nm after excitation at 267 nm (third harmonic of a mode-locked Ti-sapphire laser). Temporal scans were made with 33.3 fs steps in both parallel and perpendicular polarization configurations of the excitation and the gating beams. The width (fwhm) of a Gaussian instrumental response function (IRF) is about 350 fs at 330 nm and decreases to about 300 fs at 360 nm due to the decreasing group velocity mismatch between the fluorescence and the fundamental in the sum-frequency crystal. We

judge that the time resolution of our setup is better than 100 fs after analysis, depending on the signal-to-noise ratio.

All experiments were performed at room temperature (20 ± 1 °C) under aerated conditions. Solutions ($\sim 2.5 \times 10^{-3}$ mol/dm³) were kept flowing through a 0.4 mm quartz cell, which itself was in continuous motion perpendicular to the excitation beam. The power density is estimated to 0.2 ± 0.1 GW/cm² for a 40 mW output from the tripler unit.

Total fluorescence kinetics $F(t)$ shown below (Figure 1) were constructed from the parallel ($I_{par}(t)$) and perpendicular ($I_{perp}(t)$) signals according to the equation

$$F(t) = I_{par}(t) + 2I_{perp}(t) \quad (1)$$

Our recordings also allowed us to determine the fluorescence anisotropy $r(t)$.

$$r(t) = \frac{I_{par}(t) - I_{perp}(t)}{F(t)} \quad (2)$$

To characterize the fluorescence decays obtained a merged nonlinear fitting/reconvolution process was performed using the convolution of a model function and the IRF. As a model function either mono- or biexponential functions were used. Neither mono nor bi-exponential fits do necessarily correspond to the true physical picture, but are used in a phenomenological way to obtain a satisfactory fit. In order to quantify the relative contributions of the two components to the total fluorescence, their time-integrated relative contribution were also calculated as explained elsewhere [42].

Results

Alcohols The kinetic parameters of the fluorescence decay obtained by using the procedure described in the previous section are collected in Table 1. All zero-time anisotropies r_0 are close to 0.4, indicating that no change in electronic structure occurs between the absorbing and the emitting state.

(Table 1)

The fluorescence of uracil in the different alcohols show ultrafast monoexponential decay like in the case of acetonitrile [30,31] or in aqueous solution [29]. In all the solvents examined the fluorescence lifetimes of uracil are ~ 0.1 ps (Table 1), i.e. limited by the time-resolution of our experimental setup, not providing any hint about solvent effect on the decay mechanism.

(Figure 1)

On the contrary, the fluorescence decays of thymine and 5-fluorouracil in alcohols are slow enough to study the effect of the solvent (Figure 1). As can be seen in Figure 1 mono-exponential functions do not describe the experimental data satisfactorily.

Figure 2 presents the fluorescence decays of T and 5FU in acetonitrile, 1-propanol and water. The decays obtained for methanol and ethanol solutions are not shown here because they are very similar to those observed for 1-propanol (Figure 1 and Table 1). For both T and 5FU, the average fluorescence lifetime, $\langle \tau \rangle$, increases in the order:

$$\text{Acetonitrile} < \text{methanol} \approx \text{ethanol} \approx \text{1-propanol} < \text{water}$$

(Figure 2)

In the case of thymine, the fluorescence decays in all three alcohols are well described by bi-exponential functions, as was also found earlier for aqueous and acetonitrile solutions [29-31]. In all the solvents examined the fast components have similar values ($\tau_1 \sim 0.2$ ps). The values of the slower component (τ_2) range between 0.4 and 0.6 ps in the protic solvents, whereas in acetonitrile it is significantly larger ($\tau_2 \sim 1.1$ ps). However, the relative contribution of the short time constant to the total emission is dominant in acetonitrile ($\sim 80\%$), whereas for alcohols the two components have comparable weight. Finally, in the case of aqueous solutions, the slower component has clearly a more important contribution.

As a consequence, the shortest $\langle \tau \rangle$ value is found for acetonitrile solution (0.24 ps) and is about half of that found for aqueous solution (0.39 ps). The average lifetime is gradually increasing in different alcohols from methanol to 1-propanol, even if the differences are small (Table 1).

5-fluorouracil shows a similar behavior to that of thymine, although the excited state decay occurs on a significantly slower time-scale (Figure 2b). The fluorescence signals recorded for 5FU are clearly non-exponential in all solvents. The two time constants determined by bi-exponential fits for 5FU in acetonitrile and in alcohol solutions have comparable values ($\tau_1 \sim 0.3\text{-}0.4$ ps, $\tau_2 \sim 1$ ps), whereas they are significantly slower in water. However, also for 5FU the most significant difference between the solvents examined concerns the relative weight of the two components. As a matter of fact, for acetonitrile the relative weight of the fast component is much larger than that of the slow one, whereas for the protic solvents the opposite is found. For aqueous solutions, more than 80% of the excited state population follows the slow path; as a consequence, the average lifetime is almost four times longer than that obtained in acetonitrile, and twice that found in alcohols, where very similar $\langle \tau \rangle$ are obtained (Table 1).

Deuterium Isotope Effect: The fluorescence decays of thymine and 5-fluorouracil in H_2O and in D_2O are compared in Figure 3. While the decay of T is only slightly shorter in D_2O than in H_2O , a 20 % decrease of the average lifetime for 5FU was observed when going from water to heavy water. Not surprisingly, also in D_2O the initial anisotropies r_0 are close to 0.4, indicating that the absorbing and the emitting state are identical.

(Figure 3)

Discussion

As mentioned in the introduction, it is today relatively well established that pyramidalization around the 5-position and out-of-the-plane flipping of the 5-substituent in the first excited singlet are involved in the ultrafast internal conversion taking place in uracil derivatives. Before scrutinizing the details of such mechanisms on the molecular level, it is tempting to think that such large-amplitude motions will be affected by macroscopic solvent properties such as viscosity (local mechanical friction) or polarity (local dielectric friction). Various examples can be found in the literature where ultrafast excited state dynamics scale with solvent polarity [43] or solvent viscosity [44,45]. The solvents used in the present study are characterized by dielectric constants (ϵ) ranging from ~ 21 for 1-propanol to ~ 78 for water. Moreover, the hydrogen bonding ability of the alcohols studied is intermediate between that of acetonitrile and water, slightly decreasing with the chain length. Finally, while the viscosity (η) of methanol is intermediate of those of acetonitrile and water, ethanol and 1-propanol are more viscous. Values of the dielectric constant and the viscosity are taken from ref. [46] and references therein.

We have consequently tried various correlations between macroscopic solvent parameters such as the polarity or the viscosity and the characteristic times, τ_1 , τ_2 and $\langle\tau\rangle$ obtained from fits. There is no correlation between τ_1 and any solvent parameter, probably due to the relative uncertainty in the determination of the τ_1 values. The τ_2 and $\langle\tau\rangle$ lifetimes are better defined, but there is no clear-cut correlation between any of them with the solvent parameters. As an example, the $\langle\tau\rangle$ values of thymine as a function of solvent polarity and of 5-fluorouracil as a function of solvent viscosity are shown in Figure 4. As can be seen, partial correlation can be found for some solvents, but, in all cases, the average lifetimes determined for T and 5FU in strongly hydrogen bonding H_2O and D_2O differ significantly from the others.

(Figure 4)

Therefore, we will now discuss the experimental results presented in the previous section in the general framework we recently proposed on the ground of first principle quantum mechanical calculations in the condensed phase [33]. As for uracil, the observed ~ 0.1 ps fluorescence lifetime is within the time resolution of our experimental setup, which indicates that a very fast internal conversion process occurs in this molecule leading to ultrashort fluorescence lifetimes, independently on the polarity and the hydrogen bonding ability of the solvent. This could be viewed in the light of our previous proposal that the mechanism of the excited state decay is not significantly affected by the solvent, and it always involves the pyramidalization at C5 together with the out of plane displacement of the C5 substituent. Such a motion was predicted to be almost barrierless for uracil, both in the absence and in the presence of explicit solute-solvent hydrogen bonds. For uracil it is thus not easy to find evidence in the time-resolved fluorescence data supporting the existence (as predicted by QM calculation) of a crossing between the bright $S\pi$ and the dark Sn state in water. However, transient absorption experiments of uracil and uracil derivatives in different solvents confirm that a significant part of the population on $S\pi$ decays to a dark state [34].

For thymine and 5-fluorouracil an energy barrier between the minimum of $S\pi$ and the CI with S_0 is predicted by our computations. For these two compounds the existence of alternative barrierless decay channels could thus have a larger impact on the excited state lifetime. In fact, for thymine and 5-fluorouracil the observed fluorescence decays depend significantly on the solvent in spite of the fact that the computed energy barriers towards the CI with ground state are nearly solvent independent [33]. The average fluorescence lifetimes in alcohols are in between those previously measured in acetonitrile and water. This finding

can be explained by our QM calculations, which indicate that **S π** can effectively decay to the underlying dark **S n** state in acetonitrile. The presence of hydrogen bonds with the surrounding solvent molecules leads to a destabilization of **S n** , explaining why this additional decay channel is absent or much less effective in water. The fluorescence decays of T and 5FU in alcohols are in nice agreement with this picture, since the $\langle \tau \rangle$ values are larger than those determined for acetonitrile, which has a comparable polarity. Thus, the presence of hydrogen bonds should affect the stability of **S n** , and, consequently, the efficiency of the ‘**S n** route’ through the **S π /S n** conical intersection, much more than the polarity of the embedding medium. This is in line with what was observed in Figure 4.

Only purposely tailored Quantum Dynamical calculations could definitively assess the microscopic mechanisms underlying the bi-exponential character of the excited state decay curves. However, on the ground of the available experimental and computational results, it is already possible to propose some explanations for such behavior. On the one hand the ultrafast component could be related to the Franck-Condon region and the slower component to a more flat part of the **S π** surface [8], likely around a pseudo planar minimum [29,33]. Recent studies have also suggested that the presence of a wide plateau on the PES [29], can give rise to a bi-exponential decay time [47,48]. Finally, the bi-exponential character of the excited state decay curves could also be related to the presence of **S n** , since a part of the wave packet moving on **S π** , after crossing the **S π /S n** CI, could be trapped within the dark state. After spending some time around the minimum of **S n** , a part of the excitation could then re-cross the **S π /S n** CI, giving account of the slower component of the fluorescence decay. In this respect it is noteworthy that, though τ_1 and τ_2 exhibit some dependence on the solvent, the most significant factor seems to be the relative weight of the two components. This finding also supports our proposal that the solvent modulates the accessibility of the additional **S n** pathway, but it does not dramatically affect the motion on the two possible pathways.

Finally, it is noteworthy that the $S\pi$ lifetime is shorter in ethanol and in 1-propanol than in water, although these alcohols are more viscous than water. Even if a quite large-amplitude motion is required to reach the CI with S_0 , this result indicates that viscosity does not affect the motion along the relaxation path, at least for relatively small C5 substituents like methyl and fluorine groups.

In their study of solvent effects on ultrafast excited-state dynamics of adenines [49], Cohen et al. found no deuterium isotope effect. This was interpreted as a sign that proton transfer to or from the solvent plays no role in the excited state deactivation. In our case, we do observe an isotope effect for T and 5FU, even if it is modest. In fact, the fluorescence decays are faster in D_2O than in H_2O , whereas "normal" dynamic isotope effects would give the opposite result due to the heavier mass of the deuterium as well as the lower zero-point energy of the OD vibration as compared to OH [50,51]. This experimental finding can be interpreted in the framework built on the ground of our previous computational results. Indeed, solvent molecules are not involved in the motion towards the $S\pi/S_0$ CI [33]. Furthermore, calculations predict that the equilibrium geometry of the first solvation shell depends on the solute electronic state, i.e. the degrees of freedom associated to solvent molecules should be excited following $S_0 \rightarrow S\pi$ electronic transition [5,33]. We can thus expect that part of the energy absorbed in the electronic transition flows towards the solute-solvent vibrations and is then dissipated in the solvent cage. Solute-solvent vibrations are slower in D_2O than in H_2O and there are experimental evidence that the solvation dynamics is slower in the former solvent, [52] which was interpreted as due to stronger hydrogen bonding in D_2O compared to H_2O , which slows down the reorientation of the excited-state dipoles in the bulk D_2O . Moreover, in a very recent study of an adenine derivative it was shown that the vibrational cooling of hot ground state levels is 1.75 times slower in D_2O than in H_2O [53]. The dissipation of excitation energy to the 'solvent' degrees of freedom is thus expected to be

less effective in D₂O. As a consequence, in this solvent there is a larger amount of energy available for the intramolecular degrees of freedom involved in the motion towards the **S π /S₀** conical intersection, and for crossing the energy barrier existing for thymine and 5-fluorouracil on this path. Although additional theoretical work is obviously necessary to explain this feature, the above considerations can give account of the slightly shorter lifetimes found in D₂O, especially for 5-fluorouracil that exhibits the largest energy barrier on the reaction path.

Finally, it is worth considering the possibility of intersystem crossing (ISC) as an additional decay channel for the photoexcited **S π** state. ISC is known to occur in pyrimidines, notably uracil and thymine. The triplet formation of pyrimidine bases was shown to depend strongly on the solvent [54] and the excitation wavelength [55]. The ISC yield of uracil increases by one order of magnitude when the excitation wavelength is decreased from 280 nm to 230 nm. The triplet states may be formed either directly from the bright **S π** state [56,57] or via an intermediate **S_n** state [58]. However, the yield of triplet formation in uracils, especially in protic solvents, is much lower than the overall solvent independent yield of "dark" state formation (\approx 40%) as deduced from ground state recovery yields measured by femtosecond transient absorption [34]. For this reason we favor the case involving the intermediate **S_n** state as outlined in our previous work [30,31,33]. In this picture the triplet state has no direct consequence for the S₁ fluorescence decay.

Conclusion

In this paper we have presented the results of time-resolved fluorescence upconversion experiments of uracil, thymine and 5-fluorouracil in three alcoholic solvents and in D₂O. Confirming our previous experimental and computational results, solvent is shown to

remarkably affect the lifetime of the bright $S\pi$ excited state. Indeed for 5-fluorouracil and thymine, the average lifetime in alcohols is intermediate between that found in water and in acetonitrile. This result clearly points out the presence of solute-solvent hydrogen bonds as the key factors modulating the excited state behavior of $S\pi$, whereas solvent polarity and viscosity are less effective. Those experimental findings strongly support our previous proposal that the solvent modulates the lifetime of the $S\pi$ excited state in uracils by modifying the relative energy and the interaction between the bright $S\pi$ and the dark Sn states. A decreasing energy gap can provide an additional decay channel for the fluorescence signal.

For uracil, where a barrierless path towards the CI with S_0 is predicted by calculations, the fluorescence decays are always at the limit of experimental resolution (faster or equal to 0.1 ps), confirming that the internal conversion mechanism, involving out of plane motion of the 5 substituent, is not significantly affected by the solvent.

Finally, an inverse isotope effect is found for thymine and 5-fluorouracil, since the excited state decay is faster in D_2O than in H_2O , indicating that solute-solvent and solvent-solvent degrees of freedom are not directly involved in the motion leading to the CI with S_0 .

On the balance, the results hereby reported, together with previous computational results, provide a well assessed framework for discussing solvent effects on the photophysics of uracils and definite indications on the underlying microscopic mechanisms.

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Table 1. Parameters derived from the fits of the fluorescence decays using mono- or bi-exponential functions. The α value corresponds to the relative amplitude of the first (τ_1) term, $\langle\tau\rangle$ is the average lifetime defined as $\alpha\tau_1 + (1-\alpha)\tau_2$ and p is the time-integrated relative contribution of the slow (τ_2) component to the total fluorescence (see ref. [42]). Also given are the uncertainties from the fits (one standard deviation), not to be confused with the experimental uncertainty of 0.1 ps.

Compound	Solvent	α (%)	τ_1 (ps)	τ_2 (ps)	$\langle\tau\rangle$ (ps)	p (%)
Uracil	CH ₃ CN ^a	100	0.11 (0.01)	-	-	-
	MeOH	100	0.09 (0.01)	-	-	-
	EtOH	100	0.10 (0.01)	-	-	-
	PrOH	100	0.11 (0.01)	-	-	-
	H ₂ O ^b	100	0.10 (0.01)	-	-	-
Thymine	CH ₃ CN ^a	95 (1)	0.19 (0.01)	1.10 (0.18)	0.24 (0.01)	24
	MeOH	63 (6)	0.14 (0.04)	0.41 (0.04)	0.24 (0.03)	64
	EtOH	66 (9)	0.19 (0.04)	0.51 (0.06)	0.29 (0.04)	55
	PrOH	72 (6)	0.21 (0.02)	0.59 (0.06)	0.32 (0.03)	52
	D ₂ O	61 (1)	0.17 (0.01)	0.61 (0.01)	0.34 (0.01)	70
	H ₂ O ^b	56 (2)	0.20 (0.02)	0.63 (0.02)	0.39 (0.01)	72
5-fluorouracil	CH ₃ CN ^a	88 (7)	0.30 (0.03)	1.01 (0.34)	0.39 (0.05)	30
	MeOH	68 (6)	0.44 (0.03)	1.15 (0.09)	0.67 (0.04)	55
	EtOH	52 (4)	0.31 (0.03)	0.93 (0.04)	0.60 (0.03)	74
	PrOH	42 (3)	0.29 (0.03)	0.93 (0.02)	0.66 (0.02)	81
	D ₂ O	53 (9)	0.43 (0.02)	1.75 (0.03)	1.04 (0.02)	78
	H ₂ O ^b	35 (4)	0.65 (0.06)	1.67 (0.05)	1.31 (0.05)	83

^a: from Reference [31], ^b: from Reference [29].

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CHART AND FIGURE CAPTIONS:

Chart 1. The schematic structure of the substituted uracils studied in the present work, where, R denotes the different substituents on the 5-position.

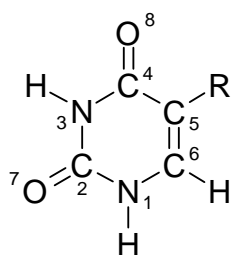
Figure 1. Fluorescence decays of thymine and 5-fluorouracil in methanol, ethanol and 1-propanol recorded at 330 nm after excitation at 267 nm. Solid lines correspond to fits with mono-exponential functions.

Figure 2. Fluorescence decays of a) thymine and b) 5-fluorouracil in acetonitrile, 1-propanol and water recorded at 330 nm after excitation at 267 nm. Also shown (dotted line) is the 0.33 ps (fwhm) Gaussian instrumental response function.

Figure 3. Fluorescence decays of a) thymine and b) 5-fluorouracil in water and D₂O recorded at 330 nm after excitation at 267 nm. Also shown (dotted line) is the 0.33 ps (fwhm) Gaussian instrumental response function.

Figure 4. The mean fluorescence lifetimes ($\langle\tau\rangle$) of a) thymine as a function of solvent polarity and of b) 5-fluorouracil as a function of solvent viscosity.

CHEMICAL STRUCTURE:



Uracil: $R = H$

Thymine: $R = CH_3$

5-fluorouracil: $R = F$

Chart 1. The schematic structure of the substituted uracils studied in the present work, where, R denotes the different substituents on the 5-position.

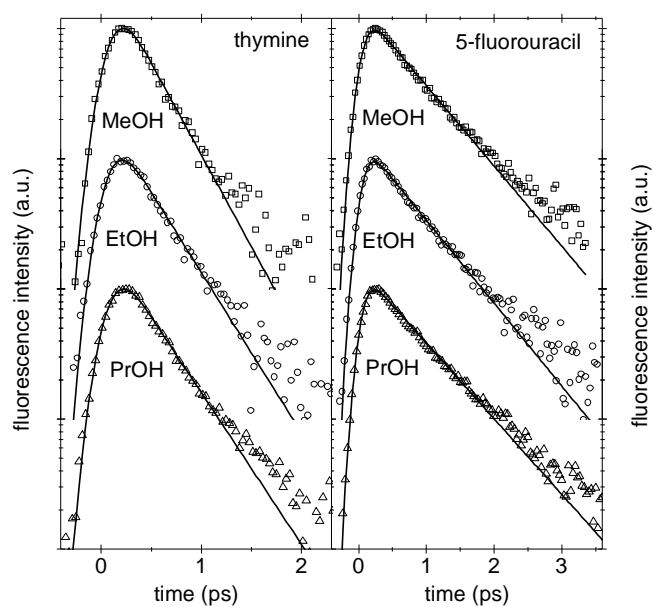


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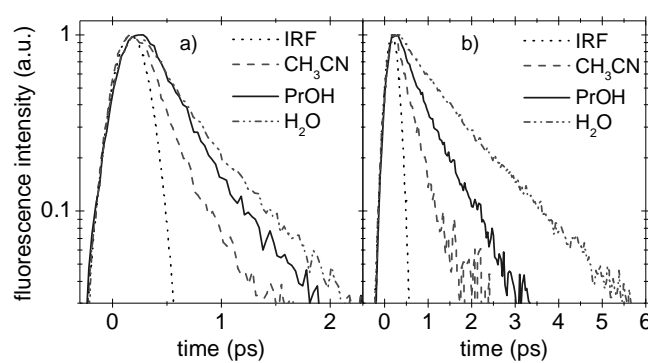


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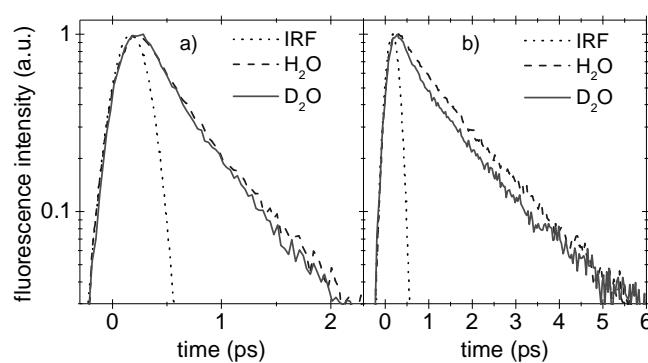


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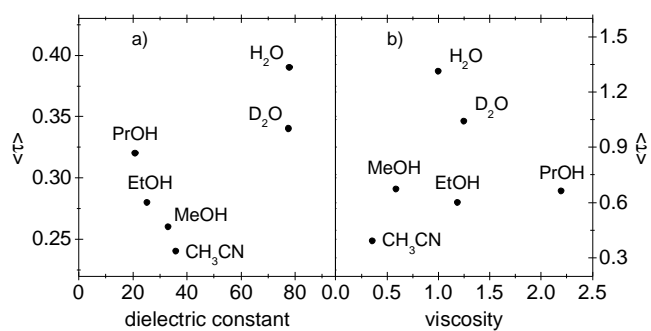


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