Supporting Information

Direct Fluorescence Monitoring of the Delivery and Cellular Uptake of a Cancer-targeted RGD Peptide-appended Naphthalimide Theragnostic Prodrug

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Contents

Additional UV/Vis absorption, fluorescence, and HPLC studies

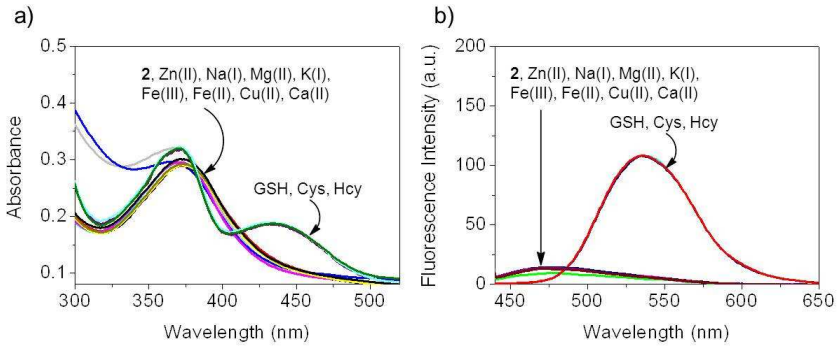


Figure S1. Absorption (a) and fluorescence (b) spectra of **2** (10.0 μM) recorded in the absence and presence of GSH, Cys, Hcy (5.0 mM, respectively) and various metal cations (1.0 mM: monovalent metal ions; 0.1 mM: divalent metal ions, respectively) with excitation effected at 430 nm. All spectra were acquired 2 h after addition of the analytes in question and were recorded in PBS buffer (pH 7.4) containing 16% (v/v) DMSO at 37 °C.

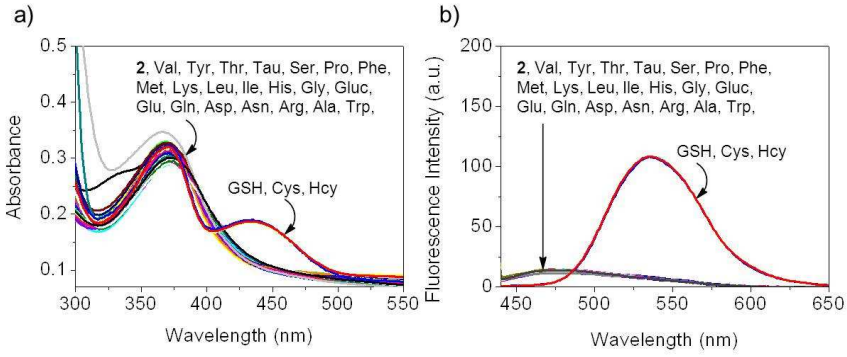


Figure S2. Absorption (a) and fluorescence (b) spectra of **2** (10.0 μM) toward GSH, Cys, Hcy (5.0 mM, respectively) and other amino acids (5.0 mM, respectively) with an excitation at 430 nm. All spectra were acquired 2 h after addition of various analytes in PBS buffer (pH 7.4) containing 16 % (v/v) of DMSO at 37 °C.

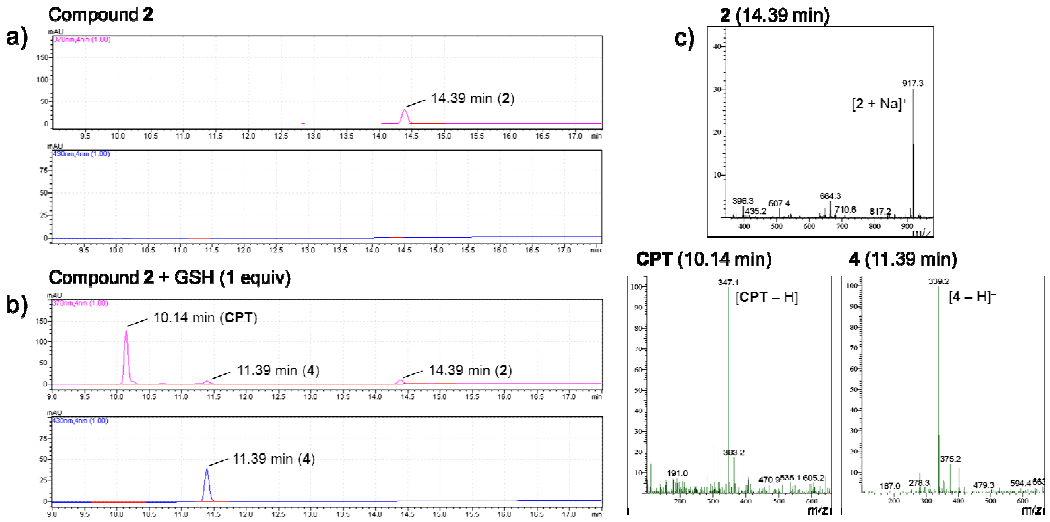


Figure S3. Reverse-phase HPLC chromatograms of **2** (0.1 mM) (a) without and (b) with GSH (0.1 mM). Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 370 (pink) and 430 nm (blue), respectively. The corresponding mass spectra as determined by ESI-MS spectrometry are shown in (c).

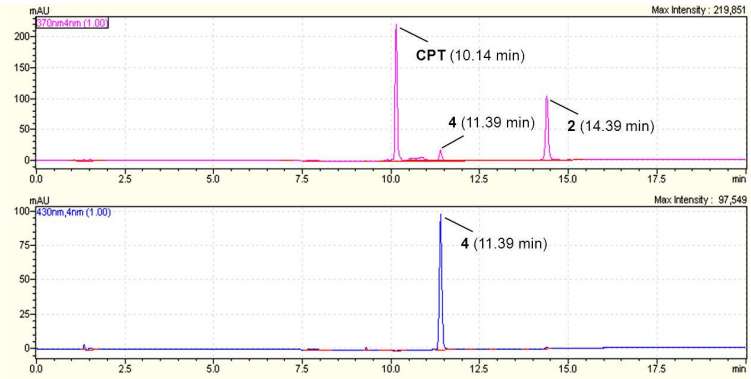


Figure S4. Reverse-phase HPLC chromatograms of a mixture solution of CPT, **2**, and **4**. Peaks in the chromatograms were detected by monitoring the UV/Vis absorption at 370 nm (pink) and 430 nm (blue), respectively. Assignments of the species in question were made using a combination of ESI-MS spectrometry, UV spectroscopy and comparisons to known standards.

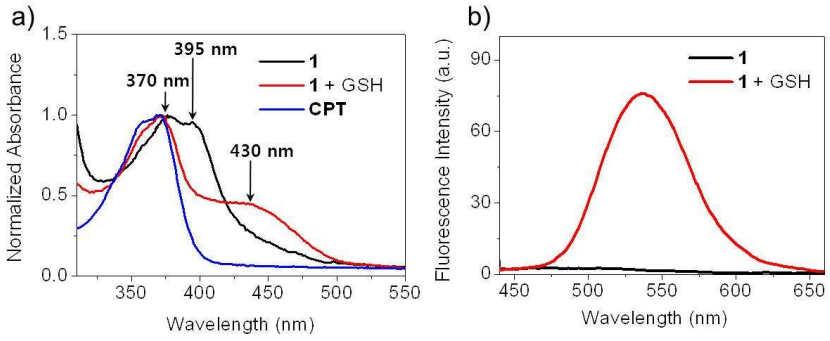
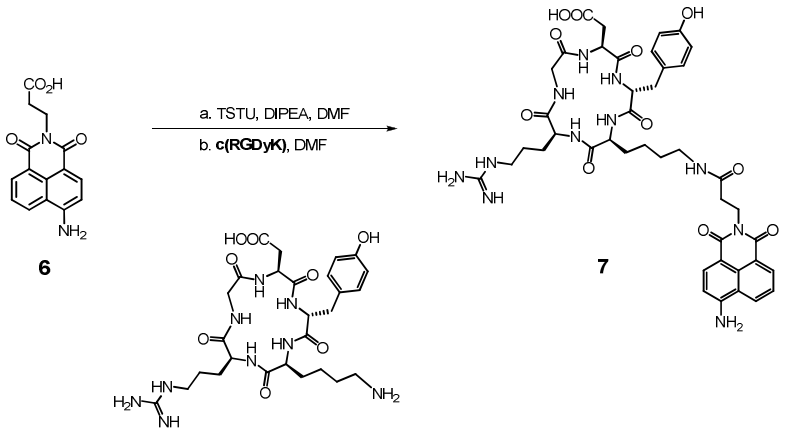


Figure S5. (a) Normalized absorption (a) and fluorescence spectra (b) of **CPT** and **1** (10.0 μM) recorded in the absence and presence of GSH (1.0 mM) with excitation effected at 430 nm. All spectra were acquired 2 h after sample preparation in PBS buffer (pH 7.4) containing 16% (v/v) DMSO and then holding at 37 °C.

As discussed in the text proper, the addition of GSH to a solution of **1** at 37 °C in PBS buffer (pH 7.4) containing 16% (v/v) DMSO, led to cleavage, as inferred from an analysis of the reaction products via reverse-phase HPLC and ESI-MS. To aid in the identification of the cleavage products, compound **7** was independently prepared (Scheme S1). Its chemical structure was confirmed by ESI-MS analysis (Figure S18). The retention time of **7** in the HPLC chromatogram was found to coincide with that of the non-CPT component present in the reaction mixture produced upon treating **1** with GSH. As seen in Figure S6, CPT (elution time: 9.8 min) was the other cleavage product (besides **7**; elution time 9.1 min), present in this reaction mixture.



Scheme S1. Synthesis of **7**.

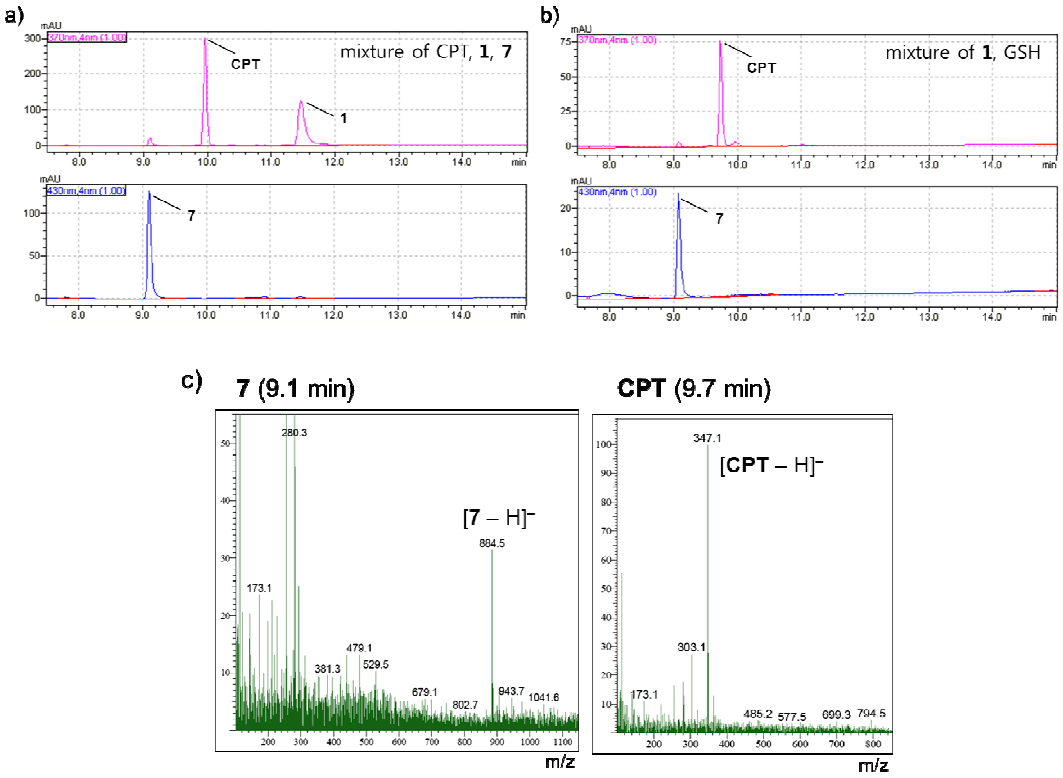


Figure S6. Reverse-phase HPLC chromatograms of a mixture solution (a) of CPT, **1**, **7** and a reaction mixture (b) of **1** with GSH. Peaks in the chromatograms were detected at 370 nm (pink) and 430 nm (blue), respectively. The chemical composition of the materials assigned to the indicated peaks was confirmed by ESI-MS spectrometry (c), as well as via UV absorption spectroscopy.

Additional cell imaging data

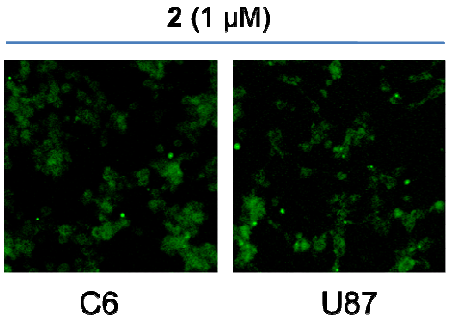


Figure S7. Confocal microscopic analysis of U87 and C6 cells treated with **2**. The cells were incubated with PBS containing **2** (1 μM), with the images being obtained after incubating for 30 min. Cell images were obtained using excitation at 458 nm and a long-path (> 505 nm) emission filter.

^{1}H-NMR, ^{13}C-NMR, ESI-MS, MALDI-TOF MS, and HPLC analyses

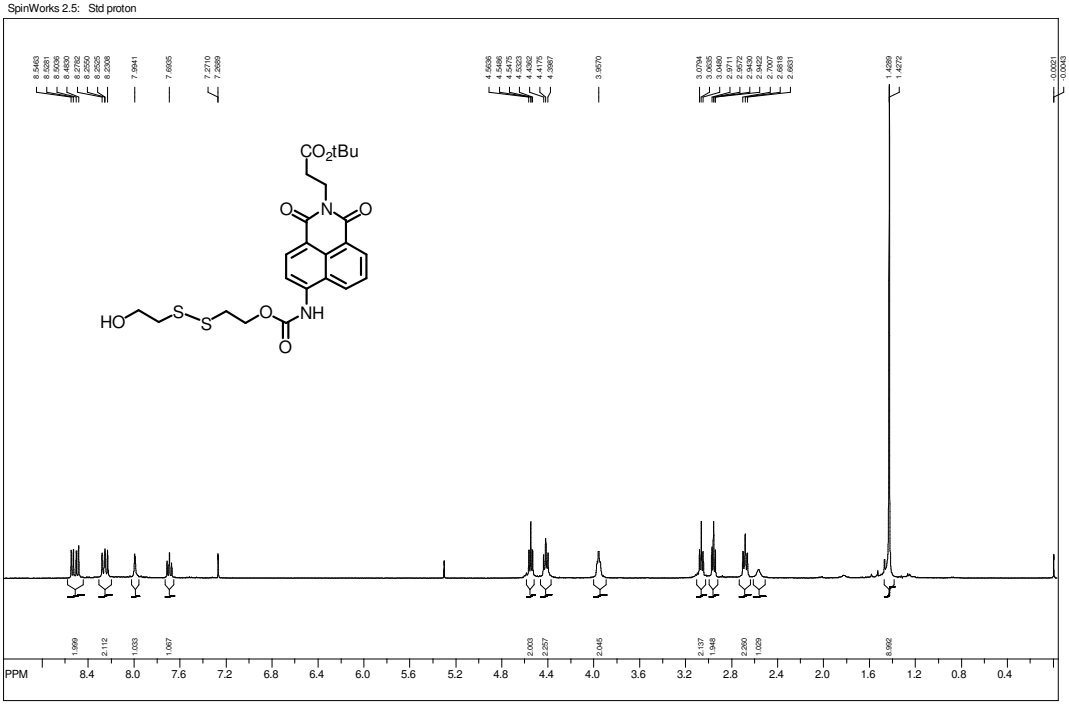


Figure S8. ^{1}H NMR spectrum of **5** recorded in $CDCl_3$.

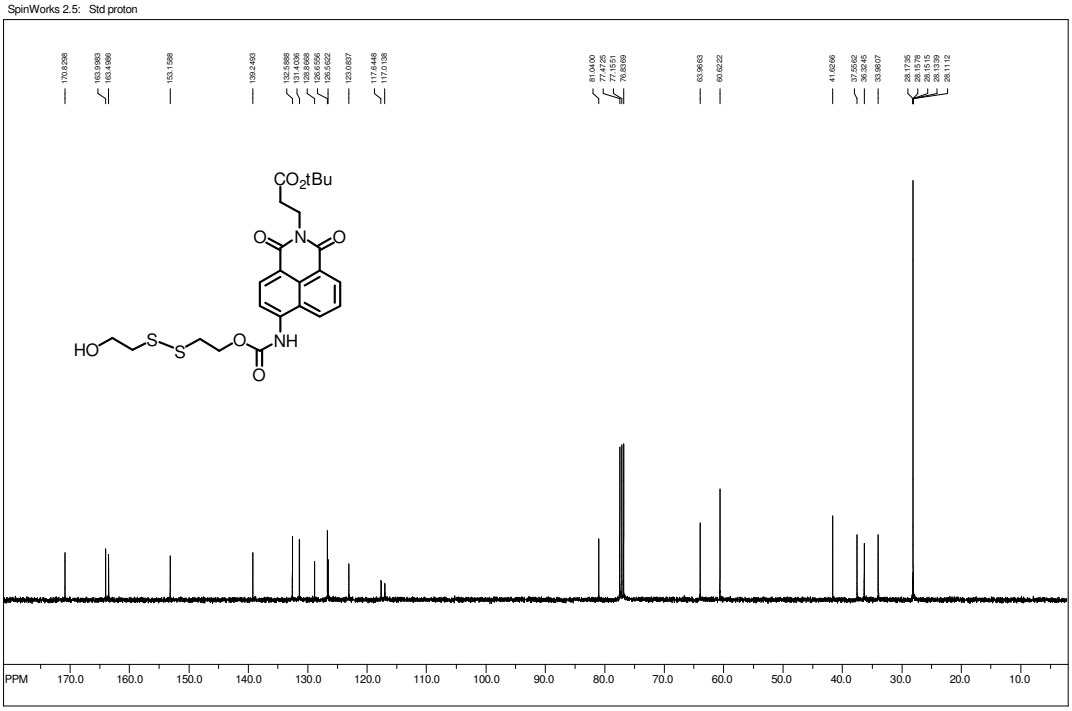


Figure S9. ^{13}C NMR spectrum of **5** recorded in $CDCl_3$.

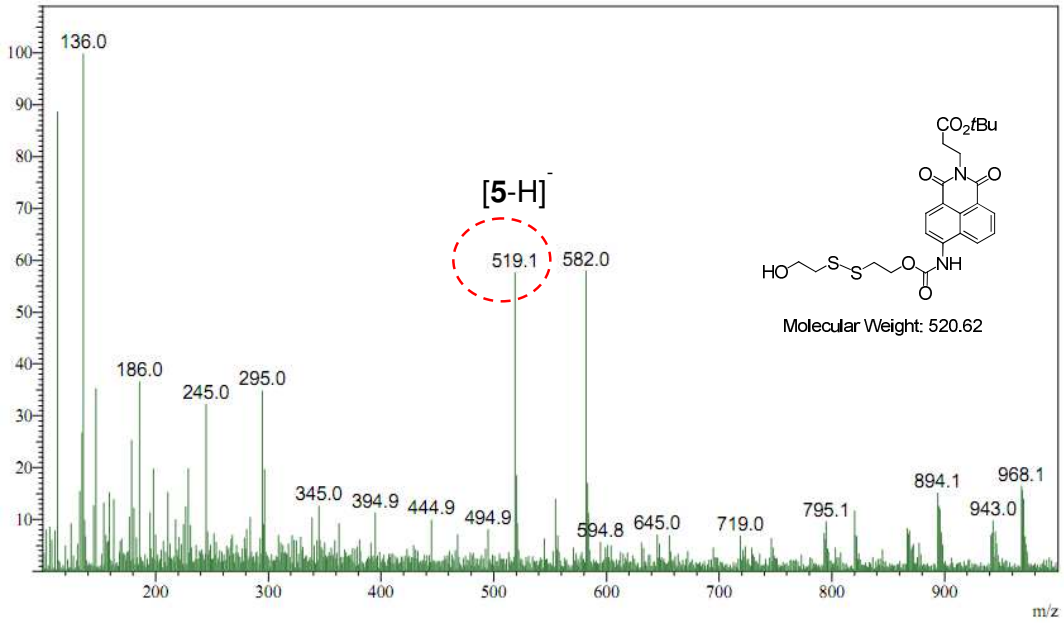


Figure S10. ESI-MS spectrum of **5**.

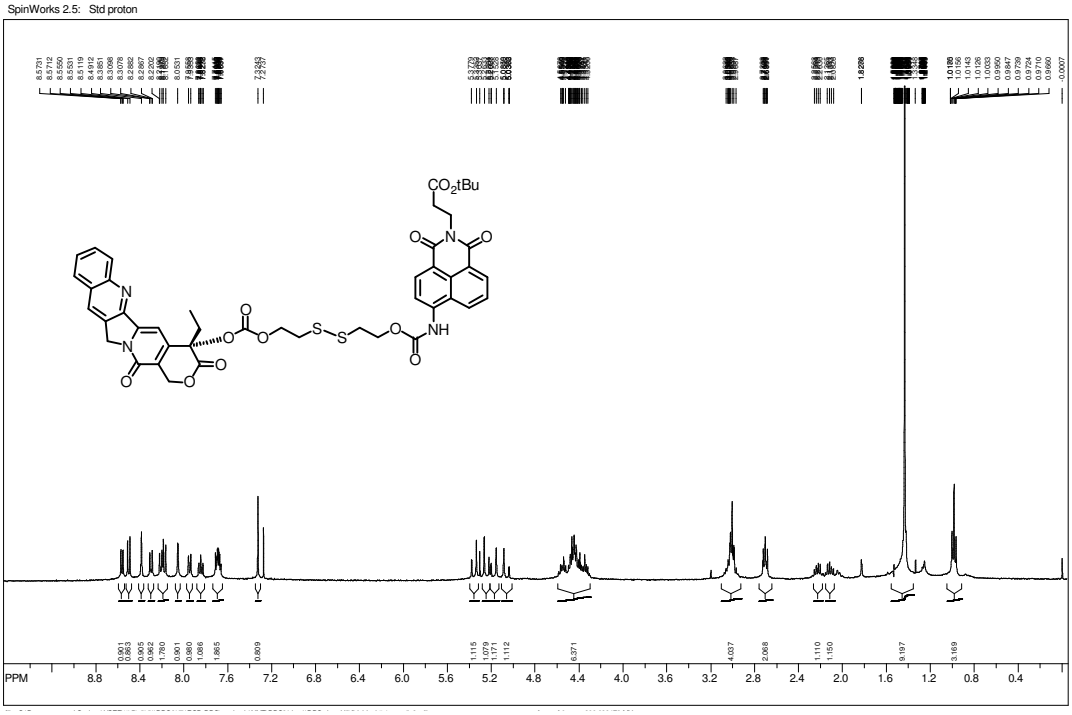


Figure S11. ^{1}H NMR spectrum of **2** recorded in $CDCl_3$.

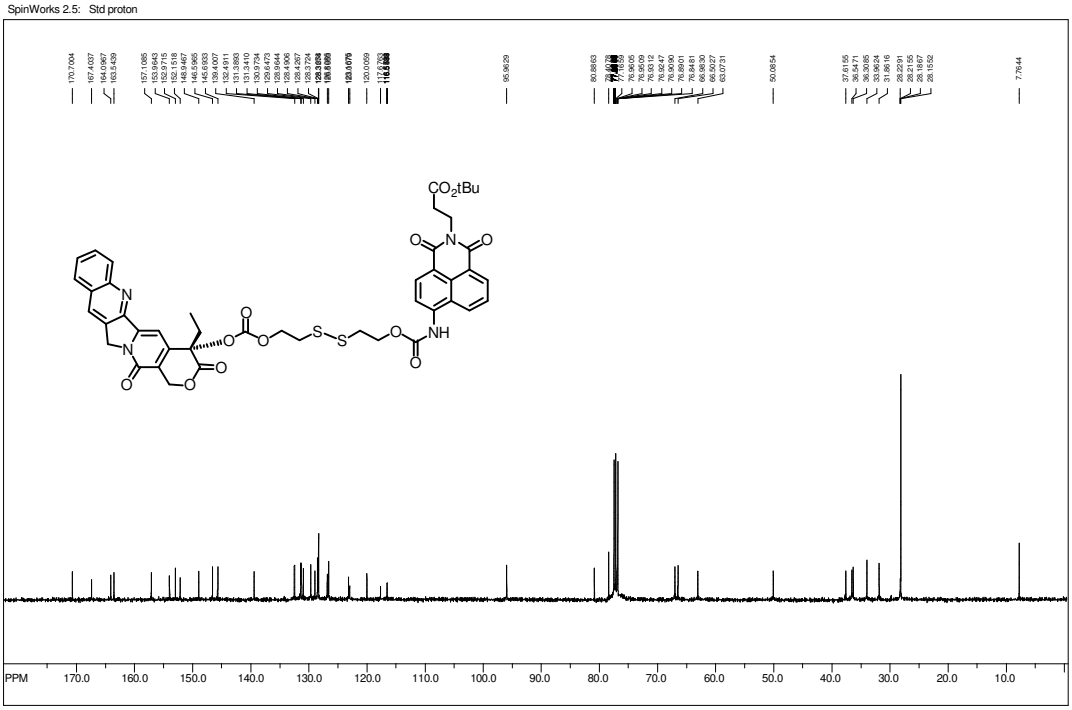


Figure S12. ^{13}C NMR spectrum of **2** recorded in $CDCl_3$.

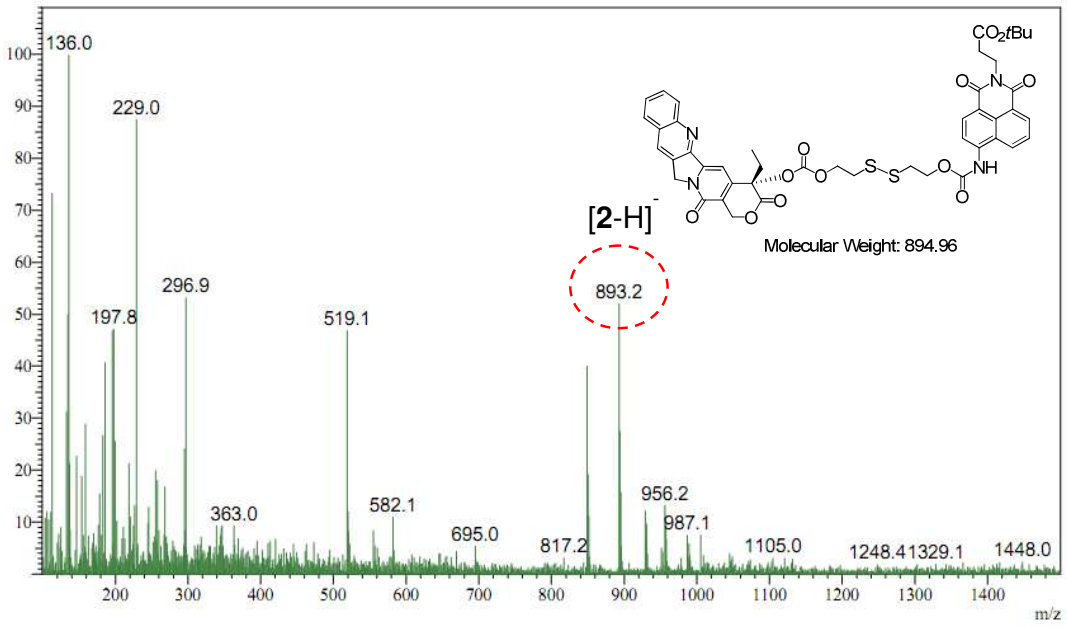


Figure S13. ESI-MS spectrum of **2**.

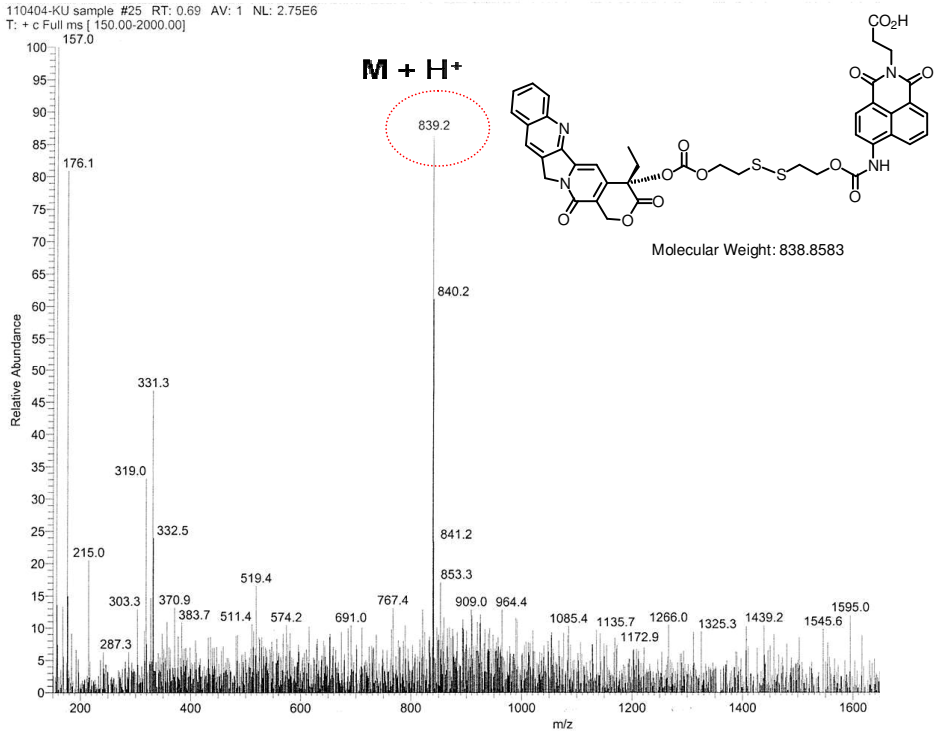


Figure S14. MALDI-TOF MS spectrum of the hydrolyzed **2** with TFA/DCM.

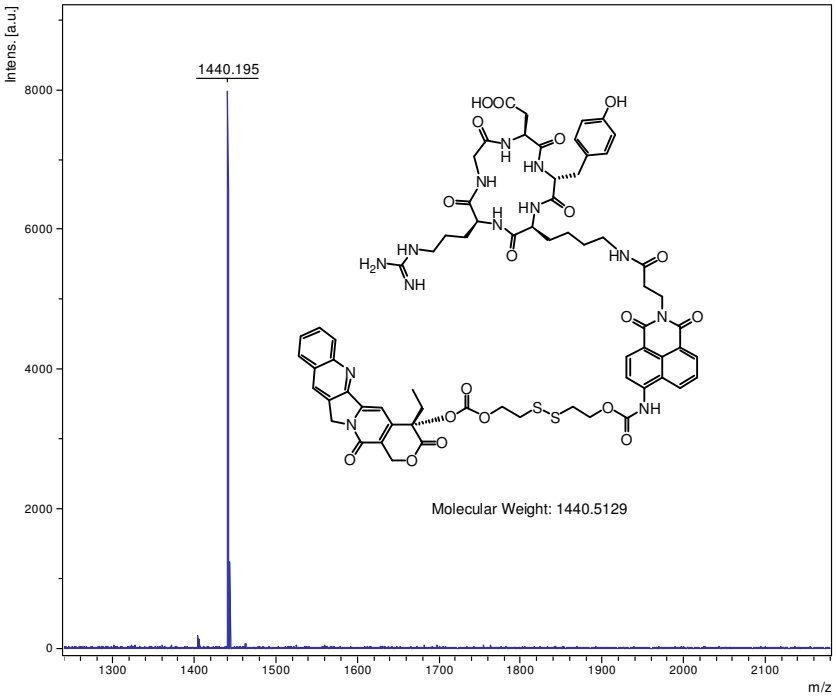


Figure S15. MALDI-TOF MS spectrum of **1**.

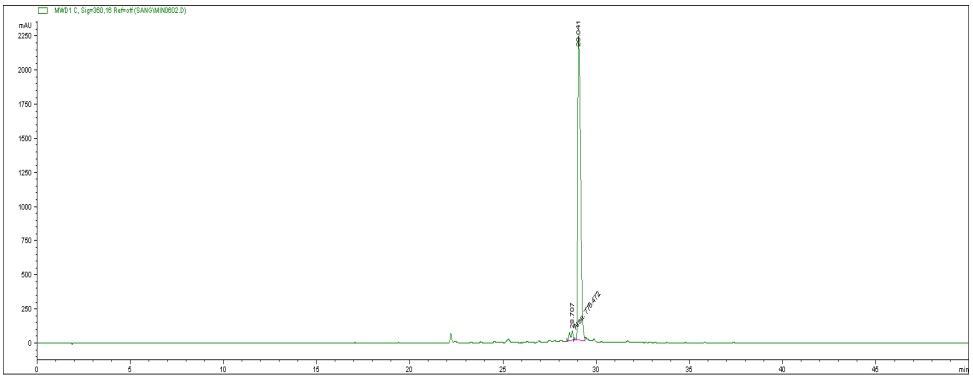


Figure S16. HPLC chromatogram of **1**.

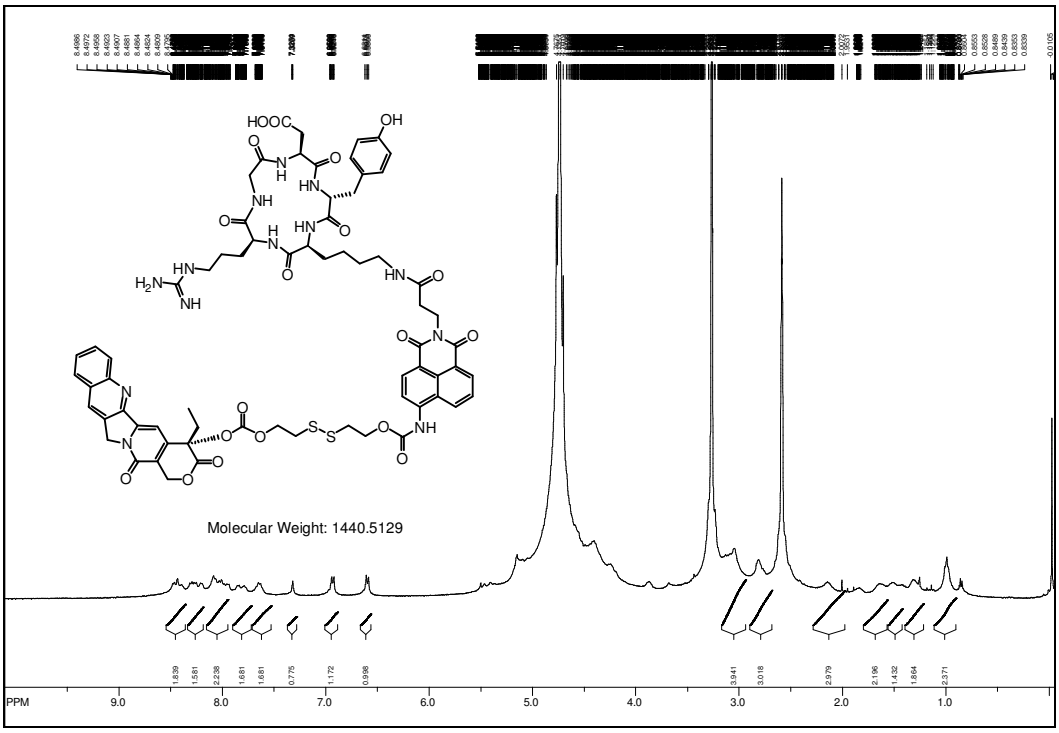


Figure S17. ^{1}H NMR spectrum of **1** recorded in CD_3OD containing 5% (v/v) DMSO-d_6.

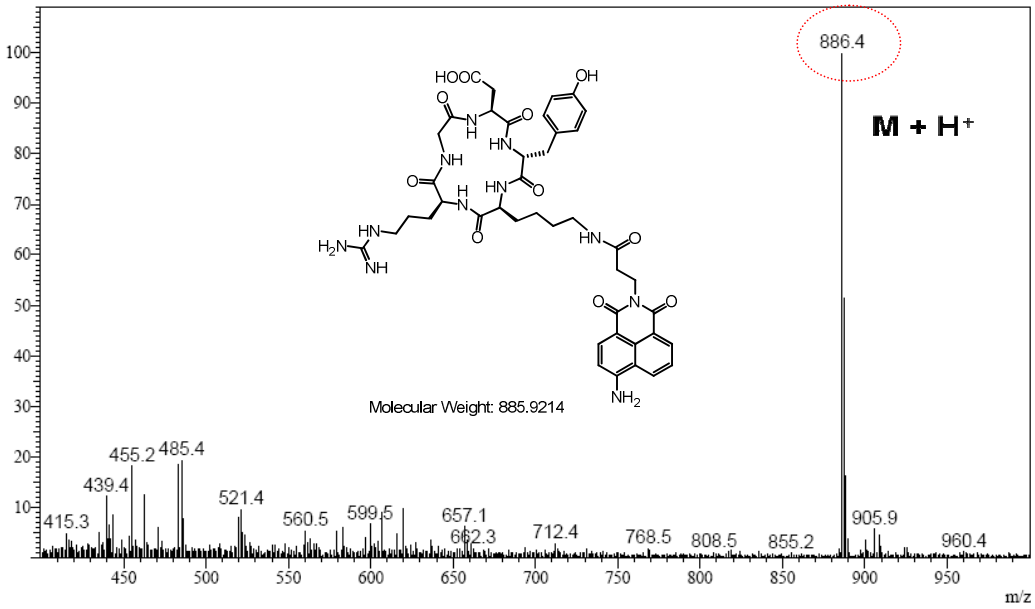


Figure S18. ESI-MS spectrum of **7**.

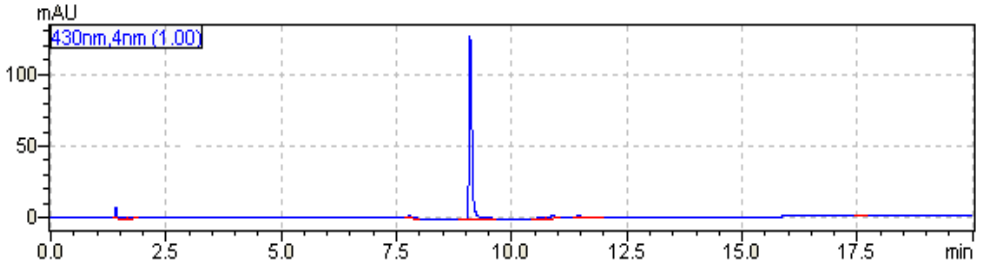


Figure S19. HPLC chromatogram of **7**.

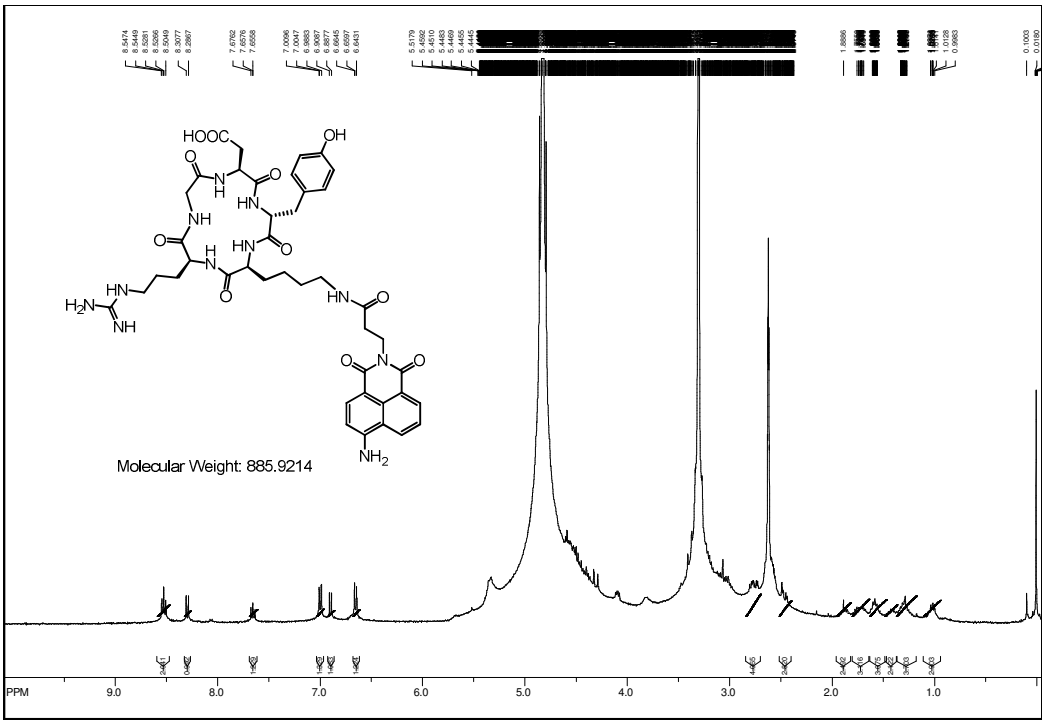


Figure S20. ^{1}H NMR spectrum of **7** recorded in CD_3OD containing 1% (v/v) DMSO-d_6.