Electronic Supplementary Information (ESI)

Heavy-atom-free BODIPY-based photodynamic therapy agents activated at long wavelengths

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1. General methods

Synthesis

All chemical reagents were commercial and used without further purification unless otherwise stated. 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. Purification of the synthesized products was performed by normal-phase flash column chromatography using silica gel (230-400 mesh, Merck, Germany) and the used eluents are specified using the proportions indicated by volume:volume ratio. ^{1}H and ^{13}C NMR spectra were recorded on 300 or 700 MHz NMR spectrometers at room temperature. Chemical shifts (δ in ppm) were referenced to internal solvent $CDCl_3$ (7.260 for ^{1}H and 77.16 for ^{13}C) o CD_3OD (3.310 for ^{1}H and 49.00 for ^{13}C). Multiplicity is indicated as follows: s = singlet; d = doublet; t = triplet; 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 performed using ESI or MALDI-TOF and ion trap (positive or negative mode) for the detection.

Spectroscopic properties

The photophysical signatures were registered from diluted solutions (around 2×10^{-6} M), prepared by adding the corresponding solvent to the residue from the adequate amount of a concentrated stock solution in ethanol, after vacuum evaporation of this solvent. UV-Vis absorption and fluorescence spectra were recorded on a Varian model CARY 4E (Agilent Technologies, USA) spectrophotometer and an Edinburgh Instruments (UK) spectrofluorometer (model FLSP 920), respectively. Fluorescence quantum yields (Φ_{fl}) were obtained using zinc phthalocyanine (Φ_{fl} = 0.30 in benzene)[1] as reference, from corrected spectra (detector sensibility to the wavelength). The values were corrected by the refractive index of the solvent. Radiative decay curves were registered with the time-correlated single-photon counting technique as implemented in the aforementioned spectrofluorometer. Fluorescence emission was monitored at the maximum emission wavelengths after excitation by means of a Fianium pulsed laser (time resolution of picoseconds) with tunable wavelength. The fluorescence lifetime (τ_{fl}) 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 (chi-square, Durbin–Watson, and the analysis of the residuals).

[1] A. M. Brouwer. Standards for photoluminescence quantum yield measurements in solution (IUPAC technical report). *Pure Appl. Chem*., 2011, **83**, 2213-2228.

The photoinduced production of singlet oxygen ($^1O_2$) was determined by direct measurement of the luminescence at 1276 nm with an NIR detector integrated in the aforementioned spectrofluorometer (InGaAs detector, Hamamatsu G8605-23). The $^1O_2$ signal was registered in front configuration (front face), 40° and 50° to the excitation and emission beams, respectively, and leaned 30° to the plane formed by the direction of incidence and registration in cells of 1 cm. The signal was filtered by a low cut-off of 850 nm. $^1O_2$-generation quantum yield (Φ_Δ) was determined using the following equation:

$$\Phi_\Delta = \Phi_\Delta^r \cdot (\alpha^r/\alpha^{PS}) \cdot (Se^{PS}/Se^r)$$

where Φ_Δ^r is the quantum yield of $^1O_2$ generation for the used reference (2,6-diiodo-3,5-dimethyl-8-methylthioBODIPY, MeSBDP, which was 0.91 in chloroform,[2] and methylene blue, which was 0.58 in deuterated methanol[3]). Factor $\alpha = 1-10^{-Abs}$ corrects the different amount of photons absorbed by the sample (α^{PS}) and reference (α^r). Factor Se is the intensity of the $^1O_2$ phosphorescence signal of the sample (Se^{PS}) and the reference (Se^r) at 1276 nm. $^1O_2$ quantum yields were averaged from at least five concentrations between 10^{-6} M and 10^{-5} M.

Quantum mechanics calculations

Ground-state geometries (S_0) were optimized using a hybrid exchange-correlation functional with the Coulomb-attenuating method (CAM-B3LYP), within Density Functional Theory (DFT), and a triple valence basis set with a polarization function (6-311g*). All the calculations were run without any geometrical constraint, and the geometries were considered to be at minimum energy when the corresponding frequency analysis did not give any negative value. The time-dependent (TD) method at the above detailed calculation level (functional and basis set) was used to simulate the absorption spectra as vertical Franck–Condon transitions. The solvent effect (chloroform) was also simulated during the calculations by the Self-Consistent Reaction Field (SCRF) using the Polarizable Continuum Model (PCM). All calculations were run on the Gaussian 16 software implemented in the “Arina” informatics cluster of the UPV/EHU.

Cell culture conditions

For *in vitro* experiments, human melanoma cell line SK-Mel-103 was used, which was maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated in an atmosphere of 20% O_2 and 5% CO_2 at 37 ^{0}C. 3-3'-**1**/**7** compounds were completely dissolved in DMSO (> 99.9%) to prepare a stock solution (10 mM) from which serial dilution with culture medium were done to obtaine the corresponding

[2] R. Prieto-Montero, R. Sola-Llano, R. Montero, A. Longarte, T. Arbeloa, I. López-Arbeloa, V. Martínez-Martínez, S. Lacombe. Methylthio BODIPY as a standard triplet photosensitizer for singlet oxygen production: A photophysical study. *Phys. Chem. Chem. Phys*., 2019, **21**, 20403-20414.

[3] R. W. Redmond, J. N. Gamlin. A compilation of singlet oxygen yields form biologically relevant molecules. *Photochem. Photobiol*., 1999, **70**, 391-475.

working concentration. Considering the possible toxicity of DMSO to cells, a maximum concentration of 1% of DMSO was considered to avoid any cell toxicity.

Phototoxicity evaluation in cell culture

The phototoxic capacity of **1**-**7** compounds was evaluated in SK-Mel-103 cells by using the WST-1 Assay for Cell Proliferation and Viability (Roche, Switzerland). For this, $5x10^3$ cells/well were seeded in a 96-well plate and were kept in the incubator for 24 h prior to treatment. Next, each of **1**-**7** compounds was then incorporated in the medium at the concentrations indicated in the text and incubated with the cells for 24 h. Afterwards, cells were rinsed with a PBS solution and replaced with fresh medium and irradiated with a 36 W LED device at 10 cm (900 mW/cm^2) of distance during 30 min (16 mW/cm^2, light dose irradiating the cells). To allow the passage only of the wavelength capable of exciting the **1**-**7** compounds a Newport Colored Glass Longpass filter (λ > 550 nm) was incorporated between the light source and the 96-well plate. After irradiating the 96-well plate, the cells were incubated for another 24 h and then 7 µl of WST-1 was added to each well and were further incubated for 2 h at 37 ^{0}C. Finally, the absorbance of the cellular medium was measured at a wavelength of λ = 450 nm with the spectrophotometer Wallac 1420 Victor2 Microplate Reader (PerkinElmer, USA). The intrinsic toxicity of the compound, without the effect of light exposure, was evaluated under the same conditions but omitting the irradiation treatment step. The EC_{50} which refers to the concentration of compound which reduced 50% of cell viability compared to the untreated controls was calculated from a sigmoidal curve fit of the photocytotoxicity data for at least three independent measurements for the same compound.

Subcellular co-localization studies

To carry out the subcellular co-localization studies, 2.5 x 10^4 SK-Mel-103 cells were seeded on 24 nm φ glass coverslips previously introduced, under sterile conditions, in a 6-well plate and incubated at 37 ^{0}C for 24 h. Then, cells were treated with the compounds **2**, **4** and **7** at a concentration of 5 µM, 10 µM and 10 µM respectively, for 24 h. Next, cells were rinsed with 2 mL of PBS saline solution and incubated with different subcellular-specific trackers (Molecular Probes ® by Life Technologies, USA) in green. For specific labeling of lysosomes, cells were incubated with 75 nM Lysotracker green for 2 h and for labeling of lipid droplets (LDs) with 10 µM PM546 (1,3,5,7,8-pentamethylpyrromethene-difluoroborate complex, Luxottica / Exciton, USA) green for 30 min. In addition, the nuclei of the cells were stained by incorporating the fluorescent dye Hoechst 33258 (2 µg/mL) for 15 min prior to visualization in the confocal microscopy Leica (Germany) TCS SP8 HyVolution II. To visualize the nuclei, the Hoechst 33258 was excited with the 405 nm laser and the fluorescence emitted in the range of 415-475 nm was collected. To excite the cellular markers in green, the 488 nm wavelength laser was used, and the fluorescence emitted between 490-530 nm was collected and finally

2, **4** and **7** compounds were excited with the 532 laser and the emission was collected in the range of 640-780 nm. To analyse the degree of co-localization between the specific subcellular markers and **2**, **4** and **7** compounds, the Person's correlation coefficient (R) was calculated from the images obtained by confocal using the LAS X program. It is considered that there is a co-localization when R values are ≥ 0.7.

ROS production in cells

To evaluate the capacity to generate reactive oxygen species (ROS) of **7** in SK-Mel-103 cell line, the marker CM-H2DCFDA green (General Oxidative Stress Indicator, Invitrogen, ThermoFisher, USA) was used. Firstly, SK-Mel-103 cells were seeded in a density of $25x10^4$ cells/well on 24 nm φ glass coverslips previously introduced, under sterile conditions, in a 6-well plate and incubated at 37 ^{0}C for 24 h. Afterwards, 10 μM of **7** was added to the culture medium and left for 24 h, then the cells were rinsed with 2 mL of PBS and places in fresh medium. Then, cells were irradiated following the same protocol used to analyse the phototoxic capacity and the marker CM-H2DCFDA in green was incorporated at a final concentration of 500 nM during 15 min incubation. Confocal images were taken in the confocal microscopy Leica TCS SP8 HyVolution II and mean fluorescence intensity of the probe was obtained with ImageJ/Fiji. CM-H2DCFDA was exited at 488 nm and emission read at 511 ± 23 nm.

Statistical analysis

The *in vitro* experiments using cell lines were performed at least three times independently, with the results shown in the text being representative for each of the studies. Data is presented in the graphs and in the text as mean ± standard deviation of the mean (SEM) obtained by the software GraphPad Prism 8.0. Statistical significance was assessed using the appropriate method according to the data analysing, in this case either the two-tailed Student's t-test or the one-way ANOVA was used. It is considered that there is a significant difference between two populations when the p-value < 0.05.

2. Synthesis and characterization

BODIPY dimer **1**[4] was synthesized by the corresponding described methods.

General procedure for Negishi reactions

The corresponding halogenated BODIPY (0.04-0.22 mmol), $PdCl_2(PPh_3)_2$ or $PdCl_2(PPh_3)_2$/ X-Phos (10 mol%) and dry toluene (4 mL) were placed into a Schlenk tube capped with a rubber septum under argon atmosphere. This tube was previously air-evacuated and backfilled

[4] Z. Kang, Q. Wu, X. Guo, L. Wang, Y. Ye, C. Yu, H, Wang, E. Hao, L. Jiao. $FeCl_3$-promoted regioselective synthesis of BODIPY dimers through oxidative aromatic homocoupling reactions. *Chem. Commun*., 2021, **57**, 9886-9889.

with argon (this sequence was repeated three times). Then, the corresponding organozinc reagent (0.16-1.31 mmol) was drop-wise added over the mixture and the resulting reaction mixture stirred at r.t. for 1 or 24 h. The reaction was monitored by thin layer chromatography (TLC). Once the reaction was monitored as finalized, the mixture was filtered (CH_2Cl_2 was used for elution and washing), and the obtained solution submitted to solvent evaporation under vacuum. The obtained residue was purified by flash chromatography on silica gel.

Synthesis and characterization of compounds

2: According to above described general procedure, dimer **1**[4] (27 mg, 0.04 mmol), $PdCl_2(PPh_3)_2$ (3 mg, 0.004 mmol) and 1 M $ZnMe_2$ in toluene (0.20 mL, 0.20 mmol) in dry toluene (4 mL) were reacted for 60 min. Flash chromatography using hexane/EtOAc (97:3) afforded **2** in 93% isolated yield (24 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 4.5 Hz, 2H, 2CH), 6.95 (s, 4H, 4CH), 6.60-6.57 (m, 4H, 4CH), 6.28 (d, *J* = 4.5 Hz, 2H, 2CH), 2.65 (s, 6H, $2CH_3$), 2.37 (s, 6H, $2CH_3$), 2.16 (s, 12H, $4CH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1 (C), 146.4 (C), 142.8 (C), 138.7 (C), 137.0 (C), 136.9 (C), 136.4 (C), 130.6 (C), 130.3 (CH), 128.2 (CH), 127.6 (CH), 123.1 (CH), 121.6 (CH), 21.3 (CH_3), 20.3 (CH_3), 15.6 (CH_3) ppm. FTIR ν 2924, 1562, 1524, 1440, 1328, 1255, 1149, 1110, 1013 cm^{-1}. HRMS-MALDI-TOF *m/z* calcd for $C_{38}H_{36}B_2F_4N_4$ $[M]^+$ 646.3062, found 646.3084.

3: According to above described general procedure, dimer **1**[4] (55 mg, 0.08 mmol), $PdCl_2(PPh_3)_2$ (6 mg, 0.008 mmol) and $BrZn(CH_2)_5CO_2Et$ (0.32 mL, 0.16 mmol) in dry toluene (4 mL) were reacted for 60 min. Flash chromatography using hexane/EtOAc (97:3) afforded dimer **3** in 38% isolated yield (24 mg). ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.51 (d, *J* = 4.2 Hz, 1H, CH), 7.50 (d, *J* = 4.2 Hz, 1H, CH), 6.962 (s, 2H, 2CH), 6.957 (s, 2H, 2CH), 6.70 (d, *J* = 4.2 Hz, 1H, CH), 6.66 (d, *J* = 4.2 Hz, 1H, CH), 6.56 (appt t, *J* = 4.2 Hz, 2H, 2CH), 6.39 (d, *J* = 4.2 Hz, 1H, CH), 6.33 (d, *J* = 4.2 Hz, 1H, CH), 4.10 (q, *J* = 7.0 Hz, 2H, CH_2O), 3.04 (t, *J* = 7.7 Hz, 2H, CH_2), 2.37 (s, 6H, $2CH_3$), 2.30 (t, *J* = 7.7 Hz, 2H, CH_2), 2.16 (s, 6H, $2CH_3$), 2.14 (s, 6H, $2CH_3$), 1.76 (quint, *J* = 7.7 Hz, 2H, CH_2), 1.67 (quint, *J* = 7.7 Hz, 2H, CH_2), 1.46 (quint, *J* = 7.7 Hz, 2H, CH_2), 1.23 (t, *J* = 7.0 Hz, 3H, CH_3) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 173.9 (COO), 167.1 (C), 150.4 (C), 144.4 (C), 143.5 (C), 143.1 (C), 139.0 (C), 138.8 (C), 137.9 (C), 137.3 (C), 137.0 (C), 136.9 (C), 136.7 (C), 134.4 (C), 131.6 (CH), 130.1 (CH), 130.0 (C), 129.5 (C), 128.9 (CH), 128.4 (CH), 128.3 (CH), 127.2 (CH), 124.8 (CH), 123.5 (CH), 120.8 (CH), 118.6 (CH), 60.4 (CH_2O), 34.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.0 (CH_2), 24.9 (CH_2), 21.3 (CH_3), 20.3 (CH_3), 14.4 (CH_3) ppm. FTIR ν 2921, 2852, 1732, 1555, 1522, 1387, 1245, 1146, 1094, 975 cm^{-1}. HRMS-MALDI-TOF *m/z* calcd for $C_{44}H_{45}B_2ClF_4N_4O_2$ $[M]^+$ 794.3353, found 794.3344.

4: According to above described general procedure, dimer **1**[4] (150 mg, 0.22 mmol), $PdCl_2(PPh_3)_2$ (16.5 mg, 0.022 mmol) and $BrZn(CH_2)_5CO_2Et$ (378 mg, 1.31 mmol) in dry toluene

(4 mL) were reacted for 60 min. Flash chromatography using hexane/ EtOAc (97:3) afforded **4** in 71% isolated yield (141 mg). ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.42 (d, *J* = 4.2 Hz, 2H, 2CH), 6.95 (s, 4H, 4CH), 6.61 (d, *J* = 4.2 Hz, 2H, 2CH), 6.57 (d, *J* = 4.2 Hz, 2H, 2CH), 6.34 (d, *J* = 4.2 Hz, 2H, 2CH), 4.10 (q, *J* = 7.0 Hz, 4H, $2CH_2O$), 3.03 (t, *J* = 7.7 Hz, 4H, $2CH_2$), 2.37 (s, 6H, $2CH_3$), 2.29 (t, *J* = 7.7 Hz, 4H, $2CH_2$), 2.15 (s, 12H, $4CH_3$), 1.75 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.67 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.45 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.23 (t, *J* = 7.0 Hz, 6H, $2CH_3$) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 173.9 (COO), 165.5 (C), 146.3 (C), 142.9 (C), 138.6 (C), 136.9 (C), 136.1 (C), 130.6 (CH), 130.3 (C), 128.2 (CH), 127.5 (CH), 123.1 (CH), 119.8 (CH), 60.4 (CH_2O), 34.3 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.0 (CH_2), 24.9 (CH_2), 21.3 (CH_3), 20.4 (CH_3), 14.4 (CH_3) ppm. FTIR ν 2924, 2854, 1733, 1561, 1524, 1378, 1297, 1149, 1101, 987 cm^{-1}. HRMS-MALDI-TOF *m/z* calcd for $C_{52}H_{60}B_2F_4N_4O_4$ $[M]^+$ 902.4737, found 902.4749.

5: To a solution of **4** (130 mg, 0.14 mmol) in dry CH_2Cl_2/DMF (5:5 mL) was added NBS (153 mg, 0.84 mmol), and the reaction was stirred to r.t. for 24 h. Progress of the reaction was monitored by TLC. After disappearance of the starting material, the reaction was washed with H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and the obtained solution submitted to solvent evaporation under vacuum. The residue was purified by flash chromatography on silica gel using toluene/EtOAc (99:1) as eluent, to yield **5** in 43% (73 mg). ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.97 (s, 2H, 2CH), 6.96 (s, 2H, 2CH), 6.74 (s, 2H, 2CH), 6.65 (s, 2H, 2CH), 4.10 (q, *J* = 7.0 Hz, 4H, $2CH_2O$), 2.99-2.89 (m, 4H, $2CH_2$), 2.37 (s, 6H, $2CH_3$), 2.28 (t, *J* = 7.7 Hz, 4H, $2CH_2$), 2.21 (s, 6H, $2CH_3$), 2.15 (s, 6H, $2CH_3$), 1.74 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.65 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.45 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.23 (t, *J* = 7.0 Hz, 6H, $2CH_3$) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 173.9 (COO), 165.2 (C), 144.5 (C), 141.6 (C), 139.4 (C), 136.8 (C), 136.6 (C), 135.5 (C), 134.6 (C), 132.0 (CH), 129.1 (C), 128.5 (CH), 128.4 (CH), 127.1 (CH), 111.7 (CBr), 109.5 (CBr), 60.3 (CH_2O), 34.3 (CH_2), 29.6 (CH_2), 28.2 (CH_2), 28.1 (CH_2), 24.6 (CH_2), 21.3 (CH_3), 20.5 (CH_3), 20.3 (CH_3), 14.4 (CH_3) ppm. FTIR ν 2924, 2856, 1734, 1562, 1524, 1421, 1335, 1229, 1121, 1098, 1061, 1027, 997 cm^{-1}. HRMS-MALDI-TOF *m/z* calcd for $C_{52}H_{56}B_2Br_4F_4N_4O_4$ $[M]^+$ 1218.1116, found 1218.1136.

6: According to above described general procedure, dimer **5** (70 mg, 0.06 mmol), $PdCl_2(PPh_3)_2$ (4 mg, 0.006 mmol), X-Phos (3 mg, 0.006) and 1 M $ZnMe_2$ in toluene (1.20 mL, 1.20 mmol) in dry toluene (4 mL) were reacted for 24 h. Flash chromatography using hexane/EtOAc (7:3) afforded **6** in 15% isolated yield (8.6 mg). ^{1}H NMR (700 MHz, $CDCl_3$) δ 6.95 (s, 2H, 2CH), 6.93 (s, 2H, 2CH), 6.31 (s, 4H, 4CH), 4.10 (q, *J* = 7.0 Hz, 4H, $2CH_2O$), 2.89-2.82 (m, 4H, $2CH_2$), 2.36 (s, 6H, $2CH_3$), 2.27 (t, *J* = 7.7 Hz, 4H, $2CH_2$), 2.22 (s, 6H, $2CH_3$), 2.15 (s, 6H, $2CH_3$), 1.95 (s, 6H, $2CH_3$), 1.88 (s, 6H, $2CH_3$), 1.70-1.62 (m, 8H, $4CH_2$), 1.42 (quint, *J* = 7.7 Hz, 4H, $2CH_2$), 1.22 (t, *J* = 7.0 Hz, 6H, $2CH_3$) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 174.0 (COO), 164.3 (C), 144.4 (C), 141.5 (C), 138.3 (C), 137.2 (C), 136.8 (C), 134.6 (C), 134.3 (C), 130.8 (C), 129.9

(C), 129.1 (CH), 128.1 (CH), 128.0 (CH), 125.3 (CH), 60.3 (CH_2O), 34.4 (CH_2), 29.7 (CH_2), 28.4 (CH_2), 27.8 (CH_2), 24.9 (CH_2), 21.3 (CH_3), 20.6 (CH_3), 20.3 (CH_3), 14.4 (CH_3), 11.5 (CH_3), 11.42 (CH_3), 11.38 (CH_3) ppm. FTIR ν 2923, 2855, 1732, 1560, 1524, 1380, 1287, 1144, 1091, 988 cm^{-1}. HRMS-MALDI-TOF *m/z* calcd for $C_{56}H_{68}B_2F_4N_4O_4$ $[M]^+$ 958.5363, found 958.5375.

7: To a solution of **1**[4] (22 mg, 0.03 mmol) in THF/H_2O (10 mL, 8:2) were added MESNA (21 mg, 0.13 mmol) and $NaHCO_3$ (11 mg, 0.13 mmol) under argon atmosphere, and the reaction mixture was stirred at r.t. for 4 h. Progress of the reaction was monitored by TLC. Upon complete consumption of starting material, the solvent was removed under reduced pressure. The residue was purified by flash chromatography on alumina using CH_3CN/H_2O (9:1) as eluent, to yield **7** in 66% (19 mg). ^1H NMR (700 MHz, CD_3OD) δ 7.34 (d, *J* = 4.2 Hz, 2H, 2CH), 7.05 (s, 4H, 4CH), 6.81 (d, *J* = 4.9 Hz, 2H, 2CH), 6.74 (d, *J* = 4.9 Hz, 2H, 2CH), 6.49 (d, *J* = 4.2 Hz, 2H, 2CH), 3.53-3.50 (m, 4H, $2SCH_2$), 3.21-3.19 (m, 4H, $2SCH_2$), 2.38 (s, 6H, $2CH_3$), 2.16 (s, 12H, $4CH_3$) ppm. ^{13}C NMR (176 MHz, CD_3OD) δ 162.5 (C), 146.4 (C), 140.5 (C), 140.2 (C), 138.9 (C), 138.1 (C), 138.0 (C), 131.8 (CH), 131.1 (C), 129.3 (CH), 127.1 (CH), 123.9 (CH), 119.6 (CH), 52.3 (CH_2S), 28.3 (CH_2S), 21.2 (CH_3), 20.1 (CH_3) ppm. FTIR ν 2917, 2851, 1558, 1520, 1438, 1378, 1179, 1092, 1047, 981 cm^{-1}. HRMS (ESI) *m/z* calcd for $C_{40}H_{38}B_2F_4N_4O_6S_4^{2-}$ 448.0904, found 448.0898.

3. ^{1}H NMR, ^{13}C NMR and HMRS spectra

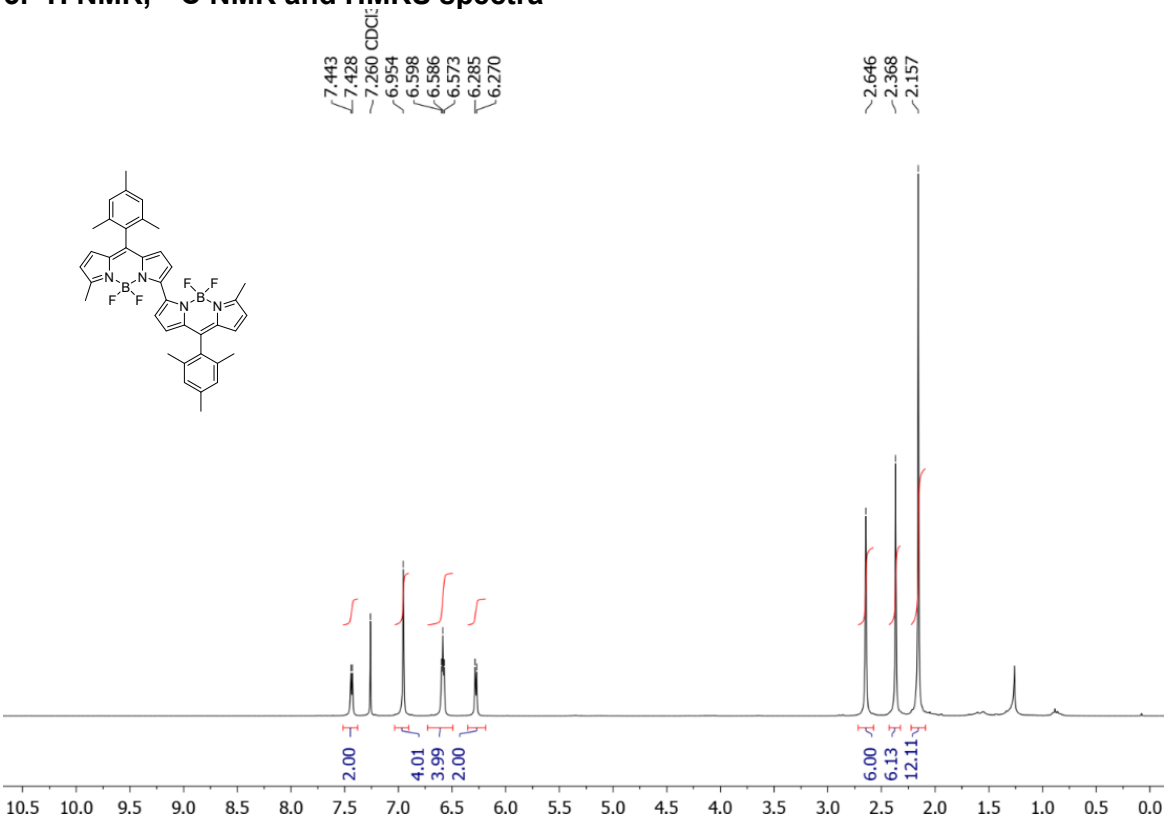

Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of **2.**

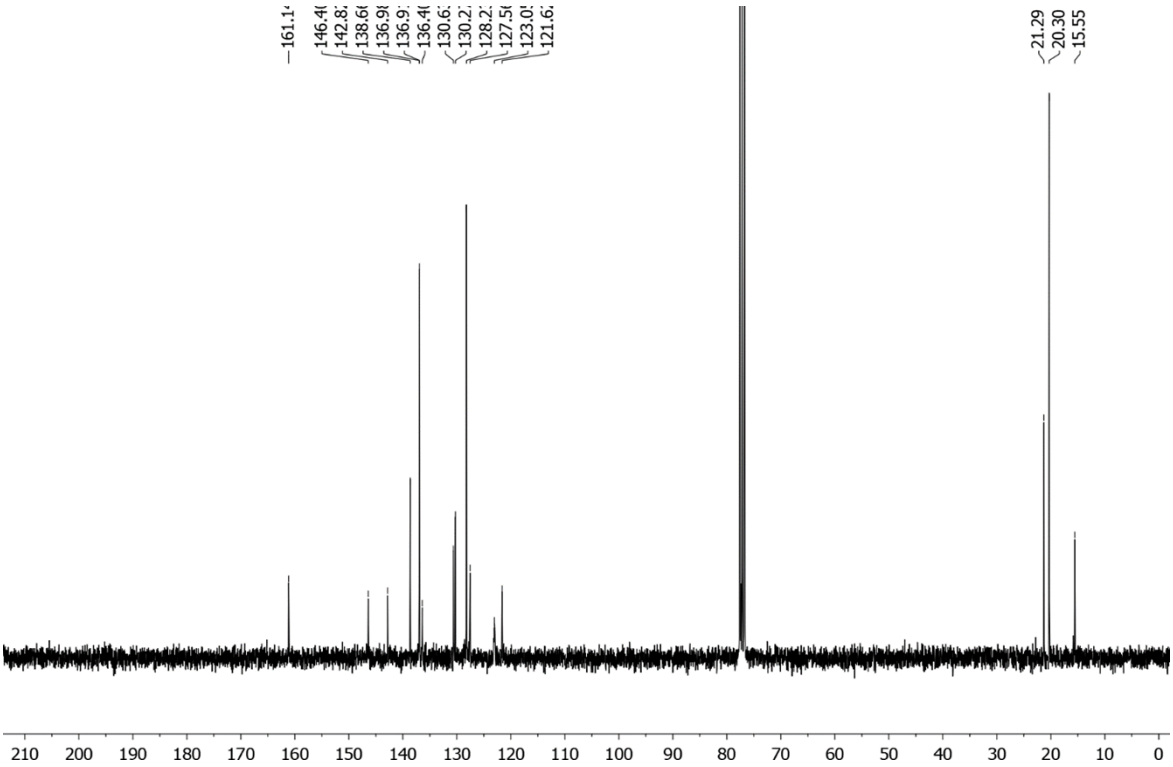

Figure S2. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of **2.**

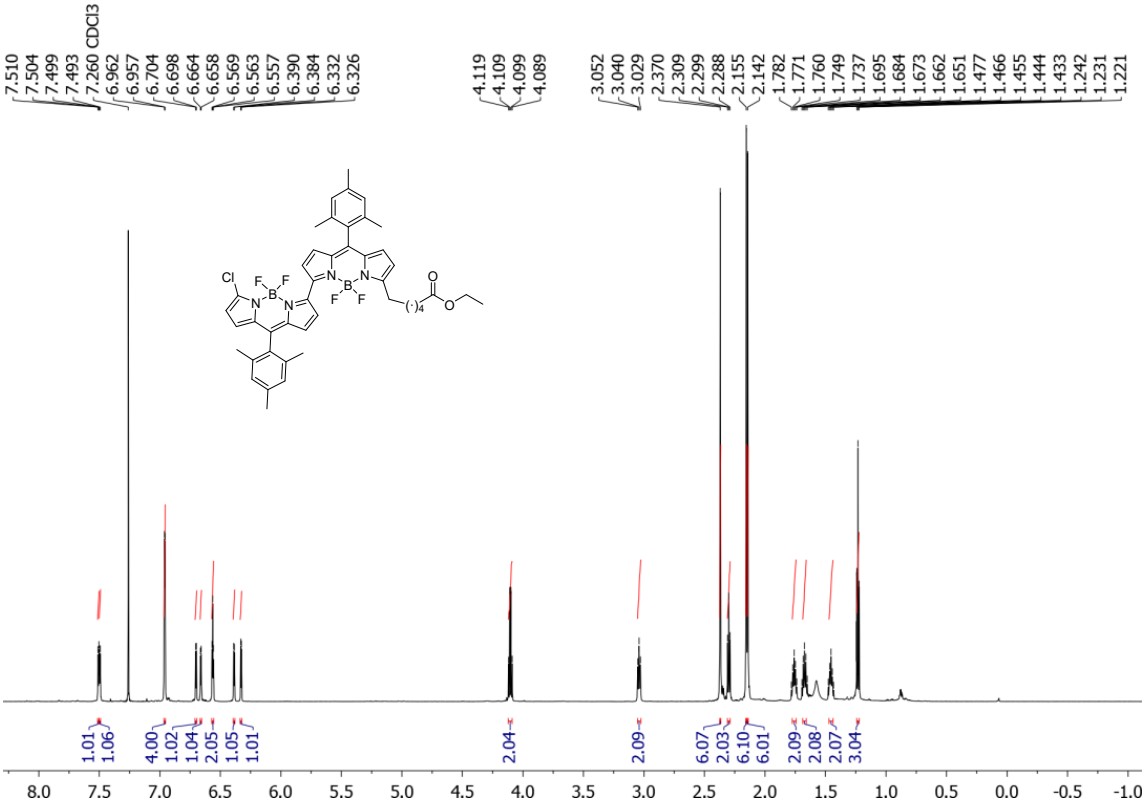

Figure S3. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of **3.**

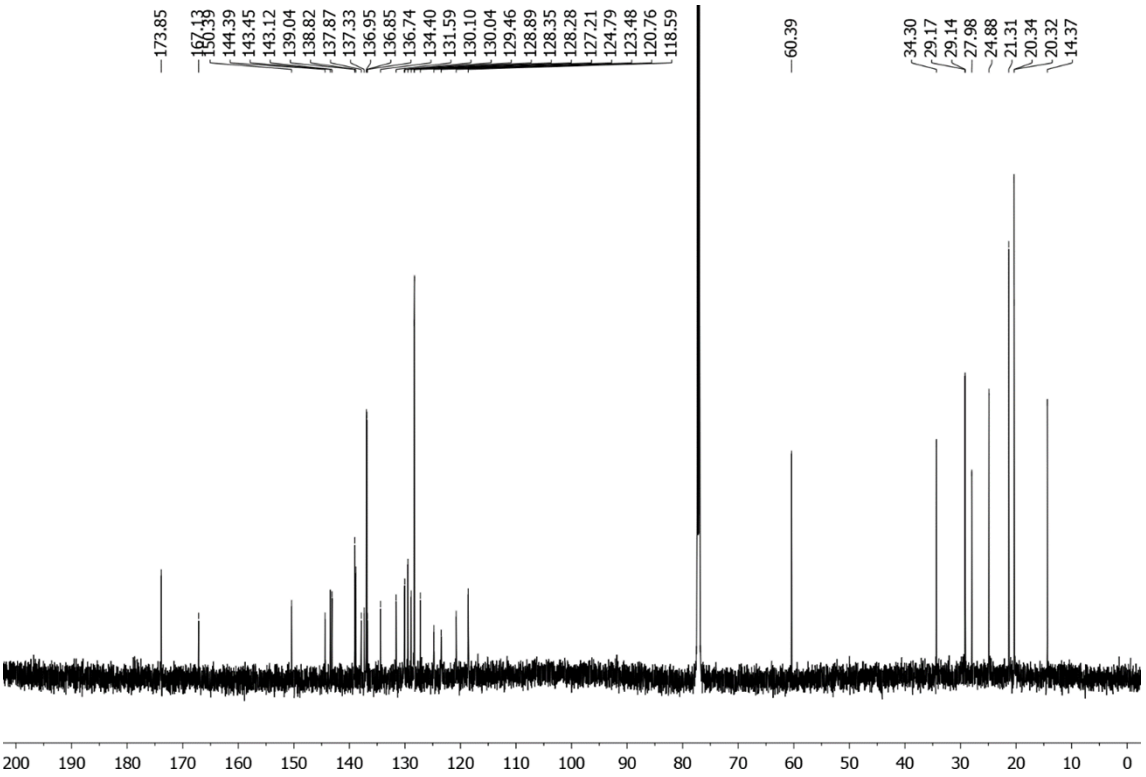

Figure S4. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of **3.**

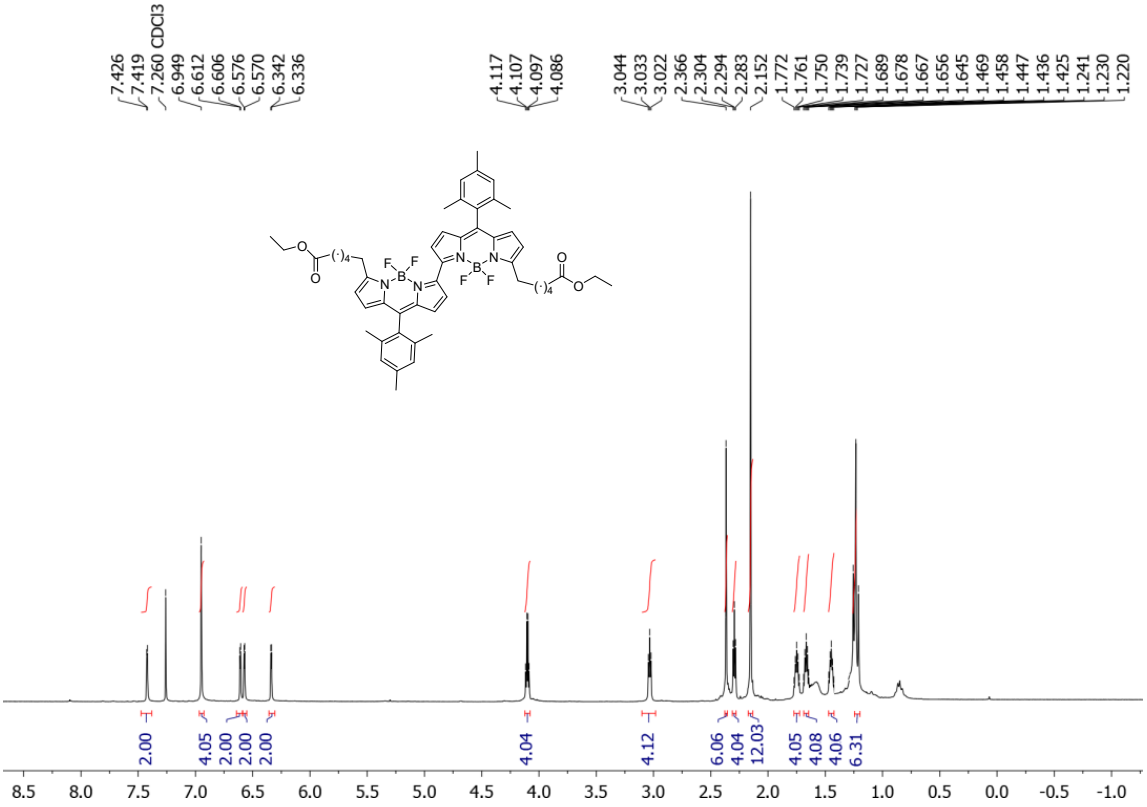

Figure S5. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of **4.**

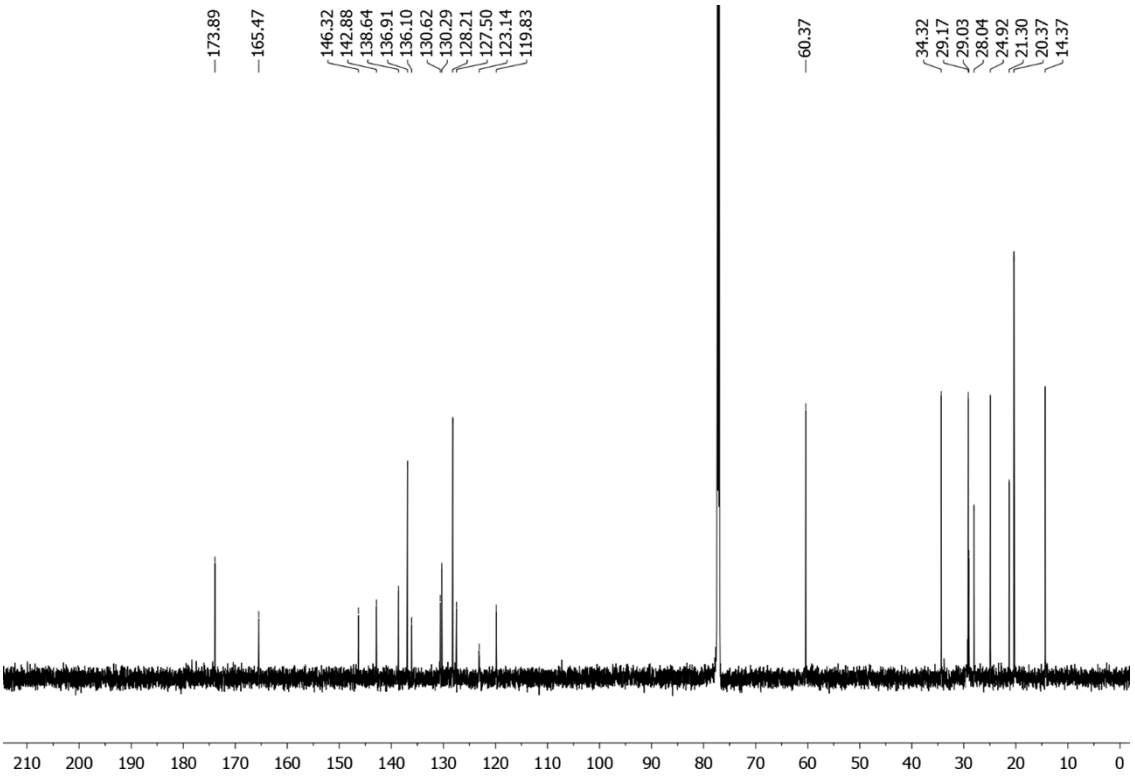

Figure S6. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of **4.**

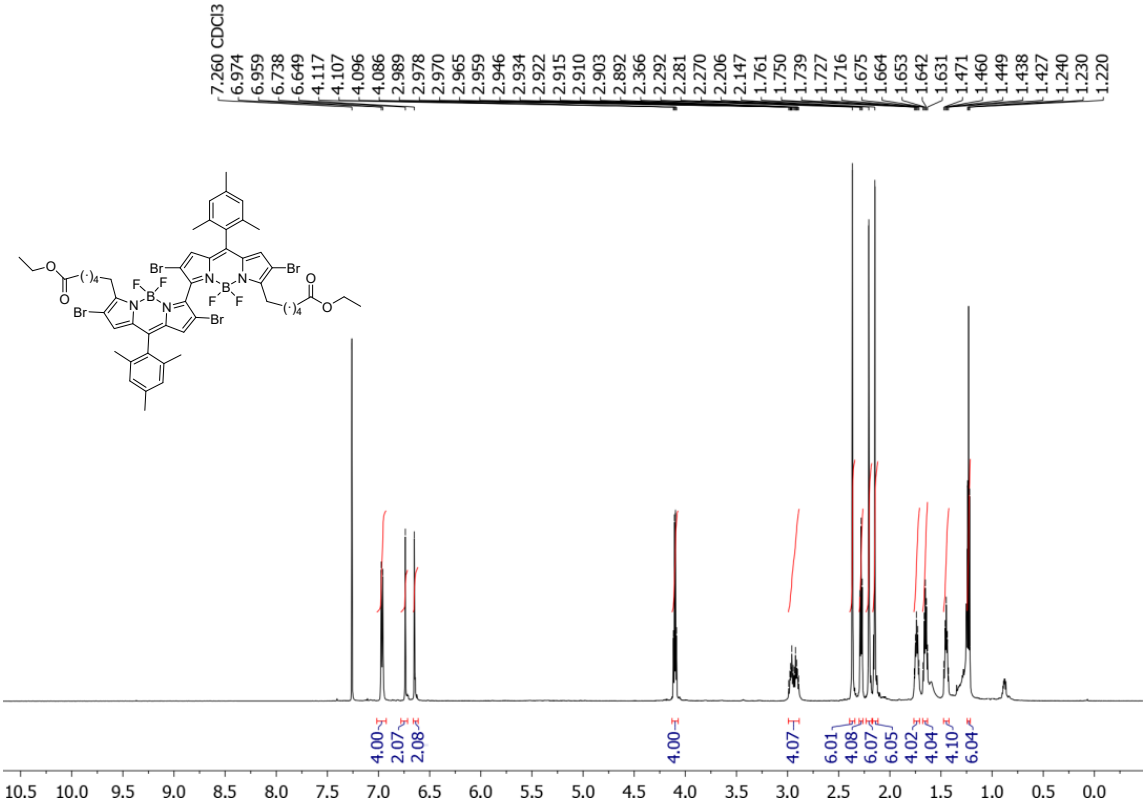

Figure S7. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of **5.**

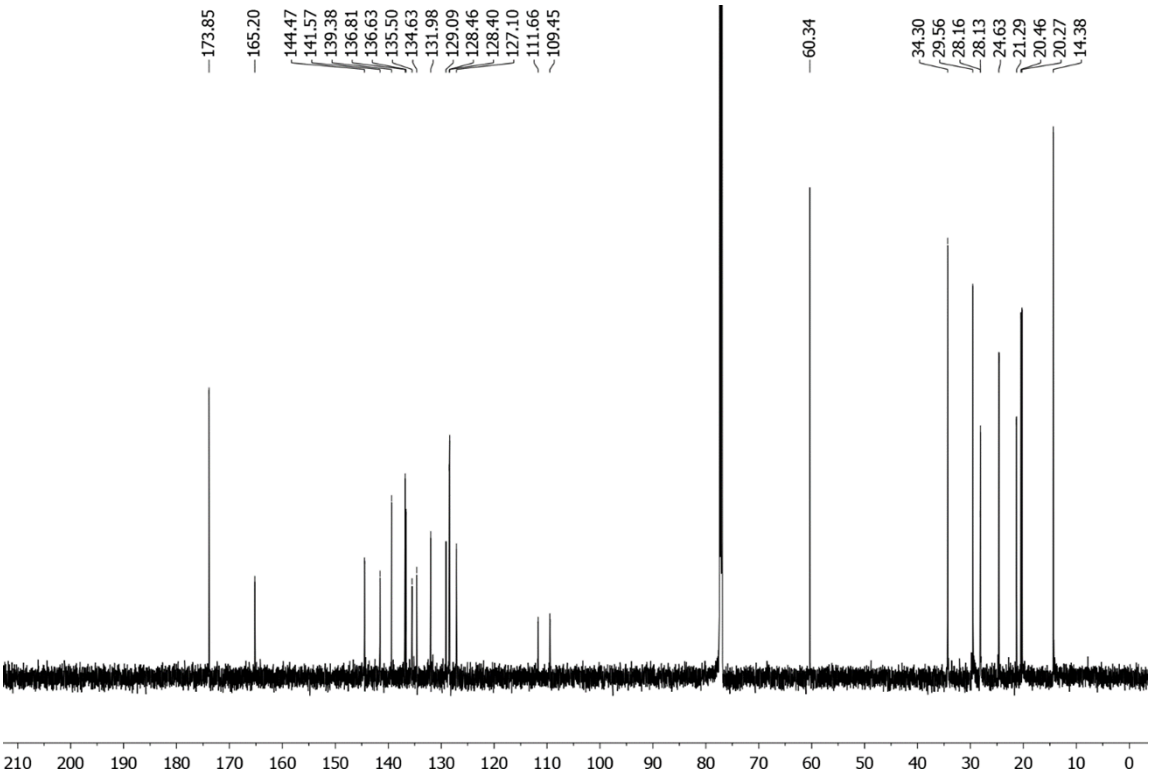

Figure S8. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of **5.**

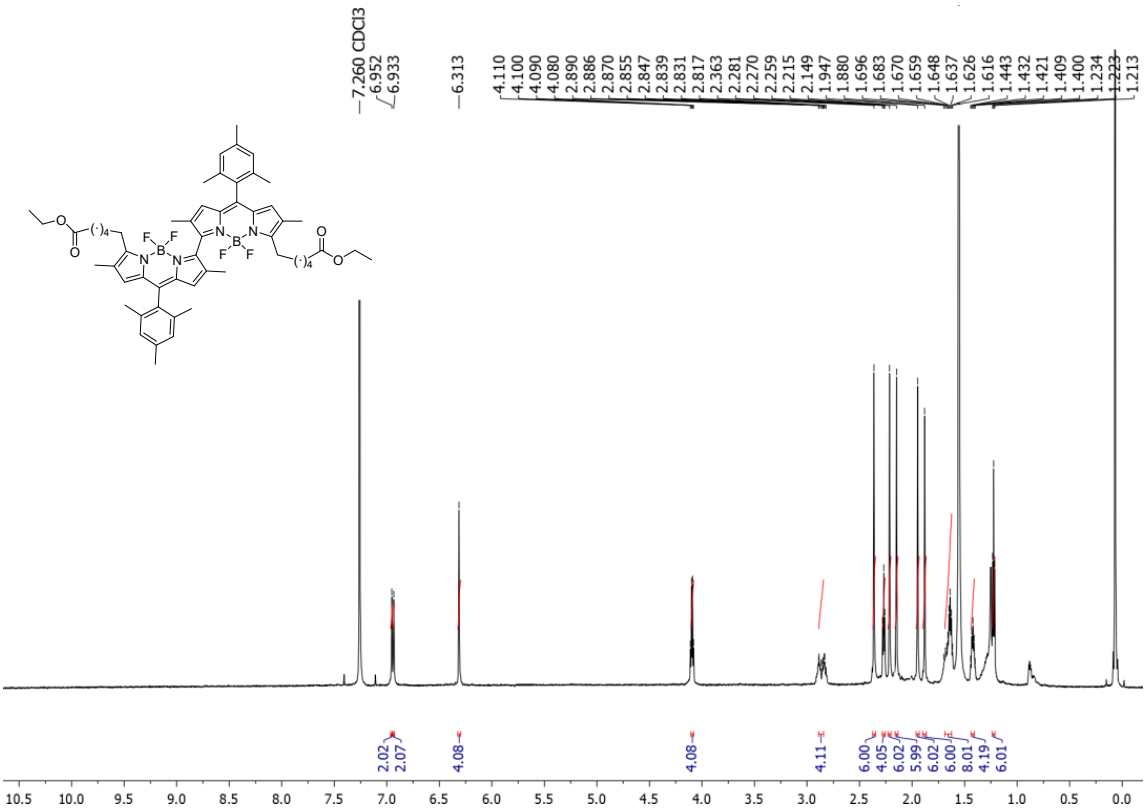

Figure S9. ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of **6.**

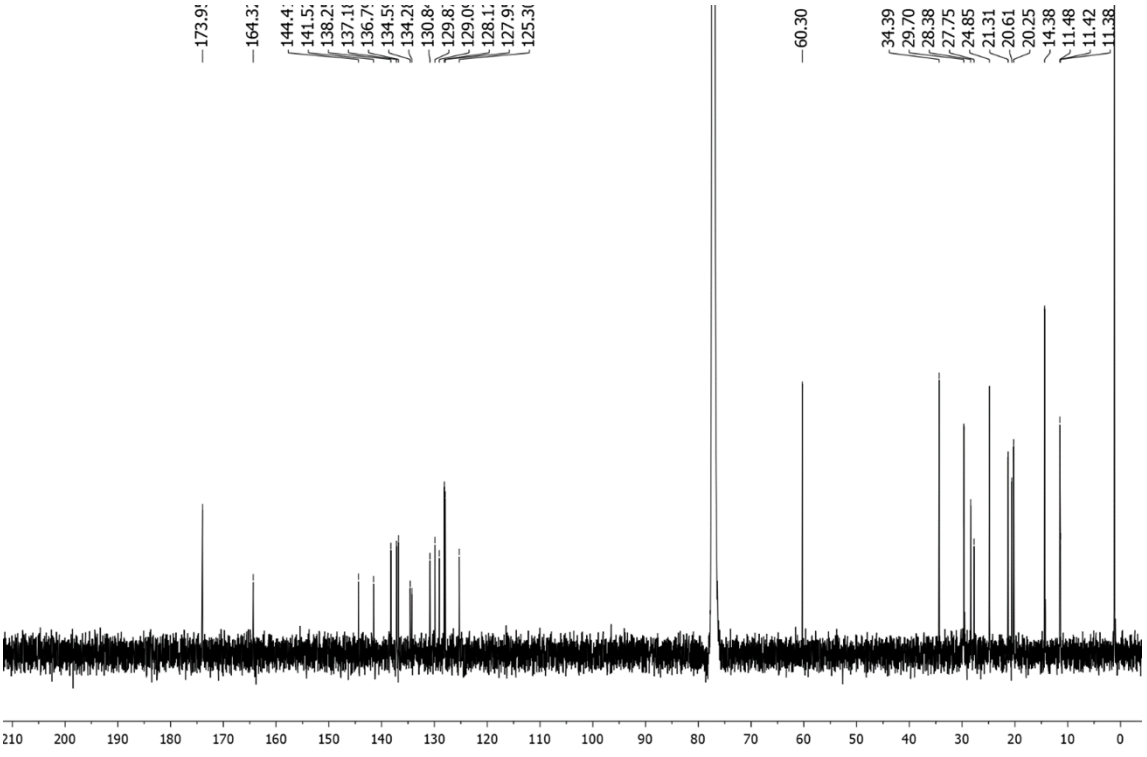

Figure S10. ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of **6.**

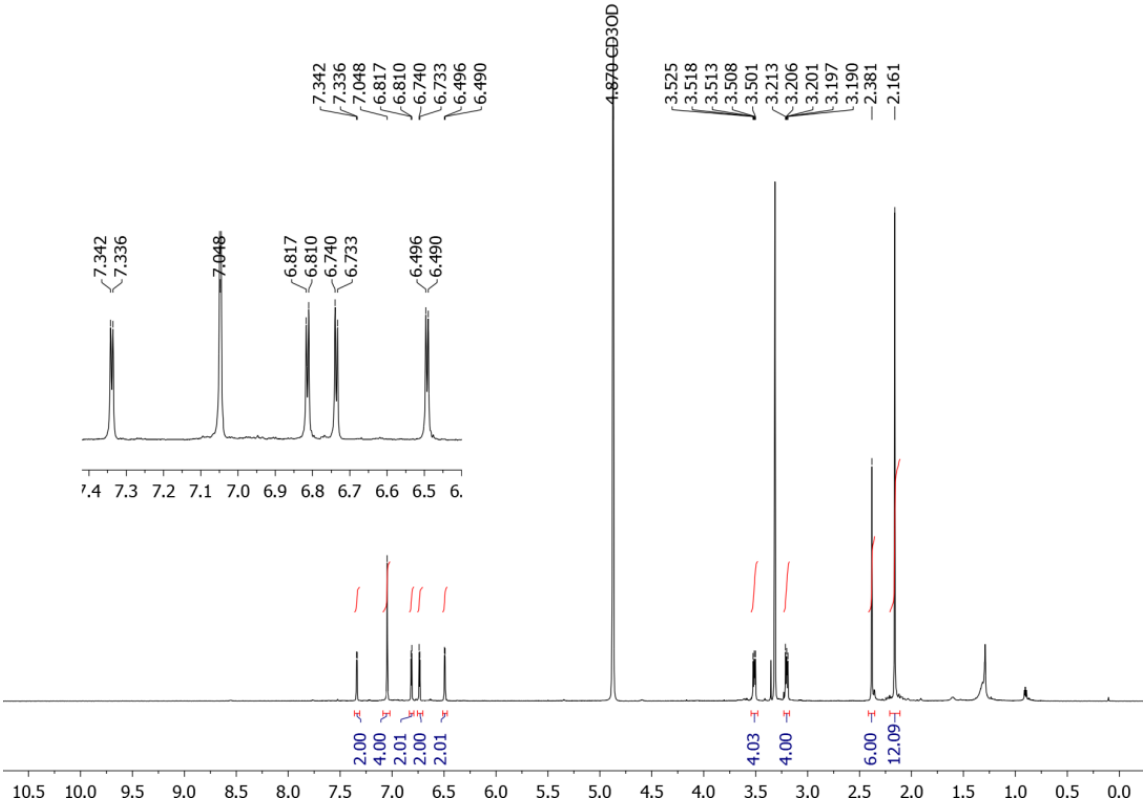

Figure S11. ^{1}H NMR (700 MHz, CD_3OD) spectrum of **7.**

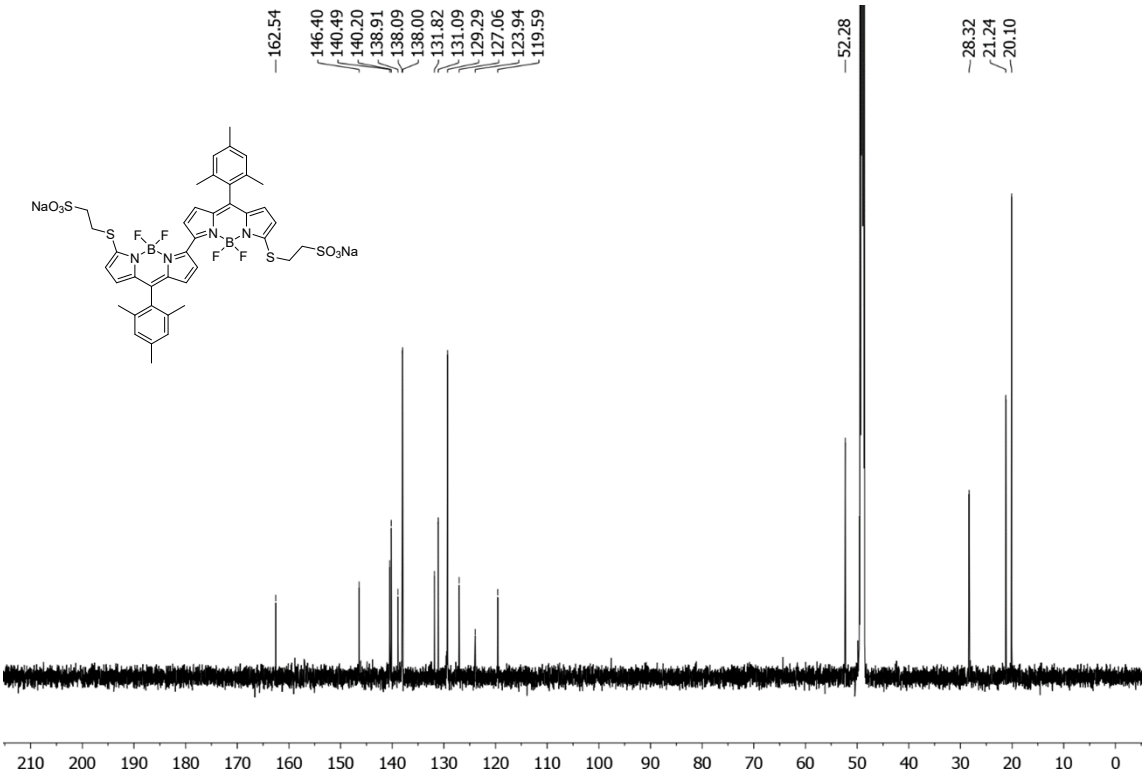

Figure S12. ^{13}C NMR (176 MHz, CD_3OD) spectrum of **7.**

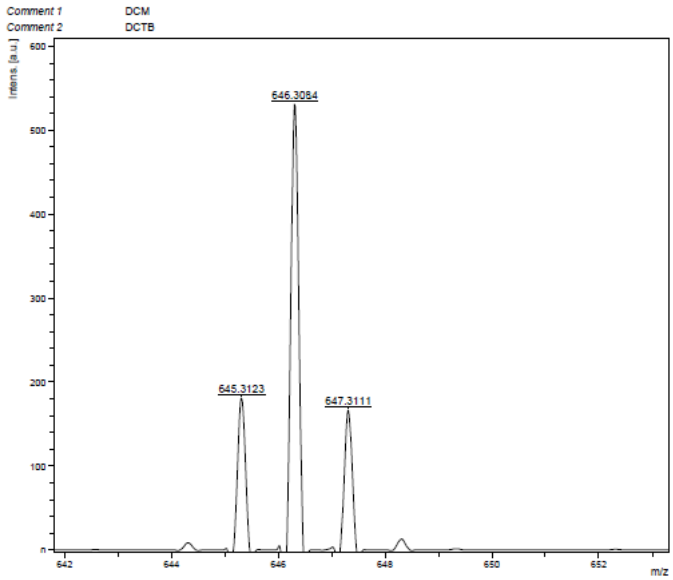

Figure S13. HRMS-MALDI-TOF spectrum of **2.**

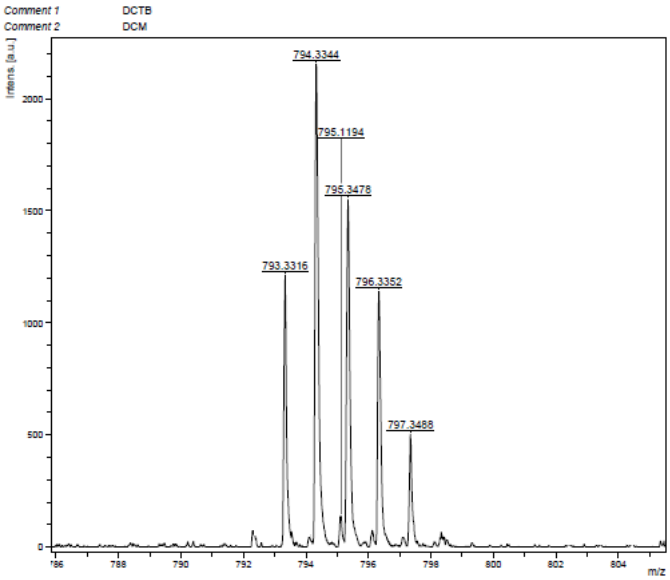

Figure S14. HRMS-MALDI-TOF spectrum of **3.**

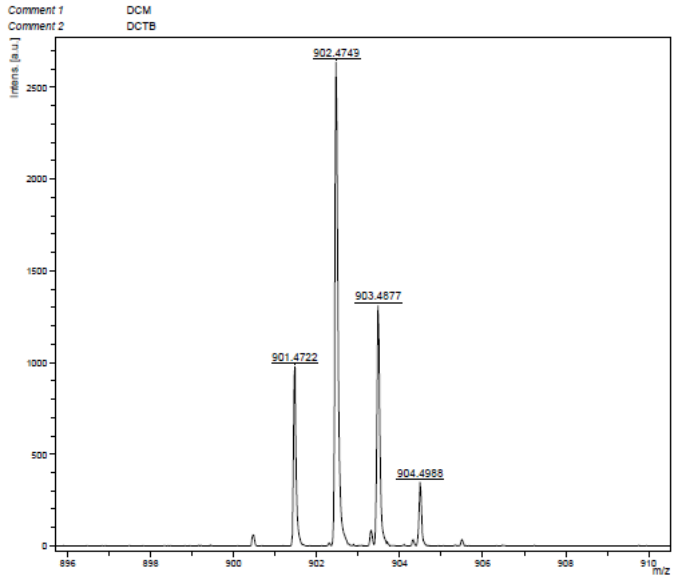

Figure S15. HRMS-MALDI-TOF spectrum of **4.**

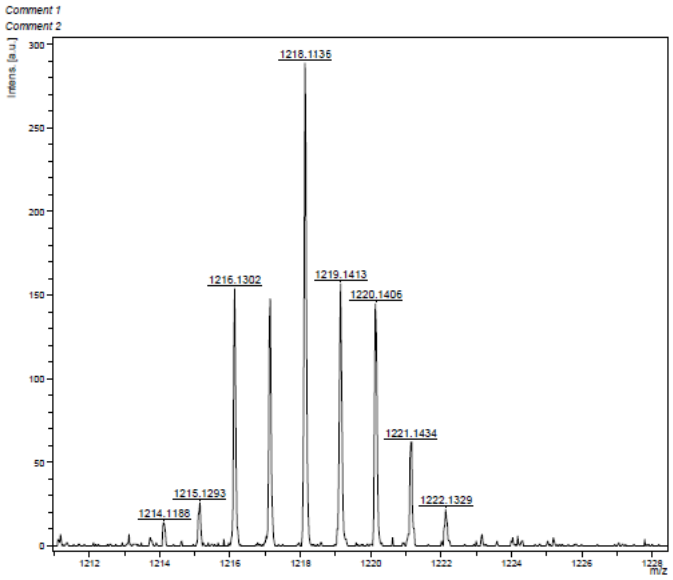

Figure S16. HRMS-MALDI-TOF spectrum of **5.**

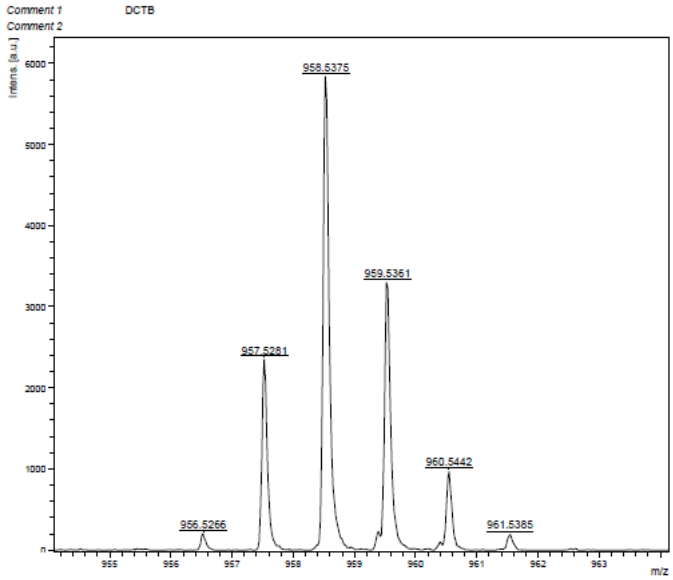

Figure S17. HRMS-MALDI-TOF spectrum of **6**.

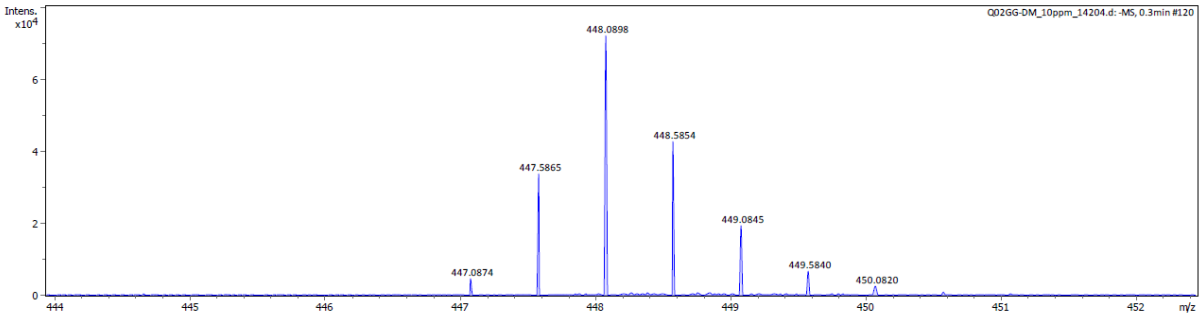

Figure S18. HRMS-ESI NEG. spectrum of **7.**

4. Photophysical properties

Table S1. Photophysical properties of the 3-3´ BODIPY dimers in diluted solutions (2 μM) of solvents of different polarity.

	λ_{ab} (nm)	ε_{max} (10^4 $M^{-1}\cdot cm^{-1}$)	λ_{fl} (nm)	Φ_{fl}	$\Delta\nu_{St}$ (cm^{-1})	τ_{fl} (ns)	k_{fl} ($10^8 s^{-1}$)	k_{nr} ($10^8 s^{-1}$)
1								
Toluene	675.0	6.1	723.0	0.55	985	2.96	1.85	1.52
$CHCl_3$	674.5	5.4	720.5	0.57	945	3.23	1.76	1.33
MeCN	648.0	5.4	701.5	0.51	1175	3.08	1.65	1.59
2								
Toluene	687.5	5.9	731.5	0.52	875	2.75	1.89	1.74
$CHCl_3$	686.0	5.4	731.5	0.51	905	2.91	1.75	1.68
MeCN	664.5	5.2	714.0	0.38	1045	2.26	1.68	2.74
3								
Toluene	684.0	4.0	728.5	0.49	895	2.83	1.73	1.80
$CHCl_3$	685.5	3.8	726.0	0.49	815	3.05	1.61	1.67
MeCN	660.0	3.5	711.5	0.38	1095	2.56	1.48	2.42
4								
Toluene	678.5	5.2	726.5	0.54	975	3.06	1.76	1.50
$CHCl_3$	681.0	5.2	724.5	0.55	880	3.33	1.65	1.35
MeCN	654.5	4.6	707.0	0.52	1135	3.21	1.62	1.49
5								
Toluene	578.0 504.0	9.1 11.3	677.0	0.03	2530 5070	0.19(97%)-0.70(3%)	-	-
$CHCl_3$	575.0 501.5	8.0 10.7	676.5	0.03	2610 5160	0.18(91%)-0.35(9%)	-	-
MeCN	568.0 496.5	7.6 9.6	690.5	0.01	3125 5660	0.13(76%)-0.37(24%)	-	-
6								
Toluene	577.5 497.5	3.8 3.6	675.5	0.57	2510 5295	3.40	1.67	1.26
$CHCl_3$	575.0 497.5	3.5 3.0	676.0	0.61	2600 5310	3.66	1.66	1.06
MeCN	569.5 493.5	3.4 2.8	682.5	0.23	2910 5610	1.67	1.38	4.61
7*								
EtOH	723.0	6.3	773.0	0.14	895	1.33	1.05	6.46
MeCN	710.0	6.5	768.5	0.19	1070	1.59	1.20	5.09

$CHCl_3$: chloroform; EtOH: ethanol; MeCN: acetonitrile

Absorption (λ_{ab}) and fluorescence (λ_{fl}) wavelength, molar absorption at the maximum wavelength (ε_{max}), fluorescence quantum yield (Φ_{fl}) and lifetime (τ_{fl}), Stokes shift ($\Delta\nu_{St}$), and radiative (k_{fl}) and non-radiative (k_{nr}) rate constants

*not soluble neither in toluene nor in chloroform, even at low concentrations

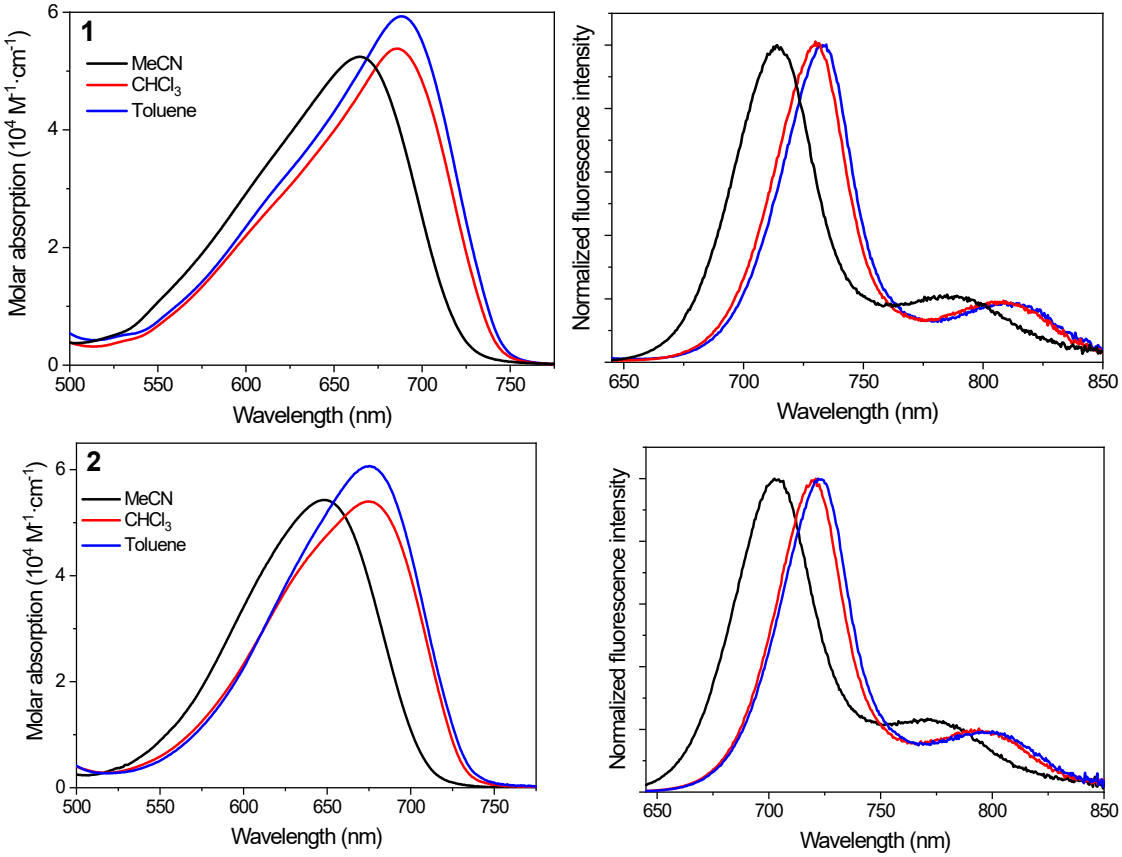

Figure S19. Absorption and normalized fluorescence spectra of the simplest chlorinated precursor 3-3´ dimer **1** and its methylated derivative **2** in diluted solutions (2 μM) of solvents with different polarity.

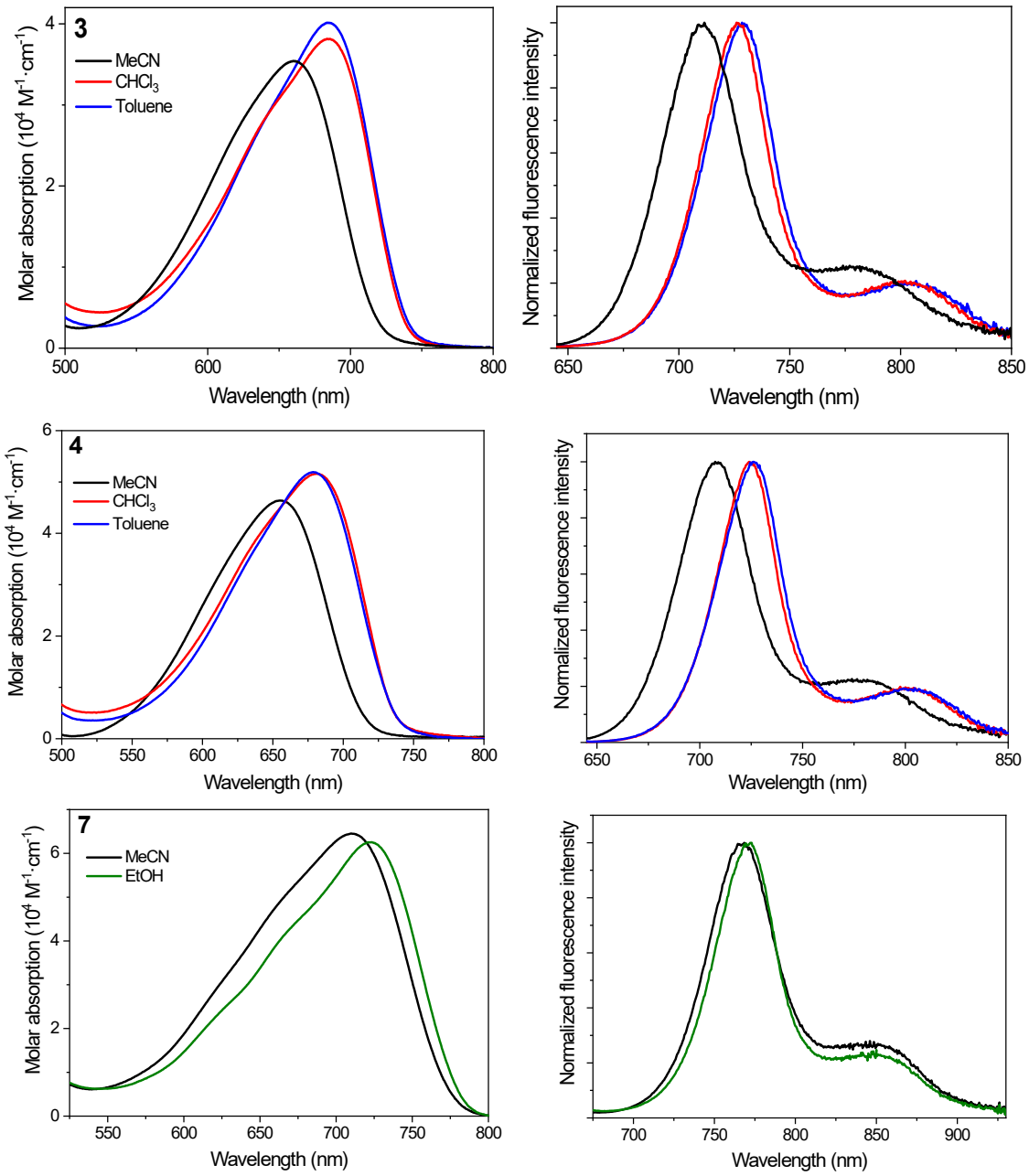

Figure S20. Absorption and normalized fluorescence spectra of 3-3´ dimers functionalized at the opposite position 5 (mono and disusbtituted with polimethylene chains bearing ester end group, **3** and **4**, and sulphurated with anioinic terminal sulfonate **7**) in diluted solutions (2 μM) of solvents with different polarity.

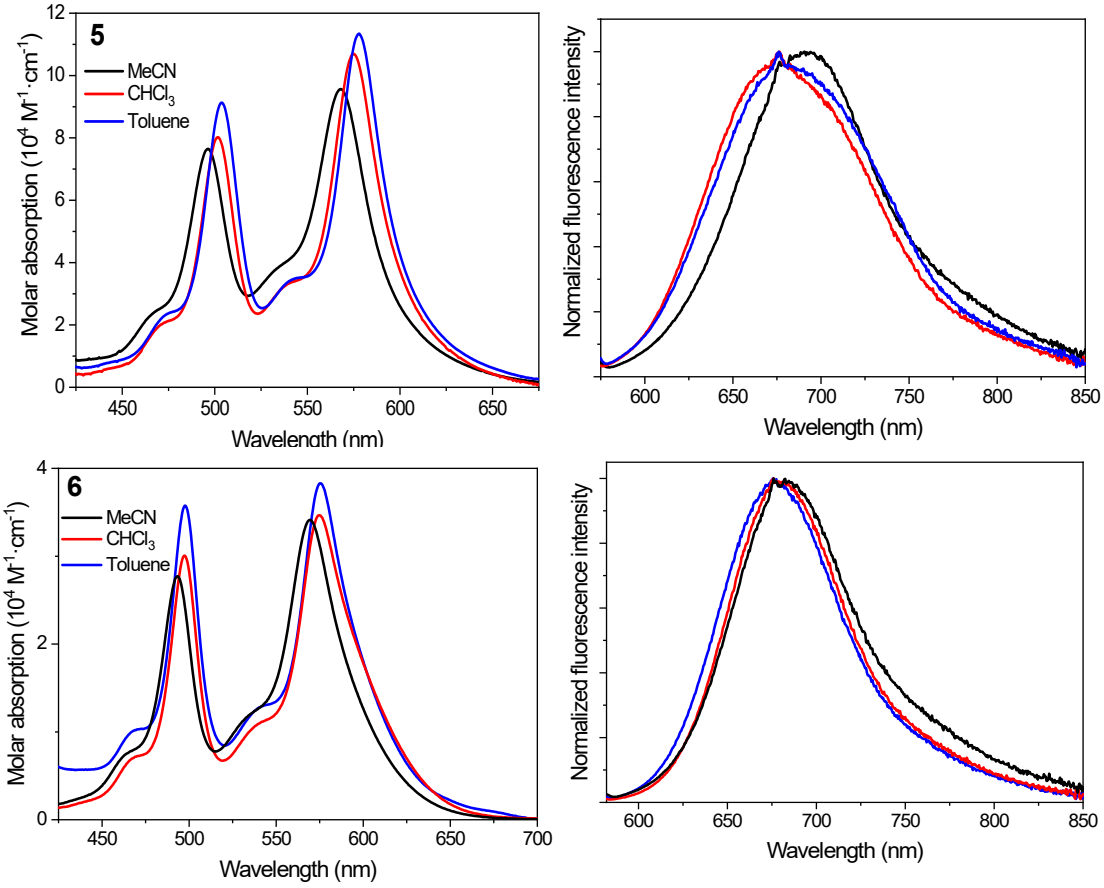

Figure S21. Absorption and normalized fluorescence spectra of the sterically hindered and constrained 3-3′ dimers (2 and 6 positions brominated, **5**, or methylated, **6**) in diluted solutions (2 μM) of solvents with different polarity.

5. Cell viability

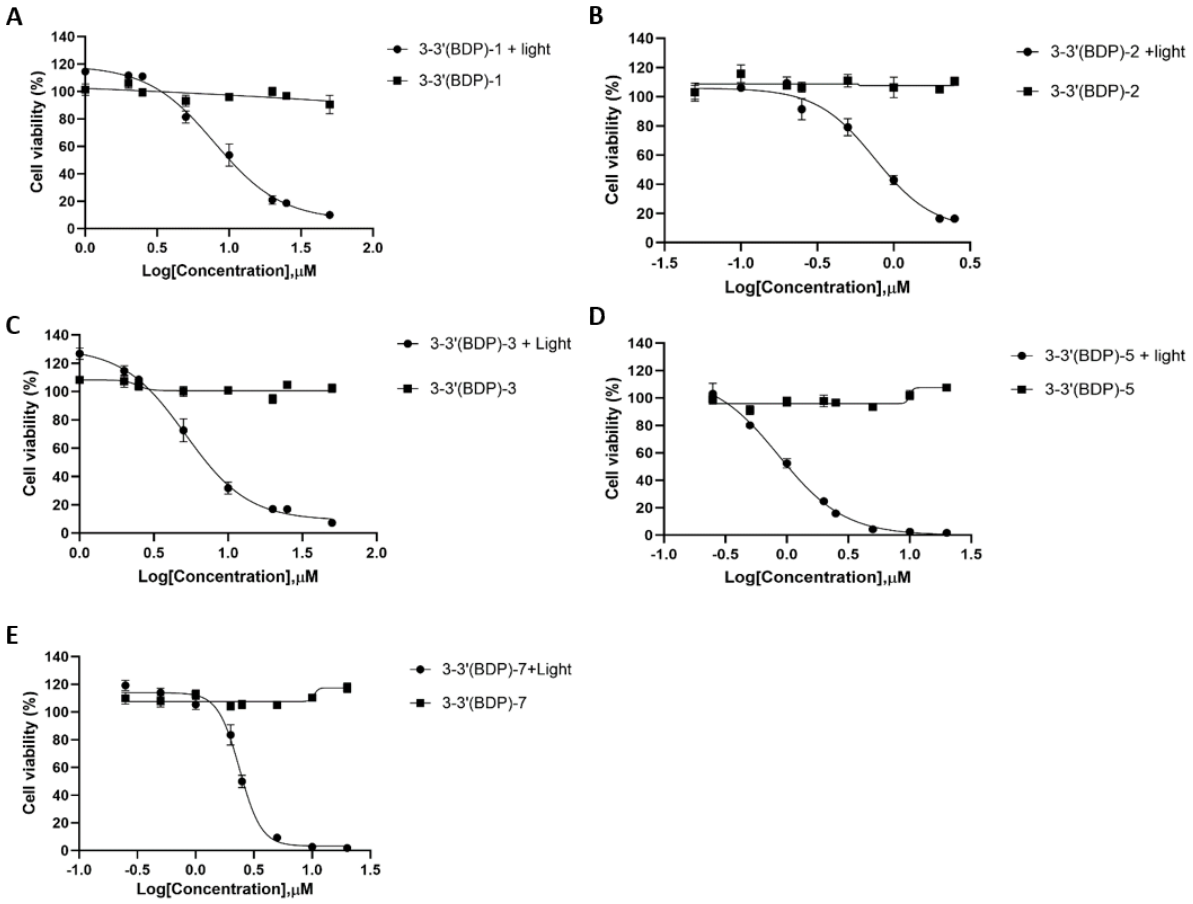

Figure S22. Representation of the sigmoidal fitting of concentration-cell viability curves of the photocytotoxic assay in SK-Mel-103 cancer cells of 3-3'(BDP)-**1 (A)**, 3-3'(BDP)-**2 (B)**, 3-3'(BDP)-**3 (C)**, 3-3'(BDP)-**5 (D)** and 3-3'(BDP)-**7 (E)**. Cells were treated with increasing concentrations of each of the compounds within the range between 0.05 mM and 50 mM for 24 h before irradiation with visible light (λ > 475nm) for 30 min. Cell viability was determined 24 h after the irradiation step and with the WST method. Values are expressed as mean ± SEM.

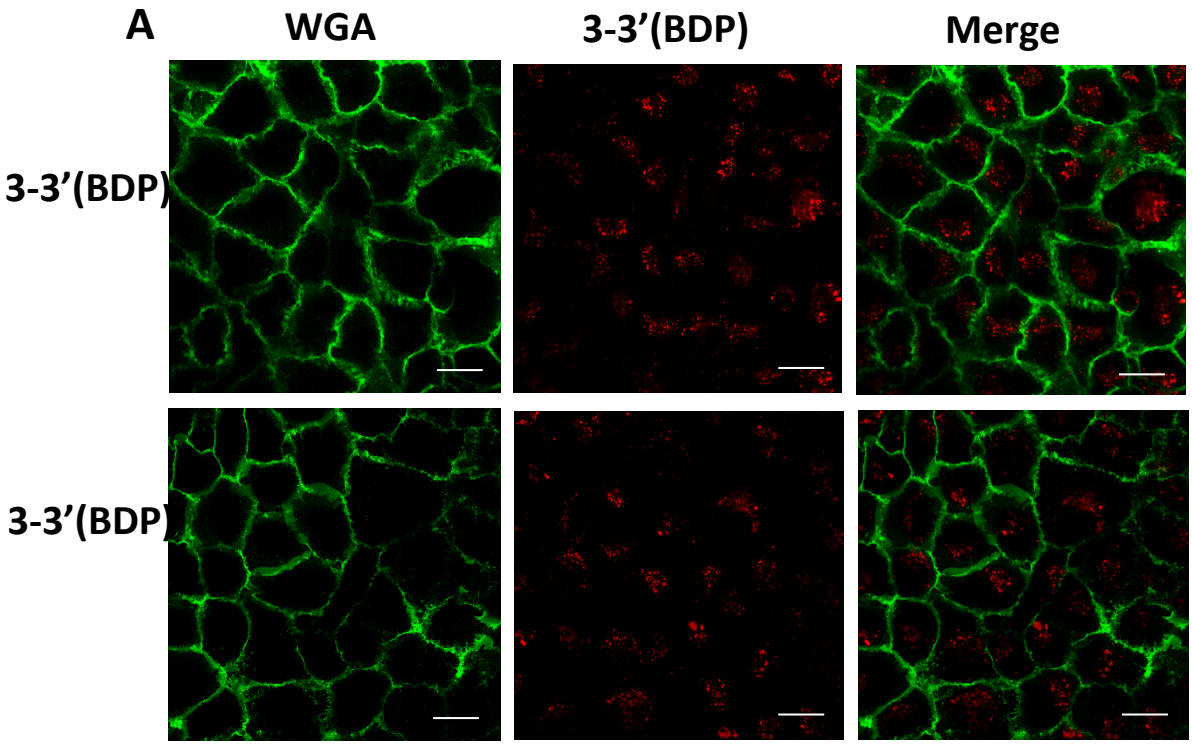

Figure S23. Subcellular co-localization of 3-3'(BDP)-**4** and 3-3'(BDP)-**7** in SK-Mel-103 following 24 h incubation with 10 μM (red) and stained with the green tracker WGA (plasma membrane). Images were adquired using the confocal microscope Leica TCS SP 8 HyVolution II. 3-3'(BDP)-**4** and 3-3'(BDP)-**7** were excited at 562 nm and the emission was collected at 640-780 nm. Green tracker WGA was excited at 488 and the emission was collected at 490-530 nm. Scale bar 20 μm.

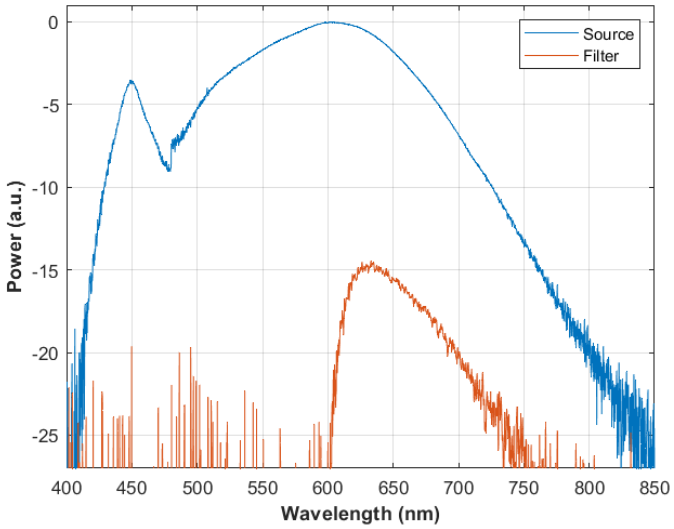

Figure S24. Emission spectrum of the used LED light source without filter (blue curve) and with applied filter (red curve). The spectra were obtained using an AQ6373E Visible Wavelength Optical Spectrum Analyzer (350 nm–1200 nm) coupled with a Liquid Light Guide (Ø3 mm core, 6' [1.8 m] length).