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The synthesis of polarity-sensitive fluorescent dyes based on the BODIPY chromophore

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ABSTRACT

Two novel BODIPY dyes were synthesized, and their fluorescence characteristics studied in different solvents. Both dyes were extremely sensitive to solvent polarity. Their fluorescence intensities were very low in DMSO but increased more than 80 times in chloroform; similar behaviour was found in fluorescence quantum yield, which was enhanced ~60-fold. Both dyes displayed fluorescence enhancement with bovine serum albumin, which illustrated that the two probes were very sensitive to polarity change and, therefore, may be valuable as microenvironment-sensitive probes in biochemical research.

1. Introduction

In recent years, dipyrrometheneboron difluoride (difluoroboradiaza-*s*-indacene, BODIPY) has attracted much interest because of its excellent high molar extinction coefficient, high fluorescence quantum yield and good stability towards light and chemical [1]. Many BODIPY derivatives have been synthesized and utilized in fields as diverse as fluorescent dyes [2], chemosensors [3–8], light harvesting systems [9] and photodynamic therapy [10]. This work describes two BODIPY dyes (**1** and **2**) which can be used as polarity-sensitive probes. It is well known that environment-sensitive fluorescence probes are widely used in biochemical research [11–14], their fluorescence properties being dependent upon the polarity of the environments. They generally possess low fluorescence quantum yield in polar solvents, but become highly fluorescent in apolar solvents or exhibit a longer emission wavelength in polar solvents but a shorter wavelength in nonpolar solvents [15]. Although many polarity probes [16–18] have been reported, there are still some disadvantages that exist; for example, either they display a short emission wavelength or the changes in different solvents are not marked enough. Hence, a challenge remains to develop polarity-sensitive probes with longer emission wavelength. This paper concerns two, environment-sensitive dyes with good chemical stability and excellent photostability, whose fluorescence properties are greatly influenced by different polarity solvents and which is attributed to a photoinduced electron transfer (PET) mechanism. The two dyes were also applied to bovine serum albumin (BSA) to examine the polarity changes.

2. Experimental

2.1. Chemicals and instruments

All solvents and reagents were either of analytical grade or HPLC grade. All chemicals were purchased from commercial sources. Fluorescence spectra were obtained with FLS-920 Edinburgh Fluorescence Spectrometer with a Xenon lamp and 1.0 cm quartz cells at the slits of 2/2 nm. Absorption spectra were measured on a pharmaspect UV-1700 UV–Visible spectrophotometer (SHIMADZU). All pH measurements were made with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. ^{1}H NMR, ^{13}C NMR spectra were taken on a Bruker 300 MHz spectrometer. Rhodamine B was selected as the fluorescence quantum yield standard ($\phi_f = 0.69$ in methanol [19]).

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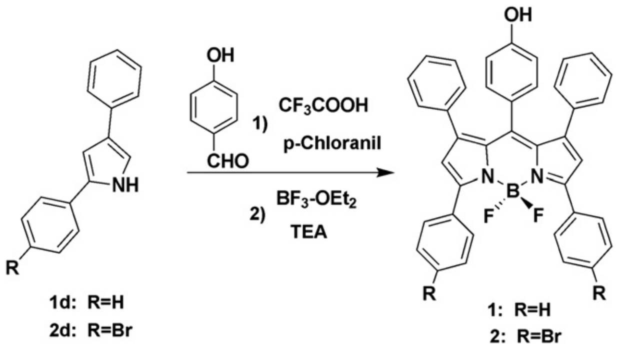

Fig. 1. The synthesis of dye **1** and dye **2**.

2.2. *Synthesis of 1: 4,4-difluoro -8-[4-hydroxyphenyl] –1,3,5,7-tetraphenyl -4- bora- 3a,4a -diaza-s-indacene (**1**)*

Compound **1d** (0.40 g, 1.8 mmol) and p-hydroxy benzaldehyde (0.10 g, 0.9 mmol) were dissolved in 100 mL of absolute dichloromethane. A single drop of trifluoroacetic acid and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.2 g, 9.0 mmol) were added, and the solution was stirred at room temperature for 5 h. The reaction mixture was condensed to 10 mL and filtered to provide a green solid. Without purification, the green solid and 15 mL of triethylamine were dissolved in 100 mL of abs. dichloromethane under an Ar atmosphere, and the mixture was stirred at room temperature for 15 min; 10 mL of borontrifluoride etherate were then added and stirring was continued overnight. The ensuing solution was washed with water and dried over anhydrous $MgSO_4$, filtered, and evaporated. The crude product was purified by silica gel chromatography eluting with $CHCl_3$ to afford dye **1** as a red solid. (0.20 g, 37.8%) ^1H NMR (300 MHz, DMSO): δ 5.84–5.87(d, $J = 9.0$ Hz, 2H), 6.69–6.73(t, $J = 4.5$ Hz, 4H), 6.80–6.83(d, $J = 9.0$ Hz, 4H), 6.89–6.98(m, 6H), 7.45(s, 6H), 8.30(s, 4H), 9.28(s, 1H, –OH). ^{13}C NMR (75 MHz, DMSO): 156.9, 156.3, 147.9, 136.0, 133.8, 132.8, 129.6, 129.5, 129.0, 128.3, 127.4, 126.2, 124.3, 123.4, 113.7. IR (KBr disc) cm^{-1}: 1164(B–F), 3441 (–OH). Anal. Calcd for $C_{39}H_{27}BF_2N_2O$: C, 79.60; H, 4.62; B, 1.84; F, 6.46; N, 4.76. Found: C, 79.94; H, 4.73; B, 2.01; F, 6.69; N, 4.95. HRMS [Found: *m/z* 588.2136, Calcd for $C_{39}H_{27}BF_2N_2O$: M, 588.4520].

2.3. *Synthesis of 2: 4,4-difluoro-8-[4-hydroxyphenyl]-1,7-diphenyl-3,5-dibromo phenyl4-bora- 3a,4a-diaza-s-indacene (**2**)*

2 was synthesized in a similar way to that described for the synthesis of dye **1**. Yield 44.7%, violet red solid. ^1H NMR (300 MHz, DMSO): δ 5.83–5.86(d, $J = 9.0$ Hz, 2H), 6.68–6.71(d, $J = 9.0$ Hz, 2H), 6.78–6.82(m, 6H), 6.92–6.94(m, 6H), 7.65–7.68(d, $J = 9.0$ Hz, 4H), 7.75–7.78(d, $J = 9.0$ Hz, 4H), 9.31(s, 1H, –OH). ^{13}C NMR (75 MHz, DMSO): 159.6, 155.1, 148.6, 135.7, 134.2, 132.8, 131.9, 131.8, 129.1, 127.8, 126.7, 123.9, 121.9, 114.1. IR (KBr disc) cm^{-1}: 1167(B–F), 3422 (–OH). Anal. Calcd for $C_{39}H_{25}BBr_2F_2N_2O$: C, 62.77; H, 3.38; B, 1.45; Br, 21.41; F, 5.09; N, 3.75. Found: C, 63.13; H, 3.72; B, 1.71; Br, 21.71; F, 5.37; N, 4.02. HRMS [Found: *m/z* 746.1457, Calcd for $C_{39}H_{25}BBr_2F_2N_2O$: M, 746.2415].

2.4. *Determination of quantum yields*

Fluorescence quantum yield measurements were performed on a fluorometer and UV/vis instrument. The slit width was 1.2 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield.

$$\Phi_x = \Phi_s \times (F_x/F_s) \times (n_x/n_s)^2 \times (A_s/A_x)$$

Where Φ_s is the reported quantum yield of the standard, F is the integrated emission spectrum, A is the absorbance at the excitation wavelength, and n is the refractive index of the solvents used. The subscript x denotes unknown and s denotes standard.

3. Results and discussion

3.1. *Synthesis of the BODIPY derivatives*

For the synthesis of dye **1** and dye **2**(Fig. 1), we first prepared compound **1d** and **2d** according to a known procedure [20,21], then compound **1d** and p-hydroxy benzaldehyde were dissolved in CH_2Cl_2 at room temperature in the presence of TFA as catalyst, followed by oxidation with p-chloranil, finally reacted with TEA and $BF_3 \cdot OEt_2$ to afford dye **1**. Dye **2** was prepared in the similar way.

3.2. *Spectroscopic properties of the BODIPY derivatives*

To research their spectroscopic properties, the two new BODIPY dyes were dissolved in different solvents to be studied (Table 1).

Table 1
Spectroscopic data of dye **1** and dye **2** in different solvents at 298 K.

Dye	Solvent	λ_{abs} [nm]	λ_{em} [nm]	ϕf	τ_f [ns]	k_f [10^8 s^{-1}]	kn_f [10^8 s^{-1}]
1.	CH_2Cl_2	552 ± 1	590 ± 1	0.61 ± 0.05	6.06 ± 0.1	1.01 ± 0.06	0.64 ± 0.04
	$CHCl_3$	555 ± 1	591 ± 1	0.62 ± 0.03	4.71 ± 0.1	1.32 ± 0.05	0.80 ± 0.05
	THF	555 ± 1	590 ± 1	0.44 ± 0.01	3.65 ± 0.1	1.21 ± 0.02	1.53 ± 0.08
	EtOAc	551 ± 2	587 ± 2	0.37 ± 0.02	4.09 ± 0.2	0.90 ± 0.09	1.54 ± 0.11
	CH_3CN	548 ± 1	587 ± 1	0.21 ± 0.01	2.95 ± 0.1	0.71 ± 0.01	2.68 ± 0.09
	EtOH	551 ± 1	587 ± 1	0.20 ± 0.01	2.71 ± 0.1	0.74 ± 0.01	2.95 ± 0.09
	CH_3OH	549 ± 2	584 ± 2	0.09 ± 0.003	2.79 ± 0.1	0.32 ± 0.02	3.26 ± 0.08
	DMS0	560 ± 2	598 ± 2	0.01 ± 0.001	5.64 ± 0.2	0.02 ± 0.001	1.75 ± 0.19
2.	CH_2Cl_2	556 ± 1	598 ± 1	0.55 ± 0.03	5.86 ± 0.2	0.93 ± 0.02	0.78 ± 0.18
	$CHCl_3$	556 ± 1	600 ± 1	0.60 ± 0.02	5.57 ± 0.1	1.08 ± 0.01	0.72 ± 0.09
	THF	557 ± 2	599 ± 2	0.45 ± 0.03	5.45 ± 0.1	0.83 ± 0.03	1.00 ± 0.07
	EtOAc	555 ± 1	596 ± 1	0.44 ± 0.01	5.66 ± 0.1	0.80 ± 0.02	0.97 ± 0.08
	CH_3CN	553 ± 1	594 ± 1	0.16 ± 0.01	5.17 ± 0.2	0.31 ± 0.01	1.62 ± 0.19
	EtOH	555 ± 2	595 ± 1	0.10 ± 0.004	3.56 ± 0.1	0.28 ± 0.004	2.53 ± 0.09
	CH_3OH	551 ± 1	594 ± 1	0.05 ± 0.003	5.04 ± 0.2	0.10 ± 0.001	1.88 ± 0.19
	DMS0	567 ± 2	605 ± 2	0.01 ± 0.001	5.91 ± 0.2	0.02 ± 0.002	1.67 ± 0.19

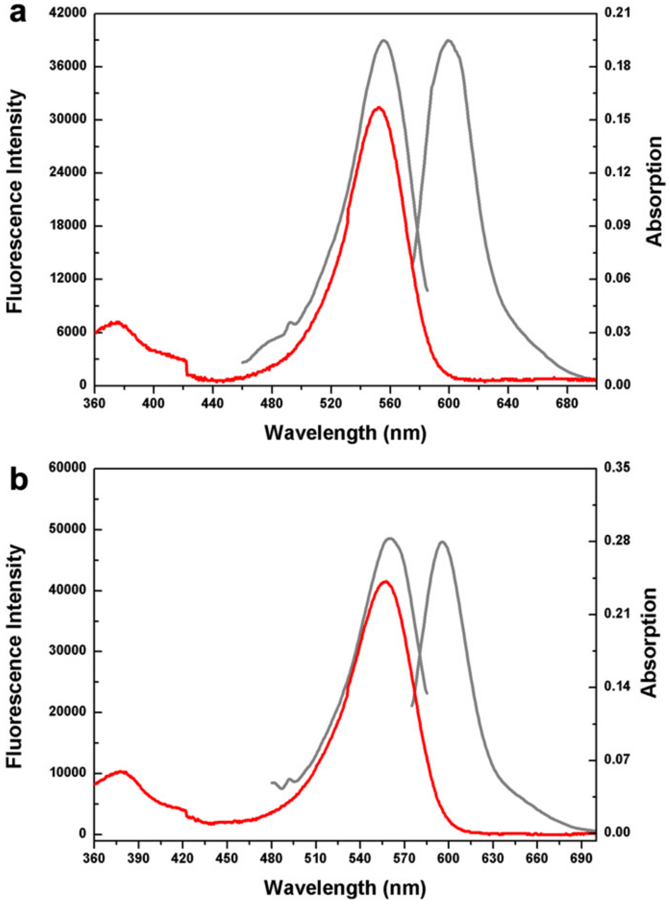

Fig. 2. The overlay of the excitation, emission (gray lines) and absorption spectra (red lines) in ethanol($c = 1.0 \times 10^{-5}$M). (a): dye **1** (b): dye **2**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The overlay of the excitation, emission and absorption spectra was shown in Fig. 2. Besides, the fluorescence intensities of dye **1** and dye **2** in different solvents were separately tested (Fig. 3). Finally, we investigated their responses to bovine serum albumin (Figs. 6 and 7, 1% DMSO as cosolvent).

We could find that their fluorescence intensities depended greatly on the polarity of solvents (Fig. 3). Dye **1** showed very low fluorescence in dimethylsulfoxide. Along with the increase of polarities, the fluorescence enhanced gradually, which reached 84 times stronger in chloroform. Similar results were observed in dye **2** and 168 times fluorescence intensity increase was shown in chloroform.

Moreover, their fluorescence quantum yields were obviously influenced by the solution polarities (Table 1). Their fluorescence quantum yields were both about 0.01 in dimethylsulfoxide, but in chloroform they increased around 60-fold, which reached to 0.62 and 0.60 respectively.

Next, we investigated their responses to different pH in water solution (Fig. 4, 1% DMSO as cosolvent), the results illuminated that neither of them was sensitive to pH in physiological condition [22]. Furthermore, the kinetics experiments were carried out (Fig. 5). The time-evolution of the fluorescence intensity keep changeless after continuous illumination, which displayed they had excellent photostability. So they would be used with high stability in biological system.

It was obvious that dye **1** and dye **2** were highly sensitive to the solvent polarity. We attributed the polarity-dependence phenomenon to the photoinduced electron transfer (PET) mechanism.

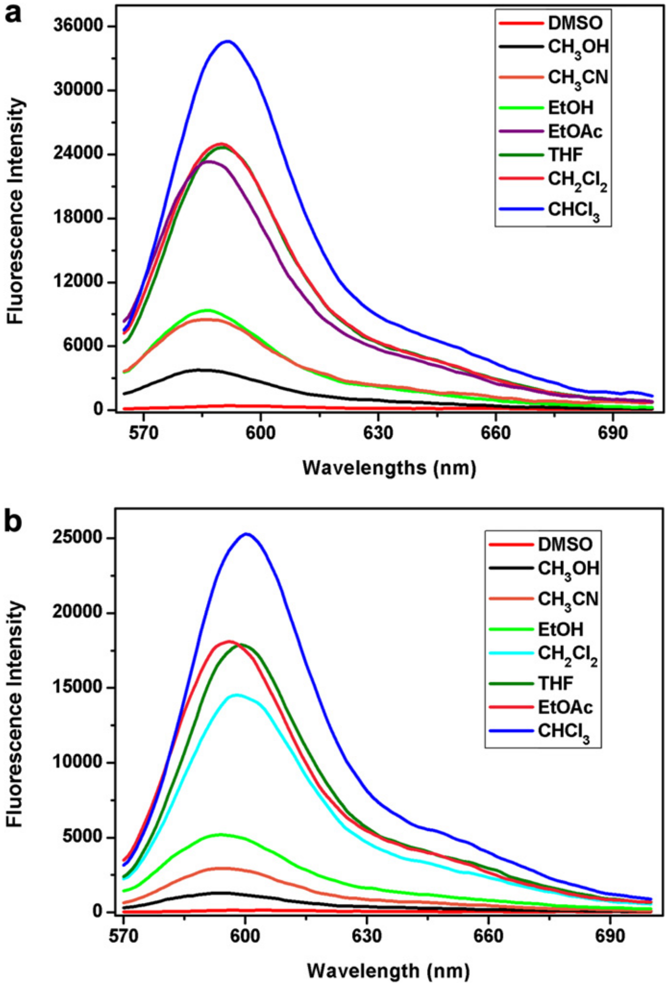

Fig. 3. (a) The fluorescence intensity of dye 1 ($c = \mathbf{1} \times 10^{-5}$M, $\lambda_{ex} = 550$ nm) in different solvents. (b) The fluorescence intensity of dye **2** ($c = 1 \times 10^{-5}$M, $\lambda_{ex} = 555$ nm) in different solvents.

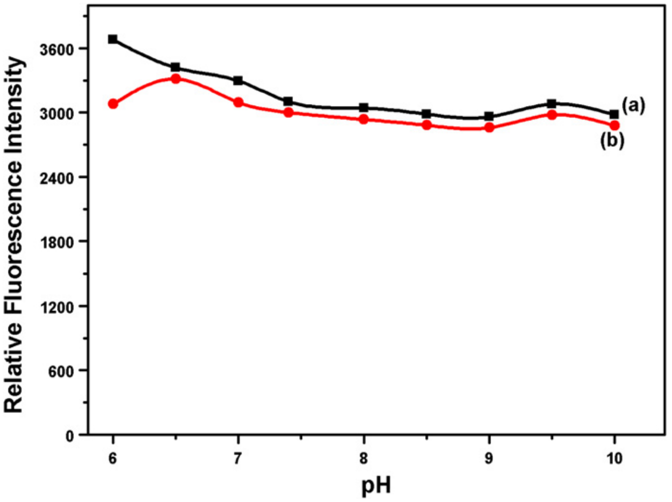

Fig. 4. (a) The Fluorescence intensity response of dye **1** (a) ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 607$ nm) and dye **2** (b) ($\lambda_{ex} = 571$ nm, $\lambda_{em} = 617$ nm) to different pH. ($c = 5.0 \times 10^{-6}$ M, 1% DMSO as cosolvent in sodium phosphate buffer).

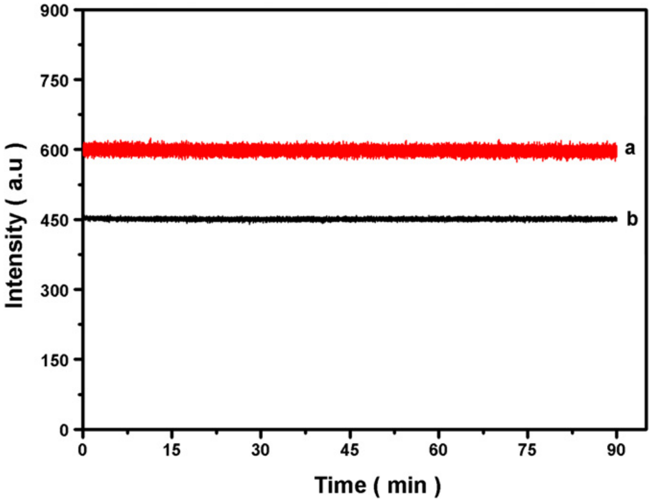

Fig. 5. The kinetics experiments of the dye **1** (a) and dye **2** (b) at 298 K in EtOH ($c = 5.0 \times 10^{-6}$ M, $\lambda_{ex} = 550$ nm and 555 nm respectively).

It was proposed that the BODIPY fluorophore acted as the electron acceptor and the phenol moiety as the electron donor. It is reported that the feasibility of electron transfer can be judged from the change in free energy (ΔG_{eT}). The ΔG_{eT} value can be calculated by the Rehm–Weller equation [23],

$$\Delta G_{eT} = E_{ox} - E_{red} - E_{00} - C$$

where E_{ox} and E_{red} are the oxidation potential of the electron donor and the reduction potential of the acceptor, respectively, E_{00} is the singlet excited energy of the fluorophore, and C is the work term for the charge separation state.

The group of Nagano [18] suggest that the redox potential affects ΔG_{eT} and determines the rate of electron transfer. Therefore, we measured E_{red} of dye **2** in every solvent.

3.3. Electrochemistry

Cyclic voltammetry was carried out to determine the redox potentials of dye **2** in various solvents (Table 2). The reduction potential of the BODIPY fluorophore decreased (from −0.69 V in DMSO to −0.84 V in CH_2Cl_2). These results indicated that it was harder for the fluorophore to be reduced in less polar solvents. Electron transfer should be favoured in polar media because of the stabilization of the CT state in polar environment. Therefore, PET processes occurred more easily in polarity solvents.

3.4. Biological applications of the BODIPY dyes to serum albumin

Finally, we examined the responses of the two dyes to bovine serum albumin. The two BODIPY dyes were dissolved in sodium phosphate buffer (pH 7.40) with or without BSA. As shown in Fig. 6, the absorption maximum of them showed a blue-shift in the presence of BSA. Both showed a marked fluorescence increase in the presence of BSA. Their fluorescence emission were quenched in buffer by PET, but when BSA was added, the fluorescence increase because PET is less favoured at the surface of BSA. Then BSA of different concentrations was added to the solution. The fluorescence intensity increased continuously with varying amounts of BSA (Fig. 7). Dye **1** showed a nearly 30-fold fluorescence increase when 8.75 μM BSA was added, and the enhancement of dye **2** reached to about 20 times with BSA. The results suggested that the two dyes were extremely sensitive to the polarity change. They could be used to examine the protein polarity change.

Table 2
The Reduction Potential of dye **2** in Various Solvents.[a]

	DMSO	CH_3CN	MeOH	CH_3Cl_2
	E_{red}(V vs SCE)			
Dye **2**	−0.69	−0.81	n.d.[b]	−0.84

[a] The redox potentials were measured by cyclic voltammetry in DMSO, CH_3CN, MeOH, CH_2Cl_2, containing 0.10 M TBAP with a scan rate of 0.1 V/s^{-1}.
[b] n.d. = not detectable.

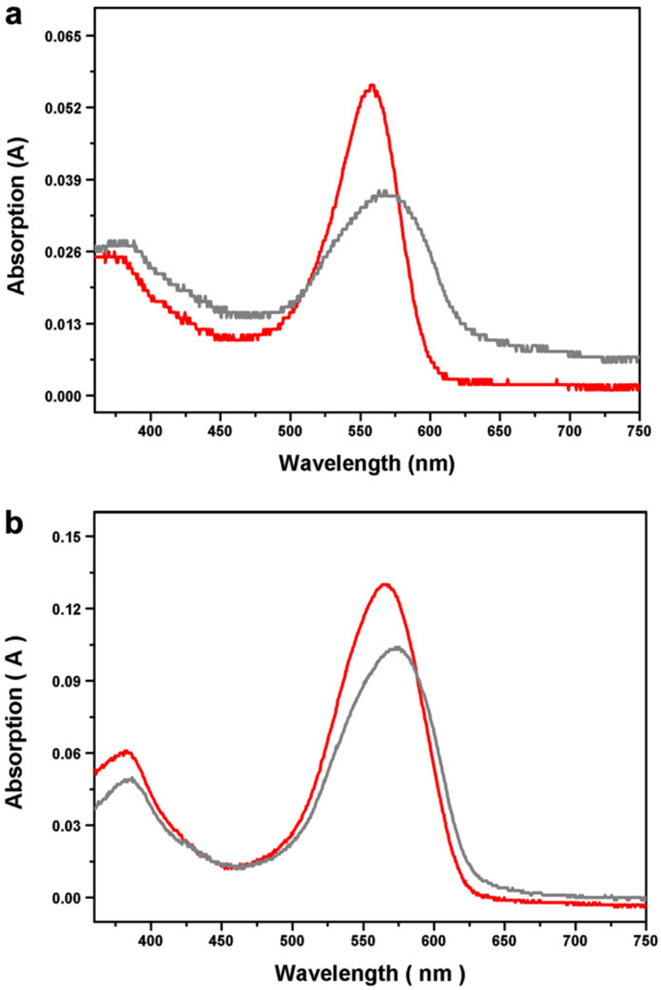

Fig. 6. Absorption spectra change of dye **1** (a) and dye **2** (b) (6.25 μM, containing1% DMSO as cosolvent) with (red lines) or without (gray lines) BSA (8.75 μM) in sodium phosphate buffer (pH 7.40). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

It was obvious that dye **1** and dye **2** showed high sensitivity to the solvent polarity. Thus, dye **1** and dye **2** could be used as polarity-sensitive fluorescence probes. Although a library of BODIPY-based environmental polarity sensors [18] were synthesized, the emission wavelength of their compounds is range from 507 nm to 519 nm. Among them, the longest emission wavelength is 519 nm, but the emission wavelengths of our dyes were all above 580 nm, dye **2** even reached 605 nm, which could reduce the background fluorescence better in biosystem. Besides, the two dyes both showed marked fluorescence increase with only 8.75 μM BSA addition, which illustrated they had high sensitivity. Moreover, we are planning to modify dye **1** and dye **2** with some functional groups,

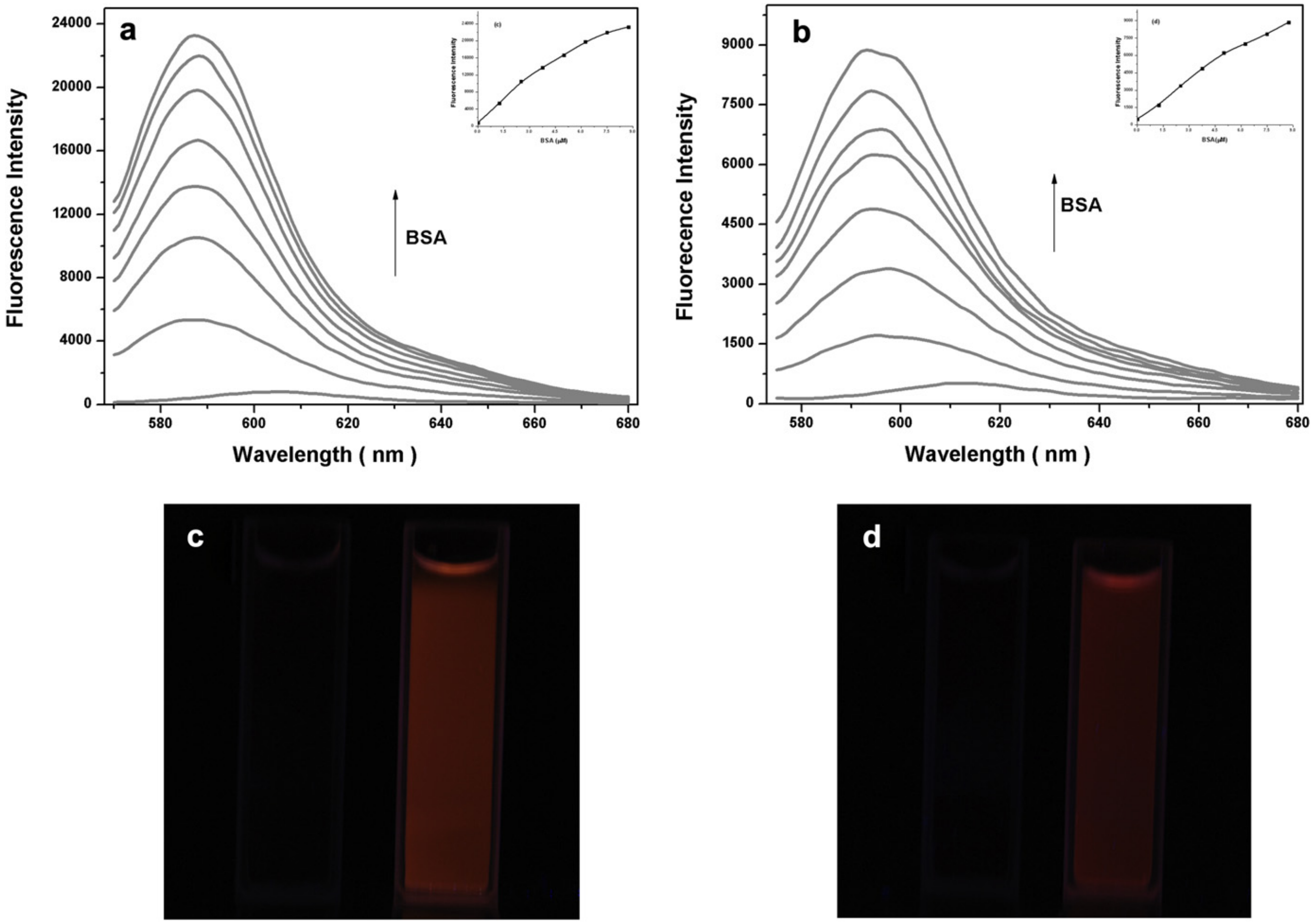

Fig. 7. The fluorescence intensities and the emission wavelengths maximum of dye **1** (a) and dye **2** (b) are altered by BSA (excited at 560 nm and 570 nm respectively). The molar concentrations of BSA were 1.25, 2.50, 3.75, 5.00, 6.25, 7.50, 8.75 μM respectively. BSA shifted the maximal emission wavelength of dye **1** from 607 nm to 588 nm and dye **2** from 617 nm to 608 nm. Fluorescence change of dye **1** (c) and dye **2** (d) excited at 365 nm (left: without BSA and right: with BSA).

and they can be attached to the proteins to study the conformational changes. The related work is being in progress.

4. Conclusion

We synthesized two new BODIPY dyes (dye **1**, dye **2**). They all have good chemical stability, excellent photostability. Significantly, they were quite sensitive to solvent polarity. Their fluorescence intensity and fluorescence quantum yields were greatly influenced by solvent polarity. They were very low in dimethylsulfoxide and enhanced notably in chloroform. Their application to the bovine serum albumin illuminated they had high sensitivity and were suitable for biological research. Therefore, they would be greatly valuable as microenvironment-sensitive probes in biochemical research. Their biological applications are being further studied.

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