

A Near-Infrared Fluorescent and Photoacoustic Probe for Visualizing Biothiols Dynamics in Tumor and Liver

Weizhong Ding ^{1,2}, ShanKun Yao ^{1,2}, Yuncong Chen ^{1,2,3,*}, Yanping Wu ^{1,2}, Yaheng Li ^{1,2},
Weijiang He ^{1,3,*} and Zijian Guo ^{1,2,3,*}

¹State Key Laboratory of Coordination Chemistry, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

²Chemistry and Biomedicine Innovation Center (ChemBIC), Nanjing University, Nanjing 210023, China

³Nanchuang (Jiangsu) Institute of Chemistry and Health, Jiangsu, Nanjing 210000, China

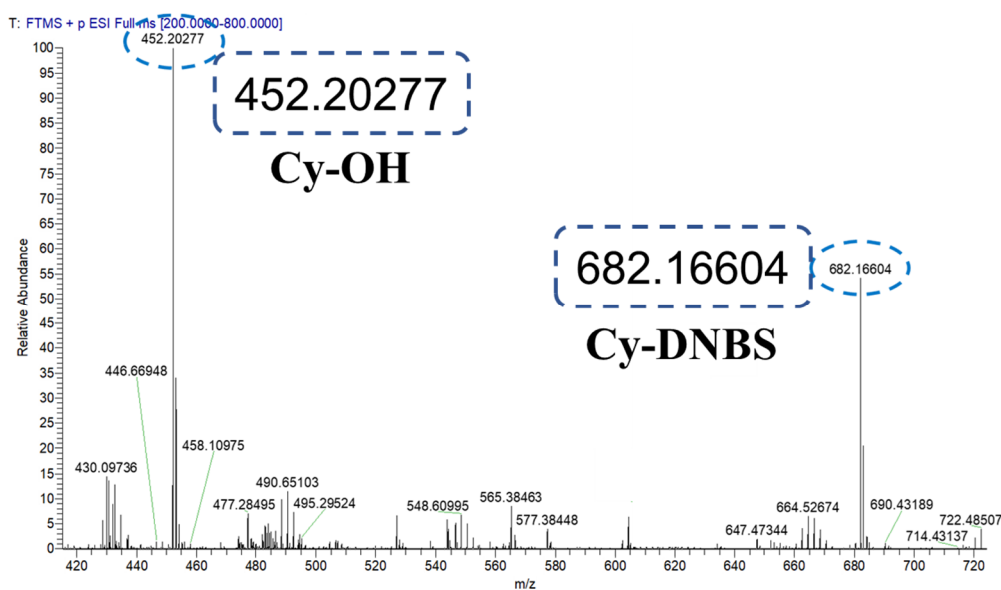


Figure S1. Mass spectrum acquired by mixed Cy-DNBS (10 μ M) with GSH (20 μ M) for 2 min and then determined by mass spectrometry with positive mode.

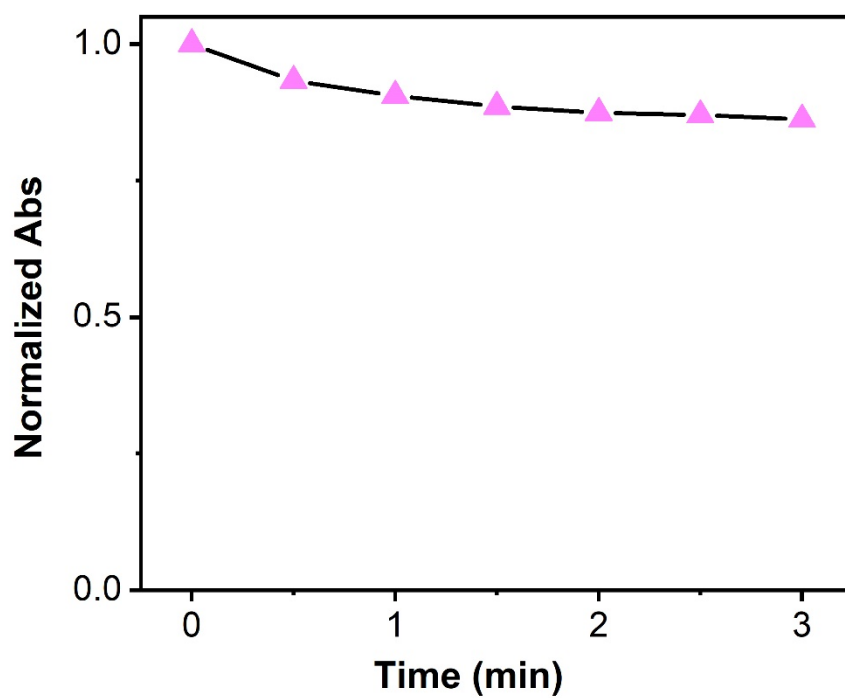


Figure S2. The photostability of Cy-DNBS (10 μ M) in methanol was estimated by irradiating solutions in a quartz cuvette with a 635 nm laser (50 mW/cm²) for 3 min at a 30 second interval.

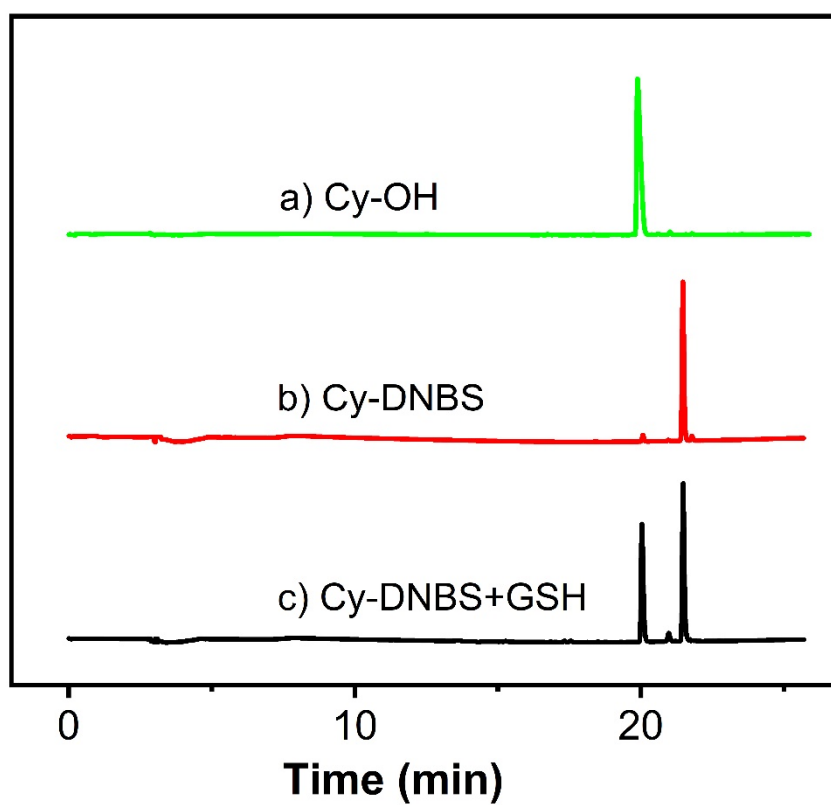


Figure S3. HPLC chromatograms of a) Cy-OH, b) Cy-DNBS and c) the mixtures of Cy-DNBS with GSH over 2 min. The mobile phase was acetonitrile and water.

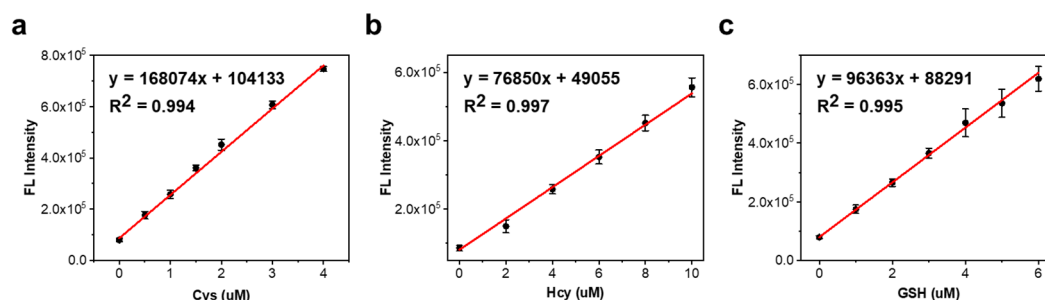


Figure S4. Linear relationship between fluorescence intensity of Cy-DNBS at 762 nm and concentration of (a) Cys, (b) Hcy and (c) GSH. $\lambda_{\text{ex}} = 680 \text{ nm}$, $\lambda_{\text{em}} = 700\text{-}900 \text{ nm}$. slit, 5 nm/5 nm.

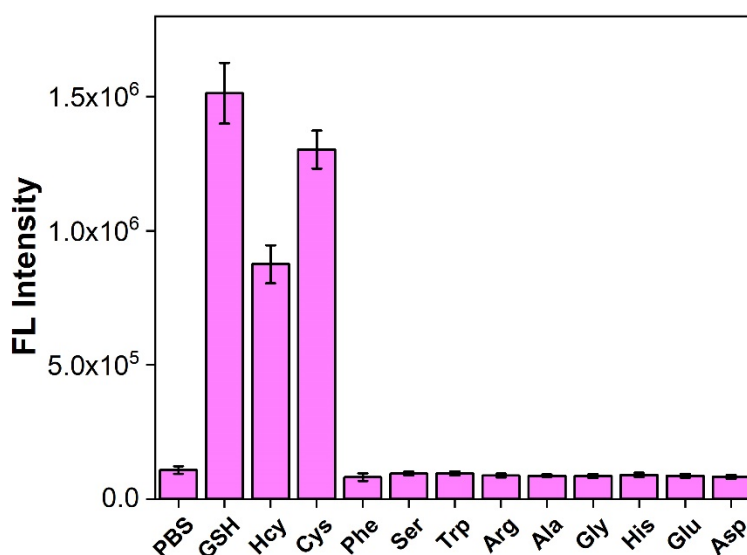


Figure S5. Histogram of the fluorescence intensity of Cy-DNBS (10 μM) towards Cys, Hcy, GSH and other amino acids including Phe, Ser, Trp, Glu, Gly, His, Ala, Arg, Asp (20 μM) at 762 nm in PBS solution (pH 7.40). $\lambda_{\text{ex}} = 680 \text{ nm}$, $\lambda_{\text{em}} = 700\text{-}900 \text{ nm}$. slit, 5 nm/5 nm.

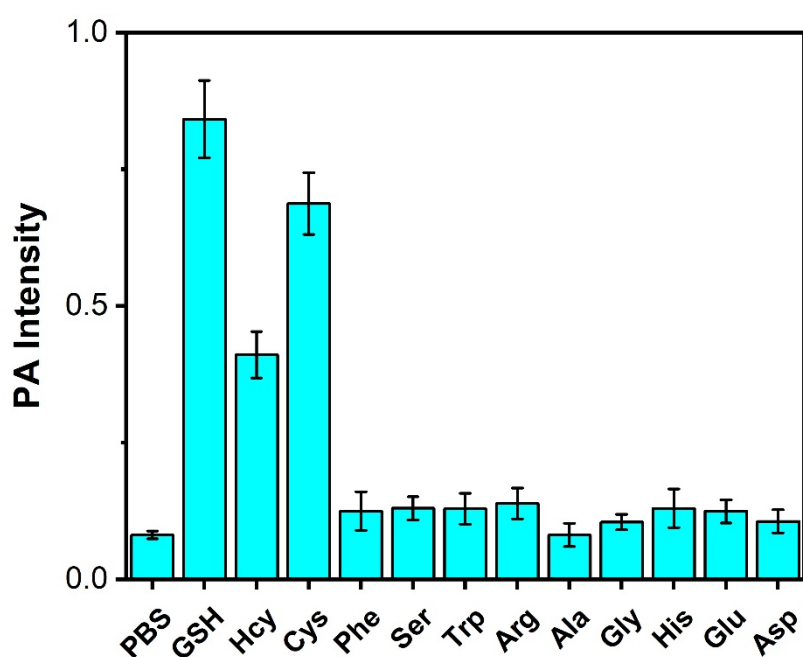


Figure S6. Histogram of the PA intensity of Cy-DNBS (10 μ M) towards Cys, Hcy, GSH and other amino acids including Phe, Ser, Trp, Glu, Gly, His, Ala, Arg, Asp (20 μ M) at 725 nm in PBS solution (pH 7.40).

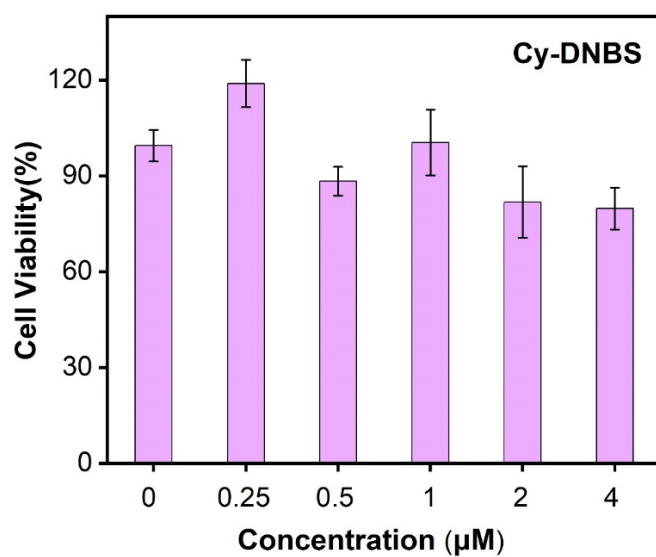


Figure S7. The cell viability of HepG2 cells after incubating with different concentration of Cy-DNBS were measured by MTT assay. The error bar represented the standard deviation of three individual measurements.

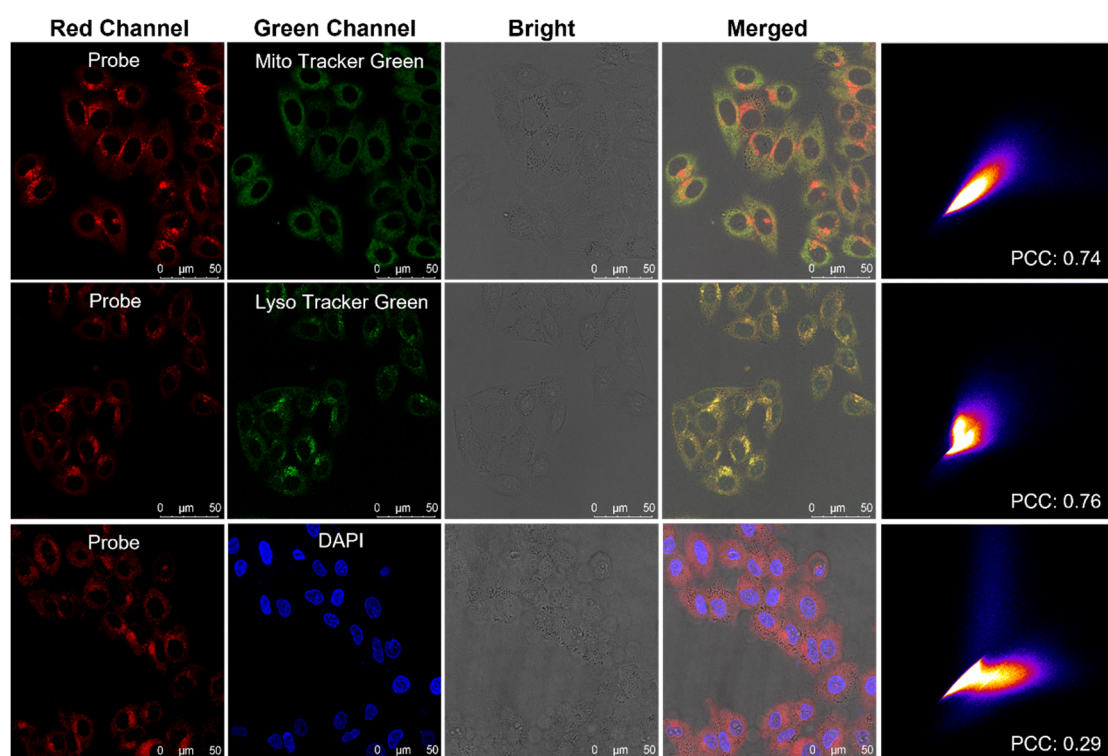


Figure S8. HepG2 cells were pretreated with exogenous Cys (2 mL, 4 μ M) for 30 min and then Cy-DNBS (2 mL, 4 μ M) were co-incubated with three commercial organelle dyes (Mito-Tracker, Lyso-Tracker and DAPI, 1 μ M) for 30 min. Green channel ($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 500\text{-}620 \text{ nm}$), Red channel ($\lambda_{\text{ex}} = 633 \text{ nm}$, $\lambda_{\text{em}} = 680\text{-}800 \text{ nm}$), Blue channel ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 420\text{-}550 \text{ nm}$). Scale bar: 50 μ m.

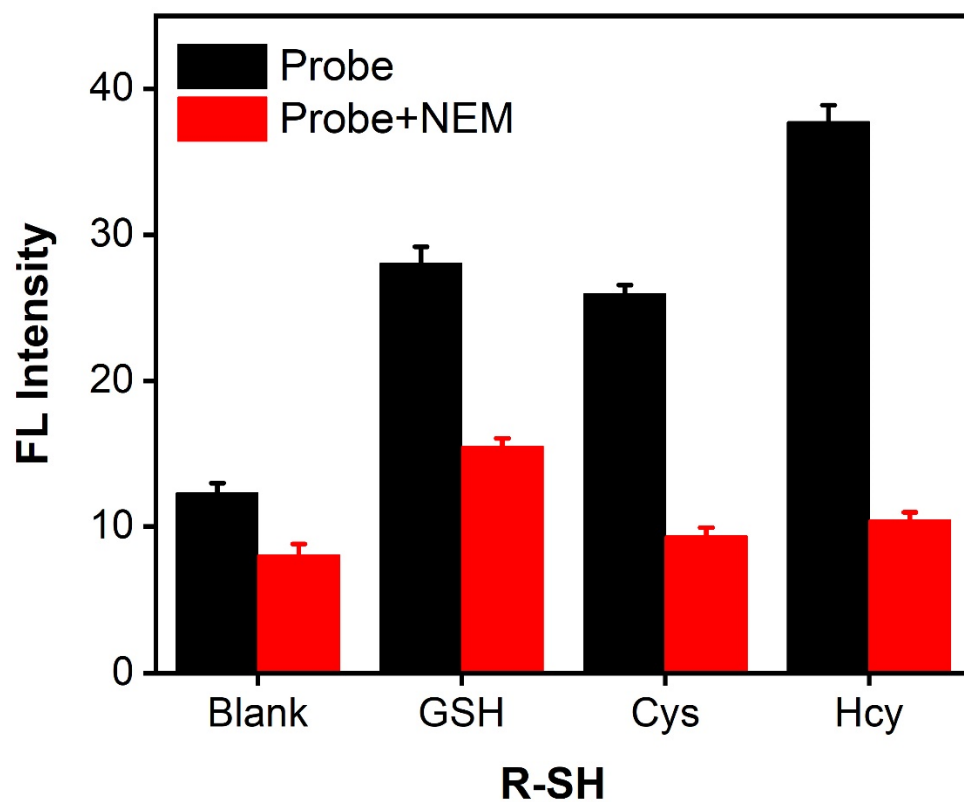


Figure S9. Fluorescence intensity data were calculated from Figure 3.

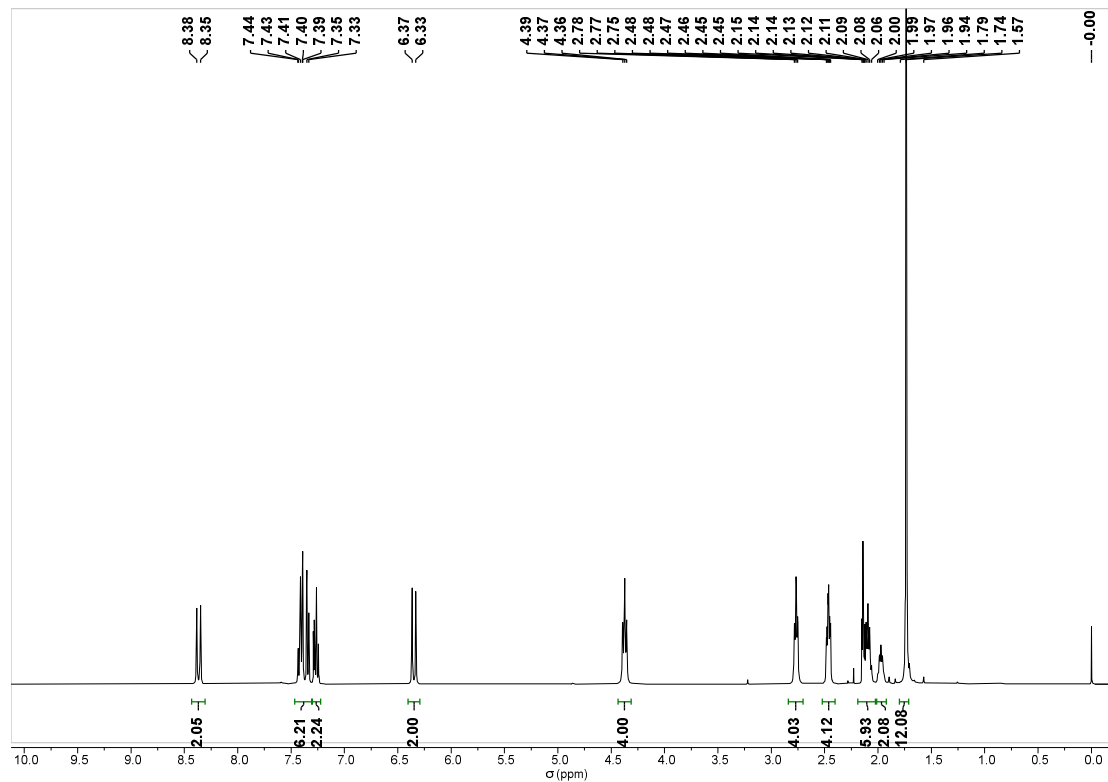


Figure S10. ^1H NMR spectrum of Cy7 in CDCl_3 .

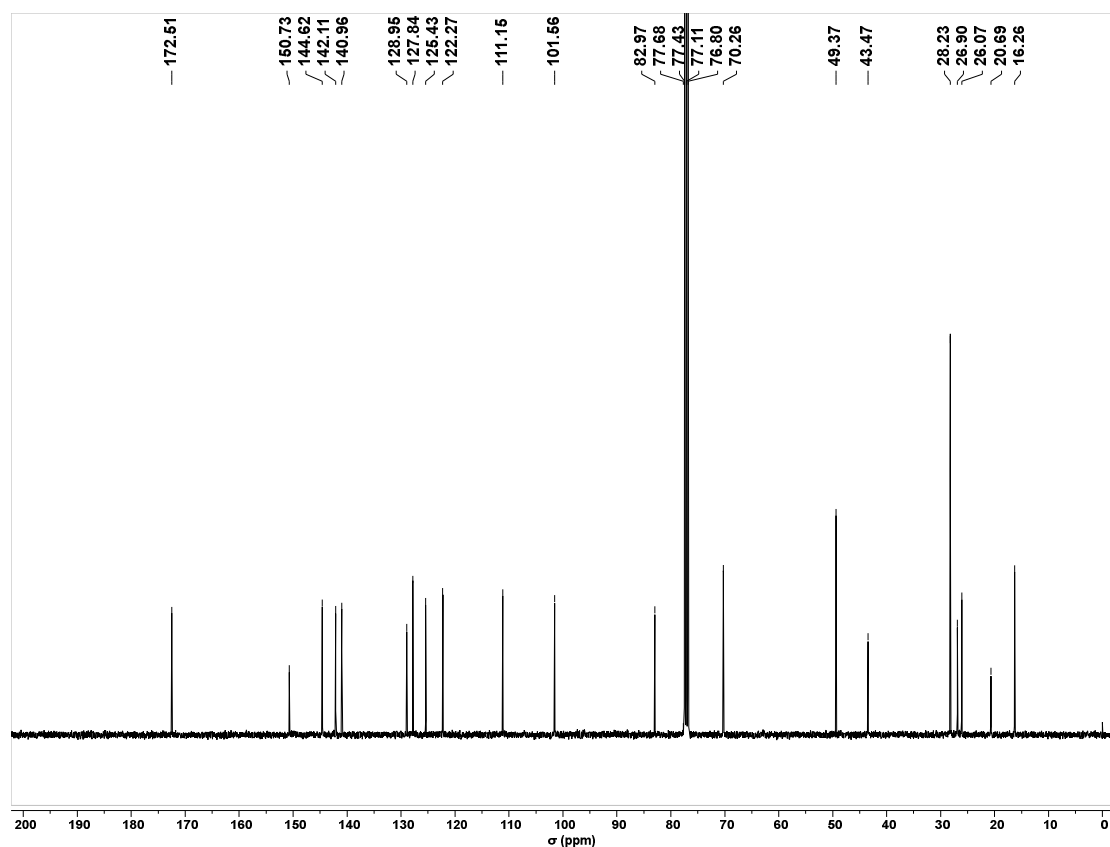


Figure S11. ¹³C NMR spectrum of Cy7 in CDCl₃.

0420-CL#12-22 RT: 0.11-0.21 AV: 11 NL: 6.96E8
T: FTMS + p ESI Full ms [200.0000-1000.0000]

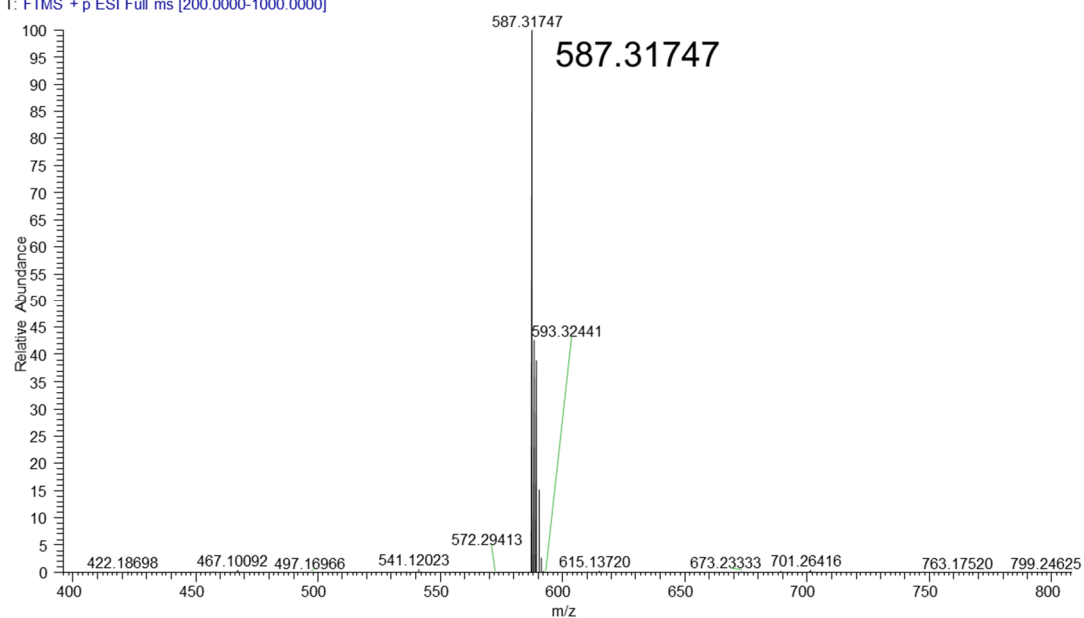


Figure S12. HR-MS spectrum of Cy7.

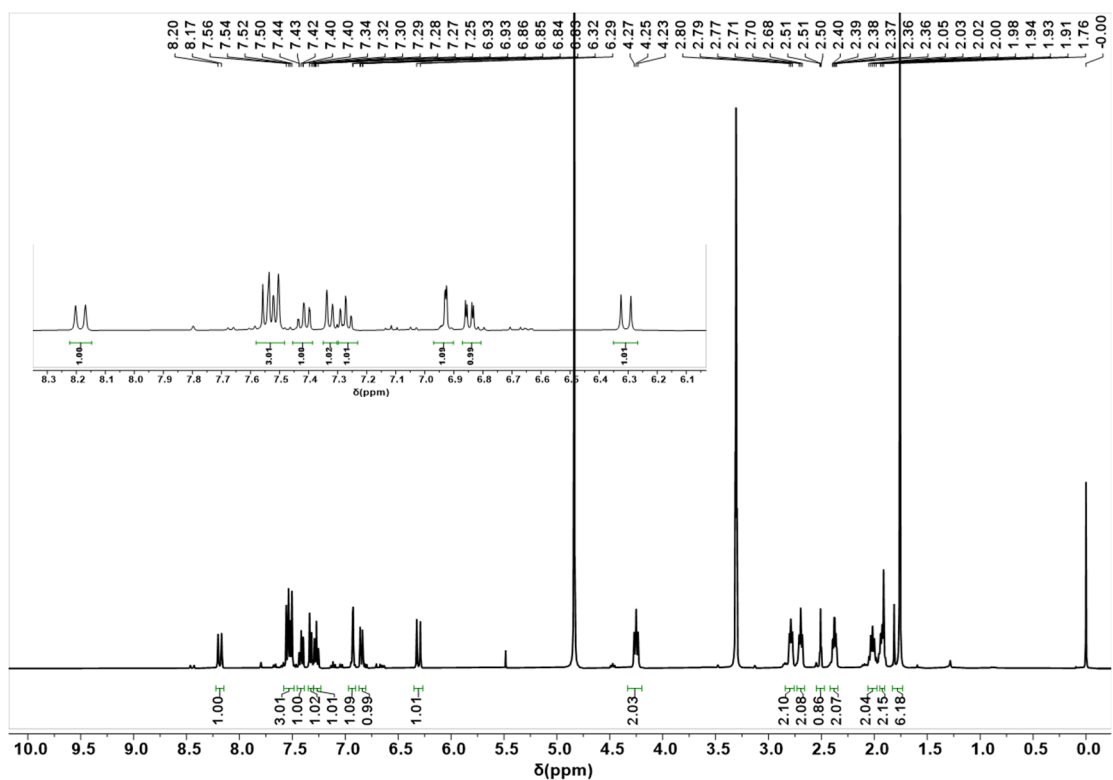


Figure S13. ¹H NMR spectrum of Cy-OH in CD₃OD.

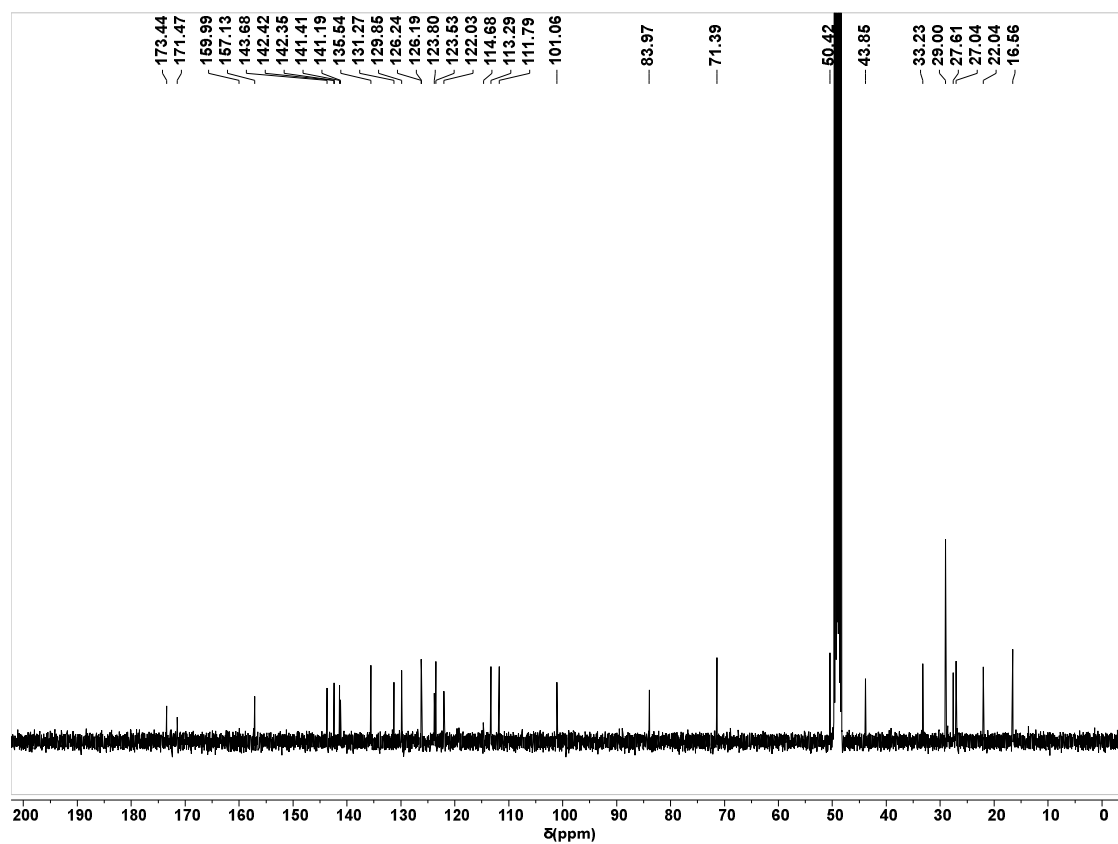


Figure S14. ¹³C NMR spectrum of Cy-OH in CD₃OD.

T: FTMS + p ESI Full ms [50.0000-750.0000]

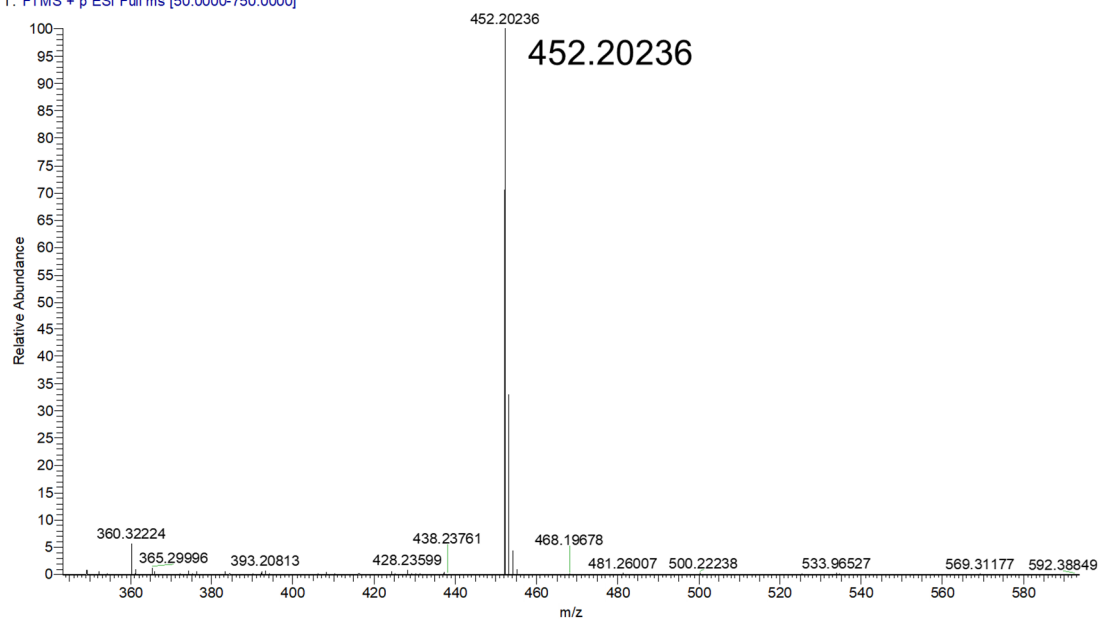


Figure S15. HR-MS spectrum of Cy-OH.

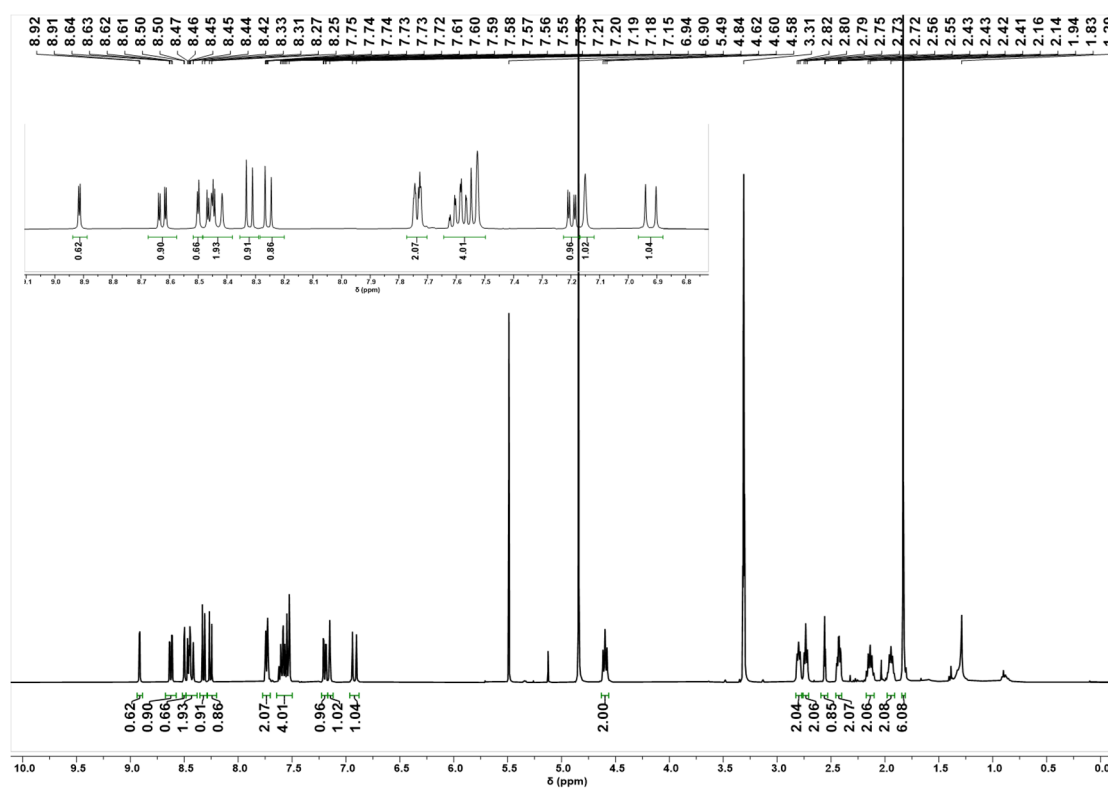


Figure S16. ¹H NMR spectrum of Cy-DNBS in CD₃OD.

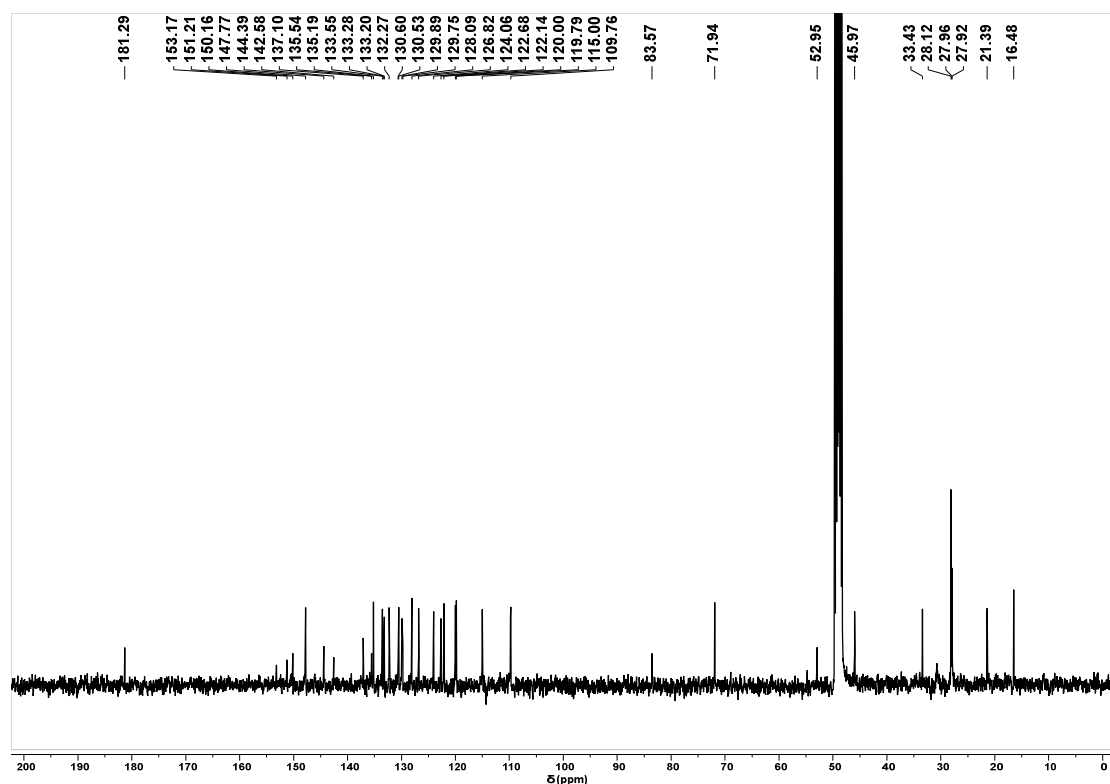


Figure S17. ^{13}C NMR spectrum of Cy-DNBS in CD_3OD .

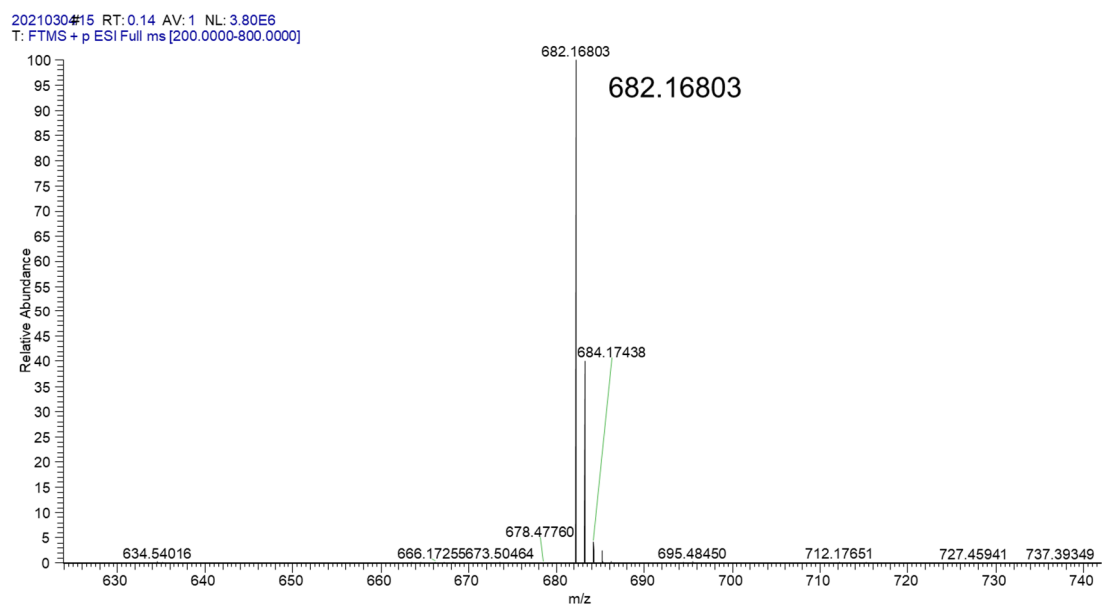
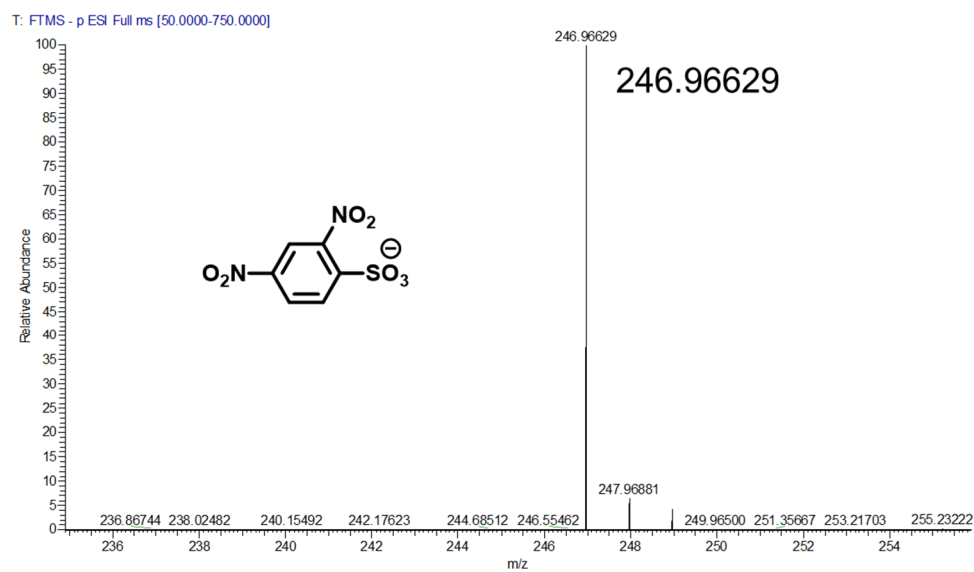


Figure S18. HR-MS spectrum of Cy-DNBS.



11

Figure S19. HR-MS spectrum of Cy-DNBS with negative mode.