Electronic Supplementary Information (ESI)

Mitochondria selective trackers for long-term imaging based on readily accessible neutral BODIPYs

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1. General methods

1.1. Synthesis

Anhydrous solvents were prepared by distillation over standard drying agents according to common methods. All other solvents were of HPLC grade and were used as provided. Starting chemical substrates and reagents were used as commercially provided unless otherwise indicated. Thin-layer chromatography (TLC) was performed with silica gel plates, and the chromatograms were visualized by using UV light (λ = 254 or 365 nm).Flash chromatography was performed using silica gel (230-400 mesh). NMR spectra were recorded using $CDCl_3$ at 20 ºC. ^{1}H NMR and ^{13}C NMR chemical shifts (δ) were referenced to internal solvent $CDCl_3$ (δ = 7.260 and 77.03 ppm, respectively). Multiplicity is indicated as follows: s = singlet; d = doublet; t = triplet; q = quadruplet; m = multiplet. Coupling constants (*J*) are dated in hertz (Hz).DEPT 135 experiments were used to determine the type of carbon nucleus (C *vs.* CH *vs.* CH_2*vs.* CH_3). FTIR spectra were obtained from neat samples using the attenuated total reflection (ATR) technique. High-resolution mass spectrometry (HRMS) was performedusingelectronicimpact (EI) and ion tramp (positive mode) for the detection.

1.2. Spectroscopic measurements

Photophysical properties were recorded using diluted dye solutions (ca. $2 \cdot 10^{-6}$ M) prepared from a concentrated stock solution in ethanol (ca. 10^{-3} M), after solvent evaporation under reduced pressure, and subsequent dilution with DMSO (spectroscopic grade) and the same aqueous buffered culture media using to incubate the cells. Few drops (just two) of DMSO were added prior to the addition of the culture media to ensure the correct solubilization of the dye. UV-vis absorption and fluorescence spectra of fresh solutions were recorded on a Varian (model CARY 4E) spectrophotometer and an Edinburgh Instrument spectrofluorimeter (model FLSP 920), respectively. Fluorescence quantum yields (ϕ) were determined from corrected spectra (detector sensibility to the wavelength) by the optically dilute relative method, using commercial PM546 (1,3,5,7,8-pentamethylBODIPY) dye in ethanol (ϕ_r = 0.85) as reference.[1] The aforementioned spectrofluorimeter is also equipped with a wavelength-tunable pulsed Fianium laser. Thus, the Time Correlated Single-Photon Counting (TCSPC) technique was used to record the fluorescence decay curves.

Fluorescence emission was monitored at the maximum emission wavelength after excitation by the said Fianium at the maximum absorption wavelength. The fluorescence lifetime (τ) was obtained from the slope of the exponential fit of the decay curve, after the deconvolution of the instrumental response signal (recorded by means of a ludox scattering suspension) by means of an iterative method. The goodness of the exponential fit was controlled by statistical parameters (chi-square and the analysis of the residuals).

1.3. Dye photostability

The photostability of the dyes and MitoTracker Red dissolved in ethyl acetate was evaluated by monitoring the decrease of its laser-induced fluorescence emission upon laser excitation at 532 nm of optically matched solutions. Comparing to MitoTracker Red, the lower molar absorptivity of **1b**, **2-4** at the irradiation wavelength enforced a concentration ($8x10^{-4}$ M) higher than that of the own MitoTracker Red (10^{-4} M) in order to assure same photon absorption at 532 nm. To this aim, dye solutions were transversely pumped with 5 mJ, 8 ns FWHM pulses from the second harmonic (532 nm) of a Q-switched Nd:YAG laser (Lotis TII 2134) at a repetition rate of 10 Hz. The exciting pulses were line-focused onto the cell using a combination of positive and negative cylindrical lenses (f = 15 cm and f = -15 cm, respectively) perpendicularly arranged. Spectroscopic quartz cuvettes with 0.1 cm optical were used to allow for the minimum solution volume (40 µL) being excited. The lateral faces were grounded, whereupon no laser oscillation was obtained. The fluorescence emission was monitored perpendicular to the exciting beam, collected by an optical fiber, and imaged onto a spectrometer (Acton Research corporation) and detected with a charge-coupled device (CCD) (SpectruMM:GS128B). The fluorescence emission was recorded by feeding the signal to the boxcar (Stanford Research, model 250) to be integrated before being digitized and processed by a computer. The estimated error in the photostability measurements was 10%.

1.4. Cell cultures

Human prostate epithelial PC-3 cancer cells were purchased to American Type Culture Collection (Rockville, MD, USA) (ATCC CRL-1435). Cells were grown in sterile conditions and maintained in RPMI 1640 medium supplemented with 10% fetalbovine serum and 1% penicillin/streptomycin/amphotericin B from Sigma (St. Louis, MO,

USA). The culture was performed in a humidified 5% CO_2 environment at 37°C. For treatment experiments, cells were plated and grown for 48 h, the medium was then replaced with serum-free RPMI 1640 for 1 h before starting experiments.

1.5. Viability assays

Cells were seeded in a 24-well plate at 15000 cells/well, incubated for 24h, and then treated according to the experiments. Percentages of DMSO were 0.4% maximum (control studies showed no solvent toxicity).
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml in PBS) (100 µl) was added to each well, and the cells were incubated for 4 h at 37°C. The MTT solution was then discarded by aspiration and 200 µL of isopropanol were added to dissolve formazan crystals. Absorbance was measured at 490 and 650 nm using a microplate reader (ELX 800 Bio-Tek Intruments, INC, Bad Friedrichshall, Germany). Relative viabilities were expressed as the percentages of the viability of the vehicle-treated control.

1.6. Confocal microscopy

Cells plated in glass bottom disses were incubated first with MitoTracker or LysoTracker (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) and then with BODIPYs. Imaging was captured with a Leica TCS SP5 laser-scanning confocal microscope with LAS-AF imaging software, using a 40X oil objective, at the Confocal Microscopy Service (ICTS 'NANBIOSIS' U17) of the Biomedical Research Networking Centre on Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN at the University of Alcalá, Madrid, Spain) (www.uah.es/enlaces/investigacion.shtm). The temperature on the microscope stage was held stable during time-lapse sessions and images were taken every 7 s for a maximum 40 min.

Fixed cells imaging

Cells were first incubated for 30 min with 250 nM MitoTracker (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) to visualize mitochondria, then they were treated for 1 h with 1 µM BODIPYs, after this they were fixed in 4% paraformaldehyde in PBS 5 min. And finally they were permiabilized in 0.01%.Triton X100 in PBS Imaging was performed with a Leica TCS SP5 laser-scanning confocal microscope with LAS-AF imaging software, using 63x oil objective.

2. Supplementary figures

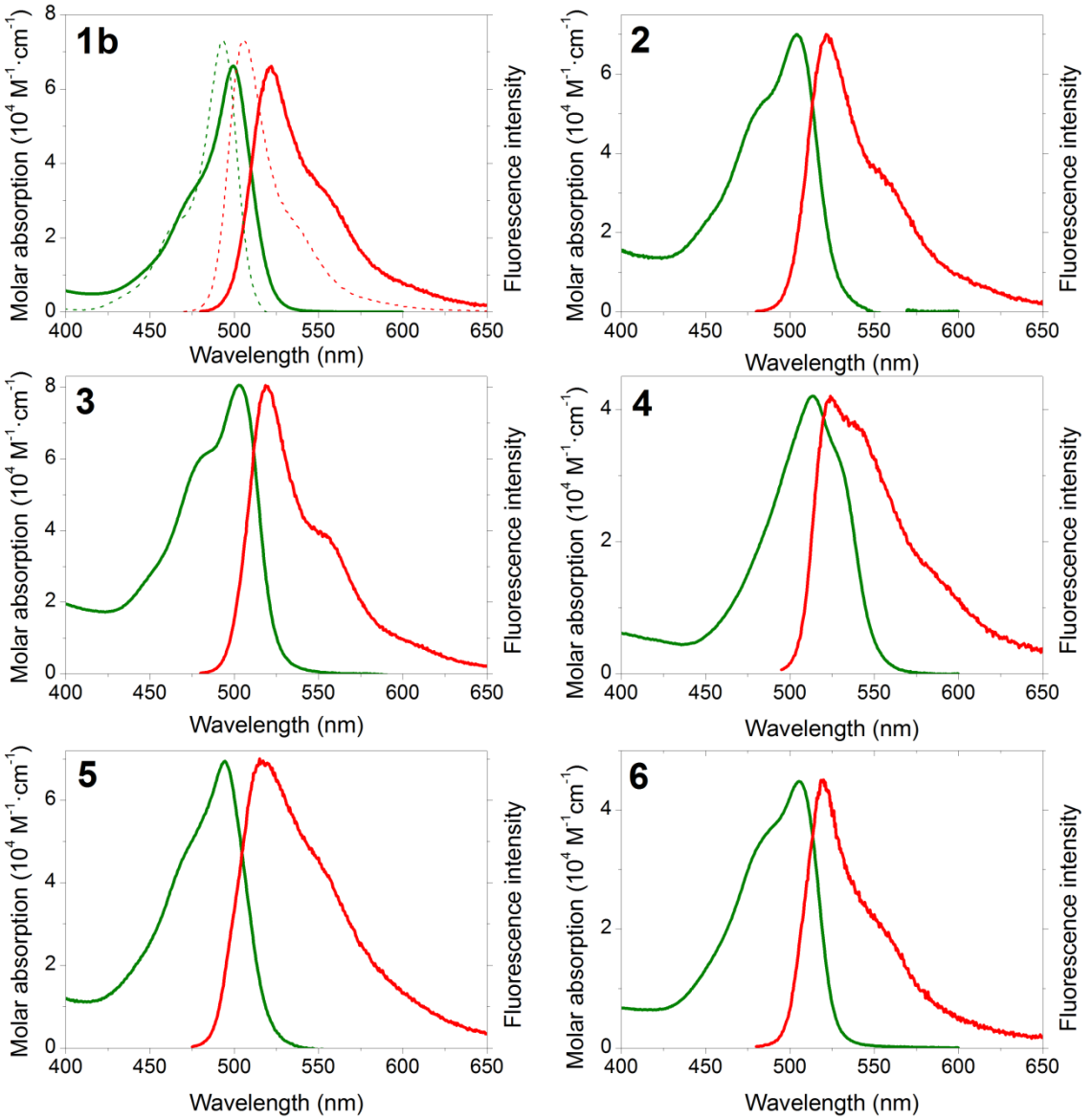


Fig. S1. Absorption and normalized fluorescence spectra of the monoformylated BODIPYs at 3 position in diluted solutions of ethanol. The corresponding spectra for the 2-formylated BODIPY **1d** (dashed) are added for comparison together with those of the 3-formylated **1b**.

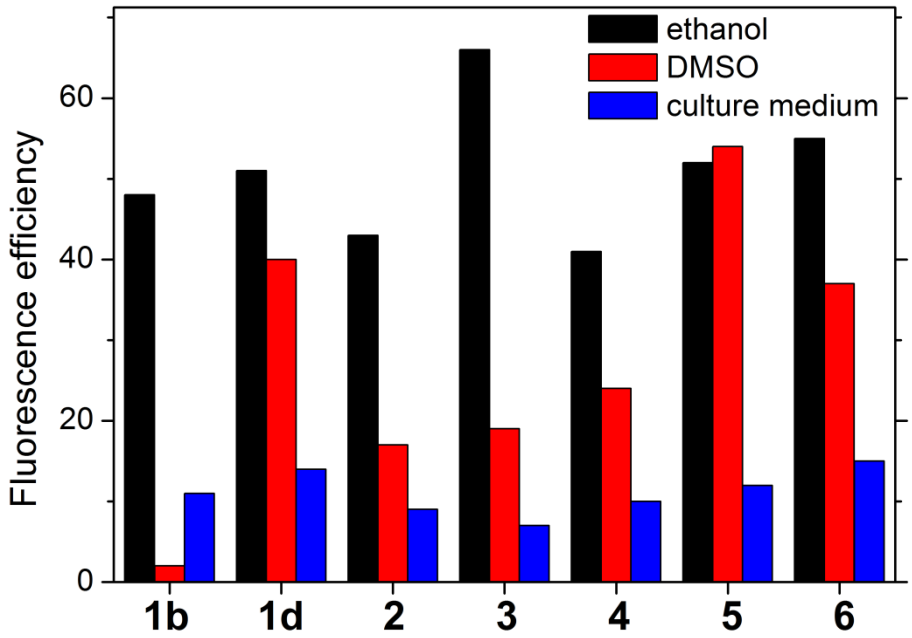


Fig. S2. Fluorescence efficiency of the 3-monoformylated BODIPYs in organic solvents and culture medium. The corresponding data for the 2-formylated BODIPY **1d** are added for comparison.

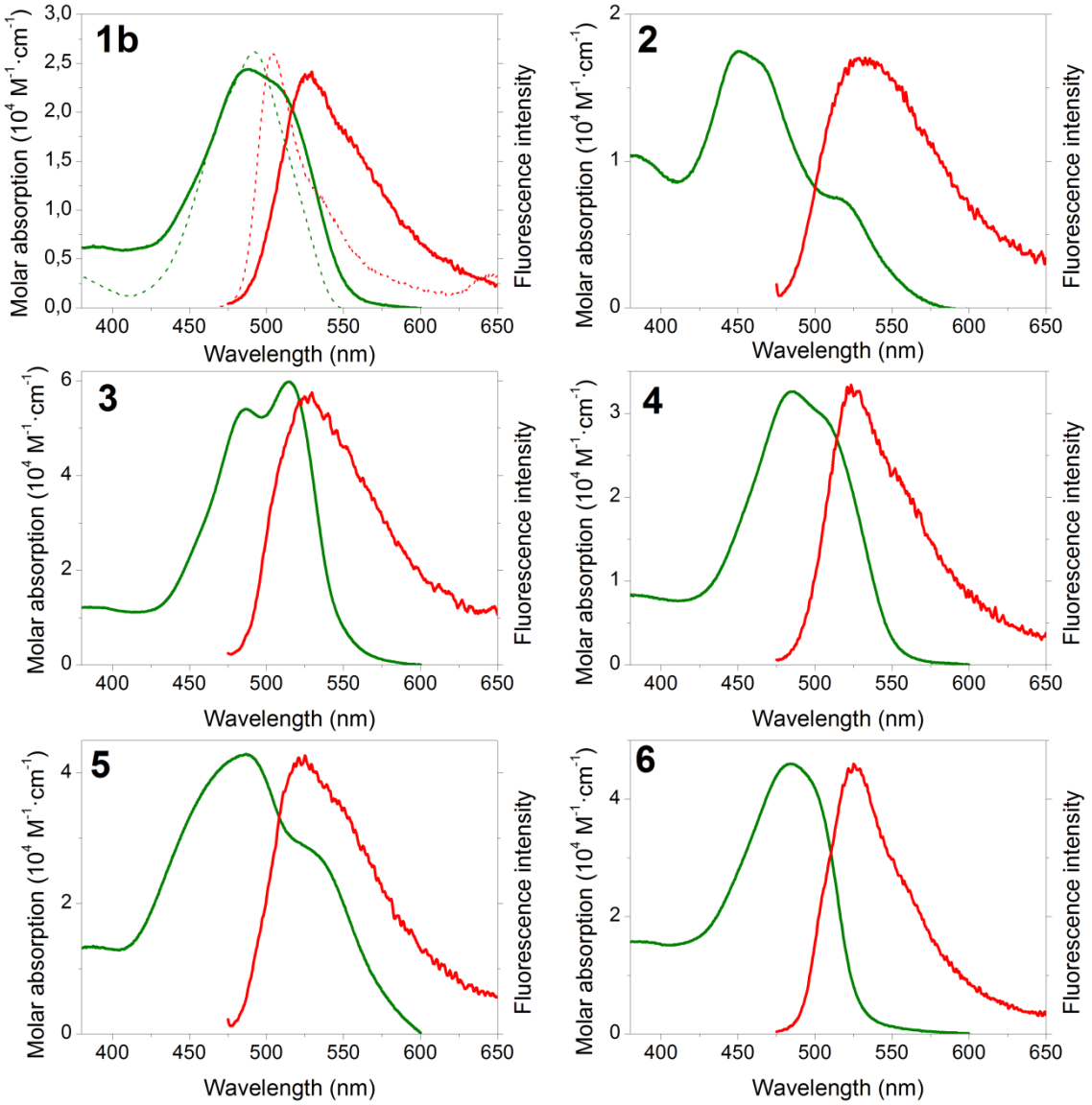


Fig. S3. Absorption and normalized fluorescence spectra of the monoformylated BODIPYs at 3 position in diluted solutions of the culture media for the cells growing just after preparing the solutions. The corresponding spectra for the 2-formylated BODIPY **1d** (dashed) are added for comparison together with those of the 3-formylated **1b**.

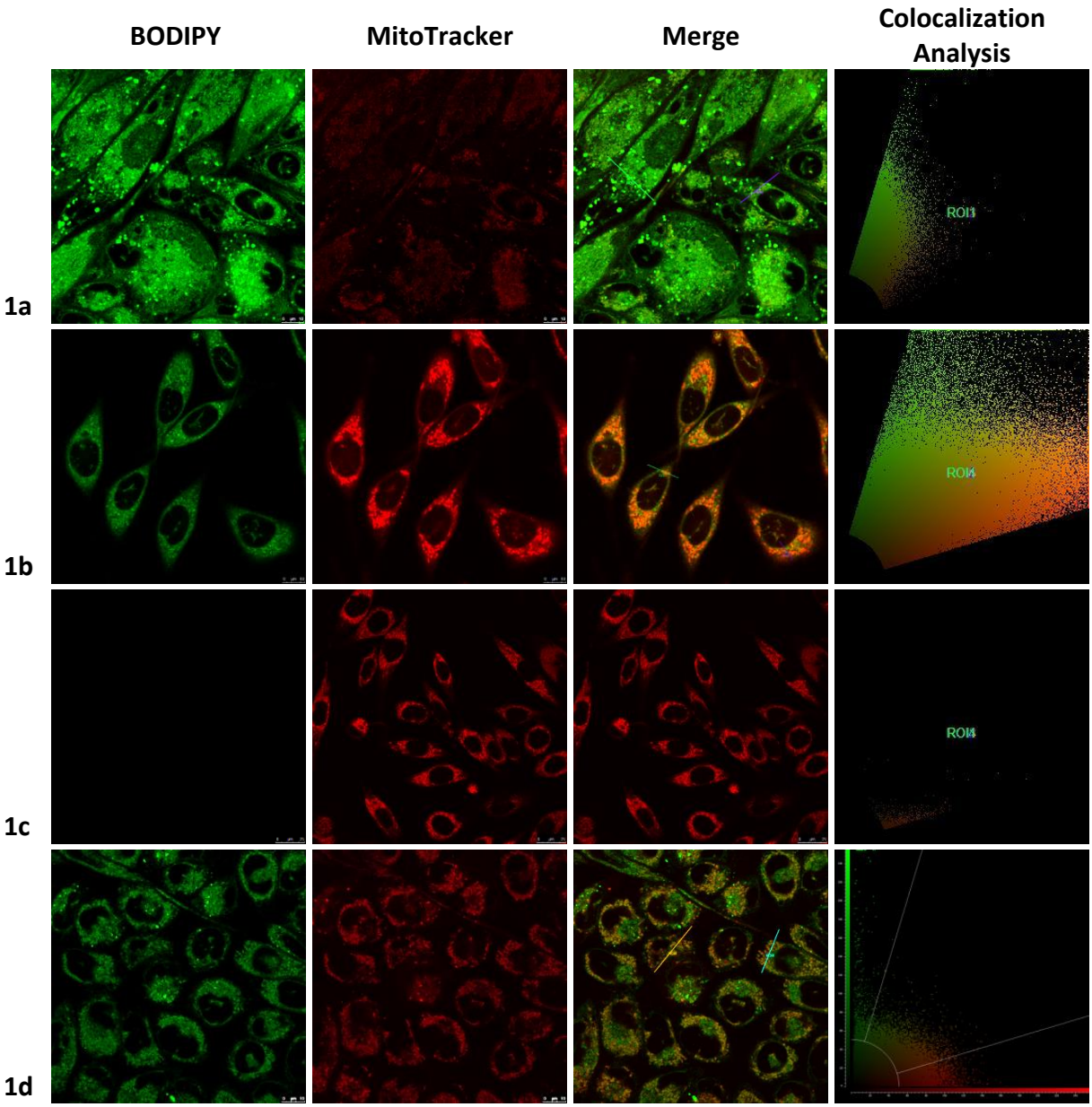


Fig. S4. Fluorescent intensity and co-localization studies of BODIPYs **1a-d** after 40 min incubation with PC-3 cells.

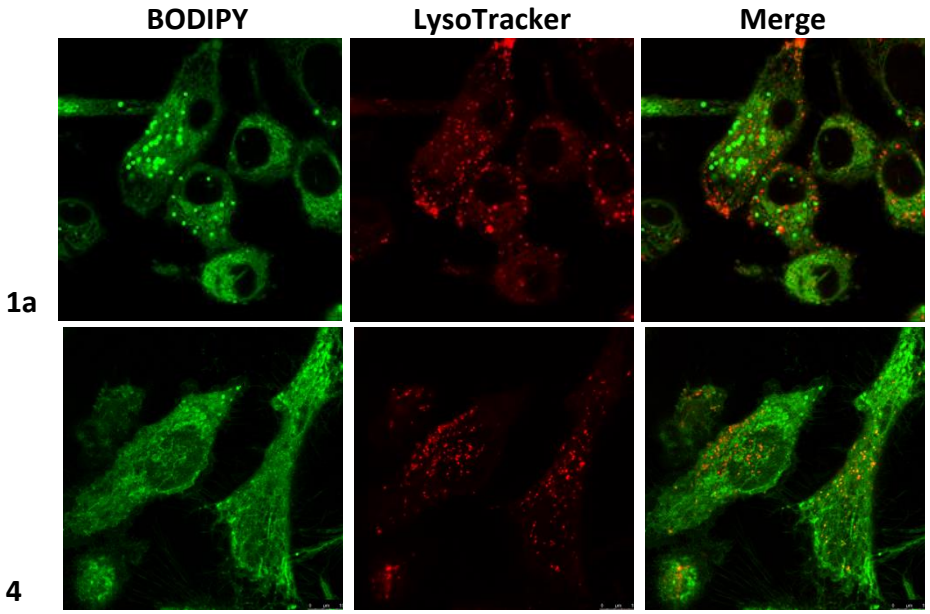


Fig. S5. BODIPYs **1a** and **4** do not colocalize with lysosomes**.** PC-3 prostate cancer cells were incubated for 30 min with 100nM LysoTracker Red (a red-fluorescent dye that stains lysosomes in live cells) and then they were pulsed with 125 nM BODIPYs. Pearson correlation coefficient are lower than 0.5.

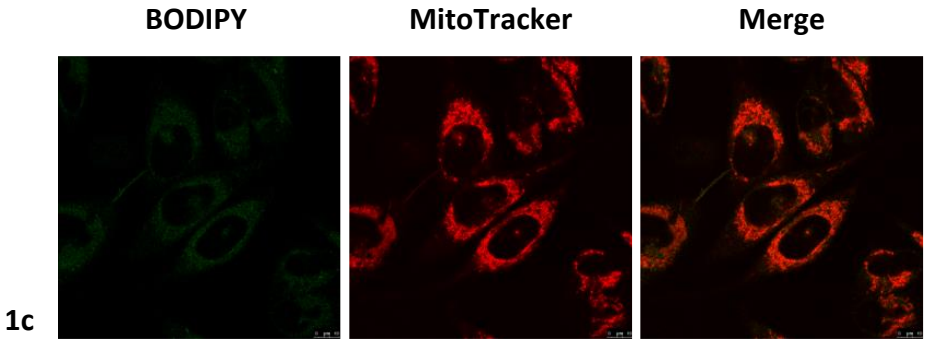


Fig. S6. 3,5-Diformyl-8-arylBODIPY **1c** does not colocalize with mitochondria.PC-3 prostate cancer cells were incubated for 15 min with 250 nM MitoTracker Red (a red-fluorescent dye that stains mitochondria in live cells) and then they were pulsed with 500 nM BODIPY **1c**. Dye incorporation into cells (green fluorescence) was followed for up to 30 min.

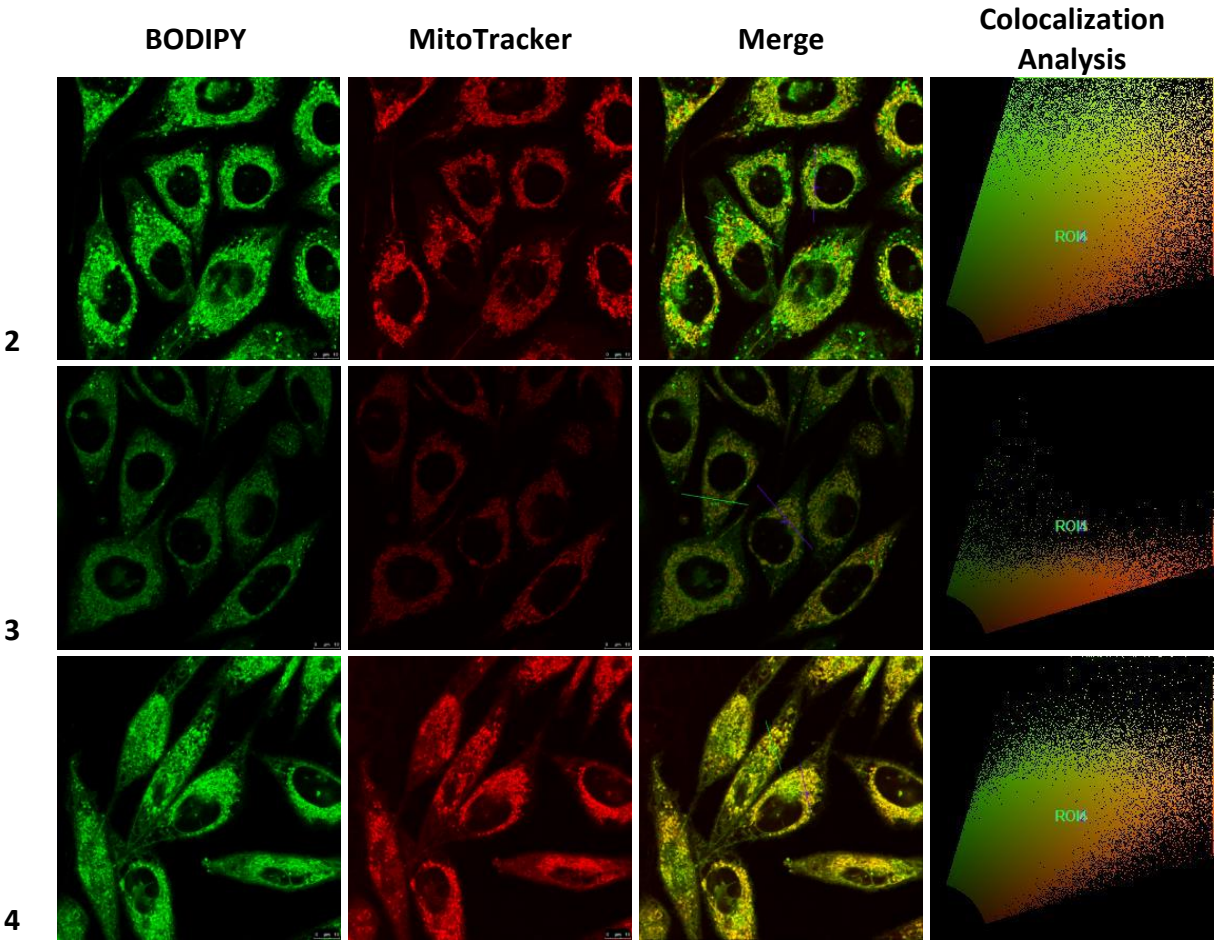
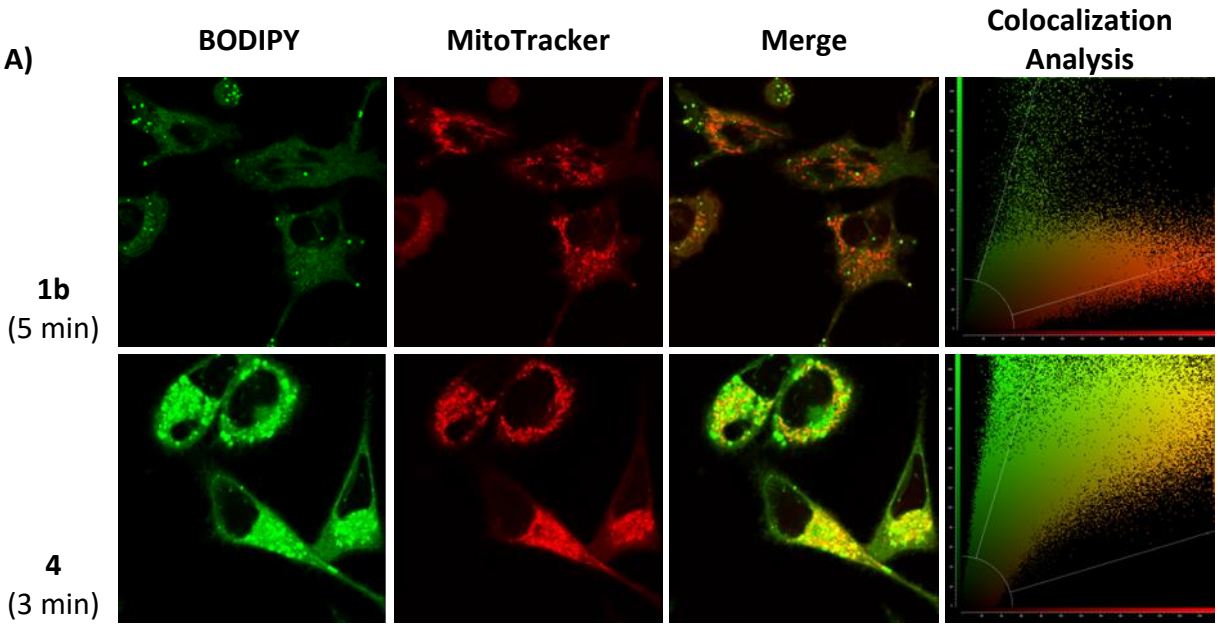


Fig. S7. Fluorescent intensity and co-localization studies of BODIPYs **2-4** after 40 min incubation with PC-3 cells.



B) VIDEO:

BODIPY 1b
https://drive.google.com/file/d/1SMrv5n_42CElHLpNUmK2QCLXdGsGmsUQ/view?usp=sharing

BODIPY 4
https://drive.google.com/file/d/14bnNUKD_MHZetZw0WMz8ifDs4NCNJmUp/view?usp=sharing

Fig. S8. A) Co-localization of 3-formyl-8-arylBODIPY **1b** and **4** with mitochondria is shown at higher magnification (63 x). **B)** Video recording of the internalization of BODIPY **1b** and **4**. Images were taken every 7 s during 15 min.

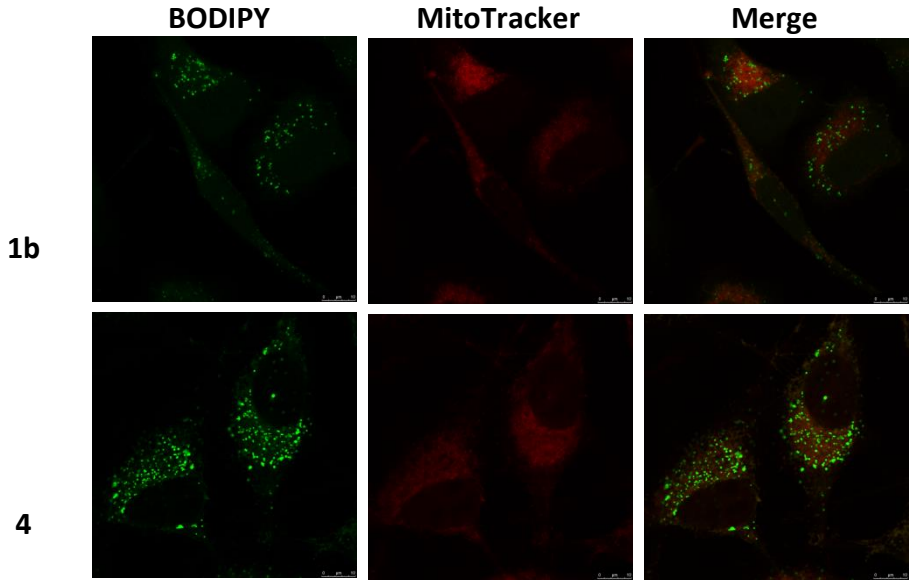


Fig. S9. 3-formyl-8-arylBODIPY **1b** and **4** still bind to mitochondria once cells are washed out of non-covalent dyes after permeabilization with Triton-X-100. PC-3 prostate cancer cells were incubated for 15 min with 250 nM MitoTracker Red and then they were treated with 1 μM BODIPYs. Thereafter, cells were fixed and permeabilized with 0.01% Triton-X-100. Although colocalization coefficients for compounds **1b** and **4** were 58% and 75%, respectively (i.e, lower than those found in non-permeabilized cells), the fixation and permeabilization procedure also resulted in an even greater loss of MitoTracker binding to mitochondria and lower fluorescence intensity

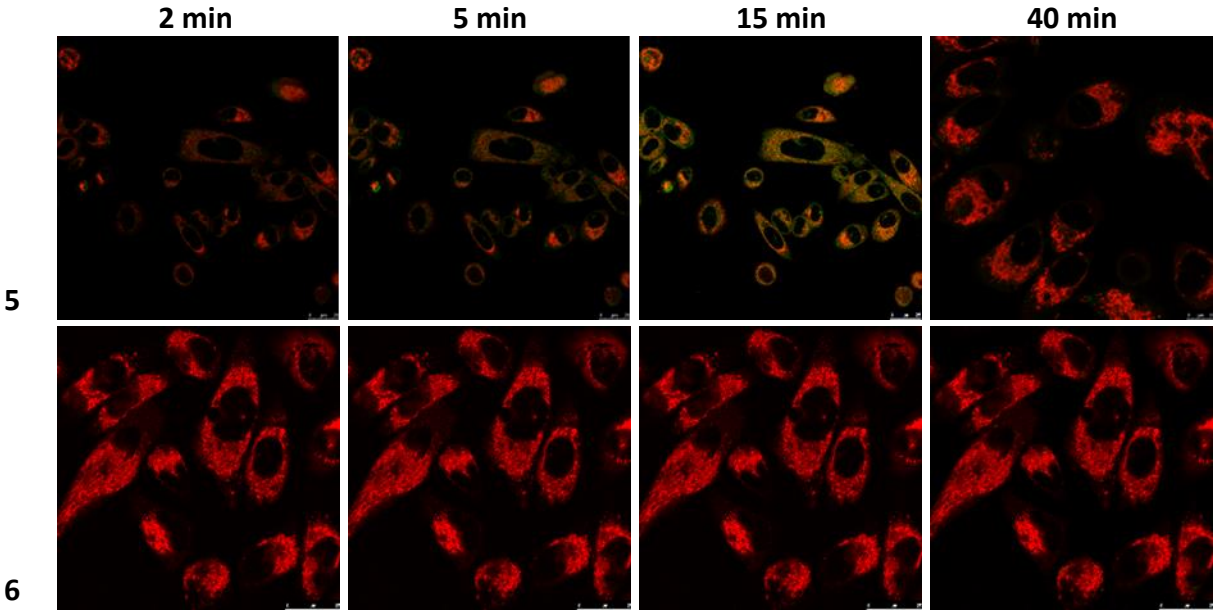


Fig. S10. 3-Formyl BODIPYs **5** and **6** do not colocalize with mitochondria**.** PC-3 cells were incubated for 15 min with 250 nM MitoTracker Red for mitochondrial staining and then they were pulsed with 125 nM BODIPYs. Dye incorporation into cells was followed for up to 40 min. BODIPY **5** fluorescent intensity (green) was maximal at 15 min and it decreased thereafter.

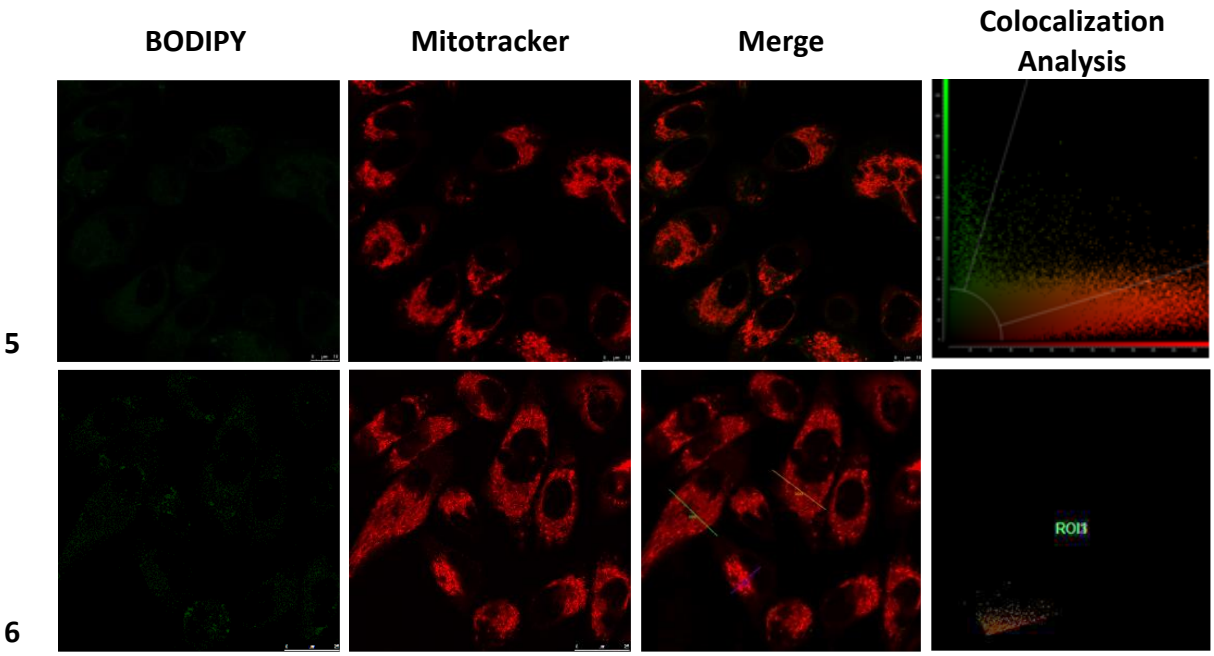


Fig. S11. Fluorescent intensity and co-localization studies of BODIPYs **5** and **6** after 40 min incubation with PC-3 cells.

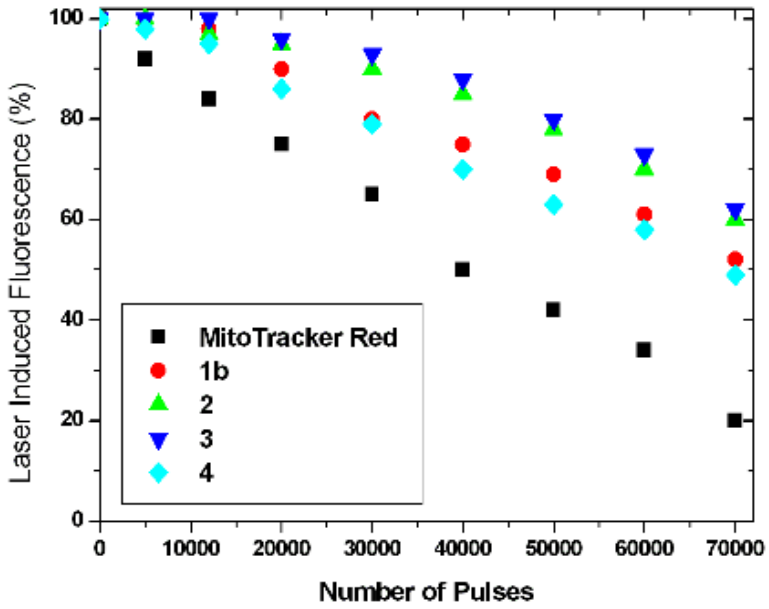


Fig. S12. Photostability of compounds **1b**, **2-4** *versus* MitoTracker, in terms of PFEI, in ethyl acetate solution after 70,000 pump pulses. PFEI is the percent laser-induced fluorescent emission intensity after n pumping laser pulses, referred to the initial intensity. Optically matched solutions at 532 nm were used.

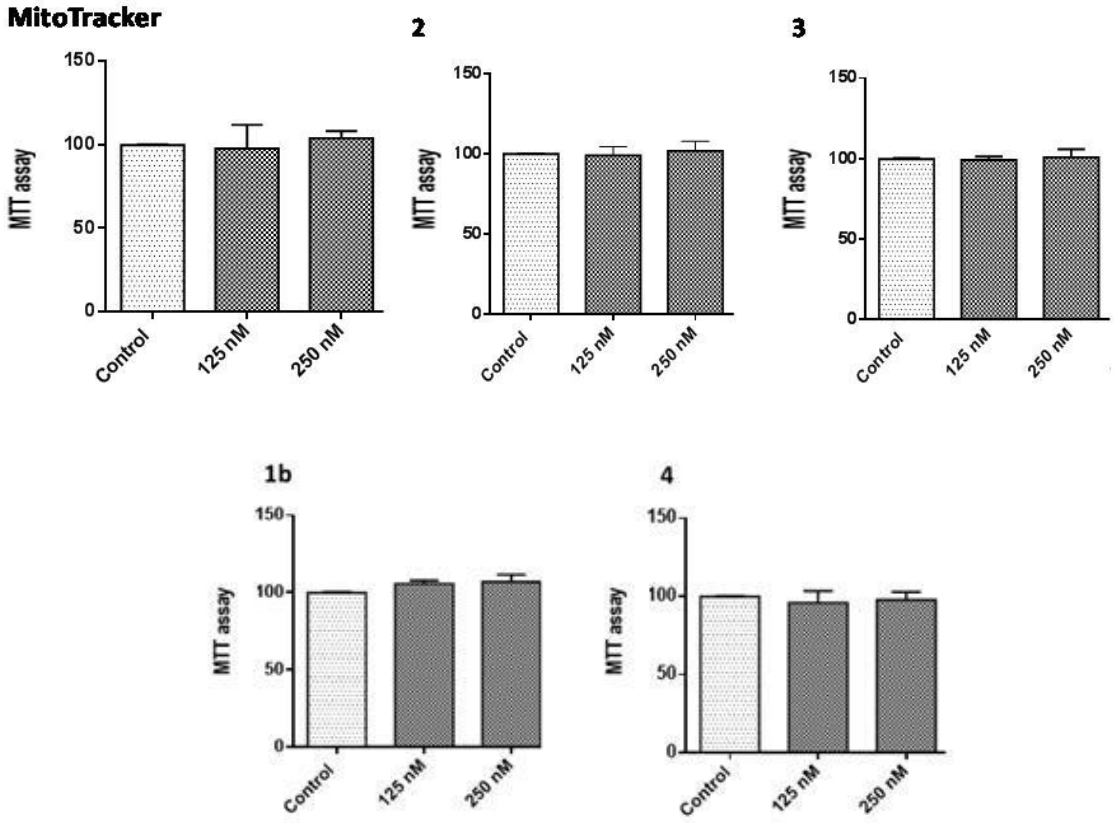


Fig. S13. Cell viability is not affected by 3-formylBODIPYs **1b**, and **2-4**. PC-3 cells were treated for 24 h with MitoTracker Red and 3-formylBODIPYs **1b**, and **2-4** for 24 h and cell viability was assessed by the ability to reduce MTT. Results were referred to control measurements (100% viability) and they were expressed as the mean ± S.D. of three experiments performed in duplicate. Student's test did not show any statistically significant difference.

3. Supplementary table

Table S1. Photophysical properties of the monoformylated BODIPYs in diluted solutions (2 μM) at organic solvents and the culture media. To avoid solubility problems in this last physiological environment the minimal amount of DMSO (just two drops) was added to solubilize the dye prior to the addition of the culture media. The photophysical properties were measured in fresh solutions, just after preparing the samples (mainly in DMSO and the culture media). The corresponding data of the compounds **1a** and**1c**are previously published.[2]

	λ_{ab} (nm)	ε_{max} (10^4 $M^{-1}\cdot cm^{-1}$)	λ_{fl} (nm)	ϕ	τ (ns)
1b					
Ethanol	499.0	6.6	522.0	0.48	3.46
DMSO	491.0	3.9	515.5	0.02	0.11(80%) – 1.47(6%) – 3.23(14%)
Medium	488.0	2.4	524.5	0.11	0.88(81%) – 4.16(21%)
1d					
Ethanol	493.0	7.4	505.0	0.51	2.54
DMSO	497.5	7.4	510.5	0.40	2.81
Medium	492.5	2.6	503.5	0.14	2.46(88%) - 3.59(12%)
2					
Ethanol	504.0	7.0	521.5	0.43	3.15
DMSO	490.5	1.2	509.0	0.17	2.23(18%) – 3.23(82%)
Medium	451.5	1.8	538.0	0.09	0.63(25%) – 4.65(75%)
3					
Ethanol	503.0	8.0	518.5	0.66	4.55
DMSO	505.0	6.7	511.0	0.19	0.22(33%) – 4.99(67%)
Medium	515.0	6.0	525.0	0.07	0.63(81%) – 4.67(19%)
4					
Ethanol	503.0	4.2	521.5	0.41	3.47
DMSO	492.5	3.1	506.5	0.24	3.76
Medium	485.0	3.3	521.0	0.10	0.19(85%) – 4.07(15%)
5					
Ethanol	494.5	6.9	516.5	0.52	4.57
DMSO	489.5	4.3	502.5	0.54	4.78
Medium	486.5	4.3	528.0	0.12	1.23(81%) – 4.64(19%)
6					
Ethanol	505.5	4.5	520.0	0.55	4.55
DMSO	489.5	3.6	502.0	0.37	2.94(55%) – 4.34(45%)
Medium	484.0	4.6	525.0	0.15	0.82(91%) – 4.67(9%)

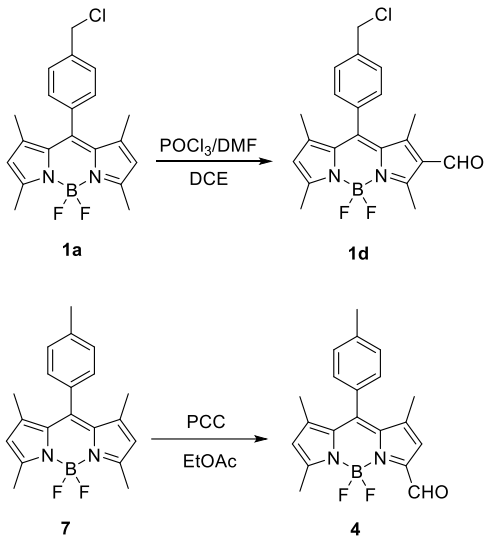
Absorption (λ_{ab}) and fluorescence (λ_{fl}) wavelength; molar absorption at the maximum (ε_{max}); fluorescence quantum yield (ϕ) and lifetime (τ)
DMSO: dimethylsulfoxide; see experimental sections for the conditions of the culture medium

Table S2. Co-localization coefficients

BODIPY	Pearson'sCorrelation	OverlapCoefficient	Co-localizationRate
1a	0.4981	0.7464	33.98%
1b	0.8162	0.8622	95.42%
1c	0.1791	0.2646	0.47%
1d	0.3423	0.5001	40.12%
2	0.7764	0.8316	82.16%
3	0.7757	0.8182	76.90%
4	0.8863	0.9070	97.34%
5	0.6888	0.7133	51.03%
6	0.3860	0.4988	0.38%

4. Synthetic procedures and characterization data

BODIPYs **1a**,[3] **1b-c**,[2] **2-3**[2] and **5-6**[2] (see Fig. 1 in manuscript) were synthesized by the corresponding described methods. The synthesis procedures of BODIPYs **1d** and **4** are illustrated in Scheme S1



Scheme S1. Synthesis of BODIPYs **1d** and **4**.

4.1. Synthesis of 1d

A mixture of $POCl_3$ (0.37 mL, 4 mol) and DMF (0.62 mL, 8 mol) was stirred in an ice bath for 5 min under argon. After being warmed to rt, it was stirred for additional 30 min, and then, BODIPY **1a**[3] (100 mg, 0.27 mmol) in 1,2-dichloroethane (DCE, 8 mL) was added. The reaction was raised to 60 ºC and stirred for an additional 45 min. The mixture was then cooled to rt and slowly poured into saturated aqueous $NaHCO_3$ (100 mL) under ice-cold conditions and stirring maintained for 1h. Finally, the reaction mixture was diluted with dichloromethane (DCM) and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography, using silica gel and hexane/EtOAc (95:5 to 9:1), to afford **1d** (69 mg, 64%) as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H, CHO), 7.56 (d, *J* = 8.1 Hz, 2H, 2CH), 7.29 (d, *J* = 8.1 Hz, 2H, 2CH), 6.15 (s, 1H, CH), 4.67 (s, 2H, CH_2Cl), 2.81 (s, 3H, CH_3), 2.61 (s, 3H, CH_3),1.65 (s, 3H, CH_3),1.42 (s, 3H, CH_3) ppm.^{13}C NMR (75 MHz, $CDCl_3$) δ 185.8 (CHO), 161.9 (C), 156.6 (C), 147.2 (C), 142.8 (C), 139.3 (C), 134.2 (C), 134.0(C), 129.6

(CH and C), 128.2 (CH and C), 126.4 (C), 124.2 (CH), 45.4 (CH_2Cl), 15.1 (CH_3), 15.0 (CH_3), 13.0 (CH_3), 11.7 (CH_3) ppm. FTIR ν2924, 2853, 1670, 1543, 1515, 1439, 1314, 1189, 1046, 987cm^{-1}. HRMS-EI *m/z* 400.1317 (400.1325 calcd for $C_{21}H_{20}BClF_2N_2O$).

4.2. Synthesis of 4

According to above described general procedure, 1,3,5,7-tetramethyl-8-tolylBODIPY **7**[4] (30 mg, 0.089 mmol) and PCC(115 mg, 0.532mmol) in EtOAc (10 mL) were reacted for 20 h. Flash chromatography using hexane/EtOAc (95:5 to 9:1) afforded **4** (23 mg, 74%) as an orange solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (t, *J* = 2.1 Hz, 1H, CHO), 7.34 (d, *J* = 8.1 Hz, 2H, 2CH), 7.16 (d, *J* = 8.1 Hz, 2H, 2CH), 6.77 (s, 1H, CH), 6.22 (s, 1H, CH), 2.66 (s, 3H, CH_3), 2.45 (s, 3H, CH_3),1.50 (s, 3H, CH_3),1.42 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.6 (CHO), 165.8 (C), 149.9 (C), 144.4 (C), 143.6 (C), 139.8 (C), 137.8 (C), 135.8 (C), 133.1 (C), 130.8 (C), 130.2 (CH), 127.4 (CH), 125.1 (CH), 119.8 (CH), 21.5 (CH_3), 15.6 (CH_3), 15.2 (CH_3), 14.2 (CH_3) ppm. FTIR ν2920, 2857, 1668, 1548, 1371, 1198, 1071, 982cm^{-1}. HRMS-EI *m/z*352.1551 (352.1559 calcd for $C_{20}H_{19}BF_2N_2O$).

5. Experimental evidence supporting the covalent linkage of 3-formylBODIPYs with an amino group on the proteins

It was carried out the reactions of **1b** and **4** with a primary amine in EtOH/PBS (9:1) at 37 ºC, and in both cases the reaction is already observed within 1 h by TLC, obtaining a practically total conversion after 12 h, as it is shown in the ^{1}H-NMR spectrum of the reaction crudes, which implies the formation of the corresponding imines. This result indicates that 3-formylBODIPY can be covalently linked to the amino groups present in the organelle.

On the other hand, to check the reactivity of these compounds with amino acids, we have carried out the reaction of **4** with cysteine in a 1:10 ratio in EtOH/PBS at 37 ºC, observing the formation of the corresponding thiazolidine derivative (see ^{1}H-NMR of the reaction crude), which is captured by reaction with ethyl chloroformate (according to the procedure described by Ziessel et al.[5]) yielding a stable derivative.

In addition, we measured the photophysical properties of **4** in absence and presence of cysteine (ratio 1:10 and 1:100) at different aging times (up to 24 hours) in the same aqueous media (see spectra in Figure S14). The recorded changes in the intensity of the absorption and fluorescence spectral bands (slight at low contents but stronger at high excess of electron donor cysteine) support the viability aforementioned reaction between amine and aldehyde. It is noteworthy that both spectral bands remain in the same position regardless of the cysteine amount and the aging time, explaining why no change in the emission colour was detected during the visualization of the mitochondria by fluorescence imaging. In other words, the reaction takes place, being the driving force for the selective recognition of mitochondria by probe **4**. However, this reaction is unsuited for the sensing of amino-containing biomolecules because the formed imine does not change drastically the photophysical properties (i.e., spectral shift) of the probe.

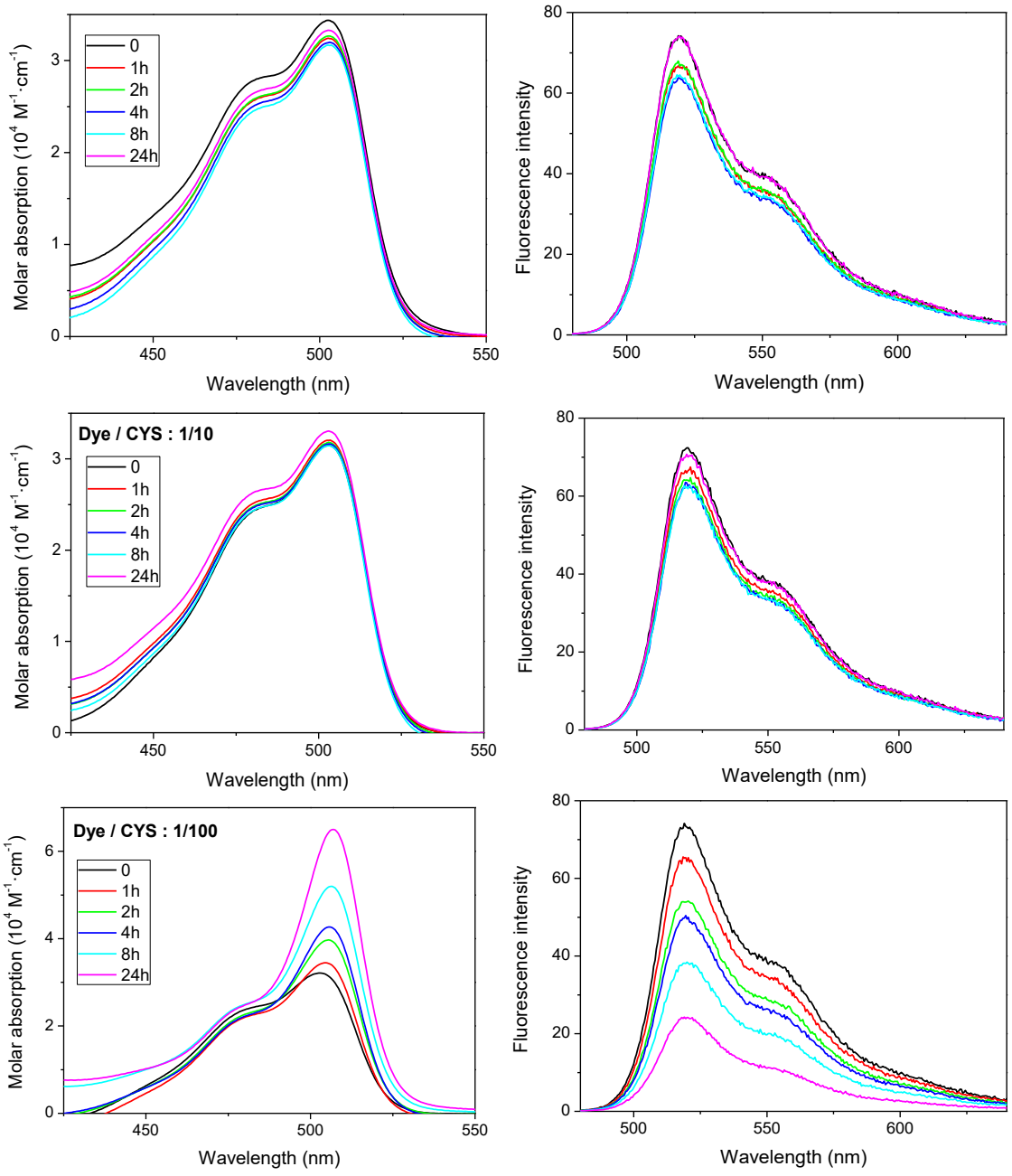
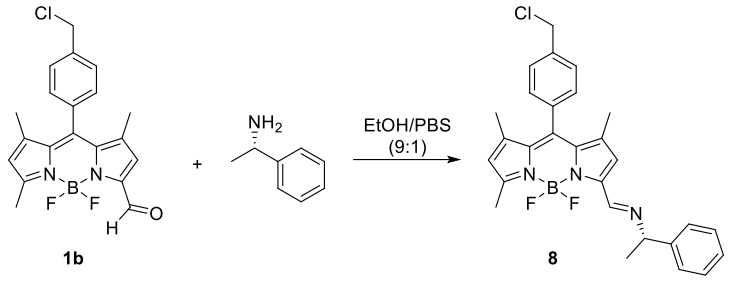


Fig. S14. Absorption and fluorescence spectra of dye 4 (concentration 4 μM) in EtOH/PBS mixtures (volumen ratio 9/1) in absence of cysteine (top) and in the presence of different amounts of cysteine (40 μM and 400 μM, middle and bottom, respectively).

5.1. General procedure for reaction of BODIPYs with amines

To a solution of BODIPY (1 equiv) in EtOH/PBS (9:1, 5 mL) was added amine (1.5 equiv) or cysteine (10 equiv), and the mixture was stirred at 37 ºC for 12 h. The reaction was followed by TLC.

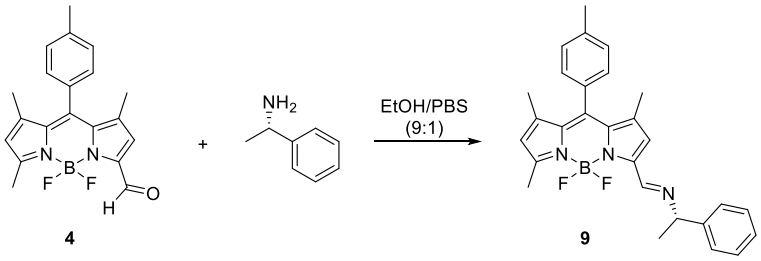
5.2. Reaction of 1b with (S)-(-)-1-phenylethylamine



According to the general procedure described in section 5.1., **1b** (23 mg, 0.06 mmol) and (*S*)-(-)-1-phenylethylamine (11 μL, 0.09 mmol) in EtOH/PBS (5 mL) were reacted. The formation of imine **8** was detected by TLC and confirmed by ^{1}H-NMR (reaction crude).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H, CH=N), 7.55 (d, *J* = 7.5 Hz, 2H, 2CH), 7.43-7.23 (m, 7H, 7CH), 6.86 (s, 1H, CH), 6.10 (s, 1H, CH), 4.66 (s, 2H, CH_2Cl), 4.63 (q, *J* = 6.6 Hz, 1H, CH), 2.61 (s, 3H, CH_3), 1.59 (d, *J* = 6.6 Hz, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.41 (s, 3H, CH_3) ppm.

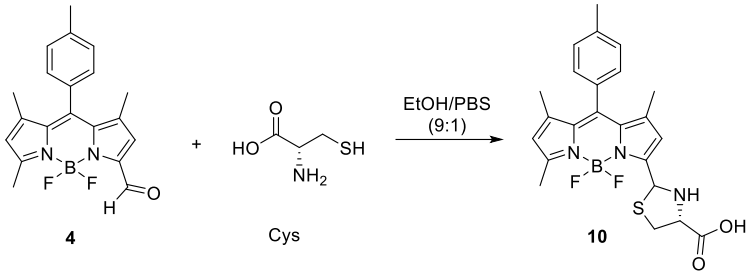
5.3. Reaction of 4 with (S)-(-)-1-phenylethylamine



According to the general procedure described in section 5.1., **4** (20 mg, 0.06 mmol) and (*S*)-(-)-1-phenylethylamine (11 μL, 0.09 mmol) in EtOH/PBS (5 mL) were reacted. The formation of imine **9** was detected by TLC and confirmed by ^{1}H-NMR (reaction crude).

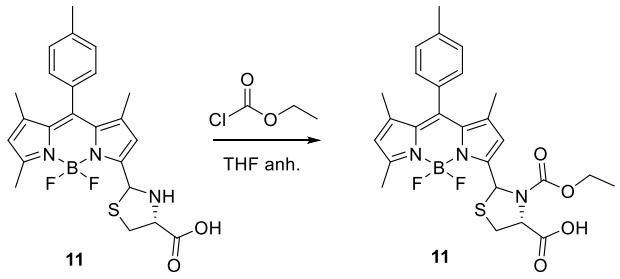
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H, CH=N), 7.46-7.15 (m, 9H, 9CH), 6.87 (s, 1H, CH), 6.10 (s, 1H, CH), 4.65 (q, *J* = 6.6 Hz, 1H, CH), 2.62 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 1.61 (d, *J* = 6.6 Hz, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.44 (s, 3H, CH_3) ppm.

5.4. Reaction of 4 with cysteine (Cys)



According to the general procedure described in section 5.1., **4** (20 mg, 0.06 mmol) and (*L*)-cysteine (73μL, 0.60 mmol) in EtOH/PBS (5 mL) were reacted. The formation of possible thiazolidine **10** was detected by TLC and ^{1}H-NMR (see spectrum reaction crude in section 7, pag. S28).

5.5. Reaction of thiazolidine 10 with ClCO$_2$Et



The residue obtained in the reaction described in section 5.4., was dissolved in dry THF (7 mL). To this mixture was added dropwise ethyl chloroformate (33 μL, 0.34 mmo). The solution was stirred at rt for 1.5 h, and the solvent was removed under vacuum. The residue was purified by column chromatography, using silica gel and DCM/EtOAc/AcOH (85:15:0.1%), to afford *N*-protected thiazolidine **11** (13 mg, 43%) as an orange solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30-7.12 (m, 4H, 4CH), 6.65 (s, 1H, CH), 6.54 (s, 1H, CH), 6.02 (s, 1H, CH), 4.78 (t, *J* = 7.8 Hz, 1H, CH), 4.25-4.11 (m, 2H, CH_2O), 3.50-3.32 (m, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.21 (t, *J* = 6.9 Hz, 3H, CH_3) ppm.

6. Hydrolysis tests of the benzyl chloride group

To check the possible hydrolysis of the benzyl chloride group, we have carried out three additional experiments. Hydrolysis has not been observed in any case.

6.1. Reaction of 1a with THF/H_2O

A solution of **1a** (15 mg, 0.04 mmol) in THF/H_2O (1:1, 4 mL) was stirred at rt for 5 days. Unaltered starting material was detected by TLC.

6.2. Reaction of 1a with a dilute NaOH solution in THF/H_2O

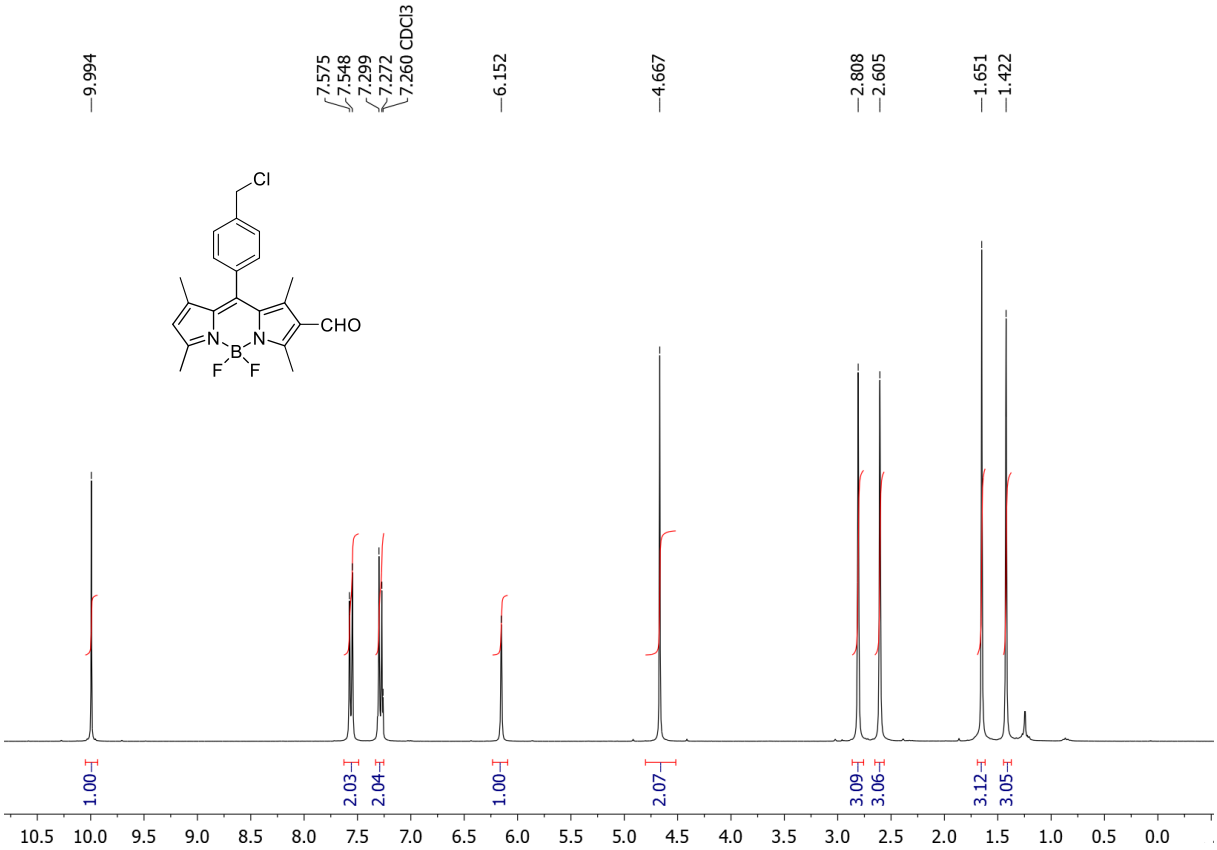
To a solution of **1a** (15 mg, 0.04 mmol) in THF (2 mL) was added a solution of NaOH (2 mL, 8.10^{-3} M, 0.016 mmol) and the mixture was stirred at rt for 5days. Unaltered starting material was detected by TLC.

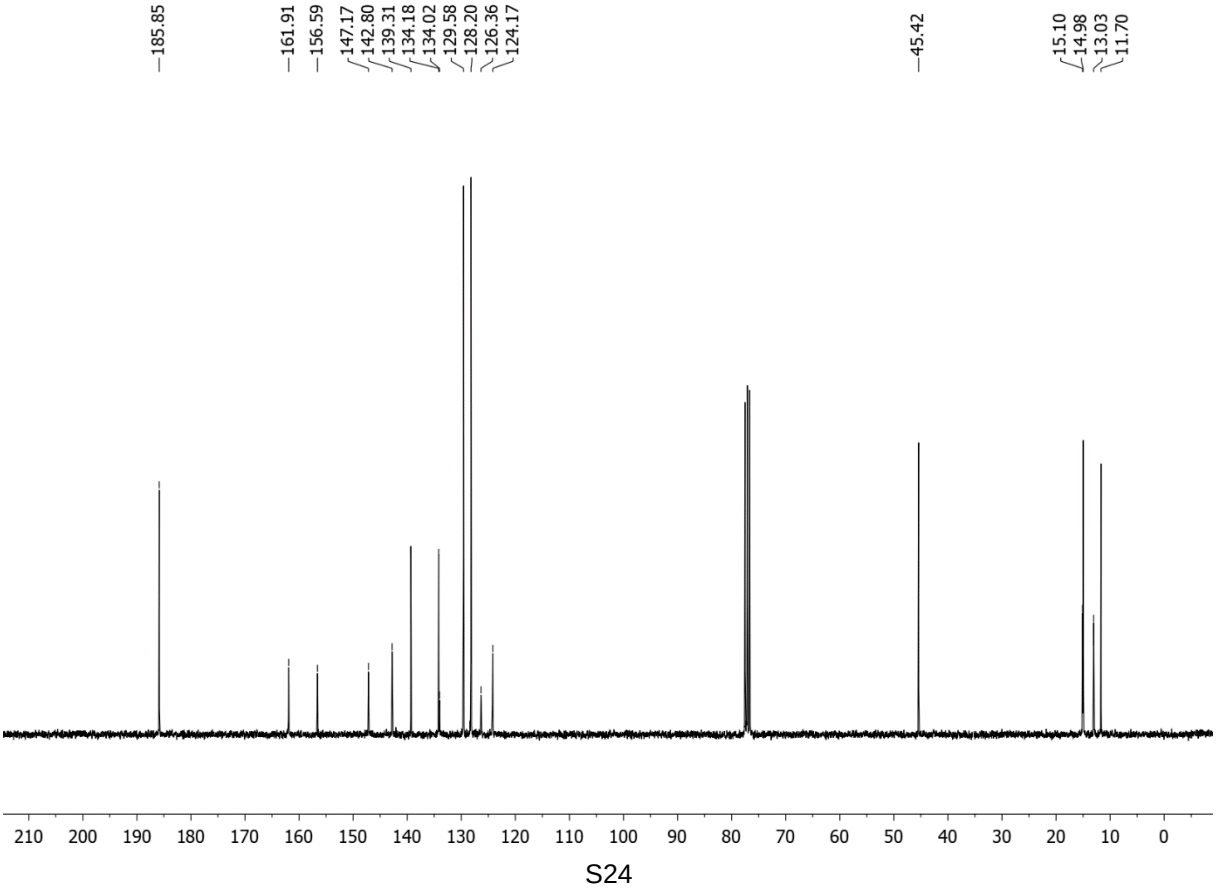
6.3. Reaction of 1a with a concentrated NaOH solution in THF/H_2O

To a solution of **1a** (15 mg, 0.04 mmol) in THF (2 mL) was added a solution of NaOH (2 mL, 2.5 M, 5 mmol) and the mixture was stirred at rt for 10days. Unaltered starting material was detected by TLC.

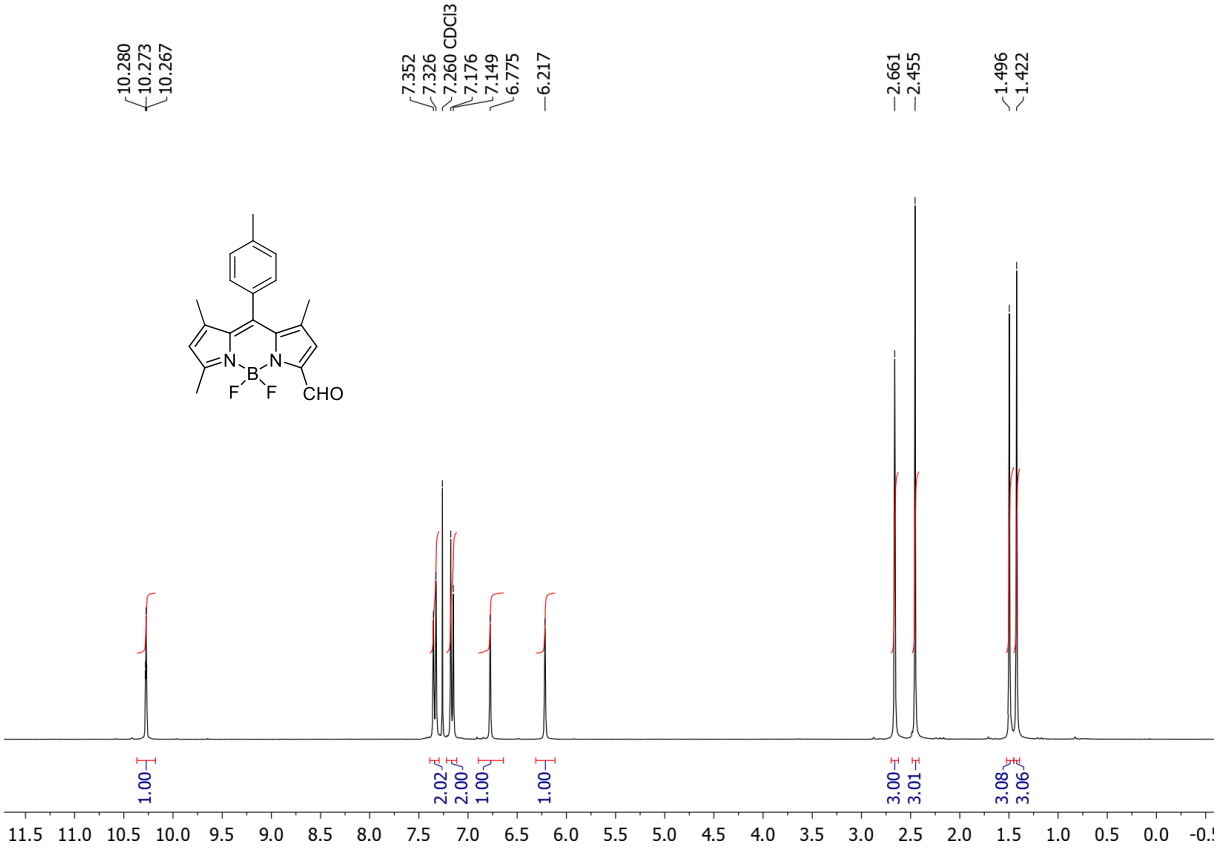
7. ^{1}H NMR and ^{13}C NMR spectra

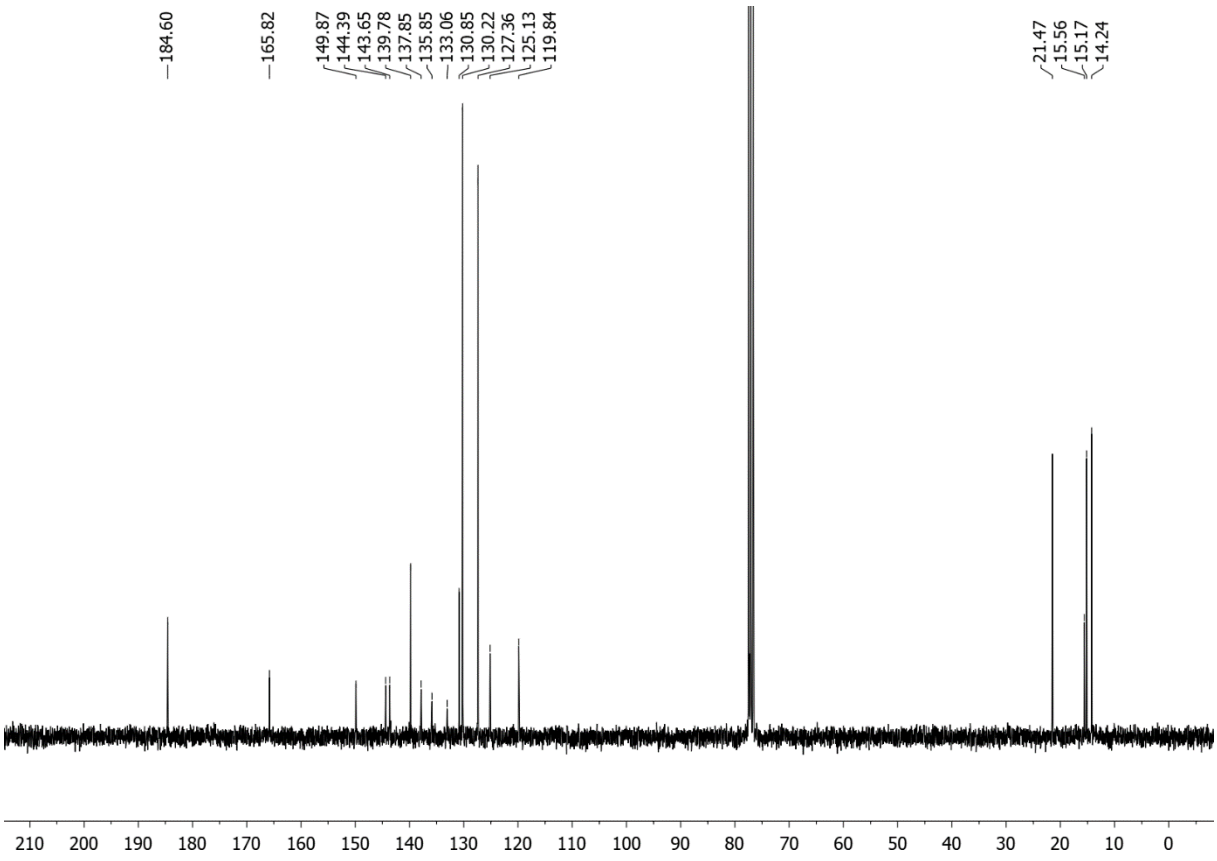
^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectra of **1d**



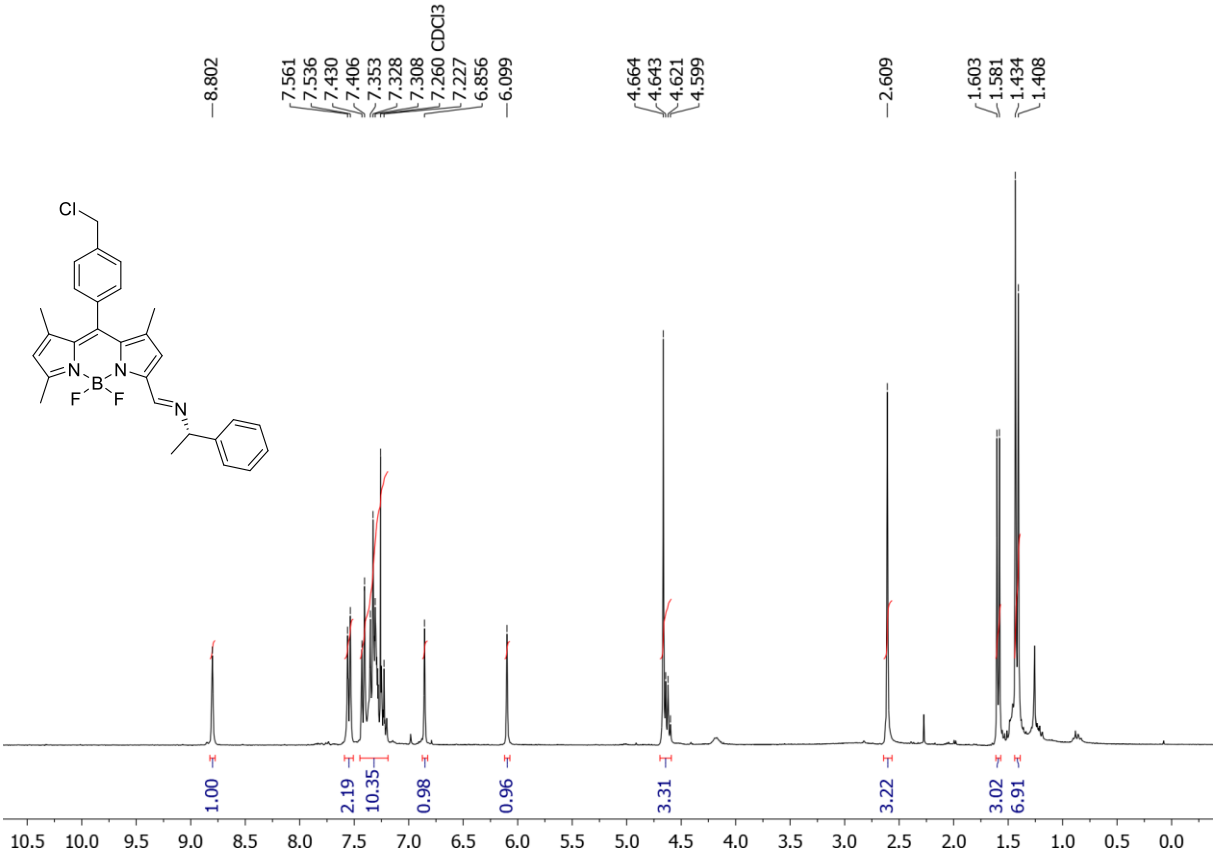


^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectra of **4**

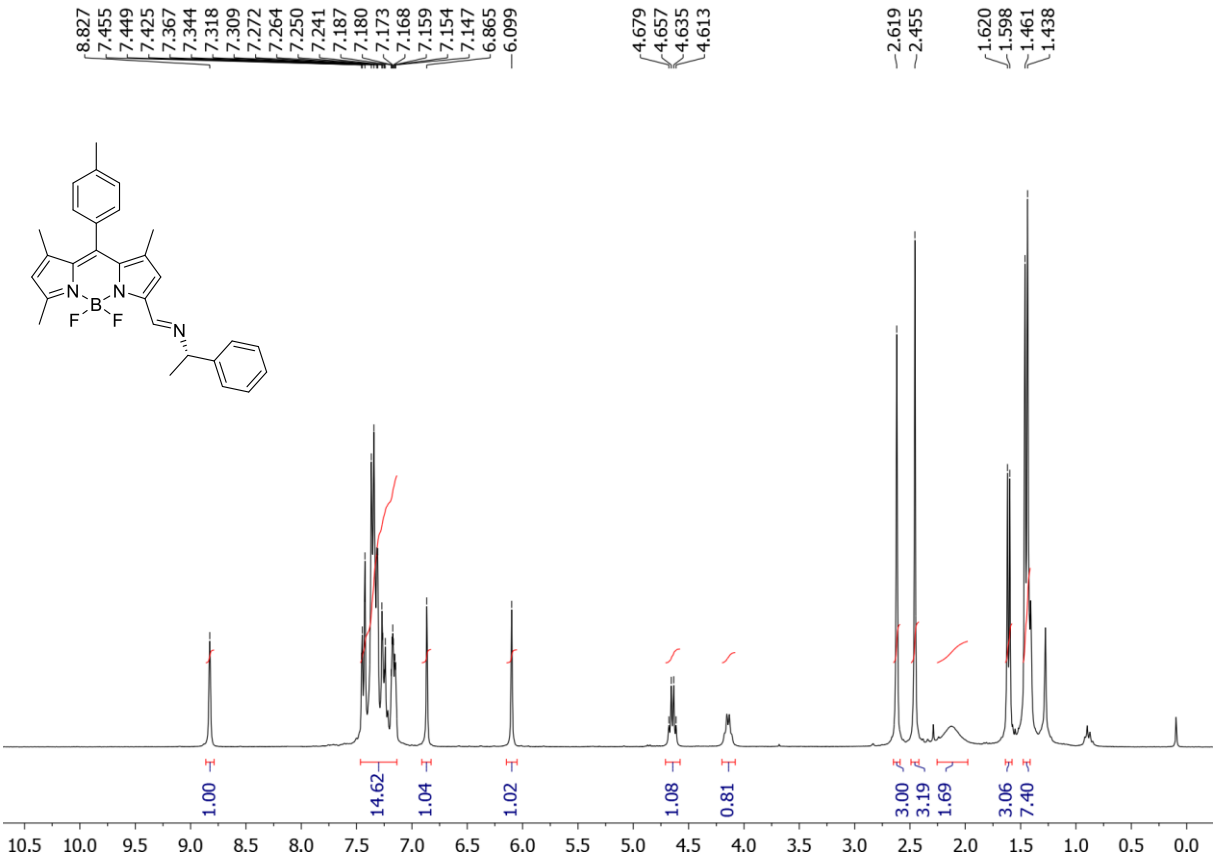




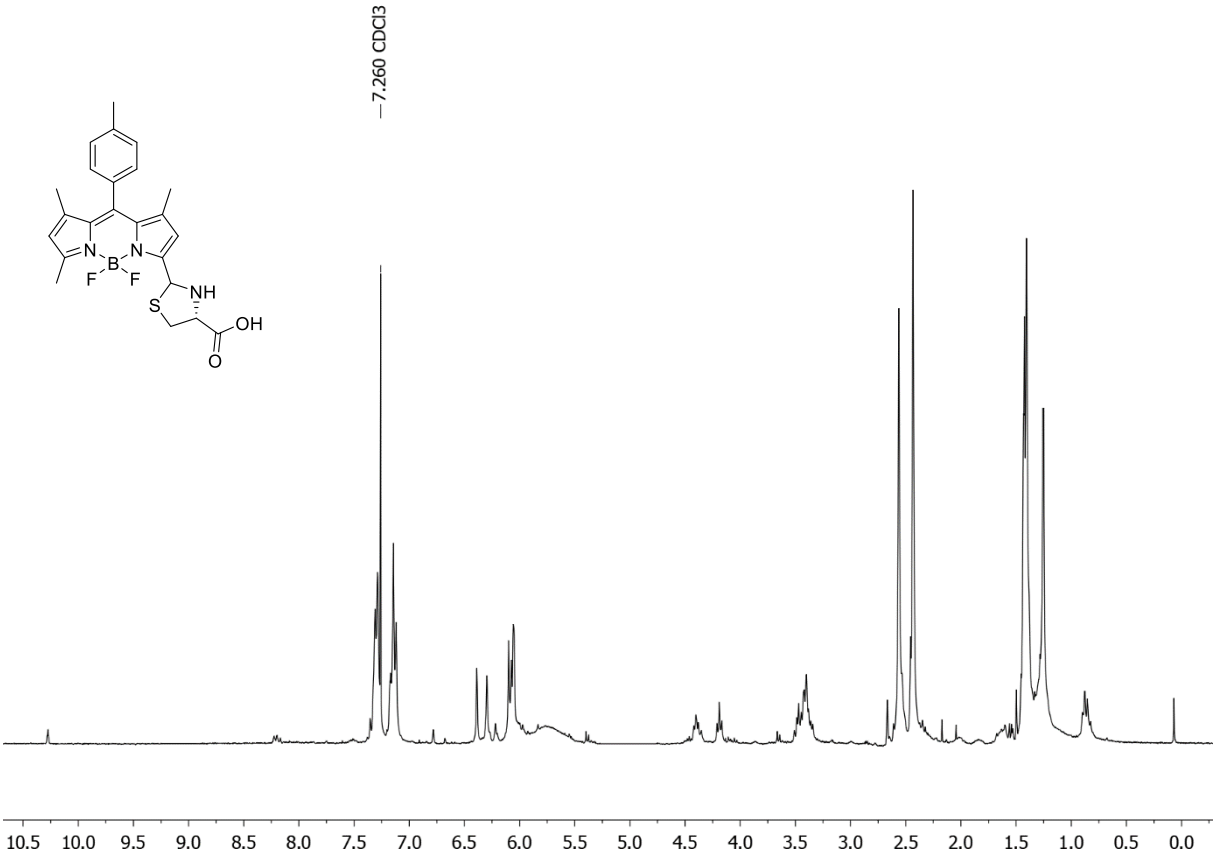
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of imine **8**(reaction crude)



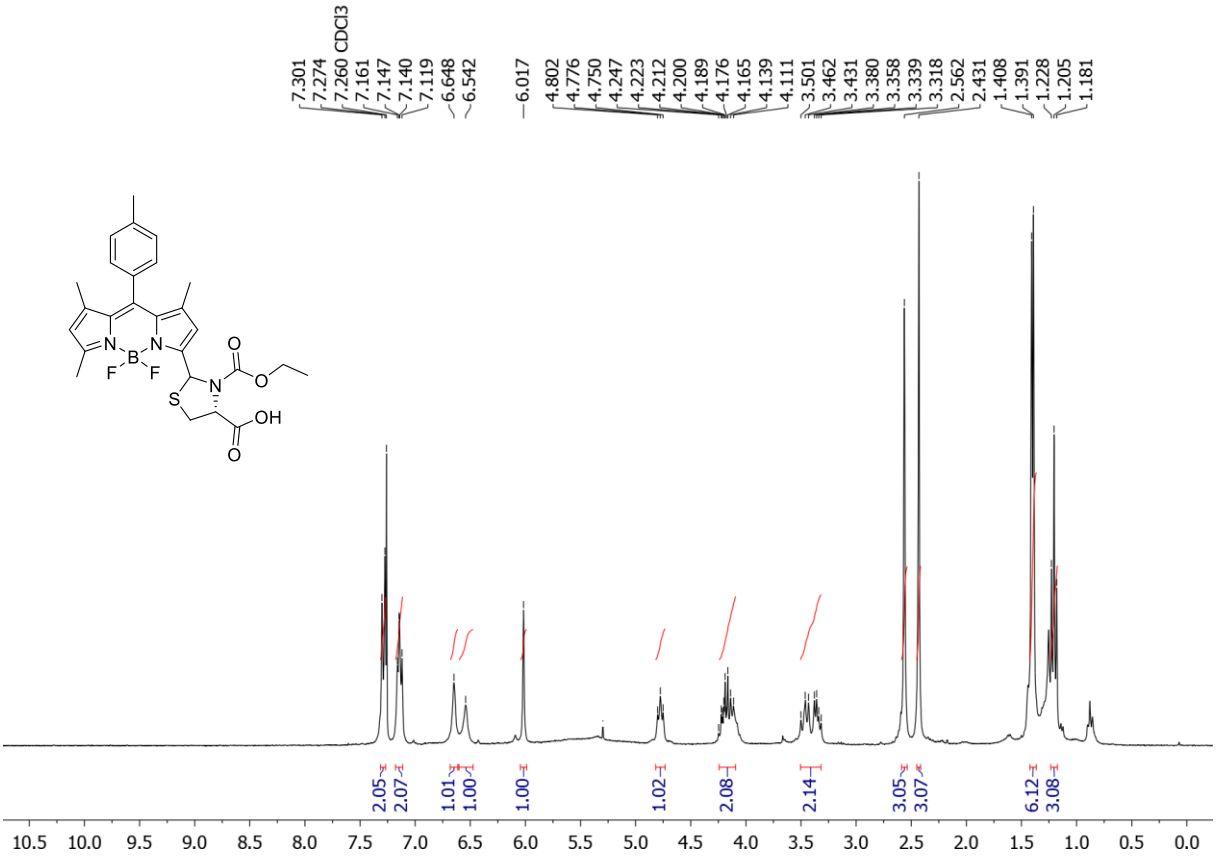
^{1}H NMR (300 MHz, $CDCl_3$) spectrum of imine **9**(reaction crude)



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of thiazolidine **10**(reaction crude)



^{1}H NMR (300 MHz, $CDCl_3$) spectrum of *N*-protected thiazolidine **11**



8. References

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