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Synthesis and spectral properties of new boron dipyrromethene dyes

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Abstract

New BODIPY dyes have been synthesized via the condensation of R–CH_2COCl (R = H, Br, Cl and Et_2N) with 2,4-dimethylpyrrole and the coordination with BF_3 etherate. The acetyl chlorides with electron-withdrawing substituents (R = Br or Cl) are easier to react with the 2,4-dimethylpyrrole to form the related dyes and make a red-shift in absorbance and fluorescence maximum. Dye **4** (R = Et_2N), based on PET mechanism, exhibits good properties as a pH fluorescent sensor with a pK_a of 8.74 and a 15-fold fluorescence enhancement in the pH range of 9.38–7.60.

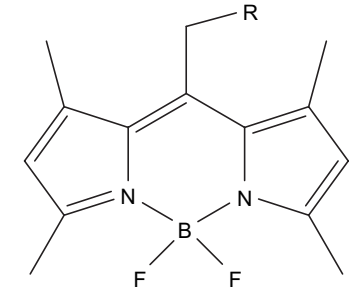
Keywords: BODIPY; Fluorescent dyes; PET; pH probe; Biological label

1. Introduction

In the past decades, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes [1] have been greatly developed and their applications in biochemistry and molecular biology also made a lot of progress [2]. BODIPY dyes have shown excellent photophysical properties such as high absorption coefficients, high quantum yields, little sensitivity to solvent polarity and pH, and narrow band width [3]. The maximum absorption and emission wavelengths of the dyes range from 500 nm to 700 nm, and vary with the different substituents on the pyrrole-moieties. Kang and copartners [4,5] have synthesized a lot of BODIPY dyes from aldehydes and two equivalents of pyrroles. Kevin Burgess' group has synthesized a series of long wavelength BODIPY dyes [6,7] through enlarging the conjugate of pyrroles and described another synthetic way in which acyl chlorides, instead of aldehydes, were taken as starting materials. The acyl-chloride way was a one-spot reaction with much higher yields and did not need a successive oxidizing step.

Based on BODIPY fluorophores, some of sensors or switches have been studied, which have been used for the detection of alkali and alkali-earth metal ions (e.g. Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Na^{+} [8]), heavy and transition metal ions (e.g. Hg^{+}, Ag^{+}, Cu^{2+}, Zn^{2+} [9–12]), and neutral molecular (e.g. nitric oxide [13]). For their narrow fluorescent bands, these types of dyes are more suitable for DNA sequencing [14,15]. A pH probe, based on BODIPY reporter, has been studied by Baki and exhibited fluorescent change near neutrality with a pK_a of 6.5 [16].

In this paper, the synthetic reactivity of the acetyl chlorides with different electron-withdrawing or -donating groups and the spectral properties of the resulted dyes (**1**–**4**, Scheme 1)



	1	2	3	4
R =	H	Br	Cl	$N(CH_2CH_3)_2$

Scheme 1. BODIPY dyes.

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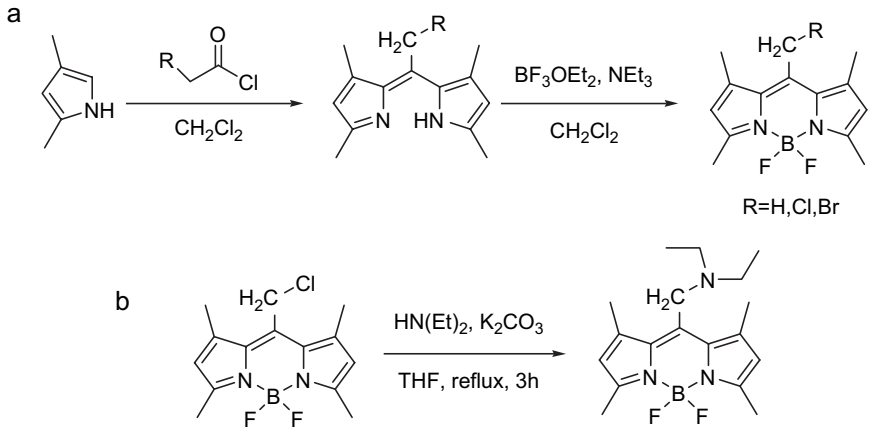

Scheme 2. Synthesis of the BODIPY dyes.

Table 1
The reactivity of acyl chlorides with 2,4-dimethylpyrrole

Dye	R	Reaction conditions	Yield (%)
1	H	Refluxing in CH_2Cl_2, 10 h	40
		r.t., 10 h	0
2	Br	r.t., 2 h	36
3	Cl	r.t., 2 h	34

are reported. A pH sensor (**4**) with a larger pK_a (8.7) is also described.

2. Experimental

2.1. Instruments and reagents

Mass spectral studies were carried out using HP1100 API-ES mass spectrometer. NMR spectra were obtained using a Varian 400 MHz spectrometer. Steady-state emission and excitation spectra were recorded using PTI-C-700 Felix and Time-Masters system. 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 using an HP-8453 spectrophotometer. All the pH values were measured with a Model PHS-3C meter (Shanghai Leici Equipment Factory, China).

All solvents and reagents used were reagent grade. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions. Tetrahydrofuran was distilled from sodium-benzophenone, and methylene chlorine was distilled from calcium hydride. Silica gel (100–200 meshes) and neutral aluminum oxide (100 meshes) were used for flash column chromatography.

2.2. Synthesis

Dye **1** (R = H): Acetyl chloride (0.14 ml, 1.95 mmol) and 2,4-dimethylpyrrole (0.4 ml, 3.89 mmol) were added in an absolute dichloromethane solvent under argon. On refluxing the mixture for 10 h, the reaction mixture turned black. The reaction of the pyrrole was monitored by thin layer chromatography. When the reaction was over, BF_3-etherate (8 ml, 7.52 mmol) and triethylamine (4 ml, 28.65 mmol) were added to the above mixture subsequently and stirred for 4 h. On removing the solvent by evaporation in vacuum, a dark residue was obtained which was purified via chromatography on silica gel column, with the eluting solvent of 3:1 hexane/dichloromethane, giving a red powder. Recrystallizing from ethyl

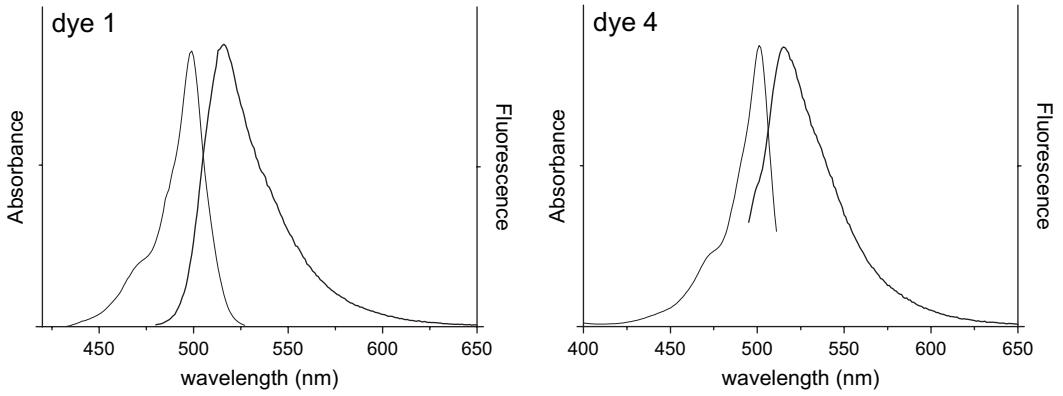

Fig. 1. The absorption and emission spectra of dye **1** and dye **4** in $CHCl_3$.

Table 2
Photophysical characteristics of the five dyes in different solvents

Dye	Solvent	Absorbance λ_{max} (nm)	Emission λ_{max} (nm)	ε[a] ($\times 10^5$)	Φ_f[b]
1	Chloroform	499	516	0.819	
	Ethanol	495	511	0.762	1.000
2	Chloroform	529	545	0.902	
	Ethanol	521	538	0.812	0.316
3	Chloroform	529	542	0.500	
	Ethanol	524	535	0.548	0.262
4	Chloroform	501	515	0.700	
	Ethanol	497	513	0.644	0.015

[a] Molar extinction coefficients are in cm^{-1} M and in the maximum of the highest peak.
[b] The fluorescence quantum yields were determined, fluorescein ($\Phi_f = 0.90$, ethanol as the solvent) as the reference.

acetate, 0.204 g of dye **1** was obtained with 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.41 (s, 6H, CH_3), 2.52 (s, 6H, CH_3), 2.58 (s, 3H, CH_3), 6.05 (s, 2H, CH). MS, *m/z* (M^-): 262.1.

Dye **2** (R = Br): Bromo-acetyl chloride (0.17 ml, 1.95 mmol) and 2,4-dimethylpyrrole (0.4 ml, 3.89 mmol) were dissolved in dichloromethane and kept stirring at room temperature under argon. After thin layer chromatography showed that pyrrole was consumed over (ca. 2 h), BF_3-etherate (8 ml, 7.52 mmol) and triethylamine (4 ml, 28.65 mmol) were added to the above mixture. Removing the solvent after 4-h stirring by evaporation in vacuum, dark residue was obtained and purified via simple chromatography on silica gel column with eluting solvent of 6:1 hexane/ethyl acetate. Recrystallizing from ethyl acetate, 0.239 g of pure dye **2** obtained with the yield of 36%. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.53 (s, 12H, CH_3), 4.77 (s, 2H, CH_2), 6.09 (s, 2H, CH). Anal. Calcd. for $C_{14}H_{16}BBrF_2N_2$: C, 49.31; H, 4.73; B, 3.17; Br, 23.43; F, 11.14; N, 8.22. Found: C, 49.39; H, 4.77; B, 3.10; Br, 23.48; F, 11.14; N, 8.15.

Dye **3** (R = Cl): Similarly to dye **2**, from chloro-acetyl chloride (0.15 ml, 1.95 mmol), 0.196 g of dye **3** was obtained with 34% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 2.53 (s, 12H, CH_3), 4.77 (s, 2H, CH_2), 6.08 (s, 2H, CH). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 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. MS, *m/z* (ES) (M^+): 296.0. Anal. Calcd. for $C_{14}H_{16}BClF_2N_2$: C, 56.70; H, 5.44; B, 3.65; Cl, 11.96; F, 12.81; N, 9.45. Found: C, 56.80; H, 5.49; B, 3.60; Cl, 11.93; F, 12.80; N, 9.48.

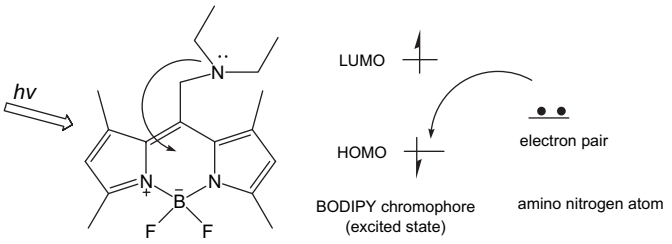

Fig. 3. Photo-induced electron transfer of **4**.

Dye **4** (H = $N(CH_2CH_3)_2$): Dye **3** (0.100 g, 0.338 mmol) and diethylamine (0.035 ml, 0.338 mmol) were mixed together in dried THF and refluxed for 2 h. After thin layer chromatography shows that dye **3** disappeared, the reaction mixture was concentrated and separated by alumina column with dichloromethane as eluting solvent, giving 0.090 g of pure dye **4** with the yield of 90%. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.05−1.08 (t, 6H, CH_3), 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 2.60−2.65 (q, 4H, CH_2), 3.90 (s, 2H, CH_2), 6.05 (s, H, CH), 6.44 (s, H, CH). ^{13}C NMR (400 MHz, $CDCl_3$), δ: 12.18, 14.60, 16.57, 17.48, 17.73, 48.25, 51.126, 120.41, 121.49, 128.98, 131.08, 132.25, 140.98, 141.31, 141.95, 153.94. ^{19}F NMR (400 MHz, $CDCl_3$), δ: −145. MS, *m/z* (ES) (M^+): 333.3. Anal. Calcd. for $C_{18}H_{26}BF_2N_3$: C, 64.88; H, 7.86; B, 3.24; F, 11.40; N, 12.61. Found: C, 64.95; H, 7.87; B, 3.20; F, 11.47; N, 12.55.

3. Results and discussion

3.1. Reactivity of acyl chlorides

The traditional synthetic route to BODIPY dyes is from the condensation of aldehydes with pyrrole, via oxidation, to the coordination with BF_3-etherate. This process is complicated, especially the oxidation may produce many byproducts and the yields of the reactions are low. Burgess and co-workers [6,7] have developed acyl chloride−pyrrole route to synthesize

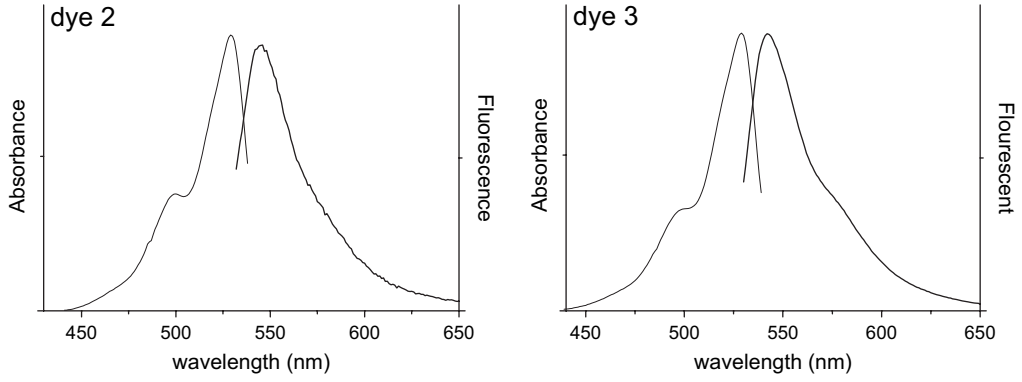

Fig. 2. The absorption and emission spectra of dye **2** and dye **3** in $CHCl_3$.

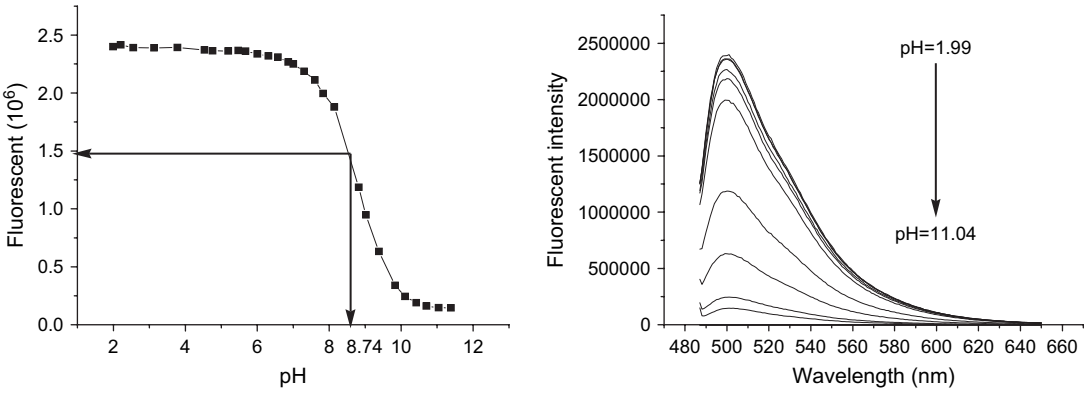

Fig. 4. Fluorescence emission spectrum of compound **4** in aqueous solution with different apparent pH: 1.99, 5.19, 5.67, 6.86, 7.30, 7.83, 8.82, 9.38, 10.11, 10.04. λ_{ex}: 482 nm; λ_{em}: 500 nm.

this kind of dyes in which acyl chlorides replace the aldehydes as starting materials (Scheme 2). As no oxidation step is needed, the total yield is much higher.

As the coordination step of BF_3 always has high yield, the condensation of acyl chloride with pyrrole is decisive to the total yield. Although the detailed mechanism of the condensation is not clear, electron-withdrawing groups in the acyl chlorides are helpful to synthetic reaction (Table 1). Bromoacetyl chloride or chloro-acetyl chloride can react with pyrrole easily in dichloromethane at room temperature. Acetyl chloride, however, needs a 10-h refluxing condition to finish the reaction and does not react with the pyrrole at room temperature. In a further run, propionyl chloride reacted even more slowly. So, electron-donating substituents in acetyl chloride decrease the reactivity with the pyrrole.

3.2. Spectroscopic properties

Similar to other BODIPY dyes, **1**–**3** have a narrow peak in emission spectra (Fig. 1) and high fluorescence quantum yield (Φ_f) (Table 2). The $\lambda_{max\ (abs)}$ and $\lambda_{max\ (em)}$ of **2** and **3** exhibit a red-shift of about 25–30 nm as compared to **1** (Fig. 2). Dye **1** emits the strongest green fluorescence with a Φ_f ($CHCl_3$) of almost 1. Dye **4**, however, exhibits poor fluorescence quantum efficiency (0.015 in $CHCl_3$). This might result from the quenching of intramolecular electron pair of its amino nitrogen atom, via a PET mechanism (photo-induced electron transfer) (Fig. 3).

The photo-induced electron transfer process is elucidated in Fig. 3. One electron in the lone pair of electrons of amino nitrogen atom, with higher energy level than the HOMO of BODIPY, gets transferred to the HOMO so that it prohibits the electron in the LUMO from going back and quenches the emission.

3.3. Fluorescence intensity change with pH

No notable variation in the fluorescence of **1**–**3** was observed in aqueous buffer solutions with different pH from 2 to 11. For dye **4**, however, the fluorescent intensity changed remarkably: the fluorescence in low pH was 15-fold larger than that in high pH (Fig. 4). The fluorescence change was fully reversible and took place within the pH range from 9.38 to 7.60 with an apparent pK_a of 8.74.

In the low pH (<pK_a), the amino nitrogen in the molecule of **4** is protonated. The protonation changes the electron state of the amino nitrogen atom and decreases the energy level. Subsequently, PET is stopped and fluorescence recovers. In comparison with the pH probe (pK_a 6.5) by Baki [16], **4** gives a higher detective pH range with pK_a 8.74.

4. Conclusion

New BODIPY dyes (**2**–**4**) were synthesized from acyl chlorides and 2,4-dimethylpyrrole. The acetyl chlorides with electron-withdrawing substituent easily react with the pyrrole to form the related dyes and make a red-shift in absorbance and fluorescence maximum. Dye **4**, based on PET mechanism, exhibits good properties as a pH fluorescent sensor with pK_a 8.74 and a 15-fold fluorescence enhancement.

Acknowledgments

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