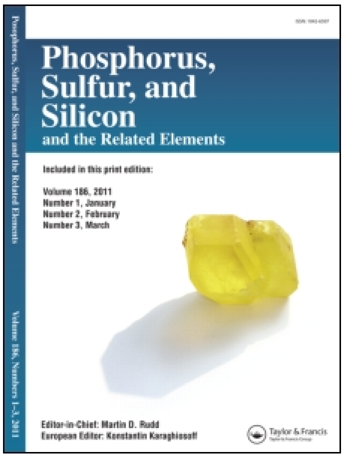

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Mimetics of the Selenoenzyme Glutathione Peroxidase: Novel Structures and Unusual Catalytic Mechanisms

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Mimetics of the Selenoenzyme Glutathione Peroxidase: Novel Structures and Unusual Catalytic Mechanisms

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Glutathione peroxidase (GPx) mimetics comprise an important class of selenium-containing antioxidants that catalyze the destruction of biologically harmful peroxides in the presence of stoichiometric thiol reductants. The synthesis of two novel cyclic selenium compounds and their evaluation as GPx mimetics was achieved. The first is a cyclic seleninate ester that is formed in situ *from the oxidation of allyl 3-hydroxypropyl selenide. The second is a spirodioxyselenurane that is similarly formed from di(3-hydroxypropyl) selenide. Both compounds were shown to be remarkably active catalysts in an assay based on the reduction of t-butyl hydroperoxide with benzyl thiol. The mechanisms of the catalytic cycles of the two novel selenium compounds were elucidated and were found to be distinct from each other and from that of GPx.*

Keywords Cyclic seleninate esters; glutathione peroxidase mimetics; spirodioxyselenuranes

INTRODUCTION

Aerobic metabolism sustains most living organisms, including humans. Unfortunately, peroxides are formed as minor byproducts of this process and they as well as other reactive oxygen species contribute to oxidative stress, a deleterious condition that has been implicated in various degenerative processes and disease states.[1–3] These include inflammation, atherosclerosis and other cardiovascular diseases, mutagenesis and cancer, dementia, and possibly the aging process. A certain degree of protection against oxidative stress is afforded by endogenous antioxidants, as well as by those obtained in the diet. GPx is a selenoenzyme

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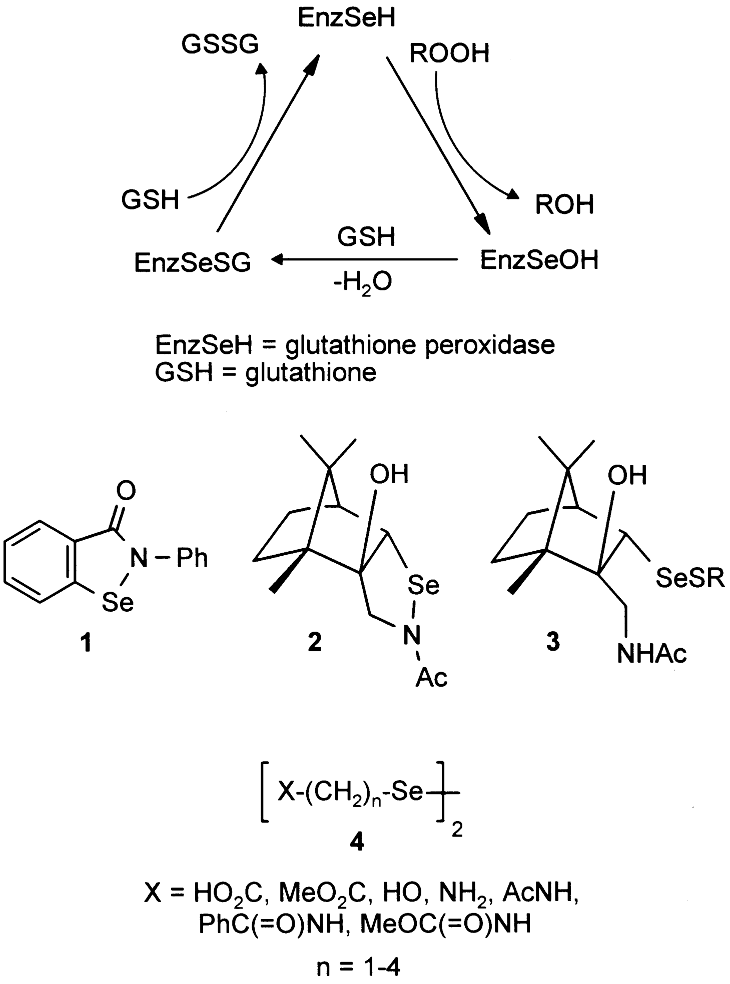
that catalyzes the reduction of harmful peroxides with the tripeptide glutathione (GSH).[4–10] The latter functions as a stoichiometric reductant that is in turn oxidized to the corresponding disulfide (GSSG). GPx possesses a selenocysteine residue in each subunit of its tetrameric structure and the redox chemistry of the selenium moiety is responsible for the catalytic properties of the enzyme. The catalytic cycle for GPx is shown in Scheme 1. Thus, the selenol function of a selenocysteine residue (EnzSeH) is oxidized by the peroxide to the corresponding selenenic acid (EnzSeOH), which is then reduced to the corresponding



SCHEME 1 Catalytic cycle of glutathione peroxidase.

selenenyl sulfide (EnzSeSG) with GSH. The original selenol is restored by reaction of EnzSeSG with a second mole of GSH.

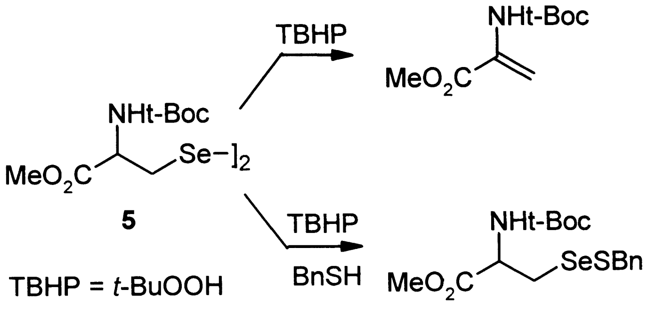
The fundamental importance of this process has prompted numerous efforts to design small-molecule selenium compounds that mimic the redox chemistry of GPx and its antioxidant properties.[11–13] Special emphasis has been placed on N—Se heterocycles and other selenium compounds with pendant amino groups, since N—Se interactions appear to modulate the redox properties and enhance the catalytic activity of the selenium function.[14,15] One such compound, ebselen (**1**), has undergone clinical trials for its antioxidant properties.[16–24] Unfortunately, **1** exhibits relatively poor catalytic activity, prompting attempts to design more efficacious GPx mimetics that would retain the low toxicity of ebselen while manifesting improved catalytic properties. The following is an overview of some of the work in our laboratory that is directed toward the discovery of improved GPx mimetics.

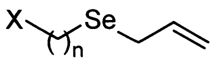
RESULTS AND DISCUSSION

Several years ago, during attempts to design camphorseleno groups that could be used as chiral auxiliaries in asymmetric selenium reactions, we discovered the cyclic selenenamide **2**.[25] It occurred to us that this compound might behave as an efficient GPx mimetic and subsequent investigation revealed that it indeed was considerably more active than **1** in this regard. Moreover, we observed that **2** functioned as a procatalyst, whose rapid reaction with a thiol afforded the corresponding selenenyl sulfide **3**, thus providing entry into the same type of catalytic manifold as seen in Scheme 1 for GPx itself.

Since the camphor moiety of **2** was presumably extraneous to the new role discovered for this compound, we embarked on a search for simpler structures that would function in a similar manner to **2**, and that would ultimately emulate the selenoenzyme itself. In particular, a paucity of existing data for aliphatic selenium compounds in this regard prompted our selection of such compounds for special scrutiny. In order to perform structure-activity studies of the catalytic activity of a series of organoselenium compounds, we required a standardized assay that would provide a quantitative basis for comparing them. This involved the use of excess *t*-butyl hydroperoxide (TBHP) as the oxidant and benzyl thiol (BnSH) as the sacrificial reductant in dichloromethane-methanol (95:5) solution at 18°C. The conversion of this thiol to its disulfide can be easily followed by HPLC or NMR, while the half-life of the reaction ($t_{1/2}$; defined as the time required for oxidation of 50% of the thiol to its disulfide) comprises a convenient parameter for comparing the activities of various catalysts. Under these conditions, a control reaction with no catalyst displayed $t_{1/2}$ of >300 h. On the other hand, the presence of 10% mole of ebselen (**1**) or selenenamide **2** lowered the $t_{1/2}$ to 42 h and 18 h, respectively.[26,27]

We thus evaluated a series of diselenides **4** containing coordinating groups at the ω-position. However, all but one of the compounds in this series showed inferior activity compared to **2**, with $t_{1/2}$ ranging from 33–170 h. The exception was **4** (X = AcNH; n = 3), which had $t_{1/2}$ = 6 h. Unfortunately, this compound proved difficult to purify and was abandoned for further studies. The selenocystine derivative **5** was surprisingly ineffective ($t_{1/2}$ = 300 h) and further investigation revealed that, in the presence of excess TBHP, it underwent a facile, quantitative selenoxide elimination to afford the corresponding dehydroalanine derivative (Scheme 2). In the presence of both TBHP and BnSH, it instead formed the corresponding selenenyl sulfide, which proved almost devoid of catalytic activity. This was surprising in view of the key role played by the selenenyl sulfide derivative of GPx (EnzSeSG) in Scheme 1 and of **3** in the catalytic cycle of **2**.





6

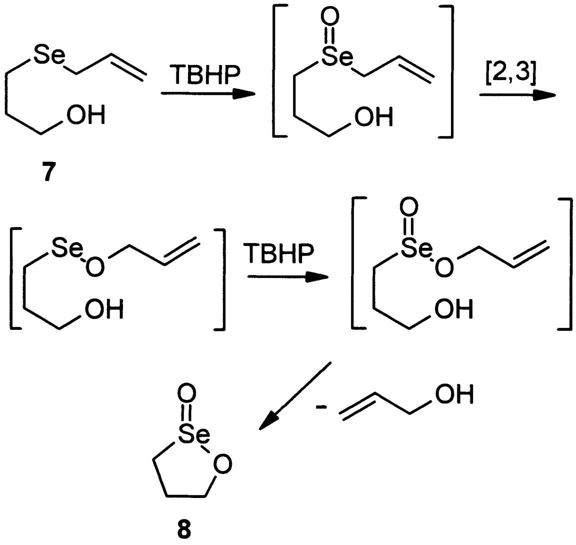
X = MeO_2C, AcNH, PhC(=O)NH
MeOC(=O)NH, CF_3C(=O)NH

n = 1-3

SCHEME 2 Oxidation of a selenocystine derivative with TBHP with and without added BnSH.

We next evaluated a series of ω-substituted alkyl allyl selenides **6** in our standard assay.[26,27] Our reasoning was that they would undergo rapid oxidation to their selenoxides, followed by even more rapid

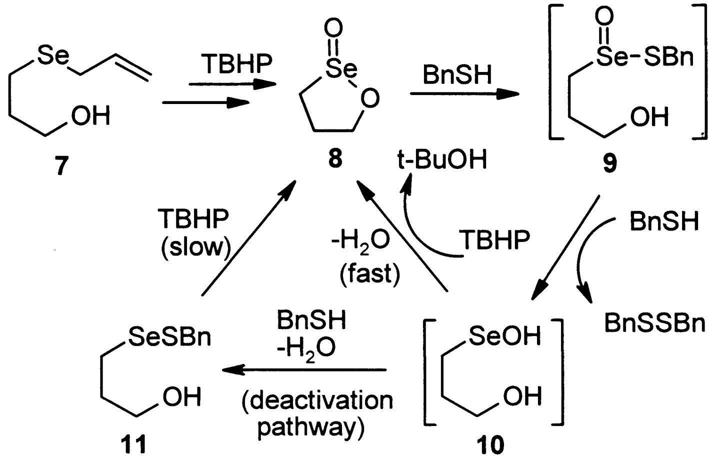
[2,3]sigmatropic rearrangements to generate reactive selenenate esters that we hoped would provide entry into the desired catalytic cycle in the presence of BnSH and TBHP. This hypothesis proved correct, with **6** giving improved half-lives, compared to those of the earlier diselenides, of 5.6–28 h. It is noteworthy that the most active allyl selenide **7** ($t_{1/2} = 4.8$ h) contained an ω-hydroxyalkyl substituent instead of the usual ω-aminoalkyl group. The use of hydroxyl groups as coordinating substituents in GPx mimetics had been little studied in the past[28] and we selected this compound for further scrutiny. We found that, as expected, **7** was inert toward BnSH. However, oxidation with TBHP was complete within 15 min and, when an excess of the oxidant was present, the selenide was converted into the cyclic seleninate ester **8**, which was isolated in nearly quantitative yield (Scheme 3). It proved to be a stable compound, which fully was characterized by spectroscopic and analytical methods. Only a few cyclic seleninate esters have been reported to date, all of which contain stabilizing substituents or other rings fused to the cyclic seleninate structure.[29–32] Thus, it appears that **8** is the first example of a simple, unsubstituted, monocyclic seleninate ester.



SCHEME 3 Formation of cyclic seleninate ester **8** from the oxidation of selenide **7**.

When the seleninate ester **8** was subjected to our assay, it produced an even faster reaction rate ($t_{1/2} = 2.5$ h) than its precursor selenide **7**. We postulated the mechanism in Scheme 4 to account for its remarkable catalytic activity and offer the following observations in its support.

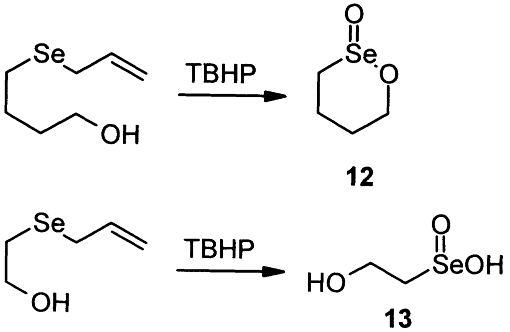
Compound **8** was inert toward the hydroperoxide, but reacted rapidly with BnSH in separate control experiments. Thus, the thioseleninate **9** in the first step and the selenenic acid **10** are plausible intermediates. When TBHP also is present, the oxidation of **10** regenerates seleninate ester **8**. However, treatment of **8** with excess thiol in the absence of the hydroperoxide afforded a nearly quantitative yield of selenenyl sulfide **11**, which was isolated and characterized. Furthermore, analysis of the reaction mixture that was produced under the normal assay conditions of either selenide **7** or seleninate ester **8** revealed that **8** and a small amount of the selenenyl sulfide **11** were the only selenium-containing products remaining after exhaustion of the thiol. We therefore conclude that, when allyl selenide **7** is employed in the assay, it is rapidly converted to the seleninate ester **8** via Scheme 3, which then serves as the true catalyst and is regenerated at the end of the process shown in Scheme 4.[26,27]



SCHEME 4 Catalytic cycle of cyclic seleninate ester **8**.

In view of the important role that selenenyl sulfides play in the GPx cycle shown in Scheme 1 and in the similar mechanism associated with the catalytic properties of selenenamide **2**, we also subjected **11** to the assay. To our surprise, **11** gave $t_{1/2} = 35$ h, ca. 14 times longer than that of **8**. This rules out any significant role of **11** in the catalytic cycle of Scheme 4 and indicates that the formation of the selenenyl sulfide represents a deactivation pathway in this present process. We also observed that the oxidation of authentic **11** with excess TBHP regenerated the cyclic seleninate ester **8**, but at a much slower rate. Thus, catalytic activity eventually is restored to the system even when the deactivation pathway in Scheme 4 is followed.

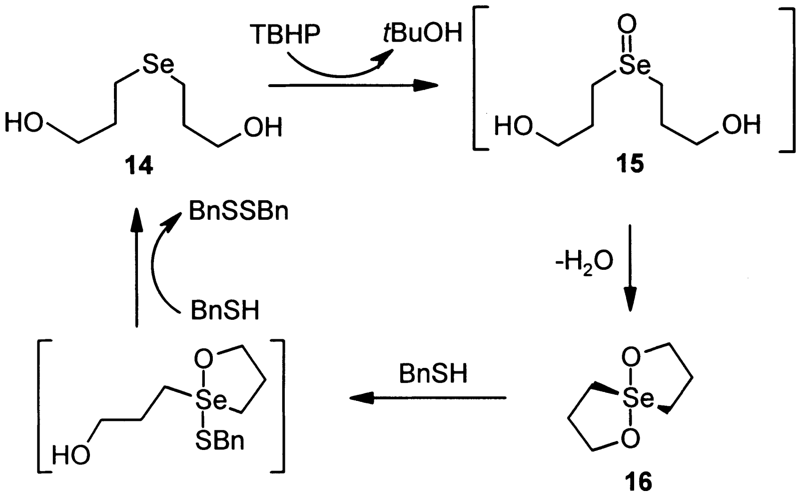
Similar examination of the 4-hydroxybutyl and 2-hydroxyethyl allyl selenide homologues of **7** revealed that the former also afforded the corresponding cyclic seleninate ester **12**, while the latter produced only the open-chain seleninic acid **13** (Scheme 5), presumably because of strain in the corresponding 4-membered ring. The longer $t_{1/2}$ of these two selenides (9.8 h and 7.7 h, respectively, compared with 4.8 h for the 3-hydroxypropyl derivative **7**) indicates that the 3-hydroxypopyl side chain of **7** and the five-membered ring of **8** comprise the optimum chain length and ring size for catalytic activity of these species.



SCHEME 5 Oxidation of allyl 4-hydroxybutyl selenide and allyl 2-hydroxyethyl selenide with TBHP.

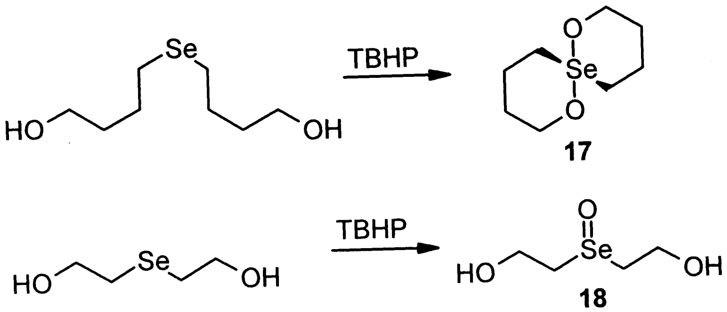
Prompted by the success of the above investigations of allyl ω-hydroxyalkyl selenides, we turned our attention to structurally related di(ω-hydroxyalkyl) selenides in order to see if they would act as effective catalysts in our assay.[33] Although there is no possibility for a [2,3]sigmatropic rearrangement in dialkyl selenides, we intended to see if the corresponding selenoxides would undergo *syn*-eliminations to generate selenenic acids which might also provide entry into the catalytic cycle. By analogy, the GPx catalytic cycle in Scheme 1 also was reported to proceed via a selenenic acid intermediate (EnzSeOH). When selenide **14** was subjected to our assay, it gave $t_{1/2} = 2.9$ h, comparable with that observed earlier with cyclic seleninate ester **8** ($t_{1/2} = 2.5$ h). This encouraging result prompted more careful scrutiny of the process. As expected, **14** proved inert when treated with BnSH alone. However, when oxidized with excess TBHP, it was rapidly consumed (<5 min) and was converted quantitatively to the corresponding novel spirodialkoxyselenurane **16**. The latter could be isolated and was fully characterized by spectroscopic methods and by X-ray crystallography.[33] Although a few dioxyselenuranes have been reported, they typically contain aromatic moieties attached to the Se atom or acyloxy instead of alkyloxy

Se substituents.[34–40] Thus, as with cyclic seleninate ester **8**, **16** also represents the first example of its respective ring system that is unadorned by additional rings or substituents. Furthermore, when a sample of **16** was tested for catalytic activity in the usual manner, the $t_{1/2}$ was identical to that measured with its precursor selenide **14**. Finally, we observed that at the end of the assay, when all of the BnSH had been consumed, **16** was the only selenium-containing species present in the reaction mixture when an excess of TBHP was present. On the other hand, when **16** was treated with excess BnSH in the absence of the hydroperoxide, it regenerated the selenide **14**. These observations are consistent with the catalytic cycle in Scheme 6, where oxidation of **14** first produces the corresponding selenoxide **15**, which spontaneously cyclizes to **16**. The latter then undergoes substitution of one alkoxy group by the thiol, followed by a reductive elimination to regenerate the original selenide **14**. The rapid cyclization of **15** evidently does not permit the corresponding selenoxide *syn*-elimination of **15** to compete effectively.



SCHEME 6 Catalytic cycle of selenide **14**.

Similar experiments were conducted with the homologous di(4-hydroxybutyl) and di(2-hydroxyethyl) selenides, both of which proved inferior catalysts to **14**. The former produced spirodialkoxyselenurane **17** upon oxidation, with $t_{1/2} = 5.1$ h, whereas the latter afforded only the corresponding selenoxide **18** (Scheme 7). However, as in the case of the cyclic seleninate, the three-carbon chains of 3-hydroxypropyl selenide **14** and five-membered rings of the corresponding spiro compound **16** resulted in the most efficacious catalysts.



SCHEME 7 Oxidation of di(4-hydroxybutyl) selenide and di(2-hydroxyethyl) selenide with TBHP.

These results therefore provide some interesting new insights into the design of new GPx mimetics. First, it is clear that several distinct mechanisms for the reduction of hydroperoxides with thiols are possible, in addition to the one shown in Scheme 1 for GPx. Thus, the catalytic cycles in Schemes 4 and 6 involve the novel and unusual cyclic seleninate ester **8** and spirodialkoxyselenurane **16**, respectively, as intermediates. Second, the formation of selenenyl sulfides is not necessary to establish the catalytic cycle, and the competing formation of such intermediates can even comprise a deactivation pathway in the overall process, as seen in Scheme 4. Third, coordinating oxygen substituents even can be more effective than their more widely studied nitrogen analogues in generating highly active catalysts. Finally, the previous neglect of aliphatic selenium compounds as GPx mimetics appears to be unjustified. While simple dialkyl selenides normally show a propensity for selenoxide elimination, we have discovered two types of aliphatic selenides that provide entry into their respective catalytic manifolds by [2,3]sigmatropic rearrangement, ultimately producing a key cyclic seleninate ester intermediate, and by cyclization to spirodialkoxyselenuranes, respectively.

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