

Supplementary Information

**Substituent Directed Cellular Imaging in the 800-850 nm range with  
BF<sub>2</sub>-Azadipyrrromethene Fluorophores**

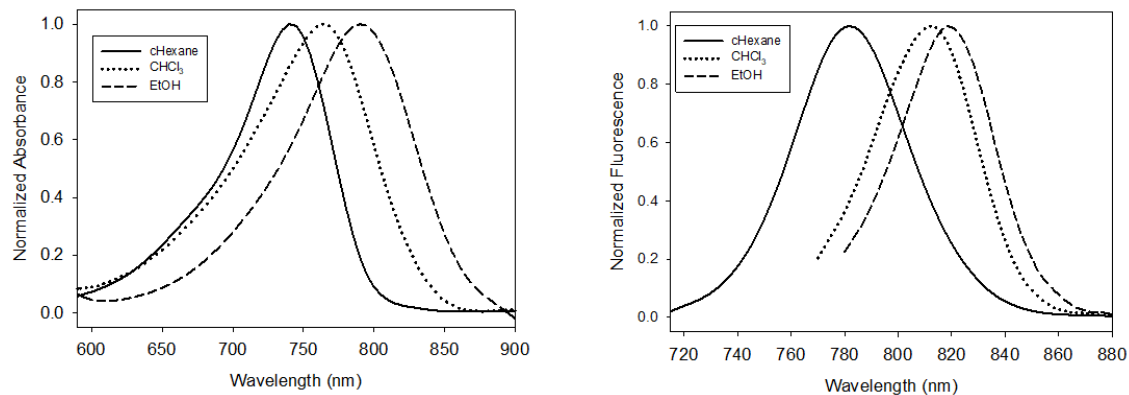
Cathal Caulfield, Dan Wu, Massimiliano Garre and Donal F. O'Shea\*

Department of Chemistry, RCSI, 123 St. Stephen's Green, Dublin 2, Ireland.

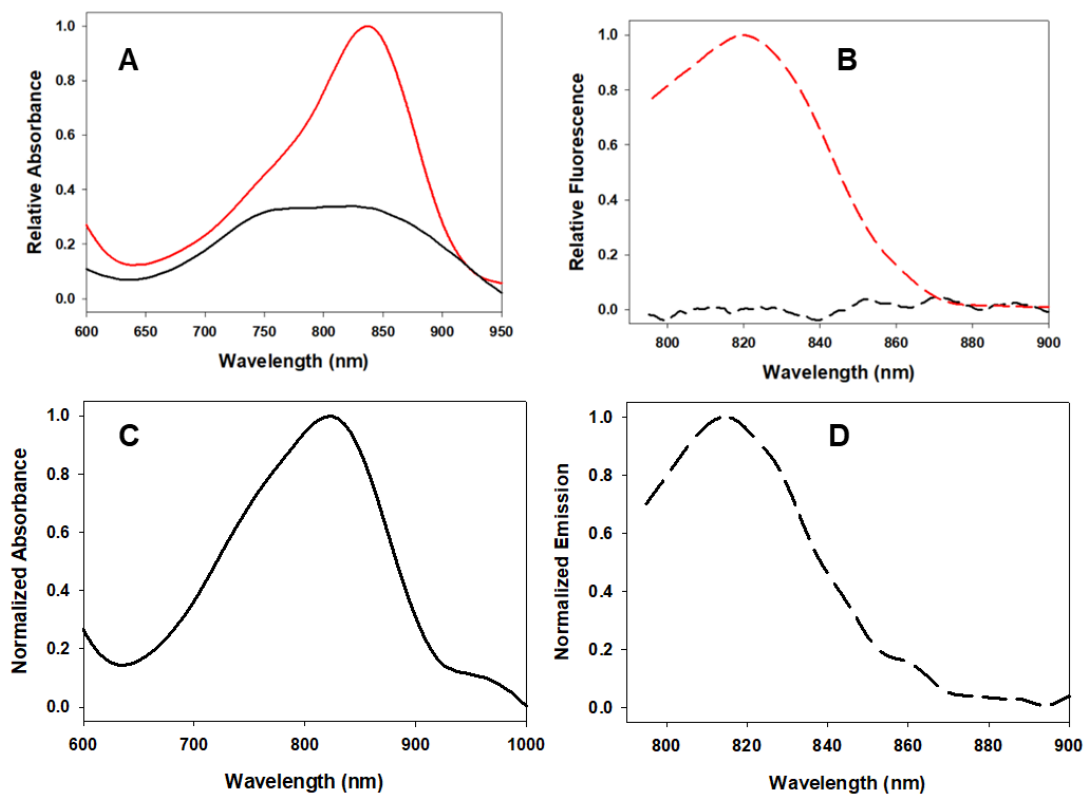
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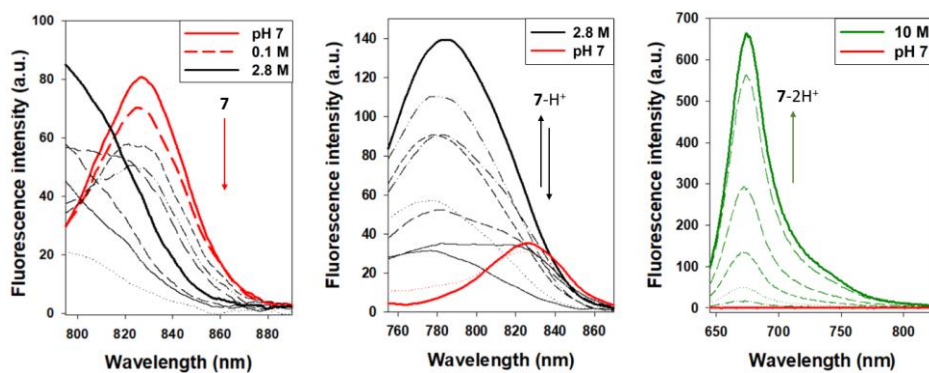
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**Fig. S1.** Normalized absorbance and fluorescence spectra of **11** (4  $\mu$ M) in *c*-hexane (solid traces) CHCl<sub>3</sub> (dotted traces) and EtOH (dashed traces).



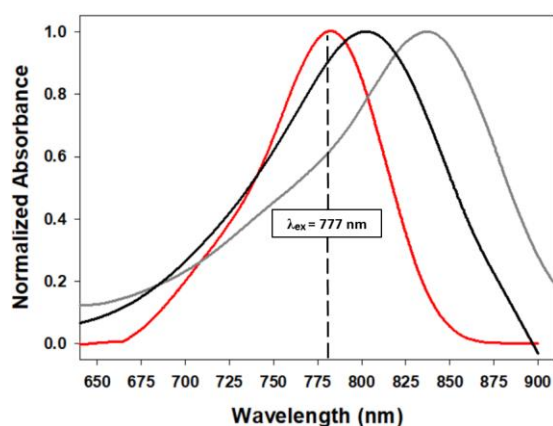
**Fig. S2.** Representative absorbance and emission spectra of **6**. (A) Absorbance and (B) fluorescence in aqueous CTAB (1% w/v; red) and H<sub>2</sub>O (bottom) (4  $\mu$ M; 10 nm slit width). (C) Absorbance and (D) fluorescence spectra recorded in aqueous fetal-calf serum.



**Fig. S3.** HCl titration of **7** in water (4  $\mu\text{M}$ ). Fluorescence spectra showing  $N$ -protonations of **7** below the pH scale. 10 nm slit widths used for red and black profiles (**7** and **7-H<sup>+</sup>** respectively); 5nm slit widths for green (**7-2H<sup>+</sup>**).

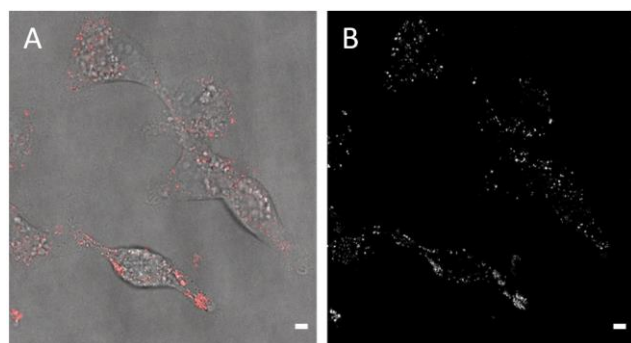
**Table S1.** Photophysical characteristics of **7** and  $N$ -protonated species of **7** in aqueous HCl.

Entry	Comp.	HCl conc. (M)	$\lambda_{\text{max}}$ abs (nm)	$\lambda_{\text{max}}$ flu (nm)	Excitation (nm)
1	<b>7</b>	$1 \times 10^{-7}$	806	826	780
2	<b>7-H<sup>+</sup></b>	2.8	744	784	740
3	<b>7-2H<sup>+</sup></b>	10	654	675	630

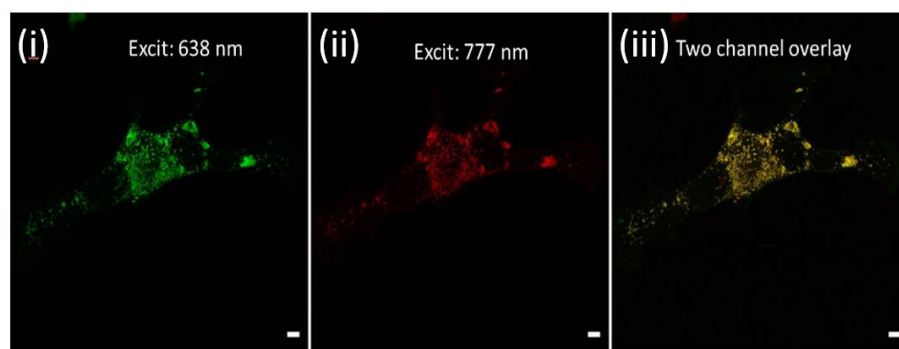


**Fig. S4.** Normalized absorbance spectra of lipophilic **5** in triolein (red), amphiphilic **6** in aq. CTAB (1% w/v; grey) and hydrophilic **7** in  $\text{H}_2\text{O}$  (pH 7; black) with excitation wavelength used for cell imaging highlighted.

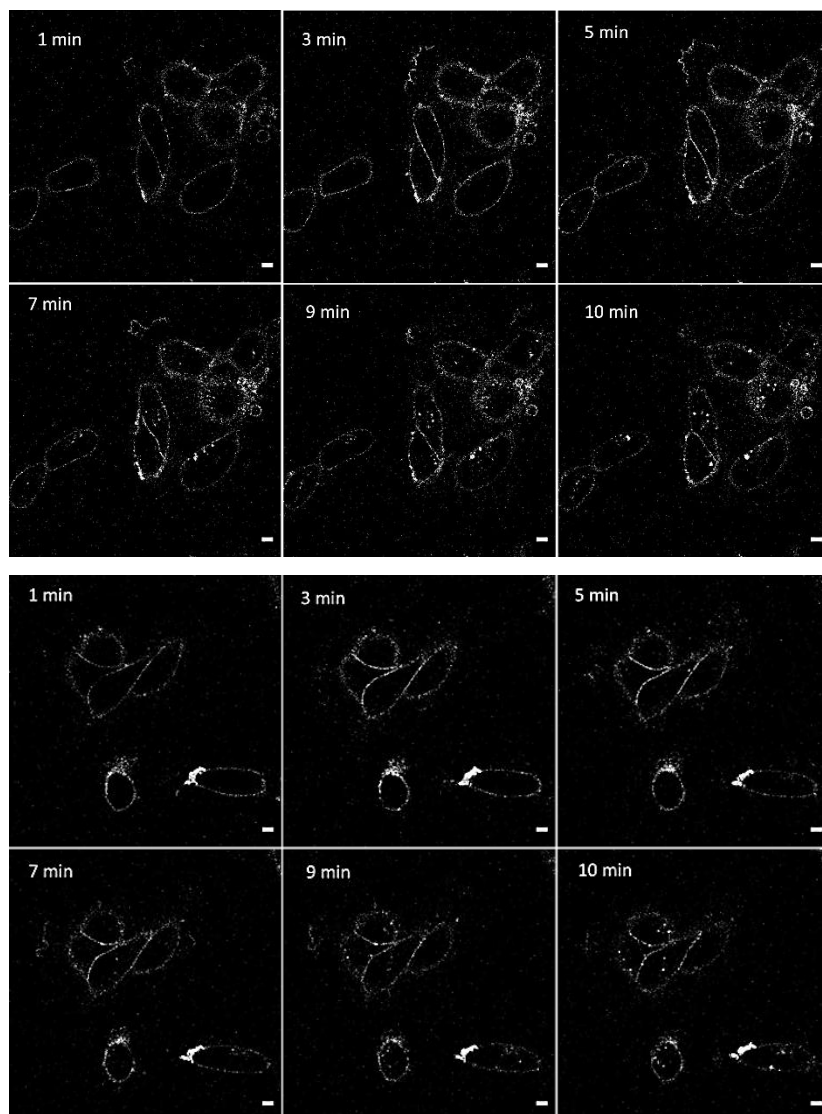
## MDA-MB 231 Cell Imaging SI



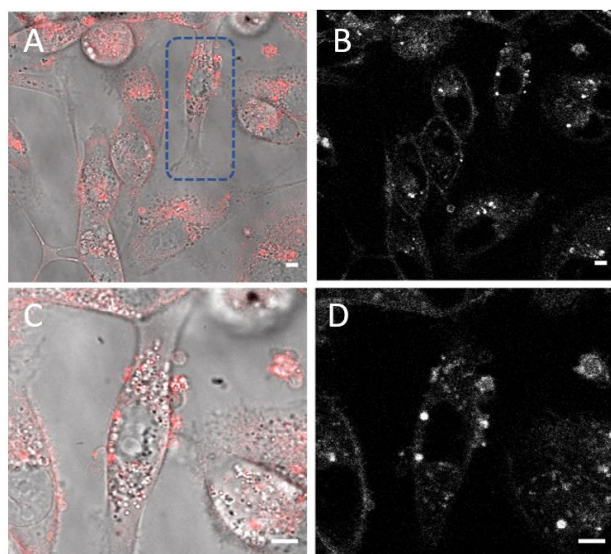
**Fig. S5.** CLSM imaging of MDA MB-231 live cells following 60 min incubation with **5** (5  $\mu\text{M}$ ). (A) CLSM image (fluorescence in red) with bright field overlay. (B) Fluorescence shown in black and white for clarity. Scale bars 5  $\mu\text{m}$ .



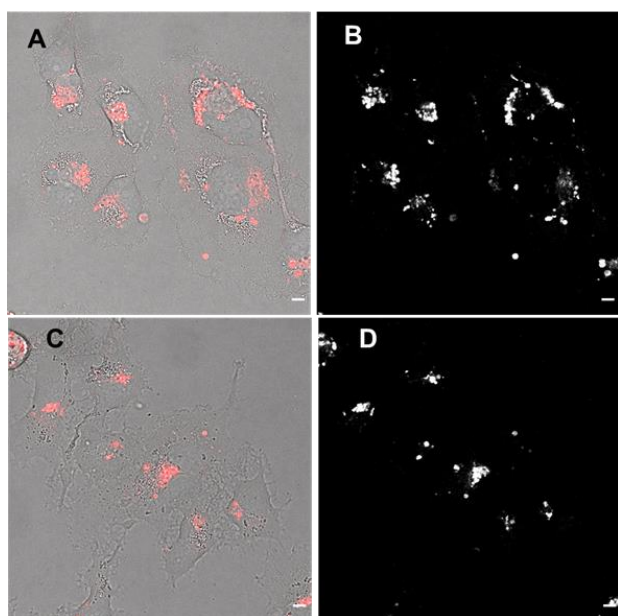
**Fig. S6.** CLSM imaging of MDA MB-231 live cells co-incubated with (i) **1b** and (ii) **5** for 1 h with (iii) showing overlaid images with co-localisation (yellow) of both fluorophores in LDs. Scale bars 5  $\mu\text{m}$ .



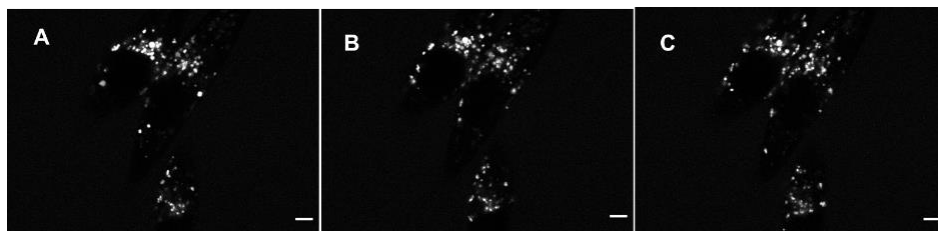
**Fig. S7.** Two representative examples of CLSM imaging of MDA MB-231 live cells over 10 min following incubation with **6** (5 μM). Fluorescence shown in black and white for clarity with time points 1, 3, 5, 7, 9 and 10 min showing increasing visualisation of plasma membranes. Scale bars 5 μm.



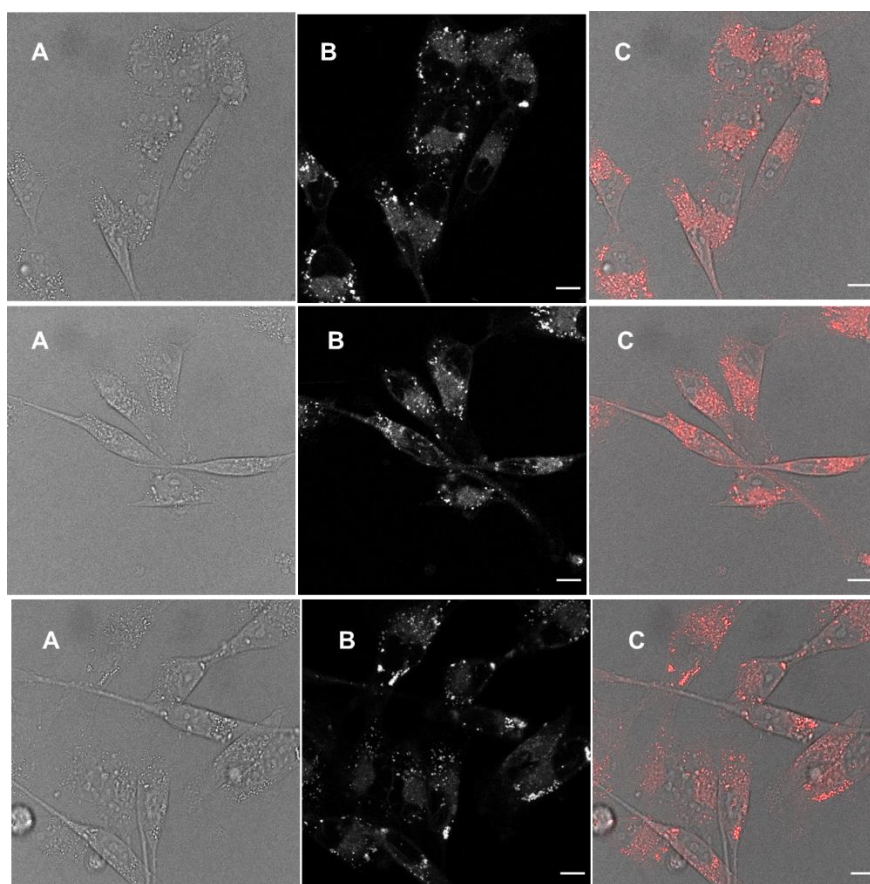
**Fig. S8.** CLSM imaging of MDA MB-231 live cells at 60 min following incubation with **6** (5  $\mu$ M). (A) CLSM image (fluorescence in red) with bright field overlay taken 60 min following the addition of **6**. (B) Fluorescence shown in black and white for clarity. (C) Expansion of image A (fluorescence in red) with bright field overlay. (D) Fluorescence shown in black and white for clarity. Scale bars 5  $\mu$ m.



**Fig S9.** Two representative examples of CLSM imaging of MDA MB-231 live cells at 4 h following incubation with **6** (5  $\mu$ M). (A and C) CLSM image (fluorescence in red) with bright field overlay taken 60 min following the addition of **6**. (B and D) Fluorescence shown in black and white for clarity. Scale bars 5  $\mu$ m.



**Fig. S10.** CLSM imaging of MDA MB-231 live cells following 24 h incubation with **7** (5  $\mu$ M). CLSM images from 5 min time lapse showing motion of vesicles with fluorescence shown in black and white for clarity. (A) CLSM image at 0 min. (B) CLSM image at 2.5 min. (C) CLSM image at 5 min. Scale bars 5  $\mu$ m.



**Fig. S11.** Three representative examples of CLSM imaging of MDA MB-231 live cells following 60 min incubation with **11** (5  $\mu$ M). (A) Brightfield images (B) CLSM images shown in black and white for clarity. (C) CLSM images, fluorescence in red, with bright field overlay. Scale bars 5  $\mu$ m.



NMR and Mass Spec Data for Compounds 8, 9, 10, 11, 5, 6, 12, 13 and 7.

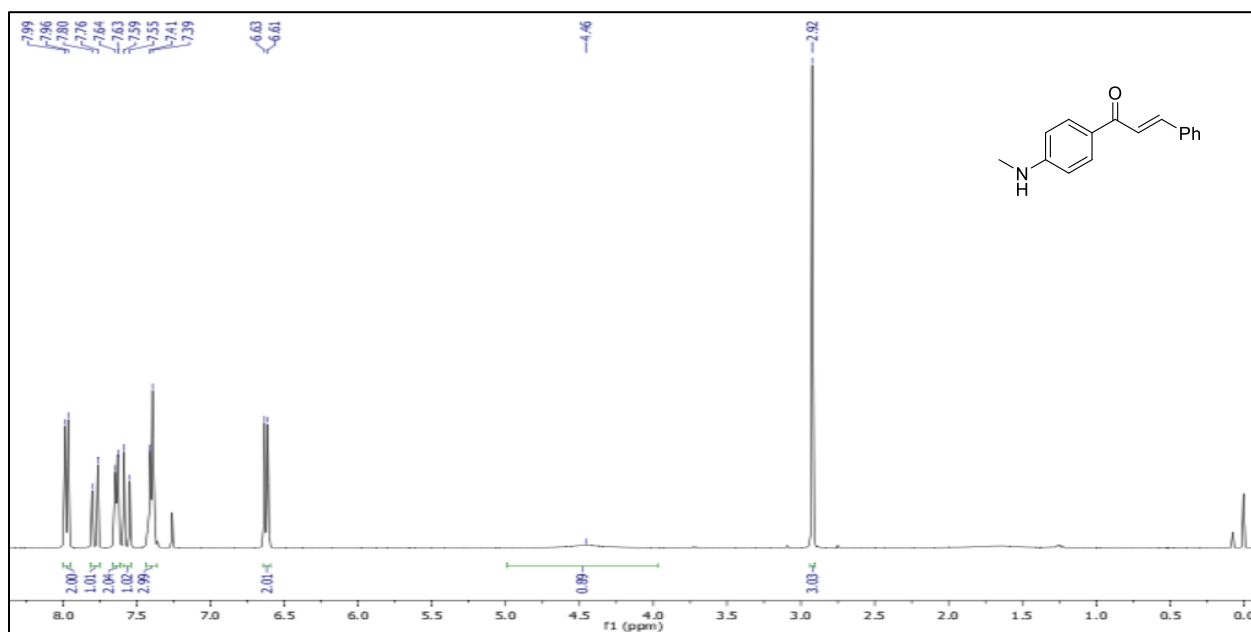


Fig. S12.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for (8).

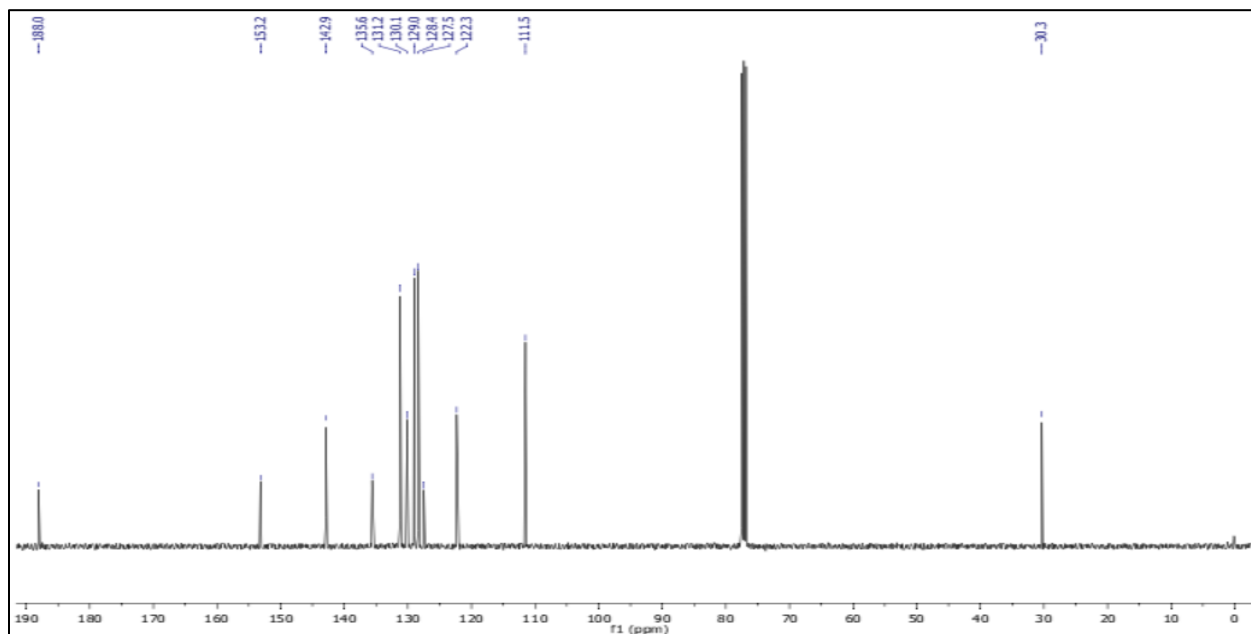
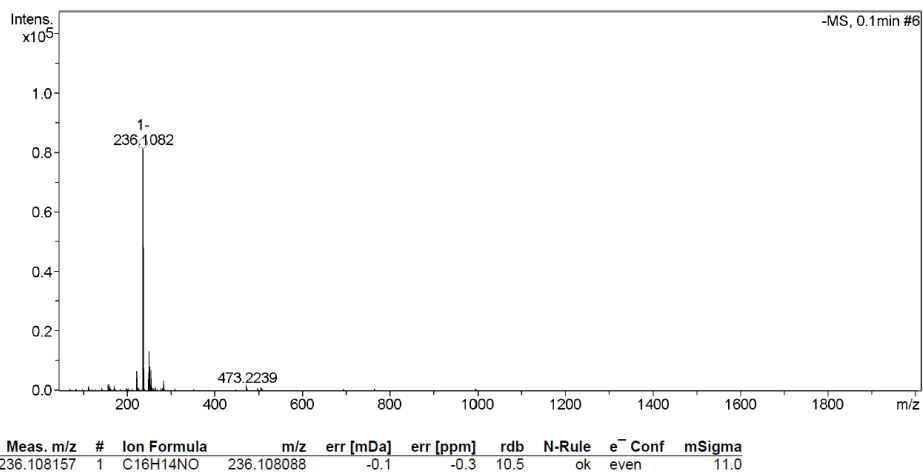
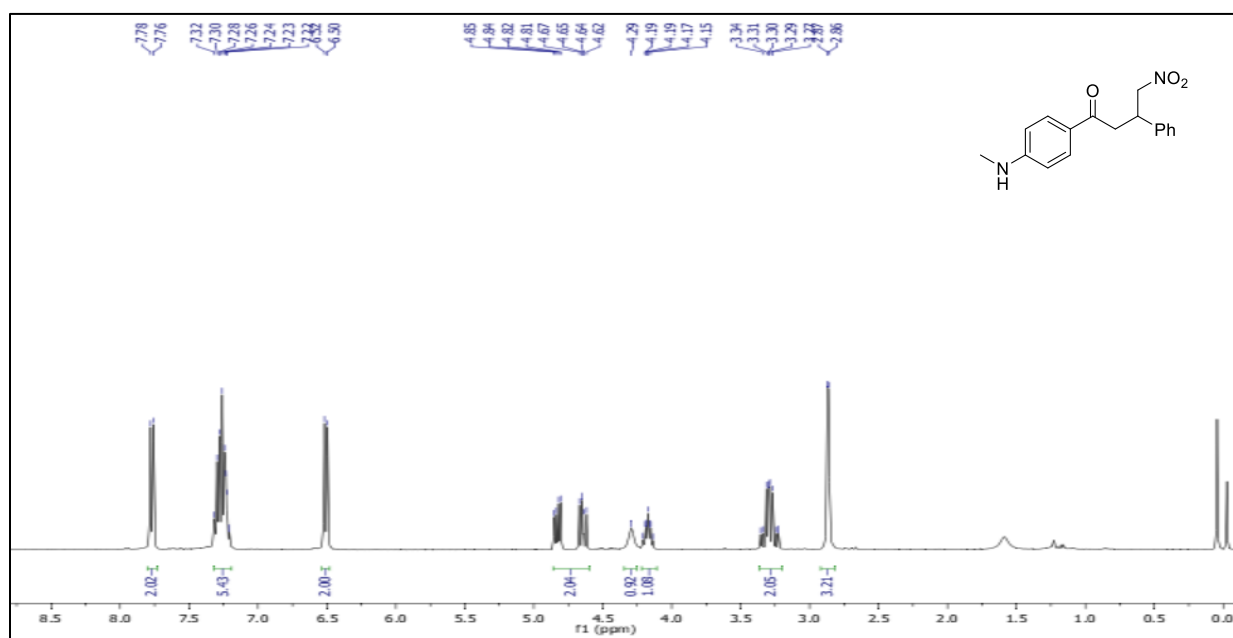


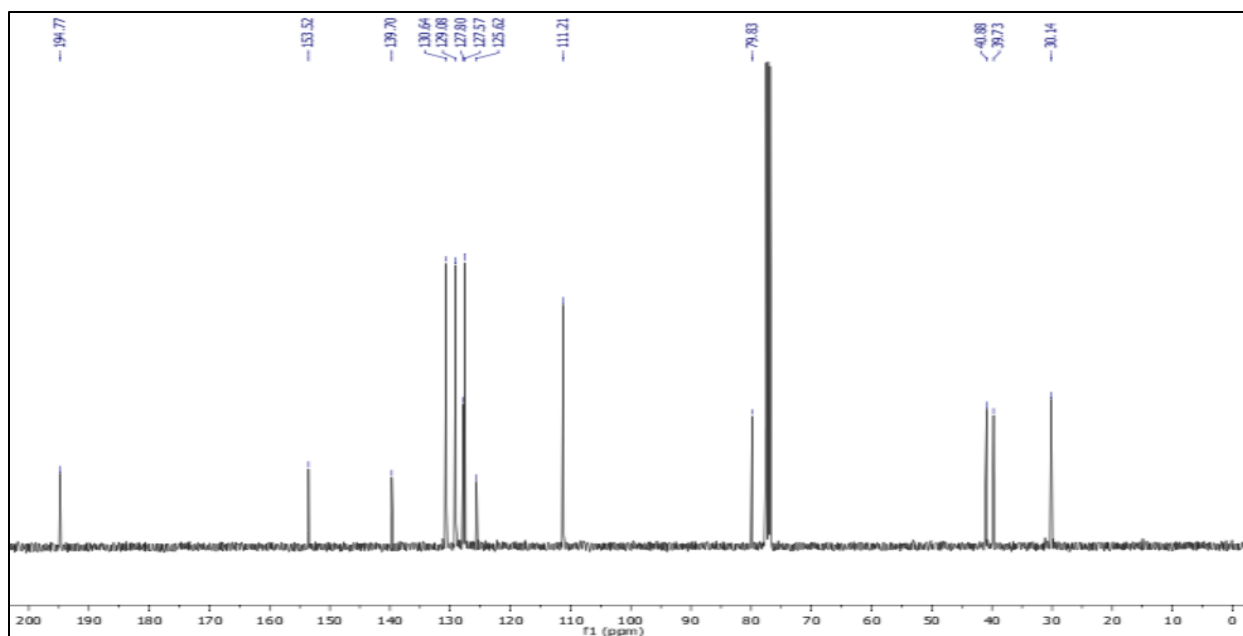
Fig. S13.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for (8).



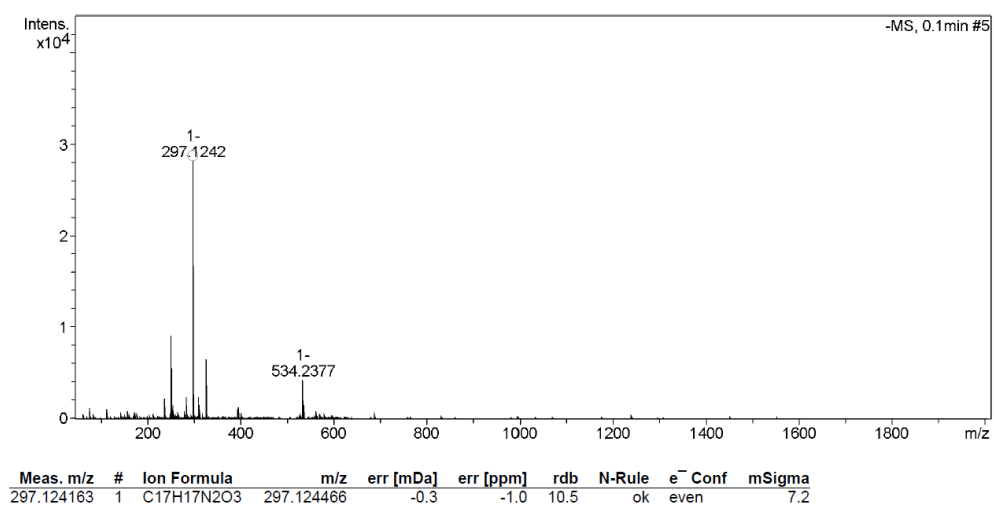
**Fig. S14.** HRMS ESI<sup>-</sup> of (8).



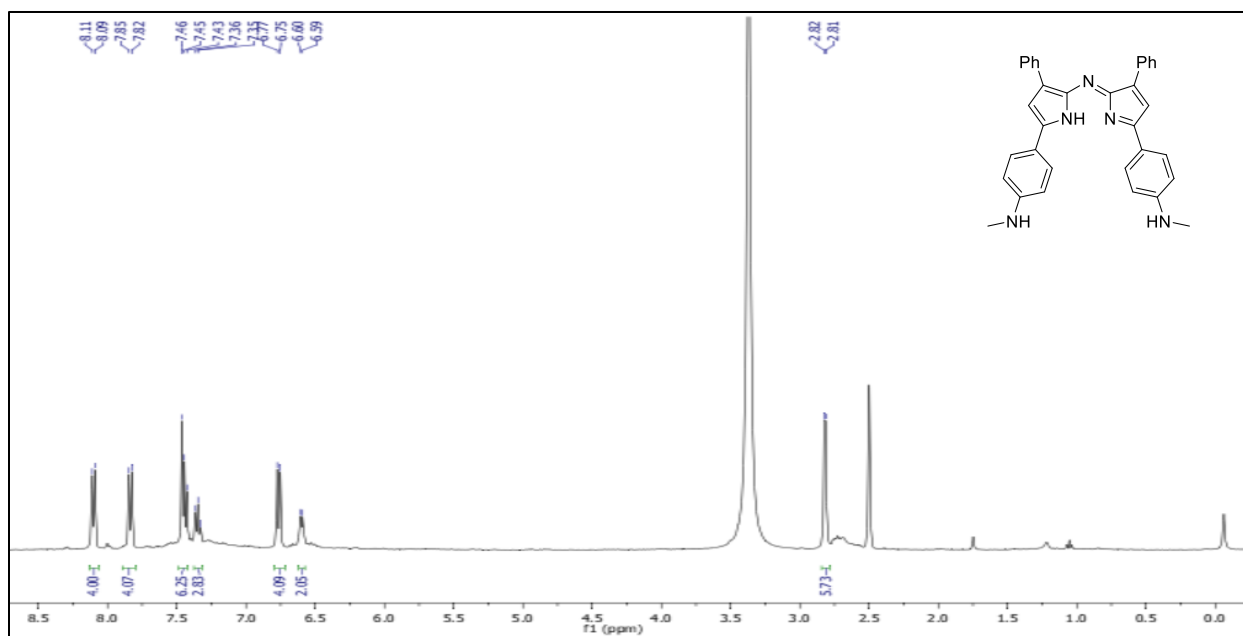
**Fig. S15.** <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum for (9).



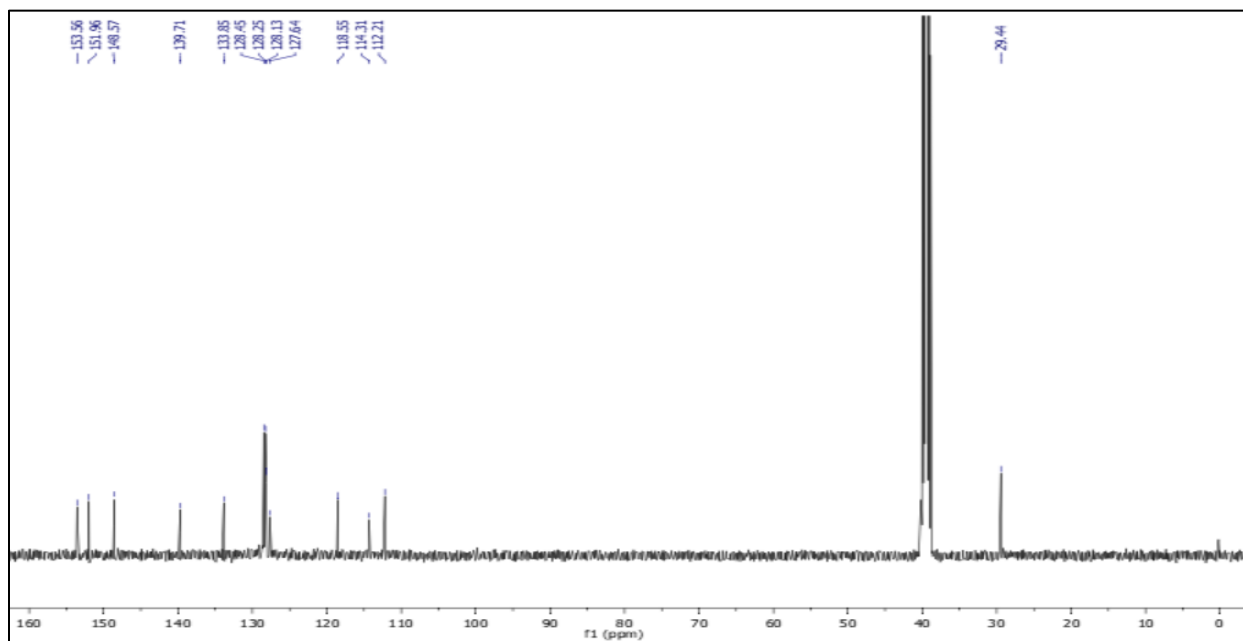
**Fig. S16.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for (9).



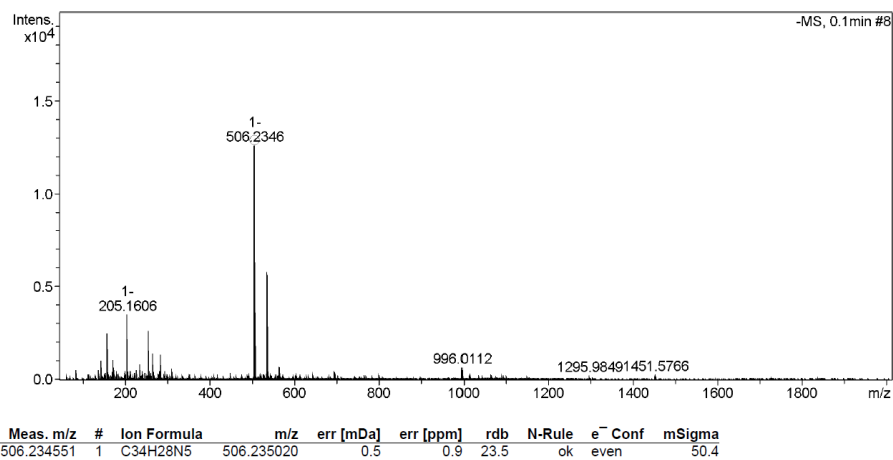
**Fig. S17.** HRMS ESI<sup>-</sup> of (9).



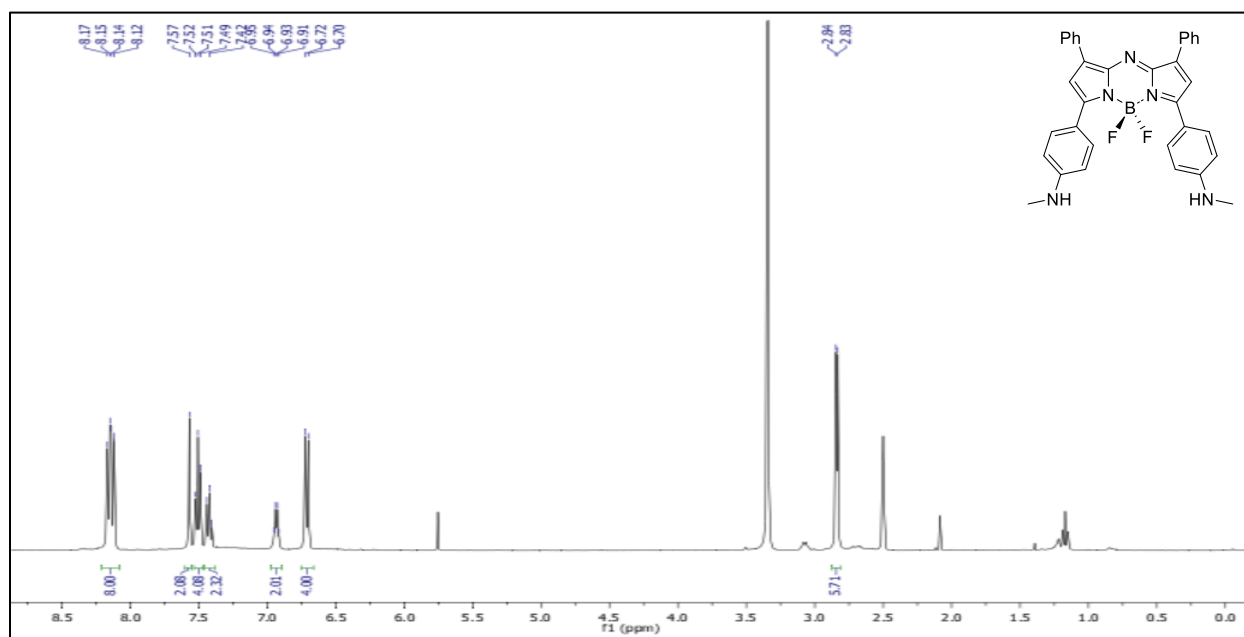
**Fig. S18.**  $^1\text{H}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for (10).



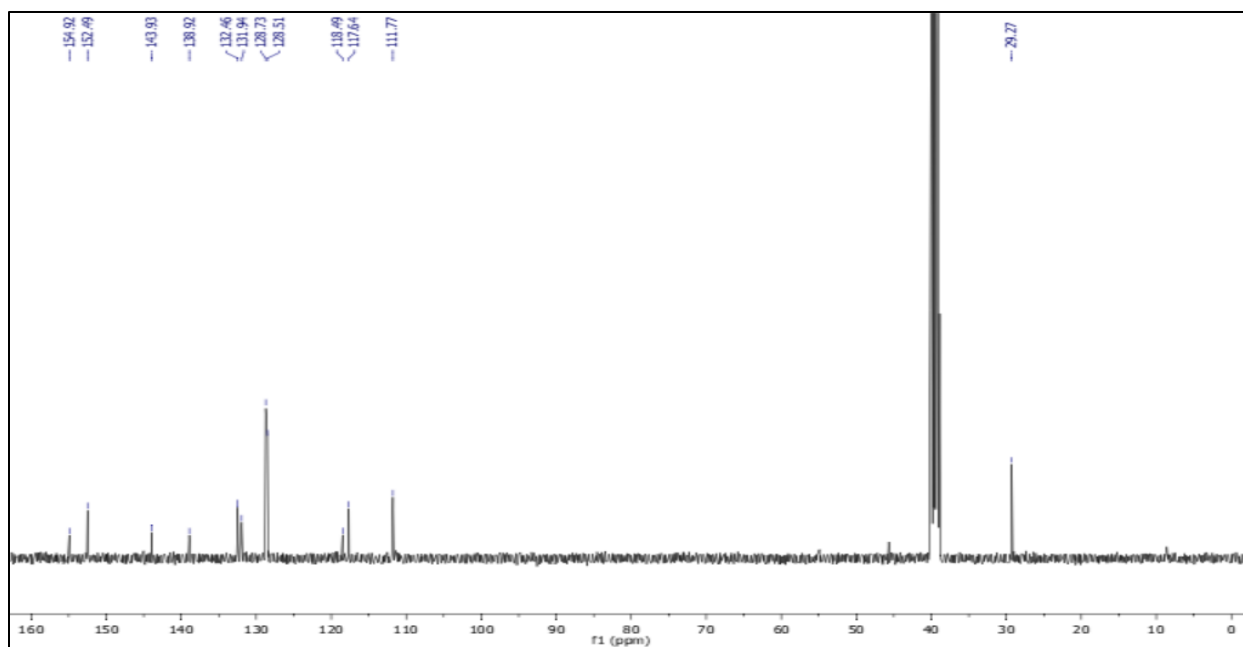
**Fig. S19.**  $^{13}\text{C}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for (10).



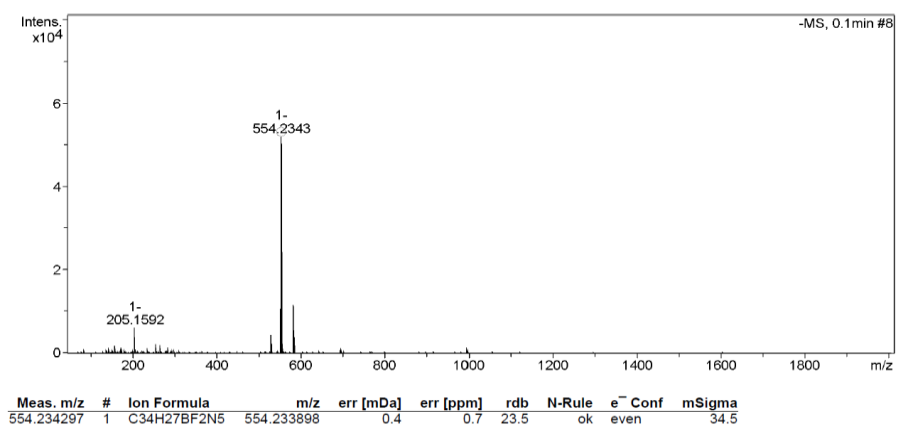
**Fig. S20.** HRMS ESI<sup>-</sup> of (10).



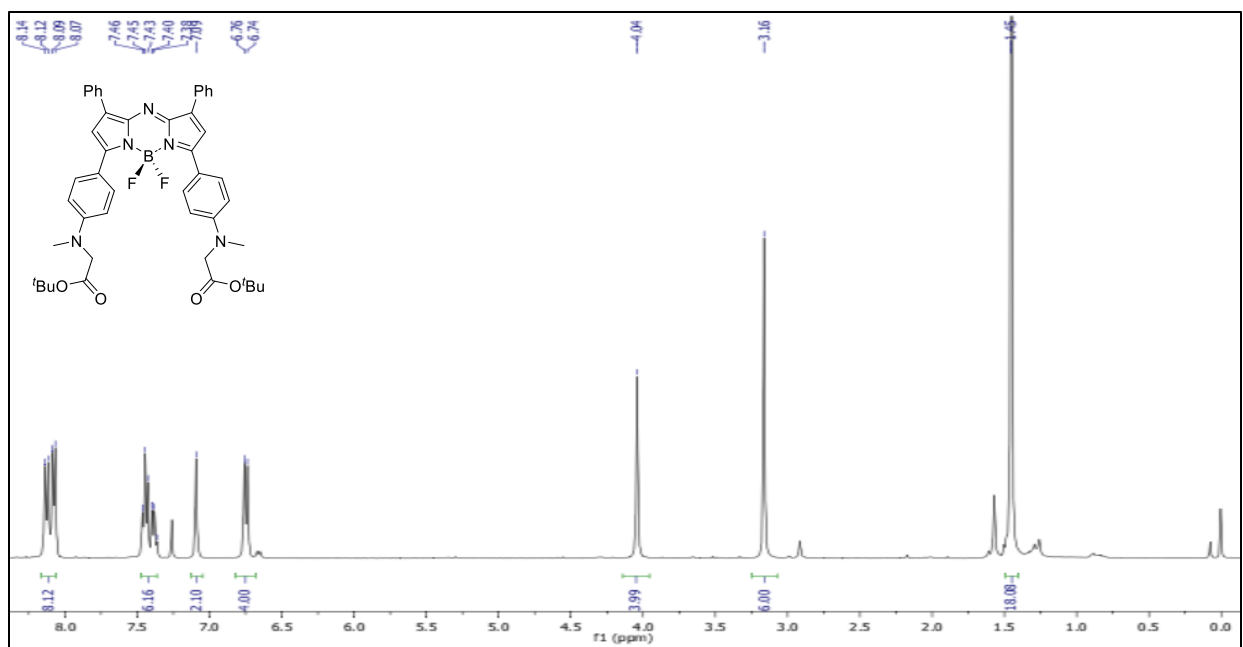
**Fig. S21.** <sup>1</sup>H NMR (DMSO-*d*<sup>6</sup>) spectrum for (11).



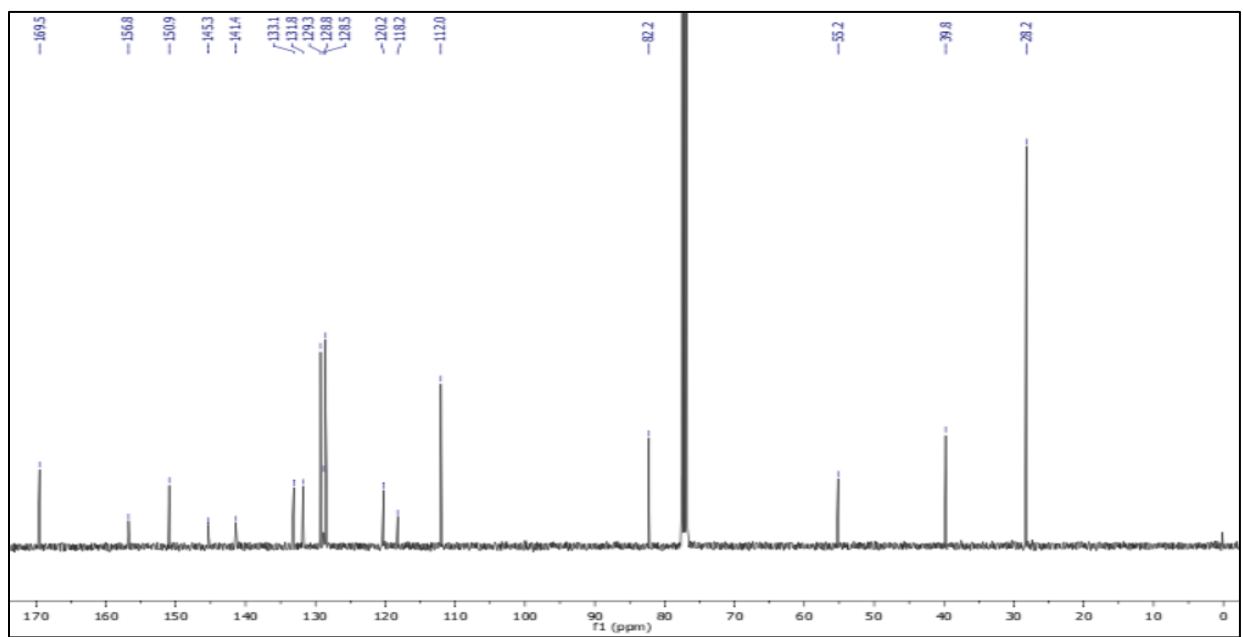
**Fig. S22.**  $^{13}\text{C}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for **(11)**.



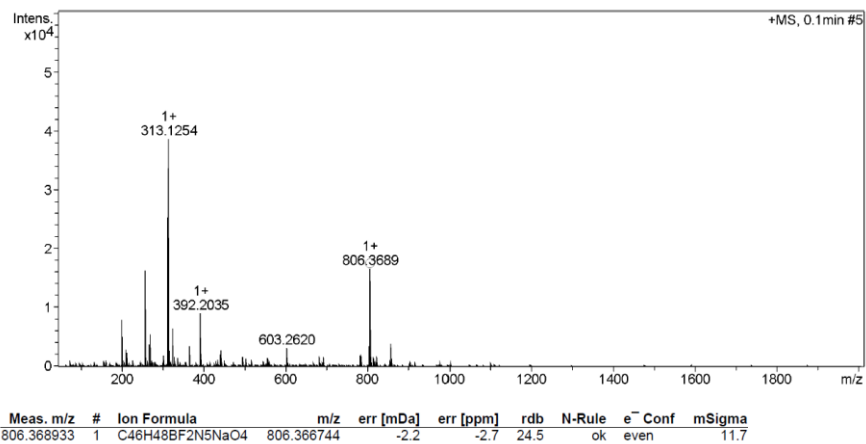
**Fig. S23.** HRMS ESI<sup>-</sup> of **(11)**.



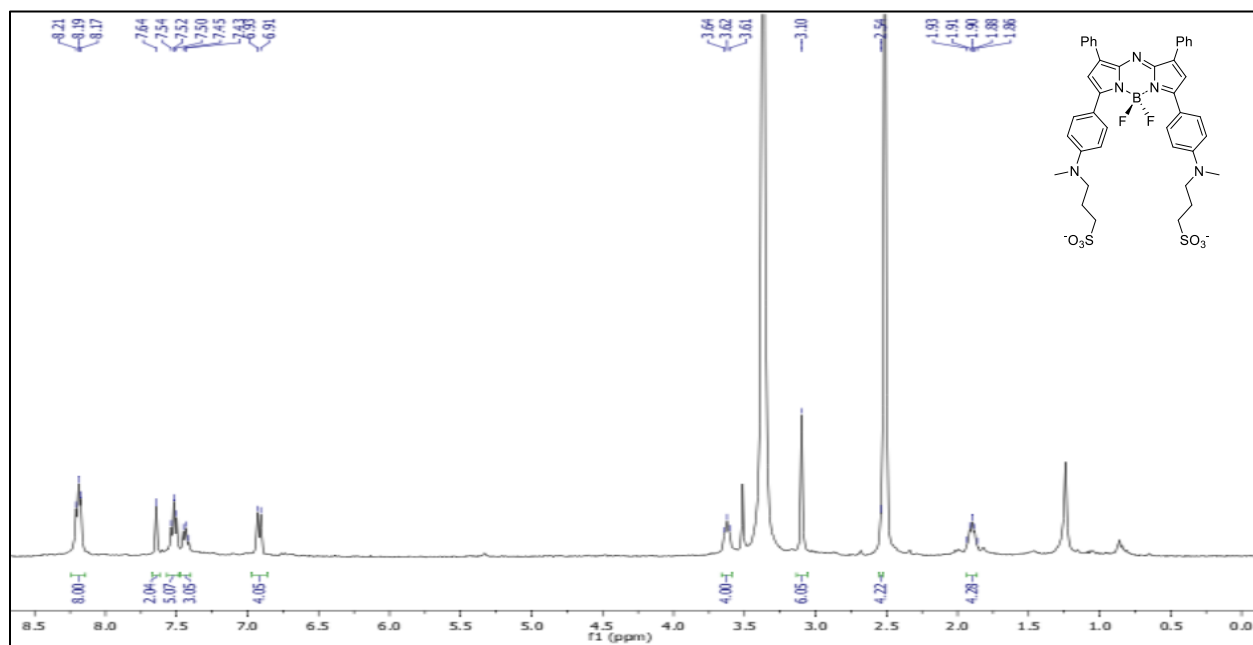
**Fig. S24.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for (5).



**Fig. S25.**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) spectrum for (5).

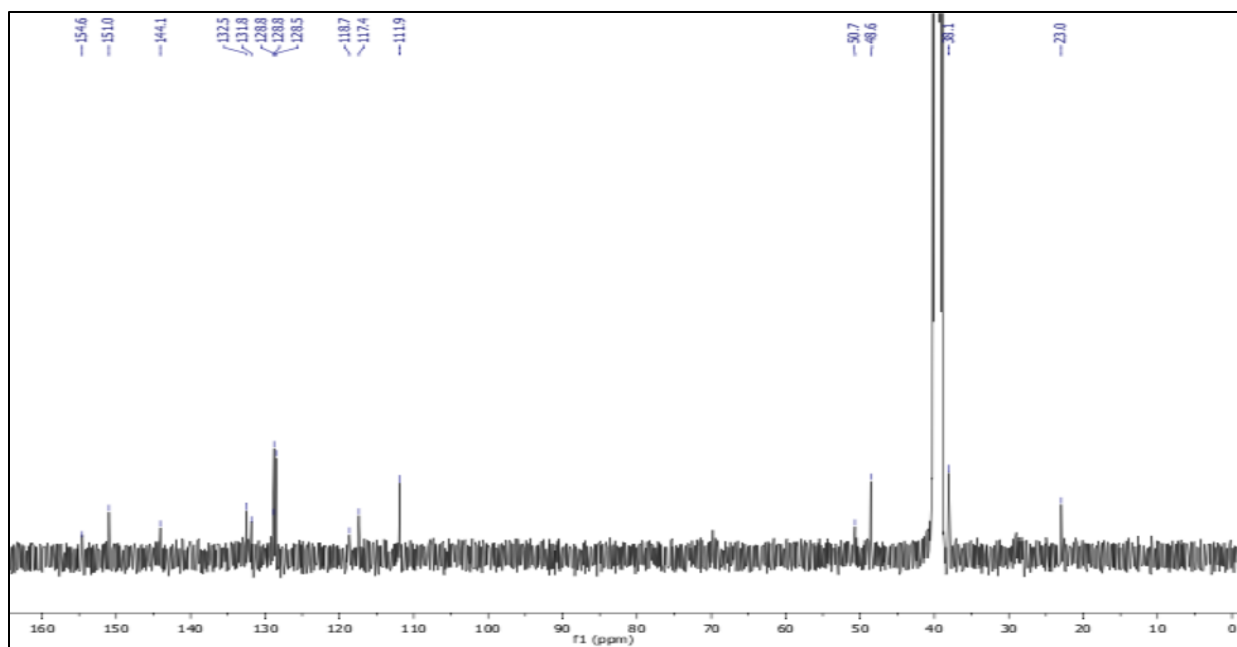


**Fig. S26.** HRMS ESI<sup>+</sup> of (5).

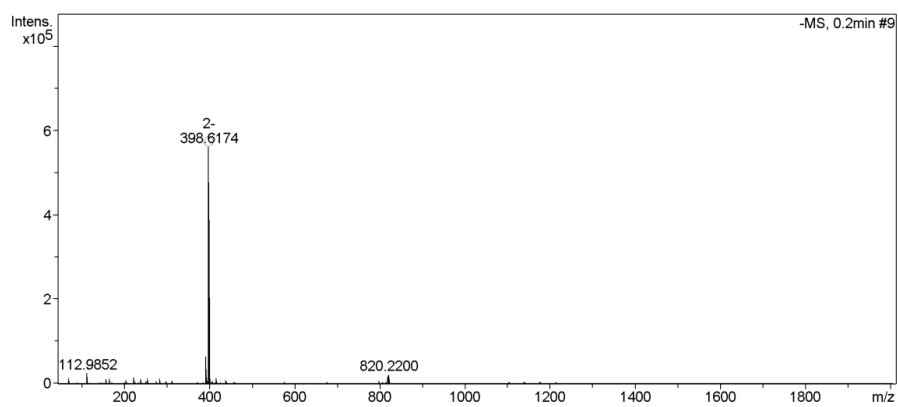


**Fig. S27.** <sup>1</sup>H NMR (DMSO-*d*<sup>6</sup>) spectrum for (6).



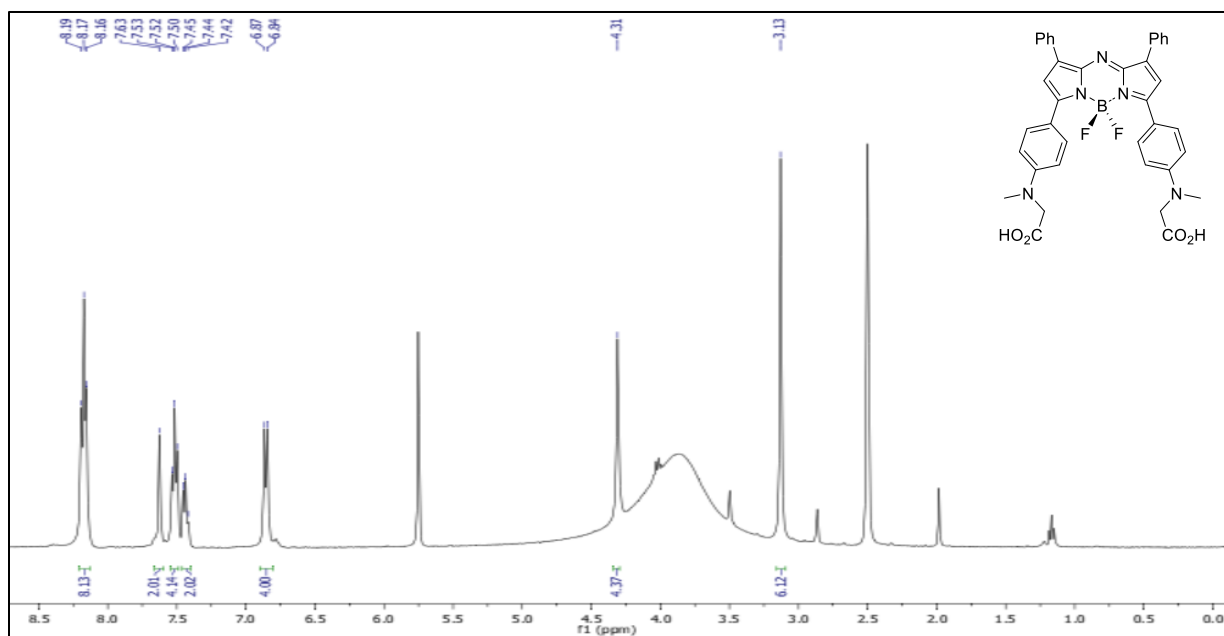


**Fig. S28.**  $^{13}\text{C}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for **(6)**.

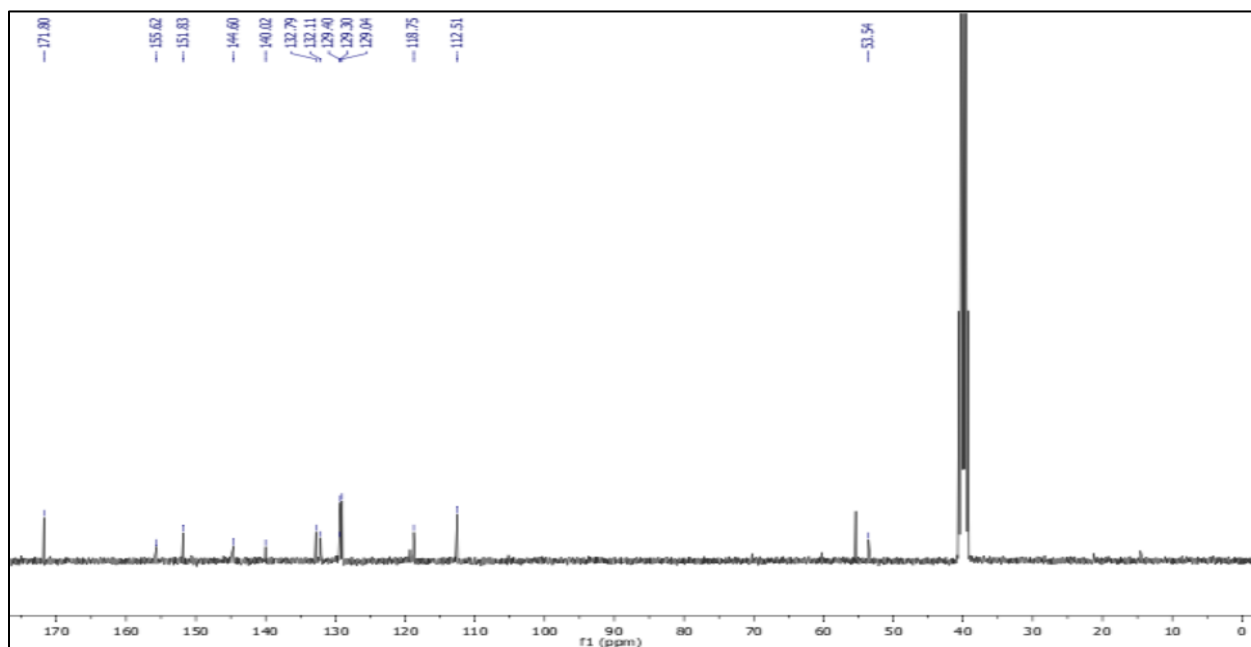


Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	rdb	N-Rule	e <sup>-</sup> Conf	mSigma
398.617366	1	C40H38BF2N5O6S2	398.617129	-0.2	-0.6	24.0	ok	even	2.6

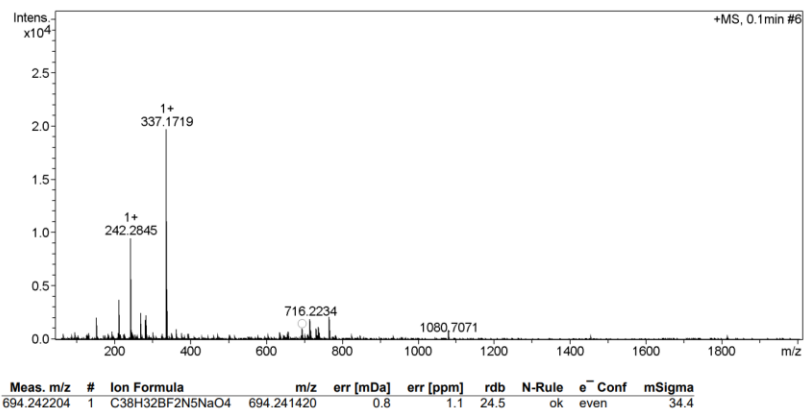
**Fig. S29.** HRMS ESI<sup>2-</sup> of **(6)**.



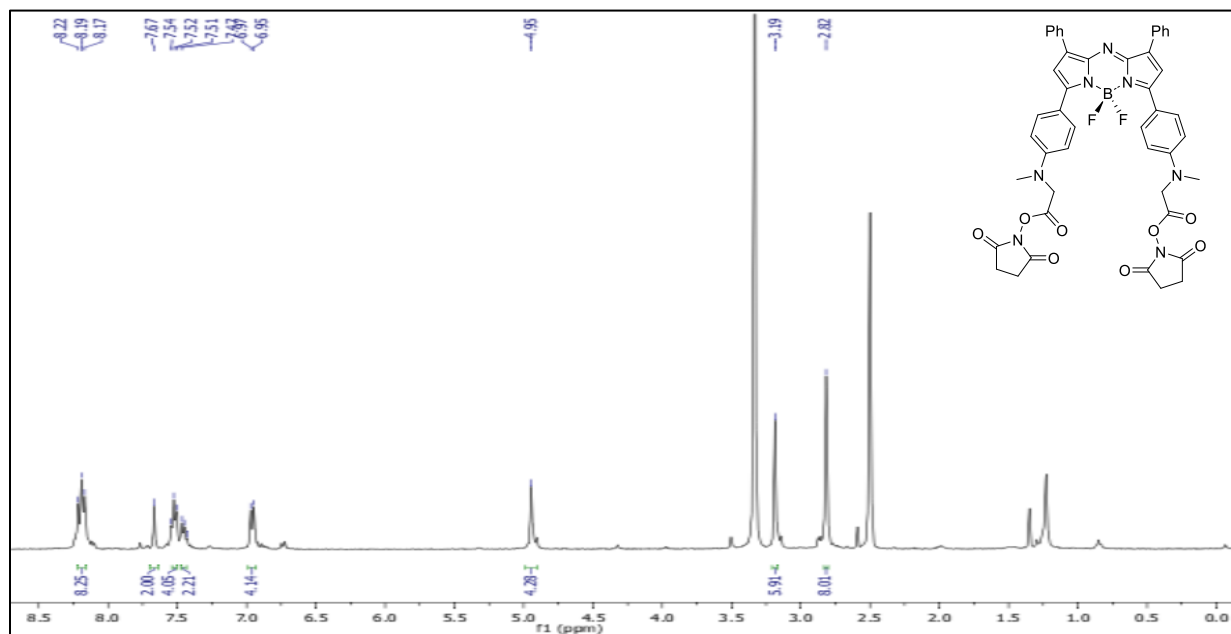
**Fig. S30.**  $^1\text{H}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for (12).



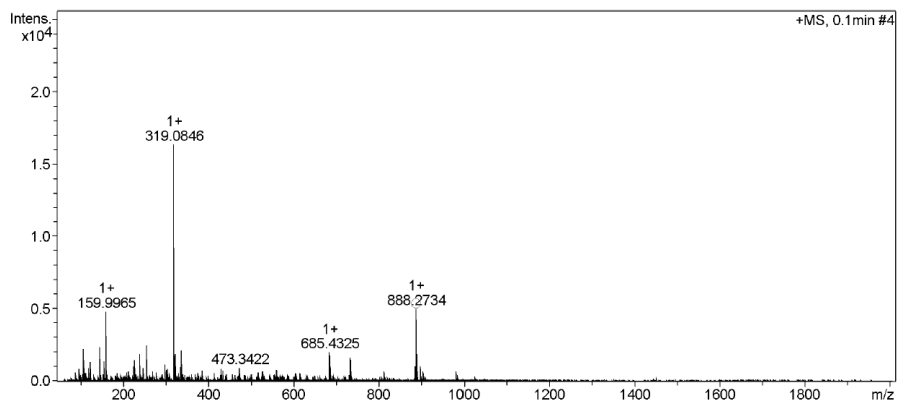
**Fig. S31.**  $^{13}\text{C}$  NMR ( $\text{DMSO-}d^6$ ) spectrum for (12).



**Fig. S32.** HRMS ESI<sup>+</sup> of (12).



**Fig. S33.** <sup>1</sup>H NMR (DMSO-*d*<sup>6</sup>) spectrum for (13).



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	rdb	N-Rule	e <sup>-</sup> Conf	mSigma
888.273364	1	C <sub>46</sub> H <sub>38</sub> BF <sub>2</sub> N <sub>7</sub> NaO <sub>8</sub>	888.274300	0.9	1.1	30.5	ok	even	32.8

Fig. S34. HRMS ESI<sup>+</sup> of (13).

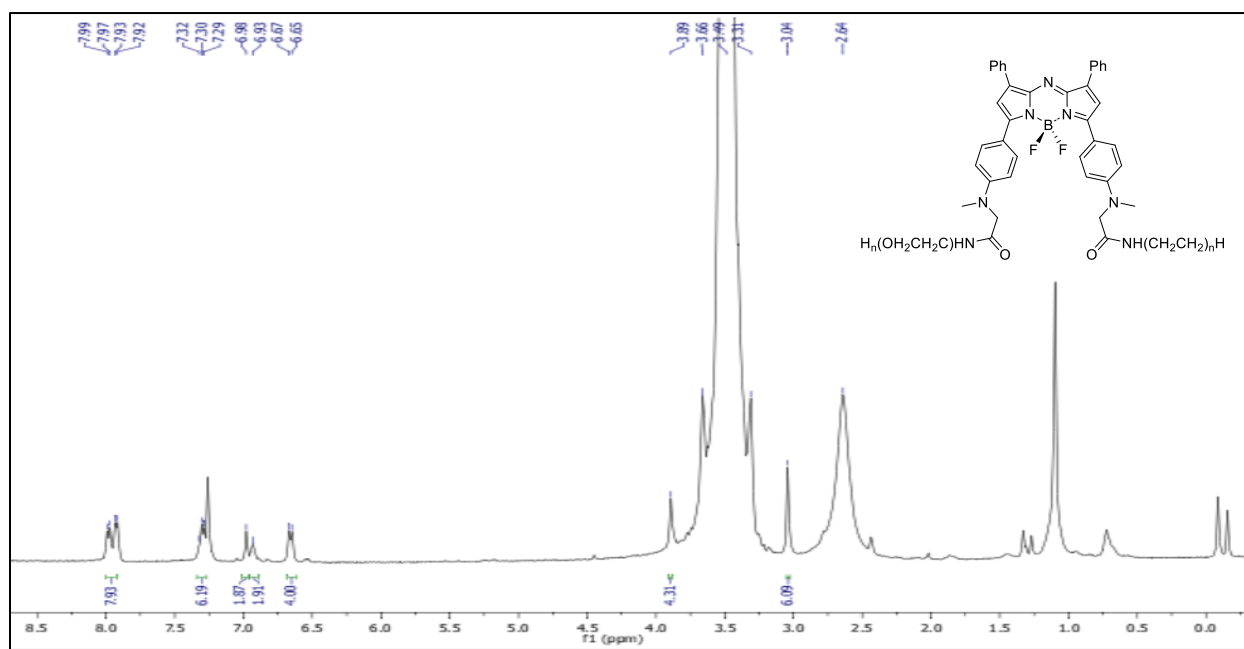
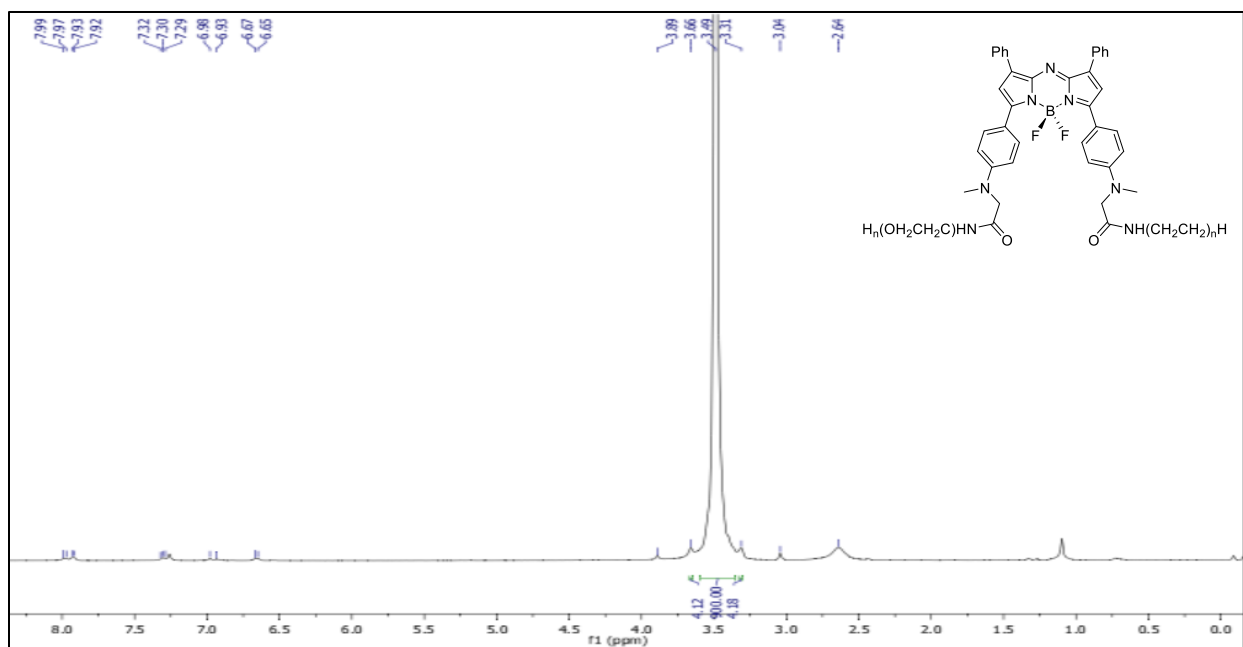
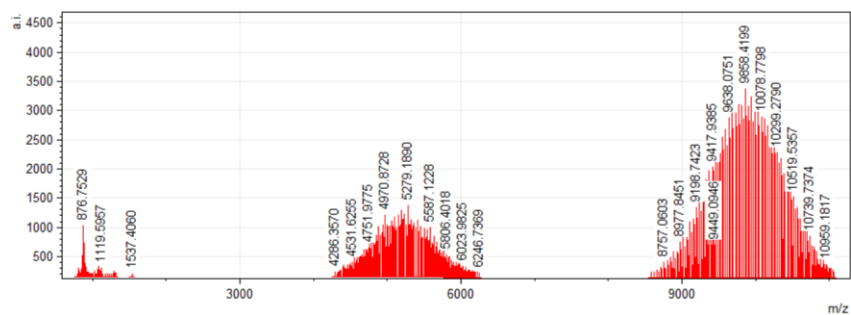


Fig. S35. <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum for (7).

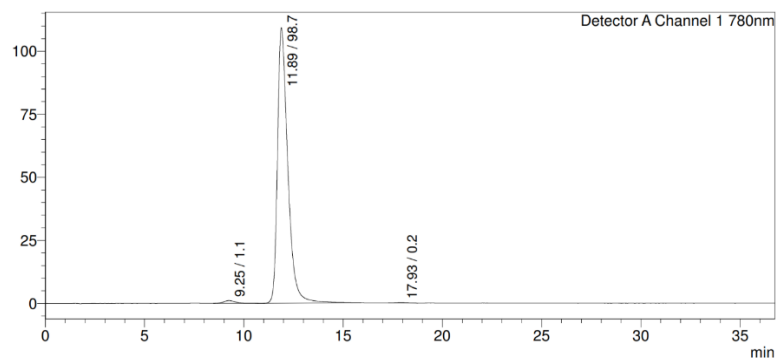


**Fig. S36.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum for (7).



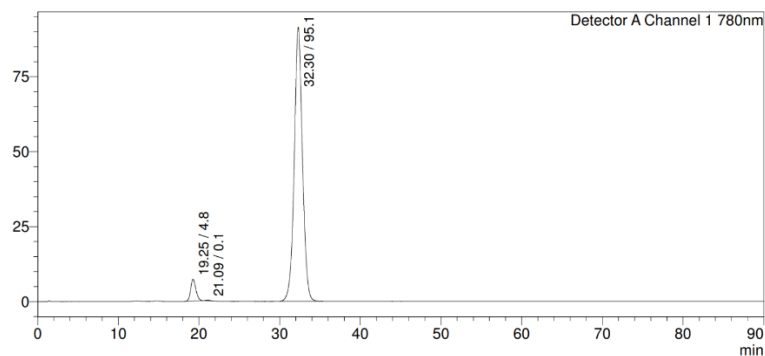
**Fig. S37.** MS MALDI-TOF of (7).

## HPLC traces of 11, 5, 6 and 7.

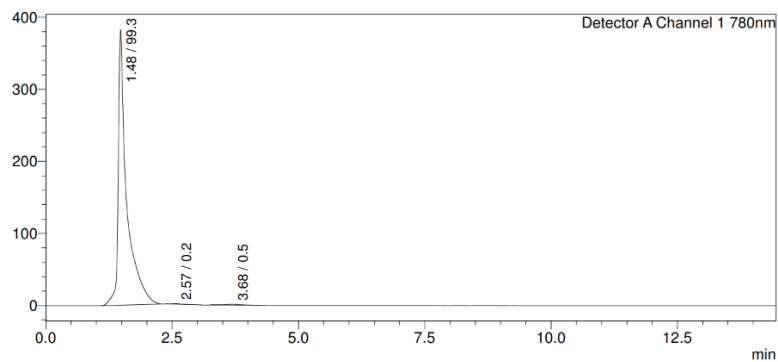


**Fig. S38.** HPLC Trace of **11**.

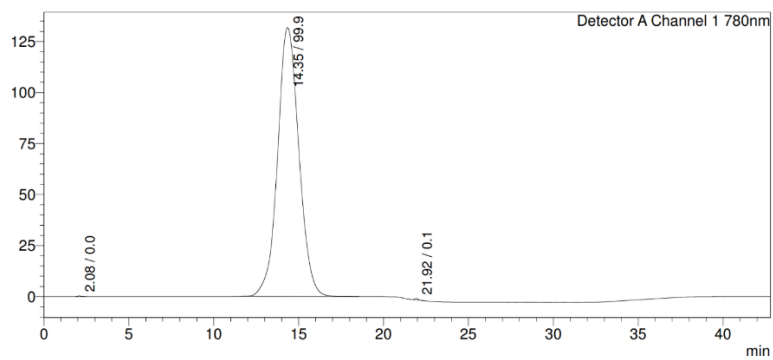
Conditions: Reverse phase-HPLC with YMC triart phenyl column and size:  $150 \times 4.6$  mm I.D., particle size: S- $5\mu\text{m}$ , 12 nm hole, detection method: UV-Vis and wavelength for detection: 780 nm. Eluent MeCN:H<sub>2</sub>O 70:30 with a flow rate at 1 mL/min.



**Fig. S39.** HPLC Trace of **5**. Conditions: As for **11** above. Eluent MeCN:H<sub>2</sub>O 70:30 with a flow rate at 1 mL/min.



**Fig. S40.** HPLC Trace of **6**. Conditions: Eluent MeCN:H<sub>2</sub>O 45:55 with a flow rate at 1 mL/min.



**Fig. S41.** HPLC Trace of **7**. Conditions: Eluent gradient of MeCN:H<sub>2</sub>O 40:60 going to CH<sub>3</sub>CN :H<sub>2</sub>O =70:30 with a flow rate at 1 mL/min.