Proton-Transfer Tautomerism of β-Carbolines Mediated by Hydrogen-Bonded Complexes

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The carboxylic acid catalyzed excited-state amino-imino tautomerism for β-carboline (β-CB) and its analogues has been investigated. Thermodynamics and microsolvation (i.e., stoichiometry of the complex formation) of various β-CB/acetic acid complexes in nonpolar solvents have been studied by means of absorption and emission titration experiments. Supplementary support of the stoichiometric ratio and structure for the hydrogen-bonding formation was provided by molecular design and syntheses of various β-CB analogues incorporating either only one hydrogen bonding site or dual hydrogen bonding sites where interplay between two sites are sterically prohibited. The results in combination with time-resolved measurements and theoretical approaches suggest the 1:2 β-CB/acetic acid complex with a structure of triple hydrogen bonding formation to be responsible for the excited-state proton-transfer tautomerism in cyclohexane. The proton transfer time is beyond the response limit of the detecting system of 15 ps, indicating that only a negligibly small geometry adjustment is required for the guest molecule (i.e., acetic acid) to a correct geometry, i.e., a hydrogen-bond relay configuration, for the triple proton transfer to proceed. In comparison, for the 1:1 β-CB/acetic acid non-hydrogen-bond relayed complexes, amino−imino tautomerism is prohibited during the excited-state lifetime, giving rise to a normal Stokes shifted emission. The results provide detailed ground-state thermodynamics of β-CB HB complexes as well as the dynamics of proton-transfer tautomerism mediated by the hydrogen-bonding structures.

1. Introduction

Spectroscopy and dynamics of host/guest hydrogen-bonding (HB) type of excited-state proton-transfer reaction have long received considerable attention. Prototypes include 7-azaindoles,[1−11] 7-hydroxyquinolines,[12−20] 2-(2′-pyridyl)indoles,[21−24] mono- and di-pyrido[2,3a] carbazoles[25−29] and β-carbolines[30−40] where the excited-state proton-transfer (ESPT) tautomerism is mediated either by self-association or by adding guest molecules (including solvents) forming host/guest types of HB complexes. From the structural viewpoint, the proton donating and accepting sites can be adjacent to each other so that a complex possessing intact dual hydrogen bonds is formed through a perfect-fitted, 1:1 (host: guest) stoichiometric ratio in the ground state. For example, the conjugated dual hydrogen bonding (CDHB) effect[41] in the case of 1:1 7-azaindole (7AI)/acetic acid complex mutually induces the π electron charge transfer from the pyrrolic nitrogen to the pyridinic nitrogen (see Figure 1), resulting in additional stabilization energy. As a result, an association enthalpy of −14 kcal/mol has been reported.[3] Upon electronic excitation, the 7AI/guest CDHB complex undergoes an ultrafast rate of double proton transfer (e.g., ∼1 ps^{-1} in the case of 7AI dimer[8,9]), resulting in an imine-like tautomer fluorescence. In contrast, for other type of systems the proton donating and accepting sites may be far separated from each other. Thus, the occurrence of ESPT possibly incorporates *n* (n ≥ 2) guest molecules. A prototype example is β-carboline (β-CB, see Figure 2).[42] From the molecular structure point of view β-CB is composed of a π-electron deficient pyridinic ring fused to a π-electron excessive indole ring and has been shown to reveal interesting photophysical properties. In the ground state, the electron charge density in the pyrrolic ring was calculated to be higher than its adjacent benzene and pyridine moieties. Upon $S_0 \rightarrow S_1$ (π, π^*) excitation, the electronic charge density migrates to the pyridinic nitrogen,[30] resulting in a drastic change in the acid−base property.[31,32] In water and alcohols, equilibrium among the neutral, cation and zwitterion was observed in the excited state.[32−34] In aprotic solvents such as dichloromethane and dioxane, experiments have been performed to study the photophysical properties of the β-CB/acetic acid complex.[35−37] Unfortunately, the requirement of a large amount of acetic acid due to the weaker HB association makes the thermodynamics as well as microsolvation studies of the HB formation inaccessible. Furthermore, the requirement of a large fraction of acetic acid creates an inhomogeneous local polar environment, which induces an additional competitive channel based on the protonation of β-CB. As a result, spectra and dynamics were complicated by multiple fluorescence, consisting of neutral, cation and zwitterionic species. Conversely, studies of β-CB/

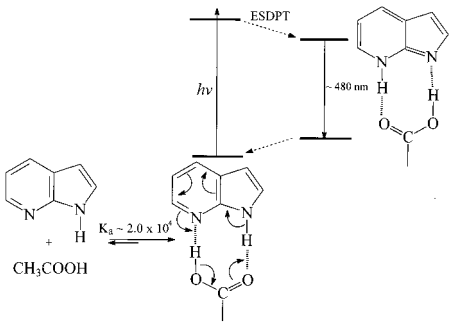

Figure 1. Conjugated dual hydrogen-bonding (CDHB) formation for 7AI, its associated redistribution of the electron density and the corresponding ESDPT reaction in cyclohexane.

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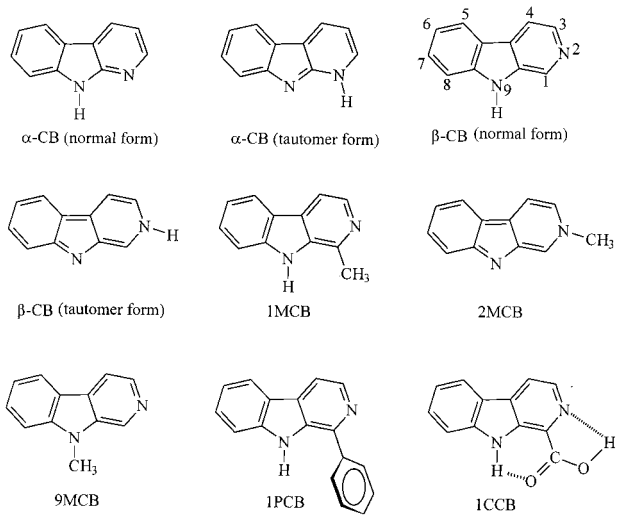


Figure 2. Structures of the proton-transfer isomers and derivatives of β-CB.

guest HB complexes in nonpolar, hydrocarbon solvents such as cyclohexane are expected to provide more detailed insight regarding the guest molecule coupled ESPT reaction. Recently, ground-state HB formation and phototautomerism of 1-methyl-β-carboline in cyclohexane titrated by the guest molecule have been reported by Balón and co-workers.[38−40] In their series of papers, comprehensive studies have been performed to differentiate the proton donor and acceptor guest molecules affecting the HB formation as well as the corresponding excited-state proton-transfer reaction.

In this study, acetic acid catalyzed excited-state amino-imino tautomerism for β-CB and its analogues in nonpolar solvents has been investigated. Thermodynamics and microsolvation (i.e., stoichiometry of the complex formation) of various β-CB/acetic acid complexes in cyclohexane have been studied by means of absorption and emission titration experiments. Owing to the minimization of solvent perturbation the results have been simulated by theoretical approaches. Supplementary support of the stoichiometric ratio and structure for the hydrogen-bonding formation was provided by molecular design and syntheses of various β-CB analogues incorporating either only one hydrogen bonding site or dual hydrogen bonding sites where interplay between two sites are sterically prohibited. The results in combination with dynamic studies render details in terms of thermodynamics, microsolvation as well as ESPT dynamics of β-CB/acetic acid HB complexes.

2. Experimental Section

2.1 Materials. Figure 2 depicts various structures of commercially available and/or synthesized β-CB derivatives. β-CB (Aldrich) and 1-methyl-β-carboline (1MCB,[43] Aldrich) were purified by column chromatography (eluent 1:3 (v/v) *n*-hexane: ethyl acetate) followed by recrystallization from acetonitrile. α-Carboline[44] was synthesized according to the previously reported method.[45] The final product was twice recrystallized from spectrograde ethanol and once from cyclohexane. The purity of these compounds was checked by fluorescence excitation spectra in dilute solution of $<1.0 \times 10^{-5}$ M where only monomer exists.

Synthesis of 9-*Methyl*-β-*carboline* ($C_{12}H_{10}N_2$, 9*MCB*). 9MCB was synthesized by adding sodium hydride (0.5 g) to the THF solution containing β-CB (0.1 g) followed by the addition of methyl iodide (0.15 g). The resulting product was purified by column chromatography (eluent 1:1 (v/v) ethyl acetate: hexanes). H^1NMR ($CDCl_3$, 200 MHz), δ 4.36 (s, 3H), 6.91 (d, $J =$ 6.8 Hz, 1H), 7.09 (d, $J =$ 6.8 Hz, 1H), 7.30−7.53 (m, 2H), 7.95 (d, $J =$ 6.8 Hz, 1H), 8.45 (d, $J =$ 6.8 Hz, 1H), 8.67 (s, 1H).

Synthesis of 2-*Methyl*-2*H*-β-*carboline* ($C_{12}H_{10}N_2$, 2*MCB*). 2MCB was synthesized by the reaction of β-CB (0.1 g) and methyl iodide (1.0 g) in THF under N_2 atmosphere. NaOH (2.5 N,10 mL) was then added and the mixture was stirred for∼20 min to obtain product which was further purified by column chromatography (eluent 1:4 (v/v) ethyl acetate: hexanes). H^1-NMR ($CDCl_3$, 200 MHz), δ 4.37 (s, 3H), 7.24 (t, $J =$ 14.0 Hz, 1H), 7.62 (t, $J =$ 14.0 Hz, 1H), 7.64 (d, $J =$ 6 Hz, 1H), 7.89 (d, $J =$ 8 Hz, 1H), 8.18 (d, $J =$ 6 Hz, 1H), 8.32 (d, $J =$ 6 Hz, 1H), 9.01 (s, 1H).

Synthesis of 1-*Phenyl*-1,2,3,4-*tetrahydro*-β-*carboline* ($C_{17}H_{16}N_2$, 1*PTCB*). 1PTCB, a precursor to synthesize 1-phenyl-β-carboline, was synthesized according to the Pictet−Spengler type of reaction.[46] Briefly, benzaldehyde (20 mmol) was added in one portion to the stirred mixture containing tetra tryptamine (5 mmol in 50 mL dichloromethane) and trifluoroacetic acid (5 wt % in aqueous solution) at room temperature. The reaction was allowed to proceed until tryptamine was completely consumed as monitored by TLC (∼2−4 h). Upon completion of the reaction, the reaction mixture was concentrated to dryness under reduced pressure and then purified by column chromatography using ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$), δ 2.91−3.27 (m, 4H), 5.58 (s, 1H), 7.13−7.23 (m, 4H), 7.29−7.38 (m, 4H).

Synthesis of 1-*Phenyl*-β-*carboline* ($C_{17}H_{12}N_2$, *IPCB*). A solution of 1PTCB (2 mmol) in dioxane (75 mL) was refluxed for 4 h in the presence of 2,3-dichloro-5, 6-dicyano-1, 4-benzoquinone (4 mmol). The reaction mixture was cooled to room temperature, and concentrated to dryness under reduced pressure to obtain 1PCB. ^{1}H NMR (400 MHz, CD_3OD), δ 7.39 (m, H), 7.66−7.71 (m, 5H), 7.92−7.98 (m, 2H), 8.38 (d, $J =$ 8.16 Hz, 1H), 8.44 (d, $J =$ 5.92 Hz, 1H), 8.50 (d, $J =$ 5.92 Hz, 1H).

Synthesis of 1-*Carboxylic*-β-*CB* ($C_{12}H_8N_2O_2$, 1*CCB*): 1-Methyl-β-carboline (0.5 mmol), potassium permanganate (2 mmol) and dicyclohexyl-18-crown-6 (0.5 mmol) in benzene (20 mL) were stirred in a ball mill for 72 h. The product potassium benzoate was filtered along with manganese dioxide and then was dissolved in 5% sodium hydride solution. After filtration of manganese dioxide the aqueous solution was extracted with ethyl ether to remove traces of crown ether followed by the acidification to pH $=$ 6 to obtain 1CCB. ^{1}H NMR (200 MHz, $CDCl_3$), δ 7.48−7.55 (m, 2H), 7.69 (d, $J =$ 6.4 Hz, 1H), 7.93 (t, $J =$ 7.6 Hz, 1H), 8.12 (d, $J =$ 2.2 Hz, 1H), 8.37 (d, $J =$ 2.2 Hz, 1H), 9.21 (s, 1H).

Acetic acid (ACID, Merck Inc.) was purified according to the previously described method.[47] Cyclohexane (Merck Inc.) was of spectrograde quality and was refluxed several hours over calcium hydride under a nitrogen atmosphere and was transferred, prior to use, through distillation to the sample cell.

2.2 Measurements. Steady-state absorption and emission spectra were recorded by a Cary 3E (Varian) spectrophotometer and a F4500 (Hitachi) fluorimeter, respectively. Both wavelength-dependent excitation and emission response of the fluorimeter have been calibrated according to a previously reported method.[47] Because the titration experiment is critical on the standpoint of the interpreting hydrogen-bonding equilibrium (vide infra), we have carefully performed our absorption and fluorescence titration studies where each data point was taken by the average of three to five measurements. The sensitivity for the absorption

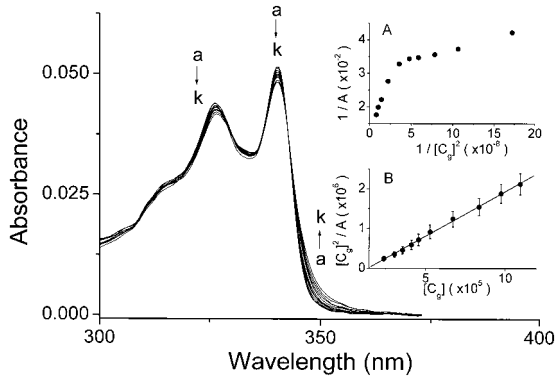


Figure 3. Concentration-dependent absorption spectra of β-CB (6.5×10^{-6} M) in cyclohexane at various ACID concentrations $[C_g^0]$ of (a) 0, (b) 3.3×10^{-5}, (c) 6.7×10^{-5}, (d) 1.0×10^{-4}, (e) 1.7×10^{-4}, (f) 2.6×10^{-4}, (g) 4.0×10^{-4}, (h) 5.0×10^{-4}, (i) 7.0×10^{-4}, (j) 8.0×10^{-4}, and (k) 1.0×10^{-3} M. Insert A: Plot of $1/A_{350}$ vs $1/[C_g]^2$ assuming the existence of 1:2 β-CB/ACID complexes exclusively in equilibrium, B. Plot of $[C_g]^2/A_{350}$ vs $[C_g]$. Note that $[C_g]$ depicted in the plot has been corrected by using eq 4 from the originally prepared $[C_g^0]$. Similar notation of ACID was used in the rest of the figure captions.

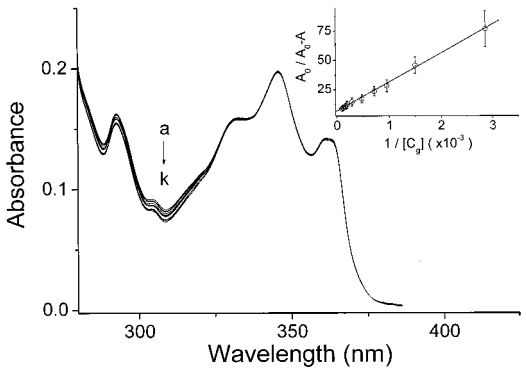


Figure 4. Concentration-dependent absorption spectra of 9MCB (7.8×10^{-6} M) in cyclohexane at various ACID concentrations $[C_g^0]$ of (a) 0, (b) 3.5×10^{-4}, (c) 6.7×10^{-4}, (d) 1.1×10^{-3}, (e) 1.4×10^{-3}, (f) 2.1×10^{-3}, (g) 3.5×10^{-3}, (h) 5.3×10^{-3}, (i) 6.3×10^{-3}, (j) 8.4×10^{-3}, and (k) 1.1×10^{-2} M. Insert: Plot of $A_0/(A_0 - A)$ vs $1/[C_g]$ at 310 nm and its best linear least-squares fitted curve.

measurement is approximately 2×10^{-4} in absorbance under constant temperature (25 ± 0.1 °C) throughout the measurement. Picosecond lifetime measurements were achieved by using either the second or third harmonic of a femtosecond Ti−Sapphire oscillator (82 MHz, 100 fs) as an excitation source. An Edinburgh OB 900-L time-correlated single photon counter was used as a detecting system. The fluorescence decays were analyzed by the sum of exponential functions with an iterative convolution method reported previously.[48] This procedure allows partial removal of the instrument time broadening and consequently renders a temporal resolution of ~15 ps.

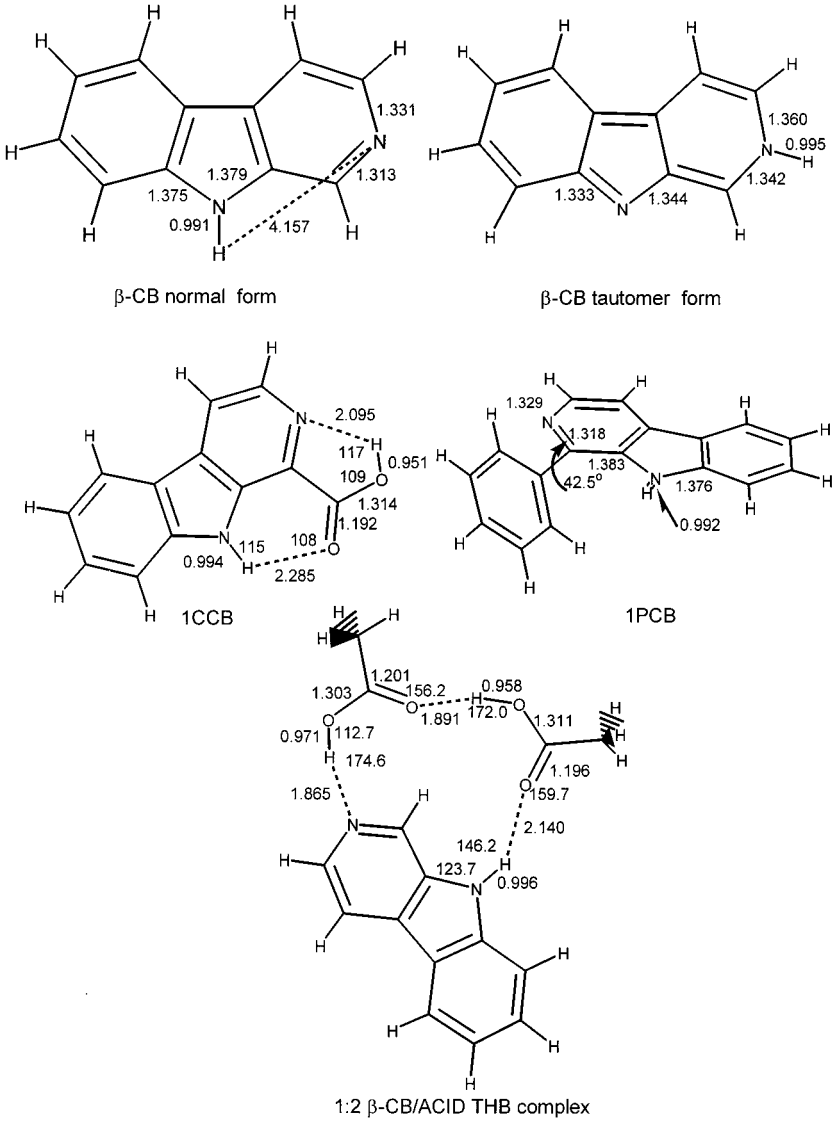
2.3 Theoretical Calculations. Gaussian 98 Rev D.3 programs were used to perform the ab initio calculation on the molecular structure. Geometry optimizations for all structures were carried out with the 6-31G(d, p) basis set at the Hartree−Fock (HF) level. This basis set has proven to be suitable for the dimer (or complex) formation incorporating hydrogen bonding formation.[49] Hessians, and hence vibrational frequencies were also performed to check whether the optimized geometrical structure for those dimeric and complex forms is at an energy minimum, transition state, or higher order saddle point. The directly calculated zero-point vibrational energies were scaled by 0.9181[50] to account for the overestimation of vibrational frequencies at the HF level. Because of the large HB systems being studied, a counterpoise correction has been incorporated to account for deviations from the basis-set superposition (BSSE).[51]

3. Results

3.1 Absorption Titration Studies. Unlike α-carboline (α-CB, see Figure 2), an isomer of β-CB with the interchange of C(1) and N(2) positions, which exhibits significant dimerization formation with a self-association constant of as high as ~1.8×10^3 M^{-1},[52−54] the absorption spectra ($\lambda_{max} \approx 340$ nm, $\epsilon_{340} \approx 7690$ M^{-1} cm^{-1}) of β-CB reveal concentration independence in cyclohexane, indicating negligible HB association. Molecular geometry analyses presented in the following section of theoretical approaches indicate that the formation of β-CB cyclic dual hydrogen-bonded dimer is thermally unfavorable due to a rise in the repulsion energy between two C(1)-hydrogen atoms toward dimerization (vide infra). Figure 3 shows the absorption spectra of β-CB upon adding various concentrations of ACID in cyclohexane. Although self-dimerization is negligible the initial concentration of β-CB, C_0, was still prepared to be as low as 6.5×10^{-6} M in this experiment to simplify further derivation of equilibrium constants among various hydrogen-bonded species. The formation of hydrogen-bonded complexes between β-CB and ACID can be clearly shown by the growth of an absorption shoulder at > 340 nm throughout the titration. The appearance of isosbestic points located at ~340 and 287 nm indicates the existence of equilibrium with common intermediates.

According to the molecular structural analysis β-CB provides two hydrogen-bonding sites. Therefore, the HB formation of β-CB incorporating ACID molecules may stoichiometrically be in the form of 1:1 and 1:2 β-CB/ACID complexes. The far separation between two HB sites (see Scheme 1 for the optimized structure) eliminates the possibility of forming a 1:1 cyclic dual HB β-CB/ACID complex that has been widely accepted in 7AI analogues[1−3,8−11] as well as in α-CB.[52] We first make an attempt to analyze the results incorporating exclusively only one hydrogen-bonding site. Empirically, the hydrogen-bonding strength between pyridinic nitrogen and carboxylic hydrogen should be stronger than that between the pyrrolic hydrogen and carbonyl oxygen due to their corresponding higher acidity and basicity. The weaker hydrogen bond in the pyrrolic hydrogen site can also be supported by the negligible spectral change of carbazole in the same range of added ACID concentrations (not shown here). Further support for the correlation between hydrogen-bonding strength and acid−base property of the proton donor and acceptor will be elaborated in the later section. In a comparative study, upon increasing the ACID concentration the model compound 9MCB where the pyridinic nitrogen is the only hydrogen bonding site revealed a small spectral change (<3 nm) in the $S_0 \rightarrow S_1$ ($\pi\pi^*$, 330−360 nm) absorption region (see Figure 4). On the basis of the structural similarity we therefore tentatively conclude that the substantial change of the β-CB $S_0 \rightarrow S_1$ ($\pi\pi^*$) absorption during the ACID titration cannot be mainly attributed to the formation of the 1:1 β-CB/ACID complex. The results on one hand may indicate that there exists a single hydrogen-bond formation between β-CB and ACID, but the redistribution of the electron density induced by the single hydrogen bonding effect is small so that the resulting $S_0 \rightarrow S_1$ ($\pi\pi^*$) absorption remains nearly unchanged during the titration. On the other hand, it is also plausible that the formation constant of 1:1 β-CB/ACID complex is small so that the concentration of the 1:1 HB complex is not large enough to cause appreciable spectral change within the

SCHEME 1: Optimized Geometries Based on the HF/6-31G(d, p) Basis Set (in Å and Degrees, Only Critical Angles Are Shown) for the Ground States of β-CB, 1CCB, 1PCB, and 1:2 β-CB/ACID THB Complex



added acetic acid concentrations. The former explanation can be supported by a nonnegligible spectral change of 9MCB at the $S_0 \rightarrow S_n$ ($n > 1$) absorption region of ~280−320 nm upon the titration of acetic acid. On the basis of the formation of a 1:1 9MCB/ACID complex the relationship between the measured absorbance at a selected wavelength where both monomer and 1:1 9MCB/ACID HB complex absorb (e.g., 310 nm) as a function of the initially prepared ACID concentration, $[C_g]$, can be expressed by

$$\frac{A_0}{A_0 - A} = \left(\frac{\epsilon_M}{\epsilon_M - \epsilon_C}\right)\left[\frac{1}{K_a[C_g]} + 1\right] \qquad (1)$$

where ϵ_M and ϵ_C are molar extinction coefficients of the 9MCB monomer and hydrogen-bonded complex monitored at a specific wavelength, respectively. A detailed derivation of (**1**) has been elaborated on ref 10b. The insert of Figure 4 shows the plot of $A_0/(A_0 - A)$ as a function of $1/[C_g]$ at a selected wavelength of 310 nm where the change of the spectral features is significant upon adding ACID. Straight-line behavior supports the validity of the assumption of 1:1 9MCB/ACID formation, and a linear least-squares fit using eq. 1 deduces a K_a value of $(2.2 \pm 0.5) \times 10^2$ M^{-1}. It is therefore reasonable to predict that the 1:1 β-CB/ACID complex, if it exists, should also have a similar K_a value due to its structural similarity with respect to the 1:1 9MCB/ACID complex. The existence of a nonnegligible amount of the 1:1 β-CB/ACID HB complex can also be supported by a nonlinear plot of the reciprocal of absorbance at > 350 nm vs $1/[C_g]^2$ (see insert A of Figure 3). Theoretically, the plot should be linear if equilibrium exists exclusively between uncomplexed β-CB and 1:2 β-CB/ACID HB complex.

The above results lead us to suggest a competitive equilibrium between 1:1 and 1:2 β-CB/ACID complexes. In addition, one has to consider the self-association of acetic acid due to its large dimerization constant in cyclohexane. The corresponding multiple equilibrium in solution can thus be depicted as follows

$$\beta\text{-CB} + \text{ACID} \overset{K_1}{\rightleftharpoons} \beta\text{-CB/ACID}$$

$$\beta\text{-CB/ACID} + \text{ACID} \overset{K_2}{\rightleftharpoons} \beta\text{-CB/(ACID)}_2$$

$$2\text{ACID} \overset{K_3}{\rightleftharpoons} (\text{ACID})_2$$

The acetic acid concentration used in this study is in an excess amount relative to β-CB. Consequently, the equilibrium con-

centration of 1:1 β-CB/ACID complex ($[C_{1:1}]$), 1:2 β-CB/ACID complex ($[C_{1:2}]$), ACID self-associated dimer and free ACID ($[C_g]$) simultaneously existing in the solution can be derived and expressed by eq (2)−(4), respectively

$$\frac{[C_g]}{[C_{1:1}]} = \frac{K_2}{[C_0]}[C_g]^2 + \frac{1}{[C_0]}[C_g] + \frac{1}{[C_0]K_1} \quad (2)$$

$$\frac{[C_g]^2}{[C_{1:2}]} = \frac{1}{[C_0]}[C_g]^2 + \frac{1}{K_2[C_0]}[C_g] + \frac{1}{K_1K_2[C_0]} \quad (3)$$

$$[C_g] = [C_g{}^0] - \left[\frac{(4K_3[C_g{}^0]+1) - \sqrt{8K_3[C_g{}^0]+1}}{4K_3}\right] \quad (4)$$

where $[C_0]$ denotes the initially prepared concentration of β-CB, $[C_g^0]$ is the initially added acetic acid concentration, $[C_g]$ represents the apparent concentration that has been corrected by considering the self-association of acetic acid in cyclohexane. In *n*-heptane the self-association constant of acetic acid K_3 has been reported to be 3.7×10^4 M^{-1} [55] which was used in the case of cyclohexane due to their similar solvent polarity. It has been concluded that the growth of the absorbance at >340 nm mainly originates from the 1:2 β-CB/ACID complex where the resonance induction effect through a relay of hydrogen bonds alters the electron density distribution. Incorporating the Beer−Lambert law with an absorption cell of 1 cm path length, eq 3 associated with the existence of 1:2 β-CB/ACID complex can be rewritten as

$$\frac{[C_g]^2}{A_{350}} = \frac{1}{\epsilon_{350}[C_0]}[C_g]^2 + \frac{1}{\epsilon_{350}K_2[C_0]}[C_g] + \frac{1}{\epsilon_{350}K_1K_2[C_0]} \quad (5)$$

In eq 5 A_{350} denotes the absorbance at 350 nm and is mainly attributed to the absorbance of the 1:2 β-CB/ACID complex. We have made an attempt to plot $[C_g]^2/A_{350}$ vs $[C_g]$. Interestingly, instead of a quadratic plot between $[C_g]^2/A_{350}$ and $[C_g]$ which is expected from eq 5, the results reveal near straight-line behavior (see insert B of Figure 3). A tentative but rational explanation is that the coefficient $1/K_2$ in front of the $[C_g]$ term is much larger than $[C_g]$ in the studied ACID concentrations so that the quadratic term associated with $[C_g]^2$ can be neglected. $[C_g]$ applied in the absorption titration experiment of β-CB is typically within 2.4×10^{-5} and 1.1×10^{-4} M. To fulfill the requirement of $1/K_2 > [C_g]$, K_2 should be $\ll 9000$ M^{-1} to have a negligible contribution of the $[C_g]^2$ term. From another angle of view one can rewrite eq 3 to $\{1/[C_{1:2}] - 1/[C_0]\}[C_g]^2 = (1/K_2[C_0])[C_g] + 1/K_1K_2[C_0]$. Throughout the titration study the concentration of the 1:2 complex, $[C_{1:2}]$, may always be much less than $[C_0]$ when both added ACID concentration and association constants are relatively small. For this case $1/[C_{1:2}]$ is expected to be $\gg 1/[C_0]$ throughout the titration experiment, and the contribution of $(1/[C_0])[C_g]^2$ term in eq 5 can be neglected. According to insert B of Figure 3 the best linear fit gives a slope of 0.022 which is theoretically equivalent to $1/(\epsilon_{350}K_2[C_0])$ (see eq 5). Although ϵ_{350} is not known at this stage its value can be estimated qualitatively by the synthesis of 1-carboxylic-β-carboline (1CCB, see Figure 2 for the molecular structure). 1CCB exhibits an S_0-S_1 absorption band (> 320 nm) similar to that of the excitation spectrum of β-CB/ACID complex (see Figure 5, details will be discussed in the section of the fluorescence titration). The results indicate that the formation of two hydrogen bonds in 1CCB also mutually induce each other through a carboxylic resonance structure (see Scheme 1). Thus, the absorptivity of 1CCB at 350 nm can be qualitatively used to simulate that of the 1:2 β-CB/ACID complex, and a value of ϵ_{350} was then estimated to be 3600 M^{-1} cm^{-1}. Because C_0 was prepared to be 6.5×10^{-6} M, a value of 1945 ± 50 M^{-1} was deduced for K_2, consistent with the early assumption of $1/K_2 > [C_g]$ when $[C_g]$ was prepared to be within 2.4×10^{-5}− 1.1×10^{-4} M in the titration study. The result rationalizes the straight-line behavior obtained in insert B of Figure 3. Our results also revealed a deviation from straight-line behavior by plotting $[C_g]^2/A_{350}$ vs $[C_g]$ when $[C_g]$ was prepared higher than 2×10^{-3} M, indicating that the contribution from the $[C_g]^2$ term becomes significant. However, to prevent such a nonlinear plot and the possible cationic formation due to the accumulation of local polar environments,[35−37] low ACID concentrations of $<1.0 \times 10^{-3}$ M were prepared throughout the study. According to the linear behavior upon plotting $[C_g]^2/A_{350}$ vs $[C_g]$, K_1 was then extracted to be 180 ± 20 M^{-1} by dividing the slope with respect to the intercept, which is on the same magnitude as that of the K_a value of ~220 M^{-1} derived from the 1:1 9MCB/ACID complex.

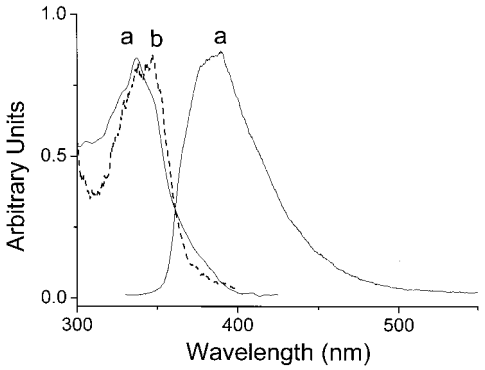


Figure 5. **a**. (—) Absorption and emission spectra of 1CCB in cyclohexane, **b**. (- -) the excitation spectra of β-CB monitored at 500 nm by adding ACID (5.0×10^{-4} M) in cyclohexane.

3.2 Fluorescence Titration Study. Table 1 lists the steady-state spectral properties of absorption and emission as well as relaxation dynamics for β-CB, β-CB/ACID HB species and its corresponding analogues in cyclohexane. Figure 6 shows the fluorescence spectra as a function of the added ACID concentration in cyclohexane. The uncomplexed β-CB (i.e., without ACID added) shows a normal Stokes shifted emission maximum at 360 nm of which the relaxation dynamics were well fit by single-exponential kinetics with a lifetime of 2.8 ± 0.2 ns ($\Phi_f \approx 0.2$). Upon increasing the ACID concentration, excitation at 350 nm, where the β-CB/ACID HB complexes absorb predominantly, results in an increase of dual emission maxima at 365 and 510 nm, respectively. The 365 nm band is defined as the F_1' component in order to distinguish it from the 360 nm band (the F_1 band) associated with the uncomplexed β-CB. The F_2 band (i.e., the 510 nm emission) exhibits emission-wavelength independent relaxation dynamics, which can be well fitted by a single-exponential decay rate of $\sim 1.7 \times 10^8$ s^{-1} ($\tau_f \approx 5.9$ ns), whereas the rise time is limited by the system response of 15 ps. In a comparative study the synthesized imino-tautomer analogue 2-methyl-2H-β-carboline (2MCB, see Figure 2) revealed a 538 nm emission ($\tau_f \approx 2.41$ ns) of which the spectral features (i.e., the emission maximum, fwhm, etc., see Figure 6) are similar to those of the F_2 band. The results unambiguously conclude that the 510 nm emission band is attributed to the tautomer emission resulting from ACID catalyzed N(9)−H to N(2) proton-transfer reaction. The ~25 nm blue shift of the F_2 band relative to 2MCB can be

TABLE 1: Thermodynamic and Photophysical Properties of β-CB and Its Methylated Derivatives, ACID Complexes and β-CB Analogues in Cyclohexane

	absorption λ_{max} (nm)	emission λ_{max} (nm)	Φ_{obs}	τ_f (ns)	K_1 or K_2 (M^{-1})[a] (298 K)
β-CB	326, 340	344, 360	0.20	2.80	
1MCB	325, 339	345, 359	0.27	2.58	
2MCB	338, 346, 464	538	0.017	2.41	
9MCB	332, 346, 361	365, 382, 402	0.30	2.24	
1PCB	340, 351	367, 380	0.034	1.33	
1CCB	338	389	0.14	2.05	
1:2 β-CB/ACID	347[b]	348, 365, 510	7.0×10^{-3}[e]	F_1: 2.7, F_1': 3.8 F_2: 5.9[f]	K_1: 165[c] K_2: 1.95×10^3
1:2 1MCB/ACID	327, 342[b]	363, 495	1.0×10^{-2}[e]		K_1: 120[c] K_2: 420
1:1 9MCB/ACID	330, 345, 360	367, 387, 407	–		K_1[d]: 220

[a] See text for the definition of K_1 and K_2. [b] Values were obtained from the fluorescence excitation spectrum. [c] Data were obtained from the fluorescence titration experiment. [d] K_1 is equivalent to K_a defined in the text. [e] The quantum yield of imine tautomer emission. [f] See text for the definition of F_1, F_1', and F_2.

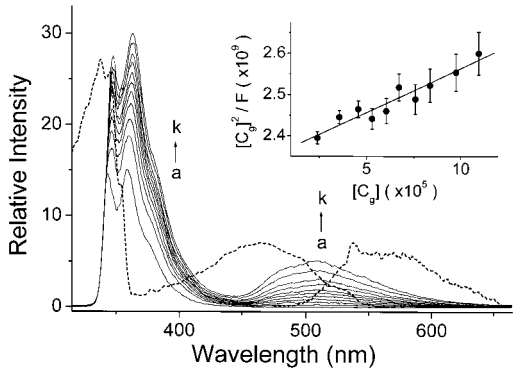


Figure 6. (—) Fluorescence spectra of β-CB in cyclohexane at various ACID concentrations $[C_g^0]$ of (a) 0, (b) 6.7×10^{-5}, (c) 1.3×10^{-4}, (d) 2.0×10^{-4}, (e) 2.6×10^{-4}, (f) 3.3×10^{-4}, (g) 4.0×10^{-4}, (h) 5.0×10^{-4}, (i) 6.0×10^{-4}, (j) 8.0×10^{-4}, and (k) 1.0×10^{-3} M. (- - -) Absorption and emission spectra of 2MCB (1×10^{-5} M) in cyclohexane. Insert: Plot of $[C_g]^2/F$ against $[C_g]$ at the F_2 band (520 nm).

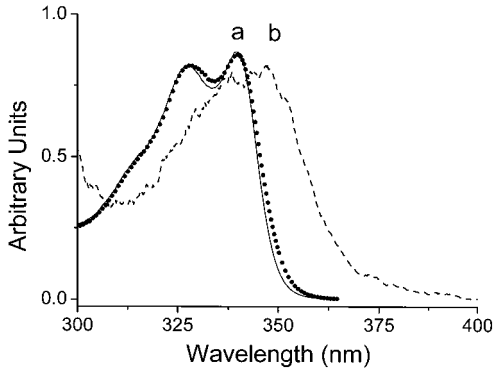


Figure 7. (—) Fluorescence excitation spectrum of the β-CB monomer (6.5×10^{-6} M) monitored at 370 nm. Fluorescence excitation spectra of β-CB in 5×10^{-4} M acetic acid monitored at **a**. (·····) 390 nm, **b**. (- - -) 530 nm.

rationalized by the hydrogen-bonding formation in the β-CB (tautomer)/ACID complexes, which is commonly observed in 7AI and its corresponding analogues.[3,10,11]

For the 1:1 β-CB/ACID complex, it has been concluded in an earlier section that the hydrogen-bonding pair consists of carboxylic hydrogen (in ACID) and pyridinic nitrogen (in β-CB). This configuration leads to the shortest distance between the carbonyl oxygen and pyrrolic proton calculated to be as far as ~5.0 Å (vide infra). Thus, it is very unlikely that the double proton transfer can take place through such a long-distance, orientation-specified migration within the excited-state life span. The system-response-limited (~15 ps) rise of the F_2 band also discounts the possibility of the proton-transfer reaction through the excited 1:1 β-CB/ACID complex in which an additional carboxylic acid has to migrate in to form a proton relay configuration, i.e., a precursor, for the proton transfer to proceed. Detailed discussion will be elaborated in the following section. Alternatively, because the increase of tautomer emission intensity correlates well with an increase of the absorbance at > 340 nm attributed to the 1:2 β-CB/ACID complex, it is more plausible that the ground-state precursor responsible for the proton transfer is ascribed to the 1:2 β-CB/ACID complex.

For a small concentration of the 1:2 β-CB/ACID complex formation the observed tautomer fluorescence intensity is theoretically proportional to the absorbance at e.g. 350 nm. Thus, eq 5 can be rewritten to give eq 6

$$\frac{[C_g]^2}{F_2} = \alpha\left[\frac{1}{\epsilon_{350}[C_0]}[C_g]^2 + \frac{1}{\epsilon_{350}K_2[C_0]}[C_g] + \frac{1}{\epsilon_{350}K_1K_2[C_0]}\right] \quad (6)$$

where α is the instrument factor, including sensitivity, alignment, etc., of the detecting system. As shown in Figure 6 the plot of $[C_g]^2/F_2$ vs $[C_g]$ revealed straight-line behavior. The results further support the absorption titration study concluding that the $(1/\epsilon_{350}[C_0])[C_g]^2$term in eq 6 can be neglected due to its small contribution in the studied ACID concentrations. Without an absolute α value K_2 cannot be extracted to make a comparison with that obtained from the absorption titration study. However, due to a better signal-to-noise ratio in the fluorescence titration study a more reliable K_1 value of ~165 M^{-1} has been deduced by dividing the slope with respect to the intercept.

The growth of the F_1' band upon increasing the acetic acid concentration is intriguing. Figure 7a and b show the excitation spectra of F_1' and F_2 bands, respectively, upon adding 5.0×10^{-4} M of acetic acid. In comparison, the excitation spectra are different in which the excitation spectrum monitored at the F_1' band is only slightly red shifted (<3 nm) with respect to that obtained from uncomplexed β-CB. In contrast, the excitation maximum of the F_2 band is shifted by as large as ~10 nm, indicating that F_1' and F_2 bands do not originate from a common ground-state species. This viewpoint can be further supported by a lifetime study. Detailed time-resolved studies indicate that F_1' and F_2 bands undergo different relaxation dynamics. In stead of a well fitted single-exponential decay rate of 1.7×10^8 s^{-1} for the F_2 band, an attempt to fit the F_1' band fitted by a single-exponential decay component rendered a χ value that deviated significantly from 1.0. Alternatively, it can be well fitted by a double exponential decay, which is theoretically expressed as

$$F(t) = A_1e^{-k_1t} + A_2e^{-k_2t}$$

where A_1 and A_2 are the emission intensity at $t \approx 0$ for the

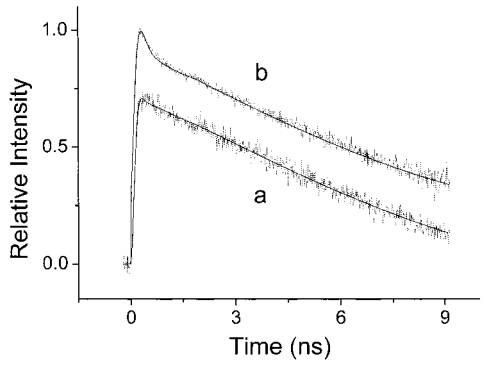


Figure 8. Time-dependent fluorescence of β-CB in cyclohexane at various ACID concentrations of **a**. 1.0×10^{-4} M, **b**. 5.0×10^{-3} M. the fluorescence intensity was monitored at 410 nm with a spectral bandwidth of ~5 nm.

decay components 1 and 2, respectively. Although the ratio for A_1 vs A_2 is concentration dependent, k_1 and k_2, within ACID concentrations of $1.0 \times 10^{-5}-2.0 \times 10^{-4}$ M, were found to be constant, with two close decay components of $(3.7 \pm 0.1) \times 10^8$ s^{-1}($\tau \approx 2.7$ ns) and $(2.6 \pm 0.2) \times 10^8$ s^{-1} ($\tau \approx 3.8$ ns), respectively. The rise time for both components cannot be resolved. The decay component with an τ_f of 2.7 ns is similar to the decay dynamics of the uncomplexed β-CB. In addition, the initial preexponential A_1 factor decreases upon increasing the ACID concentration. Thus, its assignment to the uncomplexed species is unambiguous. Accordingly, the 3.8 ns component, of which the A_2 factor increases as increasing the ACID concentration, should be associated with the β-CB/ACID complexes. Because the decay of 3.8 ns is much longer than the system-response-limited rise time of the F_2 band it is impossible to assign the F_1' band to be the precursor for the F_2 band. In addition, the assignment of the F_1' band to the β-CB cationic emission can be discarded due to its emission maximum of 365 nm that is drastically different from the reported cationic emission maximum at 450 nm in other organic solvents containing concentrated ACID.[35−37] Accordingly, in the titration study containing small ACID concentrations ($<10^{-3}$ M) it is reasonable to propose that the F_1' band results from an increase of the 1:1 β-CB/ACID HB complex in which the proton transfer is prohibited due to the far separation between ACID and pyrrolic hydrogen.

In this study, when the ACID concentration was prepared to be higher than 5×10^{-3} M, the contribution from an additional decay component gradually appeared. Figure 8a shows the decay of β-CB 410 nm emission ($\Delta\lambda \approx 5$ nm) upon adding 1.0×10^{-4} M ACID in cyclohexane, which reveals a major decay component resulting from the 1:1 β-CB/ACID HB complex. In comparison, upon adding the ACID concentration of as high as 5×10^{-3} M a fast component with a decay rate of 7.1×10^9 s^{-1} ($\tau_f \approx 140$ ps, see Figure 8b) appeared. The amplitude of the 140 ps decay component increases as the ACID concentration increases. In the strictly nonpolar, hydrocarbon solvents such as cyclohexane the assignment of this fast decay component to the protonated pyridinic nitrogen (β-CB)/carboxylate anion (ACID) ionpair emission is reasonable, which can be distinguished from the solvated β-CB cationic emission in the highly concentrated ACID and polar environment.[35−37] In the higher concentration of > 5% ACID by weight, similar to β-CB titrated by ACID in other organic solvents, a solvated cationic emission maximum at 450 nm gradually appeared. The high ACID concentration apparently makes the interpretation of thermodynamics (i.e., equilibrium) and dynamics of β-CB HB complexes more complicated and was hence avoided in this study.

4. Discussion

4.1 β-CB/Acetic Acid HB Structures. An intriguing question is immediately raised regarding the structure of the 1:2 β-CB/ACID HB complex. Because of the highly unfavorable entropy factor, large exothermicity is expected upon forming a stable 1:2 $\beta-$CB/ACID complex. We here tentatively propose that the structure of 1:2 β-CB/ACID complex be dominated by a triple-hydrogen-bonded (THB) configuration in which two ACID molecules forming hydrogen bonds with β-CB are linked to each other by an additional hydrogen bond (see Scheme 1). Although a direct experimental proof of the 1:2 β-CB/acetic acid cyclic hydrogen-bonded complex in the solution phase is not feasible at this stage, time-resolved studies and experiments on β-CB derivatives have provided indirect evidence to support this viewpoint. Global analyses of relaxation dynamics of the tautomer fluorescence have been carried out over the studied acetic acid concentrations. The results clearly revealed that independent of acetic acid concentrations the relaxation dynamics were well fitted by a single-exponential decay component. No negative preexponential factor can be resolved, indicating that the rate of acetic acid-catalyzed excited-state proton-transfer reaction is beyond our current system response of ~15 ps^{-1}. For the 1:2 β-CB/ACID noncyclic complex where two acid molecules are individually hydrogen bonded to the pyridinic nitrogen and pyrrolic hydrogen, respectively with a lack of relay configuration ESPT requires a diffusive migration of the acid molecules, forming a precursor for the proton transfer to take place. The time scale of diffusive migration, depending on the solvent viscosity, normally takes place within several hundred picoseconds or longer in the low and medium viscous solvents (for example, see the cases of 7-azaindole in alcohols[5,6]). Similar standpoint holds for the 1:2 β-CB/ACID noncyclic complex where the acetic acid molecule is hydrogen bonded with pyridinic nitrogen and the other acetic acid simultaneously. On the other hand, the possibility of proton-transfer mechanism where a 1:2 noncyclic β-CB/ACID complex is catalyzed by another guest (i.e., acetic acid) molecule is also considered. In this case the rate of proton transfer can be expressed as $k_{pt} = k_d$[guest] where the upper limit of k_d is assumed to be the diffusion-controlled rate. This proposal is discounted in the case of β-CB titrated by acetic acid. For a simplified approach, the tautomer emission was observed at the ACID concentration as low as 5×10^{-5} M. Taking k_d to be $\sim 2 \times 10^{10}$ M^{-1} s^{-1} in cyclohexane k_{pt} is calculated to be $\sim 10^6$ s^{-1}, which is too slow to compete with other nonproton-transfer decay processes and is contradictory to the observed ACID concentration independent, system-response-limited rise time of the tautomer emission. It is thus conceivable for us to propose the formation of a 1:2 β-CB/ACID cyclic complex possessing a THB structure where ESPT takes place through a conduit of relayed hydrogen bonds. A similar 1:2 (host/guest) cyclic THB complex has been proposed in the case of 7-hydoxyquinoline titrated by methanol.[16] For the 1:2 β-CB/ACID THB complex it is further proposed that the proton donor and acceptor sites are mutually induced through a conjugated triple hydrogen-bonding (CTHB) effect, resulting in a redistribution of electron density from indole to pyridine ring. As a result, a significant change in the spectral properties is expected on the $S_0 \rightarrow S_1$ ($\pi\pi^*$) absorption, consistent with the experimental results. This viewpoint is similar to that proposed in the case of 1:1 7AI/guest cyclic HB complexes in which the CDHB effect results in a drastic change of the absorption spectral features.[1−3] The linkage of the third hydrogen bond in combination with the CTHB effect stabilizes

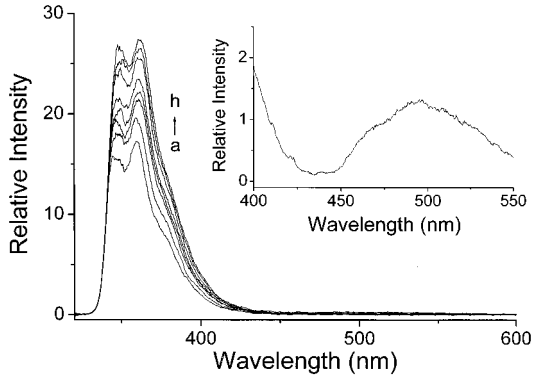


Figure 9. Fluorescence spectra of 1MCB in cyclohexane titrated by ACID in which the ACID concentration is prepared to be (a) 0, (b) 1.1×10^{-4}, (c) 2.1×10^{-4}, (d) 3.5×10^{-4}, (e) 4.2×10^{-4}, (f) 5.3×10^{-4}, (g) 6.5×10^{-4} and (h) 8.0×10^{-4} M Insert: A concentration of as high as 1.0×10^{-2} M has to be added in order to obtain an optimum tautomer emission intensity.

the 1:2 β-CB/ACID complex, explaining the relatively large K_2 value derived from the experimental results.

A strong support for the proposed CTHB effect can also be given by the synthesis of 1PCB (see Figure 2). The geometry optimized (6-31G(d, p)) structure of 1PCB reveals the C(1) substituted phenyl ring to be ~42.5° (see Scheme 1) with respect to the molecular plane of parent β-CB due to its steric effect with the α hydrogen. Although 1PCB provides dual hydrogen bonding sites the bulky phenyl substitution blocks any possible linkage between two ACID molecules (i.e., the formation of a third hydrogen bond). Experimentally, upon increasing the ACID concentration the results show negligible spectral change on the $S_0 \rightarrow S_1$ ($\pi\pi^*$, > 320 nm) absorption except for the increase of the absorptivity on the $S_0 \rightarrow S_n$ ($\pi\pi^*$, $n > 1$) absorption at 280−320 nm (not shown here), an indication of the possible 1:1 1PCB/ACID HB complex formation. Upon excitation the tautomer emission was completely obscured throughout the ACID titration experiment. Further evidence can be given by 1MCB where the methyl group, to a certain extent, hinders the formation of 1:2 1MCB/ACID THB complex. To achieve a similar spectral change as that of the 1:2 β-CB/ACID THB complex (Figure 3) more ACID concentration (i.e., 5.0×10^{-3} M) has to be added in the ACID titration study for 1MCB. Accordingly, a much smaller K_2 value (~420 M^{-1}) for the 1:2 1MCB/ACID formation has been deduced from the absorption titration study. The fluorescence titration study of 1MCB shown in Figure 9 revealed that the F_2 band was obscured at similar added ACID concentrations as in Figure 6. Higher ACID concentrations have to be added to obtain optimum F_2 emission (see insert of Figure 9). The result clearly indicates that a steric hindrance introduced by the phenyl or methyl group at the C(1) position either prohibits or hampers the THB formation. Further supplementary supports of the proposed HB structures are provided by the theoretical approaches discussed in the following section.

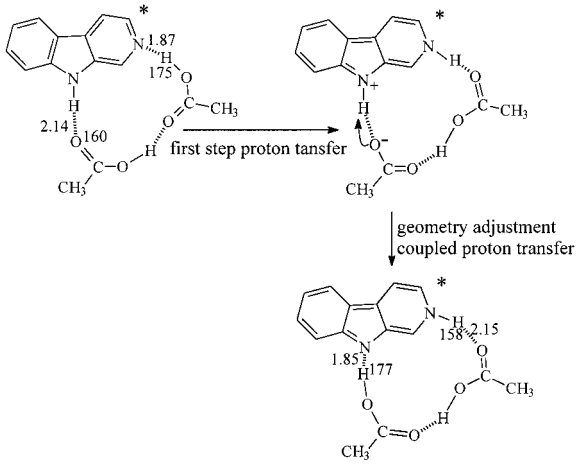
4.2 Theoretical Approach. The geometrically optimized (6-31G(d,p)) β-CB exists predominantly as an amino-like form (see Figure 2 and Scheme 1) which is more stable than its corresponding imino tautomer by ~24 kcal/mol. In comparison, the difference in free energy between amino and imino forms is calculated to be ~11.8 and 12.3 kcal/mol for α-CB and 7AI, respectively. The higher endergonic β-CB (amino form) → β-CB (imino form) tautomerism can be rationalized by destroying two aromatic rings upon forming the tautomer (i.e., imino) form, whereas it only requires the destruction of single aromaticity in the case of α-CB and 7AI (see Figure 2 for the comparison). Certainly, as proposed in several literature,[35−40] there is other possibility of forming a zwitterionic tautomer form that might possess a large dipolar change and thus can be stabilized in the polar solvents. Ab initio calculation focusing on the zwitterion formation incorporating the solvation effect is complicated and was not performed in this study. The calculation also reveals a far separated distance (4.157 Å, see Scheme 1) between proton donor (the indolic hydrogen) and acceptor (the pyridinic nitrogen) sites in β-CB (amino form). All attempts to resolve the dimeric form gave rise to imaginary vibrational frequencies, indicating that no stable dimeric form exists based on the ab inito approach (HF/6-31G(d, p) method). The failure in obtaining a geometry optimized β-CB CDHB dimeric form is possibly due to the buildup of an enormously large repulsion energy between two C(1)−H hydrogens when two β-CB molecules approach each other in a C_{2v} symmetry, rationalizing the failure in detecting any β-CB self-association in both absorption and fluorescence titration studies.

Unlike 7AI/ACID and α-CB/ACID hydrogen-bonded complexes incorporating a strong conjugated dual hydrogen-bonding effect, the formation of a 1:1 β-CB/ACID hydrogen-bonded complex, if it exists, should involve only a single hydrogen bond even though acetic acid possesses bifunctional hydrogen bonding sites. On the other hand, the far separation between proton donor and acceptor sites in β-CB leads to a favorable enthalpy factor toward the formation of 1:2 β-CB/ACID HB complexes. For the 1:2 β-CB/ACID complex possessing a THB configuration (see Scheme 1) the enthalpy of association ΔH_{ac} was calculated to be −20.78 kcal/mol. The strong triple hydrogen bond and its corresponding CTHB effect should be responsible for the highly exothermic reaction toward forming a relay type of HB complex. Although such a configuration requires a specific configuration of hydrogen bonds, the large exothermicity compensates a less favorable entropy effect. As a result, the free energy was calculated to be 2.5 kcal/mol lower than that of the free β-CB and ACIDs. Conversely, the 1:2 β-CB/ACID nonproton-relayed DHB complex is calculated to be ~9 kcal/mol less stable in enthalpy than that of the THB complex, and hence a free energy of 4.7 kcal/mol higher than that of the THB complex. The calculation also indicates that the 1:1 β-CB (pyridinic nitrogen)/ACID (carboxylic hydrogen) HB complex is more stable than the 1:1 β-CB (pyrrolic hydrogen)/ACID (carbonyl oxygen) HB complex by 3.5 kcal/mol. The result predicts the hydrogen-bonding site in the 1:1 ACID/β-CB complex to be located at the pyridinic nitrogen, consistent with the experimental result. Further evidence can be given by the similar enthalpy of association calculated between 1:1 β-CB (pyridinic nitrogen)/ ACID (carboxylic hydrogen) ($\Delta H_{ac} \approx -7.2$ kcal/mol) and 1:1 9MCB/ACID ($\Delta H_{ac} \approx -7.0$ kcal/mol) HB complexes. Note that 9MCB provides only one hydrogen-bonding site at pyridinic nitrogen. This site selective hydrogen-bonding formation can be rationalized qualitatively through the acid−base property in the formation of a hydrogen bond. According to the pH titration experiment the carboxylic hydrogen ($pK_a \approx 4.74$) is much more acidic than that of the pyrrolic hydrogen ($pK_a \approx 14.5$[56]) in β-CB. Conversely, the pyridinic nitrogen ($pK_b \approx 8.9$[58]) in β-CB is more basic than the carbonyl oxygen (C═O, $pK_b \approx 22.2$[58]). Treating the hydrogen-bonding formation as an acid (proton donor)-base (proton acceptor) type of reaction, the sum of pK_a and pK_b is equivalent to $-\log K_{eq}$ where K_{eq} denotes an equilibrium constant for the acid−base reaction. Hence, under negligible influence of the steric effect a lower $pK_a + pK_b$ value indicates a large

acid−base equilibrium constant, and the reaction favors the product formation, consistent with both experimental and theoretical approaches for the hydrogen-bonding structure of the β-CB/ACID complex. Note that this conclusion is qualitative because the hydrogen-bonded complex is in its local minimum energy where the proton is neither accepted nor donated completely. In addition, the correlation was made where the difference in $pK_a + pK_b$ between various hydrogen-bonding configurations is generally quite large (normally $\gg 5$). Once the difference becomes small the correlation may be discounted due to an uncertainty by applying the pK_a (or pK_b) value obtained in the aqueous solution to a nonpolar (or gas) environment. When the proton affinity between donor and acceptor is close enough, the formation of an unusually strong hydrogen bond has been reported in several enzymatic reactions and subsequently verified by theoretical approaches,[60−62] for which the aforementioned empirical correlation may no longer be valid. Fortunately, the proton affinity for various functional groups applied in the case of β-CB hydrogen-bonded complex is substantially different from each other, excluding the possibility of an unusually strong hydrogen-bonding formation.

4.3 Proton-Transfer Dynamics. Both experimental and theoretical approaches have drawn the conclusion that the increase of the proton-transfer tautomer emission is mainly associated with the formation of a 1:2 β-CB/ACID THB complex. On the basis of a nanopicosecond time-resolved study, the rate of excited-state proton transfer for the 1:2 β-CB/ACID THB complex was estimated to be > 15 ps^{-1} at room temperature. In the cases of the 7AI dimer or 7AI/ACID HB complex where cyclic dual hydrogen bonds are intrinsically formed, the double proton transfer in the excited state may only require a negligibly small displacement of the hydrogen atom and/or molecular skeleton. The rate of such a cooperative proton-transfer reaction, either taking place stepwise or simultaneously, is fast ($\sim$1 ps^{-1}) and perhaps mainly dominated by the proton tunneling mechanism.[8,9] In comparison, the occurrence of ESDPT in the 1:2 β-CB/ACID THB complex is intriguing from the viewpoint of the proton-transfer dynamics. Could the THB complex be in a perfect geometrical configuration so that triple proton transfer occurs instantaneously without any diffusive adjustment? Although this question still remains unanswered at this stage it is believed that a small geometrical adjustment (or even solvent perturbation) associated with the hydrogen-bonding-relay configuration might be crucial in dealing with the triple-hydrogen-bond relay type of proton transfer. This viewpoint has been supported by the synthesis of 1CCB. As shown in Scheme 1, the geometry optimized structure of 1CCB based on 6-31G(d, p) basis sets reveals the formation of intramolecular dual hydrogen bonds in a relay type of configuration. Such a configuration has been spectrally identified by its $S_0 \rightarrow S_1$ ($\pi\pi^*$) absorption spectrum which correlates well with the fluorescence excitation spectra of the imine-tautomer emission resulting from the THB complex (see Figure 5). Surprisingly, despite the relay type of dual hydrogen bonds where an excited state proton-transfer tautomerism is expected, only a normal Stokes shifted emission maximum at 390 nm was observed in 1CCB (see Figure 5). Detailed analyses indicate that the C═O·····H−N(9) hydrogen bond of $\sim$2.3 Å is much longer than that of 2.1 Å for the O−H·····N(2) site (see Scheme 1). Thus, ESPT may require a slight adjustment of the C═O·····H−N hydrogen bond to an optimum distance for the proton transfer reaction to proceed. Such an adjustment from an "incorrect" to a "correct" geometry may incorporate a large energy barrier due to the rigidity of the carboxylic group in 1CCB. Accordingly, the rate of proton transfer may be too slow to compete with other relaxation dynamics in the excited state. In comparison, the proton-relay configuration in the 1:2 β-CB/ACID THB complex is relatively much more flexible than 1CCB. As a result an ESPT mechanism incorporating triple proton transfer is proposed and depicted in Scheme 2. The π-electron excitation of the 2:1 β-CB (−N−)/ACID (−OH) THB complex mutually induces a charge redistribution of β-CB, resulting in an increase of the acidity and basicity for the pyrrolic hydrogen and pyridinic nitrogen, respectively. A ΔpK_a (pK_a*-pK_a) as much as −5.5 and 6.5 for pyrrolic hydrogen and protonated pyridinic nitrogen, respectively, has been reported.[32] The COOH·····N(2) hydrogen bond of $\sim$1.87 Å is much shorter than that of 2.14 Å for the C═O·····H−N(9) site (see Scheme 1). Whereas for the 1:2 β-CB (tautomer)/ACID THB complex the relative HB distance is reversed with COO−H·····(9) of 1.85 Å < C═O- - -H−N(2) of 2.15 Å (see Scheme 2). Thus, a triggering step for the ESDPT may incorporate the proton transfer through a preexisting β-CB(−N(2)−)/ACID(−OH) hydrogen bond, which leads to an increase of the basicity, i.e., a buildup of partial negative charge density, at the carbonyl oxygen site of the other ACID molecule through a proton relay. This in combination with the partial positive charge of the pyrrolic proton creates an electrostatic attraction, which acts as a driving force for a slight adjustment (or displacement) of both β-CB and ACID to a correct conformation. Thus, the second-step in the proton transfer can take place through a lowest potential energy surface. For this case, two ACID molecules linked with a hydrogen bond act as a proton bridge to achieve an autocatalytic ESPT process. This proposed mechanism incorporates hydrogen-bonding-relay coupled proton transfer (or tunneling) dynamics and, hence, is expected to reveal deuterium-isotope dependence. Unfortunately, upon deuteration on both β-CB and ACID, the rise time of the tautomer emission was still beyond the response of our photon counting system of $\sim$15 ps. Further study focusing on the β-CB proton-transfer dynamics in a pico-femtosecond time scale should be performed to verify the proposed mechanism.

SCHEME 2: Proposed Triple-proton-transfer Mechanism for the 1:2 β-CB/ACID THB Complex. *Indicates the Excited Singlet State ($^1\pi\pi^*$). Critical Bond Distances and Angles Are in Å and Degrees



5. Conclusion

In conclusion, we have studied thermodynamics of β-CB/ACID hydrogen-bonded complexes by means of absorption, fluorescence titration experiments. In cyclohexane, the ground-

state equilibrium for β-CB titrated by trace amounts of ACID consists of uncomplexed β-CB, 1:1 β-CB/ACID single HB and 1:2 β-CB/ACID complexes. Specific hydrogen-bonding sites and structures of the HB complex have been deduced by applying various derivatives of β-CB incorporating either only a hydrogen site or dual hydrogen bonding sites where interplay between two sites are sterically prohibited. The results in combination with time-resolved measurements and theoretical approaches suggest that the 1:2 β-CB/ACID complex consists of a triple-hydrogen-bonding formation where fast ESPT (<15 ps^{-1}) takes place through a conduit of relayed hydrogen bonds. For the 1:1 β-CB/ACID non-hydrogen-bond relayed complexes, amino−imino tautomerism is prohibited during the excited-state lifetime, giving rise to a normal Stokes shifted emission. The results provide detailed ground-state thermodynamics and dynamics of excited-state proton transfer tautomerism for β-CB/ACID HB complexes. Focus on the molecular dynamics approach of the β-CB/ACID hydrogen-bonding formation in cyclohexane is currently in progress in attempt to render more substantial evidence for the structures of β-CB/ACID HB complexes in cyclohexane.

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References and Notes

(1) Taylor, C. A.; El-Bayoumi, M. A.; Kasha, M. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 253.

(2) Ingham, K. C.; El-Bayoumi, M. A. *J. Am. Chem. Soc.* **1974**, *96*, 1674.

(3) (a) Chang, C. P.; Hwang, W. C.; Kuo, M. S.; Chou, P. T.; Clements, J. H. *J. Phys. Chem.* **1994**, *98*, 8801. (b) Chou, P. T.; Wei, C. Y.; Chang, C. P.; Chiu, C. H. *J. Am. Chem. Soc.* **1995**, *117*, 7259. (c) Chou, P. T.; Wei, C. Y.; Chang, C. P.; Kuo, M. S. *J. Phys. Chem.* **1995**, *99*, 11 994.

(4) Koijnenberg, J.; Huizer, A. H.; Varma, C. A. O. *J. Chem. Soc. Faraday Trans*. 2 **1988**, *84* (8), 1163.

(5) Moog, R. S.; Maroncelli, M. *J. Phys. Chem*. **1991**, *95*, 10 359. (b) Chapman, C. F.; Maroncelli, M. *J. Phys. Chem*. **1992**, *96*, 8430. (c) Mente, S.; Maroncelli, M. *J. Phys. Chem. A* **1998**, *102*, 3860.

(6) (a) Negrerie, M.; Gai, F.; Bellefeuille, S. M.; Petrich, J. W. *J. Phys. Chem.* **1991**, *95*, 8663. (b) Gai, F.; Chen, Y.; Petrich, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 8343. (c) Chen, Y.; Rich, R. L.; Gai, F.; Petrich, J. W. *J. Phys. Chem*. **1993**, *97*, 1770. (d) Smirnov, A. V.; English, D. S.; Rich, R. L.; Lane, J.; Teyton, L.; Schwabacher, A. W.; Luo, S.; Thornburg, R. W.; Petrich, J. W. *J. Phys. Chem.* **1997**, *101B*, 2758.

(7) Mente, S.; Maroncelli, M. *J. Phys. Chem. A* **1998**, *102*, 3860.

(8) Takeuchi, S.; Tahara, T. *J. Phys. Chem. A* **1998**, *102*, 7740.

(9) (a) Chachisvilis, M.; Fiebig, T.; Douhal, A.; Zewail, A. H. *J. Phys. Chem. A* **1998**, *102*, 669. (b) Fiebig, T.; Chachisvilis, M.; Manger, M.; Zewail, A. H.; Douhal, A.; Garcia-Ochoa, I.; de La Hoz Ayuso, A. *J. Phys. Chem. A* **1999**, *103*, 7419.

(10) (a) Chou, P. T.; Wei, C. Y.; Wu, G. R.; Chen, W. S. *J. Am. Chem. Soc*. **1999**, *121*, 12186. (b) Chou, P. T.; Wu, G. R.; Wei, C. Y.; Cheng, C. C.; Chang, C. P.; Hung, F. T. *J. Phys. Chem. B* **1999**, *103*, 10 042.

(11) (a) Chou, P. T.; Wu, G. R.; Wei, C. Y.; Cheng, C. C.; Chang, C. P.; Hung, F. T. *J. Phys. Chem. B* **2000**, *104*, 7818. (b) Chou, P. T.; Liao, J. H.; Wei, C. Y.; Yang, C. Y.; Yu, W. S.; Chou, Y. H. *J. Am. Chem. Soc.* **2000**, *122*, 986.

(12) Mason, S. F.; Philp, J.; Smith, B. E. *J. Chem. Soc. A* **1968**, 3051.

(13) Thistlethwaite, P. J.; Corkill, P. J. *Chem. Phys. Lett.* **1982**, *85*, 317.

(14) Thistlethwaite, P. J. *Chem. Phys. Lett.* **1983**, *96*, 509.

(15) Itoh, M.; Adachi, T.; Tokumura, K. *J. Am. Chem. Soc.* **1984**, *106*, 850.

(16) Konijnenberg, J.; Ekelmans, G. B.; Huizer, A. H.; Varma, C. A. G. O. *J. Chem. Soc. Faraday Trans. 2* **1989**, *85*, 39.

(17) Bohra, A.; Lavin, A.; Collins, S. *J. Phys. Chem.* **1994**, *98*, 11 424.

(18) Fang, W. H. *J. Am. Chem. Soc.* **1998**, *120*, 7568.

(19) García-Ochoa, I.; Bisht, P. B.; Sánchez, F.; Martinez-Atáz, E.; Santos, L.; Tripathi, H. B.; Douhal, A. *J. Phys. Chem. A* **1998**, *102*, 8871.

(20) Chou, P. T.; Wei, C. Y.; Wang, C. R. C.; Hung, F. T.; Chang, C. P. *J. Phys. Chem. A* **1999**, *103*, 1939.

(21) Rodríguez-Prieto, F.; Mosquera, M.; Novo, M. *J. Phys. Chem.* **1990**, *94*, 8536.

(22) Herbich, J.; Hung, C. Y.; Thummel, R. P.; Waluk, J. *J. Am. Chem. Soc.* **1996**, *118*, 3508.

(23) Kyrychenko, A.; Herbich, J.; Wu, F.; Thummel, R. P.; Waluk, J. *J. Am. Chem. Soc*. **2000**, *122*, 2818.

(24) Rios Rodríguez, M. C.; Mosquera, M.; Rodríguez-Prieto, F. *J. Phys. Chem. A* **2001**, *ASPA*.

(25) Kyrychenko, A.; Herbich, J.; Izydorzak, M.; Wu, F.; Thummel, R. P.; Waluk, J. *J. Am. Chem. Soc.* **1999**, *121*, 11 179.

(26) Marks, D.; Zhang, H.; Borowicz, P.; Waluk, J.; Glasbeek, M. *J. Phys. Chem. A* **2000**, *104*, 7167.

(27) De Valle, J. C.; Dominguez, E.; Kasha, M. *J. Phys. Chem. A* **1999**, *103*, 2467.

(28) Kyrychenko, A.; Stepanenko, Y.; Waluk, J. *J. Phys. Chem. A* **2000**, *104*, 9542.

(29) Waluk, J. Conformational Aspects of Intra- and Intermolecular Excited State Proton Transfer In. *Conformational Analysis of Molecules in Excited States*; Waluk, J., Ed.; Wiley-VCH, 2000, and references therein.

(30) Draxler, S.; Lippitsch, M. E. *J. Phys. Chem.* **1993**, *97*, 11 493−11 496.

(31) Ghiggino, K. P.; Skilton, P. F.; Thistlethwaite, P. J. *J. Photochem.* **1985**, *31*, 113.

(32) Balón, M.; Hidalgo, J.; Guardado, P.; Muñoz, M. A.; Carmona, C. *J. Chem. Soc. Perkin Trans. 2* **1993**, 99.

(33) Varela, A. P.; Miguel, M. da G.; Macanita, A. L.; Burrows, H. D.; Becker, R. S. *J. Phys. Chem.* **1995**, *99*, 16 093.

(34) Dias, A.; Varela, A. P.; Miguel, M. da G.; Macanita, A. L.; Becker, R. S. *J. Phys. Chem.* **1992**, *96*, 10 290−10 296.

(35) Reyman, D.; Pardo, A.; Poyato, J. M. L. *J. Phys. Chem.* **1994**, *98*, 10 408−10 411.

(36) Reyman, D.; Viñas, M. H.; Poyato, J. M. L.; Pardo, A. *J. Phys. Chem. A* **1997**, *101*, 768.

(37) Reyman, D.; Viñas, M. H. *Chem. Phys. Lett.* **1999**, *301*, 551.

(38) Balón, M.; Muñoz, M. A.; Guardado, P.; Carmona, C. *Photochem. Photobiol*. **1996**, *64*, 531.

(39) Balón, M.; Carmona, C.; Guardado, P.; Muñoz, M. A. *Photochem. Photobiol*. **1998**, *67*, 414.

(40) Carmona, C.; Galán, M.; Angulo, G.; Muñoz, M. A.; Guardado, P.; Balón, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 5076.

(41) Chou, P. T.; Wei, C. Y.; Hung, F. T. *J. Phys. Chem. B* **1997**, *101*, 9119.

(42) Norharman is an alternative name for β-CB, which is commonly used in the literature. However, the name of β-CB is preferred due to a series of synthesized β-CB derivatives presented in this study. An IUPAC name 9H-pyrido[3,4-b]indole has also been used in Chemical Abstracts.

(43) Alternatively, harmane is a common name for 1-Methyl-β-carboline.

(44) 1-Azacarbazole has been commonly used in the literature. In this study the alternative name α-CB was applied in order to have a clear comparison with β-CB.

(45) Stephenson, L.; Warburton, W. K. *J. Chem. Soc.* **1970**, 1355.

(46) Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797.

(47) Wei, C. Y.; Yu, W. S.; Chou, P. T.; Hung, F. T.; Chang, C. P.; Lin, T. C. *J. Phys. Chem. B* **1998**, *102*, 1053.

(48) Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.

(49) (a) Wang, J.; Boyd, R. J. *Chem. Phys. Lett.* **1996**, *259*, 647. (b) Wang, J.; Boyd, R. J. *J. Phys. Chem.* **1996**, *100*, 16 141.

(50) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 1645.

(51) (a) Stewart, J. J. P. *J. Comput. Chem*. **1989**, *10*, 221. (b) Buemi, G.; Zuccarello, F.; Raudino, A. *THEOCHEM* **1988**, *41*, 379.

(52) Chang, C. P.; Yen, F. H.; Chou, P. T.; Wei, C. Y. *J. Chin. Chem. Soc.* **1996**, *43*, 463.

(53) Chang, C. P.; Shabestary, N.; El-Bayoumi, M. A. *Chem. Phys. Lett.* **1980**, *75*, 107.

(54) Waluk, J.; Grabowska, A.; Pakula, B.; Sepiol, J. *J. Phys. Chem.* **1984**, *88*, 1160.

(55) Pimentel, G. C., McClellan, A. L., Eds. *The Hydrogen Bond*; W. H. Freeman and Co.: **1960**, p 368.

(56) The pK_a value was obtained through the average of data obtained from ref 32 and ref 57.

(57) Perez, M. A. M.; Guzman, M. C. C.; Toledo, J. H.; Almeida, M. B. *J. Chem. Soc. Perkin Trans. 2* **1986**, 1573.

(58) These values are obtained from $pK_b = pK_{H_2O} - pK_a$ where pK_a can be obtained from the corresponding conjugated acid of pyridinic nitrogen (in β-CB[32]) and carbonyl oxygen (in ACID[59]).

(59) Gordon, Arnold, J.; Ford, R. A. *The Chemist's Companion*; John Wiley & Sons: New York, 1972.

(60) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11 552.

(61) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887.

(62) Frey, P. A.; Whitt, S. A.; Tobin, J. B. *Science* **1994**, *264*, 1927.