

Internally stabilized selenocysteine derivatives: syntheses, ^{77}Se NMR and biomimetic studies†

Prasad P. Phadnis and G. Mugesh*

Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, 560 012, India. E-mail: mugesh@ipc.iisc.ernet.in; Fax: +91 80 2360 1552/2360 0683

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Selenocystine ($[\text{Sec}]_2$) and aryl-substituted selenocysteine (Sec) derivatives are synthesized, starting from commercially available amino acid L-serine. These compounds are characterized by a number of analytical techniques such as NMR (^1H , ^{13}C and ^{77}Se) and TOF mass spectroscopy. This study reveals that the introduction of amino/imino substituents capable of interacting with selenium may stabilize the Sec derivatives. This study further suggests that the oxidation–elimination reactions in Sec derivatives could be used for the generation of biologically active selenols having internally stabilizing substituents.

Introduction

Selenium, an essential trace element,¹ exerts its biological effect through several selenoenzymes, which include glutathione peroxidase (GPx),^{2,3} iodothyronine deiodinase (ID)⁴ and thioredoxin reductase (TrxR).⁵ Although these enzymes have selenocysteine (Sec), the 21st amino acid, in their active site,^{6–10} their substrate specificity and cofactor systems are strikingly different. GPx is an antioxidant selenoenzyme¹¹ that protects various organisms from oxidative stress by catalyzing the reduction of hydroperoxides at the expense of thiols.¹² ID, particularly the type I enzyme (ID-I), on the other hand, is responsible for the activation of thyroid hormones by deiodination reactions. The role of TrxR is to reduce thioredoxin (Trx) by NADPH, which is important for a variety of biological functions such as DNA synthesis.^{5,13} However, the synthetic and biological studies on Sec are hampered by instability of Sec derivatives as compared with the cysteine (Cys) analogues. In general, Sec derivatives undergo fast and mild oxidative elimination to produce dehydroalanines.¹⁴

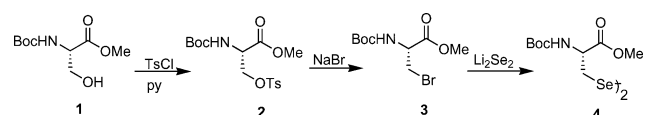
Initial attempts to synthesize Sec derivatives in the laboratory met with limited success. Soda *et al.* synthesized selenocysteine by treating β -L-chloroalanine with disodium diselenide¹⁵ whereas Pete Silks *et al.* approached the synthesis from tosylated *N*-Boc-serine methylester. The conversion of this serine ester into an iodide or a bromide derivative followed by treatment with dilithium diselenide and then deprotection with TFA afforded the required Sec.^{16,17} Walther and coworkers reported one of the first syntheses of optically pure Sec derivatives.¹⁸ A key step in the synthesis involved the nucleophilic displacement of an *O*-tosylated L-serine derivative. An alternative approach has been reported by Shirahama and coworkers, in which diphenyl diselenide was first reduced with sodium metal and the resulting selenolate was treated with *tert*-butyloxy carbonyl (Boc) protected serine β -lactone.¹⁹ Recently, this approach has been modified by performing an *in situ* reduction of diphenyl diselenide with sodium trimethoxyborohydride $[\text{NaBH}(\text{OMe})_3]$.^{14,20} More recently, a modified synthetic route to Sec derivatives using Fmoc strategy has also been reported.²¹

Recent studies on aryl substituted Sec conjugates show that the oxidative elimination reactions could lead to the generation of catalytically active selenols.²² Therefore, the fast and mild oxidative elimination of selenocysteine derivatives can be utilized for further catalytic reactions. However, the instability of such

derivatives poses a major problem in synthetic and purification procedures. To overcome this difficulty we employed an aryl selenium moiety having internally chelating substituents, which would increase the stability by $\text{Se} \cdots \text{N}$ non-covalent interactions. These substituents may also modulate the reactivity of selenols that are generated *in situ* by elimination reactions. In this article, we report the first examples of Sec derivatives having basic amino/imino groups in the close proximity to selenium.

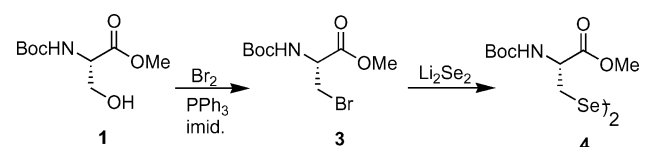
Results and discussion

The synthesis of selenocysteine derivatives was approached with protected L-serine. Our initial route to these derivatives focused on modification to literature preparations of selenocysteine and the corresponding phenyl derivative. Boc-protected serine ester (**1**) was converted into bromoalanine methyl ester (**3**), which was treated with Li_2Se_2 to afford the protected selenocystine (**4**) (Scheme 1).



Scheme 1 Synthetic route to diselenide **4**.

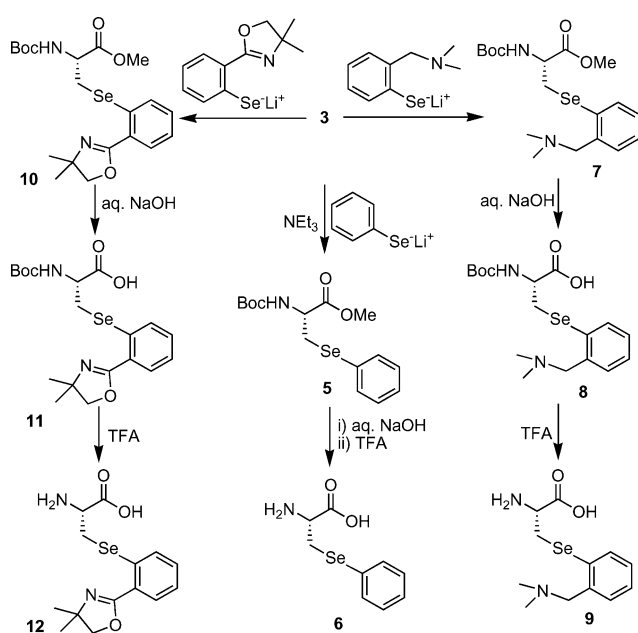
Although this reaction afforded the expected compound in low yield, our initial attempts towards scale up did not work. The ^{77}Se NMR spectrum showed a number of signals which were probably due to different diastereomers. The activation of serine $-\text{OH}$ in **1** with phenylmethanesulfonyl fluoride (PMSF) or tosyl chloride (TsCl) followed by reaction with NaHSe and subsequent oxidation also did not look promising. The major problem in these reactions lies in the tosylation step, which leads to the formation of dehydroalanine derivatives. In view of these results, we decided to find an alternative synthetic approach to **4** that does not involve the tosylation and would be amenable to scale up. Scheme 2 depicts the conversion of the protected serine into selenocystine, in which the $-\text{OH}$ group can be converted directly to a bromide without involving the tosylation step.²³



Scheme 2 Modified route for the synthesis of **4**.

† Electronic supplementary information (ESI) available: ^{77}Se NMR data for selected compounds. See <http://www.rsc.org/suppdata/ob/b5/b505299h/>

According to this method, compound **1** was treated with triphenyl phosphine, bromine and imidazole in CH_2Cl_2 to convert the $-\text{OH}$ function into the corresponding bromide. After this step, the bromide was added to a THF solution of Li_2Se_2 to afford the protected selenocysteine (**4**). Deprotection of ester and Boc groups in **4** afforded the free amino acid in the diselenide form. All analytical data obtained for this compound are in accordance with the data reported in the literature. From these results, it is clear that the *N*-Boc-*L*-Serine methyl ester may be an ideal starting material for the preparation of substituted selenocysteine derivatives. By employing the above mentioned methodology, Boc-Ser-OMe (**1**) was transformed into the corresponding bromide, and subsequent reaction with benzene selenolate (PhSe^-Li^+), generated *in situ* by reducing diphenyl diselenide (PhSeSePh) by superhydride, proved to be an effective way to produce phenyl substituted selenocysteine (**5**). It is noteworthy to mention that competing side products stemming from elimination or ester saponification were suppressed by the use of triethylamine along with the bromide (Scheme 3).

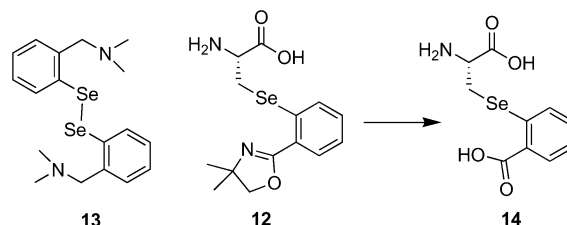


Scheme 3 Synthetic routes to internally chelated Sec derivatives.

The improvement of yield in this reaction by the addition of NEt_3 led to an assumption that a selenium moiety containing an internal amino/imino group would provide an interesting strategy to synthesize selenocysteine derivatives containing heteroatoms in close proximity to selenium. It should be mentioned that basic amino/imino groups in close proximity to selenium have been shown to play a significant role in modulating the biological activity of natural and synthetic selenium compounds. In light of this we approached the synthesis of **7** starting from diselenide **13**.²⁴ As in the case of phenyl derivative, reduction of diselenide **13** by super hydride, followed by reaction with the bromo derivative **3**, afforded the expected selenocysteine derivative **7**. Although this reaction produced the expected compound, the yield was found to be very poor. Therefore we approached the synthesis starting from *N,N*-dimethyl benzylamine. It is known that the heteroatom directed metallation of *N,N*-dimethyl benzylamine with *n*-BuLi, followed by selenium insertion, affords the corresponding lithium selenolate. Treatment of the lithium selenolate with **3** at low temperatures afforded compound **7** in good yield. Deprotection of the ester group by aq. NaOH and the removal of Boc group by TFA afforded compounds **8** and **9**, respectively. It is worth mentioning that the stability of these derivatives was found to be much higher than those derived from unsubstituted phenyl derivatives. This indicates that the presence of an amino group near selenium enhances the stability of these

compounds, possibly by non-covalent $\text{Se} \cdots \text{N}$ interactions. The variable temperature ^1H NMR spectra of compound **7** show that the $\text{Se} \cdots \text{N}$ interactions do exist in solution as evidenced by the AB pattern observed for the benzylic protons.

By following the above method the oxazoline based compound (**10**) was synthesized (Scheme 3). The deprotected derivatives were also synthesized by following similar procedures. These compounds were also found to be stable in solution, as proved by ^{77}Se NMR spectroscopy. However, during the deprotection of the Boc group by TFA, cleavage of oxazoline ring leads to the formation of acid **14**.



One of the important objectives of this work was to study these compounds by ^{77}Se NMR spectroscopy. Although a few methods have been described in the literature for the synthesis of selenocysteine derivatives, only a small amount of information is available regarding the ^{77}Se NMR chemical shifts. The ^{77}Se nucleus has a very wide chemical shift range (over 3000 ppm) and is extremely sensitive to changes in bonding and conformation. The relatively large chemical shift anisotropy (CSA) of ^{77}Se provides a very efficient relaxation mechanism and consequently broad ^{77}Se resonances in the NMR spectra of biomolecules. It is, therefore, very difficult to detect sharp ^{77}Se NMR signals for biomolecules due to the relatively low percentage of the ^{77}Se isotope in elemental selenium. ^{77}Se NMR data for D-*L*-selenocysteine in the solution phase were reported by Pan and Fackler.²⁵ However, the ^{77}Se NMR studies on compounds in the present study show that it is possible to obtain good ^{77}Se NMR spectra at appropriate concentrations. Table 1 summarizes the ^{77}Se NMR chemical shift for Sec and related derivatives (see electronic supplementary information (ESI) for spectra†).

All compounds exhibited ^{77}Se NMR signals in the range ~ 200 – 300 ppm with respect to Me_2Se . The diselenide (**4**) exhibited a signal at 294 ppm, which is greatly shifted upfield compared with that of aromatic diselenides, but it is comparable with other dialkyl diselenides.²⁶ The phenyl derivative (**5**) exhibited a signal at 252 ppm. This signal appears to be shifted upfield as compared with those of PhSeR ($\text{R} = n$ -alkyl), which normally occur around 290 ppm. Although compound **5** can be readily synthesized from **3**, this compound was found to be unstable in solution as evidenced by the appearance of a new signal at 460 ppm for diphenyl diselenide. This indicates that compound **5**, similar to the parent $[\text{Sec}]_2$, undergoes β -elimination reactions.

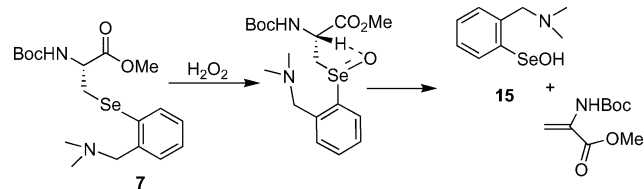
The benzylamine derivative, on the other hand, exhibited a signal at 216 ppm, which is shifted significantly upfield as compared with that of the phenyl derivatives. The signal at 216 ppm disappeared completely upon treatment with aq. NaOH and a new signal appeared at 212 ppm for the corresponding

Table 1 ^{77}Se NMR data of Sec derivatives

No.	Compound	^{77}Se NMR chemical shift (ppm)	Solvent
1	4	295	CDCl_3
2	5	252	CDCl_3
3	6	240	D_2O
4	7	216	CDCl_3
5	8	212	D_2O
6	9	191	D_2O
7	10	273	CDCl_3
8	11	258	CDCl_3
9	12	249	D_2O

acid. Furthermore, the signal also disappeared completely upon treatment with TFA, exhibiting a new signal at 191 ppm for the completely deprotected derivative **9**. The oxazoline based selenocysteine derivative (**10**) exhibited a signal at 273 ppm, which is shifted downfield as compared with that of phenyl and benzylamine derivatives. The deprotected derivative exhibited a signal at 249 ppm, which is also shifted downfield as compared with the corresponding phenyl and benzylamine derivatives. However, the chemical shifts for the protected compounds cannot be directly compared with those of the deprotected derivatives because the ^{77}Se NMR spectra for the protected and deprotected derivatives were recorded in different solvents and the solvent shift can be substantial for ^{77}Se measurements. The benzylamine and oxazoline based compounds were found to be more stable than the corresponding phenyl derivatives due to the presence of heteroatoms in the close proximity to selenium.

Because the selenocysteine derivatives can undergo fast oxidation–elimination reactions under mild conditions,^{22,27} these compounds could be used to generate catalytically active selenols. Therefore, compound **7** was treated with H_2O_2 and the progress of the reaction was followed by ^{77}Se NMR spectroscopy. Interestingly, compound **7** underwent facile oxidation–elimination reactions to produce the corresponding dehydroalanine derivative and seleninic acid (**15**) (Scheme 4). This suggests the possibility that the substituted selenocysteine derivatives could be used as precatalysts for GPx-like activity. It has been shown previously that the diselenide (**13**) derived from *N,N*-dimethyl benzylamine reduces hydroperoxides with thiols, mimicking the selenoenzyme GPx *in vitro*. Therefore, we studied the GPx-like catalytic activity by using PhSH as a thiol. The conversion of PhSH to PhSSPh was followed by reverse-phase HPLC, using methanol–water as the eluent (Fig. 1). The activities were compared with ebselen, a well known GPx mimic, and are summarized in Table 2.



Scheme 4 Oxidation–elimination reaction of **7**.

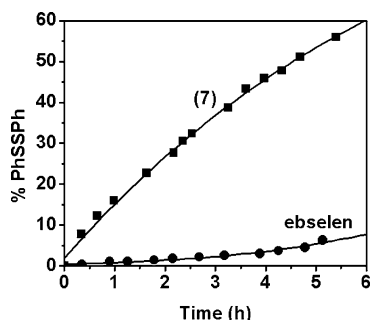
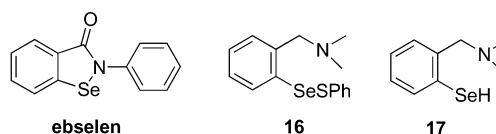


Fig. 1 Rates of formation of PhSSPh from oxidation of PhSH (5 mM) with H_2O_2 (7 mM) in the presence of catalysts ebselen and **7** (0.47 mM) in CH_2Cl_2 –MeOH (95 : 5) at 23 °C.

Table 2 Initial Rate (v_0) and activity for the reduction of H_2O_2 (7 mM) by PhSH (5 mM) in the presence of selenium catalysts (0.47 mM) at 23 °C

Compound	$v_0 / \mu\text{M min}^{-1}$	Activity
Ebselen	0.59×10^{-3}	1.0
5	1.10×10^{-3}	1.8
7	9.59×10^{-3}	16.1

The GPx activity of the phenyl derivative was only marginally higher than ebselen. The benzylamine derivative, however, exhibited an almost 16-fold increase in the GPx activity as compared with ebselen. This indicates that the selenenic acid generated during the reaction with H_2O_2 may be reduced by PhSH, exerting a catalytic cycle. The addition of H_2O_2 to compound **7** produced a signal at 1018 ppm in the ^{77}Se NMR, which could be ascribed to the selenenic acid **15**. Further reaction of this species with one equivalent of PhSH produced a signal at 565 ppm for the corresponding selenenyl sulfide **16**, which is confirmed by comparison with the chemical shift of an authentic sample. The treatment of **16** with an excess amount of PhSH then produced the corresponding selenol **17** along with the oxidized product **13**. Addition of an excess amount of H_2O_2 to **7** produced another signal at 1181 ppm, which disappeared upon addition of PhSH. The ^{77}Se NMR chemical shift observed at 216 ppm for compound **7** has never been detected during the entire process, indicating that the selenide **7** acts only as a precatalyst. Although compound **17** has been previously shown to be a catalyst in the reduction of H_2O_2 ,²⁸ capping it on a biogenic moiety such as Sec would increase its biological applications.



Conclusions

Aryl-substituted Sec derivatives containing basic amino/imino groups in close proximity to selenium could be synthesized from commercially available starting materials. The presence of a heteroatom near selenium increases the stability of Sec derivatives by intramolecular $\text{Se} \cdots \text{N}$ interactions. These interactions appear to modulate the reactivity of selenium. This study further suggests that the internally chelated aryl-substituted Sec derivatives could be used as precatalyst for generating catalytically active selenols. The fast and mild oxidative elimination of Sec derivatives to produce dehydroalanines, therefore, may give easy access to biologically active selenium compounds.

Experimental

Materials and methods

The commercially available Boc-L-serine, di-*tert*-butyl dicarbonate [(Boc) $_2$ O], *N,N*-dimethylbenzylamine, 4,4-dimethyl-2-oxazoline and *n*-BuLi, LiEt $_3$ H (super hydride) were purchased from Aldrich/Sigma Chemicals. All reactions were carried out under a nitrogen atmosphere by using standard vacuum line techniques. Solvents were purified by standard procedures²⁹ and were freshly distilled prior to use. The products were purified by column chromatography on silica gel 60/120 mesh size washed with 2% Et $_3$ N in order to avoid the cleavage of Boc group under mild acidic conditions. The compounds were characterized by NMR spectroscopy on a Bruker AVANCE 400 MHz spectrometer operating at 400 MHz (^1H), 100.56 MHz (^{13}C) and 76.29 MHz (^{77}Se). ^1H and ^{13}C chemical shifts are cited with respect to SiMe $_4$ as an internal standard. The ^{77}Se NMR spectra were recorded using diphenyl diselenide as an external standard and are shown in the ESI†. Chemical shifts are reported relative to dimethyl selenide (0 ppm) by assuming that the resonance of the standard is at 461.0 ppm. IR spectra were recorded on Perkin Elmer Spectrum One FTIR Spectrometer. Mass spectral studies were carried out on a Q-TOF Micro Mass Spectrometer with an ESI MS mode of analysis. In the case of isotopic patterns, the value given is for the most intense peak.

Synthesis of *N*-Boc-L-serine methyl ester (1**)³⁰.** To a solution of L-serine (10.029 g, 95.44 mmol) in 1 N NaOH (200 ml, 8.020 g, 200.50 mmol), a solution of di-*tert*-butyl dicarbonate [(Boc) $_2$ O]

(22.0 ml, 20.900 g, 95.76 mmol) in 1,4-dioxane (100 ml) was added at 0 °C. The resulting turbid two phase solution was stirred at ~5 °C for 30 min, allowed to warm up to room temperature and stirred for an additional 4 h. The mixture was concentrated and then acidified to pH 2–3 by addition of 1 N KHSO₄ (200 ml, 27.245 g, 200.09 mmol). The product was extracted with ethyl acetate (4 × 150 ml) and then the combined extracts were dried (Na₂SO₄), filtered and concentrated to give *N*-Boc-L-serine as a colorless oil. The essentially pure *N*-Boc-L-serine was used for further reactions without any purification. Yield: 18.429 g (94.3%), ¹H NMR (CDCl₃): δ 1.46 (s, 9H, CMe₃); 3.79–3.86 (dd, *J* 22.4, 9.6 Hz) (1H), 4.01–4.08 (dd, *J* 14.8, 10.0 Hz) (1H) (CH₂); 4.20 (s, 1H, CH); 4.38 (s, 1H, OH); 5.86 (d, *J* 7.6 Hz, 1H, NH); 7.13 (s, 1H, OH); 7.65 (br, 1H, COOH).

To a solution of *N*-Boc-L-serine (18.429 g, 89.81 mmol) in DMF (200 ml), solid K₂CO₃ (13.099 g, 94.78 mmol) was added at 0 °C. The resulting white suspension was stirred for 15 min and then methyl iodide (18.0 ml, 40.950 g, 288.56 mmol) was added. The reaction mixture was stirred for ~20 h and the progress of the reaction was monitored by TLC. The reaction mixture was filtered through celite and the filtrate was partitioned between ethyl acetate (4 × 150 ml) and distilled water (400 ml). The ethyl acetate extracts were combined and dried (Na₂SO₄), filtered and concentrated *in vacuo* to give a pale amber viscous oil, which was purified by column chromatography using ethyl acetate–petroleum ether (1 : 2). Yield: 15 g (72.3%), IR (KBr): *ν* = 3436 (O–H); 2980 (NH); 1746 (C=O ester); 1705 (C=O, Boc); ¹H NMR (CDCl₃): δ 1.44 (s, 9H, CMe₃); 3.2 (s, 1H, OH); 3.78 (s, 3H, OCH₃); 3.92 (d, *J* 17.6 Hz, 2H, CH₂); 4.37 (s, 1H, CH); 5.6 (d, 1H, NH). ¹³C NMR (CDCl₃): δ 28.3 (CMe₃); 52.6 (OCH₃); 55.7 (CH); 63.4 (CH₂); 80.3 (3 °C); 155.8 (NHC=O); 171.5 (O=C–O).

Synthesis of methyl-[2-(*tert*-butoxycarbonyl)amino]-3-bromopropionate (3). To a solution of PPh₃ (0.646 g, 2.47 mmol) in CH₂Cl₂ (15 ml), bromine (0.13 ml, 0.399 g, 2.50 mmol) was added dropwise and the resulting orange–yellow solution was stirred for 15 min. To this was added a solution of *N*-Boc-L-serine methyl ester (0.269 g, 1.23 mmol) and imidazole (0.169 g, 2.48 mmol) in 20 ml CH₂Cl₂ at 0–5 °C. This resulted in an immediate formation of a white precipitate. The reaction mixture was stirred for 3 h, the precipitate was filtered off and the filtrate was concentrated *in vacuo* to give a viscous pale yellow oil. This product was further purified by silica gel column using ethyl acetate–petroleum ether (1 : 3) to give the expected product as a colorless oil. Yield = 0.135 g (39%). The NMR data are in accordance with the literature values.

Synthesis of *N*-Boc-L-selenocysteine methyl ester-diselenide (4)¹⁶. To a suspension of selenium powder (0.349 g, 4.43 mmol) in THF (15 ml) was added carefully a solution of LiBEt₃H (super hydride) (4.90 ml, 0.234 g, 4.89 mmol) in THF at 0 °C. This resulted in the formation of a dark brown suspension of Li₂Se₂, which was boiled at reflux for 30 min and then allowed to cool to room temperature. This solution was used for the next step without any manipulation.

In another flask, methyl-[2-(*tert*-butoxycarbonyl)amino]-3-bromo-propionate (3) (0.626 g, 2.22 mmol) was prepared by treatment of *N*-Boc-L-serine methyl ester (0.540 g, 2.46 mmol) and imidazole (0.335 g, 4.93 mmol) mixture with PPh₃ (1.292 g, 4.92 mmol) and bromine (0.25 ml, 0.800 g, 5.00 mmol) in CH₂Cl₂ at 0–5 °C as previously mentioned. The bromo compound was dissolved in dry THF, cooled to –78 °C and treated with Li₂Se₂ solution (0.381 g, 2.22 mmol). The reaction mixture was allowed to warm to room temperature and the stirring was continued for additional 3 h. The resulting suspension was filtered through celite. The clear yellow solution was concentrated *in vacuo* to give yellow oil. All attempts to directly purify the product by chromatographic techniques were unsuccessful. However, the expected diselenide was obtained in pure form when the crude product was treated with I₂ in CH₂Cl₂ at room temperature. This resulted in the formation of a brown solid, which was

further purified by column chromatography using ethyl acetate and petroleum ether (1 : 2). Yield: 0.601 g (48%), IR (KBr): *ν* = 2962 (NH); 2925 (NH) (C=O, ester); 2874 (s); 2855 (s); (C=O, Boc); 1495 (s); 1465 (s); 1456 (s); MS (TOF MS ES⁺) *m/z* (%): 587 (100), 585 (87.0), 583 (51.3) [M⁺Na].

***O*-Phenyl-*N*-Boc-L-selenocysteine methyl ester (5)²⁷.** This compound was synthesized by following the method used for the synthesis of diselenide 4. The bromo derivative was added dropwise to a solution of PhSeLi (0.235 g, 1.44 mmol) (prepared separately by treatment of diphenyldiselenide (Ph₂Se₂) (0.225 g, 0.72 mmol) with LiBEt₃H (super hydride) (1.4 ml, 0.067 g, 1.4 mmol)) in dry THF at 0 °C. The reaction mixture was stirred for 20 h at this temperature. The solvent was evaporated *in vacuo* and the product was purified by column chromatography using ethyl acetate–petroleum ether (1 : 25) to give the desired compound. Yield: 0.235 g (45%), IR (KBr): *ν* = 2979 (NH), 1752 (C=O, ester); 1715 (C=O, Boc); 1500 (s); 1162 (s); ¹H NMR (CDCl₃): δ 1.42 (s, 9H, CMe₃); 3.33–3.48 (d, *J* 4.8 Hz, 2H, CH₂); 3.48 (s, 3H, OCH₃); 4.67 (m, 1H, CH); 5.36–5.38 (d, *J* 8.0 Hz, 1H, NH) 7.46–7.70 (m, 5H, Ph). ¹³C NMR (CDCl₃): δ_C = 28.3 (CMe₃); 30.7 (CH₂); 52.3 (OCH₃); 53.3 (CH); 80.1 (3 °C); 127.6 (HC-3, 4, 5); 129.2 (C of Ph); 133.8 (HC-2, 6); 156.0 (HNC=O); 171.1 (O=C–O). MS (TOF MS ES⁺) *m/z* (%): 382 (100), 380 (48), 378 (18.9) [M⁺Na]; 398 (100), 396 (48), 394 (18.9) [M⁺K].

***O*-*N,N*-Dimethylbenzyl-*N*-Boc-L-selenocysteine methyl ester (7).** This compound was synthesized by following the method used for the synthesis of diselenide 4. The bromo derivative was added dropwise to a solution of lithium selenolate (0.731 g, 3.32 mmol) generated *in situ* from *N,N*-dimethylbenzylamine in ether at 0 °C. The resulting yellowish brown suspension was allowed to warm up to room temperature and the stirring was continued for an additional 20 h, leading to an orange yellow solution. The compound, after evaporation of the solvent, was purified by column chromatography using ethyl acetate–petroleum ether (1 : 20) to give the desired compound. Yield: 0.676 g (49%), IR (KBr): *ν* = 2983 (NH); 1754 (C=O, ester); 1708 (C=O, Boc); ¹H NMR (CDCl₃): δ 1.37 (s, 9H, CMe₃); 2.25 (s, 6H, NMe₂); 3.22 (dd, *J* 13, 4.8 Hz, 1H), 3.33 (d, *J* 12.4 Hz, 1H), 3.46 (dd, *J* 13.2, 4.8 Hz, 1H) (CH₂), 3.55 (s, 3H, COOCH₃); 3.63 (d, *J* 12 Hz, 2H, CH₂); 4.4 (m, 1H, CH); 6.5 (d, *J* 8.0 Hz, 1H, NH); 7.19–7.57 (m, 4H, Ph). ¹³C NMR (CDCl₃): δ 28.3 (CMe₃); 30.5 (SeCH₂); 45.0 (NMe₂); 52.3 (OCH₃); 52.8 (CH); 64.8 (NCH₂); 79.7 (3 °C); 126.9, 128.2, 130.4, 132.2, 134.2; 140.8; 155.4 (HNC=O); 171.5 (OC=O). MS (TOF MS ES⁺) *m/z* (%): 417 (100), 415 (48.0), 418 (20.2) [M⁺Na].

4,4-Dimethyl-2-oxazoline-*N*-Boc-L-selenocysteine methyl ester (10).

Method A. This compound was synthesized by following the method used for the synthesis of diselenide 4. The bromo derivative was added dropwise to a solution of lithium selenolate (0.502 g, 1.93 mmol), generated *in situ* from 4,4-dimethyl-2-oxazoline, in ether at 0 °C. The resulting yellowish brown suspension was stirred for 18 h and then concentrated to give the expected product as an orange oil, which was further purified by column chromatography using ethyl acetate–petroleum ether (1 : 20). Yield: 0.410 g (47%), IR (KBr): *ν* = 2975 (NH); 1748 (s), 1720 (C=O, ester); 1715 (s); 1647 (w) (C=O, Boc). ¹H NMR (CDCl₃): δ 1.42 (s, 9H, CMe₃); 1.58 (s, 6H, NCMe₂); 3.28 (m, 2H, SeCH₂); 3.68 (s, 3H, OCH₃); 4.09 (s, 2H, CH₂); 4.69 (m, 1H, CH); 5.52 (d, *J* 8.0 Hz, 1H, NH); 7.2–7.75 (m, 4H, Ph). MS (TOF MS ES⁺) *m/z* (%): 457 (100), 455 (50.8), 454 (21.9) [M⁺Na].

Method B. The title compound can also be synthesized from the diselenide³¹ derived from 4,4-dimethyl-2-oxazoline. In this method, the oxazoline diselenide (0.206 g, 0.41 mmol) was reduced by LiBEt₃H (0.82 ml, 0.039 g, 0.82 mmol) in dry THF at 0 °C to give the corresponding lithium selenolate (0.106 g, 0.41 mmol) which upon treatment with the bromo derivative

at -78°C afforded the expected compound in low yield. The product was purified by column chromatography using ethyl acetate–petroleum ether (1 : 20).

Deprotection of *O*-phenyl-*N*-Boc-*L*-selenocysteine methyl ester (5). To a stirred solution of *O*-phenyl-*N*-Boc-*L*-selenocysteine methyl ester (0.150 g) in MeOH (25 ml) at $0-5^{\circ}\text{C}$, 1 N NaOH_{aq} (~20 ml) solution was added dropwise. The reaction mixture was stirred for 30 min at this temperature and neutralized with aqueous 1 N KHSO₄ solution (~20 ml). The product was extracted with ethyl acetate and the combined organic layer was dried (Na₂SO₄), filtered and concentrated to give the desired acid-deprotected derivative as a pale yellow oil. MS (TOF MS ES⁺) m/z 343 (M⁺H⁻). This compound was dissolved in CH₂Cl₂ and treated with TFA (2 ml). After stirring for 2 h at room temperature, the solvent was evaporated completely. The residue was washed with CH₂Cl₂ and petroleum ether to remove Ph₂Se₂ that resulted from the elimination reactions. The resulting water soluble off-white solid was dried *in vacuo* to yield the desired compound **6**. IR (KBr): $\nu = 3433$ (OH); 2980 (NH); 1689 (C=O, ester); 1578 (C=O, Boc); 1427 (m); 1210 (s); 1188 (s); 1140 (s) ¹H NMR (D₂O): δ 1.76 (s, 2H, NH₂); 2.98–3.03 (m, 1H, CH₂), 3.11–3.15 (m, 1H, CH₂); 3.32 (s, br, 1H, CH); 7.25 (m, 3H); 7.47 (m, 2H) (Ph). ¹³C NMR (D₂O): δ 23.2 (CH₂), 56.5 (CH); 127.1 (HC-3, 4, 5); 129.3 (C of Ph); 132.6 (HC-2, 6); 181.5 (O=C–O).

Deprotection of *O*-*N,N*-dimethylbenzyl-*N*-Boc-*L*-selenocysteine methyl ester (7). To a solution of *O*-*N,N*-dimethylbenzyl-*N*-Boc-*L*-selenocysteine methyl ester (0.140 g) in MeOH (~25 ml) was added 1 N NaOH solution (~20 ml) at $0-5^{\circ}\text{C}$. The reaction progress was monitored by TLC. The mixture was partitioned between distilled water and ethyl acetate. The organic phase was separated and the aqueous phase was further extracted with CH₂Cl₂ to remove any organic impurities. The aqueous phase was filtered and concentrated to give yellowish green solid. This solid was dissolved in MeOH (20 ml) and TFA (10 ml) was added dropwise at $0-5^{\circ}\text{C}$. The stirring was continued for 2 h and then the solution was concentrated *in vacuo* to afford **9**. IR (KBr): $\nu = 2977$; 2354; 1739; 1470; 1182; 1110. ¹H NMR (D₂O): δ 2.57 (s, 2H, NH₂); 2.74 (s, 6H, NMe₂); 3.31 (d, J 3.6 Hz, 2H, CH₂); 4.09 (s, 1H, CH); 4.44 (m, 2H, SeCH₂); 7.32–7.4, 7.1–7.73 (m, 4H, Ph). ¹³C NMR (D₂O): δ 28.3 (SeCH₂); 42.4 (NMe₂); 52.5 (H₂NCH); 61.2 (NCH₂); 129.9 (C3–Ph); 130.1 (C4–Ph); 131.2 (C5–Ph); 132.4 (Se–C1–Ph); 132.7 (C2–Ph); 137.1 (C6–Ph); 170.5 (C=O). MS (TOF MS ES⁺) m/z (%): 303 (100), 301 (47.9), 299 (18.9) [M⁺H].

Deprotection of 4,4-dimethyl-2-oxazoline-*N*-Boc-*L*-selenocysteine methyl ester (10). 4,4-Dimethyl-2-oxazoline-*N*-Boc-*L*-selenocysteine methyl ester was deprotected by following a similar method to that employed for the phenyl derivative. During the deprotection, the oxazoline ring is hydrolyzed to give the corresponding acid derivative **4**. [¹³C{¹H}] in D₂O: δ 56.3 (SeCH₂); 60.2 (OC=CHNH); 111.9 (SeC=); 114.8 (C4–Ph); 117.7 (C5–Ph); 120.6 (C2–Ph); 162.5 (C3–Ph); 162.8 (C6–Ph); 163.1 (O=COH attached to Ph ring); 163.5 (O=COH)].

HPLC Assay. The GPx-like activity of ebselen and Sec derivatives was studied with H₂O₂ as a substrate and PhSH as thiol co-substrate by following a method reported by Back *et al.* for BnSH.³² In this assay, we employed a mixture containing a 1 : 1.2 molar ratio of PhSH and H₂O₂ in dichloromethane–methanol (95 : 5) at room temperature as our model system. Runs with and without 10 mol% of added catalyst were carried out under the same conditions and the column was eluted with a methanol–water (90 : 10) mixture. Periodically, aliquots were removed and the concentrations of the product diphenyl disulfide (PhSSPh) were determined from the detector response, using pure PhSSPh as an external standard. The initial rates

were calculated from the first 5–10% of the reaction to avoid any reverse reaction from the products.

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