Electronic Supplementary Material (ESI) for Analyst.
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Imaging Lipophilic Regions in Rodent Brain Tissue with Halogenated BODIPY Probes

David Hartnell,[a,b] Bradley J. Schwehr,[a] Kate Gillespie-Jones,[c] Dasuni Alwis,[c] Ramesh Rajan,[c] Huishu Hou,[d] Nicole J. Sylvain,[d] Michael J. Pushie,[d] Michael E. Kelly,[d] Massimiliano Massi,[a,*] and Mark J. Hackett[a,b,*]

[a] School of Molecular and Life Sciences, Curtin Institute of Functional Molecules and Interfaces, Curtin University, Perth 6845 WA, Australia

[b] School of Health Sciences, Curtin Health Innovation Research Institute, Curtin University, Perth 6845 WA, Australia

[c] Department of Physiology, Biomedicine Discovery Institute, Monash University, Clayton 3168 VIC, Australia

[d] Department of Surgery, Division of Neurosurgery, College of Medicine, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan S7N 5E5, Canada

* Corresponding Author: m.massi@curtin.edu.au; mark.j.hackett@curtin.edu.au

Electronic Supporting Information

Synthetic details

General methods

All reagents were purchased from Sigma Aldrich or Combi Blocks and used as received, without further purification. Column chromatography used silica gel as stationary phase. Nuclear magnetic resonance spectra were recorded using a Bruker Avance 400 spectrometer.

Synthesis

The compounds BODIPY-Ph[1], BODIPY-PhF[2], BODIPY-PhCl[3], BODIPY-PhBr[1], BODIPY-PhI[4] were synthesised following the previously reported literature procedures. The structures of the targeted compounds was confirmed by matching ^{1}H-NMR spectra with those previously published.

Photophysical measurements

Absorption spectra were recorded at room temperature using a Perkin Elmer Lambda 35 UV/Vis spectrometer. Molar absorptivity determination was obtained from a single solution with a concentration of 10^{-7} M in DCM with absorbance of less than 1.0 for all absorption bands. Uncorrected steady state emission and excitation spectra were recorded using an Edinburgh FLSP980-stm spectrometer equipped with a 450 W xenon arc lamp, double excitation and emission monochromators and a Peltier cooled Hamamatsu R928P photomultiplier (185–850 nm). Emission and excitation spectra were corrected for source intensity (lamp and grating) and emission spectral response (detector and grating) by a calibration curve supplied with the instrument. Spectroscopic grade, non-degassed solvents and 10 mm quartz cuvettes were used.

Quantum yields were measured with the use of an integrating sphere coated with BenFlect, following the method proposed by De Mello [5]. Excited-state decays (τ) were determined with the single photon counting technique (TCSPC) using pulsed picosecond LEDs (EPLED 375, FHWM<800ps) as the excitation source, with repetitions rates between 10kHz and 1 MHz, on the same Edinburgh FLSP980-stm spectrometer. In all cases, the fluorescence lifetime was fitted with single exponential decay. The goodness of fit was assessed by minimising the reduced χ^2 function and by visual inspection of the weighted residuals.

Staining protocols

Tissue Preparation

Tissue samples (for protocol development) were generated from excess sham operated (10-12 week old) male Sprague Dawley rats from our previously published traumatic brain injury study.[6] Animal tissue was generated with approval from Monash University Standing Committee on Ethics in Animal Experimentation. Coronal brain tissue sections (10 µm thick) containing the corpus callosum, or cerebellum, were cut from on a cryo-microtome (-18 °C) and transferred to glass slides for CaF_2 discs for BODIPY staining, luxol fast blue staining, or FTIRM analysis respectively. Slides were air-dried prior to staining or FTIRM analysis.

Tissue samples to investigate lipid distribution after ischemic stroke were obtained from excess tissue generated in our previous studies investigating ischemic stroke, using a photothrombotic mouse model.[7,8] The model uses male Balb/c mice, aged 11 weeks and was approved by the University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Tissue was taken from mice 72 hours post thrombotic stroke. Coronal brain tissue sections (14 µm thick) were cut from on a cryo-microtome (-18 °C) and transferred to glass microscope slides for BODIPY staining, or immuno-fluorescence.

Tissue Staining

Histology: Tissue sections on glass slides were fixed in 10% buffered formalin (Sigma) for 10 minutes. For Luxol fast blue staining and H&E histology routine protocol was followed, as previously described. For BODIPY staining slides were washed with phosphate buffered saline (PBS, 10 nM) for 10 minutes, and then stained with the halogenated BODIPY intermediate solution (250 µL per tissue section, 0.5-50 µg/mL in DMSO) and incubated for 30 minutes at room temperature. Stain solution was then rinsed off with deionised water and then washed in PBS for 10 minutes. Slides were then rinsed with deionised water and allowed to dry at room temperature prior to fluorescence microscopy analysis.

IBA1 immuno-fluorescence: Tissue sections were fixed post sectioning using 1 mL of 10% buffered formalin dropped on top of the tissue section. After fixation, slides were rinsed in phosphate buffered saline (PBS, 10 mM), and blocked in bovine serum albumin for 1 hour at room temperature. Following blocking, the tissue sections were incubated overnight in rabbit anti-IBA1 at a dilution of 1:200 (Abcam, ab40390, Cambridge, USA) at 4 °C. , Following incubation with primary antibody, slides were rinsed in PBS, and incubated with the secondary antibody (goat anti-rabbit IgG Alex Fuor 488) at a concentration of 1:200, for 2 hours at room temperature. Lastly, the slides were rinsed with PBS, mounted with aqueous antifade medium, coverslipped, and stored at -20°C. Fluorescence microscopy images were collected at 20x magnification using a Olympus Bx51 microscope with Olympus dp70 camera and cellSans Standard software, using blue light excitation and collection of emitted fluorescence using a green filter (IBA1).

CD68 immuno-fluorescence: Tissue sections were fixed in 4% buffered paraformaldehyde, then washed with phosphate buffered saline (PBS, 0.1M). Tissues were then incubated for 1h at room temperature in blocking solution (1% bovine serum albumin, 0.02% secondary host serum, 0.001% Triton X, PBS), followed by overnight incubation at 4ºC in anti-CD68 (Abcam, Cambridge, UK) at 1:400 in blocking solution. After being washed in PBS, tissues were incubated in donkey anti-rabbit IgG Alexa Fluor 647 secondary antibody (1:200; Invitrogen). Images were collected using a 20x objective lens on a Leica DM6000 B confocal microscope, fitted with a motorized stage and a Leica DFC365FX camera.

DAPI nucleus staining: Tissue sections were incubated in DAPI (1:10 000; Molecular Probes) in blocking solution for 2h at room temperature. Tissues were then incubated in 0.05% Sudan Black B (Sigma) in 70% ethanol for 5mins to block tissue auto fluorescence, followed by coverslipping with Prolong gold antifade mountant (Invitrogen). Images were collected using a 20x objective lens on a Leica DM6000 B confocal microscope, fitted with a motorized stage and a Leica DFC365FX camera.

Fluorescence Microscopy of BODIPY Staining

Fluorescence microscopy images of BODIPY stained tissue were collected at 4x magnification using an Olympus Bx51 microscope with an Olympus dp70 camera, a mercury broadband fluorescence lamp and cellSens standard software. Imaging parameters for the images presented in this study were gathered using a blue excitation filter and a green emission filter at ISO200 and a dwell time of 500 ms.

FTIR Spectroscopic Analysis

FTIR spectroscopic images of brain tissue sections were gathered using an 8x2 pixel liquid nitrogen cooled linear array detector paired with a Nicolet iN 10MX FTIR microscope at 25 µm spatial resolution as previously described [9]. Spectroscopic images were then generated using Cytospec v2.00.03 for lipid methylene groups $\nu_s(CH_2)$ (2865 – 2840 cm^{-1}) generated using area under the curve. Images were further optimised using imageJ.

Data and Statistical Analysis

Fluorescence images were analysed using imageJ where they were background corrected. Average fluorescence intensities regions of white matter and molecular layer where determined by drawing regions of interesting (ROIs) and calculated using imageJ. Plots and statistical analysis were then generated using Graphpad Prism 8. To determine statistical differences between dTBI and sham animals Mann Whitney tests were completed.

BODIPY Staining Concentration Optimisation

To determine the concentration that afforded the highest white matter to molecular layer intensity ratio healthy sham cerebellum tissue sections were stained (0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/mL DMSO). Fluorescence images were then analysed and optimal concentrations were then selected for each probe and the respective concentration was used for all further staining applications.

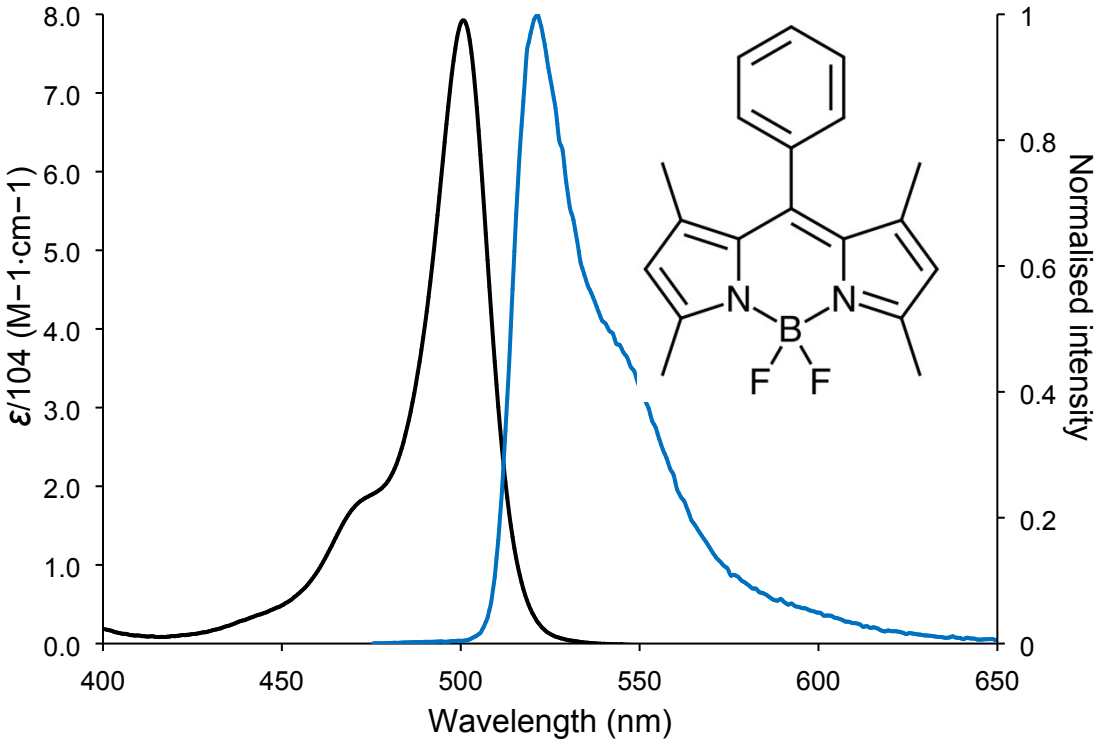

Figure S1: Absorption (black) and emission (blue) of **1** in DCM. λ_{ex} = 460 nm.

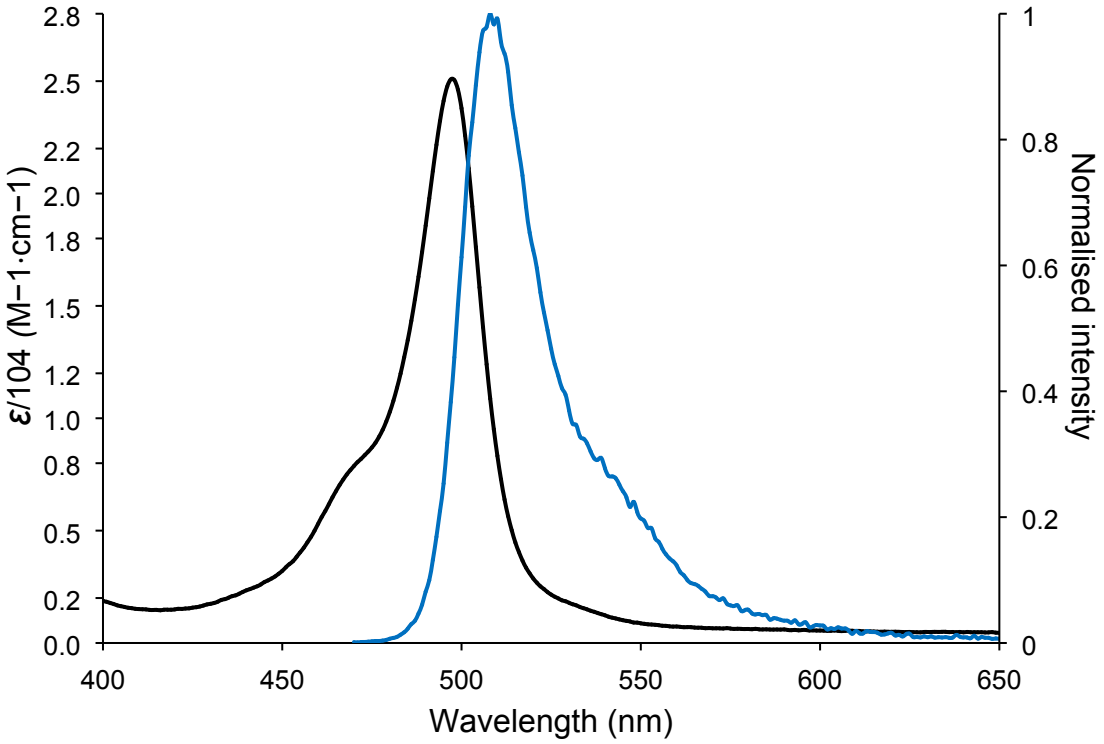

Figure S2: Absorption (black) and emission (blue) of **1** in water (5% DMSO). λ_{ex} = 460 nm.

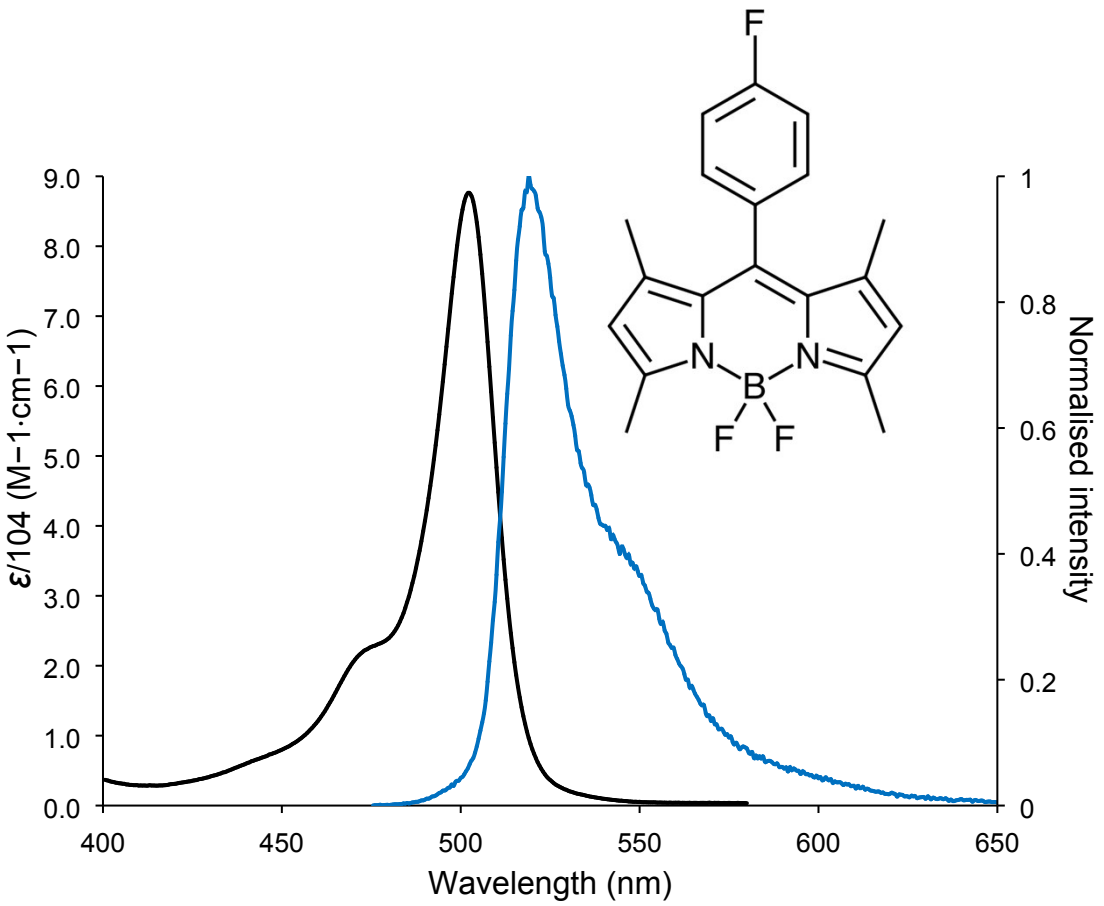

Figure S3: Absorption (black) and emission (blue) of **2** in DCM. λ_{ex} = 460 nm.

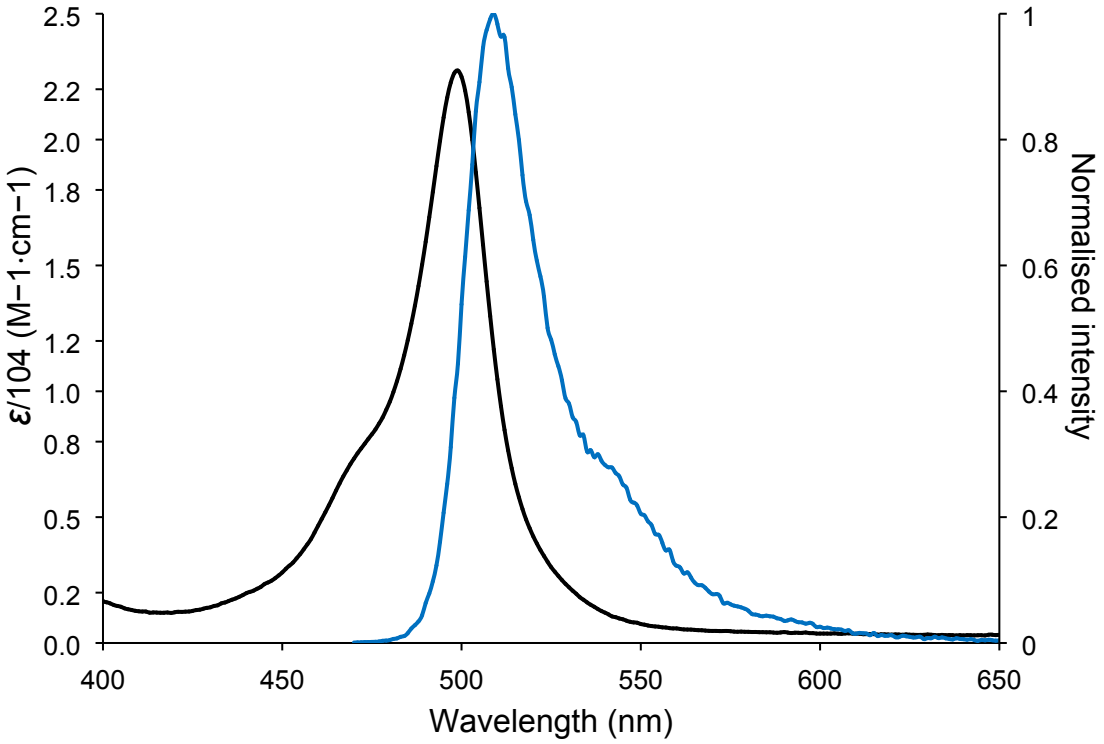

Figure S4: Absorption (black) and emission (blue) of **2** in water (5% DMSO). λ_{ex} = 460 nm.

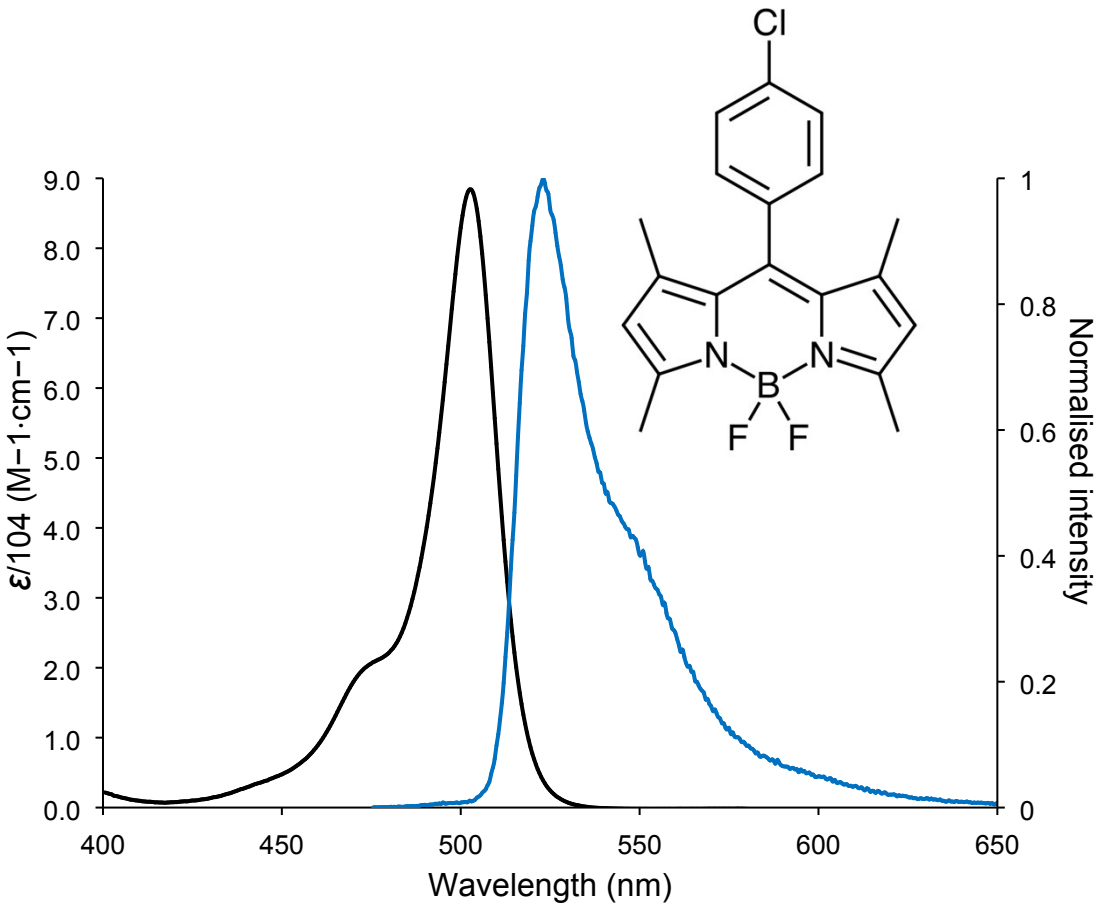

Figure S5: Absorption (black) and emission (blue) of **3** in DCM. λ_{ex} = 460 nm.

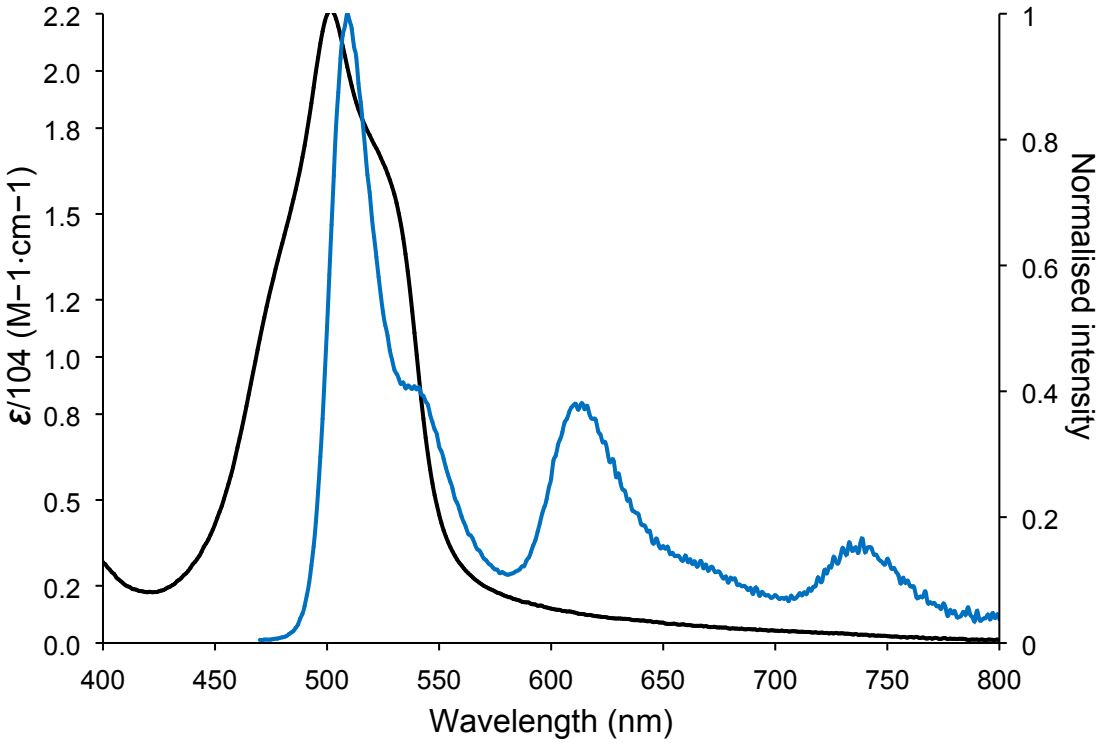

Figure S6: Absorption (black) and emission (blue) of **3** in water (5% DMSO). λ_{ex} = 460 nm.

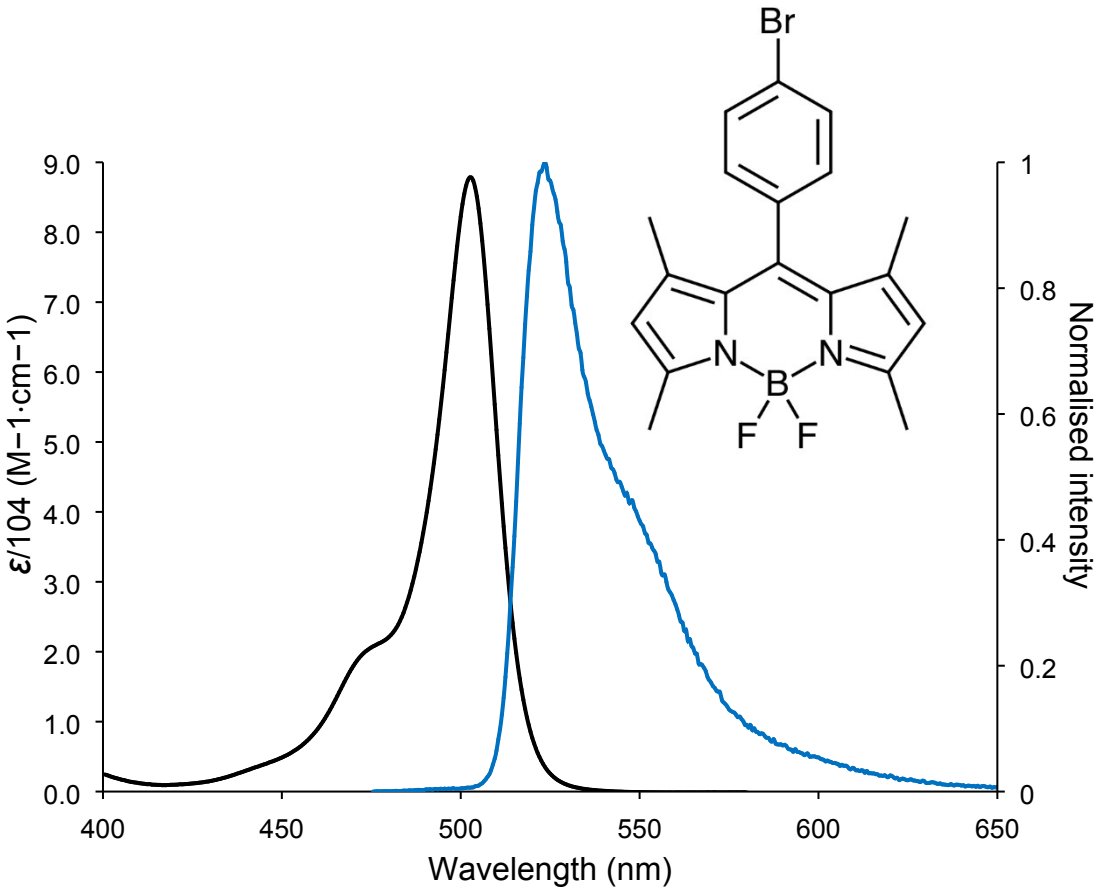

Figure S7: Absorption (black) and emission (blue) of **4** in DCM. λ_{ex} = 460 nm.

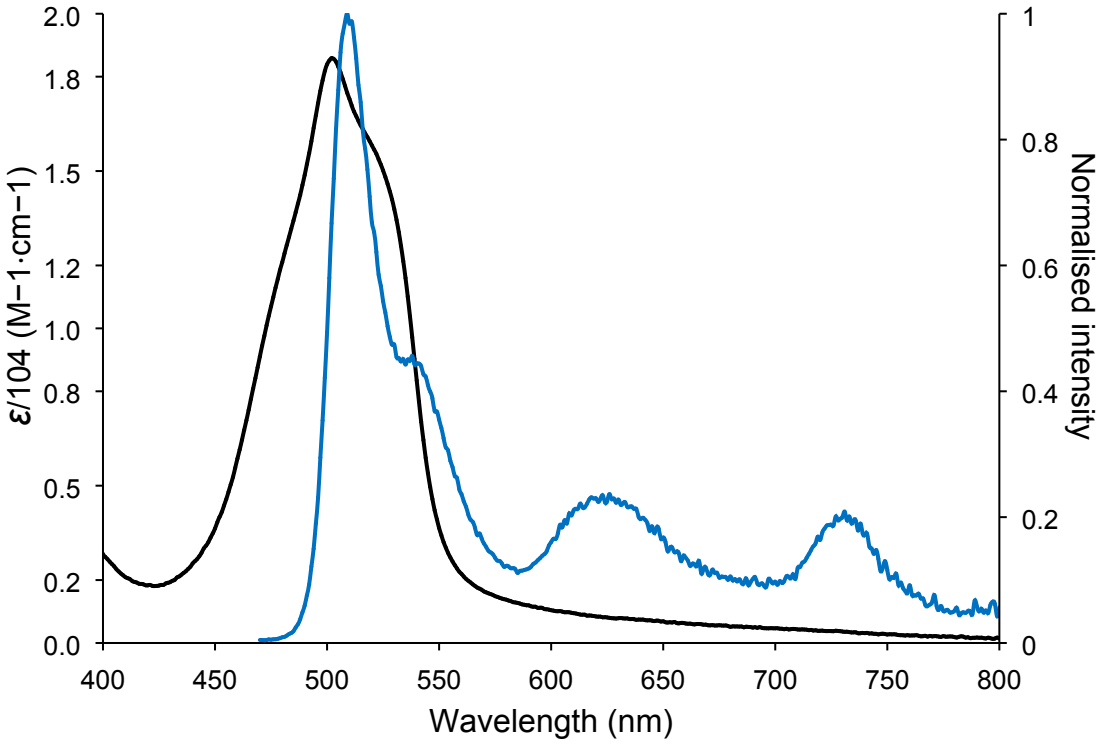

Figure S8: Absorption (black) and emission (blue) of **4** in water (5% DMSO). λ_{ex} = 460 nm.

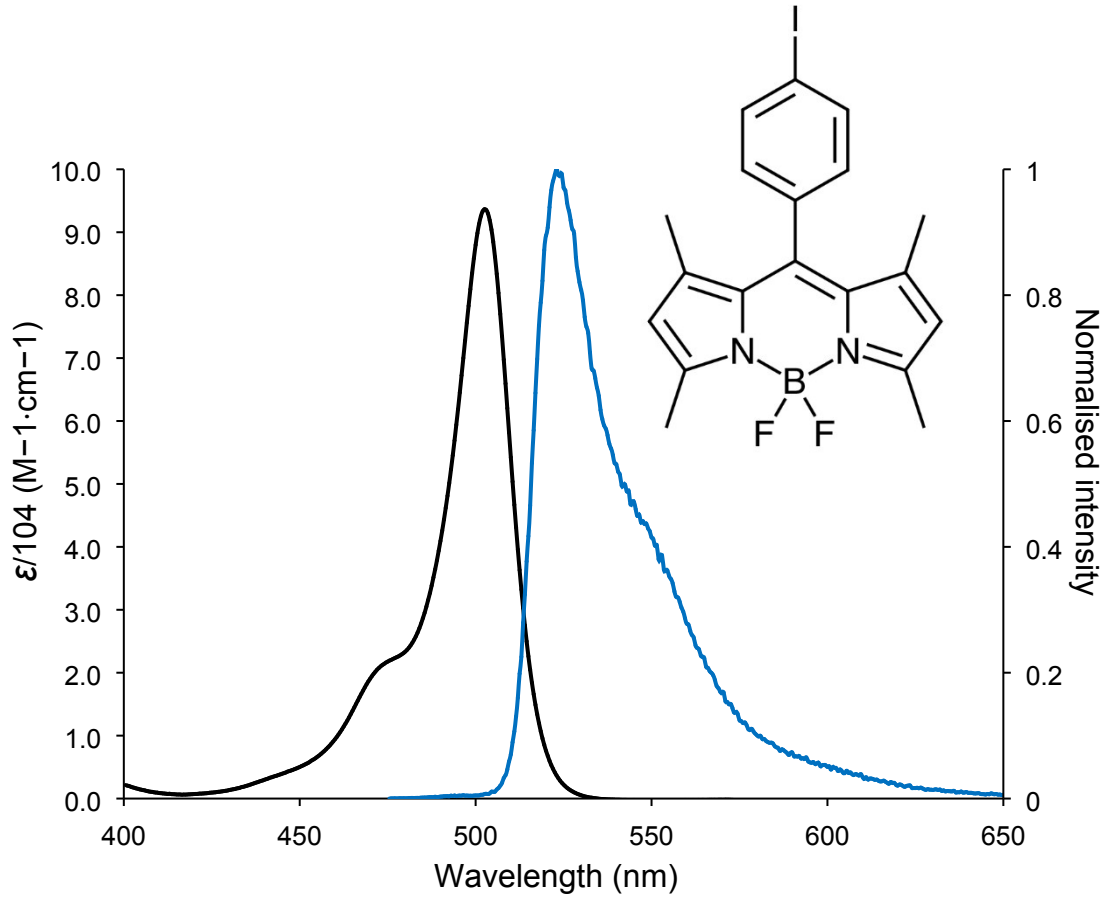

Figure S9: Absorption (black) and emission (blue) of **5** in DCM. λ_{ex} = 460 nm.

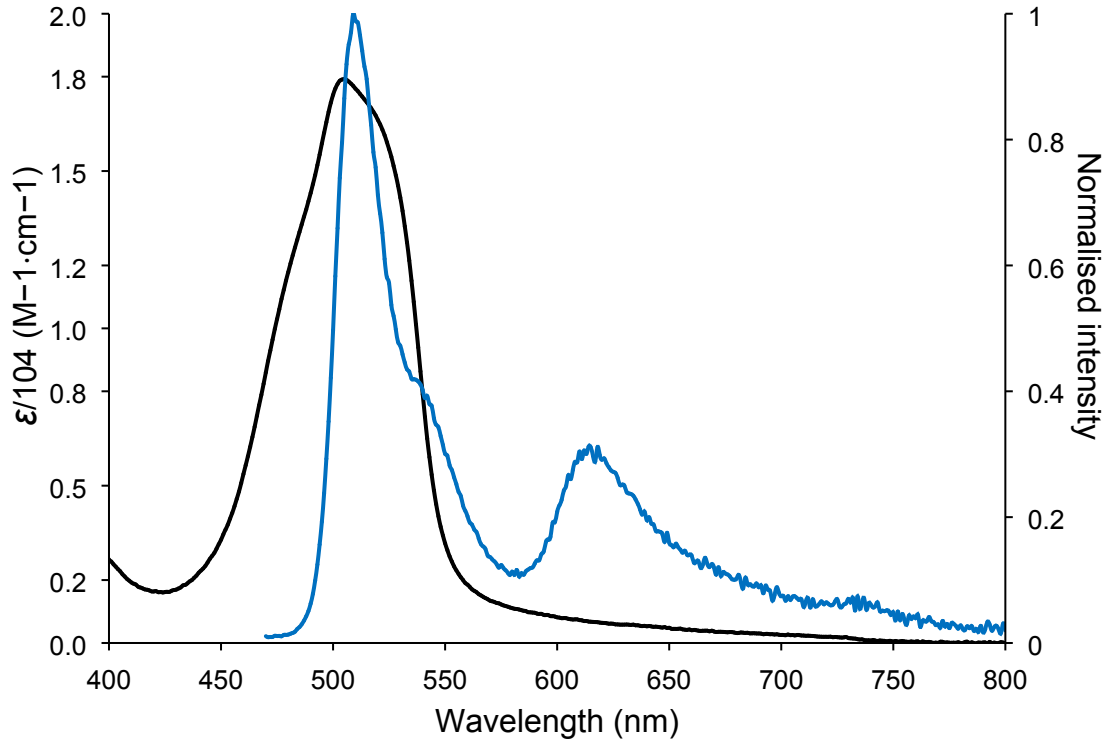

Figure S10: Absorption (black) and emission (blue) of **5** in water (5% DMSO). λ_{ex} = 460 nm.

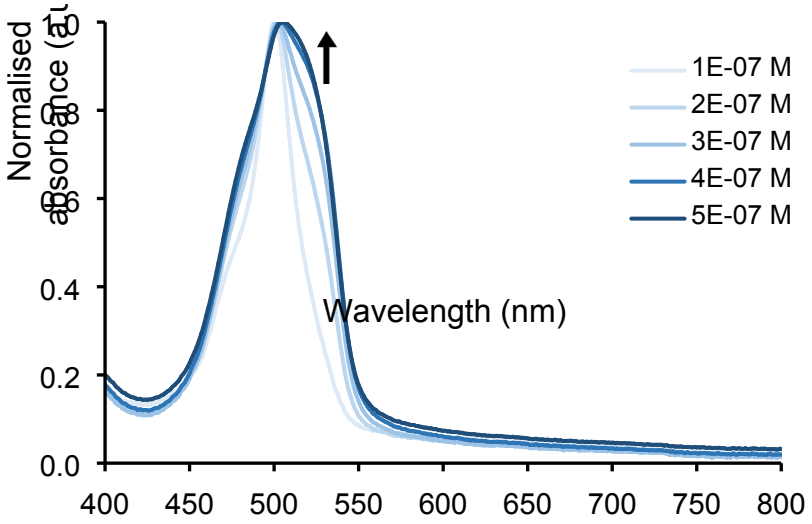

Figure S11: Normalised absorption spectra of **5** in water (5% DMSO v/v). Concentrations between $1x10^{-7}$ M and $5x10^{-7}$ M.

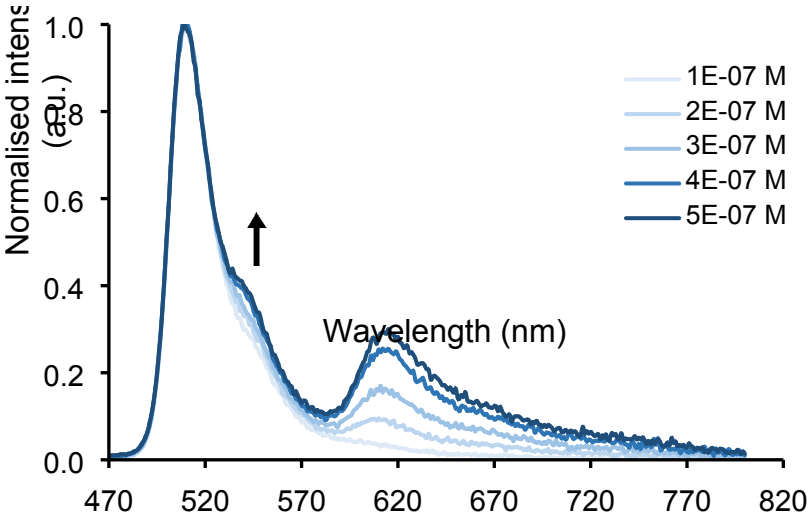

Figure S12: Normalised emission spectrum of **5** in water (5% DMSO v/v). Concentrations between $1x10^{-7}$ M and $5x10^{-7}$ M. λ_{ex} = 460 nm.

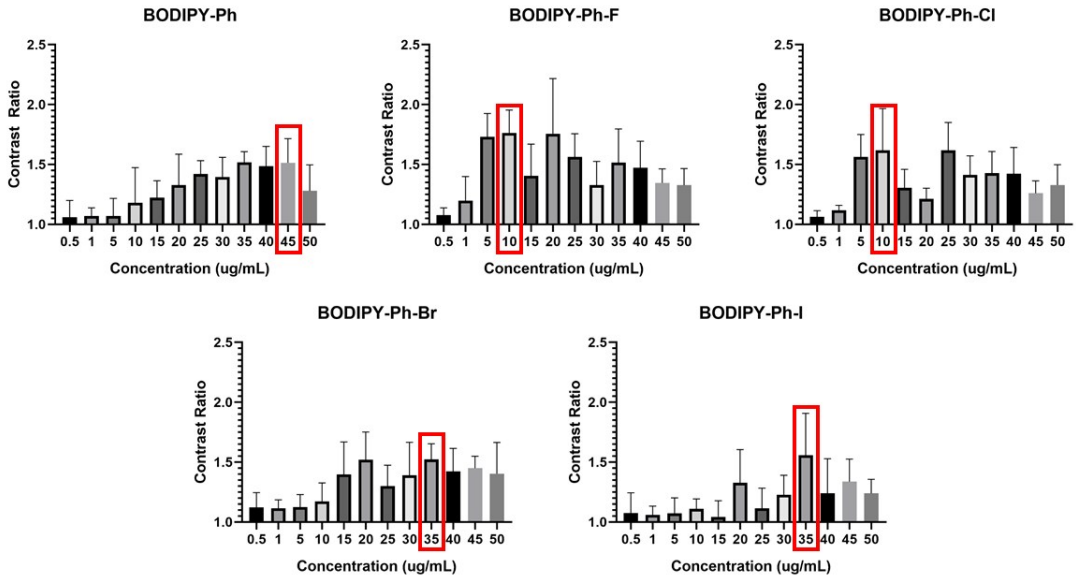

Figure S13: Summary of white matter to molecular layer fluorescence intensity ratio results for BODIPY halogenated compounds. Red boxes indicate optimal staining concentration.

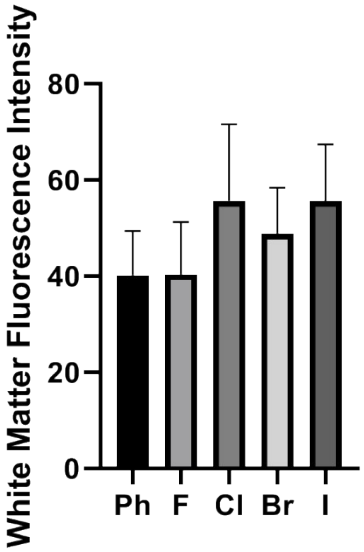

Figure S14: Comparison of white matter intensities in serial tissue sections. (Representative images presented in figure 1). Values displayed are average white matter intensities for one tissue section and error bars are the standard deviation.

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