Excited-State Electronic Properties of 6-Methylisoxanthopterin (6-MI): An Experimental and Theoretical Study

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Supporting Information

ABSTRACT: 6-Methylisoxanthopterin (6-MI) is a pteridine-based guanine analog that has a red-shifted absorption and high fluorescence quantum yield. Its Watson−Crick base-pairing and base stacking properties are similar to guanine. The fluorescence quantum yield of 6-MI is sensitive to its nearest neighbors and base stacking, making it a very useful real-time probe of DNA structure. The fundamental photophysics underlying this fluorescence quenching by base stacking is not well understood. We have explored the excited-state electronic structure of the 6-MI in frozen 77 K LiCl glasses using Stark spectroscopy. These measurements yielded the direction and degree of charge redistribution for the S0 → S1 transition as manifested in the difference dipole moment, Δμ01, and difference static polarizability, TrΔμ. TDDFT (time-dependent density functional theory) was employed to calculate the transition energy, oscillator strength, and the dipole moments of the ground and lowest optically bright excited state of 6-MI (S0 → S1). The direction of Δμ01 was assigned in the molecular frame based on the Stark data and calculations. These results suggest that the C4=O and C2-NH2 groups are electron-deficient in the excited state, a very different outcome compared with guanine. This implies that Watson−Crick hydrogen bonding in 6-MI may be modulated by absorption of a photon so as to strengthen base pairing, if only transiently. Solvatochromism was also obtained for the absorption and emission spectra of 6-MI in various solvents and compared with the Stark spectroscopic results using both the Lippert-Mataga and Bakhshiev models.

INTRODUCTION

6-Methylisoxanthopterin (6-MI), a pteridine-based guanine analog (Scheme 1), has been used to probe nucleic acid structure and dynamics. Its high fluorescence quantum yield (Φf = 0.70), combined with its red-shifted absorption (λmax = 345 nm in water), provides for selective excitation in the presence of other native nucleic acids.1,2

Scheme 1. Chemical Structure of 6,8-Dimethylisoxanthopterin Used for the QM Calculations

*The molecule used in the experiments was not the free base but 6-methylisoxanthopterinoside, where the sugar replaces the C8 methyl group.

6-MI is a fluorescent base pairing analog (FBA) of guanine: it forms Watson−Crick base pairs with cytosine. These base-pairing analogs (another example is 2-aminopurine (2AP), a fluorescent adenine analog3−5) tend to be dimmer than the larger pendant dyes often used to tag nucleic acids (e.g., fluorescein6). However, FBAs have more native base-like physical properties such as duplex melting point, steric footprints, and hydrogen bond patterns. Therefore, they are uniquely suited to act as fluorescent reporters of nucleic acid behavior.7

The emission quantum yield of 6-MI is sensitive to base stacking in DNA. We and others have examined this kind of behavior in other fluorescent adenine analogs like 2AP8−13 the pteridines 6MAP14 and DMAP,15 and 8-vinyladenine.16,17 A common conjecture about the mechanism of fluorescence quenching in these FBAs is that excited-state electron transfer occurs with *FBA as the donor or acceptor depending on the redox potentials of its neighbors. We have shown this to be likely with 6-MI based on an electrochemical analysis, at least in the proximity of purines.18 Steady-state fluorescence quenching experiments show that 6-MI is not quenched by pyrimidines.19 This theme of purines quenching 6-MI more than by pyrimidines seems to be true whether in ssDNA or dsDNA.

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Electron transfer is not the only mechanism for emission quenching. It is now becoming apparent that other mechanisms may be at play. For example, Matsika et al. have shown that the accessibility of excited states to conical intersections leading to quenching may be unavailable to the monomer FBA because of large potential barriers. These barriers can be lowered due to structural changes to the FBA that occur upon base stacking. These structural changes begin with charge redistribution of the molecule in the excited state after the absorption of a photon. Indeed, vectorial changes in excited-state charge density are responsible for initiating a number of important photobiological processes. These include the cis–trans photoisomerization of retinal, hydrogen bond switching seen in BLUF domains (photoregulation of gene expression), pyrenes, where the details of the excited electronic structure orders. A simple example is found in the photoacidity of the excited-state charge density can lead to specific changes in bond orders. Therefore, the excited-state electronic structure of each atom. Therefore, the excited-state electronic structure of 2-aminopurine and from this analysis suggested Stark spectroscopy to obtain the excited-state electronic structure of 6-MI as a function of solvent dielectric. TD-DFT calculations were used for ground- and excited-state charge densities, and were used for ground-state dipole moment, but in a frozen glass the angle between the direction of electric field vector of the molecule possessing a field-induced poling of a molecule possessing a

\[ \Delta \epsilon / \bar{\nu} = \left( \epsilon / F \right)^{2} \left\{ A_{\chi} \epsilon(\bar{\nu}) / \bar{\nu} + B_{\chi} d(\epsilon(\bar{\nu}) / \bar{\nu}) / d\bar{\nu} \right\} \]

The term \( \epsilon(\bar{\nu}) \) represents the energy-weighted unperturbed extinction coefficient as a function of the energy \( \bar{\nu} \). The electric field \( F \) represents the electric field applied on the sample, and \( f_{e} \) is the local field correction factor for an elliptical solvent cavity. An enhancement of the applied field is expected due to the cavity field of solvent matrix, which is dependent on the dielectric constant of the solvent. Because of this, \( f_{e} \) is always greater than one. For LiCl, \( e_{0} = 15 \). On the basis of the ground-state optimized structure (Figure S1 of the Supporting Information), the major axis \( a_{e} = 8.8 \) Å and the minor axis \( a_{m} = 6.2 \) Å, with \( a_{z} = 2.4 \) Å, corresponding to the width of two hydrogen atoms. The local field correction factor was estimated to be \( f_{L} = 1.63 \) based on an average of the \( a_{e}, a_{m}, \) and \( a_{z} \).

The derivative scaling factors \( A_{\chi}, B_{\chi} \), and \( C_{\chi} \) are related to intrinsic electronic properties of the chromophore and these properties are tied to the laboratory frame through \( \chi \), which is the angle between the direction of electric field vector of the linearly polarized light and that of the applied electric field \( F \). All of the Stark experiments reported here were performed with \( \chi = 54.7^{\circ} \) (magic angle) or 90°, after correcting for the refractive indices of liquid nitrogen and the ITO-coated quartz slides. \( A_{\chi} \) reflects the field-induced poling of a molecule possessing a ground-state dipole moment, but in a frozen glass \( A_{\chi} \approx 0 \).
Information about the change in polarizability and the change in permanent dipole moment can be obtained from the coefficients $B_d$ and $C_d$. The $B_d$ term is related to change in the polarizability, where

$$B_d \approx \frac{5}{2} \Delta \bar{\alpha} + (3 \cos^2 \chi - 1) \left( \frac{3}{2} \bar{m} \cdot \Delta \bar{\alpha} \cdot \bar{m} - \frac{1}{2} \Delta \bar{\alpha} \right)$$

(2)

Here $\bar{m}$ is the transition dipole moment. The difference polarizability, $\Delta \bar{\alpha} = \bar{\alpha}_g - \bar{\alpha}_0$, is a tensor, but for our purpose we consider only the trace of this polarizability, $\text{Tr} \Delta \bar{\alpha}$, which is a scalar representing the polarizability volume. The trace of a tensor is invariant with respect to a change in coordinate system. Therefore, $\text{Tr} \Delta \bar{\alpha}$ is a good estimate of the change in the polarizability volume upon excitation. The applied electric field will produce a second-derivative component of the absorption spectrum when the difference dipole moment, $\Delta \mu$, is nonzero

$$C_d = \left| \Delta \bar{\mu} \right|^2 \left( 5 + (3 \cos^2 \chi - 1)(3 \cos^2 \zeta_d - 1) \right)$$

(3)

where $\zeta_d$ represents the angle between $\Delta \bar{\mu}$ and transition dipole moment $\bar{m}$, $\zeta_d = \angle \bar{m}_0 \bar{m}_g$ for the $S_0 \rightarrow S_3$ transition. When $\chi$ is the magic angle ($54.7^\circ$), all $\chi$-dependent values vanish and $\bar{m}$ can be obtained directly. A detailed description of the fitting procedure can be found in previous papers.17,30 All determined values of $\Delta \bar{\mu}$ and $\text{Tr} \Delta \bar{\alpha}$ are reported in terms of debye and $\text{Å}^3$, respectively, where 1 debye (D) = $3.36 \times 10^{-30}$ coulomb-meter and 1 $\text{Å}^3 = 1.113 \times 10^{-40}$ coulomb-meter$^2$/volt.

**Solvatochromism: Experiment and Analysis.** Methanol (MeOH), ethanol (EtOH), isopropanol (PrOH), butanol (BuOH), acetone (ACET), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), chloroform (CHL), and water (H$_2$O) were spectrophotometric grade and used as received. A 0.40 cm $\times$ 1.0 cm fluorescence quartz cell was used. The maximum optical density of the 6-MI solution was $\sim 0.01$.

Excitation and emission spectra of 2 $\mu$M solutions of 6-MI in each of these solvents were taken using a SPEX Fluoromax-2 fluorimeter (Horiba Jobin Yvon). The excitation spectra were collected with the emission wavelength set to 430 nm, and the emission spectra were collected with excitation at 345 nm unless otherwise noted. All spectra were collected with an integration time of 0.4 s/pont, a 1 nm step size, and 2 nm excitation and emission slit widths. The spectra were solvent-corrected by subtracting excitation and emission spectra of the corresponding solvent. All spectra were corrected for any wavelength bias of the fluorimeter.

The Ooshika–Lippert–Mataga (OLM) equation defines the correlation between the solvent-dependent absorption and emission frequencies at their maximum intensities and the solvent properties as shown in eqs 4 and 5

$$\bar{\nu}_{\text{abs}} - \bar{\nu}_{\text{em}} = \frac{2 f_{\text{OLM}}(\varepsilon, n)}{h \bar{c}^3} \left( \bar{\mu}_e - \bar{\mu}_g \right)^2 + C$$

$$= \frac{2 f_{\text{OLM}}(\varepsilon, n)}{h \bar{c}^3} \left| \Delta \bar{\mu} \right|^2 + C = \Delta \bar{\mu \text{OLM}}(\varepsilon, n) + C$$

(4)

where $\bar{\nu}_{\text{abs}}$ and $\bar{\nu}_{\text{em}}$ are the frequencies of the peak absorbance and fluorescence, respectively, $h$ is Planck’s constant, $c$ is the velocity of light in vacuum, $C$ is the energy difference at $f(\varepsilon, n) = 0$, and $a$ is the radius of the solvated fluorophore including the first solvation sphere. In the OLM treatment, the dielectric properties of the solvent are found in the $f(\varepsilon, n)$ term

$$f_{\text{OLM}}(\varepsilon, n) = f(\varepsilon) - f(n) = \frac{\varepsilon - 1}{2 \varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}$$

(5)

where $n$ is the solvent refractive index and $\varepsilon$ is the dielectric constant. This treatment ignores the polarizability of the fluorophore and assumes that the ground and excited state dipole moments point in the same direction. A complete description of the terms in eqs 4 and 5 can be found in a previous reference.15

A more general treatment was formulated by Bilot and Kawski40 based on Bakhshiev’s analysis41 (BKB model). They assumed that the polarizability of the fluorophore is the same as that of the solvent but that $\bar{\mu}_e$ and $\bar{\mu}_g$ are not necessarily parallel. Equation 4 then becomes

$$\bar{\nu}_{\text{abs}} - \bar{\nu}_{\text{em}} = \Delta \bar{\mu}_BKB(\varepsilon, n) + C$$

(6)

where

$$f_{\text{BKB}}(\varepsilon, n) = \frac{\varepsilon - 1}{\varepsilon + 2} - \frac{n^2 - 1}{n^2 + 2}$$

(7)

and

$$\Delta \bar{\mu}_{BKB} = \frac{2}{h \bar{c} a} \left( \left| \bar{\mu}_g \right|^2 + \left| \bar{\mu}_e \right|^2 - 2 \left| \bar{\mu}_g \right| \left| \bar{\mu}_e \right| \cos \theta \right)$$

(8)

where $\theta$ is the angle between $\bar{\mu}_e$ and $\bar{\mu}_g$. In either model, a plot of the Stokes shift ($\bar{\nu}_{\text{em}} - \bar{\nu}_{\text{abs}}$) versus the solvent polarization function $f(\varepsilon, n)$ will then yield the slope of the eq 4 or 6 and the excited-state dipole moment, $\bar{\mu}_e$, can be computed based on an estimate of $\bar{\mu}_g$ from computational methods.

**Quantum Chemical Calculations.** The ground-state geometry of 6,8-dimethylisoxanthopterin (6,8-DMI) was optimized using the TD-DFT package in Gaussian 03 at the B3LYP/6-311+G(d,p) level of theory. The solvent was modeled using the polarizable continuum model (PCM) developed by Mennucci et al.44 The minimum energy structure was further confirmed by calculating vibrational frequencies at the same level of theory. The excited-state energies, transition moments $\bar{m}_0^{\text{exc}}$ and ground- and excited-state permanent dipole moments $\bar{\mu}_0^{\text{exc}}$ were calculated at the TD/B3LYP/6-311+G(d,p) level of theory. Permanent and transition dipole moment vectors using water as the PCM solvent were used in refining the assignment of the Stark parameters in the molecular frame. The $\bar{\mu}_e$ was used to calculate $\Delta \bar{\mu}_0^{\text{exc}} = \bar{\mu}_e - \bar{\mu}_0^{\text{exc}}$. The PCM was used to include the effect of the dielectric response of the solvent (chloroform, methanol, ethanol, and water) on $\Delta \bar{\mu}_0^{\text{exc}}$. The difference electron density was calculated by subtracting the SCF ground-state density from the one-particle Rho-density for the corresponding excited states. Chemcraft (http://www.chemcraftprog.com) was used to generate and visualize the excited-state dipole moment vectors and difference density.

Difference dipole moments, $\Delta \mu_0^{\text{exc}}$, were calculated using the finite-field hexapole method where an external electric field propagating along the $\pm x$, $\pm y$, and $\pm z$ directions in the center of mass coordinates is applied in silico as previously described17 and given by $E(\vec{F}) = E(0) - \sum \Delta \alpha_i \vec{F}_i - \frac{1}{2} \sum \Delta \alpha_{ij} \vec{F}_i \vec{F}_j$. $E$ is the vertical excitation energy and $\Delta \mu_i$ and $\Delta \alpha_{ij}$ are the vector and
Simulated Stark spectra were calculated by summing finite-field spectra computed for each axis (±X, ±Y, ±Z) and subtracting the zero-field spectra to generate field-on minus field-off spectra. The transition energies from the TD-DFT calculations were used to create a single Gaussian spectral line of 5 nm FWHM for each bright transition. However, vibronic structure has been omitted from the simulation so that the finite-field spectra represent only the overall effect of charge redistribution on the spectral shape.

## RESULTS

### Low-Temperature and Room-Temperature Absorption Spectra

The absorption spectra of 800 μM 6-MI at 77 and 298 K in 8 M LiCl are shown in Figure 1a. At room temperature, the maximum absorbance occurs at 29 400 cm⁻¹ (340.1 nm) for the S₀→S₁ transition, and this transition begins to overlap with the S₀→S₂ transition at ~32 500 cm⁻¹. The spectrum is relatively featureless, but its numerical second derivative spectrum (Figure 1b) shows a well-defined positive feature at 32 800 cm⁻¹ and negative features at 27 600 and 29 060 cm⁻¹. The Stark spectrum is dominated by second derivative feature of the absorption spectrum, suggesting that there is a large difference dipole component contributing to charge redistribution. Interestingly, the small portion of the S₀→S₂ transition taken in these scans is relatively featureless. The Stark spectra show a polarization dependence, suggesting that the angle between transition dipole and difference dipole must be smaller than 54°.

The fitting of the Stark spectra was done assuming that there was no contribution below 32 000 cm⁻¹ from the S₀→S₁ transition. A simultaneous fit of the absorption and Stark spectra was performed using zeroth, first, and second derivatives of the absorption spectrum. The fit (Figure 2a) shows a large second derivative contribution and nonzero first and zeroth derivative contributions. These components are shown in Figure 2b. The fitted Cₐ term gives |μ₀₁| = 5.1 ± 0.3 Dfₒ, and the fitted B₂ gives yields TrΔμ₀₁ = 5 ± 1 Å² for the lowest energy optical transition. The angle between transition dipole and difference dipole is ζ = 28 ± 3°. These data, along with the solvatochromic results, are tabulated in Table 1.

Even if the transition dipole moment |m₀₁| is known, there is still an infinite number of Δμ₀₁ that satisfy ζ ≤ Δμ₀₁|m₀₁|. To assign Δμ₀₁ uniquely, TD-DFT was used to compute the ground-state permanent dipole and the transition dipole moments, Δμ₀₁ and m₀₁. These vectors suggest that both ground-state and transition dipoles are in the plane of the molecule, leaving four possibilities for assigning Δμ₀₁ in the molecular frame. However, only one of these remaining vectors is in agreement with the Δμ₀₁ calculated using the finite-field approach as well as Δμᵣᵢ (Figure 3) and shown in Figure 4.

### Stark Spectroscopy Results

The Stark spectra of 6-MI (800 μM) in 8 M LiCl solutions taken at 77 K at two different polarizations of light are shown in Figure 1c. They show a
computations using the B3LYP/6-311+G(d,p) basis shows that $\vec{m}_{01}$ lies in the plane of the molecule, directed along C6–C2 axis, with components $m_x = -1.66$ D, $m_y = 0.322$ D, and $m_z = 0.0086$ D with an oscillator strength $f_{osc} = 0.274$. $\vec{m}_{01}$ makes an angle of $8^\circ$ with the computed $\vec{\mu}_{01}$. Interestingly, Seibert et al. obtained $f_{osc} = 0.153$ for the $S_0\rightarrow \pi\pi^*$ transition using MCSCF, in good agreement with our result, but the transition energy places the transition at 270 nm, clearly too low. They also used TD-DFT with a 6-31+G(d) basis set to obtain $f_{osc} = 0.032$ for the $S_0\rightarrow \pi\pi^*$ transition (348 nm).

The ground-state and excited-state dipole moments directions did not change from vacuum to water, however their magnitudes increased by $\sim 25\%$, consistent with prior observations. Both the ground- and excited-state dipole moments were directed toward C6 (Table 2). The angle between the two vectors was $10.5^\circ$. The difference dipole moment, $\Delta \vec{\mu}_{01}$, is 4.79 D and points from the center of mass to the middle of the C2–N3 bond.

The difference density from these calculations is shown in Figure 5. A significant intramolecular charge separation occurs upon photoexcitation. There is net positive charge on the N1 nitrogen, the nitrogen of the amino group, and the oxygen of the C4 carbonyl group. Net negative charge appears on N5, C6, C7, and N8 atoms. This is consistent with the $\Delta \vec{\mu}_{01}$ direction given above.

$\Delta \vec{\mu}_{01}$ was also calculated using wave functions derived from B3LYP and BHandHLYP functionals as input to the finite-field procedure (Table 3). The components of the $\Delta \vec{\mu}_{01}^{FF}$ dipoles are given in Table 3. $\langle \vec{\mu}_{01}^{FF} \rangle$ values are 3.48 and 3.58 D for B3LYP and BHandHLYP, respectively. This is about 1 D lower compared with the $\Delta \vec{\mu}_{01}$ value calculated from ground-state and excited-state dipoles; however, the direction is same. Overestimation of the excited-state dipole using TD-DFT when charge transfer is significant has been well-documented.

The computed $S_0\rightarrow S_1$ Stark spectra of 6-MI in H$_2$O, EtOH, and CHCl$_3$ were obtained as described above and shown in Figure 3b. The absorption spectra are shown for comparison in Figure 3a. The spectra for H$_2$O (red line) and EtOH are very similar, with the lowest energy transition of 6-MI centered at $\sim 326$ nm dominated by a second-derivative feature. This suggests that the experimental spectra are consistent with a large difference dipole contribution.

Solvatochromism Results. 6-MI emission spectra were taken in various solvents to explore the effect of solvent polarity.

**Table 1. Experimental Difference Moments for 6-MI**

| method | $|\Delta \vec{\mu}_{01}|$ (D) | $\text{Tr}(\Delta \vec{\mu}_{01}^{2})$ (Å$^2$) | $\xi_\Delta$ ($^\circ$) |
|--------|-----------------|-----------------|-----------------|
| Stark  | 3.2(0.2)$^{a}$  | 16(3)           | 28(3)           |
| BKB: HB | 7.2            | NA              | NA              |
| OLM: HB | 11.6           | NA              | NA              |
| BKB: All | 5.4            | NA              | NA              |
| OLM: All | 9.7            | NA              | NA              |

$^{a}f_e = 1.63$

**Table 2. TD-DFT Computed Components of the Permanent Dipole Moments in Water (in Debye)**

<table>
<thead>
<tr>
<th></th>
<th>$\vec{\mu}_x$</th>
<th>$\vec{\mu}_y$</th>
<th>$\vec{\mu}_z$</th>
<th>$\vec{\mu}$</th>
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</thead>
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<tr>
<td>$\vec{\mu}_0$</td>
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<td>$-6.49$</td>
<td>0.051</td>
<td>12.44</td>
</tr>
<tr>
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<td>$-6.14$</td>
<td>0.048</td>
<td>16.57</td>
</tr>
<tr>
<td>$\Delta \vec{\mu}_{01}$</td>
<td>4.78</td>
<td>0.35</td>
<td>0.003</td>
<td>4.79</td>
</tr>
</tbody>
</table>

**Figure 3.** (a) Simulated absorption spectra ($\lambda_{abs}$) in three solvents from the TD-DFT/finite-field approach. Only the $E_{00}$ transition is shown for the $S_0\rightarrow S_1$ band with a width of 5 nm. (b) Stark spectra ($\Delta \lambda_{abs}$) simulated from the finite-field difference approach.
and hydrogen bonding ability on the emission yield and band shape. These data are shown in Figure 6. Fluorescence excitation spectra and emission spectra of 6-MI in the various solvents were peak-normalized (data not shown). The corresponding Stokes shifts in the various solvents are listed in Table 4. The excitation maximum for 6-MI in water was found at 342 nm. A red shift in the excitation maximum was observed as a function of decreasing solvent polarity, although acetone showed a modest deviation from this expected trend. 6-MI fluorescence emission is the most red-shifted at 428 nm in water. The emission maxima blue shift with decreasing solvent polarity as expected. However, in n-butanol, the fluorescence emission red shifts to 425 nm. (See Table 4.) Again, this deviation is modest.

In the case of non-H-bonding solvents, no clear trend is observable as a function of changing solvent polarity. 6-MI in CHCl₃, DMSO, and THF exhibit the most blue-shifted excitation maxima at 346–350 nm, whereas the most red-shifted maxima were observed at 368 and 366 nm for DMF and ACN respectively (data not shown). Chloroform exhibits the most blue-shifted emission spectra with the λ_max at 412 nm, whereas that in DMF and ACN exhibited the largest red shifts with λ_max at 436 nm (data not shown). DMF and acetonitrile (as well as dioxane, studied here but not shown) are considered to be anomalous solvents with regard to their solvatochromism, and we omit them from further consideration. However, a positive slope was obtained when plotting the solvent polarity function against the Stokes shifts using either the OLM or the BKB solvatochromism models, as shown in Figure 7.

The magnitudes of the difference dipole moment, |Δμ₀₁|, based on these solvatochromism models are shown in Table 1. The fit of the Stokes shifts against the solvent polarity function for 6-MI was done in two ways. In one case, only the H-bonding solvents were included. A second treatment included all solvents. |Δμ₀₁| values from both the OLM and BKB analysis are strongly dependent on the radius of the solvated molecule. The computed radius of the solvated 6-MI nucleoside molecule is 5 Å (using DFT in Gaussian 03). If we assume that μ₉ and μₑ are in the same direction (OLM model), then |Δμ₀₁| = 9.4 D. For the BKB model, |Δμ₀₁| = 5.4 D is found for all solvents. However, it is not necessary for μ₉ and μₑ to be in the same direction. The angle between μ₉ and μₑ can be calculated directly from the magnitudes of μ₉ = 12.44 D and μₑ = 16.57 D (computed using TDDFT, see Table 2) using equation 8, which returns an angle of 13.7°. This is in very good agreement with the angle from the vectorial analysis of our theoretical results and the work by Seibert et al. for only the H-bonding solvents from the OLM and BKB models.
were 11.6 and 7.2 D, respectively. The $|\Delta \mu_{01}|$ values from the analysis of all solvents and H-bonding solvents are over-estimates compared with those measured by Stark spectroscopy and the computational results that we have reported in this work. The BKB model appears to be higher by a factor of 1.7 for all solvents and 2.3 for H-bonding solvents, whereas the OLM model is higher by a factor of 3.0 (all) and 3.6 (HB) for all solvents and 2.3 for H-bonding solvents, whereas the computational results that we have reported in this work show that the OLM approach can be attributed to the lower values of the dielectric function ($f(\varepsilon, n)$) in the OLM equation.

**DISCUSSION**

In this study, the lowest energy optically allowed transition of the fluorescent guanine analog 6-MI has been characterized by UV/vis absorption Stark spectroscopy and solvatofluorochromism. The results were interpreted with the aid of TD-DFT calculations that returned permanent and transition dipole moments for the $S_n$ states ($n = 0,1$). The magnitude and direction of the difference dipole $\Delta \mu_{01}$ have been determined. In addition, finite-field calculations were performed to simulate the Stark spectra for $S_0 \rightarrow S_1$.

Beyond its initial characterization by Hawkins et al., a small number of groups have explored the excited-state properties of 6-MI monomer. Seibert et al. have measured the pH dependence of the fluorescence emission of the analog, showing that the N3 proton is labile. These studies also gave absorption and emission maxima in aqueous solution at pH 5. Poulin et al. explored the quenching of excited state 6-MI monomer by nucleobase monophosphates and found results roughly similar to our own Stern–Volmer study.

**Charge Redistribution in 6-MI Compared with Guanine Monophosphate.** Luchowski and Krawczyk have measured the Stark spectra of purine and pyrimidine native nucleic acid bases in ethylene glycol–water glasses at 100 K. For 6 mM 2+ for GMP, they obtained $\Delta \mu_{01} = 2.7(0.2)$ D if $\Delta \mu_{01}$, which gives 1.7 D when $f_c = 1.63$, compared to 3.2 D for 6-MI. This difference can be ascribed to the polarizing effect of the $C7=O$ group of 6-MI. $\zeta_A = 26 \pm 2^\circ$, is in good agreement with the value for 6-MI. The difference polarizability, $\Delta \mu_{01} = 15 \pm 2 \ Å^4$, is identical to that for 6-MI within experimental error. The difference in the transition dipoles of GMP and 6-MI, 7.1 and 12.4 D respectively, most likely is due to the above-mentioned polarizing effect.

One interesting difference is the relative directions of the transition dipole moment. In GMP, the dipole is along the short axis of the molecule, whereas in 6-MI it is closer to the long axis. In guanine, this charge redistribution would have little impact on hydrogen bonding between it and its Watson–Crick complement cytosine, partially due to the significantly lower $\Delta \mu_{01}$ but, more importantly, due to the fact the direction of charge displacement is essentially orthogonal to the H-bonding triad. In 6-MI, as shown in Figure S, the C4=O and C2-NH$_2$ groups of the triad show significantly lower electron density in the $S_1$ state. The C2-NH$_2$ group supplies 2 H-bond donor sites and the C4=O two H-bond acceptor sites (the lone pairs). A decrease in electron density at these sites will weaken the H-bond acceptor capacity at C4=O and strengthen the H-bond donor capacity at C2-NH$_2$. The unchanged HB-donor capacity at N3-H makes the excited state an overall better H-bond donor, suggesting that H-bonding in 6MI*:C is stronger than that in the ground state of 6MI-C or compared with the native G*:C. We have seen similar behavior in 2AD and suggest that perhaps a transient change of hydrogen bonding may occur in these FBAs while the molecule is optically excited.

A further consequence of the large vectorial change in the excited-state electronic structure of 6-MI* is that the charge distribution in this state is the starting (Franck–Condon) point for the evolution of the excited-state potential. How the system evolves from this point will, in part, determine the electronic relaxation mechanism. Indeed, when incorporated into single-stranded or duplex DNA, this Franck–Condon initial excited-state charge distribution will see a very different potential energy landscape due to electronic and structural influences induced by neighboring bases, as suggested by Matsika and others.

The large change in the difference dipole moment is a clear indication that the electron density of the excited state is quite different from the ground state. Because the molecule is probed at its ground-state equilibrium nuclear configuration, the dipole moment change will correlate with higher bond orders for some parts of the 6-MI framework and lower bond orders for other parts of the molecule. As mentioned in the Introduction, this change in excited-state bond energies can result in changes in excited-state pK$_a$, redox potentials, and hydrogen bond energies. The relative change, in both magnitude and direction, in the difference dipole moment is a valuable indicator of what mechanistic pathways can be justified on the basis of electronic structure. Indeed, we and others have used this same argument to rationalize the electron-transfer pathways in DNA photocleavage.

**Comparison with Other QM Calculations.** In an extensive computational study, Seibert, Ross, and Osman explored the structure of the electronic ground and excited states of 6-MI. These calculations were performed using a variety of approaches, including second-order MP2 calculations to obtain an optimized ground-state geometry and TD-DFT (6-31G(d) and 6-31+G(d) bases) as well as CASSCF (6-31G(d) basis) calculations to characterize the excited states. Solvation was handled by the PCM.

Our B3LYP/6-311+G(d,p) TD-DFT optimized ground-state geometry is in excellent agreement with MP2-optimized ground-state geometry of Seibert et al. The largest structural deviation is the N1–C9 bond length, which is larger by +0.05 Å in the MP2 structure. The largest negative deviation is the –0.01 Å for the N3–H bond. A comparison of the various dipole moments of 6-MI is not as straightforward. The transition dipole moments were calculated in water using the PCM. Using a 6-31G(d) basis for TD-DFT calculations with the B3LYP functional produced a low oscillator strength ($f_{osc} = 0.03$) at 343 nm, close to the observed wavelength. We obtained an oscillator strength of $f_{osc} = 0.27$ centered at 326.1 nm, well blue-shifted from the observed.

The experimental values of $\Delta \mu_{01} = 3.2 \pm 0.2$ D with $\zeta_A = 28 \pm 3^\circ$ provide a basis of comparison with the quantum mechanical calculations. Seibert et al. found $\Delta \mu_{01} = 2.86$ D with $\zeta_A = 6.9^\circ$ (using CASSCF) compared with our value of $\Delta \mu_{01} = 4.79$ D with $\zeta_A = 10.5^\circ$ (using TD-DFT). The finite-field approach gave $\Delta \mu_{01} = 3.5$ D (B3LYP and BandHLYP functionals), significantly lower than the TD-DFT approach but higher than the CASSCF calculation.

Beyond issues of specific basis sets and quantum mechanical procedures, all computational approaches applied to understanding
the electronic structure of 6-MI fail to include specific solvent interactions such as hydrogen bonding. This is also the case in the solvatochromism results where the correlation of the solvent polarity with the Stokes shift is good but not robust. Therefore, in our view, the Stark experimental results give the most accurate picture of charge redistribution in the excited state. QM calculations support these results and provide a rational basis for assigning the direction of charge transfer in the molecular frame. Even here the experimentally determined $\zeta_A$ differs from the QM results by more than 15°.

## CONCLUSIONS

The degree and direction of electronic charge redistribution in 6-MI in a low-temperature glass has been measured by absorption Stark spectroscopy. Charge redistribution in the lowest optically bright state is dominated by dipole moment changes. The difference dipole moment of the guanine analog is about twice that of GMP, whereas the difference polarizability and direction of charge displacement relative to the transition dipole moment are about the same. High-level TD-DFT and finite-field difference calculations were used to refine the experimental observations. The resulting picture of the excited state suggests that significant charge displacement occurs along the long axis of the molecule, with possible modulation of hydrogen bonding during excitation. Two solvatochromism models were also used to obtain the difference dipole moment of 6-MI and were compared with the Stark spectroscopy result.

## ASSOCIATED CONTENT

*Supporting Information*

Optimized ground state geometry of 6-MI. This material is available free of charge via the Internet at http://pubs.acs.org.

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