

# Development of molecular cassettes for the excitation energy transfer in the red region of the spectrum

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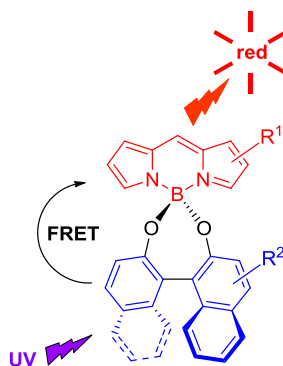
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## ABSTRACT

The development of multichromophoric systems able to achieve efficiently excitation energy transfer (EET) from donors to covalently linked acceptor chromophores (i.e., molecular cassettes) has gained great interest in the last few years, since they are extremely useful in the development of advanced materials for outstanding photonic technologies (including optoelectronics), such as solar harvesting, fluorescence microscopy or biomolecular probing.

On the other hand, the emission at longer wavelengths has important applications in Medicine, and thus it is called “the biological or therapeutic window”: In the 650-1000 nm region autofluorescence, and absorption by water, tissues and cells are minimized, and there is less light scattering. This means that deeper penetration by incident laser light can be achieved.

In our group we have introduced a new design for the achievement of energy transfer in molecular cassettes. This design is based on spiranic *O*-BODIPY having a polyarene as the donor moiety. Based on this new design, we have developed a series of *O*-BODIPY dyes showing cassette behavior and emitting in the red region of the spectrum.



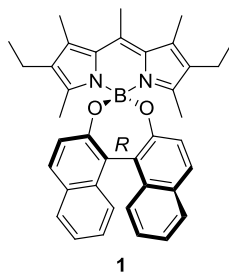
## INTRODUCTION

The development of multichromophoric systems able to achieve efficiently excitation energy transfer (EET) from donors to covalently linked acceptor chromophores (i.e., molecular cassettes) has gained a great interest in the last few years, since they are extremely useful in the development of advanced materials for outstanding photonic technologies (including optoelectronics), such as solar harvesting, fluorescence microscopy or biomolecular probing.<sup>1</sup>

4,4-Difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPYs) constitute a recognized family of fluorophores with noticeable utility in the development of photonic tools.<sup>2</sup> This is due to the excellent photophysical properties of the BODIPY chromophore, high solubility in organic-solvent systems improving dyed-material processability (e.g., in the preparation of organic films), and possibility of selective functionalization to finely modulate the dye physical properties.<sup>2a-c</sup> The usually large molar-absorption coefficients ( $\epsilon$ ) and high fluorescence quantum yields ( $\phi$ ) of BODIPYs have promoted their application as fluorescent dyes for bioimaging,<sup>3</sup> chemosensing<sup>2d,4</sup> and lasing,<sup>5</sup> among other interesting applications.<sup>2,4a</sup> However, the small Stokes shifts (around  $600\text{ cm}^{-1}$ ) of these dyes may cause re-absorption of the emitted light or effects from excitation-light scattering, which are important limits in the mentioned applications, mainly in advanced bioimaging technologies based on multicolor labeling.<sup>6</sup> This handicap can be solved by developing efficient energy-transfer cassettes with large pseudo-Stokes shifts, based on BODIPY as the acceptor chromophore.<sup>2f,3c,d,f,4b,c,5a,b</sup> However, a fine molecular engineering is needed for it, since the involved chromophores (BODIPY and donor/s) must be electronically isolated (non-conjugated), and photophysically and structurally proper to achieve an EET mechanism.<sup>7</sup> Thus, orbital overlap is needed for the Dexter mechanism (electronic exchange by short-range interaction),<sup>8</sup> whereas rigid and twisted unsaturated linkers are demanded for the through-bond energy transfer (TBET, which seems to involve electronic exchange as well).<sup>3c,4c</sup> On the other hand, Förster resonance energy transfer (FRET), also known as through-space, requires short distances, spectral overlap of donor emission and

acceptor absorption, and adequate orientation on the involved transition dipole moments to enable dipole-dipole coupling (long-range interaction).<sup>9,4c</sup>

In our group we have introduced a new design for the achievement of excitation energy transfer in molecular cassettes. This design is based on a spiranic *O*-BODIPY, which involves the boron as the spiranic shared atom, and has a polyarene as the donor moiety (Figure 1). The goal of this simple design is to keep the conformational motion of the involved donor/acceptor chromophores restricted, and tightly fixed in an almost orthogonal arrangement to ensure an efficient EET via FRET. Besides, the straightforward synthetic access to these *O*-BODIPY cassettes from BODIPYs and donor-based diols assures an excellent potential for developing future smarter BODIPY cassettes for valuable fluorescence applications (bioimaging, lasing, chemosensing, optoelectronics, etc.).<sup>10</sup>



$\lambda_{\text{exc}} = 250 \text{ nm}$ ;  $\lambda_{\text{flu}} = 546 \text{ nm}$ ;  $\phi = 89\%$ ;  
 $\Delta\nu_{\text{St}} = 26130 \text{ nm}$ ; EET ca. 100%  
(hexane)

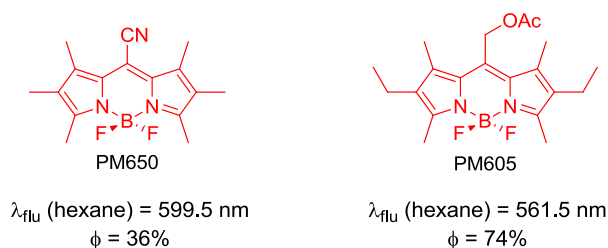
**Figure 1.** Example of molecular cassette for EET developed by de la Moya and col.

On the other hand, the organic chromophores with fluorescence bands in the Vis-red/near-IR region have applications in optical techniques for imaging, in microarrays, and in electrophoresis, and as labels and optical sensors for biological and medical applications.<sup>11,12</sup> For the latter, it is advantageous to work in the red/NIR region of the spectrum because of the so-called biological window in the 650–1000 nm region, where autofluorescence, and absorption by water, tissues and cells are minimized, and there is less light scattering. This means that deeper penetration by incident laser light (2–5 cm) can be achieved in what is sometimes also described as the therapeutic window.<sup>13</sup>

Given the interest of fluorescence emission in the red region, we decided to develop molecular cassettes for the EET with emission in this region of the spectrum, using our recently introduced spiranic-BODIPY design.

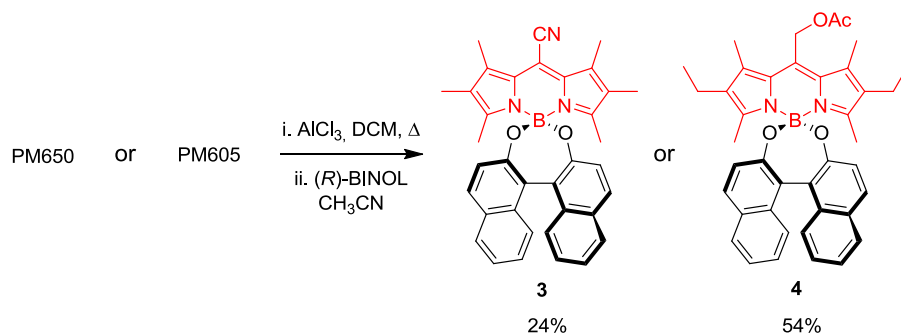
## RESULTS

Our approach for the development of molecular cassettes with red emission was to apply our reported design to commercial *F*-BODIPYs with emission at high wavelengths. Thus, we took commercial dyes PM650 and PM605, with emission maxima at  $\lambda = 599.5$  and 561.5 nm, respectively, as starting materials (Figure 2).



**Figure 2.** Starting *F*-BODIPYs.

The synthesis of *O*-BODIPYs **3** and **4** was accomplished following the procedure described for the synthesis of **1**, that is, substitution of the fluorine atoms by the *O*-binaphthyl unit of BINOL, activated by  $\text{AlCl}_3$  (Scheme 1).



**Scheme 1.** Synthesis of *O*-BODIPYs **3** and **4** from PM650 or PM605, respectively, and (*R*)-BINOL.

Before studying the potential cassette behavior of **3** and **4**, the photophysical properties of the new dyes were measured. Unfortunately, as it can be seen from Table 1, the fluorescence of the new *O*-BODIPY dyes dramatically decreased when compared to the starting *F*-BODIPYs. In the case of the cyano-derived **3**, we didn't even detect any fluorescence signal. We also observed that the fluorescence was lower in polar than in apolar solvents for the compound **4** derived from PM605 (acetone, see Table 1). We attribute this fluorescence deactivation to the participation of an intramolecular charge transfer (ICT) state, which would be more stabilized in polar solvents and reinforced by the replacement of fluorine atoms by *O*-binaphthyl groups. The origin of this effect lies in the higher electron-donor (ED) character of the *O*-binaphthyl moiety (when compared to fluorine), together with the electron-withdrawing (EW) character of the *meso* group in the BODIPY chromophore. This deactivation pathway is more important in **3**, due to the stronger EW character of the cyano group. In fact, the ICT must exist in *F*-BODIPY PM650, as well, as deduced from its low fluorescence quantum yield (0.36 in *n*-hexane) and the fact that it is lower in polar solvents (see Table 1).

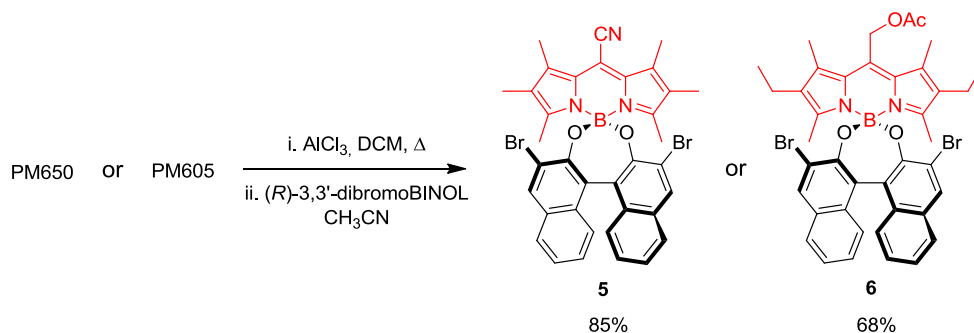
**Table 1.** Photophysical properties of new *O*-BODIPYs **3-6**. Starting PM650 and PM605 are included for comparison.

BODIPY	Solvent	$\lambda_{\text{abs}}$ (nm)	$\epsilon_{\text{max}} \cdot 10^{-4}$ ( $\text{M}^{-1} \cdot \text{cm}^{-1}$ )	$\lambda_{\text{flu}}$ (nm)	$\Delta\nu_{\text{St}}$ ( $\text{cm}^{-1}$ )	$\phi$
PM650	<i>c</i> -hexane	589.5	5.3	599.5	285	0.36
	acetone	588.0	3.5	606.0	505	0.11
<b>3</b>	<i>n</i> -hexane	587.0	3.6	*	*	*
	acetone	587.5	3.1	*	*	*
<b>5</b>	<i>n</i> -hexane	589.5	4.9	603.5	395	0.25
	acetone	591.0	4.4	619.0	765	0.02
PM605	<i>n</i> -hexane	547.5	8.3	561.5	455	0.74
	acetone	542.0	7.1	559.0	560	0.70
<b>4</b>	<i>n</i> -hexane	549.5	5.6	579.5	940	0.13
	acetone	545.5	5.3	563.5	585	0.011
<b>6</b>	<i>n</i> -hexane	552.5	6.1	575.0	710	0.60
	acetone	549.0	6.0	572.0	730	0.45

\* No signal was detected.

With the objective of increasing the fluorescence of the *O*-BODIPY dyes, our strategy to cut the ICT or, at least decrease its fluorescence quenching, was to reduce the ED

character of the BINOL moiety. To that end, we decided to introduce an EW group in said unit. Therefore, we prepared two new spiranic *O*-BODIPYs, **5** and **6**, having an EW bromine attached to the BINOL, by reaction of the same starting *F*-BODIPYs (PM650 and PM605) with (*R*)-3,3'-dibromo-1,1'-bi(2-naphthol) [(*R*)-3,3'-dibromoBINOL], as depicted in Scheme 2.

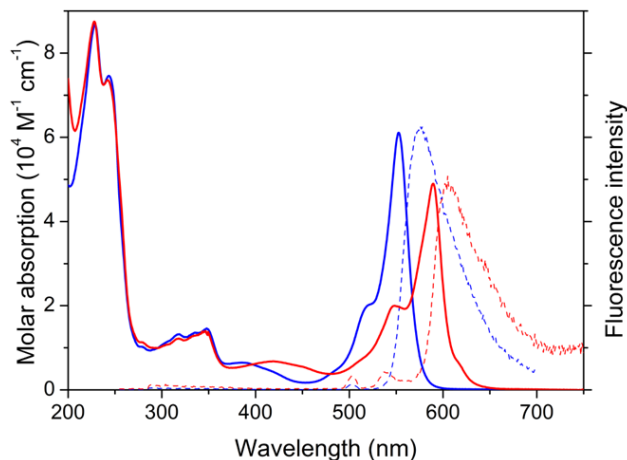


**Scheme 2.** Synthesis of *O*-BODIPYs **5** and **6** from PM650 or PM605, respectively, and (*R*)-3,3'-dibromoBINOL.

We were glad to observe that, as we had predicted, the fluorescence quantum yields of dyes **5** and **6** almost recuperated the values from parent dyes PM650 and PM605, respectively, in apolar hexane (albeit they were still lower in polar acetone, see Table 1). Given this good result in fluorescence, we proceeded to study their cassette behavior (Table 2 and Figure 3). Thus, when the binaphthyl chromophore of **5** was irradiated (*n*-hexane, 250 nm), fluorescence emission was observed from its BODIPY chromophore, with the same  $\phi$  as the obtained from direct excitation (see table 1 and 2). An analogous behavior was observed from **6**. This implies an EET with an efficiency of *ca.* 100%, having fluorescent emission at ~600 nm.

**Table 2.** Cassete behavior of **5** and **6** in hexane.

BODIPY	$\lambda_{\text{abs}}$ (nm)	$\lambda_{\text{flu}}$ (nm)	$\phi$	$\Delta\nu_{\text{St}}$ (cm <sup>-1</sup> )	ETT (%)
<b>5</b>	570	604	0.25	395	-
	250	604	0.25	27900	100
<b>6</b>	510	575	0.60	710	-
	250	575	0.60	27050	100



**Figure 3.** Absorption spectra (bold) and fluorescence spectra (dashed) of **5** (red) and **6** (blue) upon UV irradiation (250 nm).

## CONCLUSION

Two new molecular cassettes for EET have been synthesized, following the spiranic *O*-BODIPY structure introduced previously by us. These BODIPYs show high efficient EET (*ca.* 100%) with fluorescence emission towards the red region of the spectrum (*ca.* 600 nm). The new dyes contain a BODIPY unit with an EW group at *meso* position, as the acceptor chromophore with an emission in the red region of the spectrum, and an *O*-binaphthyl unit as the donor chromophore, in which an EW group has been introduced to minimize deactivation of the fluorescence by ICT. These red-emitting dyes are promising for the development of future generations of smarter BODIPY cassettes with emission in the Vis-red / NIR region of the spectrum.

## ACKNOWLEDGEMENTS

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