Electronic Supplemental Information (ESI)

NIR light controlled release of caged hydrogen sulfide based on upconversion nanoparticles

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Experimental Section

1. Experimental procedures

1.1 Materials

3,4-Dimethoxy-6-nitrobenzaldehyde, methoxypolyethylene glycol (mPEG, average molecular weight 1900) PBr_3, thiourea, oleic acid (OA), octadecylene (ODE) and dansyl chloride (DNS-Cl) were purchased from Adamas (Shanghai, China). 2,2,6,6-Tetramethyl-1-piperidinyloxyl (TEMPO), trimethylaluminium solution in toluene (2.0 M) and sodium metabisulfite were obtained from Aladdin-Reagent Inc. (Shanghai, China). Octadecylamine (ODA), $TiCl_4$ and NaN_3 were provided by Xiya Chemical Reagent Co. (Chengdu, China). Hydroxybenzotriazole (HOBt) and *o*-benzotriazole-*N,N,N′,N′*-tetramethyluronium-hexa-fluoro-phosphate (HBTU) were purchased from GL Biochem Ltd. (Shanghai, China). L929 cell line, MCF-7 cell line and Kunming mices were obtained from Xiangya Hospital of Central South University (Changsha, China). Fetal bovine serum (FBS), DMEM cell culture medium and RPMI 1640 cell culture medium were purchased from HyClone (Beijing, China). MTT cell proliferation assay kits were purchased from Beyotime Inst Biotech (Haimen, China). Other reagents and solvents were of analytical purity and used without further purification.

1.2 Synthesis and characterization of $LiYF_4$:Yb, Tm nanoparticles

$LiYF_4$:Yb, Tm nanoparticles were synthesized according to the literature method with slight modification. Briefly, Ln_2O_3 (Y_2O_3:Yb_2O_3:Tm_2O_3 = 74.5:25:0.5, molar fraction) was dissolved in 50% TFA aqueous solution by heating at 80 ^oC for 5 h. After

evaporation of the solvent $Ln(CF_3COO)_3$ was given as a white powder and re-dissolved by OA/ODE (10 mL/10 mL). Then LiF (0.52 g, 20 mmol) was added and the mixture was degassed at 110 ^oC for 1 h under vacuum. With the atmosphere of N_2 the mixture was heated to 320–330 ^oC at the rate of 25 ^oC/min and stirred vigorously for 1 h. After cooling below 50 ^oC the crude product was precipitated by acetone, centrifuged at 6000 rpm for 10 min, washed twice with acetone and redissolved in chloroform. The excess LiF was removed by centrifugation at 2000 rpm for 1 min. The $LiYF_4$:Yb, Tm nanoparticles were preserved in chloroform for further use.

Hydrodynamic diameter (D_h) of nanoparticles was determined by the dynamic light scattering (DLS) techniques using a Malvern Zetasizer Nano-ZS instrument (ZEN3600, Malvern Instruments) equipped with a temperature controller and a 633 nm He-Ne laser at a scattering angle of 173° at 25 °C. Transmission electron microscopy (TEM) images were acquired on a JEM-2100F electron microscope operated at 200 kV.

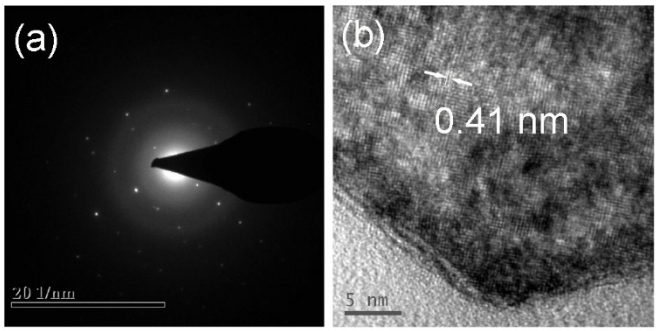


Figure S1. (a) Electron diffraction pattern and (b) HR-TEM image of $LiYF_4$:Yb,Tm nanocrystals.

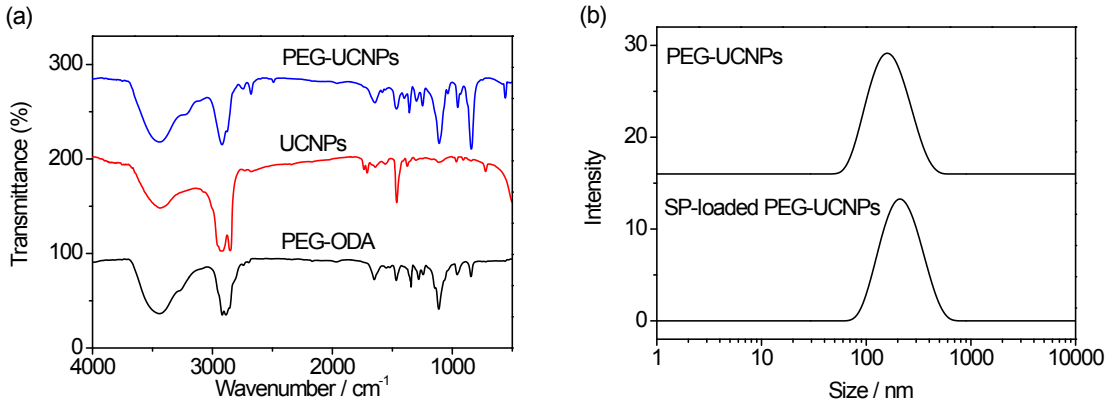


Figure S2. (a) IR of PEG-ODA, UCNPs and PEG-UCNPs; (b) size distributions of PEG-UCNPs and SP-loaded PEG-UCNPs in water.

1.3 Synthesis and characterization

1.3.1 Synthesis of mPEG-COOH

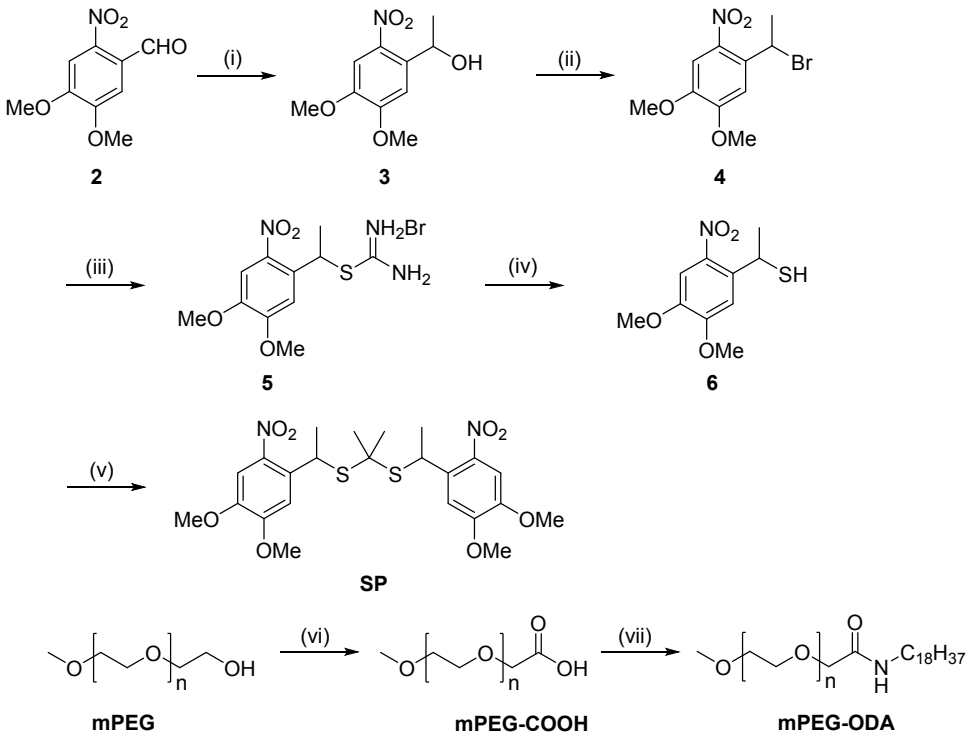
mPEG-COOH was synthesized by the carboxylation of mPEG similar to the procedure described previously.[1] In brief, mPEG (9.5 g, 5 mmol), NaBr (0.052 g, 0.5 mmol) and TEMPO (5 mg, 0.032 mmol) were dissolved in 50 mL water and kept at 0 ^{o}C. Then 15 mL of 10% NaClO solution was added, and the mixed solution was maintained at pH 10 by adding diluted hydrochloric acid. After stirring for 5 h, the mixture was washed with Et_2O for three times, followed by DCM extraction for five times and solvent evaporation to give a white solid (9.38 g, yield 98%).

1.3.2 Synthesis of PEG-ODA

To a solution of mPEG-COOH (3.83 g, 2 mmol) in 100 mL DCM were added Et_3N (0.5 mL, 3.6 mmol), HOBt (0.27 g, 2 mmol) and HBTU (0.76 g, 2 mmol). The mixture was stirred for 1 h at 0 ^{o}C. Subsequently, ODA (0.54 g, 2 mmol) was added and the reaction was transferred to room temperature and kept for 20 h. When the reaction was completed the mixture was subjected to the standard aqueous work-up,

followed by flash chromatography (DCM/EtOAc/CH_3OH, 9:3:1, v/v/v) purification to give a white solid (3.59 g, yield 83%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.98 (s, 2H), 3.87 – 3.44 (m, 157H), 3.38 (d, *J* = 1.0 Hz, 3H), 3.26 (dd, *J* = 13.6, 6.7 Hz, 2H), 1.50 (dd, *J* = 14.0, 7.0 Hz, 2H), 1.27 (d, *J* = 18.7 Hz, 30H), 0.88 (dd, *J* = 7.0, 6.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.80, 71.89, 70.94, 70.48, 70.21, 59.01, 38.92, 31.90, 29.67, 29.31, 26.94, 22.67, 14.11.



(i) $AlMe_3$, DCM; (ii) PBr_3, DCM; (iii) thiourea, THF; (iv) $Na_2S_2O_5$, H_2O/DCM; (v) $TiCl_4$, acetone, $CHCl_3$; (vi) TEMPO, KBr, NaClO, pH 10, 0 ^oC; (vii) ODA, HOBt, HBTU, Et_3N, DCM

Scheme S1. Synthetic routes for SP and mPEG-ODA

*1.3.3 Synthesis of **3***

3,4-Dimethoxy-6-nitrobenzaldehyde (3.17 g, 15 mmol) was dissolved in dry DCM (100 mL) and treated dropwise with trimethyl aluminium in toluene (2 M, 7.5 mL) at 0 ^oC. After reaction for 4 h, the mixture was transferred to ice-cold aqueous

hydrochloric acid under stirring. The product was extract with DCM and purified by flash chromatography (DCM/EtOAc, 1:1, v/v) to give compound **3** (2.45 g, yield 72%).

*1.3.4 Synthesis of **4***

Compound **3** (2.45 g, 10.8 mmol) was dissolved in 100 mL of DCM at 0 ^{o}C, followed by PBr_3 (5.41 g, 20 mmol) addition. After stirring under for 3 h at 0 ^{o}C, the mixture was dropped into ice-cold water, followed by extraction and solvent removal. Crude product was given as a light yellow solid without further purification.

*1.3.5 Synthesis of **5***

Thiourea (0.76 g, 10 mmol) was added into a solution of **4** (2.9 g, 10 mmol) in THF (120 mL). The reaction mixture was stirred at RT overnight. The product was insoluble in THF and formed white precipitates. After filtration, washed with EtOAc and dried under vacuo, compound **5** was given without further purification (3.14 g, yield 86%).

*1.3.6 Synthesis of **6***

Compound **5** (2.92 g, 8 mmol) was dissolved in 100 mL of DCM, then 100 mL of $Na_2S_2O_5$ aqueous solution (0.32 M) was added. The mixture solution was stirred vigorously for 12 h at RT under N_2 atmosphere. After removal of the water phase and evaporation of organic solvent under vacuum, compound **6** was given as a light yellow oil without further purification (1.54 g, yield 79%).

*1.3.7 Synthesis of **SP***

To a solution of compound **6** (1.54 g, 6.32 mmol) in 100 mL $CHCl_3$ at 0 °C, acetone (0.47 mL, 6.32 mmol) and $TiCl_4$ (0.22 mL, 2 mmol) were added. The reaction was kept at 0 °C for 1 h and then transferred to RT to continue for 36 h. The reaction mixture was then treated with the standard aqueous work-up and purified by flash chromatography, providing product SP as a light yellow oil (1.4 g, yield 42%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (s, 2H), 7.03 (s, 2H), 4.94 (q, *J* = 7.2 Hz, 2H), 3.99 (s, 6H), 3.90 (s, 6H), 1.62 (d, *J* = 7.2 Hz, 6H), 1.32 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.31, 147.47, 140.52, 136.48, 111.45, 106.99, 58.11, 56.47, 56.37, 39.03, 31.25, 31.11, 24.30. HRMS (TOF MS EI+) *m/z*: calcd. for $C_{23}H_{30}N_2NaO_8S_2^+$ 549.1341 $[M+ Na]^+$, found 549.1337. Melting point 136-139 °C.

1.3.8 Synthesis of DNS-Az

DNS-Az was synthesized according to a reported procedure.[2] DNS-Cl (0.27 g, 1mmol) was dissolved in 15 mL of ethanol, then 15 mL of NaN_3 (0.13 g, 2mmol) solution in water was added. The mixture reacted at RT for 3h, followed by solvent evaporation and flash chromatography purification (hexane/DCM, 3:1, *v*/*v*). The product was given as a yellow oil (0.12 g, 44%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (d, *J* = 8.5 Hz, 1H), 8.33 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.20 (d, *J* = 8.6 Hz, 1H), 7.63 (dd, *J* = 8.5, 7.7 Hz, 1H), 7.57 (dd, *J* = 8.4, 7.5 Hz, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 2.90 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.15, 133.70, 132.71, 130.13, 130.06, 129.65, 129.28, 122.98, 118.78, 115.86, 45.42. MS (ESI+)

m/z: calcd. for $C_{12}H_{13}N_4O_2S^+$ 277.07 $[M + H]^+$, found 277.05.

1.4 Preparation of PEG-UCNPs

PEG-ODA (0.11 g, 0.05 mmol) was mixed with UCNPs (~20 mg) in 1 mL of $CHCl_3$. Then the mixture solution was added into 6 mL of water and stirred at 50 °C for 3 h to evaporate $CHCl_3$. After that the solution was centrifuged at 6000 rpm for 5 min, excess PEG-ODA in supernatant was removed. The nanoparticles were re-dispersed with water and filtered through a 0.45 μm syringe filter to remove large aggregates.

1.5 Preparation of SP-loaded PEG-UCNPs

SP-loaded PEG-UCNPs were prepared by a procedure similar to that described in section 1.4, except that 5 mg of SP was mixed with PEG-ODA and UCNPs at the beginning. To measure the SP encapsulation capacity, 0.25 mL SP-loaded PEG-UCNPs solution was mixed with 0.75 mL CH_3CN, treated with sonication and centrifugation. Then the absorption of the supernatant was determined. The SP encapsulation capacity was calculated based on Figure S5 and S6.

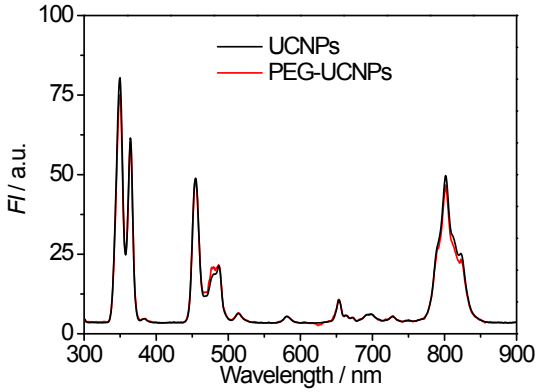


Figure S3. Emission spectra of UCNPs and PEG-UCNPs with 980 nm laser irradiation.

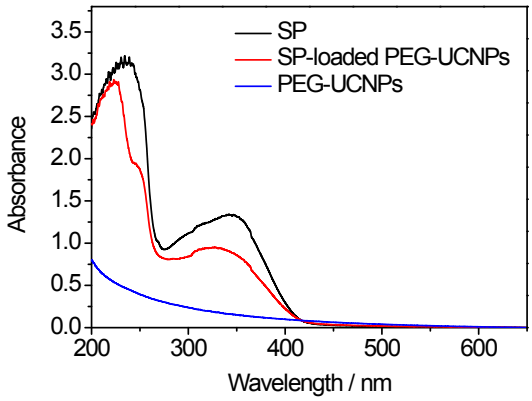


Figure S4. UV-vis absorption spectra of SP in $CHCl_3$, PEG-UCNPs and SP-loaded PEG-UCNPs in water.

1.6 Calculation the loading capacity for SP on PEG-UCNPs

For UCNPs: the volume of one particle of UCNP = 58 × 58 × 51 = 1.72×10^{-16} cm^{-3} and the density of $LiYF_4$ is about 4.0 g cm^{-3}, hence, the mass of one particle of UCNP is 6.88×10^{-16} g. So, we can obtain the concentration of UCNPs in the solution is 8.05 nM.

For SP: because the molar concentration is 0.276 mM, we can calculate the loading amount of SP on PEG-UCNPs is about 34285 molecules per one particle of PEG-UCNP.

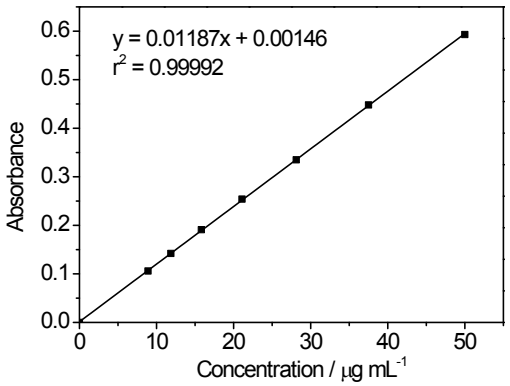


Figure S5. The standard curve for SP based on the absorption at 350 nm.

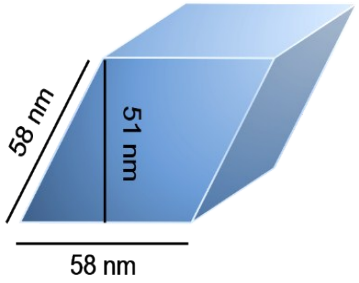


Figure S6. The model of $LiYF_4$:Yb,Tm nanoparticle.

1.7 Reaction mechanism

The H_2S release mechanism was confirmed by RP-HPLC analysis. SP (1.0 mg) was dissolved in 5 mL of methanol, and then treated with UV irradiation (wavelength 365 nm, intensity 3.5 mW cm^{-2}) for 10 min. The reaction was analysed on a HPLC apparatus using C18 reversed-phase column (dimension of 250 × 4.6 mm i.d.) with UV detection at 254 nm (Shimadzu 6AD, Japan). The mobile phase of a linear binary gradient at a flow rate of 1.0 mL min^{-1} was adopted: CH_3CN/H_2O from 50% to 80% for 20 min.

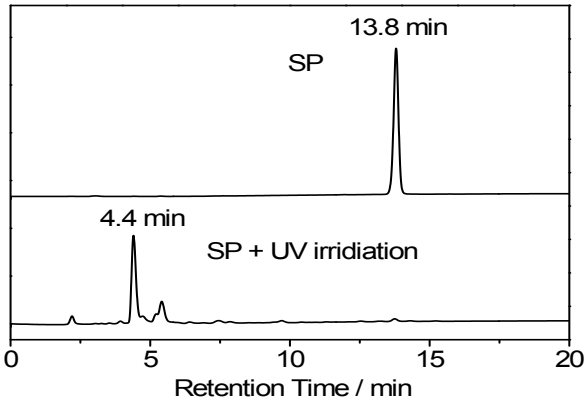


Figure S7. The RP-HPLC analysis of SP treated with UV irradiation for 10 min. The signals were monitored with UV detection at 254 nm.

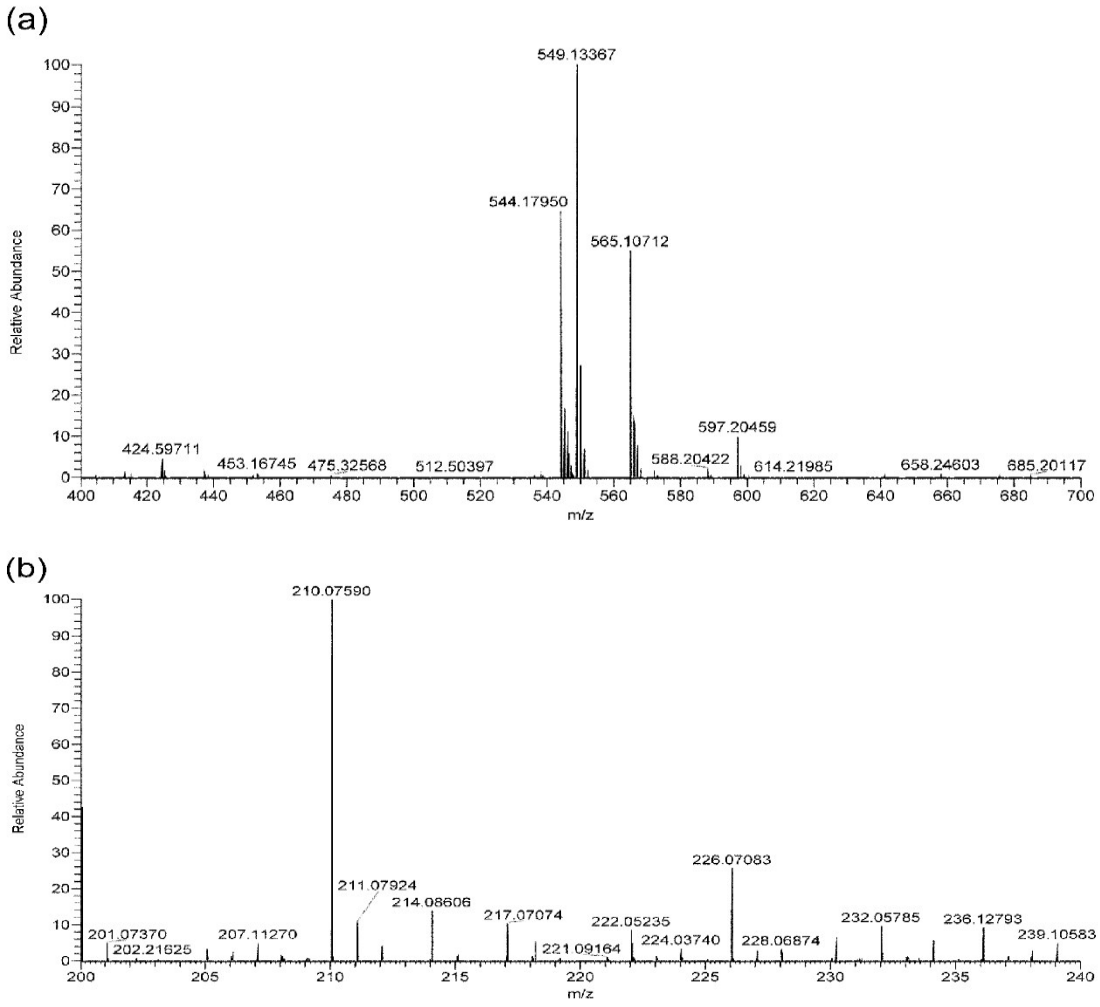


Figure S8. HRMS spectra of SP (a) before and (b) after UV irradiation.

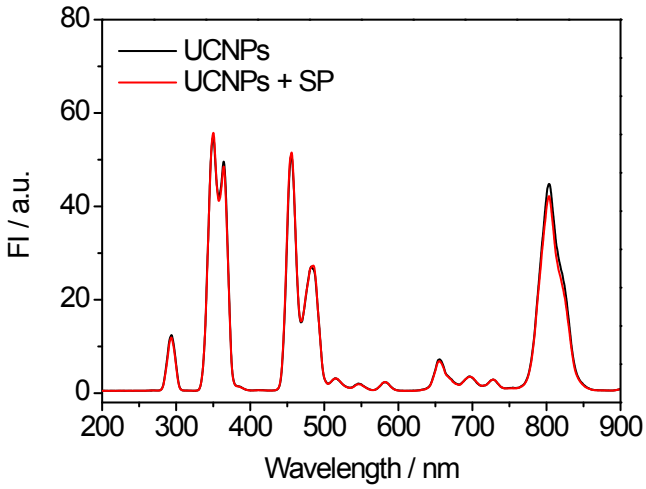
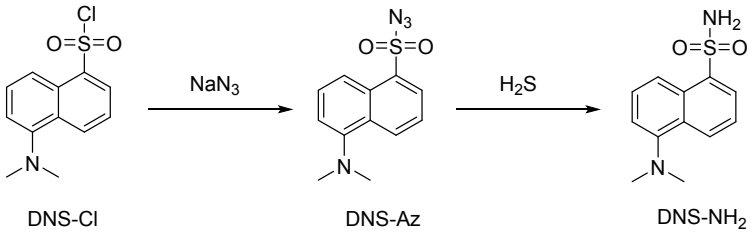


Figure S9. The fluorescence emission of PEG-UCNPs with or without SP in water.

1.8 H_2S release

SP-loaded PEG-UCNPs (2.0 mL) was added into a glass tube with cork. Continuous wave 980 nm NIR laser (Beijing Viasho Technology Co., Ltd.) was used for irradiation. To determine the relationship between H_2S release and irradiation time, the 980 nm laser light intensity was fixed at 20 W cm^{-2}. At different time intervals aliquots of reaction solution was taken out and the release H_2S was determined by fluorescence method with DNS-Az as probe.[2] The effect of the irradiation intensity of 980 nm laser on H_2S release was carried. The irradiation time was fixed at 2 h, then the reaction solution was taken out and the release H_2S was determined. The final concentration of DNS-Az was 200 μM with 0.5% Tween-20 in PBS (20 mM, pH 7.4).



Scheme S2. Synthetic route for H_2S probe (DNS-Az) and the detection mechanism.

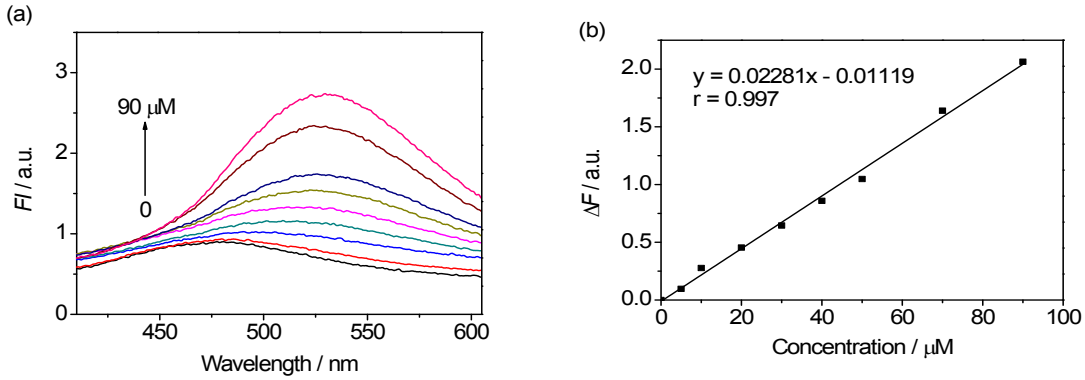


Figure S10. (a) The fluorescence spectrum of DNS-Az changed with H_2S concentration. (b) The standard curve for H_2S based on the fluorescence intensity of DNS-Az at 530 nm.

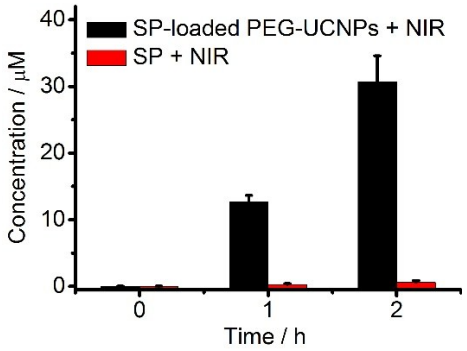


Figure S11. H_2S release from SP or SP-loaded PEG-UCNPs under 980 nm laser irradiation for different time.

1.9 Cell culture

L929 cell lines were cultured in RPMI 1640 medium supplemented with 15% FBS, 1% streptomycin/penicillin. MCF-7 cells were cultured in DMEM medium with 10% FBS, 1% streptomycin/penicillin. Cells were kept at 37 °C in a humidified atmosphere with 5% CO_2.

1.10 MTT assay

The cytotoxicity of SP and SP-loaded PEG-UCNPs to L929 cells and MCF-7 cells was determined by MTT assay. Cells in the logarithm growth period were planted in 96-well plates at a density of 5×10^4 cells per milliliter and incubated in culture medium (100 μL) in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. SP was

first dissolved in DMSO and then diluted with cell culture medium (the final concentration of DMSO < 0.5%). Then the culture medium in each well was replaced with 200 μL of SP or SP-loaded UCNPs solution diluted with culture medium. The final concentrations of SP were 3, 6, 12, 25, 50 and 100 μM, respectively. The final concentrations of SP-loaded PEG-UCNPs were 0.0625, 0.125, 0.25, 0.5, 1 and 2 mg mL^{-1}. To determine the cytotoxicity of NIR light, cells were irradiated under 980 nm laser for 2 h (intensity: 20 W cm^{-2} with 5 min break after irradiation for 20 min). After incubation for another 48 h, the culture medium in each well was removed. MTT solution (0.5 mg mL^{-1}, 100 μL) in culture medium was added into each well and incubated with cells at 37 ^{o}C for 4 h. Subsequently, the supernatant was carefully removed, followed by addition of DMSO (200 μL) to dissolve the blue formazan crystals. Then the absorbance at 490 nm (OD_{490nm}) was measured with 630 nm (OD_{630nm}) as reference on a microplate reader (Bio-Tek ELx800, USA). Cells incubated with medium alone were set as a control experiment. Results were obtained from the mean of five parallel experiments. Cell viability (%) was calculated by the following equation,

$$\text{Cell viability}(\%) = \frac{OD_{490\text{nm/sample}} - OD_{630\text{nm/sample}}}{OD_{490\text{nm/control}} - OD_{630\text{nm/control}}} \times 100 \quad (1)$$

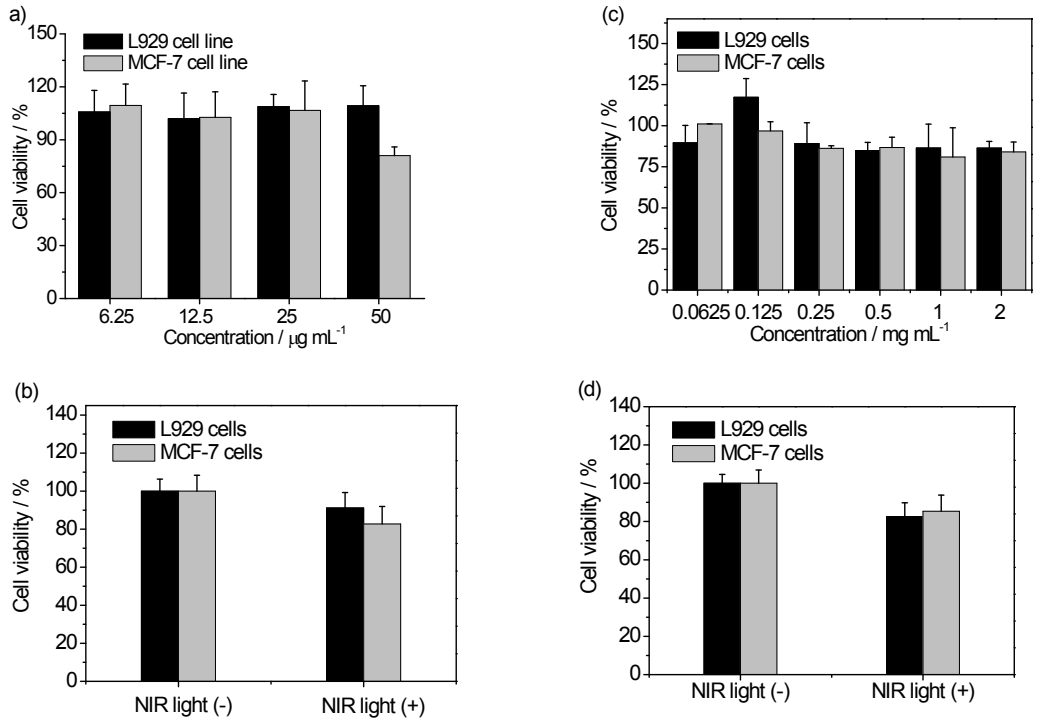


Figure S12. (a) Cytotoxicity of SP against L929 cells and MCF-7 cells at various concentrations. (b) Cell viabilities of L929 cells and MCF-7 cells before and after irradiation with 980 nm laser for 2 h. (c) Cytotoxicity of SP-loaded UCNPs against L929 cells and MCF-7 cells at various concentrations. (d) Cytotoxicity of PEG-UCNPs against L929 cells and MCF-7 cells before and after irradiation with 980 nm laser for 2 h. Error bars mean S.D. (n =3).

1.11 Intracellular H_2S release

The intracellular H_2S release was carried out according to the previously reported procedure.[3] L929 cells in the logarithm growth period were planted in coverglass bottom dishes at a density of 5×10^4 cells per milliliter and incubated in culture medium (500 μL) in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. Then SP-loaded PEG-UCNPs were added with the final concentration at 2 mg mL^{-1} and the cells were cultured for another 8 h. After that the cells were irradiated with 980 nm laser for 2 h (intensity: 20 W cm^{-2}, with 5 min break after irradiation for 20 min). Subsequently, 500 μL of DNS-Az (50 μM) and CTAB (100 μM) in PBS was added and the cells were cultured for another 30 min to detect the intracellular H_2S. Finally

the medium was removed and washed with PBS for twice. Confocal fluorescence imaging of living L929 cells was performed on an Olympus FV-1000 confocal microscope equipped with a multi-Argon laser and a 40 × objective lens. The fluorescence emission images were collected at 505–525 nm. The excitation wavelength of laser was 405 nm. Images were processed with the ImageJ-based open-source Fiji software package (http://fiji.sc/Fiji).

1.12 Ex vivo H_2S release

SP-loaded PEG-UCNPs (2.0 mL) was added into a glass tube with cork. A piece of pork skin with ~ 2 mm of thickness was plated at the surface of the glass tube. Then 980 nm NIR laser or UV (365 nm) light was used for irradiation, and the laser intensity was fixed at 20 W cm^{-2}. After irradiation for 0.5 h or 1 h 100 μL of reaction solution was taken out and the release H_2S was determined by fluorescence method with DNS-Az as probe.

1.13 In vivo Bioluminescent Imaging under NIR light irradiation

Kunming mice experiment was performed under protocols approved by the Ethical Committee of Xiangya Hospital, Central South University. SP-loaded UCNPs (1 mg mL^{-1}) in 0.9% NaCl saline solution were injected subcutaneously into the Kunming mice, followed by bioluminescence scanning using IVIS Lumina and analyzed with Living Image software (Caliper Life Sciences, USA) with the 980 nm laser as the excitation light. The laser power was 2 W cm^{-2} and an 850 nm short-pass emission filter was used.

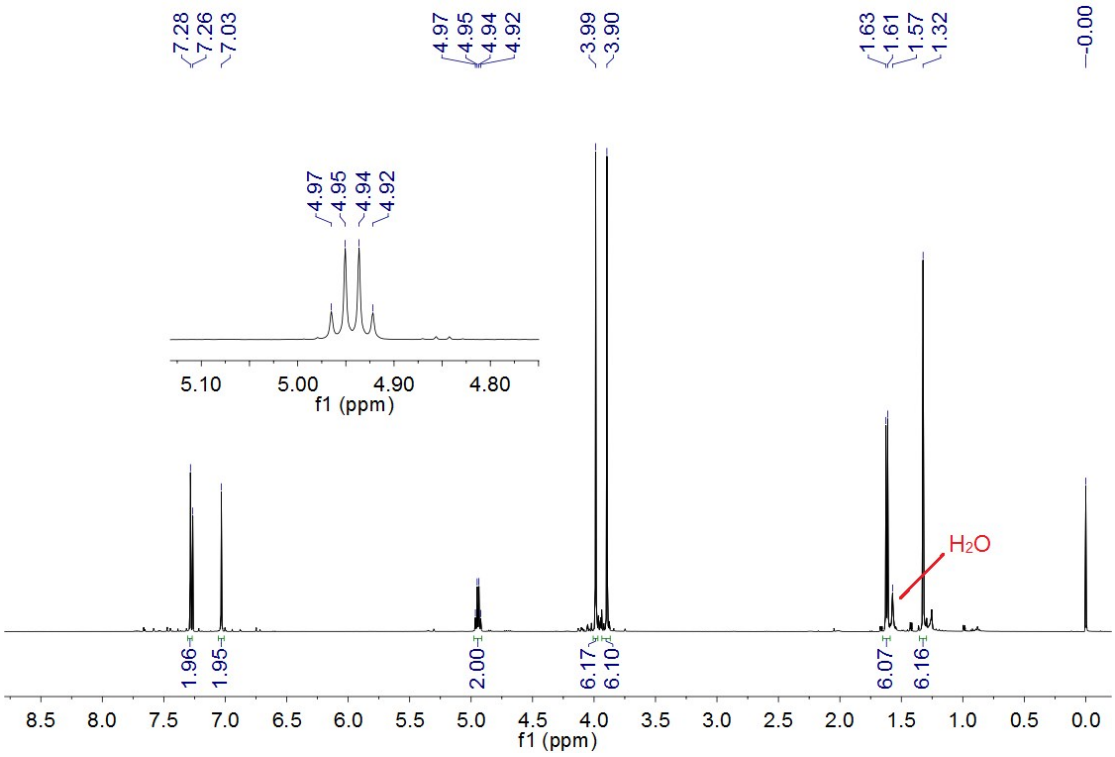


Figure S13. ^{1}H NMR spectrum of SP

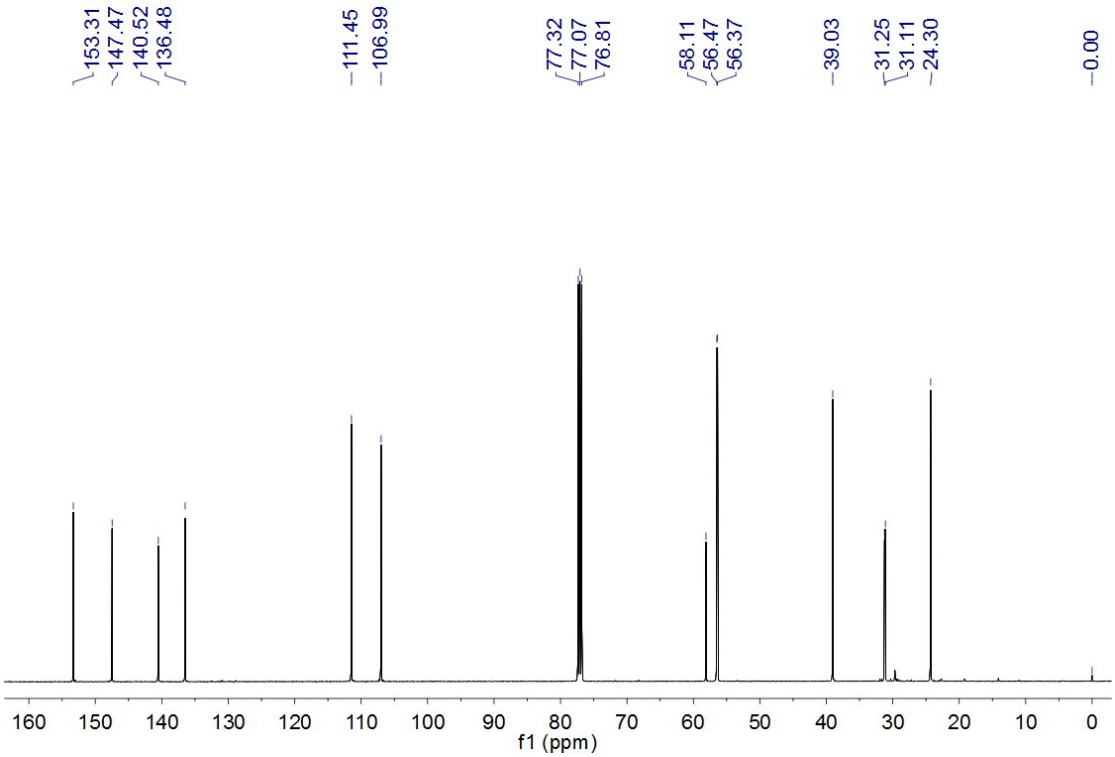


Figure S14. ^{13}C NMR spectrum of SP

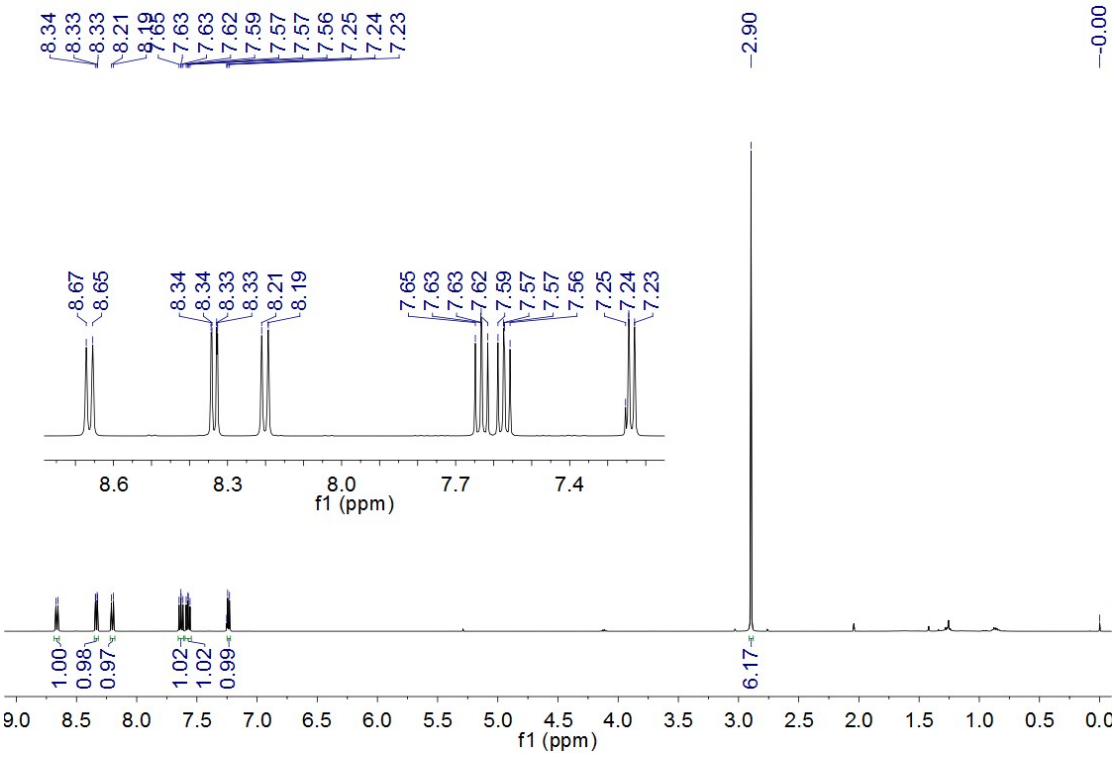


Figure S15. ^{1}H NMR spectrum of DNS-Az

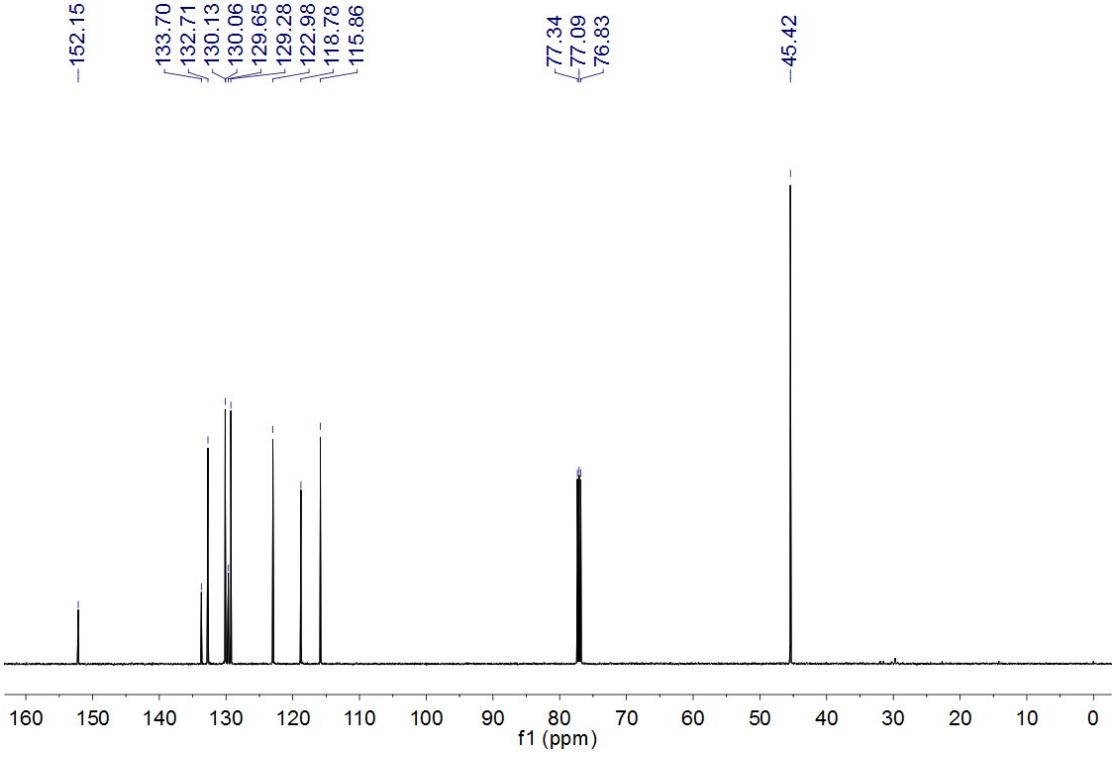


Figure S16. ^{13}C NMR spectrum of DNS-Az

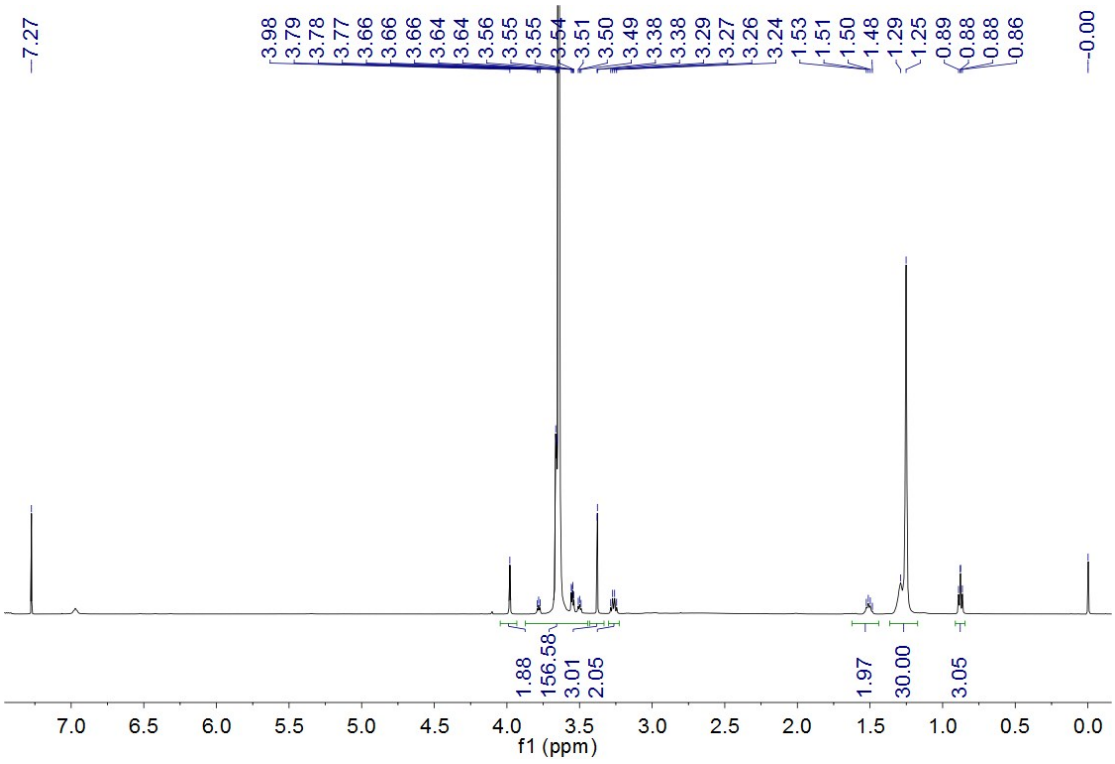


Figure S17. ^{1}H NMR spectrum of PEG-ODA

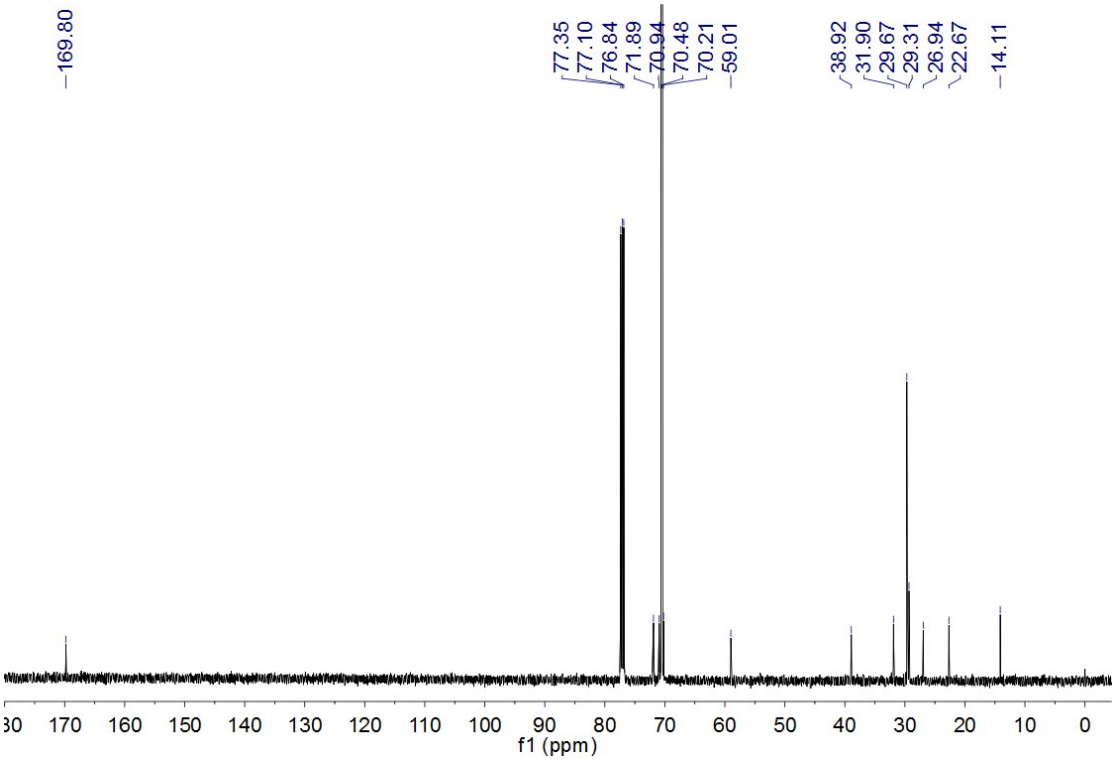


Figure S18. ^{13}C NMR spectrum of PEG-ODA

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