Supporting information

# An organelle-directed Staudinger reaction enabling fluorescence-on resolution of mitochondrial electropotentials via a self-immolative charge reversal probe

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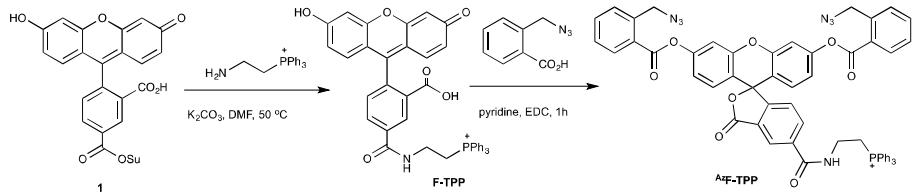
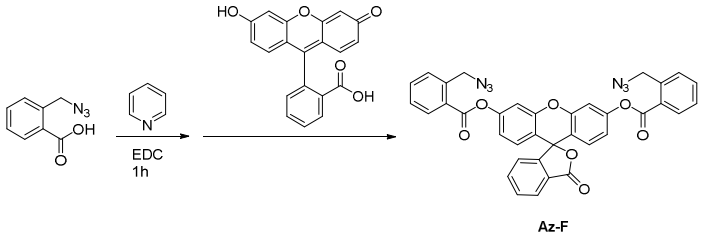
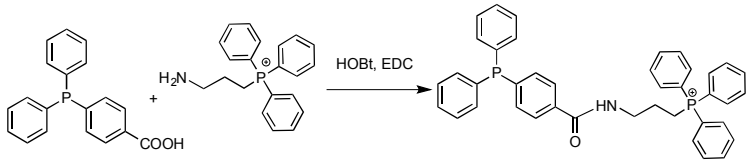
# Content




**Scheme S1.** Synthesis of F-TPP and $^{Az}$F-TPP




**Scheme S2.** Synthesis of F-TPP and $^{Az}$F




**Scheme S3.** Synthesis of $PPh_3$-TPP

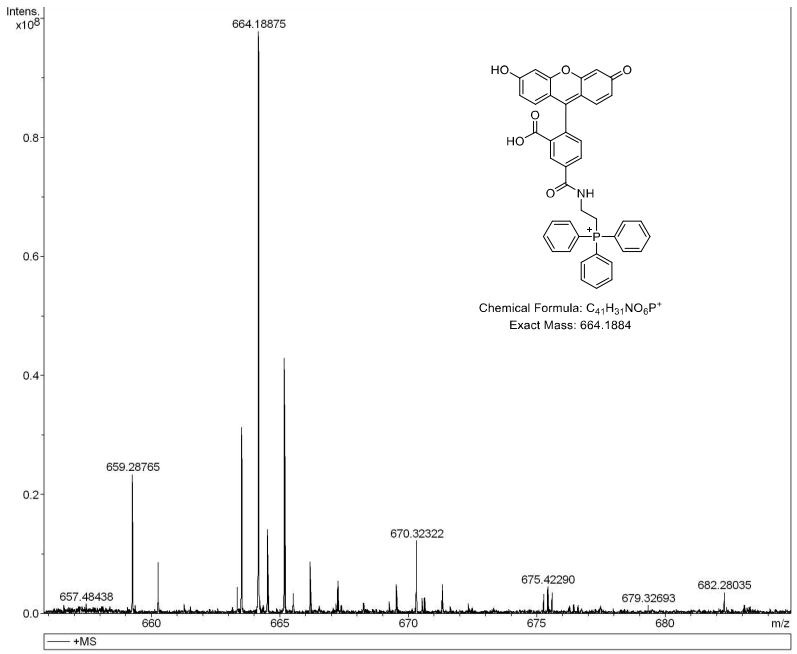



**Figure S1.** HRMS confirms genesis of F-TPP by Staudinger reduction of $^{Az}$F-TPP.

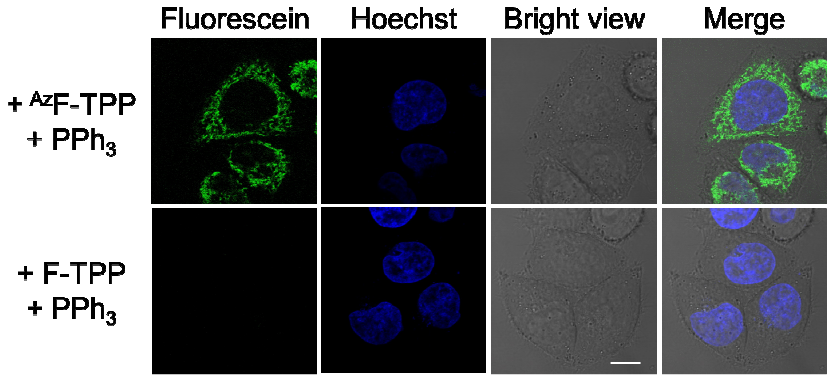



**Figure S2.** Incapability of mitochondria to uptake F-TPP. HeLa cells were respectively cultivated with F-TPP or $^{Az}$F-TPP in the presence of $PPh_3$ and then analysed by confocal fluorescence microscopy. Bar, 10 μm.

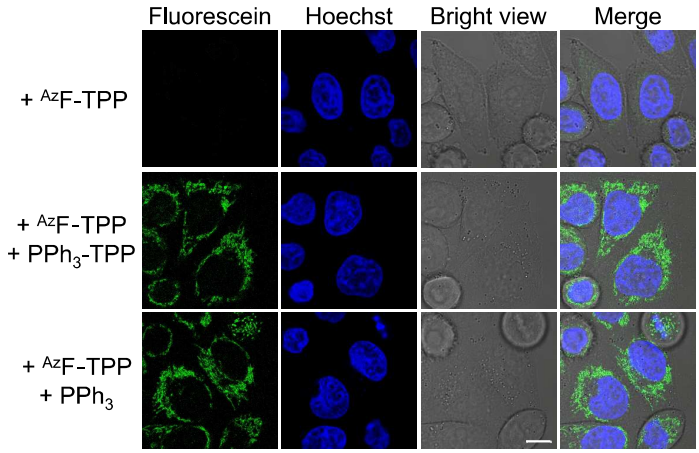



**Figure S3.** Phosphine dependent staining of mitochondria. HeLa cells were respectively cultivated with $^{Az}$F-TPP (5 μM) together with $PPh_3$ (8 μM), $PPh_3$-TPP (8 μM), or no addtion for 1 h and then analysed by confocal fluorescence microscopy. Bar, 10 μm.

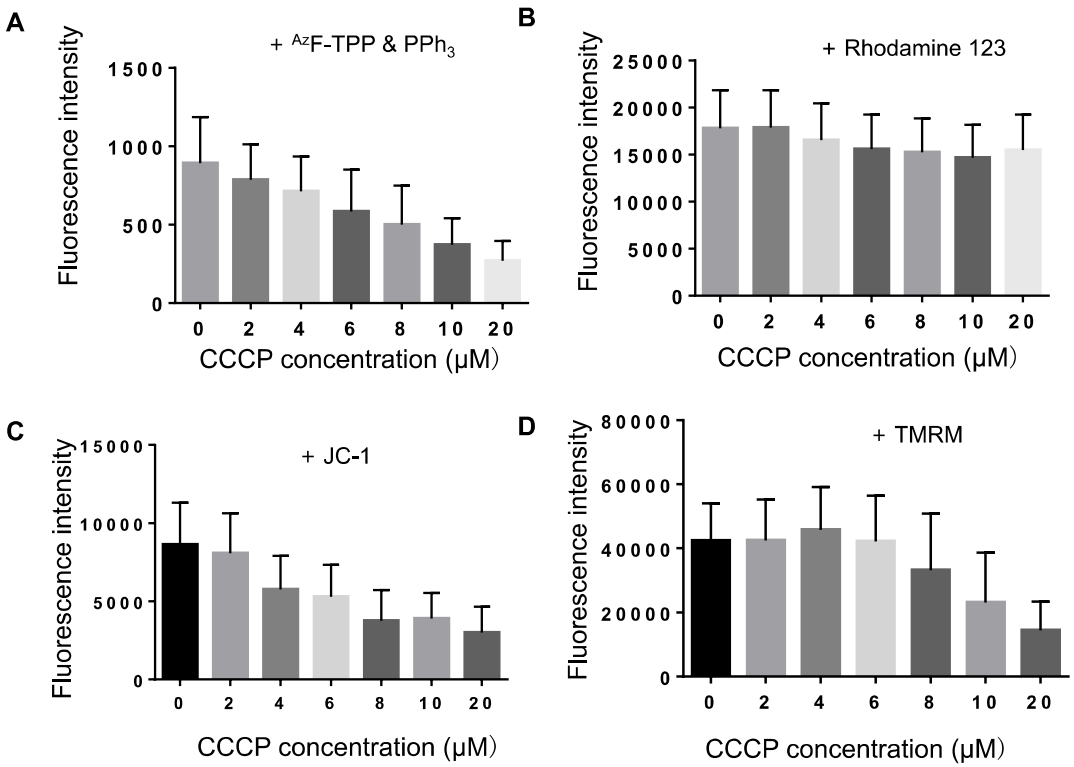



**Figure S4.** CCCP-dose dependent ΔΨm changes detected by intra-mitochondrial Staudinger reaction. HeLa cells were cultured with various doses of CCCP and then stained with $^{Az}$F-TPP/$PPh_3$ (A), rhodamine 123 (B), JC-1 (C) or TMRM (D), and then anaylyzed by flow cytometry for intracellular fluorescence. Error bars represent standard deviation of 10000 cells.

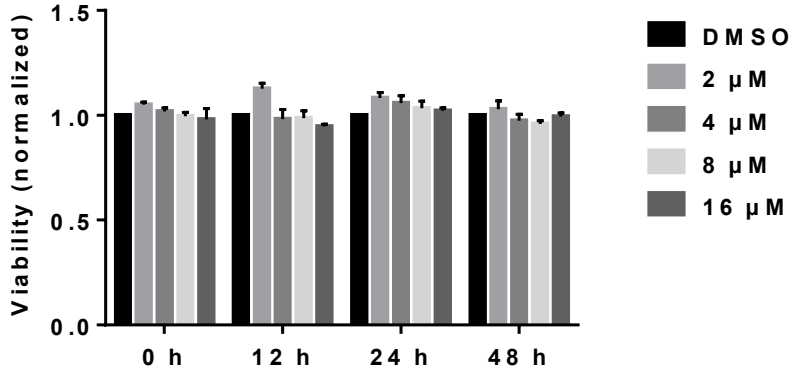



**Figure S5.** Cytotoxicity of $PPh_3$ on $^{Az}$F-TPP treated cells. HeLa cells were incubated with $^{Az}$F-TPP and various doses of $PPh_3$ and then cells were incubated for 0, 12, 24, and 48 h. The cell viability were determined by MTT assay.

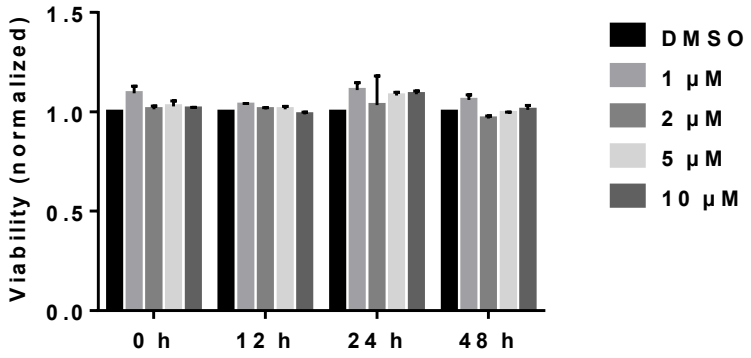



**Figure S6.** Cytotoxicity of $^{Az}$F-TPP on $PPh_3$-treated cells. HeLa cells were incubated with $PPh_3$ and various doses of $^{Az}$F-TPP and then cells were incubated for 0, 12, 24, and 48 h. The cell viability were determined by MTT assay.

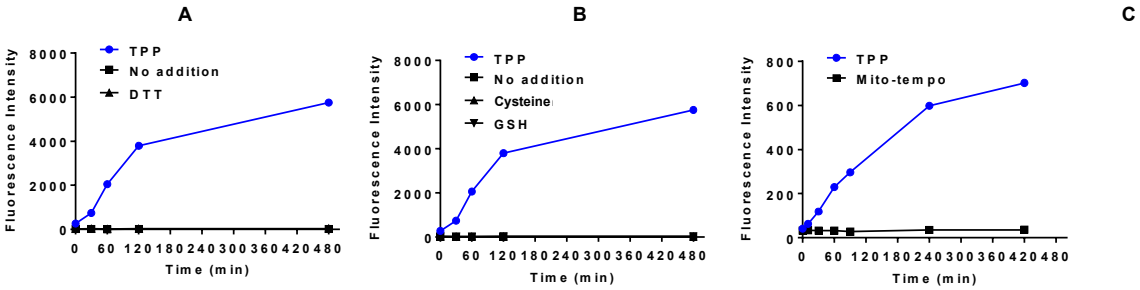



**Figure S7.** Selectivity of $^{Az}$F-TPP towards $PPh_3$ over biological reductants. To $^{Az}$F-TPP (10 mM) in Tris-HCl buffer (pH 8, 100 mM, 40% DMF) were added dithiothreitol (DTT) (25 mM) (A), cysteine (25 mM), GSH (25 mM), Mito-Tempo (0.6 mM), $PPh_3$ (0.6 mM) or no addition. Fluorescence emission of the the samples (Ex: 515 nm) were monitored and recorded as a function of incubation time.

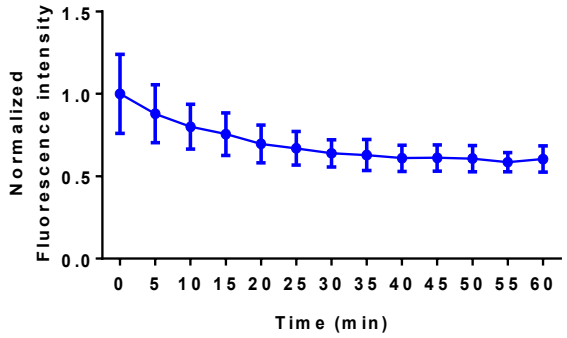



**Figure S8.** Photophysical properties of F-TPP in HeLa cells. Cells preincubated with $^{Az}$F-TPP/$PPh_3$ were constantly illuminated and scaned for 12 times with a confocal fluorescence microscope (Ex@488 nm). The fluorescence of cells were calculated by ImagingJ and normalized. Error bars represent standard deviation of 10 cells.

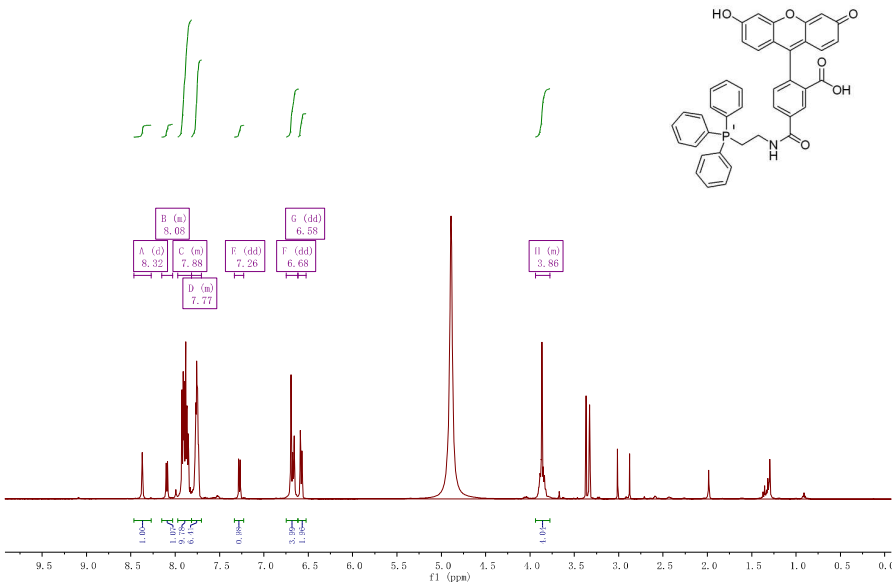



**Figure S9.** $^{1}$H-NMR of F-TPP

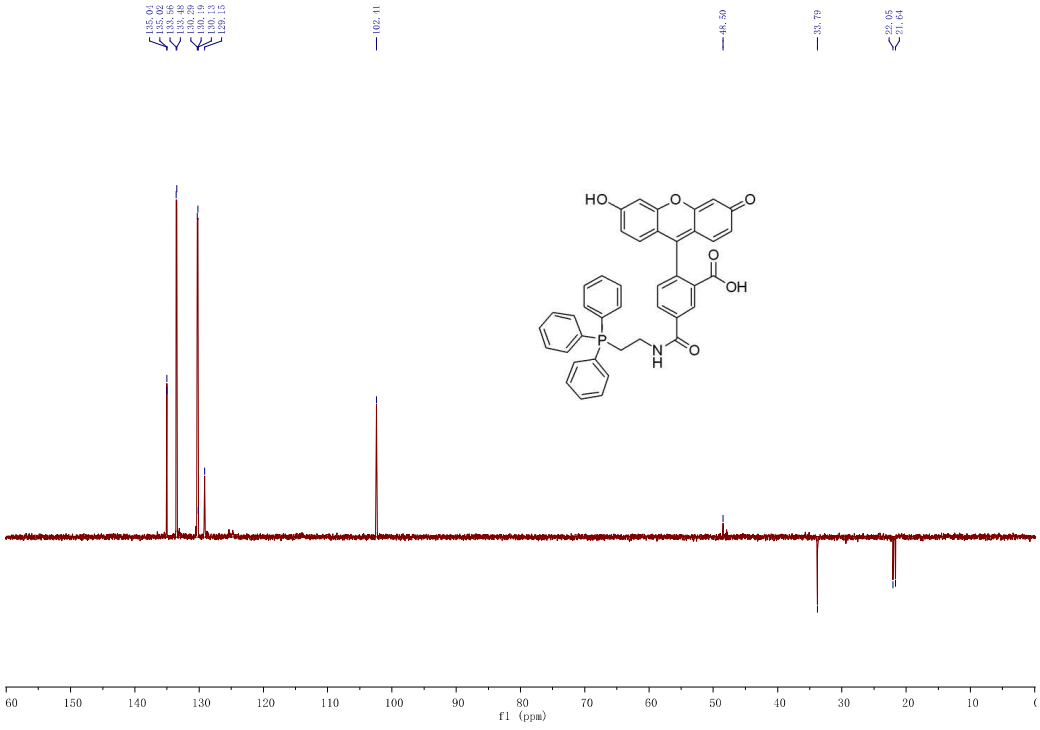



**Figure S10.** $^{13}$C-DEPT NMR of F-TPP

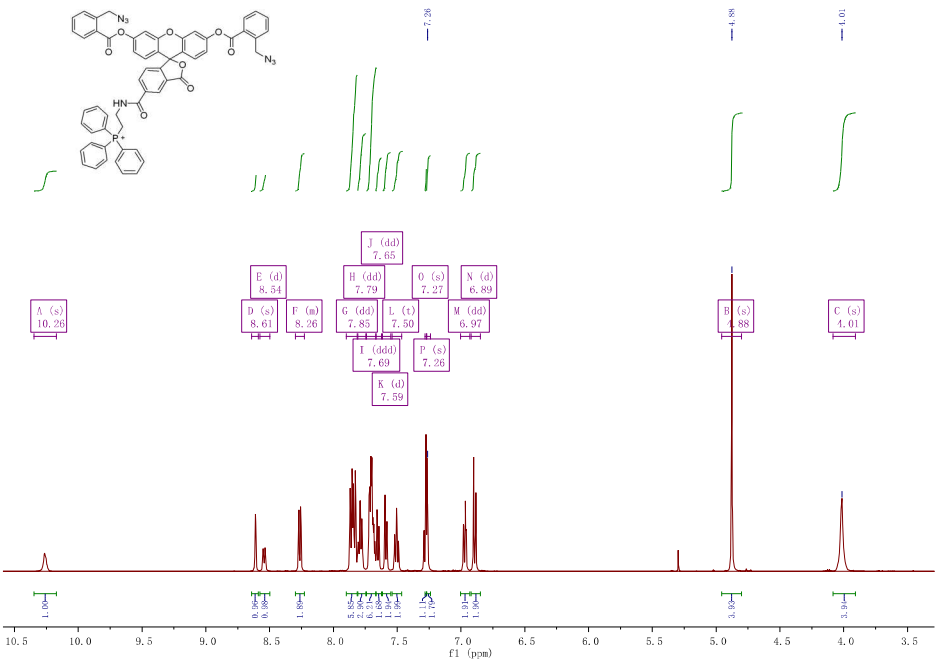



**Figure S11.** $^{1}$H-NMR of $^{Az}$F-TPP

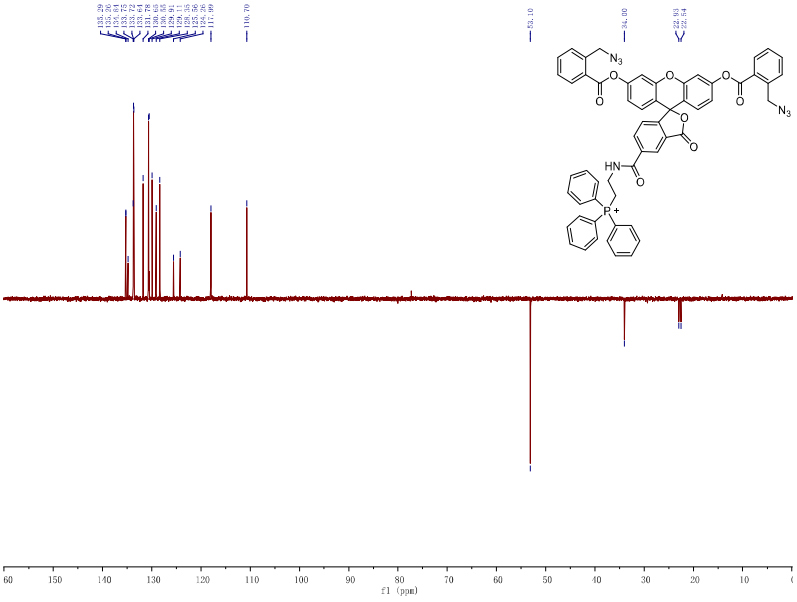



**Figure S12.** $^{13}$C-DEPT NMR of $^{Az}$F-TPP

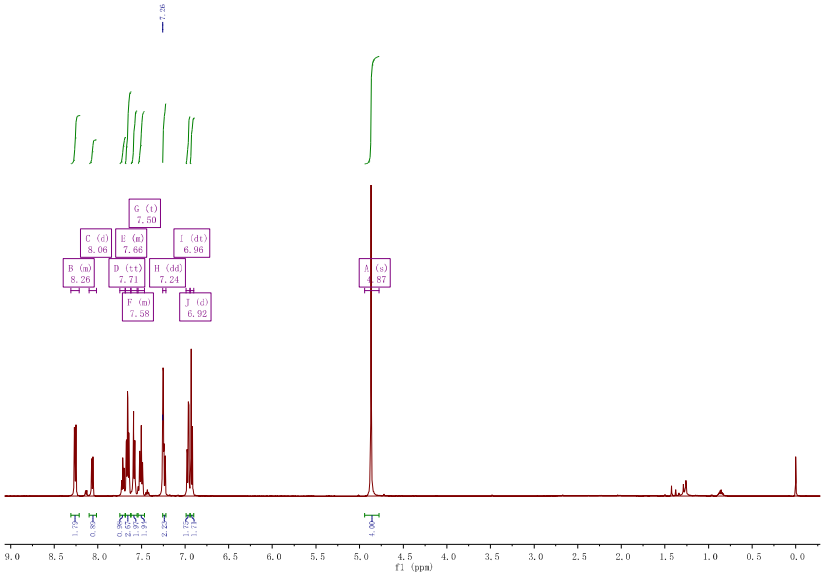



**Figure S13.** $^{1}$H-NMR of $^{Az}$F

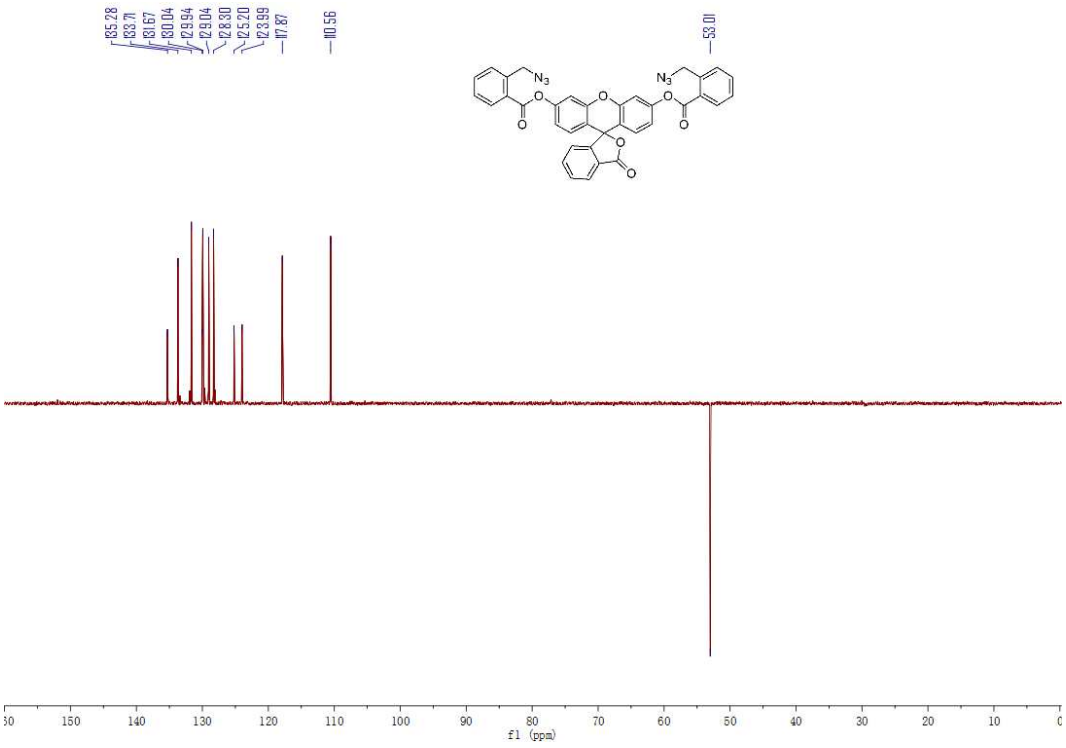



**Figure S14.** $^{13}$C-DEPT NMR of $^{Az}$F

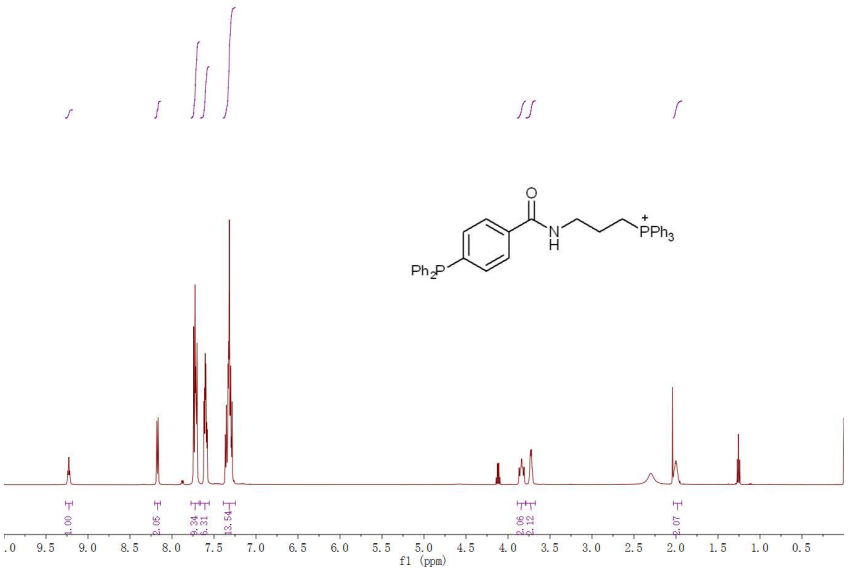



**Figure S15.** $^{1}$H-NMR of $PPh_3$-TPP

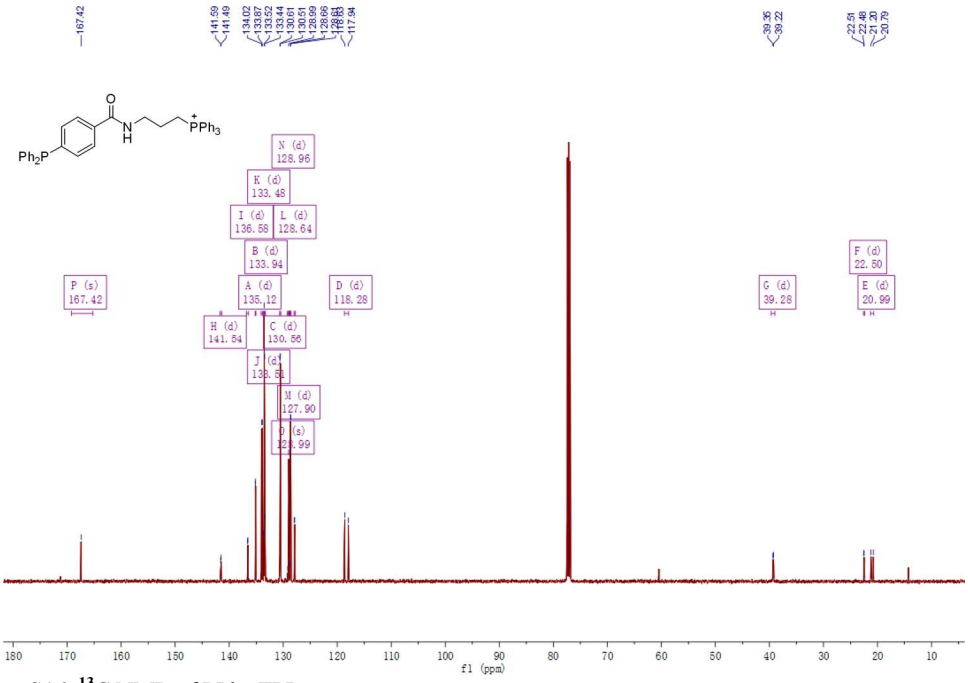



**Figure S16.** $^{13}$C NMR of $PPh_3$-TPP