Supporting Information

for

A Fluorescent Probe for Stimulated Emission Depletion Super-Resolution Imaging of Vicinal-Dithiol-Proteins on Mitochondrial Membrane

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Materials and methods for the synthesis.

All chemicals were purchased from the companies, such as Sigma-Aldrich, TCI and Aladdin (China) etc. The HeLa cells were bought from the American Type Culture Collection (ATCC) (VA, USA). All chemicals including cell culturing, MitoTracker Deep Red FM, ER-Tracker Red (BODIPY TR Glibenclamide) and LysoTracker Red DND-99 were supplied by Invitrogen (Oregon, USA). And methanol, dithiothreitol (DTT), 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) and Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) from Sigma–Aldrich were directly used as received without further purification. Amyloid β-Protein (1-42) was purchased from Bachem, Antibodis, Lamin B (H-90) and β-Actin (C4) were bought from Santa Cruz Biotechnology (Santa Crus, US). And anti-VDAC1 and VDAC2 were provided by Cambridge, UK (Abcam). Sequencing-grade trypsin was obtained from Promega (Wisconsin, US).

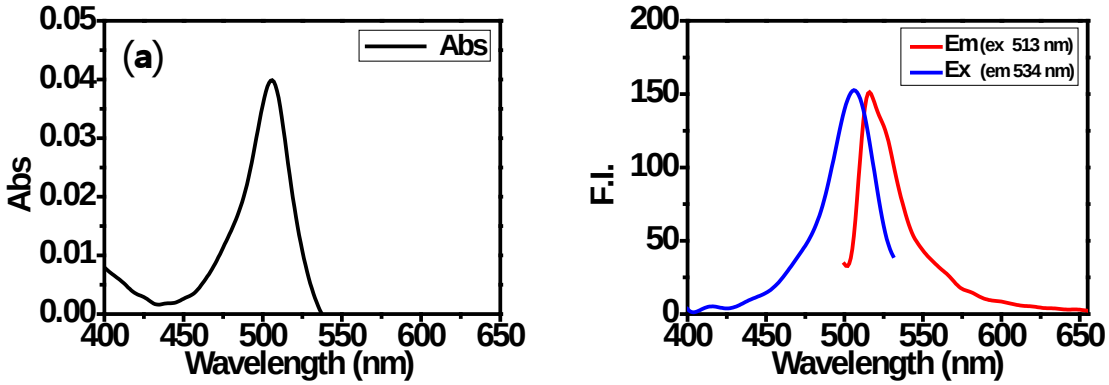


Figure S1. Absorption (a), fluorescence (red in b) and excitation (blue in b) spectra of **2 (**1 μM) in methanol. The emission was excited at 534 nm and the excitation spectrum was obtained with emission at 514 nm.

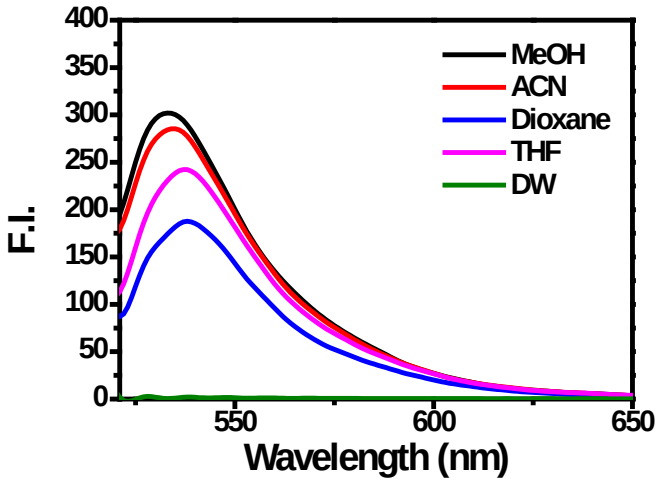


Figure S2. The emission spectra of **1** (1 μM) in different solvents such as acetonitrile (ACN), dioxane and de-ionized water (DW); Excitation wavelength was 514 nm.

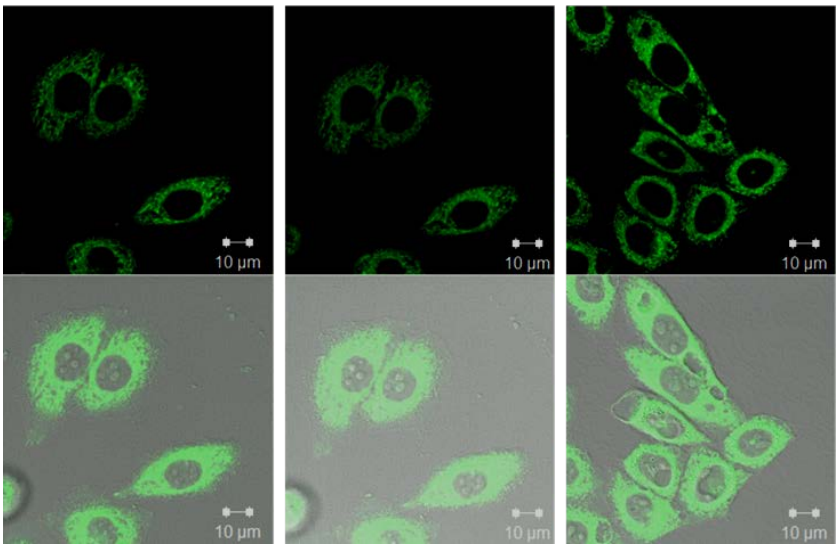


Figure S3. The confocal microscopic images of **1**-treated HeLa cells before and after washing with PBS and 4% formaldehyde; the cells were treated with Probe **1** (1 µM) for 1 h at 37 °C. After 1×PBS washing, the cells were treated with 4% formaldehyde for 30 min at -20 °C. The images were obtained at 505 nm using long pass emission filters upon excitation at 488 nm.

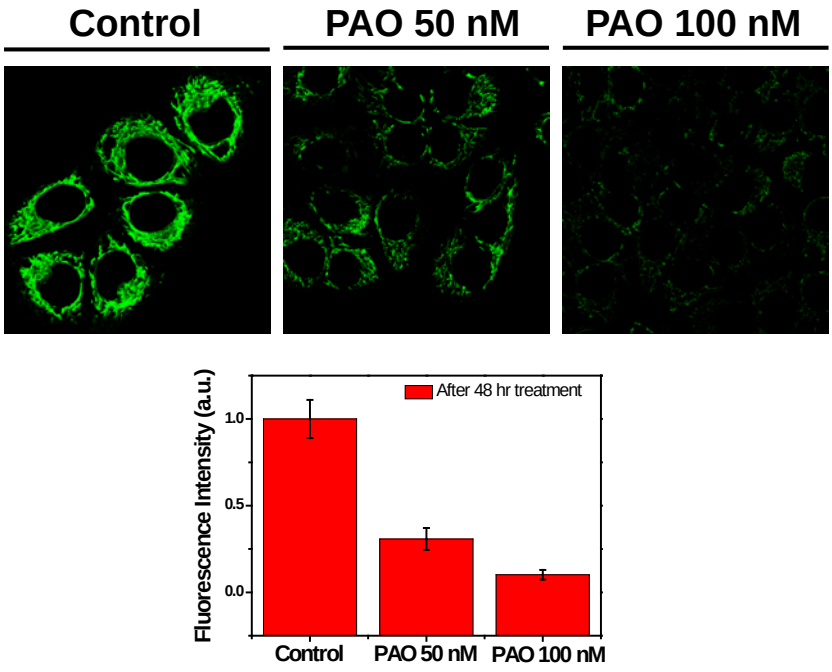


Figure S4. Fluorescence images of HeLa cells treated with Probe **1** (1 µM) after 48 h the post treatment with PAO. The images were obtained following 505 nm long pass emission filter upon 488 nm excitation.

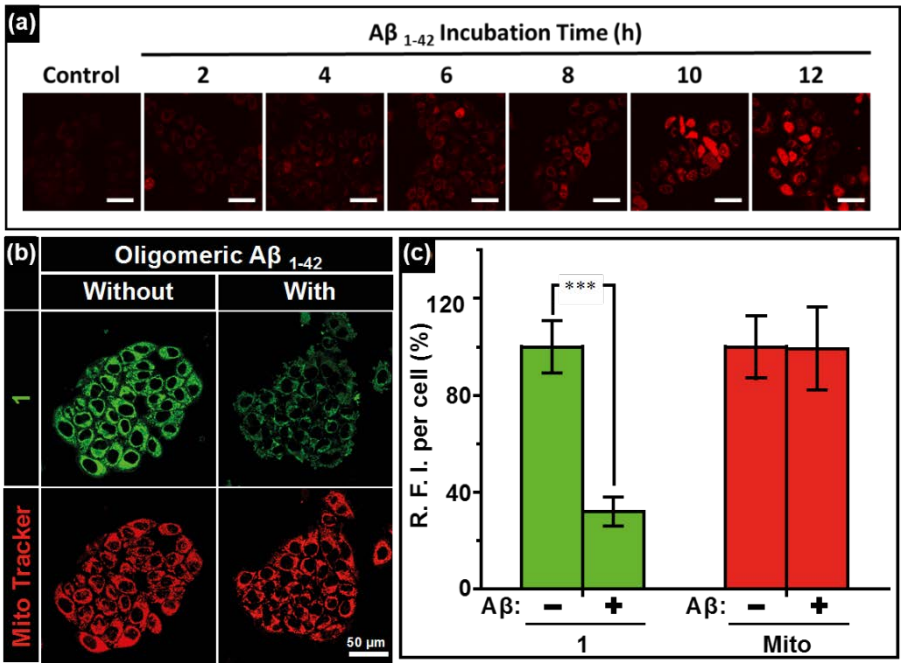


Figure S5. Confocal microscopic images of HeLa cells co-labelled with oligomeric $A\beta_{1-42}$ and (a mitochondrial damaging agent) MitoSOX (red emissive mitochondrial ROS indicator) as function of incubation time. Cells were seeded at 35 mm confocal dish. Cells were pretreated with oligomeric $A\beta_{1-42}$ (10.0 µM) for 2, 4, 6, 8, 10 and 12 h in incubator (37 °C, 5% CO_2). After each time of incubation, cells were co-labelled with MitoSOX (2.0 µM) in 1×PBS for 20 min at 37 °C in dark. The fluorescent confocal images were collected using an excitation wavelength of 488 nm and a long-path 530 nm emission filter and compared with oligomeric $A\beta_{1-42}$ untreated control cells. Scale bar indicate 50 µm. (b) Fluorescent images of HeLa cells were obtained before and after 1×PBS washing followed by ethanol (1:1 ratio) and three rounds of 4% formaldehyde solution; (c) Histogram of relative fluorescence intensity (R. F. I.) per cell was represented using image J program. Results represent the mean (± SEM) of three independent experiments (n = 3). The statistical signification was marked as *** for $p < 0.001$, compared with each of the fluorescence intensity before washing.

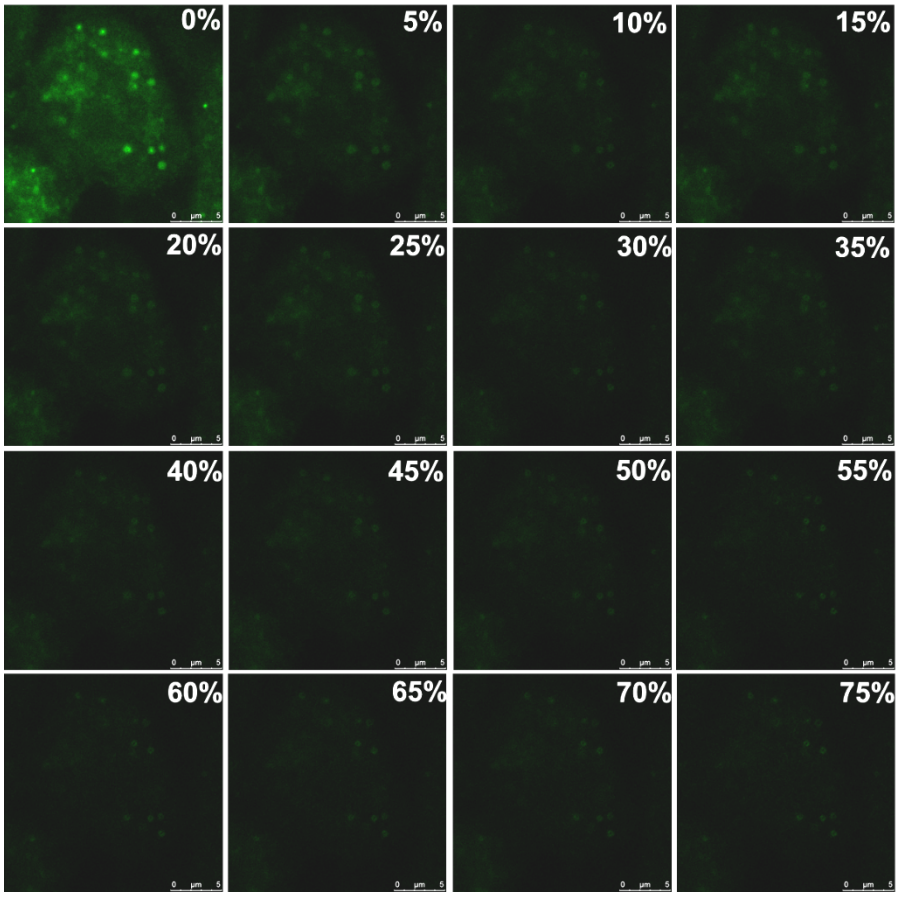


Figure S6. Fluorescent imaging of **1** (2 μM) in HeLa cells under the illumination of different STED laser power, excitation wavelength was 514 nm and STED laser wavelength was 592 nm, using a Leica SP8 suite microscopy system.

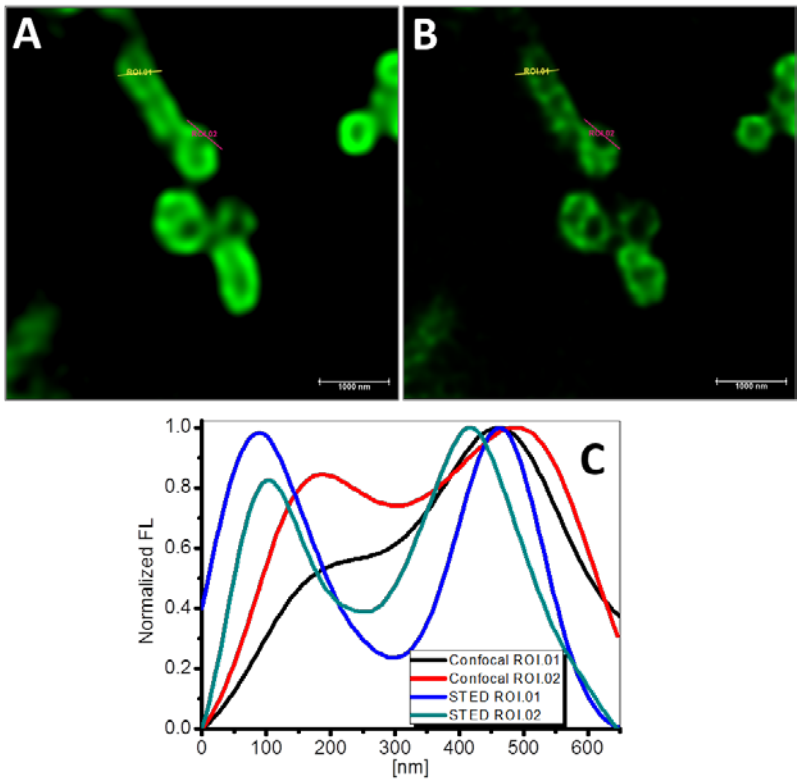


Figure S7. Magnified fluorescent imaging of mitochondria labelled by **1** (2 μM), A) Confocal imaging; B) STED super resolution imaging; C) Line traces through the single mitochondrial segment show the markedly improved resolving power of STED super resolution imaging. The excitation wavelength was 514 nm, and STED wavelength was 592 nm with 10% of the CW laser power.

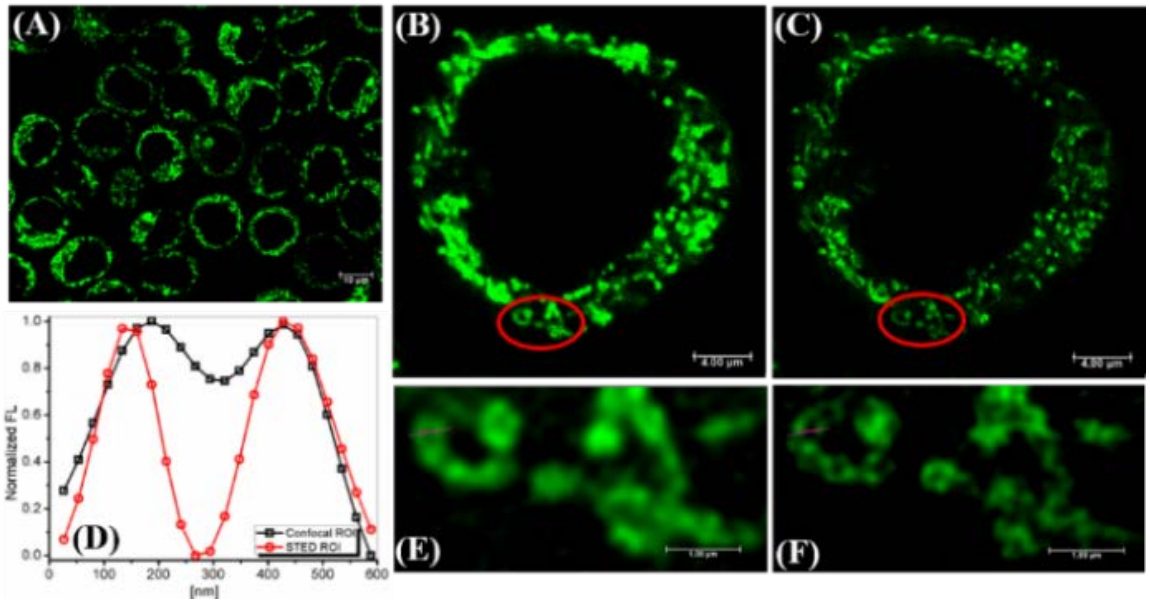
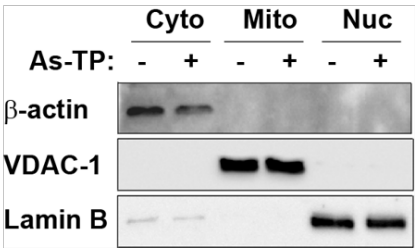
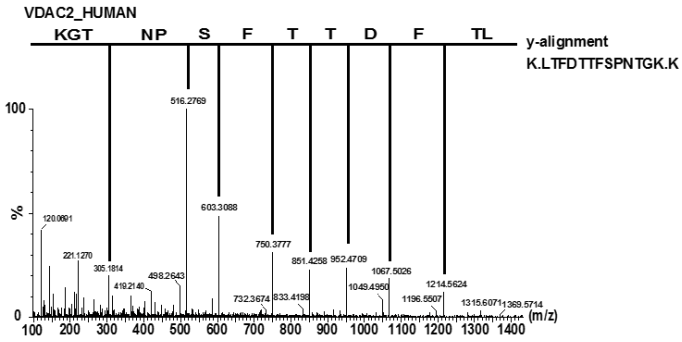


Figure S8. STED super resolution imaging of **1** (1 μM) in HeLa cells; (A) confocal fluorescence imaging; (B) confocal fluorescent imaging of a single cell; (C) STED super resolution imaging of a single HeLa cell; (D) Line traces through the single mitochondrial segment show the markedly improved resolving power of STED (Red); (E), (F) magnified images of confocal and STED super resolution fluorescent imaging in the ROI regions of B and C, respectively;



Protein name	UniProt assession No.	MW(Da)	Coverage(%)	Mascot score
Voltage-dependent anion-selective channel protein 2 (VDAC2)	P45880	38069	24	204



Protein name	UniProt assession No.	MW(Da)	Coverage(%)	Mascot score
Elongation factor Tu, mitochondrial precursor	P49411	49510	21	233
Protein disulfide-isomerase A6 precursor	Q15084	48091	20	220
Nuclease sensitive element-binding protein 1	P67809	35903	32	207
Trifunctional enzyme subunit beta, mitochondrial precursor	P55084	51262	20	193
Fumarate hydratase, mitochondrial precursor	P07954	54602	19	157
Citrate synthase, mitochondrial precursor	O75390	51680	9	133
Eukaryotic translation initiation factor 3 subunit 4	O75821	35589	12	124
Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial precursor	P22695	48413	13	115
DnaJ homolog subfamily A member 1	P31689	44839	14	112
26S protease regulatory subunit 7	P35998	48603	27	107
Nuclear migration protein nudC	Q9Y266	38219	26	102

Figure S9. Western blots analysis for confirming the purity of organelle fractions using organelle-selective makers. The markers for cytosol, mitochondria and nucleus are β-actin, VDAC1 and lamin B, respectively; MS/MS spectra of $[M+2H]^{2+}$ ions of the peptides derived from the protein band corresponding to VDAC2 are shown.

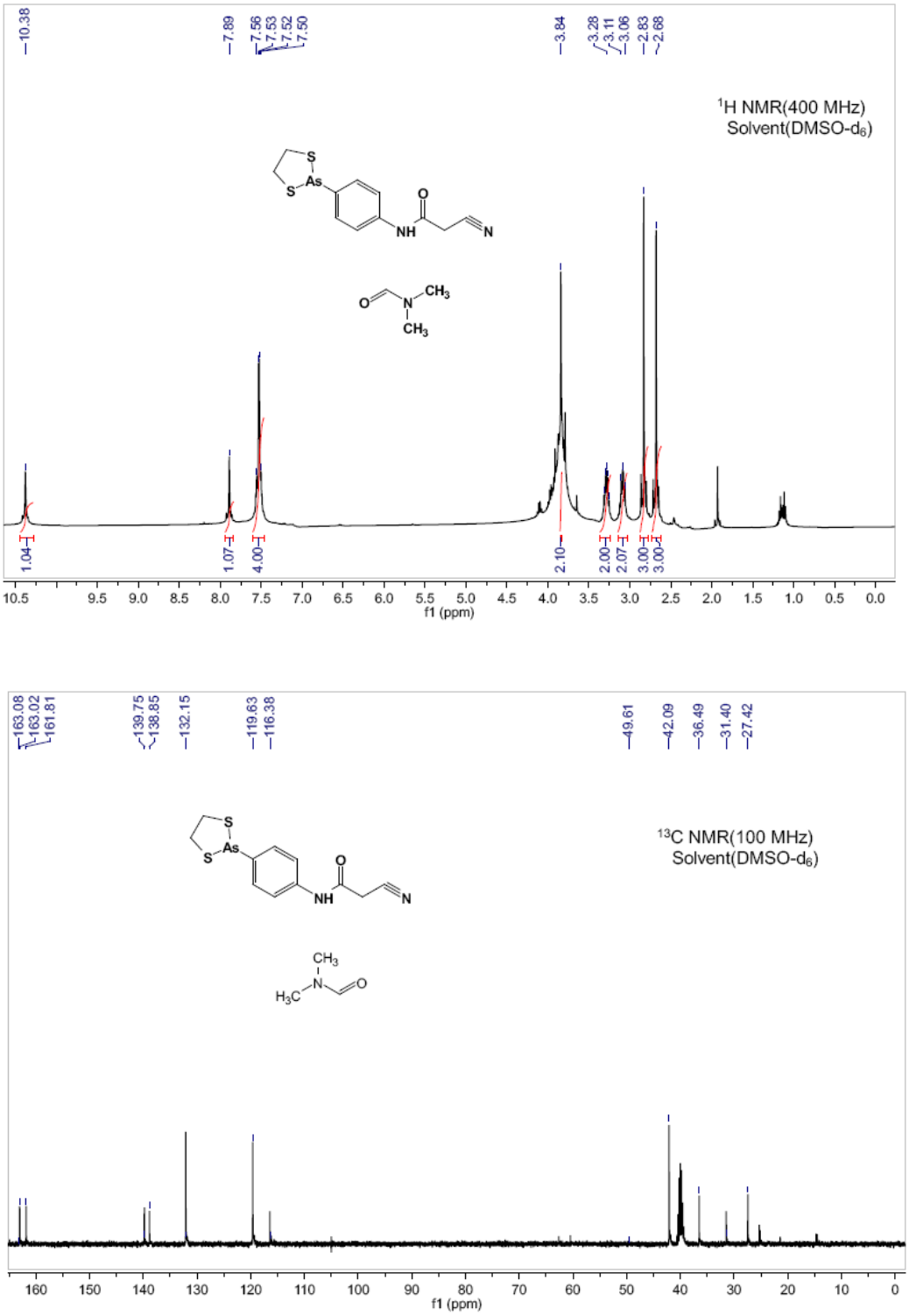


Figure S10. ^{1}H-^{13}C NMR of **8**

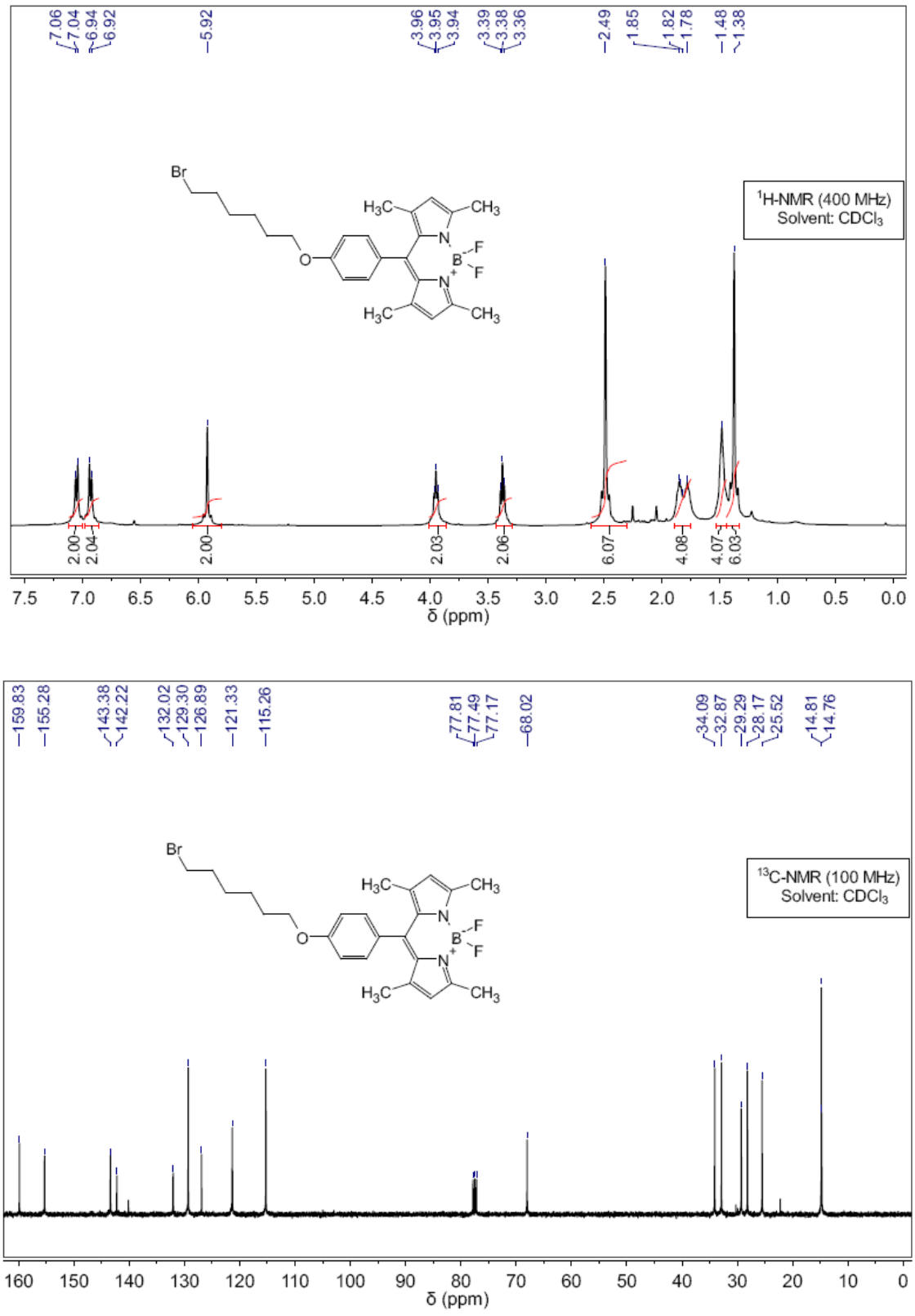
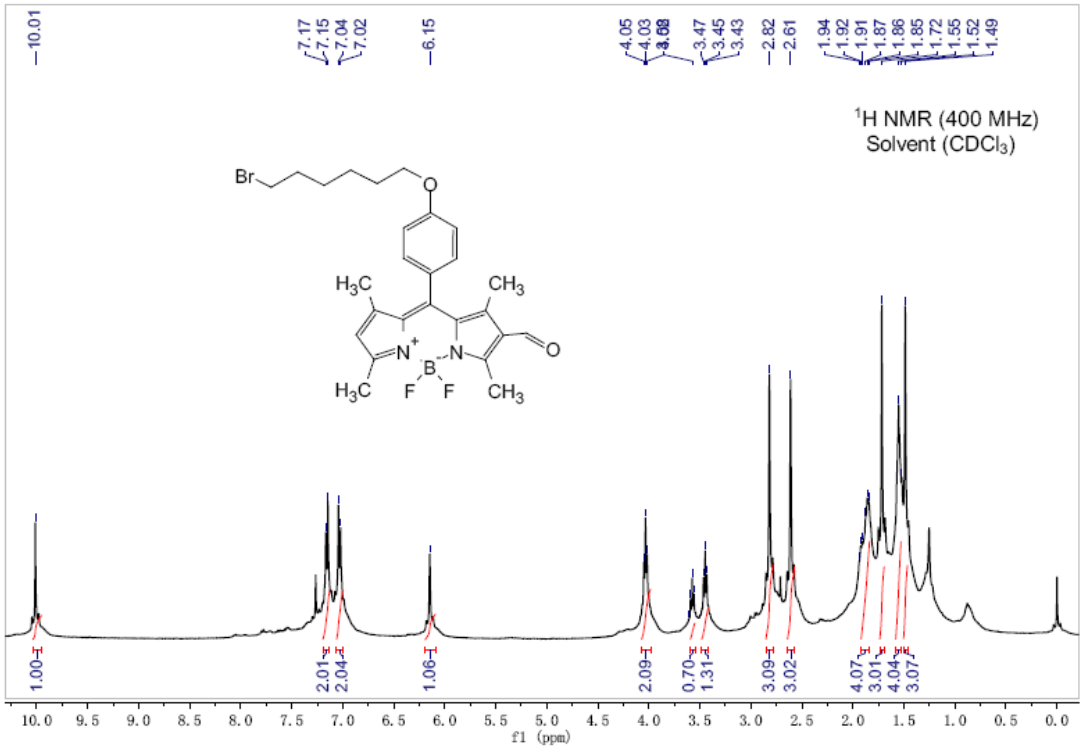


Figure S11. ^{1}H-^{13}C NMR of **5**



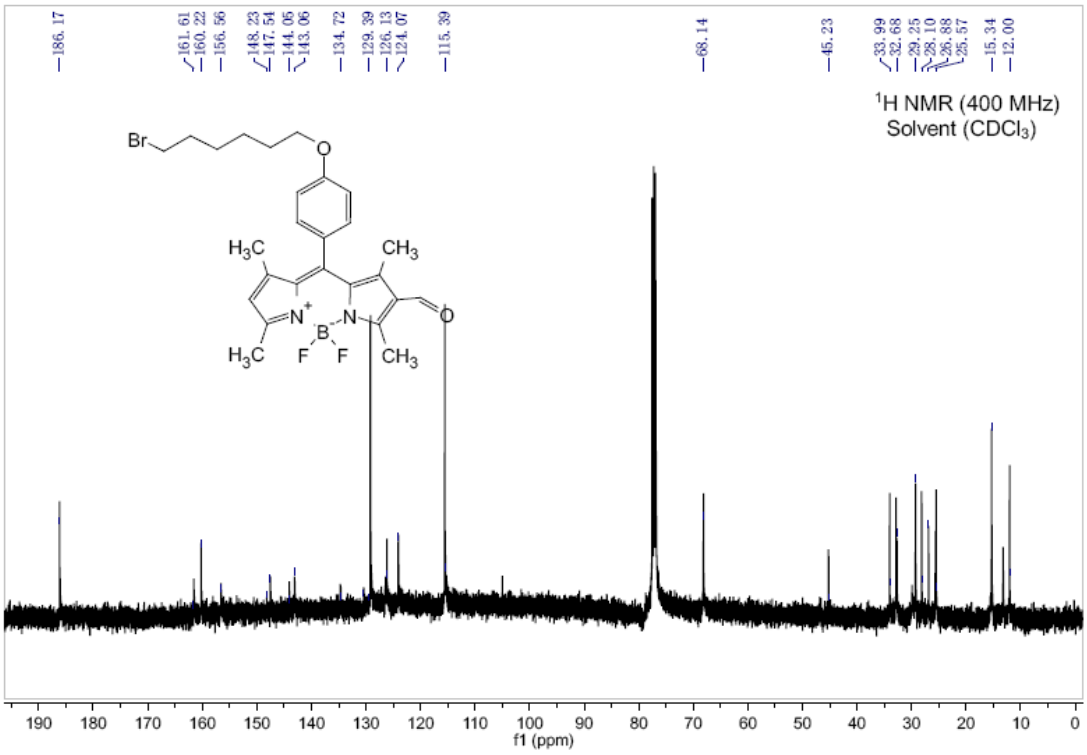


Figure S12. ^{1}H-^{13}C NMR of **4**

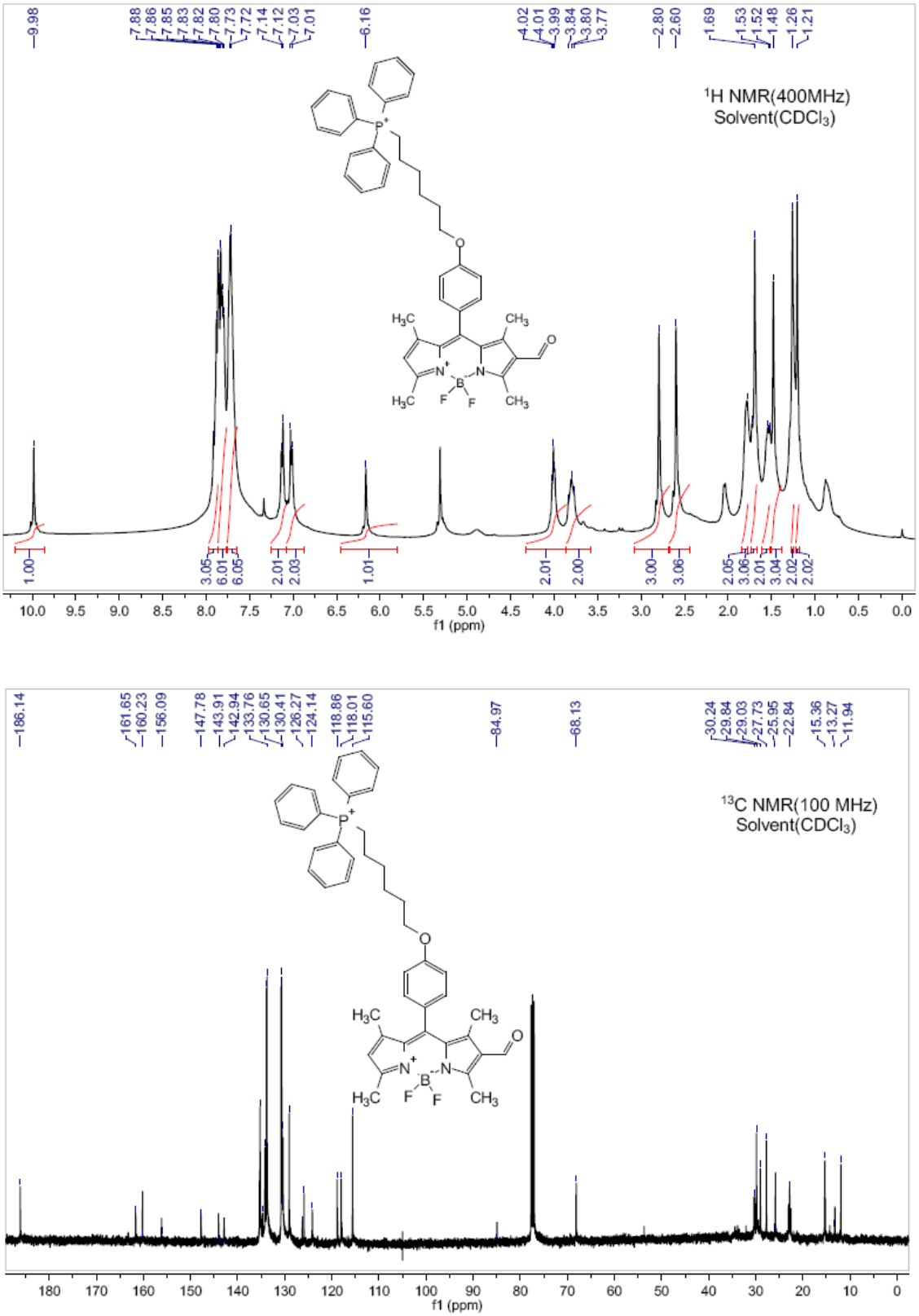
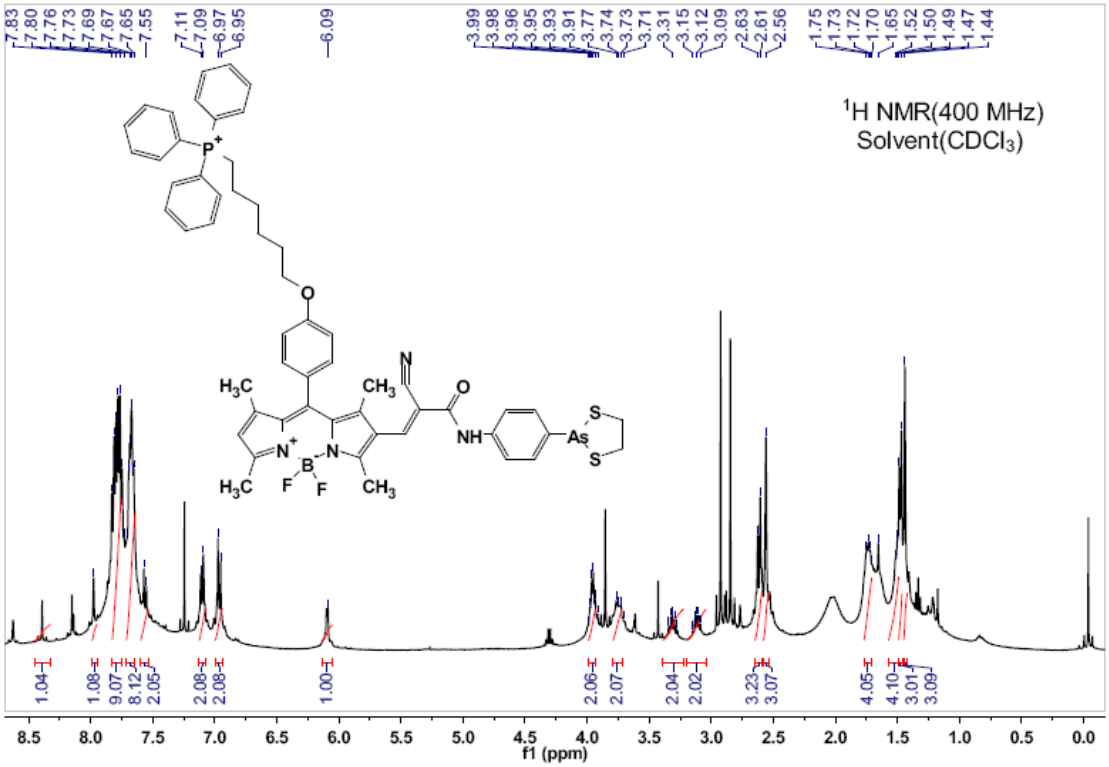
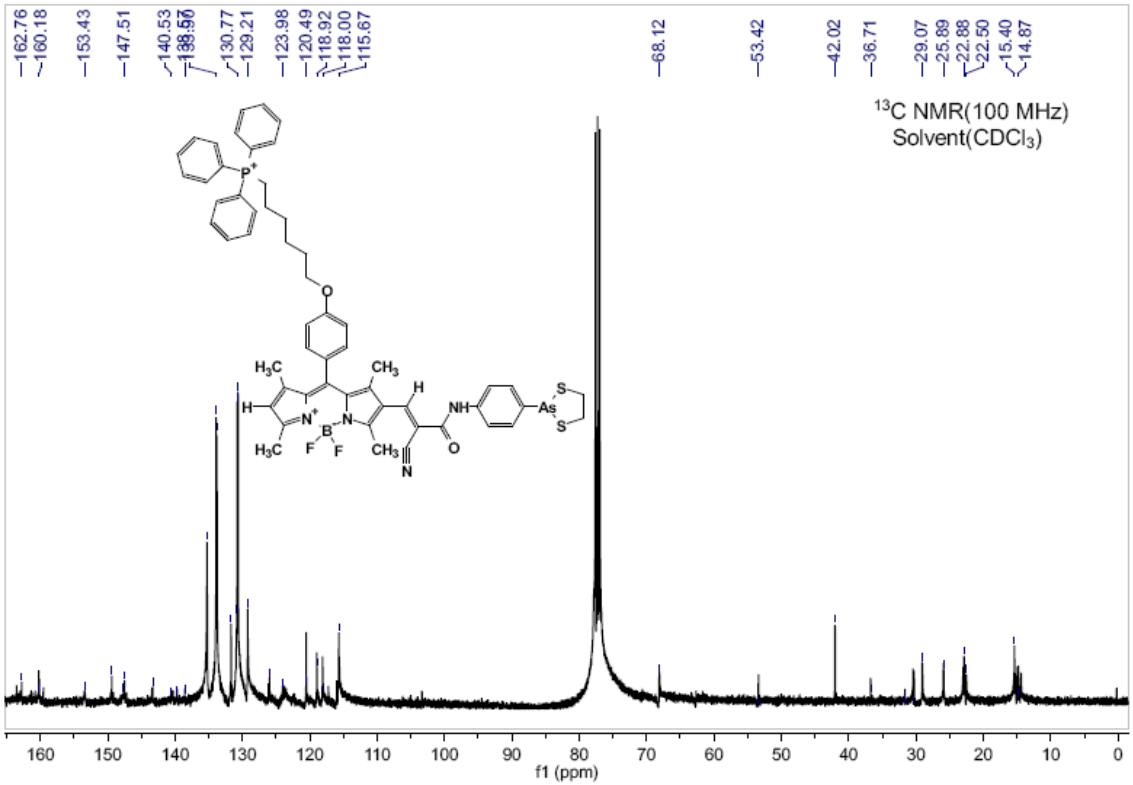


Figure S13. ^{1}H-^{13}C NMR of **3**


^{1}H NMR(400 MHz)
Solvent($CDCl_3$)
f1 (ppm)


^{13}C NMR(100 MHz)
Solvent($CDCl_3$)
f1 (ppm)

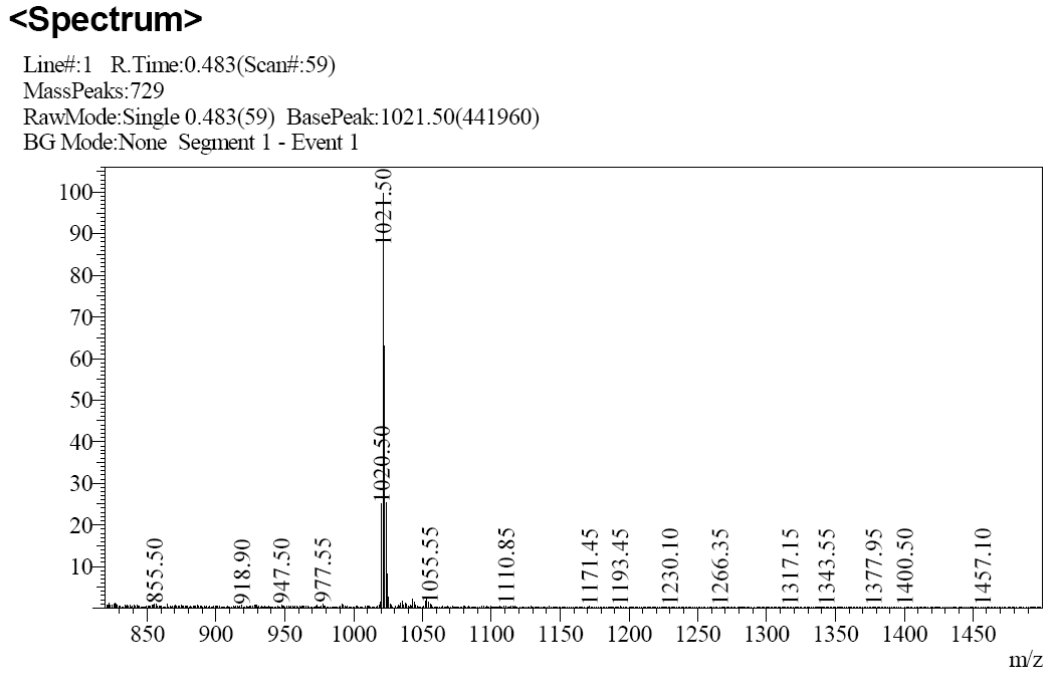


Figure S14. ^{1}H-^{13}C NMR and Mass spectra of **1**

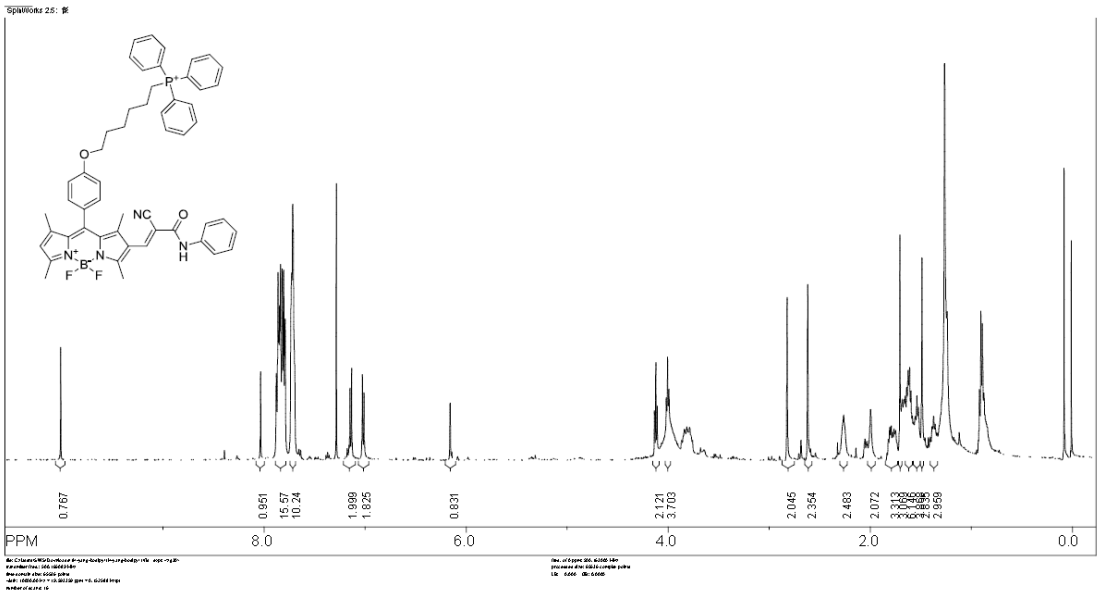


Figure S15. ^{1}H NMR spectra of reference compound **2**