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Supporting Information

Constructing a FRET-based molecular chemodosimeter for cysteine over homocysteine and glutathione by naphthalimide and phenazine

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Instrumentation and materials

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400MHz spectrometer with tetramethylsilane (TMS) as internal reference. Absorption spectra were measured on a Varian Cary 500 UV-Vis spectraphotometer. Fluorescence spectra were measured on a Cary Eclipse Fluorescence spectrometer. Electrospray ionization and time-of-flight analyzer (ESI-TOF) mass spectra were recorded with a Waters Micromass LCT mass spectrometer. Matrix assisted laser desorption ionization and time-of-flight analyzer (MALDI-TOF) were recorded by an Applied Biosystems 4700 Proteomics Analyzer.

N, *N*-dimethylformamide (DMF) was refluxed with calcium hydride and distilled before use. All other reagents and reactant including phenazine were purchased as commercial products from Aldrich and used as received without further purification. All synthetic reactions were conducted under protection of Argon shield.

Absorbance and fluorescence spectra were measured in HEPES buffer which is 20 mM with 50% EtOH (v/v) at 37.4 ^{o}C.

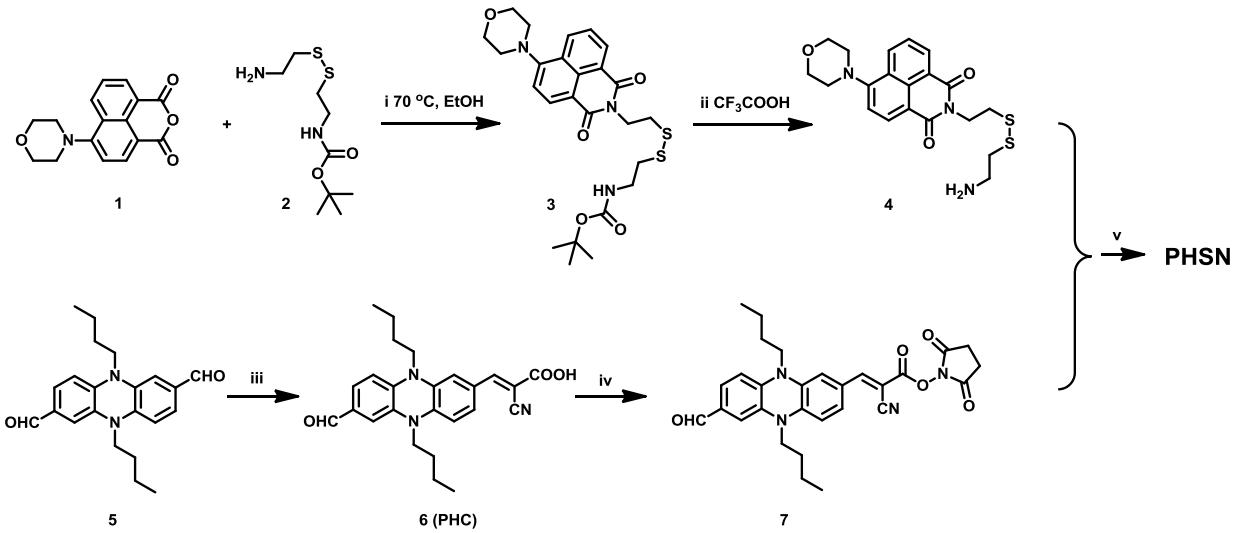
Selectivity Measurements

The changes in the absorption and fluorescence spectra caused by Cys, Hcy, GSH and various other amino acids including Ala, Arg, Asp, Glu, Gly, Leu, Met, Pro, Ser, Thr and Try in water solutions were recorded.

Cell imaging

Hela cells were cultured at 37 ^{o}C, in 5 % CO_2 air condition and maintained one day before imaging by the confocal laser scanning microscopy. The cells were plated on 14 mm glass coverslips and incubated with **PHSN** for 40 min then thiols for 60 min respectively, at 37 ^{o}C in PBS (pH = 7.4).

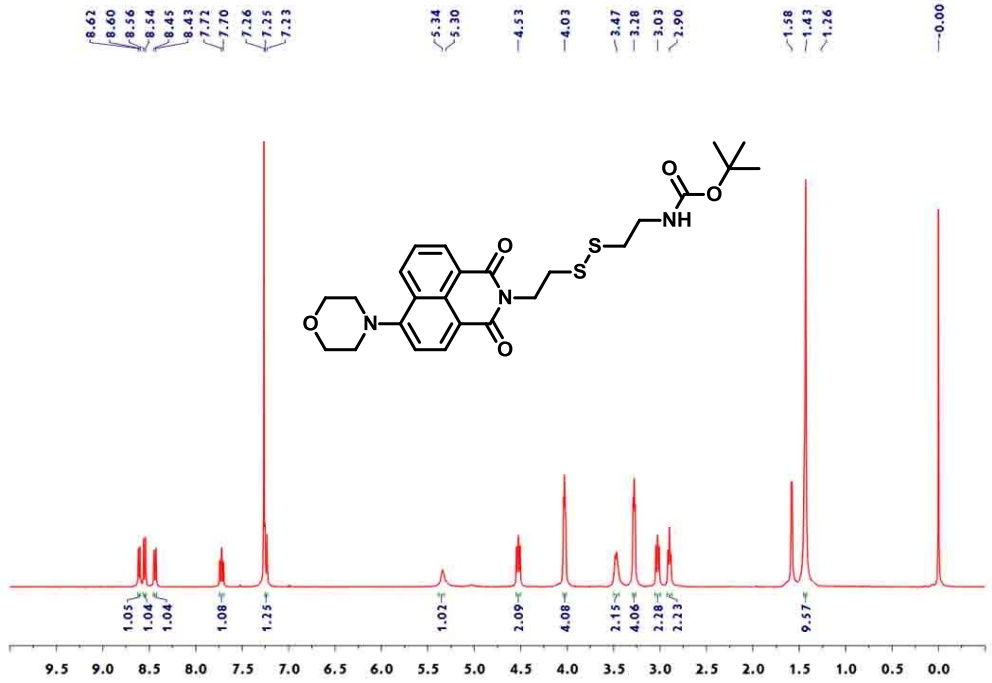
Synthesis of PHSN

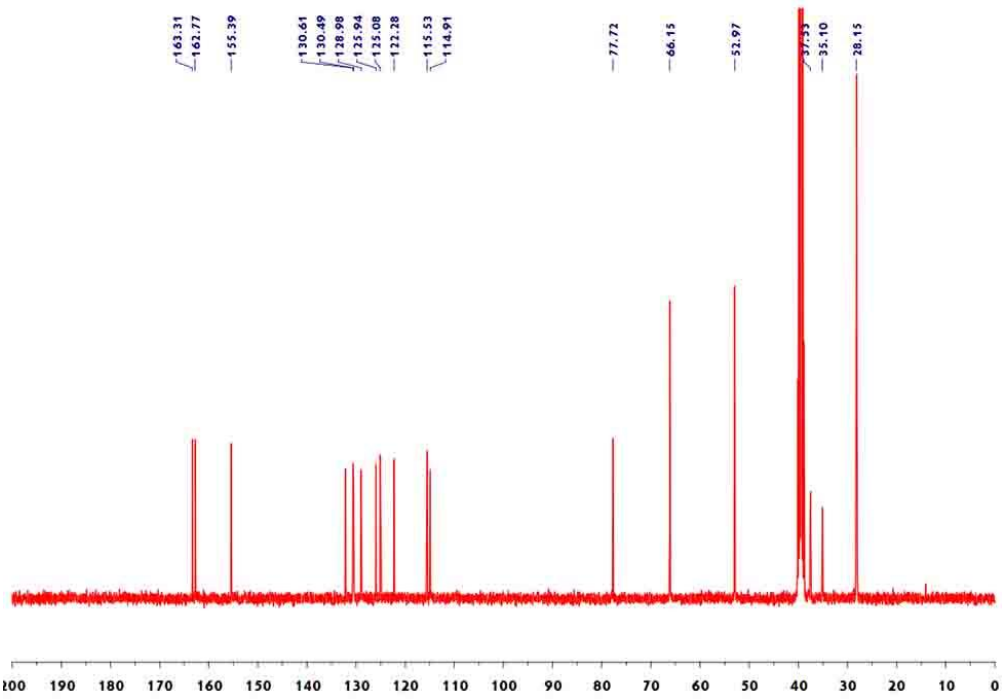


Scheme S1 The synthesis procedures of **PHSN**: (i) EtOH, 70 ^{o}C, overnight; (ii) trifluoroacidic acid, room temperature, 10 min; (iii) acetonitrile, piperidine, cyanoacidic acid, 12 h; (iv) DMF, EDC, DMAP, NHS, ice bath to rt, 6 h; (v) DMF, Et_3N, 0 ^{o}C to rt, 4h.

Characterization of PHSN

Compound 3: ^{1}H in $CDCl_3$ and ^{13}C NMR in DMSO-*d*^{6} spectrum, high-res ESI-TOF mass spectrum



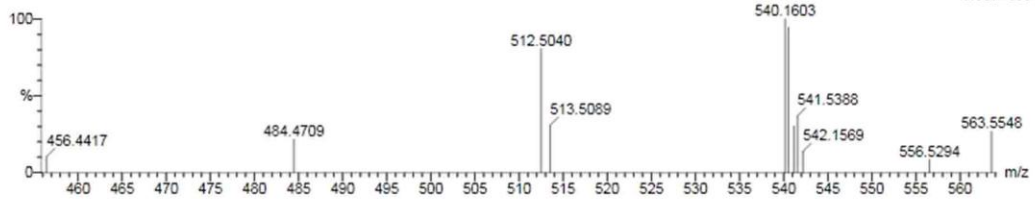


Elemental Composition Report **Page 1**

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

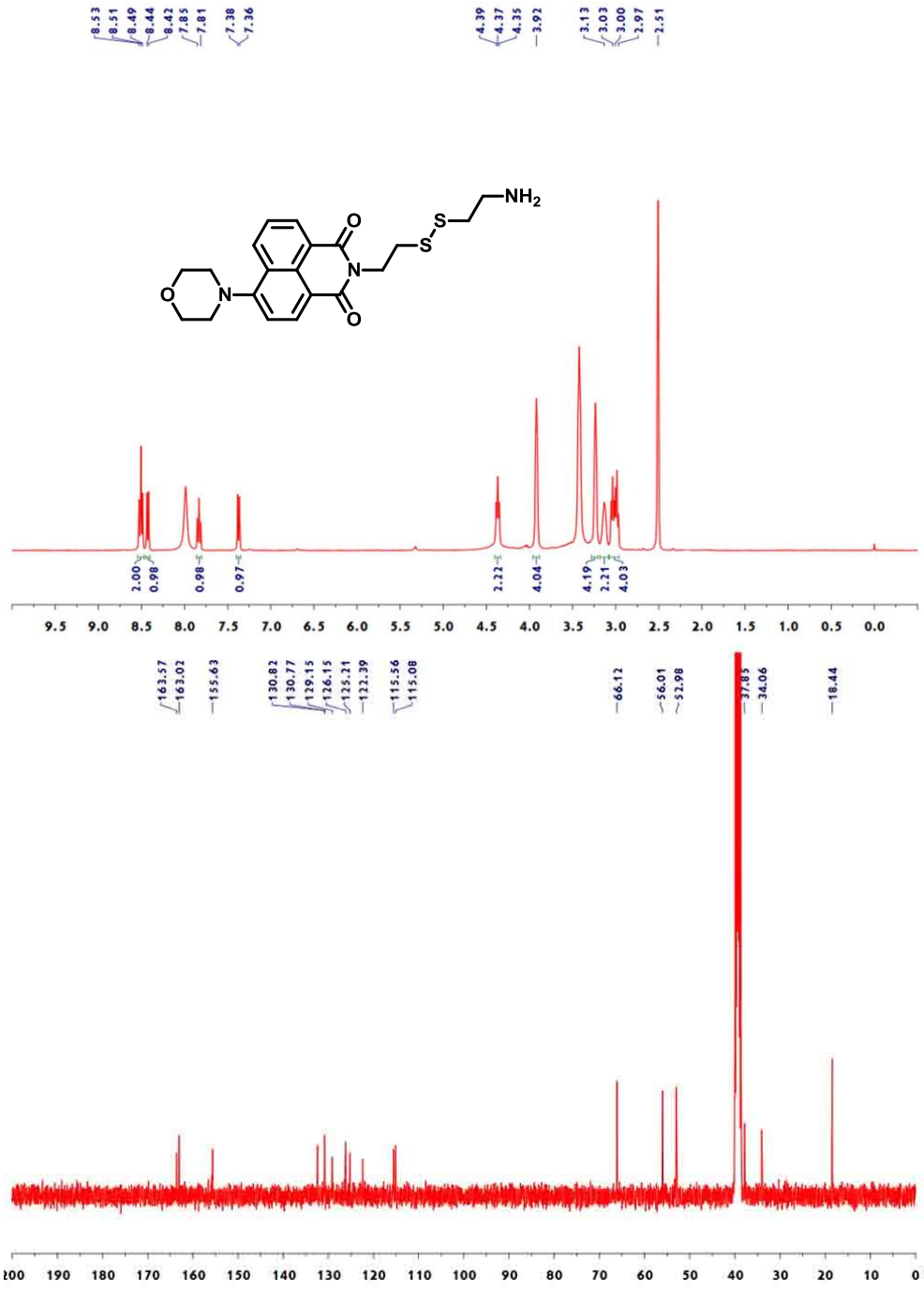
Monoisotopic Mass, Even Electron Ions
177 formula(e) evaluated with 11 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-30 H: 0-50 N: 0-3 O: 0-5 S: 0-2 Na: 0-1

JL-HUAN ECUST institute of Fine Chem 10-Sep-2014 16:10:54
HJL-YL-404 12 (0.446) Cm (1:17) 1: TOF MS ES+ 6.85e+003



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
540.1603	540.1603	0.0	0.0	11.5	40.8	0.0	C25 H31 N3 O5 S2 Na

Compound 4: ^{1}H and ^{13}C NMR spectrum in DMSO-d^{6}, high-res ESI-TOF mass spectrum

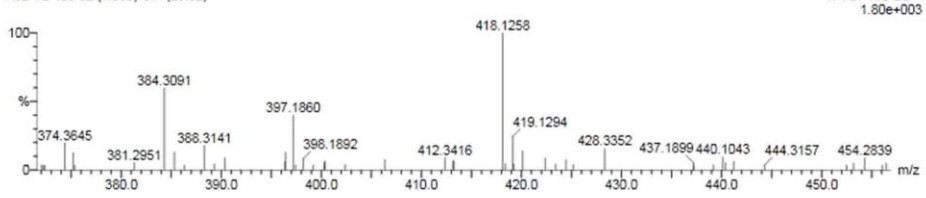


Elemental Composition Report **Page 1**

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

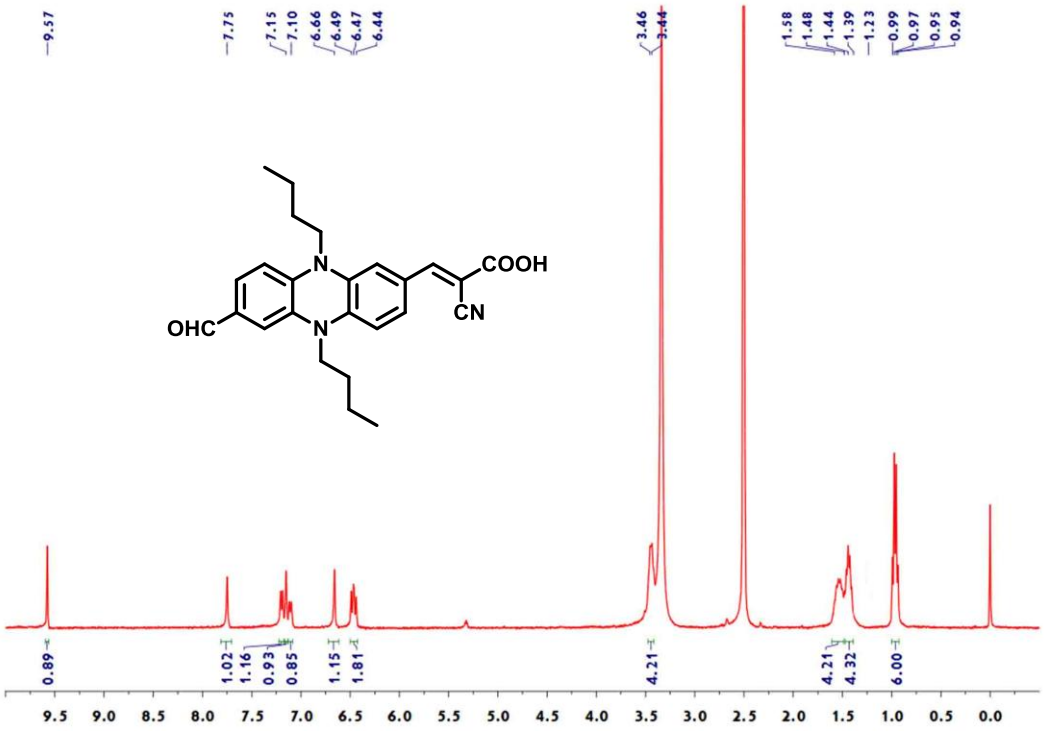
Monoisotopic Mass, Even Electron Ions
52 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 0-20 H: 0-100 N: 0-3 O: 0-3 S: 0-2

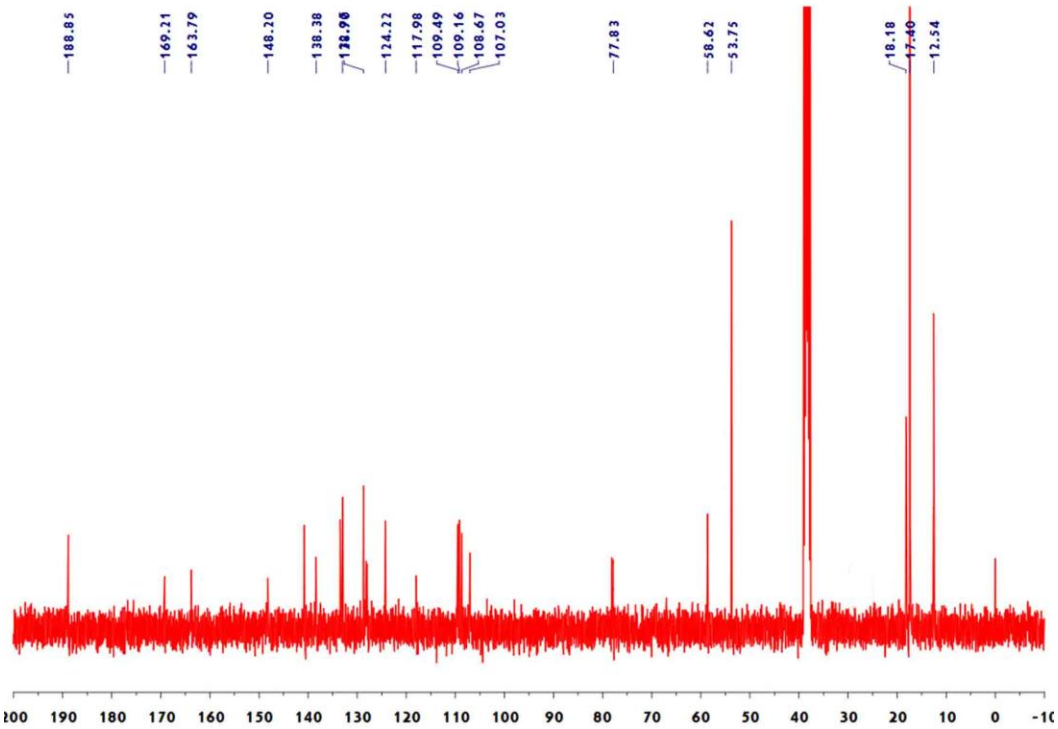
JL-HUA ECUST institute of Fine Chem 11-Sep-2014 20:32:27
HJL-YL-405 32 (1.060) Cm (29:32) 1: TOF MS ES+ 1.60e+003



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
418.1258	418.1259	-0.1	-0.2	10.5	24.3	0.0	C20 H24 N3 O3 S2

Compound 6: ^{1}H and ^{13}C NMR spectrum in DMSO-*d*^{6}, ESI-TOF mass spectrum





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
168 formula(e) evaluated with 11 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-30 H: 0-80 N: 0-5 O: 0-5

HUA-JL ECUST institute of Fine Chem 23-Sep-2013 16:15:53

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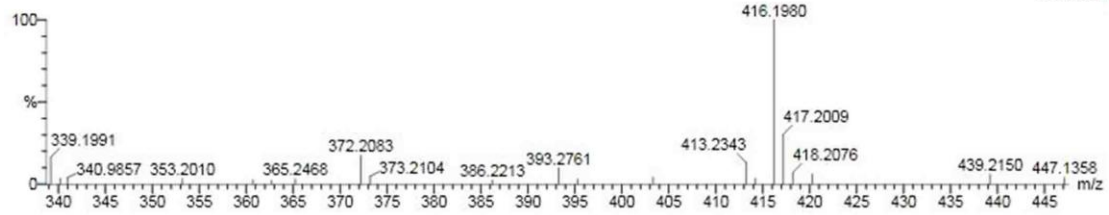
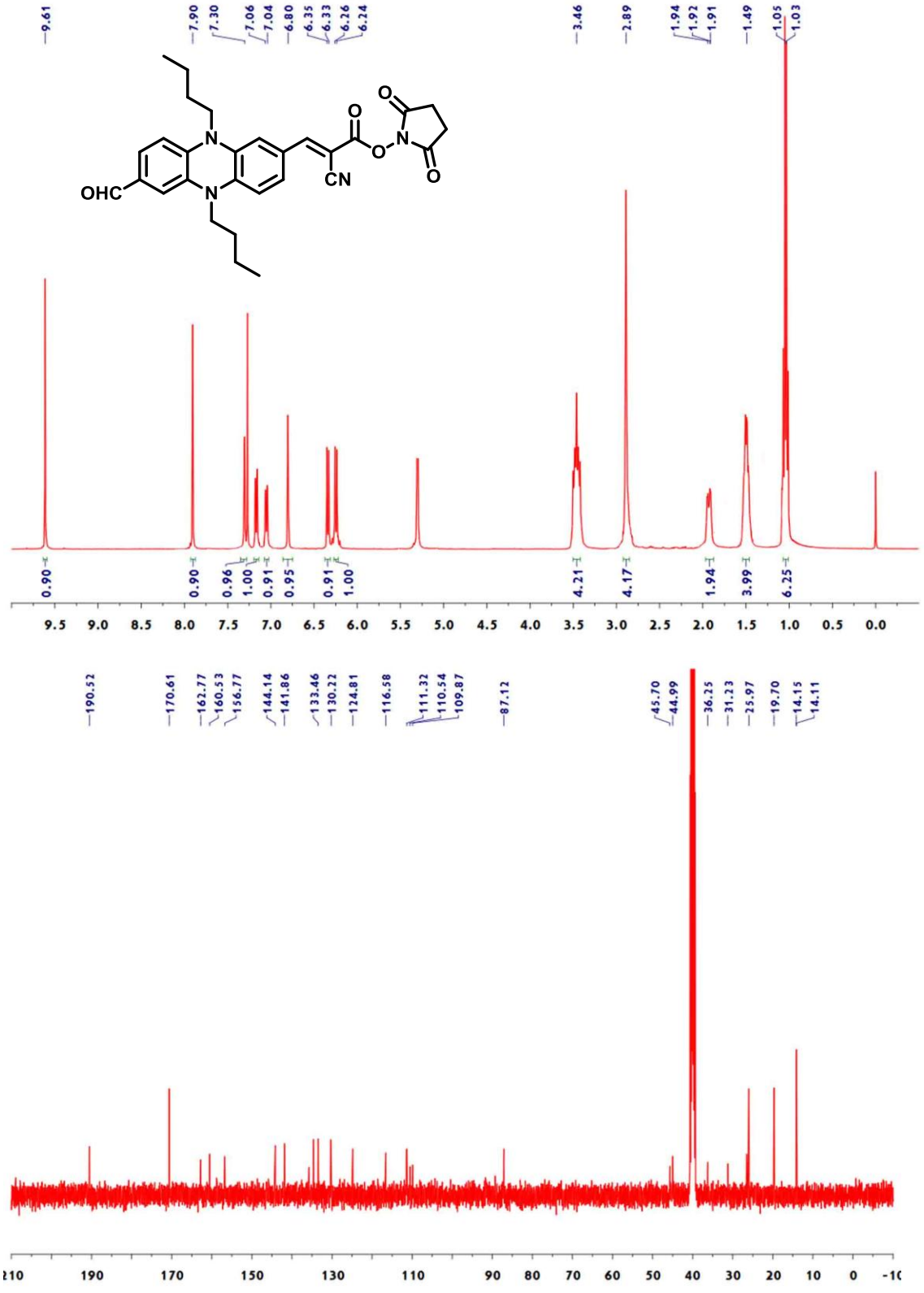


Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
416.1980	416.1974	0.6	1.4	14.5	7.5	0.0	C25 H26 N3 O3

Compound 7: ^{1}H NMR in $CDCl_3$, ^{13}C NMR spectrum in DMSO-d^6, ESI-TOF mass spectrum

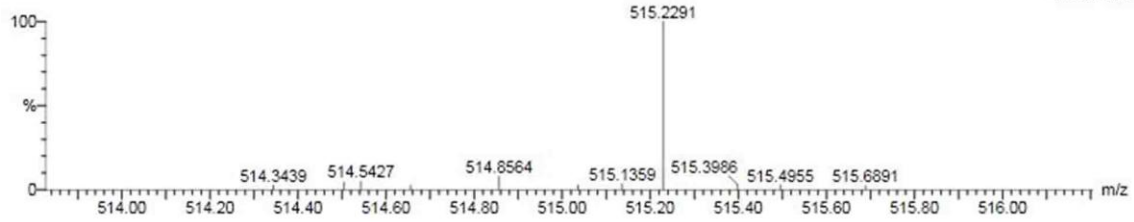


Single Mass Analysis
Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
249 formula(e) evaluated with 18 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-34 H: 0-60 N: 0-4 O: 0-12

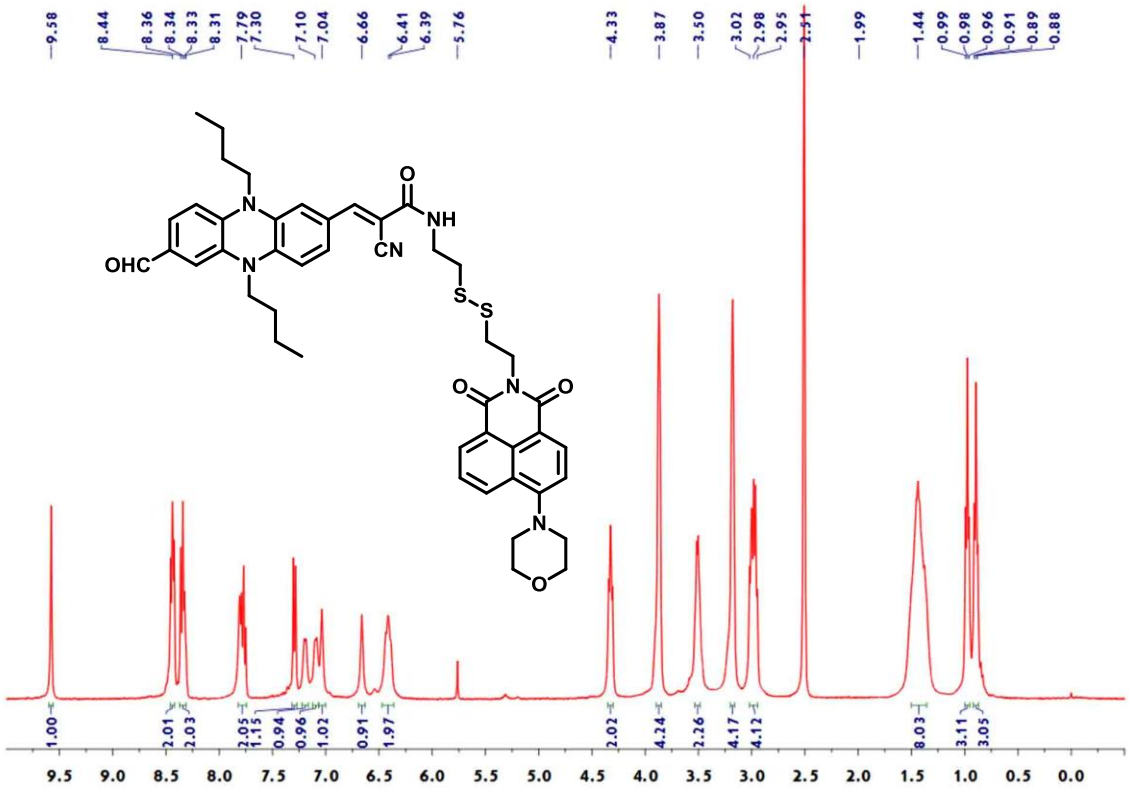
JL-HUA ECUST institute of Fine Chem 21-Sep-2013 16:41:22

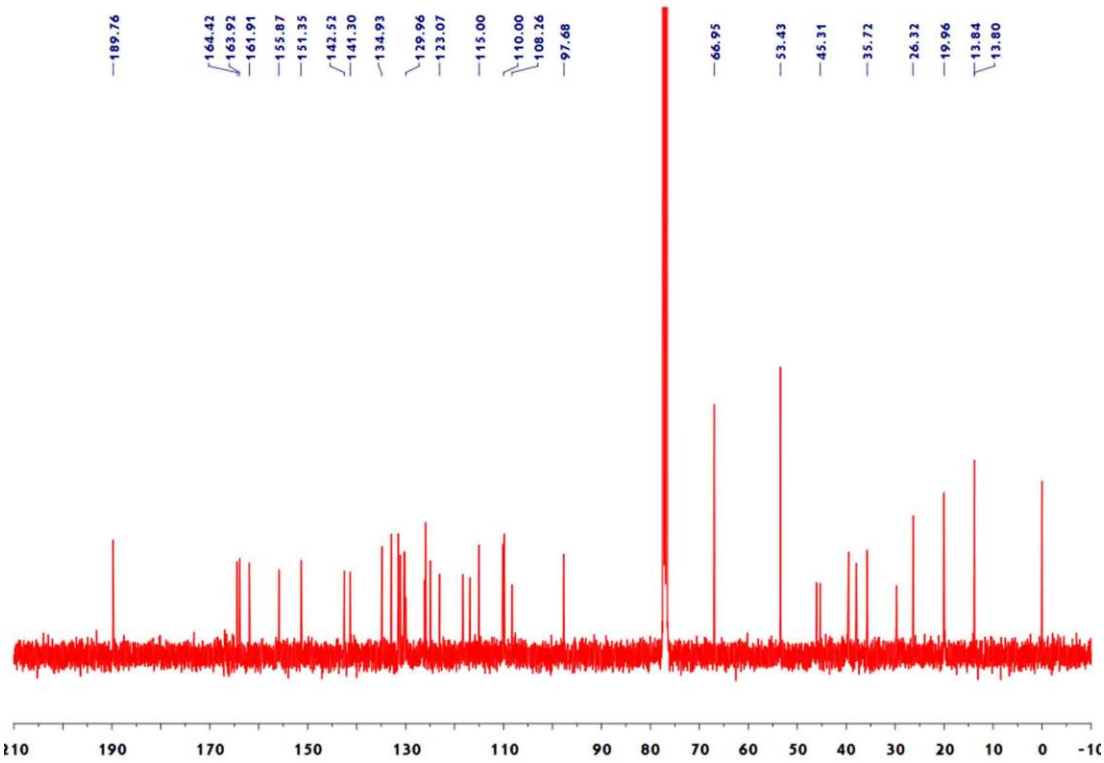
HJL-YL-322 7 (0.298) Cm (5:10) 1: TOF MS ES+ 6.13e+002



Minimum:				-1.5				
Maximum:		30.0	50.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula	
515.2291	515.2294	-0.3	-0.6	16.5	36.4	0.0	C29 H31 N4 O5	

PHSN: ^{1}H NMR in DMSO-*d*^{6}, ^{13}C NMR in $CDCl_3$ and MALDI-TOF mass spectrum

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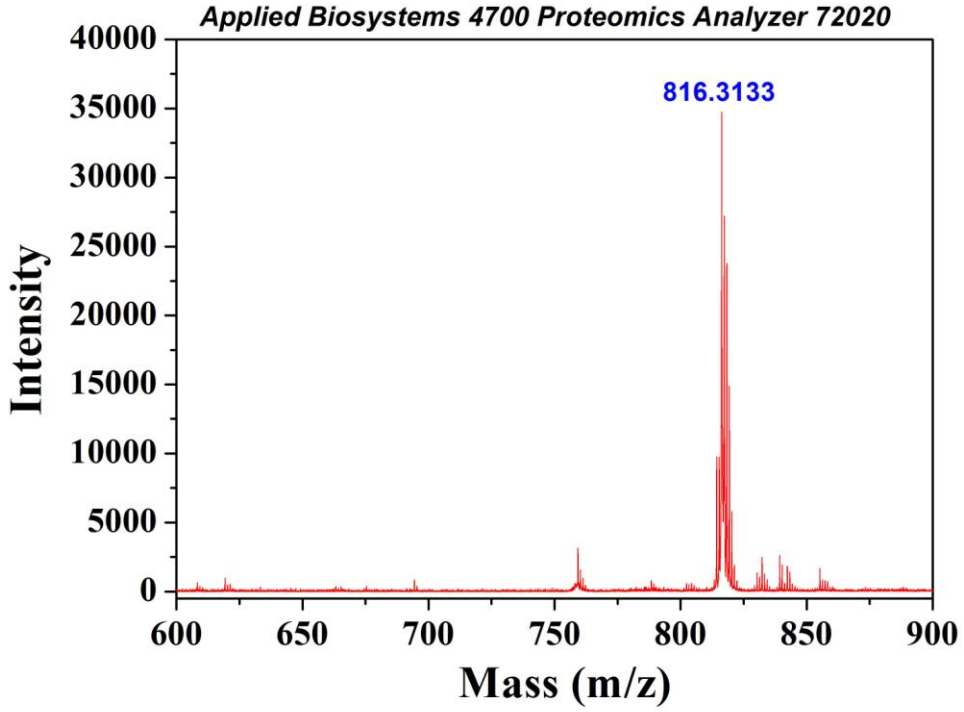
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650
700
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900
Mass (m/z)

Excitation spectra of PHSN when treated with cysteine

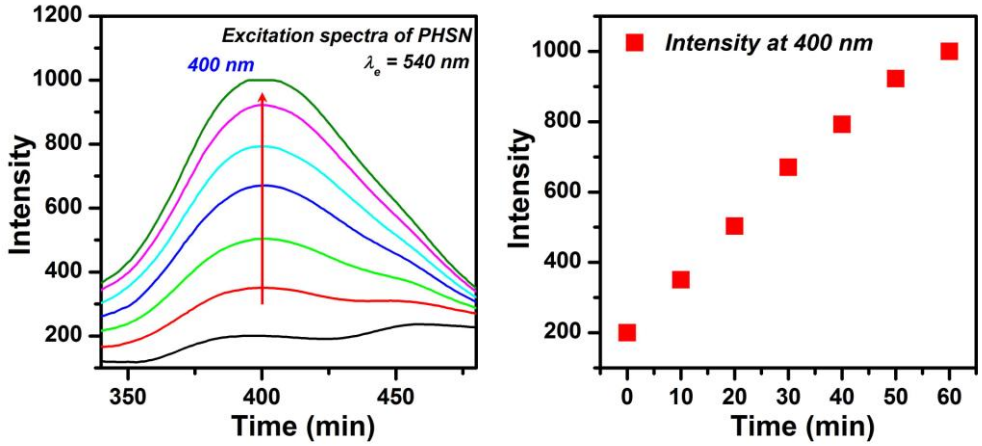


Figure S1. Left: Excitation spectra of **PHSN** with 40.0 equivalents of cysteine at 37 ^{o}C. Right: the corresponding dot plot of intensity at 400 nm against reaction minute. **PHSN** (5 μM) was in HEPES buffer (20 mM, 50% EtOH, pH 7.4). Cysteine was dissolved in distilled water and added by microsyringe.

Absorption spectra of PHSN when treated with cysteine

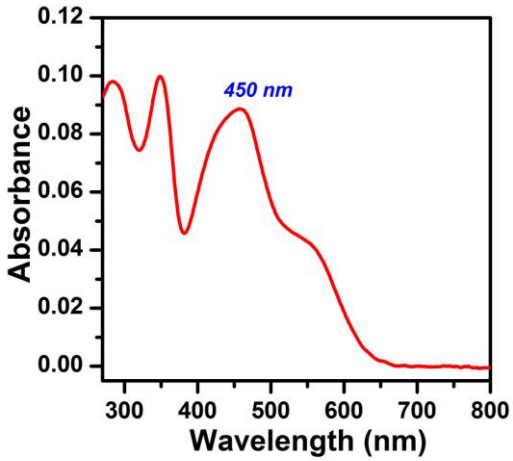


Figure S2. Unchanged absorption spectra of **PHSN** when treated with 40.0 equivalents of cysteine at 37 ^{o}C.

Emission spectra of PHSN when treated with Hcy and GSH

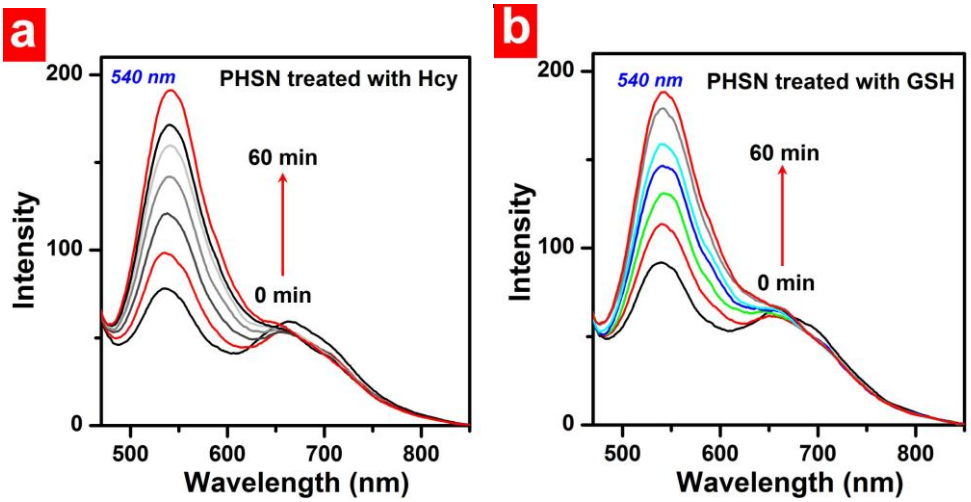


Figure S3. Time-dependent fluorescence emission spectra of **PHSN** (5 μM) toward Hcy (a) and GSH (b), respectively, from 0 – 60 min in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 ^{o}C.

Comparison of ^{1}H NMR spectra of PHSN, compounds 4 and 6

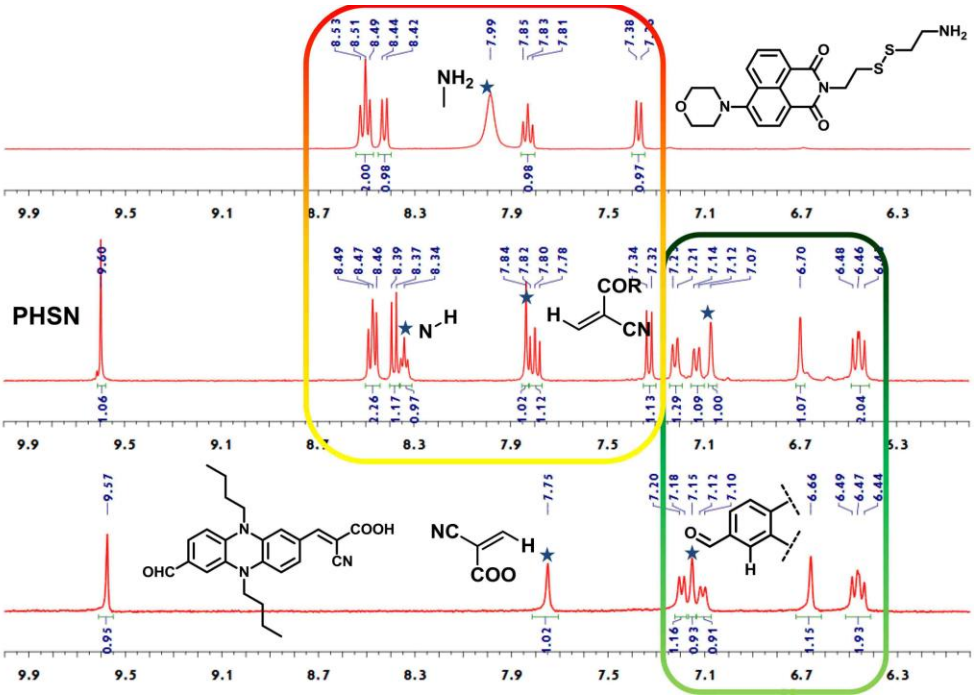


Figure S4. ^{1}H NMR spectra of compound **4** (above), compound **6** (below) and **PHSN** (middle) from 9.0 to 6.0 ppm.

^{1}H NMR titration spectra of PHSN with MPA

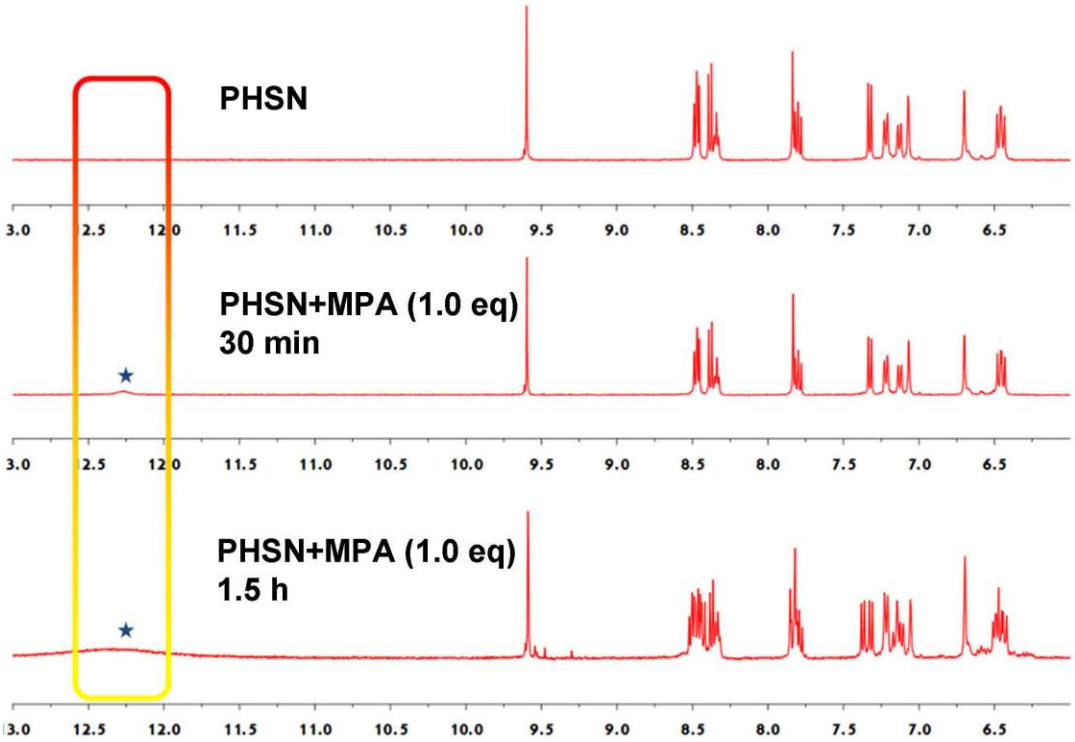
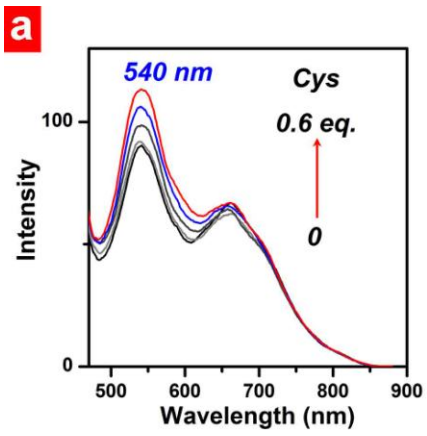


Figure S5. ^{1}H NMR spectra of **PHSN** with MPA for 30 min and 1.5 h, the spectra was shown from 13.0 to 6.0 ppm. The small peaks marked by stars showed the appearance of carboxyl proton.

Emission and excitation titration spectra of PHSN when treated with 0-0.6 eq. cysteine



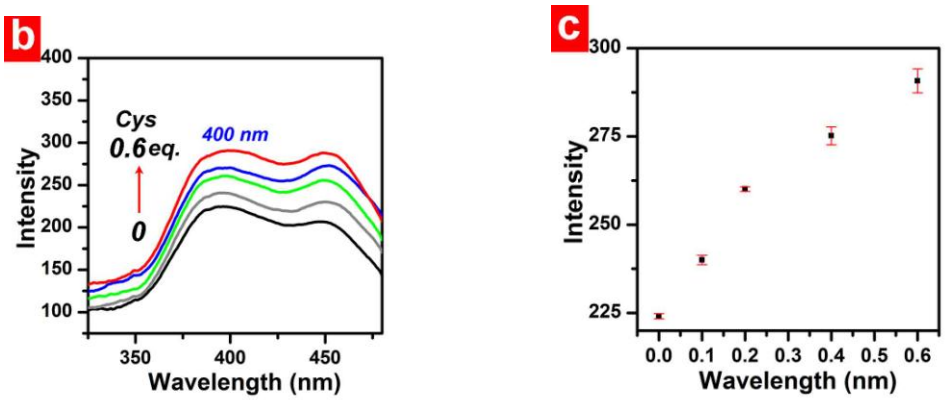


Figure S6. Emission spectra (a) and excitation spectra (b) of **PHSN** (5 μM) reacted with 0 – 0.6 eq Cys in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 ^{o}C. Each spectrum was recorded after 1 h of titration; c) excitation corresponding scattered plot of intensity at 400 nm against Cys equivalents.

Excitation spectra of PHSN when treated with various amino acids

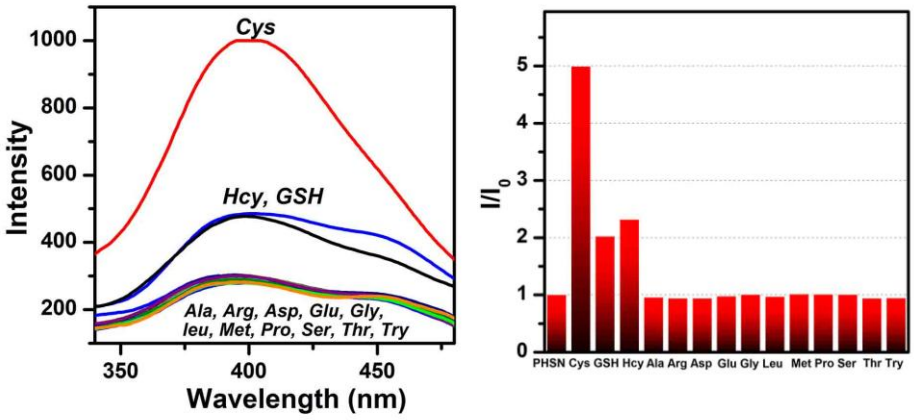


Figure S7. Left: Fluorescence excitation spectra of **PHSN** (5 μM) against 40 equivalents of Cys, Hcy, GSH and various other amino acids in HEPES buffer (50% EtOH v/v, pH 7.4) at 37.4 ^{o}C. Right: Corresponding histogram demonstrated the selectivity of **PHSN** for Cys over other amino acids.

Cell imaging

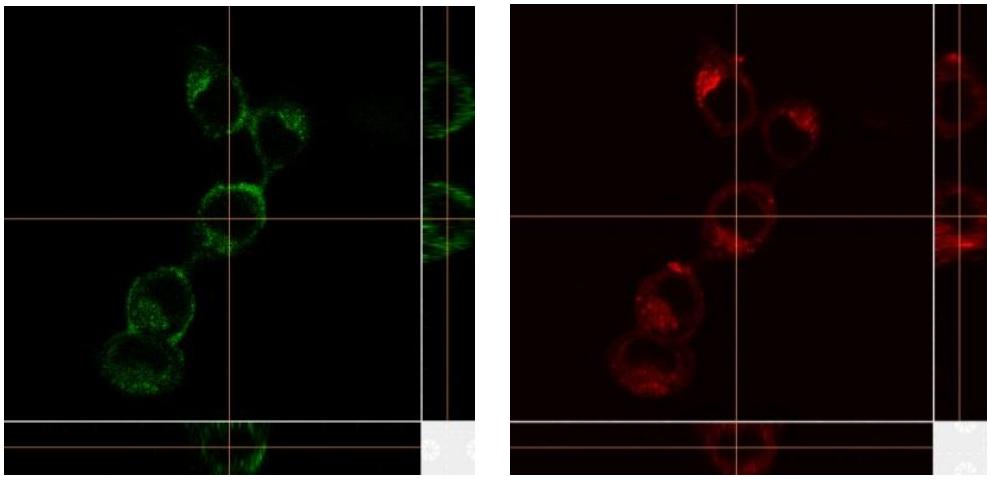


Figure S8. Confocal fluorescence Z-scan images of Hela cells in two fluorescence channels (Green Channel: λ_{ex} = 488.0 nm, λ_{em} = 525-575 nm; Red channel: λ_{ex} =561 nm, λ_{em} = 600-700 nm). Hela cells were incubated with **PHSN** (10^{-5} M) for 40 min then added and incubated with Cys (about 10^{-5} M) for another 60 min.

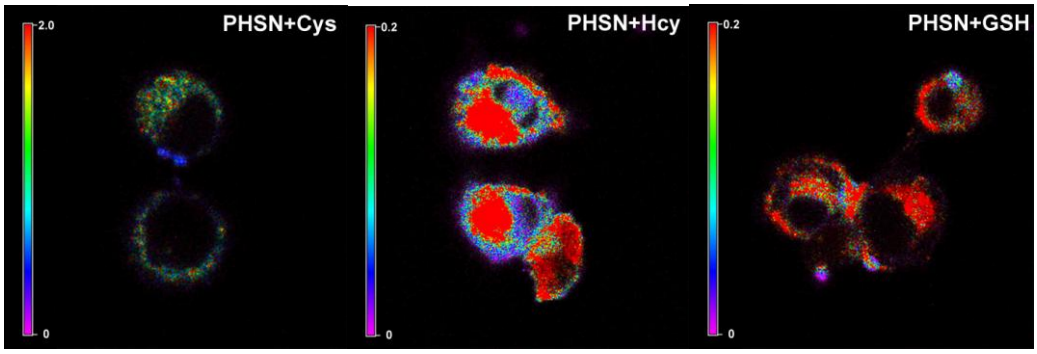


Figure S9. Confocal laser scan fluorescence ratio images of Hela cells incubated with PHSN (10 μM) in PBS buffer pH 7.4 at 37.4 ^{o}C for 40 min then added and incubated with cysteine (10 μM), homocysteine (10 μM) or glutathione (10 μM) respectively for another 60 min. The colorful bar indicated the ratio scale of Green / Red from 0 to 2.0 for **PHSN + Cys** and 0 to 0.2 for **PHSN + Hcy** and **PHSN + GSH**. Green channel (λ_{ex} = 488 nm, λ_{em} = 525-575 nm), red channel (λ_{ex} = 561 nm, λ_{em} = 600-700 nm).