



**HAL**  
open science

## Quantum-Chemistry Study of the Photophysical Properties of 4-Thiouracil and Comparisons with 2-Thiouracil

Miriam Navarrete-Miguel, Angelo Giussani, Mercedes Pozuelo-Rubio, Martial Boggio-Pasqua, Antonio Carlos Borin, Daniel Roca-Sanjuán

► **To cite this version:**

Miriam Navarrete-Miguel, Angelo Giussani, Mercedes Pozuelo-Rubio, Martial Boggio-Pasqua, Antonio Carlos Borin, et al.. Quantum-Chemistry Study of the Photophysical Properties of 4-Thiouracil and Comparisons with 2-Thiouracil. *Journal of Physical Chemistry A*, 2024, 128 (12), pp.2273-2285. 10.1021/acs.jpca.3c06310 . hal-04542328

**HAL Id: hal-04542328**

**<https://hal.science/hal-04542328>**

Submitted on 11 Apr 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

# Quantum-Chemistry Study of the Photophysical Properties of 4-Thiouracil and Comparisons with 2-Thiouracil

Published as part of *The Journal of Physical Chemistry A* virtual special issue “Roland Lindh Festschrift.”

Miriam Navarrete-Miguel, Angelo Giussani, Mercedes Rubio, Martial Boggio-Pasqua, Antonio Carlos Borin, and Daniel Roca-Sanjuán\*



Cite This: *J. Phys. Chem. A* 2024, 128, 2273–2285



Read Online

ACCESS |



Metrics & More

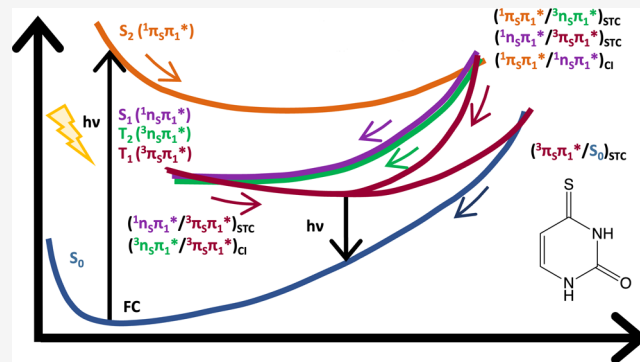


Article Recommendations



Supporting Information

**ABSTRACT:** DNA in living beings is constantly damaged by exogenous and endogenous agents. However, in some cases, DNA photodamage can have interesting applications, as it happens in photodynamic therapy. In this work, the current knowledge on the photophysics of 4-thiouracil has been extended by further quantum-chemistry studies to improve the agreement between theory and experiments, to better understand the differences with 2-thiouracil, and, last but not least, to verify its usefulness as a photosensitizer for photodynamic therapy. This study has been carried out by determining the most favorable deactivation paths of UV-vis photoexcited 4-thiouracil by means of the photochemical reaction path approach and an efficient combination of the complete-active-space second-order perturbation theory//complete-active-space self-consistent field (CASPT2//CASSCF), (CASPT2//CASPT2), time-dependent density functional theory (TDDFT), and spin-flip TDDFT (SF-TDDFT) methodologies. By comparing the data computed herein for both 4-thiouracil and 2-thiouracil, a rationale is provided on the relatively higher yields of intersystem crossing, triplet lifetime and singlet oxygen production of 4-thiouracil, and the relatively higher yield of phosphorescence of 2-thiouracil.

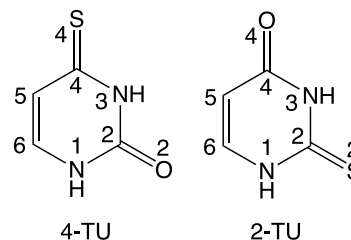


## INTRODUCTION

Chemically modified nucleobases have been used in different applications, from chemotherapy<sup>1–4</sup> and fluorescence markers<sup>5–14</sup> to nanomaterials.<sup>15–18</sup> Among them, thiated nucleobases (thiobases or thionucleobases) have received considerable attention due to their potential applications in photodynamic therapy.<sup>19–25</sup> Thionucleobases differ from the canonical nucleobases because one (or more) oxygen atom(s) is (are) replaced by a sulfur atom(s), which greatly increases the intersystem crossing yields, conferring them the characteristic of being species with very high quantum yield for triplet state population, which can be employed to stimulate in situ generation of reactive singlet oxygen (<sup>1</sup>O<sub>2</sub>). This excited state of molecular oxygen is a toxic species employed in photodynamic cancer therapy to damage biomolecules and produce cell death. <sup>1</sup>O<sub>2</sub> is generated when a photosensitizer (photodynamic drugs), after being exposed to a specific wavelength and excited to a triplet excited state, deactivates to the ground state and the energy released is transferred to the oxygen in the ground state (<sup>3</sup>O<sub>2</sub>). The photosensitizer is a key component in this therapy, and therefore, most of the research focuses on the design of more efficient photosensitizers and understanding

their physicochemical and medical applications.<sup>26–28</sup> Thionucleobases are prospective photosensitizers.

Among thionucleobases, 2-thiouracil (2-TU) (Figure 1, right) is a very interesting species because of its high triplet



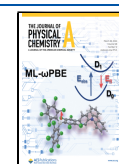
**Figure 1.** Chemical structure and labeling numbering for 4-TU and 2-TU.

**Received:** September 20, 2023

**Revised:** February 16, 2024

**Accepted:** March 3, 2024

**Published:** March 20, 2024



quantum yield ( $\Phi_{\text{ISC}} = 0.75 \pm 0.20$ ),<sup>29</sup> high phosphorescence yield ( $\Phi_{\text{ph}} = 0.65\text{--}0.70$ ),<sup>30,31</sup> and short triplet lifetime ( $\tau = 0.07 \pm 0.02 \mu\text{s}$ ).<sup>32</sup> As it has a limited absorption at 355 nm, no attempt was made to measure its singlet oxygen yield,<sup>33</sup> but it has been supposed to be similar to that observed for 2-thiothymine ( $\Phi_{\Delta} = 0.36 \pm 0.02$ ).<sup>29</sup>

2-TU deactivation pathways have been investigated computationally by different authors. Cui and Fang,<sup>34</sup> by employing the complete-active-space second-order perturbation theory//complete-active-space self-consistent field (CASPT2//CASSCF) protocol and imposing  $C_s$  symmetry in most calculations, were the first to propose three different deactivation mechanisms ( $S_2^1(\pi_S\pi_1^*) \rightarrow S_1^1(n_S\pi_1^*) \rightarrow T_1^3(\pi_S\pi_1^*)$ ,  $S_2^1(\pi_S\pi_1^*) \rightarrow T_2^3(n_S\pi_1^*) \rightarrow T_1^3(\pi_S\pi_1^*)$ , and  $S_2^1(\pi_S\pi_1^*) \rightarrow T_3^3(n_S\pi_1^*) \rightarrow T_2^3(n_S\pi_1^*) \rightarrow T_1^3(\pi_S\pi_1^*)$ ). However, these mechanisms are compromised due to symmetry constraints imposed by the authors. Later on, Gobbo and Borin<sup>35</sup> employed the same computational protocol, but without symmetry constraints, and proposed two similar deactivation pathways ( $S_2^1(\pi_S\pi^*) \rightarrow S_1^1(n_S\pi^*) \rightarrow T_1^3(\pi_S\pi^*)$  and  $S_2^1(\pi_S\pi^*) \rightarrow T_2^3(n_S\pi^*) \rightarrow T_1^3(\pi_S\pi^*)$ ). Shortly afterward, Mai et al.,<sup>36</sup> employing several levels of theory (CASSCF, MRCIS—multireference configuration interaction including single excitations, SS-CASPT2—state-specific CASPT2, and MS-CASPT2—multistate CASPT2), optimized minima and crossing points, obtaining strongly distorted geometries, and proposed new photophysical deactivation mechanisms as, for instance, the  $S_2 \rightarrow S_1 \rightarrow T_2 \rightarrow T_1$  sequence of population transfers. They also reported a spin–orbit coupling (SOC) between the  $S_1$  and  $T_2$  states of about  $150 \text{ cm}^{-1}$ , even though the  $S_1$  and  $T_2$  states are of the same  $n_S\pi^*$  nature, which is in apparent contradiction to El-Sayed's rule;<sup>37</sup> nonetheless, they detected in the photo-dynamics that the  $T_2^3(n_S\pi^*)$  and  $T_1^3(\pi\pi^*)$  states reversed their energetic order temporarily, consequently determining that the above-reported  $S_2 \rightarrow S_1 \rightarrow T_2/T_1$  pathway can be represented as  $^1\pi_S\pi^* \rightarrow ^1n_S\pi^* \rightarrow ^3\pi_S\pi^*$  decay, where the electronic states are labeled by their nature.<sup>38</sup> Consequently, from now on we will refer to the deactivation path of this molecule as  $S_2^1(\pi_S\pi^*) \rightarrow S_1^1(n_S\pi^*) \rightarrow T_2/T_1^3(\pi_S\pi^*)$ .

4-Thiouracil (4-TU) (Figure 1, left) is another important thionucleobase but less explored computationally. Its absorption spectrum is characterized by a strong band in the UVA region, with a maximum of around 330 nm, and by a weaker one in the UVB region, at 240 nm.<sup>39</sup> Its lowest-lying triplet state is of  $\pi\pi^*$  nature ( $T_1^3(\pi\pi^*)$ ), while  $T_2$  is of  $n\pi^*$  character ( $T_2^3(n\pi^*)$ ). In comparison to 2-TU, it has a higher (near unity) triplet population quantum yield ( $\Phi_{\text{ISC}} = 0.90 \pm 0.15$ ),<sup>33,39,40</sup> with the highest singlet oxygen formation yield ( $\Phi_{\Delta} = 0.49 \pm 0.02$ )<sup>33,41</sup> among thiobases. At 77 K, 4-TU shows a low phosphorescence yield ( $\Phi_{\text{ph}} = 0.15$ ).<sup>42</sup> Finally, a triplet lifetime of  $\tau = 0.23 \pm 0.02 \mu\text{s}$  was measured in anaerobic conditions and  $\tau = 0.17 \pm 0.02 \mu\text{s}$  in aerobic conditions.<sup>39</sup> A more recent study on 4-TU<sup>43</sup> has shown, by means of sub-30 fs broadband transient absorption spectroscopy in the UV with state-of-the-art QM/MM simulations, that its most relevant decay paths are  $S_2^1(\pi_S\pi^*) \rightarrow S_1^1(n_S\pi^*) \rightarrow T_1^3(\pi_S\pi^*)$  and  $S_2^1(\pi_S\pi^*) \rightarrow T_2^3(n_S\pi^*) \rightarrow T_1^3(\pi_S\pi^*)$ , with the former being the most probable. The study also explained why populating the triplet state of 4-TU is two times faster than that of 2-TU. This is attributed to the planar geometry of 4-TU in contrast to 2-TU and the fact that, in 4-TU, the minimum energy region

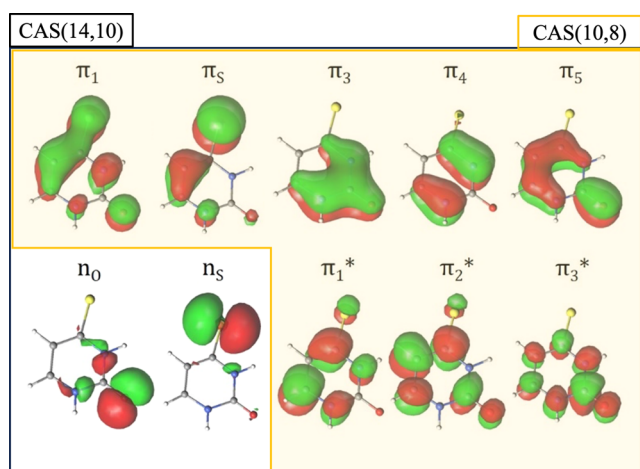
of the singlet  $n\pi^*$  state crosses the potential energy hypersurface of the triplet state of  $\pi\pi^*$  nature enhancing the possibility of population transfer to the triplet state, whereas 2-TU needs to overcome a barrier of ca. 0.1 eV in order to populate the triplet manifold. However, a detailed knowledge about the factors influencing the experimentally observed phosphorescence yields and triplet lifetimes was still missing. Therefore, the present contribution is devoted to providing deeper details on the intrinsic photophysical properties of the 4-TU molecule by using the SS-CASPT2 method and different flavors of the density functional theory (DFT) method with the B3LYP functional. Furthermore, by comparing the photophysical properties and the geometry differences between 4-TU and 2-TU, we achieve a more comprehensive interpretation of their behavior and characteristics.

## COMPUTATIONAL DETAILS

Geometry optimizations of excited-state minima, conical intersections (CIs), singlet–triplet crossings (STCs), and minimum energy paths (MEPs) have been mainly performed with the CASPT2//CASSCF protocol, which consists of geometry optimizations at the CASSCF<sup>44</sup> level, followed by single-point energy calculations at the state-specific CASPT2<sup>45</sup> (SS-CASPT2; here after CASPT2) level on top of the converged geometries. Calculations were performed without symmetry constraints and the ANO-S-VDZP basis set, since the CASPT2 method does not depend much on the basis set's size in the case of thiouracils<sup>46</sup> and also in larger systems.<sup>47</sup> However, in order to test this dependence on the basis set, CASPT2 benchmark calculations were performed in this study with the ANO-S-VDZP, ANO-L-VDZP, and ANO-L-VTZP basis sets (see Tables S1–S5 on the Supporting Information), obtaining the same conclusions for energies and SOCs. CASSCF and CASPT2 calculations were carried out with MOLCAS 8 software.<sup>48</sup> CIs and STCs were computed as the minimum energy crossing points (MECPs) on the singlet–singlet, triplet–triplet, and singlet–triplet manifolds obtained with the restricted Lagrange multiplier technique,<sup>49</sup> imposing the constraint of degeneracy between the two states considered. In order to perform a geometrical analysis and comparison, some specific geometry optimizations (indicated in Results and Discussion) were also carried out: (1) at the CASPT2/ANO-S-VDZP level and (2) with the spin-flip time-dependent DFT (SF-TDDFT),<sup>50</sup> (3) time-dependent DFT (TDDFT), and/or (4) Tamm–Dancoff DFT approach (TDA)<sup>51</sup> methods, using the B3LYP functional and the 6-311 + G\*\* basis set, with Q-Chem 5 software.<sup>52</sup>

MEPs were computed with a modified version of the steepest descent algorithm developed by Anglada and Boffil, as proposed by Müller-Brown.<sup>53</sup> Mass-weighted coordinates (in bohr (amu)<sup>1/2</sup>) were used, which means that they are equivalent to the so-called intrinsic reaction coordinate (IRC).

The CASSCF active space for ground state (GS) geometry optimization comprises the  $\pi$  and  $\pi^*$  orbitals and electrons (CASSCF(10,8)) only (Figure 2, highlighted in yellow). For this geometry optimization, state-specific CASSCF wave functions were employed; that is, a single root was employed in the CASSCF step.  $S_0/T_1$  MECP geometry optimization was obtained at the CASPT2 level due to the differential correlation initially detected when performing CASSCF geometry optimization of this point. This CASPT2 optimization was performed with an active space of CAS(10,8)



**Figure 2.** Valence natural orbitals computed at the SA(7)-CASSCF-(14,10)/ANO-S-VDZP (highlighted in black) and SS-CASSCF-(10,8)/ANO-S-VDZP (highlighted in yellow) levels of theory at the ground-state equilibrium geometry for 4-thiouracil.

including the  $\pi$ -system orbitals. Remaining geometry optimizations were carried out with a larger active space, including 14 electrons distributed over 10 molecular orbitals (MOs; 8  $\pi$  and  $\pi^*$  orbitals, plus the oxygen and sulfur lone pairs; CASSCF(14,10)) (Figure 2, highlighted in black), including the necessary roots to consider the desired states.

In order to add dynamical correlation effects to all of the geometries corresponding to the previously optimized minima, crossing points, and MEP points, the CASPT2 method<sup>49,53,54</sup> was used with an active space that includes all  $\pi$ ,  $\pi^*$  and lone pair orbitals, that is, CASSCF(14,10). Seven singlets and seven triplets were computed using as zeroth-order reference for each spin multiplicity a state-average CASSCF (SA-CASSCF) wave function (with equal weights for each state). The zeroth-order Hamiltonian with an IPEA<sup>55</sup> value of zero and an imaginary level shift<sup>56</sup> of 0.2 au have been used in CASPT2 calculations. Reference weights of the CASSCF wave functions in the CASPT2 computations for each root are compiled in Table S6, indicating that it does not include intruder states. Associated oscillator strengths ( $f$ ) were computed using transition dipole moments (length representation) obtained with the CAS state interaction (CASSI) method<sup>57</sup> and state energy differences calculated at the CASPT2 level as described above.

Benchmark computations were conducted on the different minima and the MECPs by comparing CASPT2//CASSCF and MS-CASPT2//CASSCF protocols (see Tables S7 and S8 in the Supporting Information). The outcomes consistently offer a comparable perspective in both scenarios, with the SS-CASPT2 values establishing a lower limit of 0.01 eV for the energy difference between the states on the degeneracy regions, and MS-CASPT2 values establishing an upper limit of 0.3 eV in these points where we would also expect degeneracy. The use of CASSCF for geometries and CASPT2 for the final energies also introduces a certain degree of differential correlation specially in the crossing points. However, almost degeneracies are kept when recomputing energies at the CASPT2 level on top of the CASSCF structures with gaps lower than 0.2 eV. All of these differences are not too high and allow providing a reliable description with the CASPT2//CASSCF protocol.

## RESULTS AND DISCUSSION

**Franck–Condon Region Spectroscopy.** The CASPT2 vertical absorption energies ( $E_v^{\text{abs}}$ ) computed at the CASSCF ground state optimized geometry (FC region), corresponding nature (CSF),  $f$  values, dipole moments ( $\mu$ ), and weight of the most important configurations in the CASSCF wave function for the seven lowest-lying singlet and triplet electronic states of 4-TU are collected in Table 1. We also added our computed data at TDDFT and TDA levels, and the data obtained by Borrego-Varillas et al.<sup>43</sup>

As we can notice, the 4-TU brightest state is the  $S_2$  ( $^1\pi_s\pi_1^*$ ) state, derived from the ground state by a single electron excitation from the  $\pi_s$  to the  $\pi_1^*$  orbital, with a smaller contribution from the  $\pi_1 \rightarrow \pi_1^*$  single excitation, which has also a contribution from the S atom (Figure 2) and also a small influence of double excitations from the  $\pi_s$  orbital. The  $S_1$  ( $^1n_s\pi_1^*$ ) appears at 2.75 eV vertically above the ground state, being derived from it by a single excitation from the  $n_s$  lone pair orbital, mainly localized on the sulfur atom, to the  $\pi_1^*$  orbital; the associated oscillator strength is computed to be null. Below the  $S_1$  ( $^1n_s\pi_1^*$ ) state, we found two lowest-lying triplet electronic states,  $T_1$  ( $^3(\pi_s\pi_1^*, \pi_1\pi_1^*)$ ) and  $T_2$  ( $^3(n_s\pi_1^*)$ ). The former has an electronic wave function mainly dominated by the  $^1\pi_s \rightarrow \pi_1^*$  single excitation and is localized at 2.59 eV vertically above the ground state, and the latter is mainly described by the  $n_s \rightarrow \pi_1^*$  single excitation and appears at 2.62 eV above the ground state energy. It is worth noticing the presence of triplet states below the  $S_1$  ( $^1n_s\pi_1^*$ ) and  $S_2$  ( $^1\pi_s\pi_1^*$ ) singlet excited states, a favorable condition for triplet state population via intersystem crossing mechanisms.

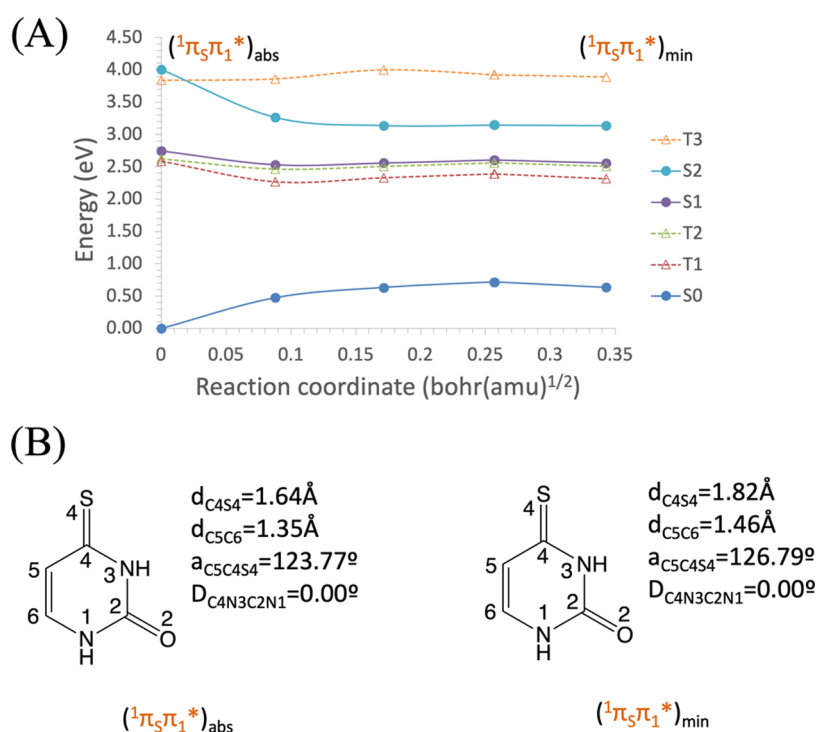
Regarding TDDFT and TDA-computed data, at the TDDFT level, the  $S_2$  ( $^1\pi_s\pi_1^*$ ) is 0.22 eV higher in energy than that at the CASPT2 level. This state is vertically placed at 4.49 eV at the TDA level, namely, about 0.5 eV overestimated with respect to the CASPT2 value. Both DFT values are much higher than the experimental one, obtained in water and in acetonitrile (3.79 eV).<sup>39,41</sup> This discrepancy on the  $S_2$  ( $^1\pi_s\pi_1^*$ ) state energy between DFT-based and multiconfigurational methods might be due to the multiconfigurational nature with certain contribution of double excitations, as identified in Table 1. Furthermore, the TDDFT and TDA methods were not able to reproduce the CASPT2 energetic order, in particular for the states situated above the  $S_2$  ( $^1\pi_s\pi_1^*$ ) state, where also some double excitations were found in the description of the electronic states. This suggests a limitation in the ability of the TDDFT and TDA methodologies mentioned here to provide an accurate description of the spectroscopic data of 4-TU.

The  $E_v^{\text{abs}}$  computed herein are now compared with those obtained experimentally.<sup>39</sup> The experimental absorption spectrum exhibits two bands, the most intense peaking at  $\sim 327$  nm (3.79 eV, ranging from 280 to 360 nm) and the weakest at 240 nm (5.12 eV). According to our results, the strongest absorption band can be associated with the electronic transitions to the  $S_2$  ( $^1\pi_s\pi_1^*$ ) and  $S_3$  ( $^1\pi_1\pi_1^*$ ) excited states. The electronic transition to the  $S_2$  ( $^1\pi_s\pi_1^*$ ) can be associated with the maximum of the strongest band at 3.79 eV, while the  $S_3$  ( $^1\pi_1\pi_1^*$ ) state contributes to the higher energy region. The weakest band can be assigned to the transition to the  $S_4$  ( $^1\pi_s\pi_2^*$ ) state, located at 4.90 eV (253 nm) above the ground state.

**Table 1. 4-Thiouracil Vertical Absorption Energies ( $E_v^{\text{abs}}$ , eV), Oscillator Strengths ( $f$ ), Nature and Weights (%) of the Main Excitations, and Dipole Moments ( $\mu$ , Debye) for the Seven Lowest Singlet and Triplet States at the Franck–Condon Region at Different Levels of Theory Data from Ref 43 and Experimental Studies are also Provided, Both Obtained in Water Solution (cs = Closed Shell, de = Double Excitation)**

state	$E_v^{\text{abs}}$	$f$	CASPT2			TDDFT			TDA			ref 43		exp. data	
			nature	weight	$\mu$	state	$E_v^{\text{abs}}$	$f$	nature	weight	state	$E_v^{\text{abs}}$	$f$		$E_v^{\text{abs39}}$
$S_0$			cs	78	4.28	$S_0$									
			$^1(\pi_S\pi_1^*)$	11											
$T_1$	2.59		$^3(\pi_S\pi_1^*)$	57	3.02	$T_1$	50	2.38	0.000	$^3(\pi_S\pi_1^*)$	49	2.49	0.000	$^3(\pi_S\pi_1^*)$	49
			$^3(\pi_1\pi_1^*)$	30											
$T_2$	2.62		$^3(n_S\pi_1^*)$	86	2.87	$T_2$	49	2.48	0.000	$^3(n_S\pi_1^*)$	49	2.55	0.000	$^3(n_S\pi_1^*)$	49
$S_1$	2.75	0.000	$^1(n_S\pi_1^*)$	88	3.10	$S_1$	50	2.79	0.000	$^1(n_S\pi_1^*)$	50	2.80	0.000	$^1(n_S\pi_1^*)$	50
$T_3$	3.84		$^3(\pi_S\pi_2^*)$	34	3.64	$T_3$	39	3.78	0.000	$^3(\pi_1\pi_1^*)$	41	3.88	0.000	$^3(\pi_1\pi_1^*)$	41
			$^3(\pi_1\pi_1^*)$	33			6.9			$^3(\pi_S\pi_2^*)$					
$S_2$	4.00	0.419	$^1(\pi_S\pi_1^*)$	47	4.55	$S_2$	46	4.22	0.298	$^1(\pi_S\pi_1^*)$	37	4.49	0.347	$^1(\pi_S\pi_1^*)$	37
			$^1(\pi_1\pi_1^*)$	18											
			de $^1(2\pi_S\pi_1^*)$	3											
			de $^1(\pi_S\pi_1^* + \pi_S\pi_2^*)$	2											
$S_3$	4.41	0.158	$^1(\pi_1\pi_1^*)$	37	8.13	$T_4$	49	4.55	0.000	$^3(n_S\pi_2^*)$	49	4.55	0.000	$^3(n_S\pi_2^*)$	49
			$^1(\pi_S\pi_1^*)$	21											
			de $^1(2\pi_S\pi_1^*)$	11											
$T_4$	4.56		$^3(n_S\pi_2^*)$	52	3.04	$S_3$	49	4.61	0.000	$^1(n_S\pi_2^*)$	49	4.61	0.000	$^1(n_S\pi_2^*)$	49
			de $^3(\pi_S\pi_1^* + n_S\pi_1^*)$	17											
$S_4$	4.90	0.128	$^1(\pi_S\pi_2^*)$	42	3.83	$S_4$	48	4.82	0.008	$^1(\pi_1\pi_1^*)$	45	4.93	0.018	$^1(\pi_1\pi_1^*)$	45
			$^1(\pi_1\pi_1^*)$	18											
$T_5$	4.93		$^3(\pi_1\pi_2^*)$	50	3.95										
$S_5$	5.10	0.000	$^1(n_S\pi_2^*)$	57	3.14										
			de $^1(\pi_S\pi_1^* + n_S\pi_1^*)$	13											
			de $^1(\pi_1\pi_1^* + n_S\pi_1^*)$	11											
$T_6$	5.19		$^3(\pi_S\pi_2^*)$	29	5.69										
			$^3(\pi_1\pi_1^*)$	17											
			$^3(\pi_S\pi_1^*)$	14											
			$^3(\pi_1\pi_2^*)$	10											
$S_6$	6.05	0.000	$^1(n_O\pi_1^*)$	70	11.45										
$T_7$	6.19		$^3(n_O\pi_2^*)$	63	5.37										
			$^3(n_O\pi_3^*)$	14											





**Figure 5.** (A) Energies of the ground and lowest-lying singlet and triplet electronic excited states of 4-thiouracil along the minimum energy path (MEP) of the  $S_2$   $(^1\pi_s\pi_1^*)$  state from the  $S_0$  equilibrium structure toward the  $S_2$   $(^1\pi_s\pi_1^*)$  equilibrium geometry, computed at the CASPT2//CASSCF(14,10)/ANO-S-VDZP level of theory. (B) Main geometrical changes related with the MEP coordinate.

(i.e., ANO-S-VDZP), optimizations of the  $S_1$ ,  $S_2$ , and  $T_1$  minima were performed at the CASSCF(14,10)/ANO-LVTZP level. In this case, the same planar structures were obtained as with the previous basis set. Additionally, a pyramidalized geometry was found for the minimum of the  $S_2$  excited state. This pyramidalized structure is located 0.63 eV higher in energy compared to the planar minimum. Consequently, we infer that it will not be relevant in our proposed scheme and that geometries obtained with ANO-S are trustworthy enough. The accuracy of the ANO-S basis set was demonstrated by Merchán et al. in related systems.<sup>58</sup> The same tests were performed for the  $S_2$  and  $S_1$  minima of the 2-TU molecule obtaining, in both cases, both a planar and a pyramidalized structure. Energies can be found in Table S11. For the  $S_2$  equilibrium structure, both geometries are degenerated in energy, while for the  $S_1$  minima, the planar geometry is located 0.15 eV above the distorted one. These calculations agree with the vision described by Mai et al.,<sup>36</sup> confirming that our level of theory is adequate for describing these systems.

Duan et al. used the TDDFT CAM-B3LYP/6-31G\* approach to study the photochemistry and photophysics of 2-TU,<sup>59</sup> obtaining similar geometries to those obtained by the MS-CASPT2 method. To analyze the performance of TDDFT in 4-TU, we also optimized the  $T_2$   $(^3n_s\pi_1^*)$  state using the CAM-B3LYP/6-31G\* calculation level. The out-of-plane distortion obtained at the CASSCF and CASPT2 levels is not reproduced at the CAM-B3LYP level (Figure S3). This indicates that TDDFT and TDA methods, at least with the mentioned functional and basis set, are not accurate for the 4-TU photochemical pathways determination.

In contrast to TDDFT and TDA, SF-TDDFT provides a good agreement with CASSCF and CASPT2 in the  $T_1S_0$  ( $(^3\pi_s\pi_1^*/GS)_{STC}$ ) crossing structure computed at such a level

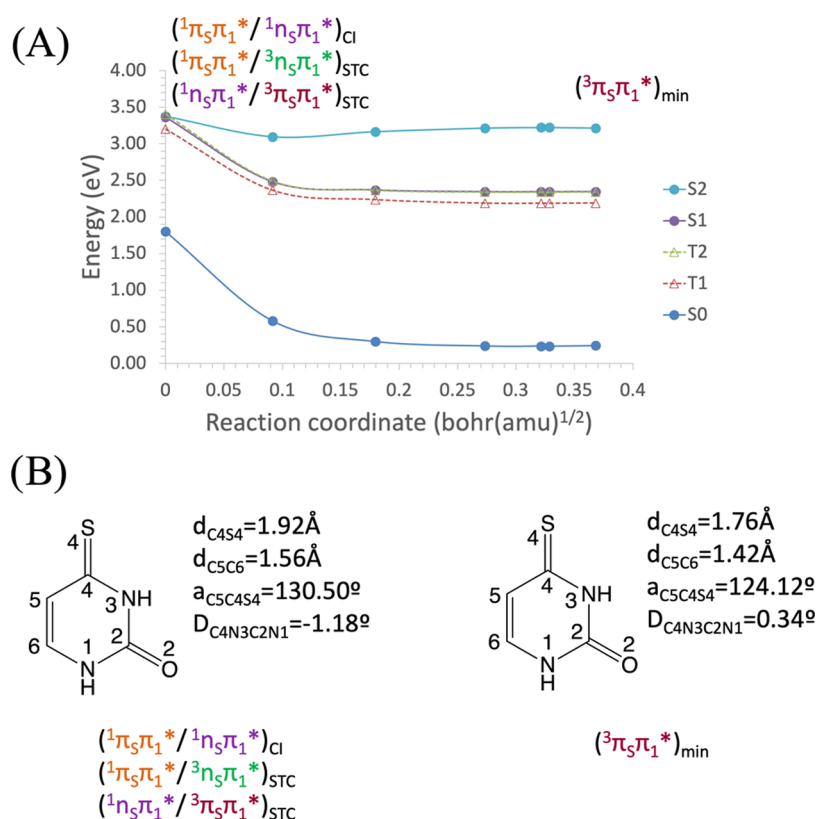
(see Figure S7), similar to the observations found in the literature for 2-TU.<sup>1,26</sup>

Overall, we chose the CASSCF method, which is in good agreement with CASPT2 results for 4-TU, to obtain the geometries of the decay channels with some data computed with the SF-TDDFT method to provide comparisons with 2-TU from the literature using such a method.

**Photophysics of 4-Thiouracil.** To understand the deactivation mechanisms of 4-TU excited states, we have performed MEP computations and CI and STC searches at the CASPT2//CASSCF level involving the  $S_0$ ,  $S_1$ ,  $S_2$ ,  $T_1$ ,  $T_2$ , and  $T_3$  electronic states. CASSCF and CASPT2 energies of all states at the most relevant points of the photophysics of the system are shown in Tables S9 and S10.

The initial step was to compute the MEP along the  $S_2$  bright excited-state potential energy hypersurface, starting from the FC region. The relative energies to the GS of the lowest singlet and triplet states along the MEP of the  $S_2$  state are shown in Figure 5A. In Figure 5B, the main geometrical changes that take place along the MEP coordinate are represented. As can be noticed, the  $S_2$  state evolves barrierless from the FC region to its minimum structure (last point of the MEP), which lies at 3.14 eV. When we remove the MEP constraint and optimize the geometry of this point, a nearby structure is found, slightly more stable (3.02 eV), which only differs in the  $N_3C_3S_4$  angle, which changes from 112.37 to 110.86°. It is important to note that this structure remains planar. It is also worth noticing the existence of an STC between the  $S_2$  and  $T_3$  states at the FC region with an SOC of  $<0.5$  cm<sup>-1</sup>, very small to be responsible for a population transfer to  $T_3$  in this region. Therefore, this STC can be safely ignored as a viable triplet state population mechanism.

As the  $S_2$   $(^1\pi_s\pi_1^*)$  state does not cross other potential energy hypersurfaces along its MEP from the FC region, we



**Figure 6.** (A) Energies of the ground and lowest-lying singlet and triplet electronic excited states of 4-thiouracil along the minimum energy path (MEP) of the T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) state from the S<sub>2</sub>S<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) conical intersection toward the T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) equilibrium geometry, computed at the CASPT2//CASSCF(14,10)/ANO-S-VDZP level of theory. (B) Main geometrical changes related with the MEP coordinate.

continued searching for a possible MECF (CI and STC) in other regions. A multistate crossing region was found involving the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>), S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), and T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) states. From there on, several possible paths are open for the decay. The CI between S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) is found at 3.37 eV. This crossing point is located ~0.24 eV above the S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) minimum and is similar to the CI reported for 2-TU in ref 43. The connection between the minimum and the CI is confirmed by an MEP computation on the S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) state starting at the S<sub>2</sub>S<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) CI geometry (see Figure S10). It is worth mentioning that other S<sub>2</sub>S<sub>1</sub> CIs were found at much higher energies, which makes them photochemically irrelevant. The computed geometries are displayed in Figure S5.

At the multistate crossing region, the STC between the S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) states is found at 3.41 eV, that is, 0.27 eV above the S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) minimum. In this region, the SOC is computed to be 107.4 cm<sup>-1</sup>, a relatively large value, which can be attributed to the different nature of the states involved.

At the multistate crossing region, we also computed the SOC between other nearby singlet and triplet states. For the STC between the T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) states, the SOC is computed to be 7.3 cm<sup>-1</sup>, in agreement with ref 43. In the case of T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>), the SOC is 169.3 cm<sup>-1</sup>. Finally, for S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), it is 6.0 cm<sup>-1</sup>. As can be seen, the lower or higher SOC follows El-Sayed's rules.

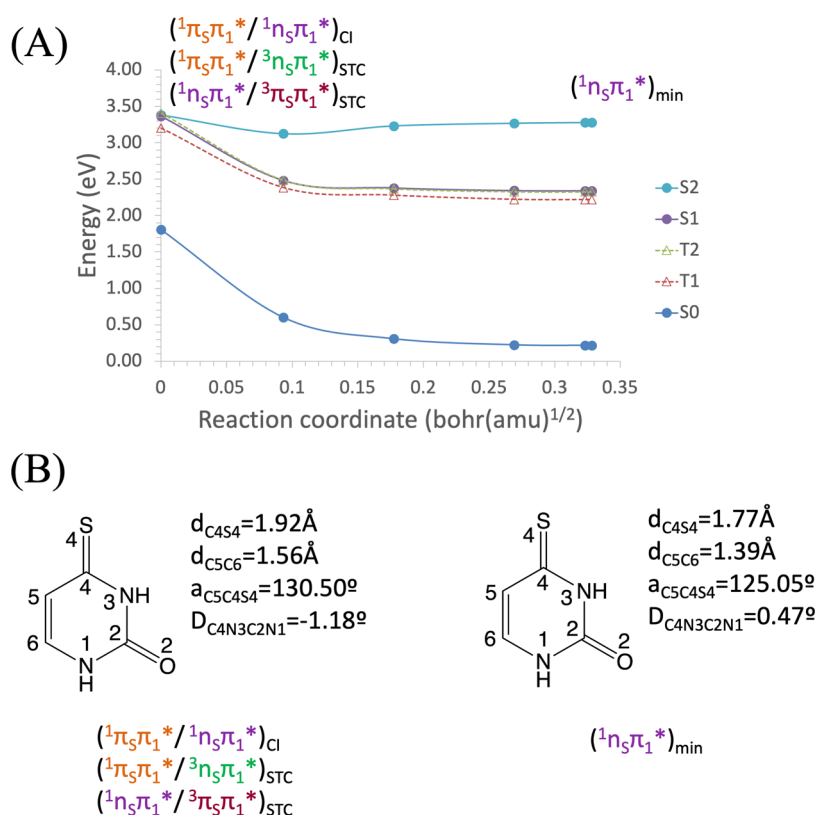
From the multistate crossing region involving the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>), S<sub>2</sub><sup>1</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), and T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) electronic states and considering the computed SOC, three plausible deactivation pathways are foreseen: (i) along the S<sub>1</sub> state via

the S<sub>2</sub>S<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) CI, (ii) along the T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) state via the S<sub>2</sub>T<sub>2</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/3n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) STC, and (iii) along the T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) state via, first, the S<sub>2</sub>S<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) CI crossing and, next, the S<sub>1</sub>T<sub>1</sub>(<sup>1</sup>n<sub>s</sub>π<sub>1</sub><sup>\*</sup>/3π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) STC (note that these CIs and STCs appear to be near-degenerate). Thus, we decided to calculate the corresponding MEPs along the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>), T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>), and T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) potential energy hypersurfaces.

The T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) MEP from the S<sub>1</sub>T<sub>1</sub>(<sup>1</sup>n<sub>s</sub>π<sub>1</sub><sup>\*</sup>/3π<sub>s</sub>π<sub>1</sub><sup>\*</sup>)<sub>STC</sub> ends in the minimum of that state at 2.19 eV, as shown in Figure 6. The S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) MEP from the S<sub>2</sub>S<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>)<sub>CI</sub> (Figure 7) and the T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) MEP from the S<sub>2</sub>T<sub>2</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/3n<sub>s</sub>π<sub>1</sub><sup>\*</sup>)<sub>STC</sub> (Figure 8) show an evolution in parallel, as expected for nπ\* states of carbonyls in π-conjugated molecules. The S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) MEP ends barrierless at the equilibrium geometry of the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) state at 2.33 eV, which coincides with the S<sub>1</sub>T<sub>1</sub>(<sup>1</sup>n<sub>s</sub>π<sub>1</sub><sup>\*</sup>/3π<sub>s</sub>π<sub>1</sub><sup>\*</sup>)<sub>STC</sub>. Meanwhile, the T<sub>2</sub><sup>3</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) MEP also ends barrierless at its minimum, which is nearby the T<sub>2</sub>T<sub>1</sub>(<sup>1</sup>π<sub>s</sub>π<sub>1</sub><sup>\*</sup>/1n<sub>s</sub>π<sub>1</sub><sup>\*</sup>)<sub>CI</sub> at 2.31 eV. For each of these MEPs, geometry optimizations without the MEP constraint were performed, affirming that the final point in each case corresponds to the minimum of the respective state. As expected from the fact that the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) states are of different nature, the computed SOC between them in the STC region is large (155.0 cm<sup>-1</sup>), in agreement with Borrego-Varillas et al.<sup>43</sup>

Finally, from the STC between the S<sub>1</sub><sup>1</sup>(n<sub>s</sub>π<sub>1</sub><sup>\*</sup>) and T<sub>1</sub><sup>3</sup>(π<sub>s</sub>π<sub>1</sub><sup>\*</sup>) states, the lowest-lying triplet state decays to its minimum, placed at 2.19 eV (see Figure 9). From this point, it can either transfer its energy to the triplet oxygen or deactivate itself to the GS through a radiative or a nonradiative process.





**Figure 7.** (A) Energies of the ground and lowest-lying singlet and triplet electronic excited states of 4-thiouracil along the minimum energy path (MEP) of the  $S_2S_1$  ( $^1\pi_s\pi_1^*/^1n_s\pi_1^*$ ) state from the  $S_2S_1$  ( $^1\pi_s\pi_1^*/^1n_s\pi_1^*$ ) conical intersection toward the  $S_1$  ( $^1n_s\pi_1^*$ ) equilibrium geometry, computed at the CASPT2//CASSCF(14,10)/ANO-S-VDZP level of theory. (B) Main geometrical changes related with the MEP coordinate.

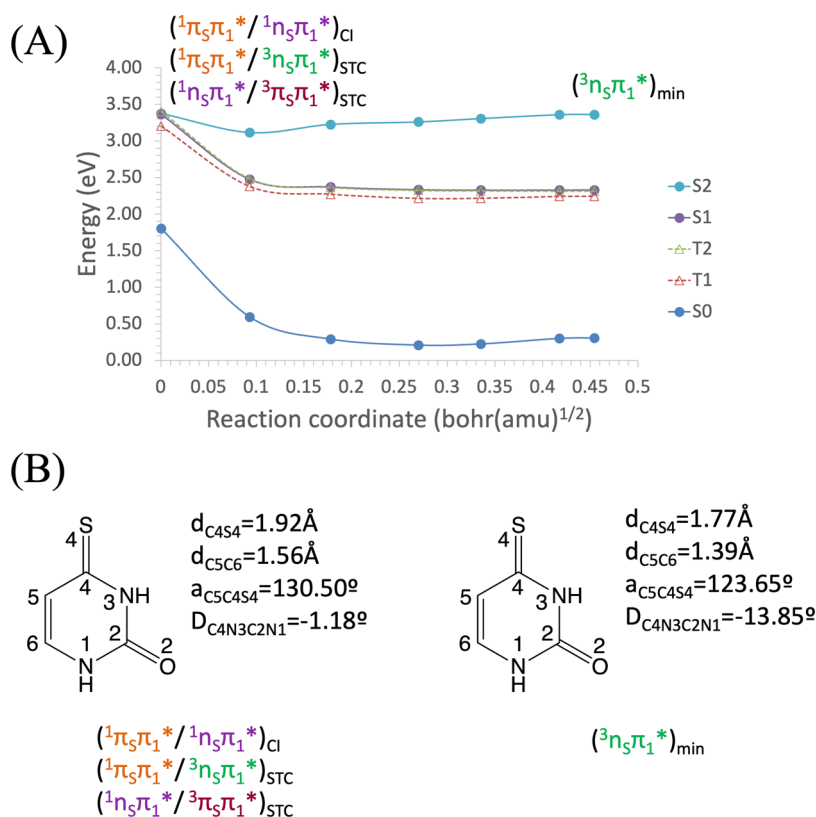
To characterize the latter, we computed the  $T_1S_0$  ( $^3\pi_s\pi_1^*/GS$ )<sub>STC</sub>, which was found at 3.17 eV. The MEP from the  $T_1S_0$  ( $^3\pi_s\pi_1^*/GS$ )<sub>STC</sub> along the  $T_1$  ( $^3\pi_s\pi_1^*$ ) potential energy surface connects the STC with the  $T_1$  ( $^3\pi_s\pi_1^*$ ) minimum, as shown in Figure S11. The relative energy from the  $T_1$  minimum to reach the  $T_1S_0$  ( $^3\pi_s\pi_1^*/GS$ )<sub>STC</sub> and therefore the point for nonradiative decay to the GS is  $\sim 0.98$  eV, which is relatively high. Furthermore, the SOC at this STC between  $T_1$  and  $S_0$  has a relatively low value of  $5\text{ cm}^{-1}$ . Both features make this process unfavorable.

The crossing between the  $S_0$  and  $S_1$  ( $^1n_s\pi_1^*$ ) electronic states was also computed for 4-TU, displaying energy barriers of about 1.15 eV from the  $S_1$  ( $^1n_s\pi_1^*$ ) minimum. Such a barrier is too high to play a relevant role in the deactivation mechanism of the molecule to the GS.

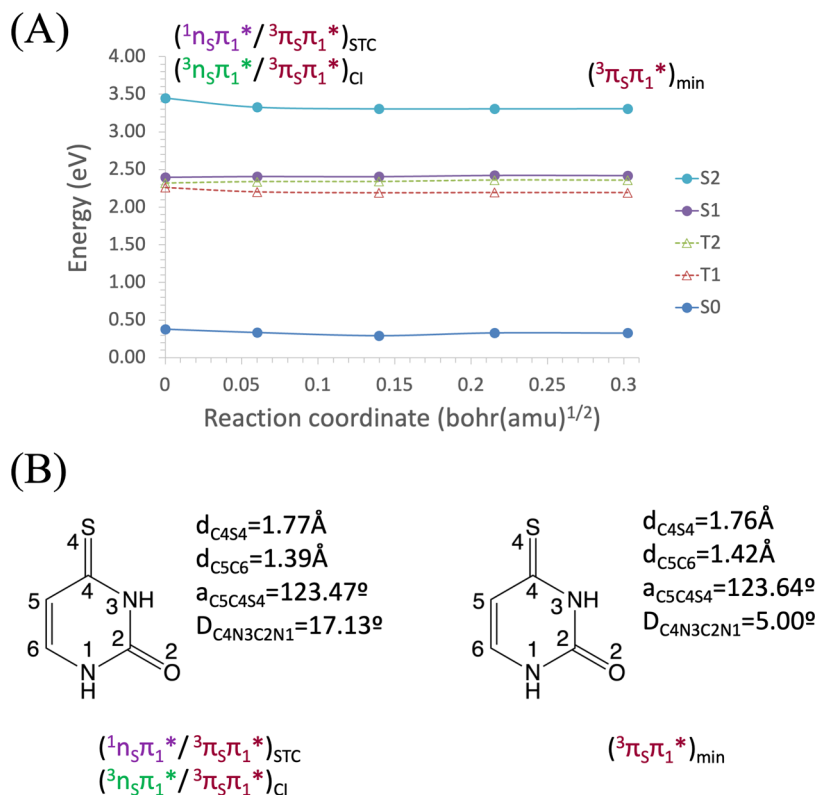
The most relevant geometry changes along the deactivation paths involve the  $S_4-C_4$  and  $C_5-C_6$  bonds, the  $S_4-C_4-C_5$  angle, and dihedral angles related to distortions from the molecular plane, namely,  $S_4-C_4-C_5-C_6$ ,  $C_4-C_5-C_6-N_1$ , and  $C_4-N_3-C_2-N_1$  (see Figures 3 and S1–S8). Along the  $S_2$  ( $^1\pi_s\pi_1^*$ ) state evolution from the FC toward the multistate crossing region, the above-mentioned chemical bonds elongate and the angles increase, while the molecule remains mostly planar. Then, from the CI between the  $S_2$  ( $^1\pi_s\pi_1^*$ ) and  $S_1$  ( $^1n_s\pi_1^*$ ) electronic states, the  $S_1$  ( $^1n_s\pi_1^*$ ) MEP evolves similarly toward the STC with  $T_1$  ( $^3\pi_s\pi_1^*$ ), shortening the bonds and decreasing the angles, but in this case, the molecule becomes slightly more distorted. The same happens from the STC between  $S_2$  ( $^1\pi_s\pi_1^*$ ) and  $T_2$  ( $^3n_s\pi_1^*$ ) on the  $T_2$  ( $^3n_s\pi_1^*$ ) MEP toward the CI with  $T_1$  ( $^3\pi_s\pi_1^*$ ). Finally, from the end of such MEPs on the  $T_1$  ( $^3\pi_s\pi_1^*$ ) potential energy hypersurface

toward its minimum, the chemical bonds and angles remain almost invariant, but the molecule returns to a flatter configuration.

Figure 10 shows an illustrative scheme of the decay paths derived from these SS-CASPT2 computations. The same scheme has been also represented with MS-CASPT2 energy values, see Figure S12 in the SI, with no significant differences on the main features of the photophysics mechanism, which reinforces the accuracy of the description provided. Three deactivation pathways can be proposed for photoexcited 4-TU. The first one will be  $S_{2,FC}$  (4.0 eV)  $\rightarrow$   $S_{2,min}$  (3.1 eV)  $\rightarrow$   $S_{1,(S_2S_1)CI}$  (3.4 eV)  $\rightarrow$   $S_{1,min}$  (2.3 eV)  $\rightarrow$   $T_{1,(S_1T_1)STC}$  (2.3 eV)  $\rightarrow$   $T_{1,min}$  (2.2 eV), with an SOC of  $155\text{ cm}^{-1}$  between the  $S_1$  ( $^1n_s\pi_1^*$ ) and  $T_1$  ( $^3\pi_s\pi_1^*$ ) states. The second path will be  $S_{2,FC}$  (4.0 eV)  $\rightarrow$   $S_{2,min}$  (3.1 eV)  $\rightarrow$   $S_{1,(S_2S_1)CI}$  (3.4 eV)  $\rightarrow$   $T_{1,(S_1T_1)STC}$  (3.4 eV)  $\rightarrow$   $T_{1,min}$  (2.2 eV), with an SOC of  $169\text{ cm}^{-1}$  between the  $S_1$  ( $^1n_s\pi_1^*$ ) and  $T_1$  ( $^3\pi_s\pi_1^*$ ) states. The last one will be  $S_{2,FC}$  (4.0 eV)  $\rightarrow$   $S_{2,min}$  (3.1 eV)  $\rightarrow$   $T_{2,(S_2T_2)STC}$  (3.4 eV)  $\rightarrow$   $T_{1,(T_2T_1)CI}$  (2.3 eV)  $\rightarrow$   $T_{1,min}$  (2.2 eV), with an SOC of  $107\text{ cm}^{-1}$  between the  $S_2$  ( $^1\pi_s\pi_1^*$ ) and  $T_2$  ( $^3n_s\pi_1^*$ ) states. The large SOC observed along the second path points out that it would be the most likely to happen. On the other hand, in the first path, the spin inversion occurs in the minimum of the  $S_1$  ( $^1n_s\pi_1^*$ ) state, where the molecule will be trapped, increasing the probability for ISC even with lower SOC values. Thus, both regions shall allow for a significant ISC mechanism. However, as they correspond to the same mechanism, we will refer to them as  $S_2$  ( $^1\pi_s\pi_1^*$ )  $\rightarrow$   $S_1$  ( $^1n_s\pi_1^*$ )  $\rightarrow$   $T_1$  ( $^3\pi_s\pi_1^*$ ) and the last one as  $S_2$  ( $^1\pi_s\pi_1^*$ )  $\rightarrow$   $T_2$  ( $^3n_s\pi_1^*$ )  $\rightarrow$   $T_1$  ( $^3\pi_s\pi_1^*$ ) paths. Therefore, we will have, in general, two deactivation pathways, with the first being the most probable.



**Figure 8.** (A) Energies of the ground and lowest-lying singlet and triplet electronic excited states of 4-thiouracil along the minimum energy path (MEP) of the  $T_2$   $^3(n_s\pi_1^*)$  state from the  $S_2T_2$  ( $^1\pi_s\pi_1^*/^3n_s\pi_1^*$ ) singlet–triplet crossing toward the  $T_2$  ( $^3n_s\pi_1^*$ ) minimum, computed at the CASPT2//CASSCF(14,10)/ANO-S-VDZP level of theory. (B) Main geometrical changes related with the MEP coordinate.



**Figure 9.** (A) Energies of the ground and lowest-lying singlet and triplet electronic excited states of 4-thiouracil along the minimum energy path (MEP) of the  $T_1$   $^3(\pi_s\pi_1^*)$  state from  $S_1T_1$  ( $^1n_s\pi_1^*/^3\pi_s\pi_1^*$ ) toward the  $T_1$   $^3(\pi_s\pi_1^*)$  equilibrium structure, computed at the CASPT2//CASSCF(14,10)/ANO-S-VDZP level of theory. (B) Main geometrical changes related with the MEP coordinate.



with an SOC of  $107.4 \text{ cm}^{-1}$  between the  $S_2 \ ^1(n_s\pi_1^*)$  and  $T_2 \ ^3(n_s\pi_1^*)$  states. The energy barriers along the paths are very low in both cases and therefore the SOC values will determine the ISC efficiencies and, consequently, the most probable deactivation path. Therefore, 4-TU will most probably decay through the path via  $S_1 \ ^1(n_s\pi_1^*)$  as an intermediate state.

A difference between the lowest-lying triplet state in both systems was found. In the case of 4-TU, there is only a minimum in the  $T_1 \ ^3(\pi_s\pi_1^*)$  potential energy hypersurface, while in 2-TU, it shows two minima of different nature ( $^3(\pi_s\pi_2^*)$  and  $^3(\pi_s\pi_6^*)$ ). In the 2-TU molecule, the  $T_1S_0$  crossings are, in general, more accessible and have higher SOCs than in 4-TU. Therefore, the deactivation to the GS will be more favorable for 2-TU.

These mentioned findings were already obtained by Borrego-Varillas et al.<sup>43</sup> modeling the excited-state dynamics of 4-TU in aqueous solution. Herein, differences in experimental data between 4-TU and 2-TU were further rationalized. Thus, the longer triplet lifetimes and lower phosphorescence quantum yields for the former can be explained due to the less energetically accessible  $T_1S_0$  crossing, lower SOCs at such crossing, and a lower radiative rate at the  $T_1$  minimum. The larger ISC quantum yield of 4-TU with respect to that of 2-TU can be associated with the fact that the SOCs along the decay path toward the  $T_1$  equilibrium geometry are higher for 4-TU. The high ISC quantum yield of 4-TU is also linked to a high singlet oxygen formation and a lower phosphorescence yield measured under aerobic conditions.

Thus, with the present study, we reinforce the conclusion that 4-TU is a better photosensitizer compared to 2-TU and is deemed a more suitable candidate for photodynamic therapy.

## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpca.3c06310>.

Geometrical parameters of interest for 4-TU; CASSCF active space for the 2-TU system; complementary MEPs for the 4-TU deactivation mechanism with their respective geometrical changes; schematic representation of the deactivation pathway of 4-TU with MS-CASPT2 energies; schematic representation of the deactivation pathway of 2-TU; ANO-S and ANO-L basis set comparison at the Franck–Condon region and MECPs for energies and spin–orbit couplings; SS and MS-CASPT2 methods comparison at equilibrium structures and MECPs for energies and oscillator strengths; reference weights for SS-CASPT2 calculations; CASSCF and CASPT2 energies at relevant points of the 4-TU molecule; Cartesian coordinates (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

Daniel Roca-Sanjuán – Instituto de Ciencia Molecular, Universitat de València, ES-46071 Valencia, Spain; [orcid.org/0000-0001-6495-2770](https://orcid.org/0000-0001-6495-2770); Email: [daniel.roca@uv.es](mailto:daniel.roca@uv.es)

### Authors

Miriam Navarrete-Miguel – Instituto de Ciencia Molecular, Universitat de València, ES-46071 Valencia, Spain

Angelo Giussani – Instituto de Ciencia Molecular, Universitat de València, ES-46071 Valencia, Spain; [orcid.org/0000-0002-9452-7641](https://orcid.org/0000-0002-9452-7641)

Mercedes Rubio – Departament de Química Física, Universitat de València, 46100 Burjassot, Spain

Martial Boggio-Pasqua – Laboratoire de Chimie et Physique Quantiques, IRSAMC, CNRS et Université Toulouse 3, 31062 Toulouse, France; [orcid.org/0000-0001-6684-5223](https://orcid.org/0000-0001-6684-5223)

Antonio Carlos Borin – Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, São Paulo CEP 05508-000, Brazil; [orcid.org/0000-0003-3047-2044](https://orcid.org/0000-0003-3047-2044)

Complete contact information is available at: <https://pubs.acs.org/10.1021/acs.jpca.3c06310>

## Funding

The Spanish Agencia Estatal de Investigación of the Ministerio de Ciencia e Innovación (MICINN) and the European Regional Development Fund (FEDER), project no. PID2021-127199NB-I00. Generalitat Valenciana, project no. CIAICO/2022/121. Universitat de València for the predoctoral grant “Atracció de Talent 2020.” Conselho Nacional de Desenvolvimento Científico e Tecnológico (The National Council for Scientific and Technological Development; CNPq), project no. 311821/2021-9.

## Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work is supported by the Spanish Agencia Estatal de Investigación of the Ministerio de Ciencia e Innovación (MICINN) and the European Regional Development Fund (FEDER) through project no. PID2021-127199NB-I00. D.R.-S. is also thankful to the Generalitat Valenciana for funding support with the project CIAICO/2022/121. M.N.-M. acknowledges Universitat de València for the predoctoral grant “Atracció de Talent 2020.” A.C.B. thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (The National Council for Scientific and Technological Development; CNPq) for research fellowship (Project number 311821/2021-9), FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the research grant 2018/19454-5 (ended in February/2021), and STI (Superintendência de Tecnologia da Informação) of the University of São Paulo and Centro Nacional de Processamento de Alto Desempenho em São Paulo (CENAPAD-SP) for services and computer time. M.B.P. thanks the CALMIP supercomputing center for granting access to HPC resources (allocation p12158). M.N.-M. and D.R.-S. are thankful to Juan Aragón and Jesús Cerdá from the Universitat de València for helpful discussions on the topic.

## ■ REFERENCES

- (1) Burke, M. P.; Borland, K. M.; Litosh, V. A. Base-Modified Nucleosides as Chemotherapeutic Agents: Past and Future. *Curr. Top. Med. Chem.* **2016**, *16* (11), 1231–1241, DOI: [10.2174/1568026615666150915111933](https://doi.org/10.2174/1568026615666150915111933).
- (2) Berdis, A. Nucleobase-Modified Nucleosides and Nucleotides: Applications in Biochemistry, Synthetic Biology, and Drug Discovery. *Front. Chem.* **2022**, *10*, 1051525 DOI: [10.3389/fchem.2022.1051525](https://doi.org/10.3389/fchem.2022.1051525).
- (3) Araie, Y.; Ohtsuki, S.; Park, S.; Nagaoka, M.; Umemura, K.; Sugiyama, H.; Kusamori, K.; Takahashi, Y.; Takakura, Y.; Nishikawa,

- M. Combined Use of Chemically Modified Nucleobases and Nanostructured DNA for Enhanced Immunostimulatory Activity of CpG Oligodeoxynucleotide. *Bioorg. Med. Chem.* **2021**, *29*, No. 115864.
- (4) Chan, K. Y.; Kinghorn, A. B.; Hollenstein, M.; Tanner, J. A. Chemical Modifications for a Next Generation of Nucleic Acid Aptamers. *ChemBioChem* **2022**, *23* (15), No. e202200006.
- (5) Xu, W.; Chan, K. M.; Kool, E. T. Fluorescent Nucleobases as Tools for Studying DNA and RNA. *Nat. Chem.* **2017**, *9* (11), 1043–1055.
- (6) Michel, B. Y.; Dziuba, D.; Benhida, R.; Demchenko, A. P.; Burger, A. Probing of Nucleic Acid Structures, Dynamics, and Interactions With Environment-Sensitive Fluorescent Labels. *Front. Chem.* **2020**, *8*, 112 DOI: 10.3389/fchem.2020.00112.
- (7) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. Fluorescent Analogs of Biomolecular Building Blocks: Design, Properties, and Applications. *Chem. Rev.* **2010**, *110* (5), 2579–2619.
- (8) Shin, D.; Sinkeldam, R. W.; Tor, Y. Emissive RNA Alphabet. *J. Am. Chem. Soc.* **2011**, *133* (38), 14912–14915.
- (9) Rovira, A. R.; Fin, A.; Tor, Y. Chemical Mutagenesis of an Emissive RNA Alphabet. *J. Am. Chem. Soc.* **2015**, *137* (46), 14602–14605.
- (10) Fluorescent Analogs of Biomolecular Building Blocks, Design and Applications. In *Fluorescent Analogs of Biomolecular Building Blocks, Design and Applications*; Wilhelmsson, M.; Tor, Y., Eds.; John Wiley: Hoboken, NJ, 2016; p 448.
- (11) Dziuba, D.; Didier, P.; Ciaco, S.; Barth, A.; Seidel, C. A. M.; Mély, Y. Fundamental Photophysics of Isomorphous and Expanded Fluorescent Nucleoside Analogues. *Chem. Soc. Rev.* **2021**, *50* (12), 7062–7107.
- (12) Karimi, A.; Börner, R.; Mata, G.; Luedtke, N. W. A Highly Fluorescent Nucleobase Molecular Rotor. *J. Am. Chem. Soc.* **2020**, *142* (34), 14422–14426.
- (13) Rovira, A. R.; Fin, A.; Tor, Y. Expanding a Fluorescent RNA Alphabet: Synthesis, Photophysics and Utility of Isothiazole-Derived Purine Nucleoside Surrogates. *Chem. Sci.* **2017**, *8* (4), 2983–2993.
- (14) Ludford, P. T., III; Yang, S.; Bucardo, M. S.; Tor, Y. A New Variant of Emissive RNA Alphabets. *Chem. - Eur. J.* **2022**, *28* (13), No. e202104472, DOI: 10.1002/chem.202104472.
- (15) Saaem, I.; LaBean, T. H. Overview of DNA Origami for Molecular Self-Assembly. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2013**, *5* (2), 150–162, DOI: 10.1002/wnan.1204.
- (16) Tintoré, M.; Eritja, R.; Fàbrega, C. DNA Nanoarchitectures: Steps towards Biological Applications. *ChemBioChem* **2014**, *15* (10), 1374–1390.
- (17) Bell, N. A. W.; Keyser, U. F. Nanopores Formed by DNA Origami: A Review. *FEBS Lett.* **2014**, *588* (19), 3564–3570.
- (18) Schreiber, R.; Do, J.; Roller, E.-M.; Zhang, T.; Schüller, V. J.; Nickels, P. C.; Feldmann, J.; Liedl, T. Hierarchical Assembly of Metal Nanoparticles, Quantum Dots and Organic Dyes Using DNA Origami Scaffolds. *Nat. Nanotechnol.* **2014**, *9* (1), 74–78.
- (19) Ortiz-Rodríguez, L. A.; Crespo-Hernández, C. E. Thionated Organic Compounds as Emerging Heavy-Atom-Free Photodynamic Therapy Agents. *Chem. Sci.* **2020**, *11* (41), 11113–11123.
- (20) Taras-Goślińska, K.; Burdziński, G.; Wenska, G. Relaxation of the T1 Excited State of 2-Thiothymine, Its Riboside and Deoxyriboside-Enhanced Nonradiative Decay Rate Induced by Sugar Substituent. *J. Photochem. Photobiol., A* **2014**, *275*, 89–95, DOI: 10.1016/j.jphotochem.2013.11.003.
- (21) Reelfs, O.; Karran, P.; Young, A. R. 4-Thiothymidine Sensitization of DNA to UVA Offers Potential for a Novel Photochemotherapy. *Photochem. Photobiol. Sci.* **2012**, *11* (1), 148–154.
- (22) Pridgeon, S. W.; Heer, R.; Taylor, G. A.; Newell, D. R.; O'Toole, K.; Robinson, M.; Xu, Y.-Z.; Karran, P.; Boddy, A. V. Thiothymidine Combined with UVA as a Potential Novel Therapy for Bladder Cancer. *Br. J. Cancer* **2011**, *104* (12), 1869–1876.
- (23) Gueranger, Q.; Li, F.; Peacock, M.; Larnicol-Fery, A.; Brem, R.; Macpherson, P.; Egly, J.-M.; Karran, P. Protein Oxidation and DNA Repair Inhibition by 6-Thioguanine and UVA Radiation. *J. Invest. Dermatol.* **2014**, *134* (5), 1408–1417.
- (24) Gemenetzidis, E.; Shavorskaya, O.; Xu, Y.-Z.; Trigiante, G. Topical 4-Thiothymidine Is a Viable Photosensitizer for the Photodynamic Therapy of Skin Malignancies. *J. Dermatol. Treat.* **2013**, *24* (3), 209–214, DOI: 10.3109/09546634.2011.631978.
- (25) Brem, R.; Daehn, I.; Karran, P. Efficient DNA Interstrand Crosslinking by 6-Thioguanine and UVA Radiation. *DNA Repair* **2011**, *10* (8), 869–876.
- (26) Dolmans, D. E.; Fukumura, D.; Jain, K. R. Photodynamic Therapy for Cancer. *Nat. Rev. Cancer* **2003**, *3* (5), 380–387.
- (27) Korbelik, M. Cancer Vaccines Generated by Photodynamic Therapy. *Photochem. Photobiol. Sci.* **2011**, 664–669, DOI: 10.1039/c0pp00343c.
- (28) Correia, J. H.; Rodrigues, J. A.; Pimenta, S.; Dong, T.; Yang, Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions. *Pharmaceutics* **2021**, *13* (9), 1332 DOI: 10.3390/pharmaceutics13091332.
- (29) Ashwood, B.; Pollum, M.; Crespo-Hernández, C. E. Photochemical and Photodynamical Properties of Sulfur-Substituted Nucleic Acid Bases. *Photochem. Photobiol.* **2019**, *95* (1), 33–58.
- (30) Pownall, H. J.; Schaffer, A. M.; Becker, R. S.; Mantulin, W. W. The Electronic Spectroscopy of Pyrimidines: The Effect of Covalently Bonded Sulfur on the Phosphorescence and Absorption Spectra. *Photochem. Photobiol.* **1978**, *27* (5), 625–628.
- (31) Vendrell-Criado, V.; Sáez, J. A.; Lhiaubet-Vallet, V.; Cuquerella, M. C.; Miranda, M. A. Photophysical Properties of 5-Substituted 2-Thiopyrimidines. *Photochem. Photobiol. Sci.* **2013**, *12* (8), 1460–1465.
- (32) Koyama, D.; Milner, M. J.; Orr-Ewing, A. J. Evidence for a Double Well in the First Triplet Excited State of 2-Thiouracil. *J. Phys. Chem. B* **2017**, *121* (39), 9274–9280.
- (33) Pollum, M.; Jockusch, S.; Crespo-Hernández, C. E. Increase in the Photoreactivity of Uracil Derivatives by Doubling Thionation. *Phys. Chem. Chem. Phys.* **2015**, *17* (41), 27851–27861.
- (34) Cui, G.; Fang, W. H. State-Specific Heavy-Atom Effect on Intersystem Crossing Processes in 2-Thiothymine: A Potential Photodynamic Therapy Photosensitizer. *J. Chem. Phys.* **2013**, *138* (4), No. 044315.
- (35) Gobbo, J. P.; Borin, A. C. 2-Thiouracil Deactivation Pathways and Triplet States Population. *Comput. Theor. Chem.* **2014**, *1040–1041*, 195–201.
- (36) Mai, S.; Marquetand, P.; González, L. A Static Picture of the Relaxation and Intersystem Crossing Mechanisms of Photoexcited 2-Thiouracil. *J. Phys. Chem. A* **2015**, *119* (36), 9524–9533.
- (37) El-Sayed, M. A. Spin-Orbit Coupling and the Radiationless Processes in Nitrogen Heterocyclics. *J. Chem. Phys.* **1963**, *38* (12), 2834–2838.
- (38) Mai, S.; Marquetand, P.; González, L. Intersystem Crossing Pathways in the Noncanonical Nucleobase 2-Thiouracil: A Time-Dependent Picture. *J. Phys. Chem. Lett.* **2016**, *7* (11), 1978–1983.
- (39) Zou, X.; Dai, X.; Liu, K.; Zhao, H.; Song, D.; Su, H. Photophysical and Photochemical Properties of 4-Thiouracil: Time-Resolved Ir Spectroscopy and DFT Studies. *J. Phys. Chem. B* **2014**, *118* (22), 5864–5872.
- (40) Milder, S. J.; Kliger, D. S. Spectroscopy and Photochemistry of Thiouracils: Implications for the Mechanism of Photocrosslinking in tRNA. *J. Am. Chem. Soc.* **1985**, *107* (25), 7365–7373.
- (41) Shukla, M. K.; Leszczynski, J. Electronic Transitions of Thiouracils in the Gas Phase and in Solutions: Time-Dependent Density Functional Theory (TD-DFT) Study. *J. Phys. Chem. A* **2004**, *108* (46), 10367–10375.
- (42) Favre, A.; Saintomé, C.; Fourrey, J. L.; Clivio, P.; Laugâa, P. Thionucleobases as Intrinsic Photoaffinity Probes of Nucleic Acid Structure and Nucleic Acid-Protein Interactions. *J. Photochem. Photobiol., B* **1998**, *42* (2), 109–124, DOI: 10.1016/S1011-1344(97)00116-4.
- (43) Borrego-Varillas, R.; Teles-Ferreira, D. C.; Nenov, A.; Conti, I.; Ganzer, L.; Manzoni, C.; Garavelli, M.; Maria De Paula, A.; Cerullo, G. Observation of the Sub-100 fs Population of a Dark State in a

Thiobase Mediating Intersystem Crossing. *J. Am. Chem. Soc.* **2018**, *140* (47), 16087–16093.

(44) Siegbahn, P. E. M.; Almlöf, J.; Heiberg, A.; Roos, B. O. The Complete Active Space SCF (CASSCF) Method in a Newton–Raphson Formulation with Application to the HNO Molecule. *J. Chem. Phys.* **1981**, *74* (4), 2384–2396.

(45) Andersson, K.; Malmqvist, P.Å.; Roos, B. O. Second-Order Perturbation Theory with a Complete Active Space Self-Consistent Field Reference Function. *J. Chem. Phys.* **1992**, *96* (2), 1218–1226.

(46) Nenov, A.; Conti, I.; Borrego-Varillas, R.; Cerullo, G.; Garavelli, M. Linear Absorption Spectra of Solvated Thiouracils Resolved at the Hybrid RASPT2/MM Level. *Chem. Phys.* **2018**, *515*, 643–653.

(47) Corral, I.; González, L. Theoretical Investigation of Anthracene-9,10-Endoperoxide Vertical Singlet and Triplet Excitation Spectra. *J. Comput. Chem.* **2008**, *29*, 1982–1991.

(48) Aquilante, F.; Autschbach, J.; Carlson, R. K.; Chibotaru, L. F.; Delcey, M. G.; De Vico, L.; Fdez-Galván, I.; Ferré, N.; Frutos, L. M.; Gagliardi, L.; et al. Molcas 8: New Capabilities for Multiconfigurational Quantum Chemical Calculations across the Periodic Table. *J. Comput. Chem.* **2016**, *37*, 506–541.

(49) De Vico, L.; Olivucci, M.; Lindh, R. New General Tools for Constrained Geometry Optimizations. *J. Chem. Theory Comput.* **2005**, *1*, 1029–1037.

(50) Harabuchi, Y.; Keipert, K.; Zahariev, F.; Taketsugu, T.; Gordon, M. S. Dynamics Simulations with Spin-Flip Time-Dependent Density Functional Theory: Photoisomerization and Photocyclization Mechanisms of Cis-Stilbene in  $\Pi\pi^*$  States. *J. Phys. Chem. A* **2014**, *118* (51), 11987–11998.

(51) Jensen, F. *Introduction to Computational Chemistry*; John Wiley & Sons: Chichester, UK, 2016.

(52) Shao, Y.; Gan, Z.; Epifanovsky, E.; Gilbert, A. T. B.; Wormit, M.; Kussmann, J.; Lange, A. W.; Behn, A.; Deng, J.; Feng, X.; et al. Advances in Molecular Quantum Chemistry Contained in the Q-Chem 4 Program Package. *Mol. Phys.* **2015**, *113* (2), 184–215.

(53) Müller, K.; Brown, L. D. Location of Saddle Points and Minimum Energy Paths by a Constrained Simplex Optimization Procedure. *Theor. Chim. Acta* **1979**, *53*, 75–93, DOI: 10.1007/BF00547608.

(54) Anglada, J. M.; Bofill, J. M. A Reduced-Restricted-Quasi-Newton-Raphson Method for Locating and Optimizing Energy Crossing Points between Two Potential Energy Surfaces. *J. Comput. Chem.* **1997**, *18* (8), 992–1003.

(55) Ghigo, G.; Roos, B. O.; Malmqvist, P.Å. A Modified Definition of the Zeroth-Order Hamiltonian in Multiconfigurational Perturbation Theory (CASPT2). *Chem. Phys. Lett.* **2004**, *396* (1–3), 142–149.

(56) Forsberg, N.; Malmqvist, P.Å. Multiconfiguration Perturbation Theory with Imaginary Level Shift. *Chem. Phys. Lett.* **1997**, *274* (1–3), 196–204.

(57) Malmqvist, P.-Å.; Roos, B. O. The CASSCF State Interaction Method. *Chem. Phys. Lett.* **1989**, *155* (2), 189–194.

(58) Merchán, M.; González-Luque, R.; Climent, T.; Serrano-Andrés, L.; Rodríguez, E.; Reguero, M.; Peláez, D. Unified Model for the Ultrafast Decay of Pyrimidine Nucleobases. *J. Phys. Chem. B* **2006**, *110* (51), 26471–26476.

(59) Duan, J. X.; Zhou, Y.; Xie, Z. Z.; Sun, T. L.; Cao, J. Incorporating Spin-Orbit Effects into Surface Hopping Dynamics Using the Diagonal Representation: A Linear-Response Time-Dependent Density Functional Theory Implementation with Applications to 2-Thiouracil. *Phys. Chem. Chem. Phys.* **2018**, *20* (22), 15445–15454.

(60) Sánchez-Rodríguez, J. A.; Mohamadzade, A.; Mai, S.; Ashwood, B.; Pollum, M.; Marquetand, P.; González, L.; Crespo-Hernández, C. E.; Ullrich, S. 2-Thiouracil Intersystem Crossing Photodynamics Studied by Wavelength-Dependent Photoelectron and Transient Absorption Spectroscopies. *Phys. Chem. Chem. Phys.* **2017**, *19* (30), 19756–19766.