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Tailoring the Photophysical Signatures of BODIPY Dyes: Toward Fluorescence Standards across the Visible Spectral Region

Rebeca Sola Llano, Edurne Avellanal Zaballa,
Jorge Bañuelos,
César Fernando Azael Gómez Durán,
José Luis Belmonte Vázquez,
Eduardo Peña Cabrera and Iñigo López Arbeloa

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.74848>

Abstract

The modern synthetic routes in organic chemistry, as well as the recent advances in high-resolution spectroscopic and microscopic techniques, have awakened a renewable interest in the development of organic fluorophores. In this regard, boron-dipyrroin (BODIPY) dyes are ranked at the top position as luminophores to be applied in photonics or biophotonics. This chromophore outstands not only by its excellent and tunable photophysical signatures, but also by the chemical versatility of its core, which is readily available to a myriad of functionalization routes. In this chapter, we show that, after a rational design, bright and photostable BODIPYs can be achieved along the whole visible spectral region, being suitable as molecular probes or active media of lasers. Alternatively, the selective functionalization of the dipyrroin core, mainly at *meso* position, can induce new photophysical phenomena (such as charge transfer) paving the way to the development of fluorescent sensors, where the fluorescent response is sensitive to a specific environmental property.

Keywords: fluorescent probes, laser dyes, BODIPY, organic synthesis, charge transfer, fluorescent sensors

1. Introduction

The recent advances in high-resolution fluorescence microscopy have played a key role in the success and boom of bioimaging techniques in the last few years. Bioimaging is a noninvasive

method to visualize biological processes by means of fluorescence emission in real time. Nowadays, thanks to super resolution microscopy (nanoscopy, awarded with the Nobel Prize of 2014 in chemistry), the diffraction resolution limit is surpassed, allowing the detection at the single molecular level [1–3]. The development of this revolutionary technological tool has awakened a renewed interest in the design of organic luminophores with improved properties as fluorescent probes or markers to detect or monitor biochemical events [4]. These molecules should fulfill some requirements to fit the requests of these advanced techniques. They should be biocompatible and photostable, and outstand by a high light absorption and emission probability, to guarantee long-lasting and bright fluorescence images. Note that these characteristics are almost the same than those required for the active media of tunable dye lasers [5, 6]. In other words, those molecules suitable as fluorescent probes are also valid as laser dyes.

The modern avenues in organic synthesis allow the development of organic fluorophores fulfilling the above requirements as well as with the required functionality to recognize selectively the target biomolecule [7]. Actually, there is a wide pool of commercially available families of organic dyes with absorption/emission bands covering almost the whole ultraviolet/visible spectral region. Among them, boron-dipyrromethene (BODIPY) dyes are likely at the “pole” position, owing to their excellent photophysical signatures [8, 9], but mainly due to the chemical versatility of its boron-dipyrroin core, readily available to a multitude of synthetic routes (**Figure 1**) [10, 11]. As a consequence, the BODIPY dyes can be exhaustively and specifically functionalized with a plethora of substituents [12–14]. At the same time, such substitution pattern rules the photophysical properties of the resulting dye or can induce new photophysical phenomena, such as charge or electron transfer, energy transfer or triplet state population. In other words, after a rational molecular approach, tailor-made BODIPYs can be designed with the specific requirements of any application demanding an organic dye. In fact,

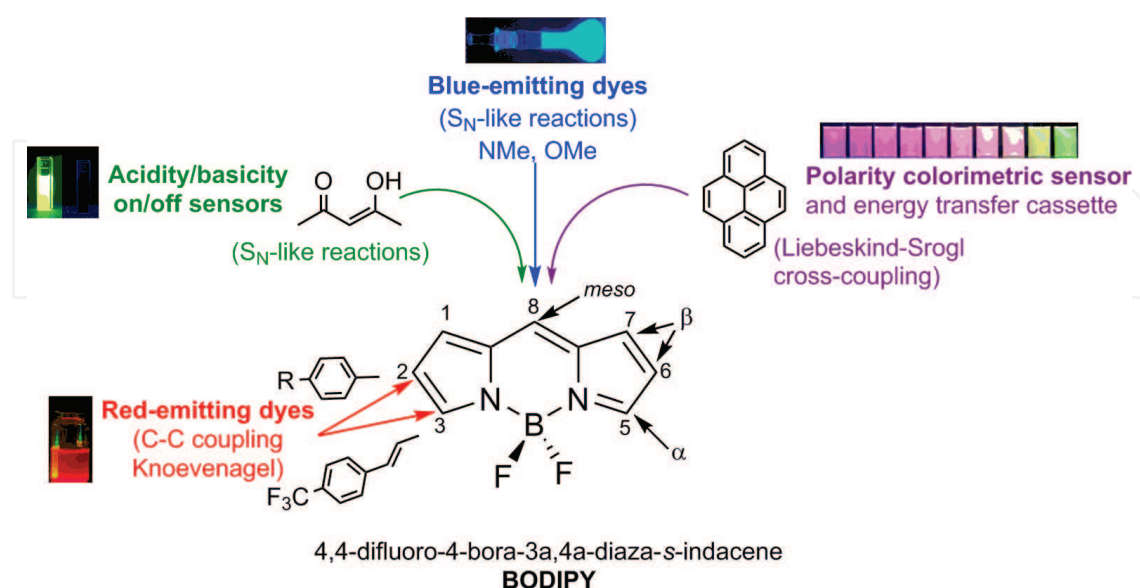


Figure 1. Basic molecular structure of the BODIPY dye. Schematic view of the structural modifications and chemical reactions tested herein.

a fast look at the bibliography reveals the popularity of these dyes since the number of publications dealing with this kind of dyes in many different applications fields is growing unstoppable during the last years. As a matter of fact, BODIPYs are not only applied in bioimaging [15] or as lasers [16], but also in many other scientific areas, such as photovoltaic devices as photosensitizers [17, 18], electrochemistry [19], sensors for recognition at the molecular level [20], or biomedicine in photodynamic therapy for the treatment of cancer [21, 22], just to cite some of the most relevant application fields.

In this chapter, we describe suitable molecular strategies to finely modulate the photophysical properties of BODIPY dyes (**Figure 1**). In the first part, we highlight some structural guidelines to shift the spectral bands to both edges (blue and red) of the visible spectral region, while keeping their characteristic high fluorescent efficiencies. In this way, fluorescent probes and laser dyes based on BODIPY can be attained covering the whole visible spectral region just choosing the adequate substitution pattern at the right chromophoric position. In the second part, we modify the molecular structure to induce new photophysical phenomena (energy and mainly charge transfer). To this aim, we selected the *meso* position since it is highly sensitive to the substituent effect. As a result, the fluorescent efficiency can response selectively to a specific property of the surrounding environment (such as polarity and acidity/basicity) leading to fluorescent sensors [23–25].

2. Modulation of the spectral shift

The structure of the BODIPY core features two pyrroles linked by a methine unit and a difluoroboron bridge (**Figure 2**). These dyes can be classified as polymethine dyes with a cyclic cyanine delocalized π -system leading to a quasi-aromatic backbone since the said boron center does not take part in the electronic delocalization [26]. Owing to symmetric reasons, the molecular dipole moment is oriented along the transversal axis and the negative charge is located around the fluorine atoms, whereas the positive charge around the opposite *meso* position (**Figure 2**). Thus, the chromophore core is fully planar and rigid. Such molecular structure ensures a high radiative deactivation probability, and a low nonradiative deactivation relaxation, since the internal conversion (related with the molecular flexibility), as well as the intersystem crossing probability (related with the spin-orbit coupling feasible in aromatic frameworks where the electron flow makes a loop), are largely truncated [27]. As a consequence, and taking as reference the simplest BODIPY with just hydrogens in the dipyrroin core [28], sharp and strong absorption and fluorescence bands are recorded in the green-yellow part (500–510 nm) of the visible (Vis) spectrum (HOMO \rightarrow LUMO transition with the corresponding dipole moment oriented in the long molecular axis, **Figure 2**), with absorption coefficients approaching $10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and fluorescent efficiencies approaching the 100%, regardless of the solvent [9]. Besides, it shows stable laser emission at 540 nm with efficiencies approaching the 55% [29].

In the following sections, we show the tested strategies to induce deep hypsochromic and bathochromic shifts of the absorption and fluorescence spectral bands, while keeping high

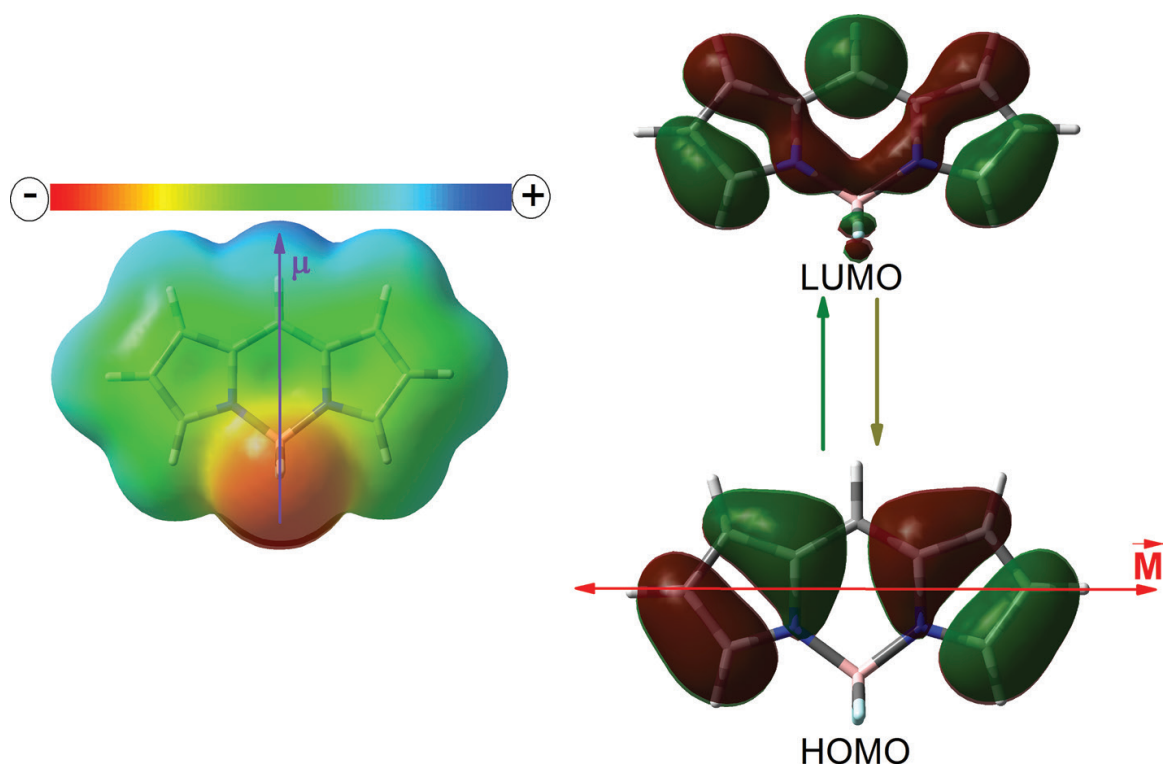


Figure 2. Frontier molecular orbitals and charge distribution by means of the electrostatic potential mapped onto the electronic distribution. The direction of the molecular dipole moment (μ) as well as the transition dipole moment (M) is also depicted.

fluorescent efficiencies (see molecular structures in **Figure 3**). The main aim is to attain fluorophores based on BODIPY with optimal photophysical properties along the visible spectral region, outperforming those commercially available as standard dyes, in terms of their fluorescence and laser performance, in each spectral region.

2.1. Blue-emitting BODIPYs

In the bibliography, some attempts have been made to achieve blue-emitting dyes based on BODIPY, but the core was drastically changed and the fluorescent response was not the best [30, 31]. However, strikingly, the sole incorporation of heteroatoms (nitrogen or oxygen, **Figure 3**) directly linked to the *meso* position led to the searched spectral hypsochromic shift (**Figure 4**) [29]. Such chromophoric position is highly sensitive to the substituent effect since a marked change in the electronic density takes place there upon excitation (note that in the HOMO, there is a node at such position, while it contributes much to the LUMO, **Figure 2**). This kind of 8-heteroatomBODIPYs can be straightforwardly attained after nucleophilic substitution at the 8-methylthioBODIPY precursor (provided by “cuantico” enterprise) [29]. Such starting precursor plays a key role in the chemistry of BODIPY since undergoes organic reactions unavailable until recently and open the door to new functionalization routes [32]. Thus, amines and alcohols can react specifically at *meso* position via nucleophilic substitution. The recorded spectral bands show that the hypsochromic shift depends on the electronegativity

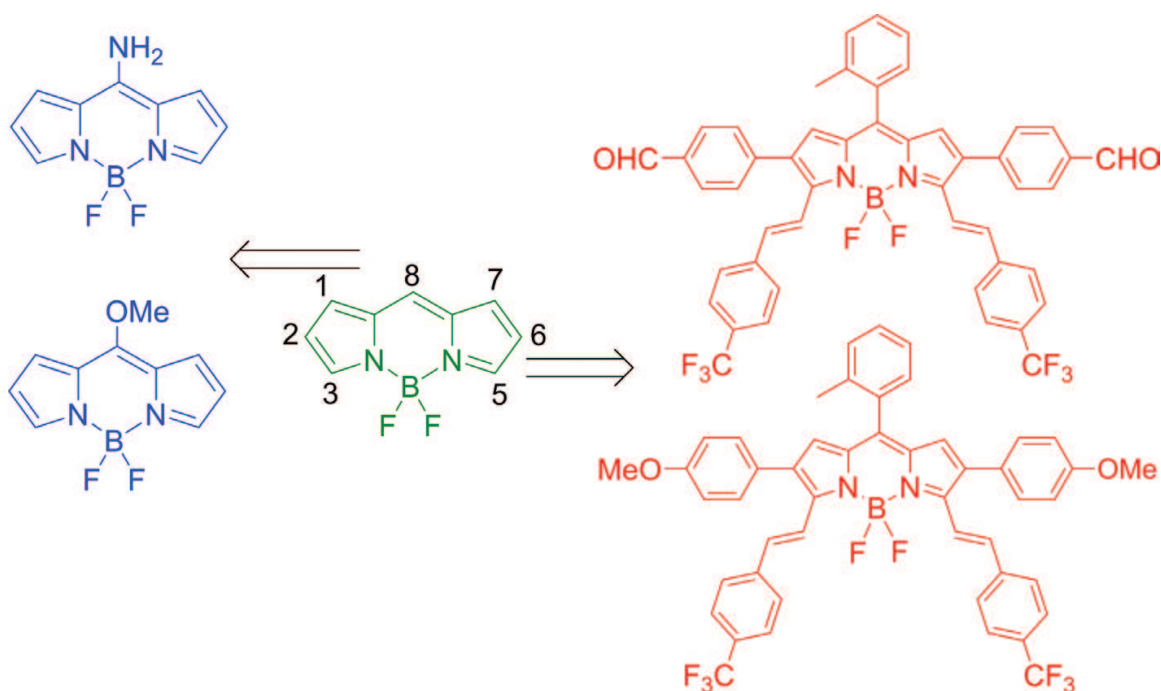


Figure 3. Molecular structure of the reference BODIPY dye and its blue-emitting (8-heteroatoms) and red-emitting (π -extended) derivatives.

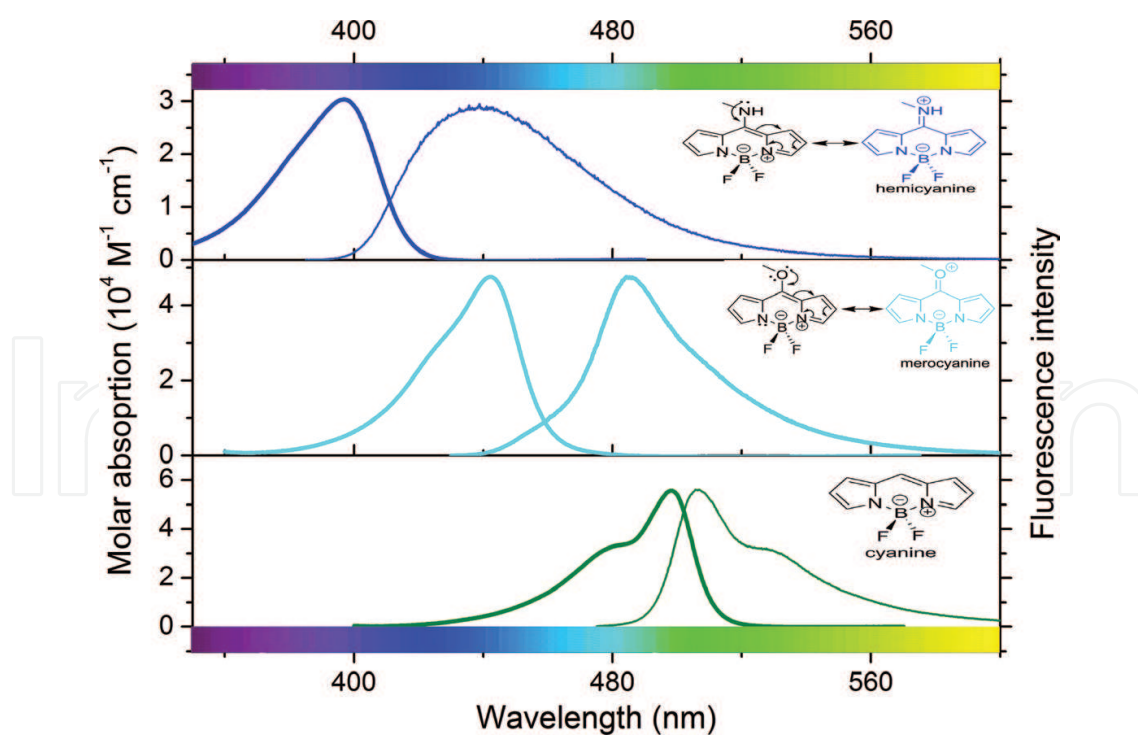


Figure 4. Absorption (bold line) and normalized fluorescence (thin line) spectra of the simplest BODIPY and its derivatives bearing methylamine and methoxy at *meso* position in a common solvent (ethanol). The main resonant structures resulting from the electronic coupling between the 8-heteroatoms and the dipyrin core are also enclosed.

(or the electron releasing ability) of the attached heteroatom. Thus, while oxygen induces a moderate spectral shift (around 50 nm in absorption and 25 nm in fluorescence with regard to the reference simplest BODIPY), the nitrogen provokes a much more marked spectral shift (90 and 50 nm, respectively) placing the absorption/emission bands of the BODIPY in the blue-edge (420 and 460 nm, respectively) of the visible spectrum (**Figure 4**). Quantum mechanics calculations anticipate a hypsochromic shift upon the presence of heteroatoms, since their electron donor character raises the energy of the LUMO, increasing the energy gap. However, the theoretically predicted shift is much lower than the experimentally recorded, suggesting that additional processes are taking place. Indeed, an electronic coupling between the heteroatom and one chromophoric pyrrole is feasible. Such resonant interaction seems to prevail mainly in the ground state and implies a reorganization of the electronic density leading to the stabilization of new delocalized π -systems, alternatively to the expected cyanine one; a hemicyanine and merocyanine, formed after electron coupling of the electron donor amine and methoxy, respectively (**Figure 4**). Thus, the hemicyanine is the responsible of the blue absorption, whereas the merocyanine of the greener one, owing to the lower electronegativity (more electron donor ability) of the nitrogen atom.

The BODIPYs bearing 8-methoxy are highly fluorescent regardless of the properties of the surrounding environment (**Figure 5**). In contrast, the fluorescent response of the 8-aminated analogs depends on the solvent polarity, being much lower in polar media. Such fluorescence quenching (from 70% in apolar to 15% in polar solvents, **Figure 5**) suggests that non-fluorescence intramolecular charge transfer (ICT) phenomena are switched on. The highly electron donor amine pushed the spectral bands toward the blue edge; however, such electron releasing ability can induce also the formation of an ICT state where the dipyrroin acts an electron acceptor. Note that the electron donor ability of the methoxy seems not high enough

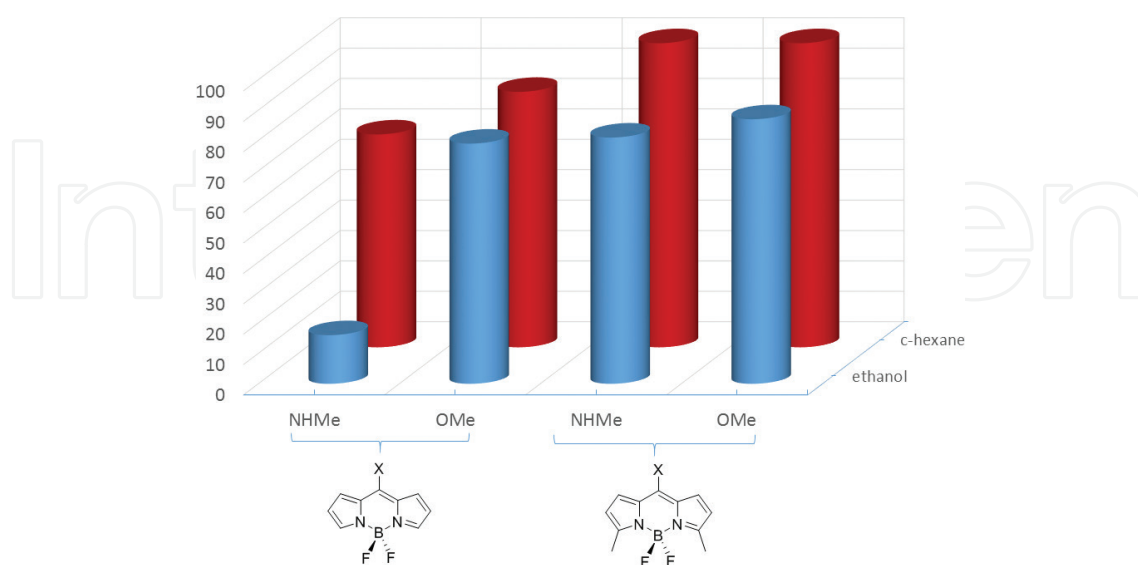


Figure 5. Fluorescent efficiencies of the BODIPYs bearing methylamine and methoxy at *meso* position in apolar (cyclohexane) and polar (ethanol) solvents. The corresponding data for their dimethylated derivatives at 3,5-positions are also included to highlight the ability of this alkylation to regulate the fluorescent response.

as to activate ICT since the fluorescent efficiency is always higher than 80% (**Figure 5**). Such ICT hampers the behavior of 8-aminoBODIPYs as fluorescent probe or laser dye. However, the simple methylation at key positions 3 and 5, hinders the population of the fluorescence quenching ICT and high fluorescent responses are recovered regardless of the media (always higher than 80%, and even approaching the 100% in apolar media, **Figure 5**) [29]. Such selective alkylation decreases the electron acceptor character of the BODIPY and reduces the probability of formation of ICT, allowing the recovering of a high fluorescent efficiency.

As a result, the laser performance of such dimethylated 8-heteroatomBODIPYs is excellent, not only in terms of efficiency but also in photostability [29]. We should bear in mind that one of the main drawbacks of commercially available blue-emitting dyes (such as coumarins, considered as the benchmark in this spectral region) is their low photostability under prolonged irradiation. The herein reported blue BODIPYs surpass the laser performance of these dyes maintaining the 45% and 70% of the laser emission after 40,000 pulses for the dyes bearing amine or methoxy, respectively, and reaching efficiencies up to 30% at 455 nm and 50% at 490 nm, respectively.

2.2. Red-emitting BODIPYs

The development of red-emitting BODIPYs is an active seeking subject. Fluorophores working in this spectral region are highly desirable and appealing for bioimaging purposes, owing to the ability of this kind of irradiation to penetrate deeper into tissues [4, 15]. Besides, the background autofluorescence of biomolecules (usually blue or green) is avoided, which is reflected in a more sensitive detection process. Most of the available dyes in the red spectral region are poorly fluorescent or has a low photostability. Thus, and in analogy to the strategy carried out in the preceding section, there is a great interest to shift the emission of BODIPYs to the red-edge, and thereby to circumvent in this way such photonic limitations taking advantage of the brightness and robustness of BODIPYs. In the bibliography, four major strategies have been reported to get the desired bathochromic shift [33]: (1) replacement of the central carbon at *meso* position by an aza group (aza-BODIPYs) [34]; (2) arylation of the dipyrroin core (fused BODIPYs) [35]; (3) extension of the aromatic π -system through aromatic framework; and (4) attachment of electron rich groups.

In this section, we have chosen the combination of the last two options as the strategy to develop bright and photostable red-emitting BODIPYs (**Figure 3**). To this aim, the aforementioned versatile 8-thiomethylBODIPY was taken again as scaffold. In this way, specific functionalization was added with orthogonal selectivity, which means that we can modulate the substitution patterns at positions 8, 2 and 6, and 3 and 5 independently, using non-interfering synthetic routes specific for each functionalization (nucleophilic substitution at *meso* position, Liebeskind-Srogl cross-coupling (LSCC) from easily accessible 2,6-halogenated compounds, and Knoevenagel condensation, from methylated 3,5-positions) [36]. By this synthetic approach, the dipyrroin core was decorated with a sterically hindered *ortho*-methylphenyl at *meso* position, a styryl arm bearing *para*-trifluoromethyl at positions 3 and 5, and finally a *para* substituted (with electron donor methoxy or withdrawing formyl) phenyl ring at positions 2 and 6 (**Figure 3**).

These fully functionalized dyes show absorption and emission bands placed deeply in the red/near infrared spectral window (absorption centered at around 660 nm, whereas fluorescence at around 700 nm, **Figure 6**). The frontier molecular orbitals reveal that the delocalized π -system is extended through the aromatic frameworks at positions 2 and 6, and mainly through positions 3 and 5, explaining the huge bathochromic shift recorded (around 160 nm with regard to the reference simplest BODIPY, **Figure 6**). Note that the constrained 8-aryl group does not take part in the delocalization since it is almost orthogonally disposed with regard to the dipyrin plane. This is an important fact in the molecular design, since it is well known that *meso*-aryl moieties with free motion switch on non-radiative deactivation channels, which drastically quench the fluorescence signal [37, 38]. The steric hindrance provided by the *ortho* methyl is enough to avoid such quenching pathway.

As a result of the rational design, high red fluorescent efficiencies are recorded in apolar media (reaching almost the 90% in cyclohexane, **Figure 7**). However, the evolution of the fluorescence quantum yield with the solvent polarity drastically depends on the functionalization at the 2,6-phenyl rings [36]. Thus, upon formylation of such aryl, the fluorescent efficiency slightly decreases in polar media (down to 73% in methanol, **Figure 7**). However, the replacement of such group by methoxy implies a clear decline on the fluorescent efficiency (down to 13%, **Figure 7**), together with a further bathochromic shift (emission placed at 710 nm, **Figure 6**). In the former compound, with the weak electron withdrawing formyl group, the trend is the expected one with BODIPYs; thus, this compound is an ideal candidate as fluorescent probe or laser dye in the red-edge. However, in the latter compound, bearing the strong electron donor methoxy, a quenching process is activated in polar media. Considering the push-pull character of the molecule (from 2,6-methoxy to 3,5-trifluoromethyl), an ICT is stabilized in polar media limiting the fluorescent response of the dye.

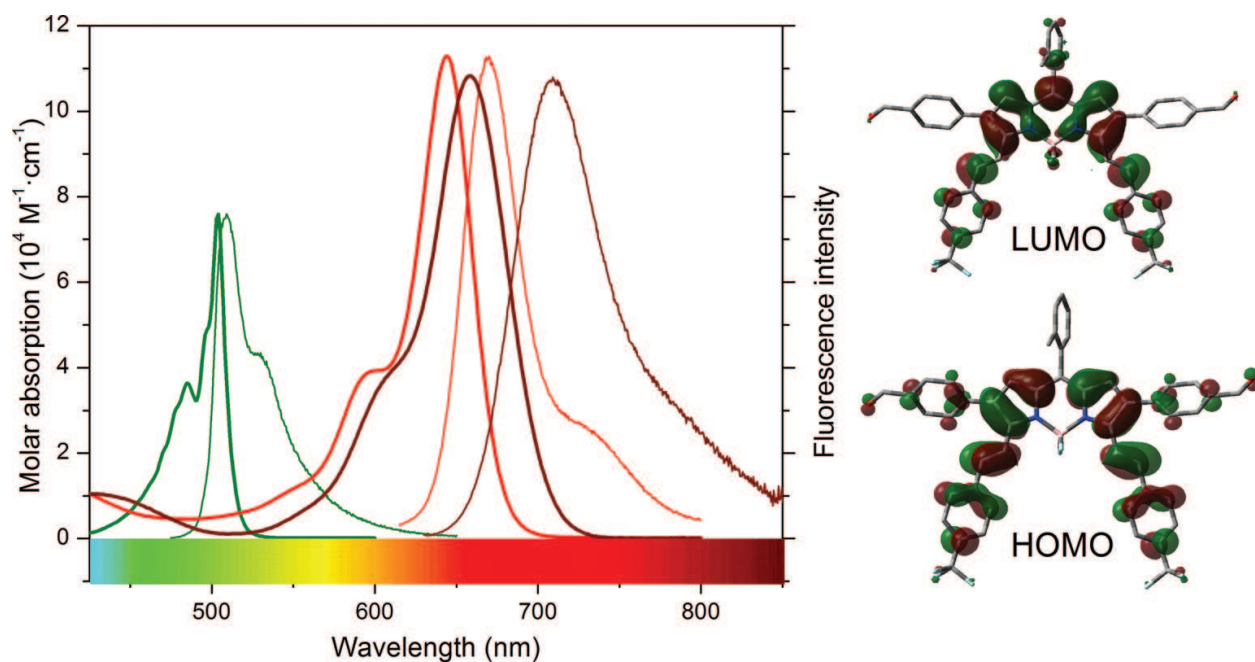


Figure 6. Absorption (bold line) and normalized fluorescence (thin line) spectra of the red-emitting dyes with regard to its reference unsubstituted counterpart in cyclohexane. The corresponding frontier contour maps of a representative red BODIPY is also enclosed.

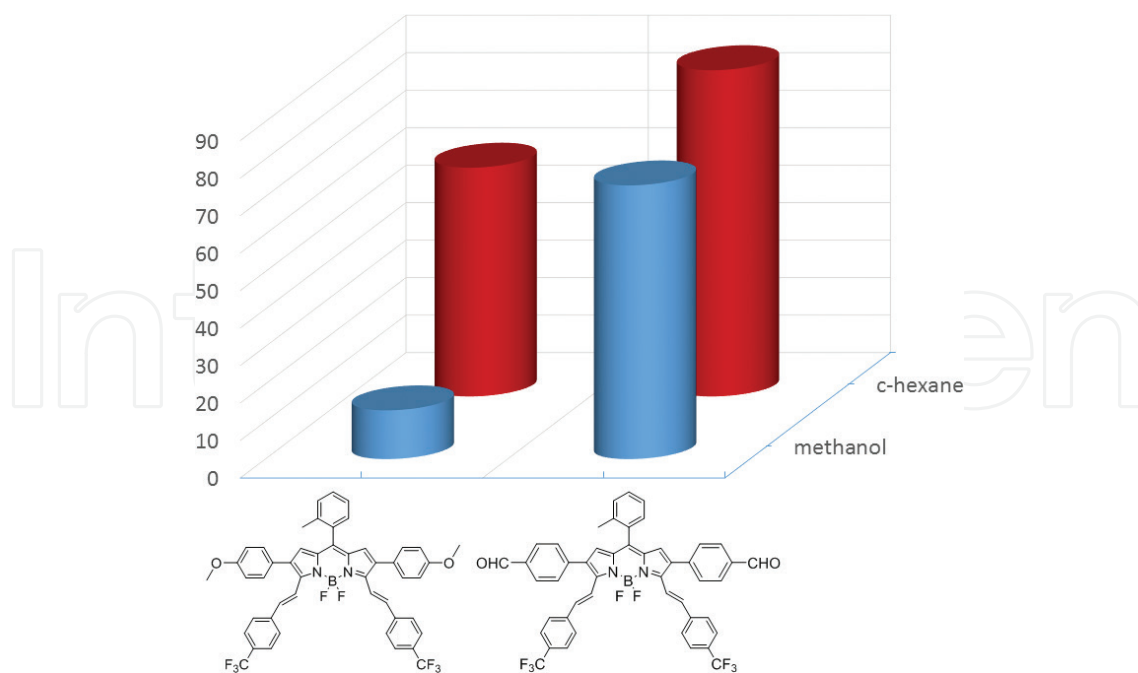


Figure 7. Fluorescent efficiencies of the red-emitting BODIPYs in apolar (cyclohexane) and polar (methanol) solvents.

Again, we tested the laser performance of these two dyes, and it was excellent [36]. The laser efficiencies reached the 60% in the formylated dye, being lower for the derivative bearing methoxy instead (43%, in agreement with the fluorescence measurements) and the laser emission is tunable from 700 to 740 nm, remaining high (90–100%) even after 50,000 pulses. Such performance is much better than that of styryls (LDS dyes), which provide much lower efficiencies, or oxazines, which are more unstable. Therefore, these BODIPYs should behave nicely as red fluorescent probes or laser dyes.

Summing up, and taking into account the herein developed fluorophores in both sections (2.1 and 2.2), we have been able to translate the excellent photophysical signatures of BODIPYs to both sides of the visible spectral region, leading to fluorophores which can be considered standards or benchmark in terms of photonic behavior, such as fluorescent and lasing efficiency and photostability.

3. Induction of new photophysical processes

In the preceding sections, we have demonstrated the deep impact of the substitution pattern in the spectral shift. Moreover, in some cases, such functionalization was able to induce new photophysical phenomena such as ICT processes, which should be avoided to ensure high fluorescent response. Indeed, depending on the environmental conditions, it becomes the low-lying excited state and quenches efficiently the emission from the locally excited state. Therefore, usually dyes ongoing ICT are not suitable as fluorescent probes for bioimaging or active media of tunable dye lasers. However, and owing to the sensibility of the ICT to the surrounding environment properties, fluorophores undergoing ICT can be ideal as fluorescent sensors to monitor a specific characteristic of the media, following the fluorescent efficiency

(on/off switches, if the ICT is not emissive) or the change in the fluorescence color (colorimetric sensors, if the ICT becomes fluorescence) [23–25]. In the next sections, we describe acidity/basicity and polarity sensors based on BODIPYs ongoing ICT processes (see molecular structures in **Figure 8**).

3.1. Sensor of acidity/basicity

In the previous section, we have shown the chemical versatility of the 8-methylthioBODIPY. For instance, in Section 2.1, the 8-heteroatomBODIPYs were attained by reaction of such scaffold with amine or alcohol and the ensuing displacement of the thiomethyl owing to the undergoing S_NAr -like reaction. A similar protocol can be used to attach acetylacetonate (acac) groups to the said *meso* position of the BODIPY (**Figure 8**) [39]. These dyes substituted with active methylene groups offer a wide assortment of post-functionalization opportunities, since it can act as ligand for metals or semiconductors, and even itself can become a fluorophore after chelation of the acac with difluoroboron. The acac can exist in the keto or enol form, but in the herein reported compounds, theoretical calculations suggest that the tautomeric equilibrium is shifted to the enolic form, thereby providing an ionizable hydroxyl group.

The acac group has a scarce impact in the spectral band positions, being those typical of the BODIPY. Indeed, the optimized geometry predicts that the acac is disposed perpendicular to the dipyrroin plane avoiding an electronic coupling. As a consequence, the 8-acacBODIPY displays a bright green emission with fluorescent efficiencies approaching 100% (**Figure 9**). Such orthogonal disposition is a key feature to ensure high fluorescent deactivation. In fact, those BODIPY derivatives bearing sp^2 hybridized carbons linked at *meso* position (such as vinyl) are non-fluorescent owing to the undergoing planarity distortion in the dipyrroin plane upon selective electron coupling with one chromophoric pyrrole in the excited state [40–42]. One way to avoid such quenching is to induce high enough steric hindrance, as happens

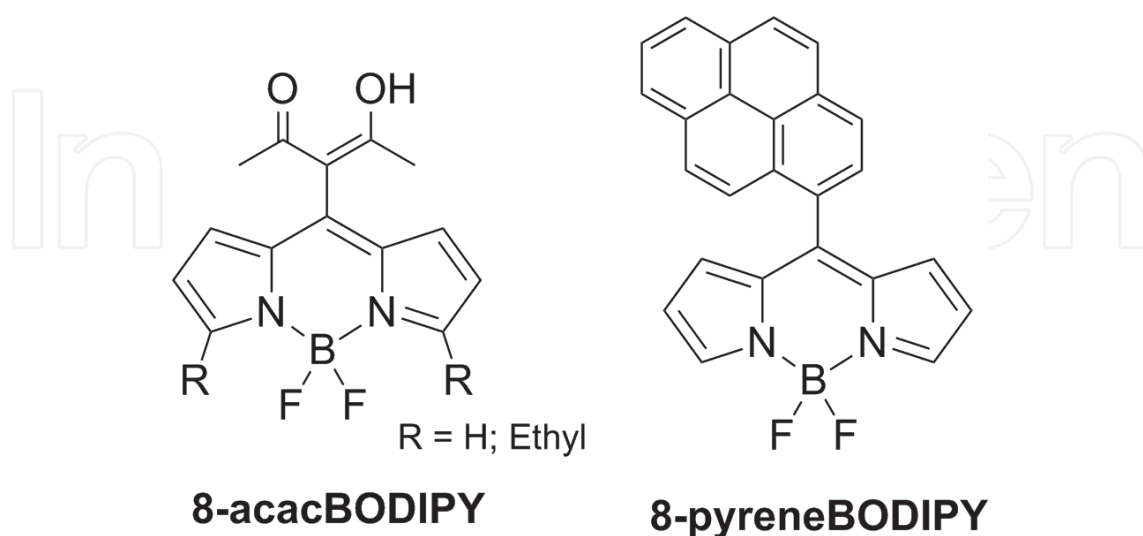


Figure 8. Molecular structure of the herein designed fluorescent sensors based on BODIPY.

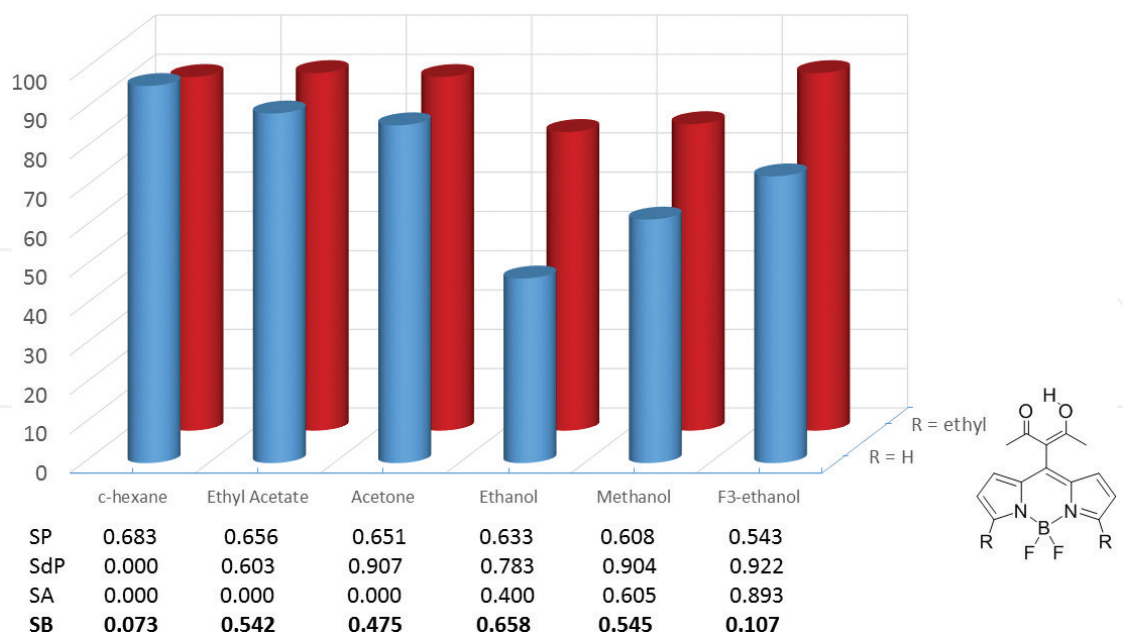


Figure 9. Evolution of the fluorescent efficiency of the 8-acacBODIPYs with the solvent properties described by the Catalan solvent scale (SP – Polarizability; SdP – Dipolarity; SA – Acidity; SB – Basicity).

with the acac. However, a closer inspection to the evolution of the fluorescent response with the solvent discloses that in alcohols, the efficiency drops more than expected (down to 47% in ethanol, **Figure 9**), whereas the lifetime remained high (around 7 ns). Using the solvent scales to account for the contribution of each solvent property (provided by Catalan) [43], we find that there is a correlation between the solvent basicity (SB scale) and the decrease in fluorescence (**Figure 9**). This is why we tested the influence of the pH of the media in the fluorescence signatures. Usually, the BODIPY dyes are rather hydrophobic and are not soluble in water. However, the presence of acac increases its hydrophilic character, and the dye becomes soluble in water, at least at low concentrations (around μM).

The fluorescence intensity decreases progressively as the pH of the media increases, thus at pHs more basic than 8 the fluorescence emission is almost completely lost (**Figure 10**). From the evolution of the fluorescence quantum yield with the pH a pK_a of 6.9 is attained. Therefore, a quenching process takes place in basic media being reversible since acidification of basic solution implies a recovering of the fluorescence signal. Therefore, this dye behaves as an on/off switch to monitor the pH of the environment by measuring the evolution of the fluorescent response. Thus, at low pHs, the sensor is highly fluorescent (on), but at high pHs, the sensor is almost dark (off). Such sensing ability should be related with the ionization of the hydroxyl group of the enolic form of the 8-acac in basic conditions. Theoretical simulations show that the electronic distribution and energies of the HOMO and LUMO in acid media (acac as enol) is the typical of BODIPYs, thus being unaltered by attachment of the 8-acac (**Figure 10**). However, at basic media (pH higher than the pK_a where the hydroxyl is ionized and the corresponding enolate is formed), the contour maps change markedly. The geometrical arrangement of the acac as enolate is no longer orthogonal with the dipyrroin, but it

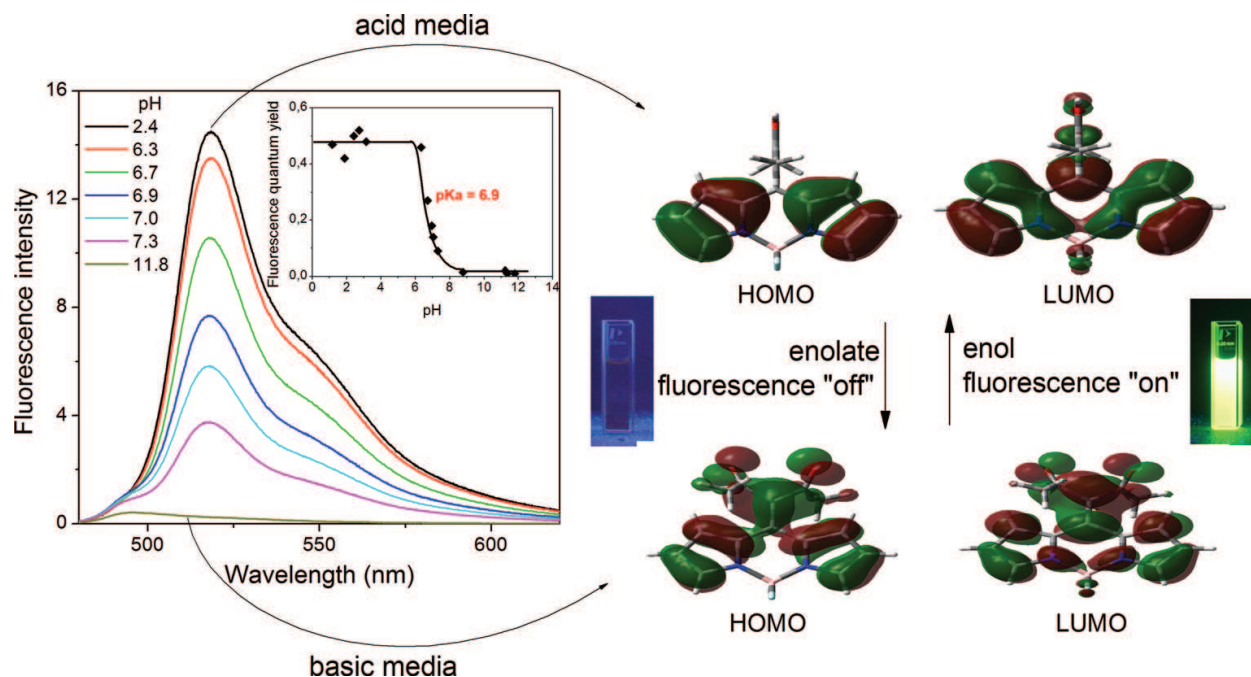


Figure 10. Evolution of the fluorescence band and quantum yield (inset) of the 8-acacBODIPY with the pH in aqueous solutions. The frontier contour maps before and after ionization of hydroxyl of the 8-acac are also enclosed.

becomes more coplanar (twisting dihedral angle around 30°), enabling an electron coupling. In this state, the negative charge of the enolate interact by resonant with the dipyrin (see the electronic density spanned through the whole structure in both frontier orbitals), causing a planarity distortion in the chromophore (**Figure 10**). Therefore, the ICT from the ionized hydroxyl of the enolate to the BODIPY implies a drastic increase in the internal conversion probability and a loss of the aromaticity explaining the lack of fluorescence signal in basic media and allowing the sensing ability of this dye.

The effect of the ethylation of positions 3 and 5 is worthy of being outlined [39]. In agreement with the aforementioned results for 8-aminoBODIPYs in Section 2.1, the sole alkylation at these key positions enhances the fluorescent efficiency mainly in basic media (up to 76% in ethanol, **Figure 9**). It seems that the inductive donor effect of the ethyl groups infers a lower electron acceptor character to the BODIPY, being less predisposed to accept the electronic density from the 8-enolate, and hence lowering the quenching ICT pathway in basic media.

The laser measurements correlate well with the photophysical findings [39]. Indeed, the 8-acacBODIPY displays a green laser emission at 550 nm with an efficiency up to 55%, maintaining the 90% of the laser emission after 50,000 pulses. Ethanol is the solvent with the lower lasing efficiency (down to 43%) in agreement with the recorded decrease in the fluorescence quantum yield (**Figure 9**). Moreover, the 3,5-ethylation implies not only an increase in the laser efficiency (up to 70%, in concordance with the evolution of the fluorescent efficiency in **Figure 9**) but also it becomes more photostable (keeping the 100% of the emission under the same pumping regime). Therefore, these dyes bearing acac at *meso* position are rather versatile since they behave as laser dye and as on/off pH sensor as well.

3.2. Sensor of polarity

Another exploited strategy with BODIPYs is based on the development of multichromophoric dyes. In particular, that is related with the molecular design of excitation energy transfer (EET) cassettes [44]. The idea is to link covalently a suitable chromophore acting as energy donor to a complementary fluorophore playing the role of energy acceptor. This kind of dyads allows an efficient light harvesting over a broad spectral region, but provides almost exclusively the emission of the energy acceptor via a highly efficient intramolecular excitation energy transfer (intra-EET) owing to the short donor-acceptor distance imposed by the covalent linkage. The most common EET mechanism is mediated by the Förster formalism (FRET, also known as through-space), which demands a spectral overlap between the energy donor fluorescence band and the energy acceptor absorption spectrum to enable the required dipole-dipole coupling. Other reported EET mechanisms are Dexter (electronic exchange) or through-space (TBET, superexchange mechanism) [45, 46]. Following this approach, we tested the photophysical signatures of a dyad featuring a pyrene (blue emitting chromophore) linked to the *meso* position of the BODIPY (green emitting fluorophore) core (**Figure 8**). The pyrene was chosen since it can be grafted to the *meso* position of the 8-methylthioBODIPY platform via a LSCC reaction from the adequate boronic acid [47]. Besides its fluorescence profile, it is suitable to undergo FRET with the BODIPY since the required spectral overlap is feasible [48].

The absorption profile of the dyad shows the characteristic bands of both chromophores (**Figure 11A**). Thus, the typical vibrational resolution of the electronic band of the pyrene is recorded in the UV, followed by the sharp absorption of the BODIPY at the Vis region. Therefore, both chromophores contribute additively to the overall broadband absorption covering the UV-blue-green spectral region. In fact, the electron coupling between both moieties is disabled in the ground state since the pyrene is twisted around 65° due to steric reasons [48]. Regardless of the excitation wavelength (directly at the BODIPY Vis absorption or at the pyrene UV absorption), the Vis emission outcoming from the BODIPY prevails in the fluorescence profile, whereas the emission from the pyrene is strongly quenched (**Figure 11A**). This experimental finding supports that the dyad undergoes intra-EET from the donor pyrene to the BODIPY acceptor and final light emitting moiety. Likely, the ongoing mechanism is FRET due to the available spectral overlap, but also TBET could be viable since both chromophores are directly linked enabling the required electronic exchange [45, 46]. The fluorescent efficiency (30% in apolar cyclohexane) and lifetime (around 2 ns) of the dyad are lower than the expected in BODIPYs. This moderate fluorescence loss can be attributed to the free motion of the bulky pyrene at the key and substituent sensitive *meso* position, which enhances the internal conversion probability.

Nonetheless, the above described fluorescence signatures in apolar media dramatically change in polar media (**Figure 11B**). Whereas no changes are detected in the absorption features, the fluorescence profile is deeply altered. The emission at 520 nm (assigned to the locally excited (LE) state) is strongly quenched, and a new broad and red-shifted emission is recorded (around 640 nm and reaching 700 nm in the most polar media). Besides, the fluorescent efficiency dramatically decreases in the more polar media where such new emission becomes rather weak and almost negligible. These experimental findings reveal complex

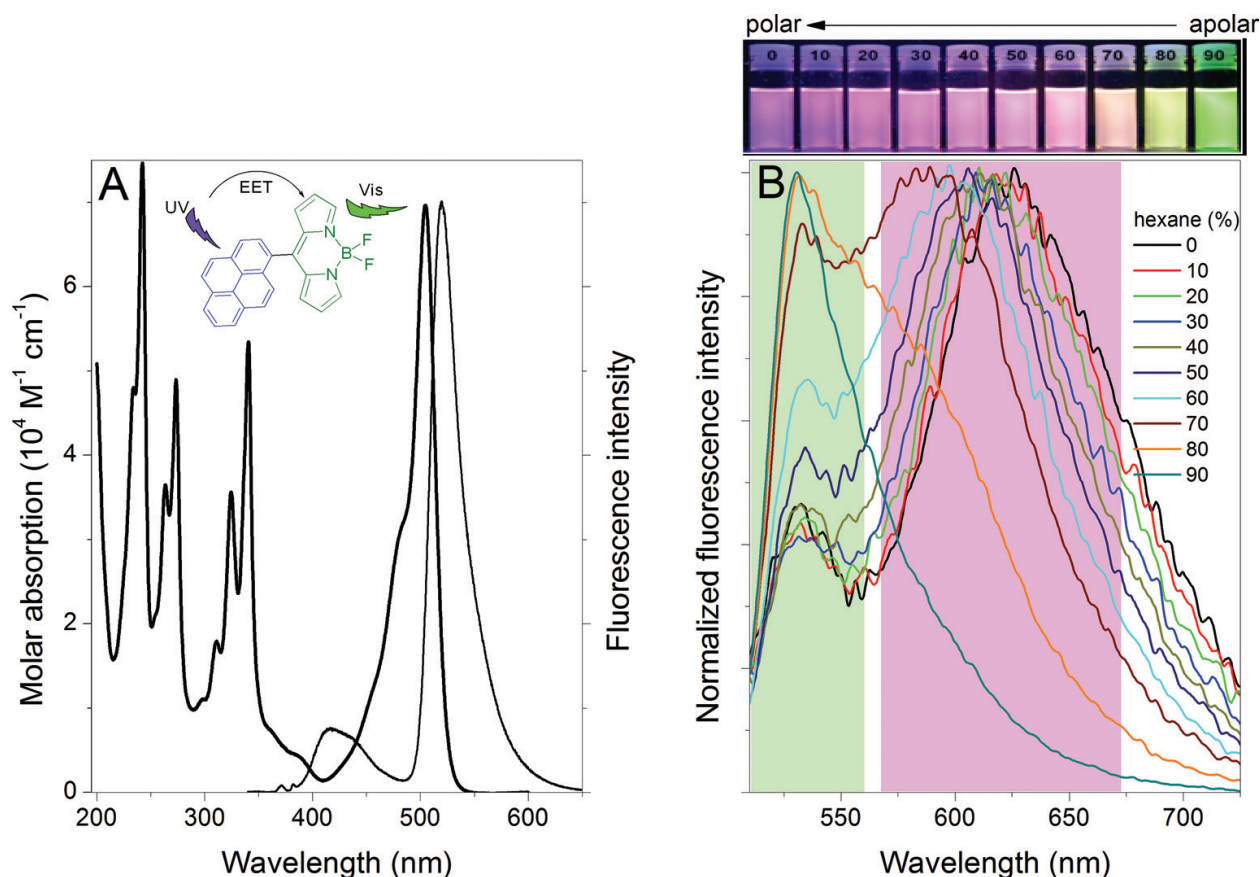


Figure 11. (A) UV-Vis absorption (bold line) and fluorescence (thin line, upon UV excitation at 340 nm) spectra of the 8-pyreneBODIPY in cyclohexane. A sketch of the ongoing energy transfer is also enclosed. (B) Evolution of the fluorescence profile with the solvent polarity (normalized spectra in hexane-tetrahydrofuran mixtures). The two detection channels and the corresponding fluorescence images in each mixture proportion are included to highlight the sensing ability.

molecular dynamics in the excited state (EET still takes place in polar media) since a new low-lying emitting state is formed upon excitation in polar media. All these trends pinpoint to the activation of an ICT state from the pyrene to the BODIPY. The high dipole moment of the ICT owing to its inherent charge separation explains why it is further stabilized in polar media. In the preceding sections, a non-fluorescence ICT was already claimed to explain the recorded fluorescence quenching in polar media. However, in this case, the ICT becomes fluorescence displaying a long-wavelength emission very sensitive to the solvent polarity. An increase of the polarity of the media implies a more efficient quenching of the emission from the LE state, and also from the ICT as well, since the charge recombination probability is lower and the charge separation is further favored [49, 50].

Although this dyad is not valuable as laser dye or fluorescent probe, it can be applied as polarity sensor of the surrounding environment owing to the ICT formation and its high sensitivity to this solvent property. As a matter of fact, we have systematically varied the medium polarity using mixtures of apolar solvent (hexane) and a polar one (tetrahydrofuran) (**Figure 11B**). Thus, in apolar media, just a green emission is recorded from the LE state. However, a progressive increase of the solvent polarity implies that the emission from the ICT

is detected in detriment of that from the LE state. Thus, in polar environment, both emissions are simultaneously detected, leading to a yellow emission. Finally, in the more polar media, the emission from the ICT exceeds that from the LE and prevails, leading to a purple emission. Summing up, in this dyad, where the ICT fluorescence deactivation is feasible, two channels for detection are provided; one at the green part of the visible (around 520 nm) and other at the red part (around 640 nm). Therefore, the solvent polarity can be monitored by measuring the change of the fluorescence intensity at these wavelength, or alternatively just by the naked eye by visualizing the emission color under UV excitation owing to the ongoing intra-EET.

4. Conclusions

The rich and versatile chemistry of the robust boron-dipyrrin core allows the design and synthesis of a myriad of molecular structures with diverse photophysical signatures, which can be finely modulated depending on the target application field. This tailoring ability is likely the main reason of the success and reputation of these chameleonic fluorophores. It is not risky to envisage that nowadays BODIPYs are the most recommended luminophore for optoelectronics and biophotonics, or whatever utility in which an organic dye could be involved. After a rational design, a molecule fitting the requirements of a specific application can be customized, guaranteeing a nice and accurate performance.

In this chapter, we settle some structural guidelines to orient the synthesis of novel BODIPYs as candidates to be applied as fluorescent probes, laser dyes or fluorescent sensors along the entire visible spectral region. All the herein tested BODIPY derivatives are attained from the 8-methylthioBODIPY scaffold. This versatile platform is able to undergo different synthetic routes selectively in orthogonal positions, in particular at *meso* position, at β (2 and 6) and α (3 and 5) pyrrolic positions. Such reactivity enables a rich and exhaustive functionalization of the dipyrin core.

One of the main pursue goals was to translate the excellent stability and fluorescent response of BODIPYs working usually in the middle part of the visible spectrum (green-yellow) to both edges (blue and red), giving rise to standard and optimal fluorophores across this spectral region. On the one hand, to push the spectral bands toward the red-edge aromatic frameworks bearing electron rich groups were grafted to the BODIPY core, mainly at positions 3 and 5. Push-pull chromophores (bearing electron donor and acceptor functionalities) provide more pronounced red spectral shift, but the fluorescence emission is damaged owing to the induced charge separation (ICT). On the other hand, the opposite blue shift was achieved changing the electronegativity of the heteroatom attached at the *meso* position. The electron coupling of these heteroatoms with the dipyrin switches on new delocalized π -system responsible of the hypsochromic shift. To ensure high fluorescent efficiencies, it is recommended to alkylate the key positions 3 and 5.

Taking into account that the *meso* position is the most sensitive one of the backbone to the substituent effect, it was selected to attach functionalities able to induce ICT processes. These phenomena should be avoided if a high fluorescent response is required, but it is ideal to

develop sensors due to its sensitive to specific environmental properties. On the one hand, the attachment of active methylene at such position leads to bright green dyes. However, such emission is lost in basic media. Such reversible quenching allows the monitorization of the pH of the surrounding environment following the change of the green fluorescence intensity (on/off switch). On the other hand, the grafting of pyrene enables excitation far away from the emission region owing to energy transfer processes. At the same time, such functionalization induces an ICT state characterized by red emission. The ratio between the green and red fluorescence is very sensitive to the solvent polarity. Thus, this sensor allows to visualize the polarity of the environment just by the color of fluorescence.

Although the number of reports dealing with BODIPYs is huge and still growing fast, we foresee that the future of these dyes is far from being over, and still plenty of applications need to be explored with this marvelous and amazing organic luminophore.

Acknowledgements

Financial support from MICINN (MAT2017-83856-C3-3-P), Gobierno Vasco (IT912-16), and CONACyT (Mexico, Grant 253623) are acknowledged. R. S-L. and E.A-Z. thank Gobierno Vasco for a postdoctoral contract and predoctoral fellowship, respectively. C. F. A. G.-D. and J. L. B.-V. thank CONACyT for doctoral scholarship.

Author details

Rebeca Sola Llano¹, Edurne Avellanal Zaballa¹, Jorge Bañuelos^{1*}, César Fernando Azael Gómez Durán², José Luis Belmonte Vázquez², Eduardo Peña Cabrera² and Iñigo López Arbeloa¹

*Address all correspondence to: jorge.banuelos@ehu.eus

¹ Department of Physical Chemistry, University of the Basque Country (UPV/EHU), Bilbao, Spain

² Department of Chemistry, University of Guanajuato, Guanajuato, Mexico

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