

Electronic Supplementary Information

A biotin-conjugated glutathione-responsive FRET-based fluorescent probe with a ferrocenyl BODIPY as the dark quencher

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Fig. S1 (a) UV-Vis and (b) fluorescence ($\lambda_{\text{ex}} = 610 \text{ nm}$) spectra of **7**, **10** and **13a** (all at $1 \text{ }\mu\text{M}$) in DMF. The inset of figure (b) shows the fluorescence observed for these compounds upon excitation at 365 nm .

Fig. S2 Change in (a) UV-Vis and (b) fluorescence ($\lambda_{\text{ex}} = 610 \text{ nm}$) spectra of **13a** ($2 \text{ }\mu\text{M}$) in the presence of GSH (2 mM) in PBS with 0.5% Tween 80 over 10 h at $37 \text{ }^{\circ}\text{C}$. The inset of figure (b) shows the time-dependent fluorescence intensity at 650 nm .

Fig. S3 Comparison of the relative intracellular fluorescence intensity of **15** in CHO-K1 and A549 cells. Data are expressed as the mean $\pm$ standard deviation of three independent experiments.

Fig. S4 (a) Bright field and confocal fluorescence images of A549 cells after incubation with GSH-OEt (10 mM) for 30 min , followed with **13a** or **13b** ($2 \text{ }\mu\text{M}$) for 4 h . (b) Comparison of the relative intracellular fluorescence intensity of **13a** and **13b** with the pre-treatment with GSH-OEt. Data are expressed as the mean $\pm$ standard deviation (number of cells = 50).

^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of all the new compounds

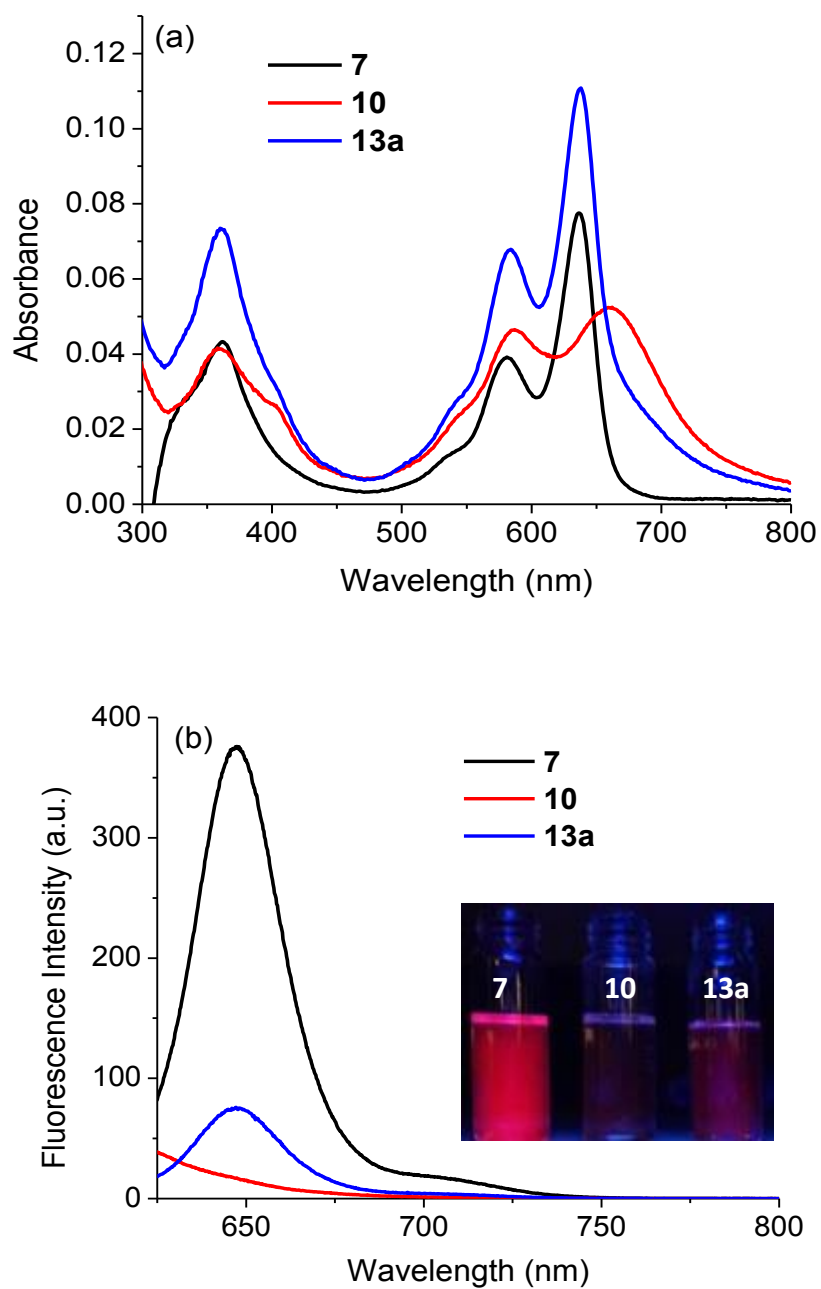


Fig. S1 (a) UV-Vis and (b) fluorescence ($\lambda_{\text{ex}} = 610$ nm) spectra of **7**, **10** and **13a** (all at 1 μM) in DMF. The inset of figure (b) shows the fluorescence observed for these compounds upon excitation at 365 nm.

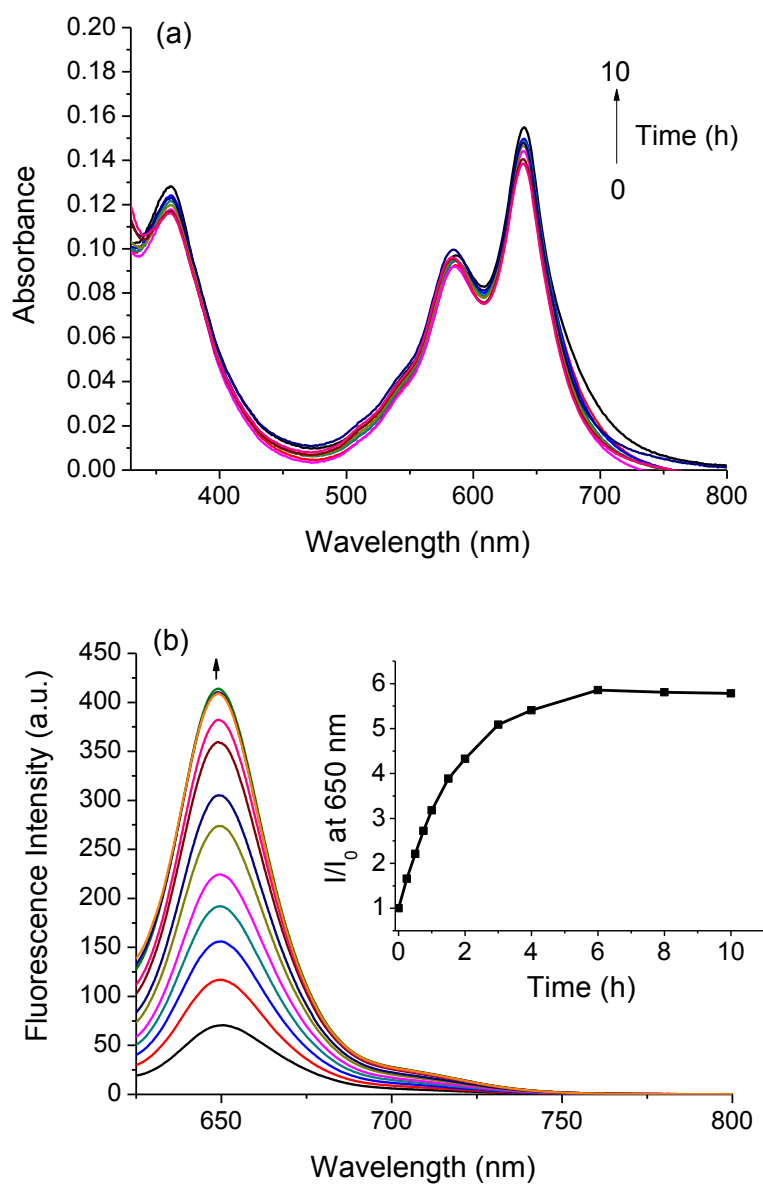


Fig. S2 Change in (a) UV-Vis and (b) fluorescence ($\lambda_{\text{ex}} = 610$ nm) spectra of **13a** (2 μ M) in the presence of GSH (2 mM) in PBS with 0.5% Tween 80 over 10 h at 37 $^{\circ}$ C. The inset of figure (b) shows the time-dependent fluorescence intensity at 650 nm.

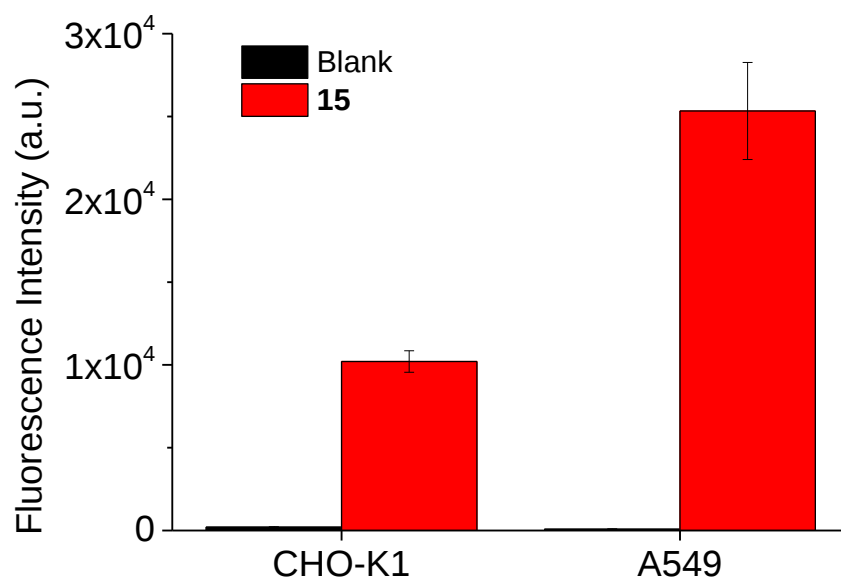
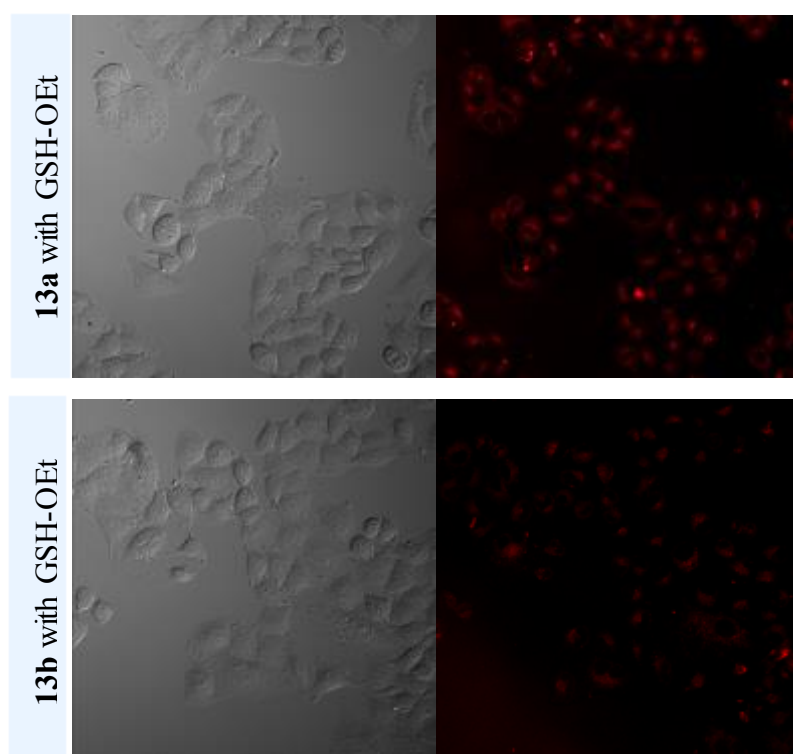


Fig. S3 Comparison of the relative intracellular fluorescence intensity of **15** in CHO-K1 and A549 cells. Data are expressed as the mean $\pm$ standard deviation of three independent experiments

(a)



(b)

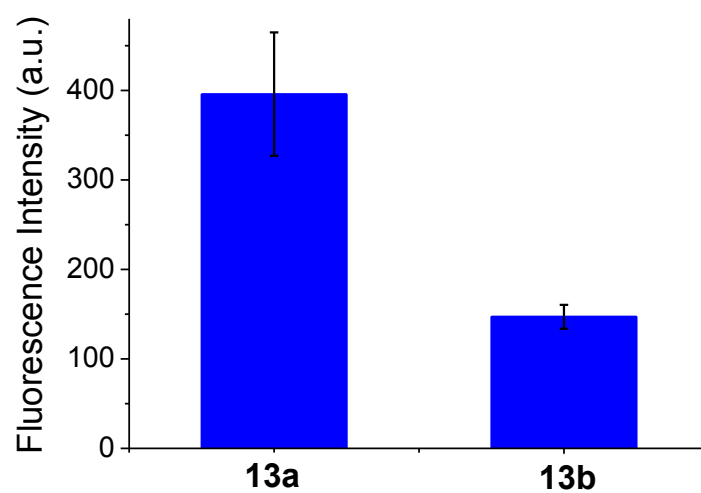
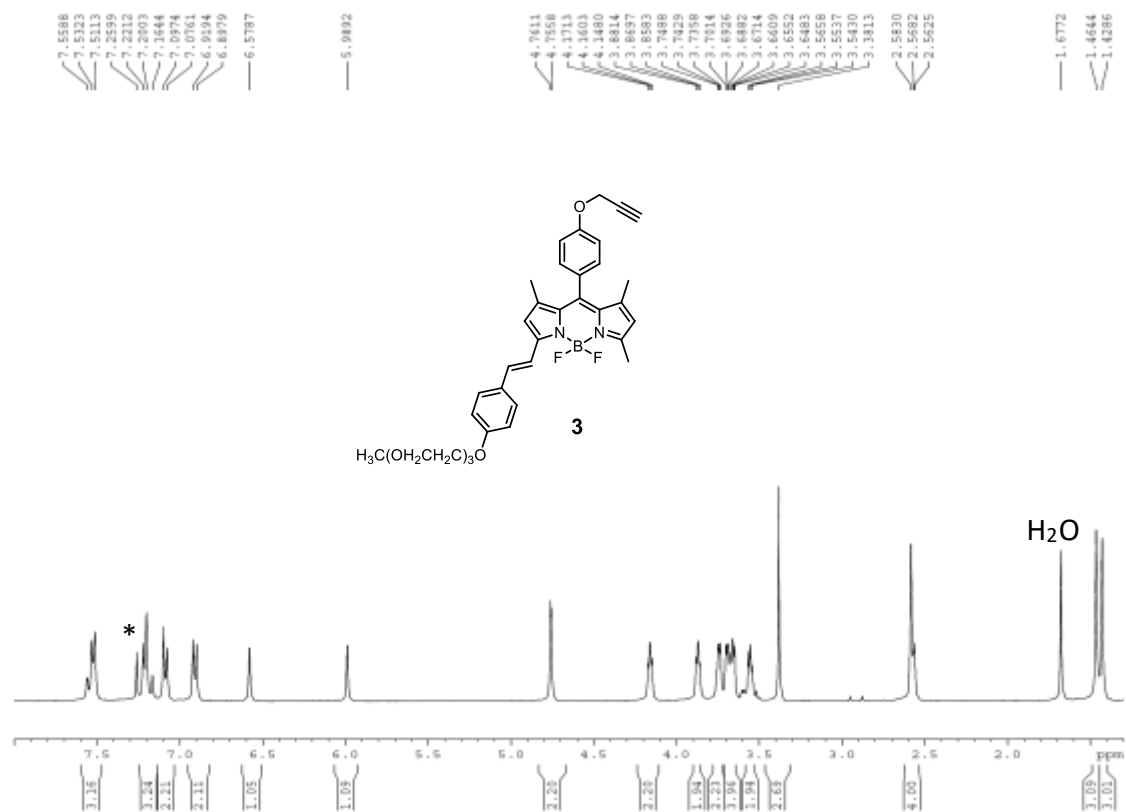
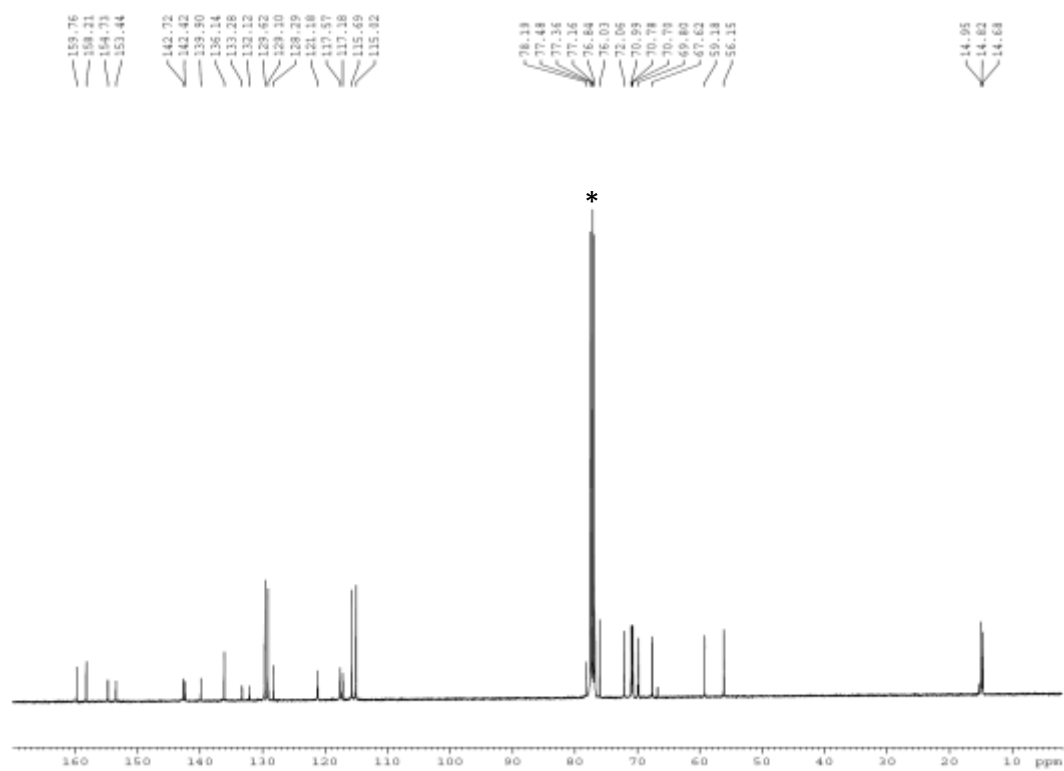


Fig. S4 (a) Bright field and confocal fluorescence images of A549 cells after incubation with GSH-OEt (10 mM) for 30 min, followed with **13a** or **13b** (2 μ M) for 4 h. (b) Comparison of the relative intracellular fluorescence intensity of **13a** and **13b** with the pre-treatment with GSH-OEt. Data are expressed as the mean $\pm$ standard deviation (number of cells = 50).

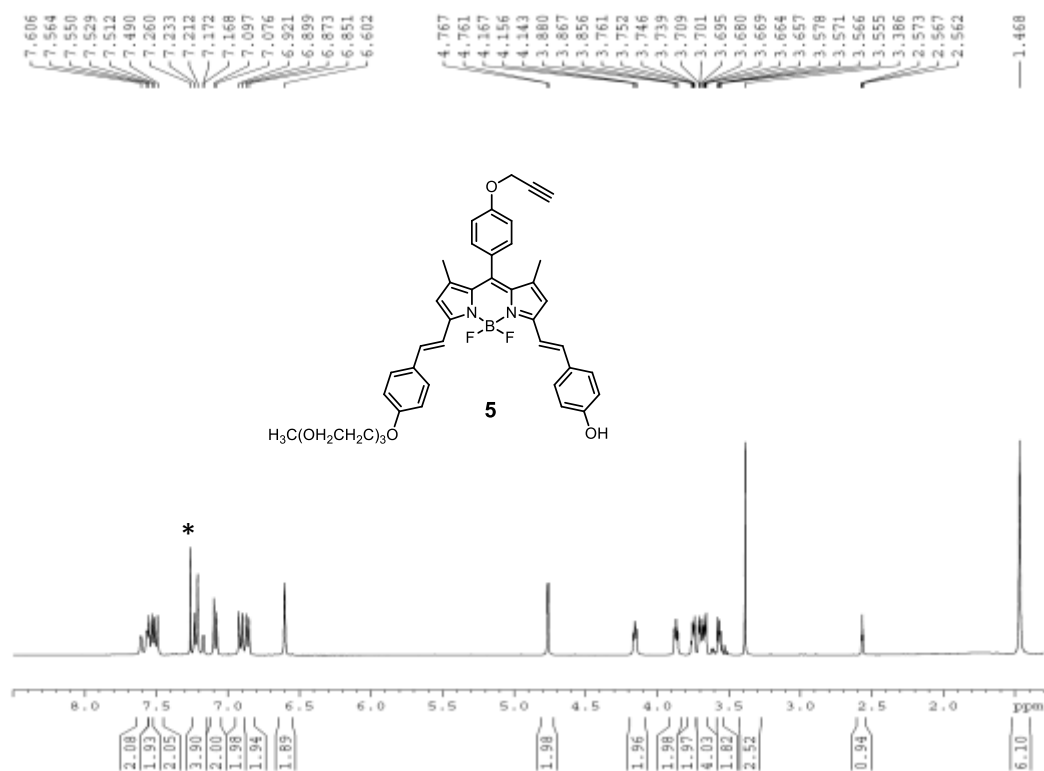
^1H NMR spectrum of **3** in CDCl_3



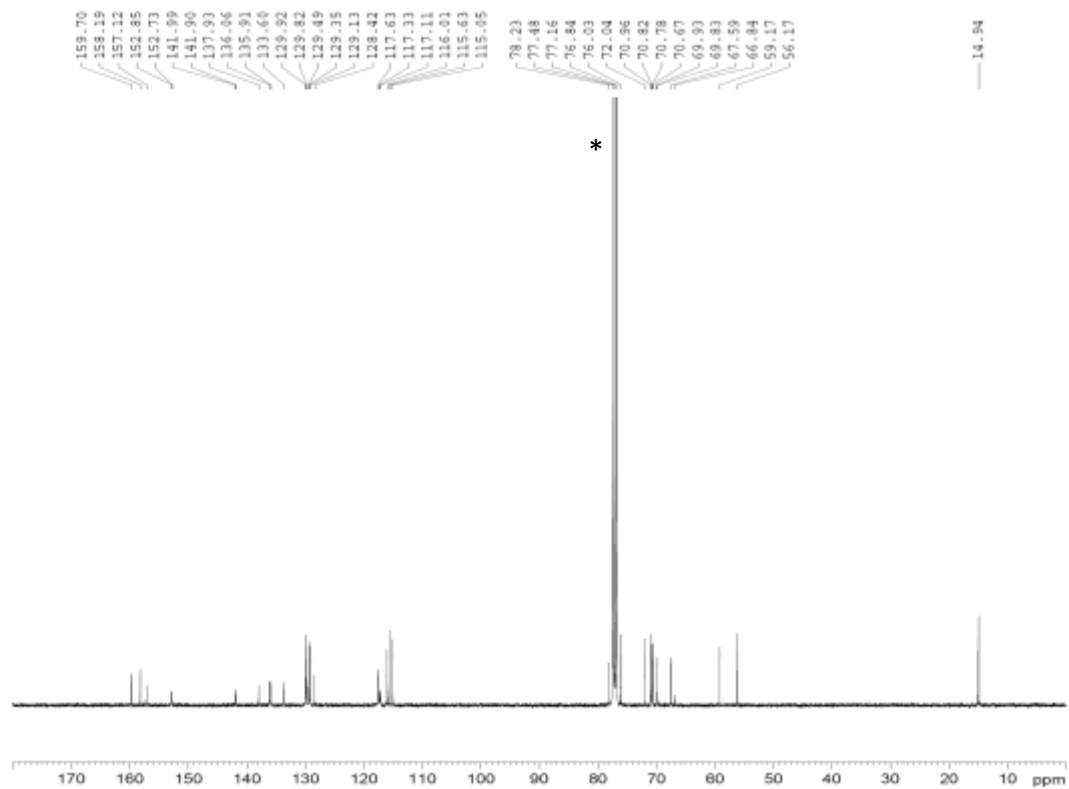
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **3** in CDCl_3



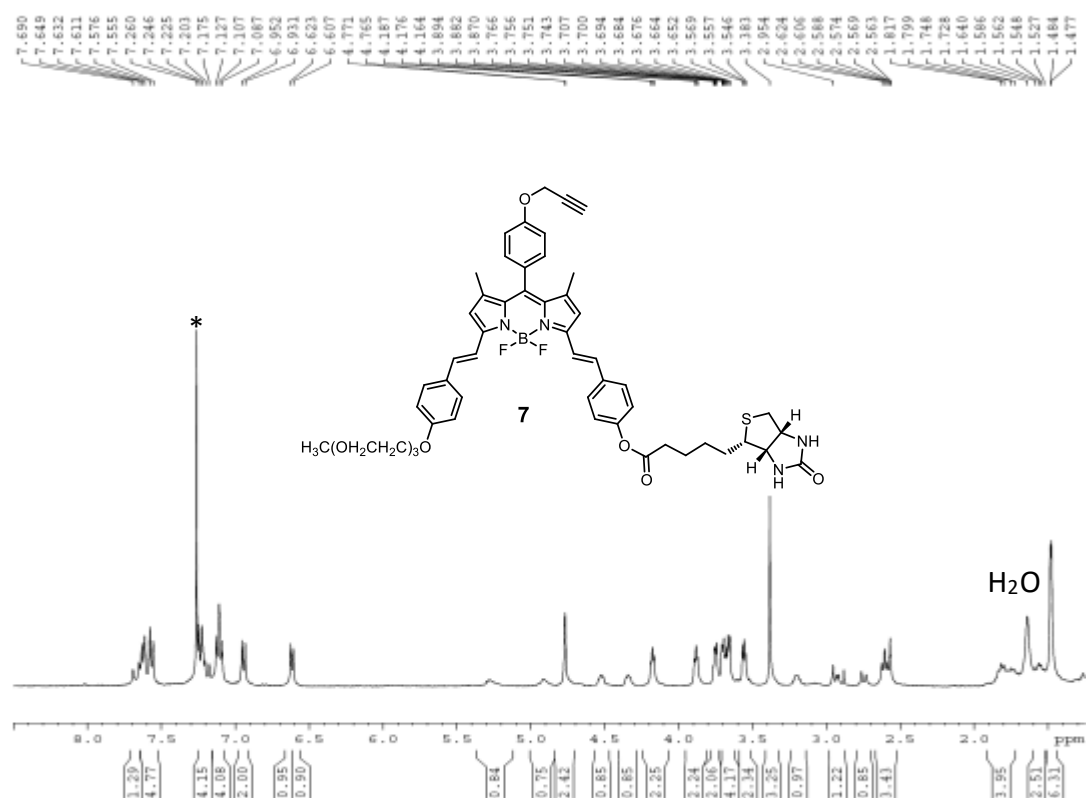
^1H NMR spectrum of **5** in CDCl_3



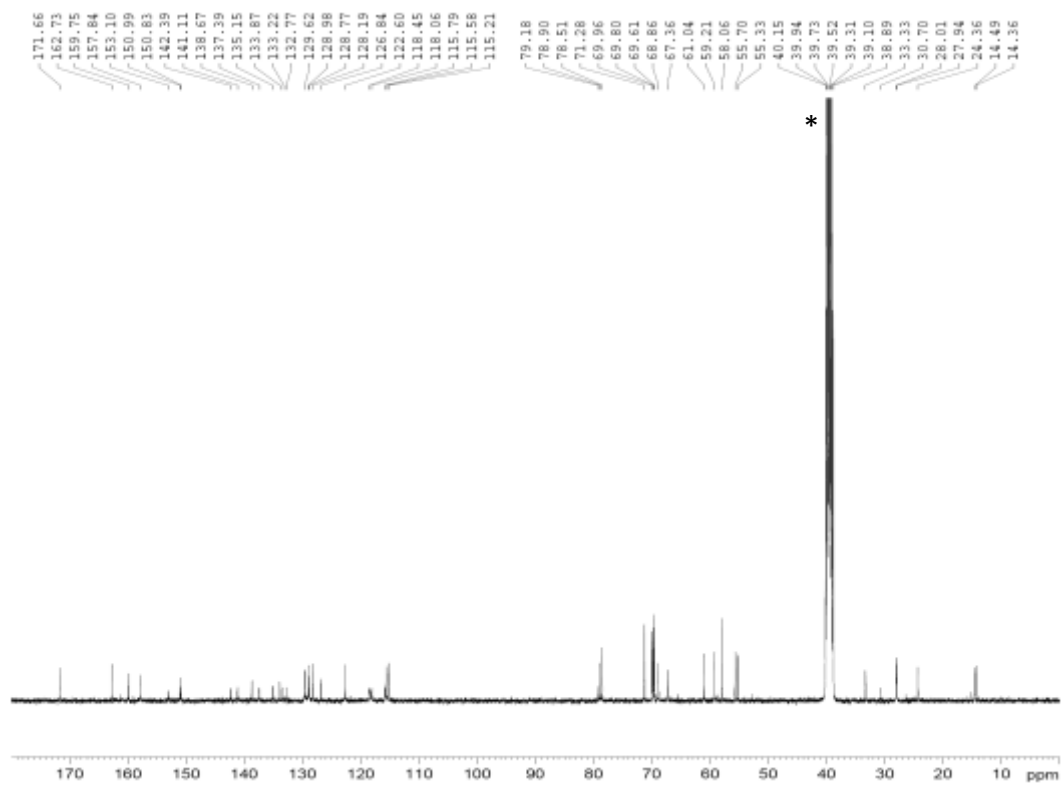
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **5** in CDCl_3



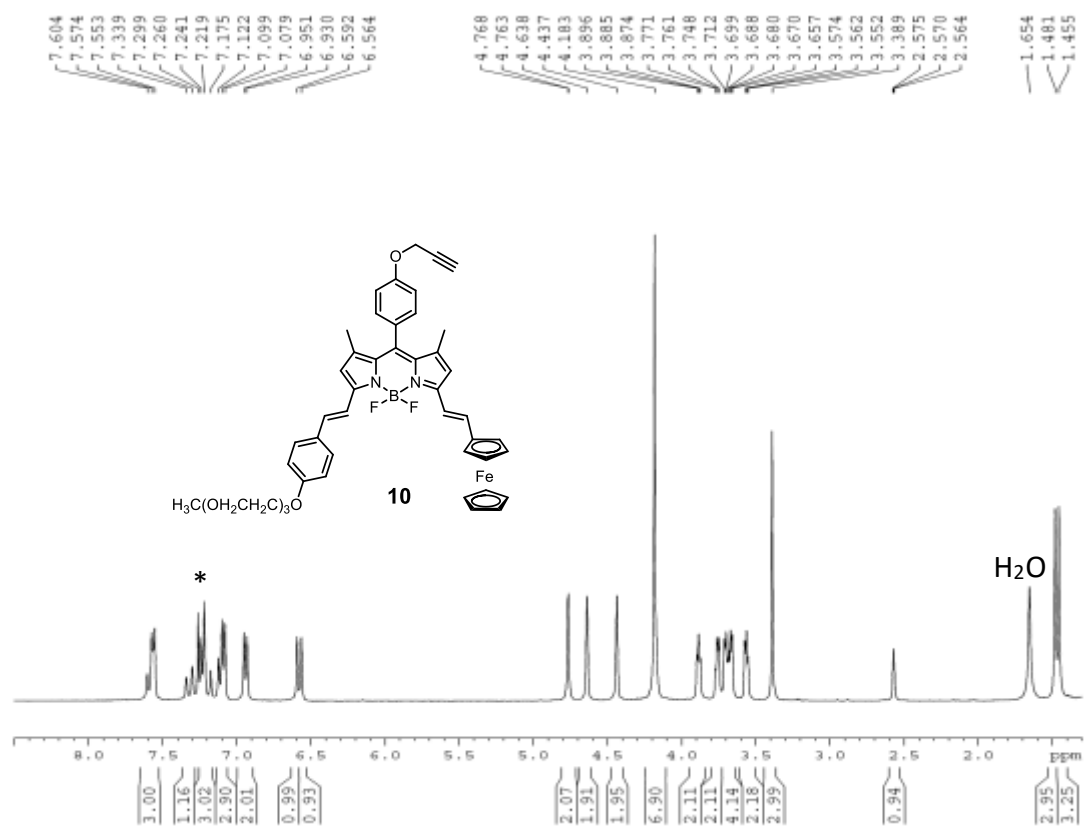
^1H NMR spectrum of **7** in CDCl_3



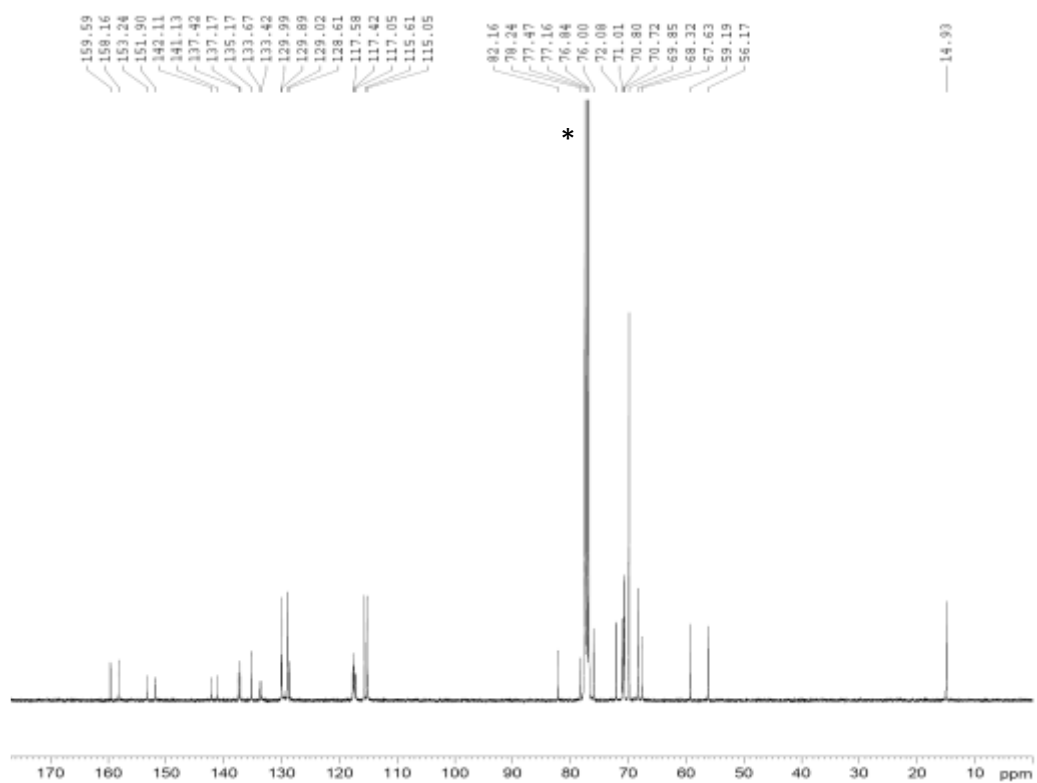
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **7** in $\text{DMSO}-d_6$



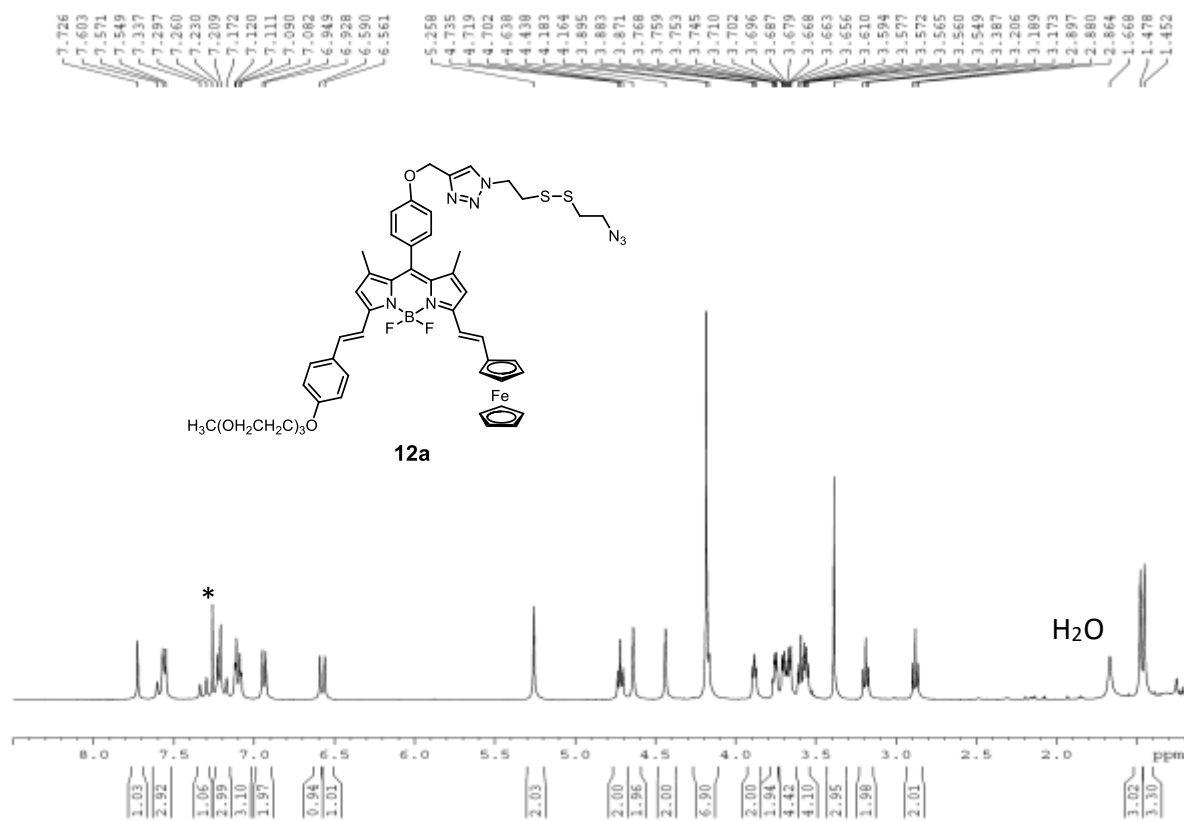
^1H NMR spectrum of **10** in CDCl_3



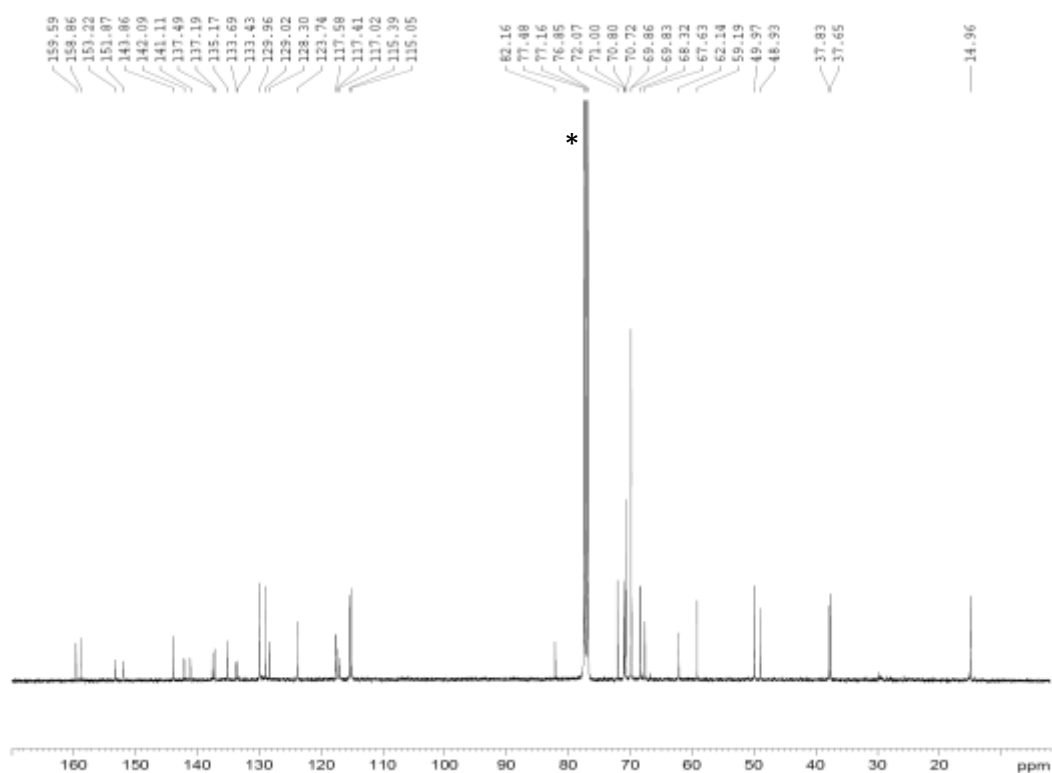
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **10** in CDCl_3



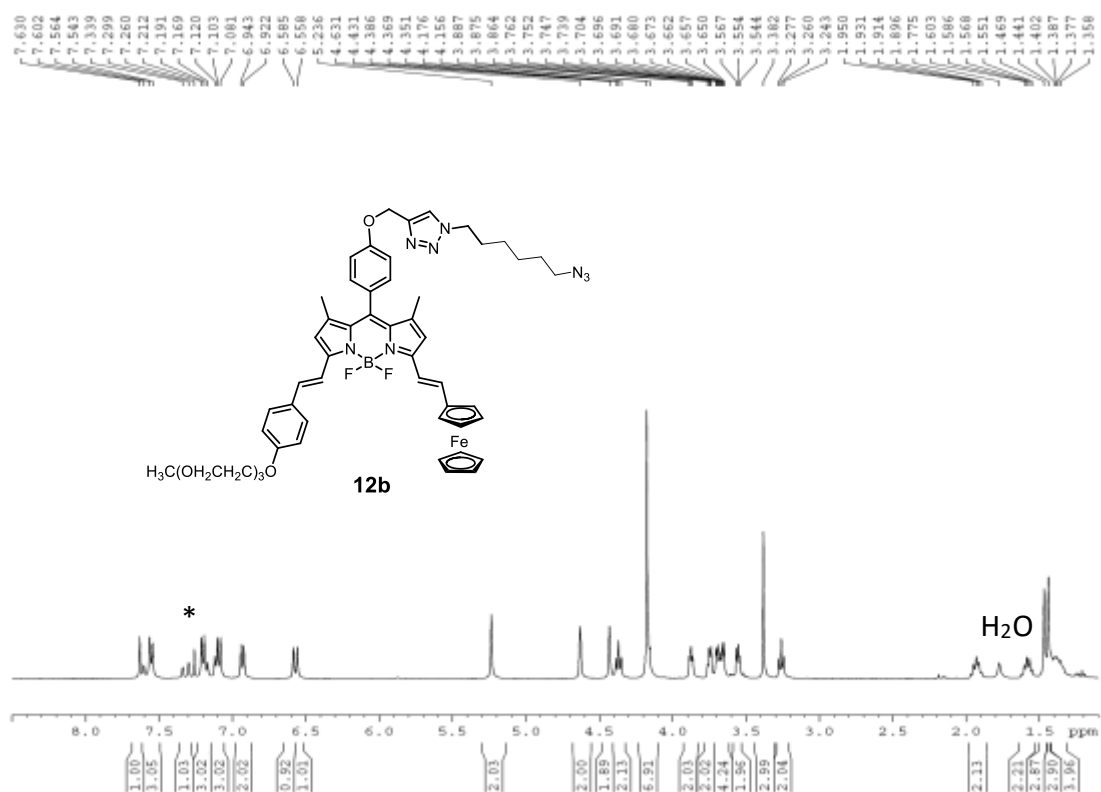
^1H NMR spectrum of **12a** in CDCl_3



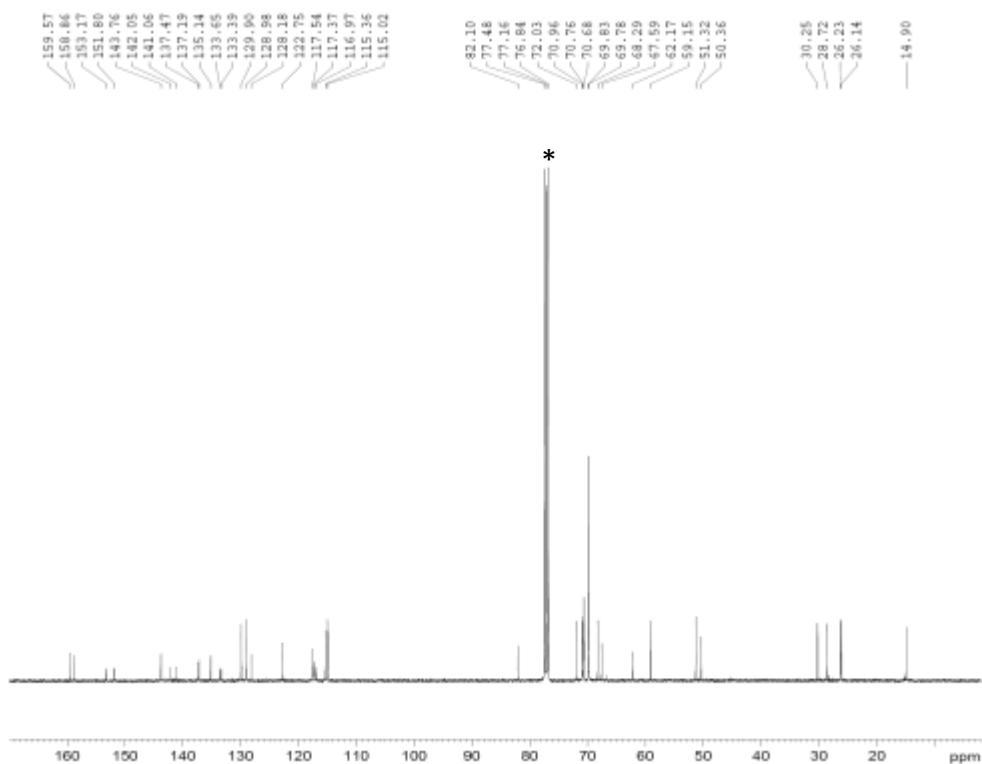
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **12a** in CDCl_3



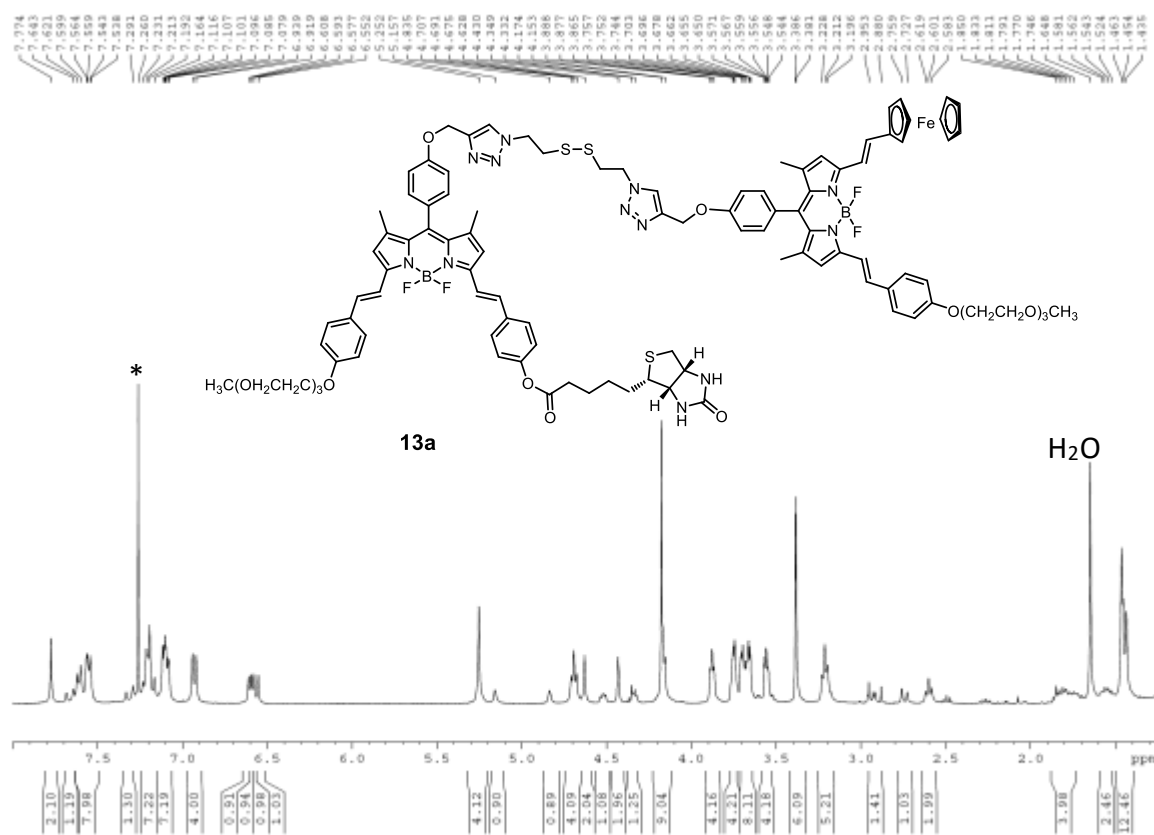
^1H NMR spectrum of **12b** in CDCl_3



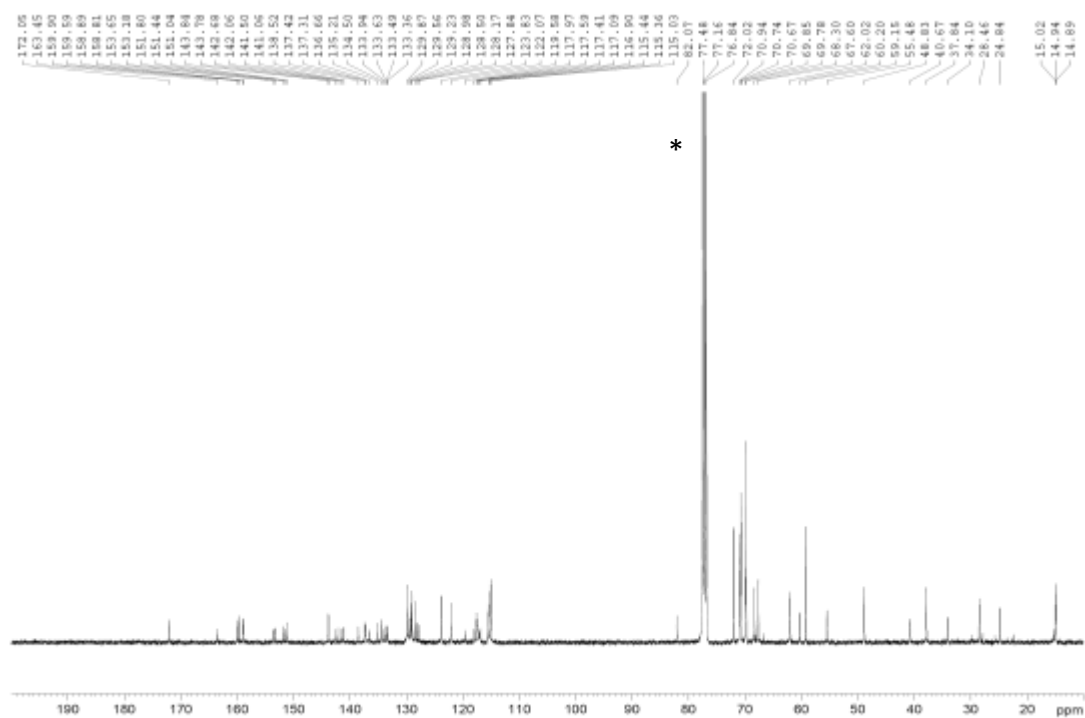
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **12b** in CDCl_3



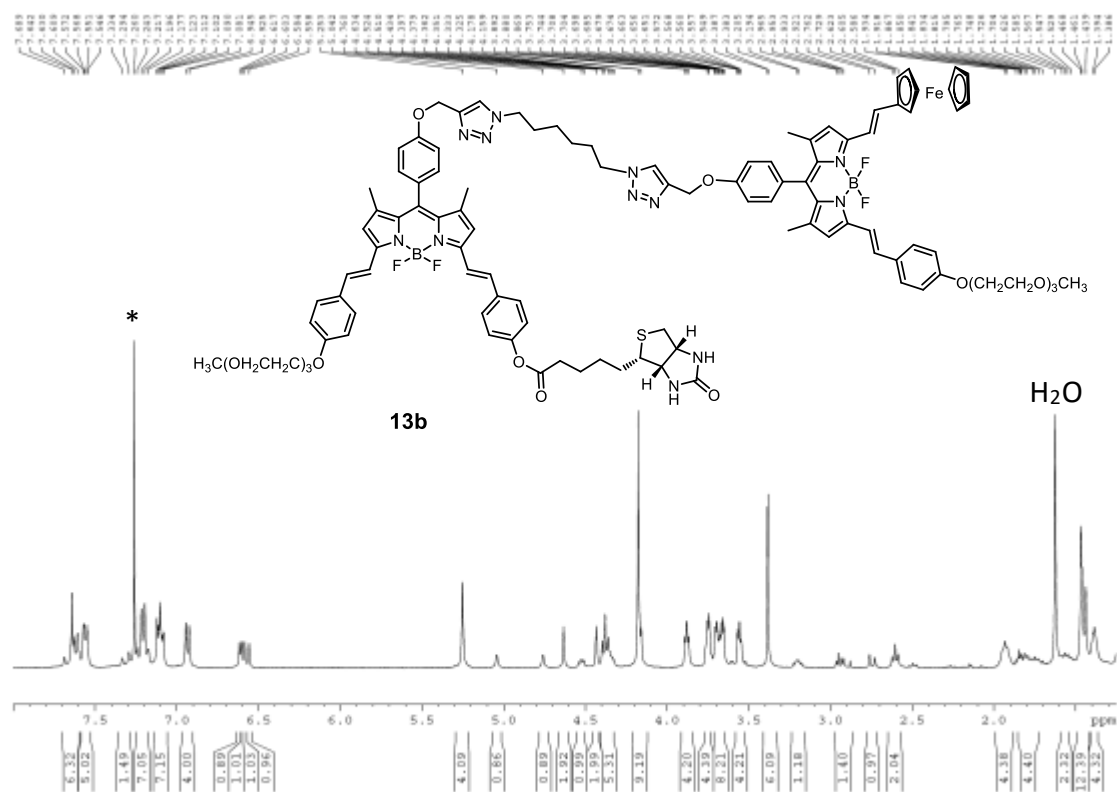
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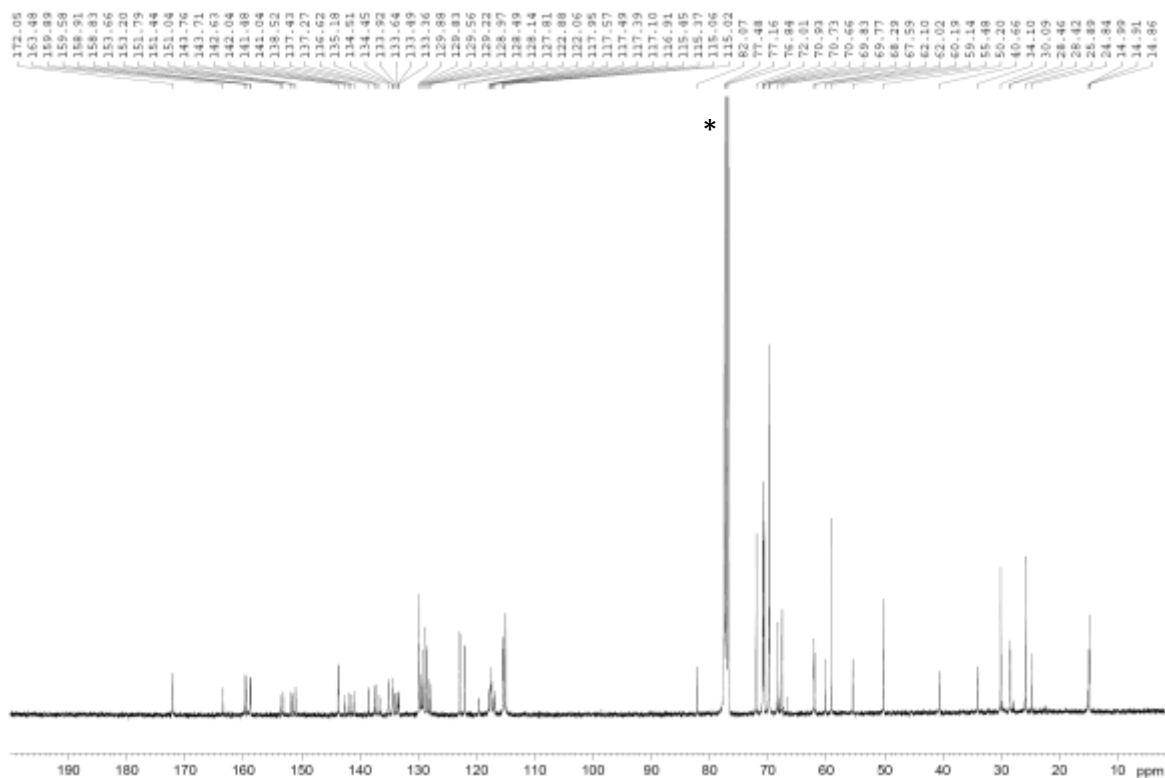
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **13a** in CDCl_3



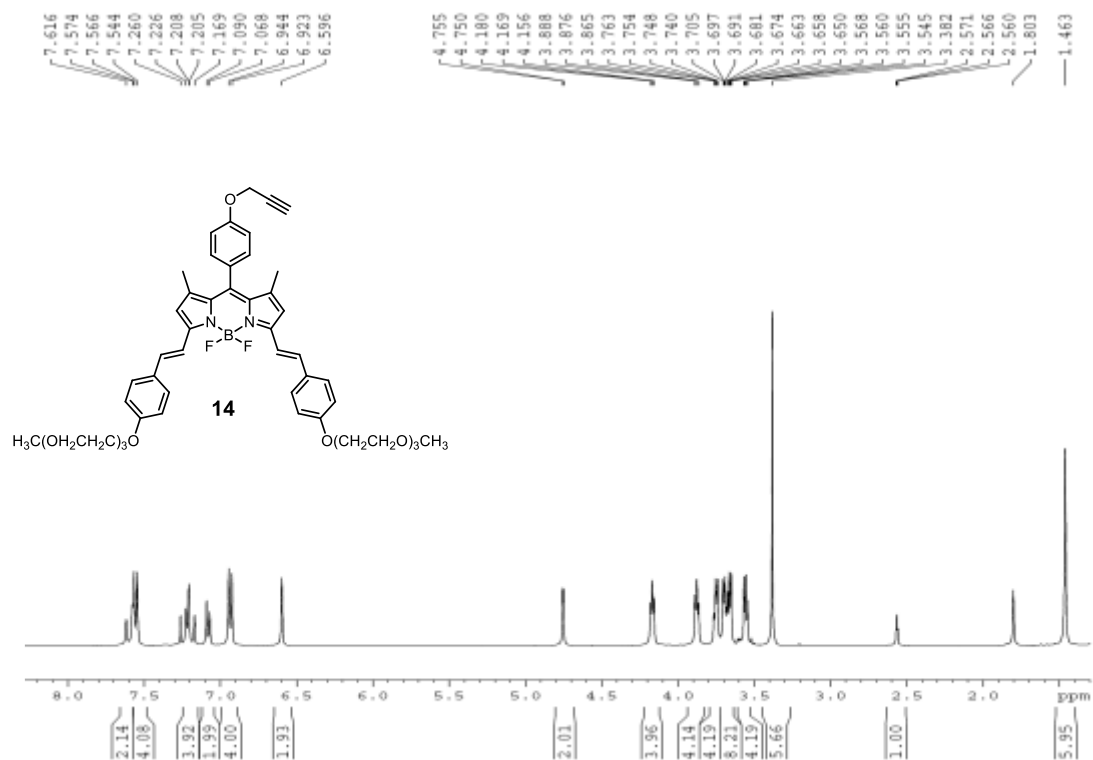
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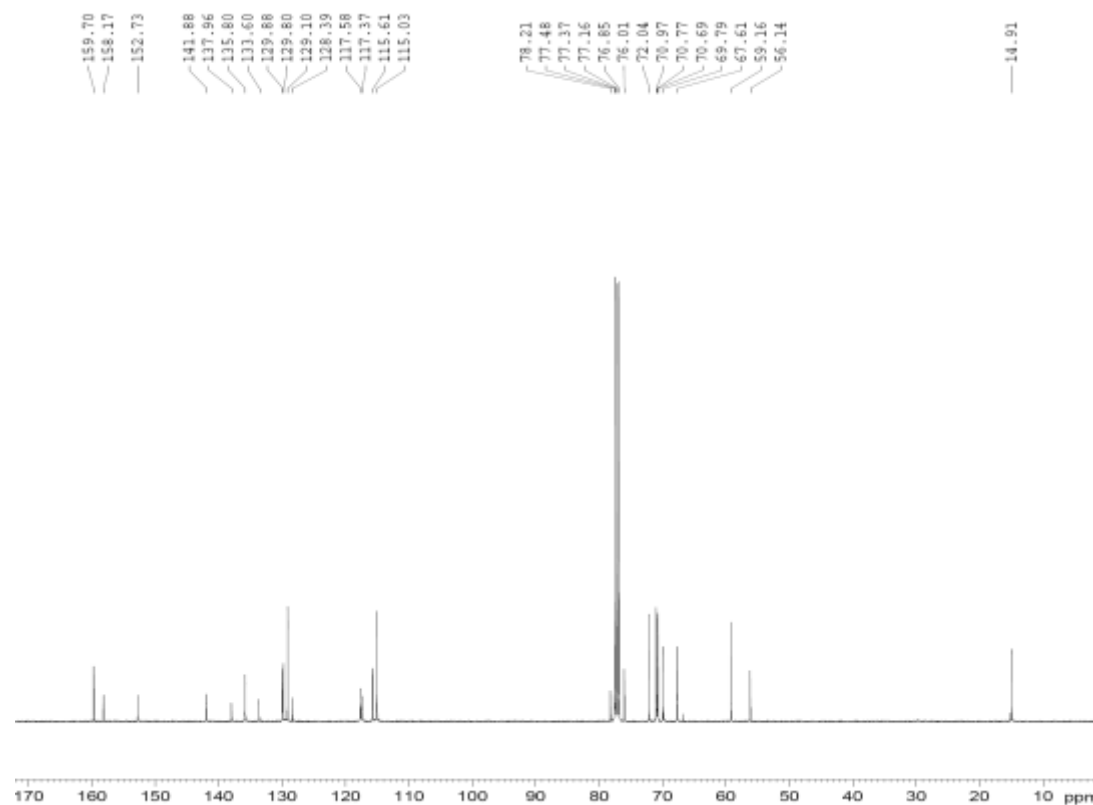
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **13b** in CDCl_3

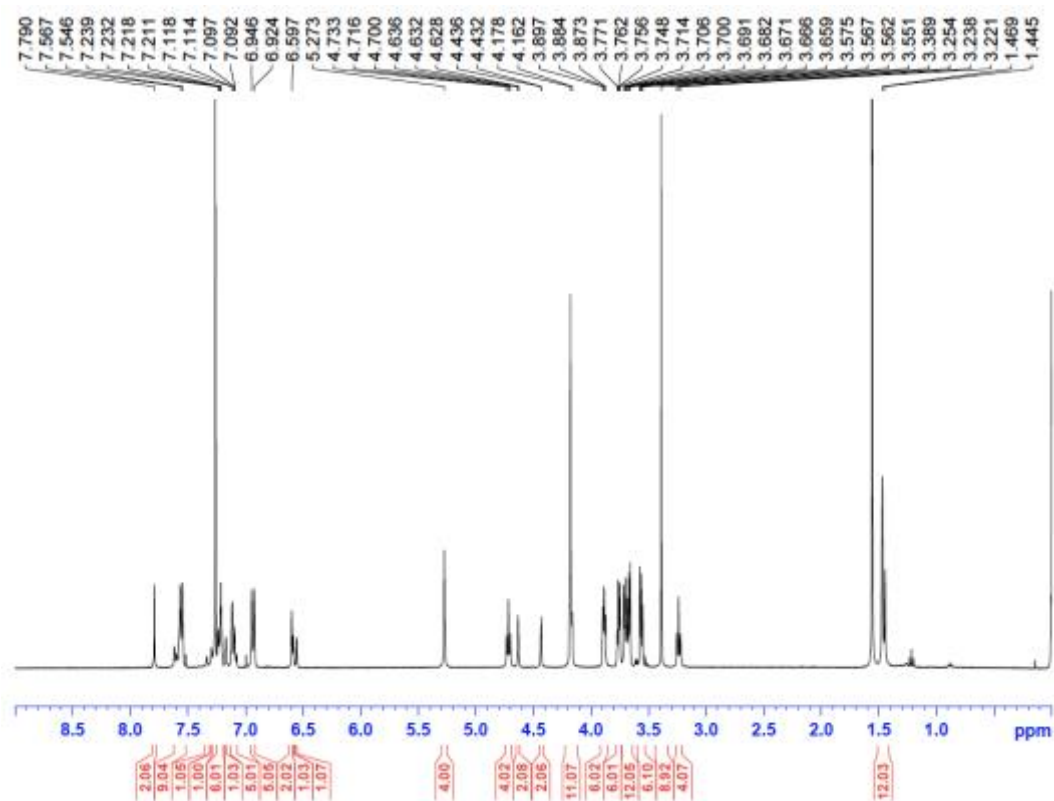


^1H NMR spectrum of **14** in CDCl_3



$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **14** in CDCl_3



¹H NMR spectrum of **15** in CDCl₃ $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **15** in CDCl_3 