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

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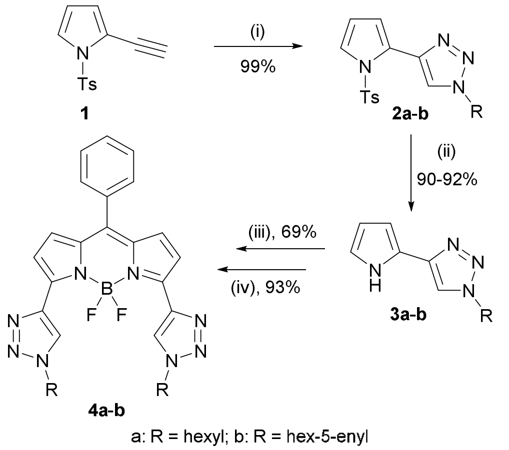
The versatile synthesis of an unprecedented bis-triazolyl BODIPY dye displaying a high quantum yield even in water is described. X-Ray diffraction analyses and NMR measurements demonstrate that the simultaneous presence of the two triazole moieties helps re-enforcing the rigidity of the BF_2 complex.

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-dipyrrins (BODIPY) 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 versatile 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'-diaryl dipyrrins,[5] we have been interested in the preparation of 5,5'-bis-triazolyl dipyrrins which extended π-conjugated system could result in some promising photophysical properties. Interestingly, 5,5'-bis-triazolyl dipyrrins had not been reported to date, even if the monotriazolyl analogues had already been claimed very useful for Live Neuron or cell Imaging.[6]

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

As terminal-(bis)-α-ethynyl dipyrrins appeared unstable in our reaction conditions, we envisioned the synthesis of 5,5'-bis-triazolyl dipyrrins starting from the pyrrole-triazole building block **2**. This synthon was readily prepared from 2-ethynyl-1-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 NEt_4Br as a phase transfer agent. The corresponding pyrrole-triazole moiety **3** was subsequently reacted with benzaldehyde using the Lindsey's conditions[9] to afford the expected dipyrrins 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) RN_3, $CuSO_4$,-$5H_2O$, Na ascorbate, $PhCO_2H$, tBuOH/H_2O, rt, 30 min; (ii) 5M $NaOH_{aq}$, NEt_4Br, dioxane, reflux, 40 hr; (iii) 1) PhCHO, BF_3-Et_2O, anhydrous CH_2Cl_2, rt, 2 hr, 2) DDQ, 2 hr, rt; (iv) $BF_3.Et_2O$, DIPEA, anhydrous CH_2Cl_2, rt, 2 hr.

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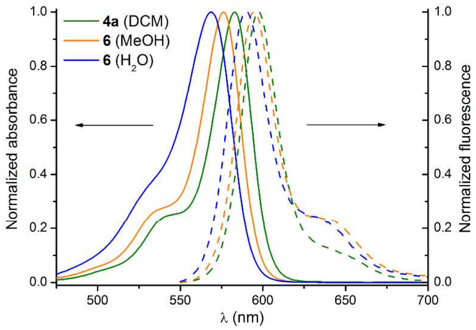
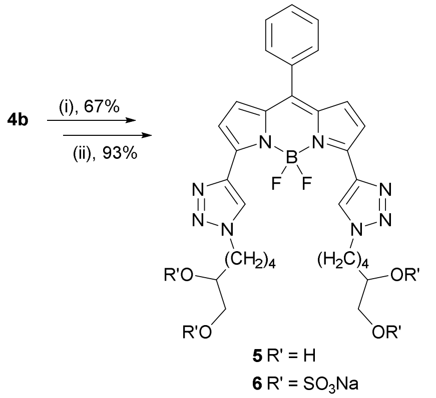


Fig. 1 Normalized absorption (plain line) and normalized emission (dotted line) spectra for BODIPYs **4a** and **6**.

Eventually, the targeted boron complex was isolated in 93% yield after reaction with $BF_3.Et_2O$ using *N*,*N*-diisopropylethylamine (DIPEA) as a proton sponge (Scheme 1). Overall, **4a-b** could be isolated in four steps with 58% overall yield from the 2-ethynyl-1-tosyl-1H-pyrrole **1** building block.

Aiming at developing water soluble adducts, the diene **4b** was further functionalized by reaction with $K_2Os(OH)_2(H_2O)_2$/NMO to afford the expected tetraol **5** in 67% yield (Scheme 2). The solubility of **5** in water still being poor, it was converted to the corresponding tetrasulfate sodium salt **6** in 93% yield using $TEA.SO_3$ and NaOH in DMF (Scheme 2). The water solubility of **6** exceeds 10 $mmol.L^{-1}$.

Next the photophysical properties of the BODIPY dyes were investigated (Table 1). The chromophores display sharp and intense absorption transition ($\varepsilon > 60{,}000$ $L.mol^{-1}.cm^{-1}$) centered in the orange-red spectral range with the typical shape of BODIPY dyes (Figure 1 + ESI). The emission is localized in the red (570-590 nm) and is perfectly mirror compared with the absorption. Remarkably, the hexyl- or hex-5-enyl derivative **4** revealed an optimal fluorescence quantum yield (> 0.95) in CH_2Cl_2. Tetraol **5** being not soluble in water, the photophysical measurements were determined in MeOH and revealed a quantum yield of 0.86. Similarly, the tetrasulfate sodium salt **6** exhibited a quantum yield of 0.91 in MeOH that is almost maintained in pure water (0.81).



Scheme 2 Access to the highly water soluble dye **6**. *Reagents and conditions*: (i) $K_2Os(OH)_2(H_2O)_2$, NMO, Me_2CO/*t*BuOH/H_2O, rt, 12 hr; (ii) 1) $TEA.SO_3$, anhydrous DMF, rt, 12 hr; 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} [c] (10^6 s^{-1})
4a	CH_2Cl_2	583	67000	598	>0.95	6.9	138	7.2
4b	CH_2Cl_2	583	83900	596	>0.95	6.5	146	7.7
5	MeOH	575	72700	590	0.86	6.8	126	20.6
6	MeOH	576	64000	595	0.93	7.8	119	8.9
6	H_2O	569	61000	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 $k_{NR}=(1-\phi)/\tau$.

Thus, the quantum yields of the bis-triazolyl 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.[10] 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 moieties, a five-membered ring resembling thiophene that was proved to allow a better electronic delocalization[11] than the phenyl analogues because of a smaller steric hindrance and a more planar structure and ii) the formation of stronger intramolecular CH…F interactions (*vide infra*).[12,13]

The close proximity of the triazole **C**H and the BF_2 residue was ascertained on the basis of the ^{13}C NMR analyses (Figure 2). Indeed, the triazole **C**H 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.*[14] with "blocked" benzannulated BODIPY dyes (J^{TS} = 12 Hz) and higher than values measured with rather planar systems like thiophene.[11] Hence, the two triazole moieties act synergistically to rigidify the BF_2 complex *via* strong intramolecular CH…F interactions Consequently the free rotation of the triazole is forbidden limiting non radiative desexcitations.

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 $CHCl_3$ (Figure 3).

4a crystallizes in the monoclinic system ($P2_1/c$ centrosymmetric space group).[15] The X-ray crystal structure reveals a planar dipyrrin (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 dipyrrin 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···HC

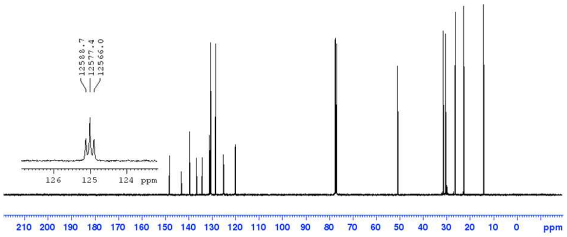


Fig. 2 ^{13}C NMR spectrum of **4a** revealing the B-F···H-C a "Through Space" coupling of 11.3 Hz.

interactions with B-F···H distances ranging from 2.287(2) Å to 2.571(3) Å.

Next, two representative (lipophilic *vs* hydrophilic dyes) 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 µM. Interestingly, both dyes displayed very little cytotoxicity, after 1 h incubation at concentrations used for the staining experiment (ESI Fig. S1). Thanks to the easy cell penetration and the highly fluorescent character of the bis-triazolyl-BODIPY dyes, high quality images could be obtained after incubation. Two representative views highlight the high fluorescence observed (Figure 4). 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 hydrophilic nature of **6**.

In conclusion, we have developed the efficient synthesis of unprecedented bis-triazolyl 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 rigidifying 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.

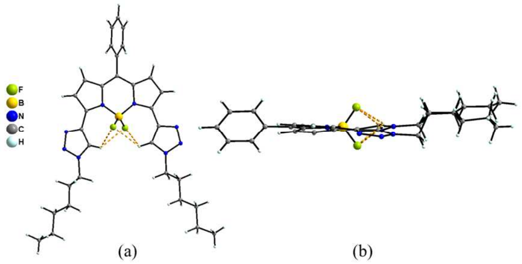


Fig. 3 (a) Crystal structure of **4a** revealing (b) the planar Triazole-BODIPY sub-structure and short B-F···H distances (orange dashed lines).

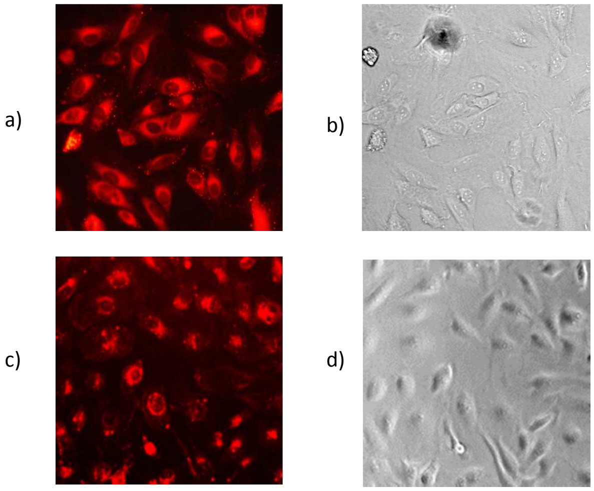


Fig. 4 Epifluorescence images of HeLa cells incubated for 15 min with 50 nM of **4a** in DMEM + DMSO 1%: a) Fluorescence signal in the red channel b) Bright field. Images of HeLa cells incubated for 15 min with 50 µM of **6** in DMEM: c) Fluorescence signal in the red channel d) Bright field.

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15 CCDC 1405386; Empirical formula: $C_{31}H_{37}BF_2N_8$; molecular weight = 570.5 g.mol^{-1}; crystal system: monoclinic; space group: P2_1/c; a = 11.183(1) Å; b = 9.737(1) Å; c = 27.817(4) Å; β = 94.45(1)°; V = 3019.5(6) Å^3; crystal description: plate; crystal color: red; crystal size: 0.099×0.279×0.331 mm^3; 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.