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Convenient one-pot procedure for synthesizing 4,4′-dimethoxy-boradiaza-*s*-indacene dyes and their application to cell labeling

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ABSTRACT

We succeeded in developing a convenient one-pot pathway for synthesizing 4,4′-dimethoxy-boradiaza-*s*-indacene dyes **4a**–**d**. The structures feature two methoxy groups in place of the fluorine atoms in 4,4′-difluoro-4-boradiaza-*s*-indacene. These novel dyes emitted green fluorescence and possessed moderate to high fluorescence quantum yields (Φ=0.32–0.93). We demonstrated that these dyes have applicability to cell labeling.

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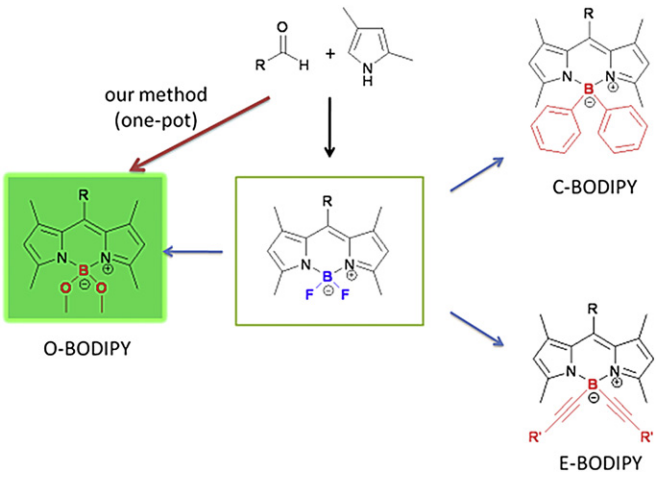
1. Introduction

Derivatives of 4,4′-difluoro-4-boradiaza-*s*-indacene (F-BODIPY) have been proven to be useful fluorophores on account of their high molar absorptivity, fluorescence quantum yield, and stability against light and chemical reactions. Because of these excellent characteristics, they have also been extensively utilized as biomolecular labels;[1–6] fluorescent chemosensors for metal ions,[7–16] protons,[17,18] and superoxides;[19] and dye-sensitized solar cell (DSSC) material.[20] Recently, non-fluorinated BODIPY dyes have been developed rapidly and extensively by replacing 4,4′-difluorine atoms with other substituents, such as carbocycle groups (C-BODIPY), ethynyl groups (E-BODIPY), or alkoxy groups (O-BODIPY).[21–23] However, till date, only a few O-BODIPY dyes have been reported.[24,25] Treatment of F-BODIPY with $AlCl_3$ in dichloromethane and subsequent addition of alcohol resulted in the total substitution of the two fluorine atoms to afford 4,4′-dialkoxyl-substituted BODIPY, reported by Mély and Bonnet.[26] They found that the O-BODIPY dyes have similar or even better spectroscopic properties than the parent, F-BODIPY. In this paper, we provide an alternative one-pot procedure for the synthesis of 4,4′-dimethoxy-boradiaza-*s*-indacene dyes **4a**–**d**, which have different aryl substituents at the *meso* position.

As shown in Scheme 1, the BODIPY derivatives C-BODIPY, E-BODIPY, and O-BODIPY, which are different from each other in terms of the substituents on the boron atom, are always prepared from the parent, F-BODIPY, and different nucleophiles via nucleophilic substitution reactions. In other words, at least two steps are required to synthesize these BODIPY molecules. Herein, we propose an alternative one-pot method for synthesizing the O-BODIPY framework. In our synthesis, the target O-BODIPY molecules were prepared directly from pyrrole, aldehyde, and trimethyl borate in one flask. This straightforward approach allowed us to shorten the



Scheme 1. Different types of BODIPY derivatives (C-, E-, and O-BODIPY) and their synthetic pathways.

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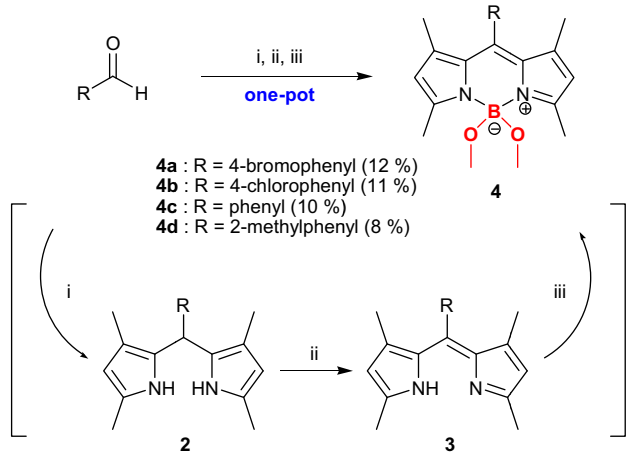
reaction procedure and reduce the reaction time by not isolating the F-BODIPY intermediate, which was a necessary step in the traditional synthesis of O-BODIPY.

2. Results and discussion

The general reaction procedure for the synthesis of 4,4′-dimethoxy-boradiaza-*s*-indacene by the one-pot pathway is as follows: 2,4-dimethylpyrrole is reacted with aldehyde under trifluoroacetic acid (TFA) catalysis, which is followed by oxidation of the resulting dipyrromethane with tetrachloro-*p*-benzoquinone and, finally, addition of triethylamine and trimethyl borate. In this experiment, we used THF as the reaction solvent because tetrachloro-*p*-benzoquinone has low solubility in CH_2Cl_2. We synthesized the well-known compound, 4,4′-difluoro-8-(4-bromophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene, in dry THF instead of CH_2Cl_2, which is the most commonly used solvent in the synthesis of BODIPY, and found that the yield was much better (33% vs 18%). Therefore, we selected THF as the solvent throughout the experiment. In addition, the reaction time, temperature, and quantity of the reagents are critical to the success of the reaction. A better yield was obtained when the reaction intermediate, dipyrromethene, was first treated with Et_3N (5 equiv). Green fluorescence was immediately observed; this was followed by the addition of $B(OMe)_3$ (10 equiv) and refluxing for 24 h. Another serious problem encountered in the experiment was that the products were easily hydrolyzed during silica gel column chromatography. This problem can be solved by using neutral Al_2O_3 instead of silica gel and eluting with ethyl acetate/hexane.

Compounds **4a**–**d** were obtained in moderate yield and all of them emitted a green fluorescence (512–516 nm) under excitation at 500 nm (Scheme 2). The Stokes shift of these four compounds was not large (11–13 nm), but their fluorescence quantum yields were moderate to high (0.32–0.93). Table 1 lists the spectroscopic data of dyes **4a**–**d**.



Scheme 2. Synthetic pathway and plausible intermediates in the synthesis of O-BODIPY dyes **4a**–**d**. (i) 2,4-dimethylpyrrole, TFA, dry THF; (ii) tetrachloro-*p*-benzoquinone; (iii) Et_3N, trimethyl borate, reflux.

Table 1
Absorption and fluorescence data for **4a**–**d** in THF

Dye	λ_{max}/nm	λ_{em}/nm[b]	Φ_F[a]	Stokes shift/nm	ε (M^{-1} cm^{-1})
4a	504	515	0.47	11	38,350
4b	504	516	0.32	12	37,440
4c	500	513	0.48	13	45,870
4d	500	512	0.93	12	18,300

[a] 3-(2-Benzothiazolyl)-7-(diethylamino) coumarin (coumarin-6) in THF (Φ_F=0.99) as the reference.
[b] Excited at 500 nm.

The significantly high quantum yield of compound **4d** was attributed to the steric restriction against rotation between the *ortho*-methyl phenyl substituent at C8 and the methyl groups at C2 and C6 of the BODIPY framework. It is well known that steric restriction increases the dihedral angle between the core and the *ortho*-methyl phenyl group and diminishes the excited state energy lost through nonradiative modes, resulting in enhanced quantum yield and blue-shifted absorption and emission.[27]

Because of its high quantum yield, compound **4d** is thought to have potential applications in bioscience. It has the highest quantum yield among these four compounds; therefore, it was selected to test its potential to bind cells. U937 cells treated with compound **4d** were detected by flow cytometry on the basis of green fluorescence on their cell membrane, as shown in Fig. 1. DC2.4 and THP-1 cell lines were also tested, and they showed similar behavior (Supplementary data).

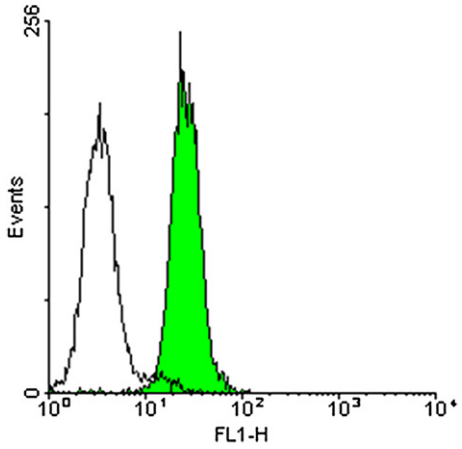


Fig. 1. Compound **4d** could bind on cell surface. U937 cells were stained with compound **4d** and then determined the green fluorescent intensities using a flow cytometer with 488 nm excitation.

In addition, the intracellular binding efficacy was determined by fluorescent microscopy. As Fig. 2A showing, **4d** could label on the cell surface and intracellular constituents with a dose dependent manner. Interestingly, **4d** was especially bound on cytosolic area but not nuclei. The mean fluorescent intensity levels of different staining conditions including group of cell surface labeling with 2 μM **4d** (Fig. 2B left panel), or both extra- and intracellular staining with 2 μM or 4 μM **4d** groups (medium and right panels of Fig. 2B) were 36.18, 48.03, and 87.52, respectively. These data revealed that the fluorescent compound **4d** had the potential for cell labeling applications. In the future, we intend to modify the structure of this dye and to label the desired target specifically.

3. Conclusions

In conclusion, we developed a convenient one-pot procedure for synthesizing new 4,4′-dimethoxy-boradiaza-*s*-indacene dyes **4a**–**d**. Among these new dyes, **4d** exhibited a very high fluorescence quantum yield. The proposed approach is an efficient, straightforward, and versatile way of synthesizing a new class of fluorescent BODIPY dyes with potential applications in biochemistry and molecular biology.

4. Experimental

4.1. General materials and methods

Unless otherwise specified, all reactions were performed under nitrogen atmosphere using standard Schlenk techniques. THF was

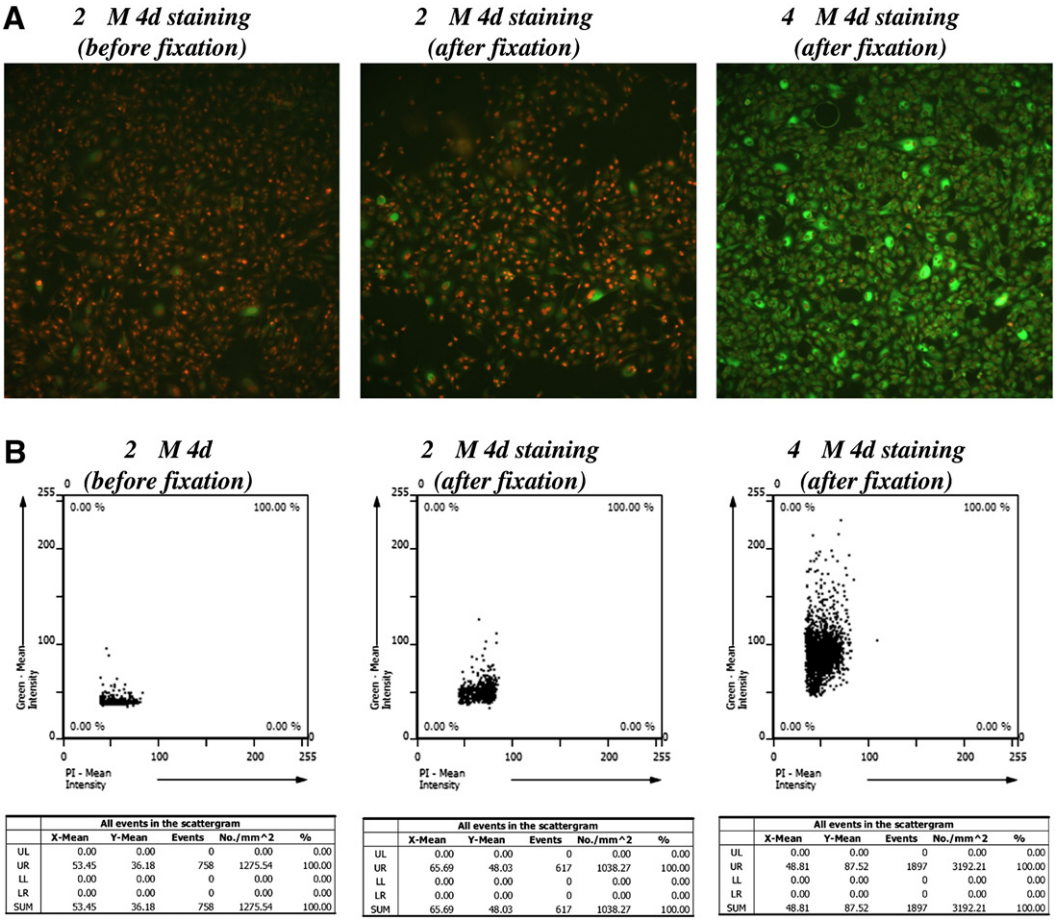


Fig. 2. (A) Qualification and (B) quantification of fluorescence of A549 cells after **4d** labeling.

distilled from sodium and benzophenone under nitrogen atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. Absorption and fluorescence spectra were recorded on a Cary 50 probe UV–vis spectrophotometer. All chromatographic separations were carried out on MN-Aluminum oxide (activated neutral, 70–230 mesh). Mass spectra (FAB) were recorded on a VG70-250S mass spectrometer.

4.2. General procedure for the synthesis of compounds 4a–d

2,4-Dimethylpyrrole (2.25 mmol) and aldehyde (1 mmol) [4-bromobenzaldehyde (**1a**), 4-chlorobenzaldehyde (**1b**), benzaldehyde (**1c**), *o*-tolualdehyde (**1d**)] were dissolved in dry THF (15 mL) under nitrogen. One drop of trifluoroacetic acid (TFA) was added and the solution was stirred at room temperature for overnight (14–18 h). Tetrachloro-*p*-benzoquinone (1 mmol) was added once directly and the mixture was stirred for additional 3 h. The reaction mixture was then treated with triethylamine (5 mmol) and stirred for additional 1 h. Trimethyl borate (10 mmol) was added and the mixture was refluxed for 24 h. After the solution was cooled down to room temperature, H_2O (5 mL) was added and the mixture was extracted with ethyl acetate (80 mL); the organic phase was washed with water (3×60 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated at reduced pressure. The crude product was purified by aluminum oxide column chromatography (ethyl acetate/hexane).

*4.2.1. Dimethyl 2-[(Z)-(3,5-dimethyl-2H-pyrrol-2-ylidene)(4-bromophenyl)methyl]-3,5-dimethyl-1H-pyrrol-1-yl boronate (**4a**).* Dark brown solid (51 mg, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J=8.0 Hz, 2H), 7.18 (d, J=8.0 Hz, 2H), 5.95 (s, 2H), 2.92 (s, 6H), 2.50 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 141.0, 139.8, 134.6, 132.6, 132.2, 130.1, 123.0, 121.2, 49.1, 14.8, 14.7; MS (EI) m/z (%) 428.1 (9), 426.1 (9, M^+), 398.1 (17), 396.1 (100), 381.1 (62), 300.2 (12), 143.1 (23); HRMS (EI) m/z calcd for $C_{21}H_{24}BBrN_2O_2$ 426.1114, found 426.1123.

*4.2.2. Dimethyl 2-[(Z)-(3,5-dimethyl-2H-pyrrol-2-ylidene)(4-chlorophenyl)methyl]-3,5-dimethyl-1H-pyrrol-1-yl boronate (**4b**).* Dark brown solid (42 mg, 11%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J=8.4 Hz, 2H), 7.23 (d, J=8.4 Hz, 2H), 5.95 (s, 2H), 2.92 (s, 6H), 2.50 (s, 6H), 1.40 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 141.1, 140.0, 135.0, 134.1, 132.7, 129.8, 129.3, 121.2, 49.1, 14.7, 14.6; MS (EI) m/z (%) 384.2 (4), 382.2 (12, M^+), 353.2 (32), 350.2 (100), 335.1 (59), 295.1 (22), 143.1 (10); HRMS (EI) m/z calcd for $C_{21}H_{24}BClN_2O_2$ 382.1619, found 382.1614.

*4.2.3. Dimethyl 2-[(Z)-(3,5-dimethyl-2H-pyrrol-2-ylidene)(phenyl)methyl]-3,5-dimethyl-1H-pyrrol-1-yl boronate (**4c**).* Dark brown solid (35 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.26 (m, 5H), 5.94 (s, 2H), 2.94 (s, 6H), 2.51 (s, 6H), 1.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 141.6, 141.2, 135.6, 132.9, 128.9, 128.7, 128.2, 120.9, 49.1, 14.5, 14.4; MS (EI) m/z (%) 348.2 (14, M^+), 317.2 (81), 316.2 (100), 301.2 (55), 158.6 (12), 143.1 (6); HRMS (EI) m/z calcd for $C_{21}H_{25}BN_2O_2$ 348.2009, found 348.2002.

*4.2.4. Dimethyl 2-[(Z)-(3,5-dimethyl-2H-pyrrol-2-ylidene)(2-methylphenyl)methyl]-3,5-dimethyl-1H-pyrrol-1-yl boronate (**4d**).* Dark brown solid (29 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.15 (m, 4H), 5.94 (s, 2H), 2.97 (s, 3H), 2.90 (s, 3H), 2.51 (s, 6H), 2.15 (s, 3H), 1.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6,

141.1, 140.1, 135.3, 135.1, 132.4, 130.6, 128.9, 128.0, 126.7, 120.7, 49.1, 49.0, 29.7, 14.6, 13.8; MS (EI) m/z (%) 362.2 (5, M^+), 330.2 (78), 315.2 (25), 143.1 (9), 91.1 (100), 57.1 (35); HRMS (EI) m/z calcd for $C_{22}H_{27}BN_2O_2$ 362.2166, found 362.2168.

4.3. General procedure for cell culture and labeling

Cell culture. The U-937, THP-1, and DC2.4 cell lines were cultured in complete RPMI medium (RPMI-1640 medium; Invitrogen) containing 10% heat-inactivated fetal calf serum (FCS; Biochemical Industries), 50 U/ml penicillin, and 50 mg/ml streptomycin. The human lung epithelial cell lines A549 were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% FCS, 50 U/ml penicillin, and 50 mg/ml streptomycin. All the cultures were maintained at 37 °C in 5% CO_2 at 95% humidity, and the medium was changed every 3 days.

Cell labeling. For cell surface labeling, U-937, THP-1 or DC2.4 cells were washed once with PBS and stained with compound **4d** on ice for 30 min. After washing twice, cells were resuspended in 0.5 mL FACScan medium (RPMI-1640 medium containing 2% FBS and 0.1% sodium azide), and then analyzed using flow cytometer (FACScan; BD Biosciences). For intracellular labeling, A549 cells were cultured in wells of chamber slide (Thermo Scientific, NUNC Lab-Tek™ Chamber Slide™ System) at 37 °C in 5% CO_2 at 95% humidity for one day and then fixed with 4% formaldehyde in PBS for 30 min. After washing, the fixed cells were stained with compound **4d** at room temperature for 30 min and were followed by fluorescence microscopy detection.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.08.043.

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