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Fluorescent pH probes based on boron dipyrromethene dyes

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ABSTRACT

Two novel fluorescent pH probes based on 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene dyes were configured as a "fluorophore-spacer-receptor" system. Their absorption and fluorescence properties were investigated in various solvents; in aqueous solution, based on their photo-induced electron transfer, the probes exhibited 3-fold (pH 8.70–4.93) and 14-fold (pH 9.02–3.29) fluorescence enhancement upon increase in the acidity of the solution, respectively. The probes can be used as fluorescent pH probes which are excitable with visible light; the pK_a values of the probes were determined from the fluorescence changes.

1. Introduction

The determination of pH is of great importance because it usually plays a crucial role in a variety of systems. There are many methods for pH measurement at present. However, fluorescent pH probes are especially interesting due to significant advantages over other techniques, such as nondestructive character, high sensitivity and specificity [1,2].

In recent years, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) fluorescent dyes [3] have been greatly developed [4–17]. They are a class of highly rigidized, polymethine-like highly fluorescent dyes, which has many attractive spectral characteristics, such as high absorption coefficient, high fluorescence quantum yield, and long-wavelength emission. Other useful properties of the BODIPY fluorophore are its photochemical stability and insensitivity to changes in experimental conditions, such as the polarity and oxygen content of the medium. This kind of fluorophore has been applied in the construction of selective and efficient fluorescent pH probes recently [18–23]. The review of some fluorescent pH probes based on BODIPY dyes are listed in Table 1.

The photo-induced electron transfer system using the "fluorophore-spacer-receptor" format, developed by de Silva [24,25], is one of the most popular approaches to the design of fluorescent sensors and switchers [26–31]. In this model, the excited state of the fluorophore can be quenched by intramolecular electron transfer from the receptor to the fluorophore (or vice versa) prior recognition. Upon recognition of species such as cations, the oxidation potential of the receptor is increased and this causes the electron transfer to be "switched off" and in turn the emission to be "switched on".

In this paper, based on PET mechanism two BODIPY fluorescent dyes **1** and **2** were synthesized (Scheme 1). Their spectroscopic properties were studied in different solvents. The influence of protons on the fluorescent intensity of them was discussed. The effect of substituents at an amino nitrogen atom on pK_a of both dyes was also studied.

2. Experimental

2.1. Instruments and reagents

Mass spectral studies were carried out using HP1100 APIES mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian INVOA 400 MHz spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard in $CDCl_3$. Steady-state emission and excitation spectra were recorded on Perkin Elmer LS55 instrument. All fluorescence emission spectra had been corrected for the spectral response of the detection system (emission correction file provided by instrument manufacturer). Visible absorption spectra were determined on Perkin Elmer Lambda 35 spectrophotometer. All the pH values were measured with a model PHS-3C meter (Shanghai Leici Equipment Factory, China).

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Table 1
Review of pH fluorescence probes based on BODIPY dyes

Dyes	Solvent	p*Ka*	Ref.
8-[4-Hydroxyphenyl]-4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethoxycarbonyl-4-bora-3a,4a-diaza-*s*-indacene	MeOH–H_2O (1:1, v/v)	10.4	[18]
8-[4-*N*,*N*-Dimethylaminophenyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	MeOH–H_2O (1:1, v/v)	3.3	[20]
8-Calix[4]arene-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	EtOH–H_2O (1:1, v/v)	6.5	[21]
8-[4-Hydroxyphenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene	H_2O	8.69	[22]
8-[3-Hydroxyphenyl]-4,4-difluoro-3,5-dimethyl-4-bora-3a,4a-diaza-*s*-indacene	H_2O	9.34	[22]
8-[6-Hydroxy-2-naphthalenyl]-4,4-difluoro-3,5-dimethyl-4-bora-3a,4a-diaza-*s*-indacene	H_2O	9.20	[22]
8-[4-Carboxyphenyl]-4,4-difluoro-3-[3-chloro-4-hydroxystyryl]-1,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	H_2O	7.60	[23]
8-[*N*,*N*-Diethylaminomethyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	H_2O	8.74	[10]
8-[*N*-Propylaminomethyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	EtOH–H_2O (1:1, v/v)	7.75	This article
8-[*N*,*N*-Dipropylaminomethyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene	EtOH–H_2O (1:1, v/v)	7.38	This article

All reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions. Tetrahydrofuran was distilled from sodium–benzophenone, and methylene chlorine was distilled from dry P_2O_5. All the materials were obtained from commercial suppliers and were used without further purification. Silica gel (200–300 meshes) was used for flash column chromatography. Boron trifluoride etherate (BF_3-OEt_2) was ca. 48% BF_3.

The pH titration was run in an ethanol/water mixture (1:1 v/v). In a typical experiment, a solution of the dyes containing small amounts of 1 M HCl was prepared. Diluted NaOH solution was added to achieve the appropriate pH change. The overall volume change did not exceed 2%.

2.2. Synthesis

2.2.1. 8-[Chloromethyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**3**)

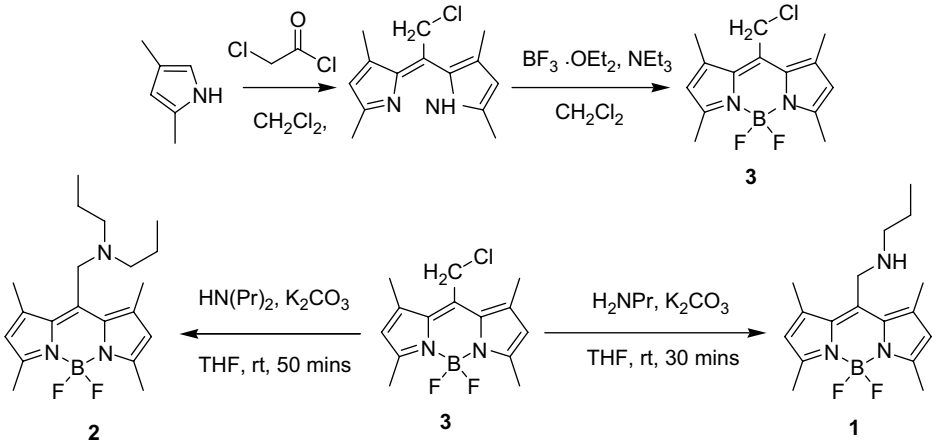
Chloro-acetyl chloride (0.15 mL, 1.95 mmol; note: incompatible with strong bases, alcohols, strong oxidizing agents, may decompose on exposure to water or moisture) and 2,4-dimethylpyrrole (0.4 mL, 3.89 mmol) were dissolved in dichloromethane and the ensuing mixture was stirred at room temperature under a N_2 atmosphere. After thin layer chromatography showed that the pyrrole had been consumed, BF_3-OEt_2 (8 mL, 7.52 mmol) and triethylamine (4 mL, 28.65 mmol) were added to the above mixture. Removing the solvent after 4 h by evaporation in vacuum, dark residue was obtained and purified by flash column chromatography (6:1 petroleum ether-ethyl acetate) to give 0.196 g compound **3** in 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ_H: 2.53 (s, 12H, CH_3), 4.77 (s, 2H, CH_2), 6.08 (s, 2H, CH). ^{13}C NMR (100 MHz, $CDCl_3$), δ_C: 14.6, 16.0, 37.3, 122.5, 131.6, 136.1, 141.3, 156.8. ^{19}F NMR (400 MHz, $CDCl_3$), δ: −146. ESI-MS positive: m/z $[M]^+ = 296.0$.

2.2.2. 8-(N-Propylaminomethyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**1**)

Compound **3** (201.3 mg, 0.68 mmol), *n*-propylamine (40.1 mg, 0.68 mmol), potassium iodide (16.6 mg, 0.10 mmol) and potassium carbonate (93.8 mg, 0.68 mmol) in 30 mL THF were stirred for 30 min under N_2 atmosphere. After filtration, THF was removed by evaporation. The crude compound was purified by flash column chromatography (1:5 petroleum ether–acetic ester) to afford 0.127 g of compound **1** in 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.25 (s, 1H, CH), 5.87 (s, 1H, CH), 3.72 (s, 2H, CH_2), 2.67 (t, 2H, $J = 7.3$ Hz, CH_2), 2.49 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 1.33–1.61 (m, 2H, CH_2), 1.05 (t, 3H, $J = 7.2$ Hz, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 153.2, 137.93, 135.56, 119.91, 113.57, 112.39, 55.35, 53.98, 23.13, 21.41, 15.37, 13.13, 12.15. ESI-MS positive: m/z $[M + H]^+ = 320.2$.

2.2.3. 8-[N,N-Dipropylaminomethyl]-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**2**)

Compound **3** (201.3 mg, 0.68 mmol), dipropylamine (68.7 mg, 0.68 mmol), potassium iodide (16.6 mg, 0.10 mmol) and potassium carbonate (93.8 mg, 0.68 mmol) in 30 mL THF were stirred for 50 min under N_2 atmosphere. After filtration, THF was removed by evaporation. The crude compound was purified by flash column chromatography (1:2 petroleum ether–acetic ester) to afford 0.106 g of compound **2** in 43% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.45 (s, 1H, CH), 6.05 (s, 1H, CH), 3.87 (s, 2H, CH_2), 2.58 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.41 (t, 4H, $J = 7.3$ Hz, CH_2), 1.49 (m, 4H, CH_2), 0.88 (t, 6H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 152.9, 146.37, 140.96, 141.82, 132.76,



Scheme 1. Synthesis of the BODIPY-based pH probes.

131.88, 120.46, 118.30, 65.75, 57.17, 52.11, 22.88, 17.79, 15.37, 14.32, 12.03. ESI-MS positive: m/z $[M+H]^+ = 362.3$.

3. Results and discussion

3.1. Design and synthesis of dyes

The two dyes (**1** and **2**) were designed for determining pH changes. They are based on the "fluorophore-spacer-receptor" model, where the BODIPY moieties are the fluorophores, and *n*-propylamine or dipropylamine is the proton receptor. Methylene fragment serves as spacer that covalently separates the two units. In these particular cases, it was predicted that a PET process would quench fluorescence emission of the BODIPY unit. The protonation of the *n*-propylamine or dipropylamine would disallow the electron transfer. Consequently the emission would be "switched on". Thus, we expect the fluorescence to be strong in acidic media.

The structures of the synthesized compounds were characterized by ^{1}H NMR spectra, ^{13}C NMR spectra, and MS. ^{1}H NMR spectra of the boradiazaindacence moieties in **1** and **2** show great changes from that of intermediate **3**. The singlet peak at 2.53 ppm of the four CH_3 groups in **3** splits into four peaks in **1** or **2**; the 4.77 ppm peak of the CH_2 spacer in **3** shifts upfield to 3.72 ppm for **1** and 3.87 ppm for **2**, respectively; the singlet peak at 6.08 ppm of the protons on the pyrrole rings in **3** splits into two peaks: 6.25 and 5.87 ppm in **1** (6.45 and 6.05 ppm in **2**). The distinguishing spectrum differences between the same parts of their structures imply that the boradiazaindacence moiety of **1** or **2** is no longer symmetric.

3.2. Spectroscopic properties

The new BODIPY dyes were dissolved in different solvents to study their spectroscopic properties (Table 2). For both dyes, the excitation and emission maxima shift to the red wavelength region with decreasing solvent polarity, indicating the more polar character of the ground state [22]. Both of the dyes have their maxima of excitation (493–503 nm) and emission (506–515 nm) within a very narrow wavelength range in the five solvents.

The Stokes shift indicates the difference in the properties and structure of the fluorophores in the ground state S_0 and in the first exited state S_1. The Stokes shift is given in Eq. (1). The Stokes shifts for both dyes are small ($\Delta_{max}\nu = 389$–$829\ cm^{-1}$).

$$\nu_A - \nu_F\left(cm^{-1}\right) = \left(\frac{1}{\lambda_A} - \frac{1}{\lambda_F}\right) \times 10^7 \quad (1)$$

The fluorescence efficiencies of the dyes were estimated by measuring their fluorescence quantum yield using Eq. (2) on the basis of the absorption and fluorescence spectra taken in different solvents. Fluorescein ($\Phi = 0.90$) in 0.1 M NaOH was used as a standard [32].

$$\Phi_{sample} = \Phi_{st}\frac{S_u}{S_{st}}\frac{\lambda_{st}}{\lambda_u}\frac{n_{Du}^2}{n_{Dst}^2} \quad (2)$$

where Φ_{st} is the emission quantum yield of the standard, λ_{st} and λ_u represent the absorbance of the standard and sample at the excited wavelength, respectively, while S_{st} and S_u are the integrated emission band areas of the standard and sample, respectively, and n_{Dst} and n_{Du} are the solvent refractive index of the standard and sample, u and s refer the unknown and standard, respectively.

As seen from the data in Table 1, the fluorescence quantum yields (Φ_f) of them are relatively poor in the solvents. This might result from the quenching of intramolecular electron pair of its amino nitrogen atom via a PET mechanism.

The molar absorption coefficients for both BODIPY dyes are high and lie in the 53 200–91 100 $L\,mol^{-1}\,cm^{-1}$ range. The fact that the molar absorption coefficients are higher than $10^4\,L\,mol^{-1}\,cm^{-1}$ indicate that the long-wavelength band of the absorption spectra is a band of charge transfer which occurs as a result from a $\pi \rightarrow \pi^*$ electron transfer during the $S_0 \rightarrow S_1$ transition.

Fig. 1 illustrates the PET process in terms of molecular orbitals. Upon excitation of the BODIPY fluorophore, an electron of the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), which enables PET from the HOMO of the donor (amino nitrogen atom) to that of the BODIPY fluorophore, causing fluorescence quenching of the latter. Upon protonation, the HOMO of the donor becomes lower in energy than that of the BODIPY fluorophore; therefore, PET is no longer possible, and the fluorescence intensity of the probes was enhanced.

3.3. Dependence of pH on the absorbance and fluorescent intensity of the dyes

Fig. 2 shows that there is no significant shift of the absorption maxima for compounds **1** and **2** at different pH values. The absorbance of **1** and **2** decreased slightly with decreasing pH values.

As shown in Fig. 3, the fluorescence emission intensities of **1** and **2** increased significantly at lower pH values, whereas the wavelength of emission remains unchanged as a function of pH. In the low pH ($<pK_a$), the amino nitrogen atom in the molecules of the dye was protonated. The protonation changes the electron state of the amino nitrogen atom. Subsequently, PET was stopped and fluorescence recovered. The fluorescence intensity in low pH (4.93) was ca. 3-fold larger than that in high pH (8.70) for probe **1**. The fluorescence intensity in low pH (3.29) was ca. 14-fold larger than that in high pH (9.02) for probe **2**. Therefore, the fluorescent enhancement factor of **2** was larger than that of **1** upon proton recognition. The fluorescence change of both of the probes was fully reversible and took place mainly within the pH range from 4.93 to 8.70 for probe **1** and from 3.29 to 9.02 for probe **2**.

Table 2
Photophysical characteristics of the two dyes in different solvents

Dyes	Solvent	λ_{Abs} (max/nm)	λ_{em} (max/nm)	λ_{ex} (max/nm)	$\Delta_{max}\nu$ (cm^{-1})	Φ_f	$\varepsilon \times 10^4$ ($L\,mol^{-1}\,cm^{-1}$)
1	MeCN	493	506	493	521	0.019	7.38
	EtOH	495	508	495	517	0.096	5.32
	THF	496	509	496	515	0.053	6.78
	CH_2Cl_2	499	510	499	433	0.067	6.23
	Cyclohexane	502	512	502	389	0.099	6.08
2	MeCN	493	514	493	829	0.001	7.45
	EtOH	496	512	496	630	0.018	8.33
	THF	497	512	497	589	0.033	7.56
	CH_2Cl_2	500	513	500	507	0.040	8.31
	Cyclohexane	503	515	503	464	0.043	9.11

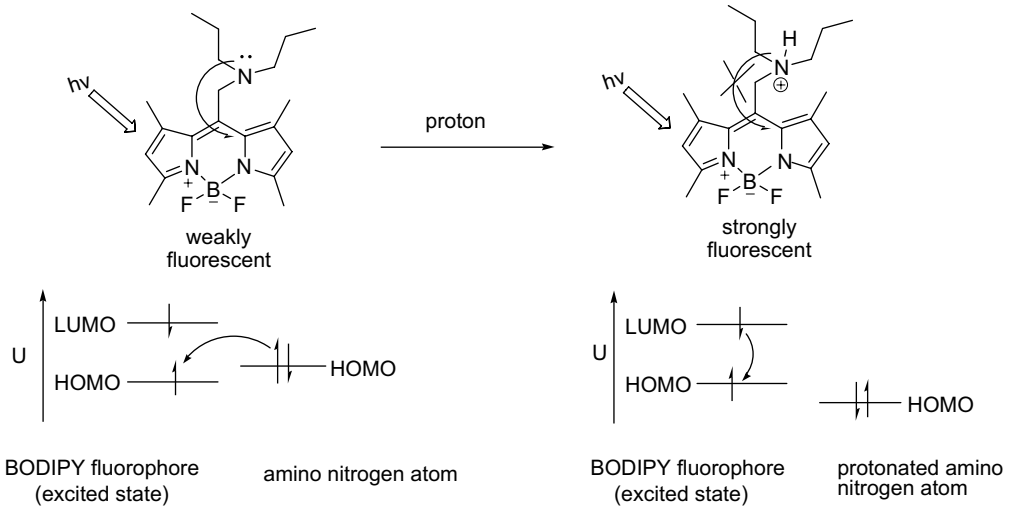


Fig. 1. The principle of proton recognition by fluorescent PET molecular probe **2**.

3.4. The acidity constants (K_a)

The acidity constants K_a of probe **1** and probe **2** were determined in ethanol/water (1:1, v/v) solution by fluorimetric titration as a function of pH using the fluorescence emission spectra. Sigmoidal curve fitting of Eq. (3) to the normalized fluorescence intensity I/I_{max} recorded as a function of pH yielded values of K_a, the fluorescence intensity I_{min} and I_{max} at minimal and maximal [H^+], respectively.

$$pK_a = pH - \log\frac{I - I_A}{I_B - I} \quad (3)$$

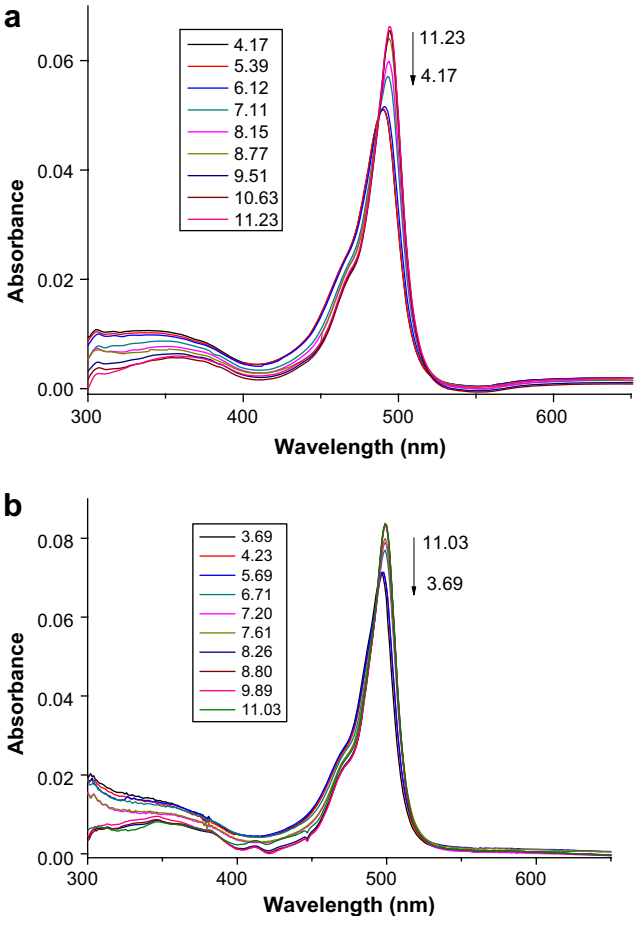


Fig. 2. Absorption spectra of **1** and **2** in ethanol/water (1:1, v/v) solutions as a function of pH. (a) Probe **1**; (b) probe **2**.

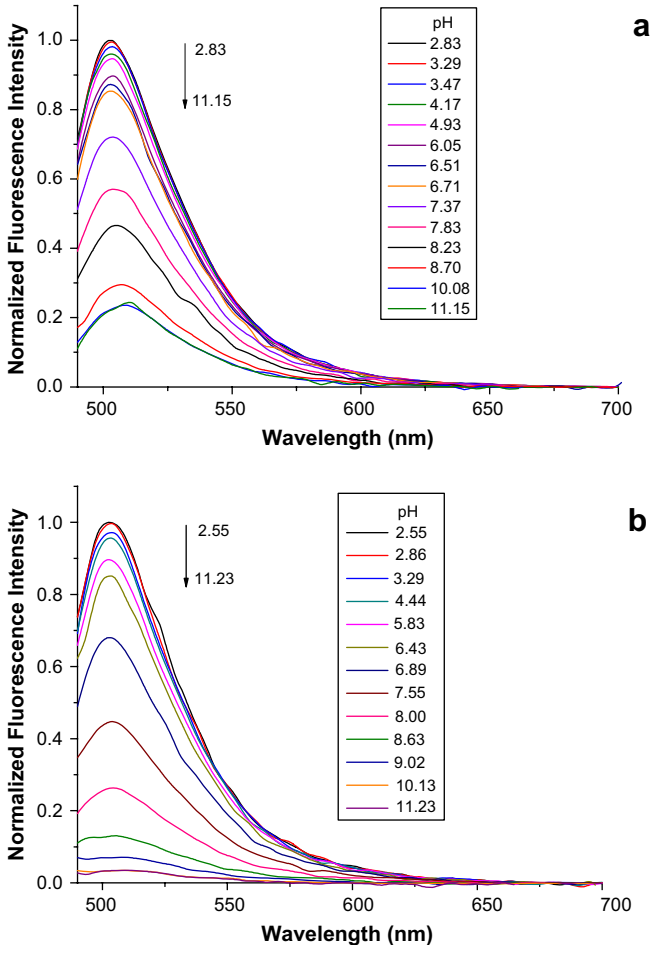


Fig. 3. Normalized fluorescence spectra of **1** and **2** in ethanol/water (1:1, v/v) solutions of different pH values. Excitation was at 480 nm. (a) Probe **1**; (b) probe **2**.

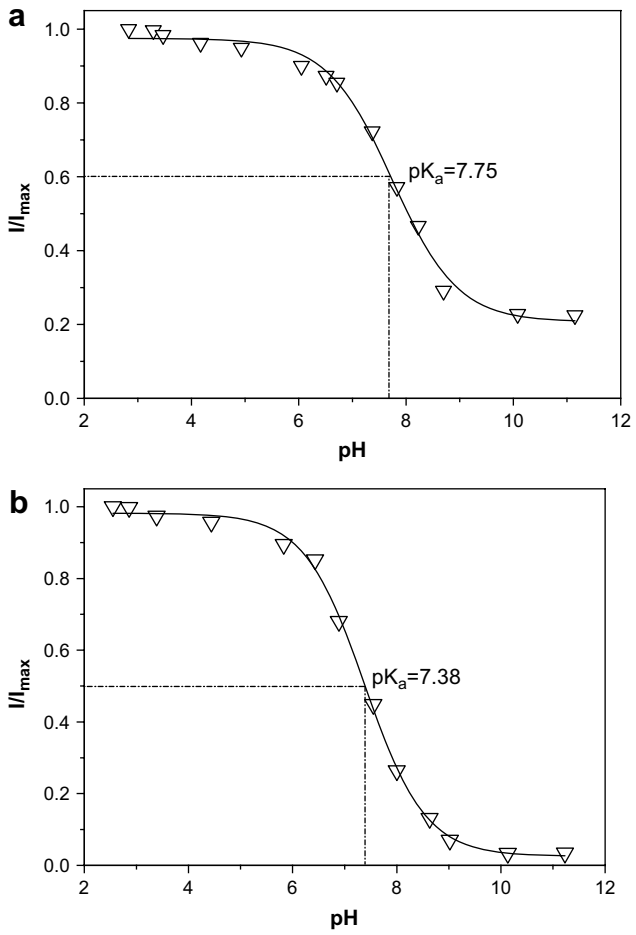


Fig. 4. Normalized fluorescence intensity (emission maxima wavelength) of the probes in ethanol/water (1:1, v/v) solutions versus pH. Excitation was at 480 nm. (a) Probe **1**; (b) probe **2**.

The pK_a of the BODIPY probes can be tuned by varying the substituents on the amino nitrogen atom. When only one hydrogen atom stays on the amino nitrogen atom of the probe, the pK_a of **1** is 7.75. When both hydrogen atoms on the amino nitrogen atom are substituted with *n*-propyl group, the pK_a of **2** drops to 7.38 (Fig. 4). This may be due to the stress structure of the molecule and the protonation of the tertiary nitrogen atom will lead to greater steric strain [33]. Therefore, the affinity of a proton to the tertiary nitrogen atom becomes more difficult and the pK_a of probe **2** is lower than that of probe **1**.

4. Conclusion

Two new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene dyes (**1** amd **2**) were synthesized. Their absorption and fluorescence properties were investigated as a function of solvents. Fluorescent dyes have absorption maxima in the 493–503 nm region and fluorescence emission maxima around 506–515 nm, depending on the solvents. Based on PET mechanism, probe **2** shows a significant fluorescent enhancement (14-fold) upon increasing the acidity of the solution. They can be used in aqueous solution as fluorescent pH probes excitable with visible light, with pK_a values 7.75 for probe **1** and 7.38 for probe **2**, respectively, depending on the substituents on the amino nitrogen atom.

Acknowledgments

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