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# Functional Mimics of Glutathione Peroxidase: Bioinspired Synthetic Antioxidants

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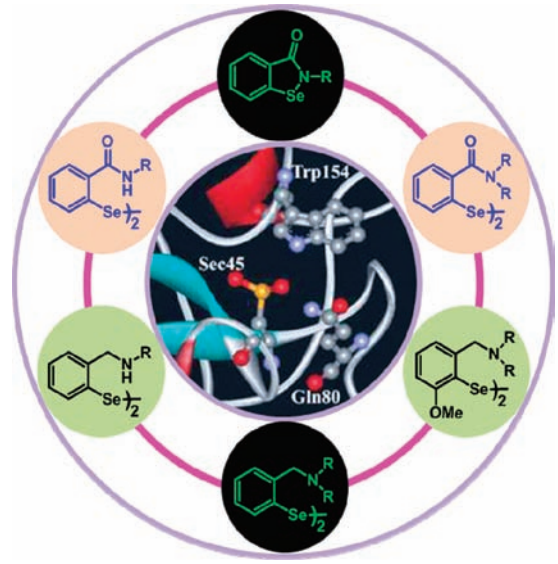
## CONSPECTUS




Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify these reactive intermediates. Mammalian cells have elaborate antioxidant defense mechanisms to control the damaging effects of ROS. Glutathione peroxidase (GPx), a selenoenzyme, plays a key role in protecting the organism from oxidative damage by catalyzing the reduction of harmful hydroperoxides with thiol cofactors. The selenocysteine residue at the active site forms a "catalytic triad" with tryptophan and glutamine, which activates the selenium moiety for an efficient reduction of peroxides.

After the discovery that ebselen, a synthetic organoselenium compound, mimics the catalytic activity of GPx both in vitro and in vivo, several research groups developed a number of small-molecule selenium compounds as functional mimics of GPx, either by modifying the basic structure of ebselen or by incorporating some structural features of the native enzyme. The synthetic mimics reported in the literature can be classified in three major categories: (i) cyclic selenenyl amides having a Se−N bond, (ii) diaryl diselenides, and (iii) aromatic or aliphatic monoselenides. Recent studies show that ebselen exhibits very poor GPx activity when aryl or benzylic thiols such as PhSH or BnSH are used as cosubstrates. Because the catalytic activity of each GPx mimic largely depends on the thiol cosubstrates used, the difference in the thiols causes the discrepancies observed in different studies.

In this Account, we demonstrate the effect of amide and amine substituents on the GPx activity of various organoselenium compounds. The existence of strong Se···O/N interactions in the selenenyl sulfide intermediates significantly reduces the GPx activity. These interactions facilitate an attack of thiol at selenium rather than at sulfur, leading to thiol exchange reactions that hamper the formation of catalytically active selenol. Therefore, any substituent capable of enhancing the nucleophilic attack of thiol at sulfur in the selenenyl sulfide state would enhance the antioxidant potency of organoselenium compounds. Interestingly, replacement of the *sec*-amide substituent by a *tert*-amide group leads to a weakening of Se···O interactions in the selenenyl sulfide intermediates. This modification results in 10- to 20-fold enhancements in the catalytic activities. Another strategy involving the replacement of *tert*-amide moieties by *tert*-amino substituents further increases the activity by 3- to 4-fold. The most effective modification so far in benzylamine-based GPx mimics appears to be either the replacement of a *tert*-amino substituent by a *sec*-amino group or the introduction of an additional 6-methoxy group in the phenyl ring. These strategies can contribute to a remarkable enhancement in the GPx activity.

In addition to enhancing catalytic activity, a change in the substituents near the selenium moiety alters the catalytic mechanisms. The mechanistic investigations of functional mimics are useful not only for understanding the complex chemistry at the active site of GPx but also for designing and synthesizing novel antioxidants and anti-inflammatory agents.



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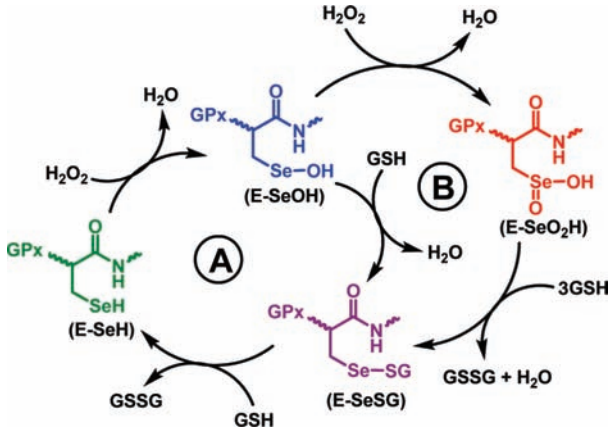
## Introduction

Glutathione peroxidase (GPx),[1,2] iodothyronine deiodinase (ID),[3] and thioredoxin reductase (TrxR)[4] are the three major mammalian selenoenzymes that contain selenocysteine (Sec) in their active sites. GPx is an antioxidant enzyme that protects various organisms from oxidative stress by catalyzing the reduction of hydroperoxides by using thiol cofactors.[1,5] The deiodinases are responsible for the activation/inactivation of thyroid hormones.[3] TrxR plays an important role in a variety of biological functions such as DNA synthesis.[6] Although all three enzymes contain Sec in their active sites, their catalytic mechanisms, substrate specificity, and cofactor systems are strikingly different.[7] While glutathione (GSH) is an efficient cofactor for GPx, a dithiol such as dithiothreitol (DTT) is required for the catalytic activity of the deiodinases.[3,7] The TrxRs, on the other hand, have cysteine moieties in different subunits, and they prefer to utilize these cysteines as thiol cofactors. The catalytic cycle of TrxR involves the formation of an internal selenenyl sulfide, which undergoes redox reactions with additional cysteine residues.[7,8]

The catalytic mechanism of GPx involves the initial oxidation of selenol (E-SeH) to produce the corresponding selenenic acid (E-SeOH). The E-SeOH thus produced reacts with glutathione (GSH) to produce selenenyl sulfide (E-SeSG). A second molecule of GSH then attacks at the sulfur center of E-SeSG to regenerate the active form of the enzyme (E-SeH) (Scheme 1, cycle A). In the overall process, 2 equiv of GSH is oxidized to the corresponding disulfide (GSSG), while the hydroperoxide is reduced to water.[1,5] When the peroxide concentration is higher than that of the thiol, the selenium center in the selenenic acid (E-SeOH) may undergo further oxidation to produce the seleninic acid (E-$SeO_2H$) (Scheme 1, cycle B). However, the seleninic acid may lie off the main catalytic cycle in the presence of thiol cofactor. It has been reported that the selenium center of Sec residue forms a "catalytic triad" with two other amino acid residues, tryptophan (Trp) and glutamine (Gln), by noncovalent interactions.[9] These interactions activate the Sec moiety for an efficient reduction of peroxides. Although the GPx cycle involves selenol, selenenic acid, and selenenyl sulfide as the major intermediates, the crystal structure of native GPx reveals the existence of the enzyme in its seleninic acid form (Figure 1a).[10,11] The crystal structure of the semisynthetic enzyme selenosubtilisin also shows that the Sec moiety exists in a seleninic acid form (Figure 1b).[12] These observations suggest that the seleninic acid is the most stable form and may represent the resting state of the enzyme.

Although successful mimics of ID and TrxR are not available, the chemistry at the active site of GPx has been extensively studied with the help of synthetic mimics.[13,14] Since the discovery that ebselen (**1**) mimics the activity of GPx,[15] several research groups have developed a number of small-molecule selenium compounds either by modifying the basic structure of ebselen or by incorporating some structural features of the native enzyme. The synthetic mimics reported in the literature can be classified into three major categories: (i) cyclic selenenyl amides having Se−N bond, (ii) diaryl diselenides, and (iii) aromatic or aliphatic monoselenides (Figure 2).[13,14,16−29] In addition, a number of tellurium compounds have been shown to exhibit interesting antioxidant activity.[30] The mechanism for the antioxidant activity of these compounds depends mainly on the relative reactivity of the selenium or tellurium centers toward thiol and peroxide substrates. In this Account, we describe the effect of amide and amine substituents on the reactivity of diaryl diselenides toward thiols and peroxides and discuss how the changes in the reactivity alter the catalytic mechanisms.

**SCHEME 1.** Proposed Catalytic Mechanism for the Antioxidant Activity of GPx




## GPx Activity of Ebselen and Related Compounds

The anti-inflammatory compound ebselen (**1**) exhibits significant antioxidant activity in the reduction of $H_2O_2$ and other hydroperoxides in vivo.[31,32] Ebselen exhibits interesting therapeutic properties for a number of disease states including anti-inflammatory activities. These properties are due to the ability of ebselen to catalyze the reduction of hydroperoxides by GSH.[33] However, the catalytic cycle of ebselen is controversial probably due to the differences in the nature of peroxides and thiols employed for measuring the GPx activity. Recent studies have shown that ebselen is a relatively inefficient catalyst for the reduction of hydroperoxides with aryl

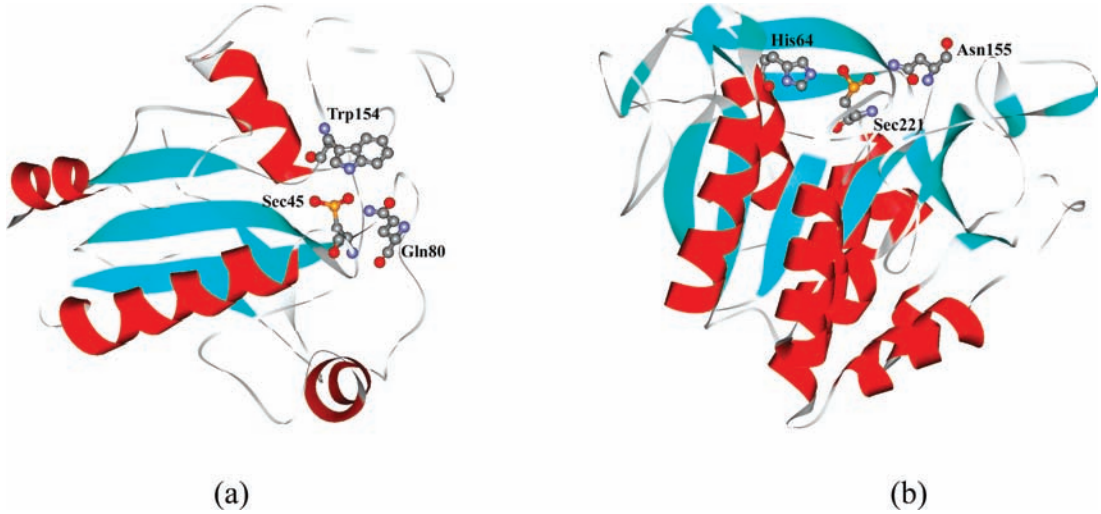



**FIGURE 1.** X-ray crystal structures of (a) GPx (PDB: 1GP1)[10,11] and (b) selenosubtilisin (PDB: 1SEL).[12]

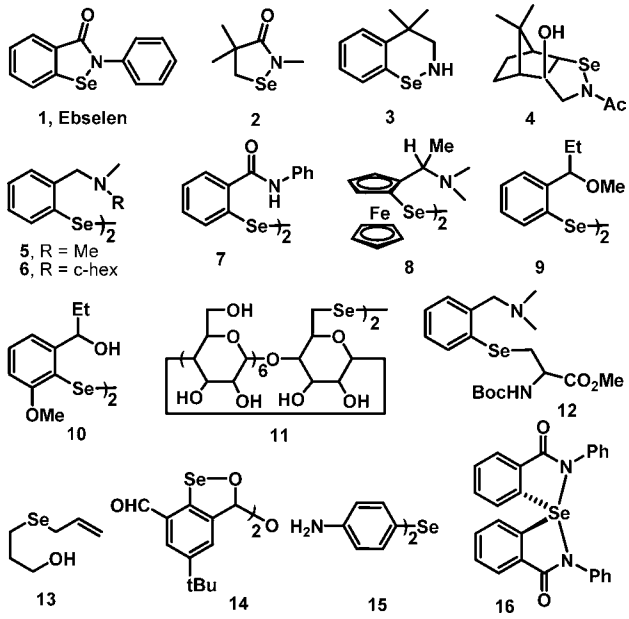
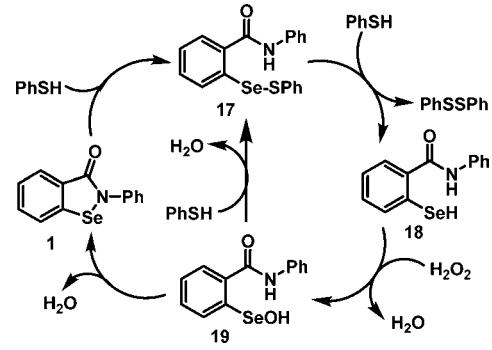
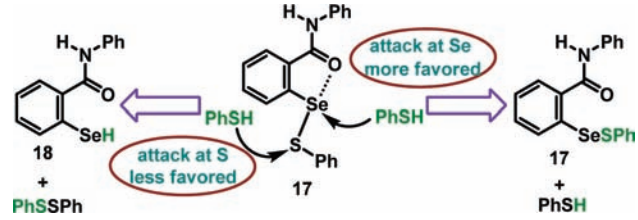



**FIGURE 2.** Chemical structures of some GPx mimics reported in the literature.

**SCHEME 2.** One of the Initially Proposed Catalytic Cycles of Ebselen




**SCHEME 3.** Se···O Interactions Modulate the Nucleophilic Attack of Thiol in Selenenyl Sulfide




or benzylic thiols such as PhSH or BnSH as cosubstrates.[16,33] The facile ring-opening of ebselen by thiols led to the assumption that the catalytic cycle of ebselen may involve the selenenyl sulfide (**17**), selenol (**18**), and selenenic acid (**19**) as shown in Scheme 2.

We have studied the GPx activity of ebselen and related compounds to understand the reason for the relatively poor catalytic activity of these compounds in aromatic thiol assays.[34−36] The reaction of ebselen with PhSH does not generate any selenol even when an excess amount of thiol is used. Although the Se−N bond in ebselen is readily cleaved by PhSH to produce the selenenyl sulfide **17**, the reaction of **17** with PhSH does not produce the selenol **18**. This is due to the presence of strong Se···O nonbonded interactions in the selenenyl sulfide, which facilitates an attack of thiol at selenium rather than at sulfur, leading to thiol exchange reaction (Scheme 3).[34] This undesired thiol exchange reaction hampers the formation of selenol **18**.

To understand the effect of various substituents at the nitrogen atom in ebselen on the strength of Se···O interactions and the thiol exchange reactions,[37] we have studied a number of ebselen derivatives (**20**−**30**) (Figure 3).[35,36] These studies indicate that the carbonyl-oxygen interacts with selenium in the selenenyl sulfides irrespective of the substituents on nitrogen. Therefore, all the selenenyl sulfides derived from **20**−**30** invariably undergo thiol exchange reactions when

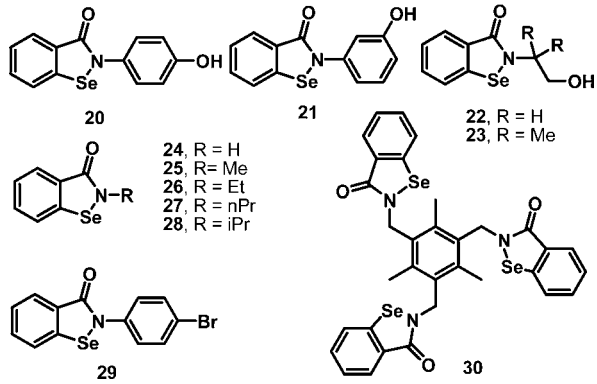


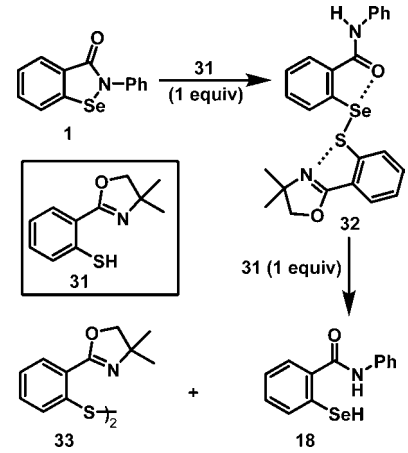

**FIGURE 3.** Ebselen analogues having different substituents on nitrogen atom.

aromatic thiols are used as cosubstrates. These studies also indicate that the nature of thiol has a dramatic effect on the catalytic activities of these ebselen analogues. On the other hand, the nature of peroxides does not appear to have any significant effect on the catalytic efficiencies. Therefore, the discrepancies in the activities of ebselen and related compounds in various assays should arise mainly from the variation in thiol used for the reduction of hydroperoxides.

As the strong Se···O interactions in the selenenyl sulfides reduce the antioxidant activity, the introduction of substituents that can weaken the Se···O interactions may increase the activity. In view of this, we have used the oxazoline-based thiol **31** to introduce an additional S···N interaction in the selenenyl sulfide.[34] As expected, the reaction of ebselen with thiol **31** produces the selenenyl sulfide **32** having an S···N interaction. Although there is a significant Se···O interaction, the strength of S···N interaction is found to be sufficient to modulate the attack of incoming thiol at the sulfur center. Addition of 1 equiv of **31** to compound **32** produces the selenol **18** and the disulfide **33** (Scheme 4). These observations suggest that the introduction of coordinating amine or other groups in the thiols could enhance the catalytic activity of ebselen and the related compounds. Further improvements in the catalytic activity may be obtained by completely preventing the Se···O interactions.

In the absence of thiols, ebselen reacts readily with peroxides to oxidize the selenium center. Interestingly, this reaction does not produce the corresponding selenoxide as previously reported,[38] but it produces the corresponding seleninic acid **34** in nearly quantitative yield.[36] Similar reactions occur with other ebselen analogues **20**−**30** (Figure 3). The crystal structures of two seleninic acids **34** and **35**, derived from **1** and **28**, respectively, show that the carbonyl-oxygen atom noncovalently interacts with selenium as shown in Figure 4.[36,39] The reaction of seleninic acid **34** with an excess

**SCHEME 4.** S···N Interactions in **32** Modulate the Nucleophilic Attack of **31** at the Sulfur Center[34]




amount of PhSH produces the selenenyl sulfide **17**. The formation of **17** may proceed via the formation of a thiol-seleninate and selenenic acid (**19**) intermediates.[36] When the concentration of PhSH is not sufficient to perform the conversion of seleninic acid **34** to the selenenyl sulfide **17**, compound **19** undergoes a rapid cyclization to produce ebselen. The addition of an excess amount of PhSH to the reaction mixture containing ebselen and other oxidized compounds leads to the formation of **17**. Therefore, the selenenyl sulfide **17** is found to be the only selenium-containing product when a large excess (∼5 equiv) of PhSH is added to the seleninic acid **34**.

The disproportionation of the selenenyl sulfide **17** to the corresponding diselenide **7** is found to be the major reaction pathway for the GPx activity of ebselen. The rate of this disproportionation depends on the nature of thiol used for the formation of the selenenyl sulfide. The diselenide **7** produced in this reaction reacts further with $H_2O_2$ to generate a mixture of selenenic acid **19** and seleninic acid **34**. While compound **19** either reacts with PhSH to produce the selenenyl sulfide **17** or undergoes a cyclization to produce ebselen, the seleninic acid **34** reacts with PhSH or diselenide **7** to generate the selenenic acid **19**. Based on these observations, we have proposed a revised mechanism for the GPx activity of ebselen (Scheme 5).[36] In this catalytic cycle, the formation of the diselenide **7** is the rate-determining step.

As the diselenide **7** was found to be a key intermediate in the GPx cycle of ebselen, we extended our work to study a number of diselenides having *sec*-amide moiety. The *sec*-amide-based diselenides **36**−**39** can be readily prepared by treating the selenenyl amides **25**−**28** with triphenyl phosphine (Scheme 6).[39] While ebselen and compounds **25**−**28** readily react with PhSH to produce the corresponding selene-

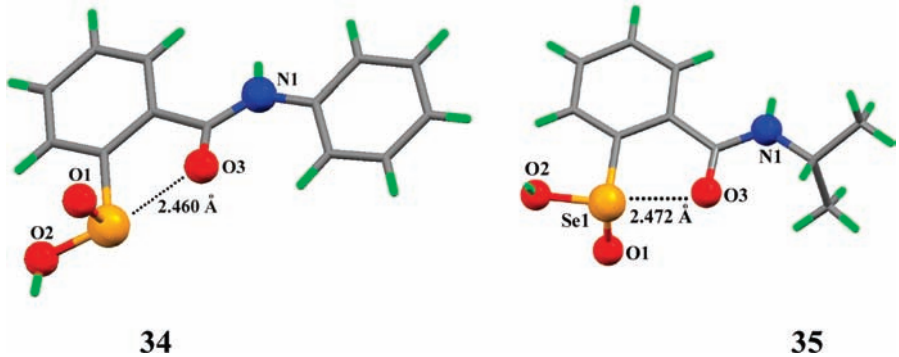
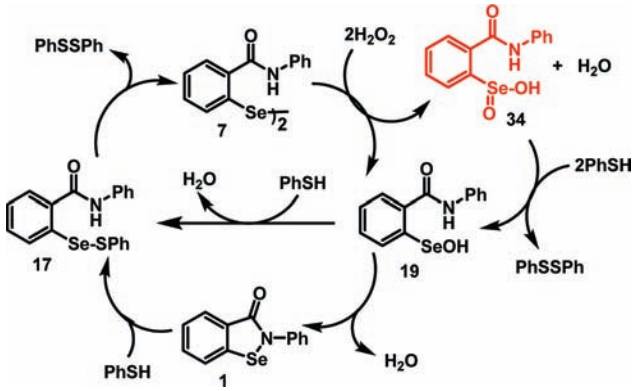
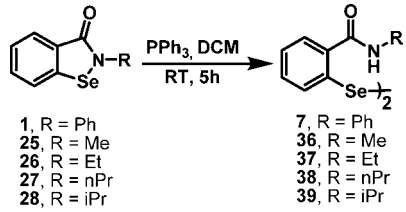



**FIGURE 4.** X-ray crystal structures of seleninic acids **34** and **35**.[36,39]

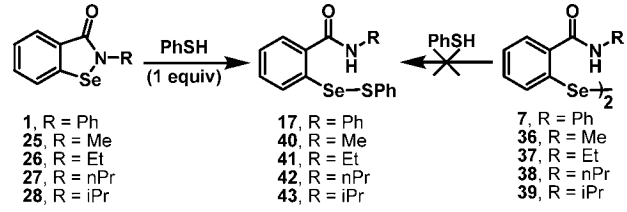
**SCHEME 5.** Revised Catalytic Mechanism for the GPx Activity of Ebselen[36,39]




**SCHEME 6.** Synthesis of *sec*-Amide-Based Diselenides from Selenenyl Amides

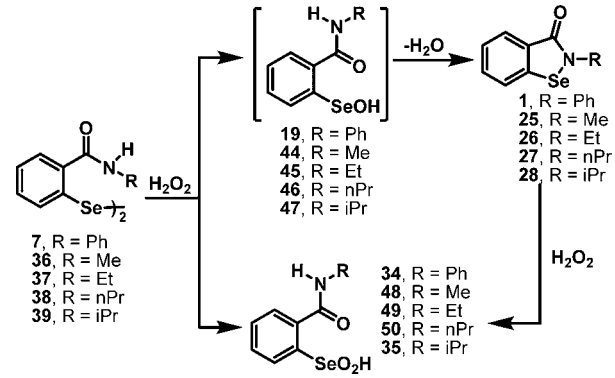



**SCHEME 7.** Reactivity of Selenenyl Amides and Diselenides toward PhSH




nyl sulfides **17** and **40**−**43**, the diselenides are essentially unreactive toward PhSH (Scheme 7). Therefore, the reaction of diselenides **7** and **36**−**39** with $H_2O_2$ is important for the GPx activity of these compounds. In fact, diselenides **7** and **36**−**39** react readily with $H_2O_2$ to produce the corresponding selenenic acid and seleninic acids (Scheme 8).[39] Therefore, it is not surprising that the selenenyl amides and *sec*-amide-based

**SCHEME 8.** Reactions of *sec*-Amide-Based Diselenides with $H_2O_2$




diselenides follow similar mechanisms for their catalytic activities.[36,39] It should be noted that the diselenide bonds may be cleaved by more reactive dithiols such as lipoic acid or dithiothreitol.[40,41]

A key feature in the catalytic mechanism of selenenyl amides and *sec*-amide-based diselenides is the regeneration of selenenyl amides under a variety of conditions via the formation of selenenic acids as shown in Schemes 5 and 8. This cyclization has a lot of biological significance. Although the cyclization of a selenenic acid to a selenenyl amide has not yet been detected in proteins, there are indications that such a transformation may occur in selenoenzymes. It has been shown that the selenenic acid (E-SeOH) produced in the GPx cycle reacts readily with a yet undefined X−H group with elimination of $H_2O$ to produce an oxygen-free Se(II) compound that instantly reacts with thiols.[42] Flohé has proposed that the E-SeOH intermediate may react with proximal Gln residue to form an Se−N bond.[43] The assumption that a selenenyl amide could be generated in proteins is further supported by the recent reports that the redox regulation of protein tyrosine phosphatase 1B (PTP1B; Figure 5) involves a sulfenyl amide intermediate.[44,45] In this redox mechanism, the sulfenic acid generated in response to PTP1B oxidation by $H_2O_2$ is rapidly converted to a sulfenyl amide species (Scheme 9).

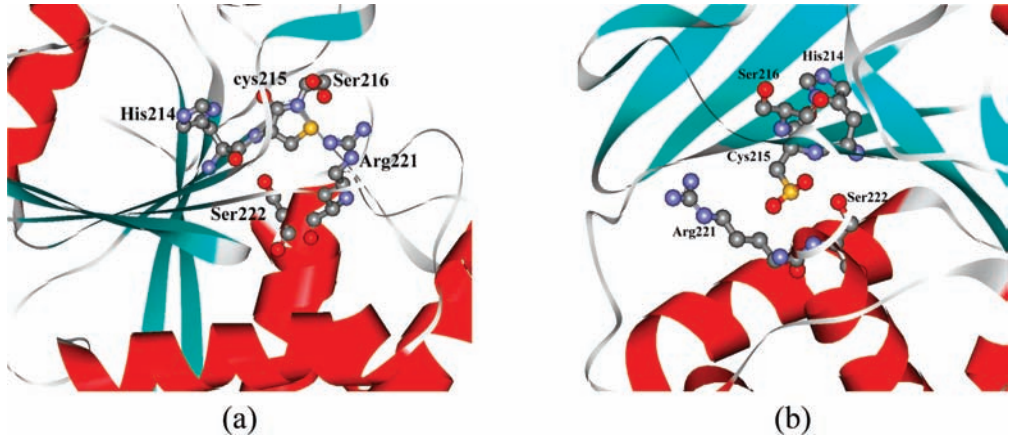
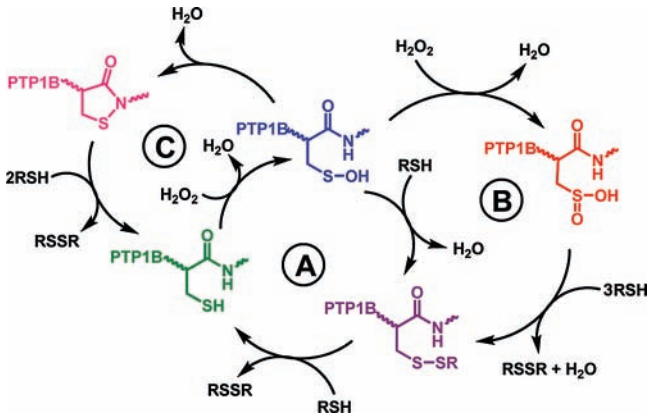



**FIGURE 5.** X-ray crystal structures of PTP1B in its (a) sulfenyl amide (PDB: 1OES) and (b) sulfinic acid (PDB: 1OEU).[44,45] Some peptide residues are removed for clarity.

**SCHEME 9.** Redox Regulation of PTP1B Involving a Sulfenyl Amide




The formation of sulfenyl amide is reversible as the cleavage of S−N bond by cellular thiols (e.g., GSH) converts the inactivated protein back to its catalytically active form. Recently, we have shown that S···O/N nonbonded interactions in the sulfenic acid intermediate may modulate the cyclization process.[46] As the selenium moiety in selenenic acids is generally more electrophilic than the sulfur moiety in sulfenic acid, the attack of amide-nitrogen at selenium is expected to be more facile than a similar attack at the sulfur center.

Similar to the oxidation of selenenyl amide to produce the corresponding seleninic acids, the reactions of sulfenyl amides with $H_2O_2$ have been shown to produce the corresponding sulfinic acids.[47] A comparison of GPx catalytic cycle (Scheme 1) with the redox regulation of PTP1B (Scheme 9) indicates that the chemistry at the active site of PTP1B is very similar to that of GPx. However, it is not clear whether any amino acid residue at the active site of GPx can modulate the cyclization similar to the role of histidine in PTP1B.

**TABLE 1.** Reduction of $H_2O_2$ by PhSH in the Absence and Presence of Diselenides

| compound | $t_{1/2}$ values (min)[a] | relative activity[b] |
|---|---|---|
| control | 2905 | 1.0 |
| **36** | 1675 | 1.7 |
| **37** | 1640 | 1.8 |
| **38** | 1721 | 1.7 |
| **39** | 1892 | 1.5 |
| **51** | 82 | 35.4 |
| **52** | 136 | 21.4 |
| **53** | 175 | 16.6 |
| **54** | 168 | 17.3 |

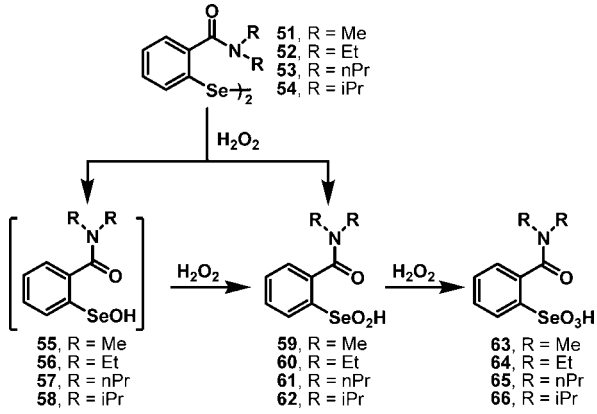
[a] The time required for 50% conversion of PhSH to PhSSPh. [b] The relative activities are given with respect to the control values. For reaction conditions, see ref 39.

## Diaryl Diselenides Having *tert*-Amide Substituents

The main reason for the poor catalytic activity of *sec*-amide-based compounds is the presence of strong Se···O nonbonded interactions in the selenenyl sulfide intermediates that leads to undesired thiol exchange reactions.[7,34,35] It has been shown previously that the GPx activity of *tert*-amine-based diselenides depends on the strength of Se···N interactions.[17,18,21] The compounds that exhibit weak Se···N interactions in the selenenyl sulfide intermediates show much better activity than the ones having strong Se···N interactions.[21] Therefore, it was thought worthwhile to see whether the replacement of *sec*-amide group in compounds **36**−**39** by a *tert*-amide substituent would enhance the catalytic activity. In view of this, we have synthesized diselenides **51**−**54** having *tert*-amide substituents and compared the GPx activities of these compounds to that of *sec*-amide-based diselenides **36**−**39** (Table 1).[39]

From Table 1 it is clear that the *tert*-amide-substituted diselenides **51**−**54** are almost 10−20 times more active than the corresponding *sec*-amide-based compounds **36**−**39**.[39] In

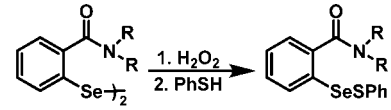
**SCHEME 10.** Reaction of *tert*-Amide-Based Diselenides with $H_2O_2$[39]




contrast to the GPx activity of *sec*-amide-based diselenides, which is not affected by the nature of peroxides, the catalytic activities of the *tert*-amide-based diselenides highly depend on the nature of peroxides used for the assay.[35,39] While the *sec*-amide-based diselenides exhibit strong Se···O interactions, such interactions are relatively weak in the case of *tert*-amide-based diselenides. However, diselenides **51**−**54** are unreactive toward PhSH, indicating that the replacement of *sec*-amide by the *tert*-amide substituent does not alter the reactivity of the diselenides toward thiols. When compounds **51**−**54** are treated with $H_2O_2$, they produce the seleninic acids **59**−**62** as the major products along with some unreacted diselenides. This is in contrast to the reactions of **36**−**39** with $H_2O_2$ in which the diselenides are almost quantitatively converted to the corresponding seleninic acids and selenenyl amides (Scheme 8). The cyclization of the selenenic acids **44**−**47**, due to the presence of free −N−H groups, leads to the formation of selenenyl amides (Scheme 8).[36,39] Furthermore, in contrast to the seleninic acids **35** and **48**−**50** that do not undergo any further oxidation, the *tert*-amide-based seleninic acids **59**−**62** react with an excess amount of $H_2O_2$ (∼25 equiv) to produce the overoxidized selenonic acids **63**−**66** (Scheme 10). This indicates that the cyclization of selenenic acids **44**−**47** to the corresponding selenenyl amides **25**−**28** protects the selenium moiety from overoxidation.

In the presence of PhSH, all the oxidized selenium compounds **59**−**66** react rapidly with PhSH to produce the corresponding selenenyl sulfides (**67**−**70**, Scheme 11). The $^{77}$Se NMR chemical shifts for these selenenyl sulfides are shifted almost 60 ppm upfield as compared to that of the *sec*-amide-based selenenyl sulfides **40**−**43**, indicating that the Se···O interactions in compounds **67**−**70** are relatively weak.[39] The theoretical calculations on the selenenyl sulfides indicate that the Se···O distances in compounds **67**−**70** are significantly larger than that of compounds **40**−**43**. For example, the

**SCHEME 11.** Reactions of Diselenides **51**−**54** with $H_2O_2$ and PhSH




**51**, R = Me; **52**, R = Et
**53**, R = nPr; **54**, R = iPr

**67**, R = Me; **68**, R = Et
**69**, R = nPr; **70**, R = iPr

Se···O distance of 2.93 Å in compound **70** is much higher than that observed for compound **43** (2.47 Å) (Figure 6).[39] The weak Se···O interactions in compounds **67**−**70** are partly responsible for the higher activities of the diselenides **51**−**54** as compared to that of the corresponding *sec*-amide analogues **36**−**39**. As the Se···O interactions are relatively weak, the *tert*-amide-based selenenyl sulfides become unstable in solution, and therefore, they undergo rapid disproportionation to produce the corresponding diselenides. This results in an enhancement in the catalytic activity of *tert*-amide-based diselenides. It should be noted that the formation of diselenides by disproportionation reaction is the rate-determining step. The detailed mechanistic studies indicate that the catalytic mechanism of *tert*-amide-based diselenides is identical to that of the *sec*-amide-based compounds except the fact that the selenenic acids derived from the *sec*-amide-based diselenides undergo a cyclization to produce the corresponding selenenyl amides (Scheme 5).[39]

## Amine-Based Diaryl Diselenides as GPx Mimics

It is known that two amino acid residues Trp and Gln that are present in all known members of the GPx family constitute a "catalytic triad" with Sec in which the selenol moiety is both stabilized and activated by the hydrogen bonding with the imino group of Trp and amido group of the Gln residue.[10,48] This concept led to the design and synthesis of diaryl diselenides having basic amino groups in close proximity to selenium. The reductive cleavage of −Se−Se− bonds in compounds **5**, **6**, and **8** produces the corresponding selenols as the catalytically active species.[17,18,21] The deprotonation of selenols by the proximal amino groups produces more reactive selenolates that readily react with peroxides to produce the corresponding selenenic acids. Reactions of these selenenic acids with thiols produce the corresponding selenenyl sulfides, which upon reactions with second equivalents of thiols regenerate the selenols. In this way, the catalytic cycle of the amine-based selenols is identical to that of GPx (Schemes 1 and 12). A complete inhibition of the catalytic activity of **71** by gold(I) chlorides[49] indicates that the selenol is the key compound in the catalytic cycle.

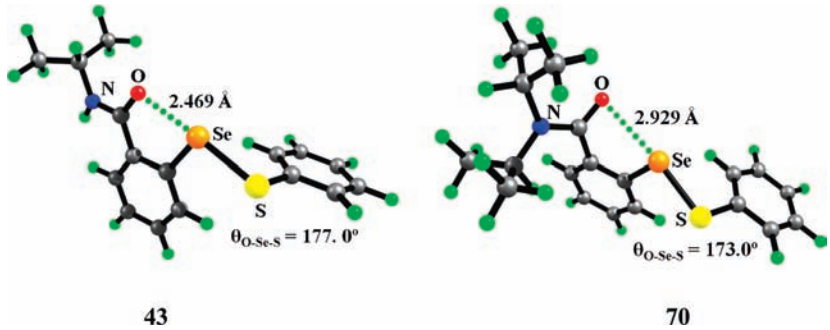
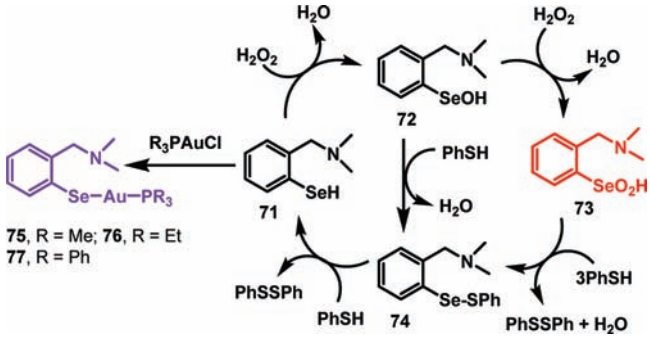
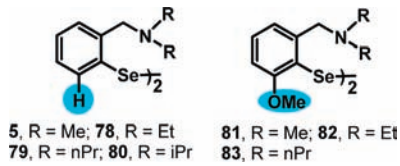



**FIGURE 6.** Energy optimized geometries of selenenyl sulfides **43** and **70**.[39]

**SCHEME 12.** Catalytic Cycle of Selenol **71** (Derived from **5**) and Inhibition by Gold(I) Chlorides




The amino-substituted diselenide **5** exhibits much better GPx activity than ebselen (**1**) in the presence of aromatic thiols.[50] Although the Se···N interactions in the selenenyl sulfide (**74**) derived from **5** are weaker than the Se···O interactions in compound **17**, a large excess of thiol is generally required to overcome the thiol exchange reaction in the *tert*-amine-based selenenyl sulfides.[50] These observations suggest that the prevention of Se···N interactions in selenenyl sulfides might lead to the development of better GPx mimics. Recently, we have reported that the replacement of an aryl proton in compounds **5** and **78**–**79** by a methoxy group (compounds **81**–**85**) prevents the Se···N interactions in the key intermediates and dramatically enhances the GPx activity.[50]




Although the catalytic mechanisms of the diselenides **81**–**83** are identical to that of **5** and **78**–**79**, our studies reveal that the introduction of the methoxy group leads to dramatic changes in the reactivity of selenium in all the intermediates. The first major change is observed in the reactivity of diselenides toward thiols. While the diselenides **5** and **78**–**79** with 2 equiv of PhSH produce a mixture of selenols (**71**, **84**–**85**) and selenenyl sulfides (**74**, **87**–**88**), the reactions of compounds **81**–**83** with 2 equiv of PhSH produced the selenols (**90**–**92**) quantitatively (Scheme 13).[50] The $^{77}$Se NMR chemical shifts for the selenenyl sulfides (**93**, 470 ppm; **94**, 461 ppm; **95**, 451 ppm) showed a dramatic upfield shift (~100 ppm) with respect to those of the selenenyl sulfides (**74**, **87**–**88**), indicating the absence of any strong Se···N interactions in compounds **93**–**95**. In agreement with these observations, the theoretical studies on the selenenyl sulfides indicate that the interaction energies for the Se···N interactions in compounds **74** and **87**–**88** are almost two times higher than those of the corresponding methoxy-substituted compounds.[50] The Se···N distance observed for compound **74** is much shorter than that in compound **93** (Figure 7). The weakening of Se···N interaction due to the introduction of methoxy-substituent increases the positive charge on sulfur atoms in the selenenyl sulfides. This enhances the possibility of a nucleophilic attack of incoming thiol at the sulfur center.

As the reactions of selenenyl sulfides with thiols should produce the corresponding selenols for the catalytic activity, it is important to understand the effect of amino groups on the reactivity of selenols **71**, **84**–**85**, and **90**–**92**. Our experimental and theoretical studies indicate that the introduction of a methoxy-substituent significantly enhances the zwitterionic nature of the selenols (Scheme 14). Because of the large negative charge on selenium in compounds **90**–**92**, the $^{77}$Se NMR chemical shifts of these compounds are shifted almost 100 ppm upfield relative to those of compounds **71** and **84**–**85**. Therefore, the selenium centers in compounds **90**–**92** are more nucleophilic than those in compounds **71** and **84**–**85**.[50]

In contrast to the change in the reactivity of selenium in selenenyl sulfides and selenols upon the introduction of the 6-methoxy group, such changes in the selenenic acids appear to have a very little effect on the catalytic activities of compounds **5**, **78**–**79**, and the corresponding 6-methoxy-substituted diselenides. However, all the *tert*-amine-based

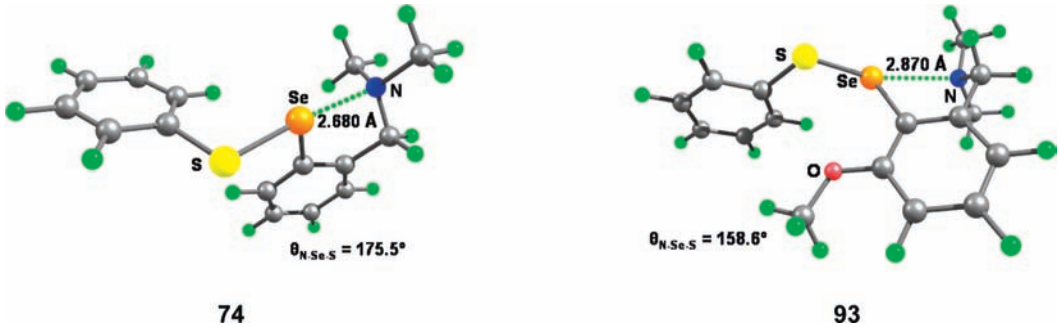
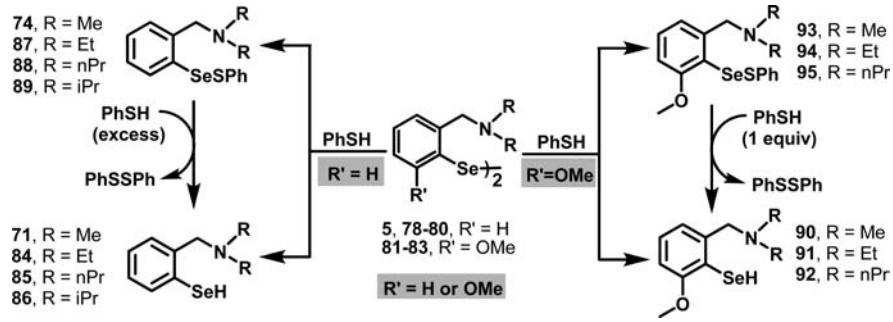
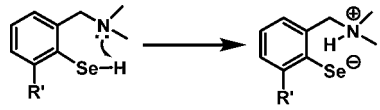



**FIGURE 7.** Energy optimized geometries of selenenyl sulfides.[50]

**SCHEME 13.** Reaction of *tert*-Amine-Based Diselenides with PhSH to Produce the Corresponding Selenenyl Sulfides and Selenols




**SCHEME 14.** Deprotonation of the Selenol Moieties by the *tert*-Amino Groups in Compounds **71** and **90**




diselenides (**5** and **78**−**83**) react with $H_2O_2$ to produce seleninic acids (**100**−**103** and **111**−**113**) as the major products. This is similar to the reactivity of the amide-based diselenides (**36**−**39** and **51**−**54**) toward $H_2O_2$.[36,39] As the reactions of diselenides with thiols are much faster than those with $H_2O_2$, the first step of the catalytic cycle of amine-based diselenides should be the cleavage of −Se−Se− bonds by thiols. It should be noted that the methoxy-substituent prevents the overoxidation of seleninic acids **111**−**113** to the corresponding selenonic acids **114**−**116** (Scheme 15). While the 6-methoxy group prevents the overoxidation mainly by steric protection, the *sec*-amide group prevents the overoxidation of seleninic acids by cyclization (Scheme 5).

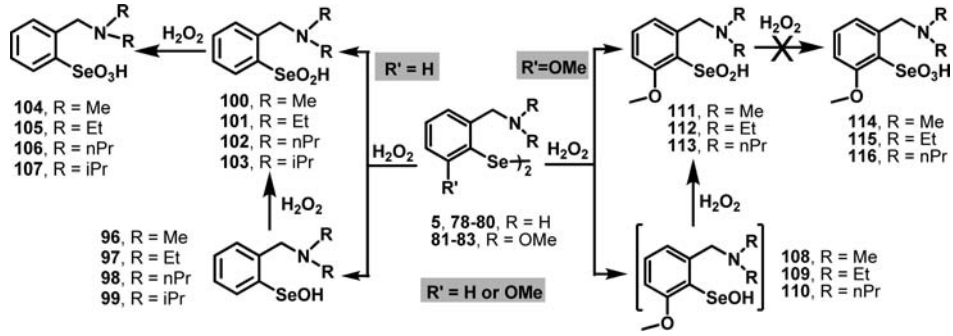
Our further studies on amine-based diaryl diselenides indicate that the replacement of *tert*-amino groups by the *sec*-amino-substituent significantly enhances the GPx activity.[51] A detailed mechanistic study on the formation of various catalytic intermediates indicates that the *sec*-amine-based diselenides **117**−**120** behave very similarly to the methoxy-substituted diselenides **81**−**83**. Similar to the methoxy-substituted diselenides **81**−**83**, but in contrast to diselenides **5** and **78**−**80**, the *sec*-amine-based compounds **117**−**120** react readily with PhSH (2 equiv) to produce the corresponding selenols (**125**−**128**) quantitatively (Scheme 16).[50,51] The *sec*-amine groups not only increase the solubility of these compounds in water but also deprotonate the thiol to provide a high local concentration of nucleophilic thiolate anion.

## Conclusions and Outlook

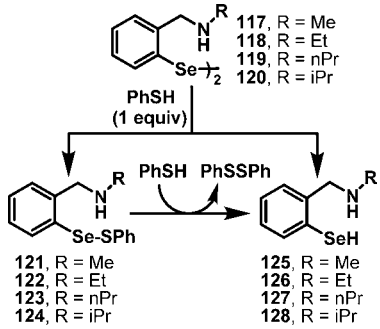
The results discussed in this Account demonstrated the effect of various amide and amine substituents on the glutathione peroxidase activity of organoselenium compounds. The experimental and theoretical studies on ebselen and related compounds suggest that strong Se···O/N interactions in the selenenyl sulfide intermediates are detrimental to the biological activity of synthetic selenium compounds due to thiol exchange reactions. Therefore, any substituent that is capable of enhancing the nucleophilic attack of thiol at sulfur in the selenenyl sulfide state would enhance the antioxidant potency of organoselenium compounds. The use of highly reactive thiol cosubstrates appears to be important to overcome the thiol exchange reactions.

In *tert*-amine-based GPx mimics, the introduction of additional substituents that can prevent strong Se···N interactions in the selenenyl sulfide state significantly enhances the catalytic activity. The replacement of *tert*-amino substituents by *sec*-amino groups also increases the activity, as the *sec*-amino

**SCHEME 15.** Reactions of *tert*-Amine-Based Diselenides with $H_2O_2$




**SCHEME 16.** Reaction of *sec*-Amine-Based Diselenides **117**−**120** with PhSH




groups are better than the *tert*-amino moieties in generating the catalytically active selenols. This is due to the absence of any significant thiol exchange reactions in the selenenyl sulfides derived from *sec*-amine-based diselenides. A comparison of the activities of *sec*-amino-substituted compounds with those of *sec*-amide-based diselenides indicates that the *sec*-amino-substituted compounds are more sensitive to the nature of peroxide than the *sec*-amide-based GPx mimics. As the catalytic activity of GPx mimics largely depends on the thiol employed in the reactions, the difference in the thiol cosubstrates is responsible for the discrepancies observed in the outcomes of different studies. It is interesting to note that the selenoenzymes employ remarkably different cosubstrate systems to exert various biological reactions. While the GPx enzyme uses glutathione as cosubstrate, iodothyronine deiodinase and thioredoxin reductase utilize internal thiols (cysteine residues) for their catalytic activity. It would be of particular interest to check whether an in-built thiol can be used in a synthetic selenium compound to enhance its antioxidant activity.

Another aspect that has been debated for several years is the nature of intermediates produced in the GPx cycle. Although selenol, selenenyl sulfide, and selenenic acid were generally accepted as common intermediates, the formation of a selenenyl amide species cannot be completely ruled out. In the GPx cycle of ebselen, it has been demonstrated that the cyclization of the selenenic acid is similar to the formation of a sulfenyl amide at the active site of protein tyrosine phosphatase 1B (PTP1B). This, however, leads to an assumption that the formation of a selenenyl amide is feasible in selenoproteins, which may be an active area of research in the future.

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## BIOGRAPHICAL INFORMATION

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## FOOTNOTES

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