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

Supporting Information

N-annulated Perylene-Based Colorimetric and Ratiometric Near-Infrared Fluorescent Probes for the Selective Detection of Hydrogen Sulfide in Mitochondria, Lysosomes, and Serum

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1. Linear correction between the absorbance ratio and concentrations of NaHS

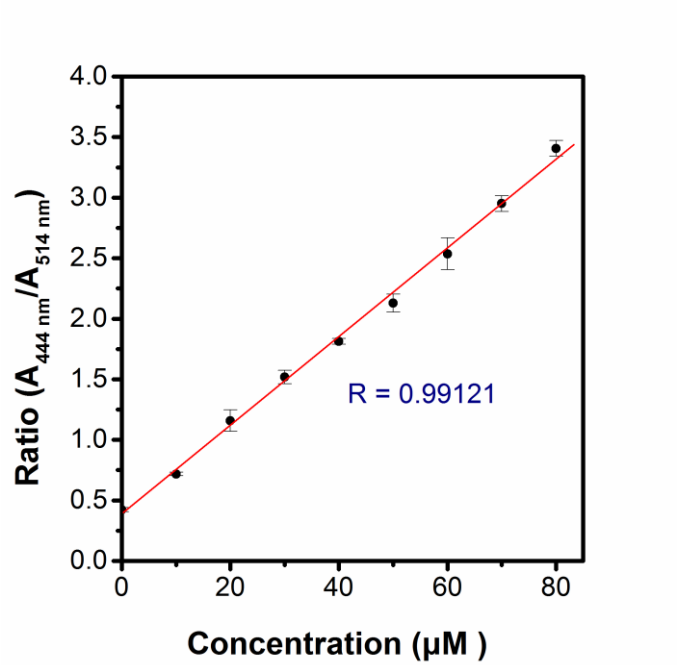


Figure S1. Linear correction between the absorbance ratio ($A_{444\ nm}/A_{514\ nm}$) of **NPNM** and concentrations of NaHS (0-80 μM). The absorbance was measured in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃.

2. Time-dependent absorption spectra of NPNM towards NaHS

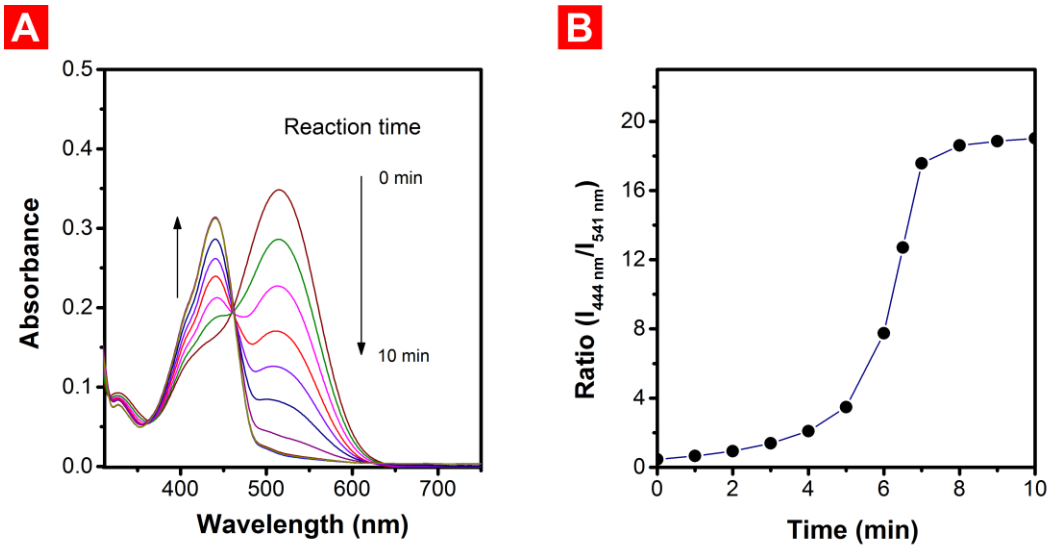


Figure S2. (A) Time-dependent (0-10 min) absorption spectra of **NPNM** (10 μM) towards NaHS (100 μM) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃. (B) The curve of time-dependent absorbance ratio ($I_{444\ nm}/I_{514\ nm}$) responses towards NaHS.

3. Time-dependent fluorescence spectra of NPNM towards NaHS

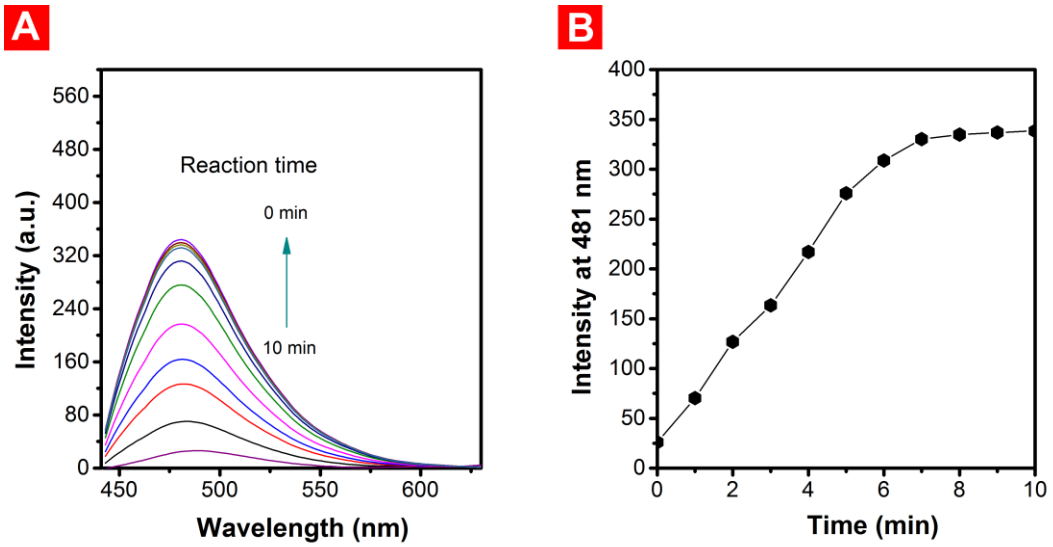


Figure S3. (A) Time-dependent (0-10 min) fluorescence emission spectra of **NPNM** (10 μM) towards NaHS in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃. (B) The variation curve of fluorescence intensity at 481 nm with the reaction time (0-10 min). λ_{ex} = 435 nm.

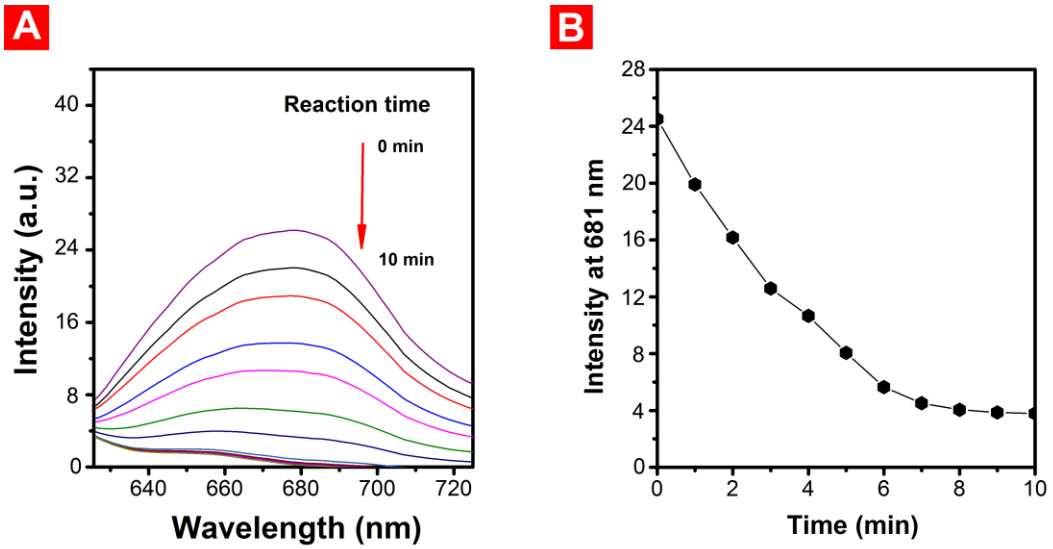


Figure S4. (A) Time-dependent (0-10 min) fluorescence emission spectra of **NPNM** (10 μM) towards NaHS in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃. (B) The variation curve of fluorescence intensity at 681 nm with the reaction time (0-10 min). λ_{ex} = 510 nm.

4. Absorption and fluorescence response of Mito-NPNM towards NaHS

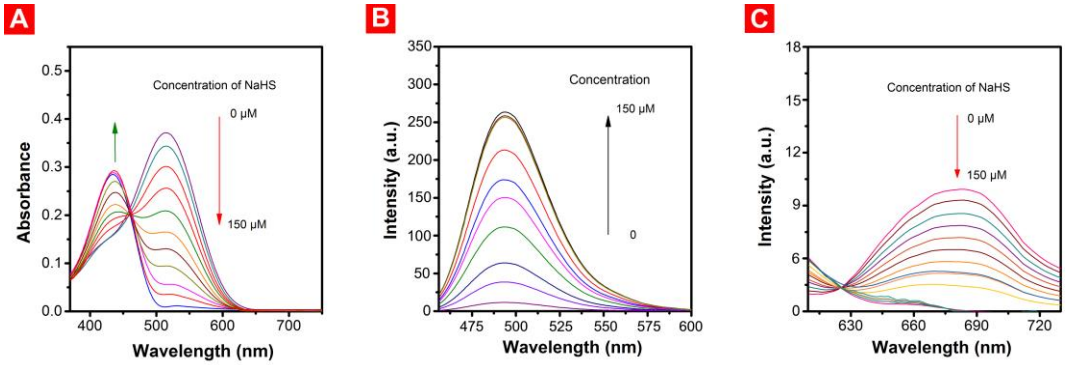


Figure S5. The absorption (A) and fluorescence emission (B and C) changes of **Mito-NPNM** (10 μM) with the various concentration of NaHS (0-150 μM) in PBS buffer solution (pH = 7.4, 37 ℃). (B) λ_{ex} = 435 nm. (C) λ_{ex} = 510 nm.

5. Absorption and fluorescence response of Lyso-NPNM towards NaHS

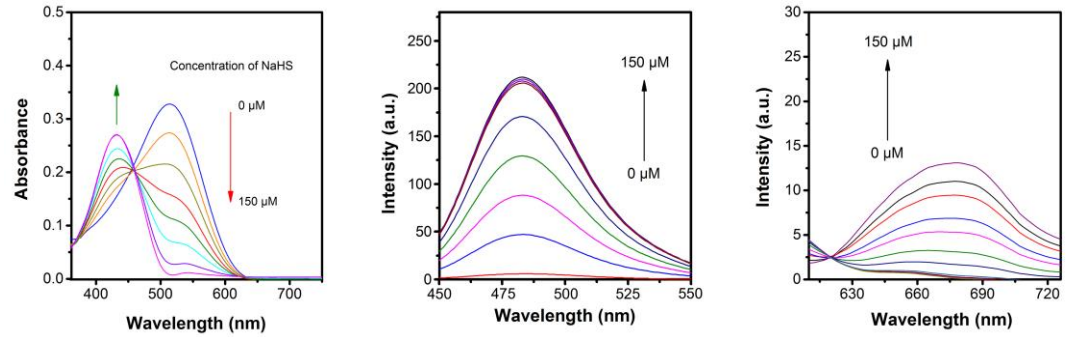


Figure S6. The absorption (A) and fluorescence emission (B and C) changes of **Lyso-NPNM** (10 μM) with the various concentration of NaHS (0-150 μM) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃. (B) λ_{ex} = 435 nm. (C) λ_{ex} = 510 nm.

6. Selectivity

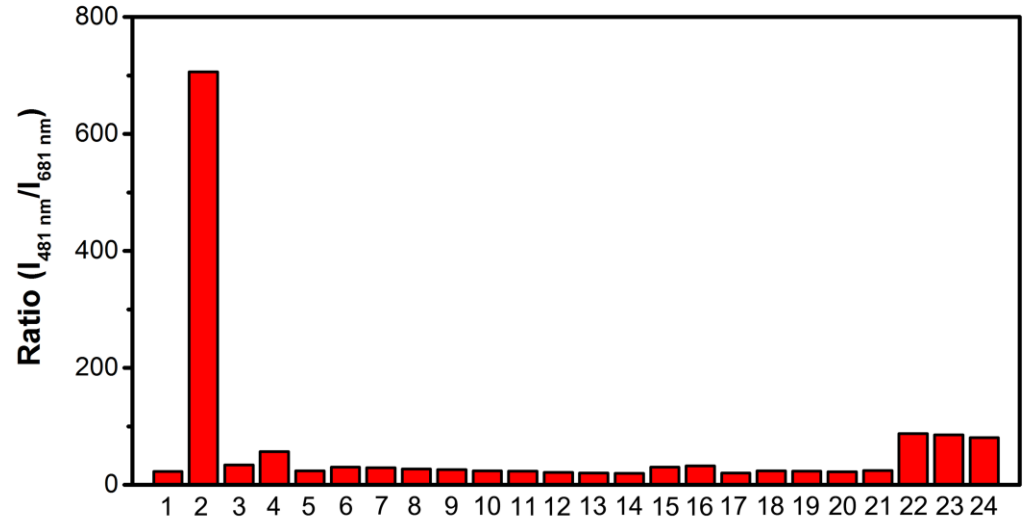


Figure S7. The selectivity of **Mito-NPNM** (10 μM) towards different molecules (1: blank; 2: NaHS; 3: HSO_3^-; 4: SO_3^{2-}; 5: SO_4^{2-}, 6: $S_2O_3^{2-}$; 8: $S_2O_4^{2-}$; 9: F^-; 10: Cl^-; 11: Br^-; 12: I^-; 13: AcO^-; 14: SCN^-; 15: CO_3^{2-}; 16: HCO_3^-; 17: $H_2PO_4^-$; 18: NO_2^-; 19: OH^-; 20: ClO^-, 21: H_2O_2; 22: t-BuOOH; 23: Cys; 24: Hcy and 25: GSH) in PBS buffer solution at 37 ℃. The concentrations of biological thiols (Cys, Hcy and GSH) were 5 mM, and the concentrations of other molecules were 200 μM.

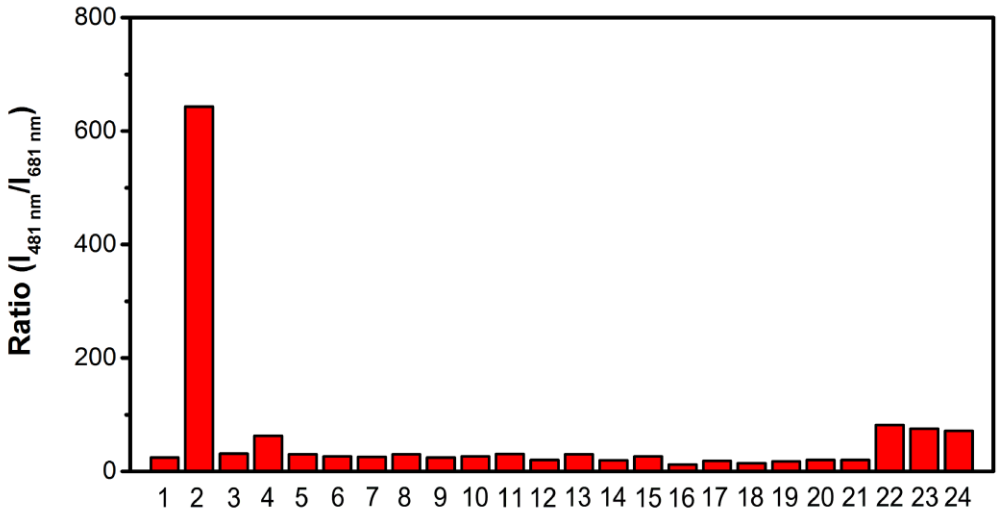


Figure S8. The selectivity of **Lyso-NPNM** (10 μM) towards different molecules (1: blank; 2: NaHS; 3: HSO_3^-; 4: SO_3^{2-}; 5: SO_4^{2-}, 6: $S_2O_3^{2-}$; 8: $S_2O_4^{2-}$; 9: F^-; 10: Cl^-; 11: Br^-; 12: I^-; 13: AcO^-; 14: SCN^-; 15: CO_3^{2-}; 16: HCO_3^-; 17: $H_2PO_4^-$; 18: NO_2^-; 19: OH^-; 20: ClO^-, 21: H_2O_2; 22: t-BuOOH; 23: Cys; 24: Hcy and 25: GSH) in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH=7.4) at 37 ℃. The concentrations of biological thiols (Cys, Hcy and GSH) were 5 mM, and the

concentrations of other molecules were 200 μM.

7. The fluorescent emission of the core of N-annulated perylene

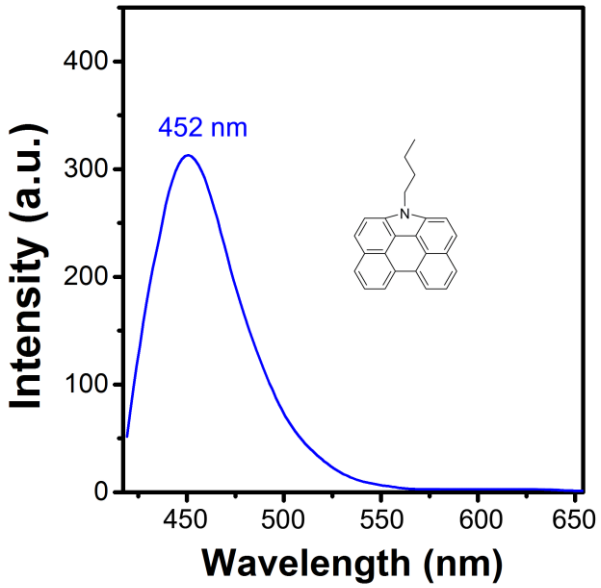


Figure S9. The fluorescence emission spectra of the core of N-annulated perylene in DMSO-PBS buffer solution (DMSO: PBS = 3: 7, v/v, pH = 7.4) at 37 ℃. λ_{ex} = 400 nm.

8. The stability of NPNM

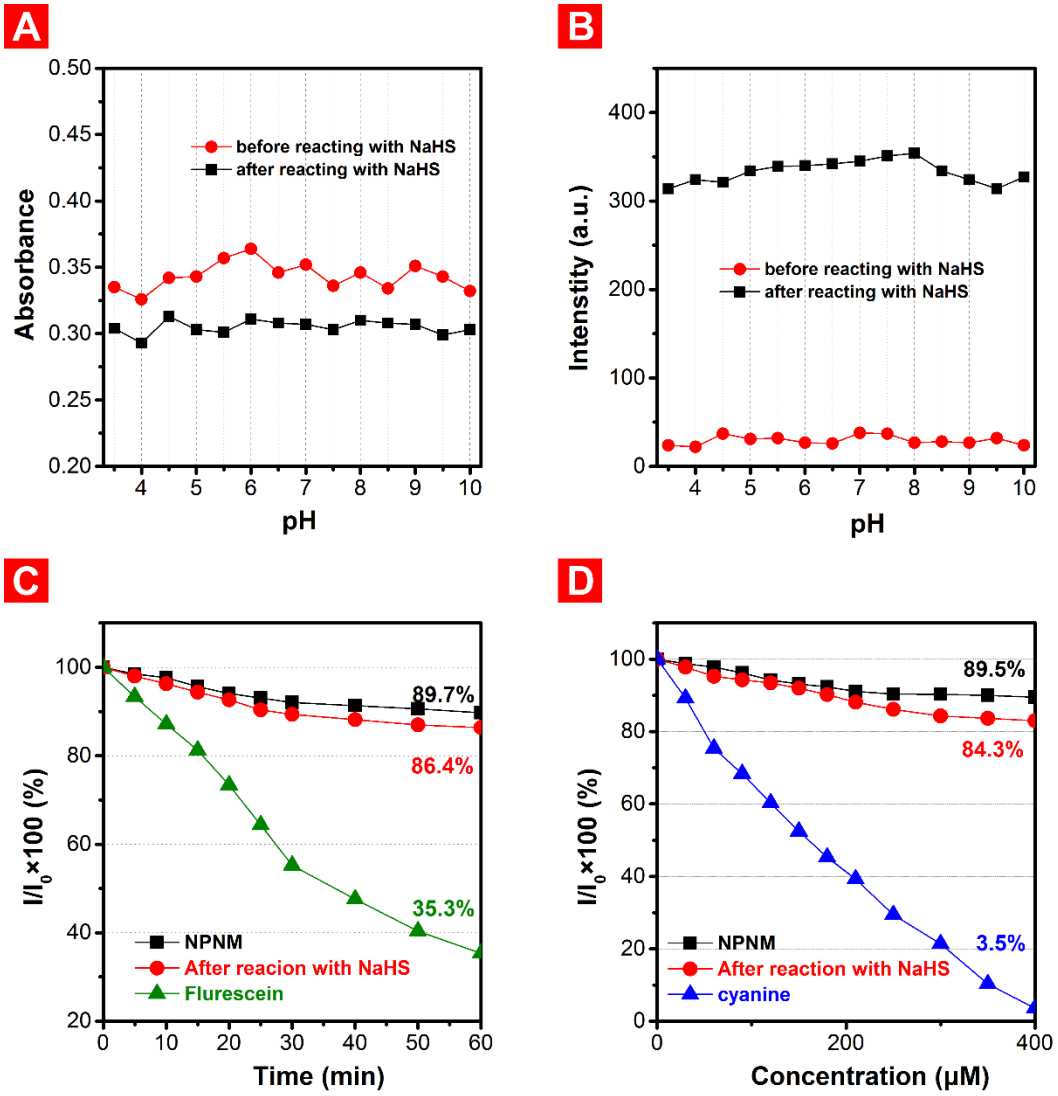


Figure S10. The absorption (A) and fluorescence intensity (B) of **NPNM** (10 μM) before and after reacting with NaHS (100 μM) for 7 minutes at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction. (C) Photostability of **NPNM** (10 μM) and the product after reacting with NaHS (100 μM) compared with fluorescein isothiocyanate were studied under 1 kW/m^2 light irradiation for 0-60 minutes. (D) Chemostability of **NPNM** (10 μM) and the product after reacting with NaHS (100 μM) compared with cyanine were also studied after the addition of different concentrations (0-40 equivalents) of NaClO. I_0 represented the initial fluorescence intensity and I represented the intensity after the treatment.

9. The pH stability of Mito-NPNM and Lyso-NPNM

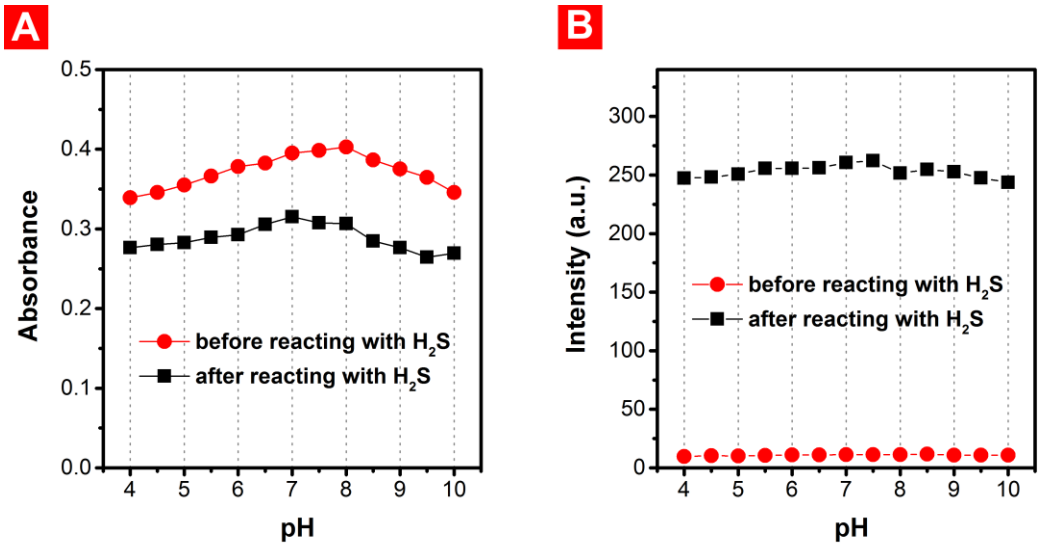


Figure S11. The absorption (A) and fluorescence intensity (B) of **Mito-NPNM** (10 μM) before and after reacting with NaHS (100 μM) for at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction.

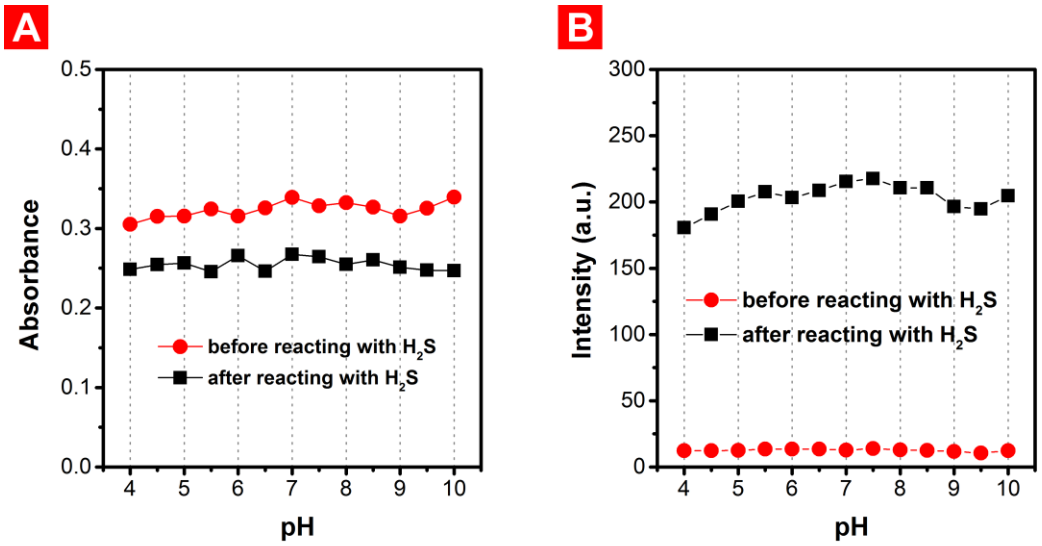


Figure S12. The absorption (A) and fluorescence intensity (B) of **Lyso-NPNM** (10 μM) before and after reacting with NaHS (100 μM) at different pH values. The maximum absorption wavelength at 514 nm and 444 nm were measured respectively before and after the reaction. The fluorescence emissions at 681 nm and 481 nm were also measured before and after the reaction.

10. Photostability of Mito-NPNM and Lyso-NPNM

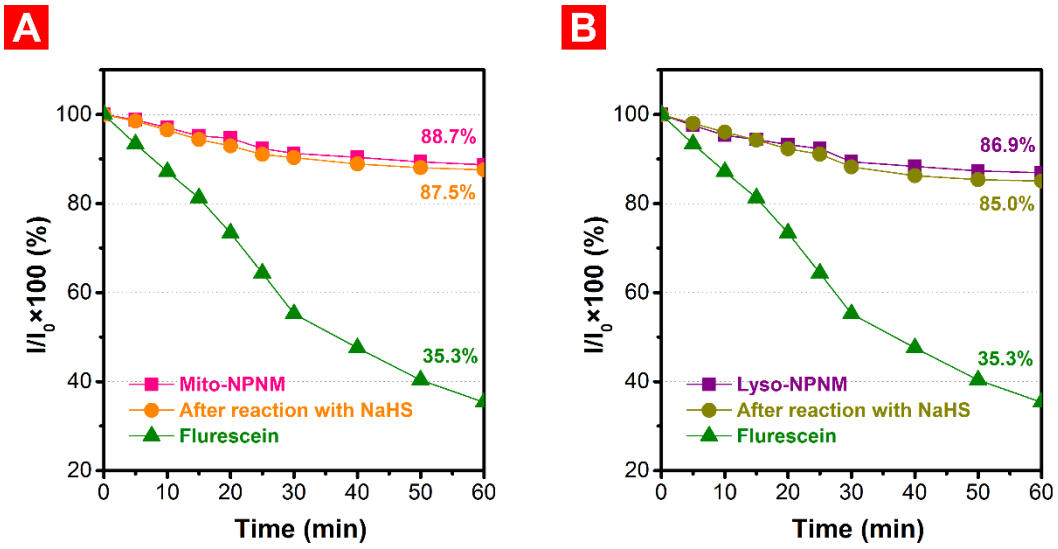


Figure S13. (A) Photostability of 10 μM **Mito-NPNM** (A), **Lyso-NPNM** (B) and their products after reacting with NaHS (100 μM) compared with fluorescein isothiocyanate were studied under 1 kW/m^2 light irradiation for 0-60 minutes. I_0 represented the initial fluorescence intensity at 481 nm and I represented the intensity after the treatment at 681 nm.

11. Chemostability of Mito-NPNM and Lyso-NPNM

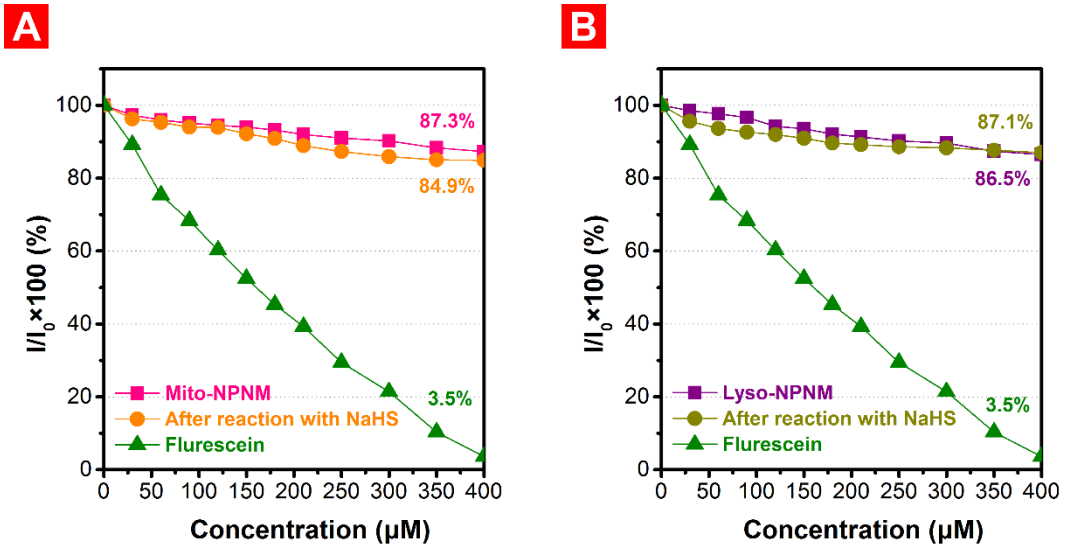


Figure S14. Chemostability of 10 μM **Mito-NPNM** (A), **Lyso-NPNM** (B) and their products after reacting with NaHS (100 μM) compared with cyanine were also studied after the addition of different concentrations of NaClO (0-40 equivalents). I_0 represented the initial fluorescence intensity and I represented the intensity after the treatment.

12. The cytotoxicity of Lyso-NPNM and Mito-NPNM

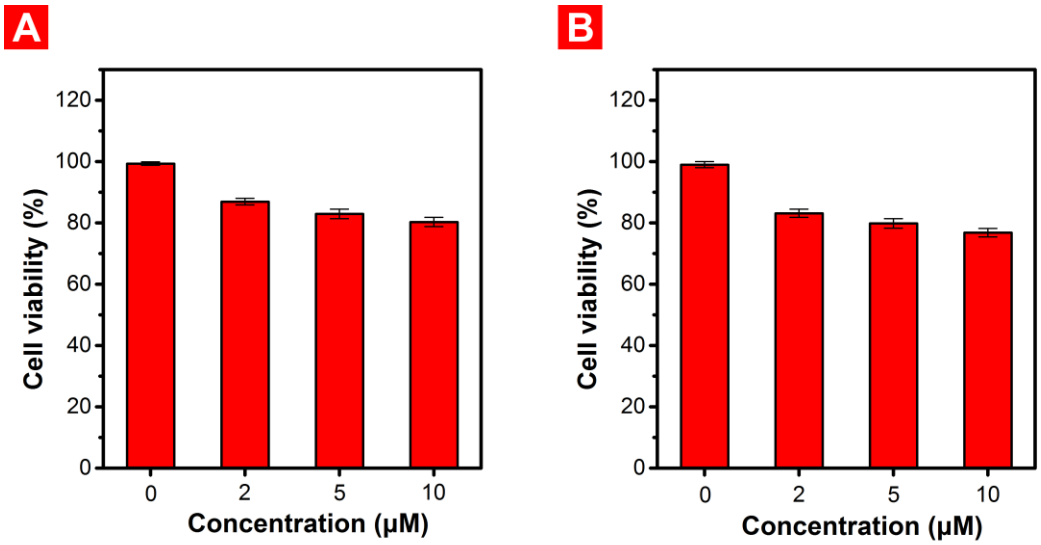


Figure S15. Cell viability (%) estimated by MTT proliferation tests versus incubation concentration of **Lyso-NPNM** (A) and **Mito-NPNM** (B). Hela cells were incubated with 0-10 μM **Lyso-NPNM** or **Mito-NPNM** at at 37 ℃ for 24 hours.

13. Detecting H_2S in fetal bovine serum

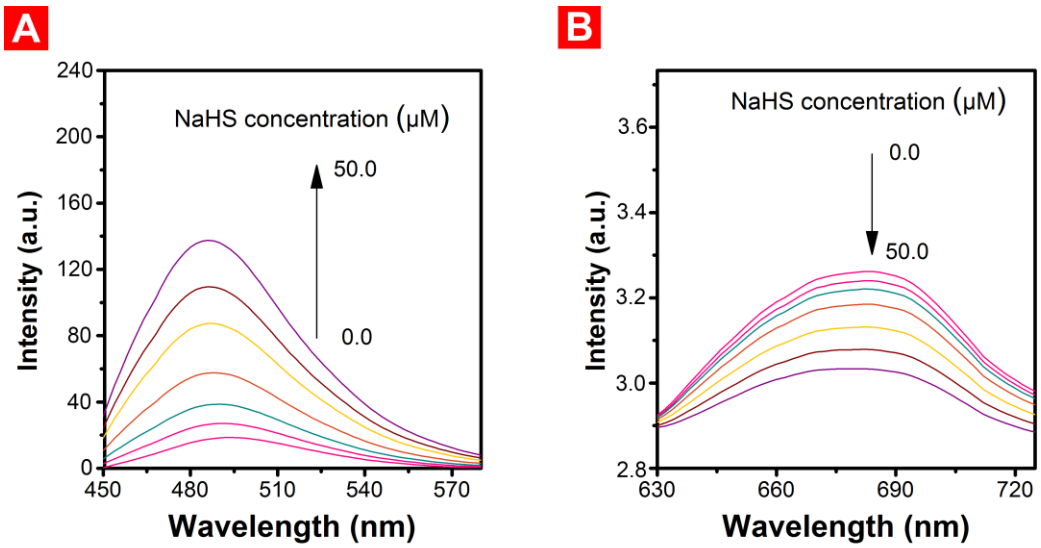


Figure S16. The changes of fluorescence spectra of **NPNM** in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) λ_{ex} = 435 nm; (B) λ_{ex} = 510 nm.

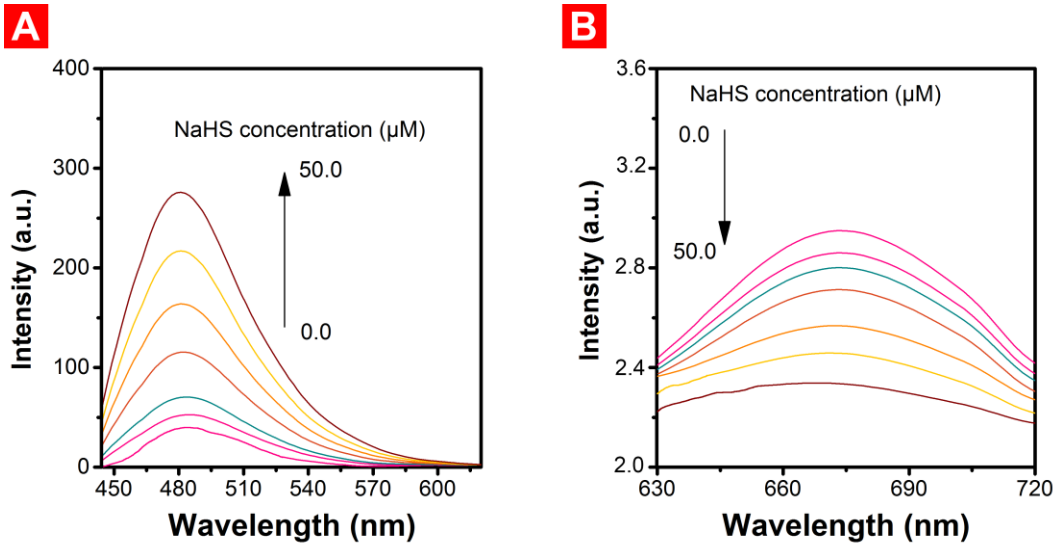


Figure S17. The changes of fluorescence spectra of **Mito-NPNM** in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) λ_{ex} = 435 nm; (B) λ_{ex} = 510 nm.

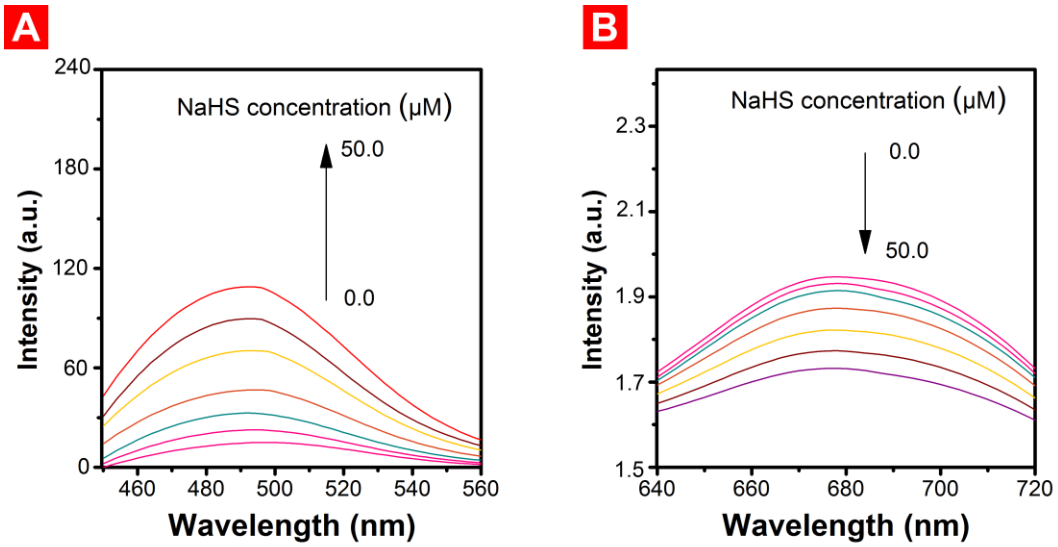


Figure S18. The changes of fluorescence spectra of **Lyso-NPNM** in the presence of different concentration (0.0, 5.0, 10.0, 17.0, 20.0, 40.0, and 50.0 μM) of NaHS in the FBS serum. (A) λ_{ex} = 435 nm; (B) λ_{ex} = 510 nm.

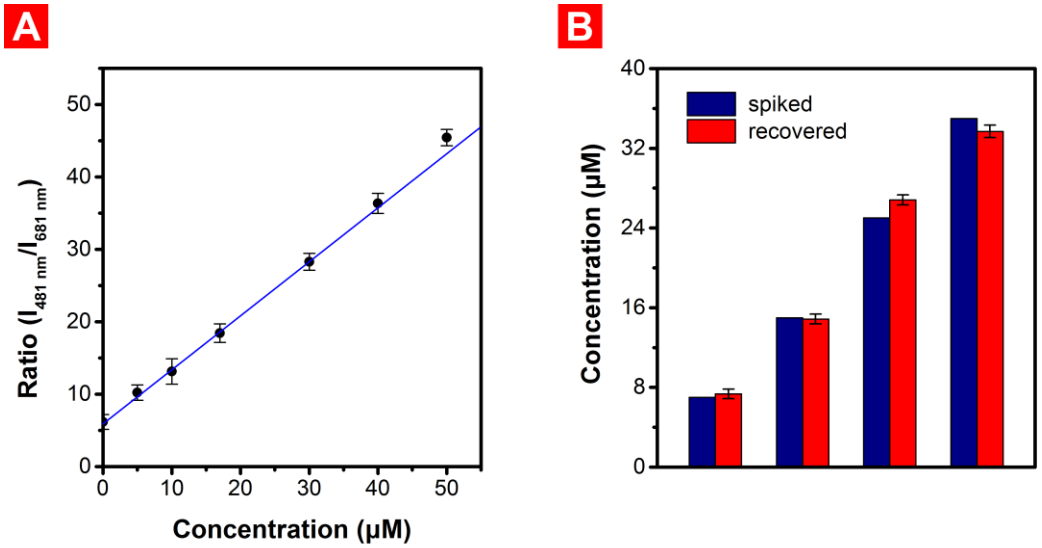


Figure S19. (A) Plotting the ratiometric fluoresecne intensity ($I_{481\ nm}/I_{681\ nm}$) as a function of low NaHS concentration (0-50 μM) for **NPNM** (10 μM). (B) The spiked (7.0, 15.0, 25.0 and 35.0 μM) and measured concentrations (7.55, 14.76, 27.63 and 32.70 μM) of NaHS in diluted serum solution.

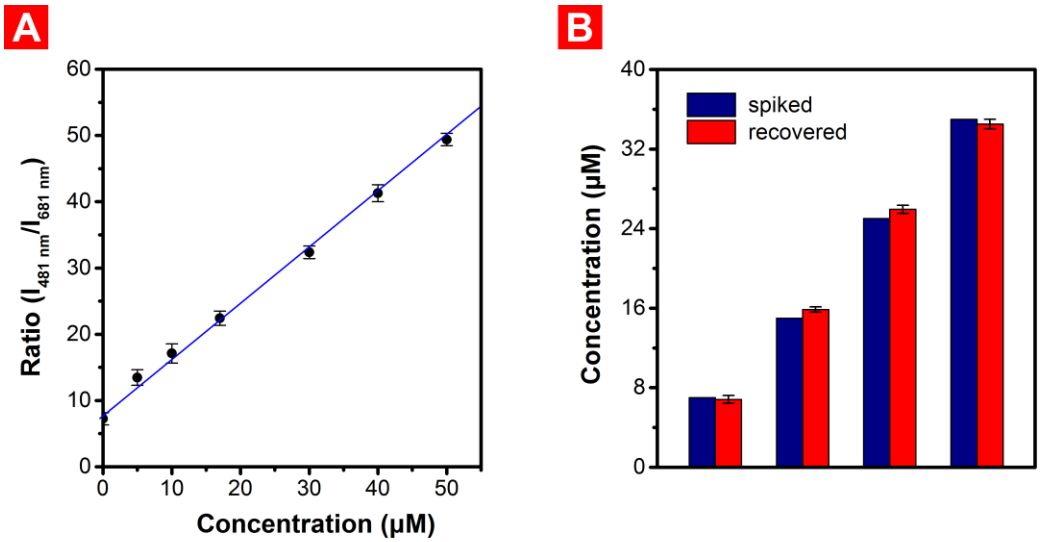


Figure S20. (A) Plotting the ratiometric fluoresecne intensity ($I_{481\ nm}/I_{681\ nm}$) as a function of low NaHS concentration (0-50 μM) for **Lyso-NPNM** (10 μM). (B) The spiked (7.0, 15.0, 25.0 and 35.0 μM) and measured concentrations (6.62, 15.86, 27.03 and 34.02 μM) of NaHS in diluted serum solution.

14. Characterization of new compounds

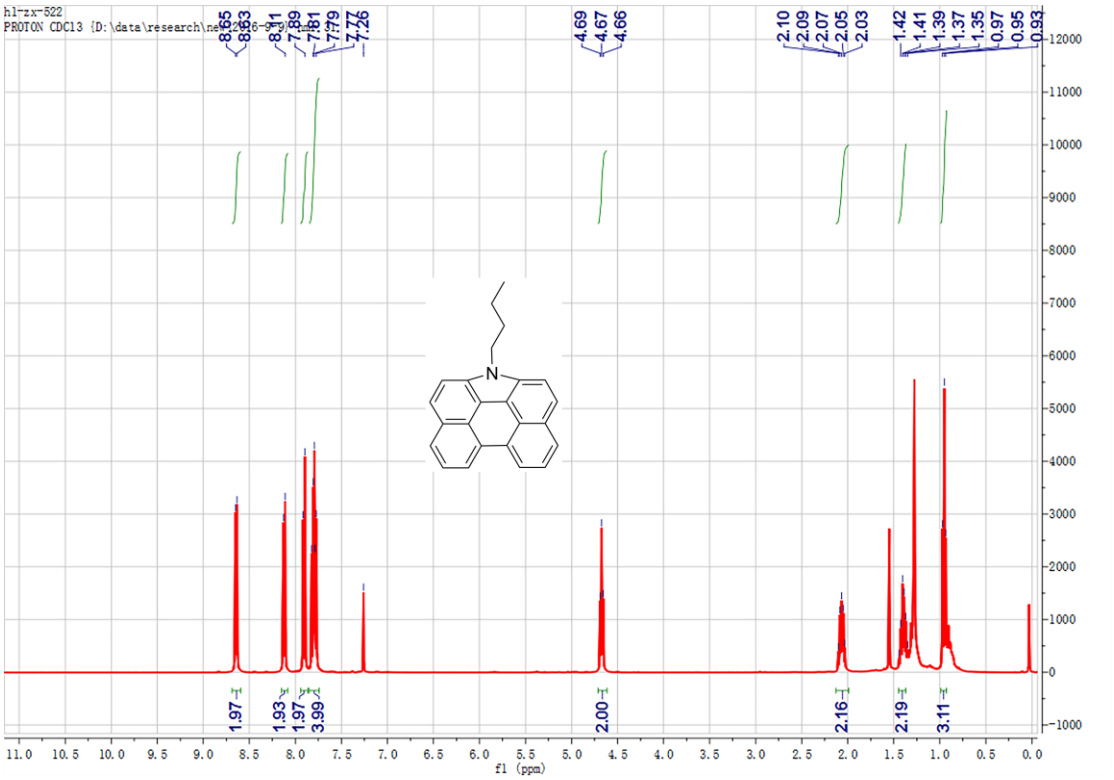


Figure S21. ^{1}H NMR spectrum of intermediate compound **2** in $CDCl_3$

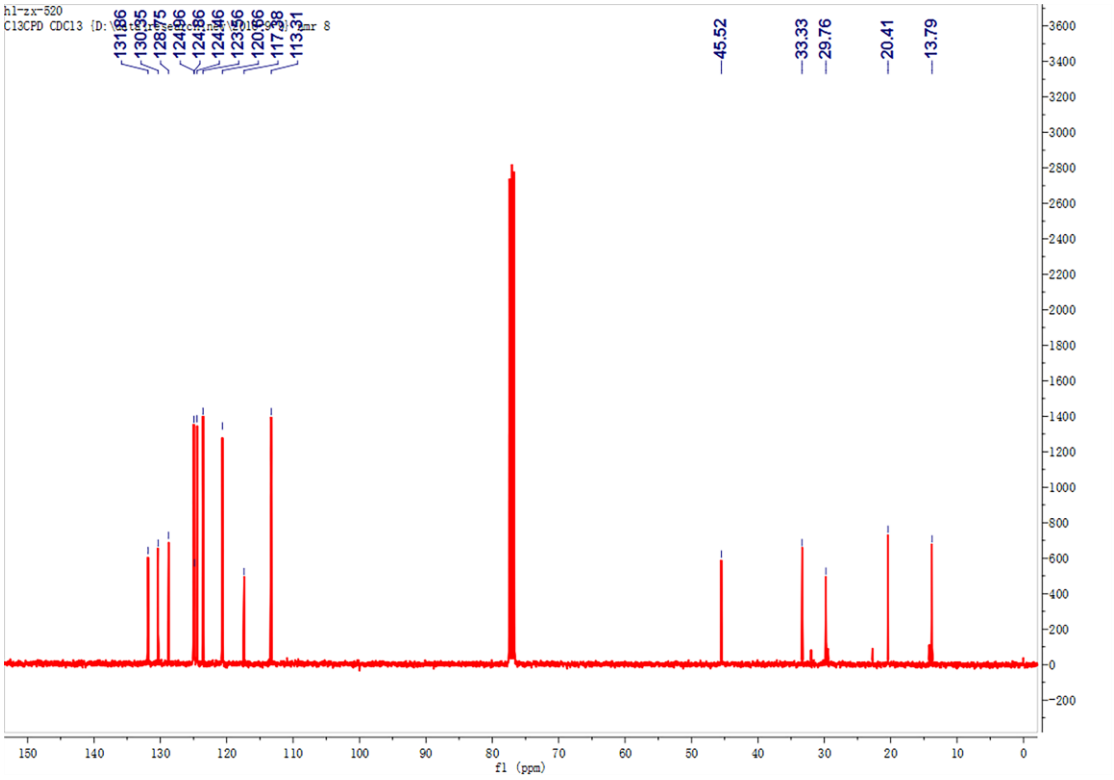
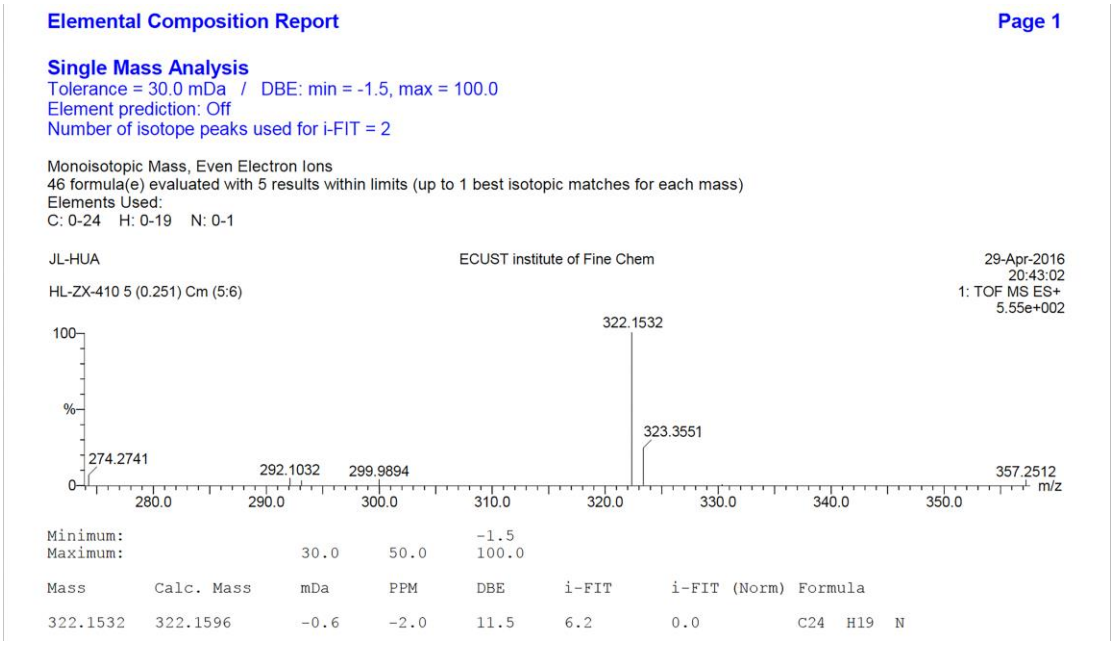


Figure S22. ^{13}C NMR spectrum of intermediate compound **2** in $CDCl_3$



Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
46 formula(e) evaluated with 5 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-24 H: 0-19 N: 0-1

JL-HUA ECUST institute of Fine Chem 29-Apr-2016 20:43:02
HL-ZX-410 5 (0.251) Cm (5:6) 1: TOF MS ES+ 5.55e+002

Minimum:		30.0	50.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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Figure S23. HRMS of intermediate compound **2**

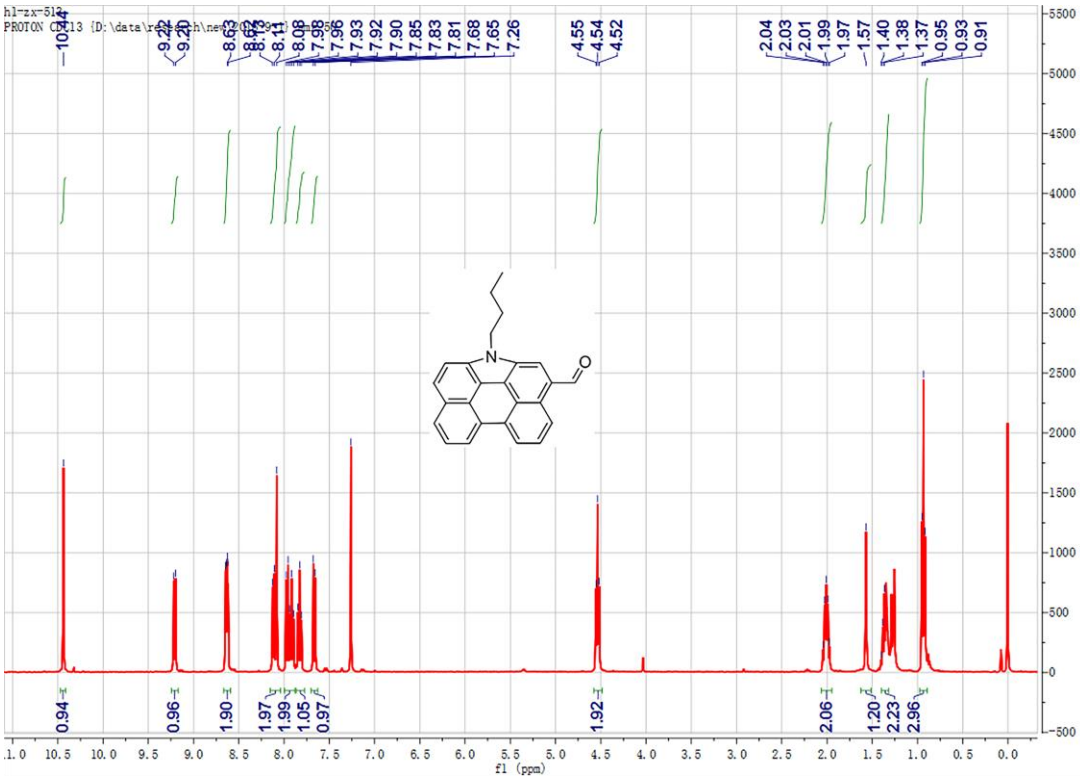


Figure S24. ^{1}H NMR spectrum of intermediate compound **3** in $CDCl_3$

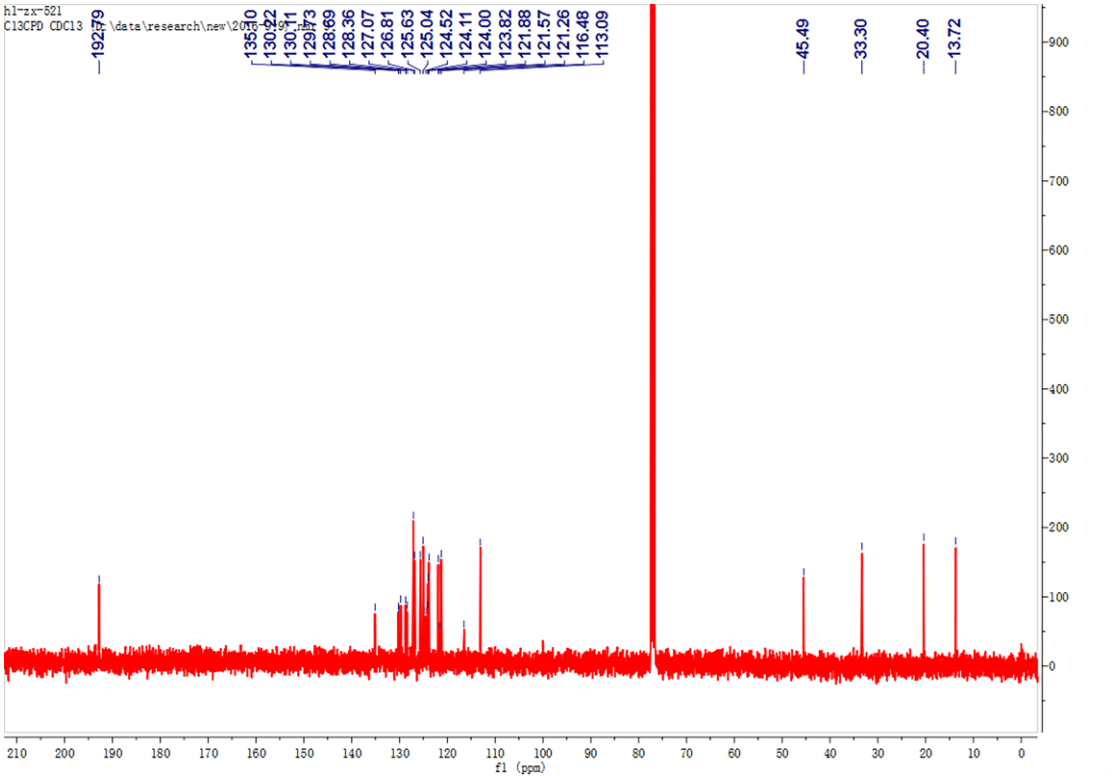


Figure S25. ^{13}C NMR spectrum of intermediate compound **3** in $CDCl_3$

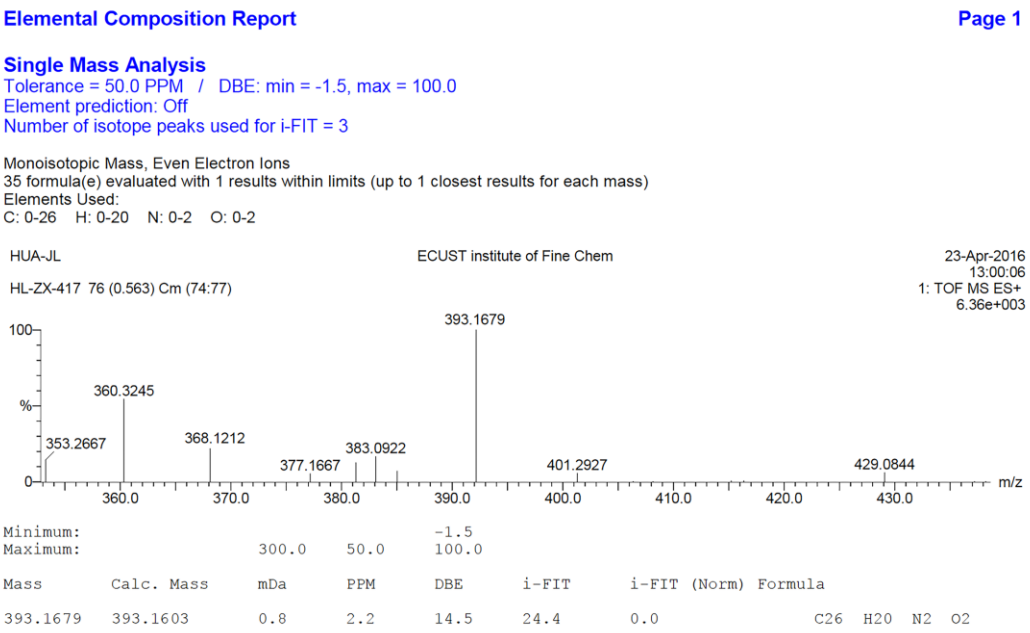


Figure S26. HRMS of intermediate compound **3**

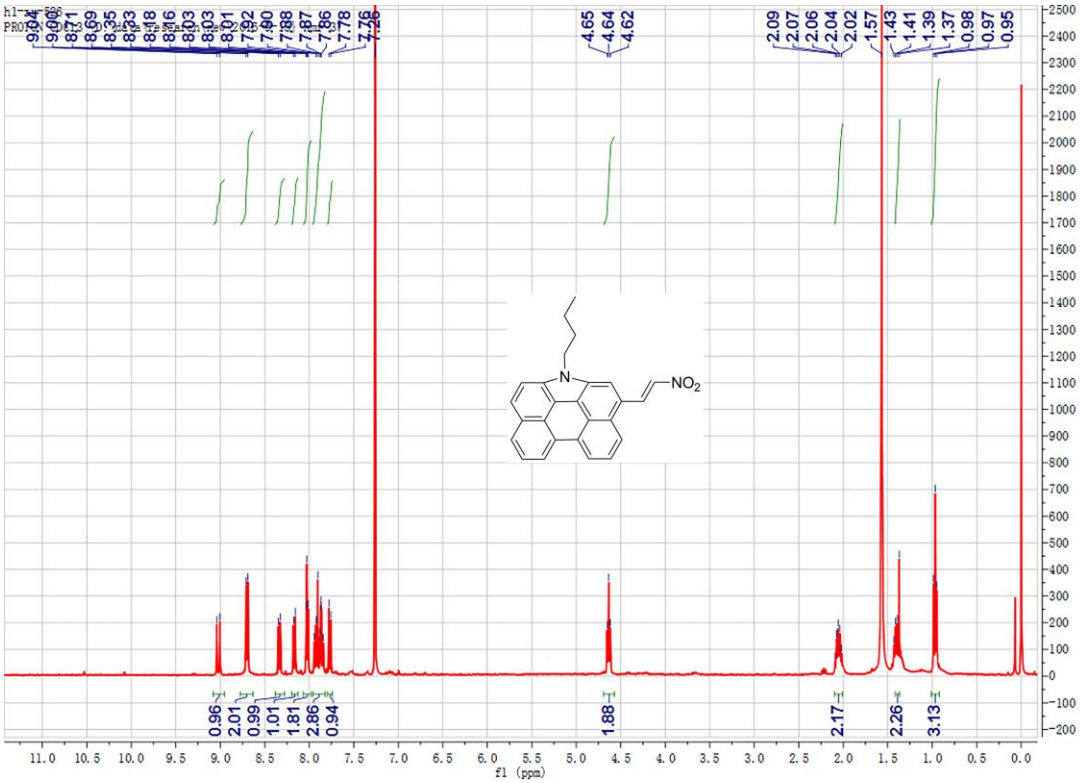


Figure S27. ^{1}H NMR spectrum of **NPNM** in $CDCl_3$

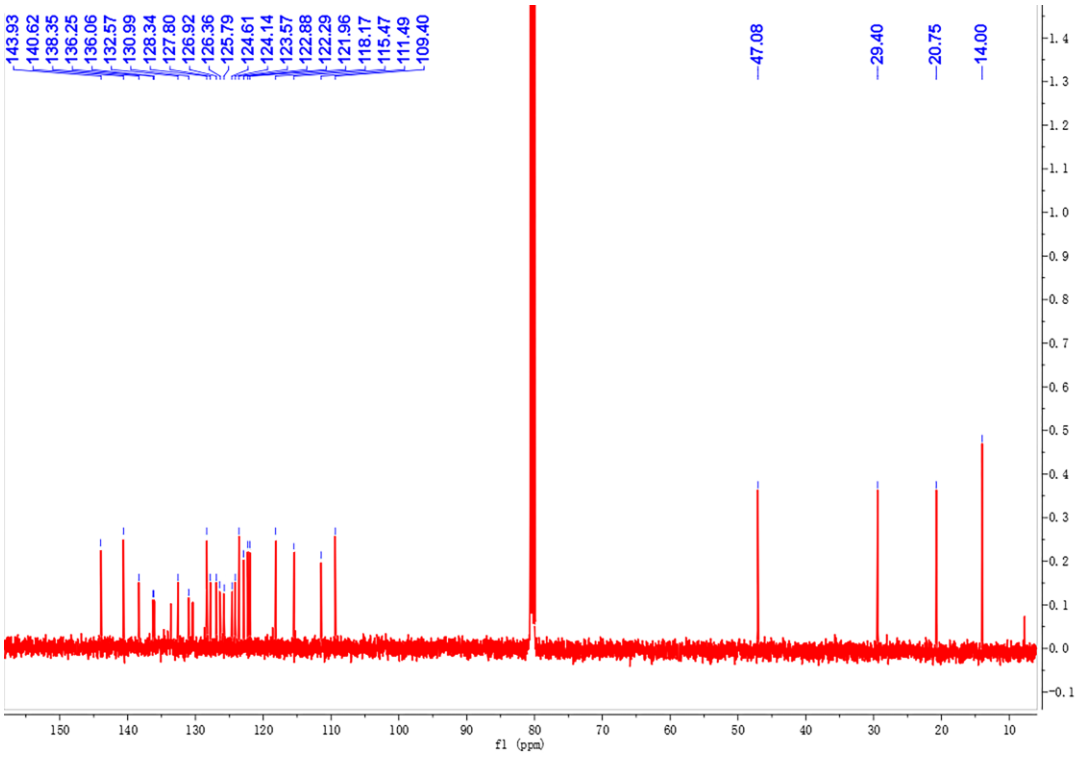
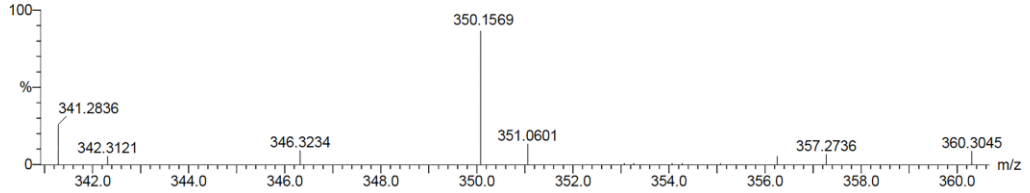


Figure S28. ^{13}C NMR spectrum of **NPNM** in $CDCl_3$

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
7 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-25 H: 0-19 N: 0-1 O: 0-1

HUA-JL ECUST institute of Fine Chem 23-Apr-2016 12:50:30
HL-ZX-437 37 (0.316) Cm (36:38) 1: TOF MS ES+ 4.34e+002



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
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Figure S29. HRMS of **NPNM**

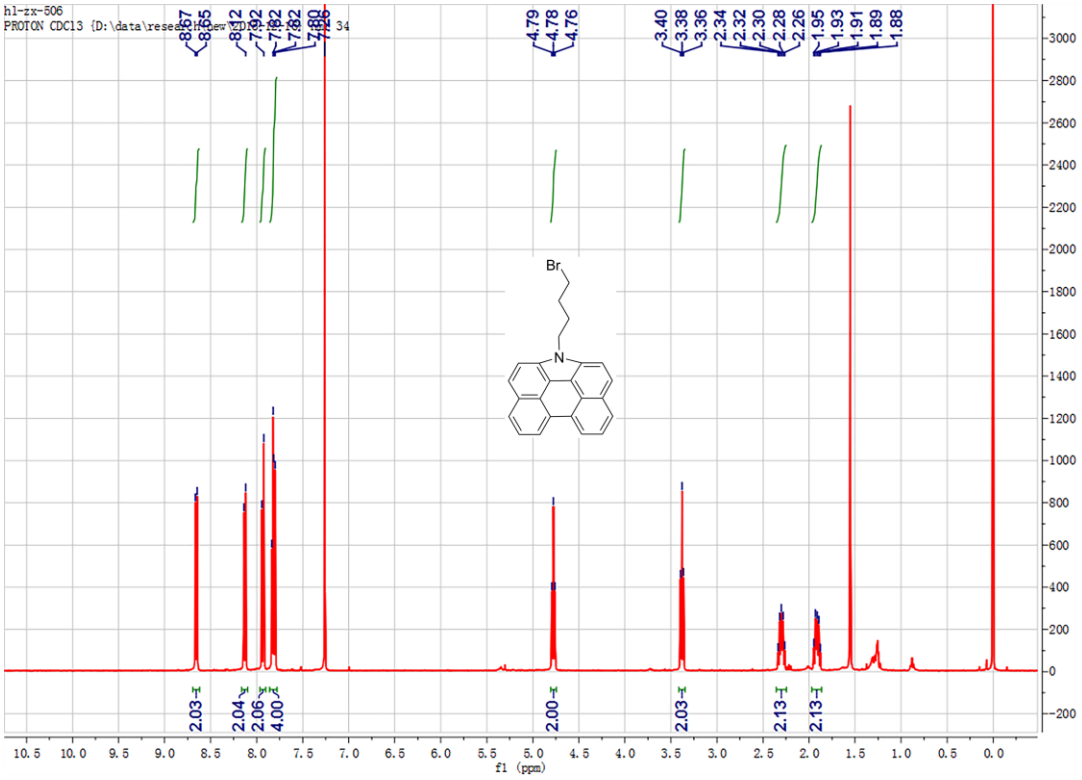


Figure S30. ^{1}H NMR spectrum of intermediate compound **4** in $CDCl_3$

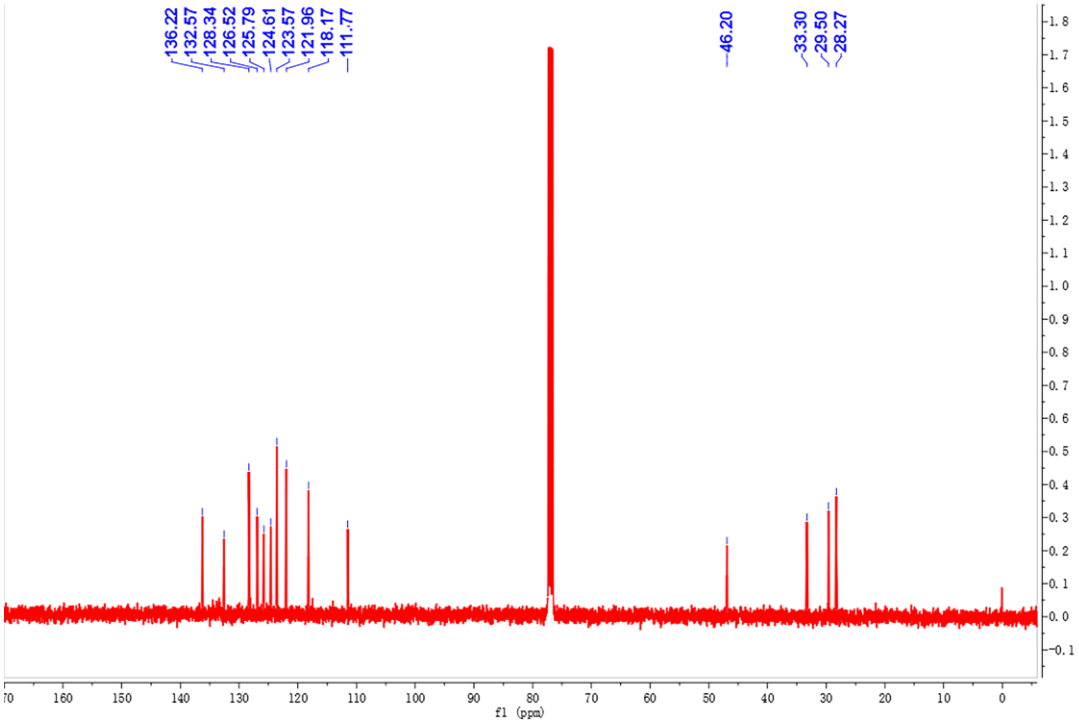


Figure S31. ^{13}C NMR spectrum of intermediate compound **4** in $CDCl_3$

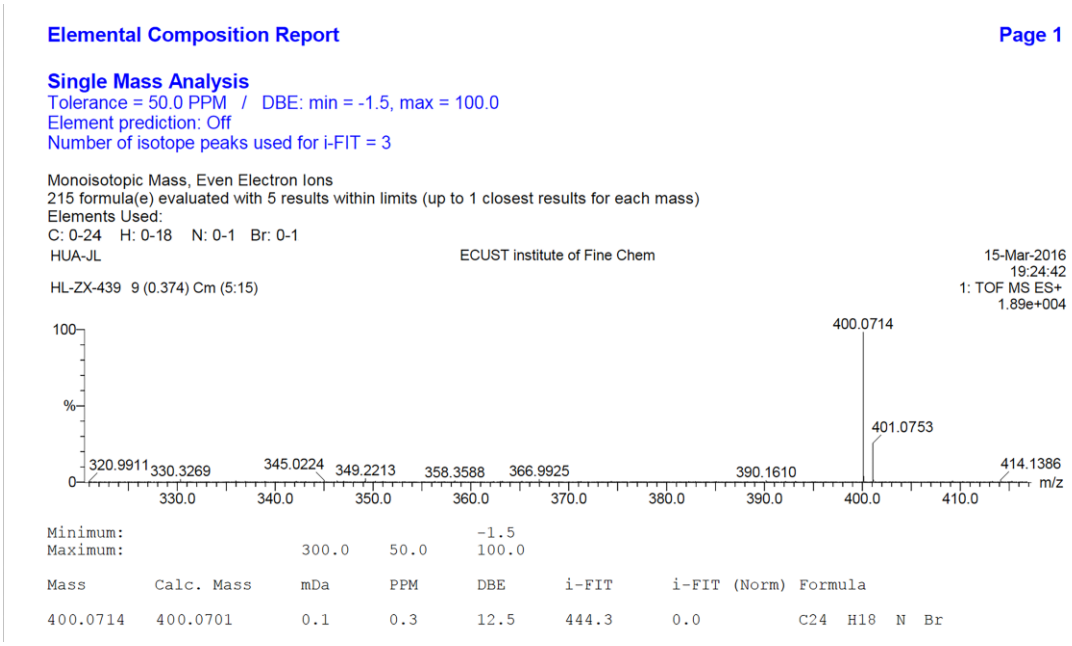


Figure S32. HRMS of intermediate compound **4**

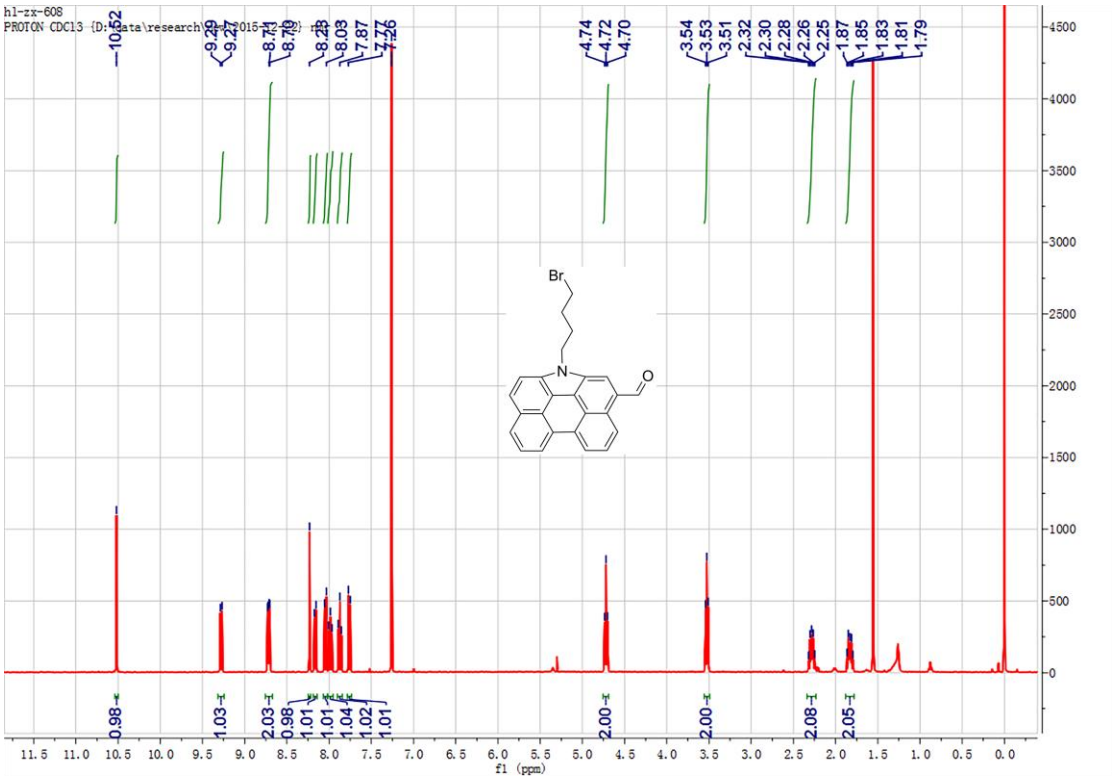


Figure S33. ^{1}H NMR spectrum of intermediate compound **5** in $CDCl_3$

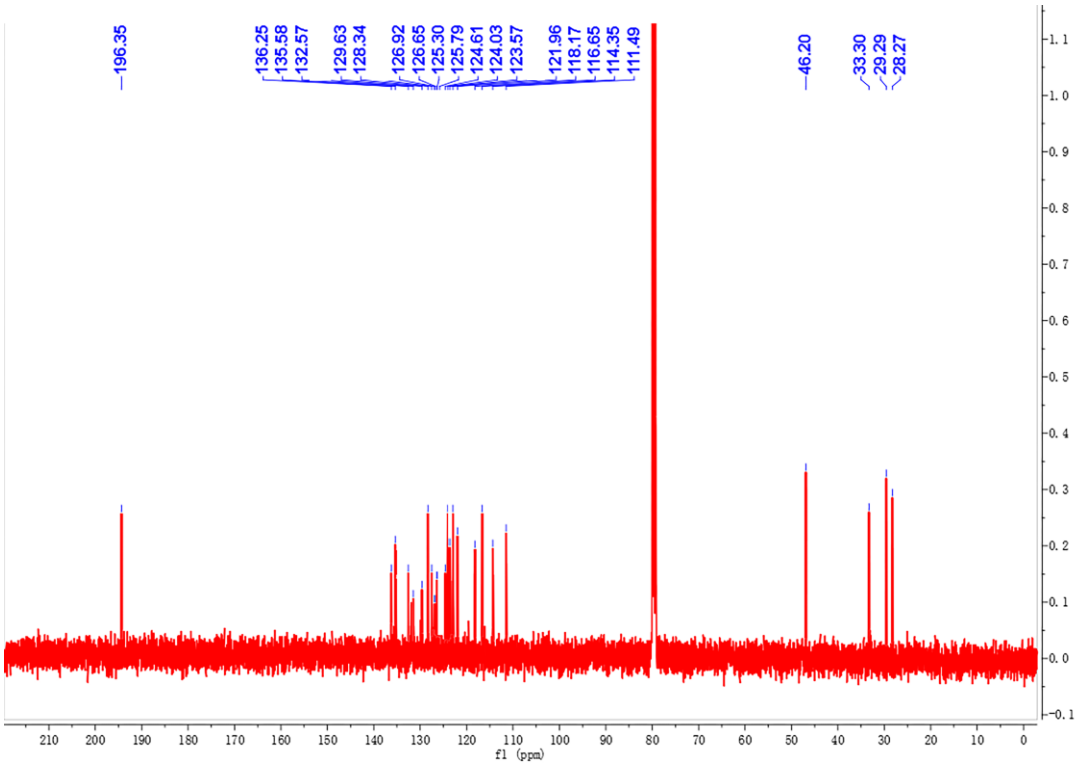


Figure S34. ^{1}H NMR spectrum of intermediate compound **5** in $CDCl_3$

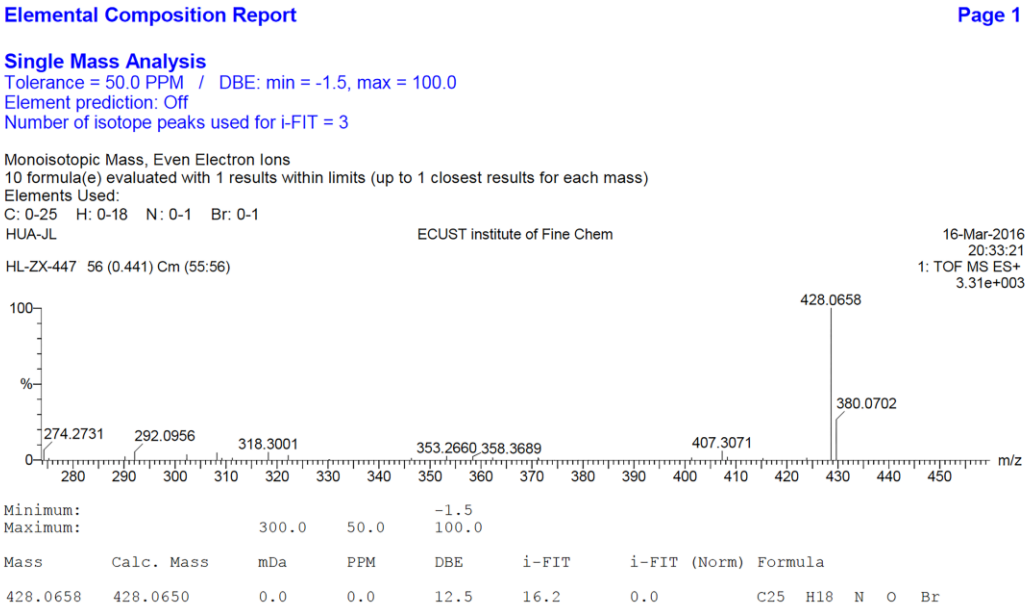


Figure S35. HRMS of intermediate compound 5

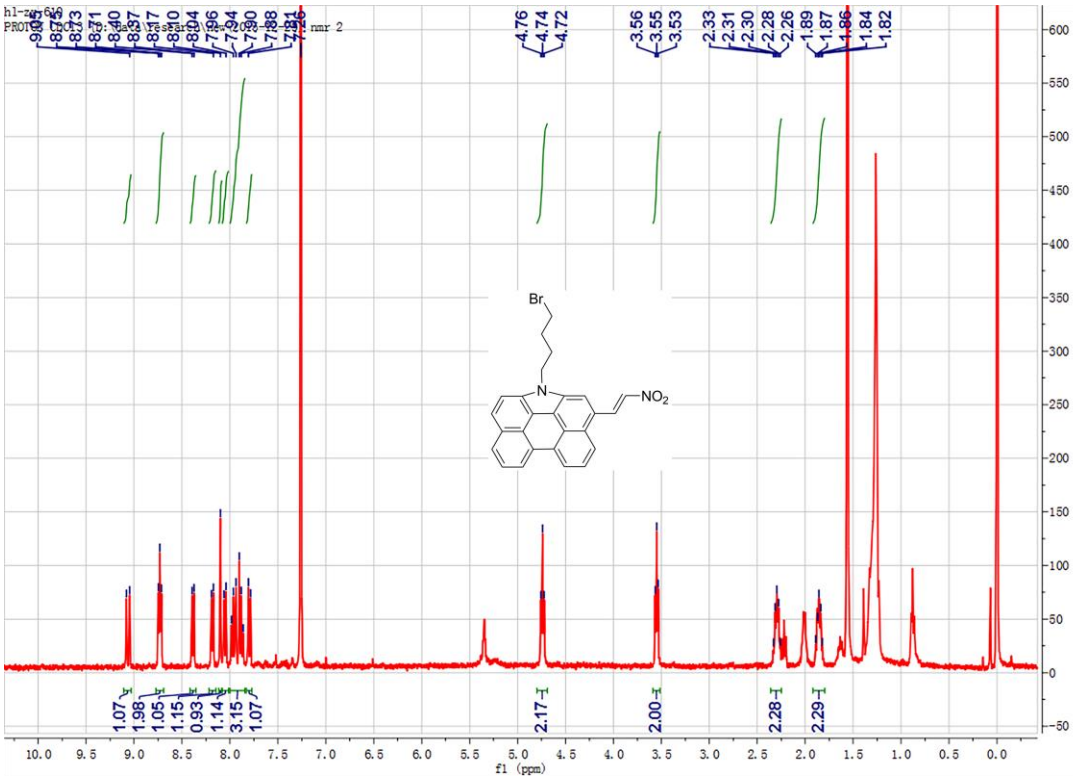


Figure S36. ^{1}H NMR spectrum of intermediate compound **6** in $CDCl_3$

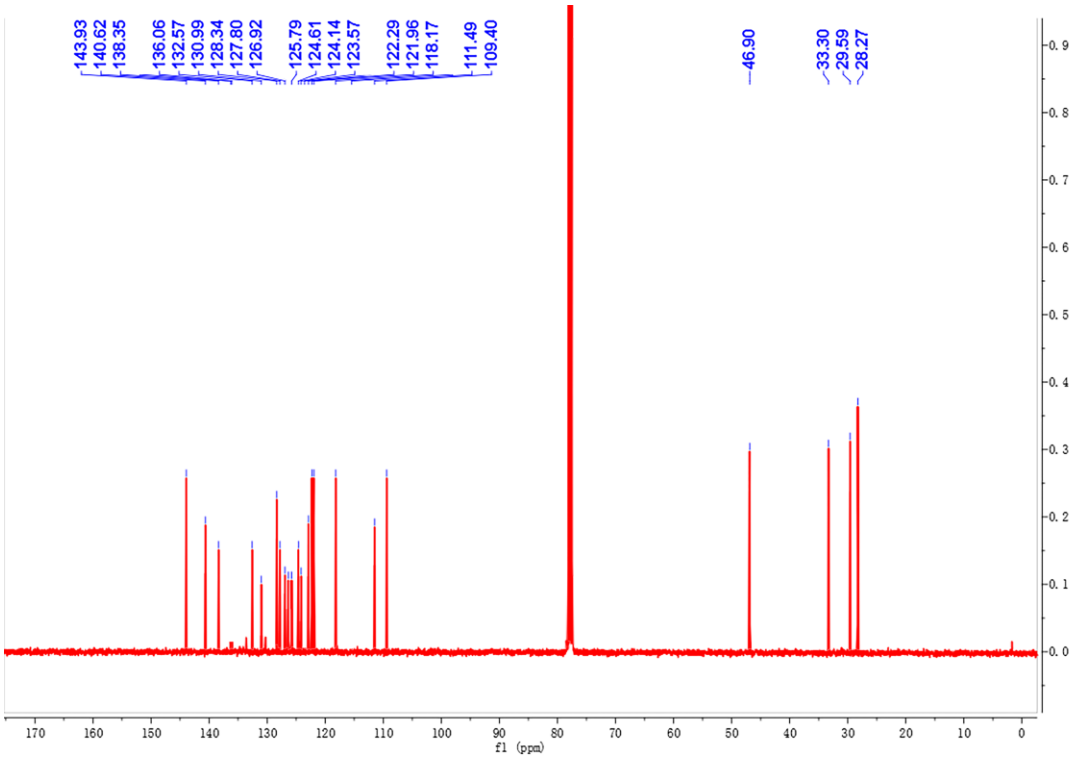


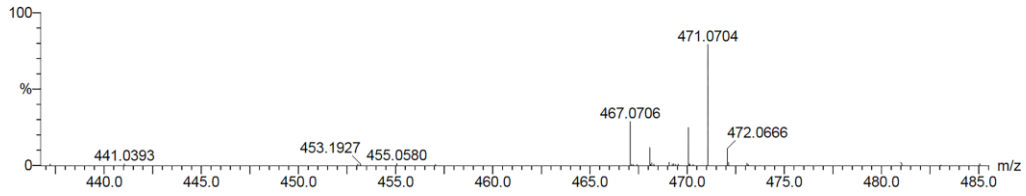
Figure S37. ^{13}C NMR spectrum of intermediate compound **6** in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-26 H: 0-19 N: 0-2 O: 0-2 Br: 0-1

JL-HUA ECUST institute of Fine Chem 09-Mar-2016 16:13:26
HL-ZX-412 20 (0.692) Cm (16:24) 1: TOF MS ES+ 5.70e+004



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
471.0704	471.0708	0.9	1.9	7.5	138.4	0.0	C26 H19 N2 O2 Br

Figure S38. HRMS of intermediate compound **6**

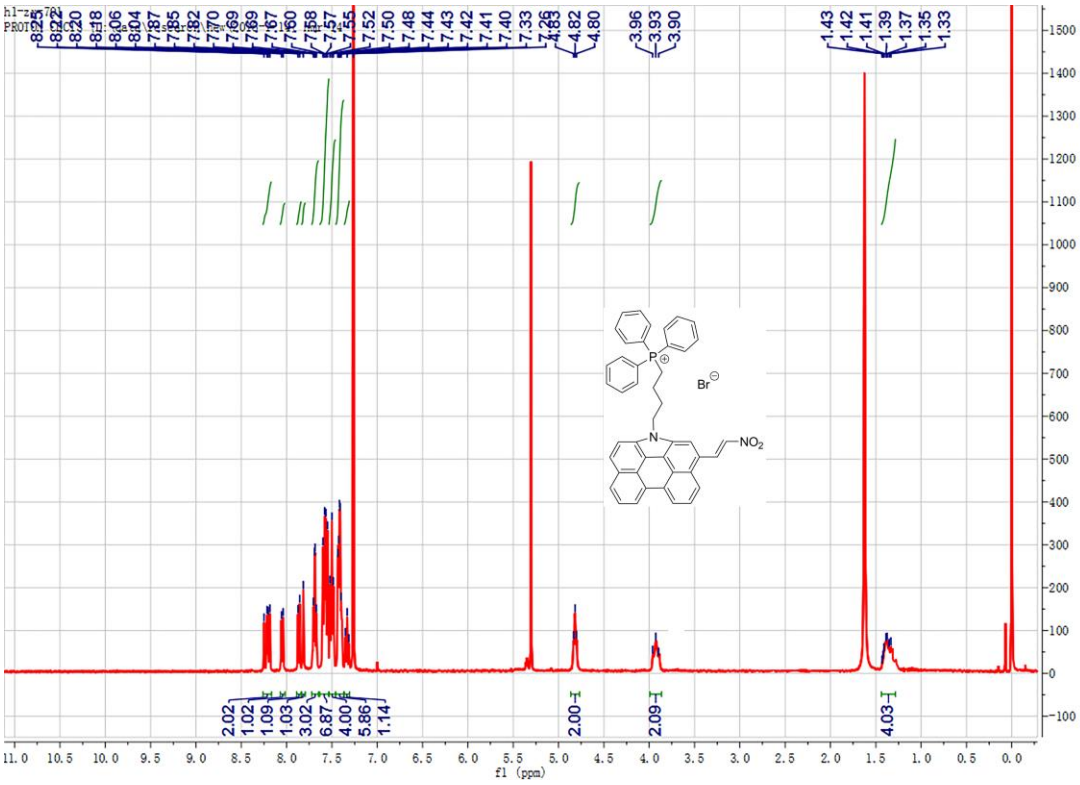


Figure S39. ^{1}H NMR spectrum of **Mito-NPNM** in $CDCl_3$

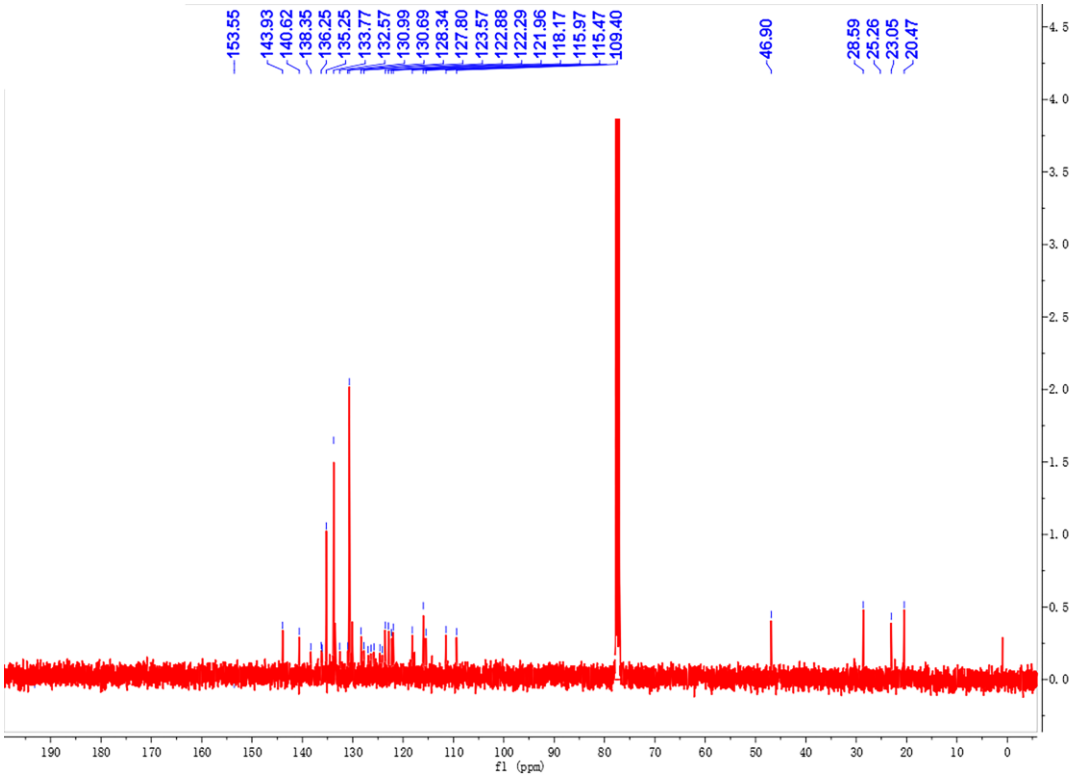


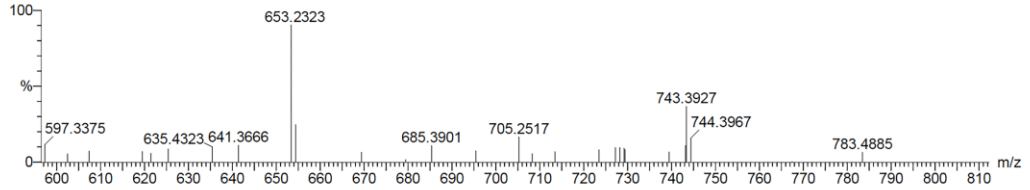
Figure S40. ^{13}C NMR spectrum of **Mito-NPNM** in $CDCl_3$

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
387 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-44 H: 0-34 N: 0-2 O: 0-2 P: 0-1

HUA-JL ECUST institute of Fine Chem 20-Apr-2016 20:19:30
HL-ZX-415 91 (0.650) Cm (90:93) 1: TOF MS ES+ 2.59e+003



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
653.2323	653.2352	-0.1	-0.1	9.5	20.4	0.0	C44 H34 N2 O2 P

Figure S41. HRMS of **Mito-NPNM**

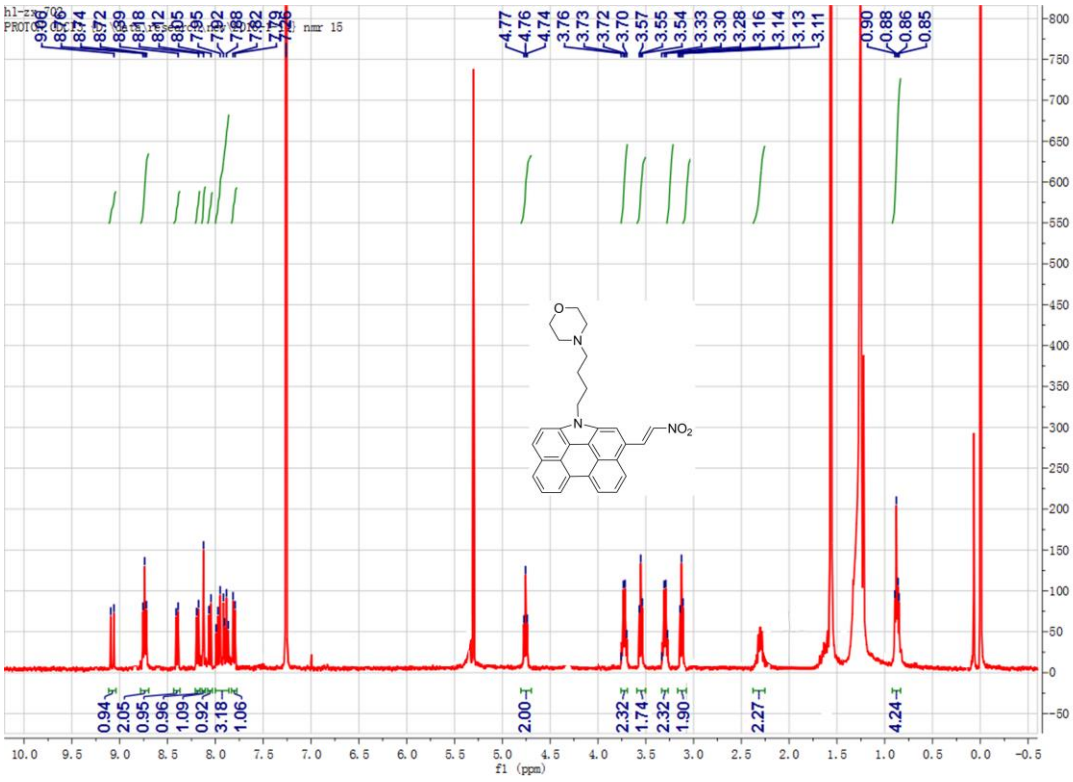


Figure S42. ^{1}H NMR spectrum of **Lyso-NPNM** in $CDCl_3$

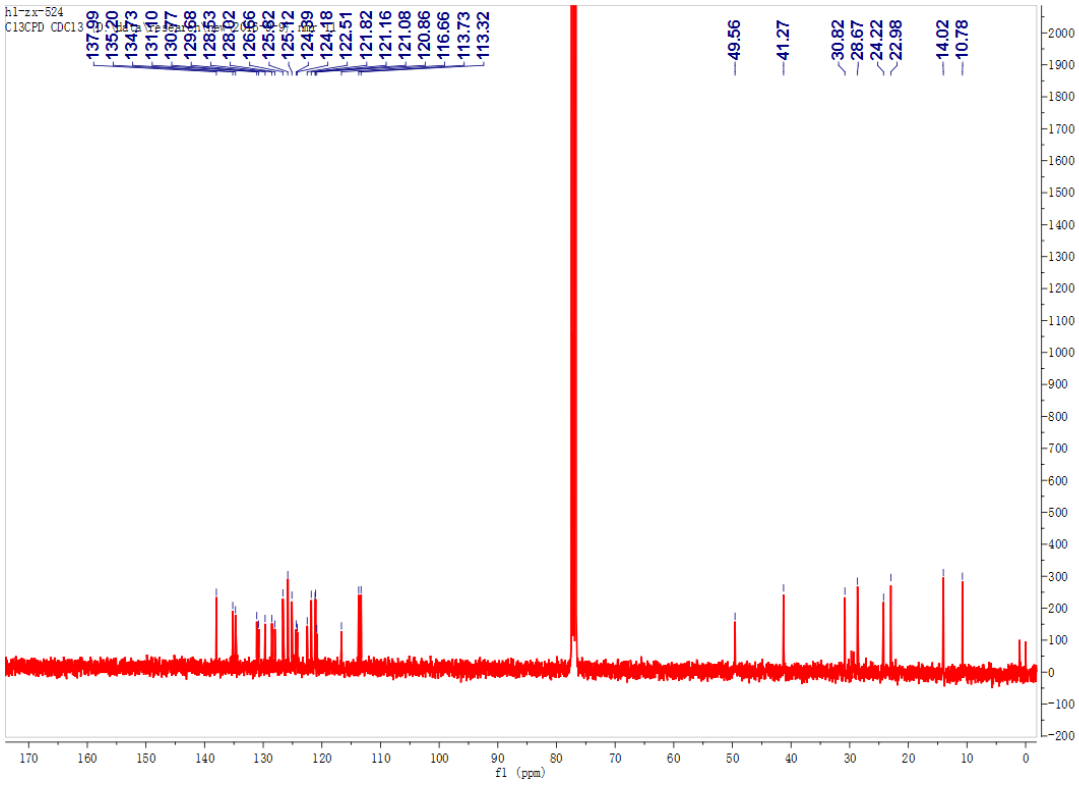


Figure S43. ^{13}C NMR spectrum of **Lyso-NPNM** in $CDCl_3$

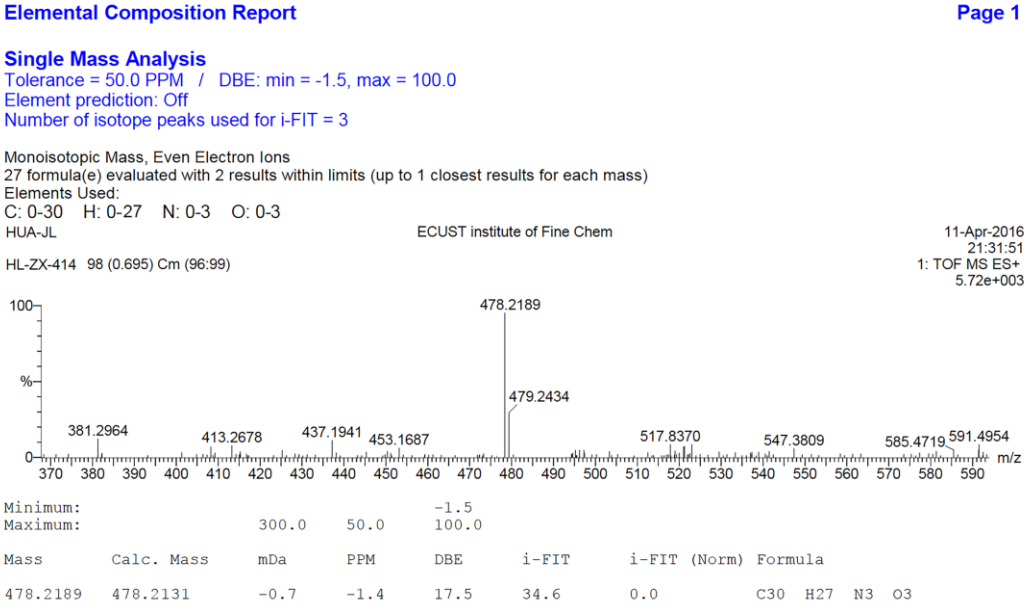


Figure S44. HRMS of **Lyso-NPNM**