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

Supporting Information for

A Targetable Fluorescent Probe for Imaging Exogenous and Intracellular Formed Nitroxyl at Mitochondria in Living Cells

Mingguang Ren, Beibei Deng, Kai Zhou, Jian-Yong Wang, Xiuqi Kong, Weiying Lin *

Institute of Fluorescent Probes for Biological Imaging, School of Materials Science and Engineering, School of Chemistry and Chemical Engineering, University of Jinan, Jinan, Shandong 250022, P.R.

*Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Fluorescent Probes for Biological Imaging, School of Materials Science and Engineering, School of Chemistry and Chemical Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China. Email: weiyinglin2013@163.com.

Table of contents

Page

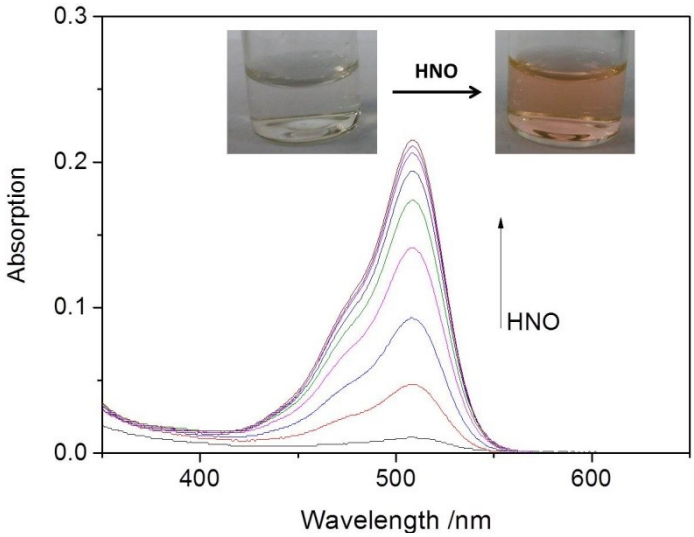


Figure S1 The absorption spectral changes of **Mito-HNO** (10 μM) upon addition of increasing concentrations of AS (0-10 equiv) in PBS buffer, pH 7.4, containing 5 % DMF as a cosolvent. Inset: Photographs showing the color changes of the probe **Mito-HNO** (1 mM) before and after addition of 10 equiv. AS to the solution.

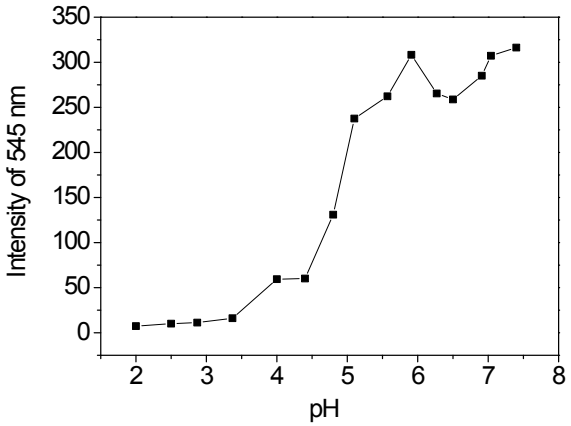


Figure S2 The emission intensity changes (at 545 nm) of compound **3** at different pH PBS buffer, containing 5 % DMF as a cosolvent (λ_{ex} = 488 nm).

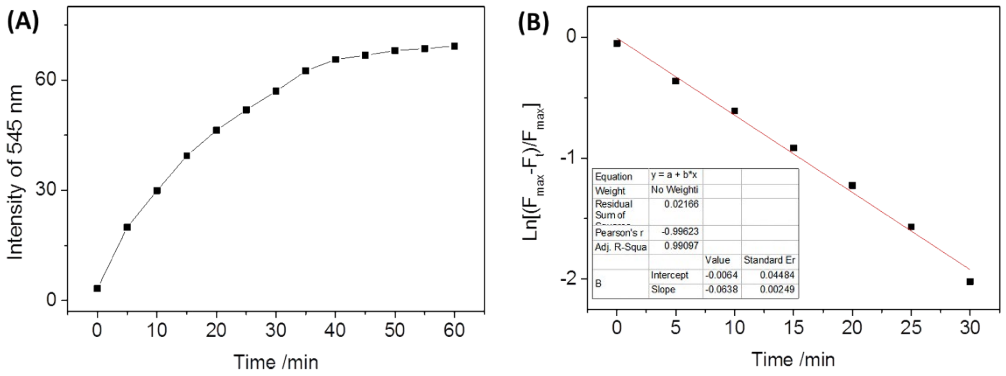


Figure S3 (A) The fluorescence intensities at 545 nm of **Mito-HNO** (5 μM) in the presence of AS (30 μM) at room temperature (25 °C) for continuously monitored at time intervals periods (0-60 min) in PBS buffer (pH 7.4, containing 5 % DMF as a cosolvent). (B) *Pseudo*-first-order kinetic plot of the reaction of **Mito-HNO** in the presence of AS at 25 °C.

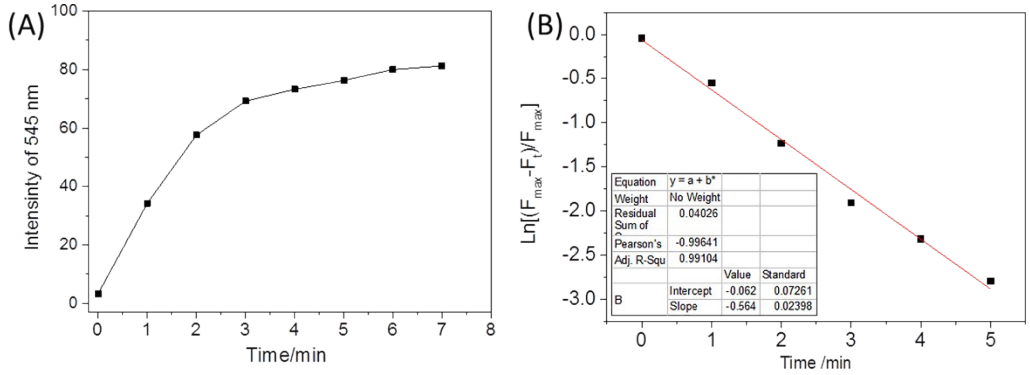


Figure S4 (A) The fluorescence intensities at 545 nm of **Mito-HNO** (5 μM) in the presence of AS (30 μM) at 37 °C for continuously monitored at time intervals periods (0-7 min) in PBS buffer (pH 7.4, containing 5 % DMF as a cosolvent). (B) *Pseudo*-first-order kinetic plot of the reaction of **Mito-HNO** in the presence of AS at 37 °C.

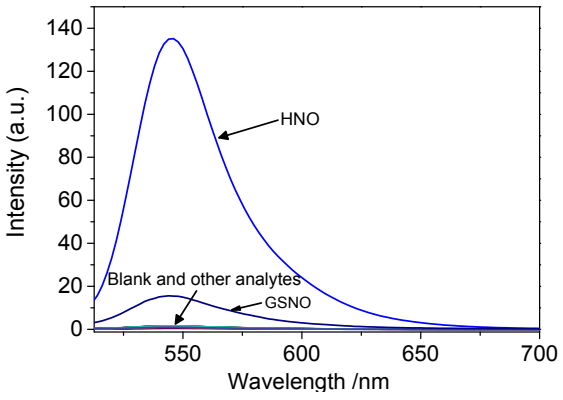


Figure S5 The fluorescence responses of the probe **Mito-HNO** (10.0 μM) to various relevant species (100 μM) in pH 7.4, PBS buffer (5 % DMF) at 37 °C for 20 min (λ_{ex} = 488 nm).

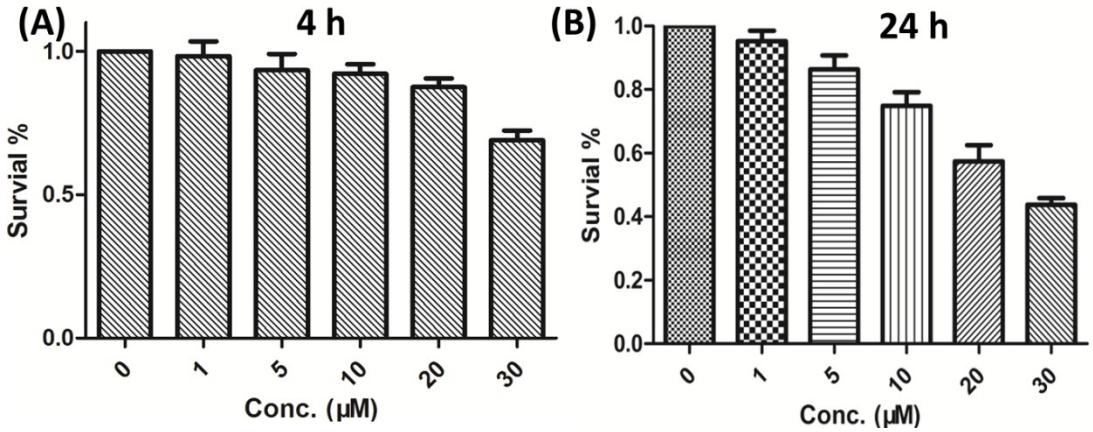


Figure S6 Cytotoxicity assays of **Mito-HNO** at different concentrations (0 μM; 5 μM; 10 μM; 20 μM; 30 μM) for HeLa cells in different time periods (A) 4h and (B) 24h.

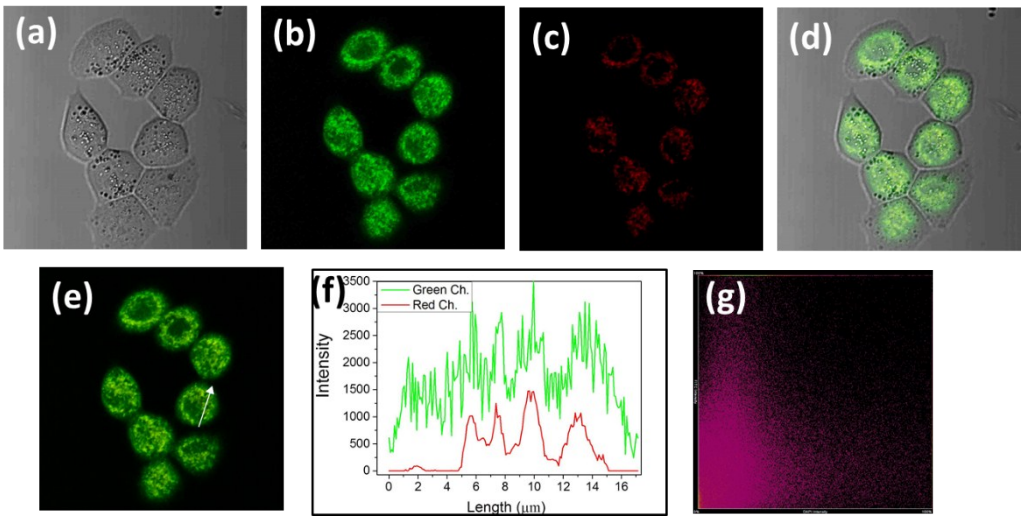


Figure S7 Brightfield and fluorescence images of HeLa cells stained with compound **4** and MitoTracker Red a) brightfield image; b) from green channel; c) from the red channel (mitochondria staining); d) overlay of brighfield, green and red channels; e) overlay of green and red channels ; f) Intensity profile of linear region of interest across the HeLa cell costained with green channel of compound **4** imaging of HNO and red

channel of Mito Tracker Red; g) Intensity scatter plot of green and red channels.

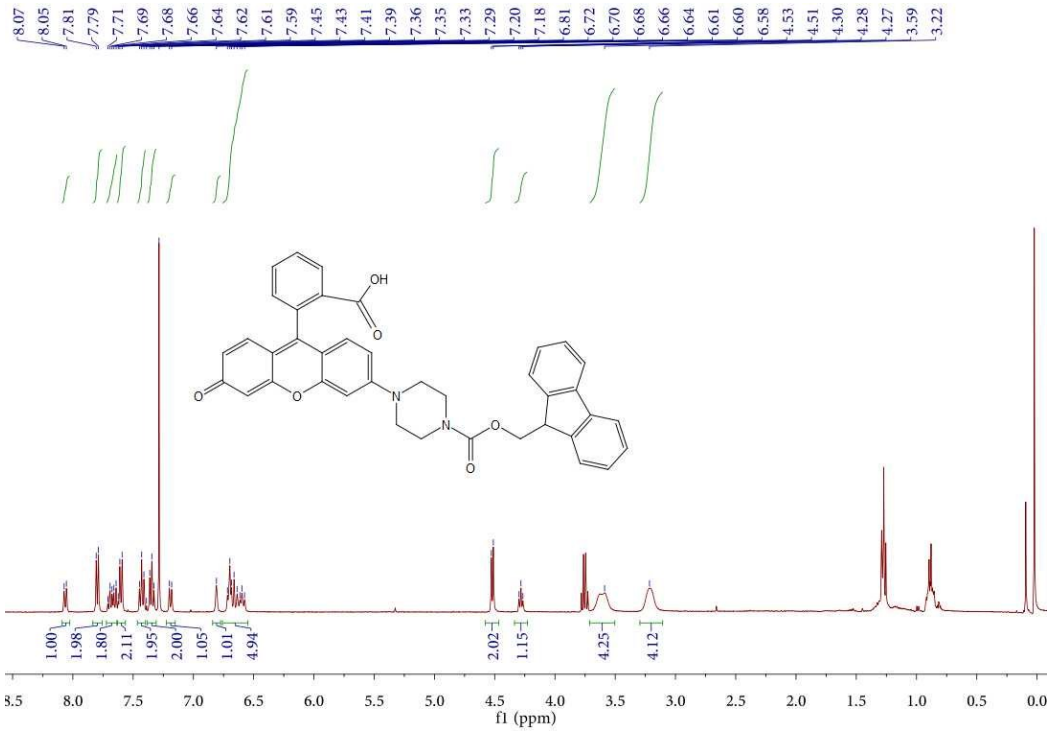


Figure S8 ^{1}H-NMR ($CDCl_3$) spectrum of compound **3**

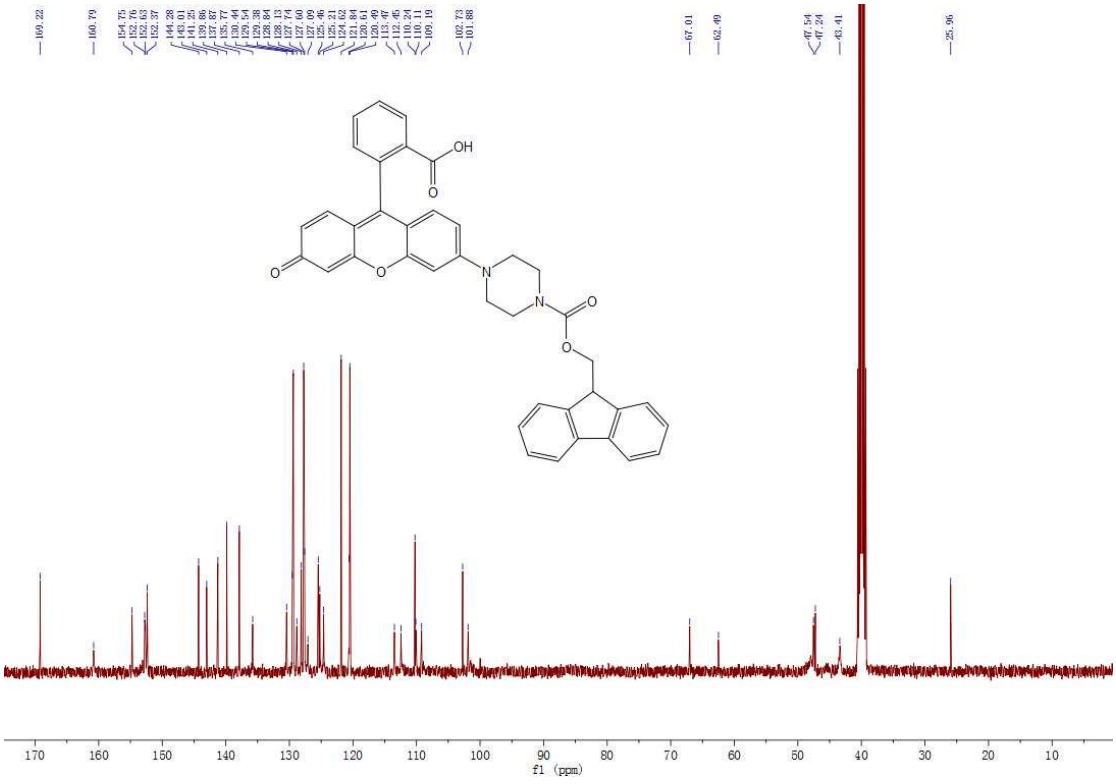


Figure S9 ^{13}C-NMR (DMSO-d_6) spectrum of compound **3**

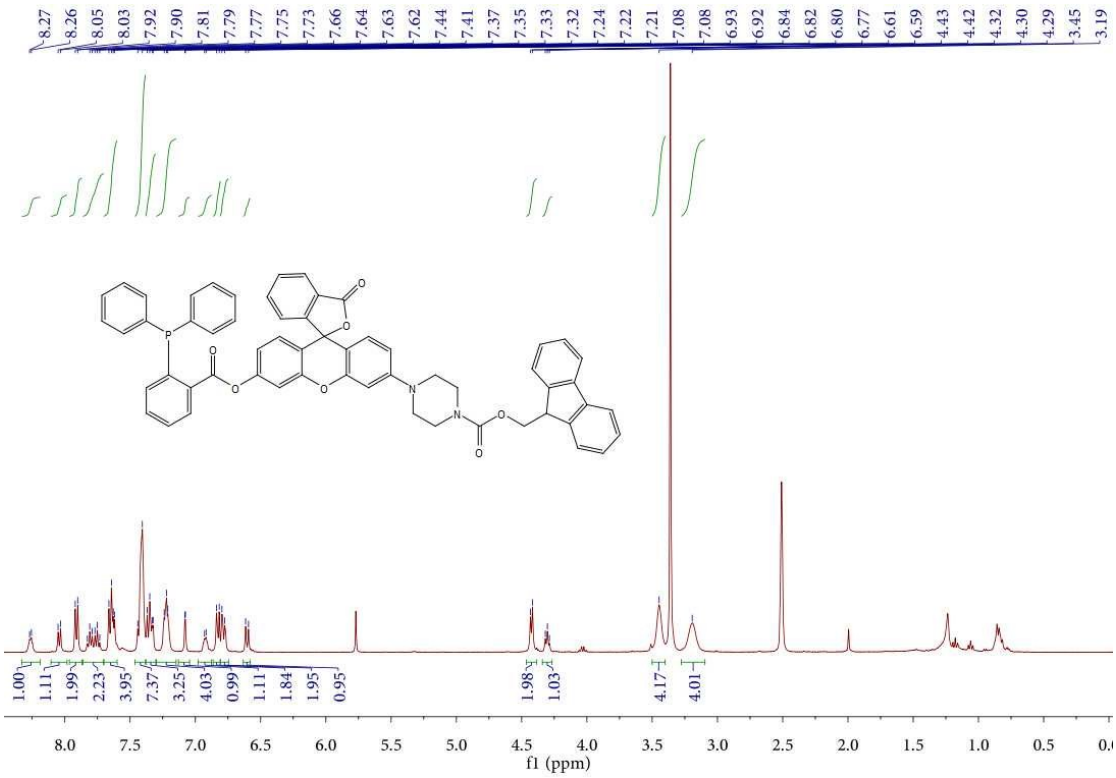


Figure S10 ^{1}H-NMR (DMSO-d_6) spectrum of compound **4**

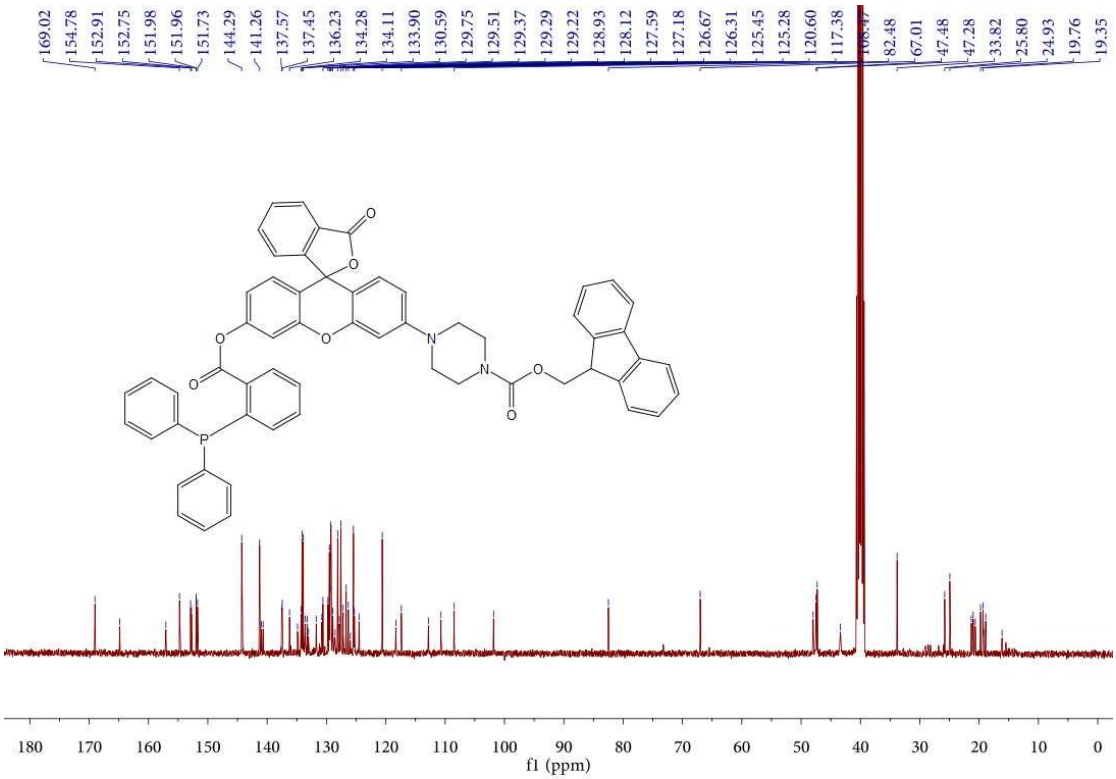


Figure S11 ^{13}C-NMR (DMSO-d_6) spectrum of compound **4**.

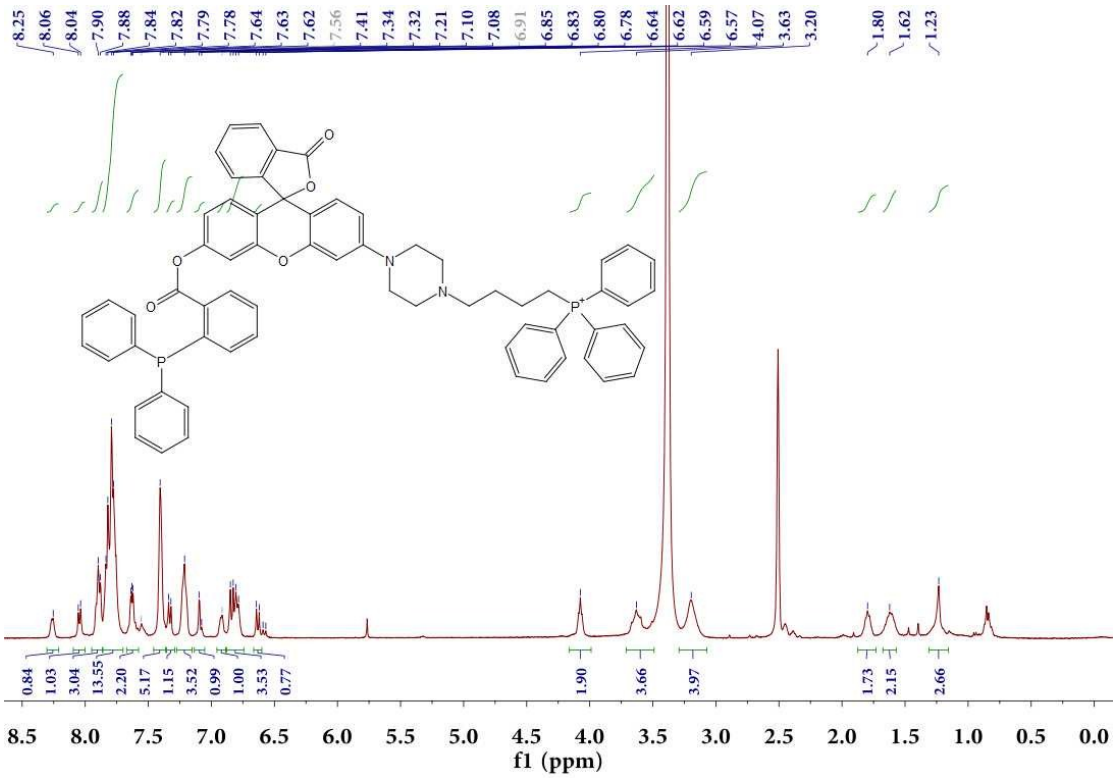


Figure S12 ^{1}H-NMR (DMSO-d_6) spectrum of compound **Mito-HNO**.

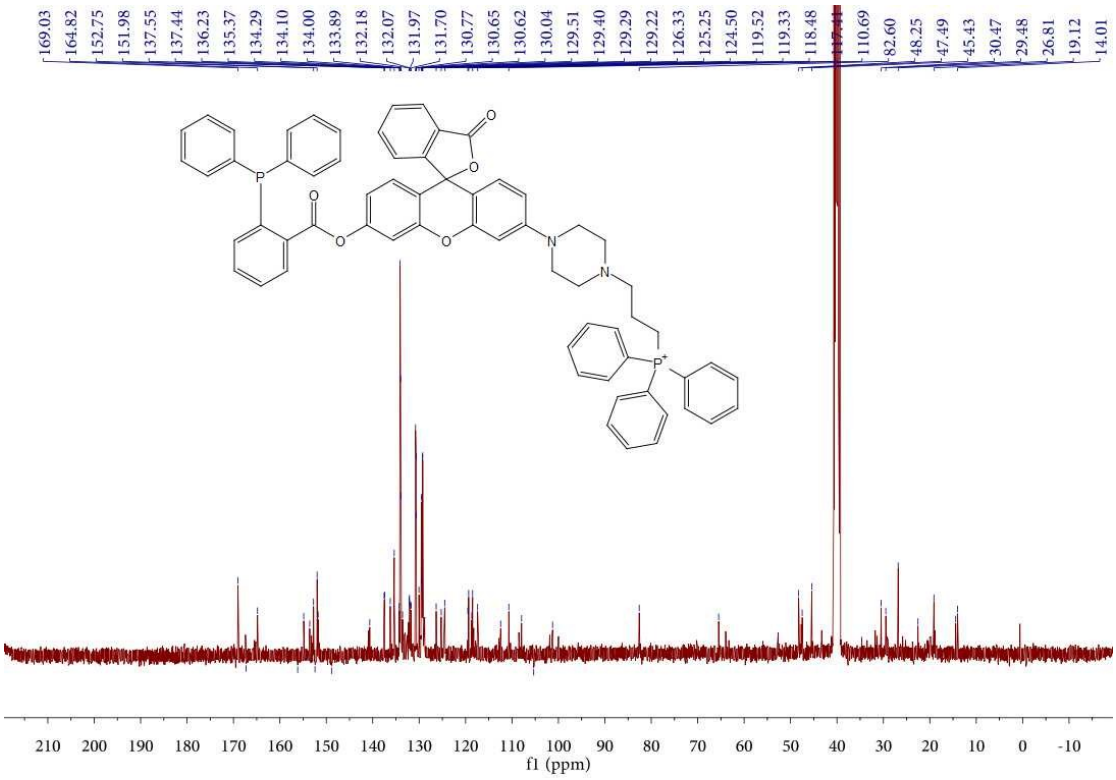


Figure S13 ^{13}C-NMR (DMSO-d_6) spectrum of compound **Mito-HNO**.

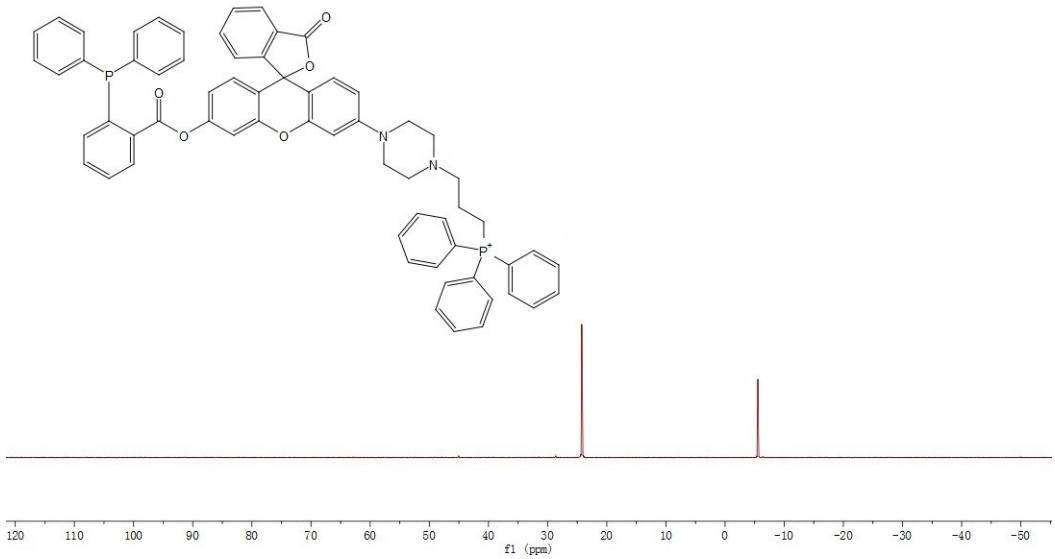
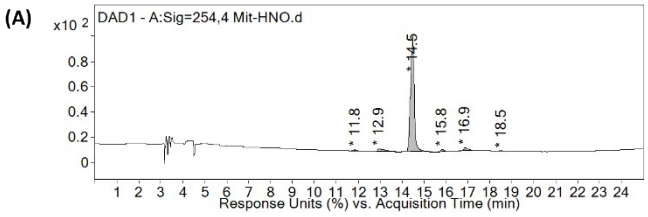


Figure S14 ^{31}P-NMR (DMSO-d_6) spectrum of compound **Mito-HNO** without reference.



(B) **Integration Peak List**

Start	RT	End	Height	Area	Area %
11.7	11.8	12	0.9	6.26	0.60
12.8	12.9	13.8	1.21	13.92	1.34
14.2	14.5	15.4	67.85	993.85	95.89
15.6	15.8	16.1	1.44	10.1	0.97
16.7	16.9	17.2	1.78	10.68	1.03
18.4	18.5	18.6	0.27	1.54	0.15

Figure S15 The purity of the probe **Mito-HNO** was analyzed by HPLC. (A) Typical HPLC chromatogram with UV/vis detection (254 nm). The retention times of **Mito-HNO** is 14.5 min. (B) Integration peak list of the results of HPLC chromatogram.