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The Design and Properties of Organoselenium Compounds with Glutathione Peroxidase-Like Activity

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The Design and Properties of Organoselenium Compounds with Glutathione Peroxidase-Like Activity

by

David James Press

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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Abstract

The selenoenzyme glutathione peroxidase protects against oxidative stress by reducing peroxides in the presence of glutathione, a tripeptide thiol. Under conditions of extreme oxidative stress, for example during ischemic reperfusion, this process can become overwhelmed. Glutathione peroxidase mimetics are organoselenium compounds which catalytically destroy peroxides and can be used to treat oxidative stress associated with ischemic reperfusion and related conditions. The majority of this Thesis describes the design of efficacious glutathione peroxidase mimetics.

An investigation of substituent effects upon the glutathione peroxidase-like activity of aromatic cyclic seleninate esters and spirodioxyselenuranes was initiated. It was found that para-substitution with electron-donating groups resulted in the greatest increase in catalytic activity. Hammett analysis established that the rate-determining step in each compound’s catalytic cycle was the oxidation of Se(II) to Se(IV). Overall, para-methoxy substitution caused the greatest increase in thiol peroxidase activity, while dimethoxy and trimethoxy substitution did not ensure superior thiol peroxidase activity. Included in this study were 3-hydroxypropyl and 2,3-dihydroxypropyl (2-hydroxymethyl)phenyl selenides, which are new classes of glutathione peroxidase mimetics that displayed strong peroxide destroying activity. The former class of compounds includes the most active organoselenium glutathione peroxidase mimic prepared in our group to date, while members of the latter class are sufficiently water soluble to allow for their activity to be monitored in aqueous environments. Additionally, naphthalene peri-diselenides were found to have significantly improved thiol peroxidase activity relative to acyclic diselenides. This was due to the severely reduced dihedral
angle found in the naphthalene peri-diselenides, which leads to a destabilized ground state, lower ionization potential and an increased rate of reaction with peroxides. These diselenides also produced stable charge-transfer complexes with tetracyanoquinodimethane that are of potential interest as photovoltaic materials.

Finally, configurational stability of the cyclic seleninate esters and spirodioxselenuranes was investigated by variable-temperature NMR spectroscopy. These compounds were configurationally stable at high temperatures, while the spirodioxselenuranes displayed unexpected proton NMR behaviour, caused by temperature-dependant chemical shifts.
Acknowledgements

First and foremost, I would like to thank my supervisor, Professor Thomas G. Back. Without his guidance and encouragement this work would not have been possible. Professor Back allowed me the freedom to pursue avenues of research that neither of us fully predicted nor appreciated at the beginning of my studies.

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For my Grandparents;

Grandpa Press, the playing Grandpa, who was an early model and mentor

Grandma Waddell, who pointed out that those we love never leave, and often proves it so

Grandpa Waddell, who ‘wasn’t bad, for an old man’, to the last breath

Grandma Press, who is loved dearly and still lives in a place of personal refuge
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<table>
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<tr>
<th>Symbol</th>
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<tr>
<td>$^{125}$Te NMR</td>
<td>tellurium-125 nuclear magnetic resonance</td>
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CuZnSOD  copper-zinc superoxide dismutase
δ        chemical shift in ppm
Δν       difference in chemical shift
ΔG_{298} Gibbs free energy difference at 298 Kelvin
[D]      concentration of donor
d        doublet
DCM      dichloromethane
dd       doublet of doublets
ddd      doublet of doublets of doublets
ddt      doublet of doublets of triplets
DEPT     distortionless enhancement by polarization transfer
DIO      iodothyronine deiodinase
DIPEA    diisopropylethylamine
DMF      dimethylformamide
DMSO     dimethyl sulfoxide
DNA      deoxyribonucleic acid
dt       doublet of triplets
ε        molar absorptivity
EDG      electron-donating group
EDTA     ethylenediaminetetraacetic acid
EI       electron impact
ESI      electrospray ionization
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<th>Abbreviation</th>
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<td><em>endo</em> hydrogen</td>
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<tr>
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</tr>
<tr>
<td>HO$^+$</td>
<td>hydroxyl radical</td>
</tr>
<tr>
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</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
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<td><em>exo</em> hydrogen</td>
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</tr>
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</tr>
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<td>Kelvin</td>
</tr>
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</tr>
<tr>
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<td>lowest unoccupied molecular orbital</td>
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μg  microgram
M  molarity
m  meta
m  multiplet
m/z  mass to charge ratio
M+  molecular ion
MCPBA  meta-chloroperoxybenzoic acid
mg  milligrams
MHz  mega hertz
min  minutes
mL  millilitres
mmol  millimoles
MnSOD  manganese superoxide dismutase
mol  moles
MOM  methoxymethyl
mp  melting point
mtDNA  mitochondrial deoxyribonucleic acid
n  normal chain
NADP+  nicotinamide adenine dinucleotide phosphate
NADPH  reduced nicotinamide adenine dinucleotide phosphate
NBS  N-bromosuccinimide
Na  alpha amine of amino acid
<table>
<thead>
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<tr>
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<tr>
<td>ORTEP</td>
<td>Oak Ridge Thermal Ellipsoid Plot Program</td>
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<td>3,3',5'-triiodothyronine</td>
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<tr>
<td>$\sigma$</td>
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<tr>
<td>Tc</td>
<td>convergence temperature</td>
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<tr>
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<td>tetracyanoquinodimethane</td>
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<td>tetrahydrofuran</td>
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<td>tryptophan</td>
</tr>
<tr>
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<td>tetrahyafulvalene</td>
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<td>(v_0)</td>
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Chapter One: Introduction

1.1 Brief History of Organoselenium Chemistry

Selenium was first discovered in 1818 by the Swedish chemist Jön Jacob Berzelius\(^1\) upon examining a foul-smelling red mud found in the lead chambers of a Swedish sulfuric acid factory. The name selenium was chosen to reflect Selene, the goddess of the moon, a parallel to the naming of the related element tellurium which derives its name from the Latin *tellus* (Earth). The first organoselenium compound was reported by Löwig\(^2\) in 1836 when he prepared diethyl selenide. Progress in organoselenium chemistry was very slow for over a century as these compounds are notoriously malodorous and are sometimes sensitive to oxygen and light. Even with the advent of modern techniques for stabilizing and handling these compounds, advances were slow in the first half of the 20\(^{th}\) century due to the demonstrated toxicity of inorganic selenium compounds and presumed toxicity of organoselenium compounds. It was not until the 1970s that organoselenium chemistry really gained interest, driven by the utility of various selenium reagents and reactions in synthesis. The observation that selenoxides with \(\beta\)-hydrogen atoms could decompose into olefins under mild conditions\(^3\) led a number of groups to investigate this reaction further. The versatility of this transformation, now known as selenoxide *syn*-elimination, and related reactions was demonstrated in the 1970s by Sharpless,\(^4\) Clive\(^5\) and Reich.\(^6\) The study of selenium in biochemistry started to develop significantly around the time of these initial forays into the use of selenium in synthesis.\(^7\) Organoselenium chemistry has now grown into a large field with many subdisciplines. For example, the polarizable nature of selenium allows for organoselenium reagents to be used as nucleophiles\(^8\) or as electrophiles.\(^9\)
Additionally, the weak carbon–selenium bond makes organoselenium reagents a reliable source of radicals. These features have resulted in a wide array of selenium reagents for use in synthesis. Organoselenium reagents also find application in enantioselective reactions, ligand design and catalysis, as well as conducting materials and semiconductors.

The work described in this Thesis involves the design and synthesis of organoselenium reagents for use as biological antioxidants. These compounds emulate the glutathione peroxidases, a class of enzymes known to contain selenium and used by higher organisms to destroy harmful peroxides. For this purpose, small molecule organoselenium compounds have been investigated and have been shown to act as glutathione peroxidase (GPx) mimetics. Application of such compounds involves the treatment of oxidative stress, especially as related to ischemic reperfusion of stroke and heart attack patients. Oxidative stress can also lead to disease states such as dementia, cardiovascular damage, mutagenesis and cancer if left untreated. We wished to design more efficacious GPx mimetics for evaluation as antioxidants for preventing oxidative stress. This involved studying the effect of electron-donating and electron-withdrawing substituents upon two classes of GPx mimetics that our group had designed. A relationship was defined which showed that electron-donating groups improved catalytic efficiency for all classes of GPx mimetics studied. Further efforts focussed on the preparation of effective and potentially non-toxic GPx mimetics to be tested in an in vitro assay. In order to place this work in an appropriate literature context, Section 1.2 describes relevant biochemistry of selenium, Section 1.3 discusses the toxicity of
organoselenium compounds, Section 1.4 provides a review of GPx mimetics and Section 1.5 summarizes in vitro assays currently used by research groups for testing the antioxidant activity of GPx mimetics

1.2 Aspects of Selenium in Biology and Biochemistry

1.2.1 Background

Initial evidence in the 1930s suggested that selenium present in the diet of animals could be deleterious. Farm animals and laboratory rats fed grain from the same area in South Dakota displayed disease to the central nervous system and liver, and investigations suggested that selenium incorporated into the grain was the culprit. For a number of years selenium was considered to be an environmental toxin and the origin of its toxicity was researched. On the other hand, in the 1950s, selenium was shown to be a component of the dietary agent Factor 3, which was known to protect against necrotic degeneration of various organs. In 1973 selenium was presented by Ganther and coworkers as being involved in the glutathione-mediated metabolism of hydroperoxides. Their work suggested that selenium might be the active constituent of an enzyme responsible for this metabolic pathway. Later that same year, Flohé and coworkers determined, through neutron activation analysis, that selenium was indeed a component of the enzyme glutathione peroxidase. Contrary as the early suggestion of toxicity and the apparent dietary requirement of selenium seem, it is now accepted that selenium, although toxic in high doses, is an essential dietary trace element. The American Institute of Medicine set a minimum recommended dietary allowance for selenium at 55 µg per day for adults, with an upper limit of safe consumption at 400 µg.
per day.\textsuperscript{21} Forms of selenium present in the diet include: selenomethionine \textbf{1},
selenocysteine \textbf{2}, 2-selenyl-\textit{N},\textit{N},\textit{N}-trimethylhistidine \textbf{3}, \textit{Se}-methylselenocysteine \textbf{4}, \textit{γ}-glutamyl-\textit{Se}-methylselenocysteine \textbf{5}, sodium selenite \textbf{6} and sodium selenate \textbf{7} (see Figure 1.1).\textsuperscript{22}

![Chemical structures of selenium compounds](image)

**Figure 1.1 Major Dietary Sources of Selenium**

Best known for its role as an antioxidant\textsuperscript{23} in the glutathione peroxidases and for catalysing the production of active thyroid hormone (iodothyronine deiodinases), selenium has been identified as an active component of twenty-five enzymes within the human proteome.\textsuperscript{24} In addition to the glutathione peroxidases (an enzyme family containing seven known proteins, five of which contain selenium) and the three identified deiodinases, the remaining seventeen selenoproteins are the 15-kDa selenoprotein, thioredoxin reductases 1 and 2, thioredoxin glutathione reductase, selenophosphate synthetase 2 and selenoproteins H, I, K, M, N, O, P, R, S, T, V and W. The activity of the deiodinases will be briefly covered in the next subsection and glutathione peroxidases will be covered in Subsection 1.2.4.
1.2.2 Iodothyronine Deiodinases

The first iodothyronine deiodinase (DIO1) was discovered to be a selenoprotein in 1990. This was followed later that decade by the characterization of DIO2 and DIO3 as selenoproteins. Deiodinases are responsible for regulating the hormonal activity of the thyroid by cleaving specific iodine – carbon bonds present in these hormones, which regulate normal growth along with basal metabolism. The thyroid gland has the highest level of selenium content of all the organs due to the presence of both the deiodinases and the glutathione peroxidases. Specifically the prohormone thyroxine (T4) is deiodinated at the 5’ position by DIO1 and DIO2 to produce 3,5,3’-triiodothyronine (T3). In addition, DIO1 and DIO3 can convert thyroxine into 3,3’,5’-triiodothyronine (reverse T3, rT3). Both T3 and rT3 can undergo further deiodination to form primarily 3,3’-diiodothyronine (T2) via DIO1 or DIO3 and DIO1 or DIO2 respectively. These processes are illustrated in Scheme 1.1.

Scheme 1.1
1.2.3 Oxidative Stress and Antioxidants

Of special relevance to this Thesis is the enzyme family of the glutathione peroxidases (GPx) and their role as biological antioxidants. It should be noted that glutathione peroxidases are present in all eukaryotic and also in some prokaryotic organisms.\textsuperscript{30} Specifically, GPx catalyses the reduction of lipid and hydrogen peroxides in the presence of the tripeptide glutathione, which acts as a stoichiometric reductant. As peroxides are part of a group of small molecules known as reactive oxygen species (ROS) it is prudent to discuss their relationship to one another and to the antioxidant defence mechanisms of living organisms.

An accumulation of ROS and their interaction with biomolecules within a living system is known as oxidative stress.\textsuperscript{31} Over time, this condition can lead to a variety of disease states including mutagenesis and cancer, atherosclerosis, other cardiovascular disease, neurological damage and dementia. Reactive oxygen species are also a significant component of the aging process.\textsuperscript{32} A major contributor to the formation of ROS is the reduction of oxygen by the mitochondrial electron transport chain during aerobic metabolism.\textsuperscript{33} Under normal conditions, reduction of oxygen to two molecules of water occurs as a four electron process in complex IV of the electron transport chain. It is during the transport of electrons towards complex IV (via enzyme complexes I, II and III) that inadvertent leakage of electrons results in the single electron reduction of oxygen to superoxide anion ($\text{O}_2^-$). Within the mitochondria, superoxide can be reduced further by superoxide dismutase (SOD) to hydrogen peroxide ($\text{H}_2\text{O}_2$).\textsuperscript{34} It is estimated that via these pathways, up to 2\% of all oxygen molecules consumed are converted into $\text{O}_2^-$ and finally
H₂O₂. An additional source of hydrogen peroxide, although not a result of respiration, are the monoamine oxidases found in the outer mitochondrial membrane. Finally, hydrogen peroxide can be reduced in the presence of transition metals to produce hydroxyl radical (HO’), one of the most reactive radicals known in nature. This occurs by the process known as the Fenton reaction (Equation 1), where one molecule of hydrogen peroxide is reduced by Fe(II) to produce HO’, HO’ and the oxidized Fe(III). In addition to other cellular reductants, superoxide anion can actually facilitate this reaction by acting as a reductant to return Fe(III) to Fe(II), while producing a molecule of O₂ (Equation 2). Superoxide is known to cause the release of Fe(II) ions from iron-sulfur clusters found in dehydratases, resulting in increased production of hydroxyl radical.

\[
\begin{align*}
H₂O₂ + Fe(II) & \rightarrow HO^- + HO' + Fe(III) \quad (1) \\
Fe(III) + O₂ & \rightarrow Fe(II) + O₂ \quad (2)
\end{align*}
\]

Other contributors to oxidative stress include peroxynitrite (ONOO⁻) which can be formed by the reaction of superoxide with nitric oxide. Furthermore, hypochlorous acid (HOCl) is generated from hydrogen peroxide and chloride by myeloperoxidases (MPO), while singlet oxygen is produced during the subsequent breakdown of hypochlorous acid by hydrogen peroxide and finally, lipid peroxides are formed by the oxidation of lipids by various ROS (Scheme 1.2).
Mitochondrial biomolecules are especially prone to degeneration by reactive oxygen species. Lipids in the mitochondrial membrane can become oxidized, which leads to increased permeability of the membrane. Amino acids can react with various ROS, resulting in proteins displaying impeded function. Oxidation of DNA can occur within the mitochondria also; in fact, it is known that mitochondrial DNA (mtDNA) undergoes deletions and mutations at a higher rate than nuclear DNA. Damage to mtDNA is of particular concern because this results in damage to the polypeptides encoded by mtDNA which are present in the respiratory chain. This leads to reduced mitochondrial function and integrity, resulting in the release of more ROS, which in turn causes further damage to DNA, proteins and lipids.

There are a number of mechanisms by which organisms respond to ROS, both with dietary antioxidants (e.g. vitamins C and E, polyphenolic compounds) and via enzymatic pathways. As mentioned above, superoxide is converted to hydrogen peroxide by superoxide dismutases (SOD). Two main isozymes are responsible for this
conversion, manganese SOD (MnSOD) and copper-zinc SOD (CuZnSOD). The mitochondrial matrix contains MnSOD which is responsible for the destruction of O$_2^-$ in the matrix and on the interior side of the inner membrane.$^{41}$ The intermembrane space contains CuZnSOD$^{42}$ as the main enzyme for the removal of superoxide. Hydrogen peroxide is converted into two molecules of water by GPx in conjunction with glutathione, or it can be converted into a molecule of oxygen and a molecule of water by catalase. Additionally there are transport proteins which bind redox-active metals, thus preventing these metals from catalysing the formation of HO’ from H$_2$O$_2$.

### 1.2.4 The Glutathione Peroxidases

As mentioned above, hydrogen peroxide and lipid hydroperoxides are reduced to water or alcohol by the glutathione peroxidases. This activity occurs via a catalytic cycle where the selenium centre undergoes a sequence of oxidation and reduction events, respectively facilitated by peroxides and the tripeptide thiol glutathione (8) or other biological thiols.

\[
\begin{align*}
\text{HSe} & \quad \text{HO} \quad \text{NH}_2 \\
\text{HO} & \quad \text{O} \quad \text{NH}_2 \\
\hline
\text{2} & \quad \text{8}
\end{align*}
\]

There are currently seven known human glutathione peroxidases, five of which contain the amino acid selenocysteine (2) while GPx5 and GPx7 contain no selenium residue at all. The first selenoprotein identified was GPx1,$^{20}$ which is also known as cytosolic GPx or classical GPx. This homotetrameric isozyme is expressed in the liver
and erythrocytes and is known to reduce hydrogen peroxide and, under some circumstances, fatty acid hydroperoxides. The gastrointestinal tract contains an epithelium-specific isozyme known as GI-GPx or GPx2 which is also present in the liver in humans. Its location suggests that it is a first line of defence against intestinal organic peroxides in addition to the activity that it displays against hydrogen peroxide. A glycosylated GPx known as plasma GPx (p-GPx) or GPx3 is found in extracellular compartments of the tissues where it is expressed (predominantly the kidney). The concentration of glutathione in the plasma is low enough that reduction is expected to occur via electron donation from thioredoxin. A monomeric glutathione peroxidase known as GPx4 is expressed ubiquitously through a variety of tissues. As GPx4 is the only GPx known to reduce phospholipid hydroperoxides it is also referred to as PH-GPx. In addition to reducing these substrates, GPx4 catalyses the reduction of a number of lipid peroxides, including cholesterol hydroperoxides and thymine hydroperoxide. It is also unique in its ability to reduce hydroperoxides still integrated in membranes. Under conditions of low glutathione concentration, protein thiol groups may be used as reducing agents with GPx4. There is little known about the final selenocysteine-containing GPx except that GPx6 is thought to be restricted to expression in the developing embryo and the olfactory epithelium in adults.

Of the four most studied, GPx1-3 are homotetrameric while GPx4 is active in its monomeric form. The refined crystal structure of bovine erythrocyte glutathione peroxidase presented by Epp, Ladenstein and Wendel clearly shows a tetrameric form (Figure 1.2). The active sites are found in surface depressions on two faces of this protein.
(i.e. two on each side) and binding studies demonstrated that negative cooperativity of substrate glutathione molecules results in a half-site reactivity for this enzyme. Ladenstein later presented a crystal structure of human GPx3 and compared the two interspecies enzymes, showing that there were no significant differences of secondary structure elements and only minor differences in amino acid sequences.\textsuperscript{55}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure12.png}
\caption{Bovine Erythrocyte GPx\textsuperscript{54}}
\end{figure}

The catalytic cycle of the glutathione peroxidases proposed by Ganther\textsuperscript{56} is illustrated in Scheme 1.3. The mechanism involves a two electron redox sequence where the selenium atom in selenocysteine is cycled through states of oxidation and reduction. In its reduced form the enzyme exists as selenol 9 which can react with organic peroxides or hydrogen peroxide, converting the selenocysteine to its oxidized counterpart, selenenic acid 10. This step demonstrates the antioxidant role of the enzyme as the peroxide is converted to either a molecule of water or an alcohol. The following step in the catalytic
cycle involves a molecule of glutathione (GSH) reacting with the selenenic acid to form selenenyl sulfide 11 and water. Completion of the catalytic cycle involves a second molecule of glutathione reacting at the sulfur atom of 11 to form glutathione disulfide and the enzyme in the reduced state. Pathway A is the dominant cycle under regular physiological conditions. If oxidative stress becomes extreme it is possible that the enzyme could enter pathway B. For example, selenenic acid 10 could be oxidized to seleninic acid 12, which would require three equivalents of GSH to return it to selenenyl sulfide 11.

Scheme 1.3

It has been demonstrated that during the catalytic cycle a number of amino acids in close proximity to the selenocysteine residue likely have important structural and
functional roles. The selenium atom of the active site is kept in place and activated by nearby tryptophan and glutamine residues.\textsuperscript{54} As evidenced by their Se-N bond distances,\textsuperscript{57} there are expected to be hydrogen bonds between the amino group of the tryptophan and the amido group of glutamine (Figure 1.3, complex A). This undoubtedly results in deprotonation to the selenolate, making the selenium more nucleophilic and prone to oxidation.

![Diagram A](image1.png)

**Figure 1.3 Important Complexes of GPx and GSH\textsuperscript{57}**

Molecular modelling\textsuperscript{57} has shown that a large number of peripheral amino acids aid in the fast reaction of glutathione with the selenenic acid in the second step of the cycle. As depicted in complex B of Figure 1.3, the proximity of tryptophan and arginine
residues results in hydrogen bonding to the thiol functional group, facilitating dissociation to the thiolate. The glutathione molecule is held in place by other electrostatic interactions between additional GPx residues and the carboxylic acid groups of glutathione’s glycyl and γ-glutamyl units.

The final step of the catalytic cycle has also been modelled to help elucidate binding interactions during reaction of the second molecule of glutathione with the selenenyl sulfide. Significantly, there is coordination of a threonine residue with the covalently bound sulfur of the first glutathione molecule (complex C, Figure 1.3). This results in polarization of the selenium-sulfur bond, which leads to nucleophilic attack of the incoming, second glutathione molecule at the sulfur of the first. This is important, as without this interaction the dominant pathway would be thiol exchange rather than reduction of selenium.

As described by Mugesh and du Mont, many of the interactions observed in natural glutathione peroxidases have been the inspiration for the design of small-molecule organoselenium compounds for both structure–activity studies and in the hope of creating efficient GPx mimetics (see Section 1.4).

1.3 Toxicity of Organoselenium Compounds

Selenium toxicity (selenosis) is relatively rare but has been reported in humans and animals. In humans, the usual cause of selenium poisoning is accidental consumption of high doses of dietary supplements while, in animals, selenium poisoning most often
occurs via consumption of plants known to accumulate high levels of selenium. Selenosis can be the cause of a variety of symptoms including nail damage, hair loss, ‘garlic breath’, gastrointestinal irritation and neurological damage. Excess levels of selenium can become a physiological problem by actually increasing oxidative stress (see below) or by substituting selenium for sulfur atoms in methionine, cysteine and cystine. The nail damage and hair loss associated with selenosis is a direct result of selenium substitution in the structural protein keratin which also results in damage to hooves and horns in animals.\textsuperscript{59} The toxicity of organoselenium compounds depends on a number of factors including chemical form, animal species, nutritional and dietary interactions, age, physiological state and route of administration.\textsuperscript{60}

A review of the toxicology of both organoselenium and organotellurium species has been published by Nogueira, Zeni and Rocha.\textsuperscript{61} There appear to be two general modes of toxicity of organoselenium compounds: degradation resulting in the release of inorganic selenium species and glutathione oxidase activity of selenols or selenolates. The first mode of toxicity can be mitigated by preparing organoselenium compounds that are less likely to undergo metabolic degradation. For example, there is a relationship between the toxicity of the organoselenium compound and the strength of the carbon – selenium bond. Alkyl diselenides are known to be more toxic than aryl diselenides because $C_{sp2} – Se$ bonds are more resistant to cleavage than $C_{sp3} – Se$ bonds.

With respect to the second mode, both organic and inorganic selenium compounds are known agents for the oxidation of biologically relevant thiols. As early as
1941, Painter suggested that the oxidation of glutathione, cysteine and other thiols might be caused by selenite induced poisoning. Later, biochemical evidence for this was reported in laboratory animals with selenite poisoning that also had significantly reduced levels of plasma and tissue glutathione. In the early 1990s sodium selenite and selenocystine were found to react in the presence of a number of thiols to reduce oxygen to superoxide and hydrogen peroxide. At the same time, Chaudière and coworkers demonstrated the mechanism of this glutathione oxidase activity in the presence of organic diselenides and selenols. In their study, selenocystamine was shown to facilitate the oxidation of glutathione via one-electron reduction of dioxygen and subsequent involvement of reactive oxygen species. This process was observed to occur under neutral pH and ambient oxygen pressure. It is paradoxical that selenium compounds can not only reduce harmful ROS, but also catalyse their formation.

Scheme 1.4
The mechanism that they reported is depicted in Scheme 1.4, starting with the heterolytic reduction of the diselenide bond of selenocystamine with glutathione (step i). This effectively produces two equivalents of selenolate ion, one equivalent from step i and the second equivalent from reaction of the resulting selenenyl sulfide with glutathione (step ii). Under anaerobic conditions these were the only reactions observed and the selenolate persisted. In the presence of oxygen the selenolate ion promotes the three step reduction of oxygen to water. A molecule of oxygen first reacts with selenolate to produce superoxide anion and selenyl radical (step iii). In the presence of a second selenolate anion (and a source of protons) superoxide can be reduced further to produce a molecule of hydrogen peroxide (step iv). The initial diselenide might be regenerated in the presence of a second selenyl radical (step v), but under physiological conditions it is likely that glutathione could reduce the selenyl radical back to a selenolate ion (step vi). Selenolate ions are obviously also very reactive towards hydrogen peroxide and so can be oxidized to produce selenenic acids and a molecule of water (step vii). Under physiological conditions and in the presence of glutathione, the selenenic acid quickly converts to the corresponding selenenyl sulfide and a second molecule of water (step viii).

1.4 Organoselenium Compounds as GPx Mimetics

There is considerable interest in the design of small-molecule organoselenium compounds that can act as glutathione peroxidase mimics. One of the main applications of such molecules could be the treatment of oxidative stress related to reperfusion of ischemic stroke or heart attack patients. Immediately following ischemic reperfusion
there is a burst of ROS which can be attributed to an immune response involving an increase in neutrophils, released to invade previously hypoxic tissue. A further contributor to oxidative stress associated with ischemic stroke is increased lactic acid concentration. The associated pH change results in a pro-oxidant environment as the rates of conversion of superoxide to hydrogen peroxide and hydroperoxyl radical increases. Acidosis also increases the amount of iron available for free radical formation because the decreased pH promotes dissociation of protein-bound iron. The brain is particularly susceptible to reactive oxygen species because it contains a larger amount of polyunsaturated fatty acids, it consumes the highest amount of oxygen per body mass, it contains high levels of iron and ascorbate which can act as pro-oxidants and it is not particularly enriched in antioxidants.

Many groups have prepared compounds which can reduce peroxides in the presence of stoichiometric amounts of glutathione or other thiols. One such compound that has been studied extensively for neuroprotective ability and a host of other applications is the selenazolone ebselen (13, Scheme 1.5). This compound has been known in the literature for nearly a century and was tested both in vitro and in vivo in pharmacological studies in the mid-1980s. Ebselen has shown neuroprotective activity in a number of animal models and the Daiichi-Sankyo Pharmaceutical company in Japan has been investigating ebselen clinically in humans, including Phase III trials.

Although ebselen is one of the most widely studied GPx mimetics, its catalytic mechanism has been the topic of debate and has resulted in the generation of a number of
independent studies. An overview of the literature reveals that the exact catalytic mechanism for the reduction of peroxides by ebselen depends strongly on the level of peroxide present (excess or limiting) and the nature of the reducing thiol. Fischer and Dereu prepared a number of potential intermediates and characterized them by $^{77}$Se NMR spectroscopy to investigate their role in the reduction of peroxides by ebselen.\textsuperscript{71} Using hydrogen peroxide as an oxidant and benzyl thiol as a reductant, they proposed a catalytic cycle for ebselen composed of two parts. In the case of high peroxide concentration (see Scheme 1.5) ebselen (13) is quickly oxidized to seleninamide 14. Two pathways were proposed for reduction of 14 back to ebselen. Thiolysis of the selenium – nitrogen bond could occur to produce thiol seleninate 15 or the thiol could add to the selenium – oxygen bond resulting in intermediate 17. Reduction of either intermediate with a second molecule of thiol and production of water would complete the cycle.

\textbf{Scheme 1.5}

Additionally Fischer and Dereu investigated ebselen’s antioxidant behaviour under conditions of excess thiol (Scheme 1.6). When this was the case, a fast reaction
occurred to produce selenenyl sulfide 18. Disproportionation of this compound to benzyl disulfide and diselenide 19 was observed to be a slow but important step of the catalytic cycle. Oxidation of 19 resulted ultimately in the reformation of ebselen along with two molecules of water. This presumably occurs via the selenenic anhydride 20 although this intermediate could not be observed. The stabilization of the selenium atom as a cyclic selenenamide moiety led these authors make the suggestion that similar stabilization may also be present in the glutathione peroxidases via proximal peptidic NH-bonds from tryptophan and glutamine residues (see Figure 1.3).

**Scheme 1.6**

Alternatively, a pathway excluding disproportionation of 18 was proposed by Engman. Only trace conversion of selenenyl sulfide 18 (R = C₈H₁₇) to the corresponding
disulfide and diselenide was observed by his group when 18 was left in the presence of excess 1-octyl mercaptan. Faster reaction times were recorded when this mixture was exposed to hydrogen peroxide.\textsuperscript{72} It is possible that oxidation of selenenyl sulfide 18 to thioseleninate 15 could occur much faster than disproportionation. Intermediate 15 would react with a second molecule of thiol to generate the corresponding disulfide and selenenic acid 16, which could reform the selenenyl sulfide in the presence of more thiol (Scheme 1.7).

\textbf{Scheme 1.7}

A third proposal was presented by Haenen and coworkers,\textsuperscript{73} involving the selenol derivative of ebselen. During the course of their studies, they observed that diselenide formation proceeds via a second-order reaction, dependant on both the concentration of
free thiol and the concentration of selenenyl sulfide (Scheme 1.8). Similarly to other pathways, ebselen was found to react quickly with thiols to form selenenyl sulfides. The next step in their proposed cycle is the formation of selenol 21 after reaction of 18 with a second molecule of thiol. Although 21 was not directly observed, diselenide 19’s proposed formation is via either nucleophilic attack by the selenium atom of 21 on a subsequent molecule of 18 or reaction of the selenol directly with another molecule of ebselen. Oxidation of diselenide 19 back to ebselen was proposed to occur via selenenic acid anhydride 20 (see Scheme 1.6) in the same way that Fischer and Dereu had suggested previously.

**Scheme 1.8**
More recently Mugesh has proposed a mechanism of catalytic activity involving seleninic acid $22$. This compound was observed to be formed when two molecules of hydrogen peroxide were reacted with diselenide $19$ to produce $22$ and selenenic acid $16$ (Scheme 1.9). Seleninic acid $22$ re-enters the catalytic cycle after reduction to $16$ by two molecules of thiol. Seleninic acid $22$ was also observed to oxidize diselenide $19$ to produce solely selenenic acid $16$, but details of this pathway were not discussed, although this presumably occurs by hydrolysis of an anhydride-type intermediate. Similarly to Fischer and Dereu, Mugesh observed that formation of diselenide $19$ was essential to this particular catalytic cycle. Diselenide $19$ was formed via disproportionation of $18$ after the reaction of thiol with ebselen ($13$) to form the selenenyl sulfide. This is in contrast to Haenen’s proposal of selenol intermediates and to Engman’s suggestion that the selenenyl sulfide is oxidized to a thioseleninate. The presence of $22$ in the proposed catalytic cycle shown in Scheme 1.9 highlights an important point. The selenium atom in this species is in a higher oxidation state than in ebselen and would therefore require a large peroxide to thiol ratio to form. As this scenario is unlikely to occur \textit{in vivo}, molecules like $22$ are biologically less relevant than their reduced counterparts.
Further investigation of ebselen’s catalytic cycle and its mode of action has been carried out through in-depth computational work by Antony and Bayse.\(^7\) Demonstration of the sensitive nature of the catalytic cycle to the precise type of thiol and oxidative conditions resulted in the proposed mechanism shown in Scheme 1.10. The contribution of diselenide 19 to the cycle was ruled out by Bayse for two reasons. First, the rate of disproportionation of a selenenyl sulfide to 19 and the corresponding disulfide was deemed to be insufficient to sustain catalysis. Second, the formation of diselenide by any pathway would be second order in concentration of intermediate selenium compounds, which is a scenario unlikely to occur *in vivo*. 
The mechanism proposed can be broken down into three distinct modes of action depending on whether conditions mimic extreme oxidative stress, whether the thiol is peptidic or a dithiol, and whether the thiol is a simple thiol. Under conditions of extreme oxidative stress, ebselen is expected to become oxidized to seleninamide 14, which can react with a molecule of thiol to produce 15 (pathway A). As proposed in other mechanisms, this would react with a second molecule of thiol to produce selenenic acid 16. Under conditions which are potentially more relevant to a physiological environment, ebselen is expected to react with a molecule of thiol to produce selenenyl sulfide 18. If the thiol is simple (not a dithiol and no pendant coordinating groups capable of S⋯X interactions), the next step which can occur is oxidation to the thioseleninate 15 (pathway
B). Simple thiols react in this manner because the selenium – sulfur bond is polarized in such a way that nucleophilic attack of a second thiol molecule occurs at selenium, which results in thiol exchange rather than formation of disulfide and selenol. However, if the thiol is part of a peptide (as is the case for glutathione) the selenium – sulfur bond becomes polarized such that nucleophilic reaction at sulfur by a second molecule of thiol will occur (pathway C). This is a result of significant S···O interaction possible via the carboxyl functionalities present in these thiols. Reaction of 18 with such a thiol will produce selenol 21 along with a molecule of disulfide. Peroxide oxidation of 21 to selenenic acid 16 followed by reaction with another molecule of thiol reforms 18. Pathway C also dominates for dithiols because the formation of an intramolecular disulfide bond is kinetically favoured. It is notable that pathway C, involving peptidic thiols or in cases where a dithiol is present, closely imitates that of the enzymatic pathway of glutathione peroxidases (see Scheme 1.3).

The catalytic cycle of ebselen proposed by Antony and Bayse demonstrates an important feature of organoselenium chemistry as it relates to designing GPx mimetics. Polarization of the selenium – sulfur bond in selenenyl sulfides results in modulated reactivity of such compounds, which depends on the dipole direction. This is especially important if a compound’s catalytic cycle involves conversion of a selenenyl sulfide into other intermediates as a rate-determining step. Depending on the exact nature of the selenenyl sulfide, either of the sulfur or selenium atoms can be the more electropositive of the two. In the case where selenium is more electropositive, the dominant reaction that can occur with a second molecule of thiol is an exchange of one thiol for the other.
Computational evidence suggests that, in simple selenenyl sulfides (MeSeSH, HSeSMe and HSeSH), nucleophilic attack of thiol at selenium is both kinetically and thermodynamically more favorable than at sulfur. Sarma and Mugesh investigated whether this would also apply to ebselen and a number of other GPx mimetics with known selenenyl sulfide intermediates. Using calculated Se–•••O or Se–•••N distances, electron densities on selenium and sulfur along with theoretical and experimental Se NMR chemical shifts, the reactivity of various selenium species was explored. In selenenyl sulfide 18 (derived from ebselen) there is a moderate Se–•••O interaction (r_{Se–O} = 2.47 Å; E_{Se–O} = 19.01 kcal/mol) and the calculated Se NMR chemical shift for this compound is shifted substantially downfield compared to that of PhSeSPh (604 ppm vs 437 ppm, respectively). This suggests that electron density at the selenium atom in 18 is considerably reduced by the pendant amide group. The possibility that the electron-withdrawing nature of the amide group causes the observed chemical shift difference between 18 and PhSeSPh was ruled out by calculating the positive charge on the sulfur atom of each compound. If mesomeric effects were causing the increased electropositive nature of the selenium atom in 18 then the sulfur atom would also be expected to be electron-deficient. Calculations demonstrated that the sulfur atom in this compound carries less positive charge than the sulfur in PhSeSPh (0.029 vs 0.089 respectively). Experimentally, Sarma and Mugesh found that addition of PhSH to ebselen readily produced selenenyl sulfide 18 but excess PhSH did not produce the corresponding selenol 21 (the expected product if nucleophilic attack occurred at sulfur). In a classic thiol crossover experiment, selenenyl sulfide 23 (derived from ebselen and thiocresol) was exposed to ethanethiol producing a new selenenyl sulfide (24) and no selenol 21 (see
Scheme 1.10) or related products. This demonstrates that the thiol exchange pathway is one that requires serious consideration when designing GPx mimetics. Selenenyl sulfides 25 and 26 were also included in Mugesh’s study, which showed the electropositive nature of Se in each of these molecules (calculated $^{77}$Se NMR chemical shifts of 547 ppm and 525 ppm, respectively; Se positive charges: 0.352 and 0.289, respectively), although the effect is greater in 18 (calculated $^{77}$Se NMR: 604 ppm; Se positive charge: 0.377).

![Chemical structures](Image)

**Figure 1.4 Selenenyl Sulfides and Thiol in Mugesh's Study**

Also investigated by Mugesh was the role of functional groups with the ability to chelate to the sulfur atom of a selenenyl sulfide. For example, compound 27 was reacted with excess thiol 28 readily producing the disulfide and benzeneselenol. Computationally, an elongation of the selenium – sulfur bond was observed along with a change in the polarity of this bond. Even for compound 29, where both sulfur and selenium have coordinating groups, it was demonstrated that nucleophilic attack at sulfur occurred preferentially. As pointed out by Antony and Bayse, these findings support
their computationally derived catalytic cycle for ebselen. In their cycle, compounds such as 18 do not react by nucleophilic attack at the sulfur, whereas compounds with a peptidic thiol component (i.e. 30) are inclined to undergo disulfide and selenol formation.

![Figure 1.5 Effect of Peptidic Thiol on Selenenyl Sulfide Intermediate](image)

Other organoselenium compounds containing coordinating groups close to selenium have been investigated for GPx-like activity. For example, Spector and coworkers\textsuperscript{78} investigated symmetrical diselenides substituted with tertiary benzyl amines at the ortho-position. They hypothesized that compounds like 31 (Figure 1.6) would display GPx-activity due to the basic amino group deprotonating any approaching thiol molecules, a structural design inspired by the theory that similar reactions could be occurring in the actual enzyme. Also of interest to these authors was the opportunity for the amino group to interact with the selenium in some way. It was this suggestion and a later observation by Tomoda and Iwaoka\textsuperscript{79} that resulted in a number of research contributions detailing the nature and strength of non-covalent Se•••X interactions. They found that 32 displayed obvious Se•••N interactions in the solid state as the distance between the selenium and nitrogen atoms was less than the sum of their van der Waals radii. To study if this also existed in solution, they investigated the \textsuperscript{1}H NMR spectrum of this compound. The presence of an AB-quartet for the benzylic methylene group would
indicate a slow inversion about the nitrogen atom caused by Se•••N interactions. Upon finding that this was not the case, they prepared selenenyl bromide 33 and showed that the latter compound did contain diastereotopic benzylic methylene signals as measured by \(^1H\) NMR spectroscopy. Additionally, the \(^{77}\text{Se}\) NMR spectrum of 33 had a peak at 1019 ppm which is significantly downfield from the peak of benzeneselenenyl bromide at 869 ppm, also indicating significant Se•••N interactions. Demonstration of GPx activity for compound 32 led Tomoda and Iwaoka to study identified intermediates presumed to be responsible for its catalytic activity.\(^{80}\) They found that selenol 34 only existed in its zwitterionic form, demonstrating the amine’s role in deprotonating the selenol and making the selenium atom more susceptible to oxidation to the selenenic acid. Once oxidized to the corresponding selenenic acid, the Se•••N interaction was expected to stabilize the selenium atom against further oxidation until it could react with thiol to form a selenenyl sulfide.

Figure 1.6 Bis(2-dialkylaminomethyl) Diselenides and Related Compounds

In contrast to the later suggestions of Mugesh,\(^{77}\) these authors suggested that the non-covalent Se•••N interaction might enhance the reactivity of the selenenyl sulfide towards attack by a second molecule of thiol at the sulfur atom rather than the selenium atom, although this was not elaborated in detail. Using a variety of methods, Tomoda and Iwaoka suggested that the Se•••N interaction arises from a non-bonding stabilization of
the lone pair of nitrogen into the anti-bonding orbital of the Se-R bond \((n(N)\rightarrow\sigma^{\ast}(Se-R))\).\(^{81}\) In addition to Se-\(\cdots\)N interactions, Tomoda and coworkers also studied the nature of Se-\(\cdots\)O\(^{82}\) and Se-\(\cdots\)X\(^{83}\) (X = F, Cl, Br), where the pendant coordinating group was attached as a benzyl alcohol or benzyl halide at the position ortho to the selenium atom. The studied organoselenium species included selenenyl halides, cyanides, sulfides, methyl selenides and aryl diselenides. In all cases they found that there were significant but variable non-bonding stabilizations \((n(O/X)\rightarrow\sigma^{\ast}(Se-R))\) between the selenium atom and the coordinating heteroatom. As this has implications for the reactivity of selenenyl sulfides, selenenic acids and other intermediates involved in the catalytic cycles of GPx mimetics it is obviously a factor that needs to be considered when designing such molecules.

Although, as Mugesh has demonstrated,\(^{77}\) coordinating groups near selenium can promote thiol exchange rather than disulfide formation, it is clear that heteroatoms near the selenium atom can increase the reactivity of GPx mimetics and it is likely that heteroatom interactions are present and important in the enzyme.\(^{57}\) The focus of a large amount of the research on GPx mimetics is on compounds with nonbonding selenium-heteroatom interactions. The main arguments for incorporating these interactions and the heteroatoms involved are: i) if the heteroatom is basic and there is expected to be a selenol intermediate, the heteroatom can deprotonate the selenol thereby activating the catalyst towards oxidation; ii) the heteroatom, through nonbonding interactions, can stabilize oxidized states against further oxidation and potential deactivation; iii) nonbonding interactions can increase the electrophilicity of selenium, increasing the probability of nucleophilic attack of thiols at selenium in selenenic acids and other
oxidized states; iv) heteroatoms which are basic can deprotonate incoming thiols, making them more reactive.\textsuperscript{84}

During the course of studies towards chiral catalysts, Braga and coworkers\textsuperscript{85} recently disclosed that ephedrine derived selenium compounds display promising GPx activity (Figure 1.7). They found that diselenide 35 could efficiently convert hydrogen peroxide to water in the presence of benzenethiol, while compound 36 was an order of magnitude less efficient. Compound 37, containing a labile selenoester, was also active as a catalyst for reducing hydrogen peroxide. This is presumably due to the ability of the amine to interact with intermediates formed from the corresponding selenolate.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.7}
\caption{Braga's Ephedrine Based GPx Mimetics}
\end{figure}

Recently the group of Jain and coworkers presented the synthesis and GPx-activity of compounds with 2,5-dimethylpyrazole rings attached to the selenium through an ethylene bridge.\textsuperscript{86} Also substituted on the selenium atom were a variety of short chain (two to four carbon) aliphatic groups which generally terminated in amine or carboxyl functionalities. Through X-ray crystallography it was found that there were no Se---N interactions between the selenium atom and the nitrogen atoms on the pyrazole rings, although compounds of the general structure 38 did display catalytic activity via selenoxide intermediates. Very recently, this group prepared aromatic derivatives 39 with the same R groups installed on the selenium atom\textsuperscript{87} (Figure 1.8). In this case X-ray
crystallography demonstrated weak Se•••N interactions. Upon comparing the aliphatic analogues with their aromatic derivatives, it was found that the activity of this group of compounds was twice as fast for 39 relative to 38 (R = CH₂CH₂NH₂). Although it is possible to observe important non-covalent interactions with X-ray crystallography, it should be pointed out that this is only a guarantee that these exist in the solid state. The Se•••N interaction was characterized as weak by Jain’s group and it is a stretch to expect its existence in the same manner when 39 is in solution.

![Figure 1.8 Jain's Structural Modification Forces Se•••N Interaction](image)

Similarly to Spector and coworkers, Bhabak and Mugesh found that compounds 40, shown in Figure 1.9 where R = Me, Et or n-Pr, displayed better GPx activity than that of ebselen, but that (due to Se•••N interactions) these compounds still underwent thiol exchange reactions that retarded their activities. To modulate the Se•••N interaction these authors introduced methoxy groups *ortho* to the selenium atom (see compounds 41). It was found that introduction of this substituent to the aromatic ring had the effect of increasing the activity of this class of compounds by an order of magnitude when the nitrogen atom was substituted as dimethyl. It has been demonstrated that these diselenides enter into their specific catalytic cycle by reaction with thiol to form a selenenyl sulfide and that the Se•••N interaction facilitates this by weakening the selenium – selenium bond. Although the methoxy group prevented the thiol exchange reaction
from occurring due to a reduced Se•••N interaction, it was found that this interaction was sufficient to allow for thiolysis of the diselenide bond. Natural bond orbital calculations confirmed that the Se•••N of the selenenyl sulfide derivatives of 40 and 41 were indeed weaker in the case of the latter.

Figure 1.9 Diselenides Investigated by Mugesh and Singh

Diselenides 42, where there is a methoxy substituent para to the selenium atom, were also investigated. The purpose of investigating diselenides 42 was to discover if the enhancement in catalytic activity was electronic or steric in nature. If the enhancement was only steric in nature, one would expect that compounds 42 would not be better GPx mimetics than compounds 40 because there would be no steric influence on the selenium – nitrogen interaction by a substituent across the ring. It was found that compounds 42 were similar in reactivity to the unsubstituted compounds 40 and far worse catalysts than 41, which suggests that there is minimal electronic influence on the reactivity of 41. We have also investigated the effect of para-methoxy substituents (among other substituents) upon the reactivity of GPx mimetics developed in our lab. Our work was initiated in 2005, several years prior to that of Mugesh, and investigated
further during the course of my studies; it is discussed fully in Chapters Two and Three of this Thesis.

Additionally, Bhabak and Mugesh demonstrated that sec-amine diselenides 43 display better catalytic activity than their tert-amine counterparts.90 Kumar and Singh91 have prepared a naphthyl derivative of compound 40 (see compound 44) which also displays GPx activity, while naphthyl diselenide 45 displays weak activity due to a strong Se•••N interaction.92

Singh’s group has demonstrated numerous times that aryl diselenides with pendant oxazoline (46) or oxazine (47) rings to provide Se•••N interactions display very poor GPx activity due to the strength of this nonbonding interaction.92 Paradoxically, the strength of this interaction stabilizes triselenide 48 which displays GPx activity up to an order of magnitude greater than that of 47.93 Although molecules like 48 are interesting, a triselenide would be a very unlikely drug candidate due to its relative instability.

Figure 1.10 Singh's Oxazolines and Oxazines

Many groups have focused attention on ebselen derivatives where the organoselenium compound (or associated catalytic species) has a covalent Se–N bond
and often a Se---O interaction through an amide linkage. These compounds generally take the form of diselenides with pendant amide groups or contain some form of heterocycle with a labile selenium – nitrogen bond. Cyclic selenenamide 49 was prepared by Reich and Jasperse to investigate if this structural element could play a role in the catalytic activity of GPx itself. It was found that 49 did display catalytic activity via a catalytic cycle very similar to that of GPx. Back and Dyck also reported a cyclic selenenamide which displayed thiol peroxidase activity. They found that 50 acts as a procatalyst which is consumed upon exposure to thiol, producing selenenyl sulfide 51 as an intermediate in a catalytic cycle that mimics that of the glutathione peroxidases exactly.

![Figure 1.11 Cyclic Selenenamides as GPx Mimetics](image)

While investigating cyclic selenenamide 50, our group developed an HPLC based protocol for evaluating compounds for GPx activity. Using this *in vitro* assay, all new GPx mimetics developed in our laboratory are assessed for their ability to destroy peroxides in a catalytic manner. This assay involves the reduction of tert-butyl hydroperoxide to tert-butanol and water or the reduction of hydrogen peroxide to two molecules of water. This occurs in the presence of benzyl thiol, used as a stoichiometric reductant and catalytic amounts of selenium compounds, for example 50. Catalytic activity is measured as $t_{1/2}$, the time required for a catalyst to promote the conversion of benzyl thiol to dibenzyl disulfide to 50% completion under our standard conditions. All
of our catalysts are evaluated in the same manner so that we can easily compare activity for the large number of compounds developed and tested in our laboratory. The experimental set-up for this assay is further elaborated in Subsection 1.5.4.

Chaudière and coworkers have investigated the GPx activity of a number of ebselen analogues where the carbonyl group has been replaced by a gem-dimethyl quaternary carbon atom (Figure 1.12). Specifically, compound 52 was found to display similar activity to that of ebselen, while the homologous compound 53 was found to be more than twice as potent.\textsuperscript{65} Both compounds 52 and 53 have been prepared via routes allowing for multi-kilogram scale production and have been clinically investigated for treatment of a variety of conditions.\textsuperscript{96} Most notably, 53 has been investigated for the prevention of ischemic-reperfusion injury.\textsuperscript{97}

![Figure 1.12 Chaudière’s GPx Mimetics](image)

A number of groups have prepared analogues of ebselen where the core benziselenazolone structure is intact but contains various substitutions around the ring or on the nitrogen atom. For example, the $N$-methyl derivative 54 (see Figure 1.13) was found to be only mildly more potent than ebselen but far more toxic. Compound 55, with a nitro-coordinating group \textit{ortho} to selenium, was found to be more toxic than ebselen but displayed nearly an order of magnitude better GPx activity.\textsuperscript{98} Singh and coworkers also investigated ebselen derivatives with \textit{ortho}-coordinating groups. They found that
ortho-amido groups could participate in Se•••O nonbonding interactions, improving catalytic activity by as much as three times for N-methyl derivative 56. Bhabak and Mugesh reported a study of different structural features on the nitrogen atom of ebselen and what effect this had on activity. They found that derivative 57, with no nitrogen substituent, displayed reduced GPx activity. If the nitrogen was substituted with an electron deficient aromatic ring (para-bromo) catalytic activity was again reduced. On the other hand, if the same substituent was an electron rich aromatic ring (para-hydroxyl) the activity of the catalyst was higher than that of ebselen. Interestingly, ebselen derivative 58 was also found to have increased activity relative to that of ebselen. Compound 59, with three ebselen functionalities per molecule displayed GPx activity at a level only twice that of ebselen. The solid state structure of this compound was determined by X-ray crystallography demonstrating that the ebselen moieties were arranged perpendicularly to the mesitylene ring. Each selenium atom was sterically encumbered which likely led to a reduction in the expected activity for this compound.

![Diagram of structural derivatives of ebselen](Figure 1.13 Structural Derivatives of Ebselen)
Nicotinic acid-based ebselen derivatives have been prepared and tested for GPx activity by Jain’s group.\textsuperscript{101} They found that 60 displayed the best activity of their series, while compound 61 was not as active, but still more active than ebselen. Further structural modification of ebselen has seen limited success. Galet and coworkers found that 62 displayed very little catalytic activity relative to ebselen,\textsuperscript{102} while Messali, Christiaens and their colleagues found that 63 displayed catalytic activity that was nearly identical to ebselen.\textsuperscript{103}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{images/chemistry.png}
\caption{Further Structural Modification of Ebselen}
\end{figure}

In addition to their work describing the difference in activity between aromatic diselenides with pendant tertiary or secondary benzyl amines, Bhabak and Mugesh have also studied the corresponding secondary and tertiary amides.\textsuperscript{104} They found that, unlike for the amino derivatives, the diselenide bonds in the amido compounds were not easily cleaved by thiol to form the selenenyl sulfides required for entry into the ebselen catalytic cycle. This was the case even in the presence of twenty equivalents of thiol and it was suggested that this demonstrates the role of the basic nitrogen in the amino compounds where the incoming thiol molecule is made more nucleophilic by deprotonation to the thiolate. Both the secondary and tertiary amides (64 and 65, respectively) undergo quick reaction with peroxides to yield the corresponding selenenic acids. Further reaction with thiol produces the selenenyl sulfides which can disproportionate to reform the diselenides
and product disulfides. It is this step where the activity of the tertiary amides (65) is increased relative to their secondary counterparts (64). The selenenyl sulfides derived from 64 contain a much stronger Se•••O interaction relative to those derived from 65. Presumably the Se•••O coordination is stronger for the secondary amides because the alkyl groups on the nitrogen atom can become planar more easily than the tertiary amide, which would be sterically repelled by the aromatic ring hydrogen atom. Bhabak and Mugesh claimed that a stronger Se•••O interaction stabilizes the selenium – sulfur bond against disproportionation, which happens more readily in 65. They also found that, in the tertiary compounds where R was methyl, the catalytic activity was greatest.

\[
\begin{align*}
\text{64} & \quad \text{65}
\end{align*}
\]

Figure 1.15 Mugesh's Secondary and Tertiary Amides

A number of research groups have investigated other functionalities capable of Se•••O nonbonding interactions. For example, Wirth demonstrated that diselenides substituted with benzyl alcohols or benzyl ethers had GPx activity. Specifically he found that 66 and 67, both with free hydroxyl groups, were good GPx mimetics (see Figure 1.16). Diselenide 68 with a methyl ether rather than free hydroxyl group was only about half as active as either 66 or 67.\textsuperscript{105} While investigating the reactions of diselenide 69, Singh’s group demonstrated the ability of Se•••O interactions to stabilize selenenic esters when compound 70 was isolated as the sole product after a number of attempts to prepare selenenyl halides. Both compounds 69 and 70 display considerable thiol peroxidase
activity which Singh attributes to the strong covalent and nonbonding selenium – oxygen interactions formed in these molecules.  

![Chemical structures](image)

**Figure 1.16 GPx Mimetics Containing Oxygen Coordinating Groups**

With the aim of incorporating selenium – heteroatom interactions in mind, a number of research groups have prepared selenium-containing amino acids, peptides or synthetic enzymes. The semisynthetic selenoenzyme known as selenosubtilisin was prepared by converting the serine active site of the protease subtilisin into a selenocysteine moiety.  

Hilvert and Wu found that this synthetic enzyme was able to catalyse the reduction of tert-butyl hydroperoxide quite efficiently in the presence of thiol and that the catalytic cycle was analogous to that of GPx. They also surmised that stabilizing interactions might be involved when this compound is in its oxidized form. Braga and coworkers found that selenocystine homologues 71 (n = 0 – 2) catalysed the conversion of benzenethiol to diphenyl disulfide in the presence of hydrogen peroxide. By comparing these diselenides to diphenyl diselenide, they found that the presence of the coordinating amido groups had a negligible accelerating effect. Interestingly,
selenoglutathione diselenide (72) was found to catalytically break down hydrogen peroxide in the presence of the native tripeptide glutathione at a rate similar to that of diphenyl diselenide.\textsuperscript{109} Bhuyan and Mugesh recently prepared a number of peptides with selenocysteine – proline linkages where the peptide was presented as a dimer through the selenocysteine residue. Their findings show that peptides designed like this were capable of catalytic hydrogen peroxide reduction in the presence of glutathione, where the GPx activity was on the same level as that of ebselen.\textsuperscript{110} Both the groups of Mugesh and Singh have independently reported the synthesis and moderate GPx activity of a number of ebselen analogues that feature peptide or amino acid substitution on the nitrogen atom of ebselen.\textsuperscript{111} Mugesh found that amongst the compounds that were prepared in his study, 73 was the best catalyst, while Singh’s group found that 74 displayed activity at a rate slightly better than ebselen.

![Figure 1.17 GPx Mimetics Encorporating Amino Acids](image)

A number of groups have investigated selenides as potential GPx mimetics with varying success. For example, Spector prepared 75 along with 76 and found that both of
these compounds were only able to catalyse the reduction of peroxides at about 5% the efficiency of ebselen.\textsuperscript{78} Another diaryl selenide (77) was found to have little activity, whereas the tellurium analogue of this compound displayed considerable activity.\textsuperscript{112} Selenides with a core $\alpha$-(phenylselenenyl) ketone structure (78) were found to be good catalysts for the reduction of a variety of peroxides in the presence of glutathione. It was found that the active species in this catalyst system was not actually a selenide, but phenyl selenolate which was released from the $\alpha$-(phenylselenenyl) ketones upon exposure to glutathione.\textsuperscript{113} Recently, vinyl selenides 79 and 80 were found to act as GPx mimetics although the mechanism of their activity is yet to be established.\textsuperscript{114} Research by Braga\textsuperscript{115} and coworkers found that the catalytic activity for selenide 81 likely involves hydroxy perhydroxy selenuranes 82 which can form under conditions of excess hydrogen peroxide or if the catalyst enters the catalytic cycle as the corresponding selenoxide. It was found that compounds with potential chelating groups, especially amino groups as in 83, were the best GPx mimetics.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{selenides.png}
\caption{Selenides Investigated as GPx Mimetics}
\end{figure}
A number of structurally different organoselenium compounds have been prepared and studied as GPx mimetics. For example, while retaining a selenide motif Engman’s group has investigated aryl chalcogenides with functional groups capable of scavenging free radicals. They have found that these potentially multifunctional catalysts are quite efficient as free radical scavengers, but that they are generally poor thiol peroxidases unless tellurium is used as the redox-active atom. Compounds that have shown promising free radical scavenging ability and variable peroxidase activity include 84, 85, 86 and 87. Compound 87 was designed to have GPx activity along with the capability of sequestering heavy metals. Unfortunately 87, along with 1,4,7,10-tetraazacyclododecane and porphyrin derivatives were weak catalysts for the destruction of peroxides.

![Chemical Structures](image)

**Figure 1.19 Multifunctional GPx Mimetics**

A number of groups have shown interest in nitrogen heterocycles containing selenium, either as part of the ring or as part of a functional group attached to the heterocycle. While 88 was found to be a better GPx mimetic than ebselen, structural modification of this compound (full aromatization, regioisomers, sulfonamide reduction) resulted in diminished activity. Eleven different imidazole diselenides (89) with differing substitution about the nitrogen heterocycle have been prepared and observed to display glutathione peroxidase activity between 1.6 and 3.9 times greater than that of...
Although dipyridyl selenide and dipyrimidyl selenide were found to have weak thiol peroxidase activity when dithiothreitol was used as the stoichiometric reductant of the peroxide, compounds 90 (n = 0 or 1) had strong thiol peroxidase activity. However, this activity disappeared completely when a monothiol was used as the reductant. Recently, selenoureas, selenocarbamates and selenohydantoins with various substituents have been investigated for both peroxidase activity and free radical scavenging ability. Some groups have investigated macromolecules containing either selenides or diselenides for GPx activity. Luo’s group has prepared cyclodextrin based diselenides, finding that they display moderate activity, while Smet’s group has investigated dendrimeric polymers with either selenide or diselenide linkages.

![Chemical structures](image)

**Figure 1.20 GPx Mimetics with Nitrogen Heterocycles**

Following the early discovery of GPx-like activity of selenenamide 50 by Back and Dyck in 1997, our group became interested in GPx activity investigations of simple aliphatic diselenides and selenides containing potential coordinating groups. To determine catalytic efficiency, the assay that had been developed for measuring the efficiency of compound 50 was again employed by our group. It will be recalled that catalytic activity is reported as the time required for a 50% conversion of benzyl thiol to
dibenzyl disulfide (recorded as $t_{1/2}$) in the presence of the catalyst under investigation and peroxide (see Subsection 1.5.4 for an elaboration of our assay).

Our goal was a systematic investigation of structure-activity relationship of the simple aliphatic diselenides and selenides containing coordinating groups. Specifically, symmetrical two to four carbon alkyl diselenides with carboxylic acid, methyl ester, amido, carbamate, amino or hydroxyl termini were prepared. It was found that, in contrast to their diaryl counterparts, these compounds were generally poor catalysts for the reduction of tert-butyl hydroperoxide in the presence of benzyl thiol. The only compound that displayed significantly better activity than ebselen ($t_{1/2} = 42$ hours) and also better activity than the previously reported camphor-based GPx mimetic 50 ($t_{1/2} = 18$ hours) was acetamide 91 ($t_{1/2} = 6$ hours). Unfortunately, 91 was difficult to prepare in a pure state and was not pursued further.

![Chemical structure of 91](image)

After observing that alkyl selenides reacted more rapidly with tert-butyl hydroperoxide than diselenides, our group prepared a number of allyl selenides which could undergo rapid [2,3]sigmatropic rearrangements of their corresponding selenoxides to prepare transient selenenic esters. As selenenic esters are known to be thiophilic, it was presumed that these allyl selenides could potentially offer routes to catalytically active species. Thus, alkyl selenolates functionalized with a variety of coordinating groups were treated with allyl bromide to prepare the corresponding alkyl allyl selenides. The studied
compounds included two to four carbon alkyl groups terminating in methyl ester, amide, carbonate or alcohol functionalities, all of which displayed better GPx activity than ebselen ($t_{1/2} = 42$ hours) in the conditions of our assay. The most efficient catalysts were found to be 92, 93 and 94. These catalyse the oxidation of benzyl thiol to dibenzyl disulfide with $t_{1/2}$ values of 7.7, 4.8 and 9.8 hours, respectively. A control experiment found that the uncatalysed reaction had a $t_{1/2}$ greater than 300 hours. Hydroxyl groups as coordinating substituents for GPx mimetics had been unstudied to this point, with the exception of a number of diaryl diselenides studied by Wirth.\textsuperscript{105}

As oxygen coordinating groups were relatively unstudied and 92, 93 along with 94 were remarkably efficient at reducing tert-butyl hydroperoxide, our group investigated the mechanism of their catalytic activity further. As expected, it was found that 93 did not react with thiol at all in the absence of peroxide. When 93 was subjected to tert-butyl hydroperoxide in the absence of benzyl thiol, oxidation to 95 occurred rapidly, followed by [2,3]sigmatropic rearrangement to the unstable product 96 (identified by a downfield shift of the allyl signals in the $^1$H NMR spectrum of the reaction mixture). Excess oxidizing agent resulted in the formation of the novel cyclic seleninate ester 98 via the oxidation of 96 to 97, followed by cyclization and concomitant release of allyl alcohol. The conversion of allyl selenide 93 to cyclic seleninate ester 98 is depicted in Scheme 1.11.

![Figure 1.21 Allyl Selenides as GPx Mimetics](image-url)
Catalytic activity of 98 was measured in our standard assay and it was discovered that this cyclic seleninate ester had a $t_{1/2}$ of only 2.5 hours, which was faster than that of the parent allyl selenide 93. It was determined that 93 acted as a procatalyst for the more active compound 98. To probe the mode of action of these compounds further, 98 was subjected to the same control experiments as 93. It was found that 98 did not react in the presence of only the oxidizing agent, but reaction with benzyl thiol produced thioseleninate 99. Reaction with a second equivalent of benzyl thiol produced dibenzyl disulfide and reduced the selenium to selenenic acid 100. In the presence of more tert-butyl hydroperoxide, 100 was converted back to 98 after oxidation and cyclization. If 100 reacted with a third molecule of benzyl thiol then selenenyl sulfide 101 was formed. The catalytic cycle for 98 is depicted in Scheme 1.12.
As selenenyl sulfides are known to be important intermediates for a wide array of GPx mimetics, 101 was prepared separately and subjected to the same assay conditions as 93 and 98. This compound displayed a $t_{1/2}$ of 35 hours, which is considerably slower than the cyclic seleninate 98 ($t_{1/2} = 2.5$ hours). In contrast to other catalytic cycles with known selenenyl sulfide intermediates, 101 can be ruled out as a significantly contributing component to the catalytic cycle. In fact, formation of 101 is considered to be a deactivation pathway for this catalyst system. Although a similar, but slightly less active cyclic seleninate ester was observed when 94 was reacted with tert-butyl hydroperoxide, the corresponding seleninate ester did not form from 92, presumably because of its strained four-membered ring.
Symmetrical alkyl selenides were also investigated by our group to test the effect of proximal coordinating groups within the dialkyl selenide system. Once again it was found that alkyl selenides with terminal hydroxyl groups were efficient catalysts in our assay. Compound 102 was found to catalyse the reduction of hydrogen peroxide with a $t_{1/2}$ of 2.9 hours. As expected, 102 did not react in the presence of only benzyl thiol, but when it was subjected to tert-butyl hydroperoxide alone, 103 formed quickly. The catalytic activity of 103 was investigated and this compound was found to have the same $t_{1/2}$ as 102. Additionally, this spirodioxyselenurane was found to be the only persistent selenium compound in solution when all reducing thiol was consumed. To confirm that 103 was a participant in the catalytic cycle of 102, the spirodioxyselenurane was reacted with two equivalents of benzyl thiol and 102 was quantitatively recovered. The spirodioxyselenurane 103 proved to be a novel compound whose structure was unequivocally established by X-ray crystallography. Additionally, 104 was prepared from the corresponding di(4-hydroxybutyl) selenide and found to have a $t_{1/2}$ of 5.1 hours while 105 did not cyclize.

![Figure 1.22 Symmetrical Selenides as GPx Mimetics](image)

The catalytic cycle for 103, depicted in Scheme 1.13, was proposed after considering the control experiments described above. Initially a molecule of thiol substitutes one of the alkoxy groups to form intermediate 106, which can react with an
additional thiol molecule to form the reduced catalyst 102 and dibenzyl disulfide. Oxidation of 102 is presumed to form the corresponding transient selenoxide 107, which cannot be detected, but undergoes a very rapid cyclization and loss of water to complete the catalytic cycle.

Scheme 1.13

The discovery of seleninate ester 98 and spirodioxyselenurane 103 was a major breakthrough for a number of reasons. First and foremost, both catalysts perform better than ebselen by, at minimum, an order of magnitude with respect to the rate of their reduction of tert-butyl hydroperoxide with benzyl thiol. Second, we were able to demonstrate that both compounds operate by catalytic modes different from each other, from those of the GPx family, or of any other mimetic presented in the literature at the
time. Third, both 98 and 103 are remarkably simple and easily prepared from inexpensive starting materials. Both are stable and nearly odourless compounds that can be stored for extended periods at 0 °C. Finally and also of importance, the catalytic cycles of both compounds are devoid of selenol and selenolate containing intermediates, which are known to facilitate glutathione oxidase activity as was discussed in Section 1.3.

It is known that aromatic selenium compounds tend to have lower toxicities than their aliphatic counterparts due to greater metabolic stability of the carbon – selenium bond in the former compounds. Our group therefore set out to incorporate an aromatic backbone for the cyclic seleninate ester and spirodioxyselenurane structures. A route was developed which allowed for access to aromatic derivatives 108 – 111, all of which could be accessed from anthranilic acid.

![Figure 1.23 Aromatic Derivatives of 98 and 103](image)

Although all of the above aromatic compounds still had thiol peroxidase activity, both 108 and 110 were poorer catalysts than their aliphatic counterparts. Benzoyl derivatives 109 and 111, which contain electron-withdrawing substituents in the position ortho to the selenium atom, had even lower catalytic activity. While compounds 98 and 103 had activity much better than that of ebselen, we had found that their benzo derivatives were, at best, only slightly better than ebselen. At the same time that we
published our findings on the aromatic derivatives of the spirodioxselenurane and cyclic seleninate ester, Singh’s group presented similar work on 108 and 110. Singh’s group later also prepared seven-membered cyclic seleninate ester 112 along with seleninate ester 113 which contains an ortho nitro group capable of Se-O coordination. Both 112 and 113 display good GPx activity under the conditions of Singh’s assay.

It is of interest to point out that Singh determined catalytic activity by the relatively common ‘coupled reductase’ assay (see Subsection 1.5.3). Many investigations, including for 112 and 113, only report initial reaction rates, \( v_0 \), measured during the first 5-10% of the catalytic reduction of peroxides when using this assay. If, as in the case for 112 and 113, the catalyst is present in 5 mol % relative to peroxide, it is not possible to determine if the GPx mimetic being studied continues to destroy peroxides beyond 5% completion of the reaction. This can result in the reporting of anomalously high catalytic activities, specifically for compounds that enter into an assay already oxidized (consider 112 and 113) and when the rate determining step of a catalytic cycle is oxidation of the selenium atom. For this reason we monitor the catalytic activity of all our compounds to beyond 50% reaction completion.

![Figure 1.24 Cyclic Seleninate Esters Developed by Singh](image-url)
With the goal of preparing spirodiazaselenurane 114 our group investigated the oxidation of selenide 115. However, treatment of 115 with either N-chlorosuccinimide or hydrogen peroxide generated azaselenonium species 116 along with a chloride or hydroxide counterion respectively, rather than the expected spirodiazaselenurane 114. Their structures were confirmed by X-ray crystallography. Azaselenonium compound 116 (X = Cl) was tested for GPx activity in our assay and was found to be a moderately efficient catalyst for the reduction of both hydrogen peroxide and tert-butyl hydroperoxide. Mugesh and coworkers subsequently prepared spirodiazaselenurane 117 and found that substitution on the nitrogen atom was essential. This compound, along with a number of N-aryl analogues, also displays moderate GPx activity. 134

![Figure 1.25 Spirodiazaselenuranes as GPx Mimetics](image)

1.5 Various Assays for Measuring Thiol Peroxidase Activity

There are a number of in vitro assays used for measuring glutathione peroxidase or, more generally, thiol peroxidase activity. These assays are often dependent on the solubility of both the organoselenium catalyst and the thiol reductant. The common feature of all reported assays is that an organoselenium compound is assessed for its efficiency in catalysing the reduction of peroxides in the presence of thiol reductants. A variety of thiols and hydroperoxides have been used and the catalytic activity of a given
GPx mimetic is quite often sensitive to the oxidizing and reducing agents involved. Because catalyst activity is usually subject to assay conditions, most groups compare results of each new catalyst with other well studied catalysts (ebselen, diphenyl diselenide) under a standard set of conditions for whichever assay is employed.

### 1.5.1 NMR Method

Direct observation of the conversion of thiols to disulfides is one of the easiest methods for measuring a catalyst’s efficiency. A number of research groups utilize $^1$H-NMR spectroscopy to monitor the disappearance of proton peaks related to the thiol and the appearance of the associated disulfide. Engman was the first investigator to use $^1$H-NMR spectroscopy to monitor the activity of several diselenides as GPx mimetics. The three thiol substrates that were used in this system were N-acetylcysteine, tert-butyl thiol and 1-octyl thiol. Solubility of the thiol was the largest factor in deciding which solvent to use and so 4:1 D$_2$O to CD$_3$OD was employed for N-acetylcysteine while CD$_3$OD served best for the latter two thiols. Thiol peroxidase activity was monitored at a starting thiol concentration of 0.12 M while a stoichiometric equivalent of hydrogen peroxide was added for a concentration of 0.06 M and catalyst loading was 0.3 mol % relative to the thiol. In Engman’s experiment, catalyst efficiency was measured as the time required for a given catalyst to decrease the thiol concentration by 50%. Iwaoka and coworkers have also used a $^1$H-NMR-based method to measure the catalytic activity of a number of selenides with cysteamine, benzyl thiol or dithiothreitol in CD$_3$OD at various concentrations. They point out that use of a dithiol increases the rate of the reactions being monitored, although it would be unwise to assume that this reflects anything about
the catalyst being used. The use of a dithiol can result in unintended rate acceleration as the formation of a disulfide occurs intramolecularly. As there are unlikely to be dithiols present in vivo, the use of a monothiol, where disulfide formation is intermolecular, more accurately represents what occurs in biological systems.

1.5.2 UV Method with Aromatic Thiols

Hilvert and Wu were able to measure the catalytic activity of their semi-synthetic enzyme selenosubtilisin relative to the activity of diphenyl diselenide\textsuperscript{107} by monitoring the disappearance of an absorbance peak at 412 nm, resulting from 3-carboxy-4-nitrobenzene thiol, in the presence of tert-butyl hydroperoxide. This assay uses initial reaction rates to evaluate catalysts rather than 50% conversion times. In a similar assay, also utilizing initial reaction rates, Tomoda used benzenethiol as the reductant. Monitoring the reaction kinetics at 305 nm for the appearance of the disulfide was convenient, as the thiol has an extinction coefficient of 9 M\textsuperscript{-1} cm\textsuperscript{-1} at this wavelength, while diphenyl disulfide’s extinction coefficient is much larger at 1240 M\textsuperscript{-1} cm\textsuperscript{-1}. Catalysts were used at 1 mol % loadings relative to benzenethiol, while hydrogen peroxide concentration was twice that of the thiol.

Monitoring a reaction by UV detection is certainly convenient; however the use of benzenethiol is questionable. Glutathione, the thiol cofactor for glutathione peroxidase, is an aliphatic thiol. Using an aromatic thiol as a cofactor for a GPx mimetic could potentially misrepresent that catalyst’s activity. This is especially the case when the compound under study has a catalytic cycle in which the rate determining step is reaction
of the catalyst with the thiol (or thiolate). As benzene thiol has a much lower pKa than a typical aliphatic thiol, it more easily ionizes to the thiolate and might therefore react in a rate determining step more quickly than glutathione. It is also important to consider that GPx mimetics that are meant to be designed as potential drug candidates are unlikely to encounter aromatic thiols to act as reductants \textit{in vivo}.

### 1.5.3 Coupled Reductase Enzymatic Method

An indirect measurement of glutathione peroxidase activity was reported by Wendel for measuring the activity of the actual enzyme.\textsuperscript{136} This was later modified by Spector and coworkers to allow measurement of the activity of model compounds.\textsuperscript{78} The method involves the oxidation of glutathione by hydrogen peroxide in the presence of a GPx mimetic (Equation 3). The resulting glutathione disulfide is reduced back to glutathione with glutathione reductase using NADPH as a cofactor (Equation 4). Overall, the consumption of hydrogen peroxide can be monitored indirectly via UV spectroscopy by observing the decrease in the amount of NADPH in solution (Equation 5).

\[
\begin{align*}
2 \text{GSH} + \text{H}_2\text{O}_2 & \xrightarrow{\text{Catalyst}} 2 \text{H}_2\text{O} + \text{GSSG} \quad (3) \\
\text{GSSG} + \text{NADPH} + \text{H}^+ & \xrightarrow{\text{GSH Reductase}} 2 \text{GSH} + \text{NADP}^+ \quad (4) \\
\text{NADPH} + \text{H}_2\text{O}_2 + \text{H}^+ & \rightarrow 2 \text{H}_2\text{O} + \text{NADP}^+ \quad (5)
\end{align*}
\]

The assay is performed in ca. 1 mL of a solution maintained at pH 7.0 with potassium phosphate buffer (50 mM) containing 1 mM EDTA, 1 mM sodium azide, 1
mM glutathione, 0.25 mM NADPH, 1 unit of glutathione reductase and a catalytic amount of test compound. The assay is started by the addition of hydrogen peroxide and the activity of the GPx mimetic can be monitored by observing the disappearance of the UV absorption of NADPH at 366 nm (some groups use 340 nm). Catalysts tested under these conditions are usually evaluated based on initial reaction rates for 5 – 10 % reaction completion. The use of initial reaction rates raises two serious considerations. First, consider the case where the catalyst involved in a study enters the catalytic cycle at a point directly after the rate determining step. In this scenario the activity of the catalyst could be artificially inflated as the observed rate might not include the rate determining step. Secondly, if the reaction is only monitored to 5% completion and the catalyst is only present at 5 mol %, it is not actually possible to determine if the compound being investigated is acting as a catalyst. Investigators should be careful to consider both possibilities if reporting initial reaction rates for catalyst activity.

Engman and coworkers pointed out that a prerequisite condition for accurately measuring catalytic activity with this assay is that the catalyst being evaluated and any associated intermediates are inert towards the NAPDH – glutathione reductase half of the assay. For example, they found that some selenenyl sulfides can act as substrates for glutathione reductase and this ultimately inflates the observed efficacy of the catalyst under evaluation.72
1.5.4 HPLC-UV Detection

Direct measurement of thiol peroxidase activity has also been carried out by injecting reaction aliquots into an HPLC with a UV detector. Our group has used this as a primary method for monitoring GPx activity for all compounds prepared in our laboratory. The assay uses benzyl thiol as the reductant in the presence of either hydrogen peroxide or tert-butyl hydroperoxide. Catalysts are evaluated based on their ability to promote the oxidation of benzyl thiol to dibenzyl disulfide at 10 mol % loading relative to thiol. To monitor the reaction, a naphthalene internal standard is included with every run and the integrated ratio of the dibenzyl disulfide peak to that of naphthalene is determined at 254 nm as a function of time. Generally, each potential GPx mimetic is dissolved in a solution of dichloromethane and methanol (95:5), followed by the addition of the peroxide and finally the benzyl thiol. From this point the formation of dibenzyl disulfide is monitored to determine the time required for 50% conversion of thiol to disulfide. Benzyl thiol was chosen as the thiol cofactor so that the reaction and potential intermediates could also be conveniently monitored by $^1$H-NMR spectroscopy through analysis of the benzylic methylene signal. Other groups have also evaluated GPx mimetics using variations of this method.

1.6 Objectives

Previous work by our group demonstrated that cyclic seleninate ester 98 and spirodioxyselenurane 103 displayed strong GPx activity, while aromatic analogues 108 and 110 had considerably reduced activity. As aromatic compounds are likely less toxic, we wished to examine the effect of substituents on the aromatic rings of 108 and 110.
These derivatives would give us further insight into the mechanism of their catalytic cycle and might result in the discovery of catalysts with increased activity. These considerations directed efforts towards the synthesis of a number of compounds, which provided some of our best catalysts to date. These investigations are described in Chapters Two and Three.

While working with the aromatic spirodioxyselenuranes, we observed unexpected behaviour in the $^1$H-NMR spectral features of the diastereotopic benzylic methylene signals in these molecules. We became intrigued by this phenomenon and initiated an investigation into its origin. This investigation is described in Chapter Four.

Chapter Five discusses the use of a number of diselenides as GPx mimetics. A series of novel and known naphthalene 1,8-\textit{peri}-dichalcogenides was prepared with the expectation that the destabilizing effect of their constrained dihedral angle would facilitate oxidation and improve their catalytic activities. The selenium and tellurium compounds work as efficient GPx mimetics, while both these compounds and the sulfur analogue also displayed interesting photophysical properties.
Chapter Two: Substituent Effects on the Glutathione Peroxidase Activity of Aromatic Cyclic Seleninate Esters

2.1 Introduction

As described in Chapter One, early contributions by our group to the field of GPx mimetics included the discovery of three novel prototype structures 50, 98 and 103. The latter two were of special interest as their catalytic activity was 20 times greater than the benchmark compound, ebselen. The aromatic analogues 108-111 were prepared in an attempt to develop catalysts that were potentially less toxic than their aliphatic counterparts (Figure 2.1).

![Figure 2.1 Early GPx Mimetics From the Back Group](image)

These compounds were expected to be less likely to undergo metabolic degradation than their aliphatic counterparts, making them more amenable to in vivo experiments. Unfortunately catalysts 108-111 displayed severely reduced activity relative to the earlier prototype compounds. It was interesting to note that, within the aromatic subset, the benzoyl derivatives 109 and 111 were the least active catalysts. This suggested that the strongly electron-withdrawing carbonyl substituent positioned ortho to
the selenium atom had a deleterious effect on catalytic activity. It was therefore hoped that, by investigating the effects of aromatic substituents on the catalytic activity of the spirodioxyselenuranes and cyclic seleninate esters, the decrease in activity observed with the aromatic derivatives could be mitigated by judicious choice of substituents. Furthermore, we anticipated that such studies might also provide additional insight into the mechanism of these processes. A number of analogues of both 108 and 110 were prepared, all of which contained substituents at the position para to the selenium atom. Substitution at the para position can be rationalized as this allows for both mesomeric and inductive interactions with the selenium atom, while substitution meta to selenium would only result in an inductive influence. Substitution at the ortho position relative to selenium would allow for both mesomeric and inductive influence, but would introduce unintended steric interactions, thereby complicating our study. The remainder of this chapter details my work on the preparation and use of substituted aromatic cyclic seleninate esters as glutathione peroxidase mimetics. At the same time, a colleague (Eric Mercier) prepared substituted derivatives of 110 and his results are briefly summarized in Section 2.4.

2.2 Model System for Monitoring Glutathione Peroxidase Activity

Previous studies in our lab employed the in vitro assay described in Section 1.5.4 which, for the purposes of comparison and consistency, continued to be used during the course of this investigation. It will be recalled that the assay involved measuring the ability of the selenium compound to catalyse the reduction of peroxides in the presence of benzyl thiol by monitoring the formation of the corresponding disulfide by HPLC. The
justification for the choice of this thiol was provided in Section 1.5.4. The overall process is illustrated in equation 6.

\[
2 \text{BnSH} + \text{ROOH} \xrightarrow{10 \text{ mol\% Catalyst}} \frac{\text{DCM:MeOH (19:1)}}{18 \, ^\circ\text{C}} \xrightarrow{\text{BnSSBn} + \text{ROH} + \text{H}_2\text{O}} (6)
\]

\[R = \text{H or } \text{t-Bu}\]

The solvent for the reaction was a 19:1 mixture of dichloromethane and methanol. This allowed for full dissolution of all reaction components, including a diverse range of catalysts, along with the naphthalene internal standard. All catalyst trials were carried out at 18 °C with the following concentrations of 0.0031 M for the selenium compound, 0.031 M for benzyl thiol, 0.035 M for the peroxide and 0.080 M for naphthalene. Figure 2.2 shows a typical HPLC trace with well separated peaks for benzyl thiol (Rt = 3.692 min.), naphthalene (Rt = 5.748 min.) and dibenzyl disulfide (Rt = 10.42 min.) under HPLC conditions specified in Chapter Seven. Initial studies used both hydrogen peroxide and tert-butyl hydroperoxide as we wished to establish that the compounds could reduce both hydrogen peroxide and alkyl hydroperoxides.
Ebselen (13) is frequently used as a benchmark and as a reference for catalytic activity and so, in order to evaluate our catalysts, we also tested ebselen for activity. We discovered that the type and concentration of peroxide used in the assay had significant impact on catalyst activity. For example, when the source of oxidant was a 90% tert-butyl hydroperoxide solution we found that ebselen displayed a $t_{1/2}$ of 42 hours. If the concentration was reduced to 58%, the same catalyst displayed a $t_{1/2}$ of 62 hours and, when 32% hydrogen peroxide was used, the $t_{1/2}$ was 24 hours. The prolonged reaction times when tert-butyl hydroperoxide was used as the oxidant, coupled with the cessation of commercial availability of 90% tert-butyl hydroperoxide, resulted in the exclusive use of hydrogen peroxide for subsequent experiments.

In addition to testing ebselen as a benchmark in our assay, we also ran a control experiment to determine the rate of uncatalysed thiol oxidation. With no catalyst present
and 32% hydrogen peroxide as the oxidant, very slow conversion of benzyl thiol to benzyl disulfide was observed. This background reaction had a $t_{1/2}$ that was measured as 176 hours.

2.3 Aromatic Cyclic Seleninate Esters as Glutathione Peroxidase Mimetics

The decreased activity of 108 relative to that of the aliphatic analogue 98 prompted the synthesis of substituted benzo-derivatives of the aliphatic congener. To carry out the study of the effect of para-substitution on activity, the target compounds shown in Figure 2.3 were chosen for synthesis. Among them, cyclic seleninate esters 119-122 were new, while 118 and 123 had been synthesized by Dušan Kuzma, a former M.Sc. student in our group, and patented previously. Although compounds 118 and 123 had been prepared previously, comparison of their catalytic activity within a broader range of derivatives had not yet been carried out. We expected that, with 108 and 118-123 in hand, further insight into the mechanism of the catalytic cycle of the cyclic seleninate esters would be possible. Additionally, it was hoped that a wide array of derivatives would result in the identification of structural features for the synthesis of more efficacious GPx mimetics.
Subsection 2.3.1 will cover the synthesis of 108 and 118-123, while their evaluation as GPx mimetics will be discussed in Subsection 2.3.2. The use of a large number of derivatives, all substituted at the position para to the selenium atom, allowed us to make use of a Hammett-type analysis, as discussed in Subsection 2.3.3.

2.3.1 Synthetic Approaches to Aromatic Cyclic Seleninate Esters

The synthesis of the aliphatic cyclic seleninate ester 98 had been developed by Ziad Moussa, a former PhD student in our group (Scheme 2.1). The route involved forming di(3-hydroxypropyl) diselenide (124) via the reaction of 3-bromopropanol with a selenium nucleophile, prepared in situ from sodium borohydride and elemental selenium. The di(3-hydroxypropyl) diselenide could be easily converted into two equivalents of the corresponding selenolate and reacted with allyl iodide to form selenide 93. Our group expected that oxidation of allyl selenide 93 to its selenoxide would result in a [2,3] sigmatropic rearrangement. We hoped that this would ultimately end in the in situ generation of the associated selenenic acid 100 and its selenol counterpart (the latter in the presence of thiol). Both the selenenic acid and selenol functional groups are present in
the catalytic cycle of GPx. Instead, exposure of allyl 3-hydroxypropyl selenide (93) to an excess of tert-butyl hydroperoxide resulted in formation of the stable cyclic seleninate ester 98. This last step was described in more detail in Chapter One.

Scheme 2.1

Later work by Dušan Kuzma focussed on formation of the aromatic cyclic seleninate 108. His route focussed on the preparation of an aromatic analogue of allyl selenide 93 and was extended to include compounds with substituents on the aromatic ring.

The Kuzma route started with the appropriately substituted anthranilic acids (Scheme 2.2). The corresponding diazonium salts were reacted with potassium diselenide (K$_2$Se$_2$), prepared by melting elemental selenium in the presence of powdered potassium hydroxide and quenching with water at 0 °C. After air oxidation, the products of this reaction were diselenides 126. In order to prepare allyl selenides 127, these diselenides were reacted with lithium aluminum hydride, which reduced both the carboxylic acid and...
the diselenide bond. The reaction mixture was treated with allyl iodide, resulting in the formation of allyl selenides 127a and 127g. The preparation of allyl selenide 127b required a slightly modified route. In this case, lithium-halogen exchange on 2-bromo-5-methoxybenzyl alcohol (128) resulted in aryllithium 129, which could be quenched with elemental selenium to form diselenide 130b upon oxidation in air. This diselenide was reacted with sodium borohydride and finally allyl iodide to form allyl selenide 127b. The allyl selenides were then oxidized with tert-butyl hydroperoxide to form cyclic seleninate esters 108, 118 and 123.

Scheme 2.2

I prepared cyclic seleninate esters 119-122 by a slightly different route, which also worked for 108 and 123. The anthranilic acids 125a,c-g were all commercially
available. Diazotization of the anthranilic acids, followed by reaction with potassium diselenide led to product mixtures which contained diselenides 126a,c-g as the major components. Unfortunately, a significant amount of the corresponding selenide and polyselenide products were also present. These byproducts could not be separated from the mixture and so the subsequent step was carried out without purification. The reduction of diselenides 126a,c-g with lithium aluminum hydride, followed by reaction with allyl iodide resulted in low yields for allyl selenides 127a,c-g. Instead of using Dušan Kuzma’s one pot procedure, I found that carrying out each step separately provided higher yields. Lithium aluminum hydride reduction not only converts the carboxylic acid to its alcohol, but destroys any polyselenides in the mixture as all selenium – selenium bonds are also reduced. Quenching the reaction with a large volume of water allowed for the water-soluble selenolates (ArSe¯) to be easily separated from the refractory selenides, which remained in the organic phase during work up of the reaction mixture. Air oxidation of these selenolates resulted in the slow precipitation of diselenides 130a,c-g from the aqueous solution. This step occurs readily and is facilitated by stirring the selenolate solution for a period of 8-12 hours in an flask open to the atmosphere. Subsequent reduction of diselenides 130a,c-g under milder conditions and alkylation of the resulting selenium nucleophiles with allyl iodide formed allyl selenides 127a,c-g. These allyl selenides were easily converted into the desired cyclic seleninate esters 108 and 119-123. These processes are illustrated in Scheme 2.3.
Scheme 2.3

It should be noted that conversion of diselenides 130a-g directly into the cyclic seleninate esters is also possible. This likely occurs via hydrogen peroxide mediated oxidation of the diselenides to two molecules of the seleninic acids 131 (or possibly the corresponding anhydrides). Cyclisation and dehydration of these compounds forms the cyclic seleninate esters 108 and 118-123 (see Scheme 2.4). However, it was found that this route resulted in product mixtures which were difficult to purify. As the allyl selenides were prepared in good yields, were easy to purify and could be converted into compounds 108 and 118-123 in high yields, the extra step was justified.

Note: Acid 125b was not commercially available. For compound 118 (R=OMe) see Scheme 2.2.
2.3.2 Catalytic activity of Substituted Cyclic Seleninate Esters

Using the standard assay, the catalytic activity of the above series of aromatic seleninate esters was measured. The $t_{1/2}$ values reported in Table 2.1 are the average of at least two separate trials. These values were obtained from graphs of yield (\%) of dibenzyl disulfide as a function of time (see Figure 2.4 for representative graphs). The control reaction (entry 1) demonstrated that the uncatalysed reaction occurred at a slower rate ($t_{1/2} = 176$ hours) than any of the catalysed reactions. Ebselen (13) was used as a benchmark for activity, and it has modest activity under the conditions of our assay (entry 2, $t_{1/2} = 24$ hours). The activity for the aliphatic cyclic seleninate ester (98, entry 3) was similar to that of ebselen with a $t_{1/2}$ of 18 hours (this value is from a previous report\textsuperscript{129}). The unsubstituted aromatic derivative (108), as discussed earlier, displayed reduced activity relative to the aliphatic compound and had $t_{1/2} = 50$ hours (entry 4). The activity of the aromatic catalysts could be modified with substitution at the para-position relative to the selenium atom. It was found that substitution with the strongly electron-donating methoxy group in 118 improved catalytic activity and the $t_{1/2}$ was found to be 38 hours (entry 5). Although this is better than that of the unsubstituted compound, it was unfortunate that the activity did not match the activity of the aliphatic compound. Catalyst 119, with a weakly electron-donating methyl group, had catalytic activity with a
t<sub>1/2</sub> of 48 hours that was nearly identical to that of the unsubstituted derivative (entry 6). When the substituent was a phenyl ring (catalyst 120), which is mildly electron-withdrawing, the activity was decreased (entry 7, t<sub>1/2</sub> = 57 hours) relative to 108. As the substituent became more electron-withdrawing, the activity decreased further (entries 8-10). Catalytic activities for the halogen-substituted compounds 121-123 were thus 25-40% slower than that of the unsubstituted derivative. The correlation between the electronic nature of the substituents and the catalysts will be discussed further in Section 2.3.3. The results in Table 2.1 demonstrate that the catalytic activity of the cyclic seleninate esters is sensitive to substituent control. Although initial modification of unsubstituted compound 108 did not return catalytic activity to that of the aliphatic compound 98, the exploitation of substituent effects on aromatic selenium derivatives offered a route to potentially better GPx mimetics.

Figure 2.4 shows the kinetic data for the oxidation of benzyl thiol to dibenzyl disulfide with hydrogen peroxide for the control reaction (no catalyst) and separate graphs for each catalyst. Whereas the data in Table 2.1 is the average of at least two trials per catalyst, the data presented in Figure 2.4 are representative graphs for individual catalyst experiments. There is clearly a rate enhancement in the presence of any of the cyclic seleninate esters relative to the uncatalysed reaction. The greatest enhancement in rate occurs for catalyst 118. Further mechanistic insight can be gained through a Hammett analysis which is the subject of Section 2.3.3.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>$t_{1/2}$ (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nil</td>
<td>-</td>
<td>176</td>
</tr>
<tr>
<td>2</td>
<td>Ebselen (13)</td>
<td><img src="image" alt="structure_1" /></td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>98</td>
<td><img src="image" alt="structure_2" /></td>
<td>18$^{129}$</td>
</tr>
<tr>
<td>4</td>
<td>108</td>
<td><img src="image" alt="structure_3" /></td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>118</td>
<td><img src="image" alt="structure_4" /></td>
<td>38</td>
</tr>
<tr>
<td>6</td>
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<td><img src="image" alt="structure_5" /></td>
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<td>7</td>
<td>120</td>
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<td>9</td>
<td>122</td>
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<td>69</td>
</tr>
<tr>
<td>10</td>
<td>123</td>
<td><img src="image" alt="structure_9" /></td>
<td>72</td>
</tr>
</tbody>
</table>

$^a$Conditions: BnSH (0.031 M), H$_2$O$_2$ (0.035 M) and catalyst (0.0031 M) were dissolved in 19:1 DCM-MeOH. Reactions were monitored until after $t_{1/2}$. Data for entry 3 was from a previous report.$^{129}$
Figure 2.4 Single Reaction Plots in the Absence and Presence of 108 and 118-123. All graphs: x-axis in hours; y-axis in %BnSSBn.
2.3.3 Hammett Analysis for Cyclic Seleninate Esters

There are two important structural components which contribute to a molecule’s reactivity; those that are steric in nature and those that are electronic.\textsuperscript{141} Within the cyclic seleninate esters, the selenium atom plays the central role for catalytic activity. The variable substitution found in \textbf{108} and \textbf{118-123} occurs \textit{para} to the selenium atom and so any steric influence on reactivity is negligible. This means that it is possible to very accurately determine the electronic effect of substituents on the catalytic activity of the cyclic seleninate esters.

\[
\log \frac{k}{k_o} = \rho \sigma \quad (7)
\]

The Hammett equation (Equation 7) allows for a quantitative analysis of the relationship between the electronic contribution of substituents and reactivity. In the present case, \(k_o\) represents the rate constant for the catalysed reaction when \(R = H\), while \(k\) is the rate constant when \(R \neq H\). Every substituent has an intrinsic reaction constant associated with it, which is represented by \(\sigma\). These values have been determined experimentally by measuring that substituent’s effect upon the ionization of \textit{para}-substituted benzoic acids\textsuperscript{142} and are standard for the Hammett equation. The value \(\rho\) is the reaction constant for a given reaction under a standard set of conditions. It is possible to determine \(\rho\) by plotting \(\log(k/k_o)\) against the substituent constants (values of \(\sigma\)) which yields a roughly linear relationship with slope \(\rho\). Once this value is obtained, considerable insight into the effect of substituents on the reaction being investigated can be gained. A negative value of \(\rho\) signifies that an increase in positive charge occurs in the transition
state of the rate-determining step, which can be stabilized by electron-donating groups.
On the other hand, a positive value of $\rho$ suggests an increase in negative charge at the reaction centre, which would be stabilized by electron-withdrawing substituents. The Hammett plot shown in Figure 2.5 was constructed from standard values of $\sigma_{\text{para}}^{143}$ and the kinetic data available from the graphs shown in Figure 2.4 (and from the multiple trials run per catalyst). Figure 2.5 shows that the value for $\rho$ is -0.47, indicating that an increase in positive charge and stabilization by electron-donating groups is occurring in the rate-determining step of the catalytic cycle.

![Hammett Plot of Substituted Cyclic Seleninate Esters](image)

**Figure 2.5.** Hammett Plot of Substituted Cyclic Seleninate Esters

### 2.4 Aromatic Spirodi oxy selenurananes as Glutathione Peroxidase Mimetics

Parallel work was carried out by former M.Sc. student Eric Mercier on the investigation of aromatic analogues of spirodi oxy selenuranane 103. The synthesis of these aromatic compounds followed a similar strategy as that involved in the synthesis of the
cyclic seleninate esters 108 and 118-123. Briefly, substituted diselenide precursors 130 were prepared the same way as previously described (see Scheme 2.3). These were again reduced to selenolate nucleophiles which were reacted with the appropriate diazonium salt to produce symmetrical selenides 132. Oxidation of the selenides with hydrogen peroxide resulted in conversion to the desired spirodioxselenuranes 110 and 133-137 (Scheme 2.5).

Scheme 2.5

For comparison, entries in Table 2.2 show the values for reaction half times ($t_{1/2}$) for the substituted aromatic spirodioxselenuranes 110 and 133-137. It was known that aliphatic spirodioxselenurane 103 displayed markedly better catalytic activity than aliphatic cyclic seleninate 98 and this was also the case when comparing their aromatic counterparts. Catalytic activities of the spirodioxselenuranes are also subject to substituent effects. It was again found that catalytic activities were higher with electron-donating substituents, while electron-withdrawing substituents decreased activity. In the case of the spirodioxselenuranes, 133 ($R = $MeO) displayed catalytic activity nearly as high as that of the aliphatic compound 103.
## Table 2.2. GPx Activity of Spirodioxyselenuranes\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>(t_{1/2}) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nil</td>
<td>-</td>
<td>176</td>
</tr>
<tr>
<td>2</td>
<td>103</td>
<td><img src="image" alt="Structure" /></td>
<td>0.2(^{129})</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td><img src="image" alt="Structure" /></td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>133</td>
<td><img src="image" alt="Structure" /></td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>134</td>
<td><img src="image" alt="Structure" /></td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>135</td>
<td><img src="image" alt="Structure" /></td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>136</td>
<td><img src="image" alt="Structure" /></td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>137</td>
<td><img src="image" alt="Structure" /></td>
<td>22</td>
</tr>
</tbody>
</table>

\(^a\)Conditions: BnSH (0.031 M), \(\text{H}_2\text{O}_2\) (0.035 M) and catalyst (0.0031 M) were dissolved in 19:1 DCM-MeOH. Reactions were monitored until after \(t_{1/2}\). Data for entry 2 was from a previous report.\(^{129}\)

The Hammett plot for the substituted spirodioxyselenuranes (Figure 2.6) displays a relationship similar to that of the cyclic seleninate esters (Figure 2.5). For compounds 110 and 133-137 the value for \(\rho\) is again negative, which is indicative of positive charge build-up in the transition state of the rate-determining step. In this case, \(\rho = -3.1\) and is about seven times larger in magnitude than that for the cyclic seleninate esters. This indicates that the spirodioxyselenuranes are more sensitive to the nature of the \(para\)-substituent than the cyclic seleninate esters, and more susceptible to regulation of their catalytic activity through substituent effects.
2.5 Mechanism of Peroxide Reduction Catalysed by Cyclic Seleninate Esters

Dušan Kuzma investigated whether the catalytic cycle responsible for the activity of aliphatic cyclic seleninate ester 98 (see Scheme 1.13) could be applied to the aromatic compounds. His findings demonstrated that the two cycles were similar and the catalytic cycle for the cyclic seleninate ester 108 is shown in Scheme 2.6. The initial step in the catalytic cycle is reaction of benzyl thiol with 108 to form thioseleninate 138. This thioseleninate reacts with a second molecule of benzyl thiol to form selenenic acid 139 and dibenzyl disulfide. Oxidation of selenenic acid 139, followed by cyclization and loss of water completes the dominant portion of the catalytic cycle. As in the case of the catalytic cycle of 98, the corresponding selenenic acid 139 also reacts with benzyl thiol to form the corresponding selenenyl sulfide 140. However, unlike in the aliphatic series, the formation of 140 is competitive with direct oxidation of 139 back to 108, especially at higher thiol to peroxide ratios. Dušan Kuzma synthesized compound 140 independently.
and found it to be inert toward thiol and only sluggishly reactive with peroxide to eventually reform 108.\textsuperscript{144} Analogously, the aliphatic analogue of 140 displays a $t_{1/2}$ which is 14 times longer than the aliphatic cyclic seleninate ester 98.\textsuperscript{126b} It will also be recalled that Mugesh and coworkers have shown that selenenyl sulfides, with groups capable of coordinating to selenium, are poor catalysts due to thiol exchange reactions which occur for these molecules.\textsuperscript{77} Thus, the formation of 140 is a deactivation pathway for 108, although slow oxidation to thioseleninate 138 results in re-entry to the catalytic cycle. Selenenyl sulfide 140 can also slowly disproportionate to form a molecule of diselenide 130a and dibenzyl disulfide in a minor pathway.\textsuperscript{144} This diselenide can undergo oxidation\textsuperscript{140} to form cyclic seleninate ester 108 and so it too can re-enter the catalytic cycle.

\begin{center}
\textbf{Scheme 2.6}
\end{center}
Selenenyl sulfide 140 and diselenide 130a are observable intermediates that can be identified in the reaction mixture for the duration of each kinetic run as monitored by HPLC. Figure 2.7 shows an HPLC trace for the oxidation of benzyl thiol to dibenzyl disulfide in the presence of hydrogen peroxide and cyclic seleninate ester 108. The HPLC trace was recorded at approximately 7 hours into the experiment and the peaks for diselenide 130a (Rt = 2.388 min) and selenenyl sulfide 140 (Rt = 5.357 min) are clearly resolved. Also seen on the trace are the peaks for benzyl thiol (Rt = 3.926 min), the naphthalene internal standard (Rt = 5.994 min) and dibenzyl disulfide (Rt = 10.671 min).

![HPLC Trace](image_url)

**Figure 2.7. HPLC Trace of for the Rxn Catalysed by 108 at Observed at 7 Hours**

The Hammett plot for the cyclic seleninate esters (Figure 2.5), with a negative value for ρ, indicates that the catalytic cycle shown in Scheme 2.6 involves a build-up of positive charge on selenium during the rate determining step. Since it is the oxidation of the selenium atom from Se(II) to Se(IV) where an increase of positive charge occurs, we conclude that this step is rate-determining. Therefore, it is the oxidation of intermediates
like \textbf{139} which is enhanced by the presence of electron-donating groups \textit{para} to the selenium atom.

\textbf{2.6 Summary and Conclusions}

Chapter Two describes the synthesis of a variety of substituted aromatic cyclic seleninate esters and their evaluation as GPx mimetics for the reduction of hydrogen peroxide in the presence of benzyl thiol. All compounds were compared in a standard assay which involved measuring the time required for the catalysed reaction to reach 50\% completion. Kinetic data was gathered for each catalyst and a Hammett analysis was carried out for various \textit{para}-substituted derivatives. In the case of electron-donating substituents, such as methoxy (\textbf{118}) and methyl (\textbf{119}), catalytic activities were improved relative to the unsubstituted seleninate ester \textbf{108}. On the other hand, electron-withdrawing substituents, such as phenyl (\textbf{120}) and the halogens (\textbf{121-123}), reduced catalytic activity relative to \textbf{108}. The Hammett analysis thus indicated that the rate-determining step in the catalytic cycle involved an increase in positive charge on the selenium atom. Unfortunately, the most active aromatic cyclic seleninate ester (\textbf{118}) was still not as effective a catalyst as the original aliphatic cyclic seleninate ester (\textbf{98}). We found that aromatic spirodioxyselenuranes were more sensitive to electronic effects. When the substituent was methoxy for these compounds (i.e. \textbf{133}) catalytic activity was almost at the same level as that of the aliphatic congener \textbf{103}.

The accumulation of the much less catalytically active selenenyl sulfide \textbf{140} (along with similar species corresponding to catalysts \textbf{118-123}) on the HPLC trace shown
in Figure 2.7 demonstrates the presence of a deactivation pathway for these compounds. These selenenyl sulfides display weaker GPx activity than the cyclic seleninate esters. Their propensity to form from the aromatic cyclic seleninate esters, especially when thiol to peroxide ratios are high, as would be expected \textit{in vivo}, where thiols such as glutathione are abundant, precludes their use as effective GPx mimetics except in cases of extreme oxidative stress. However, the cyclic seleninate esters have an advantage over some other selenium compounds proposed for use as GPx mimetics. The catalytic cycle presented in Scheme 2.6 does not contain any selenol or selenolate species in either the main catalytic cycle or the deactivation pathway. These selenol and selenolate compounds, as discussed in Section 1.3, can actually catalyse the formation of ROS via the reduction of dioxygen to superoxide anion radical.
Chapter Three: The Synthesis and Evaluation of Methoxy-Substituted GPx Mimetics

3.1 Introduction

Chapter Two clearly demonstrates that the activity of the spirodioxyelenuranes and cyclic seleninate esters tested as GPx mimetics by our group are subject to electronic effects. The enhanced catalytic activity of compounds 118 and 133, both of which contain methoxy groups substituted para to selenium, resulted from a stabilization of increased positive charge in the rate-determining step of their respective catalytic cycles. Using this information, we hoped to improve the efficiency of both classes of compounds, while maintaining their aromatic backbones. The clear choice for doing so was to prepare compounds that had multiple electron-donating groups, capable of stabilizing the increase in positive charge involved when Se (II) gets oxidized to Se (IV).

Compounds with methoxy groups were chosen as synthetic targets of interest (Figure 3.1). For example, dimethoxy cyclic seleninate esters 141 and 142 and tetramethoxy spirodioxyelenuranes 143 and 144 along with trimethoxy compound 145 and hexamethoxy compound 146 were chosen as synthetic targets. It was of interest to see which of these compounds would display additive effects of multiple methoxy groups. In order to understand the effect of specific methoxy groups at each position on the ring, additional monosubstituted compounds with either meta-methoxy groups or with ortho-methoxy groups were also of interest. Thus, cyclic seleninate ester 147 and spirodioxyelenurane 148, containing methoxy groups at the position meta to selenium, along with ortho-methoxy compounds 149 and 150 were targeted for synthesis.
Methoxy groups ortho and para to the selenium atom were expected to enhance catalytic activity mesomerically, while ortho-methoxy substituents might also affect rates through steric or coordination effects. In the case of meta-substitution, methoxy groups cannot interact with the selenium mesomerically, but can withdraw electrons inductively, which may reduce catalytic activity. The above compounds were made to test these hypotheses. Mugesh and coworkers have also prepared GPx mimetics with methoxy groups present at either the para or ortho position relative to the selenium atom in diaryl diselenides\(^{88-89}\) (see Chapter One). In their case, para-methoxy groups had little effect on activity, while ortho-methoxy groups improved catalyst activity through steric suppression of thiol exchange reactions in selenenyl sulfide intermediates.
3.2 Synthesis of ortho-Methoxy and meta-Methoxy Analogues

Spirodioxyselenuranes and cyclic seleninate esters, which have the same aromatic substitution pattern, can generally be prepared from a single intermediate diselenide. Compounds 147 and 148 can be envisioned to arise from diselenide 151, while compounds 149 and 150 could potentially be prepared from diselenide 152. Based on our previous success in preparing catalysts from anthranilic acid derivatives,\textsuperscript{145} it was reasonable to expect that 151 and 152 could be prepared from anthranilic acids 153 and 154 (see Scheme 3.1). This was the proposed starting point for 147-150 as both 2-amino-4-methoxybenzoic acid (153) and 2-amino-3-methoxybenzoic acid (154) were commercially available.

Scheme 3.1
The first step in the forward direction was the attempted diazotization of anthranilic acids 153 and 154. Reaction of the corresponding diazonium species with potassium diselenide (prepared from potassium hydroxide and elemental selenium) was expected to provide the diselenide intermediates with the desired methoxy-substitution patterns. Unfortunately, diazotization of 153 or 154, followed by exposure to potassium diselenide did not afford the diselenide products. The corresponding 2-amino-4-methoxybenzyl alcohol (155) and 2-amino-3-methoxybenzyl alcohol (156) were treated similarly, but only 156 produced any of the desired diselenide (Scheme 3.2). The yield (10%) for this step was not high enough to support the preparation of the downstream products. Therefore, other routes were explored.

Scheme 3.2

An alternative route to diselenides 151 and 152 would be through reaction of the corresponding aryllithium species with elemental selenium. The aryl halides that would be required for lithium – halogen exchange were not commercially available. In order to prepare the required aryllithium species, directed ortho lithiation routes were investigated. A number of amide derivatives of p-anisic acid (157) and m-anisic acid
(158) were prepared. These included the \(N,N\)-diisopropyl amides 159 and 160, \(N\)-methyl amides 161 and 162, \(N\)-phenyl amides 163 and 164 and the oxazolines 165 and 166. In general, these amides were prepared by first forming the acid chloride derivatives of 157 or 158 and reacting these with the appropriate amine (Scheme 3.3). Secondary amides, tertiary amides and oxazolines are known to be strong directing groups for metallation.\(^\text{147}\)

**Scheme 3.3**

\[\text{It was possible to introduce selenium at the 2-position for all of the prepared compounds, except for 161, whose product diselenide underwent decomposition prior to isolation. Generally, compounds 159-166 were dissolved in tetrahydrofuran, lithiated with } n\text{-butyllithium and the resulting aryllithium species were reacted with elemental} \]
selenium. Upon work-up and air oxidation, diselenides 167-173 (see Figure 3.2) were isolated, although they generally contained polyselenide impurities which could not be separated. These impurities were not a concern as their eventual conversion to the desired diselenides 151 and 152 would occur.

![Chemical structures of compounds 167-173](image)

**Figure 3.2 meta-Methoxy and ortho-Methoxy Diselenides**

Hydrolysis of each of the crude derivatives 167-173 followed by reduction would form the required diselenides 151 and 152. A number of increasingly aggressive reaction conditions were used to attempt the hydrolysis of 167 and 168. For example, no hydrolysis was observed when either compound was refluxed in THF and 2 M aqueous KOH, 2 M aqueous KOH alone, a mixture of 1 M aqueous KOH and methanol or a mixture of 1 M aqueous KOH, methanol and hydrogen peroxide. Both 167 and 168 were similarly robust when subjected to para-toluenesulfonic acid in refluxing toluene, refluxing glacial acetic acid, refluxing 50% aqueous acetic acid or a mixture of 6 M aqueous HCl and methanol. Diselenide 169 was expected to be easier to hydrolyse than the N,N-diisopropyl amides. However, attempts to hydrolyse 169 in the presence of aqueous sodium hydroxide led to decomposition of the starting material. Attempted
hydrolysis of 169 under acidic conditions resulted only in recovered starting material under a variety of conditions.

Hydrolysis attempts on the N-phenyl amide 170 were severely limited by this compound’s insolubility in most solvents. When ortho-methoxy derivative 171 was treated with an excess of para-toluenesulfonic acid in refluxing toluene the only isolable product arose from the reaction of polyselenide impurities present in the starting material. This compound was identified as the corresponding benzo[1,2]diselenol-3-one (174) and similar compounds are known in the literature.\textsuperscript{148} Formation of 174 was interesting, but it was not the intended product nor was enough isolated to investigate its conversion into the required diselenide. Further attempts to hydrolyse 171 to the acid were ultimately unsuccessful.

![174](image)

The oxazoline moieties of 172 and 173 were easily hydrolysed to esters 175 and 176 (Scheme 3.4). This reaction was complete for both compounds after 2 hours of heating in a 1:1 mixture of THF and 1 M HCl. Reduction of these esters was expected to produce the desired diselenides 151 and 152. When 175 was subjected to reduction with lithium aluminum hydride, diselenide 151 was produced in moderate yield. Ester 176 did not react in the same fashion and efforts to reduce this to diselenide 152 resulted in a mixture of unidentified compounds.
Introduction of selenium was also carried out on anisaldehydes 177 and 178 using a different directed ortho lithiation approach. Comins and Brown developed an ortho-directed route for lithiation of aldehydes by first reacting the aldehyde with the lithium amide of $N,N,N'$-trimethylethlenediamine. The resulting intermediates 179 and 180 are exceptional directing groups for further lithiation. Reaction of 179 and 180 with an equivalent of $n$-butyllithium was followed by exposure to elemental selenium. Quenching the reaction mixture with 1 M HCl in the presence of air produced diselenides 181 and 182. Column chromatography of 181 and 182 resulted in decomposition and, in the case of 182, complete loss of product. It was found that we could reduce the aldehydes and recover the benzyl alcohol products 151 and 152 as pure compounds without prior purification of 181 and 182 (See Scheme 3.5). In this case, the yields of 151 and 152 were reproducibly around 50% over two steps for each diselenide.
Reliable, high yielding routes to 151 and 152 allowed for the routine preparation of meta-methoxy and ortho-methoxy derivatives of our GPx mimetics. The route to cyclic seleninate esters 147 and 149 involved the preparation of the corresponding allyl selenides (183 and 184, respectively). These were easily prepared by alkylation of the selenolates derived from 151 and 152 with allyl iodide. Oxidation of both allyl selenides 183 and 184 with hydrogen peroxide resulted in their respective conversion into cyclic seleninate esters 147 and 149. These processes are shown in Scheme 3.6.
The spirodioxyselenuranes 148 and 150 (see Figure 3.1) required the preparation of selenides 185 and 186. Reaction of the appropriate selenolates with the diazonium salts derived from 2-amino-4-methoxybenzyl alcohol (155) and 2-amino-3-methoxybenzyl alcohol (156) produced the corresponding selenides (Scheme 3.7). Unfortunately, selenides 185 and 186 were not easily separated from the starting diselenides, which were present as a 30-50\% impurity.

**Scheme 3.7**

The decision was made that, whenever possible, all GPx mimetics investigated in our lab would be measured for activity by starting with the catalyst in its reduced state. This helps eliminate the unintentional initial quick formation of dibenzyl disulfide observed for the spirodioxyselenuranes.\(^{145}\) Although this is not a large issue for catalysts measured in our assay, it can artificially inflate the measured activity of a compound if the measurement is an initial reaction rate (see Chapter One). The easiest way to purify selenides 185 and 186 was to oxidize the diselenide containing mixtures. This produced both the corresponding spirodioxyselenuranes and cyclic seleninate esters, which are more easily separated via column chromatography. Compounds 185 and 186 were finally
obtained in analytically pure form after reduction of their respective spirodioxyselenuranes back to the selenides with benzyl thiol.

The lengthy purification procedure for 185 and 186 prompted us to design a new group of derivatives which could be easily prepared in high yield and purified by routine column chromatography. The route to these compounds involved alkylation of aryl selenolates, prepared from the symmetrical diaryl diselenides, with 1-bromo-3-propanol to produce selenides expected to exhibit GPx activity which matched or exceeded that of the symmetrical aryl selenides. A complete set of alkyl aryl selenides substituted with either an ortho, meta or para methoxy-group or unsubstituted were prepared. Therefore, the reduction of diselenides 130a, 130b, 151 and 152, followed by alkylation produced 3-hydroxypropyl aryl selenides 187-190 (Scheme 3.8).

Scheme 3.8
As discussed in Chapter One, aromatic derivatives were prepared because they are expected to be more stable against metabolic degradation, which could release inorganic selenium species. The reason for this is that aromatic carbon – selenium bonds are stronger than aliphatic carbon – selenium bonds. This is not a concern for compounds 187-190 because these still retain a strong aromatic carbon – selenium bond. In fact, if the aliphatic carbon – selenium bond were to break, we expected that the resulting aryl selenium species could enter the catalytic cycle as the corresponding seleninate esters.

A preliminary solubility investigation of 187-190 demonstrated that they all dissolved in the dichloromethane – methanol mixture that we use for our \textit{in vitro} assay. These compounds were also found to be soluble in a mixture of 5\% ethanol in water for concentrations up to 2 mg/mL, above the level required for testing these compounds \textit{in vitro} or \textit{in vivo}. We anticipated that selenides with a 2,3-dihydroxypropyl group attached to selenium would be even more water soluble. Therefore, selenides 191-194 were prepared via the alkylation of selenolates with (S)-glycidol (Scheme 3.9). Solubility tests on selenides 191-194 showed that they not only dissolved in the usual dichloromethane – methanol mixture, but that they were quite soluble in water, without an organic co-solvent, at concentrations higher than 2 mg/mL. Their expected low toxicity and aqueous solubility made them promising candidates for \textit{in vivo} assays.
3.3 Positional Effect of Methoxy-Substitution on GPx Activity

The $t_{1/2}$ values reported in all tables in Chapter Three are the average of at least two separate trials carried out under the standard conditions (0.031 M BnSH, 0.035 M H$_2$O$_2$ and 0.0031 M catalyst in 19:1 DCM to MeOH, at 18 °C) of our assay.

Measuring the GPx activity for compounds 147 and 149 would complete the dataset for the aromatic cyclic seleninate esters. For purposes of comparison, the $t_{1/2}$ values for compounds 108 and 118 are included in Table 3.1. As shown earlier, compound 108 had a $t_{1/2}$ of 50 hours (entry 1), while the introduction of a methoxy group para to the
selenium atom (i.e. 118) decreased the \( t_{1/2} \) to 38 hours (entry 2). Compound 147, with a meta-methoxy group relative to the selenium atom, had a \( t_{1/2} \) of 50 hours (entry 3). When the methoxy group was at the ortho-position, the \( t_{1/2} \) extended to 70 hours (entry 4). This demonstrates that, although a para-methoxy group improves catalyst activity, when the same group is at the meta-position it has no overall effect (compare entries 1 and 3), while if it is ortho, as in 149, activity is suppressed (compare entries 1 and 4). The large decrease in activity seen for compound 149, relative to unsubstituted compound 108 is attributable to the methoxy group sterically shielding the selenium atom and slowing its oxidation. Figure 3.3 shows the kinetic plots for the reaction catalysed by compounds 147 and 149.

![Diagram](image)

**Table 3.1 GPx Activity of Cyclic Seleninate Esters**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>( t_{1/2} ) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>( R_1 = R_2 = R_3 = H )</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>( R_1 = OMe, R_2 = R_3 = H )</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>147</td>
<td>( R_1 = R_3 = H, R_2 = OMe )</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>149</td>
<td>( R_1 = R_2 = H, R_3 = OMe )</td>
<td>70</td>
</tr>
</tbody>
</table>

**Figure 3.3 Single Reaction Plots for 147 and 149. All graphs: x-axis in hours; y-axis in \%BnSSBn.**

97
The measurement of GPx activity of selenides 185 and 186 would introduce an inconsistency in our data set if we wished to compare the activity of these two compounds to the earlier spirodioxyselenuranes 110 and 133, in which the selenium atoms are in a higher oxidation state. Therefore, selenides 132a and 132b were also tested for GPx activity. The data for these compounds is reported in Table 3.2. Surprisingly, the t$_{1/2}$ values observed for 132a (t$_{1/2}$ = 20 hours, entry 1) and 132b (t$_{1/2}$ = 5.5 hours, entry 2) were much longer than for their respective spirodioxyselenuranes as measured by Eric Mercier.$^{145}$ At this time, the reason for this difference is not clear but, importantly, the results for 132a and 132b were reproducible during multiple trials on each selenide. As expected, the para-methoxy group in 132b has a significant enhancing effect upon catalytic activity relative to the unsubstituted compound. In the case of these symmetrical aryl selenides, substitution with a meta-methoxy group (i.e. 185) produces a catalyst which is considerably slower (t$_{1/2}$ = 17 hours, entry 3) than para-methoxy substituted selenide 132b (t$_{1/2}$ = 5.5 hours, entry 2) and near the same efficiency as the unsubstituted compound. This was expected as a methoxy group at the meta-position is inductively electron-withdrawing and not able to stabilize positive charge on the selenium atom through mesomeric effects. A further decrease in activity was observed if the methoxy group was substituted at the ortho-position relative to selenium, as demonstrated by selenide 186, which had a t$_{1/2}$ = 21 hours (entry 4). Similarly to the cyclic seleninate esters, this can be attributed to steric repulsions due the ortho-methoxy groups. In this case, the selenium atom is shielded by a methoxy group on each ring, which likely slows the reaction with hydrogen peroxide considerably. Figure 3.4 shows the kinetic plots in the presence of selenides 132a, 132b, 185 and 186.
Hybrid selenides 187-190 were all found to be efficient catalysts for the reduction of hydrogen peroxide. The $t_{1/2}$ values reported in Table 3.3 are in minutes rather than hours. Compound 187 was found to have a $t_{1/2}$ of 55 minutes (entry 1) and the introduction of a methoxy group para to the selenium atom resulted in superior activity.
for selenide 188 \((t_{1/2} = 35\ \text{minutes},\ \text{entry}\ 2)\). When 3-hydroxypropyl aryl selenide 189 was tested it was found that the meta-methoxy group had a slight overall enhancing effect relative to the unsubstituted compound 187 (compare entries 1 and 3), but the enhancement was not as strong as that for the para-methoxy group. Somewhat surprisingly, when the methoxy group was ortho (190, \(t_{1/2} = 48\ \text{minutes}\)) a very slight enhancement relative to the unsubstituted compound was also observed. Figure 3.5 shows the kinetic plots for compounds 187-190.

![Chemical structure](image)

**Table 3.3 GPx Activity of 3-Hydroxypropyl Aryl Selenides**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>(t_{1/2}) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>187</td>
<td>(R_1 = R_2 = R_3 = H)</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>188</td>
<td>(R_1 = \text{OMe}, R_2 = R_3 = H)</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>189</td>
<td>(R_1 = R_3 = H, R_2 = \text{OMe})</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>190</td>
<td>(R_1 = R_2 = H, R_3 = \text{OMe})</td>
<td>48</td>
</tr>
</tbody>
</table>
The 2,3-dihydroxypropyl aryl selenides also displayed high levels of activity as catalysts. The values for $t_{1/2}$ can be found in Table 3.4 and are also reported in minutes. The relationship between methoxy position and activity for these compounds was similar to their 3-hydroxypropyl counterparts, with the exception of compound 194 (ortho-methoxy, $t_{1/2} = 103$ minutes, entry 4) which was slower than the unsubstituted compound 191 ($t_{1/2} = 79$ minutes, entry 1). Generally, the 2,3-dihydroxypropyl aryl selenides had slightly lower catalytic activity than the corresponding 3-hydroxypropyl analogues. For example, the two unsubstituted (by methoxy groups) selenides 187 and 191 containing 3-hydroxypropyl and 2,3-dihydroxypropyl groups, respectively, had $t_{1/2}$ values of 55 minutes (187) and 79 minutes (191). This was the case for all pairs of selenides from Tables 3.3 and 3.4. Figure 3.6 shows the kinetic plots for compounds 191-194.
Table 3.4 GPx Activity of 2,3-Dihydroxypropyl Aryl Selenides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>t_{1/2} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>191</td>
<td>R_1 = R_2 = R_3 = H</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>192</td>
<td>R_1 = OMe, R_2 = R_3 = H</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>193</td>
<td>R_1 = R_3 = H, R_2 = OMe</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>194</td>
<td>R_1 = R_2 = H, R_3 = OMe</td>
<td>103</td>
</tr>
</tbody>
</table>

Figure 3.6 Single Reaction Plots for 191-194. All graphs: x-axis in minutes; y-axis in % BnSSBn.

The decrease in catalytic activity for the selenides with the 2,3-dihydroxypropyl side chain relative to their 3-hydroxypropyl counterparts may be attributable to a number of factors. Although weak, there might be an effect from the inductively electron-withdrawing nature of the 2-hydroxy substituent upon the selenium atom. Another possibility is that the 2-hydroxy group sterically hinders the reaction with hydrogen
peroxide, or it slows down the rate of cyclization to the spirodioxyselenurane species. Furthermore, the increased number of hydrogen bond contributors in the vicinity of the intermediate selenoxide may act to stabilize this functional group, slowing cyclization.

3.4 Synthesis of Compounds with Multiple Methoxy Groups on the Aromatic Ring

The next step in this investigation was to prepare compounds with multiple methoxy groups present on each aromatic ring. As outlined at the beginning of the chapter, the substitution patterns targeted were \textit{ortho,para}-dimethoxy, \textit{meta,para}-dimethoxy and \textit{ortho,meta,para}-trimethoxy. The key to all of the corresponding GPx mimetics was the diselenide species with the correct substitution pattern (Scheme 3.10). Diselenides 195 and 196 might be prepared from their corresponding and commercially available anthranilic acids or 2-aminobenzyl alcohols, while diselenide 197 could be prepared after lithium-halogen exchange on 2-bromo-3,5-dimethoxybenzyl alcohol (198), which was a more cost-effective route than using the corresponding anthranilic acid.

\textbf{Scheme 3.10}
Initial synthetic work focussed on the preparation of the meta,para-dimethoxy and ortho,meta,para-trimethoxy compounds from anthranilic acids 199 and 201, respectively. As it turned out, diselenide 195 could be prepared from the corresponding anthranilic acid 199, but diselenide 196 could not be prepared from anthranilic acid 201. The route to diselenide 195 involved the synthesis of diselenide 203 from the diazonium salt of anthranilic acid 199 and potassium diselenide. Isolation of diselenide 203 in a pure state was not possible and so this compound was used in the next step without further purification. Formation of diselenide 195 was completed by lithium aluminum hydride reduction of diselenide 203 followed by air oxidation (Scheme 3.11).

Scheme 3.11

Alkylation of the selenolate prepared from this diselenide with the appropriate electrophiles led to the preparation of 3-hydroxypropyl selenide 204 and 2,3-dihydroxypropyl selenide 205. Formation of the cyclic seleninate ester 141 occurred via allyl selenide 206, which was in turn prepared after alkylating the corresponding selenolate with allyl iodide. The hydrogen peroxide mediated oxidation of this compound concluded the synthesis of the desired cyclic seleninate ester. These processes are presented in Scheme 3.12.
Preparation of symmetrical diaryl selenide 207 would complete the para, meta-dimethoxy set of GPx mimetics. The attempted route for the preparation of 207 involved reaction of the selenolate derived from diselenide 195 with the diazonium salt of 2-amino-4,5-dimethoxybenzyl alcohol (see Scheme 3.13). Unfortunately, selenide 207 could not be prepared via this route, even with a six-fold excess of the diazonium salt.

Attempts to prepare the set of GPx mimetics derived from ortho, meta, para-trimethoxy substituted diselenide 196 were met with early failure. For example, multiple attempts to react the diazonium salt of 3,4,5-trimethoxyanthranilic acid with potassium
diselenide did not produce the desired product 208. An attempt to prepare diselenide 196 directly from 2-amino-3,4,5-trimethoxybenzyl alcohol (202) using the same diazotization chemistry also failed (see Scheme 3.14).

**Scheme 3.14**

It was clear that if aryl GPx mimetics with trimethoxy substitution were to be prepared, new routes needed to be explored. The 2-bromo-3,4,5-benzyl alcohol (209) was chosen as potential starting material for diselenide 196. Lithium-halogen exchange, upon exposure of 209 to two equivalents of n-butyllithium, followed by reaction of the aryllithium species with selenium could form the lithium selenolate. This intermediate would be expected to oxidize to the diselenide upon exposure to atmospheric oxygen. When lithium – halogen exchange was attempted on 209, followed by exposure to elemental selenium, none of the desired diselenide 196 was isolated (see Scheme 3.15).
It was possible that the aromatic ring was too electron rich to undergo lithium-halogen exchange to form the associated dianion. It should be noted that a similar dianion, prepared from 2-bromo-5-methoxybenzyl alcohol, was used earlier to form diselenide 130b during the preparation of compounds 118 and 133 (Chapter Two). Compound 210, with the benzyl alcohol protected as a methoxy methyl ether (MOM), was prepared. Not only did we expect that the lithium-halogen exchange would occur more readily on 210, we hoped that the MOM group would chelate to the lithium ion, which might aid in the conversion of 210 to the desired aryllithium species. When 210 was reacted with n-butyllithium, followed by exposure to elemental selenium, compound 211 was isolated after exposure of the reaction mixture to air (Scheme 3.16).

Alkylation of the selenolate prepared from diselenide 211 with the appropriate electrophiles led to the preparation of 3-hydroxypropyl selenide 212 and 2,3-dihydroxypropyl selenide 213. Mildly acidic conditions resulted in the removal of the protecting group from each of these compounds and produced selenides 214 and 215,
respectively. A similar sequence of reactions produced allyl selenide 217 via its MOM protected congener 216. Hydrogen peroxide-mediated oxidation of 217 provided the desired cyclic seleninate ester 145. These processes are presented in Scheme 3.17. Failure to form any diaryl selenide species from diselenide 211 and the diazonium salt of 2-amino-3,4,5-trimethoxybenzyl alcohol (202) precluded further efforts towards the symmetrical diaryl selenides by this route.

**Scheme 3.17**

At this point an entirely different method was explored for the synthesis of diaryl diselenides which circumvented the diselenide intermediates entirely. A report had been published on the unexpected, copper(II) oxide promoted synthesis of relatively simple
symmetrical selenides.\textsuperscript{150} The group of Rao had been attempting to prepare \(N,N'\)-disubstituted selenoureas from aryl bromides and selenourea \textsuperscript{222} in the presence of powdered copper(II) oxide. Instead of isolating the desired selenoureas, Rao and coworkers isolated symmetrical selenides and then optimized this methodology for a number of aryl halides.

The synthesis of selenides \textsuperscript{207} and \textsuperscript{218} (see Scheme 3.18), under the conditions of Rao and coworkers, required the use of aryl halides that were more complex than any other substrates presented in their paper. Both 2-bromo-4,5-dimethoxybenzyl alcohol (\textsuperscript{219}) and 2-bromo-3,4,5-trimethoxybenzyl alcohol (\textsuperscript{209}) were available to us and so we started with these aryl bromides. Rao’s conditions involve mixing two equivalents of aryl halide with one equivalent of selenourea \textsuperscript{222} in the presence of 3 mol \% of the copper oxide catalyst and two equivalents of potassium hydroxide. Unfortunately, we were unable to convert either aryl bromide into the desired selenium compounds even with 20 mol \% of copper-oxide and extended reaction times. Rao had found that aryl iodides were better substrates for this reaction and so 2-iodo-4,5-dimethoxybenzyl alcohol (\textsuperscript{220}) and 2-iodo-3,4,5-trimethoxybenzyl alcohol (\textsuperscript{221}) were both prepared. When these compounds were reacted with selenourea under Rao’s conditions, the desired tetramethoxy selenide \textsuperscript{207} and hexamethoxy selenide \textsuperscript{218} were produced, albeit in moderate yields. It was ironic that both were contaminated with large amounts of their corresponding diselenides, of which, diselenide \textsuperscript{196} had eluded previous synthetic efforts. Frustratingly, the diselenides were not easily removed from the product mixtures.
The easiest way to purify selenides 207 and 218 was to oxidize the diselenide-containing mixtures. This produced both the corresponding spirodioxyselenuranes and cyclic seleninate esters, which were more easily separated via column chromatography. Compounds 207 and 218 were finally obtained in analytically pure form after reduction of their respective spirodioxyselenuranes back to the selenides with benzyl thiol.

At this point, attention was turned to the ortho,para-dimethoxy compounds. This involved the simple bromination of methyl 3,5-dimethoxybenzoate (223) to form methyl 2-bromo-3,5-dimethoxybenzoate (224). Reduction to 2-bromo-3,5-dimethoxybenzyl alcohol (198), followed by lithium-halogen exchange and introduction of selenium produced diselenide 197 and, fortuitously, selenide 225 as a minor by-product. Luckily, selenide 225 could be isolated from the product mixtures of subsequent steps. Crude
diselenide 197 was transformed into compounds 226, 227 and 142 by the processes shown in Scheme 3.19.

Scheme 3.19
3.5  GPx Activity of the Dimethoxy and Trimethoxy Derivatives

Data from Tables 3.1-3.4 have been included for comparison with the results for the compounds presented in this section. Results for the cyclic seleninate esters, for the symmetrical diaryl selenides, for the 3-hydroxypropyl selenides and for the 2,3-dihydroxypropyl selenides are found in Tables 3.5, 3.6, 3.7 and 3.8, respectively. The standard conditions (0.031 M BnSH, 0.035 M H$_2$O$_2$ and 0.0031 M catalyst in 19:1 DCM to MeOH, at 18 °C) were used for all assays, which are averaged over at least two trials.

Information collected from studying the effect of the position of the methoxy-substituent on the activity of the various GPx mimetics showed that, when substituted para to selenium, a methoxy group could significantly increase GPx-like activity. An inductively electron-withdrawing meta-methoxy group was less or not at all effective in this regard. Although ortho-methoxy groups were expected to mesomerically stabilize positive charge on selenium, they were found to reduce catalytic activity, likely as a result of their proximity to the selenium atom and ensuing steric and hydrogen-bonding effects. The effect of combining methoxy groups at various positions relative to the selenium atom would be demonstrated by testing the compounds prepared in Section 3.4.

As seen earlier, the unsubstituted seleninate ester in entry 1 displayed $t_{1/2} = 50$ hours. The introduction of mono-methoxy substituents at either the para, meta or ortho position, resulted in improved, unchanged and suppressed catalytic activity in compounds 118, 147 and 149 (entries 2, 3 and 4), respectively. Surprisingly the incorporation of a second methoxy group to give meta,para-dimethoxy and ortho,para-dimethoxy
derivatives 141 and 142 (entries 5 and 7) respectively gave only minimal and modest enhancements of activity, respectively. Similarly, the trimethoxy seleninate ester 145 (entry 6) provided a slight improvement compared to 108. These results indicate that the introduction of second or third methoxy groups to the para-monomethoxy derivative 118 provides no advantage and actually attenuates catalytic activity. Moreover, 141, 142 and 145 were only marginally superior to the unsubstituted seleninate ester 108. Figure 3.7 shows the graphs for yield (%) of dibenzyl disulfide as a function of time for 141, 145 and 142.

![Chemical structure](image)

**Table 3.5 GPx Activity of Cyclic Seleninate Esters**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>t_{1/2} (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>R_{1} = R_{2} = R_{3} = H</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>118</td>
<td>R_{1} = OMe, R_{2} = R_{3} = H</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>147</td>
<td>R_{1} = R_{3} = H, R_{2} = OMe</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>149</td>
<td>R_{1} = R_{2} = H, R_{3} = OMe</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>141</td>
<td>R_{1} = R_{2} = OMe, R_{3} = H</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>145</td>
<td>R_{1} = R_{2} = R_{3} = OMe</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>142</td>
<td>R_{1} = R_{3} = OMe, R_{2} = H</td>
<td>43</td>
</tr>
</tbody>
</table>
The effect of multiple methoxy groups on the symmetrical diaryl selenides was similar to the cyclic seleninate esters. Both selenides 207 (meta,para-substitution) and 218 (ortho,meta,para-substitution), as well as the ortho,para-dimethoxy derivative 186 had stronger or similar activity compared to the unsubstituted compound, 132a, but were less efficient than the para-monomethoxy substituted selenide 132b. Figure 3.8 shows the kinetic plots in the presence of selenides 207, 218 and 225. It is interesting to note a slight departure from linear behaviour in the case of the hexamethoxy derivative 218 in Figure 3.8. A brief induction period of approximately 4 hours, where the reaction proceeds slowly, is followed by more rapid progress. This appears to be the result of a particularly slow rate-determining step, in which the oxidation of Se(II) to Se(IV) occurs.
Table 3.6 GPx Activity of Symmetrical Aryl Selenides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>$t_{1/2}$ (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>132a</td>
<td>$R_1 = R_2 = R_3 = H$</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>132b</td>
<td>$R_1 = \text{OMe}, R_2 = R_3 = H$</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>$R_1 = R_3 = H, R_2 = \text{OMe}$</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>186</td>
<td>$R_1 = R_2 = H, R_3 = \text{OMe}$</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>207</td>
<td>$R_1 = R_2 = \text{OMe}, R_3 = H$</td>
<td>6.3</td>
</tr>
<tr>
<td>6</td>
<td>218</td>
<td>$R_1 = R_2 = R_3 = \text{OMe}$</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>225</td>
<td>$R_1 = R_3 = \text{OMe}, R_2 = H$</td>
<td>17</td>
</tr>
</tbody>
</table>

Figure 3.8 Single Reaction Plots for 207, 218 and 225. All graphs: x-axis in hours; y-axis in %BnSSBn.

Generally, the hybrid (aromatic/aliphatic) selenides 204, 214 and 226 were found to be efficient catalysts for the reduction of hydrogen peroxide and had values for $t_{1/2}$
reported in minutes rather than hours. In the case of these selenides, the meta,para-dimethoxy compound 204 was a better catalyst than the para-methoxy compound. In fact, with a $t_{1/2} = 10$ minutes (entry 5), 204 is the best selenium-based GPx mimetic that we have so far produced. This value for $t_{1/2}$ is impressive when compared to ebselen ($t_{1/2} = 24$ hours or 1440 minutes) and even more impressive when compared to the uncatalysed reaction ($t_{1/2} = 176$ hours or 10560 minutes). Once again, the addition of an ortho-methoxy group, to form trimethoxy substituted compound 214, decreased catalyst activity, relative to all other compounds in this set of catalysts. Thus, 214 was slower than all other catalysts in this class (entry 6), including selenide 190 with only a single ortho-methoxy group (entry 4). Similarly to the cyclic seleninate esters, the ortho,para-dimethoxy compound (226) with a $t_{1/2} = 37$ minutes had catalytic activity that nearly matched that of para-methoxy compound 188 (compare entries 2 and 7). Figure 3.9 shows the kinetic plots for selenides 204, 214 and 226.

![Diagram](image_url)

Table 3.7 GPx Activity of 3-Hydroxypropyl Aryl Selenides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>187</td>
<td>$R_1 = R_2 = R_3 = H$</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>188</td>
<td>$R_1 = \text{OMe}, R_2 = R_3 = H$</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>189</td>
<td>$R_1 = R_3 = H, R_2 = \text{OMe}$</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>190</td>
<td>$R_1 = R_2 = H, R_3 = \text{OMe}$</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>204</td>
<td>$R_1 = R_2 = \text{OMe}, R_3 = H$</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>214</td>
<td>$R_1 = R_2 = R_3 = \text{OMe}$</td>
<td>102</td>
</tr>
<tr>
<td>7</td>
<td>226</td>
<td>$R_1 = R_3 = \text{OMe}, R_2 = H$</td>
<td>37</td>
</tr>
</tbody>
</table>
Figure 3.9 Single Reaction Plots for 204, 214 and 226. All graphs: x-axis in minutes; y-axis in %BnSSBn.

The activity of the 2,3-dihydroxypropyl aryl selenides followed the same general pattern, although the meta,para-dimethoxy compound was not a better catalyst than its congeners within this series. This selenide (205), with a $t_{1/2} = 70$ minutes (entry 5), had activity that was still slightly better than that of the unsubstituted compound 191 (entry 1), but it was much slower than selenide 192 (para-methoxy). Trimethoxy selenide 215 had the poorest activity of this group (entry 6), while the activity of compound 227, with ortho,para-dimethoxy substitution (entry 7), nearly matched the activity of selenide 192. This further suggests that the para-methoxy group can exert a large enough influence on activity to overcome the decrease in efficiency caused by the ortho-methoxy group.

Figure 3.10 shows the kinetic plots for selenides 205, 215 and 227.
Table 3.8 GPx Activity of 2,3-Dihydroxypropyl Aryl Selenides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Substitution</th>
<th>t_{1/2} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>191</td>
<td>R_1 = R_2 = R_3 = H</td>
<td>79</td>
</tr>
<tr>
<td>2</td>
<td>192</td>
<td>R_1 = OMe, R_2 = R_3 = H</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>193</td>
<td>R_1 = R_3 = H, R_2 = OMe</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>194</td>
<td>R_1 = R_2 = H, R_3 = OMe</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>205</td>
<td>R_1 = R_2 = OMe, R_3 = H</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>215</td>
<td>R_1 = R_2 = R_3 = OMe</td>
<td>150</td>
</tr>
<tr>
<td>7</td>
<td>227</td>
<td>R_1 = R_3 = OMe, R_2 = H</td>
<td>43</td>
</tr>
</tbody>
</table>

Figure 3.10 Single Reaction Plots for 205, 215 and 227. All graphs: x-axis in minutes; y-axis in %BnSSBn.
3.6 GPx Activity of 192 Using Glutathione as the Thiol Substrate

As mentioned above, the selenides containing a 2,3-dihydroxypropyl group were sufficiently water-soluble for use in aqueous media. In order to test the GPx activity of these compounds in water, an NMR-based assay, using $^1$H-NMR spectroscopy run in D$_2$O, was developed. Glutathione was used as the thiol cofactor for the reduction of hydrogen peroxide in the presence of selenide 192. It should be noted that selenide 192 is the only compound that has been tested under these conditions and minor adjustments may be required to make this method general for all future water-soluble GPx mimetics developed in our lab.

The experiment was carried out by dissolving glutathione and the catalyst in 5.0 mL of D$_2$O. The concentration of glutathione was 0.031 M, while the concentration of catalyst was 0.0031 M. These concentrations match those of benzyl thiol and catalyst used for our HPLC-based protocol. A proton NMR spectrum of the mixture was recorded prior to any exposure to hydrogen peroxide (see Figure 3.1).
The trial was started by adding 0.175 mmol of hydrogen peroxide (from a 33.1% w/v solution in water) and mixing vigorously. This resulted in a peroxide concentration of 0.035 M, which also matched the peroxide concentration from our previous assay. The trial was kept at ambient temperature during the course of the experiment. After 8 minutes the first $^1$H NMR spectrum was recorded, which was followed by subsequent $^1$H NMR spectra every 2 minutes for a total duration of 34 minutes. The catalytic activity was again measured as $t_{1/2}$, the time required for 50% conversion of two molecules of glutathione (GSH) to glutathione disulfide (GSSG). Figure 3.12 shows three $^1$H-NMR spectra in the region of 2.0 ppm to 4.6 ppm, recorded after 0 minutes (A), 30 minutes ($t_{1/2}$; B) and 158 minutes (after complete conversion; C).
Figure 3.12 Conversion of GSH to GSSG as Monitored by $^1$H NMR (400 MHz) Spectroscopy. Spectrum A: $t_0$, Spectrum B: $t_{1/2}$, Spectrum C: after complete conversion.

The structures of glutathione and glutathione disulfide are depicted in Figure 3.13. The peaks for glutathione (spectrum A) can be assigned as H-4 (δ 4.53, 1H), H-6 (δ 3.95, 2H), H-1 (δ 3.79, 1H), H-5 (δ 2.91, 2H), H-3 (δ 2.53, 2H) and H-2 (δ 2.14, 2H). The protons in glutathione disulfide have similar chemical shifts with the exceptions of protons H-4' and H-5'. The peak for H-4' shifts downfield (δ 4.71) relative to H-4 and, at the concentration used for this study, is completely lost under the water peak. For this reason, the spectrum has been expanded only from the range of 4.6 – 2.0 ppm in order to define the peaks of interest as clearly as possible. The peaks for glutathione disulfide can be assigned as H-6' (δ 3.93, 4H), H-1' (δ 3.78, 2H), H-5'a (δ 3.24, 2H), H-5'b (δ 2.95,
H-3' (δ 2.50, 4H) and H-2' (δ 2.13, 4H). Importantly, the diastereotopic methylene protons on carbon C-5' are well separated in glutathione disulfide. Integration of the peak for H-5'a at 3.24 ppm along with integration of the glutathione peak for H-4 at 4.53 ppm, enabled us to monitor the conversion of glutathione to glutathione disulfide and allowed for a determination of t$_{1/2}$ for catalyst 192 under these conditions.

**Figure 3.13 Glutathione and Glutathione Disulfide**

The t$_{1/2}$ for the destruction of hydrogen peroxide catalysed by 192 was found to be 30 minutes. This is faster than the t$_{1/2}$ (36 minutes) recorded for the reduction of hydrogen peroxide by 192 in dichloromethane – methanol with benzyl thiol as the stoichiometric reductant. In order to confirm that 192 was catalysing the destruction of peroxide with the glutathione reductant, a control reaction was carried out. This consisted of monitoring glutathione (at 0.031 M) in a 0.035 M solution of hydrogen peroxide in D$_2$O in the absence of the catalyst for 75 minutes. During this time, there was no detectable conversion of glutathione to glutathione disulfide. This means there is no significant background reaction contribution to the t$_{1/2}$ observed for selenide 192. Figure 3.14 shows the graph corresponding to the formation of glutathione disulfide in the presence of 192.
A systematic investigation of the effect of methoxy substitution on catalytic activity was carried out by synthesizing ortho, meta and para mono-methoxy substituted cyclic seleninate esters and symmetrical diaryl selenides. All compounds investigated were prepared after designing and optimizing routes to common diselenide intermediates. The resulting cyclic seleninate esters and symmetrical diaryl selenides were tested for catalytic activity along with two new groups of catalysts containing hydroxy- and dihydroxy-propyl side chains. These new derivatives combined the salient features of the aromatic compounds (electronic control through aromatic substitution and strong carbon – selenium bonds), as well as the relative ease of preparation of hybrid aromatic/aliphatic compounds and much improved water solubility. After measuring the activity of the GPx mimetics with ortho-methoxy and meta-methoxy groups, a number of catalysts containing dimethoxy and trimethoxy aromatic rings were prepared.

A few general statements can be made about the effects of the position and number of methoxy groups upon the catalytic activity of the cyclic seleninate esters and
the various selenides studied as GPx mimetics. As expected, the most effective location for a single methoxy substituent is the position para to the selenium atom. This is the case for all classes of GPx mimetics studied. A methoxy group at this position can stabilize positive charge increase on the selenium atom as it gets oxidized, and does so without interfering sterically. Moving the methoxy group to the meta-position usually resulted in an intermediate level of catalytic activity, which was lower than that of the para-methoxy compounds, but comparable to or slightly better than the unsubstituted compounds. Although an ortho-methoxy group can interact with the selenium atom through mesomorphic processes, compounds with this substituent displayed reduced activity relative to their unsubstituted counterparts. It is likely that the ortho-methoxy group causes unfavourable steric interactions around the selenium atom, leading to a reduction in activity for these compounds by shielding the selenium atom from oxidation by hydrogen peroxide. Alternatively, one can envision a scenario where the selenide becomes oxidized to the corresponding selenoxide but cyclization to the matching spirodioxyselenurane is sufficiently impeded to make this step rate-determining. However, these explanations are speculative and cannot be unequivocally confirmed at this time. As other groups have shown that simple selenoxides are poor GPx mimetics,\textsuperscript{112, 116-118} the formation of the spirodioxyselenurane appears to be an essential part of the catalytic cycle for these compounds.

It was found that, if a para-methoxy group was included with an ortho-methoxy group, the activity improved relative to the unsubstituted compounds and often nearly matched that of compounds with only a para-methoxy group. This suggests that the para-
methoxy group’s ability to stabilize positive charge on the selenium can partially overcome the suppressive effect related to the ortho-methoxy group. Generally, if dimethoxy substitution was meta,para the catalyst displayed activity at the level of or slightly better than the corresponding unsubstituted compound. Increasing the number of methoxy groups on each aromatic ring to three did not provide superior activity. In fact, this substitution pattern often produced the least active catalysts for a given class of GPx mimetics.

3-Hydroxypropyl and 2,3-dihydroxypropyl aryl selenides were prepared and displayed greater catalytic activity than either the cyclic seleninate esters or the symmetrical diaryl selenides. These catalysts were so efficient that the t_{1/2} values were recorded in minutes rather than hours. In particular, compound 204, the 3-hydroxypropyl selenide with meta, para-dimethoxy substitution, is the most efficient selenium-based catalyst prepared in our laboratory so far. In fact, this catalyst was more active than ebselen by two orders of magnitude. Additionally, the decomposition of hydrogen peroxide with this catalyst was more than a thousand times faster than the uncatalysed control reaction.

The water-soluble derivatives presented an opportunity for testing the catalytic activity of these compounds with glutathione, the reductant in the GPx catalytic cycle in vivo. This was an important step because all catalysts prepared by our group had, up to this point, been tested in a dichloromethane – methanol mixture which allowed for solubility of all reaction components. Although the HPLC-based in vitro assay allowed us
to thoroughly investigate the different GPx mimetics designed by our group and, as a result, gain considerable insight into the chemistry involved, the dichloromethane – methanol system does not replicate conditions found *in vivo*. Using an NMR-based assay in an entirely aqueous environment, selenide 192 was found to efficiently catalyse the destruction of hydrogen peroxide in the presence of glutathione. Under these conditions, selenide 192 was found to have a $t_{1/2} = 30$ minutes, which is slightly faster than the $t_{1/2}$ found when this compound was tested in our HPLC-based assay ($t_{1/2} = 36$ minutes). While it may not be possible to directly compare the catalysts tested in the HPLC-based assay with present and future water-soluble catalysts tested in the more recent NMR-based assay, this will likely be unimportant as the latter catalysts are much more efficient than the compounds tested previously as GPx mimetics.
Chapter Four: $^1$H NMR Studies of the Configurational Stability of Substituted Aromatic Seleninate Esters and Spirodioxyselenuranes

4.1 Introduction

The cyclic seleninate esters and the spirodioxyselenuranes presented in Chapters Two and Three have proven to be efficient GPx mimetics.\textsuperscript{126-127, 129, 145} In a reversal of their GPx-like activity, where cyclic seleninate esters were used to catalyse the reduction of hydrogen peroxide, it was also possible for these compounds to catalyse the oxidation of a variety of substrates with hydrogen peroxide. For example, our group has demonstrated that 108 can catalyse the oxidation of sulfides to sulfoxides, the epoxidation of alkenes and the conversion of enamines to $\alpha$-hydroxyketones.\textsuperscript{151} It is plausible that spirodioxyselenurane 110, along with its related selenide, could be used for similar purposes. Although both the cyclic seleninate esters and the spirodioxyselenuranes are chiral molecules, it was unnecessary to prepare molecules such as cyclic seleninate ester 108 and spirodioxyselenurane 110 as pure enantiomers for testing as GPx mimetics in our \textit{in vitro} assay. However, due to their chirality, the possibility of using pure enantiomers of the spiroseleuranes and cyclic seleninate esters in enantioselective oxidations can be envisaged. Thus, further investigation of their configurational properties was undertaken and is the subject of this chapter.

Kamigata and coworkers have resolved cyclic seleninate esters, including 108, to study their configurational stability.\textsuperscript{152} Kamigata found that chiral cyclic seleninate ester 108 was easily racemized in the presence of water via the achiral selenurane 230, as shown in Scheme 4.1. Stereochemical and chiroptical properties of the spiroseleuranes,
along with other spirochalcogenuranes, have also been the subject of recent investigations\textsuperscript{153} and several compounds of this class have been resolved and their absolute configurations determined.\textsuperscript{154}

Scheme 4.1

There have been a few reports of dynamic processes investigated by NMR spectroscopy in spirochalcogenuranes. For example, Martin and Astrologes studied tetraoxysulfuranes by NMR spectroscopy.\textsuperscript{155} Compound 231, with $R = \text{CF}_3$, was found to undergo a fast interconversion between its enantiomers. This was apparent from the appearance of only two CF$_3$ signals in the $^{19}$F NMR spectrum at 301 K.

In a static structure of 231 one would expect four separate CF$_3$ groups, which are denoted as letters in Scheme 4.2. For example, the structure on the left in Scheme 4.2 has two equivalent \textit{endo} CF$_3$ groups attached to the $\alpha$-carbon of the apical (blue) oxygen atoms. These are denoted A and A'. Similarly there are two equivalent \textit{exo} CF$_3$ groups attached to the same carbon atom (B and B'). Further inspection reveals that C and C' are


endo CF₃ groups, but on the α-carbon of the equatorial (red) oxygen atoms, while D and D’ are the corresponding exo CF₃ groups. In order to observe the presence of all four types of CF₃ groups, ¹⁹F NMR spectroscopy had to be run on a sample cooled to 123 K. To account for the fast exchange of the apical and equatorial trifluoromethyl groups, Martin and Astrologes proposed a mechanism similar to Berry pseudorotation¹⁵⁶ (Scheme 4.2).

**Scheme 4.2**

Such a mechanism results in interconversion of the enantiomers, without scrambling the distinct exo and endo trifluoromethyl groups. It is clear that while A and A’ are endo and pseudo-apical in the structure on the left, they are endo and pseudo-equatorial in the structure on the right. This mechanism renders all endo CF₃ groups equivalent (i.e. A = A’ = C = C’) and all exo CF₃ groups equivalent (i.e. B = B’ = D = D’). For this reason, Martin and Astrologes observed only two CF₃ groups by ¹⁹F NMR spectroscopy at higher temperatures.
Denney and coworkers investigated tetraoxyselenuranes and tetraoxytelluranes.\textsuperscript{157} When the $^1\text{H}$ NMR spectrum of compound 232 was recorded, only one proton resonance was observed, suggesting that the \textit{exo} and \textit{endo} hydrogen atoms were exchanging by an unspecified dissociative process. When a scavenger of both water and traces of acid was added, separate \textit{exo} and \textit{endo} peaks appeared. Both compounds 233 and 234 had two CH$_3$ resonances when observed by $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectroscopy at ambient temperatures.

![Figure 4.1 Tetraoxychalcogenuranes Investigated by Denney](image)

There is a significant difference between these tetraoxyselenuranes and the spirodioxyselenuranes studied in our group. Rather than four selenium – oxygen bonds and the lone pair of the selenium atom comprising the trigonal bipyramidal arrangement around selenium, our compounds contain two selenium – carbon bonds in place of two of the selenium – oxygen bonds. This is important for a number of reasons. It is known that, in trigonal bipyramidal structures, the more electronegative atoms preferentially occupy the apical positions.\textsuperscript{158} This is the case for the aliphatic and aromatic spirodioxyselenuranes 103 and 110, respectively, which has been confirmed via X-ray crystallography.\textsuperscript{127, 130} Thus, Berry pseudorotation of the spirodioxyselenuranes would require a transformation from a lower energy configuration to one with the oxygen atoms
in the less favourable equatorial positions. It should also be noted that any pseudorotation that places a lone pair in the apical position would be expected to have a high activation barrier.\textsuperscript{159}

Other groups have investigated the conformational stability of spirodioxysulfuranes and spirodioxselenuranes more similar to ours. For example, Reich prepared spirodioxselenurane 235 and found that the diastereotopic methyl peaks remain as separate \textit{exo} and \textit{endo} entities up to 473 K.\textsuperscript{159} Reich also prepared compound 236 and observed its equilibration to a mixture of 236 and its diastereomer, 237, at 393 K. The mechanism leading to equilibration was not fully understood.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figures/spirodioxyselenuranes.png}
\caption{Spirodioxyselenuranes Investigated by Reich}
\end{figure}

Adzima and Martin prepared spirodioxysulfuranes 238 and 239 in an attempt to rigorously study the hydrolytic stability of this class of compounds.\textsuperscript{160} Reversible hydrolysis would lead to racemization and exchange of \textit{exo} and \textit{endo} benzylic substituents. They found that 238 was relatively resistant to hydrolysis and required 2 hours of refluxing in 10\% aqueous methanol to give the corresponding achiral sulfoxide. However, compound 239, without gem-dimethyl substitution, was hydrolysed to its sulfoxide after 4 hours at room temperature when left in a mixture of D\textsubscript{2}O and CDCl\textsubscript{3}.
Diastereomeric spirodioxysulfuranes 240 and 241 were also prepared by Adzima and Martin, each produced as racemates, to study interconversion mechanisms between the two diastereomers. They found that mixtures of 240 and 241 heated in pyridine-$d_5$ at 357 K eventually equilibrated to a 78/22 ratio of the two diastereomers. This was the result when the investigation started with either a 50/50 or an 89/11 (240/241) mixture.

Although interconversion through a sulfoxide intermediate (formed after hydrolysis) was not ruled out, this route seemed unlikely as the related compound 238 required heating in the presence of water to undergo a similar process. Another route proposed by these authors involved the ionization of a sulfur – oxygen bond of 240 to produce 242, subsequent pyramidal inversion to 243 and finally recombination of the sulfur and oxygen atoms (this is depicted as pathway A in Scheme 4.3). Note that this would not produce 241, but ent-241, which also has an endo ethyl group and is a diastereomer of 240. Similarly, ent-240 would equilibrate to produce 241.
Additionally, Martin and Adzima suggested that 240 could be converted to 241 through planar transition state 244 (Scheme 4.3, pathway B) which would also result in the formation of ent-241. Martin and coworkers define this transformation, where wedge-shaped conformers are interconverted, as cuneal inversion. A final proposal involved the intermediacy of a benzylic carbocation (245). Formation of this carbocation, followed by σ-bond rotation and recombination could also convert 240 to 241 (Scheme 4.4).
Consideration of the proposals presented independently by the groups of Martin and Reich was a good starting point for investigating the configurational stability of our spirodioxyselenuranes, which was carried out using $^1$H NMR spectroscopy. This work, in addition to computations carried out by Nicole McNeil, a PhD student in our group, is the subject of Section 4.2.

4.2 Initial NMR Experiments on the Seleninate Esters and the Selenuranes

The chiral centre in the cyclic seleninate esters results from the tetrahedral arrangement around the selenium atom, consisting of three different groups and the selenium lone pair. This chiral environment around the selenium atom in 108 and, similarly, in the substituted aromatic cyclic seleninate esters (118-123) results in the non-equivalence of their benzylic methylene hydrogens. As expected, these diastereotopic benzylic hydrogen atoms lead to the appearance of AB quartets in the $^1$H NMR spectra of 108 and 118-123. Figure 4.4 shows the $^1$H NMR spectrum of cyclic seleninate 108, recorded in DMSO-$d_6$, with the AB quartet clearly defined and centred around 5.7 ppm, with $J = 15.0$ Hz and $\Delta v = 46.7$ Hz.
Variable-temperature $^1$H NMR spectroscopy was run on cyclic seleninate ester 108 to test whether the AB quartet of this compound would become a singlet at increased temperatures, which would be consistent with a rapid interconversion between its enantiomers. Dissolving 108 in DMSO-$d_6$ and heating in 15 K increments resulted in no change to the AB quartet, even at 377 K. Figure 4.5 shows the variable-temperature $^1$H NMR spectra for this compound.

**Figure 4.4 $^1$H NMR (300 MHz) Spectrum of 108 in DMSO-$d_6$**
Figure 4.5 (a) Room-temperature and (b) variable-temperature (321 – 377 K) $^1$H NMR (300 MHz) spectra of 108 in DMSO-d$_6$

In order to test if enantiomerization could be promoted under conditions of hydrolysis, as Kamigata had observed,$^{152}$ the high temperature $^1$H NMR spectrum was run again in DMSO-d$_6$ with added D$_2$O, trifluoroacetic acid or KOH. In all three cases, the AB quartet was persistent and did not disappear at temperatures as high as 377 K. This suggests that interconversion of the stereoisomers of 108 via hydrate formation (i.e. 230 in Scheme 4.1) is relatively slow on the NMR time scale in DMSO-d$_6$.

Next, the spirodioxyselenuranes were investigated for configurational stability. Compound 110 and its substituted derivatives are chiral because of the trigonal bipyramidal arrangement of the spirocycle around the selenium atom. The two oxygen
atoms are in the apical position, two of the equatorial positions are filled with the aromatic carbon atoms, while the lone pair on selenium resides in the final equatorial position. The depiction of **110** in Figure 4.6 illustrates its chirality. The benzylic methylene hydrogen atoms can be differentiated by observing that the spirodioxyselenuranes have distinct concave and convex surfaces. The hydrogen atoms that are found in the concave region are *endo* and are designated $H_n$, while the hydrogen atoms on the convex side of the molecule are *exo* and designated $H_x$.

![Figure 4.6 Spirodioxyselenurane 110 and its Enantiomer](image)

The methylene hydrogen atoms in aromatic spirodioxyselenuranes are also diastereotopic. The $^1$H NMR spectrum for the *para*-bromo derivative **135**, at room temperature, is shown in Figure 4.7. Similarly to **108**, the benzylic hydrogen atoms are present in the spectrum as an AB quartet, which is centred at 4.85 ppm, with $J = 14.7$ Hz and $\Delta \nu = 23.1$ Hz.
Intriguingly, the AB quartet observed in 135 was not present in the room temperature $^1$H NMR spectra of all of the other spirodioxyselenuranes that we had prepared previously. In addition to compound 135, the other halogen-substituted spirodioxyselenuranes (i.e. fluoro, 137 and chloro, 136) also displayed AB quartets. On the other hand, the benzylic methylene $^1$H NMR signals of the unsubstituted compound 110, and of the methyl-substituted compound 134, were sharp singlets. Figure 4.8 shows the $^1$H NMR spectra of 110 and 134-137 recorded in toluene-$d_8$ at 298 K, in the region of 4.5 – 8.0 ppm.
The appearance of singlets for the benzylic methylene protons for compounds 110 and 134 suggested that these diastereotopic protons were equilibrating by some process occurring rapidly on the NMR time scale. We were interested in determining the nature of this equilibration and we wished to ascertain whether we could observe AB quartets for these compounds at lower temperatures. Additionally, we wanted to observe if heating the halogen-substituted spirodioxselenuranes would cause their respective AB quartets to collapse into singlets. Therefore, variable-temperature $^1$H NMR experiments were run in toluene-$d_8$, which allowed for an investigation over a large temperature range allowing compounds with room-temperature singlets (110 and 134) to be cooled and compounds with potential coalescence temperatures higher than room-temperature (135-
to be heated. During the course of this study we realised that some compounds displayed coalescence temperatures above the temperature limits of the NMR spectrometer. This led us to investigate each compound’s convergence temperature, $T_c$, which we define as the temperature where the inner peaks of the AB-quartet converge to a single peak at an NMR frequency of 300 MHz. These experiments led to the observation that all of these compounds displayed well-defined AB quartets at lower temperatures for their respective diastereotopic methylene protons, which converged at increased temperatures. As expected, when the $^1$H NMR spectra of 110 and 134 were recorded at low temperature the benzylic methylene signals split into AB quartets. Figure 4.9 shows the $^1$H-NMR spectrum of 110, while the inset shows the benzylic methylene signal at temperatures between 213 and 291 K.

Figure 4.9 (a) Room-temperature and (b) variable-temperature (213 – 291 K) $^1$H NMR (300 MHz) spectra of 110 in toluene-$d_8$
Recording the $^1$H NMR spectra of 135-137 at high temperature caused the AB quartets observed for all of these compounds at room temperature to converge. To illustrate, Figure 4.10 shows the $^1$H NMR spectra of the para-fluoro derivative 137 recorded at room temperature, where an AB quartet appears, and then at increasing temperatures where the benzylic methylene signals eventually converge and finally coalesce into a singlet.

![Figure 4.10](image)

**Figure 4.10** (a) Room-temperature and (b) variable-temperature (316 – 347 K) $^1$H NMR (300 MHz) spectra of 137 in toluene-$d_8$

Table 4.1 contains the convergence temperatures (Tc) that were observed for each compound. Specifically, 110 and 134 were found to have Tc values of 267 K and 218 K,
respectively (entries 1 and 2), while 137, 136 and 135 had Tc values of 325 K, 355 K and 370 K (entries 5, 4 and 3, respectively). The low temperature values for Tc observed for 110 and 134 suggested that any processes that were equilibrating the diastereotopic hydrogens of these compounds had lower activation energies than the para-halogen derivatives.

Table 4.1 Variable-Temperature NMR Data for 110 and 134-137

| Entry | Compd | para-Substitution | Tc (K) | Δν\(^a\) (Hz) | \(|J_{AB}|\(^a\) (Hz)\) |
|-------|-------|-------------------|--------|--------------|-----------------|
| 1     | 110   | Unsubstituted     | 267    | 23.4         | 14.6            |
| 2     | 134   | Methyl            | 218    | 17.4         | 14.7            |
| 3     | 135   | Bromo             | 370    | 23.1         | 14.7            |
| 4     | 136   | Chloro            | 355    | 23.0         | 14.9            |
| 5     | 137   | Fluoro            | 325    | 19.1         | 14.9            |

\(^a\)Measured at the lowest temperature. Tc = convergence temperature; \(\Delta\nu\) = difference in chemical shift; \(|J_{AB}|\) = coupling constant.

4.3 Further Investigation of the \(^1\)H NMR Behaviour of the Spirodiioxyselenuranes

Based on the precedents described in Section 4.1, our expectations were that the NMR behaviour of our spirodiioxyselenuranes would stem from one of the following processes: a) Berry pseudorotation or a related process; b) inversion through selenium via a planar transition state; c) interconversion via hydrolysis to a selenoxide; d) selenium – oxygen bond dissociation to produce dipolar intermediates or e) benzylic carbon – oxygen bond dissociation to produce dipolar intermediates.

An initial analysis of our observations suggested that whatever equilibration was occurring proceeds slowly on the NMR time scale for compounds 135-137 at room-temperature. On the other hand, the room-temperature rate of exchange was faster than
the NMR time scale when the spirodioxyselenurane was unsubstituted (110) or had a methyl group on the aromatic ring (134). This suggested that the mechanism leading to convergence could involve increased positive charge on the selenium atom, which would be stabilized by electron-donating groups. Reversible dissociation of a Se - O bond would be such a process. Other groups have postulated similar dissociations for spirodioxysulfuranes\(^{160}\) and for spirotetraalkoxyselenuranes.\(^{157b}\) In the presence of trace amounts of water, Se – O dissociation would produce achiral selenoxide 246, resulting in the equilibration of the exo and endo hydrogen atoms. Alternatively, Se - O dissociation followed by pyramidal inversion of the resulting selenonium ion, 247, prior to selenium – oxygen recombination could also result in equilibration. Both of these possibilities are presented in Scheme 4.5.

**Scheme 4.5**

![Scheme 4.5](image-url)
If these processes were to occur, they could be promoted by adding water to the NMR solvent or could be slowed by rigorously drying the NMR solvent. Alternatively, Se – O dissociation should be promoted by using NMR solvents with higher polarity or by the addition of acid catalysts. It was found that, neither the use of rigorously dried toluene-$d_8$, nor the use of toluene-$d_8$ saturated with D$_2$O had an effect on the AB quartet convergence temperature in the variable-temperature NMR spectrum of 110. Additionally, variable temperature NMR experiments on 110 were run in pyridine-$d_5$, DMSO-$d_6$, and 25% D$_2$O-DMSO-$d_6$ to observe the effect of increased solvent polarity on the convergence temperature. It was found that the convergence temperature (Tc of 267 K in toluene-$d_8$) increased to 299, 347 and 368 K respectively (Table 4.2). This observation, where increased solvent polarity resulted in an increase in Tc, suggested that dipolar intermediates were not responsible for the equilibration of the exo and endo methylene protons.

Table 4.2 Variable-temperature NMR Data for 110 in Different Solvents

| Entry | Solvent       | Tc (K) | $\Delta \nu^a$ (Hz) | $|J_{AB}|^a$ (Hz) |
|-------|---------------|--------|---------------------|------------------|
| 1     | toluene-$d_8$ | 267    | 23.4                | 14.6             |
| 2     | pyridine-$d_5$| 299    | 15.6                | 15.6             |
| 3     | DMSO-$d_6$    | 347    | 21.0                | 15.0             |
| 4     | 25% D$_2$O–DMSO-$d_6$ | 368 | 21.0 | 15.0 |

*aMeasured at the lowest temperature. Tc = convergence temperature; $\Delta \nu$ = difference in chemical shift; $|J_{AB}|$ = coupling constant.

An additional test for Se - O bond dissociation was to run the variable temperature NMR experiments in the presence of an acid catalyst. Bond dissociation would be facilitated by the protonation of an oxygen atom in 110. In the event, there was no decrease in convergence temperature when a catalytic amount of trifluoroacetic acid was
added to compound 110 dissolved in toluene-$d_8$, which rules out acid promoted hydrolysis leading to the rapid equilibration of the diastereotopic hydrogen atoms.

A related exchange process for the conversion of the endo and exo protons in the spirodioxselenuranes is carbon-oxygen bond cleavage. The resulting benzyl cation (248) would cause a scrambling of the endo and exo positions prior to recombination of the carbon-oxygen bond. As this is also an ionic process, the experiments described above similarly rule this out. Attempts were made to trap selenonium or benzylic cation intermediates in the presence of methanol. Upon heating 110 in toluene in the presence of methanol we did not observe either trapped intermediate 249 or 250 (Scheme 4.6).

Intermolecular processes could potentially lead to the equilibration of the endo and exo methylene hydrogen atoms. For example, dimerization or polymerization at higher temperatures could render the two hydrogen atoms equivalent if an equilibration
were occurring between the monomer and a dimer or higher order species. Such processes would be expected to be sensitive to the concentration of 110. In order to test this possibility, samples of 110 were prepared at various concentrations (0.003 M, 0.035 M, 0.053 M and 0.11 M) in toluene-$d_8$. Over this concentration range there was no difference in convergence temperatures between any of the samples. An additional test was to see if two different spirodioxyselenuranes in the same solution would have any effect on convergence temperature through the formation of heterodimers or oligomers. Thus, 110 and 134 were dissolved together in toluene-$d_8$ and variable-temperature NMR spectroscopy was run on this sample. Convergence of the AB quartet occurred independently for each compound at their expected temperatures.

After ruling out the routes to equilibration of the endo and exo protons described above, non-dissociative processes were investigated. For example, cuneal inversion of 110 to its enantiomer via planar intermediate 251 would result in inversion about the selenium atom and would render the methylene hydrogen atoms equivalent. We also wished to investigate whether Berry pseudorotation,\textsuperscript{156} or the related Ugi turnstyle mechanism,\textsuperscript{161} or processes proposed by Muettterties\textsuperscript{162} could be responsible for the temperature-promoted equivalence of the diastereotopic hydrogen atoms. These processes are summerized in Scheme 4.7.
In order to explore the viability of these processes, computational experiments were conducted by Nicole McNeil, a PhD candidate in our group. The Gibbs free energy difference ($\Delta G_{298}$) between $110$ and the planar transition state $251$ was found to be 43.7 kcal mol$^{-1}$. This effectively rules out the cuneal inversion pathway in any equilibration between $110$ and $\text{ent-110}$ at the relatively low convergence temperature of 267 K observed for $110$. Next, Nicole McNeil attempted to determine the lowest energy pathway for potential stereomutations. Berry pseudorotation of $110$ to product $252$ was not expected to be feasible, as this would place carbon atoms in the apical positions, which, as mentioned earlier, would be a high energy process. Indeed, computations revealed that the lowest energy pathway involves $253$ as an initial transition state with a difference in Gibbs free energy from $110$ of 32.8 kcal mol$^{-1}$. Transition state $253$ led to a pseudo-square pyramidal intermediate ($254$) with $\Delta G_{298}$ of 19.9 kcal mol$^{-1}$. A second transition state with structure $\text{ent-255}$ was found ($\Delta G_{298} = 55.1$ kcal mol$^{-1}$) which then
could produce \textit{ent-110} to complete the transformation. When \textit{ent-110} was treated similarly, the mirror image pathway regenerated the starting material \textit{110}. These computations indicated that the modified pseudorotation mechanism comprises a relatively high energy pathway with a second transition state that is even higher than that encountered in the cuneal inversion process. Neither mechanism provides a viable exchange process for spirodioxselenurane \textit{110}. The energy profiles for both pathways are shown in Figure 4.11, while Scheme 4.8 shows their generic representation.

\begin{center}
\includegraphics[width=\textwidth]{figure4_11.png}
\end{center}

\textbf{Figure 4.11 Potential Energy Surface for Configurational Inversion of 110.}^{163} (Δ\textit{G}\textsubscript{298} in kilocalories per mole).

148
The NMR and computational experiments described above enabled us to rule out all of the possible dynamic exchange processes for the spirodioxyselenuranes that had been proposed by previous researchers for other, related compounds (see Section 4.1). A final hypothesis was that the diastereotopic endo and exo protons of the spirodioxyselenuranes had temperature-dependent chemical shifts. If this were the case, we expected that the gradual, temperature-controlled convergence of their respective chemical shifts would result in the observed coalescence of their low-temperature AB quartets into singlets. Further heating could then be followed by a crossover and divergence of their chemical shifts. The temperature-dependence of proton chemical shifts associated with hydrogen-bonded species is well known; however there are
relatively few studies of other types of compounds. To test this possibility, compounds 110 and 134 were heated beyond their respective coalescence temperatures in toluene-$d_8$. The reappearance of AB quartets for 110 and 134 at 342 K and 316 K, respectively, supported the temperature-dependent chemical shift hypothesis. We were unable to observe similar re-emergence of AB quartets for spirodioxyselenuranes 135-137, but this can be attributed to the inability to heat these samples sufficiently above their initial convergence temperatures in toluene-$d_8$. Spirodioxyselenurane 110 displayed the same NMR behaviour in DMSO-$d_6$ when it was heated beyond its DMSO-specific convergence temperature. In this case, the AB quartet became a singlet as expected, and then a second AB quartet appeared at 425 K. Figure 4.12 shows the variable temperature $^1$H NMR spectra of 110 in toluene-$d_8$ at temperatures above room temperature.

![Figure 4.12](image-url)

**Figure 4.12** (a) Room-temperature and (b) variable-temperature (316 – 377 K) $^1$H NMR (300 MHz) spectra of 110 in toluene-$d_8$
Additional computations were carried out by Nicole McNeil, with the help of Professor Arvi Rauk. They calculated the types and number of vibrational modes that would be significantly populated in the temperature ranges at which the $^1$H NMR experiments were run. Generally, the vibrational motion of the two exo-protons or the two endo-protons can be categorized as harmonic or anharmonic. If the motion is harmonic (i.e. the two exo protons are vibrating in the same way) the chemical shift of the exo-protons would be unaffected by thermal excitation of that vibrational mode. However, if a vibrational mode is anharmonic (i.e. the two exo protons are undergoing different vibrational motions; for example, in opposition to each other) then the chemical shift can be affected as this anharmonic vibrational mode becomes thermally populated. The same arguments can be applied to the vibrational modes associated with the endo-protons. The vibrational energy levels of each of the modes are populated on the basis of Boltzmann’s distribution and so the NMR spectrum is the result of the Boltzmann-averaged sum of the contributions of each of the vibrational modes. As the temperature of the sample increases, the population of the higher energy levels within the vibrational modes also increases. Nicole McNeil found that some of the vibrational modes were anharmonic and that as these vibrational modes became thermally excited the calculated chemical shifts of the exo and endo protons changed. In fact, the calculated difference in chemical shift (i.e. $\Delta\delta_{\text{exo}/\text{endo}}$) decreased eventually to zero, resulting in the appearance of a singlet. Significantly, the chemical shifts calculated for these protons at even higher thermal excitation continued to change, resulting in the reappearance of AB quartets as observed in the experimental $^1$H-NMR spectrum. It was concluded that, as the temperature rises, higher vibrational levels in each vibrational mode become populated.
and contribute increasingly to the overall motion of the molecule. This impacts the electronic and magnetic environment surrounding the diastereotopic protons and results in the temperature-dependent chemical shifts observed with the spirodioxyselenuranes.

4.4 Summary and Conclusions

The spirodioxyselenuranes display unusual variable-temperature NMR behaviour, in which their diastereotopic methylene protons appear as expected AB quartets at low temperature, converge and eventually coalesce to singlets upon heating, and finally, in the case of 110 and 134, unexpectedly regenerate new AB quartets at still higher temperature. All spirodioxyselenuranes were soluble in toluene-$d_8$, and so this solvent was generally used for the variable-temperature NMR experiments. A potential explanation for the low-temperature NMR behaviour was reversible selenium – oxygen hydrolysis to produce achiral selenoxide intermediates or heterolytic cleavage to the corresponding selenonium species, followed by their pyramidal inversion. These possibilities, as well as that of benzylic carbon – oxygen bond cleavage, were ruled out by the lack of significant solvent effects and the negligible effect of the presence or absence of added water or acid catalysts. The NMR behaviour was not concentration dependent and so associative mechanisms can also be ruled out.

Computational studies suggested that cuneal inversion through selenium, Berry pseudorotation, and turnstile rotation all proceed by high energy pathways that were inconsistent with the observed convergence at relatively low temperature. Furthermore, and most important, neither the nondissociative processes investigated by computational
methods nor the possibilities investigated through solvent and concentration effects provide satisfactory rationale for the unexpected reappearance of new AB quartets at higher temperatures for 110 and 134. Additional computations on 110 revealed that, with increasing temperature, the vibrational energy levels of vibrational modes with anharmonic motion become increasingly populated. This causes the chemical shifts of the exo and endo protons to change on a temperature-dependent basis. The result of this is that the chemical shifts of the diastereotopic protons converge, which causes the AB quartets to collapse into singlets due to coincidental chemical shift equivalence. Further heating results in crossover and divergence of the chemical shifts, which manifests itself in the reappearance of new AB quartets.

To our knowledge, this phenomenon has not been previously observed in spirodioxyselenuranes or in other classes of trigonal-bipyramidal compounds. These results suggest that caution must be exercised in attributing coalescence of NMR signals of diastereotopic protons in variable-temperature experiments to dynamic exchange processes. Temperature-dependent chemical shifts that can lead to coincidental chemical shift equivalence may be a more widespread phenomenon than is generally recognized. A reasonable experiment to perform when such behaviour is observed, providing that the sample under scrutiny is thermally stable, would be to heat as far beyond the coalescence temperature as possible to determine whether resolution of the collapsed singlet into a new multiplet occurs.
5.1 Introduction

Chapter One outlined the work of a number of groups involved in the design of glutathione peroxidase mimetics, many of which have focussed their investigations upon diaryl diselenides. The catalytic cycles of these diselenides fall into two broad categories. The first involves the diselenide only briefly as a procatalyst. A second class exists where the diselenide participates directly in the catalytic cycle.

In the first scenario, as exemplified by the work of Bhabak and Mugesh and shown in Scheme 5.1, a molecule of diselenide (31) undergoes thiolysis to produce the corresponding selenenyl sulfide 256 and selenol 257, both of which are participants in the catalytic cycle. Reduction of peroxide occurs with selenol 257, which becomes oxidized to the corresponding selenenic acid 258. Reaction of 258 with benzenethiol produces a molecule of selenenyl sulfide 256 and water. Completion of the catalytic cycle occurs after reaction of the selenenyl sulfide with another molecule of benzenethiol, reforming selenol 257 and a molecule of diphenyl disulfide.90
The second scenario (Scheme 5.2), where the diselenide participates directly in the catalytic cycle is unlikely to occur in vivo due to the concentration of selenium species required for the inevitable step involving two organoselenium molecules. However, Mugesh has reported that diselenides with pendant amido groups apparently fall into this category, at least under the conditions of their assay. In this case, the diselenide (259) is not reactive toward benzenethiol, but instead reacts with hydrogen peroxide to produce two molecules of the corresponding selenenic acid species (260). This is followed by the quick reaction of 260 with benzenethiol, which produces selenenyl sulfide 261. Bhabak and Mugesh suggested that two molecules of 261
disproportionate to reform diselenide 259. Other groups have suggested that this final step is unlikely and that oxidation of 261 to its thioseleninate, followed by reformation of the selenenic acid 260 is a more likely pathway to complete the catalytic cycle.

Scheme 5.2

Despite some improvement to catalytic activity afforded by certain ortho substituents, diaryl diselenides are generally relatively poor catalysts. We reasoned that destabilizing the diselenide bond might result in improved catalytic activity compared to other diselenides. Generally, acyclic diselenides are most stable when the dihedral C–Se–Se–C angle is acute and lies within the range of 74°-87°. It has been found that decreasing this dihedral angle by incorporating the diselenide in a cyclic structure, for example in 263, results in strong bathochromic shifts observed in the UV-vis spectra of
these compounds. In 1983, our group observed that increasing the C–Se–Se–C dihedral angle to 112.1°, as found in 264, also resulted in large bathochromic shifts. These bathochromic shifts are likely due to increases in the HOMO energy levels of the diselenides, which is manifested as a red-shift in the absorption spectra for these compounds, caused by a decreased HOMO – LUMO gap.

![Diselenides with various C-Se-Se-C dihedral angles](image)

**Figure 5.1 Diselenides With Various C-Se-Se-C Dihedral Angles**

We reasoned that the oxidation of compounds with increased HOMO energy levels with hydrogen peroxide would occur more quickly than the diaryl diselenides investigated previously by other groups. As Chapters 2 and 3 have demonstrated, the rate-determining step in the catalytic cycle for previous GPx mimetics investigated by our group is the oxidation of the selenium atom. We expected that enabling oxidation to occur more quickly, by straining the dihedral angle in diselenides, would produce compounds with increased GPx-like activity. Attention was therefore turned to the known naphtho[1,8-c,d]-1,2-diselenole 265 and its novel 2,7-dimethoxy derivative 266 (Figure 5.2). The dimethoxy compound was included as the methoxy groups were expected to facilitate the oxidation of selenium atoms, as had been seen for other methoxy-substituted compounds.
The rigid and nearly planar structure of peri-diselenide 265 results in a severely constrained C–Se–Se–C dihedral angle and longer than normal Se – Se bond length. The X-ray crystal structure of 265 has been reported and the Se - Se bond length was shown to be 2.3639(5) Å, while the C–Se–Se–C dihedral angle was not reported, but is assumed to be close to 0°. For comparison, diphenyl diselenide has a Se - Se bond length of 2.29 ± 0.01 Å and a dihedral angle of 82.0°.

5.2 Synthesis and GPx Activity of Naphthalene peri-Diselenides 265 and 266

The diselenide 265 was known and was made using a modification of the method of Meinwald and coworkers, which involved lithium-halogen exchange of commercially available 1,8-dibromonaphthalene (267), followed by exposure to elemental selenium and finally air oxidation to 265. The dimethoxy derivative 266 was prepared in a similar manner but required the synthesis of the precursor 1,8-dibromo compound, starting from commercially available 2,7-dimethoxynaphthalene (268). Bromination of this compound with NBS led to 1,8-dibromo-2,7-dimethoxynaphthalene (269), the route to which had been developed by Whiting and coworkers. Lithium-halogen exchange of 269, followed by exposure to elemental selenium and finally air oxidation produced peri-diselenide 266 in much higher yield than that of 265. These processes are shown in Scheme 5.3.
The X-ray crystal structure of 266 (Figure 5.3) was similar to that of compound 265, with an elongated selenium–selenium bond of 2.3619(6) Å. The constrained C-Se–Se-C dihedral angle (-1.60°) was expected to destabilize the diselenide and result in a more facile reaction with hydrogen peroxide.

For purposes of comparison, the ditelluride compound 270 was prepared in a manner similar to 266 after lithium-halogen exchange of 269, followed by treatment with...
tellurium powder. Also prepared for comparison was di-(2-methoxyphenyl) diselenide 271, which was produced by reacting the diazonium salt of 2-aminoanisole (272) with potassium diselenide (Scheme 5.4).\(^{171}\)

Scheme 5.4

Both diselenides 265 and 266 were tested in our HPLC-based assay, along with di-(2-methoxyphenyl) diselenide and diphenyl diselenide for comparison. The results are provided in Table 5.1, where the values are reported as an average over at least two trials carried out under the standard conditions (0.031 M BnSH, 0.035 M H\(_2\)O\(_2\) and 0.0031 M catalyst in 19:1 DCM to MeOH, at 18 °C) of our assay.

Table 5.1 GPx Activity of peri-Diselenides

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>(t_{1/2}) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nil</td>
<td>176</td>
</tr>
<tr>
<td>2</td>
<td>PhSeSePh</td>
<td>129</td>
</tr>
<tr>
<td>3</td>
<td>271</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>265</td>
<td>9.7</td>
</tr>
<tr>
<td>5</td>
<td>266</td>
<td>7.4</td>
</tr>
<tr>
<td>6</td>
<td>270</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Diphenyl diselenide provided only a slight improvement upon the uncatalysed reaction \((t_{1/2} = 129\) hours, entry 2). When di-(2-methoxyphenyl) diselenide (271) was
used as the catalyst, the $t_{1/2}$ improved slightly to 90 hours (entry 3), demonstrating the methoxy group’s ability to enhance the catalytic activity. This enhancement could be due to a dominant mesomeric effect relative to the suppressive steric effect observed with our earlier spirodioxyselenuranes.

Diselenide 265 provided an increase in catalytic activity of approximately an order of magnitude compared to diphenyl diselenide, with a $t_{1/2} = 9.7$ hours (entry 4). The dimethoxy analogue 266 showed a slight further improvement in catalytic activity with a $t_{1/2} = 7.4$ hours (entry 5). As expected, the strained, rigid and cyclic peri-diselenides 265 and 266 are considerably more effective GPx mimetics than simple diaryl diselenides. The electron-donating methoxy substituents on 266 confer a modest additional benefit to catalytic activity. Ditelluride 270 proved to be a remarkably active catalyst, producing reaction rates too fast to measure by HPLC. Separate reactions assayed after 1 and 3 minutes indicated that the yields of dibenzyl disulfide were 30% and 85%, respectively. However, after longer reaction times, or with added hydrogen peroxide, decreased disulfide yields were observed. This suggested that 270 also catalysed the further oxidation of the disulfide to mixtures of unidentified products when excess hydrogen peroxide was present. Figure 5.4 shows the kinetic plots for diphenyl diselenide, di-(2-methoxyphenyl) diselenide (271) and peri-diselenides 265 and 266.
5.3 Investigations into the Catalytic Cycle of Naphthalene peri-Diselenides

A number of control reactions were carried out in order to gain insight into the catalytic mechanism of the GPx-like activity of diselenides 265 and 266. These included observing their reactivity towards thiol in the absence of hydrogen peroxide and towards hydrogen peroxide in the absence of thiol. This helped to determine the first step in the catalytic mechanism. First, it should be noted that Kice and coworkers\textsuperscript{172} had investigated the peracid oxidation of 265 (see Scheme 5.5) and found that, with 1 equivalent of \textit{m}-chloroperoxybenzoic acid (MCPBA), it was converted into a 60:40 mixture of selenol-seleninate 273 and cyclic selenenic anhydride 274. They found that when 265 was subjected to 3 equivalents of MCPBA, the postulated cyclic seleninic anhydride 275 was
produced, but was highly insoluble, which prevented its characterization by NMR spectroscopy.

Scheme 5.5

When the novel compound 266 was subjected to control experiments it was found to react with 1 equivalent of hydrogen peroxide to form selenol seleninate 276. In the absence of thiol, this reaction was complete after 30 minutes with complete discharge of the purple colour of 266 and without the formation of the symmetrical cyclic selenenic anhydride corresponding to 274. Selenol seleninate 276 could be isolated and characterized by oxidation of 266 with 1 equivalent of MCPBA, once again without formation of any cyclic selenenic anhydride. On the other hand, treatment of 266 with excess benzyl thiol in the absence of hydrogen peroxide resulted in no significant reaction, even after 12 hours (Scheme 5.6).
It is clear that oxidation of 266 to the selenolselfeninate 276, and not ring-opening of the cyclic diselenide by thiol, is the first step in the catalytic cycle. In order to determine the next step, the selenolselfeninate 276 was subjected to the same control experiments. This compound showed no significant further oxidation after 24 hours when treated with an excess of hydrogen peroxide. Treatment of selenolselfeninate 276 with excess benzyl thiol in the absence of peroxide resulted in the reappearance of the purple colour after less than a minute, indicating the formation of 266, which was recovered in 85% yield after column chromatography. Therefore, the second step of the catalytic cycle under these conditions is reaction of the selenolselfeninate 276 with benzyl thiol and not further oxidation.

These experiments suggest the mechanism shown in Scheme 5.7, where the rate-determining and initial step is the oxidation of 266 to selenolselfeninate 276, followed by rapid reduction by benzyl thiol back to the original diselenide. When benzyl thiol was added to 276 slowly, in order to isolate further intermediates, polymeric products were formed which could not be identified. Treatment of these polymeric products with excess
benzyl thiol quickly reformed diselenide 266 and benzyl disulfide. By analogy to the previous work by Kice and coworkers, in which they reported the isolation of selenenyl sulfide compounds derived from 265, it is reasonable to postulate that transient intermediates 277 and 278 are formed during the reduction of 276 with benzyl thiol. After initial reaction of 276 with benzyl thiol to form 277, completion of the catalytic cycle could conceivably occur via reaction of a second molecule at the sulfur of the selenenyl sulfide. Subsequent reaction of the resulting selenolate with the electrophilic selenenic acid and overall expulsion of water would reform 266. Alternatively, the thiol could react with the selenenic acid of 277 to produce 278. In this case, the conversion of 278 to 266 may be catalysed by attack of the thiol at the sulfur atom of one of the selenenyl sulfide moieties, followed by intramolecular diselenide formation with regeneration of benzyl thiol. The rate-determining oxidation of 266 to 276 is consistent with the overall rate enhancement afforded by the ortho-methoxy groups.

Scheme 5.7
Additional confirmation of the role of selenolseleneinate $276$ in the catalytic cycle occurred upon measuring the GPx activity of this compound. As expected, $276$ was found to independently catalyse the reduction of hydrogen peroxide with benzyl thiol. In this case the $t_{1/2}$ was 5.9 hours, which is shorter than the $t_{1/2}$ for diselenide $266$. This is attributable to the very fast initial formation of dibenzyl disulfide upon exposure of $276$ to the assay conditions. The kinetic plot (see Figure 5.5) also shows a positive intercept of $\sim 11\%$, which is consistent with nearly instantaneous reaction with the thiol and consumption of $276$, followed by the slower subsequent reoxidation of the resulting diselenide.

![Figure 5.5 Single Reaction Plot for 276; x-axis in hours; y-axis in % BnSSBn.](image)

As mentioned above, selenolseleneinate $276$ was not oxidized further after 12 hours of exposure to hydrogen peroxide. However, when $276$ was oxidized with 2 equivalents of MCPBA, cyclic seleninic anhydride $279$ was formed, which could also be prepared directly from $266$ by treatment of the diselenide with 3 equivalents of MCPBA. In contrast to the highly insoluble seleninic anhydride $275$, obtained by Kice and coworkers,$^{172}$ $279$ was found to be sufficiently soluble in DMSO-$d_6$ to permit the recording of its $^1$H, $^{13}$C and $^{77}$Se NMR spectra. The $^1$H NMR spectrum showed the
presence of two sets of signals in the ratio of 1.0 to 0.8 (Figure 5.6), suggesting the presence of two distinct diastereomers.

Figure 5.6 $^1\text{H}$ NMR (400 MHz) Spectrum of Cyclic Seleninic Anhydride 279

Since the selenium atoms in 279 are chiral centres, the observed nonequivalence can be attributed to the presence of cis and trans diastereomers that are configurationally stable at room-temperature on the NMR time scale (Figure 5.7).

Figure 5.7 Cyclic Seleninic Anhydrides 279 and Dipotassium Salt 280
The signals did not coalesce when a sample of the mixture was subjected to variable-temperature $^1$H NMR experiments and heated to 403 K. However, the presence of cis and trans isomers was confirmed by adding excess potassium hydroxide in deuterium oxide to the NMR sample. Figure 5.8 shows the resulting $^1$H NMR spectrum, which contains a single set of signals, presumably caused by anhydride ring opening and formation of the dipotassium salt, 280, of the corresponding bis(seleninic acid).

Figure 5.8 $^1$H NMR (400 MHz) Spectrum of Dipotassium Salt 280

5.4 Photophysical Properties of the Naphthalene peri-Dichalcogenides

The planar, highly electron-rich nature of diselenide 266 and ditelluride 270 suggested to us that these molecules might be efficient donors in donor-acceptor
complexes. These donor-acceptor complexes are usually weak and result from the ground-state mixing of the HOMO of the donor and the LUMO of the acceptor. Interest in novel organic charge transfer donors was initiated by the synthesis of tetrathiafulvalene (TTF, 281), its stable radical cation and dication, along with the observation of the metallic-like conductivity of the electron transfer complex of TTF with tetracyanoquinodimethane (TCNQ, 282). The heavier and more polarizable selenium and tellurium analogues of sulfur-containing donor molecules are expected to reduce coulombic repulsions and improve charge transfer properties, in part through differences in ionization potentials and increased HOMO energy levels. Two first such examples included tetraselenafulvalene and tetramethyltetraselenafulvalene, which are both better donors than their sulfur-containing counterparts. Systems that display charge transfer bands can act as semi-conductors, which are fundamentally important components of organic photovoltaic cells, organic field effect transistors, and organic light emitting diodes. Broad charge transfer bands enhance the photon-harvesting properties for organic photovoltaic applications.

![Figure 5.9 TTF and TCNQ](image)

Although many TTF-based donors are known, relatively little has been reported on chalcogenoacenes containing the naphtha[1,8-cd]-1,2-dichalcogen core. The constraint of the dihedral angles of diaryl dichalcogenides from nearly orthoganol to either larger or smaller angles, as in the case of the nearly planar naphthalene peri-
dichalcogenides, is accompanied by a bathochromic shift that results from destabilization of the ground state and a decrease in the energy of the HOMO-LUMO transition. Electron-donating substituents, like those present on 266 and 270, are expected to increase the HOMO energies further. The photophysical properties of 266, 270 and their sulfur analogue 283 were investigated and compared to the unsubstituted naphthalene peri-diselenide 265. Donor-acceptor complexes between these dichalcogenides and TCNQ were observed to form in acetonitrile.

Absorption spectra of compounds 265, 266, 270 and 283 are shown in Figure 5.10. All compounds absorbed strongly in the UV to near-visible region with molar absorptivities near 10,000 M\(^{-1}\) cm\(^{-1}\). As expected, the unsubstituted diselenide 265 displayed the weakest absorptivity compared to the dimethoxy derivatives 266, 270 and 283. For each of these compounds, the lowest energy transition undergoes a bathochromic shift down the period from sulfur to tellurium. These compounds show extremely weak electronic transitions above 450 nm and the molar absorptivity of the UV-vis absorptions did not change over a concentration range of 10\(^{-5}\) M to 10\(^{-3}\) M, indicating that negligible intermolecular interactions occur in acetonitrile.
When TCNQ was added to any of the dichalcogenides, a new absorption feature emerged at longer wavelength, which is shown in Figure 5.11. The near-IR spectrum of each compound was recorded in the presence of TCNQ at 1:1 stoichiometry, while the overall concentration of TCNQ and donor compound was 5 x 10^{-3} M. The new absorption feature was very broad in all cases and, although weak, can be attributed to a donor-acceptor interaction with charge transfer character. Ditelluride 270 had an exceptionally broad charge-transfer peak from approximately 900-1600 nm and also had the strongest absorption. The lowest value of \( \lambda_{\text{max}} \) for TCNQ with a donor molecule was with diselenide 265 at 936 nm. All methoxy-substituted donor molecules showed bathochromic shifts, relative to 265. For the TCNQ complexes of the dimethoxy compounds, the \( \lambda_{\text{max}} \) values were 1117 nm, 1125 nm and 1205 nm for 283, 266 and 270,
respectively. The absorption features observed are remarkable for naphthalene based donor molecules. Specifically, the broad bands in the near-IR region allow for absorption of low energy photons across a large portion of the solar spectrum not often accessible with other light-harvesting materials.

![Graph of absorption bands](image)

**Figure 5.11 Charge Transfer Bands of Dichalcogenides with TCNQ**

Job plots confirmed that the donor-TCNQ complexes studied all had 1:1 stoichiometry. The Job plots shown in Figure 5.12 were prepared by using the continuous variation concentration method and measuring the absorbance value at $\lambda_{\text{max}}$ for each donor-TCNQ mixture. The near-IR plots resulting from the continuous variation concentration method for ditelluride 270 are presented in Figure 5.13, while similar plots for the other complexes are found in Appendix B.
Figure 5.12 Job Plots for TCNQ – Dichalcogenide Complexes. All graphs: y-axis in absorbance at $\lambda_{\text{max}}$ for each complex.

Figure 5.13 Continuous Variation Concentration Plot for Ditelluride 270 and TCNQ
5.5 Summary and Conclusions

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 265. A severe decrease in the C–Se–Se–C dihedral angle results in a much faster reaction of the peri-diselenide with hydrogen peroxide than that observed for diselenides without conformational restriction. The introduction of electron-donating 2,7-dimethoxy substituents on the naphthalene skeleton resulted in peri-diselenide 266. The methoxy groups provided a further improvement in catalytic activity by facilitating the rate-determining Se(II) to Se(IV) oxidation step in the catalytic cycle. A further dramatic increase of more than two orders of magnitude was realized with the tellurium analogue 270, relative to the diselenides 265 and 266.

The GPx activity was determined to originate from the catalytic cycle presented in Scheme 5.7. The peri-diselenide 266 reacts with hydrogen peroxide in the rate-determining step to form selenoseleninate 276. The reaction of this compound with benzyl thiol quickly regenerates the peri-diselenide 266. Unlike the catalytic cycles presented for diaryl diselenides, we unequivocally demonstrated that the diselenide moiety participates in the catalytic cycle of these conformationally constrained, cyclic compounds. Although compounds with selenium in a higher oxidation state than selenol seleninate 276 could be formed by reaction with MCPBA, the resulting cyclic seleninic anhydride does not participate in the catalytic cycle as the oxidation of 266 does not proceed beyond 276 in the presence of only hydrogen peroxide.
The planar structures of 265, 266 and 270, along with the increased electron-rich nature of 266 and 270, due to their dimethoxy substitution, led us to investigate their properties as donor molecules in donor-acceptor charge transfer systems. Disulfide 283 was also prepared in order to complete the series. Moving down the period from sulfur to tellurium in the dimethoxy series, there is a clear trend toward lower energy absorptions of the charge-transfer band associated with complex formation in acetonitrile. The 2,7-dimethoxy substituents are advantageous because they enhance synthetic access by increasing yields (19% yield for 265 vs 64% yield for 266, respectively, during the introduction of selenium), they improve solubilities compared to diselenide 265, and they add electron density to the aryl systems, resulting in a further increase in HOMO energy levels and the ability to absorb very low energy photons when complexed with electron-deficient acceptors.
Chapter Six: Conclusions and Future Work

6.1 Summary and Conclusions

Cyclic seleninate esters and spirodioxyselenuranes display glutathione peroxidase-like activity which is comparable to and, in many cases, much better than that of the well-known GPx mimetic, ebselen. A Hammett analysis of para-substituted compounds demonstrated that both the cyclic seleninate esters and the spirodioxyselenuranes participate in catalytic cycles where the peroxide mediated oxidation of the selenium atom from Se(II) to Se(IV) is the rate-determining step. Therefore, electron-donating groups, capable of stabilizing increased positive charge are desirable substituents for including on the aromatic backbones of these compounds. The spirodioxyselenuranes are more sensitive to substituent effects and generally display better activity than the cyclic seleninate esters. Compounds substituted with para-methoxy groups have very strong glutathione peroxidase-like activity, while substitution with meta- or ortho-methoxy groups results in lower catalytic activity. In the case of meta-substitution, this is due to the inability of the methoxy group to interact with the selenium atom through mesomeric processes, while ortho-substitution causes a decrease in activity due to steric crowding around the selenium atom.

Hybrid aliphatic/aromatic selenides where the selenium atom is substituted with either a 3-hydroxypropyl or 2,3-dihydroxypropyl chain are water soluble and display strong glutathione peroxidase-like activity. Significantly, 4,5-dimethoxy-2-(hydroxymethyl)phenyl 3-hydroxypropyl selenide (204) displays the strongest glutathione peroxidase-like activity of all of the selenium compounds prepared by our
group to date. The activity of this compound is greater than that of ebselen by more than two orders of magnitude.

Variable-temperature analysis revealed that the spirodioxyselenuranes display interesting temperature-dependent $^1$H NMR behaviour. The $^1$H NMR spectra of these compounds were found to display AB quartets for the benzylic methylene protons at low temperatures, which collapsed to singlets upon heating. Computations revealed that pseudorotation and inversion processes have high activation energies, thereby precluding their participation in potential exchange processes. Similarly, failure to trap ionic intermediates ruled out exchange processes which involve Se – O or C – O cleavage. Finally, the $^1$H NMR behaviour of the spirodioxyselenuranes was discovered to be due to temperature-dependent chemical shifts which result in coincidence of the $^1$H NMR signals for the diastereotopic hydrogen atoms and coalescence of the AB quartets, followed by the divergence of their chemical shifts and regeneration of the AB quartets upon further heating. Importantly, these results suggest that caution must be exercised in attributing coalescence of NMR signals of diasterotopic protons in variable-temperature experiments to dynamic exchange processes. It may be that temperature-dependent chemical shifts leading to coincidental chemical shift equivalence is a more widespread phenomenon than is generally recognized. To our knowledge, this is the first time that such behaviour has been noted.

Finally, naphthalene peri-diselenides display significantly improved GPx-like activity relative to diaryl diselenides. This improved activity is due to the severely
reduced dihedral angle found in the naphthalene peri-diselenides, which leads to a destabilized ground state, lower ionization potential and an increased rate of reaction with peroxides. Moreover, these naphthalene peri-diselenides and their tellurium counterparts display remarkable photophysical properties when complexed with TCNQ. These charge transfer complexes have broad absorption bands in the near-IR region, allowing for the absorption of low energy photons in a portion of the solar spectrum not often accessible with other light-harvesting materials.

6.2 Future Directions

6.2.1 Electron-Rich GPx Mimetics

The efficiency of the hybrid aliphatic/aromatic selenides as GPx mimetics, coupled with their ease of synthesis from accessible diaryl diselenides and their general water solubility make this an important class of compounds for further improvement. It would be interesting to investigate aryl analogues with para-amino substituents, which are also electron-donating. For example, the Hammett substituent constants for unsubstituted nitrogen and N,N-dimethylamino groups at the para position (\(\sigma_p\)) are -0.57 and -0.63,\(^{184}\) respectively, while that for the methoxy group is -0.28.\(^{185}\) All three substituents are able to stabilize positive charge but the nitrogen-containing substituents are stronger stabilizing groups, even when located at the meta position (i.e. NMe₂, \(\sigma_m = -0.10\), NH₂, \(\sigma_m = -0.09\), while MeO \(\sigma_m = 0.10\)).\(^{184}\) Thus, organoselenium compounds such as 284, 285 and 286 are of special interest (Figure 6.1). Not only would these compounds be expected to have strong GPx activity, they would likely be very water soluble and so could be assessed in an aqueous environment under the conditions of the \(^1\)H NMR assay.
In addition to further improvement of GPx-like activity, there will be a need to investigate potential drug candidates through \textit{ex vivo} and eventually \textit{in vivo} studies. It has been established that the best compounds are the hybrid aliphatic/aromatic analogues and so these compounds should be considered as lead candidates for further testing. Specifically, compounds \textbf{188}, \textbf{192} and \textbf{204} display particularly strong catalytic activity. Additionally, the hybrid compounds also are water soluble, which may allow for their intravenous administration during potential \textit{in vivo} studies. If it should be found that water solubility actually causes problems due to quick clearance or poor targeting, the versatility of the \textit{para}-alkoxy group may allow for fine tuning of overall lipophilicity, presumably occurring without changing the level of GPx-activity.

\textbf{6.2.2 Potential Asymmetric Oxidation Catalysis with Spirodi oxyxyselenuranes}

The 2,3-dihydroxypropyl selenides were prepared after alkylation of aryl selenolates with (S)-glycidol to produce single enantiomers, for example \textbf{191} (see Scheme 6.1, if \(R = \text{H}\)). Oxidation of such compounds and subsequent cyclisation would result in the formation of diastereomers \textbf{287} and \textbf{288}, obtained as single enantiomers (Scheme 6.1). The mixture would be expected to be enriched in \textbf{287} because the hydroxyl group (when \(R = \text{H}\)) in \textbf{287} is \textit{exo} and located on the sterically less hindered, convex face.
of the molecule. If the secondary alcohol is replaced by a bulky ether substituent, the ratio of 287 to 288 would be further enriched in favour of 287.

Scheme 6.1

This creates an exciting scenario where a molecule capable of carrying out catalytic oxidation reactions can be enantiomerically enriched by starting with a choice of either (S)-glycidol or (R)-glycidol, both of which are relatively inexpensive. The configurational stability of the fully aromatic compounds, as demonstrated by our $^1$H NMR spectroscopic and computational investigation of these molecules, suggests that once the spirodioxyselenurane forms, it is unlikely to undergo processes which would compromise the enantiopurity of molecules such as 287. Although the spirodioxyselenuranes have not been investigated as catalysts for general oxidation reactions, the seleninate esters have been shown to catalyse the oxidation of sulfides to sulfoxides, the epoxidation of alkenes and the conversion of enamines to $\alpha$-hydroxyketones.¹⁵¹ Thus, it is not unreasonable to suggest that, under the correct conditions, compounds such as 287 or its enantiomer could catalyse similar, asymmetric transformations. As compounds such as 191 are stable in water, it may even be possible to carry out such reactions in aqueous environments with environmentally benign
hydrogen peroxide, which would be a green alternative to traditional oxidation conditions.

6.2.3 Naphthalene Based Compounds

There is potential for both the materials applications of the naphthalene dichalcogenides and their use as GPx mimetics. Although the 2,7-dimethoxy substituents resulted in a modest increase in activity, relative to the unsubstituted naphthalene peri-dichalcogenide, it may be of interest to modify substituents at these positions. For example, compounds with 2,7-di(hydroxymethyl) substitution, such as 289, would be of interest for two reasons. This compound contains the strained selenium – selenium bond and, importantly, the possibility of oxidation to two cyclic seleninate esters moieties attached to the same molecule, potentially enhancing glutathione peroxidase activity. An alternative compound, which can generate two units of the spirodioxselenurane moiety per molecule, would be 290. Although both molecules look promising as GPx mimetics, they may be too sterically hindered to act as efficient GPx mimetics.

It may also be worthwhile investigating ways to polarize the selenium – selenium bond in the naphthalene peri-diselenides. This could potentially make these diselenides more reactive toward oxidation or, in a reversal of reactivity, result in reaction with thiol as a first step in catalytic activity. One way to do this would be to prepare compounds with electron-withdrawing (EWG) and electron-donating (EDG) groups at the 2 and 7
positions of the naphthalene core. However, a molecule of this sort (291) would likely be much easier to design than to synthesize.

Further utility for the naphthalene compounds exists in their use as donors for charge-transfer complexes. The weak complexes formed between TCNQ and the 2,7-dimethoxynaphthalene peri-diselenides could be improved by increasing the electron-rich nature of the naphthalene core. One way to do this would be to include a 4,5-peri-diselenide group in addition to the 1,8-peri-diselenide already found on the molecules. Naphthalene bis(diselenides) exist, although the methoxy substituted derivatives have not been reported in the literature. A second strategy to improve the electron-rich nature of the naphthalene core would be to increase the number of methoxy groups on the compound. Combining the two strategies (i.e. including a 4,5-peri-diselenide and extra methoxy groups) suggests the highly electron-rich molecule 292 or, alternatively, 293.
Chapter Seven: Experimental Section

7.1 General Comments

All reagents, unless otherwise noted, were obtained from commercial sources and purified by standard methods as necessary. THF was distilled over LiAlH$_4$ immediately prior to use, obtained from an MBraun MB-SPS solvent purification system or dried using the method of Williams and Lawton.$^{186}$ The concentrations of alkyllithium reagents were determined by titration with menthol in the presence of the indicator 2,2'-bipyridyl immediately prior to use.$^{187}$ Hydrogen peroxide was titrated prior to use by the method of Kolthoff.$^{188}$ Ebselen (13) was prepared using the method developed by Engman.$^{189}$ All reactions were carried out in ventilated fumehoods. All glassware used for reactions involving selenium compounds was washed with sodium hypochlorite solution and selenium waste was kept in segregated containers. Chromatography refers to flash chromatography on silica gel (230-400 mesh). NMR spectra were recorded in the solvent indicated. Referencing of chemical shifts for $^1$H and $^{13}$C NMR spectroscopy was relative to the residual solvent (CHCl$_3$: $^1$H at $\delta$ 7.26 ppm, $^{13}$C at $\delta$ 77.2 ppm; DMSO: $^1$H at $\delta$ 2.50 ppm, $^{13}$C at $\delta$ 39.5 ppm),$^{190}$ while $^{77}$Se and $^{125}$Te NMR spectroscopy were recorded with diphenyl diselenide ($\delta$ 463 ppm)$^{191}$ and diphenyl ditelluride (421 ppm)$^{192}$ as external standards, relative to dimethyl selenide and dimethyl telluride ($\delta$ 0.00 ppm), respectively. Assignments of primary (CH$_3$), secondary (CH$_2$), tertiary (CH) and quarternary (C) carbons, where indicated, were based upon DEPT-135 analysis. Numbering of atoms in structures shown in this Chapter was made for convenience in indicating spectral assignments and does not necessarily reflect IUPAC nomenclature. Melting points were
measured using an A. H. Thomas hot-stage apparatus. IR spectra were recorded on a Nicolet Nexus 470 spectrometer fitted with an attenuated total reflectance accessory and IR (solid) indicates use of this accessory. $^1$H NMR, $^{13}$C NMR, $^{77}$Se NMR and $^{125}$Te NMR data were collected on a Bruker DMX-300 ($^1$H, 300 MHz; $^{13}$C, 75 MHz), a Bruker AMX-300 ($^1$H, 300 MHz; $^{13}$C, 75 MHz, $^{77}$Se, 57 MHz), a Bruker DRX 400 MHz ($^1$H, 400 MHz, $^{13}$C, 101 MHz, $^{77}$Se, 76 MHz), a Bruker Avance III 400 MHz ($^1$H, 400 MHz, $^{13}$C, 101 MHz, $^{77}$Se, 76 MHz, $^{125}$Te, 126 MHz) or a Bruker Avance II 400 MHz ($^1$H, 400 MHz, $^{13}$C, 101 MHz, $^{77}$Se, 76 MHz, $^{125}$Te, 126 MHz). Low and high resolution mass spectra were obtained on a Waters GCT Premier, a Thermo Finnigan SSQ7000, a Bruker Esquire 3000, an Agilent 6520 Q-Tof or a Bruker FT-ICR-MS Apex mass spectrometer by Ms. Q. Wu, Ms. D. Fox, Mr. J. Li or Mr. W. White. All mass spectra were obtained by electron impact ionization at 70 eV with a direct probe sample introduction unless otherwise indicated. Elemental analyses were determined by Mr. J. Li using a Control Equipment Corporation 440 Elemental Analyzer or a Perkin Elmer Series II 2400 CHNS/O Analyzer. The X-ray crystal structure presented in Chapter Five was collected by Dr. M. Parvez and the corresponding report in Appendix C was provided by him.

The HPLC-based in vitro assay was carried out in the following manner. Catalytic activity was measured by adding the catalyst (0.031 mmol, 10 mol % relative to thiol) to a mixture of 33% hydrogen peroxide (0.35 mmol, 0.035 M) and redistilled benzyl thiol (0.31 mmol, 0.031 M) in 10.0 mL of dichloromethane-methanol (95:5) while maintaining the temperature at 18 ºC. The reactions were monitored by HPLC analysis, using a UV detector at 254 nm and a reversed phase column (Novapak C18; 3.9 x 150 mm). For
Chapter Two; acetonitrile–water was employed as the solvent (gradient: 60:40 to 80:20 over 15 minutes with a flow rate of 0.9 mL/min) for all compounds except ebselen (gradient: 60:40 to 100:0 over 15 minutes with a flow rate of 0.9 mL/min) and 123 (gradient: 50:50 to 80:20 over 4 minutes and continued at 80:20 for a total of 15 minutes with a flow rate of 0.9 mL/min). For Chapters Three and Five, where some $t_{1/2}$ values were very fast, the eluent system was again composed of acetonitrile–water; however, a gradient method (acetonitrile-water, 60:40 to 80:20 over 15 minutes with a flow rate of 0.9 mL/min) was used for trials involving the cyclic seleninate esters, while an isocratic method (acetonitrile-water, 90:10 with a flow rate of 0.9 mL/min) was used for all other catalysts. For all trials, the % yield of dibenzyl disulfide at a given time was determined by using naphthalene (0.0080 M) as an internal standard according to the relationship below, as determined by the calibration plot shown.\(^{144}\)

\[
\text{(Mass of BnSSBn)} = \left( \frac{\text{Peak Area of BnSSBn}}{\text{Peak Area of Naphthalene}} \right) \times (0.036 \text{ mg}^{-1})
\]

![Calibration Plot](image)

\[y = 0.036x \quad R^2 = 0.9995\]
The reaction half-life ($t_{1/2}$) was determined by plotting the yield (%) of the product disulfide versus time and represents the time required for conversion of 50% of the thiol into its disulfide.

7.2 Experiments Related to Chapter Two

7.2.1 Preparation of 2,2′-diselenobisbenzoic acid (126a)

Sodium nitrite (5.87 g, 85.1 mmol) in 45 mL of water was added dropwise to a stirred solution containing 2-aminobenzoic acid (10.0 g, 72.9 mmol) and 45 mL of concentrated hydrochloric acid in 120 mL of water at 0 °C. The solution of the resulting diazonium salt was stirred for 20 minutes while a solution of potassium diselenide was prepared separately in the following manner. Elemental selenium (11.5 g, 146 mmol) and potassium hydroxide (32.8 g, 585 mmol) were mixed together and melted in a round bottomed flask with a heat gun. Cold water (100 mL) was added to the hot melt to produce a red aqueous solution of potassium diselenide, which was subsequently cooled to 0 °C. The pH of the diazonium salt solution was adjusted to neutral with 2 M NaOH and this was added to the potassium diselenide solution. The resulting mixture was stirred at 0 °C for 30 minutes, then heated with a heat gun until the initially red, opaque solution became transparent. The mixture was filtered to remove elemental selenium and the filtrate was acidified with 2 M HCl solution. The resulting precipitate (16.5 g) was collected by filtration and was found to contain diselenide 126a, but could not be purified.
and was used directly in the next step; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 13.73 (br s, 2H, COOH), 8.05 (d, $J$ = 8.0 Hz, 2 H), 7.69 (d, $J$ = 8.0 Hz, 2 H), 7.51 (t, $J$ = 7.2 Hz, 2 H), 7.39 (t, $J$ = 7.2 Hz, 2 H).

7.2.2 4-Substituted 2,2'-diselenobisbenzoic acids 126c-g

![Structure of 2,2'-diselenobisbenzoic acids 126c-g]

In general, diselenides 126c-g were prepared similarly, but contained large amounts of impurities. Since it was easier to remove the impurities at a later stage, they were typically used in the next step without further purification or characterization.

7.2.3 Preparation of 2,2'-Diselenobis(5-methoxybenzyl alcohol) (130b)

![Structure of 2,2'-Diselenobis(5-methoxybenzyl alcohol) (130b)]

In a flame-dried 100 mL round bottom flask, 300 mg (1.38 mmol) of 2-bromo-5-methoxybenzyl alcohol (128) was dissolved in 15 mL of dry THF, cooled to −78 °C and treated with tert-butyllithium (2.5 mL, 4.2 mmol) under a nitrogen atmosphere. The mixture was stirred for 40 min, selenium powder (330 mg, 4.18 mmol) was added and the cooling bath was removed. The mixture was stirred for 5 hours under a nitrogen atmosphere at room temperature to dissolve the selenium, forming a red solution. The flask was then opened and the mixture was stirred overnight in the presence of air,
followed by quenching with 1 M HCl (8 mL), extraction with diethyl ether, washing with saturated NH₄Cl, drying and concentration in vacuo. The final product was chromatographed (hexanes-ethyl acetate, 1:1) to give 173 mg (58%) of 130b as a yellow oil, which solidified upon standing; mp 103-104 °C (from ethanol-hexane): IR (KBr) 3337, 1294, 1233, 1056, 1014, 815 cm⁻¹; ¹H NMR (300 MHz; CDCl₃) δ 7.47 (d, J = 8.7 Hz, 2 H, H-3), 7.04 (d, J = 3.1 Hz, 2 H, H-6), 6.74 (dd, J = 8.7, 3.1 Hz, 2 H, H-4), 4.65 (s, 4 H, H-7), 3.84 (s, 6 H, H-8), 2.04 (br s, 2 H, O-H); ¹³C NMR (75 MHz; CDCl₃) δ 161.3 (C-5), 145.7, 139.1, 120.5, 114.2, 114.0, 65.6 (C-7), 55.5 (C-8); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 452.6; mass spectrum, m/z (EI, relative intensity) 434 (8, M⁺), 256 (18), 200 (58), 108 (100); exact mass calcd for C₁₆H₁₈O₄₈₀Se₂: 433.9536; found: 433.9517.

7.2.4 Typical Procedure for the Preparation of Selenides 130a,c-g: 2,2'-Diselenobisbenzyl alcohol (130a)

Diselenide 126a (10.0 g, 25.0 mmol) was suspended in 50 mL of THF and carefully added to lithium aluminum hydride (5.70 g, 150 mmol) in 125 mL of THF. The mixture was left to react at room temperature. After 14 hours, the mixture was cooled to 0 °C, quenched with 100 mL of water and washed with ethyl acetate. The aqueous layer was left overnight, resulting in the precipitation of 4.2 g (45%) of the diselenide as a yellow solid with mp 98-100 °C (from water); ¹H NMR (400 MHz; CDCl₃) δ 7.68 (dd, J = 7.6, 1.2 Hz, 2 H), 7.38 (dd, J = 7.6, 1.2 Hz, 2 H), 7.30 (dt, J = 7.5, 1.3 Hz, 2 H), 7.19 (dt, 7.6, 1.3 Hz, 2 H), 4.71 (s, 4 H, H-7), 2.02 (br s, 2 H, OH); ¹³C NMR (101 MHz;
CDCl$_3$) $\delta$ 142.3, 135.1, 130.8, 129.1, 128.9, 128.5, 65.5 (C-7); $^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 431.7. Diselenide 130a is a known compound and the spectral data was consistent with literature data.$^{130}$

7.2.5 2,2’-Diselenobis(5-methylbenzyl alcohol) (130c)

![Chemical structure](image)

Prepared according to the same procedure as for 130a. Yield: 43%. Yellow solid; mp 97-99 °C (from water): IR (KBr) 3273, 1456, 1057, 1015, 812 cm$^{-1}$; $^1$H NMR (300 MHz) $\delta$ 7.53 (d, $J$ = 7.7 Hz, 2 H, H-3), 7.23 (s, 2 H, H-6), 7.02 (d, $J$ = 7.7 Hz, 2 H, H-4), 4.66 (s, 4 H, H-7), 2.36 (s, 6 H, H-8); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 143.1, 138.0, 133.2, 129.6, 129.0, 127.0, 64.2 (C-7), 21.5 (C-8); $^{77}$Se NMR (57 MHz) $\delta$ 438.0; mass spectrum, $m/z$ (EI, relative intensity) 400.0 (30), 199.1 (38), 184.0 (62), 92.1 (100); exact mass calcd for C$_{16}$H$_{18}$O$_2$Se$_2$: 401.9637, found: 401.9644. Anal. Calcd for C$_{16}$H$_{18}$O$_2$Se$_2$: C, 48.02; H, 4.53; found: C, 48.23; H, 4.71.

7.2.6 2,2’-Diselenobis(5-phenylbenzyl alcohol) (130d)

![Chemical structure](image)

Prepared according to the same procedure as for 130a. Yield: 39%. Yellow solid; mp 55-56 °C (from water): IR (KBr) 3378, 1183, 1022, 757 cm$^{-1}$; $^1$H NMR (300 MHz) $\delta$
7.78 (d, $J = 8.2$ Hz, 2 H), 7.66 (m, 2 H), 7.62-7.59 (m, 4 H), 7.48-7.43 (m, 6 H), 7.39-7.34 (m, 2 H), 4.83 (s, 4 H, H-7), 2.01 (s, 2 H, OH); $^{13}$C NMR (75 MHz) $\delta$ 142.6, 142.0, 140.1, 135.7, 129.6, 129.0, 127.9, 127.4, 127.2, 127.1, 65.6 (C-7); $^{77}$Se NMR (76 MHz) $\delta$ 429.6; mass spectrum, $m/z$ (EI, relative intensity) 526 (4, M+), 246 (35), 154 (100); exact mass calcd for C$_{26}$H$_{22}$O$_2$Se$_2$: 525.9950; found 525.9959.

7.2.7 2,2'-Diselenobis(5-bromobenzyl alcohol) (130e)

Prepared according to the same procedure as for 130a. Yield: 47%. Yellow solid; mp 120-122 °C (from water); IR (KBr) 3365, 1087, 1030, 887, 800 cm$^{-1}$; $^1$H NMR (300 MHz) $\delta$ 7.60 (d, $J = 2.0$ Hz, 2 H, H-6), 7.49 (d, $J = 8.2$ Hz, 2 H, H-3), 7.33 (dd, $J = 8.2$, 2.0 Hz, 2 H, H-4), 4.73 (d, $J = 6.2$ Hz, 4 H, H-7), 1.93 (t, $J = 6.2$ Hz, 2 H, OH); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$ 145.5, 134.8, 131.7, 130.7, 129.6, 122.0 63.7 (C-7); $^{77}$Se NMR (57 MHz, DMSO-d$_6$) $\delta$ 401.1; mass spectrum, $m/z$ (EI, relative intensity) 532 (30, M+), 263 (60), 248 (100), 156 (90), 77 (63); exact mass calcd for C$_{14}$H$_{12}$$^{79}$Br$_2$$^{81}$BrO$_2$$^{80}$Se$_2$: 531.7514; found: 531.7513. Anal. calcd for C$_{14}$H$_{12}$Br$_2$O$_2$Se$_2$: C, 31.73; H, 2.28; found: C, 31.76; H, 2.25.
7.2.8 2,2’-Diselenobis(5-chlorobenzyl alcohol) (130f)

Prepared according to the same procedure as for 130a. Yield: 41%. Yellow solid; mp 118-120 °C (from water); IR (KBr) 3330, 1096, 1013, 874, 809 cm⁻¹; ¹H NMR (300 MHz) δ 7.55 (d, J = 8.2 Hz, 2 H, H-3), 7.45 (d, J = 2.6 Hz, 2 H, H-6), 7.18 (dd, J = 8.2, 2.6 Hz, 2 H, H-4), 4.72 (d, J = 5.6 Hz, 4 H, H-7), 1.97 (t, J = 6.2 Hz, 2 H, OH); ¹³C NMR (75 MHz, DMSO-d₆) δ 145.4, 134.7, 133.6, 128.9, 128.8, 127.8, 63.7 (C-7); ⁷⁷Se NMR (57 MHz, DMSO-d₆) δ 385.5; mass spectrum, m/z (EI, relative intensity) 442 (30, M+), 220 (32), 204 (61), 112.0 (100), 77 (32); exact mass calcd for C₁₄H₁₂⁵Cl₂O₂⁸Se₂: 441.8545; found: 441.8524. Anal. calcd for C₁₄H₁₂Cl₂O₂Se₂: C, 38.12; H, 2.74; found: C, 38.46; H, 2.47.

7.2.9 2,2’-Diselenobis(5-fluorobenzyl alcohol) (130g)¹⁴⁴

Prepared according to the same procedure as for 130a. Yield: 42%. Yellow solid; mp 111-112 °C (from water); IR (KBr) 3214, 1238, 1144, 1051, 1016, 874 cm⁻¹; ¹H NMR (300 MHz) δ 7.52 (dd, J = 8.4, 5.9 Hz, 2 H, H-3), 7.24 (dd, J = 8.8, 3.1 Hz, 2 H, H-6), 6.89 (dt, J = 8.2, 3.1 Hz, 2 H, H-4), 4.69 (s, 4 H, H-7), 2.06 (br s, 2 H, OH); ¹³C NMR (75 MHz) δ 163.9 (d, J = 248.7 Hz, H-5), 146.0 (d, J = 7.3 Hz, C-1), 138.3 (d, J = 7.9 Hz, C-2).
C-3), 124.2 (d, $J = 3.6$ Hz, C-2), 115.2 (d, $J = 21.2$ Hz), 115.3 (d, $J = 22.4$ Hz), 64.9 (C-7); $^{77}$Se NMR (76 MHz) $\delta$ 441.8; mass spectrum, m/z (EI, relative intensity) 410 (10, M+), 188 (23), 96 (100); exact mass calcd for C$_{14}$H$_{12}$F$_{2}$O$_{2}$Se: 409.9136; found: 409.9132. Anal. calcd for C$_{14}$H$_{12}$F$_{2}$O$_{2}$Se: C, 41.20; H, 2.96; found: C, 41.03; H, 2.83.

7.2.10 Typical Procedure for the Preparation of Allyl Selenides 127a-g: Allyl 2-(hydroxymethyl)phenyl selenide (127a)$^{129}$

![Image of structure](image)

2,2'-Diselenobisbenzyl alcohol (130a) (0.73 g, 2.0 mmol) was dissolved in 40 mL of THF at 0 °C under a nitrogen atmosphere. Sodium borohydride (0.37 g, 9.7 mmol) was added in one portion, followed by 8 mL of absolute ethanol. After 5 minutes, allyl iodide was added (0.36 mL, 0.66 g, 3.9 mmol) and the reaction was warmed to room temperature. The mixture was quenched with 25 mL of 1 M HCl after 2 hours. This was then extracted with ether, washed with saturated NaCl solution, dried (MgSO$_4$) and concentrated. The resulting oil was chromatographed (hexanes-ethyl acetate, 2:1) to provide 0.37 g (82%) of allyl selenide 127a as a colourless oil; IR (neat) 3350, 1028, 750 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) $\delta$ 7.54 (dd, $J = 7.4$, 1.3 Hz, 1 H), 7.40 (dd, $J = 7.4$, 1.3 Hz, 1 H), 7.31–7.18 (m, 2 H), 6.00–5.86 (m, 1 H, H-9), 4.99–4.92 (m, 2 H, H-10), 4.76 (s, 2 H, H-7), 3.51 (d, $J = 7.7$ Hz, 2 H, H-8), 2.40 (br s, 1 H, OH); $^{13}$C NMR (75 MHz; CDCl$_3$) $\delta$ 143.0, 134.8, 134.3, 129.7, 128.5, 128.4, 128.0, 117.3, 65.6 (C-7), 31.1 (C-8); mass spectrum, m/z (EI, relative intensity) 228 (10), 187 (38), 157 (17), 129 (26), 105 (17), 78 (100); exact mass calcd for C$_{10}$H$_{12}$O$_{80}$Se: 228.0053. Found: 228.0060.
7.2.11 Allyl 4-methoxy-2-(hydroxymethyl)phenyl selenide (127b)

Prepared according to the same procedure as for 127a. Yield: 56%. Colourless oil; IR (neat) 3371, 1293, 1162, 1054, 1018, 915 cm\(^{-1}\); \(^1\)H NMR (300 MHz) \(\delta\) 7.49 (d, \(J = 8.7\) Hz, 1 H, H-6), 7.00 (d, \(J = 2.6\) Hz, 1 H, H-3), 6.75 (dd, \(J = 7.2\) Hz, 3.1 Hz, 1 H, H-5), 5.94-5.86 (m, 1 H, H-10), 4.88 (dd, \(J = 10.3, 1.6\) Hz, 1 H, H-11a), 4.81 (dd, \(J = 15.4\) Hz, 1.6 Hz, 1 H, H-11b), 4.76 (d, \(J = 6.2\) Hz, 2 H, H-7), 3.81 (s, 3 H, H-8), 3.39 (d, \(J = 7.2\) Hz, 2 H, H-9), 2.48 (t, \(J = 6.2\) Hz, 1 H, OH); \(^{13}\)C NMR (75 MHz) \(\delta\) 160.2 (C-4), 145.6, 138.3, 134.5, 118.9, 116.9, 114.1, 114.0, 65.8 (C-7), 55.4 (C-8), 31.9 (C-9); mass spectrum, \(m/z\) (El, relative intensity) 258 (16, M+), 217 (12), 136 (17), 108 (100); exact mass calcd for C\(_{11}\)H\(_{14}\)O\(_2\)\(^{80}\)Se: 258.0159; found: 258.0140.

7.2.12 Allyl 4-methyl-2-(hydroxymethyl)phenyl selenide (127c)

Prepared according to the same procedure as for 127a. Yield: 79%. Yellow oil; IR (neat) 3420, 1630, 1271, 1155 cm\(^{-1}\); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta\) 7.46 (d, \(J = 8.2\) Hz, 1 H, H-6), 7.24 (s, 1 H, H-3), 7.04 (d, \(J = 7.7\) Hz, 1 H, H-5), 5.97-5.85 (m, 1 H, H-10), 4.95-4.91 (m, 2 H, H-11), 4.75 (s, 2 H, H-7), 3.47 (d, \(J = 7.7\) Hz, 2 H, H-9), 2.42 (br s, 1 H, OH), 2.35 (s, 3 H, H-8); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) \(\delta\) 143.2, 138.3, 135.7, 134.5,
129.5, 129.3, 125.7, 117.1, 65.7 (C-7), 31.4 (C-9), 21.3 (C-8); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 263.2; mass spectrum m/z (EI, relative intensity) 242 (16, M+), 201 (15), 92 (10), 42 (92), 40 (100); exact mass calcd for C$_{11}$H$_{14}$O$_2$Se: 242.0210; found: 242.0195.

7.2.13 Allyl 4-phenyl-2-(hydroxymethyl)phenyl selenide (127d)

Prepared according to the same procedure as for 127a. Yield: 66%. Yellow oil; IR (neat) 3413, 1630, 1587, 1174, 1030, 752 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.68-7.60 (m, 4 H), 7.48-7.35 (m, 4 H), 6.02-5.93 (m, 1 H, H-9), 5.01-4.98 (m, 2 H, H-10), 4.84 (s, 2 H, H-7), 3.55 (d, $J = 7.2$ Hz, 2 H, H-8), 2.56 (br s, 1 H, OH); $^{13}$C NMR (75 MHz; CDCl$_3$) δ 143.1, 140.7, 140.2, 135.0, 134.1, 128.8, 128.5, 127.5, 126.9, 126.84, 126.75, 117.2, 65.5 (C-7), 31.0 (C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 267.7; mass spectrum m/z (EI, relative intensity) 304 (34, M+), 263 (23), 154 (96), 42 (100); exact mass calcd for C$_{16}$H$_{16}$OSe: 304.0366; found: 304.0368.

7.2.14 Allyl 4-bromo-2-(hydroxymethyl)phenyl selenide (127e)

Prepared according to the same procedure as for 127a. Yield: 84%. Yellow oil; IR (neat) 3200, 1630, 1091, 1039, 983, 904 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.56 (d, $J = 1.5$ Hz, 1 H), 7.38-7.30 (m, 2 H), 5.93-5.81 (m, 1 H, H-9), 4.95-4.91 (m, 2 H, H-10), 4.70
(d, J = 5.6 Hz, 2 H, H-7), 3.46 (d, J = 7.7 Hz, 2 H, H-8), 2.65 (t, J = 6.2 Hz, 1 H, OH);

$^1$H NMR (300 MHz; CDCl$_3$) δ 7.42-7.39 (m, 2 H), 7.15 (dd, J = 8.4, 2.1 Hz, 1 H, H-5), 5.91-5.80 (m, 1 H, H-9), 4.94-4.89 (m, 2 H, H-10), 4.68 (s, 2 H, H-7), 3.44 (d, J = 7.8 Hz, 2 H, H-8), 3.02 (br s, 1 H, OH); $^{13}$C NMR (75 MHz; CDCl$_3$) δ 144.8, 136.0, 134.1, 134.0, 128.2, 128.0, 127.3 117.6, 64.8 (C-7), 31.2 (C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 269.2; mass spectrum m/z (EI, relative intensity) 262 (22, M+), 221 (54), 112 (100); exact mass calcd for C$_{10}$H$_{11}^{35}$ClO$_{80}$Se: 261.9664; found: 261.9687.

7.2.16 Allyl 4-fluoro-2-(hydroxymethyl)phenyl selenide (127g)

Prepared according to the same procedure as for 127a. Yield: 81%. Yellow oil; IR (neat) 3387, 1635, 1265, 1191, 804 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.42-7.39 (m, 2 H), 7.15 (dd, J = 8.4, 2.1 Hz, 1 H, H-5), 5.91-5.80 (m, 1 H, H-9), 4.94-4.89 (m, 2 H, H-10), 4.68 (s, 2 H, H-7), 3.44 (d, J = 7.8 Hz, 2 H, H-8), 3.02 (br s, 1 H, OH); $^{13}$C NMR (75 MHz; CDCl$_3$) δ 144.8, 136.0, 134.1, 134.0, 128.2, 128.0, 127.3 117.6, 64.8 (C-7), 31.2 (C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 269.2; mass spectrum m/z (EI, relative intensity) 262 (22, M+), 221 (54), 112 (100); exact mass calcd for C$_{10}$H$_{11}^{35}$ClO$_{80}$Se: 261.9664; found: 261.9687.
Prepared according to the same procedure as for 127a. Yield: 56%. Yellow oil: IR (neat) 3304, 1626, 1600, 1579, 1268, 1146, 1028, 873, cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.52 (dd, $J$ = 8.4, 5.8 Hz, 1 H, H-6), 7.20 (dd, $J$ = 9.5, 2.7 Hz, 1 H, H-3), 6.89 (dt, $J$ = 8.3, 2.8 Hz, 1 H, H-5), 5.96-5.82 (m, 1 H, H-9), 4.91 (d, $J$ = 9.9 Hz, 1 H, H-10a), 4.86 (d, $J$ = 17.1 Hz, 1 H, H-10b), 4.76 (d, $J$ = 5.8 Hz, 2 H, H-7), 3.43 (d, $J$ = 7.6 Hz, 2 H, H-8), 2.40 (t, $J$ = 5.9 Hz, 1 H, OH); $^{13}$C NMR (75 MHz; CDCl$_3$) δ 164.6 (d, $J$ = 246.6 Hz, C-4), 147.8 (d, $J$ = 7.1 Hz), 139.4 (d, $J$ = 7.7 Hz), 135.6 , 124.6 (d, $J$ = 3.3 Hz), 118.8, 116.8 (d, $J$ = 22.3 Hz), 116.7 (d, $J$ = 21.1 Hz), 66.6 (d, $J$ = 1.2 Hz, C-7), 33.2 (C-8); $^{77}$Se NMR (57 MHz; CDCl$_3$) δ 263.0; mass spectrum, m/z (relative intensity) 246 (24, M$^+$), 205 (31), 96 (100); exact mass calcd for C$_{10}$H$_{11}$FO$_{80}$Se: 245.9959; found: 245.9941. Anal. calcd for C$_{10}$H$_{11}$FO$_{80}$Se: C, 48.99; H, 4.52; found: C, 48.89; H, 4.77.

### 7.2.17 Typical procedure for the preparation of cyclic seleninate esters 108 and 118-123: benzo-1,2-oxaselenolane Se-oxide (108)

![Cyclic seleninate ester](image)

Allyl selenide 127a (0.42 g, 1.8 mmol) and 65% tert-butyl hydroperoxide (2.5 mL, 18 mmol) were stirred in dichloromethane (25 mL) at room temperature for 14 hours. The solution was concentrated under reduced pressure and the resulting mixture was purified by chromatography (ethyl acetate-methanol, 9:1) to afford 0.33 g (88%) of cyclic seleninate ester 108 as a colourless solid; mp 139-140 °C (from ethyl acetate); IR (KBr) 1462, 1260, 966 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.81 (d, $J$ = 7.7 Hz, 1 H, H-6), 7.61-7.47 (m, 3 H), 5.97 (d, $J$ = 13.8 Hz, 1 H, H-7a), 5.61 (d, $J$ = 13.6 Hz, 1 H, H-7b);
$^{13}$C NMR (75 MHz; CDCl$_3$) δ 148.3, 143.7, 132.2, 129.3, 125.5, 122.9, 78.6 (C-7); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 1349.0; mass spectrum, m/z (EI, relative intensity) 202 (30), 106 (74), 78 (100). Exact mass calcd for C$_7$H$_6$O$_2$Se: 201.9533. Found: 201.9540. Anal. calcd for C$_7$H$_6$O$_2$: C, 41.81; H, 3.01. Found: C, 41.54; H 2.97.

**7.2.18 4-Methoxybenzo-1,2-oxaselenolane Se-oxide (118)**

Prepared according to the same procedure as for 108. Yield: 81%. Colourless solid; mp 169-170 °C (from ethyl acetate); IR (KBr) 1281, 1240, 1153, 1050, 984, 830 cm$^{-1}$; $^1$H NMR (300 MHz; CDCl$_3$) δ 7.67 (d, $J$ = 8.7 Hz, 1 H, H-6), 7.02 (dd, $J$ = 8.7 Hz, 2.0 Hz, 1 H, H-5), 6.92 (d, $J$ = 2.0 Hz, 1 H, H-3), 5.93 (d, $J$ = 13.8 Hz, 1 H, H-7a), 5.55 (d, $J$ = 13.8 Hz, 1 H, H-7b), 3.88 (s, 3 H, H-8); $^{13}$C NMR (75 MHz; CDCl$_3$) δ 163.0, 146.6, 139.8, 126.6, 115.9, 107.3, 78.2 (C-7), 55.9 (C-8); $^{77}$Se NMR (57 MHz; CDCl$_3$) δ 1348.8; mass spectrum, m/z (EI, relative intensity) 232 (5, M+), 216 (11), 136 (84), 108 (41), 43 (100); exact mass calcd for C$_8$H$_8$O$_3$Se: 231.9639; found: 231.9646. Anal. calcd for C$_8$H$_8$O$_3$: C, 41.58; H, 3.49; found: C, 41.61; H 3.60.

**7.2.19 4-Methylbenzo-1,2-oxaselenolane Se-oxide (119)**
Prepared according to the same procedure as for 108. Yield: 97%. Colourless solid; mp 164-165 °C (from ethyl acetate); IR (neat) 1230, 974, 835 cm\(^{-1}\); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta\) 7.69 (d, \(J = 7.7\) Hz, 1 H, H-6), 7.29-7.26 (m, 2 H), 5.91 (d, \(J = 13.8\) Hz, 1 H, H-7a), 5.54 (d, \(J = 13.8\), 1 H, H-7b), 2.43 (s, 3 H, H-8); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) \(\delta\) 145.2, 144.0, 142.8, 130.0, 124.8, 122.9, 78.2 (C-7), 21.5 (C-8); \(^{77}\)Se (76 MHz; CDCl\(_3\)) \(\delta\) 1347.9; mass spectrum \(m/z\) (EI, relative intensity) 216 (27, M\(^+\)), 120 (83), 91 (100); exact mass calcd for C\(_8\)H\(_8\)O\(_2\)\(^{80}\)Se: 215.9690; found: 215.9700. Anal. calcd for C\(_8\)H\(_8\)O\(_2\)Se: C, 44.67; H, 3.75; found: C, 44.63; H, 3.89.

7.2.20 4-Phenylbenzo-1,2-oxaselenolane Se-oxide (120)

Prepared according to the same procedure as for 108. Yield: 99%. Colourless solid; mp 164-165 °C (from ethyl acetate); IR (KBr) 1173, 1079, 1009, 981, 858 cm\(^{-1}\); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta\) 7.84 (d, \(J = 7.7\) Hz, 1 H, H-6), 7.73-7.67 (m, 2 H), 7.60-7.57 (m, 2 H), 7.52-7.41 (m, 3 H), 6.04 (d, \(J = 13.8\) Hz, 1 H, H-7a), 5.69 (d, \(J = 13.8\) Hz, 1 H, H-7b); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) \(\delta\) 147.0, 145.8, 144.7, 139.6, 129.2, 128.7, 128.5, 127.6, 125.5, 121.4, 78.6 (C-7); \(^{77}\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 1351.4; mass spectrum \(m/z\) (EI, relative intensity) 278 (11, M\(^+\)), 182 (100), 154 (40); exact mass calcd for C\(_{13}\)H\(_{10}\)O\(_2\)\(^{80}\)Se: 277.9846; found: 277.9855. Anal. calcd for C\(_{13}\)H\(_{10}\)O\(_2\)Se: C, 56.33; H, 3.64; found: C, 56.45; H, 4.04.
7.2.21 4-Bromobenzo-1,2-oxaselenolane Se-oxide (121)

![Chemical structure](image)

Prepared according to the same procedure as for 108. Yield: 98%. Colourless solid; mp: 162-163 °C (from ethyl acetate); IR (KBr) 1191, 983, 835 cm\(^{-1}\); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta\) 7.71-7.61 (m, 3 H), 5.94 (d, \(J = 14.3\) Hz, 1 H, H-7a), 5.60 (d, \(J = 14.4\) Hz, 1 H, H-7b); \(^13\)C NMR (75 MHz; CDCl\(_3\)) \(\delta\) 147.1, 145.9, 132.4, 127.0, 126.8, 126.0, 77.9 (C-7); \(^77\)Se (57 MHz; CDCl\(_3\)) \(\delta\) 1323.6; mass spectrum \(m/z\) (EI, relative intensity) 280 (38, M\(^+\)), 186 (98), 184 (100), 158 (47), 156 (60), 77 (44); exact mass calcd for C\(_7\)H\(_5\)\(^{79}\)BrO\(_2\)\(^{80}\)Se: 279.8638; found: 279.8630. Anal. calcd for C\(_7\)H\(_5\)\(^{79}\)BrO\(_2\)Se: C, 30.02; H, 1.80; found: C, 30.06; H, 1.93.

7.2.22 4-Chlorobenzo-1,2-oxaselenolane Se-oxide (122)

Prepared according to the same procedure as for 108. Yield: 83%. Colourless solid; mp 159-160 °C (from ethyl acetate); IR (KBr) 1204, 987, 868 cm\(^{-1}\); \(^1\)H NMR (300 MHz; CDCl\(_3\)) \(\delta\) 7.73 (d, \(J = 8.7\) Hz, 1 H, H-6), 7.50-7.47 (m, 2 H), 5.94 (d, \(J = 13.8\) Hz, 1 H, H-7a), 5.60 (d, \(J = 14.3\) Hz, 1 H, H-7b); \(^13\)C NMR (75 MHz; CDCl\(_3\)) \(\delta\) 146.5, 145.8, 138.7, 129.5, 126.7, 122.9, 77.8 (C-7); \(^77\)Se (76 MHz; CDCl\(_3\)) \(\delta\) 1343.9; mass spectrum \(m/z\) (EI, relative intensity) 236 (15, M\(^+\)), 220 (15), 156 (36), 140 (34), 112 (100); exact
mass calcd for C$_7$H$_5$$^{35}$ClO$_2$$^{80}$Se: 235.9143; found: 235.9139. Anal. calcd for C$_7$H$_5$ClO$_2$Se: C, 35.70; H, 2.14; found: C, 35.67; H, 2.28.

7.2.23 4-Fluorobenzo-1,2-oxaselenolane Se-oxide (123)$^{144}$

Prepared according to the same procedure as for 108. Yield: 79%. Colourless solid; mp 141-142 °C; IR (KBr) 1250, 1231, 989, 928, 861, 830 cm$^{-1}$; $^1$H NMR (300 MHz) $\delta$ 7.91 (dd, $J = 9.2$, 5.0 Hz, 1 H, H-6), 7.27-7.09 (m, 2 H), 5.88 (d, $J = 14.1$ Hz, 1 H, H-7a), 5.53 (d, $J = 14.1$ Hz, 1 H, H-7b); $^{13}$C NMR (75 MHz) $\delta$ 166.6 (d, $J = 251.7$ Hz, C-4), 148.5 (d, $J = 8.9$ Hz, C-2), 145.2 (C-1), 130.0 (d, $J = 9.5$ Hz, C-6), 118.2 (d, $J = 23.2$ Hz, C-3), 111.5 (d, $J = 23.9$ Hz, C-5), 79.2 (C-7); $^{77}$Se NMR (76 MHz, acetone-$d_6$) $\delta$ 1358.9; mass spectrum, m/z (EI, relative intensity) 220 (12, M+), 124 (51), 96 (100); exact mass calcd for C$_7$H$_5$FO$_2$$^{80}$Se: 219.9439; found: 219.9455. Anal. calcd for C$_7$H$_5$FO$_2$Se: C, 38.38; H, 2.30; found: C, 38.35; H, 2.52.

7.3 Experiments Related to Chapter Three

7.3.1 Preparation of 2-bromo-3,4,5-trimethoxybenzyl alcohol (209)

3,4,5-Trimethoxybenzyl alcohol (4.12 mL, 5.08 g, 25.6 mmol) was dissolved in 120 mL of ethyl acetate. NBS (5.70 g, 32.0 mmol) was added in one portion and the
mixture was left for 16 hours. The mixture was washed with water, brine, dried (MgSO₄) and concentrated under reduced pressure to afford 6.78 g (96%) of 2-bromo-3,4,5-trimethoxybenzyl alcohol as a pale yellow solid, mp 50-52 °C (from hexanes); lit.¹⁹³ mp 54.5-55.5 °C; ¹H NMR (400 MHz; CDCl₃) δ 6.84 (s, 1 H, H-6), 4.70 (d, J = 6.0 Hz, 2 H, H-7), 3.89 (s, 3 H), 3.87 (s, 3 H), 2.18 (t, J = 6.2 Hz, 1 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 153.1, 151.0, 142.5, 135.5, 108.5, 107.8, 65.2 (C-7), 61.2, 61.2, 56.3. Benzyl alcohol 209 is a known compound and the spectral data was consistent with the literature data.¹⁹³

7.3.2 Preparation of methoxymethyl 2-bromo-3,4,5-trimethoxybenzyl ether (210)

2-Bromo-3,4,5-trimethoxybenzyl alcohol (800 mg, 2.89 mmol) was dissolved in 10 mL of dichloromethane. Diisopropylethyl amine (0.610 mL, 453 mg, 3.50 mmol) was added, followed by chloromethyl methyl ether (0.265 mL, 281 mg, 3.47 mmol). After 14 hours of stirring at room temperature the mixture was poured into 20 mL of water. This aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 1:1) to afford 845 mg (91%) of the MOM-protected alcohol as a colourless oil; IR (neat) 2938, 1576, 1481, 1324, 1243, 1152, 990 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 6.87 (s, 1 H, H-6), 4.75 (s, 2 H, H-8), 4.61 (s, 2 H, H-7), 3.88 (s, 3 H), 3.86 (s, 3 H), 3.43 (s, 3 H, H-
\[ \text{C NMR (101 MHz; CDCl}_3 \text{) } \delta 152.9 \text{ (C), 151.0 (C), 142.6 (C), 133.0 (C), 109.1 (C), 108.2 (CH, C-6), 96.3 (CH}_2 \text{, C-8), 69.1 (CH}_2 \text{, C-7), 61.2 (CH}_3 \text{), 61.1 (CH}_3 \text{), 56.3 (CH}_3 \text{), 55.7 (CH}_3 \}; \text{mass spectrum } m/ z \text{ (EI, relative intensity) 320 (68, M}^+\text{), 259 (84), 181 (100); exact mass calcd. for C}_{12}H_{17}O_{579}Br: 320.0259; \text{ found: 320.0261. Anal. calcd. for C}_{12}H_{17}O_{5}Br: C, 44.88; H, 5.34; found: C, 44.60; H, 5.15.\]

7.3.3 Preparation of 2-iodo-4,5-dimethoxybenzyl alcohol (220)

2-Iodo-4,5-dimethoxybenzyl alcohol 220 was prepared according to the procedure described by Lete and coworkers.\textsuperscript{194} 3,4-Dimethoxybenzyl alcohol (1.16 mL, 1.01 g, 6.00 mmol) was dissolved in 30 mL of chloroform. Silver (I) trifluoroacetate (1.32 g, 5.98 mmol) was added followed by a solution of iodine (1.50 g, 5.91 mmol) in 180 mL of chloroform. Stirring was continued for 1 hour and the solution was filtered through celite, dried (MgSO\textsubscript{4}) and concentrated. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 2:1) to afford 1.15 g (65\%) of 2-iodo-3,4,5-trimethoxybenzyl alcohol as a white solid, mp 86-87 °C (from hexanes-ethyl acetate), lit.\textsuperscript{194} mp 83-85 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \( \delta \) 7.21 (s, 1 H), 7.00 (s, 1 H), 4.60 (s, 2 H, H-7), 3.87 (s, 3 H), 3.85 (s, 3 H), 2.05 (br s, 1 H, OH); \textsuperscript{13}C NMR (101 MHz; CDCl\textsubscript{3}) \( \delta \) 149.7, 149.1, 135.4, 121.7, 111.8, 85.5, 69.2 (C-7), 56.4, 56.1. The spectral data for methyl ester 220 was consistent with the literature data.\textsuperscript{194}
7.3.4 2-Iodo-3,4,5-trimethoxybenzyl alcohol (221)

Prepared according to the same procedure as for 220. Yield: 80%. Colourless solid, mp 54-55 °C (from hexanes-ethyl acetate); lit.\textsuperscript{194} mp 52-54 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) δ 6.92 (s, 1 H, H-6), 4.64 (s, 2 H, H-7), 3.87-3.86 (m, 9 H), 2.16 (br s, 1 H, OH); \textsuperscript{13}C NMR (101 MHz; CDCl\textsubscript{3}) δ 154.1 (C), 153.1 (C), 141.6 (C), 138.5 (C), 108.2 (CH, C-6), 84.7 (C), 69.5 (CH\textsubscript{2}, C-7), 61.1 (CH\textsubscript{3}), 61.0 (CH\textsubscript{3}), 56.3 (CH\textsubscript{3}). The spectral data for methyl ester 221 was consistent with the literature data.\textsuperscript{194}

7.3.5 Preparation of methyl 2-bromo-3,5-dimethoxybenzoate (224)

Methyl ester 224 was prepared according to the method of Danishefsky and coworkers.\textsuperscript{195} Methyl 3,5-dimethoxybenzoate (4.60 g, 23.4 mmol) was dissolved in 200 mL of acetonitrile. NBS (4.60 g, 25.8 mmol) was added along with pyridine (1.89 mL, 1.86 g, 23.5 mmol) and the mixture was left for 9 hours. The mixture was concentrated to a volume of 20 mL, diluted with 50 mL of ethyl acetate, washed with 1 M HCl, brine, dried (MgSO\textsubscript{4}) and concentrated to afford 6.19 g (96%) of methyl 2-bromo-3,5-dimethoxybenzoate as a white solid, mp 58-59 °C (from hexanes-ethyl acetate); lit.\textsuperscript{195} mp 57-59 °C; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) δ 6.79 (d, J = 2.8 Hz, 1 H, H-4), 6.57 (d, J = 2.8
Hz, 1 H, H-6), 3.91 (s, 3 H), 3.87 (s, 3 H), 3.81 (s, 3 H); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$
167.3 (C, C-7), 159.7 (C), 157.3 (C), 135.0 (C), 106.3 (CH), 102.5 (CH), 102.2 (C), 56.7 (CH$_3$), 55.8 (CH$_3$), 52.7 (CH$_3$). The spectral data for methyl ester 224 was consistent with the literature data.$^{195}$

**7.3.6 Preparation of 2-bromo-3,5-dimethoxybenzyl alcohol (198)**

![Chemical Structure](attachment:structure.png)

Lithium aluminum hydride (555 mg, 14.6 mmol) was suspended in 50 mL of THF and cooled to 0 °C under N$_2$ atmosphere. A solution of methyl 2-bromo-3,5-dimethoxybenzoate (2.0 g, 7.3 mmol) in 30 mL of THF was carefully added and the mixture was warmed to room temperature and left for 3 hours. The mixture was cooled to 0 °C and quenched with subsequent addition of 0.5 mL of water, 0.5 mL of 15 % aqueous NaOH solution and 1.5 mL of water. Ethyl acetate (50 mL) was added and after 30 minutes the mixture was dried (MgSO$_4$), filtered through celite and concentrated to afford 1.5 g (83%) of 2-bromo-3,5-dimethoxybenzyl alcohol which could be purified further by recrystallization in hexanes, mp 91-92 °C; lit.$^{196}$ mp 94-95 °C; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 6.68 (d, $J = 2.8$ Hz, 1 H, H-6), 6.41 (d, $J = 2.8$ Hz, 1 H, H-4), 4.70 (d, $J = 6.0$ Hz, 2 H, H-7), 3.85 (s, 3 H), 3.80 (s, 3 H), 2.36 (t, $J = 6.4$ Hz, 1 H, OH); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 160.1, 156.6, 141.9, 104.9, 102.3, 98.9, 65.3 (C-7), 56.4, 55.7. Benzyl alcohol 198 is a known compound and the spectral data was consistent with the literature data.$^{197}$

204
7.3.7 2-Amino-4-methoxybenzyl alcohol (155)

![Chemical structure of 2-Amino-4-methoxybenzyl alcohol](image)

Lithium aluminum hydride (680 mg, 17.9 mmol) was suspended in 50 mL of THF and cooled to 0 °C under N₂ atmosphere. A solution of 2-amino-4-methoxybenzoic acid (1.20 g, 7.18 mmol) in 30 mL of THF was carefully added and the mixture was warmed to room temperature and left for 14 hours. The mixture was cooled to 0 °C and quenched with the subsequent addition of 0.7 mL of water, followed by 0.7 mL of 15 % aqueous NaOH solution and 2.1 mL of water. Ethyl acetate (50 mL) was added and after 30 min the mixture was dried (MgSO₄), filtered through celite and concentrated to afford 980 mg (89%) of 2-amino-4-methoxybenzyl alcohol as a brown solid, mp 79-81 °C (from ethyl acetate), lit.¹⁹⁸ mp 75-76 °C; ¹H NMR (400 MHz; CDCl₃) δ 6.95 (d, J = 8.0 Hz, 1 H, H-6), 6.26 (dd, J = 8.0 Hz, 1.6 Hz, 1 H, H-5), 6.24 (d, J = 2.0 Hz, 1 H, H-3), 4.57 (s, 2 H, H-7), 4.15 (br s, 2 H, NH₂), 3.75 (s, 3 H, H-8); ¹³C NMR (101 MHz; CDCl₃) δ 160.9 (C), 147.5 (C), 130.5 (CH), 118.1 (C), 103.3 (CH), 101.8 (CH), 63.9 (CH₂, C-7), 55.3 (CH₃, C-8). Benzyl alcohol 155 is a known compound and the spectral data was consistent with the literature data.¹⁹⁸

7.3.8 Preparation of 2-amino-3-methoxybenzyl alcohol (156)

![Chemical structure of 2-Amino-3-methoxybenzyl alcohol](image)
Prepared according to the same procedure as for 155. Yield: 95%. Brown solid, mp 43-45 °C (from ethyl acetate) lit.\(^{199}\) mp 42-46 °C; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 6.79 (dd, \(J = 7.6\) Hz, 1.6 Hz, 1 H), 6.72 (dd, \(J = 7.6\) Hz, 2.0 Hz, 1 H), 6.68 (t, \(J = 7.6\) Hz, 1 H, H-5), 4.64 (s, 2 H, H-7), 4.29 (br s, 2 H, NH\(_2\)), 3.86 (s, 3 H, H-8), 2.04 (br s, 1 H, OH); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 147.6 (C), 135.8 (C), 125.2 (C), 121.3 (CH), 117.5 (CH), 110.5 (CH), 64.1 (CH\(_2\), C-7), 55.8 (CH\(_3\), C-8). Benzyl alcohol 156 is a known compound and the spectral data was consistent with the literature data.\(^{198}\)

### 7.3.9 Preparation of \(\text{N,N-diisopropyl-4-methoxybenzamide (159)}\)

4-Methoxybenzoic acid (2.5 g, 16 mmol) was suspended in 10 mL of chloroform. DMF (10 drops) and thionyl chloride (4.6 mL, 7.6 g, 64 mmol) were added and the mixture was refluxed for 4 hours. The mixture was cooled to room temperature and the chloroform and excess thionyl chloride were removed under reduced pressure. The crude 4-methoxybenzoyl chloride was dissolved in 30 mL of dichloromethane and added to a solution of diisopropylamine (8.9 mL, 6.5 g, 64 mmol) in 80 mL of dichloromethane. After 14 hours the mixture was washed with water, 1 M NaOH, dried (MgSO\(_4\)) and concentrated to leave a colourless oil (98%) \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.25 (d, \(J = 8.8\) Hz, 2 H, H-2), 6.86 (d, \(J = 8.8\) Hz, 2 H, H-3), 3.79 (s, 3 H, H-5), 3.70 (br s, 2 H, H-7), 1.32 (br s, 12 H, H-8); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 171.0 (C, C-6), 160.0 (C, C-1), 131.5 (C, C-4), 127.5 (CH), 113.8 (CH), 55.4 (CH\(_3\), C-5), 20.9 (CH\(_3\), C-8), C-7 is not
visible. Amide 159 is a known compound and the spectral data was consistent with the literature data.  

7.3.10  

**N,N-Diisopropyl-3-methoxybenzamide (160)**

![Chemical Structure of 160]

Prepared according to the same procedure as for 159. Yield: 94%. Colourless solid; mp 88-90 °C (from dichloromethane); $^1$H NMR (400 MHz; CDCl$_3$) δ 7.26 (t, $J$ = 7.8 Hz, 1 H, H-5), 6.89-6.83 (m, 3 H), 3.80 (s, 3 H, H-7), 3.62 (br s, 2 H, H-9), 1.46 (br s, 6 H, H-10a), 1.18 (br s, 6 H, H-10b); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 170.8 (C, C-10), 159.8 (C), 140.4 (C), 129.7 (CH), 117.8 (CH), 114.6 (CH), 111.2 (CH), 55.4 (CH$_3$, C-7), 20.8 (CH$_3$, C-10), C-9 is not visible. Amide 160 is a known compound and the spectral data was consistent with the literature data.  

7.3.11  

**Preparation of N-methyl-4-methoxybenzamide (161)**

![Chemical Structure of 161]

Amide 161 was prepared according to a modification of the procedure of Du, Hyster and Rovis. 4-Methoxybenzoic acid (2.5 g, 16 mmol) was suspended in 10 mL of chloroform. DMF (10 drops) and thionyl chloride (4.6 mL, 7.6 g, 64 mmol) were added and the mixture was refluxed for 4 hours. The mixture was cooled to room temperature and the chloroform and excess thionyl chloride removed under reduced
pressure. The crude 4-methoxybenzoyl chloride and potassium carbonate (4.4 g, 32 mmol) were dissolved in 20 mL of diethyl ether and cooled to 0 °C. Methyl ammonium chloride (1.6 g, 24 mmol) was added and the mixture was left for 12 hours. The mixture was quenched with 1 M HCl, and extracted with ethyl acetate, washed with saturated sodium bicarbonate, dried (MgSO₄) and concentrated to leave a colourless solid (37%); mp 117-119 °C (from ethyl acetate) lit.²⁰² mp 115-117 °C; ¹H NMR (400 MHz; CDCl₃) δ 7.73 (d, J = 9.2 Hz, 2 H, H-2), 6.85 (d, J = 8.8 Hz, 2 H, H-3), 6.66 (br s, 1 H, NH), 3.79 (s, 3 H, H-5), 2.93 (s, 1.5 H, H-7) and 2.92 (s, 1.5 H, H-7); ¹³C NMR (101 MHz; CDCl₃) δ 168.0 (C-6), 162.1, 128.8, 127.0, 113.7, 55.4 (C-5), 26.8 (C-7). The spectral data for amide 161 was consistent with the literature data.²⁰¹

7.3.12 N-methyl-3-methoxybenzamide (162)

Prepared according to the same procedure as for 161. Yield (40%). Colourless solid; mp 63-65 °C (from ethyl acetate) lit.²⁰³ mp 65 °C; ¹H NMR (400 MHz; CDCl₃) δ 7.34-7.33 (m, 1 H), 7.27-7.26 (m, 2 H), 6.99-6.96 (m, 1 H), 6.63 (br s, 1 H, NH), 3.78 (s, 3 H, H-7), 2.96 (s, 1.5 H, H-9) and 2.94 (s, 1.5 H, H-9); ¹³C NMR (101 MHz; CDCl₃) δ 168.3 (C-8), 159.8, 136.2, 129.6, 118.8, 117.6, 112.3, 55.4 (C-7), 26.9 (C-9). The spectral data for amide 162 was consistent with the literature data.²⁰¹
7.3.13 Preparation of N-phenyl-4-methoxybenzamide (163)

Prepared according to the same procedure as for 159. Yield: 29%. Colourless solid; mp 171-172 °C (from ethyl acetate) lit.\(^2\) mp 172-173 °C; \(^1\)H NMR (400 MHz; DMSO-\(d_6\)) \(\delta\) 10.09 (br s, 1 H, NH), 7.98 (d, \(J = 9.2\) Hz, 2 H, H-2), 7.79 (dd, \(J = 8.6\) Hz, 1.0 Hz, 2 H, H-8), 7.34 (dd, \(J = 8.4\) Hz, 7.9 Hz, 2 H, H-9), 7.10-7.05 (m, 3 H, H-10 and H-3), 3.84 (s, 3 H, H-5); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 164.9, 161.9, 139.4, 129.6, 128.5, 127.0, 123.4, 120.4, 113.6, 55.4 (C-5). Amide 163 is a known compound and the spectral data was consistent with the literature data.\(^3\)

7.3.14 N-Phenyl-3-methoxybenzamide (164)

Prepared according to the same procedure as for 159. Yield: 90%. Brown solid; mp 118-119 °C (from dichloromethane) lit.\(^4\) mp 117-118 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.10 (br s, 1 H, NH), 7.64 (dd, \(J = 8.6\) Hz, 1.0 Hz, 2 H, H-10), 7.41 (dd, \(J = 2.6\) Hz, 1.4 Hz, 1 H, H-2), 7.39-7.30 (m, 4 H), 7.14 (tt, \(J = 7.6\) Hz, 1.0 Hz, 1 H, H-12), 7.05 (ddd, \(J = 8.1\) Hz, 2.6 Hz, 1.1 Hz, 1 H), 3.81 (s, 3 H, H-7); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 165.9, 160.0, 138.1, 136.5, 129.8, 129.1, 124.7, 120.4, 118.9, 118.1, 112.6, 55.5 (C-7).
Amide 164 is a known compound and the spectral data was consistent with the literature data.  

### 7.3.15 Preparation of 2-(4-methoxyphenyl)-4,4-dimethyl-2-oxazoline (165)

![Chemical structure of 165]

Oxazoline 165 was prepared according to a modification of the procedure of Meyers for the synthesis of 2-(3-methoxyphenyl)-4,4-dimethyl-2-oxazoline.  

4-Methoxybenzoic acid (2.5 g, 16 mmol) was suspended in 10 mL of chloroform. DMF (10 drops) and thionyl chloride (4.6 mL, 7.6 g, 64 mmol) were added and the mixture was refluxed for 4 hours. The mixture was cooled to room temperature and the chloroform and excess thionyl chloride removed under reduced pressure. The crude 4-methoxybenzoyl chloride was dissolved in 30 mL of dichloromethane and added to a solution of 2-amino-2-methylpropanol (6.1 mL, 5.7 g, 64 mmol) in 80 mL of dichloromethane. After 14 hours the mixture was washed with water, 1 M HCl, 1 M NaOH, dried (MgSO₄) and concentrated to leave N-(1,1-dimethyl-2-hydroxyethyl)-4-methoxybenzamide (96%) as a colourless solid which was used without further purification; mp 46-48 °C; ¹H NMR (400 MHz; CDCl₃) δ 7.68 (d, J = 8.9 Hz, 2 H, H-2), 6.88 (d, J = 8.9 Hz, 2 H, H-3), 6.22 (br s, 1 H), 3.82 (s, 3 H, H-5), 3.64 (s, 2 H, H-9), 1.38 (s, 6 H, H-8); ¹³C NMR (101 MHz; CDCl₃) δ 168.1 (C), 162.4 (C), 128.9 (CH), 127.1 (C), 113.9 (CH), 70.9 (CH₂, C-9), 56.4 (C, C-7), 55.5 (CH₃, C-5), 24.8 (CH₃, C-8). The N-(1,1-dimethyl-2-hydroxyethyl)-4-methoxybenzamide (1.5 g, 6.7 mmol) was dissolved
in thionyl chloride (2.1 mL, 30 mmol) and reacted at 25 °C for 3 hours. The mixture was cooled to 0 °C and carefully quenched with 1 M NaOH until the resulting solution was neutral. The mixture was extracted with ethyl acetate, dried (Na₂SO₄) and concentrated to leave a colourless oil (85%) which could be used without further purification; ¹H NMR (400 MHz; CDCl₃) δ 7.86 (d, J = 9.0 Hz, 2 H, H-11), 6.88 (d, J = 9.0 Hz, 2 H, H-12), 4.06 (s, 2 H, H-18), 3.82 (s, 3 H, H-14), 1.35 (s, 6 H, H-17); ¹³C NMR (101 MHz; CDCl₃) δ 162.0, 161.9, 130.0, 120.7, 113.7, 79.1 (C-18), 67.5 (C-16), 55.4 (C-14), 28.6 (C-17). Oxazoline 165 is a known compound and the spectral data was consistent with literature data.²⁰⁹

7.3.16 2-(3-Methoxyphenyl)-4,4-dimethyl-2-oxazoline (166)

Prepared according to the same procedure as for 165. For N-(1,1-dimethyl-2-hydroxyethyl)-3-methoxybenzamide: Yield: 94%. Colourless solid; mp 71-72 °C; ¹H NMR (400 MHz; CDCl₃) δ 7.31-7.27 (m, 2H), 7.21 (dt, J = 7.7 Hz, 1.4 Hz, 1 H), 7.00 (ddd, J = 8.1 Hz, 2.6 Hz, 1.0 Hz, 1 H), 6.30 (br s, 1 H), 3.82 (s, 3 H, H-7), 3.65 (s, 2 H, H-11), 1.39 (s, 6 H, H-10); ¹³C NMR (101 MHz; CDCl₃) δ 168.3 (C, C-8), 156.0 (C), 136.5 (C), 129.6 (CH), 118.8 (CH), 117.8 (CH), 112.5 (CH), 70.7 (CH₂, C-11), 56.5 (C, C-9), 55.5 (CH₃, C-7), 24.7 (CH₃, C-10). For 2-(3-Methoxyphenyl)-4,4-dimethyl-2-oxazoline: Yield: 83%. Colourless oil; ¹H NMR (400 MHz; CDCl₃) δ 7.51 (ddd, J = 7.6 Hz, 1.4 Hz, 1.0 Hz, 1 H), 7.45 (dd, J = 2.6 Hz, 1.5 Hz, 1 H, H-13), 7.28 (t, J = 8.0 Hz, 1
H, H-16), 6.98 (ddd, $J = 8.3$ Hz, 2.7 Hz, 1.0 Hz), 4.07 (s, 2 H, H-20), 3.81 (s, 3 H, H-18), 1.36 (s, 6 H, H-22); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 162.0 (C), 159.6 (C), 129.5 (C), 129.4 (CH), 120.8 (CH), 118.0 (CH), 112.7 (CH), 79.2 (CH$_2$, C-20), 67.7 (C, C-21), 55.5 (CH$_3$, C-18), 28.5 (CH$_3$, C-22). Oxazoline 166 is a known compound and the spectral data was consistent with literature data.\(^{208}\)

7.3.17 Preparation of 2,2'-diselenobis(N,N-diisopropyl-4-methoxybenzamide) (167)

\[
\begin{align*}
\text{N,N-Diisopropyl-4-methoxybenzamide (0.47 g, 2.0 mmol) was dissolved in} & \text{ 20 mL of dry THF and cooled to } -78 \degree \text{C under a nitrogen atmosphere. n-Butyllithium (1.1} \\
\text{mL, 2.2 M, 2.4 mmol) was added and the mixture was warmed to 0 \degree \text{C over 45 minutes.} & \text{Elemental selenium (0.18 g, 2.3 mmol) was quickly introduced and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional} \\
\text{3 h and then quenched with 20 mL of saturated ammonium chloride solution. Air was} & \text{rapidly bubbled through the mixture for 30 min, the mixture was washed with brine, dried} \\
\text{(MgSO$_4$) and concentrated under reduced pressure. The resulting oil was purified by} & \text{flash chromatography (100\% ethyl acetate) to afford 0.51 g (82\%) of the diselenide} \\
\text{(82\%) as a yellow solid which contained a significant amount of impurities; IR (film)} & \text{2962, 1614, 1429, 1338, 1229, 1038 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 7.33 (d, } J = 2.4 \\
\text{Hz, 2 H, H-3), 7.02 (d, } J = 8.0 \text{ Hz, 2 H, H-6), 6.70 (dd, } J = 8.4 \text{ Hz, 2.4 Hz, 2 H, H-5,} & \text{3.71 (s, 6 H, H-7), 3.68 (br s, 4 H, H-9), 1.34 (br s, 24 H, H-10); $^{13}$C NMR (101 MHz;}
\end{align*}
\]
CDCl$_3$ $\delta$ 169.5 (C, C-8), 160.4 (C), 131.5 (C), 130.9 (C), 126.5 (CH), 116.6 (CH), 113.0 (CH), 55.5 (CH$_3$, C-7), 20.9 (CH$_3$, C-10), C-9 is not visible; $^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 425.8; mass spectrum $m/z$ (CI, relative intensity) 629 (86, (M+H)$^+$), 627 (100), 316 (95); exact mass calcd. for C$_{28}$H$_{41}$N$_2$O$_4$Se$_2$ (ESI, M+H): 629.13913; found: 629.13902.

7.3.18 2,2'-Diselenobis(N,N-diisopropyl-3-methoxybenzamide) (168)

![Structure Image]

Prepared according to the same procedure as for 167. Yield: 53%. Mixture of compounds; mass spectrum $m/z$ (CI, relative intensity) 629 (84, (M+H)$^+$), 627 (100), 314 (12); exact mass calcd. for C$_{28}$H$_{41}$N$_2$O$_4$Se$_2$ (ESI, M+H): 629.13913; found: 629.13902.

7.3.19 Preparation of 2,2'-diselenobis(N-methyl-3-methoxybenzamide) (169)

![Structure Image]

N-Methyl-3-methoxybenzamide (0.31 g, 1.9 mmol) was dissolved in 20 mL of dry THF and cooled to -78 °C under a nitrogen atmosphere. $n$-Butyllithium (1.7 mL, 2.4 M, 4.0 mmol) was added and the mixture was warmed to 0 °C over 45 minutes. Elemental selenium (0.17 g, 2.2 mmol) was quickly introduced and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional 3 h and then quenched with 20 mL of saturated ammonium chloride solution. Air was
rapidly bubbled through the mixture for 30 min., the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (ethyl acetate-methanol, 9:1) to afford 0.15 g (33%) of the diselenide as a yellow solid which contained a significant amount of impurities; IR (film) 3267, 2933, 1557, 1452, 1262, 1067 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.33 (dd, J = 7.8 Hz, 8.2 Hz, 2 H, H-5), 6.95 (dd, J = 7.6 Hz, 1.2 Hz, 2 H), 6.89 (dd, J = 8.4 Hz, 0.8 Hz, 2 H), 6.66 (br s, 2 H, NH), 3.84 (s, 6 H, H-7), 2.50 (s, 3 H, H-9) and 2.48 (s, 3 H, H-9); ¹³C NMR (101 MHz; CDCl₃) δ 170.3 (C, C-8), 160.1 (C), 144.5 (C), 131.3 (CH), 119.4 (CH), 117.5 (C), 111.9 (CH), 56.6 (CH₃, C-7), 26.3 (CH₃, C-9); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 404.2; mass spectrum m/z (EI, relative intensity) 488 (1, M⁺), 408 (16), 243 (100), 214 (48); exact mass calcd. for C₁₈H₂₁N₂O₄⁸₀Se₂ (ESI, M+H): 488.98263; found: 488.98121.

7.3.20 2,2'-Diselenobis(N-phenyl-4-methoxybenzamide) (170)

Prepared according to the same procedure as for 169. Yield: 80%. Colourless solid; IR (KBr) 3293, 1625, 1589, 1529, 1430, 1323, 1270, 1220, 1038 cm⁻¹; ¹H NMR (400 MHz; DMSO-d₆) δ 10.46 (br s, 2 H, NH), 8.00 (d, J = 1.6 Hz, 2 H, H-3), 7.75 (d, J = 8.0 Hz, 4 H, H-10), 7.38 (t, J = 8.0 Hz, 6 H, H-11 and H-5), 7.13 (t, J = 7.4 Hz, 2 H), 6.98 (d, J = 7.6 Hz, 2 H), 3.72 (s, 6 H, H-7); ¹³C NMR (101 MHz; DMSO-d₆) δ 165.8, 161.7, 139.0, 134.7, 130.4, 128.6, 125.7, 123.8, 120.7, 116.3, 111.1, 55.4; ⁷⁷Se (76 MHz;
DMSO-\textit{d}_6) \delta 454.6; mass spectrum \textit{m/z} (CI, relative intensity) 613 (1, (M+H)\textsuperscript{+}), 322 (34), 308 (100), 228 (15); exact mass calcd. for C\textsubscript{28}H\textsubscript{25}N\textsubscript{2}O\textsubscript{4}\textsuperscript{80}Se\textsubscript{2} (ESI, (M+Na)\textsuperscript{+}): 613.01393; found: 613.01288.

7.3.21 2,2'-Diselenobis(N-phenyl-3-methoxybenzamide) (171)

![Chemical Structure]

Prepared according to the same procedure as for 169. Yield: 67%. Yellow solid; IR (film) 3252, 3124, 3062, 2929, 1643, 1590, 1543, 1438, 1248, 1052 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \delta 8.71 (br s, 2 H, NH), 7.27-7.17 (m, 10 H), 7.09-7.05 (m, 2 H), 6.98 (dd, \textit{J} = 7.4 Hz, 1.0 Hz, 2 H), 6.68-6.65 (m, 2 H), 3.65 (s, 6 H, H-7); \textsuperscript{13}C NMR (101 MHz; CDCl\textsubscript{3}) \delta 167.9 (C, C-8), 159.9 (C), 144.3 (C), 137.8 (C), 131.2 (CH), 128.7 (CH), 124.4 (CH), 120.5 (CH), 119.5 (C), 116.6 (C), 112.2 (CH), 56.3 (CH\subscript{3}, C-7); \textsuperscript{77}Se NMR (76 MHz; CDCl\textsubscript{3}) \delta 397.8; mass spectrum \textit{m/z} (CI, relative intensity) 613 (1, (M+H)\textsuperscript{+}), 306 (100); exact mass calcd. for C\textsubscript{28}H\textsubscript{25}N\textsubscript{2}O\textsubscript{4}\textsuperscript{80}Se\textsubscript{2} (ESI, M+H): 613.01393; found: 613.01291.
7.3.22 Diselenide 172

Prepared according to the same procedure as for 167. Yield: 64%. Colourless solid; IR (KBr) 2964, 2891, 2828, 1642, 1592, 1549, 1270, 1227, 1031 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.70 (d, $J = 8.4$ Hz, 2 H, H-6), 7.43 (d, $J = 2.4$ Hz, 2 H, H-3), 6.71 (dd, $J = 8.6$ Hz, $J = 2.6$ Hz, 2 H, H-5), 4.09 (s, 4 H, H-11), 3.65 (s, 6 H, H-7), 1.43 (s, 12 H, H-10); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 161.8, 161.3, 135.6, 130.9, 119.5, 116.2, 111.8, 79.0 (C-11), 68.6 (C-9), 55.4 (C-7), 28.8 (C-10). $^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 465.0; mass spectrum $m/z$ (CI, relative intensity) 569 (52, (M+H)$^+$), 286 (100); exact mass calcd. for C$_{24}$H$_{29}$N$_2$O$_4$Se$_2$ (CI, M+H): 569.0458; found: 569.0471.

7.3.23 Diselenide 173

Prepared according to the same procedure as for 167. Yield: 58%. Yellow solid; IR (film) 2957, 2886, 1652, 1562, 1462, 1319, 1252, 1038 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.25 (t, $J = 8.0$ Hz, 2 H, H-5), 7.14 (dd, $J = 7.9$ Hz, 1.2 Hz, 2 H), 6.89 (dd, $J =$
8.2 Hz, 1.0 Hz, 2 H), 3.97 (s, 4 H, C-11), 3.67 (s, 6 H, H-7), 1.29 (s, 12 H, H-10); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 162.7 (C), 160.1 (C), 134.4 (C), 129.3 (CH), 122.6 (C), 121.2 (CH), 112.8 (CH), 79.3 (CH$_2$, C-11), 68.2 (C, C-9), 56.25 (CH$_3$, C-7), 28.4 (CH$_3$, C-10);
$^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 424.3; mass spectrum $m/z$ (CI, relative intensity) 569 (20, (M+H)$^+$), 284 (100); exact mass calcd. for C$_{24}$H$_{29}$N$_2$O$_4$Se$_2$ (CI, M+H): 569.0458; found: 569.0450.

7.3.24 Preparation of 2,2'-diselenobis(2-amino-2-dimethylethyl 4-methoxybenzoate) (175)

A solution of diselenide 172 (0.90 g, 1.6 mmol) in a 1:1 mixture of THF and 1 M HCl was heated at 70 °C for 2 hours. The mixture cooled to room temperature and quenched with water and washed with ethyl acetate. The acidic aqueous layer was neutralised with 1 M NaOH, extracted with ethyl acetate, washed with brine, dried (Na$_2$SO$_4$) and concentrated to afford 0.59 g (62%) of the product as a yellow oil; IR (film) 3352, 2952, 2190, 1681, 1595, 1557, 1476, 1238, 1114 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.99 (d, $J$ = 8.8 Hz, 2 H, H-6), 7.35 (d, $J$ = 2.4 Hz, 2 H, H-3), 6.72 (dd, $J$ = 8.8 Hz, 2.4 Hz, 2 H, H-5), 4.11 (s, 4 H, H-9), 3.64 (s, 6 H, H-7), 1.45 (br s, 4H, NH$_2$), 1.21 (s, 12 H, H-11); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 166.8 (C), 163.6 (C), 137.2 (C), 133.0 (CH), 120.6 (C), 115.7 (CH), 112.1 (CH), 74.5 (CH$_2$, C-9), 55.4 (CH$_3$, C-7), 49.7 (C, C-10), 27.4 (CH$_3$, C-11); mass spectrum $m/z$ (CI, relative intensity) 303 (6, (M+2H)$^{2+}$), 284 (34),
270 (20), 230 (28), 214 (41), 171 (22); exact mass calcd. for \( \text{C}_{24}\text{H}_{33}\text{N}_2\text{O}_6^{80}\text{Se}_2 \) (ESI, M+H): 605.06636; found: 605.06525.

7.3.25 2,2'-diselenobis(2-amino-2,2-dimethylethyl 3-methoxybenzoate) (176)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{Se} & \quad \text{Se} \\
\text{NH}_2 & \\
\end{align*}
\]

Prepared according to the same procedure as for 175. Yield: 85%. Yellow oil; IR (film) 3352, 2967, 2938, 1714, 1567, 1462, 1295, 1267, 1057 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.31-7.25 (m, 4 H), 6.95 (dd, \(J = 7.6\) Hz, 1.6 Hz, 2 H), 4.01 (s, 4 H, H-9), 3.70 (s, 6 H, H-7), 1.14 (s, 12 H, H-11); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 167.7 (C, C-8), 160.1 (C), 137.2 (C), 129.3 (CH), 122.0 (C), 121.3 (CH), 113.8 (CH), 75.0 (CH\(_2\), C-9), 56.3 (CH\(_3\), C-7), 49.6 (C), 27.4 (CH\(_3\), C-11); mass spectrum \(m/z\) (EI, relative intensity) 604 (16, \(M^+\)), 303 (34), 284 (55), 213 (20), 83 (28), 72 (100); exact mass calcd. for \( \text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_6^{80}\text{Se}_2 \): 604.0591; found: 604.0568.

7.3.26 Preparation of 2,2'-diselenobis(4-methoxybenzyl alcohol) (151)

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\cdot & \quad \cdot \\
\end{align*}
\]

A solution of N,N,N'-Trimethylethylenediamine (0.840 mL, 653 mg, 6.40 mmol) in 20 mL of dry THF was cooled to -20 \(^\circ\)C under a nitrogen atmosphere. \(n\)-Butyllithium (2.6 mL, 2.4 M, 6.3 mmol) was added followed 15 min. later by 3-methoxybenzaldehyde
(0.73 mL, 0.82 g, 6.0 mmol) and the mixture was left at -20 °C for an additional 15 min. A second portion of n-butyllithium (2.6 mL, 2.4 M 6.3 mmol) was added at this point and the mixture was warmed to 0 °C and left for 1.5 hours. Elemental selenium (553 mg, 7.0 mmol) was quickly introduced and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional 3 h and then poured into a mixture of 30 mL of 1 M HCl and 30 mL of ethyl acetate. Air was rapidly bubbled through the mixture for 30 min., the mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting red solid (1.27 g) was suspended in 10 mL of dry THF and added to a suspension of lithium aluminum hydride (750 mg, 19.8 mmol) in 40 mL of THF at 0 °C. The mixture was warmed to room temperature and left for an additional 4 hours. The mixture was cooled to 0 °C and carefully quenched with 30 mL of water. Ethyl acetate (30 mL) was added and the resulting slurry was filtered to remove the solid precipitate. The aqueous layer was washed with ethyl acetate and then allowed to stir in the presence of air for 12 hours. Over this time 595 mg (46%, 2 steps) of diselenide 151 formed as a yellow solid which could be collected by filtration, mp 101-102 °C (from hexanes-ethyl acetate); IR (solid) 3301, 2917, 1602, 1468, 1245, 1009 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.27 (d, J = 8.8 Hz, 2 H, H-6), 7.26 (d, J = 2.4 Hz, 2 H, H-3), 6.80 (dd, J = 8.4, 2.8 Hz, 2 H, H-5), 4.70 (s, 4 H, H-7), 3.73 (s, 6 H, H-8), 1.88 (br s, 2 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 159.7, 134.2, 132.2, 129.9, 119.8, 114.7, 65.1 (C-7), 55.5 (C-8); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 437.1; mass spectrum m/z (EI, relative intensity) 434 (38, M⁺), 215 (68), 201 (70), 108 (100); exact mass calcd. for C₁₆H₁₈O₄Se₂: 433.9536; found: 433.9548. Anal. calcd. for C₁₆H₁₈O₄Se₂: C, 44.46; H, 4.20; found: C, 44.50; H, 4.15.
7.3.27 2,2'-diselenobis(3-methoxybenzyl alcohol) (152)

Prepared according to the same procedure as for 151. Yield: 54% (2 steps). Yellow solid, mp 142-144 °C (from hexanes-ethyl acetate); IR (solid) 3281, 2933, 1571, 1462, 1267, 1033 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.31 (t, \(J = 8.0\) Hz, 2 H, H-5), 7.05 (dd, \(J = 7.6, 1.2\) Hz, 2 H), 6.81 (dd, \(J = 8.4, 0.80\) Hz, 2 H), 4.62 (d, \(J = 6.4\) Hz, 4 H, H-7), 3.69 (s, 6 H, H-8), 2.24 (t, \(J = 6.6\) Hz, 2 H, OH); \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 160.3 (C), 146.3 (C), 131.3 (CH), 121.1 (CH), 119.6 (C), 110.6 (CH), 65.8 (CH\(_2\), C-7), 56.3 (CH\(_3\), C-8); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 355.5; mass spectrum \(m/z\) (EI, relative intensity) 434 (48, M\(^+\)), 216 (46), 200 (100), 135 (40), 108 (98); exact mass calcd. for \(\text{C}_{16}\text{H}_{18}\text{O}_{4}\text{Se}_{2}\): 433.9536; found: 433.9540. Anal. calcd. for \(\text{C}_{16}\text{H}_{18}\text{O}_{4}\text{Se}_{2}\): C, 44.46; H, 4.20; found: C, 44.38; H, 4.32.

7.3.28 Preparation of 2,2'-diselenobis(3,4,5-trimethoxy-1-(methoxymethoxy)methyl benzene) (211)

Trimethoxyaryl bromide 210 (1.30 g, 4.56 mmol) was dissolved in 40 mL of dry THF and cooled to -78 °C under a nitrogen atmosphere. \(n\)-Butyllithium (3.0 mL, 1.9 M, 5.7 mmol) was added and the mixture was warmed to 0 °C over 1.5 hours. Elemental
selenium (450 mg, 5.70 mmol) was quickly introduced and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional 3 h and then quenched with 40 mL of saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min, the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 1:2) to afford 850 mg (58%) of the product as a yellow oil which was used in subsequent steps immediately.

7.3.29 Preparation of 2,2'-diselenobis(4,5-dimethoxybenzyl alcohol) (195)

Diselenide 195 could be prepared by two different methods. The first involved diazotization of 2-amino-4,5-dimethoxybenzoic acid and subsequent reaction with potassium diselenide to produce diselenide 203, followed by reduction of the bisbenzoic acid to the corresponding bisbenzyl alcohol with lithium aluminum hydride to produce 195 (30%, two steps). The procedure for this route is the same as described in Subsection 7.2.1 for the formation of 203 and Subsection 7.2.4 for the reduction of 203 to 195. Alternatively, and preferentially, diselenide 195 could be prepared via lithium-halogen exchange (described below). Both routes produced pure samples of 195.
Lithium-halogen exchange:

2-Bromo-4,5-dimethoxybenzyl alcohol (3.13 g, 12.7 mmol) was dissolved in 130 mL of dry THF and cooled to -78 °C under a nitrogen atmosphere. n-Butyllithium (14.3 mL, 2.00 M, 28.5 mmol) was added and the mixture was warmed to 0 °C over 45 minutes. Elemental selenium (2.25 g, 28.5 mmol) was added and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional 3 hours and then quenched with 80 mL of saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min., the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by chromatography (gradient of hexanes-ethyl acetate, 2:1 to 100% ethyl acetate) to afford 1.67 g (54%) of the diselenide as a yellow solid, mp 148-150 °C (from ethyl acetate); IR (solid) 3349, 2932, 1584, 1494, 1259, 1213, 1150, 1032 cm⁻¹, ¹H NMR (400 MHz; CDCl₃) δ 7.01 (s, 2 H), 6.98 (s, 2 H), 4.60 (s, 4 H, H-7), 3.89 (s, 6 H), 3.76 (s, 6 H), 2.08 (br s, 2 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 150.6, 148.4, 137.2, 120.7, 120.0, 111.6, 65.3 (C-7), 56.2, 56.1; ⁷⁷Se NMR (76 MHz; CDCl₃) δ 467.6; mass spectrum m/z (EI, relative intensity) 492 (9, M⁺), 245 (20), 230 (24), 166 (14), 138 (100); exact mass calcd. for C₁₈H₂₂O₆Se₂Na (ESI, M+Na⁺): 516.9639; found: 516.9642.

7.3.30 Preparation of 2,2′-diselenobis(3,5-dimethoxybenzyl alcohol) (197) and 2,2′-selenobis(3,5-dimethoxybenzyl alcohol) (225)
2-Bromo-3,5-dimethoxybenzyl alcohol (1.00 g, 4.05 mmol) was dissolved in 50 mL of dry THF and cooled to -78 °C under a nitrogen atmosphere. n-Butyllithium (3.7 mL, 2.4 M, 8.9 mmol) was added and the mixture was warmed to room temperature over 1 hour. Elemental selenium (355 mg, 4.50 mmol) was added and the nitrogen atmosphere restored. The mixture was warmed to room temperature, left for an additional 3 hours and quenched with 30 mL of saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min, the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. Flash chromatography (100% ethyl acetate) was carried out to isolate 500 mg of a mixture of diselenide and selenide species. This mixture was used immediately in subsequent steps without further purification. The selenide was separated after further operation on the diselenide and was present as about 25% of the mixture. Selenide 225: Colourless solid, mp 159-161 °C (from ethyl acetate); IR (solid) 3252, 1586, 1462, 1310, 1157 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 6.62 (d, J = 2.4 Hz, 2 H), 6.33 (d, J = 2.4 Hz, 2 H), 4.84 (d, J = 6.4 Hz, 4 H, H-7), 3.80 (s, 6 H), 3.63 (s, 6 H), 3.31 (t, J = 6.6 Hz, 2 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 161.2, 160.5, 146.2, 110.0, 106.4, 98.8, 66.4 (C-7), 56.2, 55.5; ⁷⁷Se NMR (76 MHz; CDCl₃) δ 122.8; mass spectrum m/z (EI, relative intensity) 414 (46, M⁺), 246 (44), 230 (30), 165 (100), 139 (64), 109 (32), 95 (20), 77 (20); exact mass calcd. for C₁₈H₂₂O₆⁸₀Se: 414.0582; found: 414.0598.

7.3.31 Typical procedure for the preparation of allyl phenyl selenides: allyl 5-methoxy-2-(hydroxymethyl)phenyl selenide (183)
Diselenide **151** (200 mg, 0.463 mmol) was dissolved in 18 mL of 5:1 THF-ethanol and cooled to 0 °C. Sodium borohydride (122 mg, 3.22 mmol) was added and after 5 minutes allyl iodide (0.145 mL, 270 mg, 1.61 mmol) was added. The mixture was warmed to room temperature, left for an additional 3 hours and then quenched with 15 mL of water. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 2:1) to afford 215 mg (90%) of the product as a colourless oil; IR (neat) 3438, 2933, 2833, 1600, 1476, 1233, 1038 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.26 (d, J = 8.4 Hz, 1 H, H-3), 7.06 (d, J = 2.8 Hz, 1 H, H-6), 6.76 (dd, J = 8.4, 2.8 Hz, 1 H, H-4), 5.97-5.87 (m, 1 H, H-10), 4.99 (ddt, J = 16.9, 2.6, 1.3 Hz, 1 H, H-11b) 4.96-4.93 (m, 1 H, H-11a), 4.65 (d, J = 6.0 Hz, 2 H, H-7), 3.77 (s, 3 H, H-8), 3.49 (br d, J = 7.6 Hz, 2 H, H-9), 2.54 (t, J = 6.2 Hz, 1 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 159.1, 135.0, 134.2, 131.1, 129.6, 119.7, 117.3, 112.9, 64.8 (C-7), 55.4 (C-8), 30.8 (C-9); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 269.0; mass spectrum m/z (EI, relative intensity) 258 (64, M⁺), 217 (96), 159 (30), 135 (36), 108 (100); exact mass calcd. for C₁₁H₁₄O₂⁸⁰Se: 258.0159; found: 258.0160.

**7.3.32 Allyl 6-methoxy-2-(hydroxymethyl)phenyl selenide (184)**

![Chemical Structure](image)

Prepared according to the same procedure as for **183**. Yield: 86%. Colourless oil; IR (neat) 3462, 2929, 1633, 1567, 1471, 1248, 1176 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ
7.30 (t, $J = 8.0$ Hz, 1 H, H-4), 7.03 (dd, $J = 7.6, 0.80$ Hz, 1 H), 6.84 (dd, $J = 8.2, 1.0$ Hz, 1 H), 5.90-5.79 (m, 1 H, H-10), 4.84-4.82 (m, 1 H, H-11), 4.79 (br s, 1 H, H-11), 4.77 (d, $J = 6.8$ Hz, 2 H, H-7), 3.90 (s, 3 H, H-8), 3.48 (d, $J = 7.6$ Hz, 2 H, H-9), 2.50 (t, $J = 6.6$ Hz, 1 H, OH); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 160.1 (C), 146.1 (C), 134.9 (CH), 130.0 (CH), 121.1 (CH), 117.0 (C), 116.5 (CH$_2$, C-11), 110.4 (CH, C-10), 66.1 (CH$_2$, C-7), 56.2 (CH$_3$, C-8), 30.0 (CH$_3$, C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 178.4; mass spectrum m/z (EI, relative intensity) 258 (100, M$^+$), 217 (72), 199 (32), 108 (90); exact mass calcd. for C$_{11}$H$_{14}$O$_2$Se: 258.0159; found: 258.0157. Anal. calcd. for C$_{11}$H$_{14}$O$_2$Se: C, 51.37; H, 5.49; found: C, 51.39; H, 5.50.

7.3.33 Allyl 4,5-dimethoxy-2-(hydroxymethyl)phenyl selenide (206)

Prepared according to the same procedure as for 183. Yield: 79%. Brown oil; IR (neat) 3490, 2930, 1602, 1498, 1267, 1148, 1036 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 7.04 (s, 1 H), 6.94 (s, 1 H), 5.92-5.81 (m, 1 H, H-11), 4.87-4.79 (m, 2 H, H-12), 4.69 (s, 2 H, H-7), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.36 (d, $J = 7.2$ Hz, 2 H, H-10), 2.54 (br s, 1 H, OH); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 149.3 (C), 148.1 (C), 137.0 (C), 134.6 (CH), 119.5 (CH), 118.9 (C), 116.8 (CH$_2$, C-12), 111.6 (CH, C-11), 65.3 (CH$_2$, C-7), 56.1 (CH$_3$), 55.9 (CH$_3$), 31.9 (CH$_2$, C-10); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 273.3; mass spectrum m/z (EI, relative intensity) 288 (27, M$^+$), 247 (24), 138 (100), 123 (18), 84 (71), 39 (55); mass calcd. for C$_{12}$H$_{16}$O$_3$Se: 288.02647; found: 288.02753.
7.3.34 Allyl 4,5,6-trimethoxy-2-((methoxymethoxy)methyl)phenyl selenide (216)

![Chemical Structure](image)

Prepared according to the same procedure as for 183. Yield: 72%. Colourless oil; IR (neat) 2924, 1586, 1324, 1095, 919 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 6.87 (s, 1 H, H-3), 5.92-5.81 (m, 1 H, H-14), 4.84 (d, \(J = 18.4\) Hz, 1 H, H-15b), 4.80 (d, \(J = 10.4\) Hz, 1 H, H-15a), 4.74 (s, 2 H), 4.74 (s, 2 H), 3.93 (s, 3 H), 3.88 (s, 3 H), 3.87 (s, 3 H), 3.46-3.44 (m, 2 H, H-13), 3.44 (s, 3 H, H-9); \(^1^3\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 155.3, 154.1, 142.0, 137.8, 135.0, 116.3, 115.3, 108.1, 96.3 (C-8), 70.4 (C-7), 61.1, 61.0, 56.2, 55.7, 31.0 (C-13); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 197.7; mass spectrum \(m/z\) (EI, relative intensity) 362 (80, M\(^+\)), 289 (100), 259 (25), 181 (28), 167 (35), 139 (40); exact mass calcd. for C\(_{15}\)H\(_{22}\)O\(_5\)\(^{80}\)Se: 362.0632; found: 362.0632.

7.3.35 Preparation of allyl 4,5,6-trimethoxy-2-(hydroxymethyl)phenyl selenide (217)

![Chemical Structure](image)

Allyl selenide 216 (147 mg, 0.408 mmol) was dissolved in 5 mL of methanol. Concentrated HCl (6 drops) was added and the mixture was heated at 60 °C. After 5 hours the methanol solution was poured into 5 mL of saturated bicarbonate solution. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO\(_4\)) and concentrated under reduced pressure. The resulting oil was purified by flash
chromatography (hexanes-ethyl acetate, 1:2) to afford 97 mg (75%) of the product as a slightly yellow oil; IR (neat) 3424, 2929, 1590, 1319, 1100 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 6.80 (s, 1 H, H-3), 5.91-5.80 (m, 1 H, H-12), 4.85-4.82 (m, 1 H, H-13), 4.80 (m, 1 H, H-13), 4.72 (br s, 2 H, H-7), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.45 (br d, \(J = 7.6 \text{ Hz}, 2 \text{ H, H-11}\)), 2.48 (br s, 1 H, OH); \(^1^3\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 155.3, 154.2, 141.8, 140.7, 134.9, 116.7, 113.9, 107.9, 66.1 (C-7), 61.2, 61.0, 56.1, 30.9 (C-11); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 194.4; mass spectrum \(m/z\) (EI, relative intensity) 318 (54, M\(^+\)), 277 (44), 196 (30), 168 (100), 153 (34); exact mass calcd. for C\(_{13}\)H\(_{18}\)O\(_4\)\(^{80}\)Se: 318.0370; found: 318.0364.

7.3.36 Allyl 4,6-dimethoxy-2-(hydroxymethyl)phenyl selenide (228)

Prepared according to the same procedure as for 183. Yield: 51%. Colourless oil; IR (neat) 3452, 2933, 1590, 1462, 1152, 1067 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 6.63 (d, \(J = 3.6 \text{ Hz}, 1 \text{ H}\)), 6.42 (d, \(J = 3.2 \text{ Hz}, 1 \text{ H}\)), 5.92-5.78 (m, 1 H, H-11), 4.83-4.75 (m, 4 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.40 (d, \(J = 10.4 \text{ Hz}, 2 \text{ H, H-10}\)), 2.38 (t, \(J = 9.2 \text{ Hz}, 1 \text{ H, OH}\)); \(^1^3\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 161.5 (C), 161.1 (C), 147.2 (C), 134.9 (CH), 116.3 (CH\(_2\), C-12), 107.4 (C), 105.0 (CH), 98.0 (CH), 66.1 (CH\(_2\), C-7), 56.1 (CH\(_3\)), 55.4 (CH\(_3\)), 30.1 (CH\(_2\), C-10); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 162.6; mass spectrum \(m/z\) (EI, relative intensity) 288 (52, M\(^+\)), 247 (22), 229 (26), 166 (100), 138 (78); exact mass calcd. for
C₁₂H₁₆O₃⁸⁰Se: 288.0265; found: 288.0262. NOTE: 50 mg of the symmetrical aryl selenide 225 was also isolated as it was present in the starting material mixture.

7.3.37 Typical procedure for the preparation of benzo-1,2-oxaselenolane Se-oxides: 5-methoxybenzo-1,2-oxaselenolane Se-oxide (147)

Allyl selenide 183 (186 mg, 0.723 mmol) and 28.6% H₂O₂ (0.260 mL, 2.18 mmol) were stirred in 10 mL of dichloromethane for 16 hours. The solution was concentrated and the resulting mixture was purified by flash chromatography (ethyl acetate-methanol, 9:1) to afford 137 mg (81%) of the cyclic seleninate ester as a colourless solid, mp 148-149 °C (from ethyl acetate-methanol); IR (solid) 2934, 1598, 1472, 1432, 1257, 1230, 1023, 973 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.35 (d, J = 8.8 Hz, 1 H, H-3), 7.25 (d, J = 2.4 Hz, 1 H, H-3), 7.10 (dd, J = 8.4, 2.4 Hz, 1 H, H-4), 5.90 (d, J = 12.8 Hz, 1 H, H-7a), 5.56 (d, J = 13.2 Hz, 1 H, H-7b), 3.81 (s, 3 H, H-8); ¹³C NMR (101 MHz; CDCl₃) δ 160.5, 149.3, 135.2, 123.4, 119.9, 108.9, 78.4 (C-7), 55.9 (C-8); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 1348.5; mass spectrum m/z (EI, relative intensity) 232 (38, M⁺), 215 (24), 136 (100), 108 (46); exact mass calcd. for C₈H₈O₃⁸⁰Se: 231.9639; found: 231.9642. Anal. calcd. for C₈H₈O₃Se: C, 41.58; H, 3.49; found: C, 41.40; H, 3.35.
7.3.38 6-Methoxybenzo-1,2-oxaselenolane Se-oxide (149)

Prepared according to the same procedure as for 183. Yield: 85%. Colourless solid; mp 156-157 °C (from ethyl acetate-methanol); IR (solid) 2925, 1567, 1468, 1275, 1068, 964 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.50 (t, J = 8.0 Hz, 1 H, H-4), 6.99 (d, J = 7.6 Hz, 0.80 Hz, 1 H), 6.89 (d, J = 8.0 Hz, 1 H), 5.95 (d, J = 14.0 Hz, 1 H, H-7a), 5.58 (d, J = 13.6 Hz, 1 H, H-7b), 3.91 (s, 3 H, H-8); ¹³C NMR (101 MHz; CDCl₃) δ 157.4 (C), 146.3 (C), 136.3 (C), 134.7 (CH), 114.5 (CH), 110.4 (CH), 79.2 (CH₂, C-7), 56.2 (CH₃, C-8); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 1341.7; mass spectrum m/z (CI, relative intensity) 233 (100, M+H⁺), 218 (21); exact mass calcd. for C₈H₉O₃⁸₀Se (CI, (M+H)⁺): 232.9717; found: 232.9712. Anal. calcd. for C₈H₈O₃Se: C, 41.58; H, 3.44; found: C, 41.66; H, 3.38.

7.3.39 4,5-Dimethoxybenzo-1,2-oxaselenolane Se-oxide (141)

Prepared according to the same procedure as for 183. Yield: 87%. Colourless solid; mp 168-169 °C (from ethyl acetate-methanol); IR (film) 2937, 1576, 1497, 1280, 1215, 1030 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.23 (s, 1 H), 6.86 (s, 1 H), 5.89 (d, J = 13.2 Hz, 1 H, H-7a), 5.52 (d, J = 13.6 Hz, 1 H, H-7b), 3.91 (s, 3 H), 3.86 (s, 3 H); ¹³C NMR (101 MHz; CDCl₃) δ 152.8, 150.1, 139.0, 137.0, 107.0, 104.2, 78.7 (C-7), 56.4,
56.4; $^{77}\text{Se}$ NMR (76 MHz; CDCl$_3$) $\delta$ 1355.9; mass spectrum $m/z$ (EI, relative intensity) 262 (6, M$^+$), 166 (100), 138 (26); exact mass calcd. for C$_9$H$_{11}$O$_4^{80}\text{Se}$ (ESI, (M+H)$^+$): 262.98175; found: 262.98190. Anal. calcd. for C$_9$H$_{10}$O$_4\text{Se}$: C, 41.40; H, 3.86; found: C, 41.22; H, 4.10.

7.3.40 4,6-Dimethoxybenzo-1,2-oxaselenolane Se-oxide (142)

![Structure of 4,6-Dimethoxybenzo-1,2-oxaselenolane Se-oxide](structure.png)

Prepared according to the same procedure as for 183. Yield: 89%. Colourless solid; mp 149-151 °C; IR (KBr) 2971, 2938, 2838, 1590, 1467, 1352, 1224, 1157, 1110 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 6.46 (d, $J$ = 2.0 Hz, 1 H), 6.41 (d, $J$ = 1.6 Hz, 1 H), 5.90 (d, $J$ = 13.6 Hz, 1 H, H-7a), 5.50 (d, $J$ = 14.0 Hz, 1 H, H-7b), 3.88 (s, 3 H), 3.84 (s, 3 H), 5.90 (d, $J$ = 13.6 Hz, 1 H, H-7a), 5.50 (d, $J$ = 14.0 Hz, 1 H, H-7b), 3.88 (s, 3 H), 3.84 (s, 3 H); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 165.6 (C), 158.5 (C), 148.1 (C), 128.6 (C), 98.7 (CH), 98.2 (CH), 78.9 (CH$_2$, C-7), 56.2 (CH$_3$), 56.1 (CH$_3$); $^{77}\text{Se}$ NMR (76 MHz; CDCl$_3$) $\delta$ 1355.1; mass spectrum $m/z$ (EI, relative intensity) 263 (6, (M+H)$^+$), 246 (14), 166 (100), 151 (8), 137 (11), 123 (12), 95 (10); exact mass calcd. for C$_9$H$_{11}$O$_4^{80}\text{Se}$ (ESI, (M+H)$^+$): 262.98175; found: 262.98144. Anal. calcd. for C$_9$H$_{10}$O$_4\text{Se}$: C, 41.40; H, 3.86; found: C, 41.57; H, 3.81.
7.3.41 Preparation of 4,5,6-trimethoxybenzo-1,2-selenolane Se-oxide (146)

Allyl selenide 217 (88 mg, 0.28 mmol) and 33.1% H₂O₂ (0.057 mL, 0.56 mmol) were stirred in 5 mL of dichloromethane at room temperature for 6 hours. The mixture was concentrated and the resulting solid was purified by flash chromatography (ethyl acetate-methanol, 9:1) to afford 64 mg (80%) of the cyclic seleninate ester, mp 160-161 °C (from ethyl acetate-methanol); IR (solid) 2928, 1576, 1460, 1340, 1104, 956 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 6.62 (s, 1 H, H-3), 5.91 (d, J = 13.6 Hz, 1 H, H-7a), 5.53 (d, J = 13.6 Hz, 1 H, H-7b), 4.08 (s, 3H), 3.90 (s, 3 H), 3.86 (s, 3 H); ¹³C NMR (101 MHz; CDCl₃) δ 158.6, 150.6, 140.8, 140.4, 133.0, 99.8, 79.3 (C-7), 61.9, 61.3, 56.6; ⁷⁷Se NMR (76 MHz; CDCl₃) δ 1360.5; mass spectrum m/z (EI, relative intensity) 292 (20, M⁺), 276 (12), 196 (100), 181 (36), 153 (34); exact mass calcd. for C₁₀H₁₂O₅⁸⁰Se: 291.9850; found: 291.9855. Anal. calcd. for C₁₀H₁₂O₅Se: C, 41.25; H, 4.15; found: C, 41.31; H, 4.07.

7.3.42 Preparation of 2,2'-selenobis(4-methoxybenzyl alcohol) (185)

2-Amino-4-methoxybenzyl alcohol (492 mg, 3.22 mmol) was suspended in 12 mL of water, cooled to 0 °C and treated with 1.8 mL of concentrated HCl, followed by the dropwise addition of NaNO₂ (260 mg, 3.77 mmol) in 1.8 mL of water. In a separate
round bottom flask, the diselenide (460 mg, 1.06 mmol) was dissolved in 34 mL of THF:water (1:1), cooled to 0 °C and treated with NaBH₄ (283 mg, 7.48 mmol). The pH of the diazonium salt solution was then adjusted to ~5.5 with saturated aqueous sodium acetate. This mixture was subsequently transferred in a dropwise manner to the clear selenolate solution. The reaction mixture was warmed to room temperature, left for 2 hours and quenched with 15 mL of saturated aqueous ammonium chloride. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 1:1) to afford 301 mg of a mixture of desired selenide compound and recovered starting material. The most efficient method of separating the mixture was to subject it to oxidizing conditions, separate the resulting spirodioxyselenurane and cyclic seleninate ester mixture and reduce the spirodioxyselenurane to the corresponding selenide. Thus, the mixture and 28.6% hydrogen peroxide (0.510 mL, 4.27 mmol) were dissolved in 10 mL of dichloromethane and left for 12 hours. This was next concentrated under reduced pressure and purified by flash chromatography (ethyl acetate-methanol, 9:1) to afford 145 mg of the spirodioxyselenurane. This was reduced to the desired selenide by treating it with 2 equivalents of benzyl thiol in 5 mL of dichloromethane for 1 hour. Concentration of the mixture and purification by chromatography (hexanes-ethyl acetate, 1:1) afforded (123 mg, 0.351 mmol, 5% after 3 steps) of the desired selenide as a colourless solid, mp 115-116 °C; IR (solid) 3319, 2919, 1590, 1471, 1224, 1038 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.35 (d, J = 8.4 Hz, 2 H, H-6), 6.89 (d, J = 2.8 Hz, 2 H, H-3), 6.82 (dd, J = 8.4, 2.8 Hz, 2 H, H-5), 4.68 (s, 4 H, H-7), 3.71 (s, 6 H, H-8), 2.11 (br s, 2 H, OH); ¹³C NMR (101 MHz; CDCl₃) δ 159.7 (C), 134.5 (C), 131.8 (C), 130.4 (CH), 120.1 (CH), 113.6
(CH), 65.0 (CH₂, C-7), 55.5 (CH₃, C-8); $^{77}$Se NMR (76 MHz; CDCl₃) δ 335.8; mass spectrum $m/z$ (EI, relative intensity) 354 (10, M⁺), 199 (20), 135 (82), 121 (68), 108 (75), 77 (100); exact mass calcd. for C₁₆H₁₈O₄$^{80}$Se: 354.0370; found: 354.0357. Anal. calcd. for C₁₆H₁₈O₄Se: C, 54.40; H, 5.14; found: C, 54.38; H, 5.49.

7.3.43 Preparation of 2,2'-selenobis(5-methoxybenzyl alcohol) (186)

2-Amino-3-methoxybenzyl alcohol (492 mg, 3.22 mmol) was suspended in 12 mL of water, cooled to 0 °C and treated with 1.8 mL of concentrated HCl, followed by the dropwise addition of NaNO₂ (260 mg, 3.77 mmol) in 1.8 mL of water. In a separate round bottom flask, the diselenide (460 mg, 1.06 mmol) was dissolved in 35 mL of THF:water (1:1), cooled to 0 °C and treated with NaBH₄ (283 mg, 7.48 mmol). The pH of the diazonium salt solution was then adjusted to ~5.5 with saturated aqueous sodium acetate. This mixture was subsequently transferred in a dropwise manner to the clear selenolate solution. The reaction mixture was warmed to room temperature, left for 2 hours and quenched with 15 mL saturated aqueous ammonium chloride solution. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 1:1) to afford 330 mg of a mixture of selenide and diselenide compounds. The most efficient method of separating this mixture was to subject it to oxidizing conditions.
as the diselenide underwent oxidation at a much faster rate than the selenide. Separation could then be completed on the resulting seleninate ester and selenide mixture. Thus, the mixture and 28.6% hydrogen peroxide (0.560 mL, 4.67 mmol) were dissolved in 10 mL of dichloromethane and left for 12 hours. This mixture was concentrated and purified by chromatography (ethyl acetate-methanol, 9:1) to afford 90 mg of desired selenide species, 160 mg of the corresponding cyclic seleninate ester and 54 mg of the spirodioxyselenurane. The spirodioxyselenurane could be reduced to the title compound by dissolving in 5 mL of dichloromethane and treating with two equivalents of benzyl thiol for 1 hour. The combined selenide was isolated as a colourless solid, mp 182-183 °C; IR (solid) 3214, 2929, 1557, 1462, 1267, 1000 cm\(^{-1}\); \(^1\)H NMR (400 MHz; DMSO-\(d_6\)) \(\delta\) 7.25 (t, \(J = 8.0\) Hz, 2 H, H-5), 7.09 (d, \(J = 8.4\) Hz, 2 H), 6.82 (d, \(J = 8.0\) Hz, 2 H), 5.13 (t, \(J = 5.6\) Hz, 2 H, OH), 4.54 (d, \(J = 5.2\) Hz, 4 H, H-7), 3.56 (s, 6 H, H-8); \(^{13}\)C NMR (101 MHz; DMSO-\(d_6\)) \(\delta\) 158.6 (C), 146.2 (C), 128.5 (CH), 119.1 (CH), 118.0 (C), 109.9 (CH), 62.9 (CH\(_2\), C-7), 55.8 (CH\(_3\), C-8); \(^{77}\)Se NMR (76 MHz; DMSO-\(d_6\)) \(\delta\) 144.2; mass spectrum \(m/z\) (EI, relative intensity) 354 (50, \(M^+\)) 200 (65), 121 (100), 77 (60); exact mass calcd. for \(\text{C}_{16}\text{H}_{18}\text{O}_4\text{Se}\): 354.0370; found: 354.0371. Anal. calcd. for \(\text{C}_{16}\text{H}_{18}\text{O}_4\text{Se}\): C, 54.40; H, 5.14; found: C, 54.20; H, 5.18.

7.3.44 Preparation of 2,2'-selenobis(4,5-dimethoxybenzyl alcohol) (207)
Selenide 207 was prepared according to a modification of the general procedure of Reddy, Kumar and Rao\textsuperscript{150} for the synthesis of symmetrical aryl selenides. 2-Iodo-4,5-dimethoxybenzyl alcohol (1.09 g, 3.70 mmol) and selenourea (151 mg, 1.23 mmol) were dissolved in 5 mL of DMSO. Nanopowder (<50 nm particle size) copper oxide (59 mg, 0.74 mmol) was added and the mixture was degassed with a stream of argon for 20 min. Potassium hydroxide (138 mg, 2.46 mmol) was added and the mixture was heated at 85 °C for 18 hours. The mixture was poured onto 15 mL of water, extracted with ethyl acetate, washed with brine, dried (MgSO\textsubscript{4}) and concentrated under reduced pressure. The resulting mixture was subjected to flash chromatography (hexanes-ethyl acetate, 1:1) to afford 204 mg of a mixture of the desired selenide and undesired diselenide. The most efficient method of separating the mixture was to subject it to oxidizing conditions, separate the resulting spirodioxyselenurane and cyclic seleninate ester mixture and reduce the spirodioxyselenurane to the corresponding selenide. Thus, the mixture and 33.1% hydrogen peroxide (0.058 mL, 0.56 mmol) were dissolved in 10 mL of dichloromethane, left for 6 hours and concentrated. The resulting mixture was purified by flash chromatography (ethyl acetate-methanol, 9:1) to afford 143 mg of the corresponding spirodioxyselenurane. This was reduced to the desired selenide by treating it with 2 equivalents of benzyl thiol in 5 mL of dichloromethane for 1 hour. This was concentrated and purified by chromatography (hexanes-ethyl acetate, 1:1) to afford 128 mg of selenide 207 (17% after 3 steps) as a colourless solid, mp 112-113 °C; IR (solid) 3533, 3419, 2924, 1500, 1252, 1195, 1148 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \(\delta\) 6.96 (s, 2 H), 6.83 (s, 2 H), 4.64 (s, 4 H, H-7), 3.83 (s, 6 H), 3.68 (s, 6 H), 2.67 (br s, 2 H, OH); \textsuperscript{13}C NMR (101 MHz; CDCl\textsubscript{3}) \(\delta\) 149.2, 148.8, 135.1, 120.9, 117.3, 112.1, 64.9 (C-7), 56.1, 56.0; \textsuperscript{77}Se
NMR (76 MHz; CDCl₃) δ 310.8; mass spectrum m/z (EI, relative intensity) 414 (38, M⁺), 316 (32), 151 (100) 138 (46); exact mass calcd. for C₁₈H₂₂O₆Se: 414.0582; found: 414.0583. Anal. calcd. for C₁₈H₂₂O₆Se: C, 52.31; H, 5.36; found: C, 52.38; H, 5.51.

7.3.45 Preparation of 2,2'-selenobis(3,4,5-trimethoxybenzyl alcohol) (218)

Selenide 207 was prepared according to a modification of the general procedure of Reddy, Kumar and Rao¹⁵⁰ for the synthesis of symmetrical aryl selenides. 2-Iodo-3,4,5-trimethoxybenzyl alcohol (810 mg, 2.50 mmol) and selenourea (123 mg, 1.00 mmol) were dissolved in 5 mL of DMSO. Nanopowder (<50 nm particle size) copper oxide (40 mg, 0.50 mmol) was added and the mixture was degassed with a stream of argon for 20 min. Potassium hydroxide (112 mg, 2.00 mmol) was added and the mixture was heated at 85 °C for 18 hours. The mixture was poured onto 15 mL of water, extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was subjected to flash chromatography (hexanes-ethyl acetate, 1:1) to afford 166 mg of a mixture of the desired selenide and undesired diselenide. The most efficient method of separating the mixture was to subject it to oxidizing conditions, separate the resulting spirodioxyselenurane and cyclic seleninate ester mixture and reduce the spirodioxyselenurane to the corresponding selenide. Thus, the mixture and 33.1% hydrogen peroxide (0.045 mL, 0.437 mmol) were dissolved in 7 mL of dichloromethane,
left for 6 hours and concentrated. The resulting mixture was purified by chromatography (ethyl acetate-methanol, 9:1) to afford 100 mg of the corresponding spirodioxyselenurane. This was reduced to the desired selenide by treating it with 2 equivalents of benzyl thiol in 5 mL of dichloromethane for 1 hour. This was concentrated and purified by chromatography (hexanes-ethyl acetate, 1:1) to afford 86 mg \( \text{218} \) (15% after 3 steps) as a colourless oil; IR (neat) 3457, 2929, 2848, 1581, 1481, 1314, 1152, 1105, 1010 cm\(^{-1} \); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 6.85 (s, 2 H, H-6), 4.87 (br s, 4 H, H-7), 3.88 (s, 6 H), 3.77 (s, 6 H), 3.46 (s, 6 H), 3.36 (s, 2 H, OH); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \( \delta \) 154.3, 154.2, 142.1, 139.4, 116.4, 108.9, 66.4 (C-7), 60.9, 56.2; \(^{77}\)Se NMR (76 MHz; CDCl\(_3\)) \( \delta \) 156.0; mass spectrum \( m/z \) (EI, relative intensity) 474 (40, M\(^+\)), 276 (18), 198 (36), 181 (100), 168 (38); exact mass calcd. for C\(_{20}\)H\(_{26}\)O\(_8\)\(^{80}\)Se: 474.0793; found: 474.0792.

7.3.46 Typical procedure for the preparation of 3-hydroxypropyl phenyl selenides: 3-hydroxypropyl 2-(hydroxymethyl)phenyl selenide (187)

Diselenide \( \text{130a} \) (515 mg, 1.38 mmol) was dissolved in 35 mL of 3:1 THF-ethanol and cooled to 0 °C. Sodium borohydride (261 mg, 6.90 mmol) was added and after 5 minutes 1-bromo-3-propanol (0.271 mL, 431 mg, 3.11 mmol) was added. The mixture was warmed to room temperature, left for an additional 2 hours and quenched with 25 mL of water. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO\(_4\)) and concentrated under reduced pressure. The resulting oil was purified
by flash chromatography (hexanes-ethyl acetate, 2:1) to afford 488 mg (72%) of the product as a colourless solid, mp 42-43 °C (from ethyl acetate); IR (solid) 3376, 3303, 2911, 2838, 1436, 1180, 1051 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.54 (dd, \(J = 7.6, 1.2\) Hz, 1 H), 7.39 (dd, \(J = 7.6, 1.2\) Hz, 1 H), 7.26 (td, \(J = 7.5, 1.5\) Hz, 1 H), 7.21 (td, \(J = 7.4, 1.6\) Hz, 1 H), 4.76 (d, \(J = 5.6\) Hz, 2 H, H-7), 3.70 (q, \(J = 5.3\) Hz, 2 H, H-10), 2.98 (t, \(J = 7.2\) Hz, 2 H, H-8), 2.62 (t, \(J = 6.0\) Hz, 1 H, OH), 1.95 (t, \(J = 4.4\) Hz, 1 H, OH), 1.91 (p, \(J = 6.7\) Hz, 2 H, H-9); \(^1\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 142.5, 133.8, 130.0, 128.7, 128.6, 127.7, 65.5 (C-7), 62.2 (C-10), 32.7 (C-8), 24.6 (C-9); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 230.7; mass spectrum \(m/z\) (EI, relative intensity) 246 (100, M\(^+\)), 229 (27), 187 (36), 170 (38), 107 (90), 91 (26), 78 (90); exact mass calcd. for C\(_{10}\)H\(_{14}\)O\(_2\)\(^{80}\)Se: 246.0159; found: 246.0163. Anal. calcd. for C\(_{10}\)H\(_{14}\)O\(_2\)Se: C, 48.99; H, 5.76; found: C, 49.02; H, 5.73.

**7.3.47 3-Hydroxypropyl 4-methoxy-2-(hydroxymethyl)phenyl selenide (188)**

Prepared according to the same procedure as for 187. Yield: 67%. Colourless solid; mp 44-45 °C; IR (film) 3329, 2929, 1595, 1476, 1295, 1229, 1057 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.49 (d, \(J = 8.4\) Hz, 1 H, H-6), 6.99 (d, \(J = 2.8\) Hz, 1 H, H-3), 6.74 (dd, \(J = 8.6, 3.0\) Hz, 1 H, H-5), 4.75 (s, 2 H, H-7), 3.79 (s, 3 H, H-8), 3.66 (t, \(J = 6.0\) Hz, 2 H, H-11), 2.98 (br s, 1 H, OH), 2.85 (t, \(J = 7.2\) Hz, 2 H, H-9), 2.22 (br s, 1 H, OH), 1.84 (tt, \(J = 7.2, 6.0\) Hz, 2 H, H-10); \(^1\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 160.0, 145.1, 137.3, 119.1, 114.2, 114.0, 65.6 (C-7), 62.1 (C-11), 55.5 (C-8), 32.7 (C-9), 25.5 (C-10); \(^77\)Se
NMR (76 MHz; CDCl$_3$) δ 212.6; mass spectrum $m/z$ (EI, relative intensity) 276 (100, M$^+$), 200 (38), 137 (45), 108 (90); exact mass calcd. for C$_{11}$H$_{16}$O$_3$Se: 276.0265; found: 276.0270. Anal. calcd. for C$_{11}$H$_{16}$O$_3$Se: C, 48.01; H, 5.89; found: C, 48.24; H, 5.69.

7.3.48 3-Hydroxypropyl 5-methoxy-2-(hydroxymethyl)phenyl selenide (189)

Prepared according to the same procedure as for 187. Yield: 84%. Colourless solid; mp 58-59 °C (from ethyl acetate); IR (solid) 3352, 3271, 2930, 1594, 1468, 1279, 1221, 1041 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 7.26 (d, $J = 8.4$ Hz, 1 H, H-3), 7.07 (d, $J = 2.8$ Hz, 1 H, H-6), 6.75 (dd, $J = 8.4$, 2.4 Hz, 1H, H-4), 4.67 (s, 2 H, H-7), 3.78 (s, 3 H, H-8), 3.67 (t, $J = 6.0$ Hz, 2 H, H-11), 2.96 (t, $J = 7.2$ Hz, 2 H, H-9), 2.83 (br s, 1 H, OH), 2.44 (br s, 1 H, OH), 1.89 (tt, $J = 7.0$, 6.0 Hz, 2 H, H-10); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 159.3 (C), 134.7 (C), 131.5 (C), 130.0 (CH), 119.2 (CH), 112.5 (CH), 64.9 (CH$_2$, C-7), 62.0 (CH$_2$, C-11), 55.5 (CH$_3$, C-8), 32.6 (CH$_2$, C-9), 24.5 (CH$_2$, C-10); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 228.5; mass spectrum $m/z$ (EI, relative intensity) 276 (100, M$^+$), 217 (82), 199 (24), 135 (50), 108 (62); exact mass calcd. for C$_{11}$H$_{16}$O$_3$Se: 276.0265; found: 276.0267. Anal. calcd. for C$_{11}$H$_{16}$O$_3$Se: C, 48.01; H, 5.86; found: C, 47.95; H, 5.87.
7.3.49 3-Hydroxypropyl 6-methoxy-2-(hydroxymethyl)phenyl selenide (190)

Prepared according to the same procedure as for 187. Yield: 84%. Colourless solid; mp 49-50 °C (from ethyl acetate); IR (solid) 3362, 3267, 2933, 1567, 1462, 1262, 1038 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.29 (t, J = 8.0 Hz, 1 H, H-4), 7.04 (dd, J = 7.6, 1.2 Hz, 1 H), 6.84 (dd, J = 8.0, 1.2 Hz, 1 H), 4.80 (s, 2 H, H-7), 3.88 (s, 3 H, H-8), 3.68 (t, J = 6.0 Hz, 2 H, H-11), 2.94 (t, J = 7.0 Hz, 2 H, H-9), 2.83 (br s, 1 H, OH), 2.03 (br s, 1 H, OH), 1.81 (tt, J = 7.0, 6.0 Hz, 2 H, H-10); ¹³C NMR (101 MHz; CDCl₃) δ 160.2 (C), 145.9 (C), 129.9 (CH), 121.2 (CH), 117.3 (C), 110.5 (CH), 66.1 (CH₂, C-7), 62.3 (CH₂, C-11), 56.3 (CH₃, C-8), 32.9 (CH₂, C-9), 24.5 (CH₂, C-10); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 127.3; mass spectrum m/z (EI, relative intensity) 276 (92, M⁺), 217 (28), 200 (40), 137 (92), 108 (100); exact mass calcd. for C₁₁H₁₆O₃⁸⁰Se: 276.0265; found: 276.0275. Anal. calcd. for C₁₁H₁₆O₃Se: C, 48.01; H, 5.86; found: C, 47.99; H, 5.99.

7.3.50 3-Hydroxypropyl 4,5-dimethoxy-2-(hydroxymethyl)phenyl selenide (204)

Prepared according to the same procedure as for 187. Yield: 95%. Colourless solid; mp 38-39 °C (from ethyl acetate); IR (solid) 3300, 2924, 1505, 1452, 1252, 1152; ¹H NMR (400 MHz; CDCl₃) δ 7.11 (s, 1 H), 6.97 (s, 1 H), 4.75 (d, J = 5.6 Hz, 2 H, H-7),
3.88 (s, 3 H), 3.87 (s, 3 H), 3.70 (q, \( J = 5.3 \) Hz, 2 H, H-12), 2.91 (t, \( J = 7.0 \) Hz, 2 H, H-10), 2.56 (t, \( J = 6.0 \) Hz, 1 H, OH), 1.88 (tt, \( J = 7.0, 6.2 \) Hz, 3 H, H-11 and OH); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \( \delta \) 149.4 (C), 148.5 (C), 136.6 (C), 119.4 (C), 119.0 (CH), 112.1 (CH), 65.6 (CH\(_2\), C-7), 62.2 (CH\(_2\), C-12), 56.3 (CH\(_3\)), 56.1 (CH\(_3\)), 32.8 (CH\(_2\), C-10), 25.8 (CH\(_2\), C-11); \(^{77}\)Se NMR (76 MHz; CDCl\(_3\)) \( \delta \) 228.3; mass spectrum \( m/z \) (EI, relative intensity) 306 (22, M\(^+\)), 247 (15), 165 (20), 138 (100), 123 (22), 95 (25); exact mass calcd. for C\(_{12}\)H\(_{18}\)O\(_4\)\(^{80}\)Se: 306.0370; found: 306.0372. Anal. calcd. for C\(_{12}\)H\(_{18}\)O\(_4\)Se: C, 47.22; H, 5.94; found: C, 47.46; H, 5.89.

7.3.51 3-Hydroxypropyl 4,5,6-trimethoxy-2-((methoxymethoxy)methyl)phenyl selenide (212)

Prepared according to the same procedure as for 187. Yield: 80%. Colourless oil; IR (neat) 3457, 2943, 1576, 1486, 1319, 1262, 1105, 1033, 724 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \( \delta \) 6.87 (s, 1 H, H-3), 4.77 (s, 2 H), 4.73 (s, 2 H), 3.91 (s, 3 H), 3.88 (s, 3 H), 3.86 (s, 3 H), 3.71 (t, \( J = 6.2 \) Hz, 2 H, H-15), 3.43 (s, 3 H, H-9), 2.90 (t, \( J = 7.0 \) Hz, 2 H, H-13), 1.91 (br s, 1 H, OH), 1.81 (tt, \( J = 7.0, 6.2 \) Hz, 2 H, H-14); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \( \delta \) 155.2, 154.1, 142.0, 137.5, 115.4, 108.3, 96.1 (C-8), 70.2 (C-7), 62.2, 61.2, 61.1, 56.1, 55.7, 32.9 (C-13), 25.2 (C-14); \(^{77}\)Se NMR (76 MHz; CDCl\(_3\)) \( \delta \) 146.1; mass spectrum \( m/z \) (EI, relative intensity) 380 (100, M\(^+\)), 317 (40), 289 (50), 197 (80), 181 (40), 168 (25); exact mass calcd. for C\(_{15}\)H\(_{24}\)O\(_6\)\(^{80}\)Se: 380.0738; found: 380.0729.
7.3.52 Preparation of 3-hydroxypropyl 4,5,6-trimethoxy-2-(hydroxymethyl)phenyl selenide (214)

3-Hydroxypropyl selenide 212 (214 mg, 0.564 mmol) was dissolved in 10 mL of methanol along with 6 drops of concentrated HCl. The mixture was heated to 60 °C and left for 5 hours. The mixture was poured into 10 mL of water, extracted with ethyl acetate, washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (hexanes-ethyl acetate, 1:3) to afford 157 mg (83%) of the deprotected product as a colourless oil, IR (neat) 3490, 2943, 1590, 1476, 1319, 1157, 1095, 1010 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 6.83 (s, 1 H, H-3), 4.76 (s, 2 H, H-7), 3.90 (s, 3 H), 3.86 (s, 3 H), 3.85 (s, 3 H), 3.69 (t, J = 6.0 Hz, 2 H, H-13), 2.98 (br s, 1 H, OH), 2.90 (t, J = 7.0 Hz, 2 H, H-11), 2.27 (br s, 1 H, OH), 1.81 (p, J = 6.6 Hz, 2 H, H-12); ¹³C NMR (101 MHz; CDCl₃) δ 155.2, 154.2, 141.8, 140.5, 114.2, 108.1, 66.0 (C-7), 62.1, 61.3, 61.0, 56.1 (C-13), 32.8 (C-11), 25.4 (C-12); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 138.4; mass spectrum m/z (EI, relative intensity) 336 (100, M⁺), 277 (34), 196 (28), 181 (20), 168 (76), 153 (21); exact mass calcd. for C₁₃H₂₀O₅⁸⁰Se: 336.0476; found: 336.0468.

7.3.53 3-Hydroxypropyl 4,6-dimethoxy-2-(hydroxymethyl)phenyl selenide (226)

242
Prepared according to the same procedure as for 187. Yield: 53%. Colourless solid; mp 66-67 °C; IR (solid) 3352, 3243, 2933, 1581, 1310, 1029 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 6.64 (d, $J = 2.4$ Hz, 1 H), 6.41 (d, $J = 2.8$ Hz, 1 H), 4.79 (s, 2 H, H-7), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.69 (t, $J = 6.2$ Hz, 2 H, H-12), 2.85 (t, $J = 7.2$ Hz, 3 H, H-10 and OH), 2.02 (br s, 1 H, OH), 1.79 (tt, $J = 7.2$, 6.0 Hz, 2 H, H-11); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 161.6 (C), 161.3 (C), 147.0 (C), 107.7 9C), 105.4 (CH), 98.2 (CH), 66.3 (CH$_2$, C-7), 62.4 (CH$_2$, C-12), 56.3 (CH$_3$), 55.5 (CH$_3$), 32.7 (CH$_2$, C-10), 24.6 (CH$_2$, C-11); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 109.3; mass spectrum $m/z$ (EI, relative intensity) 306 (100, M$^+$), 230 (32), 166 (96), 138 (66); exact mass calcd. for C$_{12}$H$_{18}$O$_4$Se: 306.0370; found: 306.0367. Anal. calcd. for C$_{12}$H$_{18}$O$_5$Se: C, 47.22; H, 5.94; found: C, 47.25; H, 5.76.

7.3.54 Typical procedure for the preparation of (S)-2,3-dihydroxypropyl phenyl selenides: (S)-2,3-Dihydroxypropyl 2-(hydroxymethyl)phenyl selenide (191)

Diselenide 130a (335 mg, 0.901 mmol) was dissolved in 20 mL of 3:1 THF-Ethanol and cooled to 0 °C. Sodium borohydride (239 mg, 6.32 mmol) was added and after 5 minutes (S)-(−)-glycidol (0.240 mL, 267 mg, 3.60 mmol) was added. The mixture was warmed to room temperature, left for an additional 2 hours and quenched with 20 mL of water. The mixture was extracted with ethyl acetate, washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (100 % ethyl acetate) to afford 302 mg (64%) of the product as a
colourless solid, mp 36-37 °C (from ethyl acetate); \([\alpha]_D^{20}\) 20.5 (c 2.37, MeOH); IR (solid) 3233, 2910, 1719, 1057 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.58 (dd, \(J = 7.6, 0.80\) Hz, 1 H), 7.35 (dd, \(J = 7.4, 1.4\) Hz, 1 H), 7.26 (td, \(J = 7.4, 1.2\) Hz, 1 H), 7.20 (td, \(J = 7.4, 1.5\) Hz, 1 H), 4.80 (d, \(J = 12.4\) Hz, 1 H, H-7a), 4.69 (d, \(J = 12.0\) Hz, 1 H, H-7b), 4.04 (br s, 1 H, OH), 3.68-3.62 (m, 2 H), 3.56 (d, \(J = 11.2\) Hz, 1 H), 3.43 (dd, \(J = 11.2, 6.4\) Hz, 1 H), 3.22 (br s, 1 H, OH), 2.98 (dd, \(J = 12.8, 4.4\) Hz, 1 H, H-8a), 2.84 (dd, \(J = 12.8, 8.4\) Hz, 1 H, H-8b); \(^13\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 143.0 (C), 135.4 (CH), 129.8 (C), 129.5 (CH), 129.0 (CH), 128.4 (CH), 70.8 (CH, C-9), 65.8 (CH\(_2\)), 65.7 (CH\(_2\)), 32.6 (CH\(_2\), C-8); \(^77\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 196.3; mass spectrum \(m/z\) (EI, relative intensity) 262 (42, M\(^+\)), 185 (64), 170 (62), 107 (100), 105 (66), 91 (48), 78 (89); exact mass calcd. for C\(_{10}\)H\(_{14}\)O\(_3\)Se: 262.0108; found: 262.0108. Anal. calcd. for C\(_{10}\)H\(_{14}\)O\(_3\)Se: C, 45.99; H, 5.40; found: C, 45.82; H, 5.18.

**7.3.55 (S)-2,3-Dihydroxypropyl 4-methoxy-2-(hydroxymethyl)phenyl selenide (192)**

[Chemical structure diagram]

Prepared according to the same procedure as for 191. Yield: 64%. Colourless solid; 51-52 °C (from ethyl acetate); \([\alpha]_D^{20}\) 25.6 (c 1.25, MeOH); IR (solid) 3243, 2914, 1595, 1471, 1286, 1219, 1038; \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.54 (d, \(J = 8.4\) Hz, 1 H, H-6), 6.96 (d, \(J = 2.8\) Hz, 1 H, H-3), 6.76 (dd, \(J = 8.4, 2.8\) Hz, 1 H, H-5), 4.81 (d, \(J = 11.6\) Hz, 1 H, H-7a), 4.67 (d, \(J = 12.0\) Hz, 1 H, H-7b), 3.89 (br s, 1 H, OH), 3.79 (s, 3 H, H-8), 3.61-3.56 (m, 3 H), 3.45-3.43 (m, 1 H), 3.00 (br s, 1 H, OH), 2.91 (dd, \(J = 12.6, 4.2\) Hz, 1
H, H-9a), 2.76 (dd, J = 12.8, 8.4 Hz, 1 H, H-9b); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 160.3, 145.2, 138.3, 119.2, 115.1, 114.6, 70.7 (C-10), 66.0, 65.8, 55.5, 33.3 (C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 177.5; mass spectrum m/z (EI, relative intensity) 292 (100, M$^+$), 200 (42), 137 (58), 108 (84); exact mass calcd. for C$_{11}$H$_{16}$O$_4$Se: 292.0214; found: 292.0209.

Anal. calcd. for C$_{11}$H$_{16}$O$_4$Se: C, 45.37; H, 5.54; found: C, 45.59; H, 5.38.

7.3.56 (S)-2,3-Dihydroxypropyl 5-methoxy-2-(hydroxymethyl)phenyl selenide (193)

Prepared according to the same procedure as for 191. Yield: 74%. Colourless solid; mp 78-79 °C (ethyl acetate-methanol); [α]$_D^{20}$ 25.0 (c 5.37, MeOH); IR (solid) 3269, 2914, 1589, 1479, 1247, 1018 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 7.30 (d, J = 8.4 Hz, 1 H, H-3), 7.18 (d, J = 2.4 Hz, 1 H, H-6), 6.82 (dd, J = 8.4, 2.4 Hz, 1 H, H-4), 4.82 (d, J = 12.0 Hz, 1 H, H-7a), 4.72 (d, J = 12.0 Hz, 1 H, H-7b), 3.81 (s, 3 H, H-8), 3.75-3.67 (m, 1 H), 3.57-3.51 (m, 1 H), 3.22 (br s, 1 H, OH), 3.09 (dd, J = 13.2, 4.0 Hz, 1 H, H-9a), 2.93 (dd, J = 12.8, 8.4 Hz, 1 H, H-9b), 2.35 (br s, 1 H, OH), 2.03 (br s, 1 H, OH); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 159.6 (C), 135.2 (C), 131.1 (C), 130.7 (CH), 121.0 (CH), 113.4 (CH), 70.8 (CH, C-10), 65.8 (CH$_2$), 65.2 (CH$_2$), 55.6 (CH$_3$, C-8), 32.8 (CH$_2$, C-9); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 194.8; mass spectrum m/z (EI, relative intensity) 292 (74, M$^+$), 217 (64), 137 (100), 121 (56), 108 (70); exact mass calcd. for C$_{11}$H$_{16}$O$_4$Se: 292.0214; found: 292.0227. Anal. calcd. for C$_{11}$H$_{16}$O$_4$Se: C, 45.37; H, 5.54; found: C, 45.30; H, 5.54.
7.3.57 (S)-2,3-Dihydroxypropyl 6-methoxy-2-(hydroxymethyl)phenyl selenide (194)

Prepared according to the same procedure as for 191. Yield: 84%. Colourless solid; mp 64-65 °C; \([\alpha]_{D}^{20} 38.3 \text{ (c 3.53, MeOH)}\); IR (solid) 3261, 2923, 1568, 1454, 1253, 1038 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta\) 7.30 (t, \(J = 8.0\) Hz, 1 H, H-4), 7.01 (d, \(J = 7.2\) Hz, 1 H), 6.85 (d, \(J = 8.0\) Hz, 1 H), 4.90 (dd, \(J = 11.8, 3.4\) Hz, 1 H, H-7a), 4.70 (dd, \(J = 11.8, 4.2\) Hz, 1 H, H-7b), 4.17 (br s, 1 H, OH), 3.89 (s, 3 H, H-8), 3.73 (br s, 1 H, OH), 3.54-3.51 (m, 2 H), 3.43-3.40 (m, 1 H), 3.06 (dd, \(J = 12.8, 4.8\) Hz, 1 H), 2.97 (br s, 1 H, OH), 2.69 (dd, \(J = 12.8, 9.2\) Hz, 1 H); \(^{13}\)C NMR (101 MHz; CDCl\(_3\)) \(\delta\) 160.3 (C), 146.0 (C), 130.4 (CH), 122.1 (CH), 116.8 (C), 110.9 (CH), 70.6 (CH, C-10), 66.2 (CH\(_2\)), 66.0 (CH\(_2\)), 56.4 (CH\(_3\), C-8), 31.6 (CH\(_2\), C-9); \(^{77}\)Se NMR (76 MHz; CDCl\(_3\)) \(\delta\) 105.5; mass spectrum \(m/z\) (relative intensity) 292 (55, M\(^+\)), 215 (24), 200 (38), 169 (12), 137 (100), 108 (52); exact mass calcd. for C\(_{11}\)H\(_{16}\)O\(_4\)\(^{79}\)Se: 292.0214; found: 292.0219. Anal. calcd. for C\(_{11}\)H\(_{16}\)O\(_4\)Se: C, 45.37; H, 5.54; found: C, 45.29; H, 5.07.

7.3.58 (S)-2,3-Dihydroxypropyl 4,5-dimethoxy-2-(hydroxymethyl)phenyl selenide (205)

Prepared according to the same procedure as for 191. Yield: 95%. Colourless solid; mp 74-75 °C (from ethyl acetate); \([\alpha]_{D}^{20} 25.4 \text{ (c 3.51, MeOH)}\); IR (solid) 3406,
3243, 2931, 1499, 1260, 1018; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 7.13 (s, 1 H), 6.92 (s, 1 H), 4.80 (d, $J$ = 12.0 Hz, 1 H, H-7a), 4.68 (d, $J$ = 11.6 Hz, 1 H, H-7b), 4.02 (br s, 1 H, OH), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.68-3.61 (m, 2 H), 3.57 (dd, $J$ = 11.2, 3.2 Hz, 1 H, H-12a), 3.44 (dd, $J$ = 11.4, 6.6 Hz, 1 H, H-12b), 3.11 (br s, 1 H, OH), 2.94 (dd, $J$ = 12.8, 4.0 Hz, 1 H, H-10a), 2.79 (dd, $J$ = 12.8, 8.4 Hz, 1 H, H-10b); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 149.7 (C), 148.9 (C), 136.8 (C), 119.7 (CH), 119.5 (C), 112.7 (CH), 70.9 (CH, C-11), 65.9 (CH$_2$), 65.8 (CH$_2$), 56.3 (CH$_3$), 56.1 (CH$_3$), 33.5 (CH$_2$, C-10); $^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 196.2; mass spectrum $m/z$ (EI, relative intensity) 322 (100, M$^+$), 246 (36), 167 (62), 151 (24), 138 (78); exact mass calcd. for C$_{12}$H$_{18}$O$_5$Se: 322.0319; found: 322.0311. Anal. calcd. for C$_{12}$H$_{18}$O$_5$Se: C, 44.87; H, 5.65; found: C, 45.13; H, 5.69.

7.3.59 (S)-2,3-Dihydroxypropyl 4,5,6,trimethoxy-2-((methoxymethoxy)methyl)phenyl selenide (213)

Prepared according to the same procedure as for 191. Yield: 88%. Colourless oil; IR (neat) 3433, 2933, 1576, 1476, 1333 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 6.85 (s, 1 H, H-3), 4.83 (d, $J$ = 11.6 Hz, 1 H, H-7a), 4.72 (s, 2 H, H-8), 4.72 (d, $J$ = 11.6 Hz, 1 H, H-7b), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.60 (dd, $J$ = 10.6, 3.4 Hz, 1 H, H-15a), 3.57-3.51 (m, 1 H, H-14), 3.47 (dd, $J$ = 10.6, 6.2 Hz, 1 H, H-15b) 3.43 (s, 3 H, H-9), 3.01 (dd, $J$ = 12.4, 3.2 Hz, 1 H, H-13a), 2.68 (dd, $J$ = 12.4, 8.8 Hz, 1 H, H-13b); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 155.2, 154.5, 142.1, 137.7, 114.3, 109.2, 96.0 (C-8), 70.4, 70.2,
65.9, 61.5, 61.1, 56.2, 55.8, 3 (C-13); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 103.2; mass spectrum m/z (EI, relative intensity) 396 (95, M$^+$), 289 (35), 275 (100), 181 (70), 167 (32); exact mass calcd. for C$_{15}$H$_{24}$O$_7$Se: 396.0687; found: 396.0685.

7.3.60 Preparation of (S)-2,3-dihydroxypropyl 4,5,6-trimethoxy-2-(hydroxymethyl)phenyl selenide (215)

2,3-Dihydroxypropyl selenide 213 (163 mg, 0.412 mmol) was dissolved in 8 mL of methanol along with 6 drops of concentrated HCl. The mixture was heated to 60 ℃ and left for 5 hours. The mixture was poured into 10 mL of water, extracted with ethyl acetate, washed with brine, dried (MgSO$_4$) and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (ethyl acetate-methanol) to afford 97 mg (67%) of the deprotected product as a colourless solid, mp 54-55 ℃ (from ethyl acetate-methanol); $[\alpha]_D^{20}$ 31.8 (c 2.10, MeOH); IR (solid) 3276, 2930, 1589, 1463, 1383, 1315, 1108, 1005 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) δ 6.82 (s, 1 H, H-3), 4.86 (d, $J = 12.4$ Hz, 1 H, H-7a), 4.67 (d, $J = 12.0$ Hz, 1 H, H-7b), 4.10 (br s, 1 H, OH), 3.92 (s, 3 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.57-3.55 (m, 3 H), 3.46-3.43 (m, 1 H), 3.02 (dd, $J = 12.4$, 3.2 Hz, 1 H, H-11a), 2.83 (br s, 1 H, OH), 2.67 (dd, $J = 12.6$, 9.0 Hz, 1 Hz, H-11b); $^{13}$C NMR (101 MHz; CDCl$_3$) δ 155.3, 154.6, 142.0, 140.6, 113.6, 108.9, 70.6 (C-12), 66.2, 65.9, 61.5, 61.1, 56.2, 32.6 (C-11); $^{77}$Se NMR (76 MHz; CDCl$_3$) δ 103.5; mass spectrum m/z (EI, relative intensity) 352 (100, M$^+$), 277 (12), 260 (24), 196 (62), 168 (91), 153 (28);
exact mass calcd for $\text{C}_{13}\text{H}_{20}\text{O}_{6}^{80}\text{Se}$: 352.0425; found: 352.0440. Anal. calcd. for $\text{C}_{13}\text{H}_{20}\text{O}_{6}\text{Se}$: C, 44.45; H, 5.74; found: C, 44.49; H, 5.86.

7.3.61 (S)-2,3-Dihydroxypropyl 4,6-dimethoxy-2-(hydroxymethyl)phenyl selenide (227)

Prepared according to the same procedure as for 191. Yield: 33%. Colourless solid; mp 77-78 °C; $[\alpha]_{D}^{20}$ 36.0 (c 0.96, MeOH); IR (solid) 3402, 3312, 2903, 1586, 1428, 1329, 1203, 1171, 1068 cm$^{-1}$; $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 6.61 (d, $J = 2.8$ Hz, 1 H), 6.40 (d, $J = 2.4$ Hz, 1 H), 4.86 (d, $J = 12.4$ Hz, 1 H, H-7a), 4.65 (d, $J = 12.4$ Hz, 1 H, H-7b), 4.22 (br s, 1 H, OH), 3.91 (br s, 1 H, OH), 3.85 (s, 3 H), 3.80 (s, 3 H), 3.54-3.47 (m, 2 H), 3.39 (dd, $J = 10.6$, 6.2 Hz, 1 H), 3.18 (br s, 1 H, OH), 2.95 (dd, $J = 12.6$, 3.4 Hz, 1 H, H-10a), 2.61 (dd, $J = 9.0$, 12.6 Hz, 1 H, H-10b); $^{13}$C NMR (101 MHz; CDCl$_3$) $\delta$ 161.8 (C), 161.4 (C), 147.0 (C), 107.3 (C), 106.3 (CH), 98.4 (CH), 70.6 (CH, C-11), 66.3 (CH$_2$), 65.9 (CH$_2$), 56.4 (CH$_3$), 55.6 (CH$_3$), 31.7 (CH$_2$, C-10); $^{77}$Se NMR (76 MHz; CDCl$_3$) $\delta$ 74.8; mass spectrum $m/z$ (EI, relative intensity) 322 (64, M$^+$), 230 (32), 166 (100), 138 (56); exact mass calcd. for $\text{C}_{12}\text{H}_{18}\text{O}_{5}^{80}\text{Se}$: 322.0319; found: 322.0321. Anal. calcd. for $\text{C}_{12}\text{H}_{18}\text{O}_{5}\text{Se}$: C, 44.87; H, 5.65; found: C, 44.98; H, 5.64. NOTE: 57 mg of the symmetrical aryl selenide 225 was also isolated as it was present in the starting material mixture.

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7.3.62 $^1$H NMR based assay for 2,3-dihydroxypropyl selenide 192

Glutathione (47.6 mg, 0.155 mmol) and the catalyst (4.5 mg, 0.0155 mmol) were dissolved and diluted to 5.0 mL with deuterium oxide. The $^1$H NMR spectrum was recorded prior to adding 33.1% $\text{H}_2\text{O}_2$ (0.018 mL, 0.175 mmol). After the hydrogen peroxide addition, $^1$H NMR spectra were run every 2 minutes starting at 8 minutes and continuing until 34 minutes after addition of $\text{H}_2\text{O}_2$. After 158 minutes the $^1$H NMR spectrum was recorded again and no glutathione was observed to be present.

7.4 Experiments Related to Chapter Four

7.4.1 General Comments

Spirodioxyselenuranes 110 and 134-137 were prepared as described previously.$^{145}$ Cyclic seleninate ester 108 was prepared as described in Subsection 7.2. Variable temperature proton NMR experiments were carried out at 300 MHz on a spectrometer equipped with a compressed gas heat exchanger for higher temperatures and a liquid nitrogen evaporator for low temperatures. Temperature calibration of the controller was carried out using the temperature dependent chemical shifts of methanol (low temperature) and ethylene glycol (high temperature).$^{210}$ Samples were prepared by dissolving 0.018 mmol of the compound in 0.5 mL of deuterated solvent. Complete variable temperature proton NMR spectra of 110 and 134-137 in toluene-$d_8$ with expansions near convergence are contained in Appendix A, along with similar spectra for 110 in pyridine-$d_5$, DMSO-$d_6$ and 25% $\text{D}_2\text{O}:\text{DMSO-}d_6$. 

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7.5 Experiments Related to Chapter Five

7.5.1 Preparation of Naphtho[1,8-cd]-diselenole (265)

The general procedure of Meinwald and co-workers was followed.\textsuperscript{169} A solution of 1,8-dibromonaphthalene (1.24 g, 4.34 mmol) in 150 mL of dry THF was cooled to -78°C under a nitrogen atmosphere. \textit{n}-Butyllithium (4.9 mL, 2.5 M, 12 mmol) was added and the mixture was warmed to room temperature over 1.5 h. The mixture was cooled to 0°C, elemental selenium (960 mg, 12.2 mmol) was quickly introduced and the nitrogen atmosphere was restored. The mixture was warmed to room temperature, stirred for an additional 3 h and quenched with saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min., the mixture was washed with brine, dried (MgSO\textsubscript{4}) and concentrated under reduced pressure. The resulting crude solid was dissolved in dichloromethane, adsorbed on silica and purified by flash chromatography (hexanes:ethyl acetate, 4:1) to afford 234 mg (19\%) of diselenide 3 as a purple solid, mp 123-124°C (from ethyl acetate); lit.\textsuperscript{169} mp 127-129 °C; \textit{\textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}) \& 8.42 (dd, \textit{J} = 7.3, 1.3 Hz, 2 H), 7.83 (dd, \textit{J} = 8.3, 1.2 Hz, 2 H), 7.06 (dd, \textit{J} = 8.0, 7.3 Hz, 2 H, H-3); \textit{\textsuperscript{13}C NMR (101 MHz; CDCl\textsubscript{3}) \& 144.2, 135.9, 132.3, 131.1, 127.1, 96.1; \textit{\textsuperscript{77}Se NMR (76 MHz; CDCl\textsubscript{3}) \& 418.7; mass spectrum \textit{m/z} (EI, relative intensity) 286 (100, M\textsuperscript{+}), 206 (25), 126 (35); exact mass calcd. for C\textsubscript{10}H\textsubscript{6}\textsuperscript{80}Se\textsubscript{2}: 285.8800; found: 285.8788; Anal. calcd. for C\textsubscript{10}H\textsubscript{6}Se\textsubscript{2}: C, 42.28; H, 2.13; found: C, 42.17; H, 2.11.
7.5.2 Preparation of 1,8-dibromo-2,7-dimethoxynaphthalene (269)

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\text{Br} \\
\text{Br} \\
\text{O} \\
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The general bromination procedure of Whiting et al.\textsuperscript{[170]} was followed. NBS (8.16 g, 45.8 mmol) and pyridine (2.5 mL, 2.4 g, 30 mmol) were dissolved in 70 mL of chloroform. The solution was refluxed under a nitrogen atmosphere for 1 h. A solution of 2,7-dimethoxynaphthalene (2.16 g, 11.5 mmol) in 12.5 mL of chloroform was added and refluxing was continued for an additional 9 h. After cooling to room temperature the mixture was immediately adsorbed on silica gel. Flash chromatography (hexanes:ethyl acetate, 6:1) afforded 1.88 g (47\%) of the product as a pale yellow solid, mp 133-134 °C (from hexanes); IR (KBr) 1610, 1500, 1262, 1052, 819 cm\(^{-1}\); \(^1\)H NMR (400 MHz; CDCl\(_3\)) \(\delta 7.68 (d, J = 9.1 \text{ Hz}, 2 \text{ H, } H-4), 7.10 (d, J = 9.0 \text{ Hz}, 2 \text{ H, } H-3), 3.98 \text{ (s, 6 H, } H-7)\); \(^13\)C NMR (101 MHz; CDCl\(_3\)) \(\delta 156.5, 131.7, 130.2, 127.5, 111.7, 106.0, 57.3 \text{ (C-7)}\); mass spectrum, \(m/z\) (EI, relative intensity) 346 (100, M\(^+\)), 303 (45), 237 (20), 158 (20); exact mass calcd. for C\(_{12}\)H\(_{10}\)Br\(_2\)O\(_2\): 345.9027; found: 345.9026. Anal. calcd. for C\(_{12}\)H\(_{10}\)Br\(_2\)O\(_2\): C, 41.65; H, 2.91; found: C, 41.76; H, 2.78.

7.5.3 Preparation of 2,7-dimethoxynaphtho[1,8-cd]-1,2-selenole (266)

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\begin{array}{c}
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\text{Se} \\
\text{Se} \\
\text{O} \\
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\]

A solution of 1,8-dibromo-2,7-dimethoxynaphthalene (1.50 g, 4.34 mmol) in 200 mL of dry THF was cooled to -78 °C under a nitrogen atmosphere. \(n\)-Butyllithium (5.5
mL, 2.2 M, 12 mmol) was added and the mixture was warmed to room temperature over 1.5 h. The mixture was cooled to 0 °C, elemental selenium (960 mg, 12 mmol) was quickly introduced and the nitrogen atmosphere was restored. The mixture was warmed to room temperature, stirred for an additional 3 h and quenched with saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min, the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting crude solid was dissolved in dichloromethane, adsorbed on silica gel and purified by flash chromatography (hexanes:ethyl acetate, 4:1) to afford 950 mg (64%) of the product as a purple solid, mp 155-156 °C (from ethyl acetate); IR (KBr) 1490, 1262, 1057, 810 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.55 (d, J = 8.8 Hz, 2 H, H-3), 6.97 (d, J = 8.8 Hz, 2 H, H-4), 3.97 (s, 6 H, H-7); ¹³C NMR (101 MHz; CDCl₃) δ 153.3, 140.1, 128.1, 125.8, 123.0, 111.9, 56.6 (C-7); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 407.9; mass spectrum, m/z (EI, relative intensity) 346 (100, M⁺), 331 (90), 316 (35); exact mass calcd. for C₁₂H₁₀O₂₈₀Se₂: 345.9011; found: 345.9000. Anal. calcd. for C₁₂H₁₀O₂Se₂: C, 41.88; H, 2.93; found: C, 41.80; H, 2.93. UV-Vis abs: λ_max = 383 nm (ε = 10,625 M⁻¹cm⁻¹), λ_max = 527 nm (ε = 95 M⁻¹cm⁻¹).

7.5.4 Preparation of 2,7-dimethoxynaphtho[1,8-cd]-1,2-ditellurole (270)

A solution of 1,8-dibromo-2,7-dimethoxynaphthalene (350 mg, 1.01 mmol) in 45 mL of dry THF was cooled to -78°C under a nitrogen atmosphere and n-butyllithium
(1.25 mL, 2.25 M, 2.81 mmol) was added. The mixture was warmed to room temperature over 1.5 h and again cooled to 0 ºC. Elemental tellurium (357 mg, 2.80 mmol) was quickly introduced and the nitrogen atmosphere was restored. The mixture was warmed to room temperature, stirred for an additional 3 h and quenched with saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min and the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting red crude mixture was dissolved in dichloromethane, adsorbed on silica-gel and purified by flash chromatography (hexanes:ethyl acetate, 4:1) to afford 226 mg (51%) of ditelluride 270 as a green solid, mp 210-211 ºC (from ethyl acetate); IR (KBr) 1605, 1495, 1257, 1057, 810 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 7.71 (d, J = 8.9 Hz, 2 H, H-3), 6.93 (d, J = 8.9 Hz, 2 H, H-4), 3.99 (s, 6 H, H-7); ¹³C NMR (101 MHz; CDCl₃) δ 158.0, 146.7, 130.3, 128.7, 110.6, 106.8, 56.7 (C-7); ¹²⁵Te NMR (126 MHz; CDCl₃) δ 247.1; mass spectrum, m/z (EI, relative intensity) 442 (100, M⁺), 427 (35); exact mass calcd. for C₁₂H₁₀O₂Te₂: 441.8770; found: 441.8760. Anal. calcd. for C₁₂H₁₀O₂Te₂: C, 32.65; H, 2.28; found C, 32.64; H, 2.01. UV-Vis abs: λ_max = 402 nm (ε = 10 539 M⁻¹cm⁻¹), λ_max = 653 nm (ε = 153 M⁻¹cm⁻¹).

7.5.5 Preparation of di-(2-methoxyphenyl) diselenide (271)

Diselenide 271 was prepared according to the method of Boyd. Sodium nitrite (0.65 g, 9.5 mmol) in 5 mL of water was added dropwise to a stirred solution containing
2-methoxyaniline (1.0 g, 8.1 mmol) and 5 mL of concentrated hydrochloric acid in 13 mL of water at 0 °C. The solution of the resulting diazonium salt was stirred for 20 minutes while a solution of potassium diselenide was prepared separately in the following manner. Elemental selenium (1.3 g, 16 mmol) and potassium hydroxide (3.7 g, 65 mmol) were mixed together and melted in a round bottomed flask with a heat gun. Cold water (11 mL) was added to the hot melt to produce a red aqueous solution of potassium diselenide, which was subsequently cooled to 0 °C. The pH of the diazonium salt solution was adjusted to neutral with 2 M NaOH and this was added to the potassium diselenide solution. The resulting mixture was stirred at 0 °C for 30 minutes, then heated with a heat gun until the initially red, opaque solution became transparent. The mixture was filtered to remove elemental selenium and the filtrate was extracted with ethyl acetate, washed with brine, dried (MgSO₄), concentrated and purified by chromatography (hexanes-ethyl acetate, 6:1) to afford 0.42 g (27%) of diselenide 271 mp 85-86 °C, lit mp 83-84 °C, ¹H NMR (400 MHz; CDCl₃) δ 7.58 (d, J = 7.3 Hz, 2 H), 7.22 (t, J = 7.3 Hz, 2 H), 6.89 (t, J = 7.3 Hz, 2 H), 6.83 (d, J = 7.9 Hz, 2 H), 3.91 (s, 6 H, H-7); ¹³C NMR (101 MHz; CDCl₃) δ 157.0, 130.7, 128.3, 122.0, 118.8, 110.3, 56.0 (C-7); ⁷⁷Se NMR (76 MHz; CDCl₃) δ 332.5; mass spectrum m/z (EI, relative intensity) 374 (100), 107 (21).

7.5.6 Preparation of 2,7-dimethoxy[1,8-cd]-1,2-diselenole Se-oxide (276)
2,7-Dimethoxynaphtho[1,8-cd]-1,2-diselenole (334 mg, 0.971 mmol) was dissolved in dichloromethane (10 mL) and MCPBA (173 mg, 1.00 mmol) was added. After 30 min the reaction mixture was washed with saturated sodium bicarbonate solution, dried (MgSO₄) and concentrated. Traces of residual diselenide were removed by trituration of the resulting solid with ethyl acetate until the washings were no longer purple, leaving the selenolseleninate as an orange solid (256 mg, 73%); mp 95 ºC (dec). IR (KBr) 1614, 1505, 1257, 1048, 819 cm⁻¹; ¹H NMR (400 MHz; CDCl₃) δ 8.03 (d, J = 8.9 Hz, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.24 (d, J = 8.9 Hz, 1 H), 7.19 (d, J = 8.9 Hz, 1 H), 4.10 (s, 3 H), 4.02 (s, 3 H); ¹³C NMR (101 MHz; CDCl₃) δ 159.8, 155.8, 137.4, 135.2, 132.5, 128.8, 126.2, 117.6, 112.4, 111.5, 57.2, 56.8; ⁷⁷Se NMR (76 MHz; CDCl₃) δ 1170.9, 785.5; mass spectrum m/z (ESI) 385 ((M + Na)⁺); exact mass calcd. for C₁₂H₁₀O₃⁸0Se₂ 362.9036; found 362.9026.

**7.5.7 Preparation of cyclic seleninic anhydride (279)**

Selenolseleninate (52 mg, 0.14 mmol) was dissolved in 5 mL of toluene and MCPBA (50 mg, 0.29 mmol) was added, resulting in the disappearance of the orange color and precipitation of 279. After 30 min, the precipitate was filtered and washed with toluene to leave 51 mg (90%) of the product as a white powder, mp 119 ºC (decomposition with appearance of red color) and 284-286 ºC (extensive further decomposition); IR (KBr) 1614, 1500, 1267, 1033, 895 cm⁻¹; ¹H NMR (400 MHz,
DMSO-$d_6$) major isomer: δ 8.35 (d, $J = 9.2$ Hz, 2 H), 7.62 (d, $J = 9.2$ Hz, 2 H), 4.08 (s, 6 H); minor isomer: δ 8.30 (d, $J = 9.2$ Hz, 2 H), 7.58 (d, $J = 9.2$ Hz, 2 H), 4.04 (s, 6 H); integration of the signals at δ 4.08 and 4.04 indicated a ratio of 1.22:1.00; $^{13}$C NMR (101 MHz, DMSO-$d_6$) both isomers: δ 159.09, 159.05, 136.0 (2 C), 125.0, 124.9, 124.7, 124.2, 123.9, 122.7, 112.7, 112.2, 58.0, 57.9; $^{77}$Se NMR (76 MHz, DMSO-$d_6$) both isomers: δ 1259.3, 1248.9; mass spectrum m/z (ESI) 417 (M + Na)$^+$; exact mass calcd. for C$_{12}$H$_{10}$O$_5$Se$_2$: 394.89343, found: 394.89344.

7.5.8 Preparation of dipotassium bis(seleninate) (280)

A solution of excess KOH (8 equiv) in D$_2$O was added to a solution of the cyclic seleninic anhydride 279 in DMSO-$d_6$. The NMR spectra revealed the presence of a single compound: $^1$H NMR (400 MHz) δ 7.80 (d, $J = 9.2$ Hz, 2 H), 7.18 (d, $J = 9.2$ Hz, 2 H), 3.84 (s, 6 H, H-7); $^{13}$C NMR (101 MHz) δ 160.1, 134.0, 133.4, 129.2, 125.1, 112.7, 57.1 (C-7); $^{77}$Se NMR (76 MHz) δ 1188.1.

7.5.9 Preparation of 2,7-dimethoxynaphtho[1,8-cd]-1,2-dithiole (283)

A solution of 1,8-dibromo-2,7-dimethoxynaphthalene (700 mg, 2.02 mmol) in 90 mL of dry THF was cooled to -78 °C under a nitrogen atmosphere. n-Butyllithium (2.6
mL, 2.2 M, 5.7 mmol) was added and the mixture was warmed to room temperature over 1.5 h. The mixture was cooled to 0 °C, elemental sulfur (181 mg, 5.64 mmol) was quickly introduced and the nitrogen atmosphere was restored. The mixture was warmed to room temperature, left for an additional 10 h and then quenched with saturated ammonium chloride solution. Air was rapidly bubbled through the mixture for 30 min., the mixture was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The resulting crude solid was dissolved in dichloromethane, adsorbed to silica gel and purified by flash chromatography (hexanes:ethyl acetate, 4:1) to afford 250 mg (50%) of disulfide 283 as a red solid, mp 116-117 °C (from hexanes:ethyl acetate 1:1); IR (KBr) 2957, 2933, 2833, 1619, 1505, 1419, 1262, 1057, 805; ¹H NMR (400 MHz; CDCl₃) δ 7.37 (d, J = 8.9 Hz, 2 H), 6.98 (d, J = 8.9 Hz, 2 H), 3.95 (s, 6 H, H-7); ¹³C NMR (101 MHz; CDCl₃) δ 150.1, 137.7, 126.34, 126.25, 123.6, 112.7, 56.56 (C-7); mass spectrum, m/z (relative intensity) 250 (85, M⁺), 235 (100), 220 (45), 192 (15), 164 (15); exact mass calcd. for C₁₂H₁₀O₂S₂: 250.0122; found: 250.0125. Anal. calcd. for C₁₂H₁₀O₂S₂: C, 57.58; H, 4.03; found: C, 58.08; H, 4.02. UV-Vis abs: λₘₐₓ = 378 nm (ε = 9477 M⁻¹cm⁻¹).

7.5.10 General procedure for absorption spectra of CT-complexes

Absorption spectra were recorded in CH₃CN solutions thermo-regulated to 25 °C with a total concentration of [D] + [TCNQ] equal to 5.0 x 10⁻³ M over a range of ratios of [D]:[TCNQ] 10 : 0 to 0 : 10 using a Cary 5000 UV-vis-NIR spectrophotometer. The resulting combined spectra for each donor-TCNQ complex (compounds 265, 266, 270 and 283) are found in Appendix B.
References


Appendix A: Variable-Temperature $^1$H NMR Spectra of Spirodiyneselenuranes

Full cycle (223 K ↔ 372 K) of variable-temperature NMR spectra of 110 in toluene-$d_8$
Low-temperature NMR spectra of 110 in toluene-$d_8$
Convergence of AB quartet of 110 in toluene-$d_8$
High-temperature NMR spectra of 110 in toluene-$d_8$
High-temperature reappearance of AB quartet of 110 in toluene-$d_8$
Variable-temperature NMR spectra of 110 in pyridine-$d_5$
Convergence of AB quartet of 110 in pyridine-$d_5$
Variable-temperature NMR spectra of 110 in DMSO-$d_6$
Convergence of AB quartet of 110 in DMSO-$d_6$
Variable-temperature NMR spectra of 110 in 25% D$_2$O:DMSO-$d_6$
Convergence of AB quartet for 110 in 25% D$_2$O:DMSO-d$_6$
Low-temperature NMR spectra of 134 in toluene-$d_8$
Convergence of AB quartet of 134 in toluene-$d_8$
High-temperature NMR spectra of 134 in toluene-$d_8$
High-temperature reappearance of AB quartet of 134 in toluene-$d_8$
Variable-temperature NMR spectra of 135 in toluene-$d_8$
Convergence of AB quartet of 135 in toluene-$d_8$
Variable-temperature NMR spectra of 136 in toluene-$d_8$
Convergence of AB quartet of 136 in toluene-$d_8$
Variable-temperature NMR of 137 in toluene-$d_8$
Convergence of AB quartet of 137 in toluene-d8
Appendix B: Absorption Spectra of Complexes 10D:0A to 0D:10A

Naphtho[1,8-cd]-1,2-diselenole (265) with TCNQ (concentration 5 mM)

2,7-Dimethoxynaphtho[1,8-cd]-1,2-dithiole (283) with TCNQ (concentration 5 mM)
2,7-Dimethoxynaphtho[1,8-cd]-1,2-diselenole (266) with TCNQ (concentration 5 mM)

2,7-Dimethoxynaphtho[1,8-cd]-1,2-ditellurole (270) with TCNQ (concentration 5 mM)
Appendix C: X-ray crystallographic data for 2,7-Dimethoxynaphtho[1,8-cd]-1,2-
diselenole (266)

ORTEP diagram of 266

And

Experimental:

A dark brown block crystal of C_{12} H_{10} O_2 Se_2 was coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber. All measurements were made on a Bruker APEX2
diffractometer with graphite monochromated Cu-Kα radiation. Details of crystal data and
structure refinement have been provided in Table 1. The data were collected\(^1\) using ω and
φ scans. The data were corrected for Lorentz and polarization effects and for absorption
using multi-scan method\(^1\).
The structure was solved by the direct methods\(^2\). The non-hydrogen atoms were refined anisotropically. An asymmetric unit contains two independent molecules. The second molecule, containing Se3 and Se4 atoms was disordered over two sites in a ratio 0.8968(14):0.1032(14). The H-atoms were included at geometrically idealized positions and were not refined. The final cycle of full-matrix least-squares refinement using SHELXL97\(^2\) converged with unweighted and weighted agreement factors, \(R = 0.0340\) and \(wR = 0.0985\) (all data), respectively, and goodness of fit, \(S = 1.234\). The weighting scheme was based on counting statistics and the final difference Fourier map was essentially featureless. The figures were plotted with the aid of ORTEP-3 for Windows\(^3\).

**References:**


**Table 1. Crystal data and structure refinement for C\(_{12}\)H\(_{10}\)O\(_2\)Se\(_2\).**

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Theta range for data collection  4.59 to 68.04°.  
Index ranges  -11<=h<=14, -14<=k<=13, -19<=l<=18  
Reflections collected  18979  
Independent reflections  4051 [R(int) = 0.0298]  
Completeness to theta = 68.04°  97.0 %  
Absorption correction  Multi-scan method  
Max. and min. transmission  0.6064 and 0.3631  
Refinement method  Full-matrix least-squares on F$^2$  
Data / restraints / parameters  4051 / 0 / 286  
Goodness-of-fit on F$^2$  1.234  
Final R indices [I>2sigma(I)]  R1 = 0.0340, wR2 = 0.0970  
R indices (all data)  R1 = 0.0367, wR2 = 0.0985  
Largest diff. peak and hole  0.564 and -0.552 e.Å$^{-3}$  

Table 2. Atomic coordinates (x 10$^4$) and equivalent isotropic displacement parameters (Å$^2$x 10$^3$) for C$_{12}$H$_{10}$O$_2$Se$_2$. U(eq) is defined as one third of the trace of the orthogonalized U$^{ij}$ tensor.

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Table 3. Bond lengths [Å] and angles [°] for C_{12}H_{10}O_{2}Se_{2}.

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C(10)-C(9)-C(8) 120.4(4)  
C(10)-C(9)-H(9) 119.8  
C(8)-C(9)-H(9) 119.8  
C(9)-C(10)-C(5) 120.7(4)  
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C(5)-C(10)-H(10) 119.7  
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O(1)-C(11)-H(11B) 109.5  
H(11A)-C(11)-H(11B) 109.5  
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H(11A)-C(11)-H(11C) 109.5  
H(11B)-C(11)-H(11C) 109.5  
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O(2)-C(12)-H(12B) 109.5  
H(12A)-C(12)-H(12B) 109.5  
O(2)-C(12)-H(12C) 109.5  
H(12A)-C(12)-H(12C) 109.5  
H(12B)-C(12)-H(12C) 109.5  
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C(19)-Se(4)-Se(3) 90.71(6)  
C(14)-O(3)-C(23) 117.6(3)  
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C(14')-C(13')-C(18') 120.0
C(14')-C(13')-Se(3') 118.7(9)
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O(3')-C(14')-C(13') 116.7(18)
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Table 4. Anisotropic displacement parameters ($\AA^2 \times 10^3$) for C_{12}H_{10}O_{2}Se_{2}.  
The anisotropic displacement factor exponent takes the form:  
$$ -2\pi^2 [ h^2 a^2 U^{11} + \cdots + 2 h k a^* b^* U^{12} ] $$  

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<td>32(2)</td>
<td>43(2)</td>
<td>36(2)</td>
<td>2(1)</td>
<td>-2(1)</td>
<td>-3(2)</td>
</tr>
<tr>
<td>O(4)</td>
<td>31(2)</td>
<td>39(2)</td>
<td>39(2)</td>
<td>1(1)</td>
<td>-3(1)</td>
<td>-1(1)</td>
</tr>
<tr>
<td>C(13)</td>
<td>30(2)</td>
<td>31(2)</td>
<td>24(2)</td>
<td>1(2)</td>
<td>11(2)</td>
<td>3(2)</td>
</tr>
<tr>
<td>C(14)</td>
<td>33(2)</td>
<td>37(3)</td>
<td>25(2)</td>
<td>-3(2)</td>
<td>9(2)</td>
<td>2(2)</td>
</tr>
<tr>
<td>C(15)</td>
<td>34(3)</td>
<td>41(3)</td>
<td>39(2)</td>
<td>-12(2)</td>
<td>15(2)</td>
<td>-9(2)</td>
</tr>
<tr>
<td>C(16)</td>
<td>33(3)</td>
<td>31(3)</td>
<td>73(5)</td>
<td>-26(3)</td>
<td>22(3)</td>
<td>-7(2)</td>
</tr>
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</table>
Table 5.  Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^2 x 10^3) for C_{12}H_{10}O_2Se_2.

<table>
<thead>
<tr>
<th>Atom</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>U(eq)</th>
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<tbody>
<tr>
<td>H(3)</td>
<td>343</td>
<td>8418</td>
<td>-755</td>
<td>44</td>
</tr>
<tr>
<td>H(4)</td>
<td>1855</td>
<td>9598</td>
<td>-302</td>
<td>44</td>
</tr>
<tr>
<td>H(9)</td>
<td>3559</td>
<td>11138</td>
<td>2334</td>
<td>44</td>
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<tr>
<td>H(10)</td>
<td>3084</td>
<td>10696</td>
<td>899</td>
<td>45</td>
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</table>
Table 6. Torsion angles [°] for C₁₂ H₁₀ O₂ Se₂.

<p>| | | | | |</p>
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<th></th>
</tr>
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<tbody>
<tr>
<td>C(1)-Se(1)-Se(2)-C(7)</td>
<td>-1.60(16)</td>
<td></td>
<td></td>
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<tr>
<td>Se(2)-Se(1)-C(1)-C(2)</td>
<td>177.6(3)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Se(2)-Se(1)-C(1)-C(6)</td>
<td>2.0(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(11)-O(1)-C(2)-C(1)</td>
<td>-172.3(4)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C(11)-O(1)-C(2)-C(3)</td>
<td>7.0(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

313
\begin{align*}
\text{C}(6)\text{-C}(1)\text{-C}(2)\text{-O}(1) & \quad 177.7(3) \\
\text{Se}(1)\text{-C}(1)\text{-C}(2)\text{-O}(1) & \quad 2.2(5) \\
\text{C}(6)\text{-C}(1)\text{-C}(2)\text{-C}(3) & \quad -1.6(6) \\
\text{Se}(1)\text{-C}(1)\text{-C}(2)\text{-C}(3) & \quad -177.1(3) \\
\text{O}(1)\text{-C}(2)\text{-C}(3)\text{-C}(4) & \quad -178.1(4) \\
\text{C}(1)\text{-C}(2)\text{-C}(3)\text{-C}(4) & \quad 1.2(6) \\
\text{C}(2)\text{-C}(3)\text{-C}(4)\text{-C}(5) & \quad -1.0(6) \\
\text{C}(3)\text{-C}(4)\text{-C}(5)\text{-C}(6) & \quad 1.2(6) \\
\text{C}(3)\text{-C}(4)\text{-C}(5)\text{-C}(10) & \quad 179.3(4) \\
\text{C}(2)\text{-C}(1)\text{-C}(6)\text{-C}(7) & \quad -177.2(4) \\
\text{Se}(1)\text{-C}(1)\text{-C}(6)\text{-C}(7) & \quad -1.5(5) \\
\text{C}(2)\text{-C}(1)\text{-C}(6)\text{-C}(5) & \quad 1.8(6) \\
\text{Se}(1)\text{-C}(1)\text{-C}(6)\text{-C}(5) & \quad 177.5(3) \\
\text{C}(10)\text{-C}(5)\text{-C}(6)\text{-C}(7) & \quad -0.8(6) \\
\text{C}(4)\text{-C}(5)\text{-C}(6)\text{-C}(7) & \quad 177.4(4) \\
\text{C}(10)\text{-C}(5)\text{-C}(6)\text{-C}(1) & \quad -179.7(4) \\
\text{C}(4)\text{-C}(5)\text{-C}(6)\text{-C}(1) & \quad -1.6(6) \\
\text{C}(1)\text{-C}(6)\text{-C}(7)\text{-C}(8) & \quad 179.0(4) \\
\text{C}(5)\text{-C}(6)\text{-C}(7)\text{-C}(8) & \quad 0.0(6) \\
\text{C}(1)\text{-C}(6)\text{-C}(7)\text{-Se}(2) & \quad -0.2(5) \\
\text{C}(5)\text{-C}(6)\text{-C}(7)\text{-Se}(2) & \quad -179.2(3) \\
\text{Se}(1)\text{-Se}(2)\text{-C}(7)\text{-C}(8) & \quad -177.8(3) \\
\text{Se}(1)\text{-Se}(2)\text{-C}(7)\text{-C}(6) & \quad 1.4(3) \\
\text{C}(12)\text{-O}(2)\text{-C}(8)\text{-C}(7) & \quad 176.9(4) \\
\text{C}(12)\text{-O}(2)\text{-C}(8)\text{-C}(9) & \quad -2.3(6) \\
\text{C}(6)\text{-C}(7)\text{-C}(8)\text{-O}(2) & \quad -178.5(3) \\
\text{Se}(2)\text{-C}(7)\text{-C}(8)\text{-O}(2) & \quad 0.7(5) \\
\text{C}(6)\text{-C}(7)\text{-C}(8)\text{-C}(9) & \quad 0.7(6) \\
\text{Se}(2)\text{-C}(7)\text{-C}(8)\text{-C}(9) & \quad 179.9(3) \\
\text{O}(2)\text{-C}(8)\text{-C}(9)\text{-C}(10) & \quad 178.5(4) \\
\text{C}(7)\text{-C}(8)\text{-C}(9)\text{-C}(10) & \quad -0.6(7) \\
\text{C}(8)\text{-C}(9)\text{-C}(10)\text{-C}(5) & \quad -0.2(7) \\
\text{C}(6)\text{-C}(5)\text{-C}(10)\text{-C}(9) & \quad 0.9(6) \\
\text{C}(4)\text{-C}(5)\text{-C}(10)\text{-C}(9) & \quad -177.2(4) 
\end{align*}
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<th>Bond Sequence</th>
<th>Distance (Å)</th>
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<tr>
<td>C(13)-Se(3)-Se(4)-C(19)</td>
<td>3.36(7)</td>
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<tr>
<td>Se(4)-Se(3)-C(13)-C(14)</td>
<td>178.22(10)</td>
</tr>
<tr>
<td>Se(4)-Se(3)-C(13)-C(18)</td>
<td>-3.53(12)</td>
</tr>
<tr>
<td>C(18)-C(13)-C(14)-C(15)</td>
<td>0.0</td>
</tr>
<tr>
<td>Se(3)-C(13)-C(14)-C(15)</td>
<td>178.2(2)</td>
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<tr>
<td>C(18)-C(13)-C(14)-O(3)</td>
<td>-175.5(2)</td>
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<td>Se(3)-C(13)-C(14)-O(3)</td>
<td>2.8(2)</td>
</tr>
<tr>
<td>C(23)-O(3)-C(14)-C(15)</td>
<td>5.1(5)</td>
</tr>
<tr>
<td>C(23)-O(3)-C(14)-C(13)</td>
<td>-179.7(3)</td>
</tr>
<tr>
<td>C(13)-C(14)-C(15)-C(16)</td>
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<tr>
<td>O(3)-C(14)-C(15)-C(16)</td>
<td>174.9(3)</td>
</tr>
<tr>
<td>C(14)-C(15)-C(16)-C(17)</td>
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</tr>
<tr>
<td>C(15)-C(16)-C(17)-C(18)</td>
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<tr>
<td>C(15)-C(16)-C(17)-C(22)</td>
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</tr>
<tr>
<td>C(16)-C(17)-C(18)-C(19)</td>
<td>180.0</td>
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<tr>
<td>C(22)-C(17)-C(18)-C(19)</td>
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<td>C(16)-C(17)-C(18)-C(13)</td>
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<tr>
<td>C(22)-C(17)-C(18)-C(13)</td>
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</tr>
<tr>
<td>C(14)-C(13)-C(18)-C(19)</td>
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<tr>
<td>Se(3)-C(13)-C(18)-C(19)</td>
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<td>Se(3)-C(13)-C(18)-C(17)</td>
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<td>C(13)-C(18)-C(19)-C(20)</td>
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<td>Se(3)-Se(4)-C(19)-C(20)</td>
<td>178.38(10)</td>
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<tr>
<td>Se(3)-Se(4)-C(19)-C(18)</td>
<td>-3.68(12)</td>
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<td>C(18)-C(19)-C(20)-C(21)</td>
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<td>177.9(2)</td>
</tr>
<tr>
<td>C(18)-C(19)-C(20)-O(4)</td>
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<td>Se(4)-C(19)-C(20)-O(4)</td>
<td>-2.1(2)</td>
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<tr>
<td>C(24)-O(4)-C(20)-C(19)</td>
<td>168.5(3)</td>
</tr>
<tr>
<td>C(24)-O(4)-C(20)-C(21)</td>
<td>-11.6(5)</td>
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C(19)-C(20)-C(21)-C(22)  0.0
O(4)-C(20)-C(21)-C(22)  -179.9(3)
C(20)-C(21)-C(22)-C(17)  0.0
C(16)-C(17)-C(22)-C(21)  180.0
C(18)-C(17)-C(22)-C(21)  0.0
C(13')-Se(3')-Se(4')-C(19')  1.8(7)
Se(4')-Se(3')-C(13')-C(14')  178.0(9)
Se(4')-Se(3')-C(13')-C(18')  -0.8(11)
C(18')-C(13')-C(14')-O(3')  179(2)
Se(3')-C(13')-C(14')-O(3')  0(2)
C(18')-C(13')-C(14')-C(15')  0.0
Se(3')-C(13')-C(14')-C(15')  -178.8(17)
O(3')-C(14')-C(15')-C(16')  -179(2)
C(13')-C(14')-C(15')-C(16')  0.0
C(14')-C(15')-C(16')-C(17')  0.0
C(15')-C(16')-C(17')-C(18')  0.0
C(15')-C(16')-C(17')-C(22')  180.0
C(16')-C(17')-C(18')-C(19')  180.0
C(22')-C(17')-C(18')-C(19')  0.0
C(16')-C(17')-C(18')-C(13')  0.0
C(22')-C(17')-C(18')-C(13')  180.0
C(14')-C(13')-C(18')-C(19')  180.0
Se(3')-C(13')-C(18')-C(19')  -1.2(18)
C(14')-C(13')-C(18')-C(17')  0.0
Se(3')-C(13')-C(18')-C(17')  178.8(18)
C(17')-C(18')-C(19')-C(20')  0.0
C(13')-C(18')-C(19')-C(20')  180.0
C(17')-C(18')-C(19')-Se(4')  -176.6(18)
C(13')-C(18')-C(19')-Se(4')  3.4(18)
Se(3')-Se(4')-C(19')-C(20')  -179.8(9)
Se(3')-Se(4')-C(19')-C(18')  -3.2(11)
C(18')-C(19')-C(20')-C(21')  0.0
Se(4')-C(19')-C(20')-C(21')  176.6(17)
C(18')-C(19')-C(20')-O(4')  -179(2)
Se(4')-C(19')-C(20')-O(4') -2.6(19)
C(19')-C(20')-C(21')-C(22') 0.0
O(4')-C(20')-C(21')-C(22') 179(3)
C(20')-C(21')-C(22')-C(17') 0.0
C(18')-C(17')-C(22')-C(21') 0.0
C(16')-C(17')-C(22')-C(21') 180.0
Appendix D: Presentations of this Work

Oral Presentations


Poster Presentations


Research Publications

1. **D. J. Press**, N. M. R. McNeil, A. Rauk, T. G. Back; “NMR and Computational Studies of the Configurational Properties of Spirodi oxyse lenuranes. Are Dynamic Exchange Processes or Temperature-Dependent Chemical Shifts Involved?”; *J. Org. Chem. 2012*, 77, 9268-9276. I completed the NMR studies for this publication, under the supervision of Professor Back. This research was discussed in Chapter 4 of this thesis. N.M.R. McNeil completed the computational studies with the assistance of Professor Rauk. The body of the manuscript was written by Professor Back. N.M.R. McNeil and I assembled the experimental and supporting information portion of this work.

2. **D. J. Press**, T. G. Back, T. S. Sutherland; “Charge transfer complexes of electron-rich naphthalene peri-dichalcogenides with TCNQ”; *Tetrahedron Lett. 2012*, 53, 1603-1605. I completed the synthetic research presented in this publication and the materials-type investigation, under the supervision of Professors Back and Sutherland, respectively. Some of the results from Chapter 5 of this thesis were included in this publication. I wrote some of the initial draft of the manuscript,
while Professors Back and Sutherland made revisions. I wrote the experimental portion of the work, and also assembled the supporting information.

3. **D. J. Press**, T. G. Back; “Enhanced Glutathione Peroxidase Activity of Conformationally Restricted Naphthalene peri-Dichalcogenides”; *Org. Lett.* **2011**, *13*, 4104-4107. I completed the synthetic research presented in this publication along with the investigation of GPx-like activity, under the supervision of Professor Back. Some of the results from Chapter 5 of this thesis were included in this publication. The body of the manuscript was written by Professor Back. I wrote the experimental portion of the work, and also assembled the supporting information.

4. **D. J. Press**, E. A. Mercier, D. Kuzma, T. G. Back; “Substituent Effects upon the Catalytic Activity of Aromatic Cyclic Seleninate Esters and Spirodioxy selenuranes That Act as Glutathione Peroxidase Mimetics” *J. Org. Chem.* **2008**, *73*, 4252-4255. This research was discussed in Chapter 2 of this thesis. This work was an investigation of substituents which would improve the catalytic activity of aromatic cyclic seleninate esters and spirodioxyselenuranes, developed by D. Kuzma. I completed the synthetic research on the cyclic seleninate ester derivatives, while E.A. Mercier did the same for the spirodioxyselenuranes. The investigation of their catalytic activity was carried out jointly, by both E.A. Mercier and myself. The body of the manuscript was written by Professor Back. E.A. Mercier and I wrote the experimental portion of this work and assembled the supporting information.