Long wavelength red fluorescent dyes from 3,5-diiodo-BODIPYs†

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Amphiphilic and long wavelength red fluorescent dyes (4 and 7) were prepared from the Sonogashira coupling reactions of 3,5-diiodo-BODIPYs (1 and 6). One of these compounds, BODIPY 7, readily accumulated within human carcinoma HEp2 cells and was found to localize mainly within the endoplasmic reticulum (ER).

BODIPYs have recently attracted much research interest in diverse fields,[1,2] for example as labeling reagents,[1–5] fluorescent switches,[6] chemosensors,[7,8] laser dyes,[9] photosensitizers,[10] energy transfer cassettes,[11] and harvesting arrays,[12] due to their remarkable properties,[2] including large absorption extinction coefficients, sharp fluorescence emissions, high fluorescence quantum yields, high photophysical stability, and low sensitivity to the polarity and pH of their environment. BODIPYs possessing long wavelength absorption and emission profiles in the red and near infrared region (650–900 nm) of the spectrum are particularly promising for biological applications since the background absorption, light scattering and the autofluorescence of cell components are largely reduced.[3a]

Long wavelength absorbing and emitting BODIPYs are often obtained by extending the conjugation of the BODIPY chromophore *via* functionalization of the pyrrolic positions.[13] Usually, this is achieved *via de novo* syntheses from appropriately substituted pyrroles[14] (if readily accessible), for example benzo-/naphtho-fused BODIPYs.[15] On another hand, significant breakthroughs in this area were achieved with the development of three ready-made BODIPY platforms as shown in Fig. 1: the 3,5-dimethyl-BODIPYs (**A**),[16] the 3,5-dichloro-BODIPYs (**B**),[17] and the 3,5-dithioalkyl-BODIPYs (**C**).[18] These platforms provide a convenient way for the functionalization of the 3,5-pyrrolic positions of the chromophore, and have been used to introduce various functionalities, in particular aryl, alkenyl or alkynyl groups, therefore conferring desired long wavelength absorption/emission properties on the BODIPY core. However, BODIPY **A** can only react with specific types of aryl adehydes under harsh conditions, and BODIPYs **B** and **C** generally lack β-pyrrolic substituents. Herein we report an alternative BODIPY platform complementary to **A**–**C**, bearing 3,5-diiodo substituents, and the use of this platform in Sonogashira coupling reactions leading to the efficient generation of several long wavelength emitting fluorescent BODIPYs. Preliminary *in vitro* studies on one of these dyes indicate that this type of compound is highly membrane permeable and it can selectively localize within specific subcellular organelles, suggesting promising biological applications for these molecules, namely in bioimaging.

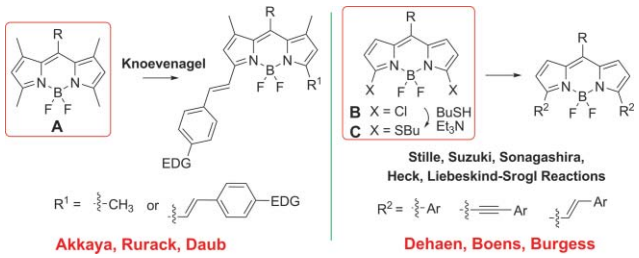
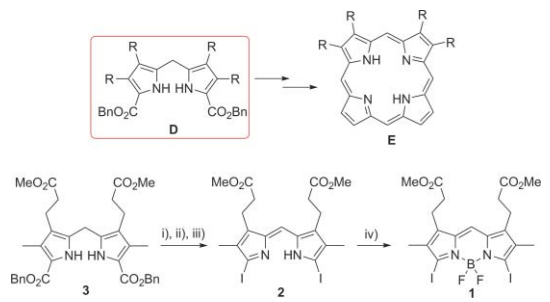


Fig. 1 Common routes for the functionalizations of the BODIPY chromophore at the 3,5-positions.

The 3,5-diiodo-BODIPY **1** was obtained in 90% yield upon $BF_3{\cdot}Et_2O$ complexation of 1,9-diiodo-dipyrromethene **2**, as shown in Scheme 1. Dipyrromethene **2** was synthesized from dipyrromethane **3** using the literature procedure.[19] This synthesis can be scaled up to a multigram scale with minimal chromatography isolation. In addition to compound **2**, a variety of 1,9-diiodo-dipyrromethenes are readily available, since dipyrromethanes **D** with various pyrrolic functionalities have been widely used in numerous [2+2] syntheses of porphyrins **E**.[20]



Scheme 1 Synthesis of 3,5-diiodoBODIPY **1** from readily available **3**. Reaction conditions: (i) Pd/C, H_2, THF, 96%; (ii) I_2, $NaHCO_3$, $MeOH–H_2O$, r.t. 80%; (iii) DDQ, 60%; (iv) Et_3N, $BF_3{\cdot}Et_2O$, 90%.

The Sonogashira coupling reactions of arylethynes and BODIPY **B** at 80 °C have been reported.[17] Consequently, we anticipated higher reactivity of the 3,5-diiodo-BODIPY platform in this reaction. BODIPY **1** did show superior reactivity in the Pd(0)-catalyzed Sonogashira coupling reactions, smoothly generating the desired long wavelength fluorescent dyes **4a–c** as dark blue solids in 66–67% yields within 2 h at 60 °C, as shown in Scheme 2.

Fig. 2 shows normalized absorbance (a) and fluorescence (b) spectra for compounds **1** and **4a–c**. The newly added arylethynyl

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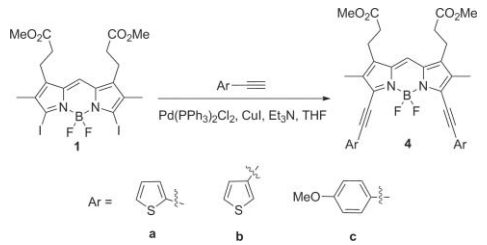


Scheme 2 Functionalization of 3,5-diiodoBODIPY **1** *via* the Sonogashira coupling reaction with arylethynes to generate BODIPYs **4a–c**.

groups caused broad absorption bands and red-shifts of both maxima, as previously described for 3,5-diarylethynyl-BODIPYs.[17] Additional spectroscopic studies were conducted for our compounds, and the results obtained are summarized in Table 1. The arylethynyl groups caused red-shifts of the maximum absorption wavelength in the order of 74–90 nm, and maximum fluorescence wavelength red-shifts in the order of 74–103 nm. The increase of fluorescent quantum yields was in agreement with previous reported 3,5-diarylethynyl-BODIPYs.[17]

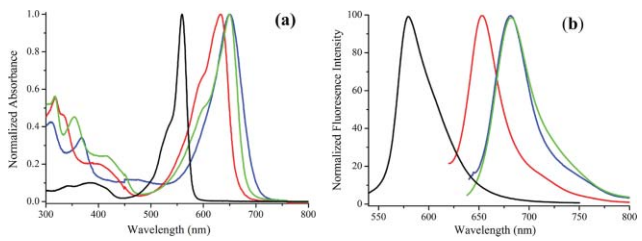


Fig. 2 Normalized UV-vis (a) and fluorescence (b) spectra of BODIPYs **1** (black), **4a** (blue), **4b** (red) and **4c** (green) in dichloromethane.

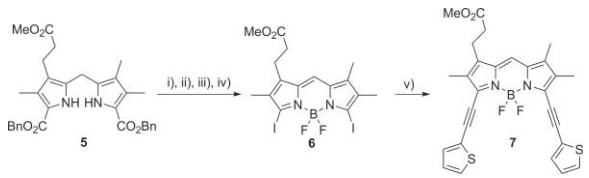
Interestingly, the newly installed 2-ethynylthiophenes in BODIPY **4a** caused significantly larger red-shifts on the maximum absorption wavelength (90 nm) and maximum fluorescence wavelength (102 nm) compared with those observed for BODIPY **4b** bearing 3-ethynylthiophene groups (74 nm red-shifts for both maxima). The nature of the solvent was found to have little effect on the photophysical properties of these compounds, as shown in Table S1.†

Under similar optimized reaction conditions as described above, we also prepared an asymmetric long wavelength BODIPY dye (**7**), in 72% yield from the corresponding diiodo-BODIPY, as shown in Scheme 3. The starting BODIPY **6** was synthesized in 33% overall yield from readily available dipyrromethane **5**,[21,22]

Table 1 Photophysical properties of BODIPY **1**, **4a–c**, **6** and **7** in dichloromethane

BODIPYs	λ_{max}/nm	lgε	λ_{em}/nm	Φ^a	Stokes shift/nm
1	559	4.66	579	0.07	20
4a	649	4.76	681	0.17	12
4b	633	4.67	653	0.39	20
4c	649	4.77	682	0.29	23
6	558	4.47	576	0.05	18
7	674	4.35	686	0.13	12

[a] For BODIPY **1** and **6**, the fluorescence quantum yields were calculated using fluorescein in 0.1 N NaOH aqueous solution (Φ = 0.90) as the standard; for BODIPY **4a–c** and **7**, methylene blue in MeOH (Φ = 0.03) was used as the standard.



Scheme 3 Synthesis and functionalization of asymmetric 3,5-diiodoBODIPY **6**. Reaction conditions: (i) Pd/C, H_2, THF, 94%; (ii) I_2, $NaHCO_3$, MeOH–H_2O, r.t. 80%; (iii) DDQ, 55%; (iv) Et_3N, $BF_3{\cdot}Et_2O$, 80%; (v) 2-ethynylthiophene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 72%.

and showed similar reactivity in the Sonogashira coupling reaction as BODIPY **1**. Furthermore, BODIPY **6** showed similar absorption and emission profiles to those of BODIPY **1** (Table 1). The low fluorescence quantum yields observed for the diiodo-BODIPYs (**1** and **6**) can be attributed to the heavy atom effect, as previously described for 2,6-dibromo-BODIPY[16c,23a] and 2,6-diiodo-azaBODIPY,[23b] both of which have been proposed as photosensitizers for the photodynamic therapy (PDT)[24] of tumors, due to this effect.

In comparison with the diiodo-BODIPY **6** and as expected, the newly installed 2-ethynylthiophenes in BODIPY **7** caused large red-shifts of the absorption and emission maxima (to 116 nm and 110 nm, respectively). Similar results have been observed using other organic solvents, as shown in Table S1.†

BODIPY **7** was found to rapidly accumulate within human carcinoma HEp2 cells. Upon incubation with HEp2 cells at 1 μM concentration for 1 h at 37 °C, BODIPY **7** gave bright red fluorescence, as seen using a Leica DMRXA microscope with

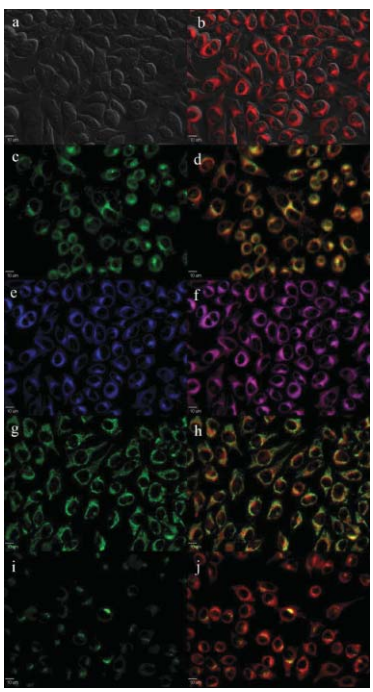


Fig. 3 Subcellular localization of BODIPY **7** in HEp2 cells at 1 μM for 1 h: (a) phase contrast, (b) overlay of **7** fluorescence and phase contrast, (c) LysoSensor Green fluorescence, (e) ER Tracker Blue/White fluorescence, (g) MitoTracker Green fluorescence, (i) BODIPY FL C5-ceramide fluorescence, and (d, f, h, j) overlays of organelle trackers with **7** fluorescence. Scale bars: 10 μm.

40× NA 0.8 dip objective lens and DAPI, FITC and Cy5 filter cubes (see Fig. 3). The overlay with phase contrast (Fig. 3b) shows that BODIPY has no cytotoxic effect under these conditions. In order to investigate the subcellular distribution of BODIPY **7**, the HEp2 cells were co-incubated with LysoSensor Green (lysosomes) at 50 nM for 30 min, ER Tracker Blue/White (ER) at 100 nM for 30 min, MitoTracker Green (mitochondria) at 250 nM for 30 min and BODIPY FL C5-ceramide (Golgi) at 50 nM for 30 min. The corresponding overlay images are shown in Fig. 3d, f, h and j. Our results indicate that the preferential sites of subcellular localization of BODIPY **7** are the cell ER and also, but to a smaller extent, the lysosomes. Further biological evaluation of these long wavelength absorbing/emitting BODIPYs is currently under way.

In summary, long wavelength fluorescent BODIPYs **4** and **7** have been synthesized in good yields from the corresponding 3,5-diiodo-BODIPYs *via* Sonogashira coupling reactions. Preliminary *in vitro* investigations indicate that these compounds are readily taken up by cancer cells, have low dark cytotoxicity and accumulate preferentially within cell ER. Our results suggest that this type of BODIPY dye could have promising biological applications.

Acknowledgements

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