Supplementary Information (SI) for ChemComm.
This journal is © The Royal Society of Chemistry 2024

Supporting Information

GSH and Viscosity Double-Locked Response Fluorescent Probe for Imaging and Surgical Navigation of Hepatocellular Carcinoma

Wen Zhang,[a] Min Zhang,[a] Mengmei Li[a], Xin Wang,[a] Ping Li,*[a,c] and Bo Tang*[a,b]

a. College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Institutes of Biomedical Sciences, Shandong Normal University, Jinan 250014, Shandong, People's Republic of China
b. Laoshan Laboratory, Qingdao 266237, Shandong, People's Republic of China
c. College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070, People's Republic China

Email: lip@sdnu.edu.cn, tangb@sdnu.edu.cn

Table of Contents

Experimental Procedures S3
Materials and instruments S3
Synthesis of PG-V S3
Cells culture S4
Cytotoxicity assays S4
Cells imaging S5
Establishment of tumour-bearing mice model S5
Fluorescence imaging experiments of mice S5
Organs and tumour tissue imaging S5
Statistical analysis S5
Supplemental Figures S6
Figure S1 Absorption spectrum of the PG-V S6
Figure S2 Fluorescence response of PG-V to GSH S6
Figure S3 Linear relationship between log F_{583} and log η S7
Figure S4 Fluorescence responses of GSH-activated PG-V to polarity S7
Figure S5 Fluorescence response of PG-V at different pH values S8
Figure S6 Cell toxicity of PG-V S8
Figure S7 Effect of PG-V on body weight of Kunming mice S9
Figure S8 HRMS of compound 1. .... S9
Figure S9 HRMS of the PG-OH S9
Figure S10 HRMS of PG-V. S10
Figure S11 ^{1}HNMR of compound 1 .... S10
Figure S12 ^{13}CNMR of compound 1 .... S11
Figure S13 ^{1}HNMR of PG-OH S11
Figure S14 ^{13}CNMR of PG-OH S12
Figure S15 ^{1}HNMR of PG-V S12
Figure S16 ^{13}CNMR of PG-V S13
References S13

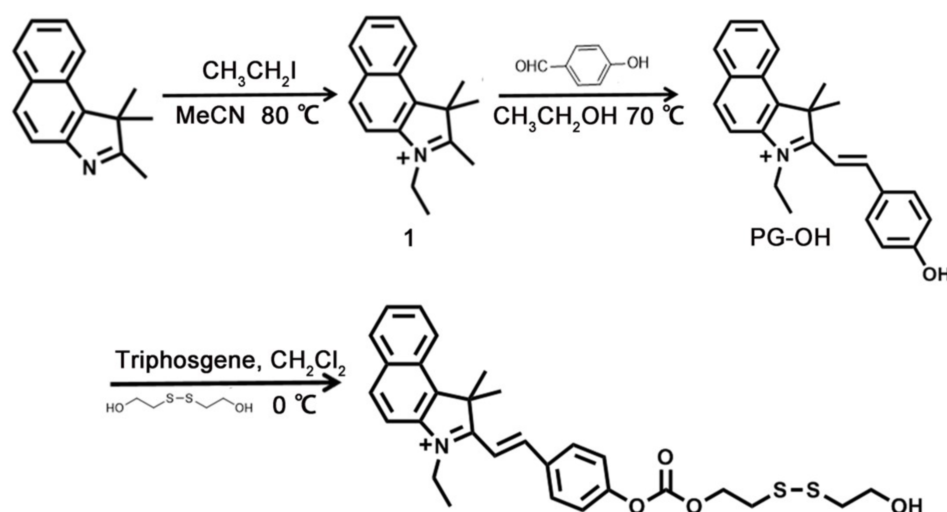
Experimental Procedures

Materials and instruments

All reagents were analytically pure, and common reagents and solvents such as methanol, dichloromethane, triethylamine, and ethanol were purchased from Sinopharm Chemical Reagent Co. 1,1,2-Trimethyl-1H-benzo[e]indole, bis(2-hydroxyethyl)disulfide, p-hydroxybenzaldehyde, and triphosgene were obtained from Shanghai Aladdin Biochemical Science and Technology Co. Ltd. The ethyl iodide was from Shanghai McLean Biochemical Science and Technology Co. Ltd. The dexamethasone and Cell Counting Kit-8 (CCK-8) reagent were obtained from MedChemExpress. ROS were prepared according to methods adapted from previous reports.[1,2] $O_2^{\bullet -}$ was produced from KO_2 in DMSO solution by an ultrasonic method. Hydrogen peroxide (H_2O_2), and hypochlorite (NaClO) were acquired from 30 %, 70 %, and 10 % aqueous solutions, respectively. The hydroxyl radical (•OH) was generated by the reaction of 1.0 mM $FeCl_2$ with a 200 μM H_2O_2 aqueous solution. Nitric oxide (NO) was obtained from a stock solution prepared with sodium nitroprusside. Singlet oxygen ($^1O_2$) was prepared with the NaClO-H_2O_2 system.

The viscosity value was recorded by an NDJ-8S rotational viscometer. Absorption spectra were recorded on a UV-visible spectrophotometer (Evolution 220, Thermo Scientific). Fluorescence spectra were obtained with an FLS-1000-SS-STM spectrometer (Edinburgh Instruments Ltd., England). Confocal imaging was performed on a Leica SP8 high-resolution fluorescence microscope. ^{1}H NMR spectra were obtained at 400 MHz using Bruker NMR spectrometers, and ^{13}C NMR spectra were recorded at 100 MHz. The mass spectra were obtained using the Bruker Maxis ultra-high-resolution-TOF MS system. The images of isolated tissues and mice were carried on the IVIS Lumina II in vivo imaging system.

Synthesis of PG-V



Scheme S1. The syntheses of PG-V.

Synthesis of Compound 1. Compound 1 was prepared according to a reported method.[3] HRMS data, m/z calculated for [$C_{17}H_{20}N^+$], 238.1591, found 238.1646. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.38 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 8.9 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 8.9 Hz, 1H), 7.79 (dd, J = 11.2, 4.0 Hz, 1H), 7.73 (t, J = 7.2 Hz, 1H), 4.64 (q, J = 7.3 Hz, 2H), 2.96 (s, 3H), 1.77 (s, 6H), 1.51 (t, J = 7.3 Hz, 3H). ^{13}CNMR (100 MHz, DMSO-d_6): δ 196.43, 138.68, 137.52, 133.52, 131.20, 130.20, 128.89, 127.74, 123.89, 113.68, 55.94, 43.86, 21.97, 14.23, 13.39.

Synthesis of PG-OH. Under the protection of N_2, p-hydroxybenzaldehyde (0.15 g, 1.2 mmol) and compound 1 (0.24 g, 1.0 mmol) were dissolved in 20 mL anhydrous ethanol, 5 μL piperidine and 5 μL acetic acid with stirring at 70 ℃ for 8 h. Then, the mixed solution was evaporated by reduced pressure distillation, and column chromatography was used to refine the PG-OH with CH_3OH/CH_2Cl_2 (v/v, 1:30) as the eluting agent. HRMS data, m/z calculated for [$C_{24}H_{24}NO^+$], 342.1853, found 342.1895. ^{1}HNMR (400 MHz, DMSO-d_6): δ 8.38 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 8.9 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 8.9 Hz, 1H), 7.79 (dd, J = 11.2, 4.0 Hz, 1H), 7.73 (t, J = 7.2 Hz, 1H), 4.64 (q, J = 7.3 Hz, 2H), 2.96 (s, 3H), 1.77 (s, 6H), 1.51 (t, J = 7.3 Hz, 3H). ^{13}CNMR (100 MHz, DMSO-d_6): δ 181.97, 164.18, 163.78, 153.80, 138.63, 138.29, 134.26, 133.42, 132.57, 131.45, 130.50, 128.84, 127.34, 126.38, 123.44, 117.07, 116.31, 113.42, 108.36, 53.86, 42.35, 26.25, 14.24.

Synthesis of PG-V. Anhydrous dichloromethane solution of the PG-OH (0.341 g, 1.0 mmol) and triphosgene (0.5935 g, 2.0 mmol) were stirred at 0 ℃. Then, anhydrous pyridine (400 μL, 5 equiv) was added to the above mixture and stirred under nitrogen for 2 h to remove excess phosgene. Bis-(2-hydroxyethyl) disulfide (500 μL, 4.0 mmol) and triethylamine (1.5 mL) were added to the reaction solution. The solution was stirred for 12 hours after it changed from yellow-green to orange. The prepared solution was extracted with water and dichloromethane. The organic phase was dried anhydrous with sodium sulfate, filtered, and steamed under pressure. The residue was purified by thin layer chromatography (CH_2Cl_2/MeOH = 8:1) to obtain a light yellow solid (yield 8%). HRMS data, m/z calculated for [$C_{29}H_{32}NO_4S_2^+$], 522.1768, found 522.1847. ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.35 – 8.29 (m, 1H), 8.18 (dd, J = 34.6, 8.6 Hz, 1H), 7.80 – 7.66 (m, 1H), 7.38 (d, J = 7.8 Hz, 1H), 5.14 (d, J = 6.5 Hz, 1H), 4.52 (t, J = 6.4 Hz, 1H), 3.89 (t, J = 5.9 Hz, 3H), 3.03 (t, J = 6.5 Hz, 1H), 2.88 (dd, J = 11.1, 5.3 Hz, 4H), 2.10 (s, 3H), 1.67 (s, 1H), 1.25 (s, 10H), 0.89 – 0.81 (m, 4H). ^{13}CNMR (100 MHz, DMSO-d_6): δ 172.93, 169.70, 167.44, 132.12, 130.10, 129.12, 122.57, 113.73, 73.34, 67.87, 64.96, 64.23, 59.87, 54.50, 43.51, 38.56, 31.75, 30.54, 30.27, 28.83, 27.02, 25.78, 23.72, 22.86, 19.02, 14.34, 13.98, 11.26.

Cells culture

Hepatic cell line (HL-7702 cells), hepatocellular carcinoma cell line (SMMC-7721) cells, and Hepa 1-6 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HL-7702 cells and SMMC-7721 cells were cultured in high-glucose DMEM supplemented with 10 % fetal bovine serum, 1 % penicillin, and 1 % streptomycin (w v^{-1}) at 37 °C in a 5 % CO_2/95 % air MCO-15AC incubator (SANYO, Tokyo, Japan).

Cytotoxicity assays

The toxicity of PG-V on HL-7702 cells was tested using CCK-8 assay. First, after the cells were divided into 96-well plates, different concentrations of PG-V (0 M, 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, and 1×10^{-9} M) were added to the well plates and incubated with the cells for 24 h. Then, the CCK-8 reagent (10 μL $well^{-1}$) was added. After 2 h, the absorbance of the solution at 450 nm was measured by using a Triturus microplate reader.

Cells imaging

Cells were seeded on 15 mm glass-bottom dishes and cultured for 24 h. The living cells were treated with 20 μM PG-V and different agents for 30 min. Then, the cells were washed three times with 1.0 mL PBS before imaging. All the fluorescence images were obtained on a Leica SP8 high-resolution fluorescence microscope. The excitation wavelength of the PG-V was 514 nm, and the corresponding green channel of 530-650 nm emission wavelength was collected. For the co-localization experiment, the excitation wavelength of PG-V was 514nm, and the collection range was 530-570nm. The imaging parameters for other commercial dyes were as follows: LysoTracker Red, 0.5 μM, Ex = 633 nm, collected from 650−800 nm; MitoTracker Red, 0.5 μM, Ex = 633 nm, collected from 650−800 nm; ER-Tracker Red, 0.5 μM, Ex = 633 nm, collected from 650−800 nm; Golgi-Tracker Red, 0.5 μM, Ex = 633 nm, collected from 650−800 nm. Each experiment was repeated at least three separate times with identical results.

Establishment of tumour-bearing mice model

All 4-week-old Kunming mice were housed in the microbarrier unit (HH-MMB-I) to adapt to the environment for one week. A mouse model of HCC was established by subcutaneous injection of Hepa 1-6 cells at a density of 1×10^7 cells per mouse. Two weeks later, tumor-bearing mice were used for imaging experiments.

Fluorescence imaging experiments of mice

PG-V (100 μM, 50 μL) was intravenously injected into the tumor-bearing or normal mice through the tail vein. All mice were transferred to IVIS Lumina II in vivo imaging system equipped with 520 nm and 570 ± 10 nm filter for in vivo imaging and image-guided lesions resection. The mice were anesthetized prior to injection and during imaging.

Organs and tumour tissue imaging

After broken neck death, a laparotomy was performed on mice to remove organ tissues and tumors and then washed with PBS three times. Images were gathered using IVIS Lumina II in vivo imaging system with an excitation of 520 nm and an open filter (570 ± 10 nm).

All the animal experiments were carried out under the relevant laws and guidelines issued by the Ethical Committee of Shandong Normal University (No. AEECSDNU2024097). After experiments, the mice carcasses were sealed and stored in a special refrigerator at -20 °C. Then, the carcasses were deeply buried after burning by Yunshui Tengyue Environmental Technology Co., Ltd. (Jinan, China).

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, $n \geq 3$. The Student's t-test was used for comparisons between two groups of experiments. Statistically significant P values are indicated

in Figures and legends as ***P < 0.001, **P < 0.01.

Supplemental Figures

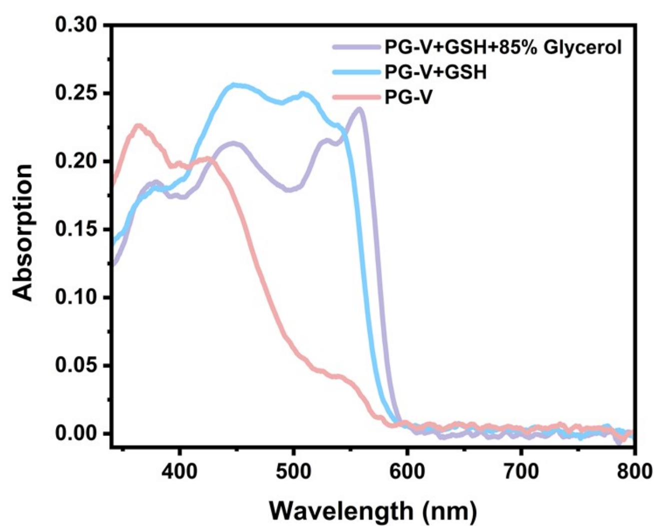


Figure S1. The absorption spectrum of 10 μM PG-V to different environments, including 10 mM PBS buffer, 200 μM GSH, and coexisting 200 μM GSH and 85% glycerol.

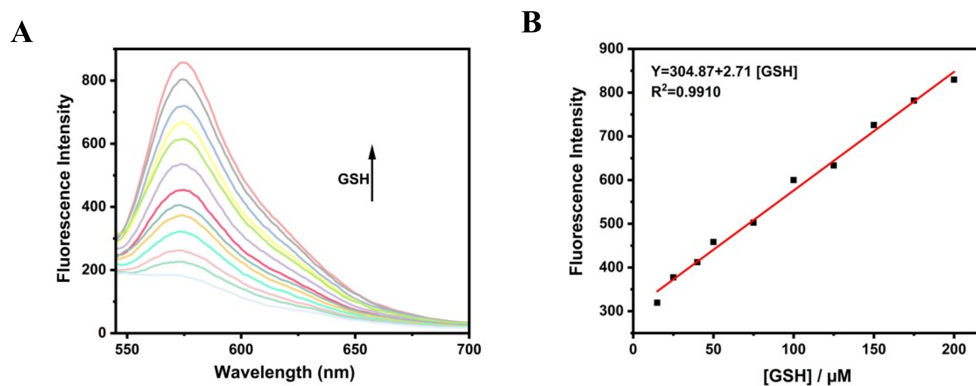


Figure S2. (A) Fluorescence response of PG-V (10 μM) in the presence of GSH at various concentrations. (B) The linear relationship between fluorescence intensity and GSH concentrations (0-200 μM). λex/em=520/583 nm.

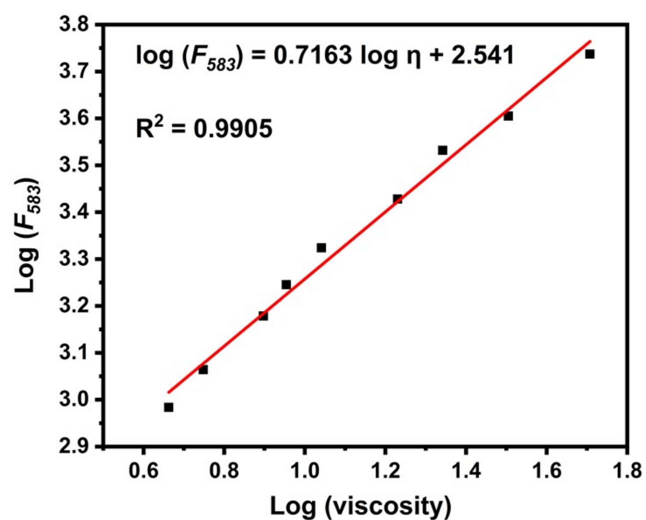


Figure S3. Linear relationship between log F_{583} and log η. $\lambda_{ex/em}$=520/583 nm.

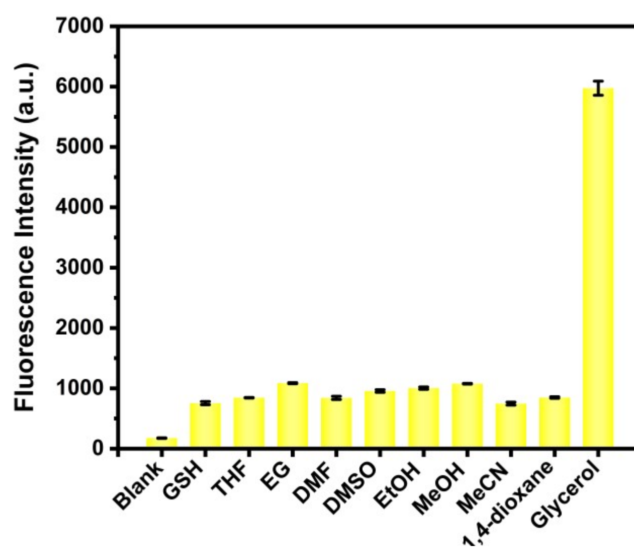


Figure S4. Fluorescence responses of PG-V (10 μM) in different polar solvents after GSH activation (Blank, 200 μM GSH, THF, Glycol, DMF, DMSO, Ethyl alcohol, Methyl alcohol, 1,4-dioxane, 85 % glycerol). $\lambda_{ex/em}$=520/583 nm.

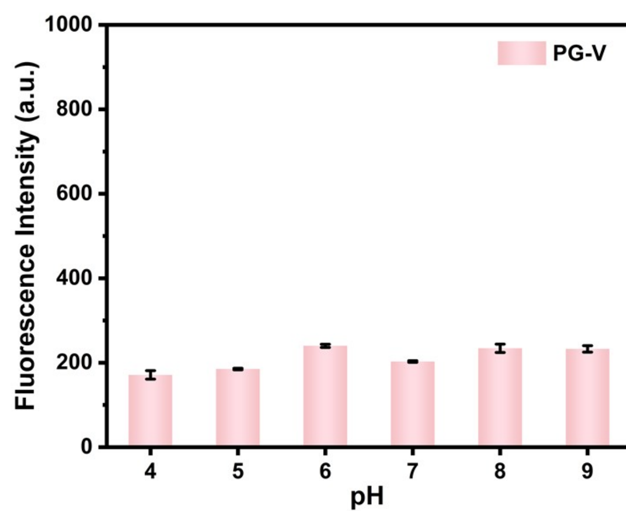


Figure S5. Fluorescence response of PG-V (10 μM) at different pH values. $\lambda_{ex/em}$=520/583 nm.

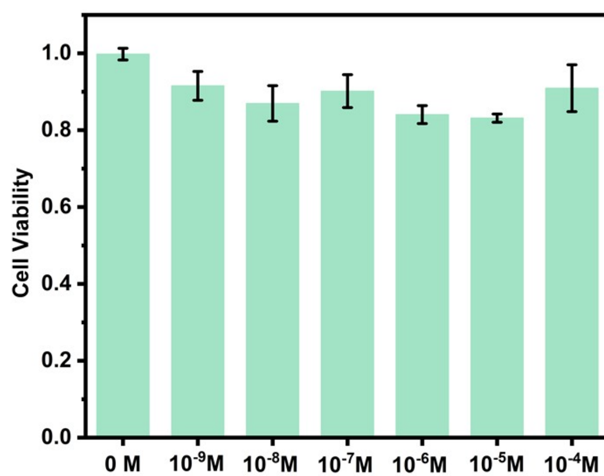


Figure S6. Cell toxicity of PG-V towards HL-7702 cells with an incubation time of 24 h.

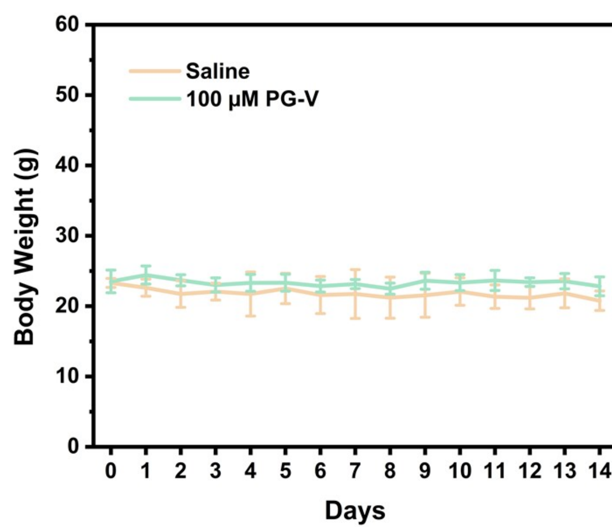


Figure S7. Effect of PG-V (100 μM) on body weight of Kunming mice over two weeks.

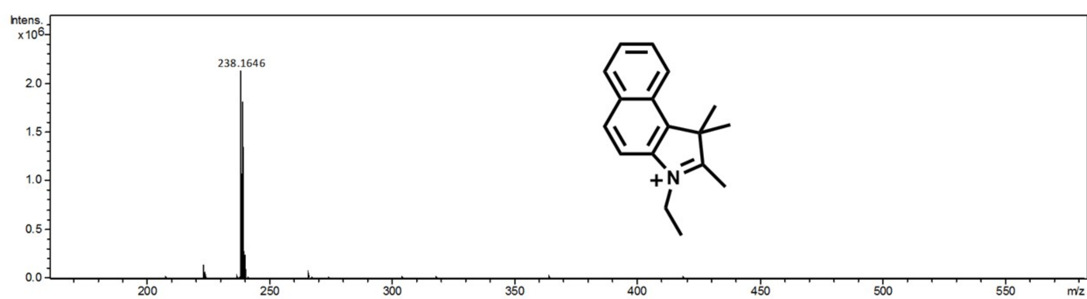


Figure S8. HRMS of compound 1.

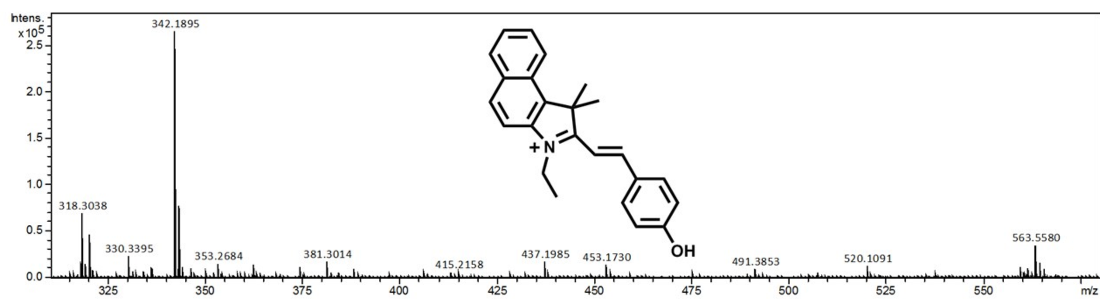


Figure S9. HRMS of the PG-OH.

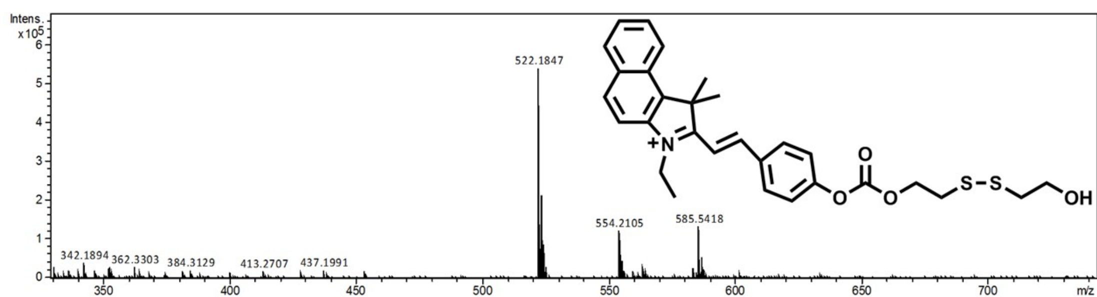


Figure S10. HRMS of PG-V.

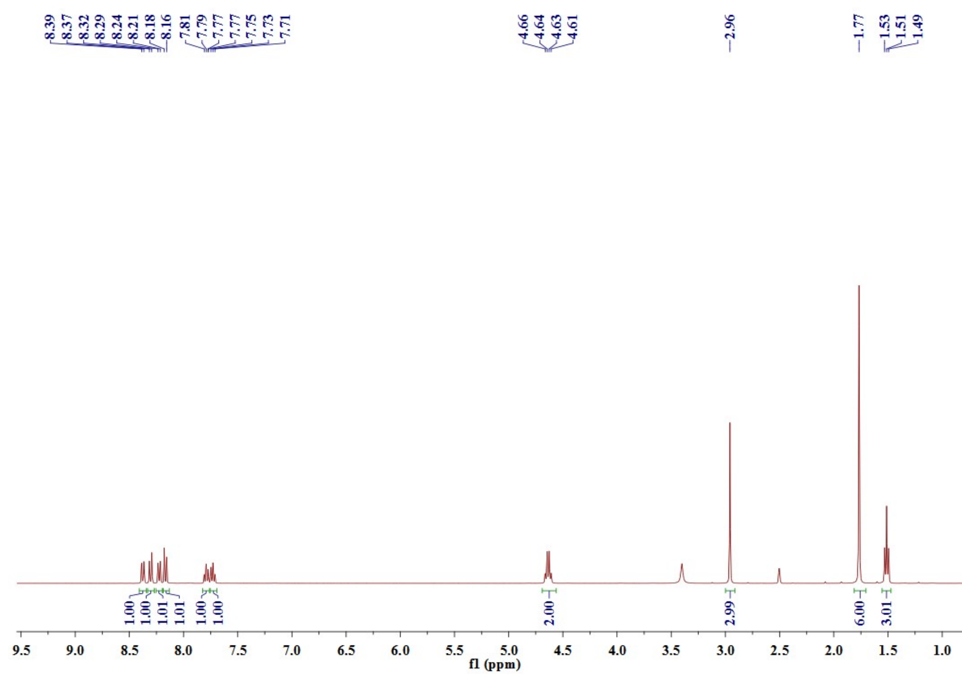


Figure S11. ^{1}HNMR (400 MHz, DMSO-d_6) of compound 1.

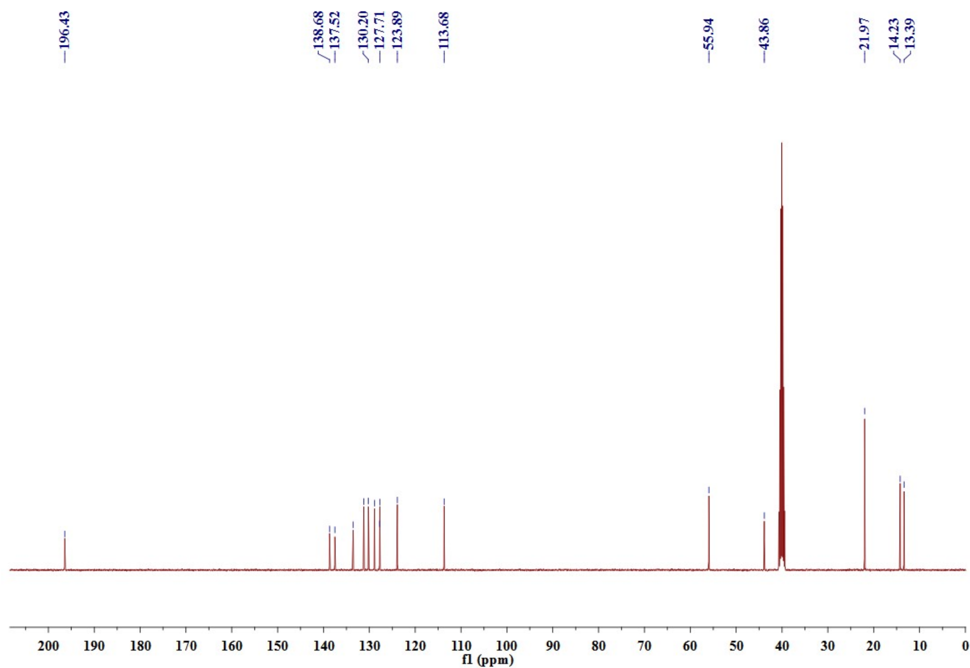


Figure S12. ^{13}CNMR (100 MHz, DMSO-d_6) of compound 1.

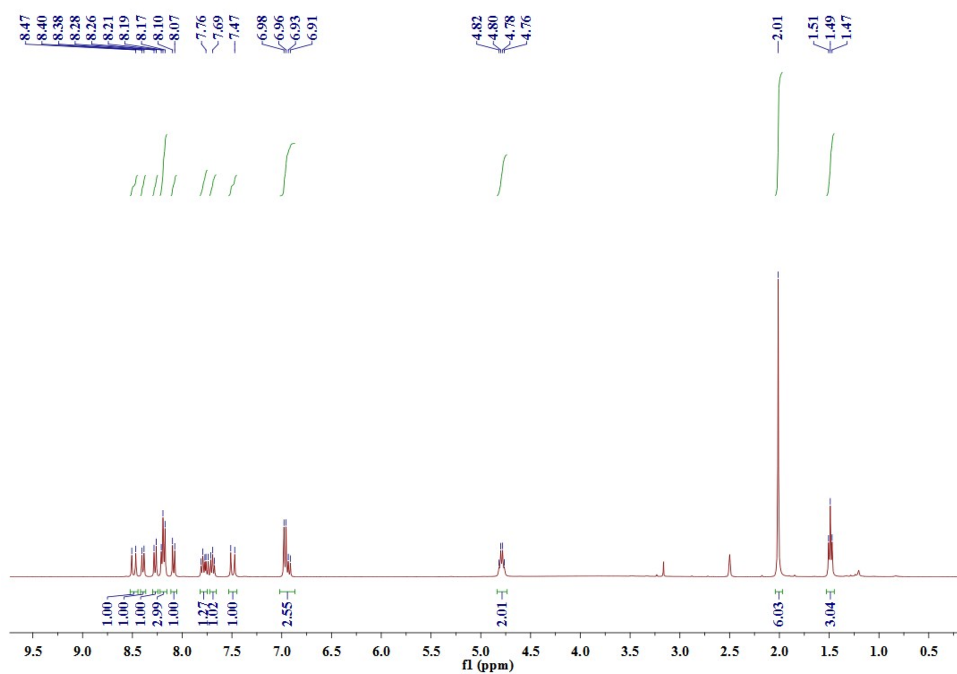


Figure S13. ^{1}HNMR (400 MHz, DMSO-d_6) of PG-OH.

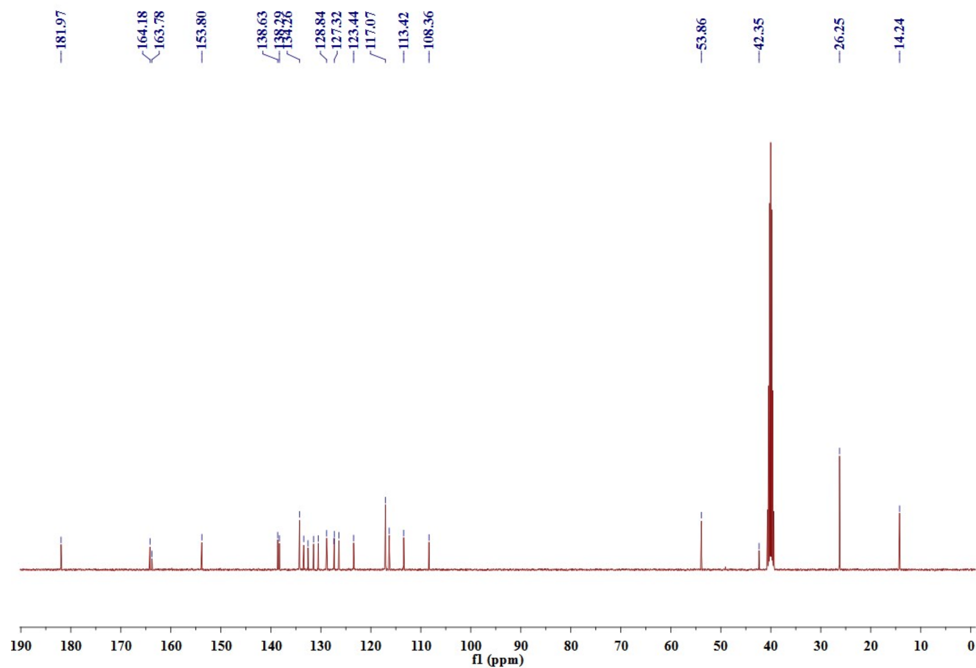


Figure S14. ^{13}CNMR (100 MHz, DMSO-d_6) of PG-OH.

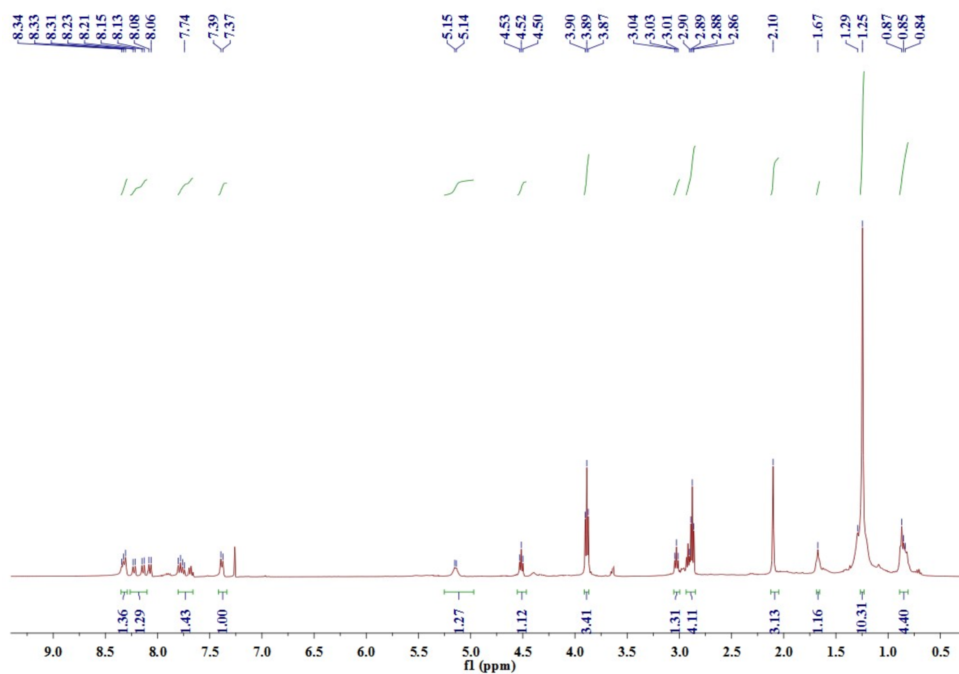


Figure S15. ^{1}HNMR (400 MHz, $CDCl_3$) of PG-V.

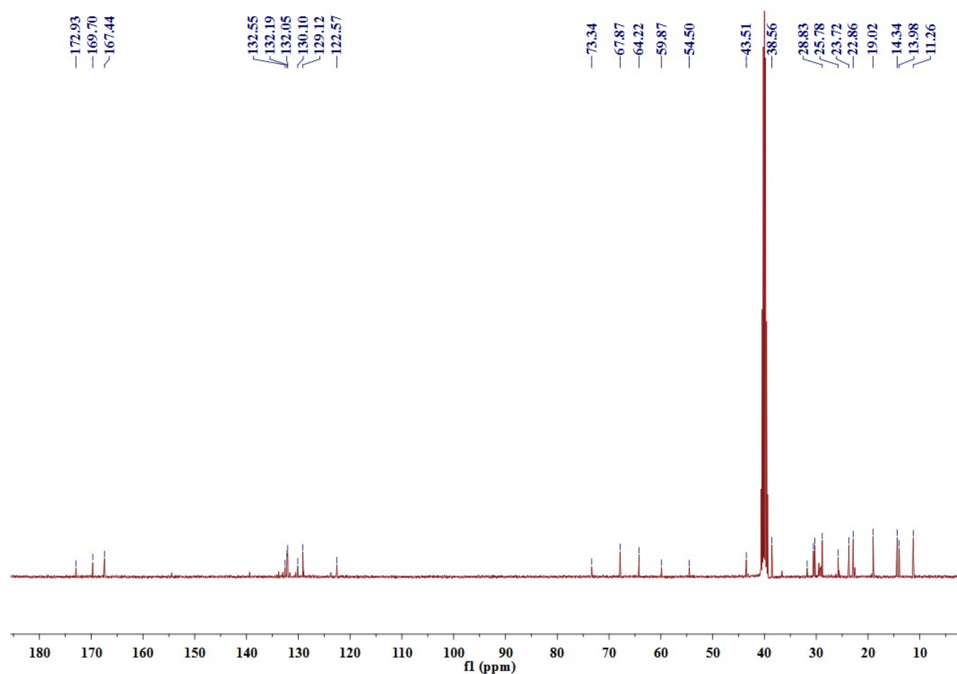


Figure S16. ^{13}CNMR (100 MHz, DMSO-d_6) of PG-V.

References

1 H. Maeda, K. Yamamoto, Y. Nomura, I. Kohno, L. Hafsi, N. Ueda, S. Yoshida, M. Fukuda, Y. Fukuyasu, Y. Yamauchi. *J. Am. Chem. Soc.*, 2005, **127**, 68-69.

2 D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano, T. Nagano. *J. Am. Chem. Soc.*, 2010, **132**, 2795-2801.

3 B. C. Chen, C. D. Li, J. Zhang, J. F. Kan, T. T. Jiang, J. Zhou and H. M. Ma, *Chem. Commun.,* 2019, **55**, 7410-7413.