# Water-Catalyzed Excited-State Proton-Transfer Reactions in 7-Azaindole and Its Analogues

**ARTICLE** *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · SEPTEMBER 2014

Impact Factor: 3.3 · DOI: 10.1021/jp506136v · Source: PubMed



**7 AUTHORS**, INCLUDING:

Jiun-Yi Shen
National Taiwan University
**33** PUBLICATIONS **356** CITATIONS

THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

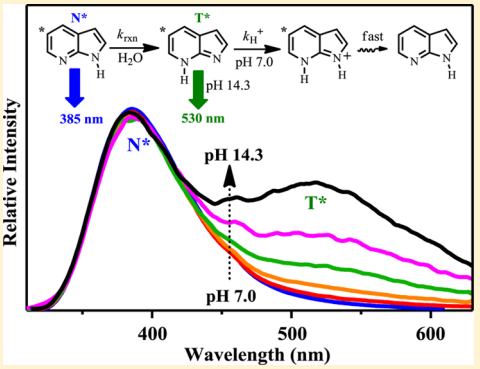
# Water-Catalyzed Excited-State Proton-Transfer Reactions in 7-Azaindole and Its Analogues

Yu-Sin Wu,‡ Huai-Ching Huang,‡ Jiun-Yi Shen, Huan-Wei Tseng, Jr-Wei Ho, You-Hua Chen, and Pi-Tai Chou*

Department of Chemistry and Center for Emerging Material and Advanced Devices, National Taiwan University, Taipei 10617, Taiwan

Supporting Information

**ABSTRACT:** The mechanism of the water-catalyzed excited-state proton-transfer (ESPT) reaction for 7-azaindole (**7AI**) has long been investigated, but there are some controversial viewpoints. Recently, owing to the superiority of sensing biowaters in proteins by a **7AI** analogue, 2,7-diazatryptophan, it is timely to reinvestigate water-catalyzed ESPT in **7AI** and its analogues in an attempt to unify the mechanism. Herein, a series of **7AI** analogues and their methylated derivatives were synthesized to carry out a systematic study on p$K_a$, p$K_a$*, and the associated fluorescence spectroscopy and dynamics. The results conclude that all **7AI** derivatives undergo water-catalyzed ESPT in neutral water. However, for those derivatives with −H (**7AI**) and a electron-donating substituent at C(3), they follow water-catalyzed ESPT to form an excited N(7)−H proton-transfer tautomer, T*. T* is rapidly protonated to generate an excited cationic (TC*) species. TC* then undergoes a fast deactivation to the N(1)−H normal species in the ground state. Conversely, protonation in T* is prohibited for those derivatives with an electron-withdrawing group at the C(2) or C(3) or with the C(2) atom replaced by an electron-withdrawing nitrogen atom (N(2) in, e.g., 2,7-diazatryptophan), giving a prominent green T* emission. Additional support is given by the synthesis of the corresponding N(7)−$CH_3$ tautomer species, for which p$K_a$* of the cationic form, that is, the N(7)−$CH_3$N(1)−$H^+$ species, is measured to be much greater than 7.0 for those with electron-donating C(3) substituents, whereas it is lower than 7.0 upon anchoring electron-withdrawing groups. For **7AI**, the previously missing T* emission is clearly resolved with a peak wavelength at 530 nm in the pH interval of 13.0−14.3 ($H_-$ 14.2).




## INTRODUCTION

Excited-state proton-transfer (ESPT) reactions have attracted considerable attention due to their occurrence in a wide variety of photophysical and photochemical processes. The mechanism of ESPT, which correlates with the strength of a hydrogen bond (H-bond) and dynamics of proton (or hydrogen atom) motion, provides valuable information on the fundamental of chemical reaction as well as mimicking proton-transfer processes that ubiquitously take place in biological systems.

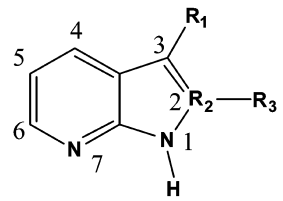
Among numerous ESPT molecules, 7-azaindole (**7AI**; see Figure 1) has shown its photophysical uniqueness and hence great biological significance. Azaindole is analogous to indole that forms the base of amino acid tryptophan and is akin to the adenine nucleobase. The photophysics of **7AI** have been widely investigated in a variety of environments. In hydrocarbon solvents, Kasha and co-workers first demonstrated that in concentrated solution, **7AI** formed dual H-bond dimers, which upon electronic excitation undergo excited-state double proton transfer (ESDPT)[1] to give a green proton-transfer tautomer emission. In this regard, the **7AI** doubly H-bonded dimer with its ESDPT has long been served as a prototype to illustrate the possible photoinduced proton transfer taking place in the DNA double helix and consequently the cause of mutation.[2−4]

The excited-state tautomerization also proceeds efficiently in protic solvents such as alcohols. The ESDPT process in alcohol has been explained in terms of a two-step model,[5−7] in which the formation of a **7AI**−solvent molecule cyclic H-bond complex is the prerequisite and rate-determining step, followed by the fast proton tunneling process. The rate of the tautomerization reaction typically requires several hundred picoseconds, which is 2 orders of magnitude slower than the reaction rate for its dual hydrogen-bonding dimer in hydrocarbon solvents.[8,9]

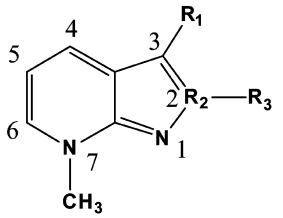
The photophysical behavior of **7AI** in water, however, appeared to be anomalous. In sharp contrast to the dual emission bands for **7AI** in alcohols, consisting of two well-separated normal (~370 nm, N*) and proton-transfer tautomer (~510 nm, T*) bands, only a single band maximized at ~385 nm is resolved in neutral water. Several research groups have

**Special Issue:** Photoinduced Proton Transfer in Chemistry and Biology Symposium

**Received:** June 20, 2014
**Revised:** August 20, 2014
**Published:** September 16, 2014



| $R_1$ | $R_2$ | $R_3$ | Compound |
|---|---|---|---|
| H | C | H | **7AI** |
| $CH_3$ | C | H | **3MAI** |
| $CH_2CHNH_2CO_2H$ | N | – | **(2,7-aza)Trp** |
| CN | C | H | **3CAI** |
| H | C | $CF_3$ | **2CF$_3$AI** |



| $R_1$ | $R_2$ | $R_3$ | Compound |
|---|---|---|---|
| H | C | H | **7M7AI** |
| $CH_3$ | C | H | **7M3MAI** |
| $CH_2CHNH_2CO_2H$ | N | – | **7M(2,7-aza)Trp** |
| CN | C | H | **7M3CAI** |
| H | C | $CF_3$ | **7M2CF$_3$AI** |

**Figure 1.** Chemical structures of various **7AI** derivatives and their methyl (N(7)−$CH_3$) tautomer analogues.

studied the emission of **7AI** in water and reached different conclusions regarding its origin and hence different mechanisms. On the one hand, Petrich and co-workers[6] interpreted the photophysics of **7AI** in water by proposing that only a small fraction (<20%) of the **7AI** molecules in pure water are capable of undergoing excited-state tautomerization on a 1 ns time scale. No appreciable emission intensity can be observed for **7AI** in water at around 530 nm because very little tautomer is generated and is rapidly protonated to give a tautomer cationic emission (~440 nm) that is buried under the major normal (385 nm) emission. On the other hand, Chapman and Maroncelli also carefully analyzed the relaxation dynamics of **7AI** in water and came up with somewhat different viewpoints.[10] They concluded that even though the emission spectra for **7AI** in alcohols (dual emission) and water (single emission band) appear to be qualitatively different, there is intrinsically nothing anomalous about the behavior of **7AI** in water. The spectral dissimilarity is because of two quantitative differences in terms of rates. In water, while the rate of N* → T* proton transfer is much slower, the nonradiative deactivation for T* is very fast. The combination of these two effects leads to a consequence of only the normal (N*) emission band being observed.

In yet another approach in chemical biology, 7-azatryptophan, an analogue of **7AI**, has long been applied as an alternative of tryptophan to probe the protein structure and dynamics.[11−13] This is mainly due to the fact that 7-azatryptophan possesses both a longer absorption spectral onset (cf. tryptophan) and a polarity-sensitive emission peak wavelength. Unfortunately, similar to that of **7AI**, 7-azatryptophan in water lacks proton-transfer tautomer emission and could not be used as a marker to probe the surrounding water environment in proteins. In proteins, water ubiquitously partakes in governing the structure and functionality. Recent advances have supplied more convincing data that water molecules in proteins are one of the key elements in activating biofunctionalities such as enzymatic reactions.[14−17]

In an aim to specifically probe the water environment, we recently replaced C(2) of 7-azatryptophan (see Figure 1 for numbering) by a nitrogen atom (N(2)), forming 2,7-diazatryptophan (**(2,7-aza)Trp**) that exhibits remarkable water-catalyzed proton-transfer properties.[18] **(2,7-aza)Trp** exists in two isomers in the ground state, that is, the N(1)−H and N(2)−H isomers, in which the N(1)−H isomer undergoes water-catalyzed ESPT, resulting in an N(7)−H tautomer emission maximized at 500 nm. This prominent proton-transfer emission thus serves as a unique fingerprint and has been successfully exploited for direct sensing of a site-specific water environment in the human thromboxane A2 synthase (hTXAS) without disrupting its native structure.[18] We also noticed that upon removing the $\alpha$-amino acid part from **(2,7-aza)Trp**, forming 2,7-diazaindole, similar proton-transfer emission was also resolved, which is in stark contrast to the lack of tautomer emission for **7AI** in water. From the fundamental point of view, a core issue is thus raised regarding factors that govern the water-catalyzed ESPT and hence the resolution of proton-transfer tautomer emission. This, together with the long-standing puzzle regarding the mechanism of ESPT for **7AI** in water, leads us to revisit water-catalyzed ESPT in **7AI**. We also carried out a systematic study on p$K_a$, p$K_a$*, and the associated fluorescence spectroscopy/dynamics for a series of **7AI** derivatives and their methylated tautomer analogues. The results conclude that the luminescence properties are harnessed by the excited-state acidity/basicity properties of the proton-transfer tautomer, and the overall ESPT mechanism in water can be unified among the titled **7AI** derivatives. Details of the results and discussion are elaborated as follows.

## METHODS

**Synthesis.** The detailed synthetic procedures and compound characterizations are elaborated in the Supporting Information.

**Spectroscopic and Dynamic Measurements.** Steady-state absorption and emission spectra were recorded using a Hitachi U-3310 spectrophotometer and an Edinburgh FS920 fluorimeter, respectively. Ultrafast spectroscopic studies were performed using a femtosecond fluorescence up-conversion system (FOG100, CDP) pumped by a femtosecond oscillator (Tsunami, Spectra-Physics). The details for measurements were described previously[19,20] and are included in the Supporting Information.


dx.doi.org/10.1021/jp506136v | J. Phys. Chem. B 2015, 119, 2302−2309

**Calculation of the pH and $H_-$ Value.** When $[OH^-]$ is greater than 1 M, the acidity function is modified to be in the form of $H_-$ for superbasic media. The detailed procedure is elaborated in the Supporting Information.

## RESULTS AND DISCUSSION

In the early approach, El-Bayoumi et al. compared the isotope effect and emission quantum yield of **7AI** with indole and $N_1$-methyl-7-azaindole in $H_2O$.[21] They found that **7AI** had a relatively low emission quantum yield of 0.03 in $H_2O$ and a relatively large isotope effect ($\Phi D_2O/\Phi H_2O$ = 3.63). They concluded that both the N(7) and N(1)−H sites were involved in the deactivation process. In addition, Chapman and Maroncelli observed nearly identical radiative decay rate constants for **7AI** in $H_2O$ ($3.7 \times 10^7$ $s^{-1}$) and in $D_2O$ ($3.6 \times 10^7$ $s^{-1}$) but a larger nonradiative decay rate constant in $H_2O$ ($1.2 \times 10^9$ $s^{-1}$) than that in $D_2O$ ($2.9 \times 10^8$ $s^{-1}$).[10] To probe the water catalytic effect on **7AI**, in an early approach, Chou and co-workers gradually added a small amount of water into the aprotic solvent containing **7AI** and observed the growth of green proton-transfer tautomer emission. The result clearly proves that water molecules are capable of catalyzing the proton-transfer reaction for **7AI** in the excited state.[22] However, when **7AI** is in an aprotic solvent with a small amount of water, it does not tend to be protonated due to the low availability of water molecules. On the basis of these observations, one would speculate that the quenching pathway of **7AI** in water is highly related to excited-state water-catalyzed ESPT that is subject to the deuterium isotope effect. Despite a number of relevant supports of ESPT for **7AI** in bulk water, the direct support of ESPT, that is, the observation of tautomer emission, is unfortunately missing (vide supra). The $pK_a$* of the **7AI** tautomer analogue 7-methyl-7*H*-pyrrolo[2,3-*b*]pyridine (**7M7AI**) (see Figure 1 and Table 1) in the protonated form

**Table 1. Ground- and Excited-State $pK_a$ for the Protonated Form of 7AI, 3MAI, and Various 7AI Methyl (N(7)−$CH_3$) Tautomer Analogues**[a]

| compounds | $pK_a$ | $pK_a$* |
|---|---|---|
| **7AI** | 4.5[6] | 4.6[6] |
| **3MAI** | 4.8[b] | 4.6[c] |
| **7M7AI** | 8.9[23] | 10.3[6] |
| **7M3MAI**[24] | 9.3[b] | 9.4[d] |
| **7M(2,7-aza)Trp** | 5.8[b] | 5.9[e] |
| **7M3CAI**[25] | 4.8 | 0.2 |
| **7M2CF$_3$AI** | 4.4[b] | −0.3[f] |

[a]See Figure 1 for their structures. [b]Determined by the half-neutralization method. [c]The excitation wavelength was 285 nm, and the emission was monitored at 375 nm for the emission intensity titration experiment. [d]The excitation wavelength was 350 nm, and the emission was monitored at 550 nm for titration. [e]The excitation wavelength was 342 nm, and the emission was monitored at 400 nm for titration. [f]The excitation wavelength was 315 nm, and the emission was monitored at 500 nm for titration.

**7M7AI** cation (N(1)−$H^+$) was measured to be 10.3.[6] Assuming similar $pK_a$* values between the **7M7AI** cation (N(1)−$H^+$) and the protonated **7AI** tautomer (N(1)−$H^+$), Petrich and co-worker thus proposed that the excited-state **7AI** tautomer should be in the protonated form at pH 7. In other words, the **7AI** tautomer in the excited state, once being produced from water-catalyzed ESPT, is promptly protonated in the neutral water so that the tautomer emission is absent.

The above thermodynamic interpretation based on the acid−base property to govern the fate of proton-transfer emission is sound. However, as for **7AI**, crucial spectroscopic evidence has to be provided to verify the mechanism. First, if the excited N(7)−H tautomer of **7AI** is in the protonated form at pH 7, the associated cationic emission should be resolvable. Second, assuming a similar $pK_a$* (10.3) with the **7M7AI** cation (N(1)−$H^+$) for the protonated **7AI** tautomer (N(1)−$H^+$), the neutral N(7)−H tautomer emission should be observable at pH > 10.3; however, no tautomer emission could be resolved up to pH 12.

Because the **7AI** tautomer emission has not been resolved so far, we suspect that the $pK_a$* of protonated the **7AI** tautomer (N(1)−$H^+$) may be higher than that (10.3) predicted by using **7M7AI** (N(1)−$H^+$). We thus extended the fluorescence titration measurement to higher pH value. Figure 2 clearly

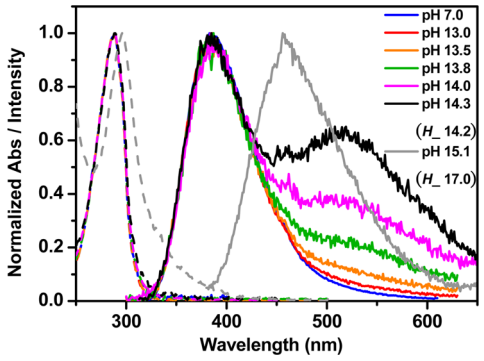



**Figure 2.** Steady-state absorption (dashed lines) and emission spectra (solid lines) for **7AI** in water at indicated pH values at room temperature.

shows that in addition to the 385 nm normal emission, the 530 nm N(7)−H tautomer emission gradually appears from pH 13.0 to 14.3 ($H_-$ 14.2). The assignment of the tautomer emission can be supported by its spectral feature being nearly identical with the emission spectrum of the tautomer analogue **7M7AI** shown in Figure 3. Further support of ESPT is rendered by identical excitation spectra for both the normal and tautomer emission bands, which are also identical with the absorption spectrum (Figure S1, Supporting Information),

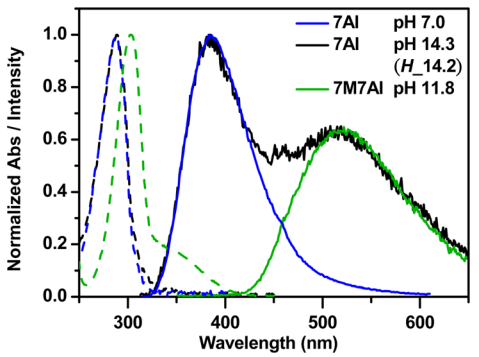



**Figure 3.** Steady-state absorption spectra (dashed lines) and emission spectra (solid lines) for **7AI** (black and blue) and **7M7AI** (green) in water at room temperature. The pH values of the solutions are indicated in the figure.


dx.doi.org/10.1021/jp506136v | J. Phys. Chem. B 2015, 119, 2302−2309

indicating that both emission bands share the common ground-state origin.

Perhaps the firmest support of ESPT is given by the relaxation dynamics of the corresponding emission. At pH 14.0, the femtosecond fluorescence upconverted signal monitored at normal emission of 430 nm consists of an instant rise (<150 fs) and a single-exponential decay component with a lifetime of 70 ps (Figure 4). Upon monitoring at the tautomer emission of

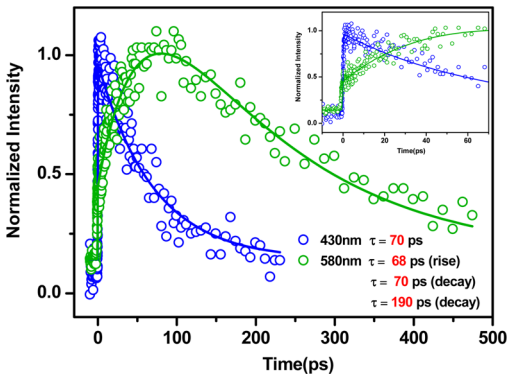



**Figure 4.** Femtosecond fluorescence up-conversion kinetic traces for the normal form emission (430 nm) and tautomer emission (580 nm) of **7AI** in the $NaOH_{(aq)}$ solution (pH 14.0) at room temperature. The excitation wavelength is 268 nm.

580 nm, the time-resolved upconverted signal consists of a small portion (<15%) of instant rise component, a relatively slow rise of 68 ps, and a population decay time of 190 ps. The rise time of 68 ps, within experimental error, is identical to the decay time (70 ps) of the normal emission, clearly demonstrating the behavior of a precursor−successor type of ESPT reaction. The instant rise component can be rationalized by the certain overlap between the normal and tautomer emissions so that decay (normal emission) and rise (tautomer emission) are canceled out, causing a net result of instant rise.

Further increasing the pH to 15.1 ($H_-$ 17.0) the deprotonation of **7AI** in the ground state takes place, which is clearly indicated by the appearance of an absorption shoulder in the region of 350−400 nm (Figure 2), giving rise to a **7AI** anion emission maximized at 465 nm. Therefore, in the steady-state measurement, the **7AI** tautomer emission can only be resolved in a narrow pH range of ~13.0−14.3 ($H_-$ 14.2), explaining why the **7AI** tautomer was obscure in the previous studies.

In the wake of resolving the **7AI** tautomer emission, another core issue lies in the verification of the tautomer cationic emission in neutral water, which, according to the above proposal, results from the protonation of tautomer in the excited state. The cationic emission of the **7AI** tautomer analogue, the **7M7AI** (N(1)−$H^+$), is at around 440 nm.[6] In neutral water, because **7AI** exhibits solely a 385 nm normal emission band at pH 7 (Figure 2), the cationic emission, if existing, must be very weak and associated with a fast nonradiative decay rate. Exploiting the fluorescence upconversion technique, we then made attempts to resolve the tautomer cationic emission of **7AI** in the region of 420−550 nm at pH 7.0. Upon monitoring the fluorescence upconverted signal at three wavelengths (420, 470, and 500 nm), the corresponding time-resolved profiles all reveal an instant rise (<150 fs) and a long population decay that remains nearly constant in the region of 50 ps (Figure 5). The long decay component is

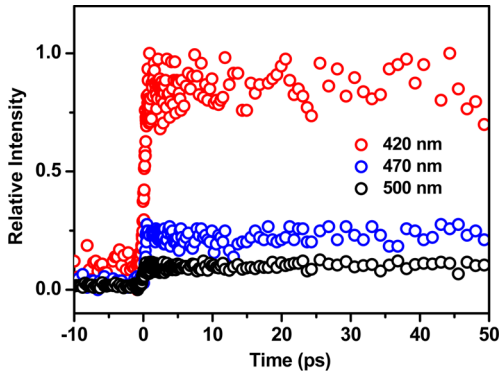



**Figure 5.** Time trace of fluorescence upconverted signals at 420, 470, and 500 nm for **7AI** in water (pH 7). The excitation wavelength is 268 nm.

further resolved to be ~900 ps and is unambiguously ascribed to the population decay of the normal **7AI** emission.[6,10] Thus, under a system response of <150 fs, we were not be able to detect any fast, resolvable component within the emission region of 420−500 nm. On the one hand, this may be rationalized by only a small population of the initial proton-transfer tautomer species in the excited state, as previously proposed by Petrich and co-workers (vide supra). However, because the 530 nm tautomer emission is well-resolved within pH 13.0−14.3 ($H_-$ 14.2) in this study, this possibility is discounted. Alternatively, it is more plausible that the excited tautomer cationic species is an intermediate, which deactivates to normal **7AI** ground-state species in a rate faster than its formation. Kinetically, this implies that the population of tautomer cation achieves a steady state and its concentration is thus too small to be resolved. We will elaborate on this viewpoint in the following section regarding the generalization of the ESPT mechanism for **7AI**.

**Unification of the Overall Mechanism of ESPT.** Generalization of the above acidity−basicity correlated proton-transfer tautomer emission requires support of other **7AI** derivatives. In 2001, we synthesized a **7AI** derivative, 3-cyano-7-azaindole (**3CAI**),[25] which revealed prominent proton-transfer emission at 480 nm (Figure 6), demonstrating that water-catalyzed ESPT indeed takes place in pure $H_2O$ (pH 7) for **3CAI**. We then synthesized the N(7)−$CH_3$ tautomer analogue of **3CAI**, namely, 3-cyano-7-methyl-7*H*-pyrrolo[2,3-*b*]pyridine (**7M3CAI**, Figure 1)[25] and measured the p$K_a$* of

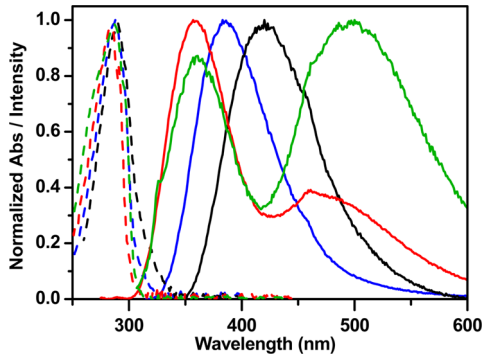



**Figure 6.** Steady-state absorption (dashed lines) and emission spectra (solid lines) for **7AI** (blue), **3MAI** (black), **2CF$_3$AI** (green), and **3CAI** (red) in neutral water at room temperature.


dx.doi.org/10.1021/jp506136v | J. Phys. Chem. B 2015, 119, 2302−2309

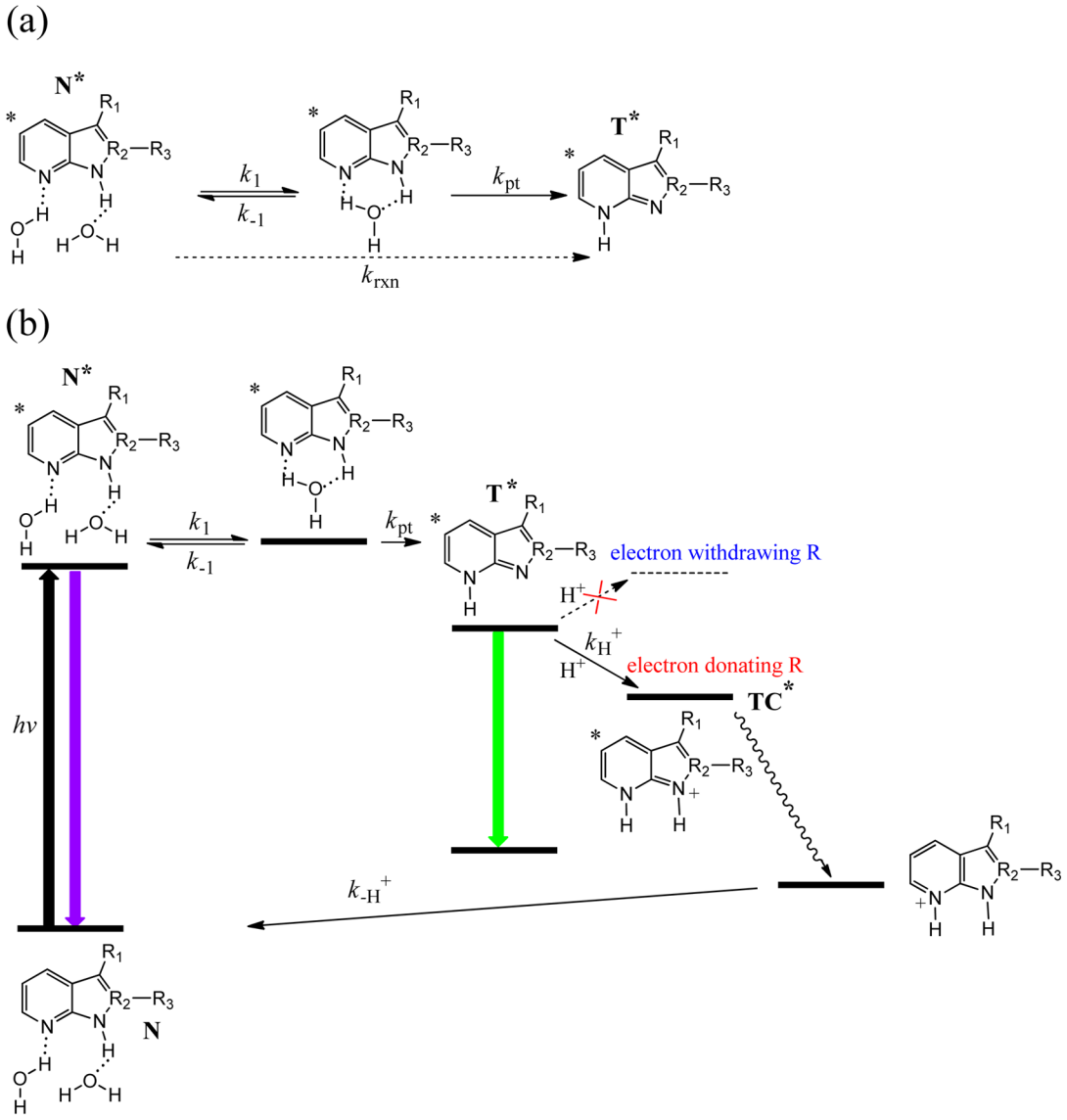



**Figure 7.** (a) The proposed water-catalyzed ESPT for **7AI** derivatives. (b) The mechanism of water-catalyzed ESPT and the following deactivation pathways for **7AI** derivatives in the neutral water. The asterisk * indicates the electronic excited state.

**7M3CAI** (N(1)−$H^+$) to be as low as 0.2 (Table 1), indicating that **7M3CAI** exists without protonation at pH 7. Likewise, assuming similar excited-state acid−base properties as **7M3CAI**, the proton-transfer tautomer of **3CAI**, the N(7)−H tautomer, is expected to exist in the excited state without protonation in neutral water, consistent with the observation of prominent green emission.[25]

Chemically, the main difference between **7AI** and **3CAI** lies in the much higher acidity due to its strong electron-withdrawing −CN group at the C(3) position, increasing the N(1)−H acidity. The water-catalyzed pyrrolic N(1)−H → pyridyl N(7)−H proton transfer essentially results in the deprotonation of N(1)−H, forming a tautomer in which the imine N(1) nitrogen is a conjugate base of the N(1)−$H^+$ acid. Accordingly, due to its strong N(1)−$H^+$ acidity, the basicity of N(1) nitrogen in the **3CAI** tautomer is expected to be much weaker (cf. 7**AI** tautomer); therefore, protonation of **3CAI** tautomer is thermally unfavorable.

To further test the above viewpoint regarding thermodynamics-governed deactivation of a proton-transfer tautomer, we then provided several other **7AI** derivatives and their N(7)−$CH_3$ tautomer analogues, which are anchored by either electron-donating or -withdrawing groups at the pyrrole site, and then carefully performed the spectroscopy and acidity (fluorescence titration) measurement. As a result, adding a methyl group at C(3) of **7AI**, forming **3MAI**, exhibits solely a normal emission band maximized at 420 nm (Figure 6) in neutral water. The p$K_a$* of its tautomer analogue **7M3MAI** (Figure 1 and Table 1) in protonated form **7M3MAI** (N(1)−$H^+$) is measured to be 9.4. Applying a similar p$K_a$* to the **3MAI** proton-transfer tautomer cation (N(1)−$H^+$), the protonation of the tautomer is expected, explaining the absence of tautomer emission in neutral water. Moreover, replacing C(2) by an electron-withdrawing N(2) nitrogen, we have reported a well-resolved proton-transfer emission in 500 nm for **(2,7-aza)Trp** in neutral water (vide supra).[18] The prohibition of protonation on the **(2,7-aza)Trp** tautomer in the excited state correlates well with a p$K_a$* of 5.9 of its tautomer analogue **7M(2,7-aza)Trp** (Figure 1 and Table 1) in the protonated form, **7M(2,7-aza)Trp** (N(1)−$H^+$). To provide an additional


dx.doi.org/10.1021/jp506136v | J. Phys. Chem. B 2015, 119, 2302−2309

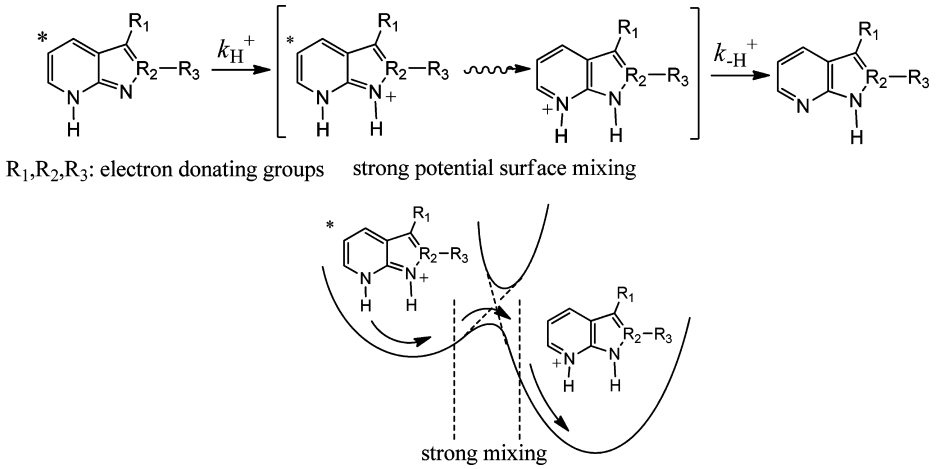



**Figure 8.** Proposed deactivation pathway of the proton-transfer tautomer cation in the excited state. The asterisk * denotes the electronic excited state.

example, we then added a substituent $CF_3$ at C(2) of **7AI** to form **2CF$_3$AI** (see Figure 1), which also gives a well-resolved tautomer emission at 500 nm (Figure 6). The existence of tautomer and hence the prominent green emission implies the low p$K_a$* (≪7) of its conjugate acid. This is firmly supported by the p$K_a$* of as low as −0.3 for the protonated form of its methyl tautomer analogue **7M2CF$_3$AI** (N(1)−H$^+$).

Therefore, the results conclude that for those derivatives with electron-donating substituent (including −H) at C(3), following water-catalyzed ESPT to form an excited N(7)−H proton-transfer tautomer, rapid protonation takes place to generate an excited cationic species at pH 7.0. Conversely, protonation of the tautomer is thermally unfavorable in the excited state for those derivatives with an electron-withdrawing substituent at C(2) or C(3), or C(2) replaced with an electron-withdrawing nitrogen atom, giving a prominent green emission.

We then make attempts to combine the water-catalyzed ESPT mechanisms for **7AI**, which were respectively proposed by Petrich[6] and Maroncelli[10] with certain dissimilarity, and make one step further to bring up a generalized mechanism applicable to all titled **7AI** derivatives in neutral water. First of all, we adopt Chapman and Maroncelli's ESPT mechanism[10] in that the entire **7AI**, rather than a small portion of **7AI** proposed by Petrich and co-workers,[6] undergoes water-catalyzed ESPT. Accordingly, polysolvated **7AI** are the predominant species in the ground state, which upon electronic excitation have to form the 1:1 **7AI**/water cyclic H-bond complex (Figure 7) prior to proton transfer. To rationalize the deuterium isotope effect, Chapman and Maroncelli[10] further proposed the existence of pre-equilibrium between polysolvated **7AI** and the **7AI**/water cyclic H-bond complex, in which forming the **7AI**/water cyclic H-bond complex is an endergonic process. This is reasonable because the molecular dynamics (MD) approach has concluded that the equilibrium toward the **7AI**/water cyclic form is negligible and has to be formed in the excited state via thermal activation prior to executing proton transfer.[26−28] Using a steady-state approach, the rate of ESPT, $k_{rxn}$, to form a tautomer in the excited can be expressed by

$$k_{rxn} = k_{pt} e^{-\Delta G^{\ddagger}/RT} \quad (1)$$

where $G^{\ddagger}$ is the difference in free energy between polysolvated **7AI** and the **7AI**/water cyclic H-bond complex. Note that $G^{\ddagger}$ is not an activation free energy but a true equilibrium free energy. $k_{pt}$ may be treated as a proton tunneling rate constant and hence is expected to be deuterium-isotope-dependent. As a result, being different from conventional wisdom, the slope of $k_{rxn}$ as a function of $1/T$ is deuterium-isotope-independent, while the extrapolation to $1/T \approx 0$ is deuterium-isotope-dependent. This pre-equilibrium reaction dynamics has been supported by the deuterium isotope studies of ESPT in the case of **3CAI**.[25]

Finally, before the above-proposed mechanism of water-catalyzed ESPT mechanism can be unified for the **7AI** and its analogues, an attempt to reconcile the proton inventory experiments reported by Pertich and co-workers[29] has to be discussed. The results led them to conclude a concerted type of ESPT reaction in alcohols, in which the prerequisite of the ESPT required a 1:1 alcohol/**7AI** H-bond complex. Conversely, in water, despite a rather small proportion of the 1:1 water/**7AI** H-bond complex that may undergo ESPT, the results show that the majority of **7AI** exists in a form of blocked species (more like a 2:1 water/**7AI** H-bond complex), in which excited-state tautomerization, that is, double proton transfer, is frustrated. Instead, a three-proton process involves the hydrogen of N(1) and the two protons of a water coordinated to it. This process serves as a major quenching pathway for **7AI** in water (pH = 7). This proposed mechanism echoes their previous studies[6,13,30] elaborated in the early section (vide supra). It is noteworthy that the analyses of the proton inventory experiment are mainly based on a rule of the geometric mean,[31] which holds true only in a symmetrical type of proton-transfer reaction. In addition, the interpretation of isotopic substitution experiments depends on whether the two-step model is appropriate. If the rate-limiting step in the double proton-transfer reaction is the formation of the cyclic complex, then Petrich and co-workers also realized that the conclusion that they made from isotope effects using a concerted reaction mode requires reinterpretation. Which model is more suitable needs additional experimental and theoretical work. In this regards, Mente and Maroncelli[26] performed the MD simulation of **7AI** in water and a series of other protic solvents such as different alcohols and concluded that the **7AI**/water (1:1) cyclic H-bond complex, if it exists in the ground state, is rather small in percentage (<2%). Therefore, they conclude that the (1:1) cyclic H-bond complex has to be formed in the excited state through the thermal equilibrium followed by ESPT, that is to say, that water-


dx.doi.org/10.1021/jp506136v | J. Phys. Chem. B 2015, 119, 2302−2309

catalyzed ESPT for **7AI** is in a two-step process as we adopt in this study, involving pre-equilibrium between the polysolvated **7AI** and the 1:1 water/**7AI** cyclic H-bond complex; the latter then undergoes proton transfer.

Upon forming the proton-transfer tautomer species in the excited state (the N(7)−H isomer denoted as T*; see Figure 7), we then adopt Petrich's model in that the thermodynamic property of the **7AI** proton-transfer tautomer T* (see Figure 7) plays a key role to decide its relaxation pathways in water. We also prove in this study that is generally applicable to all **7AI** derivatives. The core of the model lies in the acidity scale of the N(7)−H cationic form in the excited state. In neutral water (pH 7), if $pK_a$* of the tautomer cation form (denoted as TC*; see Figure 7) is <7, T* should not be protonated. One thus expects to observe tautomer T* emission. Conversely, for $pK_a$* of TC* > 7, the tautomer T* emission is not observable due to the protonation of T* at the −N(1) site (i.e., forming a TC*; see Figure 7).

Using **7AI** as a prototype, we were not able to resolve TC* emission in both steady-state and kinetic manners (vide supra). As for another case, 3-methyl-7-azaindole (**3MAI**) also exhibits solely the normal emission (Figure 6), and similarly neither tautomer (T*) nor its cationic emission could be resolved. In the early section, we tentatively proposed the excited tautomer cationic (TC*) species to be an intermediate, which deactivates to certain ground-state species in a rate much faster than its formation. Kinetically, this implies that the population of the excited tautomer cation (T*) achieves a steady state, and its concentration, and hence emission, is too low to be resolved. From a chemistry point of view, the tautomer cation (TC*) has the same canonical structure as the cation of the normal species (N(7)−$H^+$; see Figure 8). Thus, one plausible deactivation pathway is the internal conversion from TC* to the ground state of the normal cation (N(7)−$H^+$). Due to the same canonical structure and hence the great vibronic overlaps, their state mixing must be very strong. In terms of quantum mechanics, if resonance should occur, the electron motion is no longer defined as just a zero-order function alone ($\psi_1$ or $\psi_2$) but as a mixed state ($\psi_1 + \psi_2$). Such deactivation only requires the redistribution of the electron density and should be much faster than the rate of cation formation, $k_{H+}$ (Figure 8). As a result, the tautomer cationic (TC*) emission is too weak to be resolved. Upon forming the normal cation (N(7)−$H^+$) in the ground state, due to the low p*K*a values of 4.5 and 4.8 for protonated **7AI** and **3MAI**, respectively (see Table 1), further deprotonation takes place in neutral water to get back to the normal ground state, achieving a proton-transfer cycle.

## CONCLUSION

In sum, we have carried out the fluorometric pH titration, in combination with the corresponding relaxation dynamics, to resolve the proton-transfer tautomer 530 nm emission of **7AI** within a narrow basic range of pH 13.0−14.3 ($H_-$ 14.2). This explains why the tautomer emission of **7AI** has not been observed in neutral water during the past decades, whereas the tautomer emission is very prominent in, for example, alcohol solvents.[5,7] Realizing that the acid−base property of the tautomer is key for its excited-state deactivation process, we then systematically studied a series of **7AI** analogues and their methylated derivatives on $pK_a$, $pK_a$*, together with the associated fluorescence spectroscopy and dynamics. The results conclude that all **7AI** derivatives undergo water-catalyzed ESPT in neutral water. For those derivatives with an electron-donating substituent (including −H) at C(3), following water-catalyzed ESPT to form an excited proton-transfer tautomer, T*, rapid protonation takes place to generate an excited cationic species, TC*, due mainly to the $pK_a$* ≫ 7 for the cation form of T*. Conversely, protonation in T* is prohibited ($pK_a$* of TC* < 7) for those derivatives with electron-withdrawing substituent at C(2) or C(3) or C(2) replaced with an electron-withdrawing nitrogen atom (N(2) in, e.g., 2,7-diazatryptophan), giving a prominent green T* emission. For those **7AI** derivatives with an electron-donating substituent, the tautomer cation undergoes fast deactivation to the ground-state normal cationic form, followed by deprotonation to the original normal species to wrap up a proton-transfer cycle. The information gathered is thus valuable for future strategic design of the tryptophan analogues as an ideal probe for sensing water in proteins.[18]

## ASSOCIATED CONTENT

### Supporting Information

Excitation spectra for 7AI (Figure S1), syntheses and characterizations, spectroscopic and dynamic measurements, and calculation of the pH and $H_-$ value. This material is available free of charge via the Internet at http://pubs.acs.org.

## AUTHOR INFORMATION

### Corresponding Author

*E-mail: chop@ntu.edu.tw. Telephone: +886-2-33663894.

### Author Contributions

‡Y.-S.W. and H.-C.H. contributed equally to this work.

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We thank the Ministry of Science and Technology for the financial support.

## REFERENCES

(1) Taylor, C. A.; Elbayoumi, M. A.; Kasha, M. Excited-State Two-Proton Tautomerism in Hydrogen-Bonded N-Heterococlic Base Pairs. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 253−260.

(2) Watson, J. D.; Crick, F. H. C. Molecular Structure of Nucleic Acids — A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171*, 737−738.

(3) Doudney, C. O.; Rinaldi, C. N. Evidence That Ultraviolet Light-Induced DNA-Replication Death of recA Bacteria Is Prevented by Protein-Synthesis in Repair-Proficient Bacteria. *Mutat. Res.* **1989**, *217*, 33−38.

(4) Ingham, K. C.; Elbayoumi, M. A. Photoinduced Double Proton-Transfer in a Model Hydrogen-Bonded Base Pair. Effects of Temperature and Deuterium Substitution. *J. Am. Chem. Soc.* **1974**, *96*, 1674−1682.

(5) Moog, R. S.; Maroncelli, M. 7-Azaindole in Alcohols: Solvation Dynamics and Proton Transfer. *J. Phys. Chem.* **1991**, *95*, 10359−10369.

(6) Chen, Y.; Rich, R. L.; Gai, F.; Petrich, J. W. Fluorescent Species of 7-Azaindole and 7-Azatryptophan in Water. *J. Phys. Chem.* **1993**, *97*, 1770−1780.

(7) McMorrow, D.; Aartsma, T. J. Solvent-Mediated Proton-Transfer the Roles of Solvent Structure and Dynamics on the Excited-State Tautomerization of 7-Azaindole/Alcohol Complexes. *Chem. Phys. Lett.* **1986**, *125*, 581−585.

(8) Takeuchi, S.; Tahara, T. Observation of Dimer Excited-State Dynamics in the Double Proton Transfer Reaction of 7-Azaindole by Femtosecond Fluorescence Up-Conversion. *Chem. Phys. Lett.* **1997**, *277*, 340−346.

(9) Takeuchi, S.; Tahara, T. The Answer to Concerted versus Step-Wise Controversy for the Double Proton Transfer Mechanism of 7-Azaindole Dimer in Solution. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5285−5290.
(10) Chapman, C. F.; Maroncelli, M. Excited-State Tautomerization of 7-Azaindole in Water. *J. Phys. Chem.* **1992**, *96*, 8430−8441.
(11) Negrerie, M.; Bellefeuille, S. M.; Whitham, S.; Petrich, J. W.; Thornburg, R. W. Novel Noninvasive in Situ Probe of Protein-Structure and Dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 7419−7421.
(12) Negrerie, M.; Gai, F.; Bellefeuille, S. M.; Petrich, J. W. Photophysics of a Novel Optical Probe: 7-Azaindole. *J. Phys. Chem.* **1991**, *95*, 8663−3670.
(13) Gai, F.; Chen, Y.; Petrich, J. W. Nonradiative Pathways of 7-Azaindole in Water. *J. Am. Chem. Soc.* **1992**, *114*, 8343−8345.
(14) Abel, R.; Salam, N. K.; Shelley, J.; Farid, R.; Friesner, R. A.; Sherman, W. Contribution of Explicit Solvent Effects to the Binding Affinity of Small-Molecule Inhibitors in Blood Coagulation Factor Serine Proteases. *ChemMedChem* **2011**, *6*, 1049−1066.
(15) Robinson, D. D.; Sherman, W.; Farid, R. Understanding Kinase Selectivity through Energetic Analysis of Binding Site Waters. *ChemMedChem* **2010**, *5*, 618−627.
(16) Beuming, T.; Che, Y.; Abel, R.; Kim, B.; Shanmugasundaram, V.; Sherman, W. Thermodynamic Analysis of Water Molecules at the Surface of Proteins and Applications to Binding Site Prediction and Characterization. *Proteins* **2012**, *80*, 871−883.
(17) Wang, L.; Berne, B. J.; Friesner, R. A. Ligand Binding to Protein-Binding Pockets with Wet and Dry Regions. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 1326−1330.
(18) Shen, J. Y.; Chao, W. C.; Liu, C.; Pan, H. A.; Yang, H. C.; Chen, C. L.; Lan, Y. K.; Lin, L. J.; Wang, J. S.; Lu, J. F.; et al. Probing Water Micro-Solvation in Proteins by Water Catalysed Proton-Transfer Tautomerism. *Nat. Commun.* **2013**, *4*, 2611−2617.
(19) Chou, P. T.; Chen, Y. C.; Yu, W. S.; Chou, Y. H.; Wei, C. Y.; Cheng, Y. M. Excited-State Intramolecular Proton Transfer in 10-Hydroxybenzo[h]quinoline. *J. Phys. Chem. A* **2001**, *105*, 1731−1740.
(20) Chou, P. T.; Pu, S. C.; Cheng, Y. M.; Yu, W. S.; Yu, Y. C.; Hung, F. T.; Hu, W. P. Femtosecond Dynamics on Excited-State Proton Charge-Transfer Reaction in 4′-*N*,*N*-Diethylamino-3-hydroxyflavone. The Role of Dipolar Vectors in Constructing a Rational Mechanism. *J. Phys. Chem. A* **2005**, *109*, 3777−3787.
(21) Avouris, P.; Yang, L. L.; Elbayoumi, M. A. Excited-State Interactions of 7-Azaindole with Alcohol and Water. *Photochem. Photobiol.* **1976**, *24*, 211−216.
(22) Chou, P. T.; Martinez, M. L.; Cooper, W. C.; McMorrow, D.; Collins, S. T.; Kasha, M. Monohydrate Catalysis of Excited-State Double-Proton Transfer in 7-Azaindole. *J. Phys. Chem.* **1992**, *96*, 5203−5205.
(23) Robison, M. M.; Robison, B. L. 7-Azaindole. II. Conversion to 7-Methyl-7H-pyrrolo[2,3-b]pyridine and Related Compounds. *J. Am. Chem. Soc.* **1955**, *77*, 6554−6559.
(24) Yu, W. S.; Cheng, C. C.; Chang, C. P.; Wu, G. R.; Hsu, C. H.; Chou, P. T. Excited-State Double-Proton Transfer on 3-Methyl-7-azaindole in a Single Crystal: Deuterium Isotope/Tunneling Effect. *J. Phys. Chem. A* **2002**, *106*, 8006−8012.
(25) Chou, P. T.; Yu, W. S.; Wei, C. Y.; Cheng, Y. M.; Yang, C. Y. Water-Catalyzed Excited-State Double Proton Transfer in 3-Cyano-7-azaindole: The Resolution of the Proton-Transfer Mechanism for 7-Azaindoles in Pure Water. *J. Am. Chem. Soc.* **2001**, *123*, 3599−3600.
(26) Mente, S.; Maroncelli, M. Solvation and the Excited-State Tautomerization of 7-Azaindole and 1-Azacarbazole: Computer Simulations in Water and Alcohol Solvents. *J. Phys. Chem. A* **1998**, *102*, 3860−3876.
(27) Kyrychenko, A.; Waluk, J. Excited-State Proton Transfer through Water Bridges and Structure of Hydrogen-Bonded Complexes in 1*H*-Pyrrolo[3,2-*h*] quinoline: Adiabatic Time-Dependent Density Functional Theory Study. *J. Phys. Chem. A* **2006**, *110*, 11958−11967.
(28) Kyrychenko, A.; Stepanenko, Y.; Waluk, J. Molecular Dynamics and DFT Studies of Intermolecular Hydrogen Bonds between Bifunctional Heteroazaaromatic Molecules and Hydroxylic Solvents. *J. Phys. Chem. A* **2000**, *104*, 9542−9555.
(29) Chen, Y.; Gai, F.; Petrich, J. W. Solvation of 7-Azaindole in Alcohols and Water: Evidence for Concerted, Excited-State, Double-Proton Transfer in Alcohols. *J. Am. Chem. Soc.* **1993**, *115*, 10158−10166.
(30) Rich, R. L.; Chen, Y.; Neven, D.; Nbgrerie, M.; Gai, F.; Petrich, J. W. Steady-State and Time-Resolved Fluorescence Anisotropy of 7-Azaindole and Its Derivatives. *J. Phys. Chem.* **1993**, *97*, 1781−1788.
(31) Bigeleisen, J. Statistical Mechanics of Isotopic Systems with Small Quantum Corrections. I. General Considerations and the Rule of the Geometric Mean. *J. Chem. Phys.* **1955**, *23*, 2264−2267.