Gas-Phase Cytosine and Cytosine-N$_1$-Derivatives

Have 0.1-1 Nanosecond Lifetimes Near the $S_1$ State Minimum

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Abstract

UV radiative damage to DNA is inefficient because of the ultrafast $S_1 \sim S_0$ internal conversion of its nucleobases. Using picosecond pump/ionization delay measurements, we find that the $S_1(^1\pi\pi^*)$ state vibrationless lifetime of gas-phase keto-amino cytosine (Cyt) is $\tau = 730$ ps or $\sim 700$ times longer than measured by fs pump-probe ionization at higher vibrational excess energy $E_{exc}$. N$_1$-alkylation increases the $S_1$ lifetime up to $\tau = 1030$ ps for N$_1$-ethyl-Cyt, but decreases it to 100 ps for N$_1$-isopropyl-Cyt. Increasing the vibrational energy to $E_{exc} = 300 - 550$ cm$^{-1}$ decreases the lifetimes to 20 – 30 ps. The nonradiative dynamics of $S_1$ cytosine is not solely a property of the amino-pyrimidinone chromophore, but is strongly influenced by the N$_1$-substituent. Correlated excited-state calculations predict that the gap between the $S_2(^1n_O\pi^*)$ and $S_1(^1\pi\pi^*)$ states decreases along the series of N$_1$-derivatives, thereby influencing the $S_1$ state lifetime.

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The absorption of UV light by nucleobases and the ensuing photophysical and photochemical processes constitute fundamental steps of UV-induced DNA damage, the most frequent UV photolesions involving the [2+2] photodimerization of neighboring thymine or cytosine nucleobases within a DNA strand.\textsuperscript{1–3} In aqueous solution the canonical nucleobases, nucleosides and nucleotides exhibit $S_1$ state lifetimes of $\tau \sim 0.2 - 1.5$ ps when excited in the $260 - 270$ nm range, due to ultra-efficient $S_1 \rightarrow S_0$ internal conversion.\textsuperscript{1,2,4–10} The vibrational energy of the hot $S_0$ state is rapidly removed by the surrounding solvent. These properties of the nucleobase chromophores help to protect DNA and RNA from photodamage and may have allowed them to survive photolysis by the deep-UV solar radiation on prebiotic Earth, possibly leading to photochemical selection of the molecular constituents of living systems.\textsuperscript{4,11–14}

On the surface of today’s earth, however, the solar UV is cut off at $290 – 295$ nm by the ozone layer, while the long-wavelength edge of the UV absorption of cytosine (Cyt) and cytidine in aqueous solution extends to $\sim 300$ nm. Thus, the photobiologically and medically relevant region of spectral overlap\textsuperscript{1,3,11} is $290 – 300$ nm, equivalent to a range of $1150$ cm$^{-1}$. Thus, solar UV excitation of cytosine is close to adiabatic, and very far from the Franck-Condon region. Below, we show that at $S_1$ vibrational excess energy $E_{\text{exc}} < 550$ cm$^{-1}$, gas-phase amino-keto cytosine and its N$_1$-derivatives exhibit lifetimes ranging from 1.03 ns to 20 picoseconds (ps), depending both
on the N₁-substituent and on the degree of vibrational excitation. While the relation between the gas-phase spectra and the red-edge of the aqueous UV absorption of Cyt is not clear, these results suggest that the solution-phase photophysics of Cyt and its derivatives might change considerably if the excitation is moved from 260 – 270 nm to ~ 300 nm, where $E_{\text{exc}}$ is low.

The $S_1(1\pi\pi^*)$ state lifetimes of cytosine (Cyt) and its nucleosides and nucleotides dC, CMP and dCMP have been measured in room-temperature aqueous solution by femtosecond (fs) transient absorption and fluorescence upconversion techniques as $\tau = 0.2 – 1.2$ ps, using excitation at 263 – 270 nm. The nature of the efficient nonradiative decay mechanisms of gas phase Cyt has been investigated by calculations of its lowest electronically excited states, which predict three different conical intersections (CI) that connect the lowest $1\pi\pi^*$ excited state to the $S_0$ ground state; the lowest of these is denoted the “C₅-twist” CI. Spectroscopic and lifetime measurements of cold gas-phase Cyt are crucial, because they (1) allow direct comparison to the predictions of quantum chemical calculations, (2) yield detailed insight into the excited-state structures, vibrations and nonradiative dynamics and (3) can be conducted tautomer-specifically, which is very difficult or impossible in solution. Excited-state lifetimes of jet-cooled amino-keto Cyt have been measured by femtosecond (fs) pump/delayed ionization as $\tau = 0.6 – 1.5$ ps when exciting to $S_1$ state vibrational energies $E_{\text{exc}} = 1500 – 4000$ cm$^{-1}$ above the vibrationless ($\nu' = 0$) level. Here we present ps pump/ ionization lifetime measurements of the $\nu' = 0$ and lowest vibronic levels of jet-cooled keto-amino cytosine (Cyt) and its N₁-methyl, N₁-ethyl and N₁-isopropyl derivatives:

Based on a spectroscopic analysis of the Lorentzian broadening contributing to the rotational
contour widths in the 2C-R2PI spectrum of amino-keto Cyt, Lobsiger et al. determined lower lifetime limits for the $S_1 v' = 0$ level as $\tau = 45$ ps. Using nanosecond (ns) pump/ionization delay measurements, the intersystem crossing (ISC) rate constants of the $S_1 v' = 0$ and low-lying levels of amino-keto Cyt have been calculated and measured. The direct ps measurements in this work show that the $S_1 v' = 0$ lifetime of Cyt is $\sim 15$ times longer than the lower limit derived in ref. 32. The $S_1 v' = 0$ lifetime of amino-keto cytosine remains similar upon N$_1$-methyl substitution, increases further for the N$_1$-ethyl derivative, but decreases in the N$_1$-isopropyl-Cyt derivative.

Figure 1: Nanosecond two-color resonant two-photon ionization (2C-R2PI) spectra of (a) amino-keto cytosine, (b) N$_1$-methyl-Cyt, (c) N$_1$-ethyl-Cyt, and (d) N$_1$-isopropyl-Cyt; ionization at 226 nm. The $0^0$ bands are aligned at a common zero; the absolute values are (a) 31835 cm$^{-1}$, (b) 31852 cm$^{-1}$, (c) 31906 cm$^{-1}$ and (d) 31786 cm$^{-1}$. Breaking-off points indicated by red arrows.

De Vries and co-workers first investigated the two-color resonant two-photon ionization (2C-R2PI)
spectra of supersonic jet-cooled Cyt, 5-methyl-Cyt and 1-methyl-Cyt, assigning the $S_0 \rightarrow S_1$ transitions of the keto-amino tautomers to bands near 32000 cm$^{-1}$.$^{29,30}$ The analysis of the rotational band contours later confirmed the $^1\pi\pi^*$ character of the $S_0 \rightarrow S_1$ transition of Cyt.$^{31,32}$ Fig. 1 shows the $S_0 \rightarrow S_1$ vibronic spectra of jet-cooled Cyt$^{29-32}$ and its N$_1$-methyl,$^{29}$ N$_1$-ethyl, and N$_1$-isopropyl derivatives, the latter two are reported for the first time. The $0^0_0$ transition of N$_1$-methyl-Cyt was previously assigned to a band at 31916 cm$^{-1}$.$^{29}$ Based on UV/UV depletion and holeburning measurements we reassign it to the weaker band at 31852 cm$^{-1}$. Similar to Cyt, the 2C-R2PI spectra of the N$_1$-derivatives exhibit progressions in low-frequency out-of-plane vibrations.$^{31,32,36}$ The spectra break off at $E_{exc} \sim 450$ cm$^{-1}$ for Cyt, $\sim 550$ cm$^{-1}$ for N$_1$-methyl-Cyt, $\sim 450$ cm$^{-1}$ for N$_1$-ethyl-Cyt and $\sim 550$ cm$^{-1}$ for N$_1$-isopropyl-Cyt, marked by arrows in Fig. 1.

Since Cyt and its N$_1$-alkyl derivatives exhibit different breaking-off energies, we measured their $S_1$ lifetimes at the respective $0^0_0$ or lowest measurable vibronic excitation by the ps pump/ionization delay technique, which are shown in Fig. 2. The $0^0_0$ lifetime of cytosine is $\tau \sim 730$ ps (Fig. 2a), and the N$_1$-methyl- and N$_1$-ethylcytosine derivatives (Fig. 2b,c) also exhibit long decay times of $\tau = 600$ ps and $\tau = 930$ ps, respectively. Branching the alkyl side-chain in N$_1$-isopropyl-Cyt decreases the lifetime to $\tau = 100$ ps, see Fig. 2(d). The red lines are the best fits to the kinetic model described below, see also the Supporting Information (SI).

The previous lifetime estimates of $\tau = 30-45$ ps for the Cyt $0^0_0$ and $0^0_0 + 92$ cm$^{-1}$ vibronic bands were based on the assumption that Lorentzian (lifetime) broadening dominates the rotational band contours.$^{32}$ The 15x longer lifetime measured here implies that the band contours are broadened by additional mechanisms. Among these could be the large-amplitude inversion motion of the amino group of Cyt in the $S_0$ and $S_1$ states, or $J, K$-dependent Coriolis couplings that were not considered in ref. 32.

We fit the ps transients to a kinetic model in which the optically excited vibronic level $S_1, v$ decays nonradiatively to $S_0$ by internal conversion (IC) with the rate $k_{IC,v}^{S_1}$, to the triplet state (here assumed to be $T_1$) with the intersystem crossing (ISC) rate constant $k_{ISC,v}^{S_1}$, and radiatively with the radiative rate constant $k_{rad}^{S_1}$. We fit the decay of the $S_1 (^1\pi\pi^*)$ population to the differential
Figure 2: Picosecond time-resolved pump/delayed ionization transients of (a) Cyt (at its $0_0^0$ band), (b) N$_1$-methyl-Cyt ($0_0^0 + 66$ cm$^{-1}$ band), (c) N$_1$-ethyl-Cyt ($0_0^0$ band) (d) N$_1$-isopropyl-Cyt($0_0^0 + 44$ cm$^{-1}$ band), ionization at 213 nm. The lifetimes $\tau$ are defined in the text.

The equation

$$\frac{d[S_1]}{dt} = -(k_{1C,v}^{S_1} + k_{1SC,v}^{S_1} + k_{rad}^{S_1}) \cdot [S_1]$$

(1)

where $k_{1C,v}^{S_1}$ and $k_{1SC,v}^{S_1}$ depend on the vibrational level $v$ pumped by the laser, and $k_{rad}$ is assumed to be independent of $v$. The radiative lifetime $\tau_{rad} = (k_{rad})^{-1}$ of cytosine calculated at the SCS-CC2/aug-cc-pVDZ level is 18 ns, corresponding to $k_{rad} = 5.5 \cdot 10^7$ s$^{-1}$; those of the N$_1$-substituted cytosines are somewhat shorter and are given in Table S1 (Supplemental Information=SI). The triplet state is populated with the rate $k_{1SC,v}^{S_1}$ and relaxes to $S_0$ by $T_1 \sim S_0$ ISC and phosphorescence with the rate constant $k_T = k_{1SC}^{T_1} + k_{phos}$ according to:

$$\frac{d[T_1]}{dt} = k_{1SC,v}^{S_1} \cdot [S_1] - k_T \cdot [T_1]$$

(2)

Since we ionize at 213 nm, where both the $S_1$ and $T_1$ states can be ionized,$^{29-32}$ some of the low-$v$
ps transients exhibit a noticeable growing-in of the $T_1$ population, see Figs. S1-S3 (SI). On the other hand, the $T_1 \sim S_0$ decay time $1/k_T$ is on the order of $200 - 400$ ns,\textsuperscript{29-32} far beyond the time scale of our ps pump/ionization experiments. The lifetimes $\tau$ marked in Fig. 2 for increasing excess energy $E_{exc}$ correspond to the $S_1$ state decay times $\tau = (k_{IC,v}^{S_1} + k_{ISC,v}^{S_1} + k_{rad}^{S_1})^{-1}$, which are dominated by $k_{IC,v}^{S_1} + k_{ISC,v}^{S_1}$.

Fig. 3 plots the $S_1$ state lifetimes of Cyt and its N$_1$-alkyl derivatives vs. the vibrational excess energy $E_{exc}$; for detailed lifetimes and error estimates see Table S1 (SI). The ps pump/probe transients to which the lifetimes were fitted are shown in Figures S1-S3 (SI). Starting from the $S_1 0_0^+$ (or lowest measurable) vibronic band the experimental lifetimes generally decrease with increasing excess vibrational energy $E_{exc}$, reaching $\tau < 30$ ps at $E_{exc} = 350 - 550$ cm$^{-1}$. For 1-ethyl-Cyt, however, the lifetimes of the first and second out-of-plane vibronic excitations increase relative to

Figure 3: Dependence of the $S_1$ state vibronic level lifetimes of (a) Cyt, (b) N$_1$-methyl-Cyt, (c) N$_1$-ethyl-Cyt and (d) N$_1$-isopropyl-Cyt on the $S_1$ state vibrational excess energy $E_{exc}$. Note the logarithmic scale. In (a), the vibronic level lifetimes of Cyt marked by $\bigcirc$ are from line-broadening measurements.\textsuperscript{32} All other lifetimes (marked by $\bullet$) are measured by ps pump/delayed ionization.
the \( v' = 0 \) level. Fig. 3 also shows that the ns laser R2PI spectra in Fig. 1 break off at approximately the vibrational excess energy \( E_{exc} \) at which the lifetime decreases below 20 ps, as marked by a dotted line.

The ps pump/ionization transients shown in Fig. 2 and in Figs. S1-S3 exhibit minor contributions from the long-lived \( T_1 \) population, allowing to estimate ISC rate constants \( k_{ISC,v}^{S_1} \). However, these need to be corrected for the relative ionization cross sections of the \( S_1 \) and \( T_1 \) states, \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) \). In ref. 35 we have shown for Cyt that they differ by a factor of \( \sim 3 \), but for the N1-substituted cytosines they have not yet been determined. In Table S1 we therefore indicate only a few \( k_{ISC,v}^{S_1} \) values, which were calculated for \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) = 1 - 2 \) and which should be considered as rough estimates; a fuller analysis will be given elsewhere. For the lowest vibronic levels, \( k_{ISC,v} \) is in the range \( 0.3 – 4 \cdot 10^8 \) s\(^{-1} \) and \( k_{ISC,v}^{S_1} \) is in the range \( 1 – 10 \cdot 10^9 \) s\(^{-1} \).

The ps pump/ionization delay measurements show that the near-minimum region of the \( S_1 \) \( 1\pi \pi^* \) state of these cytosines must be deep enough and well enough isolated from the \( S_0 \) state (or from the perturbing \( S_2 \) state, see below) to prevent ultrafast \( S_1 \rightarrow S_0 \) internal conversion that occurs at higher vibrational excess energies \( E_{exc} = 1500 – 4000 \) cm\(^{-1} \) above the \( S_1, v = 0 \) level. As shown in Fig. 1, the ns laser \( S_0 \rightarrow S_1 \) two-color R2PI spectra break off between 300 – 550 cm\(^{-1} \) above the electronic origins. The lifetime measurements in Fig. 3 show that the spectral breaking-off correlates well with the excess energy at which the vibronic lifetimes decrease to \( \tau = 20 \) ps.

We interpret the short lifetime breaking-off in terms of the heights of barriers on the \( S_1 \) state surface that “protect” the \( S_1 \) state minimum from the lowest C5-twist conical intersection, as has been predicted by several computational studies. For Cyt, the purely electronic barriers have been calculated to lie 850 – 1600 cm\(^{-1} \) (0.1 – 0.2 eV) above the \( 1\pi \pi^* \) state minimum. However, the relevant barrier for comparison to experiment is not the electronic barrier, but the vibrationally adiabatic barrier, which includes the vibrational zero-point energies of the activated complex (at the barrier) and that of the \( S_1 \) minimum. The vibrationally adiabatic barrier could be significantly higher or lower than the electronic barrier and has not yet been calculated.

Increasing the N1-alkyl group length first increases the \( v' = 0 \) lifetime, but the chain branching
with the isopropyl substituent decreases the lifetime by 7 times, relative to Cyt. The additional methyl group of N1-isopropyl-Cyt strongly increases the density of internal-rotation states in both the $S_0$ and $S_1$ states, which is expected to enhance the $S_1 \rightarrow S_0$ internal conversion rate. However, if the density-of-states increase were the main factor determining $k_{IC}$, we expect to observe a significant lifetime decrease between cytosine and N1-methyl-Cyt, in contrast to what is observed. Malone et al. observed that 5-methylation of cytosine and cytidine actually increases the $S_1$ lifetime in aqueous solution by a factor of 7, and interpreted this in terms of the intermediacy of the close-lying $^1nO\pi^*$ excited state. Excited-state lifetime changes of uracil, 5-fluorouracil and cyclohexyluracil in solution have also been interpreted in terms of differential solvent effects acting on the close-lying $^1nO\pi^*$ and $^1\pi\pi^*$ excited states.

We investigated the influence of N1-alkylation on the excited states of Cyt using the SCS-CC2 method (see below for computational details). The calculated $^1\pi\pi^*$ and $^1nO\pi^*$ energies are shown in Fig. 4. The $^1nO\pi^*$ states of Cyt and N1-methyl-Cyt are $2700 - 3100 \text{ cm}^{-1}$ vertically above the $^1\pi\pi^*$ state; the gap decreases only slightly for N1-ethyl-Cyt. However, N1-isopropyl substitution stabilizes the $^1nO\pi^*$ state, rendering it almost degenerate with the $^1\pi\pi^*$ state. This near-degeneracy of $^1nO\pi^*$ and $^1\pi\pi^*$ states is in qualitative agreement with the decrease of lifetimes between N1-ethyl-Cyt and N1-isopropyl-Cyt, see Figs. 2 and 3. Clearly, the $^1nO\pi^*$ state energies follow a trend that depends more on the structure of the N1-alkyl chain than on its length. The $^1nO\pi^*$ state is optically “dark”, so $^1nO\pi^*/^1\pi\pi^*$ state mixing by itself should increase the excited-state lifetime if only radiative decay is considered. However, the $^1nO\pi^*/^1\pi\pi^*$ state mixing may affect the height of the barrier between the $^1\pi\pi^*$ minimum and the conical intersection, thereby increase the $S_1/S_0$ coupling and the internal conversion rate, as schematically indicated in Fig. 4. This involvement of the lowest $^1n\pi^*$ state is in agreement with the current consensus in solution-phase photophysical studies of pyrimidine nucleobases - and cytosine in particular - that the relaxation involves branching between decay to $S_0$ and population of longer-lived $^1n\pi^*$ and $^3\pi\pi^*$ states.

Summarizing, the nonradiative decay of the $S_1(^1\pi\pi^*)$ states of keto-amino Cyt and its N1-derivatives are not inevitably ultrafast. The $S_1(^1\pi\pi^*)(v' = 0)$ lifetimes of Cyt and N1-methyl-Cyt
Figure 4: Schematic potential energy surfaces of the ground and lowest singlet excited states of Cyt, N1-methyl-, N1-ethyl- and N1-isopropyl-Cyt. Adiabatic energies calculated at the SCS-CC2 level, barriers between the $^1\pi\pi^*$ minima and the C5-twist CI estimated from the R2PI spectral break-offs. Two excited-state rotamers exist for N1-isopropylcytosine; the $^1\pi\pi^*$ and $^1n_O\pi^*$ minima are those of the lowest-energy conformer.

are $\tau = 600 - 730$ ps, increasing to $\tau = 1030$ ps for N1-ethyl-Cyt at its low-lying $0^0 + 104$ cm$^{-1}$ vibronic level. For Cyt, these direct ps lifetime measurements yield lifetimes that are 15 times longer than the previous indirect measurements via Lorentzian line-broadening. The ISC rate constant for $S_1; v = 0$ Cyt is $\sim 10^8$ s$^{-1}$, see Table S1, in rough agreement with that previously determined for Cyt by nanosecond pump/ionization. It will be necessary to correct the $k_{IC}$ and $k_{ISC}$ constants for the relative $S_1$ and $T_1$ ionization cross sections. The nonradiative decay of keto-amino cytosine and its N1-derivatives depends sensitively on the amount of vibrational excess energy placed into the “bright” $S_1$ state and approaches $\tau = 20$ ps at vibrational excess energies $E_{exc} = 300 - 550$ cm$^{-1}$. These lifetimes extrapolate smoothly to the $\tau = 0.5 - 1.5$ ps lifetimes measured for keto-amino Cyt by fs pump/probe ionization at even higher excess energy $E_{exc} = 1500 - 4000$ cm$^{-1}$, and do not contradict the dynamics at higher energy. The nonradiative decay rate exhibits a marked dependence on the structure of the N1-alkyl chain. SCS-CC2 calculations suggest that this might arise from the coupling of the very close-lying $S_2(1n_O\pi^*)$ with the $S_1(1\pi\pi^*)$. 
More experiments and calculations are needed to decide whether the lifetime changes are mainly due to electronic effects, such as $1n_O\pi^*/1\pi\pi^*$ state mixing, or to vibrational effects such as vibrational/internal-rotation level densities. Extension of these measurements to the cytosine hydroxy-enol tautomers – which cannot be formed by the N$_1$ derivatives – and to complexes (cytosine base-pairs, microhydrate clusters) should also be possible.

**Experimental and Computational Methods**

The cytosine (or N$_1$-alkyl derivative) is placed in a 20 Hz internally gold-plated pulsed supersonic jet nozzle that is heated to 200 − 230° C, corresponding to vapor pressures of 0.2 − 1 mbar. The vapor is entrained in Ne carrier gas at 1.2 − 1.8 bar backing pressure and expanded into the source chamber. The nanosecond resonant two-photon ionization setup has been described in Refs. 29,30. The picosecond R2PI spectroscopic and pump/ionization delay measurements are performed with an EKSPLA PL2441 20 Hz Nd:YAG laser system producing $\sim$ 25 ps laser pulses. The 355 nm (14 mJ) output pumps an EKSPLA PG401 tunable UV optical parametric oscillator/amplifier (OPO/OPA) (60μJ/pulse, 20 ps pulse length, $\sim$ 10 cm$^{-1}$ spectral width). The Cyt (derivatives) are $S_0 \rightarrow S_1$ excited by the UV OPO/OPA and ionized by a 20 ps pulse at 213 nm (fifth harmonic of the ps Nd:YAG pump laser). The pump and ionization beams are collimated to 3 mm beam diameter by $f = 1000$ mm UV lenses and crossed with the molecular beam inside the TOF-MS ion source. For the pump/ionization delay measurements the 213 nm ionization pulse is sent over a 0 − 400 mm long translation stage, resulting in a 0 − 2.2 ns time delay, details are given in ref. 42.

The $S_0$, $S_1$ and $S_2$ state calculations were performed with the spin-component scaled coupled-cluster method with approximate treatment of doubles (SCS-CC2) and the aug-cc-pVDZ basis set; only valence electrons were correlated. The standard scaling factors 1/3 for the same-spin and 6/5 for the opposite-spin components enhances the accuracy of transition energies for both $\pi\pi^*$ and $n\pi^*$ states. Previous 2C-R2PI experiments have shown that the $S_1$ state of keto-amino Cyt is $1\pi\pi^*$ with a distinctly non-planar geometry.$^{32,35}$ In contrast to regular CC2 and to time-dependent density functional theory (TD-DFT) with the B3LYP functional, which predict the $1n_O\pi^*$ state with a near-planar minimum geometry as the $S_1$ state at its minimum, SCS-CC2 predicts the $1\pi\pi^*$ state as $S_1$, with a distinctly non-planar equilibrium structure, in agreement
with experiment. The $S_0$ and $S_1$ state minimum energies were fully optimized, as well as the $S_2$ minima of N$_1$-ethyl-Cyt and N$_1$-isopropyl-Cyt. For the latter molecules, the $1n_O\pi^*$ energy is the minimum along the internal-rotation path of the alkyl side chain. The $S_2(1n_O\pi^*)$ states of Cyt and N$_1$-methyl-Cyt could not be optimized, the energies shown in Fig. 4 are calculated vertically above the optimized $S_1$ structure. All calculations were performed with TURBOMOLE 6.4.

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**Supporting Information Available:** Details of the kinetics and fitting procedure, Table S1 with $S_1$ state lifetimes $\tau$, Figures S1-S3 with ps pump/ionization transients, Tables S2 and S3 with SCS-CC2 optimized $S_0$, $S_1$ and $S_2$ state Cartesian geometries, Table S4 with the corresponding calculated energies. This information is available free of charge via the Internet at http://pubs.acs.org.

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(44) The thresholds for SCF and one-electron density convergence were set to $10^{-9}$ a.u. and $10^{-8}$ a.u., respectively. The convergence thresholds for all structure optimizations were set to $10^{-8}$ a.u. for the energy change, $6 	imes 10^{-6}$ a.u. for the maximum displacement element, $10^{-6}$ a.u. for the maximum gradient element, $4 	imes 10^{-6}$ a.u. for the RMS displacement and $10^{-6}$ a.u. for the RMS gradient.