Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Construction of pH sensitive smart glutathione peroxidase (GPx) mimic based on pH responsive pseudorotaxane

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Contents

1. Characterization of compound 1-10

1.1. Synthesis of compound 4

Compound **4** is not a new compound. The synthesis of compound **4** has been reported many times. Generally, the synthetic route of compound **4** was as follows. 2-bromoethylamine hydrobromide (3, 10.0g, 48.8mmol) was added to 100mL tetrahydrofuran in a 500mL round bottom flask. Triethylamine (20.0mL, 143.9mmol) added into the round bottom flask dropwise to neutralize hydrobromic acid. Di-tert-butyl dicarbonate (11.0 g, 50.4mmol) was dissolved in 100mL tetrahydrofuran and added into the round bottom flask dropwise under ice-bath for 2 hours. The solution was stirred for 12 hours at room temperature. The volatiles were removed under reduced pressure and transferred the residue to a 500mL separatory funnel using 200mL. The ethyl acetate solution was extracted by 0.1M hydrochloric acid (3*100mL), 5% sodium bicarbonate solution (3*100mL), saturated sodium chloride solution (3*100mL) respectively. The organic phase was dried with anhydrous sodium sulfate and then volatiles were removed under reduced pressure to give a transparent liquid 4 (10.1 g, 92% yield). δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.45 (9H, s, $C(Me)_3$) 3.46 (2H, t, J=5.5 Hz, NCH_2) 3.53 (2H, t, J=5.0 Hz, $BrCH_2$) 4.93 (1H, s, NH) (Figure S1)

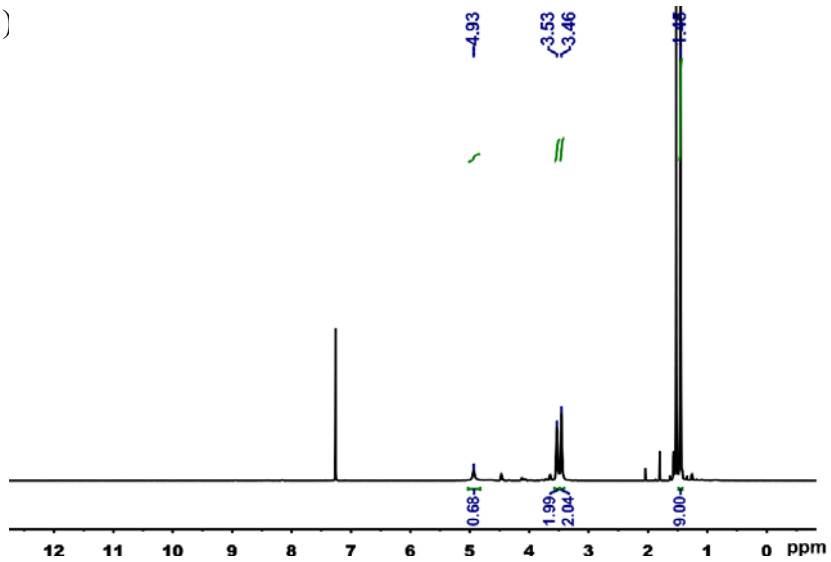

Figure S1. ^{1}H NMR spectrum of compound **4**.

1.2. ^{1}H NMR, ^{13}C NMR spectrum and high resolution ESI-TOF mass spectrum of compound 5

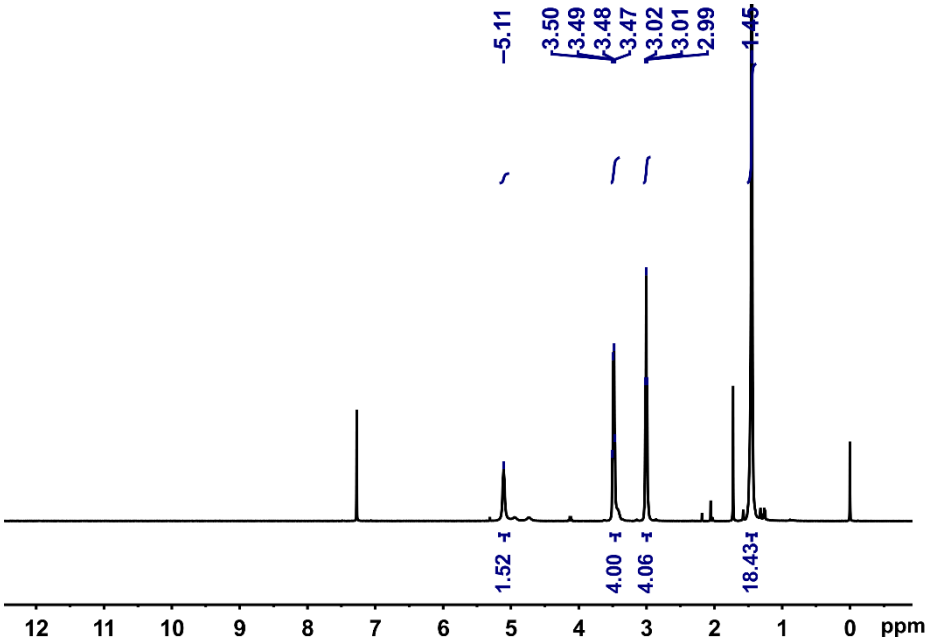

Figure S2. ^{1}H NMR spectrum of compound **5**.

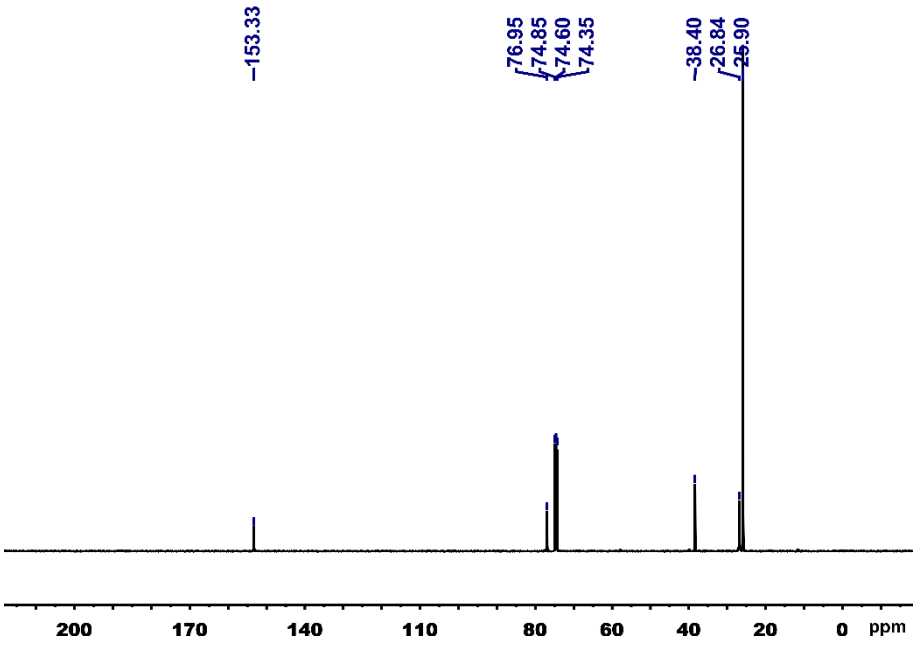

Figure S3. ^{13}C NMR spectrum of compound **5**.

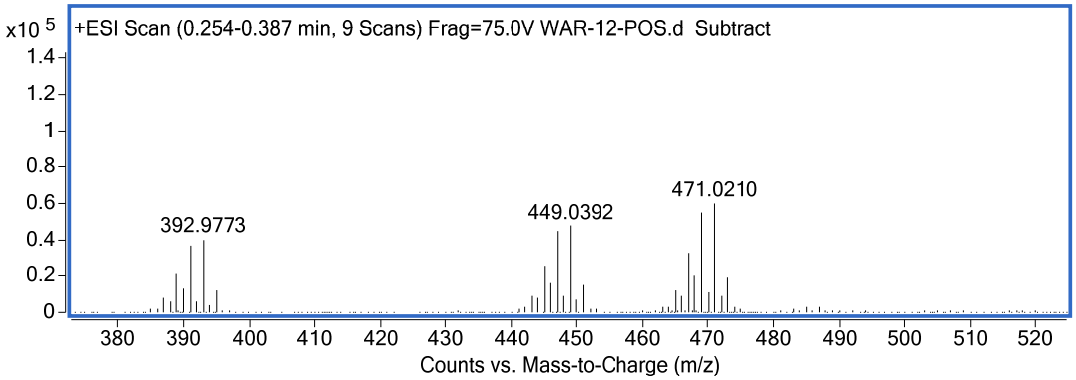

Figure S4. High resolution ESI-TOF mass spectrum of compound **5**.

1.3 ^{1}H NMR, ^{13}C NMR spectrum and high resolution ESI-TOF mass spectrum of compound 1

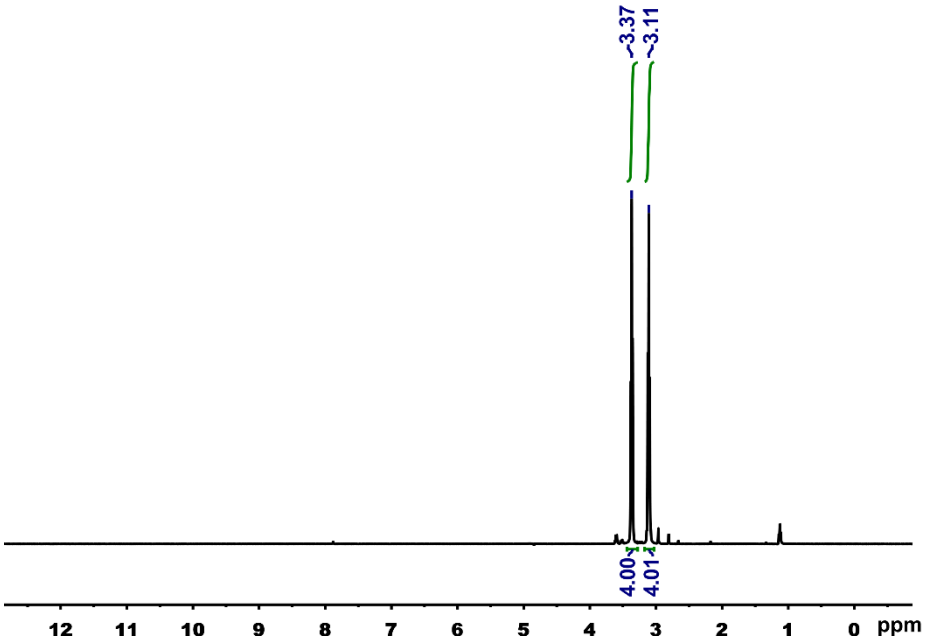

Figure S5. ^{1}H NMR spectrum of compound **1**.

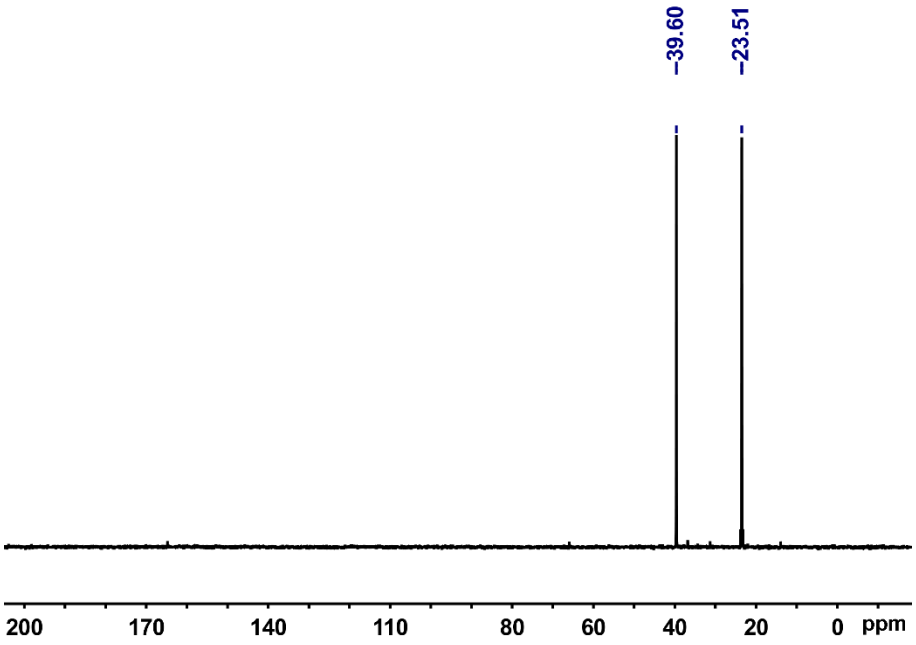

Figure S6. ^{13}C NMR spectrum of compound **1**.

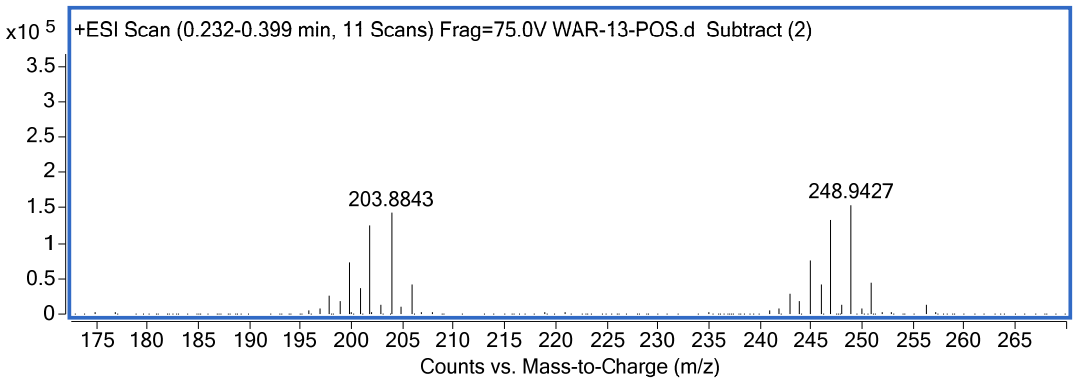

Figure S7. High resolution ESI-TOF mass spectrum of compound **1**.

1.4 ^{1}H NMR, ^{13}C NMR spectrum and high resolution ESI-TOF mass spectrum of compound 9

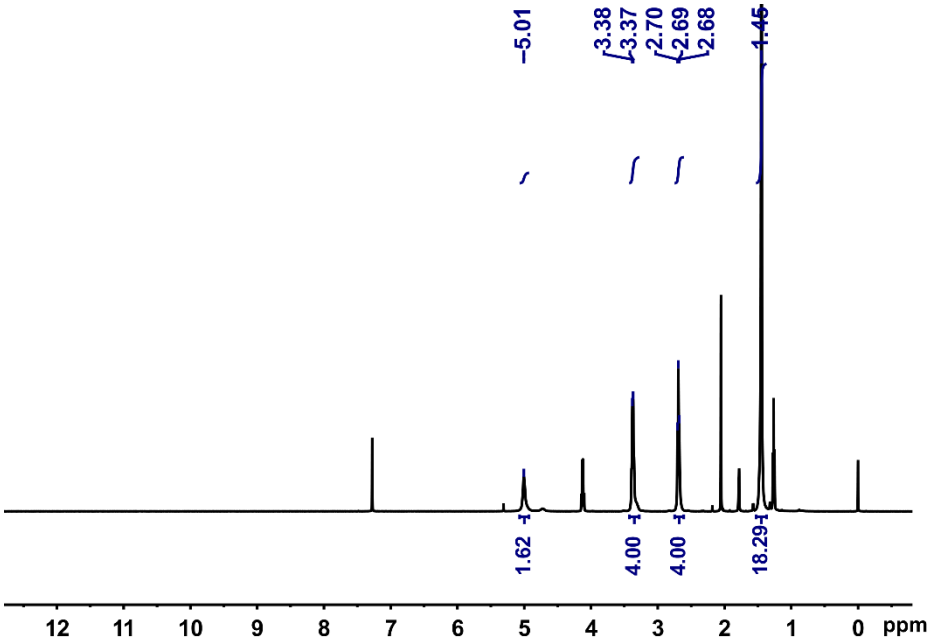

Figure S8. ^{1}H NMR spectrum of compound **9**.

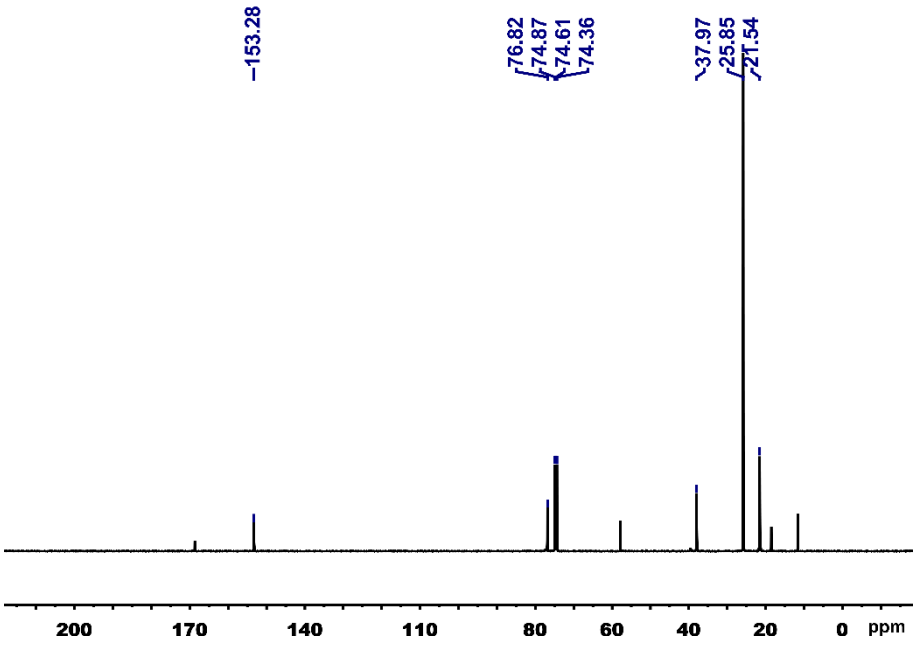

Figure S9. ^{13}C NMR spectrum of compound **9**.

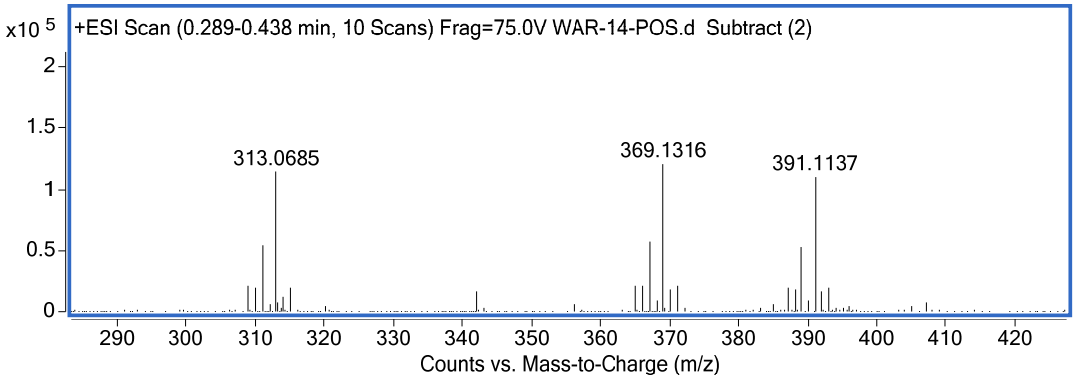

Figure S10. High resolution ESI-TOF mass spectrum of compound **9**.

1.5 ^{1}H NMR, ^{13}C NMR spectrum and high resolution ESI-TOF mass spectrum of compound 2

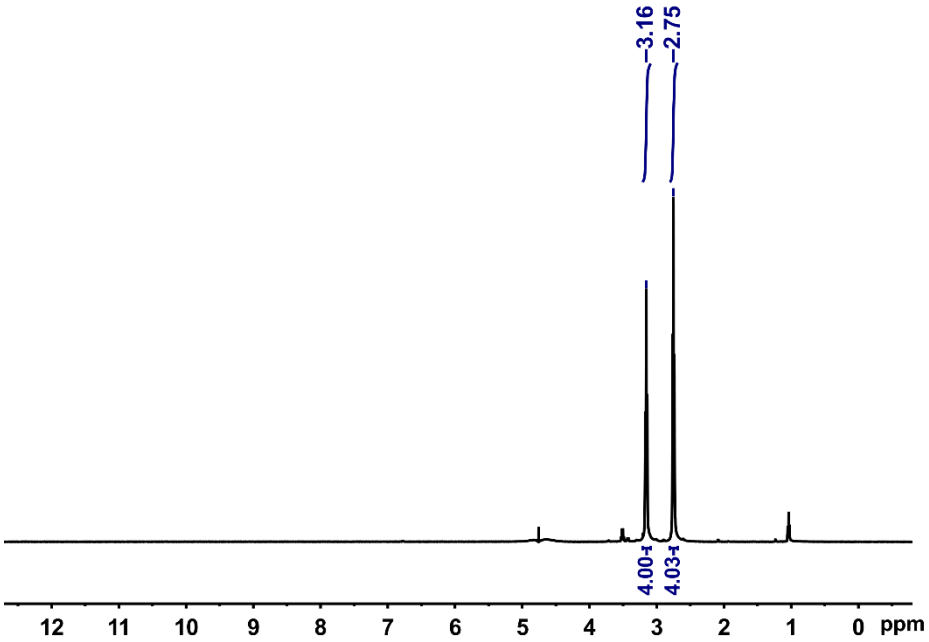

Figure S11. ^{1}H NMR spectrum of compound **2**.

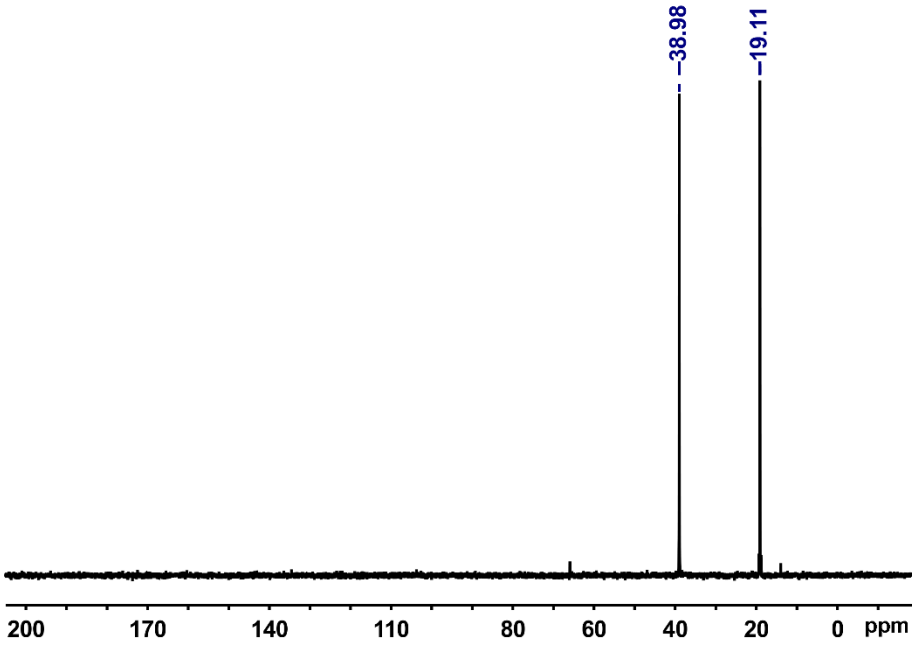

Figure S12. ^{13}C NMR spectrum of compound **2**.

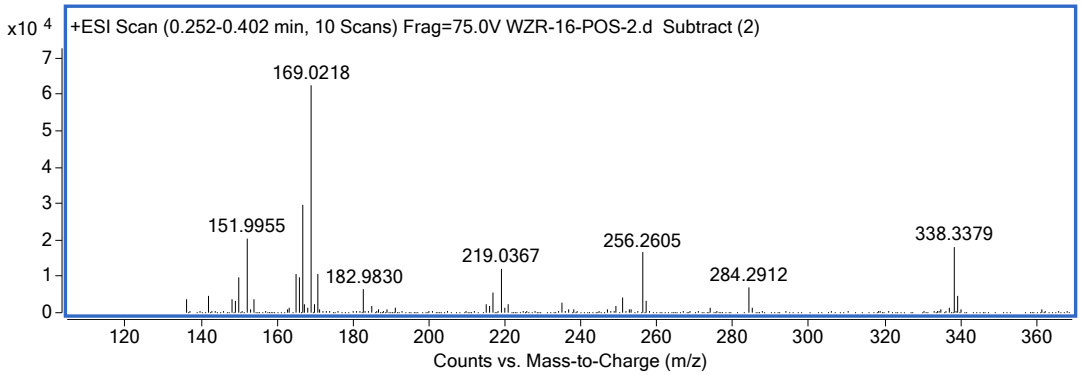

Figure S13. High resolution ESI-TOF mass spectrum of compound **2**.

1.6 Synthesis spectrum of CB[6]

CB[6] was synthesized according to the literature (K. Kim, *Chem. Soc. Rev.*, 2002, **31**, 96). Glycoluril (5.68g, 40mmol) was reacted with formaldehyde (37% w/w, 7.0ml) in 9M sulfuric acid (20ml) at 75°C for 24 h and then at 100°C for 12 h. After the reaction mixture was poured into water (200 mL), acetone (1.0 L) was added to produce precipitate. The precipitate was separated by decantation, washed with water/acetone (1:4), and filtered. 300mL water/acetone (1:2) was added to the resulting solid and stirred for a few minutes. The precipitate is the major product CB[6] that was separated by filtration and dried under vacuum. δ_H (500 MHz; D_2O, KCl) 4.32 (12H, d, J=15.5Hz, CH_2) 5.59 (12H, s, CH) 5.67 (12H, d, J=15.5Hz, CH_2) (Figure S14).

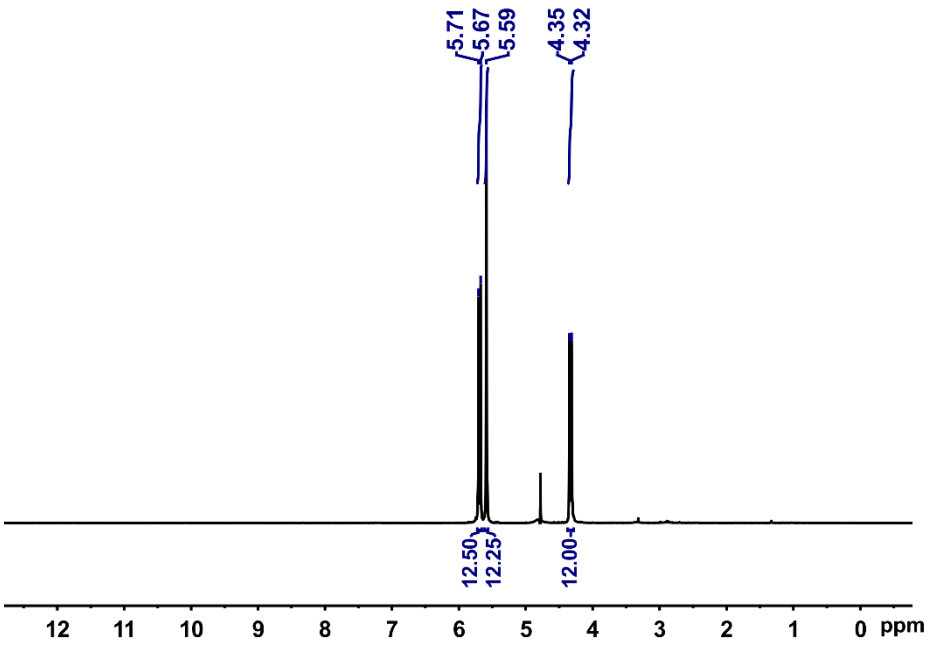

Figure S14. ^{1}H NMR spectrum of CB[6].

2. Binding constant calculation of compound 1 and 2 with CB[6]

2.1 Binding constant calculation of compound 1 with CB[6]

Generally, CB[6] and compound **1** was dissolved in D_2O at the ratio of 1:1. The experiment was performed in triplicate. The binding constants were calculated according to the integral of peaks corresponding to CB[6], compound **1** and pseudorotaxane. The binding constants between CB[6] and compound **1** for the three experiments were calculated to be 1.28×10^4, 1.17×10^4 and 1.11×10^4 M^{-1}, respectively. Thus, the final result was $1.19\pm0.09\times10^4$ M^{-1}.

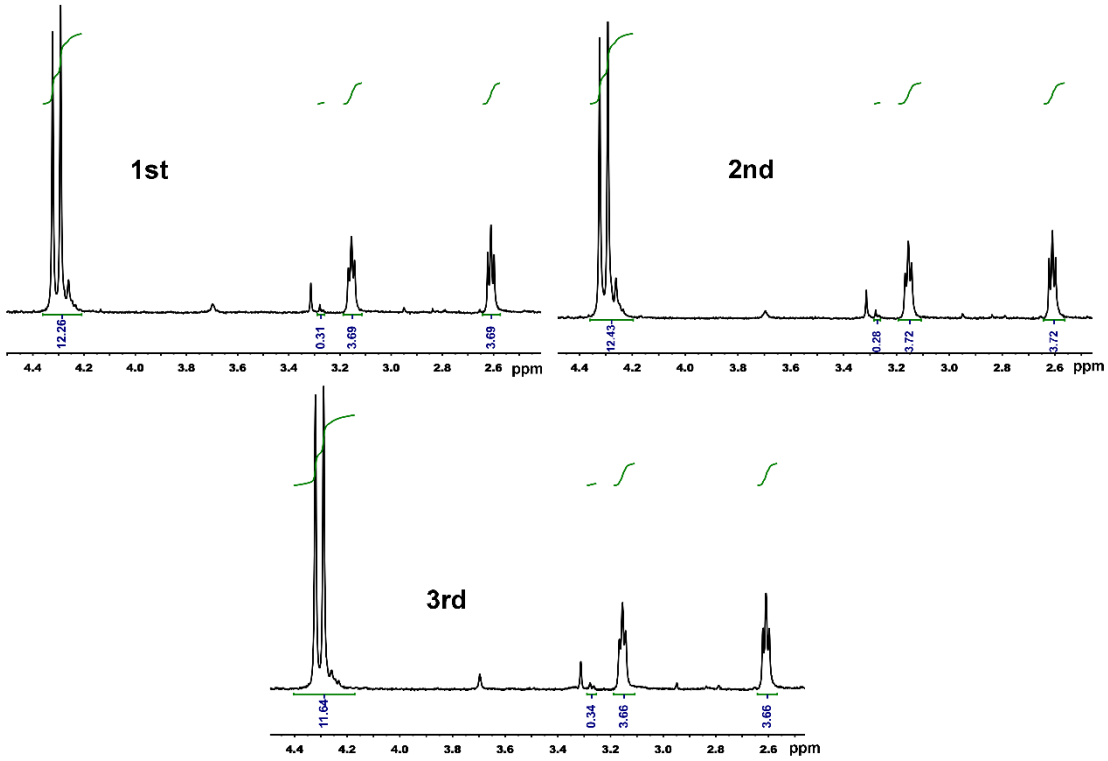

Figure S15. Partial ^{1}H NMR spectra (500 MHz) of mixtures of compound **1** with 1 equiv. CB[6] at pH=7.

2.2 Binding constant calculation of compound 2 with CB[6]

Generally, CB[6] and compound **2** was dissolved in D_2O at the ratio of 1:1. The experiment was performed in triplicate. The binding constants were calculated according to the integral of peaks corresponding to CB[6], compound **2** and pseudorotaxane. The binding constants between CB[6] and compound **2** were calculated to be 2.46×10^4, 2.43×10^4 and 2.60×10^4 M^{-1}, respectively, giving a final result of $2.50\pm0.10\times10^4$ M^{-1}.

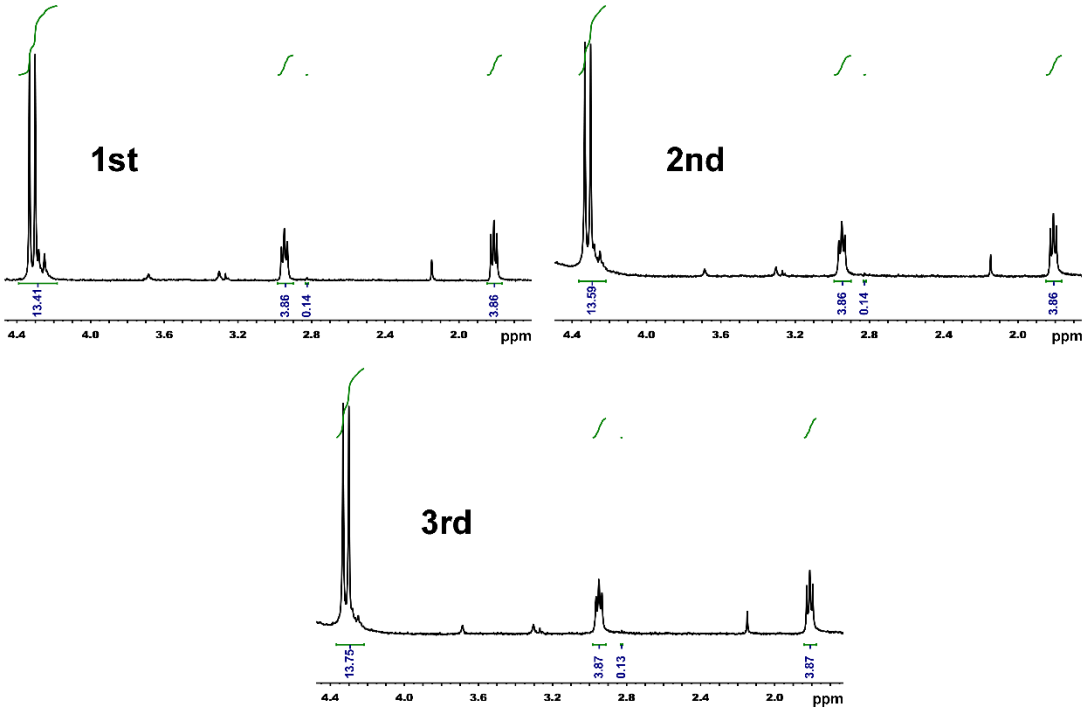

Figure S16. Partial ^{1}H NMR spectra (500 MHz) of mixtures of compound **2** with 1 equiv. CB[6] at pH=7.

3. Catalytic curves of compound 1 in enzymatic kinetics tests

3.1 Catalytic curves of compound 1 at the H_2O_2 concentration fixed to 0.5mmol/L

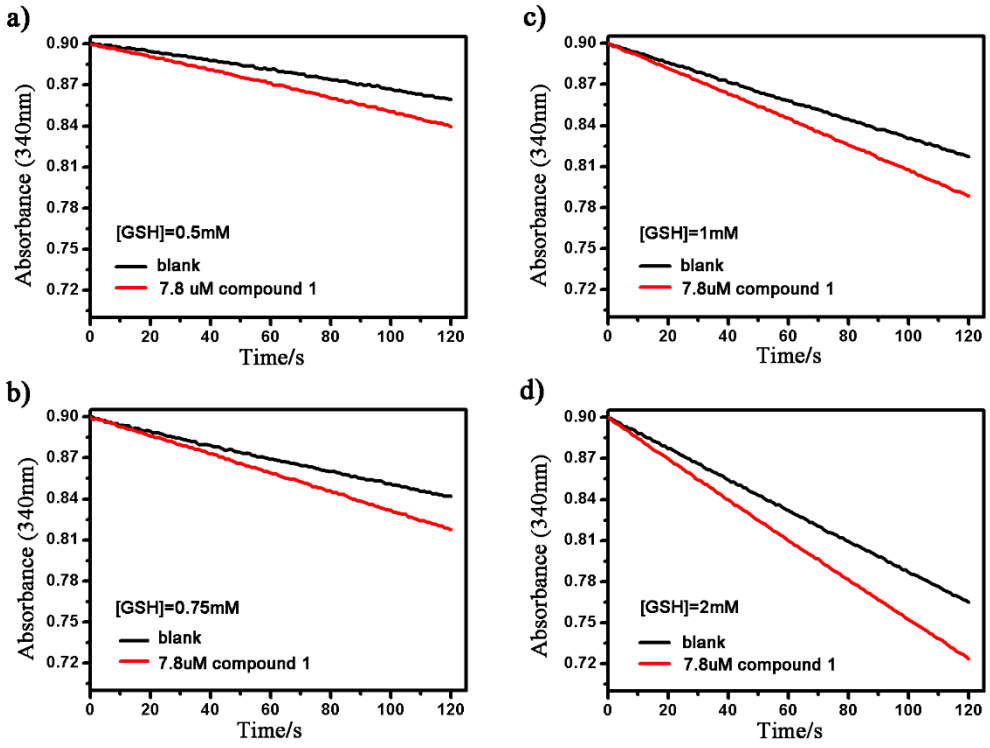

Figure S17. Catalytic curves of compound **1** at the H_2O_2 concentration fixed to 0.5mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

3.2 Catalytic curves of compound 1 at the H_2O_2 concentration fixed to 0.75mmol/L

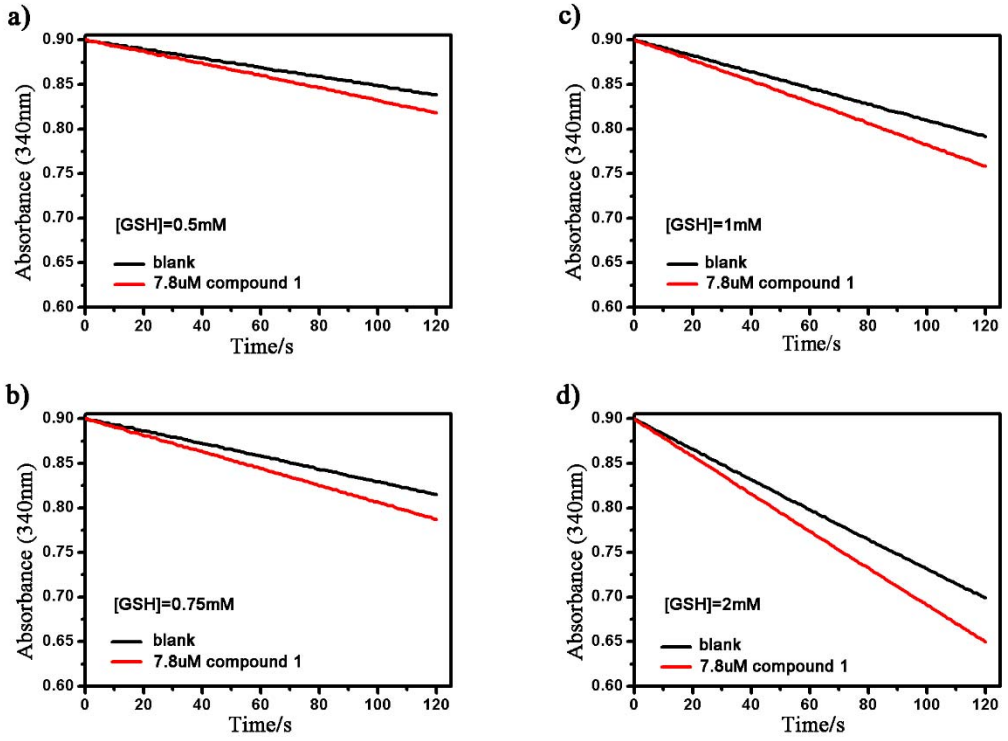

Figure S18. Catalytic curves of compound **1** at the H_2O_2 concentration fixed to 0.75mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

3.3 Catalytic curves of compound 1 at the H_2O_2 concentration fixed to 1.0mmol/L

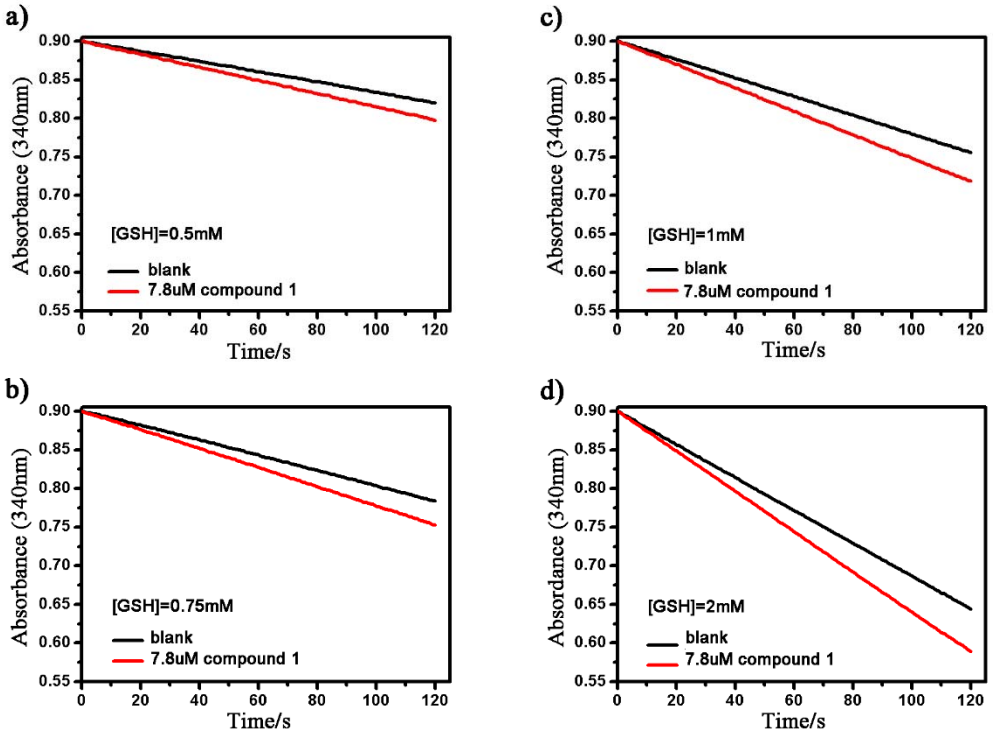

Figure S19. Catalytic curves of compound **1** at the H_2O_2 concentration fixed to 1.0mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

4. Catalytic curves of compound 2 in enzymatic kinetics tests

4.1 Catalytic curves of compound 2 at the H_2O_2 concentration fixed to 0.5mmol/L

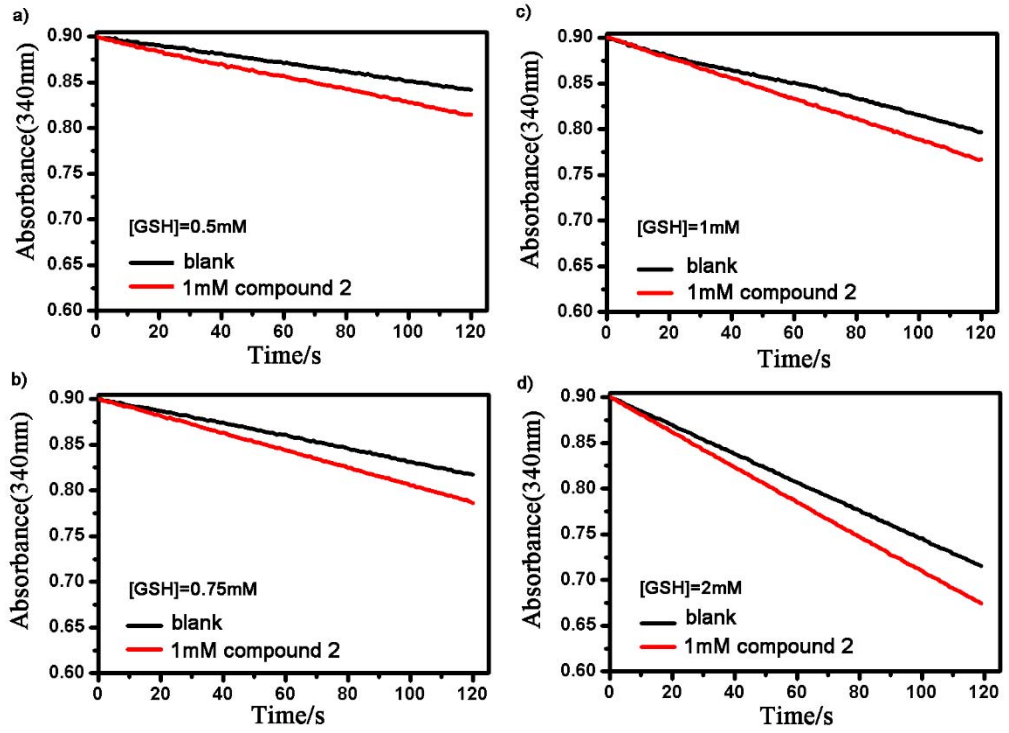

Figure S20. Catalytic curves of compound **2** at the H_2O_2 concentration fixed to 0.5mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

4.2 Catalytic curves of compound 2 at the H_2O_2 concentration fixed to 0.75mmol/L

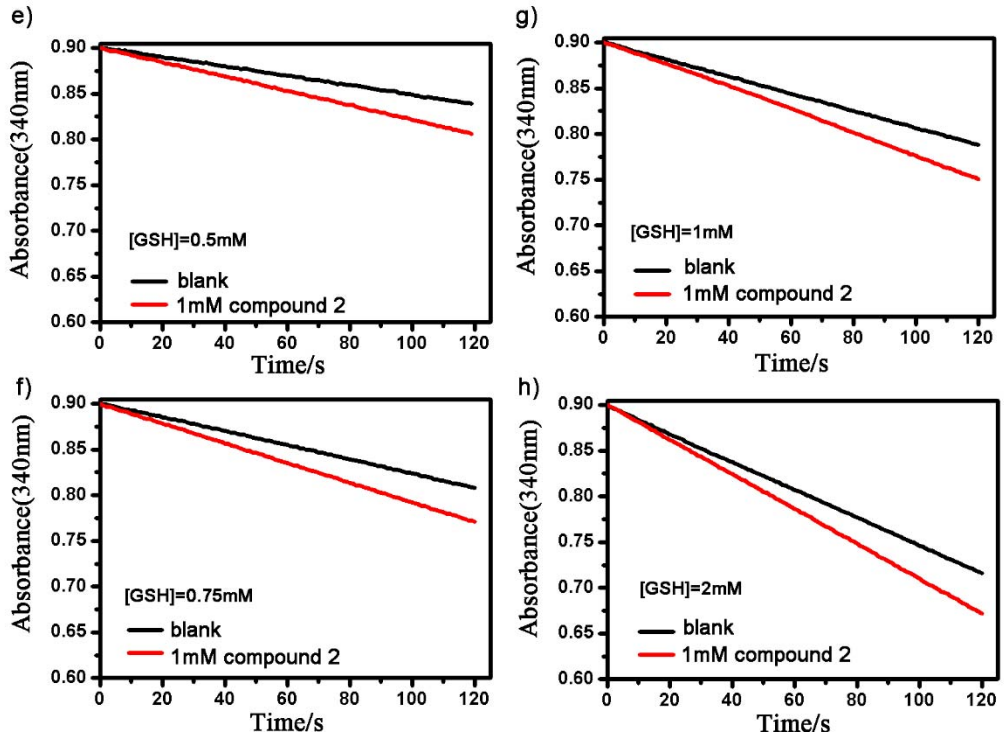

Figure S21. Catalytic curves of compound **2** at the H_2O_2 concentration fixed to 0.75mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

4.3 Catalytic curves of compound 1 at the H_2O_2 concentration fixed to 1.0mmol/L

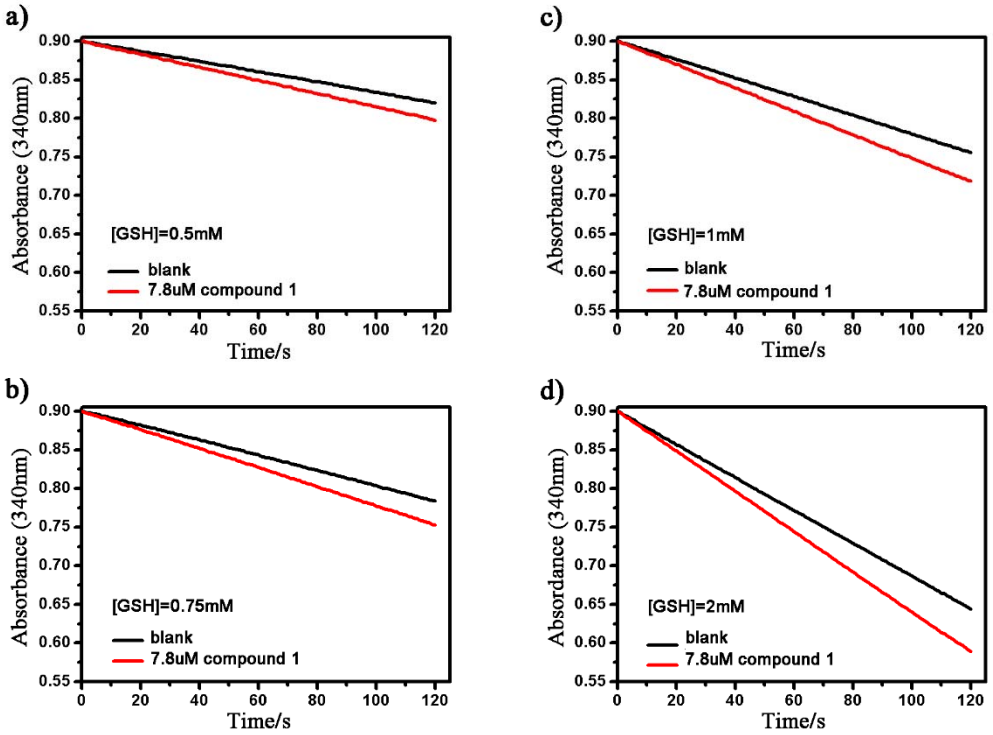

Figure S22. Catalytic curves of compound **2** at the H_2O_2 concentration fixed to 1.0mmol/L and GSH concentration of a) 0.5mmol/L; b) 1.0mmol/L; c) 1.5mmol/L; d) 1.0mmol/L.

5. Double-reciprocal plots of the reduction of H_2O_2 by GSH under the catalysis of compound 1

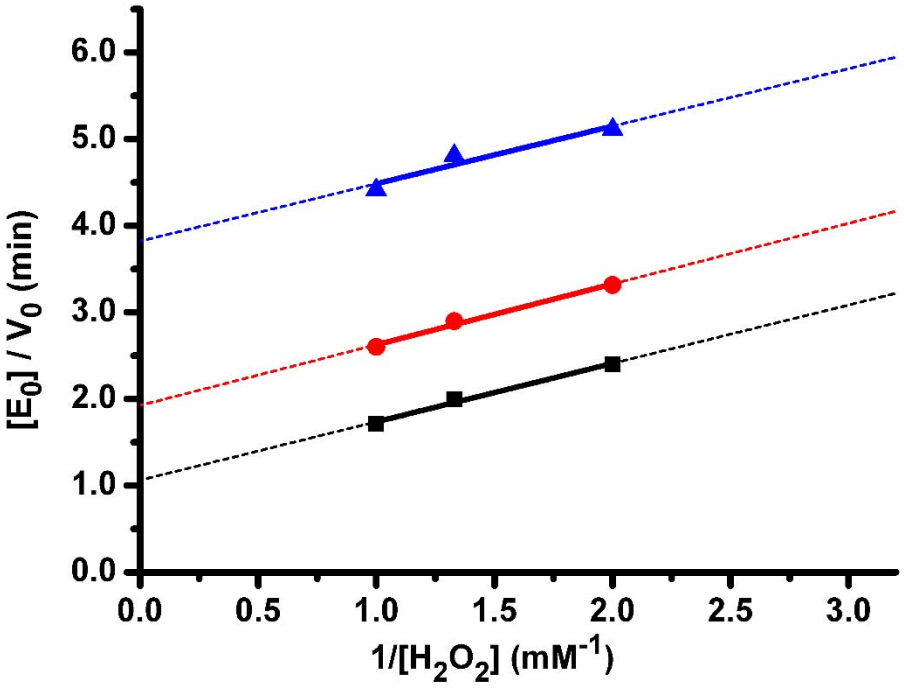

Figure S23. Double-reciprocal plots of the reduction of H_2O_2 by GSH under the catalysis of compound **1**. $[E_0]$=total enzyme concentration; $[E_0]$ / v_0 versus 1 / $[H_2O_2]$ (mM^{-1}) at [GSH]＝0.5mM (▼), 1.0mM (●) and 2.0mM (■).

6. Double-reciprocal plots of the reduction of H_2O_2 by GSH under the catalysis of compound 2

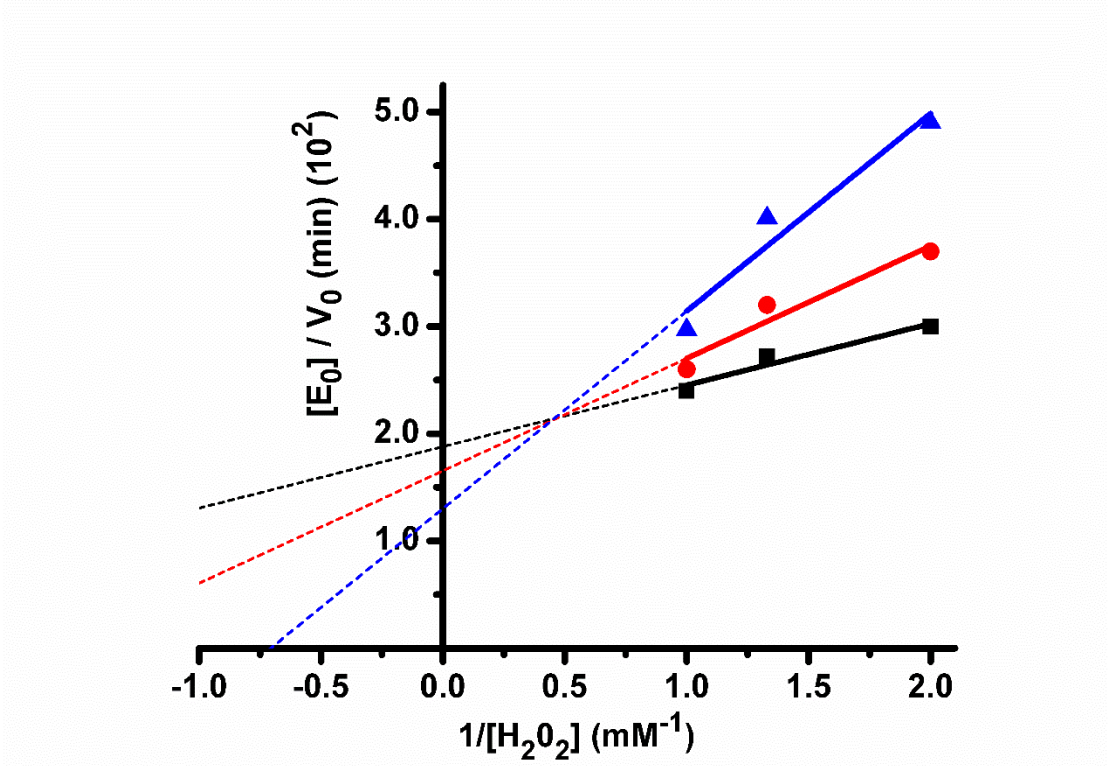

Figure S24. Double-reciprocal plots of the reduction of H_2O_2 by GSH under the catalysis of compound **2**. $[E_0]$=total enzyme concentration; $[E_0] / v_0$ versus $1 / [H_2O_2]$ (mM^{-1}) at [GSH]＝0.5mM (▼), 1.0mM (●) and 2.0mM (■).