Synthesis and biological studies of the thiols-triggered anticancer prodrug for more effective cancer therapy

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General information: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury Plus-400 (400 MHz) spectrometer with TMS as the internal standard. Chemical shifts were reported as δ values relative to the internal standard. HRMS-ESI spectra were determined on a Bruker Daltonics APEXII 47e spectrometer.

HPLC analysis

HPLC analysis were performed on Waters 1525-2998 series HPLC system (C18 column, Sun Fire, 5 μm, 4.6 mm×150 mm, UV wavelength, maximal absorbance at 274 nm; temperature, ambient; injection volume, 10 μL). To separate adducts, solvents A (H_2O) and B (MeOH) were used, delivered at a flow rate of 0.8 mL/min with the following gradient: A, 95% for 5 min; A from 95% to 60% in 1 min; A 60% for 6 min; A from 60% to 40% in 1 min; A, 40% for 5 min; A from 40% to 0% in 1 min; B 100% for 3 min.

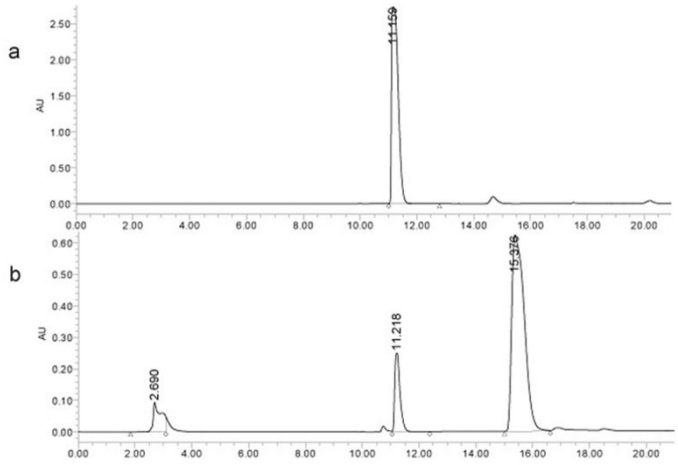


Figure S1. HPLC analysis: (a) 4-hydroxybenzyl alcohol; (b) reaction of **1** and L-Cys.

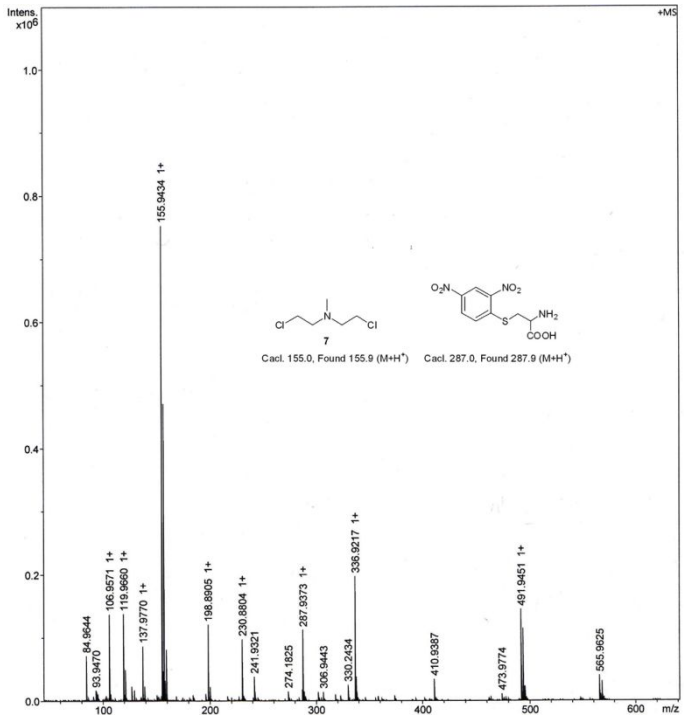


Figure S2. MS spectra of the mixture for the reaction of **1** with L-Cys.

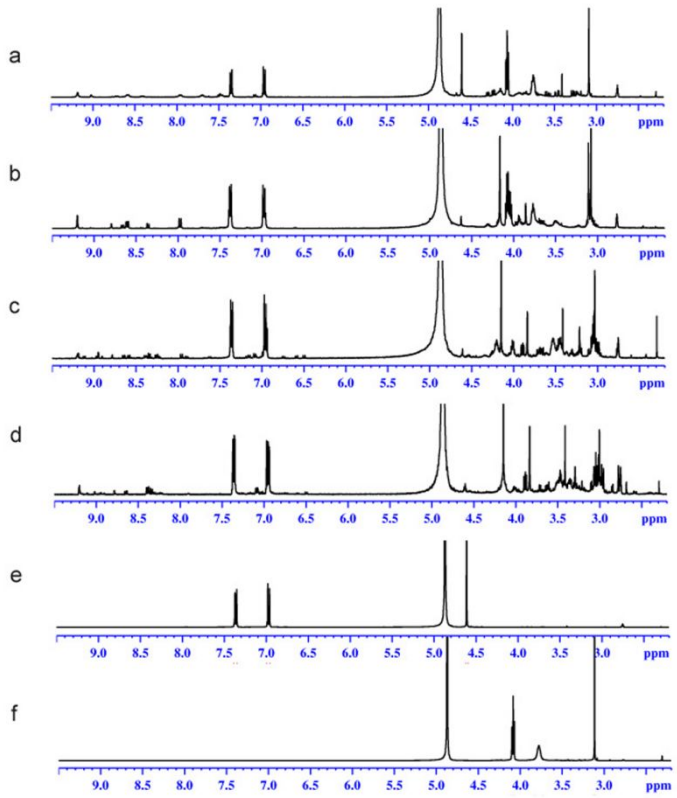


Figure S3. ^{1}H NMR analysis of the activation of **1** by L-Cys in deuterated monopotassium phosphate buffer at different pH: (a) 5.5; (b) 6.8; (c) 7.4; (d) 8.2; (e) ^{1}H NMR of **8** in D_2O at pH 5.5; (f) ^{1}H NMR of mechlorethamine with equivalent H^+ in D_2O at pH 5.5. The mixture was incubated at r.t. for 24 h.

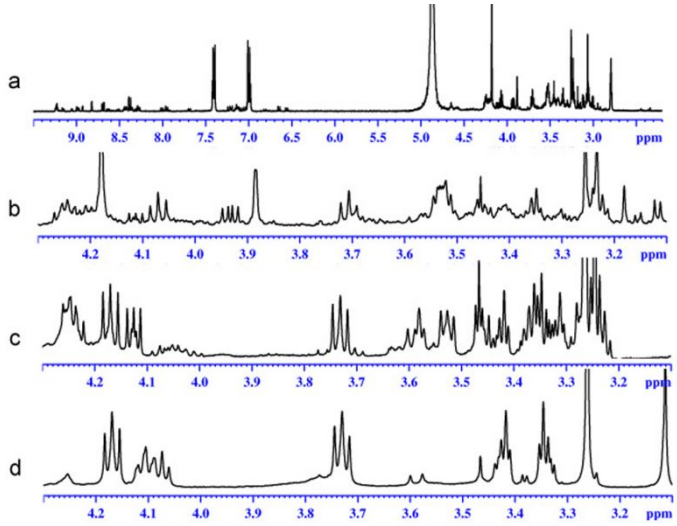


Figure S4. ^{1}H NMR analysis of the activation of **1** by L-Cys in deuterated monopotassium phosphate buffer at pH 7.4: (a) the mixture was incubated for 7 h; (b) the detailed information between δ 4.3 and δ 3.1 in (a); (c) ^{1}H NMR of mechlorethamine in alkaline condition; (d) ^{1}H NMR of mechlorethamine in neutral condition.

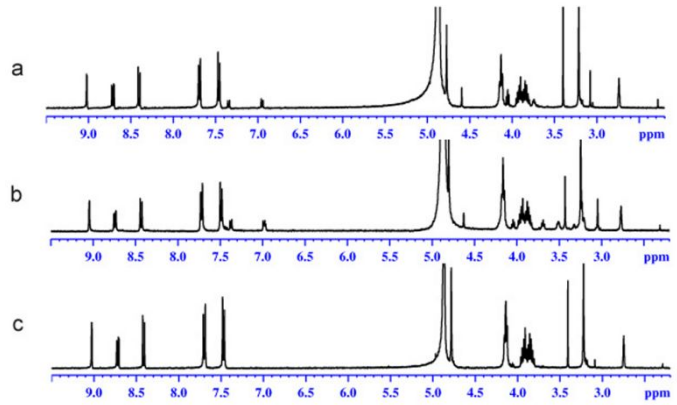


Figure S5. ^{1}H NMR analysis of the stability of **1** in deuterated monopotassium phosphate buffer at r.t.: (a) pH 5.5, incubated for 24 h; (b) pH 7.4, incubated for 7 h; (c) ^{1}H NMR of **1** in D_2O.

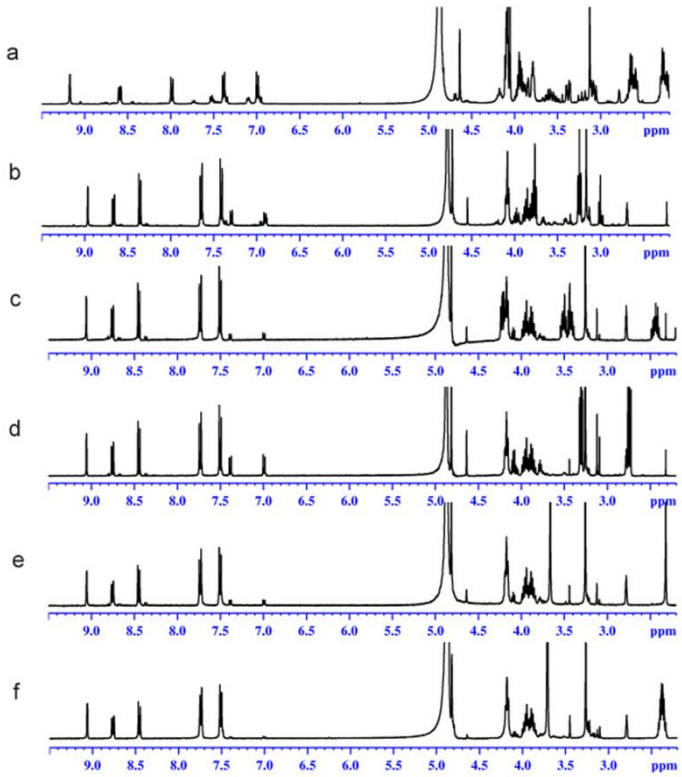


Figure S6. ^{1}H NMR analysis of the inducible activity of **1** in deuterated monopotassium phosphate buffer at pH 5.5 toward: (a) GSH; (b) L-arginine; (c) L-proline; (d) *β*- alanine; (e) glycine; (f) L-valine. The solution was incubated at r.t. for 24 h.

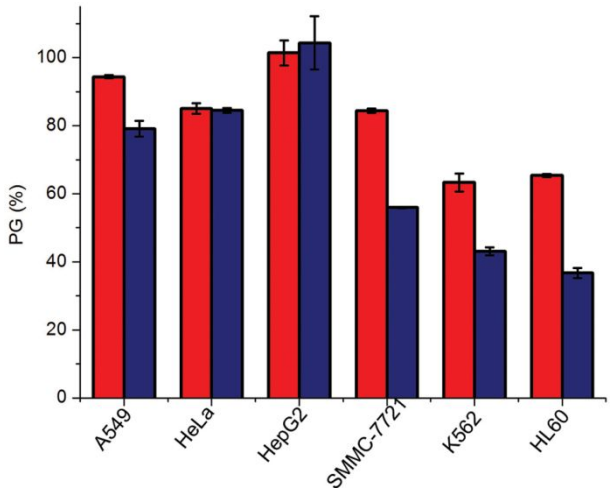
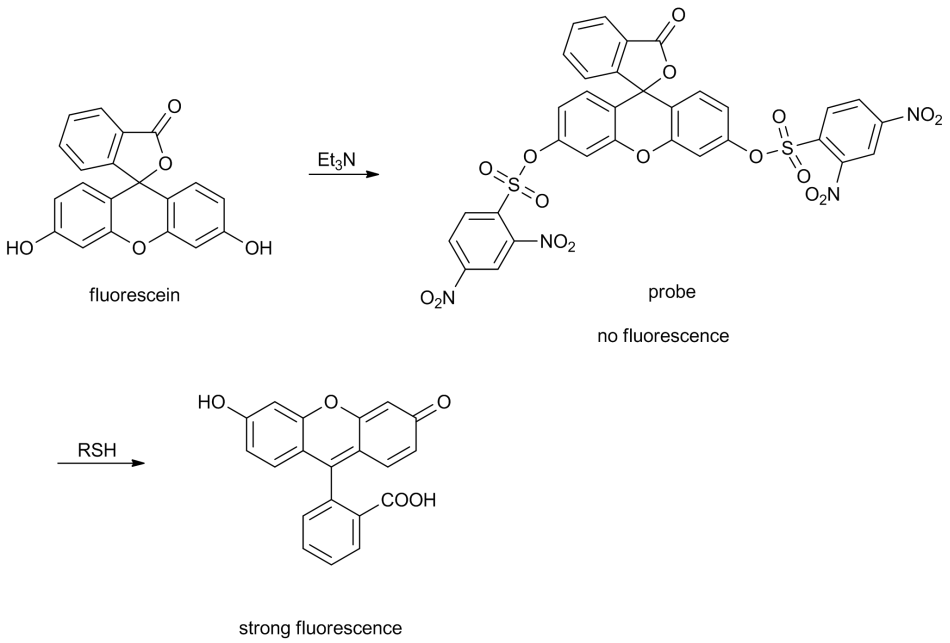
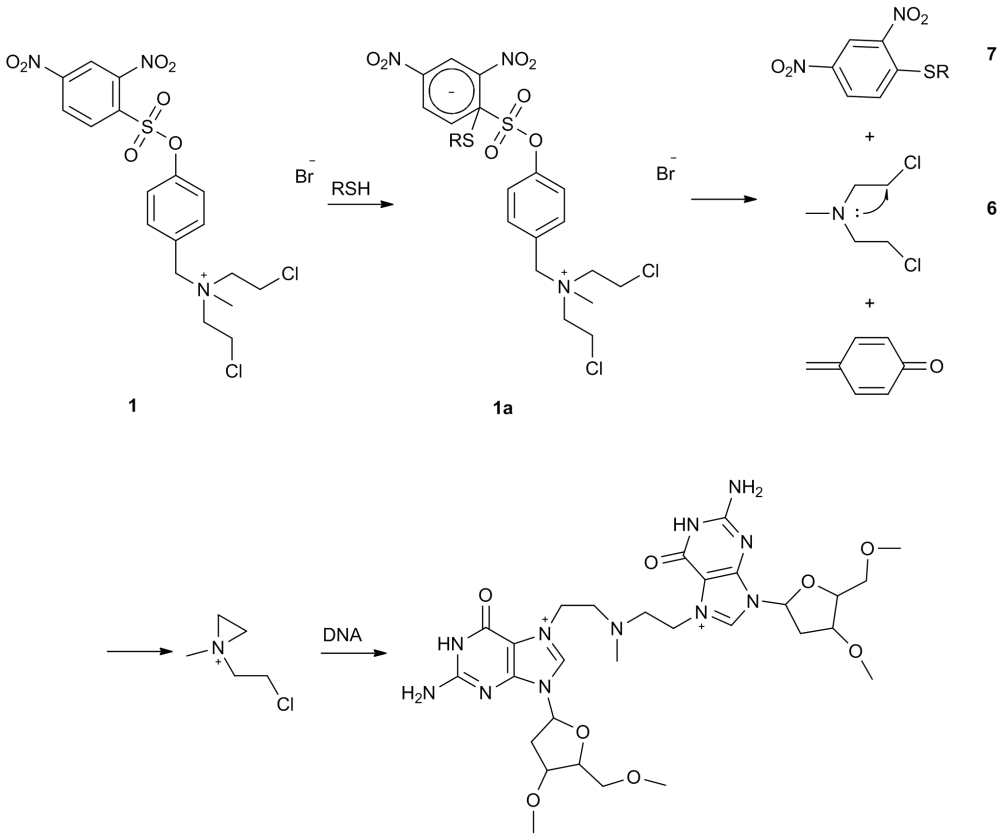


Figure S7. Effect of **5** on cancer cells. Time matched control samples are set up concurrently (without **5**). Data are expressed as the mean ± SE of three independent experiments. (red bar, 26 μM; blue bar, 52 μM). PG： percentage of growth.

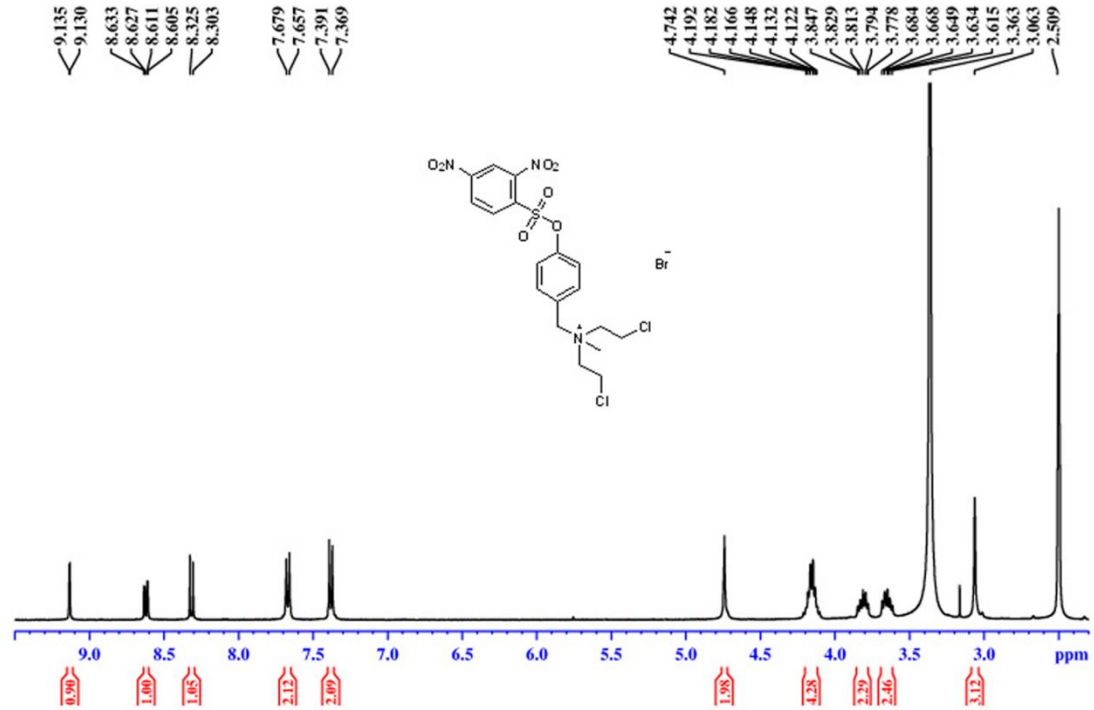
Scheme S1: the synthesis and reaction mechanism of fluorescent probe.

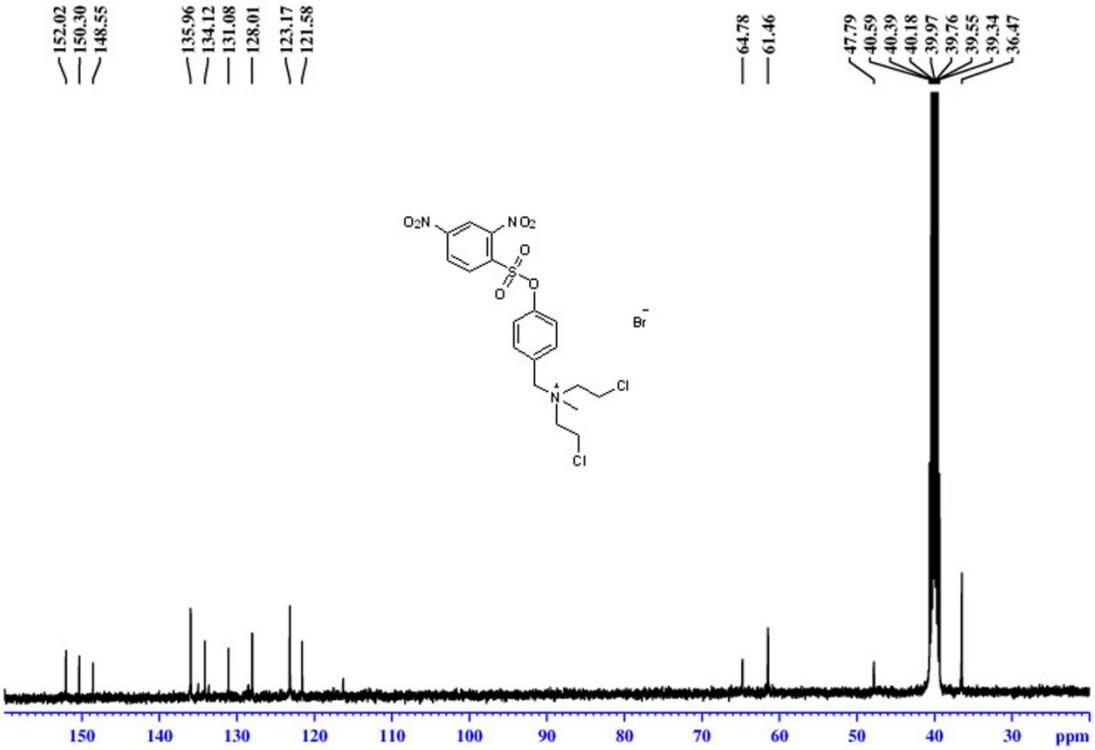


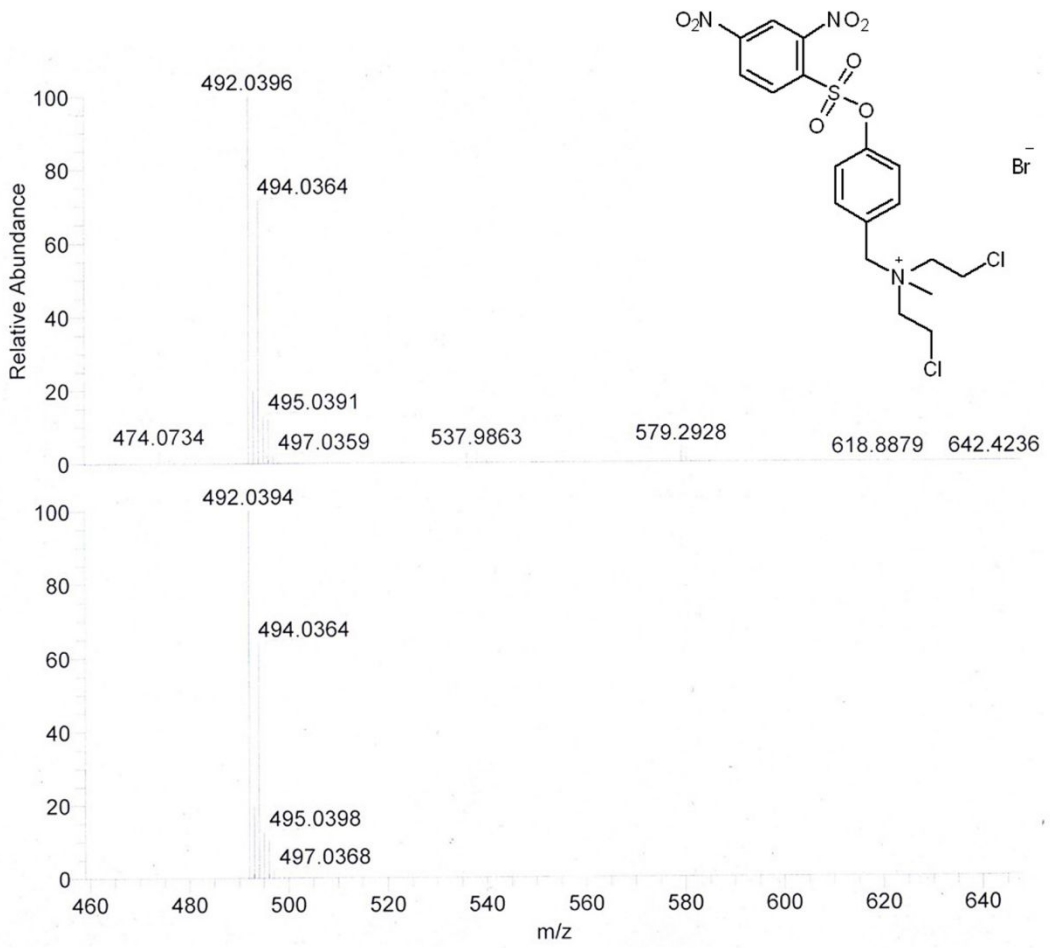
Scheme S2: cross-linking Formation Induced by **1** upon GSH Activation.



^{1}H NMR, ^{13}C NMR and HRMS-ESI spectra of **1**



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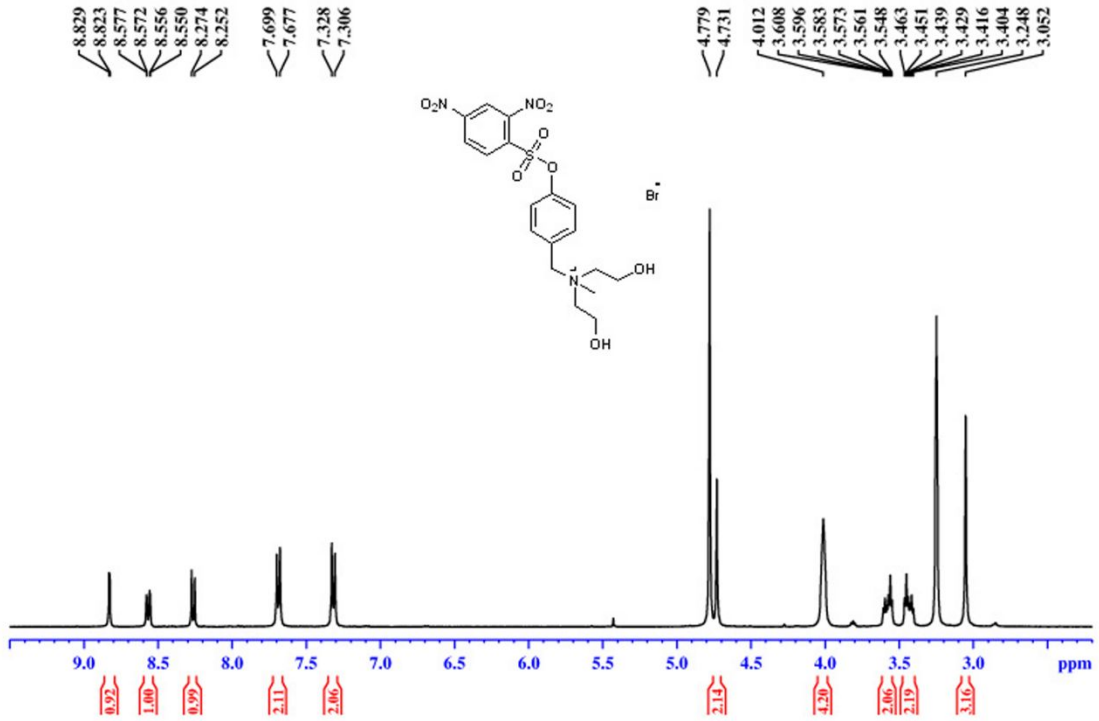
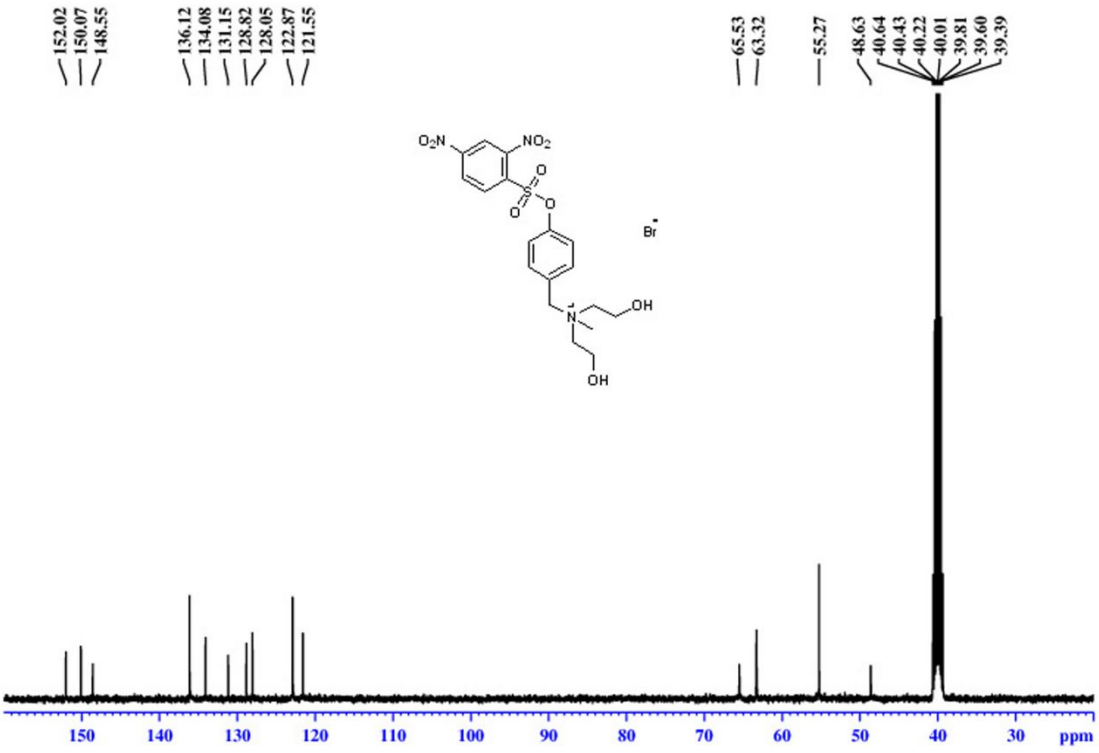
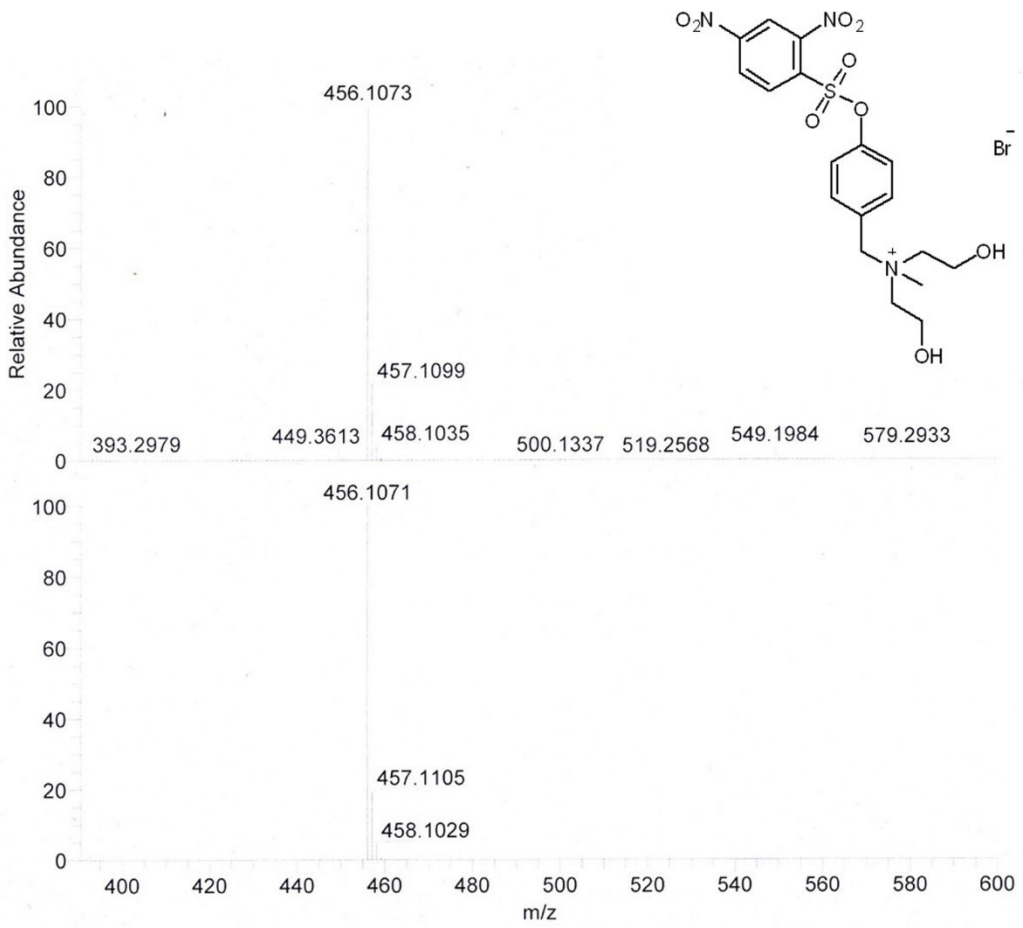
^{1}H NMR, ^{13}C NMR and HRMS-ESI spectra of **5**



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