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1 Excited State Dynamics of 6-Thioguanine

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5 Abstract

6 Here we present the excited state dynamics of jet-cooled 6-thioguanine (6-TG), using resonance-
7 enhanced multiphoton ionization (REMPI), IR-UV double resonance spectroscopy, and pump-
8 probe spectroscopy in the nanosecond and picosecond time domains. We report data on 2 thiol
9 tautomers, which appear to have different excited state dynamics. These decay to a dark state,
10 possibly a triplet state, with rates depending on tautomer form and on excitation wavelength,
11 with the fastest rate on the order of 10^{10} s^{-1} . We also compare 6-TG with 9-enolguanine, for
12 which we observed decay to a dark state with a two orders of magnitude smaller rate. At
13 increased excitation energy ($\sim +500 \text{ cm}^{-1}$) an additional pathway appears for the predominant
14 thiol tautomer. Moreover, the excited state dynamics for 6-TG thiols is different from that
15 recently predicted for thiones.

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1. Introduction

The DNA and RNA bases have a built-in protection mechanism against UV radiative damage. When they absorb UV light, they return safely to the electronic ground state in less than a picosecond by internal conversion (IC). This ultrafast IC dominates alternative relaxation pathways, such as intersystem crossing (ISC), so potentially harmful photochemical processes are largely averted. The availability of rapid IC pathways depends critically on molecular structure. This dependence results from the fact that the conical intersections that mediate IC occur at molecular geometries that differ strongly from ground state minimum geometries. Therefore the excited state dynamics of nucleobases can differ drastically for different derivatives,¹⁻⁹ analogues,¹⁰⁻¹² and even tautomers.¹³⁻¹⁵ Many derivatives and analogues of the canonical nucleobases that could serve as alternative bases lack the UV protection afforded by rapid internal conversion. This difference suggests a possible prebiotic photochemical selection of nucleobases on an early earth. In the case of guanine (G), the keto tautomer, which is prevalent in DNA, is characterized by rapid IC in contrast to enol and imino tautomers.¹⁵⁻²⁴ When the oxygen atom in DNA is replaced with a sulfur atom, the photochemistry changes dramatically due to the heavy atom effect on spin-forbidden transitions.²⁵ Canonical DNA bases absorb UVB (290-320 nm); whereas, sulfur substituted DNA analogues such as 6-thioguanine (6-TG), absorb UVA (320-400 nm).^{12, 26-28} Yu et al. reported a significant increase in intersystem crossing rate for 2-thiouracil relative to uracil in the gas phase, following excitation at 295 and 260 nm, respectively²⁹. The comparison of the excited state dynamics of G versus 6-TG has so far been limited to their keto and corresponding thione tautomers.³⁰⁻³¹ The photoproducts and photochemistry of the enol and corresponding thiol tautomers have not been studied in great detail. In this work we find significant transition rates to a dark state, most likely a triplet state, with estimated quantum yields of the order of 25% for enol G and considerably higher rates for thiol 6-TG. Here and throughout quantum yield estimates represent an upper limit as we may not be observing all decay processes in our experiment.

6-TG and other thiopurines are effective anti-inflammatory, anticancer, and immunosuppressive drugs used for over 50 years,³² however, prolonged treatment with 6-TG has been associated with a 65-250 times increased risk of skin cancer.³³ 6-TG is of interest because it can be incorporated into a patient's DNA,³⁴ increasing skin sensitivity to UVA radiation. Once 6-TG is converted into 6-TG nucleotide, it replaces the guanine in the patient's DNA.³⁵ Excitation of DNA-6TG with UVA radiation generates a series of reactions leading to the formation of reactive oxygen³⁶ species such as singlet oxygen that can damage both DNA and proteins.³⁷⁻³⁸ Other consequences of UVA interaction with DNA 6-TG includes DNA and protein oxidation, DNA-protein cross linking, DNA strand breakage, and DNA interstrand cross linking.³⁹

Guanine is known to relax to the ground state by internal conversion with lifetimes of 140 fs and 2.3 ps,²⁴ and by fluorescence on the timescale of 12 to 25 ns.⁴⁰ These fast relaxation times are indicative of a nucleobase that efficiently dissipates internal energy introduced through UV excitation. However, the fast decay was observed in femtosecond pump-probe experiments, performed with a 267 nm excitation pulse and 400 nm probe pulse, which do not map out the complete excited state dynamics. Furthermore, these femtosecond experiments lack tautomer

selectivity. In solution guanine is primarily in the keto form. In the gas phase, four guanine tautomers were observed in molecular beams by resonance enhanced multiphoton ionization (REMPI); two imino, and two enol forms.^{18, 41} Choi et al. observed keto tautomers in helium droplets which suggests the keto tautomers are present in molecular beams but unobserved by multiphoton ionization.⁴² In the present work, we evaluate the excited state lifetime of the 9-H enol guanine and compare it with that of thiol 6-TG. Research on 6-TG has focused on exploring the excited state dynamics of the thione tautomer, but no studies have been reported on the thiol tautomer.^{30, 43-45} Reichardt et al. investigated the excited state dynamics of 6-thioguanosine in phosphate buffer and in acetonitrile solutions via femtosecond broadband transient absorption spectroscopy coupled with quantum chemical calculations.⁴³ They determined that in aqueous buffer solution, ~ 80% of the S_2 ($\pi\pi^*$) excited state population decays by ultrafast intersystem crossing to T_3 ($n\pi^*$) which is ~0.1 eV below the S_2 Franck-Condon (FC) region, suggesting a strong spin-orbit coupling between these two states. However, Martínez-Fernández et al have later argued that the proposed channel is not the correct pathway to the triplet manifold.^{30, 44} Instead, they used *ab initio* calculations to show that the dominant pathway is $S_2 \rightarrow S_1 \rightarrow T_2 \rightarrow T_1$. Moreover, Martínez-Fernández et al assigned T_3 as ($\pi\pi^*$) instead of ($n\pi^*$). As a result, Martínez-Fernández et al. determined that the S_2 state ($\pi\pi^*$) and T_3 state ($\pi\pi^*$) negligibly coupled at the FC and S_2 minimum structures. Both authors however agreed that internal conversion and intersystem crossing compete in the relaxation mechanism to the ground state with the intersystem crossing pathway being the major pathway and $S_2 \rightarrow S_1 \rightarrow S_0$ being the minor pathway.

Here we present the tautomeric characterization and excited state dynamics of jet-cooled guanine in the enol form (G_e) and of 6-thioguanine (6-TG) as an alternative nucleobase and as a case study into heavy-atom effects on ISC and application of El-Sayed's rules.⁴⁶ We collected 1 and 2-color REMPI on isolated 6-TG, with IR-UV characterization, and performed nanosecond and picosecond pump-probe spectroscopy on G_e and 6-TG to construct a model of excited state decay from the origin up to ~900 cm^{-1} excess internal energy.

2. Methods

Experimental

The instrument has been previously described in detail and only a brief description of the experimental setup follows.⁴⁷ Samples (6-thioguanine, TCI 95%) are mixed with carbon black and placed on a translating graphite substrate directly in front of a pulsed molecular beam valve, based on a piezo cantilever design.⁴⁸ They are laser desorbed by a focused Nd:YAG laser (1064 nm, ~ 1 mJ/cm^2), then entrained in a supersonic molecular beam of argon (8 atm backing pressure, 15 μsec pulse width) at a repetition rate of 10 Hz. The cold, neutral molecules are ionized by REMPI and are subsequently detected in a reflectron time of flight mass spectrometer. One-color nanosecond REMPI is carried out with the frequency doubled output of a Lumonics HD-300 tunable dye laser (2 mJ/pulse , 8 ns pulse length, 0.04 cm^{-1} spectral linewidth).

The picosecond REMPI spectroscopic and pump-probe delay measurements are performed with an Ekspla PL2251 Nd:YAG laser system producing ~ 30 ps laser pulses. The 355 nm output

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3 100 pumps an Ekspla PG401 tunable optical parametric generator (OPG) (UV output of 80-120
4 101 $\mu\text{J}/\text{pulse}$, 30 ps pulse length, $\sim 6 \text{ cm}^{-1}$ spectral linewidth). 6-TG is excited by the OPG UV and
5 102 ionized by 266 nm, fourth harmonic of the ps pump laser, which is mechanically delayed up to 2
6 103 ns before colineation with the OPG beam. A variable delay between OPG UV laser and an
7 104 excimer laser (193 nm, 1.5-2 mJ/pulse) is used for pump-probe measurements in the nanosecond
8 105 range.

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11 106 For IR-UV double resonant spectroscopy a Laser Vision optical parametric oscillator/amplifier
12 107 (OPA/OPA) (mid-IR output over the range 3,200-3,800 cm^{-1} of $\sim 3\text{-}5 \text{ mJ}/\text{pulse}$, 3 cm^{-1} spectral
13 108 linewidth) precedes the ns REMPI by 200 ns. We perform double resonant spectroscopy with
14 109 two different pulse sequences in this report: in mode I we scan the IR at a fixed UV probe
15 110 wavelength and in mode II we scan the UV with a fixed IR burn wavelength. In mode I the IR
16 111 laser is scanned while the UV laser is fixed on one vibronic transition and signal depletes when
17 112 the IR laser becomes resonant with that ground state population. The resulting ion-dip spectrum
18 113 represents the ground state IR spectrum of a single tautomer, selected by the UV probe
19 114 wavelength. This IR spectrum can be compared with calculated IR frequencies to determine the
20 115 specific tautomer of the selected vibronic transition. In mode II the IR laser is set to a tautomer-
21 116 specific vibrational resonance and we scan the UV laser, comparing spectra with IR laser on and
22 117 off. The difference spectrum identifies peaks in the UV spectrum that arise from the same
23 118 tautomer.

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28 119 We performed pump-probe experiments on both the nanosecond and picosecond timescale to
29 120 determine which types of processes are occurring in this system. By combining information from
30 121 both time domains and monitoring lifetimes as a function of excitation energy, it is possible to
31 122 gain an understanding of the excited state dynamics involved in deactivation.

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34 123 The behavior as a function of time of each decay channel derives from the kinetic equations and
35 124 from solving the system of ordinary differential equations with boundary conditions shown in
36 125 equation 1, where $p(t)$ refers to a primary pathway which is populated at zero delay time and
37 126 which, with a time constant τ_n , fills a secondary pathway, $s(t)$, which in turn decays with a time
38 127 constant τ_m . Since measurements are made with a finite pulse width laser, the measured response
39 128 is a convolution of the instrument response function (IRF) with each of the pump and probe
40 129 pulses where the IRF is represented by a Gaussian centered around t_0 .

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44 130 The standard deviation of the Gaussian is fitted for each profile rather than using a single value,
45 131 as reported by Lipert et al.⁴⁹ and results in FWHM values which ranged from half to full width of
46 132 the experimental laser pulses. After convoluting the exponential decays, which gives the
47 133 excitation profile, the profile is convoluted again with the probe pulse as performed by
48 134 Spesyvtsev and shown in equations 2a-b.⁵⁰ The excitation profile, $P(t)$, consists of a sum of all
49 135 primary and secondary pathways, where the secondary pathways are multiplied by an ionization
50 136 efficiency factor (ϕ_{ion}) relative to the primary pathway, as shown in equation 2a. The excitation
51 137 profile is convoluted with 2 Gaussians centered at different t_0 with standard deviation σ in
52 138 equation 2b. This double convolution is then scaled with a factor f which accounts for the signal
53 139 intensity being in arbitrary units. The data are fit using a sum of the convolutions in 2b and a
54 140 Gaussian centered around maximum signal, as performed by Kang et al.³¹ We performed all

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3 141 fitting with the Mathematica 10 package which employed the Levenberg–Marquardt algorithm
4 142 for least squares fitting.⁵¹

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7 143 $\frac{dp}{dt} = -\frac{1}{\tau_n} p(t, \tau_n), \quad \frac{ds}{dt} = \frac{1}{\tau_n} p(t, \tau_n) - \frac{1}{\tau_m} s(t, \tau_m)$ eqn 1
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10 144 $P(t) = \sum_{n=1}^N \sum_{m=1}^M p(t, \tau_n) + \phi_{ion} s(t, \tau_m)$ eqn 2a
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13 145 $I(t) = f \int_0^t G(t - t'', t_0^{probe}, \sigma) \int_0^t G(t - t', t_0^{pump}, \sigma) P(t) dt' dt''$ eqn 2b
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15 146 Computational Methods

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18 147 Starting structures for both thiol and thione structures were optimized using the B3LYP hybrid
19 148 functional with a 6-31+G(2d,p) basis set. The shorthand notation of these structures will include
20 149 a number, 7 or 9, which indicates which nitrogen is sp³ hybridized, and a letter, e or k, which
21 150 indicates whether the structure is a thiol or thione, respectively. This nomenclature was adopted
22 151 to be consistent with the equivalent enol and keto designations for guanine. These structures
23 152 were then further optimized using MP2/6-31+G(2d,p), which were then used to perform an
24 153 anharmonic frequency analysis where ground state minima were confirmed by the absence of
25 154 imaginary frequencies. The simulated spectra arise from anharmonic frequencies with harmonic
26 155 intensities using a Lorentzian shape and FWHM of 1 cm⁻¹. The MP2 optimized structures were
27 156 used for single point energy calculations at the CCSD and EOM-CCSD level with the same basis
28 157 set. These levels of theory provide good accuracy electronic energies for the ground and
29 158 electronically excited states, respectively. These computations were performed by using
30 159 *Gaussian 09*.⁵²

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34 160 Preliminary *ab initio* molecular dynamic simulations were performed on 9e-6TG using surface
35 161 hopping including arbitrary couplings (SHARC) to augment interpretation of the experimental
36 162 excited state lifetimes with a first-order relaxation mechanism.⁵³⁻⁵⁴ The package developed by
37 163 Gonzalez and coworkers performs high accuracy *ab initio* calculations and modifies nuclear
38 164 positions as a function of time to obtain molecular dynamic information. The quantum
39 165 calculations within SHARC were performed with the *Molpro* interface.⁵⁵ A limited number of
40 166 trajectories (10) were run which included three singlet and three triplet electronic states, so that
41 167 intersystem crossing information could be obtained as well as singlet internal conversion. Once
42 168 an internal conversion (IC) or intersystem crossing (ISC) geometry was found in a *SHARC*
43 169 trajectory, the geometry was verified by performing a single point energy calculation with
44 170 *Gaussian 09*, followed by a linear interpolation of internal coordinates (LIIC) from the Frank-
45 171 Condon geometry to the intersection of interest. Both *Gaussian 09* and *Molpro* simulations
46 172 employed the state averaged complete active space self-consistent field method (SA-CASSCF).
47 173 The active space used in this method consisted of 10 electrons in 10 orbitals (10,10), and used
48 174 the 6-31G* basis set.

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53 175 It should be emphasized that the SHARC simulations and LIIC theory were done at a level
54 176 different from the aforementioned electronic state static energy calculations and anharmonic
55 177 frequency analysis. We chose this approach to achieve rigorous but preliminary theory at low
56 178 computational cost and future work on these 6-TG and G enol systems is needed with higher
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179 order perturbation methods, such as CASPT2, given the documented complexity of purine
180 excited state models.⁵⁶

181 3. Results

182 REMPI

183 Figure 1 shows the REMPI spectrum of jet-cooled 6-TG in the frequency range 32,330 - 33,330
184 cm^{-1} . The black trace shows nanosecond one-color (1C) REMPI. The spectrum exhibits a sharp
185 red-most band at 32,343 cm^{-1} , which we assume to be a 0_0^0 band and similarly sharp vibronic
186 bands up to +290 cm^{-1} , after which the spectral features lose intensity and congest. The red trace
187 shows the same spectrum with picosecond excitation. In this case the signal is lower so we used
188 two-color (2C) REMPI in which the second color consisted of 266 nm picosecond pulses. Two-
189 color ionization with nanosecond 193 nm ionization is not shown but shows features identical to
190 ionization at 266 nm. The excitation laser in the 2C-REMPI has a spectral linewidth of 6 cm^{-1} , as
191 opposed to 0.04 cm^{-1} for the laser in the 1C scan. Above 500 cm^{-1} the nanosecond signal is
192 strongly reduced in contrast to the picosecond signal. This observation points to a decrease in
193 excited state lifetime with increasing excitation energy, corresponding to a deactivation pathway
194 with a barrier of the order of 500 cm^{-1} .

195 Structural Determination

196 Figure 2 shows a double resonant spectrum recorded in mode I, with the UV probe laser fixed at
197 32,343 cm^{-1} together with calculated anharmonic IR frequencies for four different tautomers.
198 Figure 3 shows the optimized structures of the different tautomers. The simulated spectra of both
199 thiol structures fit the experimental spectrum, while none of the thione spectra fit, which was
200 previously confirmed by Kasende.⁵⁷ 6-TG thiones have 2 bands in the red part of the spectrum,
201 whereas only one band is present in the thiols (3,470 cm^{-1}), representing the $(\text{NH}_2)_{\text{sym}}$ stretch.
202 Moreover, the thione bands do not line up with the experimental data, particularly the highest
203 energy band, the $(\text{NH}_2)_{\text{asym}}$ stretch, which appears $\sim 50 \text{ cm}^{-1}$ lower than the highest frequency
204 measured. The calculated and measured intensities are well matched with the N7H/N9H stretch
205 band at 3,510 cm^{-1} , which is the most intense, followed by the $(\text{NH}_2)_{\text{sym}}$ stretch at 3,470 cm^{-1} and
206 $(\text{NH}_2)_{\text{asym}}$ stretch at 3,584 cm^{-1} with nearly identical intensities but slightly more intense at 3,470
207 cm^{-1} . We did search for the SH stretch which is calculated to be around 2,662 cm^{-1} . However, we
208 did not detect the peak in that region which is most likely due to a weak oscillator strength which
209 is calculated to be less than 10% of the least intense NH/NH₂ peaks.

210 Vibrational analysis is typically the most direct way of determining which isomers are present in
211 the molecular beam; however, Figure 2 shows that it is difficult to distinguish between 9e and 7e
212 tautomers. As calculated (Figure 3) the 9e is $\sim 9 \text{ kJ/mol}$ higher in energy than the lowest energy
213 tautomer, and the 7e is $\sim 30 \text{ kJ/mol}$ higher in energy than the lowest energy tautomer. Past work
214 in our lab on the nucleobase adenine showed that only the lowest energy isomer was present,
215 where the next lowest energy isomer was calculated to be at $\sim 33 \text{ kJ/mol}$ higher energy than the
216 one observed.⁵⁸ For this reason, we tentatively assign the UV origin species as 9e. We do not rule
217 out the possibility of 7e or perhaps a 9e-stereoisomer being present given the ambiguity of their

218 IR spectra. The discussion will proceed focusing on 9e, but keeping these alternatives in
219 consideration.

220 So far, we have shown that 9e and possibly 7e are the tautomers present when probing at the
221 origin. Typically, UV-UV hole-burning spectroscopy is used to determine the number of
222 tautomers in a REMPI scan; however, the presence of a long-lived dark state, which we discuss
223 below, interferes with this approach. For UV-UV hole-burning to work, the probe laser should
224 only produce ions from the ground state and this signal should be reduced when the ground state
225 is depleted resonantly by the burn laser. However, when the burn laser populates a long-lived
226 dark state, the probe laser can still produce ions indiscriminately from that dark state even at the
227 burn laser resonances. Since it is not practical to perform double resonant spectroscopy in mode
228 I, scanning the IR with the UV wavelength fixed, at each successive UV peak, we performed
229 double resonant spectroscopy in mode II.

230 We set the burn laser to $3,584\text{ cm}^{-1}$ 200 ns prior to the UV probe laser which was then scanned to
231 record the IR-UV hole burning spectrum. We chose the $(\text{NH}_2)_{\text{asym}}$ stretch because it is a good
232 thiol indicator (Figure 2). There is no marker frequency that can distinguish between 7e and 9e
233 tautomers as they share very similar IR spectra. We obtained spectra with IR laser off and IR
234 laser on, where a decrease in signal indicates shared IR resonance with the origin transition and
235 theoretically the same isomer. The result, shown in Figure 4, indicates that the entire UV
236 spectrum in this range correlates with the same IR marker frequency and thus exclusively with
237 thiol tautomers.

238 **Pump-Probe**

239 6-Thioguanine

240 In the following discussion, we refer to the UV excitation peaks by their energy relative to the
241 origin at $32,343\text{ cm}^{-1}$.

242 Table 1 lists fitting parameters from the picosecond and nanosecond pump-probe experiments.
243 When fitting the picosecond pump-probe traces, shown in Figure 5, all traces except for 47 cm^{-1}
244 and 290 cm^{-1} are fit with a primary decay (τ_1) on the order of hundreds of picoseconds, which
245 populates a secondary state. The lifetime of the secondary state (τ_2) can only be determined
246 within the picosecond data at excess energies greater than 462 cm^{-1} and is of the order of 1 ns.
247 The 47 cm^{-1} and 290 cm^{-1} traces are fit with a single decay of about 4 ns. It cannot be determined
248 on the picosecond timescale whether or not this 4 ns decay fills a secondary state, so we employ
249 nanosecond timescale experiments to obtain more information.

250 The nanosecond lasers are too slow to measure dynamics that occur on shorter timescales, but
251 can be used to fit longer lived pathways. Upon inspection of the nanosecond pump-probe traces
252 (Figure 6), it is evident that a long lived dark state is populated which returns to the ground state
253 on the order of a few microseconds (τ_3). This result suggests the secondary state in the
254 picosecond experiments that decays within single nanoseconds (τ_2) may be a “doorway” state
255 (DS_2) that feeds a long lived “dark” state (DS_1) which is observed in the nanosecond experiment
256 as having the microsecond lifetime τ_3 . The nanosecond pump-probe data were fit with primary

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4 257 and secondary decays, however, in this case the primary decay is from DS₂ which decays with
5 258 time constant τ_2 into DS₁ with a lifetime τ_3 . The 1 ns (τ_2) decay from DS₂ for peaks 679 cm⁻¹ and
6 259 904 cm⁻¹ is too fast to be measured by the nanosecond pump-probe, but an additional primary
7 260 decay of ~20 ns was required to fit the 679 cm⁻¹ and 904 cm⁻¹ traces, denoted τ_{2b} , implying a
8 261 double exponential decay to DS₁ at these excess energy levels. The nanosecond (τ_1) component
9 262 for 47 cm⁻¹ and 290 cm⁻¹ was fit in both the picosecond and nanosecond traces to within 4% and
10 263 we report the average value. We assume that for these two excitations the nanosecond decay is
11 264 from the bright state, not a dark state.

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16 266 9-enol Guanine
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19 267 We performed pump-probe experiments on 9e guanine to compare dynamics with 9e-6TG. The
20 268 excitation wavelength of 32,873 cm⁻¹ selects the origin of this tautomer, based on previous
21 269 results.^{41, 59} Measurements in the picosecond range show a flat line, indicating that there are no
22 270 fast dynamics to capture at that timescale. Supplemental Figure 1 shows the nanosecond trace of
23 271 the 9e guanine origin revealing two ns lifetime components. One decay path with a 40 ns lifetime
24 272 feeds a long lived dark state while the other goes directly to the ground state with a 13 ns
25 273 lifetime. Tautomer selective molecular beam LIF experiments performed by Chin et al. found a
26 274 12 ± 2 ns fluorescence lifetime when 9e guanine was excited at its origin.⁴⁰ A 50 ns collection
27 275 window was used in that work and a two-state mixing model {S₂,S₁}→S₀ was given to support
28 276 the broad dispersed fluorescence spectrum. Without a picosecond component to experimentally
29 277 confirm the S₂→S₁ hypothesis we interpret the faster 13 ns decay as fluorescence from the
30 278 optically bright state. The slower 40 ns decay then feeds a dark state with no fluorescent
31 279 signature, which could be a ¹nπ* or a triplet state. In support of the Chin branching model, it is
32 280 possible that if S₂ is the initially excited state, vibronic equilibration between S₂ and S₁ occurs on
33 281 a sub-picosecond timescale leading to fluorescence at rates that are equal or undistinguishable by
34 282 these experiments. Since in solution guanine exists predominantly in the keto form this is to our
35 283 knowledge the first observation of such a long lived dark state in enol guanine. If the 13 and 40
36 284 ns decays are the only two excited state processes, then the fluorescence quantum yield is about
37 285 75%.

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42 286 **Computational Results**
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45 287 The CCSD energy calculations for the excited states indicate that excitation is to the S₁ state
46 288 based on oscillator strengths for 9e, 7e 6-TG, and 9e guanine (Supplementary Table 1). This
47 289 conclusion suggests ruling out the option that τ_1 could be the decay of S₂ to S₁ and instead
48 290 suggests that τ_1 describes ISC, populating a DS₂ of triplet state character. Such fast ISC can be
49 291 further explained when combined with information from CASSCF calculations. The 10 SHARC
50 292 trajectories produce one conical intersection (CI) for S₁/S₀ internal conversion and two relevant
51 293 intersystem crossing geometries for El-Sayed allowed S₁(ππ*)/T₃(nπ*) and forbidden
52 294 S₁(ππ*)/T₂(ππ*) crossings. The CI involves the loss of the SH hydrogen, where the S₁/T₃ ISC
53 295 path involves a distortion of the heterocycle (ISC_{dist}), and the S₁/T₂ path corresponds to an out of
54 296 plane rotation of the thiol group (ISC_{oop}) (Figure 7). These are preliminary simulations only for
55 297 the 9e tautomer, consistent with the experimental structural findings described above. We cannot
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298 exclude the presence of a second rotamer and of a 7e tautomer so a follow-up comprehensive
299 computational treatment at a higher level will need to also consider those tautomers.

300 Figure 8a-c shows LIIC plots constructed by stepping the internal coordinates from the FC
301 geometry to the CI/ISC of interest. The path to the CI (Figure 8a) has no barrier, and can be
302 accessed from all excess energies in the FC region. The LIIC for ISC_{oop} (Figure 8b) results in a
303 barrierless path for a S₁/T₂ crossing being located well below the FC region. The transition is
304 forbidden, but with larger spin orbit coupling due to the sulfur, could be much faster than without
305 it. It also appears that this path results in the singlet and second triplet state being in close
306 proximity, in fact crossing multiple times between the 5th and 8th pathway intervals, which could
307 increase the probability of making the transition at the ISC_{oop} geometry. The ISC_{dist} pathway
308 (Figure 8c) involves a crossing seam which starts around 0 cm⁻¹ of excess energy, and extends to
309 nearly 9,000 cm⁻¹. Since this ISC transition is El-Sayed allowed, ultrafast ISC is expected if the
310 crossing is reached which may not be the case considering IVR in the excited state will distribute
311 energy across all modes rather than in the direction of the LIIC.

312 Like in the LIIC ISC pathway to T₂ (Figure 8b) there are additional crossing points on the
313 potential surface for ISC to T₃ possibly indicating that additional geometries exist for ISC which
314 over the timescale of the SHARC calculations were not populated. It could also be that a greater
315 number of trajectories would yield additional crossing geometries along with statistically
316 significant likelihoods for each. These energy crossings within the LIIC curves may also be
317 artifacts of the level of theory and of plotting a linear interpolation as opposed to a minimum
318 energy pathway.

319 4. Discussion

320 Intersystem crossing is typically a slow process due to the required spin flip, but may be ultrafast
321 when spin orbit coupling is large. Increases in spin orbit coupling may happen in various ways,
322 such as a change in symmetry or through the heavy atom effect.^{25, 46} We measured excited state
323 lifetimes with increasing excess energy to learn more about possible decay pathways in 6-TG.
324 We performed SHARC simulations initiating in the S₂ state so that internal conversion to S₁
325 produces a hot excited state. By performing simulations in this manner, we can find crossing
326 geometries that have an activation barrier, which may be accessed with larger amounts of
327 internal energy.

328 Based on the assumption that excitation of 9e-6TG results in population of S₁, the initial decay
329 can go to either the ground state (IC) or a triplet state (ISC). The picosecond pump-probe data
330 indicates initial decay to a doorway DS₂ state, suggesting that fast ISC is occurring. We do not
331 observe direct decay to the ground state on the timescales of our experiments, suggesting the
332 absence of IC. This result is interesting because Figure 8a shows a conical intersection located
333 below the Frank-Condon region. It may be that IC occurs quicker than the picosecond laser pulse
334 widths, obscuring this pathway in our experiments. Without certainty that all processes are
335 observed we cannot derive quantum yields from the rates, but we can compare observed (τ_1^{-1})
336 and (τ_2^{-1}) rates with the 40 ns decay to the long lived dark state of 9e guanine. Here it seems that
337 the sulfur←oxo substitution quenches the 75% fluorescence decay seen in 9H-G_e in favor of total
338 population redistribution to the long-lived excited state in 9e-6TG. Additionally, this long-lived

339 dark state is directly populated in the guanine case but proceeds through a doorway state in 6-
340 TG. This comparison is generalized in Figure 9 cases (a) and (c) for the 6-TG and guanine
341 models respectively.

342 Figure 9 (a,b) summarizes a tentative interpretation of the pump-probe observations of 9e-6TG
343 with two cases. Case (a) corresponds to excitation at 0, 462, 679, and 904 cm^{-1} and case (b)
344 corresponds to excitation at 47 and 290 cm^{-1} . A possible explanation involving two different
345 relaxation mechanisms is that the excess energy traces included excitations of multiple thiol
346 forms that went unresolved by mode II IR hole burning measurements. Neither the 7e tautomer
347 nor a rotational isomer, about the thiol dihedral coordinate, can be excluded. Alternatively, there
348 may be some vibronic selection criteria which strongly mediate relaxation to either case for 9e-
349 6TG. In these models the first decay τ_1 represents total population transfer from the bright state
350 S_n to DS_2 for (a) and to DS for (b). DS_2 provides a pathway to DS_1 by two measurable decay
351 rates, τ_2^{-1} and τ_{2b}^{-1} . The latter rate, τ_{2b}^{-1} , has an empirical vibronic energy barrier of +679 cm^{-1}
352 (upper limit). Case (a) then decays at τ_3^{-1} from DS_1 to below the ionization potential of our probe
353 laser, into what is likely the ground state of 6-TG. In case (b) τ_1 feeds directly into the long-lived
354 DS, which then decays at τ_3^{-1} on a microsecond timescale. The microsecond relaxation time may
355 lend credence to the identity of DS_1 and DS as triplet states.

356 A thorough theoretical model is needed to correlate the pump-probe dynamics with specific
357 electronic state identities and trajectory mechanisms. Our preliminary LIIC results provide trends
358 of IC and ISC from the 9e-6TG FC geometry, at the CASSCF level, as initial insight. Figure 8b
359 supports the notion that the S_1/T_2 crossing occurs below the Frank-Condon geometry. Figure 8c
360 shows an S_1/T_3 crossing well above the Frank-Condon geometry with S_1 and T_2 intersecting
361 along this path as well. This suggests that ISC, likened to τ_1 , is occurring quickly to T_2 at the
362 energies probed here, and that with even more energy (around +679 cm^{-1}), T_3 may be accessed
363 which would provide access to an El-Sayed allowed transition. In this case T_3/T_2 conversion
364 would be rapid and could yield the τ_2^{-1} and τ_{2b}^{-1} rates seen at higher energies.

365 5. Conclusion

366 When comparing the dynamics of the thiol system studied here with the aqueous phase
367 femtosecond study of the thione-nucleoside by Reichardt et al.⁴³ interpreted by Martínez-
368 Fernández et al.,^{30, 44} the rates for ISC are 300-1000 times lower for the thiol. Reichardt also saw
369 internal conversion occurring on the tens of picoseconds timescale, which we do not see in the
370 thiol in the gas phase. In addition to the lack of tautomer selectivity, aqueous phase experiments
371 can produce different results because the solvent affects excited state dynamics by hydrogen
372 bonding and structural changes. Possible ISC lifetimes for 9k and 7k tautomers of 6-thioguanine
373 in the gas phase for comparison are unknown. We demonstrated the effect on ISC of replacing
374 the oxygen in guanine with a sulfur by comparing the pump-probe results of 9e 6-TG with those
375 of 9e guanine. The guanine data correspond to fluorescence lifetimes and a lower decay, possibly
376 ISC, of 13 ns and 40 ns, respectively. At the origin the rate (τ_1^{-1}) for filling DS_2 in 9e 6-TG is 80
377 times greater than the decay to the long lived dark state in 9e guanine. Lastly, the presence of an
378 intermediary state (DS_2) is not seen when probing 9e guanine. Higher level computations are
379 needed for a more complete interpretation of the experimental results.

Supporting Information

Singlet and triplet excited state analysis at CCSD/6-31+G(2d,p) for ground state and EOM-CCSD/6-31+G(2d,p) for excited states, nanosecond pump probe trace of 9-enol guanine at its origin.

Acknowledgments

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537 Tables

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Fitting Parameters for 6-Thioguanine				
Excess Energy [cm ⁻¹]	τ_1 , ps	τ_2 , ns	τ_{2b} , ns	τ_3 , ns
0	481 ^a	6.1 ^b		2025 ^b
47	3920 ^{ab}			3033 ^b
290	4330 ^{ab}			2000 ^b
462	577 ^a	5.7 ^b		2590 ^b
679	87 ^a	0.9 ^a	24 ^b	878 ^b
904	168 ^a	1.1 ^a	21.5 ^b	462 ^b

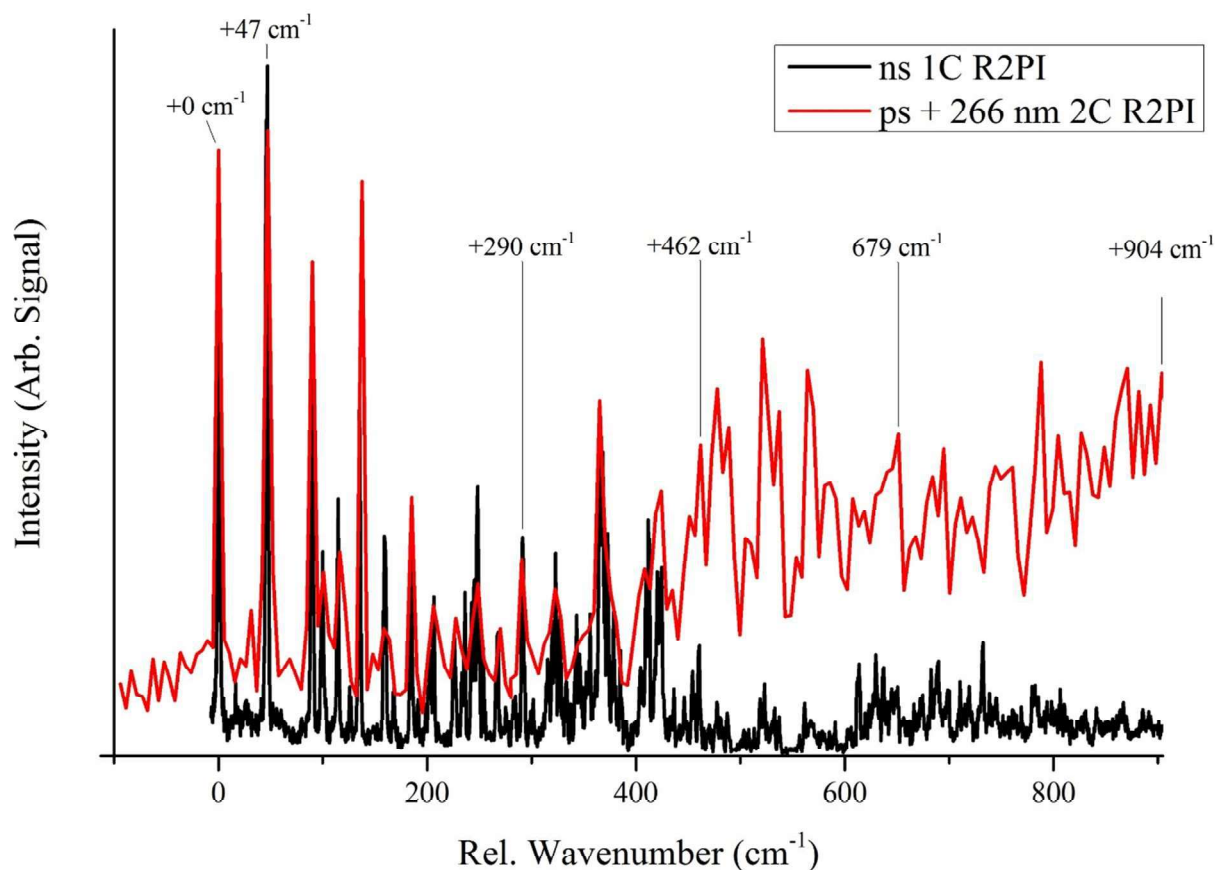
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541 **Table 1.** Summary of fitting parameters where ^a denotes a value from picosecond fit and ^b denotes a value from a
542 nanosecond fit.

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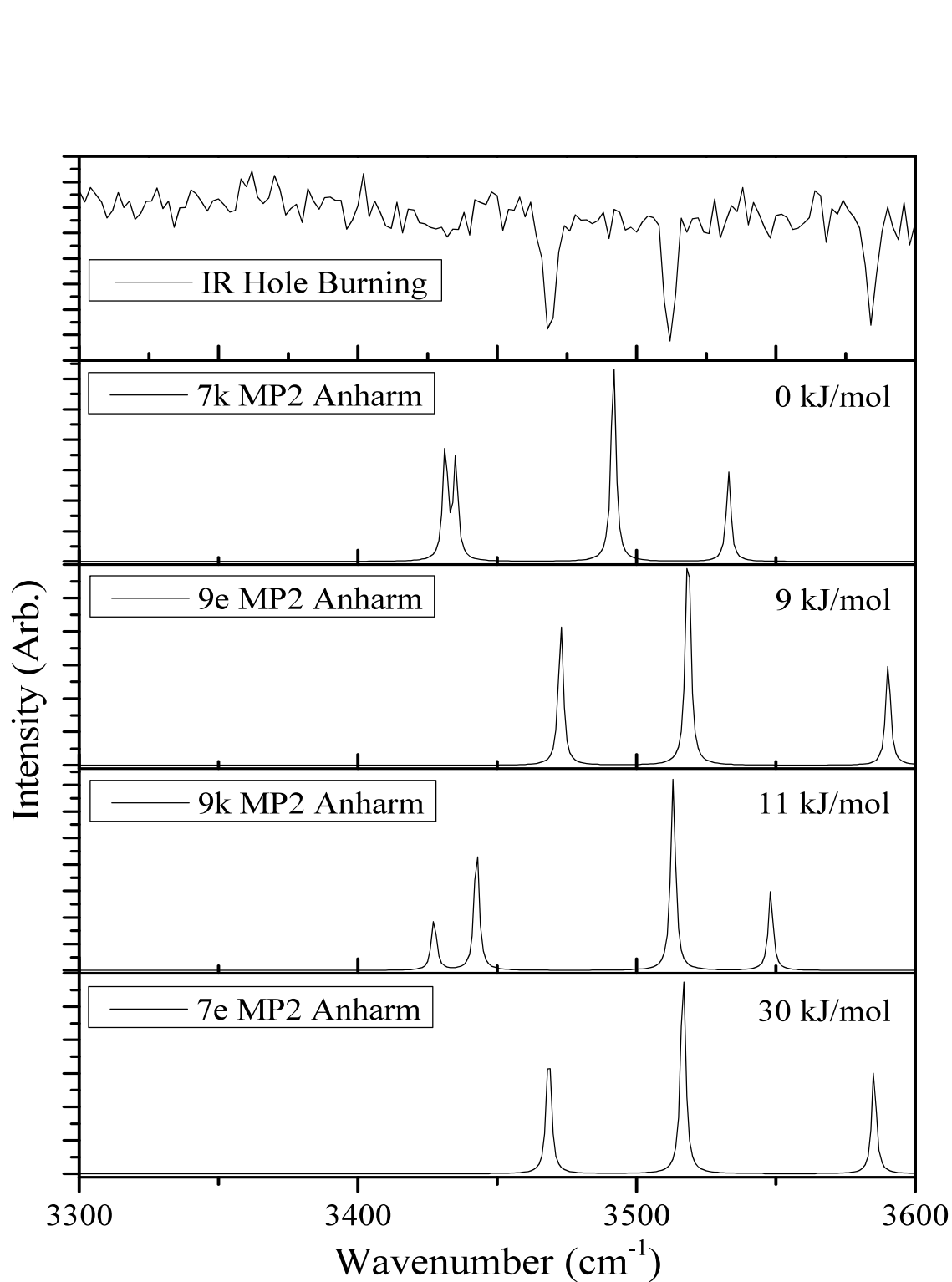
544 **Figures**

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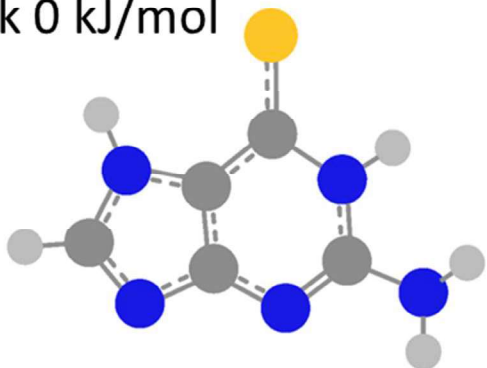
546 **Figure 1.** One-color resonant two-photon nanosecond ionization spectrum of jet-cooled 6-
547 thioguanine is shown in black and two-color resonant two-photon picosecond ionization
548 spectrum of jet-cooled 6-thioguanine is shown in red. The wavenumber scale is relative to the 0_0^0
549 band at $32,343 \text{ cm}^{-1}$ and annotations mark where pump-probe was performed. Both spectra have
550 been normalized at $+0 \text{ cm}^{-1}$.
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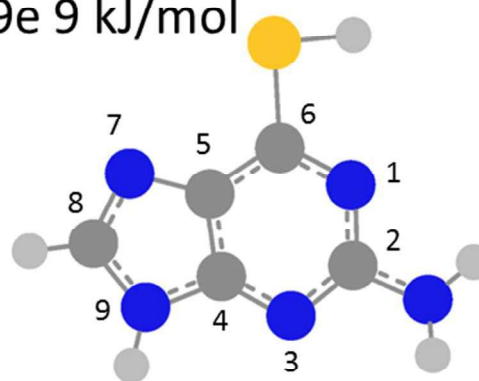


52 **Figure 2.** Experimental IR hole burning spectrum in mode I (*top*) and theoretical IR bands
53 calculated at the anharmonic MP2/6-31+G(2d,p) level. Energies shown calculated at CCSD/6-
54 31+G(2d,p).
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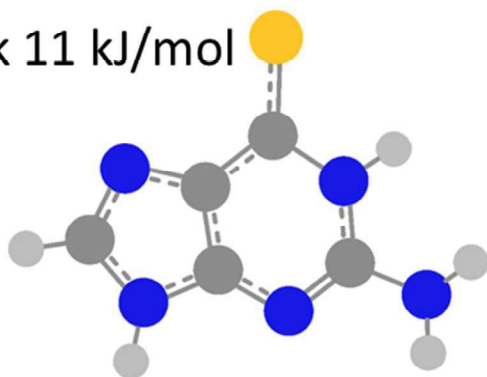
7k 0 kJ/mol



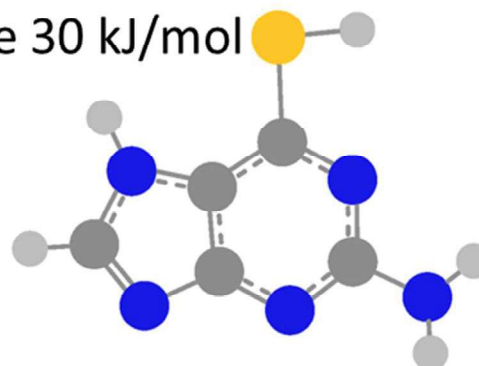
9e 9 kJ/mol



9k 11 kJ/mol



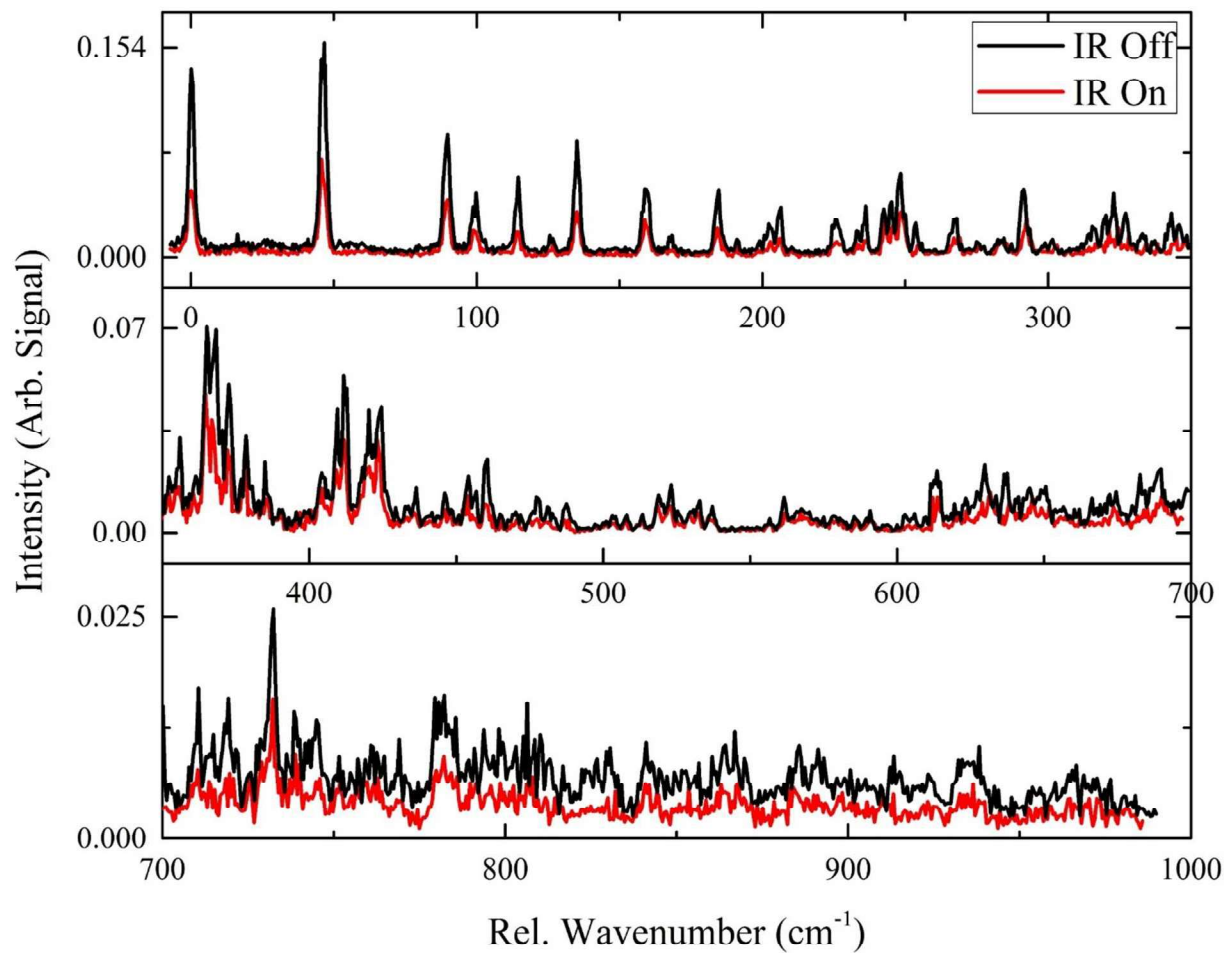
7e 30 kJ/mol



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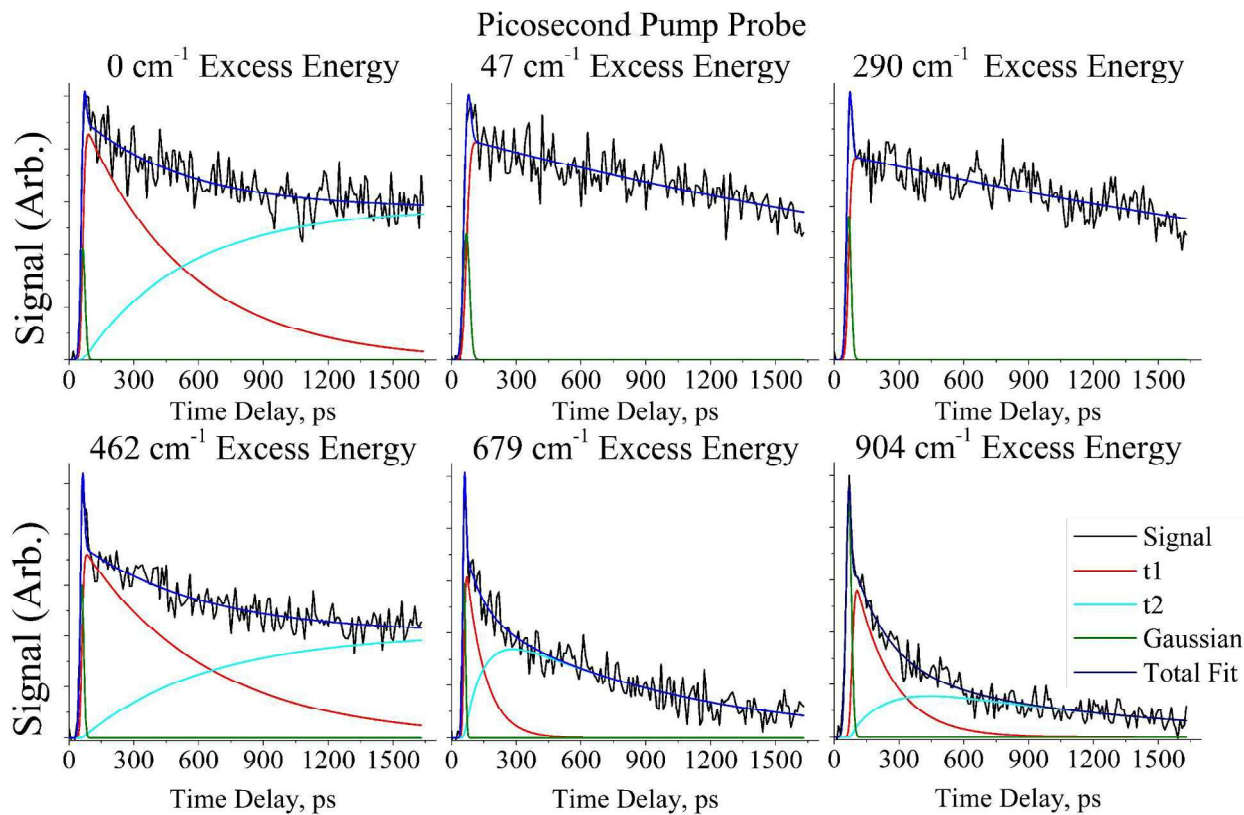
567 **Figure 3.** Ground state (Frank-Condon) structures optimized at MP2/6-31+G(2d,p). The atom
568 numbering is shown on the 9e tautomer. Energies shown calculated at CCSD/6-31+G(2d,p).

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571 **Figure 4.** IR laser set to 3,584 cm⁻¹ 200 ns prior to scanning UV. Decrease in signal indicates
572 shared IR resonance with the origin transition.

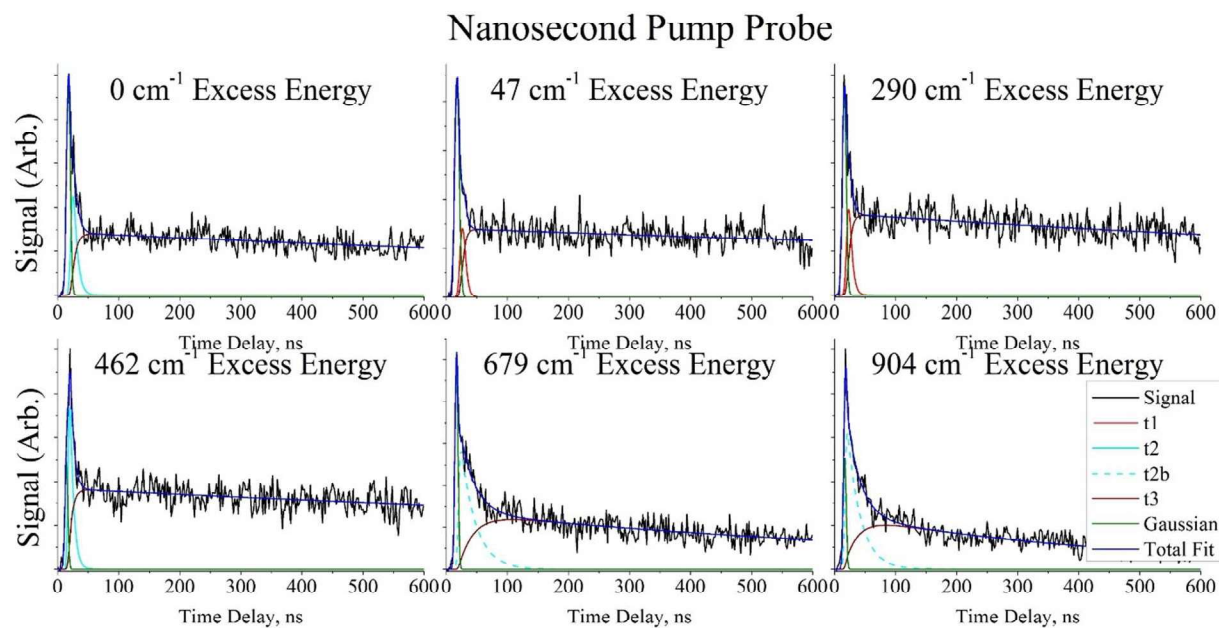


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574 **Figure 5:** Picosecond pump-probe traces with fitting for 0, 47, 290, 462, 679, and 904 cm^{-1} .
575 Fitting parameters are marked as ^a in Table 1.

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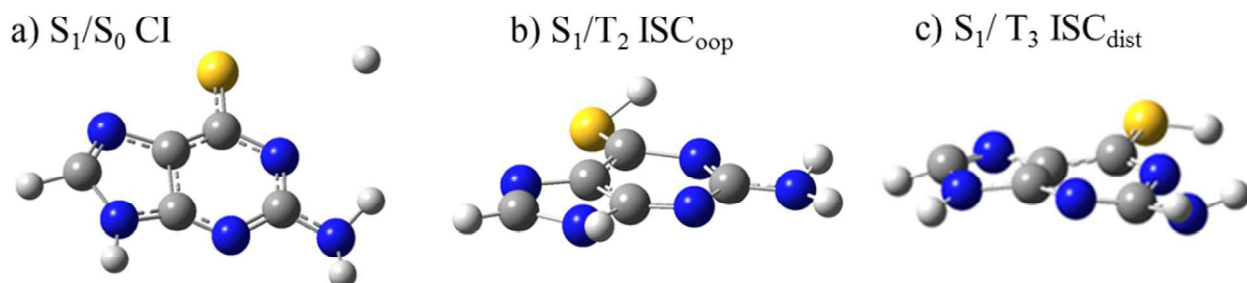
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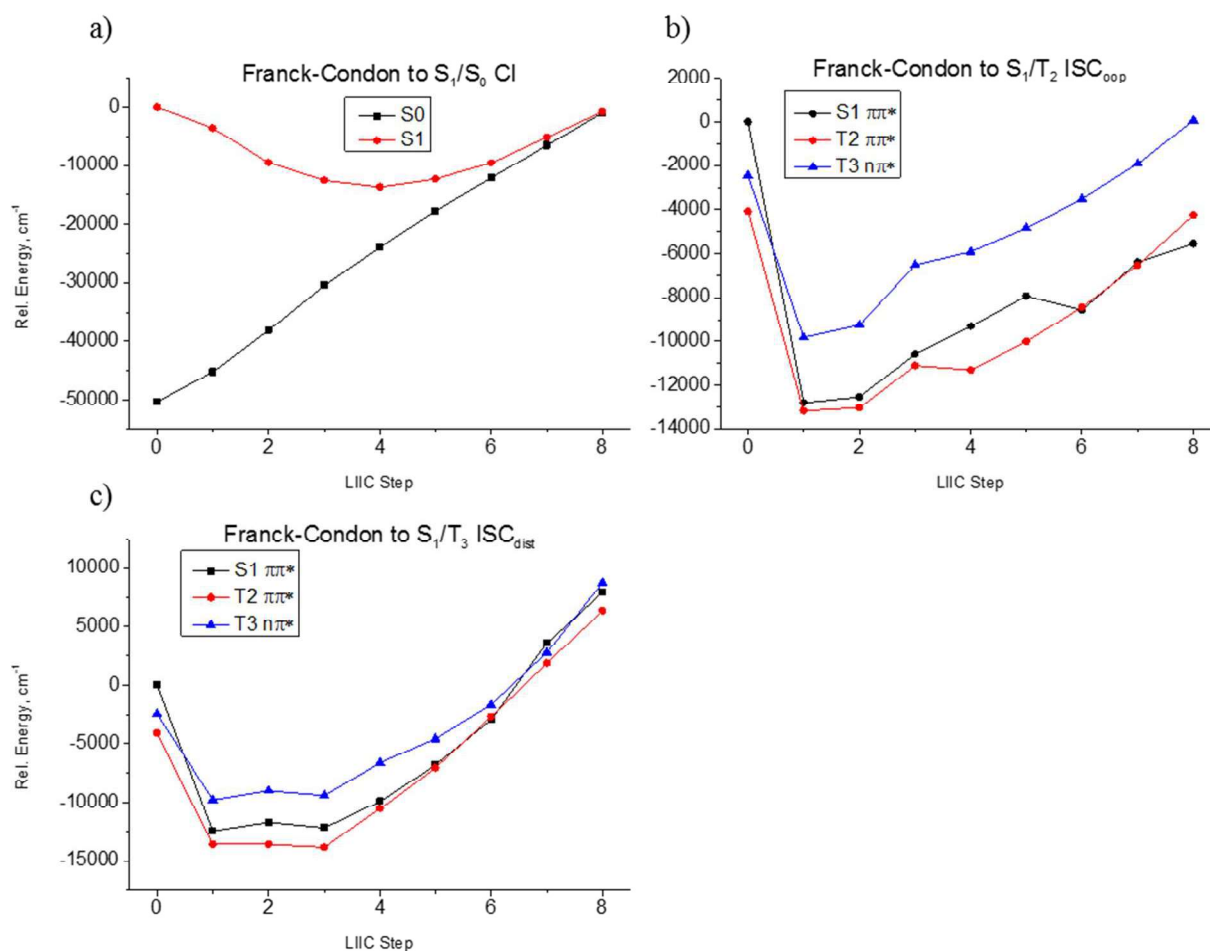
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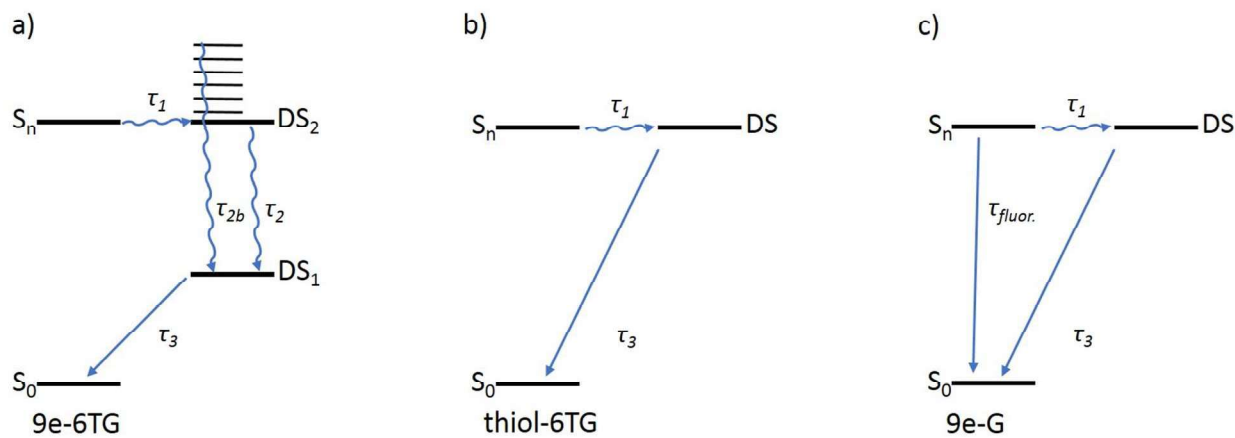
579 **Figure 6.** Nanosecond pump-probe traces with fitting for 0, 47, 290, 462, 679, and 904 cm^{-1} .
580 Fitting parameters are marked as ^b in Table 1.

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582 **Figure 7.** Structures are geometries leading to hops in the SHARC simulation. All structures are
583 calculated at SA-CASSCF(10,10)/6-31G(d) level.





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590 **Figure 9.** Proposed decay pathways where a) corresponds to traces 0, 462, 679, and 904 cm^{-1} and
 591 b) corresponds to traces 47 and 290 cm^{-1} of 6-TG and c) corresponds to the 0 cm^{-1} trace of 9e
 592 guanine. S_n is assumed to be the optically bright state probed by picosecond resolution. DS_2 ,
 593 and S_n of (b) and (c), are assumed to be the initial decay states probed by nanosecond resolution.
 594 τ_{2b} has an activation barrier of +679 cm^{-1} (upper limit).

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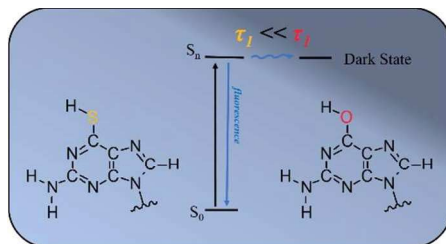
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621 TOC Graphic

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