Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Reversible pH-controlled Switching of An Artificial Antioxidant Selenoenzyme Based on Pseudorotaxane Formation and Dissociation

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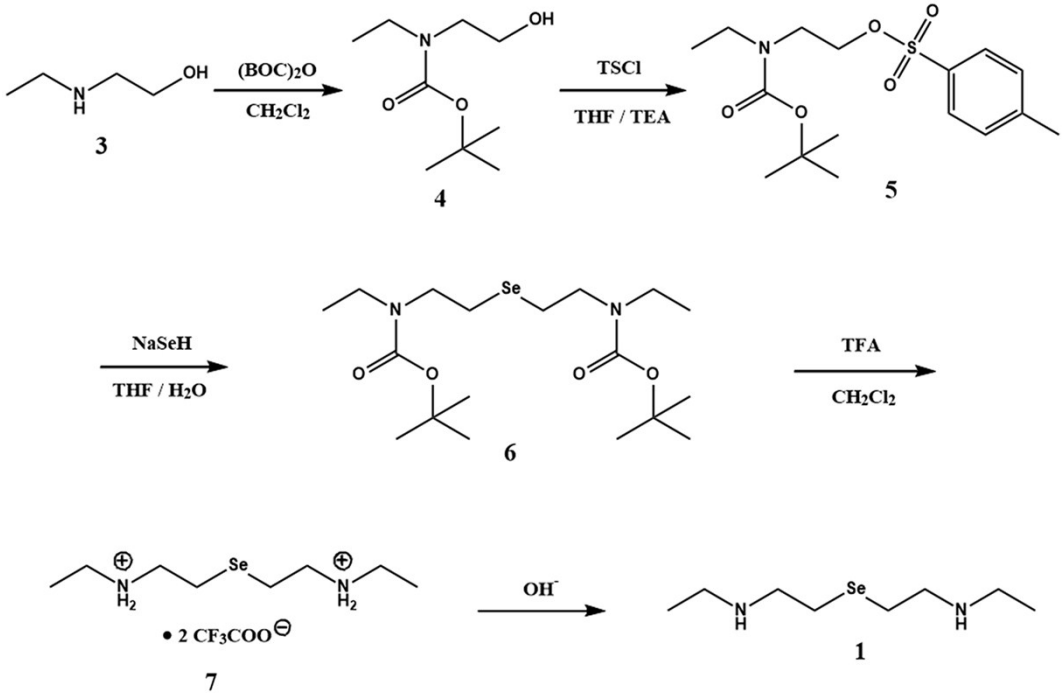
Contents

1. Synthesis of compound 1

The synthesis of organoselenium compound **1** was shown in Scheme S1. Specifically, the procedure was as follows:



Scheme S1: Synthesis procedures of compound **1**.

1.1 Synthesis of compound 4

2 - (Ethylamino) ethanol (**3**, 8.9 g, 0.1 mol) was added to 100mL dichloromethane in a 500mL round bottom flask. Di-tert-butyl dicarbonate (24 g, 0.11 mol) was dissolved in 100mL dichloromethane and added into the round bottom flask dropwise under ice-bath for 2 hours. Then stirred the solution for 12 hours at room temperature. Extracted the dichloromethane solution by 0.1M hydrochloric acid (3*100mL), 5% sodium bicarbonate solution (3*100mL), saturated sodium chloride solution (3*100mL) respectively. Dried the organic phase with anhydrous sodium sulfate and then removed the volatiles under reduced pressure to give a transparent liquid **4** (18 g, 95%). δ_H (500 MHz; $CDCl_3$) 1.11 (3 H, t, Me), 1.45 (9 H, s, $C(Me)_3$), 3.26 (2 H, q, NCH_2), 3.37 (2 H, t, NCH_2), 3.73 (2 H, t, CH_2OH). ESI-TOF mass spectrum: m/z 190.1 ($M+H^+$).

1.2 Synthesis of compound 5

Compound **4** (3 g, 15.9 mmol) was added to a mixture of 50mL dichloromethane and

7.5 mL (54.1 mmol) triethylamine in a 250mL round bottom flask. 4-toluene sulfonyl chloride (3.3 g, 17.3 mmol) was dissolved in 50mL dichloromethane and added into the round bottom flask dropwise under ice-bath for 2 hours. Then stirred the solution for 12 hours at room temperature. Extracted the dichloromethane solution by sodium chloride solution (3*50mL) and then dried the organic phase with anhydrous sodium sulfate. The volatiles were removed and the residue was purified by column chromatography (silica gel, dichloromethane/ethyl acetate: 20/1 as eluent) to give an faint yellow liquid **5** (2.3 g, 42%). δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.06 (3 H, t, Me) 1.40 (9 H, s, $C(Me)_3$) 2.45 (3 H, s, PhMe) 3.21 (2 H, q, NCH_2) 3.42 (2 H, t, NCH_2) 4.11 (2 H, t, CH_2OTs) 7.35 (2 H, d, Ph) 7.78 (2 H, d, Ph). ESI-TOF mass spectrum: m/z 344.3 ($M+H^+$).

1.3 Synthesis of compound 6

Selenium (200 mg, 2.53 mmol) and sodium borohydride (300 mg, 7.93 mmol) were added in a 250mL round bottom flask and protected by nitrogen. 10mL deoxygenated water was injected into the round bottom flask. After 30min the selenium reacted completely to give NaSeH. Compound **5** (2 g, 5.83 mmol) was dissolved in 50mL THF and deoxygenated by nitrogen. The deoxygenated solution of compound **5** was injected into the round bottom flask and stirred for 12 hours at room temperature. Removed the volatiles and transferred the residue to a 250mL separatory funnel using 100mL dichloromethane. Extracted the dichloromethane solution by sodium chloride solution (3*50mL) and then dried the organic phase with anhydrous sodium sulfate. The volatiles were removed and the residue was purified by column chromatography (silica gel, dichloromethane/ethyl acetate: 30/1 as eluent) to give an yellow liquid **6** (560 mg, 52%). δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.10 (6 H, t, Me) 1.45 (18 H, s, $C(Me)_3$) 2.69 (4 H, t, $SeCH_2$) 3.26 (4 H, q, NCH_2) 3.40 (4 H, t, NCH_2). ESI-TOF mass spectrum: m/z 425.2 ($M+H^+$).

1.4 Synthesis of compound 1

Compound **6** (560 mg, 1.32 mmol) was added to a mixture of 5 mL dichloromethane and 2 mL (27 mmol) trifluoroacetic acid in a 100mL round bottom flask and stirred for 12 hours at room temperature. Removed most of the volatiles and the residue was deposited by 50mL cold ethyl ether. The white precipitate (compound **7**) was got by filtration under reduced pressure and dissolved by 30mL saturated sodium carbonate solution. Extracted by ethyl acetate (3*30mL) and then dried the organic phase with anhydrous sodium sulfate. Removed the volatiles under reduced pressure to give a transparent liquid **1** (210 mg, 71%). δ_H (500 MHz; D_2O; Me_4Si) 1.07 (6 H, t, Me) 2.61 (4 H, q, NCH_2) 2.72 (4 H, t, $SeCH_2$) 2.81 (4 H, t, NCH_2) (Figure S1). ESI-TOF mass spectrum: m/z 225.1 ($M+H^+$) (Figure S2).

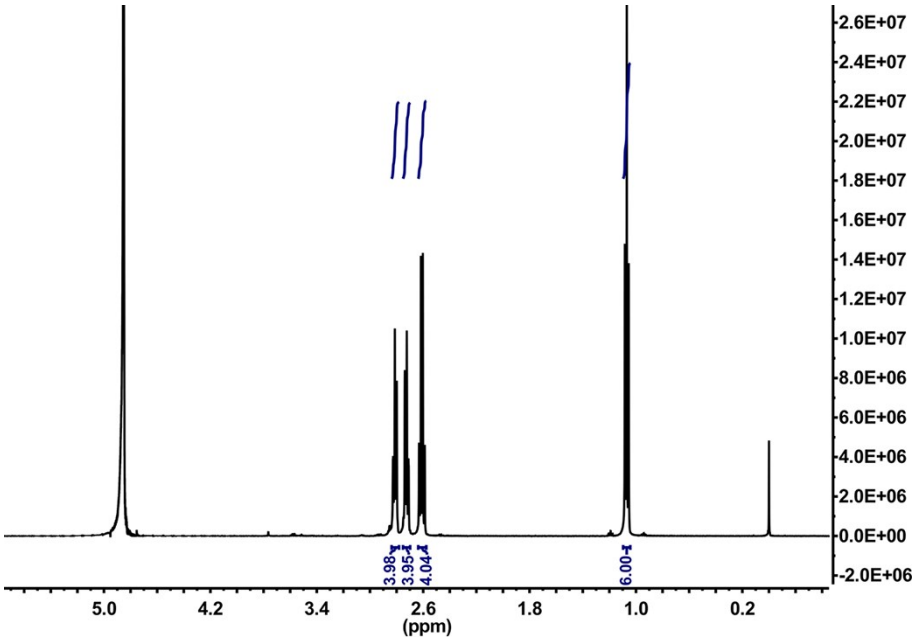


Figure S1: ^{1}H-NMR spectra of compound **1**.

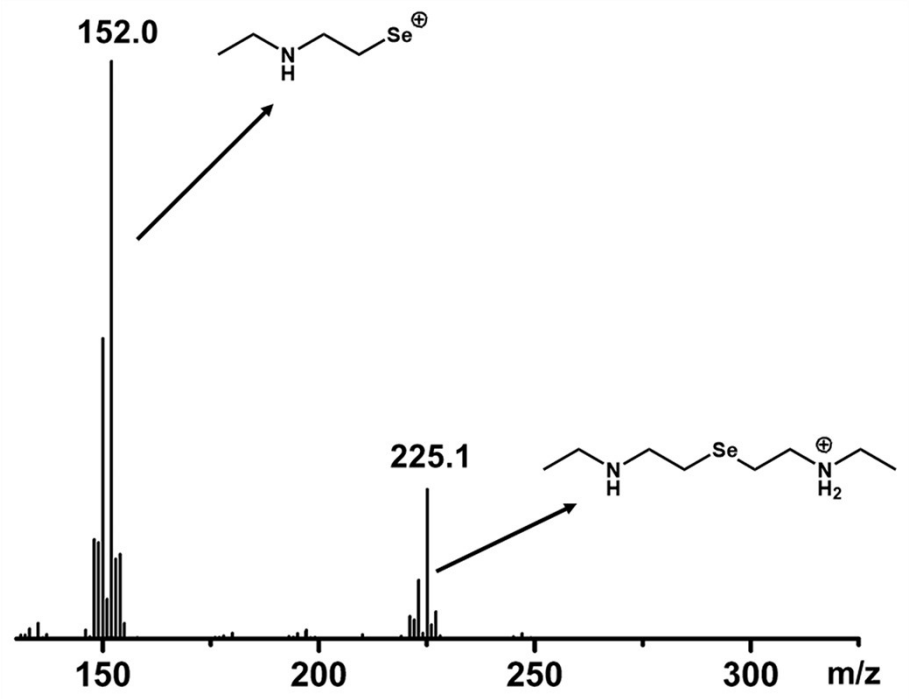
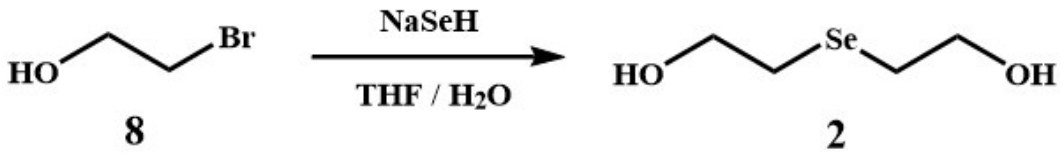


Figure S2: ESI-TOF mass spectrum of compound **1**.

2. Synthesis of compound 2

The synthesis of organoselenium compound **2** was shown in Figure S2. Specifically, the procedure was as follows:



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Scheme S2: Synthesis procedures of compound **2**.

Selenium (1.421 g, 18 mmol) and sodium borohydride (2 g, 52.9 mmol) were added in a 250mL round bottom flask and protected by nitrogen. 20mL deoxygenated water was injected into the round bottom flask. After 30min the selenium reacted completely to give NaSeH. Compound **8** (5 g, 40 mmol) was dissolved in 80mL THF and deoxygenated by nitrogen. The deoxygenated solution of compound **8** was injected into the round bottom flask and stirred for 12 hours at room temperature. Removed the volatiles and the residue was purified by column chromatography use dry method (silica gel, ethyl acetate/ methanol: 50/1 as eluent) to give a transparent liquid **2** (2.1 g, 70%). δ_H (500 MHz; D_2O; Me_4Si) 2.81 (4 H, t, $SeCH_2$) 3.82 (4 H, t, CH_2OH) (Figure S3). ESI-TOF mass spectrum: m/z 153.0 $(M\text{-}OH)^-$ (Figure S4).

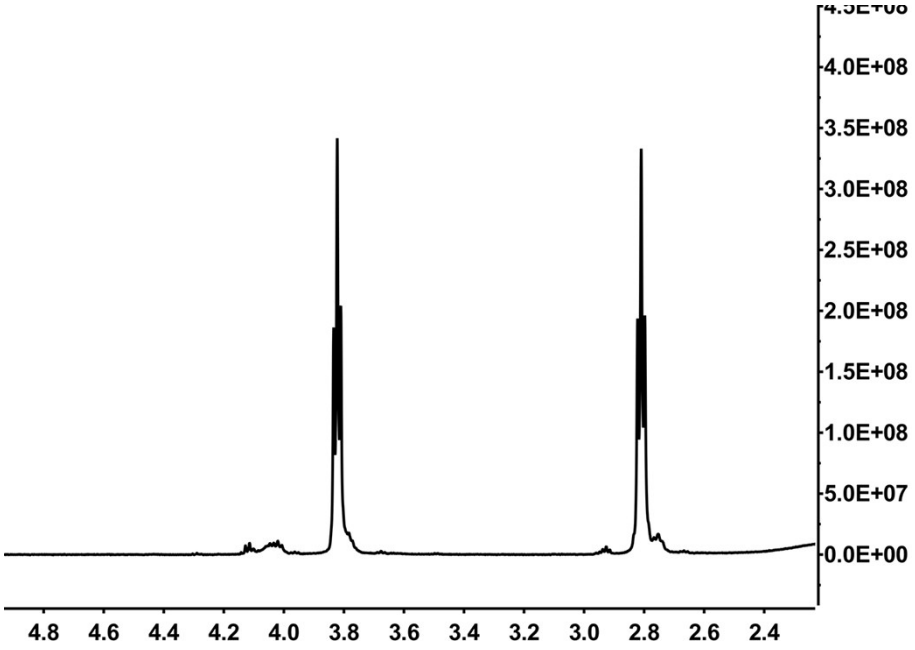

Figure S3. ^{1}H-NMR spectra of compound **2**.

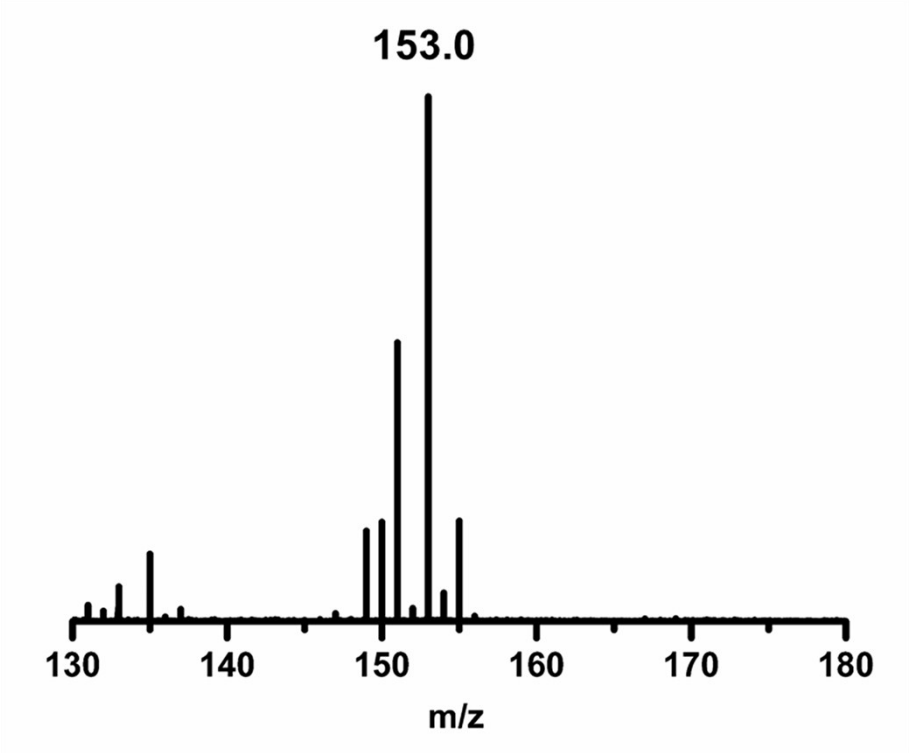

Figure S4. ESI-TOF mass spectrum of compound **2**

3. Synthesis of cucurbit[6]uril (CB[6])

Synthesis of cucurbit[6]uril (CB[6]). CB[6] was synthesized according to the method reported by Kim.[1] 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; Me_4Si) 4.32 (12 H, d, CH_2) 5.56 (12 H, s, CH) 5.71 (12 H, d, CH_2) (Figure S5).

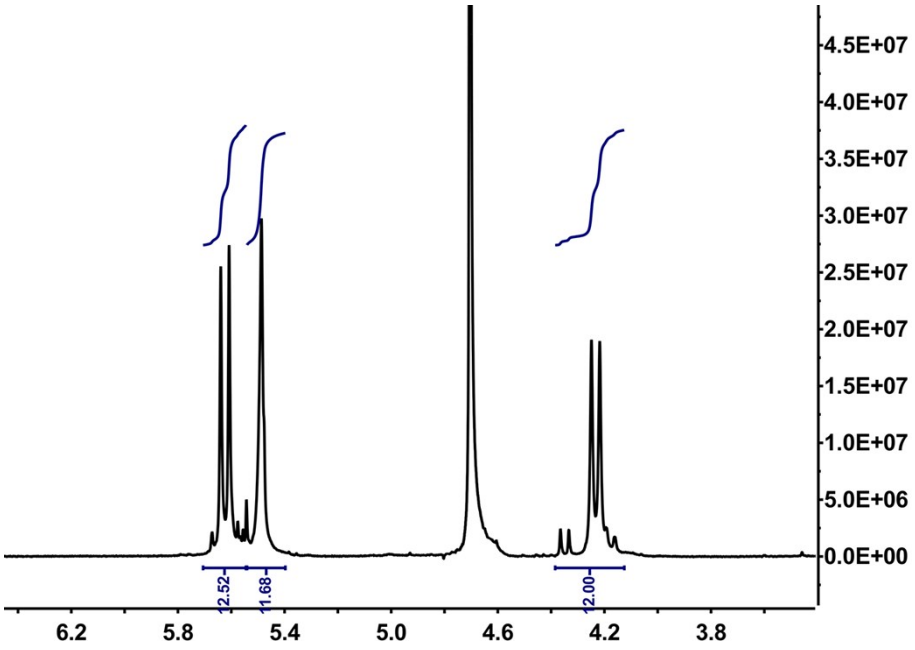

Figure S5: ^{1}H-NMR spectra of CB[6].

4. Acid-base titration experiment of compound 1

Compound **1** was dissolved in 10mL deionized water at the concentration of 1mmol/L at acid-base titration experiment. Concentrated hydrochloric acid was attenuated to 50mmol/L and added 10uL to the 10mL solution of compound **1** every time. pH of the mixtures was measured while hydrochloric acid was added by pH meter. Finally the acid-base titration curve of compound **1** was drew using amount of substance of the added hydrochloric acid as abscissa and pH of the mixtures as ordinate (Figure S6).

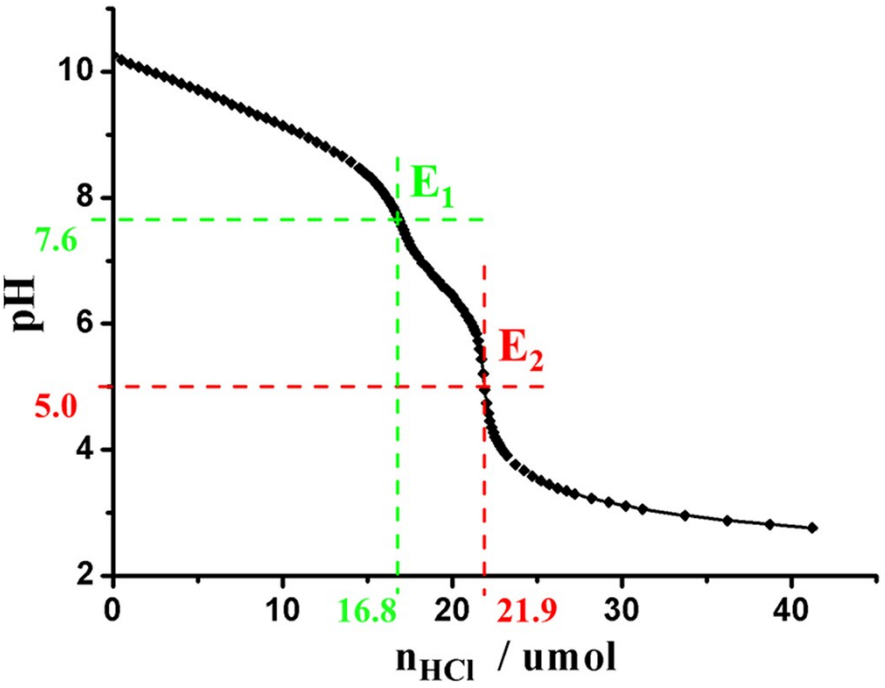

Figure S6: Acid-base titration curve of compound **1**.

There were two end points in the acid-base titration curve of compound **1**. The first end point (E_1) was at X_{E1}=16.8, Y_{E1}=7.9. The second end point (E_2) was at X_{E2}=21.9, Y_{E2}=5.0. So the abscissa of pKa_2 point was $X_2=X_{E1}/2$=8.4, the ordinate of the point was 9.3 according to the curve. So the pKa_2 of compound **1** was 9.3. In the same way, the abscissa of pKa_1 point was $X_1= (X_{E1}+X_{E2})/2$=19.4, the ordinate of the point was 6.5 according to the curve. So the pKa_1 of compound **1** was 6.5.

5. Mass spectrum of the partial dissociation of pseudo-rotaxane formed by compound 1 and CB[6] at pH=7.

The ^{1}H NMR spectrums of compound **1** and 1 equiv of CB[6] in D_2O at different pH (Figure 1B) showed that about 50% of the pseudorotaxane was dissociated when pH rised to 7. ESI mass spectrometry was also used to probe the partial dissociation of the pseudorotaxane. The mass spectrum (Figure S7) contained peaks of compound **1**, CB[6] and their pseudorotaxane. The peak of pseudorotaxane was at 421.4 (m/z) and the space of each isotopic peak was 0.33 (m/z) which correspond to (compound **1** + CB[6] + $3H^+$)$^{3+}$. The peak of CB[6] was at 537.5 (m/z) and the space of each isotopic peak was 0.5 (m/z) which correspond to (CB[6] + $2K^+$)$^{2+}$. The result further confirmed that the pseudorotaxane formed by compound **1** and CB[6] partial dissociated at pH=7.

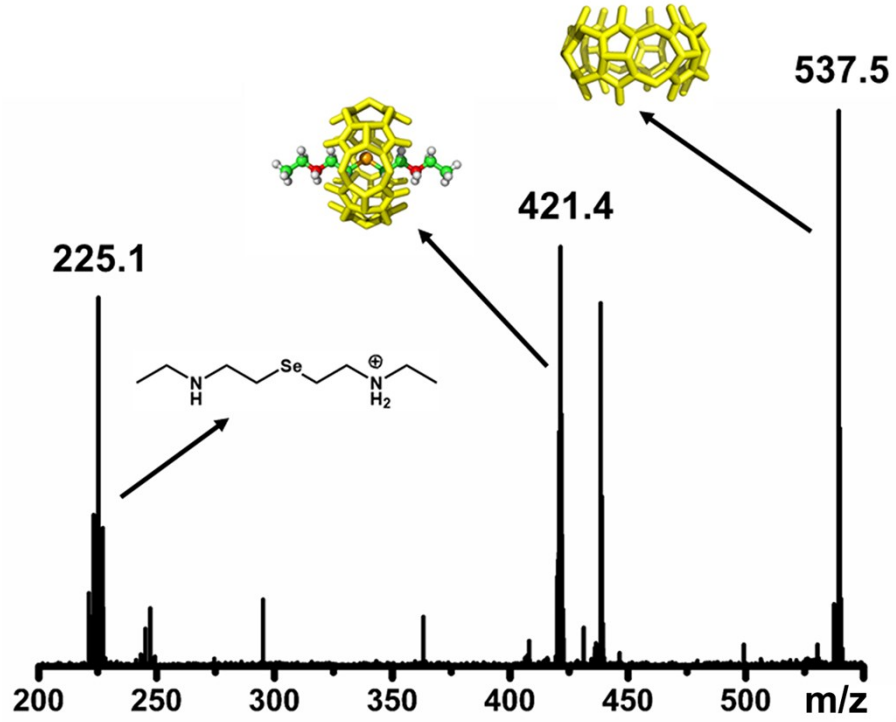

Figure S7. Mass spectrum of the partial dissociation of pseudorotaxane at pH=7.

6. ^{1}H NMR spectra of compound 1 at pH=6 and pH=11

The two nitrogen atoms of compound **1** were deprotonated at pH=6. In this case, the chemical shift of H_1 was 2.91ppm, H_2 was 3.35ppm, H_3 was 3.14ppm, H_4 was 1.30ppm (Figure S8C). However, the two nitrogen atoms of compound **1** were diprotonated at pH=11. As the two nitrogen atoms changed from deprotonated to diprotonated, the chemical shifts of H_1-H_4 had an obviously movement to upfield. H_1 moved from 2.91ppm to 2.72ppm, H_2 moved from 3.35ppm to 2.81ppm, H_3 moved from 3.14ppm to 2.61ppm, H_4 moved from 1.30ppm to 1.07ppm (Figure S8B). The chemical shifts of H_2 and H_3 had a relative big movement because they are near to the nitrogen atom. The chemical shifts of H_1 and H_4 had a relative small movement because they are relative far from the nitrogen atom. When pH rose to 11, most of the pseudorotaxane formed by compound **1** and CB[6] was dissociated. In this case, the peaks of free compound **1** at pH=11 can be observed (Figure S8A).

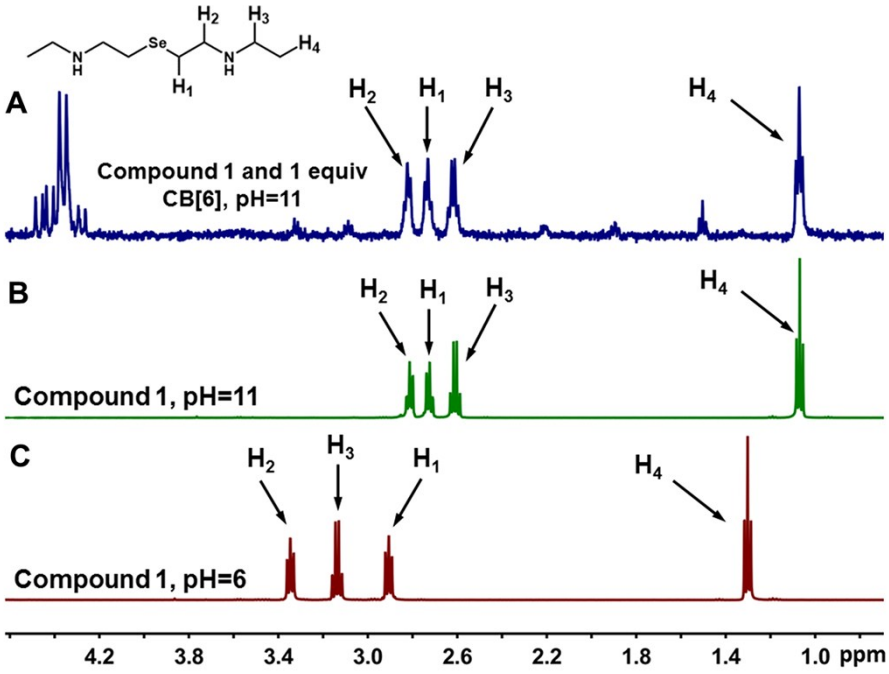

Figure S8. Partial ^{1}H NMR spectra (500 MHz) of A) compound **1** and 1 equiv of at pH=11. B) compound **1** at pH=11. C) compound **1** at pH=6.

7. Peroxidase activity investigation of compound 1

7.1 Catalytic activity of compound 1 investigated by TNB assay system

Catalytic activity of compound **1** was first investigated in an 3-carboxy-4-nitrobenzenethiol (TNB) assay system according to a modified method reported by Hilvert etal using TNB as a GSH alternative at 37 ˚C.[2] The assay mixture contained 50 mM phosphate buffer, pH 7.0, 80 μM TNB, 250 μM hydrogen peroxide (H_2O_2) and 250 - 1000 μM compound **1**. Catalytic curves of different concentration of compound **1** were tested (Figure S9). The result indicated that the slope of catalytic curves has a linear increase with the increase of concentration of compound **1**. The GPx activity was calculated to be $1.93*10^{-3}$ μmol•min^{-1}•$μmol^{-1}$ at TNB assay system.

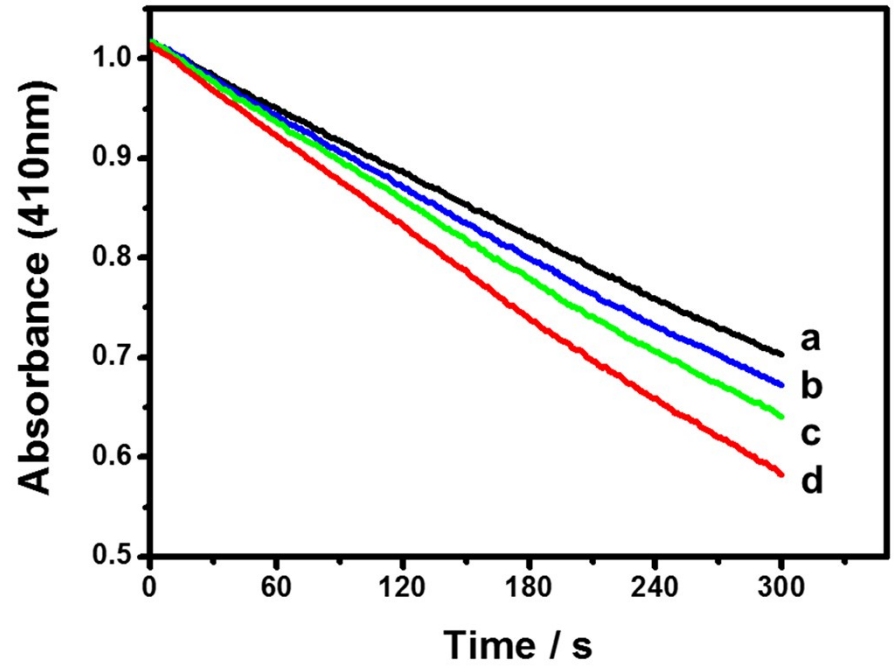

Figure S9. Catalytic curves of different concentration of compound **1** by TNB assay system. (a) negative control containing no compound **1**; (b) 250uM compound **1**; (c) 500uM compound **1**; (d) 1mM compound **1**.

7.2 Catalytic activity of compound 1 investigated by GSH reductase-reduced NADPH coupled assay system

Catalytic activity of compound **1** was also investigated using a GSH reductase-reduced nicotinamide adenine dinucleotide phosphate (NADPH) coupled assay.[3] The assay mixture contained 50 mM phosphate buffer, pH 7.0, 1 mM GSH, 500 μM hydrogen peroxide (H_2O_2), 1U glutathione reductase (GR), 300 μM NADPH and 250 - 1000 μM compound **1**. The result was similar to the result of TNB assay system (Figure S10). The GPx activity was calculated to be $4.2*10^{-2}$ μmol•min^{-1}•$μmol^{-1}$ at this system. The activity of compound **1** was relative low compared to native GPx (the activity of native pGPx which exist in human plasma is as high as 302μmol•min^{-1}•$μmol^{-1}$).[4] But it reached the same magnitude level of other organoselenium compounds. For example, in contrast to diphenyl diselenide (PhSeSePh), a typical GPx mimic, the activity of compound **1** even increases by an order of magnitude.

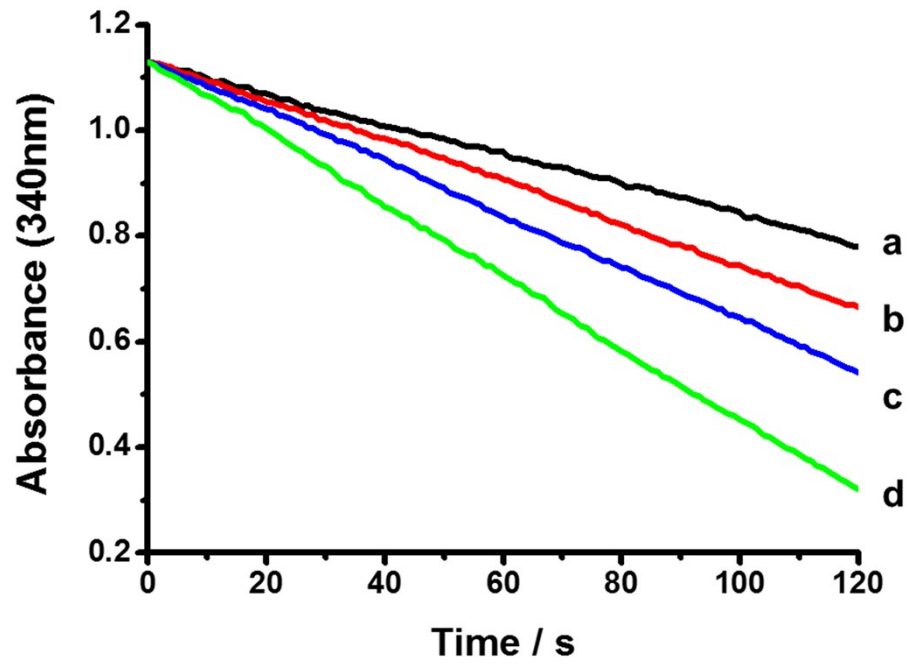

Figure S10. Catalytic curves of different concentration of compound **1** by GSH reductase-reduced NADPH coupled assay. (a) negative control containing no compound **1**; (b) 250uM compound **1**; (c) 500uM compound **1**; (d) 1mM compound **1**.

7.3 Apparent kinetic parameters of compound 1

The double-reciprocal plots of the initial velocity versus substrate concentration were tested by fixing one substrate concentration and changing another substrate concentration. The apparent kinetic parameters which calculated by double-reciprocal plots are listed below (Table S1).

Table S1. Apparent kinetic parameters for H_2O_2 reduction by GSH catalyzed by compound **1**.

[GSH] (mM)	k_{cat} ($\times 10^{-2}$ min^{-1})	$K_{M\ H2O2}$ (M)	$k_{cat}/K_{M\ H2O2}$ ($\times 10^{-2}$ $M^{-1}min^{-1}$)
0.25	8.97	5.58	1.61
0.5	4.44	0.99	4.49
0.75	3.88	0.37	10.6
[H_2O_2] (mM)	**k_{cat} ($\times 10^{-2}$ min^{-1})**	**$K_{M\ GSH}$ (M)**	**k_{cat}/K_{MGSH} ($\times 10^{-2}$ $M^{-1}min^{-1}$)**
0.5	18.3	6.33	2.89
0.75	8.89	1.76	5.06
1	6.71	1.03	6.54

8. Influence of catalytic activity by formation and dissociation of pseudorotaxane

8.1 Decrease of catalytic activity versus mixtures of compound 1 with different amounts of CB[6] at pH=6

Upon the addition of CB[6] the peroxidase activity of compound **1** decrease progressively as they form pseudorotaxane at pH=6 (Figure S11).

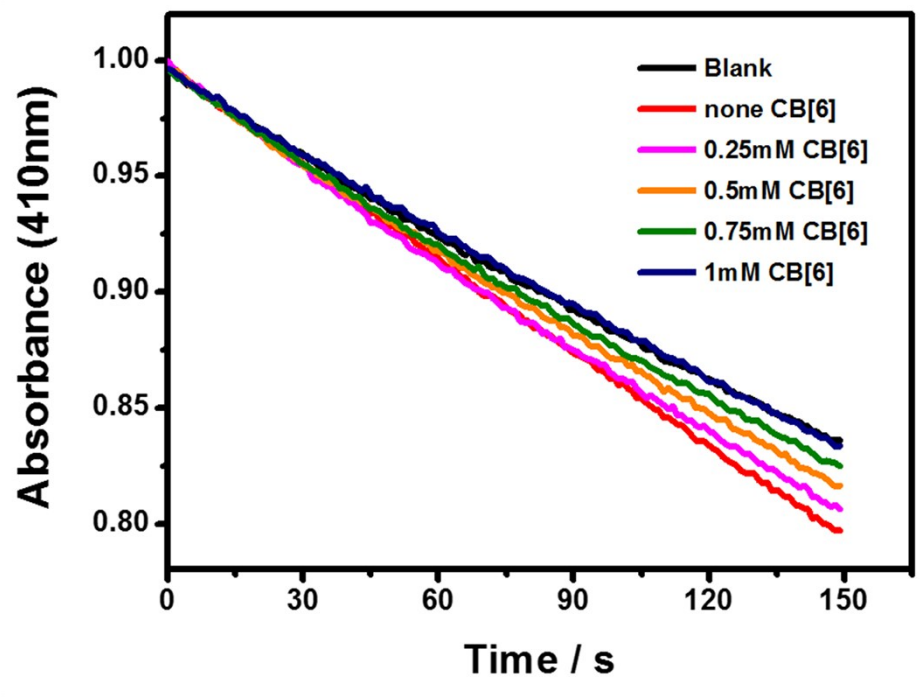

Figure S11. Catalytic curves of compound **1** (1mM) and mixtures of compound **1** (1mM) with different amounts of CB[6] at pH=6.

8.2 Decrease of catalytic activity versus mixtures of compound 1 with different amounts of CB[6] at pH=7

There was also a progressive decrease of the peroxidase activity of compound **1** upon the addition of CB[6] at pH=7. But after 1 equiv CB[6] was added there was still more than 50% activity compared to compound **1** that did not contain any CB[6] (Figure S12).

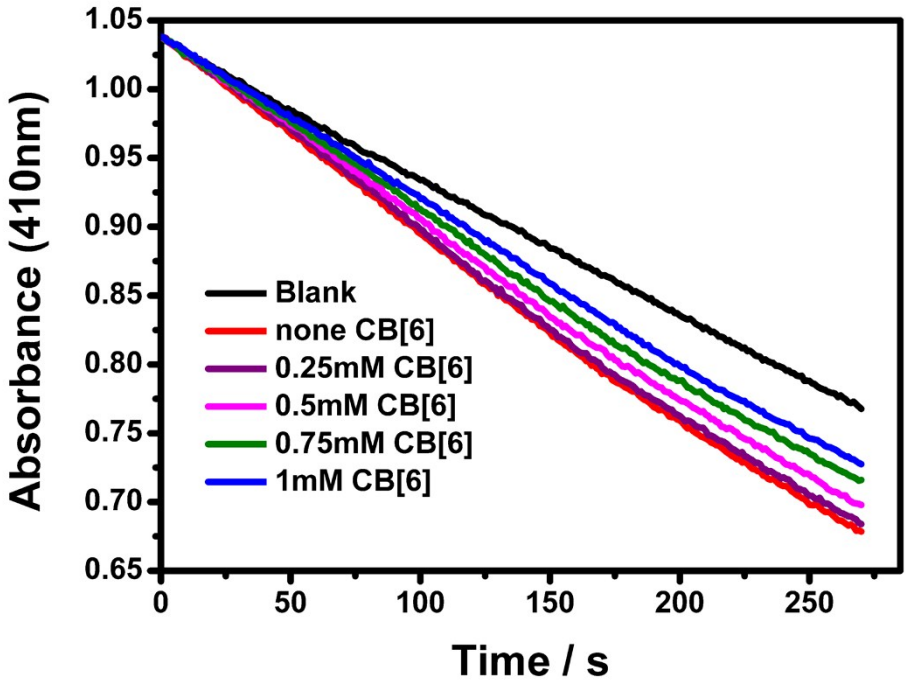

Figure S12. Catalytic curves of compound **1** (1mM) and mixtures of compound **1** (1mM) with different amounts of CB[6] at pH=7.

9. Influence of catalytic activity of compound 2 by adding CB[6]

9.1 Catalytic curves of mixtures of compound 2 with 1 equiv of CB[6] at different pH.

There is no specific binding between CB[6] and compound **2**, so the catalytic activity of compound **2** had no significant change after 1 equiv CB[6] was added (Figure S13).

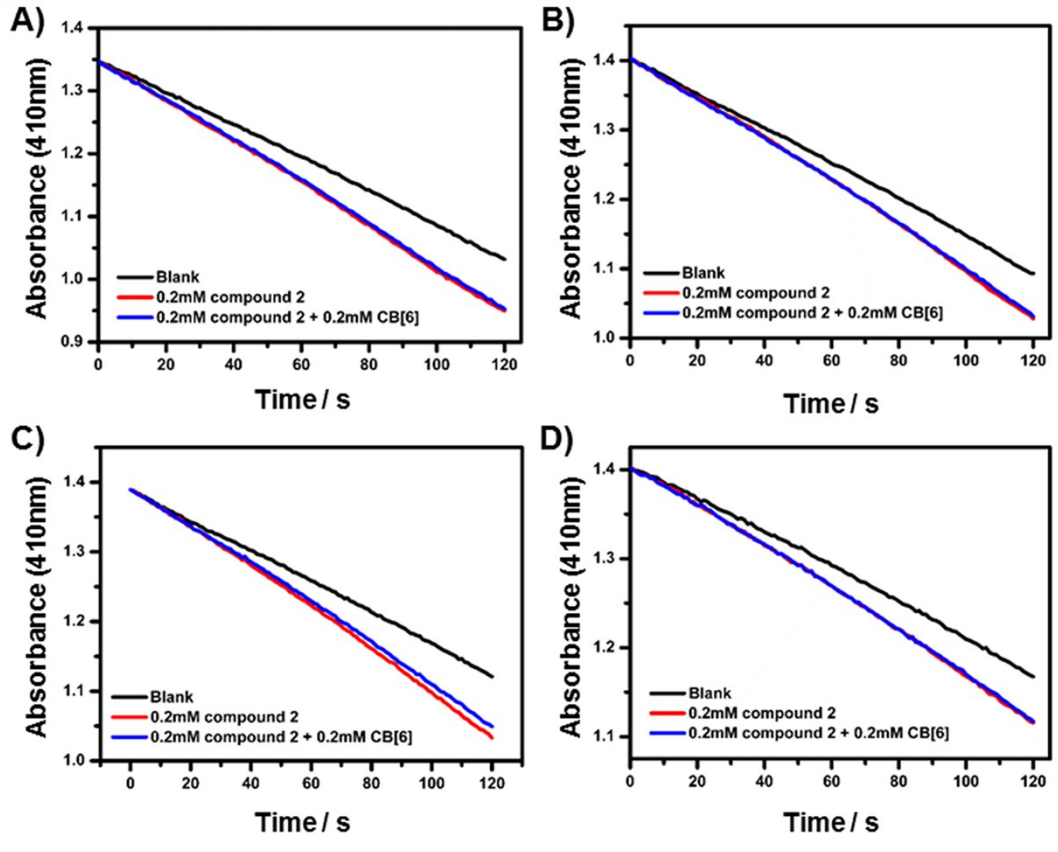

Figure S13. Catalytic curves of mixtures of compound **2** (0.2mM) with 1 equiv of CB[6] at A) pH=6; B) pH=7; C) pH=8; D) pH=9.

9.2 Relative catalytic activities of mixtures of compound 2 with 1 equiv of CB[6]

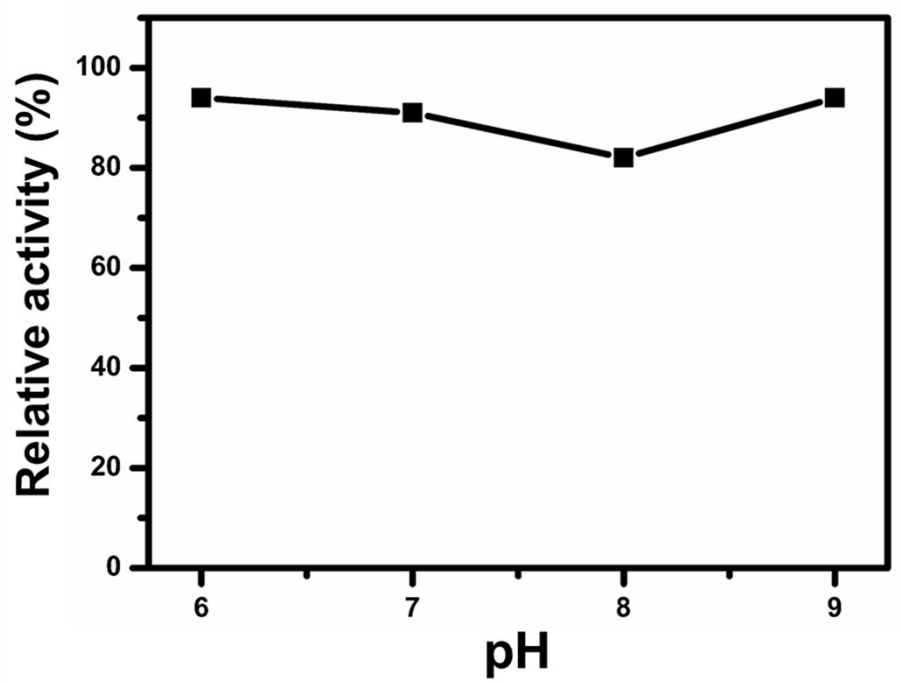

Figure S14. Relative catalytic activities of mixtures of compound **2** (0.2mM) with 1 equiv of CB[6] at a range of pH from 5 to 9. The activity of compound **2** without any CB[6] was defined as 100%.

References

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[2] Bell, I. M. and Hilvert, D. *Biochemistry*, 1993, **32**, 13969.

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[4] Chu, F. F.; Doroshow, J. H. and Esworthy, R. S. *J. Biol. Chem.* 1993, **268**, 2571.