COMMUNICATION

Glutathione peroxidase mimics based on conformationally-restricted, *peri*-like 4,5-disubstituted fluorene dichalcogenides

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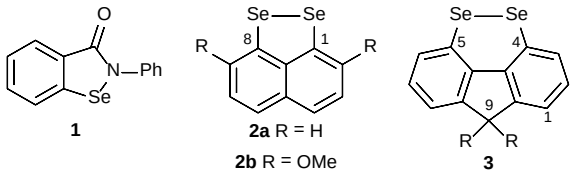
Glutathione peroxidase (GPx) regulates cellular peroxide levels through glutathione oxidation. GPx-mimics based on 4,5-disubstituted fluorene diselenides, their oxides, and ditellurides show catalytic activities comparable to related, conformationally-restricted, 1,8-naphthalene dichalcogenides.

Organoselenium compounds play a central role in biological systems and medicinal chemistry.[1] The selenocysteine-containing enzyme glutathione peroxide (GPx) catalyses the reduction of peroxides through oxidation of the endogenous thiol glutathione to glutathione disulfide.[2] The build-up of reactive oxygen species such as peroxides is associated with certain disease states, and hence small selenium-containing molecules which can mimic the function of GPx have potential in drug-development.[1,2] A wide range of GPx mimics containing diverse selenium functionality has been investigated, with the aminoselenide Ebselen **1** reaching phase 3 clinical trials for a variety of diseases associated with oxidative stress.[3]

Diselenides are promising GPx mimics,[4,5] with even the simple diphenyl diselenide showing two times greater activity than Ebselen.[6] In 2011, Back reported that 1,8-, *peri*-substituted, naphthalene diselenides **2** show an order of magnitude greater GPx-like activity compared with diphenyl diselenide.[7] Restricting the conformation around the diselenide bond to almost planar, as found in **2**, reduces the HOMO-LUMO energy gap and raises the energy of the HOMO compared with conformationally-unrestricted diphenyl diselenide, thereby increases the rate of oxidation of **2** by peroxide in the rate-determining step.

In a search for alternative conformationally-restricted aryl diselenides‡ that show enhanced GPx-like activity and which are amenable to structural variation towards medicinal chemistry applications, we considered the previously unreported 4,5-fluorene diselenides **3**. As with *peri*-substituted 1,8-naphthalenes **2**, the near planarity of fluorene[8] should constrain the geometry of the diselenide bond, and the close proximity of groups in the 4,5-(bay) region should favour Se-Se bond formation. In this paper we report the first investigation into the synthesis and properties of **3**, its mono- and trioxides, the corresponding ditelluride and their GPx-like activity.



1 **2a** R = H **2b** R = OMe **3**

Fig. 1 Selenium-containing GPx mimics: Ebselen **1**, Back's conformationally-restricted 1,8-*peri*-substituted naphthalene diselenides **2** and proposed conformationally-restricted 4,5-disubstituted fluorene diselenides **3** in this study.

Fluorene diselenides **3a** (R = Me) and **3b** (R = Bu) were synthesized from fluorenes **4a** and **4b**, through quenching the 4,5-dilithiofluorene species, generated using BuLi-TMEDA,[9] with elemental selenium (Scheme 1). Diselenide oxidation was investigated, in view of selenium oxides showing potential GPx-like activity, and to compare their behaviour with the analogous 5-membered ring naphthalene bis-selenium species reported by Kice (**2a**)[10] and Back (**2b**).[7] Oxidation with 1.2 equivalents of *m*CPBA in Et_2O gave selenolselinates **5a** and **5b** along with recovered starting material. We did not see any evidence of formation of the symmetrical selelenic anhydride in these mono-oxidations, in contrast to the oxidation of **2a**, where a mixture of isomeric monoxides is observed.[10] Use of a larger excess (3.5 equivalents) of *m*CPBA resulted in the precipitation of seleninic anhydrides **6** as single stereoisomers in excellent 85-95% yields. These were assigned as the *trans*, C_2-symmetric stereoisomers, rather than the alternative *cis*, *meso* structures, on the basis of the equivalent Me groups at C-9 in the ^{1}H and ^{13}C NMR of **6a**. Treatment of **6a** with KOH in CD_3OD formed the dipotassium salt of the bis-seleninic acid **7a**, evidenced by Se NMR, which upon acidification returned the same stereoisomer **6a** in 86% yield, suggesting the *trans*-isomer is thermodynamic preferred. In contrast, naphthalenes **2** give mixtures of diastereomeric seleninic anhydrides in both selenium oxidation and in base-mediated ring-opening - acidification.[7,10] The ditellurides **8a** and **8b** were also prepared from fluorenes **4a** and **4b** using tellurium as the quench for the dilithio species.

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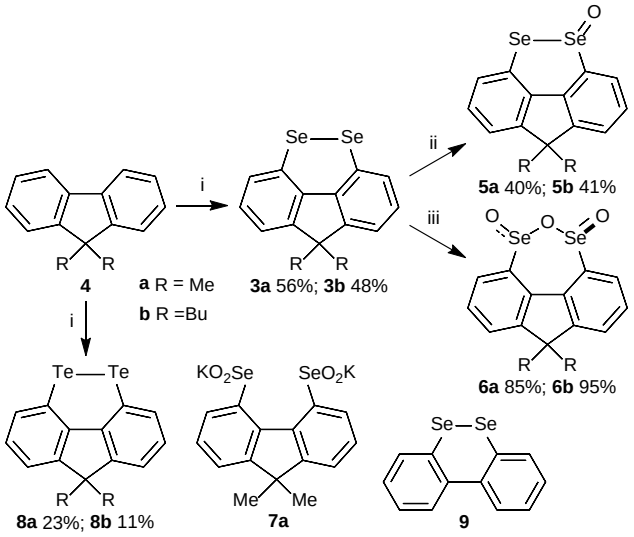
The X-ray crystal structure of **3a** is shown in Figure 2 and that of **3b** (four independent molecules in the unit cell) in the ESI (fig S1-S5).[11] In contrast to the essentially planar naphthalene diselenides **2a** and **2b** (C-Se-Se-C dihedral angle **2a**: –2.28(13),[12] **2b**: –1.50 (7)[13]), diselenides **3** are non-planar (C4-Se1-Se2-C5 dihedral angle **3a** –41.35 (12); **3b** average 42.3(20)§) and cause a twist in the fluorene plane (C4-C11-C12-C5 dihedral angle **3a** -10.9(4); **3b** average 11.3(15)§).[14] This C-Se-Se-C dihedral angle is still much smaller than in the conformationally unconstrained diphenyl diselenide (85.4(2), –85.5(3))[15] and in the less constrained biaryl diselenide, dibenzo[c,e][1,2]diselenine (**9**, Scheme 1) (59.0(3), –59.0(4), -57.0(4)).[16] The Se-Se bond length in **3b** is 2.34416(4) Å, shorter than naphthalene diselenides **2a** (2.3639(5) Å) and **2b** (2.3552(3) Å), but longer than in diphenyl diselenide (2.3066(7) Å; 2.3073(10) Å) and **9** (mean length 2.323(2) Å).



Scheme 1 Synthesis of 4,5-substituted fluorene diselenides **3**, selenolseleninates **5**, seleninic anhydrides **6** and ditellurides **8**, and structures of dipotassium salt of bis-seleninic acid **7a** and related biaryl diselenide **9**. Reagents and Conditions: (i) *n*-BuLi (4 equiv), TMEDA (4 equiv), 60 °C, 4 h, then Se or Te (8 equiv), THF, –78 °C-rt. (ii) *m*CPBA (1.2 equiv), Et_2O, 15 min. (iii) *m*CPBA (3.5 equiv), Et_2O, 15 min.

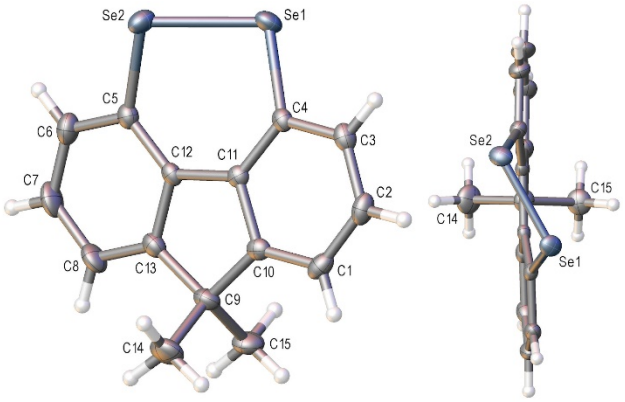


Fig. 2 Two views of the crystal structure of diselenide **3a** with ellipsoids drawn at the 50 % probability level.

The GPx-like catalytic activities of diselenides **3**, selenolseleninates **5**, seleninic anhydrides **6** and ditellurides **8** were determined using Iwoka's NMR assay,[16] which monitors the drop in concentration of dithiotheritol (**DTT^{red}**) as it is oxidized to the disulfide **DTT^{ox}** over time (Figure 3). A solvent system of 2:1 CD_3OD:$CDCl_3$ was used to maintain solubility of all components and hence compare catalytic activity under homogenous conditions, although rates in this solvent system are much slower than in the original report of D_2O.[17] The times taken for the initial concentration of **DTT^{red}** to halve (T_{50}), after addition of H_2O_2 are shown in Table 2. T_{50} allows catalysts to be compared where there is a rapid initial reaction, as is the case herein for selenolseleninates and seleninic anhydrides, prior to addition of H_2O_2. Back's naphthalene diselenide **2b**, wherein the electron-donating *ortho*-OMe groups were shown to increase catalytic activity over the non-substituted **2a**, was also included, along with a background reaction (no catalyst).

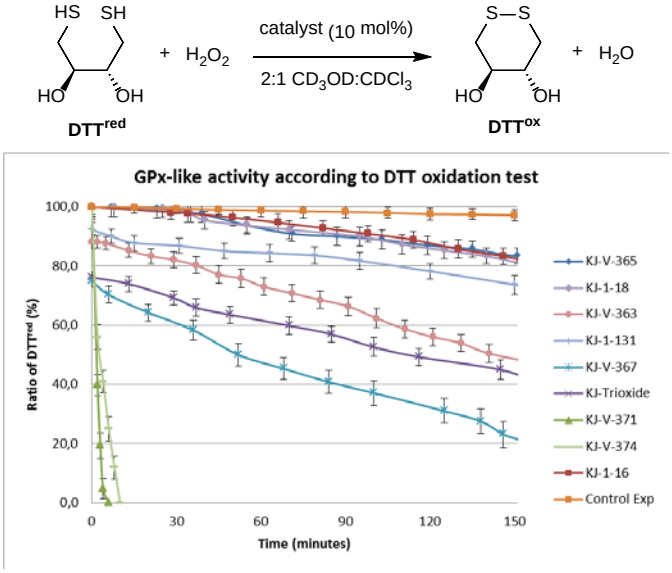


Fig. 3 Oxidation of **DTT^{red}** with H_2O_2 in the presence of selenium- or tellurium-containing catalysts (10 mol%). Reaction conditions: $[DTT^{red}]^0$ = 0.14 M, $[H_2O_2]^0$ = 0.14 M, [catalyst] = 0.014 M, 2:1 CD_3OD/$CDCl_3$ solution (0.6 mL). Reaction progress monitored by ^{1}H NMR. The mean (±) SD values of three separate experiments are reported.

Table 1 GPx-like activity of chalcogen-containing catalysts

Entry	Catalyst	Initial **DDT^{red}** (%)[a]	T_{50} (mins)[b]
1	**2b**	100	>300
2	**3a**	100	>300
3	**3b**	100	>300
4	**5a**	88	141 (±9)[c]
5	**5b**	92	253 (±17)
6	**6a**	75	52 (±8)
7	**6b**	75	105 (±11)
8	**8a**	100	<3
9	**8b**	100	<3

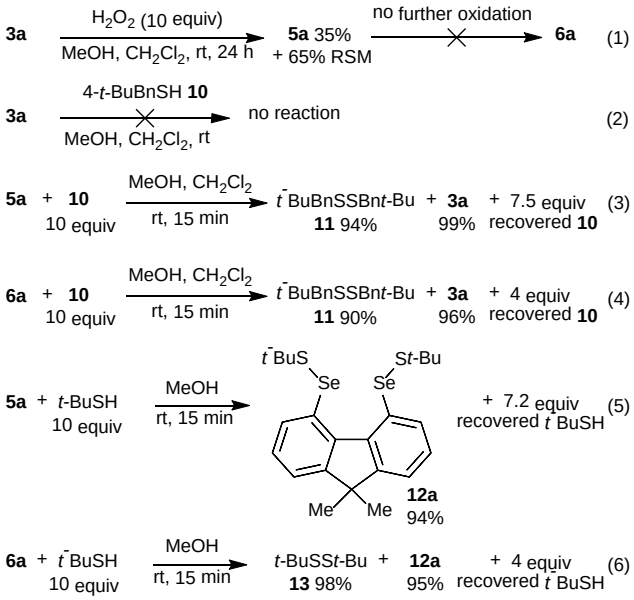
[a] After addition of 10 mol% catalyst before addition of H_2O_2; [b] T_{50} is the time required to halve the initial thiol concentration after the addition of H_2O_2; [c] data in parenthesis are the experimental error.

All of the selenium- and tellurium-containing compounds **3**, **5**, **6** and **8** catalyse the oxidation of **DTT^{red}** to **DTT^{ox}**. Delenides **3** have comparable activities to the naphthalene diselenide **2b** in this assay, despite lacking activating *ortho*-OMe substituents (Fig 1 and table 1, entries 1-3). The selenolseleninates **5** have shorter T_{50} than the corresponding diselenides **3** (table 1, entries 4 and 5). Before adding H_2O_2, approximately 10% of **DTT^{ox}** was detected, pointing to an initial fast reaction that occurs prior to the first NMR reading under these homogenous conditions. A more extensive initial reaction occurs with trioxides **6**, with approx. 25% **DTT^{ox}** detected, contributing to the overall shorter T_{50} (entries 6 and 7). In general, 9-dimethyl-substituted fluorenes catalyse the oxidation of **DTT^{red}** faster than the butyl-substituted systems (compare entries 4 vs 5, and entries 6 vs. 7). The ditellurides **8a** and **8b** were two orders of magnitude faster catalysts that the corresponding diselenides **3a** and **3b** (entries 8 and 9), with reactions complete within minutes of adding H_2O_2. However it should be noted that that tellurium-containing compounds can catalyse the further oxidation of disulfides[7,17] and hence may have adverse biological activity.

In order to gain further mechanistic insight into the catalytic cycle, stoichiometric reactions of selenium-containing catalysts were carried out (Scheme 2). Treatment of diselenide **3a** with a large excess (10 equiv.) of H_2O_2 in 2:1 MeOH:CH_2Cl_2 at room temperature gave slow oxidation to monoxide **5a** (Scheme 3, equation 2). No higher oxides were detected, and independent treatment of selenolseleninate **5a** or seleninic anhydride **6a** with H_2O_2 under these conditions gave no reaction, suggesting **6a** is not an intermediate in the catalytic cycle.

Diselenide **3a** does not react with 4-*t*-butylbenzylthiol (**10**) in CH_2Cl_2/MeOH at room temperature (Scheme 2, equation 2). However reaction of selenolseleninate **5a** with 10 equivalents of **10** gave an essentially instantaneous and quantitative transformation to diselenide **3a** and disulfide **11** (94% based on **5a**) along with 75% recovery of the thiol, showing **5a** consumes two equivalents of thiol (equation 3). Under the same conditions, seleninic anhydride **6a** underwent a similarly rapid and high-yielding transformation to **3a** and **11** (equation 4), where the 90% yield of **11** is based on theoretical consumption of 6 molar equivalents of thiol **10** and recovery of 4 equivalents of **10** (proposed intermediates and stoichiometries in the reactions of **5a** and **6a** with thiols are shown in the ESI, schemes S1-S2).

No intermediate bis-selenium species were observed in the reactions of **5a** and **6a** with **10**. However, reaction of **5a** with the bulkier thiol, *t*-BuSH, gave the bis-selenenyl sulfide **12a** (equation 5), a potential intermediate in the formation of **3a** (ESI, Scheme S1). Indeed, isolated **12a** is slowly transformed over 24 h in solution to diselenide **3a** and di-*tert*-butyldisulfide (**13**). This rate of this reaction is not changed by addition of 3 equivalents of 4-*t*-butylbenzylthiol (**10**), and no disulfides derived from **10** were formed, only **13**. The breakdown of bis-selenenyl sulfide **12a** to diselenide **3a** and disulfide **13** is thus presumably intramolecular, but given the steric hindrance provided by the *t*-Bu group, care should be taken in extrapolating these observations to all thiols. Kice reported a similar reaction of *t*-BuSH with the monoxides of naphthalene diselenide **2a** to give isolable 1,8-bis[(*tert*-butylthio)seleno]naphthalene, which led Back to propose bis-selenenyl sulfides as intermediates in the catalytic cycle of **2b**.

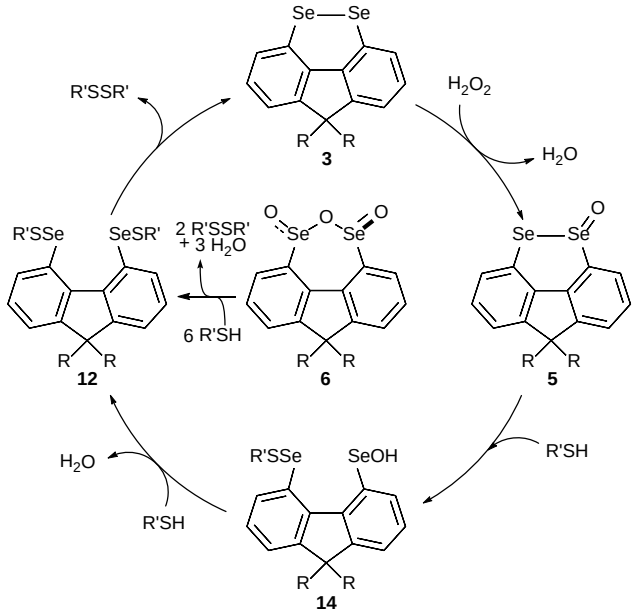


Scheme 2 Mechanistic investigations

The reaction of seleninic anhydride **6a** with *t*-BuSH also gave **12a** (equation 6), though clearly there are multiple intermediates preceding its formation (ESI, Scheme S2). These intermediates account for the formation of *tert*-butyldisulfide (**13**) (98% based on theoretical amount of *t*-BuSH consumed and recovery of exactly 4 equivalents of thiol), whereas **13** is not observed in the reaction of selenolseleninate **5a** with *t*-BuSH.§§

Based on the above observations, a catalytic cycle directly analogous to that proposed by Back for naphthalane diselenides **2** is proposed (Scheme 3): this cycle is mechanistically distinct from catalysis by other diselenides, which involve initial Se-Se bond cleavage by reaction with thiols.[20] The rate-determining step is the oxidation of diselenide **3** to selenolseleninate **5**, which in turn rapidly consumes two equivalents of thiol and forms disulfides via the intermediates **14** (not observed) and **12** (observed as **12a** for R = Me, R' = *t*-Bu). As noted above, the conversion of **12** to **3** may occur by more than one mechanism and may also be catalysed by thiol: this step is severely slowed in the case of R = *t*-Bu where nucleophilic attack at sulfur is restricted and where an intramolecular mechanism appears most likely. Both **5** and **6** act as pre-catalysts, circumventing the rate-determining oxidation, which, in the **DDT** NMR assay, results in an overall shorter T_{50}. The initial rapid reaction of **5** and **6** with **DTT^{red}** is evident in figure 1. Consumption of 1 equivalent of dithiol **DTT^{red}** (2 × SH) with the 10 mol% of catalysts **5a** and **5b** present at the start of the assay should lead to an immediate 10% reduction in the amount of **DTT^{red}**, which is consistent with the approx. 10% observed initial **DTT^{red}** (table 1, entries 4

and 5). Similarly, rapid consumption of 3 equivalents of **DDT^{red}** (6 x SH) with the starting 10 mol% of catalysts **5a** and **5b** should give a theoretical 30% reduction in the amount of **DDT^{red}**, with approx. 25% reduction observed in practice (entries 6 and 7).



Scheme 3 Proposed catalytic cycle for oxidation of thiols to disulfides

In conclusion, bay-substituted 4,5-fluorene diselenides **3** possess properties analogous to *peri*-substituted 1,8-naphthalene diselenides **2**, including increased GPx-like activity over non-conformationally constrained diselenides. Despite a greater twist in the diselenide bond, the catalytic activity of fluorenes **3a** and **3b** is similar to that of naphthalene **2b** in a homogenous **DDT** redox assay, without the need for additional activation by *ortho*-OMe groups on the aromatic rings. Moving forward, the fluorene scaffold is anticipated to be amenable to structural variation through incorporation of different groups at C-9, for example towards water-soluble GPx mimics[19,21] and application in other enzyme mimics based on naphthalene dichalcogenides.[13,22] The ease of synthesis and reactivity of seleninic anhydrides such as **6** may also hold promise in situations where rapid oxidation of thiols to disulfides may be required.

There are no conflicts to declare.

Notes and references

‡ aryl selenides are less toxic than alkyl selenides. See ref 3a.

§ For **3b** the average value calculated from molecules 1-3 for each parameter is given (see Table S1, ESI for further discussion).

§§ Ph_3CSH and 1-adamantylthiol did not produce isolable bis-selenenyl sulfides, but, like thiol **10**, gave the corresponding disulfide and diselenide **3a** directly from **6a** (see ESI).

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