Unusual spectroscopic and photophysical properties of solvatochromic BODIPY analogues of Prodan

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Abstract

Here, we report the synthesis of two isomeric BODIPY-based fluorescent molecules with a diethylamino donor group and an acetyl acceptor group at positions 5 and 2 (or 3), respectively, on the boron dipyrromethene core. The structure of the new BODIPY dyes is similar to Prodan, probably the most widely used solvatochromic probe, but with the donor and acceptor groups attached to an electron-poor heteroaromatic core. Interestingly, these BODIPY dyes exhibit unusual spectroscopic and photophysical properties, involving inverse solvatochromic behavior. An in-depth study of the solvent effects and quantum-chemical

calculations have been performed to assess the basis for such spectroscopic properties and the effect of the position of the substitution. This work points towards a rational design of new fluorescent probes, based on understanding their spectroscopic and photophysical behavior.

Keywords: Boron Dipyrromethene • Excited State • Photophysics • Quantum Chemistry • Solvatochromism

1. Introduction

It has been known for a very long time that solvents can have large effects on the spectroscopic properties of molecules.[1, 2] Compounds with solvent-dependent absorption and/or emission spectra are called solvatochromic.[3] Prodan [1-(6-(dimethylamino)naphthalen-2-yl)propan-1-one] (Figure 1) has been widely used as a fluorescent solvatochromic probe since its introduction by Weber and Farris in 1979.[4] The solvent-sensitive shifts of the fluorescence emission maxima and Stokes shifts are very large. Such an outstanding sensitivity to the environment is responsible for the success of Prodan and its naphthalene-based analogues (Acrylodan, Laurdan, Danca, Patman, Badan; Figure 1) for probing the micro-environments of a wide variety of chemical and biological systems [(reverse) micelles,[5, 6] phospholipid vesicles and cellular membranes,[7-9] protein binding sites,[6, 10] nucleosides,[11] cyclodextrines,[12, 13] etc.]. Several authors have investigated the nature of the emitting state of Prodan (locally excited and/or charge-transfer state, planar or twisted intramolecular charge-transfer state) and the origin of its high spectral sensitivity to the environment.[5, 8, 14, 15]

Environment-sensitive fluorescent probes – such as Prodan and its variants (Figure 1) – are characterized by two groups, an electron-releasing (donor, here an amine) and an electron-withdrawing (acceptor, here a carbonyl, C=O) group attached to appropriate positions of an aromatic system (here naphthalene). Upon photoexcitation an intramolecular charge transfer

(ICT) occurs from the amino group to the carbonyl group of the ketone, so that the dipole moment m in the excited (E) state becomes larger than in the ground (G) state, $m_E > m_G$, which, in turn, strongly modifies the interactions with the environment. If the ICT probe also presents hydrogen-bonding sites, specific solute-solvent interactions not only can lead to large shifts of both absorption and emission bands but also can affect the nature of the emitting species, rendering the spectroscopic signal extremely sensitive to the hydrogen-bonding characteristics of the solvent, as it happens in Brooke's merocyanine, another well-known solvatochromic dye, whose ground-state zwitterionic form is stabilized by hydrogen-bonding solvents, leading to large hypsochromic effects.

The electronic absorption spectra of Prodan and its family of 2-carbonyl-6-aminonaphthalene fluorophores (Figure 1) are located in the UV spectral region. Many analogues, such as 2- and 5- substituted naphthalene derivatives[16] or cyclopenta[*b*]naphthalene solvatochromic fluorophores,[17] still present absorption maxima, $\lambda_{\text{abs}}^{\text{max}}$, below ~400 nm. Other analogues were designed with a different fluorochromic core, such as pyrene (PA and PK),[18] anthracene (Anthradan),[19] and fluorene (FR0 and FR8).[20] Among the dyes shown in Figure 1, Anthradan has the most bathochromically-shifted absorption spectra with $\lambda_{\text{abs}}^{\text{max}}$ in the 440–460 nm range.[19] Importantly, shifting the absorption and emission bands of a fluorophore into the visible region increases its usefulness in biological applications, hence, the search for improved, red-shifted solvatochromic dyes continues.

Figure 1. Solvent-sensitive probes based on amine donor and carbonyl acceptor with naphthalene (Prodan and its derivatives Acrylodan, Laurdan, Danca, Badan, Patman), pyrene (PA and PK), anthracene (Anthradan), and fluorene (FR0 and FR8) as parent aromatic system.

To shift the spectra into the visible range, we decided to utilize the **boron dipyrromethene** (BODIPY or 4-bora-3a,4a-diaza-s-indacene) fluorophore with its intrinsically visible absorption and fluorescence spectra as parent fluorophore. BODIPY derivatives have become extremely valuable in the last decades in different research areas, owing to their versatile chemistry[21, 22] and tunable spectroscopic/photophysical properties,[23, 24] including high fluorescence quantum yields (Φ) and molar absorption coefficients (ϵ), narrow absorption and fluorescence emission bands in the visible region, and resistance towards photo-, thermal, and chemical degradation. BODIPY derivatives are among the most intensely fluorescent and most detectable fluorophores available. The rich possibilities for functionalization allow one to obtain fluorescent probes with particular features useful in applications as varied as

bioimaging,[25-27] sensing,[28] and other technological applications.[29, 30] Previously reported BODIPY dyes have been shown to behave as solvent-sensitive sensors. For instance, when an aromatic amine or an azacrown was added to the *meso*- position of the dipyrromethene, photoexcitation led to an ultrafast charge transfer from the locally excited (LE) state to a weakly emissive intramolecular charge transfer (ICT) excited state.[31] Interestingly, when the conjugation in the BODIPY ring was extended by a 3,5-distyryl modification, the characteristic BODIPY fluorescence was drastically quenched in polar solvents, compared to the high Φ value (0.92) found in hexane, but without the characteristic broad and solvent polarity-dependent emission band originating from an ICT excited state.[32] However, the ICT state was also observed when a single aminostyryl group (donor) at position 3 was conjugated to a *meso*-aryl substituted boron dipyrromethene moiety (acceptor), displaying solvent-dependent spectroscopic and photophysical characteristics, with low Φ , short fluorescence lifetime τ values and large bathochromic shifts of the emission maxima λ_{em}^{max} in polar solvents.[33, 34] Other works used BF_2 rings, such as oxadiazaborinine and naphthyridine, decorated with electron-withdrawing and electron-donating groups to achieve solvatochromic dyes and enhanced two-photon absorption cross sections.

In this work we propose BODIPY-based Prodan analogues, by introducing the dialkylamino-carbonyl donor-acceptor feature within the fluorochromic core (Figure 2). Starting from α,β -unsubstituted, *meso*-arylated boron dipyrromethenes, one can envisage thirteen different parent BODIPY molecules with resonant ICT between an amino (here diethylamino) electron donor and a carbonyl (here acetyl) electron acceptor (structures **1**, Figure 2). Unfortunately, only a limited number of these structures are synthetically accessible. At the 8-position (*meso*-position) a 2,4,6-trimethylphen-1-yl (mesityl) group was placed to ensure a high fluorescence quantum yield Φ . Indeed, it has been shown that steric hindrance of methyl groups at the *ortho*-positions of *meso*-aryl substituted BODIPYs minimizes the non-radiative rotational deactivation of the singlet-excited state.

Figure 2. General structure of amine-carbonyl 8-arylBODIPYs **1**. Derivatives **2** and **3** are studied in this paper. Compound numbers **2** and **3** refer to the position of the acetyl group.

Here, we report the synthesis and spectroscopic and photophysical properties of two isomeric BODIPY fluorescent molecules, namely 1-[5-(diethylamino)-4,4-difluoro-8-(2,4,6-trimethylphen-1-yl)-4-bora-3a,4a-diaza-s-indacen-2-yl]ethan-1-one [or 2-acetyl-5-diethylamino-8-mesitylBODIPY (**2**)] and 1-[5-(diethylamino)-4,4-difluoro-8-(2,4,6-trimethylphen-1-yl)-4-bora-3a,4a-diaza-s-indacen-3-yl]ethan-1-one [or 3-acetyl-5-diethylamino-8-mesitylBODIPY (**3**)] (Figure 2). Placing the diethylamino donor and the acetyl acceptor at positions 5 and 2 (in **2**) or 3 (in **3**), respectively, on the boron dipyrromethene core, maximizes their separation and makes an ICT excited state possible (Figure S1, in Supporting Information, SI). Importantly, the structure of **1–3** (Figure 2) differs from the previous naphthalene, anthracene, pyrene, and fluorene based probes (Figure 1), because donor and acceptor are attached to an electron-poor heteroaromatic core (*i.e.*, BODIPY) and not to an aromatic hydrocarbon.

2. Experimental Section

2.1. Synthesis and characterization

Reagents and solvents were used as received from Energy Chemicals (Shanghai, China). All reactions were performed in oven-dried or flame-dried glassware and were monitored by TLC

using 0.25 mm silica gel plates with UV indicator (60F-254). ^1H and ^{13}C NMR spectra were recorded on a 300, 400, or 500 MHz NMR spectrometer at room temperature. ^{11}B and ^{19}F NMR spectra were recorded on a 400 MHz NMR spectrometer at room temperature. Chemical shifts (δ) are given in ppm relative to CDCl_3 (7.26 ppm for ^1H and 77 ppm for ^{13}C) or to internal TMS ($\delta = 0$ ppm) as internal standard. Data are reported as follows: chemical shift, multiplicity, coupling constants, and integration. Chemical shifts of ^{11}B and ^{19}F NMR spectra are quoted in ppm relative to external $\text{BF}_3\cdot\text{OEt}_2$ (^{11}B) and CFCl_3 (^{19}F). High-resolution mass spectra (HRMS) were obtained using ESI-TOF in positive mode. Synthetic routes for compounds **2** and **3** are shown in Scheme 1. See Supplementary Materials for the details of the synthesis and characterization of the products.

2.2. Instruments

Absorption spectra and spectrally corrected steady-state fluorescence emission spectra were collected using a Perkin-Elmer Lambda 650 UV/vis spectrophotometer and a JASCO FP-8500 spectrofluorometer, respectively. All spectroscopic measurements were carried out in a Peltier temperature-controlled cell holder set at 20 °C, using undegassed samples in 10 × 10 mm cuvettes (with 10 mm optical path length for absorption and a 90° angle setup for fluorescence collection through the shortest side).

Time-resolved fluorescence decay traces were recorded using a FluoTime200 fluorometer (PicoQuant GmbH) working in single-photon timing mode.[35, 36] The pulsed excitation source was a 440 nm diode laser (LDH series from PicoQuant GmbH) operated with a PDL-800 driver (PicoQuant GmbH) at a pulse repetition rate of 20 MHz. For each compound in a given solvent, three different fluorescence decay traces were collected at different emission wavelengths, selected by a grating monochromator, after a polarizer set at the ‘magic angle’. These emission wavelengths were 525, 530, and 535 nm for **2**, and 560, 565, and 570 nm for **3**. The fluorescence decay traces were collected over 1320 channels, with a time increment of 36 ps

per channel, until they reached 2×10^4 counts in the peak channel. Histograms of the instrument response functions were collected using a LUDOX scatterer.

Time-Resolved Emission Spectroscopy (TRES) of compound **3** in different solvents was performed by collecting 45 fluorescence decay traces in the 460-640 nm emission range ($\Delta\lambda_{\text{em}} = 4$ nm) at 20 MHz excitation frequency during a fixed amount of time (500 s), to maintain the overall intensity information. For the TRES analysis and the estimation of the species-associated emission spectra (SAEMS), the fitting procedure described above was performed, by fitting globally the 45 decay traces.

Details of methods of analysis for quantum yields, intensity-weighted average decay times and SAEMS can be found in the Supplementary Material.

2.3. Computational details

Ground-state geometry optimizations were performed with DFT (PBE0).[37, 38] Excited-state geometry optimizations were carried out with TD-DFT. The nature of all stationary points was confirmed by computing the Hessian at the same level of theory. PBE0 is used in the TD-DFT calculations, since this functional provides a good balance between local excited (LE) and charge transfer states.[39, 40] TD-DFT calculations using the range-separated CAM-B3LYP functional were also performed for comparative purposes. For TD-DFT calculations, analysis of hole-electron overlaps (S_r index) and the distance between the centroids of hole and electron were performed with the Multiwfn package. The DFT and TD-DFT calculations were performed using the 6-31+G(d) basis set with Gaussian09.[41] Single-point ADC(2) calculations were performed with the TURBOMOLE program,[42] using the def2-TZVP basis set. Solvent effects were included in the ADC(2) calculations using the recent implementation of the conductor-like screening model (COSMO),[43] which include linear-response terms and state-specific effects.[44] Singlet electronic excitation energies were equilibrated to the ground state,

whereas singlet electronic emission energies were equilibrated to their respective excited states.

3. Results and Discussion

3.1. Synthesis of BODIPY analogues of Prodan dyes.

Amine–carbonyl-substituted BODIPY dyes **2** and **3** were prepared *via* different methods, both starting from *meso*-mesityl BODIPY **A** as initial reagent, as shown in Scheme 1. Key intermediate 2-acylated BODIPY **4a** was regioselectively synthesized in 62% yield from BODIPY **A** and acid chloride (here acetyl chloride) in the presence of BF₃·OEt₂ through Friedel-Crafts electrophilic aromatic substitution.[45] 3-Acylated BODIPY **4b** was synthesized in 40% yield from 2-methyl-1,3-dioxolane using Bu₄NI as catalyst and *tert*-butyl hydroperoxide (TBHP), followed by *in situ* deprotection according to our recently reported method.[46] Next, BODIPYs **2** and **3** were synthesized through the Ag(I)-mediated radical addition of an amino radical to the BODIPY core.[47] BODIPY **2** was synthesized in 90% yield from diethylamine in the presence of AgOAc in dimethyl sulfoxide (DMSO) at 80 °C for 24 minutes. Similarly, BODIPY **3** was obtained in 39% yield in 1 h. These two amine–carbonyl-substituted BODIPY dyes **2** and **3** have been fully characterized by ¹H, ¹³C, ¹¹B, ¹⁹F NMR, and HRMS (see Supplementary Material).

Scheme 1. Synthesis of amine-carbonyl BODIPY-based dyes **2** and **3**. TFA = trifluoroacetic acid, rt = room temperature.

The regiochemistry of BODIPY **2** was further confirmed by X-ray analysis (Figure 3). Crystals of BODIPY **2** suitable for X-ray analysis were obtained *via* the slow diffusion of petroleum ether into its dichloromethane solution under atmospheric pressure (Table S1). The dihedral angle of two pyrrolic rings of the dipyrin core is 2.8° (1.3° in the optimized S_0 geometry), indicating no steric hindrance of amine and carbonyl substituents to cause structural distortion of the dipyrin core. As usual, the dihedral angle between the *meso*-mesityl group and the dipyrin core is 83° (89° the computed one). The substituents at the N atom at 3-position of BODIPY are coplanar, and the C3–N3 bond length is 1.32 Å (1.34 Å the computed one), which is shorter than a typical single C–N bond, and close to partial double bonds found in amides. In contrast, the C1–N3 and C2–N3 bond lengths are 1.47 Å and 1.48 Å (both are 1.46 Å in the optimized S_0

geometry), respectively (Table S2), indicating that the lone pair of electrons on the N atom is conjugated with the planar dipyrin core. The coplanarity of the substituents can be confirmed by the deshielding (δ 3.86 ppm) and broadening of the ^1H -NMR signal of the methylene groups of the diethyleneamine. This effect is caused by long-range interaction with the fluorine atoms, and confirms close proximity of these methylene groups with the fluorine atoms of the BODIPY core. The geometry of this amino group is very striking, since previous amino-substituents showed very different conformations. For instance, a 3,5-dibenzoimidazole-substituted BODIPY exhibited longer C3-N3 distances (1.4 Å) and torsion angles N-C-N-C around 50.5° , far from a coplanar arrangement. Other amino-substituted BODIPY were more similar, for instance, in *meso*-amino-substituted BODIPYs the conjugation can be extended through the amino group, in a hemicyanine structure, but this may even alter planarity of the BODIPY core, resulting in blue-emissive dyes. A more similar situation can be found in 3-(4-morpholinyl)-substituted BODIPY, in which a short C3-N3 bond distance (1.34 Å) was reported, indicating extended conjugation of the π system to the N. However, in that fluorophore, the torsion angle N-C-N-C was 25.6° , so that the coplanarity of the three carbon atoms linked to the amine nitrogen is a remarkable feature of our BODIPY **2**. Regarding the carbonyl group, the C=O distance was xxxx Å (xxx Å the computed one), in agreement with conventional double bonds in ketones. This supports the idea that the charge separation is not large enough to backing a zwitterionic form in the ground state.

It was not possible to obtain crystals suitable for X-ray analysis for compound **3**. Nevertheless, coplanarity of the diethylamino substituent with the BODIPY moiety in **3** is well supported by the ^1H -NMR signal of the methylene protons (broad signal at δ 3.93 ppm) as described above. In contrast, the signal of the acetyl protons did not show such effect, confirming the configuration of the acetyl group with the oxygen atom closer to the fluorine atoms (as depicted in Figure 2).

Figure 3. Top (a), front (b), and side (c) views of X-ray crystal structure of BODIPY **2**. C, light gray; N, blue; B, dark yellow; F, bright green, O, red. Hydrogen atoms have been removed for clarity.

3.2. Spectroscopic and photophysical properties.

The two newly synthesized BODIPY analogues of Prodan are strongly colored solids that formed yellowish solutions in all the solvents tested. Dyes **2** and **3** were dissolved in 19 different solvents, and their UV–vis absorption and fluorescence emission spectra were collected (Tables 1 and 2). Both compounds **2** and **3** absorbed and emitted in the green region of the visible spectrum and exhibited much larger Stokes shifts than common BODIPY dyes.[23, 24] Figure 4 shows representative examples of the visible electronic absorption and fluorescence emission spectra of **2** and **3** in a selection of solvents. Further examples are shown in Figures S2 and S3. The absorption spectra of BODIPY **2** and **3** in all solvents tested exhibited a broad $S_1 \leftarrow S_0$ band, with fwhm_{abs} values around 5-fold larger than those of typical BODIPY dyes.[23, 48] The vibronic structure of the absorption band of **2** was visible in apolar solvents such as cyclohexane and toluene, although it was not as noticeable for compound **3** (with the acetyl group at the 3-position). However, the most striking feature of the absorption spectra is that a clear hypsochromic shift was observed upon increasing the solvent dipolarity.

Table 1. Spectroscopic and photophysical data of **2** as a function of solvent.

	Solvent ^[a]	$\lambda_{\text{abs}}^{\text{max}}$ [nm]	$\lambda_{\text{em}}^{\text{max}}$ [nm]	$\Delta\bar{\nu}^{[b]}$ [cm ⁻¹]	$\text{fwhm}_{\text{abs}}^{[b]}$ [cm ⁻¹]	$\text{fwhm}_{\text{em}}^{[b]}$ [cm ⁻¹]	$\epsilon^{[c]}$ [10 ⁻³ M ⁻¹ cm ⁻¹]	$\Phi^{[d]}$
1	Methanol	443	526	3560	3740	2300	42 ± 1	0.0020 ± 0.0001
2	CH ₃ CN	444	529	3620	3770	2290	41 ± 2	0.0024 ± 0.0001
3	Acetone	448	528	3380	3590	2300	45 ± 4	0.0056 ± 0.0025
4	Ethanol	446	526	3410	3720	2300	38 ± 3	0.0038 ± 0.0003
5	Ethyl acetate	454	532	3230	3590	2260	40 ± 4	0.0065 ± 0.0001
6	2-Propanol	447	528	3430	3650	2310	41 ± 1	0.0078 ± 0.0001
7	1-Propanol	447	529	3470	3670	2310	47 ± 1	0.0078 ± 0.0004
8	1-Butanol	449	529	3370	3670	2320	40 ± 4	0.0112 ± 0.0004
9	THF ^[e]	456	531	3100	3460	2220	40 ± 3	0.0073 ± 0.0004
10	1-Pentanol	450	532	3430	3660	2360	38 ± 5	0.0156 ± 0.0019
11	1,4-Dioxane	459	535	3100	3440	2150	43 ± 4	0.0289 ± 0.0005
12	Cyclohexane	495	534	1480	3160	2110	49 ± 3	0.5010 ± 0.0167
13	1-Octanol	452	531	3290	3450	2280	37 ± 8	0.0321 ± 0.0019
14	DMF ^[e]	448	530	3450	3700	2300	42 ± 2	0.0104 ± 0.0021
15	1,2-Ethanediol	443	526	3560	3780	2260	34 ± 3	0.0185 ± 0.0015
16	Chloroform	455	535	3290	3780	2210	51 ± 4	0.0565 ± 0.0024
17	Benzene	463	538	3010	3400	2150	56 ± 2	0.1100 ± 0.0026
18	Toluene	463	537	2980	3500	2170	57 ± 8	0.1299 ± 0.0032
19	Chlorobenzene	460	536	3080	3440	2190	54 ± 4	0.0609 ± 0.0015

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[a] The solvents are numbered according to increasing refractive index, *n*. [b] The values of Stokes shift, fwhm_{abs} , and fwhm_{em} are

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rounded to the nearest 10 cm⁻¹. [c] The values of absorption coefficients, ϵ , are rounded to the nearest 100 M⁻¹ cm⁻¹. [d]

256

Fluorescence quantum yield ± one standard uncertainty. Φ determined using fluorescein in 0.1 N NaOH ($\Phi_f = 0.91$) as a reference.

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[e] THF = tetrahydrofuran, DMF = dimethylformamide.

Table 2. Spectroscopic and photophysical data of **3** as a function of solvent.

	Solvent ^[a]	$\lambda_{\text{abs}}^{\text{max}}$ [nm]	Band 1 $\lambda_{\text{em}}^{\text{max}}$ [nm]	Band 1 $\Delta\nu^{\text{[b]}}$ [cm ⁻¹]	Band 2 $\lambda_{\text{em}}^{\text{max}}$ [nm]	Band 2 $\Delta\nu^{\text{[b]}}$ [cm ⁻¹]	$\epsilon^{\text{[c]}}$ [10 ⁻³ M ⁻¹ cm ⁻¹]	$\Phi^{\text{[d]}}$
1	Methanol	466	516	2080	-	-	29 ± 2	0.0136 ± 0.0005
2	CH ₃ CN	462	520	2410	554	3590	28 ± 2	0.0181 ± 0.0004
3	Acetone	466	519	2190	561	3630	31 ± 2	0.0223 ± 0.0008
4	Ethanol	469	517	1980	-	-	31 ± 3	0.0154 ± 0.0005
5	Ethyl acetate	470	519	2010	563	3520	30 ± 3	0.0285 ± 0.0016
6	2-Propanol	471	518	1930	561	3410	28 ± 2	0.0229 ± 0.0008
7	1-Propanol	471	518	1930	561	3410	27 ± 2	0.0274 ± 0.0020
8	1-Butanol	473	518	1840	563	3380	28 ± 2	0.0236 ± 0.0009
9	THF ^[e]	472	521	1990	565	3490	27 ± 3	0.0220 ± 0.0010
10	1-Pentanol	473	520	1910	564	3410	30 ± 3	0.0185 ± 0.0081
11	1,4-Dioxane	472	519	1920	566	3520	30 ± 3	0.0392 ± 0.0027
12	Cyclohexane	488	522	1340	566	2820	32 ± 3	0.2592 ± 0.0469
13	1-Octanol	475	522	1900	566	3390	30 ± 2	0.0325 ± 0.0012
14	DMF ^[e]	467	520	2180	562	3620	28 ± 3	0.0322 ± 0.0039
15	1,2-Ethanediol	466	522	2300	-	-	22 ± 3	0.0332 ± 0.0018
16	Chloroform	472	523	2070	568	3580	31 ± 3	0.0174 ± 0.0013
17	Benzene	474	525	2050	570	3550	33 ± 2	0.0577 ± 0.0030
18	Toluene	476	525	1960	569	3430	33 ± 2	0.0640 ± 0.0032
19	Chlorobenzene	473	525	2090	569	3570	32 ± 3	0.0498 ± 0.0023

259 [a] The solvents are numbered according to increasing refractive index, *n*. [b] The values of Stokes shift are rounded to the nearest
260 10 cm⁻¹. [c] The values of absorption coefficients, ϵ , are rounded to the nearest 100 M⁻¹ cm⁻¹. [d] Fluorescence quantum yield ±
261 one standard uncertainty. Φ determined using fluorescein in 0.1 N NaOH ($\Phi_r = 0.91$) as a reference. [e] THF = tetrahydrofuran,
262 DMF = dimethylformamide.

Further differences between 2- and 3-substitution arose in the emission spectra. BODIPY dye **2** exhibits a single, broad emission band in all the tested solvents (see Figures 4B, S2, and Table 1), with λ_{em}^{max} ranging from 526 (in methanol and 1,2-ethanediol) to 538 nm (in benzene); importantly, with an overall blue-shift trend upon increasing the solvent dipolarity. Surprisingly, the emission maxima of compound **2** are hardly affected by solvent dipolarity. The difference in the emission maximum wavelength of this dye dissolved in methanol or 1,2-ethanediol and benzene is only 12 nm. The blue shift in the emission spectra with solvent dipolarity indicates that, contrary to what is expected, the excited state might present a lower dipolar moment than the ground state does.

In contrast, the emission spectra of compound **3** displayed distinct variations in the different solvents. A narrow emission band, centered around 570 nm, typical for BODIPY dyes, was the major contribution in apolar solvents, such as toluene, cyclohexane, or benzene. However, this band coexisted and was overlapped with a much broader emission, appearing in the 520 nm range, which was especially noticeable in alcohols and other polar solvents (Figures 4D and S3, and Table 2). This is a striking, distinct feature. BODIPY **3** exhibited a bimodal emission from two well-defined states: the high-energy, broad band with typical features of a weakly emissive ICT state, and a narrow band, characteristic of the emission from the LE state.

Figure 4. Normalized $S_1 \leftarrow S_0$ visible absorption bands (A) and normalized fluorescence emission spectra (B) of **2** in the solvents: methanol (green); 1-propanol (magenta); 1-octanol (black); 1,4-dioxane (blue); chloroform (cyan); toluene (dark yellow); cyclohexane (red). Normalized $S_1 \leftarrow S_0$ visible absorption bands (C) and normalized fluorescence emission spectra (D) of **3** in the solvents: methanol (green); 1-propanol (magenta); 1-octanol (black); 1,4-dioxane (blue); chloroform (cyan); toluene (dark yellow); cyclohexane (red).

Other parameters in Tables 1 and 2 highlight the different behavior of BODIPYs **2** and **3**. As expected, the average Φ of both dyes is largely affected by the different solvents. As expected for the presence of weakly emissive ICT states, the fluorescence emission is much more effective in apolar solvents, with average values quantum yield of 0.50 ± 0.02 and 0.26 ± 0.05 for **2** and **3**, respectively, dissolved in cyclohexane, whereas Φ is as low as 0.002 and 0.014 for **2** and **3**, respectively, dissolved in methanol. The inspection of the quantum yield values suggests the clear presence of a weakly emissive state, in polar solvents, for **2**; whereas the dual-band emission behavior of **3** may be responsible for a not as low quantum yield in polar solvents. Those results clearly hint that at least two and three different excited-state species are responsible for the emissive characteristics of **2** and **3**, respectively.

To get more insights into the photophysics of the studied dyes, time-resolved fluorometry was employed to extract the kinetics of the excited state and fluorescence lifetimes (decay times) of the dyes dissolved in six different solvents (Table 3 and Figures S4 and S5). Fluorescence decay traces of **2** were well fitted to monoexponential functions in many of the solvents studied. This confirms that a single major emissive species can be found. Two components were found when **2** was dissolved in chlorobenzene, 1-octanol, and methanol, exhibiting an additional very short decay time, which reduced the average lifetime down to 0.451, 0.432, and 0.166 ns, respectively. This fast decaying component must be responsible of the low quantum yield in polar solvents, supporting the presence of a weakly emissive ICT state. In contrast, BODIPY **3** clearly showed fluorescence decay traces that matched bi- or tri-exponential decay functions in all the solvents studied (Table 3). A much better description of the dynamic behavior of the emitting species can be obtained with time-resolved emission spectroscopy (TRES), by which fluorescence decay traces are collected over the entire emission range (see SI for experimental details). The TRES spectra of **3** in chloroform (Figure 5A) and acetonitrile (Figure 5B) exhibited similar features: the LE, low-energy emission band was the first being depopulated, but initially, emission contributions were detected from the two bands; then, the high-energy band was responsible for the later emission. Depopulation of the LE band was definitively faster in acetonitrile than in chloroform. The low-energy band was negligible after 3 ns when **3** was dissolved in acetonitrile, whereas it still persisted after 10 ns when dissolved in chloroform. In methanol (Figure 5C), the narrow LE band was already negligible just after excitation.

Importantly, the emission spectrum associated with each of the emitters can be extracted through the species-associated emission spectra (SAEMS). As one can observe from the SAEMS of **3** in chloroform (Figure 5D), acetonitrile (Figure 5E), and methanol (Figure 5F), the short-lived component included in all three cases the narrow LE emission and a certain contribution of the high-energy band, whereas the long-lived component was exclusively composed of the

high-energy, broad band. Interestingly, the relative weight of the two components was solvent-dependent, with a higher contribution of the short component in the less polar solvents. This situation suggests a competing kinetic mechanism for **3**, evidenced in the average fluorescence decay time values (Table 3), which exhibited large values in apolar and polar solvents, due to the different emitting states in each environment.

Table 3. Fluorescence lifetimes (decay times) of **2** and **3** in selected solvents.

Compound	Solvent	$\tau_1(\text{ns})^{[a]}$	$\tau_2(\text{ns})^{[a]}$	$\tau_3(\text{ns})^{[a]}$	$\tau_{\text{ave}}(\text{ns})^{[b]}$
2	Cyclohexane	3.369	-	-	3.369
	Toluene	0.777	-	-	0.777
	Benzene	0.660	-	-	0.660
	Chlorobenzene	1.596	0.390	-	0.451 ± 0.002
	1-Octanol	2.278	0.363	-	0.432 ± 0.001
	Methanol	3.362	0.057	-	0.191 ± 0.008
3	Cyclohexane	3.791	2.644	-	3.683 ± 0.011
	Toluene	3.837	2.025	0.364	2.182 ± 0.026
	Benzene	3.770	2.001	0.289	2.240 ± 0.035
	Chlorobenzene	3.850	1.966	0.201	2.245 ± 0.038
	1-Octanol	4.307	1.770	0.173	2.691 ± 0.113
	Methanol	4.365	0.521	-	3.234 ± 0.076

[a] Fluorescence lifetimes (decay times) obtained with $\lambda_{\text{ex}} = 440$ nm, as global fits of three fluorescence decay traces collected at 525, 530, and 535 nm for **2**, and 560, 565, and 570 nm for **3**. The standard errors of the individual decay times (obtained from the global analysis fit of decay traces recorded at three emission wavelengths) were between 5 and 30 ps. [b] Average fluorescence decay time was calculated using equation S2 in SI. The error associated to the average fluorescence decay time is the standard deviation of the values obtained at the three different emission wavelengths.

Figure 5. TRES spectra of BODIPY **3** dissolved in chloroform (A), acetonitrile (B), and methanol (C) at 0, 0.5, 0.8, 1.0, 1.2, 1.5, 3.0, 5.0, 8.0, 10.0, and 15.0 ns after photoexcitation. SAEMS of the two different components (short lifetime, black line; long lifetime, red line), as obtained in TRES experiments, for BODIPY **3** dissolved in chloroform (D), acetonitrile (E), and methanol (F).

Among the unusual spectroscopic properties of BODIPYs **2** and **3**, perhaps the most striking one was the spectral shifts found in absorption and emission with solvent dipolarity, exhibiting an inverse behavior when compared to Prodan and its analogues. A hypsochromic shift with solvent dipolarity is a typical feature of merocyanine, solvatochromic dyes. In these dyes, the formation of a stable zwitterionic species leads to a further decrease in energy of the ground

state, causing blue shifts. Therefore, to rationalize solvatochromic bathochromic and hypsochromic shifts in absorption and/or emission bands of probes it is important to consider two different types of solvent contributions, namely non-specific (or general) interactions (dielectric solute-solvent interactions) and specific interactions (such as hydrogen bonding). To unravel the solvent effects on the spectral features of a dye, multi-parameter solvent scales have been proposed and applied to various physicochemical parameters.[1] A multi-parameter methodology was proposed by Kamlet and Taft, who described the solvent effect in terms of α , β , and π^* . [49-51] Kamlet and Taft's α and β take into account specific interactions involving hydrogen bonding, whereas the π^* parameter is a measure of mixed effects of solvent dipolarity and polarizability. Alternatively, Catalán and co-workers proposed a generalized treatment of the solvent effect based on four empirical, complementary, mutually independent solvent scales, *i.e.*, two general scales (dipolarity and polarizability) and two specific scales (acidity and basicity).[52] The polarizability and dipolarity of a particular solvent are characterized by the indices SP and SdP, respectively, whereas the scales SA and SB define solvent acidity and basicity, respectively. Mathematically, the solvent effect on the physicochemical observable y can be expressed by the multi-linear expression (1):

$$y = y_0 + a_{SA} SA + b_{SB} SB + c_{SP} SP + d_{SdP} SdP \quad (1)$$

where y_0 stands for the physicochemical property of interest in the gas phase; a_{SA} , b_{SB} , c_{SP} , and d_{SdP} are (adjustable) regression coefficients that reflect the sensitivity of the property y to the individual solvent-solute interaction contributions; and SA, SB, SP, and SdP are the mutually independent solvent parameters. This methodology can determine not only which solvent property is responsible for the solvatochromism, but crucially, it also quantifies the extent of the contribution of each individual solvent property to the measured sensitivity to the environment. This methodology is thus superior to other conventional solvatochromism formalisms, such as the Lippert-Mataga equation,[53] which correlates the dye's Stokes shift ($\Delta\bar{\nu}$) with the change of its dipole moment $\Delta\mu_{GE} = \mu_E - \mu_G$ upon excitation and the solvent's

orientation polarizability (Δf). Therefore, we used the generalized Catalán methodology[52] to probe which solvent properties contribute mostly to the solvent sensitivity of the observed shifts of the absorption maxima $\bar{\nu}_{\text{abs}}$ ($= 1/\lambda_{\text{abs}}^{\text{max}}$) and the fluorescence emission maxima $\bar{\nu}_{\text{em}}$ ($= 1/\lambda_{\text{em}}^{\text{max}}$) of **2** and **3**, and compare their features with Prodan and other analogues.

The results of the fits of $y = \bar{\nu}_{\text{em}}$ and $y = \bar{\nu}_{\text{abs}}$ of **2** according to equation (1) are compiled in Table S3 (Supplementary Material). The multi-linear regression using all four solvent scales for $\bar{\nu}_{\text{em}}$ and $\bar{\nu}_{\text{abs}}$ produced excellent fits, using the correlation coefficient r as goodness-of-fit criterion ($r = 0.953$ and $r = 0.925$, for the fits of $\bar{\nu}_{\text{em}}$ and $\bar{\nu}_{\text{abs}}$, respectively), resulting in equations (2) and (3), expressed in 10^3 cm^{-1} . Analysis of the F -statistic of the fit allows to confirm (at 99% of confidence level) that the correlation is significant.[#]

$$\bar{\nu}_{\text{em}} = 19.2 \pm 0.2 + (0.21 \pm 0.06) \text{ SA} + (0.02 \pm 0.05) \text{ SB} - (0.77 \pm 0.20) \text{ SP} + (0.23 \pm 0.05) \text{ SdP} \quad (2)$$

$$\bar{\nu}_{\text{abs}} = 19.9 \pm 0.8 + (0.3 \pm 0.3) \text{ SA} + (0.5 \pm 0.3) \text{ SB} + (1.2 \pm 1.0) \text{ SP} + (1.6 \pm 0.2) \text{ SdP} \quad (3)$$

By inspecting the parameter values for the fit of $\bar{\nu}_{\text{em}}$ of **2** in eqn. (2) one can conclude that those with the highest weight and most precisely determined were a_{SA} , c_{SP} , and d_{SdP} . Interestingly, two competing factors can be inferred. Whereas the increase in solvent polarizability causes red shifts in $\lambda_{\text{em}}^{\text{max}}$ (*i.e.*, lower $\bar{\nu}_{\text{em}}$ values), the solvent dipolarity and specific effects through solvent acidity promote a blue shift in $\lambda_{\text{em}}^{\text{max}}$ (*i.e.*, larger $\bar{\nu}_{\text{em}}$ values). Overall, the positive estimates of a_{SA} and d_{SdP} controlled the final blue-shift trend in $\lambda_{\text{em}}^{\text{max}}$ with increasing solvent acidity and dipolarity, observed in Table 1 in going from cyclohexane (apolar solvent, $\lambda_{\text{em}}^{\text{max}} = 534 \text{ nm}$, SA = 0.000, SP = 0.683, SdP = 0.000) to the polar solvents DMF (aprotic solvent, $\lambda_{\text{em}}^{\text{max}} = 530 \text{ nm}$, SA = 0.031, SP = 0.759, SdP = 0.977) or methanol (protic solvent, $\lambda_{\text{em}}^{\text{max}} = 526 \text{ nm}$, SA = 0.605, SP = 0.608, SdP = 0.904). These competing effects justify the low solvatochromic features of compound **2**. Similarly, the Catalán analysis of the solvatochromic shifts of $\bar{\nu}_{\text{abs}}$ of **2** [eqn. (3)] reflected that the absorption energy was predominantly controlled by changes in dipolarity of the environment of the dye, as supported

by the largest and most precise d_{SdP} estimate (*i.e.*, relatively small standard error of ca. 15%) compared with the smaller and imprecise a_{SA} , b_{SB} , and c_{SP} estimates. This is corroborated by the fact that removing the SdP solvent scale from the multi-linear fit caused a decrease in the r value down to 0.648. Likewise, the linear correlation of $\bar{\nu}_{\text{abs}}$ vs SdP gave a reasonably good fit ($r = 0.883$). The large positive d_{SdP} estimate clarifies the experimental large blue shift of $\lambda_{\text{abs}}^{\text{max}}$ (*i.e.*, larger $\bar{\nu}_{\text{abs}}$ values) with increasing solvent dipolarity, found in Table 1 in going from cyclohexane ($\lambda_{\text{abs}}^{\text{max}} = 495$ nm, SdP = 0.000) to methanol ($\lambda_{\text{abs}}^{\text{max}} = 443$ nm, SdP = 0.904). Importantly, the fact that solvent's acidity only affects noticeably to the emission and not to the absorption suggests that hydrogen-bonding-facilitated charge separation only occurs in the excited state. This hampers the vision of a merocyanine-like zwitterionic form in the ground state. A thorough discussion on the different fittings to unequivocally identify the most important solvent features defining $\bar{\nu}_{\text{em}}$ and $\bar{\nu}_{\text{abs}}$ of compound **2** can be found in the Supplementary Material and Table S3 and Figure S6.

We performed a similar correlation study with the spectral features of BODIPY **3**, namely the two emission maxima, $\bar{\nu}_{\text{em},1}$ and $\bar{\nu}_{\text{em},2}$, corresponding respectively to the high- and low-energy emission bands, and the absorption maxima, $\bar{\nu}_{\text{abs}}$, from Table 2. The Catalán analysis of the high-energy emission maxima $\bar{\nu}_{\text{em},1}$ of **3** using the four solvent scales resulted in eqn. (4), with a good correlation ($r = 0.914$). For this emission band, unequivocally, the most contributing factor was the solvent polarizability, SP. The large and well-defined negative value of the coefficient c_{SP} supports that increasing solvent polarizability causes a red shift in the emission band. This was confirmed with the very good linear correlation of the $\bar{\nu}_{\text{em},1}$ values with only the SP solvent scale ($r = 0.866$) and the high frequency polarizability function $f(n^2) = (n^2 - 1)/(2n^2 + 1)$ [2] ($r = 0.894$). This dependency suggests that the accommodation of the electrons of solvent molecules within the solvation shell is the preferred mechanism for relaxation from this emissive state. However, as there are not large polarizability differences between the chosen solvents, the solvent effects over this band were very small. In contrast, the multi-

linear analysis of the low-energy band, $\bar{\nu}_{em,2}$, indicated that both polarizability and dipolarity were the main factors controlling the spectral shifts (eqn. (5), $r = 0.936$). In all the possible correlations, using different combinations of the solvent scales, good fits were obtained as long as both the SP and SdP scales were included (Table S4). Using the two scales responding to the general solvent effects, the correlation coefficient r obtained was 0.918. Similarly to what occurred in BODIPY **2**, opposite effects were in place, with increasing polarizability resulting in red shifts and increasing dipolarity promoting blue shifts. The overall effect was a blue shift, because larger differences are found in the dipolarity of the chosen solvents. Finally, the analyses of the absorption maxima $\bar{\nu}_{abs}$ of **3** unambiguously demonstrated that solvent dipolarity is the dominant factor that affects the absorption energy (eqn. (5), $r = 0.910$). Similar to the behavior of compound **2**, the positive d_{SdP} estimate is in conformity with the experimental blue shift of λ_{abs}^{max} of **3** with increasing solvent dipolarity, seen in Table 2 in going from cyclohexane (apolar, aprotic solvent, $\lambda_{abs}^{max} = 488$ nm) to the polar solvents acetonitrile (aprotic, $\lambda_{abs}^{max} = 462$ nm) or methanol (protic, $\lambda_{abs}^{max} = 466$ nm). Likewise, further discussion on the complete analyses of compound **3** can be found in the Supplementary Material and Table S4 and Figure S7.

$$\bar{\nu}_{em,1} = 19.9 \pm 0.2 + (0.02 \pm 0.06) SA + (0.07 \pm 0.05) SB - (1.0 \pm 0.20) SP + (0.07 \pm 0.05) SdP \quad (4)$$

$$\bar{\nu}_{em,2} = 18.6 \pm 0.2 + (0.02 \pm 0.20) SA - (0.10 \pm 0.08) SB - (1.4 \pm 0.3) SP + (0.27 \pm 0.06) SdP \quad (5)$$

$$\bar{\nu}_{abs} = 20.6 \pm 0.4 - (0.06 \pm 0.15) SA - (0.09 \pm 0.13) SB + (0.2 \pm 0.5) SP + (0.9 \pm 0.1) SdP \quad (6)$$

It is interesting to compare the unusual solvatochromic behavior of BODIPYs **2** and **3**, structurally analogues of Prodan, with the features of those dyes listed in Figure 1. It must be emphasized that λ_{em}^{max} of Prodan[4] and other analogues[18, 19] moves in the opposite direction than BODIPYs **2** and **3**, involving large red shifts in going from an apolar, aprotic (hexane or toluene) to a polar and/or protic (methanol or water).

We also considered the influence of the viscosity of the solvent as a potential source of variation of the spectral features of dyes **2** and **3**. The selected aliphatic alcohol solvents that we selected are an interesting family, since they are the only acidic solvents and covering a broad range of viscosity values (between 0.5 and 16 mPa·s). The environment viscosity may have an important effect in spectral properties of BODIPY dyes with rotor-like substituents. In order to avoid this effect, we chose for the *meso*- substituent a 2,4,6-trimethyl-phenyl group, which should ensure orthogonality between the *meso*- phenyl ring and the BODIPY core. Although some spectral features, such as $\bar{\nu}_{\text{abs}}$, $\bar{\nu}_{\text{em}}$ and quantum yield, exhibited notable trends with viscosity in alcohols (Figures S8 and S9), the fact that these trends do correlate well with the intrinsic tendency of variation of viscosity with dipolarity and polarizability of the alcohol (see Figure S10) allows to infer that the effect of viscosity is negligible. This is likewise confirmed, given the spectral features of **2** and **3** are well predicted by the Catalan solvent scales, for both alcohol and non-alcohol solvents (see Figures S6 and S7), regardless of the viscosity of the environment.

In order to unveil the nature of the emissive states and the complex photophysical properties of compounds **2** and **3**, next we performed quantum chemical calculations.

4. Quantum chemical calculations

The potential energy surfaces (PES) of both low-lying and higher-lying excited states of **2** and **3** were explored with time-dependent density functional theory (TD-DFT). The PBE0 functional is used in the TD-DFT excited state optimizations. In Table S5 we report the TD-DFT results using two different functionals for **2**, i.e., PBE0 and CAM-B3LYP. PBE0 reproduces more accurately the bright $\pi\pi^*$ state (S_1) while the $n\pi^*$ state is located more accurately with CAM-B3LYP (as compared with the ADC(2) estimates, see discussion below). Thus, as expected, the range-separated CAM-B3LYP functional reproduces better the excited state characterized by a larger

amount of charge transfer character, i.e., the $n\pi^*$ state (see its large charge transfer D distance and its small hole-electron overlaps S_i in Table S5). Aiming at attaining a balanced description between both local and charge transfer excited states, single point second-order algebraic diagrammatic construction [ADC(2)] calculations were performed at relevant stationary points of the excited-state PES (see details in the Experimental Section). Due to the so-called “cyanine challenge”, [54] obtaining accurate excited-state properties of BODIPY dyes is still challenging for TD-DFT-based methodologies. In this regard, the ADC(2) method has shown to yield accurate estimates of the excited-state properties of BODIPY dyes, [55] and thus in the following we mainly describe the ADC(2) results for **2** and **3**. As mentioned above, rather low quantum yields along with complex excited-state decay kinetics involving several excited-state species have been measured for these two BODIPY dyes, especially for **3**. These characteristics are rarely found in BODIPY dyes, which often display very high Φ values and mono-exponential fluorescence decays.

We first modeled the UV-vis absorption characteristics of **2** and **3** in two different solvents: toluene and acetonitrile (see results in Table 4). Solvent effects were included in the ADC(2) calculations using the recent implementation of the conductor-like screening model (COSMO). [43] Two excited states (S_1 and S_2) contribute to the UV-vis spectrum of **3** (see the orbitals involved in these states in Figure 6) while a few more dark states are found for **2** (see Table 4). Regardless of the BODIPY dye, the spectroscopic state is always S_1 , which is a very bright $\pi\pi^*$ state involving the BODIPY core, with a small participation of the dialkylamino group (see Figure 6). S_1 is thus responsible of the absorption characteristics of these dyes. Note, that the computed COSMO-ADC(2) excitation energies perfectly match the experimental evidence, compare *e.g.* the computed absorption maxima in toluene for **2** and **3** (466 and 483 nm, respectively), with those measured experimentally (463 and 476 nm). Note that in agreement with the experiments, the computed shifts in the absorption spectra are rather small, considering the different properties of the selected solvents. Higher in energy are found dark

states, such as the $n\pi^*$ state shown in Figure 6, which involves the lone pair of the acetyl moiety and corresponds to S_2 and S_4 for **3** and **2**, respectively (see Table 4). In the case of **2**, two additional dark states of charge transfer $\pi\pi^*$ character are found below the $n\pi^*$ state (see the orbitals involved in the excited states of **2** in Figure S11). In general, dark states, although not easily populated at the Franck-Condon region, might be populated in the course of photodeactivation through internal conversion processes, and thus they might play a role in the excited decay dynamics.

Figure 6. Orbitals involved in the S_1 and S_2 states of **3**.

Table 4. COSMO-ADC(2)/def2-TZVP^[a] singlet electronic excitation energies (in eV) in toluene and acetonitrile (values between parentheses), and oscillators strengths f (in a.u.) of **2** and **3**.

Compound	State (Character)	Exc. Energy	f (gas phase)	f (toluene) ^[b]	f (ACN) ^[b]
2	$S_1 (\pi\pi^*)$	2.663 (2.631)	0.745	0.868	0.847
	$S_2 (\pi\pi^*)$	3.804 (3.762)	0.000	0.000	0.000
	$S_3 (\pi\pi^*)$	3.886 (3.857)	0.057	0.000	0.021
	$S_4 (n\pi^*)$	4.108 (4.035)	0.000	0.000	0.000
3	$S_1 (\pi\pi^*)$	2.569 (2.502)	0.624	0.727	0.690
	$S_2 (n\pi^*)$	3.706 (3.610)	0.032	0.001	0.000

[a] These COSMO calculations are equilibrated for the ground state. [b] Obtained from PCM-TD-PBE0/6-31+G(d) calculations.

Next, to model the emission processes but also to rationalize the complex photophysics, we explored the singlet excited-state PES, so to capture relevant relaxation effects upon photodeactivation. Thus, we performed TD-DFT optimizations of the first five excited states to locate excited-state minima (see computational details in the experimental section). Indeed, with this protocol, we were able to obtain four different PES minima for compounds **2** and **3**. COSMO-ADC(2) single-point emission energies were performed on the optimized excited-state structures. Exemplarily, in Figure 7 are shown the different deactivation pathways opened up upon light irradiation for **3** in toluene. We recall that **3** displays experimentally the most complex behavior among the BODIPY dyes explored herein. Note that the results presented in Figure 7 are actually a qualitative representation of the PES landscapes because no minimum energy paths connecting the different minima have been explicitly calculated. In the $\pi\pi^*$ PES three different minima were obtained, *i.e.*, $(\pi\pi^*)_{\text{min1}}$, $(\pi\pi^*)_{\text{min2}}$, and a twisted ICT minimum (*i.e.*, TICT_{min}). The $\pi\pi^*$ deactivation coordinate mainly involves the rotation of the dialkylamino group. Note that the dihedral angle characterizing such a rotation increases from 34.3° to 44.7° when going from $(\pi\pi^*)_{\text{min1}}$ to $(\pi\pi^*)_{\text{min2}}$ and it reaches a maximum value of 86.7° at the TICT_{min} minimum (see Figure 7). Large oscillator strengths are obtained at the $(\pi\pi^*)_{\text{min1}}$ and $(\pi\pi^*)_{\text{min2}}$ minima ($f = 0.562$ and 0.493 , respectively), in principle enabling fluorescence from these two minima. The COSMO-ADC(2) computed emission energies from $(\pi\pi^*)_{\text{min1}}$ is 563 nm, which is in perfect agreement with the experimental 0-0 band of **3** (569 nm), see Figure 4d. Thus, among $(\pi\pi^*)_{\text{min1}}$ and $(\pi\pi^*)_{\text{min2}}$, likely $(\pi\pi^*)_{\text{min1}}$ is only responsible for this narrow LE emission, which is responsible for the short-live component of **3**. This is the only emission band observed for **2**, and our calculations also support the fact of $(\pi\pi^*)_{\text{min1}}$ being solely responsible for its emissive characteristics (see Table 5).

Table 5. COSMO-ADC(2)/def2-TZVP^[a] singlet electronic emission energies (in eV) at different excited-state minima in toluene and acetonitrile for **2** and **3**.

		$(\pi\pi^*)_{\min 1}$	TICT _{min}	$(n\pi^*)_{\min}$
2	Toluene	$S_1(\pi\pi^*)$: 2.200	-	$S_2(n\pi^*)$: 3.343 / $S_1(\pi\pi^*)$: 2.445
	CH ₃ CN	$S_1(\pi\pi^*)$: 2.066	-	$S_2(n\pi^*)$: 2.589 / $S_1(\pi\pi^*)$: 2.442 ^b
3	Toluene	$S_1(\pi\pi^*)$: 2.202	S_1 : 0.897	$S_2(n\pi^*)$: 2.952 / $S_1(\pi\pi^*)$: 2.441
	CH ₃ CN	$S_1(\pi\pi^*)$: 2.200	S_1 : 0.095	$S_2(\pi\pi^*)$: 2.354 / $S_1(n\pi^*)$: 2.195

[a] These COSMO calculations are equilibrated for the emissive state. [b] Non-equilibrated emission energy.

In the following, we address the origin of the measured high-energy band for **3**, which is responsible for its long-lived component. Thus, in addition to the $\pi\pi^*$ channel, we successfully optimized a minimum in the $S_2(n\pi^*)$ PES (see the $n\pi^*$ deactivation pathway in Figure 7). As mentioned above, the $n\pi^*$ state is a second dark-state. The main geometrical relaxation involves the stretching of the C=O distance (compare 1.22 Å with 1.27 Å at the ground state and at the $(n\pi^*)_{\min}$ optimized minima, respectively). The $n\pi^*$ state is vertically located 4.11 and 3.71 eV above the ground state for **2** and **3**, respectively (see Table 4). Under the experimental conditions it is unlikely that this state will be directly populated. However, it can be populated in the course of photodeactivation. The well of the $n\pi^*$ state for **3** is deeper in energy than for **2** (compare e.g., 2.195 vs 2.598 eV in acetonitrile, respectively in Table 5). Thus, due to its higher energetic accessibility, the population of the $n\pi^*$ well is more likely to happen in **3** than in **2**. Furthermore, in toluene, the $n\pi^*$ state remains the second excited state, and it is thus located above the bright $\pi\pi^*$ state at $(n\pi^*)_{\min}$, (see Figure 7) whereas the order between the states is reversed in acetonitrile. Therefore, in toluene internal conversion from $n\pi^*$ to the lowest lying $\pi\pi^*$ state is likely, from where emission of a photon will predominantly occur (see Figure 7). Note that the computed $\pi\pi^*$ emission energy at $(n\pi^*)_{\min}$ is 2.44 eV (508 nm), and thus this excited-state minimum might be responsible for the high-energy band (slow component) of **3**, which peaks at 525 nm. Thus, the dual emission observed in toluene is explained for **3**. Conversely, in acetonitrile, the excited-state ordering is reversed, and the $n\pi^*$

state becomes the lowest-lying excited state (see Table 5), from where non-radiative deactivation to the ground state is the only possibility. Therefore, population of this channel in acetonitrile is likely linked to the enhanced radiationless deactivation processes experimentally observed in polar solvents, exhibiting a much lower quantum yield. Further theoretical studies (e.g., excited state dynamics simulations) are needed to corroborate these hypotheses but also to unambiguously decipher the actual mechanisms of dual photoluminescence. We also note that, for instance, in the case of toluene, where a larger adiabatic energy gap between the $\pi\pi^*$ and the $n\pi^*$ states, the emission process might be facilitated *via* vibronic coupling between these excited states.

In a nutshell, as disclosed by our calculations, different non-radiative decays (involving *i.e.*, the TICT and/or the $n\pi^*$ state) are opened up for **2** and **3** in different environments, which likely explain the differences observed experimentally.

5. Conclusions

The rational design of novel fluorophores is an important field, which combines organic chemistry, physical chemistry, and spectroscopy. Being capable of preparing new dyes, with tailored properties, will pave the way to increasingly challenging biophysical and biomedical applications.

Based on the combination of electron-releasing and electron-withdrawing groups linked to an aromatic moiety, we have synthesized and characterized two new BODIPY dyes, with diethylamino and acetyl groups in different places of the BODIPY core, maximizing the possibility of an ICT excited state, in analogy with the structural arrangement of Prodan, the best known solvatochromic dye. However, the fact that the BODIPY moiety is electron-deficient provides the new compounds with unusual spectroscopic and photophysical properties, namely, dual-band absorption and emission bands, low quantum yields, and

distinct solvent effects on the luminescence properties. Solvent dipolarity and polarizability cause opposite effects over the emission of dyes **2** and **3**. Overall, these competing effects result in a very low anti-solvatochromic behavior of these dyes, in huge contrast with Prodan and other analogues. The behavior of **2** and **3** also contrast that of hysochromic merocyanines, with stabilized ground-state zwitterions.

Importantly, state-of-the-art quantum chemistry calculations have allowed us to understand the deactivation mechanisms of the dyes, as well as the sources of the dual emission behavior of compound **3**. Such experiments have clearly shown the different effect of the position of the acetyl group within the BODIPY core on the molecular orbitals of the molecules. Importantly, as demonstrated by our theoretical investigations, the TICT state is responsible for the very low emission quantum yields of these BODIPY dyes.

These BODIPY dyes clearly exemplify the importance of understanding complex photophysics in the design of new luminescent probes.

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Notes and references

[#] **Note:** given N is the number of data points used in the fit, df represents the degree of freedom and F is the F -statistic of the fit, these parameters can be used to assess the likelihood of a larger F value occurring by chance by comparing F with critical values in published $F(v_1, v_2)$

distribution tables or obtained through dedicated software. In the fits of $\bar{\nu}_{em}$ and $\bar{\nu}_{abs}$ of compound **2**, resulting in eqns. (2) and (3), the F values were 35 and 21, respectively. The critical F value from the $F(\nu_1, \nu_2)$ distribution has ν_1 and ν_2 degrees of freedom, with $\nu_1 = N - df - 1 = 19 - 14 - 1 = 4$, and $\nu_2 = df = 14$. Assuming a probability level α of 0.01 (1%), standing for the probability of erroneously concluding that there is a relationship, the critical level of $F(\nu_1, \nu_2)$ is 5.04. Since the F values of the fits in eqns. (2) and (3) are substantially higher than 5.04, it is extremely unlikely that an F value this high occurred by chance. Hence, with $\alpha = 0.01$, the hypothesis that there is no relationship between $\bar{\nu}_{em}$ or $\bar{\nu}_{abs}$ and the parameters $\{[SA, SB, SP, SdP]\}$ must be ruled out when F exceeds the critical level, 5.04. Similar analyses can be done for all the multi-linear analyses reported in Tables S3 and S4 (Supplementary Material).

[§] **Note:** The multi-linear equation found for the $\bar{\nu}_{em}$ values of Prodan, using just the SA and SdP solvent scales, was $\bar{\nu}_{em} = [(24.8 \pm 0.3) - (3.3 \pm 0.4) SA - (3.3 \pm 0.5) SdP] \times 10^3 \text{ cm}^{-1}$ ($r = 0.979$). For Anthradan, the corresponding equation was: $\bar{\nu}_{em} = [(20.5 \pm 0.2) - (3.0 \pm 0.3) SA - (2.7 \pm 0.3) SdP] \times 10^3 \text{ cm}^{-1}$ ($r = 0.983$). These results pointed clearly to the central role of solvent acidity (SA) and dipolarity (SdP) in shifting $\bar{\nu}_{em}$.

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