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Ultra-bright intercellular lipids pseudo di-BODIPY probe with low molecular weight, high quantum yield and large two-photon action cross-sections

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ABSTRACT

Small fluorescent molecules are widely used to label compartments in biological systems; nevertheless, small molecules with high quantum yield and large two-photon absorption (2PA) cross-section (δ) are rarely reported. Herein, two novel pseudo di-BODIPY derivatives have been synthesized and fully characterized. One of which (**B1**) showed high planarity of the conjugated system from crystal structural information and can generate superior 2PEF as well as prodigious $\Phi\delta_{max}$ and high quantum value compared to another fluorescent molecules (**B2**) tested. It was found that compound **B1** possessed prodigious two-photon (2P) action cross-section ($\Phi\delta_{max}$ = 869.72 GM) in high polar solvent and higher quantum yield (Φ = 0.84), and can be capable of labeling lipid-rich endoplasmic reticulum (ER) in living cells in the range of 10^{-9} molar concentrations effectively. In addition, its recognition mechanism has been further confirmed with molecular modeling calculations and ^{1}H NMR spectral methods The high affinity of **B1** towards lipid rich organ was also explored over mouse and zebrafish tissue models. These pseudo di-BODIPY derivatives offer a new strategy to design molecules targetting universal membrane subcellular structures *in vitro* and *in vivo*.

1. Introduction

Lipids, one of the important bio-macromolecules, are virtually found in all biological systems. These macromolecules can be classified into three categories according to the biological functions: storage lipids (the primary form of energy), structural lipids (used in biomembrane structures in the form of lipid bilayer) and active lipids (biological regulating substances) [1–3]. It is believed that lipid state, which in turn affects membrane dynamics, can be altered in inflammatory diseases, and could play a significant role in the pathogenesis of age-related diseases, such as Alzheimer's disease and cardiovascular diseases [4–7]. In addition, there are a large number of structural lipids, which forms membrane-enclosed intracellular structures, distributed in eukaryotic cells. These structural lipids improve intracellular physiological and biochemical reactions in different intracellular compartments such as mitochondria, Golgi apparatus and endoplasmic reticulum (ER) [8]. Arguably, the most notable organelle is ER that accounts for almost half of the endomembrane system in eukaryotic cells [9]. This organelle is also the site of protein and lipid synthesis within the cell [10].

Light microscopy remains a powerful tool for visualization of cellular structures. However, two-photon microscopy (2PM) has progressively becoming widely used in bio-imaging applications, especially those that employ excitation source in the near infrared (NIR) region [11]. Subsequently, efforts have been made to design molecules that capable of imaging ER morphology in living cells and tissues using 2PM [12–14]. Coordination compounds for ER targeting have been researched [8]; however, their heavy metal core induce unexpected cytotoxicity and cell stress. Small organic boron compounds on the other hand, offer a very attractive alternative due to their low molecular weight and ease of functionalization. Therefore, BODIPY-based compounds that have widespread applications have been considered as a potential scaf-

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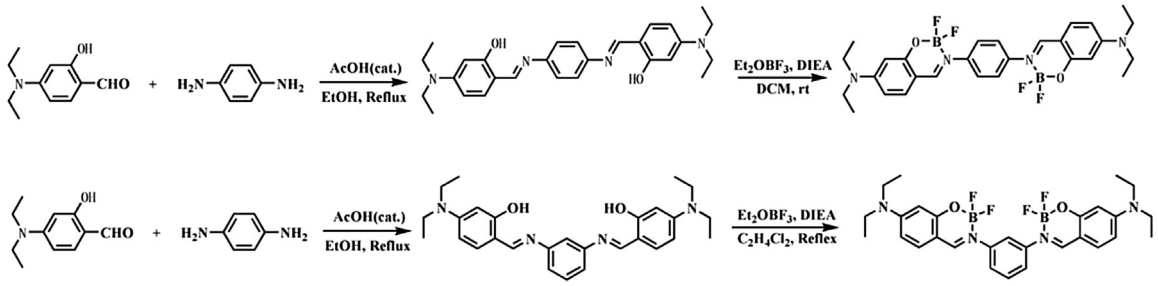

Scheme 1. The synthesis route of the title compound **B1** (a) and **B2** (b).

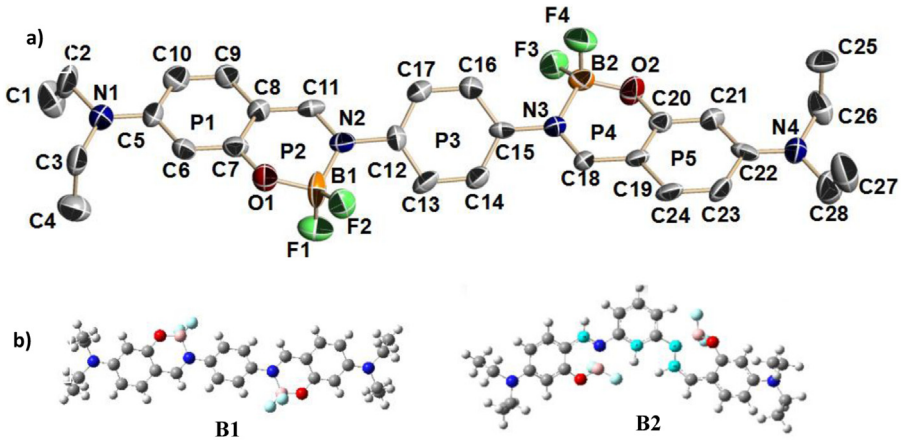

Fig. 1. (a) Side view of crystal structure of **B1, (b)** The optimized structure of **B1**-**B2**, respectively.

fold for developing functional fluorescent probe. These compounds are particularly suitable to act as fluorescent probes due to its high photo-stability, neutral total charge, high fluorescence quantum yield, strong absorption and emission spectra [15–17]. So far, BODIPY-based probes that accumulate in subcellular organelles, enzymes and peptides have been developed [10,18]. In addition, commercialized BODIPY-based trackers are available including ER-Tracker Green (Ex. 504 nm; Em. 511 nm), ER-Tracker Red (Ex. 587 nm; Em. 615 nm), ER Thermo Yellow (Ex. 559 nm; Em. 581 nm) [8,10]. However, most of the above compounds are not optimized for two-photon imaging with relatively low two-photon (2P) action cross-section ($\Phi\delta_{max}$) [19], as well as small Stokes shift (ER Tracker Green = 7 nm, ER Tracker Red = 28 nm, ER Thermo Yellow = 22 nm) that embarrassed their further unitization. Hence, a new generation BODIPY probes that not only capable of specifically labeling subcellular organelles but also with large two-photon (2P) action cross-section and high fluorescence quantum yield are particularly required [20,21].

To tackle the issues above, two analogous pseudo di-BODIPY derivatives were designed and synthesized based on Schiff base for the following considerations: (1) The 4-N, N'-dihydroxyethylsalicylaldehyde containing a strong electron donor can readily react with aniline derivatives to form the Schiff base with an extensive π-conjugated system, which has been used to develop two-photon chromophores [22]. In the structure of the Schiff base, the O atom of —OH and the N atom of —CH=N- bond can bind to the boron atom to form six-membered ring with good planarity, which may enhance 2PA response and increase the quantum yield. (2) Luminescent fluorophores containing two boron centers are more advantageous over isolated dyes, since they seem to contribute to increased brightness, luminescence intensity and molar absorption coefficient [23], which are also important requirements for cell-imaging reagents. (3) Analogous pseudo di-BODIPY derivatives consisting of differently substituted anilines may have different photophysical properties as well as bio-specificity. Although, **B1**, **B2** and many ramifications were previously reported by Gilles Ulrich's group and reviewed by the other researchers [24], at the present work, they were systematically investigated on their two-photon absorption properties and applications in bio-imaging. The data suggested that **B1** exhibited low cytotoxicity, very large $\Phi\delta_{max}$ and high quantum yield in high polar solvents, which make it a potential two-photon fluorescence probe for subcellular endoplasmic reticulum both *in vitro* and *in vivo* (Scheme 1).

2. Experimental section

Detailed methods and approaches see Supplementary files.

3. Results and discussions

3.1. Structure features of B1

Fig. 1**a** showed the crystal structure of **B1**, the sythetic route was highlight in Scheme 1, the structural parameters were displayed in **Table S1**. The selected bond distances and angles were listed in **Table S2**.

The single crystal of **B1** belonged to triclinic system with Pī space group. The Schiff base groups were located in the para-position of central phenyl ring (P3) which could reduce the inter-molecular interaction. Meanwhile, the introduction of boron bisfluoride group would decrease the planarity of **B1** with dihedral angel between P2 (P4) and P3 41.5° (40.5°). However, the main C—C/C—N bond distances (**Table S2**) were located between normal C—C/C—N single band and C=C/C=N double bond length indicating high π-electron delocalization within the molecule, which might be favorable for its good nonlinear optical response. The optimized structures of **B1**