Supporting information

Lysosome-targeted Near-Infrared Fluorescence Probe for Imaging Endogenous Cysteine (Cys) in Living Cells

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[b] *Tianjin Key Laboratory for Photoelectric Materials and Devices, and Key Laboratory of Display Materials & Photoelectric Devices, Ministry of Education, School of Materials Science & Engineering, Tianjin University of Technology, Tianjin 300384, China. Fax: (+86)22-60215226; Tel: (+86)22-60216748; E-mail: xshzeng@tjut.edu.cn*

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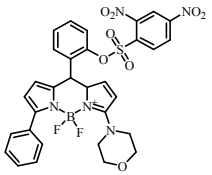
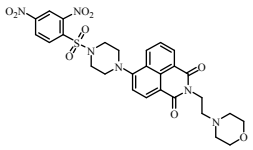
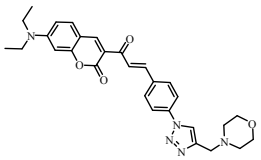
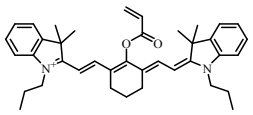
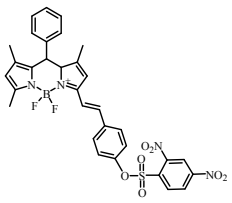
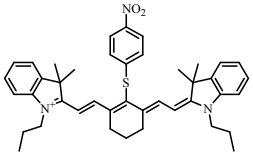
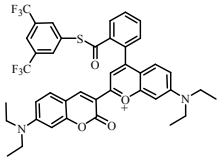
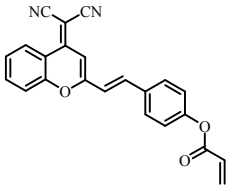
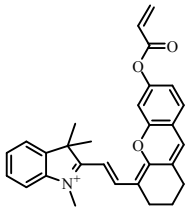
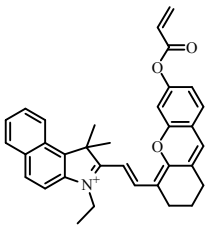
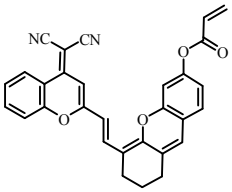
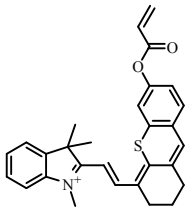
Table S1 Comparison of the reported Cys probes in the literature

Structrure of sensors	Cys selectivity	λ_{em}	Organelle-targeted ability	Reference
	Poor	584 nm	Lysosomes	*Org. Biomol. Chem.*, 2015, **13**, 8163
	Poor	540 nm	Lysosomes	*Scientific Reports,* 2016, **6**, 19562
	Good	498 nm	Lysosomes	*Anal. Chem.,* 2018, **90**, 7018
	Good	625 nm	NO	*Chem. Sci.,* 2012, **3**, 2760
	Good	590 nm	NO	*Biosens. Bioelectron.,* 2011, **26**, 3012
	Good	748 nm	NO	*RSC Adv.,* 2014, **4**, 8360
	Poor	650 nm	NO	*Anal. Chem.,* 2014, **86**, 1800

	Good	706 nm	NO	*RSC Adv.,* 2014, **4**, 46561
	Good	697 nm	NO	*Anal. Chem.,* 2015, **87**, 4856
	Good	735 nm	Mitochondria	*ACS Appl. Mater. Interfaces, 2015, 7, 27968*
	Good	760 nm	NO	*Anal. Chem.,* 2018, **90**, 1014
	**Good**	**765 nm**	**Lysosomes**	In this paper

Determination of the fluorescence quantum yield

Fluorescence quantum yields (Φ_I) of **SHCy-C** and **SHCy** were determined by using rhodamine B (Φ_B = 0.73) [s1] as the fluorescence standard. The quantum yield was calculated using the following equation.

$$\Phi_1 = \Phi_B \times \frac{Abs_B \times F_1 \times \lambda ex_B \times \eta_1^2}{Abs_1 \times F_B \times \lambda ex_1 \times \eta_B^2}$$

Where Abs is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and η is the refractive index of the solvent used. Subscripts 1 and B refer to the **SHCy-C**/**SHCy** and to the standard, respectively.

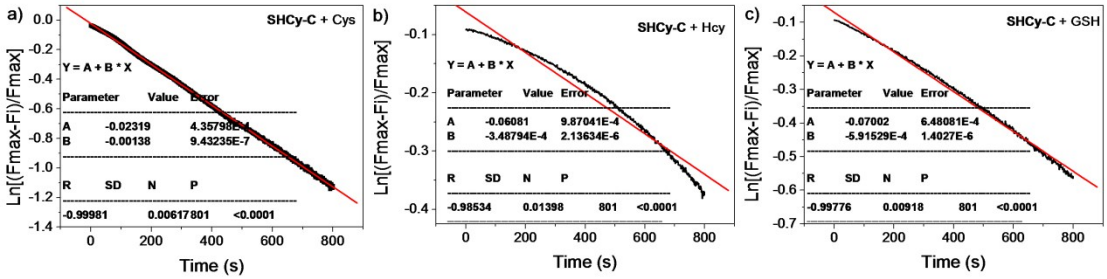


Fig. S1. Pseudo first-order kinetic plot of reaction of probe **SHCy-C** (5 μM) with (a) Cys (50 μM), for k = 276 $M^{-1}s^{-1}$; with (b) Hcy (50 μM), for k = 69.8 $M^{-1}s^{-1}$; with (c) GSH (50 μM), for k = 118.4 $M^{-1}s^{-1}$; respectively.

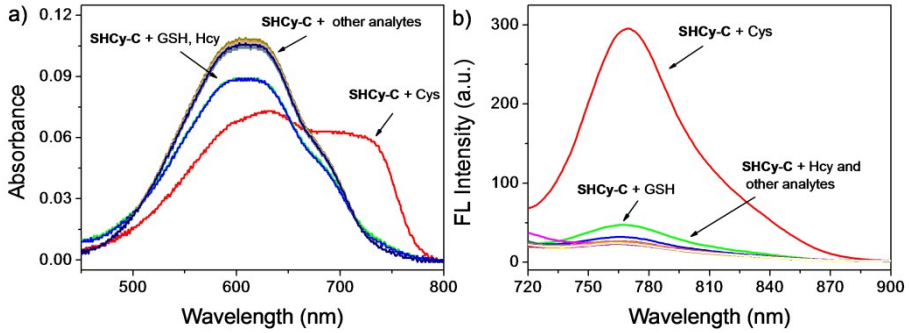


Fig. S2. The UV-Vis absorption spectra (a) and fluorescence emission spectra (b) of **SHCy-C** (5 μM) with the addition of 10 eq. various analytes (Cys, Hcy, GSH, Val, Try, Ala, Arg, Ser, Leu, His, Lys, Phe, Pro, Glu, Tyr), 1 eq. cations (Ca^{2+}, Fe^{2+}, Cu^{2+},Zn^{2+}) and 1 eq. anion (S^{2-}). Note: after interacted with analytes for 5 min, each spectrum of **SHCy-C** was recorded λ_{ex} = 700 nm.

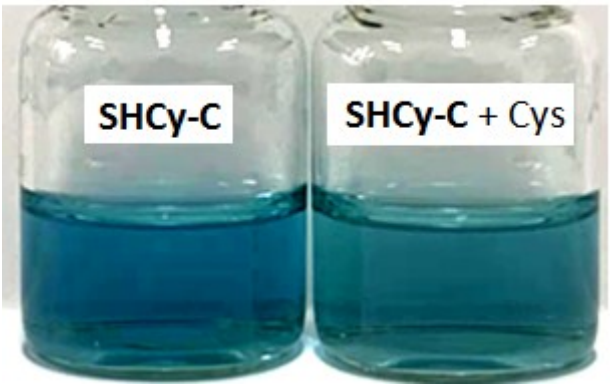


Fig. S3. The color of **SHCy-C** (5 μM) in the absence and presence of Cys (50 μM).

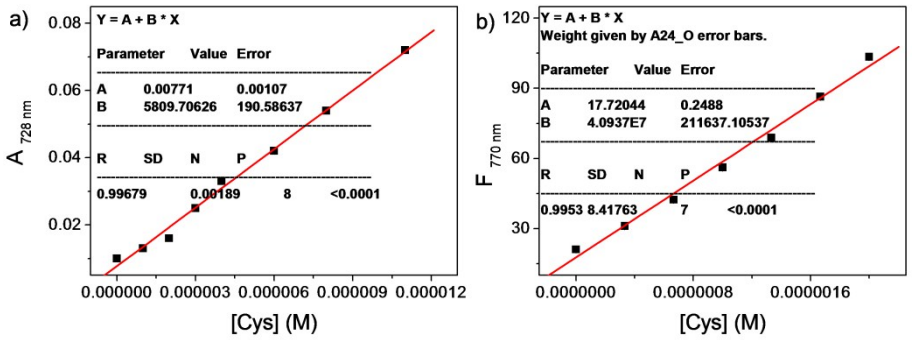


Fig. S4. The linear relationship between the absorbance a) and fluorescence intensity b) of **SHCy-C** (5 μM) and the Cys concentration ranging from 0-50 μM and 0-2.0 μM, respectively.

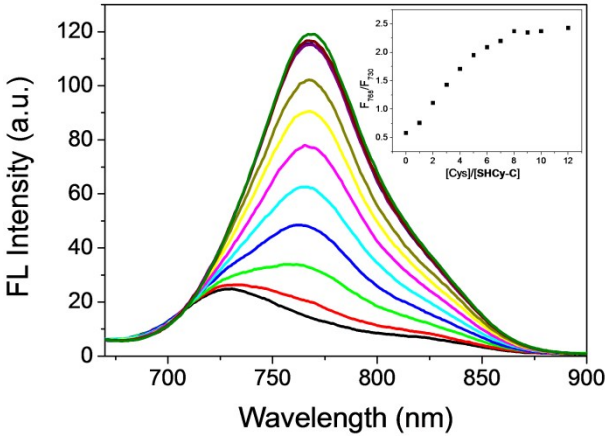


Fig. S5. The FL titration of **SHCy-C** (5 μM) upon addition of different concentrations of Cys (0-120 μM) in ethanol/PBS (1/4, v/v, 10 mM, pH 7.4). Each spectrum of **SHCy-C** was recorded at 5 min after the addition of Cys. Inset: the fluorescence ratio (F768/F730) of **SHCy-C** upon addition of Cys. λ_{ex} = 630 nm.

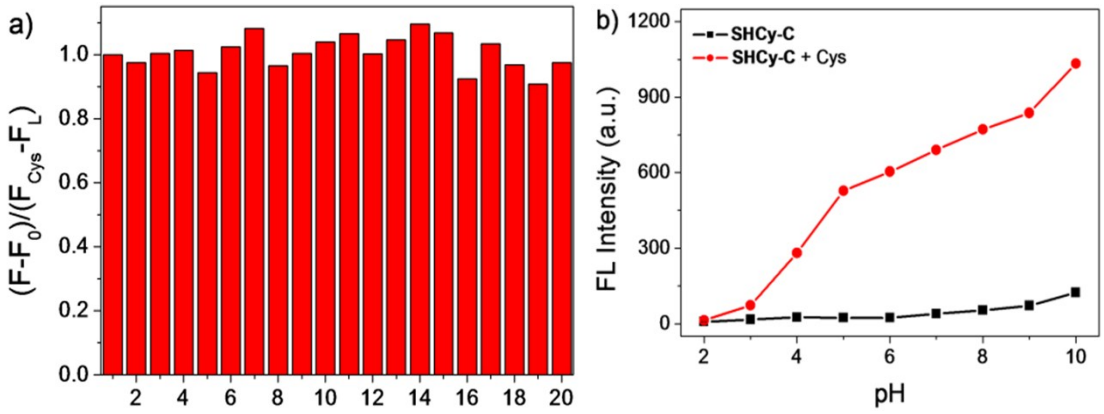


Fig. S6. a) Fluorescence ratio changes at 770 nm of **SHCy-C** (5 μM) to Cys (10 μM) in the presence of other analytes (1-20: Cys, Hcy, GSH, Val, Try, Ala, Arg, Ser, Leu, His, Lys, Phe, Pro, Glu, Tyr, Ca^{2+}, Fe^{2+},Cu^{2+},Zn^{2+}, S^{2-}); b) Fluorescence intensity at 770 nm of **SHCy-C** (5 μM) in the absence and presence of Cys (10 μM) in the pH range of 3-10. Note: After interacted with Cys for 15 min, the fluorescence intensity of **SHCy-C** were recorded. λ_{ex} = 700 nm.

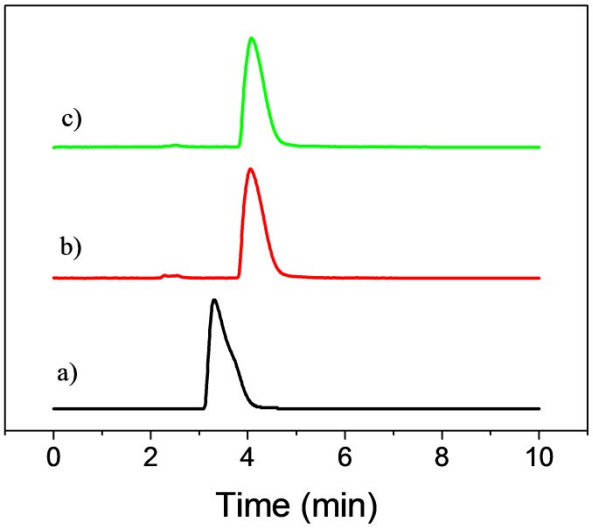


Fig. S7. HPLC chromatograms of **SHCy** and **SHCy-C**. a) **SHCy-C**; b) the reactant of **SHCy-C** with 20 equiv. Cys for 5 min; c) the reactant of **SHCy** with 20 equiv. Cys for 5 min; The mobile phase was MeOH/MeCN (v/v, 4/1).

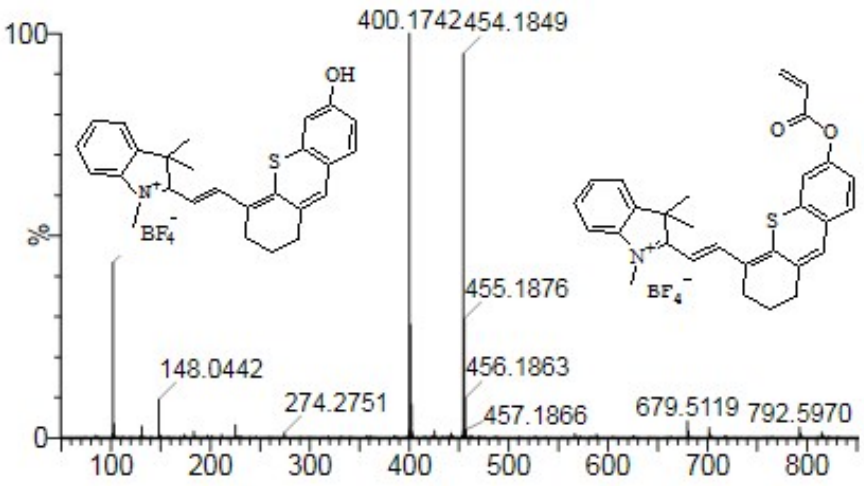


Fig. S8. HRMS of the probe **SHCy-C** with addition of 2.0 equiv. Cys for 2 hours.

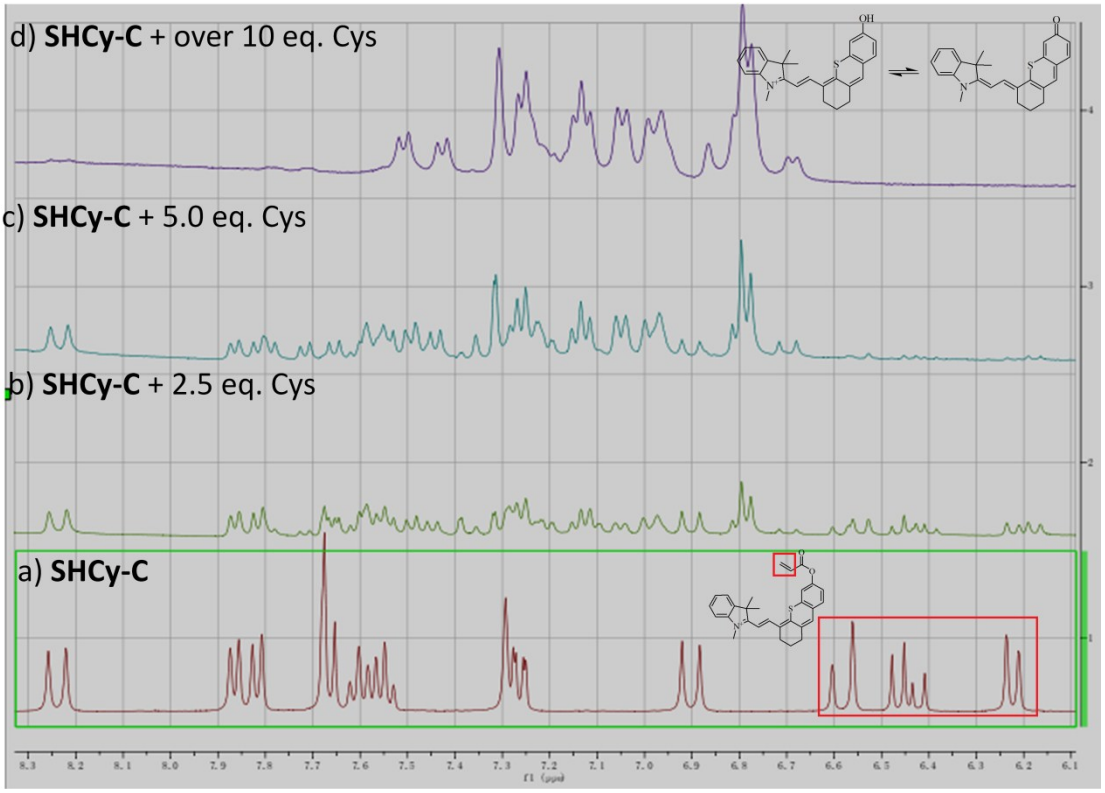


Fig. S9. ^{1}H NMR spectra of **SHCy-C** in the abaence and presence of Cys (2, 5, and over 10 equiv.).

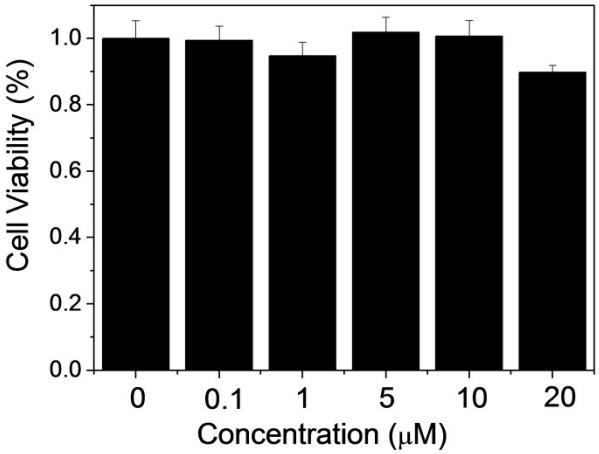


Fig. S10. Cell viability estimated by MTT proliferation tests versus incubation concentrations of **SHCy-C**.

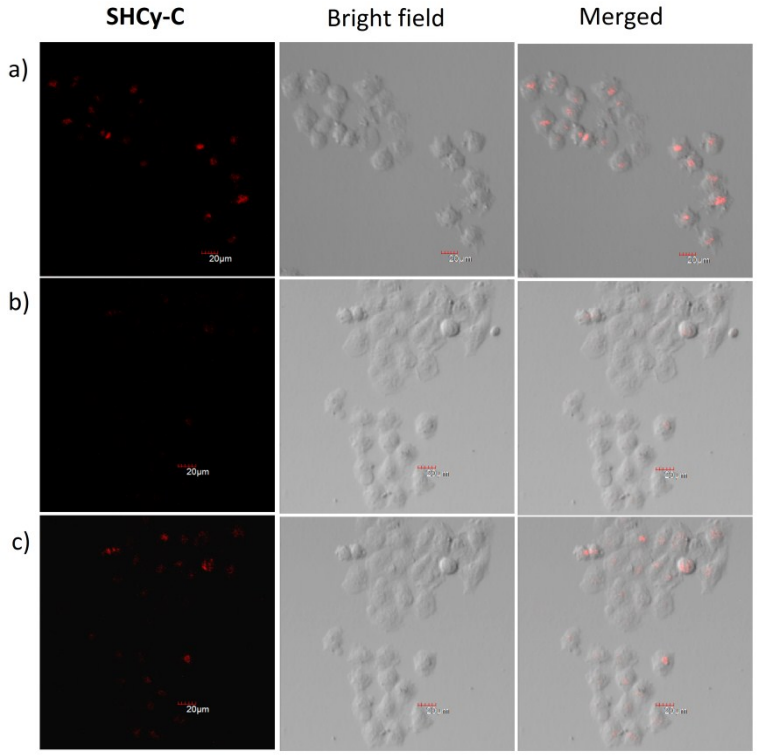


Fig. S11. The fluorescence imaging of **SHCy-C** in living Hela cells. a) Hela cells were treated with **SHCy-C** (0.2 μM) for 20 min; b) Hela cells pretreated with 1 mM NEM for 20 min and followed by incubation with **SHCy-C** (0.2 μM) for another 20 min; c) Hela cells pretreated with 1 mM NEM for 20 min and followed by incubation with **SHCy-C** (0.2 μM) for another 20 min and treated with 200 μM Cys for further 20 min. λ_{ex} = 635 nm, λ_{em} = 700-800 nm, scale bar is 20 μm.

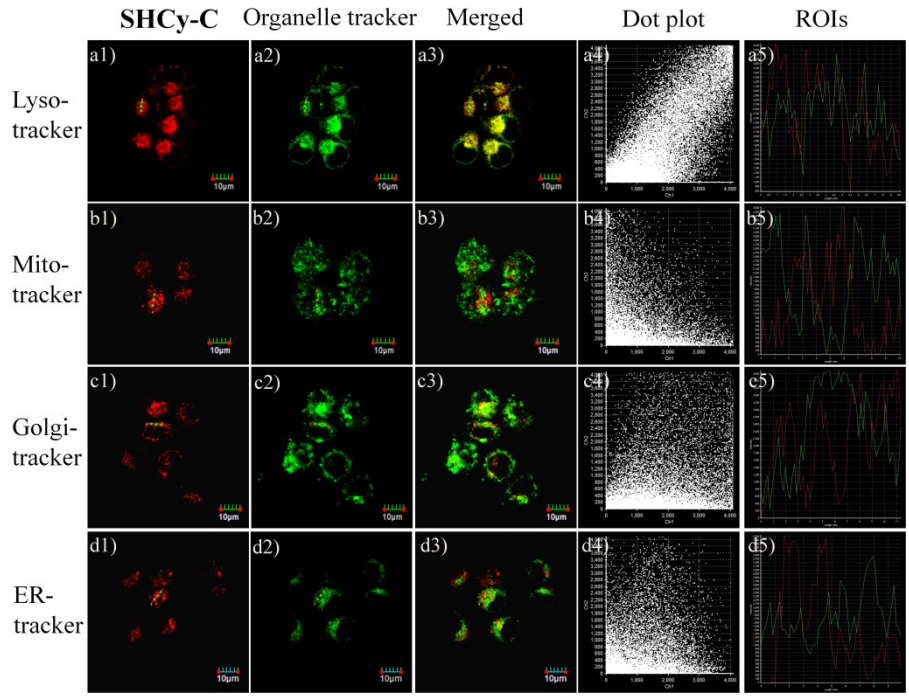


Fig. S12. Colocalization fluorescence images of **SHCy-C** with different organelle-trackers. a) Fluorescence images of **SHCy-C** (2 μM) with Lyso-Tracker Green DND-26 (0.2 μM, λ_{ex} = 488 nm, λ_{em} = 512-552 nm); b) Fluorescence images of **SHCy-C** (2 μM) with Mito-Tracker Green (0.2 μM, λ_{ex} = 488 nm, λ_{em} = 512-548 nm); c) Fluorescence images of **SHCy-C** (2 μM) with Golgi-Tracker (0.2 μM, λ_{ex} = 488 nm, λ_{em} = 503-539 nm); d) Fluorescence images of **SHCy-C** (2 μM) with ER-Tracker (0.2 μM, λ_{ex} = 488 nm, λ_{em} = 514-550 nm). Scale bar is 10 μm

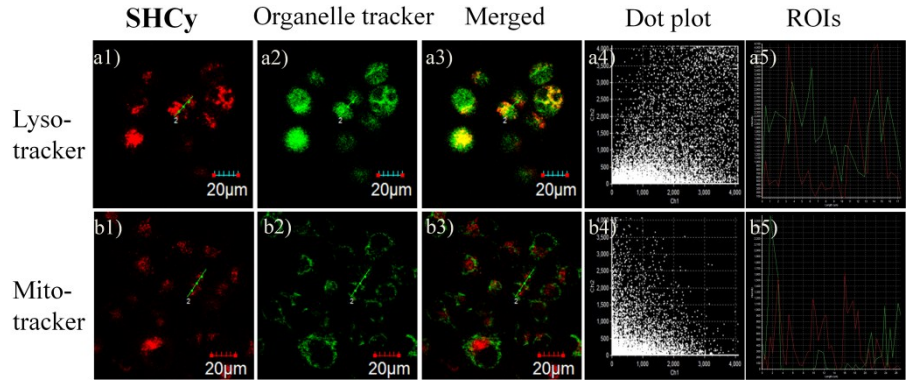


Fig. S13. Colocalization fluorescence images of the NIR dye **SHCy** with different organelle-trackers (0.2 μM). a) Fluorescence images of **SHCy** (1 μM) with Lyso-Tracker Green DND-26 (λ_{ex} = 488 nm, λ_{em} = 520-550 nm); b) Fluorescence images of **SHCy** (1 μM) with Mito-Tracker Green (λ_{ex} = 488 nm, λ_{em} = 520-550 nm). Scale bar is 20 μm.

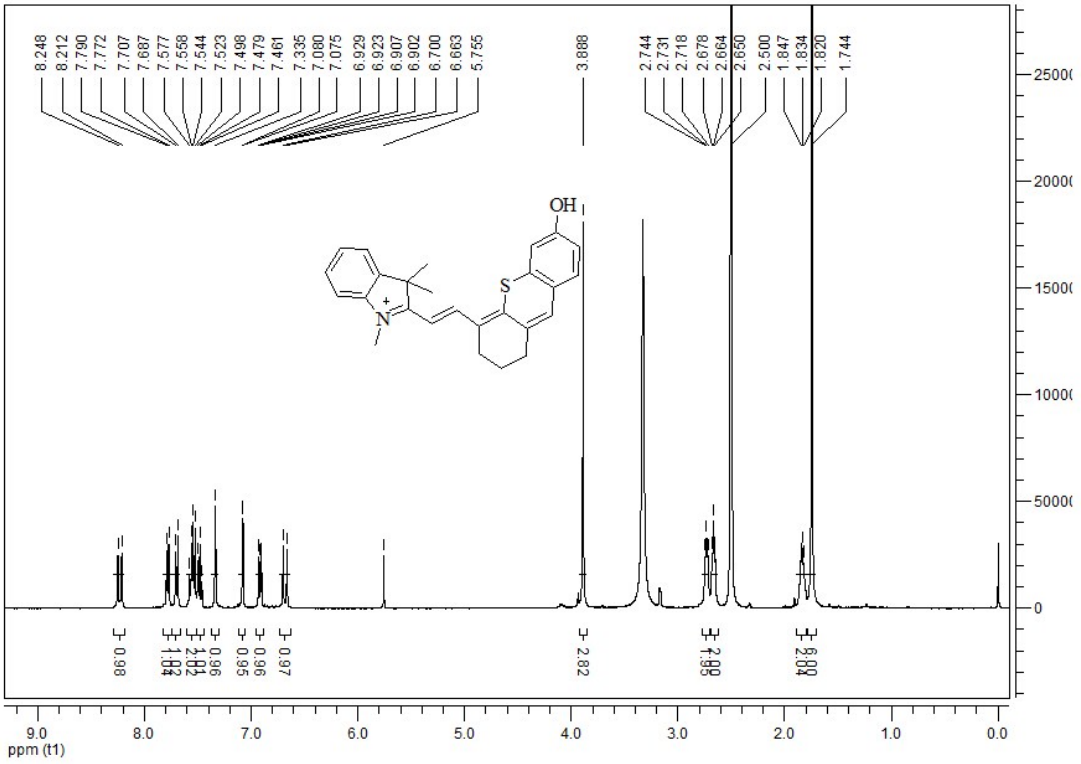


Fig. S14. ^{1}H NMR of **SHCy**

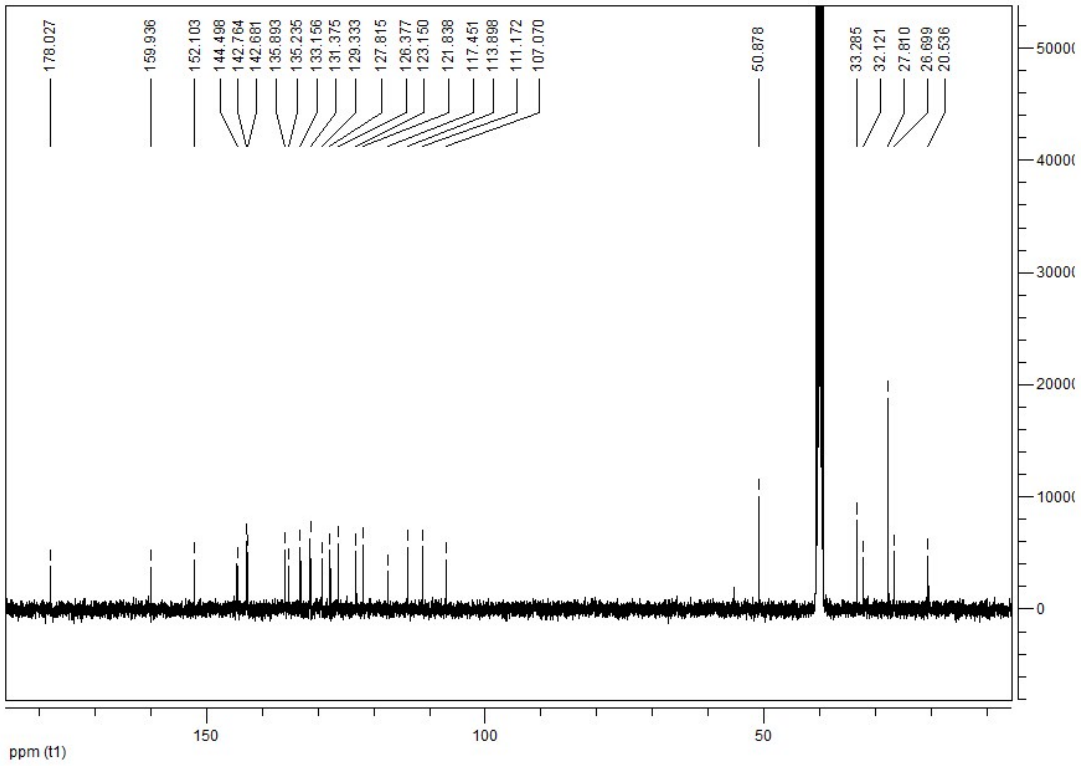


Fig. S15. ^{13}C NMR of **SHCy**

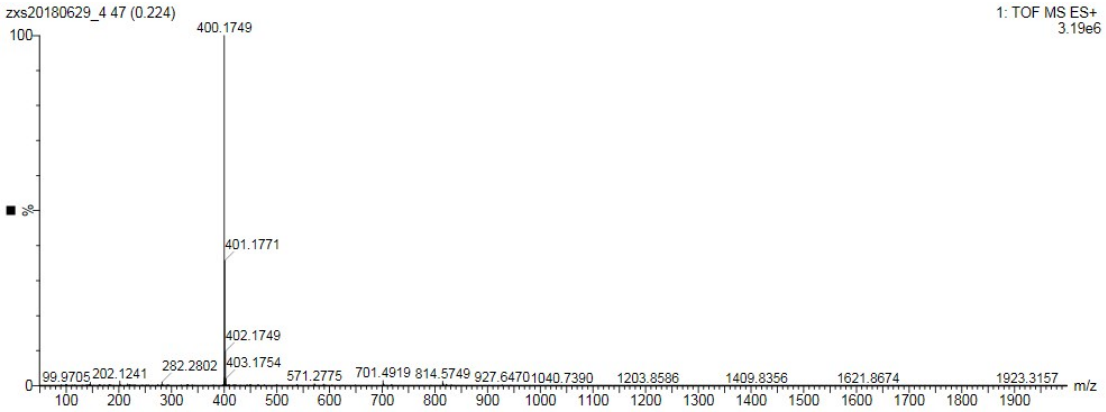


Fig. S16. HRMS of **SHCy**

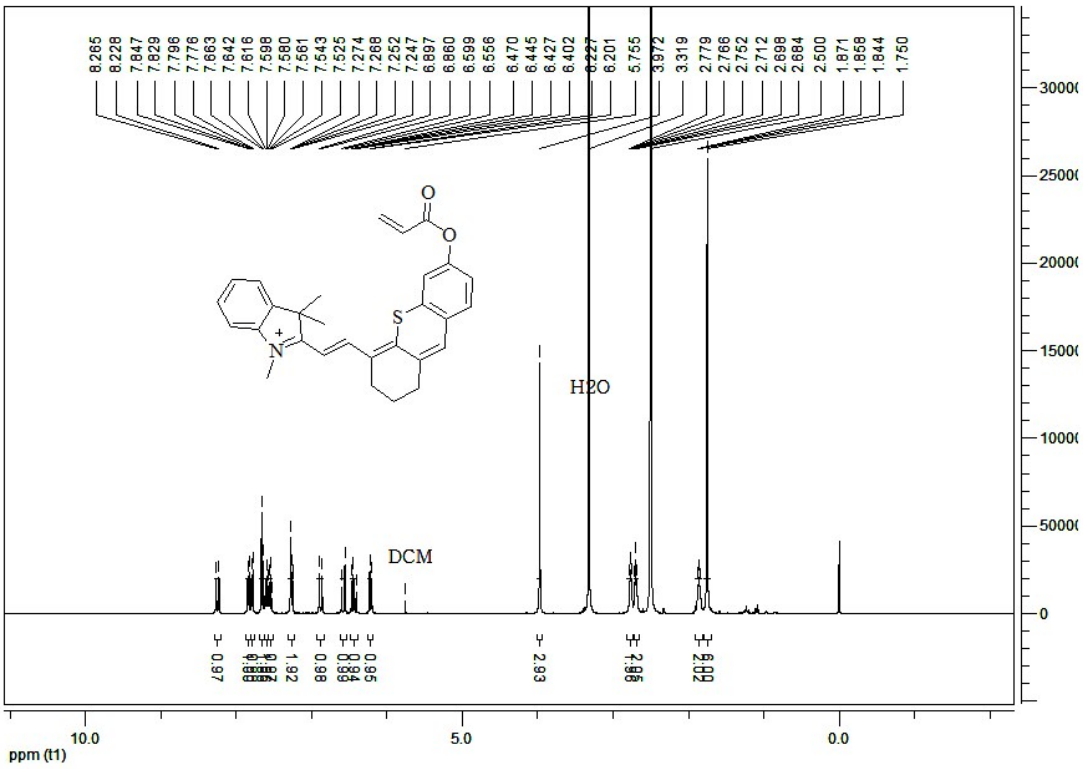


Fig. S17. ^{1}H NMR of **SHCy-C**

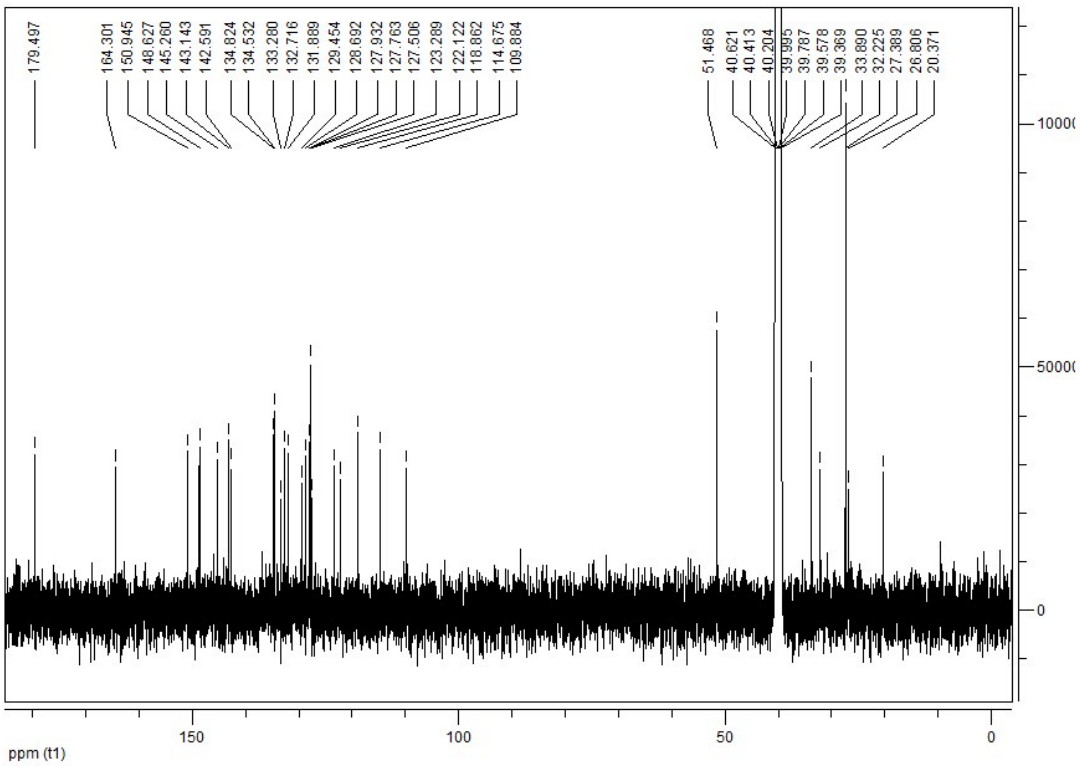


Fig. S18. ^{13}C NMR of **SHCy-C**

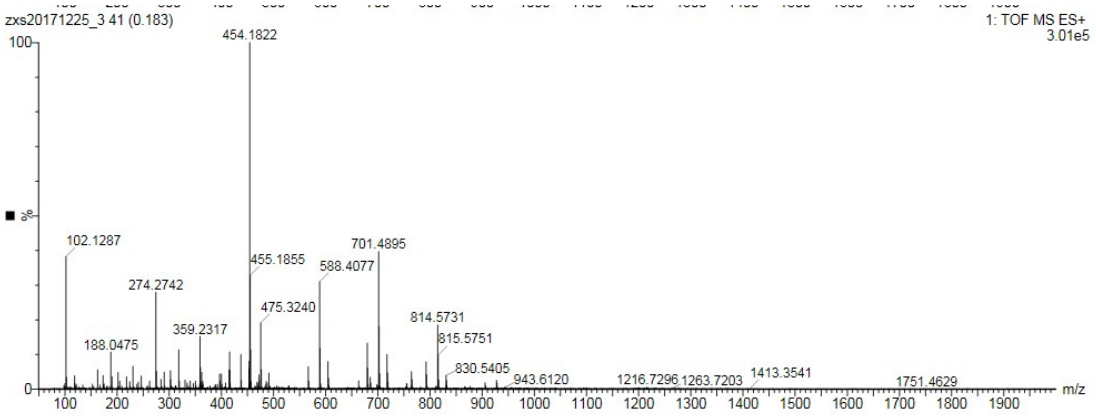


Fig. S19. HRMS of **SHCy-C**

References

S1. T. Karstens and K. Kobs, *J. Phys. Chem.*, 1980, **84**, 1871-1872.