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Near IR emitting BODIPY fluorophores with mega-stokes shifts†

Aaron Martin,[a] Conor Long,[b] Robert J. Forster[ab] and Tia E. Keyes*[ab]

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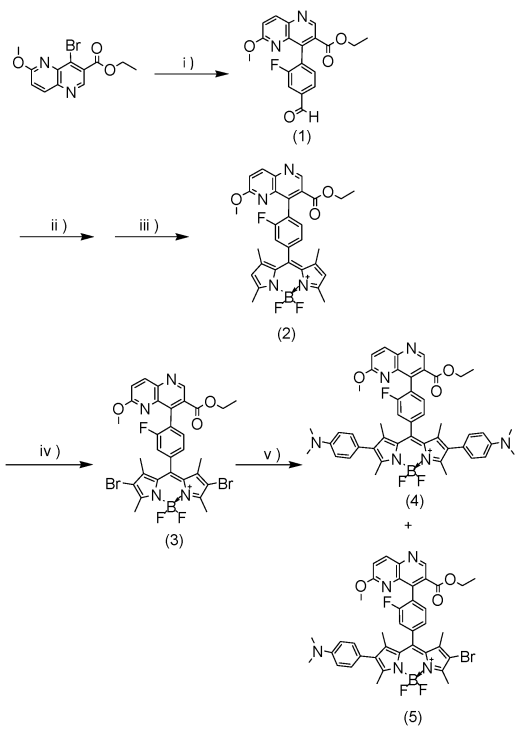
A novel Near Infra-Red emitting BODIPY derivative is presented which exhibits the largest Stokes shift thus far reported for a BODIPY compound.

The 4,4-difluoro-4-bora-3a,4a-diaza-indacene (BODIPY) derivatives are a synthetically versatile class of fluorophore.[1,2] They are of particular interest in sensing and imaging because of their notably high fluorescence quantum yields in the visible spectral region and also their photostability.[3,4] Indeed several BODIPY derivatives have been commercialized for biological labelling. Ideally, in bioimaging applications the dye should emit within the biological window (650–900 nm) where light scattering,[5] auto-fluorescence, and tissue absorption are minimised. The simple BODIPY core emits *ca.* 500 nm, and strategies to induce bathochromic shifts in these dyes have been developed which have focussed on attachment of secondary units at the pyrrole,[6,7] *meso*,[8] and *N-ortho* positions.[9]

Less widely explored for BODIPY derivatives is the implementation of a Stokes shift *i.e.* a separation between the absorbance and emission maxima in the spectral properties of the derivative.[10] Indeed, although mega Stokes shifts (Stokes shift exceeding 100 nm) are relatively common in luminescent inorganic complexes such as ruthenium polypyridyl complexes, which are formally phosphorescent,[11] shifts of comparable magnitude are unprecedented in BODIPY derivatives. Mega-Stokes shifted luminophores are nonetheless of significant value in imaging and sensing applications. They eliminate the self-quenching prevalent in high quantum yield fluorophores which restricts their use at concentrations as low as 10^{-7} molar. Self-quenching is particularly problematic in applications which require multiple labels, for example conjugation of protein or DNA or localisation of probes in sub-cellular structures such as organelles or membranes. This is particularly true in quantitative fluorescence sensing applications. Furthermore, as we have recently demonstrated, mega Stokes shifted luminophores have application in multimodal imaging.[12]

In this contribution, we describe two novel BODIPY push-pull compounds incorporating a naphthyridine acceptor site and dimethylamino aryl linked BODIPY donor (4 and 5, shown in Scheme 1). The naphthyridine unit is a well known acceptor and interesting because of its H-bond capability which has been applied in DNA sensing.[13] Compound **4** exhibits both a NIR emission and a mega-stokes shift of approximately 185 nm. To our knowledge, this is the largest Stokes shift ever reported for a BODIPY derivative.

Scheme 1 shows the synthetic steps leading to the novel BODIPY derivatives **4** and **5**. The π electron conjugation of the central pyrrole rings is extended by attachment of dimethylamino aryl groups to the 6 and 2 positions of the BODIPY core and a naphthyridine substituent at the 8 position. The detailed synthesis and structural characterisation is presented in the ESI.† Briefly, bromonaphthyridine was obtained by treating 6-methoxypyridin-3-amine with diethyl ethoxymethylenemalonate as reported previously.[14] A Suzuki-Miyaura protocol[15] was



Scheme 1 Synthetic route to NIR BODIPY dyes. (i) 2-fluoro-4-formylphenylboronic acid, K_2CO_3, $Pd(dppf)Cl_2$. DCM, dioxane/H_2O (3 : 1), 45 min, 79%; (ii) 2,4-dimethyl-1*H*-pyrrole, TFA, DCM, 4h; (iii) tetrachlorobenzoquinone, Et_3N. $BF_3{\cdot}OEt_2$, 12 h, 58%; (iv) 1,1′-azobis(cycloheaxanecarbonitrile), N-bromosuccinimide, CCl_4, 4 h, 22%; (v) 4-dimethylaminophenylboronic acid, K_2CO_3, $Pd(dppf)Cl_2{\cdot}DCM$, dioxane/H_2O (3 : 1), 45 min, (30%, **4**) and (24%, **5**).

[a] *Biomedical Diagnostics Institute, Dublin City University, Glasnevin, Dublin 9, Ireland*
[b] *School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland. E-mail: Tia.Keyes@dcu.ie*

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then followed combining 4-formylarylboronic acid (1.5 equiv) with Pd(dppf)Cl_2·DCM (4 mol%) as catalyst and K_2CO_3 (2 equiv) as a base in aqueous dioxane/H_2O (3 : 1) which was heated to reflux for *ca.* 45 min (Scheme 1). This gave 8-(4-formylaryl)-2-methoxynaphthyridine **1** in high yield.[16]

Typically reactions came to completion with no unwanted by-products (by TLC). The desired product was isolated by flash column chromatography (hexane : EtOAc 4 : 1) as a yellow oil and recrystallised from pentane to yield **1** as a white solid. A Knoevenagel reaction with 2,4-dimethylpyrrole produced 2 which was then purified on silica gel (20% EtOAc:hexane). The insertion of bromine[17] at the 2,6 positions of the BODIPY was achieved by reacting **2** with 1,1′-azobis-(cycloheaxanecarbonitrile) (4 equiv) and N-bromosuccinimide (4 equiv.) to yield **3**. The bromo derivative was then reacted with *p*-dimethylaminophenyl boronic acid using the same Suzuki-Miyuara conditions described above to afford compounds **4** and **5**. The symmetric form **4** was the dominant product (25 mg, 30%) which was isolated as a dark blue solid, following purification *via* flash column chromatography using hexane/EtOAc, 4 : 1.

Fig. 1(a) and (b) show the absorption (green), excitation and emission spectra of **4** and **5** respectively in CH_2Cl_2. BODIPY is typically characterised by intense and narrow absorption and fluorescence bands, often with mirror image vibronic structure and by notably small Stokes shifts. Whereas both **4** and **5** exhibit markedly broad absorption and emission features, which lack mirror symmetry. Furthermore, both exhibit substantial Stokes shifts, which in light of the broad FWHM of the emission maxima are more consistent with charge transfer transitions. Indeed compound **4** exhibits an absorption at 554 nm [ε, 3×10^3 M^{-1} cm^{-1}, (± 180)] and an emission with a maximum in dichloromethane at 740 nm. The Stokes shift is therefore 186 nm. This is, to our knowledge, the largest reported for a BODIPY derivative and is indeed remarkable, more generally, among organic fluorophores. **4** exhibits a fluorescence lifetime of 0.5 ns, such a short lifetime renders the complex insensitive to O_2. The quantum yield of emission is 0.06 (±0.003), which is considerably lower than conventional BODIPY compounds. However, it is significantly higher than many luminescent metal complexes, such as the Ru(II) polypyridyls which exhibit comparable Stokes shifts, but whose emissions are rarely so red-shifted. The radiative, k_f and non-radiative decay rates, k_{nr} for **4** were estimated from this data to be 1.2×10^8 and 1.9×10^9 s^{-1} respectively.[18] The asymmetric bromo compound, **5**, exhibits comparable absorption to **4**, albeit with a blue shift to 523 nm [ε, 7×10^4 M^{-1} cm^{-1} (±4800)]. However, it exhibits a more intense emission and longer emission lifetime [ϕ = 0.14 (±0.008), τ = 1.9 ns]. This data yields k_f and k_{nr} values of 7.4×10^7 and 4.5×10^8 s^{-1} respectively for **5**. Interestingly, unlike **4**, where strong correlation exists between the absorption and excitation spectra, for **5**, the maximum of the absorption spectra is red shifted by roughly 20 nm compared with the excitation spectrum. The emission and excitation spectra for **4** and **5** were studied over the concentration range 10^{-7} to 10^{-5} M and found not to change, indicating this behaviour is not due to aggregation or excimer formation. This suggests that the red end of the absorbance spectrum contains contributions from an optical transition that does not yield an emitting state in 5.

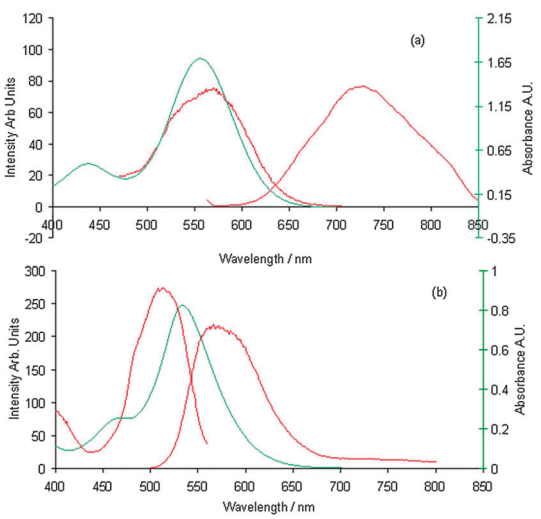

Fig. 1 Absorption (green), emission (red) and excitation (red) spectra of (a) **4** (λ_{ex} = 553 nm) and (λ_{em} = 720 nm) (b) **5** (λ_{ex} = 550 nm) and (λ_{em} = 585 nm) in CH_2Cl_2 solution. Excitation and emission spectra were collected with a slit width of 10 nm for **4** and 5 nm for **5**.

Both compounds were modelled using Density Functional Theory (DFT), full details of these calculations are provided in the ESI.† The hydrid functional B3LYP was used along with the 6-311G(d) basis set.[19–22] Structural parameters were optimised without symmetry or other constraints. Each molecule was divided into two fragments. The first (Frag 1) consisted of the boron heterocycle and associated dimethylamino aryl groups, and the second (Frag 2) consisted of the *meso*-substituent *i.e.* the fluorophenyl link and the naphthyridyl substituent. The nature of the lowest energy excited states was classified according to the contribution of the fragment orbitals to the excited state. The results of these calculations are outlined in Fig. 2 where the orbitals which are mainly centred on Frag 1 are indicated in red while those centred on Frag 2 are drawn in blue. For both compounds, the HOMO is centred on Frag 1. The HOMO is mostly localised on the dimethylaminoaryl unit on **5** whereas for **4** it is extended over both of dimethylaminoaryl fragments. Interestingly, the LUMO switches from the naphthyridine fragment, Frag 2, in **4** to Frag 1 in **5**. Calculations show the lowest energy optical transitions for

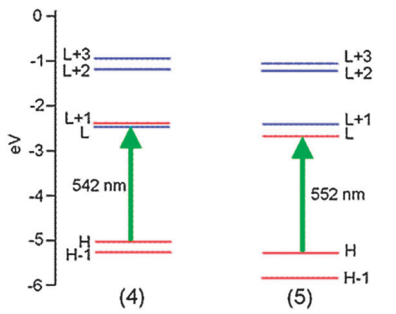

Fig. 2 The orbital energy levels for the two highest occupied orbitals and four lowest energy virtual orbitals for **4** and **5**. The green arrow indicates the transition to the lowest energy singlet excited state which in the case of **4** involves a transition from Frag 1 (red) to Frag 2 (blue), the equivalent transition in **5** is centred on Frag 2 (red).

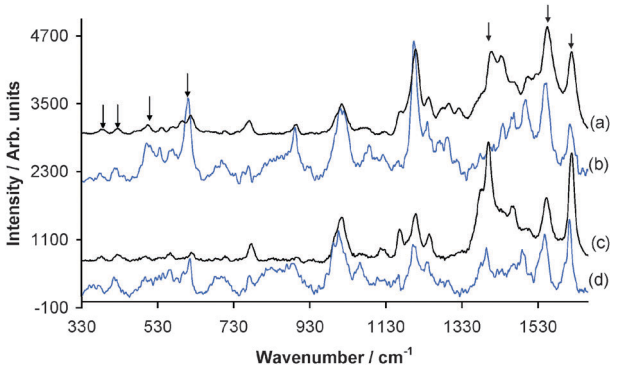

Fig. 3 Resonance Raman spectroscopy of (a) **5** excited at 473 nm, (b) **5** excited at 532 nm, (c) **4** excited at 532 nm, (d) **4** excited at 473 nm. Samples were approx. 1% w/w of compound dispersed in KBr.

both compounds have significant charge transfer character. For **5** this transition involves charge transfer from the dimethylaminoaryl group to the BODIPY whereas for **4** charge transfer occurs between fragment 1 and 2. The energy of these optical transitions are reasonably well correlated with the DFT calculations, although the red shift of the optical transition for **4** with respect to **5** is not reflected in the calculations.

To further elucidate the optical transitions and the disparity between excitation and absorbance spectra in **5**, resonance Raman spectroscopy of the BODIPY derivatives were investigated, exciting at 473 and 532 nm, Fig. 3, black and blue respectively. Resonance Raman spectroscopy is uniquely enabled in these fluorophores by their large Stokes shifts. 473 and 532 nm coincide reasonably well with the maxima of the absorbance and excitation spectra respectively of **5**. 532 nm correlates well with both the excitation and absorbance frequencies of **4** as these match. However, 473 nm is pre-resonant with the absorbance/excitation maxima of **4** and post-resonant with a higher frequency transition centred around 438 nm. Correspondingly, the similarities in the spectra shown in Fig. 3(c) and (d) indicate that both 473 and 532 nm are resonant with the same transition of **4**. Although there are some changes to relative intensity, all spectral features are present in both spectra. Vibrational bands characteristic of both Frag 1 and Frag 2 are resonantly enhanced indicating charge transfer between these fragments. In contrast, there are significant differences in the resonance Raman spectra excited at 473 compared to 532 nm for **5**. Under 473 nm excitation, which is resonant with the excitation spectrum maximum, the Raman signature is strongly reminiscent of the resonance Raman spectra shown for **4** consistent with charge transfer between Frag 1 and Frag 2. However, under 532 nm excitation, *i.e.* resonance with the absorbance maximum of **5**, a number of key modes observed under 473 nm excitation are much weaker or missing. Most notably features at 1400 and 1520 cm^{-1}, which are associated, on the basis of DFT calculations, with the naphthyridyl unit, the features are marked with arrows. The remaining bands are associated predominantly with Frag 1 suggesting a charge transfer between the dimethylaminoaryl moiety and the substituted boron heterocycle, consistent with the HOMO–LUMO transition predicted for **5**. In contrast, both DFT and resonance Raman indicate that the state excited under 473 nm irradiation is likely to be the HOMO to LUMO + 1 transition and from the excitation spectrum it appears this is the origin of the luminescence.

Overall, we conclude, that the emissive state in both cases arises from a charge transfer transition between the dimethylaminoaryl substituted boron heterocycle moiety and the naphthyridyl units in both compounds. However, for **5** this transition is not the lowest energy optical absorbance. A Stokes shift for both complexes is observed, but particularly for **4** which at 185 nm is the largest reported for a 4-Difluoro-4-bora-3a,4a-diaza-indacene derivative.

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