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Bis-triazolyle BODIPYs: a simple dye with strong red-light emission

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Abstract: The versatile synthesis of an unprecedented bis-triazolyle BODIPY dye displaying a high quantum yield even in water is described. X-Ray diffraction analyses and NMR measurements demonstrate that the simultaneous presence of the two triazole moieties helps stiffening the BF₂ complex.



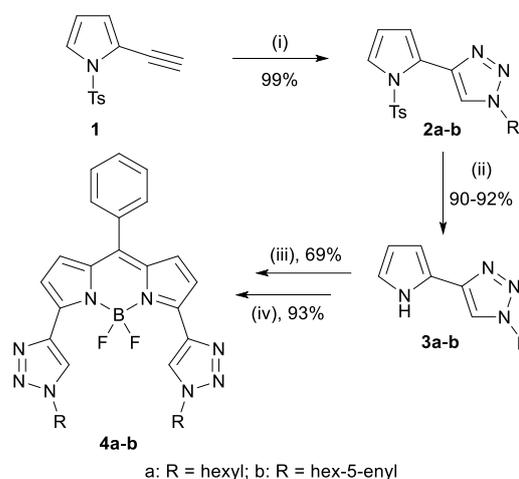
The development of highly fluorescent probes has witnessed a considerable development over the past two decades.¹ Indeed, fluorescence is a highly sensitive spectroscopy which is nowadays ubiquitous in numerous fields including -among others- medical diagnosis, material and analytical sciences and is demanding for even more performant systems. Within the myriads of fluorescent candidates, boron-dipyrromethene (BODIPY) dyes occupy a central position due to their intense and tunable absorption and emission properties.² The possibility to perform subtle chemical modifications on the BODIPY core allows to finely tune their photophysical properties or to functionalize the dye for bioconjugation purpose.³ Nevertheless, the modifications of the BODIPY scaffold have to remain cost-effective for further applicative development. The well-known CuAAC, Copper Catalyzed Azide-Alkyne cycloaddition approach is among the most appealing strategy for the efficient functionalization of substrates due to its efficiency, versatility and tolerance to many functional groups.⁴

Within the course of our investigations dealing with the development of 5,5'-diaryle dipyrromethenes,⁵ we have been interested in the preparation of 5,5'-bis-triazolyle dipyrromethenes which extended π -conjugated system could result in some promising photophysical properties. Interestingly, 5,5'-bis-triazolyle dipyrromethenes had not been reported yet, even if the monotriazolyle analogues had already been claimed very useful for Live Neuron Imaging or as "Megastokes" fluorescent dyes.⁶

Herein, we report a very straightforward synthesis of 5,5'-bis-triazolyle boron-dipyrromethenes dyes which exhibit remarkable fluorescent properties even in water and which were evaluated for cell imaging.

(bis) α -Ethynyl dipyrromethenes being inherently unstable, we envisioned the synthesis of 5,5'-bis-triazolyle dipyrromethenes starting from the pyrrole-triazole building block **2**. This synthon was readily prepared from 2-ethynyl-tosyl-1H-pyrrole **1**⁷ using a classical copper catalyzed 1,3-cycloaddition in the presence of sodium ascorbate and benzoic acid.⁸ Pyrrole detosylation proceeded

efficiently (90% yield) using aqueous NaOH in dioxane with NEt₄Br as a phase transfer agent. The corresponding pyrrole-triazole moiety **3** was subsequently reacted with benzaldehyde using the so-called Lindsey's conditions⁹ to afford the expected dipyrromethenes after oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in a satisfying 69% yield.



Scheme 1 Synthesis of the BODIPY dyes **4a-b**. *Reagents and conditions:* (i) RN₃, CuSO₄, 5H₂O, Na ascorbate, PhCO₂H, tBuOH/H₂O, rt, 30 min; (ii) 5M NaOH_{aq}, NEt₄Br, dioxane, reflux, 40 hr; (iii) **1**, PhCHO, BF₃.Et₂O, anhydrous CH₂Cl₂, rt, 24 hr, 2) DDQ, 2 hr, rt; (iv) BF₃.Et₂O, DIPEA, anhydrous CH₂Cl₂, rt, 2 hr.

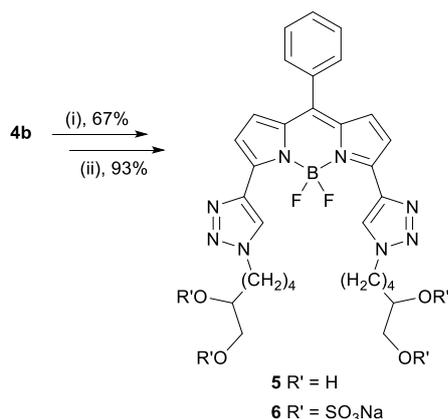
Eventually, the targeted boron complex was isolated in 93% yield after reaction with BF₃.Et₂O using *N,N*-diisopropylethylamine (DIPEA) as a proton sponge (Scheme 1). Overall, **4a-b** could be isolated in four steps with a remarkable 58% yield from the readily available ethynyl-tosylpyrrole **1**.

Aiming at developing water soluble adducts, **4b** was further functionalized by reaction with K₂O_s(OH)₂(H₂O)₂/NMO to afford the expected tetraol **5** in 67% yield (Scheme 2). The solubility of **5** in water still being poor, **5** was converted to the corresponding tetrasulfate sodium salt **6** in 93% yield using TEA.SO₃ and NaOH in DMF (Scheme 2). The water solubility of **6** exceeds 10 mol.L⁻¹.

Next the photophysical properties of the BODIPY dyes were investigated (Table 1). The chromophores display sharp and intense absorption transition ($\epsilon > 60,000$ L.mol⁻¹.cm⁻¹) centered in the orange-red spectral range with the typical shape of BODIPY dyes (Figure 1-S13, supp. information). The emission is localized in the red (570-590 nm) and is perfectly mirror compared with the absorption. Remarkably, the hexyl-derivative **4a** revealed an optimal fluorescence quantum yield (> 0.95) in CH₂Cl₂. Similarly, the tetrasulfate sodium salt **6** exhibited a quantum yield of 0.91 in MeOH that is almost maintained in pure water (0.81). Thus, the quantum

yields of the bis-triazolylo BODIPYs family are excellent especially for red-emitting chromophores, independently of the nature of the alkyl chain on the triazole moieties and of the solvent.

Last, rather long fluorescence lifetimes were measured with τ values of about 7 ns. These exceptional photophysical properties account for a very rigid system exhibiting a very little loss of energy *via* non radiative pathway. This last point is confirmed by low non radiative kinetic constants compared with the radiative ones (Table 1). The rigidity of the molecules can be explained by i) the triazole rings, less bulky than the phenyl analogues often used that allow a better delocalization¹⁰ and ii) the formation of intramolecular CH...F interactions (*vide infra*).¹¹



Scheme 2 Access to the highly water soluble dye **6**. *Reagents and conditions:* (i) K₂O₅(OH)₂(H₂O)₂, NMO, Me₂CO/*t*BuOH/H₂O, rt, 12 hr; (ii) 1) TEA.SO₃, anhydrous DMF, 12 hr, rt, 2) NaOH, 0°C.

Table 1 Photophysical properties of the dyes in various solvents

Solvent	λ_{\max} (nm)	ϵ (L.mol ⁻¹ .cm ⁻¹)	λ_{em} (nm)	ϕ^a	τ^b (ns)	k_R^c (10 ⁶ s ⁻¹)	k_{NR}^b (10 ⁶ s ⁻¹)	
4a	CH ₂ Cl ₂	583	67,000	598	>0.95	6.9	138	7.2
6	MeOH	576	64,000	595	0.93	7.8	119	8.9
6	H ₂ O	569	61,000	590	0.81	6.9	117	27.5

[a] Standard used for quantum yield measurements: cresyl violet ($\phi = 0.55$ in MeOH).

[b] NanoLED excitation at 490 nm. [c] with $k_R = \phi/\tau$ and $k_{NR} = (1-\phi)/\tau$.

The formation of intramolecular CH...F interactions was ascertained on the basis of the ¹³C NMR analyses (Figure 1). Indeed, the triazole CH signals appeared as a triplet with a coupling constant of $J^{TS} = 11.3$ Hz. This high "Through Space" coupling constant is similar to that measured by Burgess *et al.*¹² with "blocked" benzannulated BODIPY dyes ($J^{TS} = 12$ Hz) and higher than values measured with rather planar systems like thiophene.¹⁰ Hence, the two triazole moieties act synergistically to stiffen the BF₂ complex *via* strong intramolecular CH...F interactions. Consequently the free rotation of the triazole is forbidden limiting non radiative desexcitations.

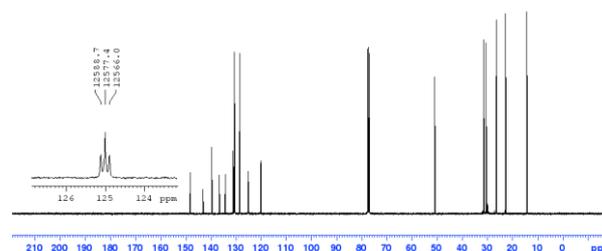


Fig. 1 ¹³C NMR spectrum of **4a** revealing the B-F...H-C a "Through Space" coupling of 11.3 Hz.

Definitive proof for this hypothesis was brought by single-crystal X-ray diffraction characterizations. Single-crystals suitable for X-ray diffraction analyses were obtained by slow diffusion of pentane in a concentrated solution of **4a** in CHCl₃ (Figure 2). **4a** crystallizes in the monoclinic system (P2₁/c centrosymmetric space group).¹³ The X-ray crystal structure reveals a planar dipyrromethene (DPM) subunit with the triazoles lying approximately in the same plane (tilt angle = -2.8° and +6.4°). The *meso*-phenyl substituent displays an angle of 49.1° vs the main DPM plane and the alkyl chains adopt the expected symmetrical zigzag conformations. The boron atom sits in the main plane with the fluorine atoms respectively on top and below. As expected after the NMR analyses, the CH of the triazoles point inward allowing stabilizing B-F...H interactions with B-F...H distances ranging from 2.287(2) Å to 2.571(3) Å.

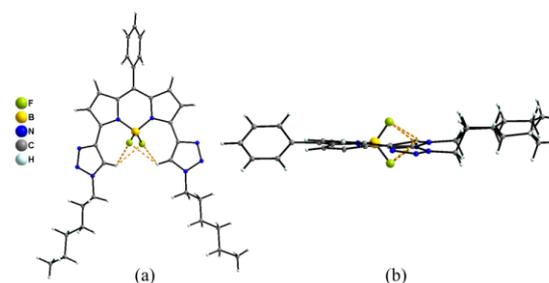


Fig. 2 (a) Crystal structure of **4a** revealing (b) the planar Triazole-BODIPY substructure and short B-F...H distances (orange dashed lines).

Next, the triazole-functionalized BODIPY dyes **4a** and **6** were evaluated for cell imaging. HeLa cells (human fibroblasts) were chosen as models. In DMEM (Dulbecco's Modified Eagle Medium), **4a** displayed a very efficient penetration as an equilibrium was observed after 10 min with a 10 nM solution of **4a**. In the same conditions, 15 min were necessary for the penetration of **6**, with an initial concentration of 10 μ M. Interestingly, both dyes displayed very little cytotoxicity, as no cell death was evidenced even with concentrations as high as 1 mol.L⁻¹ for **6**. Thanks to the easy cell penetration of the dye and the highly fluorescent character of the bis-triazolylo-BODIPY dyes, very good quality images could be obtained after incubation. Two representative views highlight the high fluorescence observed (Figure 3). Both **4a** and **6** displayed a cytoplasmic localization, with a diffuse signal in the case of **4a**, while perinuclear punctuate spots were observed with **6**. It should be noted that the optimal signal for **6** was observed with concentrations 3 orders of magnitude higher than those used for **4a**, most probably due to the highly lipophobic nature of **6**.

In conclusion, we have developed the efficient synthesis of unprecedented bis-triazolyle BODIPY dyes. Straightforward access to both lipophilic and highly hydrophilic systems is described. The simultaneous presence of the two symmetrical triazole substituents allows for the establishment of B-F...H interactions that help for stiffening of the system. Consequently, the non-radiative dissipation pathways are strongly limited and very high quantum yields were obtained. This high brightness conjugated with a low cytotoxicity allowed for cell imaging. After a short incubation time, good quality images could be collected stressing the promising interest of these new BODIPY derivatives for bio-imaging applications. Work is currently underway in our Laboratory for the development of a functionalized version of the dye.

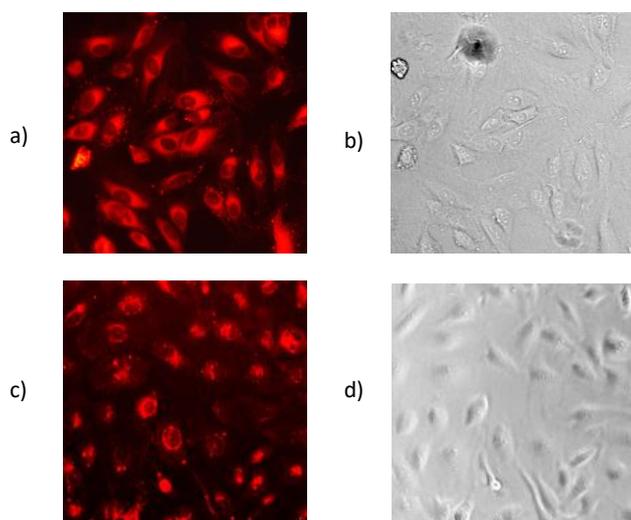


Fig. 3 Epifluorescence images of HeLa cells incubated for 15 min with 50 nM of **4a** in DMEM + DMSO 1%: a) Fluorescence signal in the red channel b) Bright field. Images of HeLa cells incubated for 15 min with 50 μ M of **6** in DMEM: c) Fluorescence signal in the red channel d) Bright field.

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- CCDC 1405386; Empirical formula: C₃₁H₃₇BF₂N₈; molecular weight = 570.5 g.mol⁻¹; crystal system: monoclinic; space group: P2₁/c; a = 11.183(1) Å; b = 9.737(1) Å; c = 27.817(4) Å; β = 94.45(1)°; V = 3019.5(6) Å³; crystal description: plate; crystal color: red; crystal size: 0.099×0.279×0.331 mm³; Z = 4; T = 293 K; d = 1.255; μ = 0.086 mm⁻¹; Number of independent reflections: 7122; R_{int} = 0.087; R(F) = 0.0782; R_w(F) = 0.0839; S = 1.08; $\Delta\rho_{\min}$ = -0.40 e⁻.Å⁻³; $\Delta\rho_{\max}$ = +0.63 e⁻.Å⁻³; Number of reflections used: 2901; Number of refined parameters: 379; absorption correction: analytical.