Supplemental Information for

BODIPY Fluorophores for Membrane Potential Imaging

Authors

Jenna M. Franke,[‡] Benjamin K. Raliski,[‡] Steven C. Boggess,[‡] Divya V. Natesan,[‡] Evan T. Koretsky,[‡] Patrick Zhang,[‡] Rishikesh U. Kulkarni,[‡] Parker E. Deal,[‡] and Evan W. Miller[‡§†]*

Departments of [‡]Chemistry and [§]Molecular & Cell Biology and [†]Helen Wills Neuroscience Institute. University of California, Berkeley, California 94720, United States.

J. Am. Chem. Soc. **2019**, *141*; DOI: 10.1021/jacs.9b05912

Table of Contents

Methods

Chemical synthesis and characterization

Chemical reagents and anhydrous solvents were purchased from commercial suppliers and used without further purification. Compounds **1**, **4**, **5**, **9**, **14**, **20**, and **23** were prepared according to literature procedures.[1–5] 2,4-dimethylpyrrole-3-carboxylic acid was purchased from CombiBlocks. All reactions were carried out in flame-dried flasks sealed with septa and conducted under an inert nitrogen atmosphere. Thin layer chromatography (TLC, Silicycle, F254, 250 μm) and preparative thin layer chromatography (pTLC, Silicycle, F254, 1000 μm) were performed on glass-backed plates pre-coated with silica gel and were visualized by fluorescence quenching under UV light. Flash column chromatography was performed on Silicycle Silica Flash F60 (230-400 Mesh) using a forced flow of air at 0.5–1.0 bar.

NMR spectra were measured on a Bruker AVQ-400, AVB-400, AV-500, AV-600, or AV-700 MHz instrument, indicated for each compound. CoC-NMR is supported in part by NIH S10-OD024998. NMR spectra measured on Bruker AVII-900 MHz, 225 MHz, equipped with a TCI cryoprobe accessory, were performed by Dr. Jeffrey Pelton (QB3). Funds for the QB3 900 MHz NMR spectrometer were provided by the NIH through grant GM68933. Chemical shifts are expressed in parts per million (ppm) and are referenced to either d_6-DMSO, 2.5 ppm, $CDCl_3$, 7.26 ppm, acetone-d_6, 2.05 ppm, or MeOD, 3.31 ppm. Coupling constants are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; sep, septet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dt, doublet of triplets; td; triplet of doublets; m, multiplet.

High-resolution mass spectra (HR-ESI-MS) were obtained by Dr. Rita Nichiporuk (QB3 Mass Spectrometry Facility at University of California, Berkeley). High performance liquid chromatography (HPLC) and low resolution ESI Mass Spectrometry were performed on an Agilent Infinity 1200 analytical instrument coupled to an Advion CMS-L ESI mass spectrometer. The column used for the analytical HPLC was Phenomenex Luna 5 μm C18(2) (4.6 mm I.D. × 75 mm) with a flow rate of 1.0 mL/min. The mobile phases were MQ-H_2O with 0.05% trifluoroacetic acid (eluent A) and HPLC grade acetonitrile with 0.05% trifluoroacetic acid (eluent B). Signals were monitored at 254, 400, and 500 nm over 13 min with a gradient of 10-100% eluent B, unless otherwise noted. Ultra-high performance liquid chromatography (UHPLC) for purification of final compounds was performed using a Waters Acquity Autopurificaiton system equipped with a Waters XBridge BEH 5 μm C18 column (19 mm I.D. x 250 mm) with a flow rate of 30.0 mL/min, made available by the Catalysis Facility of Lawrence Berkeley National Laboratory (Berkeley, CA). The mobile phases were MQ-H_2O with 0.05% formic acid (eluent A) and HPLC grade acetonitrile with 0.05% formic acid (eluent B). Signals were monitored at 400 and 500 nm over 20 min with a gradient of 10-100% eluent B, unless otherwise noted.

Spectroscopic studies

Stock solutions of BODIPY fluorophores and VoltageFluors were prepared in DMSO (250 μM–2 mM) and diluted with PBS (10 mM KH_2PO_4, 30 mM $Na_2HPO_4 \cdot 7H_2O$, 1.55 M NaCl, pH 7.4) or filtered absolute ethanol. For pH titration studies, 2 mM DMSO stocks of BODIPY VoltageFluors were diluted (1:2000 dilution) in buffers containing 40 mM NaCl, 10 mM buffer, and 0.1% (w/v) SDS. The following buffer systems were used: phosphate (pH 2.5, 7.5); carbonate (pH 10). UV-Vis absorbance and fluorescence spectra were recorded using a Shimadzu 2501 Spectrophotometer and a Quantamaster 4L-format scanning spectrofluorimeter (Photon Technologies International). The fluorimeter is equipped with an LPS-220B 75-W xenon lamp and power supply, a 1010B lamp housing with integrated igniter, switchable 814 photon-counting/analog photomultiplier detection unit, and MD5020 motor driver. Samples were measured in 1-cm path length quartz cuvettes (Starna Cells).

Relative quantum yields (Φ_{Fl}) were calculated by comparison to fluorescein (Φ_{Fl} = 0.93 in 0.1 M NaOH) and rhodamine 123 (Φ_{Fl} = 0.90 in ethanol) as references.[6,7] Briefly, stock solutions of standards were prepared in DMSO (0.25-1.25 mM) and diluted with appropriate solvent (1:1000 dilution). Absorption and emission (excitation = 470 nm) were taken at 5 concentrations. The absorption value at the excitation wavelength (470 nM) was plotted against the integration of the area of fluorescence curve (475-700 nm). The slope of the linear best fit of the data was used to calculate the relative Φ_{Fl} by the equation $\Phi_{Fl(X)} = \Phi_{Fl(R)}(S_R/S_X)(\eta_X/\eta_R)^2$, where S_R and S_X are the slopes of the reference compound and unknown, respectively, and η is the refractive index of the solution. This method was validated by cross-referencing the reported Φ_{Fl} values of fluorescein and rhodamine 123 to the calculated Φ_{Fl} using the one standard as a reference for the other and vice versa. Calculated Φ_{Fl} within 10% of the reported value for both standards ensured that Φ_{Fl} calculated for BODIPY fluorophores and VoltageFluors was reliable within 10% error. To determine extinction coefficients (ε), BODIPY fluorophore stock solutions were prepared in DMSO (0.25-1.25 mM) and diluted with appropriate solvent (1:1000 dilution). For each fluorophore, 3 independent measurements were made of the absorbance value at the wavelength of maximum absorbance. Extinction coefficients (ε) were then calculated according to Beer's law ($A = \varepsilon cl$) were c is the known concentration of fluorophore, and l is the path length. For all extinction coefficient measurements, cuvettes with a path length of 1 cm were used.

Cell culture

All animal procedures were approved by the UC Berkeley Animal Care and Use Committees and conformed to the NIH Guide for the Care and Use and Laboratory Animals and the Public Health Policy.

Human embryonic kidney (HEK) 293T cells were acquired from the UC Berkeley Cell Culture Facility. Cells were passaged and plated onto 12 mm glass coverslips coated with Poly-D-Lysine (PDL; 1 mg/mL; Sigma-Aldrich) to a confluency of ~15% and 50% for electrophysiology and imaging, respectively. HEK293T cells were plated and maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 4.5 g/L D-glucose, 10% fetal bovine serum (FBS), and 1% Glutamax.

Hippocampi were dissected from embryonic day 18 Sprague Dawley rats (Charles River Laboratory) in cold sterile HBSS (zero Ca^{2+}, zero Mg^{2+}). All dissection products were supplied by Invitrogen, unless otherwise stated. Hippocampal tissue was treated with trypsin (2.5%) for 15 min at 37 °C. The tissue was triturated using fire polished Pasteur pipettes, in minimum essential media (MEM) supplemented with 5% fetal bovine serum (FBS; Thermo Scientific), 2% B-27, 2% 1M D-glucose (Fisher Scientific) and 1% glutamax. The dissociated cells were plated onto 12 mm diameter coverslips (Fisher Scientific) pre-treated with PDL (as above) at a density of 30-40,000 cells per coverslip in MEM supplemented media (as above). Neurons were maintained at 37 °C in a humidified incubator with 5% CO_2. At 1 day in vitro (DIV) half of the MEM supplemented media was removed and replaced with Neurobasal media containing 2% B-27 supplement and 1% glutamax. Evoked activity experiments were performed on 12-15 DIV neurons. Unless stated otherwise, for loading of HEK cells, BODIPY VoltageFluors were diluted in DMSO to 1 mM, and then diluted 1:1000 in HBSS and imaging experiments were performed in HBSS. For loading of hippocampal neurons, BODIPY VoltageFluors were diluted in DMSO to 500 µM, then diluted 1:1000 in HBSS and imaging experiments were performed in HBSS.

Differentiation of hiPSC into cardiomyocytes and culture: WTC11 hiPSCs were cultured on Matrigel (1:100 dilution; Corning)-coated 6 well-plates in StemFlex medium (Gibco). When the cell confluency reached 80–90%, which is referred as day 0, the medium was switched to RPMI 1640 medium (Life Technologies) containing B27 minus insulin supplement (Life Technologies) and 8 µM CHIR99021 GSK3 inhibitor (Peprotech). At day 1, the medium was changed to RPMI 1640 medium containing B27 minus insulin supplement only. At day 3, medium was replaced to RPMI 1640 medium containing B27

supplement without insulin, and 5 µM IWP4 (Peprotech) for 2 days without medium change. On day 5, medium was replaced to RPMI 1640 medium containing B27 minus insulin supplement for 2 days without medium change. On day 7, medium was replaced toRPMI 1640 containing B27 with insulin supplement. After day 7, the medium was changed every other day. Confluent contracting sheets of beating cells appear between days 7 to 15 and are ready for dissociation after this time. Confluent sheets were dissociated with 0.25% trypsin-EDTA (8-30 minutes, depending on density and quality of tissue) and plated onto Matrigel (1:100)-coated Ibidi ® 24 well µ-plates (cat no. 82406) in RPMI 1640 medium containing B27 supplement and 10 µM Y27632. The following day, medium was exchanged for RPMI 1640 plus B27. Medium was changed every 2 days until imaging. For loading hiPSC cardiomyocytes, VoltageFluor dyes (BODIPY, VF2.1.Cl, or fVF 2) were diluted in DMSO to 500 µM, and then diluted 1:1000 in RPMI 1640 with B27 supplement minus Phenol Red. Imaging experiments were performed in RPMI 1640 with B27 supplement minus Phenol Red.

Epifluorescence microscopy

For HEK293T cells, epifluorescence imaging was performed on an AxioExaminer Z-1 (Zeiss) equipped with a Spectra-X Light engine LED light (Lumencor), controlled with Slidebook (v6, Intelligent Imaging Innovations). Images were acquired with either a W-Plan-Apo 20x/1.0 water objective (Zeiss). Images were focused onto either an OrcaFlash4.0 sCMOS camera (sCMOS; Hamamatsu) or an eVolve 128 EMCCD camera (EMCCD; photometrix). For rat hippocampal neurons, µManager (V1.4, open-source, Open Imaging) was used to control the microscope.[8] For BODIPY-VF images, the excitation light was delivered from a LED at 510/25 nm and emission was collected with a triple emission filter (473/22, 543/19, 648/98 nm) after passing through a triple dichroic mirror (475/30, 540/25, 642/96 nm).

Membrane staining and photostability in HEK293T cells

HEK293T cells were incubated with a HBSS solution (Gibco) containing BODIPY VoltageFluors (1 µM) at 37°C for 20 min prior to transfer to fresh HBSS (no dye) for imaging. Microscopic images were acquired with a W-Plan-Apo 20x/1.0 water objective (Zeiss) and OrcaFlash4.0 sCMOS camera (Hamamatsu). For image intensity measurements, regions of interest were drawn around cells and the mean fluorescence was calculated in ImageJ (FIJI, NIH).[9] Background fluorescence was subtracted by measuring the fluorescence from regions of interest containing no cells.

For photostability experiments, HEK293T cells were incubated separately with VF2.1.Cl (1 µM), fVF 2 (1 µM), Et*m*H (1 µM), TM*m*OMe (1 µM), carboxy*m*OMe (1 µM), or amide*m*H (1 µM) in HBSS at 37°C for 20 min. Data were acquired with a W-Plan-Apo 20x/1.0 water objective (Zeiss) and OrcaFlash4.0 sCMOS camera (Hamamatsu). Images were taken every 5 seconds for 6 minutes with constant illumination of teal LED (2.48 mW/mm^2; 25 ms exposure time). The obtained fluorescence curves (background subtracted) were normalized with the fluorescence intensity at t = 0 and averaged (three rafts of cells of each dye).

Voltage sensitivity in HEK293T cells and Neurons

Analysis of voltage sensitivity in HEK cells was performed using ImageJ (FIJI).[9] Briefly, a region of interest (ROI) was selected manually based on fluorescence intensity and applied as a mask to all image frames. Fluorescence intensity values were calculated at known baseline and voltage step epochs. For analysis of voltage responses in neurons, regions of interest encompassing cell bodies (all of approximately the same size) were drawn in ImageJ and the mean fluorescence intensity for each frame extracted. ΔF/F values were calculated by first subtracting a mean background value from all raw fluorescence frames, to give a background subtracted trace (bkgsub). A baseline fluorescence value (Fbase) is calculated from the median of all the frames and subtracted from each timepoint of the bkgsub trace to yield a ΔF trace. The ΔF was then divided by Fbase to give ΔF/F traces. No averaging has been applied to any voltage traces.

Voltage imaging in cardiomyocytes

Functional recordings using VoltageFluor indicators (BODIPY, fVF 2, and VF2.1.Cl) were performed on an inverted epifluoresence microscope (AxioObserver Z-1; Zeiss) equipped with a Spectra-X light engine LED light (Lumencore) and controlled with µManager software (V1.4, open source, Open Imaging). Image series were acquired using a Plan-Apochromat 20/0.8 air objective (20X, Zeiss) focused onto an OrcaFlash4.0 sCMOS camerea (sCMOS, Hamamatsu). Sampling rate of 200 Hz (4x4 binning and restricted to 512x125 pixel frame for high-speed acquisition) was used. Analysis of action potential data was performed using in-house MATLAB scripts used previously (Boggess et al., *ACS Chem Biol.* **2019**, *14*, 390-6), which are available upon request.

Electrophysiology

For electrophysiological experiments, pipettes were pulled from borosilicate glass (Sutter Instruments, BF150-86-10), with a resistance of 5–8 MΩ, and were filled with an internal solution; (in mM) 125 potassium gluconate, 1 EGTA, 10 HEPES, 5 NaCl, 10 KCl, 2 ATP disodium salt, 0.3 GTP trisodium salt (pH 7.25, 275 mOsm).

Recordings were obtained with an Axopatch 200B amplifier (Molecular Devices) at room temperature. The signals were digitized with Digidata 1332A, sampled at 50 kHz and recorded with pCLAMP 10 software (Molecular Devices) on a PC. Fast capacitance was compensated in the on-cell configuration. For all electrophysiology experiments, recordings were only pursued if series resistance in voltage clamp was less than 30 MΩ. For whole-cell, voltage clamp recordings in HEK 293T cells, cells were held at -60 mV and 100 ms hyper- and de-polarizing steps were applied from -100 to +100 mV in 20 mV increments.

Functional Imaging in Neurons

Extracellular field stimulation was delivered by a SD9 Grass Stimulator connected to a recording chamber containing two platinum electrodes (Warner), with triggering provided through the same Digidata 1332A digitizer and pCLAMP 9 software (Molecular Devices) that ran the electrophysiology. Action potentials were triggered by 1 ms 60 V field potentials delivered at 5 Hz. To prevent recurrent activity, the HBBS bath solution was supplemented with synaptic blockers; 10 µM 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; Santa Cruz Biotechnology) and 25 µM D(-)-2-Amino-5-phosphonopentanoic acid (D(-)-APV; Sigma-Aldrich). Functional Imaging of BODIPY-VFs was performed using the OrcaFlash4.0 sCMOS camera (sCMOS; Hamamatsu) and W-Plan-Apo 20x/1.0 water objective (Zeiss). Images were binned 4x4 to allow sampling rates of 0.5 kHz and 2500 frames (5 s) were acquired for each recording.

Computation

Gaussian 16 software[1] was used to draw BODIPY fluorophore structures. Bromines on the *meso*-aromatic ring were omitted because they are not present in the final VoltageFluor structure. Sodium counter-cations were added to the 1,3,5,7-tetramethyl-2,6-carboxylate BODIPY structure. All BODIPYs were assigned a –1 charge, then geometry optimizations, frequency calculations, and HOMO/LUMO orbital calculations were performed in Gaussian in an inert, gas-phase environment using WB97XD functional[2] and def2svp basis set.[3] Visualizations of BODIPY HOMOs (**Figure S12**) were generated with GaussView 6.

Supplementary Figures.

Figure S1. Reduction of molecular wire under Pd/C & H_2 benzyl removal conditions

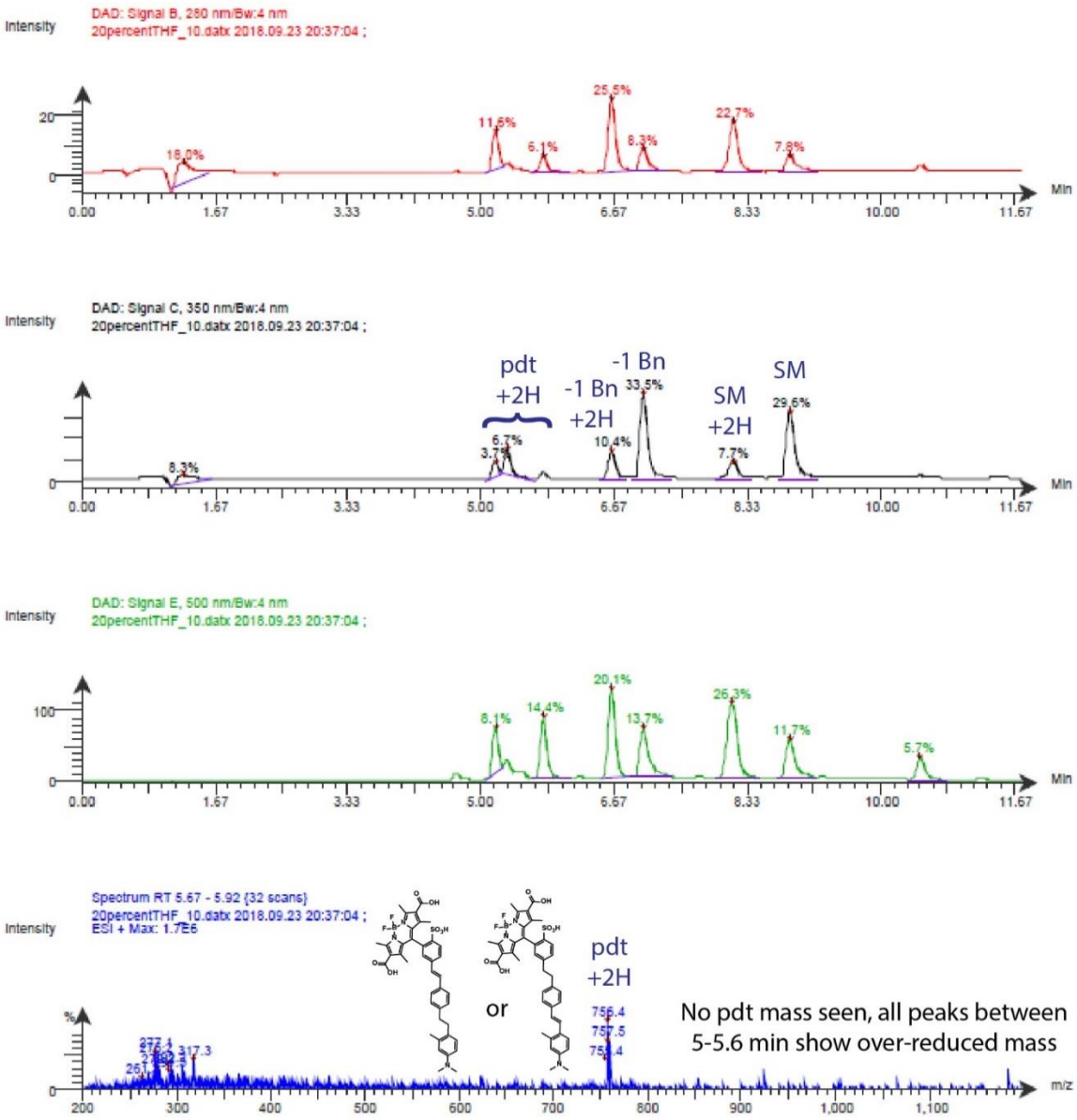


Figure S1. LC-MS of treating OBn*m*Me **26** with 20 mol% Pd/C under hydrogen atmosphere. While one benzyl group is successfully cleaved with only partial over-reduction of the molecular wire, no doubly deprotected product was obtained. All peaks between 5-6 min displayed 756 m/z, corresponding to cleaving both benzyl groups and also reducing an alkene.

Figure S2. Absorption and emission spectra of BODIPY VoltageFluors.

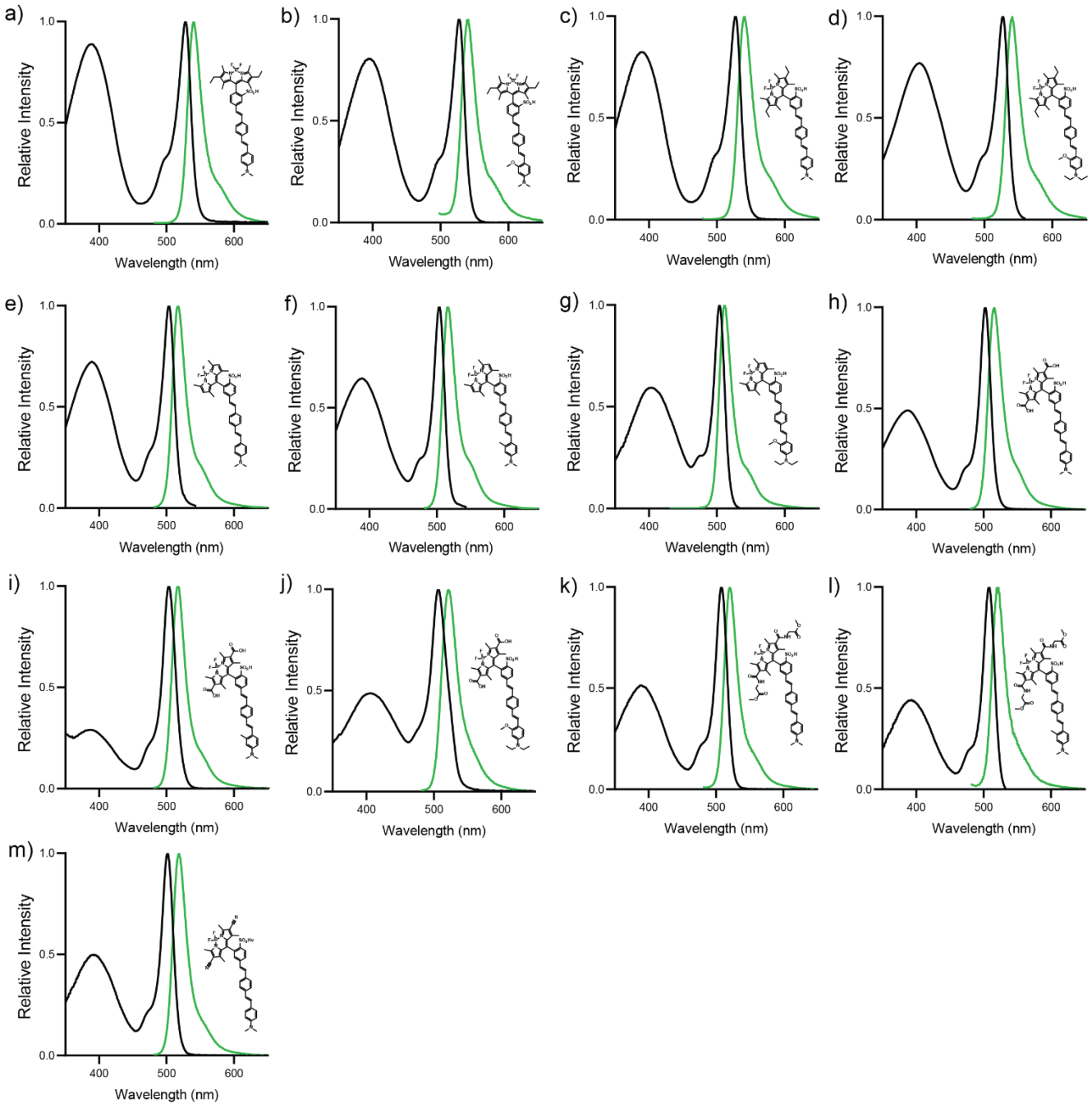


Figure S2. Absorption and emission spectra of **a)** Et*p*H, **b)** Et*p*OMe, **c)** Et*m*H, **d)** Et*m*OMe, **e)** TM*m*H, **f)** TM*m*Me, **g)** TM*m*OMe, **h)** carboxy*m*H, **i)** carboxy*m*Me, **j)** carboxy*m*OMe, **k)** amide*m*H, **l)** amide*m*Me, and **m)** cyano*m*H BODIPY VoltageFluors. Spectra were acquired in ethanol with 1 µM dye.

Figure S3. Excitation, emission, and absorption spectra of BODIPY VF dyes

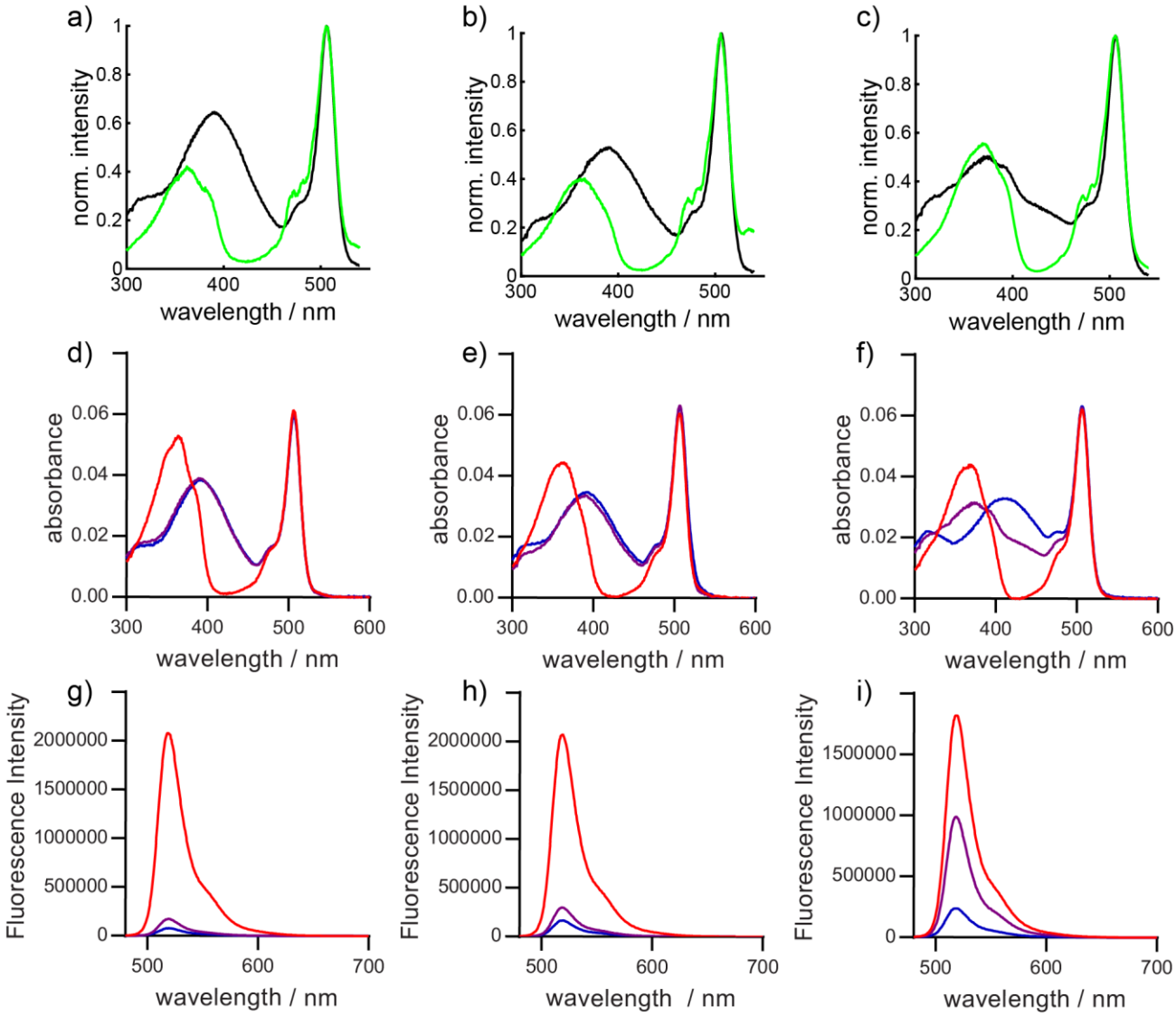


Figure S3. Excitation, emission, and absorption spectra of BODIPY VF dyes **17**, **18**, and **19**. **a-c**) Overlaid excitation (green) and absorption scans (black) of **a**) **17**, **b**) **18**, and **c**) **19**. Spectra were acquired in phosphate buffered saline (10 mM) with 40 mM NaCl and 0.1% (w/v) SDS, pH 7.5, and emission was monitored at 560 nm. Dyes were 1 μM. **d-f**) Absorption spectra of **d**) **17**, **e**) **18**, and **f**) **19**. Spectra were acquired in phosphate buffered saline (10 mM) with 40 mM NaCl and 0.1% (w/v) SDS, pH 2.5 (red), 7.5 (purple) and carbonate buffered saline (10 mM) with 40 mM NaCl and 0.1% (w/v) SDS, pH 10 (blue). **g-i**) Emission spectra of **g**) **17**, **h**) **18**, and **i**) **19**. Spectra were acquired in phosphate buffered saline (10 mM) with 40 mM NaCl and 0.1% (w/v) SDS, pH 2.5 (red), 7.5 (purple) and carbonate buffered saline (10 mM) with 40 mM NaCl and 0.1% (w/v) SDS, pH 10 (blue). Excitation was provided at 460 nm. Dyes were 1 μM.

Figure S4. Cellular characterization of ethyl-substituted (para) BODIPY VF dyes 6 and 7

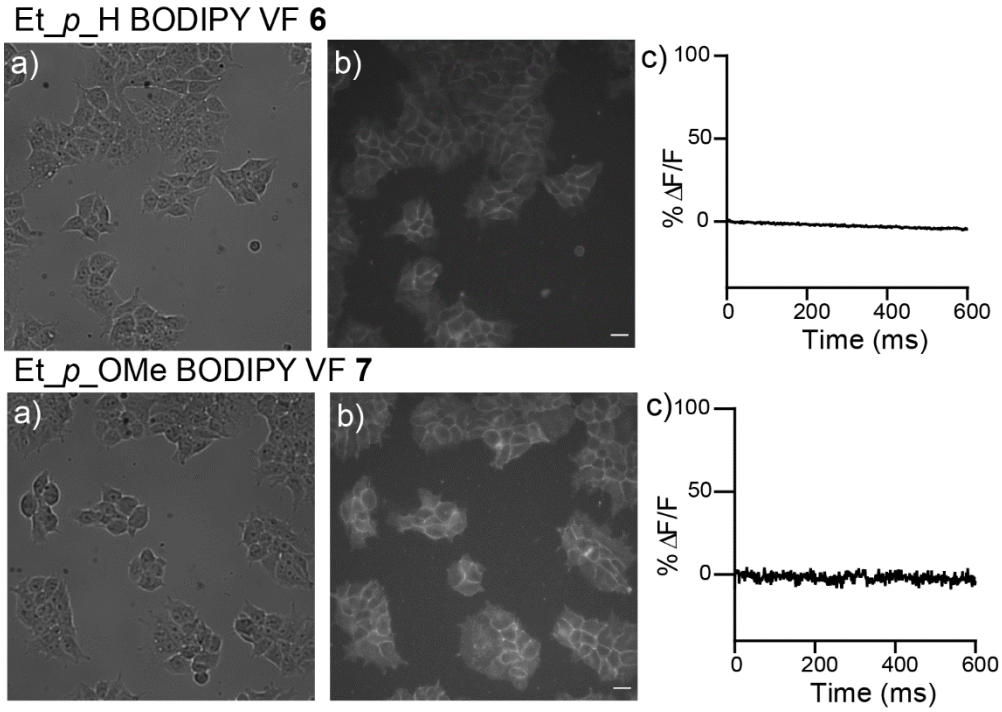


Figure S4. Cellular characterization of ethyl-substituted (*para*) BODIPY VF dyes Et*p*H **6** and Et*p*OMe **7**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. BODIPY VoltageFluors with < 5% ΔF/F are shown as unconcatenated, non-bleach corrected traces. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity.

Figure S5. Cellular characterization of ethyl-substituted (meta) BODIPY VF dyes 15 and 16.

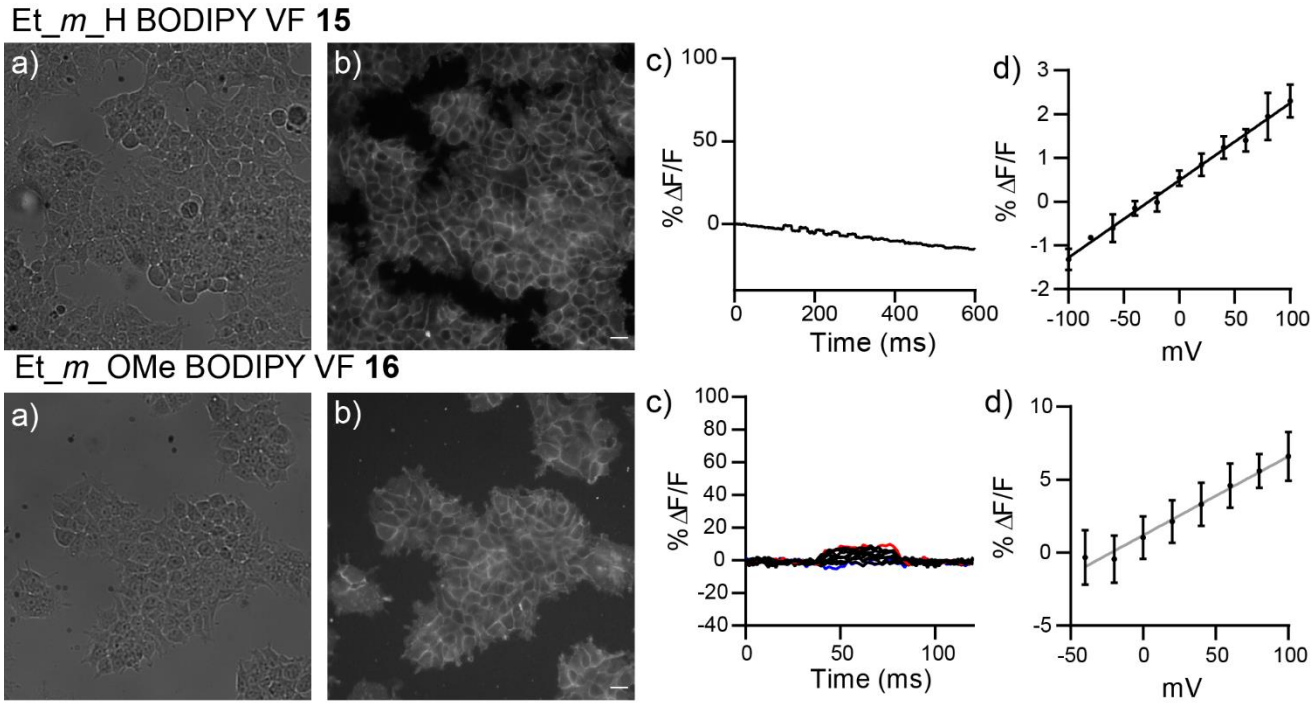


Figure S5. Cellular characterization of ethyl-substituted (*meta*) BODIPY VF dyes Et*m*H **15** and Et*m*OMe **16**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. BODIPY VoltageFluors with < 5% ΔF/F are shown as unconcatenated, non-bleach corrected traces. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity. **d)** Plot of fractional change in fluorescence (ΔF/F) vs. final membrane potential. Data represent the mean ΔF/F, ± S.E.M., for a minimum of n = 3 separate cells. Grey line is the line of best fit.

Figure S6. Cellular characterization of H-substituted BODIPY VF dyes 17, 18, and 19.

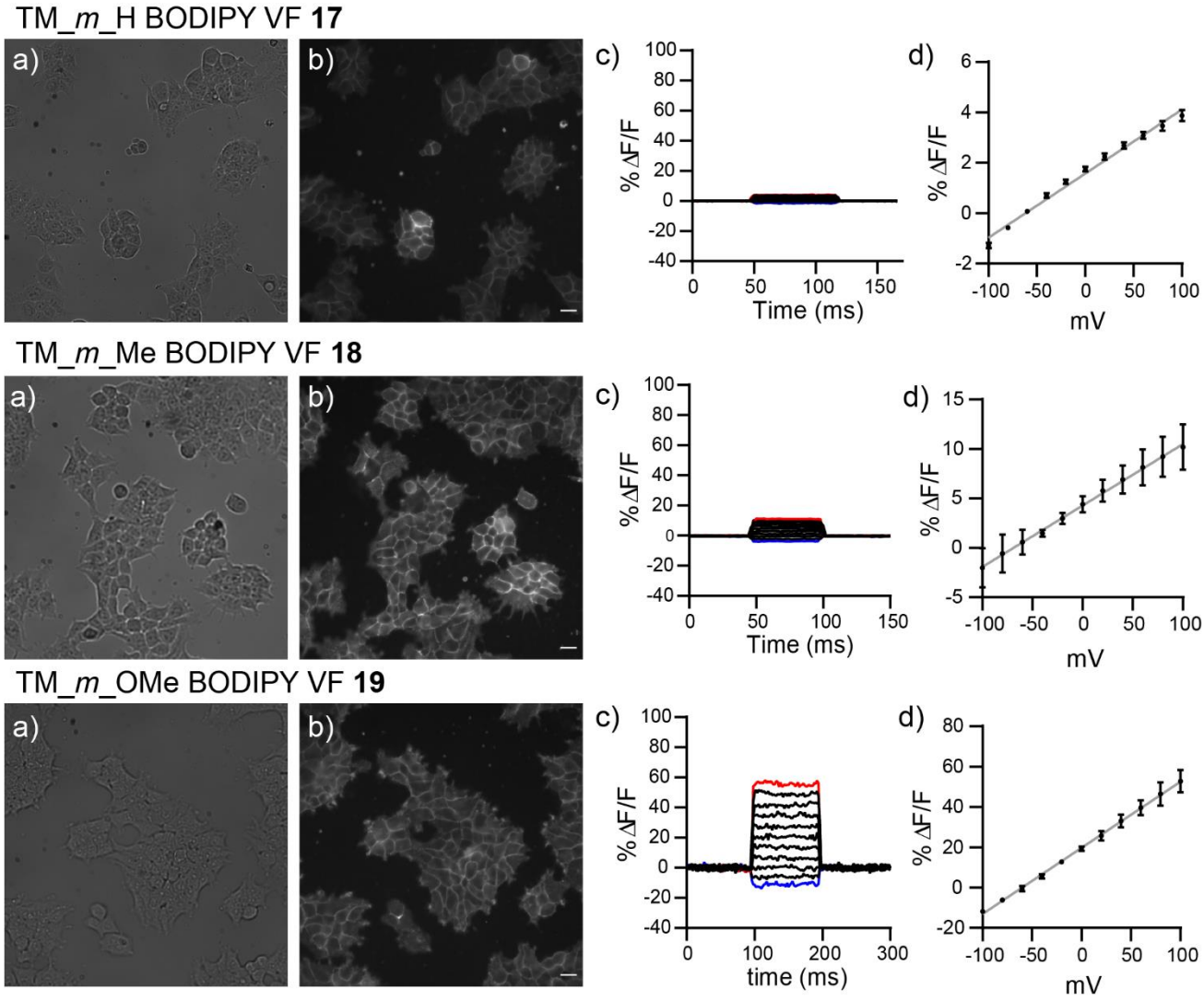


Figure S6. Cellular characterization of H-substituted BODIPY VF dyes **17**, **18**, and **19**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity. **d)** Plot of fractional change in fluorescence (ΔF/F) vs. final membrane potential. Data represent the mean ΔF/F, ± S.E.M., for a minimum of n = 3 separate cells. Grey line is the line of best fit.

Figure S7. Cellular characterization of cyano-substituted BODIPY VF dye 22.

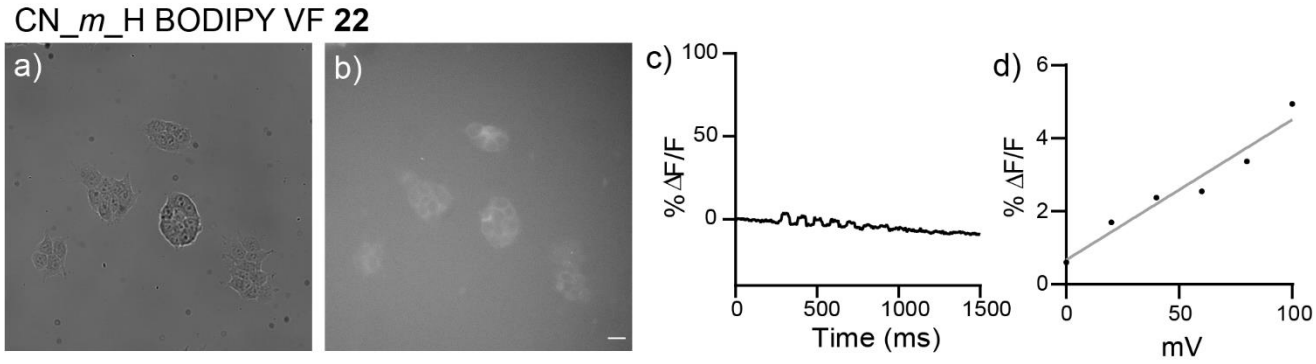


Figure S7. Cellular characterization of cyano-substituted BODIPY VF dye **22**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. BODIPY VoltageFluors with < 5% ΔF/F are shown as unconcatenated, non-bleach corrected traces. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity. **d)** Plot of fractional change in fluorescence (ΔF/F) vs. final membrane potential for n = 1 cell. Grey line is the line of best fit.

Figure S8. Cellular characterization of carboxy-substituted BODIPY VF dyes 28, 29, and 30.

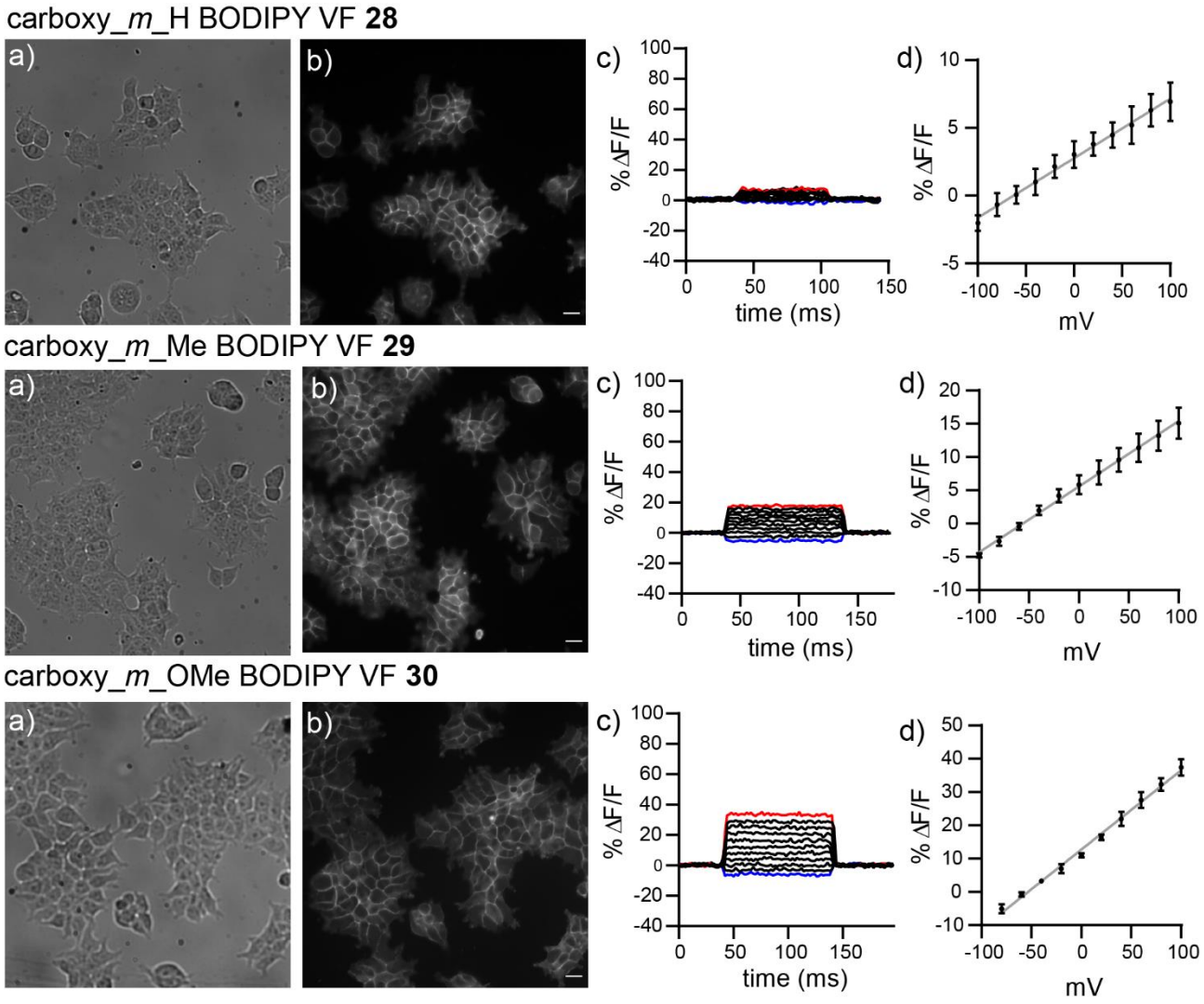


Figure S8. Cellular characterization of carboxy-substituted BODIPY VF dyes **28**, **29**, and **30**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity. **d)** Plot of fractional change in fluorescence (ΔF/F) vs. final membrane potential. Data represent the mean ΔF/F, ± S.E.M., for a minimum of n = 3 separate cells. Grey line is the line of best fit.

Figure S9. Cellular characterization of amide-substituted BODIPY VF dyes 35 and 36.

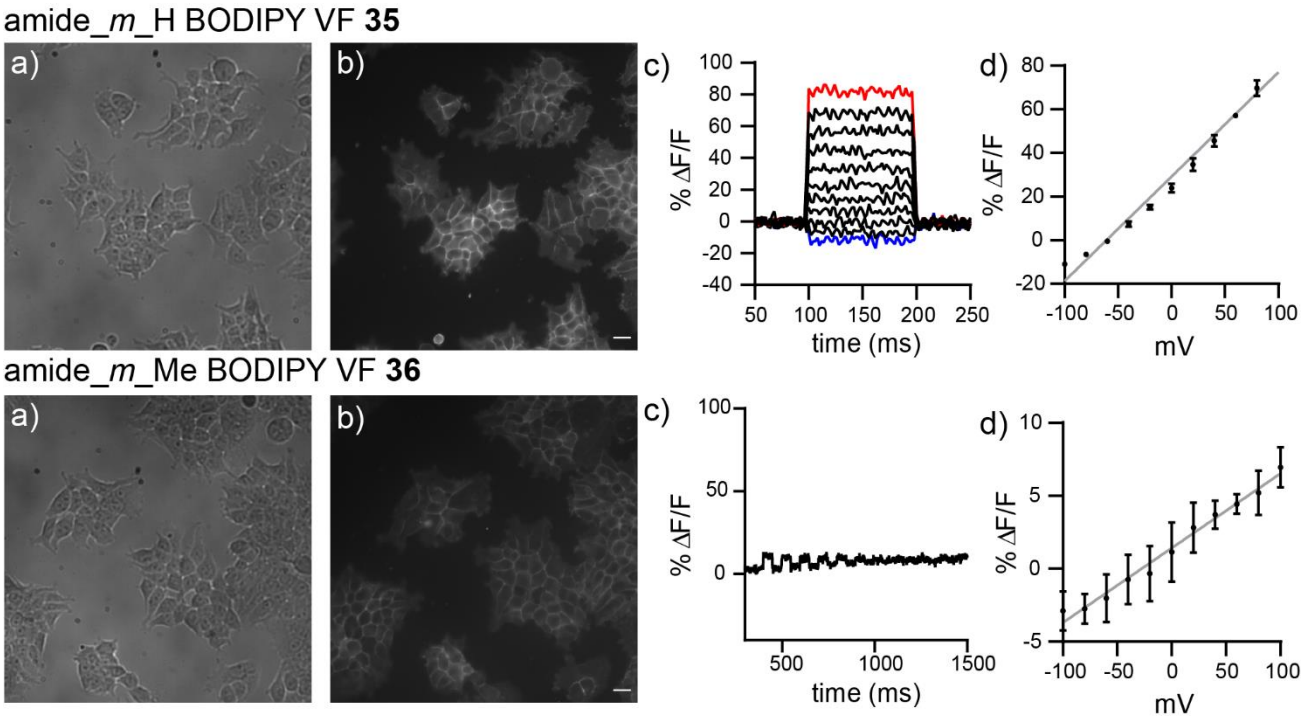


Figure S9. Cellular characterization of amide-substituted BODIPY VF dyes **35** and **36**. HEK293T cells stained with 1 μM BODIPY VF are visualized under **a)** transmitted light and **b)** widefield fluorescence microscopy. Fluorescence images are adjusted to allow membrane staining to be seen. Scale bars are 20 μm. **c)** Plot of fractional change in fluorescence (ΔF/F) vs. time for hyper- and depolarizing steps (±100 mV in 20 mV increments) from a holding potential of -60 mV in a single HEK cell under whole-cell voltage-clamp mode. BODIPY VoltageFluors with < 5% ΔF/F are shown as unconcatenated, non-bleach corrected traces. All plots are scaled from -40 to 100% ΔF/F to facilitate comparison of voltage sensitivity. **d)** Plot of fractional change in fluorescence (ΔF/F) vs. final membrane potential. Data represent the mean ΔF/F, ± S.E.M., for a minimum of n = 3 separate cells. Grey line is the line of best fit.

Figure S10. Relative brightness and photostability of BODIPY VoltageFluors

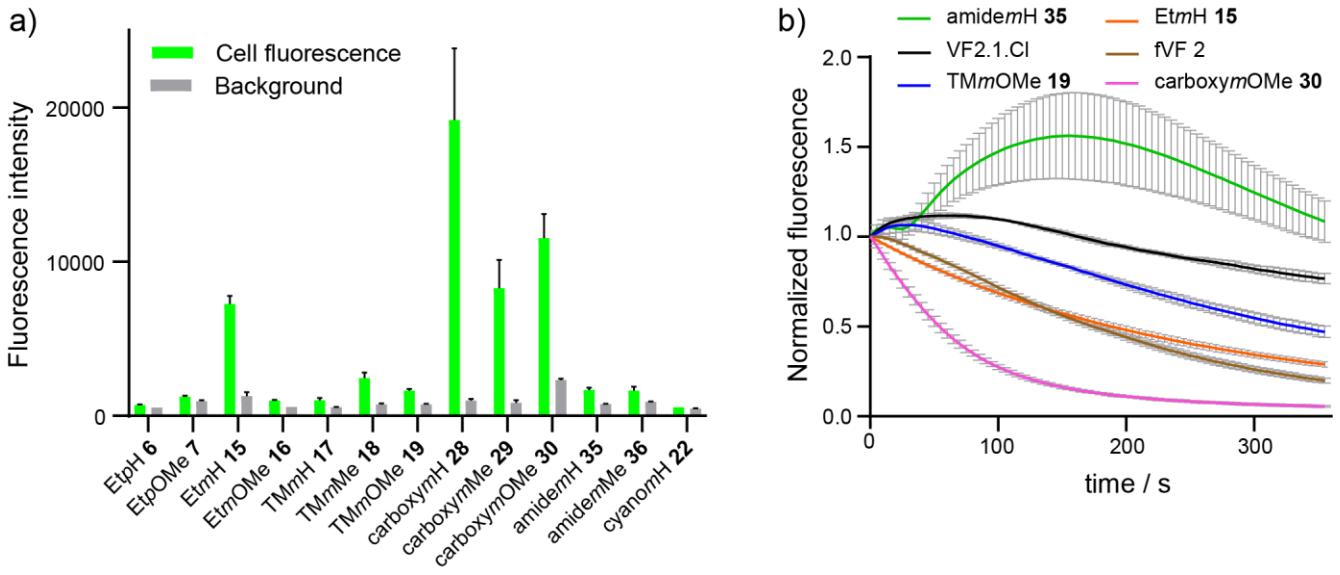


Figure S10. Relative brightness and photostability of BODIPY VoltageFluors. **a)** Average fluorescence intensity of BODIPY VoltageFluors in HEK293T cells for n = 3 images. Cells were loaded with 1 µM of each dye, and images acquired with teal LED/100 ms exposure time. **b)** Relative photobleaching of 1 µM BODIPY VoltageFluors as well as 1 µM of two dichlorofluorescein-based voltage indicators, VF2.1Cl (Miller, et al. *Proc Natl Acad Sci USA*, **2011**, *109*, 2114-2119) and fVF 2 (Boggess et al., *ACS Chem Biol*, **2019**, *14*, 390-396).

Figure S11. Voltage imaging in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with selected BODIPY VF dyes.

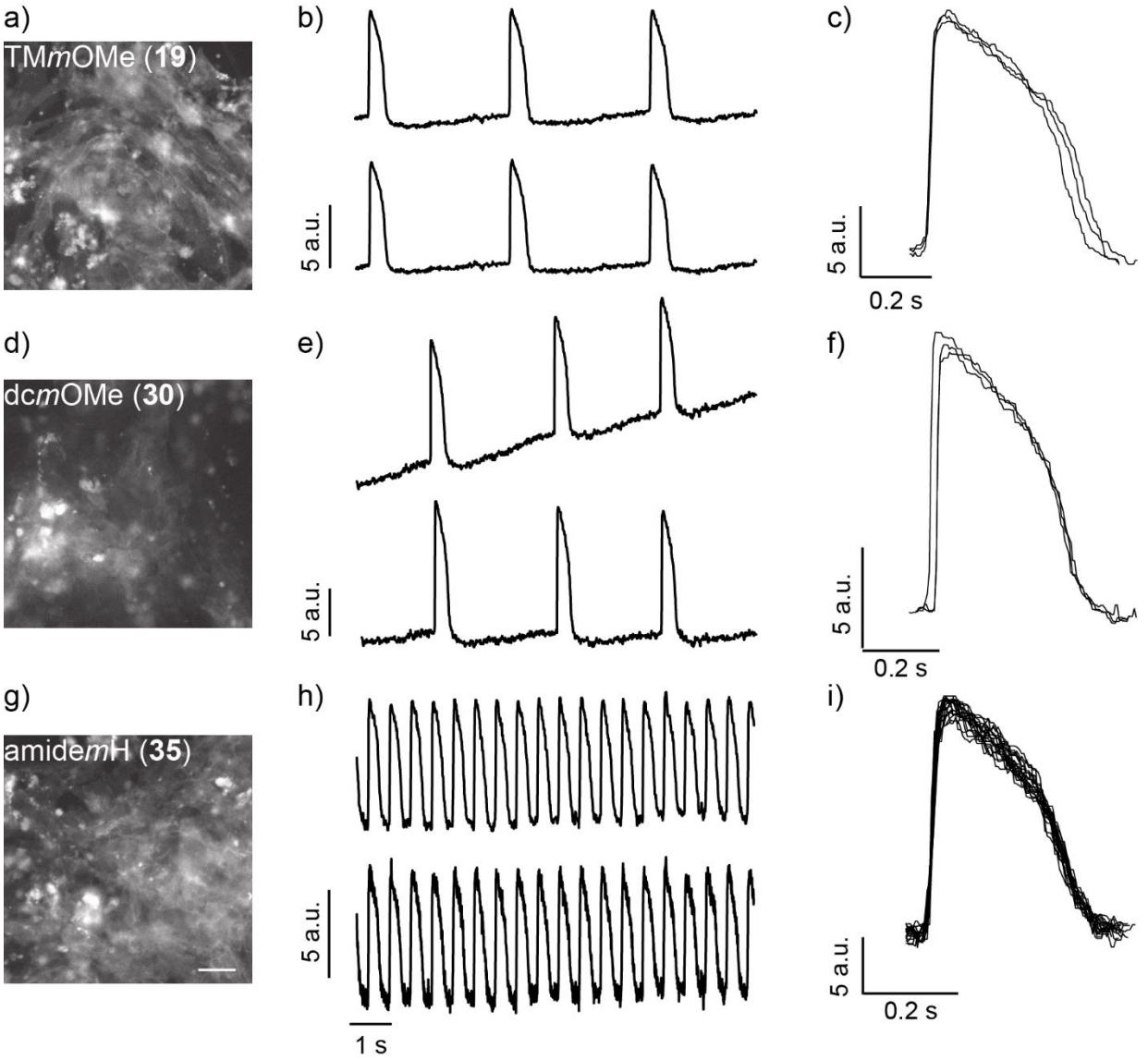


Figure S11. Voltage imaging in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with selected BODIPY VF dyes. Panels **a**, **d**, and **g** show the widefield, epifluorescence micrographs of hiPSC-CMs stained with **a)** TM*m*OMe BODIPY VF **19** (different image from that shown in **Figure 4a** of main text; provided here for comparison), **d)** carboxy*m*OMe BODIPY VF **30**, or **g)** amide*m*H BODIPY VF **35**. All dyes were loaded at a concentration of 500 nM. Scale bar for these images is 50 μm. Panels **b**, **e**, and **h** display plot of mean pixel intensity (arbitrary fluorescence units, a.u.) across an entire field of view (similar to that shown in **Figure 4b** in the main text) vs. time during a 10 second acquisition (500 frames/s). The upper trace is the raw, median-filtered signal. The lower trace has been bleach-corrected. (panel b, lower trace, is reproduced from **Figure 4c** in the main text, for comparison). Panels c, f, and i depict individual action potentials from the bleach corrected traces.

Figure S12. Extended voltage imaging in hiPSC-CMs with TMmOMe BODIPY VF, fVF 2, and VF2.1.Cl.

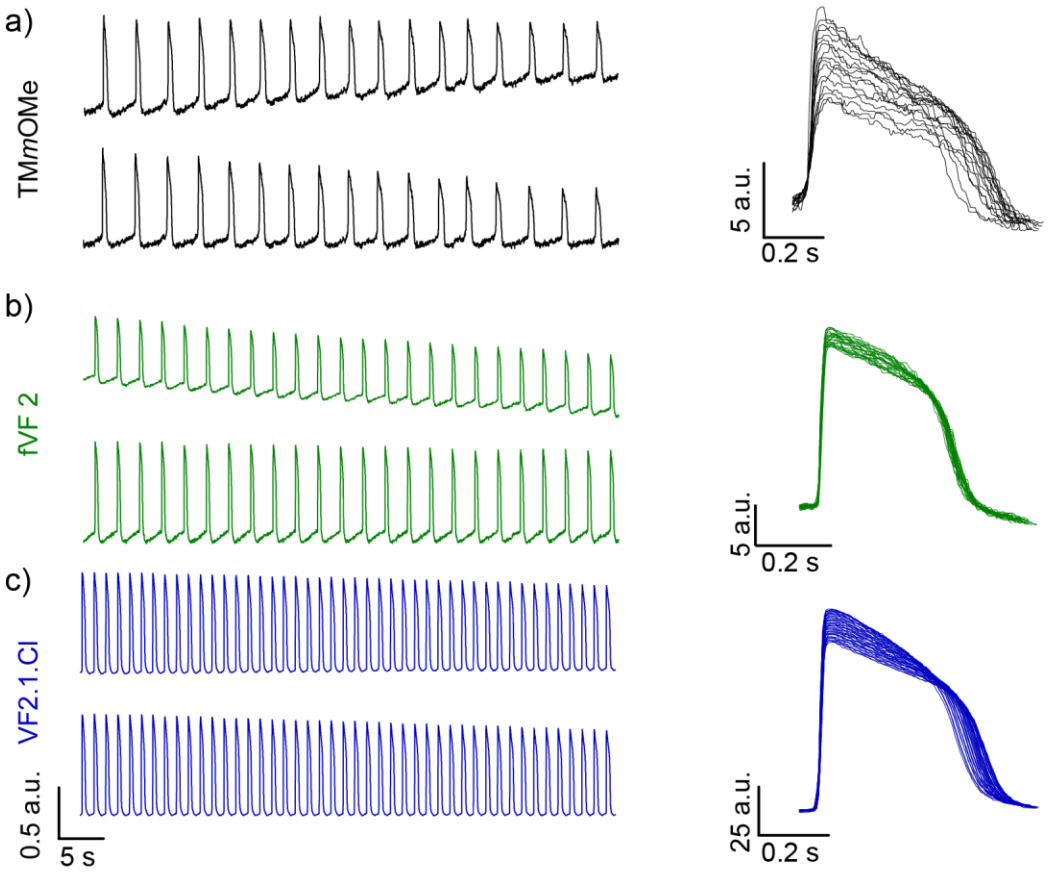


Figure S12. Extended voltage imaging in hiPSC-CMs with TM*m*OMe BODIPY VF, fVF 2, and VF2.1.Cl. Analysis of continuous voltage imaging in hiPSC-CMs for **a)** TM*m*OMe BODIPY VF **19** (black), **b)** fVF 2 (green; from Boggess et al., *ACS Chem Biol*, **2019**, *14*, 390-396), and **c)** VF2.1.Cl (blue; from Miller, et al. *Proc Natl Acad Sci USA*, **2011**, *109*, 2114-2119). The plots on the left depict the mean pixel intensity from an entire field of view (similar to **Figure 4b**, main text) vs. time during a 60 second acquisition (all dyes loaded at 500 nM, and under identical illumination conditions); upper traces are median-filtered values; lower traces are corrected for bleaching. The plots on the right are the concatenated action potentials from the bleach-corrected traces.

Figure S13. Computed HOMO energy levels for BODIPY fluorophores

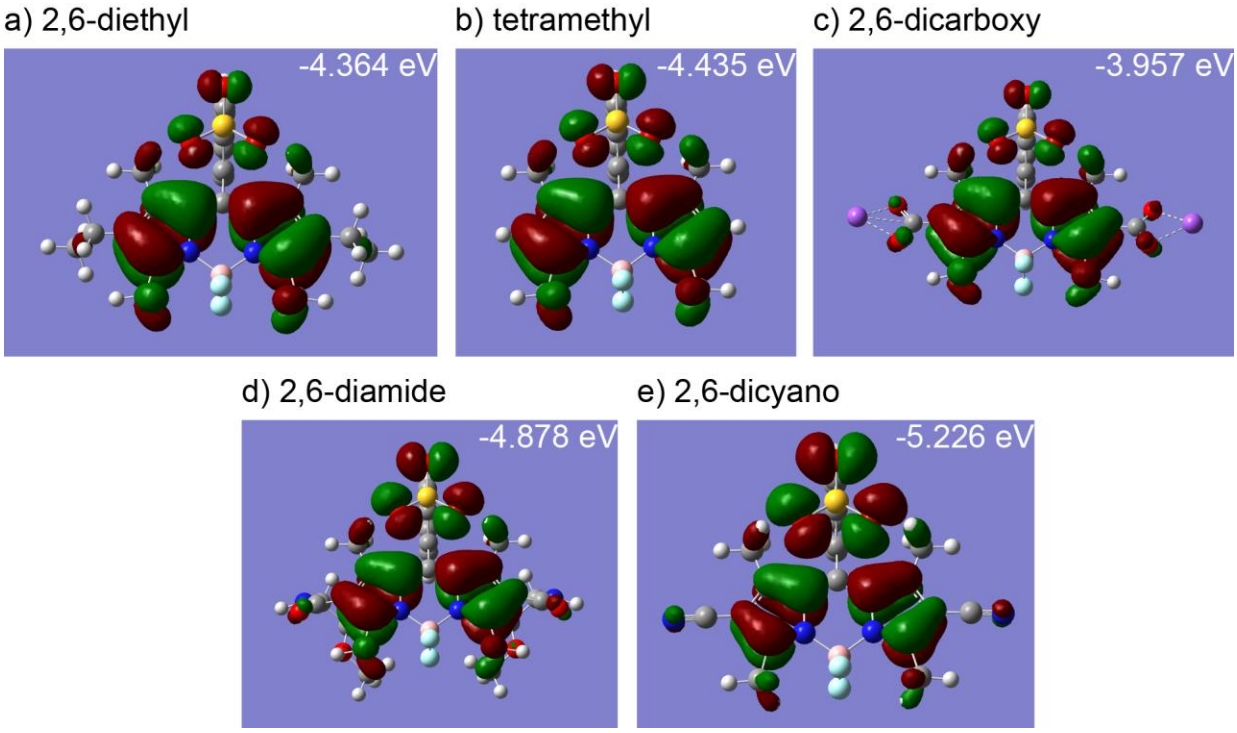


Figure S13. Computed HOMO energy levels for BODIPY fluorophores. Calculations are for sulfonated BODIPY dyes **11**, **12**, **32**, **34**, and **21**, but without Br. Calculations performed with the WB97XD functional and def2svp basis set. Calculated HOMO energies and associated orbital visualizations for **a)** 2,6-diethyl BODIPY **11**, **b)** 2,6-hydrogen (tetramethyl) BODIPY **12**, **c)** 2,6-dicarboxy BODIPY **32**, **d)** 2,6-diamide BODIPY **34** (methyl amide), and **e)** 2,6-dicyano BODIPY **21**.

Figure S14. Computational analysis of sulfonated BODIPY energy levels

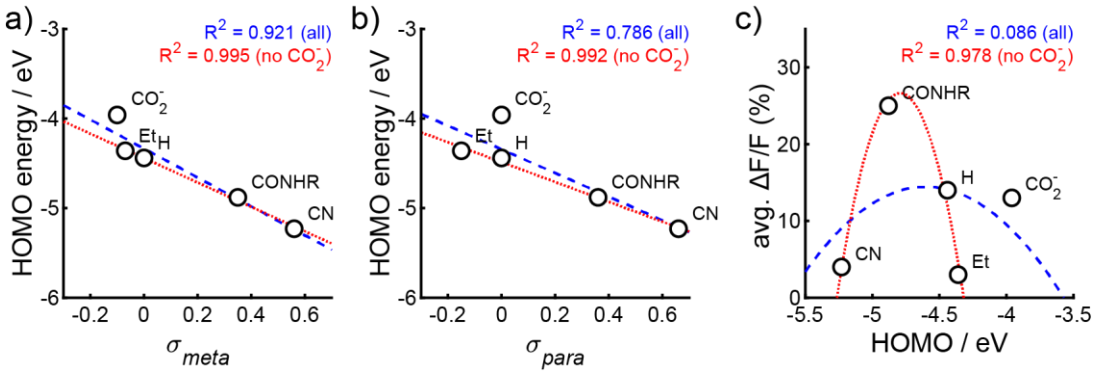
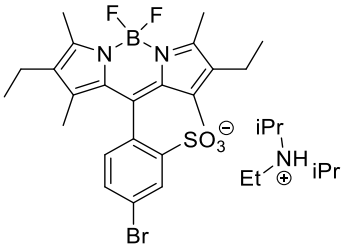


Figure S14. Computational analysis of sulfonated BODIPY energy levels. Plots of calculated HOMO energy (eV) vs. **a)** σ_{meta} or **b)** σ_{para}. Blue dashed lines indicate line of best fit including all data points. Red dotted lines depict the line of best fit, excluding σ parameters for carboxylates ($-CO_2^-$). R-squared parameters for each fit are indicated at the top of the plot. Values for σ_{meta} or σ_{para} are taken from Hansch, et al., *Chem. Rev.*, **1991**, *91*, 165-195. **c)** Plot of average voltage sensitivity (in units of ΔF/F per 100 mV) for particular 2,6-substitutions on BODIPY vs. the calculated HOMO level of that type of BODIPY. Blue dashed line indicates binomial fit including all data. Red dotted line excludes the calculated HOMO level for carboxylate-containing BODIPYs ($-CO_2^-$).

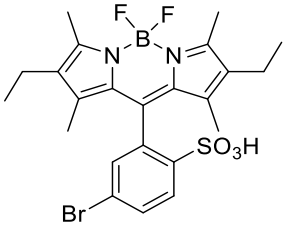
Synthesis of BODIPY dyes



1,3,5,7-tetramethyl-2,6-diethyl-*p*-bromo BODIPY (3). *Para*-bromo sulfonated aldehyde **1** (256 mg, 0.97 mmol, 1 eq) was added to a flame-dried 25 mL round-bottom flask. Flask was evacuated and backfilled with N_2 3x, then DMF (5 mL), 3-ethyl-2,4-dimethyl-*1H*-pyrrole (287 µL, 2.12 mmol, 2.2 eq) and TFA (2 drops) were added via syringe, and reaction stirred under nitrogen atmosphere overnight. DDQ (219 mg, 0.97 mmol, 1 eq) was then added, stirred for 5 min, then concentrated under reduced pressure. An optional silica plug (3-10% MeOH in DCM gradient) yielded the dipyrromethene as a pink, green iridescent solid, which was taken onto the next step directly.

The dipyrromethene was dissolved in DCM (20 mL), DIPEA (1.91 mL, 11 mmol, 11 eq) and $BF_3{\cdot}Et_2O$ (2 mL, 15.5 mmol, 16 eq) were added via syringe and the solution became green fluorescent. After 10 min, reaction was quenched by addition of water, and organics were washed with 0.25N HCl (3 x 30 mL), brine (40 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (3-7% MeOH in DCM gradient) yielded the BODIPY **3** as a pink, green iridescent solid (252 mg, 49%).

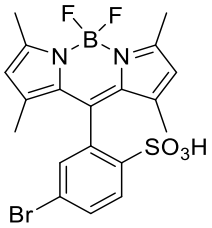
^{1}H NMR (400 MHz, MeOD) δ 8.24 (d, *J* = 2.06 Hz, 1H), 7.76 (dd, *J* = 2.06, 8.20 Hz, 1H), 7.17 (d, *J* = 8.20 Hz, 1H), 3.68 (sep, *J* = 6.64 Hz, 1H, $NEt(iPr)_2H^+$ salt), 3.18 (q, *J* = 7.41 Hz, 2H, $NEt(iPr)_2H^+$ salt), 2.45 (s, 6H), 2.33 (q, *J* = 7.52 Hz, 4H), 1.42 (s, 6H), 1.33 – 1.28 (m, 12H, $NEt(iPr)_2H^+$ salt), 0.98 (t, *J* = 7.51 Hz, 6H). **^{13}C NMR** (400 MHz, MeOD) δ 154.1, 147.2, 140.3, 140.1, 135.2, 133.64, 133.59, 133.3, 133.0, 132.5, 123.9, 55.8 ($NEt(iPr)_2H^+$ salt), 43.8 ($NEt(iPr)_2H^+$ salt), 18.0, 15.2, 13.1, 12.7, 12.1 ppm. **ESI-HR(-)**, calculated for $C_{23}H_{25}BBrF_2N_2O_3S^-$: 537.0836, found: 537.0825.



1,3,5,7-tetramethyl-2,6-diethyl-*m*-bromo BODIPY (11). *Meta*-bromo sulfonated aldehyde **9** (160 mg, 0.60 mmol, 1 eq) was added to a flame-dried 25 mL round-bottom flask. Flask was evacuated and backfilled with N_2 3x, then DMF (5 mL), 3-ethyl-2,4-dimethyl-*1H*-pyrrole (179 µL, 2.12 mmol, 2.2 eq) and TFA (2 drops) were added via syringe, and reaction stirred under nitrogen atmosphere overnight. DDQ (137 mg, 0.60 mmol, 1 eq) was then added, stirred for 5 min, then concentrated under reduced pressure. An optional silica plug (3-10% MeOH in DCM gradient) yielded the dipyrromethene as a pink, green iridescent solid, which was taken onto the next step directly.

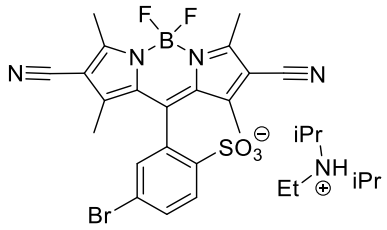
The dipyrromethene was dissolved in DCM (20 mL), DIPEA (1.2 mL, 6.9 mmol, 10.4 eq) and $BF_3{\cdot}Et_2O$ (1.19 mL, 9.7 mmol, 16 eq) were added via syringe, and the solution became green fluorescent. After 10 min, reaction was quenched by addition of water, and organics were washed with 0.25N HCl (3 x 10 mL), brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (3% MeOH in DCM) yielded the BODIPY **11** as a pink, green iridescent solid (126 mg, 33%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (d, J = 8.42 Hz, 1H), 8.16 (dd, J = 2.08, 8.48 Hz, 1H), 7.85 (d, J = 2.10 Hz, 1H), 2.91 (s, 6H), 2.76 (q, J = 7.56 Hz, 4H), 1.88 (s, 6H), 1.42 (t, J = 7.54 Hz, 6H). **^{13}C NMR** (900 MHz, DMSO-d_6) δ 162.8, 155.7, 149.7, 149.2, 145.6, 142.3, 142.2, 142.0, 141.64, 141.55, 133.5, 27.2, 24.8, 22.3, 21.5 **ESI-HR(-)**, calculated for $C_{23}H_{25}BBrF_2N_2O_3S^-$: 537.0836, found: 537.0837.



1,3,5,7-tetramethyl-*m*-bromo BODIPY (12). *Meta*-bromo sulfonated aldehyde **9** (504.9 mg, 1.9 mmol, 1 eq) was added to a flame-dried 25 mL round-bottom flask. Flask was evacuated and backfilled with N_2 3x, then DMF (5 mL), 2,4-dimethyl-*1H*-pyrrole (432 µL, 4.2 mmol, 2.2 eq) and TFA (3 drops) were added via syringe, and reaction stirred under nitrogen atmosphere overnight. DDQ (432 mg, 1.9 mmol, 1 eq) was then added, stirred for 5 min, then concentrated under reduced pressure. An optional silica plug (2-14% MeOH in DCM gradient) yielded the dipyrromethene as a pink, green iridescent solid, which was taken onto the next step directly.

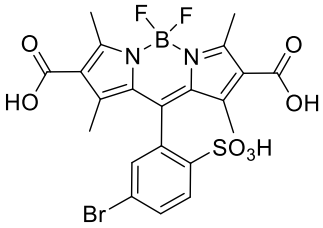
The dipyrromethene was dissolved in DCM (40 mL), DIPEA (3.6 mL, 21 mmol, 11 eq) and $BF_3{\cdot}Et_2O$ (3.8 mL, 30 mmol, 16 eq) were added via syringe and the solution became green fluorescent. After 10 min, reaction was quenched by addition of 10 mL iPrOH. Organics were washed with 0.25N HCl (2 x 20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (1-7% MeOH in DCM gradient) yielded the BODIPY **12** as a pink, green iridescent solid (350 mg, 38%). **^{1}H NMR** (400 MHz, MeOD) δ 8.47 (d, J = 8.44 Hz, 1H), 8.16 (dd, J = 2.08, 8.47 Hz, 1H), 7.87 (d, J = 7.87 Hz, 1H), 6.44 (s, 2H), 2.91 (s, 6H), 1.96 (s, 6H). **^{13}C NMR** (900 MHz, DMSO-d_6) δ 164.5, 155.8, 153.9, 151.2, 144.8, 142.3, 142.1, 141.9, 141.6, 133.4, 131.0, 24.24, 24.21. **ESI-HR(-)**, calculated for $C_{19}H_{17}BBrF_2N_2O_3S^-$: 481.0210, found: 481.0211.



1,3,5,7-tetramethyl-2,6-cyano-*m*-bromo BODIPY (21). *Meta*-bromo sulfonated aldehyde **9** (256 mg, 0.22 mmol, 1 eq) and 2,4-dimethyl-*1H*-pyrrole-3-carbonitrile (**31**) (58.3 mg, 0.49 mmol, 2.2 mmol) were added to an oven-dried 25 mL round-bottom flask. Flask was evacuated and backfilled with N_2 three times, then dissolved in DMF (680 µL) and DCM (1.01 mL). TFA (100 µL) was added via syringe and reaction stirred under inert N_2 atmosphere overnight. DDQ was then added (50.1 mg, 0.22 mmol, 1 eq), stirred for 5 min, then solution was concentrated under reduced pressure.

The dipyrromethene was dissolved in DCM (5 mL), then DIPEA (423 µL, 2.4 mmol, 11 eq) and $BF_3{\cdot}Et_2O$ (436 µL, 3.5 mmol, 16 eq) were added and reaction stirred for 1.5 hrs. Reaction was quenched by addition of water, and organics were washed with water (3 x 30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (1-7% MeOH in DCM gradient) yielded the BODIPY **21** as a pink, green iridescent solid (33.8 mg, 29%).

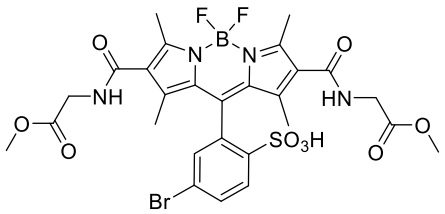
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.04 (d, J = 8.5 Hz, 1H), 7.89 (dd, J = 8.5, 2.0 Hz, 1H), 7.61 (d, J = 2.0 Hz, 1H), 3.71 (p, J = 6.6 Hz, 1H, $NEt(iPr)_2H^+$ salt), 3.20 (q, J = 7.4 Hz, 2H, $NEt(iPr)_2H^+$ salt), 2.66 (s, 6H), 1.70 (s, 6H), 1.40 – 1.25 (m, 15H, $NEt(iPr)_2H^+$ salt). **^{13}C NMR** (400 MHz, Methanol-d_4) δ 160.2, 150.9, 146.8, 144.3, 134.9, 133.4, 133.3, 132.5, 132.2, 126.5, 114.7, 106.9, 56.0 ($NEt(iPr)_2H^+$ salt), 55.0 ($NEt(iPr)_2H^+$ salt), 43.9, 14.2, 13.9, 13.3 ppm. **ESI-HR(-)**, calculated for $C_{21}H_{15}BBrF_2N_4O_3S^-$: 531.0115, found: 531.0110.



1,3,5,7-tetramethyl-2,6-carboxy-*m*-bromo BODIPY (32). *Meta*-bromo sulfonated aldehyde **9** (357 mg, 1.4 mmol, 1 eq) and 2,4-dimethylpyrrole-3-carboxylic acid **31** (412 mg, 3.0 mmol, 2.2 eq) were added to a flame-dried 25 mL round-bottom flask. Flask was evacuated and backfilled with N_2 3x, then DMF (10 mLand TFA (100 µL) were added via syringe and reaction stirred under nitrogen atmosphere overnight. DDQ (306 mg, 1.4 mmol, 1 eq) was added and solution stirred for 5 min then was concentrated under reduced pressure. An optional silica plug (15% MeOH + 1% AcOH in DCM) yielded the dipyrromethene as a pink, green iridescent solid, which was taken onto the next step directly.

DCM (50 mL) was added to 250 mL round-bottom flask containing the dipyrromethene and the solution was sonicated to suspend the material. DIPEA (2.6 mL, 15 mmol, 11 eq) and $BF_3{\cdot}Et_2O$ (2.7 mL, 22 mmol, 16 eq) were added via syringe and the solution became green fluorescent. After 10 min, reaction was quenched by addition of 10 mL iPrOH and solution was concentrated under reduced pressure. Flash chromatography on silica gel (10-20% MeOH in DCM + 1% AcOH, gradient) yielded the BODIPY **32** as a pink, green iridescent solid (380 mg, 49%). This material was >90% pure by analytical HPLC and was used without further purification in the next synthetic step. For NMR and spectroscopic characterization, was purified by reverse phase preparative HPLC (10-100% MeCN in water, 0.05% formic acid additive).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.01 (d, J = 8.5 Hz, 1H), 7.82 (dd, J = 8.5, 2.1 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 2.75 (s, 6H), 1.80 (s, 6H). **^{13}C NMR** (900 MHz, Methanol-d_4) δ 170.3, 158.9, 145.8, 144.5, 143.6, 135.6, 133.6, 133.2, 132.9, 132.0, 125.9, 14.8, 13.6 ppm. **ESI-HR(-)**, calculated for $C_{21}H_{17}BBrF_2N_2O_7S^-$: 569.0006, found: 569.0008).

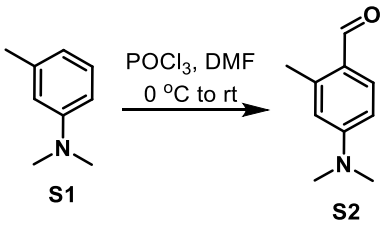


1,3,5,7-tetramethyl-2,6-amido-*m*-bromo BODIPY (34). 1,3,5,7-tetramethyl-2,6-dicarboxy BODIPY **32** (23 mg, 0.04 mmol), glycine methyl ester (11.3 mg, 0.09 mmol, 2.25 eq), and HATU (34.4 mg, 0.09 mmol, 2.25 eq) were dissolved in DMF (0.5 mL), then DIPEA (70 µL, 0.4 mmol, 10 eq) were added and reaction stirred at rt for 3.5 hrs. Reaction was concentrated to near-dryness under reduced pressure, then 10 mL DCM was added and solution was washed with water (2 x 5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Preparative TLC (15% MeOH in DCM) yielded amide BODIPY **34** as an orange, green iridescent solid (24 mg, 82%). This material was >95% pure by analytical HPLC and

was used without further purification in the next synthetic step. For NMR and spectroscopic characterization, a small amount was further purified by reverse phase preparative HPLC (10-100% MeCN in water, 0.05% formic acid additive).

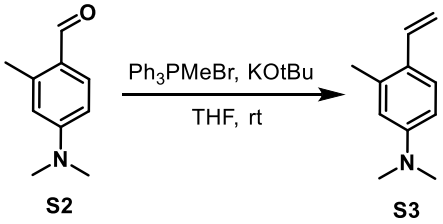
^{1}H NMR (700 MHz, Methanol-*d*$_4$) δ 8.02 (d, J = 8.5 Hz, 1H), 7.83 (dd, J = 8.5, 2.1 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 4.12 – 3.99 (m, 4H), 3.73 (s, 6H), 2.61 (s, 6H), 1.63 (s, 6H). **^{13}C NMR** (600 MHz, Methanol-*d*$_4$) δ 171.6, 168.1, 155.9, 143.9, 142.9, 133.9, 133.0, 132.7, 132.1, 129.5, 126.1, 52.6, 42.0, 13.5, 13.1 ppm. **LR-MS (ESI+)** calculated for $C_{27}H_{29}BF_2BrN_4O_9S^+$: 713.09, found 713.4. **Analytical HPLC retention time:** 5.02 min (10-100% MeCN in water, 0.05% TFA additive).

Synthesis of BODIPY VoltageFluors



4-(dimethylamino)-2-methylbenzaldehyde (S2). A flame-dried round-bottom flask was charged with *N,N*-dimethyl-*m*-toluidene **S1** (4 g, 29.6 mmol, 1 eq) and evacuated/backfilled with N_2 3x. DMF (45 mL) was added and solution was cooled to 0 °C in an ice-water bath. $POCl_3$ (4.98 mL, 53.6 mmol, 1.8 eq) was added dropwise via syringe and reaction stirred at rt 18 hr. Reaction was poured into ice water (500 mL) and adjusted to pH 9 with 1M NaOH. The resulting precipitate was filtered, washed with water (50 mL), then dried *in vacuo*, yielding **S2** as a white solid (3.19 g, 66%).

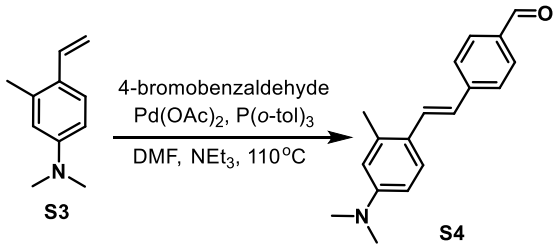
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.98 (s, 1H), 7.67 (d, *J* = 8.7 Hz, 1H), 6.57 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.43 (d, *J* = 2.6 Hz, 1H), 3.07 (s, 6H), 2.63 (s, 3H). **^{13}C NMR** (400 MHz, $CDCl_3$) δ 190.37, 153.52, 142.77, 134.60, 123.34, 113.42, 108.83, 39.96, 20.38. **ESI-HR(+)**, calculated for $C_{10}H_{14}O_1N_1^+$: 164.1070, found: 164.1068.



***N,N*,3-trimethyl-4-vinylaniline (S3).** A flame-dried round-bottom flask was charged with Ph_3PMeBr (11.17 g, 31 mmol, 1.6 eq) and evacuated/backfilled 3x with N_2. Anhydrous THF (18 mL) and KOtBu (3.5 g, 31 mmol, 1.6 eq) were added and stirred for 15 min. Aldehyde **S2** was added slowly via a funnel, which was rinsed with THF (8 mL). After 2.5 hrs, solvent was removed under reduced pressure, hexanes were added, filtered through a pad of alumina, and concentrated. Resulting residue was purified further with an alumina column (3 – 5% EtOAc in hexanes gradient), yielding styrene **S3** as a light yellow oil (2.9 g, 92%).

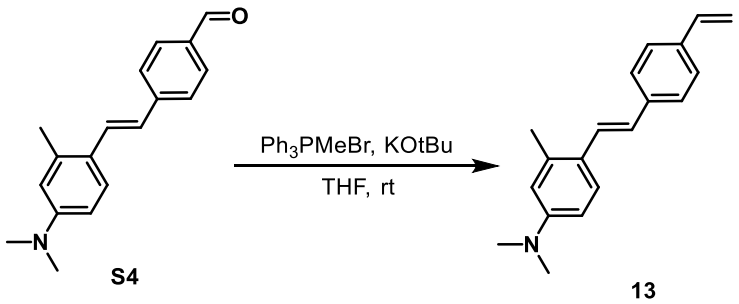
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 (dd, *J* = 8.7, 1.7 Hz, 1H), 6.89 (ddd, *J* = 17.4, 11.0, 1.9 Hz, 1H), 6.60 (dt, *J* = 8.6, 2.1 Hz, 1H), 6.52 (t, *J* = 2.1 Hz, 1H), 5.50 (dq, *J* = 17.4, 1.4 Hz, 1H), 5.10 (dq, *J* = 10.9, 1.4 Hz, 1H), 2.96 (d, *J* = 1.4 Hz, 6H), 2.35 (d, *J* = 1.8 Hz, 3H). **^{13}C NMR** (400 MHz, Chloroform-

d) δ 150.31, 137.99, 134.48, 126.23, 125.54, 114.07, 111.07, 110.69, 40.65, 20.38. **ESI-HR(+)**, calculated for $C_{11}H_{16}N_1^+$: 162.1277, found: 162.1275.



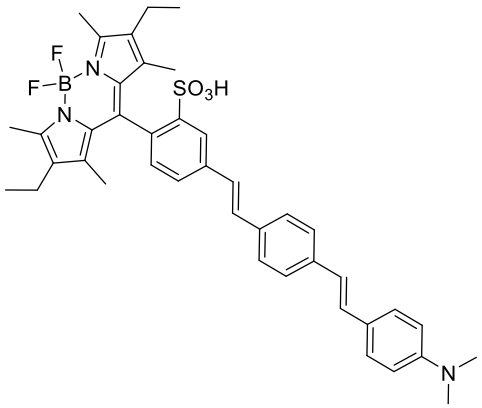
(E)-4-(4-(dimethylamino)-2-methylstyryl)benzaldehyde (S4). A flame-dried Schlenk flask was charged with **S3** (2.9 g, 18 mmol, 1 eq), 4-bromobenzaldehyde (3.3 g, 18 mmol, 1 eq), $Pd(OAc)_2$ (40.4 mg, 0.18 mmol, 1 mol%), and P(*o*-tol)$_3$ (109 mg, 0.36 mmol, 2 mol%). Flask was evacuated and backfilled with N_2 3x, DMF (20 mL) and NEt_3 (8 mL) were added, and reaction stirred at 110 °C 18 hr. Reaction was concentrated under reduced pressure, then residue was dissolved in EtOAc (200 mL) and washed with water (2 x 225 mL) and brine (200 mL). Organics were dried with Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (5 – 20% EtOAc in hexanes, gradient) yielded **S4** as an orange solid (1.55 g, 32%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.87 – 7.80 (m, 2H), 7.61 (d, *J* = 8.1 Hz, 2H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 16.1 Hz, 1H), 6.90 (d, *J* = 16.1 Hz, 1H), 6.61 (dd, *J* = 8.7, 2.8 Hz, 1H), 6.53 (d, *J* = 2.6 Hz, 1H), 3.00 (s, 6H), 2.45 (s, 3H). **^{13}C NMR** (400 MHz, $CDCl_3$) δ 191.8, 150.7, 145.0, 137.7, 134.7, 130.4, 130.1, 129.7, 126.7, 126.5, 124.1, 123.9, 114.0, 110.6, 40.5, 20.6 ppm. **ESI-HR(+)**, calculated for $C_{18}H_{20}O_1N_1^+$: 266.1539, found: 266.1526.



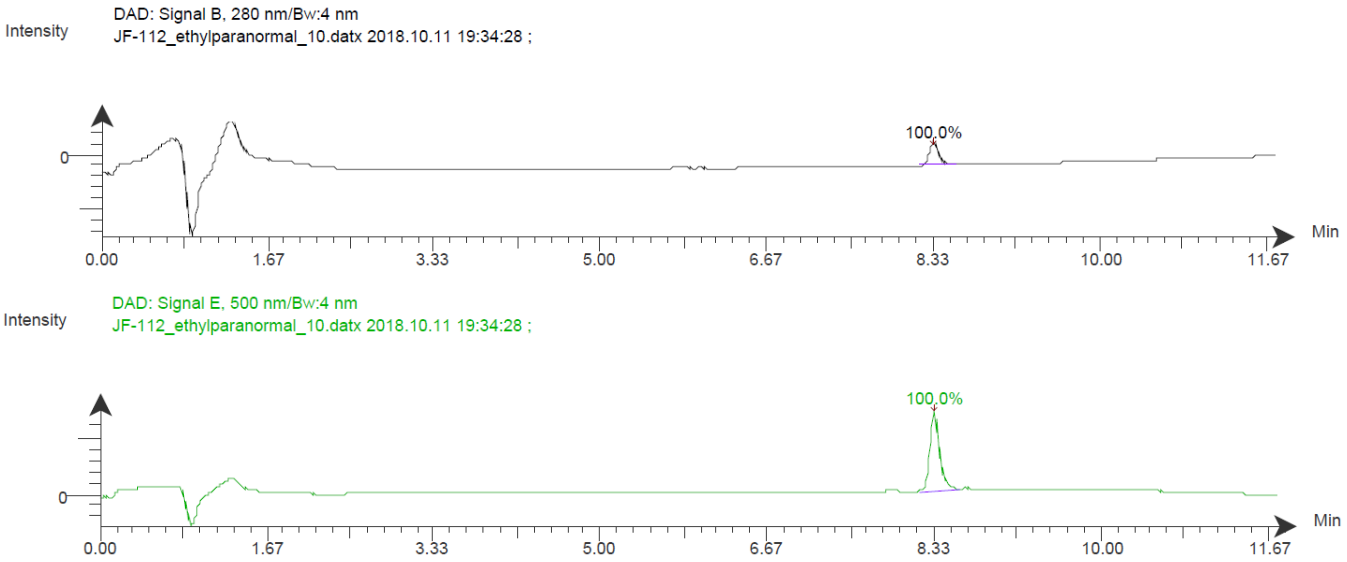
***(E)-N,N*,3-trimethyl-4-(4-vinylstyryl)aniline (13).** A flame-dried round-bottom flask was charged with Ph_3PMeBr (474 mg, 1.3 mmol, 1.6 eq) and evacuated/backfilled 3x with N_2. Anhydrous THF (1.8 mL) and KOtBu (149 mg, 1.3 mmol, 1.6 eq) were added and stirred for 15 min. Aldehyde **S4** was dissolved in THF (1 mL + 1mL rinse) and pipetted into reaction flask. After 20 hrs, solvent was removed under reduced pressure, hexanes were added, filtered through a pad of celite, and concentrated. Flash chromatography on silica (3 – 5% EtOAc in hexanes gradient), yielded **13** as a yellow solid (141 mg, 65%).

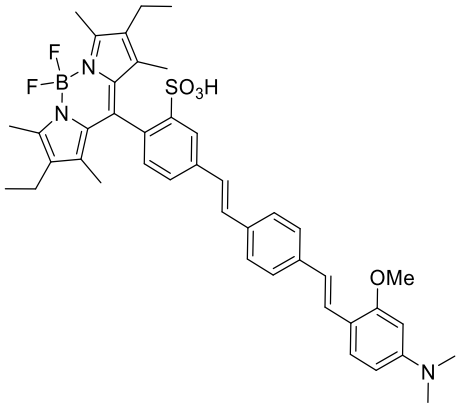
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (d, *J* = 8.7 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 16.1 Hz, 1H), 6.97 (d, *J* = 16.1 Hz, 1H), 6.83 (dd, *J* = 17.6, 10.9 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.65 (d, *J* = 2.7 Hz, 1H), 5.87 (dd, *J* = 17.6, 0.9 Hz, 1H), 5.34 (dd, *J* = 10.8, 1.0 Hz, 1H), 3.07 (s, 6H), 2.54 (s, 3H). **^{13}C NMR** (400 MHz, $CDCl_3$) δ 150.15, 138.22, 136.92, 136.71, 136.09, 126.59, 126.47, 126.32, 126.30, 125.44, 124.82, 114.08, 113.18, 110.73, 40.51, 20.58. **ESI-HR(+)**, calculated for $C_{19}H_{22}N_1^+$: 264.1747, found: 264.1742.



Ethyl BODIPY *p*-normal wire (6). To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-2,6-diethyl-*p*-bromo BODIPY **3** (87.5 mg, 0.16 mmol, 1 eq), molecular wire **4** (44.5 mg, 0.18 mmol, 1.1 eq), $Pd(OAc)_2$ (3.2 mg, 0.015 mmol, 9 mol%), and P(*o*-tol)$_3$ (8.9 mg, 0.029 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (1.1 mL) and NEt_3 (60 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (4% MeOH in DCM) yielded Et*p*H **6** as a reddish orange solid (105.7 mg, 92%).

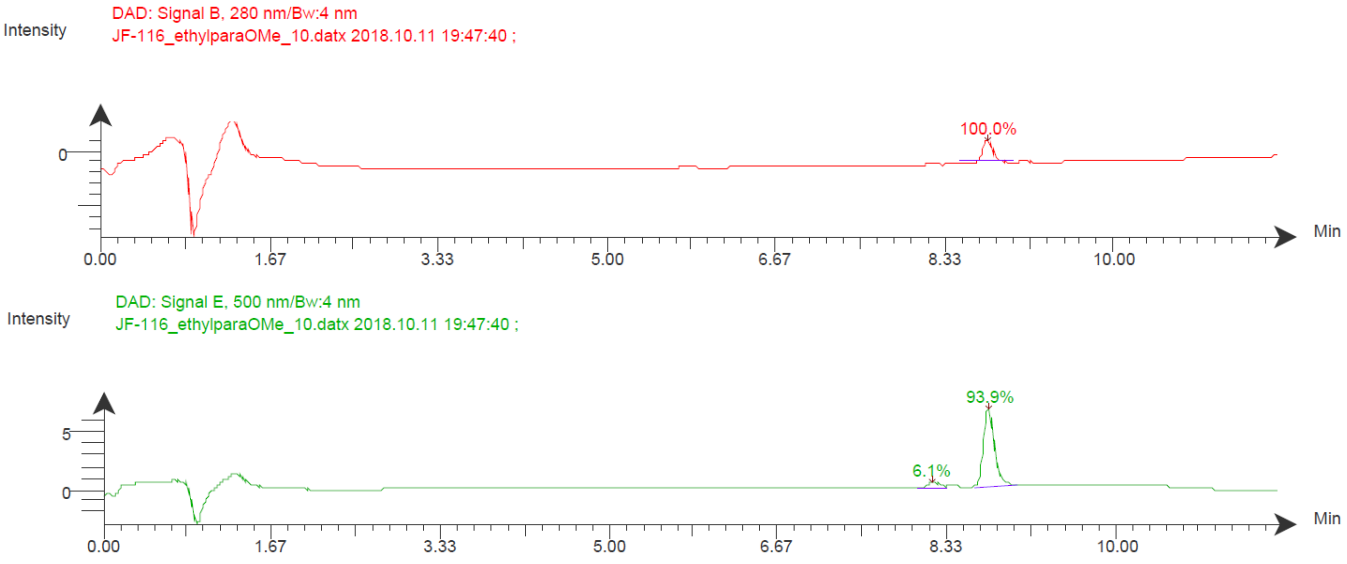
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.83 (s, 1H), 8.42 (d, *J* = 1.8 Hz, 1H), 7.63 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.54 – 7.46 (m, 4H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 16.7 Hz, 2H), 7.22 – 7.13 (m, 2H), 7.09 (d, *J* = 16.2 Hz, 1H), 6.92 (d, *J* = 16.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 2H), 2.99 (s, 6H), 2.86 (qd, *J* = 7.2, 4.3 Hz, 6H, Et_3HN^+ salt), 2.49 (s, 6H), 2.32 – 2.25 (m, 4H), 1.46 (s, 6H), 1.08 (t, J = 7.3 Hz, 9H, Et_3HN^+ salt), 0.95 (t, *J* = 7.4 Hz, 6H). **^{13}C NMR** (400 MHz, Chloroform-*d*) δ 152.4, 143.8, 140.5, 140.1, 138.6, 138.2, 135.5, 132.3, 131.4, 130.4, 129.8, 129.1, 128.4, 127.8, 127.4, 127.2, 126.6, 126.5, 124.1, 112.6, 46.2, 40.6, 29.9, 17.2, 14.9, 12.5, 11.7, 8.32 ppm. **ESI-HR(-)**, calculated for $C_{41}H_{43}BF_2N_3O_3S^-$: 706.3092, found: 706.3074. **Analytical HPLC retention time:** 8.39 min. Estimated purity >99%.

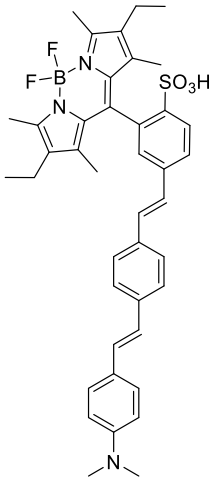




Ethyl BODIPY *p*-methoxy wire (7) To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-2,6-diethyl-*p*-bromo BODIPY **3** (44.4 mg, 0.08 mmol, 1 eq), methoxy molecular wire **5** (22.6 mg, 0.09 mmol, 1.1 eq), $Pd(OAc)_2$ (1.7 mg, 0.007 mmol, 9 mol%), and P(*o*-tol)$_3$ (4.5 mg, 0.015 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (0.6 mL) and NEt_3 (300 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (4% MeOH in DCM) yielded Et*p*OMe **7** as a reddish orange solid (17.1 mg, 25%).

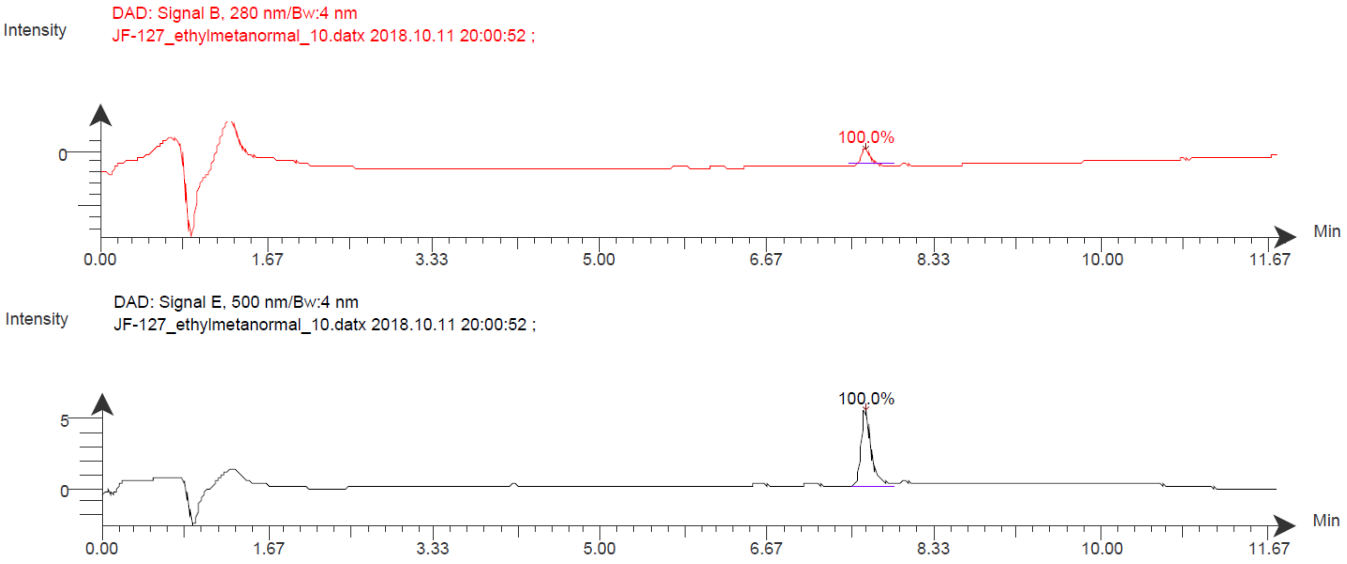
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 (s, 1H), 7.63 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.55 – 7.40 (m, 6H), 7.26 (apps, 1H), 7.19 – 7.09 (m, 2H), 6.95 (d, *J* = 16.4 Hz, 1H), 6.36 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.23 (d, *J* = 2.4 Hz, 1H), 3.90 (s, 3H), 3.00 (s, 6H), 2.91 – 2.80 (m, 5H, Et_3HN^+ salt), 2.48 (s, 6H), 2.27 (q, *J* = 7.6 Hz, 4H), 1.45 (s, 6H), 1.08 (t, *J* = 7.3 Hz, 7H, Et_3HN^+ salt), 0.95 (t, *J* = 7.5 Hz, 6H). **^{13}C NMR** (600 MHz, Chloroform-*d*) δ 158.2, 152.4, 151.5, 143.5, 140.2, 139.8, 138.8, 138.5, 135.0, 132.2, 131.3, 131.2, 130.4, 129.7, 128.2, 127.3, 127.2, 127.0, 126.4, 126.2, 124.3, 123.9, 115.2, 105.2, 95.6, 77.2, 76.8, 55.4, 46.0, 40.5, 17.0, 14.7, 12.3, 11.6, 8.2 ppm. **ESI-HR(-)**, calculated for $C_{42}H_{45}BF_2N_3O_4S^-$: 736.3197, found: 736.3183. **Analytical HPLC retention time:** 8.63 min. Estimated purity 94%.

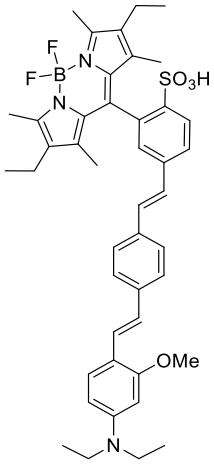




Ethyl BODIPY *m*-normal wire (15) To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-2,6-diethyl-*m*-bromo BODIPY **12** (51.6 mg, 0.09 mmol, 1 eq), molecular wire **4** (26.2 mg, 0.10 mmol, 1.1 eq), $Pd(OAc)_2$ (1.9 mg, 0.009 mmol, 9 mol%), and P(*o*-tol)$_3$ (5.2 mg, 0.017 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (660 µL) and NEt_3 (330 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (4% MeOH in DCM) yielded Et*m*H **15** as a reddish orange solid (17.3 mg, 26%).

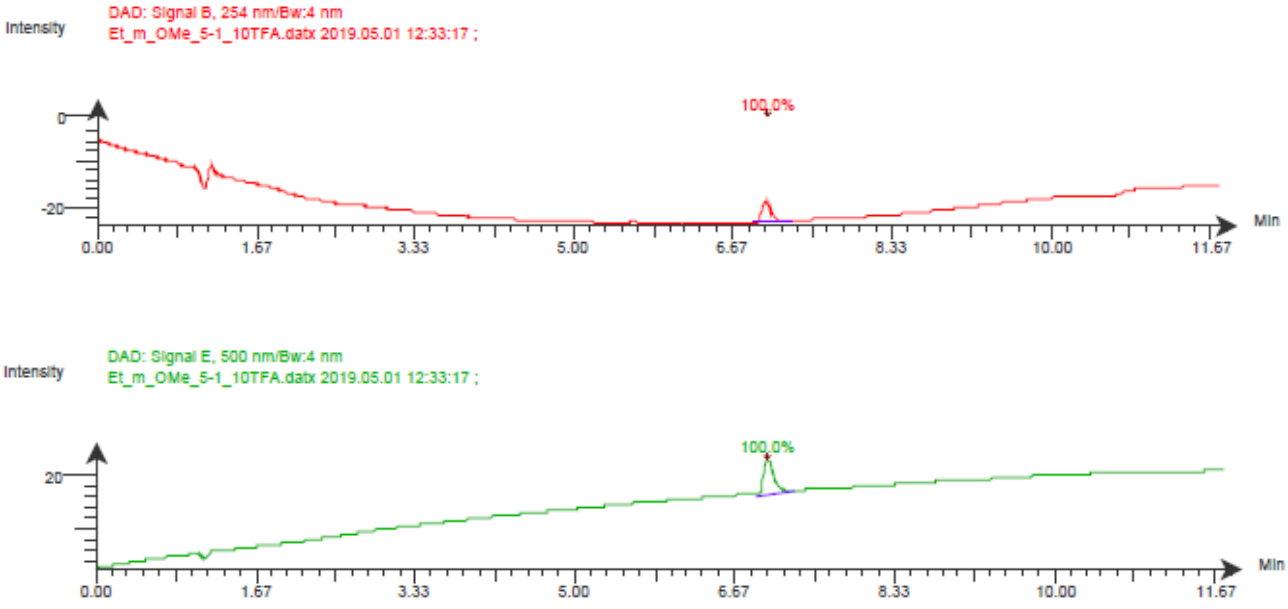
^{1}H NMR (500 MHz, Methanol-d_4) δ 8.06 (d, *J* = 8.3 Hz, 1H), 7.77 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 3H), 7.44 – 7.39 (m, 4H), 7.29 (d, *J* = 16.4 Hz, 1H), 7.19 (d, *J* = 16.4 Hz, 1H), 7.09 (d, *J* = 16.2 Hz, 1H), 6.93 (d, *J* = 16.3 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 3H), 2.96 (s, 8H), 2.47 (s, 6H), 2.34 (q, *J* = 7.5 Hz, 4H), 1.46 (s, 6H), 0.99 (t, *J* = 7.6 Hz, 6H). **^{13}C NMR** (700 MHz, Methanol-d_4) δ 153.5, 151.7, 143.6, 141.9, 141.6, 140.1, 139.7, 136.7, 134.9, 133.3, 132.6, 132.1, 130.5, 130.2, 128.7, 128.7, 128.3, 127.4, 127.3, 126.9, 124.9, 113.9, 40.8, 17.9, 15.1, 12.6, 11.93. **ESI-HR(-)**, calculated for $C_{41}H_{43}BF_2N_3O_3S^-$: 706.3092, found: 706.3077. **Analytical HPLC retention time:** 7.59 min. Estimated purity >99%.

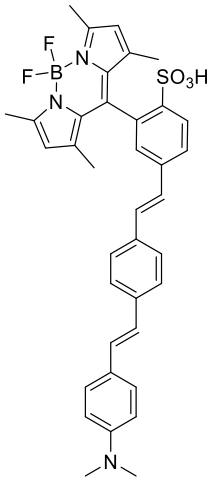




Ethyl BODIPY *m*-methoxy wire (16). To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-2,6-diethyl-*m*-bromo BODIPY **11** (49.3 mg, 0.09 mmol, 1 eq), methoxy molecular wire **14** (30.9 mg, 0.10 mmol, 1.1 eq), $Pd(OAc)_2$ (1.8 mg, 0.008 mmol, 9 mol%), and P(*o*-tol)$_3$ (5.0 mg, 0.016 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (660 µL) and NEt_3 (330 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (0 – 5% MeOH in DCM, gradient) yielded Et*m*OMe **16** as a reddish orange solid (20.0 mg, 29%).

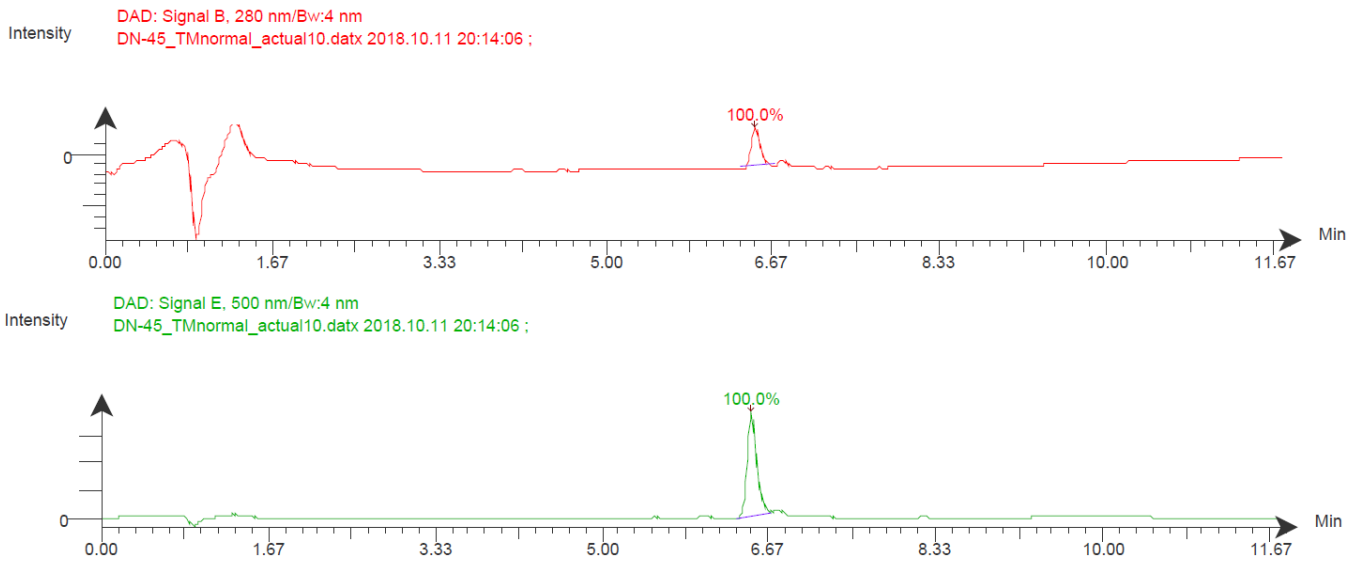
^{1}H NMR (400 MHz, Methanol-d4) δ 8.06 (d, *J* = 8.3 Hz, 1H), 7.75 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.44 – 7.34 (m, 6H), 7.26 (d, *J* = 16.4 Hz, 1H), 7.14 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 16.4 Hz, 1H), 6.33 (d, *J* = 9.1 Hz, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.40 (q, *J* = 7.1 Hz, 4H), 3.07 (q, *J* = 7.3 Hz, 4H), 2.65 (s, 10H), 2.47 (s, 6H), 2.33 (q, *J* = 7.5 Hz, 4H), 1.45 (s, 6H), 1.18 (dt, *J* = 8.6, 7.1 Hz, 12H), 0.98 (t, *J* = 7.5 Hz, 6H). **^{13}C NMR** (400 MHz, MeOD) δ 159.88, 153.62, 150.1, 143.21, 142.05, 141.56, 140.52, 140.25, 136.19, 134.78, 133.36, 132.59, 132.25, 130.43, 128.62, 128.49, 128.27, 127.46, 127.12, 126.56, 125.18, 124.35, 115.6, 106.17, 96.36, 55.87, 47.76, 45.70, 40.43 (DMSO), 17.87, 15.11, 13.00, 12.59, 11.95, 9.09 ppm. **ESI-HR(-)**, calculated for $C_{44}H_{49}BF_2N_3O_4S^-$: 764.3510, found: 764.3492. **Analytical HPLC retention time:** 6.95 min. Estimated purity >99%.

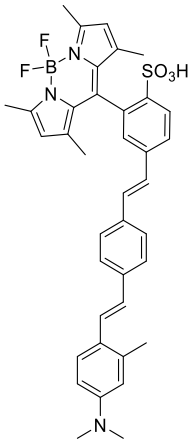




Tetramethyl BODIPY *m*-normal wire (17). To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-*m*-bromo BODIPY **12** (35.6 mg, 0.07 mmol, 1 eq), molecular wire **4** (18.1 mg, 0.07 mmol, 1.1 eq), $Pd(OAc)_2$ (0.15 mg, 0.0006 mmol, 9 mol%), and P(*o*-tol)$_3$ (0.4 mg, 0.0013 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (440 µL) and NEt_3 (220 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (0 – 4% MeOH in DCM, gradient) yielded TM*m*H **17** as an orange solid (23.1 mg, 56%).

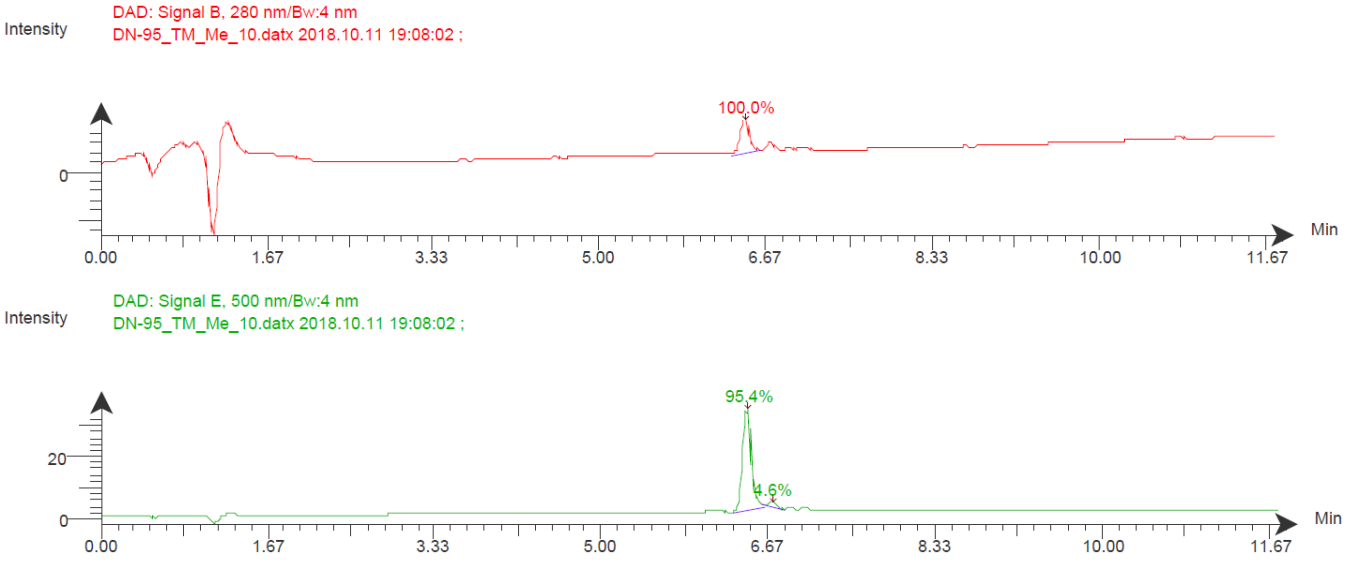
^{1}H NMR (900 MHz, Methanol-d_4) δ 8.06 (d, *J* = 8.2 Hz, 1H), 7.77 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.55 – 7.52 (m, 2H), 7.48 (dd, *J* = 9.3, 2.9 Hz, 2H), 7.43 – 7.39 (m, 3H), 7.30 (d, *J* = 16.3 Hz, 1H), 7.20 (d, *J* = 16.3 Hz, 1H), 7.09 (dd, *J* = 16.2, 2.9 Hz, 1H), 6.93 (d, *J* = 16.2 Hz, 1H), 6.77 – 6.73 (m, 2H), 5.99 (s, 2H), 2.96 (d, *J* = 1.6 Hz, 7H), 2.48 (s, 6H), 1.55 (s, 6H). **^{13}C NMR** (900 MHz, Methanol-d_4) 155.5, 151.9, 144.9, 143.4, 142.9, 142.0, 139.7, 136.6, 134.2, 133.3, 132.2, 130.5, 130.3, 128.7, 128.4, 128.3, 127.3, 126.8, 124.8, 121.6, 113.8, 40.8, 14.8, 14.6 ppm. **ESI-HR(-)**, calculated for $C_{37}H_{35}BF_2N_3O_3S^-$: 650.2466, found: 650.2457. **Analytical HPLC retention time:** 6.65 min. Estimated purity >99%.

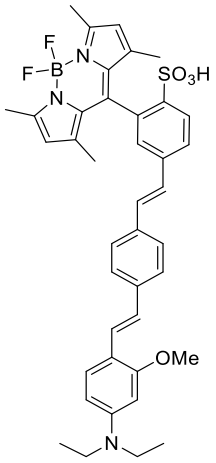




Tetramethyl BODIPY *m*-methyl wire (18). To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-*m*-bromo BODIPY **12** (50.4 mg, 0.10 mmol, 1 eq), methyl molecular wire **13** (30.2 mg, 0.11 mmol, 1.1 eq), $Pd(OAc)_2$ (1.2 mg, 0.005 mmol, 9 mol%), and P(*o*-tol)$_3$ (3.2 mg, 0.01 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (700 µL) and NEt_3 (350 µL). The Schlenk flask was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (1 – 4% MeOH in DCM, gradient) yielded TM*m*Me **18** as an orange solid (24 mg, 35%).

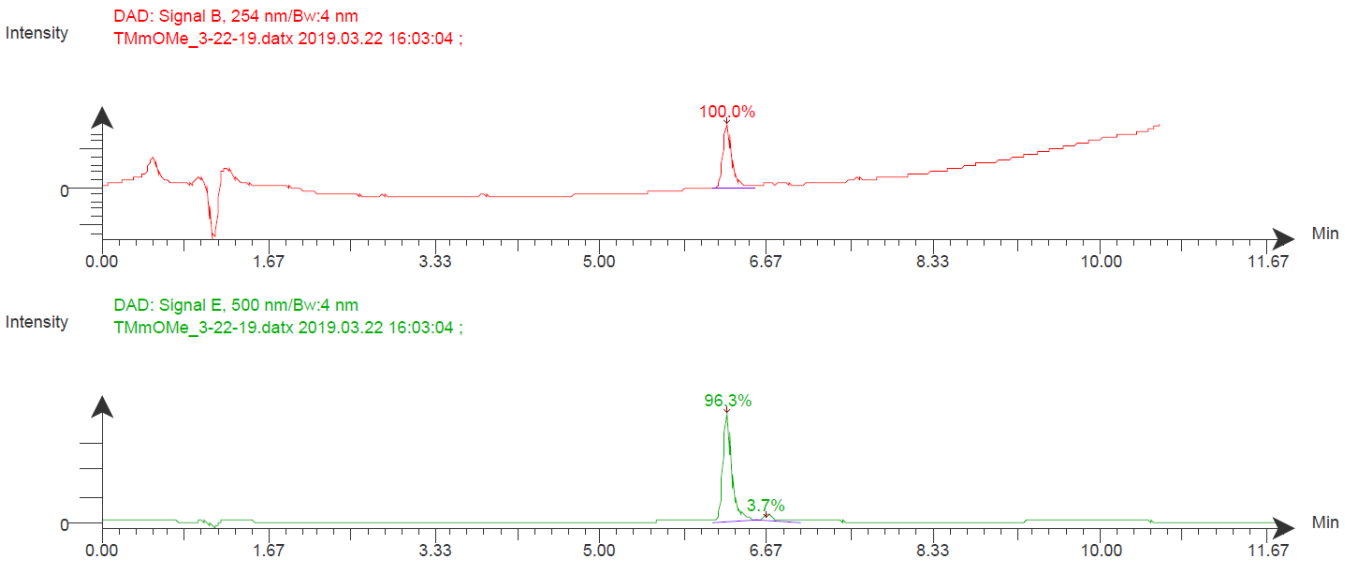
^{1}H NMR (700 MHz, DMSO-d_6) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 1H), 7.58 – 7.51 (m, 5H), 7.39 – 7.31 (m, 3H), 7.26 (d, *J* = 16.4 Hz, 1H), 6.90 (d, *J* = 16.2 Hz, 1H), 6.59 (d, *J* = 8.6 Hz, 1H), 6.54 (d, *J* = 2.7 Hz, 1H), 6.04 (s, 2H), 3.32 (s, 6H), 2.92 (s, 7H), 2.43 (s, 6H), 2.37 (s, 3H), 1.45 (s, 6H). **^{13}C NMR** (900 MHz, DMSO-d_6) δ 152.8, 149.9, 144.9, 143.0, 138.5, 137.8, 136.5, 135.2, 131.5, 129.5, 128.9, 126.9, 126.5, 126.3, 126.2, 126.1, 125.9, 124.4, 123.7, 120.2, 113.7, 110.4, 53.4, 48.6, 39.9, 20.1, 18.0, 16.70, 14.1, 13.9 ppm. **ESI-HR(-)**, calculated for $C_{38}H_{37}BF_2N_3O_3S^-$: 664.2622, found: 664.2612. **Analytical HPLC retention time:** 6.50 min. Estimated purity 95%.

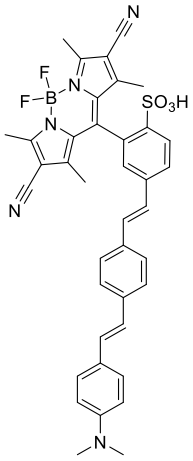




Tetramethyl BODIPY *m*-methoxy wire (19). To a flame-dried 10 mL Schlenk flask were added 1,3,5,7-tetramethyl-*m*-bromo BODIPY **12** (51.1 mg, 0.09 mmol, 1 eq), methoxy molecular wire **14** (30.2 mg, 0.10 mmol, 1.1 eq), $Pd(OAc)_2$ (1.1 mg, 0.005 mmol, 9 mol%), and P(*o*-tol)$_3$ (2.9 mg, 0.01 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (630 µL) and NEt_3 (310 µL). The Schlenk flask was sealed shut and heated to 100 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (0 – 4% MeOH in DCM, gradient) yielded TM*m*OMe **19** as an orange solid (30.5 mg, 62%).

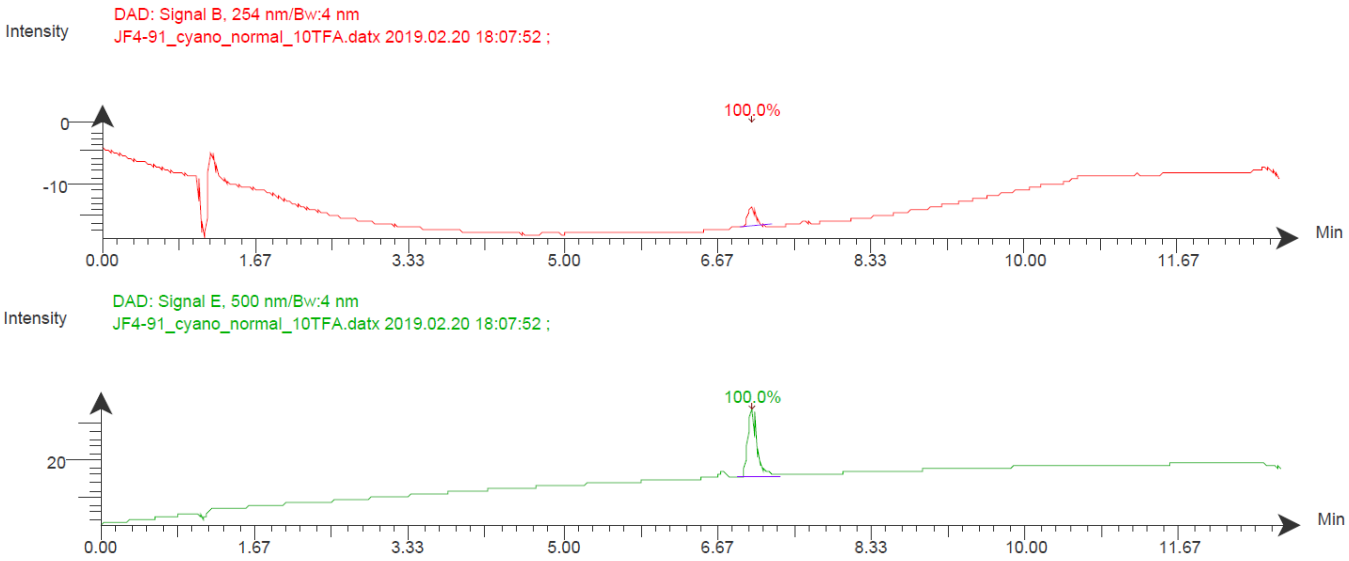
^{1}H NMR (300 MHz, Methanol-d_4) δ 8.06 (d, J = 8.3 Hz, 1H), 7.75 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.45 – 7.33 (m, 5H), 7.26 (d, *J* = 16.4 Hz, 1H), 7.14 (d, *J* = 16.3 Hz, 1H), 6.89 (d, *J* = 16.5 Hz, 1H), 6.32 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.24 (d, *J* = 2.3 Hz, 1H), 5.99 (s, 2H), 3.86 (s, 3H), 3.40 (q, *J* = 7.0 Hz, 4H), 2.48 (s, 6H), 1.53 (s, 6H), 1.18 (t, *J* = 7.0 Hz, 6H). **^{13}C NMR** (400 MHz, Methanol-d_4) δ 159.9, 155.5, 150.2, 144.9, 143.2, 142.9, 142.0, 140.6, 136.2, 134.1, 133.3, 132.3, 130.4, 128.3, 127.5, 127.1, 126.5, 125.2, 124.2, 121.6, 106.0, 96.2, 55.9, 45.6, 14.63, 14.56, 13.0 ppm. **ESI-HR(-)**, calculated for $C_{40}H_{41}BF_2N_3O_4S^-$: 708.2884, found: 708.2873. **Analytical HPLC retention time:** 6.16 min. Estimated purity 96%.

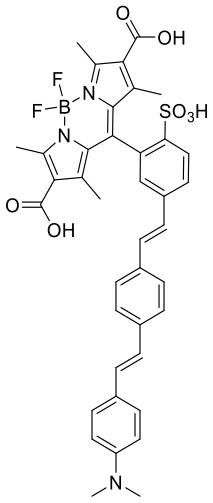




2,6-cyano BODIPY *m*-normal wire (22). To a flame-dried 4 mL dram vial were added 1,3,5,7-tetramethyl-2,6-cyano-*m*-bromo BODIPY **21** (29.2 mg, 0.05 mmol, 1 eq), molecular wire **4** (15.0 mg, 0.06 mmol, 1.1 eq) and $Pd(dba)_2$ (12.5 mg, 0.014 mmol, 25 mol%). The vial was evacuated and backfilled with N_2 3x before addition of 1M $P(tBu)_3$ in toluene (27 µL, 0.03 mmol, 50 mol%) and DMF (1.1 mL). The vial was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (7 mL) and IPA (3 mL). Washed with water (3 x 10 mL), washed with sat. aq. sodium bicarbonate (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (10% MeOH in DCM) yielded CN*m*H **22** as a yellowish solid (2.1 mg, 6%).

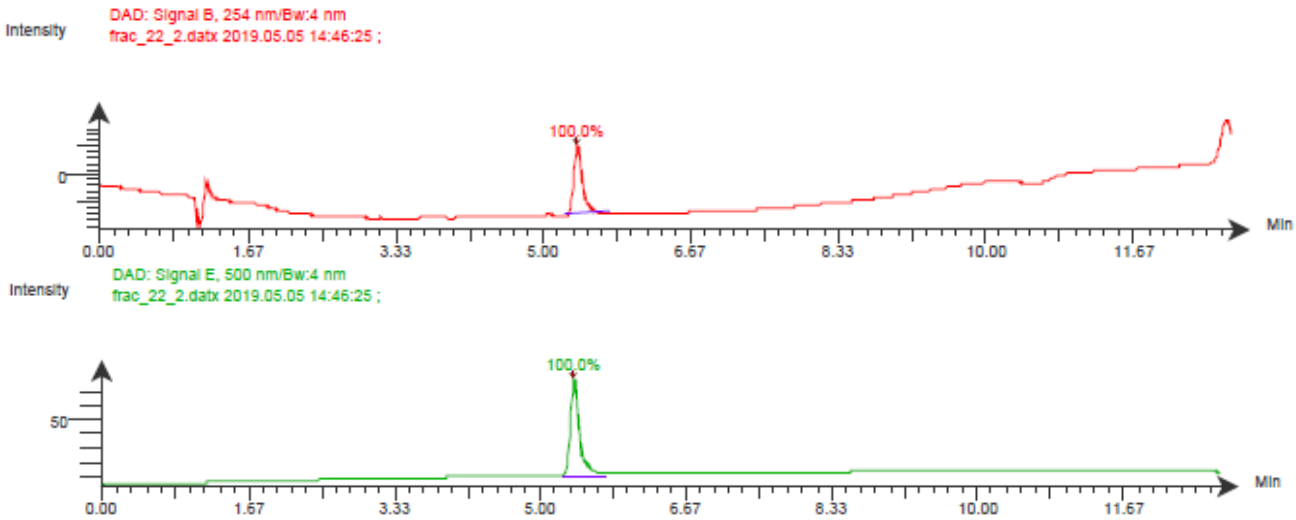
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.11 (d, J = 8.3 Hz, 1H), 7.93 – 7.86 (m, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.52 – 7.45 (m, 3H), 7.45 – 7.32 (m, 3H), 7.23 (d, J = 16.4 Hz, 1H), 7.11 (d, J = 16.2 Hz, 1H), 6.93 (d, J = 16.3 Hz, 1H), 6.76 (d, J = 8.6 Hz, 2H), 2.97 (s, 6H), 2.67 (s, 6H), 1.74 (s, 6H). **^{13}C NMR** (400 MHz, Methanol-d_4) δ 159.6, 151.9, 151.0, 143.0, 142.8, 140.0, 136.5, 133.0, 131.8, 130.8, 130.4, 128.8, 128.7, 128.4, 127.3, 127.0, 126.3, 124.7, 114.7, 113.8, 49.6, 49.4, 49.2, 48.8, 48.6, 48.4, 40.7, 14.0, 13.7 ppm. **ESI-HR(-)**, calculated for $C_{39}H_{33}BF_2N_5O_3S^-$: 700.2371, found: 700.2356. **Analytical HPLC retention time:** 6.94 min. Estimated purity >99%.

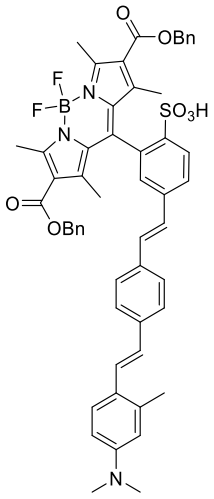




2,6-carboxy BODIPY m-normal wire (28). To a flame-dried 4 mL dram vial were added 1,3,5,7-tetramethyl-2,6-carboxy-*m*-bromo BODIPY **24** (42.6 mg, 0.07 mmol, 1 eq), molecular wire **4** (20.5 mg, 0.08 mmol, 1.1 eq) and $Pd_2(dba)_2$ (13.7 mg, 0.014 mmol, 20 mol%). The vial was evacuated and backfilled with N_2 3x before addition of 1M $P(tBu)_3$ in toluene (30 µL, 0.03 mmol, 50 mol%) and DMF (1.5 mL). The vial was sealed shut and heated to 70 °C overnight. The DMF was removed *in vacuo*. Preparatory thin layer chromatography (12% MeOH + 1% AcOH in DCM) yielded carboxy*m*H **28** as a yellow-orange solid (3.1 mg, 6%). This material was 93% pure by analytical HPLC. For NMR and spectroscopic characterization, a small amount was further purified by reverse phase preparative HPLC (10-100% MeCN in water, 0.05% formic acid additive).

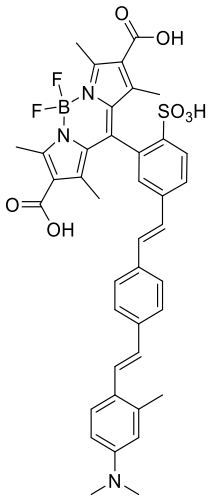
^{1}H NMR (900 MHz, DMSO-d_6) δ 7.88 (d, J = 8.1 Hz, 1H), 7.74 (dd, J = 8.4, 1.8 Hz, 1H), 7.57 – 7.50 (m, 4H), 7.46 – 7.41 (m, 3H), 7.39 (d, J = 16.4 Hz, 1H), 7.27 (d, J = 16.4 Hz, 1H), 7.16 (d, J = 16.3 Hz, 1H), 6.96 (d, J = 16.3 Hz, 1H), 6.72 (d, J = 8.5 Hz, 2H), 2.93 (s, 7H), 2.71 (s, 6H), 1.73 (s, 6H). **ESI-HR(-)**, calculated for $C_{39}H_{35}BF_2N_3O_7S^-$: 738.2262, found: 738.2241. **Analytical HPLC retention time:** 5.30 min. Estimated purity >99%.





OBn methyl wire (26). To a flame-dried 10 mL Schlenk flask were added OBn BODIPY **24** (107 mg, 0.1 mmol, 1 eq), methoxy molecular wire **14** (41.3 mg, 0.16 mmol, 1.1 eq), $Pd(OAc)_2$ (2.9 mg, 0.013 mmol, 9 mol%), and P(*o*-tol)$_3$ (7.8 mg, 0.026 mmol, 18 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (2 mL) and NEt_3 (1 mL). The Schlenk flask was sealed shut and heated to 100 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (3 – 7% MeOH in DCM, gradient) yielded OBn*m*Me **26** as an orange solid (39.3 mg, 30%).

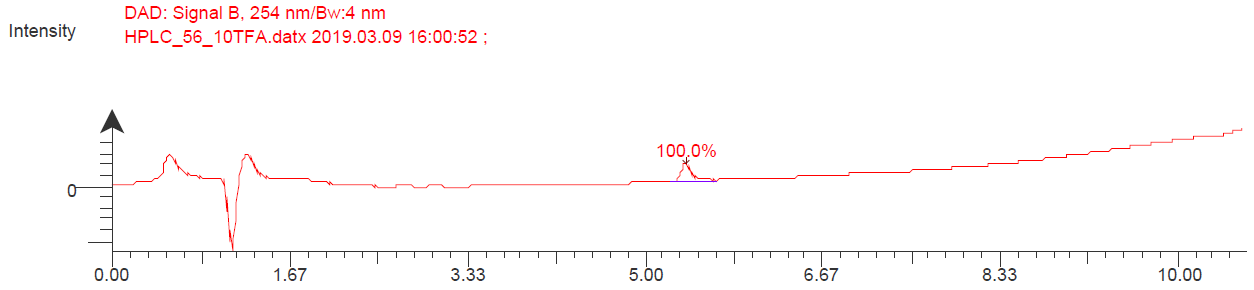
^{1}H NMR (900 MHz, Methanol-*d*$_4$) δ 7.98 (d, *J* = 8.2 Hz, 1H), 7.59 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.34 (s, 4H), 7.30 (d, *J* = 7.1 Hz, 4H), 7.27 (td, *J* = 8.9, 8.1, 2.4 Hz, 6H), 7.25 – 7.21 (m, 2H), 7.16 (d, *J* = 1.7 Hz, 1H), 7.10 (d, *J* = 16.2 Hz, 1H), 6.99 (d, *J* = 16.2 Hz, 1H), 6.75 (d, *J* = 16.0 Hz, 1H), 6.54 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.49 (d, *J* = 2.6 Hz, 1H), 5.16 (s, 4H), 2.86 (s, 6H), 2.74 (s, 7H), 2.32 (s, 3H), 1.69 (s, 6H). **^{13}C NMR** (900 MHz, Methanol-*d*$_4$) δ 165.6, 159.8, 151.6, 149.2, 147.3, 142.9, 142.2, 140.0, 138.0, 137.5, 136.2, 133.3, 133.3, 132.6, 130.7, 129.6, 129.3, 129.2, 128.3, 128.1, 127.7, 127.7, 127.4, 127.2, 126.5, 126.1, 126.0, 122.6, 118.2, 115.3, 112.0, 67.1, 49.9, 40.8, 30.8, 30.8, 30.7, 20.7, 15.42, 15.41, 15.39, 14.5, 14.2 ppm. **ESI-HR(-)**, calculated for $C_{54}H_{49}BF_2N_3O_7S^-$: 932.3358, found: 932.3369. **Analytical HPLC retention time:** 8.91 min.

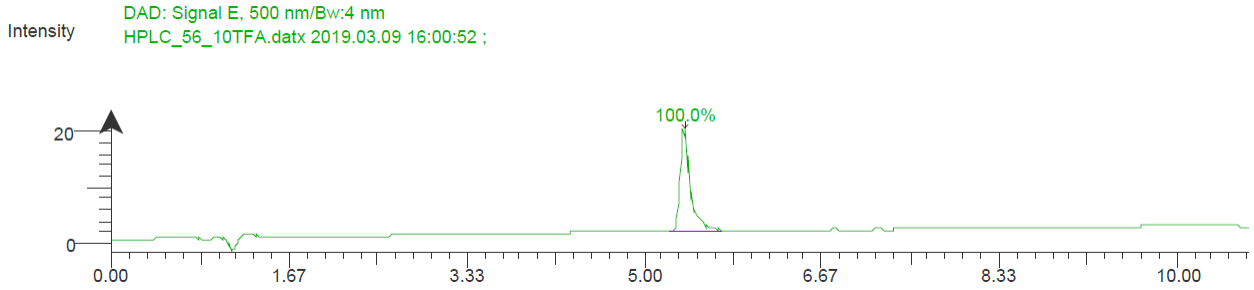


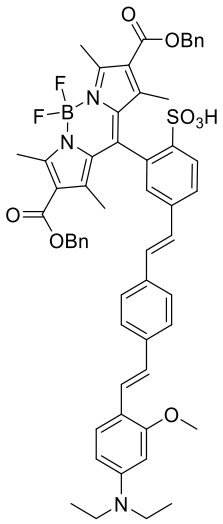
2,6-carboxy BODIPY *m*-Me wire (29). To a flame-dried 10 mL Schlenk flask were added $Pd(OAc)_2$ (1.4 mg, 0.019 mmol, 9 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DCM (230 µL), Et_3SiH (26 µL, 0.161 mmol, 2.4 eq), and NEt_3 (3 µL, 0.019, 28 mol%). The mixture was stirred for 15 minutes at rt, then **26** (62.6 mg, 0.067 mmol, 1 eq) was dissolved in DCM and transferred to the

reaction via syringe (200µL + 200µL rinse). The Schlenk flask was sealed shut at room temperature for 4 hours. The reaction mixture was quenched with saturated aqueous NH_4Cl (2 mL), extracted with DCM (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Preparatory thin layer chromatography (15% MeOH and 1% AcOH in DCM) yielded carboxy*m*Me **29** as a yellow-orange solid (8.8 mg, 31%).

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.07 (d, J = 8.2 Hz, 1H), 7.81 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (dd, J = 7.8, 5.0 Hz, 1H), 7.57 – 7.43 (m, 6H), 7.35 (d, J = 16.2 Hz, 1H), 7.32 (d, J = 16.4 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 6.87 (d, J = 16.1 Hz, 1H), 6.64 (dd, J = 8.7, 2.8 Hz, 1H), 6.57 (d, J = 2.7 Hz, 1H), 2.94 (s, 6H), 2.74 (s, 7H), 2.39 (s, 3H), 1.80 (s, 6H). **ESI-HR(-)**, calculated for $C_{40}H_{37}BF_2N_3O_7S^-$: 752.2419, found: 752.2411. **Analytical HPLC retention time:** 5.28 min. Estimated purity >99%.



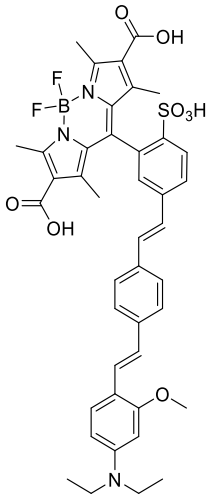




OBn methoxy wire (27). To a flame-dried 25 mL Schlenk flask were added OBn BODIPY **24** (608 mg, 0.81 mmol, 1 eq), methoxy molecular wire **14** (274 mg, 0.89 mmol, 1.1 eq), $Pd(OAc)_2$ (5.5 mg, 0.024 mmol, 3 mol%), and P(*o*-tol)$_3$ (14.8 mg, 0.049 mmol, 6 mol%). The flask was evacuated and backfilled with N_2 3x before addition of DMF (10.8 mL) and NEt_3 (5.4 mL). The schlenk flask was sealed shut and heated to 100 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash

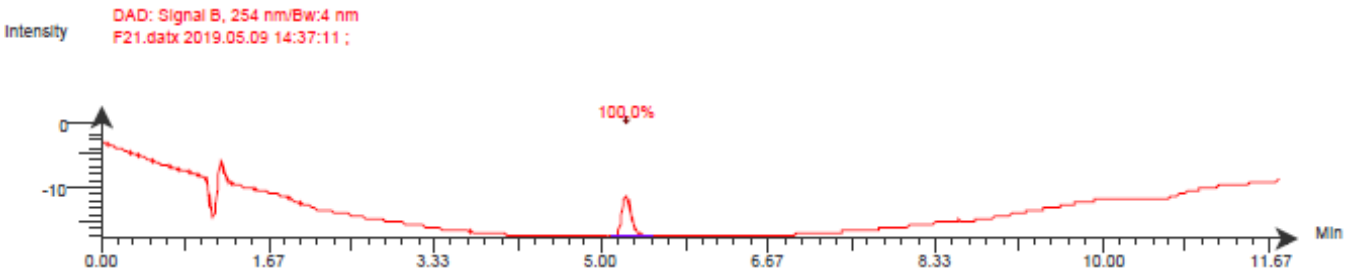
chromatography on silica gel (4 –10% MeOH in DCM, gradient) yielded OBn*m*OMe **27** as an orange solid (336.8 mg, 43%).

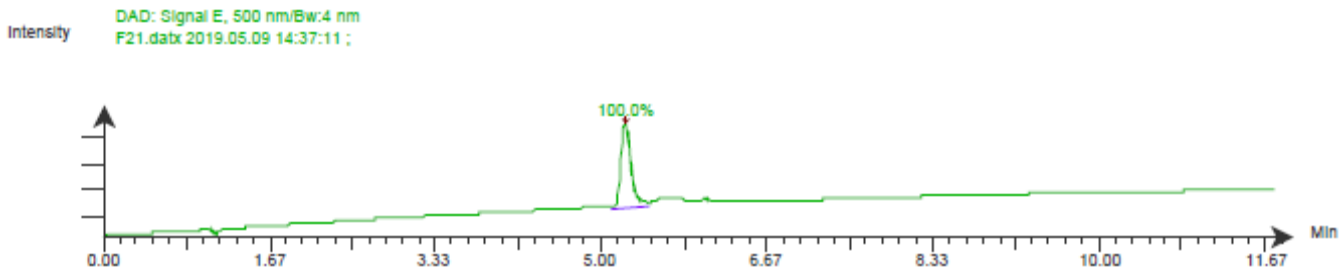
^{1}H NMR (400 MHz, Acetone-d_6) δ 8.06 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.30 (s, 17H), 7.27 (d, J = 7.9 Hz, 1H), 6.97 (d, J = 16.4 Hz, 1H), 6.33 (dd, J = 8.7, 2.5 Hz, 1H), 6.29 (d, J = 2.4 Hz, 1H), 5.27 (s, 4H), 3.88 (s, 3H), 3.44 (q, J = 7.0 Hz, 4H), 2.77 (s, 7H), 1.86 (s, 7H), 1.17 (t, J = 7.0 Hz, 6H). **ESI-HR(-)**, calculated for $C_{56}H_{53}BF_2N_3O_8S^-$: 976.3620, found: 976.3606. **Analytical HPLC retention time:** 8.27 min.

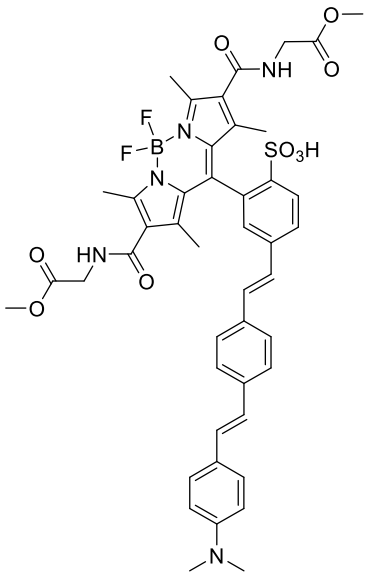


2,6-carboxy BODIPY *m*-methoxy wire (30). To a flame-dried 20 mL scintillation vial was added $Pd(OAc)_2$ (10.4 mg, 0.027 mmol). The vial was evacuated and backfilled with N_2 3x before addition of Et_3SiH (250 µL, 0.9 mmol), NEt_3 (20 µL, 0.086 mmol), and DCM (2.9 mL). This 5x stock solution was stirred at rt for 15 min. To another flame-dried 20 mL scintillation vial equipped with a stir bar was added **27** (67.2 mg, 0.07 mmol, 1 eq). The vial was evacuated and backfilled with N_2 3x before addition of the 1/5 of the stock solution total volume (640 µL). The vial was sealed and stirred at room temperature overnight. The reaction mixture was quenched with saturated aqueous NH_4Cl (3 mL), extracted with 3:1 DCM:IPA (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (5 – 15% MeOH + 1% AcOH in DCM) yielded carboxy*m*OMe **30** as a yellow-orange solid (7.5 mg, 14%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, J = 8.2 Hz, 1H), 7.73 – 7.66 (m, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.43 (t, J = 8.4 Hz, 3H), 7.38 – 7.27 (m, 3H), 7.24 (d, J = 16.5 Hz, 1H), 6.92 (d, J = 16.4 Hz, 1H), 6.28 (dd, J = 8.9, 2.3 Hz, 1H), 6.20 (d, J = 2.3 Hz, 1H), 3.83 (s, 3H), 2.69 (s, 6H), 1.72 (s, 6H), 1.10 (d, J = 7.0 Hz, 6H). **ESI-HR(-)**, calculated for $C_{42}H_{41}BF_2N_3O_8S^-$: 796.2681, found: 796.2669. **Analytical HPLC retention time:** 5.19 min. Estimated purity >99%.

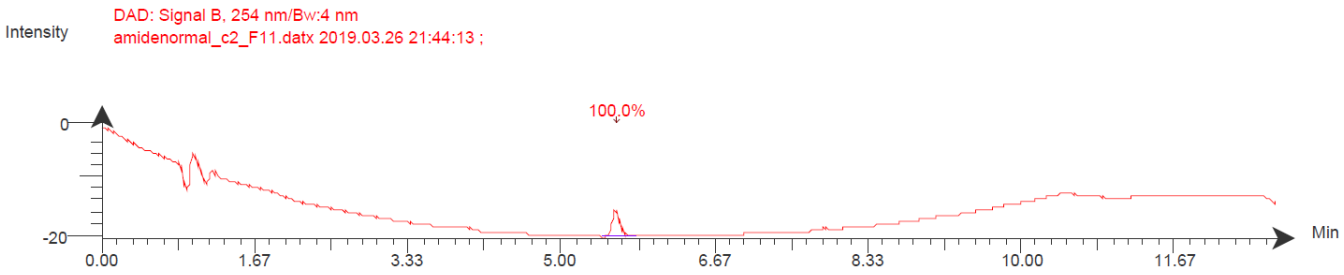


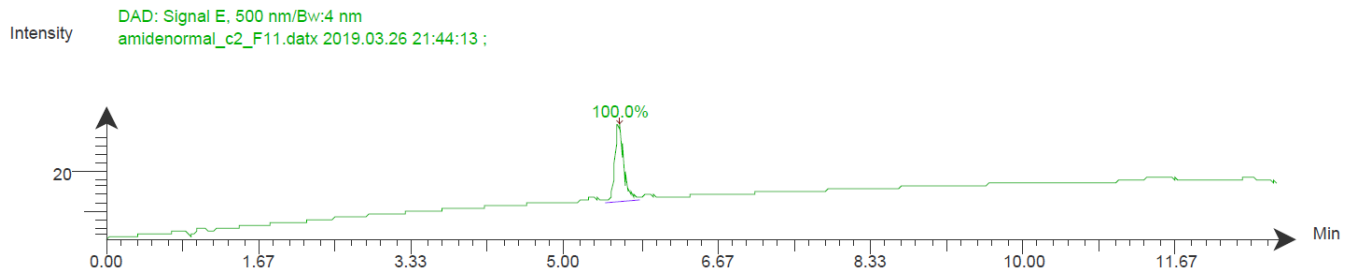


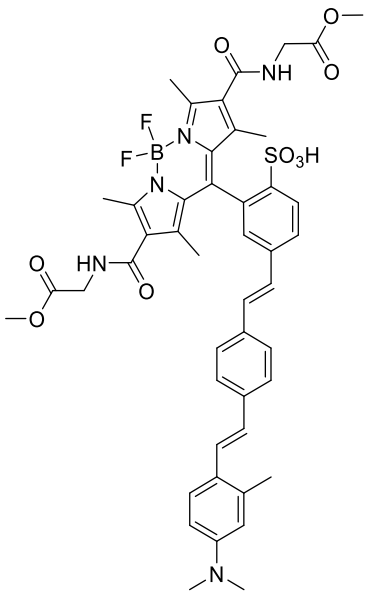


2,6-amide BODIPY *m*-normal wire (35). To a flame-dried 4 mL dram vial were added amide BODIPY **34** (15.6 mg, 0.02 mmol, 1 eq), molecular wire **4** (6.5 mg, 0.03 mmol, 1.1 eq), $Pd(OAc)_2$ (1.2 mg, 0.005 mmol, 25 mol%), and P(*o*-tol)$_3$ (3.3 mg, 0.011 mmol, 50 mol%). The vial was evacuated and backfilled with N_2 3x before addition of DMF (350 µL) and NEt_3 (150 µL). The dram vial was sealed shut and heated to 100 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with saturated NH_4Cl in water (10 mL), water (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (10% MeOH in DCM) yielded amide*m*H **35** as a yellow-orange solid (4.1 mg, 21%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (d, *J* = 8.2 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.58 – 7.50 (m, 4H), 7.43 (d, *J* = 8.8 Hz, 2H), 7.36 (s, 2H), 7.28 (d, *J* = 16.5 Hz, 1H), 7.16 (d, *J* = 16.3 Hz, 1H), 6.96 (d, *J* = 16.4 Hz, 1H), 6.72 (d, *J* = 8.8 Hz, 2H), 3.99 – 3.86 (m, 4H), 3.62 (s, 6H), 2.93 (s, 6H), 2.55 (s, 6H), 1.55 (s, 6H). **ESI-HR(-)**, calculated for $C_{40}H_{41}BF_2N_3O_4S^-$: 880.3005, found: 880.2984. **Analytical HPLC retention time:** 5.47 min. Estimated purity >99%.

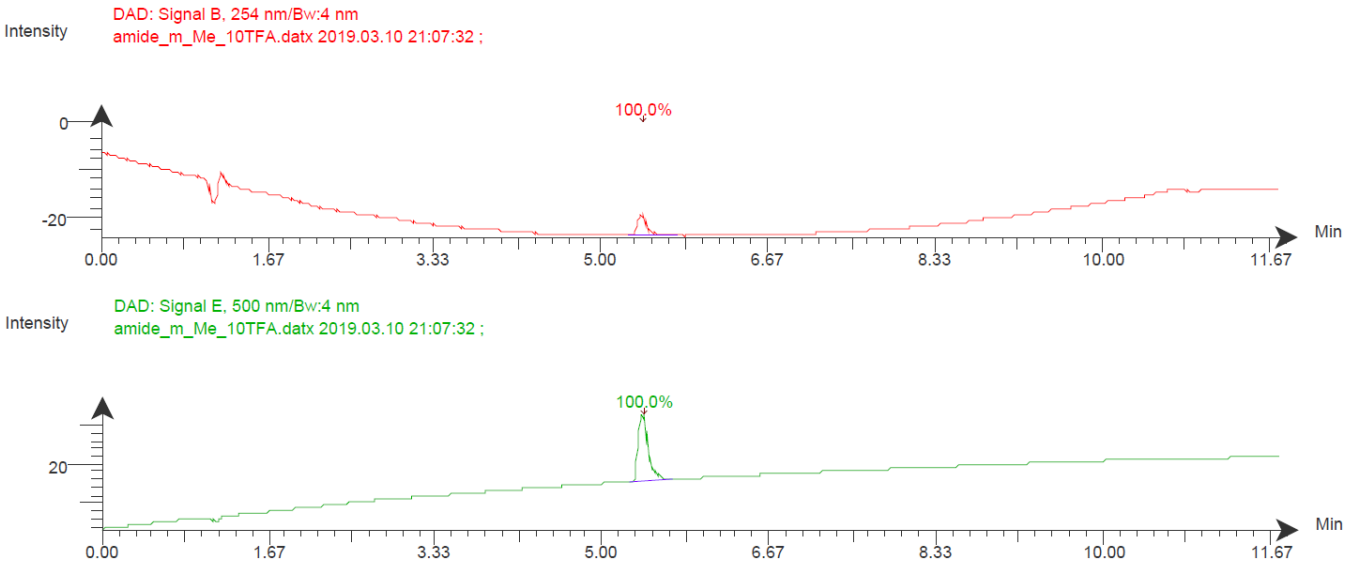






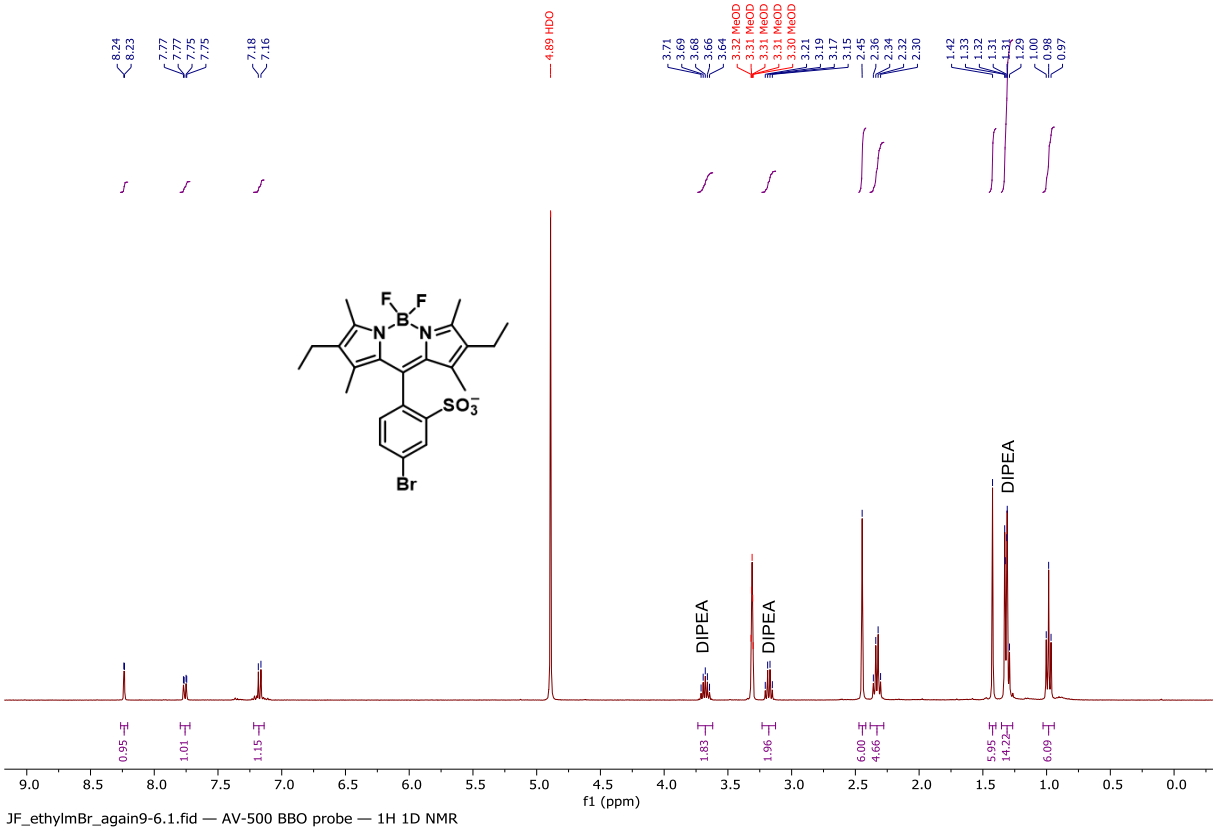
2,6-amide BODIPY m-Me wire (36). To a flame-dried 4 mL dram vial were added amide BODIPY **34** (21.0 mg, 0.03 mmol, 1 eq), methyl molecular wire **13** (9.3 mg, 0.04 mmol, 1.1 eq), $Pd(OAc)_2$ (1.7 mg, 0.007 mmol, 25 mol%), and P(*o*-tol)$_3$ (4.5 mg, 0.015 mmol, 50 mol%). The vial was evacuated and backfilled with N_2 3x before addition of DMF (400 µL) and NEt_3 (300 µL). The dram vial was sealed shut and heated to 100 °C overnight. The DMF was removed *in vacuo* and the residue dissolved in DCM (10 mL). Washed with water (10 mL), and the water layer was extracted with DCM (3 x 10 mL). The organics were combined, washed with brine (1 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Flash chromatography on silica gel (15%-20% MeOH in DCM) yielded amide*m*Me **6** as a yellow-orange solid (8.7 mg, 34%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.10 (d, *J* = 8.3 Hz, 1H), 7.84 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.59 – 7.46 (m, 6H), 7.35 (dd, *J* = 16.3, 8.3 Hz, 2H), 7.23 (d, *J* = 16.4 Hz, 1H), 6.88 (d, *J* = 16.1 Hz, 1H), 6.64 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.58 (d, *J* = 2.6 Hz, 1H), 4.05 (d, *J* = 2.5 Hz, 4H), 3.72 (s, 6H), 2.95 (s, 6H), 2.62 (s, 6H), 2.40 (s, 3H), 1.68 (s, 6H). **^{13}C NMR** (700 MHz, Methanol-d_4) δ 171.73, 168.41, 155.64, 151.91, 146.24, 143.29, 142.58, 140.22, 138.07, 136.78, 133.51, 133.15, 132.70, 129.35, 129.33, 128.49, 127.91, 127.82, 127.54, 127.26, 126.78, 126.34, 49.68, 42.17, 40.91, 13.61, 13.31, 13.29. **ESI-HR(-)**, calculated for $C_{46}H_{47}BF_2N_5O_9S^-$: 894.3161, found: 894.3149. **Analytical HPLC retention time:** 5.24 min. Estimated purity >99%.

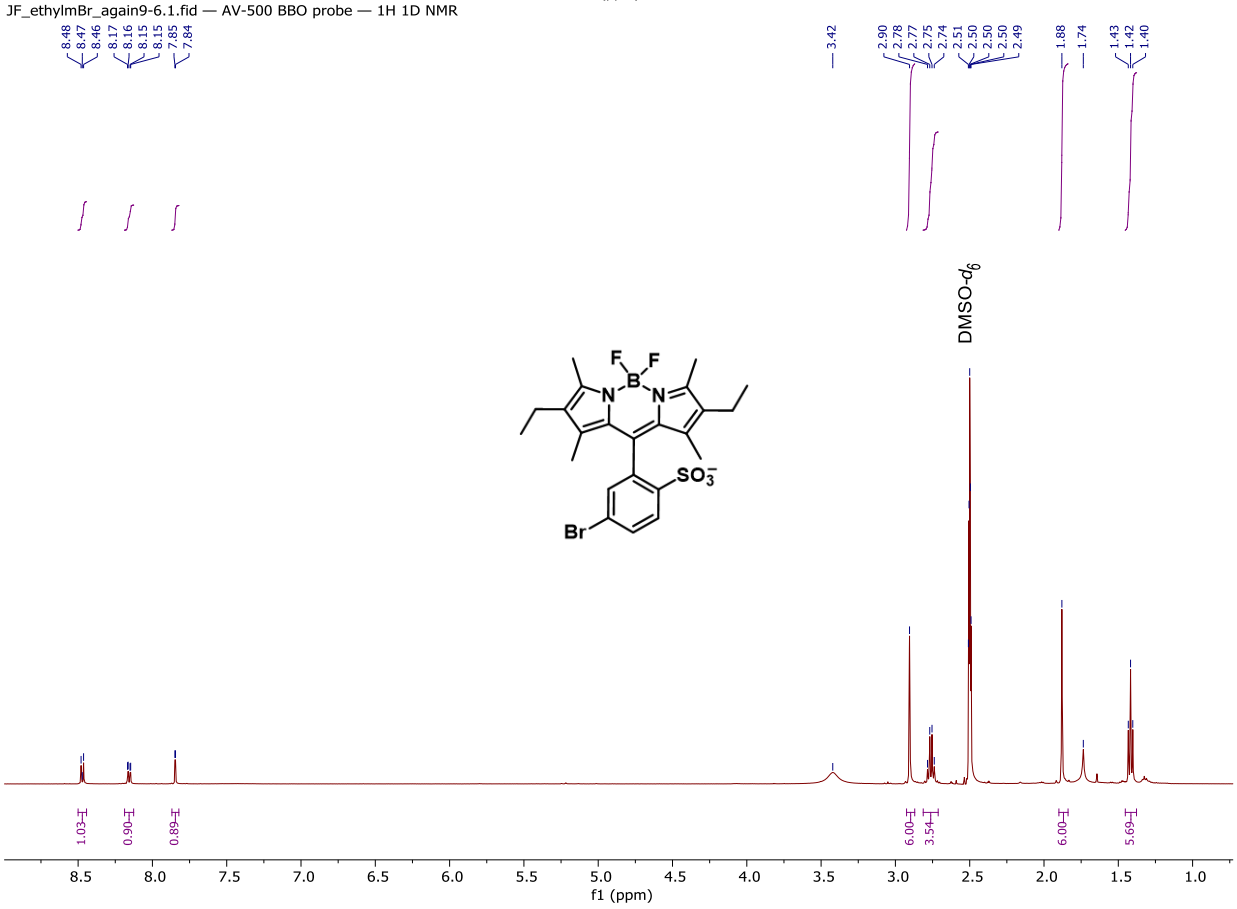

Intensity
DAD: Signal B, 254 nm/Bw:4 nm
amide_m_Me_10TFA.datx 2019.03.10 21:07:32 ;
100.0%
0
-20
0.00
1.67
3.33
5.00
6.67
8.33
10.00
11.67
Min
Intensity
DAD: Signal E, 500 nm/Bw:4 nm
amide_m_Me_10TFA.datx 2019.03.10 21:07:32 ;
100.0%
20
0.00
1.67
3.33
5.00
6.67
8.33
10.00
11.67
Min

Spectra of Compounds

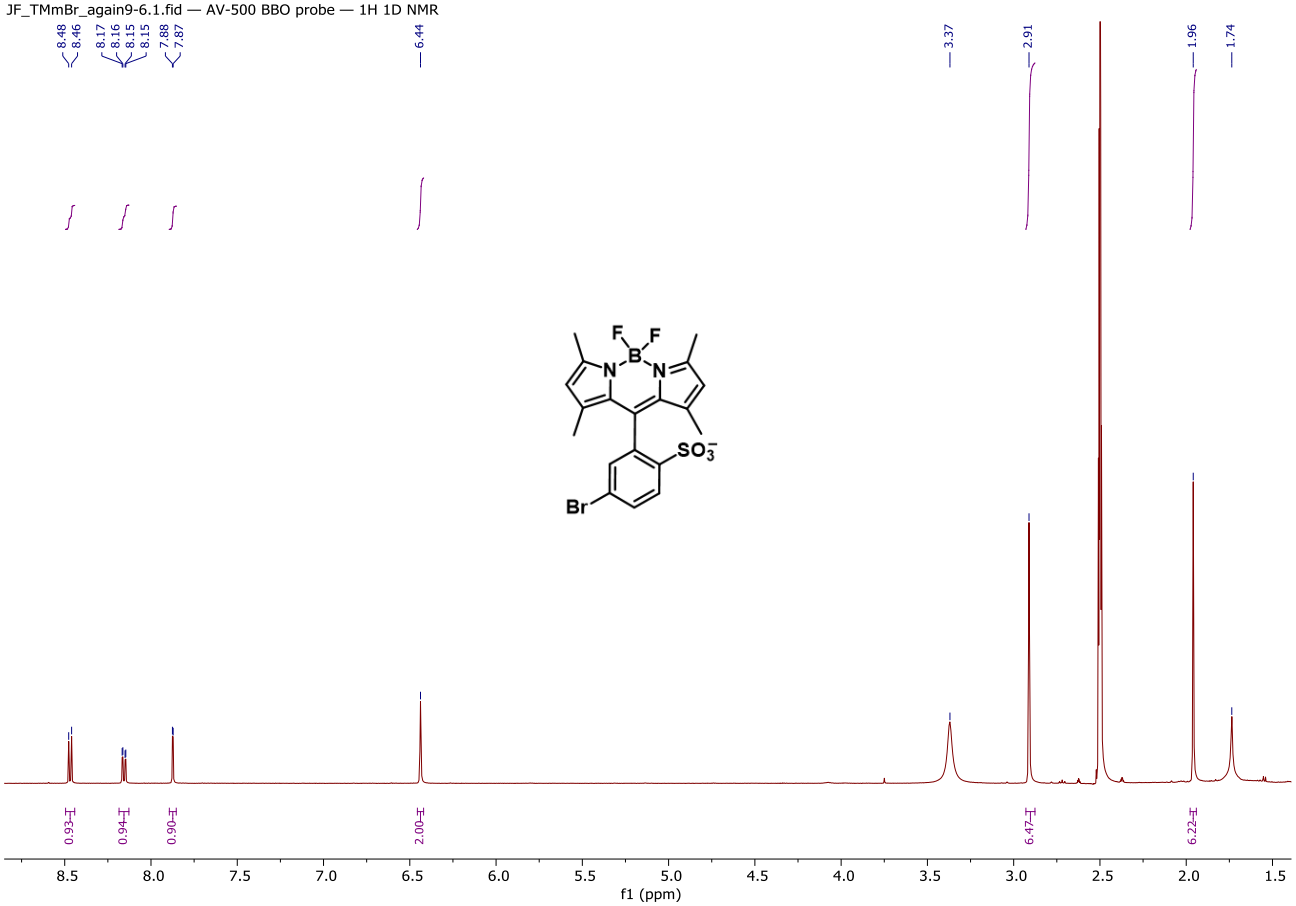
JF3-120_ethylpBrclean.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



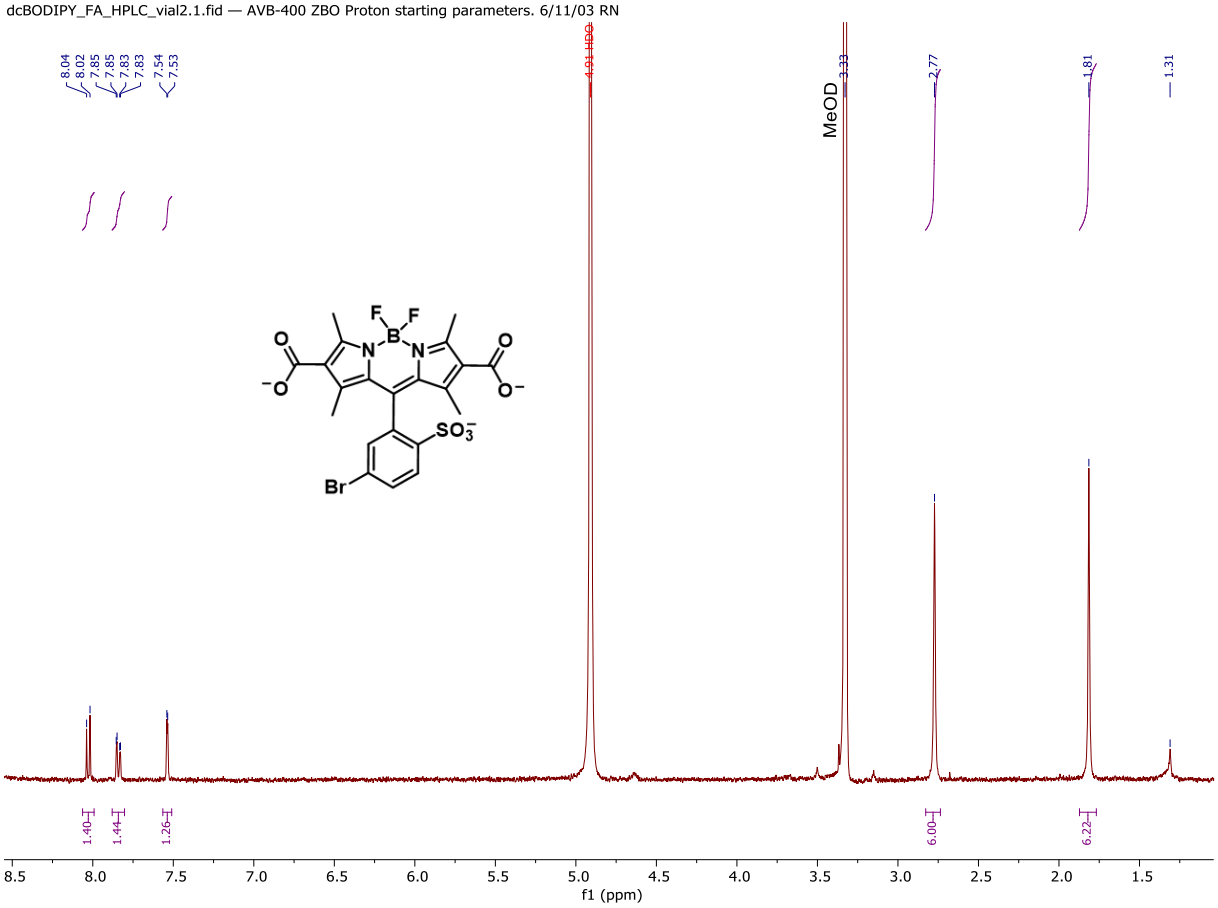
JF_ethylmBr_again9-6.1.fid — AV-500 BBO probe — 1H 1D NMR



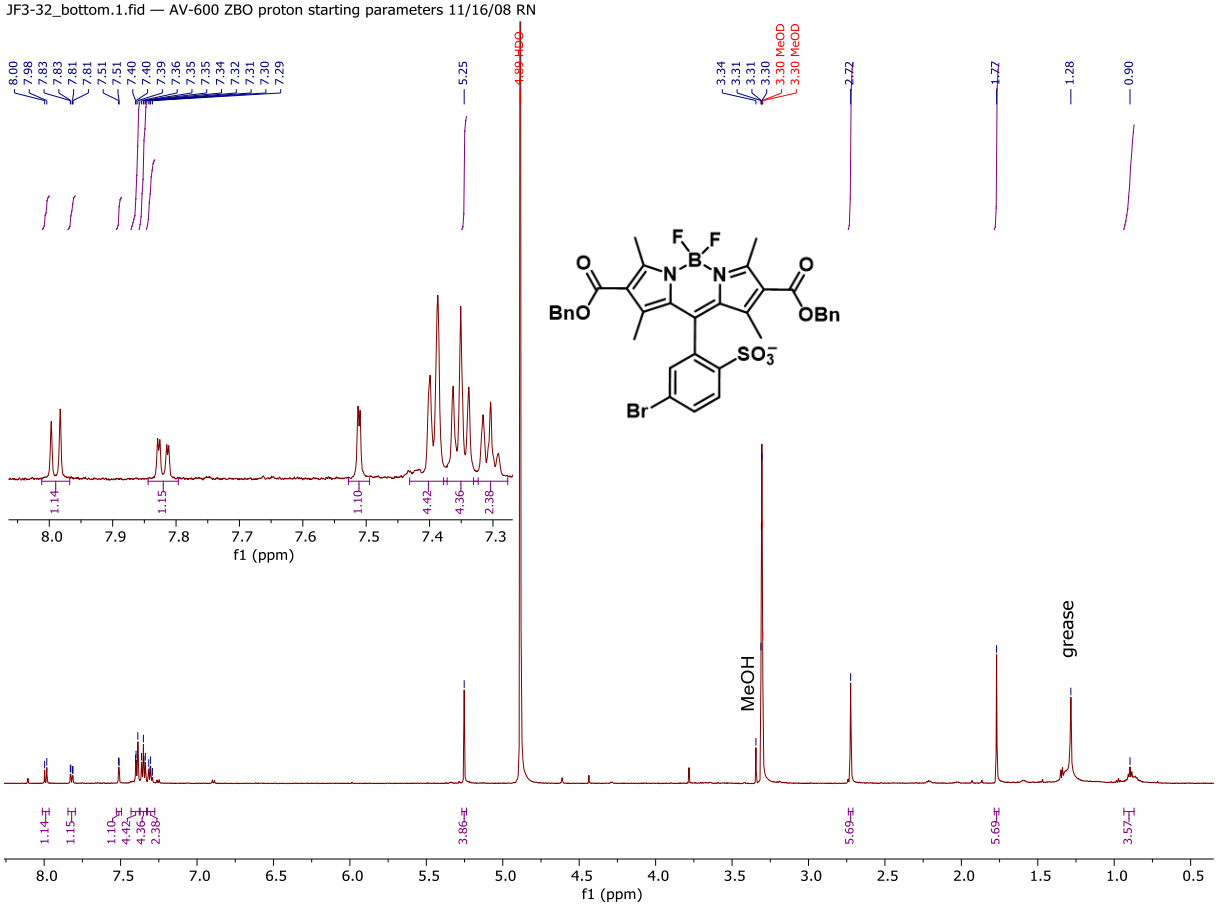
JF_TMmBr_again9-6.1.fid — AV-500 BBO probe — 1H 1D NMR



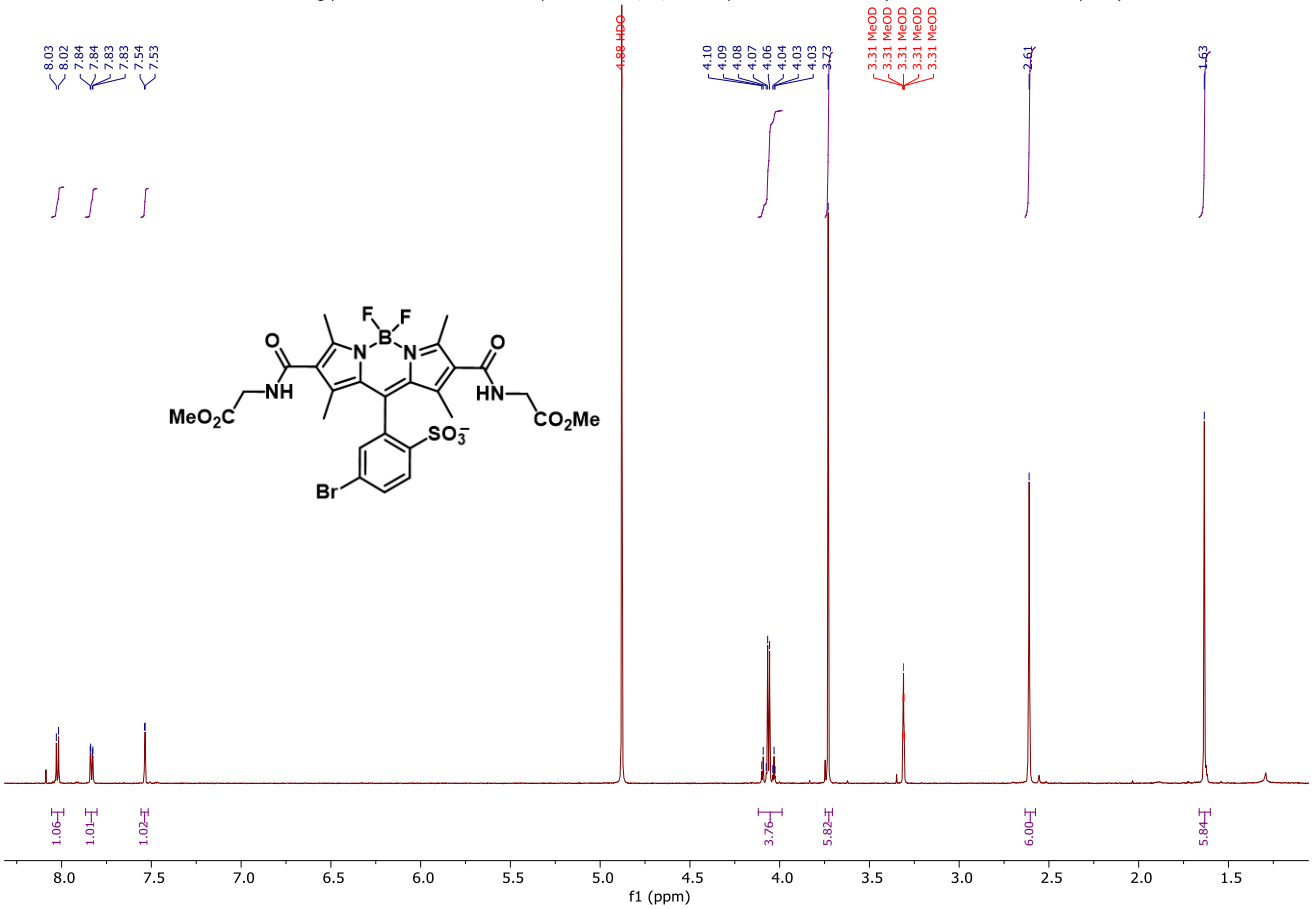
dcBODIPY_FA_HPLC_vial2.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



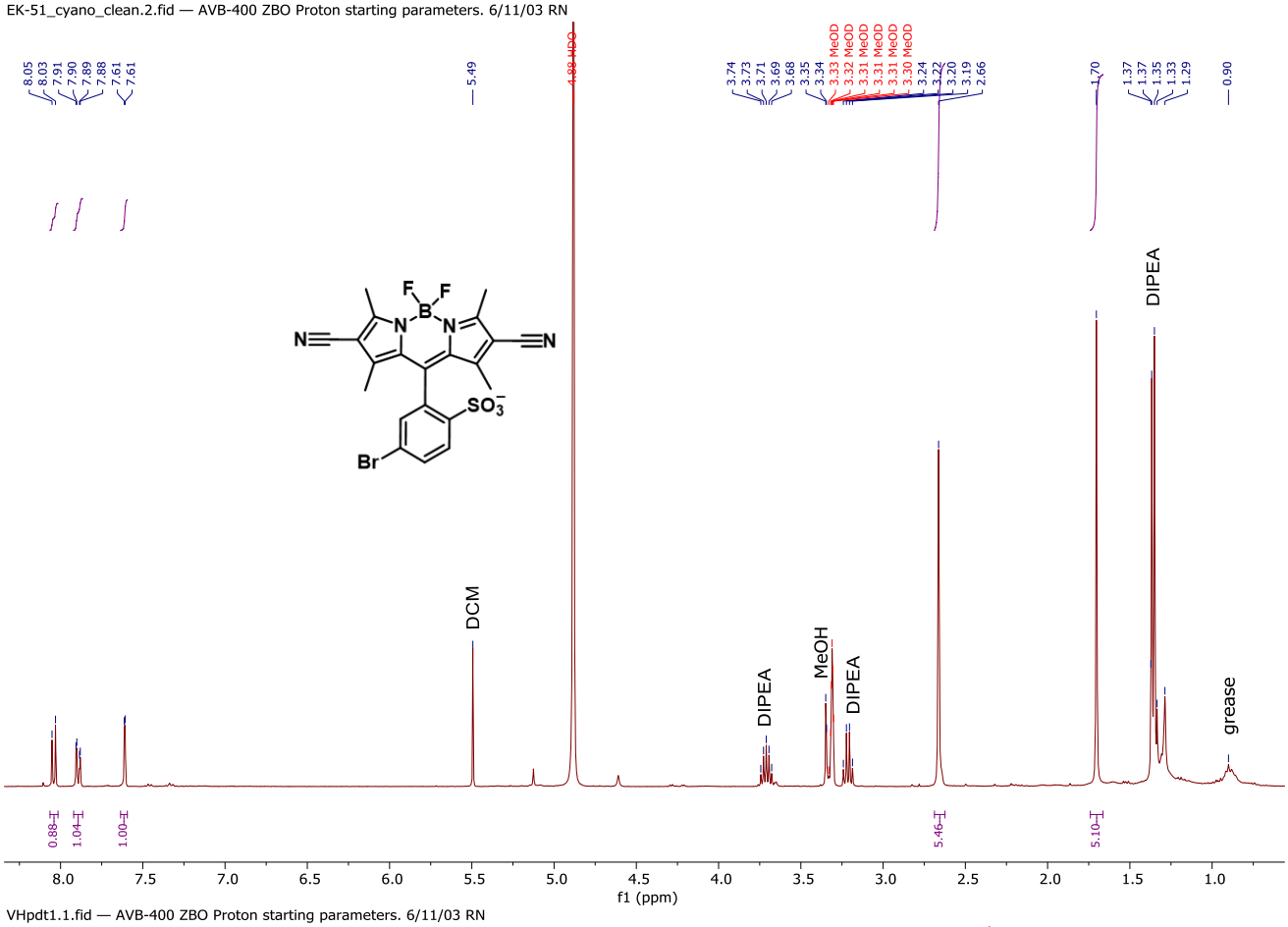
JF3-32_bottom.1.fid — AV-600 ZBO proton starting parameters 11/16/08 RN



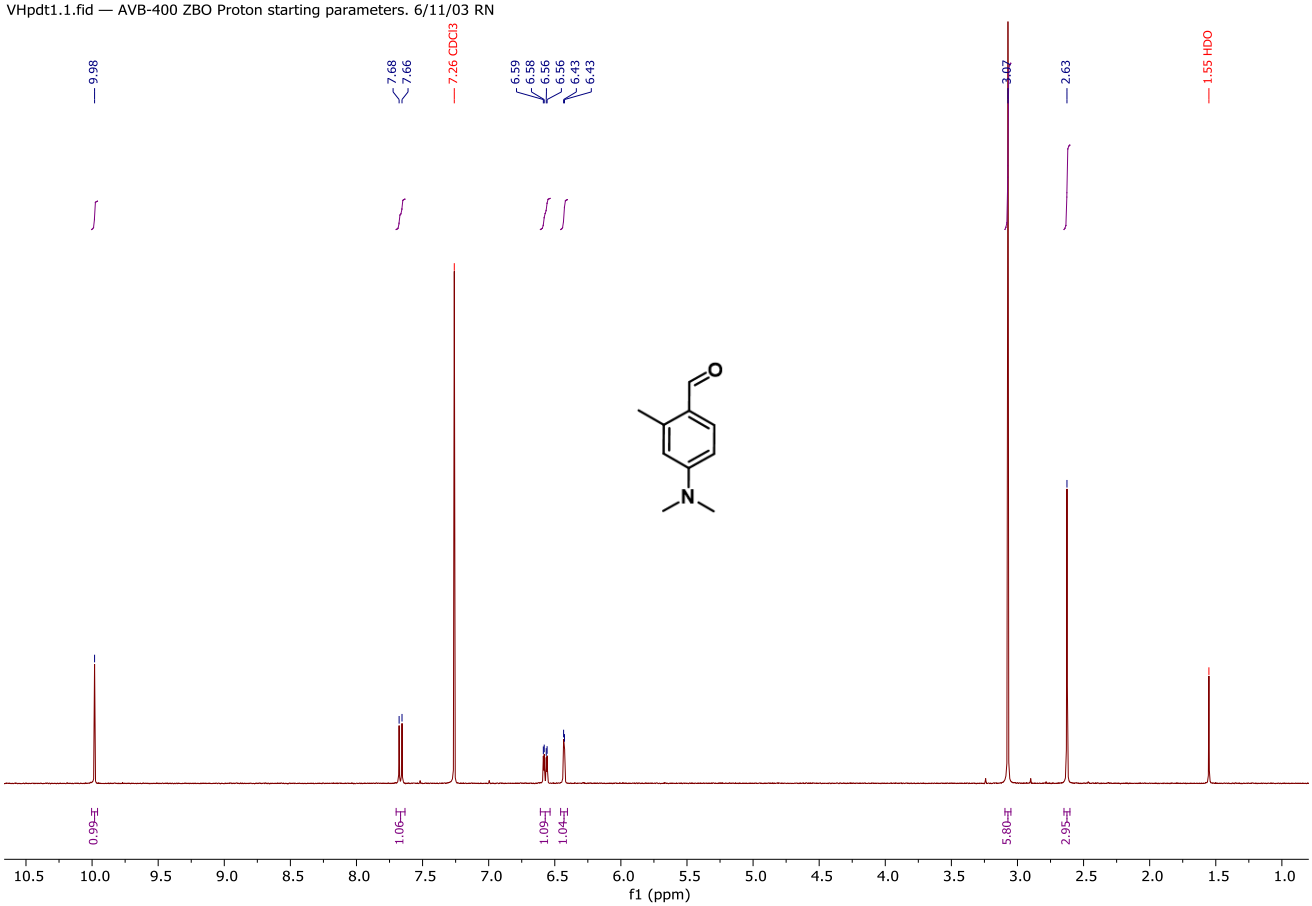
JF4-75_amideHPLCed.1.fid — AV-700 starting parameters for 1D 1H — TXI probe - HC 08/22/2018 — p1=14.88 us @ -6 dB (13 dB attenuator at the amplifier)



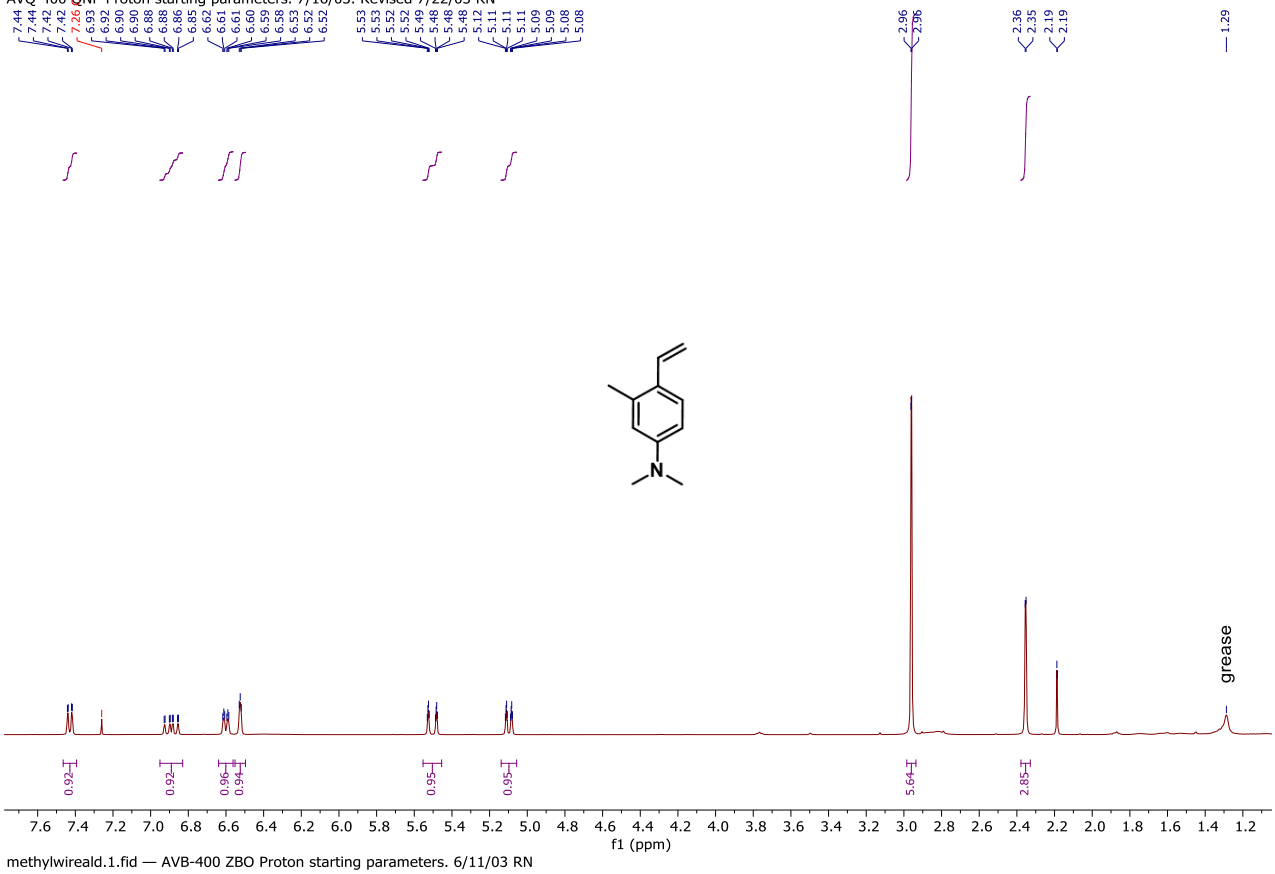
EK-51_cyano_clean.2.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



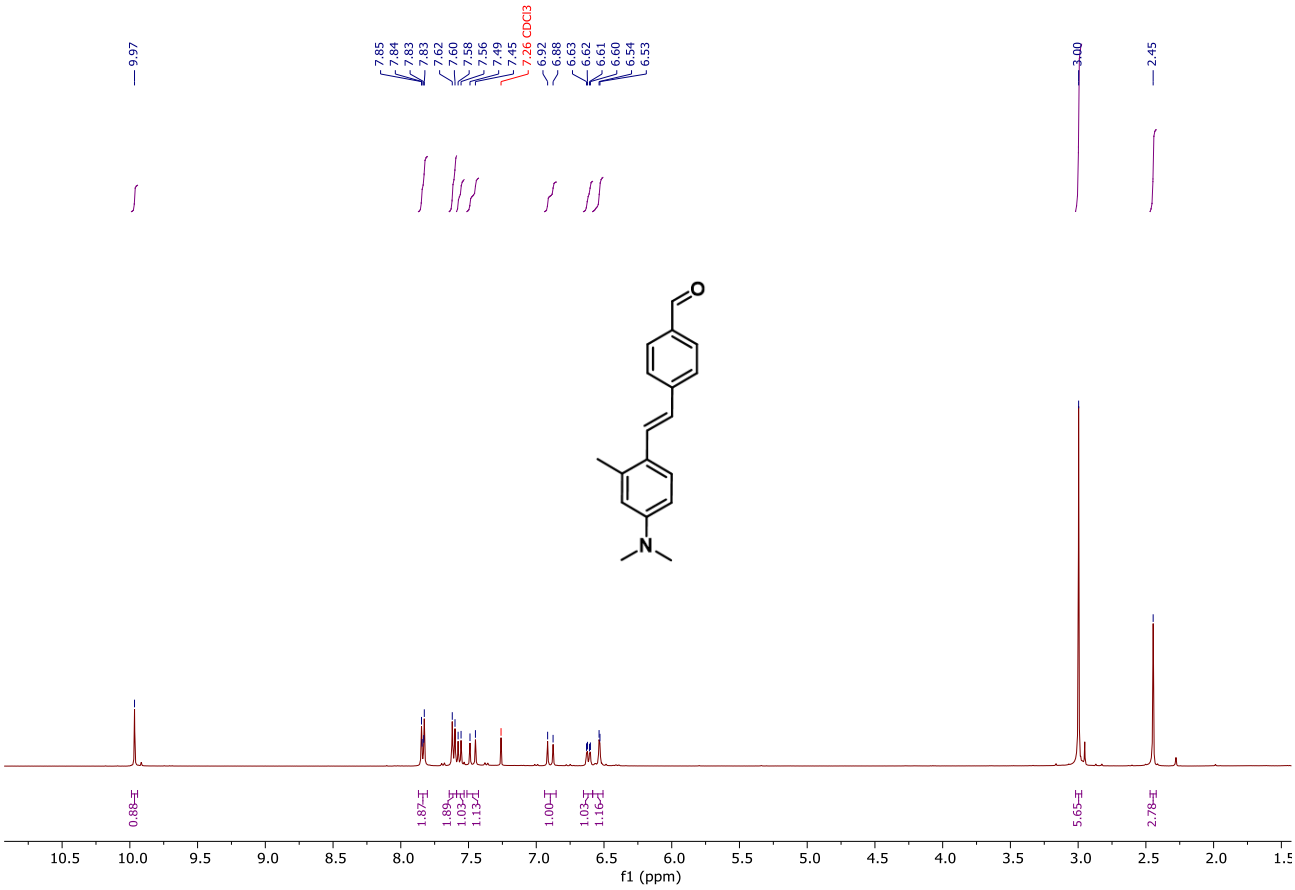
VHpdt1.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



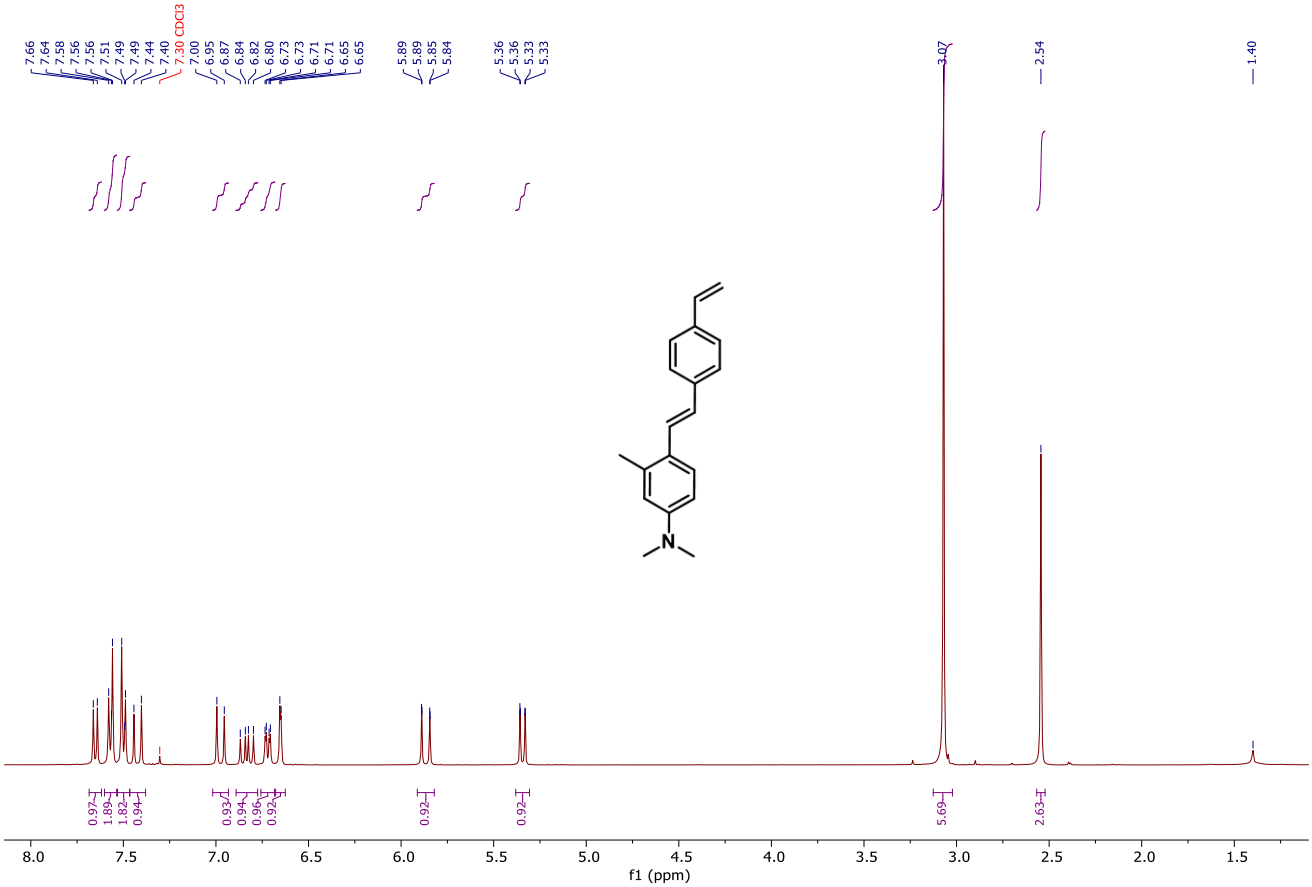
JF4-37_styrene.1.fid
AVQ-400 QNP Proton starting parameters. 7/16/03. Revised 7/22/03 RN



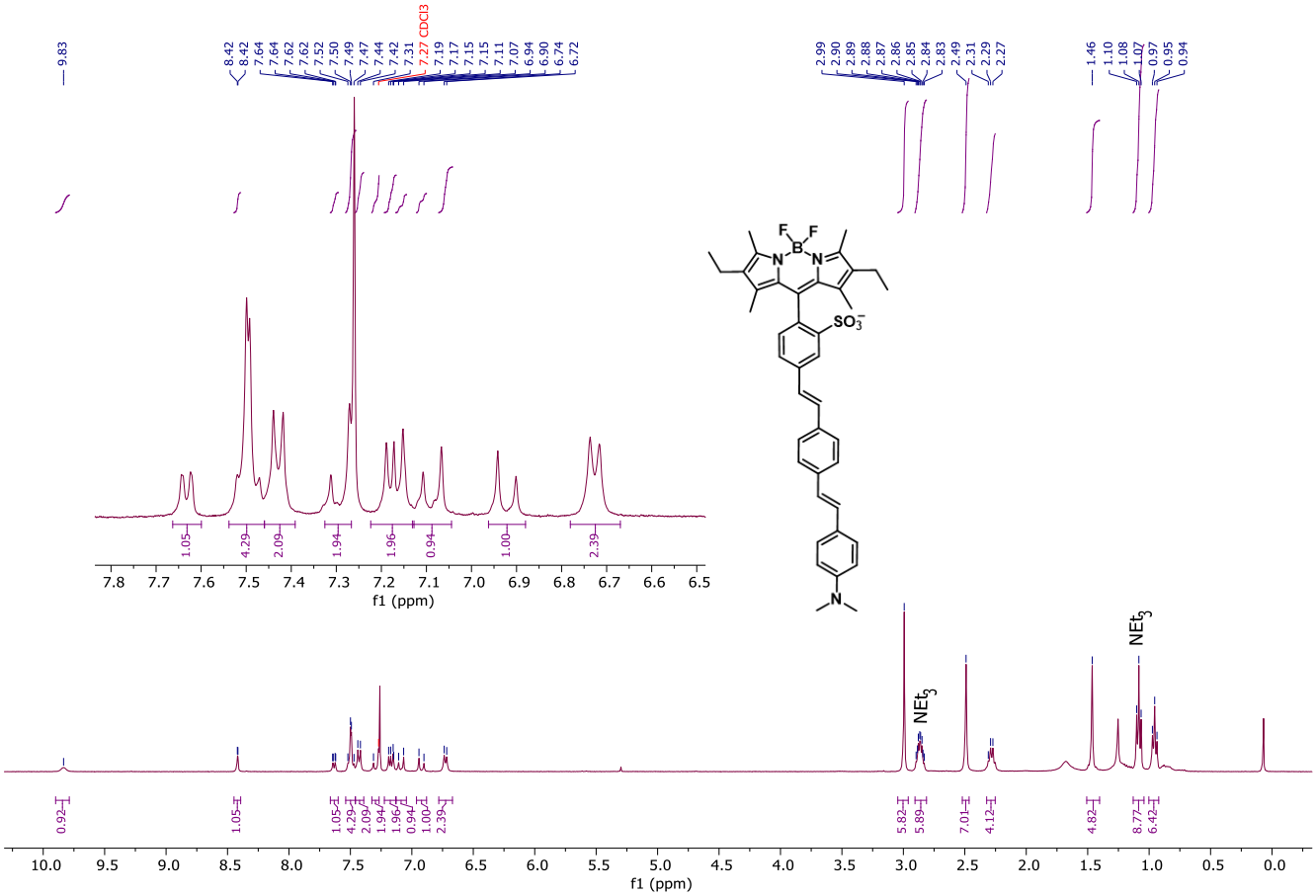
methylwireald.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



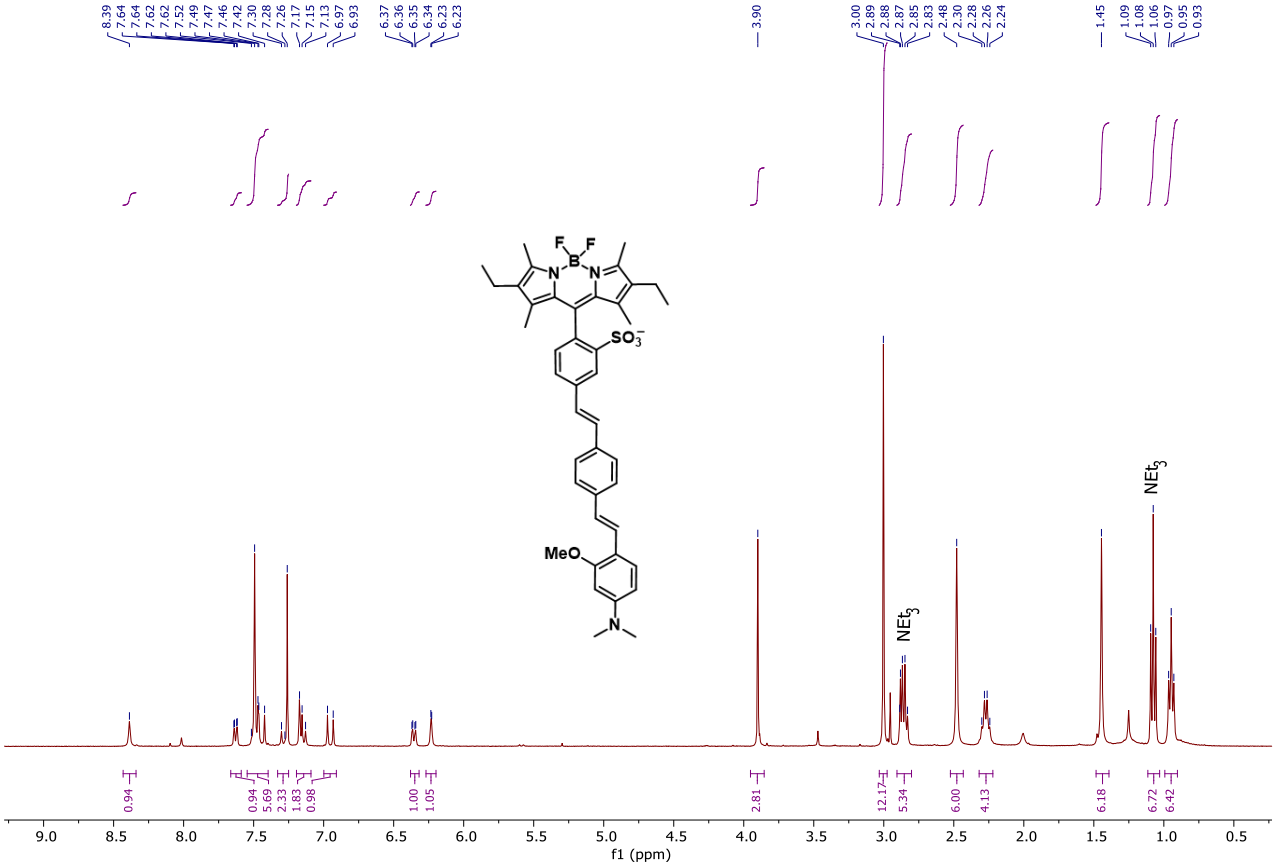
JF-methylwire.1.fid — AVB-400 ZBO Carbon Starting paramters 6/11/03 RN



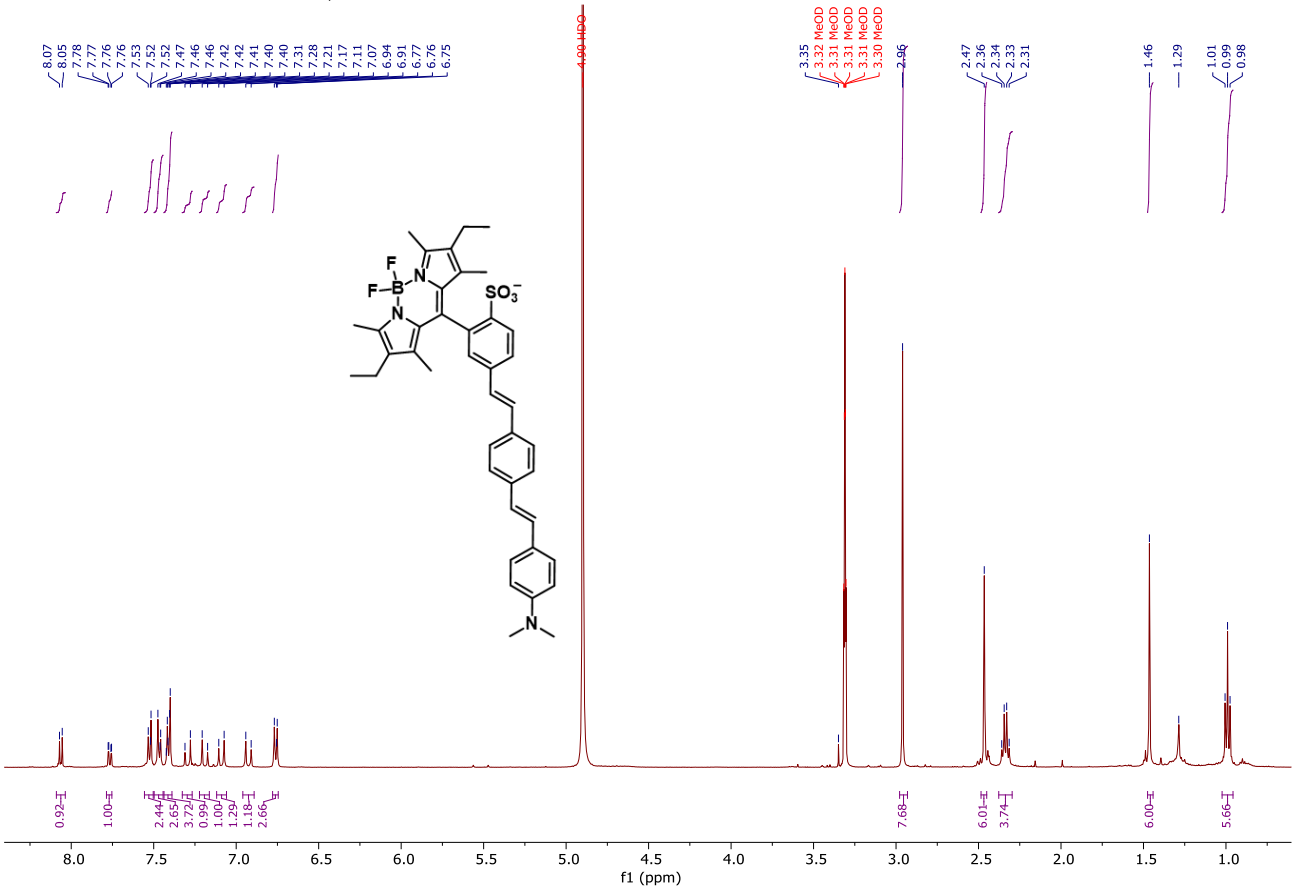
JF-112_F4-7_Etparanorm_13C.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



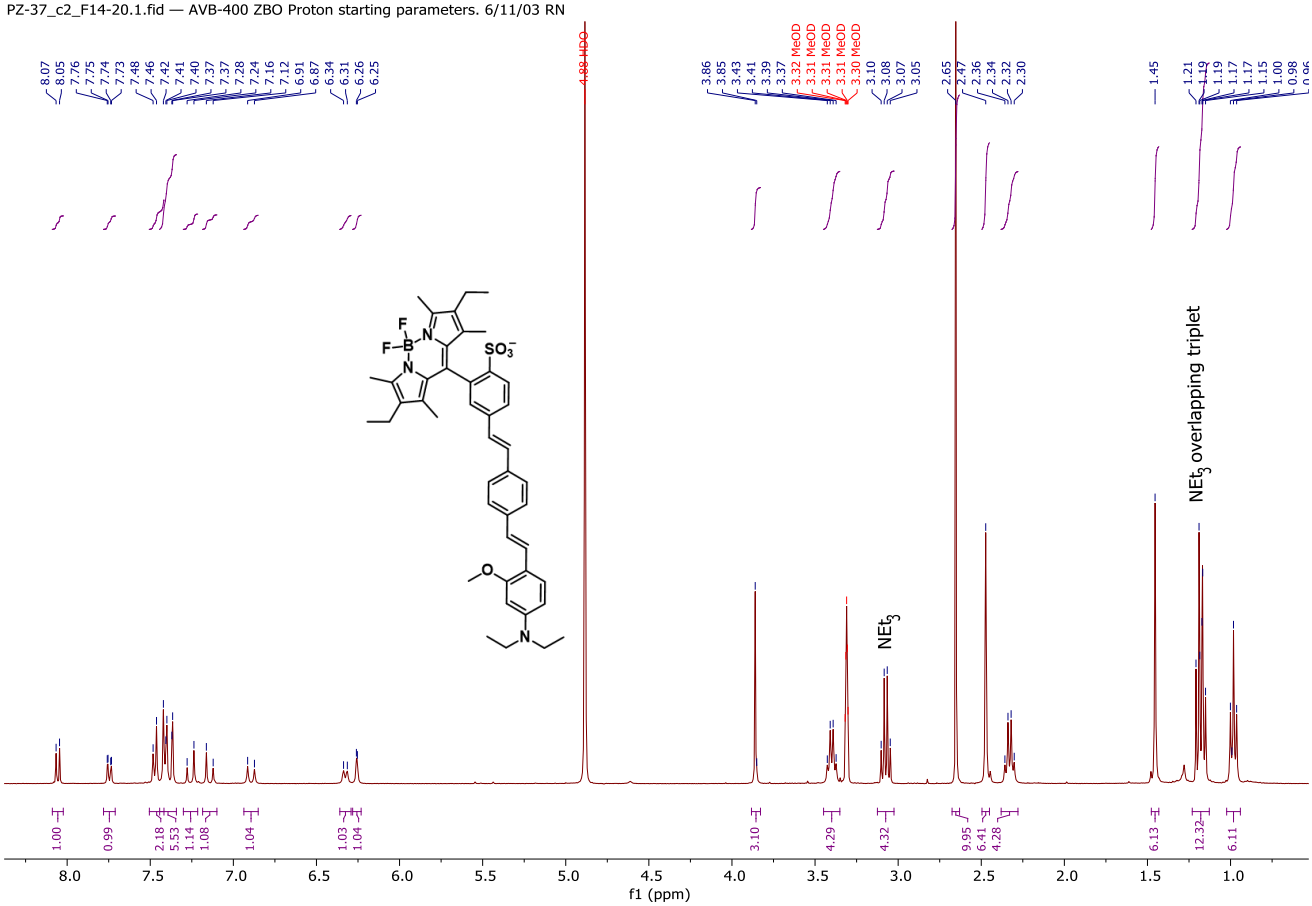
DN-78_EtpOMe.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



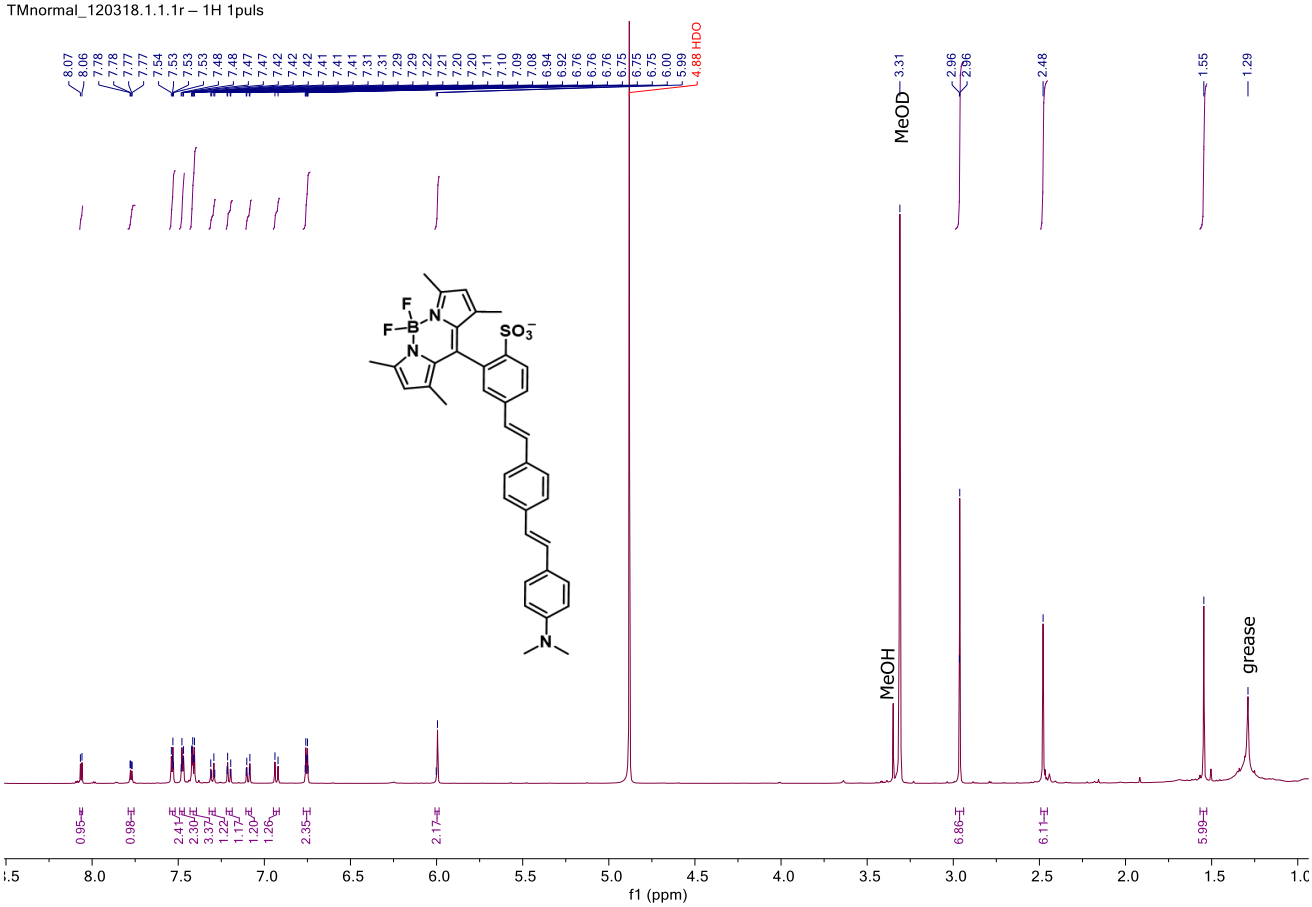
JF4-73_frac15-20_Etmnor.4.fid — AV-500 TBI probe — 1H 1D NMR



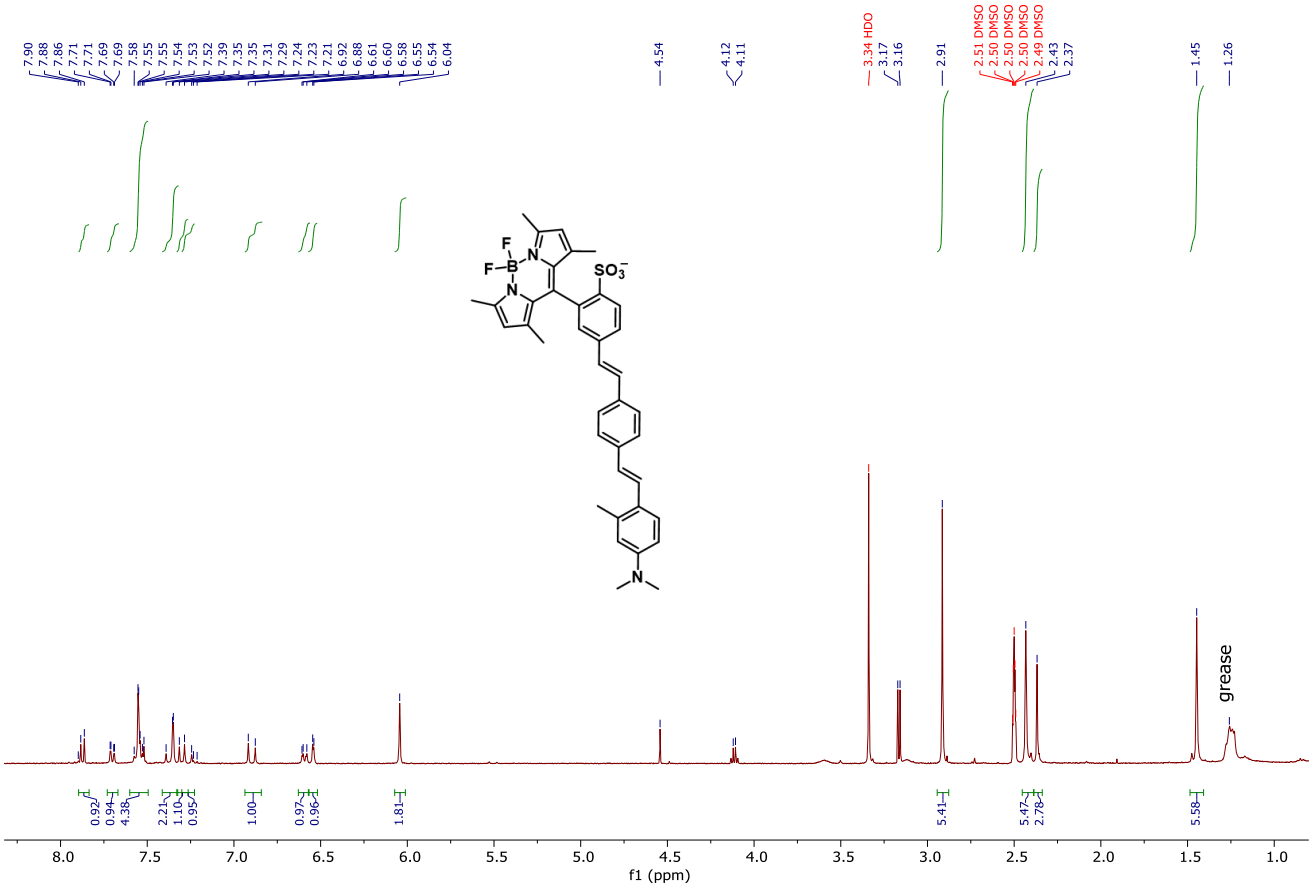
PZ-37_c2_F14-20.1.fid — AVB-400 ZBO Proton starting parameters. 6/11/03 RN



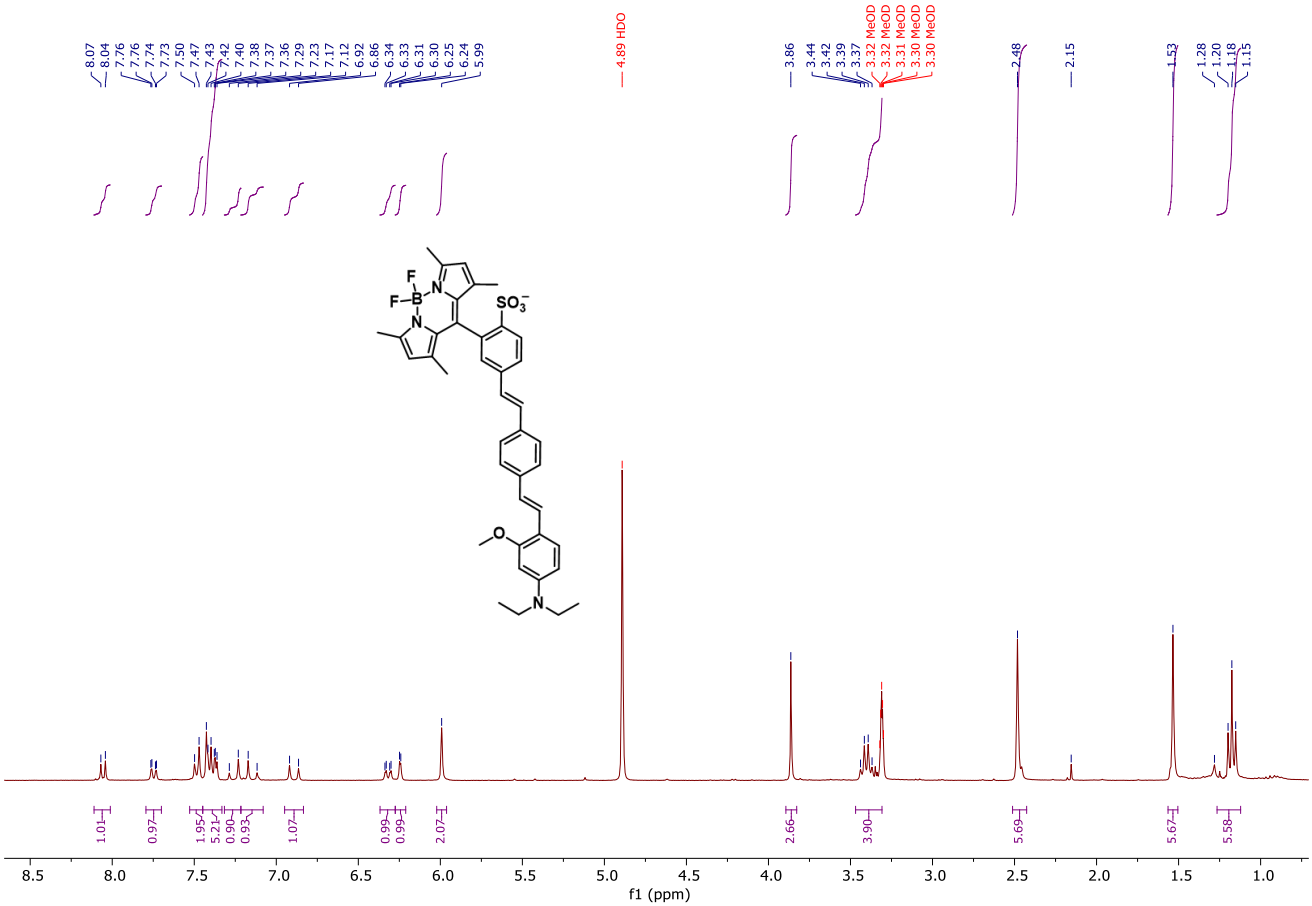
TMnormal_120318.1.1.1r – 1H 1puls

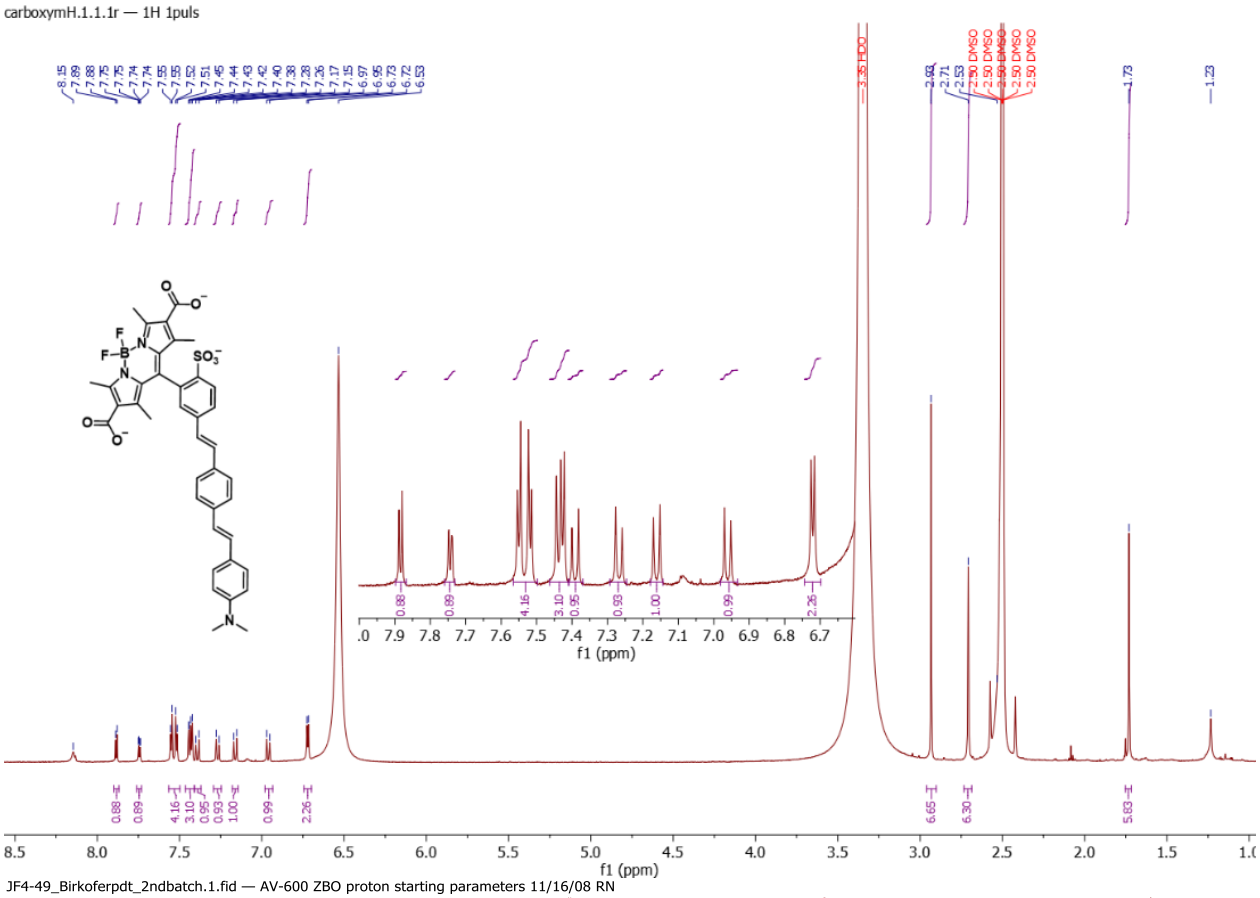


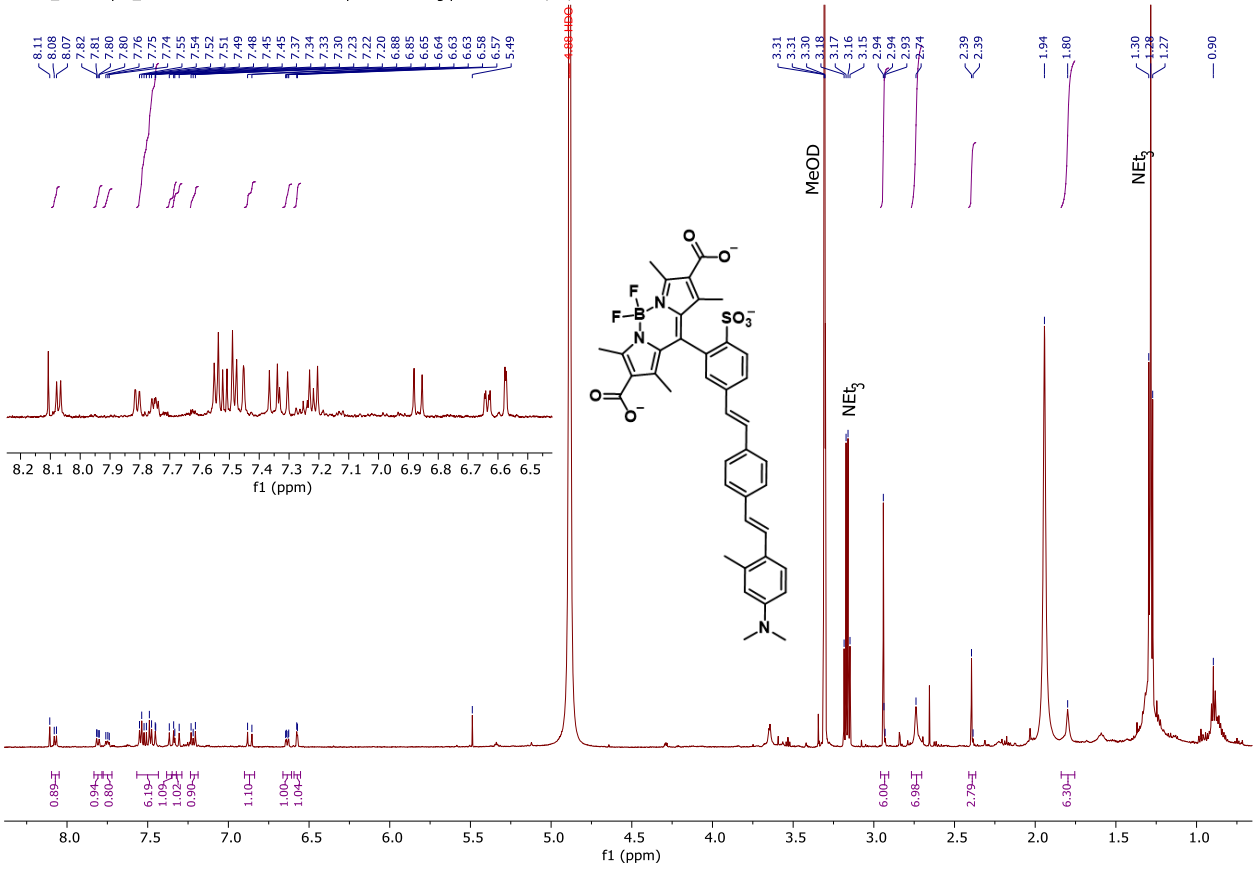
DN-95_c2_TMmMe_DMSO.2.fid — AVQ-400 QNP Proton starting parameters. 7/16/03. Revised 7/22/03 RN

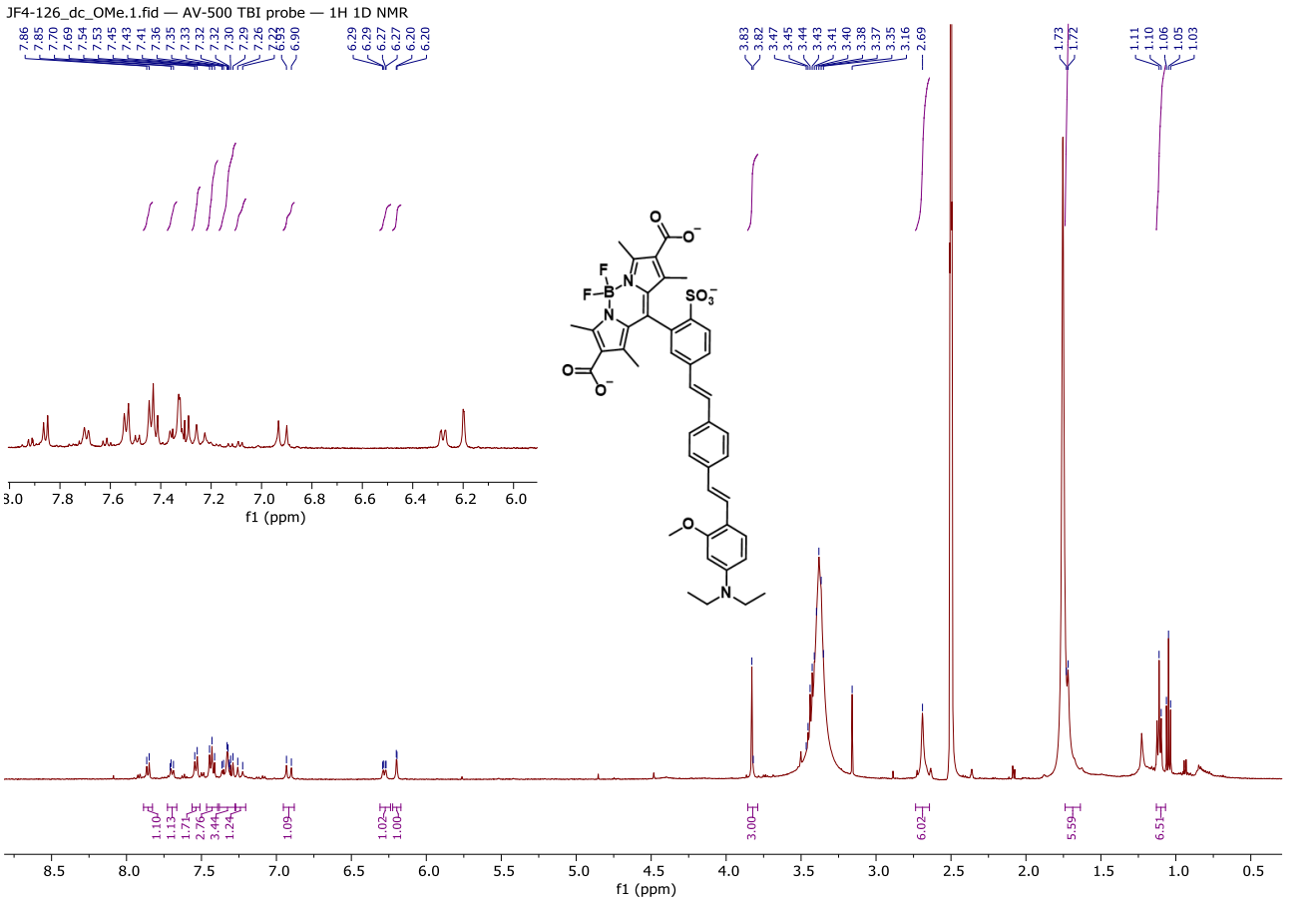


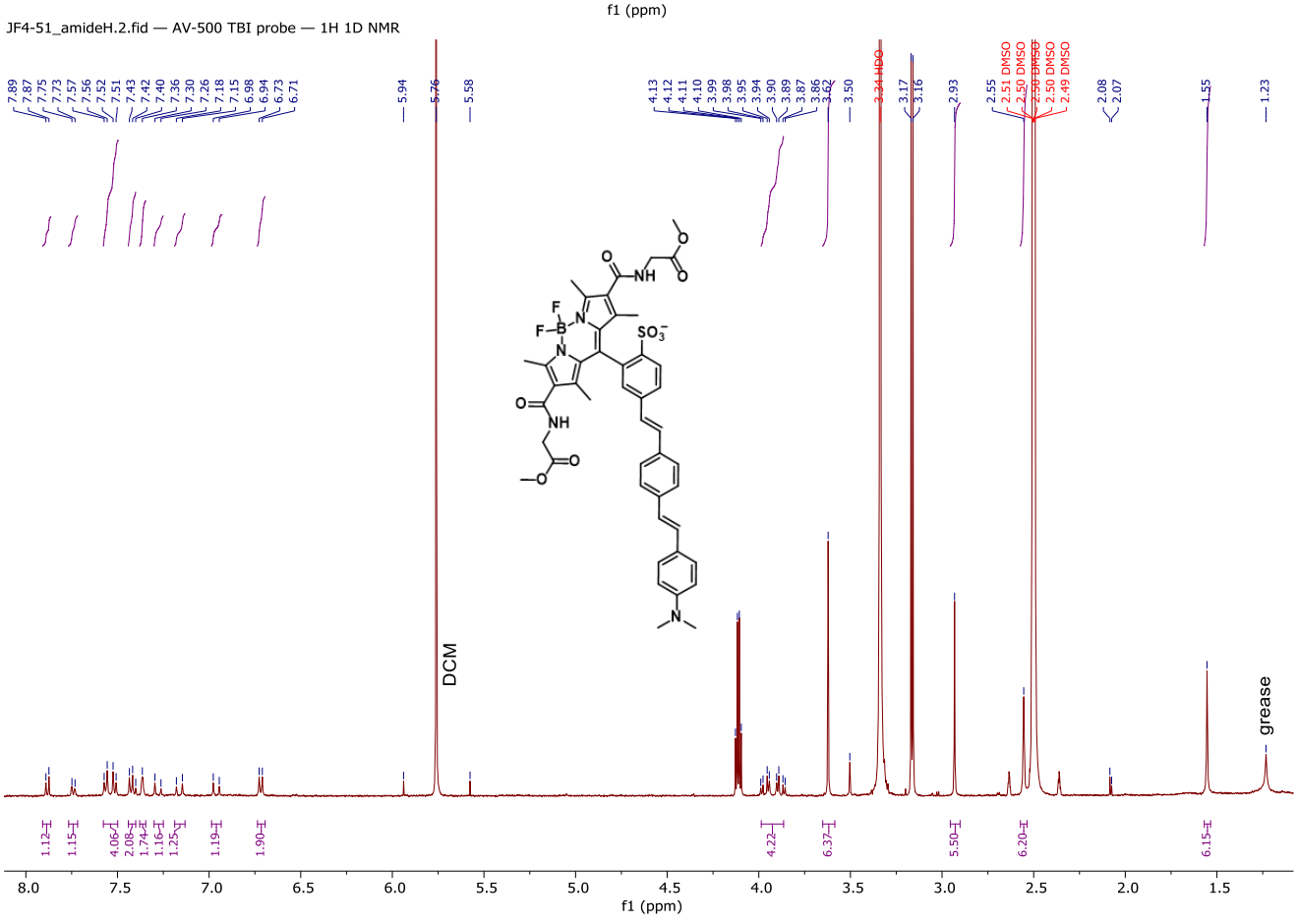
JF4-92_TMmOMe.2.fid — AV-300 Dual C-H probe proton starting parameters 7/23/03 RN.

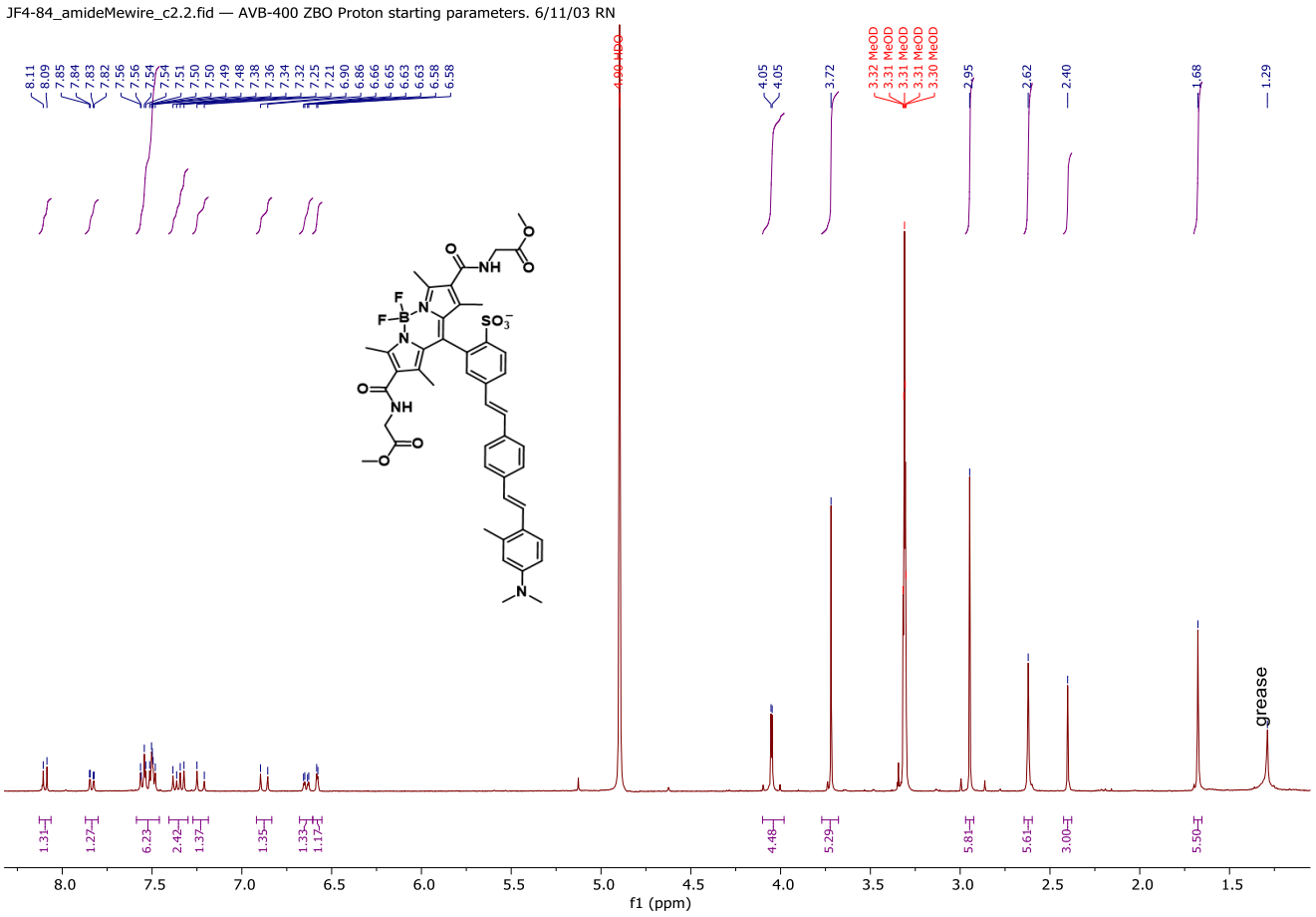



carboxymH.1.1.1r — 1H 1puls
f1 (ppm)


JF4-49_Birkoferpdt_2ndbatch.1.fid — AV-600 ZBO proton starting parameters 11/16/08 RN
MeOD
NEt3
NEt3
f1 (ppm)


JF4-126_dc_OMe.1.fid — AV-500 TBI probe — 1H 1D NMR
f1 (ppm)


JF4-51_amideH.2.fid — AV-500 TBI probe — 1H 1D NMR
DCM
grease
f1 (ppm)



References

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. B.01*, Wallingford, CT, 2016.

2. Chai, J. D.; Head-Gordon, M., Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections. *Phys Chem Chem Phys* **2008,** *10* (44), 6615-6620.

3. Weigend, F.; Ahlrichs, R., Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: Design and assessment of accuracy. *Phys Chem Chem Phys* **2005,** *7* (18), 3297-3305.