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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 consistent with conformational restriction about the dichalcogen bond.

Organoselenium compounds play a central role in biological systems and medicinal chemistry.[1] The selenocysteine-containing enzyme glutathione peroxidase (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 (Fig. 1).[2,3]

Diselenides are promising GPx mimics,[4–7] 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 (Fig. 1).[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.
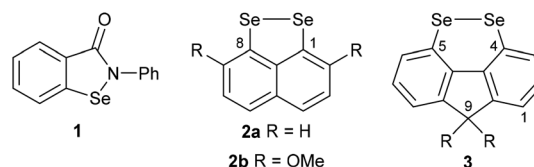

Fig. 1 Selenium-containing GPx mimics: Ebselen **1**, Back's conformationally-restricted 1,8-*peri*-substituted naphthalene diselenides **2** and proposed conformationally-restricted 4,5-disubstituted fluorene diselenides **3** in this study.

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** (Fig. 1). 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 dichalcogen bond formation.[9] 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.

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 Et₂O gave selenolseleninates **5a** and **5b** along with recovered starting material. We did not see any evidence of formation of the symmetrical selenenic 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* struc-

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†Electronic supplementary information (ESI) available: Experimental procedures and analytical data, copies of NMR spectra and X-ray crystallography for **3a** (CCDC 2099301) and **3b** (CCDC 1470801). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1ob02153b

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tures, 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$_3$OD formed the dipotassium salt of the bis-seleninic acid **7a**, evidenced by ^{77}Se NMR, which upon acidification returned the same stereo-isomer **6a** in 86% yield, suggesting the *trans*-isomer is thermo-dynamic 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 ditel-lurides **8a** and **8b** were also prepared from fluorenes **4a** and **4b** using tellurium as the quench for the dilithio species.

The X-ray crystal structure of **3a** is shown in Fig. 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 naphtha-lene 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 confor-mationally 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).

The GPx-like catalytic activities of diselenides **3**, selenolsele-ninates **5**, seleninic anhydrides **6** and ditellurides **8** were deter-mined using Iwoka's NMR assay,[17] which monitors the drop in concentration of dithiotheritol (**DTT**[red]) as it is oxidized to the disulfide **DTT**[ox] over time (Fig. 3). A solvent system of 2 : 1 CD$_3$OD : CDCl$_3$ was used to maintain solubility of all com-
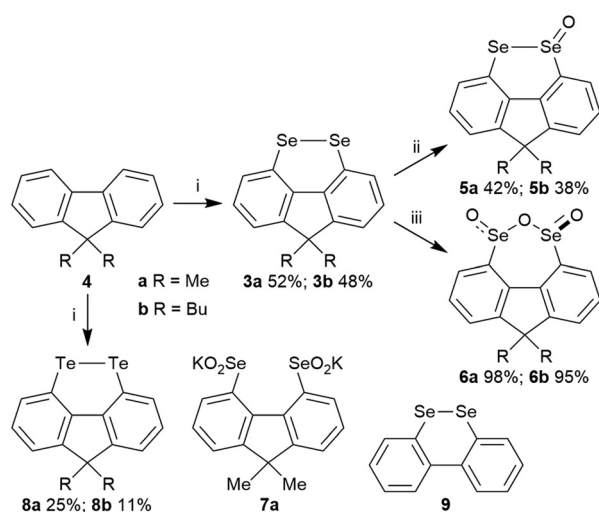
Scheme 1 Synthesis of 4,5-disubstituted fluorene diselenides **3**, sele-nolseleninates **5**, seleninic anhydrides **6** and ditellurides **8**, and struc-tures of dipotassium salt of bis-seleninic acid **7a** and related biaryl dise-lenide **9**. Reagents and conditions: (i) *n*-BuLi (4 equiv.), TMEDA (4 equiv.), 60 °C, 4 h, then Se or Te (8 equiv.), THF, −78 °C–rt. (ii) *m*CPBA (1.2 equiv.), Et$_2$O, 15 min. (iii) *m*CPBA (3.5 equiv.), Et$_2$O, 15 min.
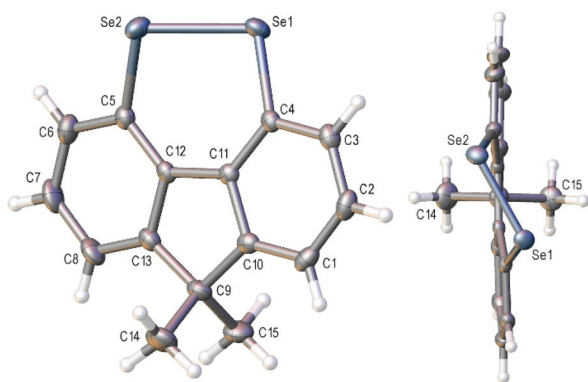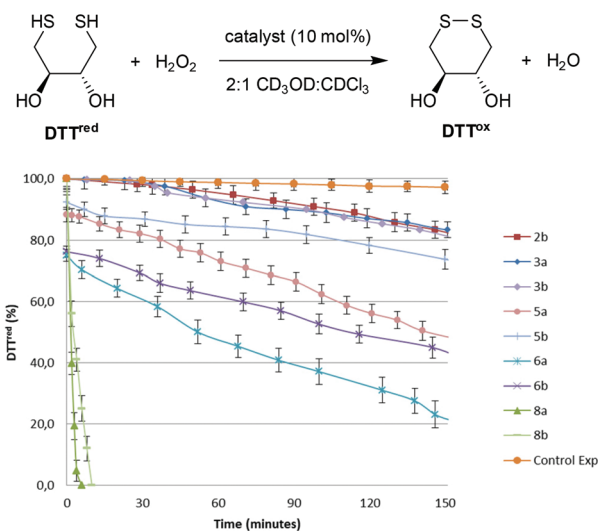

Fig. 3 Oxidation of **DTT**[red] with H$_2$O$_2$ in the presence of selenium- or tellurium-containing catalysts (10 mol%). Reaction conditions: [DTT[red]]^0 = 0.14 M, [H$_2$O$_2$]^0 = 0.14 M, [catalyst] = 0.014 M, 2 : 1 CD$_3$OD/CDCl$_3$ solution (0.6 mL). Reaction progress monitored by ^{1}H NMR. The mean (±) SD values of three separate experiments are reported.

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

Table 1 GPx-like activity of chalcogen-containing catalysts

Entry	Catalyst	Initial **DDT**[red] [a] (%)	T_{50}[b] (min)
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$_2$O$_2$. [b] T_{50} is the time required to halve the initial thiol concentration after the addition of H$_2$O$_2$. [c] Data in parenthesis are the experimental error.

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ponents 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.[18] The times taken for the initial concentration of $\mathbf{DTT^{red}}$ to halve (T_{50}), after addition of H_2O_2 are shown in Table 1. 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).

All of the selenium- and tellurium-containing compounds **3**, **5**, **6** and **8** catalyse the oxidation of $\mathbf{DTT^{red}}$ to $\mathbf{DTT^{ox}}$. Diselenides **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 $\mathbf{DTT^{ox}}$ 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% $\mathbf{DTT^{ox}}$ detected, contributing to the overall shorter T_{50} (entries 6 and 7). In general, 9,9-dimethyl-substituted fluorenes catalyse the oxidation of $\mathbf{DTT^{red}}$ faster than the butyl-substituted systems (compare entries 4 *vs.* 5, and entries 6 *vs.* 7). Oxidation using ditellurides **8a** and **8b** is two orders of magnitude faster that the corresponding diselenides **3a** and **3b** (entries 8 and 9), with reactions complete within minutes of adding H_2O_2.[19]

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 2, eqn (1)). 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.[20]

Diselenide **3a** does not react with (4-(*tert*-butyl)phenyl)methanethiol (**10**)[21] in CH_2Cl_2/MeOH at room temperature (Scheme 2, eqn (2)). However reaction of selenolseleninate **5a** with 10 equivalents of **10** gave an essentially instantaneous and quantitative transformation to diselenide **3a** and disulfide **11** along with 75% recovered thiol **10**, (eqn (3)). Under the same conditions, seleninic anhydride **6a** underwent a similarly rapid and high-yielding transformation to **3a** and **11** (eqn (4)), where the 90% yield of **11** is based on theoretical consumption of 6 molar equivalents of thiol **10** (stoichiometries in the reactions of **5a** and **6a** with thiols are shown in the ESI, Scheme S1†).

No intermediate bis-selenium species were observed in the reactions of **5a** and **6a** with thiol **10**. However, reaction of **5a** with the bulkier thiol, *t*-BuSH, gave the bis-selenenyl sulfide **12a** (Scheme 2, eqn (5)), a potential intermediate in the formation of **3a**. Indeed, isolated **12a** is slowly transformed over 24 h in
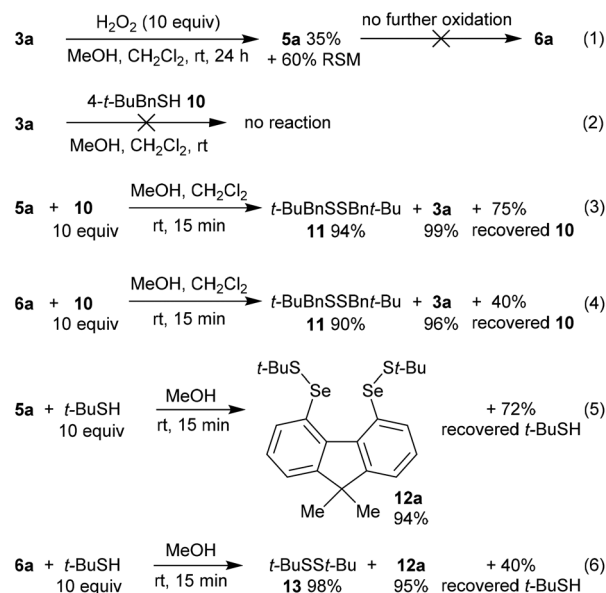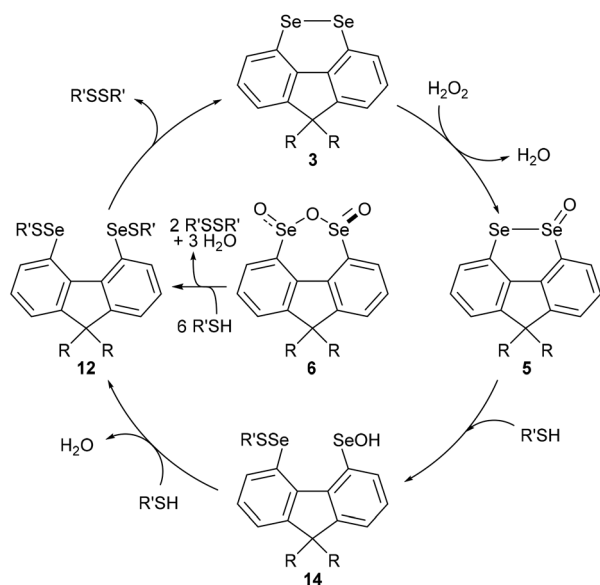
Scheme 2 Mechanistic investigations. % yields of recovered thiol are based on theoretical consumption.

solution to diselenide **3a** and di-*tert*-butyl disulfide (**13**). This rate of this reaction is not changed by addition of 3 equivalents of thiol **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,[10] which led Back to propose bis-selenenyl sulfides as intermediates in the catalytic cycle of **2b**.[7]

The reaction of seleninic anhydride **6a** with *t*-BuSH also gave bis-selenenyl sulfide **12a** (Scheme 2, eqn (6)), though clearly there are multiple potential intermediates preceding its formation. These intermediates account for the formation of disulfide **13** (98% based on theoretical amount of *t*-BuSH consumed and consistent with recovery of 4 equivalents of thiol, Scheme S1†), 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 naphthalene diselenides **2** is suggested[7] (Scheme 3): this cycle is mechanistically distinct from catalysis by other diselenides, which involve initial Se–Se bond cleavage by reaction with thiols.[22] 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. Oxides **5** and **6** initially circumvent the rate-determining oxidation, resulting in overall

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Scheme 3 Proposed catalytic cycle for oxidation of thiols to disulfides.

shorter T_{50} in the **DDT** NMR assay. The initial rapid reaction of **5** and **6** with **DTT^{red}** is evident in Fig. 3. 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 × 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).

Conclusions

In conclusion, readily synthesized, bay-substituted 4,5-fluorene diselenides **3** possess properties analogous to *peri*-substituted 1,8-naphthalenes **2**, including increased GPx-like activity compared with 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 particularly amenable to structural variation through incorporation of different functionality at C-9, for example towards water-soluble GPx mimics,[18,23] and the close proximity of groups in the 4,5-bay positions may be exploited in other applications based on 1,8-*peri*-substituted naphthalenes.[24,25]

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ Aryl selenides are less toxic than alkyl selenides. See ref. 3*a*.
§ For **3b** the average value calculated from molecules **1**–**3** for each parameter is given (see ESI†).
¶ Ph$_3$CSH and 1-adamantylthiol gave the corresponding disulfide and diselenide **3a** directly from **6a** (see ESI†).

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