Solubilization of Pyridone-Based Fluorescent Tag by Complexation in Cucurbit[7]uril

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ABSTRACT: Aimed at further exploring the hosting properties of cucurbit[7]uril (CB7), we have exploited the spectroscopic and photophysical properties of a known fluorescent label as the guest molecule, namely, 3-cyano-6-(2-thienyl)-4-trifluoromethyl pyridine (TFP), in neat solvents. The formation of an inclusion host–guest complex with CB7 was checked by UV–vis absorption spectroscopy, and the value of binding constant (9.7 × 10^4 M^{-1}) was extracted from the spectrophotometric data. The modulation of keto–enol equilibrium in TFP by the local environment is governed by the interplay between dimerization through intermolecular hydrogen bonding between individual solute molecules, favoring the enol form, and intermolecular hydrogen bonding between TFP and the surrounding solvents, favoring the keto form. Time-resolved fluorescence results established that the macromolecular CB7 host stabilizes preferentially the neutral enol form over the keto form of TFP. Unprecedently, our results reveal a linear dependence of the amplitudes of the extracted decay-associated spectra from the time-resolved fluorescence spectra of TFP on the sum of polarity/polarizability and hydrogen bonding parameters of the local environment, confirming that TFP at micromolar concentration in the CB7 complexes is experiencing a methanol-like environment. The results rationalized the 42-fold enhancement in the solubility of TFP in water media by complexation in CB7.

1. INTRODUCTION

The modulation of emission properties, including emission maxima and lifetimes, of the guest molecules utilizing host–guest chemistry has become a popular approach in the literature, with applications in biomedicine, chemical sensing, and energy conversion,1–4 being a straightforward and inexpensive approach that bypasses the synthetic burdens and cyclodextrins (CDs),3,4 was exploited to prevent fluorescence quenching by dimer dissociation and protection against molecular aggregation.5,6 This has also led to alleviation of the solubility of potential guests in water at neutral pH and enhancement in their thermal and photochemical stability.7,8

The present study was specifically conducted to explore the effects of hydrogen bonding of excited states in CB host–guest inclusion complex molecules on the fluorescence of the guest molecules by analogy to those interactions in CD complexes.9 Fluorescence enhancement in several CB host–guest complexes has already been rationalized through the effects of host-induced polarity and rigidity (confinement) effects, assuming mostly electrostatic and hydrophobic non-covalent intermolecular interactions10–16 yet rarely hydrogen bonding effects were mentioned.17,18 Our research group, among others,10–21 has exploited non-covalent supramolecular interactions to enhance the emission of several guests inside the CDs and CBs in water, such as derivatives of coumarin drugs through hydrogen bonding interactions,19 benzimidazole drugs through ion–dipole interactions,20 and luciferin bioluminescent fluorophores through dipole–quadrupole interactions.21 We have also, among others, published other examples on fluorescence enhancement upon which the CB has suppressed photo-induced electron transfer22 or twisted-intramolecular charge transfer in some dyes.11,13,23

In the present work, 3-cyano-6-(2-thienyl)-4-trifluoromethyl pyridine (TFP)24 was specifically selected as a model dye that displays keto–enol equilibrium, with the aim of further exploring the hosting potential of cucurbit[7]uril (CB7) macrocycles (Figure 1) for enhancing the emission of the selected tag and consequently its water solubility. Supramolecular shifting of keto–enol equilibrium by CBs toward the keto forms was reported to be governed by either polarity21,25 or polarizability effects.26 However, the role of CB hydrogen bonding interactions in modulating keto–enol equilibria in the excited states has never been addressed to the best of our
knowledge. Not only CB7 shifts the keto–enol equilibrium in TFP toward the enol forms, but also it enhances both its emission and water solubility. Thus, our findings in the present work offer TFP as a probe to unfold more information on the structure and dynamics of different biological media, such as luciferase.21 Moreover, the TFP fluorophore itself has been employed to stain the mitochondria surface,22 to inhibit metallo-β-lactamase thus restoring the function of carbapenem antibiotics in the treatment of antibiotic-resistant bacteria,28 and to inhibit hepatitis C virus.29 The very low water solubility of TFP has limited its use because of formation of dots in the microscope.27

2. RESULTS AND DISCUSSION

2.1. Interaction of TFP with CB7 and pK_a Determination in Water. The inclusion of TFP by CB7 is evidenced by absorption and emission spectroscopic measurements in pure water. The acid-dissociation constant (pK_a) value for TFP was extracted from the evolution of the UV–vis absorption spectra of TFP as a function of pH (Figure 2; Figures S1 and S2 in the Supporting Information). The value has shifted by 1.3 units in the presence of CB7 (pK_a = 5.82 vs 7.11), because of the preferential binding of CB7 toward the neutral species over the anion forms.

The formation of an inclusion host–guest complex with CB7 at pH 3 was checked by UV–vis absorption spectroscopy, and the value of binding constant (9.7 × 10^5 M^-1) was extracted from the spectrophotometric data (Figure 3). Contrarily, a very weak binding was observed between CB7 and TFP at pH 11 (Figure S3 in the Supporting Information), confirming the preferential binding of CB7 toward the neutral species over the anion forms. Upon the addition of CB7 to neutral TFP, the absorption band around 400 nm was broadened and red-shifted (from 399 to 402 nm), while the emission intensity increased by 1.5-fold with no peak shift at about 465 nm (excitation at 395 nm). TFP has partially entered the cavity of CB7 from its thiophene ring in a 1:1 ratio as confirmed by the NMR titration of TFP by CB7 in 50% DMSO in water at pH 3 (Figure 4). All NMR peaks were shifted to lower ppm, except H-a proton, which remained unchanged.

2.2. Spectrophysics of TFP in Neat Solvents and CB7. Table 1 summarizes the collected major absorption and emission maxima for keto and enol structures (see Figure 5 and Figure S4 in the Supporting Information), along with the corresponding Stokes shifts in different neat solvents and inside the CB7 cavity. While the keto species absorbs and emits around 400 and 450 nm, the enol form has its absorption and emission bands centered about 365 and 400 nm. The results confirmed that a non-hydrogen bonding solvent, such as chloroform, stabilizes the enol form, whereas DMSO stabilizes the keto form. The 1H-NMR spectra were also measured for TFP at different concentrations in DMSO-d_6 (Figure S6). Assignments of the major peaks are fully explained in the Experimental Section. It transpires that TFP exists mainly in its keto form in this solvent, regardless of the concentration. Contrarily, the concentration-dependent NMR measurements in CDCl_3 (Figure S7) confirm that TFP’s structure has shifted mostly from enol to keto form upon lowering the concentration. Specifically, the singlet-broad peak, which belongs to proton H-a, appears to be most sensitive to the structure of the individual form. In CDCl_3 at high concentration, the NMR resonance of this proton appears at 6.9 ppm. However, the peak shifts to higher ppm, when the concentration of TFP was lowered in CDCl_3. The new position at 7.6 ppm was also observed in the NMR spectrum of TFP in DMSO-d_6 confirming the partial conversion of enol to keto form when the concentration of TFP is lowered in CDCl_3 because of dissociation of the enol dimer. Noticeably, neither the absorption/emission peak positions nor their intensities were sensitive to solvent properties, such as polarity, polarity/polarizability, and rigidity, which are manifested by several solvent parameters in Table 2, such as orientational polarizability (Δf), Kamlet and Taft (π*), and viscosity (η) parameters. The measured absorption and emission spectra were also not correlated with the hydrogen bonding donating (α) or accepting abilities (β) of the solvents. This means that any of these solvent parameters—polarity, polarizability, confinement, or hydrogen bonding effects—does not alone play a role in affecting the photophysical properties of TFP.

We turned to time-resolved emission studies being more useful for better understanding the origin of luminescence and assessing which solvent property controls the keto–enol equilibrium in TFP in the excited states at micromolar concentration. In the present study, the emission decays were measured every 10 nm across the entire emission spectra for each sample when excited at 375 nm. In the global analysis, the data measured at all wavelengths are fitted simultaneously by the sum of exponential decays convoluted with the instrument response function (IRF). The lifetimes are assumed to be the same across the whole data set, while the pre-exponential factors are left free. The estimated pre-exponential factors/
amplitudes represent the estimated spectra, with a lifetime given by the inverse of the rate constant of the exponential decay. The global analysis of the present data was performed utilizing Edinburgh FAST software, which uses a model of a number of parallel decaying components, assuming no kinetic growth. In this case, the estimated amplitudes at each wavelength are termed decay-associated spectra (DAS), representing the true spectra for each species originating from a different ground state. Specifically, DAS (Figure 6 and Figure S5 in the Supporting Information) were constructed from the extracted intensity-contribution fraction ($f_i$) calculated from the pre-exponential amplitudes ($B_i$) (see the Experimental Section). All emission traces were globally fitted to a triexponential model function after being convoluted with an IRF of $\sim 30$ ps. The measurements were carried out under nitrogen purging. The fluorescence lifetime experiments were repeated several times. The estimated experimental error was 4%.

Excited-state lifetimes alongside amplitudes and maxima of the extracted DAS are listed in Table 3. All observed DAS amplitudes were positive, confirming no sign of evolution in the excited state. The enol species emits at about 400 nm (DAS peak), whose excited-state lifetime is about 1−3 ns, whereas the keto form shows long and short-lived components with lifetimes of about 1−3 (DAS peak at 440 nm) and 0.1−0.5 ns (DAS maximum around 470 nm), respectively, both originating from a separate ground state. Further experiments are needed to explore the origin of the two transient keto species, yet the average lifetime was calculated in Table 4 (see the Experimental Section). To get an insight into the effect of solvent on the photophysical properties of TFP, the radiative

Figure 3. Binding titration of TFP with CB7 at pH 3: Panel (A) shows the evolution of the UV−vis absorption spectra of TFP (22 μmol/L) with the addition of CB7. The inset shows the nonlinear fitting according to a 1:1 binding model (solid line, see the Experimental Section). Panel (B) shows the effects of binding to CB7 (0−300 μmol/L) on the emission spectra of TFP (22 μmol/L) at the same pH, $\lambda_{ex} = 395$ nm. OD = optical density and $I_f$ = fluorescence intensity.

Table 1. Absorption ($\lambda_a$) and Steady-State Emission ($\lambda_f$) Maxima along with Stokes Shifts for Enol and Keto Forms of TFP at 25 μM in Neat Solvents and Inside the CB7 Cavity (10 equiv)$^{ac}$

| solvent  | $\lambda_a$ (nm) | $\lambda_f$ (nm) | Stokes shifts (cm$^{-1}$) |
|----------|-----------------|-----------------|--------------------------|
| $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ | 357 | 396 | 426 | 2758.7 | 1525.8 |
| $\text{CHCl}_3$ | 369 | 400 | 398 | 465 | 1974.6 | 3494.6 |
| $\text{CH}_2\text{Cl}_2$ | 369 | 405 | 399 | 471 | 2037.6 | 3459.9 |
| $\text{C}_4\text{H}_8\text{O}_2$ | 399 | 396 | 430 | 2602.6 | 1744.2 |
| THF | 361 | 398 | 433 | 2575.2 | 1905.3 |
| $\text{CH}_3\text{CN}$ | 362 | 405 | 399 | 450 | 2561.7 | 2469.1 |
| $\text{CH}_3\text{OH}$ | 364 | 398 | 445 | 2346.9 | 2528.1 |
| $\text{H}_2\text{O}$ (pH 3) | 399 | 400 | 465 | 2777.8 | 3557.3 |
| CB7 (pH 3) | 402 | 400 | 465 | 3370.2 |

$^a$Enol and keto forms were excited at 350 and 390 nm, respectively. $^b$C$_2$H$_5$OC$_2$H$_5$ = diethyl ether. $^c$C$_4$H$_8$O$_2$ = 1,4-dioxane.

Figure 4. $^1$H-NMR titration of TFP with CB7 (0−3.5 equiv) in 50% DMSO-d$_6$ in D$_2$O at pD 4.0 (400 MHz). CB7 peaks are indicated.
Figure 5. Absorption (black) and emission (red) spectra ($\lambda_{\text{exc}} = 350 \text{ nm}$ for enol and $390 \text{ nm}$ for keto) of TFP chromophores in $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ (A) and water (pH 3) (B) in their enol (red dashed lines) and keto (red solid line) forms.

Table 2. Solvent Properties Including Hydrogen Bonding Accepting Ability, Hydrogen Bonding Donating Ability, and Polarity/Polarizability Parameter

| solvent       | refractive index, $n$ | dielectric constant, $\varepsilon$ | orientational polarizability, $\Delta f$ | viscosity (cP), $\eta$ | $\alpha$ | $\beta$ | $\pi^*$ | $\alpha + \beta + \pi^*$ |
|---------------|-----------------------|----------------------------------|----------------------------------------|------------------------|--------|--------|--------|----------------------|
| $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ | 1.352 | 4.33 | 0.17 | 0.24 | 0 | 0.47 | 0.27 | 0.74 |
| $\text{CHCl}_3$ | 1.446 | 4.81 | 0.15 | 0.57 | 0.44 | 0 | 0.58 | 1.02 |
| $\text{CH}_2\text{Cl}_2$ | 1.424 | 8.93 | 0.22 | 0.41 | 0.33 | 0 | 0.82 | 1.12 |
| $\text{C}_4\text{H}_8\text{O}_2$ | 1.422 | 2.25 | 0.02 | 1.37 | 0 | 0.37 | 0.55 | 0.92 |
| THF           | 1.407 | 7.58 | 0.21 | 0.48 | 0 | 0.55 | 0.58 | 1.13 |
| $\text{CH}_3\text{CN}$ | 1.344 | 37.5 | 0.31 | 0.34 | 0.19 | 0.31 | 0.75 | 1.25 |
| $\text{CH}_3\text{OH}$ | 1.328 | 32.7 | 0.31 | 0.54 | 0.93 | 0.62 | 0.60 | 2.15 |
| $\text{H}_2\text{O}$ (pH 3) | 1.333 | 80.1 | 0.37 | 1.0 | 1.17 | 0.18 | 1.09 | 2.44 |
| DMSO          | 1.477 | 48.9 | 0.27 | 1.99 | 0 | 0.76 | 1.00 | 1.76 |
| $\text{C}_6\text{H}_6$ | 1.501 | 2.28 | 0.01 | 0.60 | 0 | 0.10 | 0.59 | 0.69 |
| $\text{C}_6\text{H}_6\text{CH}_3$ | 1.496 | 2.38 | 0.01 | 0.56 | 0.11 | 0 | 0.54 | 0.65 |

Figure 6. DAS of the three-component mixture of fluorophores for TFP (25 $\mu$M) in $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ (A) and water (pH 3) (B) upon excitation at 375 nm and room temperature. The corresponding steady-state spectra of each solution are also shown for comparison (see the Experimental Section).

Table 3. Amplitudes and Maxima of the DAS for Enol and Keto (Major) Forms of TFP at 25 $\mu$M in Neat Solvents and Inside the CB7 Cavity (10 equiv)

| solvent       | r (ns) | DAS maximum | DAS amplitude |
|---------------|--------|-------------|---------------|
|               | enol   | keto long-lived | keto short-lived | enol   | keto long-lived | keto short-lived | % keto |
| $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ | 1.04 | 2.74 | 0.25 | 400 | 430 | 470 | 0.96 | 0.41 | 0.04 | 29.9 |
| $\text{CHCl}_3$ | 2.10 | 0.69 | 0.13 | 405 | 445 | 475 | 0.89 | 0.39 | 0.40 | 31.0 |
| $\text{CH}_2\text{Cl}_2$ | 2.31 | 0.71 | 0.17 | 390 | 440 | 470 | 0.90 | 0.21 | 0.48 | 34.8 |
| $\text{C}_4\text{H}_8\text{O}_2$ | 1.68 | 2.80 | 0.35 | 390 | 440 | 470 | 0.91 | 0.52 | 0.02 | 36.4 |
| THF           | 1.50 | 2.81 | 0.56 | 400 | 440 | 470 | 0.89 | 0.61 | 0.05 | 40.7 |
| $\text{CH}_3\text{CN}$ | 2.88 | 1.77 | 0.17 | 390 | 440 | 470 | 0.62 | 0.59 | 0.08 | 48.8 |
| $\text{CH}_3\text{OH}$ | 1.03 | 1.85 | 0.19 | 410 | 440 | 470 | 0.35 | 0.79 | 0.02 | 69.3 |
| $\text{H}_2\text{O}$ (pH 3) | 1.88 | 0.29 | 0.11 | 400 | 440 | 470 | 0.21 | 0.91 | 0.05 | 81.3 |
| CB7 (pH 3)  | 3.19 | 1.00 | 0.40 | 420 | 440 | 470 | 0.34 | 0.17 | 0.73 | 68.2 |

$^a$The population for keto forms was calculated using the relationship $\% \text{keto} = \frac{\text{DAS amplitude for keto(major)}}{\text{DAS amplitudes for enol + keto}}$, in which the amplitudes for the major keto species were considered (see Figure S5 in the Supporting Information). For time-resolved fluorescence measurements, the time resolution was $\sim 30$ ps and the excitation wavelength was 375 nm.
Table 4. Fluorescence Quantum Yield, Excited-State Lifetimes, Radiative Rate Constant, and Nonradiative Rate Constant for TFP-Keto and TFP-Enol Forms in Different Media

| solvent               | \( \Phi^a \) | \( \tau^b (\text{ns}) \) | \( k_r \times 10^4 \text{ s}^{-1} \) | \( k_n \times 10^4 \text{ s}^{-1} \) |
|-----------------------|-------------|--------------------------|---------------------------------|---------------------------------|
| \( \text{C}_2\text{H}_5\text{OC}_2\text{H}_3 \) | 0.015       | 1.04                     | 1.43                            | 9.47                            |
| CHCl\(_3\)            | 0.204       | 2.10                     | 9.73                            | 3.78                            |
| CH\(_2\)Cl\(_2\)      | 0.176       | 2.31                     | 7.63                            | 5.36                            |
| C\(_6\)H\(_6\)O\(_2\) | 0.060       | 1.68                     | 3.58                            | 5.58                            |
| THF                   | 0.081       | 1.50                     | 5.43                            | 6.12                            |
| CH\(_2\)OH            | 0.055       | 1.28                     | 1.93                            | 3.27                            |
| H\(_2\)O (pH 3)       | 0.081       | 1.03                     | 3.43                            | 9.36                            |
| CB\(_7\) (pH 3)       | ND\(^c\)   | 3.19                     | ND\(^c\)                        | ND\(^c\)                        |

\(^a\)\( \Phi \) values were measured using Coumarin 2 (C 450) in acetonitrile as the standard (\( \Phi_\text{std} = 0.8 \))\(^1\) and calculated using the known equation: \( \Phi_{\text{unk}} = \Phi_{\text{std}} \left( \frac{n_{\text{unk}}}{n_{\text{std}}} \right) \left( \frac{\lambda_{\text{unk}}}{\lambda_{\text{std}}} \right)^2 \), where \( n \) is the refractive indices for the standard (std) and experimental (unk) solvents, \( I \) is the fluorescence integral of the emission between 400 and 600 nm, and \( A \) is the absorbance at the excitation wavelength. The error estimated as the standard deviation of the mean was approximately 10\% for the fluorescence quantum yields. \( k_r \) and \( k_n \) were calculated using the known equations: \( \Phi = \frac{k_r}{k_r + k_n} \), where \( k_r \) and \( k_n \) are the radiative and nonradiative rate constants, respectively. This result is also not surprising and agrees with previous findings on similar keto--enol systems.\(^30\)

However, to correct the effects of concentration on the keto/enol ratio, we have used the data in Figure S7 to create Table S1 in the Supporting Information, in which the relative integral values for H-a protons at peak positions 7.6 and 6.9 ppm were used to estimate the keto/enol ratio at different TFP concentrations in CDCl\(_3\). The ratio at 0.05 mM in Table S1 in the Supporting Information matches the ratio from the DAS experiment in Table 3 at 0.025 mM (41 versus 31\%). The slope of the plot of this keto percentage as a function of TFP concentration (Figure S9 in the Supporting Information) was used to extrapolate the calculated keto/enol ratio in Figure S8 for the 0.05 mM concentration. The data in Figure S8 were corrected and replotted in the same graph for comparison. It transpires that the keto/enol ratios from the NMR analysis at micromolar concentrations match the ratios obtained from DAS experiments in Figure 7 and Table 3 within 14\% error (for example, 56 versus 41\% at 0.05mM in CDCl\(_3\)), which is plausible considering that the two different approaches for calculating keto/enol ratios from NMR data would have different accuracies.

Despite the high binding affinity between TFP and CB\(_7\), only 1.5-fold fluorescence enhancement was observed upon complexation to CB\(_7\). This can be explained by the NMR titration results in Figure 4 that the fluorescent tag in the complex is partially exposed to water.

From the correlation in Figure 7, it appears that the sum of polarity/polarizability and hydrogen bonding interactions experienced by the probe in CB\(_7\) is equivalent to that in methanol. In the present study, this conclusion was implemented on enhancing solubility of TFP in water (Figure 9). Notice the fluorescence enhancement is attributed to the confinement effects by complexation in CB\(_7\), which has reduced the nonradiative rate constant in the free fluorophore more significantly than the radiative rate constants (compare values in CB\(_7\) to that in water in Table 4). This conclusion is supported by observing no shift in the emission peak position at 465 nm upon complexation (Figure 3B).
2.3. Implication for Solubilization of TFP in Water by CB7 and Other Applications. The effect that the addition of CB7 has on the solubility of the guests was determined by UV spectrophotometry (see Figure 9) at pH 3. In the presence of 3.7 mmol/L CB7, the solubility of TFP increased by a factor of 42. In terms of absolute solubilities (these were determined from the OD values), they increased upon complexation from 0.18 to 0.65 mmol/L. It is plausible to assume the enhancement is governed by the methanol-equivalent solvent effects (see above). The use of macrocycles for solubilization in aqueous solutions is consequently particularly promising for TFP fluorescent tags.

Certainly, the position of the functional groups and their location in the complex with respect to the receptor may put some limitation on the potential benefits of TFP as a fluorescent probe. From another perspective, one could expect that different probes report contrasting properties for the CB7 receptor, that is, while TFP “is experiencing a methanol-like environment” in CB7, other probes could experience other solvent-like environments, and the extrapolation of the results here for other probes must be treated with caution.

3. CONCLUSIONS

It is concluded that time-resolved emission measurements evaluated by a global analysis method while assuming an appropriate kinetic model are more useful in understanding the local environment effects (represented here by CB7) on the water solubility and emission properties of some fluorescent tags (such as TFP) that belong to the pyridone family. Inclusion of TFP in the CB7 cavity has led to a 1.5-fold enhancement in its fluorescence quantum yield and an increase by 45-fold in its water solubility. The observations were rationalized on the basis of the spectrophysical behaviors of TFP in neat solvents. The emission signals of TFP were found to be well correlated with the sum of solvent hydrogen bonding abilities and polarity/polarizability parameters, establishing that CB7 has solvent properties equivalent to those in the methanol solvent. This methanol-like micro-environment enables CB7 to stabilize preferentially the enol form over the keto form at the ground state regardless of the concentration of TFP whether in micromolar (DAS results) or millimolar (NMR results).

4. EXPERIMENTAL

4.1. Samples. CB7 (purity > 99.9%) was purchased from Sigma-Aldrich and used as received. However, the host was assumed to contain 20% water in the calculation of
concentrations as notified by the supplier. Millipore water was used (conductivity less than 0.05 μS).

4.2. Synthesis of TFP. A mixture of 4,4,4-trifluoro-1-(thiophen-2-yl)butane-1,3-dione (10 mmol, 2.22 g), 2-cyanoacetamide (11.0 mmol, 0.92 g), and potassium hydroxide (12.0 mmol, 0.67 g) in ethanol (50 mL) was heated under reflux for 6 h. The mixture was cooled, and the resultant mixture was acidified with 0.1 M HCl. The precipitate formed was crystallized from an appropriate solvent and dried to give the desired 2-pyridone. Yellow powder; yield 89%; from ethanol, mp 293–294 °C; IR (KBr, cm⁻¹): 1661 (C=O), 2230 (CN), 3419 (NH); ¹H NMR (DMSO-d₆, 400 MHz, δ ppm): 7.23 (t, 1H, H-c, J = 4.0 Hz), 7.63 (bs, 1H, H-a), 7.88, 7.89 (dd, 1H, H-d, J = 4.9 Hz), 8.15, 8.16 (dd, 1H, H-b, J = 3.7 Hz), 13.57 (bs, 1H, NH, exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 100 MHz, δ ppm): 105.9 (C₅), 113.8 (C₃), 120.5 (C₁), 129.7 (C₂), 164.8 (C₄). Anal. Calcd for C₁₁H₅F₃N₂OS: C, 48.89; H, 1.97; N, 10.28; S, 11.95. ¹H NMR values of the solutions were adjusted (±0.2 units) by adding adequate amounts of HCl or NaOH and recorded using a pH meter (WTW 330i equipped with a WTW SenTix Mic glass electrode).

4.4. Photochemistry. The fluorescence lifetimes were measured by time-correlated single-photon counting on a LifeSpec II spectrometer (Edinburgh Instruments) by using an EPL-375 picosecond diode laser (λₑₓ = 375 nm, repetition rate = 20 MHz, and instrument function = 30 ps) for excitation. The time-resolved emission (intensity of ∼1000–3000 counts/s) was collected (with a constant dwell time of 10 s) every 10 nm across the entire emission spectrum by a red-sensitive high-speed PMT (Hamamatsu, HS773-04) detector. The data were analyzed by the iterative reconvolution method using the instrument’s software that utilizes the Levenberg–Marquardt algorithm to minimize χ². The fluorescence decay was analyzed in terms of the multiplexponential model

\[ I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \]  

where \( \tau_i \) are the lifetimes with amplitudes \( \alpha_i \) and \( \sum \alpha_i = 1.0 \). The contribution of each component to the steady-state intensity is given by

\[ f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \]  

where all the decay times and amplitudes are over the their sum in the denominator. The mean decay time (average lifetime) is given by

\[ \tau = \sum_i f_i \tau_i \] 

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02761.

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