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# A Simple and Efficient Strategy To Enhance the Antioxidant Activities of Amino-Substituted Glutathione Peroxidase Mimics

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**Abstract:** The glutathione peroxidase (GPx) activities of some diaryl diselenides incorporating tertiary amino groups were studied with $H_2O_2$, CumOOH, and *t*BuOOH as substrates and with PhSH as thiol co-substrate. Simple replacement of a hydrogen atom with a methoxy group dramatically enhances the GPx activity. The introduction of methoxy substituents *ortho* to selenium in *N*,*N*-dialkylbenzylamine-based compounds makes the basicity of the amino groups perfect for the catalysis. The presence of 6-OMe groups prevents possible Se···N interactions in the selenols, increasing their zwitterionic characters. The methoxy substituents also protect the selenium in the selenenic acid intermediates from overoxidation to seleninic acids or irreversible inactivation to selenonic acid derivatives. The additional substituents also play a crucial role in the selenenyl sulfide intermediates, by preventing thiol exchange reactions—which would normally lead to an inactivation pathway—at the selenium centers. The strengths of Se···N interactions in the selenenyl sulfide intermediates are dramatically reduced upon introduction of the methoxy substituents, which not only reduce the thiol exchange reactions at selenium but also enhance the nucleophilic attack of the incoming thiols at sulfur. The facile attack of thiols at sulfur in the selenenyl sulfides also prevents the reactions between the selenenyl sulfides and $H_2O_2$ that can regenerate the selenenic acids (reverse-GPx cycle). These studies reveal that the simple 6-OMe groups play multiple roles in each of the catalytically active intermediates by introducing steric and electronic effects that are required for efficient catalysis.

**Keywords:** antioxidant activity · GPx mimics · noncovalent interactions · selenium · selenoenzymes

## Introduction

Glutathione peroxidase (GPx) is a selenocysteine-containing mammalian antioxidant enzyme that protects various organisms from oxidative damage by catalyzing the reduction of harmful peroxides in the presence of glutathione (GSH).[1] The GPx redox cycle involves the oxidation of the catalytically active selenol (E-SeH) moiety by the peroxides to produce the corresponding selenenic acid (E-SeOH), which upon reaction with the thiol cofactor GSH generates the key intermediate selenenyl sulfide (E-SeSG). The attack of a second GSH moiety at the -Se-S- bond regenerates the active site selenol with release of the cofactor in its oxidized form, GSSG (Scheme 1).[1e–g] When the GSH cofactor is depleted in the reaction mixture, the selenenic acid produced in response to GPx oxidation may undergo further oxidation to seleninic (E-$SeO_2H$) or selenonic (E-$SeO_3H$) acids that disturb the main catalytic pathway. In the catalytic cycle, the rapid reactions of the selenenic acid with GSH and of the resulting selenenyl sulfide (E-SeSG) with a second GSH to produce the selenol appear to be very important, because these reactions ensure that the selenium moiety in the enzyme is not irreversibly inactivated.

Because of the importance of GPx as a natural antioxidant enzyme that plays a crucial role in oxidative stress,[2] several groups have been working toward the design and synthesis of small-molecule organoselenium compounds that mimic the peroxide-reducing ability of GPx in the presence of thiols. After the successful identification of ebselen (**1**) as a clinically useful antioxidant and anti-inflammatory drug,[3] several other selenium compounds displaying GPx-like activities were reported in the literature (Figure 1.). These compounds can be classified into two categories depending upon their reaction behavior with thiols and peroxides. In the first category, the compounds (e.g., **1**, **2**, **4**–**8**, **11**, and **12**) undergo one- or two-step reactions with excess amounts of

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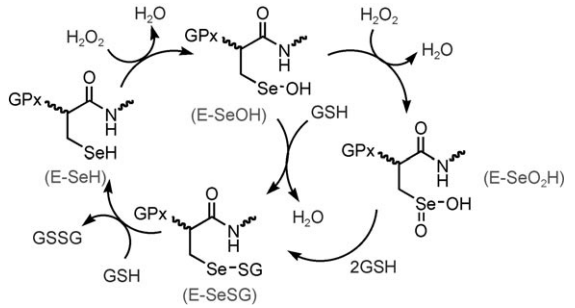



Scheme 1. Proposed catalytic mechanism of GPx, involving selenol, selenenyl sulfide, and selenenic acid intermediates. In the absence of thiols, the selenenic acid undergoes overoxidation to produce the seleninic acid species.

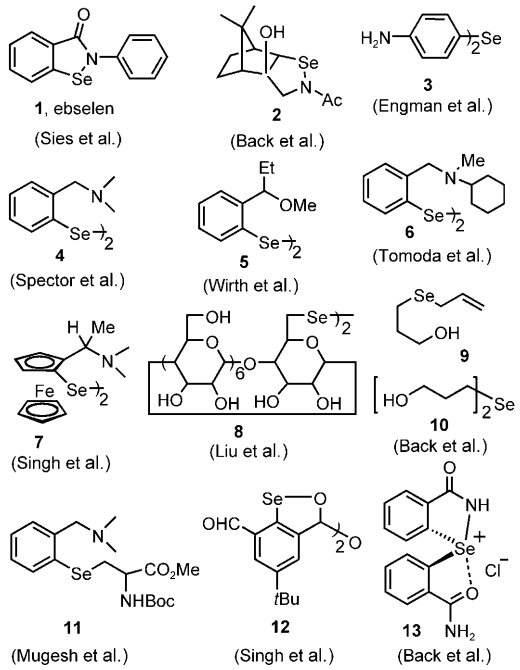
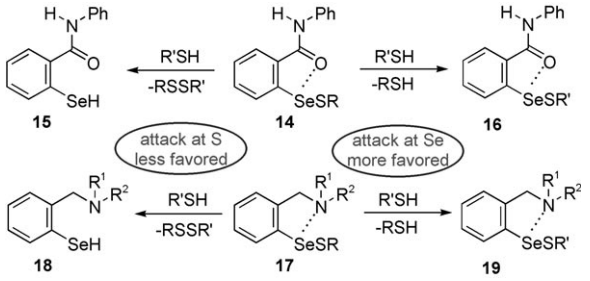



Figure 1. Ebselen and some other important synthetic GPx mimics reported in the literature.

thiols to generate reactive selenols, which undergo reactions with the peroxides to produce the corresponding selenenic acids.[3a,4] The selenenic acids thus produced undergo further reactions with thiols to regenerate the selenols through the formation of selenenyl sulfides as the key intermediates. The second class of compounds (e.g., **3**, **9**, **10**, **13**), on the other hand, do not produce any selenols, but they effectively reduce the peroxides in the presence of thiols through $Se^{II}$–$Se^{IV}$ redox cycles.[5]

If we consider the GPx catalytic cycle, the reaction of E-SeOH with GSH would be expected to be facile, due to the electrophilic nature of selenium. On the other hand, the reaction of E-SeSG with GSH to produce the selenol form of the enzyme is an unusual and unexpected reaction, because the selenium center in the E-SeSG intermediate would also be expected to display electrophilic reactivity.[1e] This would lead to a thiol exchange reaction at the selenium center rather than a nucleophilic attack of the incoming thiol at the sulfur center. Recent model studies on low-molecular-weight selenium compounds show that reductions of selenenyl sulfides to selenols need to overcome large energy barriers, and so the nucleophilic attack of thiols (or thiolates) at the selenium centers of the Se–S moieties are therefore more favorable than at sulfur.[6] The thiol attack at the selenium center in a selenenyl sulfide is further enhanced by nonbonding interactions between selenium and other heteroatoms such as O and N.[7,8b–d] The effect of thiols on the GPx activity of ebselen is one of the interesting examples in which these nonbonding interactions play an important role. These thiol exchange reactions, hampering the regeneration of the catalytically active selenol species, may account for the relatively low catalytic activities of synthetic selenium compounds with certain thiol cofactors. Ebselen, for example, has been found to be an inefficient catalyst in the reduction of hydroperoxides with aryl and benzyl thiols (such as PhSH and BnSH) as cofactors,[5c,d,8] due to the thiol exchange that takes place at the selenium center in the selenenyl sulfide intermediate (**14**, Scheme 2).[8b]




Scheme 2. The two possible reactions of the selenenyl sulfides derived from ebselen (**1**) and of diaryl diselenides incorporating tertiary amino groups.

The amino-substituted diselenide **4** exhibits much higher GPx activity than ebselen in the presence of aromatic thiols.[4b,e] This is probably due to the relatively weak Se···N interactions in compound **17** relative to the Se···O interactions in compound **14**. However, the selenenyl sulfide **17** does undergo a thiol exchange reaction in addition to the reaction that leads to the formation of selenol **18**. Furthermore, the strength of Se···N interactions in **17** is sufficient to perform a reverse-GPx cycle (i.e., the reaction of selenenyl sulfides with peroxides to regenerate the corresponding selenenic acids), which considerably reduces the GPx activity.[4e] These observations suggest that the prevention of strong Se···N interactions in amino-substituted diaryl diselenides might lead to the development of better GPx mimics.

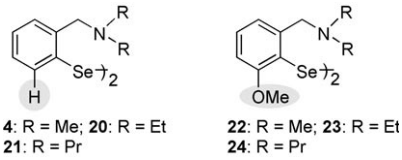
Unfortunately, it has not been possible to design and synthesize diaryl diselenides that have basic amino groups in close proximity to selenium but do not exhibit any strong Se···N interactions in the selenenyl sulfide intermediates. In this paper we report that the replacement of an aryl proton in compound **4** by a methoxy group prevents the Se···N in-

teractions in the key intermediates and dramatically enhances the GPx activities of **4** and of some related compounds. We also show that the strong Se···N interactions reduce the basicity of the tertiary amino group and that the introduction of a methoxy substituent helps the tertiary amino group to act as a general base during the catalytic cycles of benzylamine-based compounds.

## Results and Discussion

The amino-substituted diselenides (**4**, **20**–**24**) required for this study were synthesized from *N*,*N*-dialkylbenzylamines, 2-bromo-*N*,*N*-dialkylbenzylamines, or 3-methoxy-*N*,*N*-dialkylbenzylamines by the well-established heteroatom-directed *ortho*-lithiation methodology. Metallation of substituted benzylamines with *n*BuLi in diethyl ether afforded the corresponding *ortho*-lithiated compounds. Subsequent treatment with selenium powder and oxidative workup afforded diselenides in moderate yields. The GPx-like activities of these compounds were studied with hydrogen peroxide ($H_2O_2$), cumene hydroperoxide (Cum-OOH), and *tert*-butyl hydroperoxide (*t*BuOOH) as the substrates and with PhSH as the thiol co-substrate. The catalytic reduction of peroxides by PhSH in the presence of various selenium compounds was studied by a method similar to that of Back and co-workers.[4c,5c,d] The formation of PhSSPh in the reactions was studied by a reversed-phase HPLC method, and the times required for 50% conversion of PhSH into PhSSPh ($t_{1/2}$) were calculated by determining the peak areas at different time intervals. A calibration plot was used to calculate the amounts of PhSSPh formed during the reactions.




**4**: R = Me; **20**: R = Et
**21**: R = Pr

**22**: R = Me; **23**: R = Et
**24**: R = Pr

As expected, the diselenides based on *N*,*N*-dialkylbenzylamine moieties (**4**, **20**, **21**) exhibited much higher GPx activities than ebselen in all three peroxide systems used. It has previously been shown that the high GPx activity of **4** is probably due to the presence of the dimethylamino group, which can deprotonate the selenol **18** to generate a more reactive selenolate.[4b,e] Similar activation may occur in compounds **20** and **21**, containing a diethyl- and a dipropylamino group, respectively.

Interestingly, remarkable enhancements in the activity were observed when the protons at the 6-positions in compounds **4**, **20**, and **21** were replaced by simple methoxy substituents. Wirth and co-workers had previously reported an interesting observation that the substitution of aryl protons by methoxy groups in certain chiral diselenides incorporating alcohol moieties improved the stereoselectivity in asymmetric selenenylation reactions.[9] They showed the increased transfers of chirality to be due to the presence of the methoxy substituents, which exhibit Se···O noncovalent interactions. The additional substituents may also provide the necessary steric environments around the selenium moieties.[10]

From Table 1 and Figure 2, it is clear that the methoxy-substituted diselenides (**22**–**24**) are much better catalysts than the diselenides **4**, **20**, and **21** and that compounds **22**–**24** show very high activities in all three peroxide ($H_2O_2$, Cum-OOH, *t*BuOOH) systems. In particular, the diselenide **22** was found to be a remarkably active catalyst, with the $t_{1/2}$ value (3.8 min) obtained for this compound at 5 μM concentration being much lower than that found for **4** (19.2 min) at 10 μM concentration. This indicates that the catalytic activity of the methoxy-substituted compound **22** is almost one order of magnitude higher than that of **4**. Furthermore, quantitative conversion of PhSH into PhSSPh over the given time period was observed only with compounds **22**–**24**. The times required for complete conversion were found to be 12.5 min for **22**, 23 min for **23**, and 30 min for **24** (Figure 2). In contrast, only 90%, 77%, and 86% conversions were observed after 58 min, 44 min, and 69 min, respectively, when compounds **4**, **20**, and **21** were used as catalysts. In the presence of ebselen, only 11.5% conversion was observed after a reaction time of 100 min (Figure 2). This is

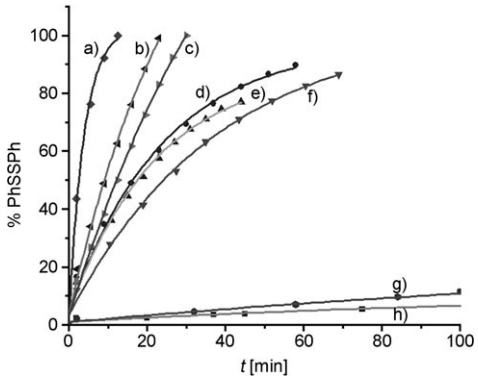



Figure 2. Catalytic reduction of Cum-OOH by PhSH in the presence of various selenium compounds. The formation of PhSSPh was followed by reversed-phase HPLC, and the % conversions were calculated from calibration plots: a) **22**, b) **23**, c) **24**, d) **4**, e) **20**, f) **21**, g) **1**, and h) control. Assay conditions: catalyst (10.0 μM, except compound **22**; [catalyst **22**] = 5 μM), PhSH (1.0 mM), Cum-OOH (2.0 mM) in MeOH at 22 °C.

due to the thiol exchange reactions that take place at the selenium centers in the selenenyl sulfides derived from compounds **4**, **20**, **21**, and ebselen, which lead to the accumulation of the corresponding selenenyl sulfide species in the solutions.[8b] Our HPLC experiments indicated that the selenenyl sulfides were the predominant species in the reaction mixtures, particularly when higher concentrations of the selenium compounds (**4**, **20**, **21**, and ebselen) were used. In the case of compound **4**, the accumulation of the selenenyl sulfide species was observed even at lower concentrations of

catalyst (10 μM). In contrast, no such species was detected for compound **22** during the entire catalytic cycle (Figure S2, Supporting Information).

Table 1. Values of $t_{1/2}$ for the reduction of peroxides by PhSH in the presence of compounds **1**, **4**, and **20**–**24** at 22 °C.

| Compound | $t_{1/2}$ values [min][a] | | |
|---|---|---|---|
| | $H_2O_2$ | Cum-OOH | *t*BuOOH |
| control | 1460.0 | 1053.0 | 780.0 |
| **1** (ebselen) | 821.0 | 522.0 | 744.0 |
| **4** | 19.2 | 16.5 | 24.4 |
| **20** | 19.9 | 17.6 | 35.2 |
| **21** | 22.7 | 24.3 | 49.7 |
| **22**[b] | 3.8 | 2.6 | 6.3 |
| **23** | 12.5 | 9.1 | 29.0 |
| **24** | 13.2 | 12.1 | 35.2 |

[a] Assay conditions: the reactions were carried out in MeOH at 22 °C [catalyst (10.0 μM, except compound **22**), PhSH (1.0 mM), peroxide (2.0 mM)]. [b] The conversion was too fast to be measured at 10 μM concentration, and so a 5 μM concentration of the catalyst was used.

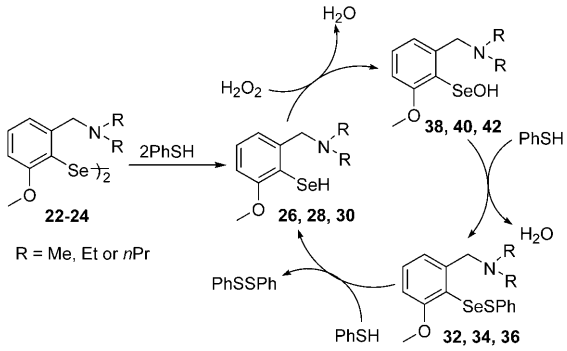
To understand the higher GPx activities of compounds **22**–**24** in relation to those of the parent diselenides (**4**, **20**–**21**), we have undertaken a detailed study to probe the role of amino and methoxy substituents. We have studied the catalytic cycles of compounds **4**, **20**, and **21** individually and have compared them with those of **22**–**24** (Scheme 3) to understand the role of amino substituents in each catalytically active intermediate. Although the catalytic mechanisms of diselenides **22**–**24** were found to be identical with those of **4**, **20**, and **21**, these studies revealed that the introduction of the methoxy substituents leads to dramatic changes in the reactivity of selenium. Our DFT calculations and experimentally measured $^{77}$Se NMR chemical shifts (Table 2) suggest that the strengths of the Se···N interactions in compounds **22**–**24** are significantly less than those in **4**, **20**, and **21**, which is consistent with the report by Wirth et al. that the presence of a methoxy substituent in the 6-position prevents interaction between selenium and the alcohol sidechain.[9] Although the strong Se···N interactions in compound **4** and related diselenides have been shown to be important for the reductive cleavage of the -Se-Se- bond by thiols,[4b,e] we have found that the relatively weak Se···N interactions in compounds **22**–**24** are sufficient for facile reduction of the diselenide bonds by thiols (Scheme 3).

Table 2. The theoretical data for **14** and **31**–**36** obtained by DFT calculations at the B3LYP/6-31+G(d)//B3LYP/6-311+G(d,p) levels, along with the experimentally measured and theoretically calculated $^{77}$Se NMR chemical shifts.

| Compd | $r_{Se\cdots O/N}$ [Å] | $\theta_{O/N\text{-}Se\text{-}S}$ [°] | $^{77}$Se [ppm] (calcd)[a] | $^{77}$Se [ppm] (exptl) |
|---|---|---|---|---|
| **14** (R = Ph) | 2.456 | 176.2 | 660 | 588 |
| **31** | 2.680 | 175.5 | 556 | 564 |
| **32** | 2.869 | 158.5 | 419 | 470 |
| **33** | 2.690 | 168.6 | 494 | 558 |
| **34** | 2.819 | 158.3 | 414 | 461 |
| **35** | 2.663 | 176.2 | 522 | 555 |
| **36** | 2.846 | 158.7 | 423 | 451 |

[a] The values are cited with respect to $Me_2Se$.

Treatment of the diselenides **4**, **20**, and **21** with PhSH (1 equiv in each case) produced the corresponding selenols




Scheme 3. Proposed catalytic mechanism for the reduction of $H_2O_2$ by PhSH in the presence of compounds **22**–**24**.

**25**, **27**, and **29**, together with the selenenyl sufides **31**, **33**, and **35**, respectively, in nearly 1:1 ratios. The addition of further PhSH (a second equivalent in each case) does not lead


to complete conversion of the selenenyl sufides into the selenols. This is due to the presence of Se···N interactions in the selenenyl sulfides **31**, **33**, and **35**, which lead to thiol exchange reactions rather than to the formation of the corresponding selenols. The $^{77}$Se NMR chemical shifts (Table 2) for compounds **31** (564 ppm), **33** (558 ppm), and **35** (555 ppm) show significant downfield shifts relative to that of PhSeSPh (526 ppm). Large excesses of thiol are therefore required for the conversion of the selenenyl sulfides into the selenols. However, the selenenyl sulfides **31**, **33**, and **35** were found to be the major species during the catalytic cycle. The addition of 4-Me-$C_6H_4SH$ to solutions containing **31**, **33**, or **35** produced new selenenyl sulfides through thiol exchange reactions (Figure S3, Supporting Information). Interestingly, the reactions of compounds **22**–**24** with PhSH (1 equiv in each case) readily produced the corresponding selenols (**26**, **28**, and **30**) with the formation of only trace amounts of the corresponding selenenyl sulfides (**32**, **34**, and **36**). As the amounts of thiol were not sufficient for quantitative conversion of **22**–**24** into the selenols, we detected some unreacted diselenides in the reaction mixtures. However, the addition of further PhSH (a second equivalent in each case) to the reaction mixtures converted the diselenides and selenenyl

sulfides completely into the selenols. The $^{77}$Se NMR chemical shifts for the selenenyl sulfides **32** (470 ppm), **34** (461 ppm), and **36** (451 ppm) show dramatic upfield shifts (≈100 ppm) with respect to those of the selenenyl sulfides **31**, **33**, and **35**, indicating the absence of strong Se···N interactions. The absence of any strong Se···N interactions and the presence of the methoxy groups at the 6-positions prevent the thiol exchange reactions at the selenium centers. Therefore, the addition of one equivalent of thiol to a selenenyl sulfide **32**, **34**, or **36** is sufficient to generate the corresponding selenol.

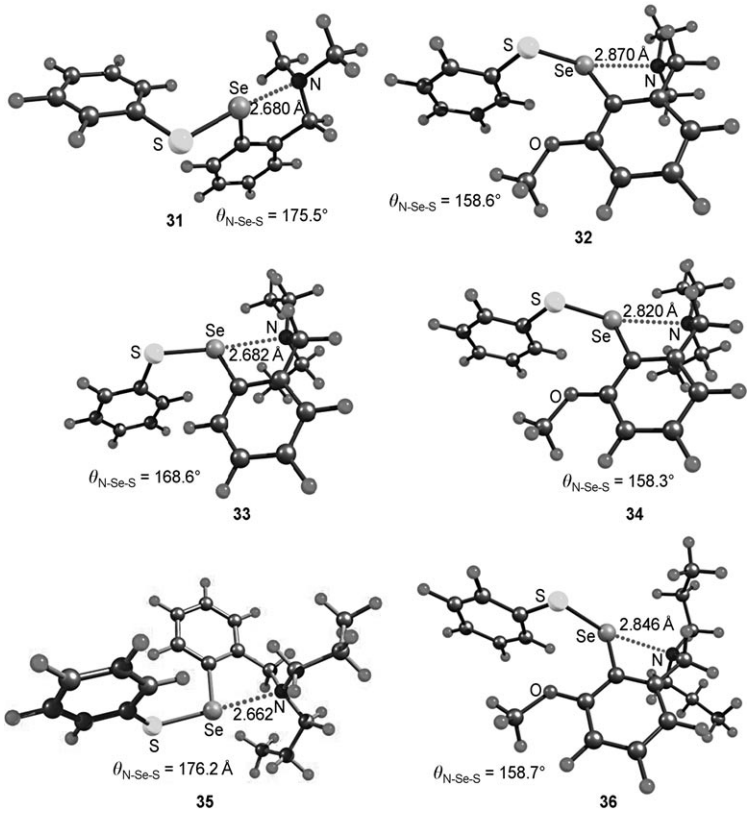



Figure 3. Energy-optimized geometries of the selenenyl sulfide intermediates **31**–**36**. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.

To complement the experimental findings, we have also carried out detailed DFT studies on the selenenyl sulfide intermediates. The geometries were fully optimized at the B3LYP level with use of the 6-31+G(d) basis set. The interaction energies between the selenium and nitrogen atoms were calculated by natural bond orbital (NBO) calculations.[11] These studies have revealed that the strengths of Se···N interactions in compounds **31**, **33**, and **35** are dramatically decreased upon incorporation of methoxy groups at the 6-positions (Table 3, Figure 3). As an example, the Se···N distance in compound **32**, with a methoxy substituent (2.869 Å), is significantly longer than that in **31** (2.680 Å). Similarly, the Se···N interaction energy in compound **32** ($E_{Se\cdots N}$: 5.78 kcal mol$^{-1}$) is much lower than that in **31** ($E_{Se\cdots N}$: 11.20 kcal mol$^{-1}$). Consistently with our experimental data, the calculated $^{77}$Se NMR chemical shift values for **32**, **34**, and **36** are shifted upfield by ≈120 ppm relative to those of **31**, **33**, and **35**. This indicates that the selenium centers in compounds **31**, **33**, and **35** are more deshielded than in the methoxy-substituted compounds, due to the strong Se···N interactions. The NBO analysis shows that the introduction of the methoxy substituents and the subsequent weakening of the Se···N interactions lead to increases in the positive charges on the sulfur atoms in the selenenyl sulfides (Table 3), which would enhance the possibility of nucleophilic attack of incoming thiol/thiolate at the sulfur centers. These changes in the electronic properties of selenium and sulfur and the increases in the steric hindrance around selenium upon introduction of methoxy substituents drive the conversion of selenenyl sulfides into the corresponding selenols.[12]

Table 3. Theoretical data for **14** and **31**–**36** obtained by NBO analysis at the B3LYP/6-31+G(d)//B3LYP/6-311+G(d,p) level of theory.

| Compd | $q_{Se}$ | $q_S$ | $E_{Se\cdots O/N}$ [kcal mol$^{-1}$] |
|---|---|---|---|
| **14** (R = Ph) | 0.408 | 0.023 | 15.20 |
| **31** | 0.309 | 0.039 | 11.20 |
| **32** | 0.304 | 0.067 | 5.78 |
| **33** | 0.297 | 0.040 | 11.23 |
| **34** | 0.301 | 0.063 | 7.02 |
| **35** | 0.306 | 0.035 | 12.57 |
| **36** | 0.305 | 0.065 | 6.59 |

Because the reactions of selenenyl sulfides with thiols should produce the corresponding selenols for the catalytic activity, we have also studied the natures of the selenols by experimental and computational methods (Figure 4). The *N*,*N*-dialkylamino groups in the methoxy-substituted selenols (**26**, **28**, and **30**) have been found to be stronger bases than those in the selenols **25**, **27**, and **29**. This can easily be explained by comparing the $^{77}$Se NMR chemical shifts. The experimentally measured $^{77}$Se NMR chemical shifts for the methoxy-substituted selenols are shifted almost 100 ppm upfield [**26** (−58 ppm), **28** (−42 ppm), **30** (−37 ppm)] relative to those for the unsubstituted selenols [**25** (35 ppm), **27** (54 ppm), **29** (56 ppm)] (Table 4), indicating that the introduction of a methoxy substituent significantly increases zwitterionic character.[13] The selenium centers in compounds **26**,

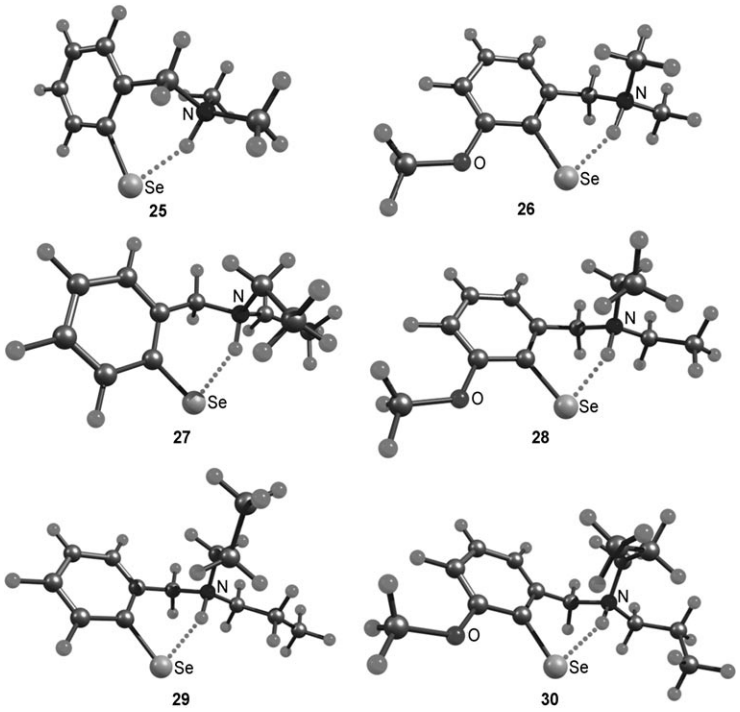



Figure 4. Energy-optimized geometries of the selenol species **25**–**30**. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.

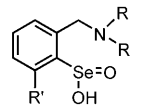
Table 4. The theoretical data for **15** and for **25**–**30** obtained by DFT calculations at the B3LYP/6-31+G(d)//B3LYP/6-311+G(d,p) levels, along with the experimentally measured and theoretically calculated $^{77}$Se NMR chemical shifts.

| Compd | $r_{Se\cdots O/N}$ [Å] | $q_{Se}$ | $^{77}$Se [ppm] (calcd)[a] | $^{77}$Se [ppm] (exptl)[a] | $E_{Se\cdots O/N}$ [kcal mol$^{-1}$] |
|---|---|---|---|---|---|
| **15** | 2.577 | 0.269 | 206 | 232 | 7.10 |
| **25** | 3.028 | −0.289 | −30 | 35 | 0.00 |
| **26** | 3.016 | −0.246 | −94 | −58 | 0.00 |
| **27** | 3.062 | −0.312 | 84 | 54 | 0.00 |
| **28** | 3.055 | −0.279 | −2 | −42 | 0.00 |
| **29** | 3.059 | −0.314 | 63 | 56 | 0.00 |
| **30** | 3.078 | −0.295 | 0 | −37 | 0.00 |

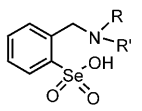
[a] The values are cited with respect to $Me_2Se$.

**28**, and **30** are, therefore, more nucleophilic than those in **25**, **27**, and **29**. Furthermore, the selenol moieties in **26**, **28**, and **30** are significantly different from that in the ebselen-selenol (**15**), which shows a large downfield shift in the $^{77}$Se NMR (232 ppm, Table 2), due to the strong Se···O interactions ($E_{Se\cdots O}$ = 7.10 kcal mol$^{-1}$). We have previously shown that the zwitterionic form of **25** (i.e., **25b**) in water is about 2.37 kcal mol$^{-1}$ more stable than its counterpart with weak Se···N interactions, and that the zwitterionic form of **15** is about 5.45 kcal mol$^{-1}$ less stable than its counterpart with weak Se···O interactions.[8b] In this study, we have found that the zwitterionic form **25b** is about 5.0 kcal mol$^{-1}$ more stable than the undissociated form (**25a**), which is consistent with the experimentally measured $^{77}$Se NMR data. The zwitterionic form of **26** (i.e., **26b**), on the other hand, is ≈9.0 kcal mol$^{-1}$ more stable than the undissociated **26a**, indicating that the prevention of Se···N interactions through the introduction of a methoxy substituent increases the stability of the zwitterionic form.[13]

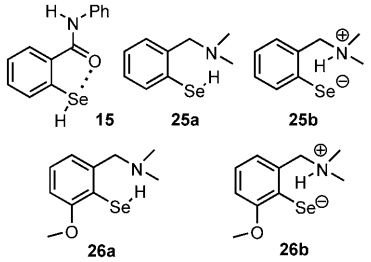
Having established the role of methoxy substituents in the selenols and selenenyl sulfides, we have also studied the effect of Se···N interactions in the selenenic acids, one of the crucial intermediate types in the GPx catalytic cycle. Treatment of compound **25** with hydrogen peroxide produced two signals in the $^{77}$Se NMR spectrum, at 1168 and 1347 ppm, that can be ascribed to the selenenic acid **37** and the seleninic acid **43**, respectively.



**43**: R = Me; R' = H
**44**: R = Me; R' = OMe
**45**: R = Et; R' = H
**46**: R = Et; R' = OMe
**47**: R = Pr; R' = H
**48**: R = Pr; R' = OMe



**49**: R = Me, R' = Me
**50**: R = Me, R' = *c*-$C_6H_{11}$




Treatment of selenol **27** with $H_2O_2$ produced a mixture of selenenic acid **39** (1171 ppm) and seleninic acid **45** (1349 ppm), although the required selenenic acid **39** was detected only in trace amounts. On the other hand, compound **29** did not produce any detectable quantities of the selenenic acid (**41**), but produced only the overoxidized seleninic acid **47** (1349 ppm).[14,15] The selenenic acid **37** was converted completely into the seleninic acid **43** and the selenonic

acid **49** upon addition of an excess amount of $H_2O_2$. This is consistent with previous reports on the GPx activities of amino-substituted compounds.[4b,e] The $^{77}$Se NMR chemical shift for the selenonic acid **49** (1019 ppm) is almost identical with that of the structurally characterized compound **50** (1022 ppm) reported by Iwaoka and Tomoda.[4b] Interestingly, treatment of selenols **26**, **28**, and **30** with $H_2O_2$ produced the selenenic acids **38** (1170 ppm), **40** (1174 ppm), and **42** (1172 ppm), respectively, and the formation of the overoxidized seleninic acids (**44**, **46**, and **48**) or selenonic acids was not observed even at very high peroxide concentrations.[14,15]

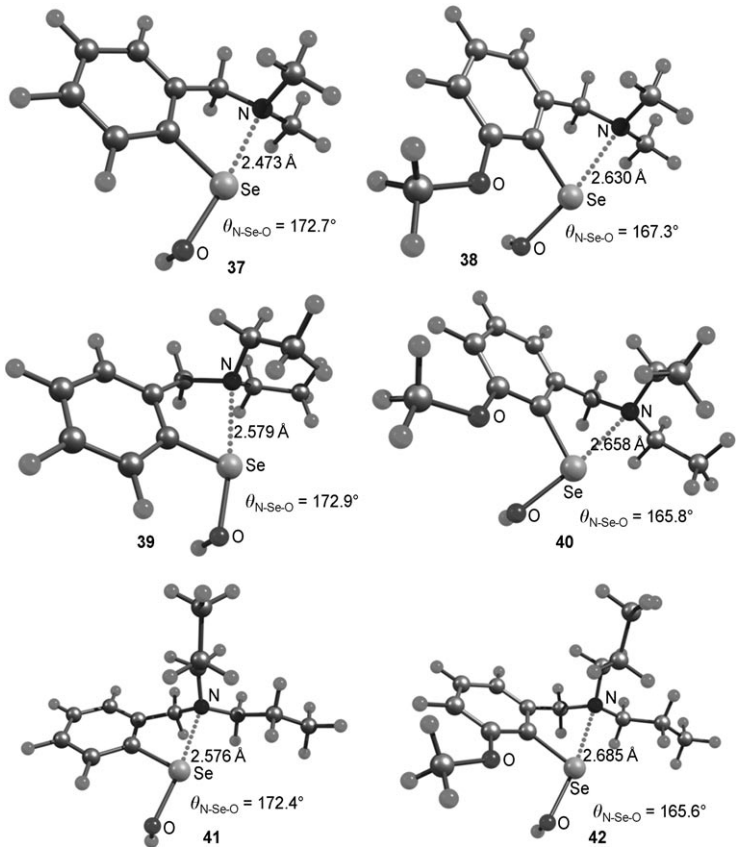



Figure 5. Energy-optimized geometries of the selenenic acid (**37**–**42**) intermediates. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.

The optimized geometries (Figure 5) and NBO analysis (Table 5) indicate that all the selenenic acids (**37**–**42**) exhibit strong Se···N interactions, which help nucleophilic attack by incoming thiols at the selenium centers. Interestingly, the addition of PhSH (1 equiv in each case) led to clean conversions of the selenenic acids **38**, **40**, and **42** into the corresponding selenenyl sulfides. No species other than the selenols, selenenyl sulfides, and selenenic acids were observed during the entire catalytic cycles of the diselenides **22**–**24**, and this type of selectivity appears to be remarkable in amino-substituted benzylic compounds. The DFT calculations on the selenenic and seleninic acids have revealed that the introduction of methoxy substituents at their 6-positions prevents the overoxidation of the selenenic acids to the corresponding seleninic acids. The energy difference between **37** and **43** is almost 4.0 kcal mol$^{-1}$ lower than that between **38** and **44**, indicating that the conversion of **37** into **43** is more favored than the conversion of **38** into **44**.

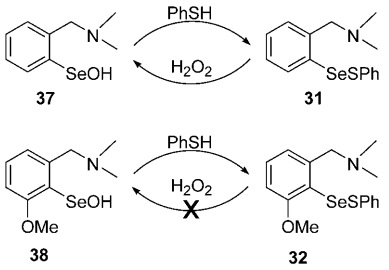
Table 5. Theoretical data for **37**–**42** obtained by DFT calculations at the B3LYP/631+G(d)//B3LYP/6-311+G(d,p) levels, along with the experimentally measured and theoretically calculated $^{77}$Se NMR chemical shifts.

| Compd | $r_{Se\cdots O/N}$ [Å] | $\theta_{O/N\text{-}Se\text{-}O}$ [°] | $q_{Se}$ | $^{77}$Se (ppm) (calcd)[a] | $E_{Se\cdots O/N}$ [kcal mol$^{-1}$] |
|---|---|---|---|---|---|
| **37** | 2.473 | 172.7 | 0.631 | 1047 (1168) | 20.47 |
| **38** | 2.630 | 167.3 | 0.625 | 1068 (1170) | 12.84 |
| **39** | 2.579 | 172.9 | 0.629 | 1056 (1171) | 16.02 |
| **40** | 2.658 | 165.8 | 0.622 | 1060 (1174) | 12.41 |
| **41** | 2.576 | 172.4 | 0.626 | 1062 (n.d) | 16.24 |
| **42** | 2.665 | 165.6 | 0.620 | 1062 (1172) | 12.04 |

[a] The $^{77}$Se chemical shifts were calculated in the gas phase and are cited with respect to $Me_2Se$. The experimentally measured $^{77}$Se chemical shifts are given in parentheses.

In addition to the thiol exchange, the reactions of selenenyl sulfides with $H_2O_2$ to produce the corresponding selenenic and/or seleninic acids (reverse-GPx cycle) also reduce GPx activity. The addition of one equivalent of $H_2O_2$ to the selenenyl sulfide **31** readily produced the selenenic acid **37** (Scheme 4), which underwent further reaction with $H_2O_2$ to generate the seleninic acid **43**. The presence of strong Se···N interactions and subsequent stabilization of the Se–S bond by thiol exchange reactions permit the selenenyl sulfide **31** to stay longer in the solution. As a result, a facile cleavage of the Se–S bond by $H_2O_2$ occurs, leading to the production of the selenenic and seleninic acids. Because of the backward reaction, the reaction between selenenic acid **37** and PhSH always produces a mixture of the selenenyl sulfide **31** and the selenenic acid **37**. The reverse-GPx cycle has been observed previously with compounds **4**, **6**, and related diselenides.[4b,e] The introduction of methoxy substituents in the 6-positions makes the selenenyl sulfides short-lived, and the reactions therefore proceed in the forward direction. As an




Scheme 4. The reaction between selenenic acid **37** and PhSH produces the selenenyl sulfide **31**, which upon treatment with $H_2O_2$ regenerates **37** through a reverse-GPx cycle. Compound **38** undergoes only the forward reaction, with the reverse reaction not being observed because of the presence of the OMe group.

example, the $^{77}$Se NMR signal at 1170 ppm due to the selenenic acid **38** disappeared completely upon addition of one equivalent of PhSH. This reaction produced a new signal for the selenenyl sulfide **32** at 470 ppm, which was unaffected by the addition of an excess amount of $H_2O_2$. Similar reactivity was observed with compounds **40** and **42**. This clearly indicates that the backward reaction involving the selenenyl sulfides and $H_2O_2$ would reduce the GPx activity.

To understand the effect of thiol co-substrate on the catalytic activities of these amine-based compounds, we determined the initial rates at various concentrations of PhSH with fixed catalyst and peroxide concentrations. The double reciprocal or Lineweaver–Burk plots obtained for compounds **4** and **20**–**24** by plotting the reciprocals of initial rates ($1/v_0$) against the reciprocals of substrate concentrations (1/[substrate]) were used to determine the catalytic parameters (Table 6). The $K_M$ (Michaelis constant) values obtained for **22**–**24** are much lower than those for **4**, **20**, and **21** under similar experimental conditions, suggesting that the thiol exchange reactions significantly increase the $K_M$ values. The catalytic efficiencies ($\eta$) for the methoxy-substituted compounds are found to be much higher than those of the parent benzylamine-based compounds. The catalytic efficiency of **22** ($9.75\times10^3\,M^{-1}min^{-1}$) is almost eight times higher than that of **4** ($1.24\times10^3\,M^{-1}min^{-1}$). Similarly, the catalytic efficiencies of compounds **23** and **24** are found to be around two and around five times higher than those of **20** and **21**, respectively. The higher catalytic efficiencies of compounds **22**–**24** relative to the parent compounds suggest that the methoxy groups at the 6-positions play a crucial role in modulating the reactivities of the key intermediates in the GPx cycle.

To gain further insight into the effect of Se···N/O interactions on the kinetic behavior of synthetic GPx mimics, we have carried out detailed kinetic studies on compounds **4** and **22**. The initial rates were measured with increasing concentrations of thiol and fixed concentrations of catalyst and hydrogen peroxide. The reaction rate for compound **22** increased rapidly at the beginning with an increase in the thiol concentration, and after a certain concentration of thiol, the rate became constant. This indicates that compound **22** follows typical saturation kinetics similar to those of the native GPx.[16] Interestingly, a linear increase in the reaction rate was observed for compound **4** with an increase in the concentration of PhSH, indicating non-saturation kinetics up to a concentration of 3 mM. The change in the kinetics pattern can be attributed to the various degrees of thiol exchange reactions in the selenenyl sulfides derived from compounds **4** and **22**. When there are thiol exchange reactions due to strong Se···N interactions, the Se–S bonds become readily exchangeable, and the selenium centers in such compounds therefore do not get saturated with thiols. This leads to relatively high $K_M$ values, as observed in the case of **4**. In contrast with **4**, compounds **20** and **21** exhibited saturation kinetics (Tables S27 and S28, Supporting Information), indicating that the replacement of the methyl groups in **4** by ethyl or propyl groups had considerably reduced the thiol exchange reactions at the selenium center. This is evident from the reactions of **31**, **33**, and **35** with 4-Me-$C_6H_4SH$, in which the thiol exchange reaction rate for compound **31** is significantly higher than those for **33** and **35** (Figure S3, Supporting Information). However, the introduction of sterically more demanding substituents at the nitrogen does not appear to be sufficient for enhancing the catalytic activity, because the strengths of Se···N interactions in **33** and **35** were found to be almost identical with those in **31** (Table 3).

In contrast with the diselenides, ebselen exhibited completely different kinetics in the presence of various concentrations of PhSH. When ebselen was treated with PhSH, a decrease in the reaction rate with increasing thiol concentration (up to $\approx$2 mM) was observed. After this, a rapid increase in the reaction rate with increasing PhSH concentration was observed. For example, the difference in the rate ($1.94\,\mu M\,min^{-1}$) due to the change in thiol concentration from 7 mM to 14 mM was found to be much higher than the difference in the rate ($0.62\,\mu M\,min^{-1}$) over the first 7 mM (Figure 6B). This indicates that a very high concentration of PhSH is required to overcome the thiol exchange reaction in the selenenyl sulfide derived from ebselen, which is consistent with our

Table 6. Catalytic parameters [maximum velocities ($V_{max}$), Michaelis constants ($K_M$), catalytic constants ($k_{cat}$), and catalytic efficiencies ($\eta$)] for the reduction of $H_2O_2$ by PhSH in the presence of diselenides **4** and **20**–**24**.[a]

| Compd | $V_{max}$ [$\mu M\,min^{-1}$] | $K_M$ [mM] | $k_{cat}$ [$min^{-1}$] | $\eta$ [$M^{-1}\,min^{-1}$] |
|---|---|---|---|---|
| **4** | 158.7[b] | 12.76[b,c] | 15.87 | $1.24\times10^3$ |
| **20** | 21.9 | 1.04 | 2.19 | $2.10\times10^3$ |
| **21** | 17.9 | 1.70 | 1.79 | $1.05\times10^3$ |
| **22** | 42.9 | 0.44 | 4.29 | $9.75\times10^3$ |
| **23** | 9.7 | 0.22 | 0.98 | $4.45\times10^3$ |
| **24** | 8.7 | 0.16 | 0.87 | $5.43\times10^3$ |

[a] Assay conditions: PhSH (0.0–4.0 mM), $H_2O_2$ (1.0 mM), and catalyst (10.0 $\mu$M) in MeOH at 22 °C. [b] The extremely high $V_{max}$ and $K_M$ values are due to the use of saturation kinetics method for a compound that follows non-saturation kinetics. [c] The $K_M$ value is much higher than the largest concentration of PhSH.

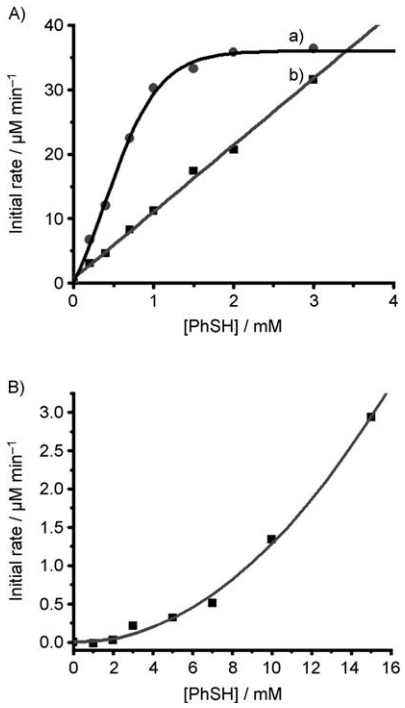



Figure 6. Effect of thiol co-substrate on initial rates for the reduction of $H_2O_2$ (1.0 mM) by PhSH in the presence of **4** and **22** (10 μM) or of ebselen (**1**, 20 μM). The reactions were carried out in methanol at 22 °C. A) Line a) **22**; line b) **4**. B) **1** (ebselen).

previous observations.[8b] As a result of this unusual behavior, the kinetic parameters for the reduction of $H_2O_2$ by PhSH could not be determined for ebselen. This is consistent with the report by Shi et al. that the effect of ebselen in cells is beneficial only when thiols are present in sufficiently high concentrations, and that the detrimental effects of ebselen may dominate in a system with thiols in low concentrations.[17]

Although compounds **4** and **22** exert their catalytic cycle through the formation of the selenols, selenenyl sulfides, and selenenic acids, comparison of the initial rates for different selenium compounds can lead to unreliable results when the initial rates are measured at only one thiol concentration. As an example, the initial rates for 10 μM concentrations of compounds **4** and **22** are identical at a 3.4 mM concentration of PhSH (Figure 6A). This is due to the difference in the kinetics behavior (saturation vs. non-saturation kinetics), which does not allow a reliable comparison. The activity of **22** must therefore be compared with that of **4** at PhSH concentrations of 1 mM or less, at which both compounds exhibit linear increases in their activities with increasing thiol concentrations (Figure 6A). These observations suggest that the determination of initial rates at only one thiol concentration can be erroneous even for compounds with similar structures if the reactivities of the selenium centers are altered by Se···N or other noncovalent interactions.

## Conclusion

In this study we have shown that simple replacement of hydrogen atoms by methoxy substituents in *N,N*-dialkylbenzylamine-based diselenides can lead to dramatic increases in the catalytic activity. The methoxy substituents enhance GPx-like activity by altering the steric and electronic environments around selenium and sulfur atoms in the key intermediates. Protection of the selenium moieties from overoxidation by peroxides and the prevention of thiol exchange reactions at the selenium atoms in the selenenyl sulfide intermediates upon introduction of the methoxy substituents have been found to be the crucial factors for the enhancement of catalytic activity. These studies have revealed that the basic amino groups in close proximity to selenium in diselenides possessing tertiary amino groups play more positive roles when methoxy groups are present at the 6-positions. From our present study and the literature data, we propose the following revised roles for the basic amino groups in GPx mimics. The tertiary amino substituents: i) should not be involved in any Se···N interactions in the selenols, but should be sufficiently basic to deprotonate the selenols to produce more reactive selenolates, ii) should not participate in strong interactions with selenium in the selenenyl sulfide intermediates, and iii) should exhibit some noncovalent interactions with selenium in the selenenic acid intermediates to increase the electrophilic reactivity of selenium.

## Experimental Section

**General procedure**: *n*-Butyllithium (*n*BuLi) was purchased from Acros Chemical Co. (Belgium). Methanol was obtained from Merck and dried before use. All other chemicals were of the highest purity available. All the reactions were carried out under nitrogen with use of standard vacuum-line techniques. Because of the unpleasant odors and toxic nature of several of the reaction mixtures involved, most manipulations were carried out in a well-ventilated fume hood. $Et_2O$ was dried over sodium metal with benzophenone. Thin-layer chromatography analyses were carried out on pre-coated silica gel plates (Merck), and spots were visualized with UV irradiation. Column chromatography was performed on glass columns loaded with silica gel or on automated flash chromatography systems (Biotage) with use of preloaded silica cartridges. $^1H$ (400 MHz), $^{13}C$ (100.56 MHz), and $^{77}Se$ (76.29 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shifts are cited with respect to $SiMe_4$ as internal ($^1H$ and $^{13}C$) and $Me_2Se$ as external ($^{77}Se$) standard. Mass spectral studies were carried out on a Q-TOF micro mass spectrometer with ESI MS mode analysis. The synthetic procedures for the ligands are described in the Supporting Information. Compound **4** was synthesized by the literature method.[18]

**Synthesis of 20**: *n*BuLi (1.4 mL of a 1.6 M hexane solution) was added dropwise with stirring to a cooled (−78 °C) solution of 2-bromo-*N,N*-diethylbenzylamine (0.50 g, 2.06 mmol) in dry $Et_2O$ (15 mL), which was then allowed slowly to attain room temperature. After 1.5 h, the solvent was removed completely under reduced pressure, to remove butyl bromide produced in the reaction. Freshly distilled $Et_2O$ (15 mL) was added, followed by the addition at 0 °C of finely ground selenium powder (0.16 g, 2.06 mmol). After the addition of selenium powder the color turned brownish. After the reaction mixture had been stirred for another 3 h, the mixture was poured into an ice-cooled saturated sodium bicarbonate solution. Oxygen was passed through the solution at a moderate rate for 15 min. The compound was extracted with ether and dried over

sodium sulfate. The solvent was evaporated to obtain a yellow colored liquid, which was purified by flash chromatography. The expected compound was eluted with ethyl acetate in petroleum ether (5%). Yield 0.48 g (49%); $^1$H NMR ($CDCl_3$): $\delta$=1.02–1.06 (m, 6H), 2.54–2.60 (m, 4H), 3.63 (s, 2H), 7.07–7.10 (m, 1H), 7.25–7.29 (t, $J$=8 Hz, 1H), 7.49–7.51 (d, $J$=8 Hz, 1H), 7.54–7.56 ppm (d, $J$=8 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=12.0, 47.2, 57.2, 124.2, 127.2, 128.0, 130.6, 132.5, 139.6 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=424 ppm; HRMS: $m/z$: calcd for $C_{22}H_{32}N_2Se_2$ $[M+H]^+$: 485.0974; found: 485.0977.

**Synthesis of 21**: *n*BuLi (1.4 mL of a 1.6 M solution in hexane) was added dropwise with stirring at −78 °C to a solution of 2-bromo-*N*,*N*-dipropylbenzylamine (0.50 g, 1.85 mmol) in dry $Et_2O$ (15 mL), and the mixture was allowed to reach room temperature slowly over 1.5 h. The solvent was removed under reduced pressure to remove the butyl bromide produced in the reaction. Freshly distilled $Et_2O$ (15 mL) was then added, followed by the addition at 0 °C of finely ground selenium powder (0.15 g, 1.85 mmol). After the addition of selenium powder the color turned brownish. After the reaction mixture had been stirred for another 3 h, the mixture was poured into an ice-cooled saturated sodium bicarbonate solution. Oxygen was passed through the solution at a moderate rate for 15 min. The compound was extracted with ether and dried over sodium sulfate. The solvent was evaporated to provide a yellow colored liquid, which was purified by flash chromatography. The expected compound was eluted with ethyl acetate in petroleum ether (2–3%). Yield 0.52 g (52%); $^1$H NMR ($CDCl_3$): $\delta$=0.74–0.80 (m, 6H), 1.36–1.45(m, 4H), 2.32–2.39 (m, 4H), 3.54 (s, 2H), 6.97–7.02 (m, 1H), 7.17–7.21 (m, 1H), 7.41–7.43 (d, $J$=8 Hz, 1H), 7.50–7.52 ppm (d, $J$=8 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=12.9, 21.3, 57.2, 125.0, 128.1, 128.8, 131.4, 133.4, 140.7 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=420 ppm; HRMS: $m/z$: calcd for $C_{26}H_{40}N_2Se_2$ $[M+H]^+$: 541.1600; found: 541.1597.

**Synthesis of 22**: *n*BuLi (4.0 mL of a 1.6 M solution in hexane) was added dropwise with stirring at ≈5 °C to a solution of 3-methoxy-*N*,*N*-dimethylbenzylamine (0.75 g, 4.55 mmol) in dry THF (25 mL), and the mixture was allowed slowly to attain room temperature. After 1.5 h, finely ground selenium powder (0.43 g, 5.46 mmol) was added at 0 °C. After the addition of selenium powder the color turned brownish and the reaction mixture was stirred overnight. The mixture was then poured into an ice-cooled saturated sodium bicarbonate solution. Oxygen was passed through the solution at a moderate rate for 15 min. The compound was extracted with ether and dried over sodium sulfate, and the solvent was evaporated to provide a yellow-colored liquid, which was purified by flash chromatography with ethyl acetate and petroleum ether. Yield 1.28 g (58%); $^1$H NMR ($CDCl_3$): $\delta$=2.15 (s, 6H), 3.31(s, 2H), 3.72 (s, 3H), 6.71–6.73 (d, $J$=8 Hz, 1H), 6.79–6.81 (d, $J$=8 Hz, 2H), 7.12–7.16 ppm (t, $J$=8 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=45.4, 55.2, 64.4, 112.8, 114.3, 121.5, 129.2, 140.5, 159.7 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=374 ppm; HRMS: $m/z$: calcd for $C_{20}H_{28}N_2O_2Se_2$ $[M+H]^+$: 489.0559; found: 489.0559.

**Synthesis of 23**: *n*BuLi (1.9 mL of a 1.6 M solution in hexane) was added dropwise with stirring at ≈5 °C to a solution of 3-methoxy-*N*,*N*-diethylbenzylamine (0.50 g, 2.59 mmol) in dry $Et_2O$ (15 mL), and the mixture was allowed slowly to attain room temperature, by which time the colorless solution had turned yellow. After the reaction mixture had been stirred for 1.0 h, finely ground selenium powder (0.20 g, 2.59 mmol) was added at 0 °C. Soon after the addition of selenium powder, the color turned brownish. After the reaction mixture had been stirred for 2 h at room temperature, the mixture was poured into an ice-cooled saturated sodium bicarbonate solution, and oxygen was passed through the solution at a moderate rate for 15 min. The compound was extracted with ether and dried over sodium sulfate. The solvent was evaporated to provide a yellow colored liquid, which was purified on an active neutral alumina column with ethyl acetate and petroleum ether as eluents. Yield 0.74 g (53%); $^1$H NMR ($CDCl_3$): $\delta$=0.93–0.96 (t, $J$=7.2 Hz, 6H), 2.40–2.45 (q, $J$=7.2 Hz, 4H), 3.44 (s, 2H), 3.70 (s, 3H), 6.67–6.69 (d, $J$=7.2 Hz, 1H), 6.81–6.83 (d, $J$=6.8 Hz, 1H), 7.09–7.13 ppm (t, $J$=8 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=10.7, 45.7, 54.1, 56.5, 111.1, 113.2, 120.1, 128.0, 140.7, 158.5 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=375 ppm; HRMS: $m/z$: calcd for $C_{24}H_{36}N_2O_2Se_2$ $[M+H]^+$: 545.1107; found: 544.9161.

**Synthesis of 24**: *n*BuLi (1.7 mL of a 1.6 M solution in hexane) was added dropwise with stirring at ≈5 °C to a solution of 3-methoxy-*N*,*N*-dipropylbenzylamine (0.50 g, 2.26 mmol) in dry $Et_2O$ (15 mL), and the mixture was allowed slowly to attain room temperature. After 1.5 h, finely ground selenium powder (0.18 g, 2.26 mmol) was added at 0 °C. After the addition of selenium powder the color turned brownish, and the system was stirred for another 2 h at room temperature. The mixture was then poured into an ice-cooled saturated sodium bicarbonate solution. Oxygen was passed through the solution at a moderate rate for 15 min. The compound was extracted with ether and dried over sodium sulfate. The solvent was evaporated to provide a yellow-colored liquid, which was purified on an active neutral alumina column with ethyl acetate and petroleum ether as eluents. Yield 0.62 g (46%); $^1$H NMR ($CDCl_3$): $\delta$=0.75–0.79 (t, $J$=7.2 Hz, 6H), 1.36–1.41 (q, $J$=7.2 Hz, 4H), 2.25–2.29 (t, $J$=7.6 Hz, 4H), 3.43 (s, 2H), 3.70 (s, 3H), 6.66–6.68 (d, $J$=7.6 Hz, 1H), 6.80–6.82 (d, $J$=7.2 Hz, 1H), 7.08–7.12 ppm (t, $J$=7.2 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=10.9, 19.2, 54.0, 54.8, 57.6, 111.0, 112.9, 120.0, 127.9, 141.2, 158.5 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=371 ppm; HRMS: $m/z$: calcd for $C_{28}H_{44}N_2O_2Se_2$ $[M+H]^+$: 601.1733; found: 601.0029.

**Synthesis of 31**: Thiophenol (20 μL, 0.17 mmol) was added at room temperature to the stirred solution of **4** (50 mg, 0.12 mmol) in dichloromethane (5 mL). The reaction mixture was stirred for 30 min, and the solvent was then evaporated. The expected compound was purified by flash chromatography on a silica gel column with ethyl acetate and petroleum ether as eluents to provide a pale yellow-colored oil. Yield: 21 mg (56%); $^1$H NMR ($CDCl_3$): $\delta$=2.32 (s, 6H), 3.61 (s, 2H), 7.11–7.16 (m, 3H), 7.94–7.25 (m, 3H), 7.51–7.53 (d, $J$=7.6 Hz, 2H), 7.96–7.98 ppm (d, $J$=7.6 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=44.3, 64.6, 126.3, 126.4, 128.1, 128.7, 129.2, 129.4, 136.2, 138.9 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=564 ppm; HRMS: $m/z$: calcd for $C_{15}H_{17}NSSe$ $[M+H]^+$: 324.0247; found: 323.8765.

**Synthesis of 33**: Thiophenol (17 μL, 0.15 mmol) was added at room temperature to the stirred solution of **20** (50 mg, 0.10 mmol) in dichloromethane (5 mL). The reaction mixture was stirred for 30 min, and the solvent was then evaporated. The expected compound was purified by flash chromatography on a silica gel column with ethyl acetate and petroleum ether as eluents to provide a pale yellow-colored oil. Yield: 18 mg (51%); $^1$H NMR ($CDCl_3$): $\delta$=0.97–1.00 (t, $J$=6.8 Hz, 6H), 2.55–2.60 (q, $J$=6.8 Hz, 4H), 3.64 (s, 2H), 7.01–7.03 (m, 3H), 7.08–7.17 (m, 3H), 7.41–7.43 (d, $J$=7.6 Hz, 2H), 7.85–7.87 ppm (d, $J$=8.0 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=9.2, 43.4, 57.8, 124.6, 124.8, 126.7, 127.0, 127.7, 134.1, 137.4, 138.0 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=558 ppm; HRMS: $m/z$: calcd for $C_{17}H_{21}NSSe$ $[M+H]^+$: 352.0560; found: 351.9353.

**Synthesis of 35**: Thiophenol (16 μL, 0.14 mmol) was added at room temperature to the stirred solution of **21** (50 mg, 0.09 mmol) in dichloromethane (5 mL), the reaction mixture was stirred for 30 min, and the solvent was then evaporated. The expected compound was purified by flash chromatography on a silica gel column with ethyl acetate and petroleum ether as eluents to provide a yellow-colored oil. Yield: 16.7 mg (49%); $^1$H NMR ($CDCl_3$): $\delta$=0.75–0.79 (t, $J$=7.2 Hz, 6H), 1.41–1.51 (m, 4H), 2.41–2.45 (t, $J$=8.0 Hz, 4H), 3.65 (s, 2H), 7.02–7.05 (m, 3H), 7.09–7.17 (m, 3H), 7.40–7.42 (d, $J$=7.6 Hz, 2H), 7.83–7.85 ppm (d, $J$=7.6 Hz, 1H); $^{13}$C NMR ($CDCl_3$): $\delta$=12.6, 18.9, 54.2, 60.6, 126.2, 126.3, 128.3, 128.6, 129.2, 135.6, 138.8, 139.6 ppm; $^{77}$Se NMR ($CDCl_3$): $\delta$=556 ppm; HRMS: $m/z$: calcd for $C_{19}H_{25}NSSe$ $[M+H]^+$: 380.0873; found: 379.9544.

**GPx activity—HPLC assay**: GPx-like activity was measured by high-performance liquid chromatography (HPLC) with use of a 2695 separation module and a 2996 photodiode-array detector and a fraction collector. The assays were performed in sample vials (1.8 mL), and a built-in autosampler was used for sample injection. In this assay, we employed mixtures containing a 1:2 molar ratio of PhSH and peroxide in methanol at room temperature (22 °C) as our model system. Runs with and without catalyst were carried out under the same conditions. Periodically, aliquots were injected onto the reversed-phase column (Lichrosphere 60, RP-select B, 5 μm) and eluted with methanol and water (85:15), and the concentrations of the diphenyl disulfide (PhSSPh) product were determined at 254 nm with the aid of pure PhSSPh as an external standard. The amount of disulfide formed during the course of the reaction was calculated from the calibration plot for the standard (PhSSPh). The plots for

kinetic parameters were obtained by use either of linear or of sigmoidal curve fitting.

**Computational methods**: All calculations were performed by use of the Gaussian98 suite[19] of quantum chemical programs. The hybrid Becke 3-Lee–Yang–Parr (B3LYP) exchange correlation functional was applied for DFT calculations.[20] Geometries were fully optimized at the B3LYP level of theory with use of the 6-31+G(d) basis sets. The NMR calculations were performed at the B3LYP/6-311+G(d,p) level on B3LYP/6-31+G(d) level optimized geometries by the GIAO method.[21] Orbital interactions were analyzed by the natural bond orbital (NBO) method at the B3LYP/6-311+G(d,p) level, and charges were calculated by natural population analysis (NPA).[11] To examine the effect of solvent on the geometries of the selenol intermediates, single-point energy calculations were performed in aqueous medium on the B3LYP/6-31+G(d)-level-optimized gas-phase geometries by use of the isodensity polarized continuum model (IPCM).[22]

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[13] Although electron-donating substituents such as the methoxy group are expected to increase the positive charge on selenium, the higher stability of the methoxy-substituted zwitterion relative to the unsubstituted compound is probably due to an increase in the basicity of the tertiary amino group. Therefore, the amino groups in compounds **26**, **28**, and **30** are better bases than the amino substituents in compounds **25**, **27**, and **29**.

[14] The identification of selenenic and seleninic acids is based on literature data and a number of control experiments. Treatment of the diselenides with $H_2O_2$ (5 equiv) produced the selenenic and seleninic

  

acids in the cases of **4**, **20**, and **21**, and only the selenenic acids in the case of **22**–**24**. Treatment of selenols **25**–**30** with $H_2O_2$ produced identical signals in the $^{77}$Se NMR. This precludes the possibility of the formation of any thiol esters in the reactions. The reactions of the oxidized species with PhSH are also helpful for assignation of the signals for the selenenic acid and seleninic acid intermediates. While the selenenic acids **38**, **40**, and **42** required only one equivalent of PhSH each to produce the corresponding selenenyl sulfides quantitatively, higher concentrations of PhSH were required for complete conversions of the oxidized compounds derived from **4**, **20**, and **21** into the corresponding selenenyl sulfides. As an example, the signal due to the selenenic acid **37** disappeared completely upon addition of one equivalent of PhSH to a mixture containing selenenic acid **37** and seleninic acid **43**. The addition of an excess amount of PhSH to the mixture converted both **37** and **43** into the selenenyl sulfide **31**.

[15] It should be noted that selenenic acids are generally quite unstable and may not survive in solution for long times. They tend to disproportionate in solution to give the corresponding seleninic acids and diselenides ($3\,RSeOH \rightarrow RSeO_2H + RSeSeR + H_2O$). However, no such disproportionation was observed in the cases of selenenic acids **38**, **40**, and **42**, which were stable enough to be detected by $^{77}$Se NMR spectroscopy. This is due to the presence of the methoxy substituents, which protect the selenium moieties from overoxidation. For structurally characterized stable selenenic acids, see: a) T. Saiki, K. Goto, R. Okazaki, *Angew. Chem. 1997, 109, 2320–2322; Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2223–2224; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2223–2224; b) A. Ishii, S. Matsubayashi, T. Takahashi, J. Nakayama, *J. Org. Chem.* **1999**, *64*, 1084–1085; c) K. Goto, M. Nagahama, T. Mizushima, K. Shimada, T. Kawashima, R. Okazaki, *Org. Lett.* **2001**, *3*, 3569–3572.

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