Synthesis of Sulfur-Containing Derivatives based on Garlic Metabolites and their Biological Activities

A Thesis Submitted to Cardiff University in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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ありがとうございます

Abbreviations

°C	Degrees Celsius
µL	Microliter
AIBN	2,2'-Azobis(2-methylpropionitrile)
aq.	Aqueous
Ar	Aryl
BPR	Back-pressure regulator
CV	Column volume
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	Diisopropyl ethylamine
DMF	Dimethyl formamide
DoE	Design of Experiments
EDG	Electron donating group
equiv.	Equivalent(s)
ESI	Electrospray ionisation
Et	Ethyl
EWG	Electron withdrawing group
g	Gram(s)
GP	General procedure

H	Hour(s)
HMBC	Heteronuclear Multiple Bond Coherence
HRMS	High Resolution Mass Spectroscopy
Hz	Hertz
i.d.	Internal diameter
IR	Infrared
J	Coupling constant in Hertz
LAH	Lithium Aluminium Hydride
M	Molarity (mol/L)
m/z	Mass over charge ratio
*m*CPBA	*meta*-Chloroperbenzoic acid
Me	Methyl
MeOH	Methanol
MHz	Megahertz
MIC	Minimum inhibitory concentration
min	Minute(s)
mL	Millitre
mm	Millimetre
mmol	Millimole
MNU	*N*-Methyl-*N*-nitrosourea
mol%	Mole percentage

n-BuLi	*n*-Butyl Lithium
nm	Nanometre
NMR	Nuclear magnetic resonance
OVAT	One-Variable-At-A-Time
Ph	Phenyl
PMB	*para*-Methoxybenzyl
PMP	*para*-Methoxyphenyl
ppm	Parts per million
psi	Pounds per square inch
PTFE	Polytetrafluoroethylene
r.t.	Room temperature
Rf	Retention factor
sat.	Saturated solution
TFA	Trifluoroacetic acid
TFAA	Trifluoracetic anhydride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet
vis	Visible
vs	Versus
wt%	Weight percentage

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1. Introduction to *Allium* Chemistry

Garlic (*Allium Sativum*), along with onions, leeks, and scallions, is part of the genus *allium*. The pungent smell and its observed health benefits are indicative of *allium* chemistry, which contain many volatile and reactive organosulfur compounds.[1]

For thousands of years, garlic has been a well-known component in many household recipes and traditional medicines in the treatment of an array of conditions. It has been hypothesised that consuming garlic can relieve the symptoms of common colds, wounds, ulcers and headaches.[2] Much attention has been drawn to *allium* chemistry and its biological properties. Thus, garlic has since been labelled as an antibacterial, antifungal, antiviral and antiprotozoal agent.[3] In addition to this, there are several reports of garlic offering beneficial properties in cardiovascular disease, prevention of stroke and atherosclerosis, and in the treatment of infections.[4]

1.1. Components of Garlic

Scientific studies into garlic began in the mid-19th century, where compounds were first isolated and identified from garlic oil. Due to the nature of the reactive and volatile organosulfur compounds, a range of techniques have been applied in their isolation and characterisation. The isolation method has been seen to affect the chemical composition, as many chemical and enzymatic reactions in garlic are catalysed by crushing, cutting or damaging the garlic clove.[1]

1.1.1. Diallyl Monosulfides and Polysulfides

Wertheim pioneered the early work of isolating compounds from garlic by steam distilling garlic bulbs at 140 °C. The first reported diallyl sulfur compound was in 1844 by Wertheim, described as a garlic oil. Further to this, Wertheim determined that the chemical composition was carbon (63.33%) and hydrogen (8.80%), and this garlic oil was named allyl sulfur.[5]

In 1981, Semmler, a German scientist, obtained a pale yellow oil from garlic bulbs that had been obtained by fractional distillation under reduced pressure. From the mixture,

he deduced that diallyl disulfide (**1**), diallyl trisulfide (**2**), diallyl tetrasulfide (**3**), collectively known as diallyl polysulfides, and propyl disulfide (**4**) was present (Figure 1.1). However, the allyl sulfur obtained by Wertheim was not present in this mixture. Semmler hypothesised that the difference in oil compositions arose from the difference in distilling procedures. For example, distillation under reduced pressure and lower temperatures, versus distillation at higher temperatures, yielded a different product mixture. This suggests that the allyl sulfur reported by Wertheim is formed from the degradation induced by heat sensitive diallyl polysulfides.[2]

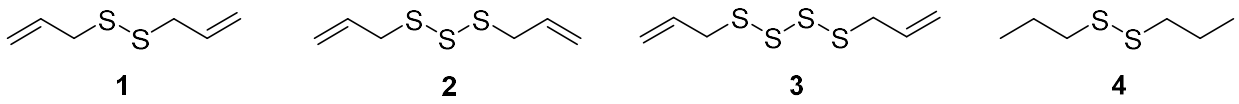


Figure 1.1: Polysulfides (**1**−**4**) obtained by Semmler in 1981.

In 1991, Lawson *et al.* reported the quantification and identification by High Performance Liquid Chromatography (HPLC) of sulfides found from steam distilled commercially available garlic. Lawson found that due to the volatility and instability of intermediate thiosulfonates and diallyl polysulfides, the mixture obtained was complex, containing numerous diallyl polysulfides (**1**−**3**), diallyl sulfide (**5**), variations of vinyl dithiins (**6**−**7**) and ajoene (**8**) (Figure 1.2).[6]

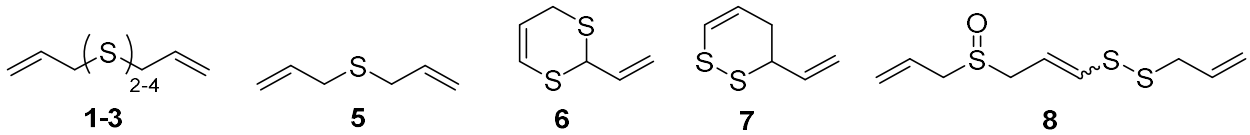
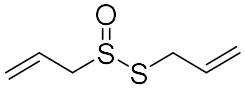


Figure 1.2: Compounds (**1**−**8**) obtained from steam distilled garlic as a mixture were separated using HPLC by Lawson *et al*. in 1991.

1.1.2. Allicin

Allicin (**9**) is the mono-oxidised version of diallyl disulfide (**1**) (Figure 1.3). Allicin has been the centre of much attention due to its reactivity and volatility, enabling the rearrangement to many other products, such as dithiins (**6**−**7**) and ajoene (**8**), otherwise difficult to synthesise (Figure 1.2). Allicin also exhibits biological properties,

such as antimicrobial[7] and antibiotic[4] properties, likely to be the result of the unstable, disulfide bond and its interaction with proteins in the cell.

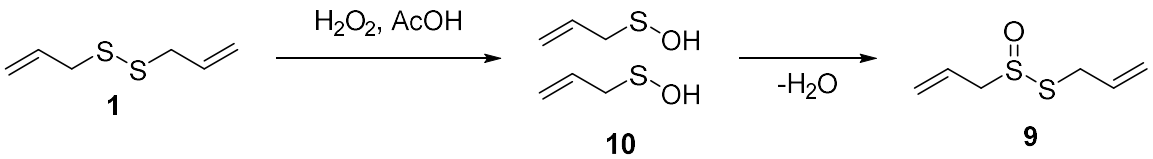


Allicin
9

Figure 1.3: Allicin (**9**) was first isolated by Cavallito in 1944.

In 1944, allicin (**9**) was isolated by Cavallito *et al.* (Figure 1.3). Different to previous distillation methods, Cavallito extracted compounds using ethanol and then removed the solvent *in vacuo,* maintaining the temperature at 50 °C. Cavallito described that the 'pungent oil' can be obtained in 0.15% yield, 6 g from 4 kg of chopped garlic using 5 L of ethanol. Cavallito hypothesised the structure of allicin by several reaction experiments where he noted which reaction products could form from allicin, and hence the structural arrangement.[8] He noted that, in some of his studies, the oily compound showed analogous antibacterial behaviour to penicillin. Further to this, it was found that allicin is not found in intact garlic cloves, but is rather a secondary metabolite that can be obtained from an enzymatic reaction, initiated by crushing and chopping the garlic.[2]

Allicin (**9**) was first chemically synthesised by mono-oxidation of diallyl disulfide in 1947 by Stoll and Seebeck (Scheme 1.1).[9] Stoll and Seebeck oxidised distilled **1** using a mixture of hydrogen peroxide and acetic acid. The peracetic acid that forms *in situ* generates two molecules of 2-propenesulfenic acid (**10**) that can spontaneously self-condense to form **9**.



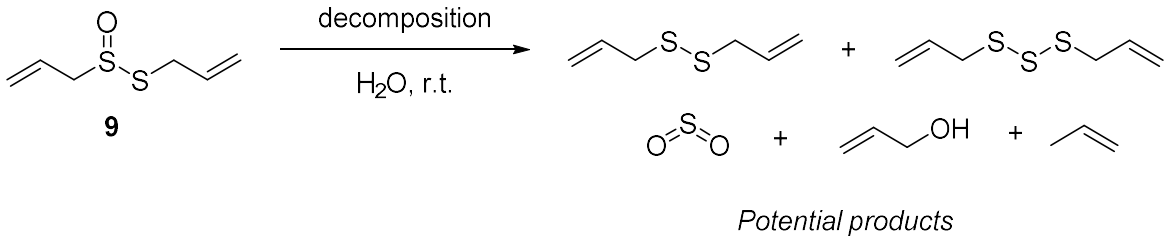
Scheme 1.1: Synthesis of allicin (**9**) from diallyl disulfide (**1**) using hydrogen peroxide and acetic acid.

Allicin can be produced from alliin in garlic (*Allium sativum L.*) in a reaction catalysed by the enzyme, *alliinase*. In a natural environment, this occurs when there is damage

to the tissue, although it can be induced by chopping or crushing the garlic cloves. The biosynthetic route by which the enzymatic reaction occurs is not yet fully understood, although Granroth *et al*. has employed much effort into radioactive labelling in an effort to determine the mechanism.[10]

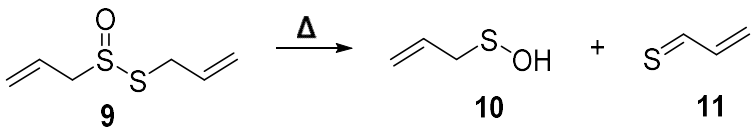
1.1.2.1. Thermal Rearrangements of Allicin

Owing to the volatility and instability of **9**, allicin can rearrange to a wide array of compounds. The products obtained have been observed to be dependent on the environment, particularly the solvent in which the rearrangement occurs. The transformations of **9** are a result of the weakened disulfide bond after oxidation. Work by Brodnitz *et al.*[11] and Yu *et al*.[12,13] reported that allicin can afford diallyl disulfide, trisulfide and other polysulfides, as well as allyl alcohol, propene and sulfur dioxide. This decomposition of **9** occurs at room temperature in water (Scheme 1.2).



Scheme 1.2: Potential products as a result of decomposition of **9**.

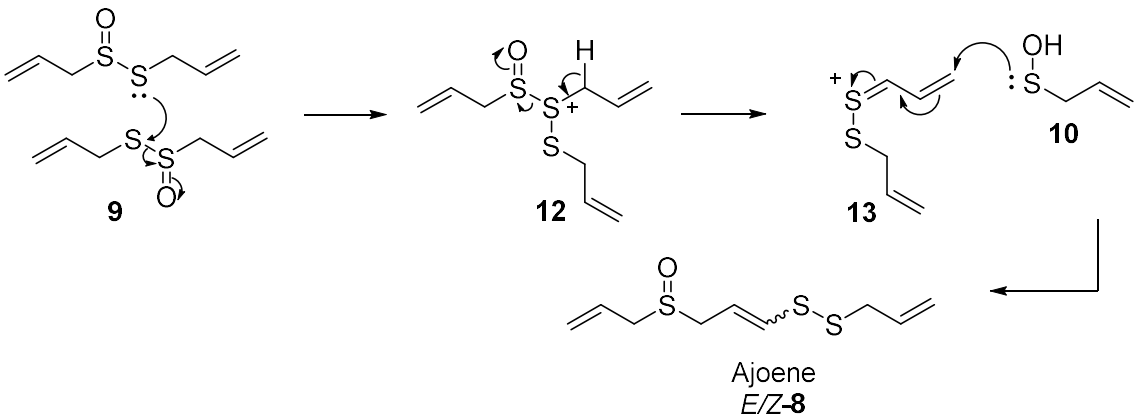
The decomposition of **9** forms 2-propenesulfenic acid (**10**) and thioacrolein (**11**) (Scheme 1.3). This is a key step in the decomposition mechanism of **9**. The newly formed volatile and reactive species can react in a number of ways, forming a wide spectrum of products.



Scheme 1.3: Key step in the decomposition of **9** to 2-propenesulfenic acid (**10**) and thioacrolein (**11**).

1.2. Ajoene

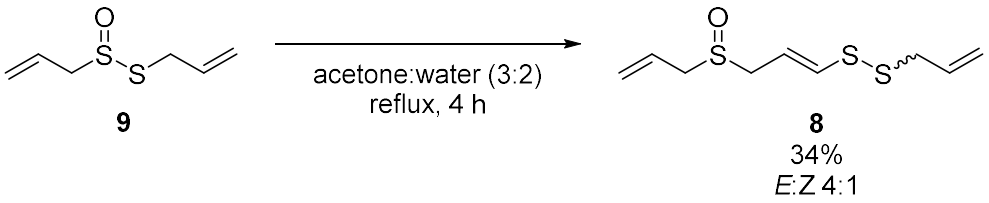
(*E*/*Z*)-Ajoene (**8**) is one of the more interesting products that **9** can form upon thermal rearrangement. This finding was first noted while boiling garlic in water, giving a product that acted as an antithrombotic agent. Although at the time this product was uncharacterised, it was later confirmed to be a novel nine carbon, three sulfur and one oxygen compound, named ajoene. A proposed mechanism is the dimerisation of two molecules of **9**, releasing a molecule of **10**. The newly formed thiosulfonium ion (**12**) rearranges to eliminate a second molecule of **10**. The newly formed thioacrolein type molecule (**13**) is now susceptible to attack in the gamma position to quench the positive charge on the sulfur atom. According to the geometry of the thio-containing diene in **13**, both *E*-**8** and *Z*-**8** can be formed (Scheme 1.4).[14]



Scheme 1.4: Rearrangement of allicin to (*E*/*Z*)-ajoene (**8**).

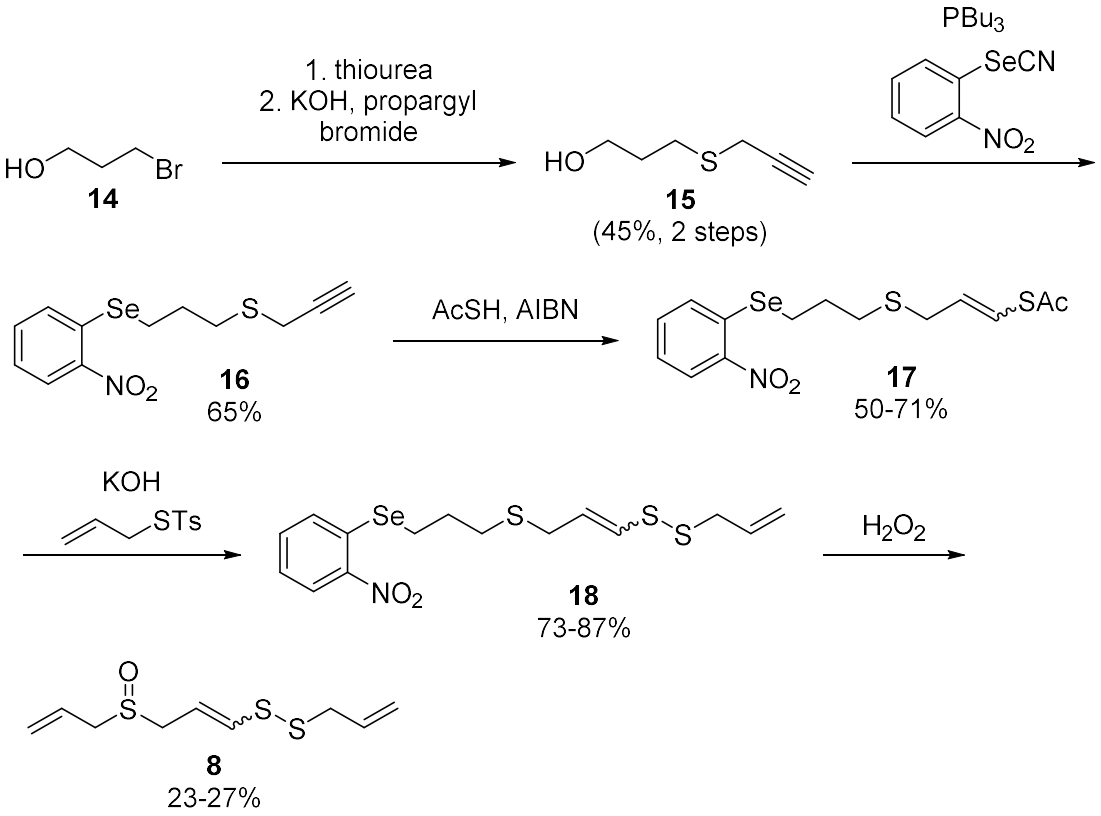
Ajoene, **8**, has been a compound of interest due to its unusual structure. Although of relatively low molecular weight, the compound encompasses a chiral sulfoxide, a central olefin giving geometric isomers, a vinylic and unsymmetrical disulfide bond, and two terminal allyl groups. Isomers of **8** have been extensively studied in biological testing. In 1984, Block *et al*. assessed ajoene as an antithrombotic agent. The potency of **8** was at such a level where 100% inhibition of platelet aggregation could be seen in rabbits for a 24 hour period after consuming **8**.[14] More recently, in 2019, Lee *et al*. determined the anti-pancreatic cancer activity of *Z*-**8**, and described the isomer as 'a lead compound for the development of anti-pancreatic cancer agents'.[15]

Previously ajoene has only been formed as a result of boiling whole garlic cloves. However, the rearrangement of **9** to **8** was investigated thoroughly by E. Block *et al.* who in 1984 reported a solvent system of 10% (v/v) of **9** in acetone/water (3:2) that gave rearrangement to **8** in 34% yield (Scheme 1.5).[14]



Scheme 1.5: Thermal rearrangement of allicin (**9**) to ajoene (**8**) in aqueous acetone.[14]

Ajoene remained a key compound of interest, however, it was not until 2018 that the first short total synthesis of **8** was reported by Silva *et al.* (Scheme 1.6).[16] In the first total synthesis, **8** could be obtained in up to 5% yield over five steps. The synthetic route starts with a simple alcohol containing a labile bromide (**14**). Treatment with thiourea gives a thiourea salt of **14** *in situ*, which can be propargylated yielding **15** in 45% over 2 steps. The addition of phenyl selenide moiety is introduced to obtain compound **16** in 65% yield. The purpose of the selenyl protecting group was to unmask the terminal allyl group. With the terminal alkyne installed, a radical addition of thioacetic acid can afford compound **17** in 50–71% yield. The acetate protecting group is deprotected with a simple base such as potassium hydroxide, and the resulting thiolate is thiolated to form the disulfide with the terminal allyl group (**18**) in 73–81% yield. Finally, oxidation of both the sulfide to sulfoxide, and the selenide to the selenoxide, affords target compound **8** in 23–27% yield. Due to the presence of β−hydrogens relative to the selenium in **18**, the selenyl elimination occurs immediately, unmasking the terminal allyl group.



Scheme 1.6: Short total synthesis of **8** reported by Silvia *et al.* in 2018.[16]

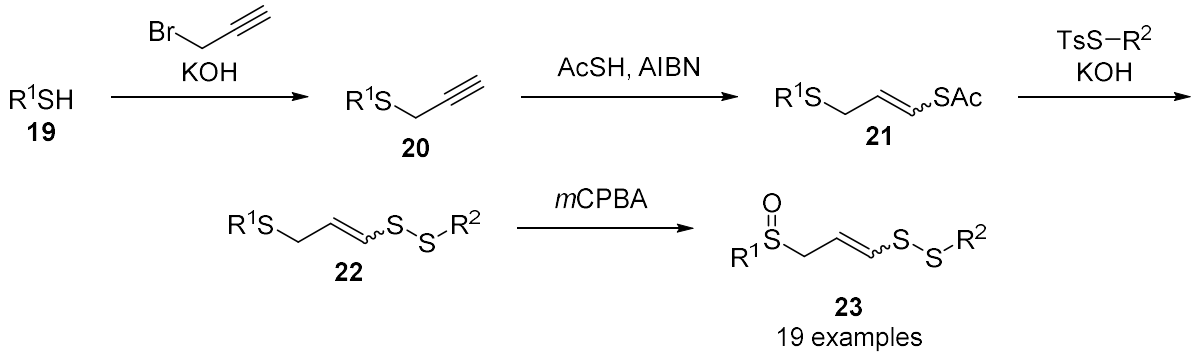
1.2.1. Ajoene Analogues

Despite efforts, until recently only the thermal rearrangement to synthesise **8** was known. Thus, over many decades, attention turned to developing analogues of **8** in an effort to obtain a molecule of similar chemical structure that possessed the same, or ideally enhanced, levels of potency and biological properties.

Block *et al*. in 1986, produced a series of analogues resembling the structure of **8**. Modifications included over-oxidation of the sulfoxide to sulfone, oxidation of one of the sulfur atoms in the disulfide bond, modifications in the nature of the terminal allyl groups to alkyl, aryl or esters, as well as changing the central double bond to an aromatic ring. Analogues were tested for their antithrombotic activity in an effort to determine the origin of the antithrombotic activity observed in **8**.[4]

In 2008 and in 2011, Hunter *et al*. reported a library of compounds (**23**) that encompassed the pharmacophore of ajoene (**8**) (Scheme 1.7).[17,18] Analogues were

developed in an investigation into novel anti-cancer agents and anti-proliferation activities. The synthetic route utilised the nucleophilicity of a thiol (**19**). Upon treatment with a base and propargyl bromide, sulfide **20** could be synthesised. Radical addition of a thioacetate realised the central olefin and vinylic sulfur atom of **21**. Treatment with a base and electrophilic sulfur source, such as a thiotosylate reagent, afforded the desired disulfide (**22**). Although a range of analogues were investigated, **8** itself could not be obtained by the developed route due to the unstable vinyl radical precursor that would be required (Scheme 1.7). Hunter also reported that a particular analogue containing *para*-methoxy benzyl (PMB) substituted terminal ends at both the sulfoxide and disulfide position, led to a compound with twelve times higher activity than *Z*-**8**.

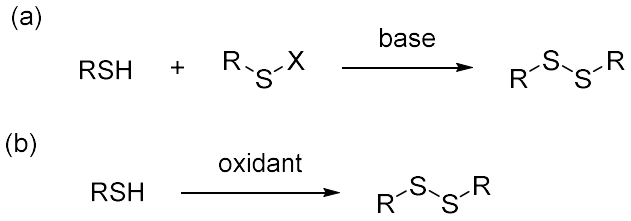


Scheme 1.7: Compound **23** as analogues of **4** synthesised by Hunter *et al*. over four steps.[17]

More recently, Block *et al*. investigated fluorinated analogues of **8** which were synthesised from the rearrangement of fluorinated versions of **9**. The reasoning behind producing fluorinated analogues was to enhance the reactivity toward thiol groups in the protein and thus influence its biological activity.[19]

1.3. Disulfides and their Biological Activities

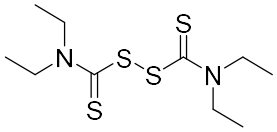
The preparation of unsymmetrical disulfides have been thoroughly investigated and two general methods exist. The first method is the reaction between an electrophilic sulfur source and a nucleophilic sulfur source, such as a thiol (Scheme 1.8a). The second method is the oxidative heterocoupling requiring an oxidising or coupling agent such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or a metal (Scheme 1.8b).[20–22]



Scheme 1.8: General methods to form disulfides from a thiol. (a): nucleophilic thiol reacting with electrophilic sulfur source, where X is a labile leaving group. (b): oxidative coupling of thiols.

Disulfide bonds are important structural motifs as a linkage in enzymes. In addition, disulfide compounds are well known for exhibiting biological activity, although their mode of action is still not completely understood. For example, a simple disulfide such as diallyl disulfide has been reported to inhibit the proliferation of human tumour cells.[23] Diallyl disulfide was found to exhibit twice the activity of *S*-allyl cystine.[24] The biological activity of disulfide compounds have been of significant interest over the past twenty years, with focus on establishing a mode of action in both eukaryotic and prokaryotic cells.[25] In addition to their inherent biological activity, disulfide bonds have also been employed to synthesise lipophilic prodrugs. Increased lipophilicity is advantageous in a biological system as it can aid adsorption and reduce the biodegradation of the compound.[26] In addition, other reports have concluded that the disulfide analogues of thiono-sulfur drugs were up to 100 times more potent in comparison to the parent drug.[27]

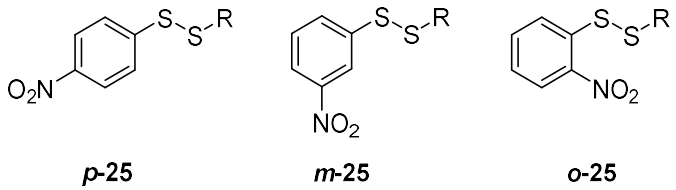
An example of a Food and Drug Administration (FDA) approved drug containing sulfur atoms is disulfiram (**24**). Drug **24** is a small molecule drug sold under the name 'Antabuse'. It is used to support the treatment of acute alcoholism, by inducing the patient to become sensitive to alcohol, thus experiencing negative side effects from small volumes of alcohol soon after consumption. In addition to this, it has also been studied as a possible anti-cancer drug (Figure 1.4).[28]



Disulfiram / Antabuse
24

Figure 1.4: Disulfiram (**24**) is a small molecule drug to treat acute alcoholism.

In 2008, Turos *et al*. investigated aryl-alkyl disulfides as growth inhibitors for *Staphylococcus Aureus* and *Bacillus Anthracis.* Several unsymmetrical disulfides synthesised exerted strong bioactivity, where the antibiotic activities were noted to be significantly higher in nitrophenyl derivatives (**25**). The proposed reason for this observation was that the arylthiol moiety is activated electronically as a leaving group, enabling the facile nucleophilic attack on the disulfide bond. In addition, where the nitrophenyl disulfide was coupled with a small alkyl residue, promising biological activities could be observed (Figure 1.5).[29]



p-**25** *m*-**25** *o*-**25**

Figure 1.5: Nitroaryl-alkyl disulfides (**25**) investigated by Turos *et al*. R = methyl, ethyl, isopropyl, *sec*-butyl, *n*-butyl.[29]

Work by Danquah *et al.* reported a series of analogues containing disulfides based on natural compounds from Persian shallots (*Allium stipitatum).*[30] Similar to those compounds discussed from *Allium sativum*, compounds from Persian shallots also possess antibacterial properties. Analogues were prepared by methylthiolation of thiols. Naturally occurring compounds isolated from Persian shallots (**26**–**28**) were determined to have a minimum inhibitory concentration (MIC) between 2.5–40 μM. In comparison, analogues prepared (**29**–**31**) were determined to have a MIC between 12.7–19.3 μM. From the series, it was found that disulfides with proximal electron withdrawing groups, such as pyridine, pyridine *N*-oxide, pyrimidine and quinoline, are required to obtain the desired biological activity (Figure 1.6).[30]

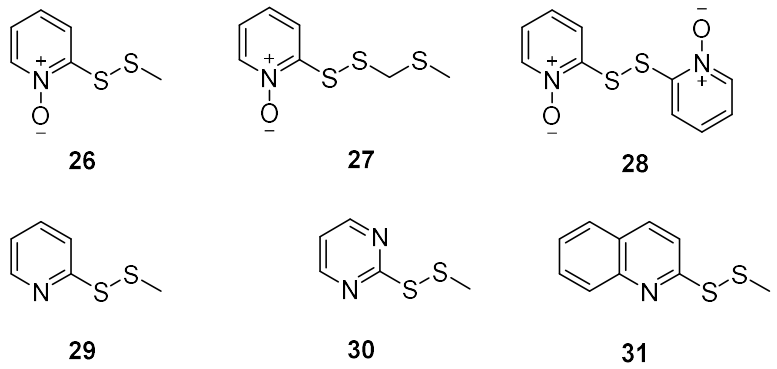
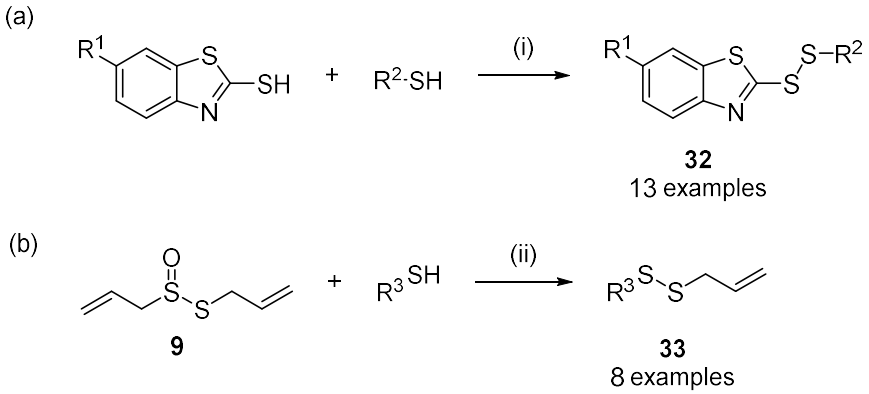


Figure 1.6: Examples of compounds isolated from *Allium stipitatum* (**26**–**28**) and a selection of synthesised analogues based on natural products (**29**–**31**).

More recently, Fong *et al*. in 2017 produced a library of 21 disulfide bond-containing analogues based on **8** and tested their inhibition levels for quorum sensing in *Pseudomonas Aeruginosa*. Structural activity studies determined that the allyl group is not essential and could be modified, whilst retaining or improving on activity. (Scheme 1.9).[31]

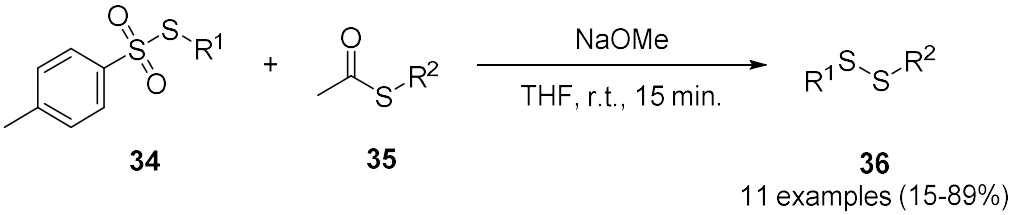


Scheme 1.9: Analogues of the type **32** and **33** produced by Fong *et al*. Reagent and conditions: (i): DDQ, CH_2Cl_2, 0 °C; (ii) MeOH. R^1: H, OEt, Cl, CF_3, Me; R^2: isopropyl, phenyl, 4-chlorophenyl, cyclohexyl, substituted thioazoles; R^3: substituted thioazoles, benzyl.[31]

1.3.1. Bis-Disulfide Compounds

Bis-disulfide compounds are well known in the literature and have been used for a variety of purposes. With the tendency to form polymers with multiple disulfide bonds, polydisulfides have found an application for surface chemistry. A range of cyclic bis-disulfides were synthesised by Houk *et al.* in 1989 to investigate their stability

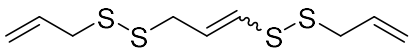
towards polymerisation, although were not biologically evaluated.[32] More recently, Mayor and co-workers in 2019 prepared a range of unsymmetrical disulfides (**36**) from thiotosylates (**34**) and thioacetates (**35**) in order to functionalise a gold surface through immobilisation (Scheme 1.10). Such disulfides are currently under investigation as an application as an anchor group in the preparation of self-assembled monolayers.[33]



34 **35** **36**
11 examples (15-89%)

Scheme 1.10: Synthesis of disulfides (**36**) through thiotosylates (**34**) and thioacetates (**35**) by Mayor *et al.* R^1: 1-adamantyl, 4-$MeOC_6H_4$, $CH_3CH_2(OCH_2CH_2)_2$, $CF_3(CF_2)_2CH_2CH_2$, pentyl, 4-NO_2-C_6H_4, 4-NH_2-C_6H_4, 3,5-$F_2C_6H_3$, 2-$CO_2MeC_6H_4$. R^2: 4-MeC_6H_4, hexyl, 4-NO_2-C_6H_4, 4-MeO-C_6H_4.[33]

With the disulfide moiety seeming to be a key factor responsible for producing the observed biological activity, it is reasoned that bis-disulfide compounds could hold superior potency due to containing two reactive functional groups. Compound **37** is a natural product which was isolated from Chinese garlic by Nohara *et al*. in 2014 (Figure 1.7). Although Nohara *et al* tested other acyclic sulfides in their abilities to supress macrophage activation, bis-disulfide **37** was extracted, but not tested.[34]



37

Figure 1.7: Allyl bis-disulfide (**37**) isolated from garlic in 2014 by Nohara *et al*.[34]

1.4. References

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2. Introduction to Flow Chemistry

Flow chemistry is a method for conducting reactions by flowing reagents through a channel, rather than stirring in a flask as in traditional methods. Flow chemistry has been developed largely over the past twenty years, where it has been applied in various syntheses of natural products, active pharmaceutical ingredients as well as other novel compounds.[35]

Major differences in flow chemistry and batch chemistry can be divided into three main areas, that is, the reaction time, the stoichiometry, and the reaction profile. Firstly, in batch chemistry, the reaction time is the time that the reagents are being exposed to a certain condition. However, in flow chemistry, residence time is the parameter of importance. Residence time can be defined as the time spent in the flow reactor, depending on the volume of the flow reactor and the flow rate. As the reaction is continuously flowing, the reaction profile will be steady, although different at each point along the reactor coil. The concentration of reactants will relate to the distance travelled, where the concentration of the starting reagents at a shorter distance, thus shorter 'reaction time', will be higher. This contrasts with batch chemistry, where the concentration is relatively uniform throughout the flask at a given point if there is efficient mixing. Lastly, in flow chemistry, it is important to consider that the flow rates of each pump in conjunction with its the concentration, is equivalent to the stoichiometry of the reactants.[36]

Flow chemistry has several benefits which are widely accepted. These benefits include vigorous mixing within the narrow channels in which the reagents are flowed, improved safety as hazardous and volatile chemicals or unstable intermediates are contained within a closed system, and an increase in heat transfer efficiency. The main advantage of flow chemistry is a direct result of the size of reaction space, which is reflected in the significantly higher surface area to volume ratio of the reactor.[37] Advantages as outlined by Hartman[38], Noël[39] and Elliott[40] also include the high concentration of reactions, if not neat, the ability to perform reactions at high temperatures and pressures unattainable in batch chemistry, and lowered operating volumes. Since flow chemistry uses initially low

operating volumes, as systems have the potential to run over a long period of time, the Space-Time-Yield (STY) can become superior over the equivalent batch process. The STY is a measure of the mass of material obtained per volume, per time frame, usually hours or days.[37] However, in addition to advantages that may arise from utilising flow chemistry, there are also a number of factors which must be considered. Such factors include the type of flow and mixing occurring between phases, the temperature of reactions, particularly where there is the potential for exothermic 'runaway' reactions, and the type of reactor required, for example, chip-based reactors and flow-based reactors.[41] In addition, the use of a back pressure regulator (BPR) controls the pressure of the system, enabling solvents to be heated to temperatures that exceed their boiling point (Figure 2.1)

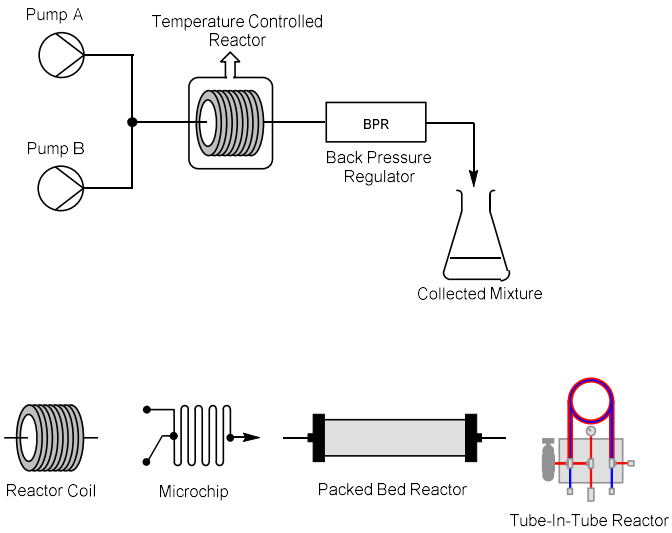


Figure 2.1: Basic flow chemistry set-up with examples of type of reactors.

2.1. Continuous Flow Chemistry

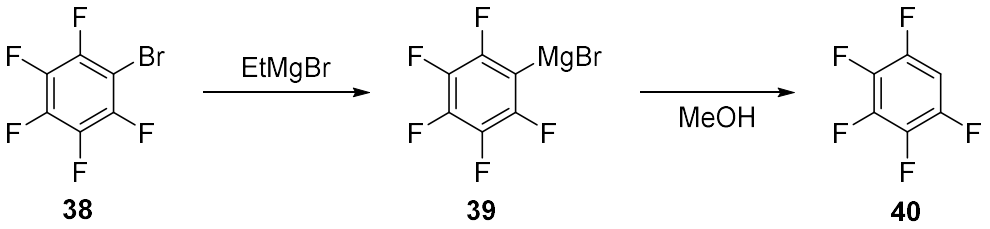
Multistep flow chemistry is when two or more single steps are combined and conducted in flow. Continuous flow chemistry is when a flow system can be run over an extended period of time without interruption. Continuous flow chemistry has been applied to many areas of chemistry, including the total synthesis of pharmaceuticals. Flow chemistry is also developing outside of academia within the manufacturing industry. Continuous flow chemistry has offered process

intensification, where the plant size can be significantly reduced at a constant volume of product output.[42]

2.1.1. Temperature Control

Temperature is an important variable providing the thermal energy that is required to overcome the activation energy barrier to yield the desired product. In addition to this, the precise control of temperature can often give the desired reactivity or selectivity to the target material over its potential by-products,[43] prevent degradation of desired product,[44] and can provide safer handling of thermally explosive intermediates.[45]

In flow chemistry, a significant advantage is the efficient heat transfer to and from the reaction mixture. When applying thermal energy to the reaction in a flow system, the high surface area to volume ratio makes heat transfer more efficient than in its comparable batch system. However, this is also important when considering exothermic reactions, particularly in cases where thermal 'runaways' are possible. The diffusion of heat from the reaction mixture to the surroundings, reduces the possibility of a thermal 'runaway' occurring, and thus the temperature is maintained within a narrow and controlled range. For example, penta-fluorobenzene (**40**) can be obtained using Grignard reagents from **38**. Grignard reagents are notoriously difficult to control due to the reactions typically proceeding exothermically. However, Wakami *et al*. proved that a flow system can produce 97% of **40** *via* **39** in 5 seconds. Under flow conditions, the runaway reaction is prevented and accurate control over the reaction temperature is possible. In comparison, this is extremely difficult to control in batch. In addition to this, the flow procedure was applied to a pilot plant scale providing 14.7 kg of **40** in 92% over 24 hours (Scheme 2.1).[46]



Scheme 2.1: Synthesis of pentafluorobenzene (**40**) by Wakami *et al*.

2.1.2. Mixing in a Flow System

Mixing is an important consideration which relates to the concentration of reactants and potential gradients. To achieve efficient mixing in batch, the whole volume of the reaction mixture should mix homogenously, rather than localised in one area.

In a flow system, the type of mixing will be partially determined by the scale, however, mixing can be described through a Reynolds number. Laminar flow, shown in Figure 2.2 (A), is when the two liquids are flowing parallel to each other, without disrupting the longitudinal flow. Slug flow (B), often achieved by using a T−piece mixer, producing a droplet of each phase, disturbing the longitudinal flow (Figure 2.2).[41]

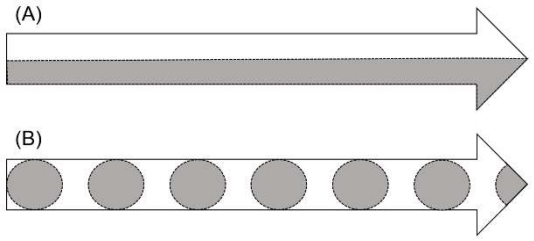


Figure 2.2: Most common type of flow between liquids. (A): Laminar flow; (B): Slug flow.

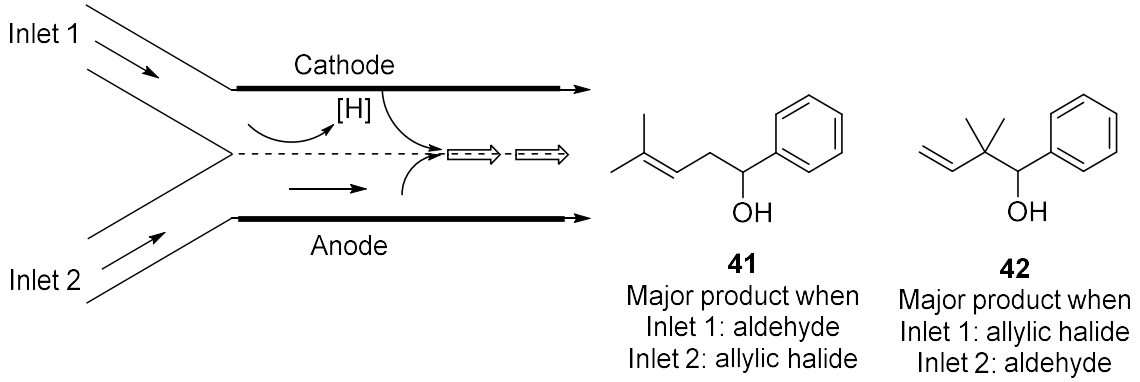
The Reynolds equation is the relationship between inertial forces (vL) and the viscous forces (υ) (Equation 2.1). Laminar flow occurs when Re < 2000, achieved by low flow rates, high viscosity liquids and large channels. Slug flow occurs when Re > 3000.

$$Re = (vL) / \upsilon$$

Equation 2.1: Reynolds Equation. Inertial forces: v: velocity (m/s); L: diameter of fluid (m). Viscous forces: υ: kinematic viscosity (m^2/s).

Flow chemistry has been applied to systems that are sensitive to the uneven mixing that often occurs in batch systems. Tanaka *et al.* reported that microfluidic conditions saw advantages such as efficient heat transfer and mixing, which allowed complex reactions to proceed in high yields due to accurate control over parameters. The functionalisation of secondary amines were investigated and allowed a significant reduction in reaction time in comparison to batch mode, from 24 hours to 2 minutes.[47]

Amemiya *et al.* also demonstrated the enhanced product selectivity in the allylation of carbonyls that could be achieved when using a flow system. The carbonyl allylation usually gives rise to two regioisomers, that is the α-adduct (**41**) and γ-adduct (**42**). Both adducts have importance in many natural products and pharmaceutical ingredients. Therefore, it is advantageous to create a synthetic strategy that illustrates chemoselectivity for either adduct over the other. Amemiya *et al.* reported that in a parallel laminar flow mode, chemoselectivity could be achieved with selective cathodic reduction of either the allylic halide or the aldehyde. Inlet 1 in laminar flow will be in contact with the cathode. Therefore, the reagent in inlet 1 will be selectively reduced by electrochemical methods. The control over adduct formation is unattainable in batch due to the mixing mode, which would inevitably lead to non-selective reduction (Scheme 2.2).[48]

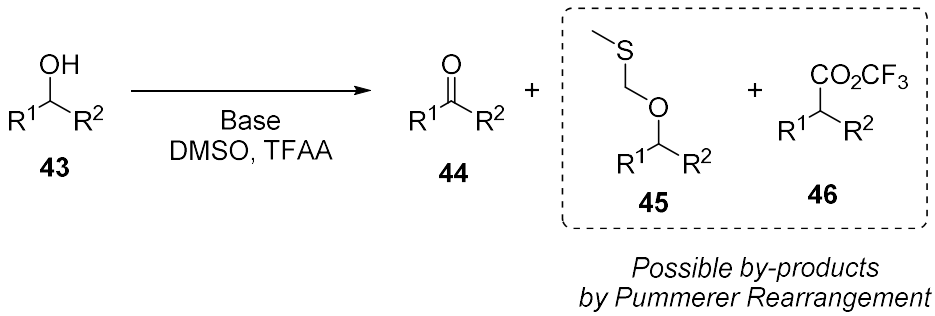


Scheme 2.2: Parallel laminar flow mode allows for the selective synthesis of α-adduct (**41**) or γ-adduct (**42**).

2.1.3. Residence Time

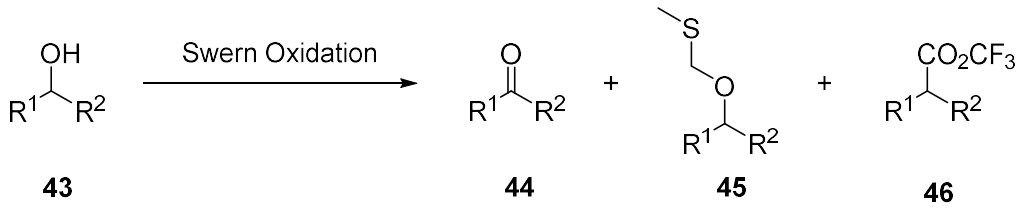
In batch mode, the reaction time is defined as the time that the reactants are in contact with each other and allowed to react. This time can range from minutes to days. Reactions that can be completed in seconds are usually difficult to control in batch as it is impractical to add all reagents together, stir the reaction and then quench the reaction after a short period of time. However, using flow systems, short reaction times are easily achieved. The reaction time, when considering a flow process, is identical to the residence time. This is usually in seconds or minutes and is controlled by the flow rate of reagents through a reactor coil or column and quenched at the end. As previously discussed, the residence time is usually much shorter than the comparable batch reaction due to such efficient mixing and heat transfer.

Using very short reaction times, Kawaguchi *et al.* reported a selective Swern oxidation of a range of alcohols **43**, such as 1-decanol, 2-octanol, cyclohexanol and benzyl alcohol, to yield products **44** in up to 95% yield. Kawaguchi and co-workers also reported that their flow system saw a reduction in by-product formation in all cases, where **45** and **46** were obtained in less than 22%, or in some cases, were not detected at all. The durability of the system was also tested and was shown to be stable for 3 hours at room temperature with no reduction in the yields or selectivity (Scheme 2.3).[49]



Scheme 2.3: Swern oxidation of generic alcohols (**43**) to their corresponding carbonyl compounds (**44**) can lead to by-products by Pummerer rearrangement.

Due to the cationic intermediate that forms between dimethyl sulfoxide and trifluoroacetic anhydride (TFAA), the first step is usually performed at temperatures not exceeding −50 °C, in order to enhance the selectivity of the desired reaction pathway over the formation of by-products. Once the cationic intermediate reacts with the alcohol, the reaction is treated with a base. However, the addition of base can also enhance the formation of by-products. Thus, in batch mode, the reaction is usually kept between −50 °C and −30 °C to allow reaction control. However, Kawaguchi *et al.* reports that for a variety of alcohols, conducting the Swern oxidation on a microfluidics scale enhanced the selectivity, despite temperatures exceeding −30 °C.



43a: $R_1 = C_9H_{19}$, $R_2 = H$
Flow: 0.01 s at 20 °C: **44a** (71%), **45a** (6%), **46a** (22%)
Batch: –20 °C: **44a** (11%), **45a** (1%), **46a** (90%)

43b: $R_1 = C_7H_{15}$, $R_2 = H$
Flow: 0.01 s at 20 °C **44b** (89%), **45b** (3%), **46b** (2%)
Batch: –20 °C: **44a** (20%), **45a** (2%), **46a** (75%)

43c: R_1 = benzyl $R_2 = H$
Flow: 0.01 s at 20 °C **44c** (75%), **45c** (-), **46c** (16%)
Batch: –20 °C: **44c** (49%), **45c** (-), **46c** (50%)

Scheme 2.4: Results of Swern oxidation conducted in flow and batch system for 1-decanol (**43a**), 1-octanol (**43b**) and benzyl alcohol (**43c**).

Kawaguchi reported that in all cases for alcohols investigated, for a range of residence times (0.01–2.4 s) between −20 °C and 20 °C, the microfluidic system gave not only higher conversion and yields, but also higher selectivity for the desired carbonyl (**44**). The feasibility of the oxidation to occur at room temperature was attributed to the short residence times and intense mixing that a microfluidic system can offer, that would otherwise be impossible to reproduce in a batch method.[49]

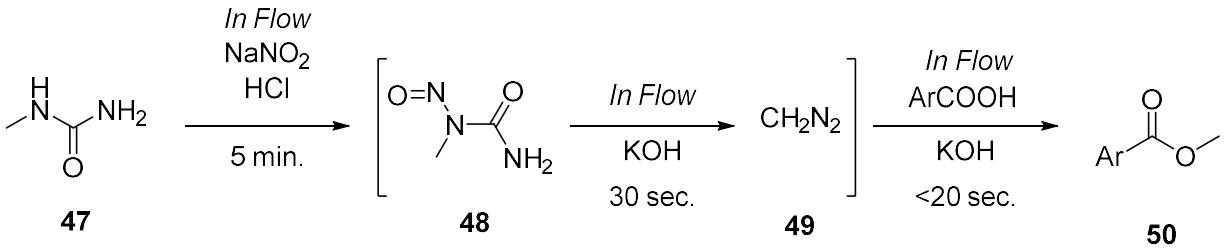
2.1.4. Handling of Toxic and Volatile Compounds

Flow chemistry conducts reactions in closed systems and therefore will allow toxic, unstable, and volatile compounds to be contained within the flow system. This not only makes for safer handling for the operator by reduced exposure, but also can lead to reactive intermediates reacting *via* the desired pathway rather than decomposing in the reaction media.

Diazomethane is widely used in the formation of carbon-carbon and carbon-heteroatom bonds. The attractiveness of using diazomethane as a reagent stems from the high atom economy and the production of nitrogen gas as by-product, which is environmentally benign and easy to remove. Despite this, the application of diazomethane to industry has been hindered by its toxicity. Diazomethane is a strong carcinogen, poison and explosive, and due to its low molecular weight (42.04 g/mol) and its low boiling point (−23 °C), controlling exposure is particularly difficult. Flow chemistry has been applied to the *in situ* generation and subsequent direct reaction of diazomethane, thus overcoming issues surrounding control of exposure. Yang *et al*. in 2018 reported many continuous flow set-ups where diazomethane could be generated and consumed. All flow set-ups were developed with an industrial perspective which could potentially allow diazomethane to be utilised on a kilogram scale.[50]

In 2017, Lehmann *et al*. also reported the continuous flow procedure for the generation and consumption of diazomethane (**49**). Compound **49** could be synthesised from in-line generated of *N*-methyl-*N*-nitrosourea (MNU, **48**), which is also a carcinogenic compound that is difficult to handle. For the consumption of **49**,

the flow system was applied to the methylation of carboxylic acid in flow to give desired ester (**50**) in excellent yields (96–99%) and high productivity (16.4–27.9 g/h) (Scheme 2.5).[51]



Scheme 2.5: Scalable and safe continuous flow system with the *in situ* generation and reaction of diazomethane (**49**).

Diazomethane synthesis in flow has been widely reported, with many recognising the beneficial switch from traditional batch chemistry towards an innovative flow synthesis from as early as 2008.[52]

Phosgene (**51**) is a synthetically useful small molecule, which can be used in chloro-carbonylation, carbonylation, chlorination and dehydration. However, phosgene is a toxic poison that disrupts a biological system to induce asphyxiation at very low levels. Being a volatile colourless gas with a boiling point of 8 °C, it is extremely challenging to handle.[53] An alternative to phosgene is often triphosgene (**52**). However, it was found that **52**, can still decompose to **51** and therefore, safety concerns are still present (Figure 2.3).

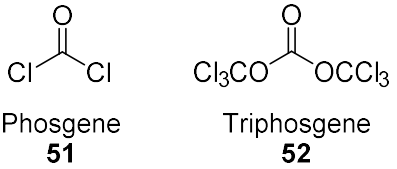


Figure 2.3: Phosgene (**51**) and its most common alternative, triphosgene (**52**).

Various reports of phosgene (**51**) incorporated flow systems have been developed. In 2001, Ajmera *et al*. demonstrated a silicon-based packed bed reactor for the in-flow synthesis of **51** from chlorine gas and carbon monoxide gas with a productivity of 1.1 g/h. In this way, the hazardous compound could be synthesised on site when required. The outlet of the developed system was reacted with a

solution of cyclohexylamine. The results of which gave complete conversion to the desired product, cyclohexylisocyanate.[54]

In 2013, Leroyer and Pratt *et al.* investigated the generation and consumption of **51** *in situ* from **52**. The objective of this research was to prove the upscaling past the laboratory scale to a pre-industrial plant scale, producing kilograms of desired product (**55**) per hour. The first flow step is the reaction between **52** and **53**, generating **51** *in situ*. The following step saw the reaction of **51** with a secondary amine (**54**) to afford **55** in high yields (>74%) in under 5 seconds. This system was transferred to three scales, initially producing product successfully at a rate of mg/h, then at 10 g/h, then at 1 kg/h. Generating **51** at 1 kg/h would be practically extremely challenging and dangerous in batch and pose serious health and safety concerns. However, through this system, **51** is consumed shortly after being generated, thus, the safety concerns are significantly reduced and the handling of this toxic compound becomes much easier (Figure 2.4).[55]

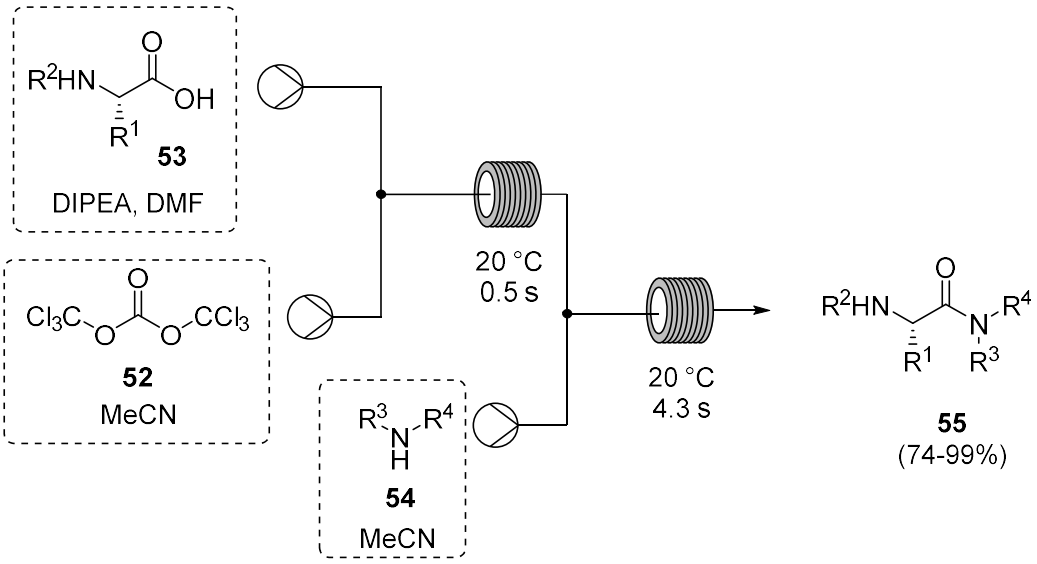


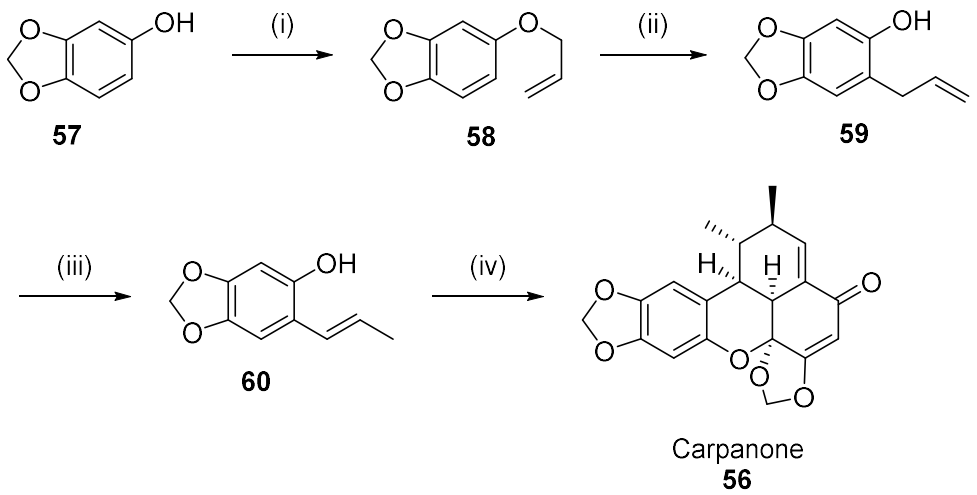
Figure 2.4: *In situ* generation of **51** from **52** and direct reaction with secondary amine (**54**) to give desired amide (**55**).

2.2. Natural Product Synthesis in Flow

Natural product synthesis has been the focal point of synthetic organic chemistry for many years, with the aim to synthesise ever more complex structures found in nature from simple starting materials. Natural product synthesis is also closely linked with drug discovery programmes where developed synthetic routes aids the

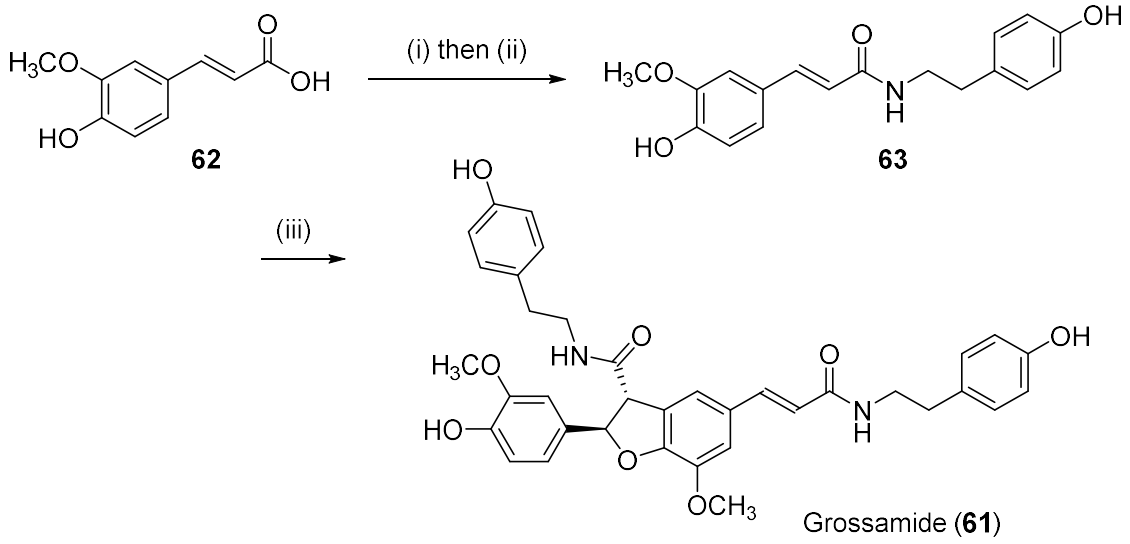
synthesis of other similar analogues that may exhibit enhanced biological activity. However, this task incurs many obstacles such as reproducible scale-up of synthetic routes, handling of unstable intermediates and complex purification of desired product from potential by-products.[56]

A recent example of natural product synthesis in flow was demonstrated by Cortés-Borda *et al.* in 2018 in their reported synthesis of Carpanone (**56**). Natural product **56** can be isolated from the bark of a carpano tree and contains a benzoxanthenone structure. The synthetic route starts with the allylation of alcohol **57** using allyl iodide and potassium hydroxide to afford ether product **58** in up to 80% yield under optimised conditions. The following step is the [3,3]-Claisen rearrangement of **60** to yield **56**. Although high temperatures are usually required for thermal activation, Cortés-Borda *et al*. reasoned that their flow system could be pressurised to allow elevated temperatures to be achieved with low boiling point solvents. Through optimisation, **59** could be obtained in a quantitative yield from a rearrangement of **58** in acetone at 222 °C for 27.6 minutes pressurised at 70 bar. The isomerisation from **59** to **60** proceeded well, where the desired *(E)*-**60** was achieved in 91% yield, with only trace amount of the *(Z)*-**60** isomer observed. Finally, **56** could be achieved in 69% from the oxidative dimerisation of **60**. Although their synthesis was based upon a previously reported synthetic route, the novelty of Cortés-Borda's work lays in the experimental aspect and the transformation of the batch synthesis into a flow system. Not only was a continuous flow synthesis developed, but the system was also automated, incorporating a self-optimising feedback algorithm. The system also included an inline NMR analysis as well as online HPLC analysis. In this way, **56** was able to be obtained over four steps in 67% overall yield (Scheme 2.6).[57]



Scheme 2.6: Synthesis of Carpanone (**56**) from **57** over four steps: (i): Allylation; (ii): [3,3]-Claisen Rearrangement; (iii): Isomerisation; (iv): Oxidative dimerisation.

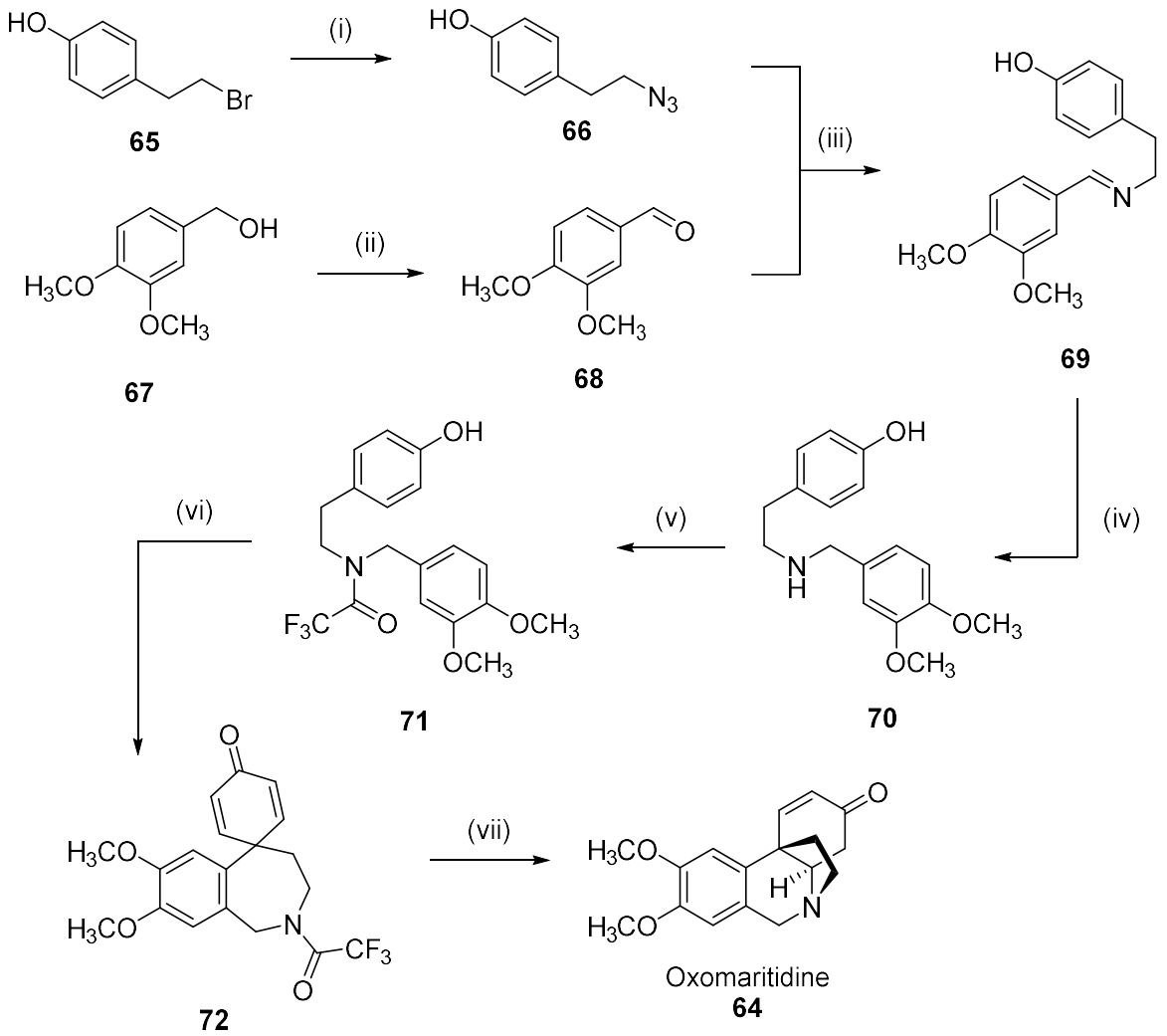
Grossamide (**61**) is a lignanamide that is found in many plants. It is believed that lignanamides regulate biological functions in the plant, with this function stemming from its structure. Grossamide and similar compounds are of significant interest in pharmaceutical industry, particularly in the search for new drugs. Baxendale *et al.* in 2006 reported the first enantioselective total synthesis of **61** in continuous flow using an automated flow reactor. Baxendale achieved this in only three steps using four columns that were packed with immobilised reagents to reproducibly and reliably synthesise **61** on a several gram scale. The synthesis initially proceeds with the amide coupling of **62** and the respective amine, followed by oxidative dearomatisation, catalysed by two acid packed columns, to yield **63**. Precursor **63** was firstly flowed through a third column which was packed with sulfonic acid as scavenger and mixed with a solution of hydrogen peroxide urea complex in a buffer mixture of pH 4.5. The combined flow was passed through the fourth and final column packed with immobilised horseradish peroxidase which facilitated the intramolecular cyclisation to yield **61**. The flow system was fitted with a simple ultra-violet/visible (UV/Vis) detector to monitor the reaction (Scheme 2.7).[58]



Scheme 2.7: Total synthesis of **61** in continuous flow by Baxendale *et al* by (i): amide coupling; (ii) oxidative dearomatisation; (iii) intramolecular cyclisation.

Oxomartidine (**64**) is an alkaloid first isolated from *Zephyranthes citrina* and first reported by Herrera *et al*. in 2000. The leaves and bulbs of the *Zephyranthes citrina*, more commonly known as 'The Yellow Witch', is known to contain cytotoxic compounds and has been used to treat various diseases, such as diabetes and tumours.[59] The first continuous multistep flow synthesis of **64** was reported by Baxendale *et al.* in 2007. Impressively, Baxendale reported that no isolation of any intermediates was required over the seven steps. In addition to this, the crude outlet of the system gave **64** in 90% purity and 40% yield. This could be achieved by using packed columns, containing reagents, catalysts and scavengers, and 'catch-and-release' agents. The synthetic route starts from 4-(-2-bromoethyl)phenol (**65**) which utilises the lability of the bromide to install an azide, to yield **66** in quantitative yields using an azide exchange resin packed column. Oxidation of **67** through flowing the reactant stream through tetra-*N*-alkylammonium perruthenate (PSP) packed column afforded desired aldehyde **68**. Azide **66** and aldehyde **68** were coupled and passed through a phosphine polymer supported column, furnishing imine **69** as major product. Imine **69** was hydrogenated in flow using a hydrogenater and a palladium on carbon catalyst. Using an inline solvent switching system, from THF to dichloromethane, allows for the final reaction steps to occur successfully. Passing amine **70** through a microfluidic reaction chip allows for the reaction with trifluoroacetic anhydride and results in the trifluoroacetylation, to yield amide **71**. The reaction stream was

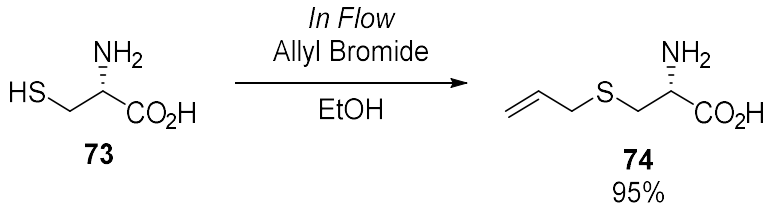
passed through a polymer supported column containing [bis(trifluoroacetoxy)iodo]benzene which afforded the oxidative phenolic coupling and the desired tricyclic product **72**. Finally, passing a stream of **72** through a base packed column enabled the cleavage of the amide bond, followed by the spontaneous 1,4-conjugate addition to yield desired compound **64** in 40% yield and 90% purity (Scheme 2.8) [60]



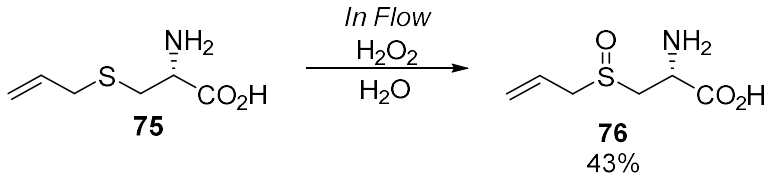
Scheme 2.8: Flow synthesis of **64** by Baxendale *et al*. (i): Azide formation; (ii): Oxidation; (iii) Imine formation; (iv): Reduction; (v) Amide formation; (vi): Oxidative phenolic coupling; (vii): 1,4-conjugate addition.

2.2.1. Synthesis of *Allium* Compounds in Flow

Synthesis of *Allium* compounds in flow has been rarely investigated. Work conducted by A. Baker in 2015 focussed on the synthesis of garlic metabolites in continuous flow.[61]



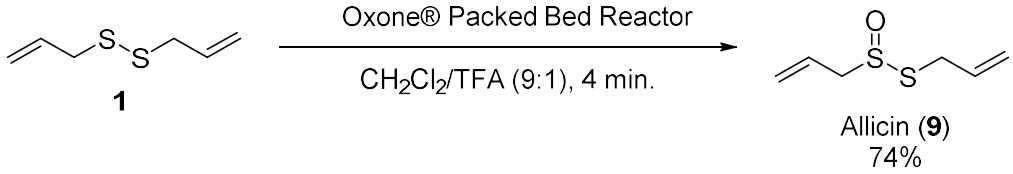
Scheme 2.9: Synthesis of **74** as a precursor of alliin amino acid (**76**) in flow (95%).



Scheme 2.10: Oxidation of **75** in flow to produce sulfoxide amino acid, alliin (**76**) in 46%.

The two steps, allylation and oxidation, were successfully completed in two separate flow systems giving 95% and 43% of **74** and **76**, respectively. Baker reports telescoping these processes, although trials did not lead to **76** being formed, likely to be the result of the solvent systems not being compatible between the first and the second step. However, these initial studies show that flow chemistry could be a useful tool to use in the synthesis of compounds that often have unstable intermediates. The flow set up was able to give desired product in a reduced time when comparing to batch systems.[61]

In 2018, Silva *et al.* reported the selective oxidation of sulfides in flow, where allicin (**9**) could be made from **1** in 74%.[62] The flow set-up used a packed bed reactor of Oxone®, where the sulfide or disulfide was passed through the column in a solvent system of dichloromethane and trifluoroacetic acid (9:1). In just 4 minutes, **9** could be obtained in good to excellent yields with high functional group tolerance.



Scheme 2.11: Synthesis of **9** in flow from the mono oxidation of diallyl disulfide (**1**).

Structurally complex *Allium* molecules, such as ajoenes, do not have a reported flow synthesis. However, as described, flow techniques can be a powerful tool to produce the desired product, potentially on a kilogram scale. Incorporating toxic, volatile, or highly reactive species into new synthetic routes *via* flow, allows for the development of new synthetic routes to complex molecules with accurate control, facile scale-up, and reduced health and safety concerns.

2.3. References

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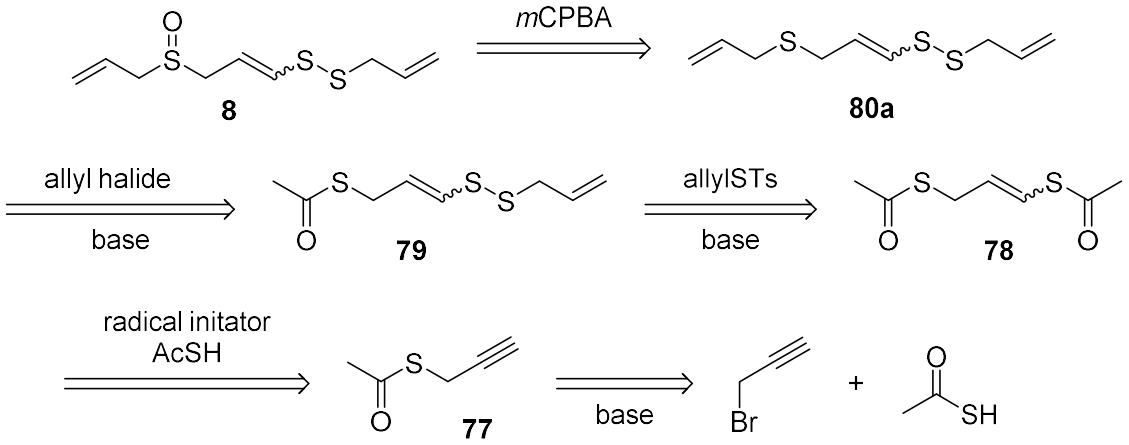
3. Synthesis of Ajoene

Neither ajoene (**8**), nor any precursor, has been synthesised in continuous flow. Continuous flow has many advantageous over traditional batch chemistries such as the increased productivity, significant reduction in reaction times and reduced exposure of hazardous chemicals to the operator (see Chapter 2). Hunter *et al*. synthesised a range of ajoene analogues in batch mode but was unable to form ajoene itself. This was due to the unavailability of the allyl mercaptan precursor that would be required. Therefore, the range of analogues Hunter synthesised over four steps (propargylation, radical addition, disulfide formation and oxidation) started from commercially available thiols or were synthesised *via* its isothiouronium salt.[17]

The aim of this project was to synthesise **8** in continuous flow by an alternative route initially investigated by F. Silva (PhD thesis). If successful, the route not only overcomes the limitation experienced by Hunter *et al*. to install a terminal allyl group at the sulfoxide, but also has several additional advantages over the reported total synthesis of ajoene.[16] The alternative route shows improved atom economy through starting with low molecular weight reagents and the elimination of using selenium to install the terminal double bond.

3.1. Retrosynthesis

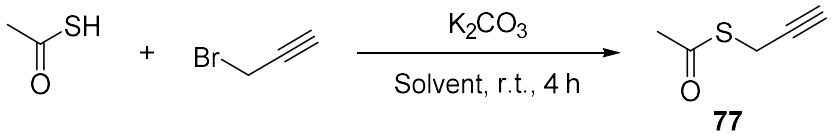
The retrosynthesis was initially investigated by F. Silva in 2019. Using propargyl bromide and thioacetic acid in combination with a base, the propargylated sulfide (**77**) can be obtained. By employing a radial initiator, thioacetate can be added onto the terminal alkyne installing the vinylic protected thiol as the desired dithioacetate product (**78**). With the thioester being a base sensitive functional group, the acetate can be cleaved and reacted with an electrophilic sulfur source to furnish the desired disulfide (**79**). A second deprotection, followed by the reaction with an allyl halide will yield the desired ajoene precursor (**80a**). A final oxidation of the sulfide to the sulfoxide will reach the target molecule **8** (Scheme 3.1).



Scheme 3.1: Retrosynthesis of **8**.

3.2. Propargylation of Thioacetic Acid

Propargylation of thioacetic acid requires basic conditions to form the thioacetate which is able to undergo an S_N2 substitution of the bromide yielding the desired product **77**. To transform this process into a flow mode, a range of bases and solvents were investigated in batch. Organic bases were initially investigated as this would enable a homogenous flow system. However, employing triethyl amine or diisopropyl ethylamine (DIPEA) in THF yielded only trace product with the formation of crystals. Crystals were presumed to be an adduct between the base and the thioacetic acid. Hoover *et al*. in 2018 were able to form **77** in 75% yield from potassium thioacetate and propargyl bromide in dimethyl formamide (DMF).[63] Using Hoover and co-workers findings, potassium carbonate was investigated to form the thioacetate potassium salt *in situ*. Fortunately, quantitative yields of **77** could be obtained in batch mode after 4 hours. After the reaction, the insoluble inorganic base was filtered and washed with dichloromethane several times. The dichloromethane and THF filtrates were combined and concentrated *in vacuo* at room temperature. (Scheme 3.2).



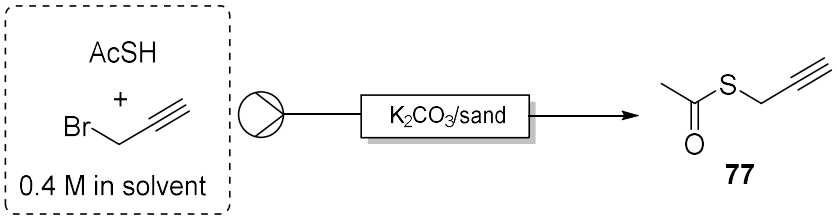
Scheme 3.2: Propargylation of thioacetic acid to **77**.

Although THF gave quantitative yields in batch, other solvents were screened as the solvent of step 1 must also be compatible with the radical addition of step 2. Toluene was investigated as it is a common solvent for radical addition reactions. However, concentrating the reaction *in vacuo* to isolate **77** from toluene proved difficult due to the high boiling point of the solvent and thus, the obtained yield is low. Dichloromethane was also investigated, however, the ratio of thioacetic acid and **58** was just 5:1 by ^{1}H NMR, indicating poor conversion. This is reflected in the low yield of 15%. Methanol was also trialled, although since potassium carbonate was slightly soluble, no definitive yield was determined (Table 3.1).

Entry	Solvent	Yield 77 (%)
1	THF	>99
2	Toluene	10
3	CH_2Cl_2	15
4	THF:CH_2Cl_2 (1:1)	>99

Table 3.1: Solvents investigated for propargylation of thioacetic acid and corresponding yields of **77**. Reagents and conditions: K_2CO_3 (1 equiv.), solvent (0.4 M), r.t., 4 h.

From this, tetrahydrofuran or its mixture with dichloromethane was chosen as suitable solvent systems to investigate in flow. Since potassium carbonate is not soluble in either of these solvents, a heterogenous system must be developed. Thus, a base-packed reactor was trialled for the flow system with an emphasis on packing of the base and a suitable solvent system that could enable the synthesis and efficient eluting of **77** (Scheme 3.3).



Scheme 3.3: Propargylation of thioacetic acid to obtain thioester **77** in flow.

Entry	Solvent	K_2CO_3/sand (wt%)	Residence time (min)	Yield 77 (%)
1	THF:CH_2Cl_2 (1:1)	7	20	19
2	THF:CH_2Cl_2 (1:1)	7	40	42
3	THF:CH_2Cl_2 (1:1)	7	60	45
4	THF:CH_2Cl_2 (1:1)	14	30	49
5	THF:CH_2Cl_2 (1:1)	21	30	46
6	THF	7	20	50
7	THF	21	30	79
8	THF	42	18	84
9	THF	56	14	57
10[a]	THF	42	24	82

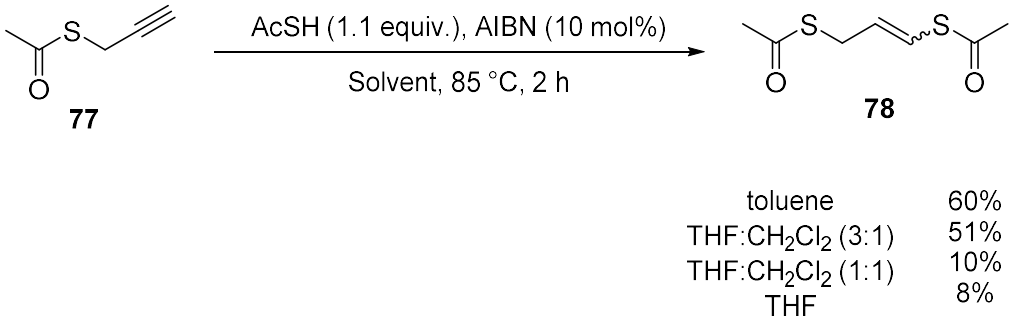
Table 3.2: Propargylation of thioacetic acid according to Scheme 3.3. Conditions and reagents: HPLC K120 Knauer Analytical pump with thioacetic acid and propargyl bromide (0.4 M) in dry solvent, column: K_2CO_3/sand (20 g total) in Omni-Sep® column (15 x 150 mm). [a] column: repacked Biotage® Snap Ultra cartridge (30 g total). The column volume and therefore flow rate was varied between each run due to the percentage of base.

In the development of flow process, an Omni-Sep® column was packed with a mixture of potassium carbonate and sand. The addition of sand reduced the build-up of pressure as the base is compacted as it is consumed over time due to smaller particle size. The addition of sand therefore allowed the flow rate to remain consistent. As a starting point, a mixed solvent system of THF:CH_2Cl_2 (1:1) was used comprising a 7 weight percentage (wt%) of base with a residence time of 20 minutes. However, the eluted reaction mixture had not achieved complete conversion and only 19% yield of **77** could be achieved. Increasing the residence time further to 40 minutes, resulted in a significant increase in the yield of **77** to 42%. However, increasing the percentage of base and residence time further to 21 wt% and up to 60 minutes respectively, saw no significant increase in yield (Table 3.2, entries 1–5). In contrast, where only THF was

employed as solvent, the reaction was able to achieve complete conversion (Table 3.2, entries 7–10) where **77** was obtained in yields of up to 84% in 18 minutes. Increasing the base percentage to 56 wt% saw a decrease in yield, likely to be the result of the product being unable to elute efficiently from the column. This conclusion was reached as in batch mode, the potassium carbonate is filtered and washed several times. Without this step, the yield of **77** suffers. Entry 10 confirmed the reproducibility of entry 8 where a larger column, and therefore an increased residence time, was used.

3.3. Radical Addition of Thioacetic Acid

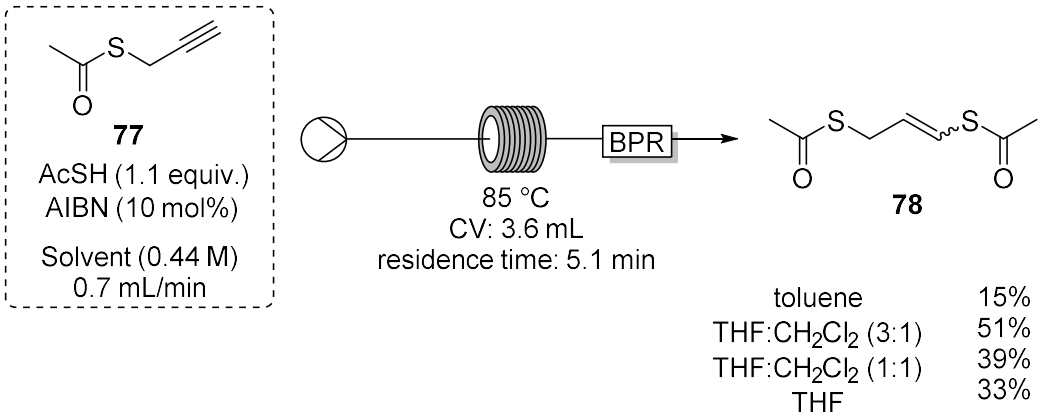
For the generation of the central double bond, a radical addition of thioacetate to **77** was employed using azobisisobutyronitrile (AIBN) as initiator. Firstly, a range of solvents were investigated in batch to yield the dithioacetate **78**. Firstly, toluene was investigated as it is an inert and high boiling point solvent. When toluene was employed as solvent, 60% yield of desired product could be achieved. Although this was a promising result and proved that compound **78** could be obtained in good yields, toluene would not be suitable in a continuous flow scheme as the previous step occurs in THF. Therefore, THF and its mixtures in dichloromethane were investigated. The presence of dichloromethane seemed to be advantageous as it increased the yield from 8% to 51% using a mixture of THF:CH_2Cl_2 of 3:1 (Scheme 3.4).



Scheme 3.4: Radical addition of thioacetic acid to **77** to afford dithioester **78** in up to 60% yield.

Translating the batch reaction scheme to a flow set-up required the use of a Uniqsis® Heated Reactor to heat the PTFE reactor coil (internal diameter (i.d.): 0.8 mm) to 85 °C. A back-pressure regulator (40 psi) was fitted to ensure that the system

remained homogenous, despite the evolution of nitrogen gas from the decomposition of AIBN. A flow rate of 0.7 mL/min was used as this was the lowest flow rate that gave a consistent observed flow rate with the back pressure regulator. Toluene was trialled in order to test the system and yielded the desired product in 15% yield. Fortunately, a mixed solvent system of THF:CH_2Cl_2 (3:1) gave the best yield of 51% of **78** (Scheme 3.5). This was advantageous as the solvents between the first two steps were compatible and the system could easily be coupled together.

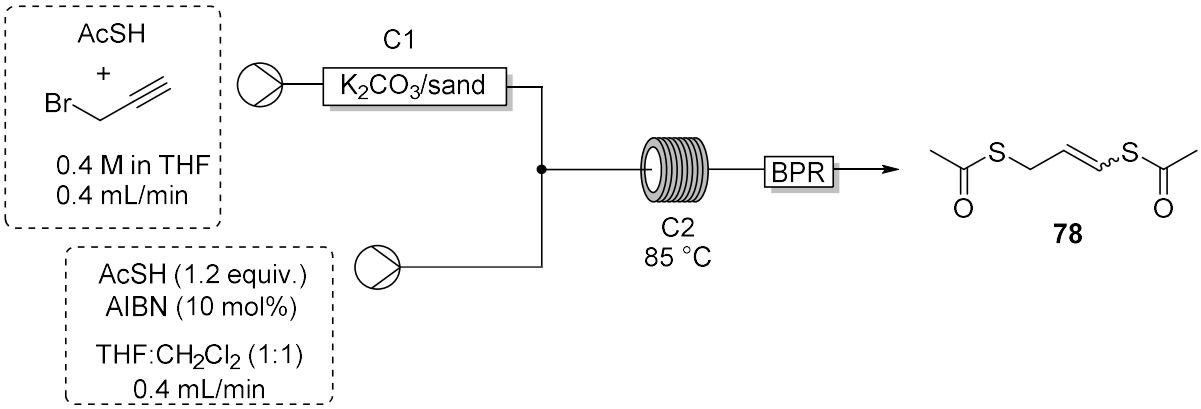


Scheme 3.5: Radical addition of thioacetic acid to **58** to afford dithioester **59** in up to 51% yield in flow.

In order to optimise the synthesis of **78**, the first two flow steps were coupled together and the column volume (CV) of step 2 was increased. With the propargylation reaction, consistently yielding 82−84% of **77**, the highest yield of **78** that could be expected over two steps was around 80%. The thioacetic acid and propargyl bromide in THF was passed through the base packed column as previously, and the outlet was connected to a T-piece mixer where a solution of thioacetic acid and AIBN in THF:CH_2Cl_2 (1:1) were added. Since the flow rates are equivalent, the mixing of the two solutions resulted in a solvent mixture of THF:CH_2Cl_2 (3:1). The resulting flow rate in column 2 (C2) during the radical addition was 2 x 0.4 mL/min (Scheme 3.6).

For the combined system, residence times for C2 were investigated from 6–38 minutes, where the yield of **78** increased from 44 to 61%. Residence times above 38 minutes were not investigated due to the combined residence times of C1 and C2 exceeding one hour; this was deemed not practical. Moreover, a yield of 61% of **78** over two steps was satisfactory as it calculates to an estimate of approximately 74% yield for the radical addition, based on an 82% yield for step 1 (Table 3.3). In addition,

PTFE tubing with a reduced internal diameter was attempted (0.2 mm). It was thought that a reduced internal diameter would lead to more vigorous mixing and could lead to higher yields. However, reduced internal diameters gave inconsistent flow rates and leaking at fittings due to increased pressure, therefore, only internal diameters of 0.8 mm have been used.



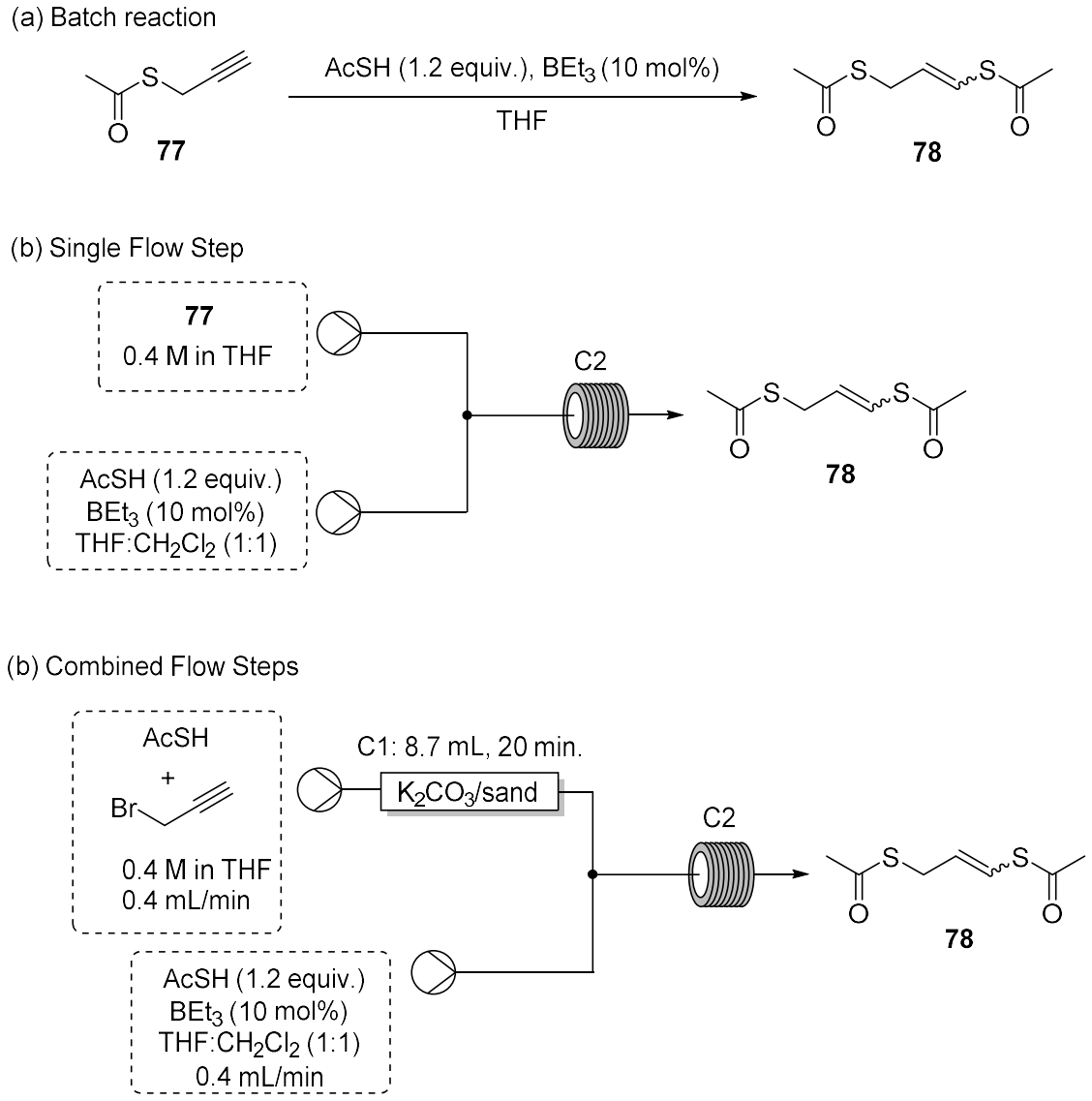
Scheme 3.6: Combined flow system of the first two steps to synthesis **78**. Investigating the effect of residence time of C2. Conditions: C1: 20 g of K_2CO_3/sand (42 wt%), 8 mL, 20 min. C2: i.d. 0.8 mm

Entry	Residence time of C2 (min)	Volume of C2 (mL)	Yield 78 (%)
1	6	4.8	44
2	25	20	47
3	38	32	61

Table 3.3: Results from set-up given in Scheme 3.6.

Triethyl boron as a boron-based radical initiator was also investigated, although gave inferior yields with a less clean reaction. The radical addition with triethyl boron as radical initiator was investigated in batch, single flow step and combined flow steps (Scheme 3.7). Trialling triethyl boron in batch mode for the radical addition of thioacetic acid to **78** gave full conversion after just 1 hour at room temperature. However, the yield of desired product was just 18% (Table 3.4, entry 1). This was thought to be the result of decomposition occurring. Therefore, translation to a flow set-up may show a more selective reaction for the desired pathway due to intense mixing regimes. The

single flow step investigations were conducted and although giving higher yields of **78** than in batch mode, the yields did not exceed 25%. A range of residence times and temperatures were trialled, from 20 – 40 min and −78 – 30 °C, respectively (Table 3.4, entries 2 – 4). The combined flow step was conducted, giving 37% yield of **78** over 2 steps. This leads to an estimate of 45% yield over the radical addition step on the basis that the propargylation in flow gives an average of 83% yield of **77**. This indicates that decomposition of the **77** could be occurring in the single flow step before the radical reaction takes place. Therefore, it is more accurate to synthesise **77** and consume it directly as in the combined flow step-up (Table 3.4, entry 5). Since triethyl boron did not give promising yields, AIBN was determined to be the radical initiator of choice.



Scheme 3.7: Synthesis of **78** in batch, single flow step and combined flow step.

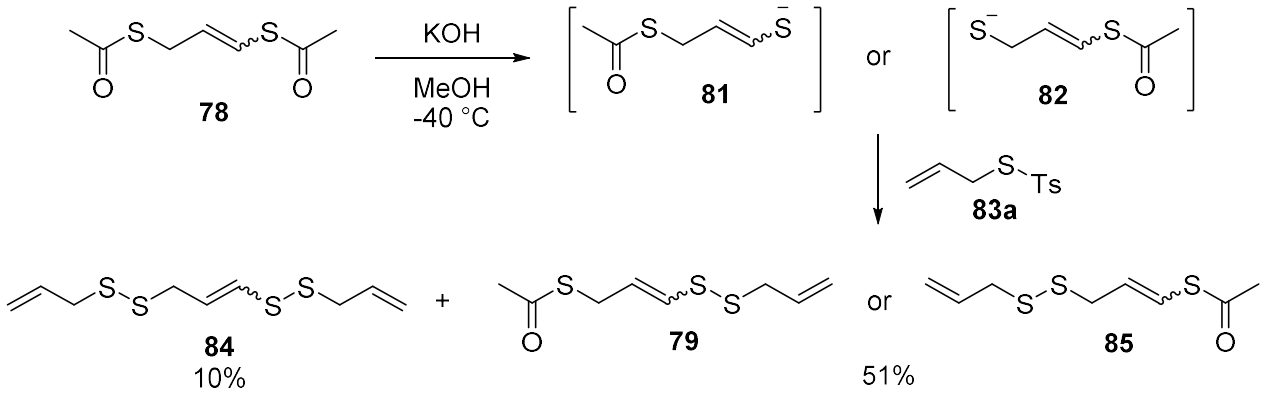
Entry	Scheme 3.7	Comment	Yield 78 (%)
1	(a)	Batch mode – r.t. 1 h	18%
2	(b)	C2: 4.75 mL, 20 min at 30 °C	20%
3	(b)	C2: 6.5 mL, 30 min at 0 °C	25%
4	(b)	C2: 3.6 mL, 40 min at −78 °C	18%

5	(c)	C2: 8.7 mL, 11 min at r.t.	37% (2 steps)

Table 3.4: Batch and flow results for the synthesis of **78** using triethyl boron as radical initiator.

3.4. Deprotection and Disulfide Formation

Treating dithioacetate **78** with potassium hydroxide and allyl thiotosylate (**83a**) results in 51% yield of either **79** or **85** and 10% of the bis-disulfide (**84**) as side product. However, the reaction could result in deprotection at the vinylic position or allylic position. It would be expected that the vinylic thioacetate is more readily cleaved. This can be rationalised by the stabilisation of the central double bond that is present in thiolate **81** but absent in **82**. This renders thiolate **81** more acidic and more likely to form over its isomeric compound **82**. Addition of **83a** furnished the disulfide bond (Scheme 3.8).



Scheme 3.8: Treatment of **78** with a base and allyl thiotosylate (**83a**) could yield isomeric product **85** as well as desired product **79** *via* thiolate intermediates **81** and **82**. Conditions and reagents: KOH (1.05 equiv.), **83a** (1.0 equiv.), −40 °C to r.t. 1 h.

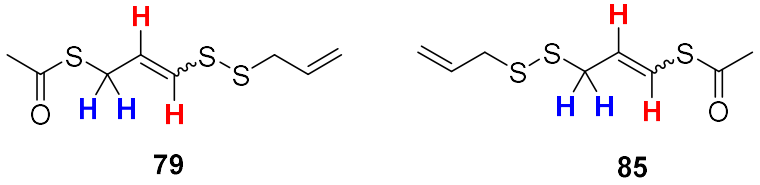


Figure 3.1: ^{1}H NMR analysis of **79** and **85** shows coupling between blue and red protons.

The protons labelled in blue of the CH_2 in the (*E*/*Z*)-product have a chemical shift of 3.57 and 3.65 ppm and appear as doublets of doublets (dd) due to coupling to the protons of the central olefin labelled in red (Figure 3.1). Analysis from the 1−dimensional ^{1}H NMR does not show definitive proof that one compound is formed

over the other. However, when analysing the $^{1}H–^{13}C$ Heteronuclear Multiple Bond Coherence (HMBC), analysis showed that **79** is formed rather than its isomer **85**. In the HMBC spectra, the protons at 3.57 and 3.65 ppm show a clear correlation with the carbonyl carbon (red) at 195.3 and 195.0 ppm. This would be unlikely to be observed if **85** was formed as this is a 5 bond correlation, and more likely to be the result of the 3 bond correlation that is seen in the case of **79** (Figure 3.2).

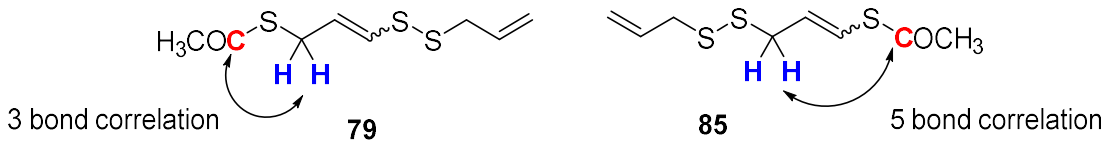
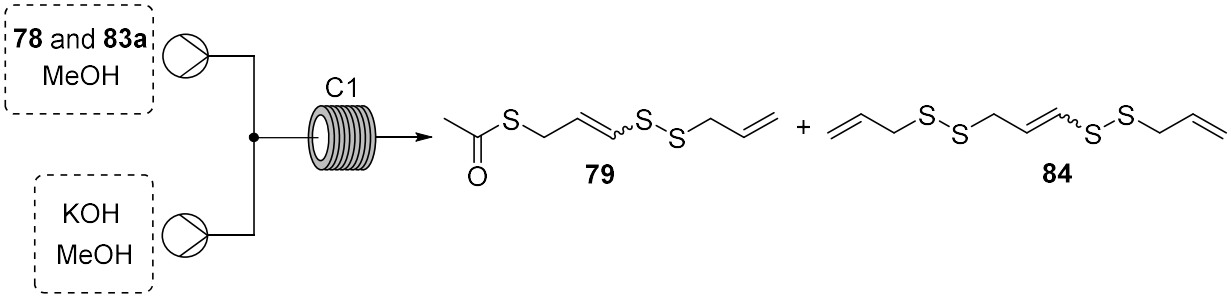


Figure 3.2: ^{1}H NMR analysis of **79** and **85** shows coupling between blue and red protons.

With the correct thioacetate in **78** being cleaved and resulting in 51% yield of **79**, attention turned to the optimisation of the process in flow. The flow scheme used a Dual Fusion 100 Touch Syringe Pump pumping at equivalent flow rates, containing a solution of **78** and **83a** in methanol and a separate solution of potassium hydroxide in methanol (Scheme 3.9). Over a number of trials, the concentration of the reaction, temperature and residence time were investigated. Concentration was shown to have some effect where 0.1 M afforded a higher yield of the **79** than 0.2 M, although the yield of the side product **84** remained constant (Table 3.5, entries 1–2). Reducing the temperature to 0 °C gave a significant increase in yield of **79** to 41–46% with 6–9% formation of **84** for residence times between 5 and 10 minutes (Table 3.5, entries 3−4). Decreasing the concentration and temperature further, whilst increasing the residence time was shown to have little effect, with side product formation in line with previous attempts at 11% (Table 3.5, entry 5). In order to reach full conversion of **78**, the equivalents of potassium of hydroxide was increased to 1.4 equivalents. However, although full conversion of **78** was achieved, the increase in base significantly increased the yield of the bis-disulfide side product (**84**) (Table 3.5, entry 5). Nohara *et al.* isolated **84** from Chinese garlic in 2014 and thus, the synthetic route that has so far been developed, stands as the first short total synthesis of **84**.[64]



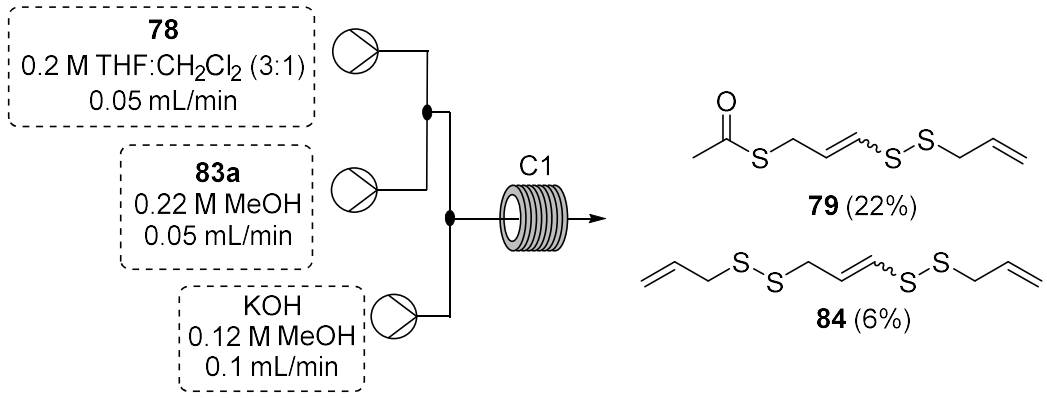
Scheme 3.9: Flow set-up for the deprotection and subsequent thioallylation to yield desired product **79** and side product **84**.

Entry	Scheme 3.7 (M)	Temperature (°C)	Residence time (min)	Yield 79 (%)	Yield 84 (%)
1	0.1	r.t.	5	35	9
2	0.2	r.t.	5	22	10
3	0.1	0	5	46	9
4	0.1	0	10	41	6
5[a]	0.05	−10	15	46	11
6[a,b]	0.1	−40	10	34	31

Table 3.5: Flow results for the formation of **79** from **84**. Conditions and reagents: **83a** (1.1 equiv.), KOH (1.05 equiv.) in MeOH; C1: 3.6 mL, i.d. 0.8 mm. Residence times achieved through adjust flow rates. [a] C1: 1 mL [b] KOH (1.4 equiv.)

In order to combine step 3 with the previous two steps, the solvents must be compatible with each other. The deprotection of the vinylic thioacetate has been conducted in methanol as solvent. Since all the starting materials, including the inorganic base, are soluble in the solvent, a homogenous solution is achieved. With an initial set of conditions which yields **79** in good yields, a trial run was conducted using a mixed solvent system which would be a more accurate representation of the final continuous system. The system where **78** and **83a** were added in separately and in their respective solvents, yielded **79** in 22% yield and **84** in 6% yield (Scheme 3.10). Although the yields for this system were low, the result proves that the deprotection

can take place selectivity in a mixed solvent of THF, dichloromethane and methanol (3:1:12).



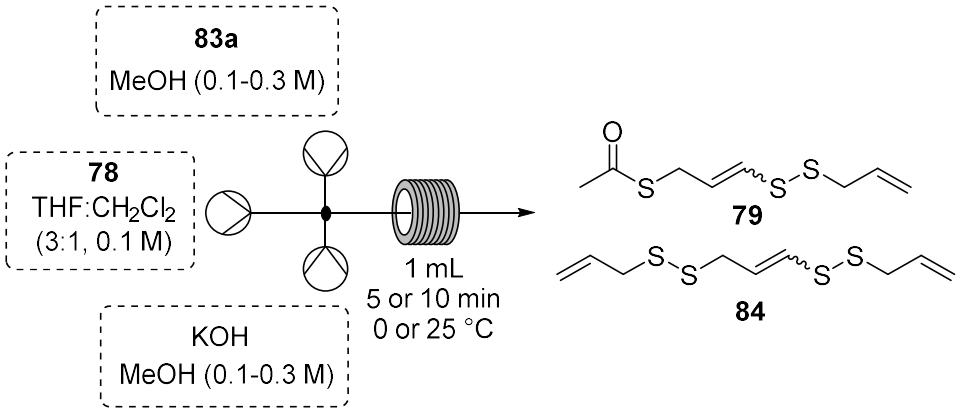
Scheme 3.10: Synthesis of **79** in flow using mixed solvent system. Conditions: C1: 1 mL, 5 minutes, 0 °C.

3.4.1. Design of Experiments

Reactions in the mixed solvent system of THF, dichloromethane and methanol (3:1:12) required optimisation to overcome the suppressed reactivity observed. Therefore, a simple fractional, optimised design using two quantitative factors at two levels (temperature and residence time), and two quantitative factors at three levels (equivalents of potassium hydroxide and **83a**) was conducted using the Design Wizard of Umetrics Modde Pro 12.1 Design of Experiment (DoE) software. The design was a randomised 21 experiments including 3 repeats (Table 3.6).

The 1−3 equivalents of potassium hydroxide and **83a** were investigated. The equivalents were controlled by the concentration of the solution whilst keeping the flow rate constant (0.1−0.3 M) (Scheme 3.11). Equivalents between 1−3 were chosen as less than 1 equivalents would not be sufficient to ensure complete conversion of **78**, and greater than 3 equivalents favoured formation of **83a**, and thus an optimal reaction space should lay within this range. The first one and a half column volumes of each run were discarded to ensure that the steady state had been reached and the result of the run was an accurate representation of the system. Approximately 1 mL of each run was collected into a vial containing 3 drops of saturated ammonium chloride

solution. From the quenched reaction mixture, a 50 µL aliquot was diluted into 1 mL of acetonitrile and analysed by reverse phase HPLC.



Scheme 3.11: Design of experiments Set-Up to optimise synthesis of **79**. Flow rate: 3 x 0.03333 mL/min to achieve 10 minute residence time or 3 x 0.06666 mL/min to achieve 5 minute residence time using syringe pump. Column: PTFE i.d. 0.8 mm, CV: 1 mL submerged in ice bath at 0 °C or water bath at 25 °C

.

Run Order	Experiment Number	Residence Time (min.)	Temp. (°C)	83a (equiv.)	KOH (equiv.)
1	5	10	25	2	1
2	15	5	0	3	3
3	17	10	0	2	3
4	16	10	25	1	3
5	1	5	25	1	1
6	9	10	25	3	2
7	21	10	0	1	2
8	8	10	0	2	2

9	11	5	25	2	2
10	12	5	0	3	2
11	18	10	25	3	3
12	14	5	25	2	3
13	10	5	25	1	2
14	13	5	0	1	3
15	2	5	0	2	1
16	4	10	0	1	1
17	3	5	25	3	1
18	6	10	0	3	1
19	7	10	0	1	2
20	20	5	25	2	2
21	19	10	25	3	3

Table 3.6: Design of Experiments randomised experiments for the deprotection of **78** to **79**. HPLC Method: Column: C18 4.6 x 150 mm, 5 micron; solvent A (30%): water 0.1% trifluoroacetic acid; solvent B (70%): acetonitrile 0.1% trifluoroacetic acid; flow rate: 1.5 mL/min; stop time: 15.0 min; injection volume: 20.0 µL; temperature: 30.00 °C; wavelength: 254.00 nm.

The percentage of product peak area for each run was entered into the Modde software. A series of response contour plots were generated for each temperature and residence time. The plots indicate higher product formation in red areas, and lower product formation in blue areas. The contour maps at 25 °C predict that the highest percentage of product formation as peak area at 254 nm is 36% and 41% at a residence time of 5 minutes and 10 minutes, respectively. This is indicated by the white arrow in Figure 3.3. The optimum equivalents predicted to reach these maxima are 2.6 equivalents of **83a** and 1.9 equivalents of potassium hydroxide for a residence time of 5 minutes, and 2.7 equivalents of **83a** and 2.0 equivalents of potassium hydroxide

for a residence time of 10 minutes. The difference between the two residence times is small and the equivalents of both **83a** and base are similar, where 10 minutes predicts requiring 0.1 equivalents more of each.

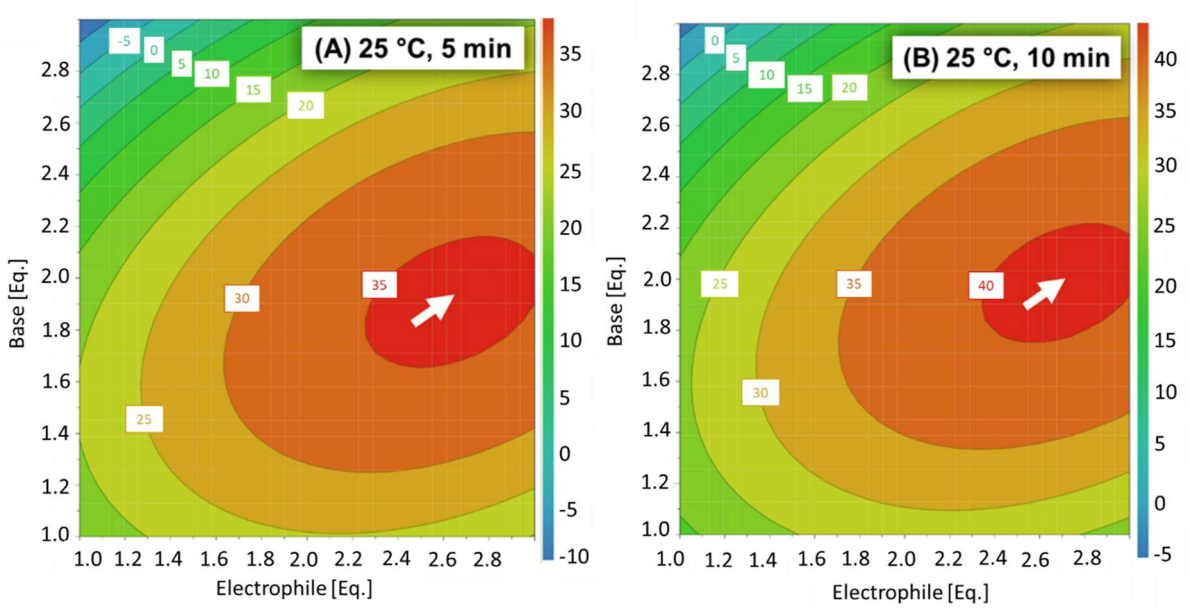


Figure 3.3: Response contour plots of product formation as percentage of peak area against base equivalents (y axis) and electrophile 83a equivalents (x axis). Plot A: 25 °C at 5 minutes; plot B: 25 °C at 10 minutes.

Contour plots at reduced temperatures indicate more promising results, where the maxima of product formation as percentage of peak area is predicted to reach up to 49% at 2.5 equivalents of **83a** and 1.7 equivalents of potassium hydroxide, at 0 °C with a 5 minute residence time (Figure 3.4, A).

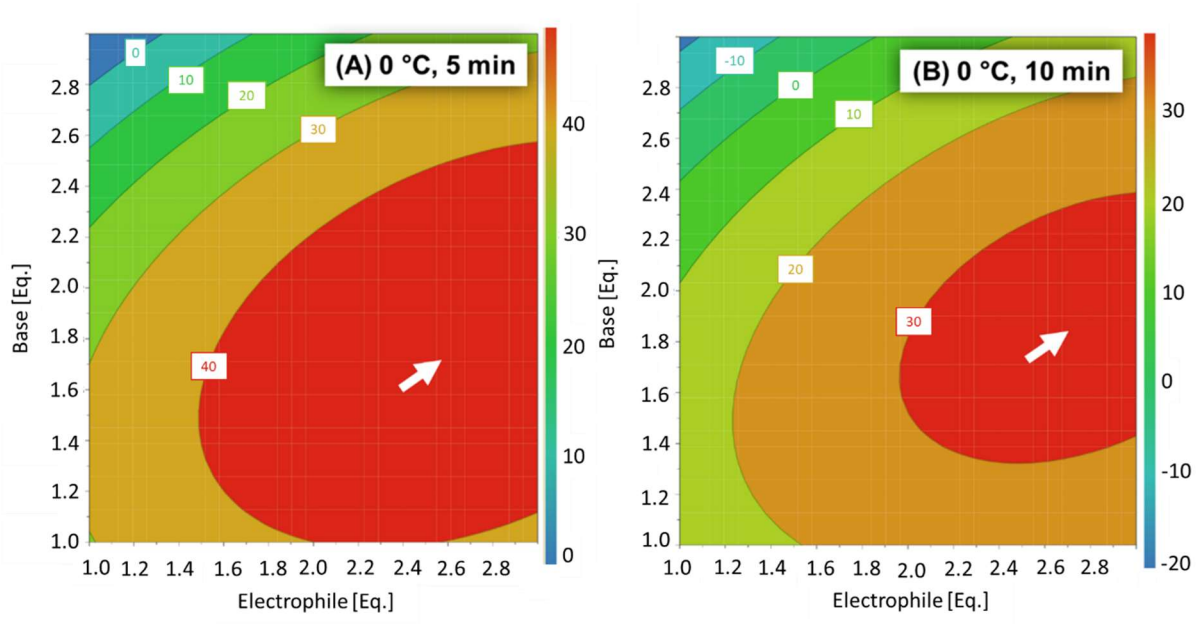
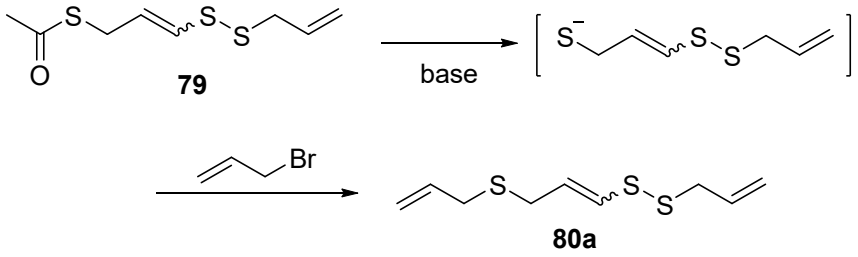


Figure 3.4: Response contour plots of product formation as percentage of peak area against base equivalents (y axis) and electrophile **83a** equivalents (x axis). Plot A: 0 °C at 5 minutes; plot B: 0 °C at 10 minutes.

The predicted conditions of 2.5 equivalents of **83a** and 1.7 equivalents of potassium hydroxide were applied to the flow system to afford **79** and **84** in 56% and 10% isolated yield, respectively. This was a significant improvement on current conditions which were reached by a One-Variable-At-A-Time (OVAT) approach. Using an OVAT approach was key for initial study screening and to narrow the reaction space studied in DoE experiments. However, the DoE study clearly indicated which combination of conditions would lead to favourable results in much fewer experiments.

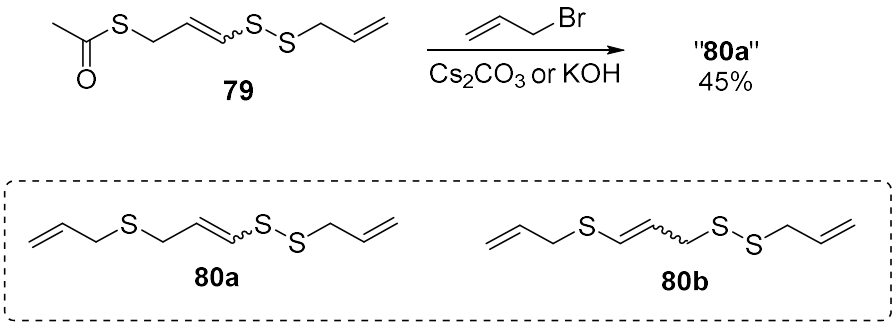
3.5. Deprotection and Allylation

With the disulfide correctly installed in **79**, the following deprotection using a base should cleave the remaining thioacetate, forming a thiolate *in situ,* which could react with allyl bromide as an electrophile in an S_N2 reaction forming **80a** (Scheme 3.12).



Scheme 3.12: Deprotection of thioacetate and allylation of **79** to afford **80a**.

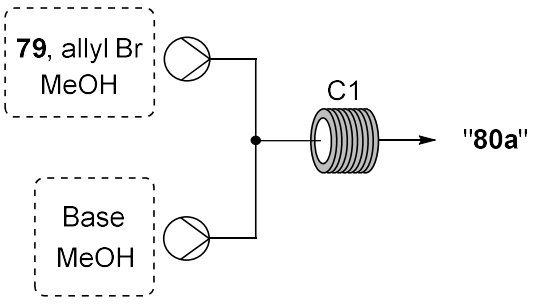
The formation of **80a** was attempted using caesium carbonate and potassium hydroxide as base. Both bases yielded what was thought to be desired product **80a** in 45% yield, although was later confirmed as **80b** (Scheme 3.13).



Scheme 3.13: Deprotection of **79** and allylation to yield "**80a**" in 45% isolated yield. Conditions: allyl bromide (1 equiv.), Cs_2CO_3 (1 equiv.), −40°C to r.t.

As the batch process yielded product "**80a**" in good yield, attention turned to developing a flow process. The ideas developed in section 3.4 were translated to the second deprotection in order to allow faster optimisation. The differences in the two steps are only the nature of the electrophile, and the solvent system which would contain a higher proportion of methanol in the second deprotection.

Firstly, the flow system was studied in methanol with a 5 minute residence time (Scheme 3.14). Caesium carbonate was used as base although gave only up to 20% yield at a range of temperatures and equivalents (Table 3.7, entries 1–7). Using potassium hydroxide as base saw a promising increase in yield to 29%, although yields remained considerably lower than in batch (Table 3.7, entry 8).



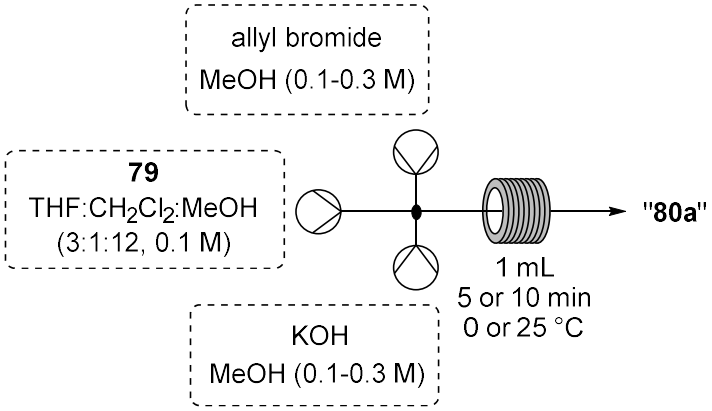
Scheme 3.14: Flow synthesis of "**80a**" using flow rate: 2 x 0.1 mL/min using syringe pumps; C1: 1 mL, 5 minute residence time.

Entry	Scheme 3.7 (M)	[Allyl Br] (M)	[Base] (M)	Temp.(°C)	Yield "80a" (%)
1	0.1	0.1	Cs_2CO_3 (0.1)	−40	7
2	0.1	0.1	Cs_2CO_3 (0.1)	0	4
3	0.1	0.1	Cs_2CO_3 (0.1)	r.t.	12
4	0.2	0.2	Cs_2CO_3 (0.2)	0	5
5	0.1	0.15	Cs_2CO_3 (0.1)	r.t.	18
6	0.1	0.2	Cs_2CO_3 (0.1)	r.t.	12
7	0.1	0.2	Cs_2CO_3 (0.2)	r.t.	20
8	0.1	0.2	KOH (0.2)	r.t.	29

Table 3.7: Flow results for the formation of "**80a**" from **79** and allyl bromide. Conditions and reagents: allyl (1−2 equiv.), base (1−2 equiv.).

3.5.1. Design of Experiments

In order to optimise the second deprotection, a DoE study in the same manner as Chapter 3.4.1 was employed using potassium hydroxide as base and allyl bromide. In the final system, **79** will be in a THF:CH_2Cl_2:MeOH (3:1:12) and therefore this was mimicked in the DoE study (Scheme 3.15, Table 3.8).



Scheme 3.15: Design of experiments Set-Up to optimise synthesis of "**80a**". Flow rate: 3 x 0.03333 mL/min to achieve 10 minute residence time or 3 x 0.06666 mL/min to achieve 5 minute residence time using syringe pump. Column: PTFE i.d. 0.8 mm, CV: 1 mL submerged in ice bath at 0 °C or water bath at 25 °C.

Run Order	Experiment Number	Residence Time (min.)	Temp. (°C)	Allyl bromide (equiv.)	KOH (equiv.)
1	5	10	25	2	1
2	15	5	0	3	3
3	17	10	0	2	3
4	16	10	25	1	3
5	1	5	25	1	1
6	9	10	25	3	2
7	21	10	0	1	2
8	8	10	0	2	2
9	11	5	25	2	2
10	12	5	0	3	2

Run Order	Experiment Number	Residence Time (min.)	Temp. (°C)	Allyl bromide (equiv.)	KOH (equiv.)
11	18	10	25	3	3
12	14	5	25	2	3
13	10	5	25	1	2
14	13	5	0	1	3
15	2	5	0	2	1
16	4	10	0	1	1
17	3	5	25	3	1
18	6	10	0	3	1
19	7	10	0	1	2
20	20	5	25	2	2
21	19	10	25	3	3

Table 3.8: Design of Experiments randomised experiments for the deprotection of **79** to "**80a**". HPLC Method: Column: C18 4.6 x 150 mm, 5 micron; solvent A (30%): water 0.1% trifluoroacetic acid; solvent B (70%): acetonitrile 0.1% trifluoroacetic acid; flow rate: 1.5 mL/min; stop time: 15.0 min; injection volume: 20.0 µL; temperature: 30.00 °C; wavelength: 254.00 nm.

The response contour plots show "**80a**" as a percentage of peak area for flow system at 25 °C (Figure 3.5) and 0 °C (Figure 3.6). Both plots show product percentage up to 60%. All plots indicate that the equivalent of base is crucial to the success of the reaction, with greater than 2 equivalents being required to achieve good yields.

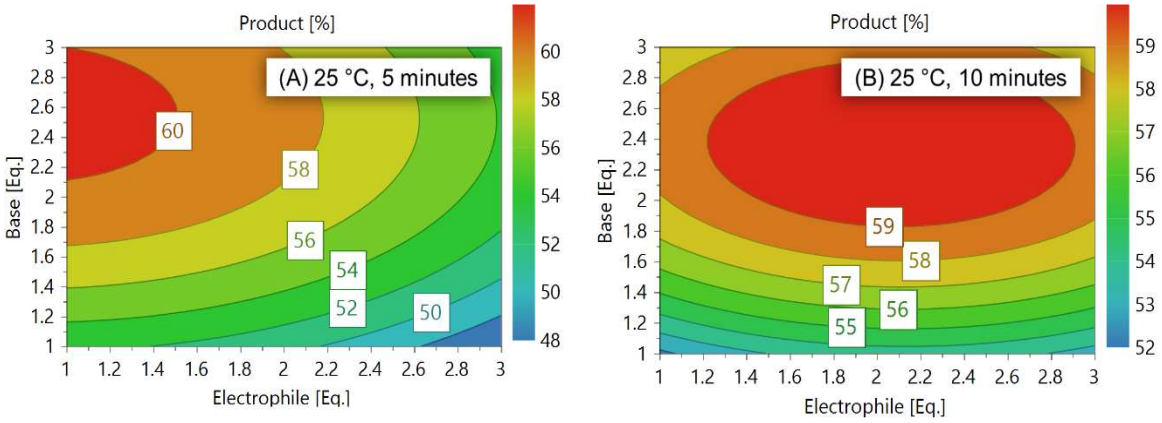


Figure 3.5: Response contour plots of product formation ("**80a**") as percentage of peak area against base equivalents (y axis) and electrophile allyl bromide equivalents (x axis). Plot A: 25 °C at 5 minutes; plot B: 25°C at 10 minutes.

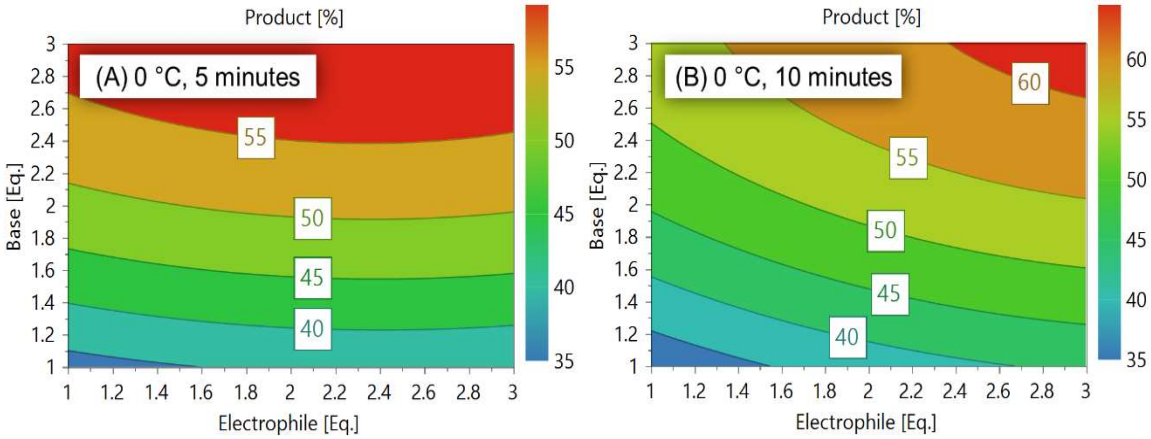
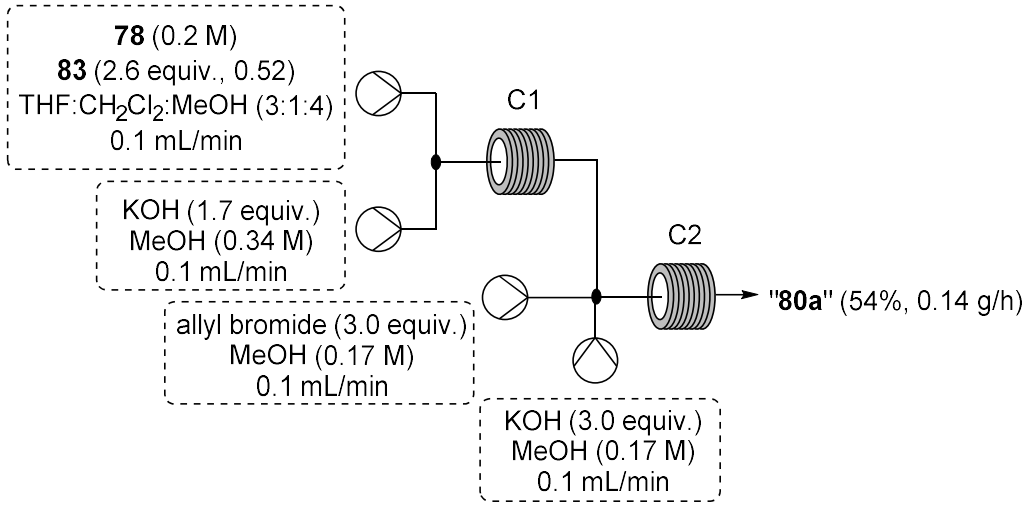


Figure 3.6: Response contour plots of product formation ("**80a**") as percentage of peak area against base equivalents (y axis) and electrophile allyl bromide equivalents (x axis). Plot A: 0 °C at 5 minutes; plot B: 0°C at 10 minutes.

A flow system at 0 °C with a 10 minute residence time was chosen, using 3 equivalents of potassium hydroxide and 3 equivalents of allyl bromide. When repeated in flow, an isolated yield of "**80a**" in 43% was achieved. This was on par with the batch set-up and it was concluded that an optimised system had been achieved. Other bases such as sodium hydroxide, caesium carbonate, potassium carbonate and ammonium hydroxide were also screened in flow using optimised conditions. However, sodium hydroxide and caesium carbonate gave inferior yields (37%), potassium carbonate was insoluble in methanol at the required concentration (0.3 M) and ammonium hydroxide gave poor conversion of starting material, where "**80a**" was isolated in 26% along with 31% of **79**.

3.5.2. Combining Both Deprotection Steps

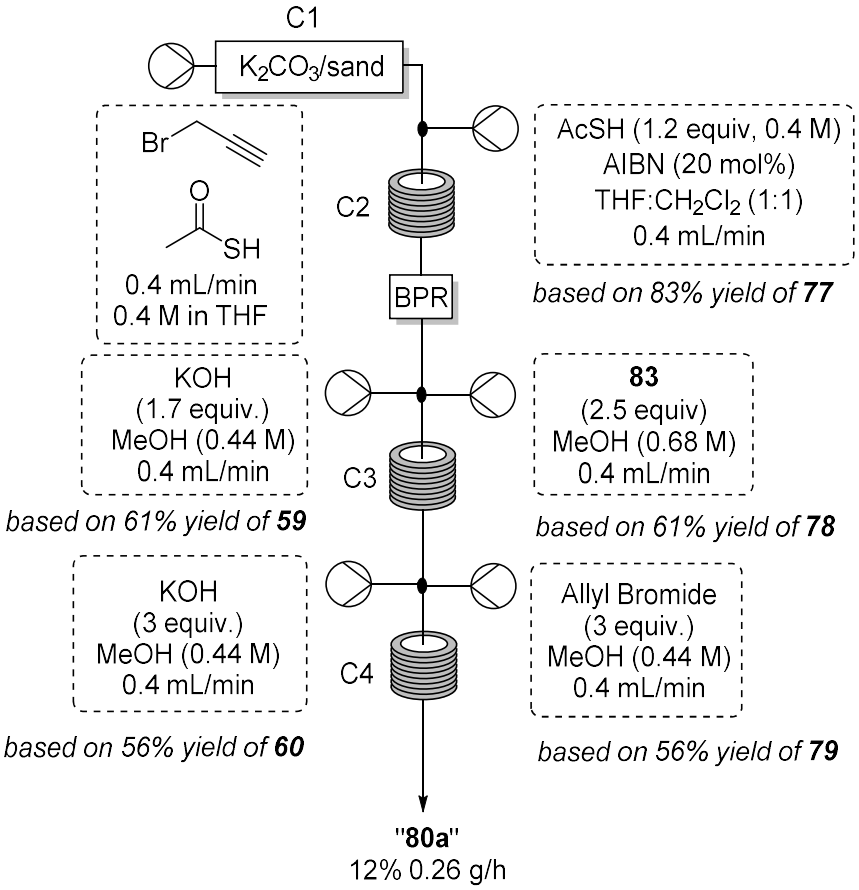
With both deprotection steps giving good yields individually, the combined system from **78** to "**80a**" was investigated to assess the feasibility of a combined system. The first deprotection yielding 56% of **79** and the second deprotection yielding "**80a**" in 43%, a yield of 24% was expected. To our surprise, 54% of "**80a**" could be achieved over two steps at a rate of 0.145 g/h. The higher yield could be due **79** not being isolated and purified, and therefore material is not lost between steps (Scheme 3.16).



Scheme 3.16: Combined continuous flow synthesis of "**80a**" from **78**. Conditions and reagents: C1: 0 °C, 5 minutes; C2: 0 °C, 10 minutes; allyl bromide and potassium hydroxide equivalents based on 56% yield in first step.

3.6. Continuous Flow from Thioacetic Acid to "80a"

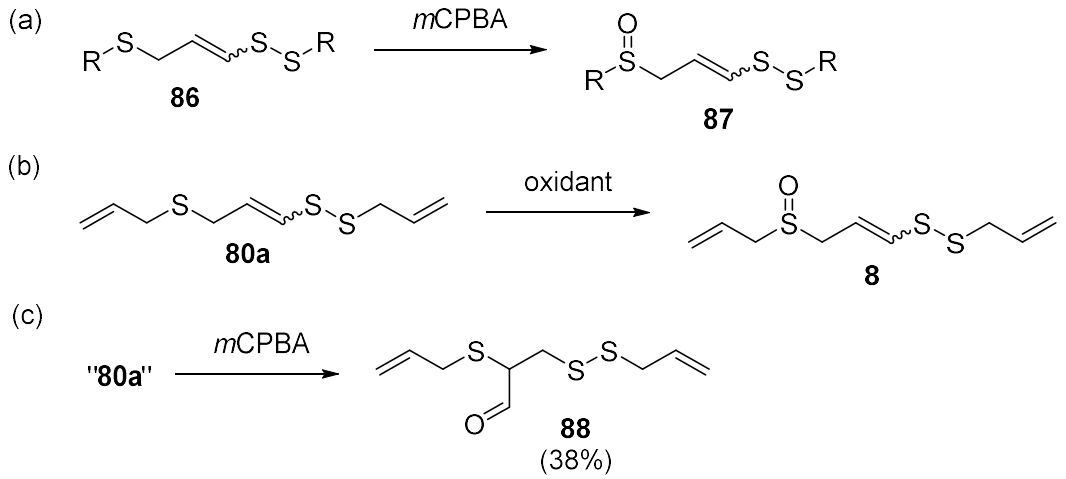
After the combination of individually optimised steps, their combination in a continuous flow system was conducted. The ratios of the reactants were calculated based upon expected yields and thus, their concentrations were adjusted. The flow system yielded "**80a**" in 12%. This was a successful result as the expected yield was calculated to be 15% over four steps. Therefore, a 12% yield provides promise as with only one purification, the product can be synthesised successfully. In order to check the flow system, **78** was also collected after the back pressure regulator which showed a 61% isolated yield. This indicated a robust and reliable system has been developed (Scheme 3.17).



Scheme 3.17: Continuous flow synthesis yielding "**80a**" in 12% over four steps (0.26 g/h). C1: 23 g, 4 wt% of K_2CO_3 in sand, 7.6 mL, 19 min; C2: 31.9 mL, 40 min at 85−90 °C, 75 psi; C3: 8.7 mL, 5.5 min at 0 °C; C4: 24 mL, 10 min at °C.

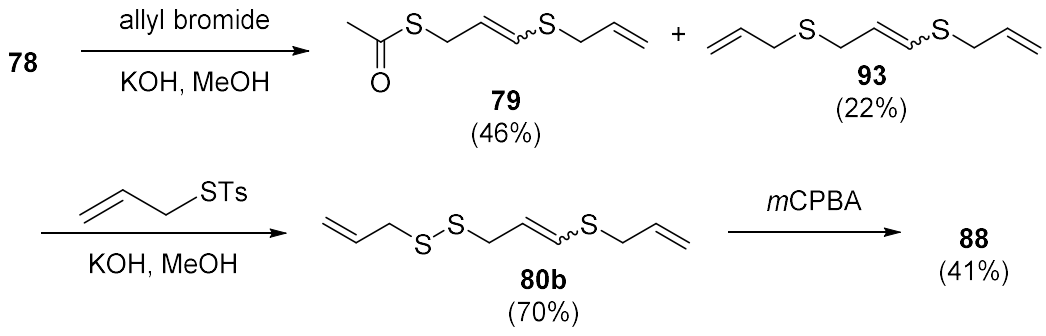
3.7. Oxidation of Sulfide to Sulfoxide

With sulfide "**80a**" in hand, a final oxidation of the sulfide should yield the desired product **8**. Literature precedence for this transformation was provided by Hunter *et al.* where it was reported that compound of the type **86** was oxidised using *m*CPBA to yield ajoene analogues of type **87** (Scheme 3.18a).[17] Thus, if "**80a**" was desired sulfide, oxidation should proceed smoothly to **8** (Scheme 3.18b). However, no batches of "**80a**", whether from flow system or batch experiments, would oxidise to **8** under a range of conditions. Oxidants trialled included *m*CPBA, Oxone®, hydrogen peroxide and peracetic acid which consistently yielded a rearranged product **88**.



Scheme 3.18: (a): Oxidation of analogous sulfides **86** to ajoene analogues **87** by Hunter *et al*.[17] (b): Oxidation of **80a** would be expected to yield **8** as major product; (c): Product from flow system "**80a**" does not yield desired product **8**, but rearrangement product **88**. Conditions: *m*CPBA (1 equiv.) in CH_2Cl_2, −78 °C to r.t., 2 h

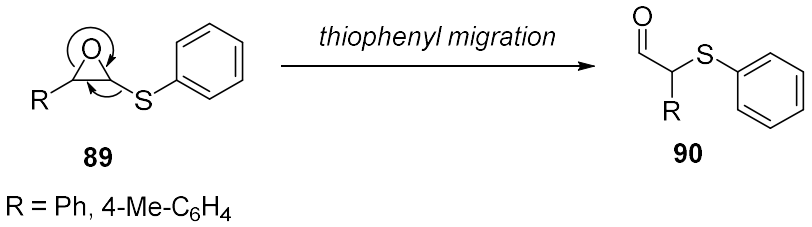
As "**80a**" would not oxidise to desired product **8**, this led to re-evaluating the product from the route developed, concluding that "**80a**" was unlikely to be the desired product **80a**. Structural analysis of **88** showed that the disulfide and the allyl thioether were correctly installed, although the oxidant had clearly reacted with the central double bond, removing the geometric isomers. Full characterisation of "**80a**" was assumed to be consistent with the correct desired structure, therefore, it was thought that the actual structure must be very similar to **80a** and could possibly be an isomer, **80b**. To prove this, **80b** was synthesised unambiguously from **79**. This was then oxidised and the product isolated in 41% was the same rearrangement product **88** that had been observed in previous attempts (Scheme 3.19).



Scheme 3.19: Synthesis of **80b**, an isomer of **80**, and subsequent oxidation to **88**.

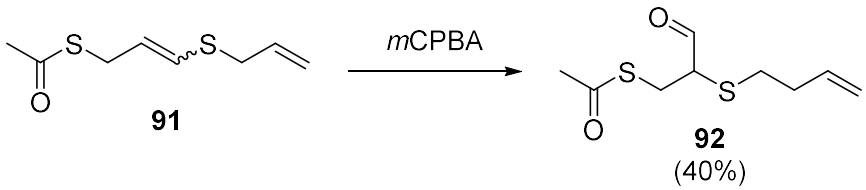
The structure of "**80a**" was confirmed as isomer **80b,** upon oxidation **88** could be formed as a result of epoxidation and subsequent rearrangement. Such

rearrangements are known and have been reported. Work by Boubia *et al*. in 1989 reported similar rearrangements (Scheme 3.20).[65] Boubia reports that brief exposure of **89** to silica gel is sufficient to catalyse the rearrangement to occur to aldehyde **90**.



Scheme 3.20: Boubia observed rearrangement of **89** to **90**.

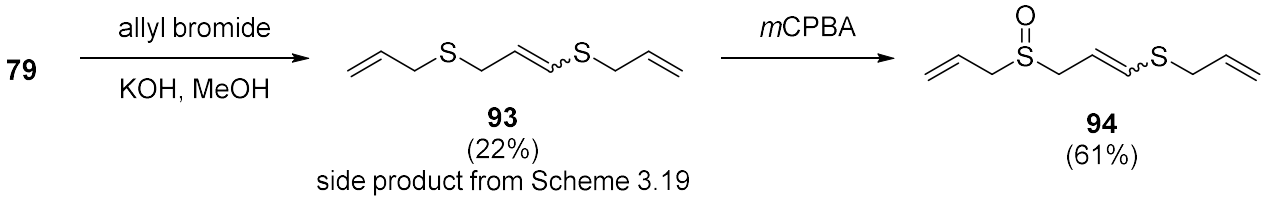
Since sulfide **80b** readily undergoes such an oxidative rearrangement to **88**, whereas **80a** was thought to undergo sulfide oxidation to **8**, it was thought that the vinylic sulfide may have an influence. Therefore, vinylic sulfide **91** was oxidised with *m*CPBA. Sulfide **92** is a simpler vinylic sulfide and therefore, the absolute configuration of its product (**92**) using two-dimensional NMR studies would provide strong evidence for the formation of the aldehyde. Oxidation of **91** yielded **92** in 40% isolated yield. This also confirmed that the terminal group (thioacetate in **91**, or allyl disulfide in **80b**) did not affect the rearrangement.



Scheme 3.21: Synthesis of **80** as a result of oxidation of **92**.

To ensure that it was not the oxidation conditions which were encouraging the oxidative rearrangement, sulfide **93** was obtained as a side product in the formation of **78** (Scheme 3.19) . Sulfide **93** contains a vinylic sulfide and an allylic sulfide, which could both be oxidised (Scheme 3.22). However, the product was not a rearrangement product and contained a sulfoxide with comparable IR stretching frequencies as ajoene (**8**). The IR frequencies were 1038, 989 and 926 cm^{-1} in **8** and 1038, 991, 926 cm^{-1} in the product obtained from the oxidation of **93**, therefore **94**. Therefore, it can be deduced that in the presence of an allylic sulfide, the oxidation to the sulfoxide is favoured, over other oxidations such as epoxidation of the double bond. This

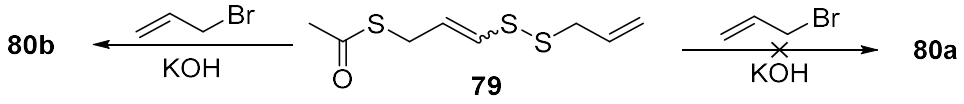
provided further evidence that the product from the original synthesis (Scheme 3.1) yielded the vinylic sulfide (**80b**) and not the desired allylic sulfide (**80a**).



Scheme 3.22: Synthesis of sulfide **93** and oxidation to **94** provides the sulfoxide with the same IR frequencies as **8**.

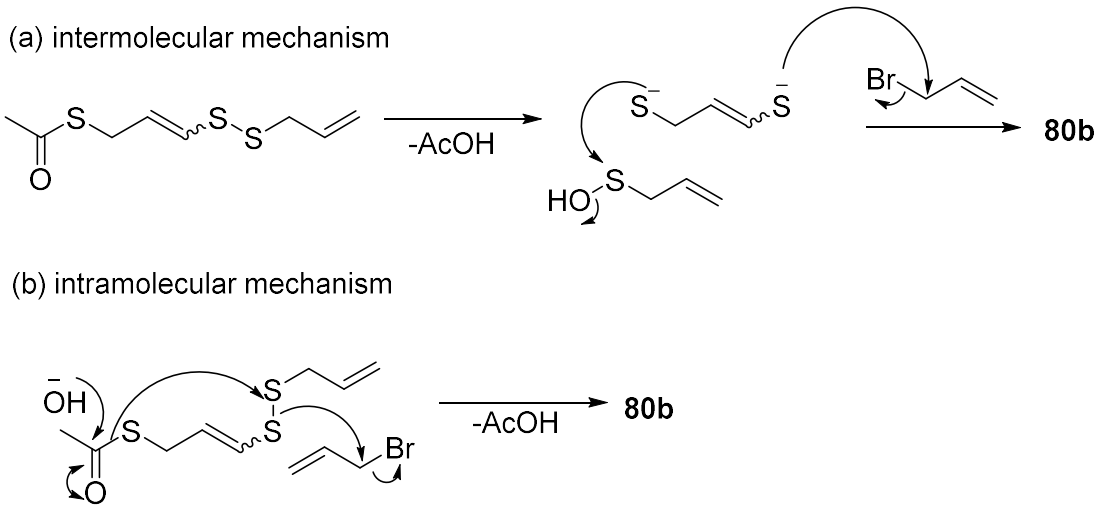
3.7.1. Scrambling

Since 2D NMR confirmed that **79** is formed from **78**, the scrambling of the disulfide and sulfide position must occur in the allylation step (Scheme 3.23).



Scheme 3.23: Deprotection and allylation of **79** results in **80b** over **80a**.

A possible intermolecular mechanism for this would be that the hydroxide anion cleaves both the acetate group, eliminating acetic acid, and the disulfide group forming 2-propenesulfenic acid. The deprotection furnishes the vinylic sulfur nucleophile which preferentially undergoes an S_N2 reaction with allyl bromide. The second sulfur nucleophile forms the disulfide bond with the sulfur atom on 2-propenesulfenic acid. This would be an intermolecular stepwise process (Scheme 3.24a). Also, an intramolecular mechanism could be possible, whereby attack of the hydroxide anion encourages a concerted type process. Here, the one sulfur of the disulfide is used to thiolate at the allylic position, and the second sulfur of the original disulfide is allylated (Scheme 3.24b). Despite which mechanism may be occurring, it can be concluded that the disulfide bond is cleaved either with an equal, or greater, preference to the acetate group.



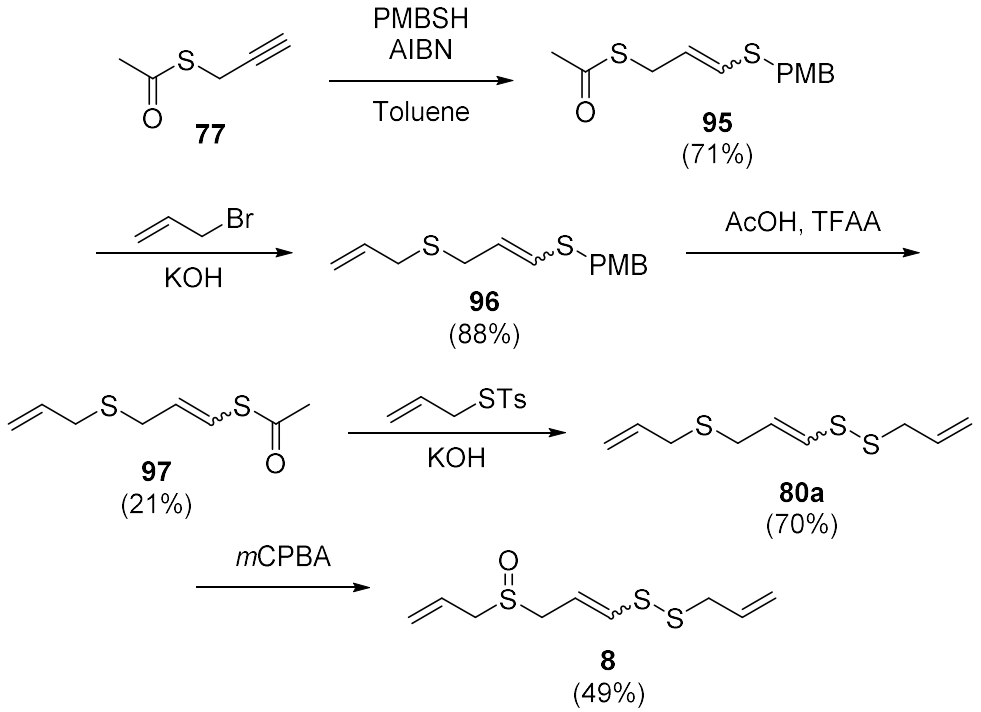
Scheme 3.24: Possible mechanism for the formation of **80b**. (a): intermolecular mechanism; (b): intramolecular mechanism.

3.8. Revised Synthesis

To overcome the scrambling of the disulfide, a revised synthesis was developed to install the disulfide at a later stage so that it would not then be exposed to basic conditions. The revised synthetic route utilises a key intermediate containing both a basic sensitive group and an acid sensitive protecting group (**95**). Having two different protecting groups, the desired sulfur atom can be functionalised selectively and unambiguously.

Alkyne **77** can be synthesised from thioacetic acid in at least 95% as previously discussed. Using AIBN as a radical initiator, *para*-methoxy benzyl (PMB) mercaptan was added to the alkyne to afford **95** in 71% yield. The allylic sulfur was functionalised first to avoid scrambling in later steps as the sulfide is stable. Deprotecting the acetate using potassium hydroxide and allylation with allyl bromide afforded the desired product **96** in good yield (88%). The PMB protecting group can be cleaved under acidic conditions and the resulting thiolate can be trapped as a thioacetate. Thioacetate **97** was obtained in 21% yield. The yield suffers due to the deprotection yielding a volatile, reactive intermediate. Since basic conditions are required in the following step, it was not possible to convert **96** to **80a** directly. With the PMB group exchanged for an acetate group, **97** can be treated with potassium hydroxide and allyl thiotosylate to yield **80a** unambiguously in 80% yield. With the correct sulfide and

disulfide installed, the allylic sulfur can be selectively oxidised to the sulfoxide using recrystallised *m*CPBA in dichloromethane to yield **8** in 49%. The yield of ajoene **8** from acetic acid is 4% over 6 steps. (Scheme 3.25).



Scheme 3.25: Synthesis of **8** in 4% yield over 6 steps from thioacetic acid.

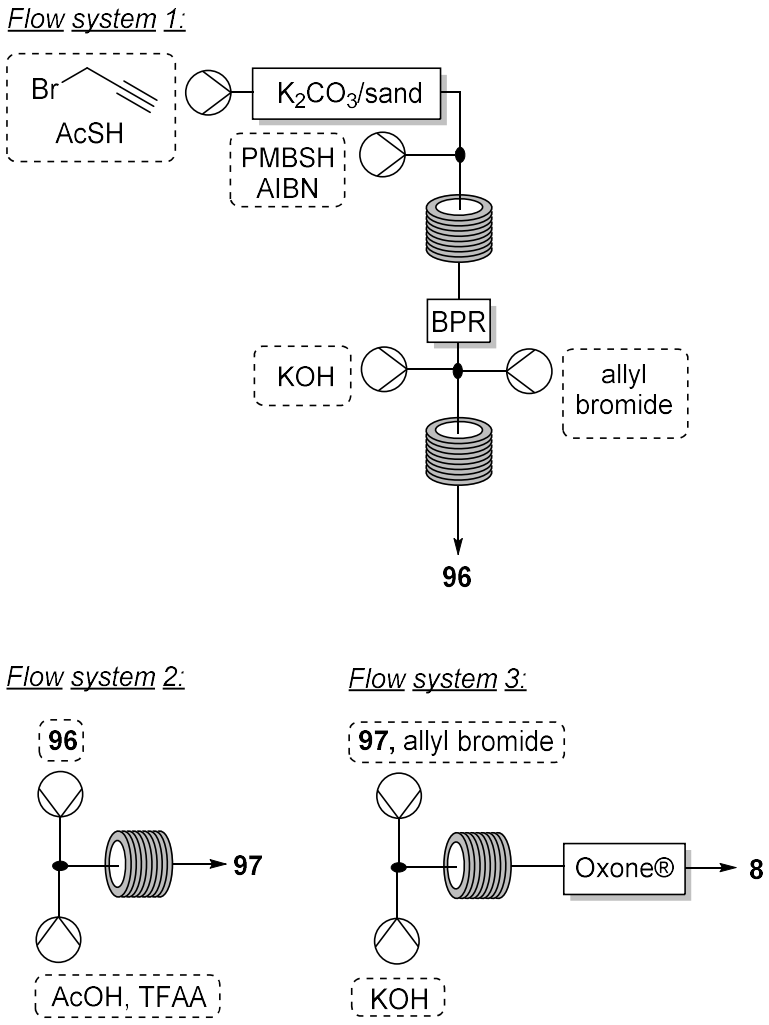
3.9. Conclusions and Future Work

In conclusion, two synthetics routes towards ajoene have been investigated. The first route focussed on the synthesis on an ajoene precursor in a continuous flow system, yielding the product in 12% at 0.26 g/h. However, the system was later determined to yield the isomeric precursor of ajoene. The product favoured oxidation to a rearranged aldehyde product over an ajoene-like product. Although not yielding the desired product, the system starts from readily available and inexpensive starting materials and requires only one silica gel chromatography purification after four steps. This provides a solid example of the benefits associated with flow chemistry and provides a route to synthetically useful materials.

The second synthesis is a revised version of the first. The synthesis overcomes the observed scrambling and subsequent oxidation limitations of the first route. In the revised sequence, ajoene can be unambiguously synthesised over six steps from simple starting materials. The discussed synthetic route stands as the third synthesis of **8** to be reported and the second total synthesis.

Future work:

Future work would encompass the further optimisation of the batch synthesis to ensure higher yields, particularly in the *p*-methoxy benzyl deprotection. The synthesis could also be transferred to a flow system. From steps 1−3 to synthesise **96** and for step 5 to synthesise **80a** from **97**, conditions from the developed flow system can be used. However, investigation for the PMB deprotection and oxidation will be required. Further efforts would see the combination of three flow systems. This may be challenging, although not impossible, due to the switching between basic and acidic reaction conditions (Scheme 3.26).



Scheme 3.26: Potential flow systems to synthesise **8**.

3.10 References

[63] G. C. Hoover, J. Ham, C. Tang, E. I. Carrera, D. S. Seferos, *Can. J. Chem.* **2018**, *96*, 929–933.

[64] T. Nohara, Y. Fujiwara, T. Ikeda, K. Yamaguchi, H. Manabe, K. Murakami, M. Ono, D. Nakano, J. Kinjo, *Chem. Pharm. Bull.* **2014**, *62*, 477–482.

[65] B. Boubia, C. Mioskowski, S. Manna, J. R. Falck, *Tetrahedron Lett.* **1989**, *30*, 6023–6026.

4. Synthesis of Cyclopropyl Ajoene Analogues

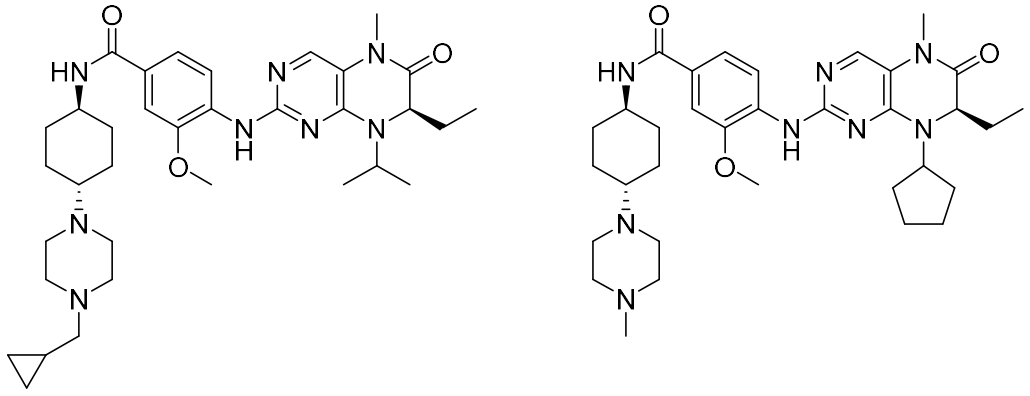
Ajoene, originally derived from garlic, is a compound known to be active in biological systems. However, owing to difficulties in its synthesis, it has not been used for commercial medicinal purposes. Therefore, it is of interest to obtain a compound with superior activity, stability, and a more facile synthesis to that of ajoene.[18]

The aim of this project was to synthesise novel ajoene analogues containing a central cyclopropyl moiety in place of the double bond. Replacing the central olefin with a cyclopropyl group will eradicate the possibility of *E*-/*Z*-isomers and significantly alter the electronic properties from its parent compound. The cyclopropyl group could be introduced in the centre of the molecule, controlling the conformation of the molecule by forming either the *cis*- or *trans*-isomer. Alternatively, the cyclopropyl group could be introduced on the terminal disulfide group or on the terminal sulfoxide group. With three different positions available to modify, three novel structures were selected to be synthesised.

4.1. Compounds Containing Cyclopropyl Moieties

The first set of analogues replaced the carbon-carbon double bond with a cyclopropyl ring. This modification was accompanied by the rationale that spatially the alkene bond and the cyclopropyl ring are similar however, electronically these two groups are very different.

Cyclopropyl moieties are known in many US Food and Drug Administration (FDA) approved drugs. The strained moiety is also present in various natural products and has shown a wide range of biological activities.[66] An example of this is given in Figure 4.1. Volasertib (**98**) is a therapeutic target for the development of antineoplastic drugs that possess high potency as well as selective inhibitory properties.[67] A structurally similar analogue, **99**, was developed where the methyl cyclopropyl group was replaced for a methyl substituent.[68] Analysis of **99** showed that the molecule had significantly reduced pharmacokinetic properties over **98**.[69]

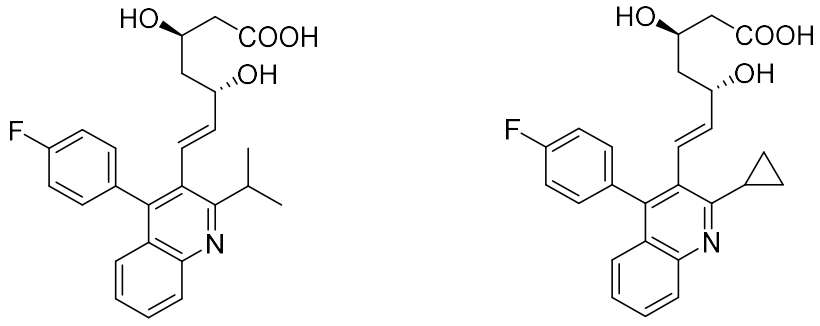


98
Volasertib

99
Volasertib-type Analogue

Figure 4.1: Volasertib (**98**) and a structurally similar analogue (**99**) were assessed for their pharmacokinetic properties.

Compound **100** is a molecule which possess inhibitory biological activity in the treatment of metabolic disorders (Figure 4.2). The isopropyl moiety in **100** was replaced for a cyclopropyl group and the biological activity was assessed and compared to its parent compound. Compound **101** was found to have a 5-fold greater activity. It was also reported that the introduction of the cyclopropyl moiety increased the bioavailability and had a longer duration of action.[70] Pitavastatin (**101**) was FDA approved in 2009 as a treatment for high serum cholesterol levels.[69]



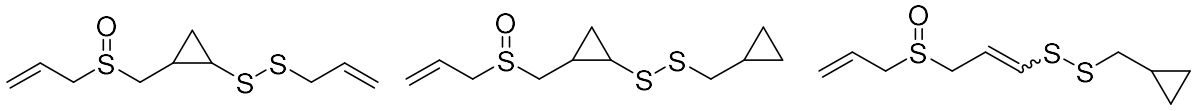
100
Pitavastatin Parent Drug

101
Pitavastatin

Figure 4.2: Replacement of the isopropyl moiety in **100** with a cyclopropyl moiety increased the biological activity of Pitavastatin (**101**) by over 5-fold was reported by Suzuki *et al*.[70]

4.2. Synthesis of Cyclopropyl Ajoene Analogues

The incorporation of a cyclopropyl ring into an ajoene analogue has not yet been reported in literature. Increased biological activity from **8** would be advantageous as the dosage of the compound to achieve the same effect could be reduced. In addition, substitution of the double bond with a cyclopropyl group would result in a structurally interesting compound that could provide insight into the mode of action of ajoene. The three novel analogues proposed are shown in Figure 4.3.



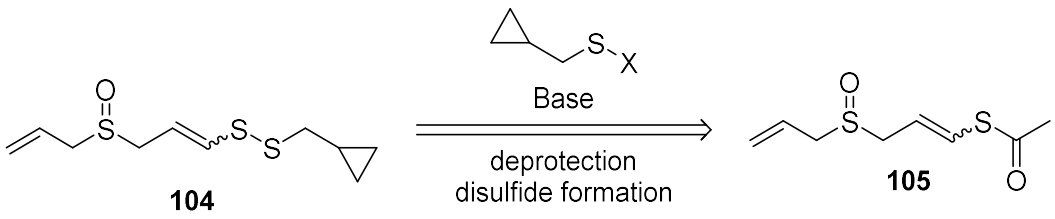
102 **103** **104**

Figure 4.3: Analogues of ajoene (**8**) containing cyclopropyl moieties in replacement of the double bonds.

Modifying the central olefin of **8** could allow for greater control in its isomers. In ajoene, *E*- and *Z*-isomers arise from the unsymmetrical central olefin. Despite efforts to gain geometric isomer control through varying synthetic routes, the selective synthesis of ajoene remains a challenge. In **102** and **103**, the central olefin has been replaced by a cyclopropyl moiety. It is possible here that *cis* and *trans* isomers of the final product can occur and so developing a novel synthesis of the proposed analogues would ideally incorporate a level of control. In addition to *cis* and *trans* isomers, **102** and **103** also has two stereogenic centres at the disubstituted cyclopropanes and thus, diastereomers of both compounds are possible.

4.3. Retrosynthetic Analysis of Analogue 104

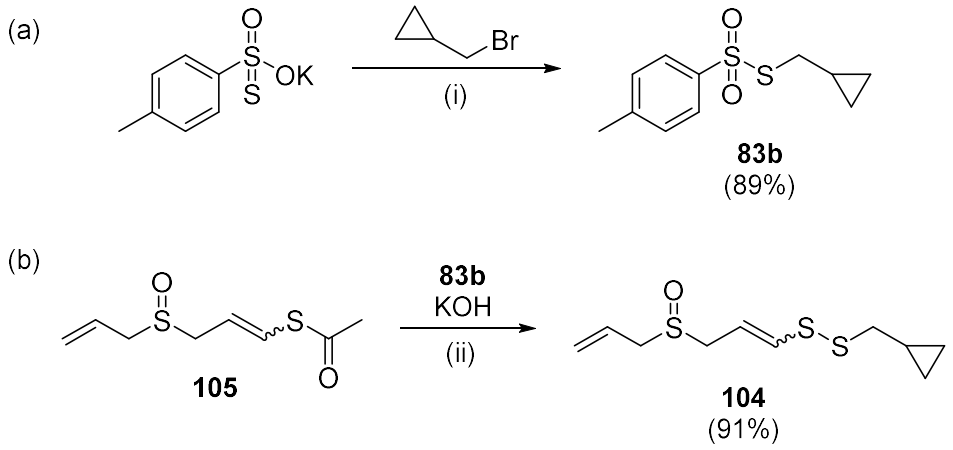
Compound **104** could be made from a precursor **105** (Scheme 4.1). Precursor **105** was obtained as a product of optimisation studies by F. Silva *et al*.[16] Treatment with a base and an electrophilic sulfur source with the desired methyl cyclopropyl group, could afford desired product (**104**).



Scheme 4.1: Compound **104** could be synthesised from precursor **105** synthesised by F. Silva.

4.3.1.1. Synthesis

Compound **104** was synthesised from **105** and the thiotosylate reagent **83b**. Thiotosylating reagent **83b** can be obtained from potassium thiotosylate and methyl cyclopropyl bromide in 89% yield (Scheme 4.2a). With reagent **83b** in hand, compound **105** could be treated with a potassium hydroxide as base to cleave the vinyl acetate *in situ* and form the desired disulfide (**104**) in 91% yield (Scheme 4.2b).

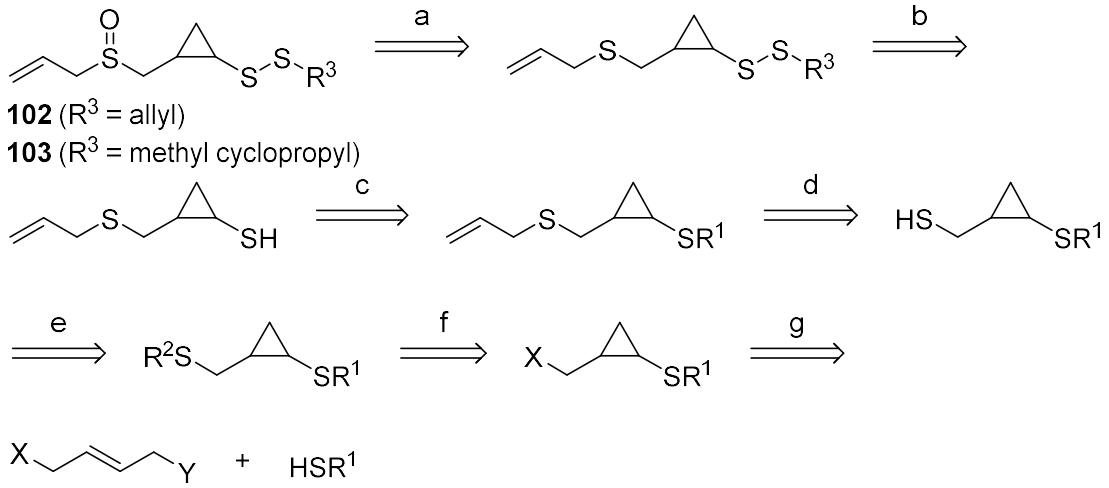


Scheme 4.2: Synthesis of **104**. Conditions: (i) methyl cyclopropyl bromide (2 equiv.) in DMF (0.1 M), r.t. (ii) **83b** (1.2 equiv.), KOH (1.1 equiv) in methanol, -40 °C to r.t., 2 h.

4.4. Retrosynthetic Analysis of Analogues 102 and 103

Retrosynthetic analysis of analogues and **102** and **103** is proposed in Scheme 4.3 over seven steps. The first retrosynthetic step a was envisioned to be the oxidation from sulfide to sulfoxide using a simple oxidant such as *m*CPBA. Step b is the disulfide formation between a nucleophilic sulfur, such as a thiol, and an electrophilic sulfur, such as a thiotosylate. Step c is the deprotection of the protecting group (R^1) to unmask the free thiol. Step d is the allylation of the sulfide. Step e is the deprotection of the sulfide. To install the second sulfide, this could be done by nucleophilic

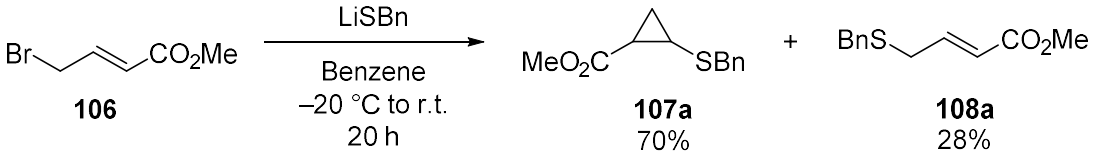
substitution with a labile leaving group (X) in step f. The final step, g, to give the cyclopropyl could be introduced by several methods, including a Michael Induced Ring Closure (MIRC). This route will be underpinned by the stability of the intermediates as well as the selective deprotection of both protecting groups (R^1 and R^2).



Scheme 4.3: Retrosynthetic analysis of analogues **102** and **103**.

4.5. Installing Central Cyclopropyl Moiety

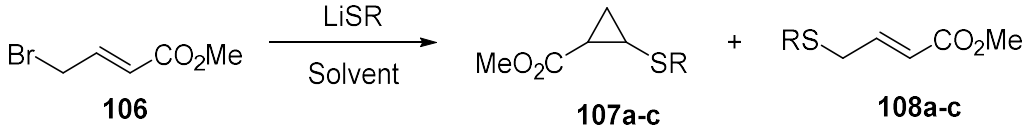
Installation of the central cyclopropyl moiety could be achieved in a variety of ways. However, the key to synthesise **102** and **103** effectively is reliant on the selective functionalisation of either side of the cyclopropyl moiety. A. Bernard *et al*. reported in 2003 a Michael Induced Ring Closure (MIRC) between methyl 4-bromocrotonate (**106**) and benzyl mercaptan to yield a product **107** containing a cyclopropyl functionality (Scheme 4.4).[71] Interestingly, Bernard *et* al. reported that the desired product was obtained in exclusively its *trans* isomer, along with 28% of side product, **108**, resulting from the direct substitution of the bromide by the thiolate. This method to obtain a compound containing a cyclopropyl thioether seemed attractive as it also incorporated isomeric control. The presence of the ester moiety also presented the opportunity for further functionalisation post MIRC.



Scheme 4.4: Synthesis of compound containing cyclopropyl (**107**) with thioether functionality in 70% yield.[71]

4.5.1. Michael Induced Ring Closure (MIRC) with Thiols

The MIRC reaction with thiol substrates of the type RSH (Scheme 4.5) included a consideration for the later step of deprotecting of the R group. The lithium thiolate was generated *in situ* using *n*-butyl lithium (*n*-BuLi) in tetrahydrofuran (THF) or benzene.



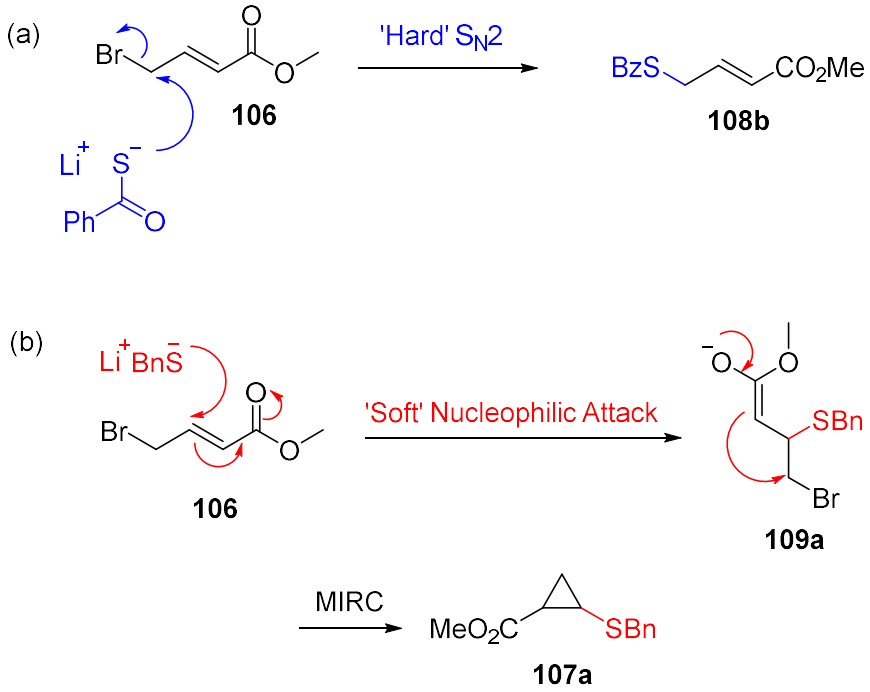
Scheme 4.5: Investigation of potential lithium thiolates for MIRC.

Entry	R	Solvent	Temperature (°C)	Desired product	Side product
1	Bn	THF	−20 to r.t.	**107a** 62%	**108a** 17%
2	Bz	Benzene	−20 to r.t.	**107b** 0%	**108b** 65%
3	para-methoxy benzyl (PMB)	THF	−40 to r.t.	**107c** 40%	**108c** 0%

Table 4.1: Isolated yields for MIRC reactions for methyl 4-bromocrotonate (**106**) with benzyl mercaptan, thiobenzoic acid and *para*-methoxy benzylmercaptan (PMB) to yield **107a-c** and **108a-c** respectively.

Benzyl mercaptan as the thiol source yielded the greatest amount of desired product in 62% (**107a**). The desired product in entries 1 and 3, **107a** and **107c** respectively, were obtained exclusively in the *trans* isomer. The conformation of the cyclopropyl product was determined by comparing of the ^{1}H NMR data reported by Bernard *et al*., who assigned the structure unambiguously by NOSEY and differential NOE experiments, also in comparison with analogous products reported in the literature.[72,73]

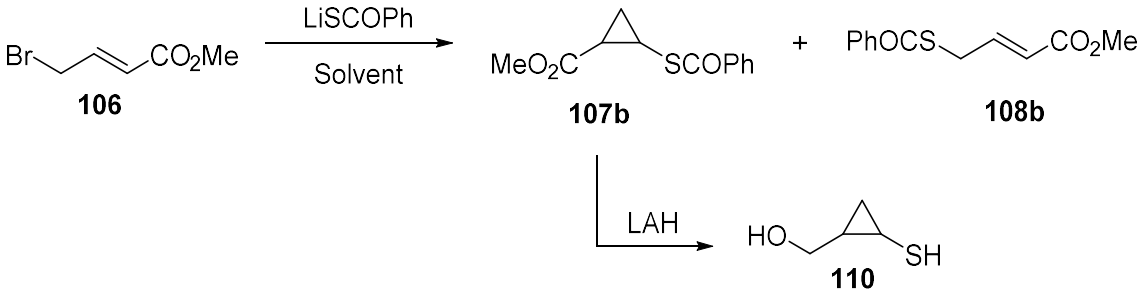
Reaction of the lithium thiobenzoic acid with **106** (entry 2) did not form the desired product and saw exclusively the direct substitution product, **108b**, in a good yield (65%). An explanation for this result may lay in the nucleophilicity of the formed thiolate. In the cases of the benzyl thiolate nucleophiles, the preferential attack is the β-position of the α, β-unsaturated carbonyl. This soft nucleophilic attack generates intermediate **109a,** which readily undergoes a ring closure reaction resulting the desired cyclopropyl product **107a**. However, where the thiolate is conjugated with a carbonyl, as in thiobenzoic acid, the preferential attack is the direct S_N2 reaction to eliminate the bromide, yielding product **108b**. The nucleophilicity of the thiolate resulting from thiobenzoic acid is harder than in the case of benzyl mercaptan that yields a much softer nucleophile. The mechanisms of both these cases are shown in Scheme 4.6a and Scheme 4.6b.



Scheme 4.6: Possible mechanisms for the nucleophilic attack of the thiolates resulting from benzyl mercaptan and thiobenzoic acid. Observations show that a hard nucleophile will proceed by S_N2 of the bromide anion yielding product **108b** (a), whereas a softer nucleophile will attack the α,β-unsaturated system in the β-position yielding product **107a** (b).

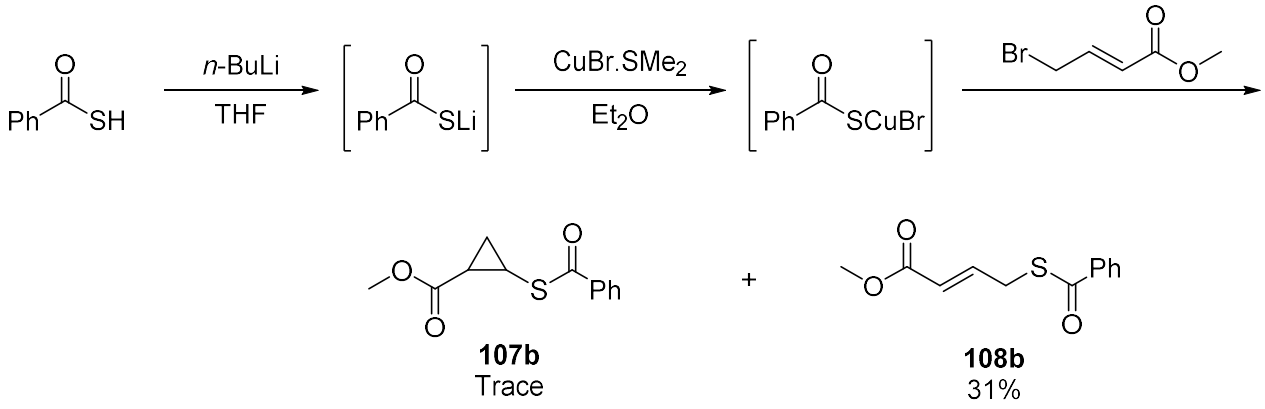
Generating **107b** was the preferred ring closed product over **107a** and **107c**. This is due to the nature of the benzoyl (Bz) protecting group. In the following step, treatment with lithium aluminium hydride (LAH) could reduce the ester and thioester to the

alcohol and thiol respectively. This would be a convenient method to afford the double deprotected product in just one step (Scheme 4.7).



Scheme 4.7: MIRC reaction with **106** and thiobenzoic acid could generate **107b** which could be reduced to cleave both protecting groups to yield **110** in one step.

As the lithium thiobenzoate was reacting by the undesired pathway to yield the substitution product **108b**, it was hypothesised that forming a softer nucleophile, such a copper thiolate, could tune the nucleophilicity in such a way to encourage the MIRC pathway (Scheme 4.8). Despite efforts, this yielded only trace amount **107b** as observed in ^{1}H NMR. Although the desired reaction was encouraged, the direct substitution dominated, and **108b** was obtained in 31% yield. Since thiobenzoic acid did not yield **108b**, the synthesis was revised to include **107a** or **107c** as viable synthetic routes.

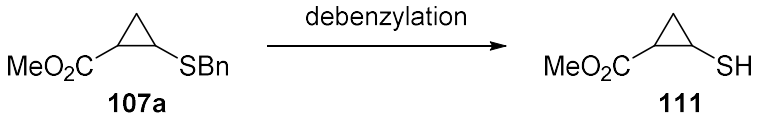


Scheme 4.8: Forming the thiobenzoate cuprate from the lithium thiolate in order to tune nucleophilicity to encourage formation of desired product **107b**.

4.6. Deprotection of Protected Thiol

4.6.1. Deprotection of Benzyl Group

Since **107a** could be synthesised in 62% yield, the deprotection of the benzyl group was examined. Although debenzylation is not the following step in the retrosynthetic route, the deprotection was investigated earlier in the synthesis to determine its feasibility (Scheme 4.9).



Scheme 4.9: Desired deprotection of thioether **107a** to yield **111**.

Reported literature procedures for debenzylating a thiol typically use harsh conditions such as sodium or lithium in liquid ammonia[74], hydrogen fluoride in anisole[75], and sodium in boiling butyl alcohol[76]. A variety of other methods were investigated, although in all cases no desired debenzylated product could be observed.

Three literature procedures have been trialled, all yielding no desired product (Scheme 4.9, Table 4.2). A literature procedure by D. Chen *et al*. where an alcohol was debenzylated was trialled (Entry 1).[77] However, since all starting material was recovered with 0% conversion, it was suspected that the sulfur poisoned the palladium catalyst. The following attempt used a method developed by K. Okano *et al*. using boron trichloride to remove a benzyl group (Entry 2).[78] The final attempt used magnesium turnings and ammonium formate to generate hydrogen *in situ* using a procedure reported by N. Narenda Babu *et al.* (Entry 3).[79] Although N. Narenda Babu *et al*. reports yields up to 90% yield for *S*-benzyl protected cystines, the reaction conditions did not give desired product. The reaction achieved 43% conversion based on recovered starting material. This may indicate that the resulting debenzylated thiol is not stable and further decomposes.

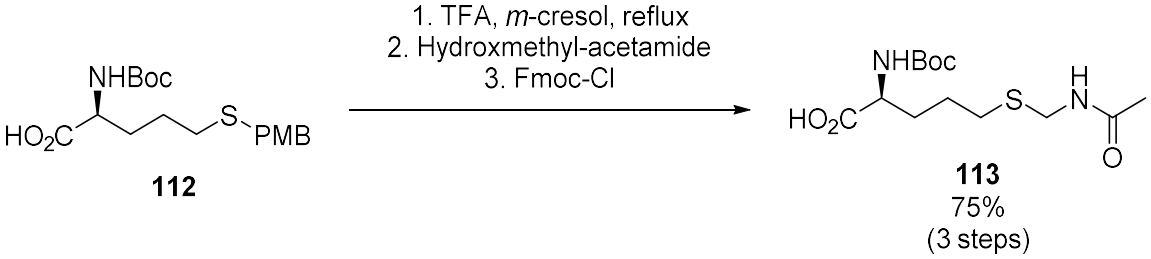
Entry	Conditions	Result
1	$Pd(OH)_2/C$, H_2, EtOAc, 20 h, r.t.	0% conversion
2	BCl_3, CH_2Cl_2, 20 h, −78 °C to r.t.	0% conversion
3	Mg turnings, $NH_4^+HCOO^-$, MeOH, r.t.	57% starting material recovered

Table 4.2: Table of results for the debenzylation of protected thiol following three literature procedures.

4.6.2. Deprotection of *para*-Methoxybenzyl Group

Since the debenzylation of the **107a** was unsuccessful, the substrate in the MIRC was altered for the PMB-thiol to yield **107c**. The presence of the *para*-methoxy group has been reported to increase the lability of the leaving group due to its electron donating effects.

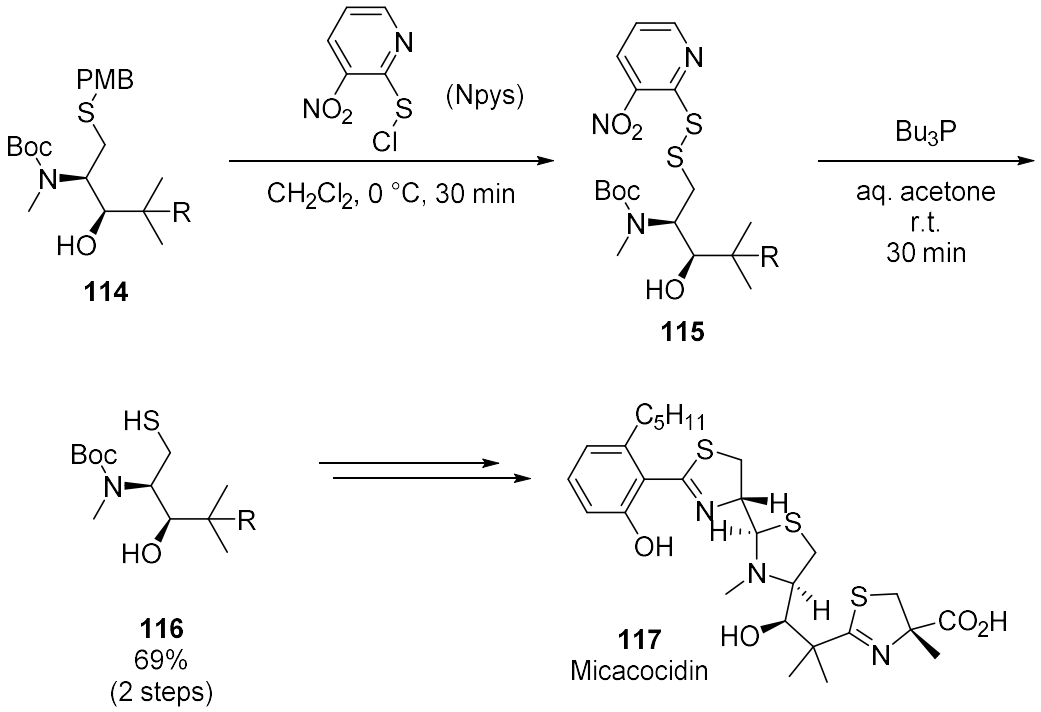
Deprotection of the *para*-methoxy benzyl (PMB) group using acidic conditions have been reported in literature. In 1993, Sujatha *et al.* reported the synthesis of cystine homologs (**113**). Sujatha and co-workers treated PMB protected thiols (**112**) with trifluoroacetic acid (TFA) to generate the intermediate thiolate before further functionalisation to yield **113** in 75% yield over two steps (Scheme 4.10).[80]



Scheme 4.10: Synthesis of cystine homologs using PMB deprotection reported by Sujatha *et al.*[80]

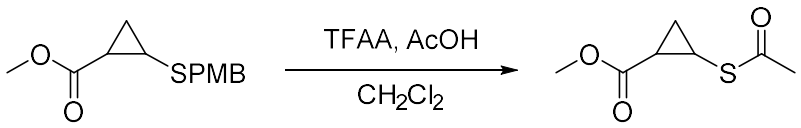
In 1998, Ino *et al*. also reported a deprotection method of PMB protected thiols using 3-nitro-2-pyridylsulfenyl chloride (Npys) in the synthesis of Micacocidin (**117**). The protected thiol **114** was treated with Npys; this deprotected the PMB group by forming disulfide **115**. Treatment with tributyl phosphine cleaves the disulfide bond to yield a free thiol **119** in 69% yield over two steps (Scheme 4.11).[81]

More recently in 2011, Jung *et al.* reported a mild selective deprotection of PMB groups using triflic acid and 1,3-dimethoxybenzene.[82] Jung demonstrated the successful deprotection on a range of PMB protected alcohols in yields up to 93%.



Scheme 4.11: Deprotection of thioether **114** using Npys followed by Bu_3P in acetone to yield **116** in the total synthesis of **117** reported by Ino *et al*.

The deprotection was briefly trialled with a model compound **107c** to ensure that the PMB protecting group was able to be cleaved and that the route posed an element of viability. Under acidic conditions and treatment with trifluoroacetic anhydride (TFAA) and acetic acid in dichloromethane, the thiolate generated *in situ* was trapped as thioacetate **118** in 24% yield (Scheme 4.12). Despite aiming for **118** as its free thiol, the thioacetate is a viable product as it can be easily deprotected with base to form the desired disulfide functionality.



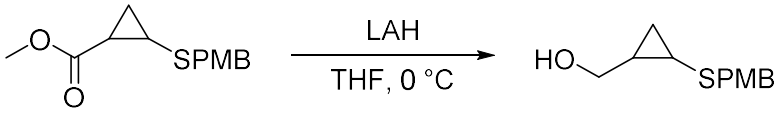
107c **118**
24%

Scheme 4.12: Deprotection of **107c** to yield **118** in 24% using 5 equiv. of TFAA and 5 equiv of AcOH.

4.7. Reduction of Ester to Alcohol

Reduction of an ester to an alcohol is well reported in the literature using a hydride source. The reagent of choice for this simple transformation is lithium aluminium hydride (LAH). Another common reducing agent is sodium borohydride ($NaBH_4$). In this case, sodium borohydride does not have the sufficient strength to reduce the ester. This is due to the electronegativity of the metal. Aluminium is less electronegative than boron and thus, the hydride in LAH is more electron rich, and therefore a stronger nucleophile than the hydride in sodium borohydride.

The reduction of **107c** to its corresponding primary alcohol, **119**, proceeded smoothly by reduction using LAH. The desired product **119** could be isolated in 80% with no further purification required after aqueous work up (Scheme 4.13). With the alcohol functionality in place, attention turned towards building the allylic thioether which can be oxidised to the sulfoxide, such as in target molecules **102** and **103**.



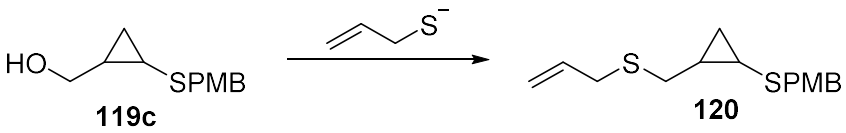
107c **119**
80%

Scheme 4.13: Reduction of MIRC ester (**107c**) to alcohol **119** using one equivalent of lithium aluminium hydride.

4.8. Conversion of Ester to Sulfide

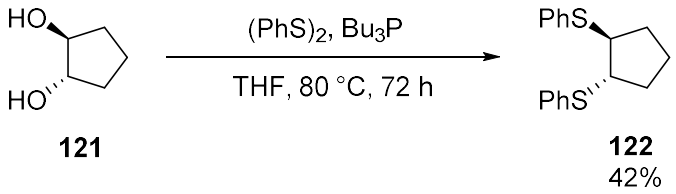
With the primary alcohol **119** in hand, attention turned to converting the alcohol to the desired thioether **120**. The desired transformation would afford the allylated thioether with elimination of the hydroxide (Scheme 4.14). However, since the alcohol is not a

group that will readily eliminate from the molecule, several routes were explored to increase the leaving group ability of the alcohol.



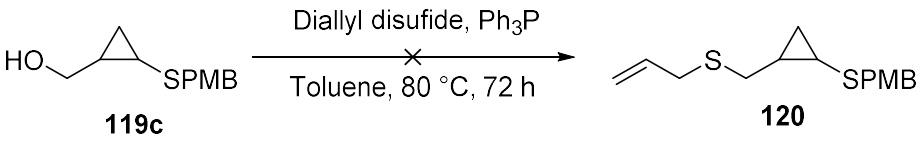
Scheme 4.14: Desired transformation of alcohol **119** to thioether **120**.

A literature example of a primary alcohol transformed into a thioether in one step was described by Skarzewski *et al.* in 2003, where the diol **121** was converted to the dithioether **122** in one step using a diphenyl disulfide (Scheme 4.15).[83] This work was then further extended in 2008, where the same group reported the mono substitution to produce mixed alcohol-thiol products with central cyclohexane rings.[84]



Scheme 4.15: Treatment of diol **121** with diphenyl disulfide yields dithioether **122** in 42% yield.

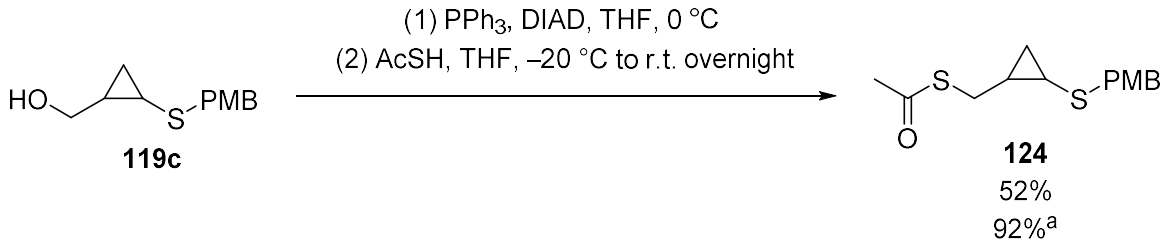
The literature procedure was applied to the model alcohol **119c** in an effort to achieve the sulfide **125** (Scheme 4.16). Converting the alcohol directly into an allylated thioether would be a convenient method and a time saving strategy. In addition, using diallyl disulfide would be an inexpensive and readily source of allylated thiolate. Unfortunately, no desired product could be achieved, and the starting material was recovered after work-up.



Scheme 4.16: Attempt to convert alcohol **119c** to **120** using diallyl disulfide.

Since the thioether could not be directly achieved from the primary alcohol, the alcohol was converted to a thioacetate *via* a standard Mitsunobu reaction.[85] A literature

procedure converting an alcohol to a thioacetate was followed. The literature procedure used high equivalents, where all reagents were at 4 equivalents. Product **124** could be obtained from **119c** in 52% isolated yield. The purification of the desired product from unreacted triphenyl phosphine, triphenyl phosphine oxide, unreacted diisopropyldiazocarboxylate and diisopropylhydrazine-1,2-dicarboxylate were likely to be the cause of inefficient purification. There was a dramatic increase in yield of **124** to 92% when the equivalents of all reagents were halved (Scheme 4.17).



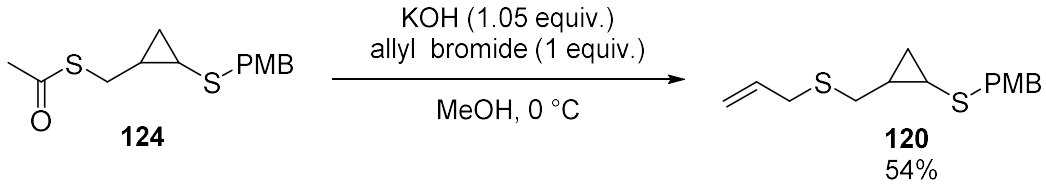
Scheme 4.17: Mitsunobu reaction to transform primary alcohol **119c** to thioacetate **124**. Reagents and conditions: (1) Triphenyl phosphine (4 equiv.), diisopropylazocarbozylate (4 equiv.) in THF at 0 °C vigorously stirred 30 min; (2) Thioacetic acid (4 equiv.), **119c** (1 equiv.) in THF, -20 °C to room temperature, overnight. [a]PPh_3 (2 equiv.), DIAD (2 equiv.), AcSH (3 equiv.)

Having installed a labile base sensitive protecting group in **124**, this would allow the selective deprotection, thus installing the disulfide bond on the correct sulfur atom, omitting the possibility of isomers.

4.9. Deprotection

4.9.1. Deprotection and Allylation

Deprotection of **124** and allylation proceeded smoothly under mild basic conditions to afford the desired product **120** in 54% yield. The PMB protected thiol was unaffected in this transformation. The main advantage of installing the thioether before the disulfide is that the thioether is more stable and so should retain its integrity in the following steps.

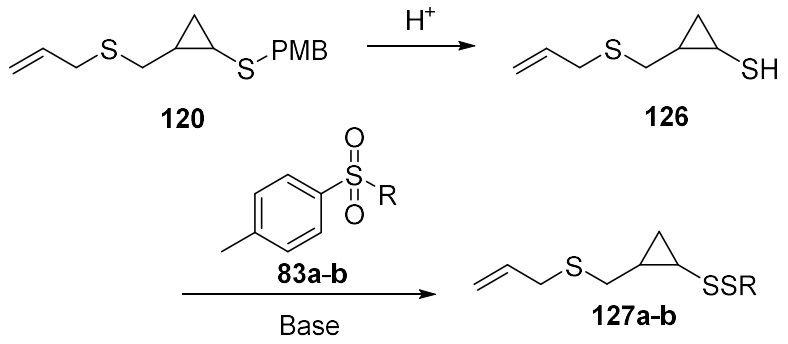


Scheme 4.18: Deprotection of acetate in **124**, and subsequent allylation yields **120** in 54% yield.

Installing the allyl group from a commercially available and inexpensive reagent such as allyl bromide, leaves the possibility that other compounds could be made in the same way. This could be achieved by altering the allyl bromide to an alkyl or aryl bromide.

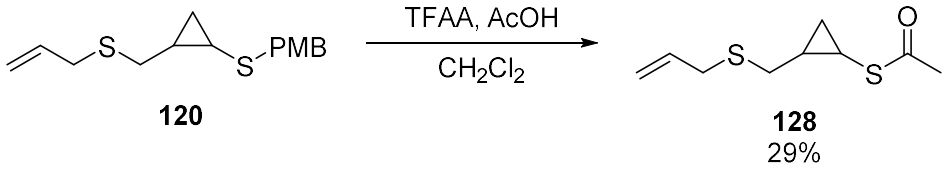
4.9.2. Deprotection of *para*-Methoxy Benzyl Group

As previously mentioned, a PMB protected thiol can be deprotected under acidic conditions. The presence of the methoxy group on the phenyl ring increases the electron density due to the electron donating nature of the ether and increases lability. As a deprotected product, thiol **126** would be desired since **126** could be reacted directly with a base fand **83a**-**b** to form the desired disulfide **127a**–**b** (Scheme 4.19).



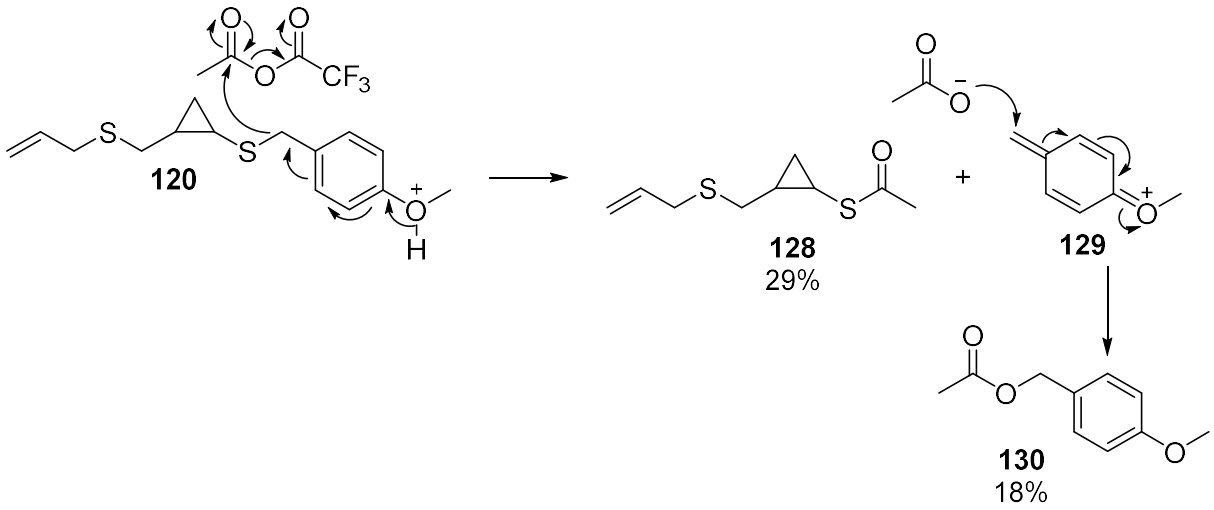
Scheme 4.19: Desired deprotection of **120** to yield thiol **126**. R = **a**: allyl or **b**: methyl cyclopropyl

As shown previously, the PMB group can be deprotected in **107c** to give **118** in 24% yield (Scheme 4.12). Trifluoroacetic anhydride in a mixture with acetic acid was applied to **120** to yield the thioacetate **128** in 29% yield (Scheme 4.20).



Scheme 4.20: Deprotection of **125** yields thioacetate **128**.

A proposed mechanism is given in Scheme 4.21. Firstly, the methoxy group of **120** is protonated under acidic conditions. The loss of the proton encourages the benzylic C−S to break by the movement of electrons through the aromatic ring. The thiolate attacks carbonyl of the mixed anhydride. This leads to **128** being obtained in 29% yield. The cleaved group intermediate (**129**) is then attacked at the most electrophilic position by an acetate which forms an ester **130**, isolated in 18% yield. This mechanism is proposed on the basis that a non-fluorinated acetate is seen in both products.



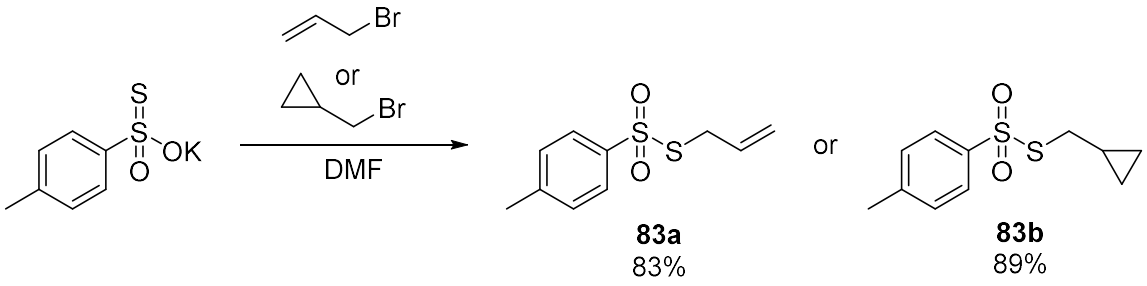
Scheme 4.21: Proposed mechanism for the deprotection of PMB yielding **128** and **130** and 29% and 18% isolated yield, respectively.

The deprotection is low yielding; reasons for this may be two-fold. Firstly, the starting material is recovered in all cases in a least 10% yield. A probable reason for this may be that the thiolate of **120** is a strong nucleophile and can attack the intermediate **129** over the mixed anhydride to yield the starting material (**120**). Secondly, the mechanism may not be concerted, and so if the thiolate is present *in situ*, it may be unstable and prone to rearrangement or side reactions *via* an undesired reaction pathway.

4.10. Disulfide formation

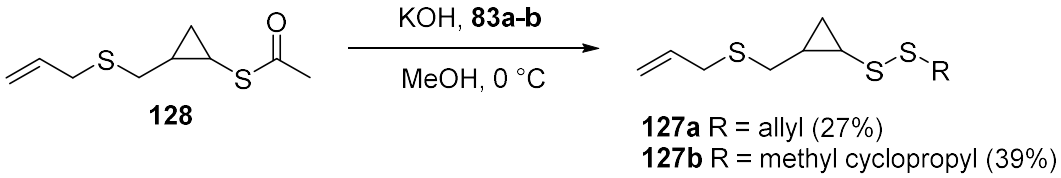
The deprotection of the thioacetate unmasks a sulfur nucleophile. Therefore, an electrophilic sulfonating reagent is required for the disulfide formation. Potassium thiotosylate is a commercially available reagent with an electrophilic sulfur present. By stirring at room temperature in dimethyl formamide (DMF) with allyl bromide or methyl

cyclopropyl bromide, the desired sulfonating reagent (**83a**–**b**) can be achieved in excellent yields (Scheme 4.22).



Scheme 4.22: Synthesis of sulfonating reagent from potassium thiotosylate. Conditions and reagents: potassium thiotosylate (1 equiv.), allyl bromide or methyl cyclopropyl bromide (2 equiv.), DMF (0.1 M), r.t, 18 h.

When **128** is treated with a base, the thioester is cleaved unmasking the thiolate *in situ*. This can then react with the electrophilic sulfur in **83a**–**b** to afford the desired disulfide product (**127a**–**b**) in 27-39% yield (Scheme 4.23). The yields for this transformation are poor, likely to be due to the instability of the thiolate intermediate.

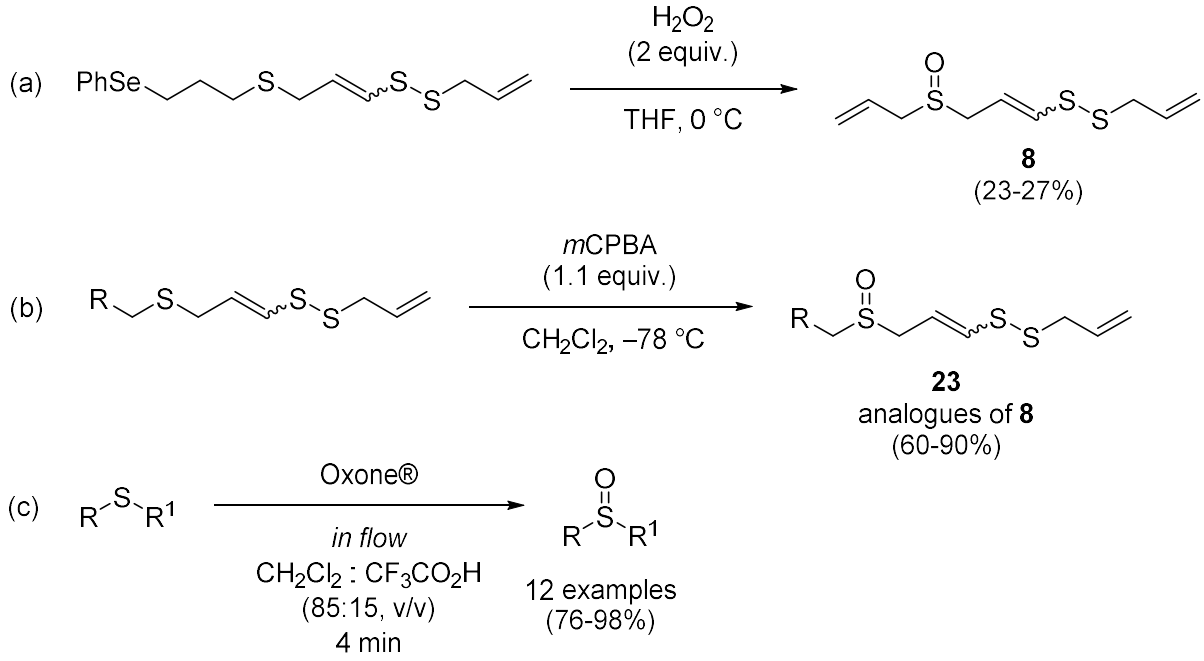


Scheme 4.23: Formation of disulfide bond to yield desired product **118**. Conditions: **83a-b** (1.2 equiv.), KOH (1 equiv.), methanol.

4.11. Oxidation

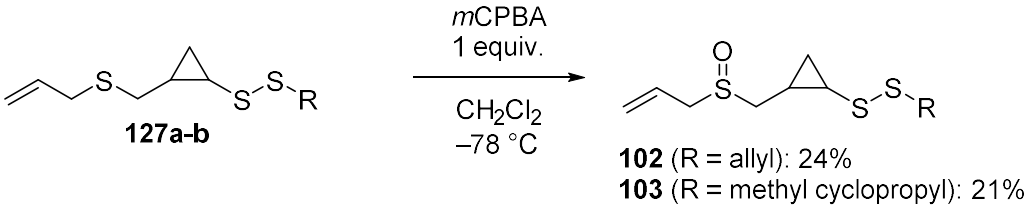
The oxidation of sulfides are well explored. An oxidation using hydrogen peroxide was used in the short total synthesis of **8** by F. Silva in 2018.[16] Using 2 equivalents of hydrogen peroxide, simultaneous oxidation of the selenide to encourage selenoxide elimination, and oxidation of the sulfide to desired sulfoxide could take place (Scheme 4.24a). In 2008, Hunter *et al*. produced a library of ajoene analogues. The sulfoxide was furnished using a simple *m*-chloroperbenzoic acid (*m*CPBA) oxidation which gave the desired products in up to 90% yield (Scheme 4.24b)[17]. In both examples, the sulfur to sulfoxide transformation seems to occur preferentially over oxidation of either the double bond or disulfide. In a third example, F. Silva in 2018 probed the oxidation of

sulfides using Oxone® in a packed column in a flow system. Twelve sulfide examples were able to be oxidised in high yields in just 4 minutes under the established conditions (Scheme 4.24c).[62]



Scheme 4.24: Literature examples for the oxidation of sulfides. (a): oxidation of sulfide and selenide by F. Silva *et al*.[16] (b): oxidation of ajoene analogues by Hunter *et al*.[17] (c): oxidation of sulfides in flow by F. Silva *et al*.[62]

For the oxidation of **127a-b**, *m*CPBA was firstly purified from a commercially available reagent which is sold at approximately 77% purity. Purification of *m*CPBA removed the non-reactive *m*-chlorobenzoic acid using a literature procedure.[86] Treating sulfides **127a-b** with purified *m*CPBA acid afforded the final target compounds in 21-24% isolated yield (Scheme 4.25).



Scheme 4.25: Oxidation of sulfides to target material **119a-b** in 21-24% yield.

Over eight steps in total, the final target compounds (**102** and **103**) could be synthesised from readily available starting materials.

4.12. Conclusions and future work

Three novel ajoene analogues containing cyclopropyl moieties in place of the central double bond and/or the disulfide terminal group in ajoene were synthesised. Two analogues were synthesised over a new sequence of eight steps, with the key step being the Michael Induced Ring Closure (MIRC) which proceeds selectively, giving only the *trans* isomer. The third analogue was synthesised over two steps in total due to the availability of the precursor that has been previously synthesised in Section 3.

Future work:

Future work would naturally involve optimising the reaction steps and scaling up to provide a proof of principle to synthesis gram quantities of the target material. The scope could also be extended to encompass other ajoene analogues with strained ring systems as the central moiety, such as cyclobutyl or cyclopentyl. In addition to optimising the synthesis for higher yields, work could also involve developing a synthesis which is diastereoselective, perhaps in the form of a chiral Michael induced ring closure or by other means.

4.13. References

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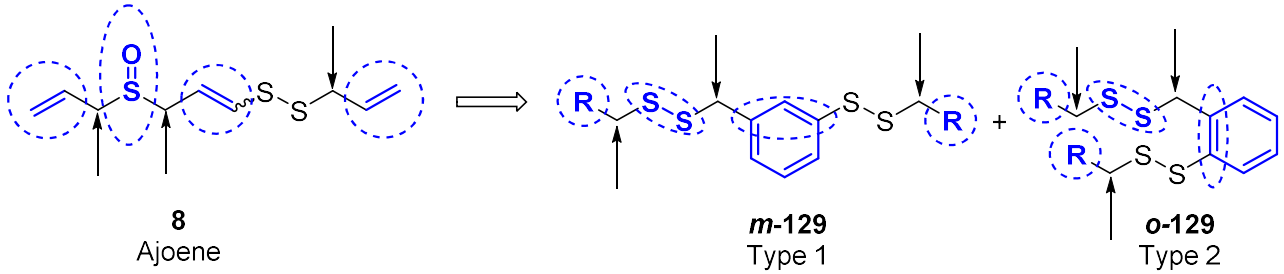
5. Introduction and Objectives

Disulfide compounds are well known for possessing biological activity.[29] Their inherent instability and their presumed ability to interact with the thiols of cystines in a biological system has been proposed as the key mechanism for the biological activity observed. Disulfide compounds, such as diallyl disulfide and diisopropyl disulfide have been isolated from *Allium* extracts,[7] along with bis-disulfide compounds such as 2,2'-(prop-1-ene-1,3-diyl)bis(1-allyldisulfane).[34] The naturally occurring bis-disulfide therefore gives precedent to a class of novel compounds which may exhibit heightened biological activity, therefore lowering the effective dose required. Ajoene analogues containing bis-disulfide moieties are still under explored. However, the difficulty in in producing ajoene analogues is the lack of control over the geometric isomers, which complicates purification and lowers yields.

The aim of this project was to synthesise a range of novel bis-disulfide based on the structure of ajoene (**8**) (Scheme 5.1). In addition to alternating the nature of the original sulfoxide moiety in **8**, the central olefin could also be modified to a central aromatic compound, thus giving complete control over the conformation of the compound. Type 1 analogues (***m*-129**) demonstrate 1,3-subsitution, mimicking *E*-ajoene, while type 2 analogues (***o*-129**) demonstrate 1,2-subsitution, mimicking *Z*-ajoene. Terminal groups (R) were also modified to a range different of groups.



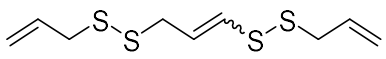
Scheme 5.1: Proposed ajoene analogues (***m*-/*o*-129**) demonstrating modified aspects of **8** and unchanged aspects. Bonds and atoms highlighted in blue illustrate points of modification. Arrows highlighting CH_2 groups have been intentionally left unchanged.

5.1. Bis-Disulfides as Analogues of Ajoene

Varying the sulfoxide group in **8** allows a set of analogues to be synthesised. The oxidation state of the sulfoxide sulfur atom in ajoene is formally 0, therefore, modifying the sulfur atom to a sulfide or disulfide would formally change the oxidation state to −2 and −1, respectively. Having the sulfur atom in a reduced state may change the reactivity or its interaction within a biological environment.

5.1.1. Bis-Disulfide with Central Olefin

The bis-disulfide (**37**) with a central olefin and two terminal allyl groups is a natural product which was first reported in 2014 by Nohara *et al.* along with five other novel acyclic sulfur compounds obtained from garlic bulbs (Figure 5.1).[34] However, its only known chemical synthesis is discussed in Chapter 3. However, as with the syntheses of ajoene analogues, the synthesis encompassed no control over the formation of one geometric isomer over the other.



37

Figure 5.1: Central olefin bis-disulfide as analogue of ajoene.

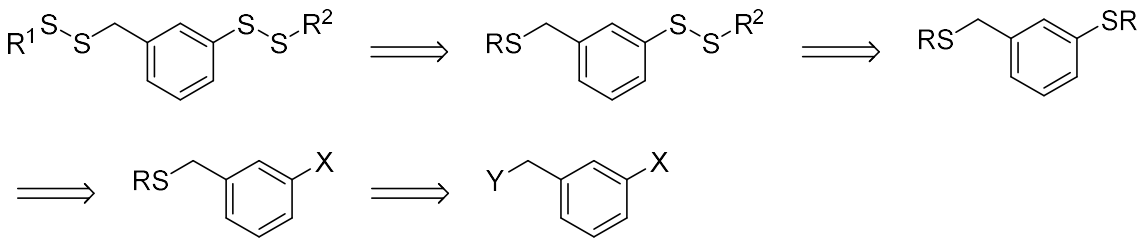
To the best of our knowledge, bis-disulfide ajoene analogues with a central olefin and varying terminal groups are not known in the literature and so stand as an unexplored class of novel compounds.

5.1.2. Bis-Disulfide with Central Phenyl

Synthesising analogues of ajoene with a central phenyl ring in place of the central olefin would allow the geometry of substituents to be fixed, giving selectively to only the *meta*- or *ortho*-substituted product (***m-/o-*129**). Starting materials with a 1,2- or 1,3-substitution pattern as starting materials are inexpensive and commercially available.

5.1.2.1. Retrosynthesis of 129

The retrosynthetic analysis of ***m-/o-*129** is given in Scheme 5.2. The disulfide can be synthesised in both cases using a suitable thiolating agent and a base. As a base is required to form the disulfide bond, R must be a base sensitive protecting group. The sulfur atoms could be introduced by reagents such as thiourea, a sulfur containing acid or a thiol. Where Y is a suitable leaving group, the sulfur atom can be introduced using a S_N2 substitution at the benzylic position. This would require a nucleophilic sulfur source, such as thiolate from its acid. In the case of using thioacetic acid as a sulfur source, it would be possible to conveniently install R as the acetate group, which can later be cleaved to unmask the thiol in the disulfide formation. A more complex reaction would be required to install the sulfur atom at the directly attached to the phenyl ring due to its reduced lability. This position is not suitable for S_N2 and would require other means such an S_NAr, electrophilic aromatic substitution, or a coupling reaction where X is a suitable leaving group. In this case, S_NAr was not a suitable pathway due to the lack of a strong electron withdrawing group that could activate the correct position for attack of the sulfur nucleophile. Although, aromatic sulfonation is well known, it is often reversible and requires harsh conditions.[87] In addition, issues with selectivity or functional group tolerance are possible. Due to this, the direct coupling of the aryl halide and sulfur is the preferred method of choice.

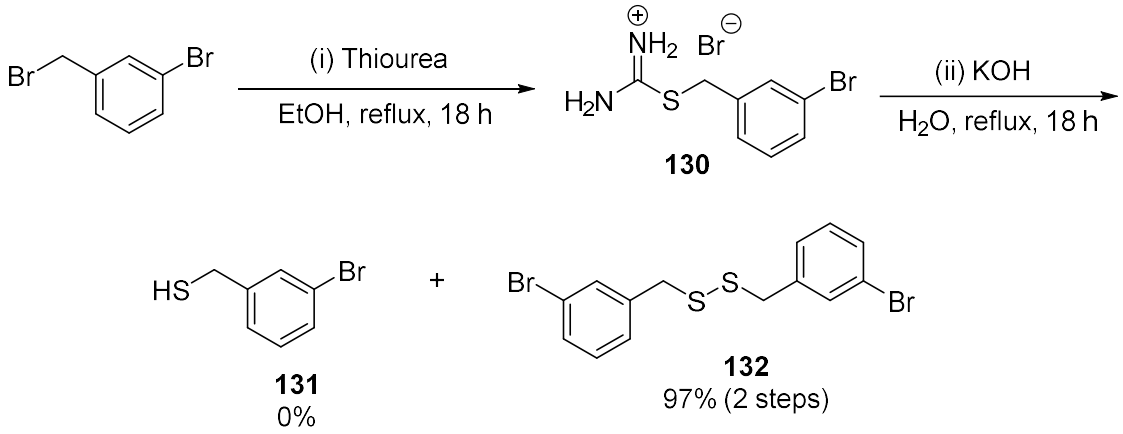


Scheme 5.2: Retrosynthetic analysis of bis-disulfide central aromatic compounds, where R^1, R^2: alkyl, allyl, aryl; R: Protecting group; X, Y: leaving group.

5.2. Synthesis of *meta*-Substituted Analogues

5.2.1. Installing Benzylic Sulfur Atom

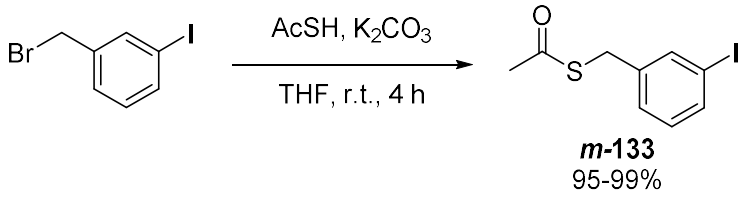
In order to install the sulfur atom at the benzylic position, several options were investigated. Firstly, thiourea was investigated as the sulfur source. Thiourea is commonly known to displace the bromide atom to form the thiourea salt.[16] Thus, the synethic route in Scheme 5.3 was followed. Starting from the commercially available 3-bromobenzyl bromide, thiourea was refluxed in ethanol to yield thiourea salt **130**. The labile benzylic bromide can be substituted by the thiourea to form **130**. This is advantageous are that the salt is easily isolated by filtration. When using potassium hydroxide as base (3 equivalents), the thiourea salt can be cleaved giving products **131** and/or **132,** with urea as by-product. However, thiol **131** was not observed and instead, its dimer disulfide **132** was isolated in 97% yield over two steps. Although the disulfide **132** could clearly be cleaved to **131** through the action of a hydride, it was concluded that three steps to achieve **131** was excessive and thus, the synthetic route starting from 3-bromobenzyl bromide was not investigated further.



Scheme 5.3: Route for introduction of sulfur at benzylic position by installing thiourea salt as key step. Conditions and reagents: (i): 3-bromobenzyl bromide (2.2 mmol), thiourea (3 equiv.), EtOH (0.37 M), reflux, 18 h; (ii): KOH (2.5 equiv.), H_2O (0.37 M), reflux, 18 h; isolated yield **132** in 97% over two steps.

In an effort to arrive at an efficient method to install the benzylic sulfur atom, a simple S_N2 reaction was employed. Potassium carbonate as base and thioacetic acid were used to form the potassium thioacetate anion *in situ,* to substitute the benzylic position of 3-iodobenzylbromide and form the desired thioacetate product (***m*-133**). Under argon atmosphere, all reagents were stirred at room temperature for four hours.

Toluene and THF were investigated as solvent, where tetrahydrofuran gave superior yields (95-99% versus 48%). Using THF as solvent, a crude product resulted that did not require further purification. This was a significant advantage of using a solid base and a volatile solvent system. Excess thioacetic acid could also be removed *in vacuo* (Scheme 5.4).



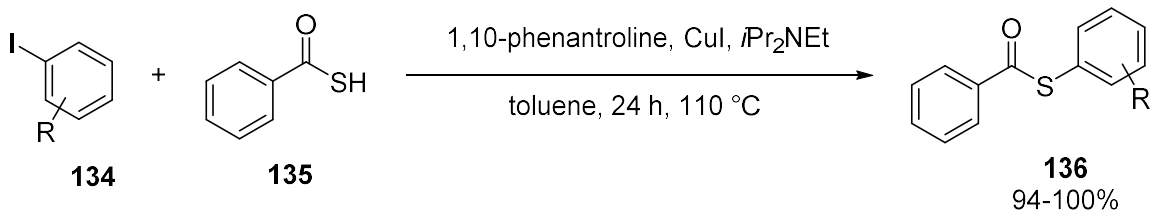
***m*-133**
95-99%

Scheme 5.4: Nucleophilic substitution of bromide in the benzyl position with thioacetate under basic conditions. Conditions and reagents: thioacetic acid (1 equiv.), potassium carbonate (1 equiv.), THF (0.17 M), r.t. 4 h.

The developed method using thioacetic acid and potassium carbonate was the preferred method due to the reaction occurring quickly and being operationally simple. Having a protected thiol is also a key advantage. The thioacetate group is stable under a wide range of conditions and so will not react or be destroyed during the subsequent reaction steps, where the phenylic sulfur atom is installed. The substitution of the phenylic iodine would require harsher conditions than that of the substitution of the benzyl bromide.

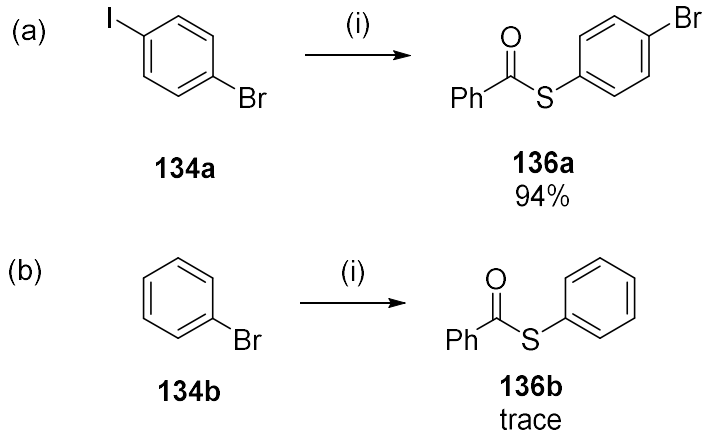
5.2.2. Coupling of Aryl-X and R-SH

Sawada *et al.* reported in 2006 the synthesis of *S*-aryl thiolates by coupling of aryl iodides (**134**) and thiobenzoic acid (**135**) to yield thioesters (**136**) in excellent yields (Scheme 5.5).[88] The conditions developed by Sawada showed a tolerance for a wide range of function groups, including electron withdrawing groups, such as nitro as well as electron donating groups, such as methoxy, providing the products in excellent yields.



Scheme 5.5: Sawada *et al.* reported the synthesis of *S*-aryl thiolates using copper catalysed coupling. Reagents and conditions: ArI (2.45 mmol), CuI (10 mol%), 1,10-phenanthroline (20 mol%), thiobenzoic acid (1.2 equiv.), *i*Pr$_2$NEt (2.0 equiv.), toluene (5 mL). R = *ortho*-methyl, *meta*-nitro, *para*-COCH$_3$/methoxy/bromo/methyl in yields 94–100% determined by HPLC.[88]

Under the conditions as stated in Scheme 5.5, 4-bromo iodobenzene (**134a**) and bromobezene (**134b**) were investigated. In the case of **134a**, **136a** could be obtained in 94% yield. As a control reaction and investigation into the feasibility of the aryl-bromide as a coupling partner, **134b** was trialled. However, this yielded only traces of product (**136b**). Both reactions indicate a high level of selectivity for the halogen atom, favouring iodine as a coupling partner (Scheme 5.6). This literature precedent also encouraged the move away from **131** and **132** as a viable route, due to the inert nature of the aryl bromide, which may later be difficult to remove. In the case of ***m*-133**, the literature examples show promise that the aryl iodide can be replaced with a sulfur in excellent yields.

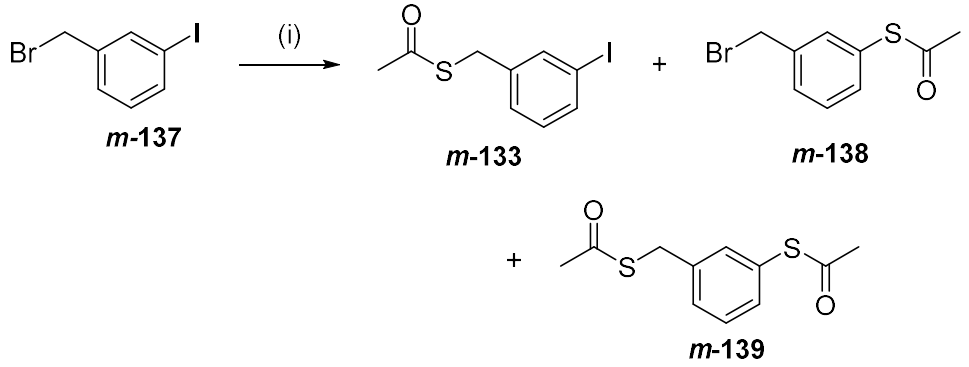


Scheme 5.6: Investigating into aryl halide coupling partners. (i): conditions as given in Scheme 5.5.

In 2013, Soria-Castro *et al*. reported a convenient method for copper catalysis in the absence of base.[89] In addition, Soria-Castro further developed the work of Sawada by exploring the nature of the thiolate, expanding to potassium thioacetate, which is

commercially available. This also negated the need to form the thiolate salt *in situ*, therefore, rending the process as base-free.

Based on the success reported by Sawada *et al.* the reaction was trialled with 3-iodobenzyl bromide (***m*-137**) in order to access whether the reaction would form either the bromide substituted product (***m*-133**), the iodide substituted product (***m*-138**), or the dithioacetate product (***m*-139**). Affording ***m*-139**, would allow the synthetic route to be shortened by installing both sulfur atoms in one step and would therefore be advantageous (Scheme 5.7).



Scheme 5.7: Reaction of substitution halides in 1,3-iodo benzyl bromide (***m*-137**) could afford either monothioacetate product (***m*-133** or ***m*-138**) or dithioacetate product (***m*-139**). Conditions: (i) conditions as given in Scheme 5.5.

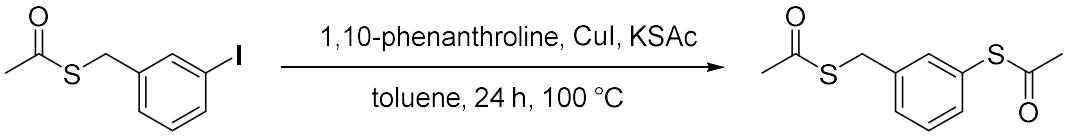
The reaction was firstly investigated without the use of copper as a coupling reagent. This was to determine whether the potassium thioacetate salt in toluene at 100 °C could effectively substitute at the benzylic position. This yielded ***m*-133** in 48% yield (Table 5.1, Entry 1). In comparison to attempts using thioacetic acid and potassium carbonate (5.2.1), this was a significant decrease in yield compared to 95-99% of ***m*-133** which had been previously achieved. This entry also stood as a background reaction, which confirmed that no aryl halide coupling occurs without copper iodide and 1,10-phenanthroline. To probe whether a one pot reaction could be achieved, conditions developed by Soria-Castro were employed with the alternation of a larger excess of potassium thioacetate, which was increased to 10 equivalents. The reaction was somewhat successful whereby thioacetate ***m*-139** was obtained in 58% yield, and thioacetate ***m*-133** in 20% yield (Table 5.1, Entry 2). In all cases product ***m*-138**, where

the bromide has not been substituted, was not observed. This was expected due to the higher lability of the benzylic bromide over the aryl iodide.

Entry	Conditions	*m*-133	*m*-138	*m*-139
1	KSAc, Toluene, 100 °C, 24 h	48%	-	0%
2	1,10-phenanthroline (0.2 equiv), CuI (0.2 equiv.), KSAc (10 equiv.), Toluene, 100 °C, 24 h	20%	-	58%

Table 5.1: Synthesis of compound **4**-**6** from 3-iodobenzyl bromide ***m*-137**.

Since a higher yield could be achieved by installing each sulfur atom stepwise, it was concluded that this would be the most efficient way of preparing ***m*-139**. In the case of ***m*-133**, base free conditions developed by Soria-Castro *et al*. are particularly advantageous as the benzylic thioacetate that was installed in the previous step, is unlikely to be tolerated under basic conditions. The reaction proceeded smoothly and cleanly affording ***m*-139** from ***m*-133** in >97% isolated yield (Scheme 5.8).



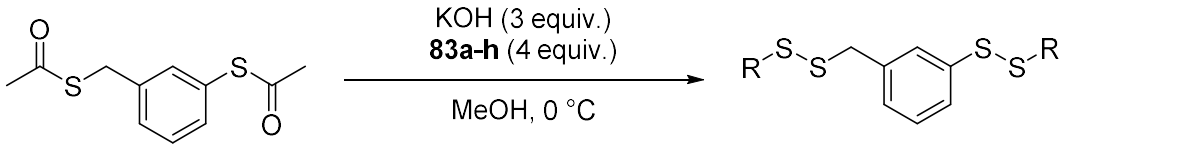
***m*-133** ***m*-139** >97%

Scheme 5.8: Copper coupling of aryl iodide with thioacetate yielding desired product in >97% yield. Conditions: 1,10-phenanthroline (0.2 equiv.), CuI (0.2 equiv.), KSAc (1.5 equiv.), 100 °C, 24 h.

5.3. Symmetric Bis-disulfide Formation

To obtain the target molecule (***m*-/*o*-129***)*, the final step is the cleavage of both protecting groups to unmask the thiolate *in situ*. Using a base such as potassium hydroxide can generate the thiolate, which can then react with an electrophilic sulfur

source to form two disulfide bonds. Since both thiolates will be functionalised with the same terminal group, there is no selectivity point to consider.



***m*-139**

***m*-129a** (R = allyl): 69%
***m*-129b** (R = methyl cyclopropyl): 61%
***m*-129c** (R = *iso*-butyl): 81%
***m*-129d** (R = benzyl): 21%
***m*-129e** (R = 4-CF_3-$C_6H_4CH_2$): 30%
***m*-129f** (R = 4-CO_2CH_3-$C_6H_4CH_2$): 1%
***m*-129g** (R = 4-CH_3-$C_6H_4CH_2$): 70%
***m*-129h** (R = 4-OCH_3-$C_6H_4CH_2$): 15%

Scheme 5.9: Synthesis of ***m*-129a-h**. Reagents and conditions: KOH (3.0 equiv.), R-thiotosylate (**83a-h**) (4.0 equiv.), MeOH, 0 °C, 1 h. Isolated yields are given (1-81%).

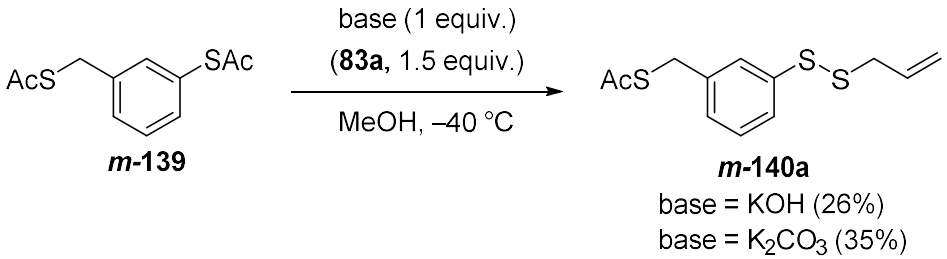
Using potassium hydroxide as base and **83a-h** as thiolating agent, eight different bis-disulfides (***m*-129a-h**) could be obtained (Scheme 5.9). Different terminal groups included were allyl, methyl cyclopropyl, *iso*-butyl, benzyl and four *para*-substituted benzyl groups. The reaction proceeded cleanly giving good yields in the cases of alkyl and allyl substituents (61-81%). However, as the terminal groups became more electron rich, the yields were lower. The yield suffered further when aryl terminal ends were introduced. Where benzyl thiotosylate was used, 33% of a mono-disulfide was obtained, with the benzylic position was unreacted. Despite the excess in **83d** and base, the reaction could not exclusively reach the ***m*-129d** and yielded only 21% of desired product. The yield suffered further with trifluoro-, methoxy- and methyl ester groups in the *para*−position. Disappointing yields of ***m*-129e**, ***m*-129f** and ***m*-129h** were the result of low conversion, abundance of degradation products and a complex purification routine. Compounds ***m*-129e**, ***m*-129f** and ***m*-129h** required several flash chromatography columns, in normal phase followed by reverse phase, and preparative thin layer chromatography. Aromatic thiotosylates were shown to give a less clean reaction, with the desired product being formed in a complex mixture of materials. Despite this, ***m*-129** was obtained in all cases to demonstrate the feasibility of using electron donating and electron withdrawing groups.

5.4. Unsymmetrical Bis-Disulfide Formation

5.4.1. Mono-Disulfide Formation

Since symmetrical bis-disulfides (***m*-129**) could be synthesised with ease through a concise synthesis from available starting materials, it was probed whether unsymmetrical disulfides with two differing terminal groups could be formed.

Allyl thiotosylate (**83a**) was chosen as the first thiolating agent due to the allyl moiety giving distinctive signals in the ^{1}H NMR from the second disulfide group. The formation of a mono disulfide (***m*-140a**) proved to be challenging with yields ranging from 26−35%. Treatment with a base would cleave the most labile acetate, in this case, this should be the thioacetate directly bonded to the aromatic ring. With 1 equivalent of base, starting material was recovered. However, adding more equivalents of potassium carbonate or potassium hydroxide to fully mono deprotect the starting material led to the fully deprotected intermediate, hence favouring the bis-disulfide formation (***m*-129a**). In addition to this, it has been noted that the disulfide formation is most successful where the equivalents of thiolating agent is greater than that of the base (See Chapter 4). This complicates the reaction as the reaction conditions favour the bis-disulfide (***m*-129a**), or the desired product (***m*-140a**), in low yields with starting material remaining (Scheme 5.9).



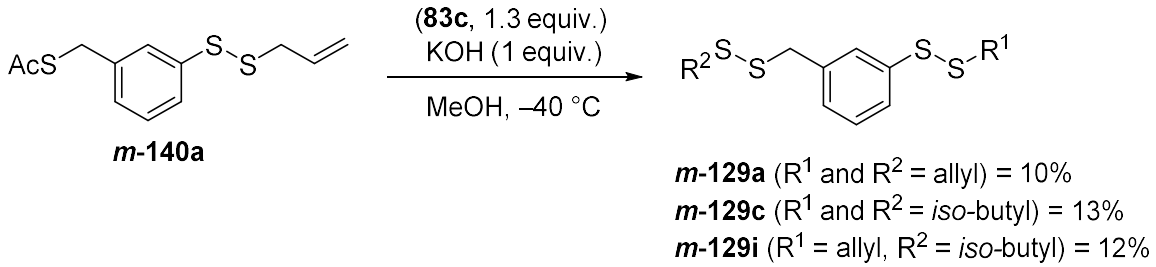
***m*-139** ***m*-140a**
base = KOH (26%)
base = K_2CO_3 (35%)

Scheme 5.10: Mono deprotection of ***m*-139** to yield ***m*-140a** with potassium hydroxide or potassium carbonate as base and **83a**.

5.4.2. Second Disulfide Formation

Unsymmetrical bis-disulfides requires that the first disulfide formed would be stable under the conditions for the second disulfide formation. This was investigated using

m*-140a** as a starting material and *iso*-butyl thiotosylate (**83c**). Although the desired product (m*-129i**) was obtained in 12% after one flash silica gel chromatography purification and three preparative thin layer chromatography purifications, two other products, (***m*-129a** and ***m*-129c**), were also obtained in similar yields, 10% and 13%, respectively. These are the symmetrical bis-disulfides which can only be formed as a result of disulfide exchange or scrambling. Scrambling could be promoted by the presence of base which cleaves the existing disulfide. For a selective unsymmetrical bis-disulfide to be formed, the route would require an amendment to ensure that the initial disulfide is unaffected in the formation of the second (Scheme 5.11).



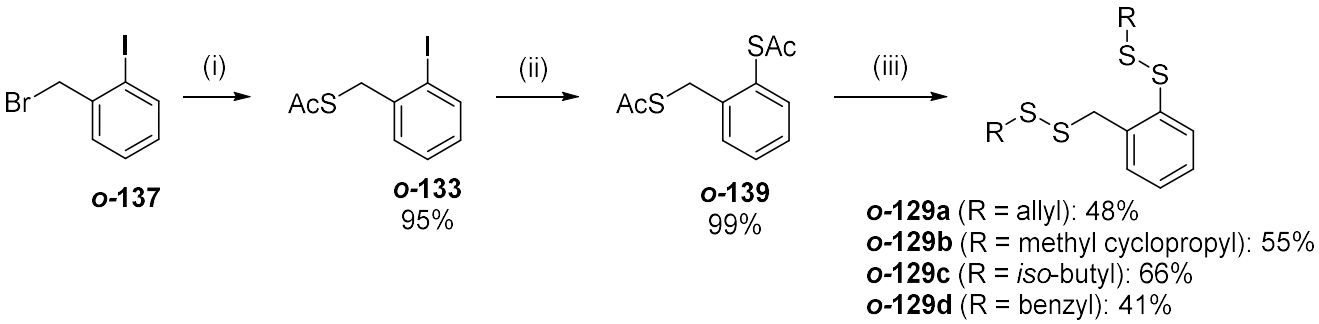
***m*-140a**

***m*-129a** (R^1 and R^2 = allyl) = 10%
***m*-129c** (R^1 and R^2 = *iso*-butyl) = 13%
***m*-129i** (R^1 = allyl, R^2 = *iso*-butyl) = 12%

Scheme 5.11: Synthesis of the unsymmetrical bis-disulfide ***m*-129i**.

5.5. *Ortho*-substituted analogues

The *ortho*-substituted analogues were intended to mimic the *Z*-isomer as the two disulfide bonds would be much closer in space. This, therefore, gives a more sterically hindered compound. For the first two steps, the yields did not differ from its *meta*-analogue and ***o*-139** could be obtained in 94% over two steps. However, due to the steric hinderance, the final yields of ***o*-129a-d** were much lower than its *meta*-analogues and thus, substituted benzyl analogues were not attempted. The route for the *ortho*-analogues were identical to the *meta*-analogues which had been developed previously. Compounds ***o*-129a-d** could be obtained in yields between 41-66%. This is within the expected yield due to the steric effects and the instability of the disulfide bond. It is likely that the yields were higher but during the course of the reaction, the ***o*-129** that has been formed has subsequently been cleaved (Scheme 5.12).



***o*-137**

***o*-133**
95%

***o*-139**
99%

***o*-129a** (R = allyl): 48%
***o*-129b** (R = methyl cyclopropyl): 55%
***o*-129c** (R = *iso*-butyl): 66%
***o*-129d** (R = benzyl): 41%

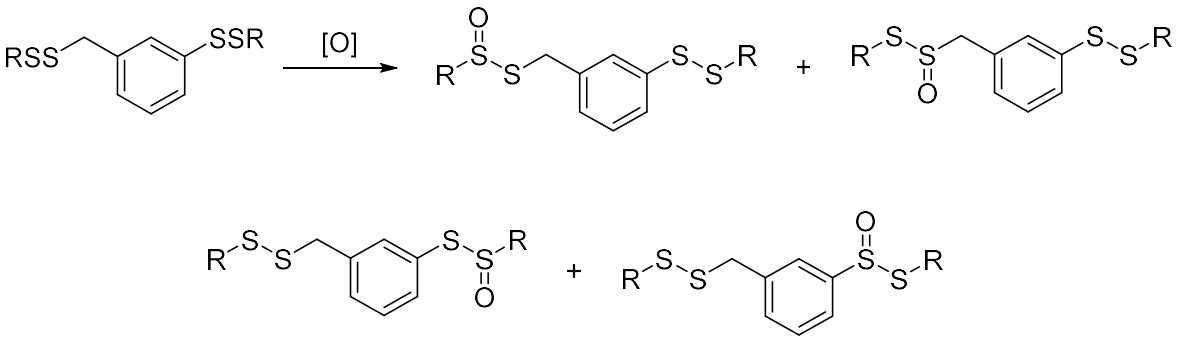
Scheme 5.12: Synthetic route from ***o*-137**. Conditions and reagents: (i) thioacetic acid (1 equiv.), ***o*-137** (1 equiv.) potassium carbonate (1.0 equiv.), THF, r.t., 4 h; (ii) 1,10-phenanthroline (0.2 equiv.), CuI (0.2 equiv.), potassium acetate (1.5 equiv.), toluene, 120 °C, 14 h; (iii) potassium hydroxide (3 equiv.), thiotosylate (4 equiv.), methanol, 0 °C, 2 h.

5.6. Conclusions and Future Work

In conclusion, a novel and short route to unsymmetrical bis-disulfide compounds has been developed. Over three to four steps, a total of nine 1,3-subsituted bis-disulfides have been synthesised, along with four 1,2-subsituted bis-disulfides. The nucleophilic substitution of the labile bromide in the benzyl position provides the product in good yields and does not require purification. The copper catalysed coupling of the iodine in the phenylic position with the thioacetate salt furnished the product in a quantitative yield. The deprotection of both thioacetates can be achieved in a one pot process providing the desired bis-disulfides in low to excellent yields. Generally, a trend was observed by which the more electron rich thiotosylates gave low yields and a complex reaction mixture, whereas less electron rich thiotosylates, such as alkyl, gave excellent yields. By installing a two different sulfur atoms, the route allows a degree of control in its substitution patterns, with the position directly attached to the benzene ring being more acidic and more likely to react first.

Future work:

Future work in this area would likely focus on the optimisation of the mono-deprotection in order to introduce two varying terminal ends with greater success and minimal scrambling of terminal groups. This control may be easier achieved within a flow system. Future work may also include investigation concerning the oxidation of the disulfide. With potentially four positions to oxidise, this may provide a novel class of allicin-like analogues (Scheme 5.13).



Scheme 5.13: Future work could include oxidation of disulfide.

5.7. References

[87] A. R. Moosavi-Zare, M. A. Zolfigol, E. Noroozizadeh, *Synlett* **2016**, *27*, 1682-1684

[88] N. Sawada, T. Itoh, N. Yasuda, *Tetrahedron Lett.* **2006**, *47*, 6595–6597.

[89] S. M. Soria-Castro, A. B. Peñéñory, *Beilstein J. Org. Chem*. **2013**, *9*, 467–475.

6. Introduction and Objectives

Ajoene analogues have been well studied by Block[4] and Hunter[17,18]. However, there is sustained interested in widening the library of compounds that we can synthetically produce. The aim of this project was to synthesise analogues with a varied position of the sulfoxide. Altering the position of the sulfoxide will influence the electronic properties. Ajoene analogues with a central olefin, thus, altering the position of the sulfoxide from the allylic to the vinylic position, were coincidentally investigated in Chapter 2 (Figure 6.1a). The reduced sulfide was shown to favour epoxidation of the central olefin and to undergo subsequent rearrangement. Therefore, the central moiety was altered to a phenyl ring (***m-/o*-142**). Four compounds of a similar type (**141**) have been synthesised by E. Block in 1986, where R is either allyl, benzyl or propyl (Figure 6.1b).[4]

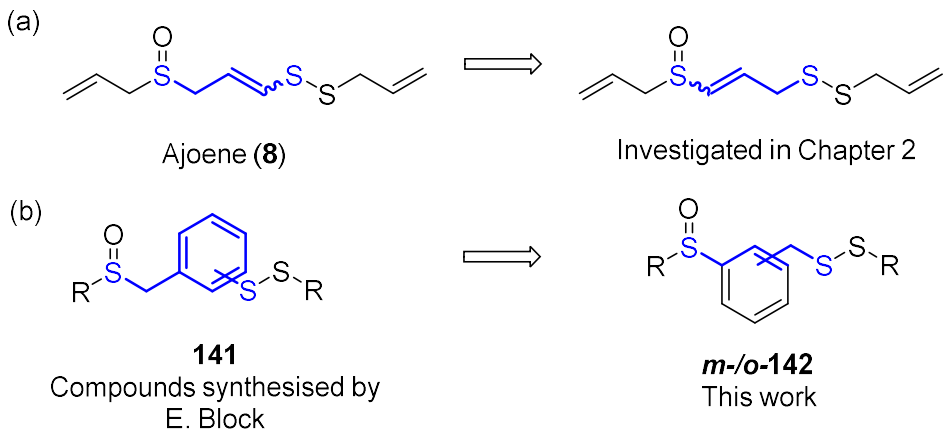
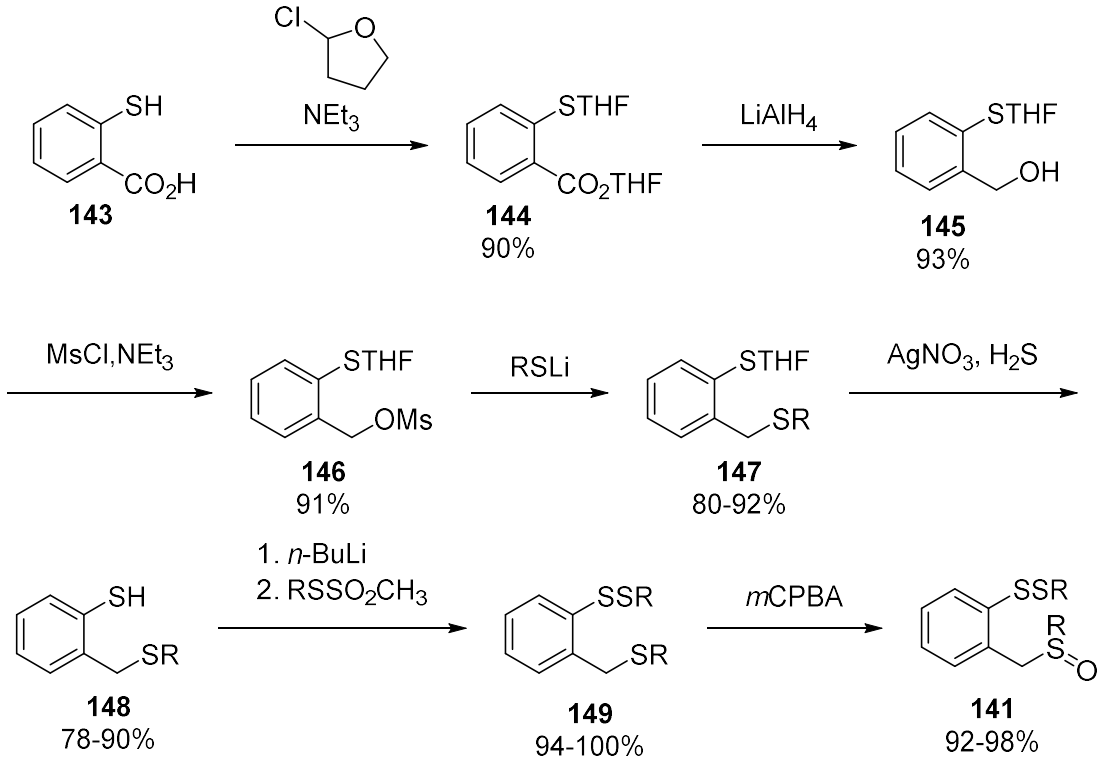


Figure 6.1: Introduction and aims of this work.

E. Block synthesised central aromatic compounds with an *ortho*-substitution pattern (**141**). To the best our knowledge, the *meta*- or *para*-compounds were not synthesised, despite the starting material, 3-mercaptobenzoic acid and 4-mercaptobenzoic acid, being commercially available. The synthetic route developed by E. Block begins with thiosalicylic acid (**143**), which is treated with 2-chlorotetrahydrofuran and triethylamine as base. Since the thiol and carboxylic acid are acidic, both will be deprotonated in the presence of base and will be protected with a tetrahydrofuran ring to yield **144** in 90% yield. Reduction with lithium aluminium hydride reduces the ester selectively to **145**, leaving the monothioacetal

unaffected. Addition of mesyl chloride with triethylamine as base, converts the alcohol into a more labile leaving group, affording **146**. This allows for the nucleophilic substitution with the lithium thiolate to form the desired benzylic sulfide (**147**). Silver nitrate and hydrogen sulfide unmasks the aromatic thiol (**148**), allowing further functionalisation. Treatment with *n*-butyl lithium as base and an electrophilic sulfur source furnishes the disulfide bond in **149** in excellent yields. Oxidation with *m*CPBA selectively oxidises the sulfide to afford **141** in up to 98% yield (Scheme 6.1).

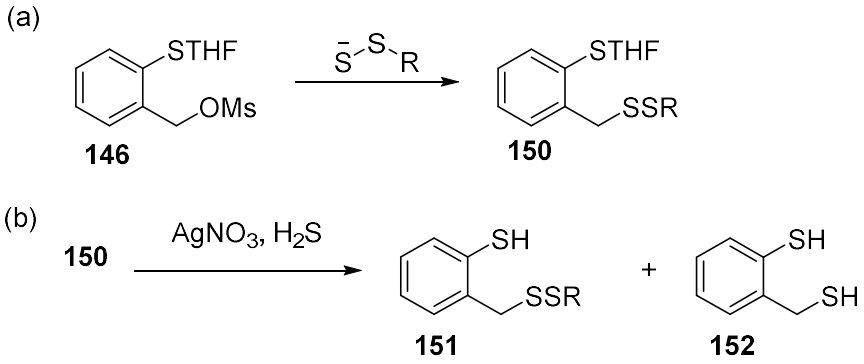


Scheme 6.1: Synthetic route developed by E. Block to achieve *ortho*-ajoene analogues (**141**) with a central aromatic moiety.[4]

6.1. Retrosynthesis

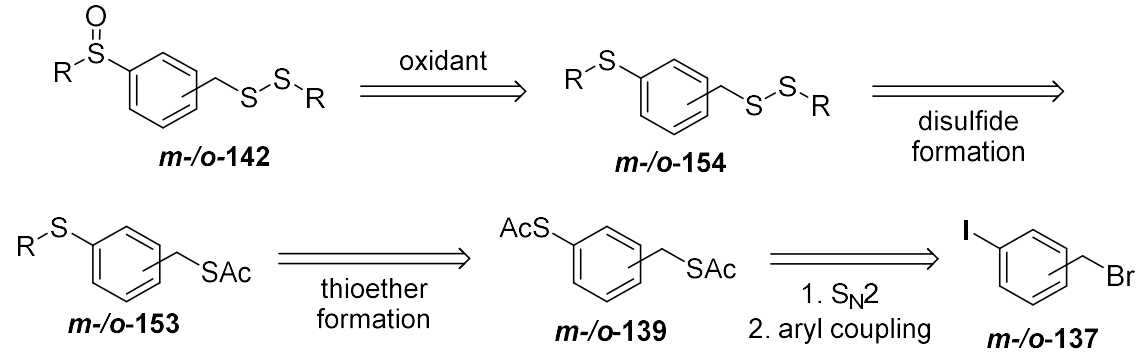
E. Block's synthesis of analogues with a central aromatic core would not be a viable route to achieve the isomeric compounds (***m-/o-*142***). The reasons for this are twofold. Firstly, functionalising the benzylic position by utilising the leaving group ability of the mesylated alcohol in **146** would mean that a nucleophilic disulfide source ("−SSR") would be required to achieve **150**. Such a reagent would likely be unstable, with a reactivity difficult to control (Scheme 6.2a). Secondly, deprotecting

the tetrahydrofuran of **150** with silver nitrate and hydrogen sulfide would most likely also reduce the newly installed disulfide to the thiol **152**. The disulfide is unlikely to survive the conditions of deprotection. In addition, the use of hydrogen sulfide gas also poses safety concerns, thus, an alternative, safer and shorter route was desired (Scheme 6.2b).[4]



Scheme 6.2: Difficulties in applying E. Block's synthetic route to product analogous compounds ***m-/o*-142**.

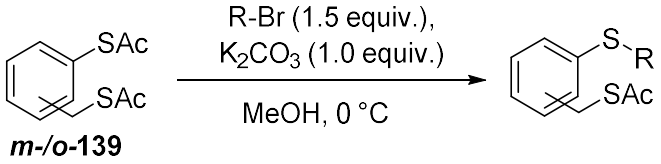
To achieve aromatic analogues ***m-/o*-142***,* a convenient method is to start from the dithioacetate (***m-/o*-139**) that was discussed in Chapter 5. From the ***m-/o*-139**, the most labile thioacetate is deprotected and transformed to ***m-/o*-153**. This thioether is then stable to basic conditions, which allows the less labile thioacetate to be deprotected, unmasking the thiolate. The thiolate *in situ* can form a disulfide bond to furnish the desired precursor (***m-/o*-154**). The sulfide can then be selectively oxidised to the sulfoxide (***m-/o*-142**). The same route will be taken for the *ortho*- and *meta*-analogues (Scheme 6.3).



Scheme 6.3: Retrosynthesis of ***m-/o*-142** *via* ***m-/o*-139** as key intermediates.

6.2. Deprotection and Thioether Formation

Deprotection of the first thioacetate was achieved in methanol using potassium carbonate as base. Potassium carbonate was chosen to deprotect the most labile thioacetate selectively, leaving the benzylic thioacetate intact. The aromatic thioacetate is the most labile thioacetate due to the stabilisation of the thiolate from the phenyl group. This is due to the lone pair of the thiolate being readily overlapped with the electron density from the aromatic ring. The benzylic thioacetate does not have this stabilisation as it not directly attached to the phenyl ring. Terminal ends such as allyl, alkyl and aryl were attempted and generally gave good yields (Scheme 6.4).



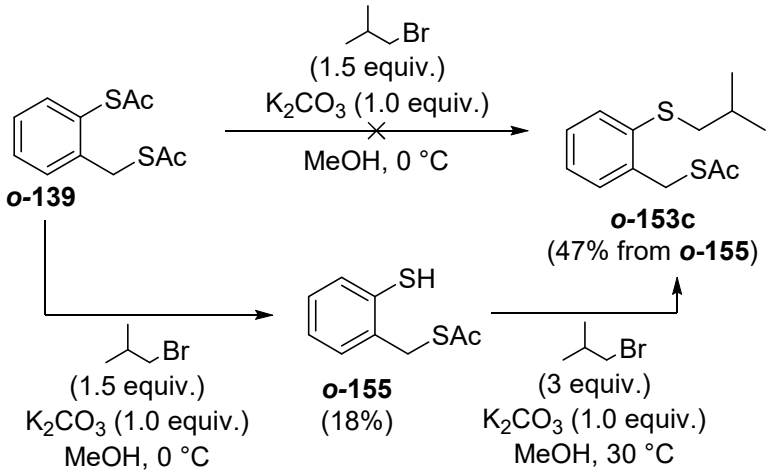
***m*-/*o*-139**

***m*-153a** (R = allyl): 73%
***m*-153b** (R = methyl cyclopropyl): 86%
***m*-153c** (R = *iso*-butyl): 70%
***m*-153d** (R = benzyl): 78%
***o*-153a** (R = allyl): 63%
***o*-153b** (R = methyl cyclopropyl): 60%
***o*-153c** (R = *iso*-butyl): 8%[a]
***o*-153d** (R = benzyl): 58%

Scheme 6.4: Installation of phenylic sulfide. [a]: intermediate thiol (***o*-155**, R = H) isolated. Thiol (1 equiv.), potassium carbonate (1 equiv.) and *iso*-butyl bromide (3 equiv.) in MeOH, 30 °C, 12 h.

In all cases, the ***m*-153a-d** were obtained in higher yields than ***o*-153a-d**. This can be rationalised through the lower steric hinderance around the thiolate in the ***m*-139** which is able to cleanly take part in the required nucleophilic substitution. The most significant difference in yields between the ***m*-153a-d** and ***o*-153a-d** can be seen when R is *iso*-butyl. In the case of ***o*-139**, no reaction to the ***o*-153c** was observed. The reaction product arising from treatment with a base and *iso*-butyl bromide was the thiol (***o*-155**) in 18%. The thiol was purified and treated with another equivalents of potassium carbonate and a high excess of *iso*-butyl bromide. The reaction was heated to 30 °C which encouraged the desired product to form. The thioether ***o*-153c** was isolated in 47%. Further base was not added, and the temperature was

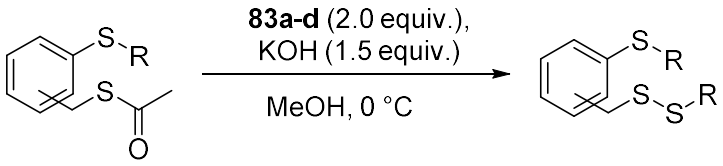
not increased above 30 °C due to concerns of side reactions such as the dimerisation of two thiolates to yield a disulfide. It is worth noting that in the case of ***m*-139**, the reaction proceeded smoothly. However, when using the more sterically hindered starting material and attempting a nucleophilic substitution on a hindered carbon, the reaction was low yielding. Over two steps, the desired thioether could be obtained in only 8% isolated yield (Scheme 6.5).



Scheme 6.5: *Iso*-butyl bromide showed lowered reactivity where the intermediate thiol (***o*-155***)* was recovered.

6.3. Deprotection and Disulfide Formation

Deprotection of the second thioacetate could be achieved using potassium hydroxide as base. The benzylic thioacetate of ***m-/o*-153** is less labile and so a stronger base than potassium carbonate was required. As the thioether is not base sensitive, an excess of base could be used to ensure complete conversion of the starting materials. Thiotosylate reagents (**83**) with the desired R group were used as an electrophilic source. Since potassium hydroxide is also able to cleave **83a-d**, the base was added in methanol and stirred for 10 minutes before the addition of the thiotosylate. This ensured that the base reacted with the acetate in ***m-/o*-153** over the more labile sulfone-sulfur bond of **83**. In this way, ***m*-154** could be obtained in very good yields between 81-85%. The yield of ***o*-154** varied between 51-88%, although generally, the yield of ***o*-154** was lower than that of ***m*-154,** with the exception of when R was *iso*-butyl.



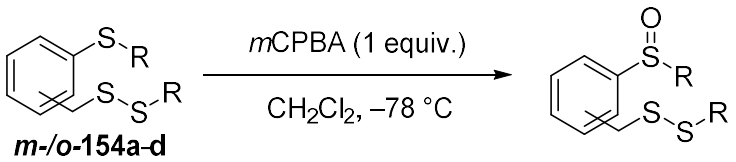
***m*-/*o*-153**

***m*-154a** (R = allyl): 81%
***m*-154b** (R = methyl cyclopropyl): 85%
***m*-154c** (R = *iso*-butyl: 80%
***m*-154d** (R = benzyl): 84%
***o*-154a** (R = allyl): 51%
***o*-154b** (R = methyl cyclopropyl): 70%
***o*-154c** (R = *iso*-butyl): 88%
***o*-154d** (R = benzyl): 58%

Scheme 6.6: Second deprotection of ***m*-/*o*-153a-d** and disulfide formation to yield ***m*-/*o*-154a-d**.

6.4. Oxidation

With eight desired precursors in hand, ***m*-/*o*-154a-d** were treated with one equivalent of *m*CPBA in dichloromethane. Oxidation of all sulfides proceeded cleanly and selectively. No side products as a result of other sites being oxidised were observed.



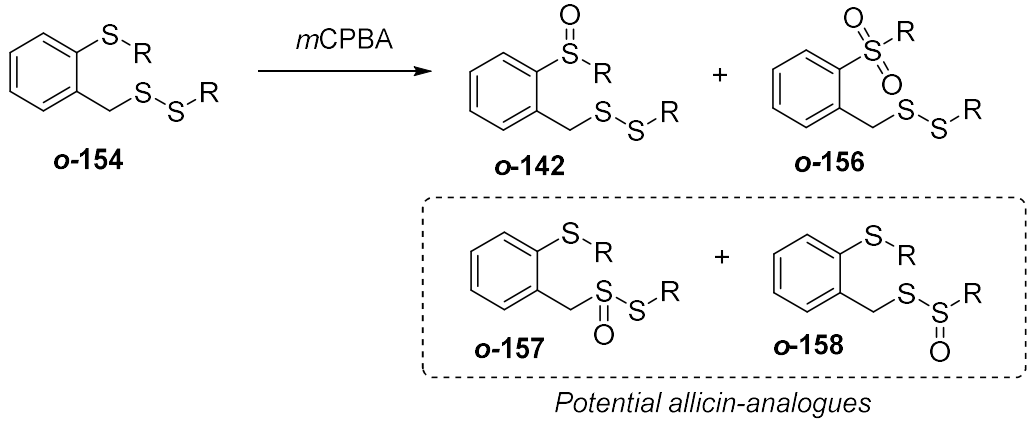
***m*-/*o*-154a-d**

***m*-142a** (R = allyl): 79%
***m*-142b** (R = methyl cyclopropyl): 71%
***m*-142c** (R = *iso*-butyl): 65%
***m*-142d** (R = benzyl): 45%
***o*-142a** (R = allyl): 40%
***o*-142b** (R = methyl cyclopropyl): 77%
***o*-142c** (R = *iso*-butyl) : 67%
***o*-142d** (R = benzyl): 78%

Scheme 6.7: Oxidation of ***m*-/*o*-154a-d** with *m*CPBA furnished desired product, ***m*-/*o*-142a-d**.

Recrystallised *m*CPBA was used and where full conversion was not reached, ***m*-/*o*-154a-d** was recovered. Despite incomplete conversion, the amount of *m*CPBA did not exceed one equivalents. This was due to the potential of side reactions occurring. Potential side reactions include oxidation of the disulfide bond to reach allicin analogues (***o*-157** and ***o*-158**), which could further rearrange, or

over-oxidation to sulfone (***o*-156**) (Scheme 6.8). At 1 equivalent of *m*CPBA, the reaction mixture was clean, containing only unreacted ***m*-/*o*-154** and desired product, ***m*-/*o*-142**, in addition to the reduced *m*CPBA.



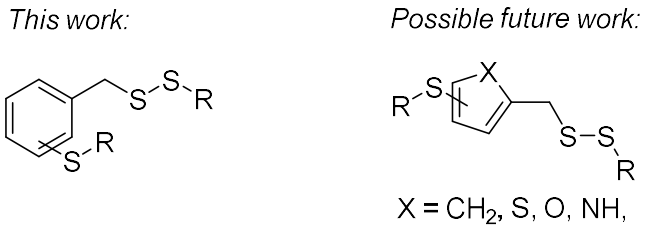
Scheme 6.8: Potential side products from oxidation with *m*CPBA.

6.5. Conclusions and Future Work

Eight examples of ajoene analogues have been synthesised. The novelty in these analogues lay in the position of the sulfoxide and the disulfide which have been reversed. Reversing the positions will affect the steric and electronic characteristics of the analogues. These compounds included four *meta*-substitution patterns and four with *ortho*-substitution patterns to imitate the geometric *E*/*Z*-isomers of ajoene. The synthetic route consists of six steps. The key intermediate is the dithioacetate which is able to be selectively cleaved.

Future work:

Future work could see the improvement of the synthetic route to cleave selectively one of the protecting groups over the other. This could be achieved using varied protecting groups which could be cleaved under different conditions. Over oxidation of the sulfoxide or oxidation of the disulfide in the final compounds could be an extension of this work to novel compounds. In addition, future work could be extended to incorporate other central moieties such a cyclopentadiene, thiophene, furan or pyrrole as examples. Particularly interesting would be a central moiety with a heteroatom as this may affect the way in which the compound interacts with the proteins in the cell through the formation of hydrogen bonds (in the case of pyrrole), or disulfide bridges (in the case of thiophene).



Scheme 6.9: Possible future work may include the alteration of the central group, or of the terminal R groups.

6.6. References

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7. Introduction and Objectives

The compounds that have been synthesised as described in Chapters 3–6 were designed as ajoene (**8**) analogues. Modifications in their structure were hoped to lead to higher biological activity than ajoene itself. The biological assays used to determine the activity were developed and conducted by D. Neef at Neem Biotech Ltd.

7.1. Minimum Biofilm Inhibition Concentration (MBIC)

The Minimum Biofilm Inhibition Concentration (MBIC) is the concentration of a drug that is required to inhibit the biofilm production of the bacteria. Biofilms are an organised group of microbial communities that are attached to each other and a surface.[90] Biofilm-producing bacteria do so as a protective mechanism, making the bacteria harder to eradicate and less accessible by antibiotics. Two examples of biofilm-producing bacteria are *Staphylococcus Aureus* (*S. Aureus*) and *Pseudomonas Aeruginosa* (*P. Aeruginosa*). *S. Aureus* is a gram-positive bacteria which is linked to the formation and persistence of chronic wounds.[91] *P. Aeruginosa* is a gram-negative bacteria which can also be found in chronic wounds, as well as causing chronic lung infections, particularly affecting those patients with cystic fibrosis.[92]

Investigating the MBIC against these bacteria will involve dosing the bacteria with known, varying concentrations of the drug. The concentration at which biofilm production is inhibited in comparison to the standard control with no drug is determined as the MBIC. From this experiment, the half-maximal inhibitory concentration (IC_{50}) can be deduced. This is the concentration of drug where half of the biological process is inhibited. The lower the IC_{50}, the higher the efficacy of the drug.[93] The IC_{50} value is often expressed as a pIC_{50} value, the negative log value of the IC_{50} (M) value. This mathematical manipulation of the IC_{50} allows pIC_{50} values to be obtained, where the higher the value, the higher the efficacy of the drug. This becomes a logarithmic scale which is deemed as easier to interpret and compare.[94]

7.2. Summary of Analogues

The structures of all analogues evaluated in MBIC studies are given Figure 7.1.

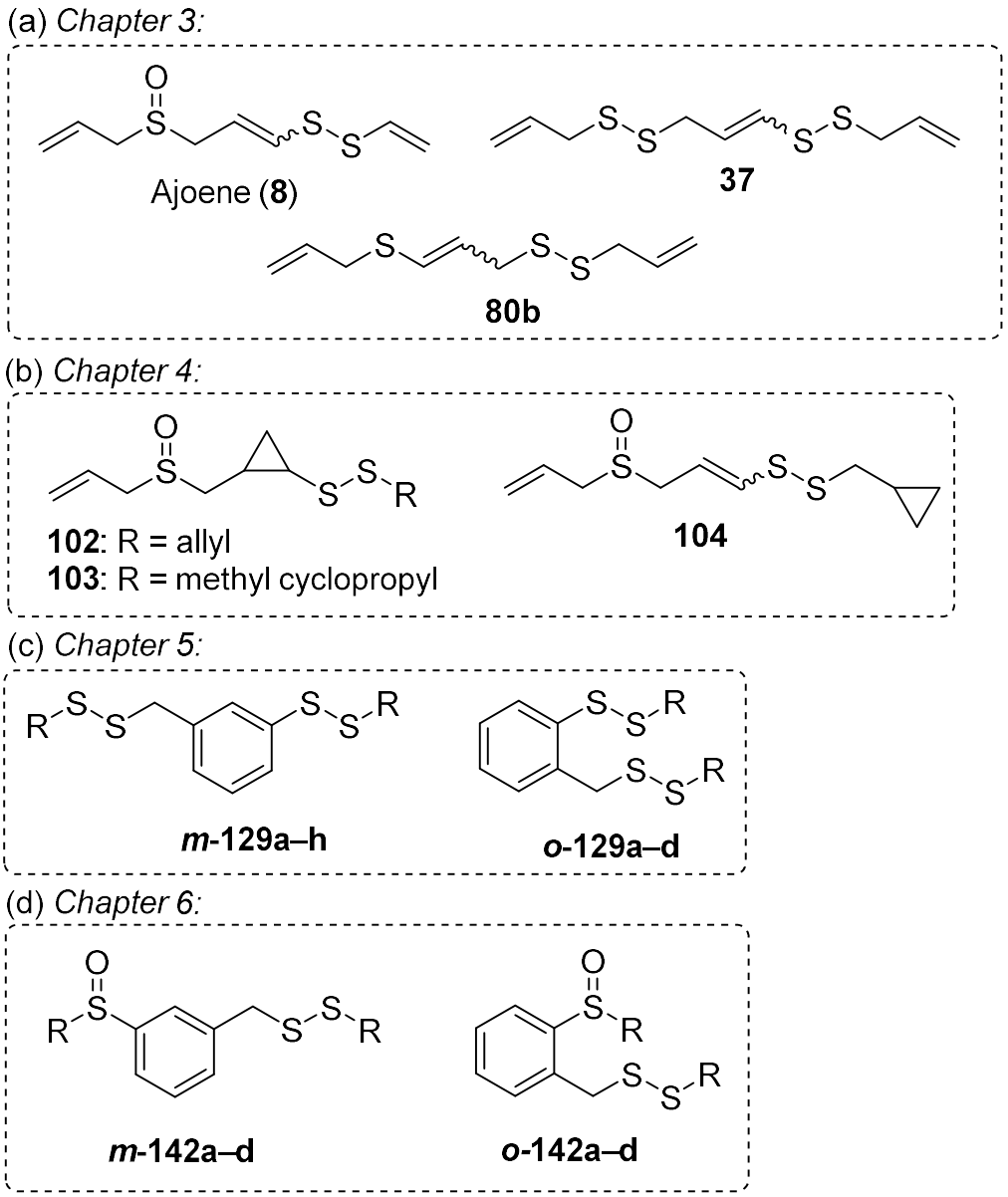

Figure 7.1: Summary of compounds synthesised as described in Chapters 3–6 which have been tested. R: **a** = allyl, **b** = methyl cyclopropyl, **c** = *iso*-butyl, **d** = benzyl, **e** = 4-CF_3-C_6H_4-CH_2, **f** = 4-CO_2CH_3-C_6H_4-CH_2, **g** = 4-CH_3-C_6H_4-CH_2, **h** = 4-OCH_3-C_6H_4-CH_2.

7.3. Biological Results

Minimum Biofilm Inhibition Concentration (MBIC) studies were conducted on all 25 compounds against *S. Aureus* and *P. Aeruginosa* using ajoene (**8**) as a standard benchmark. The 24 analogues were specifically designed bearing different groups in several positions to investigate the influence of the modification on the biological activity. The raw results expressed as concentrations (μM) are summarised in Table 7.1.

Chapter 3 focused on the synthesis of **8**. During this investigation, compounds **37** and **80b** were obtained. Comparing **8**, **37** and **80b** can give the direct comparison of the sulfoxide, disulfide and sulfide moiety (Figure 7.1a, Table 7.1, entries 1–3). Chapter 4 focused on the incorporation of the cyclopropyl moiety in the central and terminal positions. From the developed synthesis, **102**, **103** and **104** were obtained (Figure 7.1b, Table 7.1, entries 4–6). Chapter 5 saw the synthesis of novel bis-disulfide structures with a central olefin. This was investigated for a 1,3- and 1,2-substitution pattern leading to compounds ***m-/o*-129a**–**h** (Figure 7.1c, Table 7.1, entries 7–18). The final analogue series was realised in Chapter 6 where compounds (***m-/o*-142a**–**d**) contained a central olefin with a sulfoxide that is directly bonded to the aromatic ring (Figure 7.1d, Table 7.1, entries 19–26).

Entry	Compound	*S. Aureus* MBIC IC_{50} (μM)	*P. Aeruginosa* MBIC IC_{50} (μM)
1	**8**	0.565	49.2
2	**37**	<2.25	28
3	**80b**	<2.25	67.5
4	**102**	>48	>128
5	**103**	>48	131
6	**104**	0.986	12.3

7	***m*-129a**	0.223	1.64
8	***m*-129b**	44	>144
9	***m*-129c**	42	>144
10	***m*-129d**	36	>144
11	***m*-129e**	17	>128
12	***m*-129f**	1.3	>128
13	***m*-129g**	5.6	>128
14	***m*-129h**	7.2	>128
15	***o*-129a**	0.172	15.8
16	***o*-129b**	0.425	65.5
17	***o*-129c**	0.48	>128
18	***o*-129d**	1.4	63.4
19	***m*-142a**	9.08	69
20	***m*-142b**	7.9	113
21	***m*-142c**	8.28	36.3
22	***m*-142d**	0.92	>128
23	***o*-142a**	12.5	>128
24	***o*-142b**	16.2	>128
25	***o*-142c**	14.5	54.8
26	***o*-142d**	4	8.3

Table 7.1: Summary of MBIC studies against *S. Aureus* and *P. Aeruginosa*.

The IC_{50} values for *S. Aureus* and *P. Aeruginosa* have been converted to their negative log (pIC_{50}) to produce graphs as given in Figure 7.2 and Figure 7.3, respectively. As their pIC_{50} values, it is more clearly demonstrated which analogues surpass the strength of ajoene against the corresponding bacteria. The value for ajoene has been set on the graph as a red dotted line.

In Figure 7.2, four analogues give higher pIC_{50} values against *S. Aureus* than that of ajoene (**8**). These compounds are ***m*-129a**, ***o*-129a**, ***o*-129b** and ***o*-129c**. In Figure 7.3, six analogues also give higher pIC_{50} values against *P. Aeruginosa* than that of ajoene (**8**). In this case, the compounds are **37**, **104**, ***m*-129a**, ***o*-129a**, ***m*-142c** and ***o*-142d**.

Disulfide **37** and sulfide **80b** show different pIC_{50} against *P. Aeruginosa*. Since the only difference between **37** and **80b** is the presence of a secondary disulfide bond in **37**, it can be concluded that the heightened pIC_{50} value against *P. Aeruginosa* is the result of this change. Therefore, it can be concluded that the disulfide plays a key role in the inhibition mechanism. Since the same modification yielded higher IC_{50} values against *S. Aureus*, it also suggests that the inhibition mechanism may not be the same between both bacteria.

The pIC_{50} values for ***m*-142c** and ***o*-142d** clearly indicate some inhibition of biofilm production by *P. Aeruginosa*. However, since both analogues have different terminal groups and the sulfoxide is varying relative positions, further investigative work would be required to fully clarify which moiety is contributing to the method of inhibition. This result gives preliminary evidence that this general structure of compounds may lead to potent drugs should the structure be tuned further. Such modifications may include a wider range of terminal groups and varying the sulfoxide to the *para*-position.

Compounds **102** and **103** show poor performance over both MBIC testing producing disappointing IC_{50} values. Against *S. Aureus,* the IC_{50} values are at least 85 times higher than that of ajoene. When testing against *P. Aeruginosa*, the IC_{50} values are in the region of 2.6-2.7 times higher. However, **104** shows promising results against *P. Aeruginosa* and an IC_{50} value which is higher than ajoene against *S. Aureus* but significantly lower than values obtained by **102** and **103**. This clearly

illustrates that the electronic factors in ajoene, more specifically, in the central olefin ajoene, cannot be substituted with an alkyl moiety. In doing so, the biologically activity is not retained. It is worth nothing that although changing the terminal group of the disulfide in **102** and **103** does not seem to affect the inhibition, this is more than likely an effect which is being masked by the central modification. Changing the terminal group in ajoene to a cyclopropyl (**104**) leads to reduced activity against *S. Aureus* and thus, it can be concluded that there will be an effect present from this change. Since the synthesis of compounds **102** and **103** was laborious, and the biological activity was not increased, this group of central-cyclopropanted compounds can be ruled out as potential drug targets.

From both data sets, we can note that both ***m*-129a** and ***o*-129a** show promising results against both bacteria. The results obtained from ***m*-/*o*-129a** are perhaps the most exciting from this round of biological assays. The lowered IC_{50} values demonstrates the importance of the disulfide group. Since both isomers have different values, it also insinuates that the position of the disulfide group does play a role in its mode of action. Compound ***m*-129a** gives particularly low IC_{50} values against both bacteria. This suggests an element of generality with the bis-disulfide structure where one drug can potentially be used against a range of bacteria. Interestingly, *iso*-butyl, methyl cyclopropyl and benzyl terminal groups do not show promising IC_{50} values. This may be due to the steric effects, electronic effects or the inherent reactivity of the allyl group which is at an optimum for effective inhibition.

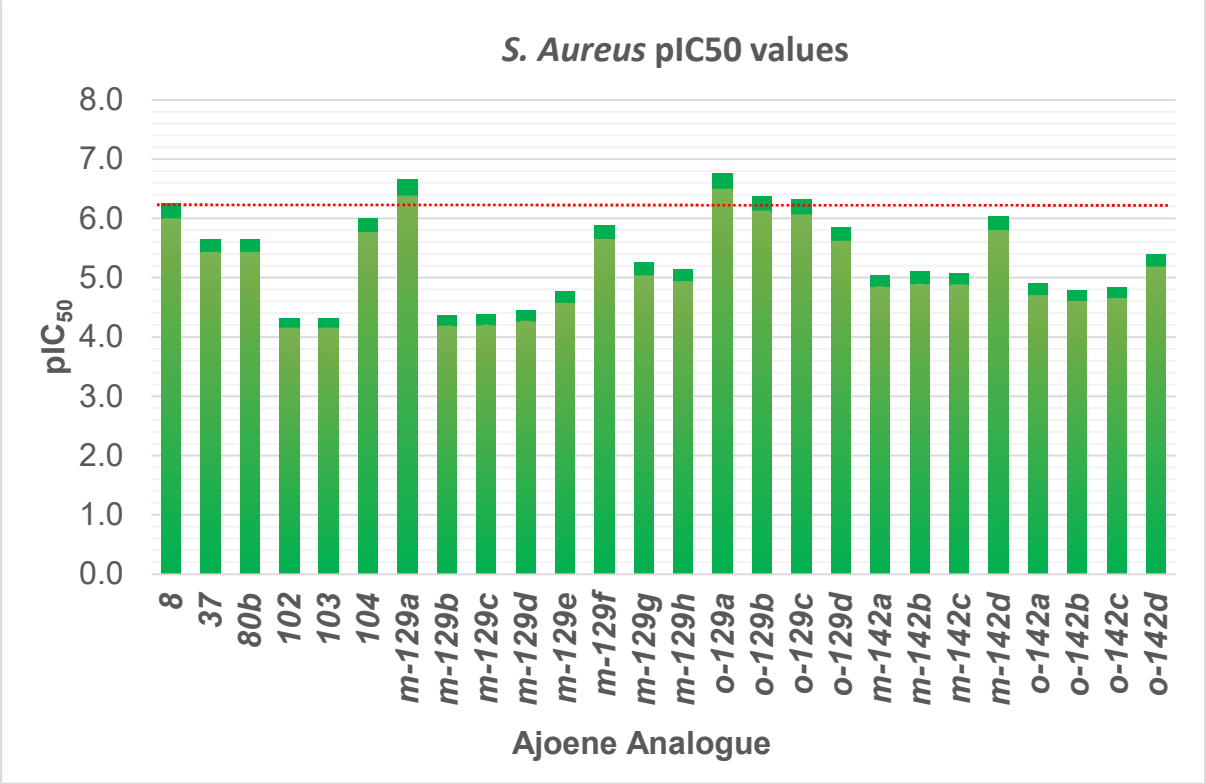

Figure 7.2: Analogues of ajoene and their corresponding pIC_{50} values when tested against *S. Aureus*.

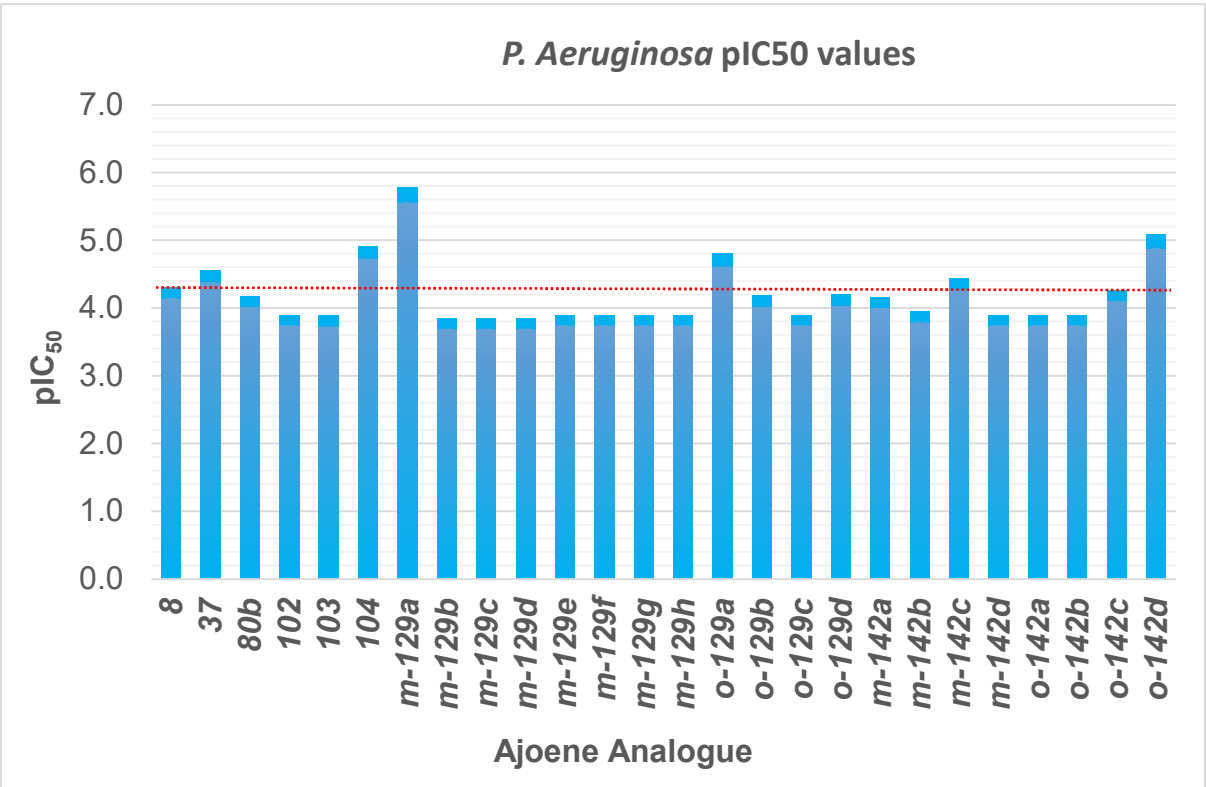

Figure 7.3: Analogues of ajoene and their corresponding pIC_{50} values when tested against *P. Aeruginosa.*

7.4. Conclusion and Future Work

The MBIC results as IC_{50} and pIC_{50} values have been given. The graphs present the pIC_{50} values in comparison to ajoene (**8**) and show good results against both bacteria. The most promising result was found in ***m*-129a** and ***o*-129a** which shows an IC_{50} value that is 2.5 and 3.3 times lower than that of ajoene against *S. Aureus,* respectively. Against *P. Aeruginosa,* the IC_{50} values of ***m*-129a** and ***o*-129a** were up to 30 and 3.1 times lower than that of ajoene, respectively. This is perhaps the greatest result as it highlights that there is a crucial role of the geometry of the molecule with the bacteria. In this case, the *meta* position was shown to be particularly potent. More generally, this demonstrates the important of the disulfide allyl group. This is advantageous as the synthesis to ***m*-/*o*-129a** has been developed as simple route which achieves a compound with no structural ambiguity, such as chiral centers or geometric isomers, to consider.

Future work:

Future work may focus on the most active analogues identified, ***m*-129a** and ***o*-129a**. These analogues could be tested in a wider range of biological assays. This may encompass range of bacteria, fungi or viruses. These analogues could also be included in stability testing and formulation experiments to explore their viability as pharmaceutical candidates.

7.5. References

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8. Experimental

8.1. General Considerations

The reactions were performed using standard laboratory equipment. Air sensitive reactions were carried out under argon atmosphere using oven-dried glassware. Reactions were stirred using magnetic stirring and heated to specified temperatures using hotplates with temperature probe control and an adapted heating block. Lower temperatures were obtained using ice/water (0 ºC), dry ice/acetonitrile (−40 ºC) and dry ice/acetone (−78ºC). Büchi B-461, B-481 or B-490 were used for solvent evaporations (reduced pressure down to 15 mbar) and high vacuum apparatus was used to further dry the products. All chemicals were purchased from Sigma Aldrich, Alfa Aesar, Fisher Scientific, TCI UK, Fluorochem and used without further purification. Dry solvents were obtained from an MBRAUN SPS-800 solvent purification system.

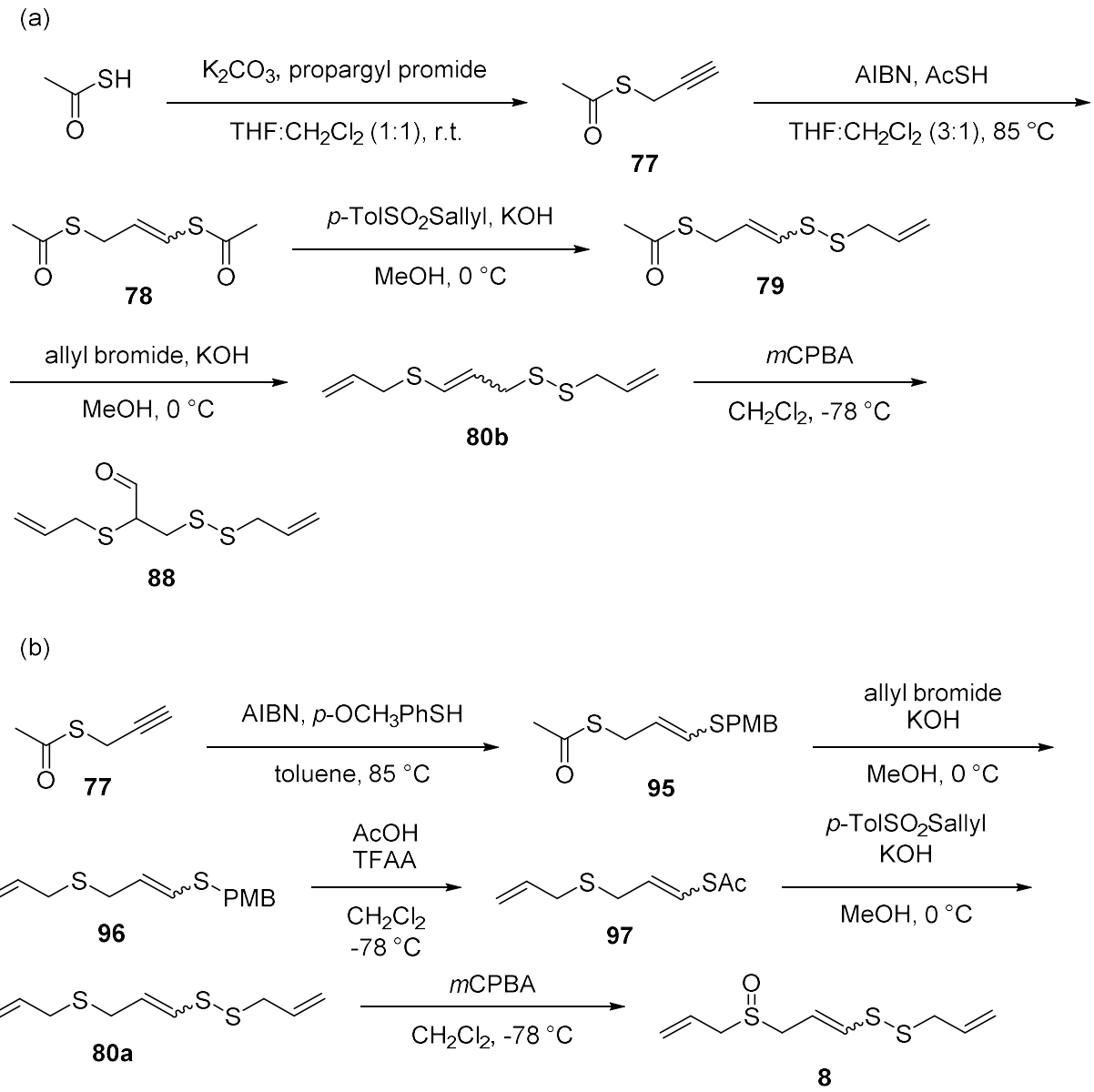
All the reactions were monitored by thin-layer chromatography (TLC), which was performed on Merck Silica gel 60 F254 (0.20 mm) and visualised by UV radiation (254 nm) or/and by staining with potassium permanganate solution (1.5 g $KMnO_4$, 10 g K_2CO_3, 1.25 mL 10% NaOH, 200 mL distilled H_2O). Automated column chromatography was performed on a Biotage® Isolera Four using Biotage® cartridges SNAP Ultra 10g, SNAP Ultra 25g, SNAP Ultra 50g, SNAP Ultra 100g. The solvents used for the purification are indicated in the text and were purchased from Fisher Scientific as laboratory grade.

^{1}H NMR and ^{13}C NMR spectra were measured on Bruker DPX 500 (500 MHz), Bruker DPX 400 (400 MHz), Bruker DPX 300 (300 MHz) instruments. The chemical shifts δ are given in ppm downfield of tetramethylsilane (δ = 0 ppm). Compounds or crude reaction mixtures were dissolved in deuterated chloroform. Coupling constants (*J*) are given in Hertz. The multiplicity of signals is designated: s = singlet, d = doublet, t = triplet, q = quartet, quin= quintet, dt = double of triplet, m = multiplet. Residual solvent peaks are 7.26 ppm for chloroform. The molecular ions peaks values quoted for either molecular ion $[M]^+$, molecular ion plus hydrogen $[M+H]^+$, molecular ion plus sodium $[M+Na]^+$, molecular ion plus ammonium $[M+NH_4]^+$ or

molecular ion plus $[M+K]^+$. IR spectra were recorded on Shimadzu IR Affinity-1S apparatus. Wavenumbers are quoted in cm^{-1}.

8.2. Chapter 3: Synthesis of Ajoene

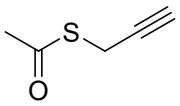
8.2.1. General Scheme



Scheme 8.1: General schemes of experimental work in Chapter 7. (a): Initial synthesis yielding rearrangement product **88**. (b) Revised synthesis yielding desired product **8**.

8.2.2. Experimental

_S_-(Prop-2-yn-1-yl) ethanethioate (77)



Batch Procedure:

To a solution of thioacetic acid (0.35 mL, 5 mmol) in THF (15 mL), propargyl bromide (0.43 mL, 5 mmol) and potassium carbonate (0.69 g, 5 mmol) were added. The mixture was stirred at room temperature for 4 hours. The mixture was filtered, and the solid washed with dichloromethane (2 x 20 mL). The mixture was concentrated *in vacuo* at room temperature to yield title product as a yellow oil (0.57 g, 95%).

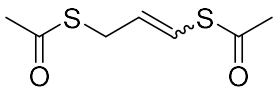
Flow Procedure:

Potassium carbonate (8.4 g, 0.06 mol) and sand (11.6 g) were shaken until a homogenous mixture was achieved by eye. The K_2CO_3/sand (20 g) was packed into a glass OmniSep® (150 x 15 mm). The packed column was weighed, and dry tetrahydrofuran (THF) was flowed through at 0.4 mL/min for 1 hour, after which, the column was reweighed. The mass difference equated to the calculated column volume (CV, 7.1 mL, residence time: 18 minutes).

To an oven dried glass, thioacetic acid (0.4 M) and propargyl bromide (0.4 M) were added to dry THF under argon. The reaction mixture was passed through the column at 0.4 mL/min and the first column volume of reactant was discarded. The following two column volumes were collected and concentrated *in vacuo* at room temperature to yield title product as a yellow oil (453 mg, 95%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (d, *J* = 2.7 Hz, 2H, C*H*$_2$C≡CH), 2.37 (s, 3H, CH_3), 2.18 (t, *J* = 2.7 Hz, 1H, CH_2C≡C*H*) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.0 (C=O), 78.9 (CH_2*C*≡CH), 71.0 (CH_2C≡*C*H), 30.3 (CH_3), 17.6 (*C*H_2C≡CH) ppm. Spectra in accordance with literature data.[95]

_S_,_S_'-(Prop-1-ene-1,3-diyl) diethanethioate (78)



Batch Procedure:

S-(Prop-2-yn-1-yl) ethanethioate (**77**) (398.1 mg, 3.5 mmol) was dissolved in dry toluene (5 mL) and the solution was heated to 85 °C under argon atmosphere. Azobisisobutyronitrile (AIBN) (57 mg, 0.35 mmol, 0.1 equiv.) was added to the solution directly, followed by the dropwise addition of thioacetic acid (0.28 mL) in toluene (5 mL) over 40 minutes using a syringe pump. The mixture was left to stir at 85 °C for a further 1 hour. The reaction was then quenched with aqueous saturated solution of sodium carbonate (5 mL) and the toluene was removed *in vacuo*. The remaining residue was dissolved diethyl ether (20 mL) and the organic layer was washed with brine (2 x 10 mL) and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 15 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **78** as a colourless oil (0.4 g, 60%, *E*:*Z* 1.2:1.0).

Single Step Flow Procedure:

S-(prop-2-yn-1-yl) ethanethioate (**77**) (753 mg, 6.6 mmol), thioacetic acid (0.522 mL, 7.3 mmol, 1.1 equiv.), AIBN (0.124 g, 0.753 mmol, 10 mol%) was dissolved in dry CH_2Cl_2 (5 mL) and dry THF (15 mL). The solution was pumped using HPLC K120 Knauer Analytical pump through PTFE tubing (i.d. 0.8 mm, volume: 3.6 mL, residence time: 5.1 minutes) at 0.7 mL/min. The system was fitted with a back pressure regulator at 40 psi. The first column volume of reactant was discarded, and the following three column volumes were collected and concentrated *in vacuo* at 40 °C. The resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 15 CV, then increased to 100%

diethyl ether over 3 CV) to afford compound **78** as a colourless oil (0.35 g, 51%, *E*:*Z* 1.1:1.0).

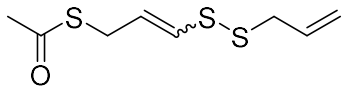
Combined Flow Procedure for steps (i) and (ii):
Potassium carbonate (8.4 g, 0.06 mol) and sand (11.6 g) were shaken until a homogenous mixture was achieved by eye. The K_2CO_3/sand (20 g) was packed into a glass OmniSep® (150 x 15 mm). The packed column was weighed, and dry tetrahydrofuran (THF) was flowed through at 0.4 mL/min for 1 hour, after which, the column was reweighed. The mass difference equated to the calculated column volume (CV, 8 mL, residence time: 20 minutes).

To an oven dried glass, thioacetic acid (0.4 M) and propargyl bromide (0.4 M) were added to dry THF under argon. The reaction mixture was passed through the base/sand column at 0.4 mL/min using a HPLC pump. The outlet of the base column was connected to one inlet a T-piece mixer. A solution of AIBN (0.04 M), thioacetic acid (0.48 M) in dry THF:CH_2Cl_2 (1:1) under argon was also connected to the T-piece mixer. To the outlet was a PTFE coil (i.d. 0.8 mm) of varying column volumes (CV: 4.74–32 mL), with a back pressure regular (75 psi). The PTFE coil was submerged in a water bath at 85–90°C. The first column volume of reactant was discarded, and the following column volumes were collected and concentrated *in vacuo* at 40 °C. The resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 15 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **78** as a colourless oil (44-61% over 2 steps).

As a mixture of *E:Z* isomers: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.70 (d, *J* = 9.5 Hz, 1H, CH_2CH=C*H*), δ 6.69 (d, *J* = 9.5 Hz, 1H, CH_2CH=C*H*), 5.78-5.86 (m, 1H, CH_2C*H*=CH), 5.83 (dt, *J* = 9.6, 7.7 Hz, 1H, CH_2C*H*=CH), 3.63 (d, *J* = 7.7 Hz, 2H, C*H*$_2$), 3.55 (d, *J* = 7.7 Hz, 2H, C*H*$_2$), 2.40 (s, 3H, C*H*$_3$), 2.34 (s, 3H, C*H*$_3$), 2.34 (s, 3H, C*H*$_3$), 2.32 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.1 (C=O), 194.9 (C=O), 192.7 (C=O), 191.1 (C=O), 128.3 (CH_2CH=*C*H), 126.7 (CH_2CH=*C*H), 120.9

(CH_2CH=CH), 120.7 (CH_2CH=CH), 31.5 (CH_2CH=CH), 31.1 (CH_2CH=CH), 30.6 (CH_3), 30.5 (CH_3), 30.5 (CH_3), 28.7 (CH_3) ppm. HRMS (ASAP) $[M+H]^+$ calc. 191.0201, found 191.0203 $[C_7H_{11}O_2S_2]^+$. IR (neat): 1632, 1352, 1124, 1103, 948, 619 cm^{-1}.

_S_-(3-(Allyldisulfaneyl)allyl) ethanethioate (79)



Batch Procedure:

The vinyl thioacetate, *S*,*S*'-(prop-1-ene-1,3-diyl) diethanethioate (**78**) (100 mg, 0.53 mmol) was dissolved in methanol (5.3 mL) and cooled to -40 °C. Potassium hydroxide (36 mg, 0.56 mmol, 1.05 equiv.) in methanol (5.3 mL) was added to the solution, followed by the direct addition of allyl thiotosylate (121 mg, 0.53 mmol, 1.0 equiv.). The solution was left at −40 °C for 1 hour and warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of ammonium chloride (5 mL), and solvent was removed *in vacuo*. The resulting residue was dissolved in diethyl ether (20 mL), and washed with water (20 mL), brine (2 x 20 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 10 CV, held at 20% diethyl ether for 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **79** as a pale yellow oil (60 mg, 51%). The bis-disulfide (**84**) was also isolated as a pale-yellow oil (13 mg, 10%).

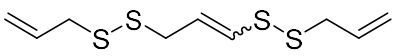
Flow Procedure:

The vinyl thioacetate, *S*,*S*'-(prop-1-ene-1,3-diyl) diethanethioate (**78**) (100 mg, 0.53 mmol) and allyl thiotosylate (**83a**, 363 mg, 1.59 mmol, 3.0 equiv.) was dissolved in methanol (5.3 mL, 0.1 M) and transferred to a syringe. Potassium hydroxide (69 mg, 1.06 mmol, 2.0 equiv.) was dissolved in methanol (4.5 M) and transferred to a syringe. Both syringes pumped at 0.1 mL/min using syringe pumps through a T-

piece mixer and to PTFE tubing (i.d. 0.8 mm, volume: 1 mL, residence time: 5 min). The PTFE coil was submerged in an ice bath at 0 °C. The first column volume of reactant was discarded, and the following seven column volumes were collected and concentrated *in vacuo* at 40 °C. The resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 10 CV, held at 20% diethyl ether for 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **79** as a pale yellow oil (43 mg, 56%). The bis-disulfide (**84**) was also isolated as a pale yellow oil (20 mg, 10%).

As a mixture of *E*/*Z*-isomers: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.23 (dt, *J* = 14.7, 1.1 Hz, 1H, CH_2CH=C*H*), δ 6.21 (dt, *J* = 9.2, 1.0 Hz, 1H, CH_2CH=C*H*), 5.77-5.88 (m, 1H, CH_2CH=CH and CH_2CH=CH_2), 5.77-5.88 (m, 1H, CH_2CH=CH and CH_2CH=CH_2), 5.63 (dt, *J* = 9.2, 7.8 Hz, 1H, CH_2CH=CH), 5.11-5.22 (m, 4H, CH_2CH=CH_2), 3.65 (dd, *J* = 7.4, 1.1 Hz, 2H, CH_2CH=CH), 3.58 (dd, *J* = 7.4, 1.1 Hz, 2H, CH_2CH=CH), 3.34-3.37 (m, 2H, CH_2CH=CH_2), 3.30-3.33 (m, 2H, CH_2CH=CH_2), 2.34 (s, 3H, CH_3), 2.34 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.2 (C=O), 194.9 (C=O), 139.3 (CH_2CH=*C*H), 137.2 (CH_2CH=*C*H_2), 132.7 (CH_2CH=*C*H_2), 129.2 (CH_2CH=*C*H), 126.6 (CH_2*C*H=CH), 125.9 (CH_2*C*H=CH), 119.1 (CH_2CH=*C*H_2), 119.0 (CH_2CH=*C*H_2), 42.0 (*C*H_2CH=CH_2), 41.2 (*C*H_2CH=CH_2) 30.8 (*C*H_2CH=CH), 30.5 (CH_3), 30.5 (CH_3), 27.2 (*C*H_2CH=CH) ppm. HRMS (ASAP) $[M+H]^+$ calc. 221.0128, found 221.0130 $[C_8H_{12}OS_3]^+$. IR (neat): 1685, 1352, 1130, 1105, 918, 612 cm^{-1}.

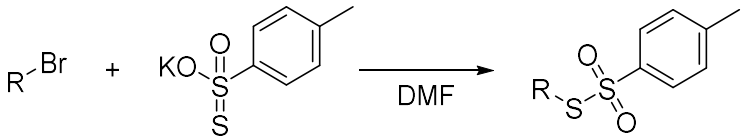
2,2'-(Prop-1-ene-1,3-diyl)bis(1-allyldisulfane) (84)



As a mixture of *E*/*Z*-isomers: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.32 (dd, *J* = 9.2, 0.6 Hz, 1H), 6.18 (dd, *J* = 14.6, 0.5 Hz, 1H), 5.94 – 5.77 (m, 6H), 5.26 – 5.11 (m, 8H), 3.51 – 3.30 (m, 12H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.7 (*C*H=CHSS), 133.5

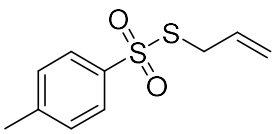
(*C*H=CHSS), 133.4 (CH_2=*C*HCH_2), 133.0 (CH_2=*C*HCH_2), 132.8 (CH_2*C*H=CH_2), 129.5 (CH_2*C*H=CH_2), 127.5 (*C*H_2=CH), 126.3 (*C*H_2=CH), 119.2 (CH=*C*H_2), 119.2 (CH=*C*H_2), 118.9 (CH=*C*HSS), 118.8 (CH=*C*HSS), 42.6 (*C*H_2SS), 42.4 (CH_2SS), 42.1 (SS*C*H_2), 41.4 (SS*C*H_2), 41.0 (SSCH_2*C*H=CH), 36.6 (SSCH_2*C*H=CH) ppm. HRMS (ASAP) $[M+H]^+$ calc. 251.0057, found 251.0056 $[C_9H_{15}S_4]^+$. IR (neat): 2361, 2432, 1217, 986, 920, 669, 650 cm^{-1}. Spectra in accordance with literature.[64]

General Procedure 1 (GP1): Synthesis thiotosylate reagents (83a–h)



Aryl/alkyl bromide (2 equiv.) and potassium *p*-toluenethiosulfonate (1 equiv.) were dissolved in DMF (0.1 M) and stirred at room temperature for 12-18 h overnight at room temperature. The solution was then suspended in water (20 mL), which was extracted with diethyl ether (2×10 mL). The combined extracts were washed with water (2 x 20 mL) to remove any residual DMF. Following drying over $MgSO_4$ and solvent evaporation the product (**83a-h**) was obtained as a colourless oil.

***S*-Allyl 4-methylbenzenesulfonothioate (83a)**



Isolated as a yellow oil in 590 mg, 83% yield from 3.1 mmol of potassium thiotosylate by GP1 in agreement with literature data.[16] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.78 (m, 2H, Ar*H*), 7.36 – 7.32 (m, 2H, Ar*H*), 5.70 (ddt, *J* = 17.0, 10.0, 7.1 Hz, 1H, C*H*=CH_2), 5.20 (ddd, *J* = 16.9, 2.5, 1.3 Hz, 1H, CH=C*H*$_2$), 5.10 (ddd, *J* = 10.0, 2.3, 1.0 Hz, 1H, CH=C*H*$_2$), 3.68 – 3.65 (m, 2H, C*H*$_2$), 2.45 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.0 (*C*$_{Ar}$), 142.2 (*C*$_{Ar}$), 130.8 (*C*H=CH_2), 130.0 (*C*$_{Ar}$), 127.3 (*C*$_{Ar}$), 120.2 (CH=*C*H_2), 39.0 (*C*H_2), 21.8 (*C*H_3) ppm

1-Allyl-2-(3-(allylthio)allyl)disulfane (80b)

Batch Procedure:

The thioacetate, *S*-(3-(allyldisulfaneyl)allyl) ethanethioate (**79**) (100 mg, 0.45 mmol) was dissolved in methanol (4.5 mL) and cooled to -40 °C. Potassium hydroxide (31 mg, 0.48 mmol, 1.05 equiv.) in methanol (4.5 mL) was added to the solution, followed by the direct addition of allyl bromide (54 mg, 0.44 mmol, 1.0 equiv.). The solution was left at -40 °C for 1 hour and warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of ammonium chloride (5 mL), and solvent was removed *in vacuo*. The resulting residue was dissolved in diethyl ether (20 mL), and washed with water (20 mL), brine (2 x 20 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 10 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **80b** as a pale yellow oil (44 mg, 45%).

Flow Procedure:

The thioacetate, *S*-(3-(allyldisulfaneyl)allyl) ethanethioate (**79**) (100 mg, 0.45 mmol) and allyl bromide (163 mg, 1.35 mmol, 3.0 equiv.) was dissolved in methanol (4.5 mL, 0.1 M) and transferred to a syringe. Potassium hydroxide (88 mg, 1.35 mmol, 3.0 equiv.) was dissolved in methanol (4.5 M) and transferred to a syringe. Both syringes pumped at 0.05 mL/min using syringe pumps through a T-piece mixer and to PTFE tubing (i.d. 0.8 mm, volume: 1 mL, residence time: 10 min). The PTFE coil was submerged in an ice bath at 0 °C. The first column volume of reactant was discarded, and the following seven column volumes were collected and concentrated *in vacuo* at 40 °C. The resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 10 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 10 CV, then

increased to 100% diethyl ether over 3 CV) to afford compound **80b** as a pale yellow oil (33 mg, 43%).

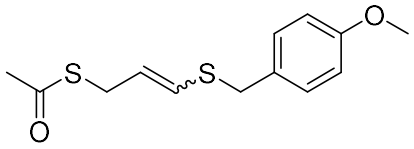
Combined Flow Procedure:

Potassium carbonate (9.7 g, 0.07 mol) and sand (14.3 g) were shaken until a homogenous mixture was achieved by eye. The K_2CO_3/sand (24 g, 42 wt.%) was packed into a 10 g Biotage® Isolera column (C1). The packed column was weighed, and dry tetrahydrofuran (THF) was flowed through at 0.4 mL/min for 1 hour, after which, the column was reweighed. The mass difference equated to the calculated column volume (CV, 7.6 mL, residence time: 19 minutes). A solution of thioacetic acid (0.4 M) and propargyl bromide (0.4 M) in THF was passed through the column using a HPLC K120 Knauer Analytical Pump. The outlet was connected a T-piece mixture, where the other inlet was from another HPLC 120 Knauer Analytical pump delivering thioacetic acid (0.4 M) and AIBN (20 mol%) in THF:CH_2Cl_2. The outlet of the two streams was a PTFE coil (C2) with internal diameter 0.8 mm and volume 32 mL with a resulting residence time of 40 minutes from resulting flow rate of 0.8 mL/min. C2 was heated by submerging in a water bath held at 85-90 °C fitted with a back pressure regulator at 75 psi. The outlet of C2 was fed into a cross mixer along with potassium hydroxide solution (0.44 M) and allyl thiotosylate (0.675 M) using a Fusion 100 Touch Syringe Pump. The outlet stream was fed into C3, a PTFE reactor coil of volume 8.7 mL with a residence time of 5.5 minutes. C3 was submerged in an ice bath at 0 °C. In a similar fashion, the outlet of C3 was fed into a cross mixer along with a solution of potassium hydroxide (0.437 M) and allyl bromide (0.437 M) in methanol using Fusion 100 Touch Syringe Pump. The outlet of which delivered the reaction mixture to C4, a PTFE coil with volume 24 mL and a resulting residence time of 11 minutes. C4 was submerged in an ice bath to allow the reaction to occur at 0 °C. The outlet of the continuous system was collected for 17 minutes (41 mL) and quenched with ammonium chloride. The organic layer was extracted using diethyl ether and washed with water and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo.* The resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 10 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 10 CV, then

increased to 100% diethyl ether over 3 CV) to afford compound **80b** as a pale yellow oil (73 mg, 12%).

As a mixture of *E*/*Z*-isomers: ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.15 (dt, *J* = 9.5, 0.9 Hz, 1H, CH_2CH=C*H*), 6.12 (dt, *J* = 15.1, 1.0 Hz, 1H, CH_2CH=C*H*), 5.77-5.94 (m, 4H, CH_2CH=CH_2 and CH_2CH=CH_2), 5.70 (dt, *J* = 9.5, 0.9 Hz, 1 H, CH_2CH=CH), 5.60 (dt, *J* = 15.1, 7.7 Hz, 1 H, CH_2CH=CH), 5.11-5.22 (m, 8H, CH_2CH=CH_2 and CH_2CH=CH_2), 3.37 (dd, *J* = 7.7, 1.0 Hz, 2H, CH_2CH=CH), 3.36 (dd, *J* = 7.7, 1.0 Hz, 2H, CH_2CH=CH), 3.33 (dt, *J* = 7.2, 1.2 Hz, 2H, CH_2CH=CH_2), 3.32 (dt, *J* = 7.2, 1.2 Hz, 2H, CH_2CH=CH_2), 3.30-3.32 (m, 4H, CH_2CH=CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.1 (CH_2CH=CH_2), 133.6 (CH_2CH=CH_2), 133.5 (CH_2CH=CH_2), 133.5 (CH_2CH=CH_2), 128.9 (CH_2CH=CH), 127.7 (CH_2CH=CH),123.5 (CH_2CH=CH), 124.8 (CH_2CH=CH), 118.7 (CH_2CH=CH_2), 118.6 (CH_2CH=CH_2), 117.9 (CH_2CH=CH_2), 117.8 (CH_2CH=CH_2), 42.7 (CH_2CH=CH_2), 42.3 (CH_2CH=CH_2), 42.1 (CH_2CH=CH), 37.5 (CH_2CH=CH), 36.8 (CH_2CH=CH_2), 35.7 (CH_2CH=CH_2) ppm. HRMS (ASAP) $[M+H]^+$ calc. 219.0336 found 219.0336 $[C_9H_{15}OS_3]^+$. IR (neat): 3080, 2978, 1633, 1423, 1205, 985, 916, 756 cm^{-1}.

S-(3-((4-Methoxybenzyl)thio)allyl) ethanethioate (95)

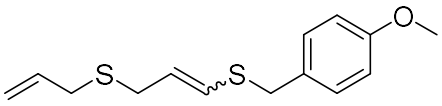


S-(Prop-2-yn-1-yl) ethanethioate (**77**) (3.64 g, 32 mmol) was dissolved in dry toluene (20 mL) and the solution was heated to 85 °C under argon atmosphere. Azobisisobutyronitrile (AIBN) (1.05 g, 6.4 mmol, 0.2 equiv.) was added to the solution directly, followed by the dropwise addition of *p*-methoxy benzyl mercaptan (7.4 mL) in toluene (20 mL) over 45 minutes using a syringe pump. The mixture was left to stir at 85 °C for a further 1 hour. The reaction was then quenched with aqueous saturated solution of sodium carbonate (20 mL) and the toluene was removed *in vacuo*. The remaining residue was dissolved diethyl ether (20 mL) and the organic layer was washed with brine (2 x 10 mL) and dried over $MgSO_4$. The

solvent was evaporated *in vacuo* and the resulting residue was purified by column chromatography using Biotage Isolera Four (gradient: 100% hexane for 3 column volumes (CV), then increased to 80:20 hexane:diethyl ether over 15 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **95** as a pale yellow oil (6.13 g, 71%).

As a mixture of *E*/Z isomers: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 4H, Ar*H*), 6.87 – 6.83 (m, 4H, Ar*H*), 6.20 (dt, J = 15.0, 1.1 Hz, 1H, HC=C*H*S), 6.08 (dt, J = 9.3, 0.9 Hz, 1H, HC=C*H*S), 5.64 – 5.50 (m, 2H, SCH_2CH=CH), 3.85 (s, 2H, SCH_2PMP), 3.83 (s, 2H, SCH_2PMP), 3.80 (s, J = 2.1 Hz, 6H, OCH_3), 3.60 (dd, J = 7.7, 0.9 Hz, 2H, SCH_2CH=CH), 3.53 (dd, J = 7.4, 1.1 Hz, 2H, SCH_2CH=CH), 2.32 (s, 3H, CH_3COS), 2.32 (s, 3H, CH_3COS) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.7 (*C*=O), 195.3 (*C*=O), 159.0 ($C_{Ar}OCH_3$), 158.9 ($C_{Ar}OCH_3$), 130.1 (C_{Ar}), 130.0 (C_{Ar}), 129.7 (C_{Ar}), 129.2 (C_{Ar}), 128.5 (CH=*C*HS), 127.9 (CH=*C*HS), 123.9 (*C*H=CHS), 123.1 (*C*H=CHS), 114.2 (C_{Ar}), 114.1 (C_{Ar}), 55.4 (OCH_3), 37.6 (SCH_2C_{Ar}), 36.6 (SCH_2C_{Ar}), 31.8 (SCH_2CH=CH), 30.6 (SCH_2CH=CH), 30.5 (CH_3C=O), 27.9 (CH_3C=O) ppm. HRMS (EI) $[M+H]^+$ calc. 268.05862, found 286.0587 $[C_{13}H_{16}O_2S_2]^+$. IR (neat): 2835, 1686, 1511, 1248, 1132, 1033, 956, 834, 626 cm^{-1}.

Allyl(3-((4-methoxybenzyl)thio)allyl)sulfane (96)

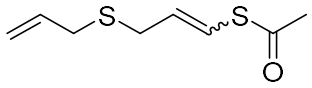


The thioacetate **95** (4.68 g, 17.5 mmol) was dissolved in methanol (100 mL) and cooled to -40 °C. Potassium hydroxide (1.7 g, 26.3 mmol, 1.5 equiv.) was added to the solution, followed by the direct addition of allyl bromide (3 mL, 35 mmol, 2 equiv.). The solution was left at -40 °C for 1 hour and warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of ammonium chloride (10 mL), and solvent was removed *in vacuo*. The resulting residue was dissolved in diethyl ether (20 mL), and washed with water (20 mL), brine (2 x 20 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was

purified using Biotage Isolera Four (gradient: 100% hexane for 10 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **96** as a pale yellow oil (3.5 g, 88%).

As a mixture of *E/Z* isomers: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 – 7.19 (m, 4H, Ar*H*), 6.85 (dd, J = 8.7, 2.7 Hz, 4H, Ar*H*), 6.07 (d, J = 9.4 Hz, 1H, HC=C*H*S), 6.01 (d, J = 15.0 Hz, 1H, HC=C*H*S), 5.85 – 5.67 (m, 2H, CH_2=C*H*CH_2), 5.62 – 5.51 (m, 2H, CH_2=C*H*CH_2), 5.18 – 4.96 (m, 4H, C*H*$_2$=CHC*H*$_2$S), 3.85 (s, 2H, SC*H*$_2$PMP), 3.83 (s, 2H, SC*H*$_2$PMP), 3.79 (s, 6H, OC*H*$_3$), 3.18 (d, J = 7.5 Hz, 2H, SC*H*$_2$CH=CH), 3.09 (d, J = 7.3 Hz, 2H, SC*H*$_2$CH=CH), 3.05 (d, J = 7.1 Hz, 2H, CH_2=CHC*H*$_2$S), 3.00 (d, J = 7.1 Hz, 2H, CH_2=CHC*H*$_2$S) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.0 (*C*$_{Ar}$OCH$_3$), 134.4 (CH_2=*C*HCH$_2$S), 130.1 (*C*$_{Ar}$), 130.0 (*C*$_{Ar}$), 129.9 (*C*$_{Ar}$), 129.4 (*C*$_{Ar}$), 127.2 (*C*$_{Ar}$), 126.3 (*C*$_{Ar}$), 126.0 (CH=*C*HS), 125.2 (CH=*C*HS), 117.3 (*C*H_2=CHCH$_2$S), 117.2, (*C*H_2=CHCH$_2$S), 114.2 (C$_{Ar}$), 55.4 (O*C*H$_3$), 55.4 (O*C*H$_3$), 37.7 (S*C*H$_2$C$_{Ar}$), 36.7 (S*C*H$_2$C$_{Ar}$), 34.3 (CH*C*H$_2$S), 33.5 (CH*C*H$_2$S), 33.0 (S*C*H$_2$CH), 29.4 (S*C*H$_2$CH) ppm. HRMS (ES) $[M+H]^+$ calc. 267.0877, found 267.0879 $[C_{14}H_{19}OS2]^+$. IR (neat): 2835, 1610, 1511, 1249, 1176, 1034, 918, 832 cm^{-1}.

_S_-(3-(Allylthio)prop-1-en-1-yl) ethanethioate (97)

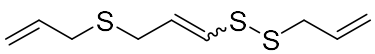


Sulfide **96** (2.26 g, 8.5 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL) under argon atmosphere and cooled to -78 °C. A premixed solution of acetic acid (3.75 mL, 65.6 mmol, 7.7 equiv.) and trifluoacetic anhydride (9.2 mL, 65.6 mmol, 7.7 equiv.) was added dropwise and the reaction left to stir at – 78°C for a further 2 hours. The reaction was quenched with a saturated solution of sodium carbonate (20 mL) and the product was extracted using CH_2Cl_2 (3 x 20 mL).The combined organic layers were washed with brine (20 mL), water (20 mL) and dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 5 column volumes (CV),

then increased to 80:20 hexane:diethyl ether over 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **97** as a pale yellow oil (329 mg, 21%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (dt, J = 9.6, 1.1 Hz, 1H, CH_2=C*H*S), 6.53 (dt, J = 15.6, 1.2 Hz, 1H, CH_2=C*H*S), 5.88 – 5.69 (m, 4H, CH_2=C*H*CH_2 and SCH_2C*H*=CH), 5.22 – 5.05 (m, 4H, C*H*$_2$=CH), 3.19 (dd, J = 7.4, 1.2 Hz, 2H, SC*H*$_2$CH=CH), 3.14 (dd, J = 7.6, 1.1 Hz, 2H, SC*H*$_2$CH=CH), 3.12 – 3.09 (m, 2H, CHC*H*$_2$S), 3.06 – 3.01 (m, 2H, CHC*H*$_2$S), 2.36 (s, J = 3.9 Hz, 3H, C*H*$_3$), 2.35 (s, 3H, C*H*$_3$) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 193.1 (*C*=O), 134.0 (SCH_2*C*H=CH_2), 130.6 (CH_2=*C*HCH_2), 130.3 (CH=*C*H_2S), 117.9 (*C*H_2=CHCH_2), 33.5 (*C*H_2S), 32.6 (S*C*H_2), 30.55 (CH_3) ppm. HRMS (CI) $[M+H]^+$ calc. 188.03241, found 188.0323 $[C_8H_{12}OS_2]^{+\cdot}$. IR (neat): 2835, 1610, 1511, 1249, 1176, 1034, 918, 832 cm^{-1}.

1-Allyl-2-(3-(allylthio)prop-1-en-1-yl)disulfane (80a)

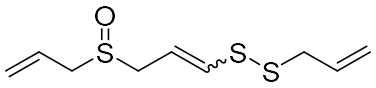


Thioacetate **97** (52 mg, 0.28 mmol) was dissolved in methanol (2.8 mL) and cooled to -40 °C. Potassium hydroxide (20 mg, 0.31 mmol, 1.1 equiv.) in methanol (2 mL) was added to the solution, followed by the addition of allyl thiotosylate (**83a**, 76.7 mg, 0.34 mmol, 1.2 equiv.). The solution was left at -40 °C for 1 hour and warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of ammonium chloride (5 mL), and solvent was removed *in vacuo*. The resulting residue was dissolved in diethyl ether (20 mL), and washed with water (20 mL), brine (2 x 20 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 10 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 10 CV, then increased to 100% diethyl ether over 3 CV) to afford compound **80a** as a pale yellow oil (43 mg, 70%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.21 (dt, *J* = 9.3, 1.1 Hz, 1H, *Z*-CH=C*H*SS), 6.09 (dt, *J* = 14.7, 1.1 Hz, 1H, *E*-CH=C*H*SS), 5.88 – 5.78 (m, *E/Z*-4H), 5.21 – 5.07 (m, 2H,

CH, *E/Z*-C*H*=CHSS), 3.40 – 3.37 (m, 4H, *E/Z*-SSC*H*$_2$), 3.37 – 3.33 (m, 6H, *E/Z*-C*H*$_2$SC*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.4 (*C*H=CHS), 134.3 (*C*H=CHS), 134.2 (CH_2=*C*H), 132.8 (CH_2=*C*H), 132.3 (CH_2*C*H=CH_2), 127.4 (CH_2*C*H=CH_2), 118.2 (CH=*C*H_2), 118.1 (*C*H_2=CH), 117.5 (CH=*C*HSS), 117.37 (CH=*C*HSS) ppm. HRMS (ASAP) $[M+H]^+$ calc. 219.0336 found: 219.0338 $[C_9H_{15}S_3]^+$. IR (neat): 2953, 2853, 986, 916, 758, 721, 648, 578 cm^{-1}.

1-Allyl-2-(3-(allylsulfinyl)prop-1-en-1-yl)disulfane (8)

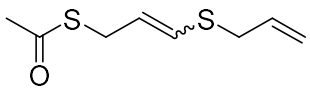


Sulfide **80a** (43 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and cooled to -78 °C. Recrystallised *m*CPBA (35 mg, 0.2 mmol, 1 equiv.) was added to the solution and left at -78 °C for one hour, then warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of sodium carbonate (5 mL), and the product was extracted using CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with water (20 mL), brine (20 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 2 column volumes (CV), then increased to 20:80 hexane:diethyl ether over 3 CV, then increased to 100% diethyl ether over 10 CV, and held at 100% diethyl ether for 20 CV) to afford compound **8** as a pale yellow oil (23 mg, 49%) in agreement with literature data.[14]

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.55 (dt, J = 9.5, 1.0 Hz, 1H, *E*-CH=C*H*SS), 6.36 (dt, J = 14.8, 1.1 Hz, 1H, *Z*-CH=C*H*SS), 5.95 – 5.70 (m, 6H, CH=C*H*C*H*$_2$), 5.47 – 5.36 (m, 4H, CH_2=CHC*H*$_2$S=O), 5.20 – 5.13 (m, 4H, C*H*$_2$=CHCH_2S), 3.59 – 3.42 (m, 8H, C*H*$_2$S(=O)C*H*$_2$), 3.39 (d, J = 7.5 Hz, 2H, SSC*H*$_2$), 3.34 (d, J = 7.3 Hz, 2H, SSC*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.7 (S(=O)CH_2*C*H=CH), 134.8 (S(=O)CH_2*C*H=CH), 132.7 (SSCH_2*C*H=CH_2), 132.6 (SSCH_2*C*H=CH_2), 125.8 (CH_2=*C*HCH_2S), 125.7 (CH_2=*C*HCH_2S), 124.0 (*C*H_2=CHCH_2S), 123.9 (*C*H_2=CHCH_2S), 119.4 (SSCH_2CH*C*H_2), 119.4 (SSCH_2CH*C*H_2), 118.2

(CH=*C*HSS), 116.9 (CH=*C*HSS), 55.0 (*C*H_2S=O), 54.5 (*C*H_2S=O), 53.1 (S(=O)*C*H_2), 49.7 (S(=O)*C*H_2), 42.2 (SS*C*H_2), 41.4 (SS*C*H_2) ppm.

_S_-(3-(Allylthio)allyl) ethanethioate (91)

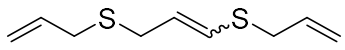


Vinyl thioacetate (**78**) (1 g, 5.3 mmol) was dissolved in methanol (53 mL, 0.1 M) and cooled to 0 °C. Potassium hydroxide (342 mg, 5.3 mmol, 1 equiv.) was added and stirred for 10 minutes. Allyl bromide (546 µL, 6.3 mmol, 1.2 equiv.) was added and the reaction was warmed to room temperature for 2 hours. After TLC indicated the full consumption of starting material, the reaction was quenched with ammonium chloride. The product was extracted in diethyl ether (3 x 50 mL) and washed with brine (20 mL) and water (20 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 2 column volumes (CV), then increased to 90:10 hexane:diethyl ether over 3 CV, then increased to 100% diethyl ether over 10 CV, and held at 100% diethyl ether for 5 CV) to afford compound **91** as a pale yellow oil (459 mg, 46% yield). Sulfide **93** was also obtained as a side product in 106 mg, 11% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (dt, *J* = 15.0, 1.1 Hz, 1H, SC*H*=CH), 6.07 (dt, *J* = 9.4, 0.9 Hz, 1H, SC*H*=CH), 5.82 (ddtd, *J* = 11.7, 8.6, 7.0, 1.6 Hz, 2H, CH_2=C*H*CH_2S), 5.65 (dt, *J* = 9.4, 7.7 Hz, 1H, SCH=C*H*), 5.57 (dt, *J* = 14.9, 7.4 Hz, 1H, SCH=C*H*), 5.21 – 5.16 (m, 2H, C*H*$_2$=CHCH_2S), 5.16 – 5.11 (m, 2H, C*H*$_2$=CHCH_2S), 3.63 (d, *J* = 7.7, 0.9 Hz, 2H, CH=CHC*H*$_2$S), 3.35 (d, *J* = 7.4, 1.1 Hz, 2H, CH=CHC*H*$_2$S), 3.32 – 3.27 (m, 4H, CH_2=CHC*H*$_2$S), 2.33 (s, 3H, C*H*$_3$), 2.32 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.7 (S*C*=OCH_3), 195.2 (S*C*=OCH_3), 134.0 (CH_2=*C*HCH_2S), 133.5 (CH_2=*C*HCH_2S), 128.3 (CH*2*=*C*HCH_2S), 127.4 (CH*2*=*C*HCH_2S), 123.7 (*C*H_2=CH), 123.3 (*C*H_2=CH), 117.9 (SCH=*C*H), 117.9 (SCH=*C*H), 36.5 (CH*C*H_2S), 35.6 (CH*C*H_2S), 31.8 (*C*H_2SCO), 30.6 (*C*H_2SCO), 30.5 (*C*H_3), 28.0 (*C*H_3) ppm. HRMS (CI) $[M+H]^+$ calc. 188.03241,

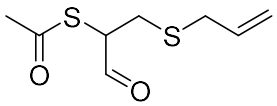
found 188.0323 $[C_8H_{12}OS_2]^+$. IR (neat): 2835, 1610, 1511, 1249, 1176, 1034, 918, 832 cm^{-1}.

Prop-1-ene-1,3-diylbis(allylsulfane) (93)



^{1}H NMR (500 MHz, $CDCl_3$) δ 6.05 (dt, *J* = 9.4, 1.0 Hz, 1H, *E*-CH=C*H*S), 6.00 (dt, *J* = 15.0, 1.1 Hz, 1H, *Z*-CH=C*H*S), 5.89 – 5.71 (m, 4H, CH_2=C*H* and C*H*=CH_2), 5.67 – 5.54 (m, 2H, C*H*=CHS), 5.22 – 5.06 (m, 8H, CH_2=CH and CH=CH_2), 3.32 – 3.28 (m, 4H, SCH_2), 3.21 (dd, *J* = 7.5, 1.0 Hz, 2H, CH_2SCH_2), 3.13 (dt, *J* = 7.1, 1.0 Hz, 4H, CH_2SCH_2), 3.09 (dt, *J* = 7.1, 1.1 Hz, 2H, CH_2SCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.4 (CH_2=*C*H), 134.4 (CH_2=*C*H), 134.2 (*C*H=CH_2), 133.8 (*C*H=CH_2), 127.1 (CH=*C*HS), 125.9 (CH=*C*HS), 125.6 (*C*H=CHS), 125.4 (*C*H=CHS), 117.8 (CH=*C*H_2), 117.7 (CH=*C*H_2), 117.3 (*C*H_2=CH), 117.3 (*C*H_2=CH), 36.7 (CHS*C*H_2), 35.8 (CHS*C*H_2), 34.4 (*C*H_2SCH_2), 33.5 (*C*H_2SCH_2), 33.0 (CH_2S*C*H_2), 29.4 (CH_2S*C*H_2) ppm. HRMS (CI) $[M+H]^+$ calc. 187.06097 found: 187.0609 $[C_9H_{15}S_2]^+$. IR (neat): 2951, 2853, 989, 920, 756, 722, 651, 576 cm^{-1}.

***S*-(1-(Allylthio)-3-oxopropan-2-yl) ethanethioate (92)**

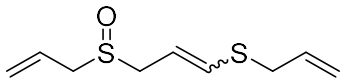


Sulfide **91** (200 mg, 1.06 mmol) was dissolved in dry CH_2Cl_2 (10.6 mL) and cooled to -78 °C. Recrystallised *m*CPBA (183 mg, 1.06 mmol, 1 equiv.) was added to the solution and left at -78 °C for one hour, then warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of sodium carbonate (5 mL), and the product was extracted using CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with water (20 mL), brine (20 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 2 column volumes (CV), then increased to 20:80

hexane:diethyl ether over 3 CV, then increased to 100% diethyl ether over 10 CV, and held at 100% diethyl ether for 20 CV) to afford compound **92** as a pale yellow oil (78 mg, 40%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, J = 10.0 Hz, 1H, C*H*O), 5.75 – 5.66 (m, 1H, C*H*$_2$=CHCH$_2$S), 5.10 – 5.08 (m, 1H, C*H*$_2$=CHCH$_2$S), 3.89 – 3.79 (m, 1H, SC*H*CHO), 3.02 (ddd, J = 22.1, 13.7, 6.8 Hz, 2H, C*H*$_2$SCOCH$_3$), 2.39 – 2.33 (m, 1H, CH$_2$=CHC*H*$_2$S), 2.31 (s, 3H, C*H*$_3$), 2.26 – 2.19 (m, 1H, CH$_2$=CHC*H*$_2$S) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.9 (S*C*(=O)CH$_3$), 177.8 (*C*HO), 133.7 (CH$_2$=*C*HCH$_2$), 118.4 (*C*H$_2$=CHCH$_2$), 37.7 (CH*C*H$_2$S), 36.7 (S*C*HCHO), 31.5 (*C*H$_2$SCOCH$_3$), 30.6 (*C*H$_3$) ppm. HRMS (ES) $[M+Na]^+$ calc. 227.0176, found 227.0187 $[C_8H_{12}O_2S_2Na]^+$. IR (neat): 2924, 1692, 1641, 1439, 1354, 1128, 955, 922, 746, 625 cm^{-1}.

Allyl(3-(allylsulfinyl)prop-1-en-1-yl)sulfane (94)

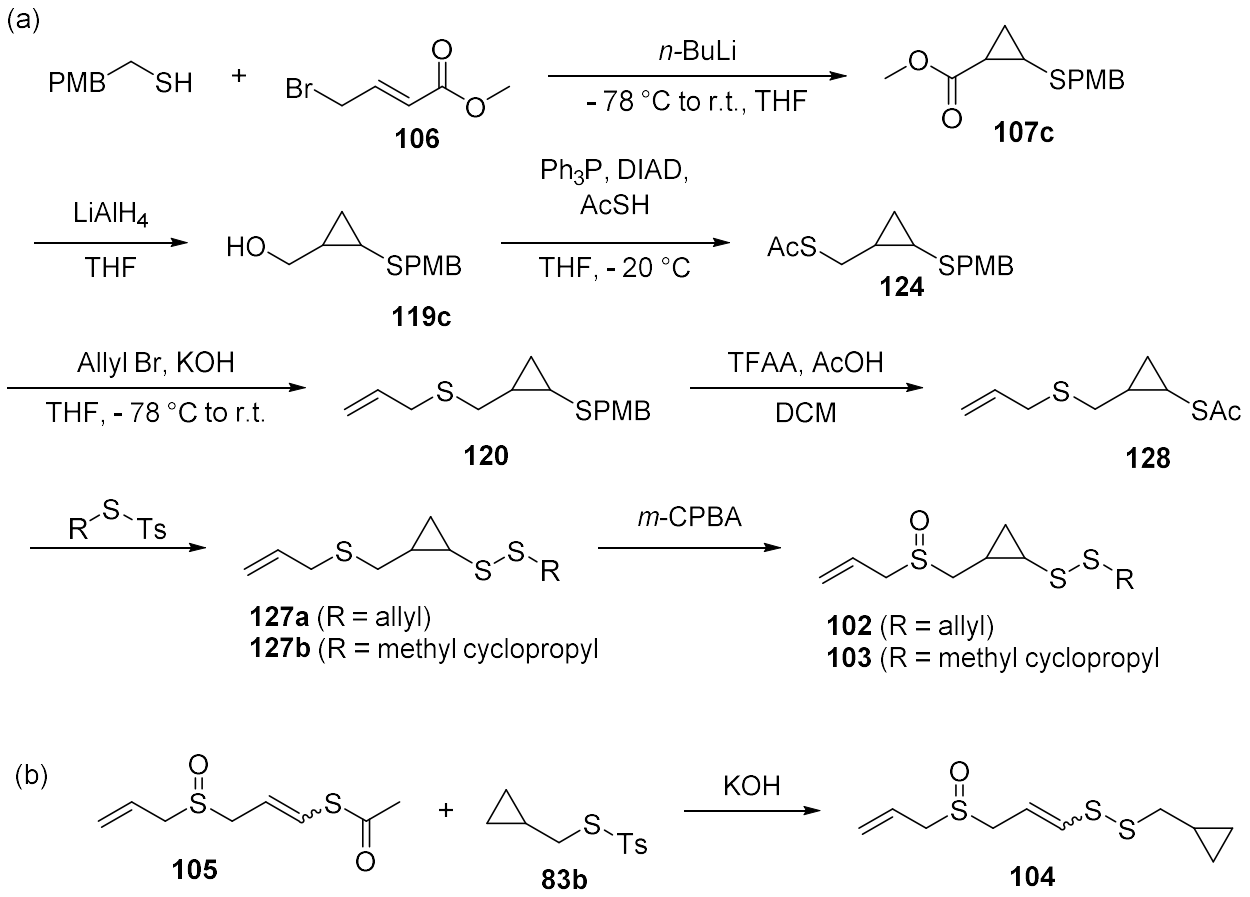


Sulfide **93** (106 mg, 0.57 mmol) was dissolved in dry CH_2Cl_2 (5.7 mL) and cooled to -78 °C. Recrystallised *m*CPBA (98 mg, 0.57 mmol, 1 equiv.) was added to the solution and left at -78 °C for one hour, then warmed to room temperature until thin layer chromatography (TLC) indicated full consumption of starting material. The reaction was quenched with a saturated solution of sodium carbonate (5 mL), and the product was extracted using CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with water (20 mL), brine (20 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified using Biotage Isolera Four (gradient: 100% hexane for 2 column volumes (CV), then increased to 20:80 hexane:diethyl ether over 3 CV, then increased to 100% diethyl ether over 10 CV, and held at 100% diethyl ether for 20 CV) to afford compound **94** as a pale yellow oil (77 mg, 67%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (dt, *J* = 9.6, 0.9 Hz, 1H, *E*-CH=C*H*S), 6.31 (dt, *J* = 15.2, 1.1 Hz, 1H, *Z*-CH=C*H*S), 5.98 – 5.76 (m, 4H, SCH_2CH=CH_2 and CH_2=C*H*CH_2), 5.74 – 5.54 (m, 2H, C*H*=CHS), 5.48 – 5.35 (m, 4H, CH_2=CH), 5.26 – 5.13 (m, 4H, CH=CH_2), 3.65 (dd, *J* = 7.7, 0.9 Hz, 2H, SOCH_2), 3.63 (dd, *J* = 7.7, 0.9 Hz, 2H, SOCH_2), 3.57 – 3.48 (m, 4H, CH_2SO), 3.45 – 3.33 (m, 4H, SCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.8 (*C*H=CH_2), 133.3 (*C*H=CH_2), 133.2 (CH=*C*HS), 133.0 (CH=*C*HS), 126.1 (CH_2=*C*H), 125.9 (CH_2=*C*H), 123.8 (*C*H_2=CH), 123.7 (*C*H_2=CH), 118.3 (CH=*C*H_2), 118.2 (CH=*C*H_2), 115.6 (C*H*=CHS), 114.3 (C*H*=CHS), 55.0 (SO*C*H_2), 54.2 (SO*C*H_2), 53.4 (*C*H_2SO), 50.6 (*C*H_2SO), 36.7 (S*C*H_2), 35.5 (S*C*H_2) ppm. HRMS (ES) $[M+Na]^+$ calc. 225.0384 found: 225.0393 $[C_9H_{14}ONaS_3]^+$. IR (neat): 3082, 3008, 2914, 1425, 1404, 1038, 991, 926, 758, 658, 577 cm^{-1}.

8.3. Chapter 4: Synthesis of Cyclopropyl Ajoene Analogues

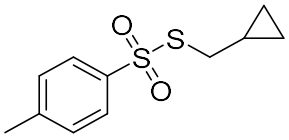
8.3.1. General Scheme



Scheme 8.2: General scheme illustrating overview of work in chapter 4. (a): Synthesis of central cyclopropyl ajoene analogues (**102** and **103**). (b): synthesis of terminal cyclopropyl ajoene analogue **140**.

8.3.2. Experimental

_S_-(Cyclopropylmethyl) 4-methylbenzenesulfonothioate (83b)

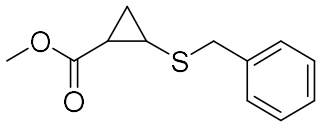


Synthesised by GP1 and isolated as a colourless oil in 1.0 g, 89% from 4.75 mmol of potassium thiotosylate in agreement with literature data.[96] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H, Ar*H*), 7.34 (d, *J* = 8.0 Hz, 2H, Ar*H*), 2.94 (d, *J* = 7.4 Hz, 2H, SCH_2), 2.45 (s, 3H, ArCH_3), 1.04 – 0.91 (m, 1H, cpr-C*H*), 0.62 – 0.51 (m, 2H, cpr-CH_2), 0.21 (m, 2H, cpr-CH_2) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 144.8 (C_{Ar}), 142.3 (C_{Ar}), 136.7 (C_{Ar}), 130.0 (C_{Ar}), 127.8 (C_{Ar}), 127.1 (C_{Ar}), 42.2 (CH_2), 21.8 (CH_3), 9.9 (cpr-*C*H), 6.3 (cpr-*C*H_2) ppm.

General Procedure 2 (GP2): Michael Induced Ring Closure

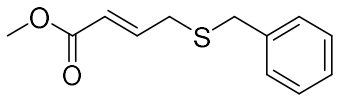
To a solution of aryl thiol (60 mmol, 1 eq.) in dry THF (80 mL) was added *n*-butyl lithium solution (2.5 M in hexanes, 29 mL, 72 mmol, 1.2 eq.) slowly at −78 °C under argon atmosphere. After 20 minutes, the solution of the so formed lithium thiolate was added dropwise to a solution of methyl bromocrotonate (7 mL, 60 mmol, 1 eq.) in dry THF (100 mL) at −40 °C and the reaction was allowed to warm to room temperature. The reaction was stirred for 20 hours, quenched with NaOH solution (10%, 60 mL) solvent removed *in vacuo*. The remaining residue was extracted with diethyl ether (3 x 50 mL), washed with NaOH solution (10%, 60 mL), then with brine. The organic extracts were dried over $MgSO_4$. The solvent was removed under vacuum and the resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 70:30 (cyclohexane:diethyl ether) over 4 CV, and then to 100% diethyl ether over 20 CV) to afford desired compound.

Methyl 2-(benzylthio)cyclopropane-1-carboxylate (107a)



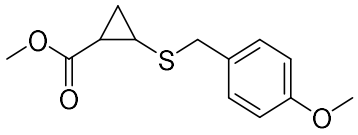
Compound **107a** was synthesised by GP2 and isolated in 8.3 g, 62% yield as a colourless oil in agreement with literature data.[71] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.19 (m, 5H, Ar*H*), 3.76 (s, 2H, SC*H*$_2$), 3.63 (s, 3H, CH_3), 2.29 – 2.23 (m, 1H, cpr-C*H*), 1.65 (ddd, *J* = 8.7, 5.3, 3.6 Hz, 1H, cpr-C*H*$_2$), 1.41 (dt, *J* = 8.4, 5.0 Hz, 1H, cpr-C*H*), 1.08 – 1.03 (m, 1H, cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1 (*C*=O), 129.0 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 127.2 (*C*$_{Ar}$), 52.0 (O*C*H$_3$), 38.0 (S*C*H$_2$), 23.9 (cpr-*C*H), 22.8 (cpr-*C*H), 17.1 (cpr-*C*H$_2$) ppm.

Methyl (*E*)-4-(benzylthio)but-2-enoate (108a)



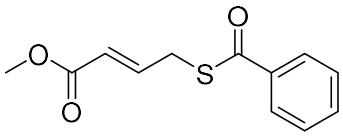
Compound **108a** synthesised by GP2 as a side product in 2.4 g, 17% yield as a colourless oil in agreement with literature data.[97] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 – 7.18 (m, 3H, Ar*H*), 6.89 – 6.82 (m, 3H, Ar*H* and CH=C*H*CH$_2$), 5.86 – 5.79 (m, 1H, C*H*=CH), 3.75 (s, 3H, C*H*$_3$), 3.61 (s, 2H, C*H*$_2$Ar), 3.09 (dd, *J* = 7.4, 1.3 Hz, 2H, C*H*$_2$S) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6 (*C*=O), 158.9 (CH=*C*HCH$_2$S), 144.0 (*C*$_{Ar}$), 130.2 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 122.7 (*C*$_{Ar}$), 114.11 (*C*H=CH), 51.73 (*C*H$_3$), 34.8 (S*C*H$_2$), 31.9 (*C*H$_2$S) ppm.

Methyl 2-((4-methoxybenzyl)thio)cyclopropane-1-carboxylate (107c):



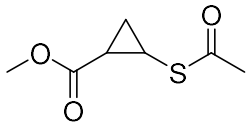
Compound **107c** was synthesised by GP2 and isolated in 5.5 g, 46% yield as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 – 7.17 (m, 2H, Ar*H*), 6.85 – 6.81 (m, 2H, Ar*H*), 3.80 (s, 3H, OC*H*$_3$), 3.75 (s, 2H, SC*H*$_2$), 3.66 (s, 3H, CO_2CH_3), 2.29 (ddd, *J* = 8.4, 5.8, 3.6 Hz, 1H, Cpr-C*H*), 1.68 (ddd, *J* = 8.8, 5.3, 3.6 Hz, 1H, Cpr-C*H*), 1.46 – 1.40 (m, 1H, Cpr-C*H*), 1.08 (ddd, *J* = 8.6, 5.8, 4.7 Hz, 1H, Cpr-C*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1 (*C*=O), 158.8 (C_{Ar}-OCH_3), 130.1 (SCH_2CAr), 130.0 (C_{Ar}), 114.1 (C_{Ar}), 55.4 ($C_{Ar}OCH_3$), 52.0 (CO_2CH_3), 37.4 (SCH_2), 23.9 (C_{Cpr}), 22.9 (C_{Cpr}), 17.1 (C_{Cpr}) ppm. HRMS (ES+) $[M+Na]^+$ calc. 275.0718, found 275.0721 $[C_{13}H_{16}O_3SNa]^+$. IR (neat): 1724, 1510, 1240, 1032, 831 cm^{-1}.

Methyl (*E*)-4-(benzoylthio)but-2-enoate (108b)



Compound **108b** was synthesised by GP2 on a 0.4 mmol scale and isolated in 62 mg, 65% yield as a colourless oil in agreement with literature data.[98] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.91 (m, 2H, Ar*H*), 7.62 – 7.56 (m, 1H, Ar*H*), 7.49 – 7.42 (m, 2H, Ar*H*), 7.00 – 6.89 (m, 1H, CH=C*H*CH_2S), 6.07 (dt, *J* = 15.5, 1.3 Hz, 1H, C*H*=CH_2), 3.85 – 3.80 (dd, *J* = 7.1, 1.4 Hz, 2H, SC*H*$_2$), 3.73 (s, 3H, OC*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.5 (*C*=O), 166.6 (*C*=O), 142.7 (CH=*C*HCH_2), 136.6 (C_{Ar}), 133.9 (C_{Ar}), 128.9 (C_{Ar}), 127.5 (C_{Ar}), 123.7 (*C*H=$CHCH_2$), 51.8 (OCH_3), 31.1 (SCH_2), 29.9 (CH_2S) ppm.

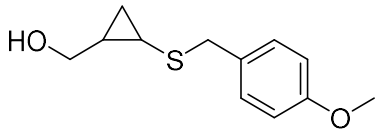
Methyl 2-(acetylthio)cyclopropane-1-carboxylate (118)



Compound **107c** (403 mg, 1.6 mmol) was dissolved in distilled dichloromethane (16 mL) under argon atmosphere. The reaction mixture was cooled to −78 °C and a pre-mixed solution trifluoroacetic acid (1.13 mL, 8 mmol, 5 equiv.) and acetic acid (458 µL, 8 mmol, 5 equiv.) was added dropwise. The reaction mixture was kept at −78 °C for 2 hours and warmed to room temperature. The reaction was quenched with a saturated solution of sodium carbonate and the product was extracted with dichloromethane (3 x 10 mL). The organic layers were washed with brine (20 mL), water (10 mL) and dried over magnesium sulfate. The organic layer was concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 40:60 (cyclohexane:diethyl ether) over 4 CV, and then to 80% diethyl ether over 20 CV) to afford **118** in 67 mg, 24% yield as a pale yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72 (s, 3H, CO_2CH_3), 2.66 (ddd, *J* = 8.6, 5.9, 3.8 Hz, 1H, cpr-C*H*), 2.31 (s, 3H, $SCOCH_3$), 1.79 – 1.75 (m, 1H, cpr-C*H*), 1.67 – 1.60 (m, 1H, cpr-CH_2), 1.14 – 1.10 (m, 1H, cpr-CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.9 (CH_3OC=O), 172.7 (*S*C=O), 52.4 (CO_2CH_3), 30.4 ($SCOCH_3$), 22.7 (cpr-*C*H), 19.9 (cpr-*C*H), 15.9 (cpr-CH_2) ppm. HRMS (ES) $[M+H]^+$ calc. 175.0429 found: 175.0433 $[C_7H_{11}O_3S]^+$. IR (neat): 1726, 1695, 1439, 1383, 1263, 1173, 957, 905, 619 cm^{-1}.

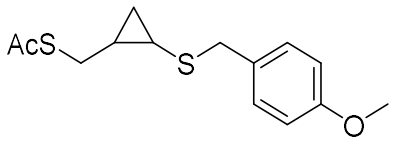
(2-((4-Methoxybenzyl)thio)cyclopropyl)methanol (119):



To a solution of **107c** (10 g, 40 mmol) in dry THF (100 mL) stirred under argon at 0 °C was added lithium aluminium hydride (1M in THF, 44 mL, 44 mmol, 1.1 eq.) was added dropwise. The ice bath was removed, and the reaction was left at room temperature for 4 hours. Sodium sulfate (saturated solution) was added dropwise until gas evolution ceased. The solvent was removed *in vacuo* and the remaining residue was extracted with diethyl ether (3 x 50 mL), washed with water (3 x 50 mL) and concentrated *in vacuo*. Compound **119** was obtained without further purification as an off low melting point white solid (7.91 g, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (q, *J* = 2.0 Hz, 2H, Ar*H*), 6.87 – 6.82 (m, 2H, Ar*H*), 3.79 (s, 3H, OCH_3), 3.73 (s, 2H, SCH_2), 3.43 (ddd, *J* = 18.7, 11.3, 6.8 Hz, 2H, CH_2OH), 1.68 – 1.63 (m, 1H, Cpr-C*H*), 1.30 (s, 1H, O*H*), 1.28 – 1.20 (m, 1H, Cpr-C*H*), 0.80 – 0.73 (m, 2H, Cpr-CH_2) ppm.^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7 (C_{Ar}-OCH_3), 130.8 (SCH_2C_{Ar}), 130.0 (C_{Ar}), 114.0 (C_{Ar}), 65.7 (CH_2OH), 55.4 (OCH_3), 37.6 (CH_2S), 25.1 (C_{Cpr}), 17.7 (C_{Cpr}), 12.9 (C_{Cpr}) ppm. HRMS (CI) $[M]^+$ calc. 224.08655 found: 224.0868 $[C_{12}H_{16}O_2S]^+$. IR (neat): 3333, 1609, 1582, 1510, 1304, 1236, 1031 cm^{-1}, m.p.: 58 – 60 °C.

***S*-((2-((4-Methoxybenzyl)thio)cyclopropyl)methyl) ethanethioate (124):**

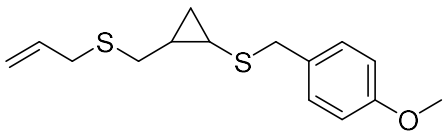


To a solution of triphenyl phosphine (16.4 g, 63 mmol, 2 eq.) in THF (100 mL) was added diisopropyl azodicarboxylate (12.4 mL, 63 mmol, 2 eq.) at 0 °C. The reaction was stirred vigorously for 30 minutes. A mixture of thioacetic acid (6.75 mL, 94.5 mmol, 3 eq.) and alcohol **119** (7g, 31.3 mmol) in THF (50 mL) was added slowly at

– 20 °C. The mixture was warmed to 0 °C over 30 minutes and then allowed to stand at 0 °C for 30 minutes. The reaction was left to stir over night then quenched with water and solvent removed *in vacuo*. The remaining residue was washed with extracted with diethyl ether (4 x 50 mL), washed with brine (2 x 50 mL), dried over magnesium sulfate and concentrated *in vacuo*. The pale yellow oil was dissolved in a mixture of hexanes : diethyl ether (10:1) at kept at – 20 °C for 3 hours, where excess triphenyl phosphine, triphenyl phosphine oxide and diisopropyl hydrazine-1,2-dicarboxylate crashed out as an off white solid. The filtrate was then concentrated and purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 80:20 (cyclohexane:diethyl ether) over 10 CV, then increased to 100% diethyl ether over 15 CV) to afford **124** as a colourless oil (9.16 g, 92% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.23 (m, 2H, Ar*H*), 6.89 – 6.85 (m, 2H, Ar*H*), 3.82 (s, 3H, OCH_3), 3.73 (d, *J* = 4.3 Hz, 2H, SCH_2), 2.84 (dd, *J* = 7.3, 4.3 Hz, 2H, CH_2SCOCH$_3$), 2.35 (s, 3H, SCOCH_3), 1.75 – 1.69 (m, 1H, Cpr-C*H*), 1.33 – 1.26 (m, 1H, Cpr-C*H*), 0.84 – 0.79 (m, 2H, Cpr-CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.7 (*C*=O), 158.7 (C_{Ar}OCH$_3$), 130.8 (SCH$_2C_{Ar}$), 130.0 (C_{Ar}), 114.0 (C_{Ar}), 55.4 (O*C*H$_3$), 37.6 (*C*H$_2$S), 33.0 (COS*C*H$_2$), 30.7 (CO*C*H$_3$), 22.7, (C_{Cpr}), 20.4, (C_{Cpr}), 15.9 (C_{Cpr}) ppm. HRMS (CI+) $[M+NH_4]^+$ calc. 300.1086, found 300.1088 $[C_{14}H_{22}O_2NS_2]^+$. IR (neat): 2917, 1690, 1034, 1242, 833, 625, 517 cm^{-1}.

Allyl((2-((4-methoxybenzyl)thio)cyclopropyl)methyl)sulfane (120):

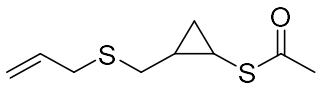


Thioacetate **124** (9.16 g, 32 mmol, 1 eq.) was dissolved in dry methanol (50 mL) and the solution was cooled to −78 °C and stirred under argon atmosphere. Potassium hydroxide (2.5 g, 44.3 mmol, 2.5 eq.) in dry methanol (20 mL) was added slowly. The mixture was left to stir for 45 min and then allyl bromide (3.1 mL, 35 mmol, 2 eq.) was added. The cooling bath was removed, and the reaction was

stirred for 2 hours at room temperature. The reaction was quenched with ammonium chloride (saturated solution, 20 mL) and solvent was removed *in vacuo*. The remaining residue was extracted with diethyl ether (3 x 20 mL), washed with water (3 x 20 mL), dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 30% diethyl ether over 10 CV, then increased to 100% diethyl ether over 15 CV) to afford **120** as a colourless oil (4.8 g, 54% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 – 7.24 (m, 2H, Ar*H*), 6.87 – 6.83 (m, 2H, Ar*H*), 5.85 (ddt, *J* = 17.2, 9.9, 7.4 Hz, 1H, CH_2=C*H*CH_2S), 5.24 – 5.17 (m, 1H, C*H*$_2$=CHCH_2S), 5.16 – 5.13 (m, 1H, C*H*$_2$=CHS), 3.80 (s, 3H, OC*H*$_3$), 3.74 (s, 2H, SC*H*$_2$C$_{Ar}$), 3.35 (d, *J* = 7.3 Hz, 2H, CH_2=CHC*H*$_2$S), 2.64 (qd, *J* = 13.2, 7.3 Hz, 2H, S*CH*$_2$C$_{Cpr}$), 1.74 – 1.68 (m, 1H Cpr-C*H*$_2$), 1.33 – 1.25 (m, 1H, Cpr-C*H*), 0.88 – 0.79 (m, 2H, Cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.7 (*C*$_{Ar}$OCH$_3$), 133.5 (*CH*$_2$=*CHCH*$_2$), 130.8 (SCH$_2$*C*$_{Ar}$), 130.1 (*C*$_{Ar}$), 118.6 (*C*H$_2$CHCH$_2$), 114.0 (*C*$_{Ar}$), 55.4 (O*C*H$_3$), 43.4 (S*C*H$_2$C$_{Cpr}$), 42.6 (S*C*H$_2$C$_{Ar}$), 37.6 (*C*H$_2$SCH$_2$), 22.8 (*C*$_{Cpr}$), 20.6 (*C*$_{Cpr}$), 15.8 (*C*$_{Cpr}$) ppm. HRMS (CI+) $[M+H]^+$ calc. 281.1028, found 281.1035 $[C_{15}H_{21}OS_2]^+$. IR (neat): 1512, 1246, 1034, 918, 833, 748, 548 cm^{-1}.

_S_-(2-((Allylthio)methyl)cyclopropyl) ethanethioate (128):

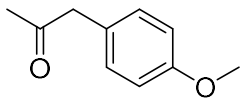


To trifluoroacetic anhydride (2.6 mL, 18.4 mmol) was added acetic acid (1 mL, 17.5 mmol) under argon atmosphere. The solution was stirred for 10 minutes. The trifluoroacetic anhydride/acetic acid solution (0.5 mL) was added to a solution of **120** (0.272 g, 0.97 mmol) in dry dichloromethane (0.5 mL) dropwise at – 78 °C. After 1 hour, trifluoroacetic anhydride/acetic acid solution (0.5 mL) was added. The reaction was allowed to warm and stirred until thin layer chromatography indicated consumption of starting material. The reaction was quenched by the dropwise addition of sodium bicarbonate (saturated solution) until gas evolution ceased. The

mixture was extracted with dichloromethane (2 x 10 mL), washed with water (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography using Biotage Isolera (gradient: 100% petroleum ether for 3 column volumes (CV), then increased to 40% diethyl ether over 20 CV, then increased to 100% diethyl ether over 10 CV) to afford compound **128** as a colourless oil (0.104 g, 29% yield) and side product **130** as a colourless oil in 31 mg, 18% yield in agreement with literature data.[99]

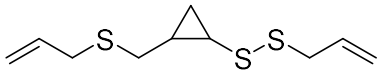
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.89 – 5.72 (m, 1H, CH_2=C*H*CH_2S), 5.14 – 5.04 (m, 2H, C*H*$_2$=CHCH_2S), 3.17 (d, *J* = 7.0 Hz, 2H, CH_2=CHC*H*$_2$S), 2.54 (ddd, *J* = 21.1, 13.5, 6.8 Hz, 2H, SC*H*$_2$C$_{Cpr}$), 2.28 (s, 3H, C*H*$_3$), 2.00 (dt, *J* = 8.4, 4.4 Hz, 1H, Cpr-C*H*), 1.22 – 1.13 (m, 1H, Cpr-C*H*), 0.99 (tt, *J* = 12.4, 6.1 Hz, 1H, Cpr-C*H*), 0.83 (dt, *J* = 10.1, 5.2 Hz, 1H, Cpr-C*H*) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.5 (*C*=O), 134.6 (CH_2=*C*HCH_2), 117.4 (*C*H_2=CHCH_2), 35.1 (S*C*H_2C$_{Cpr}$), 34.3 (CH_2=CH*C*H_2S), 30.5 (*C*H_3), 21.6 (*C*$_{Cpr}$), 17.5 (*C*$_{Cpr}$), 15.0 (*C*$_{Cpr}$) ppm. HRMS (CI+) $[M+H]^+$ calc. 203.0559, found 203.0556 $[C_9H_{15}OS_2]^+$. IR (neat): 1697, 957, 918, 625, 417 cm^{-1}.

4-Methoxybenzyl acetate (130):



^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (t, *J* = 5.8 Hz, 2H, Ar*H*), 6.88 – 6.80 (m, 2H, Ar*H*), 4.08 (s, 2H, OC*H*$_2$), 3.78 (s, 3H, OC*H*$_3$), 2.34 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (*C*=O), 158.9 (*C*$_{Ar}$), 129.6 (*C*$_{Ar}$), 127.6 (*C*$_{Ar}$), 113.7 (*C*$_{Ar}$), 65.8 (S*C*H_2), 55.0 (O*C*H_3), 20.9 (CO*C*H_3) ppm.

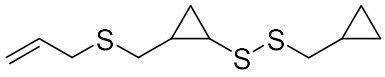
1-Allyl-2-(2-((allylthio)methyl)cyclopropyl)disulfane (127a)



The thioacetate **128** (115 mg, 0.57 mmol) was dissolved in dry methanol (200 µL). The solution was cooled to – 78 °C and stirred under argon atmosphere. Potassium hydroxide (32 mg, 0.57 mmol, 1 eq.) in dry methanol (200 µL) was added slowly and the reaction was left stirring for 30 minutes. *S*-Allyl 4-methoxybenzenesulfonothioate (156 mg, 0.68 mmol, 1.2 eq.) was dissolved in dichloromethane (200 µL) and added slowly and the reaction was allowed to warm to room temperature. Once thin layer chromatography indicated consumption of starting material, the reaction was quenched with ammonium chloride (saturated solution, 10 mL). The solvents were removed *in vacuo* and the resulting residue was extracted with dichloromethane (2 x 10 mL), washed with brine (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo.* The crude product was purified by silica gel chromatography using petroleum ether and diethyl ether 80:1, then increased to 60:1 to afford **127a** as a yellow oil (36.3 mg, 27% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (dtd, *J* = 45.5, 17.1, 7.3 Hz, 2H, CH_2=C*H*CH_2 and $SSCH_2CH$=CH_2), 5.15 (ddd, *J* = 23.0, 19.6, 11.6 Hz, 4H, C*H*$_2$=$CHCH_2$ and $SSCH_2CH$=C*H*$_2$), 3.42 (d, *J* = 7.3 Hz, 2H, SSC*H*$_2$), 3.21 (d, *J* = 7.1 Hz, 2H, C*H*$_2$S), 2.45 (qd, *J* = 13.3, 7.0 Hz, 2H, SC*H*$_2$Cpr), 2.13 (dt, *J* = 7.9, 4.0 Hz, 1H, Cpr-C*H*), 1.39 – 1.31 (m, 1H, Cpr-C*H*), 1.01 – 0.95 (m, 1H, Cpr-C*H*), 0.92 – 0.86 (m, 1H, Cpr-C*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.5 (CH_2=*C*HCH_2), 133.9 ($SSCH_2$*C*H=CH_2), 118.6 (*C*H_2=$CHCH_2$), 117.3 ($SSCH_2CH$=*C*H_2), 41.9 (SS*C*H_2), 34.8 (S*C*H_2Cpr), 34.2 (*C*H_2SCH_2), 26.1 (C_{Cpr}), 24.0 (C_{Cpr}), 17.2 (C_{Cpr}) ppm. HRMS (CI+) $[M+H]^+$ calc. 233.0487 found 233.0485 $[C_{10}H_{17}S_3]^+$. IR (neat): 3001, 2908, 2361, 1230, 964, 914, 790, 671, 582 cm^{-1}.

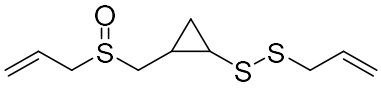
1-(2-((Allylthio)methyl)cyclopropyl)-2-(cyclopropylmethyl)disulfane (127b)



The thioacetate **128** (105 mg, 0.519 mmol) was dissolved in dry methanol (200 µL). The solution was cooled to – 78 °C and stirred under argon atmosphere. Potassium hydroxide (29 mg, 0.519 mmol, 1 eq.) in dry methanol (200 µL) was added slowly and the reaction was left stirring for 30 minutes. *S*-(Cyclopropylmethyl) 4-methylbenzenesulfonothioate (150 mg, 0.62 mmol, 1.2 eq.) was dissolved in dichloromethane (200 µL) and added slowly and the reaction was allowed to warm to room temperature. Once thin layer chromatography indicated consumption of starting material, the reaction was quenched with ammonium chloride (saturated solution, 10 mL). The solvents were removed *in vacuo* and the resulting residue was extracted with dichloromethane (2 x 10 mL), washed with brine (2 x 10 mL), dried over magnesium sulfate and concentrated *in vacuo.* The crude product was purified by silica gel chromatography using petroleum ether and diethyl ether 80:1, then increased to 60:1 to afford **127b** as a colourless oil (49.5 mg, 39% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (dd, *J* = 17.0, 9.9 Hz, 1H, CH_2=C*H*CH_2), 5.11 (dd, *J* = 12.0, 11.3 Hz, 2H, C*H*$_2$=CHCH_2), 3.21 (d, *J* = 7.1 Hz, 2H, CH_2=CHC*H*$_2$S), 2.75 (d, *J* = 7.2 Hz, 2H, SC*H*$_2$), 2.46 (qd, *J* = 13.4, 7.0 Hz, 2H, SSCH_2), 2.14 (dt, *J* = 8.1, 4.1 Hz, 1H, Cpr-C*H*), 1.36 (dd, *J* = 5.9, 2.9 Hz, 1H, Cpr-C*H*), 1.15 – 1.07 (m, 1H, Cpr-C*H*), 1.03 – 0.96 (m, 1H, Cpr-C*H*$_2$), 0.90 (dd, *J* = 13.4, 5.7 Hz, 1H Cpr-C*H*$_2$), 0.60 (q, *J* = 4.9 Hz, 2H, Cpr-C*H*$_2$), 0.29 (q, *J* = 4.9 Hz, 2H, Cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.5 (CH_2=*C*H), 117.2 (*C*H_2=CH), 45.1 (SS*C*H_2), 34.8 (S*C*H_2), 34.2 (*C*H_2S), 26.2 (*C*$_{Cpr}$), 23.8 (*C*$_{Cpr}$), 17.1 (*C*$_{Cpr}$), 11.2 (*C*$_{Cpr}$), 5.8 (*C*$_{Cpr}$), 5.7 (*C*$_{Cpr}$) ppm. HRMS (CI+) $[M+NH_4]^+$ calc. 264.0909 found 264.0911 $[C_{11}H_{22}NS_3]^+$. IR (neat): 2909, 2361, 1223, 988, 914, 826, 791, 752, 594 cm^{-1}.

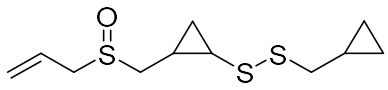
1-Allyl-2-(2-((allylsulfinyl)methyl)cyclopropyl)disulfane (102)



Sulfide (**127a**) (23 mg, 0.1 mmol) was dissolved in anhydrous dichloromethane (1 mL) and cooled to −78 °C. *m*CPBA (17.3 mg, 0.1 mmol, 1.0 equiv.) was added in one portion and the reaction was left for a further two hours until TLC indicated full consumption of starting material. Saturated sodium carbonate (5 mL) was added and the product was extracted using dichloromethane (3 x 10 mL). The combined organic layers were combined and dried over magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by preparative TLC to yield **102** (6 mg, 24%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 – 5.80 (m, 2H, CH_2=C*H*CH_2 and $SSCH_2CH$=CH_2), 5.49 – 5.37 (m, 2H, $SSCH_2CH$=CH_2), 5.22 (d, *J* = 16.9 Hz, 1H, CH_2=$CHCH_2$), 5.17 (dd, *J* = 9.9, 4.9 Hz, 1H CH_2=$CHCH_2$), 3.67 – 3.33 (m, 4H, CH_2SO and SSCH_2), 2.79 – 2.67 (m, 2H, SOCH_2), 2.35 (dt, *J* = 8.3, 4.0 Hz, 1H, Cpr-C*H*), 2.22 (dt, *J* = 8.3, 4.3 Hz, 1H, Cpr-C*H*), 1.52 – 1.36 (m, 1H, Cpr-C*H*), 1.16 – 1.06 (m, 1H, Cpr-C*H*) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.8 ($SSCH_2CH$=CH_2), 133.7 ($SSCH_2CH$=CH_2), 125.7 (CH_2=$CHCH_2$), 125.7 (CH_2=$CHCH_2$), 123.9 (CH_2=$CHCH_2$), 123.9 (CH_2=$CHCH_2$), 118.9 ($SSCH_2CH$=CH_2), 118.8 ($SSCH_2CH$=CH_2), 55.5 ($SOCH_2$), 55.1 ($SOCH_2$), 54.7 (CH_2SO), 54.5 (CH_2SO), 42.0 ($SSCH_2$), 41.9 ($SSCH_2$), 25.9 (C_{Cpr}), 25.8 (C_{Cpr}), 17.8 (C_{Cpr}), 17.6 (C_{Cpr}), 17.0 (C_{Cpr}), 16.5 (C_{Cpr}) ppm. HRMS (AP+) $[M+H]^+$ calc. 249.0442 found 249.0446 $[C_{10}H_{17}OS_3]^+$. IR (neat): 3082, 3009, 2955, 1636, 1423, 1400, 1034, 721 cm^{-1}.

1-(2-((Allylsulfinyl)methyl)cyclopropyl)-2-(cyclopropylmethyl)disulfane (103)

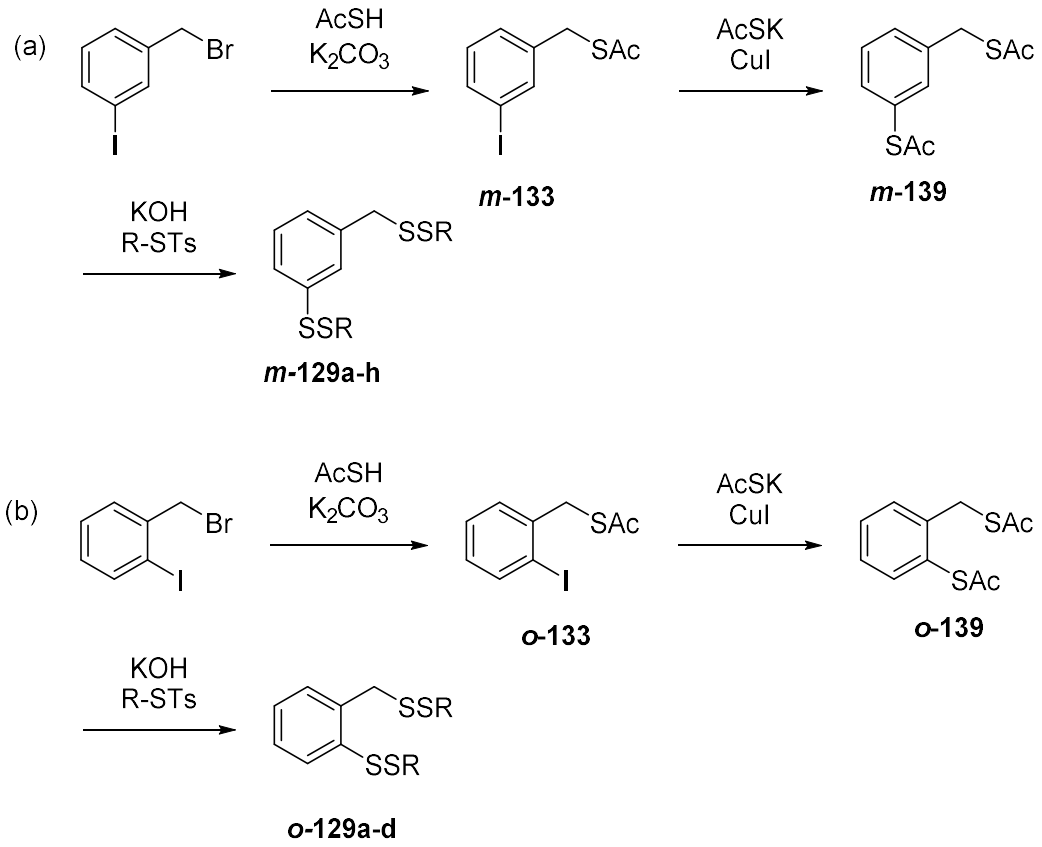


Sulfide (16b) (26 mg, 0.1 mmol) was dissolved in anhydrous dichloromethane (1 mL) and cooled to −78 °C. *m*CPBA (17.3 mg, 0.1 mmol, 1.0 equiv.) was added in one portion and the reaction was left for a further two hours until TLC indicated full consumption of starting material. Saturated sodium carbonate (5 mL) was added and the product was extracted using dichloromethane (3 x 10 mL). The combined organic layers were combined and dried over magnesium sulfate and concentrated *in vacuo*. The crude residue was purified by preparative TLC to yield **103** (5.5 mg, 21%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.98 – 5.85 (m, 1H, CH_2=C*H*), 5.48 – 5.44 (m, 1H, C*H*$_2$=CH), 5.41 (dddd, *J* = 17.1, 6.0, 2.5, 1.2 Hz, 1H, C*H*$_2$=CH), 3.69 – 3.42 (m, 2H, C*H*$_2$S=O), 2.81 – 2.68 (m, 4H, SOC*H*$_2$ and SSC*H*$_2$), 2.30 (dddd, *J* = 64.0, 8.3, 4.7, 3.8 Hz, 1H, Cpr-C*H*), 1.52 – 1.37 (m, 1H, Cpr-C*H*), 1.17 – 1.13 (m, 1H, Cpr-C*H*$_2$), 1.13 – 0.98 (m, 2H, Cpr-C*H*$_2$), 0.85 (dd, *J* = 16.5, 5.1 Hz, 1H, Cpr-C*H*$_2$), 0.63 – 0.57 (m, 2H, Cpr-C*H*$_2$), 0.33 – 0.26 (m, 2H, Cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 125.8 (CH_2=*C*H), 125.8 (CH_2=*C*H), 123.8 (*C*H_2=CH), 123.8 (*C*H_2=CH), 55.5 (SO*C*H_2), 55.2 (SO*C*H_2), 54.8 (*C*H_2SO), 54.7 (*C*H_2SO), 45.2 (SS*C*H_2), 45.2 (SS*C*H_2), 26.1 (*C*$_{Cpr}$), 26.1 (*C*$_{Cpr}$), 17.6 (*C*$_{Cpr}$), 17.4 (*C*$_{Cpr}$), 17.0 (*C*$_{Cpr}$), 16.4 (*C*$_{Cpr}$), 11.2 (*C*$_{Cpr}$), 5.8 (*C*$_{Cpr}$), 5.8 (*C*$_{Cpr}$), 5.8 (*C*$_{Cpr}$), 5.8 (*C*$_{Cpr}$) ppm. HRMS (ES) $[M+H]^+$ calc. 263.0598 found 263.0600 [$C_{11}H_{19}OS_3]^{+\cdot}$. IR (neat): 3082, 3009, 2955, 1636, 1423, 1400, 1034, 721 cm^{-1}.

8.4. Chapter 5: Synthesis of Bis-Disulfide Ajoene Analogues

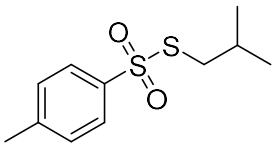
8.4.1. General Scheme



Scheme 8.3: Synthesis of bis-disulfides (a): *meta*-synthetic route; (b): *ortho*-synthetic route.

8.4.2. Experimental

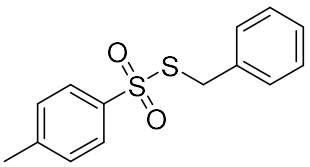
S-iso-Butyl 4-methylbenzenesulfonothioate (83c)



Synthesised by GP1 and isolated as a colourless oil in 1.7 g, 79% yield from 8.8 mmol of potassium thiotosylate.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.78 (m, 2H, Ar*H*), 7.35 – 7.31 (m, 2H, Ar*H*), 2.88 (d, *J* = 6.9 Hz, 2H, C*H*$_2$), 2.45 (s, 3H, Ar-C*H*$_3$), 1.86 (septt, *J* = 6.9, 6.7 Hz, 1H, C*H*(CH_3)$_2$), 0.92 (d, *J* = 6.7 Hz, 6H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.8 (*C*$_{Ar}$), 142.3(*C*$_{Ar}$), 123.0 (*C*$_{Ar}$), 127.2 (*C*$_{Ar}$), 44.5 (S*C*H$_2$), 28.3 (cpr-*C*H), 21.8 (*C*H$_3$), 21.8 (cpr-*C*H$_3$) ppm. HRMS (EI) $[M]^+$ calc. 244.05862 found: 244.0588 $[C_{11}H_{16}O_2S_2]^+$. IR (neat0: 2961, 1595, 1464, 1327, 1140, 1077, 812, 704, 654, 588, 521 cm^{-1}.

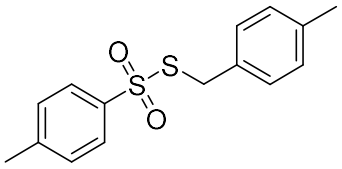
S-Benzyl 4-methylbenzenesulfonothioate (83d)



Synthesised by GP1 and isolated as a colourless oil in 3.26 g, 90% from 0.013 mmol of potassium thiotosylate in agreement with literature data.[100]

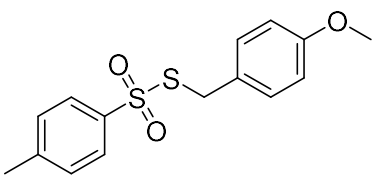
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 – 7.75 (m, 2H, Ar*H*), 7.32 – 7.29 (m, 2H, Ar*H*), 7.29 – 7.24 (m, 3H, Ar*H*), 7.24 – 7.20 (m, 2H, Ar*H*), 4.28 (s, 2H, C*H*$_2$), 2.47 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.8 (*C*$_{Ar}$), 142.2 (*C*$_{Ar}$), 133.8 (*C*$_{Ar}$), 129.9 (*C*$_{Ar}$), 129.3 (*C*$_{Ar}$), 128.9 (*C*$_{Ar}$), 128.1 (*C*$_{Ar}$), 127.2 (*C*$_{Ar}$), 40.4 (*C*H$_2$), 21.8 (*C*H$_3$) ppm.

_S_-(4-Methylbenzyl) 4-methylbenzenesulfonothioate (83e)



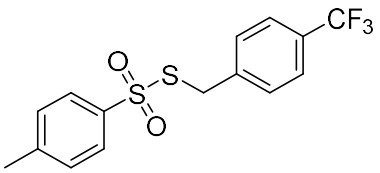
Synthesised by GP1 and isolated as a colourless oil in 1.32 g, 68% from 6.7 mmol of potassium thiotosylate in agreement with literature data.[101]
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 – 7.71 (m, 2H, Ar*H*), 7.28 (ddd, *J* = 6.7, 2.9, 0.8 Hz, 2H, Ar*H*), 7.08 – 7.02 (m, 4H, Ar*H*), 4.21 (s, 2H, SCH_2), 2.44 (s, 3H, CH_3), 2.30 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.7 (C_{Ar}), 142.2 (C_{Ar}), 138.0 (C_{Ar}), 130.6 (C_{Ar}), 129.9 (C_{Ar}), 129.6 (C_{Ar}), 129.2 (C_{Ar}), 127.2 (C_{Ar}), 40.2 (CH_2), 21.8 (CH_3), 21.3 (CH_3) ppm.

_S_-(4-Methoxybenzyl) 4-methylbenzenesulfonothioate (83f)



Synthesised by GP1 and isolated as a colourless oil in 2.8 g, 79% from 0.0116 mol of potassium thiotosylate in agreement with literature data.[100]
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.71 (m, 2H, Ar*H*), 7.28 (dt, *J* = 2.6, 1.3 Hz, 2H, Ar*H*), 7.13 – 7.07 (m, 2H, Ar*H*), 6.78 – 6.73 (m, 2H, Ar*H*), 4.21 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 2.44 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.5 (C_{Ar}), 144.7 (C_{Ar}), 142.2 (C_{Ar}), 130.5 (C_{Ar}), 129.8 (C_{Ar}), 127.1 (C_{Ar}), 125.4 (C_{Ar}), 114.3 (C_{Ar}), 55.3 (OCH_3) 40.2 (CH_2), 21.7 (CH_3) ppm.

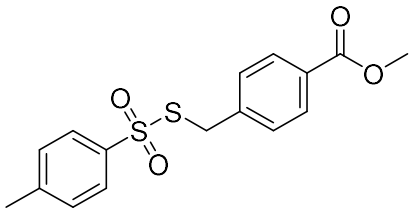
_S_-(4-(Trifluoromethyl)benzyl) 4-methylbenzenesulfonothioate (83g)



Synthesised by GP1 and Isolated as a colourless oil in 184 mg, 93% from 0.57 mmol of potassium thiotosylate.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 – 7.59 (m, 2H, Ar*H*), 7.43 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.28 (t, *J* = 6.6 Hz, 2H, Ar*H*), 7.21 – 7.17 (m, 2H, Ar*H*), 4.30 (s, 2H, CH_2), 2.40 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.0 (C_{Ar}), 142.2 (C_{Ar}), 138.4 (q, $^{4}J_{C,F}$ = 1.2 Hz, C_{Ar}), 130.2 (q, $^{2}J_{C,F}$ = 32.6 Hz, C_{Ar}), 129.8 (C_{Ar}), 129.5 (C_{Ar}), 127.1 (C_{Ar}), 125.7 (q, $^{3}J_{C,F}$ = 3.8 Hz, C_{Ar}), 124.0 (q, $^{1}J_{C,F}$ = 272.1 H, CF_3), 39.8 (CH_2), 21.7 (CH_3) ppm. ^{19}F NMR (471 MHz, $CDCl_3$) δ -62.69 (CF_3) ppm. HRMS (EI) $[M]^+$ calc. 346.0309 found: 346.0306 $[C_{15}H_{13}F_3O_2S_3]^+$. IR (neat): 2918, 1593, 1492, 1315, 1134, 1107,1067, 1016, 814, 694, 652, 582 cm^{-1}.

Methyl 4-((tosylthio)methyl)benzoate (83g)

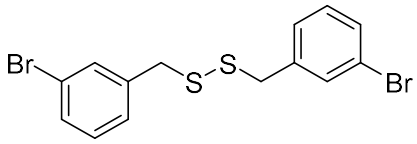


Synthesised by GP1 and isolated as a colourless oil in 1.67g, 96% from 4.9 mmol of potassium thiotosylate.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.85 (m, 2H, Ar*H*), 7.70 – 7.66 (m, 2H, Ar*H*), 7.26 – 7.21 (m, 4H, Ar*H*), 4.28 (s, 2H, SCH_2), 3.90 (s, 3H, CO_2CH_3), 2.41 (s, 3H, ArCH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (*C*=O), 145.0 (C_{Ar}), 142.1 (C_{Ar}), 139.3 (C_{Ar}), 130.1, (C_{Ar}) 129.9 (C_{Ar}), 129.8 (C_{Ar}), 129.2 (C_{Ar}), 127.1 (C_{Ar}), 52.3 (CO_2CH_3), 40.0 (SCH_2), 21.7 ($ArCH_3$) ppm. HRMS (ES) $[M+H]^+$ calc. 337.0568

found: 337.0553 $[C_{16}H_{17}O_4S_2]^+$. IR (neat): 2951, 1717, 1611, 1435, 1277, 1140, 1077, 812, 702, 652, 584, 519 cm^{-1}.

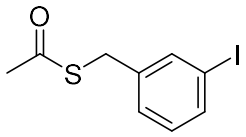
1,2-Bis(3-bromobenzyl)disulfane (132)



3-Bromobenzyl bromide (550 mg, 2.2 mmol) was dissolved in ethanol (6 mL, 0.37 M). Thiourea (502 mg, 6.6 mmol, 3 equiv.) was added and the reaction was stirred at reflux for 18 hours. The reaction mixture was cooled down to room temperature and filtered. The salt was then dissolved into distilled water (6 mL, 0.37 M) and potassium hydroxide (359 mg, 5.5 mmol, 2.5 equiv.) was added. The mixture was stirred at reflux for 18 h. After which, the title crude residue was extracted into diethyl ether (3 x 30 mL), and washed with brine (20 mL), and water (20 mL). The organic layers were dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography using Biotage Isolera (gradient: 100% petroleum ether for 3 column volumes (CV), then increased to 10% diethyl ether over 20 CV, then increased to 30% diethyl ether over 10 CV) to afford compound **132** as a colourless oil (917 mg, 97% yield) in agreement with literature data.[102]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.37 (m, 2H, Ar*H*), 7.24 – 7.14 (m, 2H, Ar*H*), 3.54 (s, 2H, CH_2) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.7 (C_{Ar}), 132.5 (C_{Ar}), 130.8 (C_{Ar}), 130.3 (C_{Ar}), 128.2 (C_{Ar}), 122.6 (C_{Ar}), 42.7 (CH_2) ppm.

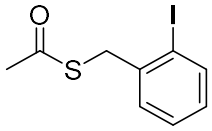
_S_-(3-Iodobenzyl) ethanethioate (_m_-133)



Thioacetic acid (238 µL, 3.37 mmol, 1.0 equiv.) was added to 3-iodo benzyl bromide (1 g, 3.37 mmol, 1.0 equiv.) and potassium carbonate (466 mg, 3.37, 1.0 equiv.) in dissolved in anhydrous THF (20 mL) under argon atmosphere. After four hours, the reaction mixture was filtered and the solid was washed with dichloromethane. The filtrate was concentrated *in vacuo* to yield ***m*-133** (935 mg, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.63 (m, 1H, Ar*H*), 7.60 – 7.55 (m, 1H, Ar*H*), 7.27 – 7.23 (m, 1H, Ar*H*), 7.03 (t, *J* = 7.8 Hz, 1H, Ar*H*), 4.04 (s, 2H, CH_2), 2.36 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.7 (*C*=O), 140.1 (C_{Ar}), 137.7 (C_{Ar}), 136.4 (C_{Ar}), 130.3 (C_{Ar}), 128.1 (C_{Ar}), 94.4 (C_{Ar}), 32.7 (CH_2), 30.4 (CH_3) ppm. HRMS (EI) $[M]^+$ calc. 291.9419 found 291.9421 $[C_9H_9OSI]^+$. IR (neat): 2922, 1686, 1128, 955, 623 cm^{-1}.

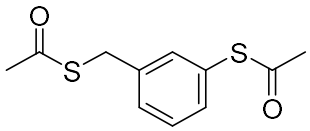
_S_-(2-Iodobenzyl) ethanethioate (_o_-133)



Thioacetic acid (238 µL, 3.37 mmol, 1.0 equiv.) was added to 2-iodo benzyl bromide (1 g, 3.37 mmol, 1.0 equiv.) and potassium carbonate (466 mg, 3.37, 1.0 equiv.) in dissolved in anhydrous THF (20 mL) under argon atmosphere. After four hours the reaction mixture was filtered and the solid was washed with dichloromethane. The filtrate was concentrated *in vacuo* to yield ***o*-133** (935 mg, 95% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 7.9, 1.2 Hz, 1H, $C_{Ar}H$), 7.46 – 7.43 (m, 1H, $C_{Ar}H$), 7.28 (td, *J* = 7.5, 1.3 Hz, 1H, $C_{Ar}H$), 6.93 (ddd, *J* = 7.6, 5.9, 1.7 Hz, 1H, $C_{Ar}H$), 4.23 (s, 2H, CH_2), 2.34 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.9 (*C*=O), 140.6 (C_{Ar}), 139.7 (C_{Ar}), 130.7 (C_{Ar}), 129.2 (C_{Ar}), 128.7 (C_{Ar}), 100.6 (C_{Ar}), 38.9 (CH_2), 30.5 (CH_3) ppm. HRMS (EI) $[M]^+$ calc. 291.9419 found 291.9421 $[C_9H_9OSI]^+$. IR (neat): 1686, 1350, 1130, 729, 621 cm^{-1}.

_S_-(3-(Acetylthio)benzyl) ethanethioate (*m*-139)

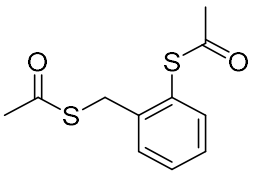


***m*-133** (1 g, 3.37 mmol), potassium thioacetate (5.1 mmol, 1.5 equiv.), copper(I) iodide (0.67 mmol, 0.1 equiv.), 1,10-phenanthroline (0.67 mmol, 0,1 equiv.) was dissolved in dry toluene (27 mL) under argon atmosphere. The reaction mixture was stirred at 100 °C for 24 h. The reaction mixture was cooled to room temperature and filtered through a short pad of silica. The solid phase was washed with diethyl ether (5 x 20 mL). The combined organic phases were concentrated *in vacuo*. The crude residue was dissolved in diethyl ether (25 mL) and washed with water (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 80:20 (cyclohexane:diethyl ether) over 10 CV, and then to 50% diethyl ether over 20 CV, then 100% diethyl ether over 5 CV) to afford compound ***m*-139** (801 mg, 99% yield) as brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.27 (m, 4H, Ar*H*), 4.11 (s, 2H, CH_2), 2.42 (s, 3H, $ArSCOCH_3$), 2.35 (s, 3H, CH_2SCOCH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.0 (ArS*C*=O), 194.0 (CH_2SC=O), 139.1 (C_{Ar}), 134.8 (C_{Ar}), 133.5 (C_{Ar}), 130.1 (C_{Ar}), 129.5 (C_{Ar}), 128.4 (C_{Ar}), 33.1 (CH_2), 30.5 ($ArSCOCH_3$), 30.4 (CH_2SCOCH_3)

ppm. HRMS (EI) $[M]^+$ calc. 240.0279 found 240.0276 $[C_{11}H_{12}O_2S_2]^+$. IR (neat): 1689, 1130, 1118, 952, 617 cm^{-1}

_S_-(2-(Acetylthio)benzyl) ethanethioate (_o_-139)



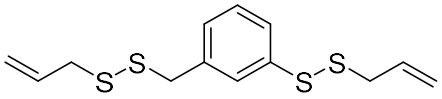
Thioacetate ***o*-133** (1 g, 3.37 mmol), potassium thioacetate (5.1 mmol, 1.5 equiv.), copper(I) iodide (0.67 mmol, 0.1 equiv.), 1,10-phenanthroline (0.67 mmol, 0,1 equiv.) was dissolved in dry toluene (27 mL) under argon atmosphere. The reaction mixture was refluxed at 120 °C for 24 h. The reaction mixture was cooled to room temperature and filtered through a short pad of silica. The solid phase was washed with diethyl ether (5 x 20 mL). The combined organic phases were concentrated *in vacuo*. The crude residue was dissolved in diethyl ether (25 mL) and washed with water (2 x 25 mL) and brine (2 x 25 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 80:20 (cyclohexane:diethyl ether) over 10 CV, and then to 50% diethyl ether over 20 CV, then 100% diethyl ether over 5 CV) to afford compound ***o*-139** (801 mg, 99% yield) as brown oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (ddd, *J* = 7.6, 1.5, 0.4 Hz, 1H, Ar*H*), 7.41 (dd, *J* = 7.6, 1.3 Hz, 1H, Ar*H*), 7.38 (td, *J* = 7.6, 1.5 Hz, 1H, Ar*H*), 7.30 (td, *J* = 7.5, 1.5 Hz, 1H, Ar*H*), 4.19 (s, 2H, CH_2), 2.45 (s, 3H, ArSCOC*H*$_3$), 2.32 (s, *J* = 3.3 Hz, 3H, CH_2SCOC*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.0 (ArS*C*=O), 193.4 (CH_2S*C*=O), 141.2 (*C*$_{Ar}$), 136.8 (*C*$_{Ar}$), 131.0 (*C*$_{Ar}$), 130.7 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 127.6 (*C*$_{Ar}$), 32.3 (*C*H_2), 30.5 (ArSCO*C*H_3), 30.4 (CH_2SCO*C*H_3) ppm. HRMS (EI) $[M+Na]^+$ calc. 263.0176 found 263.0177 $[C_{11}H_{12}O_2NaS_2]^+$. IR (neat): 1690, 1354, 1107, 953, 768, 613 cm^{-1}.

General Procedure (GP3): Disulfide formation to yield *m-/o*-129

Thioacetate (***m-/o*-139**) was dissolved in methanol (0.1 M) and cooled to 0 °C. Potassium hydroxide (3 equiv.) was dissolved in methanol (0.1 M) and added to the thioacetate. Thiotosylate reagent (**83a-h**, 4 equiv.) in dichloromethane (0.1 M) was added and the reaction was kept at 0 °C for 2 hours and then warmed to room temperature. Once TLC indicated full consumption of starting materials, the reaction was quenched with a saturated solution of ammonium chloride. The reaction mixture was concentrated *in vacuo* to remove methanol and dichloromethane. The resulting residue was extracted into diethyl ether (3 x 50 mL) and washed with brine (20 mL) and water (20 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 90:10 (cyclohexane:diethyl ether) over 10 CV, and then to 100% diethyl ether over 2CV, then 100% diethyl ether over 5 CV) to afford desired product.

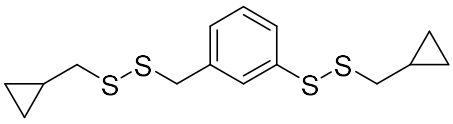
1-Allyl-2-(3-(allyldisulfaneyl)benzyl)disulfane (*m*-129a)



Synthesised by GP3 and isolated as a pale yellow oil in 45 mg, 69% yield from 0.2 mmol ***m*-139**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.49 (m, 1H, Ar*H*), 7.46 – 7.42 (m, 1H, Ar*H*), 7.29 (d, *J* = 7.7 Hz, 1H, Ar*H*), 7.20 – 7.16 (m, 1H, Ar*H*), 5.88 – 5.69 (m, 2H, CH_2=C*H*CH_2SS and SSCH_2C*H*=CH_2), 5.20 – 5.03 (m, 4H, C*H*$_2$=CHCH_2SS and SSCH_2CH=C*H*$_2$), 3.88 (s, 2H, SSC*H*$_2$Ph), 3.37 (d, *J* = 7.4 Hz, 2H, PhSSC*H*$_2$), 3.05 (d, *J* = 7.4 Hz, 2H, C*H*$_2$SSCH_2) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6 (*C*$_{Ar}$), 137.7 (*C*$_{Ar}$), 133.3 (CH_2=*C*H), 132.6 (C*H*=CH_2), 129.2 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 128.0 (*C*$_{Ar}$), 126.8 (*C*$_{Ar}$), 119.3 (CH=*C*H_2), 118.8 (*C*H_2=CH), 43.4 (SS*C*H_2Ph), 42.0 (SS*C*H_2), 41.9 (*C*H_2SS) ppm. HRMS (EI) $[M]^+$ calc. 300.0135 found 300.0134 $[C_{13}H_{16}S_4]^+$. IR (neat): 2980, 2361, 1421, 985, 918, 787, 698 cm $^{-1}$.

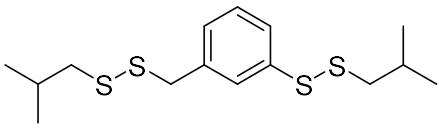
1-(Cyclopropylmethyl)-2-(3-((cyclopropylmethyl)disulfaneyl)benzyl)disulfane (*m*-129b)



Synthesised by GP3 and isolated as a pale yellow oil in 50 mg, 61% yield from 0.25 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.52 (m, 1H, Ar*H*), 7.46 – 7.44 (m, 1H, Ar*H*), 7.27 – 7.25 (m, 1H, Ar*H*), 7.18 – 7.15 (m, 1H, Ar*H*), 3.90 (s, 2H, SSC*H*$_2$Ph), 2.71 (d, *J* = 7.2 Hz, 2H, SSC*H*$_2$CH), 2.40 (d, *J* = 7.2 Hz, 2H, CHC*H*$_2$SS), 1.11 – 0.92 (m, 2H, Cpr-C*H*), 0.59 – 0.51 (m, 4H, Cpr-C*H*$_2$), 0.28 – 0.17 (m, 4H, Cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.7 (*C*$_{Ar}$), 138.3 (*C*$_{Ar}$), 129.1 (*C*$_{Ar}$), 127.8 (*C*$_{Ar}$), 127.7 (*C*$_{Ar}$), 126.1 (*C*$_{Ar}$), 45.3 (SS*C*H$_2$Ph, 45.2 (SS*C*H$_2$), 43.8 (*C*H$_2$SS), 31.1 (Cpr-*C*H), 29.9 (Cpr-*C*H), 11.0 (Cpr-*C*H), 10.9 (Cpr-*C*H$_2$), 5.9 (Cpr-*C*H$_2$), 5.7 (Cpr-*C*H$_2$) ppm. HRMS (EC) $[M]^+$ calc. 328.0448 found 328.0450 $[C_{15}H_{20}S_4]^+$. IR (neat): 3003, 2359, 1425, 789, 698 cm^{-1}.

1-*iso*-Butyl-2-(3-(isobutyldisulfaneyl)benzyl)disulfane (*m*-129c)

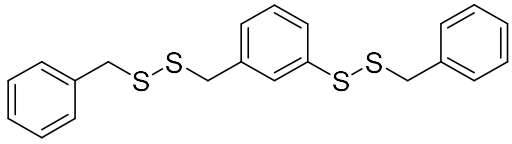


Synthesised by GP3 and isolated as a pale yellow oil in 58 mg, 81% yield from 0.22 mmol of ***m*-139*.***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (t, *J* = 1.6 Hz, 1H, Ar*H*), 7.45 – 7.41 (m, 1H, Ar*H*), 7.26 (d, *J* = 7.7 Hz, 1H, Ar*H*), 7.19 – 7.15 (m, 1H, Ar*H*), 3.87 (s, 2H, SSC*H*$_2$Ph), 2.64 (d, *J* = 6.9 Hz, 2H, SSC*H*$_2$CH), 2.31 (d, *J* = 6.8 Hz, 2H, CHC*H*$_2$SS), 2.00 – 1.76 (m, 2H, C*H*(CH$_3$)$_2$), 0.99 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$), 0.91 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.8 (*C*$_{Ar}$), 138.0 (*C*$_{Ar}$), 129.2 (*C*$_{Ar}$), 128.0 (*C*$_{Ar}$), 127.7 (*C*$_{Ar}$), 126.3 (*C*$_{Ar}$), 48.5 (SS*C*H$_2$Ph), 48.3 (SS*C*H$_2$), 43.5 (*C*H$_2$SS), 28.2 (*C*H(CH$_3$)$_2$), 28.2 (*C*H(CH$_3$)$_2$), 21.9 ((CH$_3$)$_2$), 21.2 ((CH$_3$)$_2$) ppm.

HRMS (AP) $[M+H]^+$ calc. 333.0839 found 333.0833 $[C_{15}H_{25}S_4]^+$. IR (neat): 3003, 2359, 1425, 789, 698 cm^{-1}.

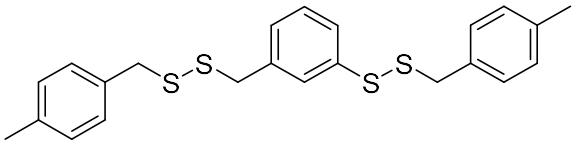
1-Benzyl-2-(3-(benzyldisulfaneyl)benzyl)disulfane (*m*-129d)



Synthesised by GP3 and isolated as a pale yellow oil in 92 mg, 21% yield from 0.23 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.19 (m, 14H, Ar*H*), 3.93 (s, 2H, SSC*H*$_2$), 3.63 (s, 2H, SSC*H*$_2$), 3.51 (s, 2H, C*H*$_2$SS) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5 (*C*Ar), 137.5 (*C*Ar), 137.4 (*C*Ar), 136.7 (*C*Ar), 129.6 (*C*Ar), 129.5 (*C*Ar), 129.1 (*C*Ar), 128.7 (*C*Ar), 128.7 (*C*Ar), 128.5 (*C*Ar), 128.0 (*C*Ar), 127.7 (*C*Ar), 127.7 (*C*Ar), 126.6 (*C*Ar), 43.6 (SS*C*H$_2$Ph), 43.5 (SS*C*H$_2$), 43.0 (*C*H$_2$SS) ppm. HRMS (AP) $[M+H]^+$ calc. 401.0526 found 401.0524 $[C_{21}H_{21}S_4]^+$. IR (neat): 3028, 2359, 2342, 1493, 1452, 696 cm^{-1}.

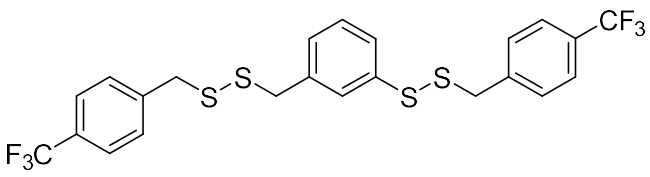
1-(4-Methylbenzyl)-2-(3-((4-methylbenzyl)disulfaneyl)benzyl)disulfane (*m*-129e)



Synthesised using GP3 and isolated as a pale yellow in 449 mg, 70% yield from 4.5 mmol of ***m*-129**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 – 7.19 (m, 12H, Ar*H*), 4.04 (s, 2H, SSC*H*$_2$), 3.74 (s, 2H, SSC*H*$_2$), 3.69 (s, 2H, SSC*H*$_2$), 2.46 (s, 3H, C*H*$_3$), 2.44 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.7 (*C*Ar), 178.7 (*C*Ar), 178.6 (*C*Ar), 178.5 (*C*Ar), 175.3 (*C*Ar), 174.7 (*C*Ar), 170.6 (*C*Ar), 170.6 (*C*Ar), 170.5 (*C*Ar), 170.5 (*C*Ar), 170.2 (*C*Ar), 169.6 (*C*Ar), 169.0 (*C*Ar), 167.7 (*C*Ar), 84.5 (SS*C*H$_2$), 84.3 (SS*C*H$_2$), 84.2 (*C*H$_2$SS), 62.4 (*C*H$_3$), 62.4 (*C*H$_3$) ppm. HRMS (EI) $[M]^+$ calc. 428.0761 found 428.0764 $[C_{23}H_{24}S_4]^+$. IR (neat): 3020, 2918, 1514, 1418, 818, 789, 698 cm^{-1}.

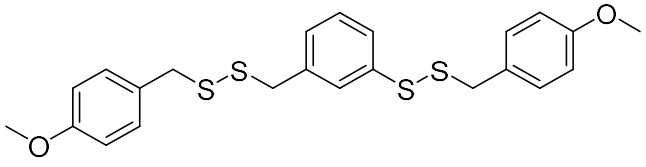
1-(4-(Trifluoromethyl)benzyl)-2-(3-((4-(trifluoromethyl)benzyl)disulfaneyl)benzyl) disulfane (*m*-129f)



Synthesised by GP3 and isolated as a pale yellow oil in 624 mg, 30% yield from 3.85 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.41 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.27 (dd, *J* = 8.0, 4.2 Hz, 2H, Ar*H*), 7.24 – 7.20 (m, 2H, Ar*H*), 7.16 – 7.11 (m, 2H, Ar*H*), 7.02 – 6.97 (m, 2H, Ar*H*), 3.88 (s, 2H, SSCH_2), 3.55 (s, 2H, SSCH_2), 3.52 (s, 2H, CH_2SS) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) 141.5 (q, $^{4}J_{C,F}$ = 1.2 Hz, C_{Ar}), 140.1 (q, $^{4}J_{C,F}$ = 1.2 Hz, C_{Ar}), 138.3 (C_{Ar}), 137.0 (C_{Ar}), 129.8 (poorly resolved, 2 x C_{Ar}), 129.6 (q, $^{2}J_{C,F}$ = 32.4 Hz, C_{Ar}), 129.5 (q, $^{2}J_{C,F}$ = 32.4 Hz, C_{Ar}), 129.1 (C_{Ar}), 128.4 (C_{Ar}), 128.0 (C_{Ar}), 126.7 (C_{Ar}), 125.5 (q, $^{3}J_{C,F}$ = 3.8 Hz, C_{Ar}), 125.4 (q, $^{3}J_{C,F}$ = 3.8 Hz, Ar*C*CF_3), 124.2 (q, $^{1}J_{C,F}$ = 272.1 Hz, *C*F_3), 124.1 (q, $^{1}J_{C,F}$ = 272.1 Hz, *C*F_3), 42.8 (SS*C*H_2), 42.8 (SS*C*H_2), 42.4 (*C*H_2SS) ppm. ^{19}F NMR (471 MHz, $CDCl_3$) δ -62.21 (C*F*$_3$), -62.31 (C*F*$_3$) ppm. HRMS (AP) $[M]^+$ calc. 536.0196 found 536.0198 $[C_{23}H_{18}F_6S_4]^+$. IR (neat): 2926, 1616, 1417, 1321, 1120, 1066, 844, 619 cm^{-1}.

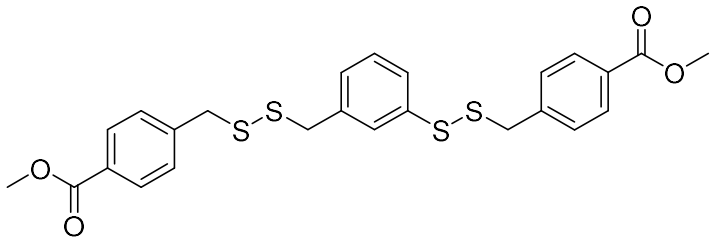
1-(4-Methoxybenzyl)-2-(3-((4-methoxybenzyl)disulfaneyl)benzyl)disulfane (*m*-129g)



Synthesised by GP3 and isolated as a pale yellow oil in 142 mg, 15% yield from 2.1 mmol of ***m*-129**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.17 (m, 7H, Ar*H*), 6.92 – 6.80 (m, 5H, Ar*H*), 3.94 (s, 2H, SSC*H*$_2$), 3.81 (s, 3H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.62 (s, 2H, SSC*H*$_2$), 3.59 (s, 2H, C*H*$_2$SS) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1 (*C*$_{Ar}$), 159.1 (*C*$_{Ar}$), 138.5 (*C*$_{Ar}$), 137.5 (*C*$_{Ar}$), 130.7 (*C*$_{Ar}$), 129.2 (*C*$_{Ar}$), 129.0 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 128.4 (*C*$_{Ar}$), 127.8 (*C*$_{Ar}$), 126.5 (*C*$_{Ar}$), 114.0 (*C*$_{Ar}$), 114.0 (*C*$_{Ar}$), 113.9 (*C*$_{Ar}$), 55.3 (O*C*H$_3$), 55.3 (O*C*H$_3$), 43.0 (SS*C*H$_2$), 43.0 (SS*C*H$_2$), 42.8 (*C*H$_2$SS) ppm. HRMS (ES) [M+Na]$^+$ calc. 483.0557 found 483.0561 [$C_{23}H_{24}O_2NaS_4$]$^+$. IR (neat): 2953, 2833, 1609, 1508, 1246, 1172, 1032, 829, 700, 546 cm^{-1}.

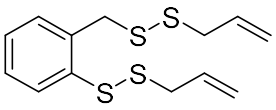
Methyl 4-(((3-((4-(methoxycarbonyl)benzyl)disulfaneyl)benzyl)disulfaneyl)methyl)benzoate (*m*-1h)



Synthesised by GP3 and isolated as a pale yellow oil in 16 mg, 1% yield from 3.6 mmol of ***m*-139**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 – 7.97 (m, 2H, Ar*H*), 7.93 – 7.89 (m, 1H, Ar*H*), 7.30 (dd, *J* = 11.3, 5.0 Hz, 6H, Ar*H*), 7.23 (dd, *J* = 3.2, 1.6 Hz, 2H, Ar*H*), 7.05 – 7.01 (m, 1H, Ar*H*), 3.95 (s, 2H, SSC*H*$_2$Ph), 3.90 (s, 3H, C*H*$_3$), 3.90 (s, 3H, C*H*$_3$), 3.62 (s, 2H, SSC*H*$_2$), 3.51 (s, 2H, C*H*$_2$SS) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9 (*C*=O), 166.8 (*C*=O), 142.7 (*C*$_{Ar}$), 141.9 (*C*$_{Ar}$), 138.4 (*C*$_{Ar}$), 137.1 (*C*$_{Ar}$), 129.9 (*C*$_{Ar}$), 129.9 (*C*$_{Ar}$), 129.6 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 129.2 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 128.1 (*C*$_{Ar}$), 126.9 (*C*$_{Ar}$), 52.3 (O*C*H$_3$), 52.3 (O*C*H$_3$), 43.1 (SS*C*H$_2$Ph), 42.9 (SS*C*H$_2$), 42.9 (*C*H$_2$SS) ppm. HRMS (ES) [M+H]$^+$ calc. 517.0636 found: 517.0634 [$C_{25}H_{25}O_4S_4$]$^+$. IR (neat): 2949, 1718, 1435, 1279, 1111, 773, 708 cm^{-1}.

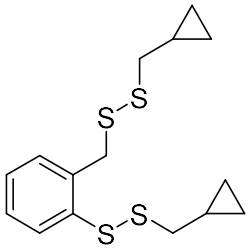
1-Allyl-2-(2-(allyldisulfaneyl)benzyl)disulfane (*o*-129a)



Synthesised by GP3 and isolated as a pale yellow oil in 299 mg, 48% yield from 2.1 mmol of ***o*-139.**

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 7.9, 1.1 Hz, 1H, Ar*H*), 7.30 (tdd, *J* = 6.0, 5.2, 1.2 Hz, 2H, Ar*H*), 7.25 – 7.19 (m, 1H, Ar*H*), 5.93 – 5.66 (m, 2H, C*H*=CH_2), 5.27 – 5.00 (m, 4H, CH=CH_2), 4.10 (s, 2H, ArCH_2SS), 3.38 – 3.35 (m, 2H, SSCH_2), 3.05 – 3.02 (m, 2H, SSCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.2 (C_{Ar}), 137.0 (C_{Ar}), 133.3 (C_{Ar}), 132.9 (C_{Ar}), 130.9 (C*H*=CH_2), 130.2 (C*H*=CH_2), 128.8 (C_{Ar}), 127.3 (C_{Ar}), 119.3 (CH=*C*H_2), 118.9 (CH=*C*H_2), 42.1 (SS*C*H_2), 41.2 (SS*C*H_2), 41.7 (Ar*C*H_2SS) ppm. HRMS (EI) $[M]^+$ calc. 300.0135 found 300.0134 $[C_{13}H_{16}S_4]^+$. IR (neat): 2980, 2361, 1421, 985, 918, 787, 698 cm $^{-1}$.

1-(Cyclopropylmethyl)-2-(2-((cyclopropylmethyl)disulfaneyl)benzyl)disulfane (*o*-129b)

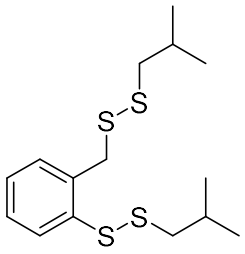


Synthesised by GP3 and isolated as a pale yellow oil in 371 mg, 55% yield from 2.1 mmol of ***o*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 – 7.83 (m, 1H, Ar*H*), 7.33 – 7.26 (m, 2H, Ar*H*), 7.18 (td, *J* = 7.4, 1.3 Hz, 1H, Ar*H*), 4.10 (s, 2H, PhCH_2), 2.71 (d, *J* = 7.2 Hz, 2H, SSCH_2), 2.39 (d, *J* = 7.3 Hz, 2H, SSCH_2), 1.10 – 0.94 (m, 2H, cpr-C*H*), 0.57 – 0.52 (m, 4H, cpr-CH_2), 0.27 – 0.21 (m, 2H, cpr-CH_2), 0.21 – 0.16 (m, 2H, cpr-CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.4 (C_{Ar}), 136.4 (C_{Ar}), 130.8 (C_{Ar}), 129.1 (C_{Ar}), 128.4 (C_{Ar}), 126.8 (C_{Ar}), 45.2 (*C*H_2), 45.2 (*C*H_2), 41.8 (*C*H_2), 10.9 (cpr-*C*H), 10.8

(cpr-*C*H), 5.8 (cpr-*C*H_2), 5.7 (cpr-*C*H_2) ppm. HRMS (EI) $[M]^+$ calc. 328.04424 found 328.0428 $[C_{15}H_{20}S_4]^+$. IR (neat): 3005, 1462, 1227, 1018, 910, 769 cm^{-1}.

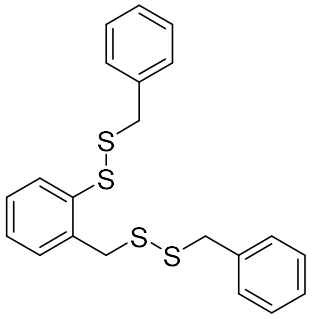
1-*iso*-Butyl-2-(2-(isobutyldisulfaneyl)benzyl)disulfane (*o*-129c)



Synthesised by GP3 and isolated as a pale yellow oil in 461 mg, 66% yield from 2.1 mmol ***o*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 7.9, 1.1 Hz, 1H, Ar*H*), 7.34 – 7.27 (m, 2H, Ar*H*), 7.19 (td, *J* = 7.4, 1.3 Hz, 1H, Ar*H*), 4.07 (s, 2H, PhCH_2), 2.63 (d, *J* = 6.9 Hz, 2H, SSCH_2), 2.30 (d, *J* = 6.8 Hz, 2H, SSCH_2), 1.95 (septt, *J* = 6.9, 6.7 Hz, 1H, C*H*), 1.84 (septt, *J* = 6.9, 6.7 Hz, 1H, C*H*), 0.98 (d, *J* = 6.7 Hz, 6H, CH(CH_3)$_2$), 0.91 (d, *J* = 6.7 Hz, 6H, CH(CH_3)$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.4 (C_{Ar}), 136.9 (C_{Ar}), 130.8 (C_{Ar}), 129.6 (C_{Ar}), 128.5 (C_{Ar}), 127.0 (C_{Ar}), 48.3 (SS*C*H_2), 48.2 (SS*C*H_2), 41.6 (Ph*C*H_2), 28.2 (*C*H), 28.2 -*C*H), 27.1 (*C*H_3), 21.9 (*C*H_3), 21.9 (*C*H_3), 21.9 (cpr-*C*H_3) ppm. HRMS (EI) $[M]^+$ calc. 332.07554 found 332.0754 $[C_{15}H_{24}S_4]^+$. IR (neat): 3003, 2359, 1425, 789, 698 cm^{-1}.

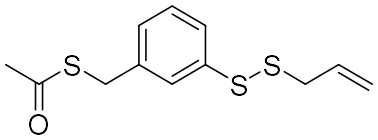
1-Benzyl-2-(2-(benzyldisulfaneyl)benzyl)disulfane (*o*-129d)



Synthesised by GP3 and isolated as a pale yellow oil in 342 mg, 41% yield from 2.1 mmol of ***o*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.38 – 7.05 (m, 11H, C_6H_5, C_6H_5 and Ar*H*), 3.95 (s, 2H, SSCH_2), 3.84 (s, 2H, SSCH_2), 3.61 (s, 2H, CH_2SS) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.3 (*C*$_{Ar}$), 136.7 (*C*$_{Ar}$), 136.6 (*C*$_{Ar}$), 136.5 (*C*$_{Ar}$), 130.8 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 129.4 (*C*$_{Ar}$), 129.4 (*C*$_{Ar}$), 128.6 (*C*$_{Ar}$), 128.4 (*C*$_{Ar}$), 127.6 (*C*$_{Ar}$), 127.5 (*C*$_{Ar}$), 127.0 (*C*$_{Ar}$), 43.5 (SS*C*H$_2$), 43.4 (SS*C*H$_2$), 41.3 (*C*H$_2$SS) ppm. HRMS (EI) $[M]^+$ calc. 400.04424, found: 400.0438 $[C_{21}H_{20}S_4]^+$. IR (neat): 3028, 2359, 2342, 1493, 1452, 696 cm^{-1}.

***S*-(3-(Allyldisulfaneyl)benzyl) ethanethioate(*m*-140a)**

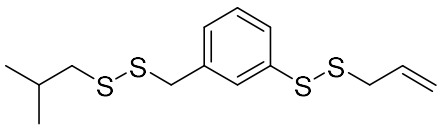


Thioacetate (***m*-139**) (200 mg, 0.83 mmol) was dissolved in methanol (8.3 mL) and cooled to −40 °C. Base (0.83 mmol, 1 equiv.) was added, followed by allyl thiotosylate (**83a**, 285 mg, 1.25 mmol, 1.5 equiv.). The reaction was monitored by TLC and left to warm to room temperature overnight. The reaction was quenched with ammonium chloride and the product was extracted into diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and water (20 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using

Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 90:10 (cyclohexane:diethyl ether) over 10 CV, and then to 30% diethyl ether over 2 CV, then 100% diethyl ether over 5 CV) to afford ***m*-140a** as a colourless oil (potassium hydroxide: 58 mg, 26%, potassium carbonate: 79 mg, 35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.45 (m, 1H, Ar*H*), 7.44 – 7.40 (m, 1H, Ar*H*), 7.26 (dt, *J* = 7.6, 2.4 Hz, 1H, Ar*H*), 7.17 – 7.13 (m, 1H, Ar*H*), 5.84 (ddt, *J* = 17.2, 9.9, 7.4 Hz, 1H, SCH_2CH=CH_2), 5.20 – 5.13 (m, 2H, SCH_2CH=CH_2), 4.12 (s, 2H, CH_2SAc), 3.39 – 3.36 (m, 2H, SCH_2CH=CH_2), 2.37 (s, 3H, CH_3) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.9 (*C*=O), 138.7 (C_{Ar}), 137.8 (C_{Ar}), 132.6 (CH=CH_2), 129.3 (C_{Ar}), 127.9 (C_{Ar}), 127.4 (C_{Ar}), 126.6 (C_{Ar}), 119.3 (CH=CH_2), 41.9 ($SSCH_2$), 33.3 (CH_2SAc), 30.5 (CH_3) ppm. HRMS (EI) $[M]^+$ calc. 270.1136 found: 270.1132 $[C_{12}H_{14}OS_3]^+$. IR (neat): 2920, 1686, 1470, 1130, 783, 630, 525 cm^{-1}.

1-Allyl-2-(3-((*iso*-butyldisulfaneyl)methyl)phenyl)disulfane (*m*-129i)



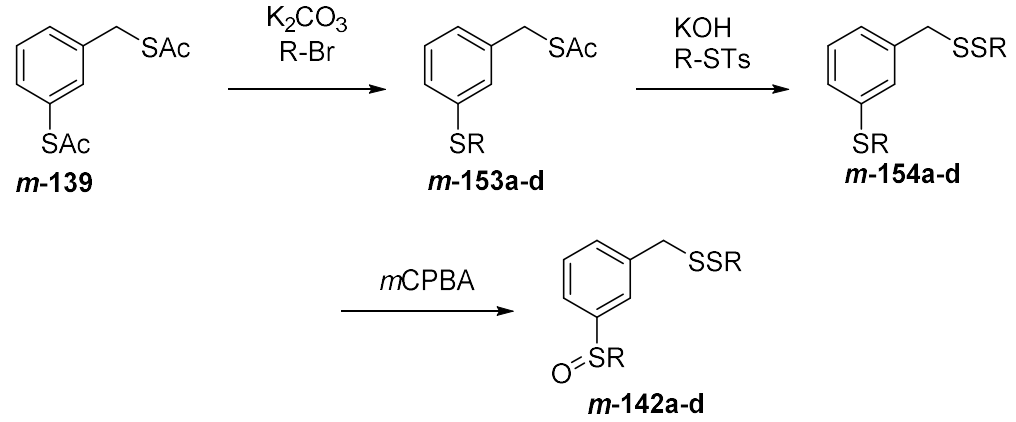
Thioacetate (***m*-140a**) (71 mg, 0.26 mmol) was dissolved in methanol (2.6 mL) and cooled to −40 °C. KOH (17 mg, 0.26 mmol, 1 equiv.) was added, followed by *iso*-butyl thiotosylate (**83c**, 3 mg, 0.34 mmol, 1.3 equiv.). The reaction was monitored by TLC and left to warm to room temperature overnight. The reaction was quenched with ammonium chloride and the product was extracted into diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and water (20 mL). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 95:5 (cyclohexane:diethyl ether) over 10 CV, and then to 80% diethyl ether over 2 CV, then 100% diethyl ether over 5 CV) to afford ***m*-129i** as a colourless oil in 10 mg, 12%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (dt, *J* = 3.8, 1.7 Hz, 1H, Ar*H*), 7.46 – 7.42 (m, 1H, Ar*H*), 7.30 – 7.27 (m, 1H, Ar*H*), 7.20 – 7.17 (m, 1H, Ar*H*), 5.88 – 5.76 (m, 1H, C*H*=CH_2), 5.20 – 5.09 (m, 2H, CH=C*H*$_2$), 3.87 (s, 2H, SSC*H*$_2$Ph), 3.40 – 3.34 (dt, 7.3, 1.3 Hz, 2H, SSC*H*$_2$CH=CH_2), 2.32 (d, *J* = 6.8 Hz, 2H, C*H*$_2$CH(CH_3)$_2$), 1.82 (septt, *J* = 6.8, 6.7 Hz, 1H, C*H*(CH_3)$_2$), 0.92 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.9 (*C*$_{Ar}$), 137.7 (*C*$_{Ar}$), 132.6 (*C*H=CH_2), 129.2 (*C*$_{Ar}$), 128.5 (*C*$_{Ar}$), 128.0 (*C*$_{Ar}$), 126.7 (*C*$_{Ar}$), 119.3 (CH=*C*H_2), 48.3 (*C*H_2SS), 43.4 (SS*C*H_2), 41.9 (SS*C*H_2), 28.2 (*C*H(CH_3)$_2$), 21.9 (*C*H_3), 21.9 (*C*H_3) ppm. HRMS (AP) $[M+H]^+$ calc. 317.0526 found: 317.0512 $[C_{14}H_{21}S_4]^+$. IR (neat): 2955, 2924, 2359, 1464, 1227, 920, 787, 698, 580 cm^{-1}.

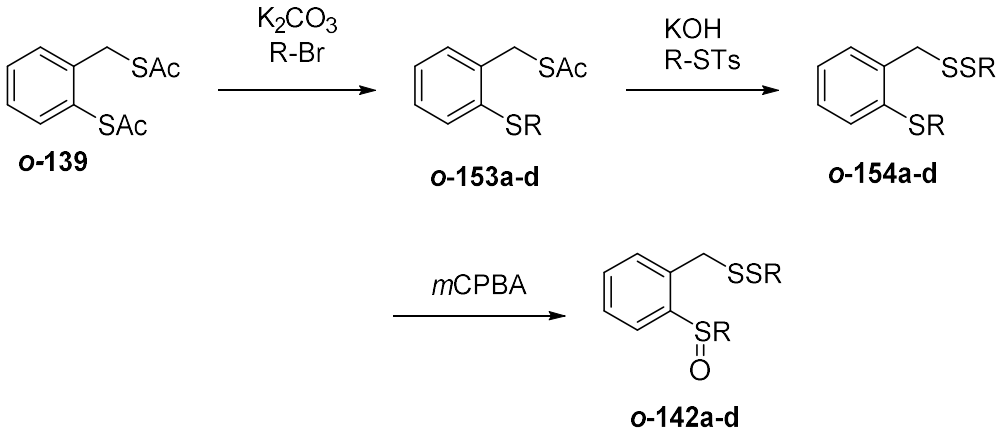
8.5. Chapter 6: Synthesis of Isomeric Ajoene Analogues

8.5.1. General Scheme

(a)



(b)



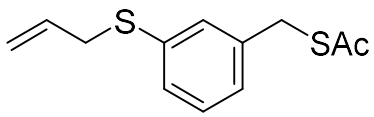
Scheme 8.4: General Scheme: (a) synthesis of ***m*-142a-d**; (a) synthesis of ***o*-142a-d**.

8.5.2. Experimental

General Procedure 4 (GP4): Synthesis of Thioether 153

Potassium carbonate (1.0 equiv.) was added to thioacetate ***m-/o-*139** (1.0 equiv.) in methanol (0.1 mL) and stirred for 10 minutes at 0 °C. Alkyl/aryl bromide (2.0 equiv.) was added and the reaction was left at 0 °C for 2 hours, and left at room temperature over night to reach full conversion. Saturated solution of ammonium chloride (5 mL) was added and methanol was removed *in vacuo*. The product was extracted using (3 x 20 mL) and the combined organic layers were washed with water (20 mL) and brine (20 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated *in vacuo.* The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 85:15 (cyclohexane:diethyl ether) over 10 CV, and then to 40% diethyl ether over 20 CV), then 100% diethyl ether over 10 CV) to afford desired product.

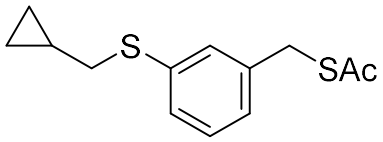
***S*-(3-(Allylthio)benzyl) ethanethioate (*m*-153a)**



Synthesised by GP4 and isolated as a pale yellow oil in 118 mg, 49% from 1.11 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (dq, *J* = 1.5, 0.9 Hz, 1H, Ar*H*), 7.21 – 7.19 (m, 2H, Ar*H*), 7.11 – 7.08 (m, 1H, Ar*H*), 5.92 – 5.82 (m, 1H, CH_2=C*H*), 5.18 – 5.05 (m, 2H, CH_2=CH), 4.08 (s, 2H, CH_2SAc), 3.57 – 3.53 (dt, 1.35, 6.87 Hz, 2H, CHCH_2S), 2.35 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8 (*C*=O), 138.4 (C_{Ar}), 136.5 (C_{Ar}), 133.4 (CH_2=*C*H), 129.6 (C_{Ar}), 129.0 (C_{Ar}), 128.2 (C_{Ar}), 126.6 (C_{Ar}), 117.8 (CH_2=CH), 36.8 (CHCH_2S), 33.2 (*C*H_2SAc), 30.3 (CH_3) ppm. HRMS (CI) $[M]^+$ calc. 238.04806 found 238.0479 $[C_{12}H_{14}OS_2]^+$. IR (neat): 2922, 1740, 1473, 1217, 916, 735, 689 cm^{-1}.

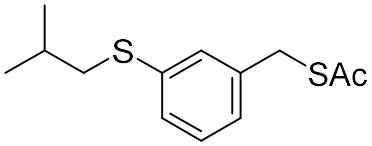
_S_-(3-((Cyclopropylmethyl)thio)benzyl) ethanethioate (_m_-153b)



Synthesised by GP4 and isolated as a pale yellow oil in 845 mg, 86% yield from 4.2 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 3H, Ar*H*), 7.16 – 7.12 (m, 1H, Ar*H*), 4.14 (s, 2H, C*H*$_2$SAc), 2.92 (d, *J* = 7.0 Hz, 2H, C*H*$_2$S), 2.41 (s, 3H, COC*H*$_3$), 1.16 – 1.07 (m, 1H, cpr-C*H*), 0.67 – 0.61 (m, 2H, cpr-C*H*$_2$), 0.34 – 0.29 (m, 2H, cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.1 (*C*=O), 138.4 (*C*$_{Ar}$), 137.8 (*C*$_{Ar}$), 129.3 (*C*$_{Ar}$), 129.1 (*C*$_{Ar}$), 127.9 (*C*$_{Ar}$), 126.3 (*C*$_{Ar}$), 39.4 (*C*H$_2$SPh), 33.3 (Ph*C*H$_2$), 30.4 (*C*H$_3$), 10.7 (cpr-*C*H), 5.7 (cpr-*C*H$_2$) ppm. HRMS (ES) $[M+H]^+$ calc. 235.0721 found 235.0718 $[C_{13}H_{17}OS_2]^+$. IR (neat) 2959, 1690, 1589, 1130, 957, 787, 623 cm^{-1}.

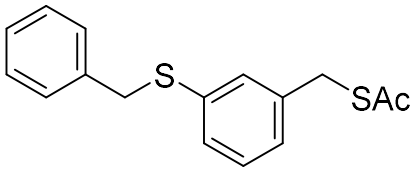
_S_-(3-(*iso*-Butylthio)benzyl) ethanethioate (*m*-153c)



Synthesised by GP4 and isolated as a pale yellow oil in 656 mg, 70% yield from 3.9 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 6.8 Hz, 1H, Ar*H*), 7.21 – 7.16 (m, 2H, Ar*H*), 7.06 (dt, *J* = 6.7, 1.9 Hz, 1H, Ar*H*), 4.07 (s, 2H, C*H*$_2$SAc), 2.80 (d, *J* = 6.8 Hz, 2H, $(CH_3)_2$CHC*H*$_2$), 2.35 (s, 3H, C*H*$_3$), 1.86 (septt, *J* = 6.8, 6.7 Hz, 1H, $(CH_3)_2$C*H*), 1.04 (s, 3H, C*H*$_3$), 1.02 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.1 (*C*=O), 138.5 (*C*$_{Ar}$), 138.2 (*C*$_{Ar}$), 129.1 (*C*$_{Ar}$), 128.9 (*C*$_{Ar}$), 127.5 (*C*$_{Ar}$), 126.1 (*C*$_{Ar}$), 42.4 (*C*H$_2$S), 33.38 (*C*H$_2$SAc), 30.5 (SCO*C*H$_3$), 28.4 (*C*H), 22.2 ((*C*H$_3$)$_2$) ppm. HRMS (ASAP) $[M+H]^+$ calc. 255.0877 found: 255.0879 $[C_{13}H_{19}OS_2]^+$. IR (neat): 2598, 1690, 1466, 1130, 957, 623 cm^{-1}.

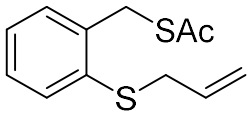
_S_-(3-(Benzylthio)benzyl) ethanethioate (_m_-153d)



Synthesised by GP4 and isolated as a pale yellow oil in 855 mg, 78% yield from 3.8 mmol of ***m*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 5H, $C_{Ar}H$), 7.25 – 7.21 (m, 2H, $C_{Ar}H$), 7.19 – 7.16 (m, 2H, $C_{Ar}H$), 7.11 – 7.08 (m, 1H, $C_{Ar}H$), 4.11 (s, 2H, CH_2SAc), 4.05 (s, 2H, CH_2S), 2.35 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.1 (*C*=O), 138.5 (C_{Ar}), 137.4 (C_{Ar}), 137.0 (C_{Ar}), 129.9 (C_{Ar}), 129.2 (C_{Ar}), 129.0 (C_{Ar}), 128.9 (C_{Ar}), 128.8 (C_{Ar}), 128.6 (C_{Ar}), 128.5 (C_{Ar}), 127.4 (C_{Ar}), 126.9 (C_{Ar}), 38.91 (PhCH_2S), 33.3 (CH_2SCOCH_3), 30.47 ($SCOCH_3$) ppm. HRMS (ES) $[M+H]^+$ calc. 289.0721 found 289.0724 $[C_{16}H_{17}OS_2]^+$. IR (neat): 2970, 2901, 1690, 1242, 1068, 714, 629 cm^{-1}.

_S_-(2-(Allylthio)benzyl) ethanethioate (_o_-153a)

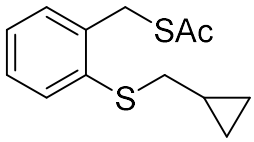


Synthesised by GP4 and isolated as a pale yellow oil in 161 mg, 63% yield from 1.1 mmol of ***o*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (dd, *J* = 7.5, 1.6 Hz, 1H, Ar*H*), 7.35 (dd, *J* = 7.8, 1.3 Hz, 1H, Ar*H*), 7.21 (td, *J* = 7.6, 1.6 Hz, 1H, Ar*H*), 7.15 (td, *J* = 7.5, 1.4 Hz, 1H, Ar*H*), 5.88 (ddt, *J* = 16.9, 10.0, 6.9 Hz, 1H, SCH_2CH=CH_2), 5.12 (d Ar*H* q, *J* = 16.9, 1.3 Hz, 1H, CH=CH_2), 5.08 (ddd, *J* = 10.0, 2.1, 1.1 Hz, 1H, CH=CH_2), 4.29 (s, 2H, CH_2SAc), 3.55 (dt, *J* = 6.9, 1.1 Hz, 2H, SCH_2CH), 2.33 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.5 (*C*=O), 138.2 (C_{Ar}), 135.4 (C_{Ar}), 133.3 (CH=CH_2), 130.9 (C_{Ar}), 130.5 (C_{Ar}), 128.1 (C_{Ar}), 126.9 (C_{Ar}), 118.2 (CH=CH_2), 37.9 (CH_2SAc), 32.3

(S*C*H$_2$CH=CH$_2$), 30.5 (CH$_3$) ppm. HRMS (EI) [M]$^+$ calc. 238.0486 found: 238.0482 [C$_{12}$H$_{14}$OS$_2$]$^+$. IR (neat): 2922, 1688, 1468, 1132, 784, 627 cm^{-1}.

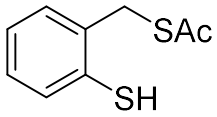
_S_-(2-((Cyclopropylmethyl)thio)benzyl) ethanethioate (_o_-153b)



Synthesised by GP4 and isolated as a pale yellow oil in 148 mg, 60% yield from 0.92 mmol of ***o*-139**.

^{1}H NMR (300 MHz, CDCl$_3$) δ 7.40 – 7.34 (m, 2H, Ar*H*), 7.17 (dtd, *J* = 20.4, 7.4, 1.5 Hz, 2H, Ar*H*), 4.31 (s, 2H, C*H*$_2$SAc), 2.85 (d, *J* = 7.1 Hz, 2H, SC*H*$_2$), 2.33 (s, 3H, COC*H*$_3$), 1.13 – 0.98 (m, 1H, cpr-C*H*), 0.63 – 0.54 (m, 2H, cpr-C*H*$_2$), 0.30 – 0.20 (m, 2H, cpr-C*H*$_2$) ppm.^{13}C NMR (126 MHz, CDCl$_3$) δ 195.6 (*C*=O), 137.8 (*C*$_{Ar}$), 136.6 (*C*$_{Ar}$), 130.4 (*C*$_{Ar}$), 130.3 (*C*$_{Ar}$), 128.2 (*C*$_{Ar}$), 126.6 (*C*$_{Ar}$), 40.6 (S*C*H$_2$), 32.3 (*C*H$_2$SAc), 30.5 (*C*H$_3$), 10.8 (cpr-*C*H), 5.9 (cpr-*C*H$_2$) ppm. HRMS (ES) [M+H]$^+$ calc. 235.0721 found 235.0718 [C$_{13}$H$_{17}$OS$_2$]$^+$. IR (neat): 2959, 1690, 1589, 1130, 957, 787, 623 cm^{-1}.

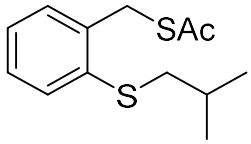
_S_-(2-Mercaptobenzyl) ethanethioate (_o_-155)



Synthesised by GP4 and isolated as a pale colourless oil in 121 mg mg, 18% yield from 3.4 mmol of ***o*-139**.

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 – 7.31 (m, 2H, Ar*H*), 7.16 – 7.11 (m, 2H, Ar*H*), 4.22 (s, 2H, C*H*$_2$), 3.45 (s, 1H, S*H*), 2.35 (s, 3H, C*H*$_3$) ppm. ^{13}C NMR (126 MHz, CDCl$_3$) δ 195.2 (*C*=O), 136.2 (*C*$_{Ar}$), 131.8 (*C*$_{Ar}$), 130.9 (*C*$_{Ar}$)fCF, 130.8 (*C*$_{Ar}$), 128.33 (*C*$_{Ar}$), 126.8 (*C*$_{Ar}$), 32.9 (*C*H$_2$), 30.5 (*C*H$_3$) ppm. HRMS (EI) [M]$^+$ calc. 198.01676 found: 198.0166 [C$_9$H$_{10}$OS$_2$]$^+$. IR (neat): 3059, 2563, 1684, 1470, 1352, 1128, 955, 764, 735, 625 cm^{-1}.

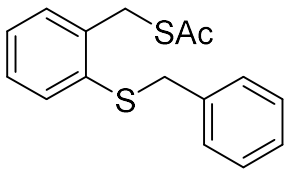
_S_-(2-(_iso_-Butylthio)benzyl) ethanethioate (_o_-153c)



Potassium carbonate (84 mg, 0.6 mmol, 1.0 equiv.) was added to thiol ***o*-155** (120 mg, 0.6 mmol, 1.0 equiv.) in methanol (6 mL) and stirred for 10 minutes at 0 °C. *Iso*-butyl bromide (155 mg, 1.8 mmol, 3.0 equiv.) was added and the reaction was heated to 30 °C until TLC indicated full consumption of starting material. Saturated solution of ammonium chloride (5 mL) was added and methanol was removed *in vacuo*. The product was extracted using (3 x 20 mL) and the combined organic layers were washed with water (20 mL) and brine (20 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated *in vacuo.* The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 85:15 (cyclohexane:diethyl ether) over 10 CV, and then to 40% diethyl ether over 20 CV), then 100% diethyl ether over 10 CV) to afford ***o*-153c** in 72 mg, 47% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.6, 1.2 Hz, 1H, Ar*H*), 7.31 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.21 (td, *J* = 7.6, 1.4 Hz, 1H, Ar*H*), 7.12 (td, *J* = 7.5, 1.1 Hz, 1H, Ar*H*), 4.29 (s, 2H, CH_2SAc), 2.81 (d, *J* = 6.8 Hz, 2H, SCH_2CH), 2.33 (s, 3H, COCH_3), 1.89 (septt, *J* = 6.8, 6.7 Hz, 1H, C*H*$(CH_3)_2$), 1.06 (s, 3H, CH(CH_3)$_2$), 1.04 (s, 3H, CH(CH_3)$_2$) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 195.5 (*C*=O), 137.25 (C_{Ar}), 137.0 (C_{Ar}), 130.4 (C_{Ar}), 129.2 (C_{Ar}), 128.2 (C_{Ar}), 126.1 (C_{Ar}), 43.2 (S*C*H$_2$), 32.2 (*C*H$_2$SAc), 30.5 (CO*C*H$_3$), 28.4 (*C*H$(CH_3)_2$), 22.3 ((*C*H$_3$)$_2$) ppm. HRMS (EI) $[M]^+$ calc. 254.07936 found: 254.0793 $[C_{13}H_{18}OS_2]^+$. IR (neat): 2927, 1688, 1466, 1130, 955, 734, 625 cm^{-1}.

***S*-(2-(Benzylthio)benzyl) ethanethioate (*o*-153d)**



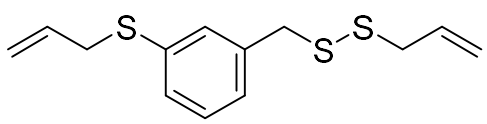
Synthesised by GP4 and isolated as a pale yellow oil in 300 mg, 58% yield from 1.8 mmol of ***o*-139**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 8H, Ar*H*), 4.19 (s, 2H, CH_2SAc), 4.09 (s, 2H, SCH_2Ph), 2.32 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 195.4 (*C*=O), 138.5 (C_{Ar}), 137.2 (C_{Ar}), 135.7 (C_{Ar}), 131.4 (C_{Ar}), 130.4 (C_{Ar}), 129.1 (C_{Ar}), 128.7 (C_{Ar}), 128.2 (C_{Ar}), 127.5 (C_{Ar}), 127.2 (C_{Ar}), 39.9 (CH_2), 32.2 (CH_2), 30.5 (CH_3) ppm. HRMS (ES) $[M+Na]^+$ calc. 311.0540 found: 311.0536 $[C_{16}H_{16}OS_2Na]^+$. IR (neat): 3059, 1686, 1466, 1130, 955, 764, 694, 623 cm^{-1}.

General Procedure 5 (GP5): Synthesis of Disulfide 154

Potassium hydroxide (1.5 equiv.) was added to the benzyl thioacetate ***m*-/*o*-153** (1.0 equiv.) in methanol (0.1 mL) at −40 °C. After 15 minutes, thiotosylate (2 equiv.) was added. After TLC indicated consumption of starting materials, saturated ammonium chloride solution (5 mL) was added and methanol removed *in vacuo*. The resulting residue was dissolved in diethyl ether and washed with water (3 x 10 mL) and brine (2 x 10 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 90:10 (cyclohexane:diethyl ether) over 10 CV, and then to 20% diethyl ether over 20 CV) to yield title product.

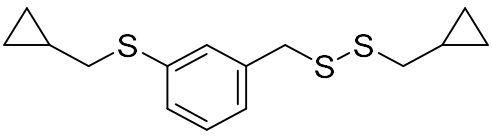
1-Allyl-2-(3-(allylthio)benzyl)disulfane (*m*-154a)



Synthesised by GP5 and isolated as a pale yellow oil in 139 mg (81%) from 0.64 mmol of ***m*-153a**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (dd, *J* = 4.2, 3.1 Hz, 1H, Ar*H*), 7.27 – 7.22 (m, 2H, Ar*H*), 7.16 – 7.11 (m, 1H, Ar*H*), 5.93 – 5.84 (m, 1H, CH_2=C*H*), 5.79 – 5.70 (m, 1H, C*H*=CH_2), 5.19 – 5.04 (m, 4H, CH_2=CH and CH=CH_2), 3.85 (s, 2H, CH_2SS), 3.57 (dt, *J* = 6.8, 1.2 Hz, 2H, CH_2=CHCH_2S), 3.07 – 3.02 (m, 2H, SSCH_2) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 138.3 (*C*$_{Ar}$), 136.4 (*C*$_{Ar}$), 133.6 (CH_2=*C*H), 133.4 (*C*H=CH_2), 130.5 (*C*$_{Ar}$), 129.0 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 127.3 (*C*$_{Ar}$), 118.7 (*C*H_2=CH), 118.0 (CH=*C*H_2), 43.5 (*C*H_2SS), 42.0 (SS*C*H_2), 37.1 (*C*H_2S) ppm. HRMS (EI) $[M]^+$ calc. 268.04086 found 268.0411 $[C_{13}H_{16}S_3]^+$. IR (neat): 3078, 978, 918, 787, 698, 582 cm^{-1}.

1-(Cyclopropylmethyl)-2-(3-((cyclopropylmethyl)thio)benzyl)disulfane (*m*-154b)

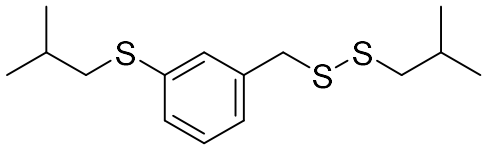


Synthesised by GP5 and isolated as a pale yellow oil in 478 mg, 85% yield from 1.9 mmol of ***m*-153b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (t, *J* = 1.6 Hz, 1H, C*H*$_{Ar}$), 7.28 – 7.19 (m, 2H, C*H*$_{Ar}$), 7.13 (dt, *J* = 7.3, 1.5 Hz, 1H, C*H*$_{Ar}$), 3.87 (s, 2H, CH_2SS), 2.87 (d, *J* = 7.0 Hz, 2H, CH_2S), 2.40 (d, *J* = 7.2 Hz, 2H, SSCH_2), 1.06 (ttt, *J* = 8.0, 7.0, 4.8 Hz, 1H, C*H*$_{Cpr}$), 1.02 – 0.93 (m, 1H, C*H*$_{Cpr}$), 0.61 – 0.53 (m, 4H, CH_{2Cpr}), 0.28 – 0.23 (m, 2H, CH_{2Cpr}), 0.21 – 0.17 (m, 2H, CH_{2Cpr}) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.4

(C_{Ar}), 137.5 (C_{Ar}), 130.0 (C_{Ar}), 129.0 (C_{Ar}), 128.2 (C_{Ar}), 126.9 (C_{Ar}), 45.2 (CH_2SS), 43.8 (CH_2S), 39.6 ($SSCH_2$), 11.0 ($C_{Cpr}H$), 10.77 ($C_{Cpr}H$), 5.80 ($C_{Cpr}H_2$), 5.72 ($C_{Cpr}H_2$) ppm. HRMS (ES) $[M+H]^+$ calc. 297.0805 found: 297.0806 $[C_{15}H_{21}S_3]^+$. IR (neat): 2955, 1464, 12249, 785, 698 cm^{-1}.

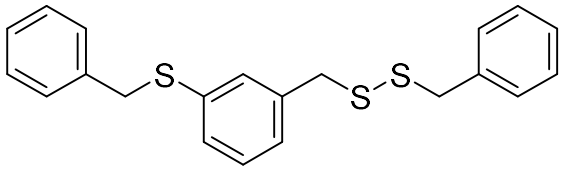
1-*iso*-Butyl-2-(3-(*iso*-Butylthio)benzyl)disulfane (*m*-154c)



Synthesised by GP5 and isolated as a yellow oil in 250 mg, 80% yield from 1.04 mmol of ***m*-153c**.

^1H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 0.6 Hz, 1H, CH_{Ar}), 7.24 – 7.19 (m, 2H, CH_{Ar}), 7.13 – 7.08 (m, 1H, CH_{Ar}), 3.84 (s, 2H, CH_2SS), 2.82 (d, J = 6.8 Hz, 2H, $SSCH_2$), 2.30 (d, J = 6.8 Hz, 2H, CH_2SS), 1.88 (td, J = 13.4, 6.7 Hz, 1H, $CH(CH_3)_2$), 1.81 (td, J = 13.4, 6.7 Hz, 1H, $CH(CH_3)_2$), 1.04 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.91 (s, 3H, CH_3) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5 (C_{Ar}), 137.9 (C_{Ar}), 129.6 (C_{Ar}), 129.0 (C_{Ar}), 127.7 (C_{Ar}), 126.7 (C_{Ar}), 48.2 (CH_2SS), 43.5 (CH_2S), 42.6 ($SSCH_2$), 28.4 ($CH(CH_3)_2$), 28.2 ($CH(CH_3)_2$), 22.2 (CH_3), 21.9 (CH_3) ppm. HRMS (ES) $[M+H]^+$ calc. 301.1118 found: 301.1118 $[C_{15}H_{25}S_3]^+$. IR (neat): 2955, 1589, 1462, 787, 698 cm^{-1}.

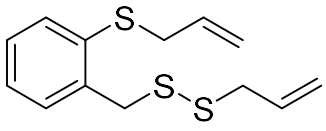
1-Benzyl-2-(3-(benzylthio)benzyl)disulfane (*m*-154d)



Synthesised by GP5 and isolated as a pale yellow oil in 911 mg, 84% yield from 3 mmol of ***m*-153d**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 12H, $C_{Ar}H$), 7.18 – 7.15 (m, 1H, $C_{Ar}H$), 7.06 – 7.01 (m, 1H, $C_{Ar}H$), 4.13 (s, 2H, CH_2SS), 3.59 (s, 2H, CH_2S), 3.50 (s, 2H, $SSCH_2$) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 138.3 (C_{Ar}), 137.5 (C_{Ar}), 137.4 (C_{Ar}), 136.8 (C_{Ar}), 130.6 (C_{Ar}), 129.6 (C_{Ar}), 129.1 (C_{Ar}), 129.0 (C_{Ar}), 128.7 (C_{Ar}), 128.6 (C_{Ar}), 127.6 (C_{Ar}), 127.5 (C_{Ar}), 127.4 (C_{Ar}), 43.4 (CH_2SS), 43.1 (CH_2S), 38.9 ($SSCH_2$) ppm. HRMS (EI) $[M]^+$ calc. 368.07216 found: 368.0720 $[C_{21}H_{20}S_3]^+$. IR (neat): 3028, 1492, 1454, 763, 698, 563 cm^{-1}.

1-Allyl-2-(2-(allylthio)benzyl)disulfane (*o*-154a)

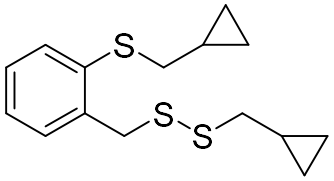


Synthesised by GP5 and isolated as a yellow oil in 92 mg, 51% yield from 0.68 mmol of ***o*-153a**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.8, 1.1 Hz, 1H, Ar*H*), 7.33 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar*H*), 7.27 – 7.23 (m, 1H, Ar*H*), 7.18 (td, *J* = 7.4, 1.3 Hz, 1H, Ar*H*), 5.88 (ddt, *J* = 16.9, 10.0, 6.9 Hz, 1H, $CH{=}CH_2$), 5.75 (ddt, *J* = 17.3, 10.0, 7.4 Hz, 1H, $CH{=}CH_2$), 5.15 – 5.04 (m, 4H, $CH{=}CH_2$ and $CH_2{=}CH$), 4.11 (s, 2H, CH_2S), 3.57 (dt, *J* = 6.9, 1.1 Hz, 2H, $SSCH_2$), 3.06 (d, *J* = 7.4 Hz, 2H, SCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.1 (C_{Ar}), 135.8 (C_{Ar}), 133.5 ($CH{=}CH_2$), 133.4 ($CH_2{=}CH$), 131.2

(C_{Ar}), 130.9 (C_{Ar}), 128.2 (C_{Ar}), 126.6 (C_{Ar}), 118.7 (CH_2=CH), 118.1 (CH=CH_2), 42.3 (SSCH_2), 42.1 (PhCH_2S), 38.1 (SCH_2CH) ppm. HRMS (EI) $[M]^+$ calc. 268.04086 found 268.0411 $[C_{13}H_{16}S_3]^+$. IR (neat): 3078, 978, 918, 787, 698, 582 cm^{-1}.

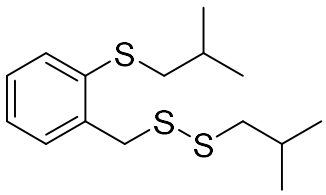
1-(Cyclopropylmethyl)-2-(2-((cyclopropylmethyl)thio)benzyl)disulfane (*o*-154b)



Synthesised by GP5 and isolated as a pale yellow oil in 105 mg, 70% yield from 0.51 mmol of ***o*-153b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (dt, *J* = 5.4, 2.7 Hz, 1H, Ar*H*), 7.32 (dd, *J* = 7.5, 1.4 Hz, 1H, Ar*H*), 7.25 – 7.21 (m, 1H, Ar*H*), 7.18 – 7.14 (m, 1H, Ar*H*), 4.15 (s, 2H, CH_2SS), 2.86 (d, *J* = 7.1 Hz, 2H, SCH_2), 2.42 (d, *J* = 7.2 Hz, 2H, SSCH_2), 1.09 – 0.96 (m, 2H, cpr-C*H*), 0.60 – 0.52 (m, 4H, cpr-CH_2), 0.27 – 0.17 (m, 4H, cpr-CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.8 (C_{Ar}), 136.8 (C_{Ar}), 130.8 (C_{Ar}), 130.7 (C_{Ar}), 128.2 (C_{Ar}), 126.2 (C_{Ar}), 45.3 (SSCH_2), 42.6 (SCH_2), 40.8 (CH_2S), 11.0 (cpr-*C*H), 10.8 (cpr-*C*H), 5.9 (cpr-*C*H_2), 5.7 (cpr-*C*H_2) ppm. HRMS (ES) $[M+H]^+$ calc. 297.0805 found: 297.0806 $[C_{15}H_{21}S_3]^+$. IR (neat): 3077, 3003, 1466, 1242, 1016, 964, 826, 758, 738 cm^{-1}.

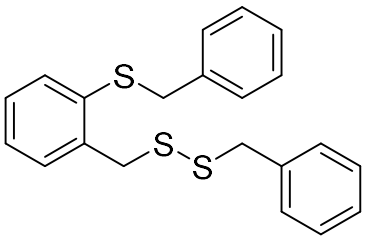
1-*iso*-Butyl-2-(2-(isobutylthio)benzyl)disulfane (*o*-154c)



Synthesised by GP5 and isolated as a pale yellow oil in 57 mg, 88% yield from 0.19 mmol of ***o*-153c**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 1H, Ar*H*), 7.26 – 7.20 (m, 2H, Ar*H*), 7.16 – 7.12 (m, 1H, Ar*H*), 4.10 (s, 2H, CH_2S), 2.83 (d, *J* = 6.8 Hz, 2H, SCH_2), 2.34 (d, *J* = 6.8 Hz, 2H, SSCH_2), 1.92 – 1.82 (m, 2H, C*H* and C*H*), 1.05 (d, *J* = 6.7 Hz, 6H, (CH_3)$_2$), 0.92 (d, *J* = 6.7 Hz, 6H, (CH_3)$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.3 (*C*$_{Ar}$), 137.2 (*C*$_{Ar}$), 130.7 (*C*$_{Ar}$), 129.8 (*C*$_{Ar}$), 128.2 (*C*$_{Ar}$), 125.8 (*C*$_{Ar}$), 48.2 (SS*C*H_2), 43.6 (SSCH_2), 42.3 (S*C*H_2), 28.5 (*C*H(CH_3)$_2$), 28.17 (*C*H(CH_3)$_2$), 22.3 (*C*H_3), 22.3 (*C*H_3), 21.9 (*C*H_3), 21.9 (*C*H_3) ppm. HRMS (ASAP) $[M+H]^+$ calc. 301.1118 found: 301.1127 $[C_{15}H_{25}S_3]^+$. IR (neat): 2955, 1464, 756, 735 cm^{-1}

1-Benzyl-2-(2-(benzylthio)benzyl)disulfane (*o*-154d)



Synthesised by GP5 and isolated as a pale yellow oil in 222 mg, 58% yield from 1.0 mmol of ***o*-153d**.

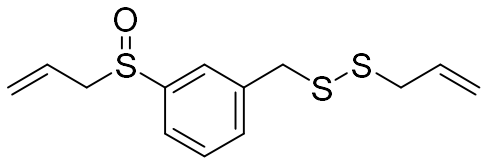
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.17 (m, 14H, Ar*H*), 4.10 (s, 2H, SCH_2), 3.79 (s, 2H, CH_2S), 3.61 (s, 2H, SSCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.4 (*C*$_{Ar}$), 137.5 (*C*$_{Ar}$), 137.4 (*C*$_{Ar}$), 136.1 (*C*$_{Ar}$), 131.7 (*C*$_{Ar}$), 130.9 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 129.1 (*C*$_{Ar}$), 128.7 (*C*$_{Ar}$), 128.6 (*C*$_{Ar}$), 128.3 (*C*$_{Ar}$), 127.6 (*C*$_{Ar}$), 127.4 (*C*$_{Ar}$), 126.8 (*C*$_{Ar}$), 43.65 (S*C*H_2), 41.88 (*C*H_2S), 40.09 (SS*C*H_2) ppm. HRMS (ES) $[M+Na]^+$ calc.

391.0625 found: 391.0609 $[C_{21}H_{20}S_3Na]^+$. IR (neat): 3059, 3028, 1494, 762, 696 cm^{-1}.

General Procedure 6 (GP6): Oxidation to Sulfoxide 142

Sulfide ***m-/o*-153** (1 equiv.) dissolved in dichloromethane (0.1 M) under argon atmosphere and cooled to -78 °C using dry ice bath. Recrystallised *m*CPBA (1 equiv.) added in one portion and reaction left at -78 °C for 2 h, then warmed to room temperature. Once TLC indicated full consumption of starting material, the reaction was quenched with sodium carbonate saturated solution. The product was extracted with dichloromethane (20 mL x 3). Combined organic layers were washed with brine (10 mL) and water (10 mL), dried over magnesium sulfate and concentrated *in vacuo*. The resulting residue was purified by column chromatography using Biotage Isolera (gradient: 100% cyclohexane for 3 column volumes (CV), then increased to 20:80 (cyclohexane:diethyl ether) over 3 CV, and then to 100% diethyl ether over 2 CV), then held at 100% diethyl ether) to yield title product.

1-Allyl-2-(3-(allylsulfinyl)benzyl)disulfane (*m*-142a)

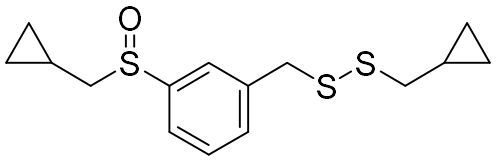


Synthesised by GP6 and isolated as a pale yellow oil in 29 mg, 78% yield from 0.13 mmol of ***m*-154a**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 – 7.53 (m, 1H, Ar*H*), 7.50 – 7.42 (m, 3H, Ar*H*), 5.75 (ddt, *J* = 17.0, 10.1, 7.5 Hz, 1H, $SSCH_2CH$=CH_2), 5.64 (ddt, *J* = 17.0, 10.1, 7.5 Hz, 1H, $CHCH_2SO$), 5.35 – 5.30 (m, 1H, CH_2=$CHCH_2SO$), 5.19 (app. dq, *J* = 17.0, 1.2 Hz, 1H, $SSCH_2CH$=CH_2), 5.14 – 5.05 (m, 2H, CH_2=$CHCH_2SO$ and $SSCH_2CH$=CH_2), 3.92 (s, 2H, $ArCH_2SS$), 3.57 (dddd, 1H, *J* = 12.9, 7.5, 1.2 Hz, CH_2CHCH_2S=O), 3.49 (dddd, 1H, *J* = 12.9, 7.5, 1.2 Hz, CH_2CHCH_2S=O), 3.10 –

3.06 (m, 2H, SSCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.4 (C_{Ar}), 139.1 (C_{Ar}), 133.2 (SSCH_2CH=CH_2), 132.2 (C_{Ar}), 129.3 (C_{Ar}), 125.2 (CH_2=CHCH_2SO), 125.1 (CH_2=CHCH_2SO), 124.2 (C_{Ar}), 123.4 (C_{Ar}), 118.9 (SSCH_2CH=CH_2), 60.9 (CH_2SO), 43.0 (CH_2SS), 42.0 (SSCH_2) ppm. HRMS (ES) $[M+H]^+$ calc. 285.0442 found: 285.0443 $[C_{13}H_{17}OS_3]^+$. IR (neat): 3015, 1366, 1217, 1090, 1045 cm^{-1}.

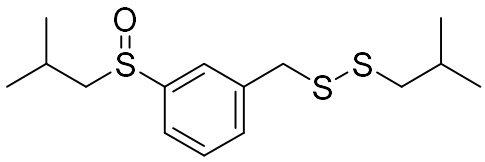
1-(Cyclopropylmethyl)-2-(3-((cyclopropylmethyl)sulfinyl)benzyl)disulfane (*m*-142b)



Synthesised by GP6 and isolated as a pale yellow oil in 93 mg, 71% yield from 0.42 mmol of ***m*−154b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 1H, ArH), 7.56 – 7.51 (m, 1H, ArH), 7.48 – 7.43 (m, 2H, ArH), 3.95 (s, 2H, ArCH_2), 2.84 (dd, 1H, J = 13.2, 7.3 Hz, cpr-CHCH_2S=O), 2.66 (dd, 1H, J = 13.2, 7.3 Hz, cpr-CHCH_2S=O), 2.42 (d, J = 7.3 Hz, 2H, SSCH_2), 1.03 – 0.93 (m, 2H, cpr-CH), 0.66 – 0.60 (m, 2H, cpr-CH_2), 0.59 – 0.53 (m, 2H, cpr-CH_2), 0.30 – 0.22 (m, 2H, cpr-CH_2), 0.20 (app. q, J = 4.8 Hz, 2H, cpr-CH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.5 (C_{Ar}), 139.4 (C_{Ar}), 132.1 (C_{Ar}), 129.4 (C_{Ar}), 125.0 (C_{Ar}), 123.3 (C_{Ar}), 63.5 (CH_2SO), 45.2 (SSCH_2), 43.4 (CH_2SS), 11.1 (SSCH$_2$C$_{Cpr}$H), 5.8 ($C_{Cpr}H_2$), 5.4 ($C_{Cpr}H_2$), 5.1 ($C_{Cpr}H_2$), 4.9 ($C_{Cpr}H_2$) ppm. HRMS (EI) $[M]^+$ calc. 312.06708, found: 312.0674 $[C_{15}H_{20}OS_3]^+$. IR (neat): 3078, 3005, 1366, 1229, 1041, 912, 792, 523 cm^{-1}.

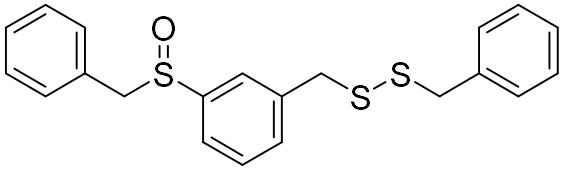
1-*iso*-Butyl-2-(3-(isobutylsulfinyl)benzyl)disulfane (*m*-142c)



Synthesised by GP6 and isolated as a pale yellow oil in 33 mg, 65% yield from 0.16 mmol of ***m*-154c**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 – 7.38 (m, 4H, Ar*H*), 3.91 (s, 2H, CH_2SS), 2.80 (dd, *J* = 13.1, 4.9 Hz, 1H, CH_2S=O), 2.45 (dd, *J* = 13.1, 4.9 Hz, 1H, CH_2S=O), 2.31 (d, *J* = 6.8 Hz, 2H, SSCH_2), 2.27 – 2.18 (m, 1H, CH(CH_3)$_2$), 1.80 (tq, *J* = 13.1, 6.8 Hz, 1H, CH(CH_3)$_2$), 1.15 (d, *J* = 6.8 Hz, 3H, CH(CH_3)$_2$), 1.05 (d, *J* = 6.8 Hz, 3H, CH(CH_3)$_2$), 0.90 (d, *J* = 6.8 Hz, 6H, CH(CH_3)$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.2 (*C*$_{Ar}$), 139.5 (*C*$_{Ar}$), 130.1 (*C*$_{Ar}$), 125.3 (*C*$_{Ar}$), 123.5 (*C*$_{Ar}$), 122.3 (*C*$_{Ar}$), 67.8 (*C*H_2SO), 48.3 (SS*C*H_2), 43.0 (CH_2SS), 28.7 (*C*H(CH_3)$_2$), 24.9 (*C*H (CH_3)$_2$), 23.8 (*C*H_3), 22.3 (*C*H_3), 22.3 (*C*H_3), 21.3 (*C*H_3) ppm. HRMS (EI) $[M]^+$ calc. 316.09838 found: 316.0978 $[C_{15}H_{24}OS_3]^+$. IR (neat): 2957, 1464, 1088, 1039, 795, 702 cm^{-1}.

1-Benzyl-2-(3-(benzylsulfinyl)benzyl)disulfane (*m*-142d)

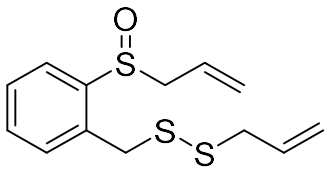


Synthesised by GP6 and isolated as a pale yellow oil in 53 mg, 45% yield from 0.31mmol of ***m*-154d**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.23 (m, 2H, Ar*H*), 7.23 – 7.12 (m, 8H, Ar*H*), 7.10 (dt, *J* = 3.2, 1.6 Hz, 2H), 6.89 (m, 2H), 3.99 (d, *J* = 12.6 Hz, 1H, CH_2S=O), 3.91 (d, *J* = 12.6 Hz, 1H, CH_2S=O), 3.63 (s, 2H, CH_2SS), 3.38 (s, 2H, SSCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.2 (*C*$_{Ar}$), 143.1 (*C*$_{Ar}$), 138.6 (*C*$_{Ar}$), 137.3 (*C*$_{Ar}$),

132.2 (C_{Ar}), 130.4 (C_{Ar}), 130.3 (C_{Ar}), 129.5 (C_{Ar}), 129.1 (C_{Ar}), 129.0 (C_{Ar}), 129.0 (C_{Ar}), 128.9 (C_{Ar}), 128.7 (C_{Ar}), 128.6 (C_{Ar}), 128.6 (C_{Ar}), 128.4 (C_{Ar}), 127.7 (C_{Ar}), 125.3 (C_{Ar}), 123.5 (C_{Ar}), 63.5 (CH_2SO), 63.5 (CH_2SO), 43.4 ($SSCH_2$), 43.0 ($SSCH_2$), 42.4 (CH_2SS), 38.8 (CH_2SS) ppm. HRMS (EI) $[M]^+$ calc. 384.06708 found: 384.0675 $[C_{21}H_{20}OS_3]^+$. IR (neat): 3059, 1494, 1452, 1080, 1043, 764, 696, 478 cm^{-1}.

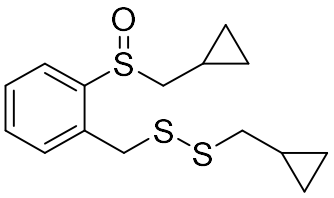
1-Allyl-2-(2-(allylsulfinyl)benzyl)disulfane (*o*-142a)



Synthesised by GP6 and isolated as a pale yellow oil in 35 mg, 40% yield from 0.31 mmol of ***o*-154a**.

^1H NMR (500 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 7.6, 1.4 Hz, 1H, Ar*H*), 7.50 (td, *J* = 7.6, 1.4 Hz, 1H, Ar*H*), 7.44 (td, *J* = 7.6, 1.4 Hz, 1H, Ar*H*), 7.36 (dd, *J* = 7.6, 1.0 Hz, 1H, Ar*H*), 5.81 – 5.70 (m, 2H, C*H*=CH_2), 5.37 – 5.34 (m, 1H, CH=CH_2), 5.26 (app. dq, *J* = 17.0, 1.2 Hz, 1H, CH=CH_2), 5.14 – 5.09 (m, 2H, CH=CH_2), 4.02 (d, *J* = 2.9 Hz, 2H, ArCH_2SS), 3.71 (dddd, *J* = 12.9, 7.5, 1.2 Hz, 1H, CH_2S=O), 3.58 (dddd, 12.9, 7.5, 1.2 Hz, 1H, CH_2S=O), 3.15 (t, *J* = 1.0 Hz, 1H, SSCH_2), 3.14 – 3.13 (m, 1H, SSCH_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.4 (C_{Ar}), 134.8 (C_{Ar}), 133.2 ($CH=CH_2$), 131.2 (C_{Ar}), 131.0(C_{Ar}), 128.95 (C_{Ar}), 125.82 (C_{Ar}), 125.4 ($CH=CH_2$), 124.0 ($CH=CH_2$), 119.1 ($CH=CH_2$), 60.8 ($S(=O)CH_2$), 42.1 ($SSCH_2$), 39.3 (CH_2SS) ppm. HRMS (ES) $[M+H]^+$ calc. 285.0442 found: 285.0443 $[C_{13}H_{17}OS_3]^+$. IR (neat): 3003, 1471, 1229, 1042, 964, 792, 700 cm^{-1}.

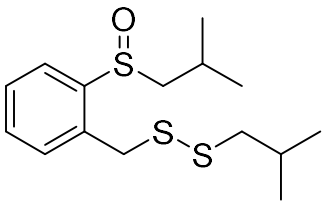
1-(Cyclopropylmethyl)-2-(2-((cyclopropylmethyl)sulfinyl)benzyl)disulfane (*o*-142b)



Synthesised by GP6 and isolated as a pale yellow oil in 80 mg, 77% yield from 0.33 mmol of ***o*-154b**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.6, 1.2 Hz, 1H, Ar*H*), 7.50 – 7.45 (m, 1H, Ar*H*), 7.40 (td, *J* = 7.6, 1.2 Hz, 1H, Ar*H*), 7.32 (d, *J* = 7.6 Hz, 1H, Ar*H*), 4.04 (q, *J* = 13.2 Hz, 2H, C*H*$_2$SS), 2.92 (dd, *J* = 13.2, 7.4 Hz, 1H, C*H*$_2$S=O), 2.75 (dd, *J* = 13.2, 7.4 Hz, 1H, C*H*$_2$S=O), 2.50 – 2.39 (m, 2H, SSC*H*$_2$), 1.11 (m, 1H, cpr-C*H*), 0.97 (m, 1H, cpr-C*H*), 0.66 – 0.59 (m, 2H, cpr-C*H*$_2$), 0.59 – 0.51 (m, 2H, cpr-C*H*$_2$), 0.33 – 0.25 (m, 2H, cpr-C*H*$_2$), 0.25 – 0.14 (m, 2H, cpr-C*H*$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.3 (*C*$_{Ar}$), 134.9 (*C*$_{Ar}$), 131.0 (*C*$_{Ar}$), 130.9 (*C*$_{Ar}$), 129.0 (*C*$_{Ar}$), 124.9 (*C*$_{Ar}$), 68.0 (S(=O)*C*H$_2$), 63.4 (S(=O)*C*H$_2$), 45.1 (SS*C*H$_2$), 39.5 (*C*H$_2$SS), 25.6 (cpr-*C*H), 10.9 (cpr-*C*H), 5.7 (cpr-*C*H$_2$), 5.7 (cpr-*C*H$_2$), 5.7 (cpr-*C*H$_2$), 5.5 (cpr-*C*H$_2$), 5.4 (cpr-*C*H$_2$), 4.8 (cpr-*C*H$_2$) ppm. HRMS (EI) $[M]^+$ calc. 312.06708 found: 312.0662 $[C_{15}H_{20}OS_3]^+$. IR (neat): 3003, 1472, 1059, 1034, 926, 768, 689 cm^{-1}.

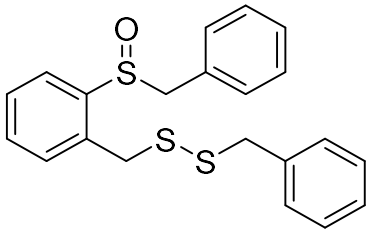
1-*iso*-Butyl-2-(2-(*iso*-butylsulfinyl)benzyl)disulfane (*o*-142c)



Synthesised by GP6 and isolated as a pale yellow oil in 35 mg, 67% yield from 0.17 mmol of ***o*-154c**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 – 7.92 (m, 1H, Ar*H*), 7.55 – 7.48 (m, 1H, Ar*H*), 7.46 – 7.40 (m, 1H, Ar*H*), 7.37 – 7.32 (m, 1H, Ar*H*), 4.11 (d, *J* = 12.9 Hz, 1H, ArC*H*$_2$SS), 3.95 (d, *J* = 12.9 Hz, 1H, ArC*H*$_2$SS), 2.82 (dd, *J* = 12.9, 7.1 Hz, 1H, C*H*$_2$S=O), 2.74 (dd, *J* = 12.9, 7.1 Hz, 1H, C*H*$_2$S=O), 2.39 (d, *J* = 2.4 Hz, 1H, SSC*H*$_2$), 2.39 (d, *J* = 2.4 Hz, 1H, SS*C*H$_2$), 2.37 – 2.32 (m, 1H, C*H*), 1.88 – 1.78 (m, 1H, C*H*), 1.20 (d, *J* = 6.7 Hz, 3H, C*H*$_3$), 1.08 (d, *J* = 6.7 Hz, 3H, C*H*$_3$), 0.93 (t, *J* = 6.7 Hz, 6H, (C*H*$_3$)$_2$) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.9 (*C*$_{Ar}$), 134.7 (*C*$_{Ar}$), 133.0 (*C*$_{Ar}$), 132.5 (*C*$_{Ar}$), 131.0 (*C*$_{Ar}$), 131.0 (*C*$_{Ar}$), 130.1 (*C*$_{Ar}$), 129.7 (*C*$_{Ar}$), 129.3 (*C*$_{Ar}$), 128.2 (*C*$_{Ar}$), 127.2 (*C*$_{Ar}$), 124.9 (*C*$_{Ar}$), 67.3 (S(=O)*C*H$_2$), 48.3 (SS*C*H$_2$), 39.4 (*C*H$_2$SS), 28.4 (*C*H), 24.7 (*C*H), 23.1 (*C*H$_3$), 21.8 (*C*H$_3$), 21.8 (*C*H$_3$), 21.6 (*C*H$_3$) ppm. HRMS (EI) [M]$^+$ calc. 316.09838 found: 316.0966 [$C_{13}H_{24}OS_3$]$^+$. IR (neat): 2957, 1464, 1077, 1034, 766, 704 cm^{-1}

1-Benzyl-2-(2-(benzylsulfinyl)benzyl)disulfane (*o*-142d)



Synthesised by GP6 and isolated as a pale yellow oil in 119 mg, 78% yield from 0.4 mmol of ***o*-154d**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 – 7.66 (m, 1H, Ar*H*), 7.42 – 7.37 (m, 2H, Ar*H*), 7.37 – 7.31 (m, 2H, Ar*H*), 7.31 – 7.22 (m, 6H, Ar*H*), 7.15 – 7.09 (m, 1H, Ar*H*), 7.05 (dd, *J* = 8.0, 1.3 Hz, 2H, Ar*H*), 4.14 (d, *J* = 12.8 Hz, 1H, S(=O)C*H*$_2$), 4.05 (d, *J* = 12.8 Hz, 1H, S(=O)C*H*$_2$), 3.72 (s, 2H, SSC*H*$_2$), 3.55 (d, *J* = 12.8 Hz, 1H, C*H*$_2$SS), 3.40 (d, *J* = 12.8 Hz, 1H, C*H*$_2$SS) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 142.3 (*C*$_{Ar}$), 137.1 (*C*$_{Ar}$), 135.2 (*C*$_{Ar}$), 131.1 (*C*$_{Ar}$), 130.8 (*C*$_{Ar}$), 130.5 (*C*$_{Ar}$), 129.8 (*C*$_{Ar}$), 129.5 (*C*$_{Ar}$), 128.9 (*C*$_{Ar}$), 128.8 (*C*$_{Ar}$), 128.6 (*C*$_{Ar}$), 128.4 (*C*$_{Ar}$), 127.8 (*C*$_{Ar}$), 125.2 (*C*$_{Ar}$), 63.26 (S(=O)*C*H$_2$), 43.5 (*C*H$_2$SS), 38.7 (SS*C*H$_2$) ppm. HRMS (EI) [M]$^+$ calc.

384.06708 found: 384.0669 $[C_{21}H_{20}OS_3]^+$. IR (neat): 3017, 1738, 1454, 1366, 1229, 1217, 1036, 765, 698, 528 cm^{-1}. Melting point range: 107−109 °C.

8.6. References

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