Supporting information

In vivo Pharmacodynamic Evaluation of Antidepressants Based on Flux Mitochondrial Cys in living Mice *via* Near Infrared Fluorescence Imaging

Xin Wang†[a], Jiali Zha†[b], Wei Zhang[a], Wen Zhang*[a], and Bo Tang[a]

[a]College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, People's Republic of China

[b]Moses Brown School, Providence, RI 02906, the United States

Corresponding authors

zhangwen@sdnu.edu.cn

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Experimental Procedures

Materials and reagents

All chemicals were purchased from Aladdin (China) Integle (China), Macklin (China), 9 ding chem. (China) and analytical grade solvents were used without further purification. The stock solution of probe CSS (1 mM) was prepared in DMSO. The stock solution of amino acids (Hcy, GSH, Cystine, $SeCys_2$, H_2S, Ile, Phe, Val, His, Met, Gly, Ser, Lys, Glu, Arg, Pro, Asp, Tyr, Cys), sodium salts (Na_2SO_3), and the stock solution of (Al^{3+}, Mn^{2+}, Na^+, K^+, Cu^{2+}, Fe^{2+}, Mg^{2+}, Fe^{3+}, Ca^{2+}) were prepared by dissolving $AlCl_3$, $MnCl_2$, NaCl, KCl, $CuCl_2$, $FeCl_2$, $MgCl_2$, $FeCl_3$ and $CaCl_2$ in deionized water. We prepared reactive oxygen species (ROS) as follows: Superoxide ($O_2^{\bullet-}$) was generated from KO_2 in DMSO solution, and the concentration of $O_2^{\bullet-}$ was determined by the concentration of KO_2. Lipid peroxyl radicals (ROO•) were generated *via* thermolysis of the 2,2'-azobis(2-methylpropionitrile) (ABIN) in acetonitrile solution at 37 °C for 30 min. Hypochlorite (NaClO) was diluted appropriately in 0.1 M NaOH (aq.) hydrogen peroxide (H_2O_2) was diluted from a 30 % aqueous solution. Hydroxyl radical (•OH) was produced by the reaction of Fe^{2+} with H_2O_2 (1:6). Singlet oxygen ($^1O_2$) was prepared using the ClO^- / H_2O_2 system (1:1).

Instruments

The mass spectra were obtained using the Bruker maXis ultra-high-resolution-TOF MS system.^{1}H NMR spectra were obtained at 400 MHz using Bruker NMR spectrometers, and ^{13}C NMR spectra were recorded at 100 MHz. Absorption spectra were measured on an Evolution 220 UV-vis spectrophotometer (Thermo Scientific Co., Ltd.). All one-photon (OP) fluorescence measurements were carried out at room temperature on an F-4600 fluorescence spectrometer. All of the spectra were acquired in 10 mM Tris buffer (pH 7.4) at λex = 670 nm, λem = 690-750 nm. Absorbance was measured in a microplate reader (RT 6000, Rayto, USA) in the MTT assay. Fluorescence imaging in cells were performed with Leica TCS SP8 Confocal Laser Scanning Microscope. The animal behavioral tests were analyzed by DepressionScan (Clever Sys. Inc.). The fluorescence imaging of mouse was using a Caliper IVIS Lumina Series III or IVIS Spectrum small animal in vivo imager.

Cell culture

PC12 cells were cultured in DMEM supplemented with 10 % fetal bovine serum, 1 % penicillin and 1 % streptomycin at 37 °C (w/v) in an MCO - 15AC incubator (SANYO, Tokyo, Japan) in 5 % CO_2 / 95 % air. One day before imaging, the cells were detached and placed in glass-bottomed dishes.

Confocal imaging experiments

Detection of endogenous Cys in cells: PC12 cells were isolated into petri dishes and cultured for 24 h before imaging. Cells pretreated with DTT (1.0 mM) and NEM (1.0 mM) were treated for 30 min. After incubation with CSS at 37 °C for 2 min, the cell culture medium was removed and the cells were washed with 1.0 mL of PBS (10 mM, pH = 7.4). Images were obtained using a Leica TCS SP8 confocal laser scanning microscope (λex: 633 nm, λem: 700-750 nm), and data analysis was performed using Leica TCS SP8 software.

Mitochondrial colocalization experiments: Probes (10 μM) and commercial dyes Mito-Green (10^{-8} M) for

mitochondrial co-incubation were used to incubate PC12 cells stimulated with DTT (1 mM) using Leica TCS SP8 confocal A laser scanning microscope was used to observe the superimposed imaging of the CSS red channel and Mito-Green green channel. Red channel: λem = 700-750 nm (λex = 633 nm). Green channel: λem = 500-550 nm (λex = 490 nm).

Oxidative stress regulation of drugs in cells: PC12 cells were isolated into petri dishes and cultured for 24 h before imaging. Cells pretreated with 2-Me (1.0 mM) at 37 °C for 30 min, then treated with amitriptyline, imipramine, and doxepin for 30 min, and incubated with CSS for 2 min, then removed the cell culture and wash the cells with 1.0 mL of PBS (10 mM, pH = 7.4). Images were obtained using a Leica TCS SP8 confocal laser scanning microscope (λex: 633 nm, λem: 700-750 nm), and data analysis was performed using Leica TCS SP8 software.

In vivo imaging experiments

At first, the mice were anesthetized by intraperitoneal injection of the anesthetic (10 % chloral hydrate, 200 μL per mouse), then the mice labeled with 0.1 mg kg^{-1} CSS via intraperitoneal injection. After 2 minutes, the mice brain images were acquired using the Caliper IVIS Lumina Series III or IVIS Spectrum small animal in vivo imager (λex = 665 nm and λem = 700-750 nm).

Determination of the limit of detection

The detection limit was determined from the fluorescence titration data. The detection limit was calculated with the following equation: Detection limit=3σ/k, where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus CSS activity.

Cytotoxicity assays

The cytotoxicity was measured by 3-(4, 5 - dimethylthiazol - 2 - yl) - 2, 5-diphenyltetrazolium bromide (MTT) assay. PC12 cells were seeded in a 96-well plate at a concentration of 1×10^5 cells $well^{-1}$ in 100 μL of Roswell Park Memorial Institute DMEM medium with 10 % fetal bovine serum, 1 % penicillin, and 1 % streptomycin and maintained at 37 °C in a 5 % CO_2 incubator for 12 h. Then, cells were exposed to different concentrations of TCS (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7} and 1×10^{-8} M) for 24 h. The total volume of 96-well microtiter plates is 200 μL $well^{-1}$. The cells were washed with 37 °C PBS and MTT solution (5 mg mL^{-1}, 20 μL) was added to each well and continuously incubated for 4 h at 37 °C. After 4 h, MTT solution was removed and DMSO (150 μL) was added to each well to dissolve the dark blue formazan crystals. Absorbance was measured at 490 nm in a Triturus microplate reader.

Mouse models with depression-like behaviours

Adult male C57 mice (age: 6 weeks; average body weight: 18 ± 2 g) were purchased from the Experimental Animal Center of Shandong University (Jinan, PR China). All animal care and experimental protocols complied with the Animal Management Rules of the Ministry of Health of the People's Republic of China and were approved by the Animal Care Committee of Shandong Normal University. Next, we constructed corticosterone-

induced depression model of C57 mice as described in reference. After 4 weeks, behavioral analysis experiments were performed to test the success of the depression model.

Sucrose preference test

Sucrose preference test (SPT) was conducted using a two-bottle choice procedure before and after model mice before and after treatment with antidepressant drugs. Before SPT, mice were habituated to drink water for 12 h with two bottles. At the start of the test, mice were given access to the two bottles, one filled with 20 mL sucrose solution and the other with 20 mL water. The position of the water and sucrose bottles (left or right) was switched every 12 h for two days. Then the mice were left undisturbed, and their overnight fluid consumption was measured after 12 h. The volume of sucrose or water of every bottle was recorded. The sucrose preference was defined as the ratio of the volume of sucrose to the total volume of sucrose and water consumed. The mice with obvious reduction in preference for sucrose were considered as the successful.

Forced swimming test

Forced swimming test (FST) were carried out following references. In the forced swimming test, each mouse was placed in a cylindrical tank (24 cm height × 10 cm diameter) filled to 6 cm with water at a temperature of 24 ± 1 °C. The mice could swim freely. The mice were subjected to 6 min of swimming, but only the last four minutes were considered in the analysis. Longer immobility time in FST are considered as an index of depression.

Tail suspension test

In the tail suspension test (TST), each mouse was suspended by the tail using adhesive scotch tape from a hook connected to a strain gauge that detected all the movements of the mouse and transmitted them to a central unit, which calculated the total duration of immobility during a 6 min test. However, only the last four minutes were considered in the analysis. Longer immobility time in TST are considered as an index of depression.

Statistical analysis

All data are expressed as the mean ± S.D. The data under each condition were accumulated from at least three independent experiments. For each experiment, unless otherwise noted, n represents the number of individual biological replicates. For each biological replicate and for all *in vitro* and *ex vivo* studies, $n \geq 3$.

Data availability

All relevant data that support the findings of this study are available from the corresponding author upon reasonable request.

Results and Discussion

HRMS (ESI) m/z calcd. for $C_{34}H_{33}N_2O_2S$ $[M+H]^+$ calculated 533.2250 found 533.2263. NMR data: ^{1}HNMR (400 MHz, d_6-DMSO): 7.154 (m, 6H), 6.743 (m, 4H), 6.69 (s, 2H), 6.61 (d, 1H), 6.57 (d, 1H), 5.77(s, 1H), 5.39(d, 1H), 4.04 (s, 1H), 1.77 (t, 4H), 1.39 (m, 4H), 1.23 (s, 6H),): δ 0.85 (t, 3H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 152.24,

150.70, 145.43， 143.19, 142.62, 141.44, 132.99, 130.09, 129.78, 127.64, 126.34, 123.46, 122.61, 119.17, 117.34, 115.69, 114.39, 113.63, 104.93, 50.90, 31.81, 29.47, 27.67, 27.01， 24.01, 13.12.

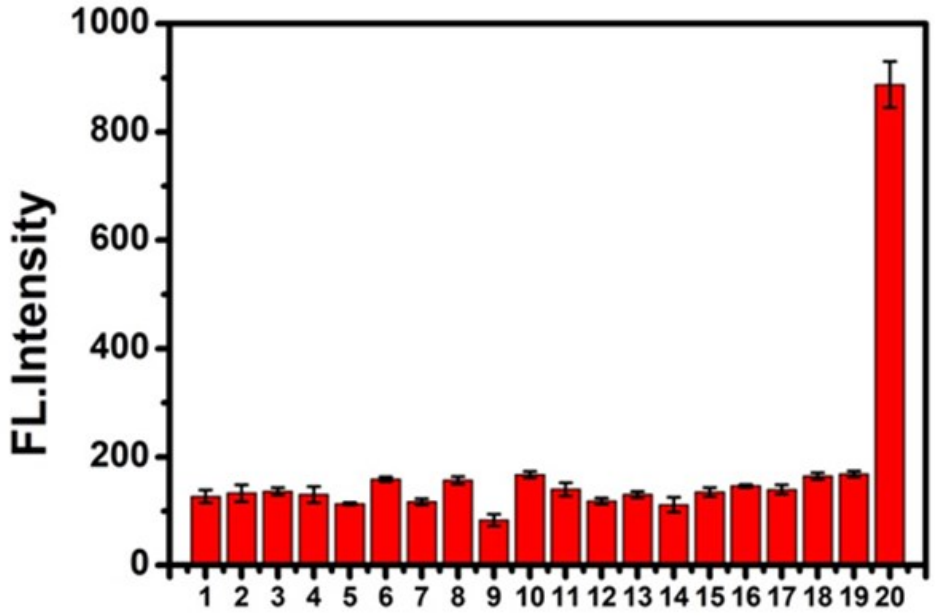

Figure S1 Flourescence changes of CSS (1) (10 μM) at 710 nm after incubation with 100 μM ROS (2. $O_2^{\cdot-}$; 3. ROO·; 4. ClO^-; 5.SO_3^{2-} 6. ·OH; 7. H_2O_2; 8. $^1O_2$), 100 μM RNS (9. $ONOO^-$;10. NO), 100 μM metal ions (11. Al^{3+}; 12. Mn^{2+}; 13. Na^+; 14. K^+; 15. Cu^{2+}; 16. Fe^{2+}; 17. Mg^{2+}; 18. Fe^{3+}; 19. Ca^{2+};) and Cys (20). (Tris buffer, 0.01 M, pH 7.4)

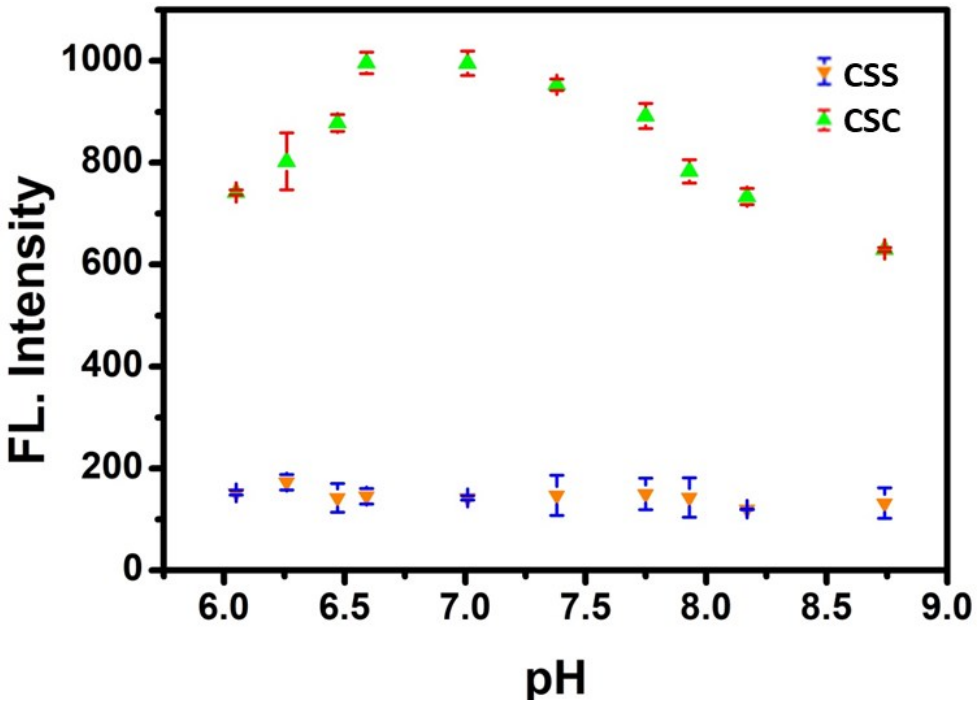

Figure S2 The fluorescence intensity of CSS and CSC at different pH (6.0 -8.7).

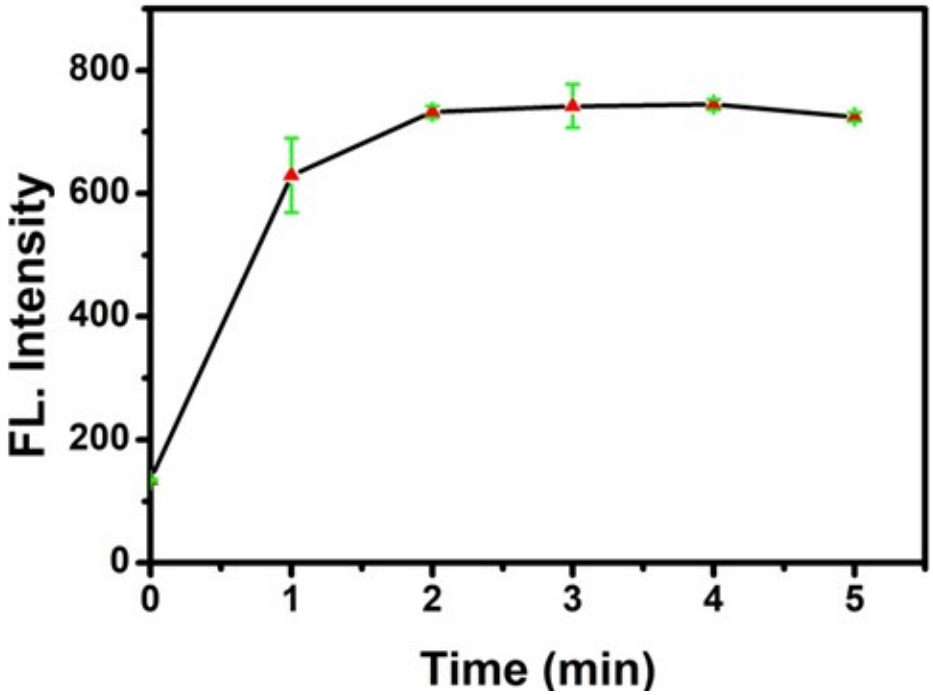

Figure S3 Time-dependent fluorescence spectra of CSS (10 μM) in the presence of Cys (50 μM) in 10 mM Tris buffer.

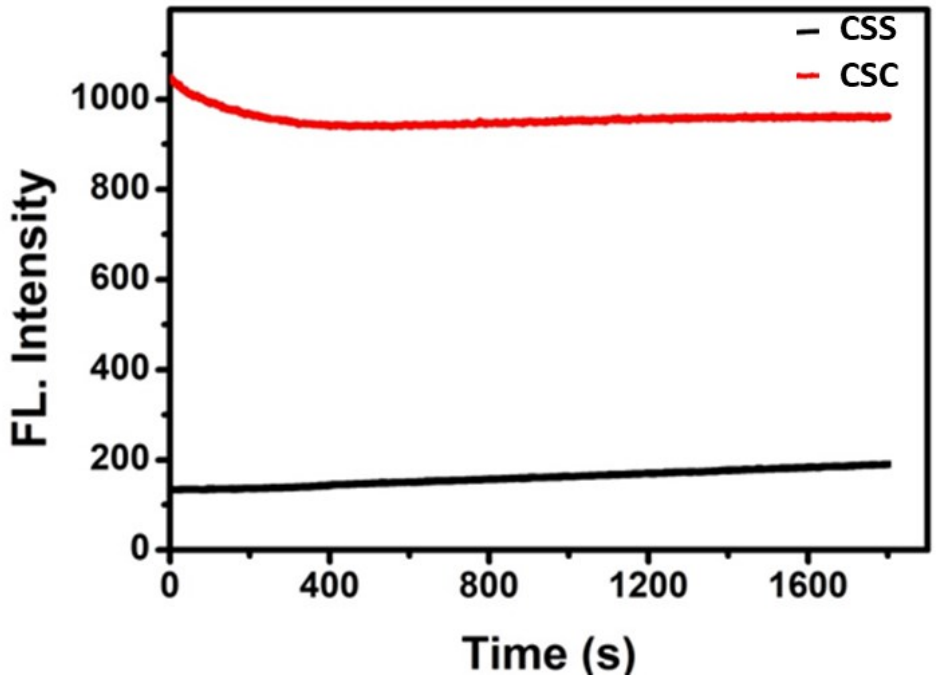

Figure S4 Photostability of CSS and CSC. Fluorescence intensity of CSS (red) and CSC (black) from 0 to 30 min of time-sequential scanning.

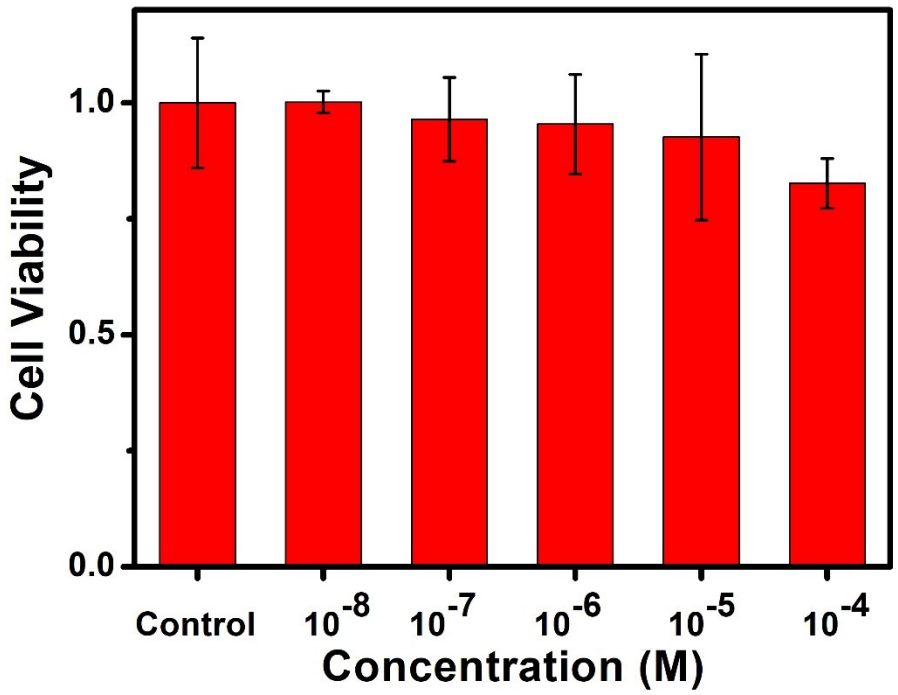

Figure S5 Cell viability (%) estimated by standard MTT assay. PC12 cells were incubated with 0-100 μM CSS at 37 °C for 24 h.

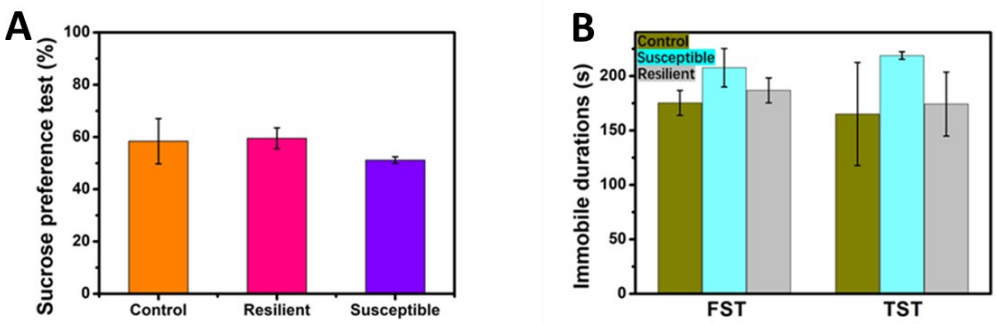

Figure S6 Depression behaviors tests. A： Sucrose preference of the control mice (orange bar), the resilient mice (rose red bar) and the susceptible mice (purple bar). B： Immobile time of forced swimming test and tail suspension test.

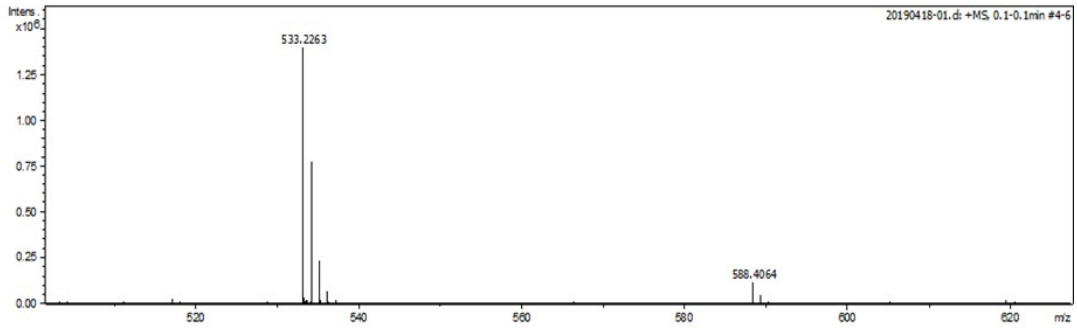

Figure S7. Mass spectrum of CSS.

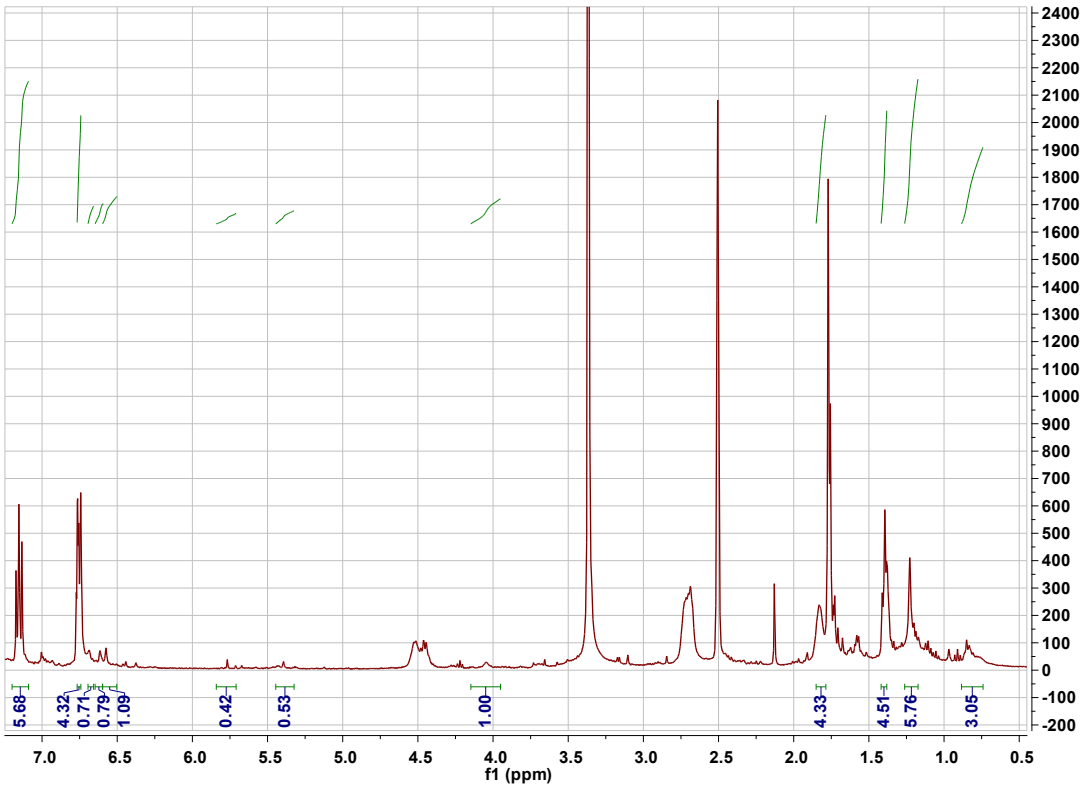

Figure S8 [1]HNMR of CSS

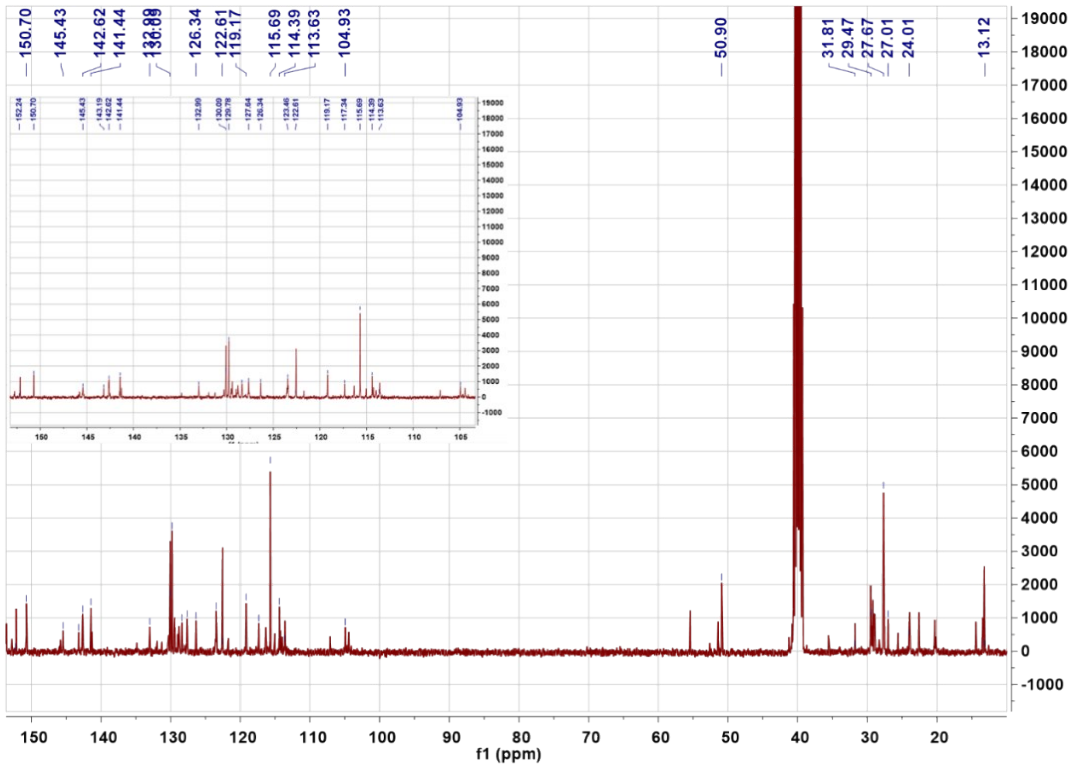

Figure S9 ^{13}CNMR of CSS

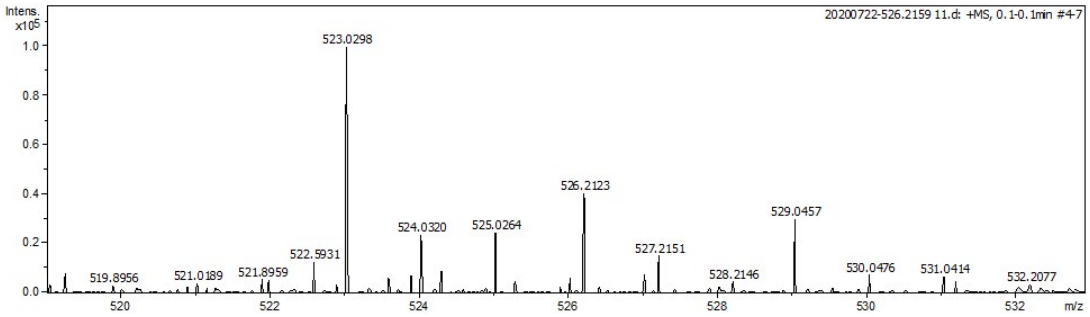

Figure S10 Mass spectrum of CSC. CSC was obtained by CSS reaction with excess Cys. HRMS (ESI) m/z calcd. for $C_{31}H_{32}N_3O_3S^+$ $[M]^+$ calculated 526.2159, found 526.2123.