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Supplementary information

Structure modulation on fluorescent probes for biothiols and the reversible imaging of glutathione in living cells

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Materials and instruments

All the solvents and reagents were obtained commercially. Fluorescence spectra were recorded on a Hitachi F-4500 or a HoribaX FluoroMax-4 spectrometer. UV–vis absorption spectra were recorded on a PerkinElmer Lambda 365 or a Persee TU-1901 spectrophotometer. The pH measurements were made with a LEITING PHSJ-3F pH meter. Fluorescence imaging was carried out by a confocal laser scanning microscope (ZEISS LSM880). ^{1}H NMR spectra were recorded on Bruker Ascend 400 MHz spectrometers, and ^{13}C NMR spectra were recorded on 100 MHz spectrometers. High-resolution mass spectra (HRMS) for ***o*-MNPy**, ***m*-MNPy** and ***p*-MNPy** were recorded on an Aglient 7250 & JEOL-JMS-T100LP AccuTOF instrument. HRMS for mechanism study were recorded on an Agilent 6224 TOF instrument.

Preparation of solutions

The probes were dissolved in DMSO, respectively, and 10 mM stock solution was prepared. Other analytes, including Cys, H_2O_2, NaClO, glycine, glutamic acid, histidine, proline, lysine, glucose, Na_2S, and Na_2SO_3, were dissolved in deionized water to afford 10 mM aqueous solution. Hcy and GSH were dissolved in deionized water to afford 1 mM and 100-500 mM aqueous solution, respectively.

Fluorescence quantum yield

The fluorescence quantum yield (Φ) indicates the ability of a substance to emit fluorescence and is a very important parameter of the probe. The calculation formula is:

$$\phi_{unk} = \phi_{std}\left(\frac{\mathrm{I}_{unk}}{\mathrm{A}_{unk}}\right)\left(\frac{\mathrm{A}_{std}}{\mathrm{I}_{std}}\right)\left(\frac{\eta_{unk}}{\eta_{std}}\right)^2$$

where, Φ_{unk} is the fluorescence quantum yield of the probe, Φ_{std} is the reference quantum yield (Quinine sulfate, dissolved in 0.1 M H_2SO_4), I_{unk} and I_{std} the integral fluorescence intensity of the probe and reference, A_{unk} and A_{std} the absorbance of the probe and reference, η_{unk} and η_{std} the refractive index of the corresponding solution of the probe and the reference, respectively.

Cytotoxicity Assays

The cytotoxicity of the probe on HeLa cells was examined by MTT assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10^3 cells/well in 100 μL of complete DMEM media and incubated at 37 ºC in an atmosphere containing 5% CO_2 and 95% air. After the cells reached about 80% confluency, the cells were incubated with 0, 2.5, 5, 10, 20 and 40 μM of the probe for 24 h, respectively. After that, 20 μL of MTT solution (5 mg mL^{-1}) in PBS was added to each well and further cultured for another 4 h at 37 ºC. Then the DMEM solution was removed and 150 μL of DMSO was added to dissolve the formed purple crystals derived from MTT. The plates were then analyzed with a microplate reader (Tecan M200 PRO, Austria) at the absorbance wavelength of 570 nm.

Cell culture and cell imaging

HeLa cells were obtained from iCell Bioscience Inc. HeLa cells were maintained under the standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37°C) in MEM medium (Corning),

supplemented with 10% FBS (Biological Industries).

Before confocal microscopy imaging of cells with probes, HeLa cells in the exponential phase were plated on 35 mm glass-bottom culture dishes for 2 days to reach around 80% confluency, respectively. Cell culture was maintained at 37 ºC under an atmosphere of 5% CO_2 and 95% air for desired time. Culture medium was changed every two days to keep the exponential growth of the cells. On the day of treatment, the cells were incubated with the appropriate concentrations of probe or additives for desired time at 37 ºC and washed with 1 mL of PBS for three times at room temperature, then they were added to 1 mL of PBS culture medium and observed under confocal microscopy with a 63× oil-immersion objective.

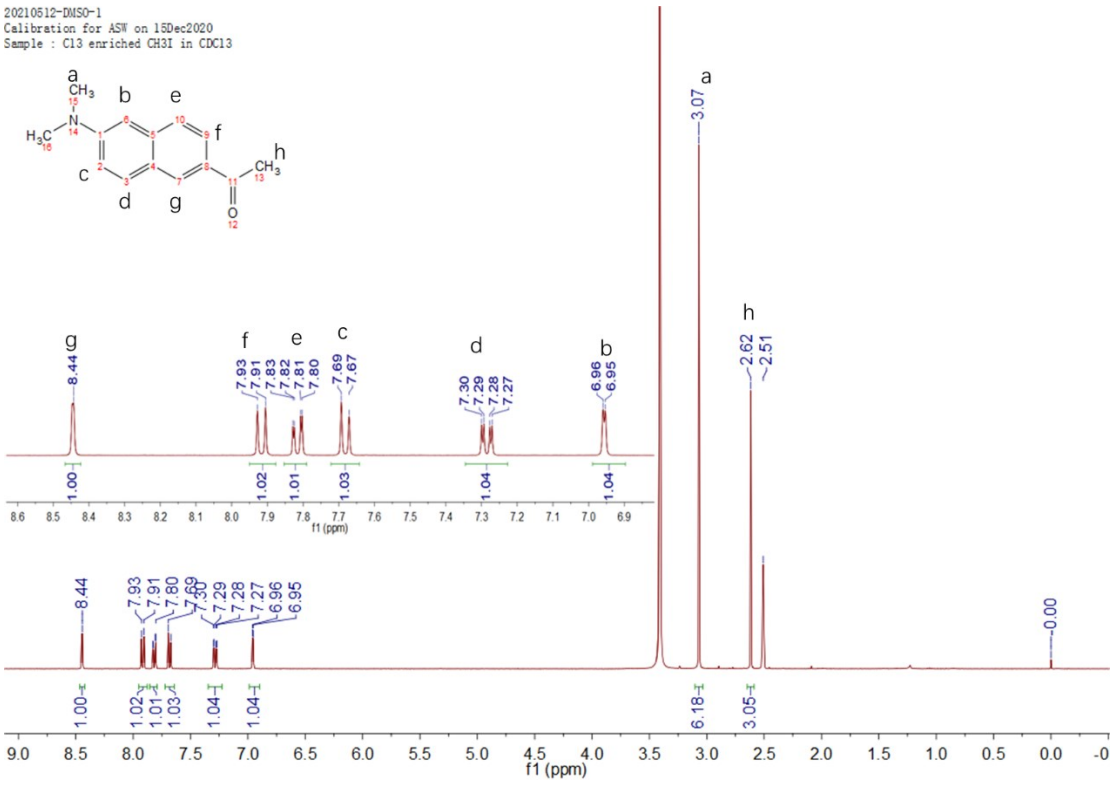

Fig. S1 The ^{1}H NMR of **MN**.

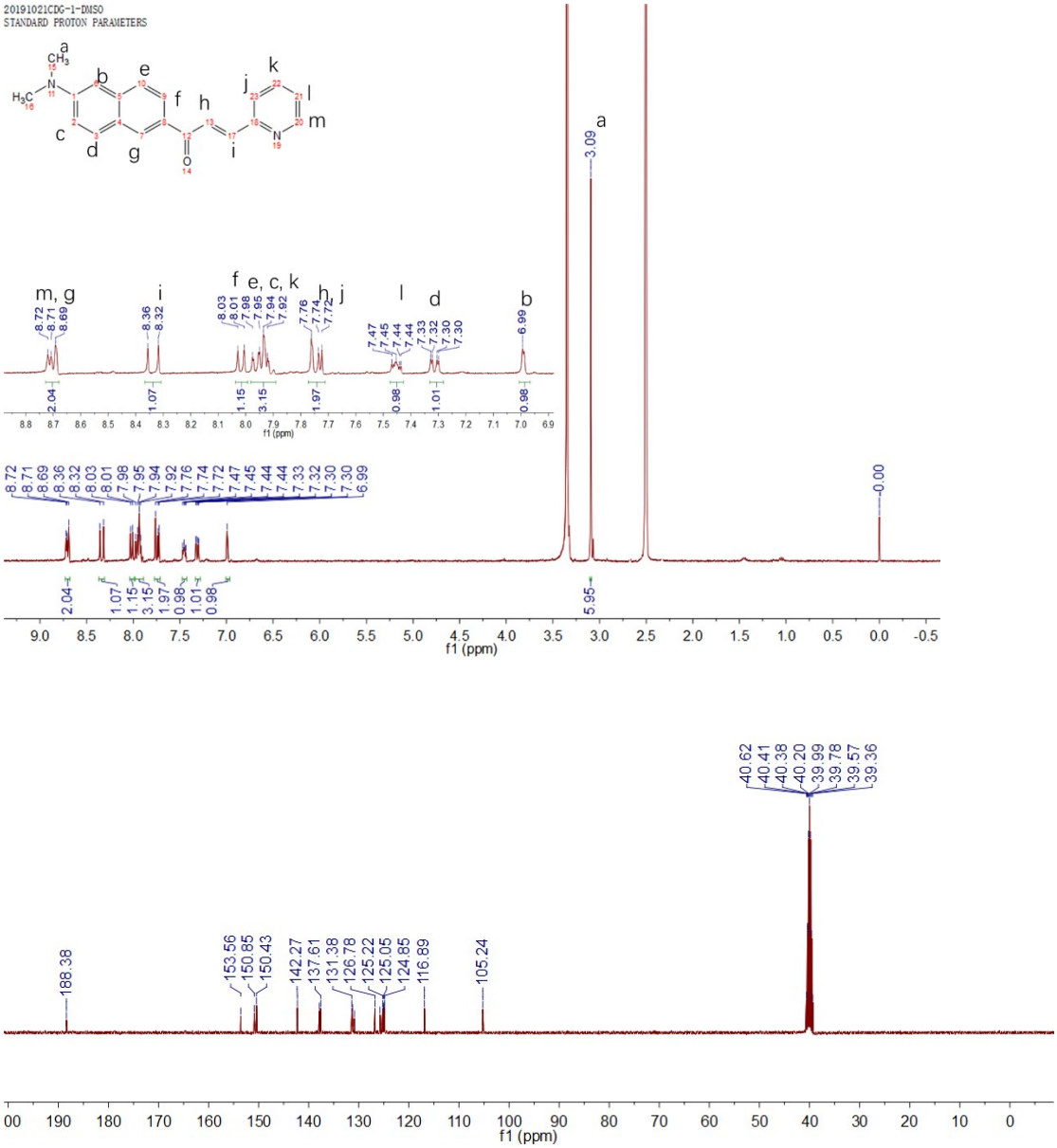

Fig. S2 The ^{1}H NMR and ^{13}C NMR of ***o*-MNPy**.

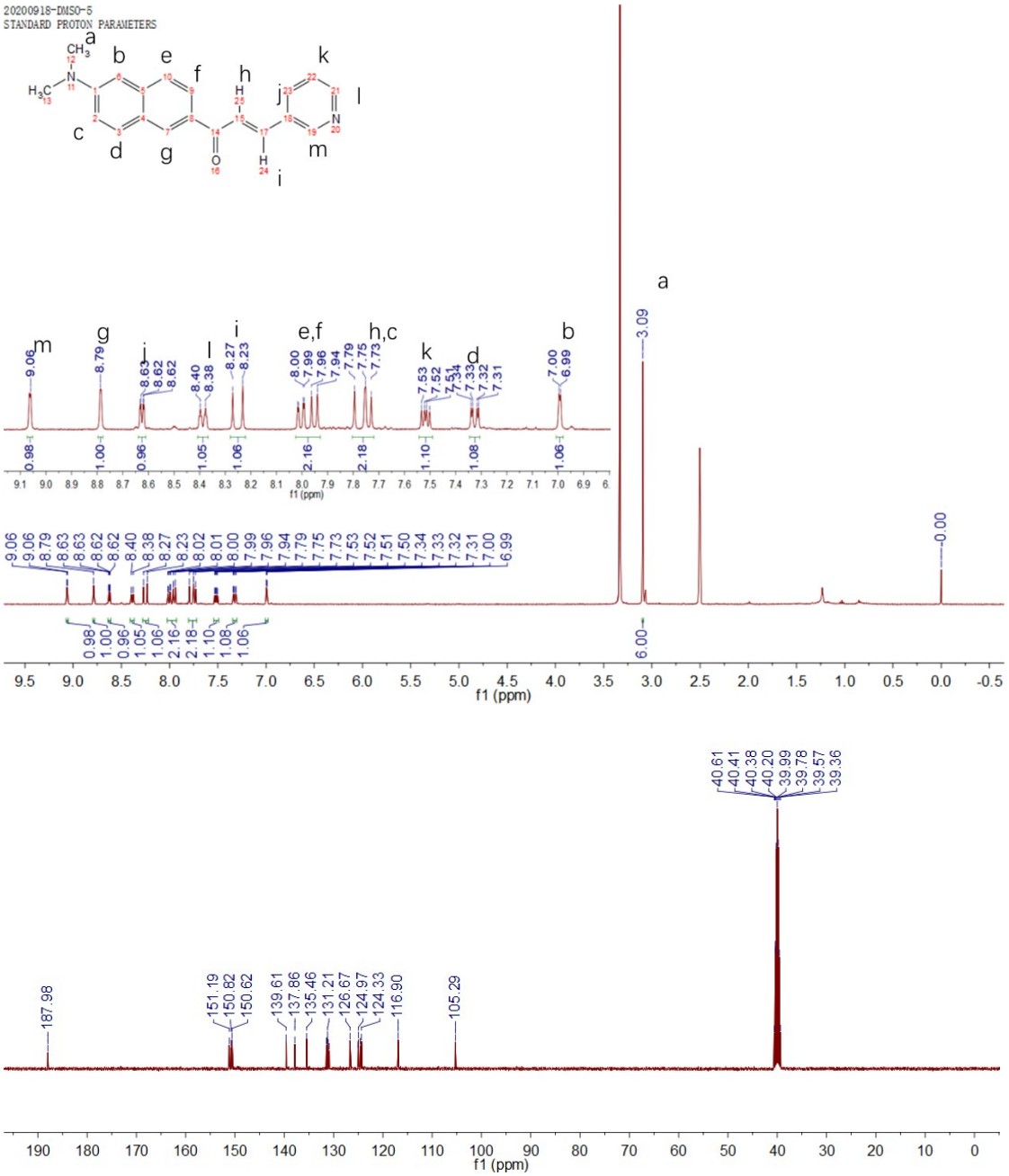

Fig. S3 The ^{1}H NMR and ^{13}C NMR of ***m*-MNPy**.

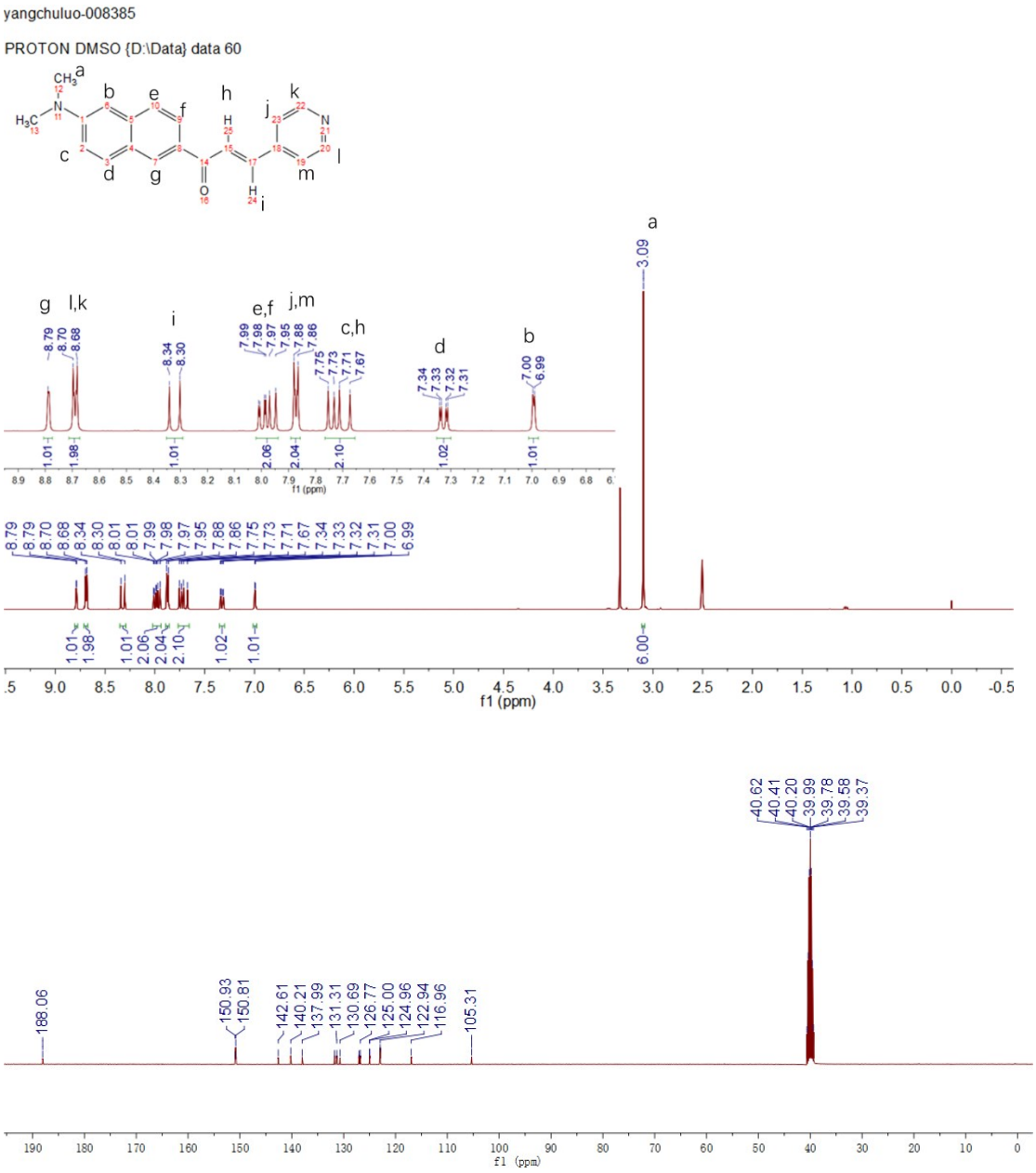

Fig. S4 The ^{1}H NMR and ^{13}C NMR of ***p*-MNPy**.

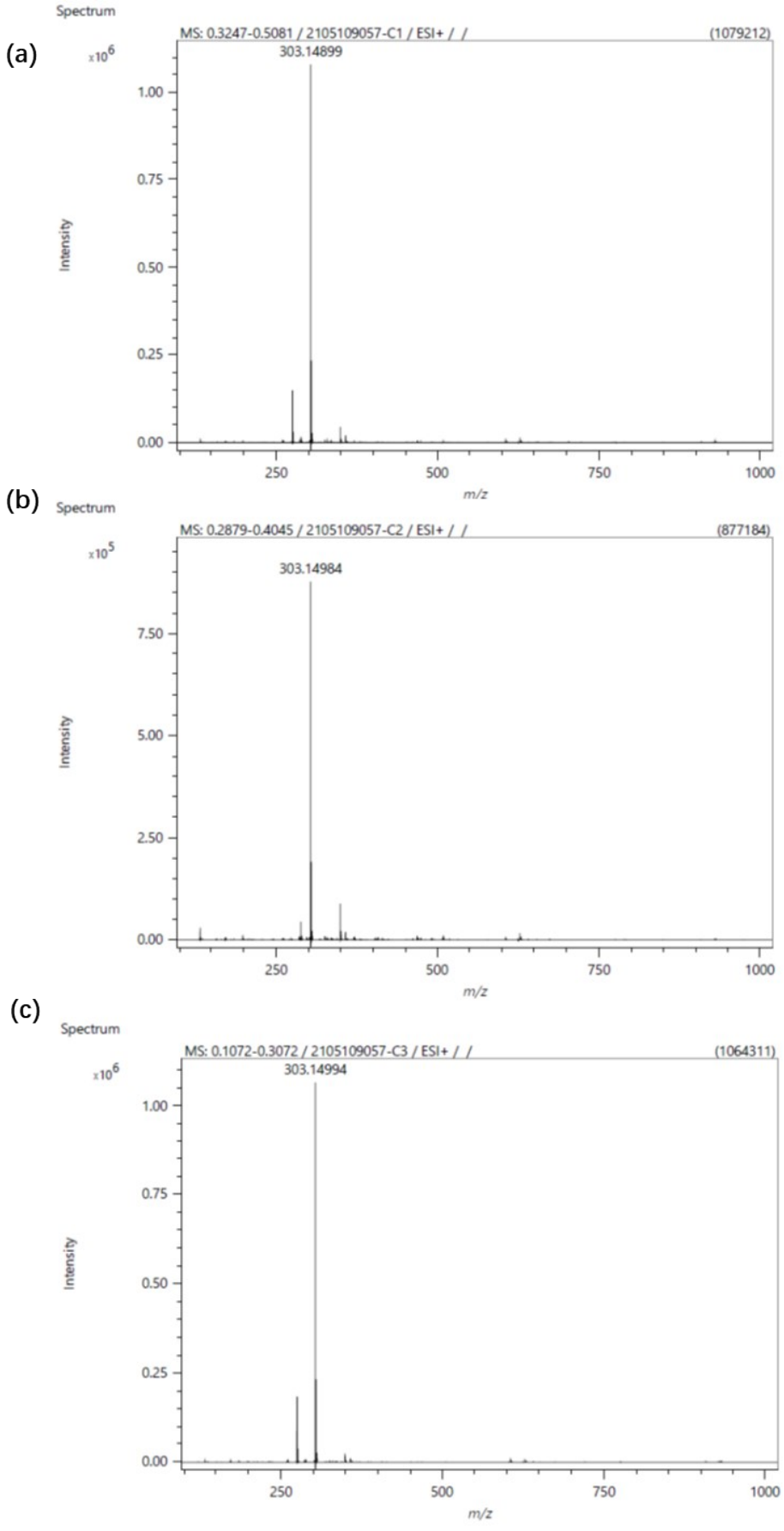

Fig. S5 HRMS of (a) ***o*-MNPy**, (b) ***m*-MNPy** and (c) ***p*-MNPy**.

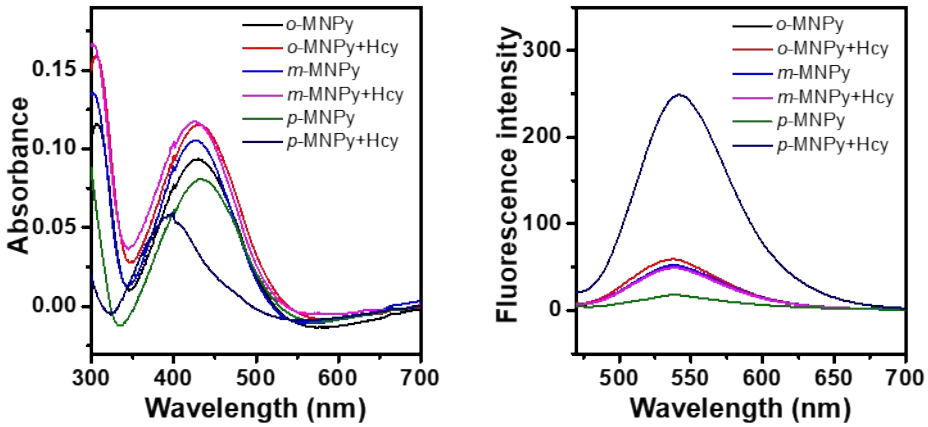

Fig. S6 (a) The absorption and (b) the fluorescence spectra of each probe (10 μM) upon treatment with Hcy (100 μM).

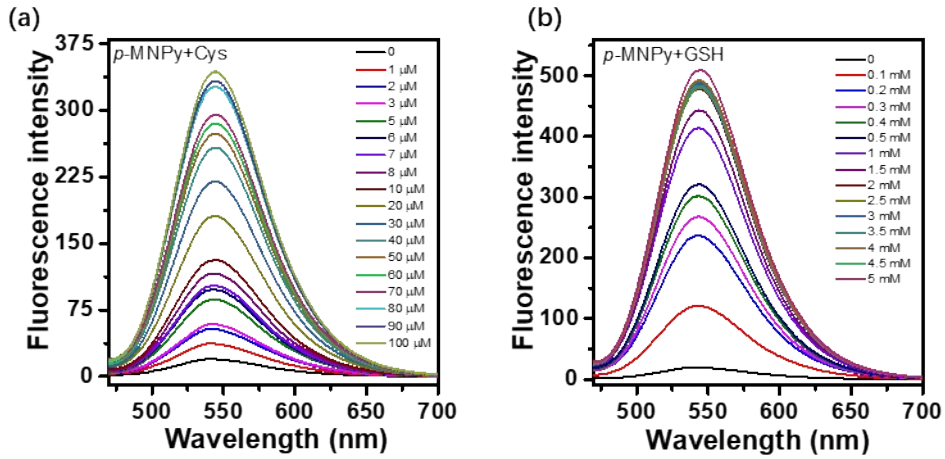

Fig. S7 The fluorescence spectra of ***p*-MNPy** (10 μM) with the addition of different amount of (a) Cys and (b) GSH.

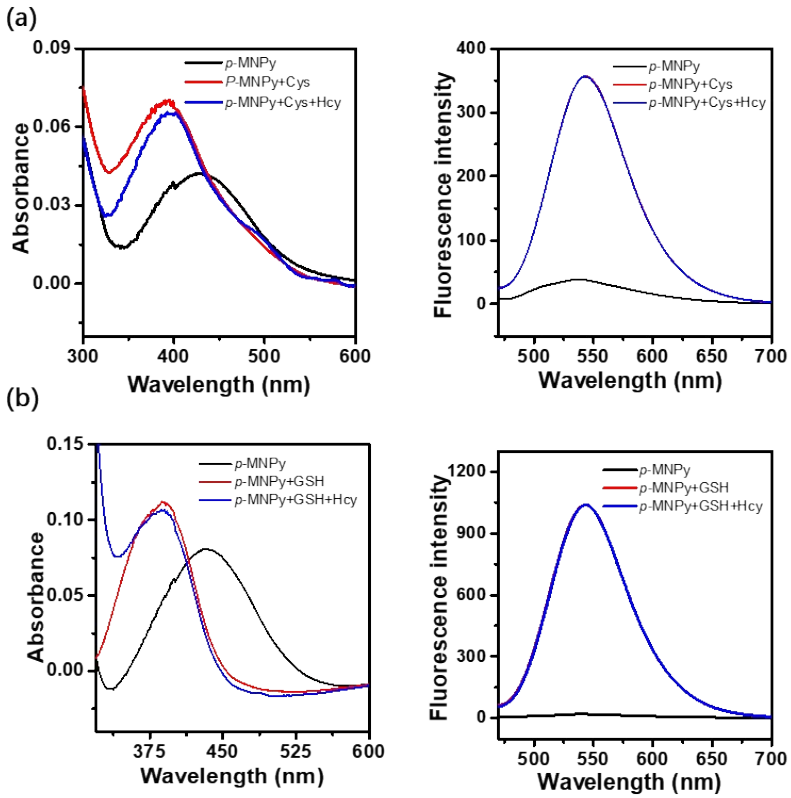

Fig. S8 The response of ***p*-MNPy** (10 μM) toward (a) Cys (100 μM) and (b) GSH (5 mM) in the absence and presence of Hcy (10 μM).

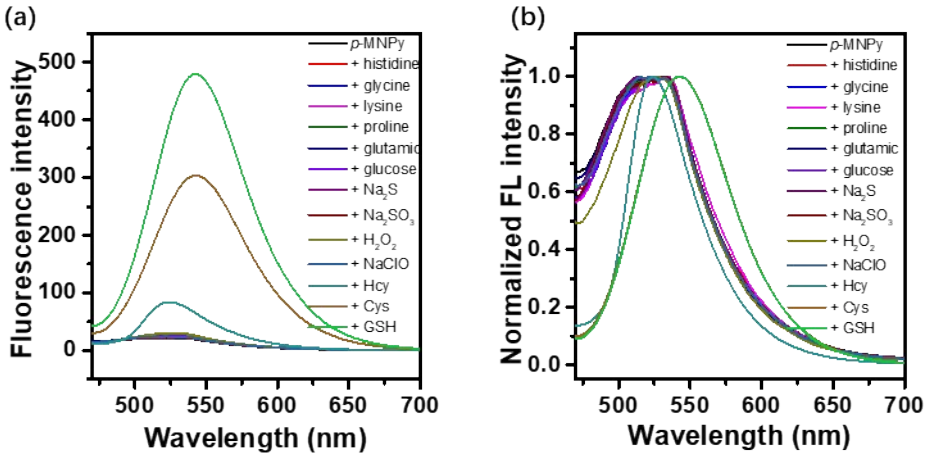

Fig. S9 The fluorescence spectra of ***p*-MNPy** with the addition of different kinds of analytes. (a) The original fluorescence spectra, and (b) normalized spectra.

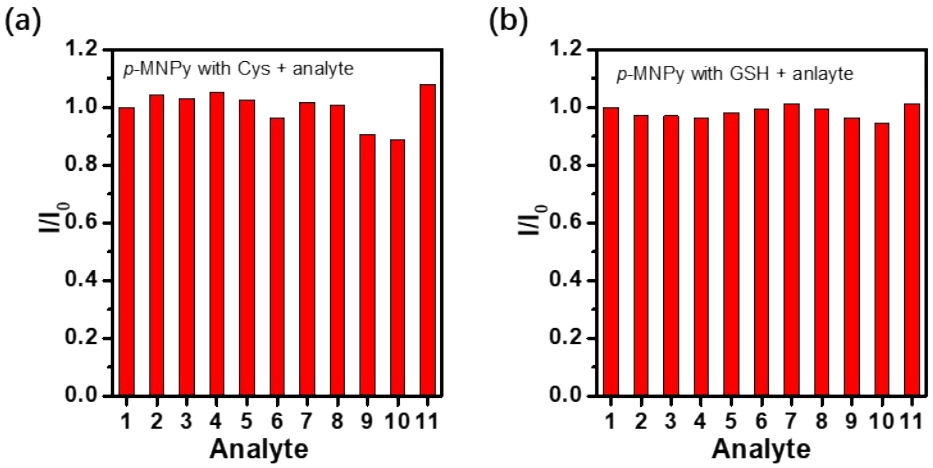

Fig. S10 The changes of the fluorescence intensity of (a) ***p*-MNPy** with Cys, and (b) ***p*-MNPy** with GSH in the presence of various bio-species. 1 control, 2 histidine, 3 glycine, 4 lysine, 5 proline, 6 glutamic acid, 7 glucose, 8 Na_2S, 9 Na_2SO_3, 10 H_2O_2, 11 NaClO. I_0 refers to the fluorescence intensity of the probe in the presence of Cys (a) or GSH (b).

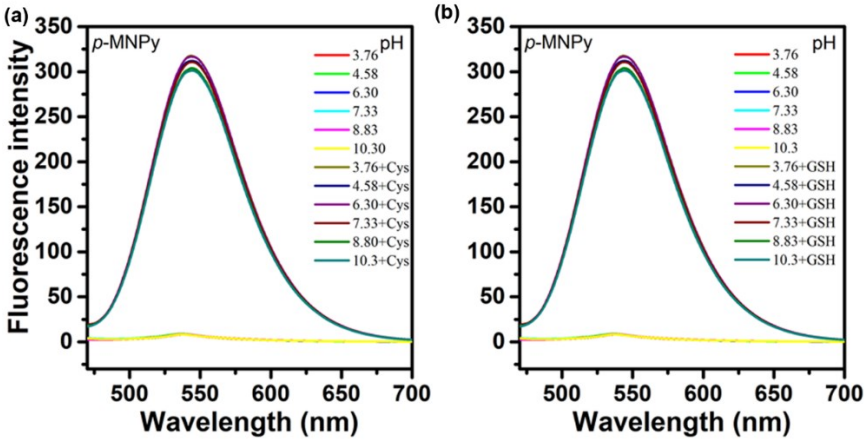

Fig. S11 The fluorescence spectra of ***p*-MNPy** in the absence and presence of (a) Cys and (b) GSH under different pH environment.

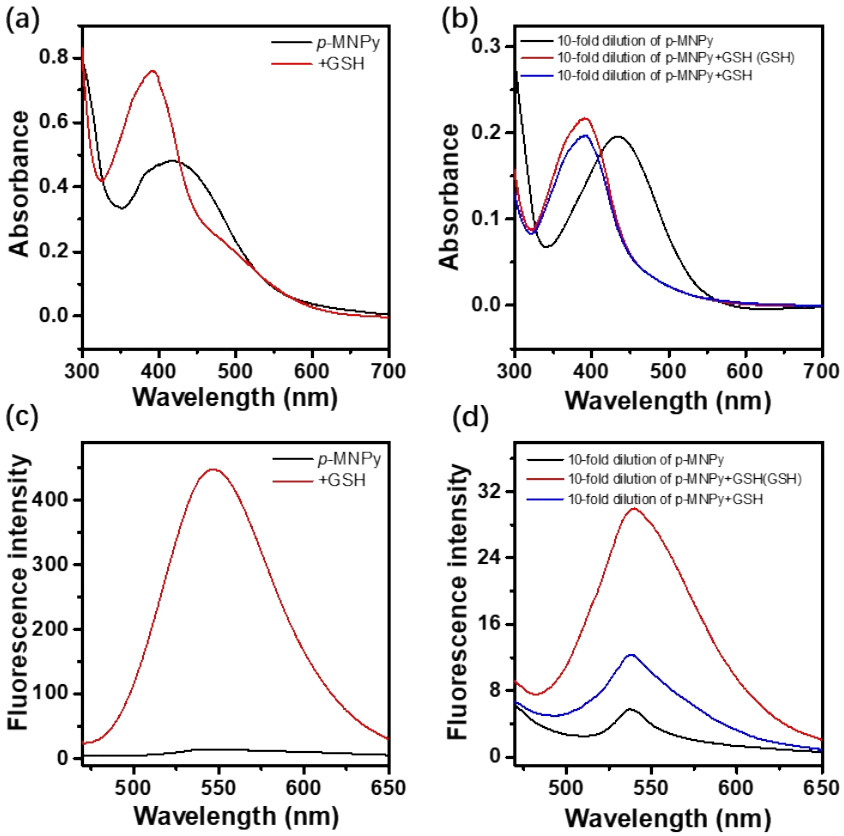

Fig. S12 (a) UV-vis spectra of ***p*-MNPy** (50 μM) and ***p*-MNPy**/GSH (50 μM/5 mM) mixture in PBS (containing 20% DMSO). (b) The initial mixture of (a) was diluted 10-fold into the buffer containing no (blue) or 5 mM GSH (red). (c) Fluorescence spectra of ***p*-MNPy** (10 μM) and ***p*-MNPy**/GSH (10 μM/1 mM) mixture in PBS (containing 10% DMSO). (d) The initial mixture of (c) was diluted 10-fold into the buffer containing no (blue) or 1 mM GSH (red).

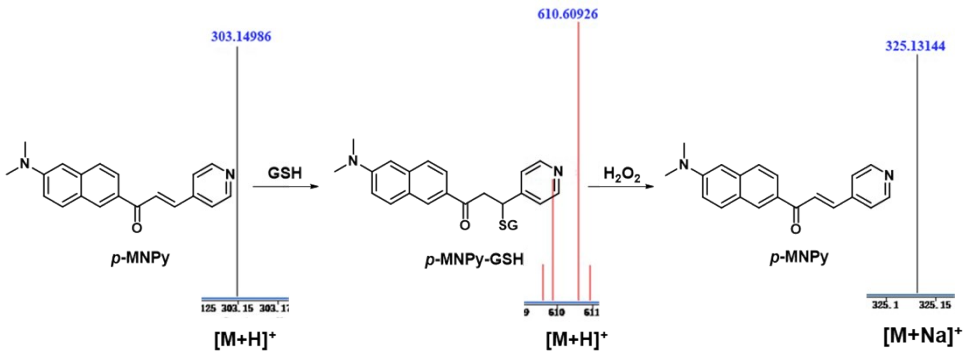

Fig. S13 Fragment peaks of the relevant compounds during the reversible recognition process.

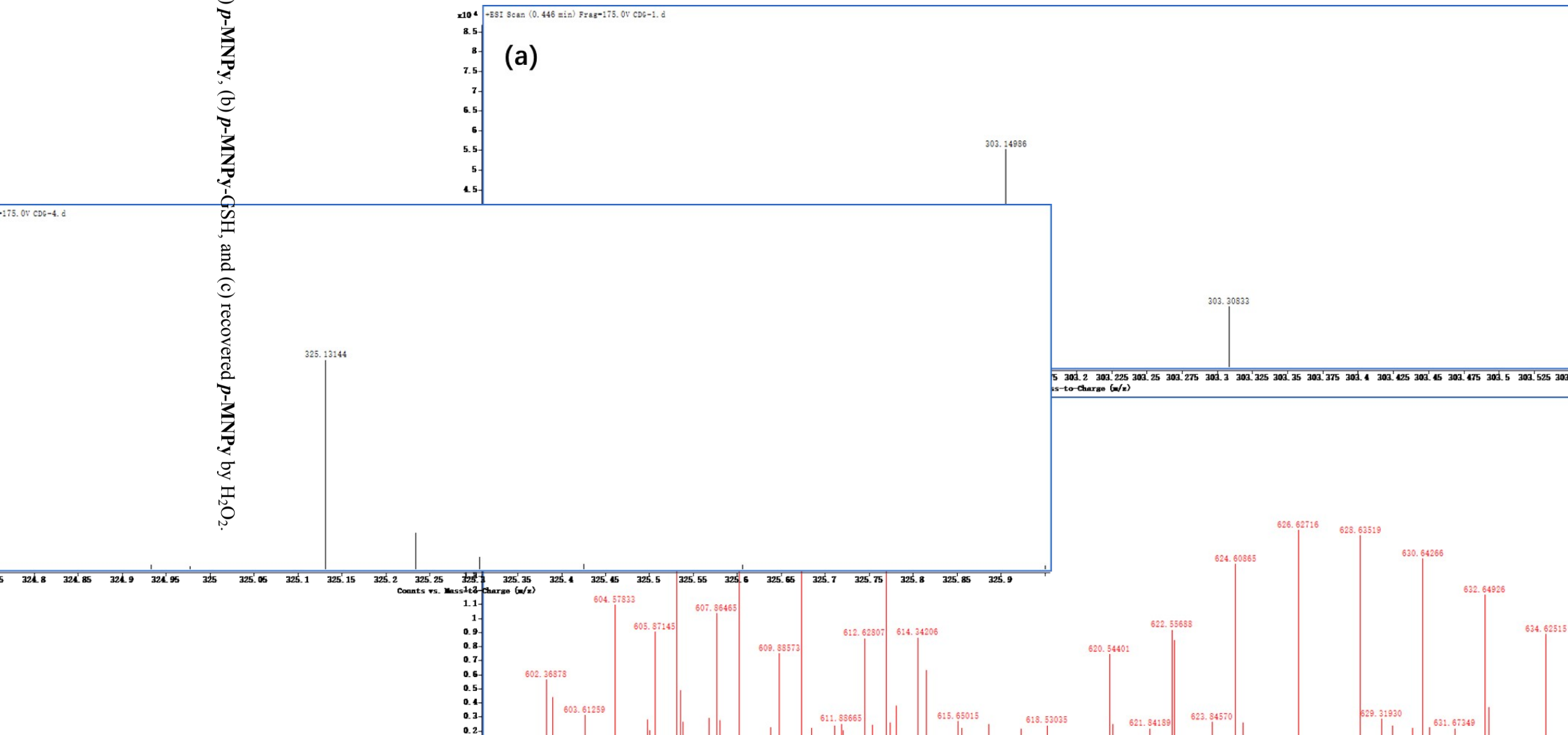

Fig. S14 The HRMS of (a) ***p*-MNPy**, (b) ***p*-MNPy-GSH**, and (c) recovered ***p*-MNPy** by H_2O_2.

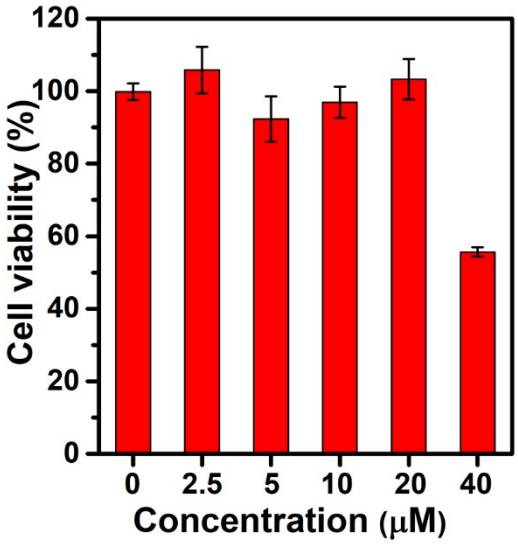

Fig. S15 The viability of HeLa cells when incubated with ***p*-MNPy** in different concentrations for 24 h.

Table S1 The spectral data of the probes in the presence of biothiols.

	The probe itself			With Cys		With Hcy		With GSH		
	λ_{ab} (nm)	λ_{em} (nm)	QY[a]	λ_{ab} (nm)	λ_{em} (nm)	λ_{ab} (nm)	λ_{em} (nm)	λ_{ab} (nm)	λ_{em} (nm)	QY[a]
***o*-MNPy**	429	538	0.16%	388	543	429	541	388	542	5.64%
***m*-MNPy**	419	538	0.19%	393	543	415	541	390	543	3.56%
***p*-MNPy**	430	538	0.13%	391	544	427	541	390	543	6.55%

[a] using quinine sulfate as the standard (QY = 0.53 in 0.1 M H_2SO_4)