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Graphic Abstract

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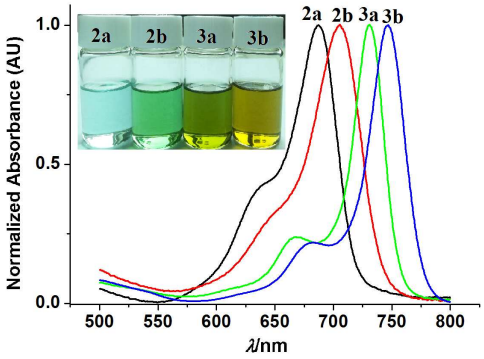

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ARTICLE TYPE

A styryl-containing aza-BODIPY as near-infrared dye†

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A novel styryl-containing pyrrole was developed by the reaction of *E*-4-phenylbut-3-en-2-one with 3-phenyl-2*H*-azirine in the presence of LDA. Utilizing this pyrrole, asymmetric styryl-containing aza-BODIPYs have been prepared in the NIR region as a result of the combined extension of conjugation. This styryl-containing aza-BODIPY was bright enough to be suited to label living cells for imaging assay in the NIR region.

Near-infrared (NIR) fluorescent dyes can greatly reduce background absorption, fluorescence, light scattering, and improve the sensitivity of the fluorescent probes and sensors.[1] More importantly, NIR fluorescent dyes with strong absorption and emissions in the low energy spectral regions (650-900 nm) have deep penetration in tissues to allow in *vivo* imaging and photodynamic therapy.[2] The safe, non-invasive nature of NIR fluorescent imaging has attracted great interests in the design and synthesis of novel NIR fluorescent dyes.[3] NIR dyes such as azo dyes,[3a] cyanine dyes,[3b] quinone dyes,[3c,d] and phthalocyanine dyes[3e] have been developed so far, however insufficient photo-stability and/or difficult modification obstructed their applications. Most NIR fluorescent dyes possess highly conjugated systems which lead to significantly reduced fluorescence quantum yield due to increased internal conversion.[3] BODIPY dyes are well-known to be highly fluorescent, very stable, and exceptionally insensitive to the polarity of solvents as well as to tunable emission wavelength, and they acted as good candidates for biological labeling applications.[4] Recently, our group focus on the BODIPYs dye.

One of the most significant strategies for shifting the spectral bands of the BODIPY to the red is the incorporation of a nitrogen atom at the meso-position to form an aza-BODIPY dye.[5] By utilizing an imine instead of a methane in BODIPY system to narrow the HOMO-LUMO band gap, a novel aza-BODIPY can simply achieve a NIR responsive dyes. Comparing to the polytype synthetic method of BODIPY,[4] the syntheticism of aza-BODIPYs is few. In more recent times, O'shea *et al* have developed new and optimized routes to a symmetric aza-BODIPY generated from 1,3-diaryl-4-nitrobutan-1-one or 3-methyl-4-nitro-1-arylbutan-1-one (Fig 1a).[5c,d,6] An aza-BODIPY reported by Shen and Luk'yanets *et al* can be prepared by reacting a phthalonitrile with an aryl magnesium bromide (Fig 1b).[7] Using an individual 2,4-diaryl pyrrole/an aryl-fused 2,4-diaryl pyrrole, Zhao and Carreira *et al* showed a more fashionable and effective method of symmetric/asymmetric aza-BODIPY (Fig 1c).[8]

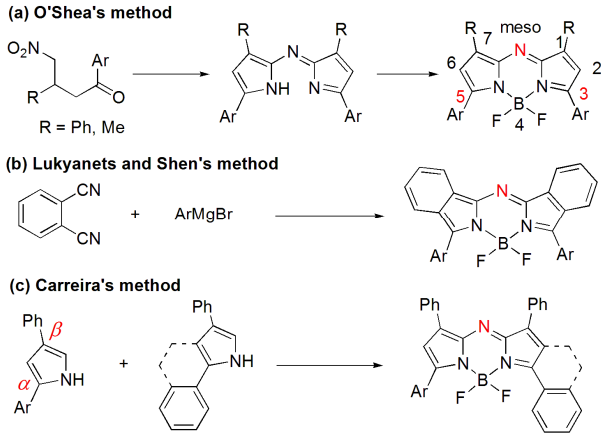
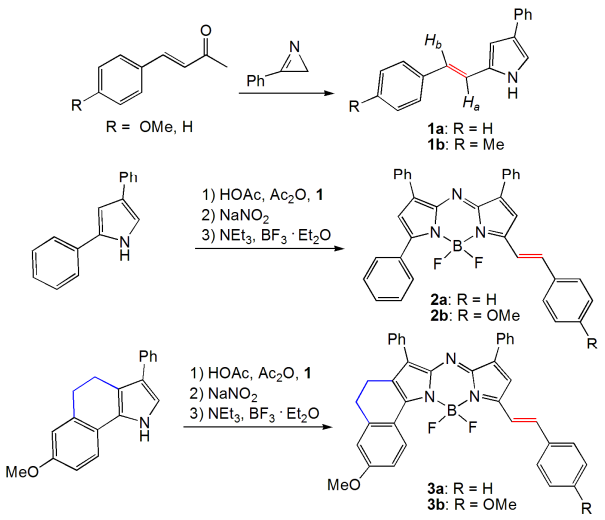


Fig. 1 Synthetic method of aza-BODIPYs.

However, the difficulty of the parent molecular modification is confronted with the major problems in aza-BODIPY system. One of the very effective methods of modification on the BODIPY core to tune the absorption to red-NIR region (upper limit: 700 nm)[4d] is an extension of the conjugated system through Knoevenagel reaction.[9] Based on the reported aza-BODIPYs, the Knoevenagel reaction is ineffective to them due to the lack of a methyl group at 3,5-positions in aza-BODIPY system.[4d, 6-8] For example, 3-ethyl-2,4-dimethyl-pyrrole was employed to expect a product of aza-BODIPY including a methyl group at 3,5-positions, but give 3-amino/3-acetamido BODIPY;[10] moreover, the aza-BODIPY with a methyl group at 3,5-positions was not also obtained from dihydronaphthalene-fused pyrrole bearing a methyl group at α-position.[11] As described above, it suggest that the basic requirement of synthesizing the aza-BODIPY should have an aryl group at α,β-position in a pyrrole in Carreira's method (Fig. 1c). In addtion, until now modifications on the methyl group at 1,7-positions in the reported aza-BODIPY are not documented.[12] Therefore, further efforts to overcome this limitation are highly required. To the best of our knowledge, no styryl-containing aza-BODIPY has been documented. Our recent research interest lies in the novel BODIPY family of fluorescent dyes, specially NIR aza-BODIPYs dye.[8a,b,13] We are now

interested in styryl-containing pyrroles derived asymmetric aza-BODIPYs as bright and tunable fluorescent dyes. To extend the Carreira's method to a versatile design platform, Herein we communicate our studies on a styryl-containing pyrrole to achieve modifications of the extension of the conjugated system in NIR asymmetric aza-BODIPY, avoiding to the use of the Knoevenagel reaction.

E-4-phenyl-2-styryl-pyrrole **1** was efficiently synthesized in moderate yield by benzylideneacetone[14] with 3-phenyl-2*H*-azirine in the presence of LDA at the first time (Scheme 1 and See ESI†). The styryl-containing pyrrole **1a** showed two sets of distinct hydrogen signals in the ^{1}H NMR spectrum (δ = 6.98 (d, $^3J_{HH}$ = 16.5 Hz, 1H$_a$) and 6.73 (d, $^3J_{HH}$ = 16.5 Hz, 1H$_b$) ppm), which were in agreement with the existence of ethylenic linkage at α-posion in the pyrrole **1a** (See ESI†). Though the failure to synthesize the aesthetic symmetric aza-BODIPY by pyrrole **1**, we fortunately found that the styryl-containing pyrrole **1** with another 2,4-diaryl pyrrole/aryl-fused 2,4-diaryl pyrrole[5d,8b] were able to successfully synthesize asymmetric conformationally styryl-containing aza-BODIPYs **2a**-**3b** under HOAc, Ac_2O and $NaNO_2$, followed by complexation with Et_3N–BF_3·Et_2O (Scheme 1).[8] This was a new aza-BODIPY structure bearing a styryl group (Scheme 1).



Scheme 1. Synthesis of the styryl-containing aza-BODIPYs.

The absorption and emission spectra of aza-BODIPYs **2a-3b** were outlined in Fig. 2 and Table 1, respectively. Aza-BODIPYs **2a-3b** absorb and emit in the NIR region, with high molar extinction coefficients. To demonstrate the spectroscopic effects of the styryl substituents, the spectral characteristics of **2a** and **3b** were compared to those of the known dyes **4**[5d] and **5**[8b], respectively (Fig. 3 and Table 1). In comparison with the emission maximum of **4** (λ_{em} = 672 nm), the effect of extending the π-conjugation in **2a** (λ_{em} = 709 nm) is considerable wherein 37 nm of bathochromic shift was achieved. Moreover, dye **2a** shows an appreciable extinction coefficients (112000 M^{-1} cm^{-1}) and higher than that (79000 M^{-1} cm^{-1}) of dye **4**. Moreover, the molecular geometries of aza-BODIPYs **2a** and **4** were optimized using density functional theory (DFT) at the B3LYP/6-31G(d) level. The calculated HOMO and LUMO orbital energy levels were summarized in Fig 4. The extension of the conjugated system in the styryl-containing BODIPY **2a** (λ_{abs} = 686 nm) resulted in a remarkable hypsochromic shift (36 nm) compared to that (λ_{abs} = 650 nm) of the aza-BODIPY **4**. It is due to the decrease in the HOMO–LUMO band gap for the lowest energy absorption bands observed for **2a** relative to that of **4** by MO calculations (Fig. 4).

In addition, the effect of extending the π-conjugation in **3b** was dramatic wherein the bathochromic shift and the high extinction coefficients were also achieved, comparing to these of the dye **5** (Table 1). Although the fluorescence quantum yield (Φ_f) of the NIR aza-BODIPY **3b** was measured to be 0.12, dye **3b** was bright enough for the following staining experiments.

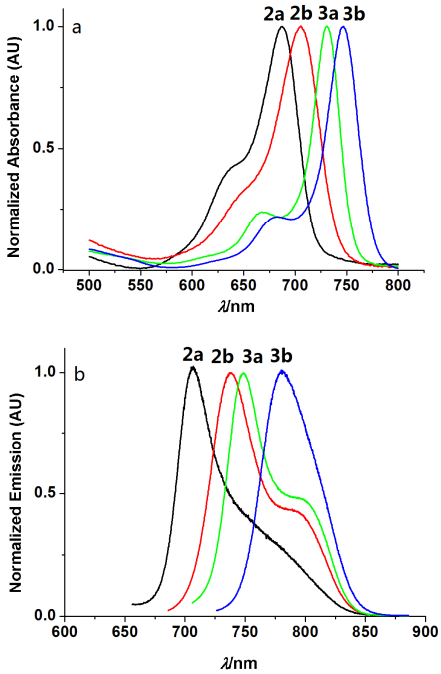


Fig. 2 (a) Normalized absorption and (b) fluorescence spectra of **2a**-**3b** in CH_2Cl_2 at 298 K.

Table 1 Photophysical properties of aza-BODIPYs **2a-3b** in CH_2Cl_2 at 298 K.

Dye	$\lambda_{abs}/\lambda_{em}$ [nm]	ε [M^{-1} cm^{-1}]	Φ_f
2a	686/709	112000	0.22
2b	704/737	98000	0.17
3a	731/749	166000	0.18
3b	746/780	153000	0.12
4	650/672	79000	0.34
5	708/732	96200	0.38

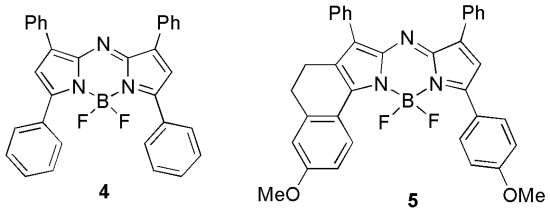


Fig. 3 Chemical structures of aza-BODIPYs **4** and **5** reported and used in the present study.

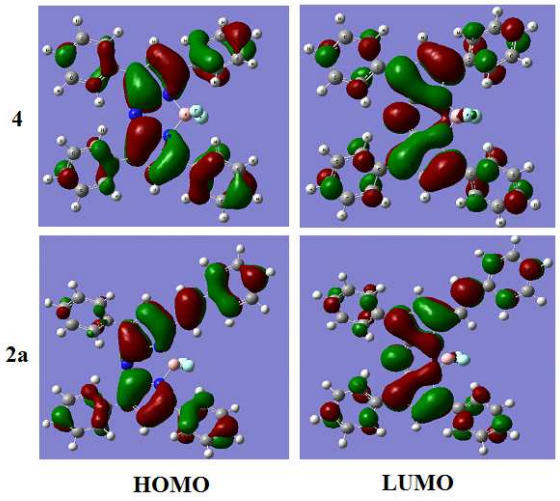


Fig. 4 Frontier molecular orbitals of BODIPYs **2a** and **4** at the B3LYP/6-31G(d) level with Gaussian 03. HOMO/ LUMO (eV) = –5.36/–3.15 for **4**; HOMO/ LUMO (eV) = –5.17/–3.15 for **2a**.

To test the effect of labeling a live cell imaging assay, we chose **3b** as a representative compound, due to its moderate fluorescence quantum yield in the NIR region, which would enable facile visualization with fluorescence microscopy. For the cell-staining experiment, 2×10^{-5} M of dye **3b** in PBS containing 0.5% (v/v) DMSO was incubated with U87 Gliomas cells for 20 min at 37 °C and the excess dye was removed by washing with PBS prior to visualization. An ArrayScan® VTI HCS Reader inverted fluorescence microscope was employed for the fluorescence image, with the excitation wavelength of the laser at 700 nm. The obtained images indicated that **3b** was efficiently internalised by living cells. Dye **3b** was membrane-permeable and was found to localize exclusively to the cytoplasm and nuclei (red color) (Fig. 5).

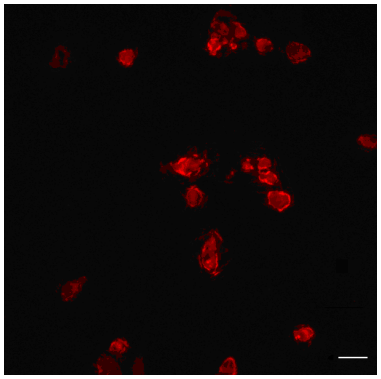


Fig. 5 Fluorescence microscope imaging of U87 Gliomas cells following incubation with 2×10^{-5} M of **3b** in PBS + 0.5% (v/v) DMSO (red color) for 20 min at 37 °C; Scale bar: 10 μm.

Conclusions

In surmmary, a novel styryl-containing pyrrole **1** efficiently synthesized in moderate yield by the reaction of *E*-4-phenylbut-3-en-2-one with 3-phenyl-2H-azirine in the presence of LDA. The asymmetric styryl-containing aza-BODIPY **2a-3b** have been prepared from pyrrole **1** absorbed and emitted in the NIR region as a result of the extension of π-conjugation. By MO calculations the decrease in the HOMO–LUMO band gap for the lowest energy absorption bands was observed in the styryl-containing aza-BODIPY. This was a new aza-BODIPY structure bearing a styryl group. The NIR aza-BODIPY dye **3b** was bright enough to be suited to label living cells for imaging assay in the NIR region. Further efforts for the water-soluble version of modifications and development of probes based on these dyes in biotechnology are ongoing in our lab.

Acknowledgements

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Notes and references

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†Electronic Supplementary Information (ESI) available: Experimental details and ^{1}H, ^{13}C NMR spectra. See DOI: 10.1039/b000000x/

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