

## Ultrafast Excited-State Dynamics of Adenine and Monomethylated Adenines in Solution: Implications for the Nonradiative Decay Mechanism

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**Abstract:** The DNA base adenine and four monomethylated adenines were studied in solution at room temperature by femtosecond pump–probe spectroscopy. Transient absorption at visible probe wavelengths was used to directly observe relaxation of the lowest excited singlet state ( $S_1$  state) populated by a UV pump pulse. In  $H_2O$ , transient absorption signals from adenine decay biexponentially with lifetimes of  $0.18 \pm 0.03$  ps and  $8.8 \pm 1.2$  ps. In contrast, signals from monomethylated adenines decay monoexponentially. The  $S_1$  lifetimes of 1-, 3-, and 9-methyladenine are similar to one another and are all below 300 fs, while 7-methyladenine has a significantly longer lifetime ( $\tau = 4.23 \pm 0.13$  ps). On this basis, the biexponential signal of adenine is assigned to an equilibrium mixture of the 7H- and 9H-amino tautomers. Excited-state absorption (ESA) by 9-methyladenine is 50% stronger than by 7-methyladenine. Assuming that ESA by the corresponding tautomers of adenine is unchanged, we estimate the population of 7H-adenine in  $H_2O$  at room temperature to be  $22 \pm 4\%$  (estimated standard deviation). To understand how the environment affects nonradiative decay, we performed the first solvent-dependent study of nucleobase dynamics on the ultrafast time scale. In acetonitrile, both lowest energy tautomers of adenine are present in roughly similar proportions as in water. The lifetimes of the 9-substituted adenines depend somewhat more sensitively on the solvent than those of the 7-substituted adenines. Transient signals for adenine in  $H_2O$  and  $D_2O$  are identical. These solvent effects strongly suggest that excited-state tautomerization is not an important nonradiative decay pathway. Instead, the data are most consistent with electronic energy relaxation due to state crossings between the optically prepared  $^1\pi\pi^*$  state and one or more  $^1n\pi^*$  states and the electronic ground state. The pattern of lifetimes measured for the monomethylated adenines suggests a special role for the  $^1n\pi^*$  state associated with the N7 electron lone pair.

### Introduction

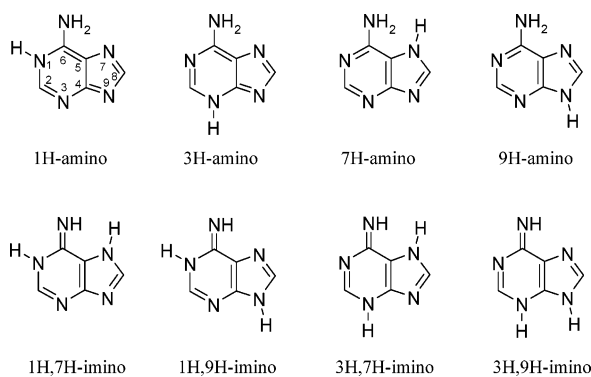
Femtosecond transient absorption<sup>1,2</sup> and fluorescence upconversion<sup>3–6</sup> measurements have recently established that the DNA and RNA bases have subpicosecond fluorescence lifetimes in aqueous solution at room temperature. Ultrafast relaxation of electronic energy provides the building blocks of life with a high degree of photostability and reduces the likelihood of photochemical damage. In particular, relaxation to the electronic ground state on the femtosecond time scale greatly reduces the yield of reactive triplet molecules. Considerable effort is currently devoted to understanding nonradiative decay by the nucleobases,<sup>7–12</sup> but consensus on the mechanism has yet to

be achieved. Only a small number of nucleobases have been studied on the ultrafast time scale, and additional experiments are sorely needed to evaluate proposed mechanisms.

Here we present a systematic study of adenine and adenine derivatives in solution at room temperature. The femtosecond transient absorption technique was used to study adenine and all four monomethylated adenines in which a methyl group is attached to a purine ring nitrogen (see Chart 1). Because the methyl group is not labile, each of these compounds provides insight into the photophysics of an adenine tautomer with hydrogen at the same position. There is longstanding interest in the photophysics of different adenine tautomers since it has been argued that most of the fluorescence is emitted from a minor tautomer.<sup>13</sup> As part of our findings, we report the first lifetime measurements for the 7H- and 9H-amino tautomers of adenine. Even though 7,9 tautomerism does not occur in DNA, adenine is an important model system for understanding

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**Chart 1.** Amino and Imino Tautomers of Adenine

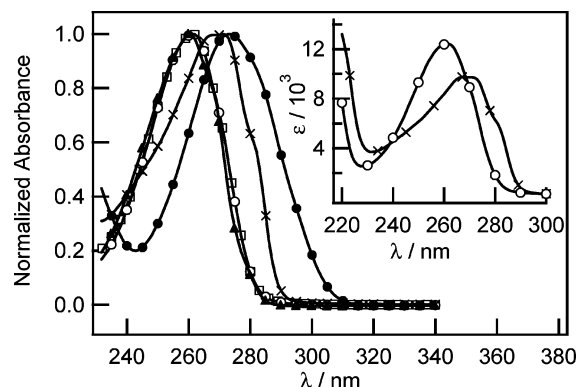
tautomerism. Rare tautomers may be responsible for substitution mutagenesis.<sup>14</sup> Additionally, slower rates of electronic energy relaxation by rare tautomers could make them more susceptible to photochemical damage. We previously pointed out the approximate correlation between excited-state lifetimes and the propensity for nucleobase photochemistry.<sup>2</sup>

An outstanding issue in nucleobase photophysics is the role played by the solvent. This is particularly important given a recent report of ultrafast lifetimes for isolated bases.<sup>15</sup> We therefore undertook the first study on the influence of the solvent on nucleobase excited-state dynamics. Specifically, we characterized  $S_1$  dynamics in  $H_2O$ ,  $D_2O$ , and in the aprotic solvent, acetonitrile. Thus, the twin aims of this report are to comment on intramolecular (tautomerism) and intermolecular (solvent effects) factors that influence nonradiative decay in adenine. The questions to be addressed include: (1) What are the consequences of tautomerism for the excited-state lifetimes? (2) What is the ground-state distribution of tautomers at room temperature? (3) Is interconversion from one tautomeric form to another on the excited-state potential energy surface the mechanism behind nonradiative decay? (4) What do the observed solvent effects imply about the mechanism of excited-state relaxation?

## Experimental Section

Transient absorption signals were recorded by the pump–probe technique using our amplified titanium sapphire laser system, as described previously.<sup>2</sup> Briefly, pump pulses with a center wavelength of  $\sim 263$  nm were obtained from the third harmonic laser output. Probe pulses were derived from a white light continuum generated in a 1-cm path length cell filled with water. Modifications to the stretcher and compressor of our chirped pulse amplifier reduced the pulse duration of the fundamental output to  $\sim 90$  fs, as measured by second-order autocorrelation in a KDP crystal. The difference-frequency mixing signal between the third harmonic pump pulse and the fundamental in the probe arm measured at the sample position had a fwhm of 270 fs. From this measurement, the fwhm of the instrument response function is  $\sim 190$  fs, assuming Gaussian pulse shapes. The induced absorbance change ( $\Delta A$ , equivalent to  $\Delta OD$ ) was recorded versus time delay between pump and probe pulses.  $\Delta A$  was calculated as  $-\log(I/I_0)$ , where  $I$  is the probe signal measured by a photodiode after the sample in the presence of the pump pulse, and  $I_0$  is the same signal with the pump blocked.

Aqueous solutions of the various adenines were studied primarily in pH 6.8 buffer. The buffer was prepared by dissolving 1.77 g of  $Na_2-$



**Figure 1.** Normalized steady-state absorption spectra at pH 6.8 for adenine ( $\blacktriangle$ ), 1-methyladenine ( $\square$ ), 3-methyladenine ( $\bullet$ ), 7-methyladenine ( $\times$ ), and 9-methyladenine ( $\circ$ ). The inset shows the extinction coefficients (units of  $M^{-1} \text{ cm}^{-1}$ ) of 7-methyladenine ( $\times$ ) and 9-methyladenine ( $\circ$ ) at pH 6.8 as a function of wavelength.

$HPO_4$  and 1.70 g of  $KH_2PO_4$  in 500 mL of ultrapurified water. 7-Methyladenine (7MA) was obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. (The standard ring numbering of adenine is shown in Chart 1.) 1-Methyladenine (1MA) and 9-methyladenine (9MA) were purchased from Acros Organics, while adenine and 3-methyladenine (3MA) were from Sigma-Aldrich. All compounds were used as received. UV/vis spectra were recorded frequently during experiments to assess photodegradation, and solutions were changed as necessary.

Solute concentrations were generally adjusted to give an absorbance between 1 and 2 for a 1-mm optical path length at our pump wavelength of 263 nm. Adenine and the monomethylated derivatives studied here are, however, significantly less soluble in acetonitrile than in water, and this led to solutions of lower absorbance. Acetonitrile solutions were prepared by adding excess solute to a quantity of solvent, stirring overnight, and finally filtering out the undissolved solid. The optical densities of these saturated solutions (measured at the pump wavelength of 263 nm for a 1-mm optical path length) were 0.24, 0.18, and 0.49 for adenine, 7MA, and 9MA, respectively. Saturated solutions of 1MA and 3MA in acetonitrile had optical densities  $< 0.1$  and were too dilute to permit pump–probe experiments.

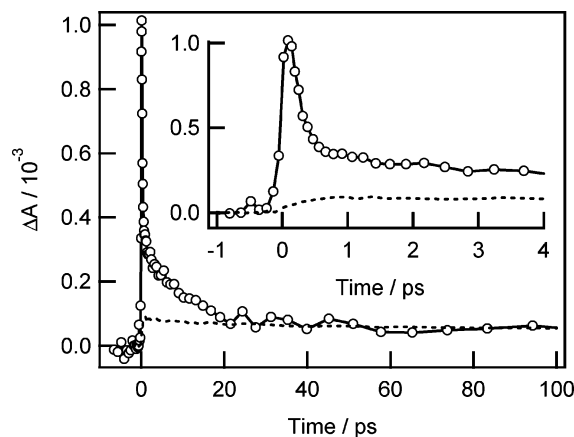
**Data Analysis.** Signals were modeled as sums of exponentials analytically convoluted with a Gaussian that represents the instrument response function of our ultrafast spectrometer. Transients at several probe wavelengths were fit simultaneously with the time constants of the exponentials as global fitting parameters, as described previously.<sup>2</sup> The fwhm of the Gaussian function was an adjustable fitting parameter. This accounts for modest changes in time resolution that occur over periods of weeks and months due to changes in laser operation. Although the fwhm was adjustable, it changed little about its average value. In no case did optimization of this parameter produce a value that was less than 190 fs, the estimate of our best time resolution from the difference frequency-mixing signal discussed above. Parameter uncertainties were obtained from the formal covariance matrix after  $\chi^2$  minimization and are twice the standard deviation ( $2\sigma$ ), unless stated otherwise.

## Results

**Adenine in  $H_2O$ .** Steady-state absorption spectra in  $H_2O$  are shown in Figure 1 for adenine and the derivatives used in this study. All compounds show strong  $\pi\pi^*$  absorption at the pump wavelength of 263 nm. The spectra are similar except for 3MA and 7MA, which exhibit significant red-shifts. Transient absorption at 570 nm for an aqueous solution of adenine is shown in Figure 2. The signals exhibit complex decays, which are identical at long delay times with signals recorded on a solvent

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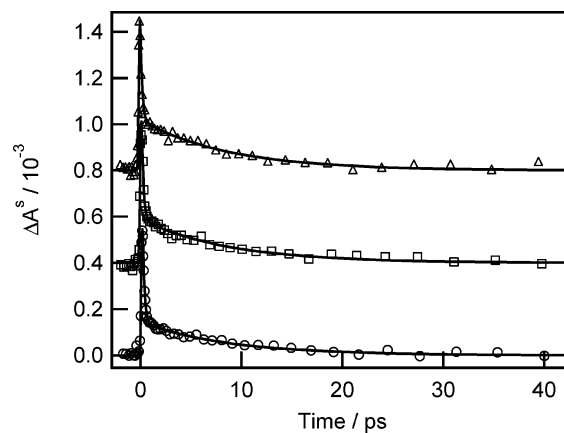


**Figure 2.** Transient absorption at 570 nm by adenine in aqueous solution (circles connected by a solid line to guide the eye). The dashed curve is the transient absorption signal at the same probe wavelength from a blank containing just water and the pH buffer. It has been scaled to agree with the signal from adenine at long times. The figure inset shows the data at short times. The pump wavelength for this and all subsequent figures is 263 nm.

blank containing just the pH buffer (dashed curve in Figure 2). These long-time signals seen in our transient absorption experiments arise from absorption by solvated electrons produced by the two-photon ionization of water.<sup>2</sup> The very small cross sections for excited-state absorption (ESA) by the nucleobases make it impossible to eliminate this signal, even at very low pump powers. As reported previously,<sup>2</sup> ESA by the purine bases is stronger than by the pyrimidine bases. As a result, the signal contribution from water is smaller for adenine than for cytosine (compare Figure 2 with Figure 1 in ref 16). For the adenines studied here, the water signal typically accounts for <20% of the maximum signal strength. On the time interval from ~1–100 ps, the water signal is nearly constant due to the slow time scale for geminate recombination by solvated electrons.<sup>17</sup>

This signal component can be removed by subtracting the signal measured on a solvent blank, after it has been scaled to agree with the nucleobase signal at long times. We verified that the long-time signal varies strictly quadratically with pump power, consistent with two-photon ionization. In this study, fits to the data used to determine lifetimes were always made to solvent-subtracted signals. This procedure results in somewhat more accurate time constants, especially when  $\tau$  is comparable to the rise time of the solvated electron signal (~300 fs; see Figure 2 inset). It also makes it easier to discern low-amplitude decays that occur on slower time scales, as seen in the solvent-subtracted data in Figure 3. In this and later figures, solvent-subtracted data is indicated by  $\Delta A^s$  on the vertical axis. The transients in Figure 3 show relaxation at three probe wavelengths across the ESA band of adenine. The maximum signal was observed near 600 nm in good agreement with the transient spectrum previously reported for cytidine.<sup>2</sup> Global fitting of the three transients in Figure 3 to a model function of two exponential decays gave time constants of  $0.18 \pm 0.03$  and  $8.8 \pm 1.2$  ps. Fit parameters are summarized in Table 1.

**Monomethylated Adenines in H<sub>2</sub>O.** Like adenine, the monomethylated adenines all exhibit a weak and broad transient

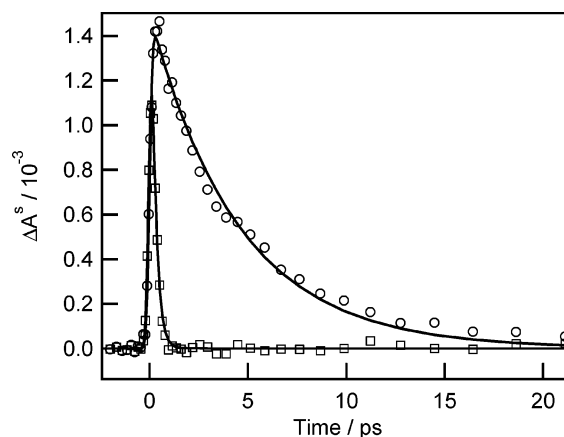


**Figure 3.** Transient absorption of adenine in pH 6.8 buffered aqueous solution at 570 nm (○), 600 nm (□), and 650 nm (Δ). Signals have been vertically offset for clarity. Solid lines are from a global fit to a biexponential decay. The solvent-only signal has been subtracted as described in the text.

**Table 1.** Adenine Global Fit Parameters<sup>a</sup>

	$\tau_1/\text{ps}$	$\tau_2/\text{ps}$	$A_1$	$A_2$
adenine in H <sub>2</sub> O	$0.18 \pm 0.03$	$8.8 \pm 1.2$	0.86	0.14
adenine in CH <sub>3</sub> CN	$0.44 \pm 0.07$	$11 \pm 5$	0.90	0.10

<sup>a</sup> Transients were fit using the function  $\Delta A = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$ . Time constants,  $\tau_1$  and  $\tau_2$ , were global-fitting parameters, but the amplitudes,  $A_i$ , were not. The  $A_i$  values in the table are for a probe wavelength of 570 nm, and they have been scaled such that  $\sum |A_i| = 1$ .



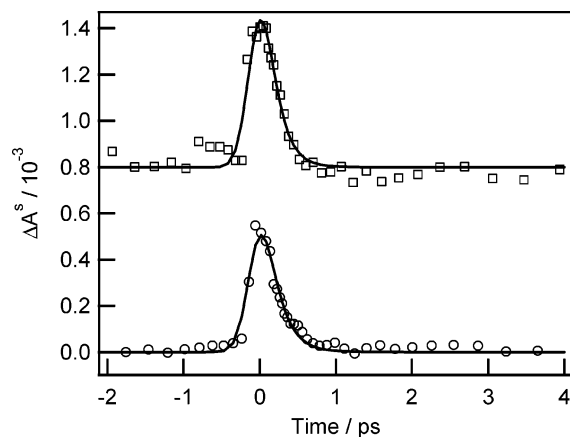
**Figure 4.** Transient absorption at 570 nm of optically matched aqueous solutions of 9-methyladenine (□) and 7-methyladenine (○) collected under identical experimental conditions. Solid lines are from a nonlinear least-squares fit to a monoexponential decay. The solvent-only signal has been subtracted as described in the text.

absorption band centered near 600 nm. Transient absorption signals for 7MA and 9MA at a probe wavelength of 570 nm were recorded in back-to-back experiments at pH 6.8 (Figure 4). For the results in Figure 4, the 7MA and 9MA solutions had concentrations of  $1.71 \times 10^{-3}$  M and  $1.53 \times 10^{-3}$  M, respectively, resulting in identical absorbance (i.e., optical density) of 1.6 at the pump wavelength of 263 nm. At constant-pump laser intensity, equal absorbance solutions have identical excited-state populations initially, allowing relative ESA cross sections to be measured (see the Discussion section). We note that the magnitude of the long-time signal (prior to subtraction of the solvent-only signal) was the same for both, as expected for equal absorbance (“optically matched”) solutions.

Signals from 1MA and 3MA are shown in Figure 5. The pH for 1MA was adjusted to pH 9, while the pH of the 3MA

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**Figure 5.** Transient absorption of 1-methyladenine at pH 6.8 (○), and 3-methyladenine at pH 8.2 (□) at 570 nm. Solid lines are from a nonlinear least-squares fit to a monoexponential function. The signal from water has been subtracted as described in the text.

**Table 2.**  $S_1$  Lifetimes (ps) of Monomethylated Adenines at Room Temperature

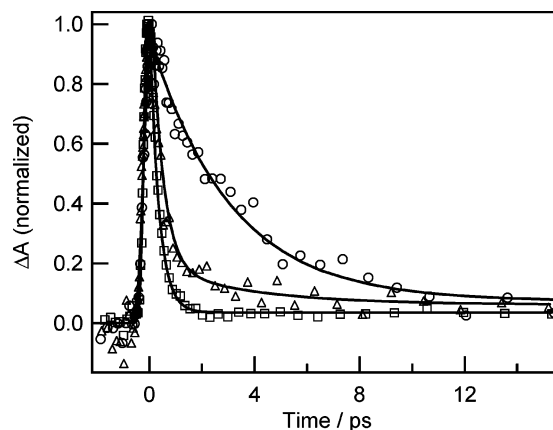
solvent	1MA (570 <sup>a</sup> )	3MA (570)	7MA (570, 600, 630)	9MA (570, 600, 630)
H <sub>2</sub> O <sup>b</sup>	0.26 ± 0.03	0.18 ± 0.06	4.23 ± 0.13	0.22 ± 0.02
CH <sub>3</sub> CN	<sup>c</sup>	<sup>c</sup>	3.3 ± 0.3	0.35 ± 0.02

<sup>a</sup> Probe wavelengths (nm) of transients used in the fits are shown in parentheses after each compound. Multiple wavelengths indicate a global fit was performed. <sup>b</sup> The pH was 9.0 for 1MA, 8.2 for 3MA, and 6.8 for the remaining compounds. <sup>c</sup> Insufficient solubility.

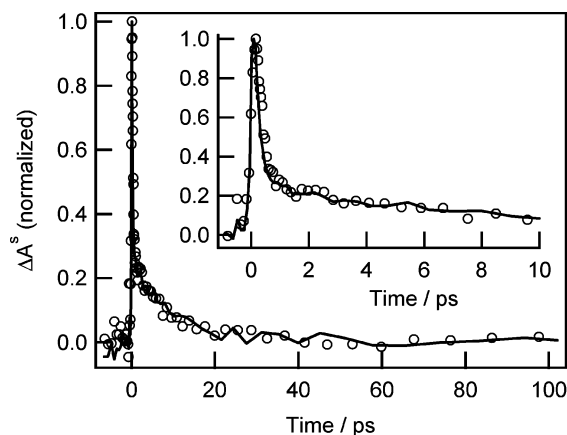
solution was adjusted to 8.2. These higher pH values were used to ensure that both compounds were present in their neutral, unprotonated forms on account of their higher basic  $pK_a$ 's of  $\sim 7$  for 1MA and  $\sim 6$  for 3MA.<sup>18</sup> Because of the very weak signals from these compounds, we recorded only a single transient at 570 nm near the maximum of the ESA band. The lifetimes determined from fits at this wavelength are shown in Table 2 for 1MA and 3MA. The decay times are equal within experimental uncertainty to each other and to the decay time of 9MA. The decay time for 7MA is significantly longer ( $\tau = 4.23 \pm 0.13$  ps).

**Solvent Effects.** Pump–probe experiments were carried out on adenine, 7MA, and 9MA in acetonitrile. 1MA and 3MA could not be studied due to their low solubility in this solvent, as mentioned earlier. The transient absorption at 570 nm from a saturated solution of adenine in acetonitrile (triangles, Figure 6) decays biexponentially as observed for water. Monoexponential decays are seen in Figure 6 for 7MA (circles) and 9MA (squares). Raw scans are shown in Figure 6 since there is no significant solvated electron population due to negligible two-photon ionization of acetonitrile. Instead, only a weak residual offset was observed in this solvent. As in the case of water, the 9MA dynamics are much faster. Decay times were determined by global fitting to transients recorded at 570, 600, and 630 nm. Table 1 lists values for adenine, while Table 2 contains lifetimes for 7MA and 9MA in acetonitrile. The trends are the same as in water, but the lifetimes are somewhat longer, particularly for 9MA and the fast component seen for adenine.

Finally, experiments were carried out on adenine in unbuffered D<sub>2</sub>O. The absence of a pH buffer should be of no



**Figure 6.** Normalized transient absorption signals for adenine (Δ), 7-methyladenine (○), and 9-methyladenine (□) in acetonitrile at 570 nm. Global, nonlinear least-squares fits are shown by the solid curves.



**Figure 7.** Normalized transient absorption signals of adenine in H<sub>2</sub>O (solid line) and D<sub>2</sub>O (○) pumped at 263 nm and probed at 570 nm. The inset shows the same data at short times.

consequence due to the low  $pK_a$  of the adenine cation. We confirmed in a separate experiment that transients for adenine in unbuffered H<sub>2</sub>O solution agree completely with transients obtained in the presence of the phosphate buffer. The transient signal at 570 nm in D<sub>2</sub>O is compared with the signal observed in H<sub>2</sub>O in Figure 7.

## Discussion

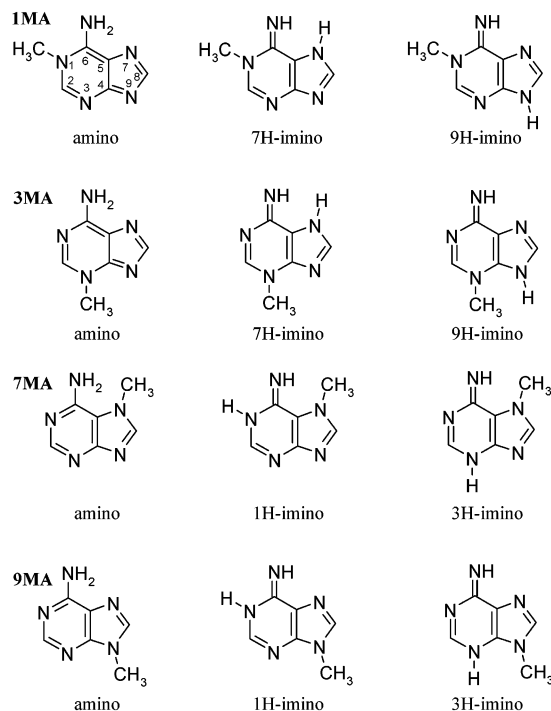
**Adenine Tautomerism.** Adenine has a total of eight structural isomers: four amino isomers in which hydrogen is attached to any one of the four ring nitrogens and four imino ones. These are shown in Chart 1 together with their abbreviations. Each of the four imino tautomers has an *E* and a *Z* stereoisomer, depending whether the hydrogen at the end of the imino C=N bond is closer to N7 or N1, respectively. There are thus 12 energetically distinguishable isomers of adenine. Following literature precedent, we will refer to these 12 isomers as *tautomers*, although tautomers are, *strictly speaking*, defined to be readily interconvertible isomers.<sup>19</sup> As will be discussed below, the energies of many of the possible isomers are sufficiently high that they do not occur to any significant extent. The tautomer distribution of adenine in aqueous solution at room temperature has been studied extensively. There is broad

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**Chart 2.** Amino and Imino Tautomers of *n*-methyladenine (*n* = 1, 3, 7, 9)



consensus from theoretical<sup>20–22</sup> and experimental<sup>23–26</sup> investigations that two tautomers coexist at significant concentrations in water: the 7H-amino and 9H-amino forms.

**Tautomerism in Monomethylated Adenines.** Methylation at a ring nitrogen reduces the number of tautomers to five: One amino and two imino forms, each with *E* and *Z* conformers. All possible structures are shown in Chart 2. Tautomerism at the 7,9 positions of the imidazole ring is blocked for 7MA and 9MA, but can still take place for the imino forms of 1MA and 3MA. As tautomerism has not been studied as extensively for these compounds as for adenine, we review the experimental and theoretical evidence for the lowest-energy tautomers in some detail.

**1MA.** IR spectra of 1MA in solid Ar indicate that the 9H-imino form is the predominant tautomer under matrix isolation conditions.<sup>27</sup> Only very small amounts of the 7H-imino and amino tautomers could be detected in a later matrix isolation study.<sup>28</sup> Ab initio calculations gave the energetic ordering 9H-imino < 7H-imino < amino.<sup>27,28</sup> The *E* conformer of 9H-imino 1MA is lower in energy by 32 kJ mol<sup>−1</sup> than the *Z* conformer. The latter structure is destabilized by steric repulsion between the imino hydrogen and the methyl group. On the other hand, the very similar energies of the *E* and *Z* conformers of 1H,7H-

imino adenine reported by Ha et al.<sup>29</sup> suggest that the two 7H-imino conformers of 1MA should have similar energies. A semiempirical study of 1MA tautomerism supports this conclusion.<sup>30</sup>

The energetic ordering of the 1MA tautomers changes as the polarity of the environment increases. A UV and IR study by Dreyfus et al. showed that the amount of the amino form increases with increasing solvent polarity.<sup>31</sup> Thus, the amino form of 1-heptyladenine increases from ~20% in chloroform to ~50% in methylene chloride.<sup>31</sup> In aqueous solution, the authors estimate that the amino form predominates with only about 1% imino form present. Ab initio calculations support the switch from a minimum energy imino to an amino form. Both the magnitude of the dipole moments ( $\mu = 3.81$  D for the (*E*)-9H-imino tautomer of 1 MA vs  $\mu = 9.59$  D for 1MA-amino)<sup>28</sup> and the comparatively small energy splitting between the amino and the minimum energy imino form in vacuo ( $\Delta E = 21.1$  kJ mol<sup>−1</sup>)<sup>28</sup> are consistent with a lower-energy amino form in aqueous solution.

**3MA.** Sharma and Lee recently studied all five tautomers of 3MA at the B3LYP/6-31+G\* level of theory.<sup>32</sup> For the isolated compounds, they found that energies increase in the order amino < (*Z*)-7H-imino < (*E*)-7H-imino < (*E*)-9H-imino  $\approx$  (*Z*)-9H-imino. The energy difference between the amino form and the next most-stable tautomer of 3MA, the (*Z*)-7H-imino form, is 43.5 kJ mol<sup>−1</sup>. The *E* conformer of the 7H-imino tautomer lies 28.0 kJ mol<sup>−1</sup> above the *Z* conformer. The *E* conformer is significantly higher in energy due to steric repulsion between the imino and the N7 hydrogens. Similar results were reported in an earlier ab initio study.<sup>33</sup> It is interesting to compare the 3MA tautomers with the five analogous tautomers of adenine, which have a hydrogen atom at N3 in place of the methyl group in 3MA. From the results of Ha et al.,<sup>29</sup> this subset of adenine tautomers has very similar relative energies as the corresponding 3MA tautomers. This indicates that replacement of N–H by N–CH<sub>3</sub> has a minor effect on the ground-state energies.

Experimental measurements confirm that the amino form of 3MA has the lowest energy in vacuo and in a nonpolar environment. Thus, the measured gas-phase acidity of 3MA supports the conclusion that the amino tautomer has the lowest energy in the gas phase.<sup>32</sup> FT-IR spectroscopy revealed the overwhelming presence of amino 3MA in an argon matrix, with only a trace amount (~0.5%) of the 7H-imino tautomer.<sup>33</sup>

There are several experimental studies on 3MA tautomerism in solution. Bergmann et al. measured the dipole moment of 3MA to be  $4.98 \pm 0.10$  D in dioxane.<sup>34</sup> Since this value was high compared with a calculated value for the 3H-amino tautomer of adenine ( $\mu = 4.2$  D<sup>35</sup>), they speculated that some amount of the 9H-imino form of 3MA is present due to its significantly higher dipole moment. However, more recent calculations have shown that the 9H-imino tautomer lies much higher in energy above the amino form of 3MA than was found

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to be the case in 1969 by Pullman et al.<sup>35</sup> This makes it highly unlikely that the 9H-imino tautomer could be present in any significant amount. Additionally, a recent estimate of the dipole moment of the amino tautomer of 3MA ( $\mu = 4.74$  D<sup>33</sup>) shows that there is no longer a significant discrepancy with the experimental value of Bergmann. In a separate study in solution, the lack of an anionic  $pK_a$  for 3-ethyladenine in water was suggested by Pal to favor the amino tautomer.<sup>36</sup> However, Dreyfus et al. have pointed out the dangers of using  $pK_a$  data to resolve questions about tautomerism.<sup>31</sup> Dreyfus et al. did not observe an imidazole N–H stretch in IR spectra of 3-methyladenine and 3-benzyladenine in chloroform.<sup>31</sup> This result and the absence of a tautomeric equilibrium in temperature-jump experiments in aqueous solution led them to conclude that 3MA exists in the amino form *regardless of environment*.

To our knowledge, there are no theoretical studies of 3MA tautomerism that include solvation. However, studies on adenine have shown that the size of the molecular dipole moment has the largest effect on the solvation energy.<sup>20,22</sup> The dipole moment of (Z)-7H-imino ( $\mu = 3.70$  D) is somewhat smaller than the value for the amino form ( $\mu = 4.74$  D), according to the calculations of Houben et al.<sup>33</sup> The (E)-7H-imino tautomer has a larger dipole moment of 5.61 D,<sup>33</sup> but lies 70–80 kJ mol<sup>−1</sup> above the amino form. Thus, solvation is not expected to significantly stabilize either of the next lowest-energy tautomers of 3MA. We conclude that available experimental and theoretical evidence indicates that 3MA is present overwhelmingly as the amino tautomer in aqueous solution.

**7MA and 9MA.** To our knowledge, there are no reports of imino tautomer energetics for 7MA or 9MA. However, replacing the methyl group of each compound by hydrogen generates an isomer of adenine, which has been studied. As shown above, calculated energies for 1MA and 3MA tautomers are very close to corresponding adenine ones. The results from Ha et al.<sup>29</sup> on all 12 adenine tautomers can therefore be used to predict energetics for the various tautomers of 7MA and 9MA. For example, the 1H,9H-imino tautomer of adenine (E and Z conformers have approximately the same energy) lies 51.3 kJ mol<sup>−1</sup> above the 9H-amino tautomer, while the 3H,9H-imino tautomer lies 136 kJ mol<sup>−1</sup> higher in energy.<sup>29</sup> The 1H,7H-imino tautomer lies 36.9 kJ mol<sup>−1</sup> above the 7H-amino tautomer of adenine.<sup>29</sup> These values, coupled with the modest dipole moments of the lowest energy tautomers, predict that the 7MA and 9MA will be present exclusively in the amino form. Experiments strongly support this conclusion.<sup>34,37,38</sup>

In summary, the evidence indicates that 1MA, 3MA, 7MA, and 9MA are each present in a single tautomeric form in aqueous solution, the amino form. In contrast, adenine is present as two amino tautomers (referred to below as the 7H and 9H tautomers) under the same conditions.

**Biexponential Dynamics of Adenine.** The signal for adenine in H<sub>2</sub>O shows a striking, biexponential decay. In contrast, transient absorption by the adenine nucleoside, adenosine, shows only a monoexponential decay with a lifetime of 290 fs.<sup>2</sup> Biexponential decay was also observed for adenine in fluorescence up-conversion experiments by Gustavsson et al., who reported lifetimes of  $0.23 \pm 0.05$  ps and  $8.0 \pm 0.3$  ps at a

temperature of  $20 \pm 1$  °C.<sup>4</sup> These values are in excellent agreement with the lifetimes in Table 1, demonstrating that our transient absorption experiments monitor the decay of the fluorescent state. Clearly, these results refute an early time-resolved report in which it was claimed that there is little change to the fluorescence lifetime of adenine upon ribosyl substitution.<sup>39</sup> From global fits at several wavelengths, decay times of  $4.23 \pm 0.13$  ps and  $0.22 \pm 0.02$  ps were determined for 7MA and 9MA in H<sub>2</sub>O, respectively. These values clearly resemble the long ( $\tau = 8.8 \pm 1.2$  ps) and short ( $\tau = 0.18 \pm 0.03$  ps) decay components seen for adenine. Since adenine is only expected to be present as the 7H and 9H tautomers in aqueous solution, we attribute the long and short decays to these species, respectively. The lifetimes for the 9H tautomer and 9MA are equal within experimental uncertainty, indicating that the electronic structure of these two species must be very similar. Recent molecular beam experiments have revealed similar REMPI spectra for 9-methyladenine and 9H-amino adenine,<sup>40</sup> suggesting that the monomethylated compounds are good models for the electronic properties of the corresponding H-substituted tautomers of adenine. In contrast, changing methyl to hydrogen at the N7 position of adenine increases the lifetime by a factor of 2. This effect is poorly understood at present, but is indicative of a special sensitivity to N7 substitution, as discussed more fully below.

**Quantitative Estimate of the Adenine Tautomer Distribution in H<sub>2</sub>O.** The two decay components seen for adenine in water are due to the presence of the 7H and 9H tautomers. In this case, the decay amplitudes can be used to estimate the amount of each tautomer. It is necessary, however, to account for differences in the ground- and excited-state cross sections for absorption. The signal from a mixture of two tautomers depends on their relative abundance, as well as on their respective ground- and excited-state absorption cross sections. The transient absorption signal,  $\Delta A(t)$ , for adenine can be modeled as

$$\Delta A(t) \propto f_7 \epsilon_7 \tilde{\epsilon}_7 \exp(-t/\tau_7) + f_9 \epsilon_9 \tilde{\epsilon}_9 \exp(-t/\tau_9) \quad (1)$$

In eq 1, the subscripts 7 and 9 label the 7H and 9H tautomers of adenine,  $f$  is the fractional population,  $\tau$  is the lifetime of each species,  $\epsilon$  is the molar absorption coefficient in the electronic ground state at the pump wavelength, and  $\tilde{\epsilon}$  is the molar absorption coefficient in the S<sub>1</sub> state at the probe wavelength. Fitting the adenine signal in H<sub>2</sub>O to two exponentials gives the prefactors for both exponentials in eq 1. The amplitude associated with the slower decay ( $A_2$  in Table 1) is proportional to  $f_7 \epsilon_7 \tilde{\epsilon}_7$ , while the amplitude of the subpicosecond decay ( $A_1$  in Table 1) is proportional to  $f_9 \epsilon_9 \tilde{\epsilon}_9$ . Thus, the fractional population of the 7H tautomer is given by

$$f_7 = \left( 1 + \frac{A_1 \epsilon_7 \tilde{\epsilon}_7}{A_2 \epsilon_9 \tilde{\epsilon}_9} \right)^{-1} \quad (2)$$

According to Dreyfus et al., extinction coefficients of the 7H and 9H tautomers of adenine are given approximately by shifting the corresponding values of 7MA and 9MA by 2.5 nm to shorter

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wavelengths.<sup>24</sup> The wavelength-dependent extinction coefficients for 7MA and 9MA are shown in the inset to Figure 1. Following this procedure, we estimate that  $\epsilon_7/\epsilon_9$  is equal to 0.85 at our pump wavelength of 263 nm. The ratio of the ESA cross sections,  $\tilde{\epsilon}_7/\tilde{\epsilon}_9$ , was estimated at a probe wavelength of 570 nm from the back-to-back scans on 7MA and 9MA shown in Figure 4. Since the solutions had the same absorbance at the pump wavelength, the ratio of the signal amplitudes is equal to the ratio of the ESA cross sections. Although the signal from 9MA appears slightly weaker than the one from 7MA in Figure 4, this is an artifact of the finite time resolution. Signals with time constants that are comparable to or shorter than the instrumental response time are strongly attenuated. The correct amplitudes are obtained from the nonlinear least-squares fit, which was carried out with convolution. The deconvoluted amplitude of the 9MA signal is  $\sim 50\%$  larger than the one from 7MA, and the ratio  $\tilde{\epsilon}_{7\text{MA}}/\tilde{\epsilon}_{9\text{MA}}$  is estimated to be 0.66. We have assumed that  $\tilde{\epsilon}_{7\text{MA}}/\tilde{\epsilon}_{9\text{MA}} \approx \tilde{\epsilon}_7/\tilde{\epsilon}_9$ . This assumption seems reasonable in view of the negligible change to the ground-state spectra upon replacing a hydrogen atom with a methyl group.<sup>24</sup> Inserting these values and the amplitudes  $A_1$  and  $A_2$  from Table 1 into eq 2 results in 22% for the fractional population of the 7H tautomer. Using a conservative estimate of 20% for the fractional error in  $\tilde{\epsilon}_7/\tilde{\epsilon}_9$ , we calculate a standard deviation of 0.036 for  $f_7$ . The quantity  $\tilde{\epsilon}_7/\tilde{\epsilon}_9$  is subject to the greatest uncertainty and dominates the error analysis.

Our best estimate for the population of the 7H tautomer ( $22 \pm 4\%$ ,  $1\sigma$ ) can be compared with several previous measurements. The distribution of adenine tautomers was studied by carbon-13 NMR spectroscopy by Chenon et al.<sup>23</sup> Prototropic tautomerism in the imidazole ring of adenine was inferred from the chemical shifts of the C4 and C5 carbon atoms. The authors determined the populations of the 7H and 9H tautomers to be 15 and 85%, respectively. Using nitrogen-15 NMR spectroscopy, Gonnella et al. likewise determined from chemical shifts that aqueous solutions are 20% 7H-amino and 80% 9H-amino.<sup>25</sup> In a separate study, Dreyfus et al. determined a value of 22% for the 7H via temperature-jump and UV absorption experiments.<sup>24</sup> The 7H tautomer was determined to be present at a concentration of  $23 \pm 3\%$  in poly(vinyl alcohol) film at room temperature by IR spectroscopy.<sup>26</sup>

**Solvent Effects and Mechanistic Implications.** Table 2 shows that decay by 9MA is faster in H<sub>2</sub>O than in acetonitrile, while the 7MA lifetime is more similar in the two solvents. Similar trends are found when comparing the fast and slow components of the adenine decays in both solvents. With the exception of 7MA, going from water to acetonitrile increases the lifetime of each compound by 50–100%. The ratio  $A_1/A_2$  (see Table 1) is slightly larger in acetonitrile than in water. This suggests that the percentage of the 7H tautomer may decrease slightly in acetonitrile. However, a more precise statement is not possible since we were unable to measure relative ESA cross sections in acetonitrile since the solubility of adenines in this solvent prevented us from preparing equal absorbance solutions. Eastman and Rosa wrote that the percent 7H tautomer should increase with increasing strength of solvent hydrogen bonds.<sup>41</sup> The lack of a dramatic change in acetonitrile suggests this view may be incorrect. Ab initio studies of solvent effects have shown that inclusion of specific water molecules does not significantly

change the tautomer energies.<sup>21</sup> Gonnella et al. argued that the 7H-amino tautomer of adenine is more strongly favored in water than in dimethyl sulfoxide.<sup>25</sup> Using alkylated adenines to model the tautomeric equilibrium in adenine, they estimated that the percentage of the 7H-amino decreases from 20% in water to 14% in DMSO. In our view, specific solute–solvent interactions such as hydrogen bonding are less important than continuum solvation of the highly polar 7H tautomer by the bulk liquid.

Changing the solvent from H<sub>2</sub>O to D<sub>2</sub>O produces no change in the transient signals within our experimental uncertainty, as shown in Figure 7. It is not surprising that the amplitudes of the slow and fast components are the same in both solvents since tautomer energetics are independent of deuteration, neglecting zero-point energies. On the other hand, the absence of a solvent kinetic isotope effect has profound implications for the nonradiative decay mechanism. Phototautomerization has been suggested as a mechanism for ultrafast excited-state decay by isocytosine,<sup>42</sup> cytosine,<sup>43</sup> uracil,<sup>44</sup> and adenine.<sup>45,46</sup> An excited-state proton-transfer reaction would presumably show a measurable isotope effect. The absence of such an effect argues strongly against phototautomerization. In addition, if solvent-assisted hydrogen transfer were important, then a larger solvent effect should be observed upon going from water to the aprotic solvent, acetonitrile. Finally, if a solvent-assisted hydrogen shift were the primary nonradiative decay mechanism, then  $S_1$  lifetimes ought to be considerably longer for isolated nucleobases due to greater energetic barriers. In fact, recent evidence suggests that ultrashort lifetimes can be observed for single bases in the complete absence of a solvent.<sup>15,40,47</sup>

Salter and Chaban recently studied minimum energy reaction paths for excited-state hydrogen atom transfer from N9 to N3.<sup>46</sup> The barrier height for this reaction was found to be lower and the shape of the reaction path to be more gently sloping for the excited state. This suggests that tautomerization on the excited-state potential energy surface is more probable than on the ground state. The authors pointed out that one or more water molecules could dramatically lower the barrier, as observed in other proton-transfer reactions. Our observation of ultrafast lifetimes ( $\tau \approx 200$  fs) for 3MA and 9MA, neither of which can undergo a 9  $\rightarrow$  3 hydrogen shift, makes this mechanism unlikely to be the dominant nonradiative decay pathway.

Domcke and Sobolewski recently proposed an intriguing mechanism for ultrafast nonradiative decay in adenine, which they suggest applies in vacuo and in solution.<sup>11,48</sup> Specifically, they identified a  $^1\pi\sigma^*$  state by ab initio calculations, which electronically predissociates the  $^1\pi\pi^*$  and  $^1n\pi^*$  states along an N–H stretching coordinate. At a large value of the N–H coordinate, the  $^1\pi\sigma^*$  state has a conical intersection with the electronic ground state, closing a pathway for ultrafast internal conversion. The authors presented reaction-path potential energy profiles for this mechanism as a function of the N9–H distance in 9H-adenine.<sup>11,48</sup> However, the present results on 9MA and our previous measurements on adenosine<sup>1,2</sup> clearly indicate that subpicosecond relaxation is possible in the absence of this

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coordinate. Adenosine, 1MA, 3MA, and 9MA all have subpicosecond lifetimes despite lacking an N–H bond on the purine ring. It is still conceivable that the Domcke-Sobolewski mechanism could instead involve one of the N–H bonds on the exocyclic amino group, but the energies of the  $^1\pi\sigma^*$  state associated with this group have not been determined. Moreover, it is difficult to envision how a  $^1\pi\sigma^*$  state associated with the amino group could interact strongly with  $^1\pi\pi^*$  and  $^1n\pi^*$  states localized on the ring(s).

We believe the most probable explanation for the femtosecond lifetimes of the DNA bases is the proximity of the  $^1n\pi^*$  and  $^1\pi\pi^*$  states.<sup>49</sup> Several authors have discussed the effect of proximal  $^1\pi\pi^*$  and  $^1n\pi^*$  states on the photophysics of adenine<sup>7,8,10,50</sup> and 2-aminopurine.<sup>9</sup> For adenine, these studies make a plausible case for a state switch from the  $^1\pi\pi^*$  surface to a  $^1n\pi^*$  state, but they do not explain ultrafast nonradiative decay to the electronic ground state. By detecting hot ground state absorption we have shown previously that  $S_0$  is repopulated on a subpicosecond time scale.<sup>1,2</sup> If the proximity mechanism is correct, then it must also explain ultrafast relaxation from the  $^1n\pi^*$  state to the electronic ground state.<sup>48</sup> The recent computational study of Ismail et al. located conical intersections between the two  $^1n\pi^*$  states of cytosine and the electronic ground state.<sup>12</sup> This mechanism appears sufficiently universal to account for ultrafast decay by the bases studied here and in ref 16. It is also consistent with the solvent effects reported here. Thus, changing the solvent polarity can differentially affect the energies of  $^1\pi\pi^*$  and  $^1n\pi^*$  states,<sup>9,10</sup> leading to changes in coupling. We believe that this polarity change and not the change in hydrogen bonding underlies the modest difference in lifetimes seen in water and in acetonitrile. Finally, the absence of a solvent kinetic isotope effect is not inconsistent with decay by conical intersections. Such features appear to be accessed by motion along complex multidimensional coordinates.<sup>12</sup>

A great deal of computational work is required to confirm the mechanistic ideas discussed here. Our hope is that this study can provide significant tests of emerging theoretical models for nonradiative decay. Interestingly, the attachment of -H, -methyl, or -ribose at N9 has minimal effect on the lifetimes. From the pattern of decay by the monomethylated adenines, it appears that substitution at N7 is the only factor correlated with longer fluorescence lifetimes. This is consistent with the dramatically increased fluorescence lifetime of the guanosine cation,<sup>2</sup> which is protonated at N7. Ismail et al. showed that the two  $^1n\pi^*$  excitations for cytosine do not lie at the same energy.<sup>12</sup> Our results suggest that the  $^1n\pi^*$  state associated with the N7 lone pair plays a decisive role in the singlet excited-state deactivation. Removal of this state in the 7-substituted compounds may extend the lifetime due to weaker coupling with a different  $^1n\pi^*$  state, which lies higher in energy. In support of this picture, we cite recent TDDFT calculations which have shown that the minimum-energy  $^1n\pi^*$  state lies higher above the lowest-energy  $^1\pi\pi^*$  state in 7H than in 9H adenine.<sup>10</sup>

Kim and co-workers recently studied adenine and several adenine derivatives under isolated molecule conditions by

femtosecond pump–probe transient ionization time-of-flight mass spectrometry.<sup>47</sup> They reported lifetimes of  $1.00 \pm 0.05$  ps,  $1.06 \pm 0.08$  ps, and  $0.94 \pm 0.03$  ps for adenine, 7MA, and 9MA, respectively, upon excitation with femtosecond pulses at 267 nm. Their signals were monoexponential in all cases. The monoexponential signal seen by these workers for adenine contrasts with the biexponential signal reported here in aqueous solution, but is not unexpected since the 9H-amino tautomer of adenine is the only form present in the gas phase.<sup>40</sup> The monoexponential decays Kang et al. observed for 7MA and 9MA are consistent with the monoexponential decays we observe in solution. The short lifetimes seen even for isolated molecules suggest that the solvent does not play a significant role in excited-state decay. The finding by Kang et al.<sup>47</sup> of identical lifetimes for 7MA and 9MA is surprising in light of our lifetimes of 4.23 and 0.22 ps for these compounds, respectively, in room temperature aqueous solution. Since solvent interactions generally quench excited states, it is difficult to explain the decreased lifetime of 7MA in the molecular beam, and further work is needed before the solution-phase measurements can be reconciled with those in the gas phase. It is worth noting that excited-state lifetimes for isolated nucleobases appear to depend sensitively on vibrational excess energy in the excited state. Thus, considerably longer lifetimes have been observed in gas-phase experiments on DNA bases for excitation much closer to the electronic origin.<sup>40,51</sup>

## Conclusions

The femtosecond transient absorption technique has been used to study the solvent- and tautomer-dependent photophysics of adenine. The room-temperature signals from adenine in aqueous solution are biexponential due to an equilibrium mixture of 7H- (~22%) and 9H-amino (~78%) tautomers. The  $S_1$  lifetime of 7H-adenine is approximately 40 times longer than that of the more abundant 9H-tautomer, and this is the reason the time-integrated emission arises principally from the former isomer. This study shows definitively that individual tautomers of the same nucleobase can differ greatly in their excited-state dynamics. Chin et al. have reached the same conclusion from gas-phase experiments on guanine.<sup>51</sup> The results for adenine and for the monomethylated adenines demonstrate that substitution at N7 in the imidazole ring has a more pronounced effect on the excited-state decay than substitution at any of the other ring nitrogens. We have shown previously that the fluorescence lifetimes of cytosine derivatives are exquisitely sensitive to chemical substitution.<sup>16</sup> The work presented here extends this sensitivity to individual prototropic tautomers. In view of the sensitivity of nucleobase excited-state dynamics to covalent modification, an open question for the future is the extent to which the noncovalent interactions (i.e., base stacking and base pairing) found in nucleic acid polymers affect their excited-state dynamics.

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