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

Xin-Dong Jiang, Dongmei Xi, Jiuli Zhao, Haifeng Yu, Guo-Tao Sun and Lin-Jiu Xiao

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 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.
A novel styryl-containing pyrrole was developed by the reaction of E-4-phenylbut-3-en-2-one with 3-phenyl-2H-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.

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. The safe, non-invasive nature of NIR fluorescent imaging has attracted great interests in the design and synthesis of novel NIR fluorescent dyes. NIR dyes such as azo dyes, cyanine dyes, quinone dyes, and phthalocyanine dyes 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. 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. Recently, our group focus on the BODIPY's 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. 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 polypeptide synthetic method of BODIPY, 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). 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). 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 and effective method of symmetric/asymmetric aza-BODIPY (Fig 1c).

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) is an extension of the conjugated system through Knoevenagel reaction. 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. 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; moreover, the aza-BODIPY with a methyl group at 3,5-positions was not obtained from dihydronaphthalene-fused pyrrole bearing a methyl group at α-position.

As described above, it suggest that the basic requirement of synthesizing the aza-BODIPY should have an aryl group at αβ-position in a pyrrole in Carreira's method (Fig. 1c). In addition, until now modifications on the methyl group at 1,7-positions in the reported aza-BODIPY are not documented. 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.
interested in styryl-containing pyroles 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 with 3-phenyl-2H-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 1H NMR spectrum (δ = 6.98 (d, 3JIH = 16.5 Hz, 1Hδ) and 6.73 (d, 3JIH = 16.5 Hz, 1Hδ) ppm), which were in agreement with the existence of ethylenic linkage at α-position in the pyrrole 1a (See ESI†). Though the failure to synthesize the asymmetric 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 were able to successfully synthesize asymmetric conformationally styryl-containing aza-BODIPYs 2a-3b under HOAc, Ac2O and NaNO2, followed by complexation with Et3N–BF3Et2O (Scheme 1). 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 4a and 5b, respectively (Fig. 3 and Table 1). In comparison with the emission maximum of 4 (λem = 672 nm), the effect of extending the π-conjugation in 2a (λem = 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 (λabs = 686 nm) resulted in a remarkable hypsochromic shift (36 nm) compared to that (λabs = 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 bathochromatic shift and the high extinction coefficients were also achieved, comparing to these of the dye 5 (Table 1). Although the fluorescence quantum yield (Φf) of the NIR aza-BODIPY 3b was measured to be 0.12, dye 3b was bright enough for the following staining experiments.

Table 1 Photophysical properties of aza-BODIPYs 2a-3b in CH2Cl2 at 298 K.

<table>
<thead>
<tr>
<th>Dye</th>
<th>λabs/λem [nm]</th>
<th>ε [M⁻¹ cm⁻¹]</th>
<th>Φf</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>686/709</td>
<td>112000</td>
<td>0.22</td>
</tr>
<tr>
<td>2b</td>
<td>704/737</td>
<td>98000</td>
<td>0.17</td>
</tr>
<tr>
<td>3a</td>
<td>731/749</td>
<td>166000</td>
<td>0.18</td>
</tr>
<tr>
<td>3b</td>
<td>746/780</td>
<td>153000</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>650/672</td>
<td>79000</td>
<td>0.34</td>
</tr>
<tr>
<td>5</td>
<td>708/732</td>
<td>96200</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Fig. 2 (a) Normalized absorption and (b) fluorescence spectra of 2a-3b in CH2Cl2 at 298 K.

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

Conclusions

In summary, 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


