

# Electronic Supplementary Information

## A Two-Photon Fluorescent Probe for Viscosity Imaging *in vivo*

Peng Ning,<sup>a</sup> Pengyu Dong,<sup>b</sup> Qian Geng,<sup>c</sup> Lei Bai,<sup>d</sup> Yaqi Ding,<sup>d</sup> Xiaohe Tian,<sup>c</sup>  
Rong Shao,<sup>b\*</sup> Lin Li<sup>d</sup> and Xiangming Meng<sup>\*a</sup>

<sup>a</sup> School of Chemistry & Chemical Engineering, Anhui University, Hefei, 230601, P. R. China.

<sup>b</sup> Jiangsu Collaborative Innovation Center for Ecological Building Materials & Environmental Protection Equipments, Yancheng Institute of Technology, Yancheng, 224051, P. R. China

<sup>c</sup> School of Life Science, Anhui University, Hefei, 230601, P. R. China

<sup>d</sup> Key Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), Nanjing, 211816, P. R. China

\*Corresponding author. Fax: +86-551-63861467; Tel: +86-551-63861467

E-mail address: mengxm@ahu.edu.cn (Xiangming Meng); sr@ycit.cn (Rong Shao).

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## 1. UV-vis spectra of MCN in water and glycerol.

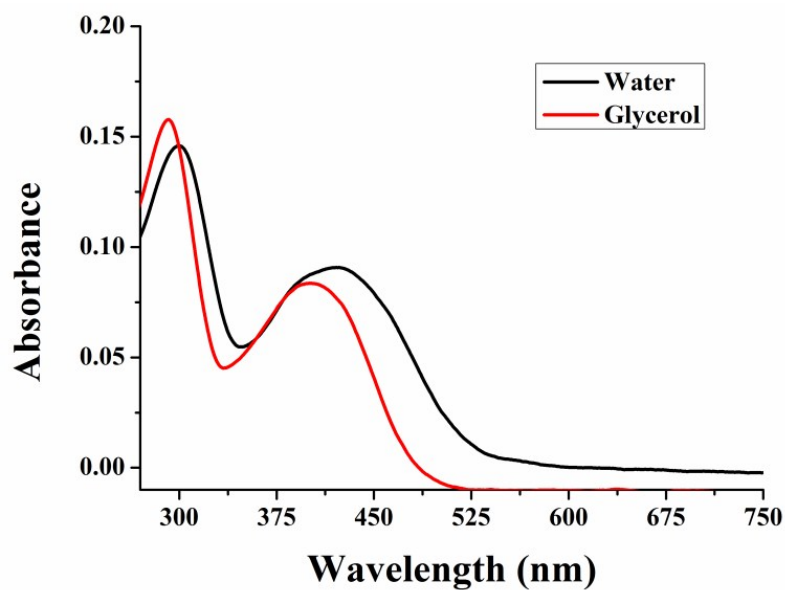


Fig. S1 UV-vis spectra of MCN (10 μM) in water and glycerol, respectively.

## 2. UV-vis and fluorescence emission spectra in different solvents.

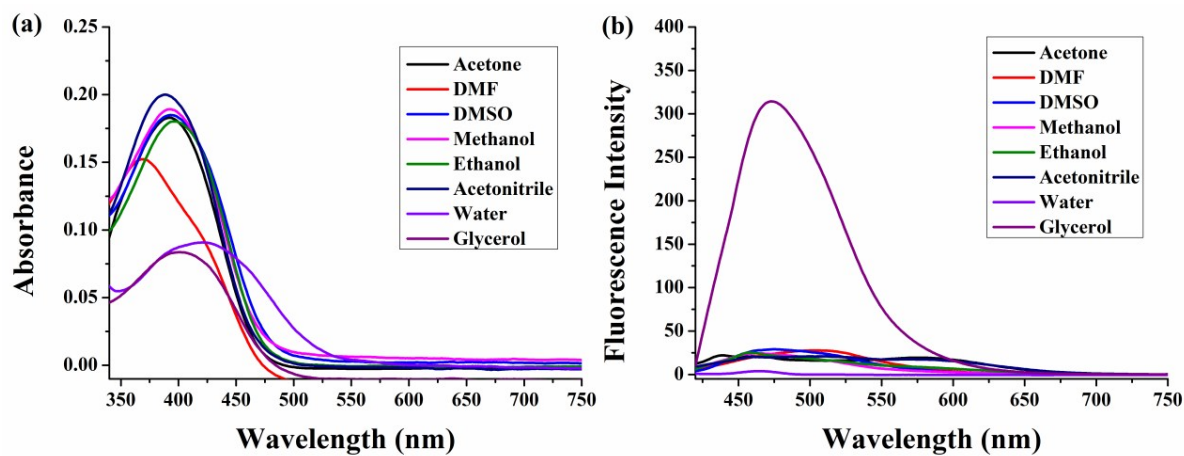


Fig. S2 (a) UV-vis spectra of MCN (10 μM) in different solvents; (b) fluorescence emission spectra of MCN (10 μM) in different solvents, excited at 400 nm.

### 3. pH stability.

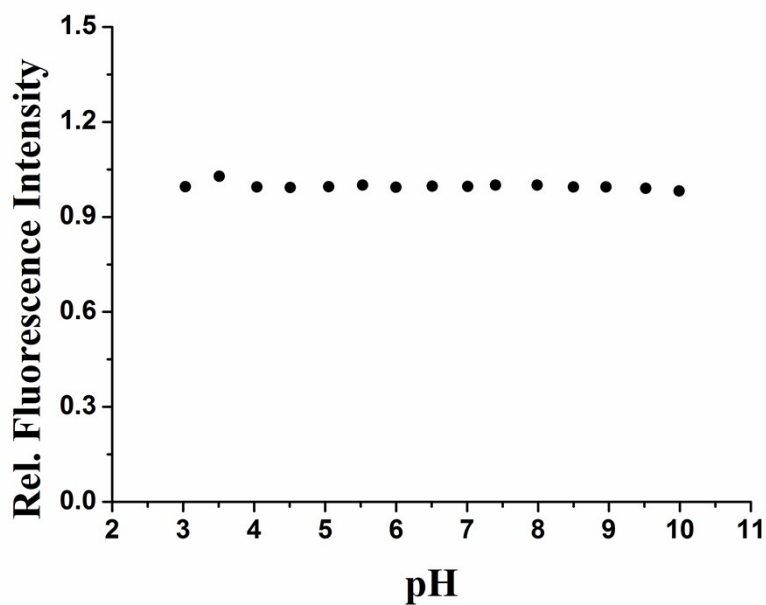


Fig. S3 Relative fluorescence emission intensity ( $I/I_0$ ,  $\lambda_{em} = 470$  nm) of MCN (10  $\mu$ M) at different pH values in water/glycerol system (7:3, v/v, 10 mM PBS buffer,  $\lambda_{ex} = 400$  nm).

### 4. Cytotoxicity assay.

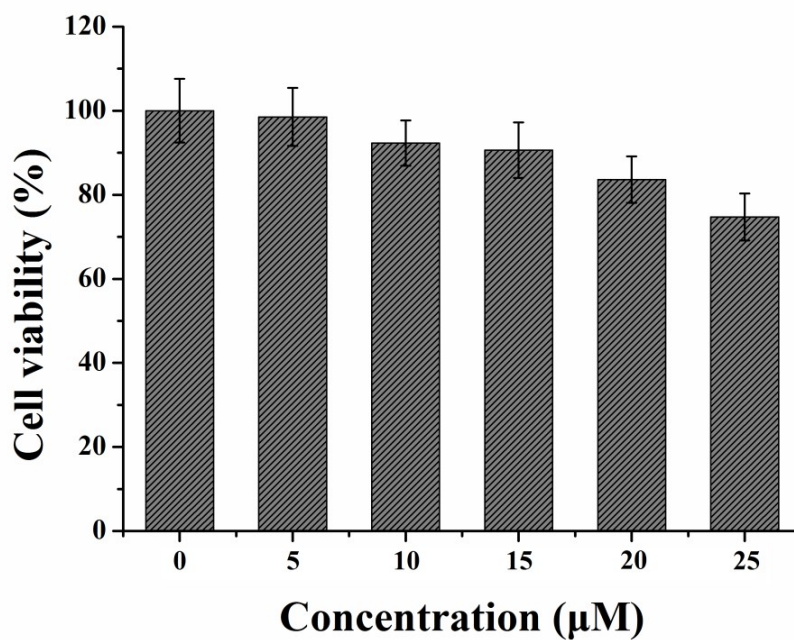
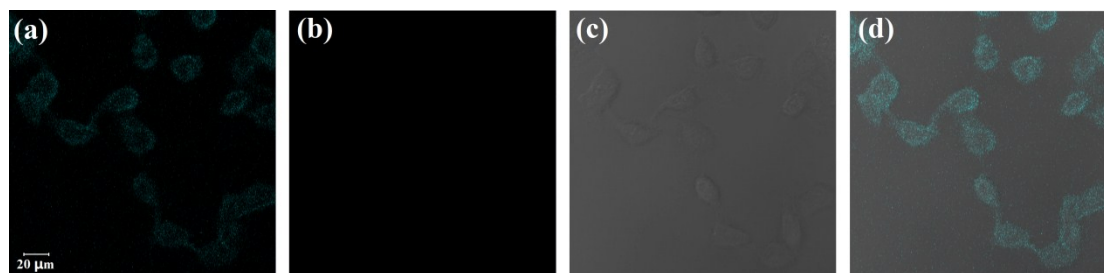


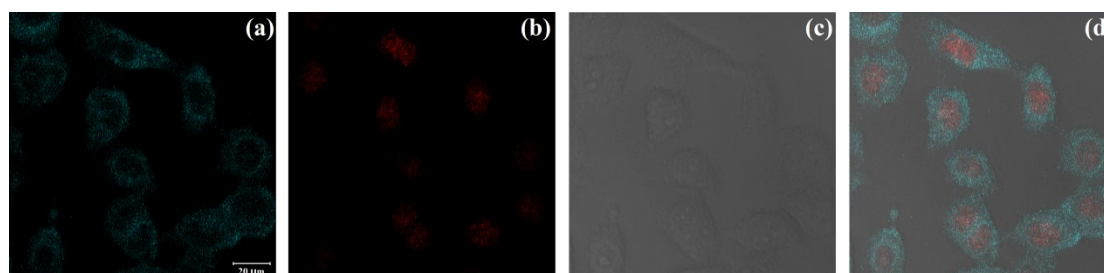
Fig. S4 Cytotoxicity data of MCN (HeLa cells incubated for 24 h).

## 5. Confocal co-localized images of MCN and PI.



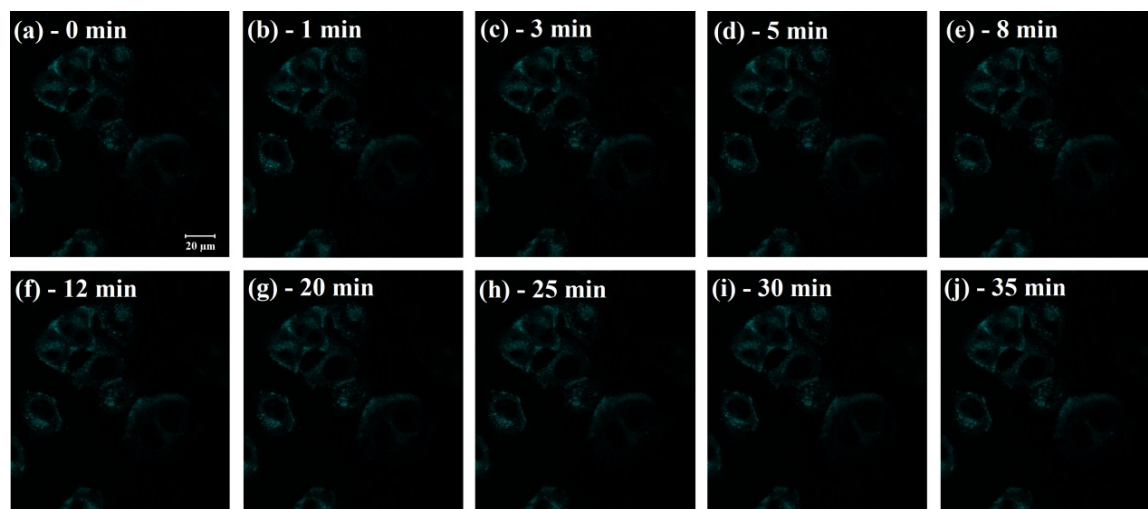
**Fig. S5** Confocal co-localized images of HeLa cells incubated with **MCN** (10  $\mu\text{M}$ ) for 30 min and **PI** (3  $\mu\text{M}$ ) for 15 min. (a) Two-photon image of HeLa cells,  $\lambda_{\text{ex}} = 800$  nm, emission wavelength from 450 nm to 490 nm; (b) one-photon image of HeLa cells,  $\lambda_{\text{ex}} = 543$  nm, emission wavelength from 605 nm to 625 nm; (c) bright-field of HeLa cells; (d) the overlay of panels (a), (b) and (c). Scale bar: 20  $\mu\text{m}$ .

## 6. Co-localized images of MCN and the nucleus dye.



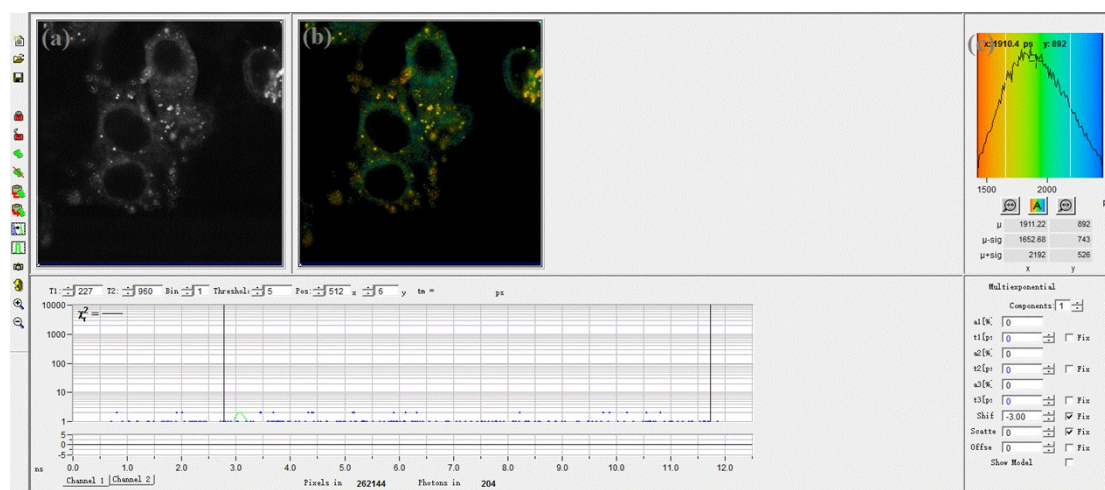
**Fig. S6** Confocal co-localized images of HeLa cells incubated with **MCN** (10  $\mu\text{M}$ ) for 30 min and **NucRed® Live 647 ReadyProbes® Reagent** (2 drops per mL of media) for 15 min. (a) Two-photon image of HeLa cells,  $\lambda_{\text{ex}} = 800$  nm, emission wavelength from 450 nm to 490 nm; (b) one-photon image of HeLa cells,  $\lambda_{\text{ex}} = 633$  nm, emission wavelength from 655 nm to 665 nm; (c) bright-field of HeLa cells; (d) the overlay of panels (a), (b) and (c). Scale bar: 20  $\mu\text{m}$ .

## 7. Two-photon fluorescence imaging of MCN without etoposide.



**Fig. S7** (a) – (j) Two-photon confocal images of HeLa cells incubated with 10  $\mu\text{M}$  MCN at different time points,  $\lambda_{\text{ex}} = 800$  nm, emission wavelength from 450 nm to 490 nm. Scale bars: 20  $\mu\text{m}$ .

## 8. Two-photon Fluorescence Lifetime Imaging (FLIM) in cells



**Fig. S8** (a) Fluorescence image obtained following 800 nm excitation and  $470 \pm 20$  nm detection from HeLa cells incubated with 10  $\mu\text{M}$  solution of MCN; (b) FLIM image obtained following 800 nm pulsed excitation of the same layer of cells; (c) Histogram of lifetimes.

## 9. Toxicity for zebrafish.

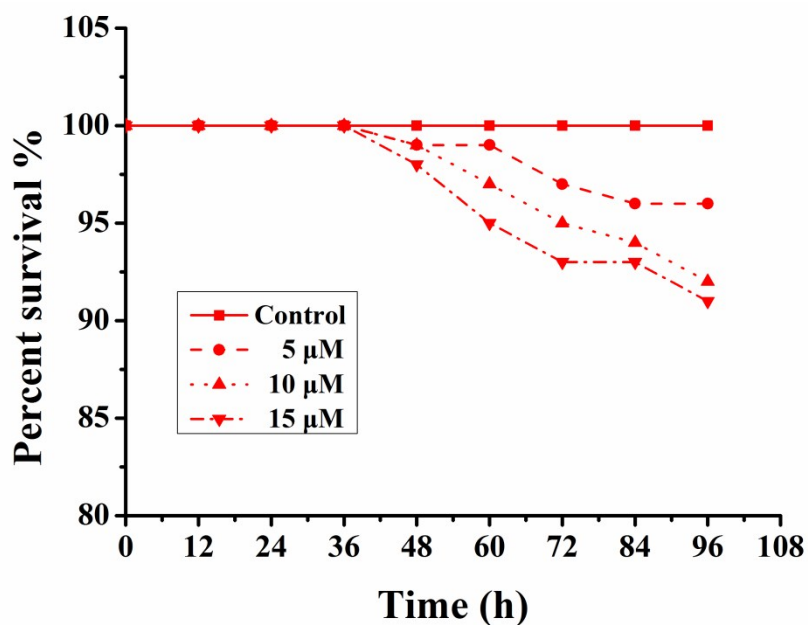


Fig. S9 Survival rate of larval zebrafish treated with MCN for up to 96 hours.

## 10. ESI-MS spectrum of MCN.

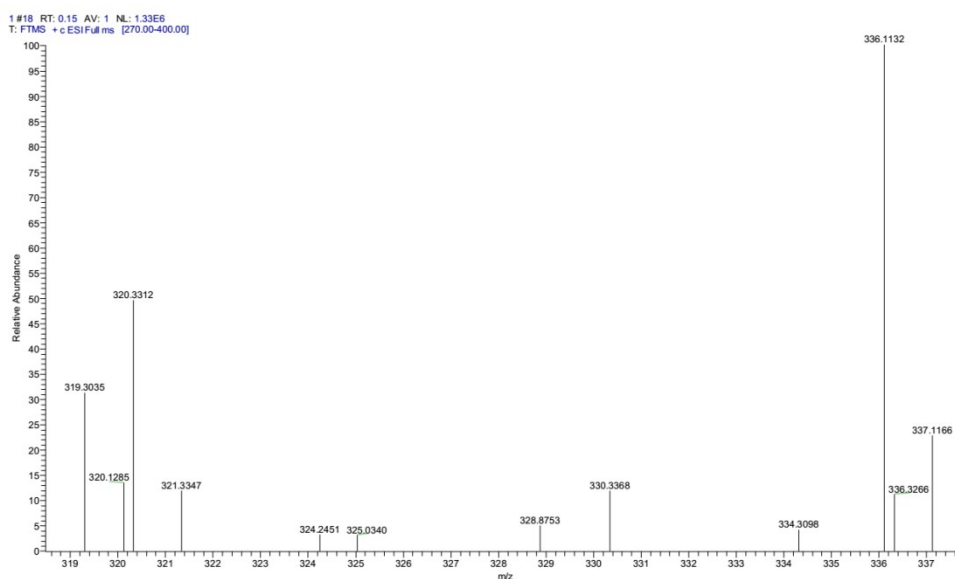


Fig. S10 ESI-MS spectrum of MCN.

## 11. NMR spectra of MCN and its intermediates.

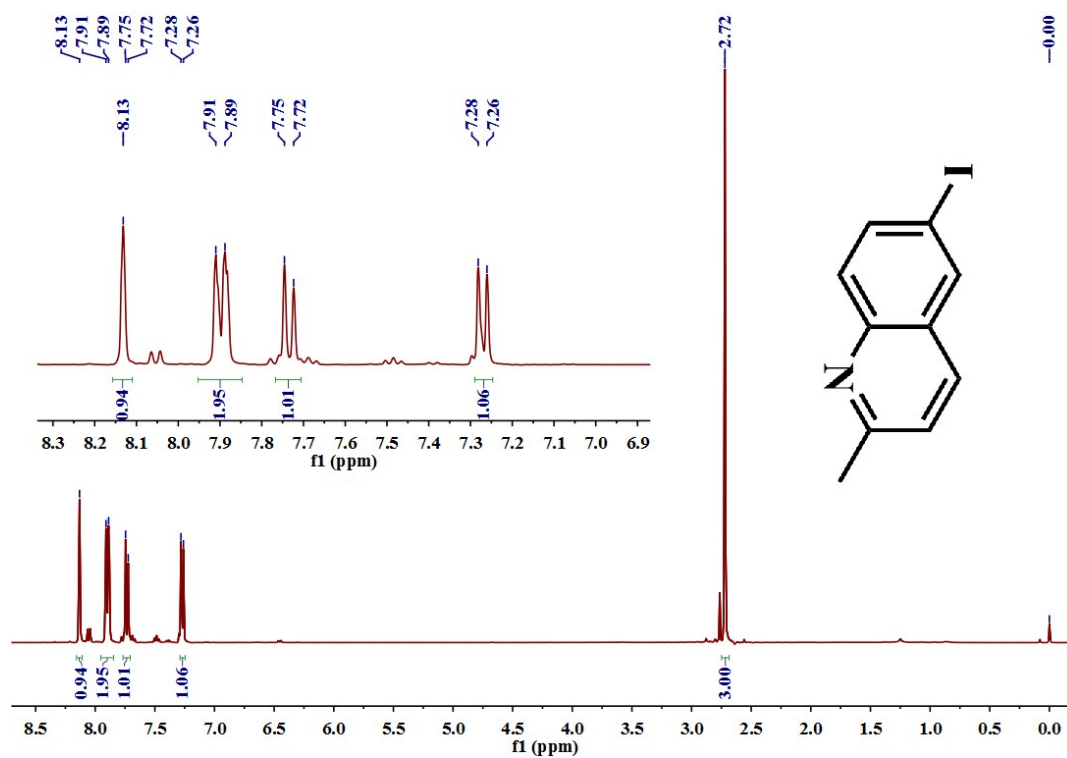


Fig. S11 <sup>1</sup>H NMR of compound 1 in CDCl<sub>3</sub>.

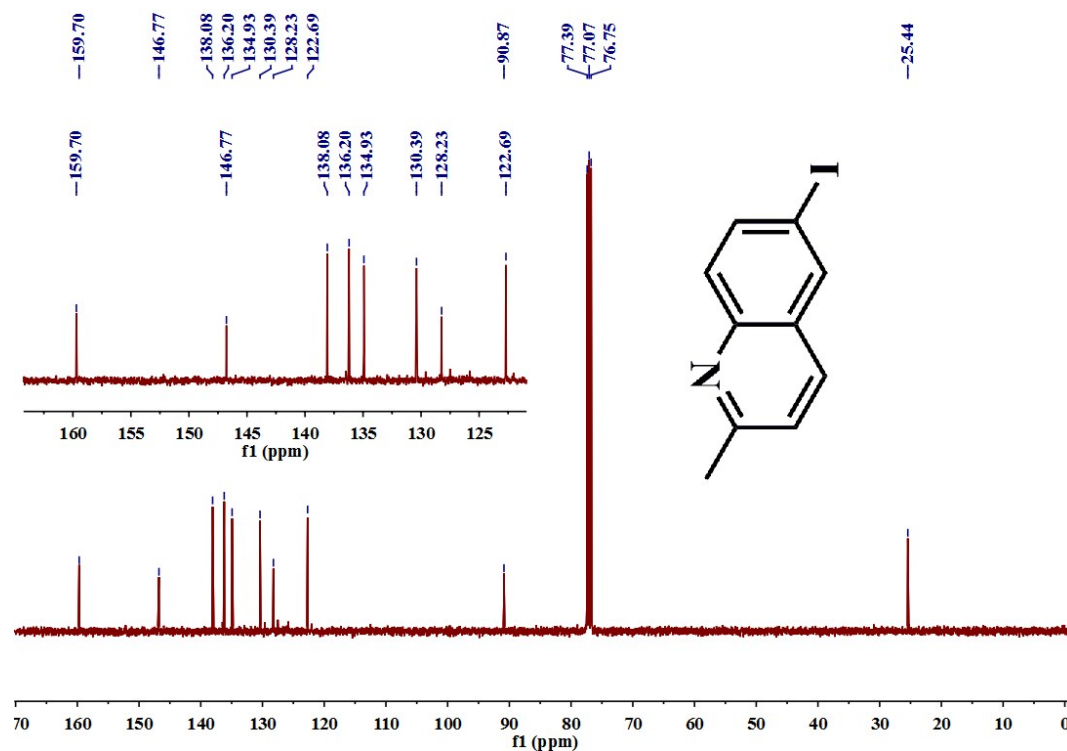


Fig. S12 <sup>13</sup>C NMR of compound 1 in CDCl<sub>3</sub>.



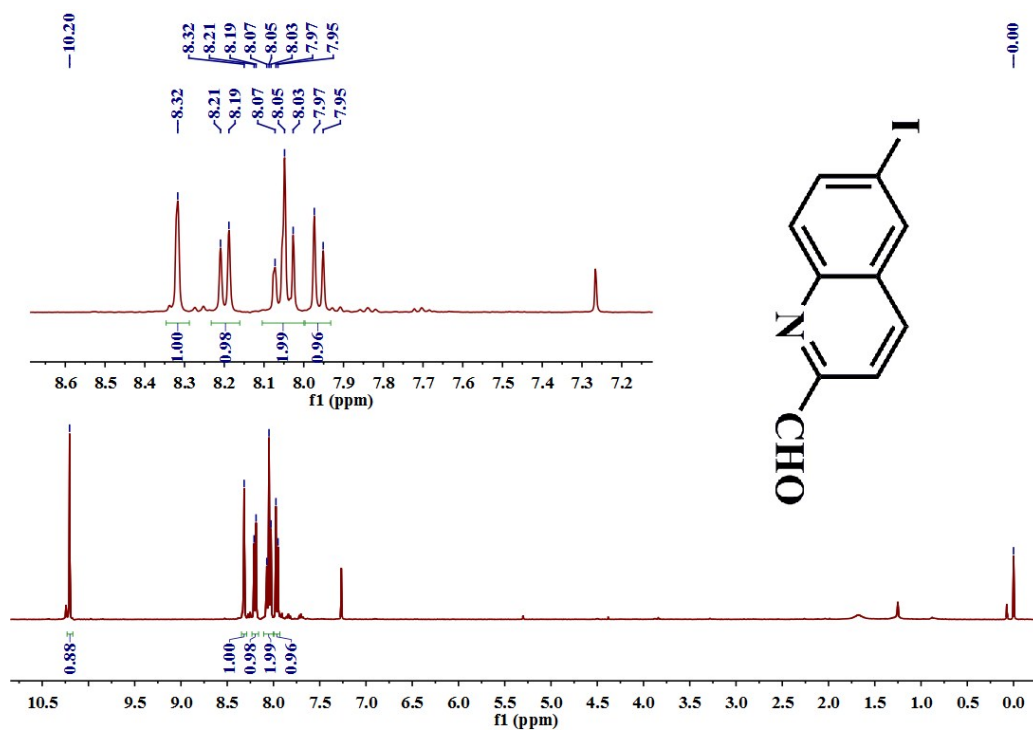


Fig. S13 <sup>1</sup>H NMR of compound 2 in CDCl<sub>3</sub>.

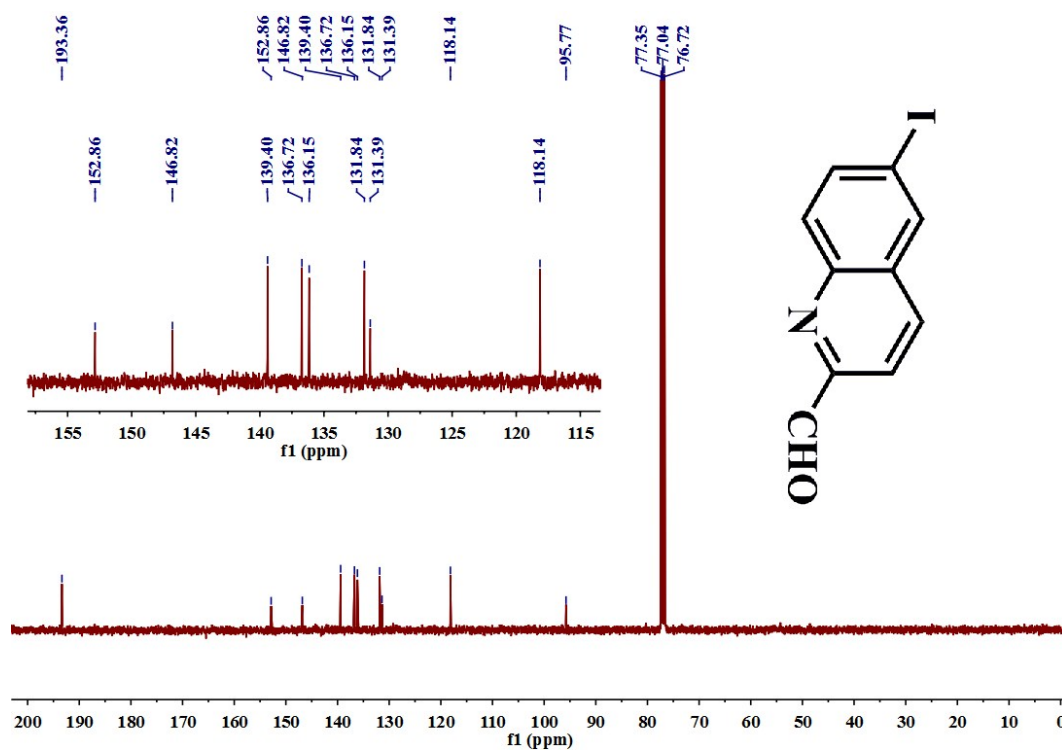


Fig. S14 <sup>13</sup>C NMR of compound 2 in CDCl<sub>3</sub>.

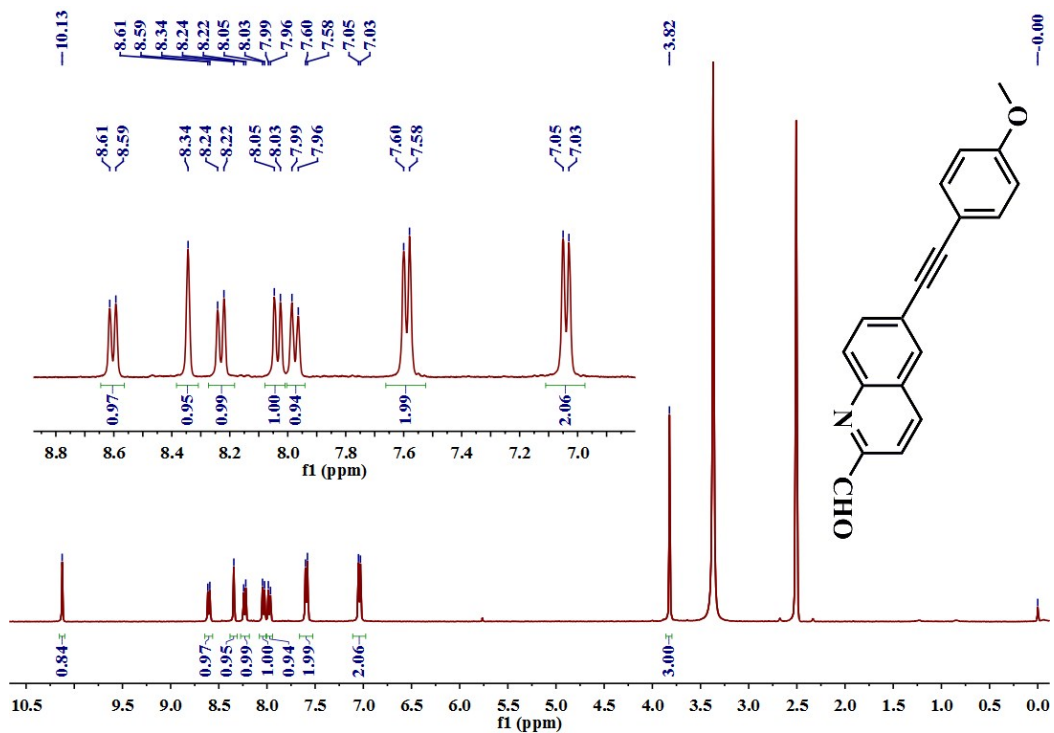


Fig. S15 <sup>1</sup>H NMR of compound 3 in DMSO-*d*<sub>6</sub>.

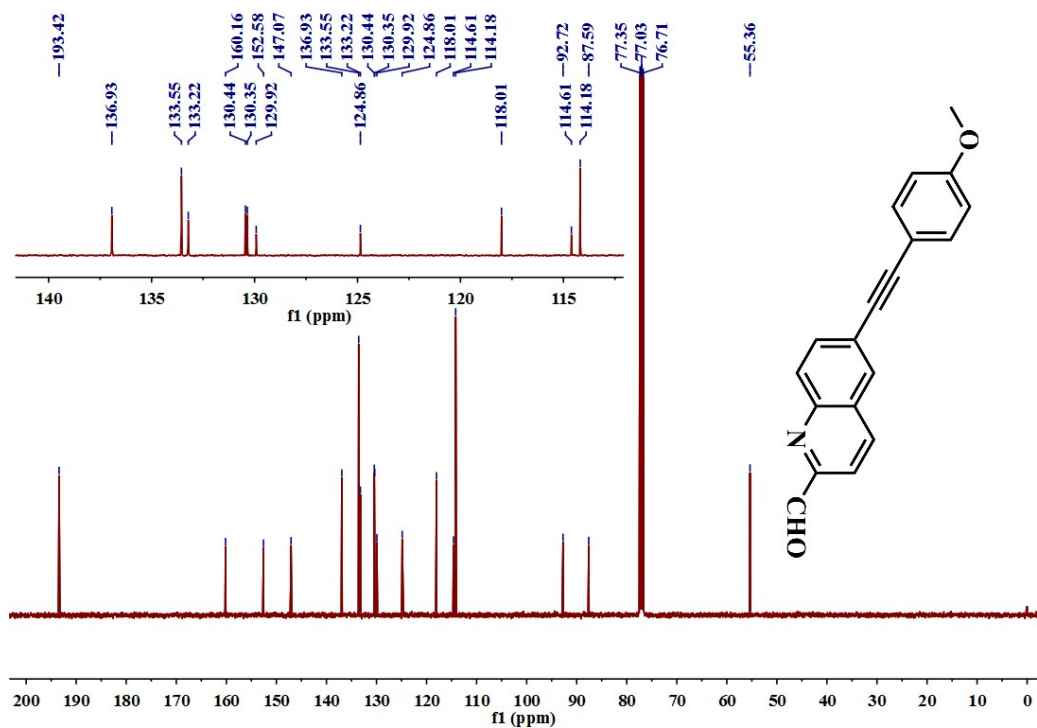


Fig. S16 <sup>13</sup>C NMR of compound 3 in CDCl<sub>3</sub>.

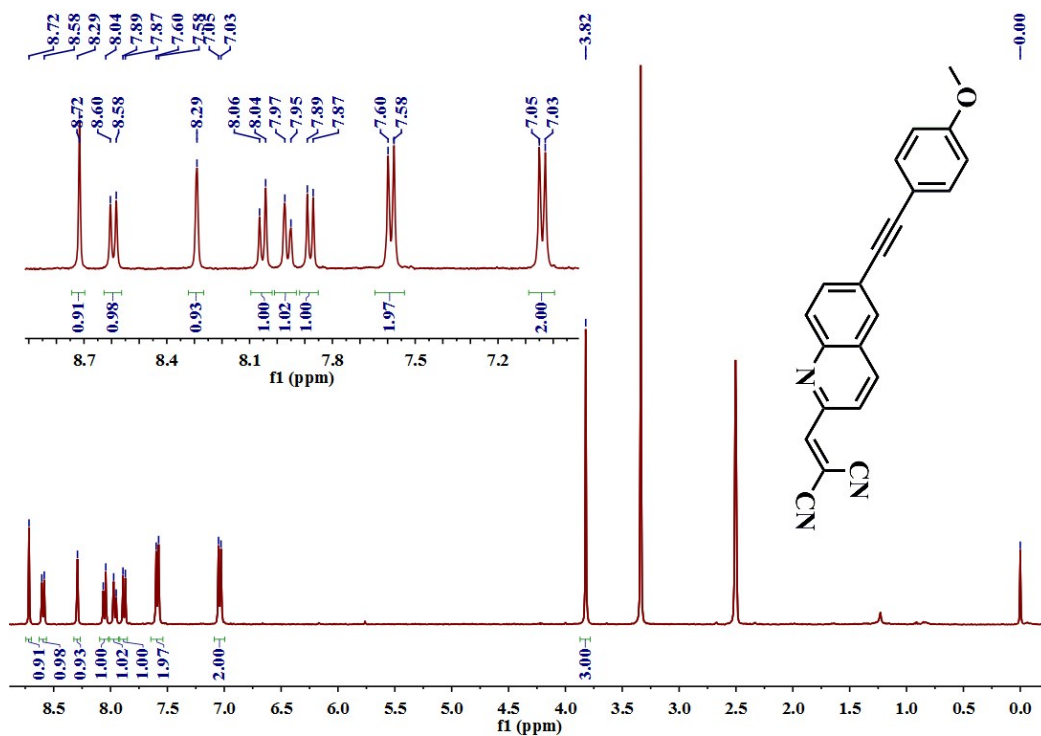


Fig. S17 <sup>1</sup>H NMR of MCN in DMSO-*d*<sub>6</sub>.

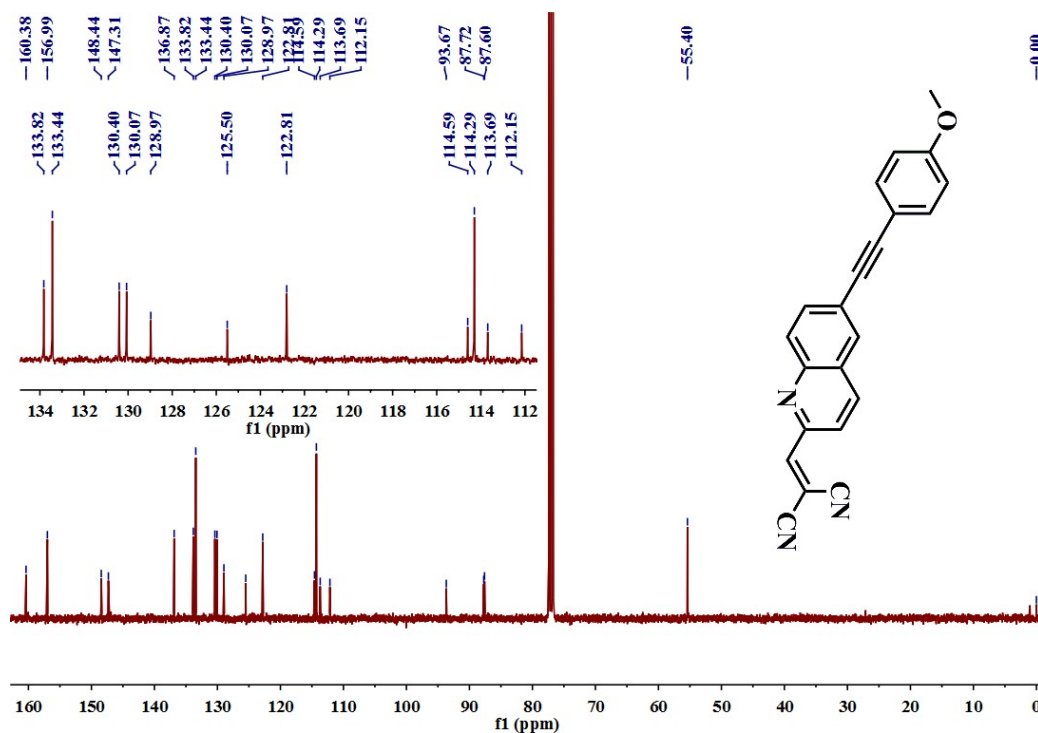


Fig. S18 <sup>13</sup>C NMR of MCN in CDCl<sub>3</sub>.