# New Phenol Benzoate Cyanine Picolinium Salt Photoacid Excited-State Proton Transfer

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**ABSTRACT:** Steady-state and time-resolved fluorescence techniques were employed to study the excited-state proton transfer (ESPT) to water and D<sub>2</sub>O of a new photoacid, phenol benzoate cyanine picolinium salt (BCyP). We found that the ground-state pK<sub>a</sub> is about 6.5, whereas the excited-state pK<sub>a</sub>\* is about -4.5. The ESPT rate constant,  $k_{\rm PT}$ , to water is ~0.5 × 10<sup>12</sup>s<sup>-1</sup> ( $\tau_{\rm PT} \approx 2$  ps) and in D<sub>2</sub>O the rate is 0.33 × 10<sup>12</sup> s<sup>-1</sup>. We determined that the BCyP photoacid belongs to the third regime of photoacids, the solventcontrolled regime.



## INTRODUCTION

Photoacids are a class of compounds that are weak acids in their ground electronic state and much stronger acids in their excited singlet or triplet states. This class of compounds has been extensively researched for the last 50 years by optical steadystate and time-resolved techniques.<sup>1-17</sup> The ground-state  $pK_a$ values range from 5 < pK<sub>2</sub> < 10 and the difference,  $\Delta pK_2$ , between their ground- and excited-state pK, values range between 3 <  $\Delta p K_a$  < 13. When the  $p K_a^*$  of a photoacid is large enough (for example, 2-naphthol with  $pK_a^* = 2.7$ ), the excitedstate proton-transfer (ESPT) rate constant,  $k_{PT}$ , is of the order of  $10^{\$} s^{-1}$  and since the radiative rate of the singlet state,  $k_{rad}$ , is usually >10<sup>8</sup>s<sup>-1</sup>, the ESPT quantum efficiency,  $\phi_{PT}$ , is smaller than 0.5. Therefore, weak photoacids like phenols, with  $pK_a^* \approx$ 3.4, do not exhibit detectable photoacidity. Photoacidity depends on the solvent. Water at room temperature is the best solvent known for ESPT processes. For weak photoacids with  $pK_a^* > 0.5$ , ESPT is not observed in methanol or longerchain alkanols, because  $k_{\rm PT}$  in methanol is smaller by about 3 orders of magnitude than its value in water for photoacids with  $pK_a^* > 0.5.$ 

In a previous paper we classified the photoacids into four groups.<sup>18</sup> Group I, "the weak photoacids", with  $0.4 < pK_a^* < 3.4$  can transfer a proton within the excited-state lifetime to water and D<sub>2</sub>O, but not to alkanols. Group II, with  $-4 < pK_a^* < 0.4$ , can transfer a proton, within the excited-state lifetime, to many protic solvents like DMSO and alkanols. The more negative the  $pK_a^*$ , the larger the ESPT rate constant. Group III constitutes a unique class of photoacids with  $pK_a^* \sim -5$ . The ESPT rate constant and the process itself depend on the solvent-reorientation relaxation time, or S(t). For water  $\langle S(t) \rangle$  is of the order of 1 ps, for methanol it is about 5 ps, and for ethanol, 15 ps. The fourth group of photoacids transfers a proton to the solvent at rates exceeding that of the solvent-reorientation

relaxation. Among such photoacids are quinone cyanine 7 (QCy7) and quinone cyanine 9 (QCy9). Up to now, the fastest ESPT rate reported is for QCy9,<sup>19</sup> with  $k_{\rm PT} \sim 10^{13} {\rm s}^{-1}$  ( $\tau_{\rm PT} \approx 100 {\rm ~fs}$ ).

*N*-Methyl-6-hydroxyquinolinium (NM6HQ<sup>+</sup>) shown in Scheme 2 belongs to group III of the photoacid class. It was studied by time-resolved fluorescence and transient pump– probe techniques.<sup>15,20,21</sup> The average decay time of the protonated form, ROH, in water is about 2 ps and in the alkanols the ESPT rate is smaller than in water and decreases with increase in the length of the alkane chain and, as expected, depends on the solvent-relaxation time.<sup>20</sup>





In the current study, we used steady-state and time-resolved fluorescence to study the ESPT rate of a newly synthesized photoacid molecule, phenol benzoate cyanine picolinium salt (BCyP), shown in Scheme 1. We found that the ESPT rate to water and  $D_2O$  is ultrafast and the photoacid belongs to group III of the photoacids, for which the ESPT rate to the solvent is

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Scheme 2. Molecular Structure (from Left to Right) of Quinone Cyanine 7 (QCy7), QCy9, and N-Methyl-6hydroxyquinolinium (NM6HQ<sup>+</sup>)



controlled by the solvent-relaxation time, S(t). We found a kinetic isotope effect KIE of about 1.5. KIE is expected to range between 1.5 and 2.1 for very strong photoacids with  $pK_a^* < -2$ .

#### MATERIALS AND METHODS

**Syntheses and General Techniques.** All reactions requiring anhydrous conditions were performed under an argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques.

Thin layer chromatography (TLC): silica-gel plates Merck 60 F254. Compounds were visualized by irradiation with UV light.

High-pressure liquid chromatography (HPLC): C18 5u, 250  $\times$  4.6 mm, eluent given in parentheses.

Preparative HPLC: C18 5u,  $250 \times 21$  mm, eluent given in parentheses.

<sup>1</sup>H NMR spectra were measured with the use of a Bruker Avance, operated at 400 MHz as mentioned. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Synthesis of Compound 1. Commercially available 3hydroxybenzaldehyde 1a (0.1 mmol), piperidine (0.13



mmol), and picolinium iodide (0.13 mmol) were dissolved in EtOH (2 mL). The reaction mixture was stirred for 60 min at 80 °C and monitored by RP-HPLC (grad. 10%-90 ACN in water, 20 min). The reaction mixture was then concentrated by evaporation under reduced pressure. The crude product was diluted with 1:1:0.1 H<sub>2</sub>O: ACN: DMSO, and purified by preparative RP-HPLC (grad. 10%–90% ACN in water, 20 min) to give compound 1: hydroxypicolinium methyl benzoate ester—merocyanine dye, in 81% yield with >99% purification. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.72 (d, *J* = 6.7 Hz, 2H), 8.13 (d, *J* = 6.8 Hz, 2H), 7.94 (d, *J* = 16.5 Hz, 1H), 7.68 (d, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 16.5 Hz, 1H), 7.44 (d, *J* = 1.4 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 4.13 (s, 3H), 3.73 (s, 3H). MS (ES+): *m*/*z* calcd for C<sub>1</sub>6H<sub>16</sub>NO<sub>3</sub>, 270.1; found, 270.2 [M]<sup>+</sup>.

Synthesis of Compound 2. 3-Hydroxybenzaldehyde 1a (0.1 mmol) and NaH (0.15 mmol) were dissolved in DMF (2 mL). After 5 min, iodomethane (0.15 mmol) was added and the solution was heated to 50 °C and monitored by TLC (85:15, Hex:EA). The mixture was then concentrated by evaporation and to the crude mixture was added, without further purification, EtOH (2 mL), piperidine (0.13 mmol), and picolinium iodide (0.13 mmol) and the reaction mixture was stirred for 60 min at 80 °C and monitored by RP-HPLC (grad.



10%–90% ACN in water, 20 min). After completion, the reaction mixture was concentrated by evaporation under reduced pressure. The crude product was diluted with 1:1:0.1 H2O: ACN: DMSO, and purified by preparative RP-HPLC (grad. 10%–90% ACN in water, 20 min) to give compound **2**; Methoxypicolinium methyl benzoate ester–merocyanine dye. 68% yield with >99% purification. <sup>1</sup>H NMR (400 MHz, DMSO):  $\delta$  8.85 (d, *J* = 6.7 Hz, 2H), 8.25 (d, *J* = 6.8 Hz, 2H), 8.06 (d, *J* = 16.5 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.62 (m, *J* = 15.5, 10.5 Hz, 3H), 4.25 (s, 3H), 3.97 (s, 3H), 3.87 (s, 3H). MS (ES+): *m*/*z* calcd for C<sub>17</sub>H<sub>18</sub>NO<sub>3</sub>, 284.1; found, 284.2 [M]<sup>+</sup>.

Spectroscopy. The time-resolved fluorescence was measured by the up-conversion technique. The fluorescence of phenol benzoate cyanine picolinium salt (BCyP), was studied in H<sub>2</sub>O and D<sub>2</sub>O at room temperature. The laser used for the fluorescence up-conversion was a cavity-dumped Ti:sapphire femtosecond laser (Mira, Coherent), which provides short, 120 fs, pulses at about 800 nm. The cavity dumper was operated with a low repetition rate of 800 kHz. The up-conversion system is a commercial system (FOG-100, CDP, Russia). The samples were excited by SHG pulses of 8 mW at wavelengths of 390-420 nm on average. The time response of the upconversion system is determined by the Raman-Stokes line of water, red-shifted by 3600 cm<sup>-1</sup>. We found that the full-width at half-maximum (fwhm) of the signal is 300 fs. To avoid photodegradation, samples were placed in a rotating optical cell, and rotated at a frequency of 10 Hz. Sample degradation was minimal and did not affect the profile of the signal decay.

Experiments were carried out on solutions at concentrations of about 0.5 mM. The steady-state fluorescence and excitation spectra were measured by a Horiba Jobin Yvon FluoroMax-3 fluorescence spectrofluorometer. The absorption spectra were measured by a Cary 5000 spectrometer.

#### RESULTS

Figure 1 shows the absorption spectra of benzoate cyanine picolinium (BCyP) in water at several pH values.

The basic-solution spectra show a broad and structureless band with a band maximum at 454 nm, which we assign to the



Figure 1. Absorption spectra of BCyP in various pH H<sub>2</sub>O.

RO<sup>-</sup> form. The RO<sup>-</sup> form also shows a second band with a maximum at 338 nm in the UV region. In acidic solutions the ROH band maximum is at 369 nm and a UV band at 332 nm also exists but is broader than the band of the basic form. The  $pK_a$  value of the ground state is about 6.5. We also notice a sharp isosbestic point at 405 nm.

With the Förster cycle calculation,<sup>22</sup> one can estimate the change in acidity upon excitation of the molecule. This calculation is based on the positions of the optical absorption or emission bands of the protonated and deprotonated forms of a photoacid. The energy cycle leads to a simple relation between band positions and change in acidity in the excited state with respect to the ground state  $pK_a$ .

$$\Delta p K_a^* = C \times \Delta \nu \tag{1}$$

$$pK_a^* = pK_a + \Delta pK_a^* \tag{2}$$

Where *C* is a factor of universal constants:

$$C = \frac{N_{\rm A}h}{\ln(10) \times RT} = 2.09 \times 10^{-3} \,\rm{cm}$$
(3)

 $\Delta \nu$  is the difference, in wavenumber units, between the positions of the ROH\* and RO<sup>-\*</sup> bands.

From the absorption of the acidic (369 nm) and basic bands (457 nm) peak position we find that  $pK_a^* = pK_a + \Delta pK_a^* = 6.5 - 11.06 = -4.56$ 

Figure 2a shows the normalized steady-state excitation spectra of BCyP in  $H_2O$  and  $D_2O$  in the acidic form (solution pH ~ 6) and in  $H_2O$ , the excitation spectrum of the basic form (solution pH ~ 10).

The fluorescence was monitored at 640 nm around the fluorescence peak of the basic  $RO^-$  form of BCyP. The excitation spectra of the acidic and basic forms of BCyP are similar to the absorption spectra of BCyP shown in Figure 1.

Figure 2b shows the normalized steady-state fluorescence spectra of BCyP in  $H_2O$  and  $D_2O$ . The ROH fluorescence band peak is at ~514 nm and the RO<sup>-</sup> band peak is at about 650 nm. In water, the ratio of the ROH-band intensity to that of the RO<sup>-</sup> band is about 0.65, and in  $D_2O$  it is about 0.85 These differences in fluorescence intensities are expected for photoacids because of the kinetic isotope effect. In  $D_2O$  the ESPT rate is slower than in  $H_2O$ .

Figure 3 shows on linear (parts a and b) and on semilogarithmic scales (parts c and d) the time-resolved fluorescence of BCyP in water at several wavelength in the spectral region of 460-610 nm.

The decay of the time-resolved fluorescence signals is nonexponential and at each wavelength the average decay time,  $(\tau_{av} = \int_0^\infty I_F(t) dt)$ , increases with increasing wavelength. Table 1 provides the values of  $\tau_{av}$  as a function of  $\lambda$ .

Photoacids that belong to regime III,<sup>18</sup> for which the ESPT rate is controlled by solvent relaxation, show a large dependence on wavelength, since both the ROH and RO<sup>-</sup> fluorescence bands shift to the red during the time that the ESPT process takes place. Previous research on 6-hydroxy-*N*-methylquinolinium ion (6HNMQ<sup>+</sup>), which belongs to regime III, reports similar behavior.<sup>16</sup> Table 2 provides the signal-fit parameters for a multiexponential fit function.

As seen in both Tables 1 and 2, the decay of the signals strongly depends on the wavelength. In water, the ROH fluorescence band peak is at about 510 nm, and that of the RO<sup>-</sup> band is at about 650 nm. The fluorescence intensity of the ROH band is relatively strong whereas that of RO<sup>-</sup> band is weaker by about a factor of 10. These characteristics were also found for QCy7 and QCy9 super photoacids shown in Scheme 2.

Figure 4 shows on linear (parts a and b) and on semilogarithmic scales (parts c and d) the time-resolved fluorescence of BCyP in  $D_2O$ .

As shown in Figure 3 for H<sub>2</sub>O as a solvent, the fluorescence decay in D<sub>2</sub>O is nonexponential. Table 1 provides the  $\tau_{av}(\lambda)$  values and Table 2b gives the multiexponential-fit parameter values. The decay times of BCyP in D<sub>2</sub>O are longer because of the kinetic isotope effect of the ESPT process.



**Figure 2.** (a) Normalized steady-state excitation spectra of BCyP in neutral and basic pH H<sub>2</sub>O and in neutral D<sub>2</sub>O, the fluorescence is monitored at  $\lambda_{em} = 640$  nm. (b) Normalized steady-state emission spectra of BCyP in neutral H<sub>2</sub>O and D<sub>2</sub>O, excited at  $\lambda = 410$  nm.

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Figure 3. Time-resolved emission of BCyP in water, measured over the wavelength range of (a) 460-510 and (b) 530-610 nm shown on a linear scale and emission shown on a semilog scale over the wavelength range of (c) 460-510 and (d) 530-610 nm.

Table 1. $ au_{av}$ Table	e of BCyP in H	H <sub>2</sub> O and D <sub>2</sub> O	
wavelength	solvent	$ au_{\mathrm{av}}$ (fs)	ratio
460	H <sub>2</sub> O	400	1.55
	$D_2O$	619	
470	H <sub>2</sub> O	475	1.74
	$D_2O$	826	
480	H <sub>2</sub> O	597	1.77
	$D_2O$	1058	
490	H <sub>2</sub> O	771	1.61
	$D_2O$	1242	
500	H <sub>2</sub> O	915	1.64
	$D_2O$	1500	
510	H <sub>2</sub> O	1068	1.62
	$D_2O$	1734	
530	H <sub>2</sub> O	1424	1.58
	$D_2O$	2247	
550	H <sub>2</sub> O	1732	1.57
	$D_2O$	2724	
570	H <sub>2</sub> O	2071	1.57
	$D_2O$	3248	
590	H <sub>2</sub> O	2389	1.52
	$D_2O$	3622	
610	H <sub>2</sub> O	2998	1.44
	D <sub>2</sub> O	4325	

Figure 5 shows the time-resolved fluorescence of BCyP in  $H_2O$  and  $D_2O$  at several wavelengths.

Each panel shows the signals in H<sub>2</sub>O and D<sub>2</sub>O at a particular wavelength over the spectral region of 500–550 nm. As seen in the figure, the average decay rate of the BCyP signal in D<sub>2</sub>O at a particular wavelength is significantly slower than that for H<sub>2</sub>O. For photoacids of regime 1 with  $pK_a \ge 0.4$ , the kinetic isotope effect (KIE) is relatively large, and its value in water is ~3. For

stronger photoacids, with  $pK_a^* < -2$ , the KIE values are smaller than 3. For the strongest photoacids, like QCy7, it is about 1.5, and for QCy9, it is close to ~2. We find that for BCyP the KIE is about 1.5 as given by the  $\tau_{av}$  ratio,  $R = \tau_{av}^{D_2O}/\tau_{av}^{H_2O}$ , shown in Table 1.

Parts a and b of Figure 6 show, on linear and semilogarithmic scales, the time-resolved fluorescence of BCyP and NM6HQ<sup>+</sup> (Scheme 1 and Scheme 2, respectively) in H<sub>2</sub>O. For NM6HQ<sup>+</sup>, the fluorescence is measured at 490 nm (ROH band maximum at 440 nm) and for BCyP, three signals, measured at 550, 570, and 590 nm, are shown in the figure. As seen in the figure, the ROH signal of BCyP at 570 nm shows good agreement with the signal of the ROH form of NM6HQ<sup>+</sup>. The signal of NM6HQ<sup>+</sup> in H<sub>2</sub>O was added to show that both NM6HQ<sup>+</sup> and BCyP photoacids belong to group III—the solvent-controlled ESPT—with  $\tau_{\rm PT} \approx 2$  ps.

**BCyP Methoxy Analogue.** An important experiment for verifying the photoacidic properties of a photoacid is the measurement the spectroscopic properties of the methoxy analogue of the hydroxyl photoacid. Therefore, the methoxy analogue shown in Scheme 3 was synthesized:

Figure 7 shows the steady-state excitation and fluorescence spectra in water of the methoxy analogue.

The excitation spectrum long wavelength ( $\lambda > 350$  nm) is similar to that of the photoacid. The short wavelength band of the excitation spectrum of the methoxy analogue is missing the second band seen in the hydroxy photoacid spectrum with a maximum at 330 nm. The fluorescence spectrum of the methoxy analogue in water consists of a single band with a maximum at 490 nm, whereas the maximum of the photoacid is at about 514 nm. As expected, the methoxy analogue is missing the RO<sup>-</sup> band at 650 nm due to the lack of an ESPT process. The intensity of the fluorescence band of the methoxy analogue is about 20 times that of the photoacid, as expected, since the Table 2. Fitting Parameters<sup>*a*</sup> of the Time-Resolved Fluorescence of BCyP in (a)  $H_2O$  and (b)  $D_2O$ , with the Use of a Multiexponential Function<sup>*b*</sup>

wavelength (nm)	<i>a</i> <sub>1</sub>	$ au_1$ (fs)	<i>a</i> <sub>2</sub>	$ au_2$ (fs)	<i>a</i> <sub>3</sub>	$ au_3$ (fs)	$a_4$	$ au_4$ (ps)
				(a) In H <sub>2</sub> O				
460	0.87	250	0.097	700	0.029	3330	0.000 97	9
470	0.83	250	0.14	840	0.029	3330	0.000 96	9
480	0.77	270	0.19	900	0.038	3330	0.000 96	9
490	0.77	350	0.17	1200	0.056	3330	0.0056	9
500	0.74	385	0.19	1400	0.065	3330	0.0065	20
510	0.71	425	0.21	1600	0.064	3330	0.011	40
530	0.76	620	0.22	3000	_	_	0.021	40
550	0.56	700	0.37	2200	0.037	3100	0.037	40
570	0.44	920	0.44	2200	0.061	3100	0.065	40
590	0.38	1000	0.47	2200	0.038	3100	0.11	40
610	0.24	1300	0.56	2400	0.032	3100	0.17	40
				(b) In D <sub>2</sub> O				
460	0.81	240	0.15	1060	0.038	3100	0	
470	0.80	300	0.17	1700	0.019	3100	0.0068	40
480	0.70	340	0.27	1650	0.0096	3100	0.019	40
490	0.64	360	0.33	1720	0		0.028	40
500	0.56	430	0.40	1870	0		0.038	40
510	0.50	470	0.45	1990	0		0.055	40
530	0.39	720	0.53	2160	0		0.078	40
550	0.31	1120	0.58	2700	0		0.11	40
570	0.54	2200	0.31	3400	0		0.15	40
590	0.19	2030	0.64	3300	0		0.17	40
					RO <sup>-</sup> signals (ps)			
610	1	200	0		~6	300		
630	0.3	200	0.7	500	~4	300		
650	0.3	200	0.7	600	~20	300		

<sup>*a*</sup>Full-width at half-maximum of the system response was  $\tau_0 = 300$  fs. <sup>*b*</sup>Multiexponential function form:

 $I_F(t) = \sum_i a_i \exp(-t/\tau_i)$ 

lifetime of the ROH form of the photoacid is determined by the ESPT rate constant and not by the radiative and nonradiative processes.

Parts a and b of Figure 8 show, on a linear and on a semilog scale, the time-resolved fluorescence of the methoxy analogue in water at several wavelengths in the spectral region of 470–550 nm.

At short wavelengths, the solvation components with time constants of a few picoseconds shift the fluorescence band to the red. At long wavelengths ( $\lambda > 510$  nm) the time-resolved signals show rise-times with time constants that fit the fast decay-times of the short-wavelength signals. At about 500 nm, near the steady-state fluorescence band peak, the signal, as expected, lacks the fast solvation components.

#### DISCUSSION

The newly synthesized photoacid BCyP exhibits properties of a very strong photoacid with a  $pK_a^*$  of ~ -4.5 calculated by the Förster cycle<sup>22</sup> formula based on the positions of the absorption bands of the ROH and RO<sup>-</sup> forms of the photoacid. At 530 nm, near the emission-band peak of the ROH, the average fluorescence lifetime  $\tau_{av} = 1.75$  ps. This time constant is close to the  $\tau_{av}$  value of the photoacid NM6HQ<sup>+</sup> which was found<sup>15,20,21</sup> to belong to the solvent-controlled ESPT regime (regime III in the classification presented in ref 18). Prior to the current study, only one photoacid had been classified as belonging to the solvent-controlled ESPT rate. In the 1980s, it

was suggested that ultrafast electron transfer might be limited by solvent relaxation.  $^{\rm 23}$ 

Sumi and Marcus<sup>23</sup> extended the Marcus electron-transfer theory to include the effect of both intramolecular-vibrational (q coordinate) and diffusive solvent-orientational motions (x coordinate) on the rate of electron-transfer reactions. Marcus wrote a similar theory for proton transfer but did not add the solvent control rate theory. They found for electron transfer that under certain conditions, the time dependence of the survival probability of the reactant is multiexponential rather than single exponential. Because of the faster relaxation time of the vibrational motion, they assumed that the solvent coordinate, x, can define a rate constant at each value of x. Thus, k(x) is given by

$$k(x) = \nu_{\rm q} \, \exp[-\Delta G^*(x)/k_{\rm B}T] \tag{4}$$

where  $\Delta G^*(x)$  is given by eq 3.6 of ref 23 when the intramolecular q coordinate motion is treated classically. The reverse reaction is omitted for simplicity. The coordinate x diffuses under the influence of a potential V(x), which represents the free energy of the reactant's surface (see Scheme 2 or Figure 1 of ref 23). During the diffusion, the reactant becomes product with a probability rate constant of k(x) at each value of x. Then, the distribution function p(x;t) at the coordinate x and at time t satisfies the Agmon–Hopfield diffusion-assisted-reaction equation.<sup>24</sup>



Figure 4. Time-resolved emission of BCyP in  $D_2O$ , measured over the wavelength range of (a) 460–510 and (b) 530–610 nm and shown on a linear scale and emission shown on a semilog scale over the wavelength range of (c) 460–510 nm (d) 530–610 nm.



Figure 5. Time-resolved emission of BCyP in H<sub>2</sub>O compared to D<sub>2</sub>O, measured at (a) 500, (b) 510, (c) 530, and (d) 550 nm.



Figure 6. Time-resolved emission of BCyP in  $H_2O$  compared to NM6HQ<sup>+</sup>, measured at several wavelengths shown on (a) a linear scale and (b) a semilog scale.

Scheme 3. Molecular Structure of the Methoxy Analogue of BCyP



**Figure 7.** Normalized steady-state excitation spectra (red, collected at  $\lambda = 520$  nm) and emission spectra (black, excited at  $\lambda = 390$  nm) of the methoxy form of BCyP in neutral H<sub>2</sub>O.

Another topic we dealt with in the current study was the kinetic isotope effect of the ESPT process of BCyP. It had been previously found that the KIE depends on the  $pK_*$ <sup>\*, 8,25</sup> For weak photoacids (group I) it was found that the KIE is about  $\sim 3.0 \pm 0.2$ .<sup>26,27</sup> The green fluorescent protein, GFP, has a hydrophobic  $\beta$ -barrel structure with a chromophore positioned at the middle of the barrel.<sup>28</sup> The chromophore is a photoacid with an ESPT rate of  $10^{11}$ s<sup>-1</sup> ( $\tau_{PT}$ ~10 ps).<sup>29</sup> The proton acceptor is a glutamate residue (glu222) which is connected to the hydroxyl group of the chromophore via a proton wire consisting of a water molecule and serine residue (S205).<sup>28</sup> The KIE of the GFP ( $\sim$ 5) is the highest known for an ESPT process at room temperature.<sup>29</sup> The KIE of BCyP is rather low, (~1.5), similar to the value of proton mobility in water and D<sub>2</sub>O. KIE is associated with proton tunneling. The probability of proton tunneling depends on both the height and width of the reaction barrier. The smaller the barrier height and/or width, the greater the tunneling probability and the smaller the KIE. The lower limit of the kinetic isotope effect for a tunneling process is the mass ratio  $\sqrt{M_D/M_H}$  i.e.  $\sqrt{2} = 1.41$ .

The solvent-controlled rate of the ROH decay of BCyP may arise from a process other than the ESPT. If it is assumed that the ground-state conformation of BCyP is in the trans configuration, then in the excited state, the electrons of the cyanine conjugated subsystem are delocalized and the barrier to trans—cis isomerism is much smaller than in the ground state. There are many such trans—cis isomerizations that take place in the excited state at fast and ultrafast reaction rates, whereas in the ground state it can take hours or more. Stilbene and its



Figure 8. Time-resolved emission of the methoxy analogue of BCyP in  $H_2O$ , measured at several wavelengths and shown on (a) a linear scale and (b) a semilog scale.

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#### Scheme 4. Molecular Structure of Thioflavin T (ThT) and Auramine-O



## Thioflavin T (ThT)

derivatives are well-known to undergo excited-state trans-cis and cis-trans isomerizations at characteristic times shorter than 100 ps. Molecular rotors like ThT and Auramine-O, shown in Scheme 4 undergo ultrafast trans-cis isomerization in the excited-state, whereas in the ground-state the potential barrier for such processes is high.

The fluorescence signals of the decay of molecular rotors are nonexponential and the average lifetime at certain wavelengths is very short and depends on the solvent viscosity. The timeresolved fluorescence of the BCyP compound shows similar behavior. The rotation-relaxation times of ThT in methanol, ethanol, propanol, and other solvents depend on the solvent viscosity. In a previous study,<sup>30,31</sup> the solvent viscosity was changed by lowering the temperature of ThT in several alkanols. It was found that the nonradiative decay followed the rotation-relaxation times that depend on the solvent viscosity over a wide range of temperatures and solvents.

#### SUMMARY AND CONCLUSIONS

In the current work, we studied the photochemistry and photophysics of a new photoacid-the phenol benzoate cyanine picolinium salt (BCyP)—shown in Scheme 1. We found, by steady-state and time-resolved fluorescence techniques, that the new BCyP photoacid belongs to the "solventcontrol" regime of photoacids.<sup>18</sup> The solvent reorientation has a profound effect on stabilizing both the reactant and product of a photoacid. It also lowers the reaction potential along the reaction coordinate during the reaction, and most importantly, it also lowers the reaction-potential barrier if the solvent follows the reaction coordinate and controls the rate of the reaction. When the solvent orientational relaxation is important in lowering the reaction-potential curve, it can limit the reaction rate. In such a case, the reaction regime is that of the "solventcontrol regime" and this regime, in electron-transfer reactions and electron-transfer theory, attracted the attention of Sumi and Marcus<sup>23</sup> and Rips and Jortner.<sup>32</sup>

Indication that a photoacid belongs to the solvent-control regime, is the fact that the time-resolved fluorescence of the ROH form of the photoacid shows a large wavelength dependence and this can be seen in Figure 3. The average time constant of proton transfer in H<sub>2</sub>O is about 2 ps, about the same as found for NM6HQ<sup>+</sup>. The kinetic isotope effect is rather small, about 1.5. We suggest in the Discussion that a nonradiative process with the same time constant as the ESPT process in  $H_2O$  and  $D_2O$  is also taking place. We believe that the nonradiative rate is a trans-cis isomerization that takes place in the excited state, as it does in many conjugate bridge systems, like that of stilbene and its derivatives.



Auramine-O

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#### Notes

The authors declare no competing financial interest.

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