Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Supporting Information

Title: *In vitro* Cytotoxicity of a Library of BODIPY-anthracene and -pyrene Dyads for Application in Photodynamic Therapy

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Contents

1. Protocol for Singlet Oxygen Quantum Yield Measurements

The procedure for the singlet oxygen quantum yield measurements was adapted from literature.[1] A solutions of 1,3-diphenylisobenzofuran (DPBF), with an optical density of 1.0 in air-saturated ethanol were formulated. The corresponding **BAD** was added to the cuvette, and its absorbance was adjusted to approximately 0.01 at the wavelength of irradiation. The solution in the cuvette was irradiated with a 532 nm laser light at a power density of 10 mW.cm^{-2}. The absorption spectra of the solutions were measured every 10 s. The slope of plots of absorbance of DPBF at 414 nm *vs.* irradiation time for each photosensitizer was calculated. Singlet oxygen quantum yields were calculated according to the following equation:

$$\Phi_{\Delta} = \Phi_{\Delta}^{ref} \times \frac{k}{k_{ref}} \times \frac{I_{abs}^{ref}}{I_{abs}}$$

where Φ_{Δ} is the singlet oxygen quantum yield, the superscript *ref* stands for **BAD-6** (0.67 in ethanol),[2] *k* is the slope of the curves of DPBF absorption (414 nm) change *vs.* irradiation time, *I* represents the absorption correction factor which is given by $I = 1–10^{-OD}$ (OD is the optical density at 532 nm).

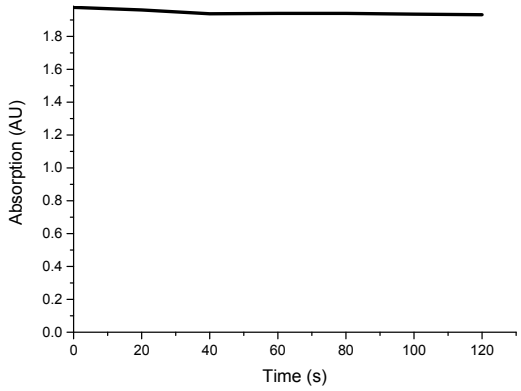


Figure S1: Change of DPBF absorption at 414 nm upon irradiation in air-saturated ethanol.

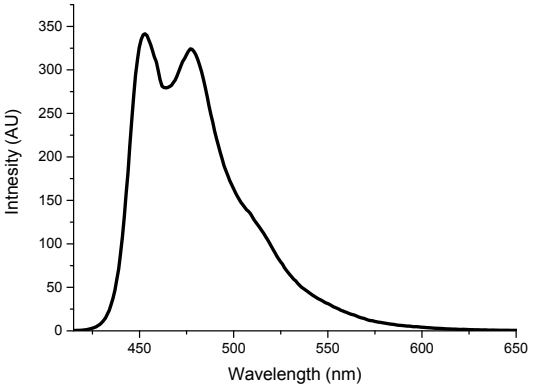


Figure S2: Fluorescence emission spectrum of DPBF at an excitation wavelength of 410 nm

2. Protocol for Phototoxicity Studies

For the photocytotoxicity studies the BADs were formulated in DMSO (Hybri-Max;Sigma-Aldrich D-2650) and diluted in appropriate medium (Dulbecco's Modified Eagle's Medium with 4.5 gL^{-1} glucose + 2 mM L-glutamine; no FCS) to give a range of concentrations (1–50 µM). The concentration of DMSO never exceeded 5.2%. The cells (MDA-MB-468) were adjusted to a concentration of 1×10^6 cells mL^{-1}, and 800 µl was added to 200 µl of BAD stock solution to achieve the desired concentrations. The cells were then incubated in the dark for an hour at 37 °C and 5 % CO_2, after which they were washed with a three times excess of media and centrifuged to eliminate any unbound BAD. The final pellets of cells were re-suspended in 1 mL medium and 100 µL (8 × 104 cells) was transferred into two 96 wells plates in quadruplicate for each concentration. One plate was subsequently irradiated with light (400–700 nm; 20 J cm^{-2}) from an Oriel quartz tungsten halogen lamp (model 66188 powered by an Oriel 1100W radiometric power supply (model 69935). The second plate was kept in the dark and served as a "no light" control. After irradiation, 5 µl of Fetal Bovine Serum was added to each well and the plates were returned to the incubator overnight. After 18 to 24 h, an MTT cell viability assay[3] was performed and the results were expressed as percentage of cell viability *vs.* compound concentration; an LD_{50} (lethal dose where 50 % of the cells are killed) was determined from the resulting curves. Each experiment was repeated in triplicate.

3. Protocol for Fluorescent Quantum Yields

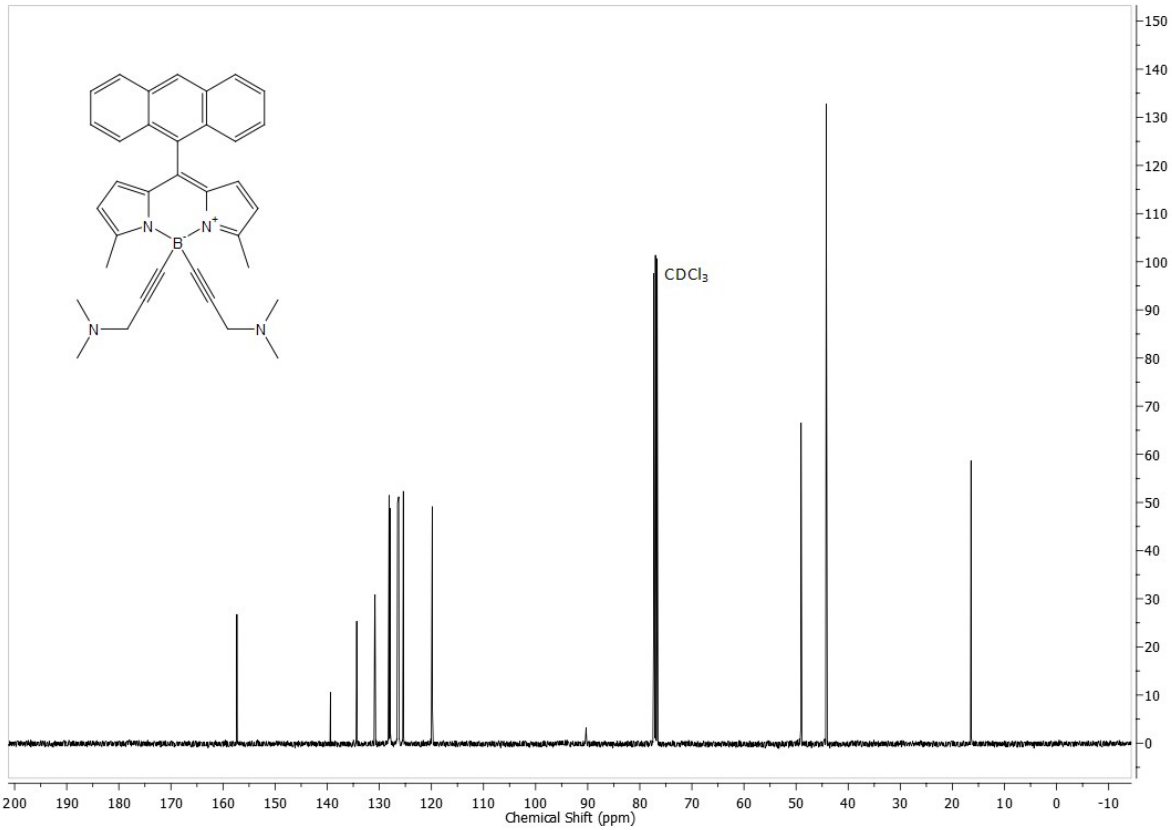
A solutions of fluorescein in 0.1 M NaOH with an absorption between 0.03-0.07 was formulated. The fluorescence quantum yield of fluorescein is reported as 0.95.[4] The maximum absorbance and area under the curves emission was noted. **BAD** solutions of concentration 1×10^{-5} M were formulated and the maximum optical density and area under the curves emission was noted. The quantum yields were calculated according to the following equation:

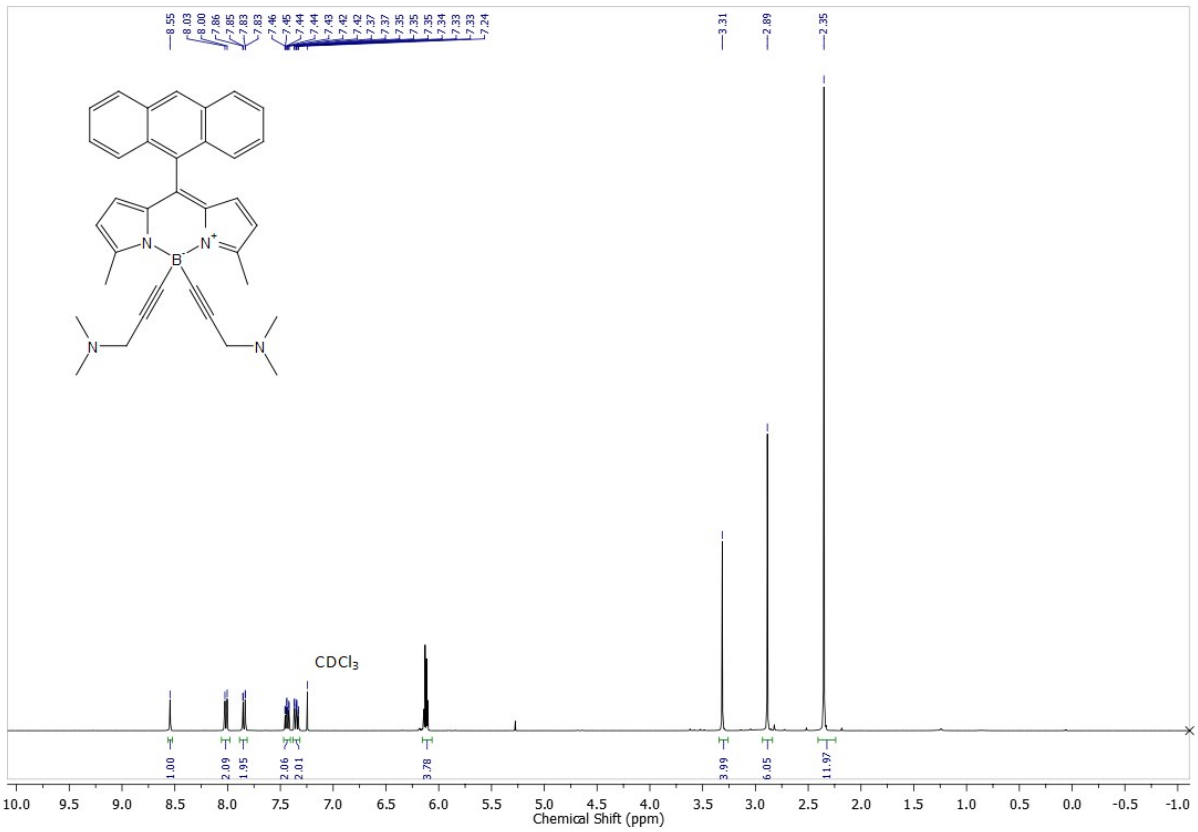
$$\Phi_f = \Phi_f^{ref} \times \frac{OD}{OD_{ref}} \times \frac{I_{abs}^{ref}}{I_{abs}} \times \frac{n^2}{n_{ref}^2}$$

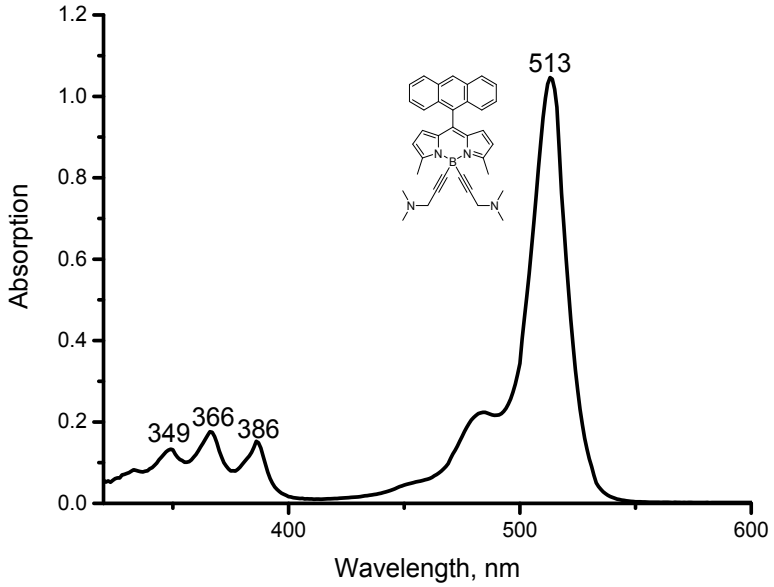
where Φ_f is the fluorescence quantum yield, the superscript *ref* stands for fluorescein, OD is the optical density, *I* is the integrated intensity, *n* is the refractive index.

4. NMR and UV/Vis Spectra

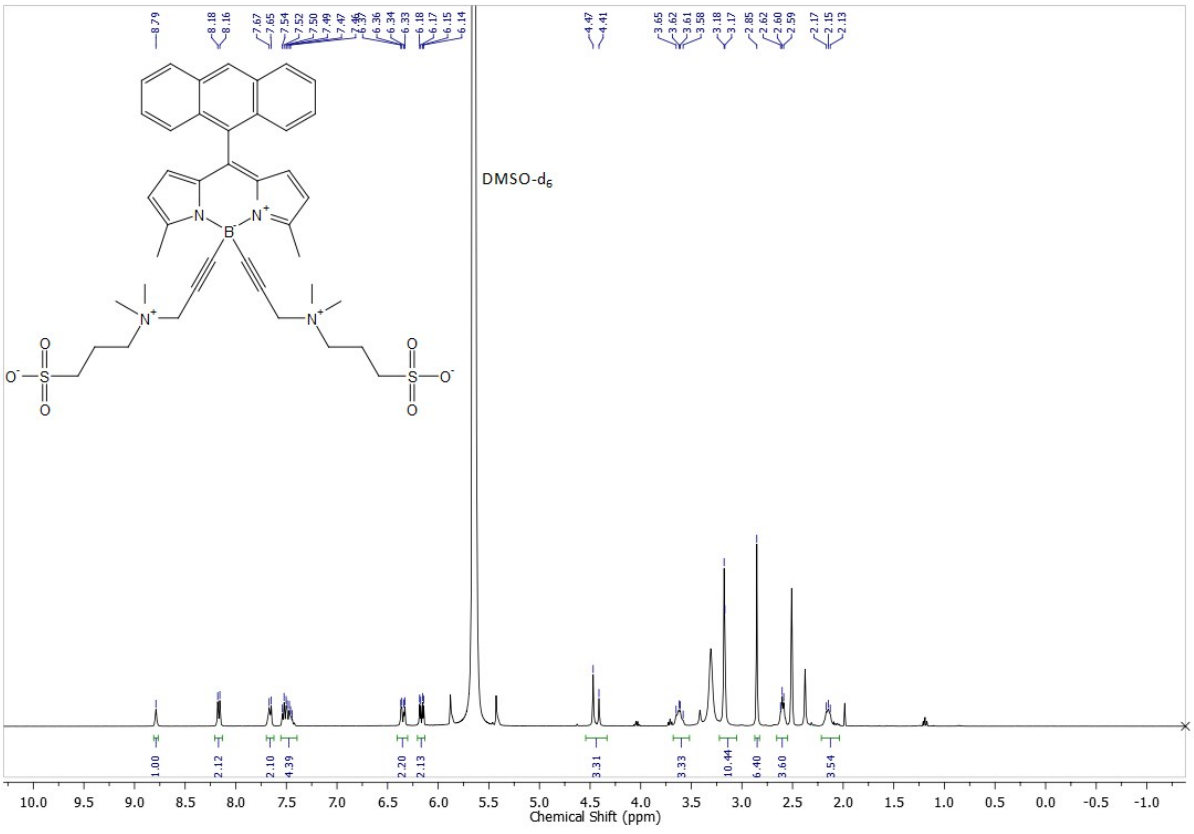
4.1 Characterization Spectra for **BAD-23**

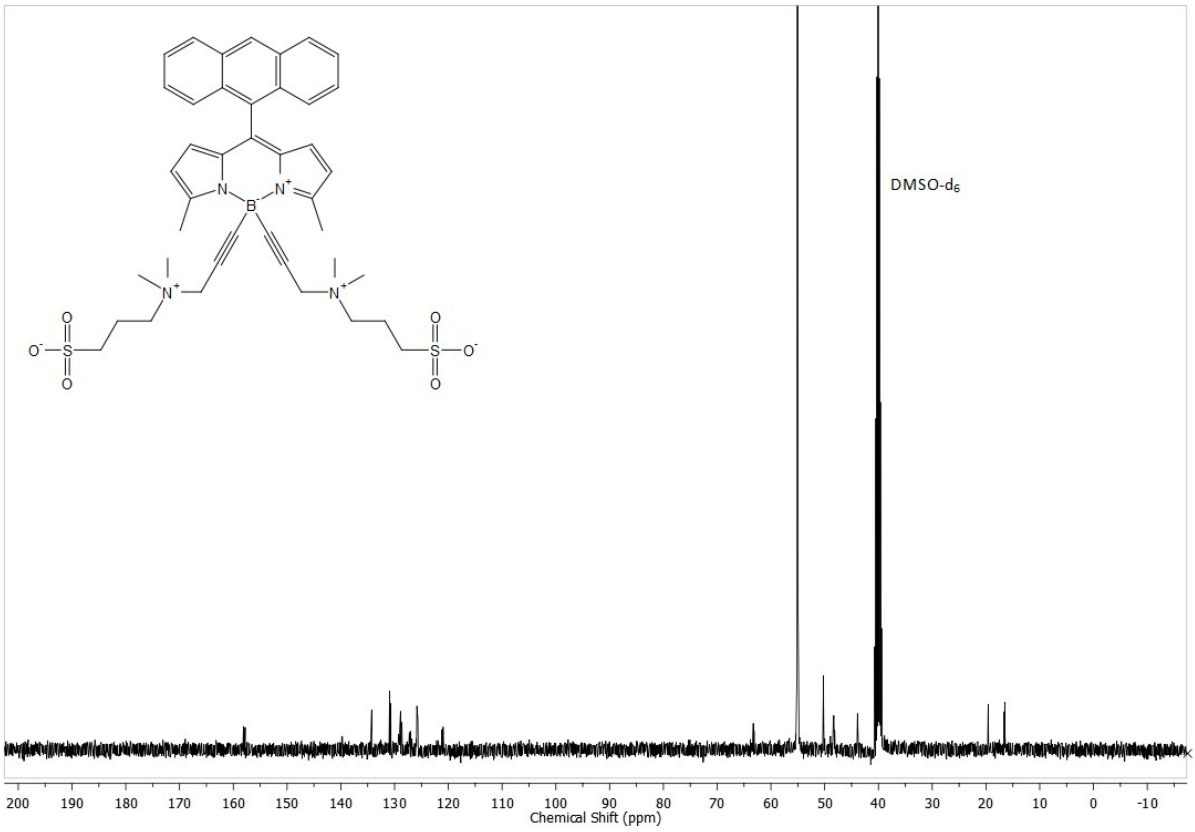






4.2 Characterization Spectra for **BAD-15**

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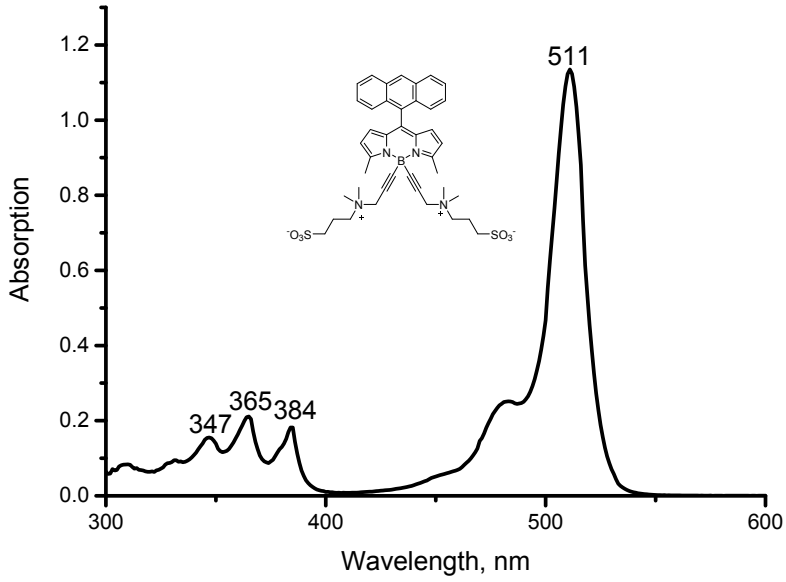
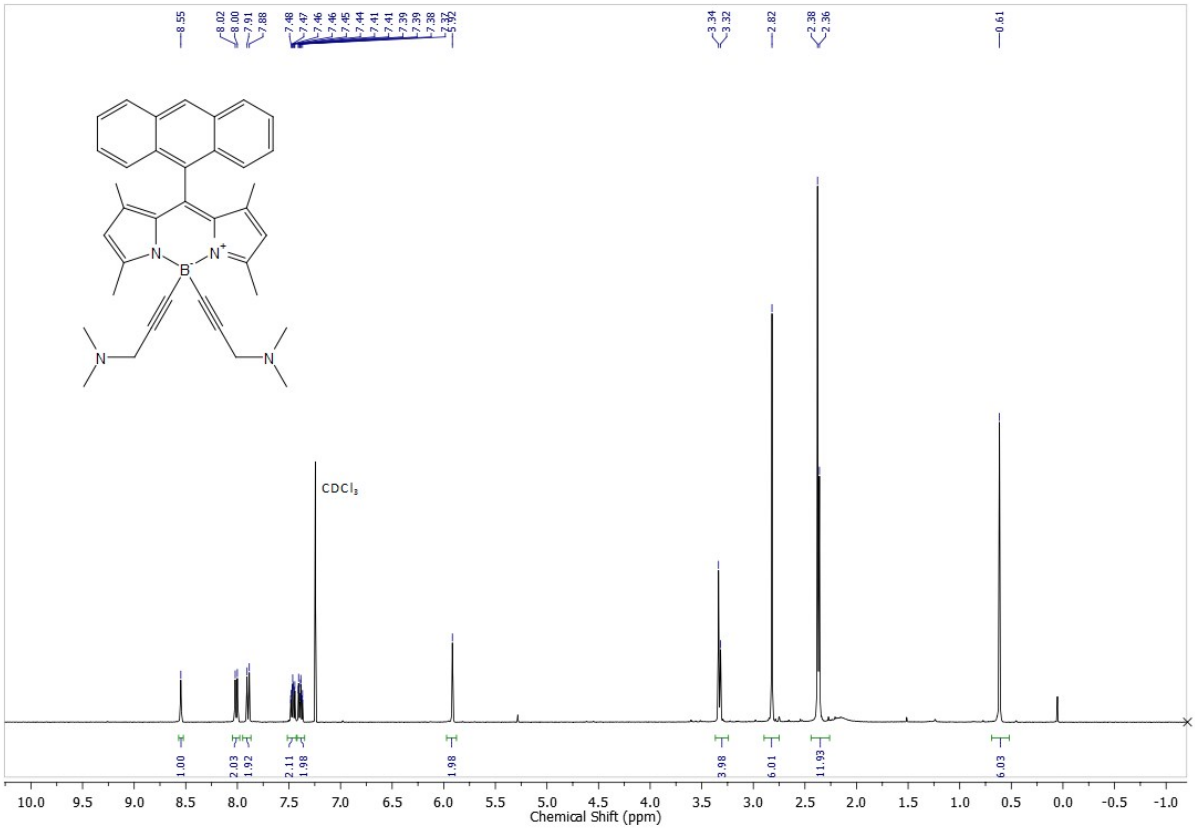
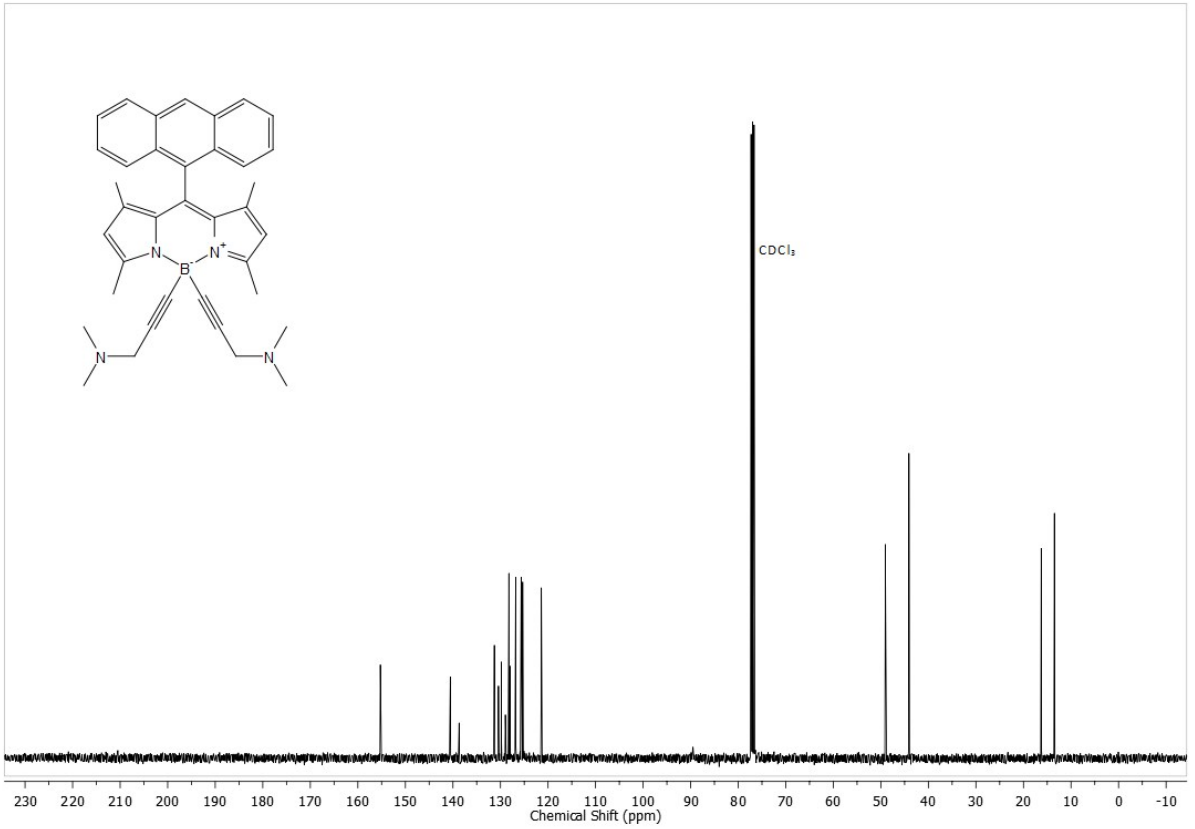
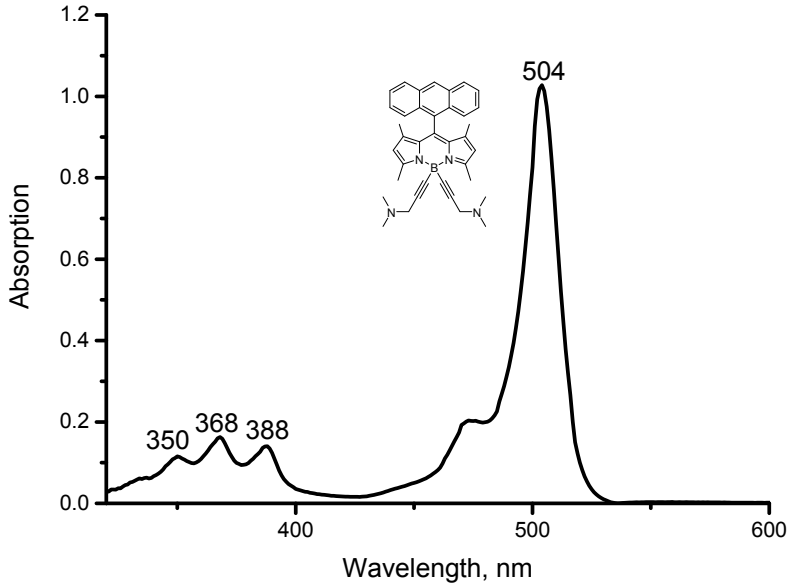
Chemical Shift (ppm)


Absorption
Wavelength, nm
511
365
384
347
1.2
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600

4.3 Characterization Spectra for **BAD-24**







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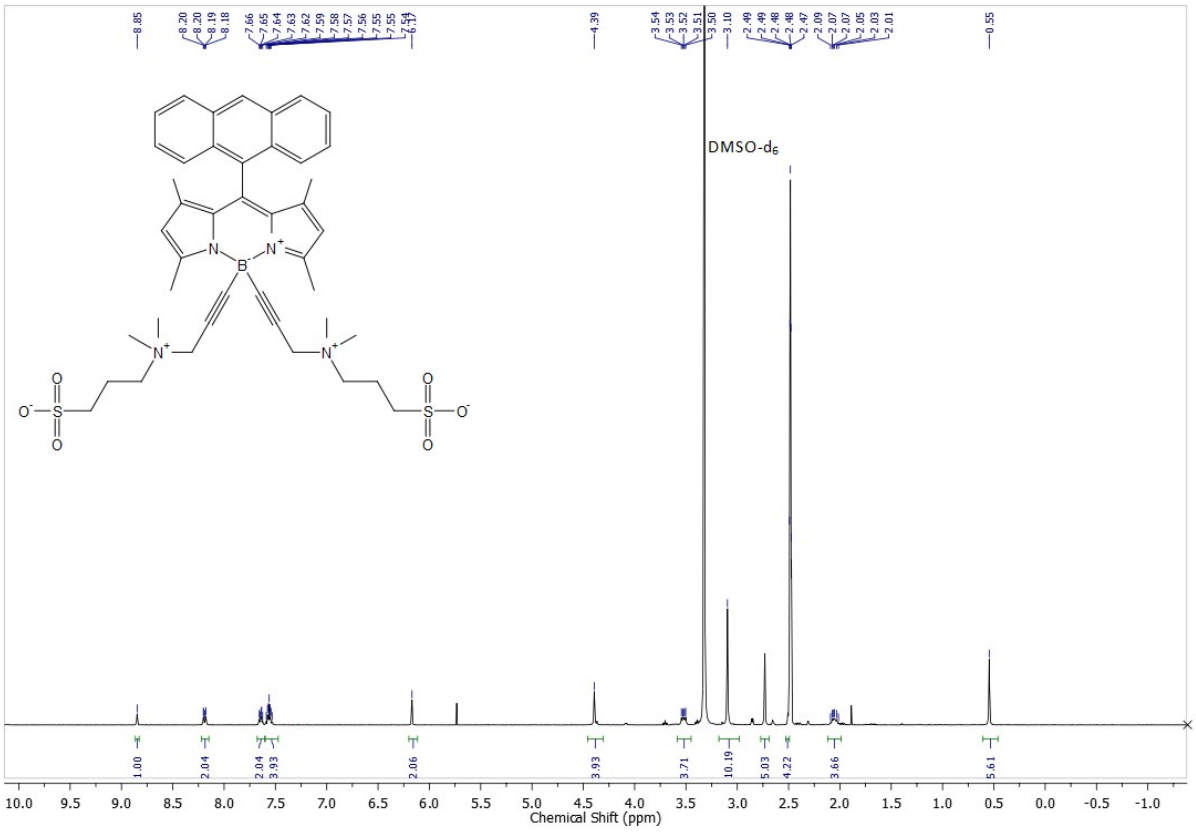
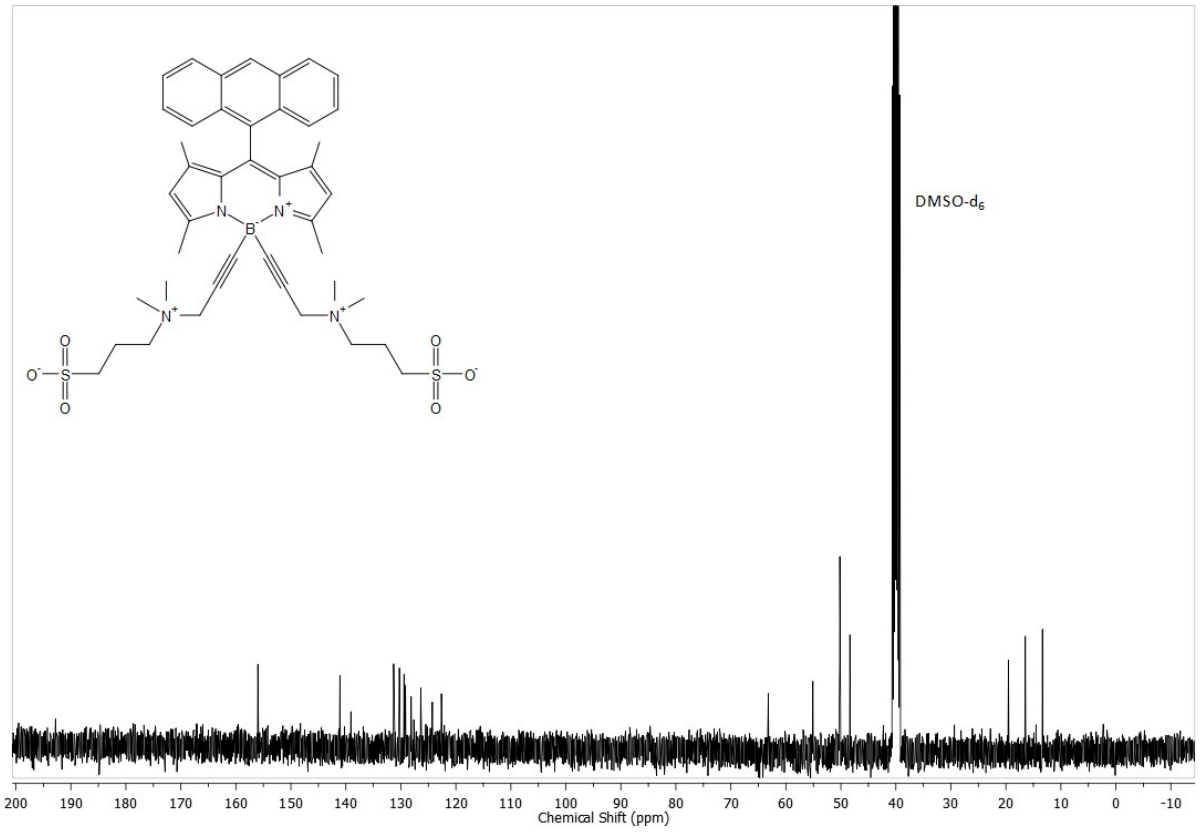
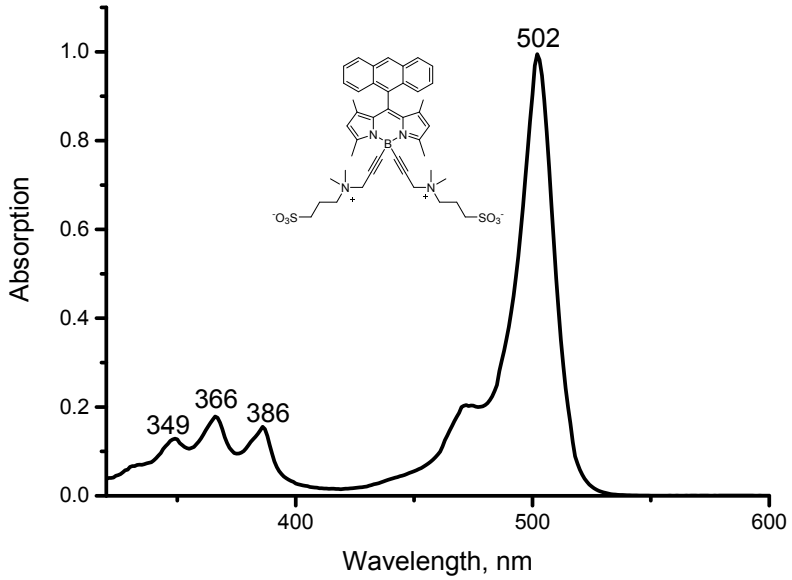
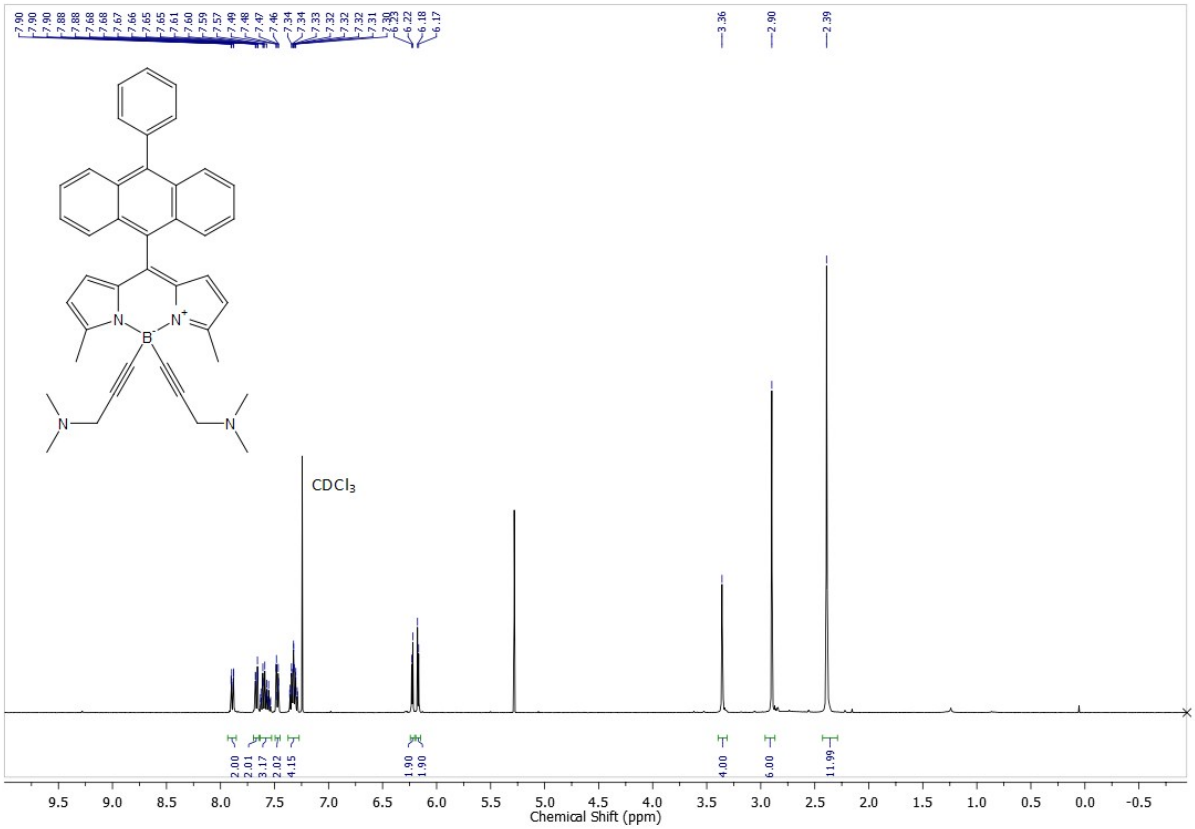
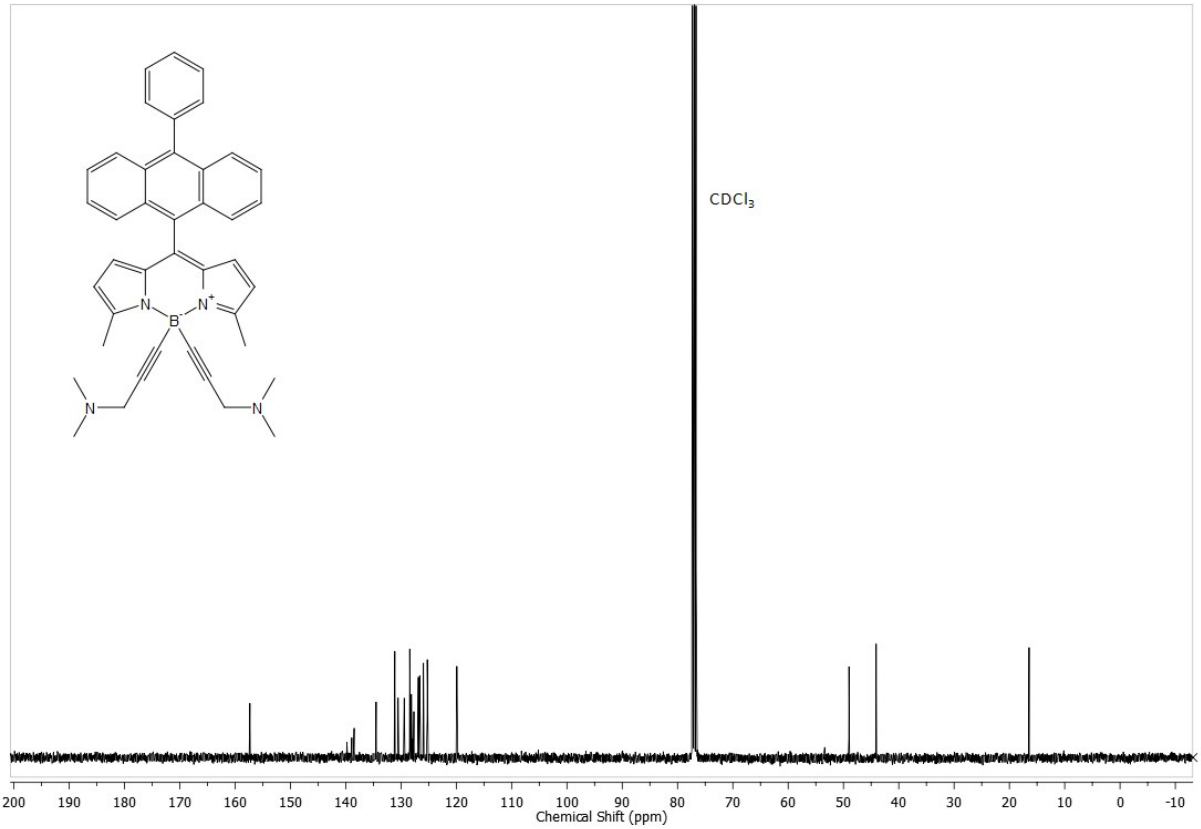


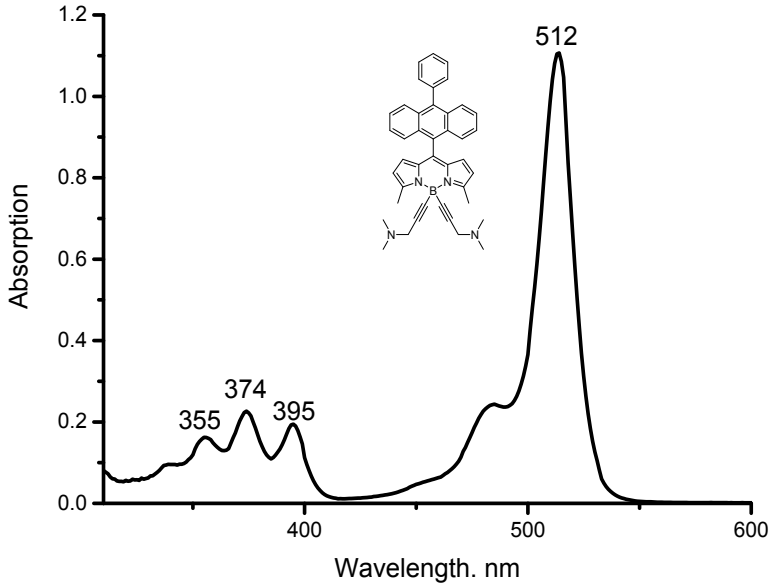

DMSO-d6
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
Chemical Shift (ppm)


502
366
386
349
Absorption
Wavelength, nm
0.0 0.2 0.4 0.6 0.8 1.0
400 500 600

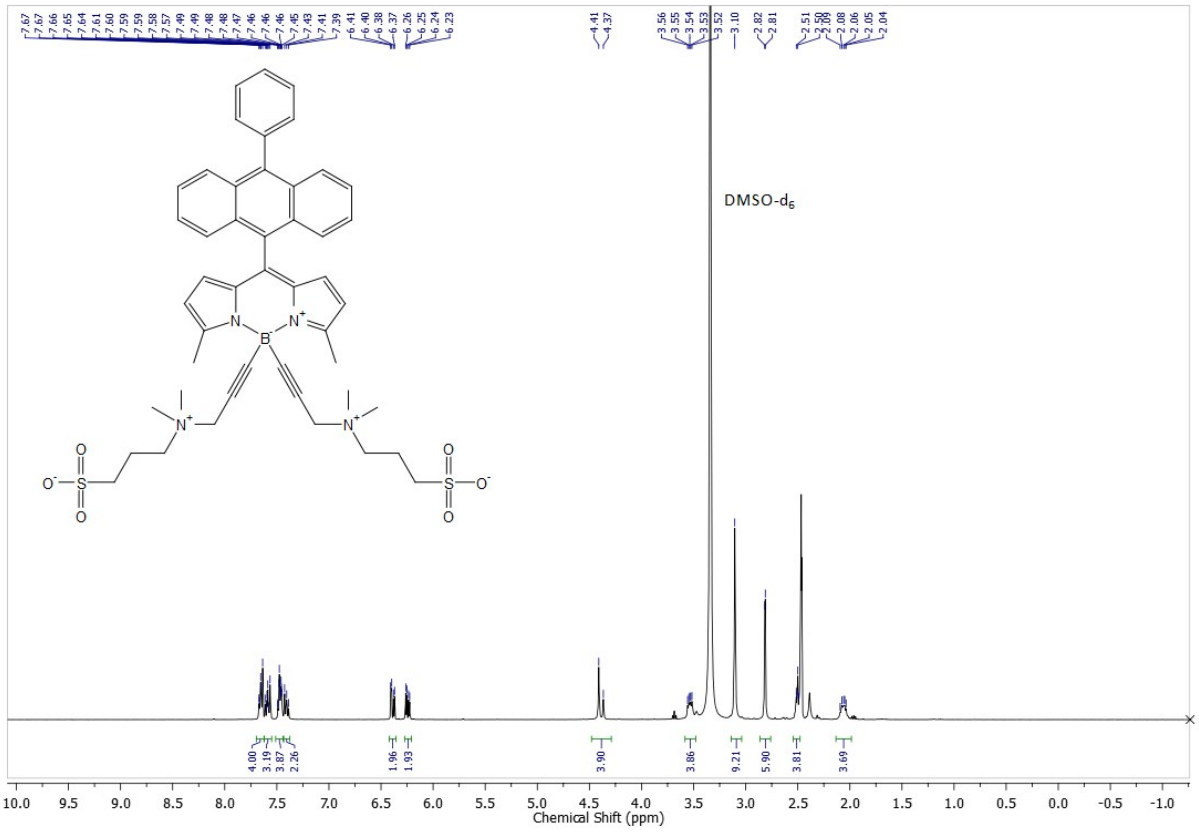
4.5 Characterization Spectra for **BAD-25**

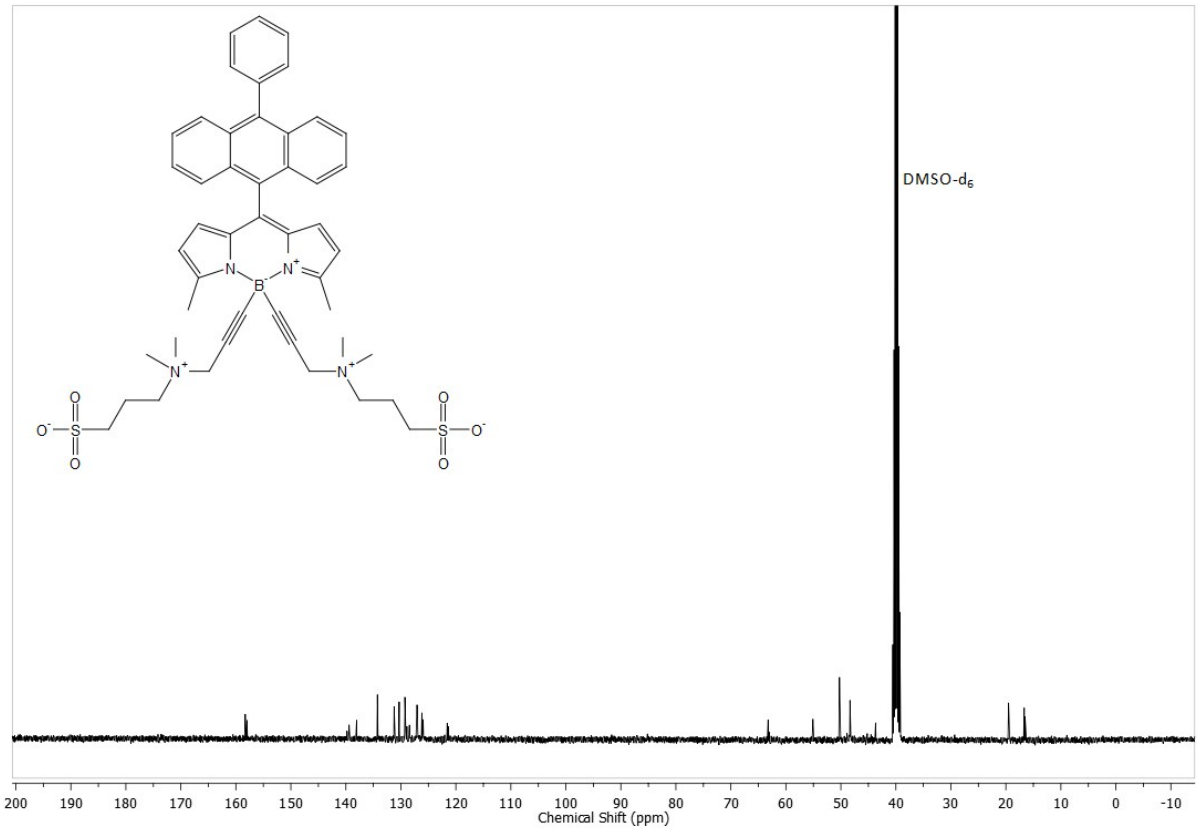






4.6 Characterization Spectra for **BAD-17**

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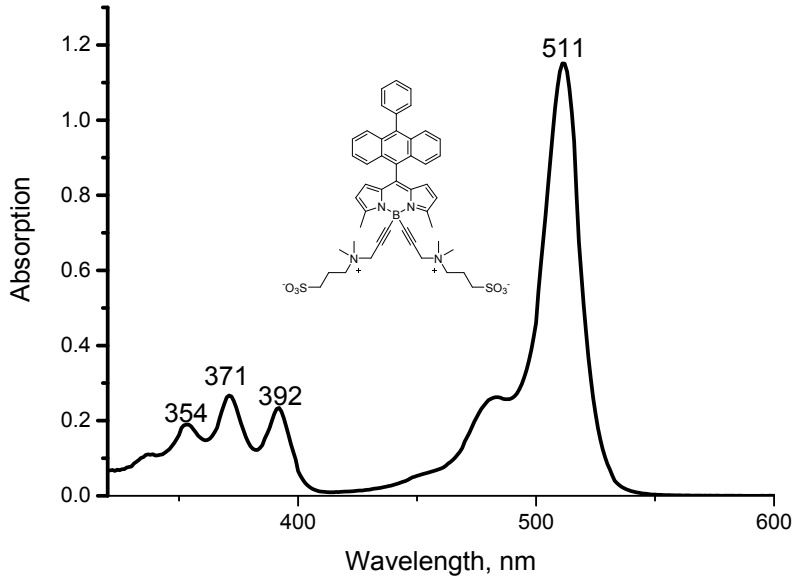
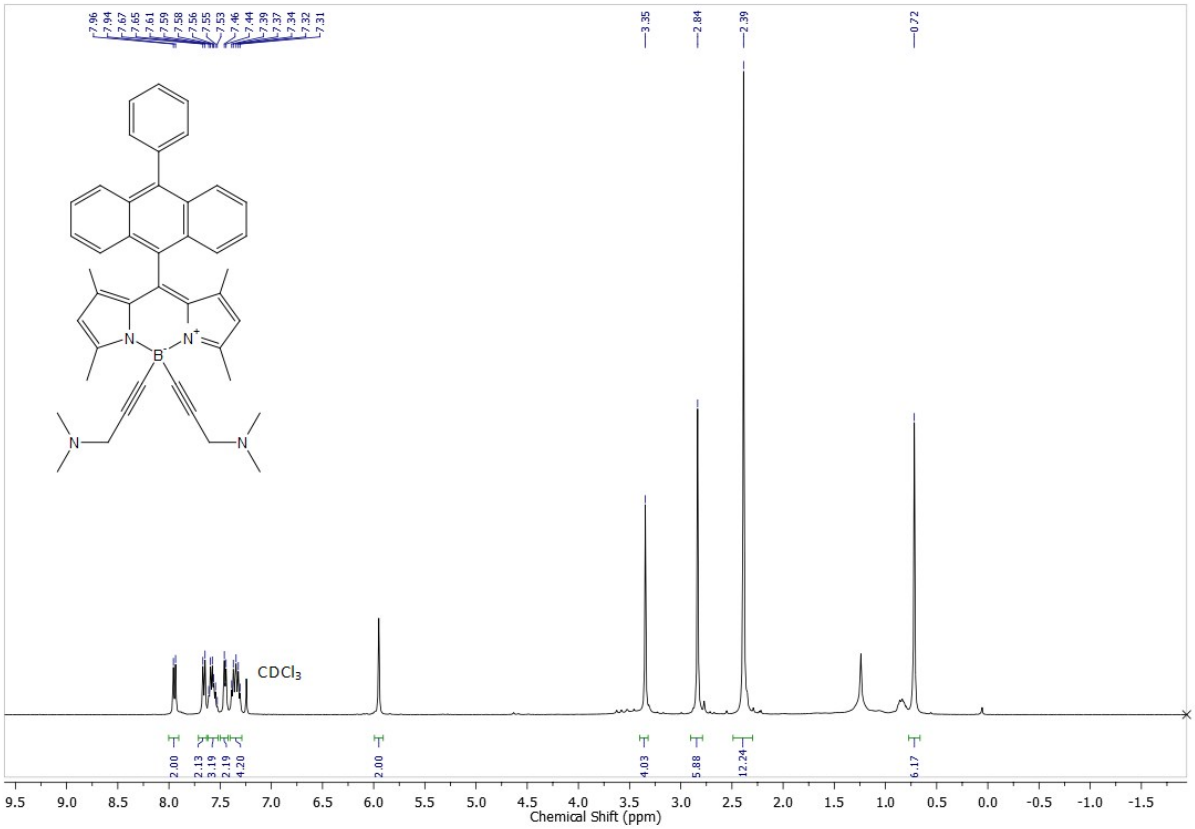
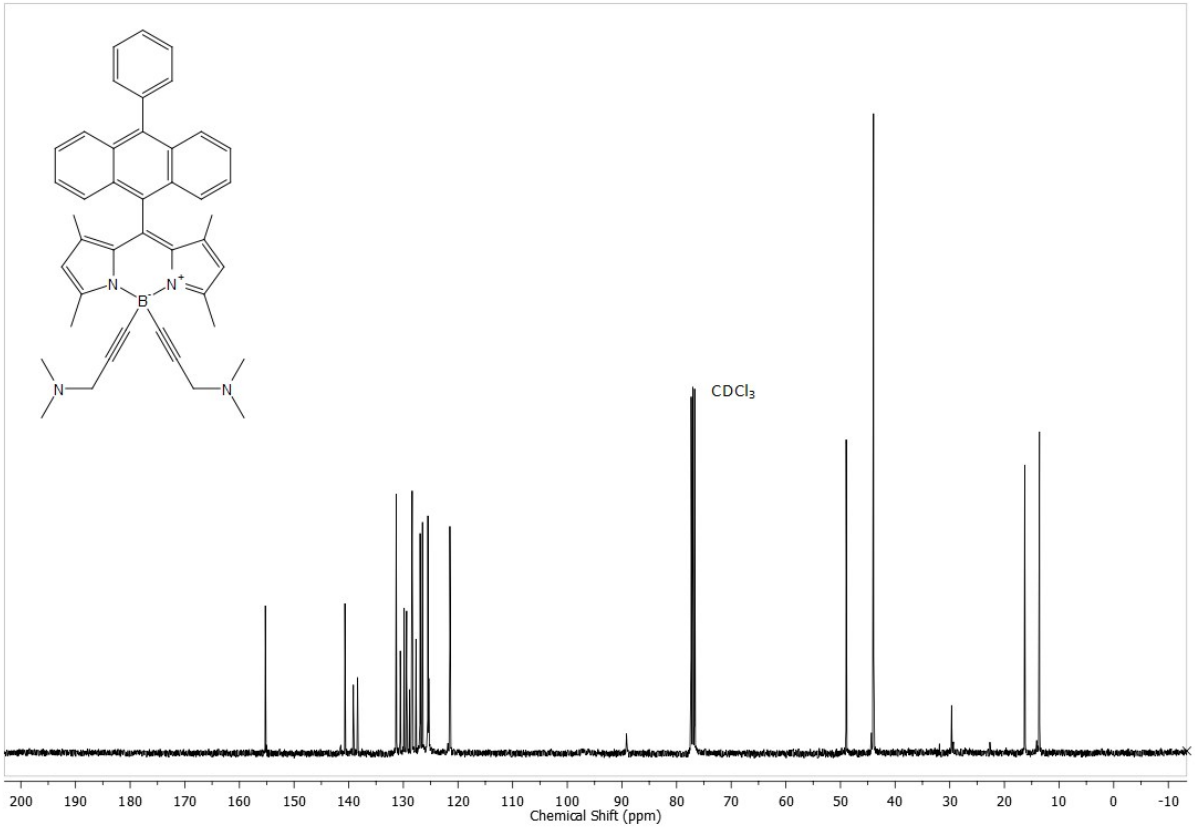
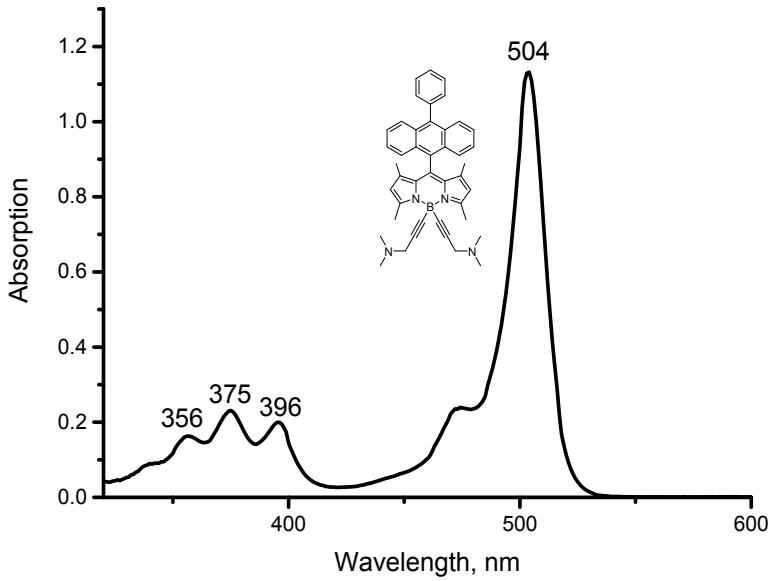
Chemical Shift (ppm)


Absorption
1.2
1.0
0.8
0.6
0.4
0.2
0.0
511
371
392
354
400
500
600
Wavelength, nm

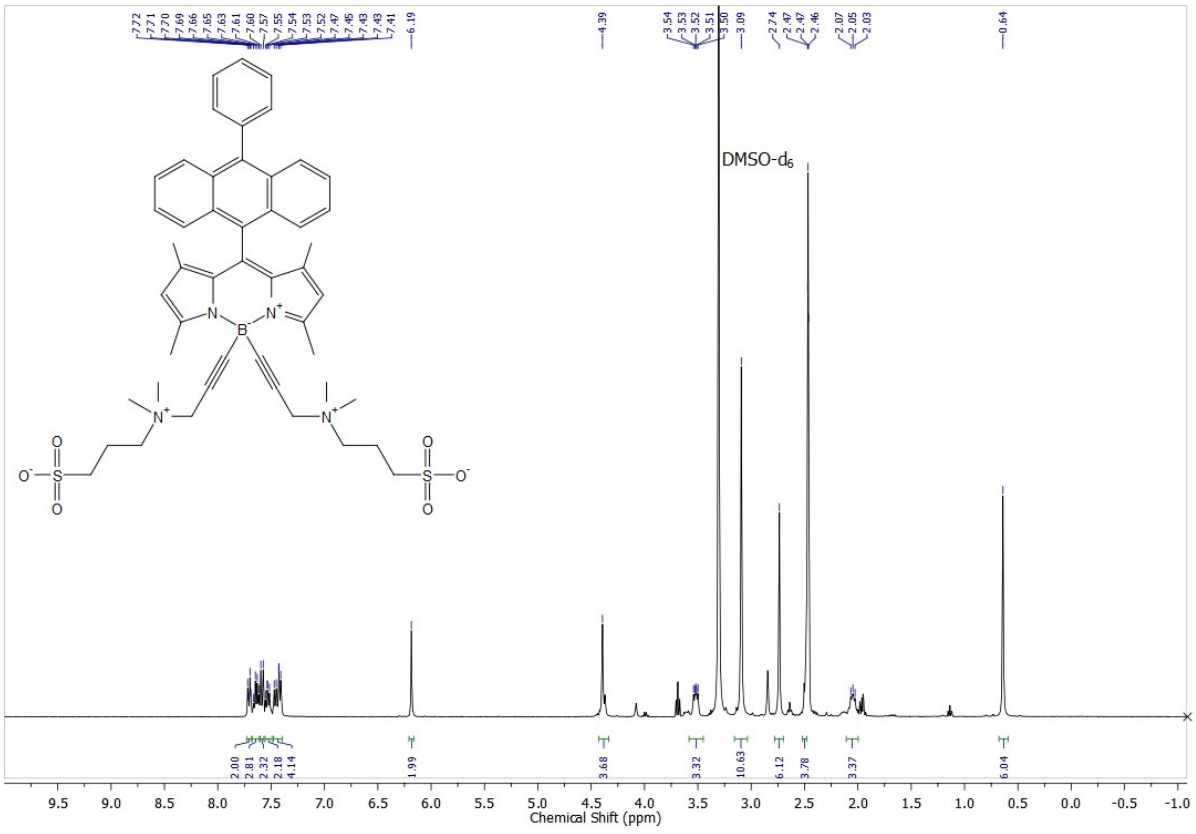
4.7 Characterization Spectra for **BAD-26**

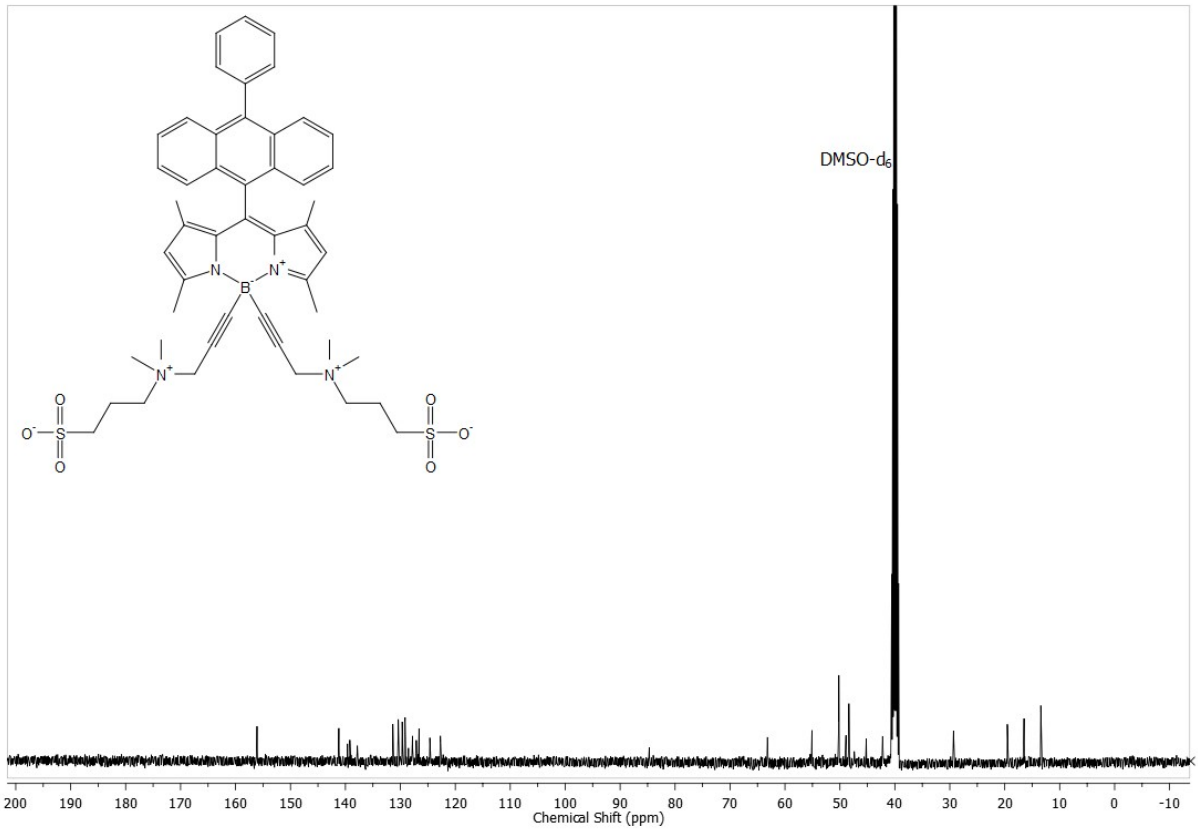






4.8 Characterization Spectra for **BAD-18**




DMSO-d6
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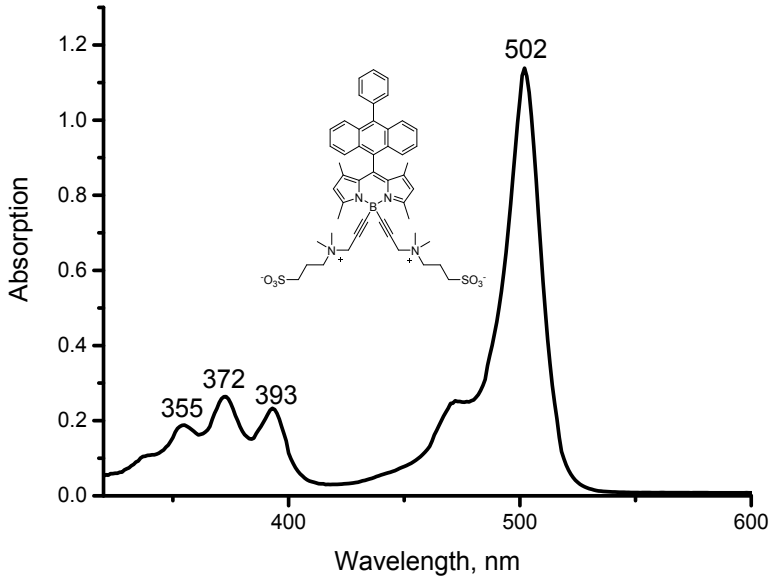
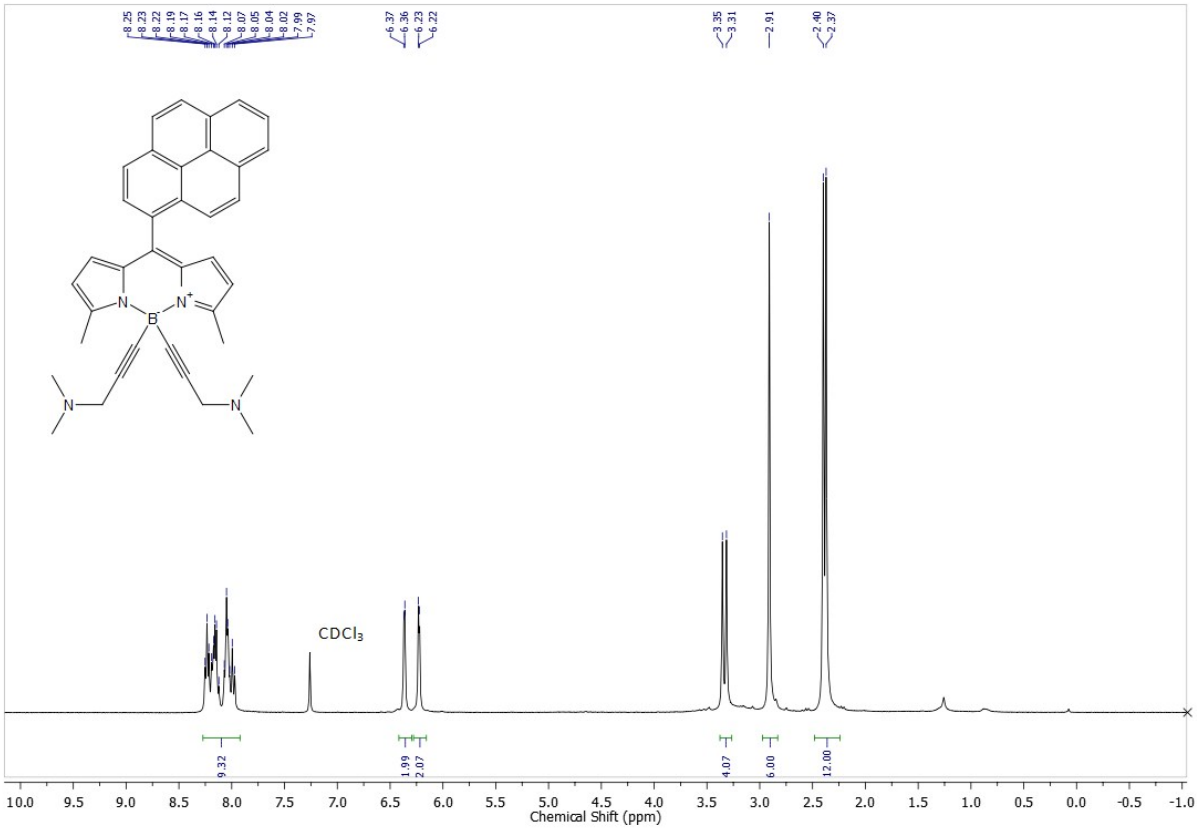
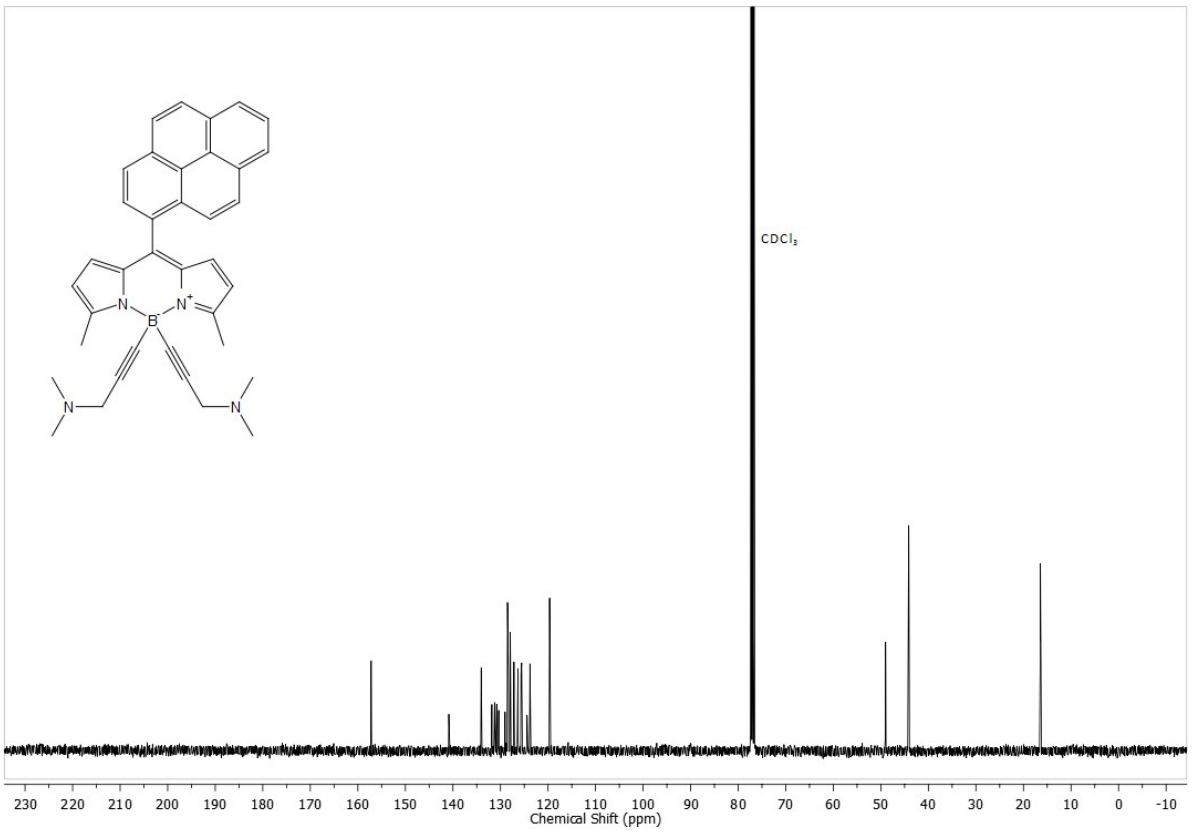
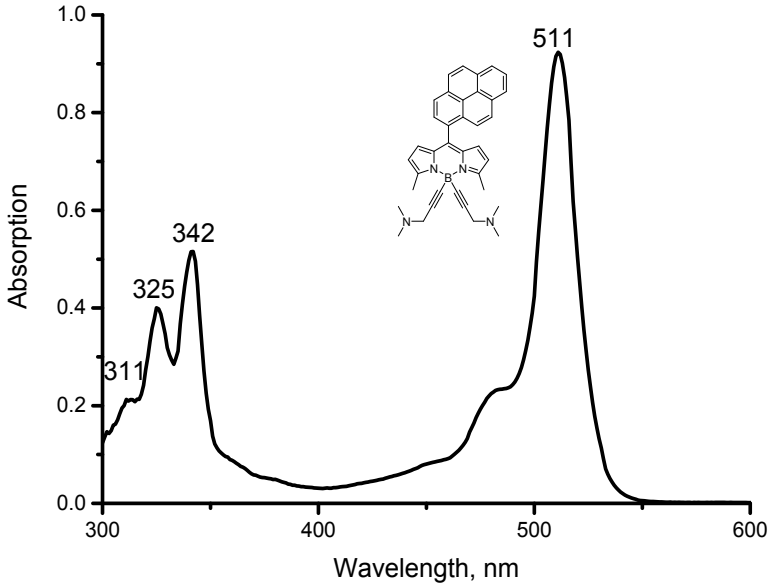
Chemical Shift (ppm)


Absorption
1.2
1.0
0.8
0.6
0.4
0.2
0.0
502
372
393
355
400
500
600
Wavelength, nm

4.9 Characterization Spectra for **BPyrD-27**







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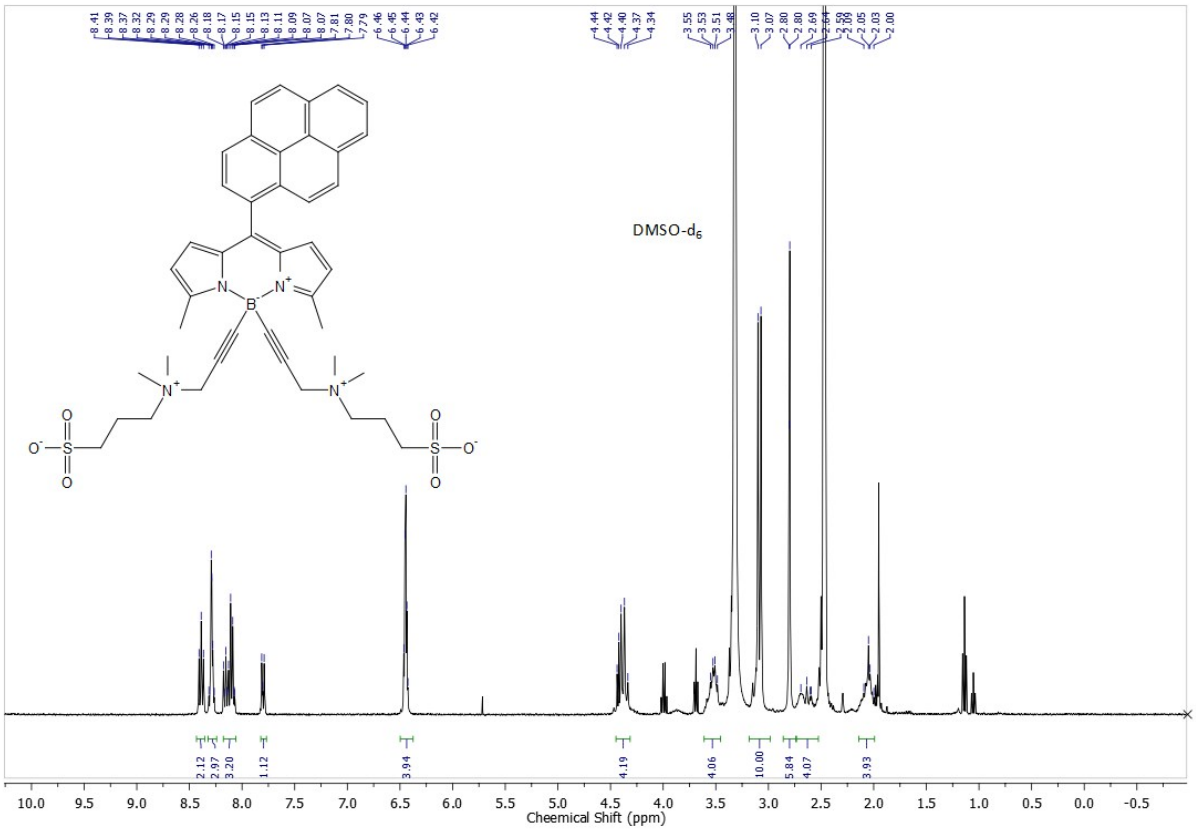
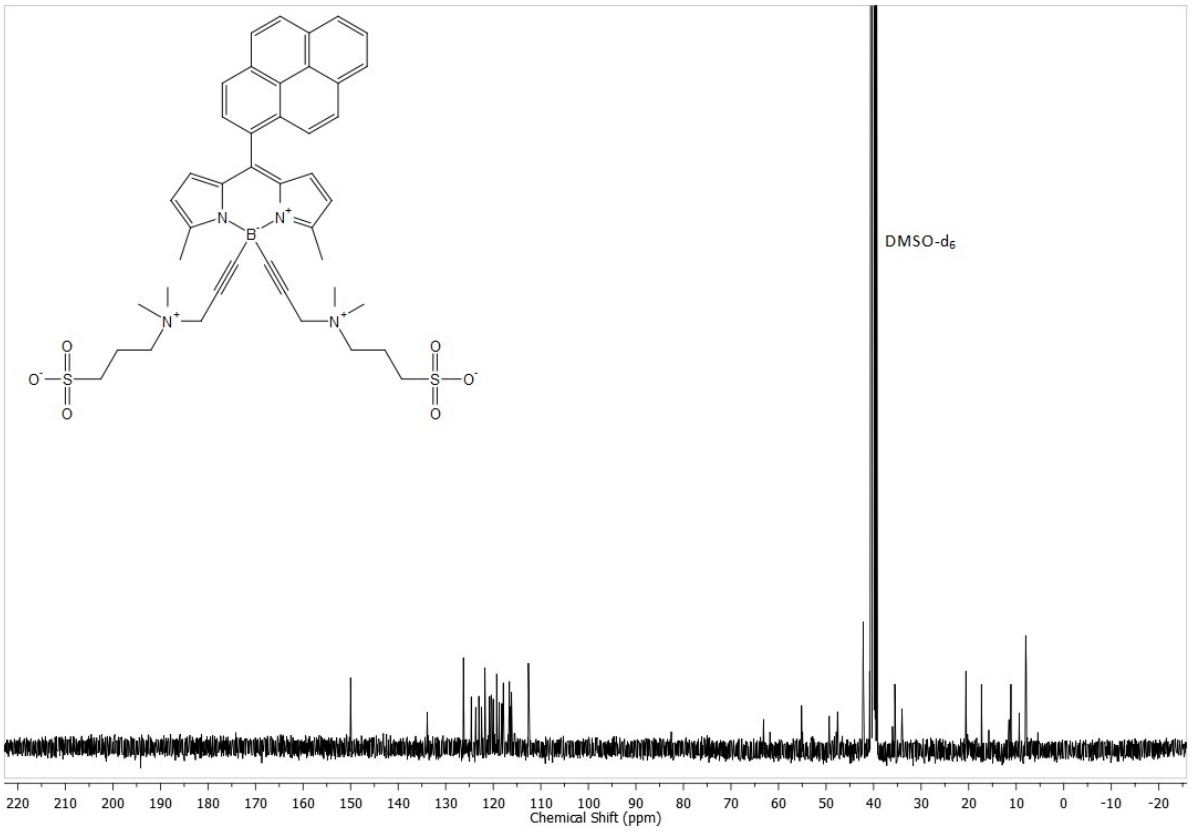
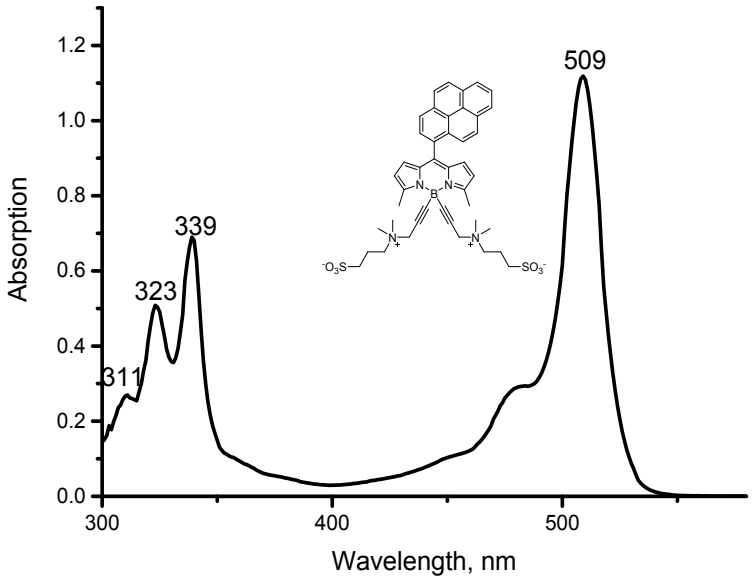
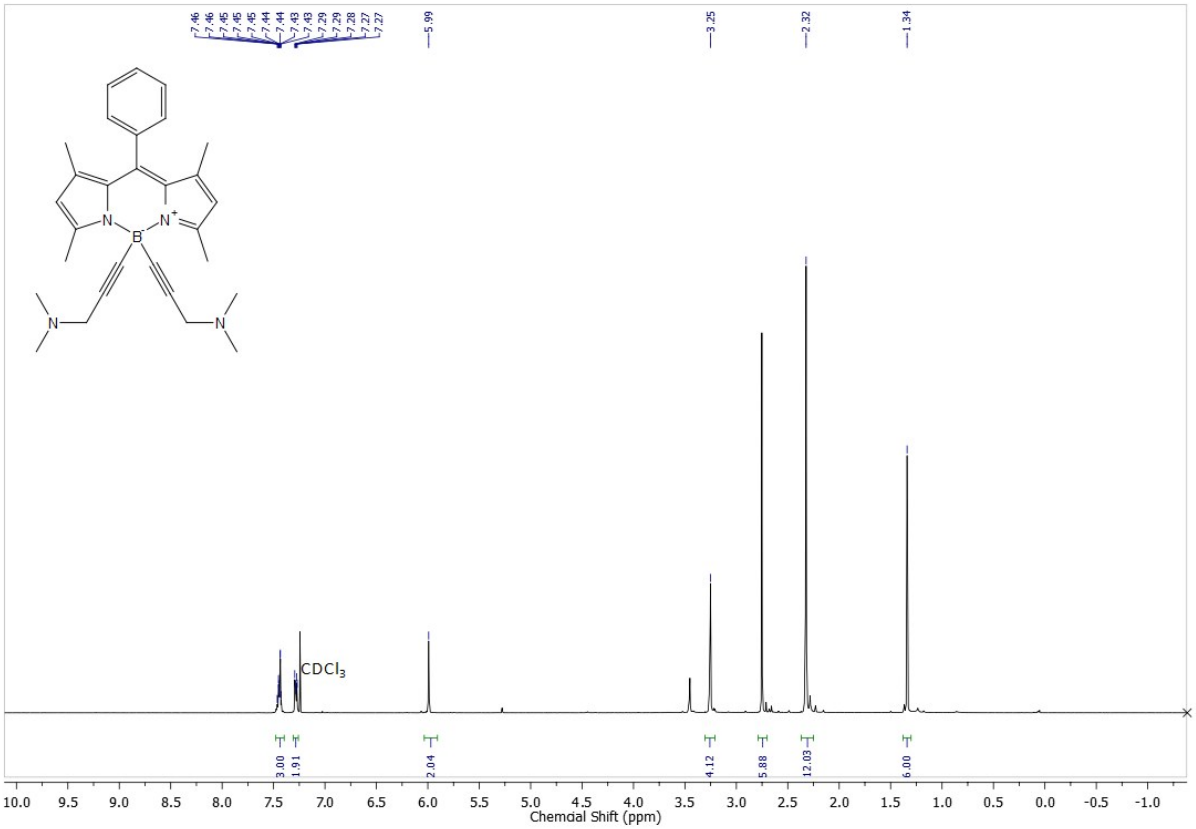
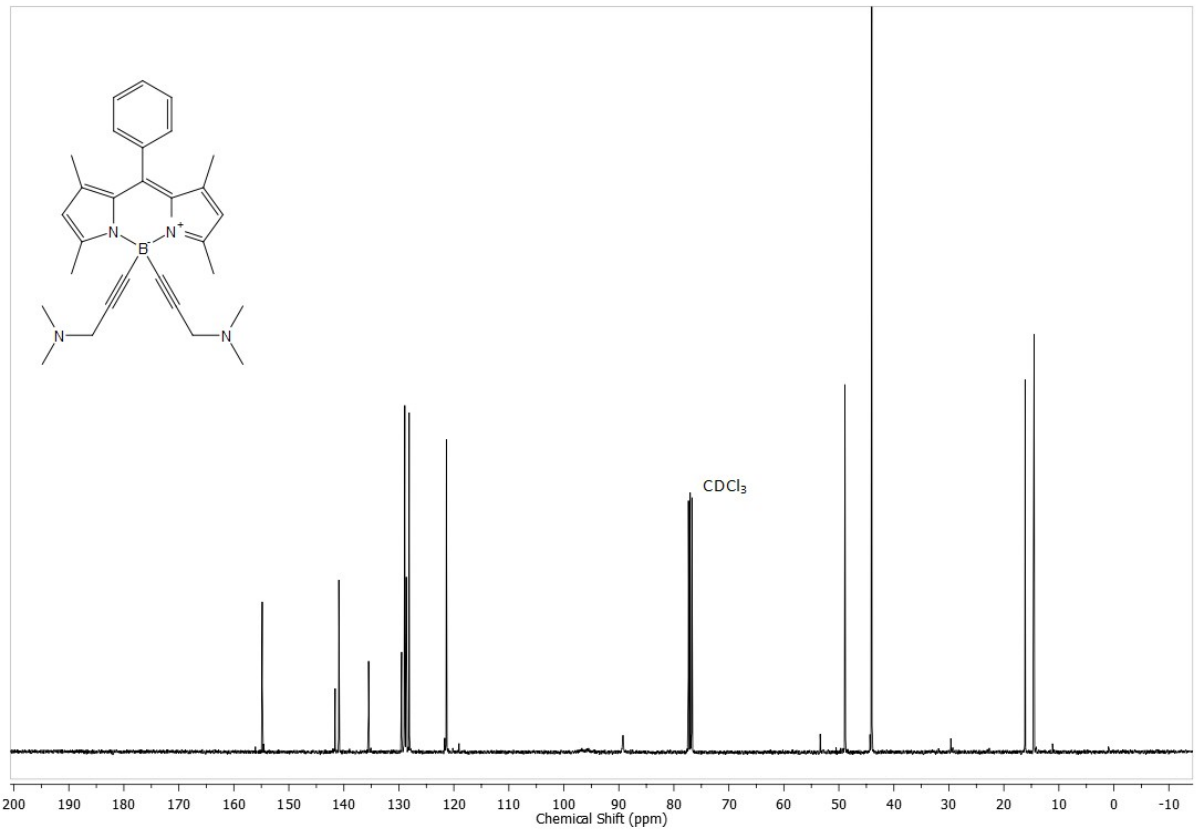


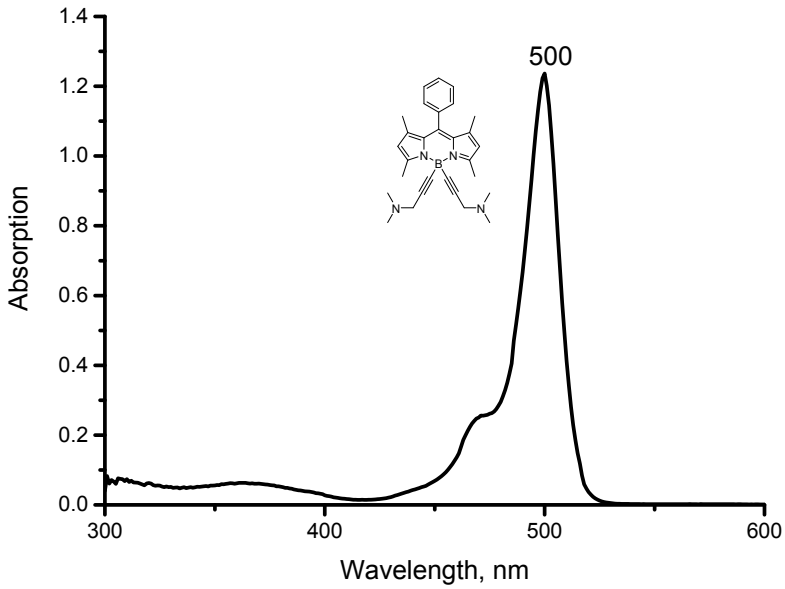

DMSO-d6
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)


Absorption
1.2
1.0
0.8
0.6
0.4
0.2
0.0
311
323
339
509
300
400
500
Wavelength, nm

4.11 Characterization Spectra for **28**







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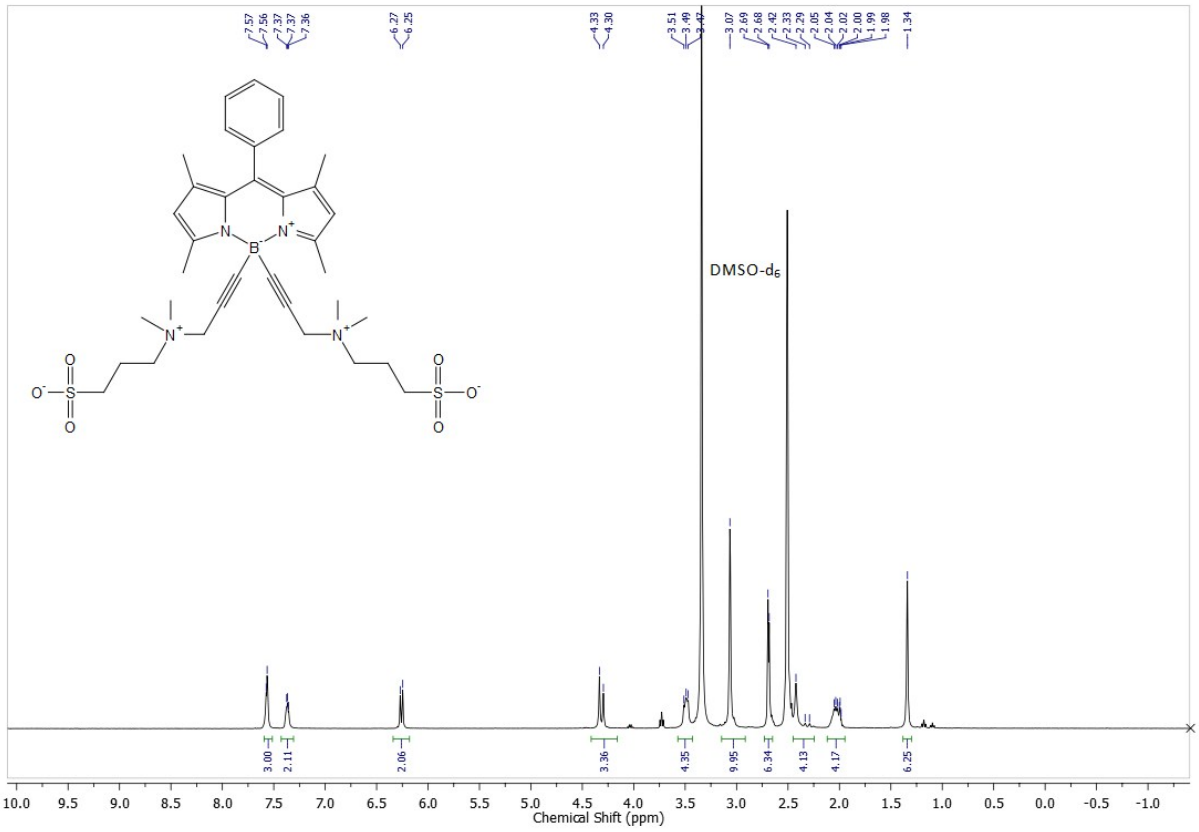

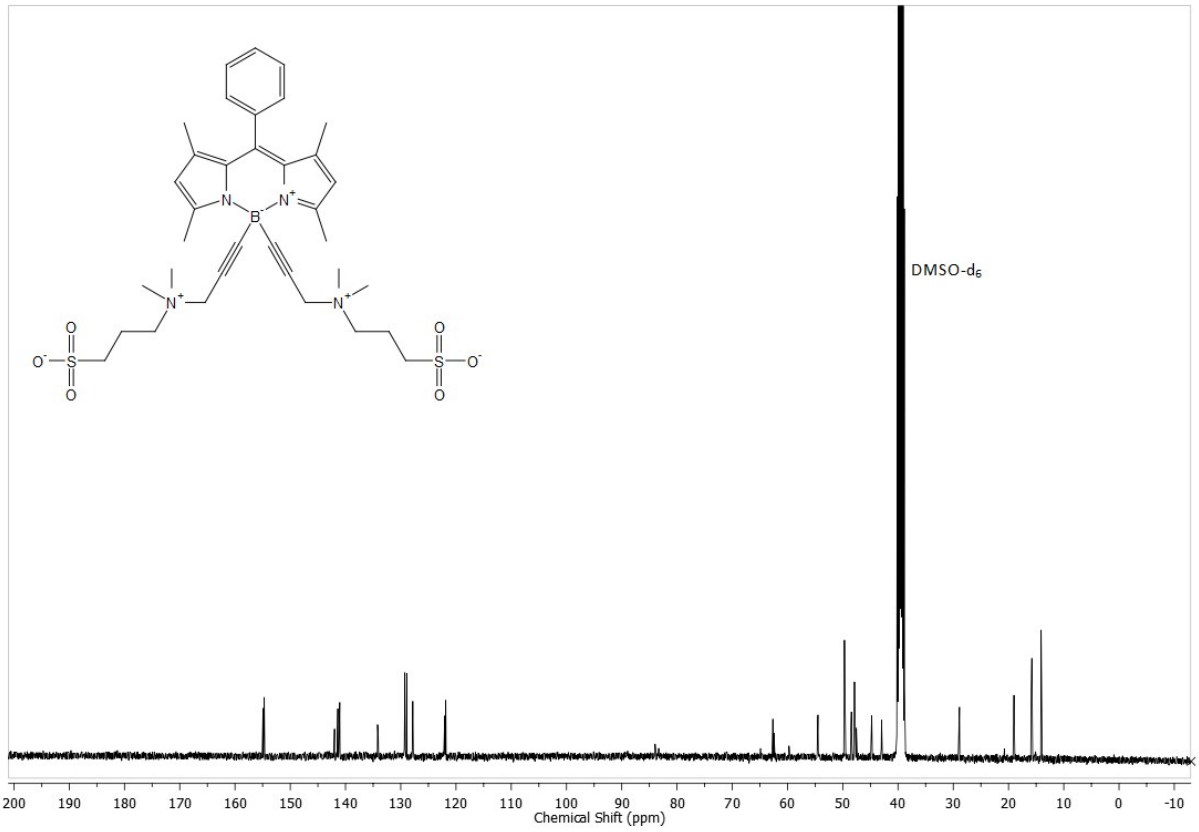
DMSO-d6
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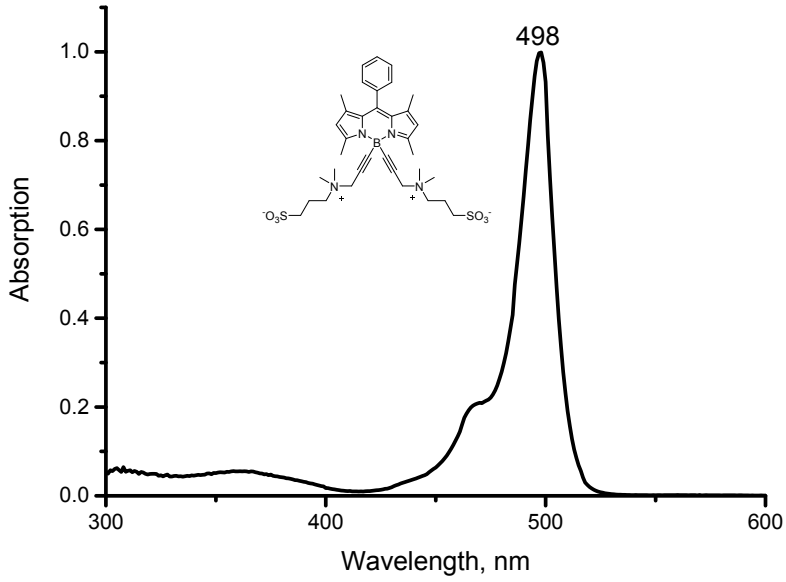
Chemical Shift (ppm)


498
Absorption
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
Wavelength, nm

5. References

[1] Y. Cakmak, S. Kolemen, S. Duman, Y. Dede, Y. Dolen, B. Kilic, Z. Kostereli, L. T. Yildirim, A. L. Dogan, D. Guc, E. U. Akkaya, *Angew. Chem. Int. Ed.* 2011, **50**, 11937–11941.

[2] M. A. Filatov, S. Karuthedath, P. M. Polestshuk, H. Savoie, K. J. Flanagan, C. Sy, E. Sitte, M. Telitchko, F. Laquai, R. W. Boyle, M. O. Senge, *J. Am. Chem. Soc*., 2017, **139**, 6282–6285.

[3] T. Mossman, J. Immunol. Meth., 1983, **65**, 55–63.

[4] J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Ed., Kluwer Academic/Plenum Publishers, New York, London, Moscow, Dordrecht, 1999.