

Supporting Information

Synthesis and Properties of Water Soluble 1,9-Dialkyl Substituted BF₂ Azadipyrrromethene (NIR-AZA) Fluorophores

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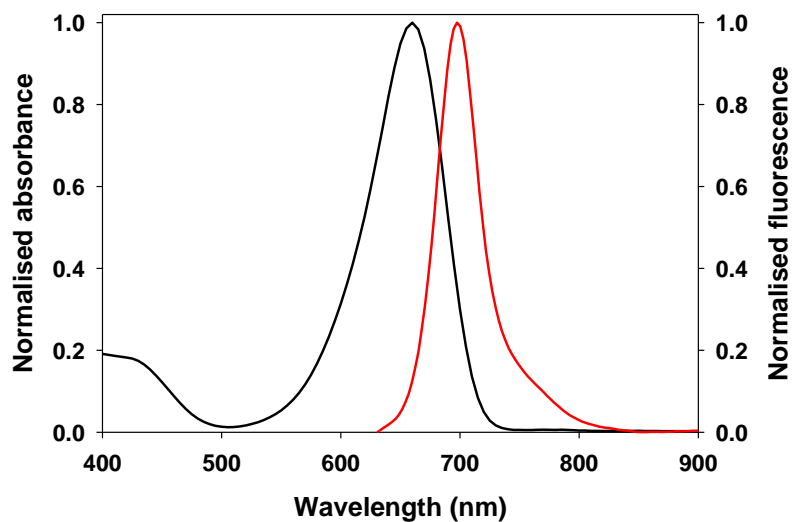
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General Information

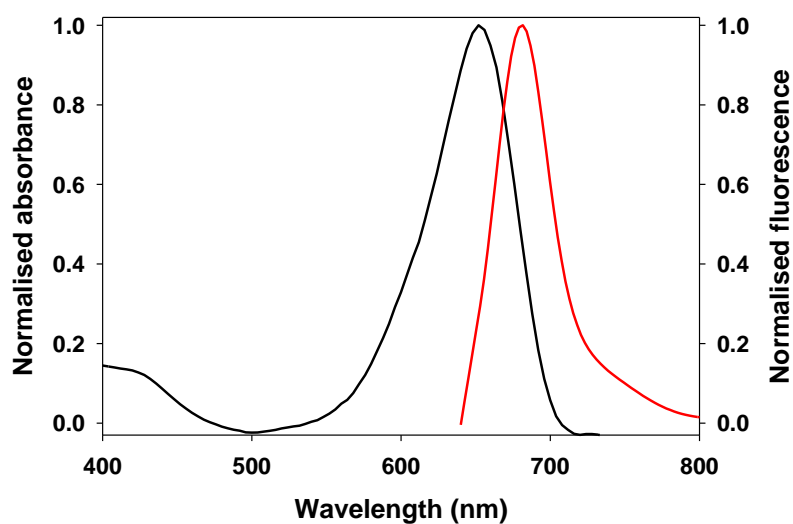
All reactions involving air-sensitive reagents were performed under nitrogen in oven-dried glassware using syringe-septum cap technique. All solvents were purified and degassed before use. Chromatographic separation was carried out under pressure on silica gel using flash-column techniques. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel coated aluminum plates using UV light (254 nm) as visualizing agent. Unless it is specified, all reagents were used as received without further purifications. ^1H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively, and calibrated using residual non-deuterated solvent as an internal reference. Chemical shifts are reported in parts-per-million (ppm). ESI mass spectra were acquired using a microTOF-Q spectrometer in positive and negative modes as required. High performance liquid chromatography (HPLC) was performed with a Shimadzu instrument equipped with a Shimadzu SPD-20AV prominence UV/vis Detector. Separations employed YMC RP Triart Phenyl 5 μm HPLC column (150 \times 4.6 mm I.D.), with a water: CH_3CN solvent gradient. Fluorescence emission spectra measurements were conducted with a Varian Cary Eclipse fluorescence spectrophotometer with the excited and emissive slit of 5 nm. UV-vis spectra were recorded using a Varian Cary 50 Scan UV-visible spectrophotometer using 1 cm path length quartz cuvettes.

Absorption and Fluorescence of **1a,b** in CHCl₃

Normalized abs and flu spectra of **1a** in CHCl₃

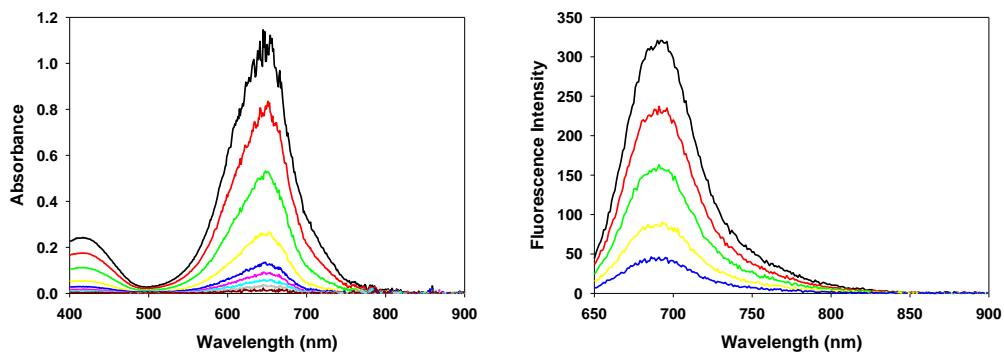


Normalized abs and flu spectra of **1b** in CHCl₃

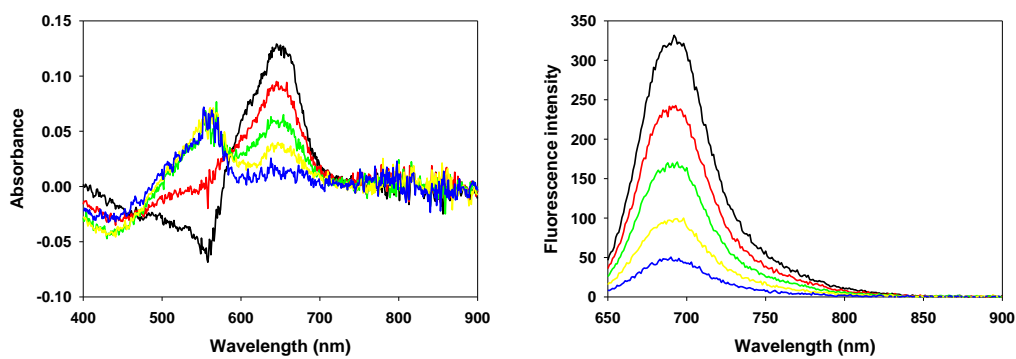


Absorption and Fluorescence of **6a,b** in PBS, DMEM, MeOH

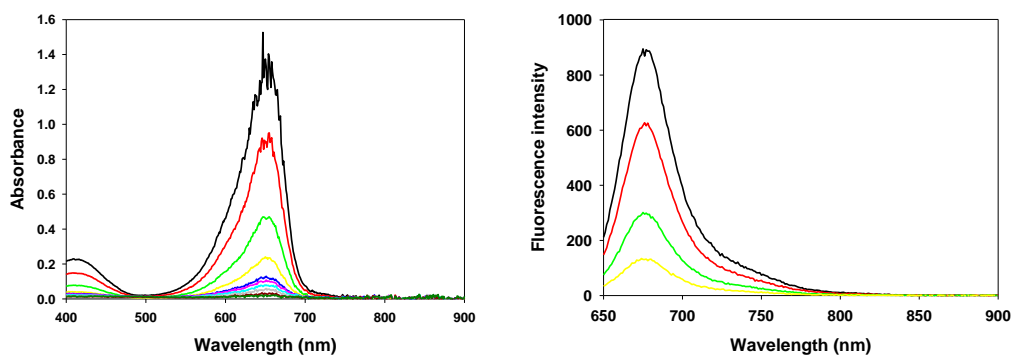
Absorbance and fluorescence spectra for a serial dilution of **6a** in PBS



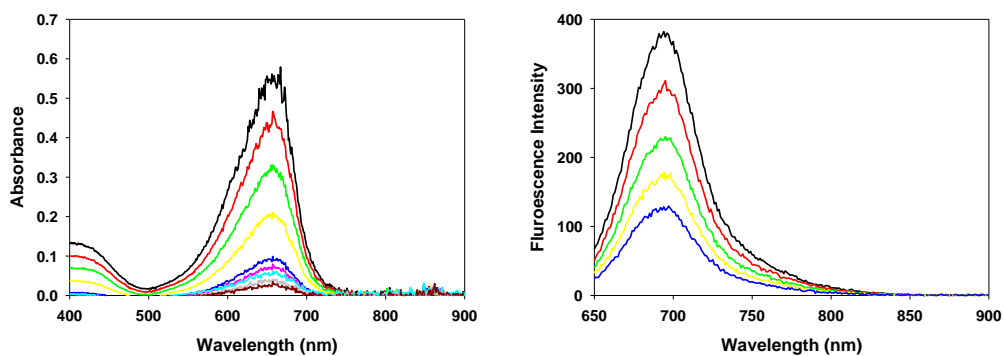
Absorbance and fluorescence spectra for a serial dilution of **6a** in cell media DMEM



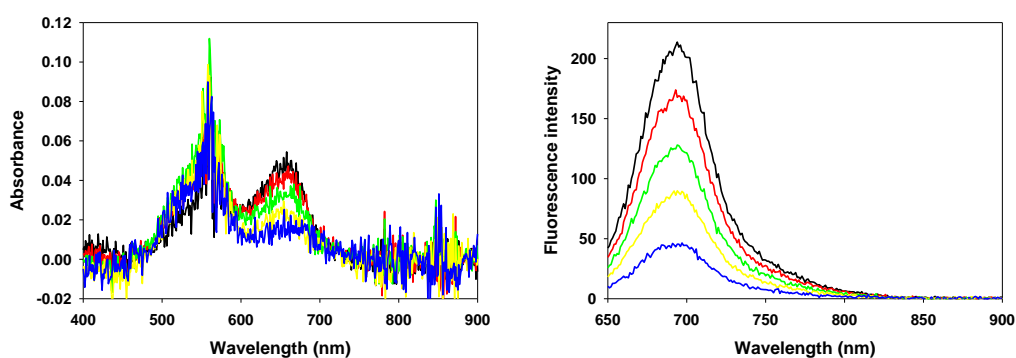
Absorbance and fluorescence spectra for a serial dilution of **6a** in MeOH



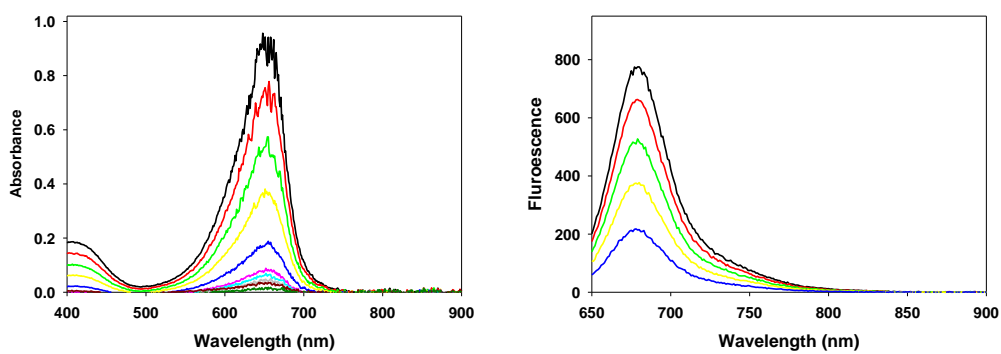
Absorbance and fluorescence spectra for a serial dilution of **6b** in PBS



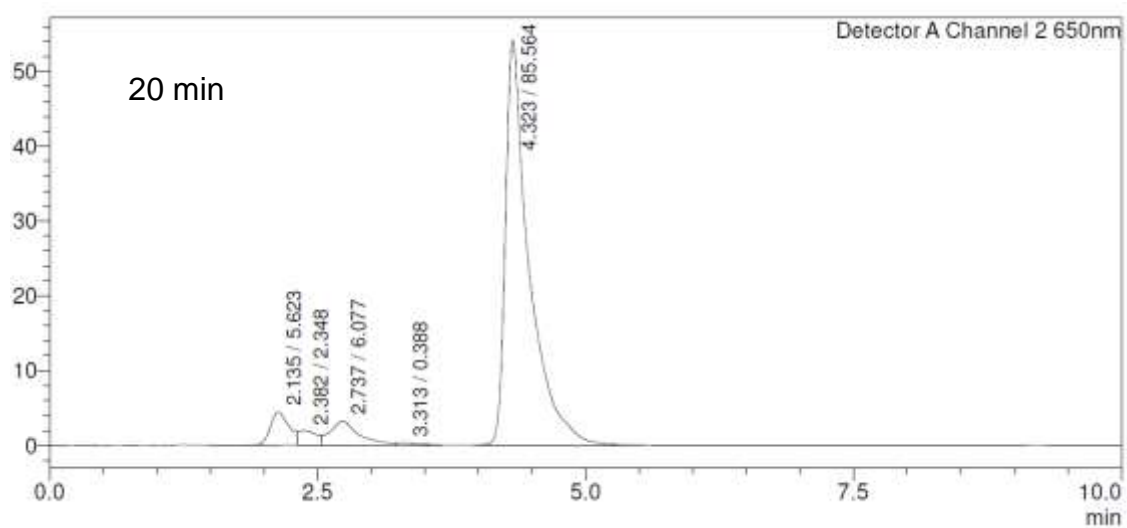
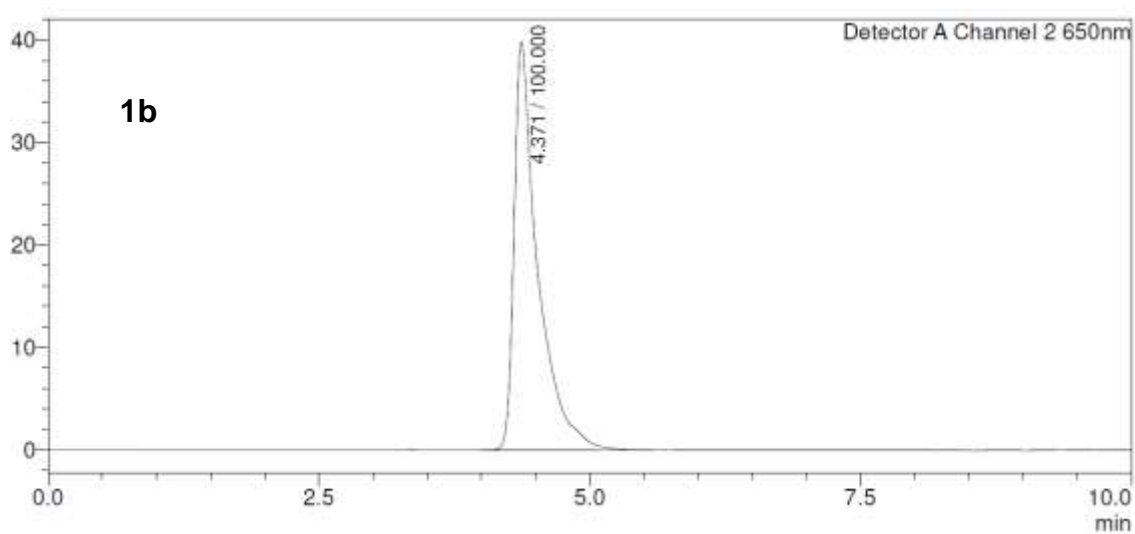
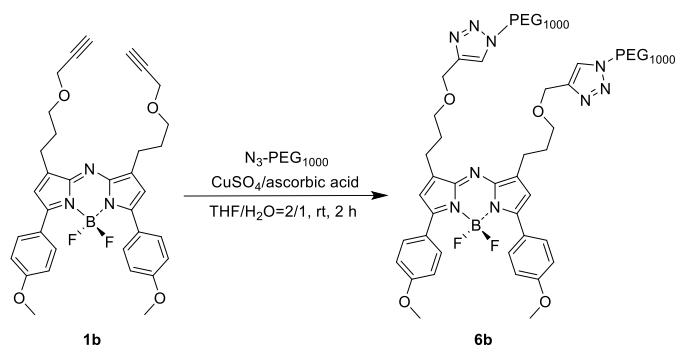
Absorbance and fluorescence spectra for a serial dilution of **6b** in cell media DMEM

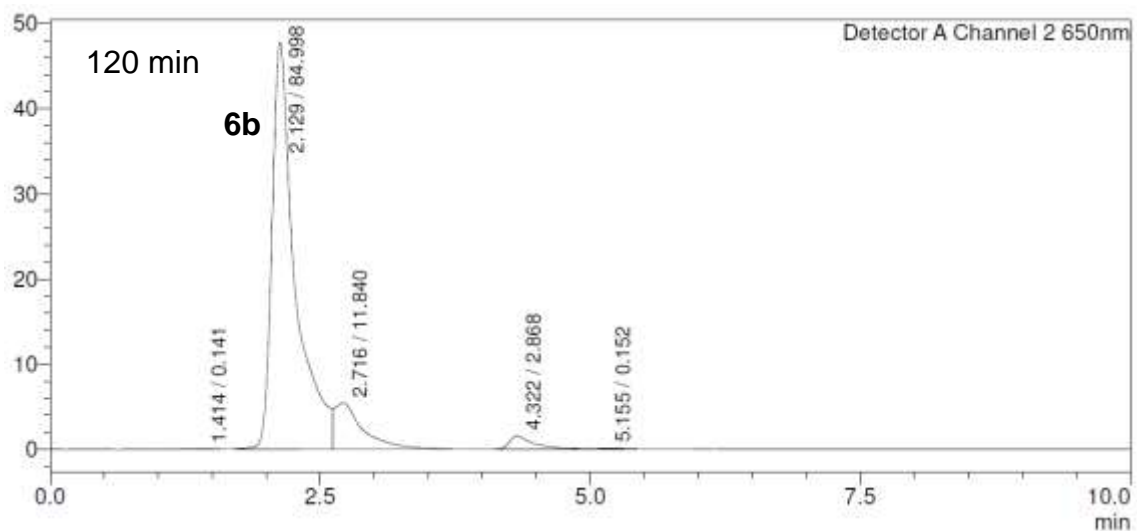
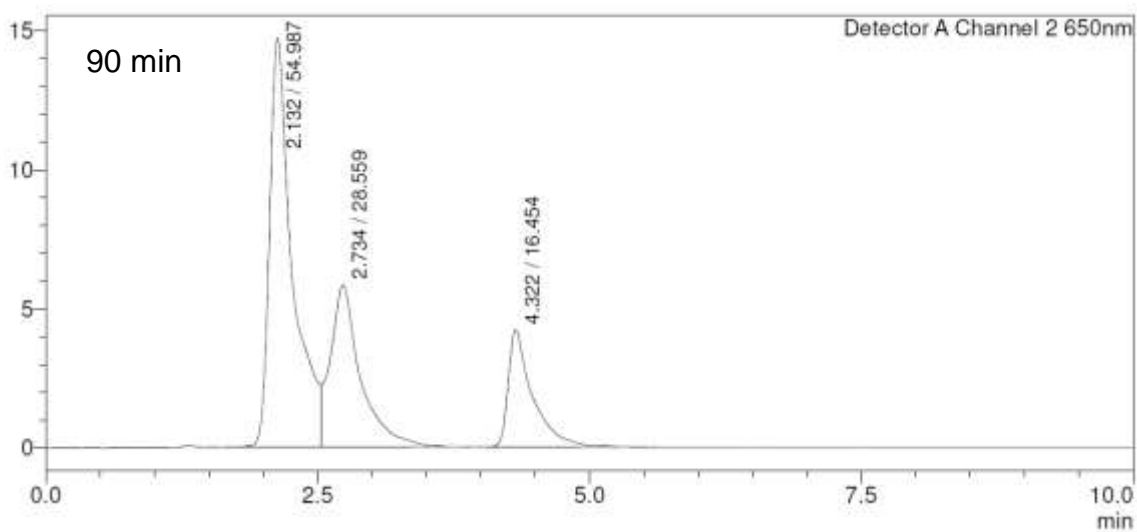
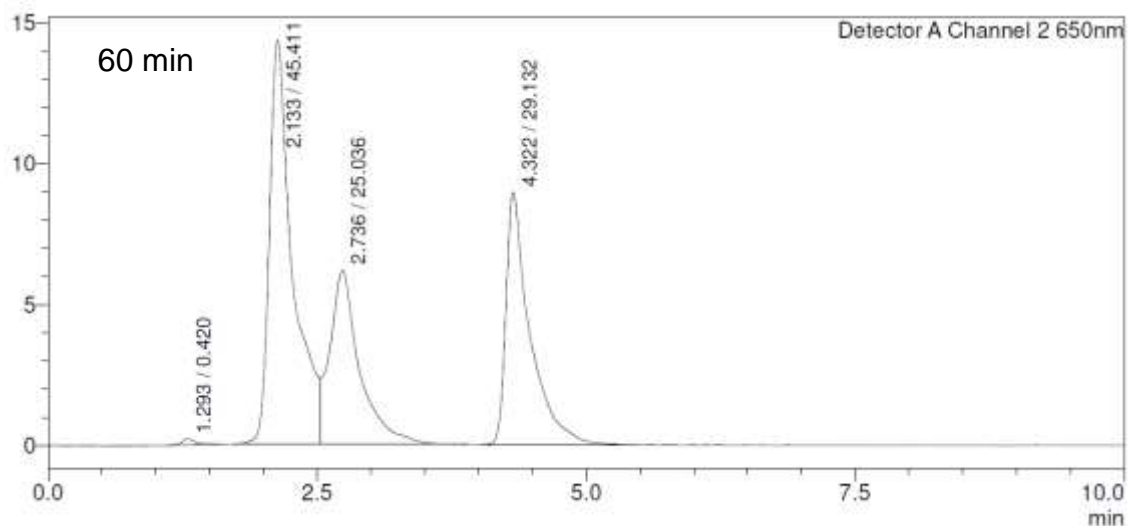


Absorbance and fluorescence spectra for a serial dilution of **6b** in MeOH



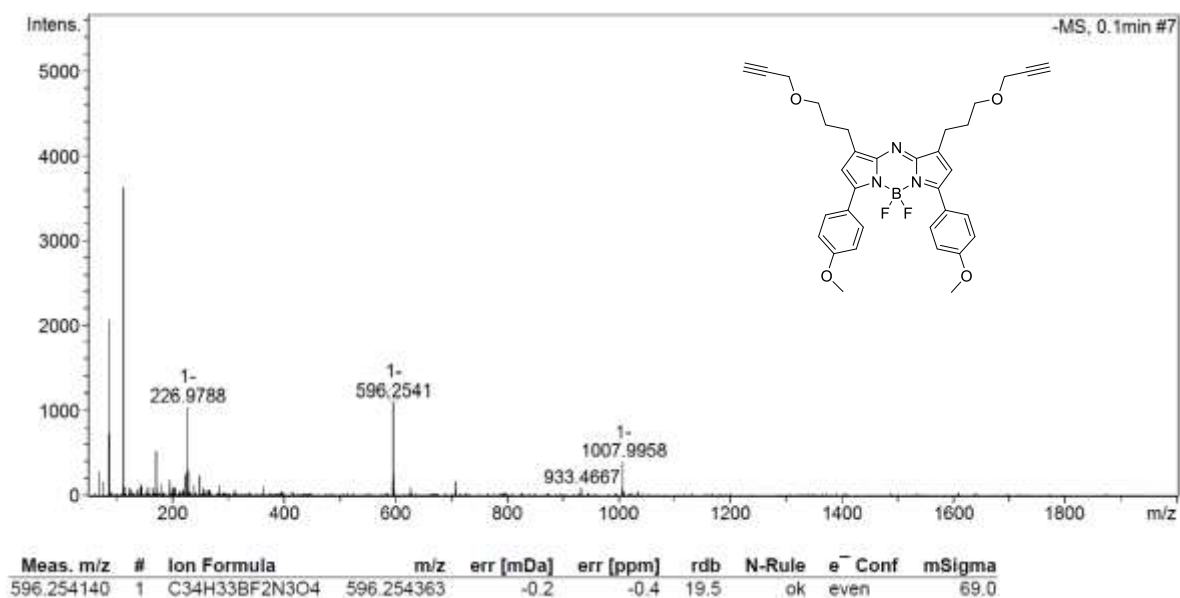
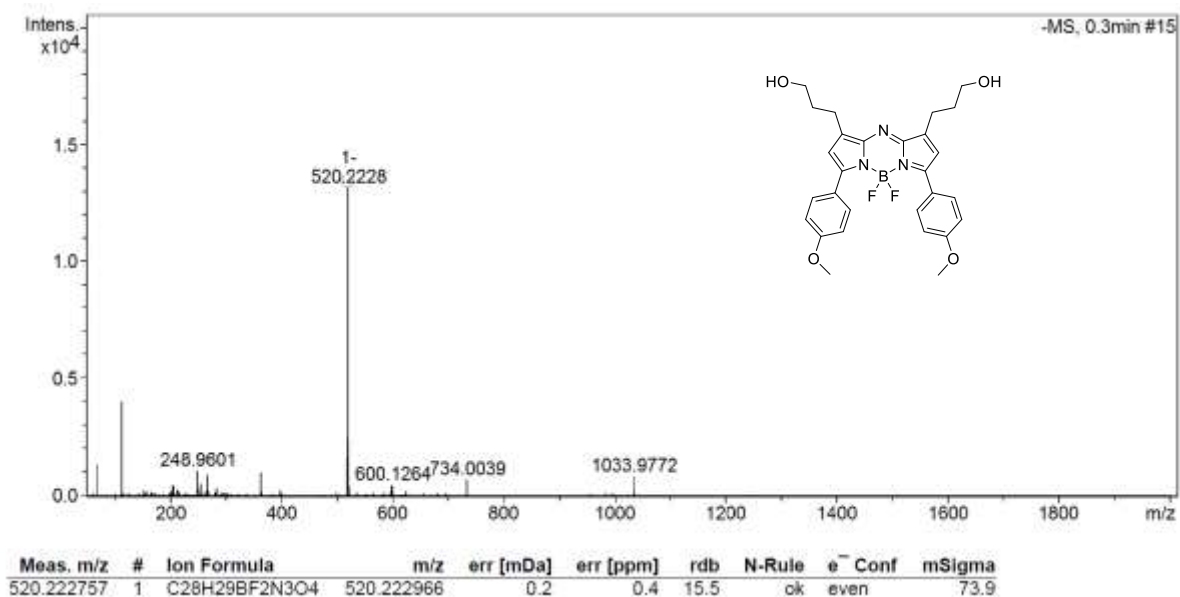
HPLC traces for monitoring click reaction of **1b**



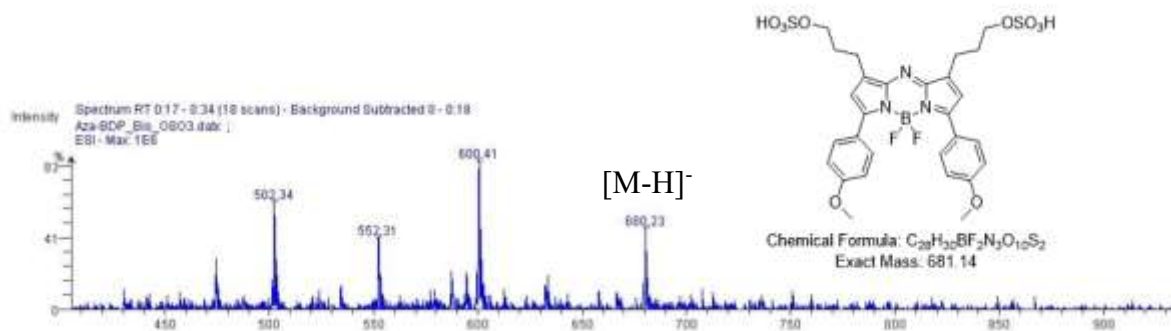


Reverse phase HPLC with YMC triart phenyl column and size: 150 × 4.6mm I.D., particle size: S-5 μ m, 12 nm hole, detection wavelength: 650 nm. Eluent CH₃CN :H₂O=80:20 with 1 mL/min flow rate.

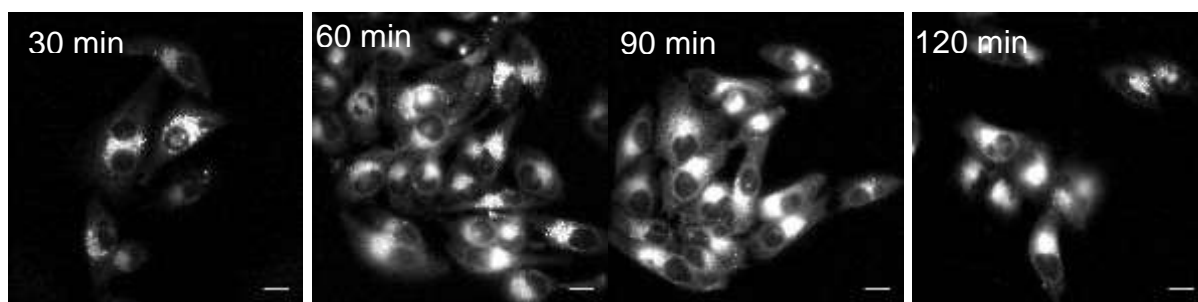
ESI-HRMS of **1a**, **1b** (negative mode)



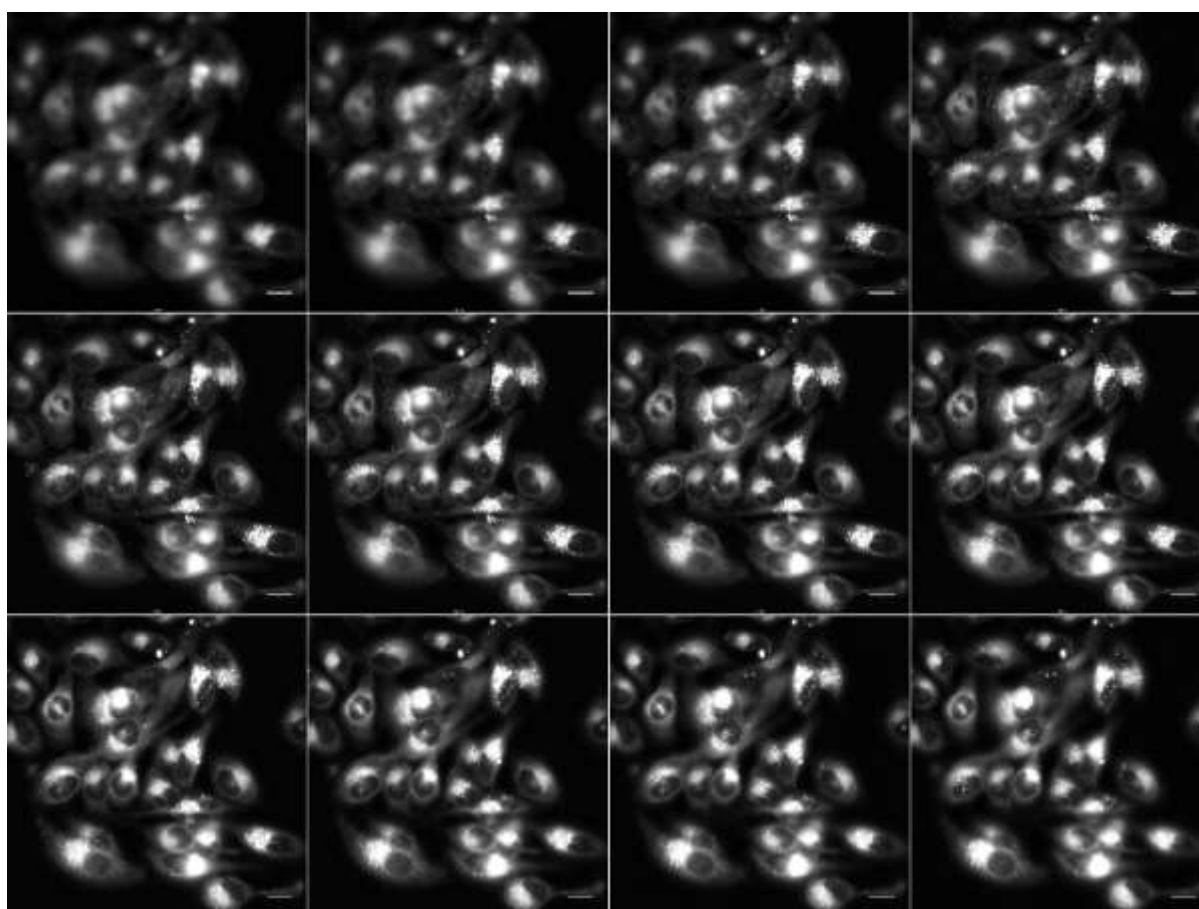
ESI-MS of **6a** (negative mode)



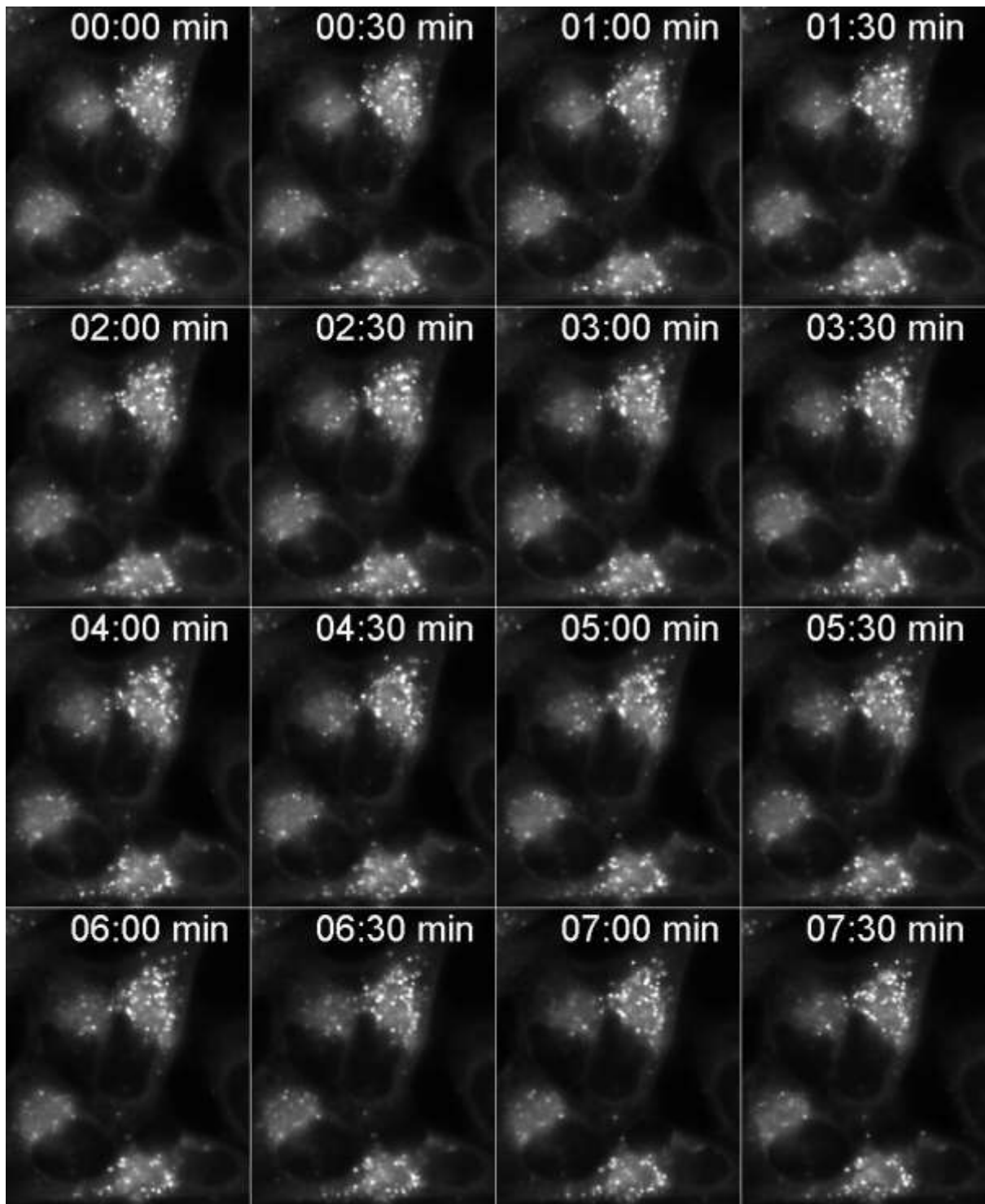
Live cell images of **6a** at 30, 60, 90, 120 min with Hela-Kyoto cell line.



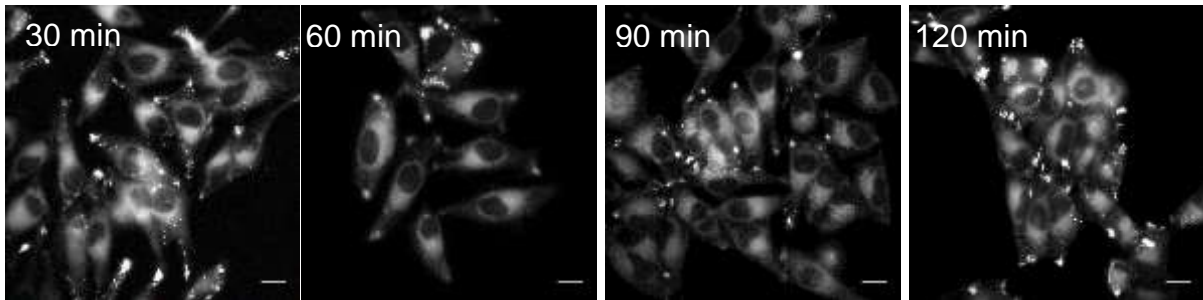
Montage of Z-stack images of **6a** at 60 min (see video)



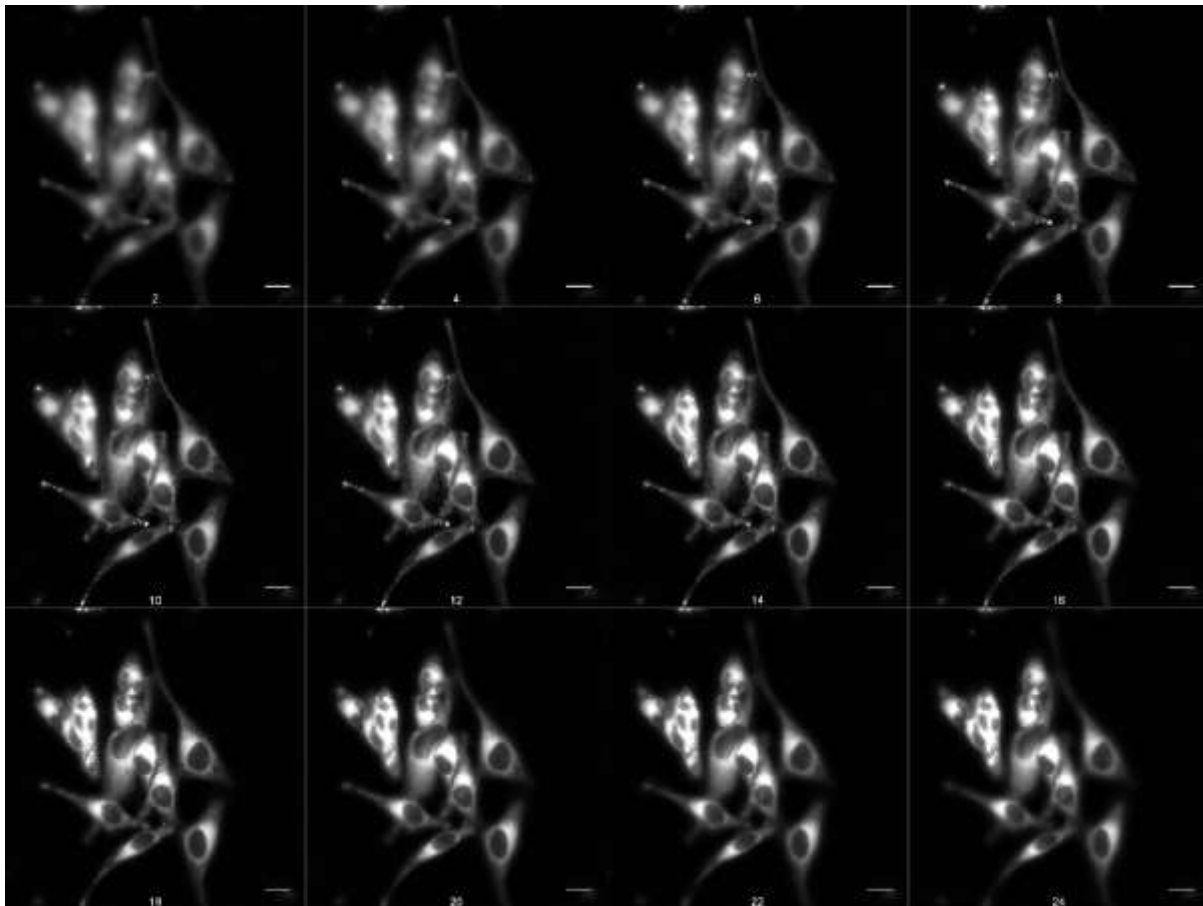
Montage of vesicles motion live images of **6a** (see video)



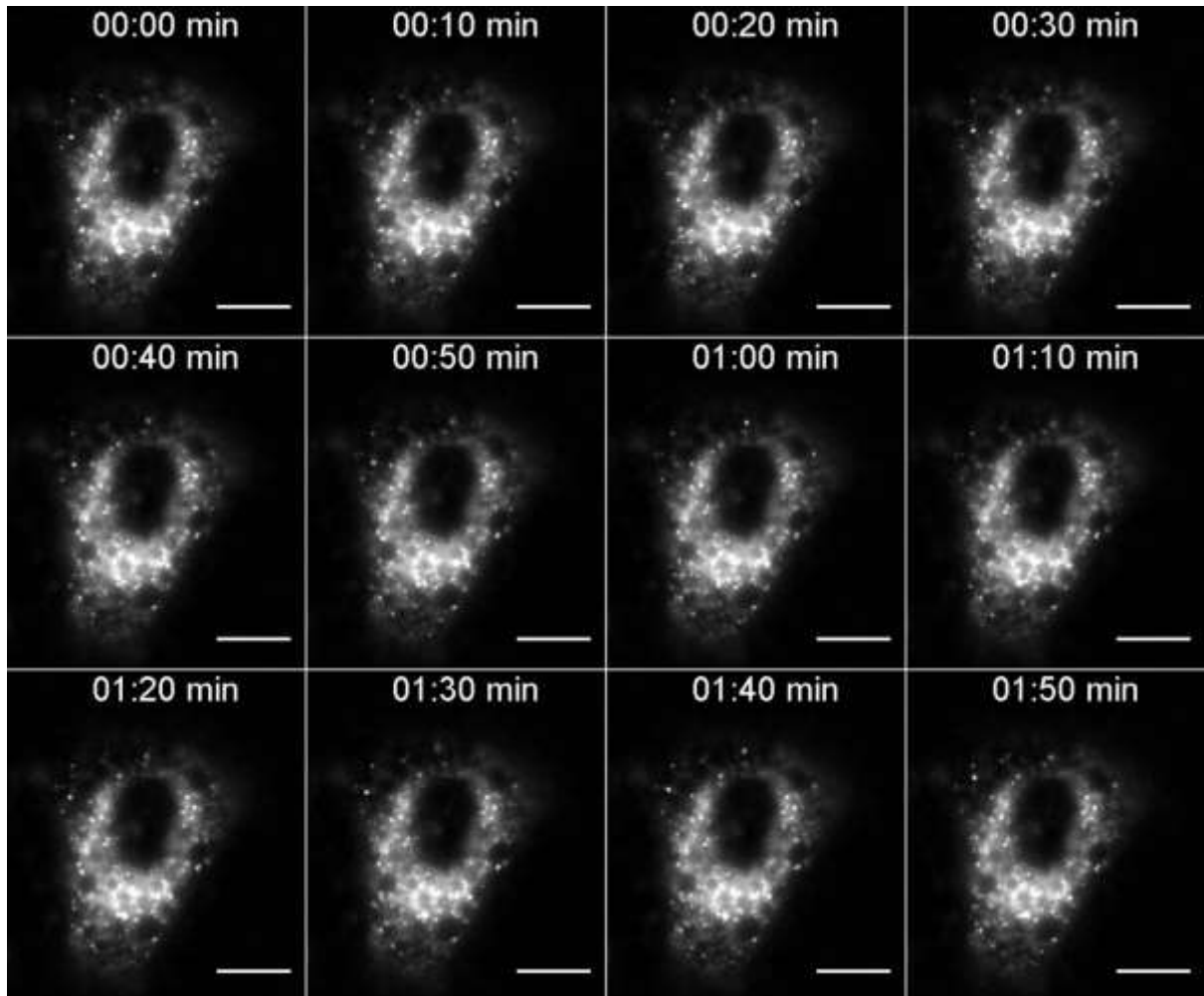
Images of **6b** at 30, 60, 90, 120 min in HeLa Kyoto cell line



Montage of Z-stack images of **6b** at 60 min (see video)

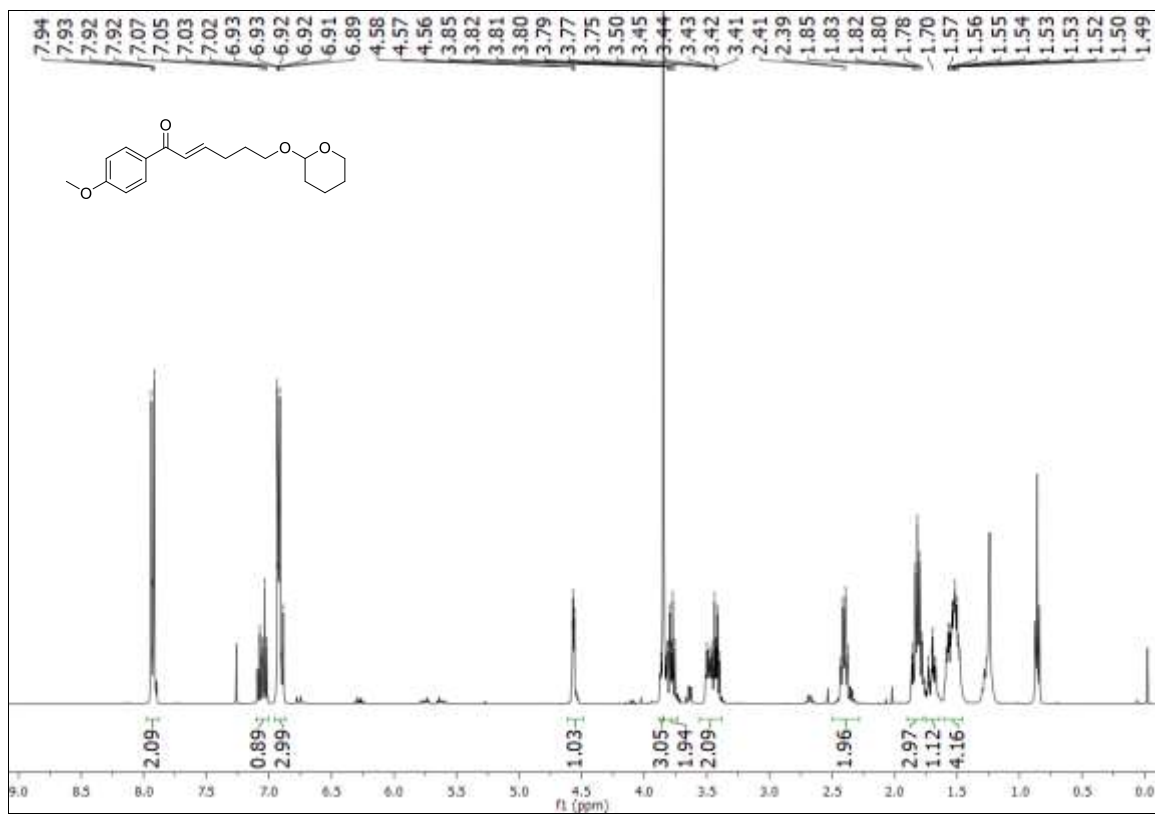


Montage of vesicles motion live images of **6b** (see video)

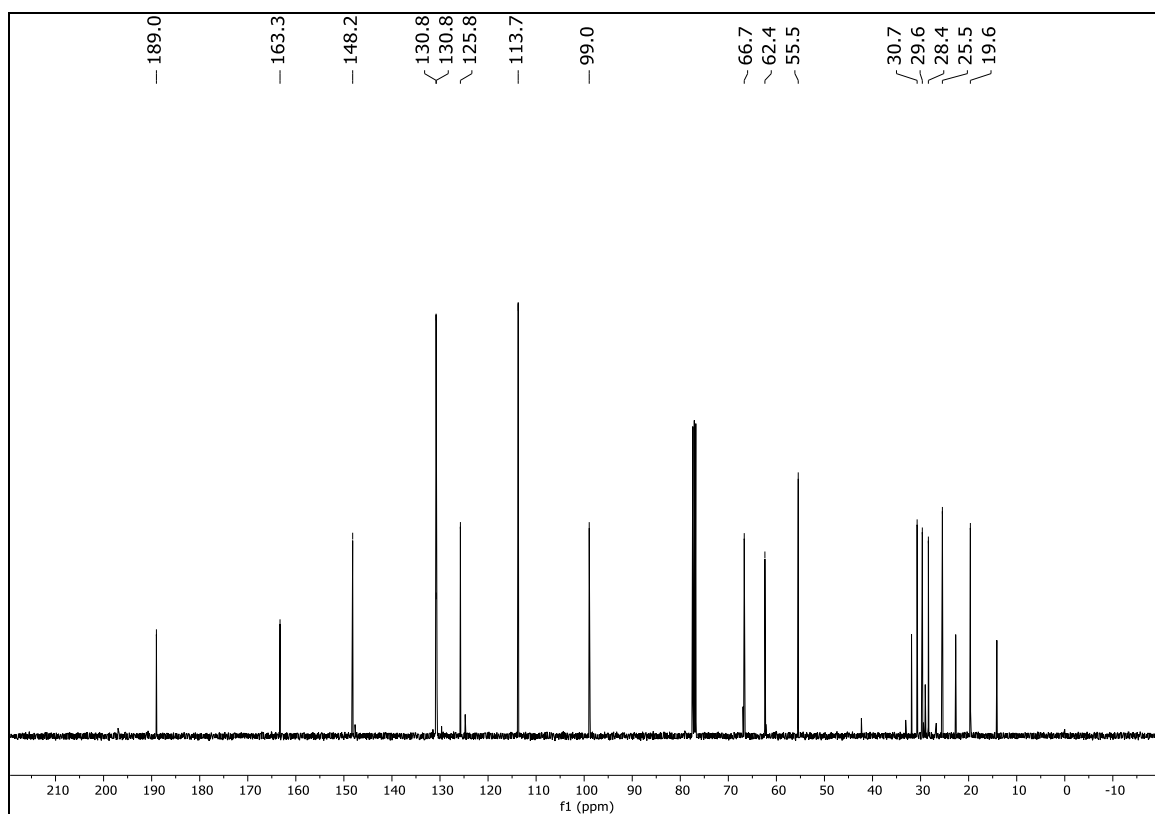


Compound 4a

^1H NMR (400 MHz, CDCl_3)

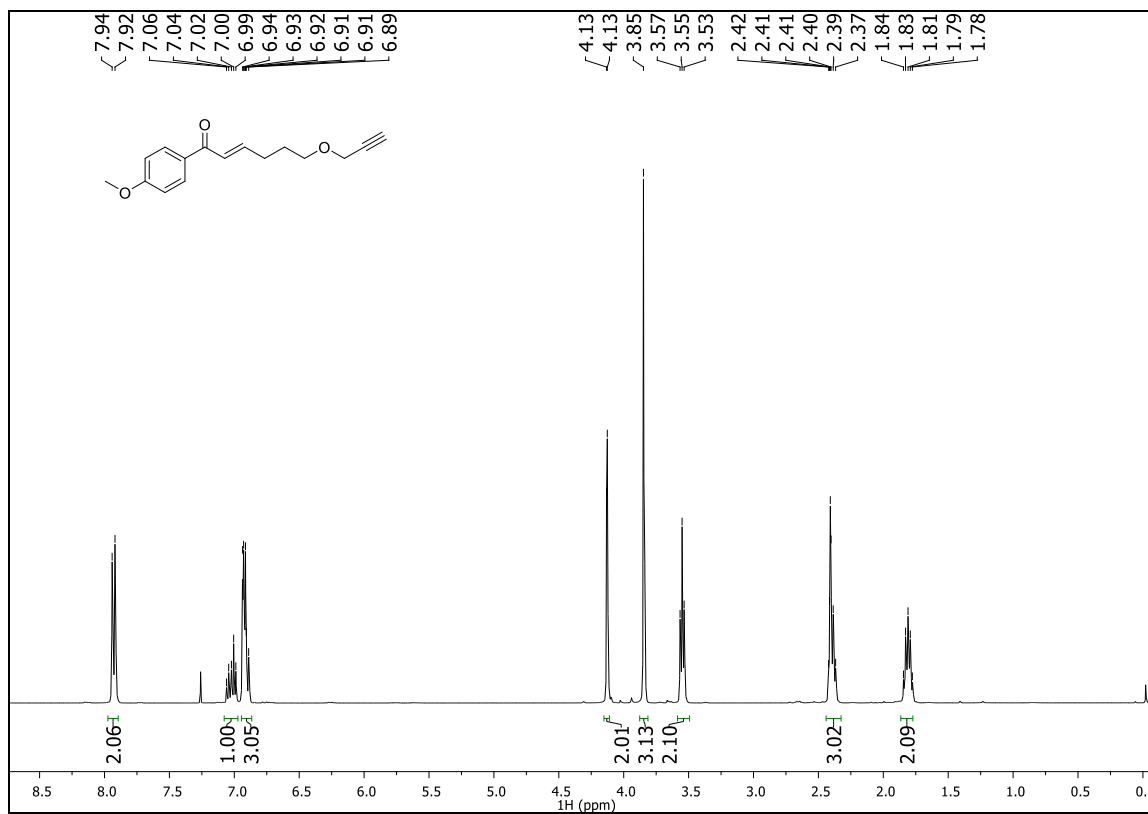


^{13}C NMR (100 MHz, CDCl_3)

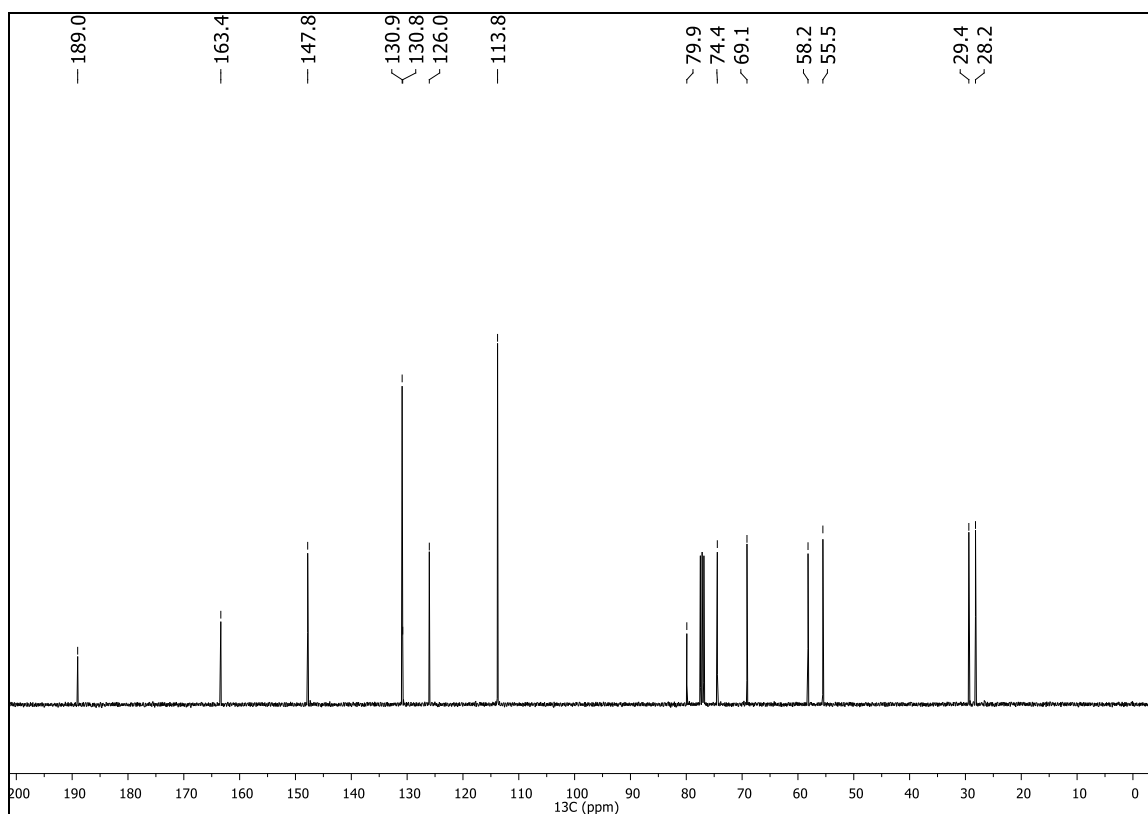


Compound 4b

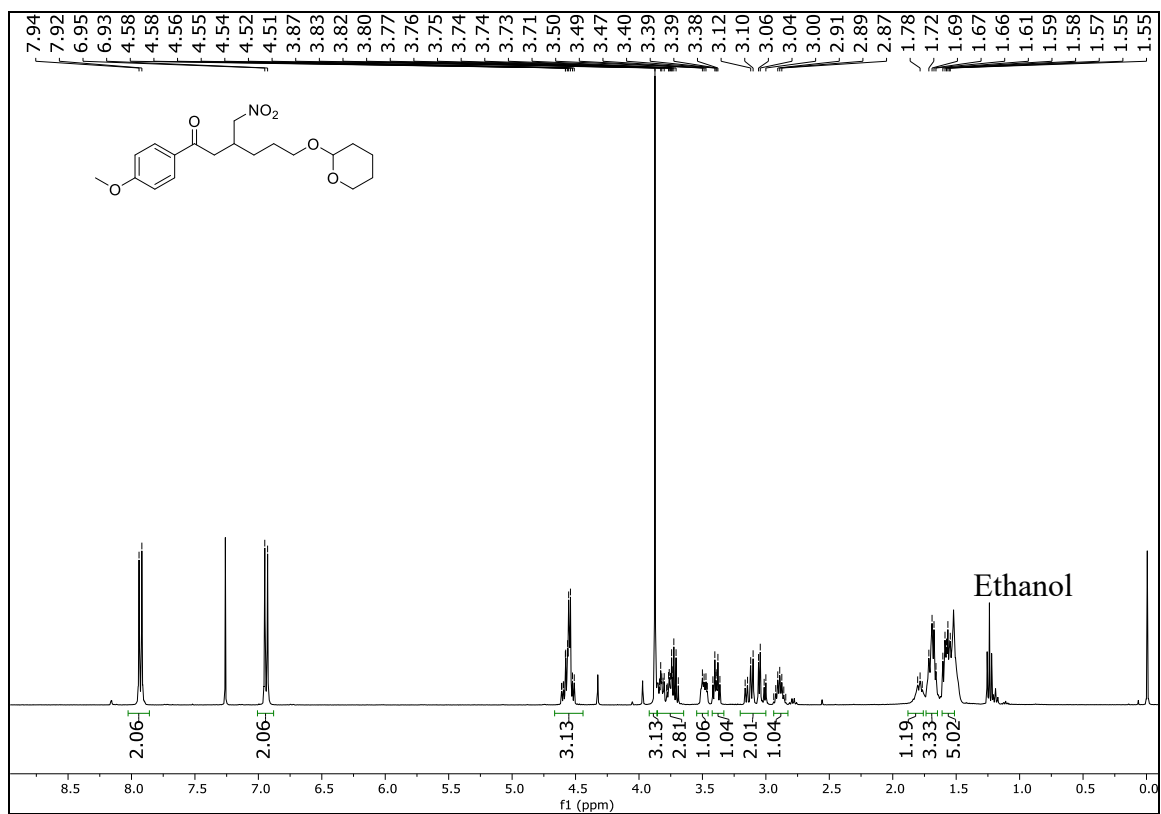
^1H NMR (400 MHz, CDCl_3)



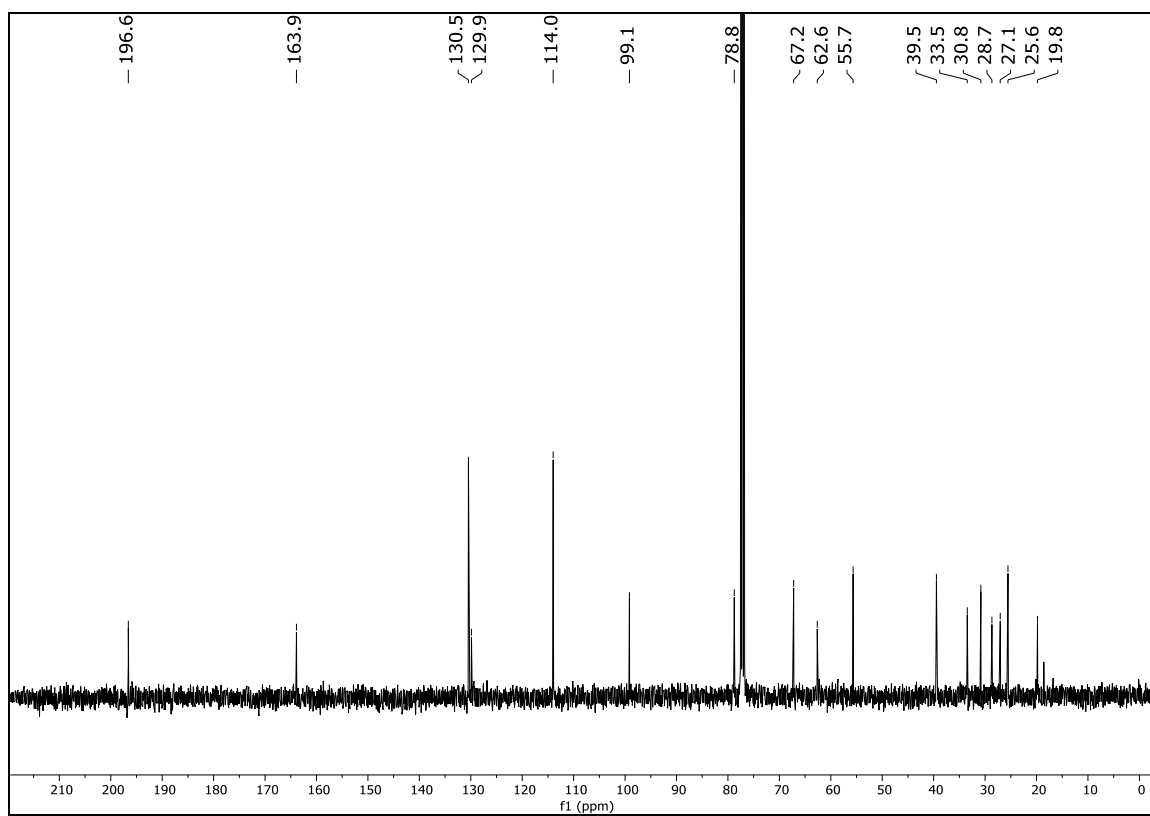
^{13}C NMR (100 MHz, CDCl_3)



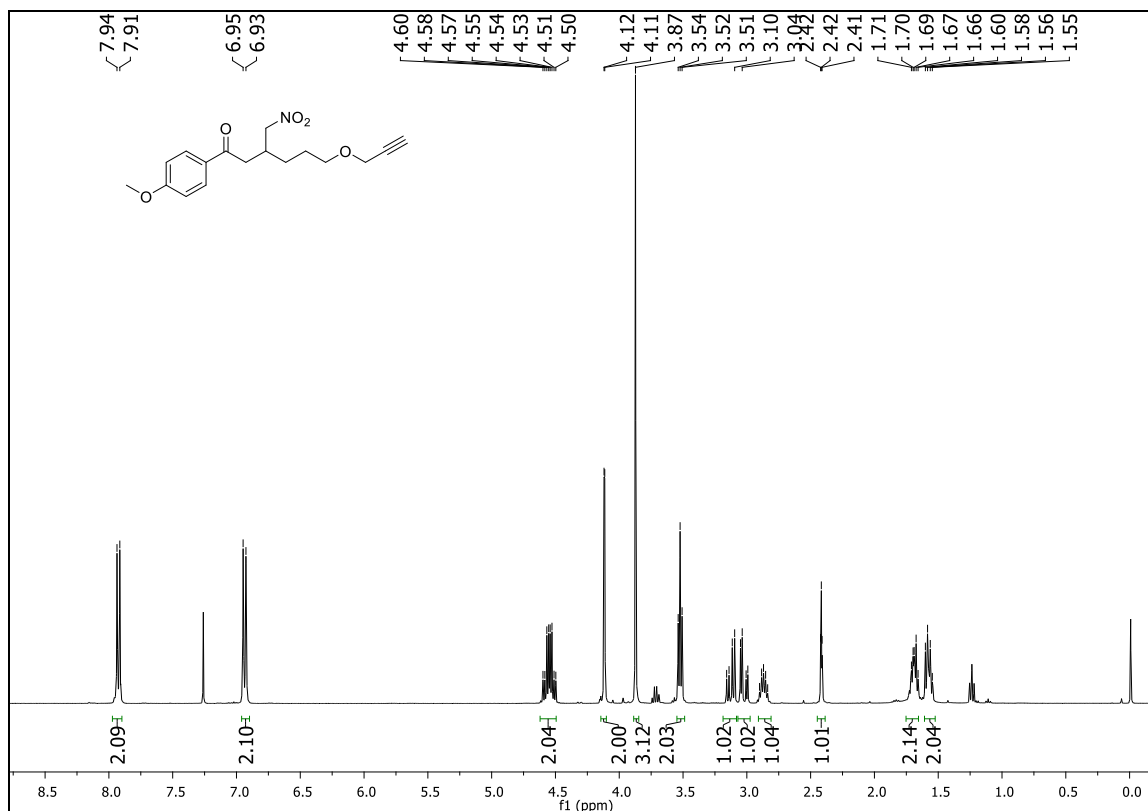
Compound 5a
¹H NMR (400 MHz, CDCl₃)



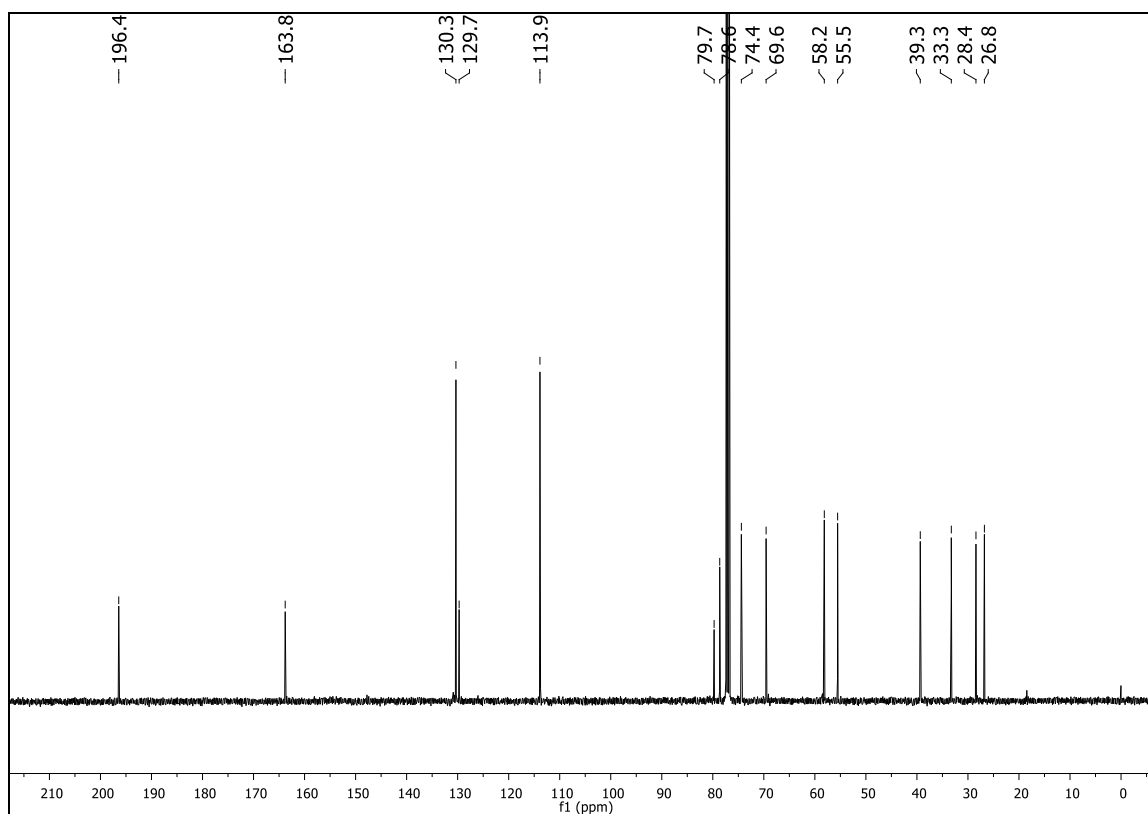
¹³C NMR (100 MHz, CDCl₃)



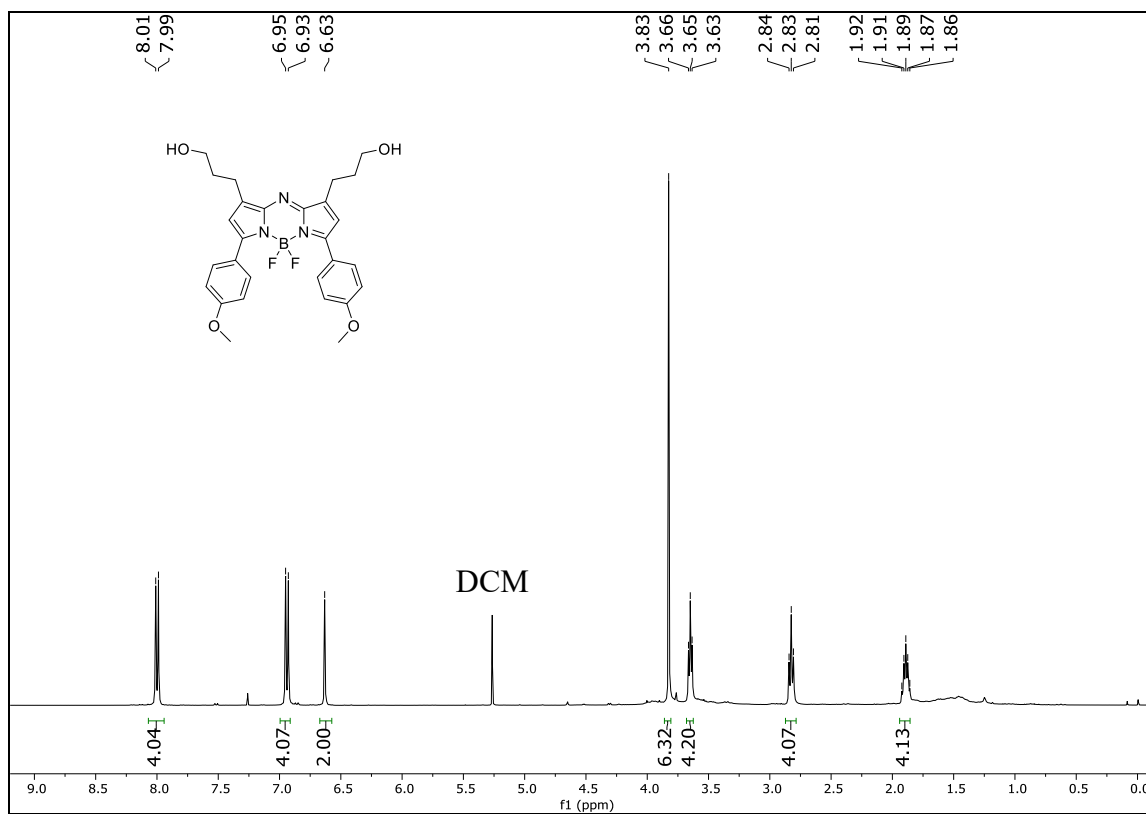
Compound 5b
¹H NMR (400 MHz, CDCl₃)



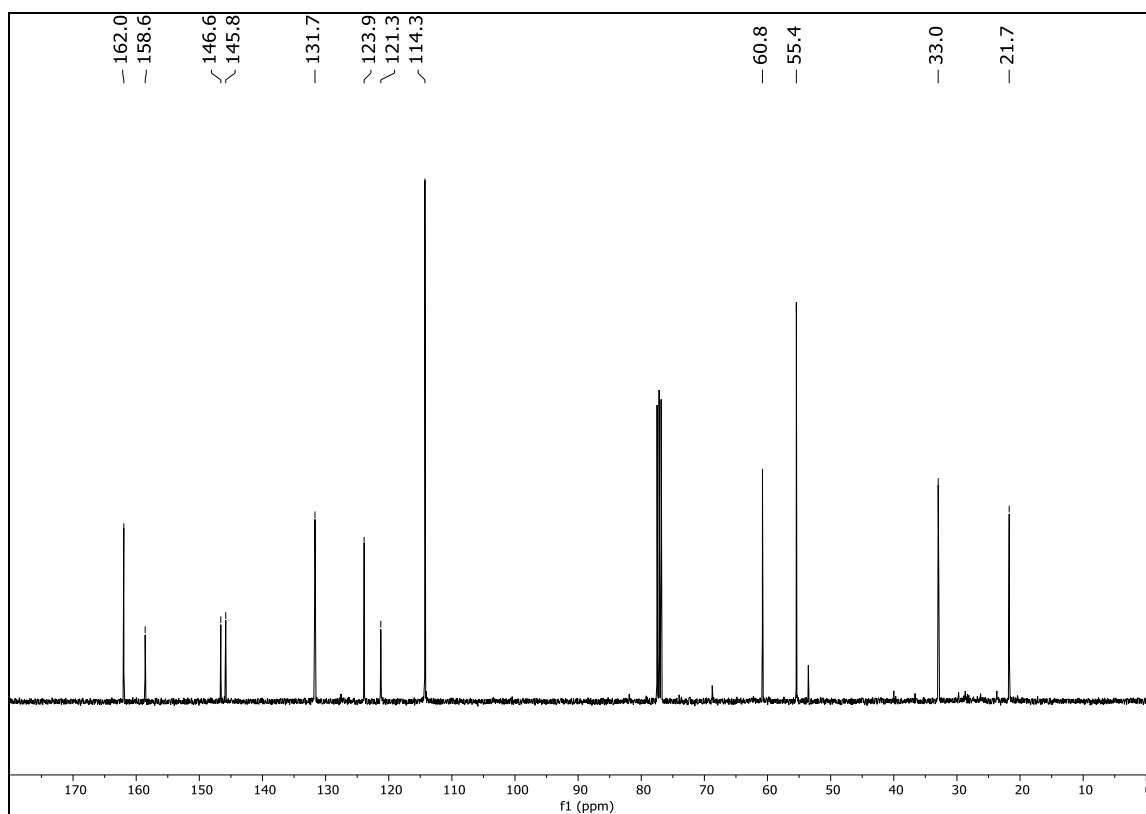
¹³C NMR (100 MHz, CDCl₃)



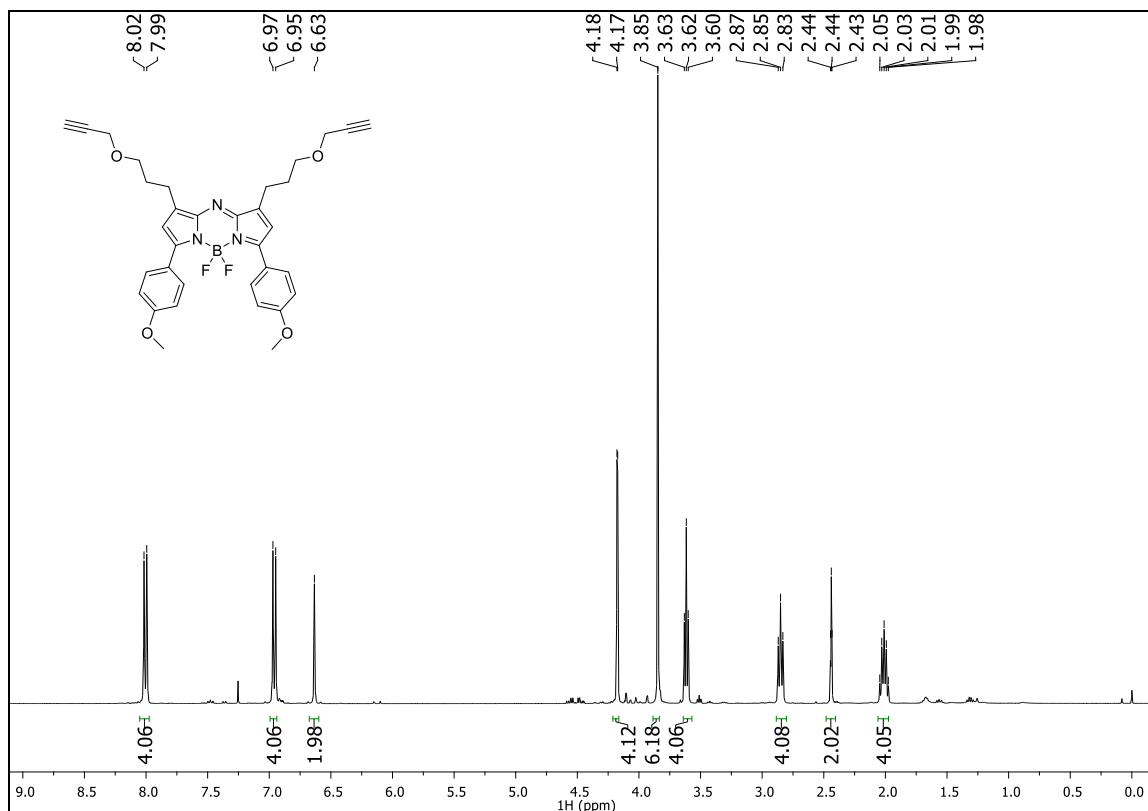
Compound 1a
¹H NMR (400 MHz, CDCl₃)



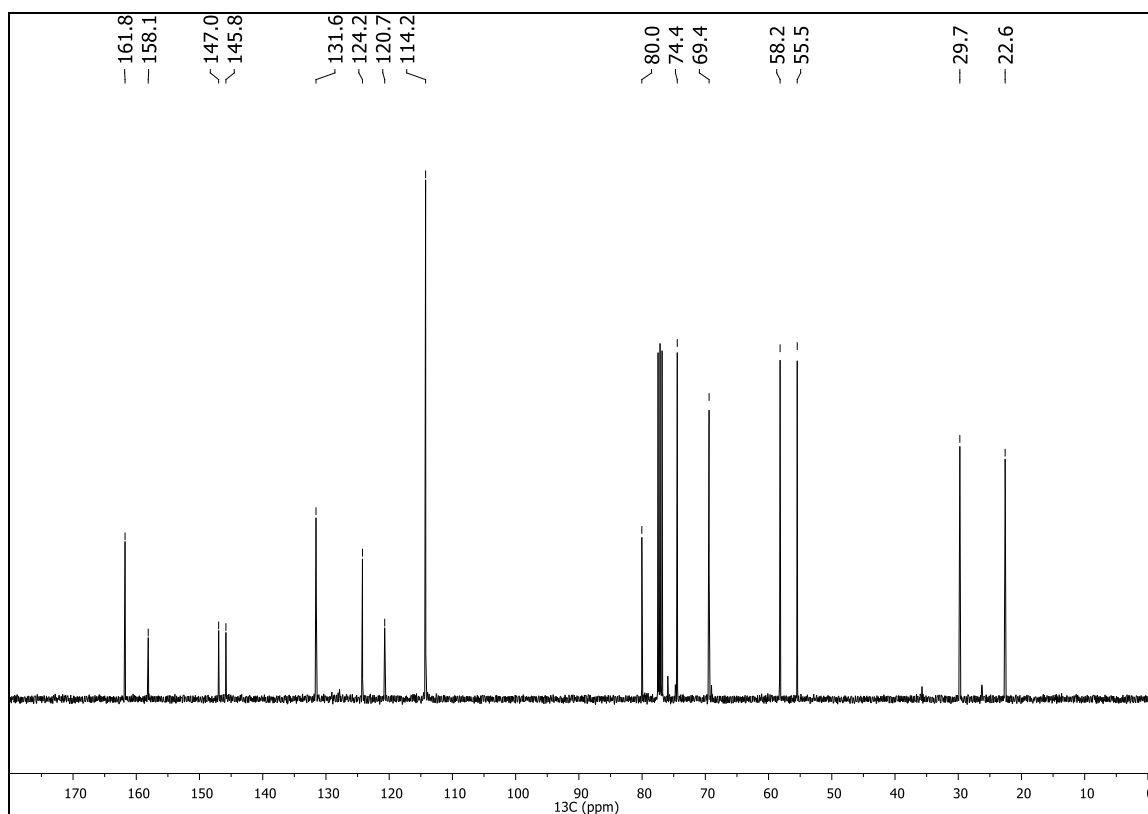
¹³C NMR (100 MHz, CDCl₃)



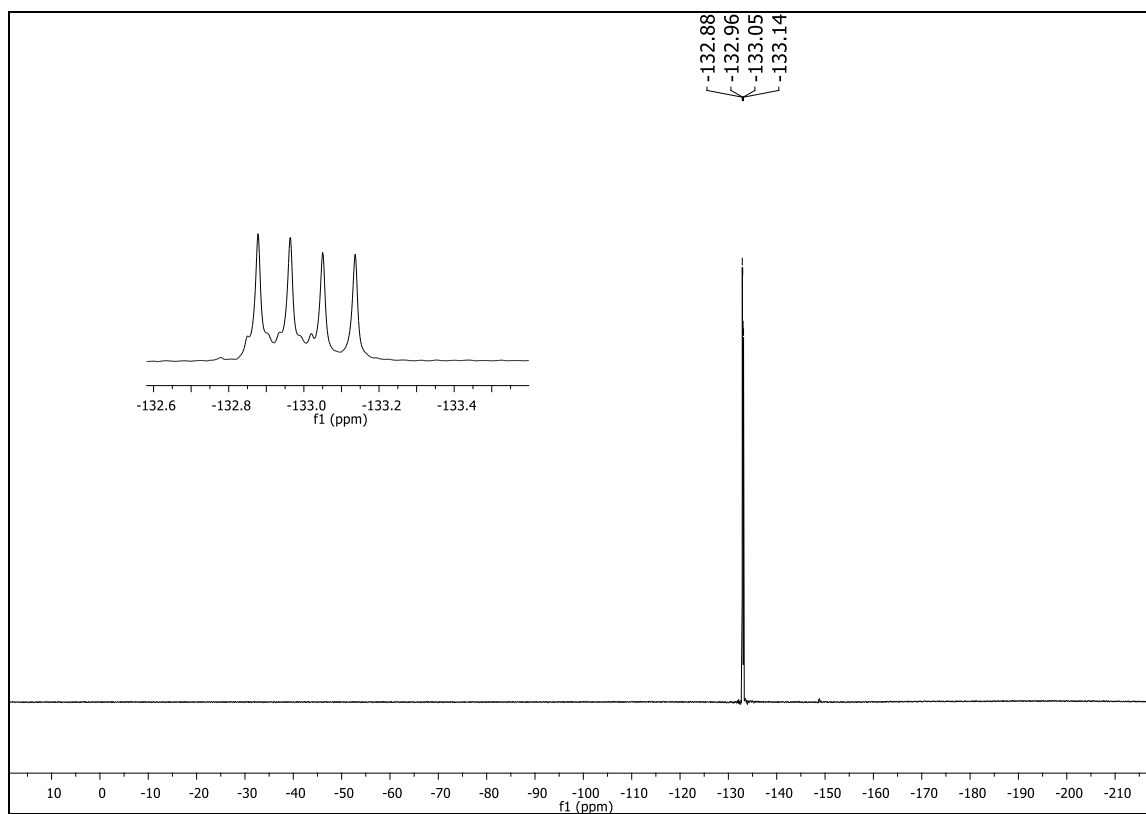
Compound 1b
 ^1H NMR (400 MHz, CDCl_3)



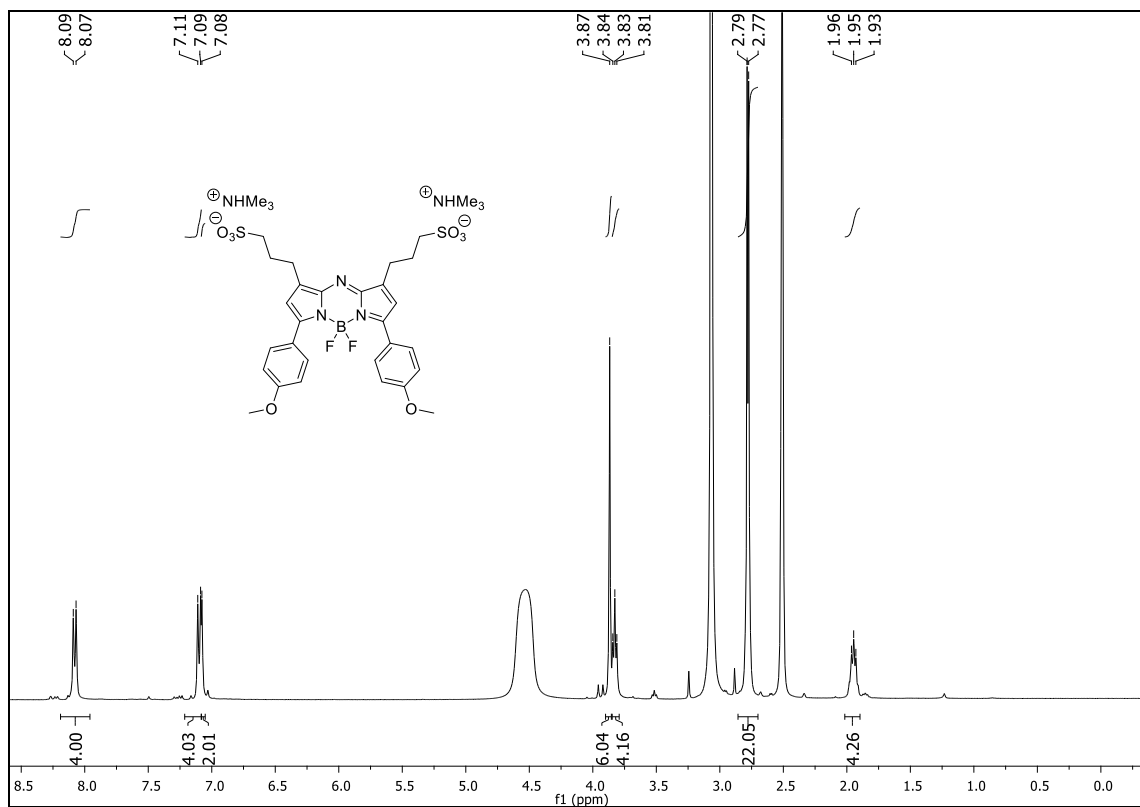
^{13}C NMR (100 MHz, CDCl_3)



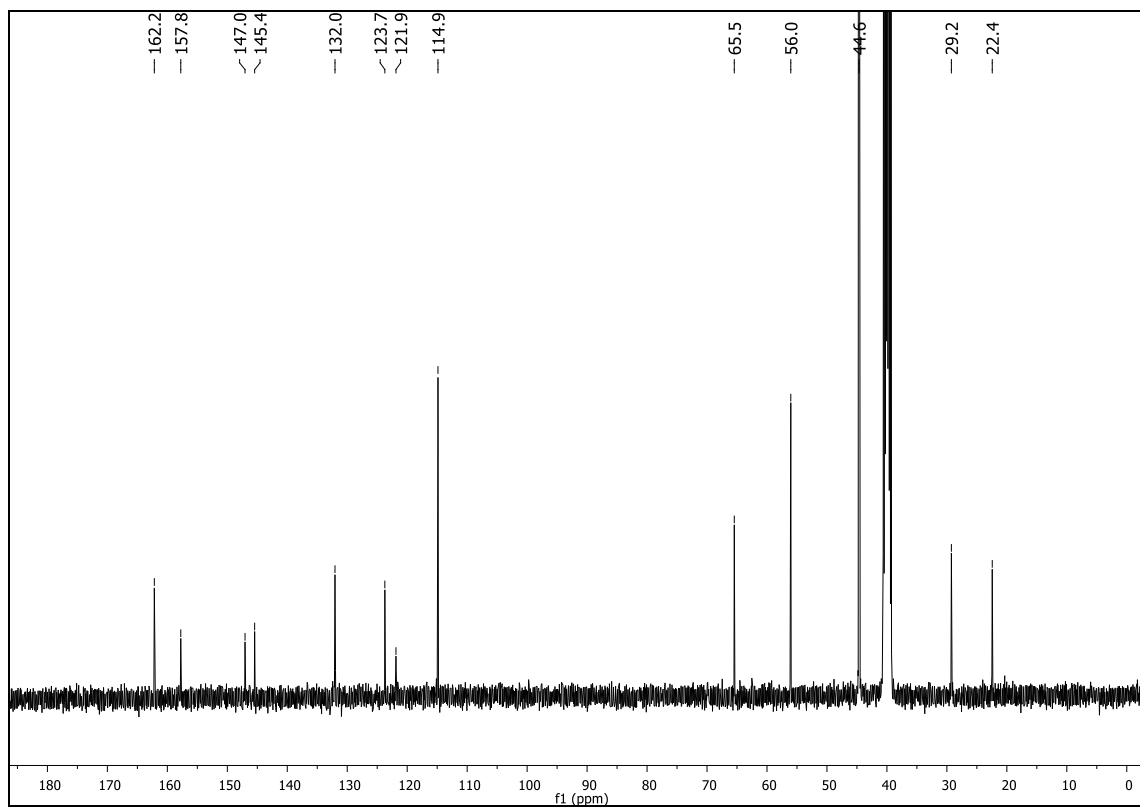
^{19}F NMR (376 MHz, CDCl_3)



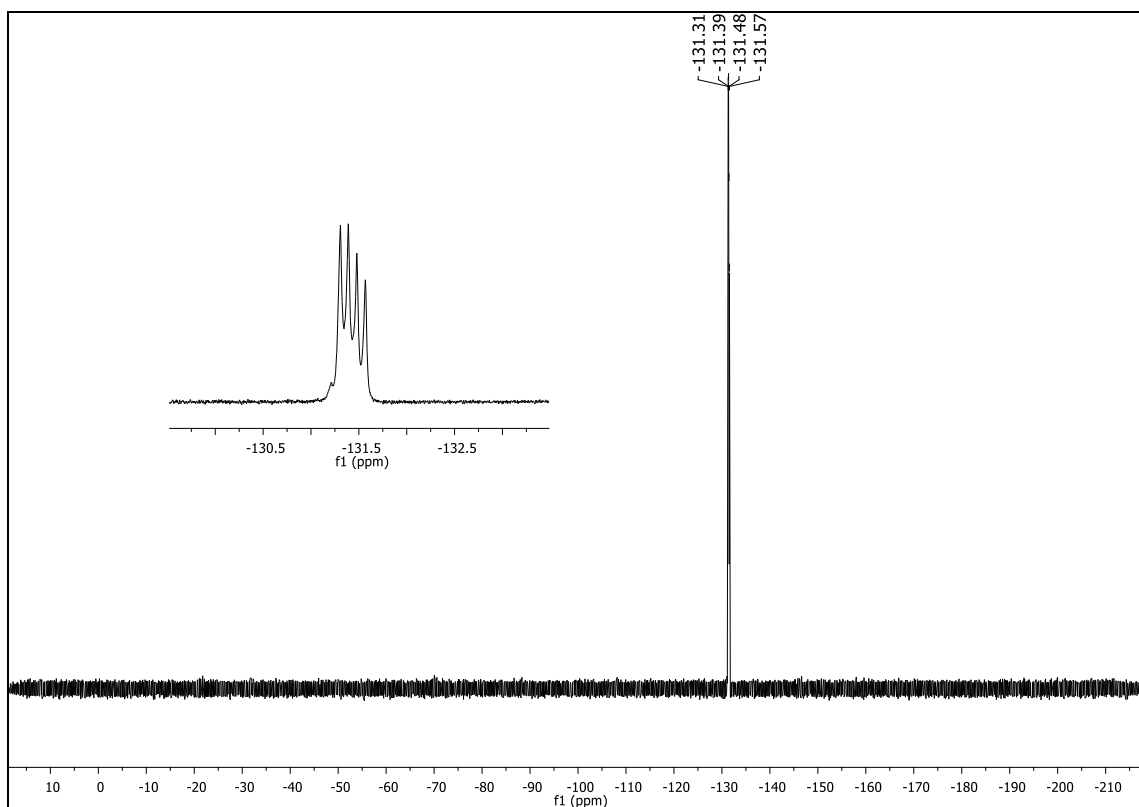
Compound 6a
¹H NMR (400 MHz, DMSO-*d*₆)



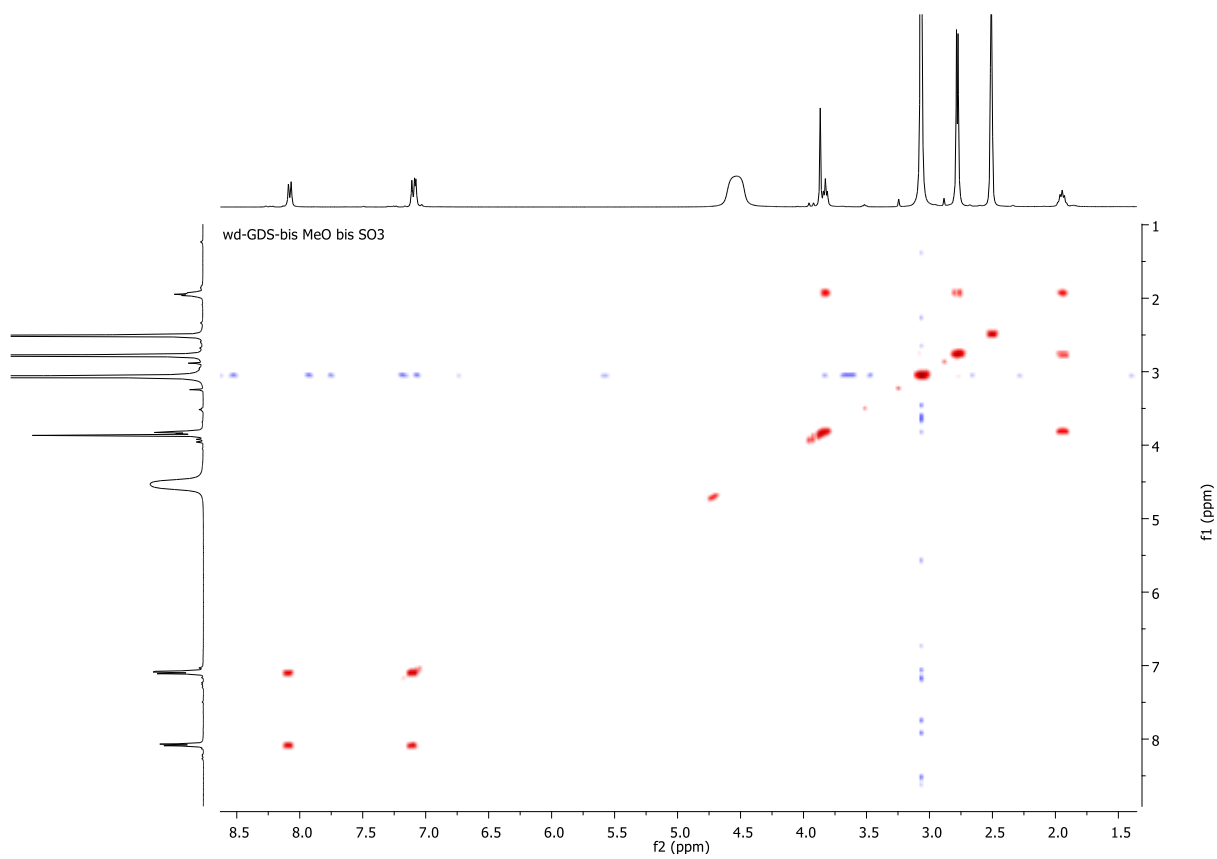
¹³C NMR (100 MHz, DMSO-*d*₆)



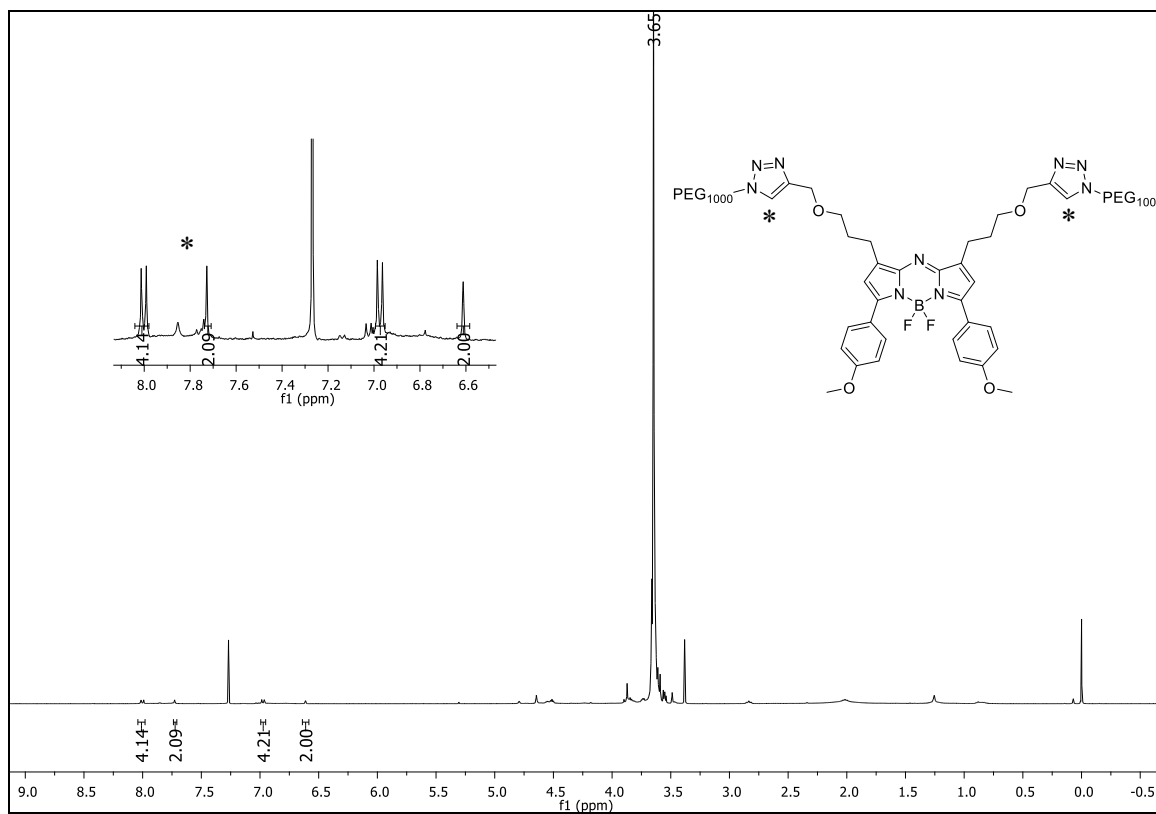
^{19}F NMR (376 MHz, $\text{DMSO-}d_6$)



^1H COSY NMR ($\text{DMSO-}d_6$)



Compound 6b
¹H NMR (400 MHz, CDCl₃)



¹⁹F NMR (376 MHz, CDCl₃)

