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Water-soluble BODIPY dyes: a novel approach for their sustainable chemistry and applied photonics†

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The BODIPY family of organic dyes has emerged as a cornerstone in photonics research development, driving innovation and advancement in various fields of high socio-economic interest. However, the majority of BODIPY dyes exhibit hydrophobic characteristics, resulting in poor solubility in water and other hydrophilic solvents. This solubility is paramount for their optimal utilization in a myriad of photonic applications, particularly in the realms of biology and medicine. Furthermore, it facilitates safer and more sustainable manipulation and chemical modification of these expansive dyes. Nevertheless, bestowing BODIPYs with water solubility while preserving their other essential properties, notably their photophysical signatures, poses a significant challenge. In this context, we present a straightforward general chemical modification aimed at converting conventional hydrophobic BODIPYs into highly hydrophilic variants, thus enabling their efficient solubilization in water and other hydrophilic solvents with minimal disruption to the dye's inherent photophysics. The efficacy of this methodology is demonstrated through the synthesis of a number of water-soluble BODIPY dyes featuring diverse substitution patterns. Furthermore, we showcase their utility in a spectrum of photonics-related applications, including in-water BODIPY chemistry and dye-laser technology, and fluorescence microscopy.

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Introduction

Water stands out as the most sustainable medium for reactions and processes due to its abundance, affordability, non-toxicity, and safe handling characteristics.¹ Nature itself has chosen water as the primary medium for biological chemistry, where all organic reactions and life-sustaining interactions occur.²

Additionally, the growing environmental awareness in recent years has heightened the importance of utilizing water as the preferred medium over organic solvents.³ Unlike organic solvents, which contribute to smog, air pollution, ozone production, and ultimately climate change, water offers a cleaner and more environmentally friendly alternative.^{1,3}

Photonics has played a pivotal role in spearheading numerous innovations that have reshaped our lifestyles in recent years, with applications spanning a broad spectrum of fields, ranging from energy to healthcare. Many indispensable photonic applications thrive in aqueous environments, extending far beyond fluorescence bioimaging⁴ and photodynamic therapy (PDT).⁵ These include optical sensing of analytes in aqueous environments,⁶ photocatalytic water purification⁷ and hydrogen production,⁸ artificial photosynthesis,⁹ underwater wireless optical communications,¹⁰ and optogenetics,¹¹ among others.

In most of these applications, particularly in biology and medicine, molecular organic dyes play a fundamental role owing to their organic nature and compact size, which offer key advantages such as high biocompatibility and membrane permeability, or facile adaptation through organic chemistry.¹² Among these dyes, BODIPYs (boron dipyrromethenes; see Fig. 1) stand out as a noteworthy family of highly-fluorescent and photophysically adaptable organic dyes.¹³

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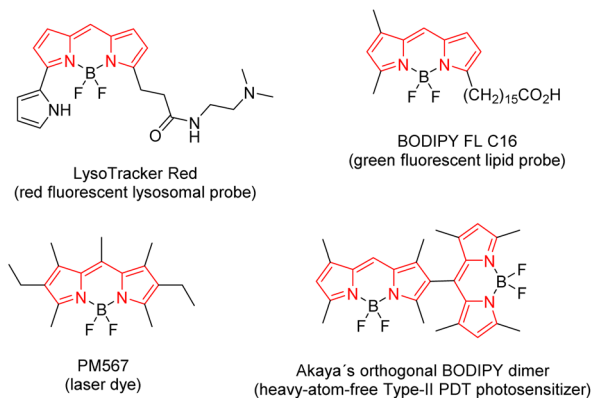
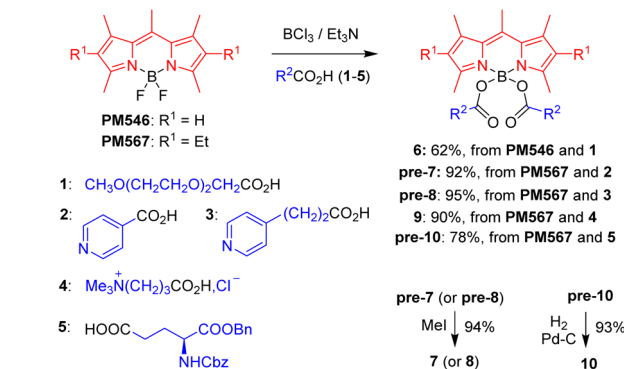


Fig. 1 Selected examples of useful BODIPY dyes (dipyrrin ligand in red).^{13a–d}

However, the hydrophobic nature of the BODIPYs poses a significant limitation in their optimal use in photonic applications that require a proper solubility in pure water or in highly hydrophilic media. In this context, several approaches have been developed to date to impart water solubility to BODIPYs.¹⁴ Unfortunately, most of them are not straightforward and involve the covalent attachment of hydrophilic moieties to the dye's π -conjugated core (dipyrrin ligand). This often leads to significant distortions in the photophysics of the original dye, as the BODIPY chromophore resides within such a π -conjugated core.

In this regard, a few recent approaches have focused on introducing the hydrophilic moieties at the BODIPY's boron centre, which does not participate in the chromophoric system, thus preventing photophysical distortion. This is usually achieved by creating B–O bonds through well-known boron fluorine substitution reactions using *O*-nucleophiles to construct water-soluble 4,4-dioxygenated BODIPYs (*O*-BODIPYs; *e.g.*, see Fig. 2).^{14e,j,o,q} Unfortunately, these methods are far from being efficient, general and/or straightforward, and the obtained BODIPY derivatives may suffer from significant chemical



Scheme 1 Synthesis of COO-BODIPYs 6–10 with hydrophilic moieties of different nature pending at boron. Bn: benzyl; Cbz: (benzyloxy) carbonyl. See the ESI† for experimental details.

instability due to the change from the strongly electron-withdrawing difluoroboron moiety in common BODIPY dyes (*F*-BODIPYs) to the less electron-withdrawing dioxygenated boron in the *O*-BODIPYs.

In connection with this interest, we have recently established a general procedure that allows for the effective transformation of BODIPY into 4,4-diaxyloxy derivatives (aka *COO*-BODIPYs) under mild reaction conditions in a single synthetic step, and using carboxylic acids as readily-available *O*-nucleophilic reagents (*e.g.*, see Scheme 1).^{15a} Notably, *COO*-BODIPYs are very robust and exhibit photophysical behaviours even superior to those of the corresponding *F*-BODIPYs,¹⁵ with their chemical stability under harsh acidic conditions being significantly greater than that of related *O*-BODIPYs. Since this soft synthetic method is compatible with a large number of functional groups, it should be easily extended to carboxylic acids functionalized with highly hydrophilic moieties, thus promoting water solubility. In this work, we demonstrate this possibility by reporting a specific methodology based on the preferential use of commercial γ -butyrobetaine hydrochloride as the solubilizing reagent, enabling the effective transformation of a wide range of *F*-BODIPYs (standard BODIPYs) into the corresponding water-soluble *COO*-BODIPYs,¹⁶ and showcasing its effectiveness and utility in a number of applications, particularly in aqueous green BODIPY chemistry and dye-laser technology, and fluorescence microscopy.

Results and discussion

Setting the method

The ideal chemical methodology for converting hydrophobic BODIPYs into hydrophilic BODIPYs should be straightforward and cost-effective, use inexpensive and readily available reagents, and efficiently enhance solubility while preserving the photophysical properties of the original dye. Accordingly, we first selected five marketed, cheap carboxylic acid derivatives possessing hydrophilic moieties, or specific functions acting as precursors of these moieties. Particularly, [2-(2-methoxyethoxy)ethoxy]acetic acid (1), isonicotinic acid (2), 3-(pyridin-4-yl)propanoic acid (3), (3-carboxypropyl)trimethylammonium

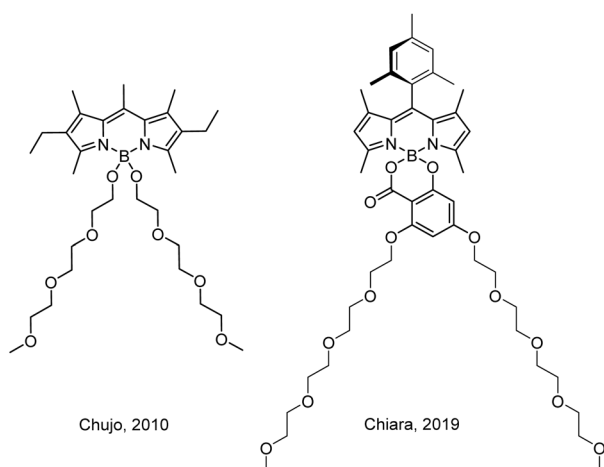


Fig. 2 Examples of hydrophilic *O*-BODIPY dyes based on highly-hydrophilic PEG chains.^{14e,q}



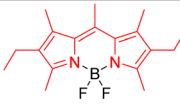

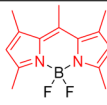
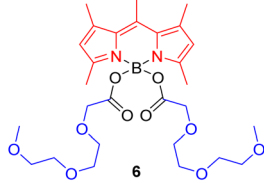
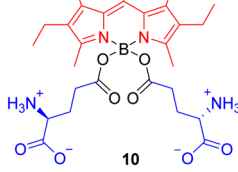
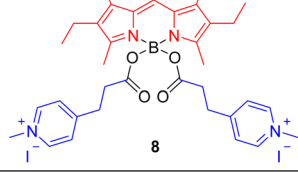
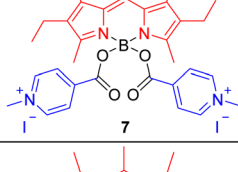
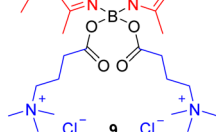
For the synthesis of **7**, **8** and **10**, a second step was needed to construct the hydrophilic group: nitrogen quarternisation in the case of **7** and **8**, or *N*-Cbz and *O*-Bn deprotections by catalytic hydrogenation in the case of **10**. These steps proceeded smoothly in almost quantitative yields (see Scheme 1).

To assess the increase in water solubility resulting from the introduction of the selected acyloxyl groups into the corresponding starting hydrophobic *F*-BODIPY, we computationally calculated log *P* values (*i.e.*, the decimal logarithm of the equilibrium partition coefficient between 1-octanol and water) at 25 °C for starting and final dyes. For this purpose, we used the popular Percepta software.¹⁷ The obtained values (see Table 1) show that all the selected hydrophilic acyloxyl moieties contribute to increasing water solubility. Thus, all the log *P* values for the new *COO*-BODIPYs **6–10** (ranging from +2.80 for **6** to −4.05 for **9**) are lower than those for parent *F*-BODIPYs **PM567** (+3.64) and **PM546** (+2.89).

Among these acyloxy moieties, the PEGylated in **6** induces the lowest increase of the water solubility (from $\log P = +2.89$ to $\log P = +2.80$), still being 1-octanol the preferred solvent against water. This should be probably improved by using longer polyether chains in the starting carboxylic acid reagent.^{141q} The amino acid moiety in **10** also increases water solubility, being the estimated partition between 1-octanol and water close to 1 : 1 ($\log P = +0.19$). However, it must be noted here that the used computational program did not allow the calculation of $\log P$ for the zwitterionic species, but just for the molecule with neutral amino and carboxyl groups. Therefore, the $\log P$ value estimated for **10** is not reliable, and the solubility in water is probably higher than the one herein calculated. Noticeably, in the case of **7-9**, having ionic acyloxy moieties, $\log P$ is estimated to be negative (see Table 1), which indicates that water is the preferred solvent against 1-octanol. In fact, $\log P$ fully reverses when transforming *F*-BODIPY **PM567** ($\log P = +3.64$) into γ -butyrobetaine-based COO-BODIPY **9** ($\log P = -4.05$).

With the aim of confirming that the substitution of fluorines by the selected, different hydrophilic acyloxy moieties increases the solubility of the BODIPY dye in water while maintaining the dye photophysics, we measured the fluorescence signatures of COO-BODIPYs **6–10** from diluted solutions (*ca.* 2×10^{-6} M) in

^a It refers to the corresponding neutral carboxyl and amino groups.

Dye	log <i>P</i>	Water solubility
 <p>PM567</p>	+3.64	
 <p>PM546</p>	+2.89	
 <p>6</p>	+2.80	
 <p>10</p>	+0.19 ^a	
 <p>8</p>	-2.82	
 <p>7</p>	-3.35	
 <p>9</p>	-4.05	

methanol and in water, and compared them with those exhibited by corresponding parent *F*-BODIPYs **PM546** and **PM567**, in almost similar experimental conditions (Table 2).

Due to their low solubility in water, hydrophobic *F*-BODIPYs **PM546** and **PM567** required the addition of a minimum amount of methanol (*ca.* 0.5% v/v) to allow accurate measurements in water. In spite of this, they underwent an effective aggregation in water, even at low dye concentration *ca.* 10^{-6} M, causing a dramatic loss of the fluorescence signal, which does not occur

Table 2 Photophysical signatures (maximum absorption, λ_{ab} , and fluorescence, λ_{fl} , wavelength; maximum molar absorption, ϵ_{max} ; fluorescence quantum yield, ϕ ; and lifetime, τ) of *F*-BODIPYs **PM546** and **PM567**, and *COO*-BODIPYs **6–10** in methanol (above) and water (below, in *italics*), respectively, at dye concentration ca. 2×10^{-6} M

Dye	λ_{ab} (nm)	ϵ_{max} (M ⁻¹ cm ⁻¹)	λ_{fl} (nm)	ϕ	τ (ns)
PM546 ^a	492.5	82 000	505.5	0.81	5.58
	<i>461.0</i>	<i>19 000</i>	<i>501.5</i>	—	—
PM567 ^a	516.0	74 000	534.0	0.81	6.10
	<i>482.5</i>	<i>25 000</i>	<i>535.0</i>	—	—
6 ^a	496.5	31 000	506.5	0.91	6.23
	<i>493.0</i>	<i>23 000</i>	<i>501.5</i>	<i>0.73</i>	<i>6.20</i>
7	517.0	65 000	536.5	0.67	6.29
	<i>517.5</i>	<i>57 000</i>	<i>538.0</i>	<i>0.41</i>	<i>6.14</i>
8	520.0	56 000	537.0	0.61	5.44
	<i>518.0</i>	<i>50 000</i>	<i>536.0</i>	<i>0.63</i>	<i>5.55</i>
9	519.5	46 000	533.5	0.74	6.87
	<i>516.5</i>	<i>44 000</i>	<i>535.0</i>	<i>0.71</i>	<i>6.89</i>
10	519.0	43 000	537.0	0.83	6.67
	<i>516.0</i>	<i>33 000</i>	<i>534.0</i>	<i>0.80</i>	<i>6.96</i>

^a Insoluble in pure water. Addition of a minimum amount of MeOH (ca. 0.5% v/v) was required to enable complete dissolution in water, albeit the dyes are prone to self-associate into non-fluorescent aggregates.

in methanol (see Table 2). This fluorescence-quenching aggregation (H-aggregates¹⁸) in water also led to noticeable flattening and broadening of the absorption profile, owing to the growth of a new and prominent band at shorter wavelengths, which is more significant for more hydrophobic peralkylated **PM567** (see Fig. S2†). Contrarily, their *COO*-BODIPY derivatives **6–10** showed no aggregation signs in water, even at concentrations higher than 10^{-3} M for some cases (*e.g.*, see Fig. S3†), and showed a photophysical behaviour comparable to that for them in methanol. It must be noted here that non-ionic *COO*-BODIPY **6** required the addition of a minimum amount of methanol to allow its photophysical characterization in water. Noteworthy, the shapes and positions of the absorption and emission bands of hydrophilic *COO*-BODIPYs **6–10** in water are similar to those for the corresponding parent hydrophobic *F*-BODIPYs in methanol (see Table 2, and *cf.* Fig. S2 and S3†), and their fluorescence quantum yields in water solution are higher than 0.60 with the exception of **7**.

Thanks to the significantly increased water solubility (Table 1), synthetic simplicity and low-cost, and maintained photophysical properties (Table 2), the one-step conversion of *F*-BODIPYs into ionic γ -butyrobetaine-based *COO*-BODIPYs (see Scheme 1) offers a practical method to impart water solubility to typically hydrophobic *F*-BODIPYs, with minimal impact on their photophysics.

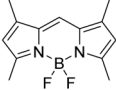
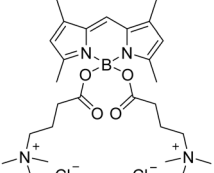
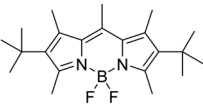
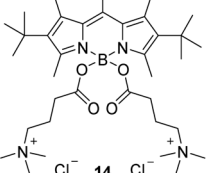
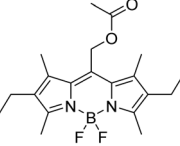
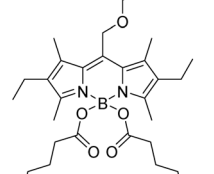
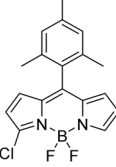
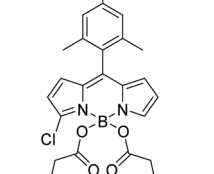
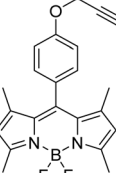
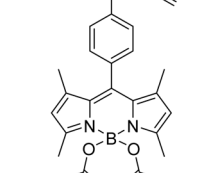
Exploring the method's scope

To explore the scope of the proposed chemical methodology, we selected a small battery of hydrophobic *F*-BODIPYs with specific instability and/or reactivity features (see **PM505**, **PM597**, **PM605**, **11** and **12** in Table 3).

Thus, marketed **PM505** (1,3,5,7-tetramethyl-*F*-BODIPY), without substitution at the BODIPY *meso* position, is prone to undergo at-*meso* nucleophilic additions to the π -conjugated

BODIPY-chromophore system.¹⁹ On the other hand, commercial, crowdedly-peralkylated **PM597** (2,6-di-*tert*-butyl-1,3,5,7,8-pentamethyl-*F*-BODIPY) easily undergoes strain-releasing de-*tert*-butylation,²⁰ while commercial **PM605** (8-(acetoxymethyl)-2,6-diethyl-1,3,5,7-tetramethyl-*F*-BODIPY) and synthetically-accessible chlorinated **11** (ref. 21) are specially featured to undergo nucleophilic substitution of acetoxyl group and aromatic nucleophilic substitution of halogen, respectively.²²

Table 3 Workable transformation of a selected set of highly-reactive hydrophobic *F*-BODIPYs (**PM505**, **PM597**, **PM605**, **11** and **12**) into the corresponding γ -butyrobetaine-based hydrophilic *COO*-BODIPYs (**13–17**). Chemical yields are into parenthesis

Starting dye	Final dye
 PM505	 13 (88%)
 PM597	 14 (54%)
 PM605	 15 (92%)
 11	 16 (72%)
 12	 17 (93%)



Finally, readily-accessible propargylated *F*-BODIPY **12** (ref. 23) was selected to test the possibility of competing at-boron substitution by the involved acidic terminal-alkyne group (formation of ethynyl *C*-BODIPYs).²⁴

Satisfactorily, all these additional *F*-BODIPYs were straightforwardly converted into the corresponding γ -butyrobetaine-based *COO*-BODIPYs **13–17** (see Table 3) following the established methodology (*i.e.*, (a) BCl_3 , (b) Et_3N , (c) γ -butyrobetaine hydrochloride (**4**), at room temperature; see Scheme 1). In all these cases, the chemical yield in isolated *COO*-BODIPY exceeded 70%, with the exception of highly unstable **PM597**, whose transformation in *COO*-BODIPY **14** took place with 54% yield (see Table 3, and the ESI† for experimental details).

Noteworthy, the calculated log *P* values for compounds **13–17** range from -2.72 for highly alkylated **14**, to -5.38 for less substituted **13** (see Table S1†), supporting a significant enhancement in water solubility due to the conducted γ -butyrobetaine derivatization. Moreover, we confirmed that this derivatization did not significantly alter the photophysical behaviour of the corresponding parent *F*-BODIPY in methanol, as the absorption and emission wavelengths, as well as the fluorescence quantum yields, remained comparable.

Most importantly, the conducted derivatization enabled similar dye photophysics in pure water (see Table S2†). The only exceptions to this rule are the absorption capabilities of γ -butyrobetaine-based *COO*-BODIPYs **14** and **15**, which were significantly lower to those recorded for their corresponding parent *F*-BODIPYs (**PM597** and **PM605**, respectively) in methanol, particularly for **14** in water (see Table S2†). This behaviour can be attributed to the previously mentioned high reactivity of the involved BODIPY cores. Indeed, the photonic performance of compound **14** resulted time-sensitive, as the dye solution in both solvents gradually bleached over time.

As a result, the obtained new set of γ -butyrobetaine-based *COO*-BODIPYs (Table 3) sustains the versatility and success of the developed method to solubilize hydrophobic *F*-BODIPYs in water, and, together with the previous ones (Table 2), constitute a valuable palette of selectable highly-bright water-soluble fluorophores with spectral bands spanning from the green to the red edge of the visible spectrum (Fig. S4†).

Showcasing some applications

The synthetic accessibility, chemical robustness, photophysical behaviour, and water solubility of the γ -butyrobetaine-based *COO*-BODIPYs make them ideal candidates to optimize and broaden the use of the commonly hydrophobic *F*-BODIPY dyes in applications where water is essential or even critical. To demonstrate their potential, we have selected three key applications: green BODIPY chemistry in aqueous media, in-water BODIPY lasing, and fluorescent biolabeling using BODIPY-dye solutions in pure water.

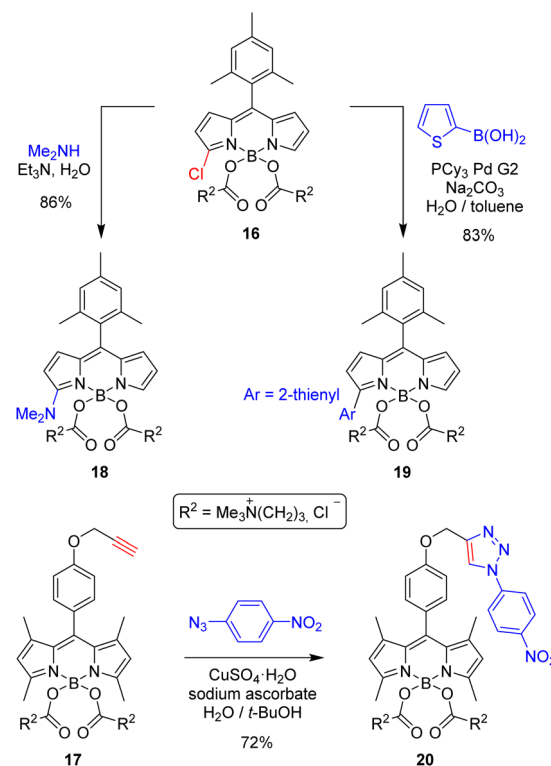
Green BODIPY chemistry in aqueous media

It is obvious that water is the most inexpensive and environmentally friendly solvent. In this context, facilitating chemical processes of hydrophobic substances in water is pivotal to

advance the implementation of more sustainable (greener) chemistry.^{1,3} On the other hand, *in vivo* organic chemistry is gaining attention in both biology and medicine, particularly in that concerning the metal-catalyzed formation of C–C bonds involving chromophoric systems,²⁵ and its employment in advancing the study and applications of optogenetics.¹¹ In this context, facilitating in-water BODIPY chemistry is essential to advance a greener BODIPY chemistry and processing, but also to harness the excellent photophysical behaviour of the tunable BODIPY chromophore in advanced biophotonic applications requiring previous chemical transformations of the dyed material in cells.

In order to explore the potential of the water-soluble γ -butyrobetaine-based *COO*-BODIPYs to facilitate in-water BODIPY chemistry, we selected three common, useful chemical transformations of the BODIPY dyes: nucleophilic aromatic substitution of halogen in 3-haloBODIPYs,^{22a–d,fg} Suzuki–Miyaura coupling of haloBODIPYs,²⁶ and “click” reaction of alkyne-based BODIPYs with azides.²⁷ For this purpose we choose chlorinated *COO*-BODIPY **16** and propargylated *COO*-BODIPY **17** (Scheme 2).

As shown in Scheme 2, 3-chloro-substituted *COO*-BODIPY **16** was able to react satisfactorily with dimethylamine in water at 50 °C, using triethylamine as proton scavenger. The expected substitution product (**18**) was obtained in just 90 min in 86% yield. Likewise, the Suzuki–Miyaura coupling of **16** with 2-thienylboronic acid in aqueous media, using chloro[(tricyclohexylphosphine)-2-(2'-aminobiphenyl)]palladium(II) (PCy₃ Pd G2) was obtained in just 90 min in 83% yield.



Scheme 2 Efficient chemical derivatizations of γ -butyrobetaine-based *COO*-BODIPYs **16** and **17** in aqueous media (see the ESI† for experimental details).



G2) as precatalyst and sodium carbonate as base, led to the expected C–C coupling product (**19**) in 83% yield.

On the other hand, propargylated *COO*-BODIPY **17** could be straightforwardly “clicked” with 1-azido-4-nitrobenzene in highly-hydrophilic aqueous *tert*-butanol, using CuSO₄/sodium ascorbate, to generate **20** in 72% yield. Noteworthy, the log *P* values calculated for the resulting final dyes (**18–20**) were negative in all cases (below –3.8; see Table S1†), remarking the capability of at-boron pending γ -butyrobetaine moieties to enhance the solubility of the BODIPY dyes in water.

BODIPY-laser operation in pure water

The thermo-optic properties of the gain-medium solvent are key in dye lasing, which along with the dye-flow rate largely determine the maximum repetition rate at which a dye laser can be operated.²⁸ Indeed, it has confirmed the superior thermo-optic properties of water in comparison to alcohols for laser operation.²⁹ However, the use of water as solvent for high-power dye lasers has been significantly restricted by the fact that several of the commonly used laser dyes are highly hydrophobic and, therefore, not easily soluble in water at the typical concentrations necessary for laser operation. This insolubility in water promotes the formation of non-fluorescent dye dimers and higher aggregates, which induces an absorptive loss of pump power in dye lasers. Conversely, aqueous gain media result in lower laser efficiency than alcoholic media in these cases.³⁰ This is especially true for the valuable but highly hydrophobic *F*-BODIPY laser dyes.³¹ Consequently, the development of aqueous active media for lasing is a topic of interest in dye laser technology.^{29b,32}

To explore the capability of the newly developed, highly-hydrophilic *COO*-BODIPYs to support dye-laser operation in pure water, we selected compounds **7–9**, **15** and **19** (see Tables 1 and 3, and Scheme 2). This selection allows us for assessing potential differential behaviours influenced by the characteristics of the BODIPY chromophore (*e.g.*, extension of the π -conjugation or different capability for light absorption/emission) and the hydrophilic acyloxy moieties at the boron position (pyridinium cation for **7** and **8**, and γ -butyrobetaine for **9**, **15**, and **19**).

For comparison, laser behaviour in alcohol (methanol) was also measured under nearly identical experimental conditions (Table 4). It must be noted here that *COO*-BODIPYs **7–9**, and **15** are hydrophilic derivatives of the well-known marketed *F*-BODIPY laser dyes **PM567** (green-emitting) and **PM605** (red-emitting), respectively.

Regardless of the solvent (methanol or pure water), under the selected operational conditions (transversal pumping and strong focusing of the incoming pump radiation), high dye concentrations (in the mM range) were needed to assure total absorption of the excitation radiation within the first millimetres of the optical cell. This is required to achieve a near circular cross-section of the emitted beam, which is essential to induce highly efficient laser action, and to allow the accurate determination of the dependence of the laser action (laser efficiency, *Eff_L*, and laser-peak wavelength, λ_L) on the dye

Table 4 Dependence of the laser action (*Eff_L* and λ_L) on dye concentration for *COO*-BODIPY dyes **7–9**, **15** and **19** in methanol and water (*Eff_L* in water is depicted in italics), respectively, under transversal laser excitation at 532 nm with 5 mJ per pulse (see the ESIs for experimental details)

Dye	Laser action ^a	Dye concentration (mM)				
		0.6	1.0	1.5	2.5	3.5
7	<i>Eff_L</i> (%)	33	43	48	34	19
	λ_L (nm)	27	37	43	29	10
8	<i>Eff_L</i> (%)	37	49	51	39	27
	λ_L (nm)	31	46	48	32	21
9	<i>Eff_L</i> (%)	38	53	58	41	26
	λ_L (nm)	33	50	55	38	19
15	<i>Eff_L</i> (%)	37	44	47	37	26
	λ_L (nm)	35	41	45	34	22
19	<i>Eff_L</i> (%)	22	35	46	55	49
	λ_L (nm)	19	30	43	50	43
	λ_L (nm)	575	580	585	587	589

^a *Eff_L* = energy conversion efficiency; λ_L : laser-peak wavelength.

concentration. In this regard, it should be noted here that less water-soluble **7** and **8**, both based on pyridinium iodide, needed to be sonicated under mild conditions to achieve their total solubilization in pure water at concentrations higher than 1.5 mM.

The recorded laser action (Table 4) is guided by the photo-physical behaviour of the corresponding dye (see Tables 2, and S2†). Thus, the higher the fluorescent quantum yield and the redder the fluorescent emission, the more efficient and redder becomes the laser action. At the dye concentration that optimizes the laser action (*ca.* 1.5 mM for **7–9** and **15**, and *ca.* 2.5 mM for **19**), the selected dyes reach high laser efficiencies in highly hydrophilic methanol, ranging from 47% for **15**, to 58% for **9**, with the laser emissions peaked in the 550–590 nm spectral region. Further increasing of the dye concentration results in a decrease of the laser efficiency, owing to the activation of reabsorption/reemission processes with remarkable deleterious effect in the laser action (see Table 4).

Noteworthy, this efficient laser action in methanol is retained in pure water (Table 4). Moreover, increasing the dye concentration in water red-shifts the laser-peak wavelength, but this displacement induces neither a laser-band broadening nor a shoulder growing (Fig. 3). These outstanding behaviours are due to the high solubility of the studied *COO*-BODIPY dyes in the selected hydrophilic media, including water, which allows reaching the required optimum operational dye concentration in the hydrophilic solvent without inducing dye aggregation, even in water at dye concentrations as high as 4 mM (Table 4 and Fig. 3).

Since dye photostability under severe, long-term laser pumping conditions is an important parameter for any advanced photonic and biophotonic application based on organic dye (*e.g.*, fluorescence imaging), the lasing photostability of the selected



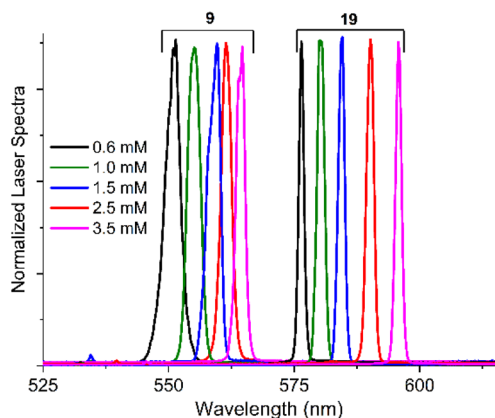


Fig. 3 Dependence of the laser spectra of COO-BODIPYs **9** and **19** on the dye concentration in pure-water solution.

COO-BODIPYs dyes in water was assessed by following the evolution of their laser-induced fluorescence emission as a function of the number of pumping laser pulses (see the ESI† for experimental details). Surprisingly, the new dyes resulted highly photostable even in water, since their laser emission intensities decrease a merely 10% after 40 000 pump pulses under the selected, drastic pumping conditions. Indeed, the studied COO-BODIPYs are, to the best of our knowledge, the first BODIPY dyes enabling highly efficient and photostable laser emission in pure water. It should be noted here that the replacement of the organic solvent by water in the gain medium does not entail modifying any other experimental variable (dye concentration, pump fluence, cavity configuration, temperature, *etc.*) to record highly efficient and stable laser emission.

Fluorescent live-cell imaging using BODIPY solutions in pure water

Fluorescence bio-imaging is pivotal for advancing biology and medicine.³³ It relies on the use of specific fluorescent markers, particularly small molecular fluorophores known as molecular bioprobes or simply molecular probes. *F*-BODIPYs are especially effective as molecular probes due to their excellent fluorescence properties, chemical and photophysical adaptability, biocompatibility, and cell membrane permeability.^{13e,f,g,s} However, a major limitation of the non-ionic molecular probes, including most BODIPY bioprobes, is their high hydrophobicity, resulting in a poor solubility in water, including the aqueous cell cultures. This often leads to undesired issues limiting biolabeling efficacy, such as dye aggregation during the staining operation, either in the aqueous dye staining solution or the cell culture.³⁴

To mitigate these problems, small amounts of organic solvents like dimethylsulfoxide (DMSO) or ethanol are typically added to the staining solutions. However, the associated cytotoxicity of these solvents constrains their use, limiting also the percentage of solvent that can be added to the cell culture.³⁵ In this context, the rapid conversion of hydrophobic *F*-BODIPYs probes into highly water-soluble γ -butyrobetaine-based COO-BODIPYs could offer an effective solution for improving their use as molecular probes. Nevertheless, the involved at-boron

chemical modification could potentially impact key properties such as biocompatibility, cell membrane permeability, and staining specificity.

To assess to what extent these properties might be affected, we have selected polyalkylated *F*-BODIPYs **PM505**, a known molecular probe,³⁶ the structurally similar but more hydrophobic **PM567**, and their corresponding γ -butyrobetaine-based COO-BODIPYs (**13** and **9**, respectively; see Table 1), and investigate their differential capability to fluorescently label live cells using pure-water solutions. For this purpose, wild-type mouse embryonic fibroblasts, MEFs, were selected.

As expected, 1 mM stock solutions of **9** and **13** in pure water could be easily obtained without detecting dye aggregation to the naked eye (see Fig. S5†). However, this is not the case for *F*-BODIPY precursors **PM567** and **PM505**, where significant aggregation remains even upon intensive vortex agitation plus sonication (Fig. S5†). Satisfactorily, **9** and **13** in pure water stained straightforwardly living MEFs without using any additional organic solvent in the cell culture, allowing the fluorescence bioimaging of live cell systems with notable efficiency (Fig. 4; see the ESI† for experimental details).

These results demonstrate that the capability of **PM567** and **PM505** for cell uptake is retained in their water-soluble γ -butyrobetaineated derivatives. Indeed, the bioimaging patterns obtained by using **PM567**, **PM505**, **9** and **13** under similar staining conditions are almost comparable, all of them staining different subcellular structures, particularly lysosomes, as demonstrated by co-localization with LysoTracker Red (see Fig. S6†).

Nonetheless, some different biospecificity should be expected upon the γ -butyrobetaine derivatization of the selected *F*-BODIPY dyes (**PM567** and **PM505**), taking into account the lack of groups for specific biorecognition in their molecular structures. Thus, in cases of *F*-BODIPY probes involving these biolabeling groups, the biospecificity should be strongly ruled by them, the influence of the different at-boron pending groups (fluorines *vs.* γ -butyrobetaine-based acyloxylys) being minimal.

Noteworthy, the study of the cell viability by the AlamarBlue reduction assay (see the ESI† for experimental details) demonstrates the conducted solubilizing BODIPY modification does not distort the high biocompatibility of the starting *F*-BODIPY dye. Thus, the γ -butyrobetaine COO-BODIPYs **9** and **13** exhibited very low cytotoxicities, which are similar to those exhibited

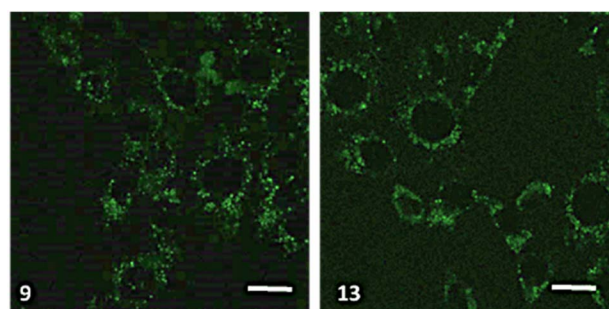


Fig. 4 Bioimaging MEFs incubated with γ -butyrobetaine-based COO-BODIPYs (**9** and **13**) in water solution at ca. 120 nM after 24 h incubation. Scale bars are 10 μ m.



by the corresponding parent *F*-BODIPYs **PM567** and **PM505**, even at dye concentrations as high as 500 nM in cell culture (see Fig. S7†). Moreover, computational prediction of the pharmacokinetic parameters of the selected paired compounds using the free web tool SwissADME³⁷ revealed similar values for γ -butyrobetaine *COO*-BODIPYs **9** and **13** and the corresponding *F*-BODIPYs **PM567** and **PM505**, except for the skin permeation parameter (K_p), which was higher for *F*-BODIPYs in agreement with their greater lipophilicity (e.g., $K_p = 10^{-5} \text{ cm s}^{-1}$ for **PM567** vs. $K_p = 10^{-6.7} \text{ cm s}^{-1}$ for **9**).

Conclusions

A straightforward chemical modification to easily convert hydrophobic BODIPYs into highly-hydrophilic BODIPYs has been developed. The transformation involves the at-boron substitution of fluorine atoms in *F*-BODIPYs by hydrophilic acyloxyl chains based on γ -butyrobetaine, yielding highly hydrophilic *COO*-BODIPYs. This chemical transformation is done in just one step using mild reaction conditions and a low-cost commercial reagent. It has been demonstrated that it can be applied to different starting *F*-BODIPYs, even to highly reactive ones, generating the corresponding *COO*-BODIPYs in moderate to high yields, which supports the generality of the methodology. The obtained chemically-modified BODIPY dyes exhibit a drastic enhancement of water solubility, as supported by their calculated log *P* values and, importantly, they retain the main photophysical signatures of the starting *F*-BODIPYs, such as position of the spectral absorption and emission bands or fluorescence quantum yield.

The potential of this chemical methodology to impart water solubility in common *F*-BODIPY dyes has been exemplified by using some of the obtained γ -butyrobetaine-based *COO*-BODIPY dyes in selected applications requiring aqueous media, such as in-water BODIPY chemistry, BODIPY-laser operation in pure water, and fluorescence biolabeling using BODIPY-dye solutions in pure water. The success in all these applications demonstrate the feasibility of the described methodology to impart water solubility to the typically highly-hydrophobic *F*-BODIPY dyes, which should expand the optimal use of these tunable dyes in specific applications requiring water or highly-hydrophilic media, particularly in the biophotonics field.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

S. d. I. M., conceptualization; S. d. I. M. and J. B. supervision; B. L. M., investigation (molecular design); C. S. and C. R., investigation (synthetic development); F. M. J., investigation (structural characterization); C. D.-N., investigation ((photo) physical behaviour); S. S.-B., investigation (in-water chemistry validation); I. G.-M., investigation (laser operation validation); M. M.-U. and I. L.-M., investigation (live-cell

imaging validation); S. d. I. M., J. B.; B. L. M., I. G.-M and I. L.-M., writing (original draft); S. d. I. M., writing (review and editing).

Conflicts of interest

There are no conflicts to declare.

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