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# Enhanced Glutathione Peroxidase Activity of Conformationally Restricted Naphthalene *peri*-Dichalcogenides

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## ABSTRACT


**Conformationally restricted naphthalene *peri*-diselenides function as glutathione peroxidase mimetics with superior catalytic activity to that displayed by typical acyclic diaryl diselenides. Their activity was increased by electron-donating methoxy substituents, while a further 100-fold increase was observed with the corresponding ditelluride.**

Oxidative stress results from the formation of reactive oxygen species (ROS) such as peroxides, the superoxide radical anion, and the hydroxyl radical as byproducts during normal aerobic metabolism. These species cause damage to cell membranes and react with various crucial biomolecules in mammalian cells.[1] The effects of oxidative stress have been implicated in a variety of degenerative processes and disease states, including inflammation, mutagenesis and cancer, dementia, and cardiovascular damage. Under normal conditions, exogenous antioxidants present in a balanced diet afford partial protection against ROS, as do endogenous enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, which catalyze the destruction of ROS. Certain conditions, such as ischemic reperfusion of stroke and heart attack victims, result in unusually high levels of oxidative stress that overwhelm the protective effects of exogenous and endogenous antioxidants. Consequently, there is considerable interest in the discovery of small-molecule compounds that could afford additional protection against the deleterious effects of ROS to such patients.

The selenoenzyme glutathione peroxidase (GPx)[2] destroys peroxides by catalyzing their reduction to alcohols or water with the stoichiometric reductant glutathione, a tripeptide that is ubiquitous and abundant in mammalian cells. Numerous types of organoselenium compounds have been investigated for their ability to emulate GPx,[3]

(1) For selected reviews, see: (a) *Oxidative Stress*; Sies, H., Ed.; Academic Press: London, 1985. (b) *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*; Rice-Evans, C., Halliwell, B., Lunt, G. G., Eds.; Portland Press: London, 1995. (c) *Free Radicals in Molecular Biology, Aging and Disease*; Armstrong, D., Sohal, R. S., Cutler, R. G., Slater, T. F., Eds.; Raven Press: New York, 1984. (d) *Oxidative Processes and Antioxidants*; Paoletti, R., Samuelsson, B., Catapano, A. L., Poli, A., Rinetti, M., Eds.; Raven Press: New York, 1994. (e) *Selenium in Biology and Human Health*; Burk, R. F., Ed.; Springer-Verlag: New York, 1994.

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(3) For reviews, see: (a) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125. (b) Mugesh, G.; Singh, H. B. *Chem. Soc. Rev.* **2000**, *29*, 347. (c) Mugesh, G.; du Mont, W.-W. *Chem.—Eur. J.* **2001**, *7*, 1365. (d) Day, B. J. *Biochem. Pharmacol.* **2009**, *77*, 285. (e) Bhabak, K. P.; Mugesh, G. *Acc. Chem. Res.* **2010**, *43*, 1408.

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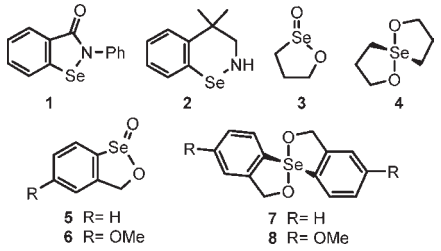
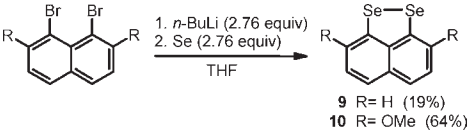
including ebselen (**1**)[4] and ALT 2074 (**2**),[5] which have undergone clinical trials for their efficacy as antioxidants and anti-inflammatory agents. We recently reported that cyclic seleninate ester **3**[6] and spirodioxyselenurane **4**[7] are highly effective catalysts for the reduction of peroxides with sacrificial thiols. Their aromatic derivatives **5** and **7**, respectively, proved less effective in this regard, although the introduction of electron-donating methoxy groups *para* to the selenium atom, as in **6** and **8**, improved their catalytic activity to levels comparable with their aliphatic counterparts.[8] This was attributed to the ability of the methoxy group to stabilize the increasing positive charge at the selenium atom during the rate-determining step of the catalytic cycle, which involves the oxidation of Se(II) to Se(IV). Singh and co-workers[9] have independently studied cyclic seleninate esters. In some cases, *ortho* substituents capable of coordinating with the selenium atom enhance the catalytic activity of GPx mimetics.[10] Since aromatic selenium compounds are generally less toxic than aliphatic ones,[3b,11] we have continued our search for efficacious aromatic GPx mimetics with other structural motifs.




Certain diselenides display significant GPx-like activity.[6,12,13] Indeed, it has been reported that, under certain assay conditions, diphenyl diselenide is a more effective catalyst than ebselen (**1**).[14] We reasoned that further improvements to the activity of diselenides might be gained by employing species where the diselenide moiety is strained or otherwise destabilized, particularly if mesomeric electron donation from a substituent is also available, assuming that oxidation at selenium is again the rate-determining step. We therefore turned our attention to the known naphtho[1,8-*cd*]-1,2-diselenole (**9**) and its novel dimethoxy derivative **10**. The rigid and nearly planar structure of *peri* diselenide **9** results in a severely constrained C−Se−Se−C dihedral angle and a longer than normal Se−Se bond length,[15] compared to the structure of acyclic diaryl diselenides, where the dihedral angle is closer to orthogonal.[16] Thus, destabilization of **9** and **10** was expected to result in a lowered oxidation potential and more facile oxidation by peroxides, accompanied by enhanced overall catalytic activity. We now report the results of our preliminary investigations into their catalytic properties in the benzyl thiol-mediated reduction of hydrogen peroxide. The diselenide **9** was obtained by a variation of the method of Meinwald et al.,[17] while **10** was obtained similarly, as depicted in Scheme 1, but in considerably higher yield.

**Scheme 1**




Both diselenides were tested in our model assay,[8,18] along with diphenyl diselenide and di(*o*-methoxyphenyl) diselenide for comparison. The results are provided in Table 1, where $t_{1/2}$ indicates the time required for oxidation of 50% of the benzyl thiol to dibenzyl disulfide and affords a convenient means for comparison of the catalysts tested in this manner. Kinetic plots are provided in the Supporting Information. Table 1 reveals that, of the compounds

(4) (a) Müller, A.; Cadenas, E.; Graf, P.; Sies, H. *Biochem. Pharmacol.* **1984**, *33*, 3235. (b) Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R. *Biochem. Pharmacol.* **1984**, *33*, 3241. (c) Parnham, M. J.; Kindt, S. *Biochem. Pharmacol.* **1984**, *33*, 3247. (d) Müller, A.; Gabriel, H.; Sies, H. *Biochem. Pharmacol.* **1985**, *34*, 1185. (e) Safayhi, H.; Tiegs, G.; Wendel, A. *Biochem. Pharmacol.* **1985**, *34*, 2691. (f) Wendel, A.; Tiegs, G. *Biochem. Pharmacol.* **1986**, *35*, 2115. (g) Fischer, H.; Dereu, N. *Bull. Soc. Chim. Belg.* **1987**, *96*, 757. (h) Haenen, G. R. M. M.; De Rooij, B. M.; Vermeulen, N. P. E.; Bast, A. *Mol. Pharmacol.* **1990**, *37*, 412. (i) Glass, R. S.; Farooqui, F.; Sabahi, M.; Ehler, K. W. *J. Org. Chem.* **1989**, *54*, 1092.

(5) For lead references to ALT 2074 (formerly BXT 51072), see: (a) Moutet, M.; D'Alessio, P.; Malette, P.; Devaux, V.; Chaudière, J. *Free Radical Biol. Med.* **1998**, *25*, 270. (b) Erdelmeier, I.; Tailhan-Lomont, C.; Yadan, J.-C. *J. Org. Chem.* **2000**, *65*, 8152. (c) Jacquemin, P. V.; Christiaens, L. E.; Renson, M. J.; Evers, M. J.; Dereu, N. *Tetrahedron Lett.* **1992**, *33*, 3863.

(6) (a) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2002**, *124*, 12104. (b) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2003**, *125*, 13455.

(7) Back, T. G.; Moussa, Z.; Parvez, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 1268.

(8) (a) Back, T. G.; Kuzma, D.; Parvez, M. *J. Org. Chem.* **2005**, *70*, 9230. (b) Press, D. J.; Mercier, E. A.; Kuzma, D.; Back, T. G. *J. Org. Chem.* **2008**, *73*, 4252.

(9) (a) Tripathi, S. K.; Patel, U.; Roy, D.; Sunoj, R. B.; Singh, H. B.; Wolmershäuser, G.; Butcher, R. J. *J. Org. Chem.* **2005**, *70*, 9237. (b) Tripathi, S. K.; Sharma, S.; Singh, H. B.; Butcher, R. J. *Org. Biomol. Chem.* **2011**, *9*, 581.

(10) For a review of the effects of coordinating substituents upon GPx-like activity, see: Mukherjee, A. J.; Zade, S. S.; Singh, H. B.; Sunoj, R. B. *Chem. Rev.* **2010**, *110*, 4357.

(11) Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255.

(12) For representative examples, see: (a) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 839. (b) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *Chem. Commun.* **1998**, 2227. (c) Wirth, T. *Molecules* **1998**, *3*, 164. (d) Engman, L.; Andersson, C.; Morgenstern, R.; Cotgreave, I. A.; Andersson, C.-M.; Hallberg, A. *Tetrahedron* **1994**, *50*, 2929. (e) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.* **1994**, *116*, 2557.

(13) Very recently, Mugesh et al. have shown that 1,8-naphthalene dithiol, diselenol and the corresponding mixed thiol-selenol emulate the behavior of iodothyronine deiodinase and are oxidized to the corresponding dichalcogenides during the deiodination process: (a) Manna, D.; Mugesh, G. *Angew. Chem., Int. Ed.* **2010**, *49*, 9246. (b) Manna, D.; Mugesh, G. *J. Am. Chem. Soc.* **2011**, *133*, 9980.

(14) It has been reported that diphenyl diselenide has ca. twice the GPx activity of ebselen: Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936.

(15) For the X-ray crystal structure of **9**, see: Aucott, S. M.; Milton, H. L.; Robertson, S. D.; Slawin, A. M. Z.; Woollins, J. D. *Heteroatom Chem.* **2004**, *15*, 530. The Se−Se bond length was reported to be 2.3639(5) Å.

(16) (a) The C−Se−Se−C dihedral angle of diphenyl diselenide was reported to be 82.0° ± 3.0°, and the Se−Se bond length was 2.29 ± 0.01 Å; see: Marsh, R. E. *Acta Crystallogr.* **1952**, *5*, 458. (b) Destabilization of the ground state of a hindered dialkyl diselenide through an increase in the dihedral angle to 112.1° has also been observed: Back, T. G.; Codding, P. W. *Can. J. Chem.* **1983**, *61*, 2749.

(17) Meinwald, J.; Dauplaise, D.; Wudl, F.; Hauser, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 255.

(18) Back, T. G.; Dyck, B. P. *J. Am. Chem. Soc.* **1997**, *119*, 2079.

**Table 1.** GPx-Like Catalytic Activity of Diselenides[a]

$$2BnSH + H_2O_2 \xrightarrow[MeOH - CH_2Cl_2 \ (95:5)]{\text{catalyst (10 mol \%)}} BnSSBn + 2H_2O$$

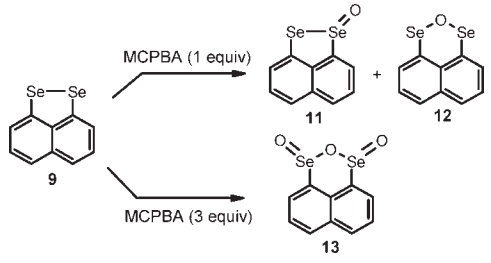
| entry | catalyst | $t_{1/2}$ (h) |
|---|---|---|
| 1 | PhSeSePh | 129 |
| 2 | [*o*-(MeO)PhSe]$_2$ | 90 |
| 3 | **9** | 9.7 |
| 4 | **10** | 7.4 |
| 5 | **14** | 5.9 |
| 6 | **19** | <0.05 |
| 7 | Nil (control) | >300 |

[a] The assay was performed with 0.31 mmol of BnSH (0.031 M), a slight excess of 0.35 mmol (0.035 M) of $H_2O_2$, and 0.031 mmol (0.0031 M) of catalyst in 10 mL of the solvent at 18 °C. Values of $t_{1/2}$ are averages of duplicate runs.

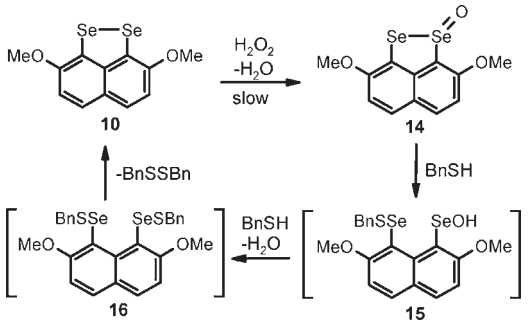
investigated, diphenyl diselenide provided the poorest catalytic activity, with a $t_{1/2}$ of 129 h (entry 1). This decreased to 90 h when the *o*-methoxy substituent was present (entry 2), again demonstrating its ability to enhance the catalytic activity. In accordance with our expectations, selenide **9** provided an increase in catalytic activity of approximately an order of magnitude, with a $t_{1/2}$ of 9.7 h (entry 3), while a slight further improvement was observed with the di-*o*-methoxy analog **10** in entry 4 ($t_{1/2}$ = 7.4 h). A control experiment with no Se-containing catalyst present produced a very slow reaction with $t_{1/2}$ > 300 h (entry 7). Evidently, the strained and rigid *peri*-diselenides **9** and **10** are considerably more effective GPx mimetics than simple diaryl diselenides and the electron-donating methoxy substituents confer a modest additional benefit. Several further experiments were conducted to elucidate the mechanism of the catalytic cycles of **9** and **10**. First, it should be noted that Kice et al.[19] had investigated the peracid oxidation of **9** and found that, with 1 equiv of MCPBA, it was converted into a 60:40 mixture of selenolseleninate **11** and cyclic selenenic anhydride **12**. Oxidation with 3 equiv of the peracid afforded the cyclic seleninic anhydride **13**, which was so highly insoluble that NMR spectra could not be obtained (Scheme 2).

In our case, a control experiment conducted with *peri*-diselenide **10** and 1 equiv of hydrogen peroxide in the absence of thiol resulted in its oxidation to the isolable selenolseleninate **14** in ca. 30 min, with complete discharge of the intense purple color of **10**, and without concomitant formation of the cyclic selenenic anhydride corresponding to **12**. On the other hand, treatment of **10** with excess benzyl thiol in the absence of hydrogen peroxide resulted in no significant reaction, even after 12 h. This indicates that the first step in the catalytic cycle of **10** is oxidation to the selenolseleninate **14** and not ring-opening of the cyclic diselenide by the thiol. Moreover, treatment of a pure sample of **14** with excess hydrogen peroxide showed no significant further oxidation after 24 h, while the reaction of **14** with excess benzyl thiol in the absence of the peroxide resulted in the rapid reappearance of the purple color, with 85% recovery of diselenide **10**.[20] Thus, the next stage in the catalytic cycle under these conditions is reduction of the selenolseleninate **14** back to diselenide **10** and not further oxidation to species such as the seleninic anhydride **17**.

**Scheme 2**




**Scheme 3**




These experiments suggest the mechanism shown in Scheme 3, where diselenide **10** is converted into selenolseleninate **14** in the rate-determining step, followed by very rapid reduction by benzyl thiol back to the original diselenide. By analogy to the previous work by Kice et al.,[19] with 2-methyl-2-propanethiol and selenolseleninate **11**, it is reasonable to postulate that transient intermediates **15** and **16** are formed during the reduction of **14** with benzyl thiol in Scheme 3. The conversion of **16** to **10** may be catalyzed by attack of the thiol at the S-atom of one of the selenenyl sulfide moieties, followed by intramolecular diselenide formation with regeneration of the thiol.[21] The mechanism in Scheme 3 is also consistent with the overall rate enhancement afforded by the *o*-methoxy groups in diselenide **10** compared to diselenide **9**, since mesomeric electron donation is expected to stabilize the increase in positive charge on Se during its oxidation from Se(II) to Se(IV) in the rate-determining step, and thus accelerate the overall process. The catalytic cycle of diselenide **9** presumably follows the same general mechanism as shown in Scheme 3 for **10**.

(19) Kice, J. L.; Kang, Y.-H.; Manek, M. B. *J. Org. Chem.* **1988**, *53*, 2435.

(20) When the thiol was added slowly to **14** in the absence of hydrogen peroxide, polymeric products were formed instead of the diselenide **10**.

A pure sample of selenolseleninate **14** was tested independently in the usual assay, producing a $t_{1/2}$ of 5.9 h (Table 1, entry 5). The shorter $t_{1/2}$ compared to that of **10** is attributed to the circumvention of the slow step in the cycle, in which the diselenide is first oxidized to the selenolseleninate, in the early stage of the process. The kinetic plot (see Supporting Information) also showed a positive intercept of 11.0−12.6%, consistent with its nearly instantaneous reaction with the thiol,[22] followed by the slower subsequent reoxidation of the resulting diselenide with the peroxide and eventual completion of the catalytic cycle.

We also subjected the selenolseleninate **14** to further oxidation with MCPBA. In contrast to the highly insoluble seleninic anhydride **13** obtained by Kice et al.,[19] the analogous product **17** in the present case was isolated in 90% yield and was sufficiently soluble in DMSO-$d_6$ to permit the recording of its $^{1}$H, $^{13}$C, and $^{77}$Se NMR spectra.[23] These spectra showed the presence of two sets of signals in the ratio of 1.0 to 0.8. Since the Se-atoms in **17** are chiral centers, we attribute the observed nonequivalence to the presence of *cis* and *trans* diastereomers that are configurationally stable at rt on the NMR time scale.[24] This hypothesis was confirmed by adding excess potassium hydroxide in deuterium oxide to the NMR sample, which resulted in collapse of the NMR spectra to single sets of signals, presumably caused by anhydride ring opening and formation of the dipotassium salt **18** of the corresponding bis(seleninic acid).

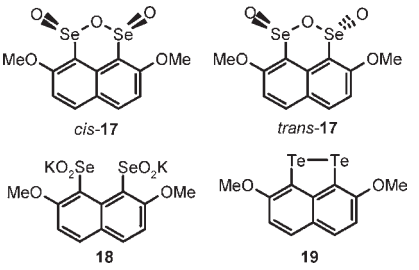
(21) Alternatively, as pointed out by a reviewer, it is possible that the selenenyl sulfide moiety of **15** reacts intramolecularly with the electrophilic selenenic acid to form a new Se−Se bond, followed by attack of the thiol at the sulfur atom of the benzylthio group to produce **10** directly, along with BnSSBn. In any case, the greater stability of the corresponding bis(selenenyl sulfide) in Kice's work is attributed to steric protection afforded by the use of *tert*-butyl instead of benzyl thiol.

(22) The 10 mol % of **14** that was added to the reaction mixture was consumed very rapidly by reaction with 2 equiv of benzyl thiol, thereby generating 1 equiv of dibenzyl disulfide. Thus, the anomalously rapid formation of ca. 10 mol % of the disulfide is expected in the initial stage of the catalytic cycle.

(23) We observed no evidence of oxidation of DMSO-$d_6$ to the corresponding sulfone by **17** in these experiments.

(24) A variable temperature $^{1}$H NMR experiment showed only partial coalescence of signals when the sample was heated to 403 K.

Finally, we prepared the novel ditelluride **19** in 51% yield in a similar manner to that of the corresponding diselenide **10** and tested it in the same assay. The ditelluride proved to be a remarkably active catalyst, producing reaction rates too fast to measure by HPLC. Separate reactions assayed after 1 and 3 min indicated that the yields of dibenzyl disulfide were 30% and 85%, respectively. However, after longer reaction times, decreased disulfide yields were observed, suggesting that **19** also catalyzed the further oxidation of the disulfide to mixtures of unidentified products when excess hydrogen peroxide was present.




In conclusion, significant enhancement of more than an order of magnitude in the GPx-like catalytic activity of simple diaryl diselenides was achieved by exploiting the conformationally restricted *peri* diselenide **10** and by introducing electron-donating methoxy substituents to facilitate the rate-determining Se(II) to Se(IV) oxidation step in the catalytic cycle. A further dramatic increase of more than 2 orders of magnitude was realized with the tellurium analog **19**, compared to the diselenide **10**.

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**Supporting Information Available.** Experimental procedures, characterization data, assays of catalytic activity, and NMR spectra of key compounds. This information is available free of charge via the Internet at http://pubs.acs.org.