Synthesis, Photophysical Study, and Biological Application Analysis of Complex Borondipyromethene Dyes

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ABSTRACT: A series of complex boronic acids were prepared through multicomponent reactions (MCRs). Both Passerini and Ugi MCRs were carried out in which one component was an arylboronic acid. The resulting highly functionalized boronic acids participated efficiently in the Liebeskind–Srogl cross-coupling reaction with meso-methylthioBODIPY derivatives to yield complex borondipyromethene (BODIPY) dyes in good yields. The joined spectroscopic and computational study points out the deep impact of the arylated chromophoric position on the photophysical signatures. Thus, unconstrained aryls grafted at the meso position did not sway the spectral band positions but switched on new nonradiative relaxation channels, whereas additional arylation at the opposite α-pyrrolic position softened such fluorescence quenching and shifted the emission to the red-edge of the visible spectrum. The conducted biological analysis revealed that peripheral blood mononuclear cells incubated with these new compounds showed reduced cytotoxicity and retained their normal activities. Additionally, the dyes remained stable inside the cells after 24 h of incubation. These results demonstrated that these novel fluorescent probes based on BODIPY can be applied for cell imaging and analysis, expanding their applications.

INTRODUCTION

Boronic acids are arguably one of the most useful functional groups in organic chemistry. Both academia and industry have greatly benefited from their properties and applications. From the synthetic point of view, one of the most important transformations where boronic acids participate is the formation of the C–C bond. Thus, in this regard, we can mention the well-known Suzuki–Miyaura cross-coupling reaction,2 the reductive coupling with tosylhydrazones,3 the transition-metal-free C–C bond formation,4 the Petasis reaction,5 the Au-catalyzed intramolecular aminoharylation of alkenes,6 the Pd- or Rh-catalyzed conjugate addition to enones,7 and their Pd-catalyzed homocoupling.8 A relatively recent example of the efficient participation of boronic acids in the formation of C–C bonds is their Pd-catalyzed, Cu(1)-mediated reaction with thioorganics, that is, the so-called Liebeskind–Srogl cross-coupling reaction (LSCC).9

Over the last few years, our research groups have exploited the commercial availability of both aryl- and heteroaryl-boronic acids to prepare a large number of borondipyromethene (BODIPY)-containing fluorophores starting from Biellmann BODIPYs10 and using the LSCC (Scheme 1).11 BODIPYs are well-known fluorophores with very interesting optical properties and varied applications.12

Even though this methodology proved to be exceptionally tolerant to the functional groups present in the initial boronic acid (functional groups, such as Cl, Br, I, CO₂H, NH₂, CH₃OH, CH₃Br, CH₂N₂O, OH, and SiMe₃ were perfectly tolerated), complex boronic acids remained to be tested. There are examples of the synthesis of boronic acids of high complexity in the literature;13 however, all of them involve a series of iterative and rather elaborate synthetic sequences for their preparation. Rather, we were interested in a quick and flexible method that would render the final complex boronic acid in, if possible, one step with a minimum purification effort. Pan’s groups in 2014 provided the answer to this challenge.14 He and his co-workers carried out the Ugi15 reaction with 4-formylphenylboronic acid, a carboxylic acid, an amine, and an isonitrile (eq 1) to yield an arylboronic acid with rich functionality.

This multicomponent reaction (MCR) takes place under very mild conditions and the final products can be isolated simply by using an acid/base treatment. We realized that this method would allow the introduction of a great deal of...
diversity by changing the nature of the carboxylic acids, amines, and isonitriles. Additionally, the starting boronic acid moiety may be attached to an arylcarboxylic acid or an aniline fragment as well. Even the possibility to have a boronic acid-containing arylisonitrile became a reality after Pan’s report of the preparation of isocyano aryl boronate esters. Herein, we wish to disclose the synthesis of complex boronic acids building upon Pan’s contribution and extend it to the Passerini reaction using not only the boronic acid fragment in the benzaldehyde component but also in the carboxylic acid and aniline partner of the MCRs. In this context, seminal contributions have been made to the synthesis of complex fluorophores using MCRs, including isocyanide-based processes by Balakirev and Vendrell. The elaborate arylboronic acids so prepared were used in the LSCC with the Biellmann BODIPYs to obtain novel fluorophores with rich functionality.

**RESULTS AND DISCUSSION**

**Synthesis.** Different aryloboronic acids analogues of type 2 were synthesized through the Passerini three-component reaction (P-3CR) between different (het)arylboronic acids (containing either the formyl or the carboxy moiety), aldehydes, benzoic acid, and t-butyl isocyanide in MeOH at room temperature for 24 h. All desired products (2a−h) were obtained in moderate to excellent crude yields ranging from 49 to 98% (Chart 1). See Supporting Information for details of the aq basic washing needed to isolate the crude products that were directly used for the LSCC.

In the reaction set studied, the yields of the products 2a−h were superior when the boronic acid fragment was attached to the benzoic acid.

In a similar way, compounds of type 3 were synthesized via the Ugi four-component reaction (U-4CR) starting from different aldehydes, amines, benzoic acids, and t-butyl isocyanide. The boronic acid fragment was connected to the aryl aldehyde, amine, or benzoic acid in each case. As before, only aq washings were needed to isolate the crude boronic acids to be used in the next reaction. The results are shown in Chart 2.

The crude yields of the final products ranged from moderate to excellent. The boronic acids thus synthesized (Charts 1 and 2) were used in the LSCC with 8-methylthioBODIPY 1a to obtain new meso-substituted BODIPY of type 4 (Chart 3).

The desired products 4a−c, 4e−k, and 4n−o were obtained in moderate to good yields ranging from 56 to 88%. On the other hand, the reaction failed to yield products 4d, 4l, and 4m. In the case of products 4l and 4m, their terminal triple bond may

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Chart 1. Synthesis of Boronic Acid Analogues through P-3CR

<chemical formula>

| R | R' | R'' | R''' |
|---|---|---|---|
| CHO | + | R'O | - NH'R' | + R'-NC |

<reaction conditions>

Crude yield after base extraction. Conditions: aldehyde (1.0 equiv), acid (1.3 equiv), isonitrile (1.3 equiv), MeOH (1 M), and rt, 24 h.
have engaged in a Cu-promoted side reaction because in the LSCC, there is an excess of CuTC. The mechanistic reason why 4d was not obtained is not clear to us at this point. The reaction times of the LSCC were typical for this process (1–3 h).11a,b Moreover, all of the products were fully characterized by confirming the structure of boronic acids 2a−h and 3a−g. Derivatives 4f and 4o have bromine atoms with which further functionalization can take place via transition-metal-catalyzed cross-coupling reactions. All new products shown in Chart 3 fluoresce in the green region of the visible spectrum (vide infra). Being aware of the importance of preparing dyes that absorb and emit in the red region of the visible spectrum for biological applications,18 a few analogues were prepared starting from our recently reported19 modified Biellmann BODIPY 1b (Chart 4).

![Diagram of Chart 2. Synthesis of Complex Boronic Acids through U-4CR](image)

The reactivity of BODIPY 1b was observed to be slightly lower than that of 1a. This resulted in somewhat longer reaction times and moderate chemical yields of the final products.

**Photophysical Properties.** The spectral bands of the meso-arylated compounds (4a−4c, 4e−4j and 4n−4o, see Chart 3) are located close to those of the corresponding unsubstituted dipyrrin core,20 regardless of the substitution pattern of the 8-phenyl ring (Table 1 and Figure S1). The twisting of such a ring with regard to the BODIPY plane (52° for para-substituted 4a and 60° for meta-substituted 4c from the ground-state-optimized geometries at the b3lyp/6-31+g* calculation level) owing to the steric hindrance with the adjacent hydrogens avoids resonant interaction with the dipyrin backbone, supporting its scarce effect on the spectral band positions and profiles. However, the exerted geometrical strain is not high enough as to lock the phenyl in a fixed conformation. Instead, the unconstrained meso-aryl group has the freedom to rotate, enabling a fluorescence quenching pathway which has a deep impact in the fluorescence parameters.21 As a result of such an enhancement of the nonradiative energy loss via internal conversion relaxation, all of this set of compounds are poorly fluorescent, with quantum yields lower than 0.06 (Table 1).

Indeed, the simulated energy landscape for the 8-phenyl motion provides a rotational barrier of 11.9 kcal/mol in the ground state, which becomes even lower upon excitation (8.1 kcal/mol, Figure 1). Thus, in the excited state, there are more energetically available conformations easily accessible owing to the low rotational barrier, supporting the higher internal conversion probability. Moreover, when the 8-phenyl group is disposed more coplanar with the dipyrrin core, an electron coupling is feasible and it leads to a deep distortion of the geometry of the chromophore (puckering along the transverse axis with bending angles up to 40°, Figure 1). Indeed, it has been theoretically proposed that after such an intramolecular interaction, a low-lying “dark” state can be populated.22 The key role of the 8-aryl free motion is supported by the slight improvement of the fluorescence response of those compounds bearing meta-substituted 8-aryl groups (4c and 4o) with regard to their para-substituted counterparts (Table 1). The exerted faintly higher steric hindrance upon functionalization at the former position slightly hinders the nonradiative deactivation pathways associated with the 8-aryl mobility.

This nonradiative deactivation is not the only ongoing quenching process in this set of compounds. Indeed, those dyes bearing electron-withdrawing moieties (such as carboxylates) directly linked to the meso-phenyl fragment feature the lowest fluorescence efficiencies (see e.g., 4e−4h and 4n in Table 1, with nearly negligible emissions). Such stronger fluorescence quenching can be assigned to the additional activation of intramolecular charge-transfer (ICT) processes. This ICT is populated from the locally excited state and is not fluorescent (or at least very weak) because no new emissions are recorded. The fluorescence lifetimes reflect all of these complex dynamics in the excited state (Table 1). Thus, the free motion of the para-substituted 8-aryl group (4a−4b and 4i−4j) induces a shortening of the lifetime (being just hundreds of picoseconds, whereas in BODIPYs, is usually in the nanosecond scale), in agreement with the recorded low fluorescence quantum yields (Table 1). This trend is ascribed to the aforementioned increase of the nonradiative pathways: on one hand, the enhanced internal conversion probability by the free motion of the 8-phenyl and on the other hand, the feasible population of a relaxed “dark” state upon excitation owing to the electronic coupling between the phenyl and the dipyrin.22 Furthermore, in 4c and 4o, the lifetimes become longer (up to 800 ps) upon meta-substitution of the 8-phenyl ring. Indeed, those dyes show the highest, albeit still low, fluorescence efficiencies among this set of compounds (Table 1). Further functionalization of the 8-aryl fragment with electron-withdrawing moieties provides more complex decay curves. In fact, in dyes 4e−4h and 4n (bearing para-carboxylated 8-phenyl rings), an additional very fast second exponential (tens of picoseconds, in the limit of the temporal resolution of our single-photon counter) is required. Such a lifetime becomes the main component of the decay curve and is assigned to the population of an ICT state, which quenches almost entirely the fluorescence response (Table 1).

In a previous work, we concluded that the attachment of a para-nitrophenyl group at the 3-position was desirable to achieve improved fluorescence response toward the red-edge of the visible spectrum.19 Therefore, we followed this strategy...
to boost the fluorescence response in the herein developed dyes leading to the compounds shown in Chart 4. As expected, the feasible resonant interaction of such an aromatic arm with the dipyrrin core leads to an extended π-system, which explains the recorded bathochromic shift of the spectral bands, owing mainly to a lowest unoccupied molecular orbital stabilization as
negative charge is mainly located just at the group, whereas without such a nitrophenyl moiety, the charge is shared between the parts (Table 2 vs 1). Indeed, the increase with regard to their respective non-nitrated counterparts (S2). Besides, in all cases, the simplicity from a computational point of view, 8-phenylBODIPY was taken as the model for the simulation.

Nevertheless, an increase of the solvent polarity clearly drops the fluorescence efficiency and the lifetimes also become faster.

Table 1. Photophysical Data of 8-Aryl-Substituted BODIPYs Collected in Chart 3 in THF

| Compound | λ_{max}^a (nm) | ε_{max}^b (M^1 cm^-1) | λ_{em}^c (nm) | ϕ^d | τ^e (ps) |
|----------|----------------|------------------------|---------------|-----|---------|
| 4a       | 501.5          | 5.2                    | 519.0         | 0.014 | 230     |
| 4b       | 504.5          | 5.4                    | 526.0         | 0.006 | 200     |
| 4c       | 502.0          | 6.3                    | 520.0         | 0.028 | 450     |
| 4e       | 504.5          | 4.5                    | 528.0         | 0.005 | 75      |
| 4f       | 505.0          | 4.1                    | 526.0         | 0.004 | 80 (92%) | 605 (8%) |
| 4g       | 504.5          | 4.6                    | 528.0         | 0.004 | 60 (91%) | 278 (9%) |
| 4h       | 504.5          | 4.6                    | 529.0         | 0.003 | 45      |
| 4i       | 501.5          | 5.8                    | 519.0         | 0.009 | 365     |
| 4j       | 501.5          | 4.5                    | 519.0         | 0.011 | 310     |
| 4k       | 502.0          | 5.3                    | 519.0         | 0.007 | 70 (60%) | 272 (40%) |
| 4l       | 501.5          | 4.0                    | 519.0         | 0.054 | 800     |

4a Absorption wavelength. 4b Molar absorption. 4c Fluorescence wavelengt. 4d Fluorescence quantum yield. 4e Fluorescence lifetime. 4f The photophysical properties of compound 4k were not measured since this dye was unstable.

Besides, such a short-living component prevails in the decay curve (Table 2). This trend was unexpected and striking because no solvent sensitiveness of the fluorescence response was recorded in previously reported related asymmetrically nitro compounds (just differing in the steric hindrance exerted around the 8-aryl, ortho-substituted). Therefore, in such nitrated BODIPYs with constrained 8-aryl, an ICT is not viable, whereas in the herein tested compounds 5a–5d (with unconstrained 8-aryl), the strong electron-withdrawing nitro moiety may be able to induce ICT processes. Recently, advanced calculations in BODIPYs bearing unconstrained meso-aryls have revealed that ICT processes can be involved in the ongoing fluorescence quenching mechanism induced by such phenyl. Indeed, upon excitation, a partial charge transfer takes place from the side pyrroles to the central six-membered ring of the dipyrin skeleton (Figure 2). The presence of unsaturated bonds at the meso-position able to couple with the BODIPY upon excitation (unconstrained vinyl or phenyl) seems to favor ICT processes. Following this line of reasoning, the 3-nitrophenyl moiety in for dyes 5a–5d could strengthen such charge separation, enabling and stabilizing ICT processes mainly in polar media. Moreover, the lowest fluorescence quantum yields and lifetimes in this set of

Table 2. Photophysical Properties of the Extended and Nitro Compounds 5a–5d (See Chart 4) in THF (Top) and Acetonitrile (MeCN, Bottom)

| Compound | λ_{max}^a (nm) | ε_{max}^b (M^1 cm^-1) | λ_{em}^c (nm) | ϕ | τ (ns) |
|----------|----------------|------------------------|---------------|---|-------|
| 5a       | 537.0          | 3.8                    | 565.0         | 0.16 | 1.46 (59%–4.19 (41%)) |
| 5b       | 538.5          | 4.6                    | 568.0         | 0.07 | 1.37 (80%–3.45 (20%)) |
| 5c       | 535.5          | 4.1                    | 564.0         | 0.16 | 1.32 (55%–4.16 (45%)) |
| 5d       | 538.5          | 6.8                    | 569.0         | 0.04 | 0.30 (98%–1.25 (2%)) |
| 5a       | 530.5          | 3.8                    | 561.0         | 0.07 | 0.44 (97%–2.35 (3%)) |
| 5b       | 533.0          | 3.8                    | 562.0         | 0.04 | 0.31 (98%–1.34 (2%)) |
| 5c       | 530.0          | 4.3                    | 559.0         | 0.09 | 0.70 (99%–2.22 (1%)) |
| 5d       | 533.5          | 6.9                    | 564.0         | 0.02 | 0.20 (99%–1.08 (1%)) |

Figure 1. Potential energy surface with regard to the 8-phenyl group rotation in the ground and first excited state in THF. For the sake of simplicity from a computational point of view, 8-phenylBODIPY was taken as the model for the simulation.

Figure 2. Normalized absorption (bold line) and fluorescence (weak line) spectra of 4a and its p-nitrophenyl counterpart 5a in THF. The corresponding calculated molecular orbitals involved in the electronic transition, as well as the electrostatic potential mapped onto the electronic density (blue for positive and red for negative charge), are also depicted.

Nevertheless, an increase of the solvent polarity clearly drops the fluorescence efficiency and the lifetimes also become faster.
Compounds are recorded for 5b and 5d, those bearing electron-withdrawing groups (meta-fluorine and para-carboxylate, respectively) directly linked to the 8-phenyl (Table 2). Likely, such a functionalization increases the charge separation and enhances the deleterious effect of the ICT in the fluorescence response of these nitrated BODIPYs.

Biological Assays. To assess the possible application of the compounds generated in the present study for biological applications such as specific cell compartment staining, we tested the fluorescence of whole cells stained with all compounds synthesized. This screening allowed us to eliminate compounds that did not render fluorescent cells. First, using human peripheral blood mononuclear cells (PBMCs), we incubated the selected compounds for 2 h and analyzed the cells by flow cytometry, recording the fluorescence emitted in different channels (fluorescence wavelengths). This strategy enabled us to discriminate compounds that were nonfluorescent inside the cells or exhibited a negative effect on the cell morphology, suggesting toxicity.

In Figure 3, we show the analysis of three compounds that showed the expected fluorescence pattern. The compounds were analyzed for fluorescence at all wavelengths available in a Beckman Coulter MoFlo high-speed cell sorter. We identified that cells retained the fluorescence up to 24 h after exposure (histogram analysis), while retaining almost 95% and up in all

Figure 3. Flow cytometry analysis of human PBMCs stained and incubated in the presence of compounds 4j, 4h, and 4n. Human PBMCs were obtained from healthy volunteers, following the institutional ethics guidelines. Cells were isolated as described previously, and cell staining was performed in 24-well plates containing $1 \times 10^6$ PBMCs per well.

Figure 4. Human PBMCs inspected under fluorescent microscopy. Aliquots containing $5 \times 10^6$ human PBMCs were incubated with 20 μg of each compound and inspected under a fluorescent microscope as reported. The scale bars represent 10 μm.
cases, the cell morphology (smoothed dot plots). Also, the PBMC samples contained not only mononuclear cells but also some granulocyte cells, which were also stained showing the same stability in the time course tested (Figure 3). These results suggested that the compounds generated are incorporated rapidly into the cells, except for compound 4n, which needed 2 h to be fully incorporated into the cells. Therefore, these data confirmed the ability of the compounds to stain all cell subpopulations present in the samples.

In the time course analysis, the synthesized BODIPYs showed intracellular fluorescence stability and cells also did not show death or apoptosis in the period tested. After analysis for 24 h, cells showed stability and fluorescence remained stable, compound 4n showed slow incorporation rate when compared with compounds 4j and 4h (Figure 3), and cells did not show indications of damage by means of losing cell shape (both size and complexity) as depicted in Figure 3, where plotted cells size versus cell complexity remains intact. We included dimethyl sulfoxide-treated cells to show that the solubilization agent used did not damage the cells.

Cell samples were stained with compounds 4h, 4j, and 4n and analyzed by fluorescent microscopy. The three compounds showed green fluorescence inside the cells: compounds 4h and 4n heterogeneously stained the PBMCs, being accumulated in patch-like structures inside the cells, whereas compound 4j clearly accumulated in the cytoplasm (Figure 4). Both 4j and 4n tended to aggregate inside cells, generating a yellowish fluorescence, also observed as the formation of small aggregates in the culture media (Figure 4). Interestingly, the three compounds were also capable of staining platelets (small dotlike structures, Figure 4).

Next, we addressed the cell cytotoxicity of compounds 4h, 4j, and 4n by measuring the release of lactate dehydrogenase (LDH), measured with a commercial kit from Thermo Scientific and as described before.26 Time-course interactions showed that compound 4h was not toxic to cells, even after 24 h of incubation (data not shown). Compounds 4j and 4n did not display cytotoxicity at short incubation times, but compound 4j showed that 62 ± 3% of cell population displayed cytotoxicity after 6 h of incubation and 100% cytotoxicity after 24 h of incubation. Interaction with compound 4n was cytotoxic only at long incubation times (24 h), where 100% of cytotoxicity was observed. This observation is in contrast with the data from flow cytometry analysis, suggesting that cell integrity is not fully compromised but partial cell lysis or metabolic interference occurs with compounds 4j after 6 h and 4n after 24 h.

Finally, we tested whether any of these three compounds could activate the ability of human PBMCs to produce TNFα and IL-10, the two gold standards of a pro- and anti-inflammatory response, respectively.27 Because of the toxicity seen in the compound tested, we analyzed the effect on cells after 6 h of incubation to minimize the long-term effect seen in longer incubations. Results presented in Figure 5 indicate that incubation for 6 h with 20 μg of each compound did not activate the immune cells, indicating that functional integrity is preserved in PBMCs in the presence of compounds 4h, 4j, and 4n. Moreover, when human PBMCs were preincubated for 6 h with 20 μg of each compound and then challenged with heat-killed 5 × 10⁵ Candida parapsilosis yeast cells, production of both cytokines was not affected by any of the three compounds tested (Figure 5). Therefore, cell functionality is preserved when human PBMCs interact with 4h, 4j, or 4n compounds in the time tested and does not activate an inflammatory or anti-inflammatory response by themselves.

Second, we analyzed the new meso-substituted BODIPYs from the Biellmann BODIPY with the red fluorescence profile in the interaction with human-derived PBMCs. Several compounds were analyzed by flow cytometry and only two compounds, 5a and 5d, showed the predicted fluorescence pattern between 616 and 670 nm. In Figure 6, human PBMCs stained with compounds 5a and 5d for 24 h were analyzed by flow cytometry, showing a clear increase in the fluorescence in the red spectrum relative to the unstained cells. Histograms shown in red correspond to the unstained cells and the histograms shown in blue and dark yellow correspond to the duplicate samples for the tested compounds. The fluorescence emission by compound 5d also showed a diminished emission at 616 nm, whereas compound 5a showed a specific emission at 670 nm. Cell morphology showed integrity as shown in the insets of Figure 6. Also, fluorescence microscopy was used to analyze these compounds to assess their intracellular localization (Figure 4). Compound 5a stained cells in yellow, whereas compound 5d labeled cells with a red fluorescence. Both compounds homogeneously stained the whole cells, including the small dots that are platelets (Figure 4). With these results, the compounds generated can be applied in a wider range of cell staining, achieving wavelengths that are useful for cell imaging and analysis.

**CONCLUSIONS**

Arylboronic acids participate in MCRs to give highly decorated products. The Passerini and Ugi MCRs were used to prepare 15 novel boronic acids with rich functionality. These boronic acids reacted with 8-methylthioBODIPY derivatives to produce 16 novel, highly functionalized BODIPY dyes in a straightforward manner.

The impact of the arylation on the photophysical signatures markedly depends on its attachment position in the dipyrrin backbone. On the one hand, the unconstrained meso-aryl, bearing the complex functionalization of the boronic acid, did not interact by resonance with the BODIPY core but enhanced

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**Figure 5.** TNFα and IL-10 production by human PBMCs. Human cells were incubated for 6 h with 20 μg 4h, 4j, or 4n and then supernatants were stored and used to quantify cytokines by ELISA. Alternatively, human PBMCs were preincubated for 6 h with the compounds, before being challenged with heat-killed 5 × 10⁵ C. parapsilosis yeast cells. Mock reactions showed threshold levels of cytokines, whereas the C. parapsilosis–human PBMC interaction stimulated known levels of both cytokines.

**Figure 6.** Human PBMCs stained with compounds 5a and 5d for 24 h by flow cytometry, showing a clear increase in the fluorescence in the red spectrum relative to the unstained cells. Histograms shown in red correspond to the unstained cells and the histograms shown in blue and dark yellow correspond to the duplicate samples for the tested compounds. The fluorescence emission by compound 5d also showed a diminished emission at 616 nm, whereas compound 5a showed a specific emission at 670 nm. Cell morphology showed integrity as shown in the insets of Figure 6. Also, fluorescence microscopy was used to analyze these compounds to assess their intracellular localization. Compound 5a stained cells in yellow, whereas compound 5d labeled cells with a red fluorescence. Both compounds homogeneously stained the whole cells, including the small dots that are platelets.
the nonradiative relaxations related with its free motion, as
probed by the different extent of the fluorescence quenching
depending on para or meta substitution. As a result, low
fluorescence efficiencies are attained in these meso-arylated
BODIPYs. On the other hand, additional unconstrained
nitrated aryl groups at the α-pyrrolic position enabled an
electron coupling and their electron-withdrawing ability
counteracted in part, such as nonradiative channels, leading
to a higher fluorescence response toward the red-edge of the
visible spectrum, albeit somehow limited owing to the
activation of charge-transfer phenomena in polar surrounding
environments.

The cell staining analysis clearly showed that the BODIPYs
generated in this work are compatible with cells showing high
enough fluorescence to be tracked by bioimaging and whole-
cell staining, along with retention of cell functionality and
reduced cell damage or toxicity in short incubation times. Our
results are encouraging for cell imaging and for cellular
structure staining for both basic and applied research. Other
MCRs to produce complex boronic acids and their cross-
couplings with different chromophores are being evaluated in
our laboratories and the results will be reported in due course.

As a matter of fact and regarding to BODIPYs, a valid
approach could be to develop such complex dyes but sterically
hindered around the key meso position (both via functional-
ization at the ortho position of arylboronic acids or upon
alkylation at the adjacent chromophoric 1 and 7 positions)
because they are expected to be brighter fluorophores.

The Experimental Section

Materials. Starting 8-(methylthio)BODIPY, CuTC, and
boronic acids are commercially available. Solvents were dried
and distilled before use.

Spectroscopic Techniques. Diluted dye solutions
(around 4 × 10^{-6} M) were prepared by adding the
Corresponding author
spectroscopic grade) to the residue
from the adequate amount of a concentrated stock solution in
acetone, after vacuum evaporation of this solvent. UV–vis
absorption and steady-state fluorescence spectra were obtained
using 1 cm path length quartz cuvettes. The emission spectra
were corrected from the monochromator wavelength depend-
ence, the lamp profile, and the photomultiplier sensitivity.
Fluorescence quantum yields (ϕ) were calculated using
commercial BODIPYs as the reference: PM546 (ϕ_r = 0.85 in
ethanol) for compounds 4a–4c, 4e–4j, and 4n–4o and
PM597 (ϕ_r = 0.43 in ethanol) for compounds 5a–5d. The
values were corrected by the refractive index of the solvent.
Radiative decay curves were registered with the time-correlated
single-photon counting technique using the same spectro-
fluorimeter (with picosecond time-resolution). Fluorescence
emission was monitored at the maximum emission wavelength
after excitation by means of a pulsed Fianium Supercontinuum
laser at an appropriate wavelength for each compound, with
150 ps full width at half-maximum pulses and working at 10
MHz. The fluorescence lifetime (τ) was obtained after the
deconvolution of the instrumental response signal from the
recorded decay curves by means of an iterative method. The
goodness of the exponential fit was controlled by statistical
parameters (x-square) and the analysis of the residuals.

Theoretical Simulations. Ground-state geometries were
optimized at the density functional theory level using the
B3LYP hybrid functional, whereas the first singlet excited-state
optimization was carried out by the configuration interaction
singles method. In all cases, the double-valence basis set adding
a polarization function (6-31+g*) was used. The energy
minimization was conducted without any geometric
restriction and the geometries were considered as energy
minimum when the corresponding frequency analysis did not give any negative value. Rotational energy barriers, in both the ground and excited states, were calculated from the potential energy surface, which was simulated by relaxed scans (steps of $10^3$) of the 8-phenylBODIPY with regard to the plane of the BODIPY core. For such an energy landscape, the simpler 8-phenylBODIPY was considered as the model because the fragment at para position of such a ring should not alter the rotational barrier but greatly increase the computational cost, resources, and time of the calculation. The solvent effect [tetrahydrofuran (THF)] was also simulated during the above calculations by the self-consistent reaction field using the polarizable continuum model. All theoretical calculations were carried out using the Gaussian 09 implemented in the computational cluster provided by the SGiker resources of the UPV/EHU.

**Synthesis and Characterization.** $^1$H and $^{13}$C NMR spectra were recorded in deuteriochloroform (CDCl$_3$), with either tetramethylsilane (0.00 ppm $^1$H, 0.00 ppm $^{13}$C) or chloroform (7.26 ppm $^1$H, 77.00 ppm $^{13}$C). Data are reported in the following order: chemical shift in ppm, multiplicities [br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), exch (exchangeable), app (apparent)], coupling constants, $J$ (Hz), and integration. Infrared spectra were recorded on a Fourier transform infrared spectrophotometer. Peaks are reported (cm$^{-1}$) with the following relative intensities: s (strong, $67\sim100\%$), m (medium, $40\sim67\%$), and w (weak, $20\sim40\%$). Melting points are not corrected. Thin-layer chromatography (TLC) was conducted in silica gel on TLC Al foils. Detection was done by UV light (254 or 365 nm). High-resolution mass spectrometry (HRMS) samples were ionized by ESI$^+$ and recorded via the time-of-flight method.

**Biological Methods.** Human PBMCs were isolated by density centrifugation using Histopaque-1077 as described. Upon human PBMC–BODIPY interaction, cytotoxicity was evaluated by measuring the release of LDH, using a commercial kit and following the manufacturer instructions.

**Typical Procedure for the Synthesis of Arylboronic Acid Analogues via the Passerini and/or Ugi MCRs (TP1).** In a round-bottomed flask equipped with a stir bar, the aldehyde (1.0 equiv), amine (1.3 equiv), and methanol (1.0 M) were first stirred for 5 min at room temperature. $\text{t-Butylisocyanide}$ (1.3 equiv) was then added to the reaction mixture. After TLC showed that the reaction went to completion, the solvent was removed under reduced pressure. The crude material was redissolved in dichloromethane (20 mL) and the resulting organic solution was then washed with a saturated aq NaHCO$_3$ solution combined with brine (3 $\times$ 20 mL). The resulting organic layers were collected, dried over MgSO$_4$, filtered, and concentrated in vacuo to afford the desired product as a white solid. This solid was triturated in petroleum ether and the residual solvent was removed in vacuo and used directly in the following reaction.

**For the Ugi MCR.** In a round-bottomed flask equipped with a stir bar, the aldehyde (1.0 equiv), amine (1.3 equiv), and methanol (1.0 M) were first stirred for 15 min at room temperature. The acid component (1.3 equiv) and $\text{t-butylisocyanide}$ (1.3 equiv) were then added to the reaction mixture. After TLC showed that the reaction went to completion, the solvent was removed under reduced pressure. The crude material was redissolved in dichloromethane (20 mL) and the resulting organic solution was then washed with 1.0 M HCl (aq) (3 $\times$ 20 mL) and a saturated aq NaHCO$_3$ solution combined with brine (3 $\times$ 20 mL). The resulting organic layers were collected, dried over MgSO$_4$, filtered, and then concentrated in vacuo to afford the desired product as a white solid. This solid was triturated in petroleum ether and the residual solvent was removed in vacuo and used directly in the following reaction.

**Typical Procedure for the Cross-Coupling of 8-Methyl-BODIPYs with Boronic Acids (TP2).** An oven-dry Schlenk tube, equipped with a stir bar, was charged with either 8-methylthioBODIPY 1a or 1b (1.0 equiv), the corresponding boronic acid (3.0 equiv), and dry THF (0.03 M) under N$_2$. The mixture was sparged with N$_2$ for 5 min, whereupon $\text{Pd}_2(\text{dba})_3$ (2.5 mol %), trifurylphosphine (7.5 mol %), and CuTC (3.0 equiv) were added under N$_2$. The reaction mixture was immersed into a preheated oil bath at 55 °C. After TLC showed that the reaction went to completion, the reaction mixture was allowed to reach room temperature and adsorbed on SiO$_2$ gel. After flash chromatography (SiO$_2$-gel, EtOAc/hexanes gradient) purification, meso-substituted BODIPYs were obtained as highly colored solids.

![BODIPY 4a](Image)

**BODIPY 4a.** According to TP2. 1a (30.0 mg, 0.1260 mmol, 1.0 equiv), 2a (134.2 mg, 0.3780 mmol, 3.0 equiv), CuTC (72.0 mg, 0.3780 mmol, 3.0 equiv), $\text{Pd}_2(\text{dba})_3$ (2.9 mg, 0.0032
mmol, 2.5 mol %), and tri-2-furylphosphine (2.2 mg, 0.0094 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 4a as a yellow solid (43.6 mg, 69% yield); mp 220–222 °C; TLC (30% EtOAc/hexanes, Rₜ = 0.30); IR (KBr, cm⁻¹): 3305 (m), 3079 (w), 2969 (w), 1727 (s), 1664 (s), 1556 (s), 1414 (s), 1387 (s), 1262 (s), 1112 (s), 1077 (s), 985 (w), 709 (m). ¹H NMR (500 MHz, CDCl₃): δ 8.13 (d, J = 7.5 Hz, 2H), 7.93 (s, 2H), 7.69 (d, J = 8.0 Hz, 2H), 7.65 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.53 (t, J = 8.0 Hz, 2H), 6.94 (d, J = 4.0 Hz, 2H), 6.53 (d, J = 3.0 Hz, 2H), 6.35 (s, 1H), 6.23 (br, 1H), 1.42 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 167.0, 164.8, 164.7, 144.5, 139.0, 135.0, 134.4, 134.1, 131.8, 130.9, 129.9, 129.2, 129.0, 127.6, 118.8, 75.5, 52.0, 28.9; HRMS (ESI⁺) m/z: calcd for C₂₆H₂₂BF₂N₃O₃ [M + H]⁺, 522.1113; found, 522.2112.

**BODIPY 4b.** According to TP2. 1a (37.0 mg, 0.1554 mmol, 1.0 equiv), 2b (174.0 mg, 0.4663 mmol, 3.0 equiv), CuTC (89.0 mg, 0.4663 mmol, 3.0 equiv), Pd₂(dba)₃ (3.6 mg, 0.0039 mmol, 2.5 mol %), and tri-2-furylphosphine (2.7 mg, 0.0116 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 4b as a red solid (45.0 mg, 56% yield); mp 95–97 °C; TLC (30% EtOAc/hexanes, Rₜ = 0.49); IR (KBr, cm⁻¹): 3435 (w), 3398 (w), 3326 (w), 2968 (w), 2932 (w), 1729 (s), 1691 (s), 1552 (s), 1414 (s), 1386 (s), 1259 (s), 1112 (s), 1078 (s), 1000 (m), 957 (m), 737 (m), 711 (m). ¹H NMR (500 MHz, CDCl₃): δ 8.12 (d, J = 7.0 Hz, 2H), 7.95 (s, 2H), 7.74 (t, J = 7.5 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 2H), 7.39 (dd, J = 8.0, 1.5 Hz, 1H), 7.33 (dd, J = 10.5, 1.5 Hz, 1H), 6.95 (d, J = 4.0 Hz, 2H), 6.55 (d, J = 4.0 Hz, 2H), 6.51 (s, 1H), 6.28 (br, 1H), 1.43 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 166.2, 164.8, 160.4 (d, 1/ₐCF = 252.0 Hz), 145.0, 144.7, 136.3 (d, 1/ₐCF = 8.4 Hz), 134.7, 134.1, 131.7, 130.7 (d, 1/ₐCF = 3.9 Hz), 130.0, 129.0, 128.6 (d, 1/ₐCF = 3.3 Hz), 126.3 (d, 1/ₐCF = 13.7 Hz), 119.1, 118.1 (d, 1/ₐCF = 23.6 Hz), 70.9, 52.1, 28.8; HRMS (ESI⁺) m/z: calcd for C₂₆H₂₂BF₂N₃O₃ [M + H]⁺, 520.2113; found, 520.2106.

**BODIPY 4c.** According to TP2. 1a (30.0 mg, 0.1260 mmol, 1.0 equiv), 2c (134.2 mg, 0.3780 mmol, 3.0 equiv), CuTC (72.0 mg, 0.3780 mmol, 3.0 equiv), Pd₂(dba)₃ (2.9 mg, 0.0032 mmol, 2.5 mol %), and tri-2-furylphosphine (2.2 mg, 0.0094 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 4c as a yellow solid (36.0 mg, 57% yield); mp 108–110 °C; TLC (30% EtOAc/hexanes, Rₜ = 0.44); IR (KBr, cm⁻¹): 3400 (m), 2968 (w), 1726 (s), 1690 (s), 1554 (s), 1413 (s), 1388 (s), 1260 (s), 1114 (s), 1078 (s), 961 (w), 735 (m), 712 (m). ¹H NMR (500 MHz, CDCl₃): δ 8.09 (d, J = 7.5 Hz, 2H), 7.93 (s, 2H), 7.78–7.76 (m, 1H), 7.73 (s, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.57–7.56 (m, 2H), 7.50 (t, J = 7.5 Hz, 2H), 6.93 (s, 2H), 6.51 (s, 2H), 6.51 (s, 1H), 6.27 (br, 1H), 1.4 (s, 9H); ¹³C NMR (126 MHz, CDCl₃): δ 167.0, 164.8, 146.7, 144.4, 137.0, 135.0, 134.2, 134.1, 131.9, 131.0, 130.4, 129.8, 129.5, 129.1, 129.0, 118.8, 75.6, 51.9, 28.8; HRMS (ESI⁺) m/z: calcd for C₂₆H₂₂BF₂N₃O₃ [M + H]⁺, 502.2113; found, 502.2107.
**BODIPY 4f.** According to TP2. 1a (15.0 mg, 0.0630 mmol, 1.0 equiv), 2f (82.0 mg, 0.1890 mmol, 3.0 equiv), CuTC (36.0 mg, 0.1890 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.4 mg, 0.0016 mmol, 2.5 mol %), and tri-2-furylphosphine (1.1 mg, 0.0047 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 4f as an orange solid (25.0 mg, 69% yield); mp 199–200 °C; TLC (30% EtOAc/hexanes, $R_f = 0.49$); IR (KBr, cm$^{-1}$): 3306 (w), 2972 (w), 1720 (m), 1661 (s), 1574 (m), 1555 (m), 1526 (s), 1513 (s), 1438 (s), 1437 (s), 1323.3, 131.5, 131.3, 130.8, 129.9, 128.0, 126.0 (q, 3J$_{CF} = 165.9$ Hz), 119.2, 119.0, 76.0, 52.1, 28.8; HRMS (ESI$^+$) m/z: calculd for C$_{39}$H$_{26}$BrF$_3$N$_3$O$_3$ [M + H]$^+$, 779.1987; found, 779.1963.

**BODIPY 4g.** According to TP2. 1a (30.0 mg, 0.1260 mmol, 1.0 equiv), 2g (160.0 mg, 0.3780 mmol, 3.0 equiv), CuTC (72.0 mg, 0.3780 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (2.9 mg, 0.0032 mmol, 2.5 mol %), and tri-2-furylphosphine (2.2 mg, 0.0085 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 15% EtOAc/hexanes afforded the desired product 4g as a red solid (51.0 mg, 71% yield); mp 170–172 °C; TLC (30% EtOAc/hexanes, $R_f = 0.49$); IR (KBr, cm$^{-1}$): 3284 (w), 3275 (w), 3108 (w), 3062 (w), 1720 (m), 1655 (m), 1574 (m), 1555 (m), 1526 (s), 1438 (s), 1323.2, 131.5, 131.3, 130.8, 129.9, 129.4, 123.6, 119.2, 76.0, 52.1, 28.8; HRMS (ESI$^+$) m/z: calculd for C$_{39}$H$_{26}$BrF$_3$N$_3$O$_3$ [M + H]$^+$, 547.1964; found, 547.1966.

**BODIPY 4h.** According to TP2. 1a (20.0 mg, 0.0840 mmol, 1.0 equiv), 2h (100.8 mg, 0.2520 mmol, 3.0 equiv), CuTC (48.1 mg, 0.2520 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.9 mg, 0.0021 mmol, 2.5 mol %), and tri-2-furylphosphine (1.5 mg, 0.0063 mmol, 7.5 mol %) for 1 h were reacted. Flash chromatography on silica gel using 15% EtOAc/hexanes afforded the desired product 4h as an orange solid (29.0 mg, 63% yield); mp 170–172 °C; TLC (30% EtOAc/hexanes, $R_f = 0.30$); IR (KBr, cm$^{-1}$): 3284 (w), 2978 (w), 1728 (m), 1655 (m), 1574 (m), 1555 (m), 1526 (s), 1438 (s), 1323.2, 131.5, 131.3, 130.8, 129.9, 128.0, 126.0 (q, 3J$_{CF} = 165.9$ Hz), 119.2, 119.0, 76.0, 52.1, 28.8; HRMS (ESI$^+$) m/z: calculd for C$_{39}$H$_{26}$BrF$_3$N$_3$O$_3$ [M + H]$^+$, 570.1963; found, 570.1963.

**BODIPY 4i.** According to TP2. 1a (27.0 mg, 0.1134 mmol, 1.0 equiv), 2i (55.0 mg, 0.2402 mmol, 3.0 equiv), CuTC (64.9 mg, 0.3402 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (2.6 mg, 0.0028 mmol, 2.5 mol %), and tri-2-furylphosphine (2.0 mg, 0.0085 mmol, 7.5 mol %) for 1.5 h were reacted. Flash chromatography on silica gel using 30% EtOAc/hexanes afforded the desired product 4i as an orange solid (55.0 mg, 82% yield); mp 120–123 °C; TLC (30% EtOAc/hexanes, $R_f = 0.15$); IR (KBr, cm$^{-1}$): 3400 (w), 3321 (w), 3108 (w), 3062 (w), 3030 (w), 2965 (w), 2924 (w), 1686 (m), 1630 (m), 1571 (s), 1544 (s), 1413 (s), 1323.2, 131.5, 130.8, 126.0 (q, 3J$_{CF} = 3.4$ Hz), 124.0 (d, 3J$_{CF} = 272.3$ Hz), 119.2, 75.9, 52.1, 28.8; HRMS (ESI$^+$) m/z: calculd for C$_{39}$H$_{26}$BrF$_3$N$_3$O$_3$ [M + H]$^+$, 779.1987; found, 779.1963.
According to TP2. **BODIPY 4j**. According to TP2. 1a (20.0 mg, 0.840 mmol, 1.0 equiv), 3b (119.5 mg, 0.2520 mmol, 3.0 equiv), CuTC (48.0 mg, 0.2520 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.9 mg, 0.0021 mmol, 2.5 mol%), and tri-2-furylphosphine (1.4 mg, 0.0063 mmol, 7.5 mol%) for 1 h were reacted. Flash chromatography on silica gel using 30% EtOAc/hexanes afforded the desired product 4j as orange crystals (46.0 mg, 88% yield); mp 125–127 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.10); IR (KBr, cm$^{-1}$): 3400 (w), 3337 (w), 3108 (w), 2965 (w), 2932 (w), 1685 (m), 1634 (m), 1572 (s), 1544 (s), 1513 (s), 1413 (s), 1388 (s), 1261 (s), 1114 (s), 1078 (s), 983 (m), 913 (m), 779 (m), 740 (m), 715 (w). $^1$H NMR (400 MHz, CDCl$_3$): δ 7.93 (s, 2H), 7.51–7.42 (m, 9H), 6.93 (d, J = 6.0 Hz, 2H), 6.79 (s, 2H), 6.68 (d, J = 7.7 Hz, 2H), 6.54 (s, 2H), 5.99 (br, 1H), 5.64 (s, 1H), 4.80 (d, J = 16.1 Hz, 1H), 4.58 (d, J = 16.3 Hz, 1H), 3.70 (s, 3H), 1.36 (s, 9H); $^{13}$C NMR (101 MHz, CDCl$_3$): δ 173.4, 168.2, 158.9, 146.5, 144.5, 138.3, 136.1, 134.9, 133.8, 151.6, 131.6, 130.7, 130.2, 129.7, 128.8, 128.5, 126.8, 118.8, 113.8, 55.3, 52.0, 28.7; HRMS (ESI+) m/z: calcd for C$_{53}$H$_{48}$BF$_2$N$_4$O$_3$ [M + H]$^+$, 811.2536; found, 811.2538.

According to TP2. **BODIPY 4n**. According to TP2. 1a (20.0 mg, 0.840 mmol, 1.0 equiv), 3f (99.6 mg, 0.2100 mmol, 2.5 equiv), CuTC (48.0 mg, 0.2520 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.9 mg, 0.0021 mmol, 2.5 mol%), and tri-2-furylphosphine (1.4 mg, 0.0063 mmol, 7.5 mol%) for 1 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 4n as an orange solid (38.0 mg, 73% yield); mp 114–115 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.10); IR (KBr, cm$^{-1}$): 3401 (w), 3109 (w), 2964 (w), 2929 (w), 1685 (m), 1635 (m), 1568 (s), 1542 (m), 1513 (s), 1413 (s), 1386 (s), 1260 (s), 1113 (s), 1078 (s), 982 (m), 912 (w), 781 (w), 739 (w). $^1$H NMR (500 MHz, CDCl$_3$): δ 7.93 (s, 2H), 7.54–7.36 (m, 9H), 6.84 (s, 4H), 6.67 (s, 2H), 6.54 (d, J = 3.0 Hz, 2H), 5.72 (br, 1H), 5.57 (br, 1H), 4.72 (d, J = 16.1 Hz, 1H), 4.41 (d, J = 16.3 Hz, 1H), 3.73 (s, 3H), 1.35 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 172.2, 168.4, 158.9, 146.2, 144.6, 139.1, 135.4, 134.9, 131.6, 130.5, 129.9, 128.9, 128.3, 126.9, 118.9, 113.9, 64.5, 55.4, 51.9, 28.8; HRMS (ESI+) m/z: calcd for C$_{53}$H$_{48}$BF$_2$N$_4$O$_3$ [M + H]$^+$, 621.2849; found, 621.2856.

According to TP2. **BODIPY 4k**. According to TP2. 1a (32.0 mg, 0.1344 mmol, 1.0 equiv), 3c (175.1 mg, 0.4032 mmol, 3.0 equiv), CuTC (76.9 mg, 0.4032 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (3.1 mg, 0.0034 mmol, 2.5 mol%), and tri-2-furylphosphine (2.3 mg, 0.0100 mmol, 7.5 mol%) for 1 h were reacted. Flash chromatography on silica gel using 30% EtOAc/hexanes afforded the desired product 4k as orange crystals (49.0 mg, 63% yield); mp 133–135 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.18); IR (KBr, cm$^{-1}$): 3323 (w), 3111 (w), 2967 (w), 1683 (m), 1635 (m), 1552 (s), 1413 (s), 1387 (s), 1261 (s), 1114 (s), 1078 (s), 957 (w), 735 (m); $^1$H NMR (500 MHz, CDCl$_3$): δ 7.93 (s, 2H), 7.56 (d, J = 6.9 Hz, 4H), 7.48–7.40 (m, 5H), 7.23 (d, J = 1.3 Hz, 1H), 6.91 (s, 2H), 6.52 (s, 2H), 6.19 (m, 1H), 6.01 (br, 1H), 5.60 (br, 1H), 4.61 (dd, J = 38.8, 16.3 Hz, 2H), 1.37 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): δ 173.2, 168.2, 149.9, 146.8, 144.0, 142.4, 136.1, 135.5, 135.0, 134.1, 131.9, 131.2, 130.8, 130.5, 130.3, 128.8, 128.7, 118.8, 110.8, 109.4, 65.3, 51.9, 28.8; HRMS (ESI+) m/z: calcd for C$_{53}$H$_{32}$BF$_2$N$_4$O$_3$ [M + H]$^+$, 591.2743; found, 591.2751.

According to TP2. **BODIPY 4o**. According to TP2. 1a (15.0 mg, 0.0630 mmol, 1.0 equiv), 6g (92.6 mg, 0.1575 mmol, 2.5 equiv), CuTC (36.0 mg, 0.1890 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.5 mg, 0.0016 mmol, 2.5 mol%), and tri-2-furylphosphine (1.1 mg, 0.0047 mmol, 7.5 mol%) for 3 h were reacted. Flash chromatography on silica gel using 20% EtOAc/hexanes afforded the desired product 4o as an orange solid (30.0 mg, 65% yield); mp 119–121 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.20); IR (KBr, cm$^{-1}$): 3401 (w), 3322 (w), 3110 (w), 2963 (w), 2925 (w), 1691 (m), 1624 (m), 1555 (s), 1412 (s), 1386 (s), 1308.8, 130.5, 130.3, 128.8, 128.7, 118.8, 110.8, 109.4, 65.3, 51.9, 28.8; HRMS (ESI+) m/z: calcd for C$_{53}$H$_{32}$BF$_2$N$_4$O$_3$ [M + H]$^+$, 621.2849; found, 621.2852.
BODIPY 5a. According to TP2. 1b (15.0 mg, 0.0418 mmol, 1.0 equiv), 2a (44.5 mg, 0.1253 mmol, 3.0 equiv), CuTC (23.9 mg, 0.1253 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.0 mg, 0.0010 mmol, 2.5 mol %), and tri-furylphosphine (0.7 mg, 0.0031 mmol, 7.5 mol %) for 2.5 h were reacted. Flash chromatography on silica gel using 15% EtOAc/hexanes afforded the desired product 5a as a purple solid (13.0 mg, 50% yield); mp 168–170 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.20); IR (KBr, cm$^{-1}$): 3426 (w), 2970 (w), 2926 (w), 1726 (m), 1672 (m), 1528 (s), 1466 (m), 1396 (m), 1341 (s), 1272 (s), 1142 (s), 1109 (s), 1077 (s), 712 (m). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.86 (s, 2H), 7.54 (s, 1H), 7.37–7.30 (m, 6H), 7.26–7.22 (m, 4H), 6.96 (s, 1H), 6.41 (s, 2H), 6.18–6.10 (m, 3H), 5.90 (s, 1H), 1.39 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 171.0, 167.6, 145.2, 144.7, 140.7, 138.4, 135.6, 134.8, 134.4, 134.3, 132.8, 132.1, 132.0, 131.3, 130.2, 129.4, 129.0, 128.9, 128.8, 128.2, 123.1, 118.8, 64.6, 52.3, 28.8; HRMS (ESI+) m/z: calcd for C$_{34}$H$_{30}$Br$_2$F$_2$N$_2$O$_2$ [M + H]$^+$, 735.0779; found, 735.0750.

BODIPY 5b. According to TP2. 1b (30.0 mg, 0.0835 mmol, 1.0 equiv), 2b (106.0 mg, 0.2506 mmol, 3.0 equiv), CuTC (47.8 mg, 0.2506 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.9 mg, 0.0021 mmol, 2.5 mol %), and tri-furylphosphine (1.5 mg, 0.0063 mmol, 7.5 mol %) for 2.5 h were reacted. Flash chromatography on silica gel using 15% EtOAc/hexanes afforded the desired product 5b as a purple solid (31.5 mg, 59% yield); mp 117–119 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.28); IR (KBr, cm$^{-1}$): 3435 (w), 3302 (w), 3081 (w), 2967 (w), 2927 (w), 1728 (m), 1667 (m), 1559 (s), 1394 (m), 1343 (m), 1261 (s), 1141 (s), 1106 (s), 1074 (s), 1002 (w), 952 (w), 708 (w). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.32 (d, $J = 8.8$ Hz, 2H), 8.13 (d, $J = 7.6$ Hz, 2H), 8.09 (d, $J = 8.8$ Hz, 2H), 7.92 (s, 1H), 7.76 (t, $J = 7.5$ Hz, 1H), 7.66 (t, $J = 7.4$ Hz, 1H), 7.53 (t, $J = 7.7$ Hz, 2H), 7.41 (d, $J = 7.8$ Hz, 1H), 7.36 (d, $J = 10.1$ Hz, 1H), 7.01 (d, $J = 4.3$ Hz, 1H), 6.99 (d, $J = 4.1$ Hz, 1H), 6.72 (d, $J = 4.3$ Hz, 1H), 6.60 (d, $J = 3.8$ Hz, 1H), 6.53 (s, 1H), 6.31 (br, 1H), 1.44 (s, 9H); $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 166.2, 164.9, 160.5 (d, $^{13}$CP = 252.1 Hz), 156.5, 148.4, 145.8, 144.4, 138.4, 137.0, 136.3 (d, $^{13}$CP = 8.4 Hz), 134.8, 134.2, 131.2, 131.8, 130.8 (d, $^{13}$CP = 3.9 Hz), 130.4 (t, $J = 4.3$ Hz), 130.0, 129.1, 129.0, 126.7 (d, $^{13}$CP = 3.2 Hz), 126.5 (d, $^{13}$CP = 13.7 Hz), 123.7, 120.9, 120.0, 118.2 (d, $^{13}$CP = 23.3 Hz), 71.0, 52.1, 28.8; HRMS (ESI+) m/z: calcd for C$_{34}$H$_{30}$Br$_2$F$_2$N$_2$O$_2$ [M + H]$^+$, 641.2183; found, 641.2149.
BODIPY 5d. According to TP2, 1b (30.0 mg, 0.0835 mmol, 1.0 equiv), 2g (106.0 mg, 0.2506 mmol, 3.0 equiv), CuTC (47.5 mg, 0.2506 mmol, 3.0 equiv), Pd$_2$(dba)$_3$ (1.9 mg, 0.0021 mmol, 2.5 mol %), and tri-2-furylphosphine (1.5 mg, 0.0063 mmol, 7.5 mol %) for 1.5 h were reacted. Flash chromatography on silica gel using 10% EtOAc/hexanes afforded the desired product 5d as a pink solid (37.5 mg, 1.0 equiv). mp 266–267 °C; TLC (30% EtOAc/hexanes, R$_f$ = 0.23); IR (KBr, cm$^{-1}$): 3282 (w), 3079 (w), 2977 (w), 1729 (s), 1665 (s), 1574 (s), 1528 (s), 1400 (m), 1325 (s), 1269 (s), 1137 (s), 1069 (s), 1018 (w), 851 (w), 729 (w).

$^{1}$H NMR (500 MHz, CDCl$_3$): δ 8.34 (d, $J = 8.8$ Hz, 2H), 8.27 (d, $J = 8.2$ Hz, 2H), 8.10 (d, $J = 8.8$ Hz, 2H), 7.97 (s, 1H), 7.72–7.70 (m, 3H), 6.91 (dd, $J = 8.8$, 4.3 Hz, 1H), 6.73 (d, $J = 4.3$ Hz, 1H), 6.62 (d, $J = 3.2$ Hz, 1H), 6.26 (s, 1H), 5.94 (s, 1H), 1.40 (s, 9H).

$^{13}$C NMR (126 MHz, CDCl$_3$): δ 166.4, 164.3, 156.6, 148.4, 146.0, 145.0, 139.5, 139.0, 138.3, 137.1, 134.8, 134.7, 131.8, 131.2, 130.9, 130.5 (t, $J = 4.3$ Hz), 130.0, 128.0, 126.1 (q, $J_{CF} = 3.7$ Hz), 123.9 (d, $J_{CF} = 273.4$ Hz), 123.7, 121.0, 120.1, 76.0, 52.2, 28.9; HRMS (ESI$^+$) m/z: calc for $C_{35}H_{29}F_5N_4O_5 [M + H]^+$, 691.2152; found, 691.2116.

## ASSOCIATED CONTENT

* Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00753.

UV–vis absorption and emission spectra in THF; $^{1}$D NMR ($^1$H, $^{13}$C); and cartesian coordinates and total energy (PDF)

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**Notes**
The authors declare no competing financial interest.

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