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Naphthalene-fused BODIPY with large Stokes shift as saturated-red fluorescent dye for living cell imaging

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ABSTRACT

A new fluorescent dye of 4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) was prepared by fusing naphthalene unit to the zig–zag edge of BODIPY core by oxidization with iron (III) chloride. The naphthalene-fused BODIPY exhibits significant bathochromic shifts in both absorption and fluorescence spectrum compared with the non-fused BODIPY. In addition, the dye emits strong saturated-red fluorescence with a quantum yield of 55% and an enlarged Stokes of 56 nm in $CHCl_3$ due to the formation of dimers and the desymmetrization of the molecule, which can be used to image living cells by fluorescence microscopy. Cell imaging experiments demonstrated its potential application as a probe in bio-organisms due to its excellent imaging effect and large Stokes shift.

1. Introduction

Fluorescent 4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes have been received great attentions for a variety of applications, such as various fluorescent sensors [1], artificial photosynthetic models [2], molecular logic gates [3], and opto-electronic devices [4]. In recent years, fluorescence microscopy has become an essential tool for investigating biologic structures and functions at the molecular and cellular level [5]. Due to their high photo stability, high fluorescent quantum yield (ΦF) and molar extinction coefficient, good biocompatibility, and relatively insensitivity to environmental conditions (i.e., solvent polarity or pH value), BODIPY dyes are regarded as one of the most promising candidates for bio-related fluorescent bioimaging [6].

However, BODIPY dyes have two disadvantages for the application in living system. One is the green fluorescence of BODIPY dyes. Normally, the emission wavelength of BODIPY dyes centers around 515 nm [6b], where the fluorescent bioimaging is often influenced by the background interference, self-absorption and scattering. In this regard, considerable attention has been given to the bathochromic shifts in both absorption and fluorescence spectrum of BODIPY dyes through chemical modification, such as fusion rings to extend π-conjugation [6b,7], substitution of *meso*-C with N atom to form aza-BODIPY dyes [6b,8], and peripherally substitution [6b,9]. Although the first chemical modification is best efficient [10], its synthetic way is often complicated. Therefore, a simply way to prepare red/NIR BODYPY dyes is in great need. The other disadvantage of classic BODIPY dyes is their generally low Stokes shift. A typically Stocks shift of BODIPY dyes is about 5–15 nm [6b], which is detrimental to the application of fluorescent bioimaging, because the fluorescence intensity might be reduced by the self-absorption and inner filter effect, which can decrease the detection sensitivity to a great extent [11]. For some applications, in particular biochemical, large Stokes shifts are highly desirable. In order to obtain large Stokes shifts of BODIPYs, a number of approaches have been reported, such as 1) excited states proton transfer [12]; 2) construction of tandem systems [13]; 3) photoinduced intramolecular charge transfer [14]; 4) oligomer [15]. Some strategies, although effective, are usually structurally complex and require significant synthetic effort [16].

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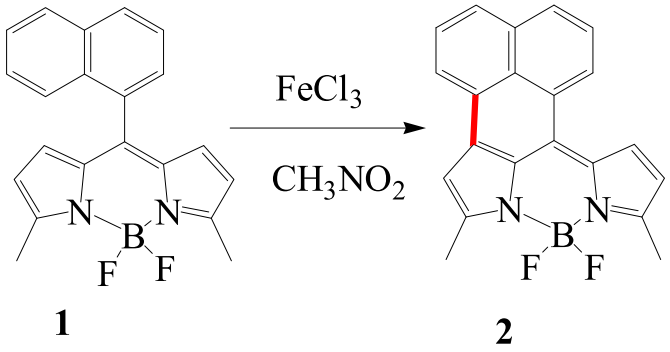

Scheme 1. Synthesis of BODIPY **2**.

In general, the free rotation of *meso*-substituents in BODIPY dyes might provide a nonradiative relaxation pathway for the excited state and result in a decrease of quantum yields. Fusion of aromatic units onto the zig-zag edge (i.e., *meso*-and β-pyrrol positions) of the porphyrin or BODIPY core has been demonstrated an efficient way to achieve bathochromic shifts in both absorption and fluorescence spectrum [17].

2. Results and discussion

Herein a new naphthalene-fused BODIPY dye **2** has been prepared by a one-step oxidization of **1**. With the fusion of naphthalenyl group onto the zig-zag edge of a BODIPY, **2** emits strong saturated-red fluorescence with a quantum yield of 55% and an enlarged Stokes of 63 nm in hexane and suitable for the application of living cell imaging.

The naphthalene-fused BODIPY **2** was synthesized as shown in Scheme 1. BODIPY **1** was synthesized in a yield of 45% by reacting 2-methylpyrrole [18] with 1-naphthaldehyde in the presence of trifluoroacetic acid (TFA), followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [19]. BODIPY **2** was synthesized by oxidizing **1** with $FeCl_3$ with a yield of 31% [17a]. All new compounds were identified by ^1H and ^{13}C NMR spectroscopy and Maldi-TOF-Mass (Figs. S1–S6 in Supplementary data). The peak ($M + H^+$) of a monomer **1** in the MALDI-TOF mass spectrum is observed as expected. However, only peak of dimer **2** is found, probably because BODIPY **2** with planar

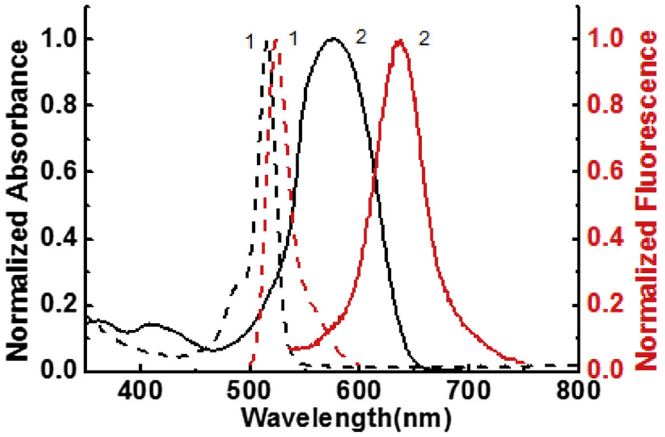

Fig. 1. Normalized absorbance (black) and emission spectra (red) for **1** (dash) and **2** (solid line) in $CHCl_3$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Absorption and emission data of BODIPY dyes.

Dye	Solvent	Absorption			Emission		SS,[c] nm
		λ, nm	ε, $M^{-1}cm^{-1}$	FWHM,[a] cm^{-1}	λ_{em}, nm	Φ_F[b]	
1	$CHCl_3$	516	88,600	748	524	0.39	8
2	$CHCl_3$	579	32,400	2371	635	0.44	56
	Hexane	571	46,000	2371	634	0.55	63

[a] FWHM: full width at half maximum of absorbance spectra.
[b] Rhodamine B was used as a standard ($\Phi_F = 0.73$ in EtOH) [14].
[c] Stokes shift.

structure has a strong tendency to formation of dimer through π-π aggregation.

Variation from **1** to **2** in ^1H NMR spectra is shown in Fig. S7 (Supplementary data). For **1**, signals (around 2.5 ppm) for two methyls exhibited one peak, but for **2**, they split up to two peaks as expected, due to the formation of unsymmetry by fusing naphthalene substituent in **2**. Similarly, there are two peaks around 6.3 ppm for β-pyrrole-Hs in **1**, which have been splitted into three peaks in **2**. Therefore, the fusion of the naphthyl to the β-pyrrole position can be confirmed by the vanish of one H signal and the desymmetrization in **2**.

Significant differences in the electronic spectra between BODIPY dyes **1** and **2** are shown in Fig. 1 and Table 1. The absorption and emission spectra of **1** are similar with those of typical *meso*-phenyl BODIPY dyes [10b], suggesting that the naphthalene ring gives little contribution to expanding π-conjugation, due to the almost perpendicular conformation between the naphthalene ring and BODIPY core. Unlike **1**, **2** displays remarkable broad peaks in absorption and emission spectrum. The full width at half maximum (FWHM) in absorption and emission spectrum of **2** is more than three-fold broader than that of **1**, revealing the desymmetrization of **2** [20]. An apparent bathochromism can be found both in absorption and emission spectrum of **2**. Compared with that of **1**, the emission spectrum of **2** exhibits a notable red shift (more than 110 nm) with $\lambda_{abs,max}$ more than 634 nm, indicating that **2** is a good candidate for saturated-red fluorescent bioimaging. Moreover, it is notable that the Stokes shift has increased from 8 nm for 1 to 56 nm for **2**, perhaps due to the formation of dimer [21] and the desymmetrization of the molecule **2** [22]. The fluorescence quantum yield (44% in chloroform) of **2** is a little higher than that (39% in chloroform) of **1** and much higher than that of other zig–zag ring fused BODIPY [16]. This can be attributed to the inhibition of the free rotation of *meso*-group and less nonirradiative energy loss [11b].

Time-dependent density function theory (TDDFT at B3LYP/6-31G*) calculations for BODIPY dyes were performed to gain better insight into the molecular geometries and absorption spectra. Selected structural data and profile were listed in Tables S1 and S2 (Supplementary data).

The geometry of the **2** has been greatly planarized by ring fusion. The dihedral between BODIPY core and its *meso*-naphthalene substituent decreases from 71.70° for **1** to 11.43° for **2**. With a plane shape, naphthyl substituent in **2** had a good contribution to the π-conjugation system, in which π-electrons can be easily extended from BODIPY core into the naphthyl substituent and delocalized throughout the whole molecule as shown in the frontier molecular orbital profiles (Fig. S9, Supplementary data). Therefore, the band gap decreases remarkably from 2.47 ev for **1** to

Table 2
Energy levels (ev), band gaps (ev) and dipole moments (Debye) of BODIPY dyes.

Dyes	HOMO	LUMO	Band gap/ev	Dipole moment/D
1	−4.59	−2.12	2.47	3.63
2	−4.47	−2.50	1.97	5.29