Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Materials

Chemically Triggered Soft Material Macroscopic Degradation and Fluorescence Detection Using Self-Propagating Thiol-Initiated Cascades

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Supplementary Materials

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Materials and instruments

1.1 Materials

Four-arm poly(ethylene glycol) azide (≥95.0%, Mn 10,000 Da) was purchased from Ponsure Biological. All chemical reagents were purchased from meryer, Aladdin Chemical Industry, and Energy Chemical, unless otherwise noted. And used without further purification. Phosphate buffers were prepared using distilled water. All cuvettes made by fused quartz were purchased from Starna Cells with standard screw and septum top. Silica gel from Qingdaohaiyang Chemical Company with 200-300 mesh was used for column chromatography.

1.2 Methods and instruments

1.2.1 Fluorescence spectroscopy

Concentration titration and fluorescence spectra in different solvents were measured on HITACHI F-7100 fluorescence spectrometer.

1.2.2 UV-vis spectroscopy

The UV-vis absorbance spectra were obtained in Cary 3500 UV-vis spectrophotometer from Aglient Technology. The spectra were run in Cary WinUV software.

1.2.3 DFT calculation

The geometry optimizations of molecules were performed using density functional theory (DFT) at the B3LYP level with the 6-31G(d) basis set, as implemented in the Gaussian 09W package.

1.2.4 Nuclear magnetic resonance (NMR)

^{1}H and ^{13}C NMR spectra were recorded on 400 MHz JEOL NMR spectrometer, Bruker Avance-400 MHz and 600 MHz NMR spectrometer. The NMR spectra were referenced to solvent and the spectroscopic solvents ($CDCl_3$, DMSO-d_6, etc.) were purchased from Cambridge Isotope Laboratories.

1.2.5 High-resolution mass spectrometry (HRMS)

High-resolution mass spec (HRMS) analysis was conducted by the WATERS I-Class VION IMS QTof mass spectrometer.

1.2.6 Hydrogels swelling

Hydrogels were prepared (150 μL/gel) and incubated for 3 h at 25 °C to ensure full gelation and fully volatilized solvent. The weight of dried hydrogel was taken as the initial value, and PBS buffer (4 mL) was added to the hydrogels, which were incubated at 25 °C. Every 30 mins, buffer was removed, using paper to absorb the liquid on

the surface of hydrogel, and hydrogel weight was recorded and replaced with PBS buffer (4 mL) until hydrogel weight no longer increase.

1.2.7 Rheometry

Rheological measurements were carried out using a Rheometer (MCR 302, Anton Paar) equipped with a parallel plate geometry (8 mm rotor). The experiments were conducted at constant temperature, i.e. 37 ^{o}C. Storage modulus G' and loss modulus G'' were obtained at constant deformation (1 % strain) with increasing frequency (from 1 Hz to 100 Hz).

1.2.8 Scanning Electron Microscopy (SEM)

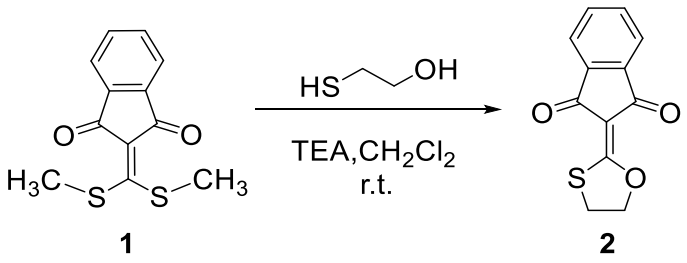
SEM images were collected by ultra-high resolution field emission scanning electron microscope (Tescan Maia3 LMH, Czech Republic). The hydrogel sample had a diameter of 10 mm and a thickness of 3 mm; after sufficient swelling, the gel was immersed in liquid nitrogen for freezing. Then the frozen sample was saved in the refrigerator at -80 ^{o}C overnight. The hydrogel was dried via freeze-drying technique. The sample was put on the objective table and sprayed by Au (Ted Pella 108A, US).

1.2.9 Tensile tests

All mechanical tests were performed by an electronic universal testing machine (UTM2203, SUNS, China) equipped with 100 N load cell and the temperature fluctuation during the test was lower than 2 ^{o}C/h to eliminate the influence of temperature fluctuations on hydrogel testing. For tensile tests, dumbbell-shaped hydrogel specimens were prepared by adding the hydrogel precursor solution to a polytetrafluoroethylene molds (designed according to type 5B of ISO 527-2 standard) and standing still. Elastic modulus was defined as the initial slope (10%) value of the stress-strain curve. The relationship between the tensile stress-strain curve and the tensile rate was obtained by stretching the hydrogel at a speed of 30 m/s, respectively.

Small molecule and the optical properties

1.3 Synthetic procedure for 2



1 **2**

To a solution of 100 mg reagent **1** and 1 equivalent of *β*-mercaptoethanol in dichloromethane (2 mL), 1 equivalent of triethylamine was added, and the resulting mixture was stirred at room temperature. The reaction was monitored by TLC and stopped after 0.5 h. After the reaction was completed, the solvent was evaporated by vacuum, the residue was purified by column chromatography on silica gel using dichloromethane/methanol (100 : 1 → 100 : 3) mixture to give the final product as a white solid (85 mg, 91% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 – 7.82 (m, 2H), 7.67 (t, *J* = 5.8 Hz, 2H), 4.86 (t, *J* = 7.3 Hz, 2H), 3.44 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.81, 186.37, 178.69, 140.13, 134.15, 133.82, 122.80, 122.06, 107.20, 75.33, 30.16. HRMS (ES+) m/z: calcd for $C_{12}H_8O_3S$ $[M+H]^+$ 233.02669 found 233.02652.

Table S1. Photophysical properties for molecules **1** and **2**

	λ_{abs}[a] (nm)	λ_{em}[a] (nm)	Stokes shift (nm)	ε[c]	QY[d]
1	400	--[b]	--[b]	1.985	--[b]
2	333	480	147	3.562	2.77

[a]The spectra were obtained in neutral aqueous buffer (1% DMSO as co-solvent) with 10 μM. [b]not detected. [c]Molar extinction coefficient (10^4 L·mol^{-1}·cm^{-1}). [d]Absolute quantum yield (QY).

1.4 Fluorescence emission for 2 in different solvents

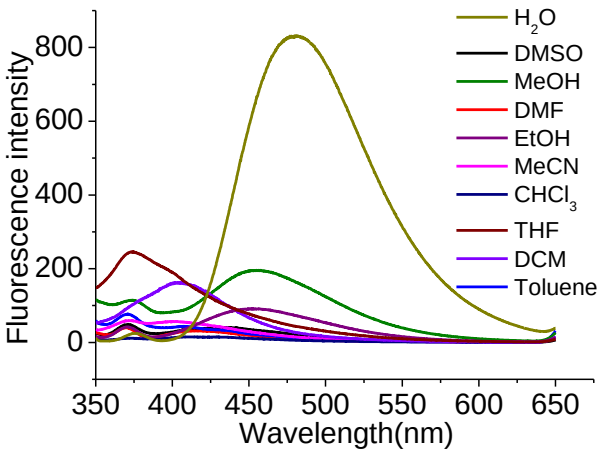


Figure S1. Fluorescence spectra for compound **2** (10 μM, λ_{ex} = 333 nm) in common solvents.

1.5 Reactions between 1 and BME in pH 9.5 buffer

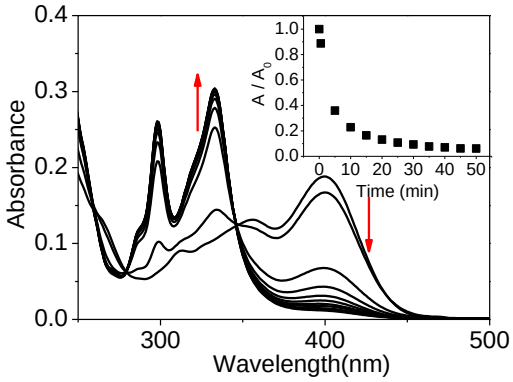


Figure S2. Time kinetics for UV-vis absorbance of **1** (10 μM) in the presence of BME (10 μM). Relationship between A/A_0 at 400 nm and time (0 – 50 minutes) (inset picture). The spectra were obtained in pH 9.5 PBS buffer (1% DMSO as co-solvent).

1.6 Reactions between 1 and BME in neutral buffer

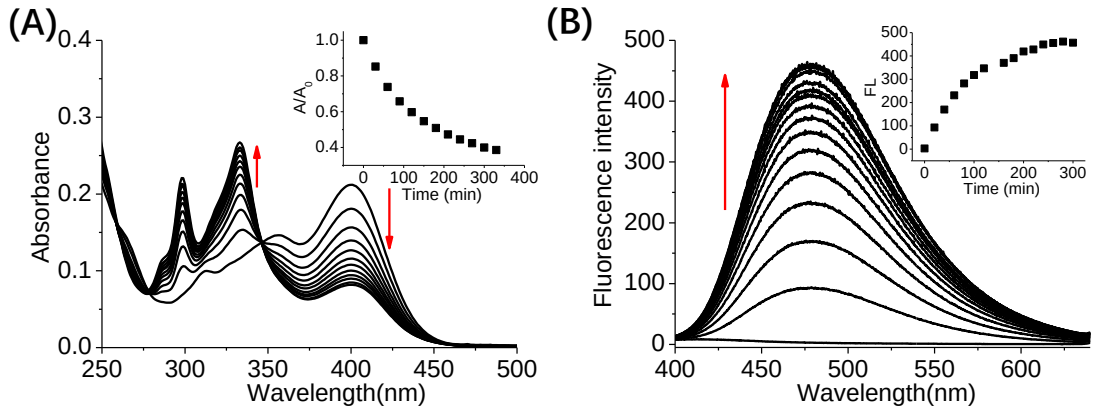
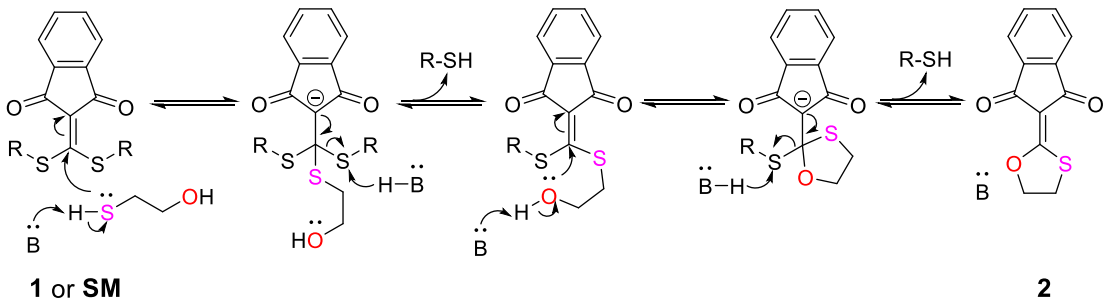


Figure S3. (A) Time kinetics for UV-vis absorbance of **1** (10 μM) in the presence of BME (10 μM). Relationship between A/A_0 at 400 nm and time (0 – 300 minutes) (inset picture). (B) Kinetics for fluorescence of **1** (10 μM) in the presence of BME (10 μM). Relationship between λ_{em} = 478 nm and time (0 – 300 minutes) (inset picture). The spectra were obtained in pH 7.4 PBS buffer (1% DMSO as co-solvent).

1.7 The proposed mechanism of degradation



Scheme S1. Proposed mechanism of degradation was promoted in basic environmental.

1.8 Stability of 1 in pH 9.5 condition

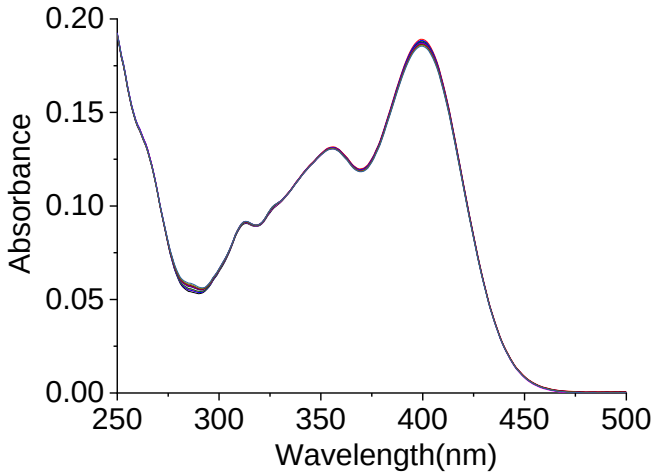
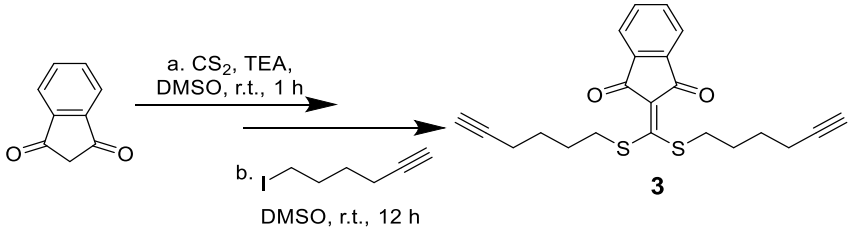


Figure S4. Time kinetics of signal changes in UV-vis spectra of **1** (0 – 60 mins) in pH 9.5 PBS buffer (1% DMSO as co-solvent).

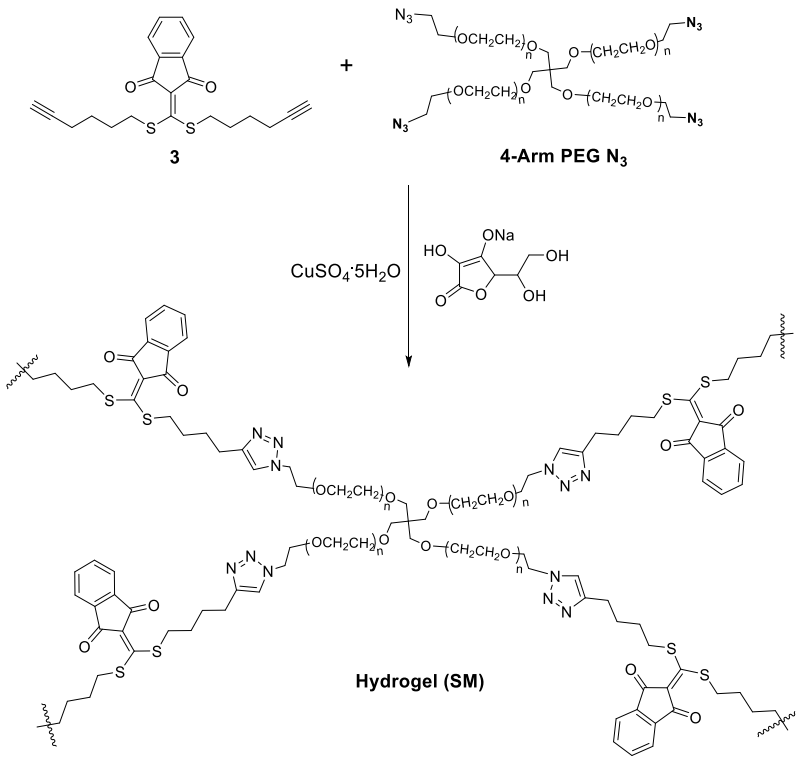
Synthesis of hydrogel and its properties

1.9 Synthesis of alkyne-terminated conjugate acceptor



Synthesis of conjugate acceptor 3. 1,3-Indandione (100 mg, 684.25 µmol, 1 eq.) was dissolved in DMSO followed by addition of triethylamine (TEA, 138 mg, 1.37 mmol, 2 eq.) and carbon disulfide (104 mg, 1.37 mmol, 2 eq.). The resulting mixture was stirred at room temperature for 1 hour, then, 6-iodo-1-hexyne (284.71 mg, 1.37 mmol, 2 eq.) was added to the solution dropwise. The reaction was monitored by TLC and stopped after 12 h. The solution was added over ice and extracted with chloromethane. Organic phase was collected and evaporated by vacuum to concentrated liquid state. The concentrated liquid product was purified by column chromatography with chloromethane as eluent to obtain yellow oily product **3** (130 mg, 50% yield). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.84 (t, *J* = 2.9 Hz, 2H), 7.68 (t, *J* = 2.9 Hz, 2H), 3.19 (t, *J* = 7.3 Hz, 4H), 2.21 (t, *J* = 6.7 Hz, 4H), 1.92 (s, 2H), 1.81 (p, *J* = 7.3 Hz, 4H), 1.63 (p, *J* = 7.3 Hz 4H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 187.06, 177.81, 140.27, 134.12, 123.04, 122.66, 83.50, 69.03, 37.94, 28.05, 27.33, 17.98. HRMS(ES+) m/z: calcd for $C_{22}H_{22}O_2S_2$ $[M+Na]^+$ 405.09534 found 405.09501.

1.10 Synthesis of hydrogel



Synthesis of SM. 4-Arm PEG azide (M_n ~ 10,000 Da, 32 mg, 3.27 μmol) and conjugate acceptor **3** (2.5 mg, 6.54 μmol) were mixed together in *tert*-butanol (100 μL), vortexing to a homogeneous liquid phase. Then the copper (II) sulfate pentahydrate (668 μg, 4.18 μmol) and sodium ascorbate (667 μg, 3.37 μmol) were added. The mixture was stirred with a vortex to form a uniform moldable gel precursor solution. Subsequently, the gelation was monitored and the hydrogel was generated in about 5 minutes. The sample was swelled in water to remove the residue reagents and dried for storage.

1.11 Degradation of SM with *β*-mercaptoethanol

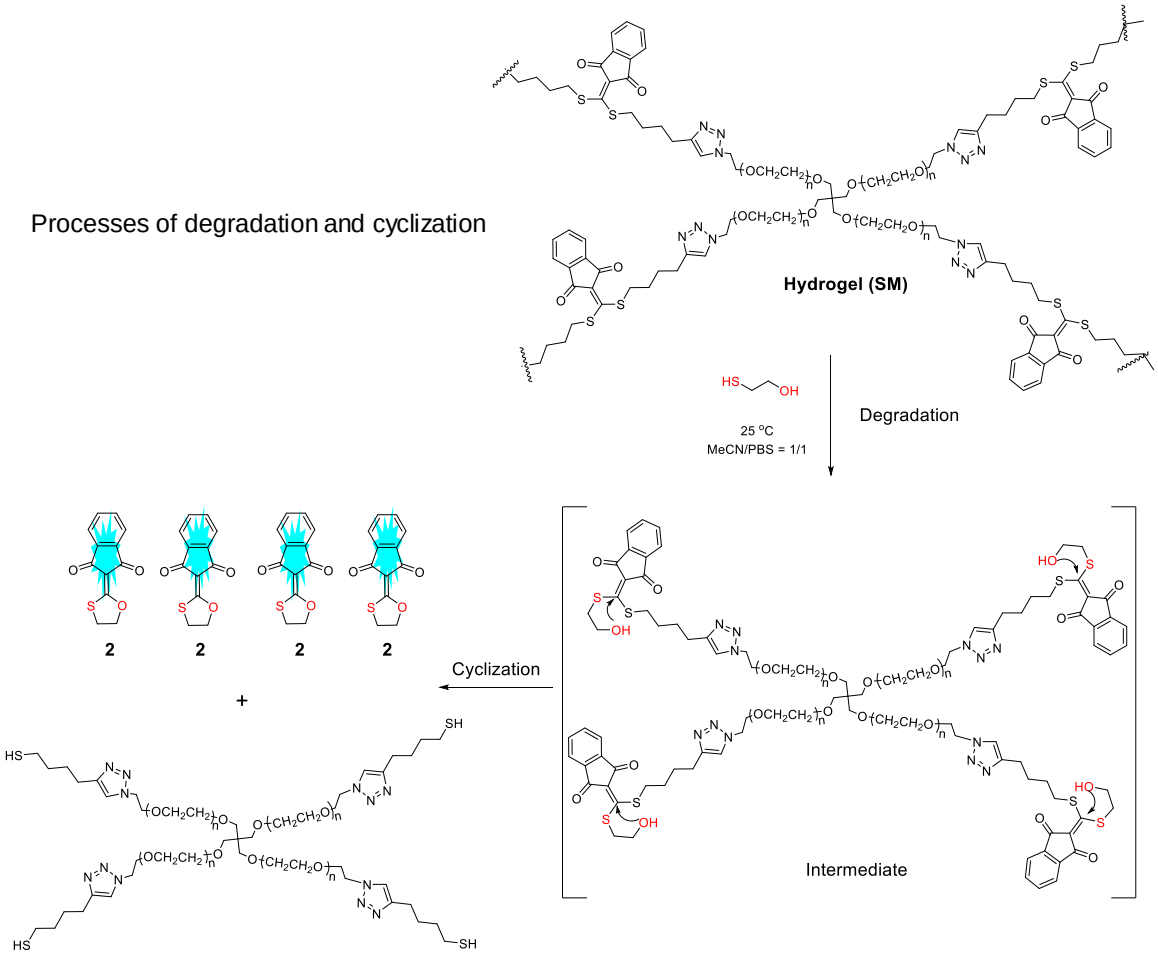


Figure S5. Degradation of **SM** by *β*-mercaptoethanol in the pH 9.5 buffer (50% MeCN as co-solvent).

Procedure: The **SM** sample synthesized by conjugate acceptor **3** (2.5 mg, 10 μmol, 1 equiv) was fully swelled in the buffer, and placed in a clean glass bottle, while the *β*-mercaptoethanol (2.2 μL, 15 μmol, 1.5 equiv) and 5 mL buffer (PBS, pH = 9.5) were added sequentially to the bottle, and then left at room temperature.

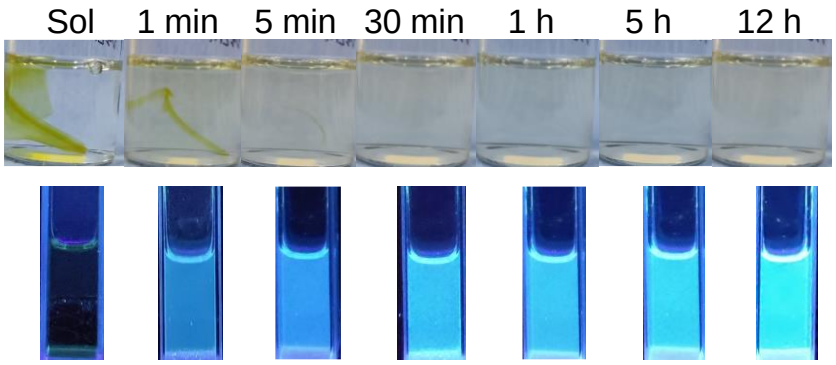


Figure S6. Photographs of the degradation process of **SM** (10 μmol) induced by *β*-mercaptoethanol (15 μmol) in different time (0 – 12 hours).

1.12 HRMS to track the generation of indicator 2

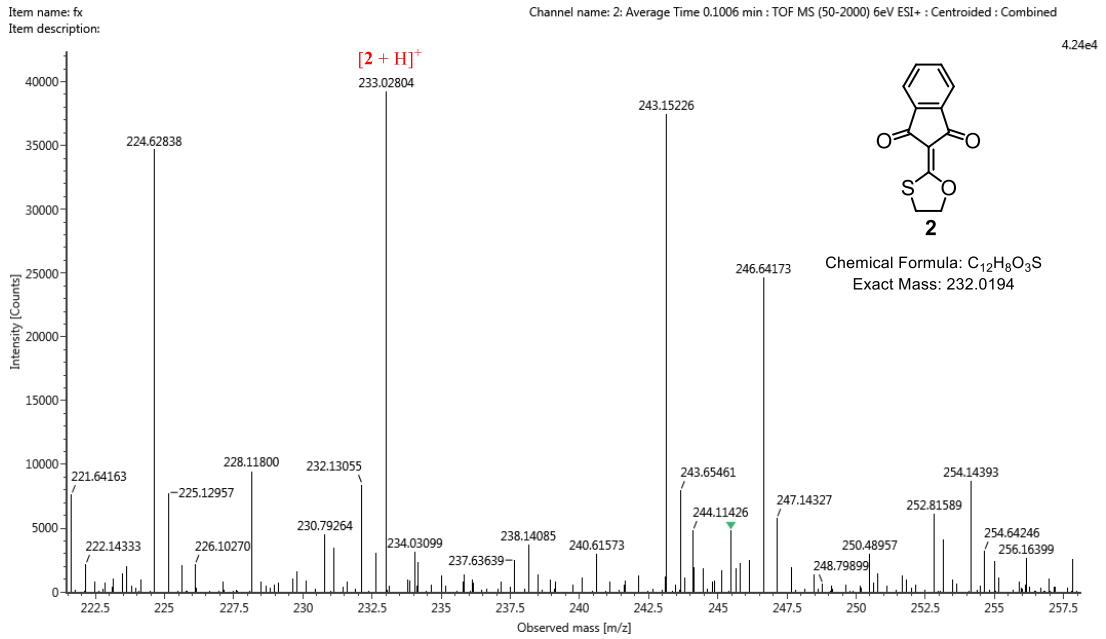


Figure S7. HRMS spectra for compound **2** after degradation of **SM** by BME.

Degradation triggered by benzyl mercaptan

1.13 Reaction between 1 and benzyl mercaptan

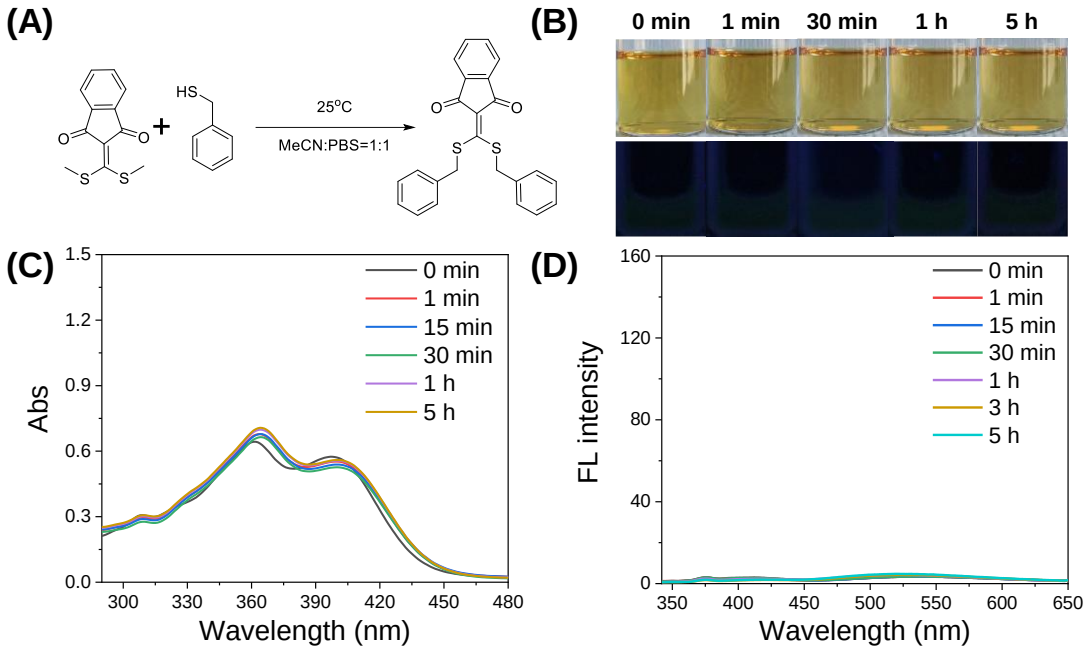


Figure S8. (A) Reaction between **1** and benzyl mercaptan in pH 9.5 buffer. (B) Photographs during the reaction process (5 hours). There was no fluorescence during the whole reaction. (C) UV-vis absorbance to record changes

over 5 hours. (D) Fluorescence spectroscopy to record changes over 5 hours.

1.14 Control experiments for the degradation in solution

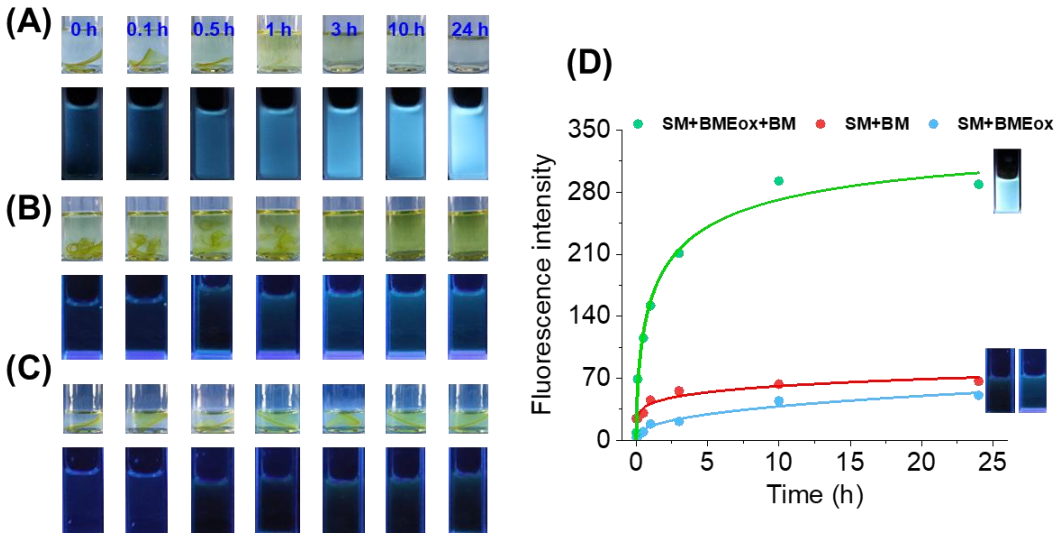


Figure S9. Images of control experiments for the macroscopic degradation of **SM** over 24 hours. (A) **SM**+BM (1:1 eq. ratio). (B) **SM**+BMEox (1:1 eq. ratio). These experiments were operated in PBS buffer (pH = 9.5, 50% MeCN as co-solvent).

1.15 Degradation in solid

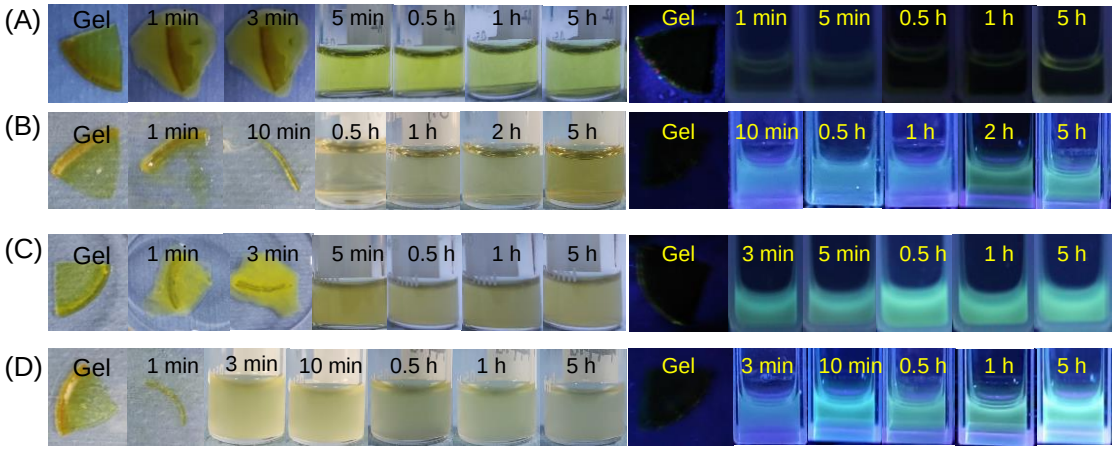


Figure S10. Degradation of **SM** and fluorescence real time tracing through self-propagating cascades in the solid state and experiments under different concentration ratio between **SM** and BM in solid state. (A) Control: **SM** + BM (1 : 1 eq. ratio); (B) **SM** + BMEox + BM (1 : 1: 0.1 eq. ratio). (C) **SM** + BMEox + BM (1 : 1 : 1 eq. ratio). (D) **SM** + BMEox + BM (1 : 1 : 10 eq. ratio). Macroscopic degradation and fluorescence images under 365 nm ultraviolet light. The samples were swelled in pH 9.5 PBS buffer (50% MeCN as co-solvent) before the test.

Degradation triggered by GSH

1.16 Reaction between 1 and GSH and photo images

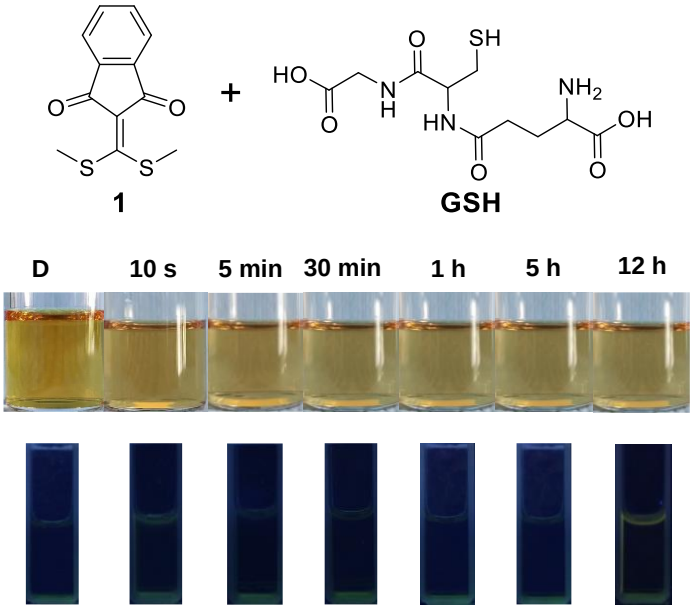


Figure S11. Mixture of **1** (2 mM, 10 µmol, 2.5 mg) and GSH (2 mM, 10 µmol, 3.2 mg) in pH 9.5 PBS buffer (50% MeCN as co-solvent) and fluorescence photo images over time (0 – 12 hours).

1.17 Optical spectroscopy to track reaction between 1 and GSH

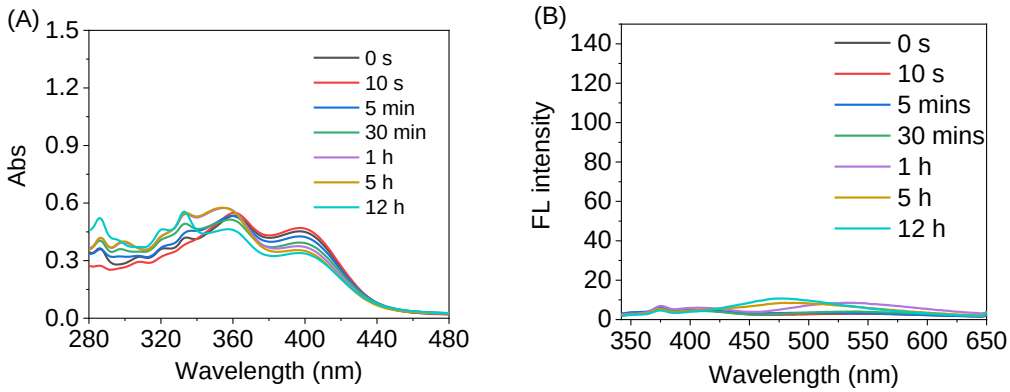


Figure S12. Reaction between **1** and GSH in pH 9.5 buffer. (A) UV-vis absorbance to record changes over 12 hours. (B) Fluorescence spectroscopy to record changes over 12 hours.

1.18 GSH induced degradation in solution

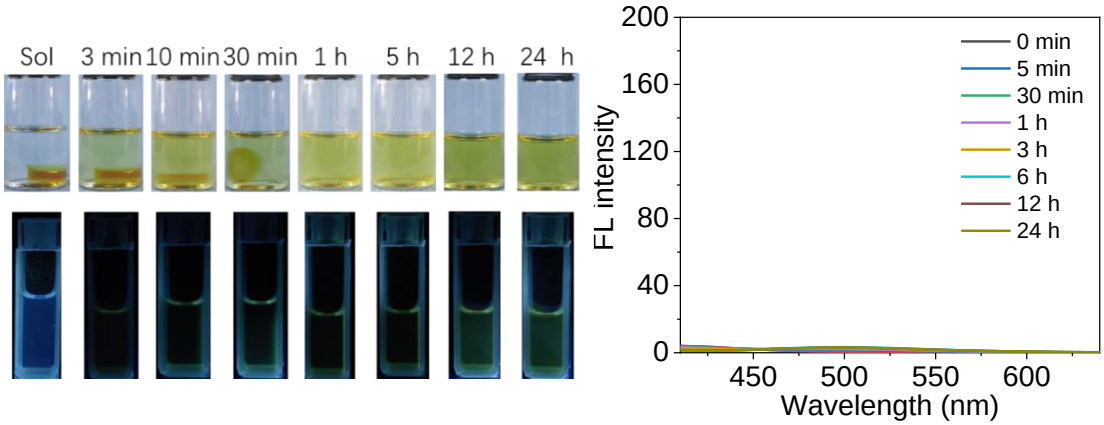


Figure S13. Control experiments for the degradation of **SM** by GSH (1:1 eq. ratio) over 24 hours. Fluorescence photo images and time kinetics in spectroscopy. These experiments were operated in PBS buffer (pH = 9.5, 50% MeCN as co-solvent).

1.19 GSH induced degradation in solid

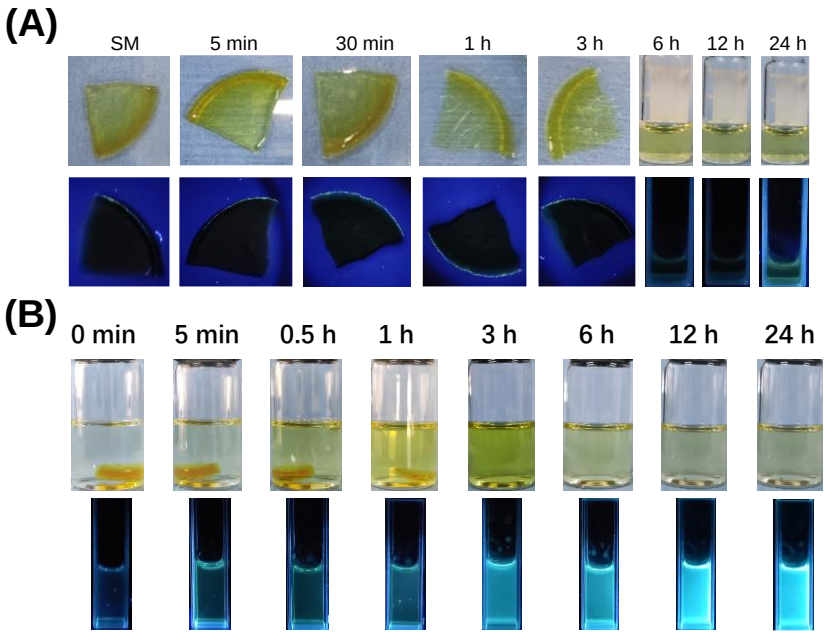


Figure S14. Control experiments for the degradation of **SM** by GSH (1:1 eq. ratio) over 24 hours in solid (A). Images for the macroscopic degradation of **SM** and fluorescence changing photos triggered by GSH (0.1 eq.) with 1.5 eq. BMEox in aqueous buffer (B).

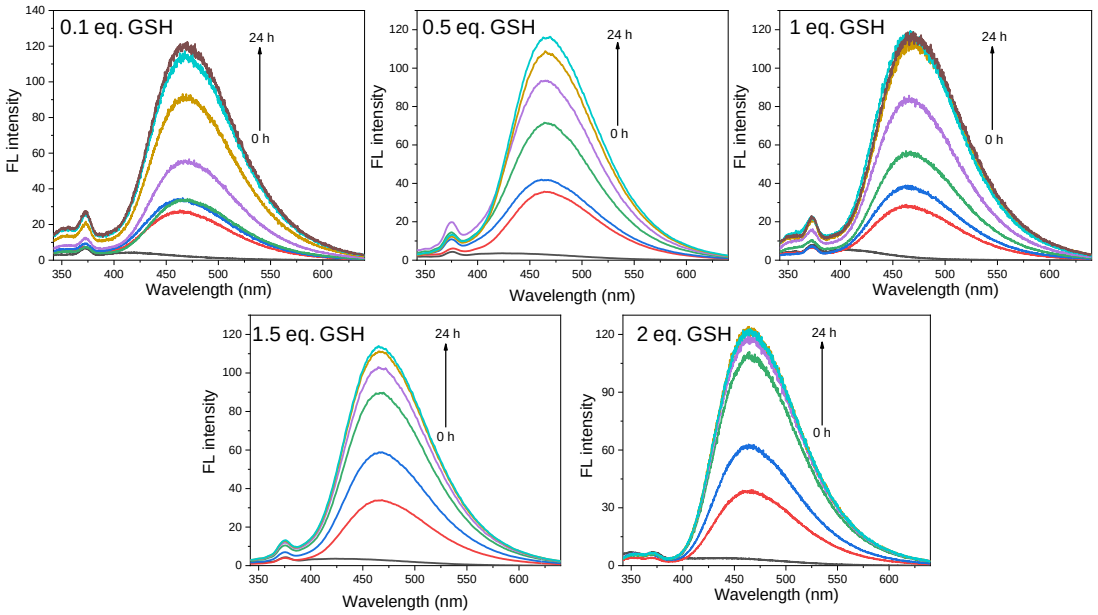


Figure S15. Fluorescence spectra tracking of the degradation of **SM**. under different concentrations of GSH (0.1, 0.5, 1, 1.5 and 2 eq. ratio) in the existence of BMEox (1.5 eq.), pH = 9.5. The samples were swelled in PBS buffer (50% MeCN as co-solvent) before the test.

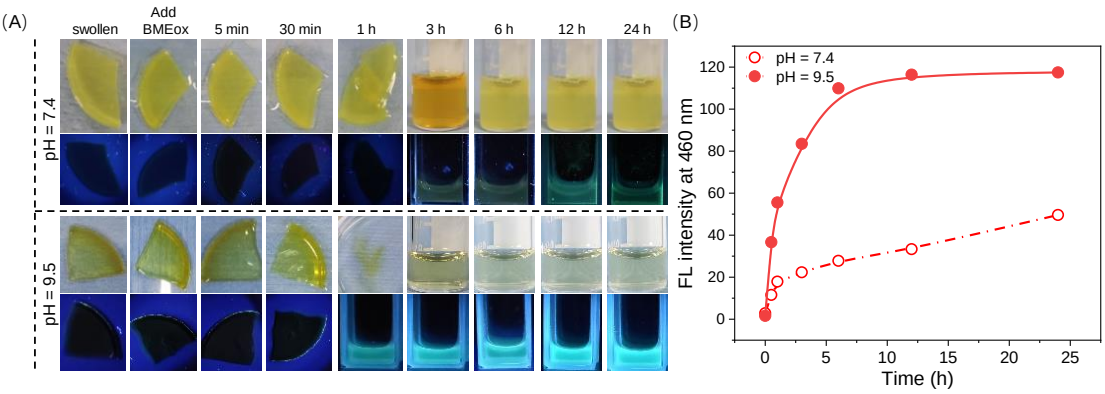


Figure S16. Degradation of **SM** and fluorescence real time tracing through self-propagating cascades in the solid state under different pH. (A) Above: **SM** + BMEox + GSH (1/1/1 eq. ratio), pH = 7.4. The samples were swelled in PBS buffer (10% MeCN as co-solvent) before the test; Below: **SM** + BMEox + GSH (1/1/1 eq. ratio), pH = 9.5. The samples were swelled in PBS buffer (50% MeCN as co-solvent) before the test; (B) Comparison of fluorescence intensities under different pH for the self-propagating GSH-initiated cascades. Macroscopic degradation and fluorescence images under 365 nm ultraviolet light.

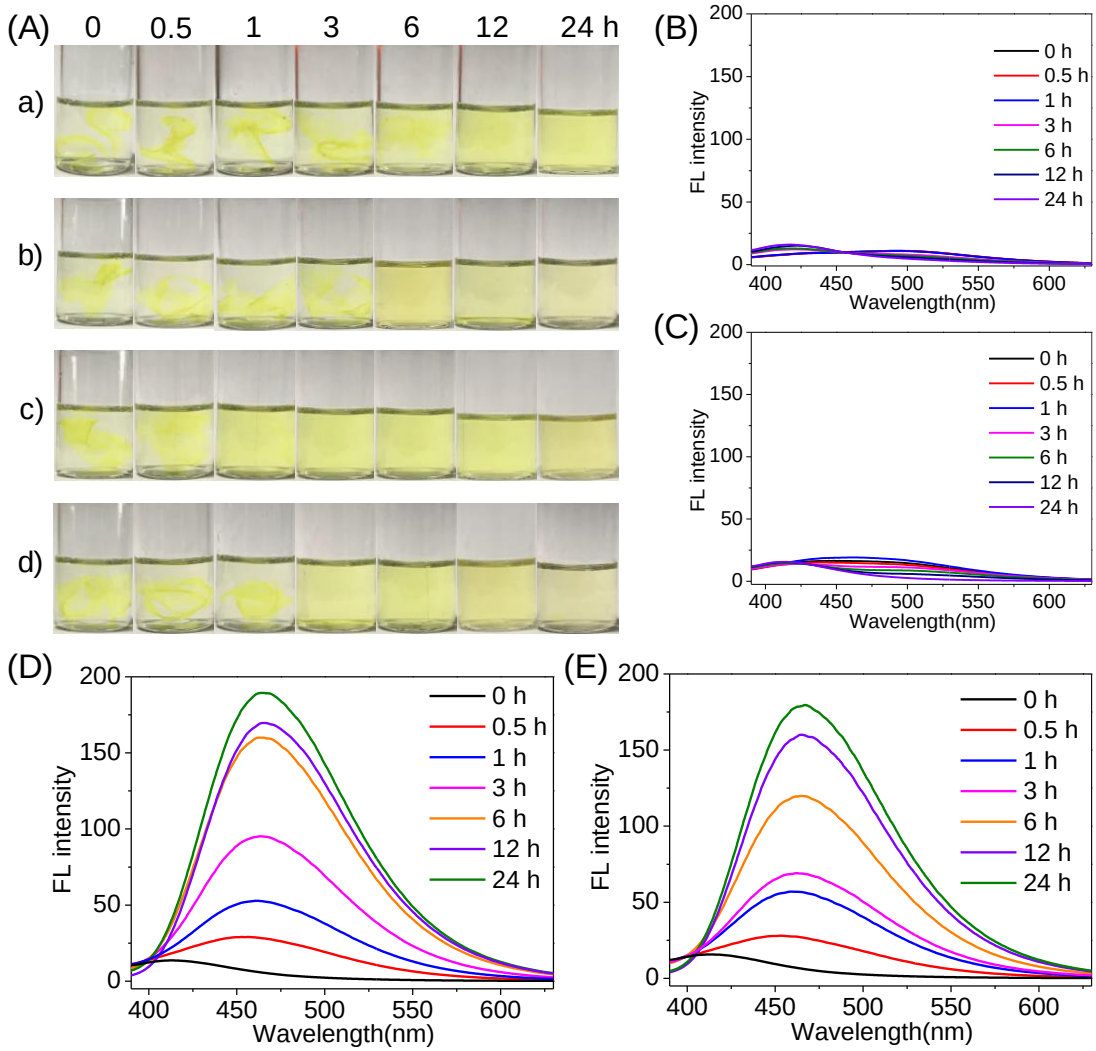


Figure S17. Selectiveness experiments of **SM** to thiols. (A) a) **SM** + Cys (1 : 0.1 eq. ratio); b) **SM** + BMEox + Cys (1 : 1 : 0.1 eq. ratio); (c) **SM** + Hcy (1 : 0.1 eq. ratio); b) **SM** + BMEox + Hcy (1 : 1 : 0.1 eq. ratio). (B) Fluorescence signal changes during degradation of **SM** by Cys (1 : 0.1 eq. ratio) without BMEox over 24 h. (C) Fluorescence signal changes during degradation of **SM** by Hcy (1 : 0.1 eq. ratio) without BMEox over 24 h. (D) Fluorescence signal changes during degradation of **SM** by Cys (1 : 0.1 eq. ratio) with BMEox (1 eq.) over 24 h. Fluorescence images taken under 365 nm ultraviolet light (bottom). (E) Fluorescence signal changes during degradation of **SM** by Hcy (1 : 0.1 eq. ratio) with BMEox (1 eq.) over 24 h (λ_{ex} = 333 nm). Fluorescence images taken under 365 nm ultraviolet light (bottom). The samples were swelled in pH 9.5 PBS buffer (50% MeCN as co-solvent) before the test.

Limit of Detection

According to the fluorescence titration data, a linear relationship between the fluorescence intensity at λ_{em} = 460 nm and GSH concentrations was observed at a certain time point of 3 h (R^2 = 0.96, Figure 4C), the detection limit was calculated with the following equation:

Detection limit = 3σ/*k*;

σ: the standard deviation of fluorescence intensity for blank measurements (n = 13);

k: the slop between the fluorescence intensity versus the concentrations of GSH.

Supplementary spectra

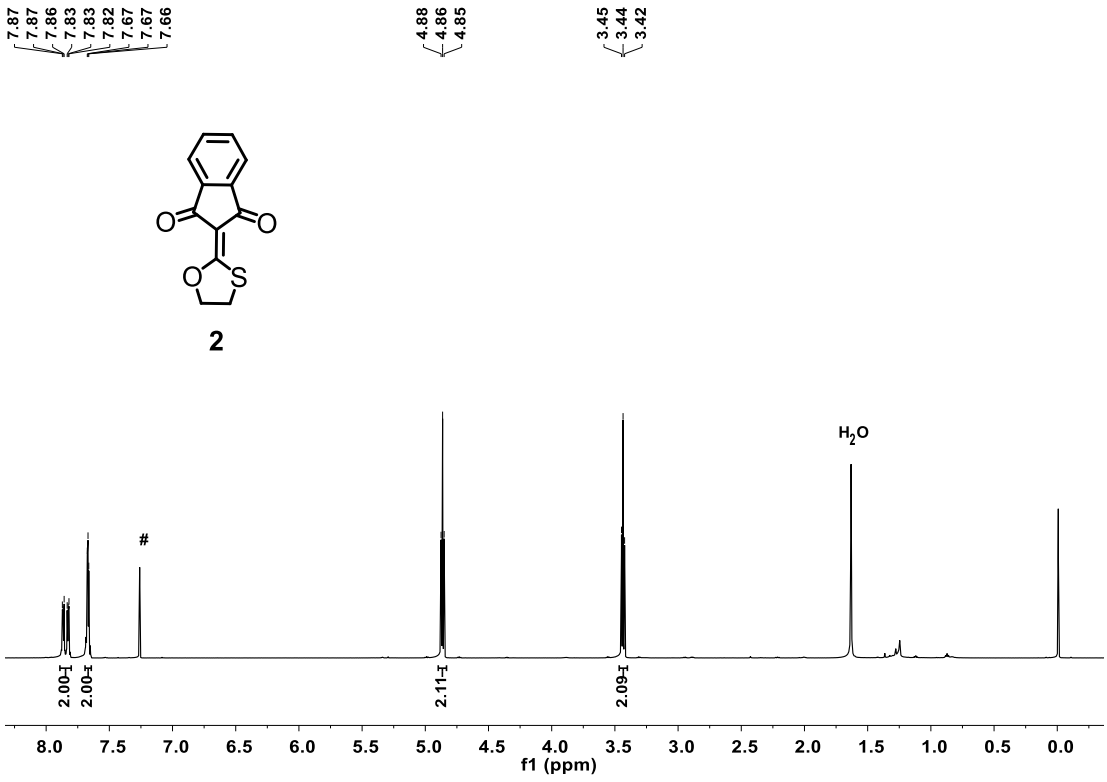


Figure S18. ^{1}H NMR spectrum for compound **2** in $CDCl_3$.

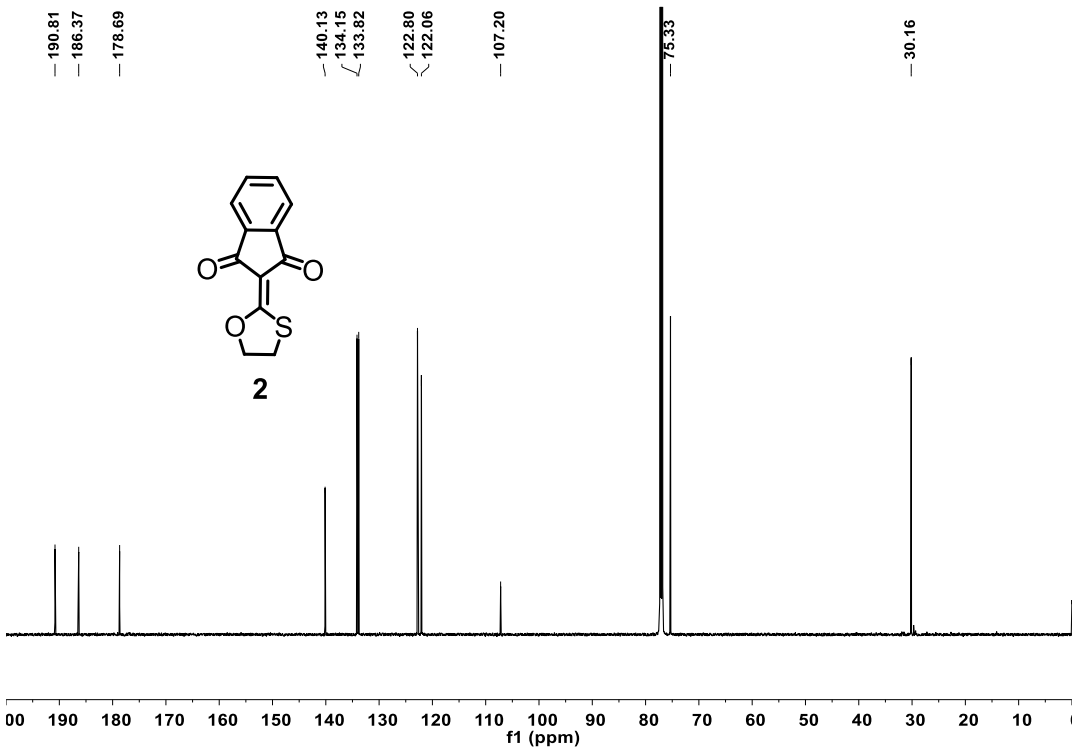


Figure S19. ^{13}C NMR spectrum for compound **2** in $CDCl_3$.

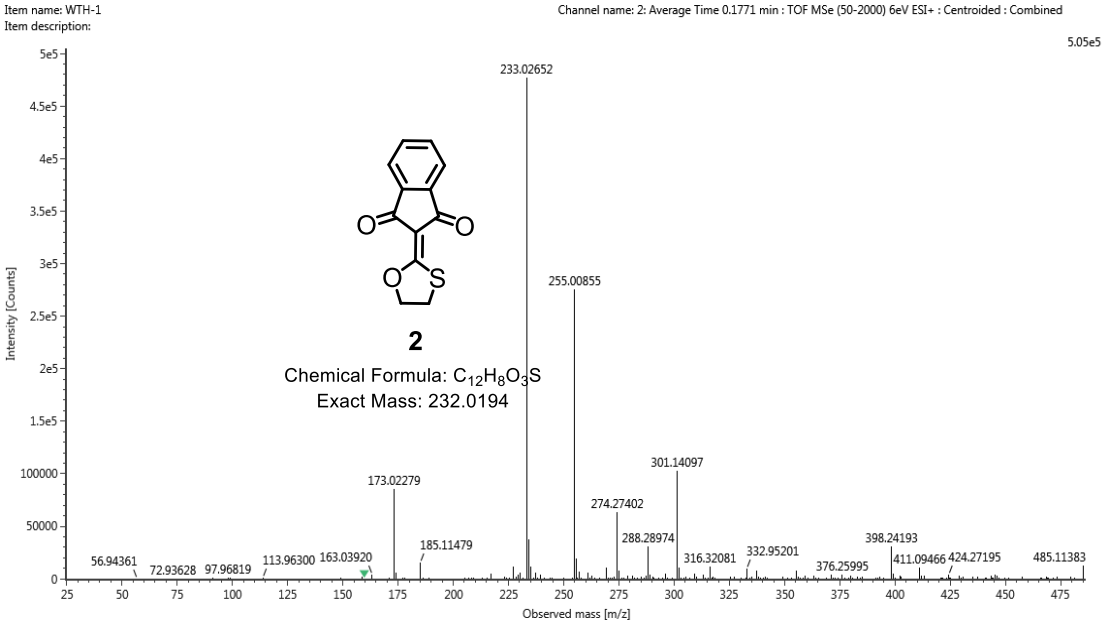


Figure S20. HRMS spectrum for compound **2**.

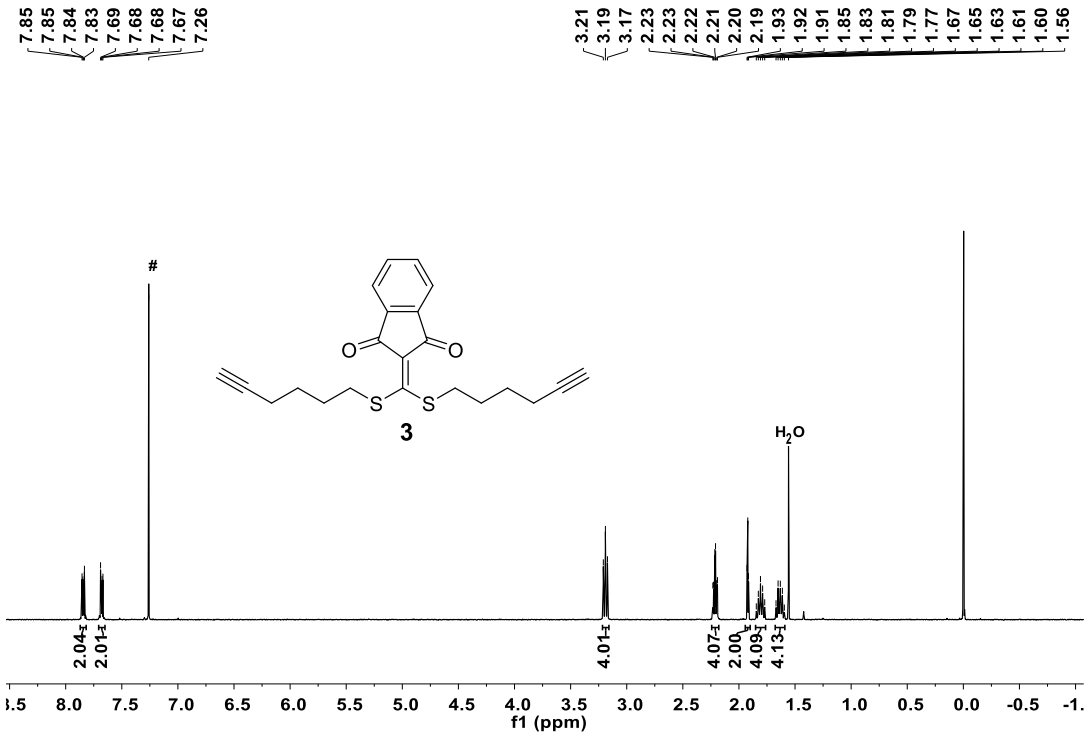


Figure S21. ^{1}H NMR spectrum for compound **3** in $CDCl_3$.

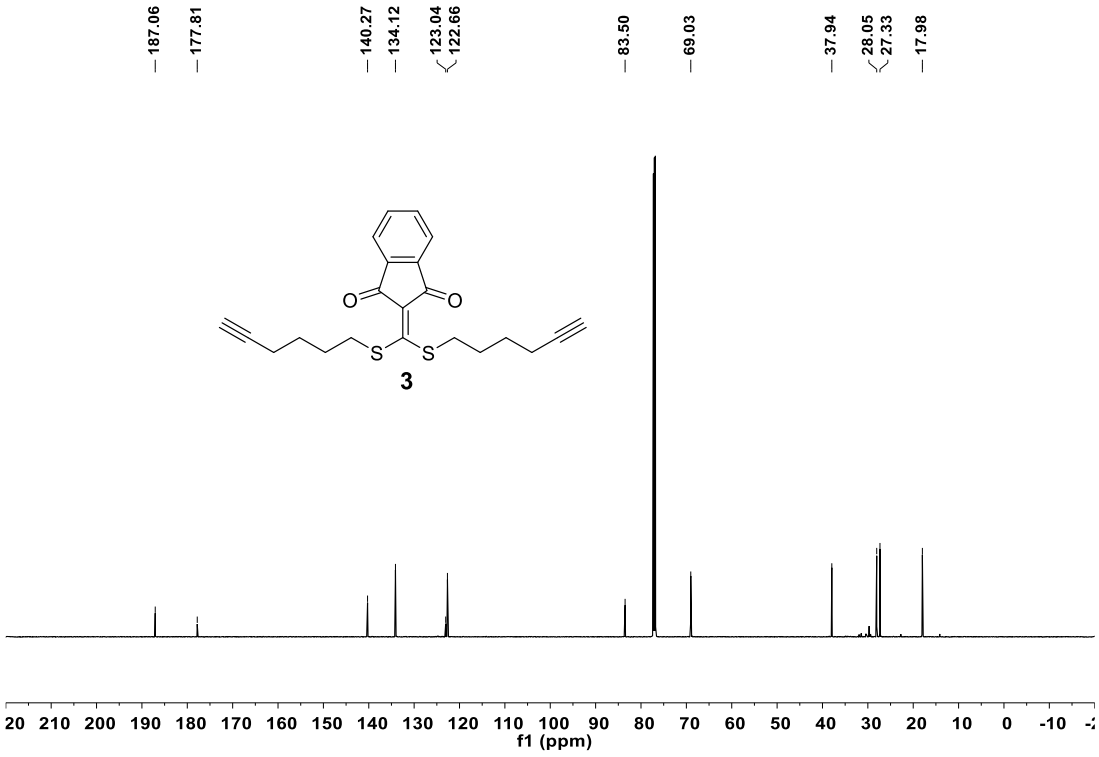


Figure S22. ^{13}C NMR spectrum for compound **5** in $CDCl_3$.

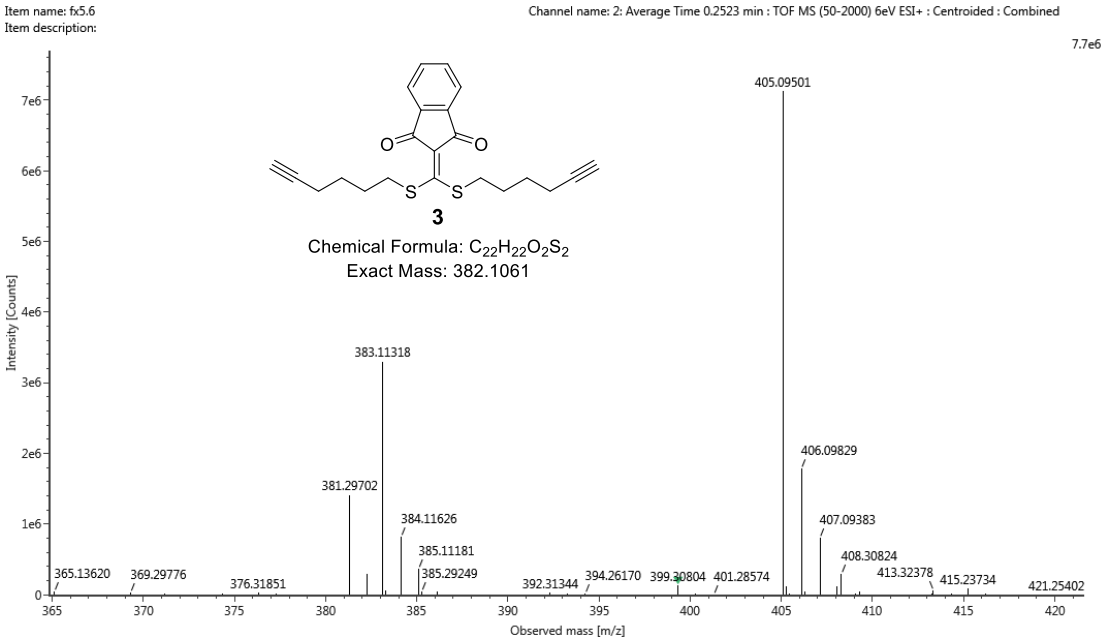


Figure S23. HRMS spectrum for compound **3**.