Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Biscarbazolylmethane-Based Cyanine: A Two-Photon Excited Fluorescent Probe for DNA and Selective Cell Imaging

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The quadratic dependence during TPA cross section measurement: To avoid any contribution from other photophysical or photochemical processes，the intensity of input pulses was adjusted to a proper regime to ensure a quadratic dependence of the fluorescence intensity versus excitation pulse energy. The plots were presented in Fig. S2. The plots were obtained in the logarithmic plots of the fluorescence intensities induced by TPA *vs.* excitation intensities at 810 nm.

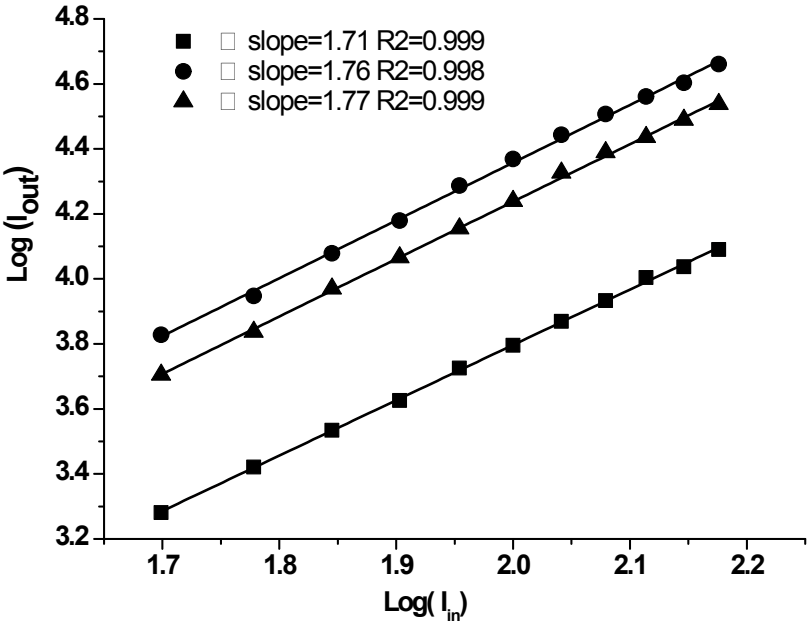


Fig. S1 The plot of quadratic dependence for Ⅰ, Ⅱ and Ⅲ at the wavelength of 810 nm.

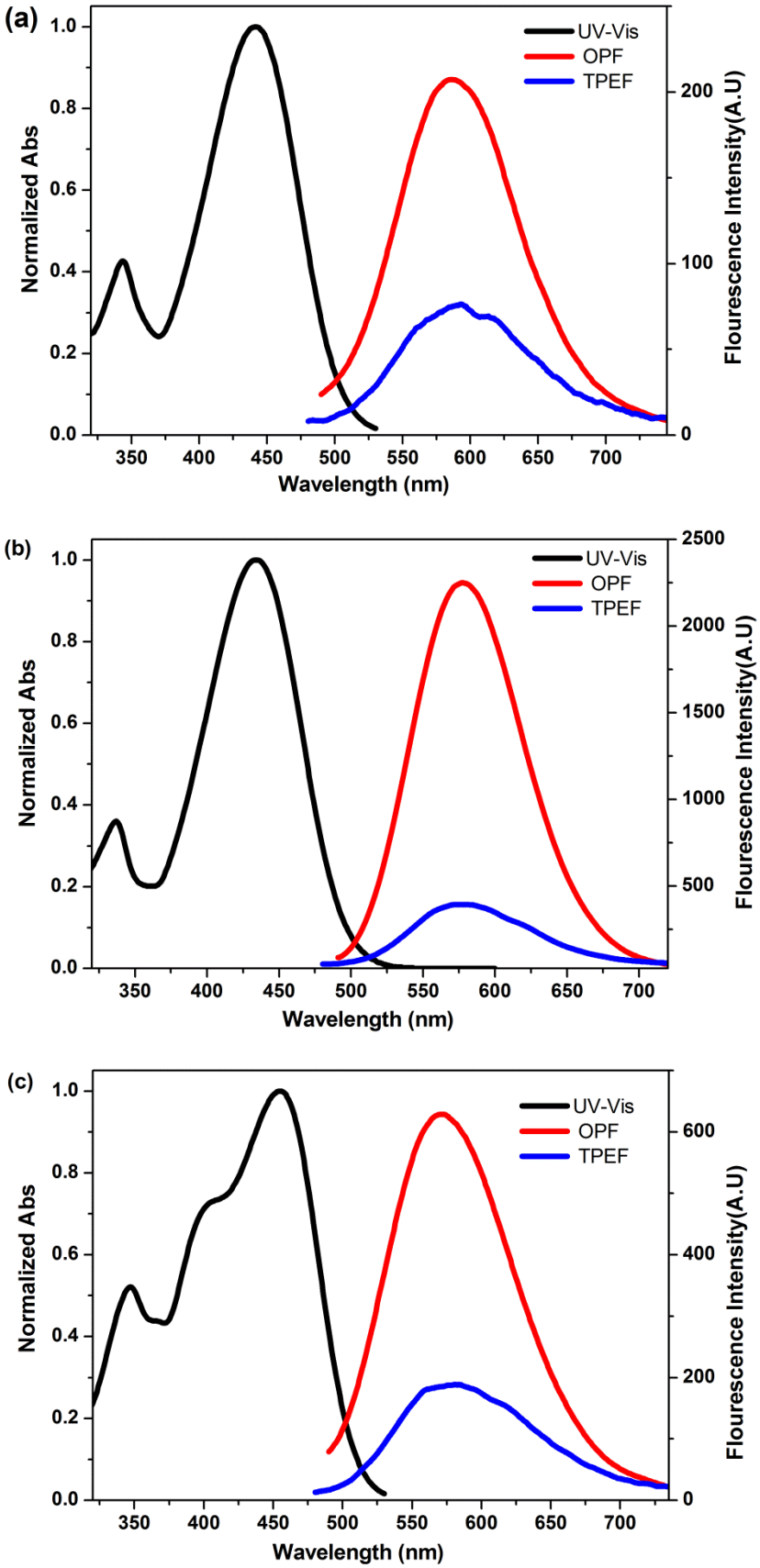


Fig. S2 Normalized absorption (black), one-photon induced fluorescence spectra (red) and two-photon fluorescence spectra (blue) of (a) Ⅰ, (b) Ⅱ and (c) Ⅲ.

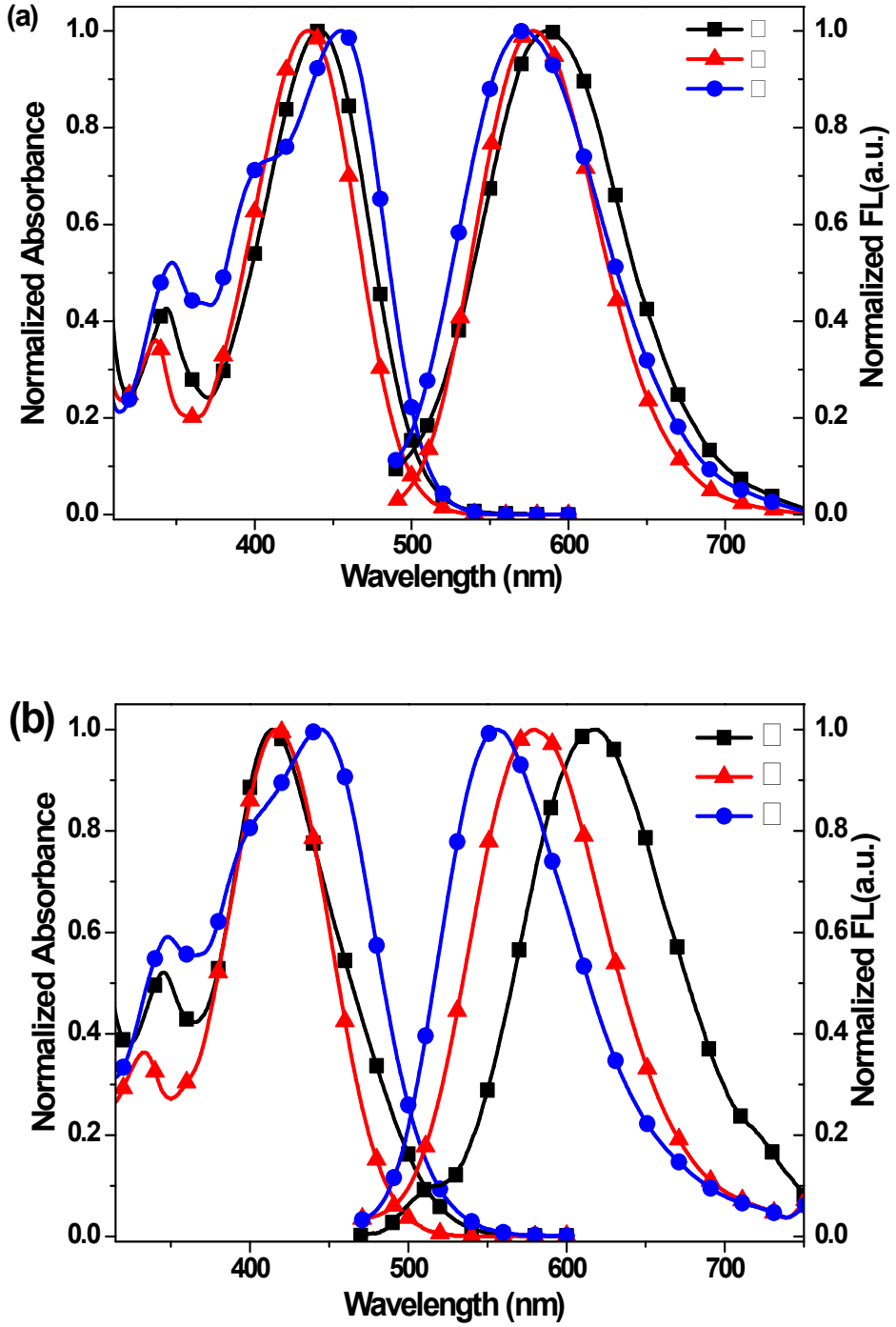


Fig. S3 Normalized absorption and one-photon induced fluorescence spectra of Ⅰ, Ⅱ and Ⅲ in glycol and Tris-HCl buffer.

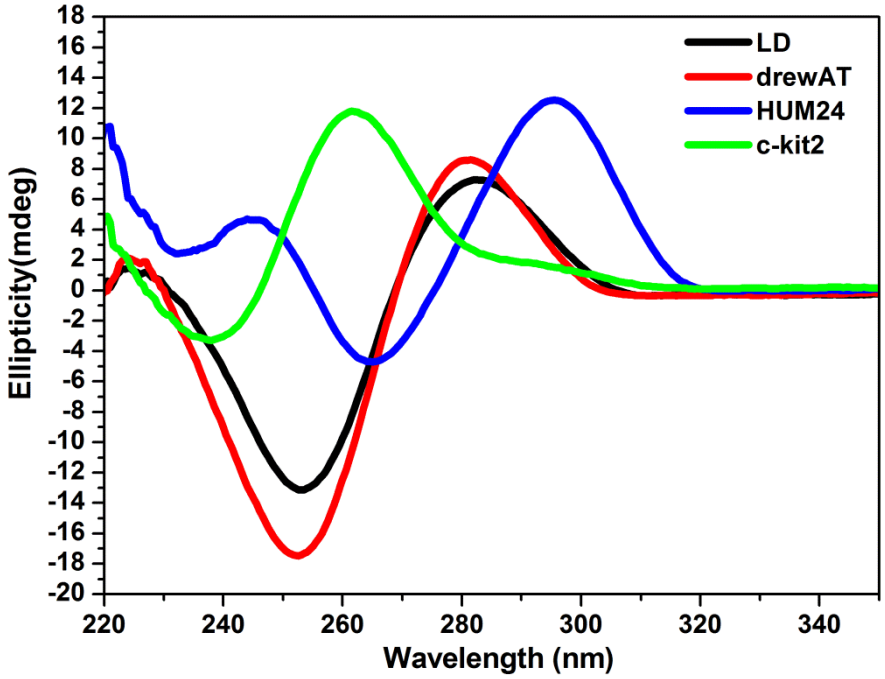


Fig. S4 Circular dichroism spectra of oligonucleotides formed in the presence of Na^+ (100 mM).

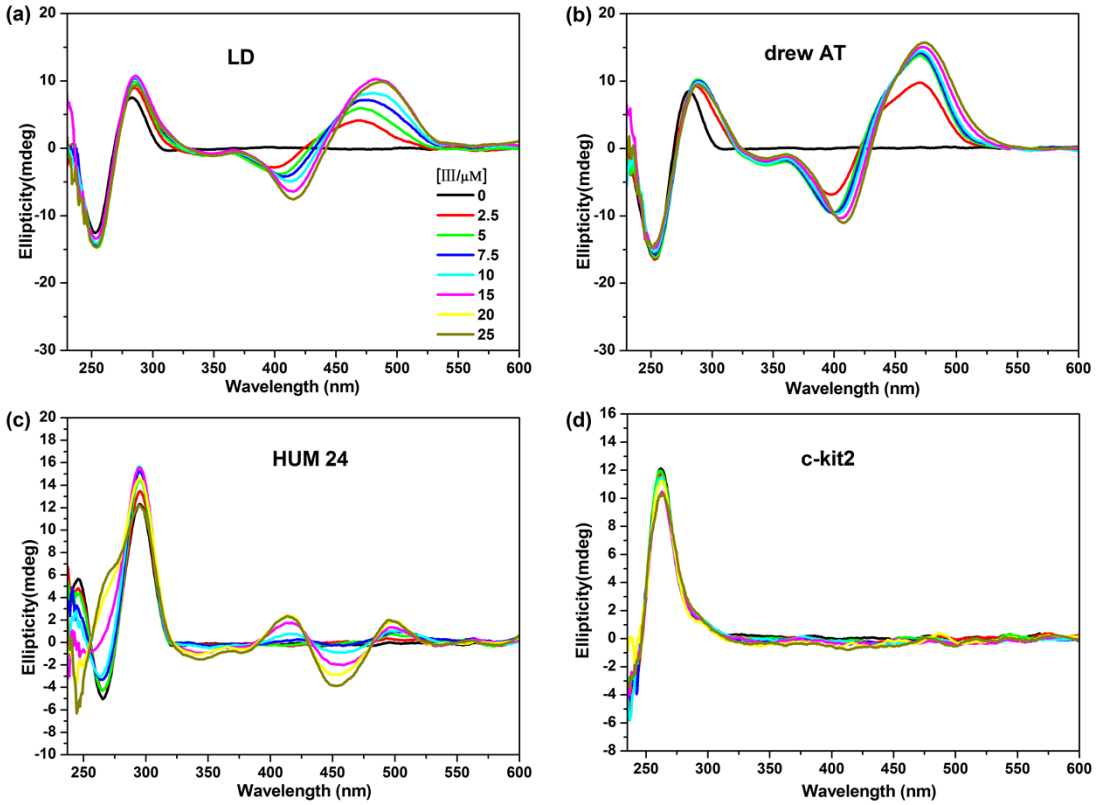


Fig. S5 Circular Dichroism spectra of 5 μM (a) LD, (b) drew AT, (c) HUM24 and (d) c-kit2 upon titration of Ⅲ (0-25 μM) in 10 mM Tris-HCl buffer containing 100 mM NaCl and 1 mM EDTA.

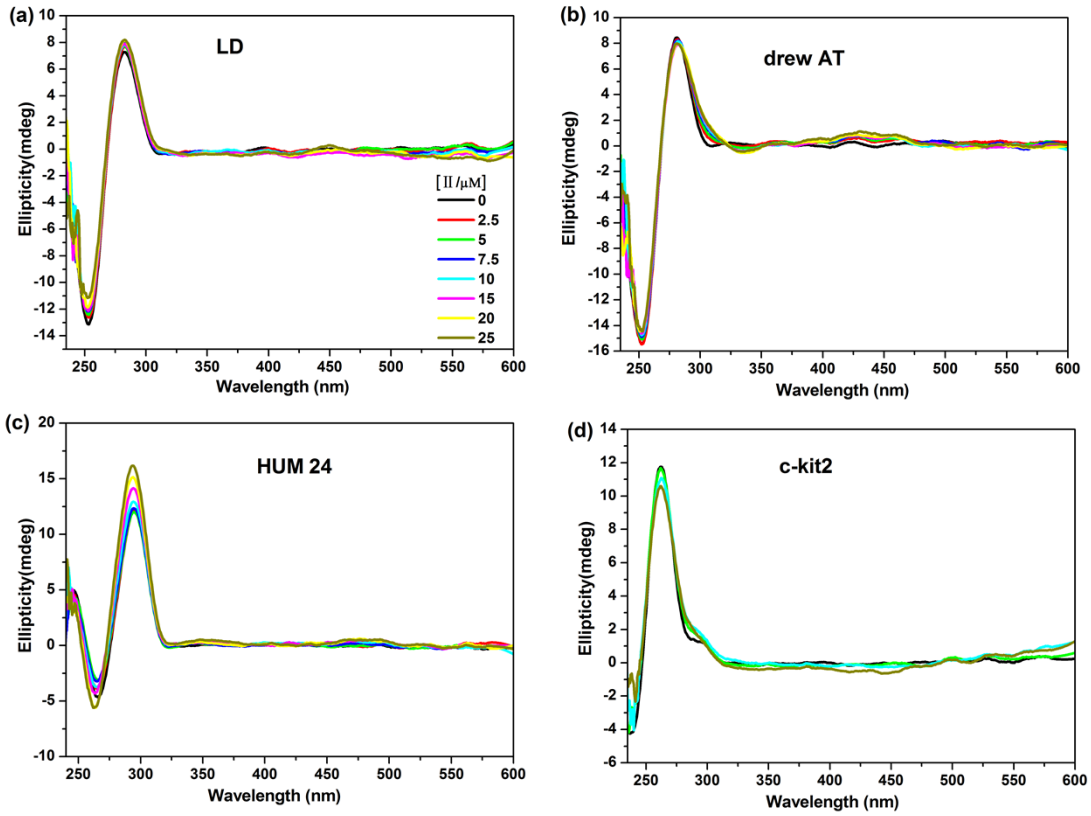


Fig. S6 Circular Dichroism spectra of 5 μM (a) LD, (b) drew AT, (c) HUM24 and (d) c-kit2 upon titration of Ⅱ (0-25 μM) in 10 mM Tris-HCl buffer containing 100 mM NaCl and 1 mM EDTA.

Confocal microscopy imaging and analysis on the staining dynamics: Confocal fluorescence imaging was performed with Nikon A1R multi-photon microscopy with a 60 × oil-immersion objective lens and living cell work station. Each image is obtained by incubating of HeLa cells with probes at a concentration of 3 μM for 2 h.

The staining dynamic analysis about the time-lapse images was carried out by the curve of the fluorescence intensity dependence on time evolution. Since living cells are always moving during the staining process, the fluorescence intensities were measured by selecting a small area in each region of approximately 20 μm^2 centered on the cell membrane, the mitochondria, and the nucleus, then the mean and standard deviation (SD) of the fluorescence were calculated from more than 10 such measurements from each region. In contrast, the intensities in the regions outside of the cells were analyzed as background. There is some signal in the background even where there are no stained subcellular structures. It is because the intensity may come from the motion of the fluorescence molecules in medium. From the curve, each probe shows different staining selectivity due to the properties of the different molecular structure. Note that probe Ⅲ may have a high affinity to lipid raft in cytoplasm after comparing with commercial lipid raft specific probe Lipid Raft Labeling Kits.

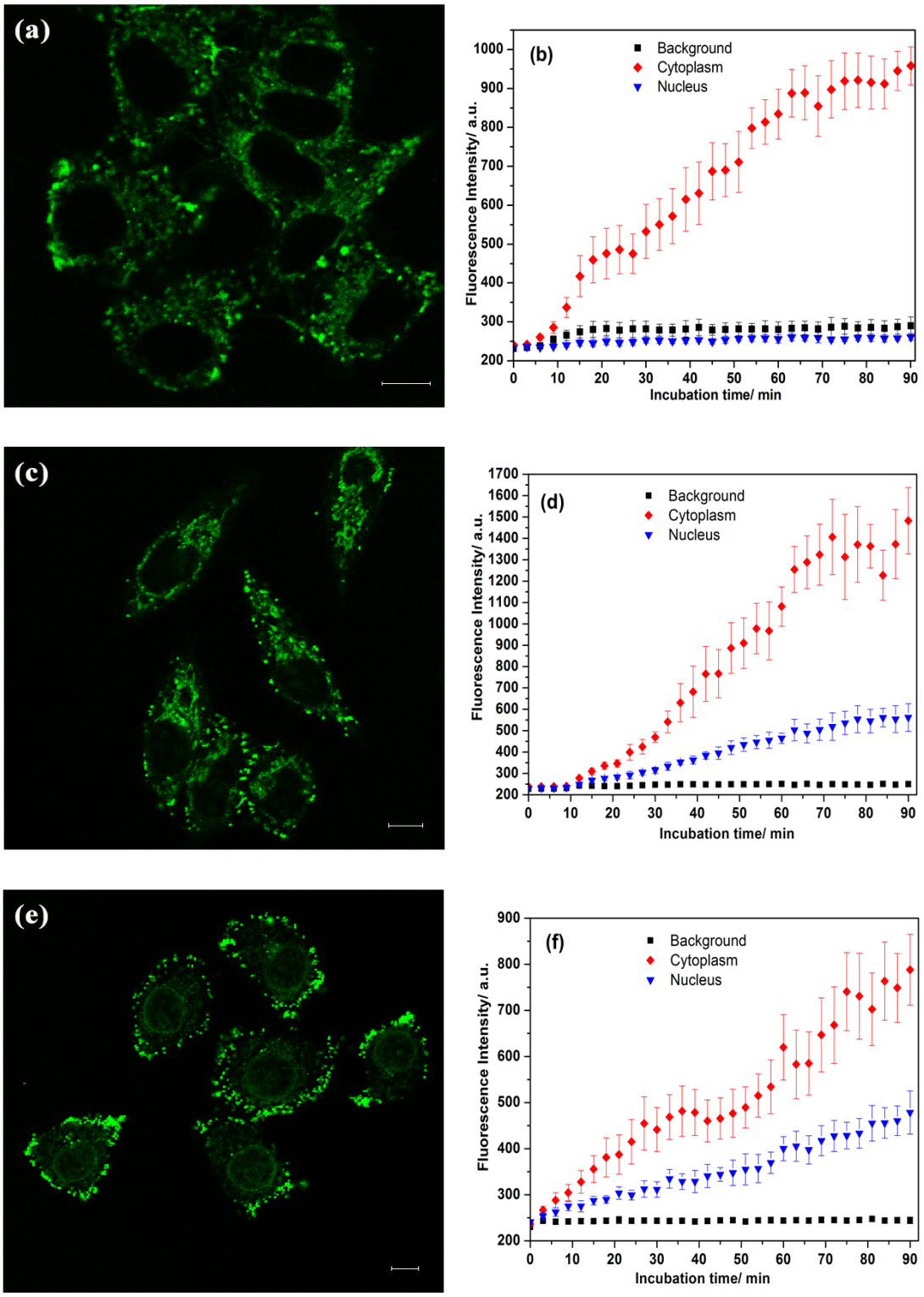


Fig. S7 Confocal fluorescence images (λ_{exc}=488 nm) obtained following incubations of HeLa cells with (a) Ⅰ, (c) Ⅱ and (e) Ⅲ (3 μM) for 2 h. Scale bar is 10 μm. Fluorescence intensity time-dependence on the incubation time for HeLa cells incubations of HeLa cells with (b) Ⅰ, (d) Ⅱ and (f) Ⅲ.

Co-localization with MitoTracker Deep Red: HeLa cells were incubated with medium containing 1 μM probes for 2 h and then the medium was removed and washed twice by PBS. After that, the cells were incubated with the medium containing 25 nM MitoTracker Deep Red for 20 min. After removal of the medium and washing with PBS twice again, fresh medium was added before imaging. The fluorescence collected ranges for probe and MitoTracker Deep Red are 500-550 nm and 670-720 nm, respectively. The overlaid confocal fluorescence image of both probe Ⅰ and MitoTracker Deep Red illustrates that probe Ⅰ mainly accumulates in the mitochondria.

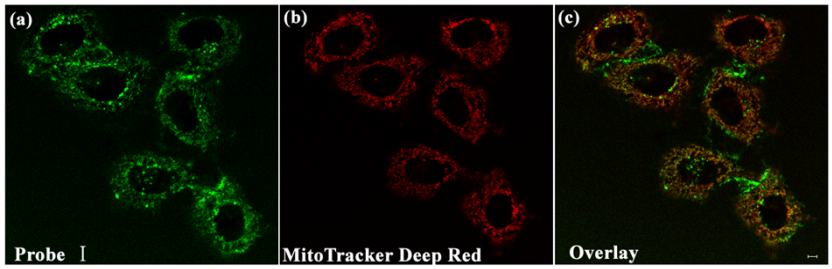
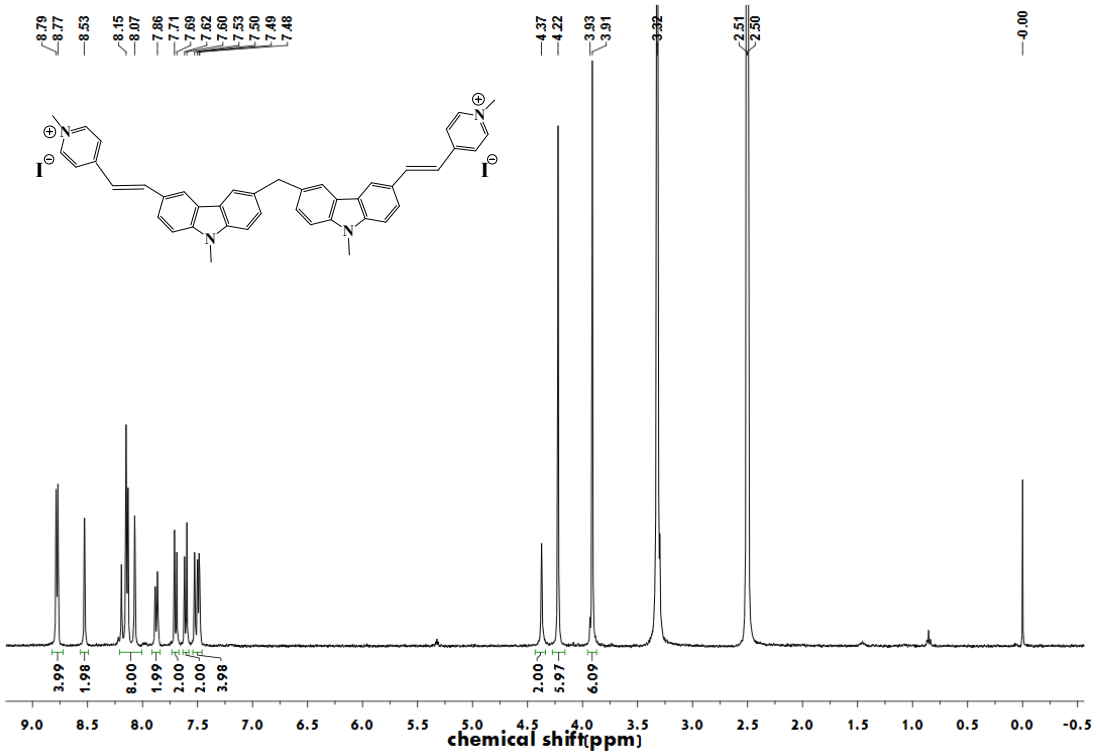


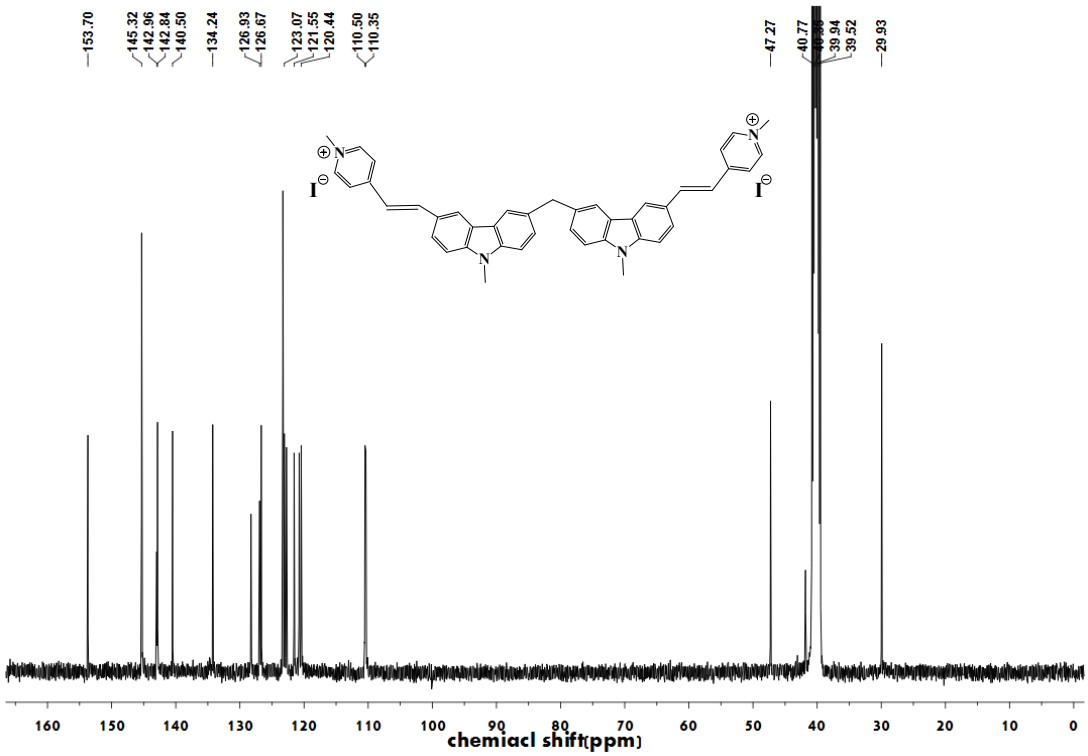
Fig. S8 Confocal fluorescence images obtained following incubations of HeLa cells with (a) Ⅰ（1 μM, excited at 488 nm, green) for 2 h and (b)MitoTracker Deep Red (25 nM, excited at 641 nm, red) for 20 min. (c) The overlapped image. Scale bar is 5 μm

^{1}HNMR, ^{13}CNMR and ESI-MS spectra of Ⅰ, Ⅱ and Ⅲ

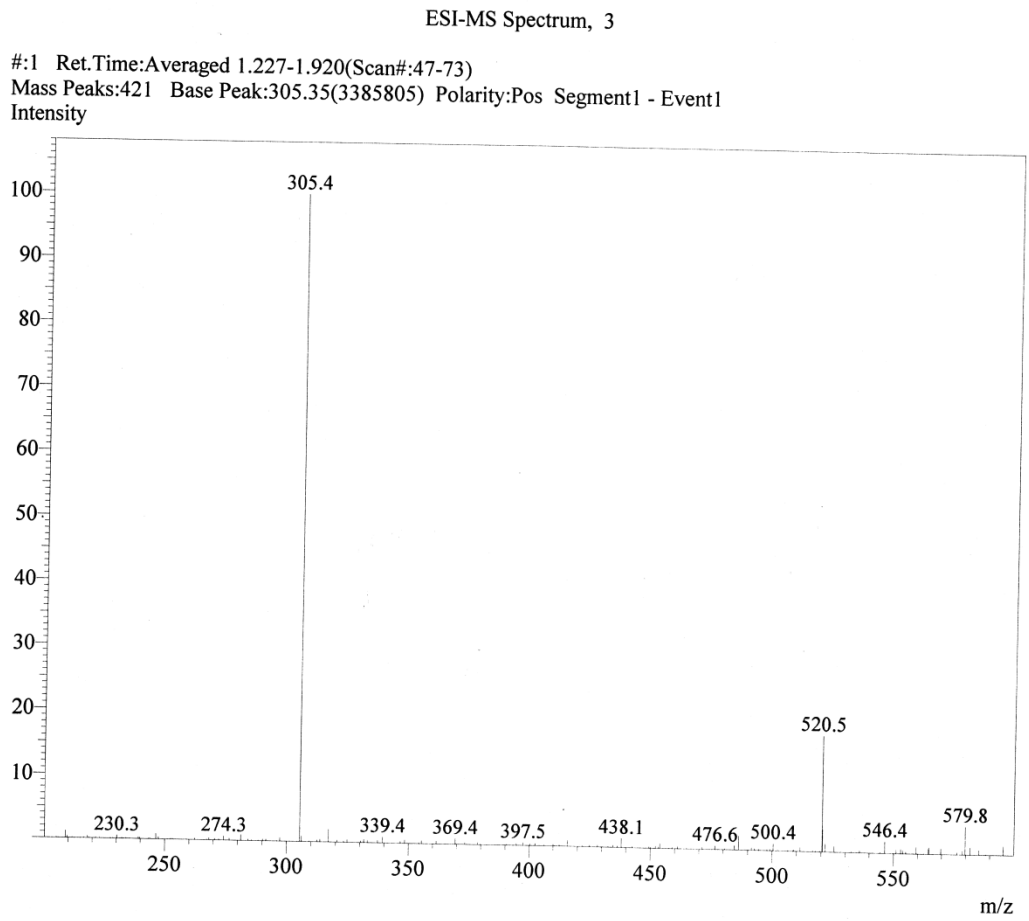
1. ^{1}HNMR spectrum of probe Ⅰ (DMSO-d6, 400MHz)



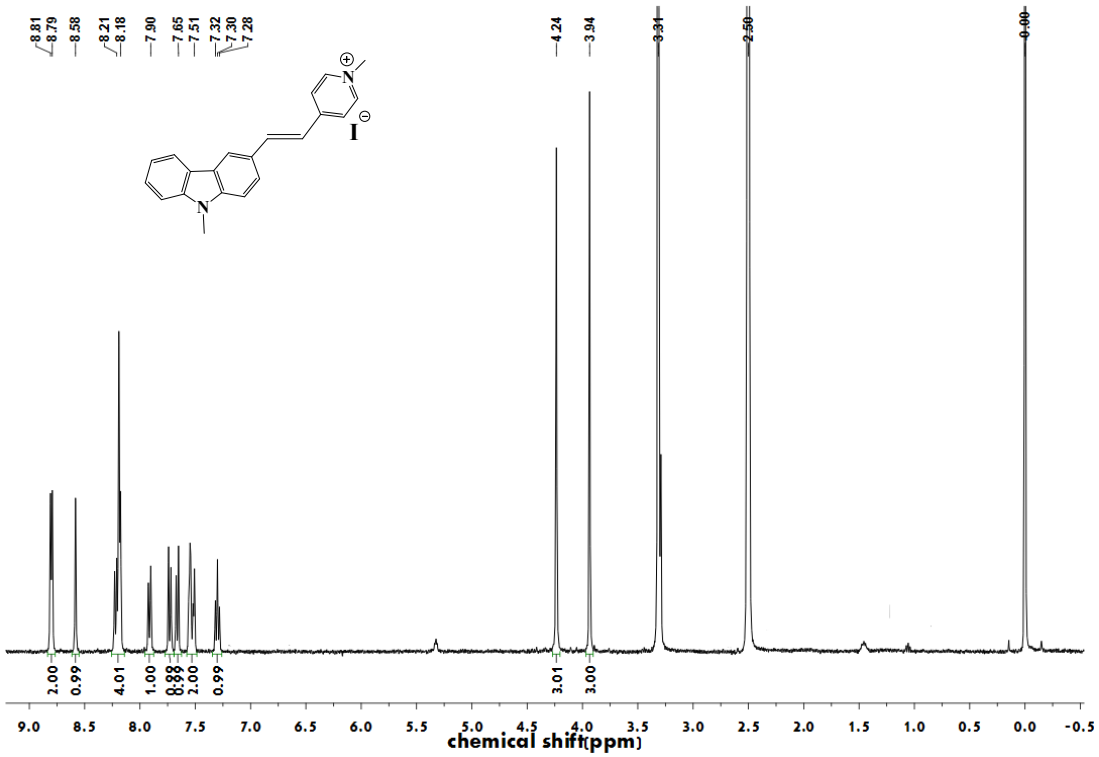
2. ^{13}CNMR spectrum of probe Ⅰ (DMSO-d6, 400MHz)



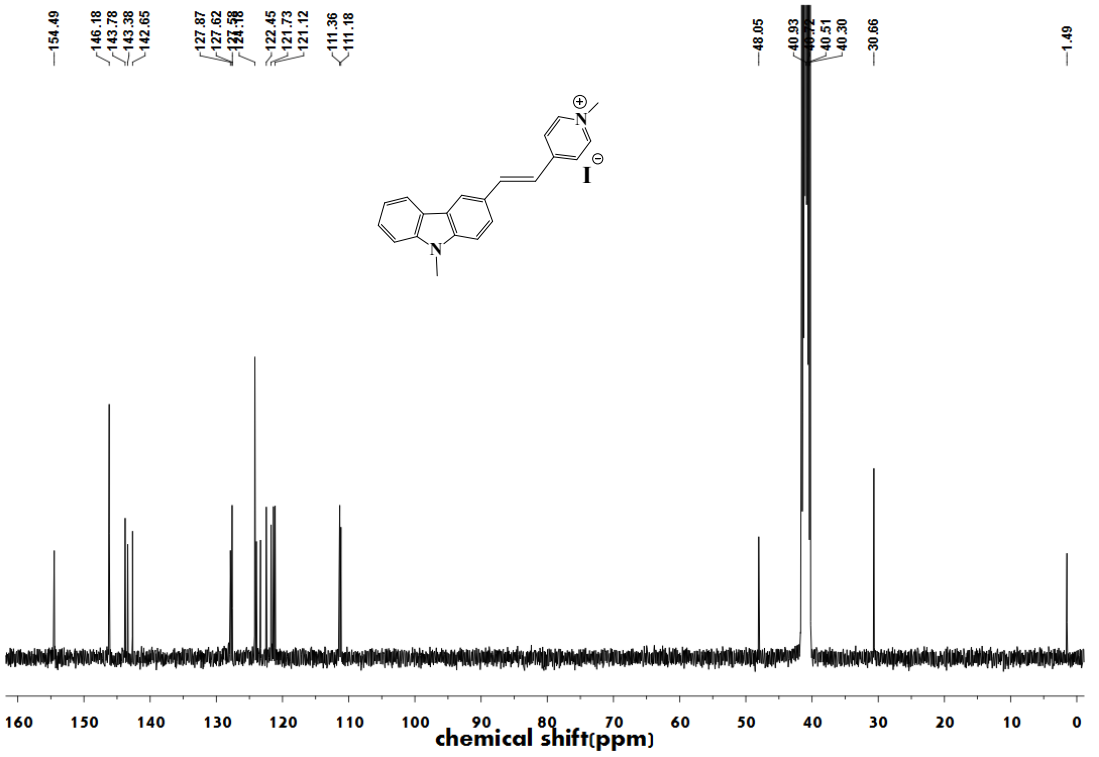
3. ESI-MS spectrum of probe Ⅰ



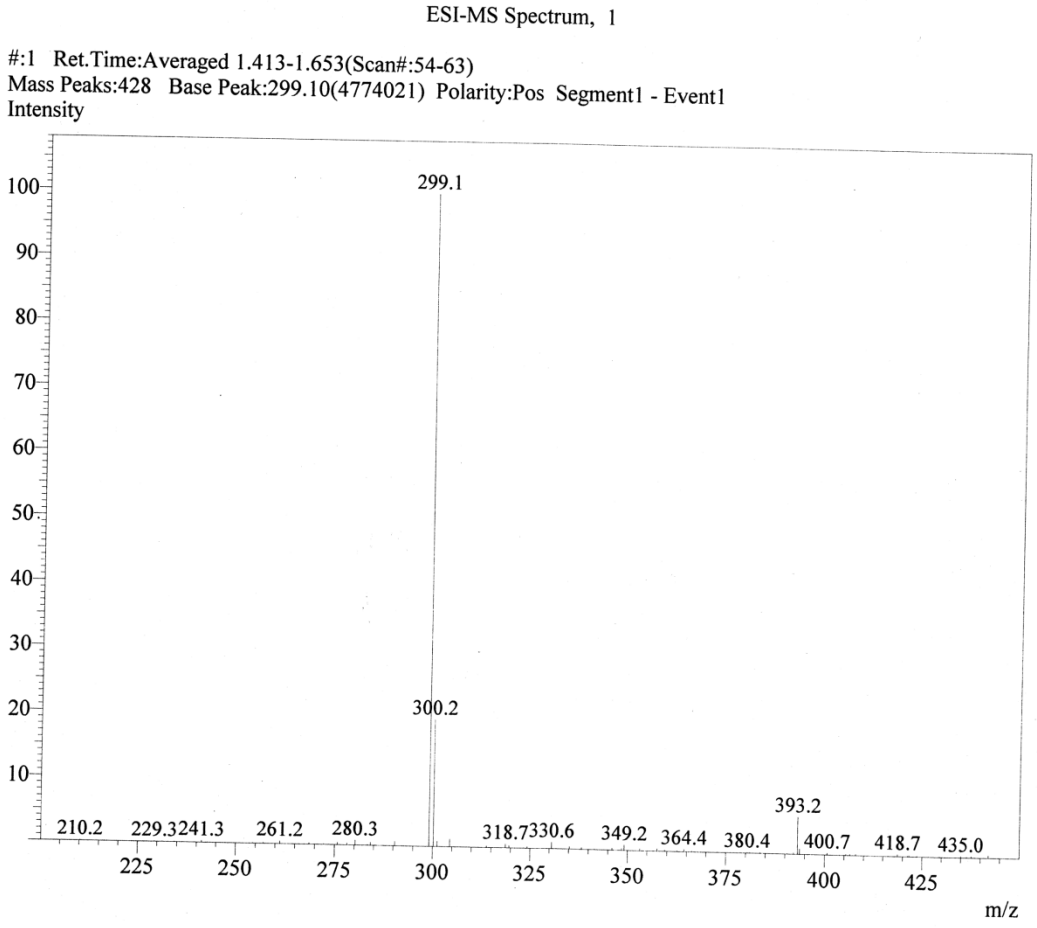
4. ^{1}HNMR spectrum of probe Ⅱ (DMSO-d6, 400MHz)



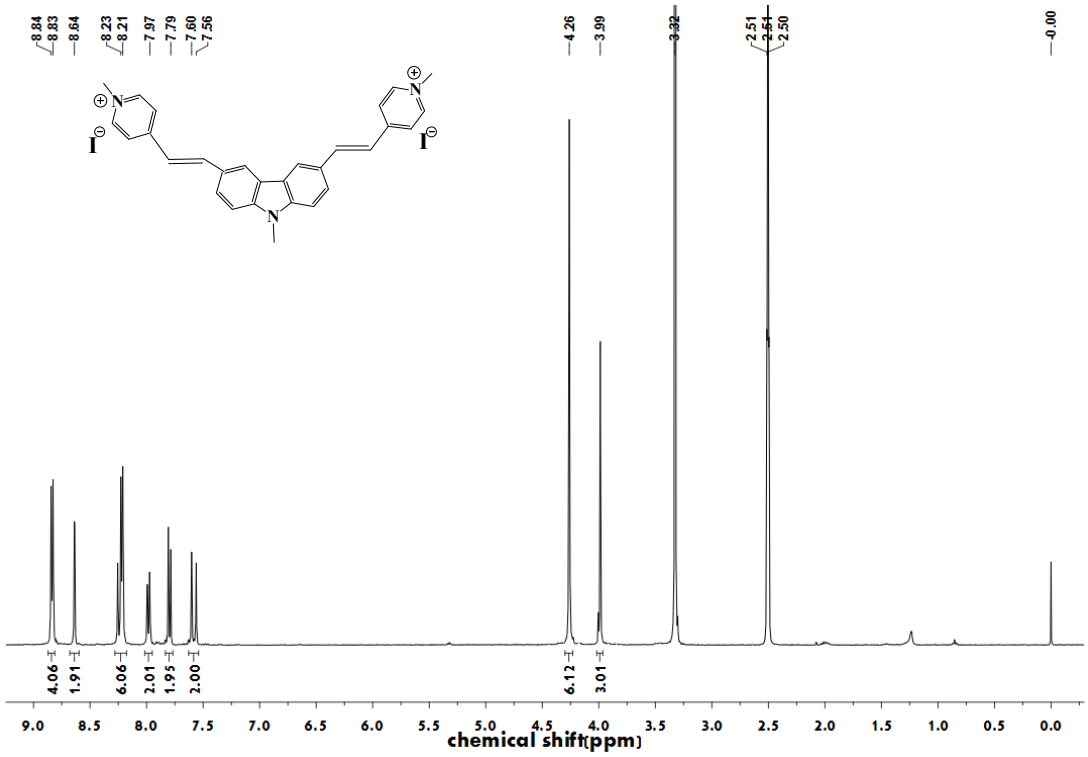
5. ^{13}CNMR spectrum of probe Ⅱ (DMSO-d6, 400MHz)



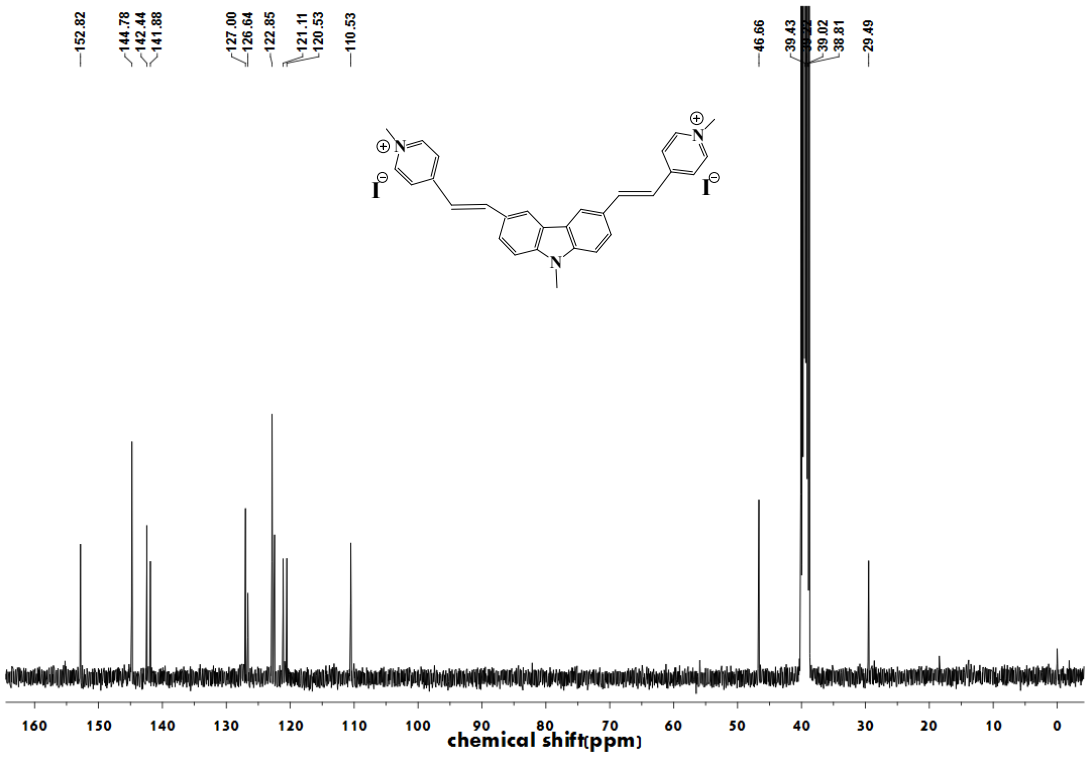
6. ESI-MS spectrum of probe Ⅱ



7. ^{1}HNMR spectrum of probe Ⅲ (DMSO-d6, 400MHz)



8. ^{13}CNMR spectrum of probe Ⅲ (DMSO-d6, 400MHz)



9. ESI-MS spectrum of probe Ⅲ

ESI-MS Spectrum, 2

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Intensity