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Planarizing cytosine: The \( S_1 \) state structure, vibrations, and nonradiative dynamics of jet-cooled 5,6-trimethylenecytosine

Maria A. Trachsel,\(^1\) Simon Lobsiger,\(^1\) Tobias Schär,\(^1\) Lluis Blancafort,\(^2,a)\) and Samuel Leutwyler\(^{1,b)}\)

\(^1\)Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland
\(^2\)Institut de Química Computacional i Catàlisi and Departament de Química, Facultat de Ciències, Universitat de Girona, C/M. A. Campanya 69, 17003 Girona, Spain

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We measure the \( S_0 \rightarrow S_1 \) spectrum and time-resolved \( S_1 \) state nonradiative dynamics of the “clamped” cytosine derivative 5,6-trimethylenecytosine (TMCyt) in a supersonic jet, using two-color resonant two-photon ionization (R2PI), UV/UV holeburning, and ns time-resolved pump/delayed ionization. The experiments are complemented with spin-component scaled second-order approximate coupled cluster (SCS-CC2), time-dependent density functional theory, and multi-state second-order perturbation-theory (MS-CASPT2) \( ab \) \textit{initio} calculations. While the R2PI spectrum of cytosine breaks off at \( \sim 500 \text{ cm}^{-1} \) above its \( 0^0 \) band, that of TMCyt extends up to \( +4400 \text{ cm}^{-1} \), with over a hundred resolved vibronic bands. Thus, clamping the cytosine \( C^5-C^6 \) bond allows us to explore the \( S_1 \) state vibrations and \( S_0 \rightarrow S_1 \) geometry changes in detail. The TMCyt \( S_1 \) state out-of-plane vibrations \( \nu'_1, \nu'_2, \) and \( \nu'_3 \) lie below \( 420 \text{ cm}^{-1} \), and the in-plane \( \nu''_1, \nu''_2, \) and \( \nu''_3 \) vibronic fundamentals appear at \( 450, 470, \) and \( 944 \text{ cm}^{-1} \). \( S_0 \rightarrow S_1 \) vibronic simulations based on SCS-CC2 calculations agree well with experiment if the calculated \( \nu'_1, \nu'_2, \) and \( \nu'_3 \) frequencies are reduced by a factor of \( 2-3 \). MS-CASPT2 calculations predict that the ethylene-type \( S_1 \rightarrow S_0 \) conical intersection (CI) increases from \( +366 \text{ cm}^{-1} \) in cytosine to \( >6000 \text{ cm}^{-1} \) in TMCyt, explaining the long lifetime and extended \( S_0 \rightarrow S_1 \) spectrum. The lowest-energy \( S_1 \rightarrow S_0 \) CI of TMCyt is the “amino out-of-plane” \((OP_X)\) intersection, calculated at \( +4190 \text{ cm}^{-1} \). The experimental \( S_1 \rightarrow S_0 \) internal conversion rate constant at the \( S_1(\nu' = 0) \) level is \( k_{IC} = 0.98-2.2 \cdot 10^8 \text{ s}^{-1} \), which is \( \sim 10 \) times smaller than in 1-methylcytosine and cytosine. The \( S_1(\nu' = 0) \) level relaxes into the \( T_1(\pi \pi^*) \) state by intersystem crossing with \( k_{ISC} = 0.41-1.6 \cdot 10^9 \text{ s}^{-1} \). The \( T_1 \) state energy is measured to lie 24 580 \pm 560 \text{ cm}^{-1} \) above the \( S_0 \) state. The \( S_1(\nu' = 0) \) lifetime is \( \tau = 2.9 \text{ ns} \), resulting in an estimated fluorescence quantum yield of \( \Phi_f = 24\% \). Intense two-color R2PI spectra of the TMCyt amino-enol tautomers appear above \( 36,000 \text{ cm}^{-1} \). A sharp \( S_1 \) ionization threshold is observed for amino-keto TMCyt, yielding an adiabatic ionization energy of \( 8.114 \pm 0.002 \text{ eV} \). © 2017 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). [http://dx.doi.org/10.1063/1.4989465]

I. INTRODUCTION

A number of femtosecond (fs) pump-probe studies of cytosine (Cyt), 1-methylcytosine (1-MCyt), and 5-fluorocytosine (5-FCyt) in the gas-phase have yielded ultra-short excited-state \( S_1 \) lifetimes that range from \( \tau = 0.2 \) to 3.2 ps.\(^1\)\(-^5\) However, more recent measurements of supersonically jet-cooled amino-keto Cyt, 5-methylcytosine (5-MCyt), and 5-FCyt based on Lorentzian lifetime broadening measurements at their respective \( S_0 \rightarrow S_1 \) electronic origins yielded lower limits on the \( S_1 \) lifetimes of 45 ps for Cyt and 75 ps for 5-FCyt.\(^6\)\(-^8\) More recent direct lifetime measurements of Cyt, 1-MCyt, and 1-ethylcytosine (1-ECyt) using the picosecond (ps) excitation/ionization delay technique revealed even longer values between \( \tau = 450-900 \) ps, which decrease rapidly with increasing vibrational excess energy (\( E_{ex} \)).\(^9\)

Computational studies of the excited-state dynamics and nonradiative decay of amino-keto Cyt\(^10\)\(-^20\) have located three different conical intersections (CIs) between the ground and lowest excited \( \pi \pi^* \) states. The lowest of these, which dominates the \( S_1 \rightarrow S_0 \) nonradiative decay, is called (Eth)\(_X\) since the intersection structure is similar to the CI structure of ethylene. This CI is characterized by a puckering of the C\(^6\) atom and a twist around the C\(^5\)-C\(^6\) bond, with a H-C-C-C-H torsional angle of \( \sim 120^\circ \).\(^10\)\(-^13,15,16,19,21,22\) The next higher CI involves an N\(^2\) out-of-plane bending and a large out-of-plane amino deformation and is called (OP)\(_X\)\(^10\)\(-^10,16,19,22\). The third CI, called (n\(_{do}, \pi^*)\)\(_X\), has a semi-planar structure with \( sp^3 \) hybridization of the C\(^6\) atom, shortening of the C\(^2\)-N\(^3\) bond, and stretching of the C\(^2\)-O bond relative to the ground state minimum.\(^10\)\(-^10,16,19,21,22\)

Zgierski \textit{et al.} have shown that covalently clamping the C\(^5\)-C\(^6\) bond of Cyt with a trimethylene bridge in 5,6-trimethylenecytosine (TMCyt) increases the \( S_1 \) state fluorescence lifetime and quantum yield in room-temperature
aqueous solution by \( \sim 1000 \) times relative to Cyt,\(^{23-25}\) to \( \tau = 1.2 \) ns and \( \Phi_{\beta} \sim 10\%. \)\(^{26}\) Their configuration interaction singles (CIS) and second-order approximate coupled cluster (CC2) excited-state calculations predicted that this clamping shifts the \((\text{Eth})_{x,y} \) conical intersection of cytosine to \( \sim 1500 \text{ cm}^{-1} \) above the \( S_1 \) minimum, making this CI energetically less accessible.\(^{26}\) The trimethylene bridge in TMCyt hardly affects the \( \pi \)-electron framework of Cyt, so the \( S_0 \rightarrow S_1 \) absorption band shifts from \( \lambda_{\text{max}} = 267 \text{ nm} \) for Cyt to 280 nm in TMCyt.\(^{26}\) In the clamped cytosine derivative pyrrolocytosine (PC), the C\(^4\)-amino group (see Fig. 1) and the C\(^5\) atom are covalently connected, resulting in a pyrrole ring fused to the Cyt chromophore.\(^{27}\) This extension of the \( \pi \)-electron framework significantly shifts the \( S_0 \rightarrow S_1 \) excitation maximum to \( \lambda_{\text{max}} = 330-345 \text{ nm} \), or about 70–80 nm to the red, compared to Cyt.\(^{28,29}\) For PC, Thompson and co-workers have measured a lifetime of \( \tau = 2.9 \) ns and a quantum yield of \( \Phi_{\beta} \sim 0.038 \) in pH 7 phosphate buffer.\(^{29}\)

Intrigued by these observations, we have measured and analyzed the \( S_0 \rightarrow S_1 \) vibronic spectrum of supersonic jet-cooled TMCyt using two-color resonant two-photon ionization (2C-R2PI), UV/UV holeburning, and depletion spectroscopies. We also measured the \( S_1 \) state lifetime and triplet-state formation kinetics as a function of \( E_{\text{exc}} \), using the nanosecond excitation/ionization delay technique, and report \( S_1 \) state nonradiative rate constants for internal conversion and intersystem crossing. In addition to the amino-keto tautomer 1 of TMCyt, we have also observed an intense R2PI spectrum that we assign to the \( S_0 \rightarrow S_1 \) transitions of the hydroxy-enol tautomers 2a/2b, see Fig. 1 for the tautomer structures. The measurements are accompanied by calculations of the lowest excited singlet (\( \pi\pi^* \)) and triplet (\( 3\pi\pi^* \)) states of TMCyt using time-dependent (TD) density functional theory (DFT) (TD-CAMB3LYP), spin-component scaled CC2 (SCS-CC2), complete-active-state self-consistent field (CASSCF), and multi-state second-order perturbation-theory (MS-CASPT2) methods.

### II. METHODS

#### A. Experimental methods

TMCyt was synthesized in three steps from adiponitrile according to Ref. 30 and characterized by \(^1\)H-NMR spectroscopy, \(^13\)C-NMR spectroscopy, and electrospray ionization (ESI) mass spectrometry as described in the supplementary material. The experimental setup for two-color resonant two-photon ionization (2C-R2PI) measurements has been previously described.\(^{31,32}\) Neon carrier gas at \( \sim 1.8 \) bar backing pressure was passed through a 20 Hz pulsed nozzle (0.4 mm diameter) heated to 230 °C that contains the TMCyt sample. 2C-R2PI spectra were measured by crossing the skinned supersonic jet with the unfocused UV excitation and ionization laser beams in the source of a linear time-of-flight mass spectrometer (TOF-MS). Excitation was performed with 0.4–0.6 mJ UV pulses from a frequency-doubled Radiant Dyes NarrowScan D-R dye laser with a bandwidth of 0.05 cm\(^{-1}\) (1500 MHz) or with an Ekspla NT342B optical parametric oscillator (UV-OPO) with a bandwidth of 4–6 cm\(^{-1}\). The frequency scale was calibrated by measuring the fundamental frequency with the WS6 wavemeter.

Ionization light of \( \sim 100 \) \( \mu \)J/pulse at 225 or 245 nm was produced by an Ekspla NT342B (UV-OPO). The measurements were typically done with 10–30 mV ion signal at the most intense vibronic band. A second frequency-doubled dye laser (\( \sim 400 \) \( \mu \)J/pulse) was used as the UV-depletion laser for UV/UV holeburning and depletion experiments. Excitation and ionization were performed as for the R2PI experiments. The depletion laser was fired 300 ns before the excitation/ionization laser pulses.

#### B. Computational methods

A uniform theoretical treatment of the ground- and excited-state potential energy surfaces of TMCyt is difficult, and we have combined several methods following a similar approach to our recent work on 1-MCyt.\(^{33}\) The electronic ground state of all 14 tautomers and rotamers of TMCyt was first optimized using density functional theory (B3LYP) with the TZVP basis set. The ground state structures of the six most stable tautomers are shown in Fig. 1; these were re-optimized at the correlated level, using the second-order Møller-Plesset (MP2) method in the resolution-of-identity (RI) approximation, the SCS-MP2 method, and the CC2 method in the RI approximation, using the aug-cc-pVTZ basis set.

The adiabatic and vertical transition energies were calculated at the SCS-CC2 level of theory with the aug-cc-pVDZ basis set. Normal-mode calculations were performed for all geometry-optimized structures to assure that they correspond to true potential energy surface minima. The transition energies were also calculated at the MS-CASPT2 level of theory. For the \( \pi\pi^* \) and \( \pi\pi\pi^* \) states, we used TD-CAM-B3LYP/6-311G** optimized geometries, whose MS-CASPT2 energy is lower than that of their CASSCF and SCS-CC2 analogues. For the optimization of \( (OP)_{\text{Min}} \), we used the CASSCF(12,12)/

*FIG. 1.* The six most stable tautomers of gas-phase 5,6-trimethylencytosine. The cytosine tautomer labels are analogous to that defined in Ref. 45.
6-311G** geometry because the other methods failed to converge to a minimum for that state.

For the calculation of the decay paths and conical intersections, we follow the MS-CASPT2/CASSCF approach, where the paths and structures are optimized at the CASSCF level of theory and the energy profiles along the paths are recalculated at the MS-CASPT2 level to include dynamic correlation energy (see our previous study of 1-MCyt). For the CASSCF and MS-CASPT2 calculations, we used, respectively, the 6-311G** basis and the ANO-L basis set contracted to 4s3p2d for C, N, and O and 3s2p1d for H. For the CASPT2 calculations, we used an imaginary level shift of 0.1 a.u. and the standard ionization potential–electron affinity correction of 0.25 a.u.

To calculate the reaction path to (OP)X, we optimized the transition structure (TS) on S1 and obtained the path by combining the intrinsic reaction coordinate and initial relaxation direction techniques. The calculated barrier includes the vibrational zero-point energy (ZPE) correction, which amounts to ∼475 cm⁻¹, based on CASSCF frequencies at (1ππ*)ₜₘᵢₙ with 3N − 6 vibrational modes and at the corresponding TS (including 3N − 7 modes). The CI was optimized using the recently developed double Newton-Raphson algorithm. The active space of the CASSCF and MS-CASPT2 calculations was specifically tailored for each path; for details, see the supplementary material. We use (10,10) and (12,12) active spaces for the ethylene- and OP-type paths, respectively. With this approach, the MS-CASPT2 S1/S0 energy gaps at the CI structures were 1973 and 2265 cm⁻¹ (0.24 and 0.28 eV) at (Eth)X and (OP)X, respectively. The path to (Eth)X has a sloped topology and does not involve a TS, was approximated with a linear interpolation in internal coordinates.

Density functional theory and CC2 calculations were performed using Turbomole 6.4. The CASSCF optimizations were performed with a modified version of Gaussian09 and the MS-CASPT2 calculations with Molcas 7.8. Vibrionic band simulations were done with the PGOPHER program. As inputs, we used the SCS-CC2 calculated S0 ground and S1 excited state geometries and the corresponding normal-mode matrices, employing conformer 1a. Additional diagonal anharmonicity constants were included for some modes. The vibrionic band intensities are based on full multidimensional Franck-Condon factors, including both mode displacements and mixing between modes (Dushinsky effect).

The vibronic simulations for conformer 1b are very similar to those for 1a.

III. RESULTS AND DISCUSSION

A. Computational results

1. Tautomers and relative energies

Figure 1 shows the six most stable calculated tautomers and rotamers of TMCyta, and Table I summarizes their relative energies calculated at different levels of theory. All the correlated wave function methods predict the trans-amino-enol 2b tautomer to be the most stable one, with the corresponding cis-rotamer 2a ∼ 0.6 kcal/mol higher. The amino-keto N1H tautomer 1 that is experimentally investigated below exists in conformer 1a, where the amino group and trimethylene ring are out-of-plane in the same direction, denoted Up-up (or Down-down), where the first (capitalized) orientation refers to the NH2 group. In conformer 1b, the NH2 group and trimethylene rings are arranged in opposite directions (Up-down or Down-up). The 1a and 1b forms are close in energy with 1b calculated to lie 3–4 cm⁻¹ above 1a. In the gas phase, both 1a and 1b are less stable than amino-enol conformers by 1.08 kcal/mol (CC2) or 1.54 kcal/mol (SCS-MP2). The B3LYP density functional method predicts the amino-keto N1H tautomer to be the most stable tautomer; however, it is known that this method predicts the order of the cytosine tautomers incorrectly.

The other TMCyta tautomers 2a, 2b, and 4 also exist as pairs of conformers analogous to 1a/1b, but only one form was calculated since the energy difference is expected to be very small. All the imino-enol forms lie >13 kcal/mol above the most stable tautomer 2b at the B3LYP/TZVP level; hence, we do not consider them any further.

2. Electronic transition energies

Table II summarizes the calculated adiabatic and vertical transition energies of the 1a, 1b, 2a, 2b, and 3a conformers predicted by the SCS-CC2 method, together with the MS-CASPT2 transitions for 1a. Both methods are in good agreement, which validates our computational approach. They predict that the S1 state minimum has 1ππ* character. The calculated adiabatic transition of 1a is 31 534 cm⁻¹ (SCS-CC2) and 31 831 cm⁻¹ (MS-CASPT2), in excellent agreement with the experimental value of 31 510 cm⁻¹. The vertical excitation energy of the 1ππ* state with the SCS-CC2 and MS-CASPT2 methods is 36 610 and 36 500 cm⁻¹, respectively. There are two low-lying 1nπ* states which arise from excitations out of the oxygen and nitrogen lone pairs, whose vertical excitation energies are 7000-10 000 cm⁻¹ higher. The structure of the 1nπ* state was optimized at the TD-CAMB3LYP level. It is a minimum on S2 with adiabatic energy of 37 597 cm⁻¹ (MS-CASPT2 single point). The structure optimization of the 1nπ*
TABLE II. SCS-CC2 and MS-CASPT2 calculated adiabatic and vertical transition energies (in cm$^{-1}$) and electronic oscillator strengths $f_{el}$ for five tautomers of 5,6-trimethylene cytosine (see Fig. 1).

<table>
<thead>
<tr>
<th>Tautomer</th>
<th>Transition</th>
<th>SCS-CC2/aug-cc-pVDZ</th>
<th>MS-CASPT2/ANO-L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adiab.</td>
<td>Vert.</td>
</tr>
<tr>
<td>1a</td>
<td>$1\pi\pi^*$</td>
<td>31 534</td>
<td>36 610</td>
</tr>
<tr>
<td></td>
<td>$1\pi\Pi^*$</td>
<td>42 349</td>
<td>0.00152</td>
</tr>
<tr>
<td></td>
<td>$2\pi\pi^*$</td>
<td>27 978</td>
<td>30 671</td>
</tr>
<tr>
<td>Ion</td>
<td></td>
<td>65 975</td>
<td>65 440</td>
</tr>
<tr>
<td>1b</td>
<td>$1\pi\Pi^*$</td>
<td>31 483</td>
<td>36 580</td>
</tr>
<tr>
<td></td>
<td>$1\pi\Pi^*$</td>
<td>42 352</td>
<td>0.00191</td>
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<td></td>
<td>$2\pi\Pi^*$</td>
<td>30 654</td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td></td>
<td>65 440</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>$1\pi\Pi^*$</td>
<td>38 631h</td>
<td>43 035</td>
</tr>
<tr>
<td></td>
<td>$1\pi\Pi^*$</td>
<td>34 508</td>
<td>38 589</td>
</tr>
<tr>
<td></td>
<td>$3\pi\Pi^*$</td>
<td>35 415</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>$1\pi\Pi^*$</td>
<td>39 319h</td>
<td>43 631</td>
</tr>
<tr>
<td></td>
<td>$1\pi\Pi^*$</td>
<td>35 008</td>
<td>38 871</td>
</tr>
<tr>
<td></td>
<td>$3\pi\Pi^*$</td>
<td>35 446</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>$1\pi\Pi^*$</td>
<td>43 312</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$1\pi\Pi^*$</td>
<td>41 401</td>
<td>41 862</td>
</tr>
<tr>
<td></td>
<td>$3\pi\Pi^*$</td>
<td>29 432</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Vertical excitation from $S_0$ equilibrium geometry, length.
$^b$At SCS-CC2 optimized geometry.
$^c$At $n_o\pi^+$ minimum optimized at the TD-CAMB3LYP level, $S_1$ energy 35 282 cm$^{-1}$.
$^d$Mixed $n_o\pi^*/n_\Pi\pi^*$ with predominant $n_o\pi^+$ character.
$^e$Mixed $n_o\pi^*/n_\Pi\pi^*$ with predominant $n_\Pi\pi^*$ character.
$^f$Optimized at the CASSCF level.
$^g$Optimized at the TD-CAMB3LYP level.
$^h$Not fully converged.

The adiabatic transition energy of conformer 1a is calculated to lie slightly above that of 1b, differing by 51 cm$^{-1}$ at the SCS-CC2 level. With this method, the $S_0 \rightarrow S_1$ transitions of the major tautomers 2b and 2a are calculated to be $1\pi\Pi^*$ and to lie at $\sim 35 000$ and $\sim 34 500$ cm$^{-1}$, respectively, or about 3500 cm$^{-1}$ further to blue than the transitions of the 1a/1b conformers. The lowest-energy electronic transition of the imino-keto tautomers 3a and 3b is predicted at 41 400 cm$^{-1}$ and 40 970 cm$^{-1}$, respectively. This is above the experimental spectral range covered in this work. On the other hand, the lowest $^1\pi\pi^*$ transition of the 4 (NH) tautomer is predicted to lie very close to that of the 1 (N1H) tautomer. However, tautomer 4 is calculated to be 5.5–5.8 kcal/mol less stable than tautomer 1; hence, we do not expect this tautomer to be observable in the supersonic jet.

3. Ground- and excited-state structures

In the SCS-CC2 $S_0$ optimized structure of 1a, the pyrimidinone framework is $C_2$ symmetric, and the amino group and the trimethylene ring are bent slightly out of the ring plane. In the $1\pi\pi^*$ excited state, the SCS-CC2 and TD-CAMB3LYP methods predict (i) a stronger pyramidalization of the amino group, (ii) an in-plane deformation of the pyrimidinone framework, and (iii) an out-of-plane bend at the C$^6$ atom (see Fig. 1 for the atom numbering). Figure 2 shows the SCS-CC2/aug-cc-pVDZ calculated geometries and geometry changes of TMCyt for both amino-keto N1H conformers. The TD-CAM-B3LYP optimized structure has similar out-of-plane deformations, see Fig. S1 in the supplementary material. This is in line with previous results for 1-MCyt, for which both methods predict a substantial deplanarization at the $^1\pi\Pi^*$ state minimum.

4. Interconversion between the 1a and 1b isomers

As shown in Table I, three correlated quantum-chemical methods (MP2, CC2, and SCS-MP2) predict the energy difference between conformers 1a and 1b to be very small (1–10 cm$^{-1}$). At the typical $T_{vib} = 5–7$ K in our supersonic jet expansions, the relative population of 1a and 1b should thus be within a factor of 2–3. Given the computed adiabatic transition frequencies of 31 534 cm$^{-1}$ for 1a and 31 483 cm$^{-1}$ for 1b (see Table II), we should observe two spectra that are mutually shifted by about 50 cm$^{-1}$. However, the R2PI and UV/UV holeburning spectra discussed below show only a single ground-state species. The reason for this is the...
FIG. 2. SCS-CC2/aug-cc-pVDZ calculated geometries and geometry changes of amino-keto 5,6-trimethylenecytosine upon $^1\pi\pi^*$ excitation (ground state is light-colored and the $^1\pi\pi^*$ state is darker). Bond length changes $\geq 0.05$ Å and bond angle changes $\geq 3^\circ$ are indicated.

large-amplitude amino-inversion of TMCyt, which interconverts the conformers 1a and 1b.

In the $S_0$ state, the SCS-CC2/aug-cc-pVDZ calculated barrier height between 1a and 1b is $\sim 30$ cm$^{-1}$. We calculated the one-dimensional (1D) inversion potential at the same level by incrementing the H–N–C$^4$–N$^3$ and H–N–C$^4$–C$^5$ angles $\theta$ in $v$ from $\theta$ in $v = 0^\circ$ by $5^\circ$ in the positive and negative directions, relaxing all other internal coordinates at every point. The resulting 1D potential is shown in Fig. 3. The inversion eigenfunctions in this potential were calculated by numerically solving the 1D vibrational Schrödinger equation. The reduced mass $\mu_{\text{red}, \theta}$ was determined by calculating the SCS-CC2/aug-cc-pVDZ amino-inversion imaginary normal-mode frequency at $\theta_{\text{inv}} = 0^\circ$; the central five points of the inversion potential were taken to represent the harmonic potential at this angle, and $\mu_{\text{red}, \theta}$ for the 1D calculation was fixed such that the calculated normal-mode and 1D frequencies in this harmonic potential were the same. Figure 3 shows that the lowest-energy $v_{\text{inv}} = 0$ level lies $\sim 130$ cm$^{-1}$ above the barrier. Its wave function is delocalized over both the 1a and 1b geometries with its maximum near planarity ($\theta_{\text{inv}} = 0^\circ$). The fact that the vibrational ground state of TMCyt is quasiplanar (delocalized over both 1a and 1b) explains why the UV/UV holeburning spectra, discussed in Sec. III B, reflect the presence of a single ground-state species only. The second amino-inversion level $v_{\text{inv}} = 1$ lies $380$ cm$^{-1}$ higher. It will be collisionally cooled out in the supersonic expansion and will not be considered further.

In the $S_0$ state, the planar ($C_s$ symmetric) structure of TMCyt is an index-2 stationary point. Normal-mode analysis at this point yields imaginary frequencies for both the NH$_2$ inversion and trimethylene-ring out-of-plane vibrations. The $S_0$-state barrier to planarity is $307$ cm$^{-1}$ at the SCS-CC2 level. In the $^1\pi\pi^*$ excited state, the barrier to planarity is much higher, 1297 cm$^{-1}$. Four imaginary frequencies are obtained at the $C_s$ stationary point.

B. Experimental results: Two-color resonant two-photon ionization, UV/UV holeburning, and depletion spectra

Figure 4(a) shows a two-color R2PI overview spectrum of jet-cooled TMCyt in the 31 300–38 500 cm$^{-1}$ range, recorded with the UV-OPO. The $0_0^0$ transition of the amino-keto 1a/1b tautomer is observed at 31 510 cm$^{-1}$. At the $\sim 5$ cm$^{-1}$ resolution of the UV-OPO, vibronic bands are resolved up to $+2100$ cm$^{-1}$ above the $0_0^0$ band. The R2PI signal extends up 4000 cm$^{-1}$ above, but due to the high density of vibronic excitations and the moderate $\sim 5$ cm$^{-1}$ resolution, the band structure...
is washed out in this range. In Fig. 4(b), we show a PGOPHER vibronic band simulation based on SCS-CC2 structures and normal modes of TMCyt. They are seen to be in qualitative agreement with the R2PI spectrum and will be discussed in more detail in Sec. III F.

Starting at 35 930 cm\(^{-1}\) or 4420 cm\(^{-1}\) above the amino-keto 0\(_{0}\) band in Fig. 4(a), further intense narrow-band absorption features are observed. Based on the SCS-CC2 calculations of Sec. III A 2, we assign these to the amino-enol tautomers 2b predicted at 34 508 cm\(^{-1}\) and 2a predicted at 35 008 cm\(^{-1}\), see also Table II.

Figure 5(a) shows a higher-resolution 2C-R2PI spectrum of the amino-keto tautomers over the lowest 1500 cm\(^{-1}\) range (31 400–32 900 cm\(^{-1}\)) using a narrow-band frequency-doubled dye laser for excitation. Because of its 0.05 cm\(^{-1}\) bandwidth, the vibronic bands are much better resolved in this spectrum. Detailed vibronic assignments are given in Sec. III C. A high-resolution UV/UV holeburning spectrum is shown in Fig. 5(b) and was recorded with the burn laser at the intense band at 0\(_{0}\) + 59 cm\(^{-1}\), marked with an asterisk in Fig. 5(a). It reproduces the 2C-R2PI spectrum in Fig. 5(a) in great detail. From this we conclude that all the observed vibronic bands originate from the ground-state level that gives rise to the transition at 0\(_{0}\) + 59 cm\(^{-1}\). Figure 5(c) shows the corresponding UV/UV depletion spectrum in which the holeburning laser is scanned with the detection laser fixed at the intense 0\(_{0}\) + 59 cm\(^{-1}\) band. The UV/UV depletion spectrum also reproduces the 2C R2PI spectrum, although the signal/noise ratio is lower than that in the UV holeburning spectrum. At 900 cm\(^{-1}\) above the electronic origin, the widths of the vibronic bands begin to increase, which indicates the onset of rapid non-radiative processes, see Sec. III F. Although no further bands can be observed in the depletion spectrum above +1000 cm\(^{-1}\), the signal remains slightly below the baseline, indicating a constant depletion of the ion signal.

**C. Vibronic band assignments**

We first attempted to assign the vibronic bands in the R2PI spectrum of TMCyt in Fig. 5(a) based on the SCS-CC2, CC2, and TD-B3LYP harmonic frequencies of the \(\pi\pi^*\) state given in Table III. The lowest-frequency in-plane vibration is predicted to be \(\nu'_1 = 254\) cm\(^{-1}\) (SCS-CC2), \(\nu'_1 = 248\) cm\(^{-1}\) (CC2), or \(\nu'_1 = 262\) cm\(^{-1}\) (TD-B3LYP); hence, the vibronic bands below \(\sim 250\) cm\(^{-1}\) must arise from out-of-plane vibrations. Experimentally, the two lowest-frequency bands at 38 cm\(^{-1}\) and 59 cm\(^{-1}\) cannot belong to the same progression, so we assign these as fundamentals of the lowest-frequency out-of-plane vibrations \(\nu'_1\) and \(\nu'_2\) (that is, as \(\nu'_1\) and \(\nu'_2\)). Table III shows that the lowest two frequencies calculated with the SCS-CC2, CC2, and TD-B3LYP methods are two to three times larger. Previous experience with SCS-CC2, CC2, and TD-B3LYP excited-state calculations of cytosine derivatives and pyrimidinones has shown that while the in-plane \(S_1\) state vibrational frequencies are well reproduced, the calculated out-of-plane vibrational frequencies are often 2–3 times higher than that observed experimentally.\(^{5,8,31,33}\)

For the PGOPHER vibronic band simulations (see Sec. II B), we therefore decreased the out-of-plane frequencies to the experimental values. Figures 6(a)–(c) show the simulated vibronic bands in red for the 0–420, 420–870, and 870–1320 cm\(^{-1}\) sections of the spectrum and compare these to the high-resolution 2C-R2PI spectrum in black. We first fitted the \(S_1\) state in-plane vibrational frequencies. The SCS-CC2 calculation predicts the lowest four in-plane fundamentals \(\nu'_1, \nu'_3, \nu'_4,\) and \(\nu'_6\) at 254, 461, 472, and 618 cm\(^{-1}\), respectively, see Table III. These normal-mode eigenvectors are shown in Fig. 7. The \(\nu'_1\) and \(\nu'_2\) normal-modes correspond to the \(\nu'_6\) in-plane vibrations that are characteristic of the \(S_0 \rightarrow S_1\) spectra of benzene and its derivatives. We therefore assigned the bands at 449 cm\(^{-1}\) and 471 cm\(^{-1}\) as \(\nu'_6\) and \(\nu'_5\), respectively, see Fig. 6(b); the order of these two vibrations was interchanged to obtain a better fit with the experimental R2PI spectrum. The \(0\)\(_{0}\) transition was fitted to the band at 257 cm\(^{-1}\); its intensity is rather small and it does not contribute further to the spectrum. The band at 615 cm\(^{-1}\) was assigned to the \(1\)\(_{0}\) fundamental. The band at 944 cm\(^{-1}\) is assigned as the in-plane fundamental \(\nu'_3\), as the overtone \(2\)\(_{0}\) had no intensity, see Fig. 6(c).

We then fitted the out-of-plane vibrations, see Fig. 6(a). The weak band at 38 cm\(^{-1}\) is assigned as the \(\nu'_1\) fundamental. Since the \(\nu'_1\) and \(\nu'_2\) vibrations involve structural changes of the trimethylene ring, see Table III, and hardly appear in the simulation, the intense 59 cm\(^{-1}\) band is assigned as the \(3\)\(_{0}\) “butterfly” vibrational fundamental. The \(3\)\(_{0}\) overtone was fitted to the band at 126 cm\(^{-1}\). The fundamentals of \(\nu'_4\) and \(\nu'_5\) were fitted to the bands at 93 cm\(^{-1}\) and 221 cm\(^{-1}\), respectively. The out-of-plane normal-mode eigenvectors \(\nu'_4, \nu'_5, \nu'_6,\) and \(\nu'_7\) are shown in Fig. 8. Note that the SCS-CC2, CC2, and TD-B3LYP harmonic frequencies in Table III differ from the fitted frequencies (Table IV) by a factor of 2–3, indicating that the \(S_1\) state potential-energy surface is much flatter and more anharmonic along these coordinates than that predicted by the excited-state calculations.

![FIG. 5.](attachment:figure5.png)
D. Photoionization efficiency curves

Figure 9 shows the photoionization efficiency (PIE) curves of the $S_1(\pi\pi^*)$ state, which were recorded at 0 ns delay of the ionization laser, and of a long-lived state, which was recorded at 50 ns delay. The PIE curve of the long-lived state shown in Fig. 9 is scaled according to the relative signal heights discussed in Sec. III E, where the $T_1$ ion signal reaches 25% of the $S_1$ signal when ionizing at 225 nm.

The PIE curve of the $S_1(\pi\pi^*)$ state in Fig. 9 exhibits a step-like ionization threshold at 33930 ± 20 cm$^{-1}$, indicating that the geometry change between the $\nu' = 0$ level of the $S_1(\pi\pi^*)$ state and the TMC$^+$ ion ground state $D_0$ is small. The Franck-Condon factor for adiabatic ionization from the $S_1$ state is sufficiently large, so the adiabatic ionization energy (AIE) threshold can be observed. The sum of the $S_0 \rightarrow S_{1\text{}\nu'0}$ excitation energy of 31510 cm$^{-1}$ and the PIE threshold in Fig. 9 is 65440 ± 20 cm$^{-1}$, giving an AIE = 8.114 ± 0.002 eV. The SCS-CC2 calculated AIE = 8.18 eV of tautomer 1a is in good agreement with this value (see Table II).

The delayed-ionization PIE curve of the long-lived state shown in Fig. 9(b) is relatively noisy; since the UV spectrum of the TMC$^+$ amino-eno1 forms begins around ~36 000 cm$^{-1}$, this contribution to the signal had to be subtracted. The PIE curve exhibits a gradual signal onset at 40 320 cm$^{-1}$ followed by a slow rise. We interpret the long-lived state as the lowest triplet state $T_1$ and this slow onset as...
FIG. 6. 2C-R2PI spectrum of jet-cooled TMCyt (upper traces, in black) and PGOPHER simulated vibronic spectrum (lower red traces, plotted in the negative direction) for (a) the region 30–420 cm\(^{-1}\), (b) the region 420–870 cm\(^{-1}\) (enhanced by factor 2), and (c) the region 870–1320 cm\(^{-1}\) (enhanced by factor 1.5). For details of the PGOPHER simulation see Fig. 4.

FIG. 7. SCS-CC2/aug-cc-pVDZ normal-mode eigenvectors of the four lowest-frequency \(S_1(\pi\pi^*)\) in-plane vibrations of 5,6-trimethylenecytosine.

photoionization of the hot vibrational levels of \(T_1\) that are formed by \(S_1 \rightarrow T_1\) intersystem crossing (ISC); the \(S_1 \rightarrow T_1\) energy difference is converted to vibrational energy of the \(T_1\) state during the ISC process. The signal onset at 40 320 cm\(^{-1}\) is thus interpreted as the lower limit to the AIE of the \(T_1\) state. The upper limit to the AIE is estimated by back-extrapolation of the linear part of the PIE curve to the zero-signal line at 41 400 cm\(^{-1}\). Subtracting these two values from the AIE of the \(S_1(\pi\pi^*)\) state \((65 440 \pm 20\) cm\(^{-1}\)) places the \(T_1\) state between 24 020 cm\(^{-1}\) and 25 140 cm\(^{-1}\) above the \(S_0\) ground state. The SCS-CC2 calculations (see Table II) support this assignment; the calculated adiabatic \(T_1\) state adiabatic energy is \(\sim 27 800\) cm\(^{-1}\), whereas the alternative of a dark \(n_0\pi^*\) state can be discarded because its estimated energy is much higher, 37 597 cm\(^{-1}\), see Table II.

E. Ns pump/ionization delay scan measurements and nonradiative kinetics

We measured the excited-state lifetime and nonradiative kinetics of TMCyt using ns laser pump/delayed ionization measurements by ionizing at 225 nm. The convolution of the pulse widths of the pump and ionization laser yields a Gaussian instrument response function (IRF) with a full width at half-maximum (FWHM) of 4.2 ns. We modeled the \(S_1(\pi\pi^*)\) state kinetics as

\[
\frac{d[S_1]}{dt} = -(k_{rad}^{S_1} + k_{IC}^{S_1} + k_{ISC}^{S_1}) \cdot [S_1],
\]

where \(k_{rad}\) is the \(S_1 \rightarrow S_0\) radiative decay rate. The SCS-CC2 calculated oscillator strength of TMCyt is \(f_{el} = 0.0918\), giving \(\tau_{rad} \approx 12\) ns or \(k_{rad} = 8.3 \cdot 10^7\) s\(^{-1}\). This value is in good agreement with the \(\tau_{rad} = 13\) ns that Zgierski \textit{et al.} estimated from the integrated \(S_0 \rightarrow S_1\) absorption spectrum of TMCyt in aqueous solution.\(^{26}\) The \(S_1\) state is assumed to decay nonradiatively to \(S_0\) by internal conversion (IC) with the rate constant \(k_{IC}^{S_1}\) and by intersystem crossing (ISC) to the \(T_1\) state with the rate constant \(k_{ISC}^{S_1}\). \(T_1\) is assumed to relax to \(S_0\) by reverse ISC and also by phosphorescence; these two pathways are combined into a single rate constant \(k_T\),

\[
\frac{dT_1}{dt} = k_{ISC}^{S_1} \cdot [S_1] - k_T \cdot [T_1].
\]
FIG. 8. SCS-CC2/aug-cc-pVDZ normal-mode eigenvectors of the S₄(1ππ*) state out-of-plane modes of 5,6-trimethylenecytosine.

However, \( k_T \) is very low (<5 \times 10^6 \text{ s}) and we cannot determine it by delay measurements on the ~50 ns time scale, so it is set to zero. The simulated time-dependent concentrations \([S_1] \) and \([T_1] \) were convoluted with the IRF and were least-squares fitted to the experimental pump/ionization signal traces.

Note that because of the 4.2 ns width of the IRF, which is similar to the inverse of the \( k_{IC} \) and \( k_{ISC} \) rate constants, the ratio of the ionization efficiencies of molecules in the \( S_1 \) and \( T_1 \) states, \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) \), can only be estimated within certain limits discussed below. If the width of the two laser pulses was significantly shorter than the inverse of the \( k_{IC} \) and \( k_{ISC} \) rates, then the experimental pump/ionization transient would exhibit a much more intense \( S_1 \) signal that would peak close to 100% on the scale as shown in Fig. 10, and the observed \( S_1:T_1 \) signal ratio would be correspondingly larger.

In Figs. 10(a)–10(c), we show the experimental pump/ionization transient with excitation at the 0\(^0\) band and ionization at 225 nm, marked by a dashed vertical line in Fig. 9. This transient is fitted for three different assumptions for the ionization efficiency ratio \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) \). In Fig. 10(a), we assume \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) = 1 \), giving the nonradiative rate constants \( k_{IC} = 2.2 \times 10^8 \text{ s}^{-1} \) and \( k_{ISC} = 4.1 \times 10^7 \text{ s}^{-1} \). Note, however, that this ratio is unrealistically low since ionization at 225 nm is 10 000 cm\(^{-1} \) above the \( S_1 \) ionization threshold but only 2700 cm\(^{-1} \) above the \( T_1 \) ionization threshold. For the fit in Fig. 10(b), we assume that the ionization efficiency ratio \( \sigma_{ion}(S_1)/\sigma_{ion}(T_1) = 4 \), which is the apparent experimental ratio between the \( S_1 \) and \( T_1 \) ion signals at 225 nm shown

![Figure 9](image-url)
in Fig. 9 and between the ion signals at 0 ns delay and 40 ns delay shown in Fig. 10. This fit gives the nonradiative rate constants that correspond to Figs. 10(a)–10(c). How-

ting the Lorentzian linewidth contribution $\sigma_{\text{ion}}(S_1):\sigma_{\text{ion}}(T_1) = 1$ and $\tau_{\text{T1}} = 1.2$ ns.

These IC and ISC rate constants of TMCyt can be compared to those of 1-MCyt, which are $k_{\text{IC}} = 2 \cdot 10^9$ s$^{-1}$ and $k_{\text{ISC}} = 2 \cdot 10^8$ s$^{-1}$ near the $S_1(\nu = 0)$ level. The main difference lies in the decrease of $k_{\text{ISC}}$ by a factor of 10–20. The ISC rate constant probably changes little upon rigidization of the material. Ns pump/ionization transients were also measured, reflecting the bandwidth of the UV-OPO. When limited array sizes of PGOPHER.

In contrast to the $S_0 \rightarrow S_1$ vibronic spectrum of Cyt and its derivatives 1-MCyt, 5-MCyt, and 5-FCy, which exhibit sharp break-offs at 450–1200 cm$^{-1}$ above the 0$^0_0$ band, indicating the onset of an ultrafast process, the $S_0 \rightarrow S_1$ 2C-2RPI spectrum of TMCyt 1a/1b extends up to 4400 cm$^{-1}$ above the 0$^0_0$ band and does not show a spectral break-off. The vibronic bands either merge or become diffuse at ~2100 cm$^{-1}$ above the 0$^0_0$ band of the amino-keto tautomer. To investigate the reason for the broadening, we modeled the complete vibronic spectrum for TMCyt using PGOPHER 8.0. The simulated spectrum is shown in Fig. 4(b). In addition to the nine optically active vibrational modes $\nu'_1, \nu'_2, \nu'_3, \nu'_4, \nu'_6, \nu'_7, \nu'_9, \nu'_3, \nu'_4$, and $\nu'_5$ that were employed for the simulation in Fig. 4(b) and that the bands broaden and merge into a semi-continuous background that is similar to the experimental spectrum in Fig. 4(a). This suggests

TABLE V. Internal conversion (IC) and intersystem crossing (ISC) rate constants, decay lifetimes, fluorescence quantum yields $\Phi_{\text{fl}}$, and ISC quantum yield $\Phi_{\text{ISC}}$, from fits to the ns excitation/ionization transients in Fig. 10 and Fig. S3 of the supplementary material, assuming the relative ionization efficiencies of the $S_1$ and $T_1$ states at 225 nm to be equal.

<table>
<thead>
<tr>
<th>Band (cm$^{-1}$)</th>
<th>$k_{\text{IC}}/10^8$ (s$^{-1}$)</th>
<th>$k_{\text{ISC}}/10^7$ (s$^{-1}$)</th>
<th>Lifetime $\tau&gt;$ (ns)</th>
<th>$\Phi_{\text{fl}}$</th>
<th>$\Phi_{\text{ISC}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0$^0_0$</td>
<td>2.2</td>
<td>4.1</td>
<td>2.9 ± 0.2</td>
<td>0.24</td>
<td>0.12</td>
</tr>
<tr>
<td>+530</td>
<td>3.4</td>
<td>6.6</td>
<td>2.0 ± 0.3</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>+1174</td>
<td>4.6</td>
<td>9.4</td>
<td>1.6 ± 0.2</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>+1646</td>
<td>12</td>
<td>12</td>
<td>0.7 ± 0.4</td>
<td>0.06</td>
<td>0.09</td>
</tr>
</tbody>
</table>
that the broadening of the spectrum at excess energies above +2100 cm\(^{-1}\) does not reflect just spectral congestion but arises from a decrease in the excited-state lifetime.

To account for the additional broadening observed in the experimental spectrum, we have calculated the two most energetically favorable excited-state decay paths that are analogous to those for cytosine and 1-MCyt.\(^{10,16,18,19,46–49}\) According to expectations and in line with the calculations of Zgierski et al.,\(^{26}\) the access to the ethylene-type intersection is hindered by the trimethylene modification. The calculated energy of the (Eth)\(_X\) CI is approximately 6800 cm\(^{-1}\) relative to the \(0^0\) transition. The path from \(1\pi\pi^*\) to that CI has a sloped topology, and the barrier for the decay is given by the energy of the CI itself, see Fig. S2 in the supplementary material.

The energetically favored decay path involves out-of-plane deformation of N\(^3\) and the amino group. The calculated energy profile along this path is shown in Fig. 11. The path leads from the \(S_1(1\pi\pi^*;\text{Min})\) structure through a transition state (TS) to a second minimum, \((OP)_{\text{Min}}\), which is similar to that previously characterized for Cyt and 1-MCyt.\(^{10,16,18,19,46–49}\) The MS-CASPT2 barrier over the TS is 1935 cm\(^{-1}\), and the energy of \((OP)_{\text{Min}}\) relative to \(1\pi\pi^*\) is 1056 cm\(^{-1}\). The decay path leads further to the \((OP)_X\) CI, which has a relative energy of approximately 4300 cm\(^{-1}\). The \((OP)_{\text{Min}}\) and \((OP)_X\) structures are characterized by a large out-of-plane bending of N\(^3\), with a C\(^2\)–N\(^3\)–C\(^4\)–C\(^5\) angle of 60.1\(^\circ\) and 58.2\(^\circ\), respectively. The ring puckering and out-of-plane bending of the amino group also increase from \((OP)_{\text{Min}}\) to \((OP)_X\) (see the O\(^6\)–C\(^2\)–N\(^3\)–C\(^5\), N\(^3\)–C\(^4\)–C\(^5\)–C\(^6\) angles in Fig. 12), which is consistent with the decay path where \((OP)_{\text{Min}}\) lies before the \((OP)_X\) CI.

The fact that broadening of the vibronic bands in the R2PI spectrum sets in at around ~2100 cm\(^{-1}\), but a semi-continuous spectrum continues up to 4400 cm\(^{-1}\) above the electronic origin, is in qualitative agreement with the calculated decay path topology. We interpret the additional broadening beyond ~2100 cm\(^{-1}\) due to the coupling between the vibrations belonging to the \(S_1(\pi\pi^*)\)- and the \((OP)_{\text{Min}}\)-minima below the barrier. The density of vibronic states belonging to both minima rises enormously when the energy exceeds this barrier (MS-CASPT2 barrier 1935 cm\(^{-1}\)). The semi-continuous spectrum that reaches up to at least 4400 cm\(^{-1}\) is also in good agreement with the calculated CI at 4300 cm\(^{-1}\).

**IV. CONCLUSIONS**

We have measured the UV vibronic spectra of jet-cooled amino-keto 5,6-trimethylenecytosine (TMCyt) using two-color resonant two-photon ionization, UV/UV holeburning, and depletion spectroscopies. The \(0^0\) band is identified at 31 510 cm\(^{-1}\). The lowest 400 cm\(^{-1}\) of the \(S_0\rightarrow S_1\) spectrum is dominated by fundamentals, overtone excitations, and combination bands of four out-of-plane vibrations. Based on the energetic sequence of the SCS-CC2 calculated vibrational frequencies and on their predicted Franck-Condon factors, we assign these as \(\nu_3\), \(\nu_5\), \(\nu_1\), and \(\nu_2\). Similar to the spectra of cytosine, 5-methylcytosine, and 5-fluorocytosine,\(^{6–8}\) the longest vibronic progression is observed for the butterfly vibration \(\nu_3\). Combination progressions in \(\nu_4\) are also built on the in-plane vibrational fundamentals \(\nu_1\) and \(\nu_2\). In contrast to unsubstituted cytosine, whose \(S_0\rightarrow S_1\) spectrum breaks off above ~500 cm\(^{-1}\), the R2PI spectrum of TMCyt extends to ~4400 cm\(^{-1}\) above, with more than 100 resolved vibronic bands. This is the most extended \(S_0\rightarrow S_1\) spectrum of any cytosine derivative measured so far. We have also observed the R2PI spectra of the amino-enol tautomers 2a and 2b starting at ~36 000 cm\(^{-1}\), but these will be discussed elsewhere.

![Fig. 11. MS-CASPT2 energy profile of the \(S_0\) and \(S_1\) singlet states along the CASSCF optimized paths for radiationless decay on \(S_1\) from \((1\pi\pi^*)_{\text{Min}}\) to \((OP)_X\) through \((OP)_{\text{Min}}\). Blue squares: \(S_1\); blue dots: \(S_0\).](image-url)

![Fig. 12. (a) Structure of the \((OP)_{\text{Min}}\) minimum in the \(S_1\) energy surface of 5,6-trimethylenecytosine. (b) Structure of the \((OP)_X\) conical intersection.](image-url)
Sharp vibronic bands can be observed up to +2100 cm$^{-1}$ above the 0$_0^0$ band. Hence, bridging of the C$_5$–C$_6$ bond with the trimethylene ring strongly raises the barrier to the ethylene-type (Eth)$_X$ conical intersection. Above +2100 cm$^{-1}$, a semi-continuous R2PI spectrum is observed up to at least +4400 cm$^{-1}$. The vibronic band simulation performed with the PGOPHER program nicely reproduces the vibronic band structure and intensities of the R2PI spectrum up to +1320 cm$^{-1}$. Towards higher frequencies, the simulations predict resolved vibronic transitions, whereas in the R2PI spectrum, an increased density of bands leads to an intense continuous signal.

From a mechanistic perspective, our computational work shows that, by blocking the twist of the C$_5$–C$_6$ bond, we not only change the energetically favored decay path but also the topology. In Cyt and 1-MCyt, the decay path leads from the 1-$\pi\pi^*$ minimum via a TS to the ethylene-type (Eth)$_X$ CI. The CI can be reached as soon as enough energy is available to go over the TSs, and this is observed as a sharp break-off of the R2PI spectrum above ~500 cm$^{-1}$ in these systems. In contrast, in TMCyt, the lowest CI is the amino out-of-plane bend to go over the TS, and this is observed as a sharp break-off of the continuous signal.

The excited-state lifetime of amino-keto TMCyt at the 0$_0^0$ band is $\tau = 2.9$ ns, which is a fourfold increase relative to that of cytosine at its 0$_0^0$ band. Additionally, the lifetime $\tau$ drops off much more slowly with increasing vibrational excess energy, being $\tau = 1.6$ ns even at a vibrational excess energy $E_{\text{exc}} = 1174$ cm$^{-1}$. From the calculated $S_1$ state radiative lifetime and experimental lifetime $\tau$, we infer that the fluorescence quantum yield at the $\nu = 0$ level is $\Phi_{\text{fl}} = 24\%$, which makes TMCyt the strongest fluorescing cytosine derivative in the gas phase known to date. $\Phi_{\text{fl}}$ drops to ~6% at $E_{\text{exc}} = 1646$ cm$^{-1}$. These fluorescence lifetimes and quantum yields are in qualitative agreement with the $\tau = 1.2$ ns and $\Phi_{\text{fl}} \sim 10\%$ values that Zgierski et al. determined for TMCyt in room-temperature aqueous solution. The availability of a strongly fluorescent gas-phase cytosine derivative opens exciting new research opportunities based on fluorescence measurements.

**SUPPLEMENTARY MATERIAL**

See supplementary material for the synthesis of 5,6-trimethylenecytosine, additional computational details on the MS-CASPT2 calculations, and additional ns pump/ionization transients.

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