Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Specific and Sensitive Imaging of Basal Cysteine over Homocysteine in Living Cells

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1.The kinetic profile of the recognition of probe 1 for Cys

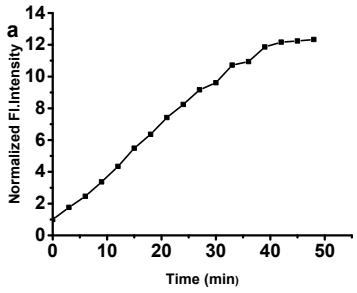


Fig.S1. The dynamic fluorescence changes at 525 nm of **probe 1** (5 μM) after the addition of either Cys in H_2O:ethanol=8:2 (v/v), pH=7.4, 10 mM PBS at room temperature. Excitation wavelength is 400 nm, excitation and emission slit widths are 5 nm and 5 nm.

2. The fluorescence responses of probe 1 with other ions

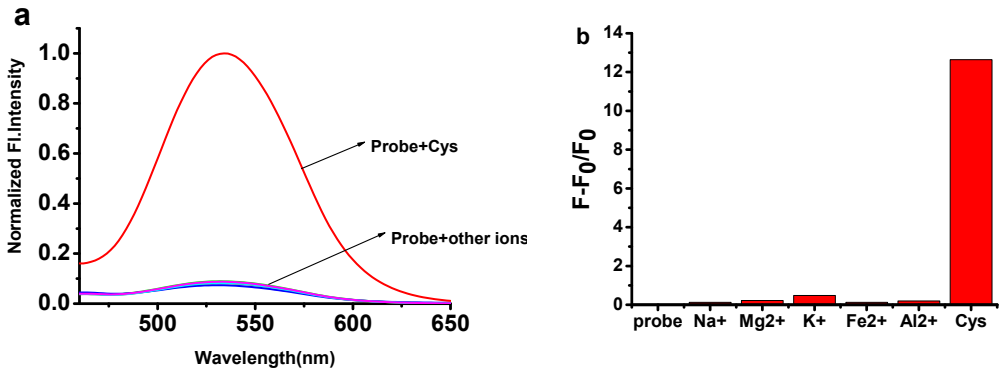


Fig.S2. The fluorescence spectra of **probe 1** (5 μM) toward Cys (10 μM) and various ions (10 μM) in H_2O:ethanol=8:2 (v/v), pH=7.4, 10 mM PBS at room temperature. (b) Fluorescence intensity changes at 525 nm. Excitation wavelength = 400 nm, excitation and emission slit widths = 5 nm and 5 nm. Each spectrum was acquired 40 min after various analytes addition at room temperature.

3. Bioimaging applications

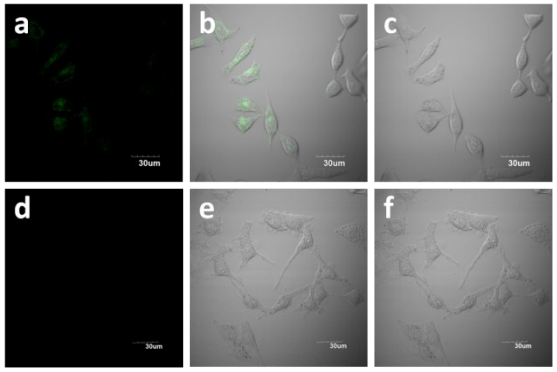


Fig.S3. Fluorescence images of Cys in living cells. (a-c) HepG2 cells stained with **probe 1** (5 μM) for 30 min. (d-f) HepG2 cells were treated with NEM (100 ng/mL) for 1 h before incubated with **probe 1** (5 μM) for 30 min. (a, d) fluorescence image. (b, e) Bright field image. (c, f) merged image. Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2. Excitation wavelength is 405 nm, emission wavelength was collected from 450 to 550nm.

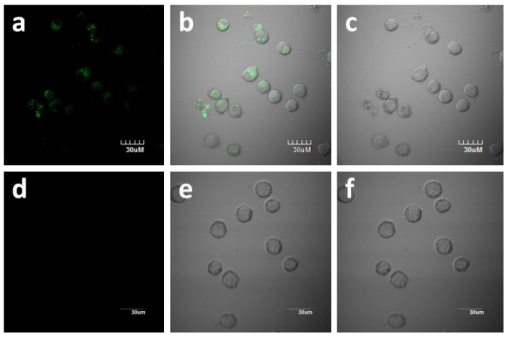


Fig.S4. Fluorescence images of Cys in living cells. (a-c) RAW 264.7 macrophage cells stained with **probe 1** (5 μM) for 30 min. (d-f) RAW 264.7 macrophage cells were treated with NEM (100 ng/mL) for 1 h before incubated with **probe 1** (5 μM) for 30 min. (a, d) fluorescence image. (b, e) Bright field image. (c, f) merged image. Incubation was performed at 37 °C under a humidified atmosphere containing 5% CO_2. Excitation wavelength is 405 nm, emission wavelength was collected from 450 to 550nm.

3. The characterization data of probe 1

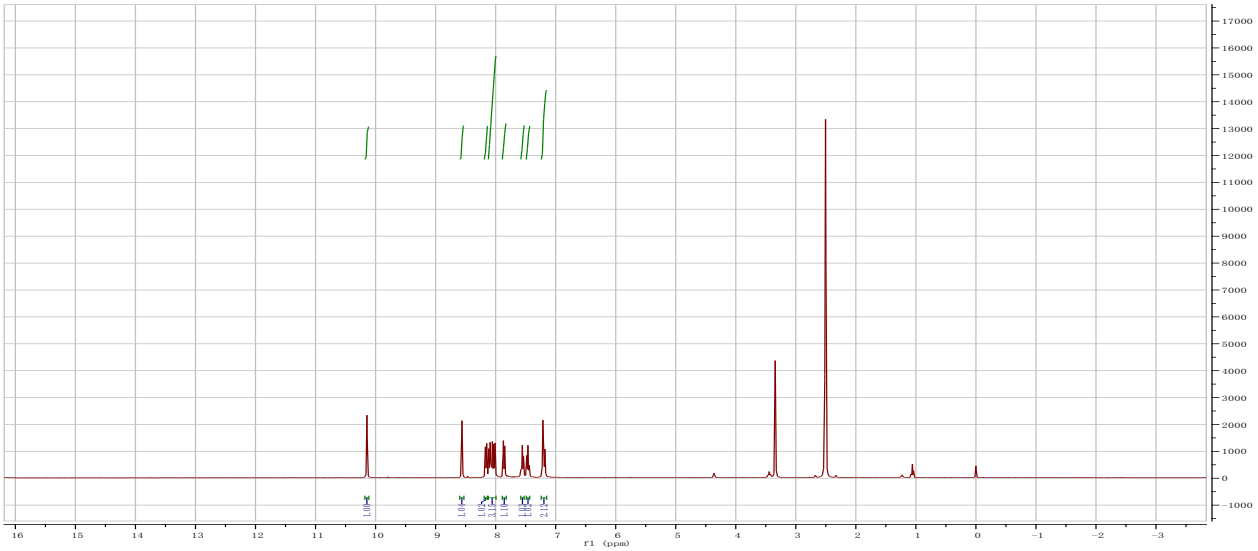


Figure S5. 1H NMR spectrum of compound P-OH.

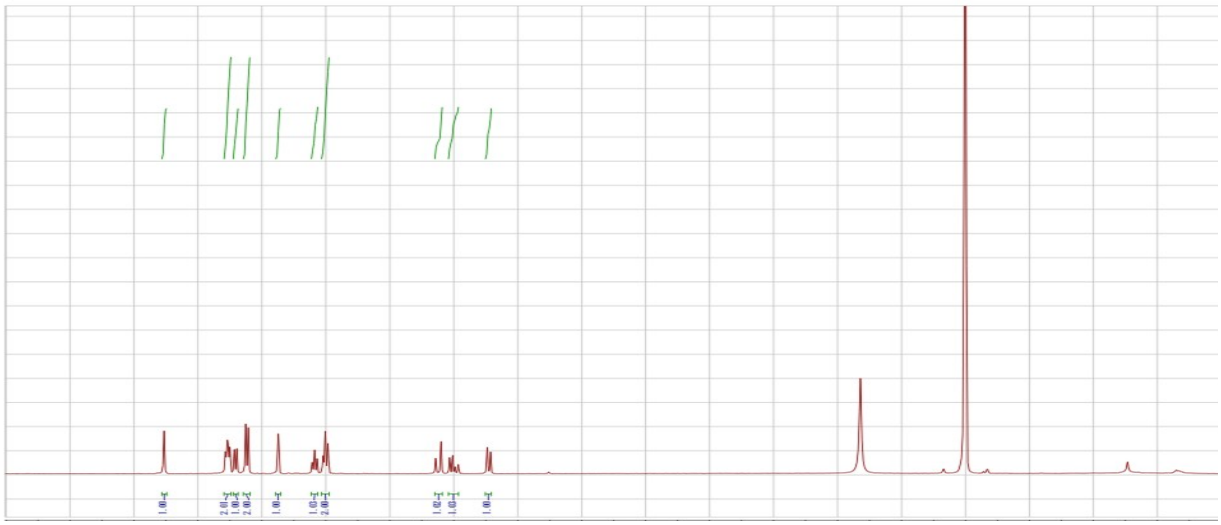


Figure S6. 1H NMR spectrum of probe 1.

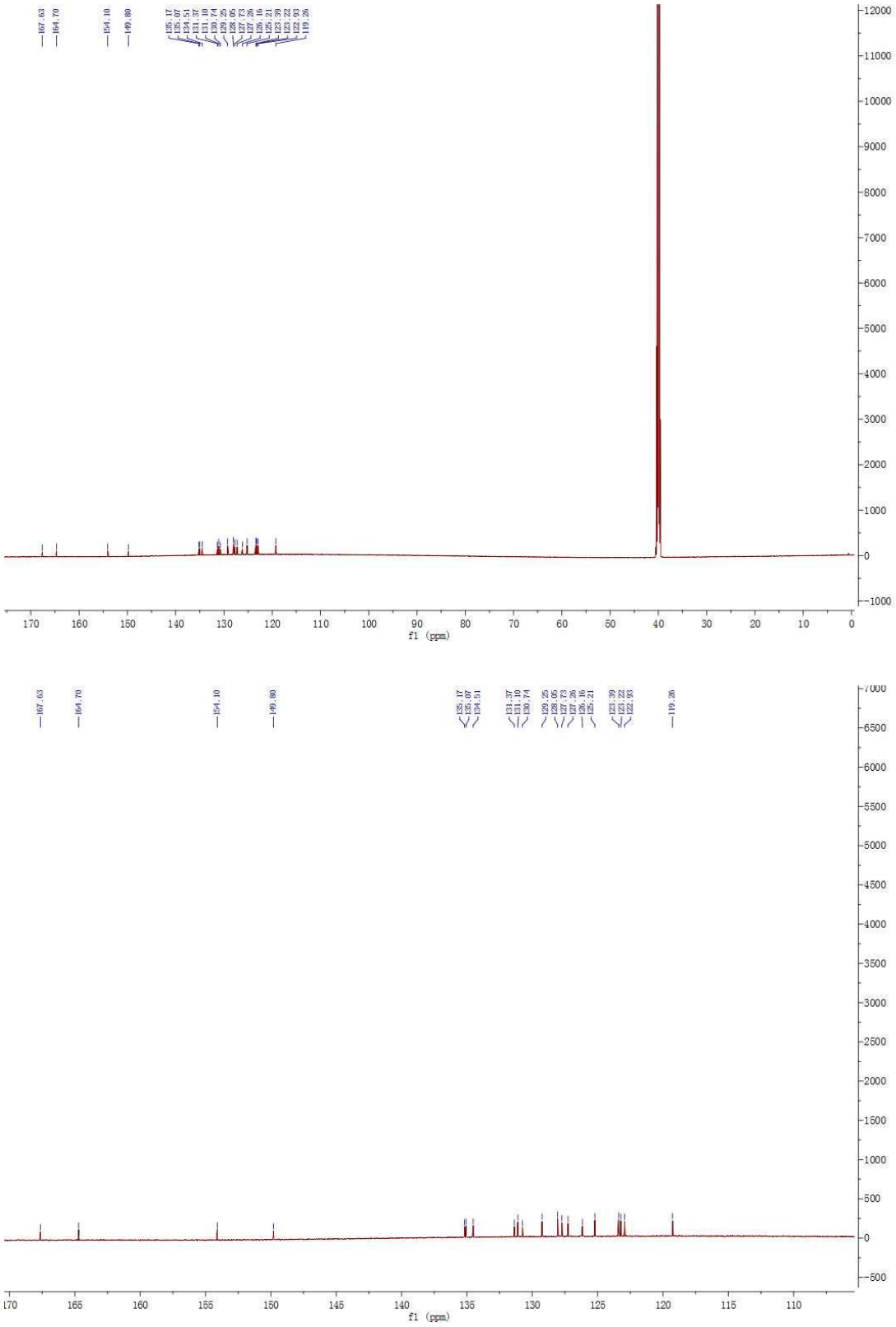


Figure S7.13C NMR spectrum of probe 1.

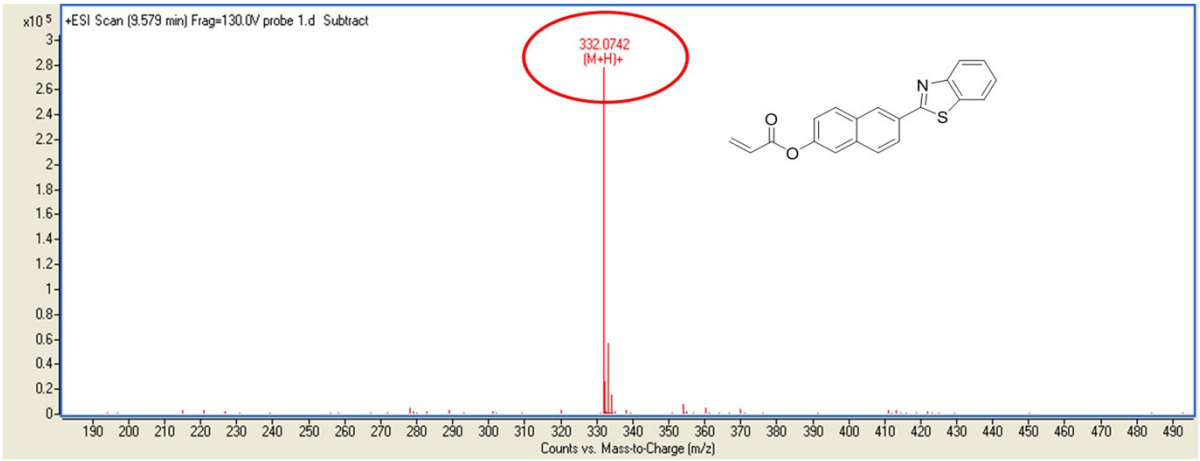


Figure S8. HRMS of probe 1.

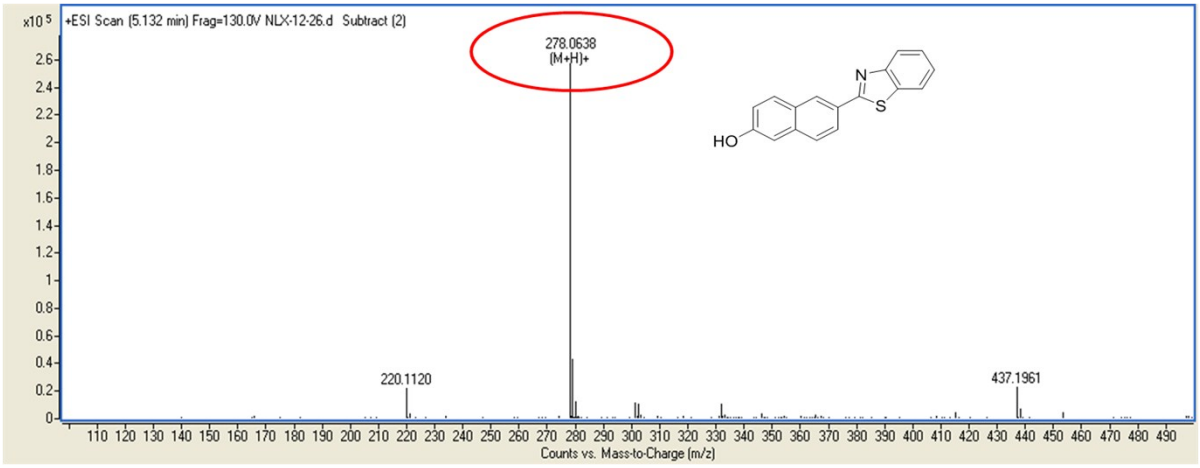
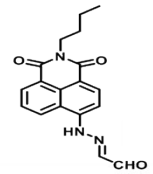
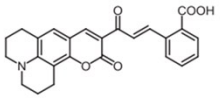
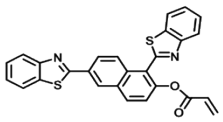
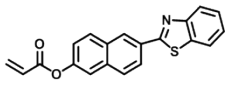


Figure S9. HRMS of probe 1 reacted with Cys.

Table S1. Comparison of fluorescent probes based on cyclizationreaction for Cys

probe	λem (nm)	Detection limit	solution	Selectivity of probe	Synthesis of probe	References
	524	—	DMSO	Cys/Hcy	2 Steps	Org. Lett., 2012,520.
	513	—	100%H_2O	Cys/Hcy	2 Steps	Biomaterials, 2012,8495.
	530	0.05 μM	50%THF	Only Cys	3 Steps	Tetrahedron letters, 2017,3214.
	525	14.8 nM	80%H_2O	Only Cys	2 Steps	This work