Synthesis of Bisisoindolomethene Dyes Bearing Anisole or Ethylthiophene Residues for Red and Near-IR Fluorescence

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Abstract: New difluorobora-diisoindolomethenes dyes were synthesized from carbohydrazide and *o*-hydroxy-acetophenone derivatives bearing phenyl, *p*-anisole or ethylthiophene substituents. The nature of the substituents allows modulating the fluorescence from 650 nm to 780 nm. Replacement of the fluoro ligands by ethynyl-aryl residues is feasible using Grignard reagents. Standard fluorescence studies prove that very efficient energy transfer, from the pyrene moiety linked to the boron center to the boradiazaindacene, is effective in providing large virtual Stokes shifts.

Key words: diisoindolomethene, ethynyl-boron, pyrene, fluorescence, energy transfer

To take full advantage of the absorption window in blood between 650 and 900 nm[1] (where both hemoglobin and water/lipid absorptions are low and autofluorescence is minimal) for fluorescence imaging and mapping deeper tissues or DNA,[2] the engineering of new red emitters is necessary. Both strong emission in this spectral region and excitation through absorption at high energies are desirable. Most common organic dyes show small Stokes shifts in emission, meaning there is only a small difference in excitation and emission energies, but Stokes shifts can be dramatically increased in tandem dyes where a high-energy chromophore is linked to a low-energy emitter.[2] One absorbing center can then be coupled to various emitters to produce multichromatic fluorescent tags, as exploited in DNA sequencing based on fluorescent derivatives of A, T, G, and C bases.[3,4] The efficiency of the intermolecular energy transfer in these dyes largely depends upon dipole–dipole interactions.[5]

Known red and near IR organic emitters include rhodamines, cyanines and some F-Bodipy (difluoro-boradiazaindacene) fluorophores.[6] In the last family, extension of the π-system of the central cyanine unit, for example by introduction of vinyl[7] or phenyl[8] substituents adjacent to the aza centers, is a tool to obtain low-energy emission, as is true for aza-Bodipy dyes as well.[9]

Our first work[10] showed the remarkable efficiency of the energy transfer from pyrene to the yellow/orange emitting Bodipy. Lately we developed green-absorbing fluorophores bearing various oligopyridine-based platforms for sensing purposes.[11] Here, we describe some new tandem molecules derived from E-Bodipy (E for ethynyl) species with a common absorption center but displaying red or NIR emissions depending on the nature of the core substituents. The difluorobora-diisoindolomethenes **4a**–**c** necessary for red emission were inspired from literature procedures (Scheme 1).[12] This method was preferred to the procedure developed by Ono et al., using a retro-Diels–Alder reaction of bicyclo[2.2.2]octadiene.[13] The R^1 and R^2 substituents, which have directly an influence on the diazaboraindacene absorption and emission wavelengths, are introduced on the starting carbohydrazide and *o*-hydroxy-acetophenone derivatives. Condensation of both compounds in refluxing propanol gives **1a**–**c** in acceptable yields. A rearrangement in presence of lead(IV)[14] salt resulting in the replacement of the phenolic hydroxyl by the acyl substituent led to the diketone **2a**–**c** precursor in high yield. The diisoindolemethene **3a**–**c** were obtained by condensation of the diketone with ammonium salt. This step likely produces a diisoindolomethane intermediate after formaldehyde elimination by a retro-aldol mechanism. Air oxidation of the diisoindolomethane to the diisoindolomethene derivatives affords the pivotal compounds **3a**–**c**.[15] These diisoindolemethenes compounds were subsequently deprotonated and stabilized in the presence of boron trifluoride etherate leading to the highly stable deep-blue (**4a**, **4b**) or deep-green (**4c**) F-Bodipy dyes.

To obtain enhanced Stokes' shifts, we opted for the use of pyrene residues a strongly absorbing chromophore around 370 nm.[10] Replacement of fluoride in an F-Bodipy by an ethynyl group, to generate E-Bodipys, can be readily achieved using ethynyl-Grignard reagents (Scheme 2).[16] The homosubstituted compounds **5a**–**c** and **6b**,**c** were isolated in excellent yields. Unfortunately, the bis(ethynyl-pyrene) derivative **6a** was extremely insoluble and difficult to characterize. Thus, to study intramolecular pyrene-to-diisoindolomethene energy transfer along this series and to increase the solubility, the ethyl-mono(pyrenyl-ethynyl) compound **7a** was synthesized by reaction of equimolar quantities of ethylmagnesium bromide and pyrene ethynyl magnesium bromide with the F-Bodipy **4a** (Scheme 3). The resulting three components (homo- and heterocoupling compounds) of the product mixture being easily separated by column chromatography. In the ^{11}B NMR spectra, the characteristic triplet at $\delta = 5.1$ ppm (t, $J_{BF} = 32$ Hz) for compounds **4a**–**c** disappeared after

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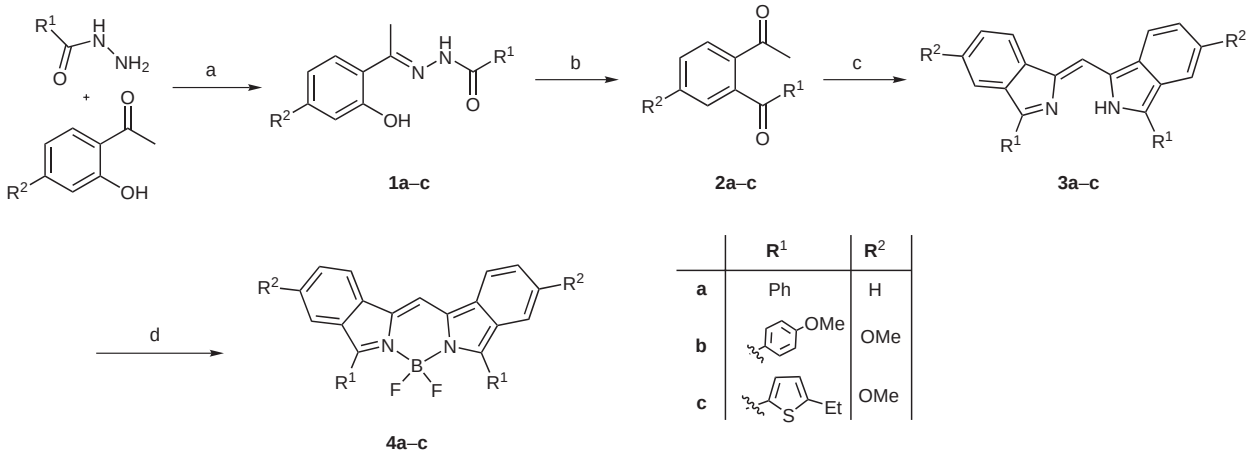
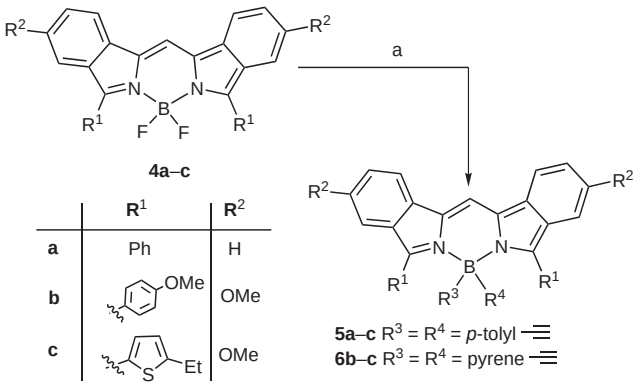


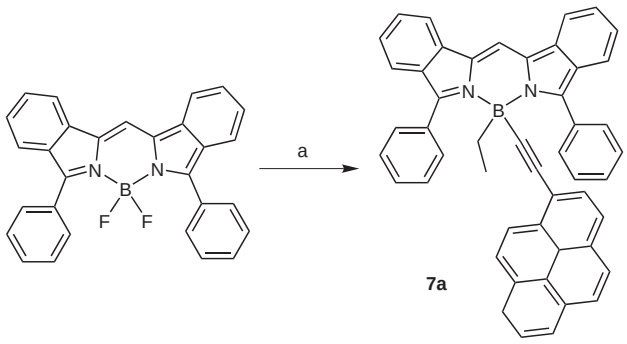
Scheme 1 *Reagents and conditions*: a) for compounds **a** and **b**: 1-PrOH, reflux, 66–88%), for compound **c** no solvent 75 °C, 87%; b) $Pb(OAc)_4$, THF, 60–95%; c) for compounds **a** and **b**: NH_4OH, MeCOOH, MeOH, r.t., 60–65%; for compound **c**: NH_4OAc, NH_4Cl, MeCOOH, EtOH, reflux, 50%.

fluorine substitution, a singlet appearing at ca. δ = –6.3 to –7.3 ppm, depending on the nature of R^3, for **5a**–**c**, **6b**,**c** and at δ = 1.1 ppm for **7a**.

Crystal structure determinations for **4a** (Figure 1),[17] **5a** (Figure 2),[18] and **6a** (Figure 3)[19] first confirm the stoichiometry and connectivity deduced from the NMR spectra. All structures reflect the almost tetrahedral geometry for the sterically hindered boron center. The bond lengths of the indacene framework are characteristic of boradiazaindacene, reflecting the entire delocalization of the cyanine. A slight distortion of the planarity of the diisoindolomethene core is observed as previously described for F-Bodipy possessing bulky substituent at the nitrogens *ortho* position.[20] This deformed system can be described as a 'butterfly' conformation; the single 'wings' consisting of two planes B–N1–C1–C2–C4–C9 and B–N2–C5–C6–C7–C9. The dihedral angle between both planes is 8.6° for **4a**, 9.0° for **5a** and 12.3° for **6a**. In the case of **7a** the boron is pointing in the ethynyl substituent direction. A tilt between the phenyl group and the indacene core is observed and seems to be dependent of the congestion induced by the boron substituent: average dihedral angles between the mean planes are 57° for **4a**, 60° for **5a** and 68° for **7a** with the larger angle facing the bulky ethyl group.



Scheme 2 *Reagents and conditions*: a) R^3–≡–MgBr (2 equiv) for **5a**–**c** and **6b**–**c**; THF, 60 °C, 41–62%.



Scheme 3 *Reagents and conditions*: a) Et–≡–MgBr (1 equiv) + pyrene–≡–MgBr (1 equiv), THF, 60 °C, 50%.

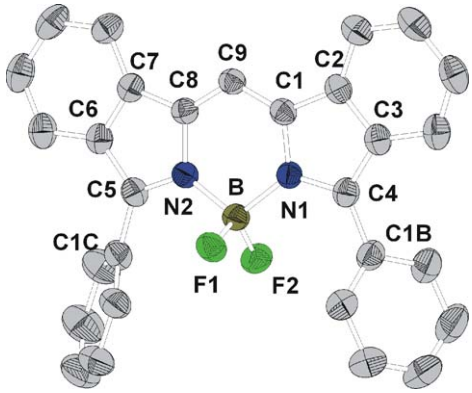


Figure 1 ORTEP view of compound **4a** (50% probability), all hydrogen atoms were removed for the sake of clarity. B–N1, B–N2, B–F1, B–F2 are 1.569(5) Å, 1.570(4) Å, 1.395(4) Å, 1.367(4) Å, respectively, with angles N1–B–N2, F1–B–F2, N1–B–F1 and N2–B–F2 of 107.95(11)°, 110.94(12)°, 108.12(11)°, and 108.05(11)°, respectively.

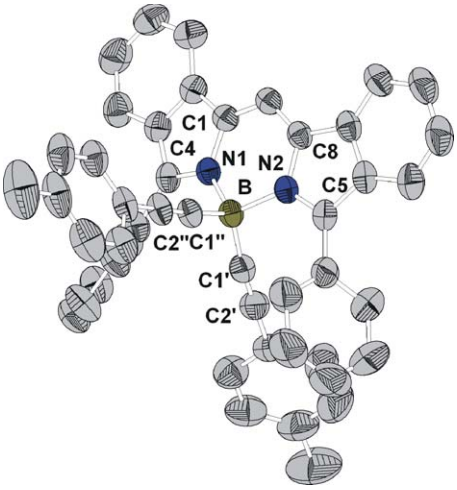


Figure 2 ORTEP view of compound **5a** (50% probability), all hydrogen atoms were removed for the sake of clarity. B–N1, B–N2, B–C1′ are 1.598(18) Å, 1.595(7) Å, 1.563(7) Å, respectively, while B–C1″ is 1.597(24) Å, with angles N1–B–N2, C1′–B–C1″, N1–B–C1″, and N2–B–C1′ of 106.62(13)°, 118.39(15)°, 106.56(15)°, and 109.00(19)°, respectively.

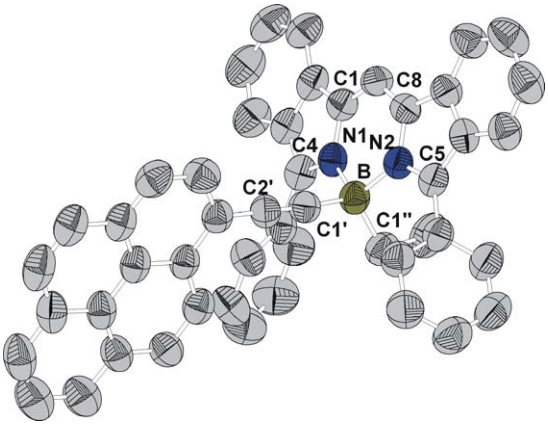


Figure 3 ORTEP view of compound **7a** (50% probability), all hydrogen atoms were removed for the sake of clarity. B–N1, B–N2, B–C1′, and B–C1″ are 1.579(13) Å, 1.603(30) Å, 1.583(11) Å, and 1.577(24)Å, respectively, with angles N1–B–N2, C1′–B–C1″, N1–B–C1″, and N2–B–C1′ of 106.60(29)°, 117.82(33)°, 105.00(32)°, and 110.44(29)°, respectively.

All dyes exhibit very high absorption in the visible (λ_{abs} = 614–634 nm for **4a**–**5a** and **7a**, λ_{abs} = 666–673 nm for **4b**–**6b** and λ_{abs} = 707–727 nm for **4c**–**6c**), assigned to the $S_0 \rightarrow S_1$ (π–π^*) transition, of the Bodipy fragment. Additional high-energy absorption bands are observed for compounds **4a**–**c** and **5a**–**c** in the 360–410 nm region, ε = approx. 20,000 to 40,000 $M^{-1}cm^{-1}$, likely due to the cyanine framework. Other intense absorptions are found at 352 nm and 372 nm (ε = 40,000 $M^{-1}cm^{-1}$ and 50,000 $M^{-1}cm^{-1}$ per pyrene unit for **6b**–**c** and **7a**), assigned to the absorption of the pyrene (Figure 4). Solution luminescence is strong for all the novel dyes in the red-near IR window, diminishing near 800 nm (Table 1). Quantum yields are slightly less for **5a** and **7a** than for the strongly red-luminescent **4a**. For **7a**, a significant hypsochromic shift is observed in absorption and emission, probably induced by steric effects that moderate electronic delocalization of the cyanine. Compounds **4b**, **5b**, and **6b** exhibit relatively strong fluorescence (quantum yields ca. 50%) around 700 nm. Selected spectroscopic data are gathered in Table 1.

Table 1 Optical Properties in CH_2Cl_2 at Room Temperature

Compd	λ_{abs} (nm)	λ_{em} (nm)	ΔS (cm^{-1})	ε (M^{-1} cm^{-1})	Φ (%)[a]
4a	634	658	575	108400	88
4b	673	704	654	118600	49
4c	727	780	934	100000	20
5a	632	656	579	82500	57
5b	666	700	729	85000	58
5c	707	746	740	90000	36
6b	667	702	748	88500	56
	370	702	12780	111000	32
6c	720	754	626	90000	33
	371	758	13762	140000	24
7a	614	643	734	78300	65
	370	643	11475	42000	64

[a] Relative quantum yields determined using Cresyl violet as shown in ref. 21 (Φ = 0.51%, λ_{exc} = 578 nm).[21] For compounds **4**–**6c**, compound **7a** was used as reference. The estimated error is fairly large in the 700–800 nm region due to apparatus sensitivity.

Compounds **4**–**5c** are weaker emitters (ϕ 20–30%) in the 750–780 nm region, the hypsochromic shifts resulting in **5c** from fluorine substitution possibly reflecting steric effects of the boron substituent on the delocalization to the ethylthiophene group. When excited in the pyrene absorption band, **5**–**6c** show only near-IR fluorescence, with no residual pyrene emission. In these cases the energy transfer is not quantitative (60% and 70%, respectively), despite the fact that no residual pyrene fluorescence is observed. For compound **7a**, photons absorbed by the pyrene subunit are channeled to the Bodipy unit, which efficiently luminesces, leading to an almost quantitative intramolecular energy transfer. The virtual Stokes shift is estimated to be 11,500 cm^{-1} for **7a** and up to 13,800 cm^{-1} for **6c**. The high quantum yields and short excited-state lifetimes (τ_{flu} = 7.0–9.0 ns) in dichloromethane at room temperature, and insensitivity to the presence of oxygen, are in keeping with emission from a singlet excited state.

Clearly, tuning of the optical properties of these tandem dyes is synthetically facile by first synthesizing the bisindolomethene core and then by replacing the fluoro ligands with ethynylaryl fragments. An added advantage of the presently described compounds is the variation in characteristics associated with the substituted alkynyl ligands on boron. These compounds have particularly useful characteristics in regard to application as fluorescent labels. They absorb and emit at exceptionally long wavelengths when compared to previously studied Bodipy species and the quantum yields remain satisfactory given the low

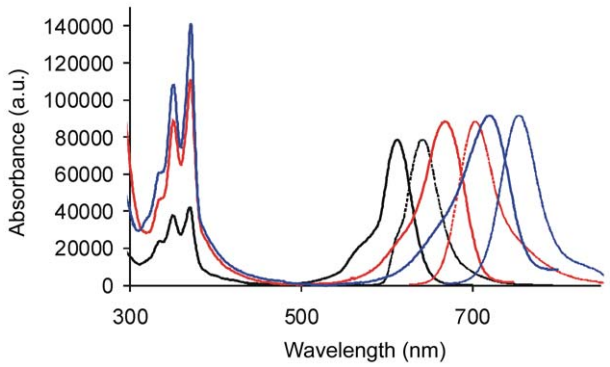


Figure 4 Absorption (full line) and emission (dotted line) of **7a** (black), **6b** (red) and **6c** (blue).

energy of the emission. We contend that the present work paves the way for the development of a new generation of stable, functionalized luminophores. For bioanalytical applications, a functional group suitable for their grafting onto biomolecules is necessary. Chemistry at boron to meet this requirement is currently under active development.

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(16) **General Procedure for Fluorine Replacement**
A Schlenk flask was charged with the ethynyl derivative and anhyd THF. A solution of EtMgBr (1 M in THF) was then added dropwise and the mixture was stirred at 50 °C for 2 h. The mixture was then added at 25 °C via a cannula to a solution of **4a–c** in anhyd THF. The mixture was stirred at 70 °C for 16 h and the solvent was removed by rotary evaporation. The residue was treated with H_2O and extracted with CH_2Cl_2. The organic extracts were washed with H_2O, and dried over $MgSO_4$. The solvent was removed, and the residue was purified by chromatography.
Compound **5a**
Prepared following general procedure from **4a** (100 mg, 0.22 mmol), 1-ethynyltoluene (104 mg, 0.9 mmol), EtMgBr (0.41 mL, 0.41 mmol, 1 M in THF), THF (8 mL). Chromatography (silica gel, CH_2Cl_2–cyclohexane, 30:70 to 50:50) gave **5a** as a blue crystalline powder (60 mg, 42%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.20 (d, 4 H, *J* = 6.6 Hz), 7.96 (d, 4 H, *J* = 6.0 Hz), 7.92 (s, 1 H), 7.61–7.39 (m, 11 H), 7.22–7.18 (m, 1 H), 6.88 (AB system, 8 H, J_{AB} = 8.0 Hz, $\Delta\delta_{AB}$ = 24.3 Hz), 2.27 (s, 6 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 151.2, 136.8, 133.7, 132.4, 131.3, 130.9, 130.3, 128.3, 128.1, 127.7, 127.6, 124.6, 123.0, 122.4, 121.7, 118.4, 115.8, 98.6, 21.3. ^{11}B NMR ($CDCl_3$, 128 MHz): δ = –7.14 (s). UV/Vis (CH_2Cl_2): λ (ε, M^{-1} cm^{-1}) = 632 (82500), 588 (27000, sh), 269 (12000) nm. MS (ES): *m/z* (nature of the peak, relative intensity) = 637.1 (100) $[M + H]^+$. Anal. Calcd for $C_{47}H_{33}BN_2$: C, 88.68; H, 5.23; N, 4.40. Found: C, 88.40; H, 4.95; N, 4.18.
(17) Crystal Data for **4a** at 293 K: $C_{29}H_{19}BF_2N_2$, *M* = 444.27, triclinic, space group P–1, *a* = 7.310 (5) Å, *b* = 11.771 (5) Å, *c* = 13.336 (5) Å, α = 86.84 (0)°, β = 79.14 (0)°, γ = 89.79 (0)°, *V* = 1125.2 (10) Å^3, *Z* = 2, λ = 0.7107 Å, D_c = 1.311 g cm^{-3}, μ = 0.057 mm^{-1}, 24014 reflections collected with θ ≤ 26.0°, 4363 unique, *R*(int) = 0.0202, and 3343 observed reflections [I ≥ 2σ(I)], 308 parameters, *R*1 = 0.0396, *wR*2 = 0.1012 refined on F^2. CCDC 637684.
(18) Crystal Data for **5a** at 293 K: $C_{47}H_{33}BN_2$, *M* = 636.56, triclinic, space group P–1, *a* = 11.119 (4) Å, *b* = 11.674 (3) Å, *c* = 15.572 (4) Å, α = 78.73 (2)°, β = 73.51 (2)°, γ = 66.73 (2)°, *V* = 1772.5 (9) Å^3, *Z* = 2, λ = 0.7107 Å, D_c = 1.193 g cm^{-3}, μ = 0.068 mm^{-1}, 12226 reflections collected with θ < 27.5°, 8071 unique, *R*(int) = 0.0241, and 5025 observed reflections [I ≥ 2σ(I)], 453 parameters, *R*1 = 0.0571, *wR*2 = 0.1438 refined on F^2. CCDC 623954.
(19) Crystal Data for **6a** at 293 K: [$C_{49}H_{33}BN_2$, 0.5 × (C_6H_{12}, C_2H_3N)], *M* = 723.19, triclinic, space group P–1, *a* = 11.294 (4) Å, *b* = 13.299 (3) Å, *c* = 14.735 (4) Å, α = 101.78 (2)°, β = 110.03 (2)°, γ = 102.17 (2)°, *V* = 1938.4 (10) Å^3, *Z* = 2, λ = 0.7107 Å, D_c = 1.239 g cm^{-3}, μ = 0.071 mm^{-1}, 10488 reflections collected with θ < 22.4°, 4585 unique, *R*(int) = 0.0351, and 3135 observed reflections [I ≥ 2σ(I)], 470 parameters, *R*1 = 0.0649, *wR*2 = 0.1879 refined on F^2. SQUEEZE macro within PLATON was used to take disordered solvent molecules (half a molecule of cyclohexane and half a molecule of MeCN in the asymmetric unit) into account during the structure refinement process. CCDC 623955.
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