

Supplementary Materials

A study on the structure, optical properties and cellular localization of novel 1,3-benzothiazole-substituted BODIPYs

Olga Kirkilessi 1, Christina Arapatzi 2, Heribert Reis 1, Vassiliki Kostourou 2,
Kyriakos C. Prousis 1,* and Theodora Calogeropoulou 1,*

1 Institute of Chemical Biology, National Hellenic Research Foundation,
48 Vassileos Constantinou Avenue, 11635 Athens, Greece;
okirkilessi@eie.gr (O.K.); hreis@eie.gr (H.R.)

2 Institute for Bioinnovation, "Alexander Fleming" Biomedical Sciences
Research Center, 34 Fleming str. Vari, 16672 Athens, Greece;
arapatzi@fleming.gr (C.A.); kostourou@fleming.gr (V.K.)

* Correspondence: kyrprousis@eie.gr (K.C.P.); tcalog@eie.gr (T.C.)

Table of Contents

<i>Experimental section</i>	S3
<i>General Information</i>	S3
<i>NMR and HR-MS spectra of the final dyes</i>	S4
<i>Optical data of the new dyes</i>	S18
<i>Theoretical calculations</i>	S22
<i>Photostability of the new dyes after long incubation</i>	S24
<i>Staining profile of dye TC514</i>	S26
<i>Staining profile of dye TC498</i>	S28
<i>References</i>	S29

Experimental section

General Information

UV–Visible spectra were recorded on a UV-1900i Shimadzu UV–Vis–NIR spectrophotometer using a 1 cm quartz cell. Steady-state emission spectra were recorded on a Fluorolog-3 JobinYvon-Spex spectrofluorometer (model GL3-21). All the fluorescence emission spectra were corrected for the wavelength response of the system. The excitation and emission slit width was set to 2 nm for quantum yield calculations. Fluorescence quantum yields of BODIPY dyes were measured in ethanol by using standard 1cm path-length quartz fluorescence cell and calculated from the equation:[63]

$$\Phi = \Phi_{ref} \frac{I}{I_{ref}} \frac{OD_{ref}}{OD} \frac{n^2}{n_{ref}^2}$$

Here, I denotes the integral of the corrected emission, OD is the optical density at the excitation wavelength and n is the refractive index of the medium. Rhodamine ($\varphi_n = 50\%$ in ethanol) and cresyl violet ($\varphi_n = 56\%$ in ethanol) were used as the references for the calculation of quantum efficiencies. A solution of each dye in EtOH (2.0×10^{-6} mol/L) was used to measure fluorescence quantum yields, absorption and fluorescence spectra. Pico-second time-resolved fluorescence spectra were measured by the time-correlated-single-photon-counting (TCSPC) method on a Nano-Log spectrofluorometer (Horiba JobinYvon), by using a laser diode as an excitation source (NanoLED, 488 and 654 nm) and a UV–vis detector TBX-PMT series (250–850 nm) by Horiba JobinYvon. Lifetimes were deconvoluted with the DAS6 Fluorescence-Decay Analysis Software, using a light-scattering solution (LUDOX) for instrument response. Molar absorption coefficient (ϵ) was obtained from the equation

$$\epsilon = A / cl$$

Here, l denotes the path length of the cuvette equal to 1 cm, c is the concentration used (2.0×10^{-6} mol/L) and A is the wavelength of maximum absorption.

NMR and HR-MS spectra of the final dyes

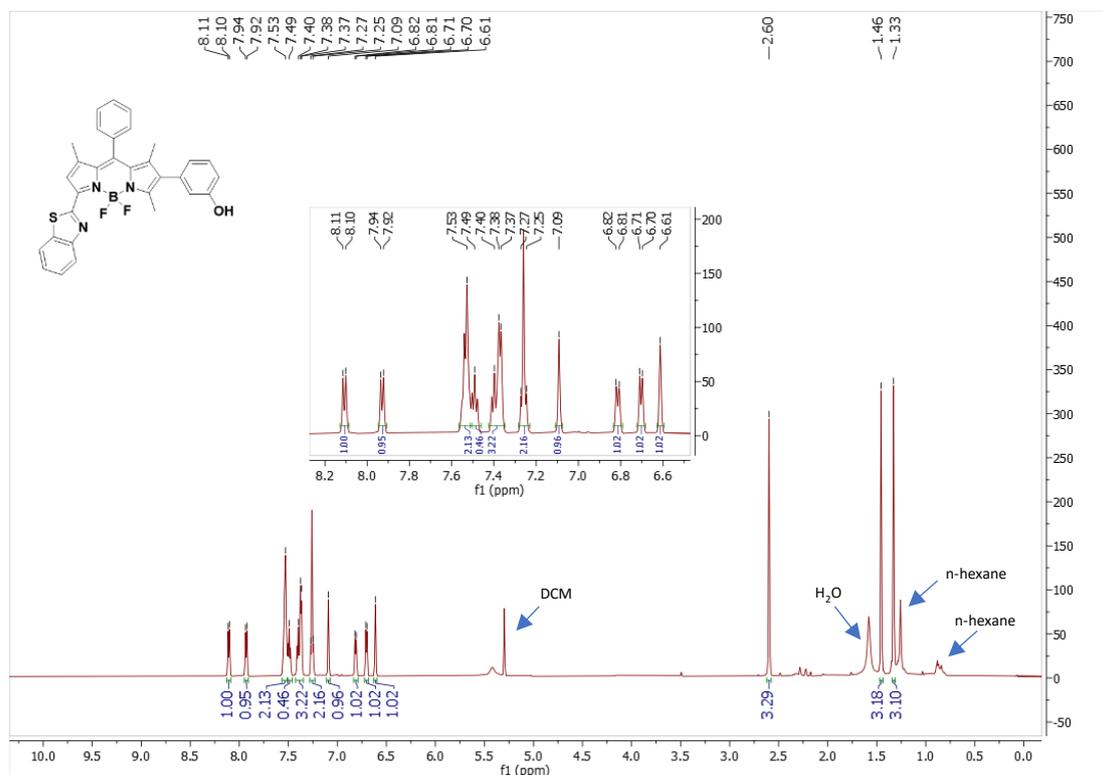


Figure S1. ¹H NMR spectrum (600 MHz, CDCl₃) of dye TC495

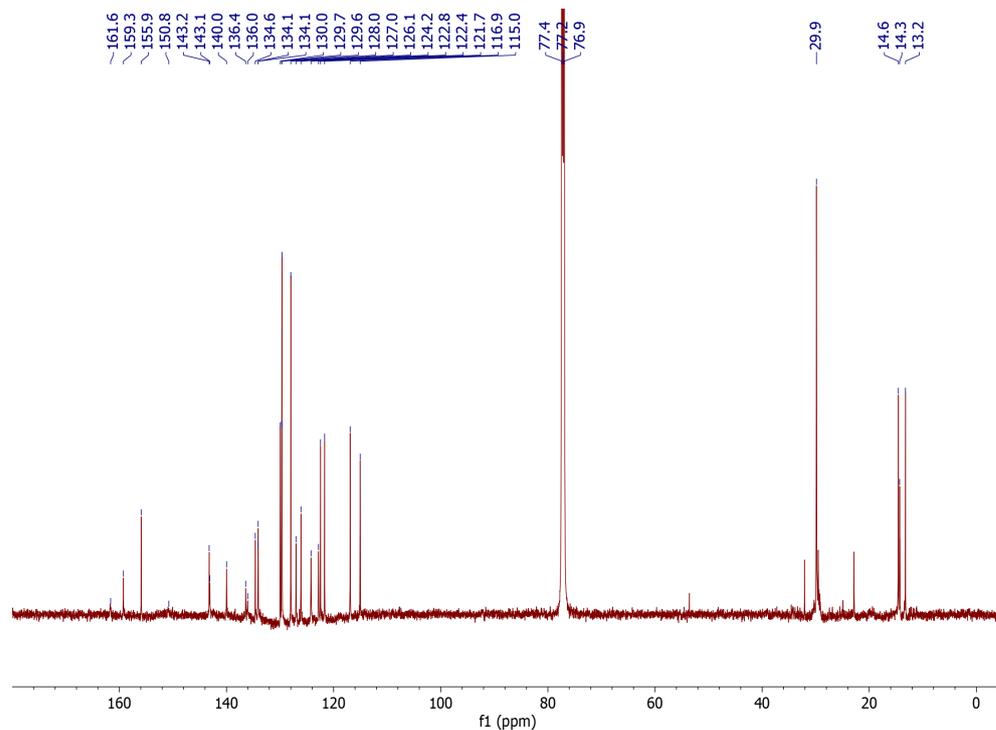
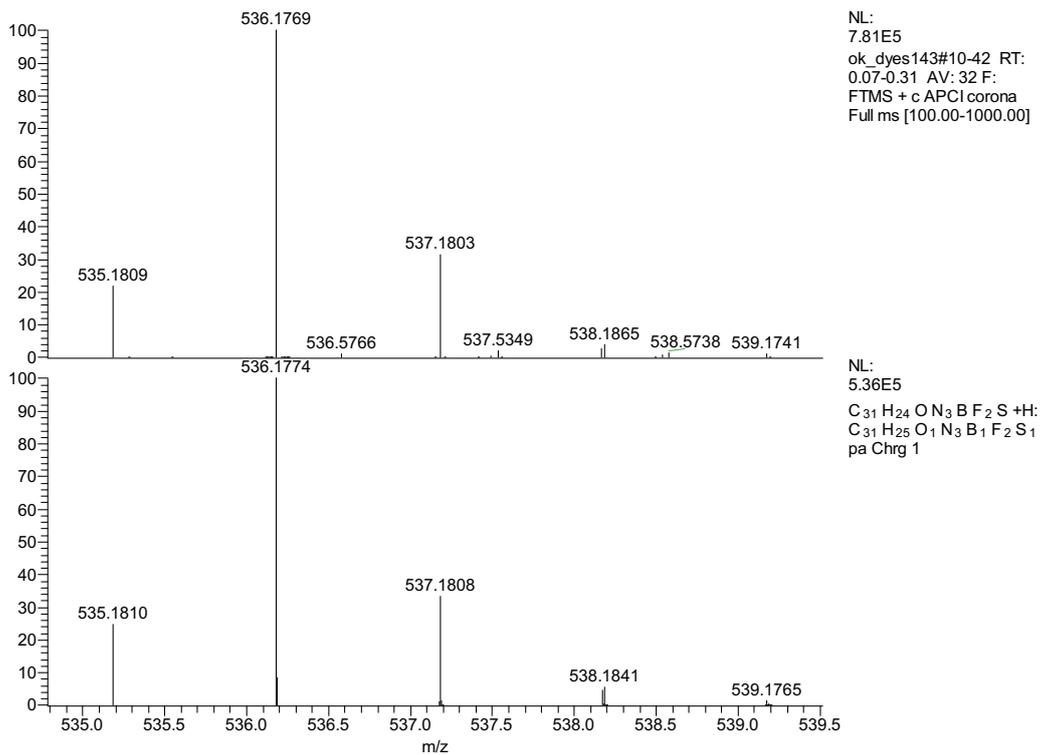


Figure S2. ¹³C NMR spectrum (150 MHz, CDCl₃) of dye TC495



Elemental composition

Single mass

Mass: 536.17694

Max. results: 10

Calculate

Idx	Formula	RDB	Delta ppm
1	C ₃₁ H ₂₅ O ₁ N ₃ B ₁ F ₂ S	20.0	-0.851

Figure S3. HR-MS (APCI⁺) of dye TC495

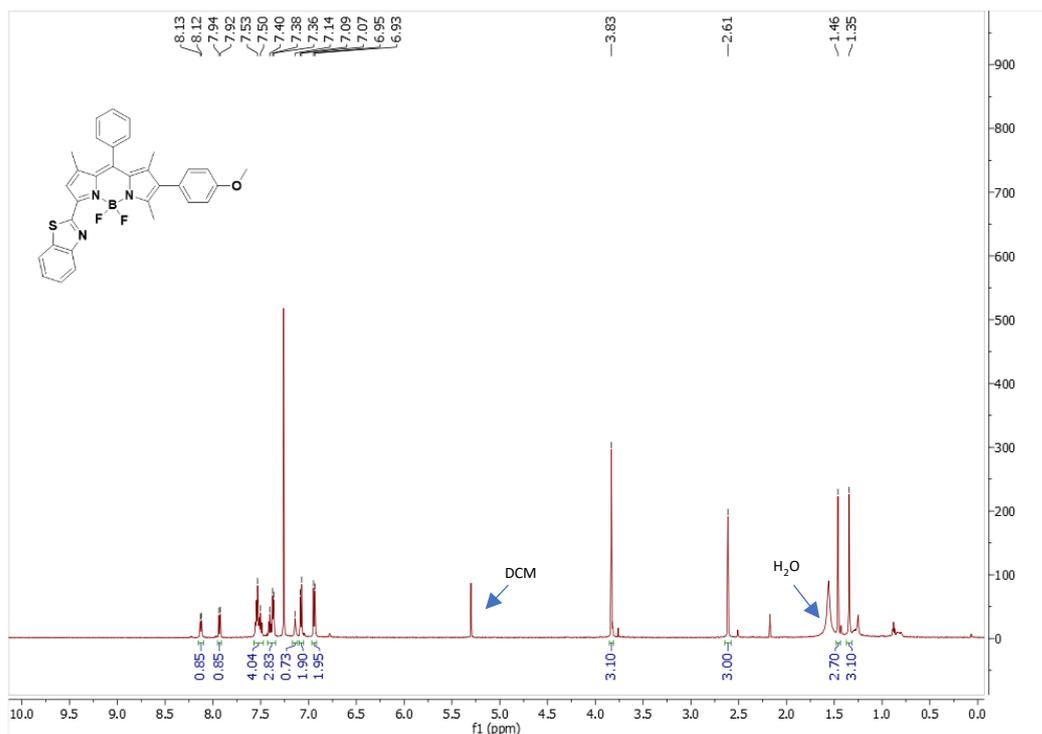


Figure S4. ^1H NMR spectrum (600 MHz, CDCl_3) of dye TC496

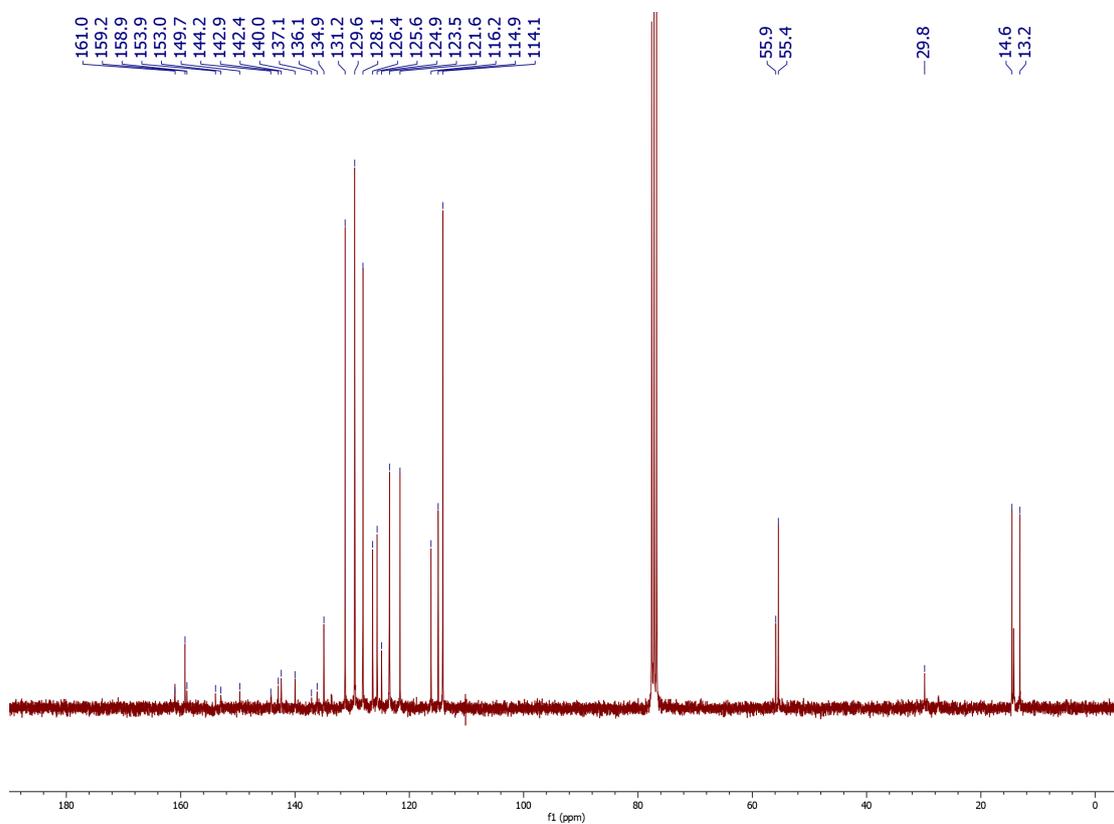
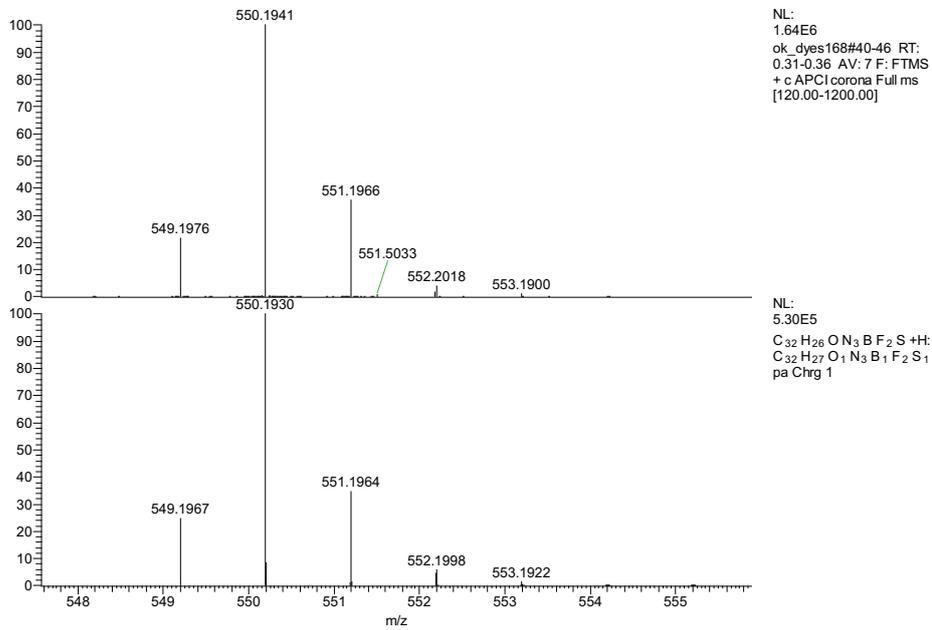


Figure S5. ^{13}C NMR spectrum (150 MHz, CDCl_3) of dye TC496



Elemental composition

Single mass

Mass:

Max. results:

Idx	Formula	RDB	Delta pp
1	C ₃₂ H ₂₇ O ₃ N ₃ B ₁ F ₂ S	20.0	1.860

Figure S6. HR-MS (APCI⁺) of dye TC496

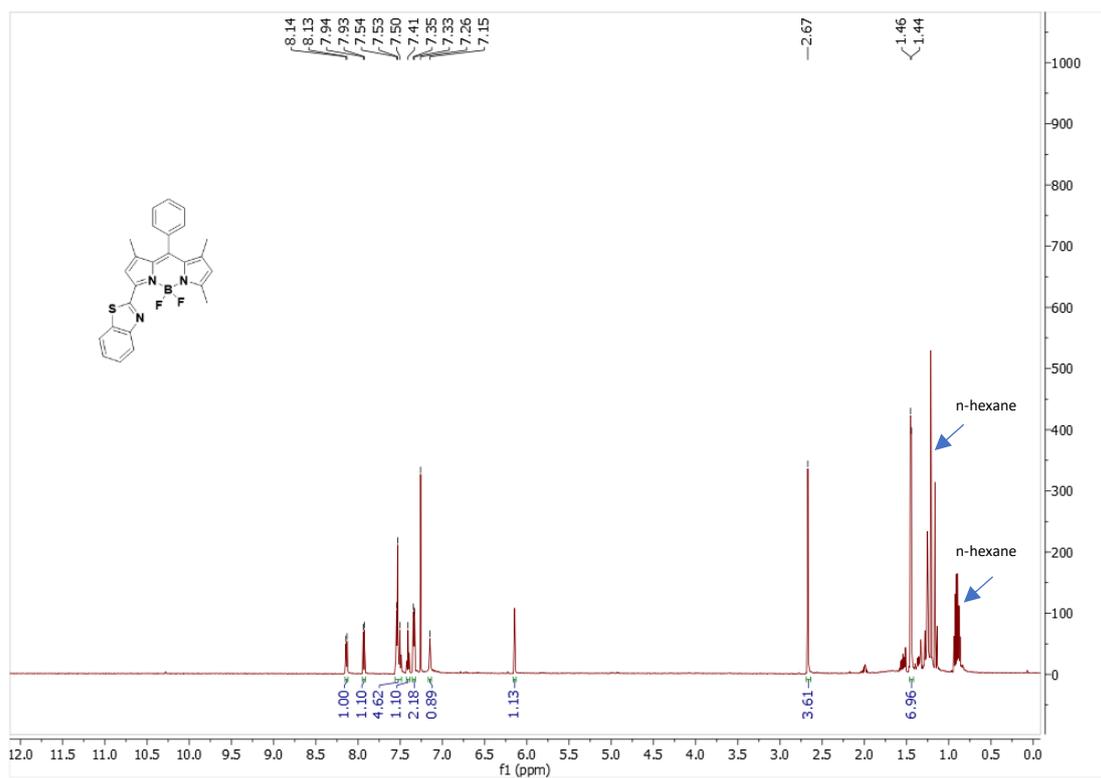


Figure S7. ¹H NMR spectrum (600 MHz, CDCl₃) of dye TC497

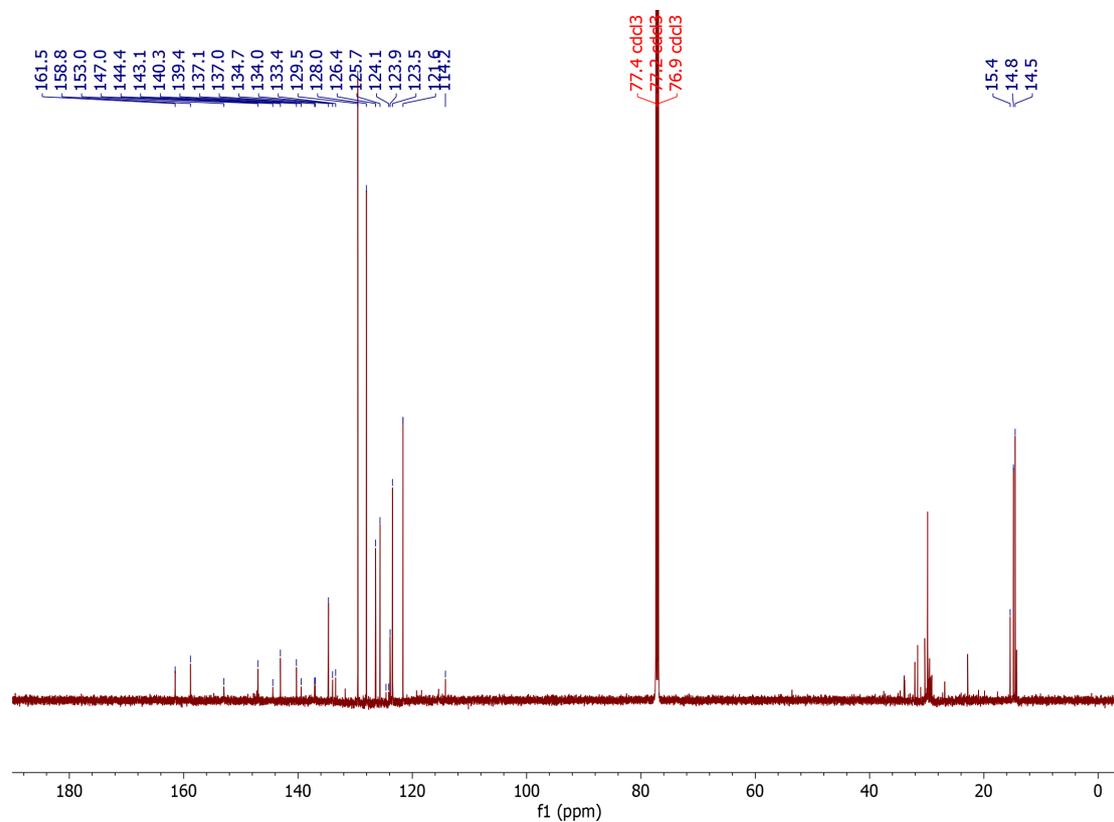
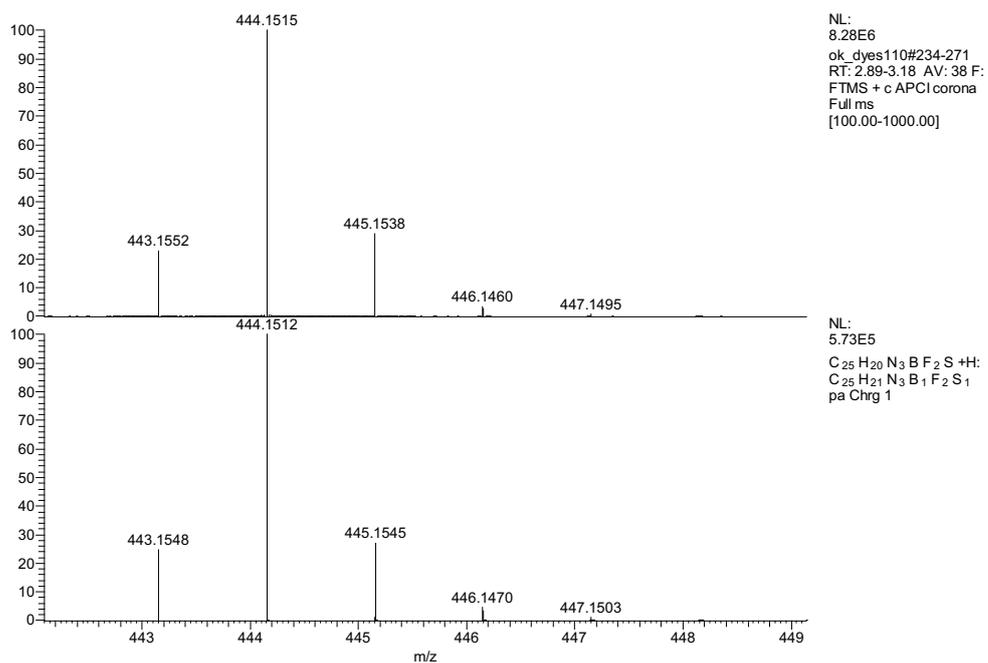


Figure S8. ¹³C NMR spectrum (150 MHz, CDCl₃) of dye TC497



Elemental composition

Single mass

Mass: 444.15149

Max. results: 1

Calculate

Idx	Formula	RDB	Delta pp
1	C ₂₅ H ₂₁ N ₃ B F ₂ S	16.0	0.694

Figure S9. HR-MS (APCI⁺) of dye TC497

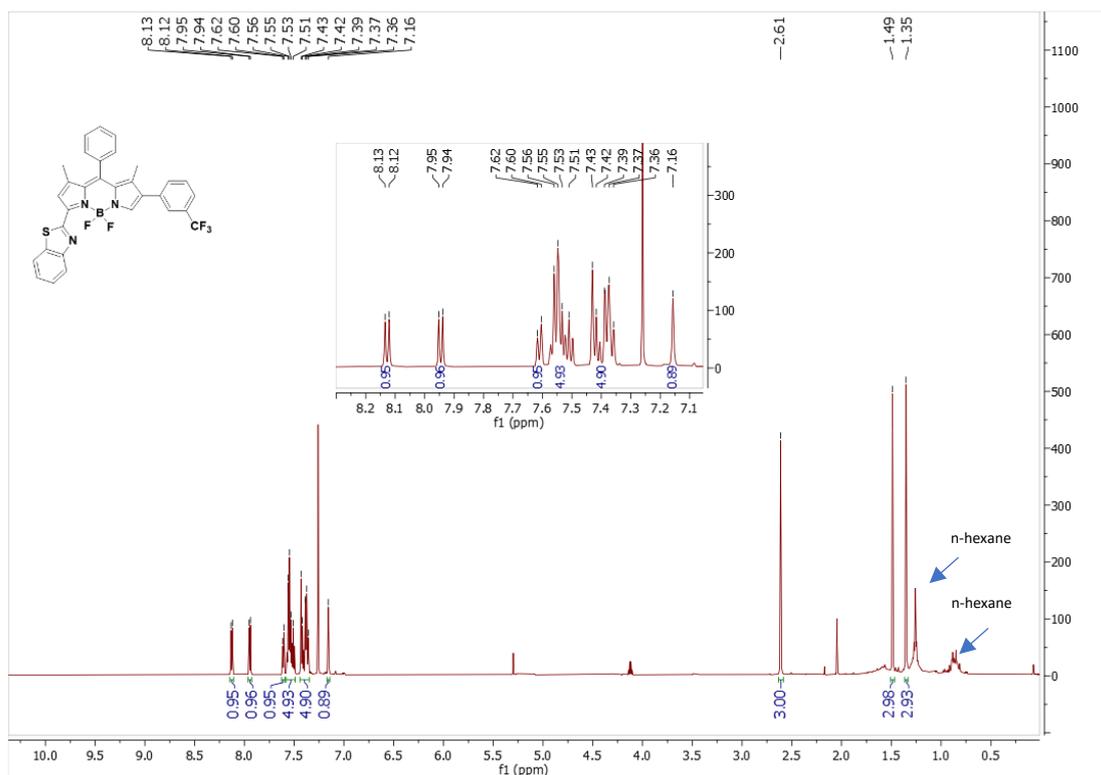


Figure S10. ¹H NMR spectrum (600 MHz, CDCl₃) of dyeTC498

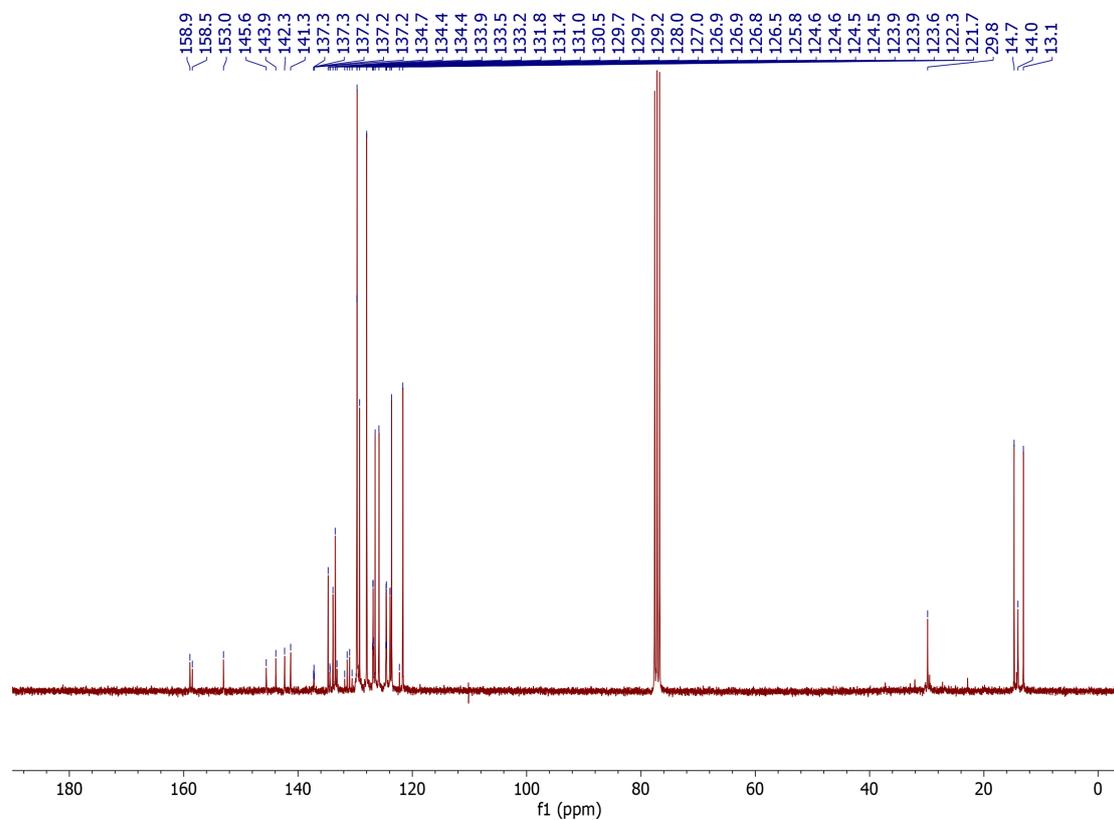
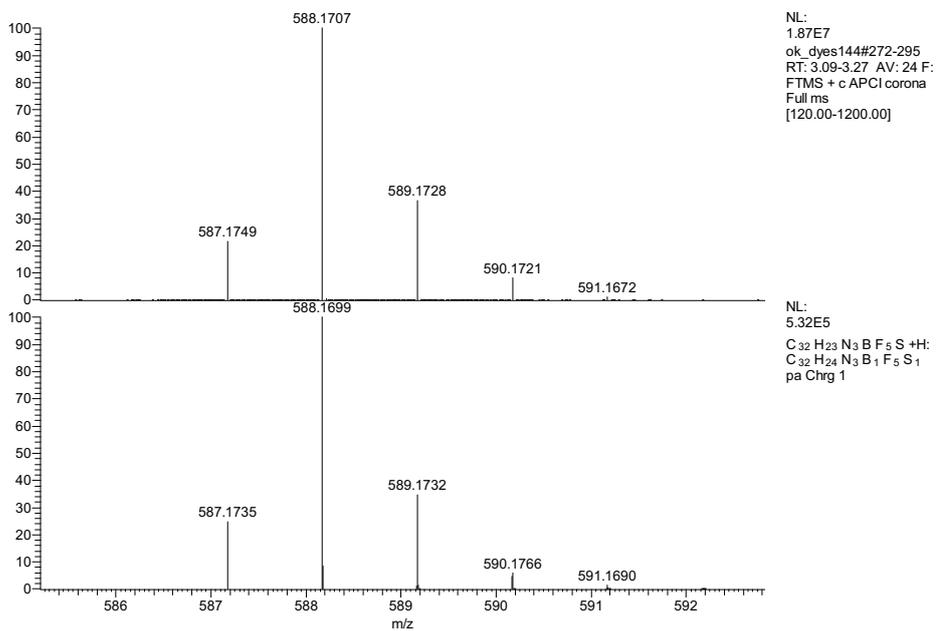


Figure S11. ¹³C NMR spectrum (75 MHz, CDCl₃) of dye TC498



Elemental composition

Single mass

Mass: 588.17067

Max. results: 1

Calculate

Idx	Formula	RDB	Delta pp
1	C ₃₂ H ₂₄ N ₃ B F ₅ S	20.0	1.366

Figure S12. HR-MS of dyeTC498

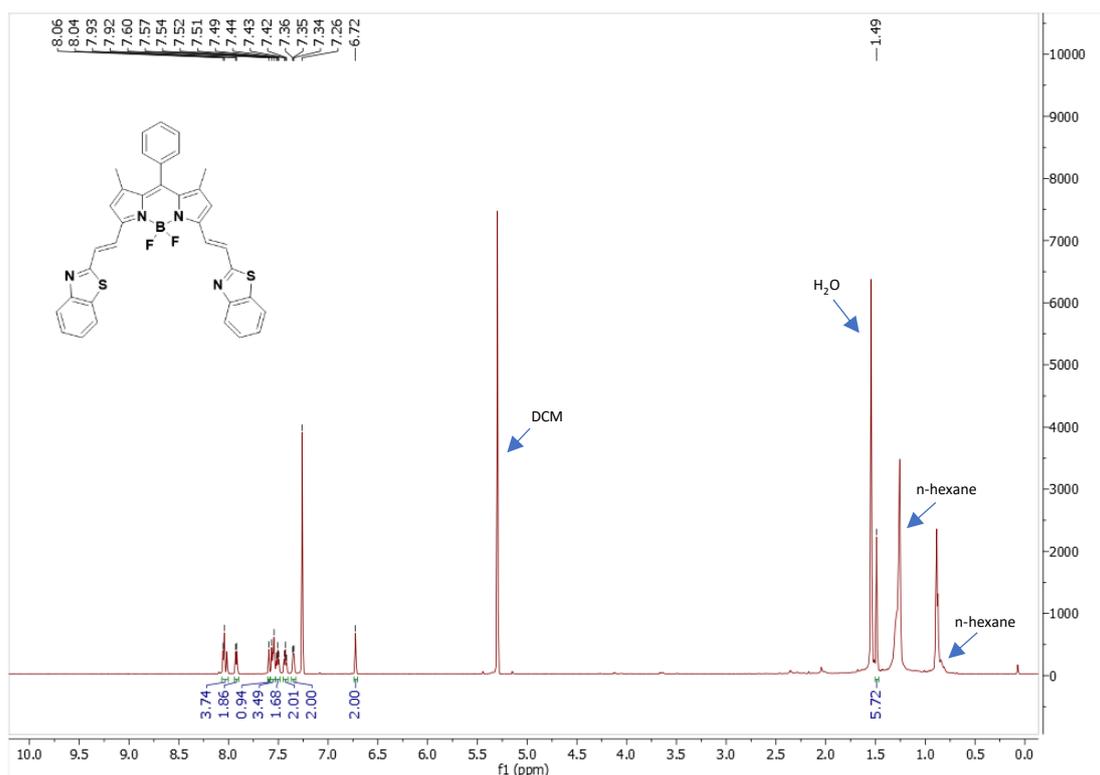


Figure S13. ^1H NMR spectrum (600 MHz, CDCl_3) of dye TC500

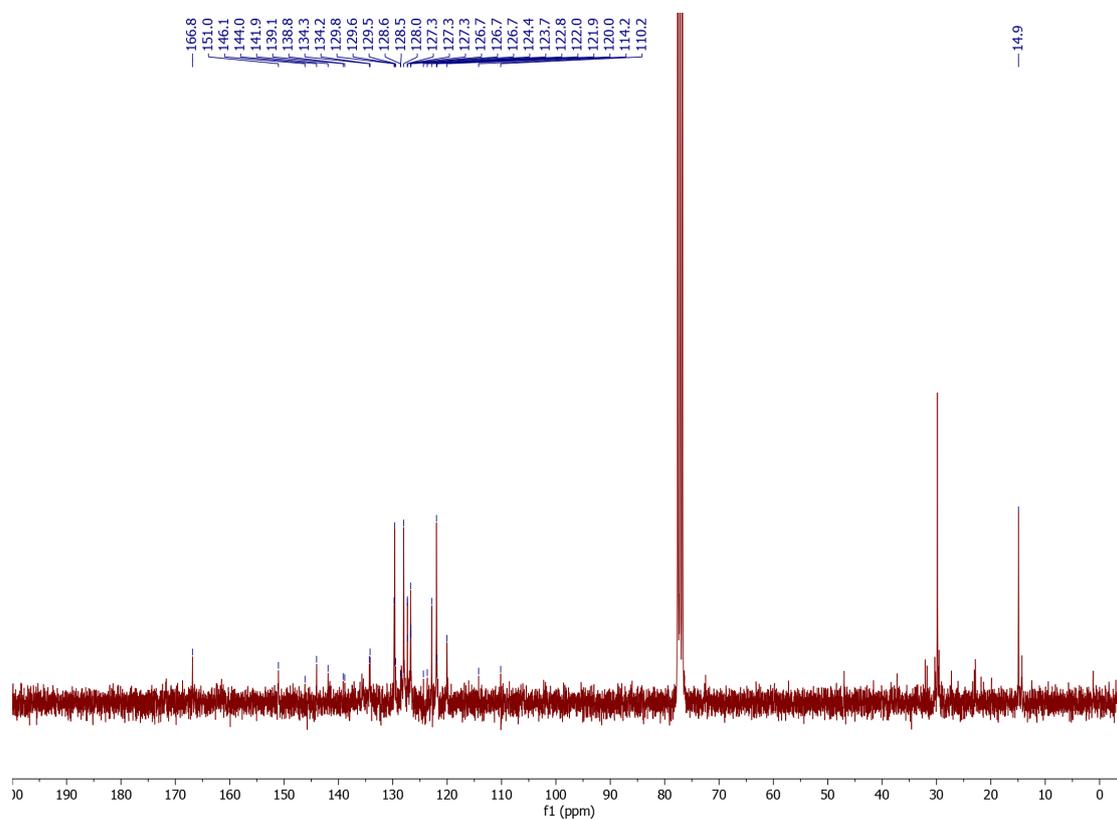


Figure S14. ^{13}C NMR spectrum (75 MHz, CDCl_3) of dye TC500

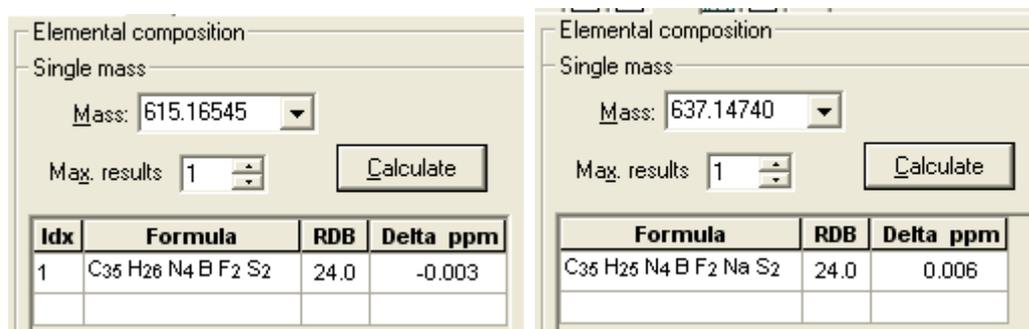
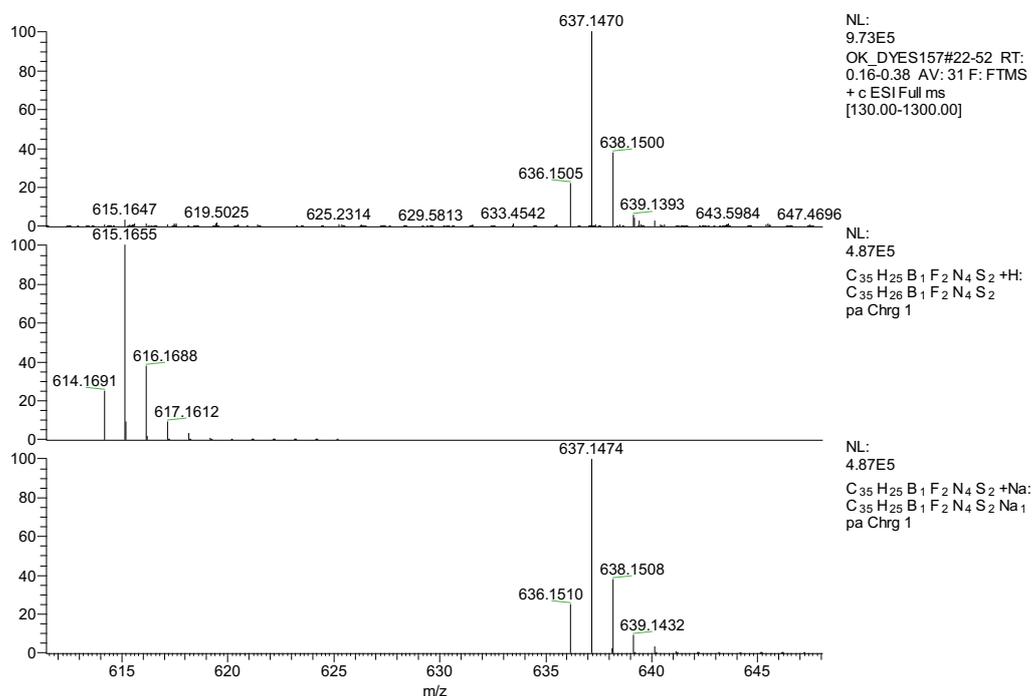


Figure S15. HR-MS (APCI⁺) of dye TC500

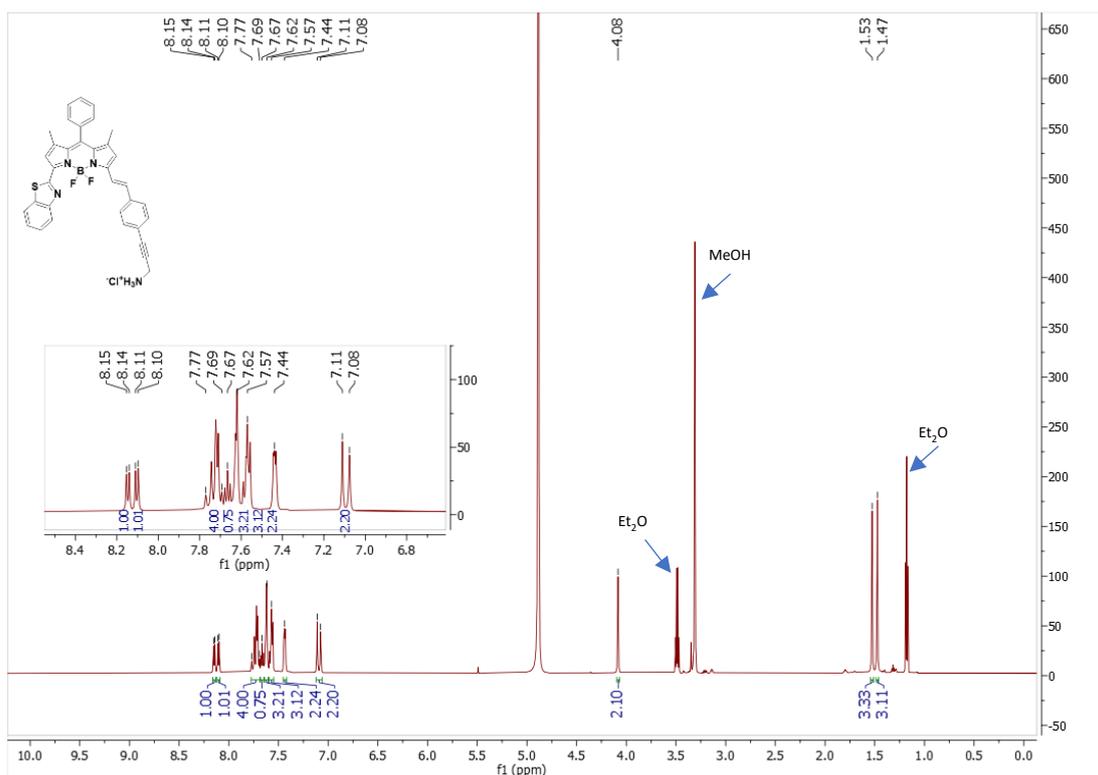


Figure S16. ^1H NMR spectrum (600 MHz, CD_3OD) of dye TC514

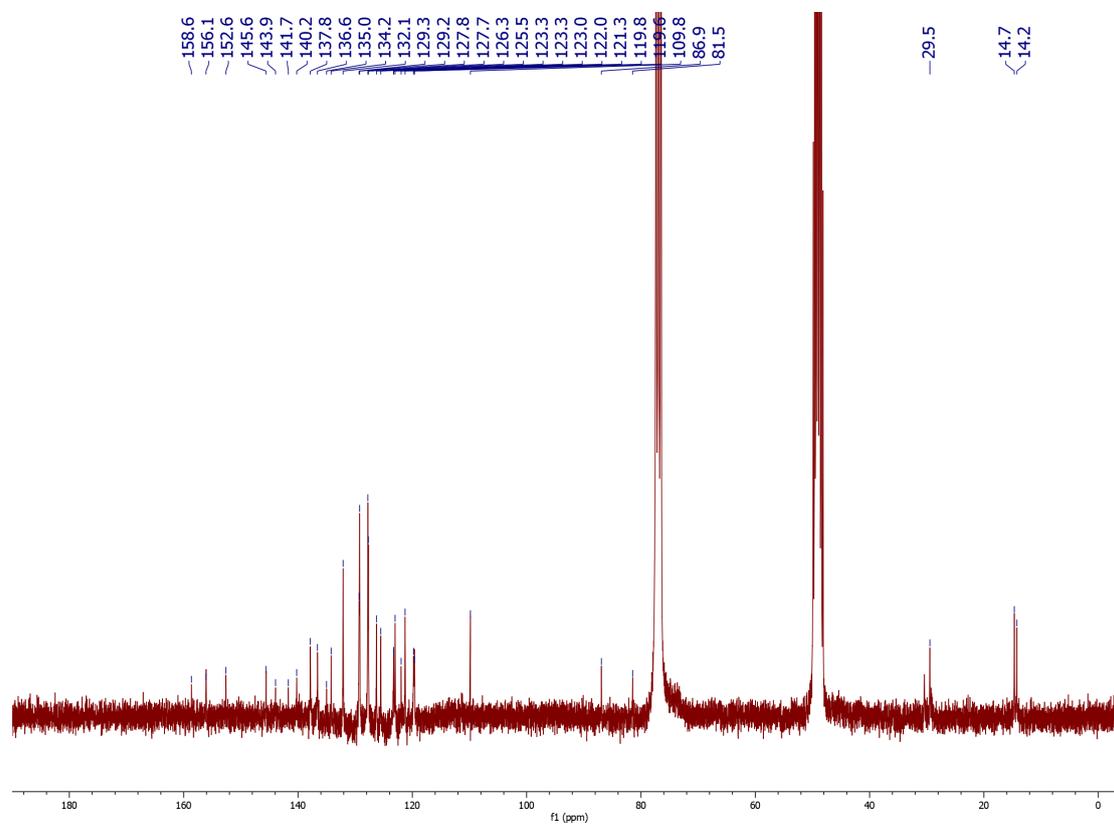


Figure S17. ^{13}C NMR spectrum (75 MHz, $\text{CD}_3\text{OD}/\text{CDCl}_3$) of dye TC514

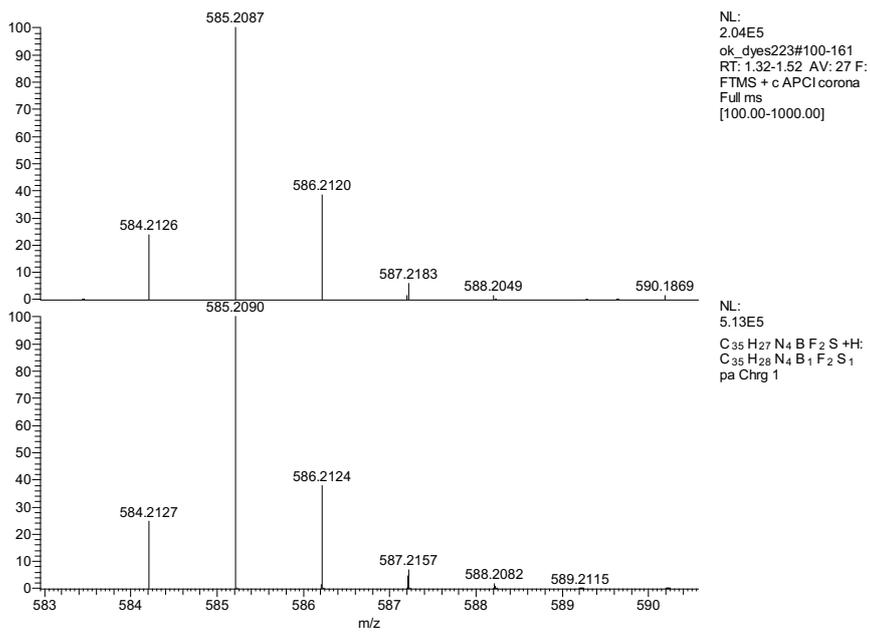


Figure S18. HR-MS (APCI⁺) of dye TC514

Elemental composition

Single mass

Mass: 585.20867

Max. results: 10

Calculate

Idx	Formula	RDB	Delta ppm
1	C ₃₅ H ₂₈ N ₄ B F ₂ S	23.0	-0.617

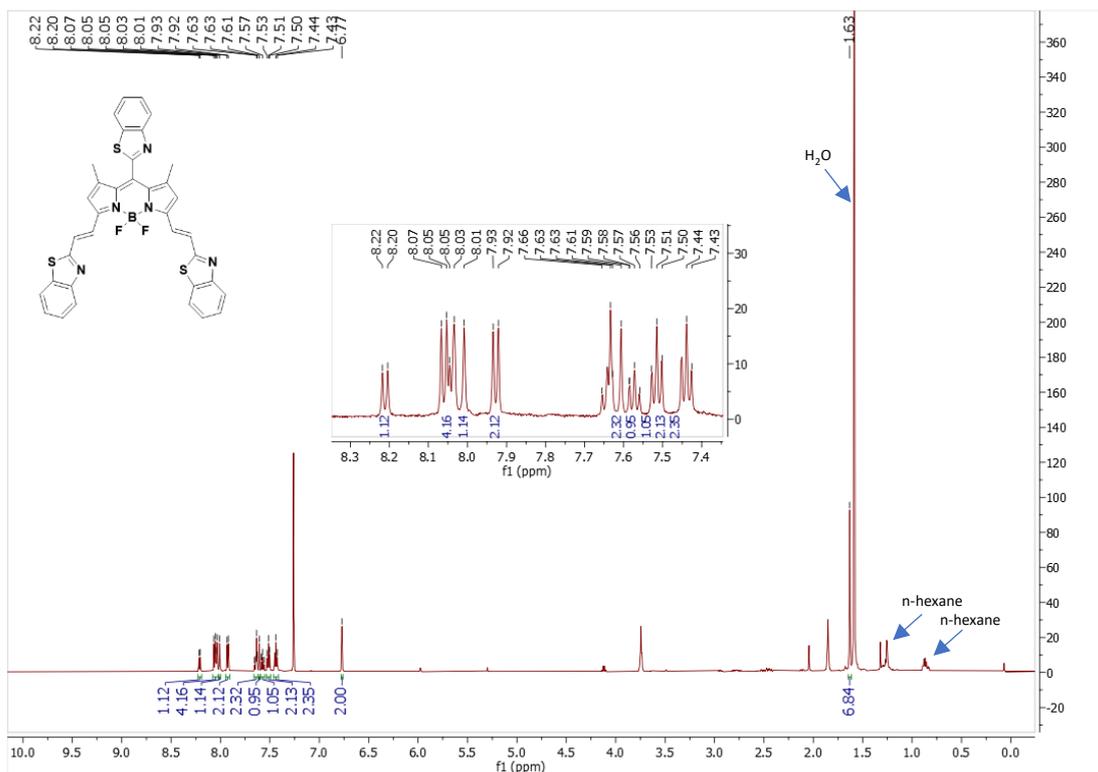


Figure S19. ¹H NMR spectrum (600 MHz, CDCl₃) of dye TC516

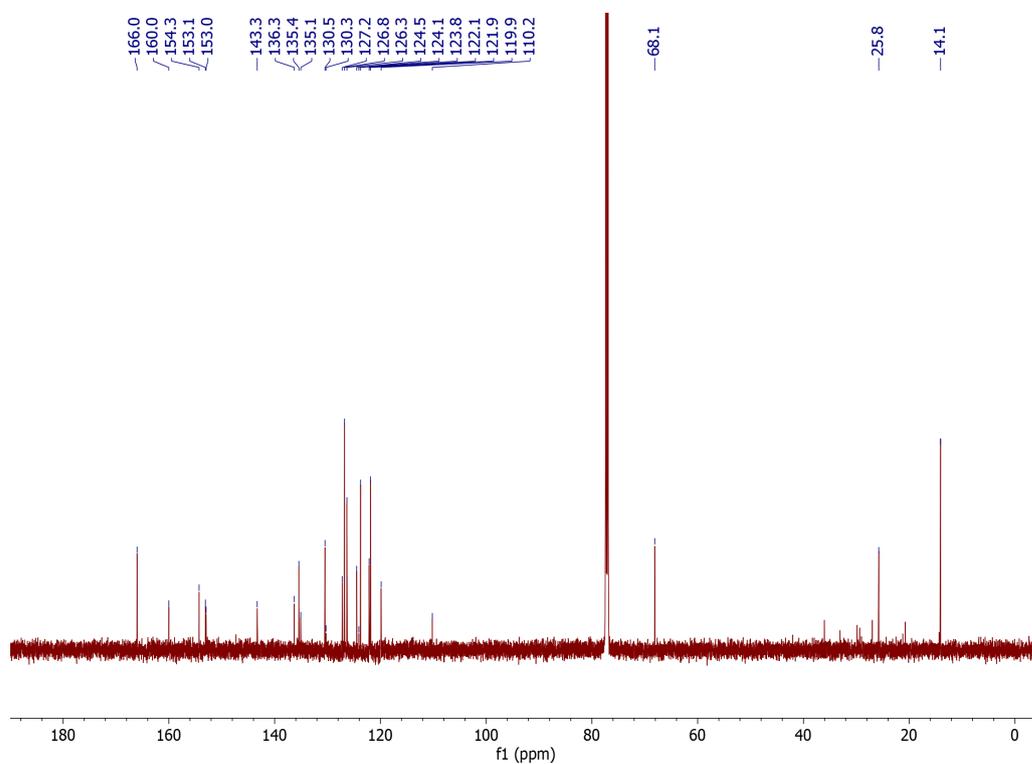
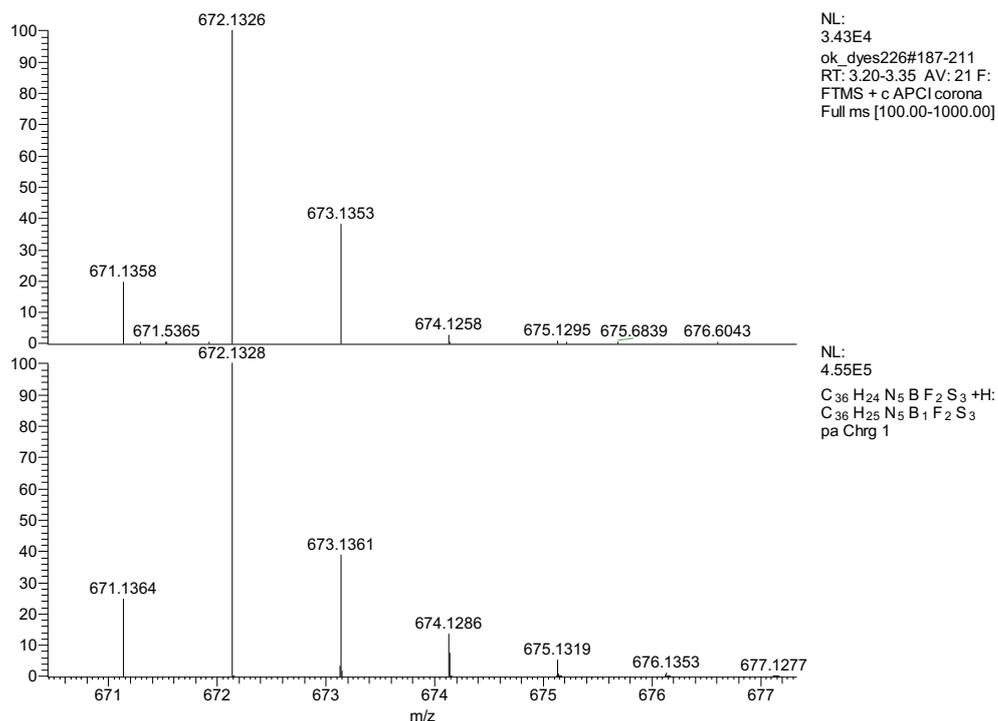


Figure S20. ¹³C NMR spectrum (150 MHz, CDCl₃) of dye TC516



Elemental composition

Single mass

Mass: 672.13264

Max. results: 10

Calculate

Idx	Formula	RDB	Delta ppm
1	C ₃₆ H ₂₅ N ₅ B F ₂ S ₃	26.0	-0.195

Figure S21. HR-MS of dye TC516

Optical data of the new dyes

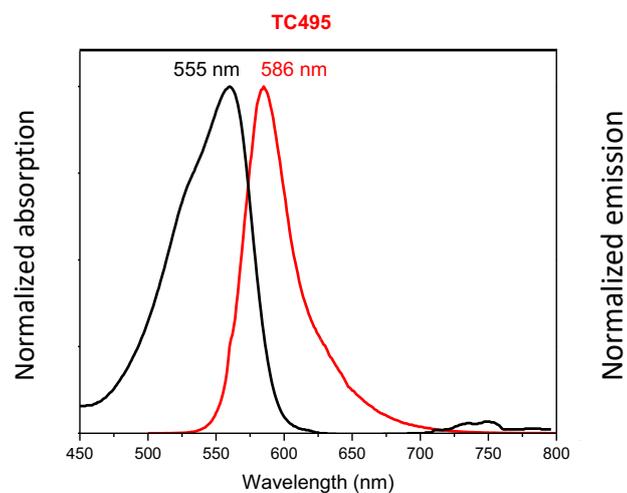


Figure S22: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye TC495

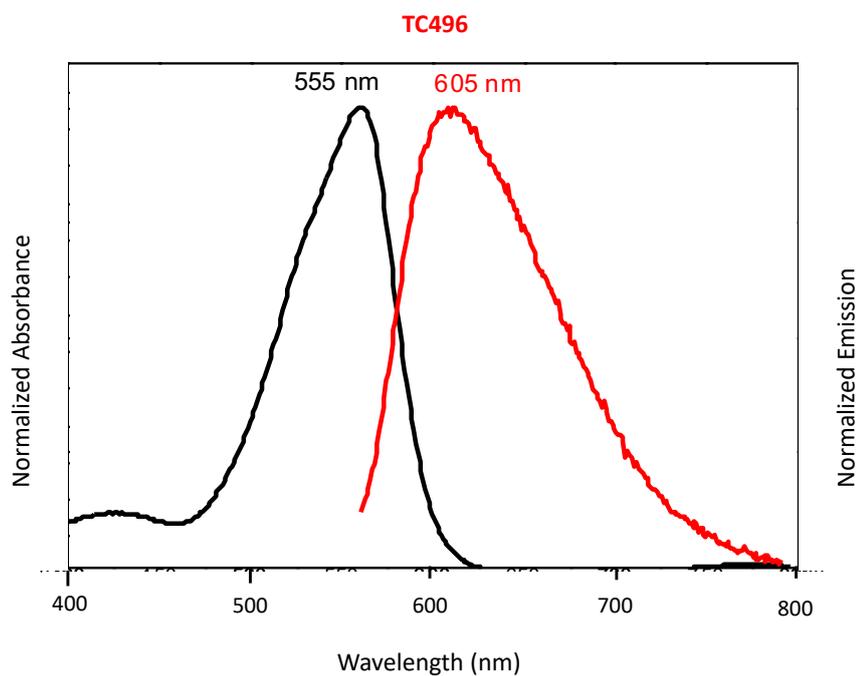


Figure S23: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye TC496

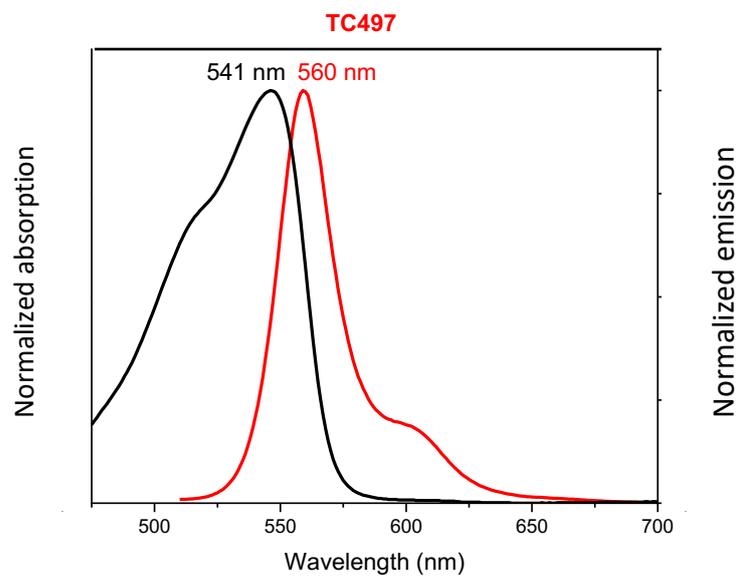


Figure S24: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye **TC497**

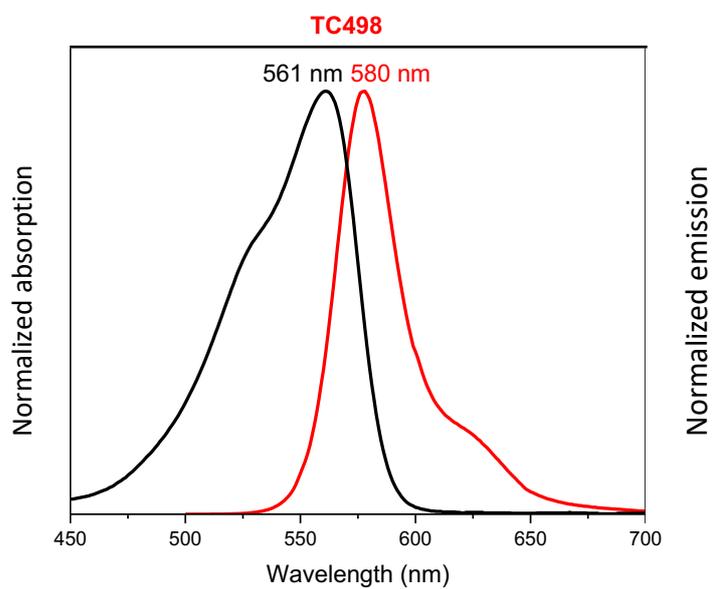


Figure S25: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye **TC498**

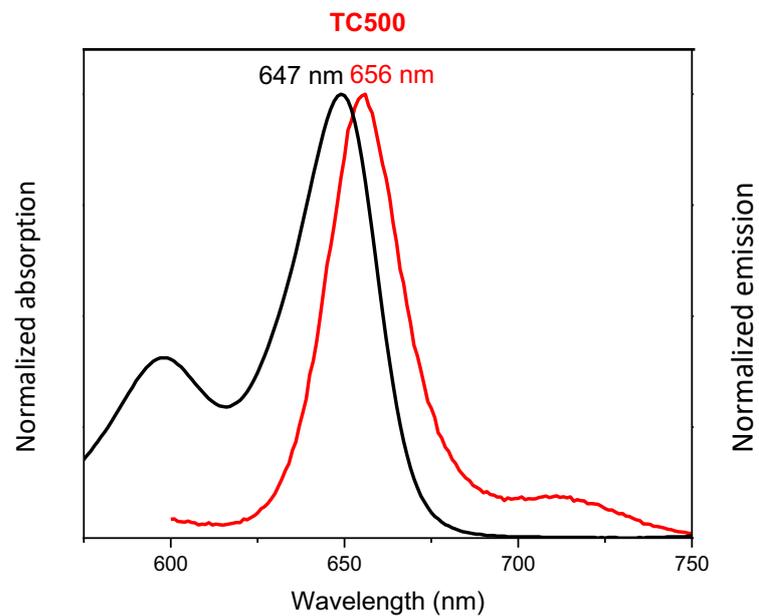


Figure S26: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye TC500

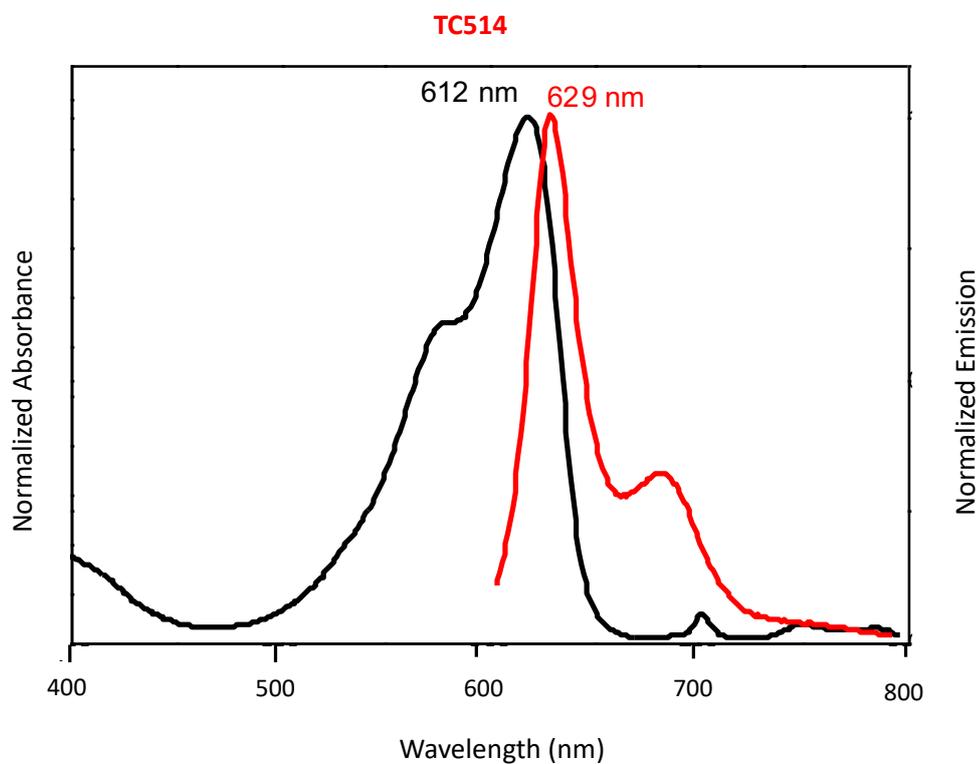


Figure S27: Normalized absorption (dark solid line) and emission (red solid line) spectra of dye TC514

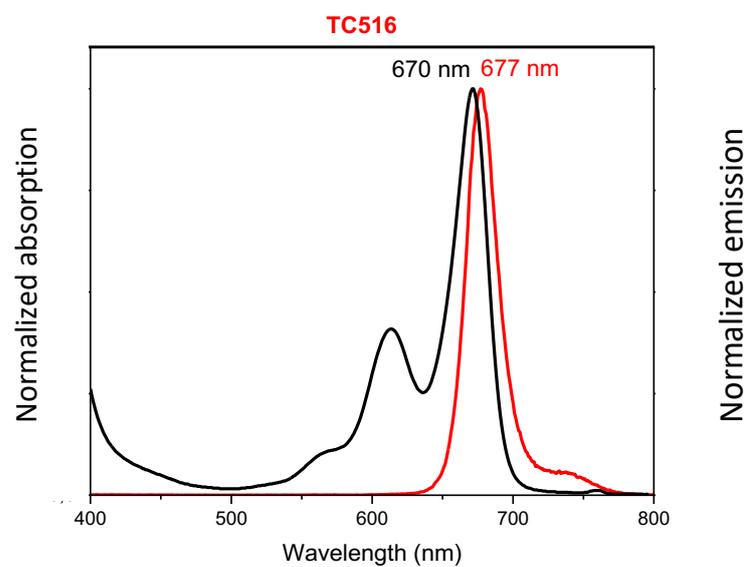


Figure S28. Normalized absorption (dark solid line) and emission (red solid line) spectra of dye **TC516**.

Theoretical calculations

Following the work of Jacquemin et al [64–66] geometries in electronic ground and first excited state were optimized with the 6-31G* basis set and the hybrid M06-2X functional [65], using the polarized continuum model in the integral equation formalism (IEFPCM) to include the solvation effect of ethanol; the occurrence of a true minimum was confirmed by the calculation of the frequencies. To validate the choice of the functional and basis set also for the further (TD)DFT calculations, electronic absorption wavelengths were computed with the M06-2X functional as well as with the Coulomb-attenuated CAM-B3LYP functional, which describes the long-range exchange interaction potential via the Hartree-Fock exchange and was specifically designed to improve the description of charge-transfer interactions of DFT with standard functionals [67]. However, the values obtained with CAM-B3LYP were very similar to those obtained with M06-2X (see Table S1), which therefore was used in the following analysis. As the use of the better basis set 6-311+G**, as advocated in Refs. [64–66] for electronic transition computations, leads only to a slight improvement, we continued to use the computationally cheaper basis set 6-31G*.

For all compounds, the transition to the first excited state S_1 was found to be strongly dipole-allowed, with a large oscillator strength f , and a large contribution of the HOMO-LUMO transition. To obtain a more quantitative measure of the orbital composition of the $S_0 - S_1$ transition, a transformation to natural transition orbitals (NTO) [68] was performed, which provides a means to analyze the orbital representation for a one-electron transition. The HOMO-LUMO transition contributes to all cases more than 95% to the two frontier NTOs, which were visually indistinguishable from the canonical frontier orbitals. Thus, the S_0-S_1 transition can be described to a very good approximation by the HOMO-LUMO transition alone.

The computed transition energies are in general agreement with the trends of the experimental values, although they are systematically overestimated, as **Table S1** shows. A general overestimation of transition energies has been observed previously for BODIPY compounds [69–71], even with the considerably more accurate and computationally expensive approach applied in Refs. [64–66], and is still the topic of current research (see, e.g. [72,73]).

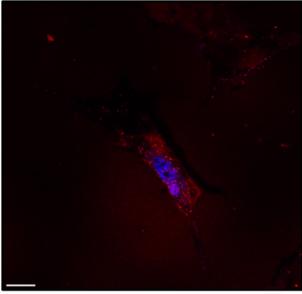
Table S1. Experimental and calculated absorption maxima λ_{abs} (in nm); oscillation strengths f in brackets.

Molecule	8-phenyl-BODIPY	TC495	TC496	TC497	TC498	TC500	TC514	TC516
CAM-B3LYP/ 6-31G*	422 (0.63)	472 (0.97)	475 (0.96)	458 (0.82)	473 (0.99)	551 (1.12)	523 (1.05)	571 (1.13)
M06-2X/6-31G*	425 (0.80)	474 (0.96)	478 (0.94)	459 (0.90)	475 (0.98)	552 (1.10)	525 (1.04)	572 (1.11)
M06-2X/6-311+G**	433 (0.62)	482 (0.96)	485 (0.94)	467 (0.81)	482 (0.98)	562 (1.08)	534 (1.03)	583 (1.10)
Exp.	499	560	560	546	561	649	618	671

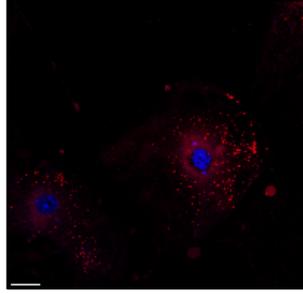
Photostability of the new dyes after long incubation

A

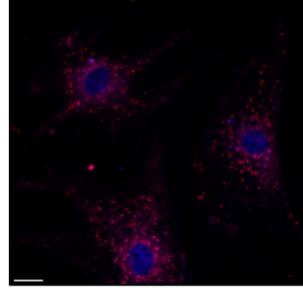
TC496



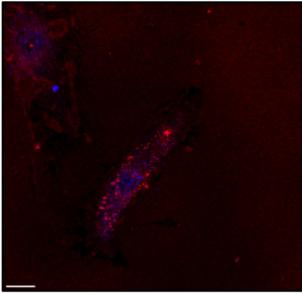
TC497



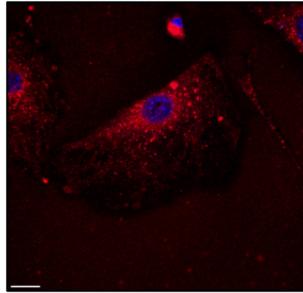
TC498



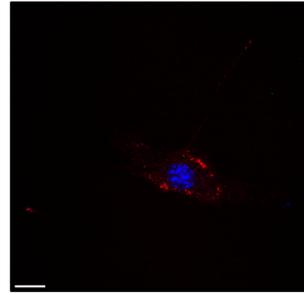
TC500



TC514

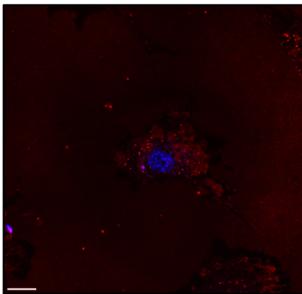


TC516

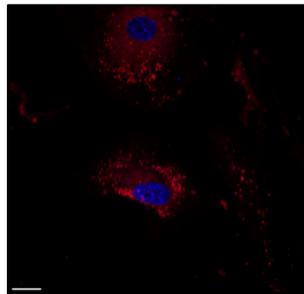


B

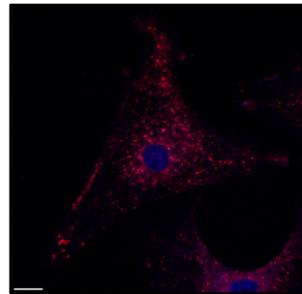
TC496



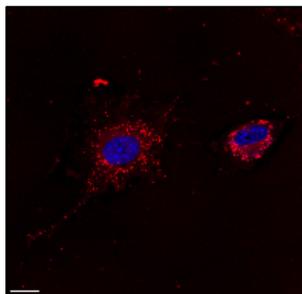
TC497



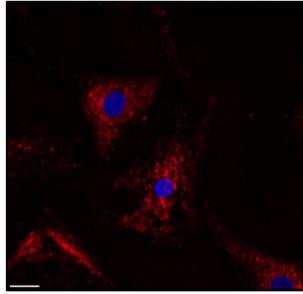
TC498



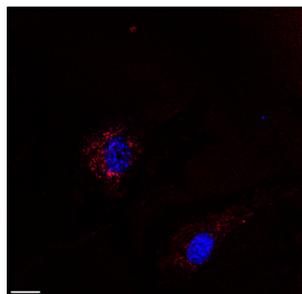
TC500



TC514



TC516



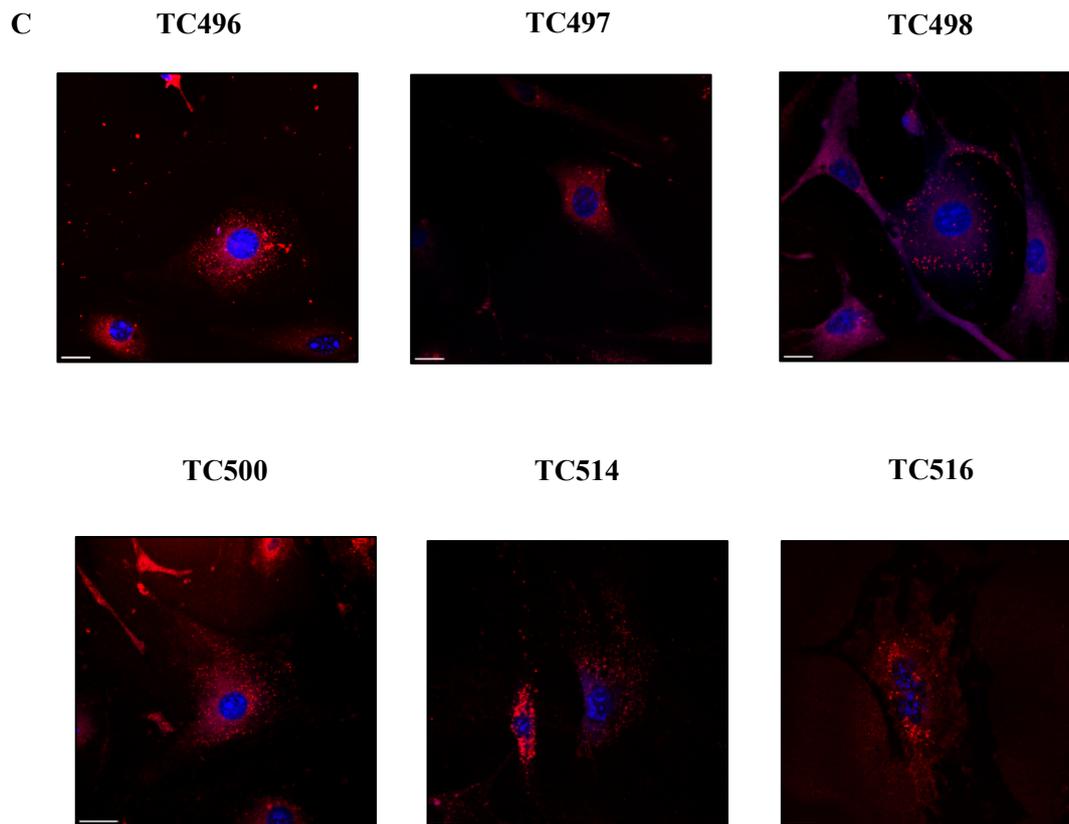


Figure S29. Photostability of the new dyes after long incubation time. The new synthesised dyes were tested for their toxicity and stability after long incubation times. The dyes were added in OptiMEM™ reduced serum medium and incubated for 30min (A), 90min (B) and 24h (C) in 37 °C, respectively. After incubation, cells were washed with PBS and fixed with PFA for 10min. Images were acquired in confocal Leica SP8X WLL system and are illustrated in red colour. The concentrations used were 1µM for **TC496**, **TC497**, **TC498** and **TC500** and 0.1µM for **TC514** and **TC516**. Cell nuclei were stained by DAPI (in blue). Scale bars, 20 µm

Staining profile of dye TC514

Lamp2 488 / TC514 excite at 590nm

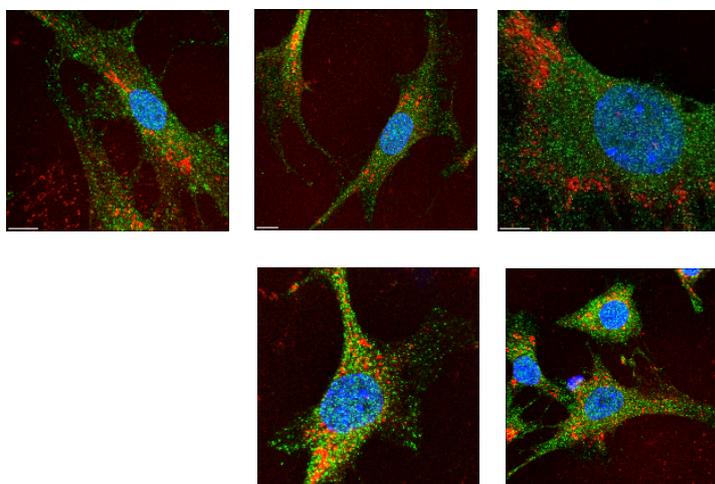


Figure S30. TC514 co-staining with lysosome marker (Lamp2). TC514 was added in live cells for 5min at 37°C, cells washed, fixed in PFA for 10min and stained with immunofluorescence with Lamp2 illustrated in green. TC514 is observed in green colour. Images acquired in confocal Leica SP8 microscope. Cell nuclei were stained by DAPI (in blue). Scale bars, 10 μ m.

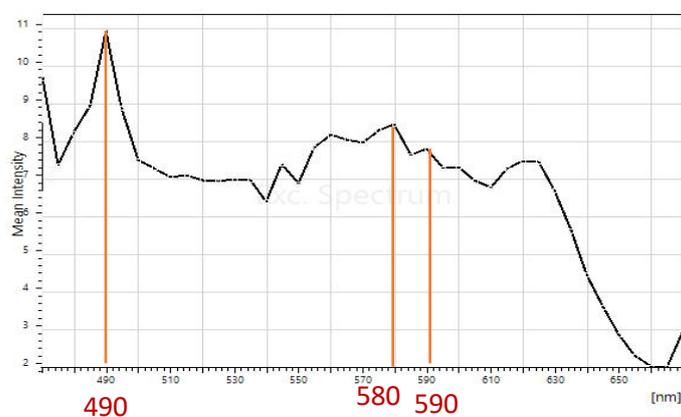


Figure S31. TC514 excitation spectrum. TC514 Δ capital scans for the excitation of the dye. Peaks at 490nm, 580nm and 590nm are noted as the as best excitation peaks in orange colour.

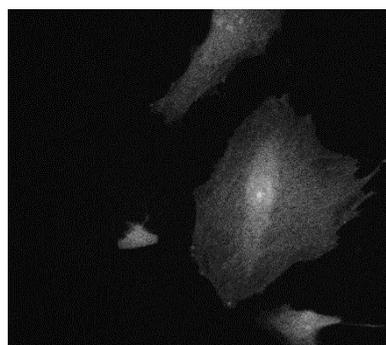
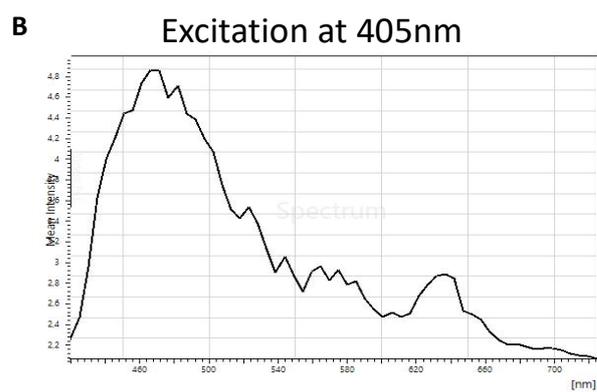
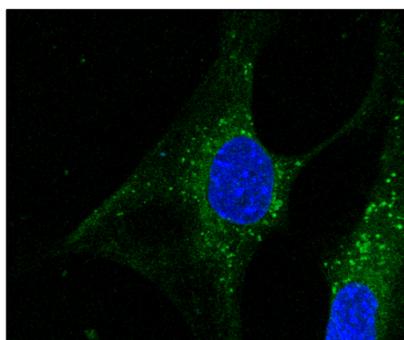
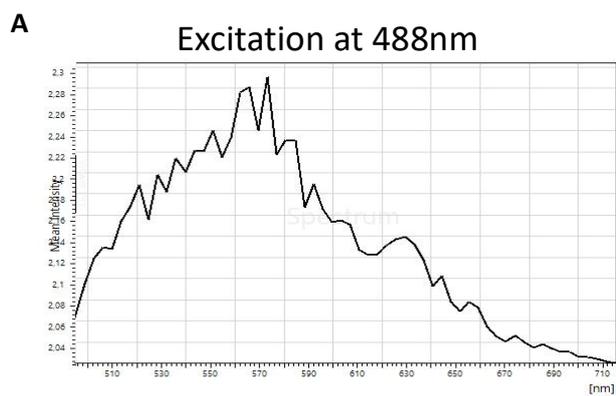


Figure S32. TC514 co-staining capacity. TC514 λ scan with excitation at 488 (A) and excitation with 405 (B).

Staining profile of dye TC498

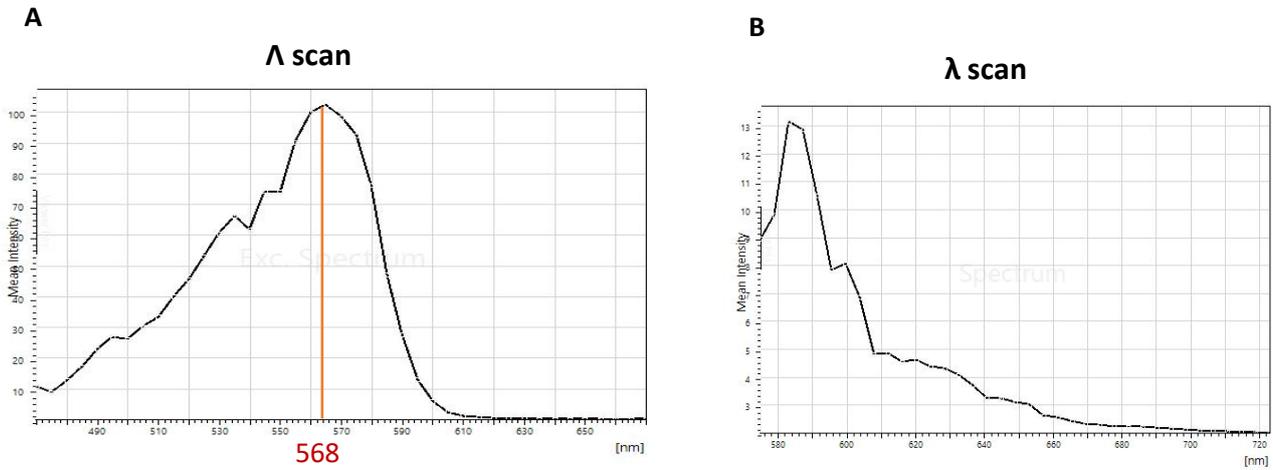


Figure S33. TC498 excitation and emission spectrum. TC498 λ capital scan reveals the best excitation peak at 568nm in orange colour (A). The emission spectrum for TC498 when excited at 568nm has a clear and narrow peak at 583nm (B).

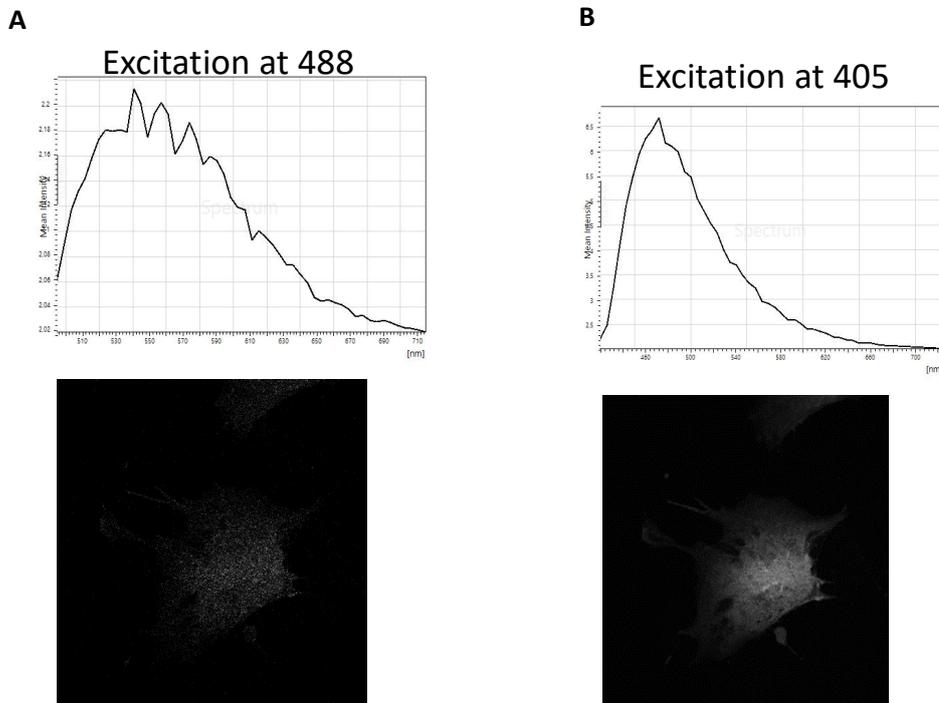


Figure S34. TC498 is suitable for co-staining with other dyes. TC498 is not excited with either 488 or 405, λ emission scan shows background signal when excited with both wavelengths 488 or 405. (A, B).

References

63. Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U. Relative and absolute determination of fluorescence quantum yields of transparent samples. *Nat. protoc.* **2013**, *8*, 1535–1550. <https://doi.org/10.1038/nprot.2013.087>
64. Chibani, S.; Laurent, A.D.; Le Guennic, B.; Jacquemin, D. Improving the Accuracy of Excited-State Simulations of BODIPY and Aza-BODIPY Dyes with a Joint SOS-CIS(D) and TD-DFT Approach. *J. Chem. Theory Comput.* **2014**, *10*, 4574–4582.
65. Chibani, S.; Le Guennic, B.; Charaf-Eddin, A.; Maury, O.; Andraud, C.; Jacquemin, D. On the Computation of Adiabatic Energies in Aza-Boron-Dipyrromethene Dyes. *J. Chem. Theory Comput.* **2012**, *8*, 3303–3313. <https://doi.org/10.1021/ct300618j>
66. Chibani, S.; Le Guennic, B.; Charaf-Eddin, A.; Laurent, A.D.; Jacquemin, D. Revisiting the optical signatures of BODIPY with ab initio tools. *Chem. Sci.* **2013**, *4*, 1950–1963. <https://doi.org/10.1021/ct500655k>
67. Yanai, T.; Tew, D.P.; Handy, N.C. A new hybrid exchange–correlation functional using the Coulomb-attenuating method (CAM-B3LYP). *Chem. Phys. Lett.* **2004**, *393*, 51–57. <https://doi.org/10.1016/j.cplett.2004.06.011>
68. Martin, R.L. Natural Transition Orbitals. *J. Chem. Phys.* **2003**, *118*, 4775–4777. <https://doi.org/10.1063/1.1558471>
69. Ngoy, B.P.; Molupe, N.; Harris, J.; Fomo, G.; Mack, J.; Nyokong, T. Photophysical studies of 2,6-dibrominated BODIPY dyes substituted with 4-benzyloxystyryl substituents. *J. Porphyr. Phtalocyanines* **2017**, *21*, 431–438. <https://doi.org/10.1142/S1088424617500420>
70. Ghai, L.; Mack, J.; Lu, H.; Yamada, H.; Kuzuhara, D.; Lai, G.; Li, Z.; Shen, Z. New 2,6-Distyryl-Substituted BODIPY Isomers: Synthesis, Photophysical Properties, and Theoretical Calculations. *Chem. Eur. J.* **2014**, *20*, 1091–1102. <https://doi.org/10.1002/chem.201303291>
71. Ni, Y.; Zeng, L.; Kang, N.-Y.; Huang, K.-W.; Wang, L.; Zeng, Z.; Chang, Y.-T.; Wu, J. meso-Ester and Carboxylic Acid Substituted BODIPYs with Far-Red and Near-Infrared Emission for Bioimaging Applications. *Chem. Eur. J.* **2014**, *20*, 2301–2310. <https://doi.org/10.1002/chem.201303868>
72. Momeni, M. R.; Brown, A. Why do TD-DFT excitation energies of BODIPY/Aza-BODIPY families largely deviate from experiment? Answers from electron correlated and multireference methods. *J. Chem. Theory Comput.* **2015**, *11*, 2619–2632. <https://doi.org/10.1021/ct500775r>
73. Postils, V.; Ruiperez, F.; Casanova, D. Mild Open-Shell Character of BODIPY and Its Impact on Singlet and Triplet Excitation Energies. *J. Chem. Theory Comput.* **2021**, *17*, 5825–5838. <https://doi.org/10.1021/acs.jctc.1c00544>