

# Thymine Dimerization Induced by Oxidative DNA Lesions and Epigenetic Intermediates via Triplet-Triplet Energy Transfer

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**KEYWORDS.** *UV light, DNA damage, cyclobutane pyrimidine dimer, time-resolved spectroscopy, CASPT2, density functional theory, exciplex, DNA oxidation, formyluracil, formylcytosine*

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Interaction of nucleic acids with light is a scientific question of paramount relevance not only in the understanding of life functioning and evolution, but also in the insurgence of diseases such as malignant skin cancer and in the development of biomarkers and novel light-assisted therapeutic tools. This work shows that the UVA portion of sunlight, not absorbed by canonical DNA nucleobases, can be absorbed by 5-formyluracil (ForU) and 5-formylcytosine (ForC), two ubiquitous oxidative lesions and epigenetic intermediates present in living beings in natural conditions. We measure the strong propensity of these molecules to populate triplet excited states able to transfer the excitation energy to thymine-thymine dyads, inducing the formation of the highly toxic and mutagenic cyclobutane pyrimidine dimers (CPDs). By using steady-state and transient absorption spectroscopy, NMR, HPLC, and theoretical calculations, we quantify the differences in the triplet-triplet energy transfer mediated by ForU and ForC, revealing that the former is much more efficient in delivering the excitation energy and producing the CPD photoproduct. Although significantly slower than ForU, ForC is also able to harm DNA nucleobases and therefore this process has to be taken into account as a viable photosensitization mechanism. The present findings evidence a rich photochemistry crucial to understand DNA photodamage and of potential use in the development of biomarkers and non-conventional photodynamic therapy agents.

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## INTRODUCTION

Nucleic acids are constantly exposed to endogenous and exogenous agents that can modify their chemical structure and therefore compromise their normal biological function.<sup>1,2</sup> Among such agents, solar light and especially UVB radiation (~290-320 nm) at high doses represents a major threat to public health. As a matter of fact, ca. 5-10% of the total UV radiation that reaches Earth's surface falls in this wavelength range<sup>3</sup> and DNA nucleobases directly absorb UVB wavelengths, triggering complex photochemical pathways leading to the appearance of DNA lesions.<sup>4-11</sup> Although DNA nucleobases are known for their high photostability,<sup>4-8</sup> dangerous DNA modifications such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (64-PP) are constantly produced and may accumulate in the skin, inducing mutation and carcinogenesis.<sup>12,13</sup> The unprotected exposure to UV light is nowadays recognized as one of the main causes of malignant skin cancer such as melanoma.<sup>14-16</sup>

The toxicity of DNA photolesions has been extensively studied: whereas CPDs are the most abundant photoproducts and are highly resistant to repair, 64-PP are less common, better repaired, but extremely mutagenic.<sup>17</sup> The high repair resistance of CPDs also results in their accumulation at the

genome level. Furthermore, the knocking down of photoleSIONS nucleotide excision repair (NER) enzymes is the main cause of highly debilitating pathologies, such as *Xeroderma Pigmentosum*, and of the exponential increase of skin cancer predisposition.

The rest of UV solar radiation, which is not filtered by the ozone layer (~90%), belongs to the less energetic UVA wavelengths (~320-380 nm).<sup>3</sup> In contrast to UVB photons, UVA light is hardly absorbed by DNA nucleobases. Instead, it can be absorbed by different chromophores, either exogenous or endogenous, located spatially close to DNA, which in turn can produce DNA lesions by indirect photochemical processes in what is known as DNA photosensitization.<sup>18</sup> These usually involve the population of triplet states of the chromophores by means of intersystem crossing (ISC) followed by several photochemical routes traditionally categorized in three classes: *i*) triplet-triplet energy transfer (TTET) to DNA, *ii*) activation of molecular oxygen to its singlet excited state, and *iii*) photoinduced electron transfer processes usually involving guanine. Other mechanisms such as photodissociation or hydrogen abstraction have also been reported.<sup>4,19</sup> While electron transfer and singlet oxygen activation usually produce oxidative DNA lesions,

triplet-triplet energy transfer has been shown, both computationally and experimentally, to favor thymine dimerization.<sup>12,20,21</sup> Here we focus on the first class of photosensitization, *i.e.* the triplet-triplet energy transfer from the chromophore to DNA.

A wide range of external photosensitizers have been studied in the last years,<sup>12,22–25</sup> a representative example being the paradigmatic case of benzophenone.<sup>26–28</sup> Nevertheless, it has been recently proposed that DNA lesions can themselves act as photosensitizers, since the modifications of their chemical structure may alter their optical and photophysical properties. This phenomenon was named as “Trojan Horse” after recognizing that 64-PP acts as an internal photosensitizer.<sup>29</sup> It was found that the presence of the pyrimidine moiety leads to absorption in the UVA range and induces CPD production after ISC and subsequent triplet-triplet energy transfer. The possibility of triplet transfer was also confirmed using molecular modeling and simulations.<sup>30</sup>

Recently, the role of oxidative lesions, namely 5-formyluracil (ForU)<sup>31</sup> and 5-formylcytosine (ForC)<sup>32,33</sup> (see Scheme 1), as potential Trojan Horses has been studied through experiments<sup>34</sup> and theoretical calculations.<sup>35–37</sup> It has indeed been recognized, both in model systems and in DNA oligomers, that the inclusion of ForU is correlated with a significant increase in the yield of CPD damages.<sup>34</sup> Furthermore, molecular modeling and simulations have shown that ForU is prone to a facile ISC, leading to an energetically favorable TTET to thymine and that those conditions are maintained in the B-DNA environment.<sup>35</sup> Subsequently, it was shown that ISC is also possible in the case of ForC, even if non-adiabatic molecular dynamics have pointed out a less-favorable phenomenon as compared to ForU.<sup>37</sup>

The behavior of ForC in comparison with ForU is indeed extremely intriguing, not only from a photophysical perspective, but also due to its biological relevance. ForC can be considered an oxidative lesion, but also an epigenetic intermediate in the demethylation process of 5-methyl-cytosine (5MetC). Indeed, the methylation of cytosine at 5 position, occurring in non-coding DNA regions with a high density of guanine and cytosine, the so-called CpG islands, results in the silencing of the specific gene expression. In eukaryotic cells, the regulation of the gene expression as a response to external stress is controlled by DNA methyltransferases (DNMT) that provide an oxidative route to demethylation involving the oxidation of 5MetC to ForC, and the subsequent participation of the base excision repair (BER) machinery. Deregulation in gene expression is found in many diseases including cancers, therefore, epigenetics intermediates are also recognized as most valuable biomarkers. The fact that an epigenetic intermediate, ForC, can potentially induce DNA photolesions, clearly opens fundamental questions concerning the subtle interplay between epigenetic phenomena and DNA photolesions, and hence can contribute to solve the molecular grounds of the problems related to the cellular tumorigenesis and the protective mechanisms that cells may have developed as a result of evolutionary pressure.

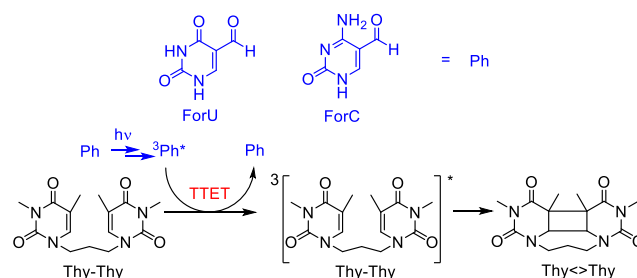
In this article, we rationalize the propensity of ForC to photoinduce thymine dimerization in model systems and DNA

oligomers, also comparing the process with the one operative in the case of ForU. The crucial differences between the two chromophores and their effects on thymine dimerization are also duly analyzed. Indeed, the description of these processes at a molecular and electronic level is of utmost importance to comprehend the naturally occurring mechanisms of DNA damage. Moreover, it also paves the way in the development of therapeutic tools such as photodynamic therapy, in which the DNA of cancerous cells in presence of man-made photosensitizers is selectively harmed by using visible or IR light.

## RESULTS AND DISCUSSION

### Triplet excited states of ForU and ForC

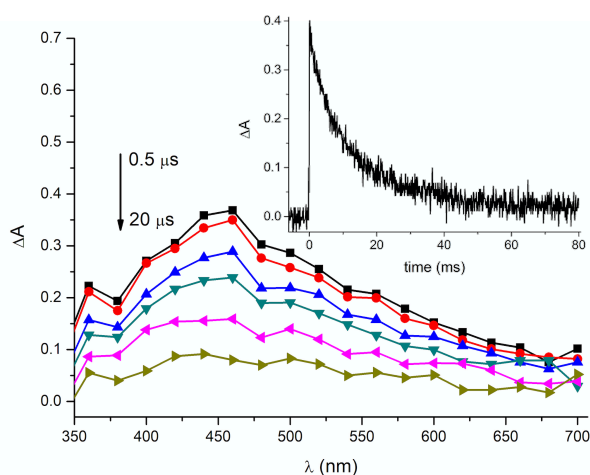
The photochemistry of ForU and ForC (Scheme 1) was tackled in aqueous media at room temperature in order to evaluate their potential to act as intrinsic photosensitizers. In this context, triplet excited states have been well-established as key intermediates in the photosensitized generation of damages.<sup>12,38</sup> Therefore, in addition to the spectroscopic characterization of the formyl derivatives, particular attention was paid here to thymine dimer formation as an analytical tool to compare the harmfulness of ForU and ForC triplet excited states. Indeed, the photosensitization of cyclobutane thymine dimer (Thy<>Thy, Scheme 1) is a clean reaction that occurs by TTET from the photosensitizer excited state to the pyrimidine base. To be efficient, this process requires a photosensitizer with a high triplet energy (higher than that of thymine), a high ISC quantum yield to allow significant population of the triplet manifold, and a triplet lifetime long enough to maximize the likelihood of the energy transfer process. Hence, monitoring Thy<>Thy formation provides valuable information on the overall strength of photosensitizing compounds.



**Scheme 1.** Structures of both photosensitizers (Ph) under study (ForU and ForC) and the photosensitized [2+2] photocycloaddition of Thy-Thy to afford Thy<>Thy

The first important property of an efficient DNA photosensitizer is absorption of light in a wavelength region where nucleobases do not (or barely) absorb, to assure the exploitation of the maximum number of incoming photons. As shown in Figure S3, in MeCN:H<sub>2</sub>O (1:1, v:v), ForC and ForU share similar absorption maxima ( $\lambda_{\text{max}}$ ) at 276-280 nm. Both compounds exhibit a band that reaches the UVA region allowing their selective excitation in the presence of canonical DNA bases. Nonetheless, the uracil derivative has a larger band, which broadens the spectral range available for

its excitation. None of the compounds show fluorescence emission, pointing toward an efficient deactivation of the singlet excited state by nonradiative pathways such as internal conversion or ISC. By contrast, phosphorescence was detected in ethanol glass at 77K with a maximum at 445 and 425 nm for ForU and ForC, respectively (Figure S4). This emission informs not only on the occurrence of ISC, which populates the triplet excited state, but also on the triplet energy ( $E_T$ ) of the uracil and cytosine derivatives determined at ca. 3.67 and 3.94 eV, respectively.<sup>37</sup> The triplet excited state behavior at room temperature was monitored using laser flash photolysis, since this technique allows the detection and characterization of transient species absorbing between 300 and 700 nm and whose lifetimes are typically in the microsecond timescale. Thus, nitrogen bubbled solutions in H<sub>2</sub>O:MeCN (1:1) of the two formyl derivatives were excited at 266 nm using the 4<sup>th</sup> harmonic of a Nd:YAG laser. The transient spectrum of ForU (Figure 1) showed a single broad band with maximum at 440-460 nm that completely disappeared after 40  $\mu$ s without giving rise to any other species. This transient signal was assigned to the triplet-triplet absorption by comparison with our previously published data in PBS, but the spectrum in aqueous acetonitrile was cleaner and much less noisy.<sup>34</sup> The decay monitored at 440 nm can be fitted with a monoexponential function,  $f(t) = A \exp(-t/\tau)$ , yielding a lifetime  $\tau$  of ca. 12  $\mu$ s (Figure 1, inset) that is much longer than the one found in PBS.



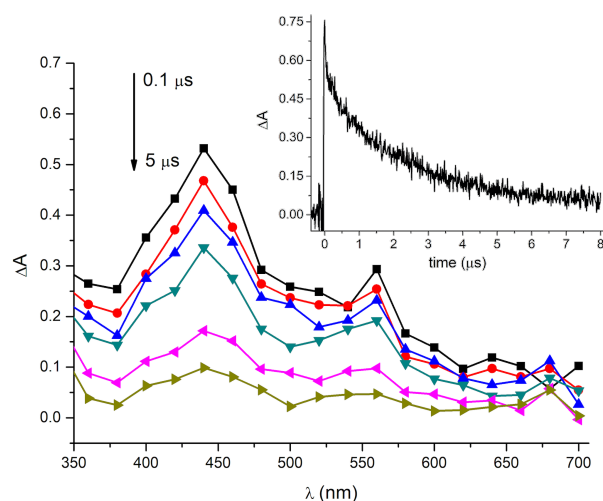
**Figure 1.** Transient absorption spectra of 5-formyluracil in MeCN:H<sub>2</sub>O (1:1, v:v) under N<sub>2</sub> at different times after the 266 nm laser pulse. Inset: decay of ForU monitored at 440 nm.

The cytosine derivative, ForC, also displayed a signal centered at 440 nm together with a shoulder at 560 nm, both bands decreasing with the same kinetics (Figure 2); this transient signal was assigned to the triplet excited state of ForC.<sup>36</sup> Concerning the kinetics, a biexponential behavior was found for the 440 and 560 nm decays, which were adjusted using the equation  $f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$ . The obtained lifetimes are  $\tau_1 = 0.07 \mu$ s ( $A_1 = 0.25$ ) and  $\tau_2 = 2.3 \mu$ s ( $A_2 = 0.75$ ) (Figure 2, inset). Both characteristic lifetimes are significantly shorter than that of <sup>3</sup>ForU\*, and thus, the dynamic range for <sup>3</sup>ForC\* intermolecular quenching is reduced, especially in the case of  $\tau_1$ . Thus, bimolecular rate

constants for <sup>3</sup>ForC\* quenching were further determined using the changes of  $\tau_2$ , only.

These intrinsic differences in the triplet lifetimes correlate very well with the nature of the emissive triplet states predicted by previous calculations for the two monomers. The emissive triplet states have been identified as <sup>3</sup>nπ\* and <sup>3</sup>ππ\*, for ForU and ForC respectively, on the basis of their vertical emissions.<sup>37</sup> Thus, the <sup>3</sup>nπ\* state of ForU has a longer lifetime given the lowest transition dipole moment with the singlet ground state and hence a smaller oscillator strength, resulting, following Einstein thumb rule, in a slower radiative decay as compared to the <sup>3</sup>ππ\* of ForC.<sup>37</sup>

The above results establish that, upon excitation, both ForU and ForC populate triplet excited states with energies higher than that of thymine and with a lifetime in the microsecond timescale, hence they can potentially induce energy transfer. Thus, the next step was to evaluate the ability of these excited states to photosensitize CPDs formation by means of time-resolved and steady-state experiments.

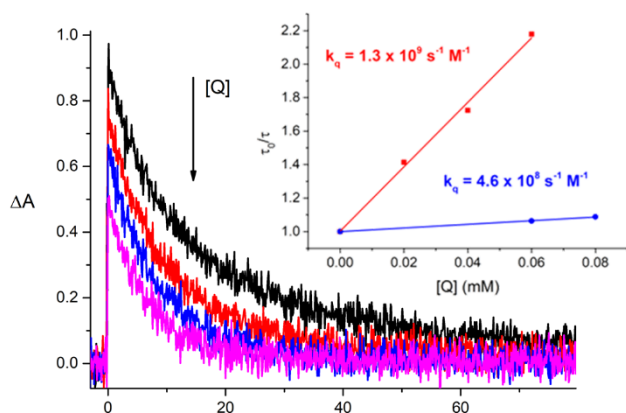


**Figure 2.** Transient absorption spectra of 5-formylcytosine in MeCN:H<sub>2</sub>O (1:1, v:v) under N<sub>2</sub> at different times after the 266 nm laser pulse. Inset: decay of ForC monitored at 440 nm.

#### Triplet-triplet energy transfer rates to Thy-Thy measured by laser flash photolysis

First, laser flash photolysis was employed to determine the rate of the triplet-triplet energy transfer from <sup>3</sup>ForU\* and <sup>3</sup>ForC\* to Thy-Thy (Scheme 1), used as a model of adjacent nucleobases. As shown in Figure 3 (and Figure S5), the addition of increasing amounts of Thy-Thy resulted in the shortening of the triplet excited state decay kinetics of the formyl derivatives, together with a decrease of the end-of-pulse signal intensity, due to the filter effect produced by the thymine chromophore at the excitation wavelength. Stern-Volmer plot representation of  $\tau_0/\tau$  as a function of the quencher concentration, reported as inlay, provided the bimolecular TTET rate constant ( $k_q$ ) for both sensitizers (see Material and Methods for more details). A value of  $1.3 \times 10^9$

$\text{M}^{-1}\text{s}^{-1}$  was obtained for the  $^3\text{ForU}^*$  quenching, while the process was almost 3 times slower for  $^3\text{ForC}^*$  with a  $k_q$  of ca.  $4.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ . Taking into account that the model system has two thymine units, the obtained values are in agreement with those previously given in the literature for TTET processes involving thymine as energy acceptor using other photosensitizers.<sup>12,39</sup>



**Figure 3.** Decays of ForU in deaerated MeCN:H<sub>2</sub>O (1:1,v:v) at 440 nm after the laser pulse at 266 nm in the presence of different Thy-Thy concentrations (from 0 to 0.08 mM). Inset: Stern-Volmer quenching of ForC (blue) and ForU (red) by Thy-Thy.

Curiously, an inverted order for the rate constants was expected based on the triplet energies, higher for ForC than for ForU, and on the Sandros' equation.<sup>40</sup> This equation establishes that, for triplet-triplet energy transfer processes, the larger the energy difference between the donor (ie. formyl derivatives) and the acceptor (ie. the Thy-Thy dyad), the higher the rate constant. The data obtained here pointed toward the occurrence of a more complex process than an typical energy transfer.

#### *Cyclobutane pyrimidine dimer production monitored by NMR and HPLC*

Next, steady-state photolysis was performed on deaerated MeCN:H<sub>2</sub>O (1:1, v:v) solutions of Thy-Thy in the presence of ForU or ForC and using monochromatic light at  $\lambda_{\text{exc}} = 310 \text{ nm}$ . All samples contained the same concentration of Thy-Thy (3.5 mM), ForC concentration was fixed at 1 mM, while in the case of ForU, the photosensitizer concentration was adjusted in order to obtain the same absorbance at  $\lambda_{\text{exc}}$  as for ForC.

The course of the photoreaction was followed by  $^1\text{H}$  NMR and HPLC (Figures 4 and S6-S8). In both cases, a clean process was observed with the formation of the *cis-syn* cyclobutane dimer of Thy-Thy (Thy<>Thy) as the only photoproduct. This assignment was based on the comparison with the HPLC elution time and with the NMR chemical shifts of synthesized Thy<>Thy (see Figure S6 and S8, up).

The NMR spectra of irradiated samples gave relevant information on the course of the photoreaction and on the nature

of the photoproducts. They show that the [2+2] photocycloaddition leading to Thy<>Thy formation results in the saturation of the C5-C6 double bond and induces characteristic changes in the chemical shifts ( $\delta$ ) of the protons belonging to the nucleobase and to the trimethylene bridge. The most pronounced change was observed for the H6 proton that shifted from 7.90 to upper fields at ca. 4.48 ppm (Figure S6). Signals of the methyl group at N3 also experienced shielding passing from 3.77 to 3.51 ppm, while those of the C5 methyl moved from 2.38 to 2.00 ppm. The ratio of the integrals of these signals (taken as pairs) can be used to evaluate the reaction course. In the case of ForC irradiation, it was observed that after 1h, 50% of Thy-Thy was decomposed to yield Thy<>Thy as the sole product (Figure S7, up). Interestingly, when using ForU as a photosensitizer, the initial dyad is completely consumed under the same conditions (Figure S7, bottom).

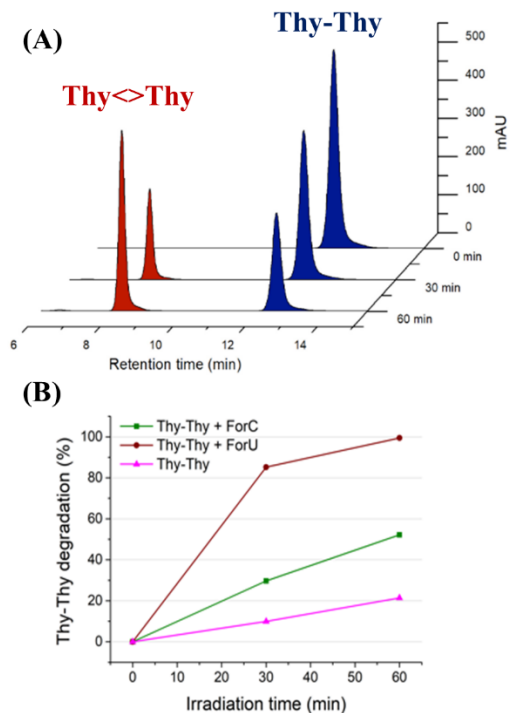
The HPLC analysis provided similar results. Figure 4A shows the chromatograms registered for different irradiation times of ForC in presence of Thy-Thy. The peak corresponding to the Thy-Thy signal, that is eluting at 12 min, decreases concomitantly with the appearance and the increase of a new peak corresponding to Thy<>Thy (8 min). A qualitatively analogous behavior was observed for the irradiation of the mixture ForU:Thy-Thy (Figure S8, up). A control experiment performed with Thy-Thy alone irradiated at  $\lambda_{\text{exc}} = 310 \text{ nm}$  (Figure S8, bottom) was also carried out and confirmed the lower yields for the direct CPD formation in the absence of photosensitizer. As expected from the difference in the bimolecular rate constants, Thy-Thy was more efficiently consumed when ForU is acting as photosensitizer. This is clearly illustrated in Figure 4B showing that the complete consumption of Thy-Thy was achieved after 1h of irradiation in presence of ForU, while only 50% of Thy-Thy is depleted in the presence of ForC, and 20% without any sensitizer.

Altogether, these experimental results showed that in spite of its higher triplet excited state energy, ForC is a less efficient Thy<>Thy photosensitizer than ForU. Thus, in order to shed more light on the molecular aspects responsible for this difference, molecular modelling was performed for a mixture of each formyl derivative and thymine.

#### *Mechanistic aspects revealed by molecular modelling*

Several factors explain the different yields of triplet-triplet energy transfer and CPD formation induced by ForU and ForC. First, the population of the triplet excited state is slower for ForC than for ForU, as shown by the respective time constants,  $\tau_{\text{ISC}} = 1940.5$  and  $3559.4 \text{ fs}$ , respectively) determined by non-adiabatic molecular dynamics.<sup>37</sup> Second, the triplet state of ForC decays faster, as evidenced by the time-resolved spectroscopic data shown in Figures 1 and 2, in coherence with the nature of the lowest triplet state ( $^3n\pi^*$  and  $^3\pi\pi^*$  for ForU and ForC, respectively).<sup>37</sup> Finally, the potential energy landscape of the electronic states involved in the photoprocess are different for ForU-Thy and ForC-Thy systems, as shown in Figure 5.



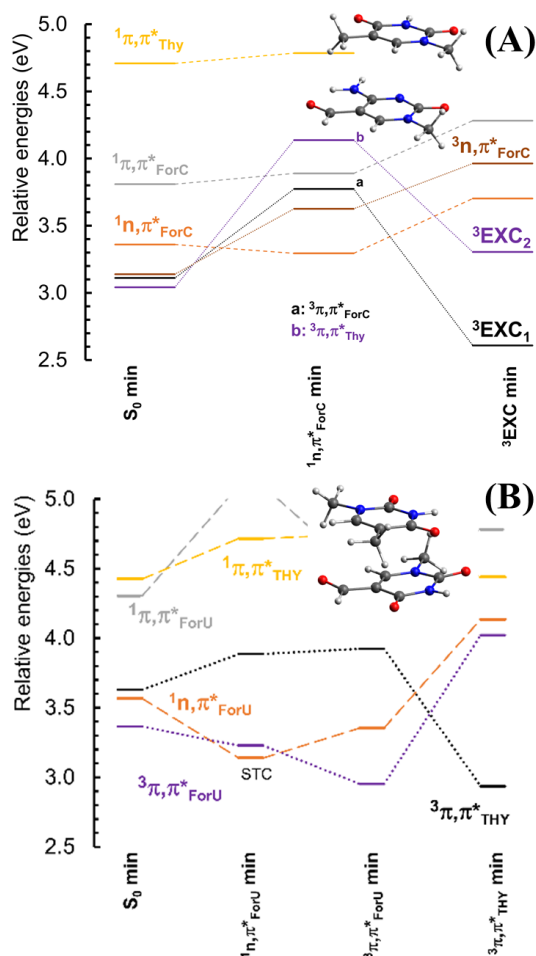


**Figure 4. (A)** HPLC chromatograms registered at 240 nm of ForC:Thy-Thy (1 mM: 3.5 mM) in H<sub>2</sub>O:MeCN (1:1, v:v) irradiated from 0 to 60 min at 310 nm, **(B)** Time-dependent photodegradation of Thy-Thy, in the presence of ForU (red), ForC (green) and alone (pink) ( $\lambda_{irr}$  = 310 nm).

Note that Figure 5 provides an overview of the energetic level of the most important electronic states calculated for relevant points of the potential energy surfaces, namely Franck-Condon, i.e. the ground state equilibrium geometry, and the optimized structures of the lowest singlet and triplet states.

In case of ForC, (Figure 5A) and as in the case of the monomer,<sup>37</sup> the first excited singlet state, of  $^1n,\pi^*$  nature and localized over ForC ( $^1n,\pi^*_{ForC}$ ), lies, at its equilibrium geometry, 0.48 eV below the triplet state of  $^3\pi,\pi^*_{ForC}$  nature, i.e. the other player involved in the ISC, justifying the slower population of the triplet manifold. Notably, and again coherently with non-adiabatic dynamics performed for the monomer,<sup>37</sup> spin-orbit couplings corroborate the role of  $^3\pi,\pi^*_{ForC}$  state as the doorway to ISC (Table S1). In contrast, in ForU, the corresponding states ( $^1n,\pi^*_{ForU}$  and  $^3\pi,\pi^*_{ForU}$ ) are quasi-degenerated at the  $^1n,\pi^*_{ForU}$  equilibrium geometry, leading to a much more efficient triplet population (Figure 5B).<sup>35</sup>

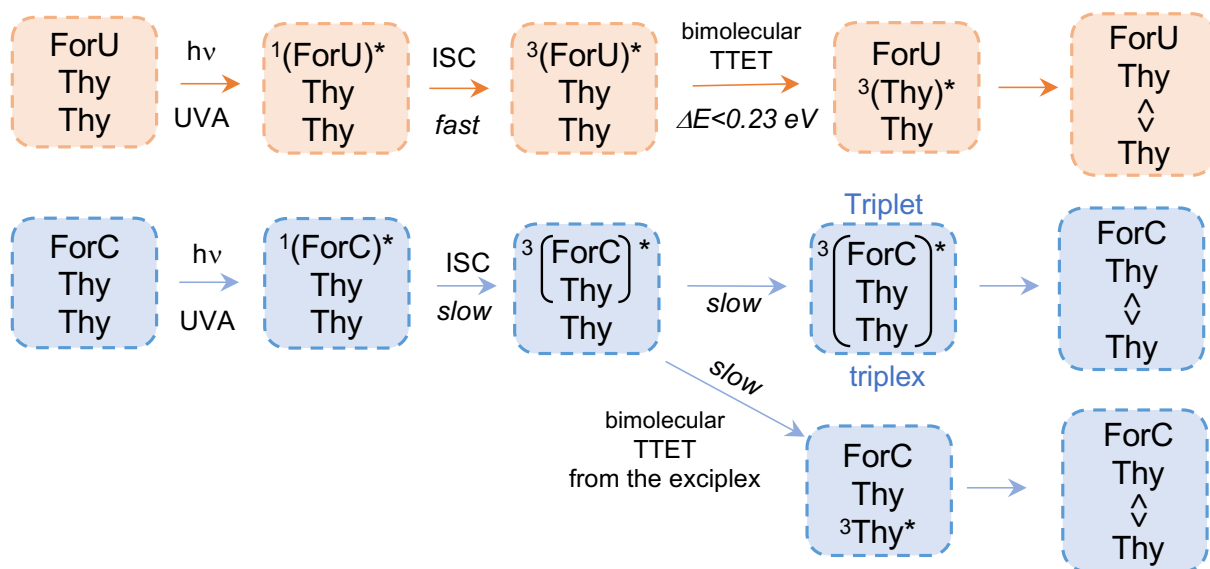
Other significant differences between the two sensitizers are due to the distribution of the spin density between the couple photosensitizer-nucleobase. The triplet states of ForC-Thy are characterized by a marked delocalization of the  $\pi$  clouds, describing the unpaired electrons, over both ForC and thymine moieties, especially involving the anti-bonding  $\pi^*$  orbitals (see Figure 6). Hence, the triplet states can be classified as exciplex (EXC<sub>1</sub>, EXC<sub>2</sub>). The delocalization is especially pronounced at the  $S_0$  min and the T<sub>1</sub> min, that in diabatic notation is labelled as  $^3EXC$  min. On the contrary, in the case of ForU, the spin density is much better localized over the two moieties, also giving rise to two well distinct



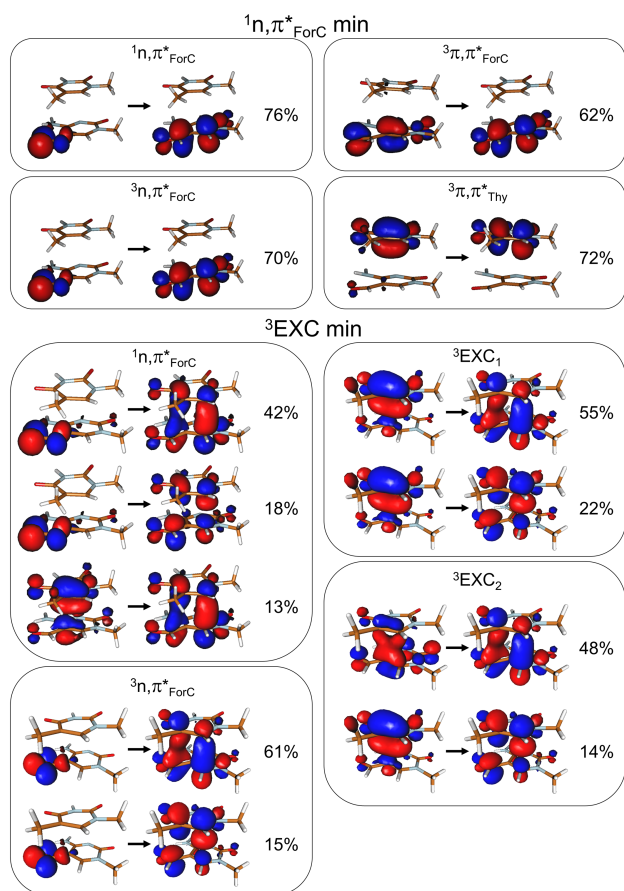
**Figure 5. (A)** CASPT2 energy profiles for the most relevant excited states of the ForC-Thy system. **(B)** CASPT2 energy profiles for the most relevant excited states of the ForU-Thy system, taken from Ref. <sup>35</sup>. STC = singlet-triplet crossing. The horizontal axis defines the equilibrium geometry of the most important states, obtained via geometry optimization, the dashed lines are used to connect the states having the same diabatic nature as a guide for the eyes.

electronic states, with a small but non-negligible driving force favoring the transfer of spin density to Thy, in a process requiring to bypass only a relatively small energy barrier.

The presence of the low energy  $^3EXC_2$  in ForC can contribute to further explain the different observed yields. Indeed, the possibility of the formation of a delocalized state involving the sensitizer and two thymine bases attached to a rigid scaffold has been previously invoked in the case of benzophenone, another photosensitizer.<sup>41</sup> Different mechanisms could be at play here (see Scheme 2) for ForC, in which (i) the exciplex state is too stable to be dissociated, and act as the energy donor for TTET to Thy, however the decreased energy of  $^3EXC_2$  (2.61 eV) locates this state below of Thy triplet excited state (at 2.87 eV)<sup>21</sup>, which makes TTET process unlikely; or (ii) the exciplex  $^3EXC_2$  can eventually further delocalize involving a second Thy unit forming a triplet triplex



**Scheme 2.** Schematic representation of the proposed CPD production from excitation of ForU (top) and ForC (bottom), according to our experimental and computational data. ISC = intersystem crossing, TTET = triplet-triplet energy transfer.



**Figure 6.** CASSCF orbitals corresponding to the highest weight electronic transitions that characterize the excited states of the ForC-Thy system. The weights of the configurations are also shown. Other transitions with much lower weights adding up to the total 100% are not reported.

that evolves forming  $\text{Thy} \rightleftharpoons \text{Thy}$ , nonetheless, such a trimolecular process can clearly be seen as slower and less efficient than the most direct route operative in the case of ForU involving localized states (Scheme 2).

Globally, the formation of the exciplex state as well as the trapping of the system in the  $^1n, \pi^*_{\text{ForC}}$  minimum, decrease the overall efficiency of the photoprocess in the ForC system.

## CONCLUSIONS

The present work characterizes the formation of dangerous CPD lesions through triplet-triplet energy transfer from ubiquitous oxidized nucleobases and epigenetic intermediates. The differences of the intrinsic photosensitization capabilities of ForU and ForC are revealed in detail by time-resolved and steady-state absorption spectroscopy, NMR, HPLC, and molecular modeling. It has been observed that the triplet-triplet energy transfer rate from ForU to thymine-thymine is 3 times faster than that from ForC ( $k_q \sim 1.3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$  for  $^3\text{ForU}^*$  and  $4.6 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$  for  $^3\text{ForC}^*$ ), leading to a complete depletion of the thymine-thymine reactant after 1 hour of irradiation in the presence of ForU. Under the same conditions, the consumption of the reactant is only about 50% when ForC acts as photosensitizer. Molecular modelling provides further evidence supporting a slower intersystem crossing and triplet-triplet energy transfer in ForC, since the relaxation of the singlet excited state leads to a region where the triplet states are less accessible. Exciplex states of  $^3\pi, \pi^*$  ( $^3\text{EXC}$ ) nature dominate the triplet state manifold in ForC-Thy and are identified as mediators of the triplet-triplet energy transfer in this system.

These results represent a step forward in the understanding of the intimate coupling between oxidative and light-induced DNA damage, responsible of the public health problem of unprotected sunlight exposure and development of

malignant skin diseases. Moreover, we also believe these results could be useful in the context of tumor detection and light-assisted therapies, since the different capability of ForC and ForU in inducing measurable CPD lesions could be used to determine or estimate the ratio of both species in DNA samples, especially relevant taking into account that their biological role is different and thus are involved in different processes.

## ASSOCIATED CONTENT

Materials and methods, full computational details, synthetic details and characterization of Thy-Thy and Thy<>Thy, phosphorescence emission, decays of ForC in the presence of Thy-Thy, NMR analysis of [2+2] photocycloaddition from irradiation of Thy-Thy, HPLC analysis of irradiation of Thy-Thy. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ACKNOWLEDGMENT

Support from the Université de Lorraine, CNRS, regional (Prometeo/2017/075) and Spanish Government (PGC2018-096684-B-I00, CTQ2017-87054-C2-2-P) is kindly acknowledged. A. F.-M. is grateful to Generalitat Valenciana and the European Social Fund for a postdoctoral contract (APOSTD/2019/149), M. L.-R. acknowledges the Universitat Politècnica de València for the FPI grant. All calculations have been performed on the local LPCT computer center and on the Explor regional center in the framework of the project "Dancing under the light".

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