Synthesis of Organoselenium-Modified β-Cyclodextrins Possessing a 1,2-Benzisoselenazol-3(2*H*)-one Moiety and Their Enzyme-Mimic Study

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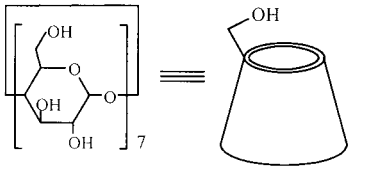
Six novel H_2O-soluble β-cyclodextrin derivatives containing a 1,2-benzisoselenazol-3(2*H*)-one moiety were synthesized by a convenient method in 25–60% yield and characterized by MS, elemental analysis, IR, ^{1}H-NMR, and UV/VIS spectroscopy. The conformations of these β-cyclodextrin derivatives **1**–**6** were analyzed by circular dichroism and fluorescence-lifetime experiments. The superoxide dismutase (SOD) activities of **1**–**6** were determined by auto-oxidation of pyrogallol at 25.0° in buffer solution (pH 8.2), giving relatively high SOD activities of up to 121–330 U/mg. Also, the glutathione peroxidase (GPX) activities of hosts **1**–**6**, determined by the method of *Wilson* at 37° in buffer solution (pH 7.0), show good GPX activities in the range of 0.34–0.86 U/μmol. The mimicking results of the bifunctional artificial enzyme models **1**–**6** were globally compared with regard to their structural and conformational difference.

Introduction. – Imitation of the highly effective catalysis by natural enzymes is currently a significant topic in chemistry and biochemistry [1][2]. A good artificial enzyme model usually has two prerequisites: one is the catalytically active center, the other is a substrate-binding-site group. Cyclodextrins possessing well-defined hydrophobic cavities can selectively bind various guests to form host-guest complexes or supramolecular species, which have been employed successfully as excellent models to mimic various enzymes [3–6]. A general method to design an effective enzyme model based on a cyclodextrin is the introduction of a suitably modified moiety at the primary or secondary rim of the cyclodextrin cavity, which can act as the catalytic functional group in an artificial mimic, accelerating the reactions of substrates accommodated in the cyclodextrin cavity. Recently, the biomimetic reactions catalyzed by cyclodextrins and their derivatives have been reviewed comprehensively by *Breslow* and *Dong* [6]. However, artificial imitation of superoxide dismutase and glutathione peroxidase with cyclodextrin derivatives has rarely been reported, except for the recent studies by *Ni* and co-workers [7] and *Shen* and co-workers [8].

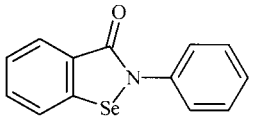
Superoxide dismutases (SODs, EC 1.15.1.1) are metalloenzymes that can protect cells from the highly reactive $O_2^{\bullet-}$ radical by accelerating the disproportionation reaction $2\,O_2^{\bullet-} + 2\,H^+ \rightarrow H_2O_2 + O_2$. Glutathione peroxidase (GPX, EC 1.11.1.9) is a selenoenzyme, which can prevent biomembranes from oxidative damage by catalyzing the reaction $2\,GSH + ROOH \rightarrow ROH + GSSG$ (GSH = glutathione). Both enzymes belong to the antioxidative enzyme family that can cooperatively compose the antioxidative defence system to dispose of free radicals and reactive oxygen species, which may be the main causes of aging, cardiovascular, tumorous, and some endemic diseases [9]. To obtain novel medicines, a great deal of effort has been devoted to the artificial imitation of GPX [10–14]. Some small molecular mimics like ebselen (PZ51) have been used as successful clinical medicines. So far, the most effective mimics are

Wilson's models, the GPX activities of which are approximately 10-fold that of PZ51 [12]. Recently, *Shen* and co-workers [8] reported that the GPX activity of a selenium-bridged bis(β-cyclodextrin) mimic is also up to 4.3 times that of PZ51. The common feature of these GPX mimics is that there exists a catalytic cycle of selenium in the catalysis procedure [12]. However, the artificial imitations of SOD have mainly focused on metal complexes of the macrocyclic compounds [15]. Extensive studies toward SOD imitation demonstrated that there is a catalytic cycle of copper in the disproportionation reaction catalyzed by natural Cu, Zn–SOD [16]. No report has appeared about imitating SOD by organoselenium-modified cyclodextrin derivatives.

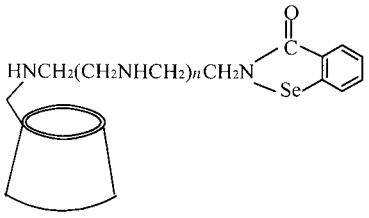
Although ebselen possessing a high GPX activity has been employed as an excellent selenoenzyme model and an effective drug, its low solubility in H_2O limits its utility. In the present study, we introduced the 1,2-benzisoselenazol-3(2*H*)-one moiety at the primary rim of β-cyclodextrin, thus obtaining the bifunctional enzyme models **1**–**6** which can simultaneously imitate SOD and GPX. As expected, the synthesized mimics **1**–**6** display excellent solubility in H_2O, with the exception of **5**. The SOD and GPX activities of these modified β-cyclodextrins were determined by autooxidation of pyrogallol at 25.0° in buffer solution (pH 8.2) and as described by *Wilson et al.* [12] at 37° in buffer solution (pH 7.0), respectively. The mimicking results of the bifunctional



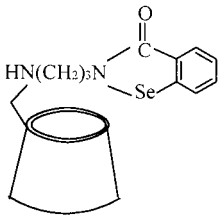
β-CD



Ebselen (PZ51)



1 $n = 0$
2 $n = 1$
3 $n = 2$



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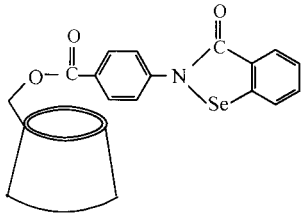

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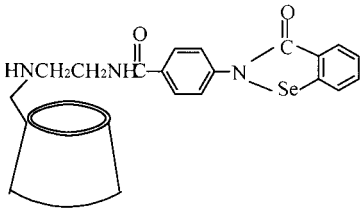

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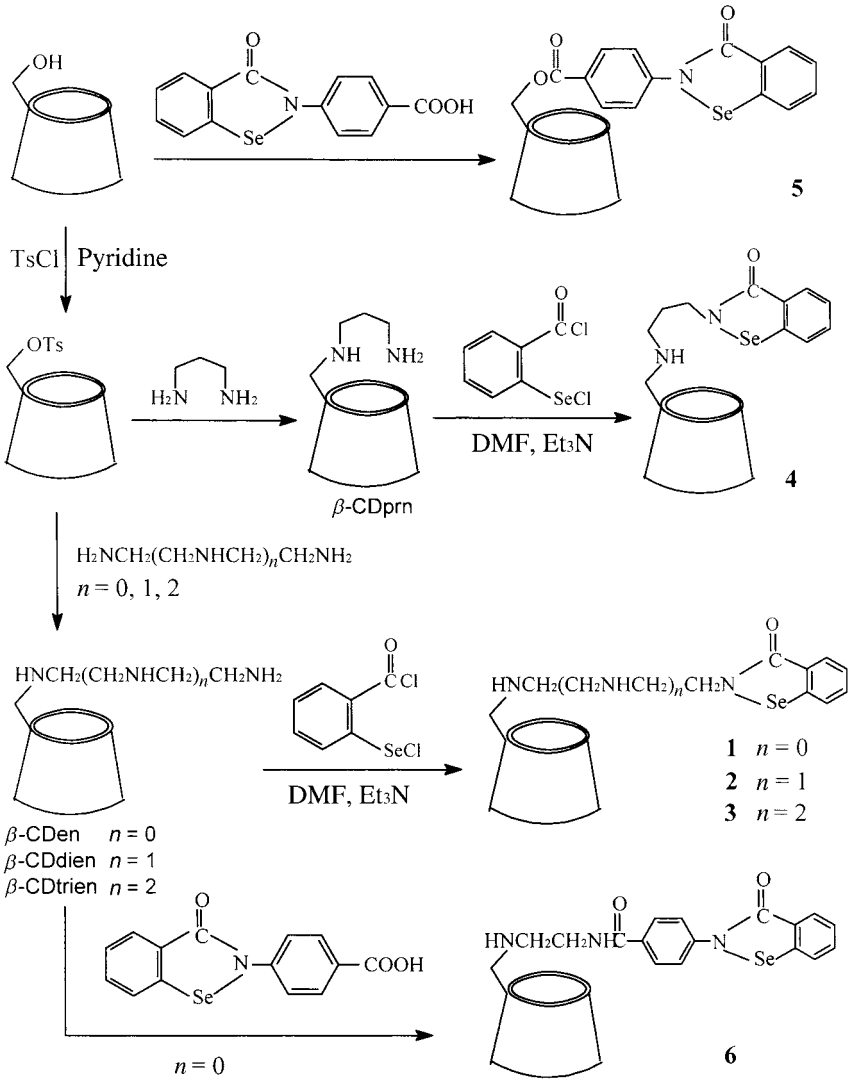
artificial enzyme models were discussed with regard to their structural and conformational differences.

Results and Discussion. – *Synthesis.* The modified β-cyclodextrins **1**–**4** and **6** were synthesized in satisfactory yields from 6-*O*-monotosyl-β-cyclodextrin, while the modified β-cyclodextrin derivative **5** was obtained by esterification of the 6-hydroxy group of β-cyclodextrin (see *Scheme*).

Conformational Analysis by Means of CD Spectra and Fluorescence Lifetimes. As can be seen from the *Figure*, the induced circular dichroism (ICD) spectra of modified

Scheme



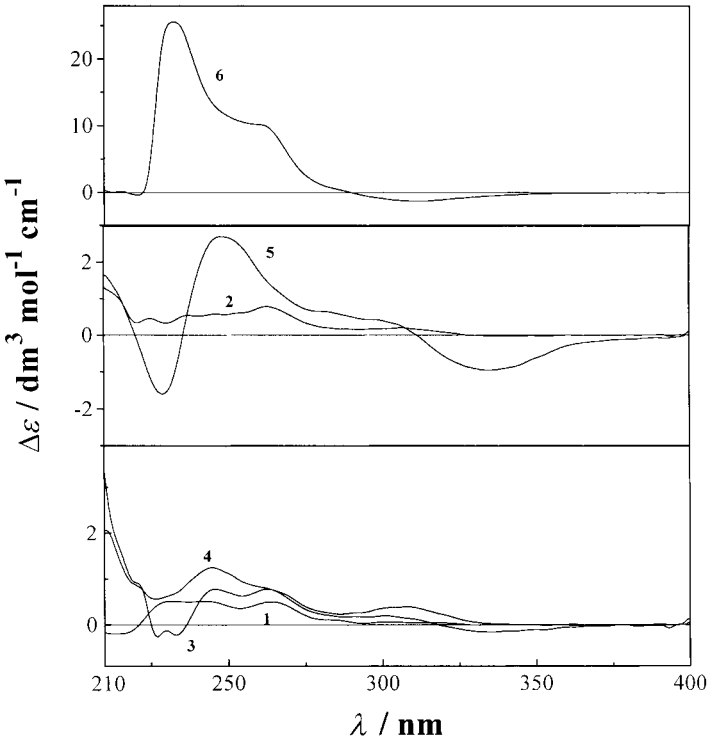


Figure. *Circular dichroism of mimics* **1**–**6** (0.1 mM) *in phosphate buffer solution* (pH 7.2) *at 25°*

β-cyclodextrins **1**–**4** and **6** in buffer solution show two positive *Cotton* effects at around 232–246 nm ($\Delta\varepsilon = 0.51 - 25.56$) and 259–264 nm ($\Delta\varepsilon = 0.50 - 10.21$). According to the results of *Harata* and *Uedaira* [17] and *Kodaka* [18], these positive *Cotton* effects indicate that the 1,2-benzisoselenazol-3(2*H*)-one moiety lies outside the chiral cavity of β-cyclodextrin, and both of the corresponding transition moments may be perpendicular to the axis of β-cyclodextrin cavity. In contrast, the ICD spectrum of β-cyclodextrin derivative **5** displays one negative *Cotton* effect at 229 nm ($\Delta\varepsilon = -1.59$) and a positive *Cotton* effect at 248 nm ($\Delta\varepsilon = 2.70$). It may be inferred that the modifying moiety is located inside the chiral cavity of β-cyclodextrin, thus the transition moments at 229 nm may be perpendicular to the axis of the β-cyclodextrin cavity, and the transition moments at 248 nm may be in parallel alignment [19].

To further analyze and confirm the conformations of the modifying moiety of **1**–**6**, time-resolved fluorescence decay of 8-anilinonaphthalene-1-sulfonate (ANS) was investigated in the presence and absence of the β-cyclodextrin derivatives. Since the rates of complexation/decomplexation are much lower than that of the fluorescence decay, the decay profile of fluorescence intensity ($F(t)$) can be described by *Eqn. 1* as a sum of unimolecular decays for all fluorescing species present in the solution, where A_i and τ_i represent the initial abundance and lifetime of the *i*th fluorescing species.

$$F(t) = \sum_{i=1}^{n} A_i \exp(-t/\tau_i) \quad (n = 1,2, \textit{etc.}) \tag{1}$$

In the absence of the host, the observed decay profile of ANS fluorescence is absolutely single-exponential in the aqueous phosphate buffer, giving a short lifetime of 0.4 ns for free ANS in the bulk solution [20]. In contrast, the decay profile in the presence of native β-cyclodextrin or modified β-cyclodextrins **1**–**4** and **6** is successfully analyzed only by a linear combination of two exponential functions, affording a short (0.5–1.5 ns) and a long (3.1–8.6 ns) lifetime assigned to free and included ANS, respectively. However, the decay curve obtained with added **5** is well-fitted to a single-exponential function, giving short lifetimes (0.6 ns). The fluorescence lifetimes and relative quantum yields thus obtained are summarized in *Table 1*. The longer lifetime of ANS (3.1–8.6 ns) in the presence of native β-cyclodextrin or mimics **1**–**4** and **6** is reasonably accounted for in terms of a more hydrophobic environment around the ANS molecule included in the cavity. Since the hydrophobic moieties of these modified β-cyclodextrins may enhance the microenvironmental hydrophobicity of host compounds, the elongated lifetimes (7.2–8.6 ns) of ANS in the presence of modified cyclodextrins are much longer than that in the presence of native β-cyclodextrin (3.1–3.2 ns). Interestingly, the addition of an excess amount of **5** does not appreciably alter the original lifetime (0.4 ns) obtained in the bulk solution. This somewhat unexpected result clearly indicates that the modified group self-included in the cavity of **5** prevents the inclusion of ANS.

Table 1. *Short and/or Long Fluorescence Lifetimes* (τ_S, τ_L) *and Relative Quantum Yields* (Φ_S, Φ_L) *for 8-Anilinonaphthalene-1-sulfonate* (ANS) *in the Presence and Absence of Host Compounds* **1**–**6** *in Aqueous Phosphate Buffer Solution* (pH 7.20; 0.1M) *at 25.0°*

ANS conc./μM	Host	Host equiv.	τ_S/ns	Φ_S/%	τ_L/ns	Φ_L/%	χ^2
10	none		0.4	100			1.42
500	none		0.4	100			1.46
10	β-CD	40	0.5	96.5	3.1	3.5	1.00
250	β-CD	10	1.5	67.6	3.2	32.4	1.24
10	**1**	21	0.8	87.0	8.6	13.0	1.38
10	**2**	21	1.5	76.8	8.1	23.2	1.51
10	**3**	21	0.7	80.5	7.2	19.5	1.48
10	**4**	22	1.1	83.6	7.9	16.4	1.32
10	**5**	22	0.6	100			1.04
10	**6**	22	0.8	79.9	8.3	20.1	1.17

SOD Activities of Mimics **1**–**6**. The superoxide dismutase reaction can be expressed as by *Eqn. 2*. The SOD activity can be calculated according to *Eqn. 3*, where $V_{total}/V_{defined} = 1:1$, v and v' are the auto-oxidation speed of pyrogallol in the absence and presence of mimics (or natural SOD enzyme), respectively, $T_{dilution}$ indicates the times of enzyme-mimic dilution, *i.e.* $T_{dilution} = V_{total}/V_{enzyme}$, and V_{enzyme} is the volume of the natural-enzyme or mimic solution. The results obtained are listed in *Table 2*. When repeated measurements were made, the SOD activity value was reproducible within an error of ± 5%.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2 \tag{2}$$

$$\text{SOD activity [U/ml]} = \frac{\dfrac{v - v'}{v}}{50\%} \cdot \frac{V_{total}}{v_{defined}} \cdot \frac{T_{dilution}}{V_{enzyme}} \tag{3}$$

Table 2. *SOD Activities of Mimics* **1**–**6**, *of a Natural SOD Enzyme Sample, as well as of Native and Oligoamino-Modified β-Cyclodextrins*

	Activity/U/mg	Activity/U/μmol
β-CD	0	0
β-CDen	0	0
β-CDdien	0	0
β-CDtrien	0	0
β-CDprn	0	0
1	179	269
2	330	510
3	221	343
4	206	301
5	121	184
6	162	262
natural SOD enzyme sample	3400	–

As can be seen from *Table 2*, native and oligoamino-modified β-cyclodextrins (including β-CDen, β-CDdien, β-CDtrien, β-CDprn; see *Scheme*) do not show SOD activities at all, which clearly indicates that the β-cyclodextrin cavity acts only as a substrate-binding site. All of the mimics **1**–**6** with the organoselenium moiety at the primary rim of β-cyclodextrin display good SOD activities in the range of 121–330 U/mg. The SOD activity of the best mimic **2** is one tenth of that of natural SOD from bovine erythrocytes. These results indicate that the organoselenium moiety is the catalytic group of these mimics. As was reported in the previous study [14], we may deduce that the underlying principle of the SOD activities of these mimics is the similarity in catalytic cycles of selenium and copper in the mechanism.

It is very interesting to compare the SOD activities of mimics possessing similar structures. For mimics **1**–**4**, the unique difference is the oligoamino chain linking the β-cyclodextrin cavity and the 1,2-benzisoselenazol-3(2*H*)-one moiety. As shown in *Table 2*, SOD activities of mimics **1**–**4** are in the following sequence: **2** > **3** > **4** > **1**, which obviously can be roughly correlated to the length of the oligoamino chain: **3** > **2** > **4** > **1**. Due to the difference of length and flexibility of the oligoamino chain, mimics **1**–**4** possess different conformations, as analyzed by the circular-dichroism and fluorescence-lifetime experiments. One possible explanation for the highest SOD activity of mimic **2** is that there may exist the best cooperation between the catalytic group (the 1,2-benzisoselenazol-3(2*H*)-one moiety) and the substrate-binding site (the β-cyclodextrin cavity), due to the suitable length/flexibility of the oligoamino chain of **2**. Mimics possessing either a longer (**3**) or a shorter (**1**, **4**) oligoamino chain show lower SOD activities than mimic **2**, because they cannot adopt the excellent cooperative relationship of **2** between the organoselenium catalytic group and the β-cyclodextrin cavity.

Both mimics **5** and **6** display relatively low SOD activities (see *Table 2*). Indeed, the self-included moiety in **5** prevents the inclusion of the substrates, which explains why **5** shows the lowest SOD activity of all of the mimics. However, it is somewhat unexpected that mimic **6** with the catalytic group located outside the β-cyclodextrin cavity also exhibits a similarly low SOD activity. This may be attributed to the rigid benzene-ring

linker between the 1,2-benzisoselenazol-3(2*H*)-one and oligoamino moiety, which might prevent the catalytic group from approaching the β-cyclodextrin cavity.

GPX Activities of Mimics **1**–**6**. The GPX activities of **1**–**6**, ebselen, and native and oligoamino-modified β-cyclodextrins were determined by the decrease of the NADPH absorption at 366 nm (see *Table 3*). When repeated measurements were made, the GPX activity value was reproducible within an error of $\pm$ 5%.

Table 3. *GPX Activities of Mimics* **1**–**6**, *of Ebselen, as well as of Native and Oligoamino-Modified* β-*Cyclodextrins*

	Activity/U/mg	Activity/U/μmol	Ref.
Ebselen (PZ51)	3.61	0.99	[12]
β-CD	0	0	[a])
β-CDen	0	0	[a])
β-CDdien	0	0	[a])
β-CDtrien	0	0	[a])
β-CDprn	0	0	[a])
1	0.25	0.37	[a])
2	0.28	0.43	[a])
3	0.31	0.48	[a])
4	0.26	0.38	[a])
5	0.22	0.34	[a])
6	0.53	0.86	[a])

[a]) This work.

Ebselen (PZ51) is one of the best small molecular mimics of GPX, but its low solubility in H_2O limits its use (see above). On the contrary, our β-cyclodextrins **1**–**6**, carrying a 1,2-benzisoselenazol-3(2*H*)-one moiety, are GPX mimics with excellent solubility in H_2O. Native and oligoamino-modified β-cyclodextrins (including β-CDen, β-CDdien, β-CDtrien, and β-CDprn) do not possess GPX activities, indicating that the β-cyclodextrin cavity acts only as a substrate-binding site. On the contrary, all of the organoselenium mimics **1**–**6** display moderate to good GPX activities in the range of 0.34–0.86 U/μmol, these values being somewhat lower than that of ebselen. Therefore, we can deduce that the organoselenium moiety plays the role of the catalytic center. It is interesting to investigate the relationship between the GPX activity and the structure of these mimics. In mimics **1**–**4**, only the 1,2-benzisoselenazol-3(2*H*)-one moiety, the most important part of ebselen, is located at the primary face of β-cyclodextrin. Nevertheless, the GPX activities of **1**–**4** are lower than that of ebselen, only 0.37–0.48 U/μmol. It can be inferred that the 2-phenyl group in ebselen might play an important role in its GPX activity. Thus, we synthesized mimic **5** possessing the whole structure of ebselen. Unfortunately, the GPX activity of mimic **5** is very weak, only 0.34 U/μmol. One possible explanation for this low activity is the self-inclusion of the modifying moiety, which is suggested by the lower solubility in H_2O and the results of the ICD and fluorescence-lifetime experiments. To obtain a well H_2O-soluble compound possessing the ebselen moiety that is well-soluble in H_2O, we designed and synthesized mimic **6** for which the results of the ICD and fluorescence-lifetime experiments established the absence of self-inclusion of the modifying moiety. As

expected, mimic **6** is easily dissolved in H_2O and shows the highest GPX activity of our mimics, up to 0.86 U/μmol, which is close to the value of ebselen (0.99 U/μmol).

Previous studies have demonstrated that there is a catalytic cycle involving organoselenium compounds as artificial GPX mimics [12][14]. Therefore, we can reasonably propose a similar cycle in the catalytic mechanism of mimics **1**–**6**, which may be the original cause of their GPX activities. Particularly, that **6** exhibits the highest GPX activity suggests that the 2-phenyl group in ebselen is very important for the high GPX activity of the latter.

Conclusion. – The above results indicate that the artificial enzyme models **1**–**6** can perform the bifunctional imitation of SOD and GPX, giving good mimicking activities. Since these enzyme models possess excellent solubilities in H_2O, they can be used as a novel medicine in place of ebselen.

Experimental Part

General. β-Cyclodextrin of reagent grade (*Suzhou Monosodium Glutamate Works*) was recrystallized twice from H_2O and dried *in vacuo* for 12 h at 100°. *N*,*N*-Dimethylformamide (DMF) was dried over CaH_2 for 2 days and then distilled under vacuum prior to use. Ethane-1,2-diamine, propane-1,3-diamine, diethylenetriamine (= *N*-(2-aminoethyl)ethane-1,2-diamine), and triethylene-tetramine (= *N*,*N*′-bis(2-aminoethyl)ethane-1,2-diamine) were dried over K_2CO_3 and then distilled under vacuum prior to use. 2-(Chloroseleno)benzoyl chloride was prepared according to [21]. Superoxide dismutase (from bovine erythrocytes, lyophilized powder) was obtained from *Sigma Chemical Company.* Commercially available *Tris*·HCl (*Beijing Yiji Chemical Company*), dimethylarsinic acid sodium salt (*Shanghai Chemical Regents Plant*), diethylenetriamine-pentaacetic acid (= *N*,*N*-bis{2-[bis(carboxymethyl)amino]ethyl}glycine; *Beijing Chemical Plant*), and pyrogallol (= benzene-1,2,3-triol; *Zhejiang Wenzhou Ouhai Chem. Plant*) were used without further purification. Glutathione (GSH), NADPH, and glutathione reductase (type III) were purchased from *Sigma*. The superoxide dismutase (SOD) activities and glutathione peroxidase (GPX) activities were measured with a *Shimadzu UV-2401* spectrometer. FT-IR Spectra: *Nicolet FT-IR-5DX*; in cm^{-1}. Circular dichroism (CD) spectra: *Jasco J-720W* spectropolarimeter. ^{1}H-NMR Spectra: at 200 MHz in $(D)_6$DMSO; *Bruker AM200* spectrometer; δ in ppm. Elemental analyses: *Perkin-Elmer 240* instrument.

4-(2,3-Dihydro-3-oxo-1,2-benzisoselenazol-2-yl)benzoic Acid. To a stirred soln. of 4-aminobenzoic acid (5 mmol) in Et_2O (50 ml) in an ice bath, 2-(chloroseleno)benzoyl chloride (5 mmol) in Et_2O (25 ml) and Et_3N (10 mmol) in Et_2O (25 ml) were added dropwise simultaneously within 0.5 h. Then the mixture was heated to r.t. with stirring for an additional 3 h. The precipitate was filtered off and washed with Et_2O, 1M HCl, and H_2O. The crude product was dissolved in 0.5M aq. Na_2CO_3 (10 ml), the soln. then filtered, the filtrate acidified with HCl soln. to pH < 1, and the precipitate filtered off, washed with H_2O, and dried *in vacuo:* white solid (85% yield). M.p. > 300° ([22] > 300°).

6-Deoxy-6-{[2-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)ethyl]amino}-β-cyclodextrin (**1**). The 6-*O*-[(4-methylphenyl)sulfonyl]-β-cyclodextrin (6-*O*-Ts-β-CD) was prepared from β-cyclodextrin with TsCl in dry pyridine [23]. The 6-[(2-aminoethyl)amino]-6-deoxy-β-cyclodextrin (β-CDen) was prepared from 6-*O*-Ts-β-CD and ethane-1,2-diamine [24]. Then, Et_3N (0.2 ml) was added to the soln. of β-CDen (0.7 g, 0.6 mmol) in dry DMF (50 ml) in an ice bath, with stirring and under N_2. Then, the soln. of 2-(chloroseleno)benzoyl chloride [21] (0.15 g, 0.6 mmol) in dry DMF (25 ml) was added dropwise, and the soln. was stirred for another 5 h at 5°. After evaporation, the yellow solid was dissolved in a minimum amount of hot H_2O, and then the soln. was poured into acetone (100 ml). The precipitate formed was filtered to give a yellow powder. This crude product was purified by column chromatography (*Sephadex G-25*, distilled, deionized H_2O): pure **1** (0.5 g, 60%). IR (KBr): 3399.5, 2914.0, 1702.6, 1635.2, 1595.6, 1541.2, 1520.9, 1444.0, 1418.3, 1381.0, 1343.9, 1298.8, 1272.4, 1238.9, 1154.4, 1074.5, 1022.6, 999.3, 940.1, 887.2, 839.6, 750.0, 701.1, 646.9, 604.7. ^{1}H-NMR: 2.9–4.1 (*m*, 44 H); 4.2–4.6 (*m*, 2 H); 4.8–5.1 (*m*, 7 H); 5.6–6.0 (*m*, 14 H); 7.3–8.0 (*m*, 4 arom. H). FAB-MS (NaI): 1358 ($[M+H]^+$), 1380 ($[M+Na]^+$). Anal. calc. for $C_{51}H_{78}N_2O_{35}Se \cdot 8\ H_2O$: C 40.78, H 6.31, N 1.86; found: C 40.67, H 5.88, N 2.04.

6-Deoxy-6-{{2-{[2-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)ethyl]amino}ethyl}amino}-β-cyclodextrin (**2**). The 6-{{2-[(2-aminoethyl)amino]ethyl}amino}-6-deoxy-β-cyclodextrin (β-CDdien) was prepared by the

reaction of 6-*O*-Ts-β-CD with diethylenetriamine [24]. Then **2** was synthesized from β-CDdien and 2-(chloroseleno)benzoyl chloride as described for **1**: 55% of **2**. IR (KBr): 3367.5, 2903.5, 1750.9, 1736.2, 1653.4, 1629.2, 1587.9, 1538.9, 1504.2, 1398.7, 1331.8, 1307.9, 1272.0, 1252.7, 1226.1, 1147.3, 1071.8, 1021.3, 940.1, 886.9, 835.4, 747.2, 691.7, 600.2. ^{1}H-NMR: 2.8–4.1 (*m*, 48 H); 4.2–4.6 (*m*, 2 H); 4.8–5.1 (*m*, 7 H); 5.6–6.0 (*m*, 14 H); 7.3–8.0 (*m*, 4 arom. H). FAB-MS (NaI): 1401 ($[M+H]^+$), 1423 ($[M+Na]^+$). Anal. calc. for $C_{53}H_{83}N_3O_{35}Se \cdot 8\ H_2O$: C 41.19, H 6.41, N 2.72; found: C 41.07, H 6.49, N 2.54.

6-Deoxy-6-{[2-{[2-{[2-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)ethyl]amino}ethyl]amino}ethyl]amino}-β-cyclodextrin (**3**). The 6-{[2-{[2-[(2-aminoethyl)amino]ethyl}amino}ethyl}amino}-6-deoxy-β-cyclodextrin (β-CDtrien) was prepared by the reaction of 6-*O*-Ts-β-CD with triethylenetetramine [24]. Then **3** was synthesized from β-CDtrien and 2-(chloroseleno)benzoyl chloride as described for **1**: 60% of **3**. IR (KBr): 3370.0, 2909.5, 1754.1, 1720.1, 1652.6, 1586.0, 1541.5, 1458.2, 1381.5, 1335.3, 1304.0, 1254.7, 1231.6, 1147.8, 1072.2, 1020.5, 940.4, 883.7, 832.3, 747.6, 697.5, 601.2, 573.4. ^{1}H-NMR: 2.8–4.1 (*m*, 52 H); 4.2–4.6 (*m*, 2 H); 4.8–5.1 (*m*, 7 H); 5.6–6.0 (*m*, 14 H); 7.3–8.0 (*m*, 4 arom. H). FAB-MS (NaI): 1442 ($[M+H]^+$). Anal. calc. for $C_{55}H_{88}N_4O_{35}Se \cdot 11\ H_2O$: C 40.19, H 6.70, N 3.41; found: C 40.28, H 6.36, N 2.98.

6-Deoxy-6-{[3-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)propyl]amino}-β-cyclodextrin (**4**). The 6-[(3-aminopropyl)amino]-6-deoxy-β-cyclodextrin (β-CDprn) was prepared from 6-*O*-Ts-β-CD and propylenediamine [24]. Then **4** was synthesized from β-CDprn and 2-(chloroseleno)benzoyl chloride as described for **1**: 58% of **4**. IR (KBr): 3367.5, 2903.5, 1750.9, 1736.2, 1653.4, 1629.2, 1587.9, 1538.9, 1504.2, 1398.7, 1331.8, 1307.9, 1272.0, 1252.7, 1226.1, 1147.3, 1071.8, 1021.3, 940.1, 886.9, 835.4, 747.2, 691.7, 600.2. ^{1}H-NMR: 2.8–4.1 (*m*, 46 H); 4.2–4.6 (*m*, 2 H); 4.8–5.1 (*m*, 7 H); 5.6–6.0 (*m*, 14 H); 7.3–8.0 (*m*, 4 arom. H). FAB-MS (NaI): 1376 ($[M+H]^+$). Anal. calc. for $C_{53}H_{83}N_3O_{35}Se \cdot 8\ H_2O$: C 41.19, H 6.41, N 2.72; found: C 41.07, H 6.49, N 2.54.

6-O-*[4-(2,3-Dihydro-3-oxo-1,2-benzisoselenazol-2-yl)benzoyl]-β-cyclodextrin* (**5**). To a soln. of DMF (100 ml) containing 4-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)benzoic acid (1.55 g) and dicyclohexylcarbodiimide (DCC; 1.65 g), β-cyclodextrin (60 g) and dry pyridine (25 ml) as well as some 4-Å molecular sieves were added. The mixture was stirred for 12 h in an ice bath and then at r.t. for another 18 h. The mixture was left for 2 or 3 days to complete precipitation. The precipitate was filtered off, the filtrate evaporated, the residue dissolved in a minimal amount of hot H_2O, and the soln. poured into acetone (150 ml). The precipitate was filtered off and the white powder washed with a large amount of acetone. The crude product was recrystallized from EtOH/H_2O 1:1 and then recrystallized twice from H_2O: pure **5** (1.5 g, 25%). Light yellow powder. IR (KBr): 3371.5, 2911.0, 1701.3, 1627.4, 1595.5, 1561.2, 1513.4, 1401.0, 1323.5, 1270.7, 1145.0, 1072.0, 1019.7, 938.2, 843.7, 794.2, 747.2, 697.6, 663.4, 573.4. ^{1}H-NMR: 3.1–3.9 (*m*, 40 H); 4.1–4.6 (*m*, 2 H); 4.8–5.2 (*s*, 7 H); 7.0–8.0 (*m*, 8 H). FAB-MS (NaI): 1461 ($[M+Na]^+$). Anal. calc. for $C_{56}H_{77}NO_{37}Se \cdot 5\ H_2O$: C 44.09, H 5.71, N 0.92; found: C 44.03, H 5.51, N 0.97.

6-Deoxy-6-{[2-{[4-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)benzoyl]amino}ethyl]amino}-β-cyclodextrin (**6**). As described for **5**, from β-CDen [24] and 4-(2,3-dihydro-3-oxo-1,2-benzisoselenazol-2-yl)benzoic acid: 35% of **6**. IR (KBr): 3391.2, 2927.6, 2150.8, 1634.2, 1591.0, 1546.4, 1450.5, 1399.3, 1387.7, 1332.1, 1262.4, 1202.3, 1155.6, 1081.0, 1029.2, 943.8, 853.0, 750.7, 704.8, 646.3, 578.3. ^{1}H-NMR: 2.8–4.0 (*m*, 44 H); 4.1–4.6 (*m*, 2 H); 4.8–5.2 (*s*, 7 H); 7.0–8.0 (*m*, 8 H). FAB-MS (NaI): 1481 ($[M+H]^+$). Anal. calc. for $C_{58}H_{83}N_3O_{35}Se \cdot 8\ H_2O$: C 43.39, H 6.17, N 2.62; found: C 43.68, H 6.19, N 2.94.

Determination of SOD Activity. Superoxide dismutase (SOD) activities of the modified β-cyclodextrins **1**–**6** were determined by a modification of the method of *Marklund* and *Marklund* [25] using autooxidation of pyrogallol at pH 8.2 and 25°. The absorption at 319 nm of the assaying mixture (1 ml), containing 54 mM *Tris*·HCl buffer (pH 8.2), 54 mM dimethylarsinic acid sodium salt, 1.07 mM diethylenetriaminepentaacetic acid, 84 μM pyrogallol, and an appropriate amount of mimic (usually 30–50 μM) or 1 mg/ml of the natural SOD sample, was recorded at 25°. One activity unit corresponds to the amount of enzyme which inhibits the rate of autooxidation of pyrogallol by 50% at pH 8.2 and 25°.

Determination of GPX Activity. The GPX activity was determined by the method of *Wilson et al.* [12] with hydrogen peroxide as the substrate in the presence of GSH. The reactions (see *Eqns. 4–6*) were performed at 37° in 700 μl of assay soln. containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM sodium azide, 1 mM GSH, 0.25 mM NADPH, 1 unit of GSSG reductase, and an appropriate amount of mimic (usually 10–50 μM final concentration). Glutathione reductase was used to reduce the oxidized GSH (GSSG) with NADPH as a cofactor (*Eqn. 4*). The decrease in NADPH absorption at 366 nm is a measure of GPX activity. The reaction was initiated by addition of 0.5 mM H_2O_2. Appropriate controls were run without enzyme or mimic and were subtracted. The GPX activity unit of enzyme is defined as the amount of enzyme that utilizes 1 μmol of NADPH per min. The activity is expressed in U/mg or U/μmol of enzyme.

$$2\,GSH + H_2O_2 \xrightarrow{\text{GSH peroxidase}} GSSG + 2H_2O \quad (4)$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{GSH reductase}} 2\,GSH + NADP^+ \quad (5)$$

$$H^+ + NADPH + H_2O_2 \rightarrow NADP^+ + 2H_2O \quad (6)$$

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