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**SYNTHESIS AND INCLUSION STUDIES OF
STABLE ALLICIN MIMICS AS
NOVEL ANTIMICROBIAL AGENTS**

BY

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ABSTRACT

Alliin, a known constituent of garlic, is a potent but unstable antimicrobial agent. Consideration of the underlying features responsible for alliin's activity, as well as its instability, prompted an investigation into substituted *S*-aryl alkylthiosulfonates as a class of potential alliin mimics with enhanced stability. Synthesis of the targets also inspired development of a novel unsymmetrical disulfide synthesis.

This thesis describes the development of a new methodology for synthesizing unsymmetrical disulfides. The synthesis involves converting a thiol to a sulfenylating agent by 1-chlorobenzotriazole (BtCl) in the presence of 1,2,3-benzotriazole (BtH). Addition of a second thiol affords unsymmetrical disulfides in excellent yields. In addition to being a one-pot methodology, the approach offers attractive environmentally friendly and cost-saving aspects. The methodology proved to be versatile, producing all types of unsymmetrical disulfides; aromatic-aliphatic disulfides, aromatic-aromatic disulfides as well as aliphatic-aliphatic disulfides including unsymmetrical cysteine disulfides.

A class of unsymmetrical disulfides (alkyl *p*-methoxyphenyl disulfides), with an activated aromatic ring, were oxidized to the corresponding thiosulfonates (*S*-alkyl *p*-methoxyphenylthiosulfonates) using *m*-CPBA, and were established to be markedly more stable than alliin itself with only ~5% structural change after 1 month at room temperature. This was in contrast to the same class of thiosulfonates with deactivated rings, which were found to be unstable. A family of relatively stable thiosulfonates was thus prepared by varying the length and fluorine content of the alkyl chain. Such compounds were subjected to a preliminary antibacterial study, which revealed that the *S*-*p*-methoxyphenyl alkylthiosulfonates exhibit activity against Gram-positive (*Mycobacterium aurum* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, qualifying them as alliin mimics at a preliminary level.

Lastly, the thesis describes the preparation and characterization of inclusion complexes formed between selected cyclodextrins and *S*-aryl alkylthiosulfonates. The complexes were investigated by thermal techniques, IR spectroscopy, powder X-ray diffraction and in three cases, single crystal X-ray diffraction. Such complexes may also offer the included molecules valuable stability and biological characteristics.

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LIST OF ABBREVIATIONS

aq.	aqueous
Ar	aromatic
br. s.	broad singlet
Boc	<i>tert</i> -butyloxycarbamate
BtH	1,2,3-benzotriazole
BtCl	1-chlorobenzotriazole
BuSH	Butylthiol
<i>t</i> -BuSH	tertiary butylthiol
BzSH	benzylthiol
cat.	catalytic
C _p	para-carbon
C _m	meta-carbon
C _o	ortho-carbon
C _s	ipso-carbon
CD	cyclodextrin
α-CD	α-cyclodextrin
γ-CD	γ-cyclodextrin
β-CD	β-cyclodextrin
d	doublet
dd	doublet of doublets
ddd	doublet of doublets of doublets
dt	doublet of triplets
DEAD	diethyl azodicarboxylate
DIMEB	heptakis(2,6-di-O-methyl)-β-CD
DSC	differential scanning calorimetry
eq.	equivalents
EI	electron ionisation
EtOAc	ethyl acetate
g	grams
GC	gas chromatography
hr.	hour
Hz	hertz
H _m	meta-proton
H _o	ortho-proton
HOMO	highest occupied molecular orbital
HPLC	high pressure liquid chromatography
HRMS	high resonance mass spectrometry
IR	infra red spectroscopy
<i>J</i>	coupling constants
LDA	lithium diisopropylamide
LUMO	lowest unoccupied molecular orbital
<i>m</i>	<i>meta</i>

m	multiplet
<i>m/z</i>	mass-to-charge ratio
mp	melting point
mg	milligram(s)
ml	millilitre(s)
mmol	millimole(s)
mol	mole(s)
Me	methyl
MeO	methoxy
MeOH	methanol
<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
NMR	nuclear magnetic resonance
<i>o</i>	ortho
<i>p</i>	para
Pet ether	petroleum ether
Ph	phenyl
ppm	parts per million
PMB-Br	para-methoxybenzyl bromide
Pr-SH	propanethiol
PrSSPr	dipropyl disulfide
PXRD	powder X-ray diffraction
q	quartet
rt	room temperature
R ¹ SH	thiol 1
R ² SH	thiol 2
R ¹ SSR ¹	homodimer 1
R ² SSR ²	homodimer 2
R ¹ SSR ²	unsymmetrical disulfide
s	singlet
SCXRD	single crystal X-ray diffractometry
t	triplet
td	triplet of doublets
TG	thermogravimetry
THF	tetrahydrofuran
TLC	thin layer chromatography
<i>p</i> -TolSH	<i>p</i> -tolylthiol
<i>p</i> -TolSBt	<i>p</i> -tolylthio-benzotriazole intermediate
TRIMEB	heptakis(2,3,6-tri- <i>O</i> -methyl)- β -CD
w/v	weight by volume
v/v	volume by volume

Chapter 1: Introduction

1.1 Overview of Garlic

Garlic, *Allium sativum*, is a plant belonging to the *Liliaceae* family and has been used as a medicinal agent for many decades.¹ It has generated much interest throughout human history as a food and medicine and is one of the most researched medicinal plants. Its folklore and therapeutic benefits date back about five thousand years to the Middle and Far East, and probably originated in the advanced civilizations of the Indus valley, from where it was imported to China before spreading to Egypt, Greece and throughout the Roman Empire into Europe.^{2,3,4} In ancient civilizations, garlic was used as a treatment for various ailments including headaches, burns and wounds, colds, worms, stings, bites, tumours, heart disease and ulcers.^{1,5} In more recent times, notably during the past two decades, garlic research has predominantly focused on the topics of atherosclerosis, cardiovascular disease, coronary thrombosis and cancer research.^{6,7} Mechanistic investigation over the last few years suggests that garlic may either prevent or decrease the occurrence of these major chronic diseases, which are mainly due to the abundance of free radicals, by virtue of its strong antioxidant properties.⁸ In particular, it has been demonstrated that garlic reduces serum cholesterol and triglyceride levels, inhibits platelet aggregation, stimulates immune effector cells and acts on bacteria, viruses and alimentary parasites.^{1,4,6} The lengthy list of biological activities and health-promoting effects of garlic is attributed to the presence or generation of sulfur-rich compounds.^{4,5} This is not surprising as many health-promoting agents contain sulfur, a good example being penicillin. The chemical structures of a few of the organo-sulfur compounds found in garlic are shown in Figure 1-1.

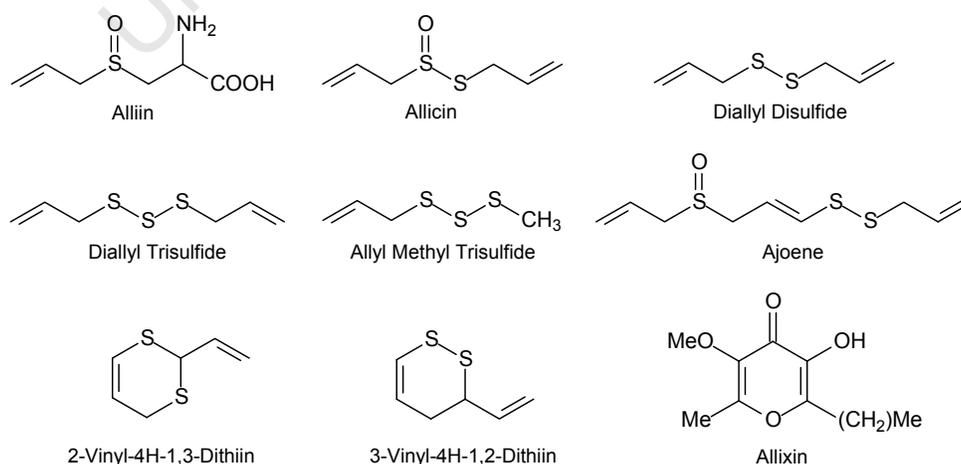


Figure 1-1 Some of the prominent biologically active compounds of garlic.

The earliest chemical studies on garlic were carried out in 1844 by the German chemist Wertheim and later by Semmler in 1892.^{9,10} Wertheim obtained a pungent-smelling oil from garlic cloves by means of steam distillation, while Semmler later reported the isolation of an oil possessing antimicrobial properties. A little over five decades later, Cavallito and Bailey reported the isolation and identification of the component responsible for the antibacterial activity of garlic.¹¹ They observed that different methods of extraction (Figure 1-2) gave different compounds, which were identified as diallyl disulfide from the ethanol extract, allicin (diallyl thiosulfinate) from the ethanol and water extract and alliin from steam distillation. The ethanol and water extract displayed the most potent antimicrobial properties.¹¹

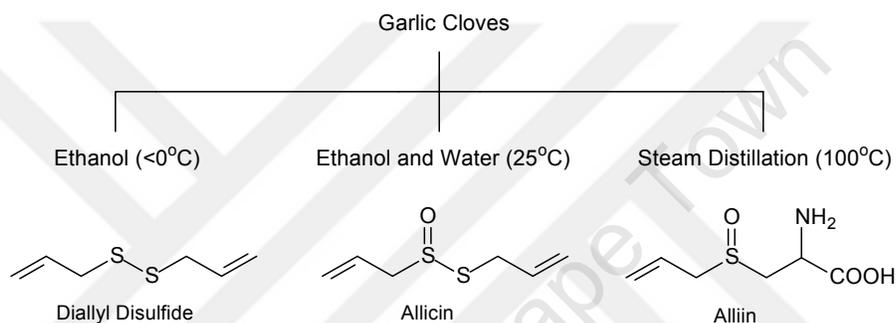


Figure 1-2 Major organo-sulfur compounds extracted from garlic cloves employing various extraction methods.¹²

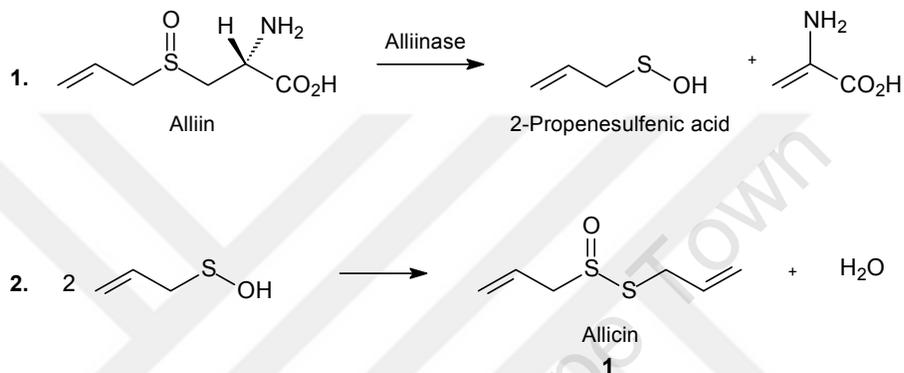
In 1947, Cavallito established the chemical structure of allicin by demonstrating that the synthetic material obtained from selectively oxidizing diallyl disulfide using perbenzoic acid was identical with that isolated from freshly-crushed garlic.¹³

1.2 Allicin

1.2.1 Chemistry and Stability

Allicin [*S*-(2-propenyl)-2-propene-1-sulfinothionate] **1** is a member of a class of unstable and reactive organosulfur compounds known as thiosulfonates and is the most abundant organic compound in freshly-crushed garlic, as well as the principal biologically-active substance. The mystery surrounding the observation that allicin is present in crushed garlic cloves and not in intact cloves baffled scientists for many years. In 1948, Stoll and Seebeck reported the isolation, identification and synthesis of an *S*-(allyl)-*L*-cysteine sulfoxide substrate named alliin, and a pyridoxal phosphate-containing enzyme, alliinase, present in intact garlic cloves.¹⁴ Cross-sectional studies of garlic have demonstrated that a cellular membrane separates the

two different compartments housing alliin and alliinase, and this design suggests a potential defense mechanism.¹⁵ Modern science has established the fascinating chemistry that goes on when a garlic clove is crushed or attacked by a pathogen. Under such circumstances, the compartment separating alliin from its enzyme alliinase is destroyed, resulting in the two substances coming into intimate contact with one another. A rapid elimination reaction ensues producing 2-propenesulfenic acid, which self-condenses to produce the thiosulfinate alliin that accounts for about 70-80 % of the organic material produced initially, Scheme 1-1.¹⁶



Scheme 1-1 Production of alliin 1 in the garlic clove.

Pure alliin has been isolated as a colourless liquid via infusion of ground garlic cloves, and contains approximately 40 % sulfur and has the typical odour of freshly-crushed garlic.¹¹ It is a moderately volatile compound with a boiling point of approximately 180°C and is poorly miscible in aqueous solutions. Alliin is unstable depending on a range of parameters including pH, concentration, temperature, solvent and the presence of additives.¹⁷ Freeman reported the half-life of alliin in blood as 2.5 minutes.¹⁷ Table 1-1 depicts the half-lives of alliin at various pH values at different temperatures, while Figures 1-3 and 1-4 show alliin's stability in various solvents at different temperatures.^{17,18} The results in Table 1-1, obtained by the work of Parkin, confirmed the results obtained by Block and Lawson.^{19,20} They established that an acidic medium is more conducive to stabilizing thiosulfinates than neutral or alkaline pH media.¹⁸

pH	~1.2	~5.5	~7.5	~9.0
20 °C	20.7 d	52.3 d	3.2 d	5.8 h
40 °C	1.7 d	2.7 d	0.57 d	1.0 h
60 °C	5.0 h	6.9 h	2.5 h	0.45 h
80 °C	0.34 h	0.82 h	0.31 h	0.06 h

Table 1-1 Half-lives of alliin in 0.1 M Tris Buffer.¹⁸

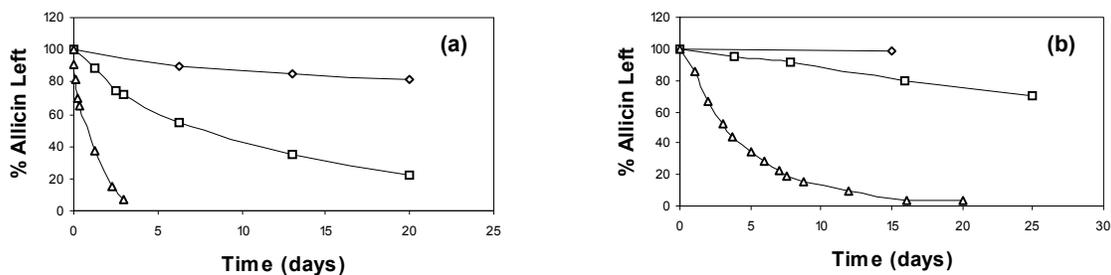


Figure 1-3 Allicin stability in various solvents at various temperatures (◇) at -16°C , (□) at 6°C , (Δ) at room temperature in (a) ethyl acetate and (b) methanol.¹⁷

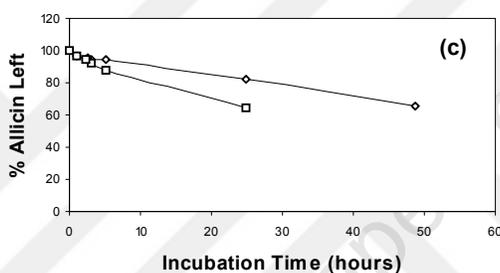
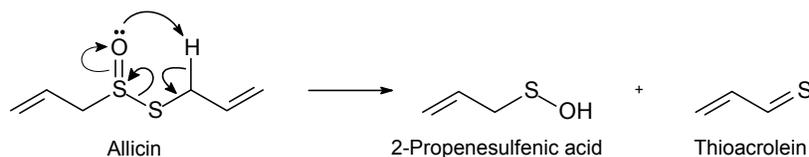
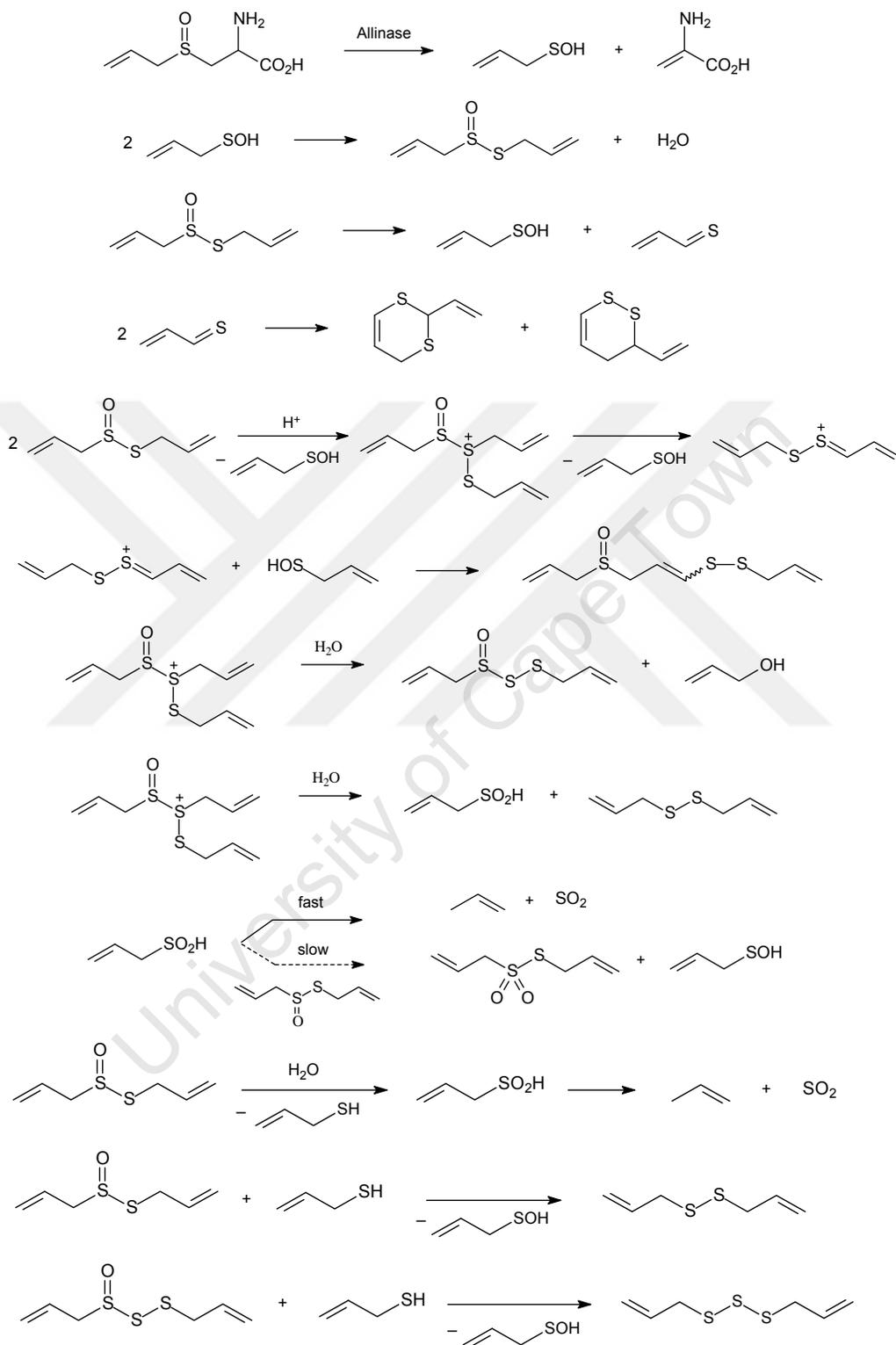


Figure 1-4 Allicin stability in water at gastric pH (◇) pH 1.2 and intestinal pH (□) pH 7.5 at 37°C .¹⁷

As noted, allicin is extremely unstable neat and the most stable in hydrogen-bonded solvents, particularly water. At low temperatures ($<0^{\circ}\text{C}$) in water, it can be stored for a number of weeks without appreciable decomposition. The explanation of allicin's instability has been elegantly provided by the work of Professor Eric Block, who in some seminal papers in the 1970's and 1980's reported that allicin fragments into thioacrolein and 2-propenesulfenic acid, the former subsequently dimerizing to 1,2- and 1,3-dithiins.^{21,22} Of crucial structural importance regarding this fragmentation reaction is the presence of an allylic hydrogen adjacent to the sulfenyl sulfur, Scheme 1-2.



Scheme 1-2 Mechanism of decomposition of allicin.

Scheme 1-5 Scheme of components formed from alliin.²³

1.2.2 Biological Activity of Allicin

Allicin has been reported to have numerous biological activities, including antimicrobial, antioxidant, antithrombotic and antiatherosclerotic. It also possesses antitumour properties, inhibits growth of *Entamoeba histolytica*,^{24,25} a major cause of intestinal amoebiasis, acts as a systematic vasodilator with an antihypertensive effect,²⁶ inhibits platelet aggregation,²⁷ inhibits alcohol dehydrogenase²⁸ and inactivates various cysteine proteinases. Allicin is also known to have serum lipid and ocular pressure-lowering capacity and affects RNA synthesis in microorganisms and lipid biosynthesis in plants and mammals.²⁹ Some of these activities are explained in the following sections.

Antimicrobial Activity

Cavallito carried out antibacterial assays on allicin and a number of its congeners, demonstrating them to be potent, yet labile, antimicrobial agents.¹³ In the years that followed, several *in vitro* studies have demonstrated allicin to have potent antibacterial, antifungal and antiparasitic activity against a range of microorganisms including methicillin-resistant *Staphylococcus aureus*.^{16,30} Antiviral activity has also been demonstrated.³¹ In spite of its potent activity, synthetic allicin, obtained either biomimetically or chemically from diallyl disulfide has not been developed for human use *in vivo* undoubtedly as a result of its instability and also because pharmaceutical companies cannot patent it in view of its having been in the public domain for so long.¹³ Various companies do sell it as an aqueous solution, but the purity of such allicin is questionable since allicin is unstable. Similarly, commercial garlic supplements can lose their potency as a result of alliinase inactivity.³² Thus, in spite of allicin's potent antimicrobial activity, its benefits to humans remain predominantly at the culinary level with no quantitative data available concerning its ability to fight human disease.³³ The various types of activity are now described.

Antibacterial Activity

It is believed that Louis Pasteur first reported the antibacterial activity of garlic in 1858 and in 1944 Cavallito and Bailey reported that the antibacterial action of garlic is mainly due to allicin.^{11,12} Subsequently, garlic was shown to be effective against a wide range of gram-positive and gram-negative bacteria, including strains of *Proteus*, *Escherichia coli*, *Pseudomonas*, *Staphylococcus aureus*, *Streptococcus*, *Clostridium*,³⁴ *Salmonella*,³⁵ *Klebsiella*, *Micrococcus* and *Bacillus subtilis*.^{12,16} Acid-fast bacteria such as *Mycobacterium tuberculosis* is also inhibited by garlic. Garlic extracts are also effective against enterobacteria,³⁶ *Helicobacter pylori*³⁷ as the cause of gastric and duodenal ulcers as well as gastric cancer, and they prevent the formation of *Staphylococcus* enterotoxins A, B and C1.³⁸

It has also been reported that allicin displays synergy with antibiotics such as streptomycin or chloramphenicol against *M. tuberculosis* and that various bacterial strains resistant to antibiotics are sensitive to allicin.¹⁶ The antibacterial activity of garlic is mainly attributed to allicin, but other active antibacterial constituents of garlic include the allicin-derived organo-sulfur compounds, diallyl sulfide, diallyl disulfide, ajoene and various thiosulfates.³⁹ It has been observed that the antibacterial activity significantly decreased when garlic extracts were stored at room temperature, while the activity was only slightly affected when stored at 0-4 °C, suggesting instability of the active compound towards heat.¹² Although the effect of allicin is astonishing and the antibacterial activity of allicin is clearly observed *in vitro*, the *in vivo* activity has not been explored to any great extent.

Antifungal Activity

Schmidt and Marquardt first established the antifungal activity of garlic in 1936.^{12,40} Since then, a broad spectrum of fungi and yeasts has proven to be susceptible to garlic extracts, including *Candida*, *Cryptococcus*, *Trichophyton*, *Rhodotorula*, *Torulopsis*, *Epidermophyton*, *Trichosporon*, *Aspergillus* and *Microsporium*.^{41,42,43} The *in vivo* activity of allicin was first reported by Osheroev who demonstrated the rapid reduction of fungi in mice infected with *A. fumigatus*.⁴⁴ The *in vitro* synergistic effect of allicin with amphotericin B, the antibiotic for treatment of fungal infections, has also been demonstrated.⁴⁵ A pure allicin sample was shown to be antifungal and Hughes and Lawson demonstrated the decrease in antifungal activity by the removal of allicin from the reaction extract.⁴⁶ Other compounds to have shown antifungal activity include diallyl sulfide, diallyl disulfide, diallyl trisulfide and ajoene. The active constituents were shown to decrease the oxygen uptake, reduce cellular growth, inhibit the synthesis of lipids, proteins and nucleic acids, damage cell membranes and inhibit the synthesis of the fungal cell wall.^{47,48,49}

Antiviral Activity

Antiviral activity of fresh garlic extracts both *in vitro* and *in vivo* has also been demonstrated. Viruses that are sensitive to garlic extracts are influenza A and B viruses, human cytomegalovirus, human rhinovirus Type 2, herpes simplex virus Types 1 and 2, parainfluenza virus Type 3, vaccinia virus, vesicular stomatitis virus, rotavirus, viral pneumonia and human immunodeficiency virus (HIV).^{50,51} Besides allicin, diallyl disulfide, diallyl trisulfide and ajoene have shown to be active.³¹ Ajoene seems to be a more effective antiviral than allicin.

Antiparasitic Activity

Very little work has been done to demonstrate the antiparasitic activity of garlic, with only a few published reports. Early work concentrated on garlic as a possible treatment for

giardiasis. This led to a clinical trial on giardiasis patients by Soffar and associates.⁵² Garlic, either as a 1 mg ml⁻¹ aqueous extract or as a 0.6 mg ml⁻¹ commercially prepared garlic capsule, removed the symptoms from all the patients within 24 hours and no indication of the infection was observed after 72 hours.⁵² The clinical trial by Soffar on giardiasis was also prompted by the finding by Mirelman who showed that allicin has a growth-inhibitory effect on *Entamoeba histolytica*.²⁴ Others have shown that garlic extracts are effective against *Giardia lamblia*, *Leishmania major*, *Leptomonas colosoma*, *Crithidia fasciculata*, *Opalina ranarum*, *Opalina dimidicita*, *Balantidium entozoon* and *Trypanosoma brucei*.⁵³ Antiprotozoals have been proven to be sensitive against allicin, ajoene and other organo-sulfur compounds. Although allicin's toxicity towards tissue-cultured mammalian cells has been noted, it has also been reported that in the presence of amoebic trophozoites, no damage to the mammalian cells was observed. This indicates that allicin has a higher affinity towards the microbial cells, which has been attributed to the fact that microbial cells only have a small amount of glutathione, if it is present at all, thereby preventing reactivation of the enzymes thiolated by allicin.²⁴

Anticancer Activity

Documentation of the anticancer properties of garlic dates back to ancient times when Hippocrates used it for cancer treatment, and ancient Egyptians used it for the treatment of tumours.^{5,54} More recently, in 1958, Weisberger and Pensky began their studies on garlic as an anti-cancer agent. They demonstrated both *in vitro* and *in vivo* that allicin inhibits the growth of tumour cells.⁵⁵ Today, the role of garlic in cancer prevention and treatment is widely discussed and studied by professionals from various disciplines.

Carcinogenesis is an intricate, multi-stage process. It involves the production of a small population of abnormal cells that increases in abnormality as a result of changes in the patterns of gene expression as well as a series of transmutations.⁸ It is well known that dietary factors play a key role in influencing malignancy and this is accounted for by potent antioxidants and nutrients from fruits and vegetables repressing the effects of oxidative DNA damage, which can lead to mutations in crucial genes, thus lowering the risk of cancer.⁵⁶ It is not surprising that The National Cancer Institute has placed garlic, which possesses strong antioxidant properties, at the top of the vegetable-pyramid representing potency in cancer prevention.⁵⁷ Marchand and co-workers and Levi and co-workers carried out separate studies in which they demonstrated the consumption of garlic to be protective against colorectal cancer.^{58,59} Studies over the past three decades have proven that garlic decreases the bioactivation of carcinogens and that garlic consumption is related to reduced cancer occurrence.⁶⁰

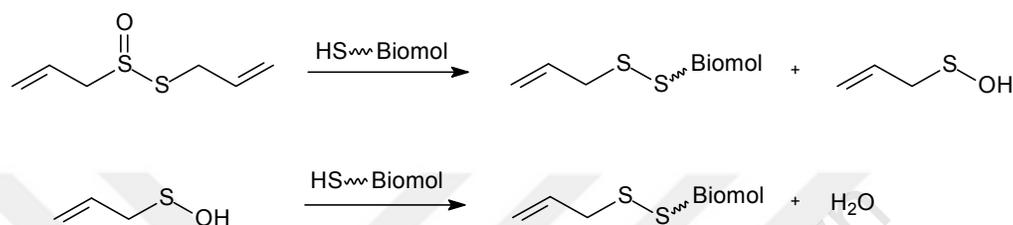
Notable examples of garlic studies undertaken over the past few years include work by Fleischauer and Arab⁶¹ who have suggested a preventative effect of garlic consumption in stomach and colorectal cancer, as well as a study by Challier and Perarnau who showed that the risk of breast cancer decreases with the consumption of garlic.⁶² Key and co-workers have also reported that garlic consumption decreases the risk of developing prostate cancer.⁶³ Studies have also concentrated on the role of pure allicin in this important field. Oommen found that allicin inhibited the proliferation of cancer cells of murine and human origin by inducing apoptosis.⁶⁴ Shalinsky and McNamara found that allicin selectively inhibited the glutathione-dependent PGH₂ to PGE₂ isomerase in the adenocarcinoma cell-line,⁶⁵ while Kang and co-workers found that allicin is an efficient immunomodulator of macrophage secretory and cellular activities.⁶⁶ Although several components of garlic have been found to inhibit and induce apoptosis of human non-leukaemia malignant cells it is known that allicin and ajoene are the most influential constituents. A recent example of the potent activity of ajoene was reported by Hassan who demonstrated the use of ajoene to chemosensitize drug-resistant tumours by enhancing the apoptotic effects of the chemotherapeutic drugs cytarabine and fludarabine by boosting their bcl-2 inhibitory and caspase-3 activities.^{6,67}

Different mechanisms have been reported for the protection afforded by garlic against cancer. They include the ability of allicin to react with glutathione in cancer cells,⁶⁸ the blocking of nitrosamine formation and bioactivation, which are suspected carcinogens,⁶⁹ and the reduction in concentration of nitrites by inhibiting nitrate reduction by bacteria.² Garlic has also been postulated as playing a role in the alteration of carcinogen metabolism, either increasing activity of the detoxification enzymatic systems or inhibiting activation of the procarcinogens.⁷⁰ Also to inhibit cellular proliferation by induction of apoptosis and to inhibit cell division in several human malignant cells, to inhibit oxidative damage, prevent chromosomal damage, and to inhibit lipoxygenase and cyclooxygenase activities.^{70,71,72} Allicin also reacts with free thiol-containing enzymes, as well as working as an effective antioxidant by trapping free radicals in which oxygen free-radicals are the chief suspects in promoting tumours.⁷³ Allicin's function as a hydroxyl radical scavenger, superoxide production inhibitor and nitrogen oxide formation inhibitor has also been reported to assist as a cancer-preventive agent in this context.^{2,36}

1.2.3 Biological Mode of Action

Allicin mode-of-action studies date back to Cavallito's seminal work of the 1940's in which he was the first researcher to demonstrate that allicin oxidises the cysteine sulfhydryl group to S-(thioallyl)-cysteine.⁷⁴ Based on this model reaction, he proposed that the thiosulfinate

grouping acts as an electrophilic pharmacophore for sulfhydryl groups of biologically active thiols such as those present in important enzymes, e.g. cysteine protease, resulting in their oxidation to disulfides.^{36,75} Attack by the soft thiol sulfur would first take place at the softer thiosulfinate sulfenyl sulfur followed by a second attack at the harder 2-propenesulfenic acid sulfur that is expelled.⁷⁶ The overall result is oxidation of two mole equivalents of thiol using one mole of allicin, Scheme 1-6.



Scheme 1-6 Oxidative action of allicin.

Cavallito postulated that allicin might function by inhibiting bacterial growth by functionalising -SH groups vital to bacterial propagation.⁷⁴ Subsequently, many researchers have corroborated these findings from the cysteine model experiment and many have shed light on the mode of action of allicin on various microbes. Later, Rabinkov confirmed the outcome of the reaction of allicin with cysteine and further carried out reactions of allicin with different thiol-containing enzymes. He showed that allicin rapidly inactivated active papain, NADP⁺-dependent alcohol dehydrogenase from *Thermoanaerobium brockii* and NADP⁺-dependent alcohol dehydrogenase from horse liver and that activity could be restored with thiol-containing compounds such as dithiothreitol, 2-mercaptoethanol or glutathione, in which exchange on the disulfide occurred with regeneration of the biologically active sulfhydryl group.⁷³ These results provide powerful evidence for the SH-modifying properties of allicin and its role in regulating enzymatic activity by thiol-disulfide exchange reactions.

As mentioned previously, Mirelman and co-workers found that allicin has a growth-inhibitory effect on *Entamoeba histolytica*, and they also reported that at low concentrations (<10 µg/ml), allicin inhibits the ability of amoebic trophozoites to damage monolayers of baby hamster kidney cells by inhibiting cysteine proteinase activity, alcohol dehydrogenases and thioredoxin reductases.^{16,24,25} These enzymes play an important role in maintaining the correct redox state within the parasite. Other SH-containing enzymes inhibited by allicin include succinic dehydrogenase, glyoxylase, urease, xanthine oxidase, triose phosphate dehydrogenase, choline oxidase, hexokinase and cholinesterase.⁷⁷ Additionally, the bacterial enzymes acetate kinase and phosphotransacetyl-CoA synthetase, were found to be inhibited by allicin, as well as DNA and RNA processing.^{78,79}

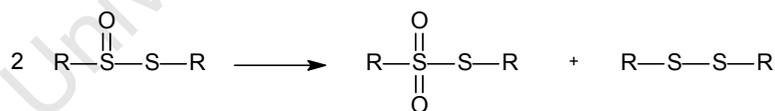
Similarly, Miron and his colleagues carried out a study to demonstrate the interaction of allicin with intracellular thiols by first showing that allicin has the ability to penetrate cell membranes. They established that allicin reacted with the thiol glutathione encapsulated in lipid vesicles.³⁶ Based on kinetic studies, they reported that the thiol was not freed from the vesicles. The expected product, *S*-allyl mercaptogluthathione, was detected in the vesicle fraction of the reaction by gel-filtration. In addition, they demonstrated that allicin can easily diffuse across the membrane into the cytoplasm of red blood cells. Observation of a colour change of the red blood cell pellets to brown during the incubation with allicin was attributed to membrane penetration.¹⁷ The results clearly demonstrated that allicin can rapidly penetrate into cells in biological systems and carry out its biological activity intracellularly.

From the plethora of research carried out on this topic it is reasonable to conclude that allicin's biological activity is attributable to the inhibitory effects it has on thiol-dependent enzymatic systems, its radical-trapping properties and its ability to enter and cross membranes.

1.3 Thiosulfinates

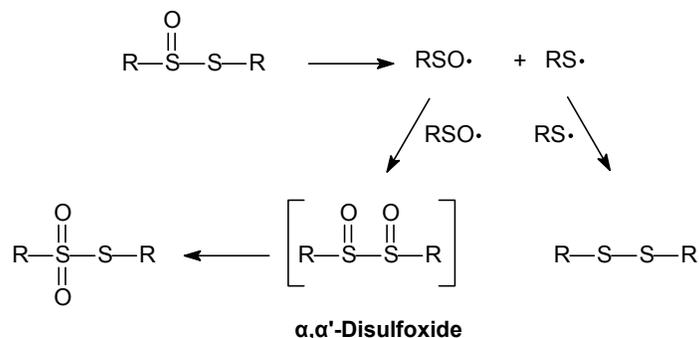
1.3.1 General Overview

Ever since the discovery of allicin, considerable interest has focused on the chemistry and properties of thiosulfinates. They are a highly unstable group of compounds, and, in addition to the fragmentation reactions already mentioned, are known to readily disproportionate to the corresponding thiosulfonate and disulfide, Scheme 1-7.



Scheme 1-7 Disproportionation of thiosulfinates.

Barnard proposed that the disproportionation of thiosulfinates may possibly occur *via* a free-radical mechanism with homolytic fission of the S-S bond.⁸⁰ Kinetic studies carried out by Koch revealed that the rate equation for the reaction contains one first-order term and one three-halves-order term, which led him to propose a unimolecular and an induced decomposition.⁸¹ The homolytic fission of the S-S bond would present one sulfinyl radical and one thiyl radical. Dimerization of two thiyl radicals would then give rise to a disulfide, while dimerization of the sulfinyl radicals would yield the thiosulfonate through a α,α' -disulfoxide (vic-disulfoxide) intermediate followed by oxygen transfer, Scheme 1-8.⁸¹



Scheme 1-8 Disproportionation of thiosulfonates.

Similarly, mechanistic studies on the thermal and catalysed disproportionation of diaryl thiosulfonates have been reported by Koch⁸¹ and Kice⁸² respectively, while thermal and photochemical studies with dialkyl thiosulfonates have been reported by Block.⁸³ Block also illustrated that thiosulfonates with alkyl substituents on the carbon atoms adjacent to each of the sulfur atoms are more stable when hydrogens for β -elimination are absent.

The highly reactive S-S bond can be cleaved either *via* electrophilic or nucleophilic scission, *via* homolytic fission or by simultaneous “push-pull” electrophile-nucleophile catalysis.⁸⁴ Bond strengths of 45 kcal/mol (methyl methanethiosulfonate) and 70 kcal/mol (dimethyldisulfide) for the thiosulfonate and disulfide S-S bond respectively have been reported, demonstrating that the thiosulfonate S-S bond is more labile than that of the disulfide.^{85,86}

Like allicin, various naturally occurring thiosulfonates have been found to possess a range of biological activities including antiviral and antifungal activity as well as possessing antitumour inhibiting properties.^{13,55} A study by Musah and co-workers demonstrated that thiosulfonates extracted from *Petiveria alliacea* L. showed significant growth inhibition of Gram-negative bacteria.⁸⁷ Furthermore, synthetic variants have been used as versatile intermediates in organic synthesis⁸⁸ and as stabilizers for synthetic rubber.⁸⁹ They have also been found to inhibit the autoxidation of polyolefins, employed for stabilizing α -radicals,⁹⁰ α -anions⁹¹ and acting as cationic synthons.⁹²

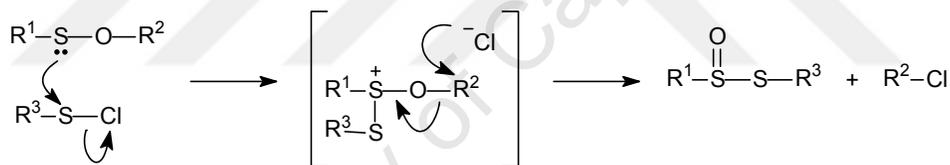
1.3.2 Biological Activity

As described previously, the biological activity of the thiosulfonates, like allicin, is associated with the reactivity of the $-\text{S}(\text{O})\text{S}-$ moiety towards biologically essential thiol groups.⁷⁴ They have been postulated to function by inhibiting bacterial growth by damaging $-\text{SH}$ groups vital to bacterial reproduction by oxidizing them to disulfides. An interesting observation was made

by Cavallito and co-workers about the activity of thiosulfonates: they reported that an increase in the carbon chain-length of each carbon group adjacent to the sulphur atoms of a dialkylthiosulfonate resulted in an increase in activity against Gram-positive bacteria, but a decrease against Gram-negative bacteria, while branching decreased activity overall.¹³ It was also reported that increasing the alkyl group's chain length promoted an increase in stability against decomposition.

1.3.3 Preparation

Thiosulfonates are usually prepared *via* two methods. The first involves the oxidation of disulfides using oxidising agents such as hydrogen peroxide or peracids such as *m*-CPBA, perphthalic acid or peracetic acid. Alternatively, they can be prepared by the reaction of a sulfinyl chloride with a thiol in the presence of a tertiary base.⁹³ Another approach involves the use of the "Thio-Arbuzov" reaction, Scheme 1-9 which involves the interaction of a sulfenyl chloride with a sulfenyl sulfide to form the thiosulfonate and the corresponding haloalkane.⁹⁴



Scheme 1-9 The "Thio-Arbuzov" reaction.

Of the various methods reported in the literature for the formation of thiosulfonates, the oxidation of disulfides is the favoured method.

1.4 The Disulfide Bond

1.4.1 General Overview

The sulfur-sulfur bond is a single covalent bond, which was first synthesized by Cahours in 1847.⁹⁵ It was not until many decades later when the S-S bond was identified in the structures of proteins and enzymes and when it was discovered that hormones such as insulin, oxytocin and vasopressin contain disulfide bridges,⁹⁶ that they assumed the eminent position they hold

today. Figure 1-5 illustrates the different types of S-S linkages at different oxidation levels of S that are found in various compounds.

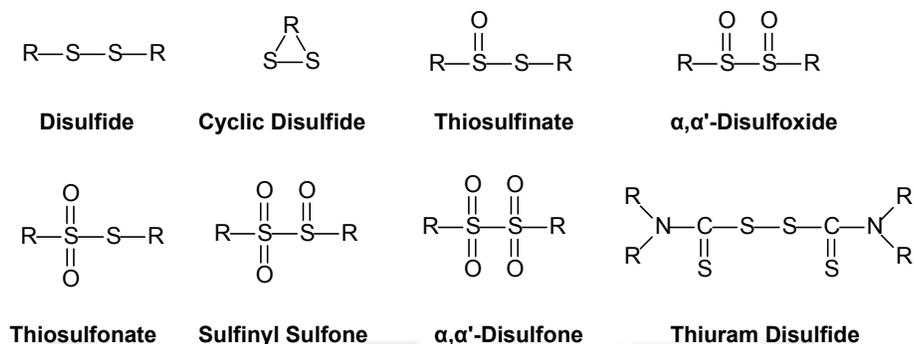


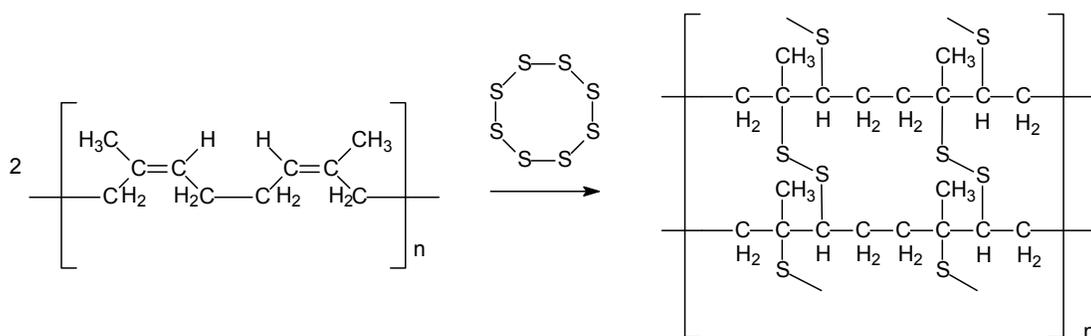
Figure 1-5 Examples of various types of sulfur-sulfur linkages.⁹⁶

1.4.2 Applications

The disulfide bond has attracted considerable interest because of its importance in pharmacological chemistry, biochemistry, industrial and agricultural chemistry and the significant role that it plays in chemical and biological processes.^{97,98} Furthermore, unsymmetrical disulfides play a pivotal role in modern medicinal chemistry research.^{99,100}

Role in Industrial and Agricultural Chemistry

Disulfide compounds fulfil an important function in the vulcanization of rubber, which is an irreversible curing process involving high heating temperatures with the addition of sulfur to form springy rubber molecules that are more durable and resistant to chemical attack. Scheme 1-10 illustrates the formation of covalent bonds in a synthetic rubber polymer. Uncured rubber cannot be used in its native state, being a sticky substance that is deformed when warm, and brittle when cold.



Scheme 1-10 Formation of a cross-linked polymer in rubber formation.

The use of accelerators or retarders in the curing process has also been introduced.¹⁰¹ Notably, the majority of these reagents are disulfide-possessing compounds. The accelerators lead to reduced cure times and energy consumption, whereas the retarders delay the process. These types of compounds are of interest to many companies, especially The Goodyear Tyre and Rubber Company. Disulfides are also used as additives for lubricating oils¹⁰² and are found in agricultural fungicides and herbicides,¹⁰³ while compounds possessing the disulfide bond have been shown to exhibit pesticidal, nematocidal, insecticidal and miticidal activity.¹⁰⁴

Role in Biochemistry

Disulfide bonds present in native proteins are formed post-translationally and mostly exist in extracellular proteins.¹⁰⁵ The disulfide functionality plays a pivotal role in the structure and function of a range of bioactive peptides. The functions of these proteins are extremely diverse, ranging from hormones, regulating metabolic processes, to providing antibodies for resistance to disease.⁹⁶ Many important enzymes including ribonuclease, lysozyme, papain, tripsin, pepsin, chymotrypsinogen A and catalase possess disulfide linkages. The disulfide grouping provides a rigidity in the structure that is responsible for the maintenance of the conformation of enzymes, and thus the activity of proteins.^{96,106} As a result, the activity of proteins can be either lost or diminished upon reduction of disulfide bonds due to the changes in conformation.¹⁰⁷

Several proteins and peptides are excreted from cells in order to carry out their functions. One of the 'defense' mechanisms of proteins against the often hostile extracellular environment is to form disulfide bonds. This is an added protective role of the disulfide bond in that they introduce conformational constraints into the polypeptide backbone, which improve the thermodynamic stability of the protein and prevent denaturation and proteolytic degradation.¹⁰⁸

Reactions of the S-S Group

The S-S moiety can undergo the following reactions, which are important in protein chemistry:

(a) Interaction between S-S and –SH groups: Nucleophilic Exchange

Although Lecher¹⁰⁹ first observed the reaction of thiols with disulfides in 1920, it was Hopkins¹¹⁰ who in 1925 recognized the biochemical importance of the reaction. Hopkins observed that glutathione actively reduces the disulfide moieties in proteins and thus helps to keep thiol groups in a reduced state.

(b) Oxidation of Disulfides

Oxidation of disulfides by halogens, organic peracids, periodic acid and peroxides is used to split disulfide bonds in proteins such as ribonuclease and insulin. The process is used in the determination of the primary structures of proteins.⁹⁶

(c) Nucleophilic attack on the S-S bond

Nucleophiles such as CN^- and SO_3^{2-} are used to break disulfide bridges in order to separate and identify different chains of proteins.⁹⁶

The formation of disulfide bonds in proteins involves a series of thiol-disulfide exchange reactions between cysteine thiolates and oxidizing disulfides. Introducing a disulfide bond chemoselectively into peptides synthetically is often a difficult procedure requiring multiple protections. However, the synthesis of unsymmetrical cysteine disulfide linkages has been explored by many researchers as a model reaction for introducing the disulfide functionality into a protein.

Role in Medicinal Chemistry

Unsymmetrical disulfides are ubiquitous in medicinal chemistry. A variety of disulfide compounds possess significant antimicrobial activity.¹¹¹ Disulfide-containing compounds have been reported to have strong antibacterial and antifungal properties, having activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*.^{112,113} Compound **3** (Figure 1-6) was isolated from the New Zealand basidiomycete (mushroom) *Cortinarius* species and was reported in 2001 to possess potent antimicrobial activity and cytotoxicity, while compound **4** (Figure 1-6) prepared by the reaction of thiols (R^1SH) with Bunte salts ($\text{R}^2\text{SSO}_3\text{Na}$) or thiosulfates ($\text{R}^2\text{SS(O)R}^2$) showed significant antifungal activity against candidosis in mice.^{111,113} Many compounds obtained from nature possess potent sulfur-containing compounds, with allicin, ajoene, diallyl disulfide and psammaphin A being just a few examples. Psammaphin A was first isolated from the *Psammaphysilla sponge* in 1987

and later an array of analogues was synthesized and shown to have marked antibacterial activity against methicillin-resistant *Staphylococcus aureus*.^{114,115}

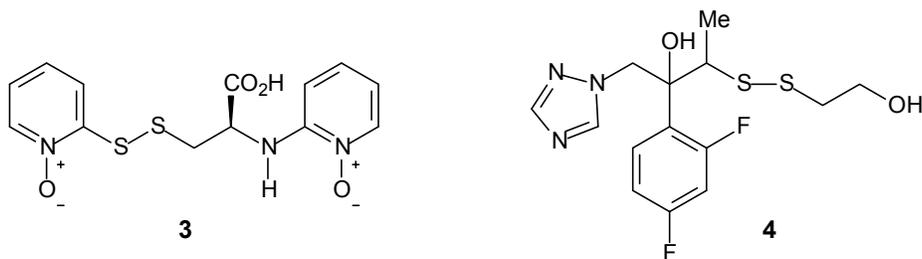


Figure 1-6 Examples of disulfide-containing compounds: **3** antimicrobial, **4** antifungal.

In addition, antitumour properties have been reported. Kono and co-workers explored the antitumour properties of mitomycin C analogues possessing a disulfide bond. They reported the synthesis and antitumour activity of various analogues against sarcoma 180 and leukemia P388 in mice.¹¹⁶ The 7-*N*-[2-[[2-(γ -L-glutamylamino)ethyl]dithio]ethyl] mitomycin C analogue **5**, shown in Figure 1-7, had the greatest activity, proving to be more potent than mitomycin C itself. It has not been determined whether it is the disulfide bond alone that increases the activity or the combination of the disulfide bond and the amino acid residue. Further studies need to be carried out to clarify this. Similarly, Stiefel synthesized a range of 6-purinylyl disulfide compounds **6** (Figure 1-7) by the reaction of 6-mercaptopurine with a variety of sulphenyl chlorides in the presence of base.¹¹⁷ These compounds have shown effective antineoplastic activity thus inhibiting the growth of cancer tissue. The final example of disulfide-containing drugs as antitumour agents was the synthesis of alkyl 2-imidazolyl disulfides **7** (Figure 1-7) reported by Kirkpatrick.¹¹⁸ These are novel antitumour agents that have shown to be cytotoxic to cancer cells *in vitro* by depleting cellular glutathione (GSH).^{118,119} Kirkpatrick not only demonstrated the antitumour activity of these compounds *in vivo* in animal models but also reported the *in vitro* inhibitory activity of these compounds against thioredoxin. This area of research is constantly being explored and will most likely present much more novel and potent antitumour agents in the years to come.

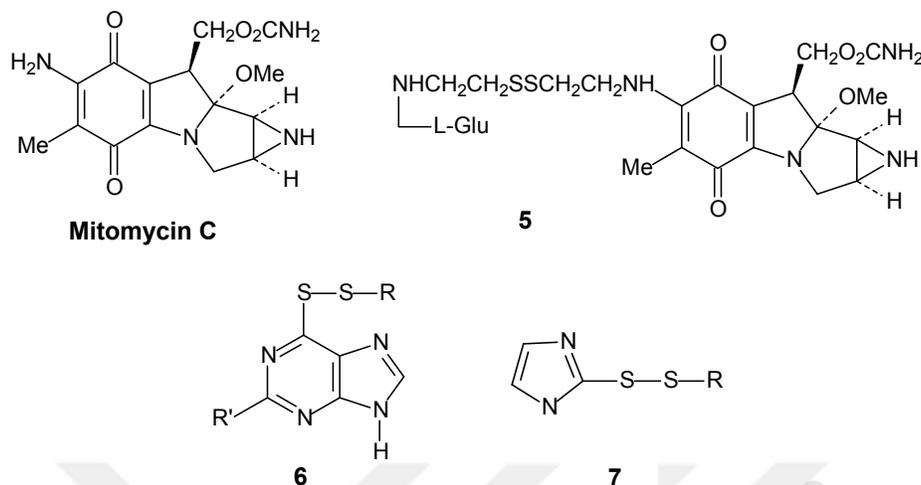
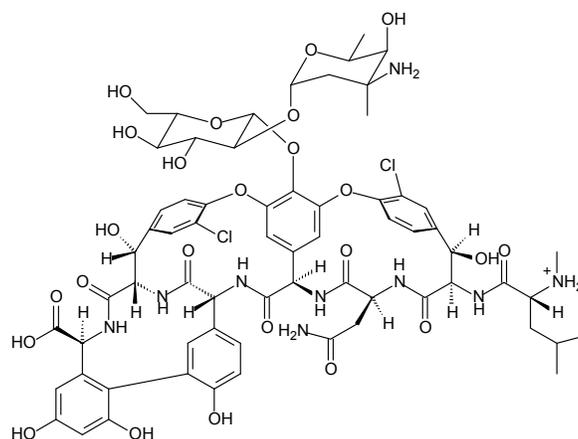
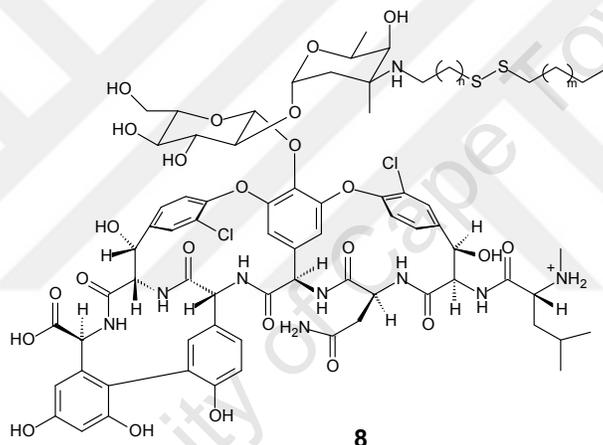


Figure 1-7 Examples of antitumour compounds. Top row: Mitomycin C and a Mitomycin analogue **5**. Bottom row: **6** ($\text{R} = -\text{CH}_3, -\text{CH}_2\text{CH}_3, -(\text{C}_6\text{H}_5)\text{CH}_3$; $\text{R}' = \text{H}, \text{amino-group}$) and **7** ($\text{R} = -\text{CH}_2\text{CH}_3, -\text{C}(\text{CH}_3)_3, -\text{CH}_2\text{C}_6\text{H}_5$).

Drug Resistance

Drug resistance has become an increasing problem in disease and over the past two decades extensive research has focused on trying to combat this issue. Vancomycin (Figure 1.8), the antibiotic used in the treatment of Gram-positive bacterial infections, is one example of a drug that has become the last line of antibiotics due to resistant organisms. Over the past two decades, vancomycin has been extensively modified to try and fight the increasing resistance it now encounters.¹²⁰⁻¹²² Such studies focus on developing prodrugs, i.e. derivatives that can be transformed *in vivo* into the active drug. Adding a hydrophobic substituent to the disaccharide moiety of vancomycin retains activity but imparts unfavourable absorption, distribution, metabolism and excretion (ADME) properties. These studies led to the hypothesis by Mu and associates that introduction of a metabolically labile linkage into the lipid chain would present the prospect of the linkage being able to degrade *in vivo* to a more hydrophilic product with an improved ADME profile.¹⁰⁰ They prepared disulfide prodrugs of vancomycin **8** by addition of a disulfide-containing lipid chain to vancomycin, Figure 1-8. These analogues have been shown to exhibit potent *in vitro* antibacterial activity against resistant strains of bacteria including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci.¹⁰⁰ In addition, an improved ADME profile was observed as a result of the presence of the disulfide linkage.

**Vancomycin****8****Figure 1- 8** Structure of Vancomycin and Vancomycin Prodrugs **8**.

Besides being used for overcoming drug-resistance problems, pro-drug methodology has also been employed for developing more easily deliverable forms of drugs. Paclitaxel is a chemotherapeutic agent used for many types of cancer but suffers from aqueous insolubility.¹²³ Hydrophilic disulfide prodrugs of Paclitaxel **9** (Figure 1-9) have been synthesized and *in vitro* studies have revealed that these pharmacologically inactive derivatives reduce cytotoxic activity in L2987 lung carcinoma cells, suggesting that the compounds are reductively activated to the active parent drug at the target site.⁹⁹ Reversed-phase analytical HPLC studies confirmed that the prodrugs underwent reduction, releasing Paclitaxel. *In vivo* evaluations carried out on athymic nude mice that had subcutaneous L2987 human lung adenocarcinoma xenografts demonstrated that certain compounds exhibited activities superior to the parent Paclitaxel.⁹⁹

There has also been significant interest in using a cysteine disulfide linkage to tag a chemotherapeutic agent such as an anti-cancer drug to a peptide carrier to generate a pro-drug form.^{99,124,125}

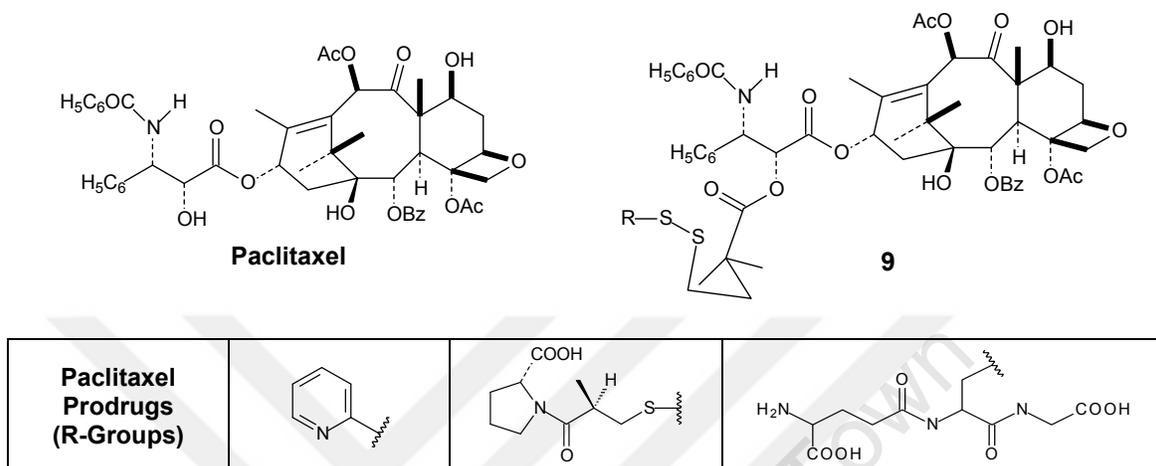


Figure 1-9 Structure of Paclitaxel and Paclitaxel Prodrugs 9.

Glutathione Redox Cycle

A premier example of the importance of the disulfide bond is in biological systems and its relevance to the glutathione redox cycle, Figure 1-10.

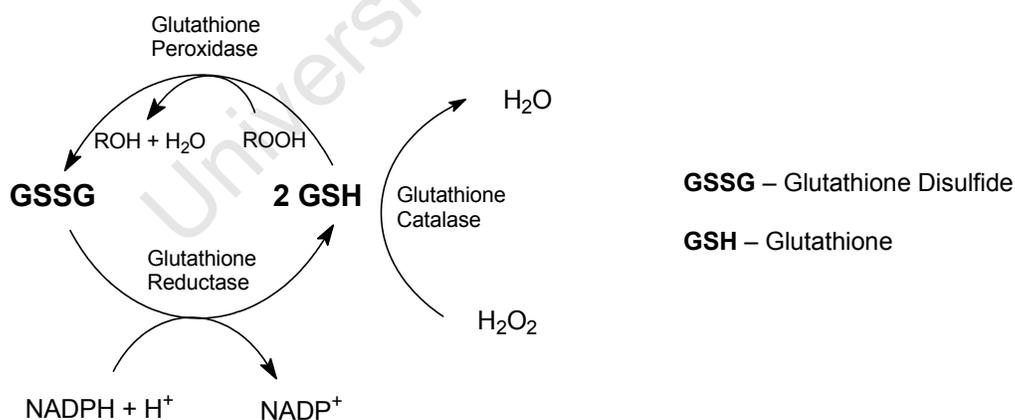


Figure 1-10 Simplified representation of the human glutathione redox cycle.

Figure 1-11 illustrates the structures of glutathione disulfide and glutathione.

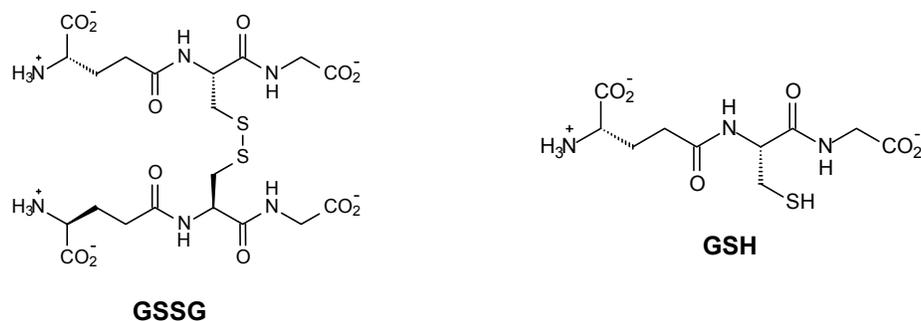
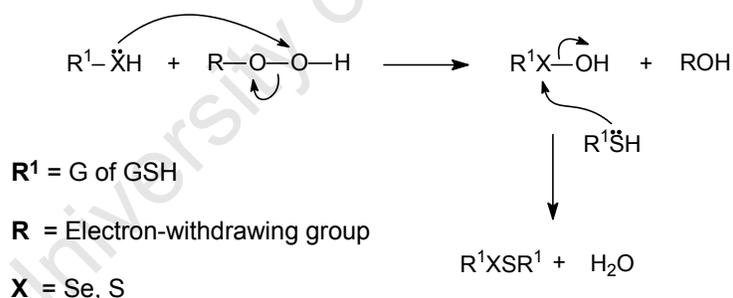


Figure 1-11 Structures of glutathione disulfide (GSSG) and glutathione (GSH).

During normal aerobic respiration, reactive oxygen species such as superoxide ($O_2^{\cdot-}$), pernitrite ($ONOO^-$), hydroxyl radicals (HO^{\cdot}), peroxy radicals (HOO^{\cdot}) and hydrogen peroxide (H_2O_2) are produced. The body's main defense system against these molecules is the glutathione redox cycle in which glutathione reduces these species (e.g. organic peroxides by means of the enzyme glutathione peroxidase).

The redox cycle illustrates an important mechanistic principle of nucleophilic reactions involving a selenium nucleophile, which can be extended to include the sulfhydryl (thiol) group of relevance to this thesis. These are depicted in Scheme 1-11.



Scheme 1-11 Diselenide and disulfide bond formation.

In view of the central importance of unsymmetrical disulfide synthesis to this thesis, a review on the various syntheses will now be presented.

1.5 Review of Unsymmetrical Disulfide Synthesis

In the early days, the synthesis of unsymmetrical disulfides was chiefly motivated by the need for functional group methodologies. In light of this, various approaches for the formation of disulfides (symmetrical and unsymmetrical) were developed and consequently the period from

1960 to 1980 was the era in which disulfide methodology development was at its height. In more recent times, the focus of disulfide methodology has changed owing to the importance of the functionality in proteins, enzymes, hormones and its diverse application in the medicinal and biological fields. Today, chemists are constantly attempting to produce methodologies for synthesizing the S-S bond under increasingly mild conditions, and in a one-pot approach. Although many methods for the synthesis of unsymmetrical disulfides are known, the synthesis of pure unsymmetrical disulfides is often a difficult and problematic process. In addition to synthesizing thiosulfonates, the motivation behind this research project was to develop an improved methodology for constructing unsymmetrical disulfides.

1.5.1 Mechanism of Unsymmetrical Disulfide Formation

A huge number of papers have been published on the synthesis of unsymmetrical disulfides. However, they all fall into one of three categories, **A**, **B** or **C** as depicted in Figure 1-12.

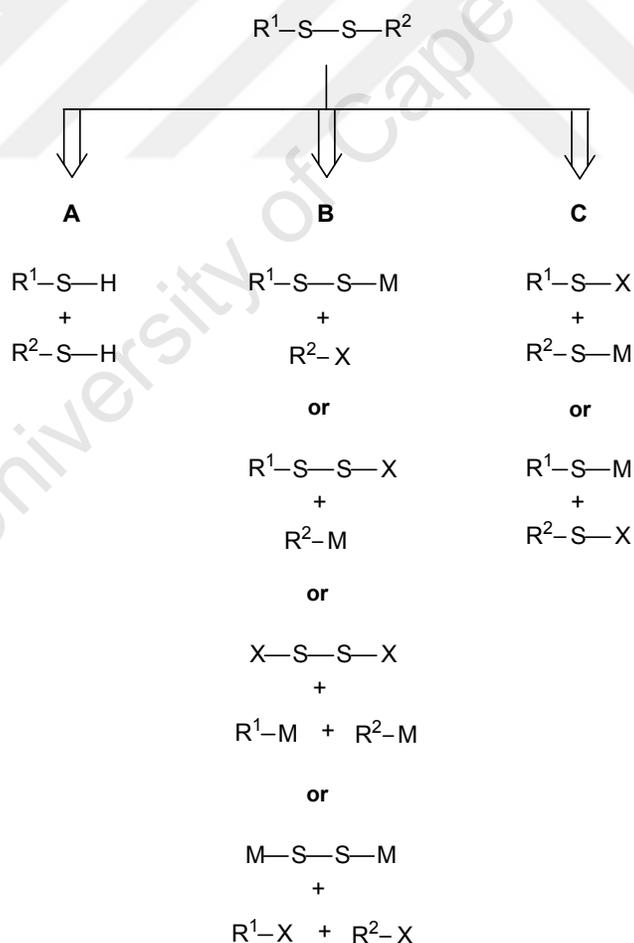


Figure 1-12 Unsymmetrical disulfide disconnections (X = a leaving group, M = hydrogen or a metal).

The oxidation of thiols (category **A**) has been utilized for many years but is more appropriate for the preparation of symmetrical disulfide synthesis, while category **B** has not been reported in the literature to any great extent. By comparison, category **C** has been exhaustively explored. The following section will give an account of the methodologies that have been thoroughly explored.

1.5.2 Categories for Unsymmetrical Disulfide Formation

Category A – Oxidation of Thiols

The oxidation of thiols dates back to the 1920's and is probably the oldest approach for obtaining the disulfide functional group, but in the case of unsymmetrical disulfide preparation, three products are possible, as illustrated in Scheme 1-12.



Scheme 1-12 Oxidation of thiols to unsymmetrical disulfides.

The literature contains a long list of available oxidants such as O_2 , I_2 , H_2O_2 , PbO_2 , Fe^{3+} salts, Br_2 , Cl_2 , $KMnO_4$, $CuSO_4$, hypohalites, SO_2 , PCl_5 , peracids and their salts, barbituric acid derivatives, azodicarbonamide, PCC, fluoride-Celite and nitrosobenzene. However, obtaining the unsymmetrical disulfide chemoselectively has not been possible. Cullum reported the following ratios of symmetrical to unsymmetrical disulfides formed when two different thiols are oxidized in a 1:1 ratio using different oxidizing agents.¹²⁶

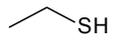
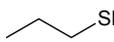
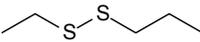
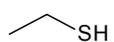
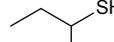
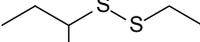
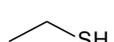
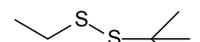
Thiol 1 (R^1SH)	Thiol 2 (R^2SH)	Oxidizing Agent	Products Obtained			Ratio 1:U:2
			Symmetrical Disulfide 1	Unsymmetrical Disulfide U	Symmetrical Disulfide 2	
		I_2	R^1SSR^1		R^2SSR^2	1:2:1
		H_2O_2	R^1SSR^1		R^2SSR^2	1:4.5:1
		$K_3Fe(CN)_6$	R^1SSR^1		R^2SSR^2	1:6:1

Table 1-2 Ratios obtained for oxidation of thiols to disulfides.

These results suggest a dependency on the type of thiol oxidized. With two primary thiols, the ratio of the unsymmetrical to symmetrical disulfides is almost equal but with more substituted

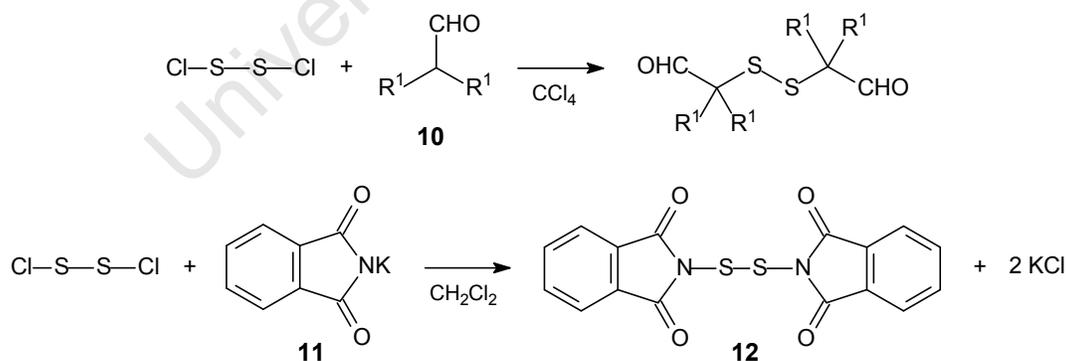
thiols, the ratio favours formation of the unsymmetrical disulfide. Production of a mixture of all three products necessitates tedious separation, which is a major drawback of the methodology. Additional disadvantages include long reaction times, low yields, the use of often expensive, rare or toxic reagents and the possibility of the disulfide being oxidized further to the sulfonic acid if reaction conditions are too harsh or the oxidant is too strong. Furthermore, there is always the chemoselectivity issue of other functional groups present being oxidized and thus this is not the methodology of choice for this transformation.

Category B – Nucleophilic Substitution with the Disulfide Bond Intact

This category involves adding R-groups to an existing S-S bond. As illustrated in Figure 1-12, category **B** can take the form of four basic types and although they are all plausible, they are either modestly reported in the literature or are absent altogether. Two types that have been reported in the literature are: (a) reaction of X-S-S-X with R-M and (b) reaction of M-S-S-M with R-X.

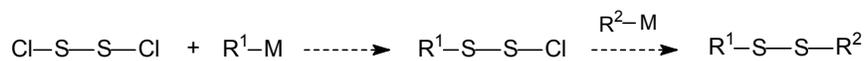
Reaction of X-S-S-X with R-M

Sulfur monochloride (S_2Cl_2), which is a highly toxic reagent, can provide an electrophilic site for reaction with various reagents (RM) in which M is a metal or a hydrogen atom, such as monoaldehydes **10** and potassium phthalimide **11**, to form disulfides, Scheme 1-13.^{127,128} The bis-sulfenamide **12** can be used for the formation of unsymmetrical disulfides.



Scheme 1-13 Symmetrical disulfide formation by reaction of S_2Cl_2 .

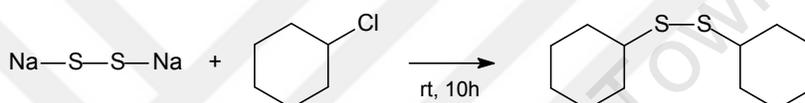
The reaction of S_2Cl_2 usually gives symmetrical products and is inappropriate for unsymmetrical targets as expressed in Scheme 1-13. Indeed, no examples exist in the literature for this approach as revealed in Scheme 1-14.



Scheme 1-14 Hypothetical reaction scheme for unsymmetrical disulfide formation by reaction of sulfur monochloride.

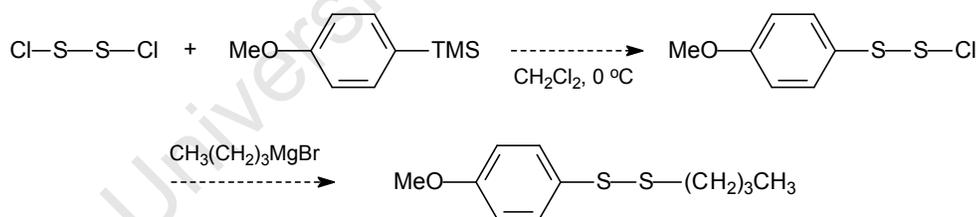
Reaction of M-S-S-M with R-X

Disodium disulfide (Na_2S_2) also reacts with various reagents to form symmetrical disulfides (Scheme 1-15) using sulfur as the nucleophilic partner.¹²⁹ However, no publications for unsymmetrical disulfide synthesis *via* M-S-S-M have appeared.

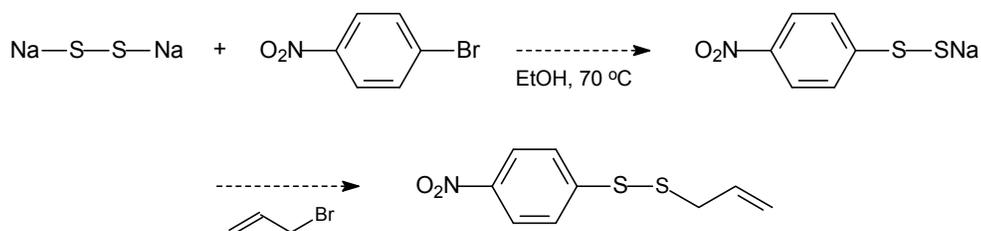


Scheme 1-15 Symmetrical disulfide formation by reaction of Na_2S_2 .

In addition, attempts to form unsymmetrical disulfides *via* these approaches in our laboratory were unsuccessful, Schemes 1-16 and 1-17. Frustratingly, a complex mixture of products resulted as shown by TLC, and no major product could be isolated by column chromatography or the isolated products could not be identified by NMR spectroscopy.



Scheme 1-16 Proposed reaction for unsymmetrical disulfide synthesis *via* sulfur monochloride.



Scheme 1-17 Proposed reaction for unsymmetrical disulfide synthesis *via* disodium disulfide.

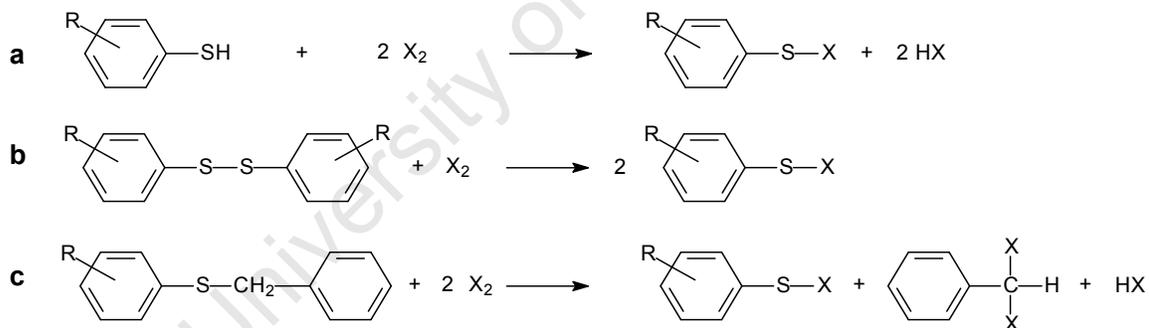
The absence of unsymmetrical disulfide synthesis *via* category **B** in the literature and the unsuccessful synthesis in our laboratory is evidence that the approach is inappropriate for routine unsymmetrical disulfide production.

Category C – Sulfenylation of a Thiol

This category is the most prolific and important. It will be presented with reference to the sulfenylating (electrophilic) reagents used.

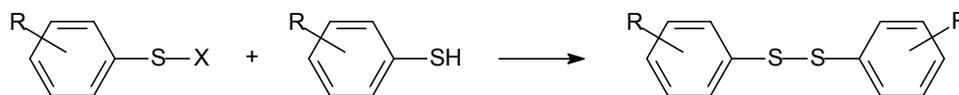
Sulfenyl Halides – RSCI

The first reported synthesis of sulfenyl halides was described by Zincke,¹³⁰ who in the early 1900's demonstrated that aromatic sulfenyl chlorides and bromides could be synthesized by reacting chlorine or bromine with an aromatic disulfide, an aromatic thiol or an aromatic benzyl sulfide at low temperature, and under anhydrous conditions as illustrated in Scheme 1-18.¹³¹⁻¹³⁴ The methods were employed according to the availability of the starting materials and essentially gave the same product. Aliphatic sulfenyl halides were only prepared and studied from the 1940's.



Scheme 1-18 Formation of sulfenyl halides *via* (a) thiols, (b) disulfides and (c) aromatic benzyl sulfides.

When reacting a thiol to make the sulfenyl halide intermediate (equation **a** in Scheme 1-18), a secondary reaction between the thiol and sulfenyl halide can take place to form the symmetrical disulfide (Scheme 1-19), which can, therefore, be the only product obtained. However, carrying out the reaction under controlled conditions of low temperatures under anhydrous conditions, and adding the thiol very slowly to a solution of the halogenating species in an inert solvent (chloroform, benzene, pentane or carbon tetrachloride) allows the formation of certain sulfenyl halides.



Scheme 1-19 Secondary reaction forming symmetrical disulfide.

The conditions required when using a disulfide to make a sulfenyl halide (equation **b** in Scheme 1-18) are essentially the same as those used in the preparation of sulfenyl halides from thiols. A limitation, though, is the possibility of halogenating the aromatic ring.

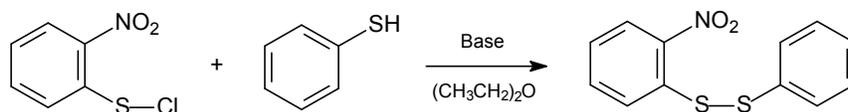
The formation of sulfenyl halides from sulfides (equation **c** in Scheme 1-18) follows similar trends to those for the disulfide and thiol approaches except that only aryl benzyl sulfides can be converted to the sulfenyl halide, with concomitant formation of the benzyl 1,1-dihalide. Aliphatic sulfides undergo the Pummerer rearrangement, thus chlorinating the α -carbon atom instead of forming the sulfenyl derivative, Scheme 1-20.



Scheme 1-20 Pummerer rearrangement of aliphatic sulfides.

Aliphatic sulfenyl halides are far less stable than aromatic ones but both are rapidly hydrolysed by water to the sulfenic acid making these compounds difficult to isolate and purify. While the aromatic variants can be distilled, the aliphatic cases are usually unstable. This may be attributed to the absence of protons on the carbon adjacent to the sulfur atom as well as resonance stabilization involving the aromatic ring.¹³⁵ Behforouz also found that aliphatic sulfenyl halides are more stable in less polar solvents such as *n*-pentane, xylene, carbon tetrachloride and cyclohexane.¹³⁵ In addition to Br_2 and Cl_2 , these days other reagents for the halogenation are also available including SCl_2 , S_2Cl_2 , SO_2Cl_2 and PCl_5 .

Sulfenyl halides readily react with thiols or thiolates to form unsymmetrical disulfides. The earliest reference to their formation dates back to the early 1920's when Lecher demonstrated that 2-nitrobenzenesulfenyl chloride dissolved in dry ether reacted with thiophenol to rapidly produce the unsymmetrical disulfide, 2-nitrophenyl phenyl disulfide, Scheme 1-21.¹⁰⁹ The presence of a base, usually triethylamine, was required to neutralize the hydrogen halide by-product formed. A plethora of examples in the literature on the subject have since been reported.



Scheme 1-21 Lecher's synthesis of an unsymmetrical disulfide.¹⁰⁹

The general sequence for unsymmetrical disulfide formation from a sulfonyl halide is thus illustrated in Scheme 1-22. After formation of the sulfonyl halide by one of the reactions described before, a nucleophilic displacement of the sulfonyl intermediate by a second thiol affords the unsymmetrical disulfide. In view of the instability of aliphatic sulfonyl halides, when making aromatic-aliphatic unsymmetrical disulfides, the aliphatic moiety is added as the second thiol. Naturally, the methodology is not appropriate for the synthesis of aliphatic-aliphatic unsymmetrical disulfides.



Scheme 1-22 General scheme for unsymmetrical disulfide synthesis *via* sulfonyl halides.

Over the years, the sulfonyl halide methodology has been extensively used for preparation of a wide variety of unsymmetrical disulfides for various purposes and applications. In the early days, scientists were only interested in making functional groups but later owing to the discovery of the importance of the disulfide functionality, scientists were striving to make disulfide compounds with potential biological significance and as potential medicinal agents. An excessive amount of literature on the topic is available and would require a separate review to cover them all, hence only a few examples will be mentioned.

In 1947, Jenkins utilized the sulfonyl halide methodology for synthesizing unsymmetrical aromatic-aromatic disulfides as possible chemotherapeutic agents. His study was based on the findings that certain symmetrical disulfides showed activity against a number of microorganisms.^{136,137} The research was limited to aromatic-aromatic unsymmetrical disulfides with a nitrogen atom attached to the aromatic ring. The reason for the preference for these compounds is unfortunately not known. Several compounds, a few of which proved to be active chemotherapeutic agents were produced in good yields (60-90%) by reaction of sulfonyl halides with thiols in the presence of a copper-bronze catalyst. Compounds that were prepared are shown in Figure 1-13.

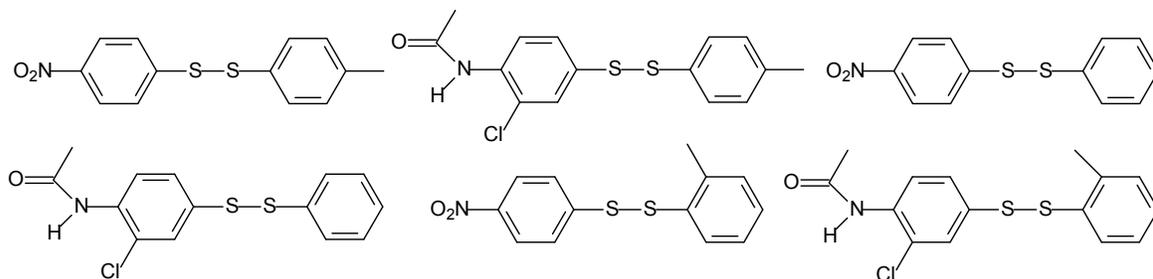


Figure 1-13 Unsymmetrical disulfides as potential chemotherapeutic agents.

Similarly, Schoberl and co-workers prepared unsymmetrical disulfides possessing a carboxylic acid moiety, as radical initiators for the polymerization of compounds such as styrene based on the polarizable S-S bond.¹³⁸ A few of the disulfides prepared by the reaction of sulfonyl chlorides with carboxylic acid thiols and obtained in low yields (20-40%) are illustrated in Figure 1-14.

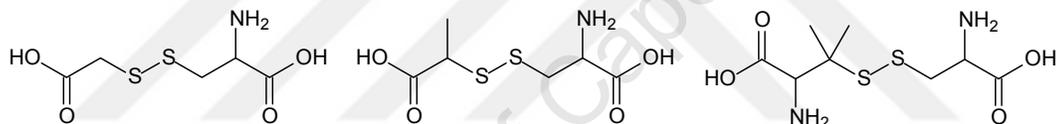
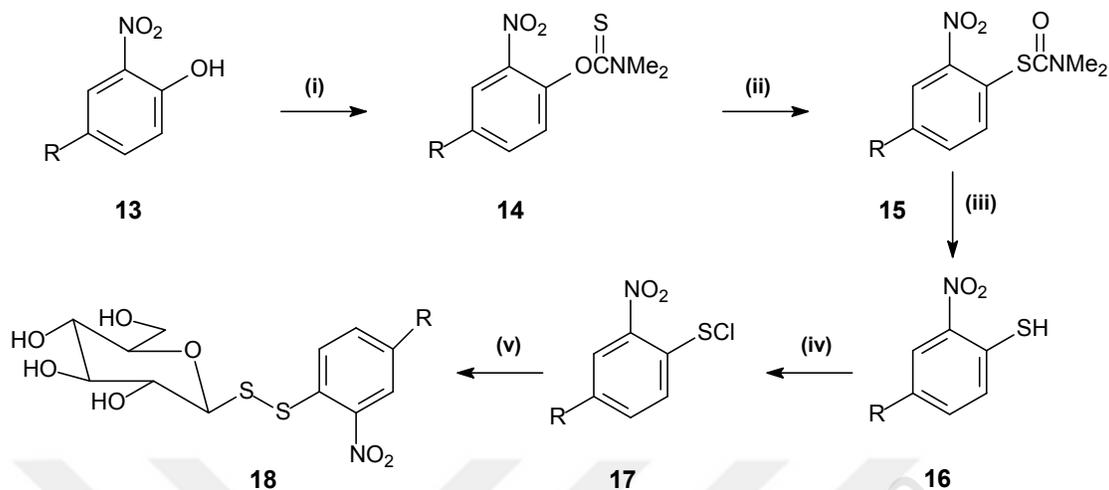


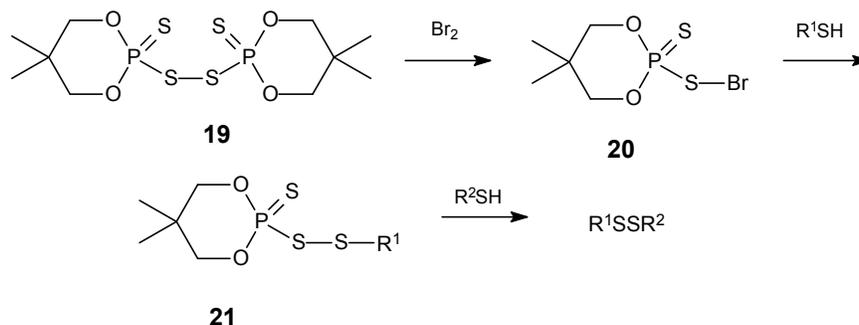
Figure 1-14 Unsymmetrical carboxylic acid disulfides.

In 1980, Keana's group constructed unsymmetrical aryl glucosyl disulfides as potential protein solubilizing agents. Solubilizing agents are used in the isolation and purification of membrane proteins.¹³⁹ The synthesis involved conversion of a substituted nitrophenol **13** to a sulfonyl halide **17** via a Newman-Kwart O→S rearrangement of an O-aryl thiocarbamate¹⁴⁰ **14**, which upon addition of a thiol sugar gave the unsymmetrical disulfide **18**, Scheme 1-23. The coupling reaction did not proceed in the absence of 15-crown-5.¹³⁹ The overall yield for the synthesis ranged between 40-67%.



Scheme 1-23 Reagents and Conditions: (i) NaH, DMF; ClCSNMe₂, 0 °C. (ii) Δ (190 °C). (iii) K₂CO₃, MeOH, reflux. (iv) SO₂Cl₂, CHCl₃, 25 °C. (v) β-D-thioglucose sodium salt, 15-crown-5, CH₃CN, 25 °C.

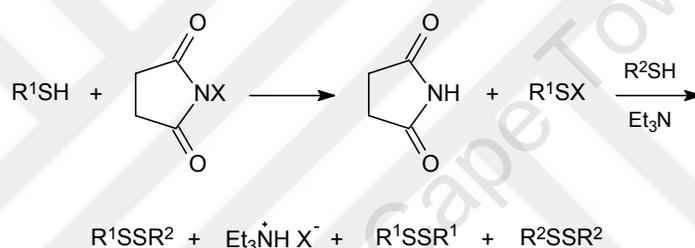
More recently, the sulfenyl halide methodology has been replaced by other procedures. However, in 2007 an example involving a sulfenyl halide as a key intermediate was reported by Witt and Antoniw.¹⁴¹ They presented an interesting approach in which the sulfenyl halide intermediate was used as a sulfur-transfer agent. The methodology was used to prepare aliphatic-aliphatic and aliphatic-aromatic unsymmetrical disulfides. The starting material, bis(5,5-dimethyl-2-thiono-1,3,2-dioxaphosphorinanyl)disulfide **19** (**19** was prepared by treatment of 5,5-dimethyl-2-thiolo-2-thiono-1,3,2-dioxaphosphorinane in aqueous alkali with NaNO₂),¹⁴⁰ was treated with bromine to give the sulfenyl halide intermediate **20**, (5,5-dimethyl-2-thiono-1,3,2-dioxaphosphorinanyl)sulfenyl bromide, which is then reacted with a thiol (R¹SH) to form a half-way product **21** that can be isolated.¹⁴¹ This compound is then reacted with a second thiol to afford the unsymmetrical disulfide product in excellent overall yield (95-100%). The complete synthesis is illustrated in Scheme 1-24.



Scheme 1-24 Unsymmetrical disulfide synthesis *via* a sulfenyl halide transfer agent.

The paper does not mention the formation of symmetrical disulfide (R^1SSR^1). However, the authors only used aliphatic thiols as R^1SH whose homodimers are easily separable from the intermediate product. Hence, the intermediate was purified before the second thiol was added, thus presenting a two-step procedure.

In an attempt to avoid using Cl_2 as the source of chlorination, Emde¹⁴² and Benn¹⁴³ demonstrated that *N*-bromo- or *N*-chloro-succinimide transforms thiols into sulfenyl halides, which upon addition of a second thiol generates the unsymmetrical disulfide, Scheme 1-25. However, symmetrical disulfides (R^1SSR^1 and R^2SSR^2) are obtained in all cases. R^1SSR^1 is obtained owing to the secondary reaction of the incoming thiol (R^1SH) with the sulfenyl halide formed while R^2SSR^2 is obtained as a result of the excess chloro-succinimide, which in a competing reaction converts the R^2SH to R^2SCI .



Scheme 1-25 Thioalkylation of thiols by succinimide reagents.

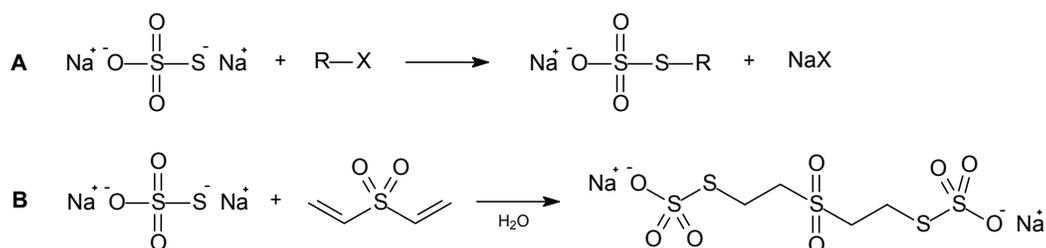
Although the approach makes use of less hazardous reagents, the need for a methodology without symmetrical disulfide formation remains a key objective in modern-day synthesis.

Over the last two decades, the use of the sulfenyl halide methodology has faded due to a number of unavoidable drawbacks as well as the availability of alternative approaches for making unsymmetrical disulfides. Drawbacks to the methodology include the use of harsh reagents to prepare the sulfenyl halides and the difficulty of purifying the sulfenyl halide derivatives owing to their thermal instability and moisture sensitivity. In addition, the majority of unsymmetrical disulfide synthesis *via* sulfenyl halides requires additional reagents such as a base or a catalyst, proceed *via* multiple steps and it is invariably impossible to avoid obtaining the symmetrical disulfide due to the reactivity of the sulfenyl halide.

Bunte Salts $RSSO_3M$

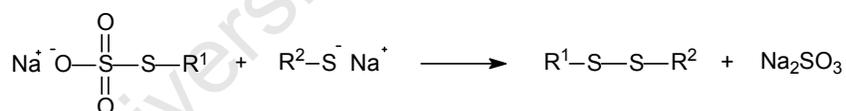
Bunte salts are alkali metal *S*-alkylthiosulfates and *S*-arylthiosulfates ($RSSO_3^-$), which were first prepared in 1874 by Bunte who synthesized sodium *S*-ethylthiosulfate by heating an aqueous solution of sodium thiosulfate with ethyl bromide.¹⁴⁴ Since then, many methods for

their preparation have been reported, including (i) reaction of thiosulfate with quinones, epoxyalkanes, activated double bonds, oxiranes and aziridines, 1° and 2° alkyl halides (Scheme 1-26 **A**) or divinyl sulfone (Scheme 1-26 **B**), (ii) reaction of sulfurous acid with sulfenyl chlorides or amides and (iii) reaction of thiols with SO₃ or ClSO₃H.¹⁴⁵⁻¹⁴⁸



Scheme 1-26 Synthesis for preparation of Bunte salts *via* (A) alkyl halides, (B) divinyl sulfones.

Bunte salts have been used as intermediates in various synthetic reactions including preparation of thiocyanates from reacting S-alkylthiosulfates with aqueous alkali metal cyanide solution, and can be hydrolyzed with acids to give the corresponding thiols, disulfides, tetrasulfides or pentasulfides.^{146,149} Of great interest to us was the popularity of the Bunte salts from the early 1920's as an approach for producing unsymmetrical disulfides *via* thiols or thiolates. Scheme 1-27 illustrates the reaction of a Bunte salt with a thiolate, which proceeds by S_N2 attack at the softer sulfur atom attached to the alkyl group of the sulfenylating thiosulfate salt with expulsion of a sulfite moiety.



Scheme 1-27 Synthesis of unsymmetrical disulfides *via* Bunte salts.

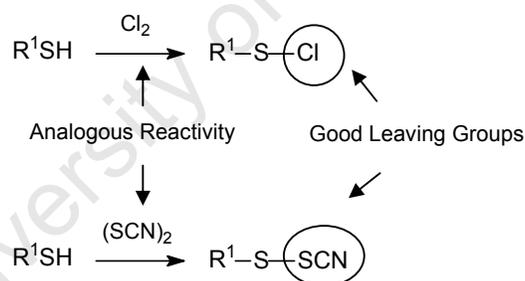
In 1925, Footner and Smiles picked up on the theme and demonstrated the ease of producing symmetrical disulfides (R¹ = R²) in alkaline aqueous solution as well as the difficulty in obtaining the pure unsymmetrical disulfides in high yields.¹⁴⁹ Reactions invariably resulted in a mixture of all three disulfides, which were often difficult or impossible to separate. This was due to the secondary reaction between the newly formed unsymmetrical disulfide and thiolate reagent.¹⁰⁹ Swan, in 1957, re-examined the thiosulfinate-thiol reaction in dilute acid solution, flushing the reaction mixture with nitrogen to remove the sulphur dioxide formed from protonation of the sulfite ion by-product, and isolated the desired unsymmetrical disulfide in high yield.¹⁵⁰ Various cysteine-containing unsymmetrical disulfides, **22** and **23** (Figure 1-15)

methodology for aliphatic disulfides is the separation of the unsymmetrical disulfide product from the added symmetrical disulfide.

In summary, the Bunte-salt method occupied for many years a popular place in the repertoire of unsymmetrical disulfide synthesis and was one of the more popular methods in the early days. However, it leads to the formation of symmetrical disulfides, and in the modern era has been by and large replaced by other methods and approaches.

Sulfenyl Thiocyanates

The disadvantages encountered while using the sulfenyl halide approach for preparing unsymmetrical disulfides encouraged chemists to search for surrogates of the halide leaving group. In 1922, Lecher described the synthesis of unsymmetrical disulfides by reacting a sulfenyl thiocyanate with a thiol.¹⁵² This study was undeniably prompted by the similarities in the properties of thiocyanogen and halogens (chlorine) (Scheme 1-29), and since some sulfenyl halides were difficult to make it was suggested that sulfenyl thiocyanates would provide a better alternative. This proved to be true. Subsequently, many papers appeared in the literature using sulfenyl thiocyanates as intermediates for forming disulfides.



Scheme 1-29 Similarities between sulfenyl halides and sulfenyl thiocyanates.

Sulfenyl thiocyanates can be prepared in two ways involving either reaction of a thiol with thiocyanogen (Scheme 1-30) or by reaction of a sulfenyl halide with a metal thiocyanate. The latter is generally unattractive in view of the need to have access to sulfenyl halides. Preparation of thiocyanogen involves converting sodium thiocyanate to lead thiocyanate by precipitation with lead nitrate followed by oxidative treatment with bromine.¹³¹ The thiocyanogen produced is used immediately without purification, and care must be taken to avoid having excess bromine. Addition of a thiol affords a sulfenyl thiocyanate intermediate **26**, which can be isolated *via* extraction but which has a limited shelf-life. Addition of a second thiol results in formation of an unsymmetrical disulfide with the displacement of thiocyanic acid, which is neutralized by a base, usually pyridine, Scheme 1-30.



26

Scheme 1-30 Unsymmetrical disulfide formation *via* sulfenyl thiocyanates.

Compared to sulfenyl halides, sulfenyl thiocyanates are reported to be slightly more hydrolytically stable, since they only slowly decompose in the presence of alcohols or water.¹³⁰ They are, however, thermodynamically unstable intermediates, and decompose rapidly above 0°C.¹⁵³ They are also difficult to isolate in a pure form, as excess thiocyanogen is required to obtain a satisfactory conversion of starting thiol and purifying the compound from the thiocyanogen and thiocyanic acid by-products is tricky experimentally.¹⁵⁴ This is normally achieved by decomposing the unwanted compounds in the reaction mixture with ice before the sulfenyl thiocyanate is isolated by extraction into an organic solvent. Once again, a drawback of the approach is the formation of symmetrical disulfide R¹SSR¹ in the first step of the synthesis owing to the possible competing reaction of the thiol with the sulfenylating agent. In addition, a major limitation for the second step (reaction of R²SH) is presence of any excess thiocyanogen which results in the formation of R²SSCN followed by conversion to the symmetrical disulfide R²SSR².

In 1961, Hiskey and his associates developed the sulfenyl thiocyanate approach extensively for synthesis of a range of unsymmetrical aliphatic disulfides including unsymmetrical cysteine derivatives.¹⁵³ However, they reported that often the desired unsymmetrical disulfide product was contaminated with symmetrical disulfide and purification usually required multiple distillations. Before the study by Hiskey, not many aliphatic unsymmetrical disulfides were reported in the literature, especially not those containing the cysteine moiety. The intense interest in making cysteine-containing disulfides stemmed from observations by scientists between 1940 and 1960 that disulfide bonds cross-linking peptide chains may be considered synthetically as unsymmetrical aliphatic disulfides. Aware of the importance of being able to form a disulfide bond for application in peptide synthesis as well as the increasing interest in the chemical behaviour of the disulfide bond in natural macromolecules, Hiskey attempted various methods to achieve aliphatic unsymmetrical disulfides.¹⁵³ Substitution of thiosulfonates with thiols and the Bunte-salt method both proved to be unsuccessful, and he thus focused on developing the sulfenyl thiocyanate method, which eventually led to the production of a range of aliphatic unsymmetrical disulfides. Hiskey later applied the method to preparing cysteine-containing polypeptides containing a range of amino acid residues.¹⁵⁵ This approach also

proved to be effective for stepwise formation of disulfide bonds in polypeptides with previously formed disulfide bonds surviving further reactions.¹⁵⁵⁻¹⁵⁷

Although the methodology proved to be a versatile one, it unfortunately has several disadvantages including; (i) the preparation of the thiocyanogen which involves the use of harsh reagents such as lead thiocyanate, sodium thiocyanate and bromine, (ii) the need for base to neutralize the thiocyanic acid formed, (iii) the need for excess thiocyanogen to drive the reaction to completion, leading to by-product formation in subsequent steps, (iv) the thermodynamic instability of the sulfenyl intermediate and (v) the formation of symmetrical disulfides.

Thiosulfonates and Thiosulfates

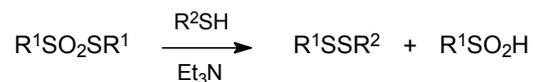
Thiosulfonates (RSO_2SR) and thiosulfates (RSOSR) have been used for many years as sulfenylating agents on account of the leaving group ability of the sulfonate and sulfinate ions respectively, Figure 1-17. The first reported examples date back to the late 1940's.



Figure 1-17 Formation of sulfonate and sulfinate ions.

Thiosulfonates

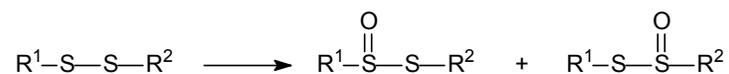
Thiosulfonates are relatively stable compounds that are fairly easily made and purified. They have the ability to thioalkylate a range of thiols and thiol derivatives in the presence of base, generally pyridine or triethylamine, to form an unsymmetrical disulfide and a sulfonic acid, Scheme 1-31.^{158,159} The base converts the thiol (R^1SH) to a thiolate which aids in the complete and rapid formation of the unsymmetrical disulfide. Excluding base, leads to extremely low yields and long reaction times. However, reactions can be accelerated by the addition of excess thiol but at the expense of the unsymmetrical disulfide yield in view of an exchange reaction with the excess thiolate.¹⁵⁹



Scheme 1-31 Unsymmetrical disulfide formation *via* thiosulfonates.

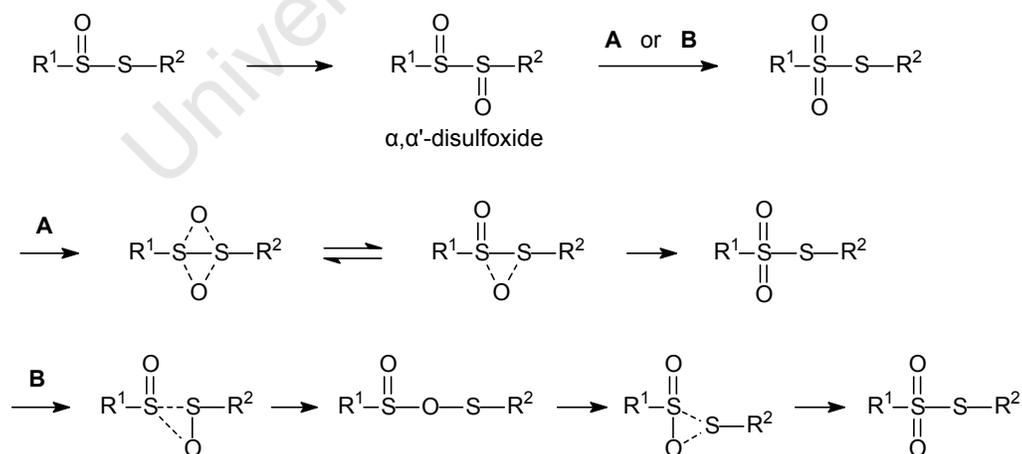
Thiosulfonates can be prepared by (i) the reaction of sulfonyl halides and thiols in the presence of pyridine, (ii) the reaction of sulfenyl halides and sulfenic acid in the presence of pyridine or (iii) the oxidation of disulfides *via* thiosulfonates.^{158,160-163}

Initial oxidation of an unsymmetrical disulfide, where R¹ is an alkyl chain and R² is an aromatic ring, can afford two regioisomeric thiosulfonates as the oxidation products, Scheme 1-32.



Scheme 1-32 Initial disulfide oxidation (R¹ = alkyl chain and R² = aromatic ring).

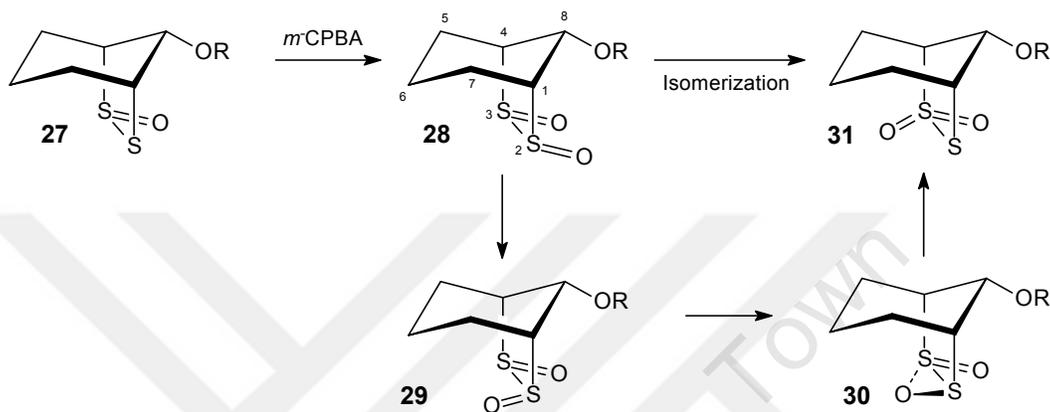
The isomer obtained or the ratio of the isomers is dependent on the relative electron density of the sulfur atoms, the more electron-rich sulfur atom being oxidized preferentially.¹⁶⁴ The product of further oxidation caused controversy for many years. It was expected that oxidation would take place at the sulfenyl sulfur atom rather than at the less nucleophilic sulfinyl sulfur atom.^{165,166} The expected product, however, was not observed until the early 1980's when Freeman's low temperature NMR study on the oxidation of aralkyl thiosulfonates confirmed the presence of the elusive α,α' -disulfoxide (see below). Interestingly, both the α,α' -disulfoxide and thiosulfonate oxidation products were observed but only the thiosulfonate was isolated. Oae and Freeman reported that the α,α' -disulfoxide rearranges at temperatures above -40 °C *via* one of two mechanisms; **A** – isomerization and **B** – intramolecular rearrangement followed by isomerization, Scheme 1-33.¹⁶⁷⁻¹⁶⁹



Scheme 1-33 Thiosulfonate formation *via* α,α' -disulfoxide (R¹ = alkyl chain and R² = aromatic ring).

Additional oxidation products such as sulfenic and sulfonic acids were also observed. However, they will not be discussed in this thesis.

Further evidence for the formation of α,α' -disulfoxide **28** was reported by Folkins and Harpp in 1991, by *m*-CPBA oxidation of a bridged bicyclic thiosulfinate **27**, Scheme 1-34.¹⁷⁰ Compound **28** was identified by low temperature ¹³C NMR studies which revealed that C1 and C4 were chemically equivalent by virtue of symmetry about the C8-C6 axis, providing proof for the symmetrical α,α' -disulfoxide structure.

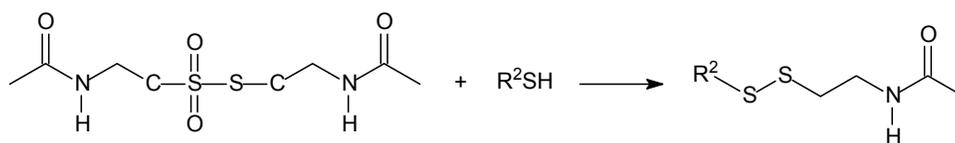


Scheme 1-34 Oxidation of bridged bicyclic thiosulfonates ($R = C(O)(CH_2)_4CH_3$).

Oxidation of **27** from the endo face to form **29** was not observed indicating that the endo face is highly hindered. The authors observed that the concentration of **28** increased until 0 °C after which it slowly isomerized to **29** and **30** to give thiosulfonate **31**, with traces of **28** still present at 10 °C. The bridged α,α' -disulfoxide **28** has been the most stable to date.

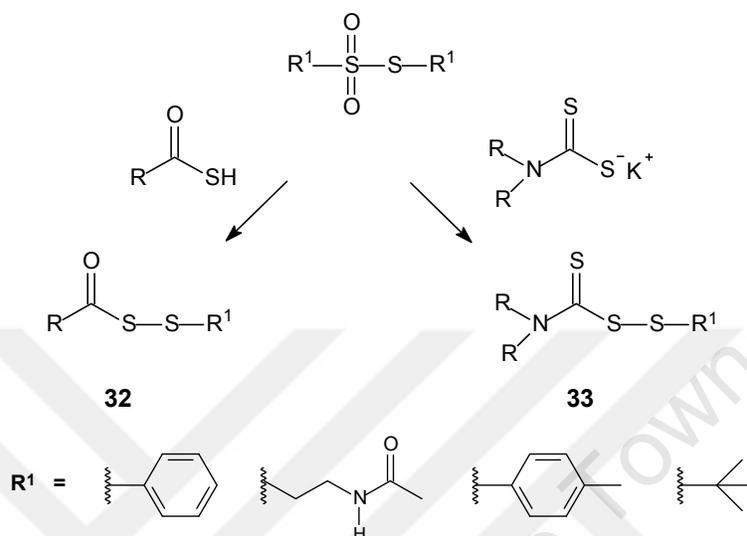
Thiosulfonates compared to other sulfonylating agents have not been utilized to any great extent for the synthesis of unsymmetrical disulfides. Nonetheless, they are notable sulfonylating agents.

In 1961, Field reported the synthesis of unsymmetrical disulfides containing a 2-aminoethylthio moiety for the use as protective agents against ionizing radiation by reacting a thiosulfonate with a thiol.¹⁷¹ The acetyl-protected 2-aminoethylthio moiety was incorporated into the thiosulfonate and rapidly reacted with thiols to give a range of unsymmetrical disulfides, Scheme 1-35.



Scheme 1-35 Unsymmetrical disulfide formation via thiosulfonates ($R^2 = t\text{-Bu, tolyl, } p\text{-NO}_2\text{Ph}$).

A few years later, Field demonstrated that carbonyl (**32**) and thiocarbamoyl (**33**) unsymmetrical disulfides could be prepared by the reaction of thiosulfonates with thiols, Scheme 1-36.^{172,173}



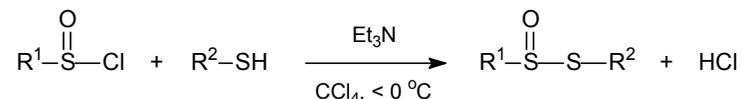
Scheme 1-36 Unsymmetrical disulfide formation via thiosulfonates.

Field demonstrated the versatility of the thiosulfonate approach and showed it to be less problematic in the purification steps. Being able to prepare and store the thiosulfonate in advance, owing to its stability, is an advantage to the approach. The drawbacks, however, outweigh the advantage of the methodology and include (i) the synthesis of the thiosulfonates which either requires the use of harsh reagents or the use of a disulfide as the starting material, (ii) half the thiosulfonate is wasted as the displaced sulfinate and (iii) the methodology requires the use of an added reagent, a base.

Thiosulfonates

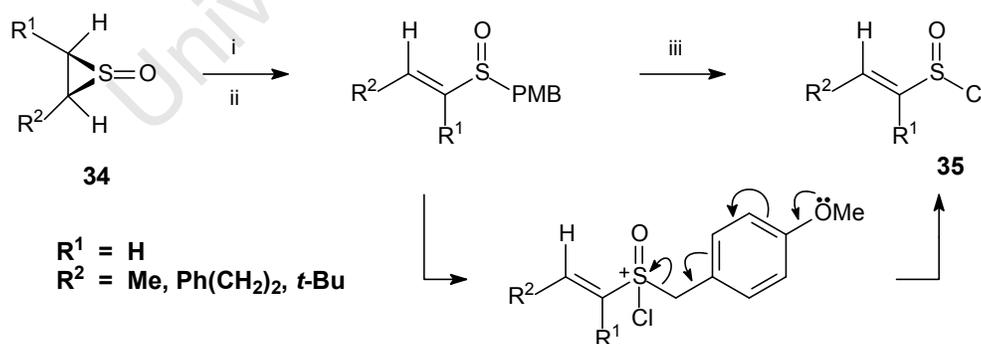
By comparison, thiosulfonates are rather unstable compounds. They are light- and temperature-sensitive, and as chiral molecules in view of an asymmetric sulfur atom, undergo rapid thermal racemization. Thiosulfonates can be prepared by (i) controlled oxidation of disulfides, (ii) reaction of sulfenic acids with various derivatives (eg. sulfonyl chloride), (iii) decomposition of sulfoxides having β -protons and (iv) reaction of sulfonyl chlorides (which need to be prepared) and thiols in the presence of a tertiary amine base.¹⁷⁴⁻¹⁷⁶ The latter was a popular approach in the early days and is still the most general and versatile method for preparing unsymmetrical thiosulfonates, with no limitation on the R groups, Scheme 1-37. However, in the absence of amine base and using a metal thiolate ($\text{R}^2\text{S}^- \text{Na}^+$) instead, no thiosulfonate is obtained.¹⁷⁷ The reaction also only proceeds when the thiol is added to a sulfonyl chloride, base mixture and not when the sulfonyl chloride is added to a thiol, base

mixture owing to the sensitivity of the thiosulfinate toward nucleophiles, resulting in the formation of the corresponding disulfide.



Scheme 1-37 Thiosulfinate synthesis *via* sulfinyl chlorides and thiols.

Sulfinyl chlorides are fundamental starting materials for a number of synthetic transformations including the preparation of chiral sulfoxides, sulfines and sulfinic acid derivatives.¹⁷⁸⁻¹⁸⁰ Preparation of sulfinyl chlorides is well documented with numerous approaches available for synthesis of arene-, alkane- and haloalkanesulfinyl chlorides. These include (i) reaction of thiols, disulfides or thioacetates with sulfuryl chloride, (ii) chlorination of thiols, thioacetates or disulfides in the presence of acetic anhydride and (iii) reaction of sulfoxides with sulfuryl chloride.¹⁸¹⁻¹⁸³ Recently, a facile synthesis of 1-alkenesulfinyl chlorides was reported.¹⁸⁴ 1-Alkenesulfinyl chlorides were formerly non-existent owing to the limited accessibility of alkenyl-substituted thiols and the reactivity of the double bond during chlorination. In 1996, Schwan and co-workers reported the first synthesis for preparation of 1-alkenesulfinyl chlorides *via* chlorination of sulfoxides, Scheme 1-38.¹⁸⁴ Regioselective LiHMDS deprotonation and consequent stereoselective ring opening of thiirane *S*-oxide **34** gave an (*E*)-1-alkenesulfenate anion which reacted with *p*-methoxybenzyl bromide (PMB-Br) to generate the corresponding sulfoxide. Reaction of the latter with sulfuryl chloride cleanly afforded 1-alkenesulfinyl chloride **35** *via* the fragmentation mechanism shown.

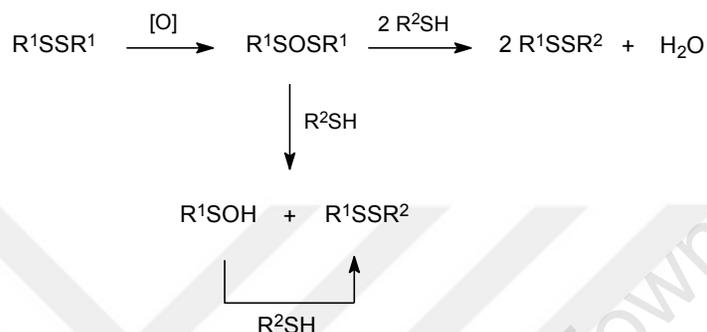


Scheme 1-38 Reagents and conditions: (i) LDA or LiHMDS, THF, -78 °C. (ii) PMB-Br, THF, -78 °C. (iii) SO₂Cl₂, THF, -78 °C to rt.

The synthesis led to the formation of a range of new 1-alkenesulfinyl chlorides for the use of various transformations. Although the sulfinyl chloride methodology is popular for the

formation of thiosulfates, the oxidation of symmetrical disulfides for the transformation is often easier and less hazardous.

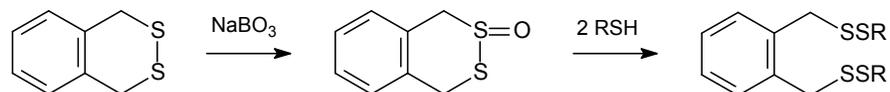
Thiols react readily with thiosulfates to give disulfides as illustrated by Scheme 1-39. An intriguing feature of the reaction is that one molecule reacts with two molecules of the thiol.



Scheme 1-39 Unsymmetrical disulfide synthesis *via* thiosulfates.

Thus, reaction of one mole of thiol with the thiosulfate produces an unsymmetrical disulfide and a sulfenic acid. In theory, the products could react to give randomization of the unsymmetrical disulfide, or alternatively the sulfenic acid could disproportionate to a sulfinic acid and thiol. In light of these possibilities, a second mole of thiol is included which then reacts with the sulfenic acid to form the second mole of unsymmetrical disulfide. Unlike the thiosulfonate method, the thiosulfate approach gives two moles of the desired unsymmetrical disulfide and can be carried out under either acidic or basic conditions.

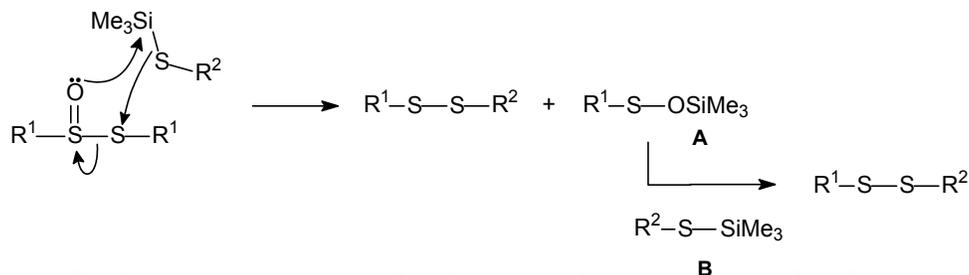
Before Field embarked on the study of thiosulfates as sulfonylating agents, the methodology was not much used or studied with only a few papers reported. In 1988, he prepared cyclic thiosulfates which reacted with aromatic or alkyl thiols to afford unsymmetrical disulfides, Scheme 1-40.¹⁸⁵ The products were unfortunately obtained in low yields (10-45 %).



Scheme 1-40 Cyclic thiosulfates used for synthesis of unsymmetrical disulfide.

Later, Capozzi demonstrated the reaction of thiosulfates with silylsulfides to afford unsymmetrical disulfides in high yields, Scheme 1-41.¹⁸⁶ It is postulated that the reaction of **A** with **B** is much faster compared to the reaction of **A** with the unsymmetrical disulfide. This is

not surprising since the sulfide sulphur is much more nucleophilic compared to the disulfide sulphur in view of silicon's relatively electropositive character; Capozzi did not observe the formation of symmetrical disulfides. The instability of the thiosulfinate, however, unfortunately makes the approach less attractive than alternatives.

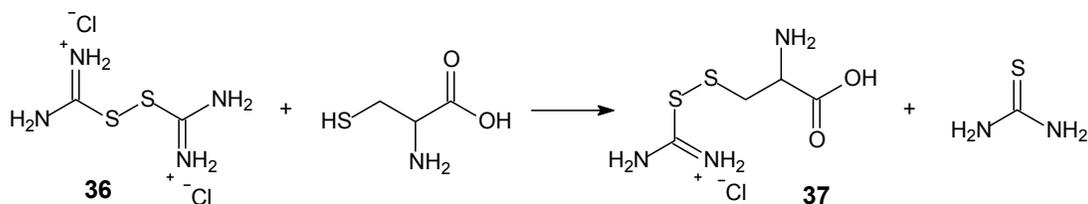


Scheme 1-41 Capozzi's reaction for formation of unsymmetrical disulfides.

Other S-Based Sulfonylating Agents

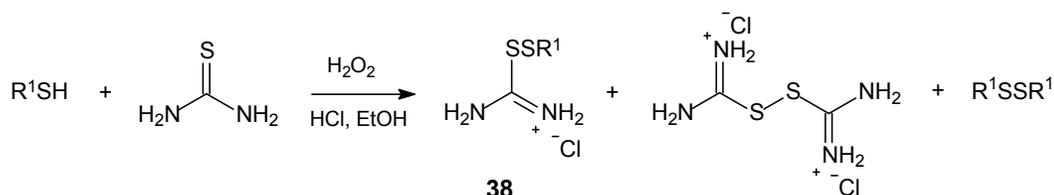
The remaining S-based sulfonylating agents comprise the sulfonylthioureas, sulfonylthiocarbonates, alkylthiodialkyl sulfonium salts and two anomalous methods; one based on sulfines and the other is a rhodium-catalyzed disulfide exchange reaction. Unlike majority of the previous examples in which the reactions needed to be carried out in a one-pot synthesis owing to the instability of the sulfonylating agent, for these cases the intermediate sulfonylating agent can be isolated and purified before the second nucleophilic substitution to form the unsymmetrical disulfide. This is desirable in view of the usage of harsh reagents, which may cause unwanted byproducts.

Toennies was the first person to prepare an S-alkylthioisothiurea salt **37** in 1937, by the reaction of dithioformamidine dihydrochloride **36** and cysteine, Scheme 1-42.¹⁸⁷ Salt **36** was prepared *via* Cl₂-oxidation of thiourea. This was followed by the work of Kopylova in 1964, who reported the synthesis of a range of S-alkylthioisothiurea salts by the reaction of either a disulfide or sulfonyl halide with thiourea.¹⁸⁸



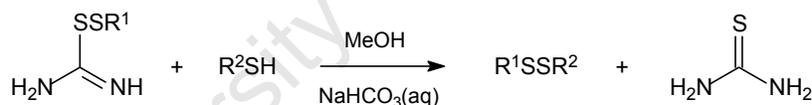
Scheme 1-42 Formation of S-cysteinethioisothiurea **37**.

In 1970, Sirakawa noted the versatility of *S*-alkylthioisothioureas **38** as sulfenylating agents and demonstrated that they could be prepared by treating a mixture of either a primary, secondary or tertiary alkylthiol and thiourea with hydrogen peroxide in an alcoholic solvent containing aqueous hydrochloric acid, Scheme 1-43.¹⁸⁹



Scheme 1-43 Alkylthioisothiourea formation.

The product **38** was isolated as crystals of the hydrochloride salt with easily removable by-products, dithioformamidine dihydrochloride (removed by filtration before product isolation) and the symmetrical disulfide (soluble in solvent). *S*-Alkylthioisothioureas are not only air stable at room temperature but also stable in acidic solutions, but they do decompose in alkaline solutions over long periods. An unsymmetrical disulfide is formed with a thiol in the presence of base *via* attack of the thiol on the more electrophilic sulfur of the *S*-alkylthioisothiourea, Scheme 1-44.

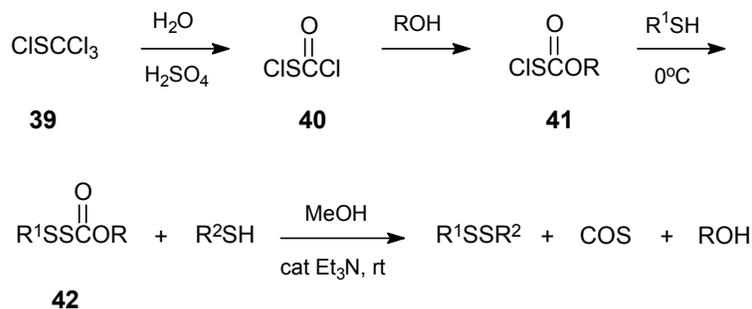


Scheme 1-44 Unsymmetrical disulfide synthesis *via* an alkylthioisothiourea.

The approach has been used to synthesize various disulfides including unsymmetrical disulfides that are often difficult to produce by other approaches such as *S*-alkylthiocysteines. The method has the advantage of being able to produce high quality (purity) unsymmetrical disulfide products in view of the isolation of the stable *S*-alkylthioisothiourea salt.

At about the same time as Sirakawa's reported work, the first sulfonyl thiocarbonates were reported by Brois, who synthesized them *via* a three-step process starting from trichloromethanesulfonyl chloride **39**, Scheme 1-45.¹⁹⁰ Chemoselective hydrolysis of the latter with aqueous H_2SO_4 to chlorocarbonylsulfonyl chloride **40** followed by addition of one molar equivalent of an alcohol gave the active reagent as a carboxysulfonyl chloride **41**. Subsequent addition of two different thiols in separate steps afforded an unsymmetrical disulfide with the

second thiol attacking the alkyl sulfenyl sulfur of **42** with expulsion of COS and ROH. The intermediate sulfenyl thiocarbonate **42** can be isolated and purified.



Scheme 1-45 Unsymmetrical disulfide synthesis from sulfenylthiocarbonates.

Mechanistically, Brois proposed a heterolytic thiol-mediated fragmentation of the sulfenyl intermediate *via* a cyclic transition state, Figure 1-18.¹⁹⁰ The fragmentation is considered to be fast with loss of COS serving as the driving force. According to Grob's conception of fragmentable systems, R¹S is the electrofugal group, COS is the unsaturated fragment and RO is the nucleofugal group.¹⁹¹

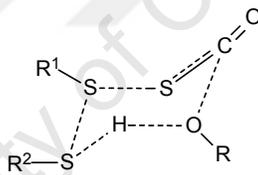


Figure 1-18 Fragmentation of the sulfenyl thiocarbonate *via* a cyclic transition-state.

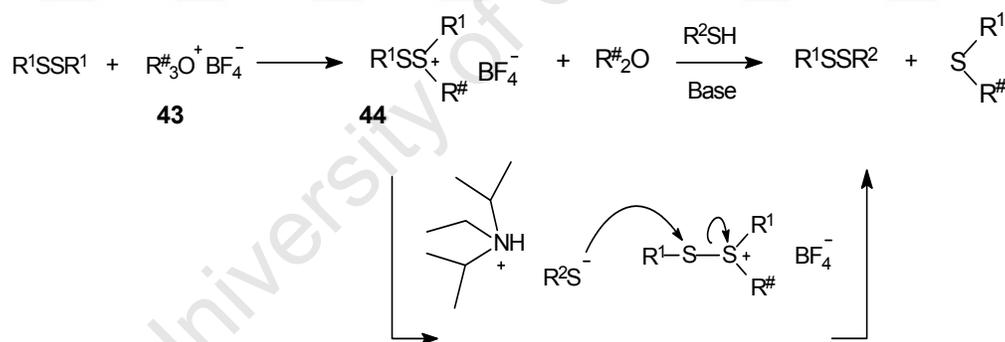
A search of the literature revealed that the approach has been successfully applied to the formation of complex peptides. An example of this was reported by Kamber who showed the formation of disulfide linkages in open-chain asymmetric cysteine peptides using this methodology.¹⁹² In addition, an interesting report by Hiskey and Schroll was reported in 1975 and 1989 respectively.^{193,194} They demonstrated that the -OR group of **42** of the sulfenylating agent could be varied to form useful derivatives, similar to protecting groups, in protein synthesis, Figure 1-19. Owing to the need for stable, readily removable protecting groups for the cysteine thiol functionality in protein synthesis, scientists are constantly attempting to find new groups for this purpose. Hence, Schroll and Hiskey showed that the thiocarbamates in Figure 1-19 are highly acid stable, stable to many conditions used for coupling of amino acids to form peptides and are easily removable under mild thiolytic conditions with dithiothreitol in the presence of *N*-methylmorpholine to afford the free cysteine.^{193,194}



Figure 1-19 Varying the OR group in peptide synthesis to a thiocarbamate.

Sulfenyl thiocarbonates are selectively reactive intermediate reagents but their preparation involves using the harsh reagent ClCOSCl and proceeds *via* a number of steps, thus detracting from being the method of choice for unsymmetrical disulfide synthesis.

A short synthesis of an *S*-based sulfonylating agent was reported in 1976 by Dubs and co-workers.¹⁹⁵ They demonstrated that a symmetrical disulfide is alkylated by triethyloxonium tetrafluoroborate **43** (Meerwein's salt) in nitromethane or dichloromethane at mild temperatures to give an alkylthiodialkylsulfonium salt **44** that could be isolated, Scheme 1-46. Subsequent addition of a thiol in the presence of a tertiary amine base such as *N*-ethyl-*N,N*-diisopropylethylamine (Hünig's base) resulted in expulsion of a sulfide due to thiolate attack on the more electrophilic sulfur of the sulfonylating intermediate to afford the unsymmetrical disulfide in satisfactory to good yields (50-80 %).

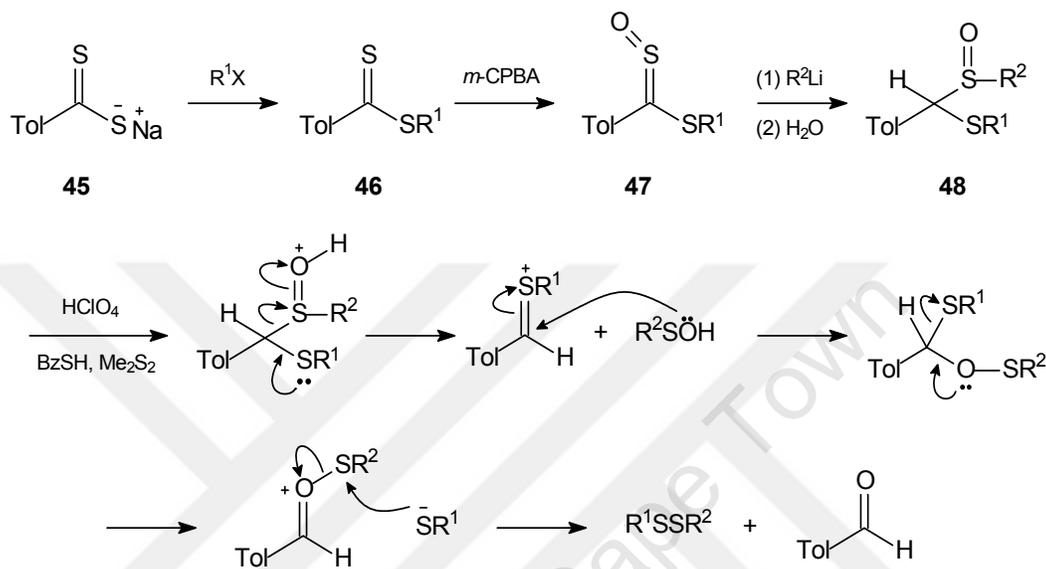


Scheme 1-46 Unsymmetrical disulfide synthesis *via* alkylthiodialkylsulfonium salts.

The preparation of the sulfonylating intermediate relies on the availability of symmetrical disulfides, which would have to be synthesized in a separate step if the disulfide is not commercially available. An excess of symmetrical disulfide would also cause purification problems in the case of aliphatic disulfides.

An interesting approach based on sulfines was reported in 1979.¹⁹⁶ A sulfine (e.g. **47**) is not the actual sulfonylating agent but may be developed into one and thus deserves a mention for unsymmetrical disulfides synthesis. After reporting the preparation of dithioacetal monoxides from sulfines and their application in acylation reactions, Zwanenburg reported the successful

synthesis of unsymmetrical disulfides using these reagents, Scheme 1-47.¹⁹⁶ This work was inspired by research done by Kishi, who reported the formation of a cyclic disulfide using dithioacetal monoxides, and Harrison who used these reagents to prepare 5-membered ring disulfides.^{197,198}



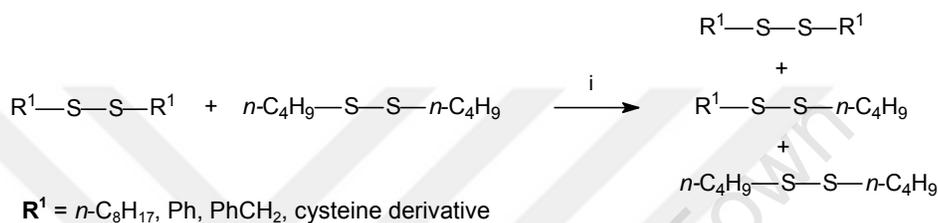
Scheme 1-47 Synthesis of unsymmetrical disulfides *via* sulfines.

A dithiocarboxylic ester **46** is prepared by alkylation of sodium dithio-*p*-toluate **45** with an alkyl halide. *m*-CPBA oxidation of it affords a mixture of *E*- and *Z*-sulfines **47**, which further react with an equimolar amount of methyl lithium to give a dithioacetal monoxide **48** upon an aqueous work-up. Compound **48** is rather unstable and requires immediate isolation and purification.¹⁹⁶ An unsymmetrical disulfide is obtained by acid catalysed substitution of **48** by perchloric acid in the presence of benzyl thiol and dimethyl disulfide. Strong acid was used for the acidolysis to accelerate the reaction, and the presence of benzyl mercaptan and dimethyl disulfide have been reported to restrict disproportionation. The acid catalysis mechanism as illustrated proceeds by protonation of the oxygen, which leads to cleavage of the C-S bond of the protonated sulfoxide and formation of an alkylsulfenic acid and a thionium ion. These newly formed compounds recombine to form a sulfenic ester. This species, according to Zwanenburg, spontaneously fragments to the corresponding unsymmetrical disulfide and aldehyde.¹⁹⁶

Although the sulfine approach produced good yields of the unsymmetrical disulfides, the synthesis comprises multiple steps and the separation and purification of the product was only achieved by exclusion chromatography. GC analysis also revealed that the symmetrical

disulfide (R^1SSR^1) was obtained, which could have been formed in the acid catalysis step.¹⁹⁶ In certain cases, the symmetrical disulfide could not be separated from the unsymmetrical disulfide *via* column chromatography.

The final example in this category is a rhodium-catalyzed exchange reaction of symmetrical disulfides to give an unsymmetrical disulfide. The reaction involves refluxing two symmetrical disulfides in the presence of $RhH(PPh_3)_4$, a phosphine and trifluoromethanesulfonic acid to give unsymmetrical disulfide and two homodimers, Scheme 1-48.¹⁹⁹



Scheme 1-48 *Reagents and conditions:* (i) $RhH(PPh_3)_4$ 3 mol%, CF_3SO_3H 6 mol%, *p*-Tol₃P 12 mol%, acetone, reflux, 15 min.

The reaction does not take place in the absence of the rhodium complex and very low yields are obtained when the phosphine or acid is omitted (<10%).¹⁹⁹ Unfortunately, complete conversion of the symmetrical disulfide to the desired unsymmetrical disulfide is impossible. Although many reports of this kind of exchange are available in the literature, the rhodium-catalyzed reaction is rapid and applicable to peptide disulfide exchange. However, even though the approach produced a number of unsymmetrical disulfides, the methodology is somewhat impractical for disulfide synthesis since one needs to have disulfides as starting materials, which could be problematic if they are not commercially available. Furthermore, the disulfide products have to be separated.

***N*-Based Sulfonylating Agents**

In addition to the sulfur-based leaving groups already mentioned, N-based ones have also found wide application. N-based sulfonylating agents are compounds with a trivalent nitrogen bonded to a divalent sulphur and undergo N-S bond scission with the formation of disulfides. They have been of interest to chemists for many years owing to their utility as synthetic reagents as well as their interesting stereochemical properties in that the S-N bond can provide a chiral axis. Four major types of sulfonylating agents make up this category as: sulfonylhydrazides, sulfonylimides (derived from phthalimide, succinimide or maleimide), sulfenamides and thionitrites, Figure 1-20.

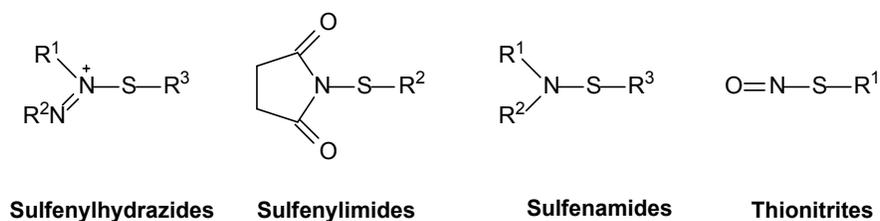
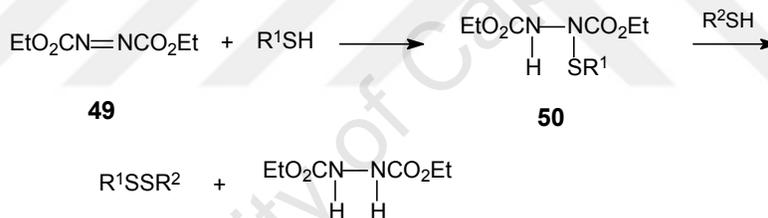


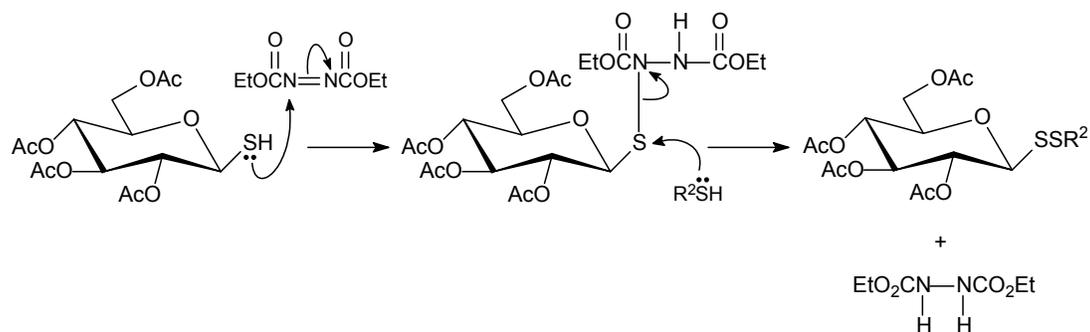
Figure 1-20 Types of N-based sulfenylating agents.

Mukaiyama was the first person, in the late 1960's, to report the synthesis of unsymmetrical disulfides *via* a N-based sulfenylating agent (sulfenyhydrazide).²⁰⁰ Mukaiyama's study was instigated by the work of Diels and Wulff who showed that dimethyl azodicarboxylate reacts with ethanethiol to form the adduct dimethyl *N*-ethylsulfenyhydrazodicarboxylate.²⁰¹ Mukaiyama showed that the readily available diethyl azodicarboxylate (DEAD) **49** reacts with thiols to give the intermediate, diethyl *N*-alkanesulfenyhydrazodicarboxylate **50**, which then reacts with a second thiol cleaving the nitrogen-sulfur bond to give an unsymmetrical disulfide, Scheme 1-49.



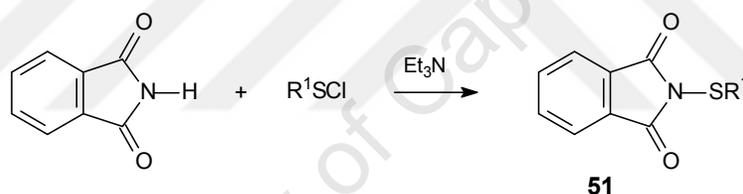
Scheme 1-49 Thioalkylation of thiols by sulfenyhydrazides.

These reactions, which share similarities with Mitsunobu chemistry using PPh₃, proceed under mild and neutral conditions to give products in moderate yields. However, this approach suffers from prolonged reaction times and the efficiency of formation of the adduct is very much sensitive to the nature of the thiol. Bulky groups hinder the formation of the adduct, and as a result secondary and tertiary thiols are normally unreactive. Amino thiols also give poor results, since the high nucleophilicity of the compounds causes formation of the symmetrical disulfide *via* reaction of the intermediate with the first thiol. This results in problems when purifying the desired compound. Mukaiyama's carbodiimide method remains one of the more popular methods for one-pot unsymmetrical disulfide synthesis for primary thiols but for more substituted thiols suffers from various drawbacks. A recent application of the methodology was reported by Falconer and co-workers in 2007, who synthesized glycosyl disulfides (Scheme 1-50) as potential *O*-glycoside mimics, as valuable tools in glycobiology research.²⁰² Two equivalents of DEAD were required to prevent the formation of the symmetrical disulfide.



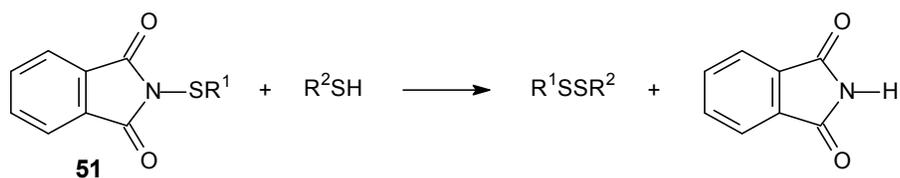
Scheme 1-50 Formation of glycosyl disulfides from DEAD.

Following Mukaiyama's work, several other papers appeared in the literature describing reagents with other N-based sulfenylating agents. In 1970, two papers were published concurrently that made use of sulfenylphthalimides **51**, derived from the condensation reaction of an imide with a sulfenyl halide in the presence of a tertiary amine base as an acid scavenger (Scheme 1-51), for thioalkylation in the synthesis of unsymmetrical disulfides.^{203,204}



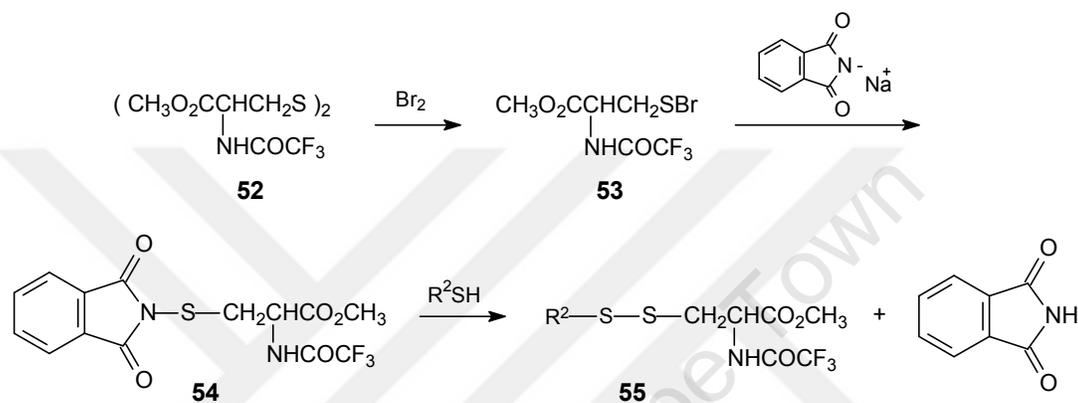
Scheme 1-51 Formation of sulfenylphthalimide.

Interestingly, Harpp and Boustany's groups independently but at about the same time during 1970 reported that thiols react with thiophthalimide intermediates to form the corresponding disulfides, Scheme 1-52.^{203,204} Both demonstrated that the reaction is carried out at temperatures between 20 and 100°C in aprotic solvents such as benzene in which the by-product (phthalimide) is insoluble, which serves as a driving force for the reaction. The advantages offered by this method include good yields, stable precursors and minimal disulfide exchange for aliphatic R-groups. This method can be used for a wide range of systems, however when applied to diaryl systems, a mixture of the three disulfides is most often obtained *via* thiol exchange onto the disulfide or *via* disulfide exchange.



Scheme 1-52 Thioalkylation of thiols by a sulfenylphthalimide.

Harpp later used this method to form unsymmetrical cysteine-containing disulfides, which were developed as model strategies for peptide synthesis, Scheme 1-53.²⁰⁵ The reaction proceeds by bromination of a protected cysteine **52** with cleavage of the disulfide bond, after which the resulting sulfenyl bromide **53** is treated with the phthalimide anion to give the cysteine sulfenamide intermediate **54**. Thiolysis of the intermediate with a thiol (in this case cysteine hydrochloride monohydrate or glutathione) gave the corresponding unsymmetrical disulfide **55**.



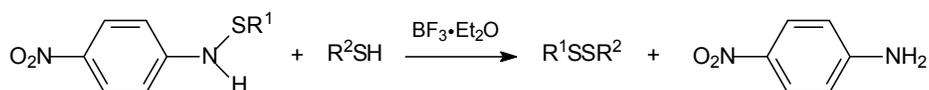
Scheme 1-53 Unsymmetrical cysteine disulfide synthesis *via* sulfenylphthalimide.

The third type of N-based sulfenylating agent, sulfenamides, are prepared under similar conditions as the sulfenylimides *via* condensation of an amine with a sulfenyl halide in the presence of an acid acceptor such as triethylamine, sodium hydride, alkali-metal hydroxide or an excess of the starting amine, Scheme 1-54.



Scheme 1-54 Sulfenamide synthesis.

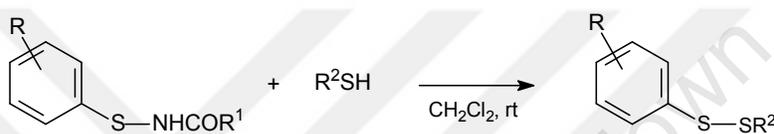
In 1986, Benati demonstrated the synthesis of unsymmetrical disulfides from reaction of 4'-nitroarenesulfenamidides with thiols in the presence of boron trifluoride etherate, which transforms the sulfenamidides into highly electrophilic species, Scheme 1-55.²⁰⁶



Scheme 1-55 Unsymmetrical disulfide synthesis *via* sulfenamides.

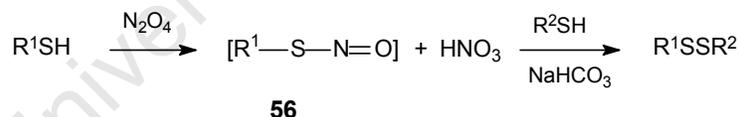
The approach gives very good yields for aralkyl disulfides; however low yields are obtained for the unsymmetrical disulfide when two aromatic thiols are employed. This could be due to disproportionation since the symmetrical disulfides were also isolated, as experienced by Harpp with the imide case.

Recently, Shimizu reported the reaction of *N*-trifluoroacetyl arenesulfenamides ($R^1 = CF_3$) with thiols to give unsymmetrical disulfides, Scheme 1-56.²⁰⁷ The reactions proceeded efficiently when aromatic thiols were added to the sulfenamide to form diaryl disulfides but higher temperatures were required to drive the reaction when aliphatic thiols were added to form aralkyl disulfides.



Scheme 1-56 Unsymmetrical disulfide synthesis *via* sulfenamides ($R = \text{H}, 2\text{-Br}, 4\text{-Me}, 4\text{-NO}_2$ and $2\text{-CO}_2\text{Me}$).

Thionitrites are the final type of N-based sulfenylating agents. Alkyl thionitrites may be prepared from thiols and alkyl nitrites, nitrosyl chloride or nitrogen trioxide, while aryl and alkyl thionitrites are prepared from thiols and nitrogen tetraoxide.^{208,209} Oae demonstrated that upon addition of a thiol to a thionitrite intermediate **56**, unsymmetrical disulfides are obtained in yields varying from 20 to 90 %, Scheme 1-57.^{210,211}



Scheme 1-57 Unsymmetrical disulfide synthesis *via* thionitrites.

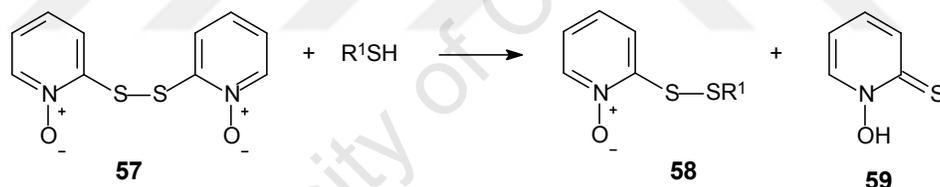
The method has not been utilized to a great extent due to the easily decomposable intermediate which is sensitive to oxidation and light, as well as the troublesome nature of working with gases.

In all four categories, the disulfide synthesis could proceed in two steps with isolation and purification of the corresponding intermediate. These were the first sulfenylating agents that were stable to isolation and stable enough to be stored for long periods.

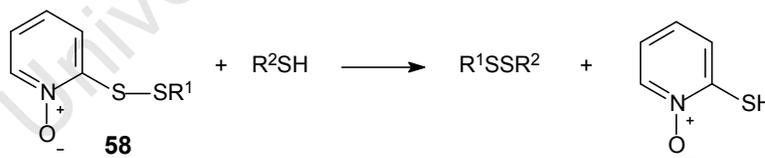
One-Pot Methodologies

In more recent years, chemists have been striving to develop one-pot syntheses with the majority of the publications in the literature utilizing a common approach involving symmetrical disulfides as sulfur transfer agents. Attractive features of the latter include their stability – they are much more stable under a variety of conditions compared to the sulfonylating agents described before – efficiency, handling and their ability to transform a wide range of thiols to unsymmetrical disulfides.

In 1991, Barton used 2,2'-dithiodipyridine-1,1'-dioxide **57**, prepared by the hydrogen peroxide oxidation of 2-mercaptopyridine N-oxide, as a transfer agent.^{212,213} The poor solubility of **57** was a major disadvantage, often requiring the use of acetic acid as the solvent. Reaction of **57** with thiols gave the unsymmetrical disulfide intermediate **58** (isolated and purified) with thiohydroxamic acid **59** as a by-product (Scheme 1-58) but frequently required long reaction times (up to 2 days) and in certain cases (e.g. compounds with short aliphatic chains) produced only the symmetrical disulfide of R¹SH. Reaction of **58** with thiol R²SH then afforded the unsymmetrical disulfide product, Scheme 1-59.



Scheme 1-58 Preparation of unsymmetrical disulfides *via* a pyridine transfer agent.

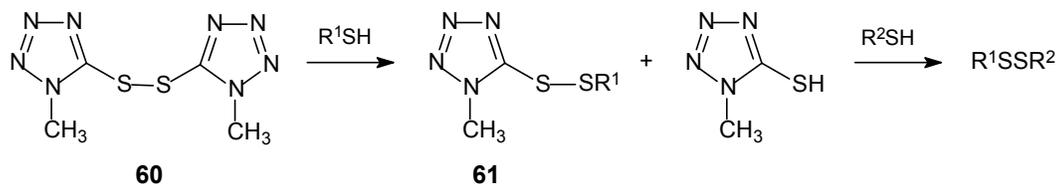


Scheme 1-59 Formation of unsymmetrical disulfides from **58**.

Barton reported that the rate of substitution on **58** is higher than that on **57**; thus the intermediate **58** must be isolated prior its reaction with a second thiol to avoid the formation of homodimer R¹SSR¹.²¹² However, this does not eliminate the formation of R¹SSR¹ since equimolar amounts of thiol (R¹SH) and **57** are employed.

In the same year, Ohtani reported the sulfonylating nature and high reactivity towards nucleophiles of the compound bis(1-methyl-1*H*-tetrazol-5-yl) disulfide **60**, prepared by a two-phase oxidation (CH₂Cl₂-H₂O) of 1-methyl-1*H*-tetrazole-5-thiol with KHCO₃-Br₂.²¹⁴

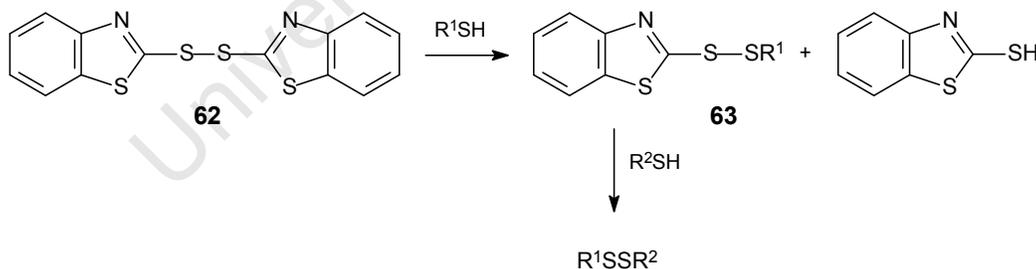
Amongst the various applications reported for **60** was the formation of unsymmetrical disulfides, Scheme 1-60.



Scheme 1-60 Unsymmetrical disulfide synthesis *via* symmetrical disulfide **60**.

The approach proved to be successful for the formation of various amino acid unsymmetrical disulfides and has the advantage of the reaction proceeding rapidly and stoichiometrically under neutral or acidic conditions. The drawback however involves the instability of the starting disulfide **60**, which decomposes gradually at pH 1.25 and rapidly at pH 7.39.

A plethora of these approaches have been reported in the literature with the major drawback for the majority of them being the need to have a separate synthesis of the symmetrical disulfide starting reagent since they are not readily available. Ternay reported the use of a commercially available symmetrical disulfide reagent, 2,2'-dithiobis(benzothiazole) **62**, for the reaction with thiols to form unsymmetrical disulfides **63** which themselves are precursors for synthesis of a second unsymmetrical disulfide. The overall scheme is shown in Scheme 1-61.²¹⁵



Scheme 1-61 Unsymmetrical disulfide synthesis from 2,2'-dithiobis(benzothiazole) **62**.

Although Ternay did not report results for the methodology as a one-pot synthesis, the methodology provides the option of either being a one-pot or a two-pot synthesis since intermediates, such as **63**, may be isolated. His approach proved to be versatile, transforming a wide range of thiols; except ones with an amine hydrochloride functionality, to unsymmetrical disulfides.

All the mentioned approaches for unsymmetrical disulfide synthesis have some distinct advantages ranging from giving high yields to being a one-pot synthesis. However, the majority of them suffer from involving highly toxic reagents or harsh conditions of pH that promote disulfide exchange leading to purification problems. Hence, alternative methods that are simple, clean, efficient and applicable to a broad range of thiols for the selective preparation of unsymmetrical disulfides are still welcome additions to the synthetic organic repertoire.

1.6 Cyclodextrins

1.6.1 Introduction

The first reference to cyclodextrins (hereinafter CDs) was published by Villiers, in 1891.²¹⁶ These substances, named cellulosines, were isolated from the enzymatic degradation of starch in the presence of *Bacillus amylobacter*.²¹⁶ Subsequently, in 1903, Schardinger reported the first detailed account for the preparation, isolation and characterisation of these so called cellulosines.^{217,218} Treatment of starch with glucosyltransferases causes hydrolysis of the starch helix, bringing about chain splitting and initiating an intramolecular reaction by which the ends of the chains are linked through rigid α -1,4 linkages, forming the cyclodextrins.²¹⁹

1.6.2 Chemical Structure

Building Block

CDs are cyclic, toroidal shaped oligosaccharides built up of glucose units that vary in number.²²⁰ The first CDs to be discovered and characterized were the parent CDs, which include α -, β - and γ -CDs (Figure 1-21) which are made up of 6, 7, and 8 α -D-glucopyranose units linked by α -1,4-glycosidic bonds. The glucose rings usually adopt the 4C_1 -chair conformation, Figure 1-22.^{221,222} Although several larger CDs exist, differing from each other by the number of glucopyranose units, CDs smaller than 6 units are sterically strained and therefore not produced enzymatically.^{223,224}

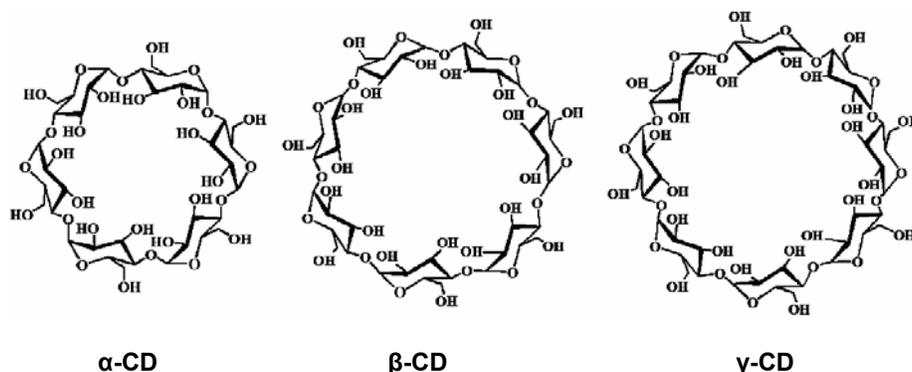


Figure 1-21 Parent cyclodextrins α -, β - and γ -CD.

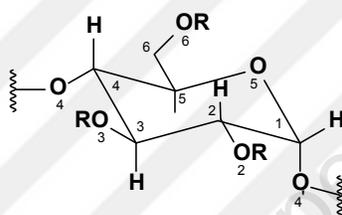


Figure 1-22 Glucopyranose unit of cyclodextrins.

Structure

The CDs have an overall shape reminiscent of a truncated cone with a slightly apolar (hydrophobic) cavity in the centre as a result of the hydrogen atoms and the glycosidic oxygen bridges that line the inside of the cavity.²²² The exterior of the molecule is polar and water soluble (hydrophilic) due to hydroxyl groups projecting from the two ends/rims of the torus. All glucose rings in the CD are usually arranged *syn*, i.e. with all of the primary, freely mobile hydroxyl groups (C6-OH) located on the narrow 'primary' rim, while all of the secondary -OH groups (C2-OH and C3-OH) are located on the wider 'secondary' rim.

Hydrogen Bonding and Solubility

The structural rigidity and stability of these macrocyclic rings are attributed to the strong intramolecular hydrogen bonding between the hydroxyl groups in positions C2-OH and C3-OH of adjacent glucose units.²²² Different degrees of hydrogen bonding are observed for the different parent CDs, with hydrogen bonds being strongest in γ -CD. The differences in hydrogen bonding are responsible for the differences observed in the aqueous solubilities exhibited by the parent CDs. The crystal structure of α -CD reveals that one glucopyranose unit is severely tilted allowing only four of the six hydrogen bonds to form, causing α -CD to be less rigid and more soluble than β -CD, which has a complete ring of hydrogen bonds thus causing it to be less soluble.^{220,225} Conversely, γ -CD possesses the strongest hydrogen bonds

but is the most soluble of the three parent CDs. This property has been attributed to its high ring flexibility.²²⁶ The solubility of the parent CDs decreases in the order 23.2 for γ -CD, 14.5 for α -CD and 1.85 for β -CD (solubility in water at 25°C (% w/v)).

1.6.3 Applications

CDs are versatile molecules and have found applications in a number of fields primarily due to their ability to form inclusion complexes with various substrates.²²⁷ CDs are used in biology (as enzyme models), agriculture (in pesticide delivery), food science (for flavour and preservation), biotechnology (cell cultivation and antibiotic production), catalysis (as substrates), technology (as chemosensors), analytical chemistry (as reagents and in separation methods) and pharmaceuticals (in drug formulations).^{220,228-232}

Pharmaceuticals and drug formulation

Our interest lies in the use of CDs in the formation of drug inclusion complexes in a pharmaceutical context. Being able to modify the pharmacokinetic properties of a drug is an important contrivance. Complexation of drugs with CDs improves their mode of delivery and minimizes adverse side effects of the active ingredient. The drug is released either by competitive displacement with endogenous lipophiles or upon dilution.²²⁶ The attractiveness of CD inclusion is that complexation does not hamper the drug's activity.²²⁶ The advantage of encapsulating drugs within CDs includes isolating them from potentially corrosive environments, shielding them from attack by various reactive molecules, reducing or preventing undesirable properties such as instability, volatility, low solubility, hydrolysis, racemization and enzymatic decomposition.²²⁶ Many drugs are available today, which are marketed as a CD formulation, a typical example being Prostaglandin E₁, which is available as an α -CD formulation.²²⁶

1.6.4 Cyclodextrin Inclusion Complexes

The most important feature of the CDs is their ability to form complexes with a variety of ionic and molecular species. Complexes often comprise two molecules, one molecule being the host, in this case the CD, and the other molecule which is included being the guest, forming the characteristic structure of an adduct.²²⁰ A key feature of the complexes is that no covalent bonds are formed between the host and the guest: the guest molecule is totally or partially situated in the CD cavity only by physical forces.^{220,228} In an aqueous solution, the CD cavity is occupied by enthalpy-rich water molecules, which are readily displaced by less polar guest molecules, which is ultimately the driving force of complexation, Figure 1-23.²²² In addition,

electrostatic interactions, van der Waals interactions, hydrophobic interactions, charge-transfer interactions, hydrogen bonding and release of conformational strain are believed to be the principal factors involved in complexation.^{228,233} Of the numerous CD applications, we are particularly interested in their use in pharmacy as drug carriers. A recent example of this application in the context of garlic has been published by Caira and co-workers in which they demonstrated that the advantage of including ajoene in CDs includes: (i) converting the compound which is an oil into a solid for easier handling and formulation purposes and (ii) suppressing the strong odours by reduction of volatility.²³⁴ Other advantages of including guests into CDs are the ability to stabilise them and act as a protective mechanism for very reactive compounds.

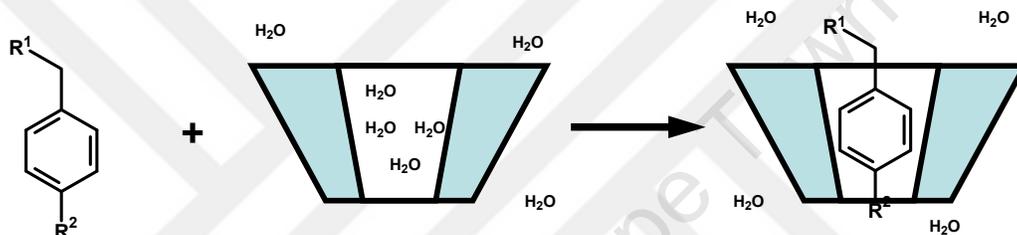


Figure 1-23 Hypothetical schematic representation of complex formation.

Cyclodextrin Complex Preparation

Although the preparation of CD inclusion complexes is usually straightforward, the conditions have to be adapted according to the type of guest and type of CD employed.²²⁸ Complexation may be carried out in a homogeneous solution or in a suspension, by melting, kneading or grinding together the components, or by simply mixing the potential guest with the CD. The methods frequently used for preparation of crystalline CD inclusion complexes are the co-precipitation and kneading techniques.

In the co-precipitation method, a saturated aqueous solution of the CD prepared, at room temperature or at elevated temperature (usually 60 to 80 °C), and an equimolar amount of guest is added while stirring. After complete dissolution, the reaction solution is filtered and complex crystal growth follows.

In the kneading method, a guest is added to a CD-water paste and kneaded for about an hour, although the kneading time for complete complexation is unpredictable. The paste is kept moist by adding a few drops of water and tested for complex formation via powder X-ray diffraction or infrared spectroscopy. Finally, the kneaded complex may be redissolved in water, filtered and left at room temperature to induce crystallization.

Methods of Analysis

It is sometimes difficult to detect the difference between a 'true' inclusion complex and an intimate physical mixture between host and guest, and since we are interested in potential medicinal agents it is necessary to demonstrate unequivocally that 'true' inclusion complexes are being produced and to characterise them structurally. Techniques available for analysing CD complexes include thin layer chromatography, IR and Raman spectroscopy, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$, UV spectrometry, mass spectrometry, powder X-ray diffraction, thermogravimetry and differential scanning calorimetry, and single crystal X-ray diffraction.²²⁰ Powder X-ray diffraction involves comparing experimental traces of peak patterns with known reference traces and thus concluding whether complexation has occurred. More definitive results are obtained from single crystal X-ray diffraction which reveals maximum detail about the complex and is the method of choice for unambiguous structural characterization.²³²

1.6.5 Cyclodextrin Derivatives

The hydroxyl groups of the CDs are amenable to modification and due to the differences in reactivity between the OH groups (C6-OH being most reactive, followed by C2-OH and the least reactive C3-OH), the selective preparation of a number of derivatised CDs is possible. Modifications result in CDs with different physical and chemical properties. Methylation of the CD hydroxyl groups is the simplest modification but the structural modification has a major effect on the manner of complexation. The effects include the increase in depth of the cavity, which allows bigger guests to be included and the change in hydrophilic character of the rims to a hydrophobic environment, which influences the manner in which the guest molecules are included.^{224,235}

Although many derivatised CDs exist and their structures, properties and inclusion properties have been extensively studied, the two most common derivatives are the partially and fully methylated derivatives of β -CD. These are heptakis(2,6-di-O-methyl)- β -cyclodextrin, more commonly known as DIMEB, and heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin, more commonly known as TRIMEB. While DIMEB has a similar conformation to that of β -CD, TRIMEB may be much more distorted due to loss of intramolecular hydrogen bonding and possible loss of the $^4\text{C}_1$ -chair conformation of the glucopyranose units, as occurs in one of its crystalline forms.²³⁶ However, the structure is stabilized by the formation of intramolecular C6-H...O5 hydrogen bonds. In addition, it has been reported that self-inclusion of the methoxyl groups from a screw-related molecule in one crystal form further stabilizes the structure of TRIMEB.²³⁴ The derivatised CDs are much more soluble than the parent CDs. The solubility of TRIMEB in water at 25 °C is recorded as 25 g in 100 ml whereas that of β -CD is 1.85 g in 100 ml.²²⁸

1.6.6 Cyclodextrin Crystal Packing

CD molecules are arranged within their crystals in one of two packing modes, either in a cage type or a channel type structure. Channel type packing is composed of head-to-head **A** or head-to-tail **B** arrangements while cage type packing is composed of herringbone type **C** or brick/layer **D** type (limited to α - and β -CD), Figure 1-24.²²⁸

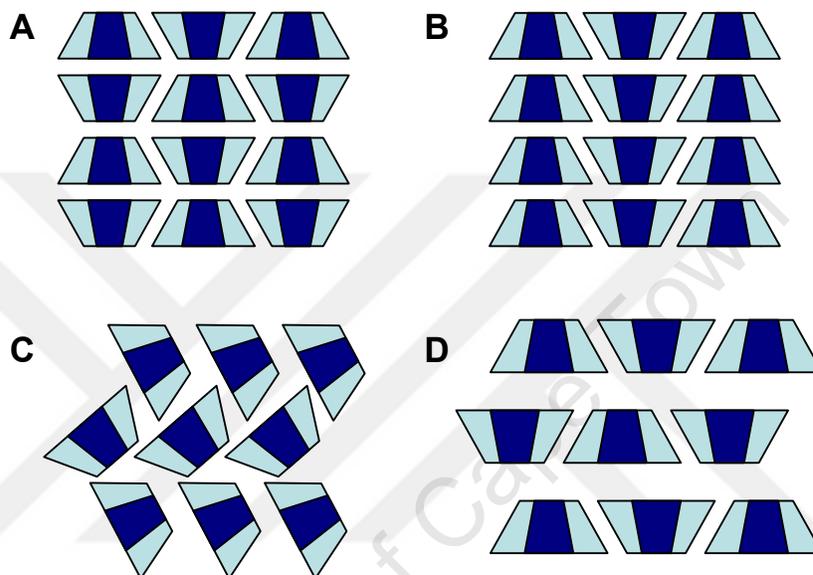


Figure 1-24 Diagrammatic representation of the packing arrangements in the parent CDs.

A = head-to-head channel type, **B** = head-to-tail channel type, **C** = herringbone cage type, **D** = brick/layer cage type.

The cage type structure is observed for complexes whose guest is completely encapsulated by the CD while the channel type is formed when the guest is large and part of the molecule projects from the CD cavity. In channel-type structures dimers stack in such a way that their cavities form infinite channels and are stabilized by hydrogen bonds formed between hydroxyl groups of adjacent units. Conversely, the cavities in cage type structures are blocked off by adjacent CD molecules, allowing no contact between guest molecules. For β -CD, cage type packing is further subdivided into monomeric structures, which are arranged in five packing arrangements (herringbone, layer type, zig zag, helical channel and brick type) and dimeric structures, which are arranged in four types of packing arrangements (channel, screw channel, intermediate and chessboard), Figures 1-25 and 1-26. The herringbone packing arrangement is the preferred arrangement as well as the most efficient packing system.

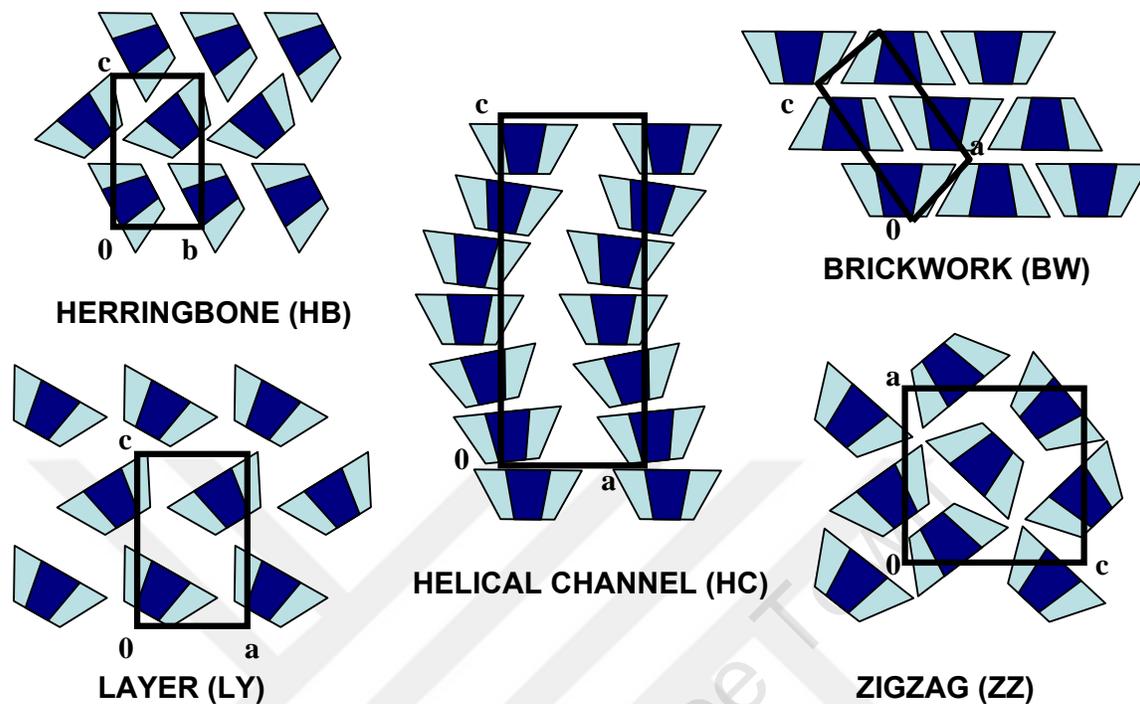


Figure 1-25 Diagrammatic representation of the five monomeric packing arrangements of β -CD in its complexes.

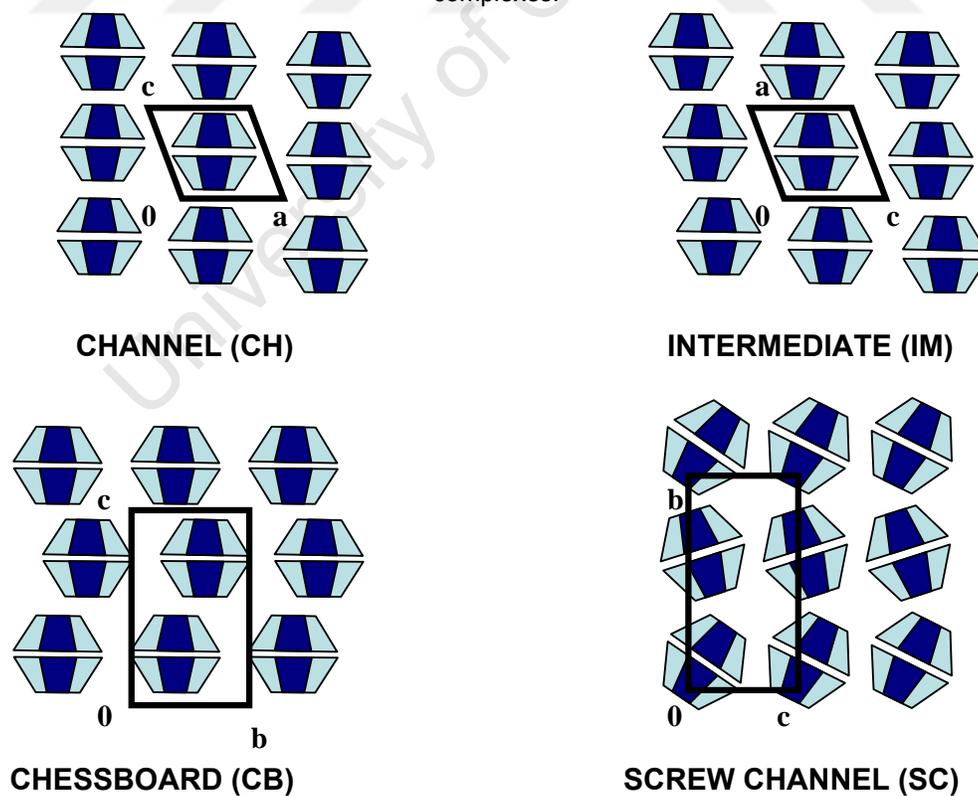


Figure 1-26 Diagrammatic representation of the four dimeric packing arrangements of β -CD in its complexes.

γ -CD complexes belong to the highly symmetric tetragonal space group $P4_21_2$ and crystallize in channel packing mode CH while DIMEB packs in various arrangements including channel type head-to-tail, modified brick type and modified herringbone arrangements. The complexes of DIMEB crystallize in the space groups $P2_1$ (monoclinic) and $P2_12_12_1$ (orthorhombic). Similarly to DIMEB complexes, TRIMEB inclusion complexes pack in a head-to-tail manner and crystallize in the preferred space group $P2_12_12_1$ with a few crystallizing in the space group $P2_1$.

1.6.7 Crystal Isostructurality

CD inclusion complexes are termed isostructural when two or more complexes crystallize with identical packing motifs, with similar unit cell dimensions, the same space group, and similar internal arrangement of molecules.²³² These parameters are easily determined *via* single crystal X-ray diffractometry (SCXRD) and powder X-ray diffraction (PXRD). Although SCXRD is the method of choice for analysis, growing single crystals suitable for crystal structure determination is often difficult and thus PXRD is employed as an initial and sometimes the only method for inclusion determination. The PXRD pattern of a presumed complex is compared to “reference patterns” (known CD inclusion complexes can be classified into a relatively small number of isostructural families, each having a characteristic PXRD pattern)²³² and a good match (presence of majority of most prominent peaks) unambiguously identifies structural features of the complex. An example of the practical application of reference patterns for definitive complex characterization is illustrated in Figure 1-27. It should be noted that the converse of isostructurality is polymorphism, which involves a single compound crystallizing in different packing arrangements.

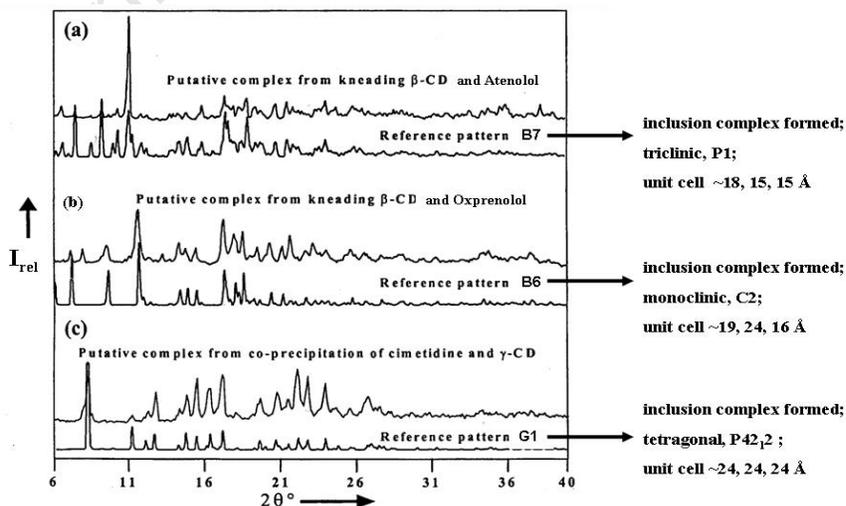


Figure 1-27 Application of reference patterns for definitive complex characterization.

1.7 Aims and Objectives

Garlic has been used for centuries by several cultures as a natural product with beneficial properties for promoting good health. The active component of fresh garlic, the thiosulfinate allicin, is unstable but possesses potent antimicrobial properties. A literature survey revealed that certain saturated thiosulfonates were both more active and more stable than allicin. In light of this, it was decided to undertake a comprehensive study to synthetically produce allicin analogues in the search for stable mimics for screening as potentially new antimicrobial agents.

Considering the mechanistic information on allicin, structural features in the targets have been chosen to preclude the possibility of a breakdown *via* a Block-type fragmentation established for allicin. The targets chosen and their SAR motif is shown below based on S-aryl alkylthiosulfonates, Figure 1-28.

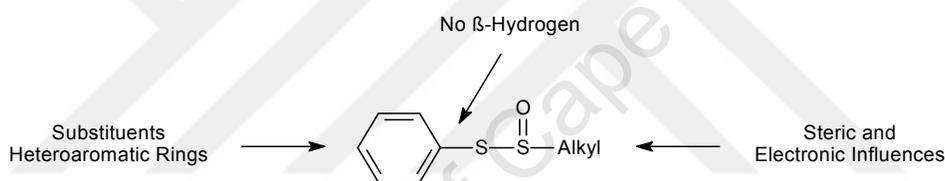


Figure 1-28 Motif for new allicin mimics, S-aryl alkylthiosulfonates

Thiosulfonates are prepared by the oxidation of disulfides, a functional grouping of interest for many years in view of its relevance to biological systems. Although many methods for unsymmetrical disulfide formation exist as described in previous sections, the majority of them suffer from a number of drawbacks. This prompted the development of a new method for synthesizing this important functional group which could be used for preparing allicin mimics.

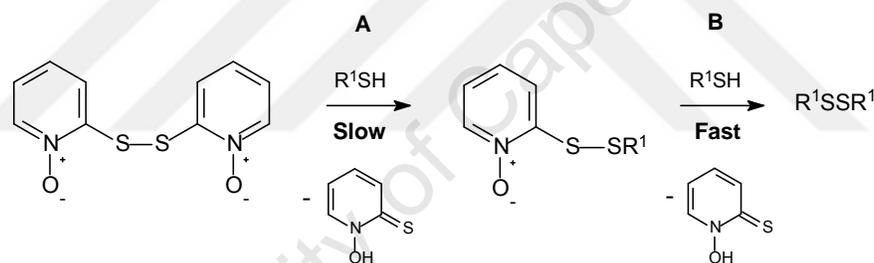
The inclusion properties of the S-aryl alkylthiosulfonates will be investigated in various cyclodextrin molecules. A practical advantage to be gained by CD inclusion complex formation in this instance is to enhance stability of the allicin mimics as well as to mask offensive odours and improve solubility of potentially water-insoluble drugs.

Lastly we will run a preliminary screen of the S-aryl alkylthiosulfonates for antimicrobial activity *via* the direct bioautography/TLC method.

Chapter 2: Unsymmetrical Disulfides

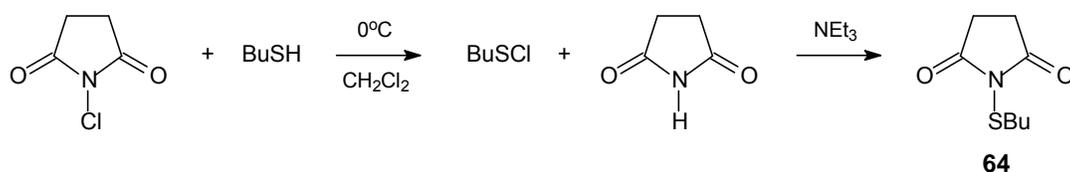
2.1 Unsymmetrical Disulfide Synthesis

A thorough literature survey revealed that the majority of the methods for preparing unsymmetrical disulfides suffer from either the use of highly toxic reagents and/or harsh reaction conditions, or that few of them meet the modern-day criteria of a one-pot transformation with green characteristics. Furthermore, many of the methods suffer from exchange reactions to produce symmetrical disulfides that are invariably difficult to separate from the desired product. Formation of the symmetrical disulfide (R^1SSR^1) is often unavoidable owing to the higher relative rate of **B** compared to **A** as illustrated by an example by Barton, Scheme 2-1.²¹² An ideal reaction for unsymmetrical disulfide synthesis would be one in which the relative rate of **A** is much higher than that of **B** thus eliminating the secondary reaction of thiol R^1SH with the newly formed sulfenylating intermediate to form homodimer R^1SSR^1 .



Scheme 2-1 Formation of symmetrical disulfides owing to relative rates of reactions.²¹²

All the above mentioned factors and the importance of the disulfide functionality were the principal motives for exploring the development of a new method for synthesis of unsymmetrical disulfides. In search of an environmentally friendly oxidant that could be recycled, we were inspired by the independent work of Emde¹⁴² and Abe²³⁷ who demonstrated in separate papers in 1952 and 1973 respectively, that *N*-chlorosuccinimide reacts with a thiol in an exchange reaction to form a sulfenyl chloride, which on addition of triethylamine subsequently reacts with the by-product succinimide to form an *N*-sulfenylsuccinimide **64**, Scheme 2-2. Over the years, sulfenamide methodology has been a favourite for disulfide synthesis and *N*-(organothio)succinimides have been useful reagents for the synthesis of unsymmetrical disulfides. We thus decided to examine the reaction and explore the possibilities for extending this type of methodology towards constructing a new one-pot synthesis of unsymmetrical disulfides.



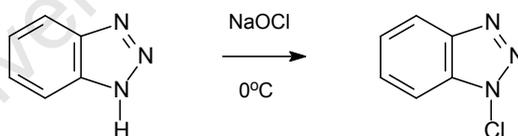
Scheme 2-2 Abe's reaction of a thiol with *N*-chlorosuccinimide

However, from the point of view of unsymmetrical disulfide synthesis, this sequence suffers from reaction of the reactive sulfenyl chloride with incoming thiol to generate the symmetrical disulfide. Nonetheless, we identified that the reaction had potential for development into a one-pot reaction, provided that experimental conditions could be identified for chemoselective formation of the *N*-sulfenyl intermediate without subsequent interception by thiol to form the symmetrical disulfide. We thus decided to explore the reagent 1-chlorobenzotriazole (BtCl) in view of the nucleophilic character of benzotriazole and the possibility of sulfenyl chloride trapping.²³⁸

2.1.1 1-Chlorobenzotriazole

Overview

BtCl was first introduced in 1968 by Rees and Storr as an air-stable oxidant of alcohols, hydrazo-compounds and 1,1- and 1,2-disubstituted hydrazines.²³⁸ It is easily prepared starting from the commercially available benzotriazole (BtH) by a simple oxidation with bleach (aqueous sodium hypochlorite) in aqueous acetic acid, Scheme 2-3.

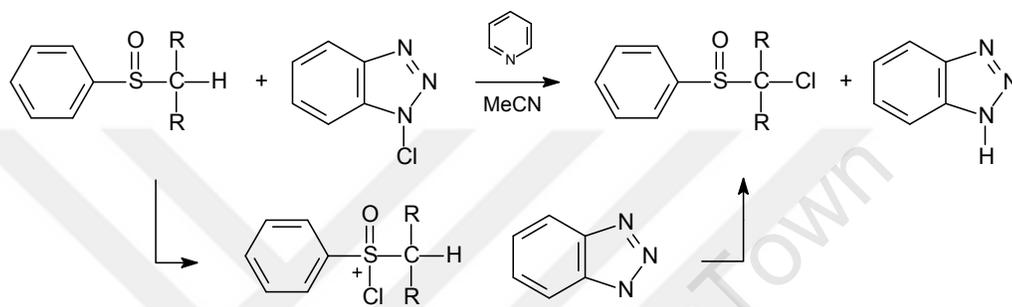


Scheme 2-3 Oxidation of benzotriazole to 1-chlorobenzotriazole with sodium hypochlorite.

The product precipitates out of solution and can be recrystallized from DCM-petroleum ether to give the pure compound with a melting point of 105-106 °C.²³⁸ BtCl is isolated as a colourless, crystalline solid that can be stored in the refrigerator for several months. This reagent has generated great interest as discussed below owing to the positive character of the chlorine atom and its resultant versatility as an oxidizing reagent as well as it being less hazardous compared to other oxidants. Its significance as a possible precursor to *N*-substituted benzotriazoles has also been attractive. Although it has been used in a variety of functional group transformations, it has found little application to thiols.

Reactions of BtCl

BtCl reacts with a wide range of functional groups under various reaction conditions including the oxidation of alcohols to aldehydes or ketones, chlorination of ketones, decarboxylation of α -hydroxy acids and oxidation of amino acids.²³⁸⁻²⁴¹ The reagent is also used for various purposes in reactions with sulfur and nitrogen compounds, including it being a chlorinating agent or an oxidizing agent.²⁴²⁻²⁴⁴ An example is illustrated in Scheme 2-4 via a Pummerer-type mechanism.



Scheme 2-4 Reaction of BtCl: an example of reaction with a sulfur compound.

2.1.2 Reaction Development

In view of our interest in developing room-temperature-stable allicin mimics, we began targeting aralkyl disulfides in which one of the allyl groups in allicin is replaced by an alkyl group and the other by an aromatic ring lacking the β -hydrogen in order to inhibit fragmentation as discussed previously, Figure 2-1.



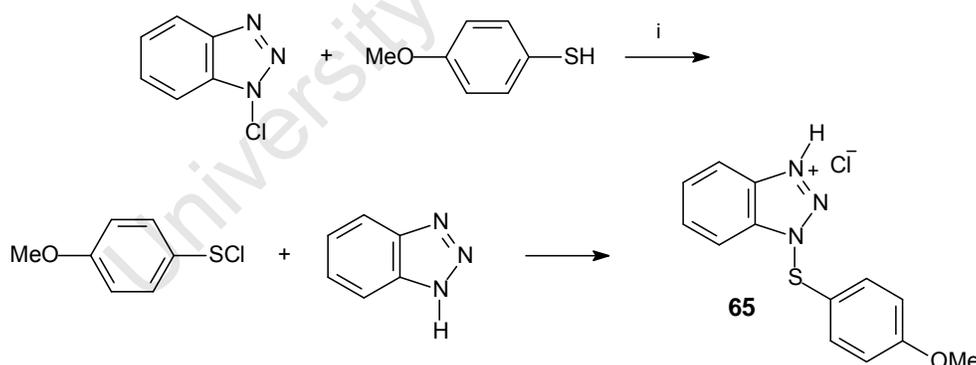
Figure 2-1 Structure design for allicin mimics.

After much consideration, we postulated an approach for unsymmetrical disulfide synthesis similar to Abe's reaction in which BtCl would react with a thiol to form an *N*-sulfenyl derivative, but with an important difference. In the case of Abe's reaction, the poorly nucleophilic neutral succinimide failed to react, i.e. 'trap out' the sulfenyl chloride without adding triethylamine to facilitate imide proton removal.²³⁷ In our case, it was perceived that the more nucleophilic and softer benzotriazole leaving group might intercept the sulfenyl chloride, thus protecting it from subsequent thiol interception by incoming thiol to form the symmetrical disulfide. However, at

higher temperature, it was considered that the adduct would be amenable to substitution by a second thiol. The development of the reaction will now be discussed.

Model System

Initially, *p*-methoxybenzenethiol and 1-propanethiol were selected as representative thiols for accessing our medicinal chemistry objective, while at the same time for developing the methodology. The study was commenced by investigating the efficacy of BtCl as an oxidant. The initial reaction involved reacting *p*-methoxybenzenethiol with 1.5 equivalents of BtCl in dry DCM at -78 °C under an inert atmosphere (N₂). BtCl was added in excess to ensure that all of the thiol would be converted, as well as to ensure that the BtCl competed favourably against the sulfenyl chloride (concentration increasing with time) for thiol. The rapid formation of a red-coloured reaction mixture upon addition of the thiol to the BtCl solution was a promising sign since it is well known that sulfenyl halide solutions have a characteristic red colour. Although the resulting product was not isolated, we were optimistic that the sulfenyl chloride was being rapidly produced *via* thiol chlorination, as in the Abe reaction, Scheme 2-2.²³⁷ The red colour faded over time, indicating that a subsequent reaction was taking place unlike the Abe reaction that requires NEt₃ to induce the sulfenyl chloride trapping reaction.²³⁷ TLC monitoring of the reaction indicated the formation of a new UV-active product of polarity in between that of the thiol and BtH. Scheme 2-5 summarizes the reaction and postulates the identity of the trapped product **65**.

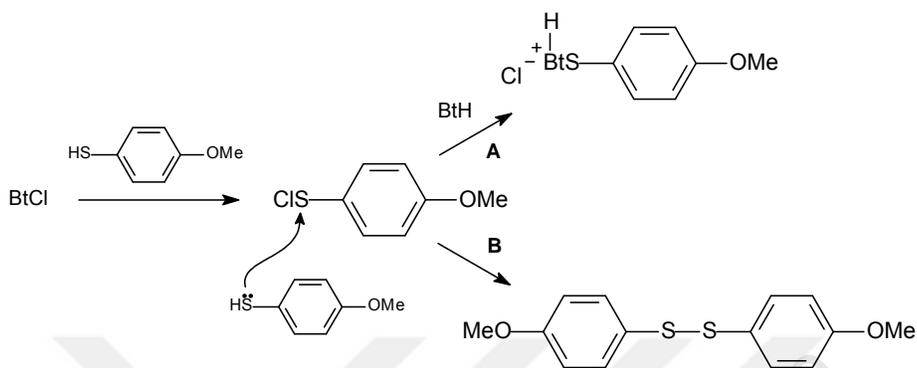


Scheme 2-5 Reagents and conditions: (i) CH₂Cl₂, -78 °C (product not isolated).

(Compound **65** is the envisaged intermediate product)

As revealed in Scheme 2-5, it was considered that the liberated benzotriazole, nucleophilic through mesomeric donation of the lone pair of the N-H nitrogen, would intercept the sulfenyl chloride via nucleophilic substitution as shown. It was decided to optimize the reaction by adding one equivalent of BtH into the reaction vessel containing the BtCl to optimize the formation of BtSR¹ and thus limit the formation of any symmetrical disulfide *via* reaction of sulfenyl chloride with incoming thiol. Scheme 2-6 illustrates the two possible pathways:

pathway **A** proceeds in the presence of BtH and thus aids the conversion of R^1SH to R^1SBt while pathway **B** without BtH could produce homodimer of the first thiol owing to a competing reaction.



Scheme 2-6 Formation of symmetrical disulfide via pathway **B**.

As mentioned previously, TLC monitoring of the reaction revealed complete conversion of *p*-methoxybenzenethiol after 1 hour and the formation of a new product. In fact, this appeared as two closely-running products and was assigned as isomeric 1-BtSR and 2-BtSR intermediates, Figure 2-2. It was discovered after chromatographically isolating the intermediates that they are rather unstable and cannot be stored. 1H NMR of the phenyl analogue (PhSBt) gave resonances consistent with the proposed structure and as two isomers.

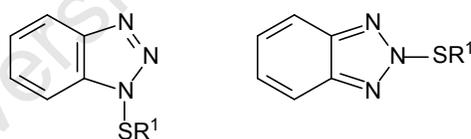
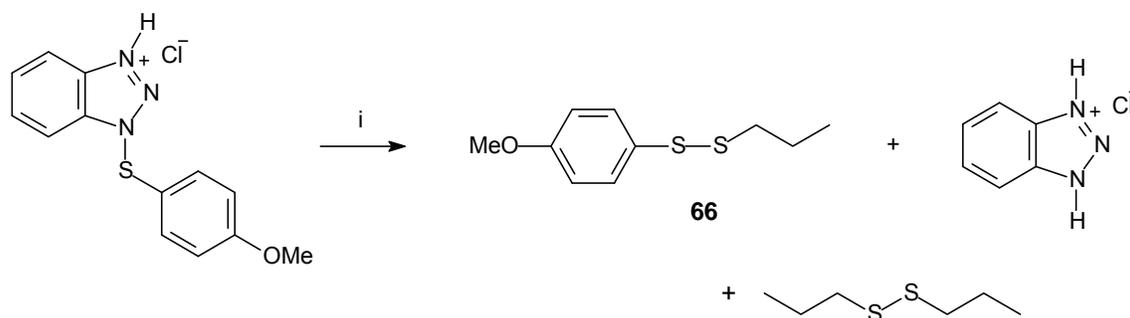


Figure 2-2 Structures of the BtSR intermediates.

The reaction was allowed to stir for an additional 1 hour and then allowed to warm to -20 °C. Importantly, no significant amounts of the homodimer disulfide di-*p*-methoxyphenyl disulfide could be detected *via* TLC, confirming that the BtH was a beneficial component in the reaction and that the resultant *N*-sulfenyl derivative $BtSR_1$ was not as reactive at -78 °C as BtCl toward the incoming thiol.

The second part of the reaction involved adding the second thiol to the reaction mixture to see if a second substitution could be effected to furnish the unsymmetrical disulfide product. In view of the lack of any appreciable reactivity of the thiol with $BtSR_1$ at -78 °C, 1-propanethiol (1.5 eq) was added slowly to the resultant solution at -20 °C and the mixture allowed to stir

while monitoring *via* TLC, Scheme 2-7. The disappearance of the isomeric BtSR intermediates and appearance of a new less polar product was rapid and complete within 10-20 min.



Scheme 2-7 Reagents and conditions: (i) Pr-SH (1.5 eq), CH₂Cl₂, -20 °C, 30 min, 91% (Step 2).

The formation of an additional highly non-polar product, which was visible by iodine staining and under UV, was also observed. This was expected since excess BtCl was added to drive the reaction to complete conversion and would be available for oxidation of the excess second thiol (PrSH) to the symmetrical dipropyl disulfide. This did not pose a problem since the very non-polar PrSSPr could easily be separated from the desired product chromatographically. The reaction work-up involved adding aqueous Na₂S₂O₃ to reduce any remaining BtCl back to BtH, which could be completely recovered (100% by mass) *via* an acid-base extraction protocol. The product was then extracted into DCM and chromatographed to give the unsymmetrical disulfide **66** as a yellow oil in high yield (91%). The IR spectrum of **66** confirmed the presence of a S-S stretch seen at 495 cm⁻¹, and no sharp peak corresponding to a -SH stretch was observed between 2600-2550 cm⁻¹. The ¹H NMR and ¹³C NMR spectra revealed the correct number of resonances as expected for the unsymmetrical disulfide product while HRMS revealed a molecular ion with fragments corresponding to fission of the disulfide bond. Scheme 2-8 outlines the overall one-pot reaction for unsymmetrical disulfide synthesis, named the “BtCl method”.



Scheme 2-8 Reagents and conditions: (i) BtCl (1.5 eq.), BtH (1 eq.), CH₂Cl₂, -78 °C, 2 hr. (ii) R²SH (1.5 eq.), CH₂Cl₂, -20 °C, 30 min. (R¹SH = *p*-methoxybenzenethiol and R²SH = Pr-SH).

BtH vs Other Additives

As mentioned before, BtH was added to the reaction to assist trapping of the very reactive sulfonyl chloride. We decided to explore the use of other additives like NEt₃ and thiourea in

place of the BtH. While the former might assist in nucleophilic trapping (base catalysis) of the sulfenyl chloride with existing BtH, the latter was perceived to offer the possibility of a trapping process like BtH. The reactions were run under the set of conditions as outlined in Scheme 2-8 and the results are reported in Table 2-1.

Entry	Additive (equiv) ^a	Yield (%)
1	None	67
2	Thiourea (1)	41
3	NEt ₃ (1.5)	18
4	BtH (1)	91

Table 2-1 Additives tested for sulfenyl halide trapping (^a Relative to R¹SH).

From the results, BtH turned out to be superior to the other additives investigated. The superior yield with one equivalent of BtH compared to omitting it (entry 1) corroborated the mechanistic view in Scheme 2-6. Other additives such as thiourea and NEt₃ proved to be detrimental to the efficiency of the reaction, particularly NEt₃, which may have facilitated reaction of thiol R¹SH to give the symmetrical disulfide (PrSSPr).

The *in situ* trapping of the sulfenyl chloride intermediate by BtH is a highlight and unique aspect of this method as it limits symmetrical disulfide formation of the first thiol, eliminating the possibility of producing all three possible disulfides as is the case with other known methods.

Order of Addition

With one aromatic thiol and one aliphatic thiol making up the allicin mimic, the question about order of thiol addition arose. It is well known that efficient construction of an aliphatic-aliphatic unsymmetrical disulfide poses a difficult problem and not many methods achieve this without producing quantities of the symmetrical disulfides, which are difficult to separate from one another. The methodology was thus tested for aliphatic thiol addition first. Disappointingly, the reaction was spectacularly unsuccessful. Adding the aliphatic thiol first resulted in formation of significant amounts of aliphatic homodimer (dipropyl disulfide), reflecting the greater nucleophilicity of the aliphatic thiol compared to the *p*-methoxybenzenethiol used in the model study towards the intermediate sulfenyl chloride. Thus it was decided to proceed with aromatic thiol as the first thiol of addition. A methodological variation would have to be developed for the preparation of aliphatic-aliphatic unsymmetrical disulfides.

Reagent Equivalents

The reaction was optimized by varying the number of equivalents of the reagents to determine the ideal conditions for maximizing the product (unsymmetrical disulfide) yield. The results are reported in Table 2-2.

Reaction	Number of Equivalents				Product Yield (%) ^a
	BtCl	BtH	R ¹ SH	R ² SH	
1	1.5	1	1	1.5	91
2	1.5	0	1	1.5	67
3	1.5	1	1	1	72
4	1	1	1	1.5	73
5	1	1	1	1	72

Table 2-2 Comparison of various equivalents (R¹SH = *p*-methoxybenzenethiol, R²SH = Pr-SH).

^a Isolated yields after column chromatography (symmetrical disulfides were not isolated).

The results clearly indicate that an excess of BtCl and the presence of BtH are both essential for obtaining the unsymmetrical disulfide in high yield as revealed by reactions 1, 2 and 4. Reaction 2, as discussed before, was carried out in the absence of BtH and gave the lowest yield. In addition, the yield of the desired product decreased when the number of equivalents of R²SH and BtCl were decreased as demonstrated by reactions 3, 4 and 5. An excess of R²SH is needed to account for the inevitable homodimerisation with the excess BtCl. Reaction 1 gave the highest yield (91%) confirming that the initial reaction conditions were efficiently selected.

Temperature Variants

The factor of temperature is crucial in all reactions and is often the determining facet of the methodology being developed. Owing to the very reactive nature of sulfenyl halides, the first step of the reaction was initially carried out at -78 °C to maximize the efficiency of that first step. At this temperature, the reaction was observed to be rapid with no side reactions observed. Substitution with the second thiol was facile at -20 °C. Although these temperature conditions worked out well, producing the unsymmetrical disulfide for the model system in high yields, it was vital to know how temperature affects the reaction. Attention was thus directed at determining the outcome of the reaction for steps 1 and 2 at higher and lower temperatures respectively. Lower temperatures were not considered for step 1 since the reaction proceeds satisfactorily at -78 °C.

(a) Temperature Evaluation of Step 1: Thiol 1 Addition

Thiol 1 was slowly added to a solution of BtCl and BtH in DCM at 0 °C. The solution rapidly went from clear to red and then slowly over time became yellow. TLC analysis revealed the formation of a new product which was different from the two spots identified as the 1-BtSR and 2-BtSR intermediates. The reaction was stopped and worked up as described before. The product was isolated by chromatography as a yellow solid which was characterized by IR, ¹H NMR and ¹³C NMR spectroscopy. The presence of a band at 495 cm⁻¹ in the IR spectrum confirmed that the compound contained an S-S bond. The ¹H NMR spectrum was similar to that of *p*-methoxybenzenethiol, although an SH singlet was absent which was observed at δ_H 3.38 in the starting material. The ¹³C NMR spectrum confirmed the presence of signals for a *p*-methoxybenzenethio-fragment and thus the compound was assigned as di-*p*-methoxyphenyl disulfide. Changing the temperature in step 1 from -78 °C to -20 °C and keeping the second step's temperature at -20 °C resulted in a drop in the yield of the unsymmetrical disulfide product and in addition, also the formation of di-*p*-methoxyphenyl disulfide. It is thus clear that higher temperatures in step 1 promote a competitive reaction of the sulfenyl chloride with incoming thiol and are thus inappropriate for the transformation, which was consequently set at -78 °C. The results obtained are shown in Table 2-3.

Temperature at Thiol Addition		Yield of Products Obtained (%)	
Step 1	Step 2	R ¹ SSR ¹	R ¹ SSR ²
0°C	<i>a</i>	70	<i>a</i>
-20°C	-20°C	25	70
-78°C	-20°C	<i>b</i>	91

Table 2-3 Results for temperature variations (*a*: reaction with R²SH not carried out, *b*: not obtained).

(b) Temperature Evaluation of Step 2: Thiol 2 Addition

The first part of the reaction was carried out as described in Scheme 2-5. The temperature was kept at -78 °C and thiol 2 was slowly added to the reaction mixture. The reaction was monitored by TLC and it was observed that the reaction proceeded at a slower rate. The reaction time was extended to one hour instead of being complete within 30 minutes. The findings suggest that the temperatures initially chosen to carry out the reaction were the best combination and thus the reaction times were set at -78 °C for step 1 and at -20 °C for step 2.

Solvent Choices

Although the solvent choice is one of the major factors contributing to reaction outcome and reaction feasibility, this aspect was not examined in this study. The reactions were chosen to

be carried out in CH_2Cl_2 owing to the nature of the reaction. The ideal system would be an aqueous system but owing to the brief formation of a sulfenyl halide intermediate it was perceived to be inappropriate. The products were obtained in high yields with CH_2Cl_2 as solvent, thus we omitted this facet. Future work is needed to investigate the effect of solvent on the reaction outcome.

Green Character

The methodology offers attractive environmentally friendly and cost-saving aspects. Firstly, bleach is used to convert BtH to BtCl as discussed before and secondly, BtH can be easily recycled. The latter is achievable by chromatography or *via* an acid/base extraction.

(a) Chromatography

BtH has an R_f value of 0.3 using 40% ethyl acetate in petroleum ether. Following reaction, the BtH by-product was isolated *via* column chromatography in 96% yield and its structure confirmed by analytical and spectral data. Its melting point of 96-97 °C agreed with the literature value²⁴⁵ of 98-99 °C, while its IR spectrum showed a broad NH band at 3080 cm^{-1} . Its ^1H NMR and ^{13}C NMR spectra were identical to those of an authentic sample of BtH which was purchased from Aldrich.

(b) Acid/Base Extraction

The following approach necessitated acidifying the reaction mixture to protonate BtH and extract its ion into the aqueous layer. Following reaction between *p*-methoxybenzenethiol and 1-propanethiol according to Scheme 2-8, the reaction was quenched with aq. $\text{Na}_2\text{S}_2\text{O}_3$ and then acidified with excess concentrated HCl to pH 1, stirred for 1 hour in a two-phase system with DCM to ensure complete extraction of the organic material. The stirring time was set at 1 hour since lower times diminished the BtH recovery yield owing to incomplete extraction into the acidified layer. The organic extracts contained the desired unsymmetrical disulfide which was isolated chromatographically. The aqueous layer was basified with aq. NaHCO_3 to pH 8 and the product (BtH) extracted with DCM. The extract was concentrated to give BtH in a high yield (99%), m.p. 96-97 °C (lit. 98-99 °C).²⁴⁵ The IR spectrum showed a broad NH band at 3080 cm^{-1} and the ^1H NMR and ^{13}C NMR spectra corresponded to the spectra of an authentic BtH sample that was purchased from Aldrich. Although the BtH was isolated quantitatively, the yield of the unsymmetrical disulfide was compromised (70%) presumably because of the relatively long stirring times with the HCl. For scale-up purposes, these two process-development factors (stirring time and efficiencies of isolation of product and by-product) would need to be evaluated.

Not many methodologies have the privilege of being one-pot, environmentally friendly and cost-effective. This is the beauty of the BtCl methodology, as it achieves all of these desirable methodological features. The key features of this new one-pot synthesis are summarized in Figure 2-3.

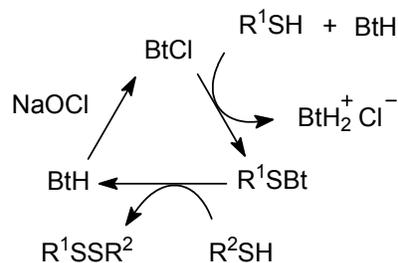


Figure 2-3 Cycle for the BtCl methodology.

2.1.3 Classes of Unsymmetrical Disulfides

Subsequent reactions to investigate the scope of the reaction were divided into the various types of disulfides. Three types of disulfides, namely aromatic-aliphatic, aromatic-aromatic and aliphatic-aliphatic, were thus investigated by this method. For the first two categories, the methodology worked well, but the last category required modification of the procedure to include a feature of an older methodology due to the difficulty encountered as described previously.

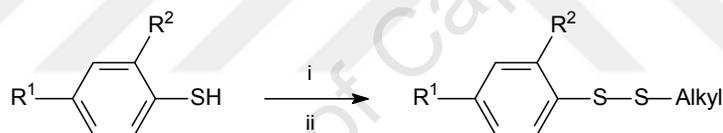
Aromatic-Aliphatic Disulfides

The literature reports several methods for the preparation of aromatic-aliphatic disulfides but many suffer from a wide range of shortcomings as discussed in Chapter 1. The newly developed method presented was thus explored to establish whether it was able to transform a range of thiols to unsymmetrical disulfides. As mentioned, adding the aromatic thiol first worked best, as the other way round resulted in the formation of large amounts of the aliphatic symmetrical disulfide. Aromatic thiols for this study were selected to address the influence of both steric and electronic factors on the efficacy of the process. Thus *p*-tolylthiol, *p*-methoxybenzenethiol and 2-hydroxyphenylthiol provided an opportunity for evaluating electron-releasing substituents, with 2-hydroxyphenylthiol also adding steric and hydrogen bonding effects (for thiosulfonates). Conversely, *p*-nitrophenylthiol and methyl thiosalicylate were chosen as electron-deficient systems. In addition, methyl thiosalicylate included an *ortho* steric effect. 2-Pyridylthiol was also included in the study as a π -deficient heteroaromatic thiol.

Reactions were run under the optimized set of conditions described for the model study (Scheme 2-8) and the unsymmetrical disulfides were obtained in excellent yields across the spectrum for all types of thiols. However, in the case of allylthiol (as R²SH) yields were consistently around 55-60%. Importantly, no symmetrical disulfide of the aromatic thiol was obtained but the excess BtCl inevitably converted the excess aliphatic thiol to its symmetrical disulfide, which could be easily separated by column chromatography. All compounds were fully characterized by IR, NMR and HRMS. The high yields and full conversion of the aromatic thiol to the unsymmetrical disulfide suggest exchange reactions post-coupling not to be a serious side-reaction as it is for other methodologies.

(a) Electron-Rich Systems

Scheme 2-9 illustrates the overall reaction sequence followed for the unsymmetrical disulfide synthesis with an electron-rich moiety: alkyl *p*-methoxyphenyl disulfides, R¹ = MeO and R² = H; alkyl *p*-tolyl disulfides, R¹ = Me and R² = H; alkyl 2-hydroxyphenyl disulfides, R¹ = H and R² = OH; alkyl *p*-aminophenyl disulfides, R¹ = NH₂ and R² = H.



Scheme 2-9 Reagents and conditions: (i) BtCl, BtH, CH₂Cl₂, -78 °C. (ii) Alkyl-SH, -20 °C.

Alkyl *p*-methoxyphenyl disulfides:

All starting thiols were commercially available. *p*-Methoxybenzenethiol was added to the reaction as R¹SH and the aliphatic thiols as R²SH. TLC analysis revealed that the reaction mixture was free of homodimer of the first thiol (R¹SSR¹) and that complete conversion of the BtSR¹ intermediate had taken place to give a more non-polar compound. The compounds were easily chromatographed and gave the corresponding unsymmetrical disulfides in excellent yields, Table 2-4. A literature survey revealed that all the newly formed disulfides are new compounds except products **69** and **72**.^{246,247} With respect to the NMR analysis, the protons and carbons are labeled as 1, 2, 3, etc from the sulfur atom side towards the methyl group of the chain, as illustrated in Figure 2-4.

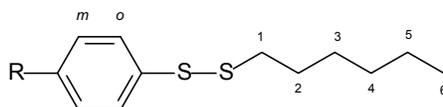


Figure 2-4 Numbering system for aromatic-aliphatic unsymmetrical disulfides.

Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
66	<i>p</i> -MeOC ₆ H ₄ SH	<i>n</i> -PrSH		91
67	<i>p</i> -MeOC ₆ H ₄ SH	<i>t</i> -BuSH		79
68	<i>p</i> -MeOC ₆ H ₄ SH	AllylSH		60
69	<i>p</i> -MeOC ₆ H ₄ SH	<i>n</i> -BuSH		93
70	<i>p</i> -MeOC ₆ H ₄ SH	<i>n</i> -HexSH		80
71	<i>p</i> -MeOC ₆ H ₄ SH	<i>n</i> -DecSH		92
72	<i>p</i> -MeOC ₆ H ₄ SH	<i>n</i> -DodecSH		93

Table 2-4 Yields obtained for alkyl *p*-methoxyphenyl disulfides.

Note: For simplicity, NMR details of compound **66** (i.e. chemical shifts, multiplicity and coupling constants) will be reported as a representative for common fragments in the structure of the compounds of this series.

Unfortunately, it was not possible to improve the yield of **68** beyond 60%. Two possible reasons could account for the low yield: (i) allylthiol, which is very reactive could have oxidized to diallyl disulfide in the sample added resulting in less allylthiol being available for the unsymmetrical disulfide formation or (ii) the compound, *p*-methoxyphenyl 2-propenyl disulfide **68** is unstable, either rearranging or decomposing at some stage of the reaction or on the acidic silica-gel column. This would be due to the allylic group since *p*-methoxyphenyl 1-propyl disulfide is stable and does not undergo any decomposition.

The ¹H NMR spectra of each compound revealed the expected disappearance of the -SH singlet at δ_H 3.38 which is observed for the starting thiol, *p*-methoxybenzenethiol, verifying that the compounds were not physical mixtures of the two starting thiols. In addition, all the compounds in the series showed the expected peaks for the aromatic protons resonating as a

pair of doublets (J 8.9 Hz) at δ_{H} 6.86 and δ_{H} 7.48 for the *meta*- (relative to C-S) and *ortho*-protons respectively. The more deshielded protons of the upfield aliphatic signals were assigned as those adjacent to the sulfur as H-1 (δ_{H} 2.71) and those for H-3 (δ_{H} 0.96) as the most upfield. In addition, the methoxy protons resonated as a sharp singlet at δ_{H} 3.80. The correct number of resonances was observed in the ^{13}C NMR for each disulfide as four aromatic signals downfield and a number of aliphatic resonances upfield including the methoxy methyl group. The four aromatic peaks revealed two large signals for the *ortho* and *meta* carbons and two much smaller signals for the relaxed quaternary carbons bearing the oxygen and sulfur heteroatoms. In this regard, the carbon bonded to the methoxy group resonated more downfield (δ_{C} 159.5) compared to that bearing the sulfur (δ_{C} 128.6). These markers provide important diagnostic references for future characterization.

Additional assignments and observations from the NMR that were noted;

- (i) C-1 of compound **67** was observed at δ_{C} 48.9 as a small, very relaxed peak,
- (ii) α -C-1 methylene of compound **68** is deshielded due to the double bond (allylic), and
- (iii) the carbon chemical shifts (assignments confirmed by HSQC) for compound **70** do not follow in numerical order as observed for compounds **66** and **69**.

For the latter point, C-2 of **70** resonates at δ_{C} 28.7 while C-3, 4 and 5, which cannot be distinguished from one another resonate at δ_{C} 22.5, 28.1 and 31.4. This is also observed for compound **71** but a further increase in alkyl-chain length made it difficult to assign the carbon peaks independently.

The IR spectra for each compound indicated the disappearance of a thiol peak at 2580 cm^{-1} and the appearance of a peak at 495 cm^{-1} for compounds **66**, **67**, **68**, **69** and **70** and 499 cm^{-1} for compounds **71** and **72**, which is diagnostic for an S-S stretch.

Alkyl *p*-tolyl disulfides:

The compounds were synthesized as described before except replacing thiol **1** with *p*-tolylthiol. A literature search revealed that the majority of the compounds in the series have been reported in earlier years when other sulfenylating agents were extensively exploited.^{206,248-250} However, compound **77** is a new compound and was fully characterized. The yields for the range of alkyl *p*-tolyl disulfides prepared for the study are illustrated in Table 2-5.

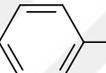
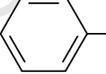
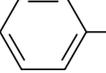
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
73	<i>p</i> -MeC ₆ H ₄ SH	<i>n</i> -PrSH	Me-  -S-S-CH ₂ CH ₂ CH ₃	85
74	<i>p</i> -MeC ₆ H ₄ SH	<i>t</i> -BuSH	Me-  -S-S-C(CH ₃) ₃	81
75	<i>p</i> -MeC ₆ H ₄ SH	AllylSH	Me-  -S-S-CH ₂ CH=CH ₂	60
76	<i>p</i> -MeC ₆ H ₄ SH	<i>n</i> -BuSH	Me-  -S-S-CH ₂ CH ₂ CH ₂ CH ₃	81
77	<i>p</i> -MeC ₆ H ₄ SH	<i>n</i> -HexSH	Me-  -S-S-(CH ₂) ₅ CH ₃	82

Table 2-5 Yields obtained for alkyl *p*-tolyl disulfides (NMR details of **73** were reported as a representative for common fragments in the structure of the compounds of this series).

Once again, the allyl-containing disulfide gave a lower yield compared to the rest of the series. However, in this series di-*p*-tolyl disulfide co-eluted with compound **75**, as observed by its NMR spectrum. Compound **75** and di-*p*-tolyl disulfide had very similar R_F values, made it thus difficult to separate them. In an attempt to obtain **75** pure, two approaches were considered; (i) a two-fold excess of BtCl was added to the reaction and (ii) isolation of the BtSR¹ intermediate was attempted. Frustratingly, both approaches were unsuccessful. The first approach continued to produce the unwanted symmetrical disulfide, while the second gave no discernible product after isolation, once again providing evidence that the intermediate is unstable. Furthermore, the lack of di-*p*-tolyl disulfide contaminating the other compounds in the series suggests that the allyl-containing product rearranges or decomposes at some stage of the reaction or the starting thiol (allylthiol) has been oxidized to diallyl disulfide. In 1994,

Leriverend prepared allyl-containing disulfides by reaction of a thiol and a dithioperoxyester obtained from rearrangement of a sulfine.²⁴⁹ He prepared 2-propenyl *p*-tolyl disulfide and benzyl 2-propenyl disulfide in 43% and 42% respectively, corroborating our findings. The ¹H and ¹³C NMR of **75** corresponded to that published.²⁴⁹

Similar trends in the ¹H and ¹³C NMR spectra as noted for the alkyl *p*-methoxyphenyl disulfides were observed for the alkyl *p*-tolyl disulfides. As expected, the ¹H NMR spectrum showed the aromatic *meta*- (relative to C-S) and *ortho*-protons resonating at δ_{H} 7.13 and δ_{H} 7.43, indicating a slightly more downfield shift for the meta protons compared to the previous series in view of methoxy's enhanced resonance shielding effect compared to that of methyl. The alkyl chain's aliphatic signals were consistent and in good agreement with previous observations and in addition, a sharp singlet at δ_{H} 2.32 corresponding to the aromatic methyl was observed. Noteworthy differences in the ¹³C NMR spectra for the compounds in this series compared to that of the alkyl *p*-methoxyphenyl disulfides were the significant shifts in the quaternary aromatic carbons. Nevertheless, the carbon attached to methyl (δ_{C} 137.1) was still more downfield compared to that attached to the sulfur atom (δ_{C} 134.5). C-2 for compound **77** was once again observed at a frequency out of numerical order. Furthermore, an S-S stretch was observed in the IR spectra at 487 cm⁻¹ for the disulfide stretch.

Alkyl 2-hydroxyphenyl disulfides:

The compounds were synthesized using the BtCl method with 2-hydroxyphenylthiol as R¹SH and the aliphatic thiol as R²SH. 2-hydroxyphenylthiol was included as a starting material to investigate the possibility of steric hindrance due to the ortho hydroxyl group on the aromatic ring as well as hydrogen-bonding effects. The reaction proceeded as before without the need for longer reaction times and thus it was concluded that the hydroxyl group did not affect the reaction, electronically or sterically. Noteworthy was the chemoselective transformation of the SH group without the need for phenolic hydroxyl-group protection, as a result of sulfur's greater nucleophilicity (softer). In addition, the activated aromatic ring was not chlorinated.

These compounds were also chosen as targets for probing possible H-bonding when converted to their thiosulfinates. These aspects will be discussed in chapter 3. However, the oxidation of these compounds proved to be problematic. The yields for two new unsymmetrical disulfides synthesized are shown in Table 2-6.

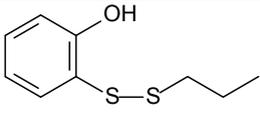
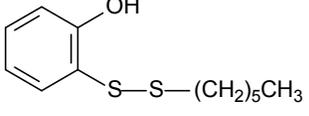
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
78	<i>o</i> -HOC ₆ H ₄ SH	<i>n</i> -PrSH		80
79	<i>o</i> -HOC ₆ H ₄ SH	<i>n</i> -HexSH		86

Table 2-6 Yields obtained for the alkyl 2-hydroxyphenyl unsymmetrical disulfides.

A full assignment of the aromatic region required the use of 2D-NMR experiments. Thus HSQC (for carbon-hydrogen correlation) and COSY (for hydrogen-hydrogen correlation) techniques were used to assign the NMR unambiguously. An example of the ¹H NMR's aromatic region for these compounds is shown below, Figure 2-5. The aromatic region of compound **78** was thus used as a model for the NMR assignments of this series.

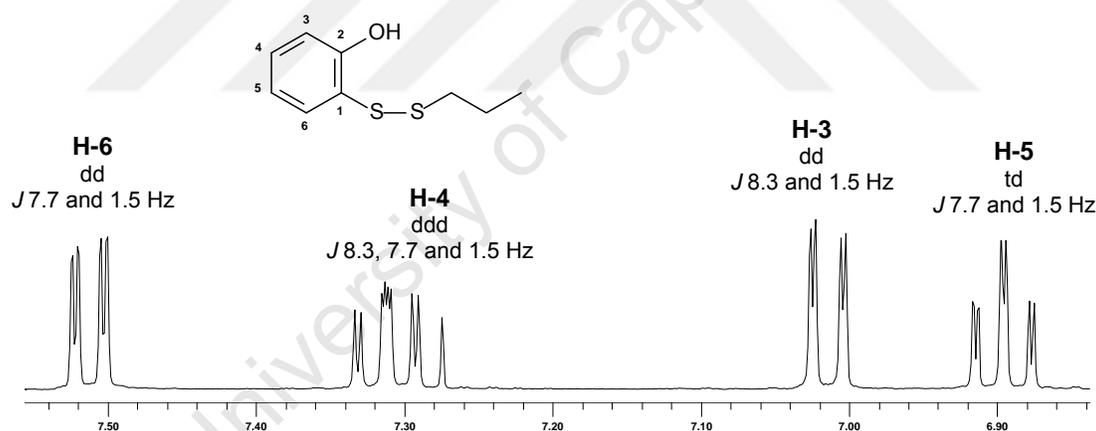


Figure 2-5 ¹H NMR spectrum of aromatic region of unsymmetrical disulfide **78**.

Examination of the aromatic signals for **78** revealed that of H-6 and H-3, both as doublet of doublets, H-6 should be the more downfield proton due to the shielding effect H-3 experiences by the OH group ortho to it. By applying this 'marker' to the COSY spectra, it was evident from an observed cross peak that an intense coupling (as indicated by the size and intensity of the cross peak) is experienced between a proton at δ_{H} 6.89 and H-6 (δ_{H} 7.51). Thus the signal at δ_{H} 6.89 was assigned as H-5. A less intense coupling, due to long-range coupling was observed, evident from a small, faint cross peak between proton H-6 and the proton at δ_{H} 7.30, which must be H-4. Hence, the final proton, resonating at δ_{H} 7.01, was assigned as H-3. This was confirmed by ¹H NMR which revealed a doublet of doublets for H-3 with a large

coupling constant of J 8.3 Hz due to H-4 and a smaller one of J 1.5 Hz due to long-range coupling with H-5. While H-5 was observed as a triplet of doublets in view of equal coupling constants with H-4 and H-6, H-4 appeared as a doublet of doublet of doublets in view of unequal couplings with H-3 and H-5. However, for compound **79**, H-5 was observed as a doublet of doublet of doublets and H-4 as a triplet of doublets. Examination of the HSQC spectrum revealed that the four signals for H-3, H-5, H-4 and H-6 in the ^1H NMR correspond to the signals found at δ_{C} 115.9, 120.8, 131.9 and 134.9 in the ^{13}C NMR respectively with C-3 and C-5 shielded by the OH group. The two quaternary carbons (C-1 and C-2) on the ring still remained to be assigned. However, from previous assignments, C-1 could be assigned as the signal at δ_{C} 121.1 and C-2 at δ_{C} 156.6.

The IR spectra confirmed the presence of a sharp S-S band at 499 cm^{-1} and a broad OH band at 3450 cm^{-1} , while high-resolution mass spectrometry confirmed the correct molecular ion.

Alkyl *p*-aminophenyl disulfide:

Owing to time constraints, *p*-aminophenyl 1-propyl disulfide **80** was the only compound synthesized for this category. The starting aromatic thiol was commercially available but only gave a moderate yield of the unsymmetrical disulfide as shown in Table 2-7.

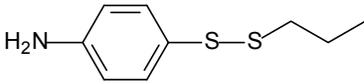
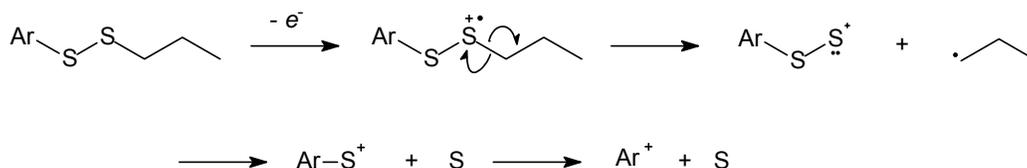
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
80	<i>p</i> -H ₂ NC ₆ H ₄ SH	<i>n</i> -PrSH		64

Table 2-7 Yield obtained for *p*-aminophenyl 1-propyl disulfide **80**.

The reaction was closely monitored by TLC, which indicated that all of the *p*-aminobenzenethiol was consumed in the first step after 3 hours of stirring (slowly warming to $-20\text{ }^{\circ}\text{C}$). Addition of 1-propanethiol resulted in a range of spots on TLC. Careful column chromatography gave no substantial masses of the by-products, dipropyl disulfide or di-*p*-aminophenyl disulfide. The compounds that were isolated could not be readily assigned a structure from their ^1H NMR spectra. However, the product was obtained but in much lower yield than the other unsymmetrical disulfides synthesized. Its ^1H and ^{13}C NMR spectra displayed all of the expected signals, including an additional broad singlet observed at δ_{H} 3.70 corresponding to the NH₂ protons. In addition, the IR spectrum confirmed the presence of a disulfide bond with a band observed at 490 cm^{-1} . Its high resolution mass spectrum revealed interesting fragments under EI mode. A correct molecular ion was observed at m/z 199.0482,

followed by sequential loss of the alkyl chain, scission of the disulfide bond with loss of one sulfur atom followed by scission of the final sulfur atom from the aromatic ring. This suggests an ionization involving one of the alkyl S lone pairs to give a radical cation, *via* the mechanism illustrated in Scheme 2-10.



Scheme 2-10 Mechanism for fragmentation of disulfides.

Hence, the mass spectrum had the following fragmentation pattern, 199.0482 $[M]^{++}$, 155.9939 $[M-CH_2CH_2CH_3]^+$, 124.0211 $[M-CH_2CH_2CH_3-S]^+$ and 93.0572 $[M-CH_2CH_2CH_3-S-S]^+$ which confirmed the presence and connectivities of the propyl chain and disulfide moiety. Table 2-8 illustrates the fragments observed by HRMS characterization.

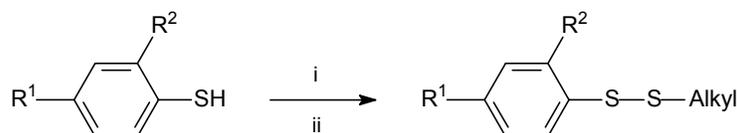
Entry	Fragment	Mass (experimental)	Mass (calculated)
1		199.0482	199.0489
2		155.9939	155.9941
3		124.0211	124.0221
4		93.0572	93.0578

Table 2-8 High resolution mass spectrum fragments observed for **80**.

(b) Electron-Deficient Systems

The literature contains little information on unsymmetrical disulfides with an electron-deficient moiety and thus two model compounds were selected for the study, namely *p*-nitrobenzenethiol and *o*-methoxycarbonylbenzenethiol (methyl thiosalicylate). The overall

reaction sequence for unsymmetrical disulfide synthesis with an electron-deficient moiety is illustrated in Scheme 2-11: alkyl *p*-nitrophenyl disulfides, $R^1 = \text{NO}_2$ and $R^2 = \text{H}$; alkyl *o*-methoxycarbonylphenyl disulfides, $R^1 = \text{H}$ and $R^2 = \text{CO}_2\text{Me}$.



Scheme 2-11 Reagents and conditions: (i) BtCl , BtH , CH_2Cl_2 , -78°C . (ii) AlkylSH , -20°C .

Alkyl *p*-nitrophenyl disulfides:

The starting aromatic thiols were commercially available with no further purification required. Pleasingly, the nucleophilic substitution for each pair of thiols was successful and gave the corresponding unsymmetrical disulfides in excellent yields as illustrated in Table 2-9.

Compound	$R^1\text{SH}$	$R^2\text{SH}$	$R^1\text{SSR}^2$	Yield (%)
81	$p\text{-O}_2\text{NC}_6\text{H}_4\text{SH}$	$n\text{-PrSH}$		88
82	$p\text{-O}_2\text{NC}_6\text{H}_4\text{SH}$	$t\text{-BuSH}$		83
83	$p\text{-O}_2\text{NC}_6\text{H}_4\text{SH}$	$n\text{-BuSH}$		95
84	$p\text{-O}_2\text{NC}_6\text{H}_4\text{SH}$	$n\text{-HexSH}$		84
85	$p\text{-O}_2\text{NC}_6\text{H}_4\text{SH}$	$\text{HO}(\text{CH}_2)_2\text{SH}$		76

Table 2-9 Yields obtained for alkyl *p*-nitrophenyl disulfides (NMR details of **85** were reported as a representative for common fragments in the structure of the compounds of this series).

Examination of the IR spectra for each compound revealed the presence of a disulfide stretch at 499 cm^{-1} as well as an asymmetric and symmetric stretch observed at 1515 cm^{-1} and 1340 cm^{-1} respectively for the nitro group. In addition, compound **85** displayed a broad OH band at 3359 cm^{-1} . High resolution mass spectrometry confirmed the correct molecular ion for

each product and revealed a fragment corresponding to S-S scission. Although the products are oils, two of the disulfides (**81** and **85**) were characterized by C, H, N, S analysis, which returned acceptable values (Table 2-10) revealing the molecular formulae for **81** and **85** as $C_9H_{11}NO_2S_2$ and $C_8H_9NO_3S_2$ respectively.

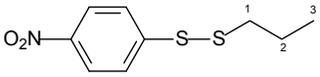
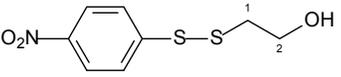
Atom	 81		 85	
	Calculated	Experimental	Calculated	Experimental
C	47.14	47.24	41.55	41.48
H	4.84	4.82	3.92	3.90
N	6.11	6.05	6.06	6.02
S	27.96	28.01	27.72	27.80

Table 2-10 Elemental analysis results for compounds **81** and **85**.

1H NMR revealed the deshielding effect of the nitro group on the aromatic protons with the meta protons (to C-S) resonating at δ_H 8.14 and the ortho ones at δ_H 7.66. This was the opposite trend to that observed for the *p*-methoxyphenyl case on resonance grounds. The 1H NMR of product **85** showed two signals in the aliphatic region. The signal resonating as a triplet (J 5.9 Hz) at δ_H 3.84 was assigned as the protons (H-2) adjacent to the hydroxyl group while the protons (H-1) adjacent to the sulfur atom resonated at δ_H 2.93 as a triplet (J 5.9 Hz). The signals at δ_C 41.3 and δ_C 60.0 in the ^{13}C NMR spectra corresponded to H-1 and H-2 in the 1H NMR spectra respectively as evident from the HSQC. The NMR assignments of the aliphatic region for each product corresponded to previous observations.

Alkyl *o*-methoxycarbonylphenyl disulfides:

Once again, the sterically bulky ortho substituent on the aromatic ring did not restrict the accessibility of the electrophile and thus the alkyl *o*-methoxycarbonylphenyl disulfides were obtained in excellent yields as shown in Table 2-11. However, the allyl-containing disulfide yet again gave an inferior yield. A thorough search of the literature failed to uncover a case in which an allyl-containing compound was obtained in yields exceeding 60%.

The NMR assignments for the aliphatic region of each compound were consistent with those reported for the corresponding alkyl chains in the other series, while the IR spectra for each compound showed the presence of a S-S stretch at 486 cm^{-1} .

Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
86	<i>o</i> -MeO ₂ CC ₆ H ₄ SH	<i>n</i> -PrSH		97
87	<i>o</i> -MeO ₂ CC ₆ H ₄ SH	<i>t</i> -BuSH		90
88	<i>o</i> -MeO ₂ CC ₆ H ₄ SH	AllylSH		55

Table 2-11 Results obtained for alkyl *o*-methoxycarbonylphenyl disulfides (NMR details of **88** were reported as a representative for common fragments in the structure of the compounds of this series).

The aromatic region of the ¹H and ¹³C NMR spectra of each product could be unambiguously assigned using 2D-NMR (COSY and HSQC spectra). The ¹H NMR spectrum (aromatic region) of compound **88** is shown in Figure 2-6 and serves to illustrate the assignments made for the aromatic protons and carbons of the alkyl *o*-methoxycarbonylphenyl disulfides.

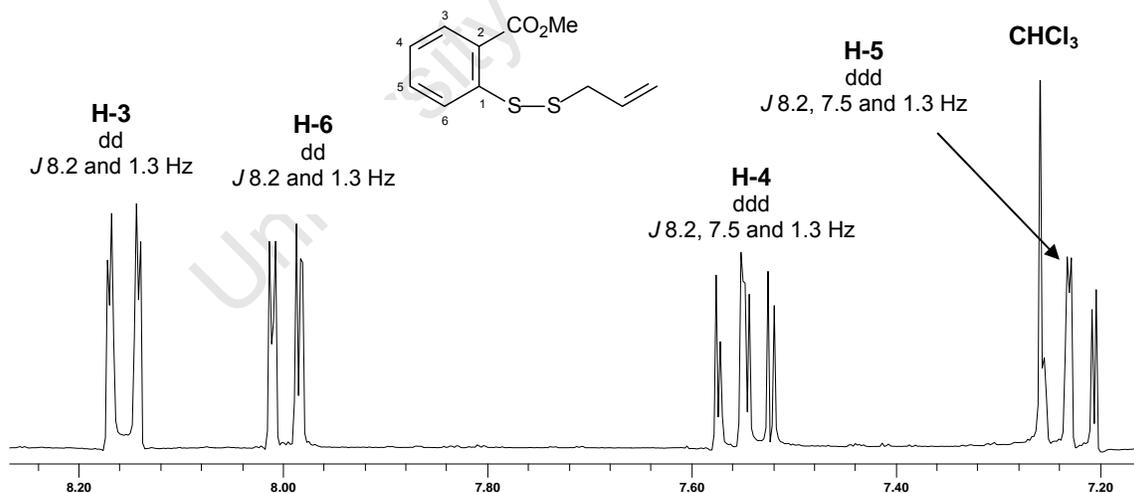


Figure 2-6 ¹H NMR spectrum of the aromatic region of unsymmetrical disulfide **88**.

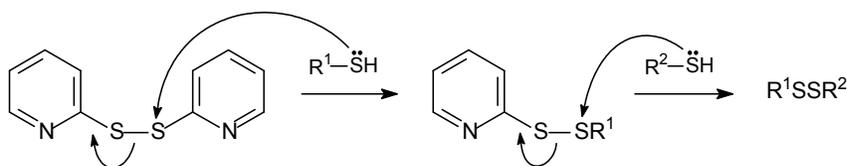
The 1,2-disubstituted array gave a similar set of resonances to those for the alkyl 2-hydroxyphenyl disulfides, except that the electron-withdrawing ester functionality resulted in different chemical shifts. Of H-3 and H-6, proton H-3 in this case was assigned as the more downfield proton due to the deshielding effect that the ester group exerts. This was used as a

marker to assign the other protons on the aromatic ring. The COSY spectrum revealed a major coupling between H-3 (δ_{H} 8.16) and the proton at δ_{H} 7.55, which was thus assigned as H-4 (J 8.2, 7.5 and 1.3 Hz). The proton H-3, however, was split as a doublet of doublets (J 8.2 and 1.3 Hz) indicating long-range coupling. A major coupling was also observed between H-4 and the same proton, resonating at δ_{H} 7.23, which thus had to be H-5. H-5 (J 8.2, 7.5 and 1.3 Hz) experienced additional coupling (major) with the proton resonating as a doublet of doublets at δ_{H} 8.00 (J 8.2 and 1.3 Hz). This was thus assigned as H-6 as the proton adjacent to H-5 on the aromatic ring. These results were consequently used to assign the carbon atoms. Hence, the carbons resonating at δ_{C} 125.1, 125.9, 131.3 and 132.6 were assigned as C-5, C-3, C-6 and C-4 respectively. As expected, the quaternary carbons resonated at δ_{C} 128.2 (C-1) and δ_{C} 141.8 (C-2). In addition, the presence of an ester was observed in the ^{13}C NMR spectrum, with a carbonyl carbon resonating at δ_{C} 166.8, and a carbonyl stretch in the IR spectrum at 1708 cm^{-1} .

(c) π -Deficient Heteroaromatic System

Alkyl 2-pyridyl disulfides:

The synthesis of this category of unsymmetrical disulfide was achieved by the BtCl reaction as described previously. Of note were the high yields observed throughout. No interference *via* N-oxide formation was observed. 2-Pyridyl disulfides are known to act as substrates for thiol exchange to generate other unsymmetrical disulfides (pyridyl-S- as the leaving group), Scheme 2-12.²⁵¹ Clearly, given the observed yields, this does not seem to be a major side reaction in this case. The compounds were purified *via* column chromatography and the yields for the range of alkyl 2-pyridyl disulfides as illustrated in Table 2-12 are for pure unsymmetrical disulfides. The products of this series have all been reported in the literature, however, often as by-products and thus they have not always been fully characterized.²⁵²⁻²⁵⁴



Scheme 2-12 Reactions of 2-pyridyl disulfides ($\text{R}^2 = \text{R}^1$ or different alkyl/aromatic/heteroaromatic groups).

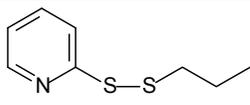
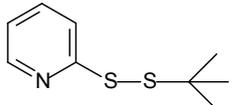
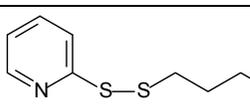
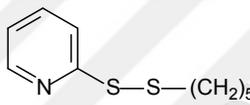
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
89	2-PyridylSH	<i>n</i> -PrSH		84
90	2-PyridylSH	<i>t</i> -BuSH		92
91	2-PyridylSH	<i>n</i> -BuSH		87
92	2-PyridylSH	<i>n</i> -HexSH		91

Table 2-12 Results obtained for alkyl 2-pyridyl disulfides (**91**'s NMR details were reported as a representative for common fragments in the structure of the compounds of this series).

Once again, the ¹H and ¹³C NMR spectra of the products could be completely assigned using HSQC and COSY experiments. Figure 2-7 illustrates the numbering system used for these compounds.

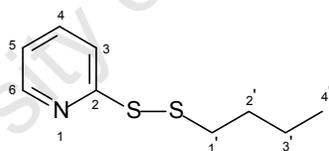


Figure 2-7 Structure and numbering of alkyl 2-pyridyl unsymmetrical disulfides.

A good 'marker' was once more identified as H-6 as the most deshielded proton δ_{H} 8.45 in the ¹H NMR spectrum. Owing to the number of long-range couplings experienced by each proton, the protons could not be assigned a multiplicity other than a multiplet. The intensity and size of the cross peaks was thus used as an indication of direct or long-range coupling; direct coupling is indicated by a large, very intense cross peak and the converse for long-range coupling. The COSY spectra revealed that H-6 experiences a large coupling due to the proton that resonates at δ_{H} 7.06 and long-range coupling due to protons resonating at δ_{H} 7.63 and δ_{H} 7.73. It is evident from the structure that the large coupling is caused by H-5, which was thus assigned at δ_{H} 7.06. Further inspection of the spectra indicated that H-5 also couples to the proton at δ_{H} 7.63, which in turn couples to the proton at δ_{H} 7.73, which were assigned as H-4 and H-3 respectively. These assignments were consistent with the ortho and para hydrogens to N being deshielded. However, the proton ortho to the disulfide grouping at

H-3 also experienced deshielding relative to H-5 (para to it). The signals in the ^{13}C NMR spectrum at δ_{C} 119.6, 120.4, 136.9, 149.5 were assigned as C-3, C-5, C-4 and C-6 by HSQC. Furthermore, HRMS confirmed the correct parent mass for each compound; $[185.0339]^+$ for **89**, $[199.0491]^+$ for **90**, $[199.0493]^+$ for **91** and $[227.0815]^+$ for **92**.

(d) Fluorinated Disulfides

Brace reported that fluorinated chains (eg. $-(\text{CH}_2)_2(\text{CF}_2)_n(\text{CF}_3)$) attached to the disulfide moiety stabilize thiosulfonates and this encouraged us to incorporate a fluorinated alkyl chain into our study.²⁵⁵ In order to give the reader an idea of what an impact fluorinated compounds have made recently on the analytical and organic chemistry fields, a brief account on fluorinated compounds and their applications will be reported.

Fluorous Techniques

The term “fluorous molecules” is used to describe molecules attached to at least one major fluorinated moiety, as illustrated in Figure 2-8. These compounds are designed to mimic the same organic molecule without the fluorine moiety, in terms of reactivity.²⁵⁶ Fluorous molecules include reagents, reactants and catalysts. Figure 2-9 shows a few examples of fluorous compounds available as reactants.

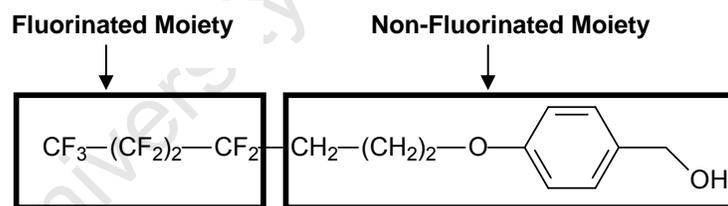


Figure 2-8 “Fluorous molecules.”

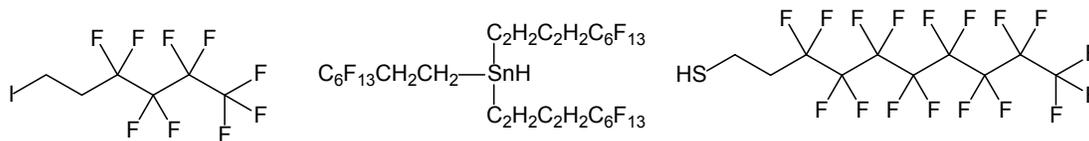


Figure 2-9 Fluorinated reactants used in synthesis.

An example of the advantages of using fluorous compounds as illustrated by Curran is shown in Table 2-13.²⁵⁶

Organic Compound (C ₄ H ₉) ₃ SnH	Fluorous Compound (C ₆ F ₁₃ CH ₂ CH ₂) ₃ SnH
mediates diverse radical reactions	mediates diverse radical reactions
- difficult to separate from organic products	- easy to separate from organic products
- difficult to recover and not reused	- recoverable and reusable

Table 2-13 An example of the differences between organic and fluorous compounds.

Separation of fluorous compounds from organic compounds is an interesting procedure. The usual two-phase system encountered during extraction becomes a tri-phase system, in which the “fluorous solvent” is immiscible with organic solvent and water. The various compounds are thus separated by their respective affinities for a particular solvent.

Fluorous techniques involve the use of fluorous molecules as strategic new options for conducting solution-phase organic reactions and purification.²⁵⁶ Fluorous chemistry has proven to improve productivity through efficient purification and is applicable to chemical discovery research as well as green, chemical-process development. The five different techniques described in the literature include; (i) fluorous biphasic catalysis, (ii) fluorous triphasic reactions, (iii) fluorous reagents and reactants, (iv) fluorous substrates and (v) fluorous mixture synthesis.²⁵⁷⁻²⁵⁹ The techniques are fairly new and not frequently used in view of expense, but with time they could become more essential in organic chemistry on account of aspects of their green character.

Fluorous Compounds

Of interest to this thesis were the fluorous compounds referred to as “fluorocarbons,” which are similar to hydrocarbons but have a number of hydrogen atoms replaced by fluorine atoms. Compared to hydrocarbons, fluorocarbons exhibit stronger intramolecular bonds and weaker intermolecular interactions, which directly result in outstanding thermal, chemical and biological inertness.²⁶⁰ This class of compounds also displays low surface tension, low solubility in water, high fluidity and high gas-dissolving capacities mainly due to the low polarizability of the fluorine atom.²⁶⁰ Fluorocarbons are hydrophobic as well as lipophilic and due to the highly polar, rigid fluorinated portion, they exhibit fluorophilic properties.^{260,261} Many fluorocarbons have been investigated and evaluated for biomedical applications such as *in vivo* oxygen delivery, liquid ventilation and drug delivery. Figure 2-10 shows two compounds that are completely fluorinated and which have been thoroughly investigated for use in therapeutic applications.²⁶⁰

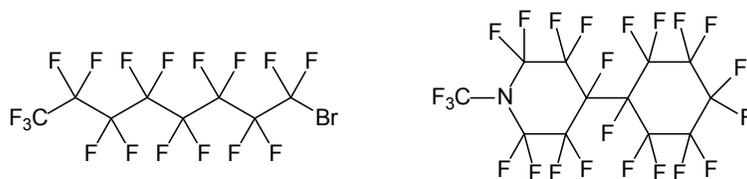


Figure 2-10 Fluorinated compounds evaluated for therapeutic applications.

Fluorinated compounds with both a fluorinated and organic moiety are shown in Figure 2-11. These mixed compounds can fulfill multiple functions including enhancing the solubility of lipophilic drugs in fluorocarbon phases of emulsions and increasing the shelf stability of liposomes when incorporated into liposomal membranes.²⁶⁰

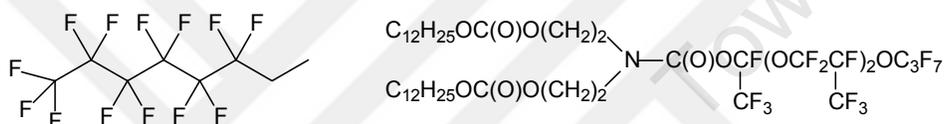


Figure 2-11 Mixed fluorocarbon-hydrocarbon compounds.

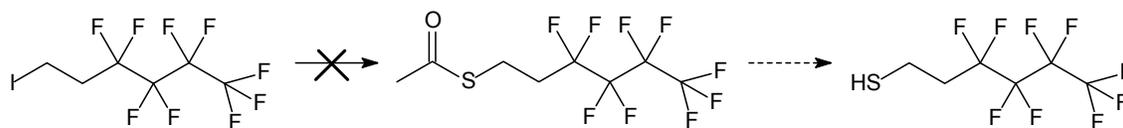
The major advantage of fluorinated systems is their potential to be delivery agents of drugs, prodrugs, genes, vaccines and a range of other materials.²⁶⁰ Our study specifically targeted fluorine's ability to stabilise thiosulfonates as demonstrated by Brace.²⁵⁵

Synthetic Approach

Two fluorine-containing aliphatic thiols were used in the study in order to make a comparison of the stability of the thiosulfonates of the hydrocarbon chains and the fluorocarbon chains. The 12-carbon fluorine-containing aliphatic thiol was commercially available and used without further purification, while the 6-carbon fluorine-containing aliphatic thiol had to be synthesized. Synthesis involved converting 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodohexane to 1,1,1,2,2,3,3,4,4-nonafluoro-1-hexanethiol.

Synthesis of 1,1,1,2,2,3,3,4,4-nonafluoro-1-hexanethiol

After many attempts to synthesise this fluorinated thiol *via* various strategies, a successful sequence was developed as evidenced by the NMR spectra of the disulfides synthesized. One of the unsuccessful synthetic routes explored involved nucleophilic substitution of 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodohexane with thiolacetic acid (Scheme 2-13), which upon treatment with lithium aluminium hydride would afford the thiol reduction product. Unfortunately no product was discernible from the substitution step.

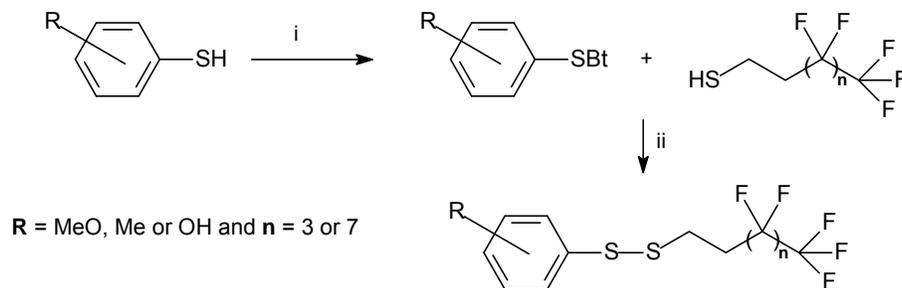


Scheme 2-13 Attempt at forming fluorinated thiol.

The successful sequence involved reacting 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodohexane with thiourea in refluxing THF. After 5 hours, the solution was cooled for 30 min and the solvent concentrated under reduced pressure. A colourless isothiuronium salt was obtained, which was dried on a vacuum pump. The salt was added to a KOH/methanol and CH_2Cl_2 solution and the reaction mixture stirred for 1 hour. Owing to the volatile nature of the thiol, it was not isolated but the complete solution directly added to the reaction as the second thiol. A notable feature was that the added MeOH had no detrimental effect on the reaction. Although solvent studies were not carried out, one can conclude that in the presence of the nucleophilic MeOH, reaction of R^1SBt proceeds as anticipated since no unwanted by-products were obtained.

Synthesis of the Fluorinated Disulfides

Using the same reaction conditions as developed for the model system, with the aromatic thiol as R^1SH and the fluorocarbon thiol as R^2SH , the desired fluorinated unsymmetrical disulfides were obtained, although in a lower overall yield compared to normal. The reaction yield could not be improved by reducing the temperature for the second step to $-78\text{ }^\circ\text{C}$ or increasing it to $0\text{ }^\circ\text{C}$. Increasing the reaction time of the second step to 2 hours also had no effect. The reaction was monitored by TLC, which revealed the formation of the BtSR^1 intermediates after addition of R^1SH and the disappearance of the Bt-intermediates and formation of a new more non-polar compound after addition of R^2SH . The reaction mixture was subjected to an aqueous work-up, and the product extracted into dichloromethane. The compounds were purified by column chromatography on silica-gel using ethyl acetate/hexane mixtures. Fluorous compounds are more efficiently purified using either/both fluorous solvents or fluorous silica. In addition, it was noticed that the unsymmetrical disulfides did not completely dissolve in organic solvents, suggesting that the organic moiety dissolved in the organic solvent while the fluorinated moiety formed a thin film at the surface of the organic solvent. This is in accordance with a report by Krafft in which he described that fluorous compounds either become adsorbed at the surfaces or at the interfaces in solution.^{255,260} The yields for the unsymmetrical disulfides synthesized (Scheme 2-14) for this category are illustrated in Table 2-14.



Scheme 2-14 Reagents and Conditions: (i) BtCl, BtH, CH₂Cl₂, -78 °C. (ii) F-AlkylSH, -20 °C.

Although many examples of symmetrical fluorinated disulfides exist in the literature, only two unsymmetrical ones have been patented. Compounds **93**, **94**, **95**, **96** and **97** have not been reported in the literature and hence were fully characterized.

Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
93	<i>p</i> -MeOC ₆ H ₄ SH	CF ₃ (CF ₂) ₃ (CH ₂) ₂ SH		54
94	<i>p</i> -MeOC ₆ H ₄ SH	CF ₃ (CF ₂) ₇ (CH ₂) ₂ SH		95
95	<i>p</i> -MeC ₆ H ₄ SH	CF ₃ (CF ₂) ₇ (CH ₂) ₂ SH		66
96	<i>o</i> -HOC ₆ H ₄ SH	CF ₃ (CF ₂) ₃ (CH ₂) ₂ SH		64
97	<i>o</i> -HOC ₆ H ₄ SH	CF ₃ (CF ₂) ₇ (CH ₂) ₂ SH		50

Table 2-14 Yields obtained for the unsymmetrical fluorinated disulfides (NMR details of **93** are reported as a representative for common fragments in the structure of the compounds of this series).

Figure 2-12 illustrates the numbering system used for the fluorinated disulfides.

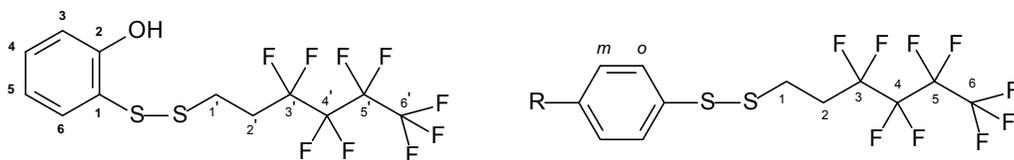


Figure 2-12 Numbering system for fluorinated disulfides (fluorine atoms will be labeled analogous to carbon and proton atoms, i.e. F-3 for fluorine atoms attached to C-3).

Characterization of the fluorinated compounds was achieved by ^1H , ^{13}C and ^{19}F NMR spectroscopy. The aromatic proton and carbon signals were analogous to those for the corresponding structures of previous compounds. Conversely, the aliphatic resonances were distinctly different due to the magnetically active fluorine.

The ^1H NMR spectrum (Figure 2-13) of each product revealed the presence of two CH_2 aliphatic signals shifted slightly downfield compared to the analogous protons of the aliphatic counterpart. The downfield shift was attributed to the deshielding effect of the fluorine atoms. The proton resonating as a multiplet at δ_{H} 2.56 was assigned as H-2 owing to the number of couplings experienced from the adjacent fluorine atoms while the multiplet at δ_{H} 2.89 was assigned as H-1. HSQC confirmed these assignments. Clearly, carbons C-3, C-4, C-5 and C-6 bearing fluorine atoms failed to give any resonances in the ^1H NMR spectrum.

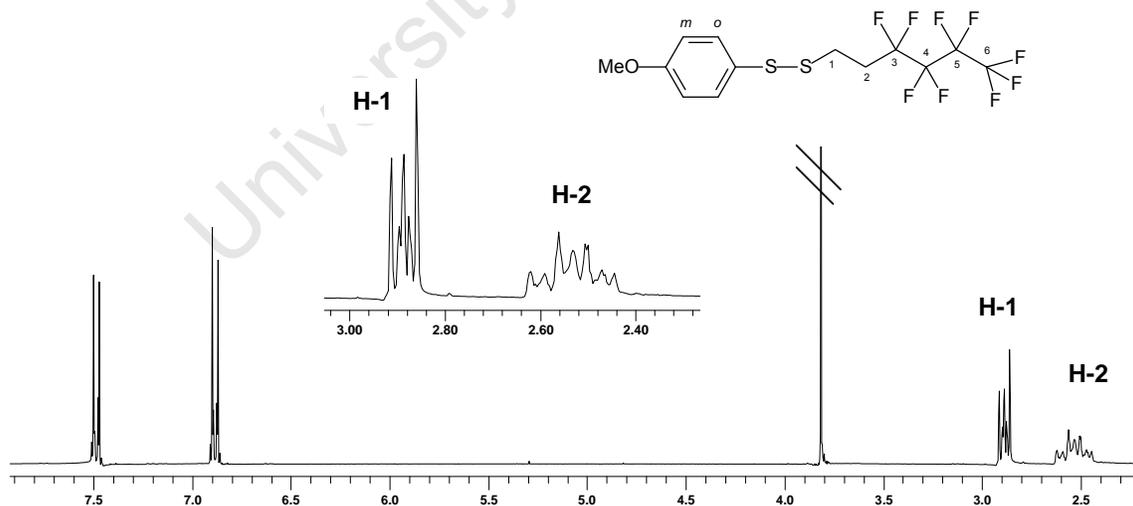


Figure 2-13 ^1H NMR spectrum of compound **93** (\backslash = truncated peak).

The aliphatic region of the ^{13}C NMR spectrum (Figure 2-14) of each product revealed the presence of a triplet (J 22.1 Hz) resonating at δ_{H} 31.4 which was assigned to the methylene carbon at C-2 adjacent to the first fluorinated carbon in the chain. The splitting observed is

due to coupling exerted by the adjacent fluorine atoms ($I_f = 1/2$; $2nI_f + 1$ gives a triplet). No signals for the carbons attached to the fluorine atoms were observed; these were expected to resonate between 80 and 120 ppm as multiplets due to extensive C-F (geminal and vicinal) couplings. It is surmised that these peaks are too relaxed and thus not detectable.

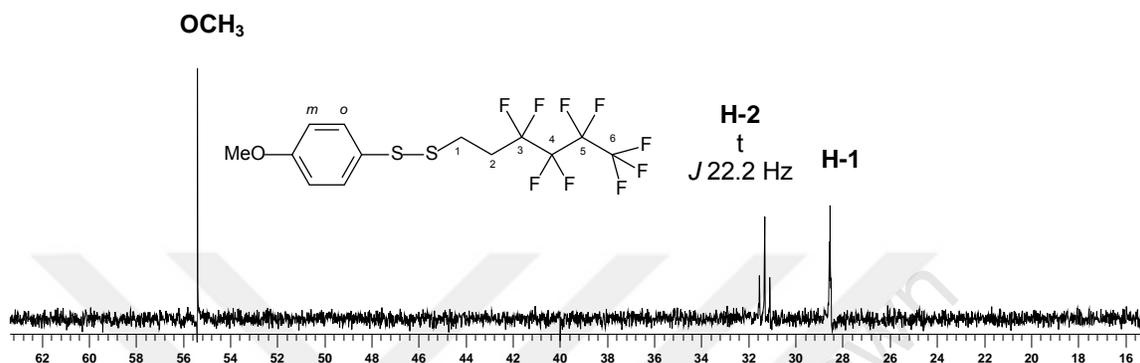


Figure 2-14 ^{13}C NMR spectrum of compound **93**.

The ^{19}F NMR spectrum of **93** revealed two interesting features, Figure 2-15. Firstly, the signals were not well resolved, showing fine splitting either resonating as a multiplet or broad singlet. This indicated that each set of fluorine atoms for each carbon (enantiotopic except C-6) experienced several small couplings.

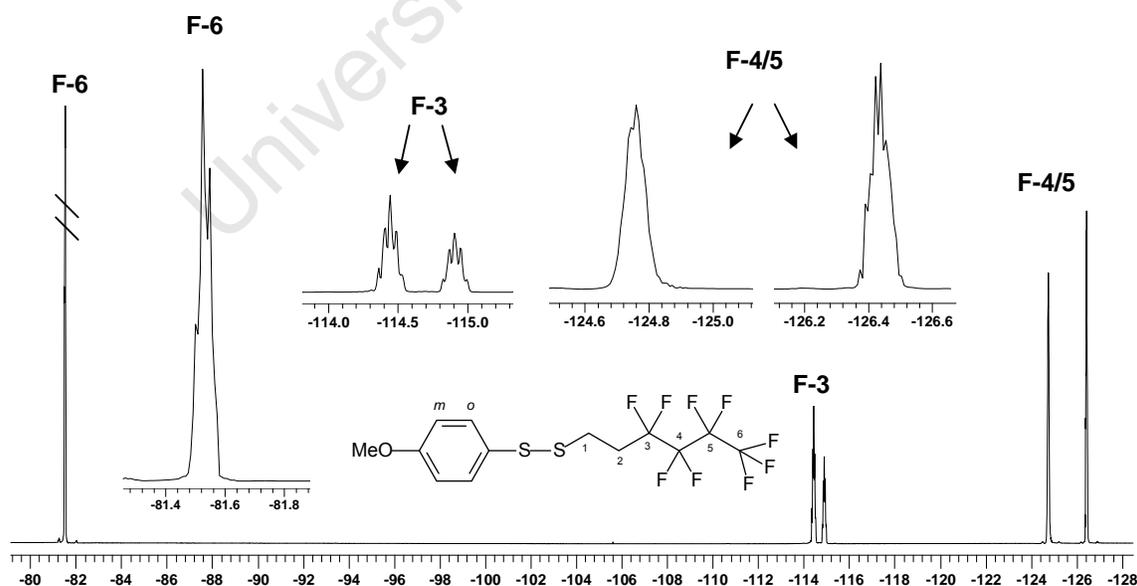


Figure 2-15 ^{19}F NMR spectrum of compound **93** (\backslash = truncated peak).

The peak resonating at δ_F -81.5 as the least deshielded was assigned as F-6 (CF_3 group), which agreed with a literature reported result for a compound possessing a fluorinated-chain.²⁵⁵ Another feature was the two peaks observed for F-3 which resonated at δ_F -114.4 and δ_F -114.9. A possible explanation is that the two fluorine atoms (F-3^a and F-3^b) are resonating in two magnetically different environments due to conformation **A** as illustrated by the Newman projections along the C-2 – C-3 axis in Figure 2-16. Interestingly, two separate peaks for F-3 were not observed for compounds **94**, **95**, **96** or **97**. Instead, F-3 resonated as a multiplet with fine couplings. All NMR assignments were in agreement with literature-reported characterization.²⁵⁵

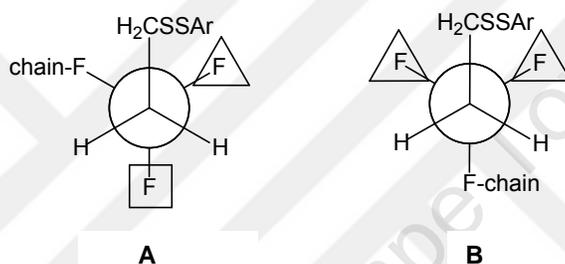
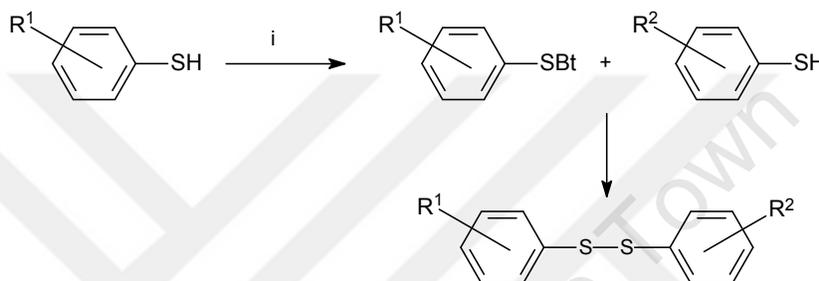


Figure 2-16 Newman projection (staggered conformers) along the C-2–C-3 axis for **93** (**A** – preferred conformation: F-atoms are in magnetically different environments, **B**: F-atoms are chemical-shift equivalent).

In addition, the IR spectra for the fluorous unsymmetrical disulfides displayed characteristic bands; a sharp band at 1265 cm^{-1} for the C-F stretch and a band at 499 cm^{-1} for the S-S stretch. Compounds **96** and **97** also displayed a broad band at 3450 cm^{-1} for the hydroxyl group.

Aromatic-Aromatic Disulfides

The reactions were carried out as described for the model system with the ratio of the various reactants maintained as before to optimize the formation of BtSR^1 and minimize the formation of R^1SSR^1 . Inevitably, though, the excess BtCl and R^2SH reacted to form the corresponding symmetrical disulfide (yields are not reported). However, in all cases, the disulfide mixture could be separated by column chromatography. The reaction scheme for synthesis of the aromatic-aromatic unsymmetrical disulfides is illustrated below, Scheme 2-15.



Scheme 2-15 Reagents and Conditions: (i) BtCl , BtH , CH_2Cl_2 , -78°C .

For this category, once again, the issue of order of thiol addition arose and hence the reactions were carried out both ways round. The order did not appear to make a difference except for compound **103**, Table 2-15. In this case, adding methyl thiosalicylate first produced large amounts of $p\text{-TolSSTol-}p$ (70% based on $p\text{-TolSH}$) indicating that the rate of interception of $o\text{-MeO}_2\text{CPhSBt}$ by $p\text{-TolSH}$ was much lower than interception of $p\text{-TolSBt}$ by $p\text{-TolSH}$; hence, the second thiol was mainly channeled through to its symmetrical disulfide. This presumably indicates a steric effect from the thiosalicyl intermediate coming into play with the less nucleophilic $p\text{-TolSH}$ since the yield went up with the more nucleophilic 2-pyridylthiol. The low yield for compound **103** could be easily rectified by simply reversing the order of addition and introducing the sterically bulkier and more acidic thiol second.

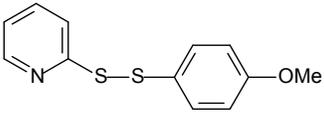
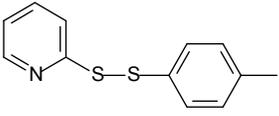
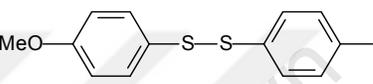
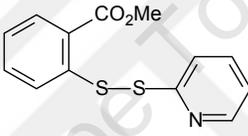
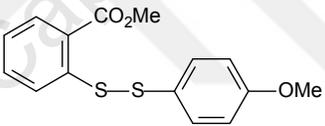
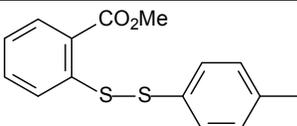
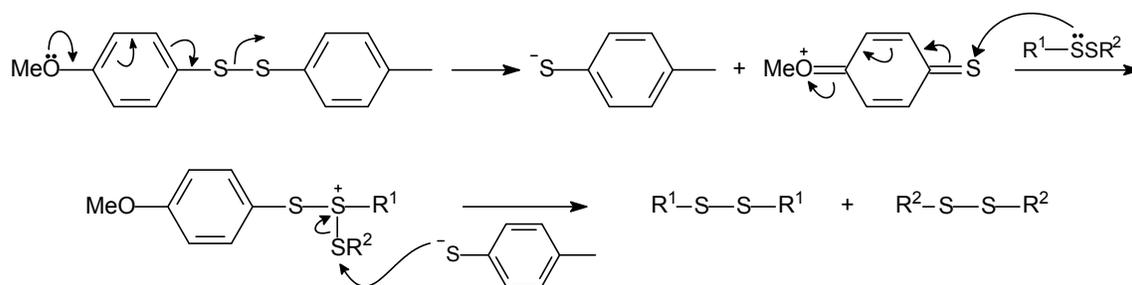
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Yield (%)
98	<i>p</i> -MeOC ₆ H ₄ SH	2-PyridylSH		80
	2-PyridylSH	<i>p</i> -MeOC ₆ H ₄ SH		78
99	<i>p</i> -MeC ₆ H ₄ SH	2-PyridylSH		85
	2-PyridylSH	<i>p</i> -MeC ₆ H ₄ SH		85
100	<i>p</i> -MeOC ₆ H ₄ SH	<i>p</i> -MeC ₆ H ₄ SH		59
	<i>p</i> -MeC ₆ H ₄ SH	<i>p</i> -MeOC ₆ H ₄ SH		58
101	<i>o</i> -MeO ₂ C ₆ H ₄ SH	2-PyridylSH		89
	2-PyridylSH	<i>o</i> -MeO ₂ C ₆ H ₄ SH		86
102	<i>p</i> -MeOC ₆ H ₄ SH	<i>o</i> -MeO ₂ C ₆ H ₄ SH		88
	<i>o</i> -MeO ₂ C ₆ H ₄ SH	<i>p</i> -MeOC ₆ H ₄ SH		86
103	<i>p</i> -MeC ₆ H ₄ SH	<i>o</i> -MeO ₂ C ₆ H ₄ SH		90
	<i>o</i> -MeO ₂ C ₆ H ₄ SH	<i>p</i> -MeC ₆ H ₄ SH		41

Table 2-15 Yields obtained for the aromatic-aromatic disulfides synthesized.

The majority of the products were obtained in good yields except for compound **100**. TLC evaluation of the results revealed that the formation of R¹SSR¹ (MeOC₆H₄SSC₆H₄OMe) was not observed in step 1, however, three new product spots were observed upon addition of R²SH. This implies that for this pair of thiols, either disproportionation occurs or upon addition of R²SH the incoming thiol attacks the unsymmetrical disulfide formed instead of the BtSR¹ intermediate. In the case of disproportionation, a possibility is that the electron releasing methoxy group helps to promote a process of ionization as depicted in Scheme 2-16. The yield for the symmetrical disulfide (di-tolyl disulfide) isolated was calculated as R¹SSR¹ 18 %.



Scheme 2-16 Proposed disproportionation of compound **100** ($R^1 = \text{MeO}$, $R^2 = \text{Me}$).

The ^1H and ^{13}C NMR spectra of compounds **98-103** were assigned without difficulty owing to previous assignments and these were confirmed by 2D-NMR (HSQC). The IR spectra of each compound confirmed the presence of the disulfide bond while elemental analysis of compounds **101**, **102** and **103**, which were isolated as solids, confirmed the correct molecular formulae, Table 2-16.

Atom	Compound 101		Compound 102		Compound 103	
	Calculated	Experimental	Calculated	Experimental	Calculated	Experimental
C	56.30	56.29	58.80	58.78	62.04	62.05
H	4.00	4.01	4.61	4.58	4.86	4.87
N	5.05	5.03	-	-	-	-
S	23.12	23.33	20.93	20.36	22.08	22.63

Table 2-16 Elemental analysis results for compounds **101**, **102** and **103**.

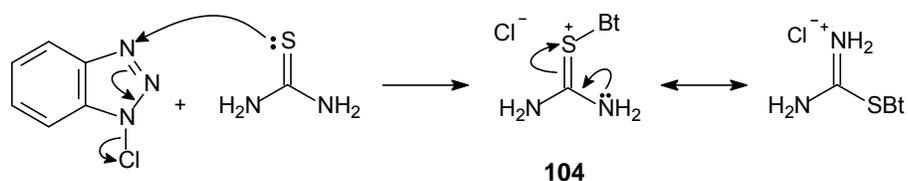
High-resolution mass spectrometry also confirmed the correct molecular ion for each aromatic-aromatic unsymmetrical disulfide and revealed scission of the disulfide bond, which was unlike previous observations in which the R (aliphatic) group of the unsymmetrical disulfide was cleaved before the disulfide bond.

Aliphatic-Aliphatic Disulfides

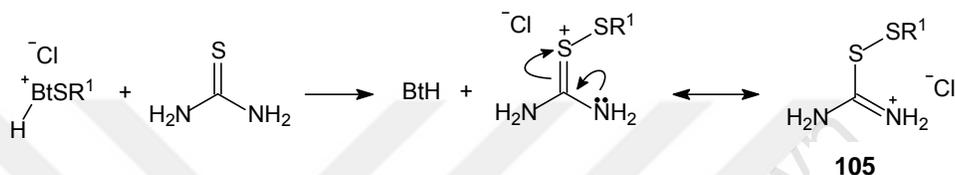
The aliphatic-aliphatic unsymmetrical disulfides were the most difficult of the three to synthesize. In earlier years (pre-1960), aliphatic-aliphatic disulfides were prepared for the petroleum industry usually by oxidation of a thiol to afford the symmetrical disulfide or oxidation of two different thiols which gave a mixture of the three possible disulfides that was invariably very difficult and sometimes impossible to separate into pure forms.²⁶² In 1951,

Douglass and co-workers attempted to prepare a range of aliphatic-aliphatic unsymmetrical disulfides by reaction of a sulfenyl chloride with a thiol but after many attempts were unable to purify the desired compounds.²⁶³ The interest in aliphatic-aliphatic disulfides increased post 1960 due to the importance of the disulfide bond in natural macromolecules. Hiskey showed that the reaction of thiocyanates with thiols afforded unsymmetrical aliphatic-aliphatic disulfides but numerous purification steps were needed and low yields were obtained (20-70%). Our modified BtCl method proved to be superior to the other methods available. However, development of an effective one-pot protocol for reliable synthesis of this category of disulfide proved to be challenging, as unsymmetrical disulfide and homodimers cannot be readily separated on column chromatography in non-polar cases. As mentioned previously, the problem faced with this system was that the reaction of an aliphatic thiol with BtCl resulted in competing interception of the intermediate sulfenyl chloride by aliphatic thiol resulting in formation of the symmetrical disulfide instead of exclusively the *N*-sulfenyl derivative. Eventually, the BtCl method was modified to include aspects of the methodology by Sirakawa based on isothiuronium salts.¹⁸⁹ As discussed in Chapter 1, Sirakawa reacted a thiol with thiourea in the presence of hydrogen peroxide to form an *S*-alkylthioisothiuronium salt, which proved to be a stable intermediate for unsymmetrical disulfide production.¹⁸⁹ Our reaction was thus modified by using an excess of BtCl in the first step to minimize competing homodimer formation, and then to convert both excess BtCl as well as BtSR¹ into isothiuronium salts using thiourea.

Pleasingly, a preliminary attempt at cleanly forming an aliphatic-aliphatic unsymmetrical disulfide by addition of thiourea was successful except longer reaction times than those used for the general Bt methodology had to be used. The reaction involved addition of a thiol (R¹SH) to a 2-fold excess of BtCl, in order to minimize the formation of R¹SSR¹, in the presence of BtH (1 equivalent). The reaction was monitored by TLC and within 10 minutes at -78 °C the reaction was complete with total conversion to the desired BtSR¹ without any homodimer (R¹SSR¹) being formed. Destruction of the excess BtCl by thiourea (3 equivalents), to form a Bt-thiourea compound **104** (Scheme 2-17), in order to avoid the formation of the symmetrical disulfide of the second thiol (R²SSR²) also resulted in reaction of the BtSR¹, presumably to form an isothiuronium salt **105**, Scheme 2-18. Although the reaction with thiourea was surprisingly fast in that a baseline product was observed after 10 min, the reaction was given 40 min at -60 °C as evidenced by TLC.

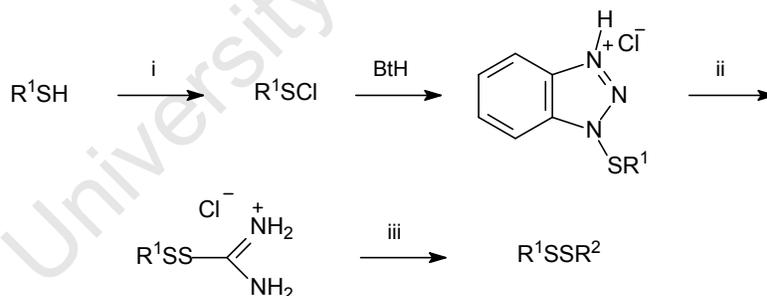


Scheme 2-17 BtCl destruction with thiourea.



Scheme 2-18 Formation of the isothiuronium salt **105**.

The reaction was allowed to stir overnight at room temperature after the addition of the second thiol (R^2SH). TLC analysis revealed the disappearance of the intermediate and the formation of a new, very non-polar product. The solvent was evaporated under reduced pressure and the product purified by column chromatography to afford high yields of the desired product, Scheme 2-19. Shorter reaction times gave inferior results indicating the lower reactivity of the isothiuronium salt compared to $BtSR^1$.



Scheme 2-19 Reagents and conditions: (i) BtCl, BtH, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$, 10 min.
(ii) Thiourea, THF, $-60\text{ }^\circ\text{C}$, 40 min. (iii) R^2SH , $-78\text{ }^\circ\text{C}$ to 0°C , 18 hours.

Subsequent to purification, the aliphatic-aliphatic unsymmetrical disulfides were obtained in high yields free from the possible symmetrical disulfides that could have formed during the reaction. The methodology was thus applied to a range of aliphatic thiols which were commercially available to establish the prestige of the approach. The yields for the aliphatic-aliphatic unsymmetrical disulfides synthesized are shown in Table 2-17.

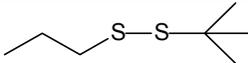
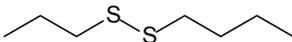
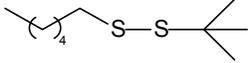
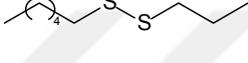
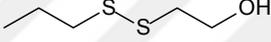
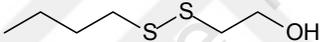
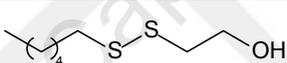
Compound	R ¹ SH	R ² SH	R ¹ SSR ²	Purity*	Yield (%)
106	<i>n</i> -PrSH	<i>t</i> -Bu		100	72
107	<i>n</i> -PrSH	<i>n</i> -BuSH		100	94
108	<i>n</i> -HexSH	<i>t</i> -Bu		100	94
109	<i>n</i> -HexSH	<i>n</i> -PrSH		100	82
110	<i>n</i> -PrSH	HO(CH ₂) ₂ SH		100	98
111	<i>n</i> -BuSH	HO(CH ₂) ₂ SH		100	78
112	<i>n</i> -HexSH	HO(CH ₂) ₂ SH		100	76

Table 2-17 Yields obtained for the aliphatic-aliphatic unsymmetrical disulfides (* as judged by NMR).

Specific assignments of resonances in the ¹H and ¹³C NMR spectra of **108**, **109** and **112** were initially challenging owing to the peaks overlapping and resonating exclusively in the aliphatic region. However, as a result of assigning the aliphatic protons and carbons of analogous alkyl chains for compounds previously reported, using HSQC and COSY experiments it was possible to assign all resonances. Purity was evaluated by ¹H NMR spectroscopy, which revealed the hetero disulfides to be >98% pure. Two spectra, one for **106** produced from using the normal BtCl methodology (“impure spectrum”) and the other for **106** prepared using the thiourea modification (“clean spectrum”) are shown in Figures 2-17 and 2-18 respectively. ¹³C NMR also revealed a clean set of resonances for the product from the modified procedure. The absence of homodimer is clearly realised by the reaction conditions. The lack of R¹SSR¹ formation unquestionably demonstrates the impressiveness of the BtCl methodology.

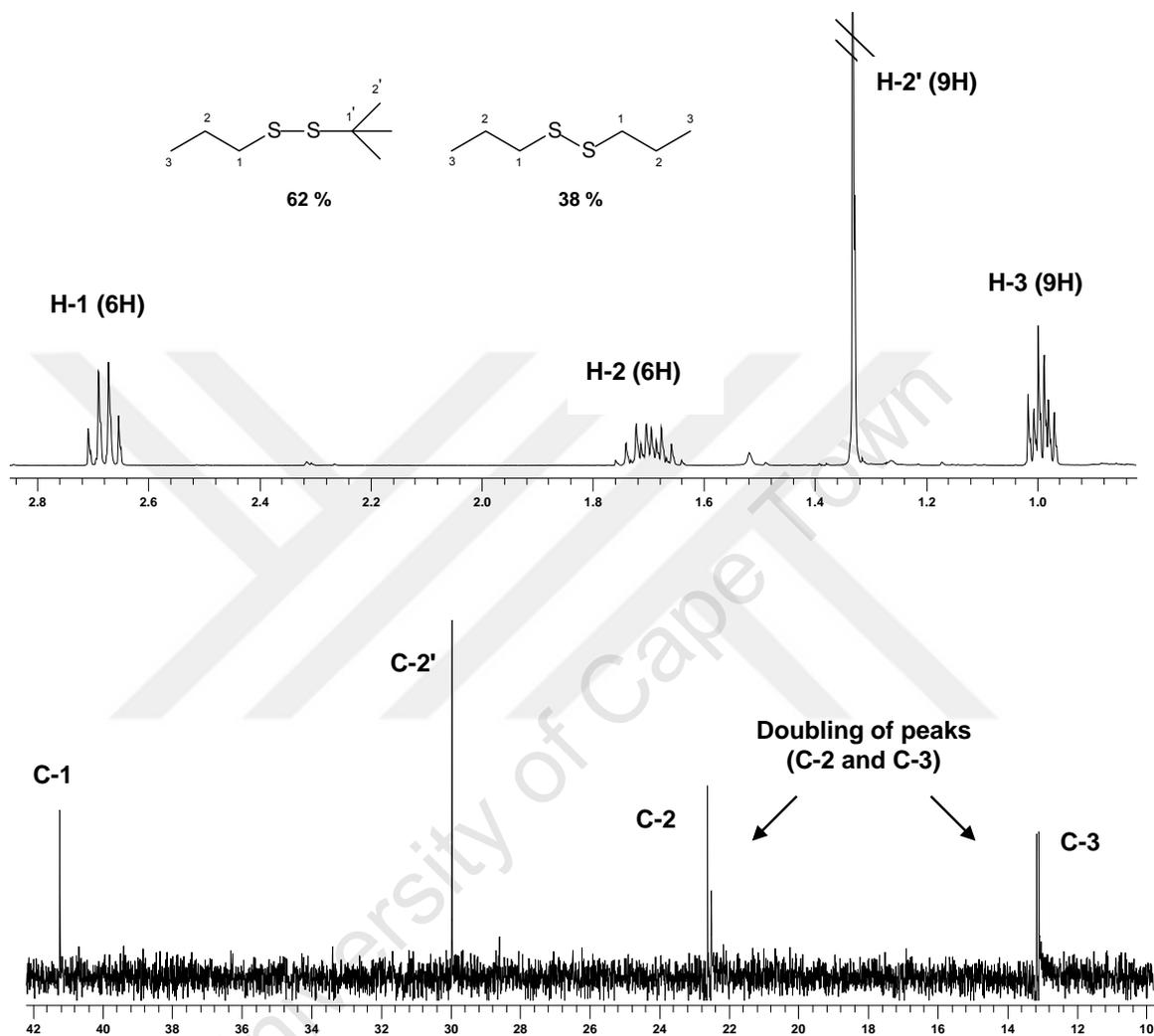


Figure 2-17 ¹H (top) and ¹³C (bottom) NMR spectra for a 62:38% mixture of *t*-butyl 1-propyl disulfide and dipropyl disulfide obtained from the normal BtCl methodology (\\ = truncated peak).

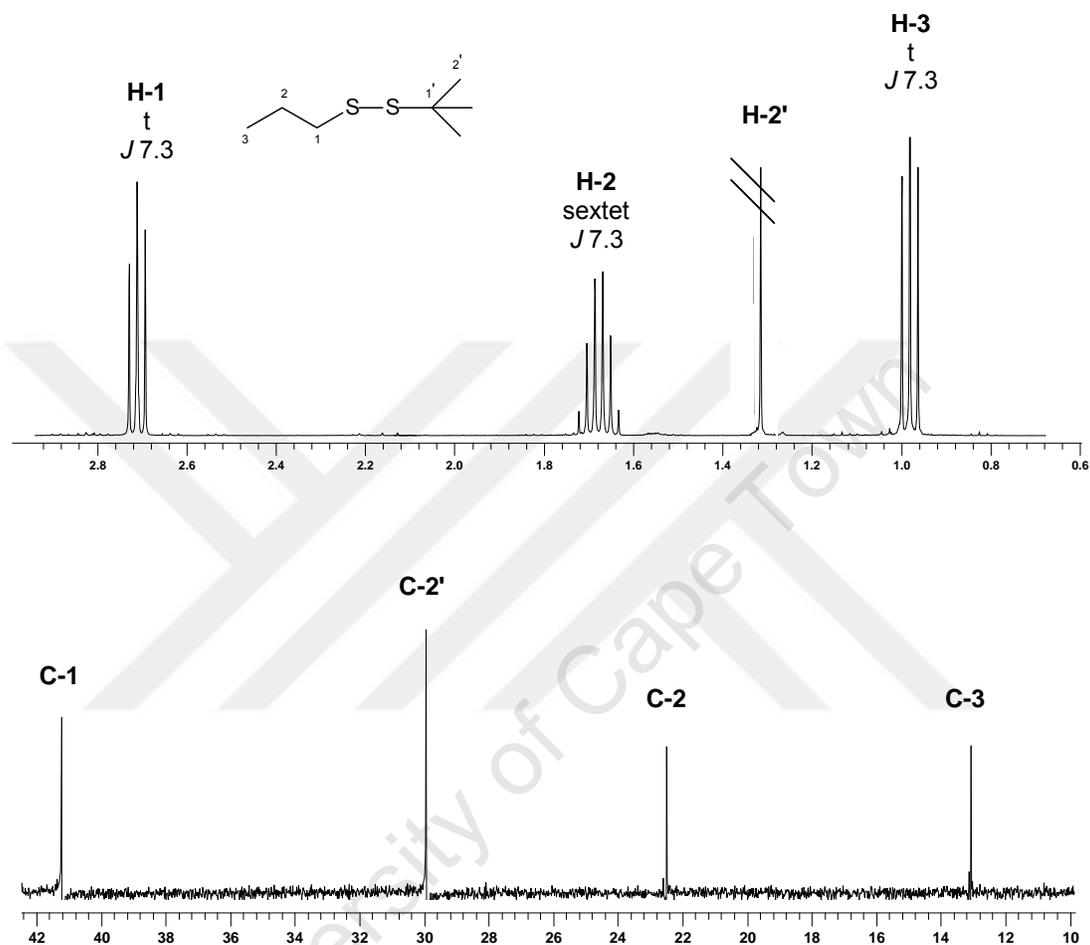


Figure 2-18 ^1H (top) and ^{13}C (bottom) NMR spectra for structure **106** obtained using the modified (thiourea) protocol ($\backslash\backslash$ = truncated peak).

The IR spectra of the products indicated the presence of a band corresponding to a S-S stretch at approximately 476 cm^{-1} for compounds **106**, **107**, **108** and **109** and at approximately 481 cm^{-1} for compounds **110**, **111** and **112**. Furthermore, the high resolution mass spectrometry confirmed the molecular ion for each compound in the series.

Producing highly pure aliphatic-aliphatic unsymmetrical disulfides is a challenging process and the ease of obtaining the desired compounds using the BtCl methodology with a range of thiols, without any harsh oxidising agents and reagents has unquestionably demonstrated the beauty of the methodology.

(a) Unsymmetrical Cysteine Disulfides

The disulfide bond plays an important role in the structure and function of a range of biologically important peptides and proteins, which includes toxins, enzymes, hormones, growth factors and immunoglobulins.^{264,265} This has inspired scientists to construct compounds of this type for various purposes with the goal of improving biological activity. A recent example as discussed in the review includes using a cysteine disulfide linkage to tag a chemotherapeutic agent, such as the reductively activated disulfide prodrugs of Paclitaxel, a folic acid-*vinca* alkaloid conjugate, EC145, and a disulfide cleavable Camptothecin prodrug.²⁶⁶⁻²⁶⁸ Yang recently demonstrated by means of fluorescence resonance energy-transfer techniques that the reduction-mediated release of a drug from a disulfide-linked folic acid conjugate occurs within the endosomes of cancer cells.²⁶⁹ This is important for drug-delivery since the reduction of the disulfide linker releases the drug at its target in an unmodified form.²⁶⁸ Moreover, it has been reported that prodrugs often have activities superior to their parents.

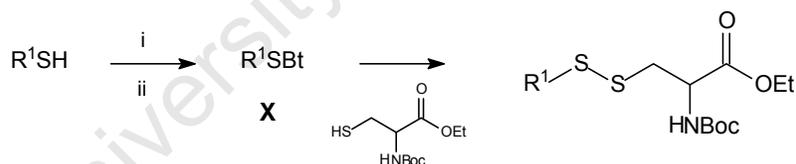
The advantages of being able to link peptide chains via intermolecular bonds include being able to develop discontinuous epitopes, the generation of active-site models and the conjugation of peptides.²⁶⁵ Consequently, several methodologies have been developed for synthesizing unsymmetrical disulfides of cysteine residues in proteins *via* intermolecular coupling of a thiol to a sulfenyl derivative. In this regard, the key sulfenyl derivatives that have emerged over the years for this purpose are the thiocarbonate functionalities,^{270,271} pyridyl variants,^{272,273} thiocyanates,²⁷⁴ sulfoxides,²⁷⁵ thiosulfonates,²⁷⁶ thiophosphates,²⁷⁷ sulfenamides,²⁷⁸ benzothiazole and tetrazole disulfides.^{279,280} An anomalous method based on disulfide exchange has also emerged recently as applicable to unsymmetrical cysteine disulfide synthesis.²⁸¹ A drawback of most of these methods is that the sulfenyl derivative is prepared from a sulfenyl halide. Moreover, such disulfide syntheses require two separate steps from the thiols. In light of this, a model study for unsymmetrical cysteine disulfide synthesis was conducted *via* the BtCl methodology, of potential application to synthesizing disulfide linkages in peptides *via* intermolecular one-pot coupling of two peptide thiols.

Synthesis

As identified in the original method, the order of addition of the thiols was considered to be an important parameter, thus both ways were studied and following several experiments, optimal procedures and yields for the two methods (Method **A** and Method **B**) were identified. *N*-Boc-L-cysteine ethyl ester **113** (prepared from L-cysteine ethyl ester hydrochloride and di-*tert*-butyl dicarbonate) was chosen as the amino acid source as it aided isolation of the products and furthermore was considered to be representative of cysteine in a peptide environment with both N- and C-termini functionalized. The other thiol was represented by a selection from the various classes already discussed.

Method A:

The first method (Scheme 2-20) involved adding the amino acid thiol second as R²SH. Initial experiments gave low and variable yields of the unsymmetrical disulfide product until it was realised that the benzotriazole hydrochloride by-product in step 1 of the sequence was promoting *N*-Boc removal on addition of the amino acid. Thus, subsequent experiments were run in the presence of triethylamine (1.2 equivalents to the first thiol), which solved the problem. The second step of the reaction was stirred for 18 hours, slowly warming from -78 °C to room temperature for complete conversion to the desired disulfide. The methodology as optimally developed was applied. Thus, for R¹ = aliphatic, the thiourea modification was utilized, whereas for R¹ = aromatic, the standard BtCl procedure was employed.

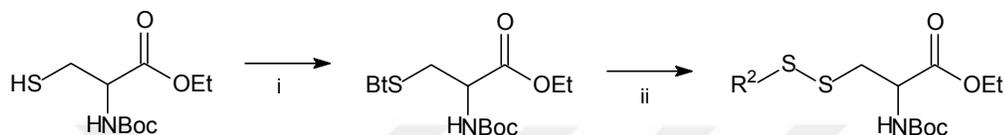


Scheme 2-20 *Reagents and conditions:* When R¹ is an aromatic thiol: (i) BtCl, BtH, CH₂Cl₂, -78 °C, 1 hr. (ii) Et₃N, -78 °C. When R¹ is an aliphatic thiol, X is an isothiuronium salt: (i) BtCl, BtH, CH₂Cl₂, -78 °C, 10 min, thiourea, THF, -78 °C, 1 hour. (ii) Et₃N, -78 °C.

The target cysteine-containing unsymmetrical disulfides were also successfully synthesized when R¹ = aliphatic without thiourea addition and gave similar yields compared to when thiourea was added.

Method B:

Conversely, the second method (Scheme 2-21) involved adding the amino acid thiol first as R^1SH , which gave high yields without necessitating the addition of triethylamine, suggesting that *N*-Boc deprotection is faster for the free cysteine thiol than its derivatives, cysteine-Bt or disulfide. Notably, reaction on the amino acid-Bt intermediate with R^2SH required longer times (3 hours) than the normal method (30 min), reflecting the relative reactivities of the two $BtSR^1$ intermediates.



Scheme 2-21 Reagents and conditions: (i) BtCl, BtH, CH₂Cl₂, -78°C, 2 hours. (ii) R²SH, -78 °C – 0 °C.

The two methods were similar experimentally, except in general, greater reaction times and temperature spans were afforded to the step involving the amino acid compared to the other thiol in view of the former's lower reaction rate. Thiols were selected to cover the various types of R groups from aliphatic to aromatic and heteroaromatic. The yields for the two methods are presented in Table 2-18 with yields as chromatographically pure material.

The yields for the two methods were similar revealing that the order of addition can be either way round, thus extending the scope of the methodology for preparing more complex targets and thus holds promise for tagging peptides onto drugs *via* a cysteine disulfide connection, as well as for the one-pot coupling of two different cysteine-containing peptides. Yields were good to very good and compared extremely well for a one-pot procedure against the alternative two-step methodologies.

Compound	R ¹ SSR ²	Method A Yield (%)	Method B Yield (%)
114		88	75
115		85	70
116		72	82
117		76	78
118		71	68
119		70	81
120		77	86
121		87	84

Table 2-18 Results obtained for unsymmetrical cysteine disulfides (NMR and IR assignments of **115** reported as a representative for the common fragment in the structure of the compounds of this series).

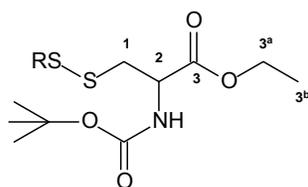


Figure 2-19 Structure and labeling of amino acid moiety.

The IR spectra for each compound indicated that there was no longer a band associated with the thiol group at 2571 cm⁻¹ as observed for the amino acid thiol. Instead, a band around 470 cm⁻¹ was observed corresponding to a S-S stretch. In addition, two carbonyl stretches

were observed at 1740 cm^{-1} and 1715 cm^{-1} for the carbamate and ester, respectively. For compound **121**, the stretch at 1710 cm^{-1} represented both ester groups. The NMR assignments for the R-groups attached to the amino acid moiety corresponded to previous assignments and hence only the amino acid moiety will be discussed.

The ^1H and ^{13}C NMR spectra could be unambiguously assigned and corresponded to L-cysteine moieties of published compounds. In the ^1H NMR spectrum, the methyl protons H-3^b resonated at δ_{H} 1.29 as a triplet (J 7.0 Hz) and the Boc-CH₃'s at δ_{H} 1.44 as a singlet. H-1 (δ_{H} 3.13) appeared as a multiplet coupling to (as observed from HSQC) H-2 (δ_{H} 4.55), which also appeared as a multiplet. Similarly, H-3^a resonated at δ_{H} 4.21 as a quartet (J 7.0 Hz). The ^{13}C NMR spectrum revealed diagnostic peaks at δ_{C} 155.0 (C=O of Boc group) and δ_{C} 170.8 (C-3) for the carbonyl carbons. Mass spectrometry confirmed the molecular mass of each product.

The high yields obtained for these often difficult-to-synthesize compounds is clear evidence that the methodology presented is versatile and applicable to a wide range of thiols.

Chapter 3: S-Aryl Alkylthiosulfinates

3.1 Overview

Thiosulfinates have gained much interest over the years and even though they tend to disproportionate due to their very unstable nature many chemists have spent a great deal of time and energy seeking new knowledge about these fascinating compounds. Their attraction and appeal is principally owed to their potent antimicrobial properties.¹³ As mentioned previously, the unsymmetrical disulfide methodology described in this thesis was developed for the preparation of allicin mimics as potentially novel antimicrobial agents. After the successful synthesis of a range of unsymmetrical disulfides using the BtCl reaction, the preparation of the corresponding thiosulfinates was embarked upon, Figure 3-1.

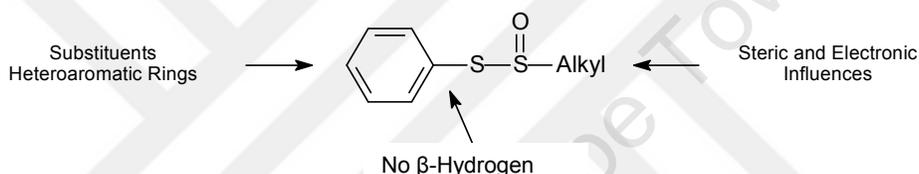
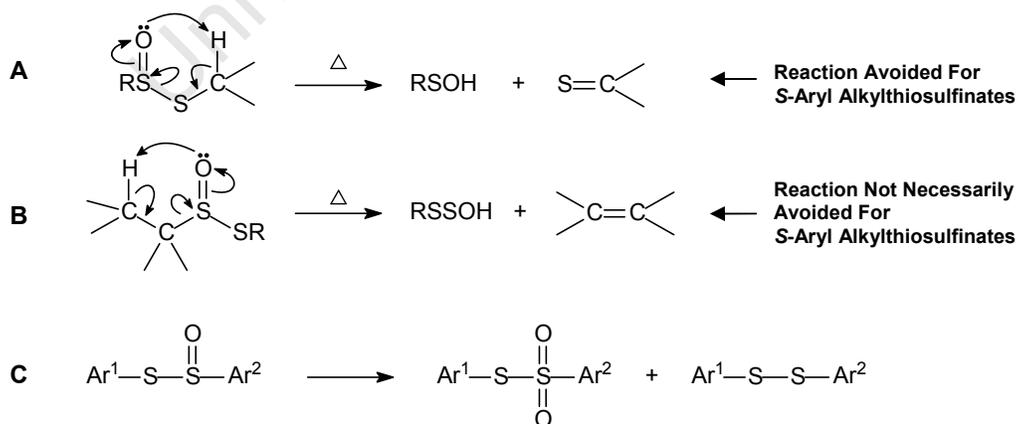


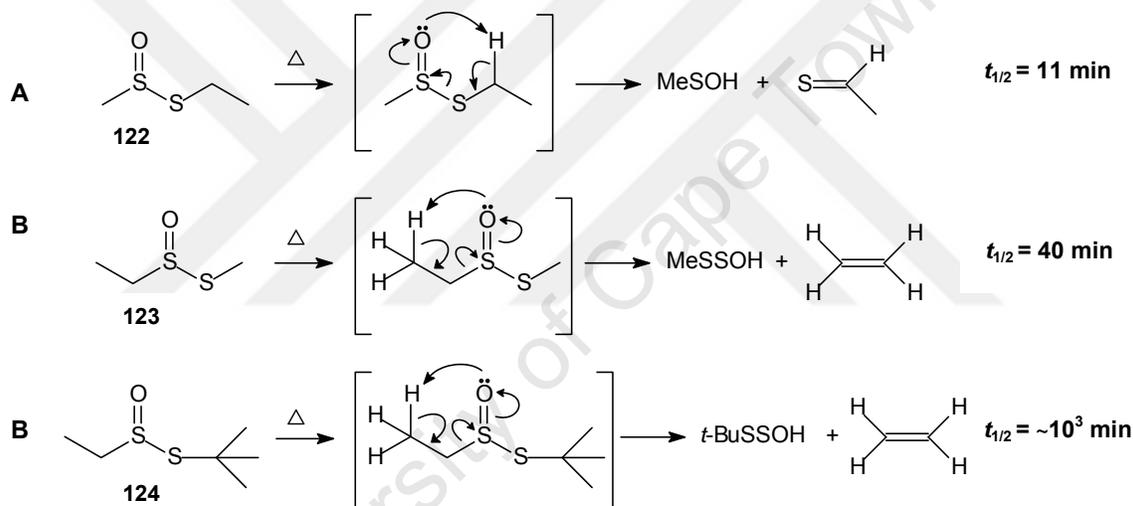
Figure 3-1 SAR for S-aryl alkylthiosulfinates.

It was decided to target S-aryl alkylthiosulfinates (Figure 3-1) in the hope of avoiding a number of rearrangement pathways. These included the Block-type rearrangements of allicin (Scheme 3-1, **A** and **B**) and disproportionation (Scheme 3-1, **C**), which is known for diaryl thiosulfinates.^{83,85,282}



Scheme 3-1 (A and B) β -Elimination processes - Block-type rearrangements, (C) Disproportionation of diaryl thiosulfinates.⁸⁵

Thus, it was considered that S-aryl alkylthiosulfinates as a medicinal chemistry target would avoid the first class (reaction **A** in Scheme 3-1) of the Block-type rearrangement pathways owing to the replacement of an alkyl chain by an aromatic ring, with no α -sulfinyl proton. The second type (reaction **B** in Scheme 3-1), however, with an available β -sulfinyl proton is not avoided but this rearrangement pathway does not occur as rapidly compared to the first type. Block reported that reaction **A** would be the favoured one in view of the enhanced acidity of the α -sulfinyl protons and the weakness of the thiosulfinate S-S bond.⁸⁵ This indicates that reaction **A** would occur under milder conditions than those required for reaction **B**. An example to demonstrate the relative rates (rate of fragmentation, measured as the thiosulfinate's half-life) of the two pathways is shown in Scheme 3-2 involving ethyl methanethiosulfinate.⁸³



Scheme 3-2 Block-type rearrangements and half-lives for thiosulfinates **122**, **123** and **124**

(half-lives – $t_{1/2}$: time for 50% decomposition at 96 °C).

Block reported that thiosulfinates lacking both α -sulfinyl and β -sulfinyl protons are the most stable. 1-Adamantyl 1-adamantanethiosulfinate (Figure 3-2) has a half-life of $>10^5$ min at 96 °C although it has a β -sulfinyl proton.⁸³ This could be suggesting that the proton is unaligned stereoelectronically for proton abstraction by the lone pair of the oxygen atom.

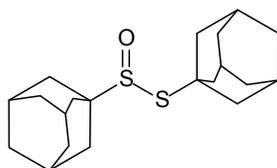


Figure 3-2 Structure of 1-adamantyl 1-adamantanethiosulfinate.

However, unsymmetrical thiosulfinates lacking both α -sulfenyl and β -sulfinyl protons are very rare if one does not consider diaryl thiosulfinates. Hence, the S-aryl alkylthiosulfinates were chosen for study.

The chemoselective oxidation of the aralkyl unsymmetrical disulfides to give the corresponding thiosulfinates was a significant and vital subject matter. It was rationalised that the sulfur atom adjacent to the alkyl chain as illustrated in Figure 3-1 would be oxidized preferentially. Mechanistically, as discussed in Chapter 1, this would be on the basis that it is the more electron-rich sulfur atom. However, there was the possibility that a regioisomeric mixture of oxidized products might be obtained from oxidation of these unsymmetrical disulfides.

Other factors identified from the literature having a stabilizing effect on thiosulfinates, which will be discussed in detail subsequently, included: (i) an increase in the length of the alkyl chain,^{83,283} (ii) fluorine substituents on the alkyl chain²⁵⁵ and (iii) hydrogen bonding.²⁸⁴ These will now be reviewed.

3.1.1 Alkyl-Chain Length of Thiosulfinates

Block demonstrated that the structure of a thiosulfinate has an effect on its thermal stability, which can be evaluated by its disproportionation half-life ($t_{1/2}$). Hence, the study by Block on the effect of the presence and absence of α -sulfenyl and β -sulfinyl protons, also demonstrated that an increase in the length of the alkyl chain increased the stability of the thiosulfinate as illustrated in Figure 3-3.⁸³

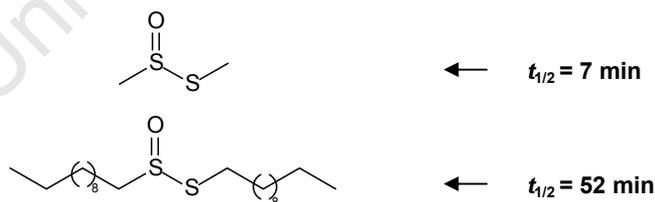


Figure 3-3 The effect of alkyl-chain length on the stability of thiosulfinates ($t_{1/2}$: time for 50% decomposition at 96 °C).

A mechanistic explanation for this phenomenon has not been reported. However, similar results were obtained from independent studies by Parkin and Allen.^{18,283} Parkin demonstrated the stability of neat thiosulfinates (as illustrated by their corresponding half-lives) of different chain lengths at various temperatures and pH values, Table 3-1. The stabilities were in the order of PropylS(O)SPropyl > EthylS(O)SEthyl > MethylS(O)SMethyl.¹⁸ Similarly, Allen

reported that thiosulfinates $RS(O)SR$ with R-groups as alkyl chains with eleven carbons or longer are much more stable than thiosulfinates with shorter chains.²⁸³

Thiosulfinate	20 °C		40 °C		60 °C	
	pH 7.5	pH 9.0	pH 7.1	pH 8.8	pH 6.8	pH 8.4
MeS(O)SMe	4.9 d	13.0 h	1.3 h	1.8 h	5.4 h	0.7 h
EtS(O)SEt	19.0 d	22.3 h	4.6 h	5.6 h	15.2 h	2.6 h
PrS(O)SPr	25.4 d	69.5 h	6.2 h	9.9 h	19.9 h	3.8 h

Table 3-1 Half-lives of thiosulfinates at various temperatures and pH values.¹⁸

3.1.2 Fluorinated Thiosulfinates

The prospect of achieving enhanced stability was further boosted by a recent paper by Brace on fluorine-containing thiosulfinates (perfluoroalkyl-segmented thiosulfinates), an example of which is shown in Figure 3-4.²⁵⁵

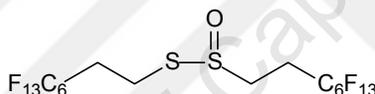
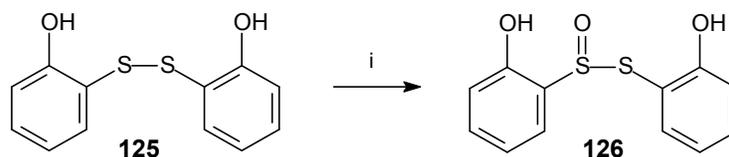


Figure 3-4 Perfluoroalkyl-segmented thiosulfinates.

Brace demonstrated that fluorinated thiosulfinates from symmetrical disulfides are significantly more stable compared to their hydrogenated analogues.²⁵⁵ Such fluorinated thiosulfinates are stable to recrystallization at 50 °C and can be stored for several months at 10 °C. Conversely, their hydrogenated analogues are thermally unstable, requiring rapid purification and cannot be stored for long periods. Krafft and Riess related the stability to the characteristics of fluorinated compounds.^{260,285} Carbon-fluorine bonds are much more stable (by approximately 75 kJ mol⁻¹) than carbon-hydrogen bonds, which *via* strong inductive effects strengthen the skeleton's C-C bonds.²⁶⁰ Furthermore, fluorine's larger size, dense electron-cloud and its electronegativity compared to hydrogen results in a compact sheath of electrons that protects the fluorinated moiety and its surroundings against various molecular species as well as reagents. Incorporating a fluorinated moiety into an unsymmetrical thiosulfinate could enhance its stability a great deal and thus this design aspect was incorporated into the study.

3.1.3 Hydrogen Bonding

In 1985, Chang and associates showed that oxidation of di-*o*-hydroxyphenyl disulfide **125** (prepared from I₂ oxidation of 2-hydroxybenzenethiol in the presence of sodium hydroxide in aq. ethanol) with *m*-CPBA gave the corresponding thiosulfinate **126** (Scheme 3-3), which proved to be stable (by melting point) after two years in a dessicator at room temperature.²⁸⁴



Scheme 3-3 Reagents and conditions: (i) *m*-CPBA, CH₂Cl₂, -45 °C.

The unusual stability of diarylthiosulfinate **126** was attributed to intramolecular hydrogen bonding between the hydroxyl group's hydrogen atom and the sulfinate's oxygen atom, Figure 3-5. This hypothesis was confirmed by ¹H NMR which revealed two independent peaks for the two hydroxyl groups, one more deshielded than the other.

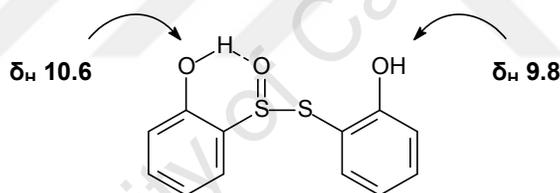


Figure 3-5 Intramolecular H-bonding observed in thiosulfinate **126**.

3.2 The Chemistry of Oxidation of Unsymmetrical Disulfides

There is a plethora of reagents available for the oxidation of disulfides including peroxyacids, hydroperoxides and hydrogen peroxide. However, the most popular reagent for the transformation is the electrophilic peroxyacid, *m*-chloroperoxybenzoic acid (*m*-CPBA). Peroxy acids are much more reactive as oxidants than H₂O₂ or hydroperoxides and thus often the oxidant of choice. The oxidation of disulfides can afford a number of products including the corresponding thiosulfinate, which is the initial product of oxidation, as well as thiosulfonate, α,α'-disulfoxide, sulfinyl sulfone and α,α'-disulfone as shown in Figure 3-6.

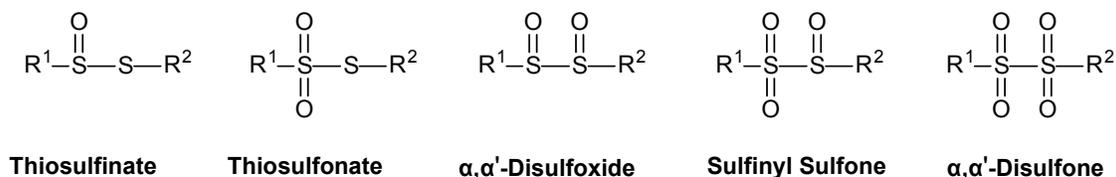
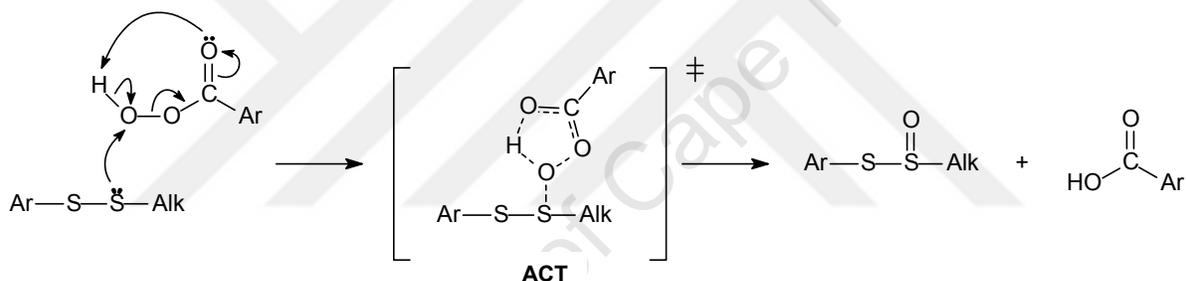


Figure 3-6 Oxidation products of disulfide oxidation.

As has already been mentioned in the introduction of this thesis, oxidation of an unsymmetrical disulfide can either give a chemoselective product or two possible regioisomeric products with the ratio dependent on the electron-density of the sulfur atoms. Mechanistically, the more electron-rich sulfur atom would be oxidized preferentially, with nucleophilic attack of the sulfur atom on the more electrophilic oxygen atom of *m*-CPBA to form the activated complex **ACT**, Scheme 3-4.

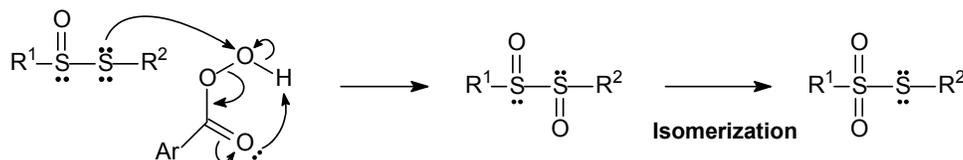


Scheme 3-4 Chemoselective oxidation of unsymmetrical disulfides.

FMO studies predict that release of the electrophilic oxygen of the peroxy acid involves the interaction of the sulfur nucleophile HOMO with the lowest vacant orbital (LUMO) of the O-O bond.²⁸⁶ The transition-state **ACT** is exceedingly rigid resulting in substantial negative activation entropies.²⁸⁷ Subsequent expulsion of *m*-chlorobenzoic acid results in formation of the corresponding thiosulfinate.

Oxidation of a thiosulfinate, however, which is much slower compared to initial oxidation, initiated much debate in the 1970's and 1980's. This resulted in the production of some interesting research, which made a considerable impact in the field. As discussed in the review, the oxidation product isolated was electronically not the expected product. According to HSAB theory, electrophilic oxidation should favour the α,α' -disulfoxide product, since the electron-rich sulfenyl sulfur would be expected to be softer (higher HOMO) than the sulfinyl sulfur, Scheme 3-5. However, according to IR and NMR data, thiosulfonates were isolated, which baffled chemists until Freeman reported NMR data to confirm the formation of the α,α' -disulfoxide as an intermediate.^{167,288} Subsequently, numerous reports have been added to

the literature to corroborate these findings. It appears that α,α' -disulfoxides are not stable compounds that rapidly isomerize to the more stable thiosulfonate.



Scheme 3-5 Isomeration of α,α' -disulfoxide to thiosulfonate.

Further oxidation of thiosulfonates results in formation of sulfinyl sulfones and α,α' -disulfones, which will not be studied or discussed here.

In this thesis, the oxidation conditions were selected to minimize the formation of thiosulfonates and the other oxidation products. Hence, the emphasis will be on the transformation of unsymmetrical disulfides to their corresponding thiosulfinates.

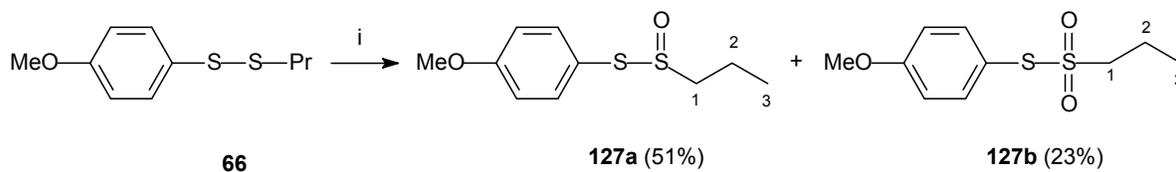
3.2.1 Model Oxidation Study

From the many published methods for the oxidation of disulfides, it was decided to test *m*-CPBA and H_2O_2 as oxidants in view of their relatively low cost, availability, the mild reaction conditions employed for the transformation and the easily removable by-products formed (*m*-chlorobenzoic acid for *m*-CPBA oxidation and H_2O for H_2O_2 oxidation). *p*-Methoxyphenyl 1-propyl disulfide was chosen as a model disulfide for oxidation and as a representative member of an electron-rich (activated) aromatic ring in the target. It was felt that a regioselective oxidation could be achieved since it is well known that the more electron-rich sulfur atom of an unsymmetrical disulfide would be oxidized predominantly.¹⁶⁴ This was considered to be the sulfur attached to the alkyl group in view of resonance involving the S-aryl sulfur and the phenyl ring. However, the influence of the electron-releasing methoxy group was unknown.

m-CPBA Oxidation

The reaction was carried out in CH_2Cl_2 as a solvent, with 1 equivalent of *m*-CPBA at -78°C , Scheme 3-6. The number of equivalents of the oxidant was kept at one to limit the formation of thiosulfonate. The reaction was stirred for 6 hours allowing to warm to room temperature and TLC confirmed the formation of two more polar compounds, which were surmised as being the thiosulfinate and thiosulfonate (or α,α' -disulfoxide which ultimately rearranged to the thiosulfonate). The reaction was quenched with saturated aqueous sodium hydrogen carbonate and the products extracted into CH_2Cl_2 .

Separation of the products to afford yellow oils was achieved by column chromatography, and the yields were 51% and 23% for thiosulfinate (the more polar product on TLC) and thiosulfonate respectively.



Scheme 3-6 Reagents and conditions: (i) *m*-CPBA, CH₂Cl₂, -78°C to rt.

NMR and IR analysis of each compound revealed them to be the thiosulfinate and thiosulfonate derived from oxidation exclusively at the alkyl sulfur. The IR spectrum for the thiosulfinate showed a significant new band at 1029 cm⁻¹ corresponding to an S=O stretch, while the thiosulfonate showed two bands at 1320 cm⁻¹ and 1130 cm⁻¹ corresponding to the asymmetric and symmetric stretches of the O=S=O respectively. The ¹H NMR and ¹³C NMR spectra of the thiosulfinate and thiosulfonate showed significant downfield shifts of the aliphatic protons compared to the NMR spectrum of the unsymmetrical disulfide, indicating that oxidation had occurred at the sulfur attached to the alkyl chain as anticipated.

Specifically, the ¹H NMR spectrum of **127a** and **127b** revealed significant downfield shifts of the protons H-1, H-2 and H-3 as indicated in Table 3-2 due to the electron-withdrawing effect of the oxidized sulfur. Naturally, these effects were most pronounced for H-1 closest to the sulfur. Their ¹³C NMR spectra confirmed these findings with a significant downfield shift observed for the carbon alpha to the oxygenated sulfurs due to the inductive effect caused by the partial positive charge on the sulfinyl sulfur atom, Table 3-3.

Proton	Disulfide 66 (ppm)	Thiosulfinate 127a (ppm)	Thiosulfonate 127b (ppm)
H-1	2.71	3.04	3.14
H-2	1.70	1.88	1.94
H-3	0.96	1.08	1.05

Table 3-2 Comparison of H chemical shifts of unsymmetrical disulfide, thiosulfinate and thiosulfonate.

Carbon	Disulfide 66 (ppm)	Thiosulfinate 127a (ppm)	Thiosulfonate 127b (ppm)
C-1	40.9	57.6	60.7
C-2	22.1	17.2	17.3
C-3	13.1	13.2	12.7

Table 3-3 Comparison of C chemical shifts of unsymmetrical disulfide, thiosulfinate and thiosulfonate.

In addition to the IR spectra of the two products, **127a** and **127b** were unambiguously distinguished from one another in view of the observation that the thiosulfonate α -protons and α -carbons shift much more downfield compared to those of the thiosulfinate. This effect is directly related to the number of oxygen atoms attached to the sulfur atom.

The trends in the chemical shifts observed in the ^1H NMR and ^{13}C NMR spectra of both the thiosulfinate and thiosulfonate are in agreement with what Oae reported in 1978 for a range of thiosulfinates and thiosulfonates.²⁸⁹ Interestingly, it was observed that the chemical shift of the carbon β to the oxygenated sulfur (C-2) of both the thiosulfinate and thiosulfonate shifted upfield compared to the unsymmetrical disulfide. This unusual shift was also observed by Oae who suggested that the carbon β to the oxygenated sulfur experiences an increase in electron density, also known as the γ -effect, due to a shielding effect by the sulfinyl oxygen.²⁸⁹ Oae illustrated this effect by means of the 5-membered ring interaction as shown in Figure 3-7. The oxygen-hydrogen interaction shown, ultimately also promotes a large downfield shift of the H_α and C_α in the ^1H and ^{13}C NMR respectively. Furthermore, the two products gave correct masses from their high resolution mass-spectra.

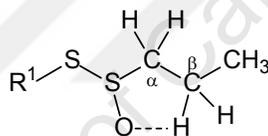


Figure 3-7 Illustration of the interaction between the oxygen attached to the sulfur atom and H_β .

H₂O₂ Oxidation

By comparison, reaction of *p*-methoxyphenyl 1-propyl disulfide **66** with 1 equivalent of H_2O_2 in acetic acid at 0 °C gave the corresponding thiosulfinate and thiosulfonate in low yields after stirring for 4 hours, see Table 3-4. In an attempt to improve the yield of the reaction, the reaction time and temperature were varied. Each case was separately chromatographed. Eventually (rt/24 hrs), a similar profile as for *m*-CPBA (-78 °C - rt) was obtained.

Temp (°C)	Time (h)	Thiosulfinate 127a (%)	Thiosulfonate 127b (%)
0	4	18	10
0	24	32	12
RT	4	34	15
RT	24	52	23

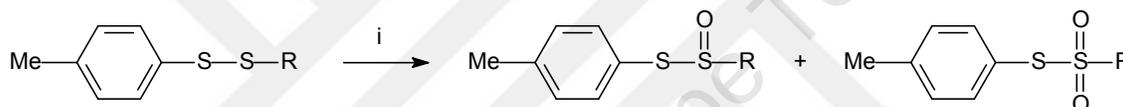
Table 3-4 Yields obtained from various conditions for oxidation of **66** with H_2O_2 .

The slow conversion of unsymmetrical disulfide to thiosulfinate by H_2O_2 resulted in the employment of *m*-CPBA as oxidant for the remainder of the study.

3.3 Electron-Rich Systems

3.3.1 Oxidation of alkyl *p*-tolyl disulfides

With the successful formation of thiosulfinate **127a**, attention was focused on oxidation of a range of alkyl *p*-tolyl disulfides to their thiosulfinates. These were also considered in view of their aromatic rings being activated albeit to a lesser extent compared to that of the *p*-methoxy case. The conversions were carried out as described for the model study using 1 equivalent of *m*-CPBA, Scheme 3-7. The corresponding thiosulfinates and thiosulfonates as identified by IR spectroscopy were isolated as yellow oils but with the former significantly predominating, Table 3-5. NMR once again indicated that the sulfur atom attached to the alkyl chain had been exclusively oxidized by virtue of downfield shifts for the alkyl signals, which will be illustrated subsequently.



Scheme 3-7 Reagents and conditions: (i) *m*-CPBA, CH₂Cl₂, -78 °C to rt.

Compound	R – Group	Thiosulfinate (a) (% yield)	Thiosulfonate (b) (% yield)
128	t-Butyl	53	10
129	Propyl	58	14
130	Butyl	60	10
131	Hexyl	70	x

Table 3-5 Yields obtained for *S*-alkyl *p*-tolyl thiosulfinates and *S*-alkyl *p*-tolyl thiosulfonates

(x: not obtained - reaction conditions as reported in a subsequent section - 3.7.1).

Unlike the majority of thiosulfinates, which require rapid purification, the thiosulfinates synthesized were stable to column chromatography and prolonged drying under reduced pressure. A search of the literature revealed that the *S*-*p*-tolyl alkylthiosulfinates (except **128a**)²⁹⁰ and all of the *S*-*p*-tolyl alkylthiosulfonates were new compounds. However, only the thiosulfinates were fully characterized by IR, NMR and HRMS. The IR spectra for each thiosulfinate showed a strong band for the sulfinate stretch at 1040 cm⁻¹ while each thiosulfonate showed two bands for the sulfonate stretch at 1330 cm⁻¹ (asymmetric) and 1140 cm⁻¹ (symmetric).

The ^1H and ^{13}C NMR spectra for the thiosulfonates and thiosulfonates (^{13}C NMR not recorded) of this series followed similar trends as those observed for *S-p*-methoxybenzene propylthiosulfinate **127a** and *S-p*-methoxybenzene propylthiosulfonate **127b** respectively. As before, H-1 and H-2 of the alkyl chain of the thiosulfonates and thiosulfonates shifted downfield the most compared to those of the unsymmetrical disulfides, Table 3-6. Similarly, the C-1 resonances of the thiosulfonates in the ^{13}C NMR spectra shifted downfield compared to the disulfide, Table 3-6. These observations gave conclusive evidence for oxidation of the alkyl sulfur atom.

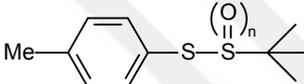
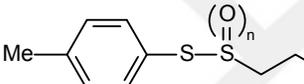
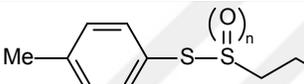
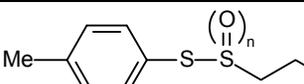
Compounds		^1H NMR		^{13}C NMR		
		H-1	H-2	C-1	C-2	
	128	(n)				
		0	-	1.30	49.0	29.9
		1	-	1.45	60.1	24.1
	129	0	2.71	1.70	40.9	22.2
		1	3.04	1.85	57.8	21.3
		2	3.16	1.93	a	a
	130	0	2.78	1.71	38.7	30.9
		1	3.09	1.81	55.8	25.4
		2	3.23	1.91	a	a
	131	0	2.76	1.70	39.0	28.8
		1	3.07	1.82	56.1	28.3
		2	b	b	b	b

Table 3-6 NMR chemical shifts for hydrogens and carbons affected by oxidation
(^a not recorded, ^b not obtained).

The thiosulfonates and thiosulfonates were distinguished from each other by IR and in view of the more downfield shifts of the H-1 in the ^1H NMR spectra for the thiosulfonates.

3.3.2 Oxidation of alkyl 2-hydroxyphenyl disulfides

The disulfides **78**, **79**, **96** and **97** were problematic to oxidize, resulting in the formation of several products that were difficult to isolate. Hence the hydrogen-bonding capability of the thiosulfonates could not be studied.

3.4 Electron-Deficient Systems

3.4.1 Oxidation of unsymmetrical disulfides with electron-withdrawing substituents

The next set considered involved changing the aromatic ring to a deactivated ring while keeping the same range of alkyl groups. Attempts to prepare the thiosulfonates shown in Figure 3-8 using the same conditions as those described above were unsuccessful. All the compounds with electron-withdrawing substituents in the aromatic ring, although producing a major product on TLC, failed to produce anything on purification involving a conventional work-up and chromatography. TLC analysis of the column fractions revealed multiple spots indicating extensive breakdown, strongly suggesting that electron deficiency in the aromatic ring leads to instability of the thiosulfonate, precluding them as potential allicin mimics. The absence of these compounds in the literature further confirms their instability.

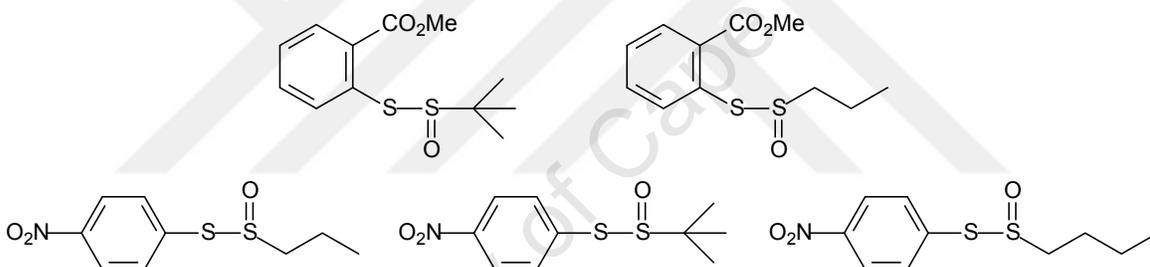
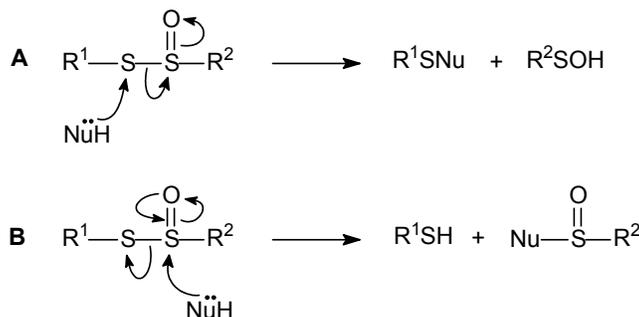


Figure 3-8 Structures of compounds with an electron-withdrawing substituent.

Electron-withdrawal in the aromatic ring would enhance electrophilicity at both sulfenyl and sulfinyl sulfurs, the sites for preferential attack by soft and hard nucleophiles respectively, Scheme 3-8 **A** and **B**.^{291,292} However, one would expect electrophilicity to increase more at the sulfinyl sulfur. Electron-withdrawal in the aromatic ring would stabilize the sulfenate leaving group, favouring sulfenyl sulfur attack, pathway **A**.



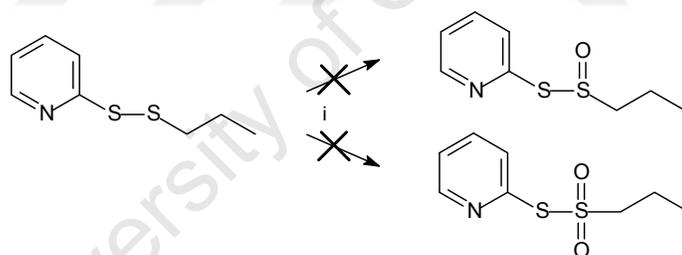
Scheme 3-8 Proposed pathways for decomposition of thiosulfonates with electron-withdrawing substituents.

The products were not isolated and characterized, hence the predominance of one pathway over the other was not delineated. However, with the experimental conditions of work-up, it is likely (*via* H₂O) that the “hard” attack at the sulfinyl sulfur predominated (pathway **B**). The instability of the target thiosulfinates precluded further study with this type of target.

3.5 π -Deficient Heteroaromatic System

3.5.1 Oxidation of 1-propyl pyridyl disulfide

An interesting and unusual result was observed for oxidation of 1-propyl pyridyl disulfide with 1 equivalent of *m*-CPBA under analogous conditions to those for the alkyl *p*-methoxyphenyl disulfide oxidation. Oxidation proceeded to form two products as observed by TLC that were stable to isolation by column chromatography. Initially, it was suspected that the products were the thiosulfinate and thiosulfonate as obtained in previous reactions, Scheme 3-9. However, the more polar of the two proved to be dipyridyl disulfide, as evidenced by TLC, melting point, IR and its ¹H NMR spectrum. The data were identical to those for a known sample of dipyridyl disulfide.



Scheme 3-9 Reagents and conditions: (i) *m*-CPBA, CH₂Cl₂, -78 °C to -10 °C.

The less polar of the two products (which will be referred to as **X**) has a structure that has not been elucidated beyond reasonable doubt as yet but which is clearly not *S*-pyridyl propylthiosulfinate or *S*-pyridyl propylthiosulfonate as evidenced by ¹H and ¹³C NMR. The IR spectrum of **X** revealed the presence of an O=S=O group as evidenced by bands observed at 1318 cm⁻¹ (asymmetrical stretch) and 1126 cm⁻¹ (symmetrical stretch). Its ¹H NMR spectrum indicated the presence of three propyl groups in the ratio 1:1:1 by integration and each one in a significantly different chemical environment, as well as one pyridyl ring, Figure 3-9. NMR assignments for the starting disulfide **89** illustrated that the methylene protons adjacent to the sulfur atom resonated at δ_{H} 2.78 (Figure 3-10), whilst the three most downfield aliphatic signals for **X** resonated at δ_{H} 2.73, 3.01 and 3.24, indicating that two are more deshielded. The ¹³C NMR spectrum (Figure 3-9) of **X** confirmed significant deshielding of one of the α -carbons

to δ_C 64.4 indicative of a $-\text{SO}_2\text{CH}_2-$ group, while the other two (δ_C 38.1 and δ_C 41.0) appeared to be $-\text{SCH}_2-$ groups. Furthermore, NMR chemical shifts indicated the pyridine nitrogen to be unoxidized.

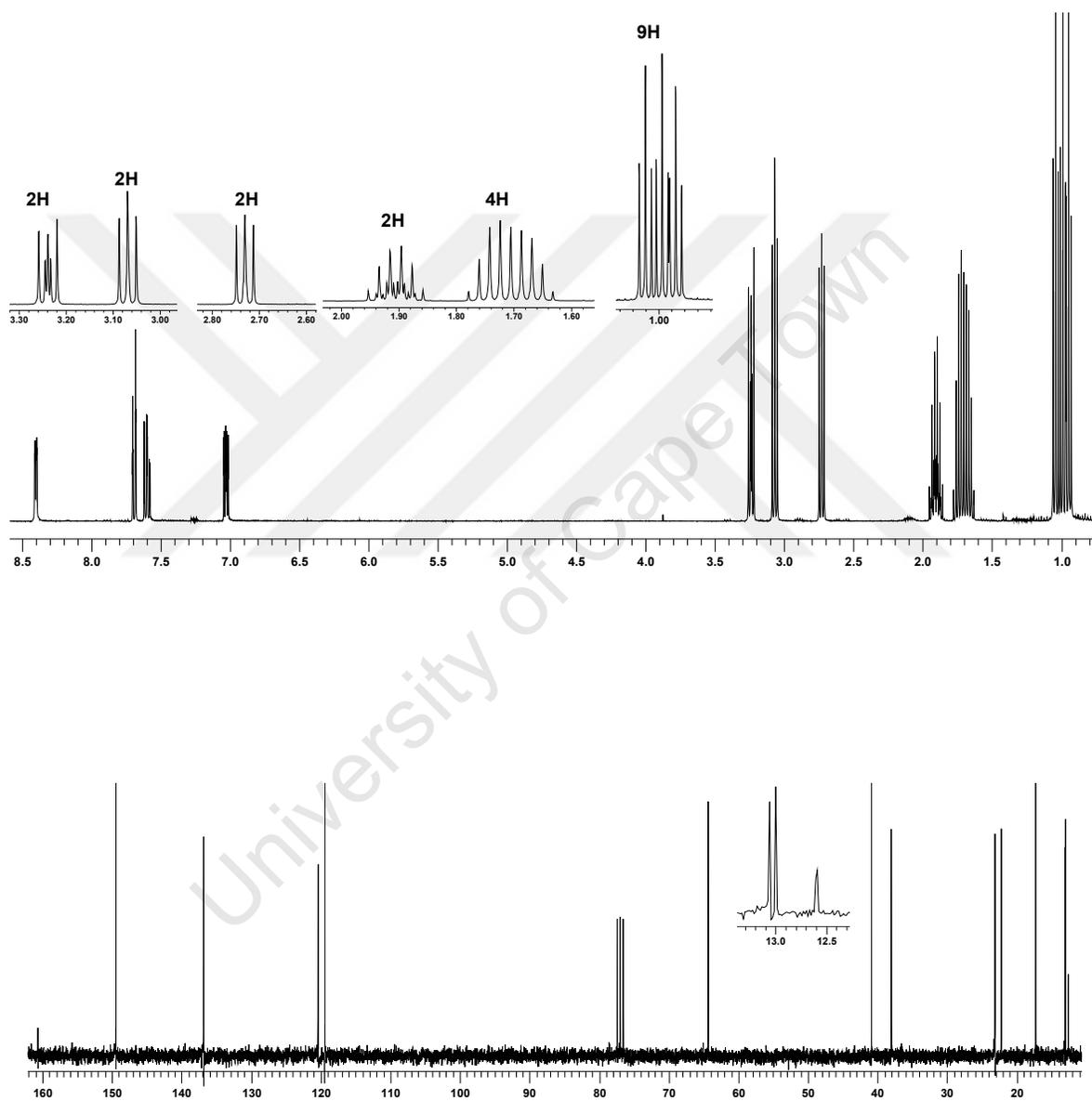


Figure 3-9 ^1H (top) and ^{13}C (bottom) NMR spectrum of X.

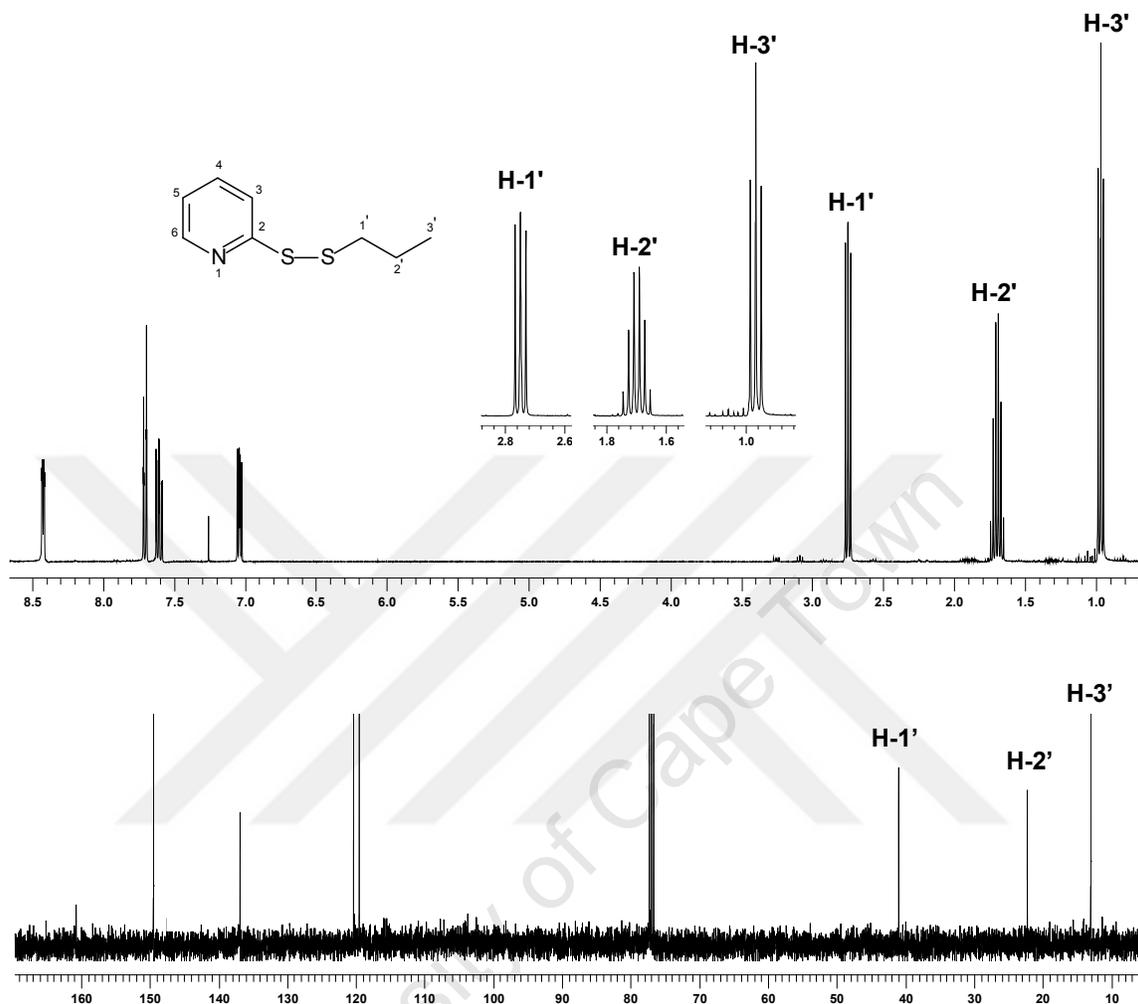


Figure 3-10 ^1H (top) and ^{13}C (bottom) NMR spectrum of **89**.

HRMS showed the starting material, 1-propyl pyridyl disulfide, as the highest molecular-weight ion, with a fragmentation pattern as: 185.0293 $[\text{M}]^{+\bullet}$, 142.9854 $[\text{M}-\text{CH}_2\text{CH}_2\text{CH}_3]^+$, 110.9999 $[\text{M}-\text{CH}_2\text{CH}_2\text{CH}_3-\text{S}]^+$ and 76.0438 $[\text{M}-\text{CH}_2\text{CH}_2\text{CH}_3-\text{S}-\text{S}]^+$, which is analogous to the patterns observed for the unsymmetrical disulfides as discussed in Chapter 2. Attempts to include **X** into a methylated cyclodextrin (TRIMEB) gave crystals, which turned out to have S-dipropylthiosulfonate as the guest as determined using single crystal X-ray diffraction. Since it is known that the product structure includes a pyridine heteroaromatic, the result suggested breakdown of **X** by the cyclodextrin. It was decided to thoroughly examine the HRMS spectrum of the product in search of a molecular-weight ion corresponding to S-dipropylthiosulfonate. Significantly, the ion 182.0452 $[\text{M}]^{+\bullet}$ was absent from the spectrum.

Compound **X** was refluxed in THF for 16 hours to investigate its stability. TLC analysis indicated no breakdown of the product. In addition, a sample stored at room temperature for two weeks was resubmitted for HRMS and NMR, to return identical results to the original.

It was then decided to postulate a structure from NMR assignments. The stoichiometry, as determined from NMR, demands a sulfurane structure as illustrated in Figure 3-11, with three sulfur ligands and one carbon ligand attached to the central sulfur atom.

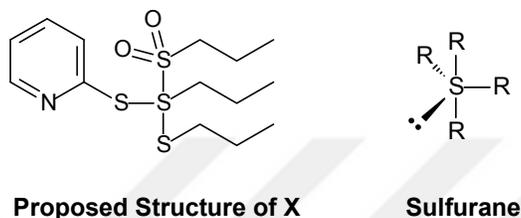
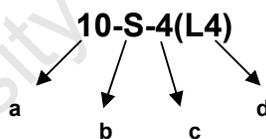


Figure 3-11 Proposed structure of **X** as determined by NMR and an illustration of a sulfurane.

Sulfurane compounds have an σ -bonded sulfur in a hypervalent state. Four ligands attached to a central sulfur atom are common with an electronic structure involving a formal expansion of the valence shell of the central atom from eight to ten electrons.²⁹³ Such sulfurane compounds are known by the nomenclature [10-S-4(L4)].



Key:

- (a) - the number of electrons, in the sulfur valence shell.
- (b) - the central atom (usually a Group 16 atom, e.g. sulfur for sulfuranes, tellurium for telluranes and selenium for selenurananes); only compounds with sulfur as the central atom will be discussed.
- (c) - the number of ligands attached to the central atom.
- (d) - the type (L = element) and number of a specific ligand (usually a strong electronegative ligand such as oxygen, nitrogen or a halogen).

This concept of hypervalent bonding was introduced in 1969 by Musher.²⁹⁴ Notably, in 1971 Martin reported the preparation of the first stable isolable sulfurane (now known as Martin's sulfurane) as a dialkoxydiarylsulfurane (a sulfurane with two S-O bonds), Figure 3-12.²⁹⁵

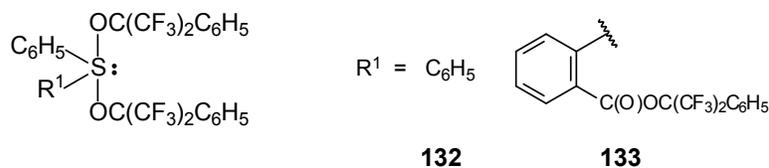


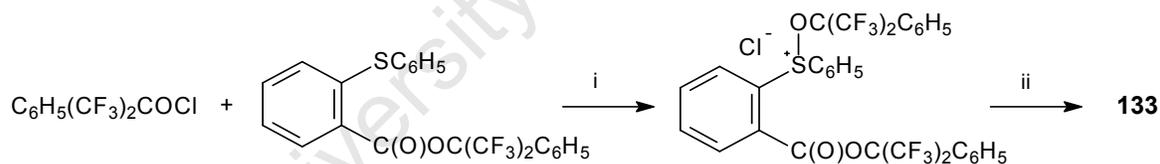
Figure 3-12 Structures of Martin's sulfuranes.

Two different methods were employed for the synthesis of these hypervalent structures. The first approach, for preparation of **132**, involved treatment of an ether solution of diaryl sulfide and alkoxide (R_FOK) with chlorine at -78°C to give the product in an 83% overall yield, Scheme 3-10.



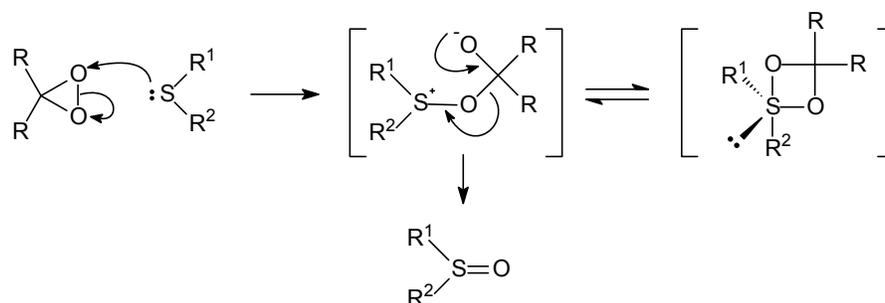
Scheme 3-10 Reagents and conditions: (i) Cl_2 , ether, -78°C .

The second approach, for preparation of sulfurane **133**, involved treatment of the corresponding sulfide with 1 molar equivalent of hexafluoro-2-phenyl-2-propyl hypochlorite (R_FOCl) to give an alkoxy-sulfonium chloride intermediate. The intermediate was not isolated but addition of alkoxide R_FOK afforded the desired compound, Scheme 3-11.



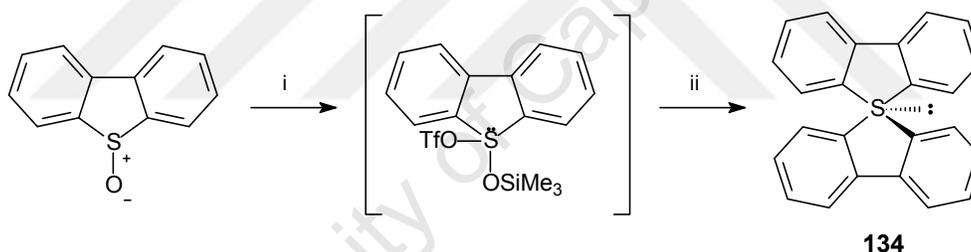
Scheme 3-11 Reagents and conditions: (i) CH_2Cl_2 , -78°C . (ii) R_FOK , ether, -78°C .

Sulfuranes with S-O bonds are normally highly reactive intermediates and have generally only been considered to be transient transition-state intermediates in nucleophilic substitution reactions at sulfur atoms, or as intermediates in oxidation of sulfides, Scheme 3-12.



Scheme 3-12 Oxidation of sulfides *via* sulfuranes.

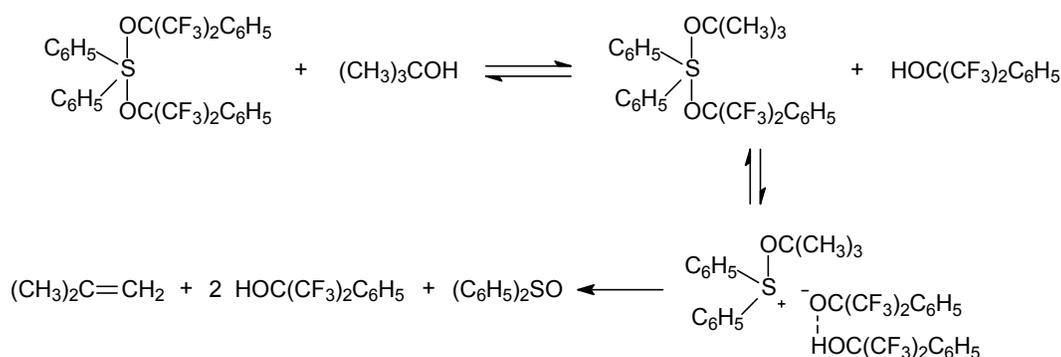
It has been reported that strong electronegative ligands stabilize a sulfuranane and aid in its isolation.²⁹⁶ Hence, the ease of isolation of **132** and **133**. Sulfurananes bearing only carbon ligands [10-S-4(C₄)] were believed to be unstable as postulated since 1962. Many years later, however, in 1992, Ogawa reported the first synthesis and structural characterization of a stable sulfuranane with four C-S bonds.²⁹⁷ Bis(2,2'-biphenylene)sulfuranane **134** was prepared as illustrated in Scheme 3-13.



Scheme 3-13 Reagents and conditions: (i) Trimethylsilyl trifluoromethanesulfonate, THF, -78 °C.
(ii) 2,2'-dilithiobiphenyl, diethyl ether/THF, -78 °C.

Today various types of sulfurananes exist, which have been successfully synthesized and isolated. The importance of these compounds as reagents has also become evident.

Sulfurananes such as **133** exhibit great reactivity toward active hydrogen compounds such as O-H, N-H, and S-H. This makes them attractive reagents for dehydrations, etherifications, oxidations and some cleavage and coupling reactions. An example of the significance of **133** in reactions involves the conversion of alcohols to alkenes in the presence of **133** by acid catalysis, Scheme 3-14.²⁹⁸

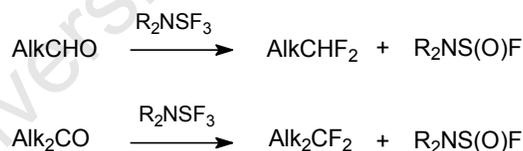


Scheme 3-14 Conversion of alcohols to alkenes by sulfurane **133**.

Sulfuranes containing fluorine atoms attached to the sulfur (fluorosulfuranes) have also found wide application in organic synthesis as fluorinating agents.²⁹⁹ Scheme 3-15 illustrates the use of SF₄, [10-S-4(F₄)], in the preparation of aminofluorosulfuranes, while Scheme 3-16 illustrates the fluorinating ability of **135** by converting aliphatic aldehydes and ketones to difluoroalkanes in high yields.²⁹⁹



Scheme 3-15 Reagents and Condition: (i) ether, -75 °C to -45 °C.



Scheme 3-16 Fluorination of aliphatic aldehydes and ketones to difluoroalkanes.

Although many types of sulfuranes have been reported, to the best of our knowledge, the [10-S-4(S₄)] type has not appeared in the literature. The proposed structure **X**, if correct, would thus be the first of its class. The structure is not implausible since sulfur is slightly more electronegative than carbon and has access to *d* orbitals, which could offer stabilization of the proposed structure.

Compound **X** will have to be characterized beyond reasonable doubt in order to publish a structure. Hence, future work will involve crystallographic analysis by some means.

3.6 Stability Study

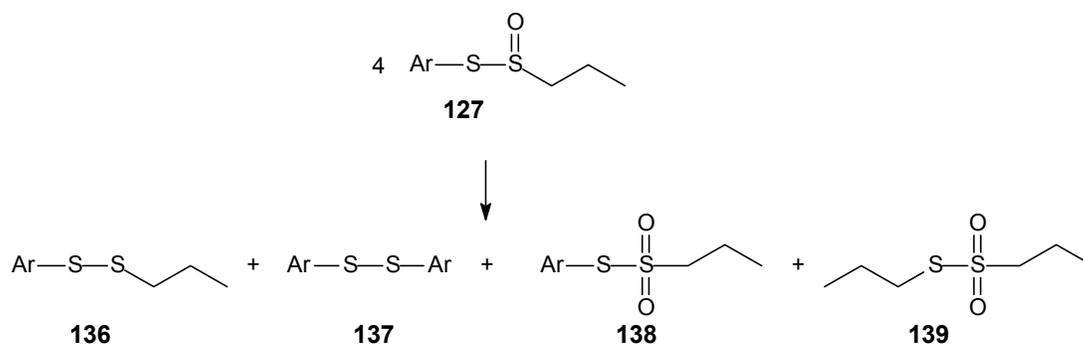
Thiosulfinates are known to be extremely unstable; neat allicin has a half-life of only 16 hours at room temperature. As mentioned, the literature reveals that they rapidly rearrange, while diaryl thiosulfinates rapidly disproportionate. However, the literature is less clear on the details of decomposition of unsymmetrical mixed aralkyl thiosulfinates. Our compounds presented an opportunity to provide details on this under-explored class of compounds.

3.6.1 Model Study of *S-p*-methoxyphenyl 1-propylthiosulfinate and *S-p*-tolyl 1-propylthiosulfinate

In light of the electron-deficient systems breaking down owing to their instability and the π -heteroaromatic system giving a completely different result than expected, a stability study (over a period of 5 months) was undertaken initially on *S-p*-tolyl propylthiosulfinate and *S-p*-methoxyphenyl propylthiosulfinate. The conditions decided on were based on drug formulations and their storage methods. Hence, the compounds were stored at; (i) -5 °C in a neat form, (ii) room temperature in a neat form and (iii) room temperature in a solvent (CDCl₃). A reliable and rapid analysis technique needed to be used to minimize the breakdown of the compounds for the period of data analysis. NMR was chosen to determine the degree of rearrangement or disproportionation of the thiosulfinates, since there was concern about column breakdown, which was considered possible in GC and HPLC techniques. Hence, NMR would provide an instant and reasonably quantitative evaluation (to within \approx 5%). TLC analysis was also used prior to NMR to detect formation of new compounds.

TLC analysis indicated the decomposition to involve disproportionation³⁰⁰ to the corresponding disulfides and thiosulfonates according to reference samples, as discussed in Chapter 1. In addition to NMR, TLC was used to assess percentage decomposition (change).

Prior to stability tests, *S-p*-methoxyphenyl propylthiosulfinate **127** was refluxed in THF for three days. Purification of the product mixture by column chromatography confirmed disproportionation to *p*-methoxyphenyl 1-propyl disulfide **136** (18%), di-*p*-methoxyphenyl disulfide **137** (33%) and *S-p*-methoxyphenyl propylthiosulfonate **138** (30%). In addition, 10% *S-p*-methoxyphenyl propylthiosulfinate (starting material) was recovered. It is surmised that the remainder was di-propylthiosulfonate **139**, which was not isolated. Scheme 3-17 illustrates the disproportionation products obtained from **127**.



Scheme 3-17 Decomposition of thiosulfinates.

In light of these results, subsequent to subjecting compound samples to the specified conditions, ^1H and ^{13}C NMR spectra were recorded to evaluate the % conversion of thiosulfinates **127** and **129** after 1, 3 and 5 months, Table 3-7. The % conversion was based on the formation of new peaks in the aromatic and methyl regions using the reference spectra and by calculating the molar ratios of the “old” and “new” aromatic, aliphatic and methoxy peaks (the thiosulfinate, thiosulfonate and 1-propyl *p*-methoxyphenyl disulfide had distinctly different chemical shifts for the aromatic and methoxy peaks).

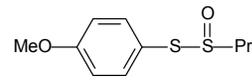
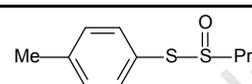
Compound	Time (months)	Neat (-5°C)	Neat (rt) ^a	In CDCl ₃ (rt) ^a
 127	1	<1	<3	60
	3	<3	<i>b</i>	100
	5	10	50	<i>b</i>
 129	1	<5	5	10
	3	6	12	75
	5	15	75	<i>b</i>

Table 3-7 Decomposition studies of thiosulfinates **127** and **129** - Values recorded as a % conversion determined by ^1H NMR and ^{13}C NMR. (^a room temperature ~ 20 °C, ^b not recorded)

After 1 month neat at room temperature, less than 5% decomposition was noted for the samples, which contrasts markedly with allicin. The samples were markedly more stable in the refrigerator with only 10-15% decomposition observed after 5 months at -5°C. Storing the samples at room temperature in deuterated chloroform, however, accelerated the rate of decomposition dramatically with 100% and 75% decomposition for thiosulfinates **127** and **129**, respectively, after 3 months. Both samples proved to be significantly more stable in a neat form at room temperature than in solution at room temperature while the samples in the

refrigerator were the most stable. Interestingly, the results indicate that whilst thiosulfinate **127** is more stable than thiosulfinate **129** in a neat form, the reverse applies in solution.

3.7 S-*p*-Methoxyphenyl Alkylthiosulfinates

With the emergence of an important structure-activity directive and the increased stability of neat *S-p*-methoxyphenyl propylthiosulfinate compared to neat *S-p*-tolyl propylthiosulfinate at room temperature, it was decided to oxidize a range of alkyl *p*-methoxyphenyl disulfides and investigate the stability and biological activity of their corresponding thiosulfinates.

3.7.1 Oxidation of alkyl *p*-methoxyphenyl disulfides

A class of seven compounds with the general structure as depicted in Figure 3-13 were chosen as candidates for stability and antimicrobial testing.

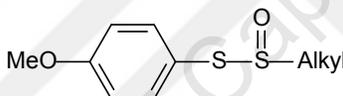


Figure 3-13 Structure of thiosulfinates (alkyl-groups defined in Table 3-8).

A further review of the literature revealed that it was likely that the thiosulfinate could be obtained exclusively. It became clear that the reaction time (6 hours) and temperature range (-78 °C to rt) were unoptimised parameters for the oxidation, hence resulting in formation of the corresponding thiosulfonate. Thus it was decided to reduce the reaction time from 6 to 3 hours and quench the reaction at -10 °C, albeit at the possible expense of conversion.

Oxidation was thus achieved by addition of 1 equivalent of solid *m*-CPBA to a solution of alkyl *p*-methoxyphenyl disulfide in CH₂Cl₂ at -78 °C. The resultant mixture was stirred for 3 hours, slowly warming to -10 °C. It should be noted that at this point only a single more polar product was observed by TLC. Subsequent to addition of aq. NaHCO₃ and the normal work-up, the mixture was purified by column chromatography. Pleasingly, no thiosulfonate was isolated after quenching the reaction at -10 °C instead of at room temperature. The corresponding thiosulfinates, as determined by IR spectroscopy, were obtained in good yields as illustrated in Table 3-8. Noteworthy, was the increase in product yield from 53% to 72% as observed for **127a**.

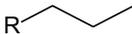
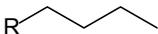
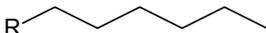
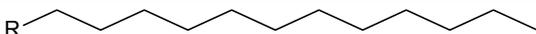
Compound	Alkyl Group (R = <i>p</i> -MeOPhSS-)	Yield (%)
127		72
140		69
141		63
142		67
143		70

Table 3-8 Structures of alkyl groups and yields obtained for *S-p*-methoxyphenyl alkylthiosulfinates.

Besides having the characteristic smell of crushed garlic, the *S-p*-methoxyphenyl alkylthiosulfinates synthesized were uniquely different compared to those (especially allicin) isolated from garlic. They were stable to an aqueous work-up, silica-gel column chromatography and prolonged drying under reduced pressure. The alkyl *p*-methoxyphenyl thiosulfinates are all new compounds and were fully characterized.

The IR spectrum of each one displayed a characteristic band at 1030 cm⁻¹ for the S=O group. NMR assignments of compound **140** will be reported as a representative for compounds in the series.

The ¹H NMR spectrum for each thiosulfinate was virtually identical, in terms of number of peaks and multiplicity, to that of the corresponding unsymmetrical disulfide but with downfield shifts for the aliphatic protons of the thiosulfinate. As mentioned before, the thiosulfinate's ¹H NMR spectrum (Figure 3-14) gave conclusive evidence of oxidation of the sulfur atom attached to the alkyl chain. The key feature was the large downfield shift of the protons (H-1) adjacent to the oxidized sulfur. The aromatic protons of the thiosulfinates, which have not been mentioned up to this point, remained at the same chemical shifts as those for the disulfides, confirming chemoselective oxidation.

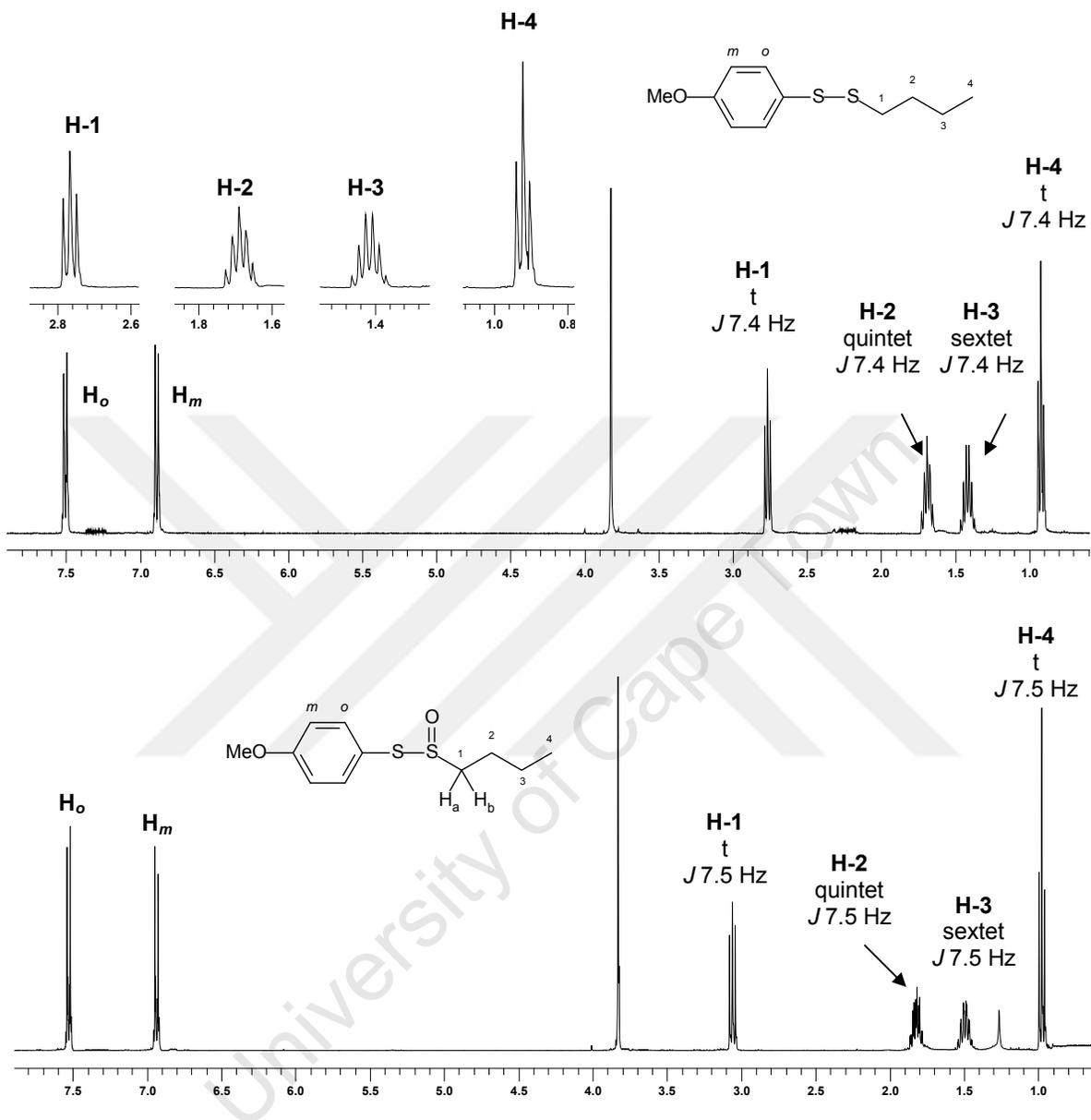


Figure 3-14 ¹H NMR spectrum of unsymmetrical disulfide **69** (top) and thiosulfinate **140** (bottom).

The magnetically non-equivalent protons H-1^a and H-1^b were expected to appear as two doublets of triplets owing to their diastereotopic nature with the chiral sulfinyl sulfur. However, chemical-shift equivalence was observed for all members in this series, and the signal appeared as a simple triplet. This is not uncommon though. Oae reported chemical shifts of a range of thiosulfonates and in some cases the α-protons were observed as chemical-shift equivalent.²⁸⁹ The ¹³C NMR spectra of the thiosulfonates were similar to those for the corresponding unsymmetrical disulfides in the aromatic region. However, downfield shifts for C-1 in the aliphatic moiety were observed from δ_C 38.6 in the disulfide to δ_C 55.6 for the

thiosulfinate (Figure 3-15). It should also be noted that for all the thiosulfinates, C_o aromatic shifted downfield while C_s shifted upfield.

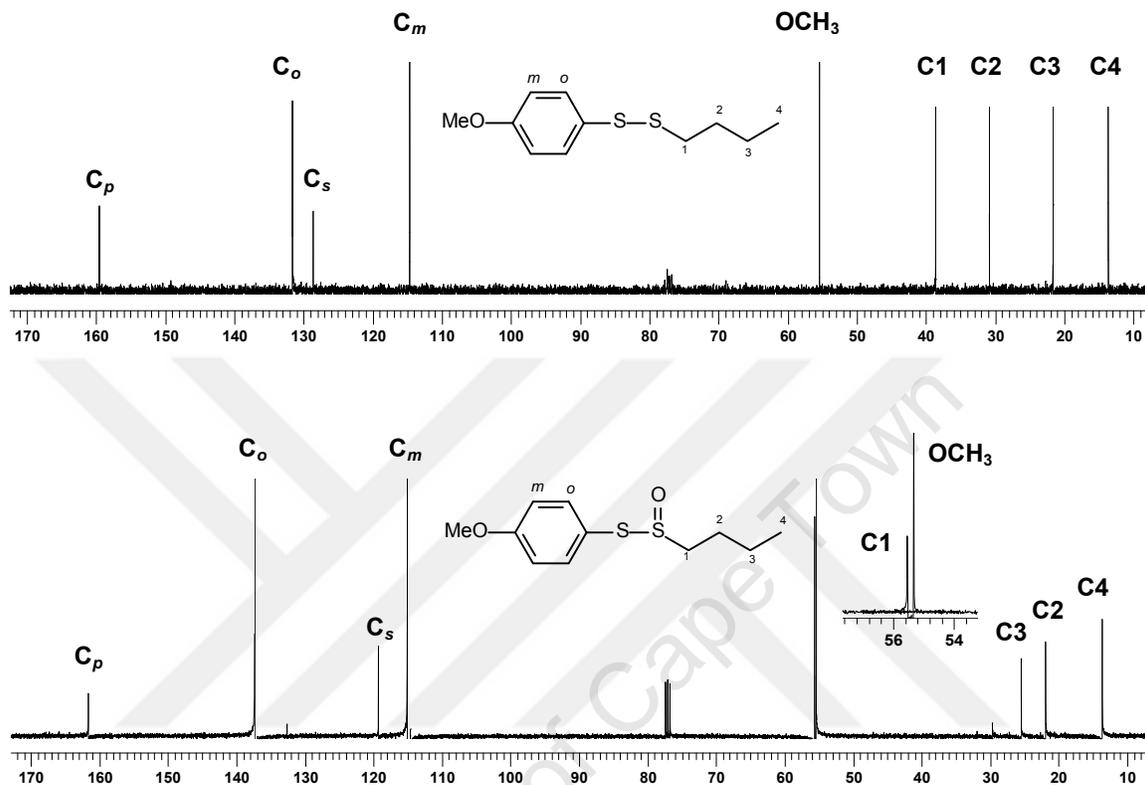


Figure 3-15 ¹³C NMR spectrum of unsymmetrical disulfide **69** (top) and thiosulfinate **140** (bottom).

Oxidation of fluorinated-alkyl *p*-methoxyphenyl disulfides

Oxidation of the fluorinated disulfides was achieved as described for the above mentioned alkyl *p*-methoxyphenyl disulfides, Table 3-9.

Compound	Alkyl Group (R = <i>p</i> -MeOPhSS-)	Yield (%)
144	R—(CH ₂) ₂ (CF ₂) ₃ CF ₃	51
145	R—(CH ₂) ₂ (CF ₂) ₇ CF ₃	50

Table 3-9 Yields obtained for the fluorinated thiosulfinates.

The NMR assignments for the two fluorinated oxidized compounds (Figure 3-16) and their respective disulfides are shown in Table 3-10.

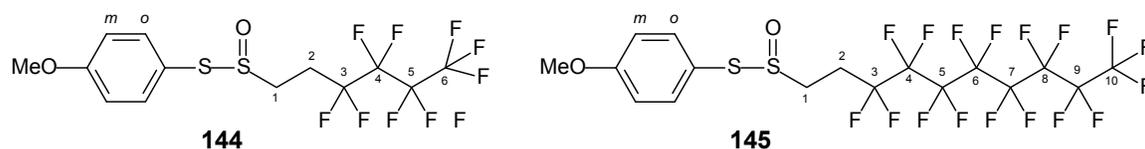


Figure 3-16 Structures of oxidized fluorinated compounds.

Compound	H or C	^1H NMR (ppm)		^{13}C NMR (ppm)	
		Disulfide	[O] Product	Disulfide	[O] Product
144	1	2.89	3.31	28.5	45.9
	2	2.54	2.69	31.3	25.3
	<i>m</i>	6.88	6.98	114.9	115.3
	<i>o</i>	7.48	7.54	132.9	137.7
145	1	2.90	3.31	28.6	45.9
	2	2.57	2.70	31.4	25.3
	<i>m</i>	6.89	6.98	114.9	115.3
	<i>o</i>	7.49	7.55	132.9	137.7

Table 3-10 Comparison of NMR assignments for disulfides **93** and **94** and their oxidized compounds **144** and **145**.

From the ^1H and ^{13}C NMR spectra of **144** and **145**, it is evident from the downfield shifts of H-1 and C-1 that the products are both oxidized. The C-1 chemical shifts suggested a thiosulfonate for both. The fluorinated disulfide and thiosulfonate were both upfield to the corresponding values for the hydrogenated analogues (see compound **66** and **127a** in Table 3-3) as a result of S=O dipole negation by the C-F bonds. The HRMS (Figure 3-17) and IR spectra confirmed the structure of **145** as a thiosulfonate with a molecular ion of $[633.9923]^+$ and a band at 1030 cm^{-1} corresponding to the S=O stretch. However, the HRMS (Figure 3-17) and IR spectra of **144** gave a different result compared to its NMR. A parent molecular ion of $[450.0001]^+$ corresponding to the thiosulfonate was observed while the IR spectrum revealed two bands at 1354 cm^{-1} and 1135 cm^{-1} corresponding to the asymmetric and symmetric stretch of the SO_2 respectively. Taking the latter results as definitive it would appear that the chemical shift of C-1 is the same for both thiosulfonate and thiosulfinate in the fluorinated series. This contrasts with the non-fluorinated case in which there was a 3.1 ppm difference in the C-1 ^{13}C NMR shifts and a 0.10 ppm difference in the ^1H NMR shifts. The reason is not

clear at this stage but may be attributable to the negation of the dipole created by the second S=O going from thiosulfinate to thiosulfonate by the fluorinated chain.

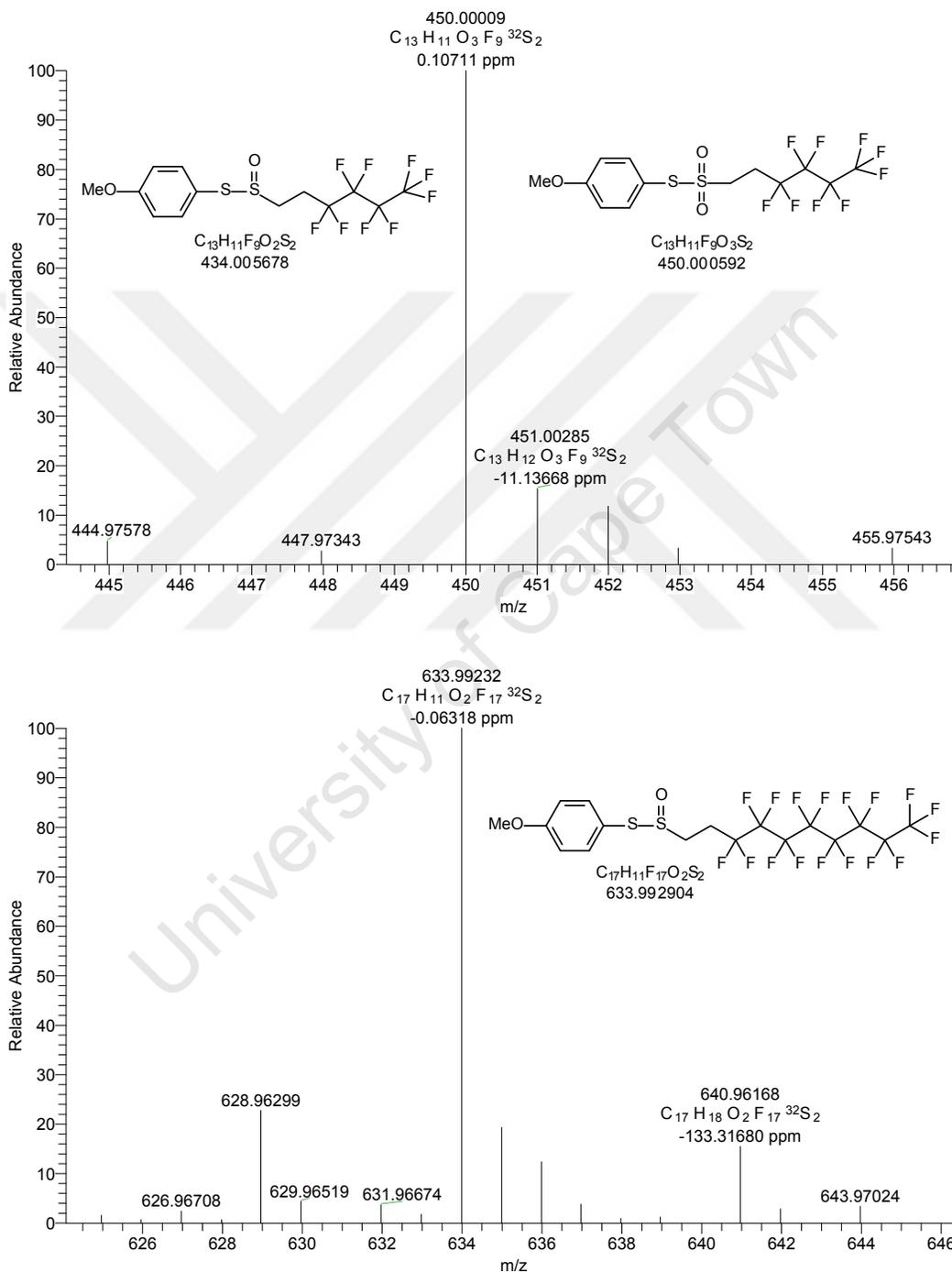


Figure 3-17 HRMS of 144 (top) and 145 (bottom).

Brace observed distinct chemical shift differences in the NMR spectra of corresponding thiosulfinate and thiosulfonates when compounds such as the ones illustrated in Figure 3-18 were studied. H-1 and C-1, in the ^1H and ^{13}C NMR spectra respectively, shifted as expected as the number of oxygen atoms on the sulfur atom increased, Table 3-11. A comparison of Tables 3-10 and 3-11 reveals a good agreement in chemical shifts of H-1 and C-1 (in the ^1H and ^{13}C NMR respectively) for thiosulfinate **145** against those of Brace (thiosulfinate **147**).²⁵⁵ However, there is a notable difference for **144** against **148**. This may be due to the fact that **148** has fluorine atoms on both sides (“symmetrical”), which could be canceling the effects observed when the fluorine atoms are only on one side-chain of the disulfide bond (“unsymmetrical”). This may be indicating that the full effect of the SO_2 dipole is experienced by **148** and not by **144**.



Figure 3-18 Structure of Brace's fluorinated compounds (disulfide **146** ($n=0$), thiosulfinate **147** ($n=1$), thiosulfonate **148** ($n=2$)).

H or C	^1H NMR (ppm)			^{13}C NMR (ppm)		
	146 ($n=0$)	147 ($n=1$)	148 ($n=2$)	146 ($n=0$)	147 ($n=1$)	148 ($n=2$)
1	2.91	3.42	3.53	27.7	46.1	53.7
2	2.57	2.68	2.65	30.9	24.5	25.7

Table 3-11 Comparison of NMR assignments for Brace's fluorinated compounds.

This unexpected result is rather interesting considering that both reactions for the transformations were carried out in exactly the same way. The discrepancy between the NMR and HRMS results could be further investigated by oxidizing similar compounds with different aromatic rings.

3.7.2 Stability Tests on the Library of S-*p*-methoxyphenyl alkylthiosulfinates

The library of S-*p*-methoxyphenyl alkylthiosulfinates were chosen as candidates for the stability tests in view of the enhanced stability observed for thiosulfinate **127** in neat form at room temperature compared to that of thiosulfinate **129** in the model study.

The library of thiosulfonates was subjected to a thermal study and their disproportionation evaluated by NMR. In order to establish the conditions at which the thiosulfonates disproportionate the fastest, the thiosulfonates were initially stored at 0 °C for 3 weeks and then stored at room temperature (~25 °C) for 3 months. Table 3-12 illustrates the percentage conversion of the thiosulfonates to their corresponding disproportionation products after 3 weeks and 3 months at 0 °C and room temperature respectively.

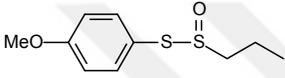
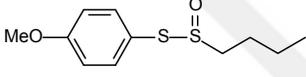
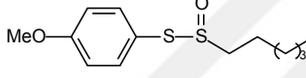
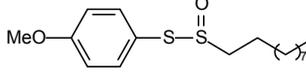
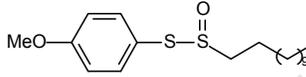
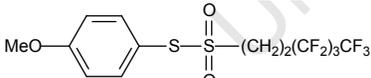
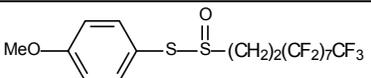
Compound	Storage Conditions					
	1 week (0 °C)	3 week (0 °C)	1 week (rt)	1 month (rt)	2 months (rt)	3 months (rt)
 127	0	0.7	1	3	18	30
 140	0	0.7	1	3	16	24
 141	0	0.5	1	3	14	22
 142	0	0	1	4	8	15
 143	0	0	0.5	1	2	9
 144	0	0	2	6	15	20
 145	0	0	2	5	7	10

Table 3-12 Decomposition studies of thiosulfonates - Values recorded as a % conversion determined by ^1H and ^{13}C NMR. (rt = room temperature)

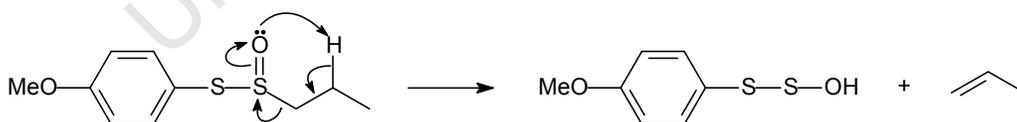
As expected, a stability pattern was observed for the thiosulfonates. Alkyl chain-length proved to have a positive effect on the stability of the thiosulfonates studied (as the length of the chain

increased the stability increased), while the fluorinated thiosulfinates indicated slightly better stability compared to its non-fluorinated counterpart.

Pleasingly, no disproportionation was observed for any of the thiosulfinates after 1 week at 0 °C. However, the thiosulfinates with the shorter chain lengths (**127** propyl, **140** butyl and **141** hexyl) started showing signs of disproportionation after 3 weeks at 0 °C. Thiosulfinates **142**, **143**, **145** and thiosulfonate **144** showed signs of decomposition only after being stored at approximately 20 °C for one week. The rate of disproportionation was slow at first but accelerated gradually after 1 month at room temperature, suggesting auto-catalysis. It was observed that **143** (12-carbon alkyl-chain attached to the sulfinyl sulfur) was the most stable, with only 9% decomposition observed after 3 months neat at room temperature. The most unstable thiosulfinate of the series after 3 months at room temperature was **127** (the shortest alkyl chain studied) with 30% decomposition observed.

Although the fluorinated thiosulfonate and thiosulfinate (**144** and **145** respectively) showed similar stability profiles as their non-fluorinated counterpart (**141** and **142**) it was observed that **144** and **145** were slightly more stable than **141** and **142**. This is contrary to Brace's²⁵⁵ observations, which once again may be indicating that fluorine atoms on either side of the disulfide bond may be significant for stability. Although the allicin mimics are not completely stable, they are to a great extent much more stable compared to allicin.

No evidence for β -sulfinyl proton abstraction as illustrated in Scheme 3-18 was observed for any of the aralkyl thiosulfinates studied. Hence, it appears that disproportionation as with diaryl thiosulfinates is a major rearrangement pathway for aralkyl thiosulfinates instead of the Block-type disproportionation as observed for dialkyl thiosulfinates.



Scheme 3-18 Block-type disproportionation of *S*-*p*-methoxyphenyl propylthiosulfinate.

3.7.3 Antimicrobial Testing of S-*p*-methoxyphenyl alkylthiosulfinates

Allicin has long been known to possess a range of biological activities against various microorganisms, including Gram-positive and Gram-negative bacteria. The principal difference between these bacteria is their cell walls. Gram-positive bacteria have a cell wall composed of a thick layer of peptidoglycan (20-80 nm thick), while Gram-negative bacteria have a cell wall composed of a thin layer of peptidoglycan (2-7 nm thick) surrounded by an outer phospholipid membrane (7-8 nm) to which lipoproteins and lipopolysaccharides are attached.³⁰¹ As a result of having two membranes, Gram-negative bacterial cells are more difficult for antibiotics to penetrate and thus it is harder to kill them with antibiotics than Gram-positives. Owing to allicin's potent antibacterial activity and its instability, it was decided to synthesize possible stable allicin mimics in order to compare their antibacterial activity to that of allicin. The activity of allicin, as discussed in Chapter 1, was proposed by Cavallito,¹¹ who reported that the thiosulfinate grouping (-S(O)-S-) is the essential pharmacophore for activity. In view of this, the compounds in this thesis, which possess the same moiety, should exhibit similar activities. Antibacterial activity was determined by TLC bioautography.

TLC Bioautography

Bioautography is used as a special detection method in the antibiotic field and is based on the growing inhibiting effects of the substances to be detected.³⁰² Compounds to be tested are spotted in various amounts onto a thin-layer chromatography (TLC) plate, which is then inoculated with microorganisms and incubated, Figure 3-19. After incubation, zones of inhibition show the amounts (in μg) at which the test compounds are active. The lowest amount at which the compound shows activity produces an inhibition zone, which is referred to as the minimal inhibitory amount (MIA), which may vary between test bacteria.

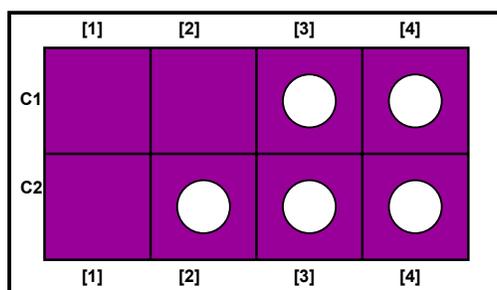


Figure 3-19 Schematic illustration of bioautography using TLC plates.

(C1 and C2 are hypothetical compounds; [1], [2], [3] and [4] is the concentration range).

The library of *S-p*-methoxyphenyl arylthiosulfinates was tested by bioautography on TLC plates using representatives of Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 strains are standard antibacterial susceptibility test strains for antibiotics used in medicine. *Mycobacterium aurum* strain A+ is a fast-growing, non-pathogenic *Mycobacterium* species that is supposed to have a similar antibiotic susceptibility profile to *Mycobacterium tuberculosis*.³⁰³

Experimental Method

All solvent extracts were spot tested for antimycobacterial activity prior to TLC. For spot testing, silica TLC plates (Merck 1.05554.0001) were divided into grids 1.5 cm by 1.5 cm squares and 10 μ l of extract (concentration range of compounds in ethyl acetate: 5-50 μ g/ μ l) was spotted per square (two 5 μ l aliquots were applied at a time). Plates were placed in a fumehood to allow the solvent to evaporate.

One loopful of each culture (*M. aurum* A+, *S. aureus* and *E. coli*) was used to inoculate a universal containing 10 ml sterile Luria broth. The culture was vortexed briefly and incubated at 37 °C for 18-24 hr with constant shaking. The OD₆₀₀ of all cultures was determined and the cultures were diluted to OD₆₀₀=0.5 with sterile Luria broth. All cultures were Gram-stained to ensure that they were not contaminated. Sterile non-absorbent cotton wool was used to dab the diluted culture onto the prepared TLC plates. The plates were incubated in sealed plastic containers, containing moistened paper towel, at 37 °C for 24 hr. Following incubation, the plates were dabbed with 0.25% thiazolyl blue tetrazolium bromide (MTT) in phosphate buffered saline (1.78 g Na₂HPO₄; 8.50 g NaCl; 1 l distilled water; pH 7.3) and incubated at 37 °C for 1 hr. The plates were then inspected for zones of inhibition.

Results

It should be noted that the bioautography method of testing presented only an indication of activity and did not give quantitative data. Thus, this was a preliminary screening aimed at identifying possibilities for further study. Table 3-13 illustrates the preliminary antibacterial results as obtained from bioautography on TLC plates. The results are recorded as MIA. Owing to the compounds being diffused on the TLC plates, the diameters of the inhibition zones could not be measured. The compounds were tested in the range 10 - 100 μ g/spot.

Compound	<i>M. aurum</i> ^a A+	<i>S. aureus</i> ^a ATCC 25923	<i>E. coli</i> ^b ATCC 25922
127	20	Inactive	50
140	20	20	20
141	Inactive	20	20
142	Inactive	Inactive	50
143	20	Inactive	20
144	50	Inactive	20
145	Inactive	Inactive	20

Table 3-13 Sensitivity of three bacterial species to thiosulfonates – values recorded as MIA ($\mu\text{g}/\text{spot}$);
^a Gram-positive, ^b Gram-negative.

The results indicated that all the compounds were active against *E. coli* (Gram-negative) either at 20 or 50 $\mu\text{g}/\text{spot}$ and a few were active against the Gram-positive bacteria (*M. aurum* A⁺ and *S. aureus*). This is an unexpected and very interesting result, as one would have expected the Gram-positive bacteria to show the greatest sensitivity to the tested compounds in view of the less complex nature of their cell wall, resulting in easier penetration of the compounds. Of the series of thiosulfonates tested, **140** was the most active, showing activity against all three bacteria, while **142** was the least active. Although **142** and **145** only killed *E. coli* bacteria, **142** was required at a higher concentration of the compound to observe killing. Hughes and Lawson reported that allicin has a MIC value of 27 $\mu\text{g}/\text{ml}$ against a single strain of *S. aureus* while Ankri reported a LD₅₀ value of 15 and 12 $\mu\text{g}/\text{ml}$ against *E. coli* and *S. aureus* respectively.

The results reported for the present study are preliminary evidence for the antimicrobial activity of the thiosulfonates prepared and support for them being allicin mimics. Further studies will be conducted on these compounds to establish quantitative data for their activities compared to allicin. In particular, it would be interesting to extend the investigation to test the antibacterial spectrum of the allicin derivatives and allicin against other Gram-negative bacteria, especially pathogenic species in genera such as *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Salmonella* and *Shigella*. These tests will need to be conducted in liquid media so that minimum inhibitory concentration (MIC) data (in $\mu\text{g}/\text{ml}$) can be obtained. In addition, the cyclodextrin-included compounds will be tested.

Chapter 4: Cyclodextrin Inclusion

4.1 Overview

The successful inclusion of the isomers of ajoene in cyclodextrins³⁰⁴ heightened the interest to establish whether the novel *S*-aryl alkylthiosulfonates would show similar affinity for these host molecules. The general interest in CD complex formation stems from including compounds with potential medicinal applications, which possess unfavourable properties such as insolubility, volatility and sensitivity. Like allicin most of the *S*-aryl alkylthiosulfonates are oils that possess very low aqueous solubility with a characteristic strong, sharp odour. Pleasingly, as mentioned, a number of *S*-aryl alkylthiosulfonates are highly active against *M. aurum*, *E. coli* and *S. aureus*. These collective properties make the *S*-aryl alkylthiosulfonates good candidates for CD inclusion. The practical advantages of CD inclusion complex formation for the *S*-aryl alkylthiosulfonates include the masking of the offensive odour and the conversion of these oils into solids to produce entities with improved handling characteristics. Other advantages of this strategy include elimination of bad tastes, improving drug delivery properties, stabilization and protection of reactive compounds, controlled release of compounds and improving bioavailability. Attempts to include allicin have been unsuccessful with evidence that the ajoene•CD complex is obtained instead of the allicin•CD inclusion complex.³⁰⁵ This further confirms the instability of allicin and its rearrangement to ajoene.

4.2 Experimental, Methods and Materials

4.2.1 Host Compounds

The host compounds, α -CD, β -CD and γ -CD, DIMEB and TRIMEB were obtained from Cyclolab [Budapest, Hungary] and were used as received.

4.2.2 Guest Compounds

The thiosulfonates were synthesized and purified as described in previous chapters and include *S*-*p*-methoxyphenyl propylthiosulfonate **127**, *S*-*p*-tolyl *t*-butylthiosulfonate **128**, *S*-*p*-tolyl propylthiosulfonate **129**, *S*-*p*-tolyl butylthiosulfonate **130**, and *S*-*p*-methoxyphenyl butylthiosulfonate **140**.

4.2.3 Complex Preparation

Several attempts were made to prepare inclusion complexes between CDs and various thiosulfonates synthesized. Frustratingly, only a few were successful. Inclusion complexes of compounds **128**, **129** and **130** with β - and γ -CD were prepared by the kneading and co-precipitation methods (general methods described in Chapter 1), a complex of compound **129** was prepared by the co-precipitation method with DIMEB, and complexes of compounds **127** and **140** with TRIMEB were prepared by the co-precipitation method.

4.2.4 Analysis

Hot Stage Microscopy (HSM)

HSM was used as a visual tool to study the morphology and physical changes of a crystal as it is heated over a given temperature range. Transformations undetected by the TGA and DSC methods such as colour and opacity changes in crystals are captured by HSM. Changes could then be related to physicochemical events such as dehydration, melting, solvent evaporation, guest release and complex decomposition. Crystals were immersed in silicone oil between two glass coverslips and placed on a Linkam THMS600 hot stage apparatus mounted on a Nikon SMZ-10 stereoscopic microscope fitted with a Linkam TP92 temperature controller. Images were captured using a real time Sony Digital Hyper HAD colour video camera and viewed and analysed using the Soft Imaging System program, analySIS.³⁰⁶

Thermogravimetric Analysis (TGA)

TGA measures the weight loss of a sample under a controlled inert atmosphere as it is subjected to a temperature programme. Measurements were made using a thermobalance which is an electronic microbalance coupled to a furnace. The technique is valuable for determination of complex thermal stability as well as determination of complex stoichiometry. All TGA runs were performed with 4-7 mg of sample placed in an alumina crucible on a Perkin Elmer PC7-Series instrument (PE-TGA7). The experimental traces were recorded at a scanning rate of 10 K min⁻¹ under N₂ gas at a flow rate of 30 cm³ min⁻¹.

Differential Scanning Calorimetry (DSC)

DSC measures the enthalpy changes as a result of the loss of guest and phase transformations which may occur upon heating the sample. A Perkin Elmer DSC7 thermal analysis system was used to record DSC traces. Sample masses ranging between 4-7 mg were placed in 50 μ l crimped, vented aluminum pans and traces recorded at a scanning rate of 10 K min⁻¹ under N₂ gas at a flow rate of 30 cm³ min⁻¹.

Elemental Analysis (Microanalysis)

Elemental analysis results served to indicate the purity of the sample and to confirm the stoichiometry of the inclusion complex (CD:drug) by measuring the carbon, hydrogen and sulfur content present in the sample. The sample is combusted at about 990 °C into CO₂, H₂O, NO_x and SO₂ using oxygen as an oxidizing agent. Masses of the oxidation products are then used to calculate the percentage of C, H and S in the sample. Elemental analysis experiments were performed on a Fison EA1108CHNS-O Elemental Analyser.

Powder X-ray Diffraction (PXRD)

PXRD experiments were performed to assess whether 'true' inclusion complexes had formed and to establish to which isostructural series they belonged. Kneaded samples were placed on X-ray insensitive Mylar® film (Chemplex – Palm City, U.S.A) and crystals were manually ground and packed into Markröhrchen non-diffracting glass capillaries (Hilgenberg, Germany), before being mounted. Samples were mounted on a Huber D-83253 Imaging Plate appliance fitted with a Huber MC 9300 power supply unit, a Philips X-ray generator and a Huber Guinier Camera 670. PXRD patterns were recorded using nickel-filtered CuK α_1 radiation ($\lambda = 1.5406 \text{ \AA}$) with generator settings kept constant at 20 mA and 40 kV. Exposure times of 10-60 min and multiscans of 10 times were used, and the 2θ range was 4-40 °. The experimental PXRD patterns were compared with reference PXRD patterns of hosts and known complexes (isostructural series).

Single Crystal X-ray Diffraction (SCXRD)***Crystal Structure Determination***

Single crystals with good extinction qualities were cut into near cubic shapes, coated with paratone N oil (Exxon Chemical Co. - TX, U.S.A) to keep the crystal rigid under low temperatures required for data-collection and prevent the loss of included water, and then mounted on a glass fibre which fitted on a goniometer head. Preliminary unit cell parameters and crystal systems were determined on a Nonius Kappa CCD Single Crystal X-ray Diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71069 \text{ \AA}$) produced by a Nonius FR590 generator operated at 53 kV and 23 mA. In addition, data were collected on a Bruker SMART Apex CCD diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$).

Data Collection

Crystal intensity data were collected on the same apparatus with the crystal cooled to 113(2) K, by a constant stream of N₂ gas to enhance diffraction quality and data collected by standard phi- and omega- scan techniques. All data were corrected for Lorentz-polarisation effects and unit cell refinement and data scaling and reduction were performed using DENZO-SMN and SCALEPACK.³⁰⁷ The space groups were assigned using the program Xprep.³⁰⁸ Xprep was also used to prepare input files (*.ins) required by SHELX programs for structure solution and refinement.³⁰⁹

Structure solution and refinement

The crystal structures were solved by the isomorphous replacement method, using published atomic coordinates of the rigid skeleton of the CD of an isostructural complex to generate an initial phasing model for refinement in the program SHELXL-97.³¹⁰ Refinement of the structures was carried out with SHELXL-97 operated through the interface X-seed.³¹¹ The process involved minimization of the function $\sum w(F_o^2 - F_c^2)^2$ using the full-matrix least-squares technique. The agreement between F_o and F_c which are the observed and calculated structure factors respectively, is expressed by the residual index R_1 or the weighted residual index wR_2 (both are low for a satisfactory model):

$$R_1 = (\sum |F_o| - |F_c|) / \sum |F_o| \quad wR_2 = [(\sum w(F_o^2 - F_c^2)^2) / (\sum w(F_o^2)^2)]^{1/2}$$

where w , the weighting scheme used for the refinement of the structure is:

$$w = 1 / [\sigma^2(F_o^2) + (aP)^2 + bP] \quad P = [\max(0, F_o^2) + 2 F_c^2] / 3$$

The terms a and b are chosen to yield a constant distribution of $w(F_o^2 - F_c^2)^2$ with $\sin\theta$ and $(F_o/F_{\max})^{1/2}$. The Goodness of Fit (S) is based on F^2 and is given by the expression:

$$S = [\sum w(F_o^2 - F_c^2)^2 / (n - p)]^{1/2}$$

where n is the number of reflections and p is the total number of parameters refined. For a well-behaved structure, S should be close to unity and the over-determination ratio should be of the order $n / p \approx 10$.

4.3 Results and Discussion

Of the ten thiosulfinate candidates presented for this study, only five formed inclusion complexes with various CD molecules. For simplicity the compounds are discussed in two sections, (i) Powder X-ray Diffraction Studies and (ii) Single Crystal X-ray Diffraction Studies.

4.3.1 Powder X-ray Diffraction Studies

Complex Preparation

The reactions of β -CD and γ -CD with the compounds shown in Table 4-1 afforded six CD complexes. However, a suitable crystal was obtained only for the β CD•*S-p*-tolyl *t*-butylthiosulfinate complex, which will be discussed later. Table 4-1 illustrates the molar quantities of the guest and CDs used in the preparation of each complex.

Guest (mg, mmol)	β -CD (mg, mmol)	γ -CD (mg, mmol)
<i>S-p</i> -tolyl propylthiosulfinate (10.0, 0.047)	53.0, 0.047 (C1)	61.0, 0.047 (C4)
<i>S-p</i> -tolyl butylthiosulfinate (10.0, 0.043)	49.0, 0.043 (C2)	57.0, 0.043 (C5)
<i>S-p</i> -tolyl <i>t</i> -butylthiosulfinate (10.0, 0.043)	49.0, 0.043 (C3)	57.0, 0.043 (C6)

Table 4-1 Molar quantities for preparation of complexes.

Kneading: Preparation of inclusion complexes of β - and γ -CD with *S-p*-tolyl *t*-butylthiosulfinate **128** involved kneading together equimolar amounts of the guest and CD. The mixture was kneaded for 1 hour and the paste kept moist by continually adding drops of distilled water.

Co-precipitation: β -CD was dissolved in 4 cm³ distilled water while γ -CD was dissolved in 2 cm³ distilled water at 65 °C. Once all the CD had dissolved, an equimolar amount of the thiosulfinate was added to the solution followed by vigorous stirring for 4 hours. The solution was filtered (0.45 μ m) and left at room temperature to induce crystallisation.

(a) β -CD Inclusion Complexes

The complexes were identified by comparing their PXRD patterns with those of known complexes. Comparisons of the PXRD traces for the expected complexes **C1**, **C2** and **C3** with that of the crystalline host and the isostructural reference patterns for β -CD complexes were made. Observations revealed that the products were not the host compound (β -CD) as their patterns did not match. However a good match for PXRD traces of **C1** and **C3** with reference pattern **B8** (Figure 4-1) and for the PXRD trace of **C2** with reference pattern **B6** was noted,

Figure 4-2. Comparing the PXRD patterns for **C1**, **C2** and **C3** formed by the kneading method with the corresponding pattern obtained for the complex prepared by the co-precipitation method gave a reasonable match, in terms of the number of peaks, the peak intensities and peak angle positions. This indicates that the same CD-inclusion complex is obtained *via* the two different preparative methods. In addition, a good agreement of the PXRD pattern of **C1** with that of **C3** was observed, Figure 4-1. This reveals the isostructural relation between these two complexes. Isostructurality as discussed in Chapter 1 refers to two or more crystalline phases sharing the same three-dimensional packing arrays, with similar unit cell dimensions and internal molecular arrangements.³¹²

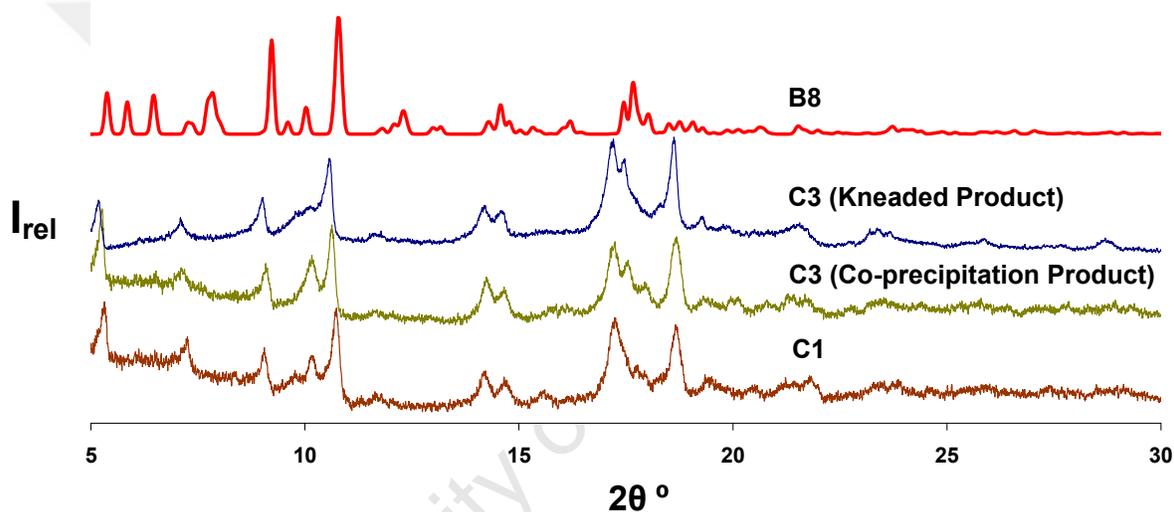


Figure 4-1 Experimental PXRD traces for **C3** and **C1** compared with reference pattern **B8**.

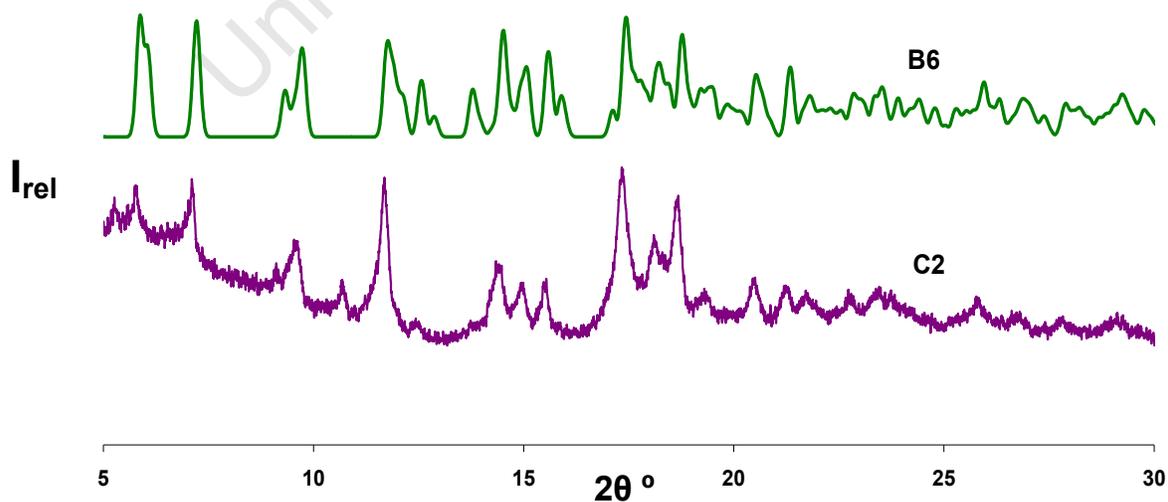


Figure 4-2 Experimental PXRD traces for **C2** compared with reference pattern **B6**.

As observed from the PXRD traces, the experimental and reference peak positions and intensities are not identical. This is attributed to differences in crystal water content, and the type, size and orientation of the guest molecules as well as the temperature at which the respective data were collected. It should also be mentioned that the reference trace is an artifact, constructed by averaging the PXRD traces of available isostructural complexes.

C1 and **C3** crystallize in the orthorhombic space group $C222_1$ with a chessboard (CB) packing arrangement while **C2** crystallizes in the monoclinic space group $C2$. The packing arrangements for complexes belonging to the space group $C2$ are associated with the channel (CH) packing arrangement in which the host molecules are stacked in a dimeric head-to-head configuration forming double volume cavities (which easily accommodate the molecules) and infinite channels. The space group and unit cell dimensions as illustrated in Table 4-2 are inferred from matching the PXRD patterns with reference traces for which these parameters are known. It may be noted that the structural information for complex **C3** deduced from PXRD was fully vindicated in the subsequent single crystal X-ray analysis of this species (Section 4.3.2). This illustrates the utility of the method.

Complex	Space Group	Isostruc. Class	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)
C1, C3	$C222_1$	B8	19.2	23.9	32.4	90.0	90.0	90.0
C2	$C2$	B6	19.3	24.1	16.0	90.0	109.0	90.0

Table 4-2 Approximate unit cell dimensions for β -CD complexes **C1**, **C2** and **C3**.

(b) γ -CD Inclusion Complexes

All known γ -CD complexes crystallize in channel packing mode CH in the tetragonal space group $P42_12$ and generally contain disordered guest molecules in the channel.³¹³ The PXRD patterns of the complexes **C4**, **C5** and **C6** were compared to the reference pattern for γ -CD complexes, which contains few peaks owing to the high symmetry of the lattice,³¹³ and as expected, closely matched the reference pattern **G1**, Figure 4-3.

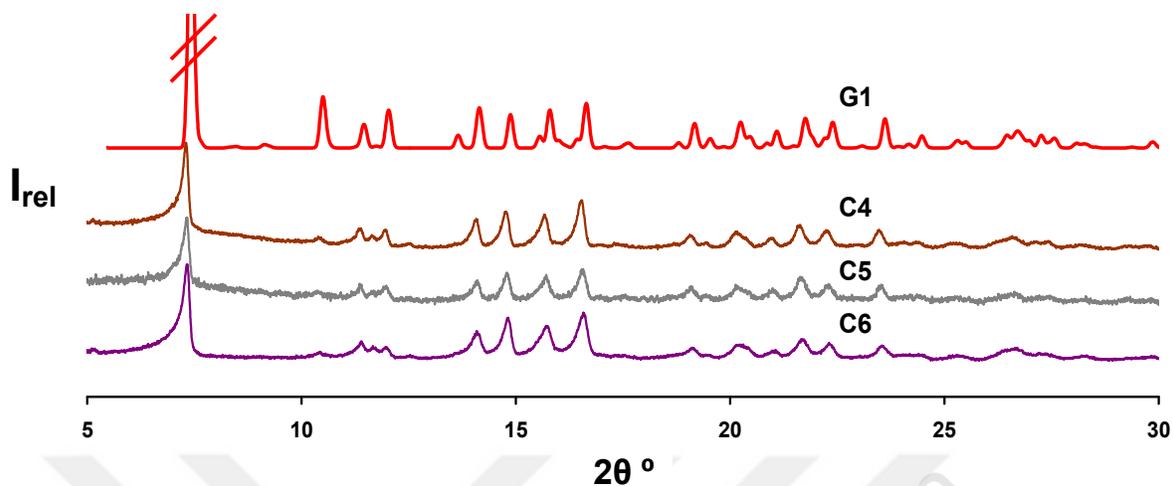


Figure 4-3 Experimental traces for complexes **C4**, **C5** and **C6** with the reference trace for γ -CD complexes **G1** (// = truncated peak).

The guests are anticipated to be located in infinite channels due to the arrangement of the γ -CD molecules. Single crystal analysis of γ -CD complexes indicates a trimeric arrangement with three CD molecules in one asymmetric unit. The CD molecules are arranged in an alternating sequence, namely in the head-to-head, head-to-tail and tail-to-tail mode, thus forming a structure built up of trimers of γ -CD. The approximate unit cell dimensions are given in Table 4-3.

Complex	Space Group	Isostruc. Class	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)
C4, C5, C6	P4 ₂ 1 ₂	G1	23.8	23.8	23.2	90.0	90.0	90.0

Table 4-3 Approximate unit cell dimensions for γ -CD complexes **C4**, **C5** and **C6**.

(c) DIMEB Inclusion Complexes

Only one inclusion complex (DIMEB•*S*-propyl *p*-tolylthiosulfinate **C7**) was formed by the reaction of DIMEB with the thiosulfates **128**, **129** and **130**.

Complex Preparation

A saturated solution of DIMEB (64 mg, 0.046 mmol) and *S*-*p*-tolyl propylthiosulfinate **129** (10 mg, 0.046 mmol) in 1 cm³ of distilled de-ionised water was stirred at 4 °C for 2 hours. The solution was filtered and the vial placed in an oven at 50 °C. Single crystals (colourless needles) were observed after 6 days of slow evaporation.

HSM

Crystals were dried, submerged in silicone oil and heated at a scanning rate of 10 K min⁻¹. The clear and translucent crystals became opaque at approximately 165 °C indicating loss of the guest molecule. This was observed at ~100 °C and at ~270 °C the crystals decomposed. No clear melting point was observed and by 320 °C the complex had turned completely dark brown indicating decomposition.

TGA

The TGA trace for **C7** showed a small mass loss (1.2 %) between 30 and 49 °C corresponding to the loss of one water molecule per host molecule. This however was not detected in the HSM analysis, but due to the minute amount of water this is expected. A thermal event in the temperature range 112-210 °C corresponds to the loss of the guest molecule, which is in keeping with HSM observations.

Elemental Analysis

C, H, S analysis confirmed a 1:1:1 DIMEB:S-*p*-tolyl propylthiosulfinate:H₂O complex ratio, with a molecular formula C₅₆H₉₈O₃₅•C₁₀H₁₄OS₂•H₂O (Experimental: %C 50.78, %H 7.28, %S 3.99 and Calculated: %C 50.69, %H 7.35, %S 4.10).

Single Crystal X-ray Diffraction

Although single crystals were obtained, no match was observed from comparison of the preliminary unit cell dimensions of the crystal with the known isostructural DIMEB series and thus isomorphous replacement could not be applied to solve the crystal structure. Frustratingly, direct methods were also unsuccessful. However, preliminary unit cell dimensions obtained from the Nonius Kappa CCD diffractometer, indicated a primitive orthorhombic lattice. The results are illustrated in Table 4-4.

Parameter	Value	Parameter	Value
a	19.55 Å	α	90.2°
b	27.53 Å	β	91.8°
c	29.81 Å	γ	91.6°

Table 4-4 Preliminary unit cell parameters for complex **C7** ($V = 16035 \text{ \AA}^3$).

4.3.2 Single Crystal X-ray Diffraction Studies

High-quality crystals were obtained for three compounds, namely β -CD•S-*t*-butyl *p*-tolylthiosulfinate (**C3**), TRIMEB•S-*p*-methoxyphenyl propylthiosulfinate (**C8**) and TRIMEB•S-*p*-methoxyphenyl butylthiosulfinate (**C9**) and their structures were thus solved by SCXRD which will now be discussed. Crystal data and refinement parameters for **C3**, **C8** and **C9** are presented in Table 4-5.

Complex Formula	$C_{42}H_{70}O_{35} \cdot C_{11}H_{16}OS_2 \cdot 12.5H_2O$ (C3)	$C_{63}H_{112}O_{35} \cdot C_{10}H_{14}O_2S_2$ (C8)	$C_{63}H_{112}O_{35} \cdot C_{11}H_{16}O_2S_2$ (C9)
Formula weight	1588.54	1659.86	1673.88
Temperature / K	113(2)	113(2)	110(2)
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic
Space group	C222 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁
a / Å	19.1873(1)	14.7750(2)	14.703(1)
b / Å	23.9921(2)	21.3329(3)	21.363(1)
c / Å	32.4473(3)	27.872(1)	27.817(2)
Volume / Å ³	14936.9(2)	8785.2(4)	8737.3(9)
Z	8	4	4
Density (calc.) / g cm ⁻³	1.413	1.255	1.272
Radiation, wavelength	MoK α , 0.71069 Å	MoK α , 0.71069 Å	MoK α , 0.71073 Å
Absorption coefficient / mm ⁻¹	0.178	0.145	0.146
F(000)	6792	3568	3600
Crystal size	0.32 x 0.26 x 0.22	0.30 x 0.30 x 0.30	0.40 x 0.35 x 0.25
Theta range / °	1.0-25.35	2.8 – 25.7	2.2 and 26.6
Index ranges	-23 ≤ h ≤ 21, -28 ≤ k ≤ 28, -39 ≤ l ≤ 39	-17 ≤ h ≤ 18, -25 ≤ k ≤ 26, -33 ≤ l ≤ 33	-19 ≤ h ≤ 19, -28 ≤ k ≤ 28, -37 ≤ l ≤ 35
Reflections collected	71011	16562	102381
Observed reflections [I>2σ(I)]	11621	11196	18979
Data/restraints/parameters	13662/24/874	16562/0/939	21027/0/889
Goodness-of-fit on F ²	1.043	1.042	1.101
Final R indices [I>2σ(I)]	R ₁ = 0.0903, wR ₂ = 0.2531	R ₁ = 0.0877, wR ₂ = 0.2674	R ₁ = 0.0917, wR ₂ = 0.2460
R indices (all data)	R ₁ = 0.1021, wR ₂ = 0.2670	R ₁ = 0.1244, wR ₂ = 0.2674	R ₁ = 0.0987, wR ₂ = 0.2536
Largest diff. peak and hole e Å ⁻³	-0.83, 1.19	-0.96, 1.58	-1.21, 2.78

Table 4-5 Crystal Data, Data Collection Parameters and Refinement Details for complexes **C3**, **C8** and **C9**.

(a) Analysis of β CD•S-*p*-tolyl *t*-butylthiosulfinate complex (C3)**HSM**

The clear crystals were removed from the mother liquor, dried on filter paper, immersed in silicone oil and heated at a rate of 10 K min⁻¹. At ca. 65 °C **C3** showed signs of dehydration through bubble formation, Figure 4-4. The crystals decompose at ca. 210 °C which was indicated by a change in the crystal colour from opaque to brown, with no clear melting point observed.

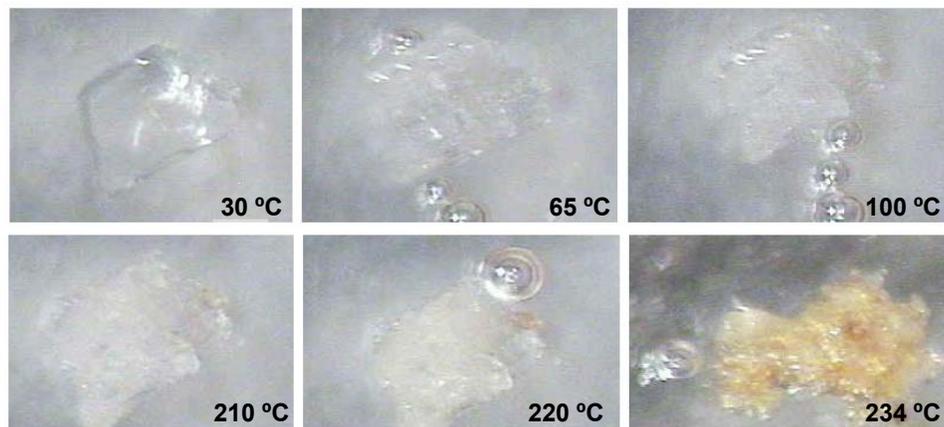


Figure 4-4 HSM images for **C3** at various temperatures.

TGA and DSC

For TGA and DSC analysis the sample was crushed and the excess water removed by drying the sample on filter paper. Figure 4-5 illustrates the combined TGA and DSC traces for the complex **C3**.

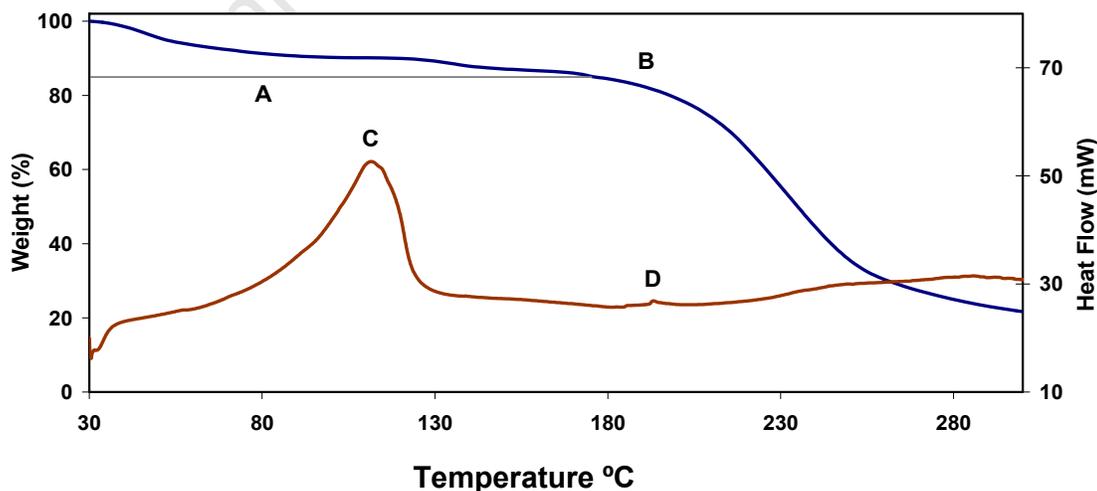


Figure 4-5 Combined TGA and DSC traces for **C3**.

The TGA trace shows a mass loss of 14.3% in the temperature range 30-168 °C (represented by event **A**) which is equivalent to 12.5 water molecules per complex unit. It is unusual for water molecules to be retained up to high temperatures and for them to be lost in a two-step process as observed in the TGA trace, with the second mass loss starting at ~120 °C. However, it can be attributed to strong interactions the water molecules may experience within the CD complex packing arrangement. Consistent with HSM analysis, the complex decomposes at an onset temperature of 185 °C represented by **B**. In agreement with the HSM and TGA experiments, a broad endotherm (**C**) was observed at 112 °C in the DSC, due to dehydration followed by decomposition at 185 °C (**D**).

Crystal Structure Analysis

Space Group Determination and Structure Solution and Refinement

Preliminary unit cell parameters and the space group were checked on the Nonius Kappa CCD diffractometer. Examination of the reciprocal lattice indicated the orthorhombic system while inspection of the reflection data revealed that the space group of the complex is $C222_1$, as predicted from the PXRD trace (Section 4.3.1). The structure of the complex was solved by isomorphous replacement using published co-ordinates of the host structure (excluding the primary hydroxyl oxygen atoms) of the isostructural *p-t*-butylbenzyl alcohol- β -cyclodextrin complex.³¹⁴ After refinement and optimization of the host structure it was observed that the guest molecule is disordered over three positions. The asymmetric unit of **C3** consists of one molecule of β -CD, a molecule of *S-p*-tolyl *t*-butylthiosulfinate and 12.5 molecules of water. It was also found that the guest crystallizes as a 2:1 mixture of stereoisomers (S:R). Crystal data and refinement parameters for **C3** are presented in Table 4-5. Refinement of the crystal structure was not trivial as it involved the unusual situation of treating a three-fold disordered guest molecule. Independent refinement of the separate components led to site-occupancy factors of 0.28, 0.32 and 0.33. In the final refinement, these were fixed at 0.333 each to ensure consistency between the model and the analytical data which had indicated one molecule of guest per host molecule. Examination of positional and thermal parameters indicated satisfactory convergence.

Structure and Conformation of the Guest Molecule

The refined electron density image **A-B-C**, including non-hydrogen guest atoms and assigned atom connectivities as well as the isolated guest models, labeled **A**, **B** and **C** are depicted in Figure 4-6.

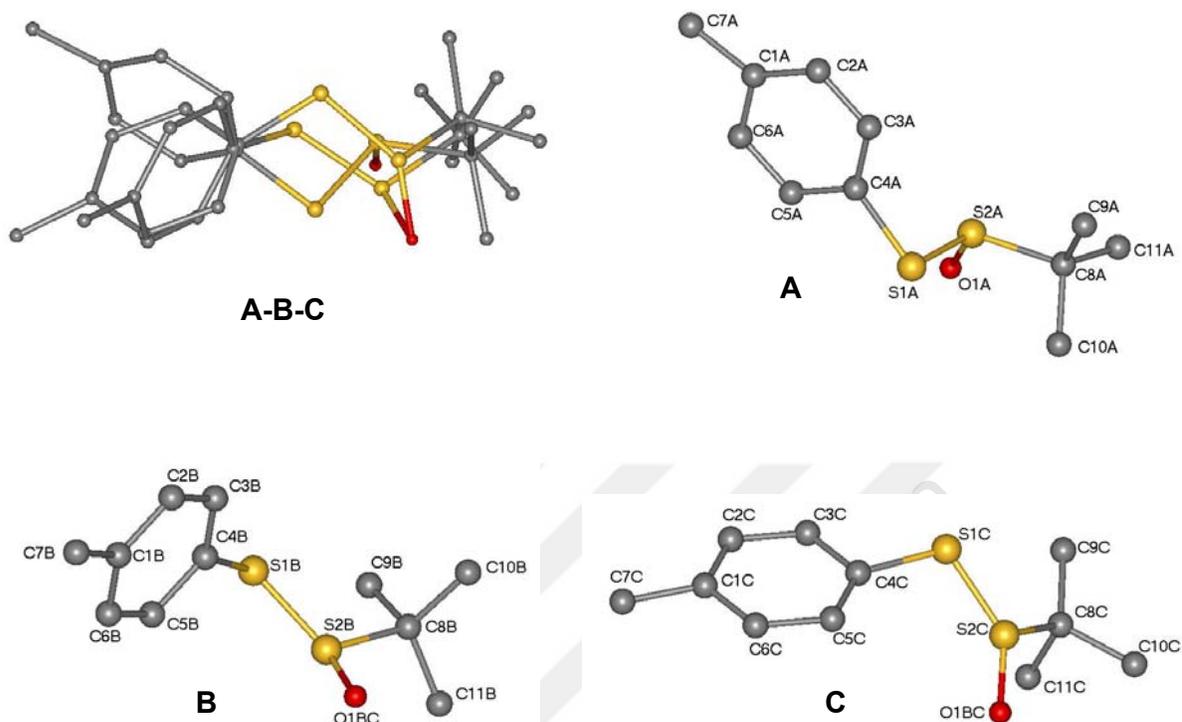


Figure 4-6 Disordered guest peaks in **C3** (**A-B-C**) and the deconvoluted models **A**, **B** and **C**.
(**A** = R-enantiomer, **B** = R-enantiomer, **C** = S-enantiomer).

Comparing the models **A**, **B** and **C** it is observed that the only common atom is O1BC, shared by components **B** and **C**. Otherwise the three models are independent and do not have common atoms. The tertiary butyl group which is adjacent to the thiosulfinate moiety is observed to be staggered with respect to the sulfoxide oxygen with torsion angles C9-C8-S2-O1 of $177(2)^\circ$ for **A**, $176(1)^\circ$ for **B** and $171(1)^\circ$ for **C**. The orientation with respect to the thiosulfinate group, defined by the torsion angle C3-C4-S1-S2 was found to be -89° , 106° and -139° for **A**, **B** and **C** respectively (e.s.d. range 0.3-1.0°). Torsion angles δ_1 : C4-S1-S2-C8 and δ_2 : C4-S1-S2-O1 are used to describe the rotation around the S1-S2 bond relative to the atoms C8 and O1 and define the conformation of the thiosulfinate group (δ_1 : $137.8(9)^\circ$, $-146.9(8)^\circ$ and $143.5(7)^\circ$ and δ_2 : $-106.6(8)^\circ$, $-7(1)^\circ$ and $-5(1)^\circ$ for models **A**, **B** and **C** respectively). Configurations at the stereogenic sulfoxide sulfur atoms are R for S2**A**, R for S2**B** and S for S2**C**. The enantiomeric ratio (R:S) present in the complex is 2:1, which suggests that β -CD displays a degree of stereo-recognition under the preparative conditions reported.

Mode of Guest Inclusion

The guest is positioned in the centre of the CD cavity and the three resolved stereoisomers are found to adopt a similar conformation, Figure 4-7.

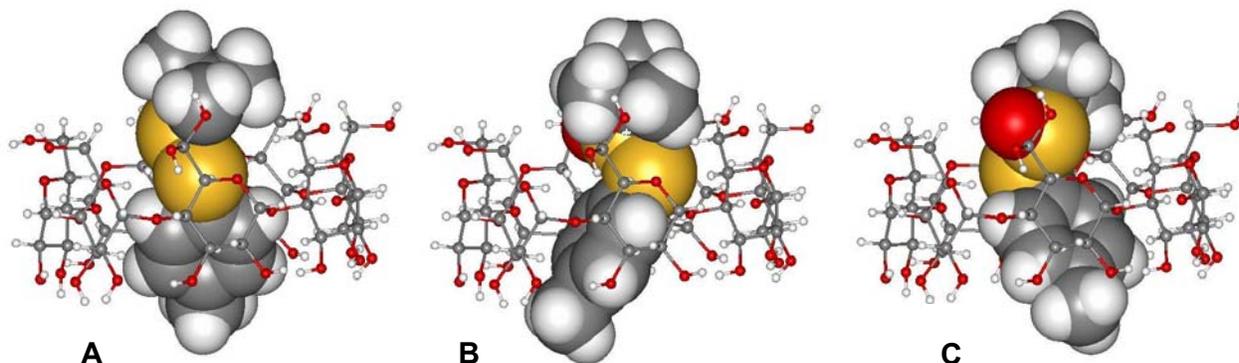


Figure 4-7 Stereoview showing the modes of inclusion of **A**, **B** and **C**

It is observed that the sulfoxide oxygen and the tertiary butyl group protrude through the primary hydroxyl side while the phenyl ring is situated inside the cavity near the secondary hydroxyl side and the thiosulfinate moiety is centrally located within the CD cavity. No hydrogen bonding between host or water molecules with the guest is observed and it is thus concluded that the guest is exclusively stabilized by hydrophobic interactions.

Crystal Packing Arrangement

C3 is present in the crystal as a dimer due to intermolecular hydrogen bonding between secondary hydroxyl groups of the host molecules and is found to crystallize in a chessboard (CB) packing arrangement. These arrangements are characterized by the presence of screw-related dimeric units aligned in 'interrupted' columns. Figure 4-8 shows the crystal packing along [001]. Isolated red spheres are water oxygen atoms. All three components of the disordered guest molecule included in the CD cavity are represented in the figure.

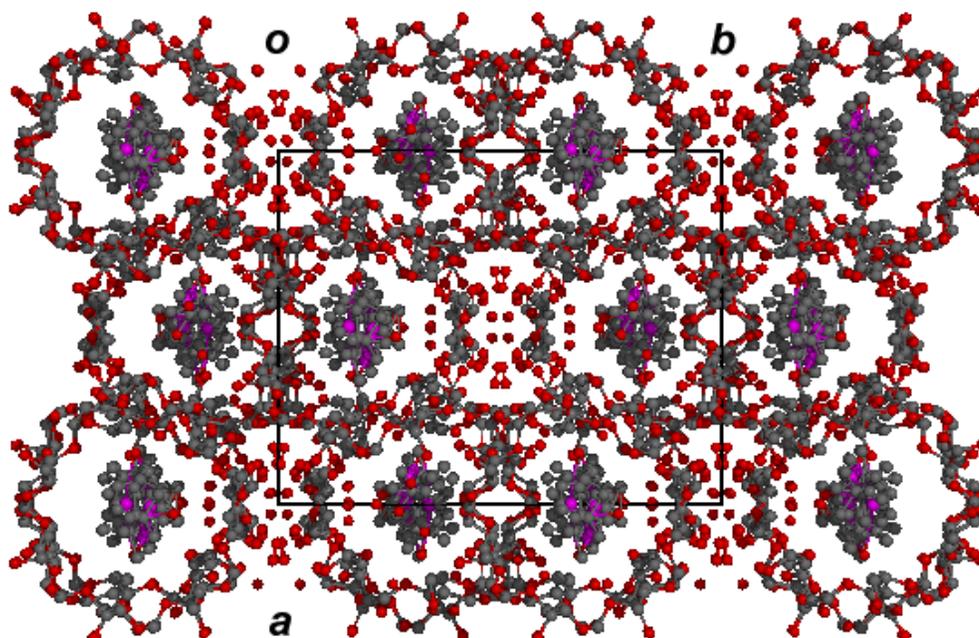


Figure 4-8 Crystal packing arrangement in C3 viewed parallel to [001].

(b) Analysis of TRIMEB•S-*p*-methoxyphenyl propylthiosulfinate complex (C8)

Complex Preparation

The complex was prepared by slowly adding the thiosulfinate (10 mg, 0.043 mmol) to a cold saturated aqueous solution (0.5 cm³) of an equimolar amount of TRIMEB (62 mg, 0.043 mmol) and the mixture was stirred at 0 °C. After 24 hours the solution was filtered (0.45 μm) and the vial incubated at 50 °C for 72 hours after which colourless prismatic crystals of the expected inclusion compound were observed.

HSM, TGA and DSC

HSM was used as a preliminary tool to establish whether complexation had taken place. The lack of a clear melt at the melting temperature of TRIMEB (156 °C) indicated that the crystals were not the host or guest. HSM revealed dehydration at approximately 100 °C by formation of bubbles. The crystals melted in the range 132-137 °C. The TGA trace showed a negligible mass loss in the temperature range 25-160 °C followed by a mass loss of ~13.2 % between 160 and 325 °C. The former corresponds to water loss while the latter corresponds to release of the guest from the inclusion complex. Above 325 °C, the host compound decomposed. In the DSC trace, onset and peak temperatures corresponding to the melting endotherm were recorded as 132.6 and 136.9 °C respectively.

Elemental Analysis

C, H, S analysis confirmed a 1:1:1 TRIMEB:*S-p*-methoxyphenyl propylthiosulfinate complex ratio, with a molecular formula $C_{63}H_{112}O_{35} \cdot C_{10}H_{14}O_2S_2 \cdot H_2O$ (Experimental: %C 52.28, %H 7.37, %S 3.90 and Calculated: %C 52.82, %H 7.65, %S 3.86).

Crystal Structure Analysis

Preliminary unit cell parameters and the space group for **C8** were determined using the Nonius Kappa CCD diffractometer. The complex was found to belong to the space group $P2_12_12_1$ (orthorhombic crystal system). The structure of the complex was solved by isomorphous replacement using published co-ordinates of the host structure of TRIMEB•(S)-Naproxen.³¹⁵ The asymmetric unit consists of one molecule of TRIMEB and one molecule of *p*-methoxyphenyl 1-propyl disulfide. Crystal data and refinement parameters are presented in Table 4-5.

A perspective view of the complex structure is shown in Figure 4-9, with the host in ball-and-stick mode and the guest in space-filling representation. The TRIMEB molecule displays the usual elliptical distortion,³⁰⁴ stabilised by several C-H...O hydrogen bonds, with the methoxy groups on the primary rim acting as a lid, presenting the guest with a cup-shaped hydrophobic surface for inclusion. The guest molecule adopts a hairpin conformation with the sulfoxide moiety located near the cavity 'roof' and the methoxyphenyl and propyl residues directed towards the secondary rim of the host molecule.

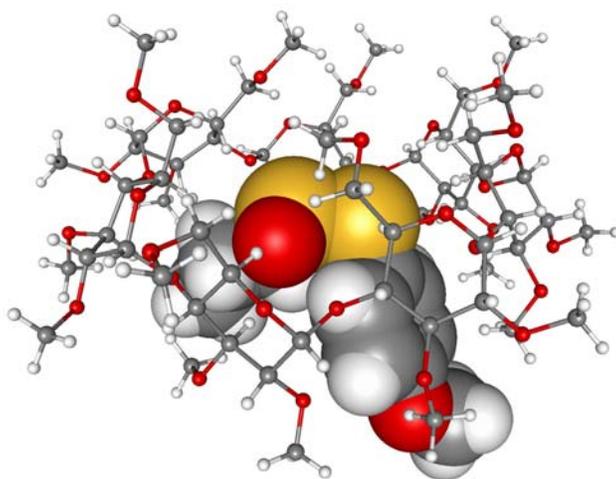


Figure 4-9 Complex **C8** illustrated by the ball-and-stick (host) and space-filling (guest) modes.

Interestingly, in complexes TRIMEB•(Z)-Ajoene and TRIMEB•(E)-Ajoene, a disordered arrangement, comprising both the *R*- and *S*-enantiomers of the guest, was identified within the host cavity.³⁰⁴ In contrast, for the crystal **C8** selected, only the *S*-enantiomer of

S-p-methoxyphenyl propylthiosulfinate was evident. The packing arrangement of **C8** is consistent with that found for complexes TRIMEB•(Z)-Ajoene³⁰⁴ and TRIMEB•(S)-Naproxen,³¹⁴ in which the complex molecules pack in a screw channel mode in a head-to-tail fashion.

(c) Analysis of TRIMEB•*S-p*-methoxyphenyl butylthiosulfinate complex (**C9**)

Complex Preparation

TRIMEB (59 mg, 0.041 mmol) was dissolved in 0.5 cm³ of distilled water at ~4 °C. Once all the CD had dissolved, an equimolar amount of the thiosulfinate (10 mg, 0.041 mmol) was added to the solution, which immediately formed precipitates. The mixture was cooled to 0 °C at which temperature the precipitate dissolved and the mixture was vigorously stirred. After 6 hours the solution was filtered (0.45 µm) and the vial placed in an oven at 50 °C for 12 hours to induce crystallization. The reaction yielded complex **C9** as beautiful colourless prismatic crystals.

HSM and DSC

Figure 4-10 presents crystal images of **C9** as they are heated. The crystals were submerged in silicone oil and heated at a scanning rate of 10 K min⁻¹. Initially (25 °C) the crystals were clear and transparent. The crystals stayed intact until they melted over the temperature range 131-134 °C. This was the only sign of activity from onset to 300 °C. DSC analysis supports the HSM results, with a melt onset of 130 °C. From the thermal analysis it is evident that the complex was unsolvated since only a melt endotherm in the DSC is observed while HSM observations revealed that the crystallinity of the sample is retained until the melting point with no evidence of dehydration.



Figure 4-10 HSM images for **C9** at various temperatures.

Elemental Analysis

C, H, S analysis confirmed a 1:1 TRIMEB:*S-p*-methoxyphenyl butylthiosulfinate complex ratio, with a molecular formula $C_{63}H_{112}O_{35} \cdot C_{11}H_{16}O_2S_2$ (Experimental: %C 53.18, %H 7.84, %S 3.75 and Calculated: %C 53.10, %H 7.71, %S 3.83).

Crystal Structure Analysis

Data were collected from a crystal of **C9** on a Bruker SMART Apex CCD diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$).

The structure of the complex was solved by isomorphous replacement using published co-ordinates of the host structure of TRIMEB·*S-p*-methoxyphenyl propylthiosulfinate (complex **C8**).³¹⁶ The asymmetric unit consists of one molecule of TRIMEB and one molecule of 1-butyl *p*-methoxyphenyl disulfide. Crystal data and refinement parameters are presented in Table 4-5. Figure 4-11 is a perspective view of the complex analogous to that obtained for **C8**, with the host represented in ball-and-stick mode and the guest in space-filling mode.

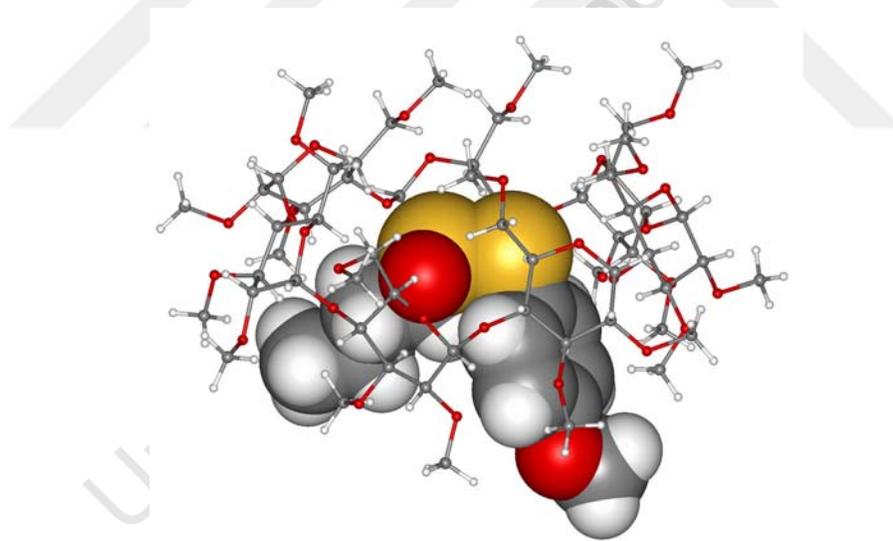


Figure 4-11 Complex **C9** illustrated by ball-and-stick (host) and space-filling (guest) modes.

In view of the fact that **C9** is isostructural to **C8** as determined by their crystal data, Figure 4-12 further demonstrates the similarity in the mode of inclusion of the guest. Evidently, the only difference is the extra methyl group protruding from the secondary rim of the host molecule, causing a bulkier appearance in the bottom left section of the diagram. An additional similarity between **C8** and **C9** is the observation that the crystal selected proved to contain the guest molecule as the *S*-enantiomer exclusively.

An overlay diagram (Figure 4-12) of the TRIMEB•*S-p*-methoxyphenyl 1-propylthiosulfinate complex **C8** (illustrated by the green host and guest) and the TRIMEB•*S-p*-methoxyphenyl butylthiosulfinate complex **C9** (illustrated by the red host and guest) highlights the similarity between these two inclusion complexes; for emphasis and clarity the extra carbon of the aliphatic chain of the guest molecule in **C9** is represented by a black sphere. The similarity found in the host conformations was expected since the TRIMEB skeleton of **C8** was used in solving the structure of **C9**. Interestingly, the guest conformations are also very similar but this is not surprising in view of the fact that the guests are similar in structure. The diagram undoubtedly illustrates that the two inclusion complexes adopt the same conformation with common atoms sharing essentially the same co-ordinates.

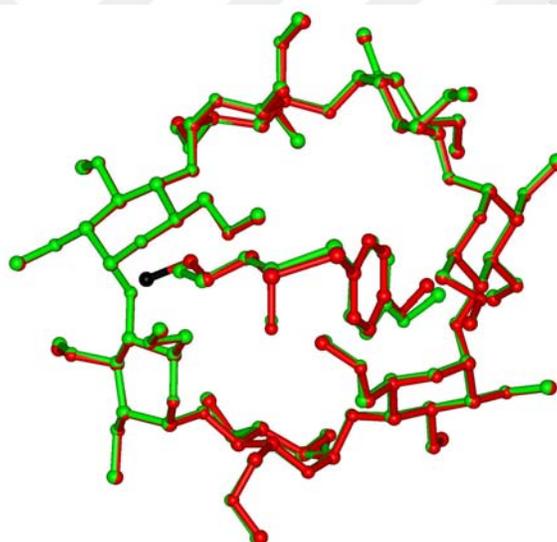


Figure 4-12 An overlay of the complexes **C8** (green) and **C9** (red).

4.4 Conclusion

Producing inclusion complexes with CD molecules has many advantages. For this study thiosulfinate oils, which are not easy to handle, have a strong odour and are not water soluble, were included into CD molecules to produce solid compounds without the strong odour characteristic of the thiosulfates. An interesting aspect of the study was the finding that only small thiosulfates are accommodated by CD molecules while thiosulfates with aliphatic chains longer than four carbon atoms are not housed by CD molecules. The reason why some molecules are able to form inclusion complexes with CD molecules and why others do not is unknown. However, in total nine CD inclusion complexes were obtained. Six of these complexes [three β -CD complexes (β -CD•*S-p*-tolyl propylthiosulfinate, β -CD•*S-p*-tolyl butylthiosulfinate and β -CD•*S-p*-tolyl *t*-butylthiosulfinate) and three γ -CD complexes (γ -CD•*S-p*-tolyl propylthiosulfinate, γ -CD•*S-p*-tolyl butylthiosulfinate and γ -CD•*S-p*-tolyl *t*-butylthiosulfinate)] were identified by the PXRD method using the published isostructural series. A DIMEB complex (DIMEB•*S-p*-tolyl propylthiosulfinate) was identified by single crystal X-ray diffraction but its structure could not be solved by isomorphous replacement or direct methods and thus only its unit cell parameters and space group are reported. The structures of two TRIMEB complexes (TRIMEB•*S-p*-methoxyphenyl butylthiosulfinate and TRIMEB•*S-p*-methoxyphenyl propylthiosulfinate) and one β -CD complex (β -CD•*S-p*-tolyl *t*-butylthiosulfinate) were fully determined by single crystal X-ray diffraction.

In conclusion, the main objective of this study has been achieved by demonstrating that CDs have an affinity for specific thiosulfates. Ongoing studies could include complex dissolution rate tests and attempts to form medicinally more acceptable inclusion complexes with the parent CDs instead of with TRIMEB, which has haemolytic properties.

Chapter 5: Summary and Conclusion

In conclusion, a new one-pot, environmentally friendly and cost-effective methodology for the synthesis of unsymmetrical disulfides R^1SSR^2 has been developed using 1-chlorobenzotriazole as the oxidant involving a range of different R groups. By modifying the procedure for aliphatic thiols using thiourea to trap out the intermediate from the first step to an isothiuronium salt, the methodology can access the difficult aliphatic-aliphatic class of unsymmetrical disulfides in a pure form. Compared to other methods, the present invention is highlighted by intramolecular trapping of the reactive sulfenyl intermediate. Temperature control achieves chemodifferentiation between the two thiols in order to avoid homodimer 1 (R^1SSR^1) formation. Further studies need to be conducted to quantify the method relative to other popular one-pot methods regarding the purity of final product and the extent of disulfide exchange.

In an attempt to develop allicin mimics that would limit Block-type fragmentation pathways while retaining the biological activity of the thiosulfinate pharmacophore, a range of substituted aralkyl thiosulfonates have been prepared. However, in the context of the overall objective, an important finding has been that thiosulfonates of this class bearing electron-withdrawing aromatic rings are relatively unstable, while those with activated aromatic rings are quite stable at room temperature. Other stabilising parameters such as length of the alkyl chain and the presence of fluorine atoms in the alkyl chain have also been shown to add a modicum of stabilization. However, the completely stable mimic still remains elusive. The introduction of hydrogen bonding to the sulfinate oxygen remains a future high-priority objective, probably achievable through a protection/deprotection protocol. Introduction of a π -excessive heteroaromatic such as furan or pyrrole would also be interesting to study. The [10-S-4(S4)] sulfurane by-product of oxidation of 2-pyridyl 1-propyl disulfide is worthy of further investigation; indeed other members of the class, e.g. butyl and hexyl, although not mentioned in this thesis, also give interesting oxidation by-products.

Finally, preliminary studies on the antibacterial activity of the most stable thiosulfonates prepared as the *S-p*-methoxy alkylthiosulfonates have given results supporting the notion of these compounds indeed being allicin mimics. Further, more detailed and quantitative studies need to be conducted in order to probe the full potential of these compounds. In this regard, cyclodextrin inclusion, where possible, may add a further positive dimension to the quest for a novel class of antibacterial agent.

Experimental

6.1 General Procedure

All solvents were freshly distilled. Dichloromethane was distilled over phosphorus pentoxide under nitrogen. Tetrahydrofuran was distilled under nitrogen and dried over sodium wire with benzophenone. Other reagents were purified according to standard procedures.³¹⁷ All reagents were purchased from Aldrich or Merck. Low temperature reactions were carried out using liquid nitrogen in acetone (-78 °C) or a slurry of water and ice (0 °C).

Thin layer chromatography (TLC) was used to monitor reactions using aluminium-backed Merck silica-gel 60 F₂₅₄ plates. Compounds on TLC were observed by a combination of ultra-violet light, iodine vapour, or by spraying with a 2.5% solution of anisaldehyde in a mixture of sulfuric acid and ethanol (1:10 v/v) and then heating at 150 °C. Column chromatography was performed using silica-gel 60 mesh (Merck 7734). All chromatography was carried out using either petroleum ether (b.p. 40-60 °C) or ethyl acetate as eluents, or a combination of these .

Melting points were measured on a Reichert-Jung ThermoVar hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer on sodium chloride plates. Elemental analyses were performed using a Fisons EA 110 CHN elemental analyzer. High-resolution mass spectrometry was performed at The University of the Witwatersrand using a VG70-SEQ micromass spectrometer or at The School of Chemistry, University of Stellenbosch on an API Q-TOF Ultima machine. All spectra were recorded in Electron Ionisation mode, unless otherwise stated.

Nuclear Magnetic Resonance spectra were recorded on either a Varian Unity 400 (at 399.95 MHz for ¹H, 100.58 MHz for ¹³C and 376.29 MHz for ¹⁹F) or a Varian VXR-300 (at 300.08 MHz for ¹H and 75.5 MHz for ¹³C) spectrometer in deuterated chloroform unless otherwise stated. Chemical shifts (δ) were recorded using residual chloroform (δ 7.26 in ¹H NMR and δ 77.00 in ¹³C NMR) as internal standards. All chemical shifts are reported in ppm and *J* values are quoted in Hz.

6.2 Synthesis and purification of disulfides

The unsymmetrical disulfides 66 to 103 were synthesized using the following method:

To a stirred solution of 1-chlorobenzotriazole (0.61 g, 4.0 mmol) and benzotriazole (0.32 g, 2.7 mmol) in CH_2Cl_2 (30 ml) under N_2 at $-78\text{ }^\circ\text{C}$ was added dropwise a solution of thiol R^1SH (2.7 mmol) dissolved in CH_2Cl_2 (2 ml). The solution was allowed to stir for 2 hours with slow warming to $-20\text{ }^\circ\text{C}$. Thiol R^2SH (4.0 mmol) in CH_2Cl_2 (2 ml) was then added slowly at $-20\text{ }^\circ\text{C}$ and the solution stirred at $0\text{ }^\circ\text{C}$ for 30 min. The reaction was then quenched with a solution of $\text{Na}_2\text{S}_2\text{O}_3$ (0.50 g in 10 ml water) together with saturated aqueous NaHCO_3 (20 ml), with rapid stirring at $0\text{ }^\circ\text{C}$ for 20 min before being extracted with CH_2Cl_2 (3×100 ml). The combined organic extracts were dried over anhydrous MgSO_4 , filtered, and evaporated under reduced pressure. The crude residue was purified by silica-gel column chromatography using petroleum ether/ethyl acetate mixtures to afford the unsymmetrical disulfides.

Formation of thiol 2 (R^2SH) for Compounds 93 and 96:

To a stirred solution of thiourea (0.38 g, 5.0 mmol) in THF (30 ml) under N_2 was slowly added 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodohexane (0.98 ml, 5.1 mmol) dissolved in THF (2 ml). The solution was allowed to reflux for 5 hours after which it was cooled to $0\text{ }^\circ\text{C}$ for 30 min. The solvent was concentrated under reduced pressure and a salt obtained. KOH (1.12 g, 20 mmol) was dissolved in cold methanol (5 ml), CH_2Cl_2 (15 ml) was added to the mixture followed by the salt. The reaction mixture was vigorously stirred for 1 hour and the resultant mixture added as R^2SH to the above described reaction.

The unsymmetrical disulfides 106 to 112 were synthesized using the following method:

To a stirred solution of 1-chlorobenzotriazole (0.61 g, 4.0 mmol) and benzotriazole (0.24 g, 2.0 mmol) in CH_2Cl_2 (20 ml) under N_2 at $-78\text{ }^\circ\text{C}$ was added dropwise a solution of R^1SH (2.0 mmol) in CH_2Cl_2 (2 ml). After 10 min, a solution of thiourea (0.46 g, 6.0 mmol) dissolved in dry THF (5 ml) was added and the solution stirred for a further 10 min. Thiol R^2SH (3.0 mmol) in CH_2Cl_2 (2 ml) was added slowly at $-78\text{ }^\circ\text{C}$ and the solution stirred for 18 hours with slow warming to room temperature. The solvent was evaporated under reduced pressure

and the crude material was purified directly by silica-gel column chromatography using petroleum ether/ethyl acetate mixtures to afford the unsymmetrical disulfides.

The unsymmetrical disulfides 114 and 115 were synthesized using the following method:

R¹SH (2.0 mmol) was added dropwise to a stirred solution of 1-chlorobenzotriazole (0.461 g, 3.0 mmol) and benzotriazole (0.238 g, 2.0 mmol) in CH₂Cl₂ (20 ml) under N₂ at -78 °C. The solution was allowed to stir for 10 min before adding thiourea (0.23 g, 3 mmol) dissolved in dry THF (5 ml). After 1 hour, Et₃N (0.34 ml, 2.4 mmol) was added and the solution stirred for 10 min. *N*-Boc-L-cysteine ethyl ester (0.748 g, 3.0 mmol) dissolved in CH₂Cl₂ (2 ml) was added at -78 °C and the mixture allowed to warm to room temperature over 18 hours. The solvent was evaporated under reduced pressure and the crude material was purified directly by silica-gel column chromatography using petroleum ether/ethyl acetate mixtures to afford the unsymmetrical disulfides.

The unsymmetrical disulfides 118 and 121 were synthesized using the following method:

R¹SH (2.0 mmol) was added dropwise to a stirred solution of 1-chlorobenzotriazole (0.461 g, 3.0 mmol) and benzotriazole (0.238 g, 2.0 mmol) in CH₂Cl₂ (20 ml) under N₂ at -78 °C. The solution was allowed to stir for 1 hour before adding Et₃N (0.34 ml, 2.4 mmol). *N*-Boc-L-cysteine ethyl ester (0.748 g, 3.0 mmol) dissolved in CH₂Cl₂ (2 ml) was added at -78 °C and the mixture allowed to warm to room temperature over 18 hours. The reaction was quenched at 0 °C with a solution of Na₂S₂O₃ (0.50 g in 10 ml water) and aqueous NaHCO₃ (20 ml) with rapid stirring over 20 min, and then extracted with CH₂Cl₂ (3 x 50 ml). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure to give a residue that was purified by column chromatography (40 g silica) using ethyl acetate/petroleum ether as eluent to give the corresponding product.

The unsymmetrical disulfides 116, 117, 119, 120 were synthesized using the following method:

N-Boc-L-Cysteine (0.498 g, 2.00 mmol) dissolved in CH₂Cl₂ (2 ml) was added dropwise to a stirred solution of 1-chlorobenzotriazole (0.461 g, 3.00 mmol) and benzotriazole (0.238 g, 2.00 mmol) under N₂ at -78 °C. The solution was allowed to stir for 2 hours (slowly warming to

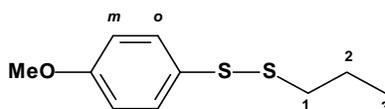
-20 °C). R²SH (3.00 mmol) was added slowly at -20 °C and the solution stirred over three hours (warming to 0 °C). The reaction was quenched by rapidly stirring at 0 °C for 20 minutes with a solution of Na₂S₂O₃ (0.500 g in 10 ml water), NaHCO₃ (20 ml) and then extracted three times with CH₂Cl₂ (50 ml). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure, to give a residue that was purified by chromatography (40 g silica) to give the corresponding product.

The thiosulfinates (127a, 128a, 129a, 130a, 131, 140, 141, 142, 143 and 145) and thiosulfonates (127b, 128b, 129b, 130b and 144) were synthesized using the following method:

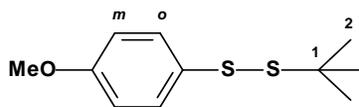
To a stirred solution of the unsymmetrical disulfide (2.0 mmol) in CH₂Cl₂ (5 ml) under N₂ at -78 °C was added *m*-CPBA (0.45 g, 1.0 eq). The reaction was allowed to stir for 3 hours slowly warming to 0 °C, then quenched with saturated aqueous NaHCO₃ (20 ml) and extracted with CH₂Cl₂ (3 × 50 ml). The combined organic extracts were dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The crude material was purified by column chromatography with petroleum ether / ethyl acetate mixtures.

6.3 Compounds

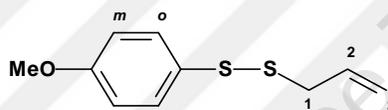
p-Methoxyphenyl 1-propyl disulfide (66)



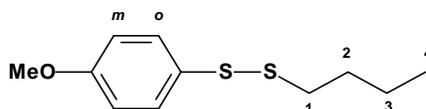
Compound **66** (0.526 g, 91%) was obtained as a pale yellow oil; IR ν_{\max} (CH₂Cl₃) / cm⁻¹ 495 (S-S); δ_{H} (400 MHz, CDCl₃): 0.96 (3H, t, *J* 7.3 Hz, H-3), 1.70 (2H, sextet, *J* 7.3 Hz, H-2), 2.71 (2H, t, *J* 7.3 Hz, H-1), 3.80 (3H, s, OCH₃), 6.86 (2H, d, *J* 8.9 Hz, H_{*m*}), 7.48 (2H, d, *J* 8.9 Hz, H_{*o*}); δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3), 22.1 (C-2), 40.9 (C-1), 55.4 (-OCH₃), 114.6 (C_{*m*}), 128.6 (C_{*s*}), 131.6 (C_{*o*}), 159.5 (C_{*p*}); HRMS: *m/z* 214.0487 (M⁺), C₁₀H₁₄OS₂ requires 214.0486.

***t*-Butyl *p*-methoxyphenyl disulfide (67)**

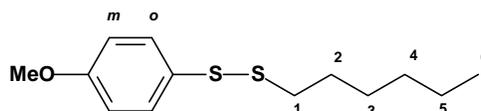
Compound **67** (0.486 g, 79%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 496 (S-S); δ_{H} (400 MHz, CDCl₃): 1.30 (9H, s, H-2), 3.80 (3H, s, -OCH₃), 6.84 (2H, d, *J* 8.9 Hz, H_{*m*}), 7.49 (2H, d, *J* 8.9 Hz, H_{*o*}); δ_{C} (75.45 MHz, CDCl₃): 29.9 (C-2), 48.9 (C-1), 55.4 (-OCH₃), 114.4 (C_{*m*}), 129.8 (C_{*s*}), 130.3 (C_{*o*}), 159.0 (C_{*p*}); HRMS: *m/z* 228.0645 (M⁺), C₁₁H₁₆OS₂ requires 228.0643.

***p*-Methoxyphenyl 2-propenyl disulfide (68)**

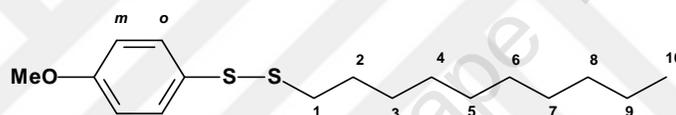
Compound **68** (0.343 g, 60%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 495 (S-S); δ_{H} (400 MHz, CDCl₃): 3.37 (2H, dt, *J* 7.2 Hz and *J* 1.0 Hz, H-1), 3.81 (3H, s, -OCH₃), 5.14 (1H, m, H-3), 5.17 (1H, m, H-3), 5.82 (1H, m, H-2), 6.86 (2H, d, *J* 8.9 Hz, H_{*m*}), 7.48 (2H, d, *J* 8.9 Hz, H_{*o*}); δ_{C} (100.58 MHz, CDCl₃): 41.7 (C-1), 55.4 (-OCH₃), 114.6 (C_{*m*}), 118.8 (C-3), 128.2 (C_{*s*}), 132.2 (C_{*o*}), 132.9 (C-2), 159.7 (C_{*p*}); HRMS: *m/z* 212.0331 (M⁺), C₁₀H₁₂OS₂ requires 212.0330.

1-Butyl *p*-methoxyphenyl disulfide (69)

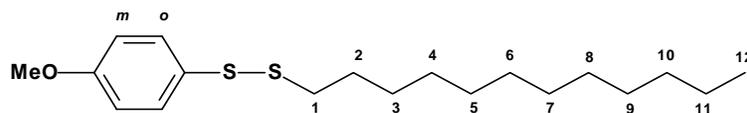
Compound **69** (0.573 g, 93%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 495 (S-S); δ_{H} (400 MHz, CDCl₃): 0.92 (3H, t, *J* 7.4 Hz, H-4), 1.42 (2H, sextet, *J* 7.4 Hz, H-3), 1.69 (2H, quintet, *J* 7.4 Hz, H-2), 2.77 (2H, t, *J* 7.4 Hz, H-1), 3.82 (3H, s, OCH₃), 6.89 (2H, d, *J* 8.6 Hz, H_{*m*}), 7.51 (2H, d, *J* 8.6 Hz, H_{*o*}); δ_{C} (100.58 MHz, CDCl₃): 13.7 (C-4), 21.7 (C-3), 30.9 (C-2), 38.6 (C-1), 55.4 (OCH₃), 114.7 (C_{*m*}), 128.6 (C_{*s*}), 131.6 (C_{*o*}), 159.6 (C_{*p*}); HRMS: *m/z* 228.0637 (M⁺), C₁₁H₁₆OS₂ requires 228.0643.

1-Hexyl *p*-methoxyphenyl disulfide (**70**)

Compound **70** (0.553 g, 80%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl_3) / cm^{-1} 495 (S-S); δ_{H} (300 MHz, CDCl_3): 0.87 (3H, t, J 6.8 Hz, H-6), 1.31 (6H, m, H-3, H-4, H-5), 1.66 (2H, quintet, J 7.1 Hz, H-2), 2.73 (2H, t, J 7.1 Hz, H-1), 3.80 (3H, s, OCH_3), 6.86 (2H, d, J 8.8 Hz, H_m), 7.48 (2H, d, J 8.8 Hz, H_o); δ_{C} (75.45 MHz, CDCl_3): 13.9 (C-6), 22.5, 28.1, 31.4 (C-3, C-4, C-5), 28.7 (C-2), 38.9 (C-1), 55.4 (OCH_3), 114.6 (C_m), 128.6 (C_s), 131.6 (C_o), 159.5 (C_p); HRMS: m/z 256.0949 (M^+), $\text{C}_{13}\text{H}_{20}\text{OS}_2$ requires 256.0956.

1-Decyl *p*-methoxyphenyl disulfide (**71**)

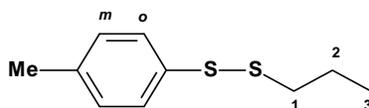
Compound **71** (0.775 g, 92%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 499 (S-S); δ_{H} (300 MHz, CDCl_3): 0.90 (3H, t, J 6.6 Hz, H-10), 1.33 (14H, m, H-9, H-8, H-7, H-6, H-5, H-4, H-3), 1.68 (2H, quintet, J 7.4 Hz, H-2), 2.74 (2H, t, J 7.4 Hz, H-1), 3.80 (3H, s, OCH_3), 6.87 (2H, d, J 9.0 Hz, H_m), 7.49 (2H, d, J 9.0 Hz, H_o); δ_{C} (75.45 MHz, CDCl_3): 14.1 (C-10), 22.6, 28.4, 29.1, 29.3, 29.4, 29.5, 31.9 (C-3, C-4, C-5, C-6, C-7, C-8, C-9), 28.7 (C-2), 38.9 (C-1), 55.3 (OCH_3), 114.6 (C_m), 128.6 (C_s), 131.5 (C_o), 159.5 (C_p); HRMS: m/z 312.1576 (M^+), $\text{C}_{17}\text{H}_{28}\text{OS}_2$ requires 312.1582.

1-Dodecyl *p*-methoxyphenyl disulfide (**72**)

Compound **72** (0.854 g, 93%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 499 (S-S); δ_{H} (300 MHz, CDCl_3): 0.90 (3H, t, J 5.9 Hz, H-12), 1.33 (18H, m, H-11, H-10, H-9, H-8, H-7, H-6, H-5, H-4, H-3), 1.68 (2H, quintet, J 7.4 Hz, H-2), 2.74 (2H, t, J 7.4 Hz, H-1), 3.81 (3H, s, OCH_3), 6.87 (2H, d, J 8.6 Hz, H_m), 7.49 (2H, d, J 8.6 Hz, H_o); δ_{C} (75.45 MHz, CDCl_3): 14.1 (C-12), 22.7, 28.4, 29.2, 29.3, 29.4, 29.5, (29.6 x 2), 31.9 (C-3, C-4, C-5, C-6, C-7, C-8,

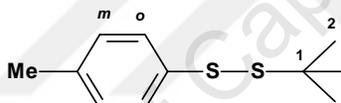
C-9, C-10, C-11), 28.7 (C-2), 38.9 (C-1), 55.3 (OCH₃), 114.6 (C_m), 128.6 (C_s), 131.5 (C_o), 159.4 (C_p); HRMS: *m/z* 340.1889 (M⁺), C₁₉H₃₂OS₂ requires 340.1895.

1-Propyl *p*-tolyl disulfide (73)



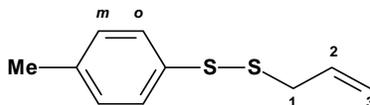
Compound **73** (0.455 g, 85%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 488 (S-S); δ_{H} (400 MHz, CDCl₃): 0.97 (3H, t, *J* 7.3 Hz, H-3), 1.70 (2H, sextet, *J* 7.3 Hz, H-2), 2.34 (3H, s, CH₃), 2.71 (2H, t, *J* 7.3 Hz, H-1), 7.13 (2H, d, *J* 8.1 Hz, H_m), 7.43 (2H, d, *J* 8.1 Hz, H_o); δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3), 21.0 (Ar-CH₃), 22.2 (C-2), 40.9 (C-1), 128.4 (C_o), 129.7 (C_m), 134.5 (C_s), 137.1 (C_p); HRMS: *m/z* 198.0511 (M⁺), C₁₀H₁₄S₂ requires 198.0537.

t-Butyl *p*-tolyl disulfide (74)

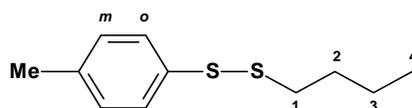


Compound **74** (0.464 g, 81%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 489 (S-S); δ_{H} (400 MHz, CDCl₃): 1.30 (9H, s, H-2), 2.31 (3H, s, CH₃), 7.10 (2H, d, *J* 8.1 Hz, H_m), 7.45 (2H, d, *J* 8.1 Hz, H_o); δ_{C} (75.45 MHz, CDCl₃): 20.9 (CH₃), 29.9 (C-2), 49.0 (C-1), 127.5 (C_o), 129.5 (C_m), 135.4 (C_s), 136.3 (C_p); HRMS: *m/z* 212.0711 (M⁺), C₁₁H₁₆S₂ requires 212.0693.

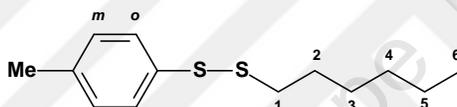
2-Propenyl *p*-tolyl disulfide (75)



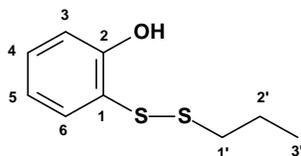
Compound **75** (0.318 g, 60%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 488 (S-S); δ_{H} (400 MHz, CDCl₃): 2.32 (3H, s, CH₃), 3.37 (2H, dt, *J* 7.2 Hz and *J* 1.0 Hz, H-1), 5.14 (1H, m, H-3), 5.16 (1H, m, H-3), 5.83 (1H, m, H-2), 7.13 (2H, d, *J* 8.1 Hz, H_m), 7.43 (2H, d, *J* 8.1 Hz, H_o); δ_{C} (100.58 MHz, CDCl₃): 21.0 (CH₃), 41.8 (C-1), 118.9 (C-3), 128.9 (C_o), 129.7 (C_m), 132.7 (C-2), 133.9 (C_s), 137.5 (C_p); HRMS: *m/z* 123.0251 (M⁺ -S-allyl), C₇H₇S requires 123.0268.

1-Butyl *p*-tolyl disulfide (76)

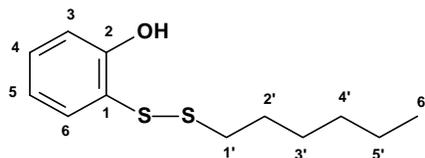
Compound **76** (0.464 g, 81%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl_3) / cm^{-1} 487 (S-S); δ_{H} (400 MHz, CDCl_3): 0.95 (3H, t, J 7.4 Hz, H-4), 1.44 (2H, sextet, J 7.4 Hz, H-3), 1.71 (2H, quintet, J 7.4 Hz, H-2), 2.38 (3H, s, CH_3), 2.78 (2H, t, J 7.4 Hz, H-1), 7.17 (2H, d, J 8.4 Hz, H_m), 7.46 (2H, d, J 8.4 Hz, H_o); δ_{C} (100.58 MHz, CDCl_3): 13.7 (C-4), 21.1 (Ar- CH_3), 21.7 (C-3), 30.9 (C-2), 38.7 (C-1), 128.4 (C_o), 129.8 (C_m), 134.4 (C_s), 136.9 (C_p); HRMS: m/z 212.0688 (M^+), $\text{C}_{11}\text{H}_{16}\text{S}_2$ requires 212.0693.

1-Hexyl *p*-tolyl disulfide (77)

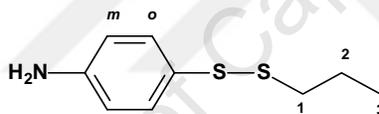
Compound **77** (0.531 g, 82%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl_3) / cm^{-1} 488 (S-S); δ_{H} (300 MHz, CDCl_3): 0.91 (3H, t, J 6.8 Hz, H-6), 1.35 (6H, m, H-3, H-4, H-5), 1.70 (2H, quintet, J 7.4 Hz, H-2), 2.36 (3H, s, CH_3), 2.76 (2H, t, J 7.4 Hz, H-1), 7.15 (2H, d, J 7.8 Hz, H_m), 7.46 (2H, d, J 7.8 Hz, H_o); δ_{C} (100.58 MHz, CDCl_3): 14.0 (C-6), 21.0 (CH_3), 22.6, 28.2, 31.4 (C-3, C-4, C-5), 28.8 (C-2), 39.0 (C-1), 128.4 (C_o), 129.7 (C_m), 134.4 (C_s), 136.9 (C_p); HRMS: m/z 240.1001 (M^+), $\text{C}_{13}\text{H}_{20}\text{S}_2$ requires 240.1006.

2-Hydroxyphenyl 1-propyl disulfide (78)

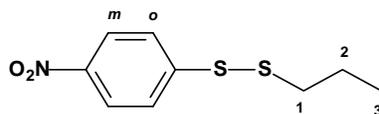
Compound **78** (0.430 g, 80%) was obtained as a yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 3500 (OH), 499 (S-S); δ_{H} (400 MHz, CDCl_3): 0.99 (3H, t, J 7.3 Hz, H-3'), 1.75 (2H, sextet, J 7.3 Hz, H-2'), 2.77 (2H, t, J 7.3 Hz, H-1'), 6.42 (1H, s, OH), 6.89 (1H, td, J 7.7 Hz and 1.5 Hz, H-5), 7.01 (1H, dd, J 8.3 Hz and 1.5 Hz, H-3), 7.30 (1H, ddd, J 8.3 Hz and 7.7 Hz and 1.5 Hz, H-4), 7.51 (1H, dd, J 7.7 Hz and 1.5 Hz, H-6); δ_{C} (100.58 MHz, CDCl_3): 13.1 (C-3'), 22.0 (C-2'), 40.4 (C-1'), 115.9 (C-3), 120.8 (C-5), 121.1 (C-1), 131.9 (C-4), 134.9 (C-6), 156.6 (C-2); HRMS: m/z 200.0325 (M^+), $\text{C}_9\text{H}_{12}\text{OS}_2$ requires 200.0330.

1-Hexyl 2-hydroxyphenyl disulfide (79)

Compound **79** (0.560 g, 86%) was obtained as a yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 3455 (OH), 499 (S-S); δ_{H} (400 MHz, CDCl₃): 0.90 (3H, t, *J* 7.3 Hz, H-6'), 1.33 (6H, m, H-3', H-4', H-5'), 1.71 (2H, quintet, *J* 7.3 Hz, H-2'), 2.79 (2H, t, *J* 7.3 Hz, H-1'), 6.41 (1H, br. s., OH), 6.89 (1H, ddd, *J* 8.3 Hz and 7.7 Hz and 1.5 Hz, H-5), 7.01 (1H, dd, *J* 7.7 Hz and 1.5 Hz, H-3), 7.31 (1H, td, *J* 7.7 and 1.5 Hz, H-4), 7.51 (1H, dd, *J* 8.3 Hz and 1.5 Hz, H-6); δ_{C} (100.58 MHz, CDCl₃): 14.0 (C-6'), 22.5, 28.1, 31.3 (C-3', C-4', C-5'), 28.5 (C-2'), 38.6 (C-1'), 115.9 (C-3), 120.8 (C-5), 121.1 (C-1), 131.9 (C-4), 134.9 (C-6), 156.7 (C-2); HRMS: *m/z* 242.0793 (M⁺), C₁₂H₁₈OS₂ requires 242.0800.

***p*-Aminophenyl 1-propyl disulfide (80)**

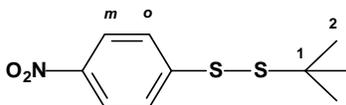
Compound **80** (0.345 g, 64%) was obtained as a dark brown oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 3370 and 3209 (NH₂ stretches), 490 (S-S); δ_{H} (400 MHz, CDCl₃): 0.96 (3H, t, *J* 7.4 Hz, H-3), 1.71 (2H, sextet, *J* 7.4 Hz, H-2), 2.71 (2H, t, *J* 7.4 Hz, H-1), 3.70 (2H, br. s., NH₂), 7.65 (2H, d, *J* 8.4 Hz, H_m), 8.16 (2H, d, *J* 8.4 Hz, H_o); δ_{C} (75.45 MHz, CDCl₃): 13.1 (C-3), 22.0 (C-2), 41.0 (C-1), 115.5 (C_m), 125.5 (C_s), 132.7 (C_o), 147.0 (C_p); HRMS: *m/z* 199.0482 (M⁺), C₉H₁₃NS₂ requires 199.0489.

***p*-Nitrophenyl 1-propyl disulfide (81)**

Compound **81** (0.544 g, 88%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1515 (asym. NO₂ stretch), 1340 (sym. NO₂ stretch), 499 (S-S); δ_{H} (400 MHz, CDCl₃): 0.99 (3H, t, *J* 7.2 Hz, H-3), 1.69 (2H, sextet, *J* 7.2 Hz, H-2), 2.74 (2H, t, *J* 7.4 Hz, H-1), 7.65 (2H, d, *J* 8.7 Hz, H_o), 8.16 (2H, d, *J* 8.7 Hz, H_m); δ_{C} (75.45 MHz, CDCl₃): 13.0 (C-3), 22.3 (C-2), 41.0 (C-1), 123.9 (C_m), 125.7 (C_o), 146.1 (C_s), 147.3 (C_p); HRMS: *m/z* 229.0239 (M⁺), C₉H₁₁NO₂S₂

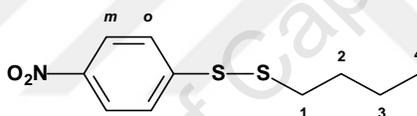
requires 229.0231; Found: C 47.24%, H 4.82%, N 6.05, S 28.01%; $C_9H_{11}NO_2S_2$ requires C 47.14%, H 4.84%, N 6.11%, S 27.96%.

***t*-Butyl *p*-nitrophenyl disulfide (82)**



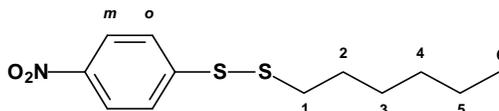
Compound **82** (0.545 g, 83%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1515 (asym. NO_2 stretch), 1340 (sym. NO_2 stretch), 499 (S-S); δ_H (400 MHz, $CDCl_3$): 1.33 (9H, s, H-2), 7.69 (2H, d, J 8.8 Hz, H_o), 8.14 (2H, d, J 8.8 Hz, H_m); δ_C (75.45 MHz, $CDCl_3$): 29.8 (C-2), 50.1 (C-1), 123.8 (C_m), 125.8 (C_o), 146.0 (C_s), 148.3 (C_p); HRMS: m/z 243.0383 (M^+), $C_{10}H_{13}NO_2S_2$ requires 243.0388.

1-Butyl *p*-nitrophenyl disulfide (83)

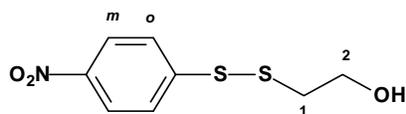


Compound **83** (0.623g, 95%) was obtained as a yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1515 (asym. NO_2 stretch), 1340 (sym. NO_2 stretch), 499 (S-S); δ_H (400 MHz, $CDCl_3$): 0.85 (3H, t, J 7.5 Hz, H-4), 1.36 (2H, sextet, J 7.5 Hz, H-3), 1.61 (2H, quintet, J 7.5 Hz, H-2), 2.73 (2H, t, J 7.5 Hz, H-1), 7.60 (2H, d, J 9.0 Hz, H_o), 8.11 (2H, d, J 9.0 Hz, H_m); δ_C (100.58 MHz, $CDCl_3$): 13.6 (C-4), 21.6 (C-3), 31.0 (C-2), 38.8 (C-1), 124.0 (C_m), 125.8 (C_o), 146.2 (C_s), 147.3 (C_p); HRMS: m/z 243.0382 (M^+), $C_{10}H_{13}NO_2S_2$ requires 243.0388.

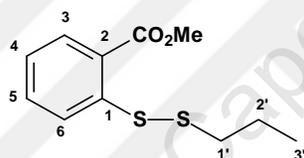
1-Hexyl *p*-nitrophenyl disulfide (84)



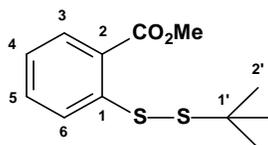
Compound **84** (0.615 g, 84%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1515 (asym. NO_2 stretch), 1340 (sym. NO_2 stretch), 499 (S-S); δ_H (300 MHz, $CDCl_3$): 0.86 (3H, t, J 6.3 Hz, H-6), 1.30 (6H, m, H-3, H-4 and H-5), 1.65 (2H, quintet, J 7.3 Hz, H-2), 2.76 (2H, t, J 7.3 Hz, H-1), 7.65 (2H, d, J 8.7 Hz, H_o), 8.15 (2H, d, J 8.7 Hz, H_m); δ_C (100.58 MHz, $CDCl_3$): 13.9 (C-6), 22.5, 28.1, 31.3 (C-3, C-4, C-5), 28.9 (C-2), 39.1 (C-1), 124.0 (C_m), 125.8 (C_o), 146.2 (C_s), 147.3 (C_p); HRMS: m/z 271.0693 (M^+), $C_{12}H_{17}NO_2S_2$ requires 271.0701.

2-Hydroxyethyl *p*-nitrophenyl disulfide (85)

Compound **85** (0.474 g, 76%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 3359 (OH), 1513 (asym. NO₂ stretch), 1340 (sym. NO₂ stretch), 469 (S-S); δ_{H} (300 MHz, CDCl₃): 2.59 (1H, br. s., OH), 2.93 (2H, t, *J* 5.9 Hz, H-1), 3.84 (2H, t, *J* 5.9 Hz, H-2), 7.66 (2H, d, *J* 9.0 Hz, H_o), 8.14 (2H, d, *J* 9.0 Hz, H_m); δ_{C} (75.45 MHz, CDCl₃): 41.3 (C-1), 60.0 (C-2), 124.0 (C_m), 125.9 (C_o), 146.2 (C_s), 146.5 (C_p); HRMS: *m/z* 231.0018 (M⁺), C₈H₉NO₃S₂ requires 231.0024; Found: C 41.48%, H 3.90%, N 6.02, S 27.80%; C₈H₉NO₃S₂ requires C 41.55%, H 3.92%, N 6.06%, S 27.72%.

***o*-Methoxycarbonylphenyl 1-propyl disulfide (86)**

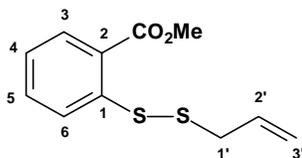
Compound **86** (0.634 g, 97%) was obtained as a pale yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 1708 (C=O), 486 (S-S); δ_{H} (300 MHz, CDCl₃): 0.99 (3H, t, *J* 7.3 Hz, H-3'), 1.70 (2H, sextet, *J* 7.3 Hz, H-2'), 2.69 (2H, t, *J* 7.3 Hz, H-1'), 3.93 (3H, s, OCH₃), 7.22 (1H, ddd, *J* 8.2 Hz, 7.5 Hz and 1.3 Hz, H-5), 7.55 (1H, ddd, *J* 8.2 Hz, 7.5 Hz and 1.3 Hz, H-4), 8.00 (1H, dd, *J* 8.2 Hz and 1.3 Hz, H-6), 8.18 (1H, dd, *J* 8.2 Hz and 1.3 Hz, H-3); δ_{C} (100.58 MHz, CDCl₃): 13.2 (C-3'), 22.3 (C-2'), 40.4 (C-1'), 52.2 (OCH₃), 124.9 (C-5), 125.7 (C-3), 127.1 (C-1), 131.4 (C-6), 132.6 (C-4), 142.1 (C-2), 166.8 (C=O); HRMS: *m/z* 242.0416 (M⁺), C₁₁H₁₄O₂S₂ requires 242.0435.

***t*-Butyl *o*-methoxycarbonylphenyl disulfide (87)**

Compound **87** (0.622 g, 90%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 1708 (C=O), 487 (S-S); δ_{H} (400 MHz, CDCl₃): 1.32 (9H, s, H-2'), 3.95 (3H, s, OCH₃), 7.19 (1H, ddd, *J* 8.2 Hz, 7.5 Hz and 1.3 Hz, H-5), 7.49 (1H, ddd, *J* 8.2 Hz, 7.5 Hz and 1.3 Hz, H-4), 7.97 (1H, dd, *J* 8.2 Hz and 1.3 Hz, H-6), 8.22 (1H, dd, *J* 8.2 Hz and 1.3 Hz, H-3); δ_{C} (100.58 MHz, CDCl₃): 30.0 (C-2'), 49.2 (C-1'), 52.1 (OCH₃), 124.9 (C-5), 126.5 (C-3), 127.1 (C-1), 131.1

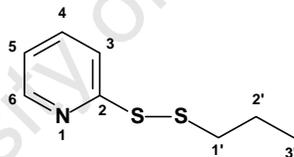
(C-6), 132.2 (C-4), 142.9 (C-2), 166.8 (C=O); HRMS: m/z 256.0597 (M^+), $C_{12}H_{16}O_2S_2$ requires 256.0592.

***o*-Methoxycarbonylphenyl 2-propenyl disulfide (88)**



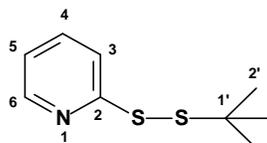
Compound **88** (0.356 g, 55%) was obtained as a pale yellow oil; IR ν_{\max} ($CHCl_3$) / cm^{-1} 1708 (C=O), 486 (S-S); δ_H (400 MHz, $CDCl_3$): 3.36 (2H, dt, J 7.3 Hz and 1.0 Hz, H-1'), 3.93 (3H, s, OCH_3), 5.09 (1H, m, H-3'), 5.13 (1H, m, H-3'), 5.85 (1H, m, H-2'), 7.23 (1H, ddd, J 8.2 Hz, 7.5 Hz and 1.3 Hz, H-5), 7.55 (1H, ddd, J 8.2 Hz and 7.5 Hz and 1.3 Hz, H-4), 8.00 (1H, dd, J 8.2 Hz and 1.3 Hz, H-6), 8.16 (1H, dd, J 8.2 Hz and 1.3 Hz, H-3); δ_C (100.57 MHz, $CDCl_3$): 41.5 (C-1'), 52.2 (OCH_3), 119.0 (C-3'), 125.1 (C-5), 125.9 (C-3), 128.2 (C-1), 131.3 (C-6), 132.4 (C-2'), 132.6 (C-4), 141.8 (C-2), 166.8 (C=O); HRMS: m/z 240.0291 (M^+), $C_{11}H_{12}O_2S_2$ requires 240.0279.

1-Propyl 2-pyridyl disulfide (89)



Compound **89** (0.420 g, 84%) was obtained as a yellow oil; IR ν_{\max} ($CHCl_3$) / cm^{-1} 478 (S-S); δ_H (400 MHz, $CDCl_3$): 1.00 (3H, t, J 7.4 Hz, H-3'), 1.73 (2H, sextet, J 7.3 Hz, H-2'), 2.78 (2H, t, J 7.4 Hz, H-1'), 7.07 (1H, m, H-5), 7.64 (1H, m, H-4), 7.74 (1H, m, H-3), 8.46 (1H, m, H-6); δ_C (MHz, $CDCl_3$): 13.0 (C-3'), 22.3 (C-2'), 41.0 (C-1'), 119.6 (C-3), 120.4 (C-5), 136.9 (C-4), 149.5 (C-6), 161.4 (C-2); HRMS: m/z 185.0339 (M^+), $C_8H_{11}NS_2$ requires 185.0333.

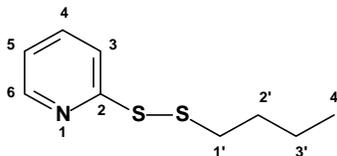
***t*-Butyl 2-pyridyl disulfide (90)**



Compound **90** (0.494 g, 92%) was obtained as a clear oil; IR ν_{\max} ($CHCl_3$) / cm^{-1} 479 (S-S); δ_H (400 MHz, $CDCl_3$): 1.34 (3H, s, H-2'), 7.04 (1H, m, H-5), 7.61 (1H, m, H-4), 7.79 (1H, m, H-3), 8.42 (1H, m, H-6); δ_C (100.58 MHz, $CDCl_3$): 29.8 (C-2'), 49.2 (C-1'), 119.7 (C-3), 120.3

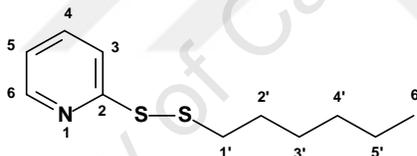
(C-5), 136.7 (C-4), 149.1 (C-6), 161.7 (C-2); HRMS: m/z 199.0491 (M^+), $C_9H_{13}NS_2$ requires 199.0489.

1-Butyl 2-pyridyl disulfide (91)



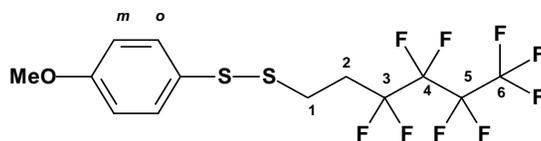
Compound **91** (0.468 g, 87%) was obtained as a pale yellow oil; IR ν_{max} ($CHCl_3$) / cm^{-1} 478 (S-S); δ_H (MHz, $CDCl_3$): 0.90 (3H, t, J 7.4 Hz, H-4'), 1.42 (2H, sextet, J 7.4 Hz, H-3'), 1.68 (2H, quintet, J 7.4, Hz, H-2'), 2.80 (2H, t, J 7.4, Hz, H-1'), 7.06 (1H, m, H-5), 7.63 (1H, m, H-4), 7.73 (1H, m, H-3), 8.45 (1H, m, H-6); δ_C (MHz, $CDCl_3$): 13.6 (C-4'), 21.6 (C-3'), 31.0 (C-2'), 38.8 (C-1'), 119.6 (C-3), 120.4 (C-5), 136.9 (C-4), 149.5 (C-6), 161.2 (C-2); HRMS: m/z 199.0493 (M^+), $C_9H_{13}NS_2$ requires 199.0489.

1-Hexyl 2-pyridyl disulfide (92)



Compound **92** (0.558 g, 91%) was obtained as a yellow oil; IR ν_{max} ($CHCl_3$) / cm^{-1} 478 (S-S); δ_H (MHz, $CDCl_3$): 0.90 (3H, t, J 6.8 Hz, H-6'), 1.32 (6H, m, H-3', 4', 5'), 1.69 (2H, quintet, J 7.4 Hz, H-2'), 2.80 (2H, t, J 7.4 Hz, H-1'), 7.06 (1H, m, H-5), 7.63 (1H, m, H-4), 7.73 (1H, m, H-3), 8.45 (1H, m, H-6); δ_C (MHz, $CDCl_3$): 13.9 (C-6'), 22.5, 28.1, 31.3 (C-3', C-4', C-5'), 28.9 (C-2'), 39.1 (C-1'), 119.6 (C-3), 120.4 (C-5), 136.9 (C-4), 149.5 (C-6), 161.5 (C-2); HRMS: m/z 227.0815 (M^+), $C_{11}H_{17}NS_2$ requires 227.0802.

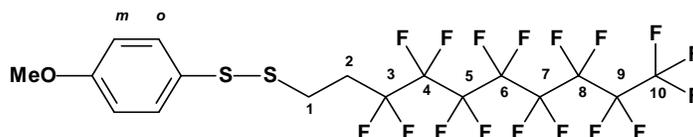
p-Methoxyphenyl 3,3,4,4,5,5,6,6,6-nonafluoro-1-hexyl disulfide (93)



Compound **93** (0.609 g, 54%) was obtained as a clear yellow oil; IR ν_{max} (CH_2Cl_2) / cm^{-1} 1265 (C-F), 499 (S-S); δ_H (300 MHz, $CDCl_3$): 2.56 (2H, m, H-2), 2.89 (2H, m, H-1), 3.82 (3H, s, OCH_3), 6.88 (2H, d, J 8.9 Hz, H_m), 7.48 (2H, d, J 8.9 Hz, H_o); δ_C (100.58 MHz, $CDCl_3$): 28.5 (C-1), 31.4 (t, J 22.1 Hz, C-2), 55.3 (OCH_3), 114.9 (C_m), 127.1 (C_s), 132.9 (C_o), 160.3 (C_p);

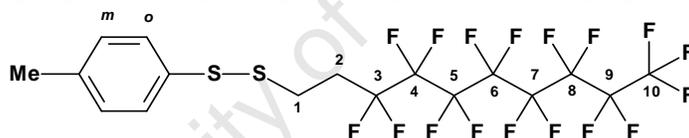
δ_F (376.3 MHz, $CDCl_3$): -126.4 (CF_2), -124.8 (CF_2), -114.9 and -114.4 (F-3), -81.5 (F-6); HRMS: m/z 418.0103 (M^+), $C_{13}H_{11}F_9OS_2$ requires 418.0108.

3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-1-decyl *p*-methoxyphenyl disulfide (94)



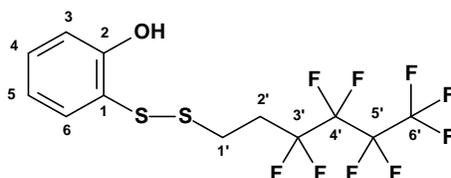
Compound **94** (1.585 g, 95%) was obtained as a white solid; IR ν_{max} (CH_2Cl_2) / cm^{-1} 1265 (C-F), 499 (S-S); δ_H (300 MHz, $CDCl_3$): 2.57 (2H, m, H-2), 2.90 (2H, m, H-1), 3.81 (3H, s, OCH_3), 6.89 (2H, d, J 8.9 Hz, H_m), 7.49 (2H, d, J 8.9 Hz, H_o); δ_C (100.58 MHz, $CDCl_3$): 28.6 (C-1), 31.4 (t, J 22.1 Hz, C-2), 55.4 (OCH_3), 114.9 (C_m), 127.1 (C_s), 132.9 (C_o), 160.2 (C_p); δ_F (376.3 MHz, $CDCl_3$): -126.4 (CF_2), -123.7 (CF_2), -123.1 (CF_2), -122.3 (2 x CF_2), -122.0 (CF_2), -114.2 (F-3), -81.2 (F-10); HRMS: m/z 617.9973 (M^+), $C_{17}H_{11}F_{17}OS_2$ requires 617.9980.

3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-1-decyl *p*-tolyl disulfide (95)



Compound **95** (1.072 g, 66%) was obtained as a yellow solid; IR ν_{max} (CH_2Cl_2) / cm^{-1} 1265 (C-F), 499 (S-S); δ_H (400 MHz, $CDCl_3$): 2.32 (3H, s, CH_3), 2.53 (2H, m, H-2), 2.89 (2H, m, H-1), 7.13 (2H, d, J 8.2 Hz, H_m), 7.40 (2H, d, J 8.2 Hz, H_o); δ_C (100.58 MHz, $CDCl_3$): 21.0 (CH_3), 28.8 (C-1), 31.4 (t, J 22.1 Hz, C-2), 129.6 (C_o), 130.0 (C_m), 132.9 (C_s), 138.1 (C_p); δ_F (376.3 MHz, $CDCl_3$): -126.4 (CF_2), -123.7 (CF_2), -123.1 (CF_2), -122.2 (2 x CF_2), -122.1 (CF_2), -114.2 (F-3), -81.2 (F-10); HRMS: m/z 602.0022 (M^+), $C_{17}H_{11}F_{17}S_2$ requires 602.0031.

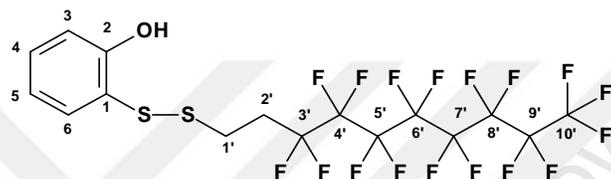
2-Hydroxyphenyl 3,3,4,4,5,5,6,6,6-nonafluorohexyl disulfide (96)



Compound **96** (0.698 g, 64%) was obtained as a yellow solid; IR ν_{max} (CH_2Cl_2) / cm^{-1} 3472 (OH), 1265 (C-F), 499 (S-S); δ_H (400 MHz, $CDCl_3$): 2.58 (2H, m, H-2'), 2.96, (2H, m, H-1'),

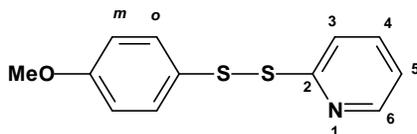
6.27 (1H, br. s., OH), 6.92 (1H, ddd, J 8.3 Hz and 7.7 Hz and 1.5 Hz, H-5), 7.03 (1H, dd, J 7.7 Hz and 1.5 Hz, H-3), 7.36 (1H, td, 7.7 Hz and 1.5 Hz, H-4), 7.48 (1H, dd, J 8.3 Hz and 1.5 Hz, H-6); δ_C (100.58 MHz, CDCl₃): 28.4 (C-1'), 31.3 (t, J 22.1 Hz, C-2'), 116.3 (C-3), 119.9 (C-1), 121.1 (C-5), 132.6 (C-4), 135.0 (C-6), 156.7 (C-2); δ_F (376.3 MHz, CDCl₃): -126.3 (CF₂), -124.7 (CF₂), -114.5 (F-3'), -81.5 (F-6'); HRMS: m/z 403.9948 (M⁺), C₁₂H₉F₉OS₂ requires 403.9951.

3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluoro-1-decyl 2-hydroxyphenyl disulfide (97)

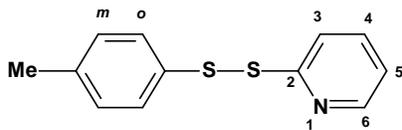


Compound **97** (0.815 g, 50%) was obtained as a yellow solid; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 3463 (OH), 1265 (C-F), 499 (S-S); δ_H (300 MHz, CDCl₃): 2.56 (2H, m, H-2'), 2.94 (2H, m, H-1'), 6.25 (1H, s, OH), 6.90 (1H, td, J 7.7 Hz and 1.5 Hz, H-5), 7.02 (1H, dd, J 8.3 Hz and 1.5 Hz, H-3), 7.34 (1H, ddd, J 8.3 Hz and 7.7 Hz and 1.5 Hz, H-4), 7.46 (1H, 1H, dd, J 7.7 Hz and 1.5 Hz, H-6); δ_C (75.45 MHz, CDCl₃): 28.4 (C-1'), 31.4 (t, J 22.0 Hz, C-2'), 116.3 (C-3), 119.9 (C-1), 121.1 (C-5), 132.6 (C-4), 135.0 (C-6), 156.7 (C-2); δ_F (376.3 MHz, CDCl₃): -126.5 (CF₂), -123.7 (CF₂), -123.1 (CF₂), -122.2 (2 x CF₂), -122.0 (CF₂), -114.2 (F-3'), -81.2 (F-10'); HRMS: m/z 603.9817 (M⁺), C₁₆H₉F₁₇OS₂ requires 603.9823.

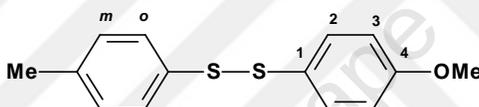
p-Methoxyphenyl 2-pyridyl disulfide (98)



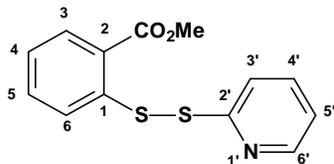
Compound **98** (0.538 g, 80%) was obtained as a yellow solid; IR ν_{\max} (CHCl₃) / cm⁻¹ 486 (S-S); δ_H (400 MHz, CDCl₃): 3.77 (3H, s, -OCH₃), 6.83 (2H, d, J 8.9 Hz, H_m), 7.08 (1H, m, H-5), 7.49 (2H, d, J 8.9 Hz, H_o), 7.62 (1H, m, H-4), 7.71 (1H, m, H-3), 8.47 (1H, m, H-6); δ_C (100.58 MHz, CDCl₃): 55.4 (-OCH₃), 114.8 (C_m), 119.8 (C-3), 120.7 (C-5), 127.2 (C_s), 131.3 (C_o), 137.1 (C-4), 149.6 (C-6), 159.9 (C_p), 160.1 (C-2); HRMS: m/z 249.0270 (M⁺), C₁₂H₁₁NOS₂ requires 249.0282.

2-Pyridyl *p*-tolyl disulfide (99)

Compound **99** (0.535 g, 85%) was obtained as a yellow solid; IR ν_{\max} (CHCl_3) / cm^{-1} 479 (S-S); δ_{H} (400 MHz, CDCl_3): 2.32 (3H, s, CH_3), 7.07 (1H, m, H-5), 7.11 (2H, d, J 8.0 Hz, H_m), 7.43 (2H, d, J 8.0 Hz, H_o), 7.61 (1H, m, H-4), 7.68 (1H, m, H-3), 8.47 (1H, m, H-6); δ_{C} (100.58 MHz, CDCl_3): 21.0 (CH_3), 119.7 (C-3), 120.7 (C-5), 128.1 (C_o), 129.9 (C_m), 132.8 (C_s), 136.5 (C_p), 137.1 (C-4), 149.5 (C-6), 160.0 (C-2); HRMS: m/z 233.0325 (M^+), $\text{C}_{12}\text{H}_{11}\text{NS}_2$ requires 233.0333.

***p*-Methoxyphenyl *p*-tolyl disulfide (100)**

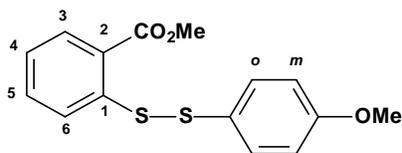
Compound **100** (0.311 g, 59%) was obtained as a yellow oil; IR ν_{\max} (CHCl_3) / cm^{-1} 488 (S-S); δ_{H} (400 MHz, CDCl_3): 2.33 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 6.83 (2H, d, J 8.9 Hz, H-3), 7.12 (2H, d, J 8.1 Hz, H_m), 7.39 (2H, d, J 8.1 Hz, H_o), 7.42 (2H, d, J 8.9 Hz, H-2); δ_{C} (75.45 MHz, CDCl_3): 21.1 (CH_3), 55.4 (OCH_3), 114.7 (C-3), 128.4 (C-1), 129.2 (C_m), 129.8 (C_o), 132.0 (C-2), 134.1 (C_s), 137.6 (C_p), 159.8 (C-4); HRMS: m/z 262.0471 (M^+), $\text{C}_{14}\text{H}_{14}\text{OS}_2$ requires 262.0486.

***o*-Methoxycarbonylphenyl 2-pyridyl disulfide (101)**

Compound **101** (0.666 g, 89%) was obtained as a yellow solid; IR ν_{\max} (CHCl_3) / cm^{-1} 1708 (C=O), 481 (S-S); δ_{H} (300 MHz, CDCl_3): 3.96 (3H, s, OCH_3), 7.06 (1H, m, H-5'), 7.23 (1H, td, J 7.5 Hz and 1.3 Hz, H-5), 7.44 (1H, ddd, J 8.2 Hz and 7.5 Hz and 1.3 Hz, H-4), 7.51 (1H, m, H-4'), 7.54 (1H, m, H-3'), 7.90 (1H, dd, J 7.5 Hz and 1.3 Hz, H-6), 8.03 (1H, dd, J 8.2 Hz and 1.3 Hz, H-3), 8.44 (1H, m, H-6'); δ_{C} (100.58 MHz, CDCl_3): 52.4 (OCH_3), 119.7 (C-3'), 120.9 (C-5'), 125.7 (C-5), 125.8 (C-3), 127.2 (C-1), 131.4 (C-6), 133.1 (C-4), 137.2 (C-4'), 140.3 (C-

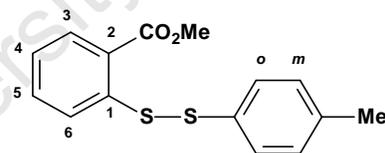
2), 149.5 (C-6'), 159.2 (C-2'), 166.8 (C=O); HRMS: m/z 277.0233 (M^+), $C_{13}H_{11}NO_2S_2$ requires 277.0231.

***o*-Methoxycarbonylphenyl *p*-methoxyphenyl disulfide (102)**

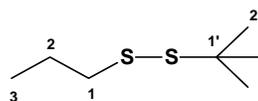


Compound **102** (0.727 g, 88%) was recrystallized from ethanol, mp. 49-50 °C; IR ν_{max} ($CHCl_3$) / cm^{-1} 1708 (C=O), 496 (S-S); δ_H (300 MHz, $CDCl_3$): 3.76 (3H, s, OCH_3), 3.93 (3H, s, CO_2CH_3), 6.81 (2H, d, J 9.0 Hz, H_m), 7.23 (1H, m, H-5), 7.44 (2H, d, J 9.0 Hz, H_o), 7.51 (1H, ddd, J 8.2 Hz and 7.5 Hz and 1.3 Hz, H-4), 8.01 (1H, dd, J 8.2 Hz and 1.3 Hz, H-6), 8.14 (1H, dd, J 8.3 Hz and 1.3 Hz, H-3); δ_C (75.45 MHz, $CDCl_3$): 52.3 ($ArCO_2CH_3$), 55.4 (OCH_3), 114.8 (C_m), 125.3 (C-5), 125.9 (C-3), 127.0 (C-1), 127.2 (C_s), 130.7 (C_o), 131.3 (C-6), 132.8 (C-4), 141.7 (C-2), 159.5 (C_p), 166.8 (C=O); HRMS: m/z 306.0360 (M^+), $C_{15}H_{14}O_3S_2$ requires 306.0362; Found: C 58.78%, H 4.58%, S 20.36%; $C_{15}H_{14}O_3S_2$ requires C 58.80%, H 4.61%, S 20.93%.

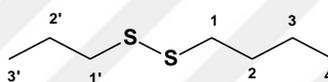
***o*-Methoxycarbonylphenyl *p*-tolyl disulfide (103)**



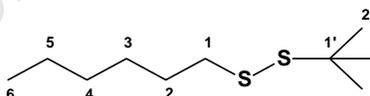
Compound **103** (0.704 g, 90%) was recrystallized from ethanol, mp. 59-62 °C; IR ν_{max} ($CHCl_3$) / cm^{-1} 1708 (C=O), 486 (S-S); δ_H (300 MHz, $CDCl_3$): 2.30 (3H, s, CH_3), 3.95 (3H, s, OCH_3), 7.08 (2H, d, J 8.1 Hz, H_m), 7.23 (1H, m, H-5), 7.38 (2H, d, J 8.1 Hz, H_o), 7.49 (1H, m, H-4), 8.03 (2H, m, H-3 and H-6); δ_C (75.45 MHz, $CDCl_3$): 21.0 (CH_3), 52.3 (OCH_3), 125.4 (C-5), 125.9 (C-3), 127.0 (C-1), 127.7 (C_o), 129.8 (C_m), 131.3 (C-6), 132.8 (C_s), 132.9 (C-4), 137.1 (C_p), 141.5 (C-2), 166.8 (C=O); HRMS: m/z 290.0433 (M^+), $C_{15}H_{14}O_2S_2$ requires 290.0435; Found: C 62.05%, H 4.87%, S 22.63%; $C_{15}H_{14}O_2S_2$ requires C 62.04%, H 4.86%, S 22.08%.

***t*-Butyl 1-propyl disulfide (106)**

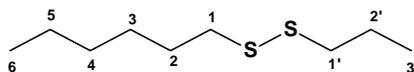
Compound **106** (0.238 g, 72%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 478 (S-S); δ_{H} (300 MHz, CDCl₃): 0.98 (3H, t, *J* 7.3 Hz, H-3), 1.33 (9H, s, H-2'), 1.68 (2H, sextet, *J* 7.3 Hz, H-2), 2.69 (2H, t, *J* 7.3 Hz, H-1), δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3), 22.6 (C-2), 30.0 (C-2'), 43.0 (C-1), 49.2 (C-1'); HRMS: *m/z* 164.0693 (M⁺), C₇H₁₆S₂ requires 164.0693.

1-Butyl 1'-propyl disulfide (107)

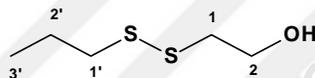
Compound **107** (0.308 g, 94%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 476 (S-S); δ_{H} (400 MHz, CDCl₃): 0.89 (3H, t, *J* 7.4 Hz, H-4), 0.96 (3H, t, *J* 7.4 Hz, H-3'), 1.38 (2H, sextet, *J* 7.4 Hz, H-3), 1.65 (4H, m, H-2' and H-2), 2.64 (4H, m, H-1' and H-1), δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3'), 13.7 (C-4), 21.6 (C-3), 22.5 (C-2'), 31.3 (C-2), 38.9 (C-1), 41.2 (C-1'); HRMS: *m/z* 164.0688 (M⁺), C₇H₁₆S₂ requires 164.0693.

***t*-Butyl 1-hexyl disulfide (108)**

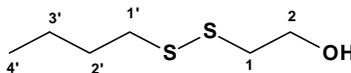
Compound **108** (0.387g, 94%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 476 (S-S); δ_{H} (300 MHz, CDCl₃): 0.88 (3H, t, *J* 6.8 Hz, H-6), 1.33 (15H, m, H-3, H-4, H-5 and H-2'), 1.63 (2H, quintet, *J* 7.4 Hz, H-2), 2.69 (2H, t, *J* 7.4 Hz, H-1), δ_{C} (75.45 MHz, CDCl₃): 14.0 (C-6), 22.5, 28.2, 31.4 (C-3, C-4, C-5), 29.2 (C-2), 29.9 (C-2'), 41.0 (C-1), 47.5 (C-1'); HRMS: *m/z* 206.1158 (M⁺), C₁₀H₂₂S₂ requires 206.1163.

1-Hexyl 1'-propyl disulfide (109)

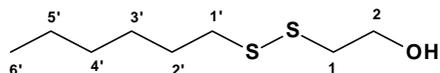
Compound **109** (0.315 g, 82%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 477 (S-S); δ_{H} (400 MHz, CDCl₃): 0.90 (3H, t, *J* 6.9 Hz, H-6), 1.00 (3H, t, *J* 7.3 Hz, H-3'), 1.35 (6H, m, H-3, H-4, H-5), 1.70 (4H, m, H-2, H-2'), 2.68 (4H, m, H-1, H-1'); δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3'), 14.0 (C-6), 22.5, 22.5, 28.2, 31.4 (C-2', C-3, C-4, C-5), 29.2 (C-2), 39.3 (C-1), 41.2 (C-1'); HRMS: *m/z* 192.1016 (M⁺), C₉H₂₀S₂ requires 192.1006.

2-Hydroxyethyl 1'-propyl disulfide (110)

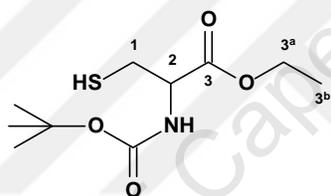
Compound **110** (0.298 g, 98%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3350 (OH), 484 (S-S); δ_{H} (400 MHz, CDCl₃): 1.00 (3H, t, *J* 7.3 Hz, H-3'), 1.72 (2H, sextet, *J* 7.3 Hz, H-2'), 1.92 (1H, s, OH), 2.70 (2H, t, *J* 7.3 Hz, H-1'), 2.85 (2H, t, *J* 5.8 Hz, H-1), 3.89 (2H, t, *J* 5.8 Hz, H-2); δ_{C} (100.58 MHz, CDCl₃): 13.1 (C-3'), 22.5 (C-2'), 41.2 (C-1'), 41.3 (C-1), 60.5 (C-2); HRMS: *m/z* 152.0334 (M⁺), C₅H₁₂OS₂ requires 152.0330.

1'-Butyl 2-hydroxyethyl disulfide (111)

Compound **111** (0.259 g, 78%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 3350 (OH), 481 (S-S); δ_{H} (300 MHz, CDCl₃): 0.91 (3H, t, *J* 7.3 Hz, H-4'), 1.40 (2H, sextet, *J* 6.3 Hz, H-3'), 1.66 (2H, quintet, *J* 5.8 Hz, H-2'), 2.18 (1H, broad singlet, OH), 2.70 (2H, t, *J* 7.3 Hz, H-1'), 2.84 (2H, t, *J* 5.9 Hz, H-1), 3.87 (2H, br. s., H-2); δ_{C} (75.45 MHz, CDCl₃): 13.6 (C-4'), 21.6 (C-3'), 31.2 (C-2'), 38.8 (C-1'), 41.2 (C-1), 60.3 (C-2); HRMS: *m/z* 166.0489 (M⁺), C₆H₁₄OS₂ requires 166.0486.

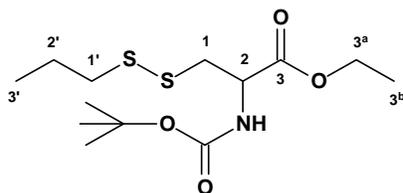
1'-Hexyl 2-hydroxyethyl disulfide (112)

Compound **112** (0.295 g, 76%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 3351 (OH), 481 (S-S); δ_{H} (300 MHz, CDCl_3): 0.88 (3H, t, J 6.8 Hz, H-6'), 1.32 (6H, m, H-3', H-4' and H-5'), 1.67 (2H, quintet, J 7.5 Hz, H-2'), 2.10 (1H, br. s., OH), 2.70 (2H, t, J 7.5 Hz, H-1'), 2.84 (2H, t, J 5.9 Hz, H-1), 3.87 (2H, t, J 5.9 Hz, H-2); δ_{C} (75.45 MHz, CDCl_3): 13.9 (C-6'), 22.4, 28.1, 31.3 (C-3', C-4', C-5'), 29.0 (C-2'), 39.1 (C-1'), 41.1 (C-1), 60.3 (C-2); HRMS: m/z 194.0794 (M^+), $\text{C}_6\text{H}_{14}\text{OS}_2$ requires 194.0799.

N-Boc-L-cysteine (113)

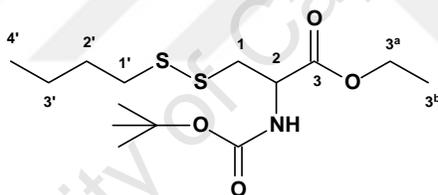
To a stirred suspension of L-Cysteine ethyl ester hydrochloride (0.93 g, 5.00 mmol) in CH_2Cl_2 (40 ml) at $-5\text{ }^\circ\text{C}$, solid NaHCO_3 (1.26 g, 15.00 mmol) was added and stirred for 10 minutes. To this suspension was added di-tert-butyl dicarbonate (1.09 g, 5.00 mmol) and the mixture allowed to stir for 12 hours, slowly warming to room temperature. The reaction was diluted with H_2O and extracted with CH_2Cl_2 (3×50 ml). The combined organic extracts were dried over anhydrous MgSO_4 , filtered and evaporated under reduced pressure. The crude material was purified by column chromatography with petroleum ether / ethyl acetate mixtures to obtain compound **113** (1.22 g, 98%) as a white solid; IR ν_{\max} (CHCl_3) / cm^{-1} 3370 (NH), 2571 (SH), 1720 (C=O), 1500 (C=O), 1393 and 1370 ($-\text{C}(\text{CH}_3)_3$); δ_{H} (400 MHz, CDCl_3): 1.29 (3H, t, J 7.2 Hz, H-3^b), 1.45 (10H, s, $-\text{C}(\text{CH}_3)_3$ and SH), 3.16 (2H, br. s., H-1), 4.21 (2H, quartet, J 7.2 Hz, H-3^a), 4.56 (1H, br. s., H-2), 5.35 (1H, br. s., NH); δ_{C} (100.57 MHz, CDCl_3): 14.1 (C-3^b), 27.2 (C-1), 28.2 ($-\text{C}(\text{CH}_3)_3$), 54.9 (C-2), 61.7 (C-3^a), 80.1 ($-\text{C}(\text{CH}_3)_3$), 155.1 (C=O), 170.2 (C-3); HRMS: m/z 249.1038 (M^+), $\text{C}_{10}\text{H}_{19}\text{NO}_4\text{S}$ requires 249.1035.

L-Alanine *N*-[(1,1-Dimethylethoxy)carbonyl]-3-(propyldithio) Ethyl Ester (114)



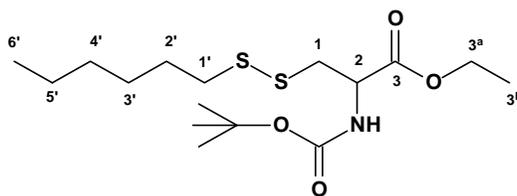
Compound **114** (0.569 g, 88%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3427 (NH), 1715 (C=O), 1500 (C=O), 464 (S-S); δ_{H} (300 MHz, CDCl₃): 0.97 (3H, t, *J* 7.3 Hz, H-3'), 1.28 (3H, t, *J* 7.3 Hz, H-3^b), 1.43 (9H, s, -C(CH₃)₃), 1.68 (2H, sextet, *J* 7.3 Hz, H-2'), 2.66 (2H, t, *J* 7.3 Hz, H-1'), 3.12 (2H, m, H-1), 4.20 (2H, quartet, *J* 7.3 Hz, H-3^a), 4.54 (1H, m, H-2), 5.32 (1H, br. s., NH); δ_{C} (75.5 MHz, CDCl₃): 12.9 (C-3'), 14.0 (C-3^b), 22.3 (C-2'), 28.2 (-C(CH₃)₃), 40.9 (C-1'), 41.2 (C-1), 53.1 (C-2), 61.6 (C-3^a), 80.0 (-C(CH₃)₃), 155.0 (C=O), 170.7 (C-3); HRMS: *m/z* 346.1119 (M⁺ + Na), C₁₃H₂₅NNaO₄S₂ requires 346.1123.

L-Alanine 3-(Butyldithio)-*N*-[(1,1-dimethylethoxy)carbonyl] Ethyl Ester (115)



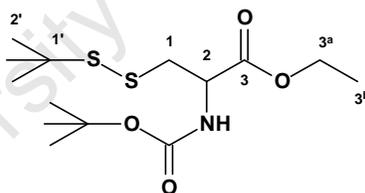
Compound **115** (0.573 g, 85%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3370 (NH), 1717 (C=O), 1500 (C=O), 464 (S-S); δ_{H} (300 MHz, CDCl₃): 0.91 (3H, t, *J* 7.4 Hz, H-4'), 1.29 (3H, t, *J* 7.0 Hz, H-3^b), 1.39 (2H, m, H-3'), 1.44 (9H, s, -C(CH₃)₃), 1.64 (2H, m, H-2'), 2.70 (2H, t, *J* 7.4 Hz, H-1'), 3.13 (2H, m, H-1), 4.21 (2H, quartet, *J* 7.0 Hz, H-3^a), 4.55 (1H, m, H-2), 5.32 (1H, br. s., NH); δ_{C} (100.6 MHz, CDCl₃): 13.6 (C-4'), 14.1 (C-3^b), 21.6 (C-3'), 28.2 (-C(CH₃)₃), 31.1 (C-2'), 38.7 (C-1'), 41.3 (C-1), 53.1 (C-2), 61.7 (C-3^a), 80.0 (-C(CH₃)₃), 155.0 (C=O), 170.8 (C-3); HRMS: *m/z* 360.1283 (M⁺ + Na), C₁₄H₂₇NNaO₄S₂ requires 360.1279.

L-Alanine *N*-[(1,1-Dimethylethoxy)carbonyl]-3-(hexyldithio) Ethyl Ester (116)

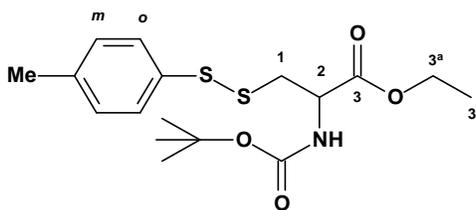


Compound **116** (0.599 g, 82%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3427 (NH), 1716 (C=O), 1500 (C=O), 464 (S-S); δ_{H} (300 MHz, CDCl₃): 0.86 (3H, t, *J* 6.8 Hz, H-6'), 1.31 (9H, m, H-3', H-4', H-5' and H-3^b), 1.43 (9H, s, -C(CH₃)₃), 1.63 (2H, quintet, *J* 7.4 Hz, H-2'), 2.67 (2H, t, *J* 7.4 Hz, H-1'), 3.11 (2H, m, H-1), 4.20 (2H, quartet, *J* 7.1 Hz, H-3^a), 4.54 (1H, m, H-2), 5.34 (1H, br. s., NH); δ_{C} (100.6 MHz, CDCl₃): 13.9 (C-6'), 14.1 (C-3^b), 22.5, 28.1, 31.3 (C-3', C-4', C-5'), 28.3 (-C(CH₃)₃), 29.0 (C-2'), 39.0 (C-1'), 41.3 (C-1), 53.1 (C-2), 61.7 (C-3^a), 80.0 (-C(CH₃)₃), 155.0 (C=O), 170.8 (C-3); HRMS: *m/z* 388.1578 (M⁺ + Na), C₁₆H₃₁NNaO₄S₂ requires 388.1592.

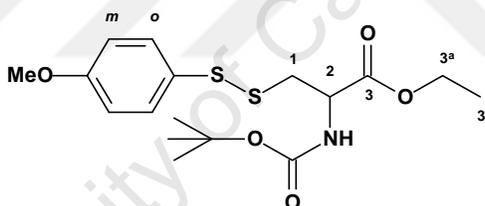
L-Alanine *N*-[(1,1-Dimethylethoxy)carbonyl]-3-[(1,1-dimethylethyl)dithio] Ethyl Ester (117)



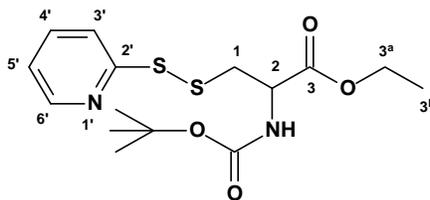
Compound **117** (0.526 g, 78%) was obtained as a clear oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3427 (NH), 1715 (C=O), 1500 (C=O), 464 (S-S); δ_{H} (300 MHz, CDCl₃): 1.28 (3H, m, H-3^b), 1.31 (9H, s, -SC(CH₃)₃), 1.44 (9H, s, -OC(CH₃)₃), 3.15 (2H, m, H-1), 4.21 (2H, quartet, *J* 7.2 Hz, H-3^a), 4.53 (1H, m, H-2), 5.35 (1H, br. s., NH); δ_{C} (100.6 MHz, CDCl₃): 14.1 (C-3^b), 28.3 (-OC(CH₃)₃), 29.8 (C-2'), 43.0 (C-1), 48.1 (C-1'), 53.4 (C-2), 61.7 (C-3^a), 80.0 (-OC(CH₃)₃), 155.1 (C=O), 170.7 (C-3); HRMS: *m/z* 360.1272 (M⁺ + Na), C₁₄H₂₇NNaO₄S₂ requires 360.1279.

L-Alanine N-[(1,1-Dimethylethoxy)carbonyl]-3-[(*p*-tolyl)dithio] Ethyl Ester (118)


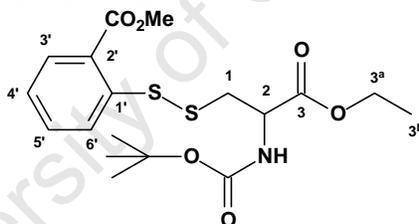
Compound **118** (0.527 g, 71%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3427 (NH), 1715 (C=O), 1500 (C=O), 488 (S-S); δ_{H} (300 MHz, CDCl₃): 1.24 (3H, t, *J* 7.4 Hz, H-3^b), 1.43 (9H, s, -C(CH₃)₃), 2.32 (3H, s, Ar-CH₃), 3.17 (2H, m, H-1), 4.18 (2H, quartet, *J* 7.4 Hz, H-3^a), 4.57 (1H, m, H-2), 5.33 (1H, br. s., NH), 7.12 (2H, d, *J* 7.8 Hz, H_m), 7.40 (2H, d, *J* 7.8 Hz, H_o); δ_{C} (100.6 MHz, CDCl₃): 14.1 (C-3^b), 21.0 (Ar-CH₃), 28.3 (-C(CH₃)₃), 40.9 (C-1), 53.0 (C-2), 61.7 (C-3^a), 80.0 (-C(CH₃)₃), 129.3 (C_o), 129.9 (C_m), 133.3 (C_s), 137.7 (C_p), 155.0 (C=O), 170.6 (C-3); HRMS: *m/z* 371.1229 (M⁺), C₁₇H₂₅NO₄S₂ requires 371.1225.

L-Alanine N-[(1,1-Dimethylethoxy)carbonyl]-3-[(*p*-anisyl)dithio] Ethyl Ester (119)


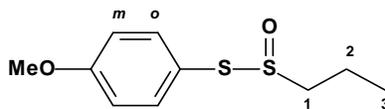
Compound **119** (0.627 g, 81%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3370 (NH), 1716 (C=O), 1500 (C=O), 497 (S-S); δ_{H} (300 MHz, CDCl₃): 1.25 (3H, t, *J* 7.1 Hz, H-3^b), 1.42 (9H, s, -C(CH₃)₃), 3.15 (2H, m, H-1), 3.77 (3H, s, OCH₃), 4.18 (2H, quartet, *J* 7.1 Hz, H-3^a), 4.57 (1H, m, H-2), 5.31 (1H, br. s., NH), 6.84 (2H, d, *J* 8.9 Hz, H_m), 7.46 (2H, d, *J* 8.9 Hz, H_o); δ_{C} (75.5 MHz, CDCl₃): 14.0 (C-3^b), 28.2 (-C(CH₃)₃), 40.6 (C-1), 52.9 (C-2), 55.3 (OCH₃), 61.6 (C-3^a), 80.0 (-C(CH₃)₃), 114.7 (C_m), 127.4 (C_s), 132.6 (C_o), 155.0 (C=O), 160.0 (C_p), 170.6 (C-3); HRMS: *m/z* 387.1185 (M⁺), C₁₇H₂₅NO₅S₂ requires 387.1174.

L-Alanine N-[(1,1-Dimethylethoxy)carbonyl]-3-[(*o*-pyridyl)dithio] Ethyl Ester (120)


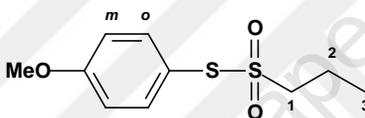
Compound **120** (0.618 g, 86%) was obtained as a white solid; IR ν_{\max} (CHCl₃) / cm⁻¹ 3370 (NH), 1713 (C=O), 1500 (C=O), 479 (S-S); δ_{H} (400 MHz, CDCl₃): 1.24 (3H, t, *J* 7.1 Hz, H-3^b), 1.44 (9H, s, -C(CH₃)₃), 3.30 (2H, m, H-1), 4.18 (2H, quartet, *J* 7.1 Hz, H-3^a), 4.53 (1H, m, H-2), 6.50 (1H, br. s., NH), 7.11 (1H, m, H-5'), 7.52 (1H, m, H-4'), 7.61 (1H, m, H-3'), 8.55 (1H, m, H-6'); δ_{C} (100.6 MHz, CDCl₃): 14.0 (C-3^b), 28.3 (-C(CH₃)₃), 42.2 (C-1), 53.2 (C-2), 61.7 (C-3^a), 79.9 (-C(CH₃)₃), 120.5 (C-3'), 121.0 (C-5'), 136.9 (C-4'), 149.9 (C-6'), 155.1 (C=O), 159.0 (C-2'), 170.7 (C-3); HRMS: *m/z* 359.1097 (M⁺ + H), C₁₅H₂₃N₂O₄S₂ requires 359.1099.

L-Alanine N-[(1,1-Dimethylethoxy)carbonyl]-3-[(*o*-methoxycarbonylphenyl)dithio] Ethyl Ester (121)


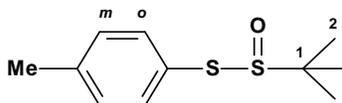
Compound **121** (0.722 g, 87%) was obtained as a clear yellow oil; IR ν_{\max} (CHCl₃) / cm⁻¹ 3370 (NH), 1711 (C=O), 1500 (C=O), 491 (S-S); δ_{H} (400 MHz, CDCl₃): 1.21 (3H, t, *J* 7.1 Hz, H-3^b), 1.40 (9H, s, -C(CH₃)₃), 3.18 (2H, m, H-1), 3.89 (3H, s, -OCH₃), 4.16 (2H, quartet, *J* 7.1 Hz, H-3^a), 4.49 (1H, m, H-2), 5.31 (1H, br. s., NH), 7.22 (1H, m, H-5'), 7.54 (1H, m, H-4'), 7.98 (1H, dd, *J* 8.0 Hz and 1.6 Hz, H-6'), 8.09 (1H, dd, *J* 8.4 Hz and 0.8 Hz, H-3'); δ_{C} (100.6 MHz, CDCl₃): 14.1 (C-3^b), 28.3 (-C(CH₃)₃), 40.9 (C-1), 52.2 (-OCH₃), 53.3 (C-2), 61.9 (C-3^a), 80.2 (-C(CH₃)₃), 125.4 (C-5'), 125.7 (C-3'), 127.2 (C-1'), 131.5 (C-6'), 132.9 (C-4'), 141.1 (C-2'), 155.0 (C=O), 166.7 (CO₂Me), 170.4 (C-3); HRMS: *m/z* 416.1198 (M⁺ + H), C₁₈H₂₆NO₆S₂ requires 416.1202.

S-*p*-Methoxyphenyl propylthiosulfinate (127a)

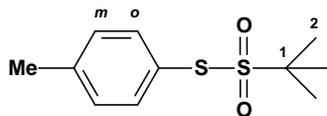
Compound **127 a** (0.331 g, 72%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1029 (S=O stretch); δ_{H} (300 MHz, CDCl₃): 1.08 (3H, t, *J* 7.5 Hz, H-3), 1.88 (2H, sextet, *J* 7.5 Hz, H-2), 3.04 (2H, t, *J* 7.5 Hz, H-1), 3.83 (3H, s, OCH₃), 6.93 (2H, d, *J* 8.9 Hz, H_{*m*}), 7.52 (2H, d, *J* 8.9 Hz, H_{*o*}); δ_{C} (100.57 MHz, CDCl₃): 13.2 (C-3), 17.2 (C-2), 55.4 (OCH₃), 57.6 (C-1), 115.0 (C_{*m*}), 119.2 (C_{*s*}), 137.3 (C_{*o*}), 161.6 (C_{*p*}); HRMS: *m/z* 230.0429 (M⁺), C₁₀H₁₄O₂S₂ requires 230.0435.

S-*p*-Methoxyphenyl propylthiosulfonate (127b)

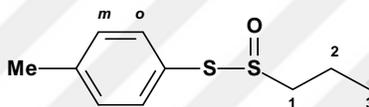
Compound **127 b** (0.113 g, 23%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1320 (asym. SO₂ stretch), 1130 (sym. SO₂ stretch); δ_{H} (400 MHz, CDCl₃): 1.05 (3H, t, *J* 7.5 Hz, H-3), 1.94 (2H, m, H-2), 3.14 (2H, m, H-1), 3.85 (3H, s, OCH₃), 6.96 (2H, d, *J* 8.9 Hz, H_{*m*}), 7.59 (2H, d, *J* 8.9 Hz, H_{*o*}); δ_{C} (100.57 MHz, CDCl₃): 12.7 (C-3), 17.3 (C-2), 55.5 (OCH₃), 60.7 (C-1), 115.4 (C_{*m*}), 118.7 (C_{*s*}), 138.0 (C_{*o*}), 162.4 (C_{*p*}); HRMS: *m/z* 246.0363 (M⁺), C₁₀H₁₄O₃S₂ requires 246.0384.

S-*p*-tolyl *t*-butylthiosulfinate (128a)

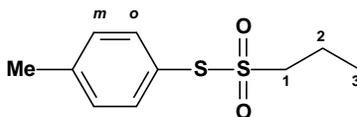
Compound **128 a** (0.242g, 53%) was obtained as a white solid; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1040 (S=O stretch); δ_{H} (300 MHz, CDCl₃): 1.45 (9H, s, H-2), 2.37 (3H, s, CH₃), 7.19 (2H, d, *J* 8.1 Hz, H_{*m*}), 7.51 (2H, d, *J* 8.1 Hz, H_{*o*}); δ_{C} (75.45 MHz, CDCl₃): 21.2 (CH₃), 24.1 (C-2), 60.1 (C-1), 126.2 (C_{*p*}), 130.2 (C_{*o*}), 135.2 (C_{*m*}), 140.2 (C_{*s*}); HRMS: *m/z* 228.0622 (M⁺), C₁₁H₁₆OS₂ requires 228.0643.

S-*p*-tolyl *t*-butylthiosulfonate (128b)

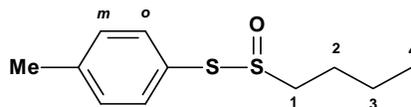
Compound 128 b (0.049 g, 10%) was obtained as a white solid; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1330 (asym. SO_2 stretch), 1140 (sym. SO_2 stretch); δ_{H} (300 MHz, CDCl_3): 1.58 (9H, s, H-2), 2.32 (3H, s, CH_3), 7.23 (2H, d, J 8.1 Hz, H_m), 7.55 (2H, d, J 8.1 Hz, H_o).

S-*p*-tolyl propylthiosulfinate (129a)

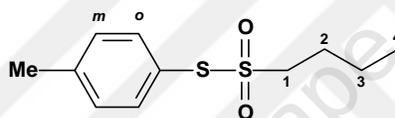
Compound 129 a (0.248 g, 58%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1040 (S=O stretch); δ_{H} (300 MHz, CDCl_3): 1.06 (3H, t, J 7.5 Hz, H-3), 1.85 (2H, sextet, J 7.5 Hz, H-2), 2.34 (3H, s, CH_3), 3.04 (2H, t, J 7.5 Hz, H-1), 7.18 (2H, d, J 8.4 Hz, H_m), 7.46 (2H, d, J 8.4 Hz, H_o); δ_{C} (75.45 MHz, CDCl_3): 13.2 (C-3), 17.2 (CH_3), 21.3 (C-2), 57.8 (C-1), 125.5 (C_p), 130.2 (C_o), 135.3 (C_m), 140.6 (C_s); HRMS: m/z 214.0478 (M^+), $\text{C}_{10}\text{H}_{14}\text{OS}_2$ requires 214.0486.

S-*p*-tolyl propylthiosulfonate (129b)

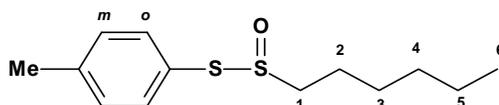
Compound 129 b (0.064 g, 14%) was obtained as a clear yellow oil; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1330 (asym. SO_2 stretch), 1140 (sym. SO_2 stretch); δ_{H} (400 MHz, CDCl_3): 1.08 (3H, t, J 7.4 Hz, H-3), 1.93 (2H, sextet, J 7.4 Hz, H-2), 2.34 (3H, s, CH_3), 3.16 (2H, m, H-1), 7.20 (2H, d, J 8.0 Hz, H_m), 7.50 (2H, d, J 8.0 Hz, H_o).

S-*p*-tolyl butylthiosulfinate (130a)

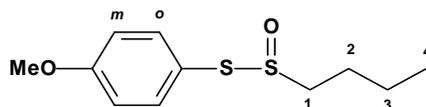
Compound **130 a** (0.310 g, 68%) was obtained as a pale yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1040 (S=O stretch); δ_{H} (300 MHz, CDCl₃): 0.97 (3H, t, *J* 7.2 Hz, H-4), 1.48 (2H, m, H-3), 1.81 (2H, m, H-2), 2.37 (3H, s, CH₃), 3.09 (2H, t, *J* 8.3 Hz, H-1), 7.21 (2H, d, *J* 8.0 Hz, H_{*m*}), 7.48 (2H, d, *J* 8.0 Hz, H_{*o*}); δ_{C} (75.45 MHz, CDCl₃): 13.6 (C-4), 21.2 (Ar-CH₃), 21.8 (C-3), 25.4 (C-2), 55.8 (C-1), 125.5 (C_{*p*}), 130.2 (C_{*o*}), 135.2 (C_{*m*}), 140.6 (C_{*s*}); HRMS: *m/z* 228.0639 (M⁺), C₁₁H₁₆S₂ requires 228.0643.

S-*p*-tolyl butylthiosulfonate (130b)

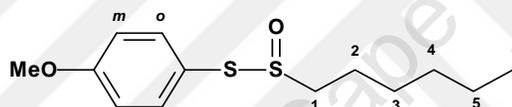
Compound **130 b** (0.049 g, 10%) was obtained as a pale yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1330 (asym. SO₂ stretch), 1140 (sym. SO₂ stretch); δ_{H} (300 MHz, CDCl₃): 0.98 (3H, t, *J* 7.3 Hz, H-4), 1.49 (2H, m, H-3), 1.91 (2H, m, H-2), 2.34 (3H, s, CH₃), 3.23 (2H, m, H-1), 7.24 (2H, d, *J* 8.0 Hz, H_{*m*}), 7.52 (2H, d, *J* 8.0 Hz, H_{*o*}).

S-*p*-tolyl hexylthiosulfinate (131)

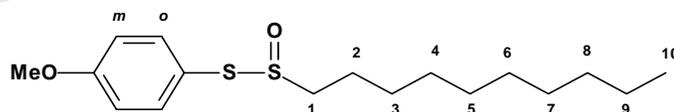
Compound **131** (0.359 g, 70%) was obtained as a pale yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1040 (S=O stretch); δ_{H} (400 MHz, CDCl₃): 0.89 (3H, m, H-6), 1.32 (4H, m, H-4, H-5), 1.47 (2H, m, H-3), 1.82 (2H, m, H-2), 2.36 (3H, s, CH₃), 3.07 (2H, t, *J* 7.6 Hz, H-1), 7.20 (2H, d, *J* 8.0 Hz, H_{*m*}), 7.48 (2H, d, *J* 8.0 Hz, H_{*o*}); δ_{C} (100.57 MHz, CDCl₃): 13.9 (C-6), 21.3 (CH₃), 22.4 (C-5), 23.5 (C-4), 28.3 (C-2), 31.2 (C-3), 56.1 (C-1), 125.6 (C_{*p*}), 130.2 (C_{*o*}), 135.3 (C_{*m*}), 140.6 (C_{*s*}); HRMS: *m/z* 256.0933 (M⁺), C₁₃H₂₀OS₂ requires 256.0956.

S-*p*-methoxyphenyl hexylthiosulfinate (140)

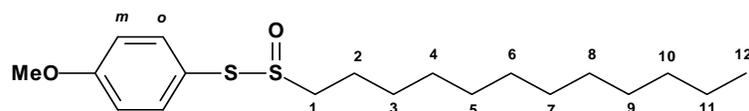
Compound **132** (0.337 g, 69%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1029 (S=O); δ_{H} (400 MHz, CDCl₃): 0.98 (3H, t, *J* 7.5 Hz, H-4), 1.49 (2H, sextet, *J* 7.5 Hz, H-3), 1.82 (2H, quintet, *J* 7.5 Hz, H-2), 3.06 (2H, t, *J* 7.5 Hz, H-1), 3.83 (3H, s, OCH₃), 6.93 (2H, d, *J* 8.8 Hz, H_{*m*}), 7.52 (2H, d, *J* 8.8 Hz, H_{*o*}); δ_{C} (100.57 MHz, CDCl₃): 13.7 (C-4), 21.9 (C-3), 25.5 (C-2), 55.4 (OCH₃), 55.6 (C-1), 115.1 (C_{*m*}), 119.3 (C_{*s*}), 137.4 (C_{*o*}), 161.6 (C_{*p*}); HRMS: *m/z* 244.0586 (M⁺), C₁₁H₁₆O₂S₂ requires 244.0592.

S-*p*-methoxyphenyl hexylthiosulfinate (141)

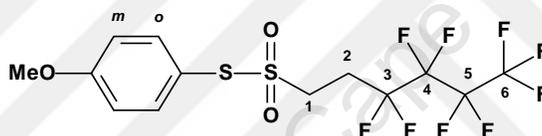
Compound **133** (0.343 g, 63%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1029 (S=O); δ_{H} (400 MHz, CDCl₃): 0.91 (3H, m, H-6), 1.33 (4H, m, H-4, H-5), 1.47 (2H, m, H-3), 1.84 (2H, m, H-2), 3.06 (2H, t, *J* 7.8 Hz, H-1), 3.83 (3H, s, OCH₃), 6.94 (2H, d, *J* 8.8 Hz, H_{*m*}), 7.53 (2H, d, *J* 8.8 Hz, H_{*o*}); δ_{C} (100.57 MHz, CDCl₃): 13.9 (C-6), 22.4, 31.3 (C-4 and C-5), 23.5 (C-2), 28.3 (C-3), 55.4 (OCH₃), 55.9 (C-1), 115.1 (C_{*m*}), 119.3 (C_{*s*}), 137.3 (C_{*o*}), 161.6 (C_{*p*}); HRMS: *m/z* 272.0899 (M⁺), C₁₃H₂₀O₂S₂ requires 272.0905.

S-*p*-methoxyphenyl decylthiosulfinate (142)

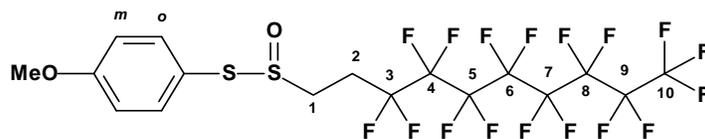
Compound **134** (0.440 g, 67%) was obtained as a clear yellow oil; IR ν_{\max} (CH₂Cl₂) / cm⁻¹ 1030 (S=O); δ_{H} (300 MHz, CDCl₃): 0.88 (3H, t, *J* 6.9 Hz, H-10), 1.31 (12H, m, H-9, H-8, H-7, H-6, H-5, H-4), 1.44 (2H, m, H-3), 1.82 (2H, quintet, *J* 7.5 Hz, H-2), 3.04 (2H, t, *J* 7.5 Hz, H-1), 3.82 (3H, s, OCH₃), 6.92 (2H, d, *J* 9.0 Hz, H_{*m*}), 7.51 (2H, d, *J* 9.0 Hz, H_{*o*}); δ_{C} (75.45 MHz, CDCl₃): 14.0 (C-10), 22.6, 28.6, 29.1, 29.2, 29.3, 29.4, 31.8 (C-9, C-8, C-7, C-6, C-5, C-4, C-3), 23.4 (C-2), 55.4 (OCH₃), 55.9 (C-1), 115.0 (C_{*m*}), 119.2 (C_{*s*}), 137.3 (C_{*o*}), 161.5 (C_{*p*}); HRMS: *m/z* 328.1525 (M⁺), C₁₇H₂₈O₂S₂ requires 328.1531.

S-*p*-methoxyphenyl dodecylthiosulfinate (143)

Compound **135** (0.499 g, 70%) was obtained as a white solid; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1030 ($\text{S}=\text{O}$); δ_{H} (300 MHz, CDCl_3): 0.88 (3H, t, J 6.8 Hz, H-12), 1.31 (16H, m, H-11, H-10, H-9, H-8, H-7, H-6, H-5, H-4), 1.45 (2H, m, H-3), 1.82 (2H, quintet, J 7.7 Hz, H-2), 3.04 (2H, t, J 7.7 Hz, H-1), 3.83 (3H, s, OCH_3), 6.93 (2H, d, J 9.3 Hz, H_m), 7.52 (2H, d, J 9.3 Hz, H_o); δ_{C} (75.45 MHz, CDCl_3): 14.1 (C-12), 22.6, 28.6, 29.1, 29.3, 29.3, 29.5, 29.6, 29.6, 31.9 (C-11, C-10, C-9, C-8, C-7, C-6, C-5, C-4, C-3), 23.4 (C-2), 55.4 (OCH_3), 55.9 (C-1), 115.0 (C_m), 119.3 (C_s), 137.3 (C_o), 161.6 (C_p); HRMS: m/z 356.1839 (M^+), $\text{C}_{19}\text{H}_{32}\text{O}_2\text{S}_2$ requires 356.1844.

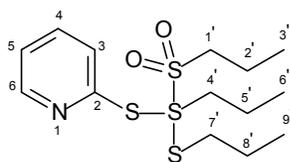
S-*p*-Methoxyphenyl 3,3,4,4,5,5,6,6,6-nonafluorohexylthiosulfonate (144)

Compound **136** (0.459 g, 51%) was obtained as a yellow solid; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1354 (asym. SO_2 stretch), 1135 (sym. SO_2 stretch); δ_{H} (400 MHz, CDCl_3): 2.69 (2H, m, H-2), 3.31 (2H, m, H-1), 3.86 (3H, s, OCH_3), 6.98 (2H, d, J 9.0 Hz, H_m), 7.54 (2H, d, J 9.0 Hz, H_o); δ_{C} (100.58 MHz, CDCl_3): 25.3 (t, J 22.1, C-2), 45.9 (C-1), 55.5 (OCH_3), 115.3 (C_m), 117.9 (C_s), 137.7 (C_o), 162.1 (C_p); δ_{F} (376.3 MHz, CDCl_3): -126.3 (CF_2), -124.4 (CF_2), -113.8 (F-3), -81.4 (F-6); HRMS: m/z 450.0001 (M^+), $\text{C}_{13}\text{H}_{11}\text{F}_9\text{O}_3\text{S}_2$ requires 450.0006.

S-*p*-methoxyphenyl 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptafluorodecylthiosulfinate (145)

Compound **137** (0.634 g, 50%) was obtained as a yellow solid; IR ν_{\max} (CH_2Cl_2) / cm^{-1} 1030 ($\text{S}=\text{O}$); δ_{H} (400 MHz, CDCl_3): 2.70 (2H, m, H-2), 3.31 (2H, m, H-1), 3.87 (3H, s, OCH_3), 6.98 (2H, d, J 8.8 Hz, H_m), 7.55 (2H, d, J 8.8 Hz, H_o); δ_{C} (100.58 MHz, CDCl_3): 25.3 (t, J 22.1, C-2), 45.9 (C-1), 55.5 (OCH_3), 115.3 (C_m), 117.9 (C_s), 137.7 (C_o), 162.1 (C_p); δ_{F} (376.3 MHz, CDCl_3): -126.5 (CF_2), -123.4 (CF_2), -123.0 (CF_2), -122.0 (2 x CF_2), -1219 (CF_2), -113.5 (F-3), -81.2 (F-10); HRMS: m/z 633.9923 (M^+), $\text{C}_{17}\text{H}_{11}\text{F}_{17}\text{O}_2\text{S}_2$ requires 633.9929.

Sulfurane Compound X



To a stirred solution of unsymmetrical disulfide **89** (2.0 mmol) in CH_2Cl_2 (5 ml) under N_2 at $-78\text{ }^\circ\text{C}$ was added *m*-CPBA (0.45 g, 1.0 eq). The reaction was allowed to stir for 3 hours slowly warming to $0\text{ }^\circ\text{C}$, then quenched with saturated aqueous NaHCO_3 (20 ml) and extracted with CH_2Cl_2 (3×50 ml). The combined organic extracts were dried over anhydrous MgSO_4 , filtered and evaporated under reduced pressure. The crude material was purified by column chromatography with petroleum ether / ethyl acetate mixtures. Compound **X** (0.382 g, 52%) was obtained as a yellow oil; IR ν_{max} (CHCl_3)/ cm^{-1} 1318 (asym. SO_2 stretch), 1126 (sym. SO_2 stretch); δ_{H} (400 MHz, CDCl_3): 0.95 (3H, t, J 7.5 Hz, H-9'), 0.99 (3H, t, J 7.5 Hz, H-6'), 1.04 (3H, t, J 7.5 Hz, H-3'), 1.71 (4H, m, H-5' and H-8'), 1.91 (2H, m, H-2'), 2.73 (2H, t, J 7.5 Hz, H-7'), 3.07 (2H, t, J 7.5 Hz, H-4'), 3.24 (2H, m, H-1'), 7.03 (1H, m, H-5), 7.60 (1H, m, H-4), 7.69 (1H, m, H-3), 8.40 (1H, m, H-6); δ_{C} (MHz, CDCl_3): 12.7, 12.7, 13.1 (C-3', C-6' and C-9'), 17.4, 22.3, 23.2 (C-2', C-5' and C-8'), 38.1 (C-7'), 41.0 (C-4'), 64.4 (C-1'), 119.5 (C-3), 120.5 (C-5), 136.9 (C-4), 149.5 (C-6), 161.4 (C-2); HRMS: m/z 185.0331 (M^+), $\text{C}_{14}\text{H}_{25}\text{NO}_2\text{S}_4$ requires 367.0768.

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Poster: New Methodology for One-Pot, Green Synthesis of Unsymmetrical Disulfides.

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International Conference on Organic Chemistry, 04-09 June 2007, Ataturk University, Erzurum, Turkey.

Poster: Synthesis of Unsymmetrical Disulfides and Thiosulfinates.

N. Stellenboom, R. Hunter, M. R. Caira.

Description of the Appended Material and Explanatory Remarks

The Crystallographic Information Files (CIF files) for the cyclodextrin inclusion complexes **C3**, **C8** and **C9** are provided on the appended CD-ROM. These may be used with a molecular graphics program (e.g. ORTEP, WebLab Viewer, POV-Ray) to generate additional views of the molecular and crystal structures to those presented in the thesis, if required.

These files have been checked with the IUCr CHECKCIF routines ('basic structural check'). All syntax errors have been removed. However, numerous ALERTS remain in the CHECKCIF reports that refer to parameters which, for one reason or another, do not satisfy the standard tests. Examples are (a) 'no chemical absolute configuration info given', (b) 'large non-solvent Ueq(max)/Ueq(min)' and (c) 'Calc. and Rep. MoietyFormula Strings Differ'. In the case of the cyclodextrin complexes analysed, such ALERTS are usually readily explained since they arise from the structural nature of these species, including the presence of disordered residues on the host molecules, and disordered water and guest molecules that complicate the crystallographic least-squares refinement, thus requiring more latitude than usual in the interpretation of the ALERTS.

The specific ALERTS listed as examples above, are explained as follows: (a) This ALERT appears because there is no explicit statement of chemical absolute configuration in the CIF file. The reason is that the absolute configuration of the cyclodextrin (composed of D-glucopyranose residues) is known and no reliance is therefore placed on the crystallographic data to determine absolute structure; (b) 'large non-solvent Ueq(max)/Ueq(min)' indicates abnormally high thermal motion detected for atoms of the main residues (host and guest). This is typical of cyclodextrins and of their included guests, even at the low temperatures (~110K) to which the reported crystals were cooled for intensity data-collection; (c) Calculated and reported molecular formulae rarely coincide for crystal structures of cyclodextrin inclusion complexes. The calculated formula of an inclusion complex is typically based on chemical/spectroscopic analysis and accounts for all atoms present, whereas the structural model invariably contains fewer atoms. In particular, H atoms of water molecules in such complexes are generally not visible in electron-density maps and so are usually omitted from the structural model. Similarly, some water oxygen atoms may be omitted from the model because they are present in the crystal at low site-occupancy and often behave unsatisfactorily on attempted refinement.

It may, however, be possible to eliminate the causes of some of the remaining ALERTS listed in the CHECKCIF reports by further refinement strategies, and it will be appropriate to attempt this before the data for complexes **C3** and **C9** are submitted for final publication (the structure of **C8** has been published and the data deposited in the Cambridge Structural Database). It should be noted that very minor changes to the structural models presented are required to achieve this and that the presence in the current data of any deficiencies does not significantly detract from the essential correctness and accuracy of the reported structures.

