Flow Reactors for the Continuous Synthesis of Garlic Metabolites

A Thesis Submitted to Cardiff University

in Fulfilment of the Requirements for the

Degree of Doctor of Philosophy

by Alastair Baker

PhD Thesis October 2015

Cardiff University

Dedicated to Caroline Baker

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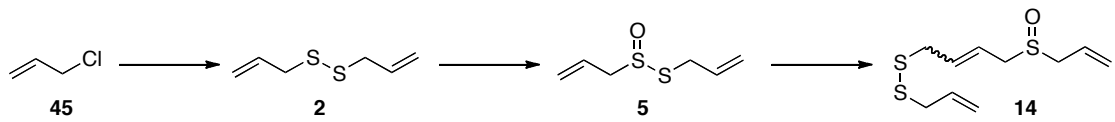
Alastair Baker

Abstract

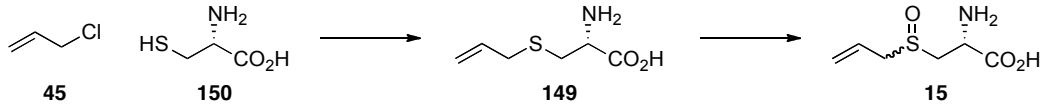
Garlic secondary metabolites are organosulfur compounds that possess prophylactic properties. The chemical composition of garlic oil extracts consists of a combination of these compounds. The instability of a major component, allicin **5**, limits the commercial viability of garlic oil extracts.

The synthesis of garlics organosulfur compounds has been performed in batch reactors. In this thesis, flow reactors were utilised to improve the throughput, reduce the operating conditions.

The thermolysis of allicin **5** is the solitary approach to produce the garlic metabolite, ajoene **14**. Ajoene **14** is has greater stability compared to allicin **5** that possesses interesting biological activity. The primary three-step synthesis investigated consisted of dialkyl polysulfide synthesis, subsequent oxidation and finally the terminal thermolysis.



In addition, other garlic metabolites have also been produced. The synthesis of unsymmetrical monosulfides and their subsequent oxidation was investigated using novel heterogeneous packed-bed flow reactors. The stable amino acid, alliin **15**, is the precursor of allicin **5**. Alliin **15** was also synthesised in homogeneous flow mode. The telescoped synthesis of alliin **15** was successfully completed using a semi-batch reactor.



Development of novel approaches to synthesise garlics organosulfur compounds is reported in this thesis. Finally, the flow reactor systems, experimental details and characterisation of the compounds are described.

List of Abbreviations

°C	Degree Celsius
Ac	Acetyl
AIBN	Azobisisobutyronitrile
Ar	Aryl
BuOH	Butanol
BPR	Backpressure regulator
CV	Column volume
dec	Decomposition
Et	Ethyl
Et_2O	Diethyl ether
ESI	Electrospray ionisation
EtOH	Ethanol
EtOAc	Ethyl acetate
GOE	Garlic oil extract
h	Hour/hours
HFIP	Hexafluoroisopropanol
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
M	Molarity (mol/L)
m-CPBA	Chloroperoxybenzoic acid
Me	Methyl
$(Me)_2CO$	Acetone
MeOH	Methanol
MHz	Megahertz
min	Minute
mL	Millilitre

mL/min	Millilitres per minute
mL/h	Millilitres per hour
mol%	Mole percent
mmol	Millimol
m.p.	Melting point
m/z	Mass over charge ratio
n-BuLi	*n*-Butyllithium
NMR	Nuclear magnetic resonance
n.r.	No reaction
o.n.	Overnight
ppm	Parts per million
Ph	Phenyl
Pr	Propyl
PTC	Phase transfer catalyst
PTFE	Polytetrafluoroethylene
RBF	Round bottom flask
r.t.	Room temperature
RV	residence volume
t-Bu	*t*-Butyl
THF	Tetrahydrofuran

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Chapter 3: Synthesis of Unsymmetrical Monosulfides

Chapter 4: Selective Oxidation of Sulfides

Chapter 5: Thermolysis of Allicin and the Synthesis of Alliin

Research Publications:

Flow Synthesis of Symmetrical Di- and Trisulfides Using Phase-Transfer Catalysis

Flow Alkylation of Thiols, Phenols, and Amines Using a Heterogenous Base in a Packed-Bed Reactor

1

Introduction to Organosulfur Chemistry of Garlic and Flow Chemistry

1.1 Garlic Introduction

Nature can employ mechanisms that cause one organism to succeed at the expense of another. At the macroscopic level, animals have teeth and claws as protection from other organisms. At the microscopic level, animals are susceptible to an array of microorganisms that invade a host to survive and may cause harm to the animal. For protection on the microscopic level, animals possess immune systems to stop the infection.

Plants do not have teeth or immune systems. A common defence mechanism employed by plants for post-infections is the secretion of antimicrobial compounds.[3] These secondary metabolites[4] are known to play an important role in plant defence against herbivores[5] and microorganisms.[6]

Allium is a genus of the *Plantae* kingdom that possesses secondary metabolites that have prophylactic potency. Typically *Alliums* grow underground in anaerobic conditions, which can also be inhabited by anaerobic bacteria. These anaerobic bacteria can be pathogenic and attack the growing root of the plant.

Figure 1.1. *Allium sativum* [7]

Alliums are known to release pungent compounds which arise from sulfur-containing molecules, known as organosulfur compounds. These compounds encompass sulfides, sulfoxides and sulfones. One particular species of this genus that possesses chemically potent organosulfur secondary metabolites is *Allium sativum,* commonly known as 'garlic.'

Garlic contains a high concentration of sulfur compounds compared to other species in the *Plantae* kingdom.

The medicinal benefits of garlic have been known for thousands of years, whilst the chemistry was not understood.[8,9,10] Garlic has been prescribed as long ago as the ancient Egyptians; in one example it was administered as a fertility test on an incorrect conjecture.[11]

Garlic possesses prophylactic properties, a term that includes antibacterial, antiviral, antifungal and antiprotozoal properties. Garlic also has beneficial effects on the cardiovascular and immune systems, which is covered in an extensive review by Harris *et al*.[12]

1.1.1. Garlic Oil Extract (GOE)

In the last three decades, there has been a substantial increase in the use of herbs/botanicals and their potent extracts as natural prophylactics, herbal medicinal products, food supplements, and in the field of livestock nutrition.[8,13]

Extracting the organosulfur compounds from garlic can be performed using several extraction techniques that are covered in the subsequent section. The isolated material is known as garlic oil extract (GOE) and the composition of the organosulfur compounds depends on the extraction technique employed. An advantage of GOE is that it contains an array of organosulfur compounds, as opposed to conventional synthetic single molecule prophylactics. GOE is cited as the most potent quorum sensing inhibitors, a property which impedes bacterial communication.[4] Having been consumed by humans for thousands of years, GOEs possess no risk to humans and has been used as a non-toxic feed component in livestock nutrition.[13] GOE has also been used as an ingredient in an insect repellent for the protection of agricultural crops[14] and food products,[15] and as a mosquito repellent for grassy recreational areas.[16]

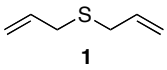
> *'Much of the previous research on garlic and its potential uses has not been specific about the active agent'*
> David Williams, founder of NeemBiotech [1]

1.2. The Precise Chemistry of Garlic

Scientific research on garlic's chemical potency began in the 19th century to identify the molecule or molecules responsible for garlic's prophylactic properties. To separate the organosulfur compounds from garlic, isolation techniques were deployed and developed.

1.2.1. Diallyl Monosulfide

The first chemist to employ an isolation technique on garlic and quantitative analysis was the German chemist Theodor Wertheim.[17] Using steam distillation, garlic oil was isolated and described as a sulfur-containing liquid. Wertheim described the garlic oil extract as 'allylschwefel,' from the Latin name of the plant *Allium sativum.* The name became allyl sulfide in English. This molecule has several modern names, however for the duration of this thesis diallyl monosulfide **1** will be used.



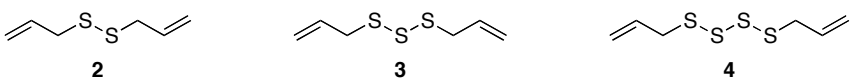
1

Figure 1.2. Diallyl monosulfide

The chemical term 'allyl' was used to describe the propen-2-yl moiety and arose from the Latin for garlic, *Allium*.[18] The 'allyl'-moiety definition was cemented in chemical terminology when French chemist, Cahours, in collaboration with the renowned German chemist Hofmann, synthesised this version of garlic oil from a newly discovered alcohol.[19] In uniformity with Wertheims publication, the alcohol was named allyl alcohol.

1.2.2. Diallyl Disulfide and Higher Polysulfides

The steam distillation employed by Wertheim was a rather harsh extraction technique and high temperatures are now known to cause spontaneous desulfurisation.[20] When milder fractional distillation was employed, different compounds were isolated (Figure 1.3).



2 diallyl disulfide | **3** diallyl trisulfide | **4** diallyl tetrasulfide

Figure 1.3. Diallyl polysulfides isolate from garlic by fractional steam distillation

In 1892, the German chemist Semmler fractionally distilled garlic, under reduced pressure. The major component was discovered to be diallyl disulfide **2**. Small amounts of diallyl polysulfides were also reported, containing several sulfur atoms that were identified as **3** and **4**.[21]

Furthermore, when the distillation was performed under reduced pressure; no diallyl monosulfide **1** was isolated. The prolonged heating at elevated temperature using Wertheim's steam distillation resulted in the polysulfides being reduced, eliminating sulfur from the polysulfides. With the development of modern analytical techniques such as high performance liquid chromatography (HPLC), it was revealed that the composition of steam distilled GOE is more complex and contains many sulfides, shown in Table 1.1 Dialkyl polysulfides have been detected containing as many as 6 sulfur atoms.

Table 1.1. Composition of steam-distilled garlic oil [22]

Compound	Steam distilled composition (%)[a]
diallyl monosulfide **1**	2
diallyl disulfide **2**	25.9
diallyl trisulfide **3**	18.5
diallyl tetrasulfide **4**	8.1
diallyl pentasulfide	2.1
diallyl hexasulfide	0.4
methyl allyl monosulfide	0.9
methyl allyl disulfide	12.5
methyl allyl trisulfide	15.2
methyl allyl tetrasulfide	6
methyl allyl pentasulfide	1.7
methyl allyl hexasulfide	0.3
dimethyl monosulfide	-
dimethyl disulfide	1.3
dimethyl trisulfide	3.4
dimethyl tetrasulfide	1.3
dimethyl pentasulfide	0.4
dimethyl hexasulfide	0.1

[a] weight percent of total sulfur compounds

Polylsulfides containing a greater number of sulfur atoms can be synthesised using liquid sulfur; a diallyl species was synthesised containing 22 sulfur atoms.[23] Some dialkyl polysulfide mixtures are patented and commercially produced by EcoSpray Limited and utilised on sports grounds as an effective nematicide.[24]

1.2.3. Allicin

Early research into the antibacterial properties of GOE against both Gram-positive and Gram-negative bacteria focussed on diallyl disulfide **2**, as this was the primary compound of GOE. In 1944, raw garlic exhibited antibacterial properties whilst **2** did not.[25] The conclusion was that **2** was not the active antibacterial molecule and that the current isolation methods of GOE were too intense and caused degradation of the prophylactic compounds.

A milder extraction technique had to be employed to isolate these compounds. Ethanolic extraction and subsequent filtration at room temperature produced garlic oil that had not been exposed to additional heating. The molecule isolated was allicin **5**. This compound possessed about 1% of the antibacterial activity of penicillin, and was also effective against the Gram-negative organisms which were practically unaffected by penicillin.[25] Its prophylactic activity is so extensive and the focus of a review,[26] and patented in several applications.[27,28]

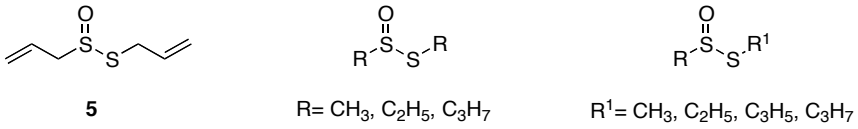


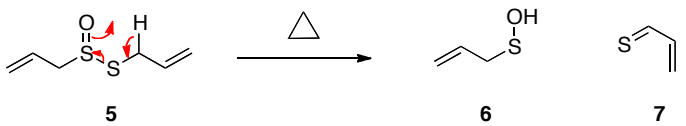
Figure 1.4. Allicin and thiosulfinate homologues found in garlic

In 1955, Fujiwara *et al*. developed a paper chromatography method for the analysis of GOE.[29] The authors were able to isolate and identify several other thiosulfinate homologues of allicin from garlic and onions (Figure 1.4). In addition, the reactions between these thiosulfinates can produce unsymmetrical thiosulfinates and will be discussed in the subsequent section.

1.2.4. Allicin Decomposition

Allicin **5** is a very reactive and unstable molecule; it can react in numerous ways and decomposes already at room temperature.[30] The previously reported thermal decomposition is described as an intramolecular process (Scheme 1.1) in which allicin **5** produces allyl sulfenic acid **6** and thioacrolien **7**.[31]

Compound **5** decomposition produces a thioacrolein **7** and allyl sulfenic acid **6**. Compound **6** condenses with another molecule of **6** to form **5** once again. Compound **7** will react via a Diels-Alder reaction to generate two structural isomers known as dithiins (**8**, **9**). The concentration of allicin exponentially decreases as stable dithiins are eliminated on each cycle.

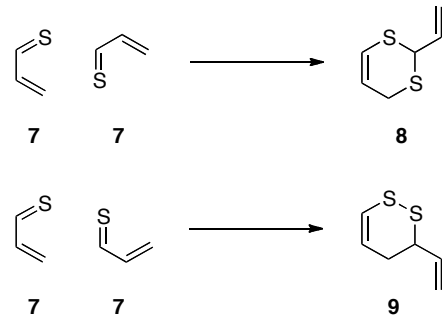


5 **6** **7**

Scheme 1.1. Thermolysis of allicin intramolecularly via a Cope-type rearrangement

1.2.4.1. Thioacrolein and Dithiins

Thioacrolein is also a very reactive molecule that rapidly dimerises with itself, proposedly through a Diels-Alder mechanism. There are two approaches through which the thioacrolien can react, producing two regioisomers. The 3-vinyl-4*H*-1,3-dithiin **8** is formed as the major product and the 2-vinyl-4*H*-1,2-dithiin **9** as the minor product with a ratio of **8**: **9** is 4.4: 1.[32]



7 **7** **8**

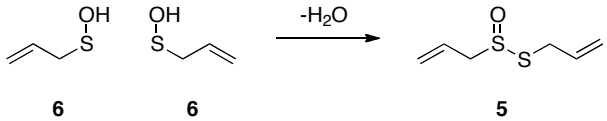
7 **7** **9**

Scheme 1.2. Thioacrolein dimerization producing two dithiin regioisomers

The dithiins possess antithrombotic activity.[33] However, compound **8** has greater pharmacokinetics as it is more lipophilic and accumulates in the fat tissues of rat systems.[34]

1.2.4.2. Allyl Sulfenic Acid and Allicin

Allyl sulfenic acid **6** generated from the breakdown of **5** contains a very reactive sulfenic acid (R-S-O-H) moiety and rapidly condense in under a minute to form allicin (Scheme 1.3).[35]



6 **6** **5**

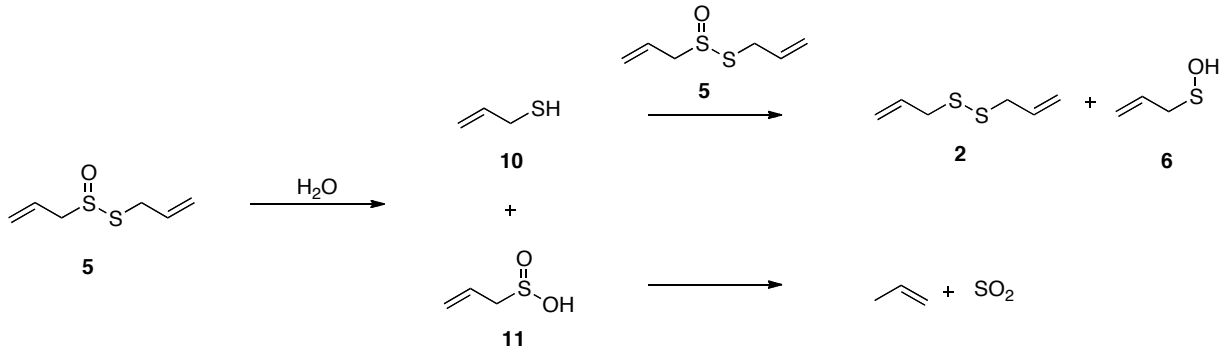
Scheme 1.3. Condensation of allyl sulfenic acid producing allicin

Vaidya and co-workers postulate that allyl sulfenic acid **6** is responsible for garlic's anti-oxidant activity.[31] The thermal initiated autoxidation of methyl linoleate by allicin in hydrogen-bond donating solvent, hexafluoro-2-propanol, was investigated. Hexafluoro-2-propanol hindered the allicin degradation, and did not inhibit the autoxidation of methyl

linoleate. The conclusion was that the degradation products of allicin, specifically **6**, have anti-oxidant activity.

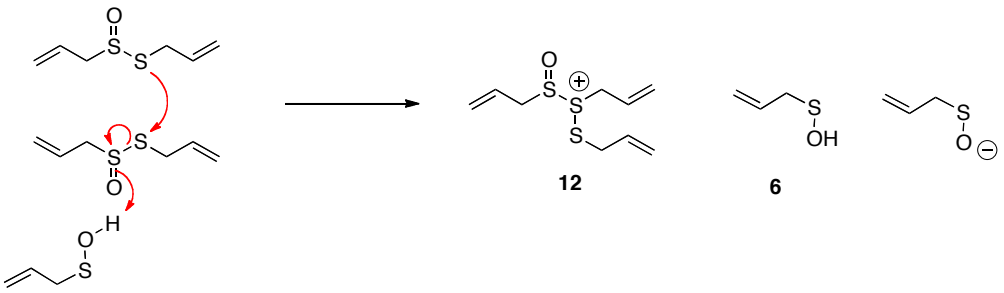
1.2.4.3. Further Allicin Decomposition Mechanisms

The thiosulfinate within **5** can hydrolyse (Scheme 1.4). This produces allyl thiol **10** and compound **11**. The latter, compound **11**, degrades to produce sulfur dioxide gas and volatile propene. Compound **10** can also react with **5**, which produces stable diallyl disulfide **2** and reactive **6**. The latter, as previously explained, condenses to produce **5** again. It is very difficult to exclude water from a reaction, as it is always present in garlic cells and soil, so these side reactions will occur.



Scheme 1.4. Allicin **5** degradation with water

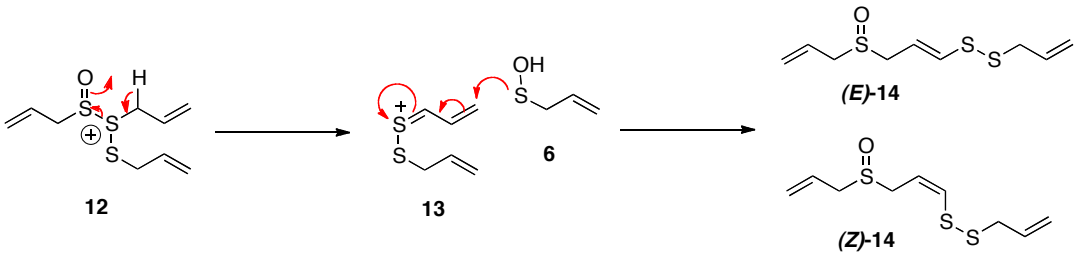
Allicin also degrades by reacting with itself. Two molecules of allicin **5** can react to form **6** and a charged trisulfide intermediate **12** (Scheme 1.5).



Scheme 1.5. Allicin **5** reacting with itself to form the charged trisulfide intermediate **12**

Compound **6** will condense with itself to form allicin again as previously discussed (Scheme 1.6).

The charged trisulfide intermediate **12** will eliminate another molecule of **6** and a charged disulfide **13**. Finally compound **6** reacts with the charged disulfide **13** at a terminal allyl group to produce ajoene **14**. The double bond produces the geometric isomers (*E*)-**14** and (*Z*)-**14**.



Scheme 1.6. Formation of ajoene **14**

1.2.5. Ajoene

In 1983, Jain and Apitz-Castro subjected GOE to silica gel chromatography. The composition of one of the fractions was unknown; however with the help of Block in 1984 the molecule was identified as **14**.[36] This organosulfur compound contains three unsaturated double bonds, a disulfide and a sulfoxide moiety.

It has also been reported that the *Z*-**14** isomer exhibits twice the antibacterial activity *E*-**14**.[37] As a result of altering the functionalities by Hunter *et al*. a structure/ reactivity hypothesis for *Z*-**14** was proposed (Figure 1.5).[38]

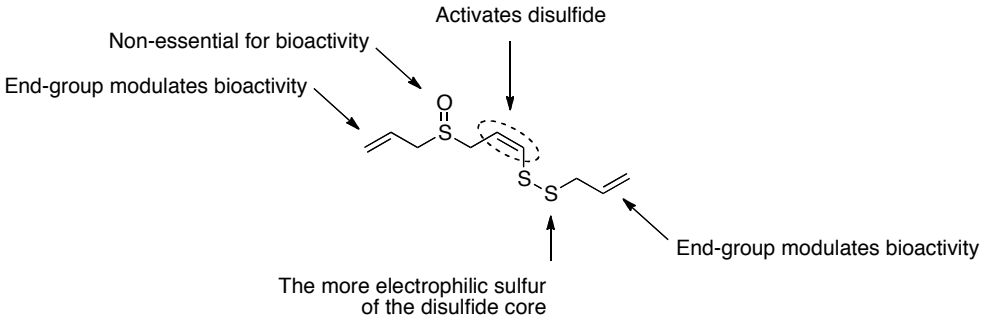


Figure 1.5. Proposed structure/ reactivity hypothesis for *Z*-**14** based on cancer-cell WHCO1 proliferation [38]

1.2.6. Alliin, *S*-alkyl-L-cysteine sulfoxides and Allicin Formation

The potent, odorous organosulfur compounds are secondary metabolites, as described in the beginning of this chapter.[4] However, these are only formed after plant cells are damaged. The reactive thiosulfinates are stored as alkyl-L-cysteine sulfoxides, which are odourless. An enzyme known as alliinase is responsible for the release of thiosulfinates. This C-S lyase enzyme is also reported as EC.4.4.1.4. In the cytoplasm of intact cells alliin is separated from the enzyme, which is stored in the vacuole.

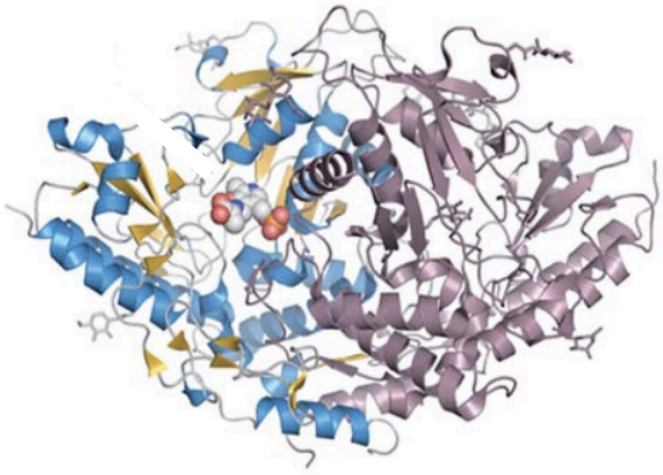
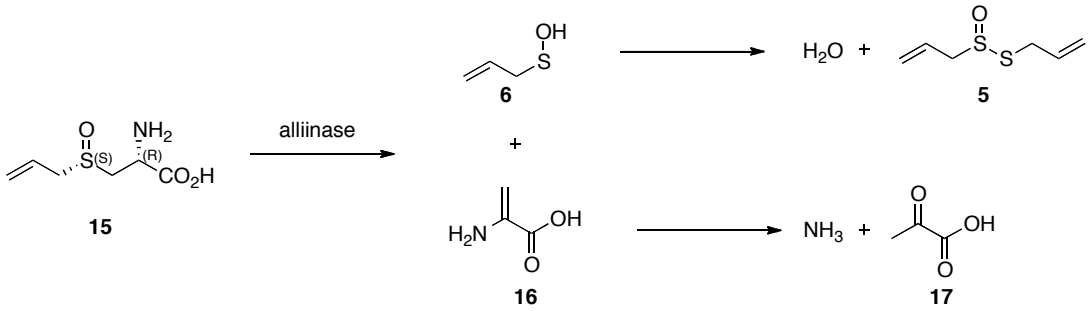


Figure 1.6. Structure of the alliinase dimer
Each monomer is coloured differently [39]

In garlic, the allicin **5** precursor is the amino acid alliin **15**, also designated ACSO. The enzymatic action of alliinase upon **15** produces 2-amino acrylate **16** and allyl sulfenic acid **6**. The latter condenses to form allicin **5**, which has already been covered earlier in the chapter.



Scheme 1.7. Alliinase reaction with alliin **15** to produce allicin **5**

In 1948 Stoll and Seebeck isolated **15** after successive extraction with ice-cold aqueous acetone.[40] In 1950, Stoll and Seebeck synthesised racemic **15**,[41] the chiral (R_CS_S)-**15** was isolated the following year performing fractional recrystallisation using 60% aqueous acetone.[42]

There are several other alkyl-L-cysteine sulfoxides in varying quantities in genus *Allium*, and the *Brassica* and *Phaseolus* genera, [43] which have also been isolated by Fujiwara *et al*. by paper chromatography (Figure 1.7).

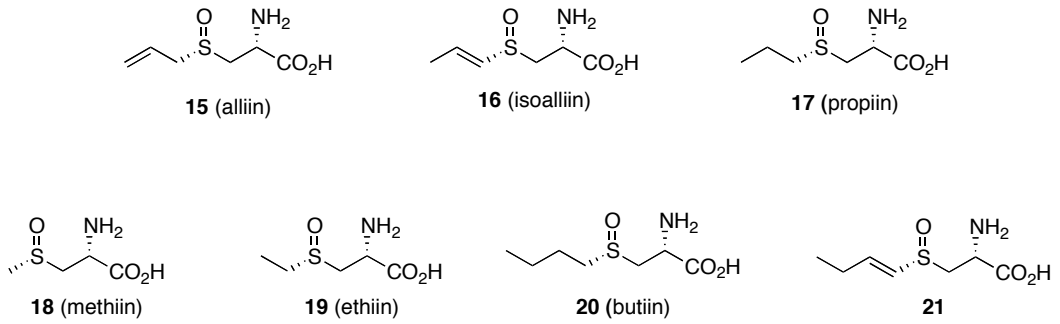
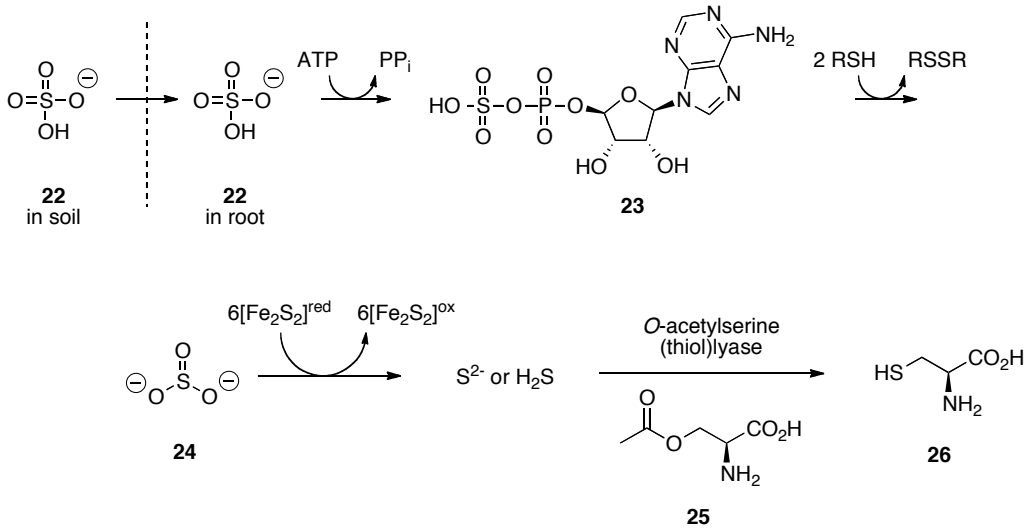


Figure 1.7. Alkyl-L-cysteine sulfoxides found in garlic

1.2.6.1. Biological Uptake of Sulfur and Synthesis of S-alkyl-L-cysteine sulfoxides

The *Allium* genus contains a high quantity of sulfur. The major sulfur sources are sulfate salts that are taken up through the roots of plants. The sulfate ions are converted into organic sulfides through the sulfur assimilation process, shown in Scheme 1.8.



Scheme 1.8. Sulfur assimilation process [44]

The inorganic sulfate **22** is adsorbed into the plant through the root by osmosis. Compound **22** is activated by adenosine triphosphate (ATP) to **23,** which is further reduced by adenylyl-sulfate (APS) reductase and glutathione to produce the sulfite **24**. Ferredoxin then reduces **24** to hydrogen sulfide or sulfide dianion, a nucleophilic source of sulfur. This reacts with *O*-acetylserine **25** to produce L-cysteine **26** by *O*-acetylserine (thiol) lyase. Then **26** is alkylated and finally oxidised selectively to produce the alkyl-L-cysteine sulfoxides.

1.3. Potency of Garlic Metabolites

The combination of the organosulfur compounds in GOE gives rise to garlic's prophylactic properties. The stability and potency vary for each organosulfur compound. An example is diallyl monosulfide, which is an essential non-toxic ingredient in an ingested insect repellent.[45]

Allicin, which is initially formed in the greatest quantity, demonstrates great biological activity against bacteria, fungi, viruses and several cancer cell lines. However, its application requires cold temperature storage, as previously stated, because it decomposes within 24 h. One of the components that it forms is allyl sulfenic acid, a very reactive compound hypothetically responsible for the anti-oxidant properties of garlic.[31] The thioacrolein is also very reactive and formers dithiin dimers which are known to have antithrombotic activity.[33] Degradation of allicin also forms polysulfides that are sold as nematicide. Ajoene **14** is known to have similar potency to allicin **5** and also inhibit quorum sensing.

1.4. Flow chemistry

1.4.1. Introduction

Continuous flow chemistry is an emerging technology that has expanded the approach to the implementation of chemistry in synthesis. Reagents are pumped continuously through a reactor, which can lead to high output. Continuous flow chemistry also encompasses microchemistry when performed in micro-reactors. Flow mode has the potential for continuous production, as opposed to batch mode where production is performed in discrete batch reactors. This emerging technology has been the focus of many reviews and the methodology is transitioning from research to industrial scale,[46] it has already been implemented in the synthesis of active pharmaceutical ingredients.[47]

The residence time within the flow reactor is the reaction time in a flow system. This is calculated based on the volume of the flow reactor and the time it takes the reagents to be pumped through. Two or more streams of reagents, for example reactant and reagent, are pumped at predetermined flow rates using pumps. Figure 1.8 shows the standard schematic diagram that will be used throughout this thesis. The streams are combined using a mixer (M_1) into a single reactor (R_1) that can be made from an array of materials (Figure 1.10).

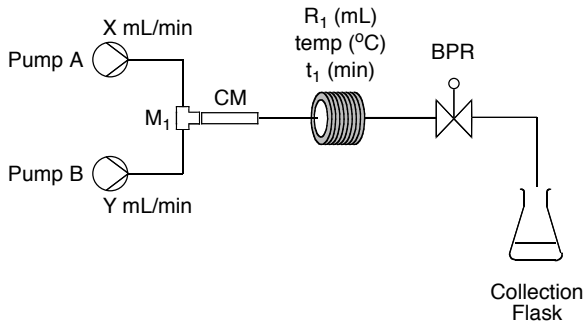


Figure 1.8. Schematic flow diagram
[M_1] t-piece mixer, [CM] Comet mixer, [R_1] PTFE coil reactor, [BPR] backpressure regulator

Traditionally, batch reactors have been glass round bottom flasks (RBFs) for laboratory scale (mL) or continuously stirred tank reactors (CSTR's) at industrial scale (L), shown in Figure 1.9. However, reaction conditions developed in batch mode at laboratory scale often require additional optimisation when performed at industrial scale. The greater bulk of material creates issues of thermal conductivity and mixing.

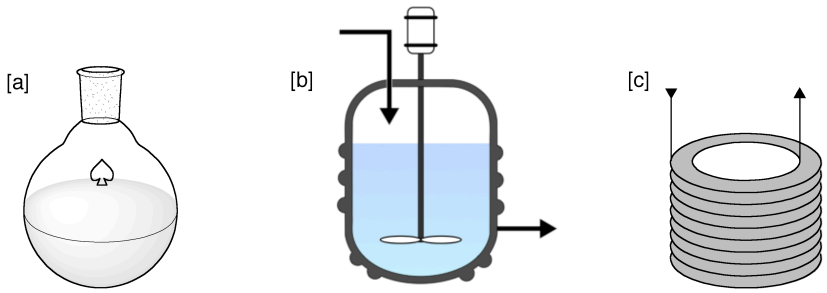


Figure 1.9. Batch and flow reactors schematics.
[a] RBF, [b] CSTR, [c] flow reactor

Flow chemistry has been a technology that utilises a high surface to volume ratio, which improves thermal conduction of the bulk solution as it is pumped through the flow reactor. The narrow diameters have also given access to properties of a flowing liquid, such as internal vortexes in biphasic solutions. Finally, the scale up of flow chemistry can avoid additional re-optimisation; a flow reaction could be run for longer or additional reactors in series to 'number up' could be used. In the subsequent section, the field of flow chemistry will be explained in greater detail.

1.4.2. Flow Reactors

There are many flow reactors that can be employed ranging from nano- to meso-scale made from an array of materials that are combined with ever improving pumps. This allows the unique properties of different materials to improve reactions performed in flow mode.[48]

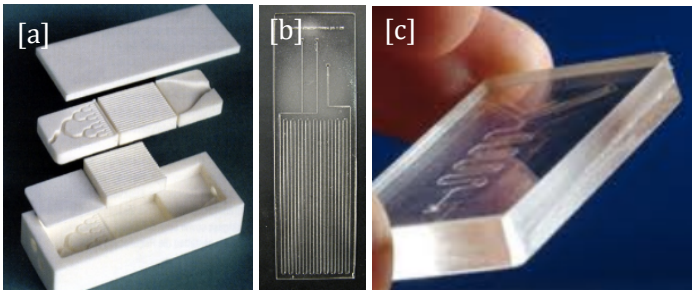


Figure 1.10. Flow reactors fabricated from various materials.
[a] ceramic, [b] glass, [c] plastic [49]

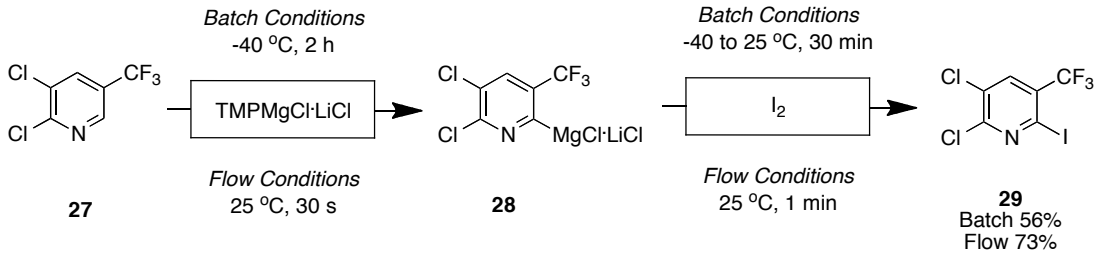
These reactors possess different benefits and are applicable to a wide array of chemistry from concentrated acid, segmented liquid-gas reactions and electrochemistry. PTFE tubing is evaluated in this thesis due to its low cost that allows for fast and inexpensive fabrication of components, such as multistep reactors (Section 4.6).

1.4.3. Temperature Control

The high surface-area to volume ratio in flow mode enables great control over heat transfer/ temperature control. Hot spots, associated with batch mode, are eliminated and more effective heating is applied to the reaction. Controlling temperature during reactions is very important; poor control can lead to degradation of products, formation of side products or, in extreme cases, exothermic runaway.

Heat is transferred to the interior of a reactor by heating the exterior. Heating the bulk of the interior is related to the surface to volume ratio. Flow reactors have much greater surface to volume ratios than batch. This results in greater heat transfer that enables more precise control of temperature, and fast heating and cooling.

Organometallic reactions often use cryogenic temperatures and long reaction times in batch mode. Work by Peterson *et al*. demonstrated that flow mode enable the metalation to be performed under more convenient conditions due to greater temperature control and improved the yield compare to the batch reaction (Scheme 1.9).[50]



Scheme 1.9. Comparison of heat transfer in flow mode and batch mode for the metalation of **27** [50]

Endothermic reactions have also benefited from the heat transfer through the high surface-area. A cooled batch vessel in a production plant that performed a reaction in 4 h was superseded by continuous micro-reactor lab plant that could complete an undisclosed reaction in less than a minute at 150 °C.[51] Super heating in flow can also be achieved when flow mode is combined with microwaves. The temperature achievable in flow mode is greater than for a microwave batch unit. This effect was demonstrated in the synthesis of two pharmaceutical relevant compounds, indacaterol and metoprolol.[52]

There are several examples of how enhanced heat transfer using flow chemistry has benefited a reaction; these are covered in a comprehensive review by Jensen *et al*.[53]

1.4.4. Mixing

Flow mode also improves mixing of reactions comparative to traditional batch mode. When reactants are added to a reactor, the initial process that mixes molecules is diffusion, which is a slow process. Further mixing involves agitation and in batch mode this is typically done using a magnetic stirrer or impeller, shown in Figure 1.11. This figure also illustrates that mixing in batch mode is time dependant. This can lead to uneven mixing in batch mode, which may induce areas of high concentrations. This, in turn, may result in hazardous hot spots being formed.

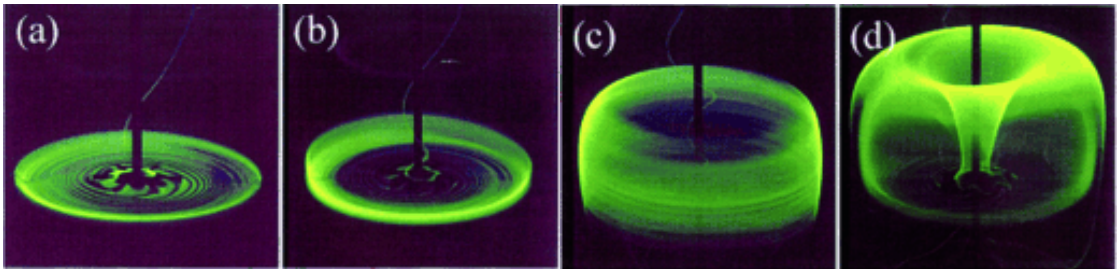


Figure 1.11. Impeller mixing of fluorescent dye (rhodamine G), injected close to the shaft of a single impeller into the 20 L vessel filled with glycerin in batch,[53] shown at a) 3s, b) 5s, c) 10s, d) 30s

Flow chemistry has been used to overcome uneven mixing, and as a result improves rate of reaction and reaction yield.[54] When two reagents streams are combined at a mixer; the combined concentration is then carried through the flow reactor. Enhanced mixing is attributed to the functionalisation of a secondary amine; in flow mode the reaction was complete in under 2 min as opposed to 24 h in a batch mode.[55] Rapid mixing in oxidation has been shown to prevent byproduct formation.[56]

However varying several factors, such as temperature, tube diameter and flow rate (the velocity of the fluids), can induce different flow regimes. Reynolds discovered that combining two fluids in a tube led to either laminar or turbulent flows.[57] Laminar flow is when a fluid's vector is very ordered as the molecules move parallel to the tube wall and is exhibited at lower flow rates (Figure 1.12 [a]). Turbulent flow is the opposite effect generated at higher flow rates, in which the flow is characterise by eddies, which perpetuates disruption to vectors of the fluid flow (Figure 1.12 [b]).

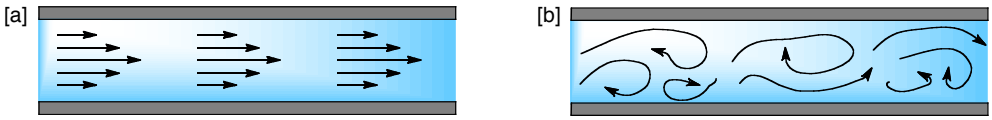


Figure 1.12. Diagram of flow vectors in tubing
[a] laminar flow, [b] turbulent flow

The variation arising from different velocities is induced by the adhesive forces of the channel wall causing the liquid nearest the wall to have laminar flow even at high flow rates. These phenomena can be characterised by a dimensionless quantity known as Reynolds numbers. Laminar flow has Reynolds numbers lower than 2000 and turbulent flow has Reynolds numbers greater than 3000.

$$Re = \frac{inertial\ forces}{viscous\ forces} = \frac{\mathrm{v}L}{v}$$

Equation 1.1. Reynolds equation
[v] mean velocity of the fluid (m/s), [L] travelled length/diameter of the fluid (m)
[v] kinematic viscosity (m^2/s)

The motion of the fluid is described as turbulent when subjected to high inertial forces. While the average vector is continuous moving through the reactor, vectors in fluid are constantly changing (Figure 1.12 [b]). When laminar flow is established at lower flow rates, the lateral mixing and eddies are not induced.

1.4.5. Liquid-Liquid Biphasic Reactions and Extractions

The hydrophobic nature of immiscible organic solvents creates a phase boundary with water (Figure 1.13 [b]). The phase boundary, known as the interface, creates a region where molecules have a different molecular environment created by the repulsion from the surface not found in the bulk. Reaction between organic reagents and aqueous reagents occur at the interface. In batch mode there is a great bulk and only one interface. To mix the phases, the interface is disturbed, agitated or stirred and droplets are formed and swirled in the adjacent phase.

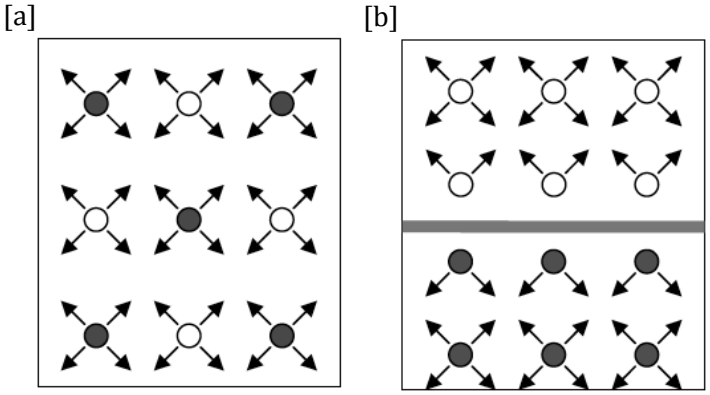


Figure 1.13. Diagrams of molecular repulsion at an interface
[a] a single phase system of two solvents as a homogenous mixture
[b] a biphasic system of two solvents as result of unbalanced forces at the boundary [58]

In flow mode, two immiscible solutions form a series of fluid packets and gives rise to a phenomenon known as segmented flow or plug flow (Figure 1.14). This creates many

packets with two interfaces; this induces internal vortices in each localised packet that increases mixing. Work has demonstrated that when the aqueous miscible solvent mixture is not segmented it exhibits laminar flow.[59]

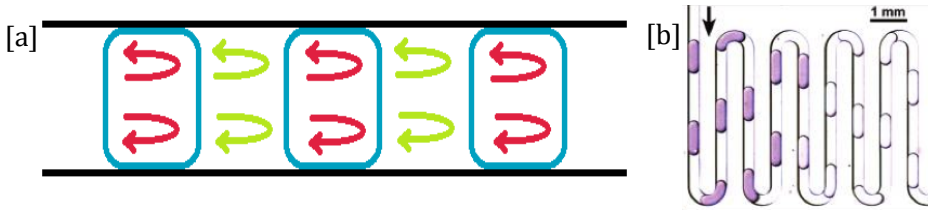


Figure 1.14. [a] diagram of interval vortices in segmented flow
[b] microphotograph of the microfluidic channel of an autocatalytic reaction [59]

This segmented flow phenomenon arises from the high surface tension. Using a droplet of oil on a bulk of water, at the interface boundary the droplet of oil will have one repulsive contact point and will 'run' across the interface. This can be exploited in flow mode; droplets of an organic phase can be formed and suspended in an aqueous solution in a micro channel, shown in Figure 1.15.

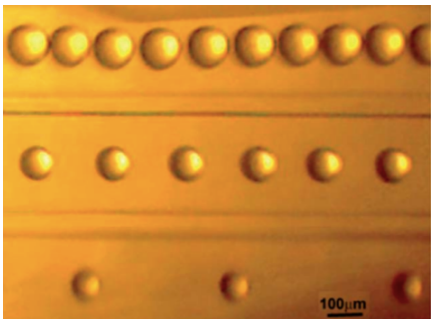
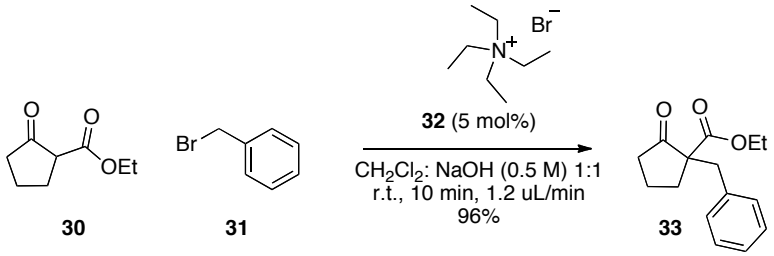


Figure 1.15. Optical micrograph of droplets formed in micro-channels with aqueous/organic phase [60]

1.4.5.1. Phase Transfer Catalysis

Many biphasic reactions require a phase transfer catalyst (PTC) to improve the reaction across a liquid-liquid interface by facilitating the migration of reagents. These are often charged quaternary nitrogen or phosphorus groups that solubilise reacting salts into the organic phase. PTC's have been known to speed up reactions when performed in flow chemistry .[61]



Scheme 1.10. Phase-transfer benzylation catalysed flow reaction of ethyl 2-oxocyclo- pentanecarboxylate [61]

1.4.5.2. Inline Phase Separation

Liquid-liquid extraction is used to isolate an organic product. It is performed using an immiscible organic solvent with greater volatility than the aqueous solvent system for practicality. Traditionally, laboratory batch mode extractions are discrete process and are performed in glass separation funnels.

Liquid-liquid biphasic reactions in flow mode have to be separated continuously. Recent developments have transformed this work-up into a continuous process to simplify the downstream processing. Flow chemistry is capable of performing this process as a continuous process.

The Ley group in 2012 developed prototype camera-and-computer based gravity separation.[62] The biphasic mixture was eluted into a separator that was monitored by the camera attached to a computer that determined the rate of extraction. The same system was developed further and demonstrated its productivity by isolating 20 g over 24 hours.[63]

Membrane separators consist of membranes with pores and have emerged as a commercially available device.[64] Jensen *et al*. developed a membrane phase separator that could be placed inline into a flow system. The membrane device was capable of complete phase separation of aqueous/organic and aqueous/fluorous systems.

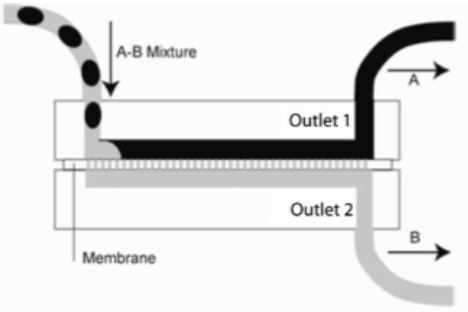


Figure 1.16. Microfluidic liquid–liquid design from Jensen *et al.*[64]

A PTFE membrane with 0.45 μm pores constructed by Cervera-Padrell *et al*. was able to perform the separation of aqueous THF up to 40 mL/min.[65] Work reported by Ley *et al.* utilised the commercially available Syrris FLLEX liquid-liquid membrane-based extraction unit to automate the work-up of alcohol alkylations.[66] Continuous inline phase separation process has been used in multistep reactions.

1.4.6. Multistep

In classic chemistry, liquid-liquid phase separations and isolations between steps are common in a multistep synthesis. Flow chemistry also suffers from these discrete processes in the terminal step between reactions. Developing phase separation inline would maintain the continuous flow and as such would be ideal for multistep synthesis. Jensen and co-workers have exhibited that liquid-liquid phase separation could be performed in flow mode using membranes and exploiting laminar flow.[67] This allowed subsequent reactions to be performed in the same continuous flow process.

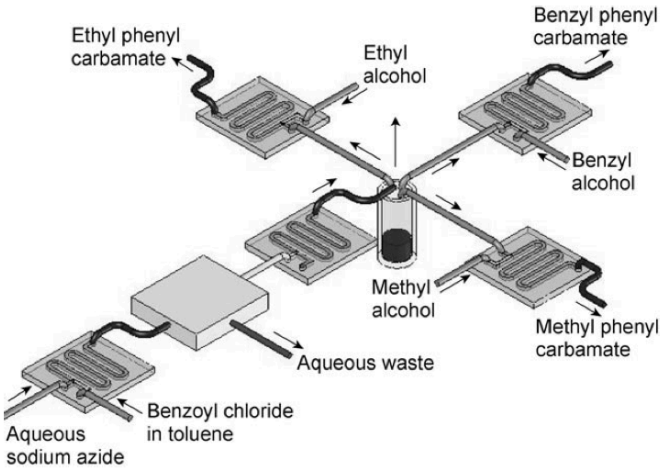


Figure 1.17. Multistep flow synthesis of carbamates from acid chlorides with an inline phase separation [67]

Inline purification by distillation was also developed (Figure 1.18). The reaction was an initial activation of phenols to the corresponding triflate performed in methylene chloride. The reaction mixture was then combined with hydrochloric acid solution (2.0 M) that

created segmented flow and extracted the salt impurities. The segmented passed through a liquid-liquid phase separator and the methylene chloride containing the purified aryl triflate was combined with dimethylformamide.[68]

Before the terminal Heck homologation was performed in dimethylformamide and required removal of the methylene chloride. This was performed by distillation at 125 °C to boil the methylene chloride that was removed by a stream of nitrogen. This solvent switch permitted the Heck reaction to be performed at higher temperatures that increased the yield and reduced the quantity of methylene chloride.

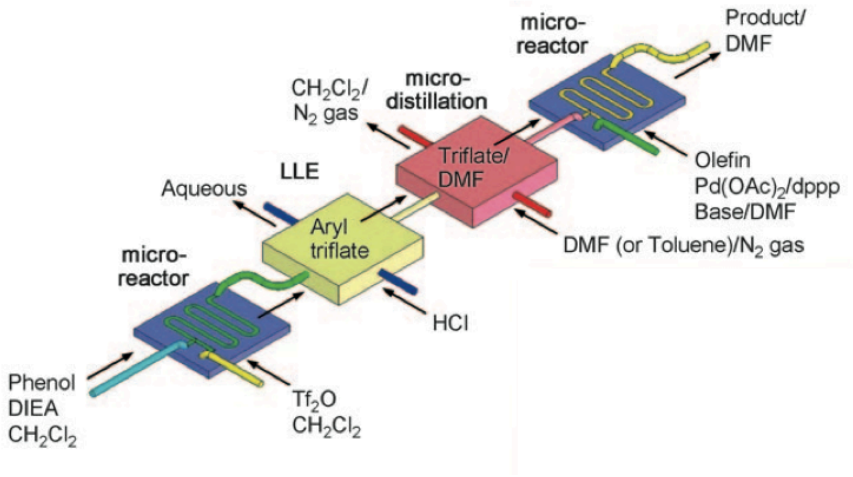


Figure 1.18. Inline micro-fluidic distillation[68]
[LLE] liquid-liquid phase separator

There is an extensive review that highlights successes in the area of multistep natural product synthesis in flow chemistry, with an emphasis on new opportunities and technological advances.[69] There are also numerous notable continuous flow syntheses that are enjoyable and worth of mentioning; Seeberger's antimalarial artemisinin synthesis,[70] Ley's breast cancer drug (E/Z)-Tamoxifen synthesis,[71] Jamison's antihistamine diphenhydramine hydrochloride synthesis,[72] McQuade's landmark ibuprofen synthesis,[73] Jamison's modified ibuprofen synthesis[74] and Trout's end-to-end continuous manufacturing of aliskiren hemifumarate.[75]

1.4.6.1. Heterogeneous (solid-liquid) Reactions

Flow mode can be further improved with the use of heterogeneous, solid-supported reagents. These would capitalise on much greater mass and heat transfer. There are three main categories; solid-supported reagents, solid-supported catalysts and solid-supported scavengers.[76] Used in combination with flow mode, clean and practical methods could be developed.

1.4.6.2. Semi-batch/ Semi-flow

The combination of flow mode and batch mode is known as semi-batch or semi-flow. The reagents are pumped from flow system into a batch reactor. This is used to maintain a concentration of a reactant and increase selectivity.[77] In the presence of a secondary reaction, slow addition keeps the concentration of the primary reagent low and form the primary product. It also allows the opportunity to add more reactant over time.

1.4.6.3. Scaling up a Flow System

Flow chemistry has been widely explored in academia, and industry employs the technologies where they are economically more favourable than batch systems. As early as 2001, an undisclosed reaction in a two-step CYTOS lab flow system was producing 59 g/hr with an 88% yield, which was scaled up to a pilot plant in 2005 and reported to be producing 20 ton/yr.[78]

1.4.6.4. Reaction Screening

Flow chemistry also lends itself to rapid reaction screening of different conditions. Goodell and co-workers made an automated multidimensional flow system that in 2009 demonstrated several reactions on a densely functionalized bicyclo(3.2.1)octanoid scaffolds. Using this setup they were able to perform 1000 reactions on analytical scale.[79] The following year Goodell worked with Treece *et al.* using a similar multidimensional screening of polycyclic iminium ethers using a flow system for 400 reactions.[80]

1.4.6.5. Reaction optimisation

As a final note on the power of flow reactors combined with computer automations and inline synthesis, it is worth mentioning the review by McMullen and Jensen which discusses all the available inline sensors and the plethora of information that could be acquired with inline reaction monitoring.[49] This rapidly emerging continuation of flow chemistry development will improve reactor development, but a number of technical challenges still remain.

1.4.7. Benefits of Flow Mode compared to Batch Mode

The parameters controlled in a chemical reaction are different in flow mode compared to batch mode. They are numerous comparisons between batch and flow mode.[81,82,83,84,85] Several of these parameters are controlled more efficiently or improved using flow mode, as will be explained in this section. The parameters include mixing, heating, safety, space allocation, automation and multistep reactions.

Reagents are mixed immediately at connectors in flow mode, which is a great improvement on diffusion in batch mode. Batch mode traditionally used additional apparatus, such as stirrer bars and mechanical mixers, to overcome this. Greater control of mixing is also exhibited and leads to greater reproducibility between batches. With flow mode, only a fraction of the reagents are within the reactor. If a reaction was to fail, only the current reactor volume is lost and the unused reagents are preserved. This can also be determined *via* inline analysis, such as Mettler Toledo FlowIR.

The temperature can also be monitored and due to the high surface area-to-volume ratio, the heat transfer is intensified. Rapid and elevated reaction heating of the small volume reactors can be achieved easier, which allows for greater control over the course of the reaction and safely generate reactive intermediates.[86] This also allows greater accessibility for heating or cooling a reaction, in endothermic or exothermic cases respectively. In the latter case, the effective heat dissipation can prevent thermal runaways.[87] Thus, flow mode improves the safety of the process.

This introduction has already demonstrated the ability of flow mode to run several reactions in a series of linked reactors. This is a paradigm shift when compared to multistep batch reactions that require discrete reaction time and isolations between reactions. This is known as 'process intensification,' which is defined as;

> *"the development of novel apparatuses and techniques that, compared to those commonly used today, are expected to bring dramatic improvement in manufacturing and processing, substantially decreasing equipment-size/production-capacity ratio, or waste production, and ultimately resulting in cheaper sustainable technologies."* [2]

Flow chemistry will be one of the ultimate tools to achieve this goal. Such systems, like inline purification systems, which can further improve the yields of multistep reactions,[68] are described in a comprehensive review by Ley *et al*.[46] There are numerous continuous

techniques that are still being developed for flow mode; liquid-liquid separation, solid phase scavenging, chromatographic separation and recrystallisation. The capacity to scale up in flow mode has also proven to be greater, with higher reproducibility over a range of scales and conditions, or by simply 'numbering up.'

Flow mode is also more amenable than batch mode for automation. Combining flow systems with inline analysis, operated reactions can self-correct.[58] Inline analysis, combined with statistical systems, has also been demonstrated to reduce time and costs to discover the optimum conditions and greater insights into the reactions.

The systems utilised in flow mode are also smaller compared to batch systems.[88] This miniaturisation reduces the space requirement in laboratories. With the combination of new inline flow technologies, process intensification is inevitable and will result in reducing the size of chemical plant required. However, size is not the only indication of process intensification. Space-time yield calculation can be used to determine reactors viability for a commercial process. The amount of product (kg) divided by the time duration of the reaction (h) and the reactor volume (L).

1.4.8. Disadvantages of Flow Mode compared to Batch Mode

Although flow chemistry is an emerging field, initial investment for the dedicated equipment needs to made, and with the diversification of reactors available extra experimentation and evaluation would have to be made. Start up and shut down procedures have to be developed with the focus on obtaining a steady state, where as batch mode simplicity and availability may be more favourable with lower investment required. The latter means batch mode has greater versatility.[89]

1.5. Overview

Garlic organosulfur compounds possess prophylactic properties. Advantageous flow mode synthesis was utilised to improve the throughput and reduce the operating conditions optimised in batch mode. Additional metabolites were synthesised for biological testing and the production of HPLC standards.

The thermolysis of allicin is the solitary approach for producing the garlic metabolite, ajoene **14**. The primary three-step synthesis investigated consisted of dialkyl polysulfide synthesis, subsequent oxidation and finally the terminal thermolysis.

In addition, other garlic metabolites have also been produced. The synthesis of unsymmetrical monosulfides and the oxidation of these compounds were investigated using novel heterogeneous packed-bed flow reactors. S-alkyl-L-cysteine sulfoxides synthesis was also achieved using homogenous semi-batch reactions.

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2

Synthesis of Symmetrical Polysulfides

2.1. Introduction

The polysulfides of garlic are formed from the thermolysis of thiosulfinates in the presence of water producing thiols. The thiols can undergo oxidation to produce polysulfides.[1] The thiosulfinate allicin **5** is reported to decompose almost completely at room temperature to diallyl monosulfide **1**, diallyl disulfide **2** and diallyl trisulfide **3**.[2] The primary constituents of steam distilled garlic oil extract (GOE) include **1**, **2** and **3**, but also dimethyl disulfide **38** and dimethyl trisulfide **39** (Figure 2.1).

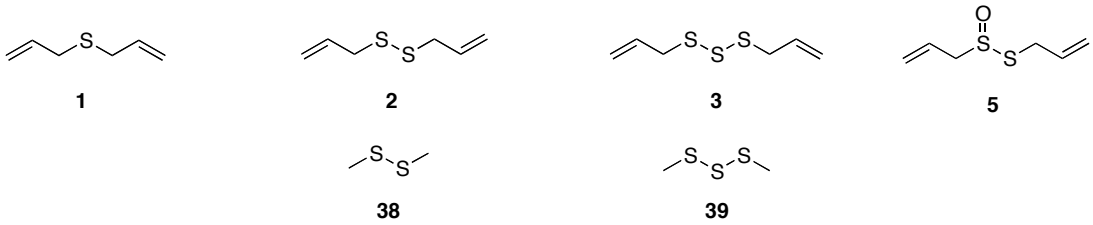


Figure 2.1. Polysulfides found in garlic oil extracts (GOE)

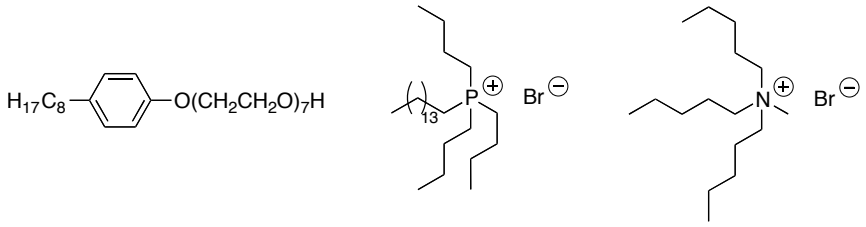
The molecules **1-3** containing allylic moieties have displayed antibacterial properties;[3] increasing the quantity of sulfur in these molecules weakens the C-S bond and increases the possibility of generating allyl thiol, a highly antibacterial molecule.[4] Diallyl trisulfide **3** has shown the greatest antibacterial activity of garlic polysulfides and is capable of releasing H_2S that can be used to treat myocardial ischemia reperfusion injury.[5]

Over 150 years ago, compound **1** was synthesised from allyl iodide and aqueous potassium sulfide.[6] In this chapter, the synthesis of mono-, di- and trisulfide garlic metabolites is described using flow chemistry to reduce the operating temperatures and shorten reaction times.

2.1.1. Synthesis of Symmetrical Monosulfides

Sodium sulfide has been used as a nucleophilic source of sulfur in the synthesis of symmetrical monosulfides from alkyl halides.[7] Shriner *et al*. published the synthesis of dibenzyl monosulfide with 83% yield by heating an aqueous methanolic solution of sodium sulfide and benzyl bromide on a steam bath with continuous stirring for 3 days.[8] Refluxing the reaction reduced the reaction time to 15 hours.[9]

Phadhan and Sharma investigated the synthesis of monosulfides using sodium sulfide and discovered that the reaction was controlled by the rate of mass transfer.[10] To overcome this, additives or phase transfer catalysts (PTC's) have been used to improve the reaction. In 1969, an emulsifier **40** was added which reduced the reaction time to 4 hours.[11] The development of PTC's led to shorter reaction times (**41**) and cheaper alternatives (**42**).[12]



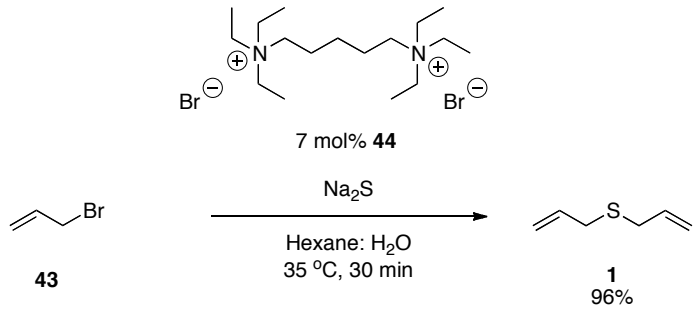
40
1969
90 °C, 4 h

41
1974
70 °C, 10 min

42
1975
2 h

Figure 2.2. PTC's used to improve the rate of reaction between sodium sulfide and benzyl chloride

Wang and Tseng improved the synthesis of the garlic metabolite **1** using hexaethylpentane-1, 5-diaminium bromide **44** as a novel PTC, which reduced the reaction time and operating temperature (Scheme 2.1). Compound **44** is unusual as it contains two terminal quaternary centres and required synthesis.



43

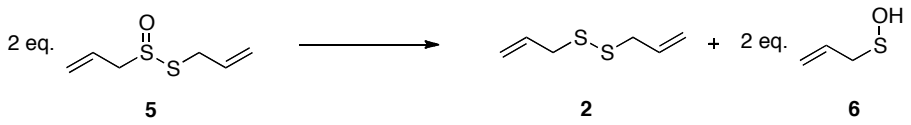
1
96%

Scheme 2.1. Synthesis of **1** using **44** as a PTC

Phase transfer catalysis was combined with flow chemistry in this chapter to further improve the synthesis of symmetrical monosulfides found in garlic.

2.1.2. Synthesis of Symmetrical Disulfides

Disulfides are major components of steam distilled GOE. Diallyl disulfide, compound **2**, is the main component, produced through the breakdown of **5**, however, there is uncertainty/inconsistency in their identity and the reaction pathways.



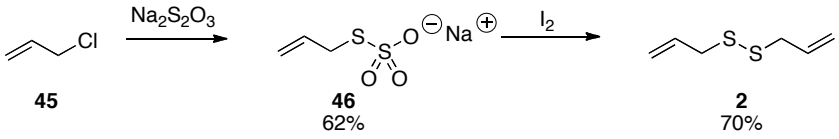
Scheme 2.2. Proposed mechanism for the formation of diallyl disulfide **2** from allicin **5** [4]

In this thesis, compound **2** is required in large quantities for the synthesis of ajoene. However, compound **2** is not commercially available in pure form. A flow methodology with short reaction times and low operating temperatures has been developed. The method has to be accessible for industry and have the potential to be combined in a multistep flow synthesis to produce ajoene.

The synthesis of **2** has been performed using two approaches; the oxidation of thiols, and thiolysis that reduces alkyl halides using sulfur reagents. The next section discusses the benefits and disadvantages of the existing synthesis of diallyl disulfide **2**.

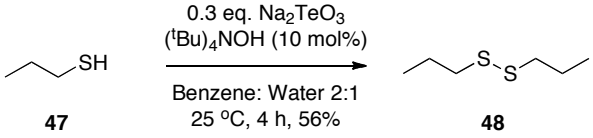
2.1.2.1. Synthesis using Thiols

After Bailey and Cavallito first isolated allicin **5**, they worked with Small to produce **2** from allyl chloride **45**. Compound **45** was reacted with sodium thiosulfate to form the Bunte salt intermediate **46**, that was then coupled via oxidation with iodine.[13]



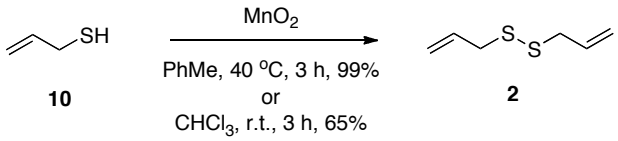
Scheme 2.3. Bunte salt oxidative coupling of thiols to synthesis **2**

An early example of thiol oxidation to produce dialkyl disulfides was performed using sodium tellurite (Scheme 2.4). The dipropyl disulfide **48** synthesised is a major component of the essential oil of onion. The onion is a member of the *allium* genus that also contains high quantities of organosulfur compounds.[14]



Scheme 2.4. Oxidative coupling of thiols to synthesis disulfides

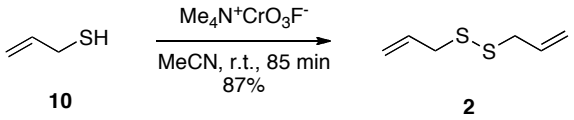
A straightforward oxidation method used MnO_2 nanoparticles on clay. The synthesis of **2** proceeded in good yields (Scheme 2.5) and only required filtration to remove the reagent. The solvents are unfavourable for industrial use and evidence for the claims of reusability of the clay were not found.[15]



10 **2**

Scheme 2.5. Oxidative coupling using MnO_2 nanoparticles to produce **2**

Other oxidants have performed the reaction with shorter reaction times and at lower temperatures. Tetramethylammonium fluorochromate could perform the oxidative coupling in acetonitrile at ambient temperatures (Scheme 2.6).[16]

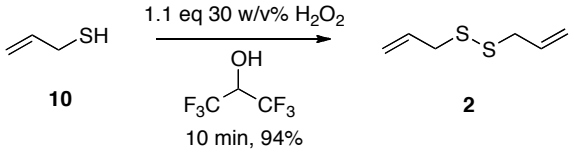


10 **2**

Scheme 2.6. Oxidative coupling using tetramethylammonium fluorochromate to synthesise **2**

This reaction has also been reported using cobalt chloride as a catalyst.[17] The authors proposed that there is a reaction with acetonitrile, however aerial oxidation is more likely and there are a wide range of catalysts used for the aerial oxidation of thiols.[18]

Kesavan *et al.* performed the oxidation rapidly using cheap hydrogen peroxide in fluorinated solvents, such as 1,1,1,3,3,3-hexafluoroisopropanol or 2,2,2-trifluoroethanol (Scheme 2.7).[19] Using expensive solvents is undesirable, however the authors claim this could be recovered, by azeotropic distillation.



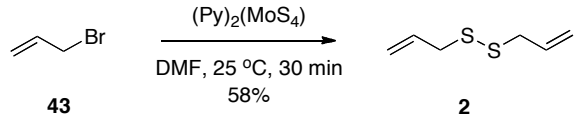
10 **2**

Scheme 2.7. Oxidative coupling using hydrogen peroxide in hexafluoroisopropanol to synthesis **2**

There are several classical methods for the synthesis of disulfides from allyl thiol alongside more recent methods developed over the last decade.[18] Although oxidation procedures for the synthesis of diallyl disulfide **2** have improved, allyl thiol **10** as a starting material in this project was deemed unreasonable. This was decided on its reactivity and instability,[1] furthermore handling large volumes at industrial scale would be undesirable. Furthermore, allyl thiol is only commercially available approximately 70% purity and should be stored at -40 °C.

2.1.2.2. Synthesis using Alkyl Halides

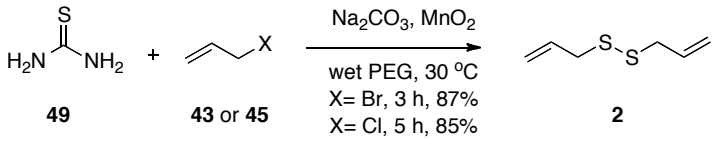
An alternative allylic starting material is an allyl halide, which could be reacted with a sulfur source. In 1989, Dhar and Chandrasekaran developed a facile synthesis from alkyl halides using piperidinium tetrathiomolybdate (MoS_4^{2-}) as the source of sulfur. The synthesis of **2** was performed quickly at room temperature with good yields (Scheme 2.8).[20] The approach using alkyl halides had much room for improvement.



43 **2**

Scheme 2.8. Synthesis of **2** using piperidinium tetrathiomolybdate

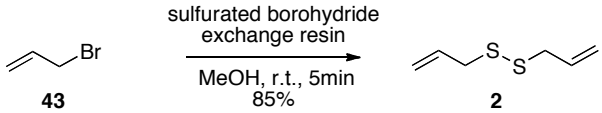
Manganese dioxide has also been used in a one-pot procedure catalysed by a micellar solution of sodium dodecyl sulfate.[21] The sulfur reagent was thiourea and both reagents are readily available. The reaction temperature was reduced when poly ethylene glycol (PEG) was used (Scheme 2.9).[22]



49 **43** or **45** **2**

Scheme 2.9. Synthesis of **2** using thiourea

Other syntheses have used alkyl halides and sodium thiosulfate with subsequent oxidation, the two-step procedure suffered from very poor atom efficiency.[23,24] An interesting sulfur source is a sulfur exchange resin. This has produced **2** in a clean and rapid reaction (Scheme 2.10).[25] As the sulfur exchange resin is heterogeneous, the reaction only required filtration to isolate the product. The sulfur exchange resin is prepared from reacting equimolar quantities of sodium borohydride with sulfur. This reagent has the potential to be used in a heterogeneous packed-bed flow reactor.

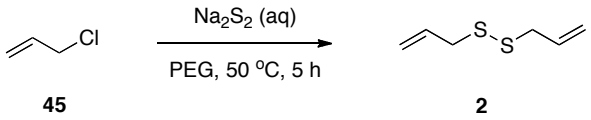


43 **2**

Scheme 2.10. Synthesis of **2** using sulfurated borohydride exchange resin

Tajbakhsh *et al.* generated quaternary diammonium borohydrides, which could also prepare disulfides rapidly from sulfur and alkyl halides.[26] But again the quaternary diammonium borohydrides required synthesis.

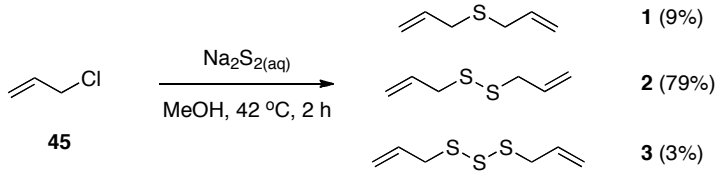
The simplest sulfur reagent that has been used is disodium disulfide (Na_2S_2), which is synthesised from sodium sulfide solution reducing elemental sulfur. The disodium disulfide preparation has been described in a patent from sodium sulfide nonahydrate to produce **2** as a precursor to a novel optical material (Scheme 2.11).[27] Sodium sulfide nonahydrate is completely dissolved in water at 50 °C, and then an equivalent of elemental sulfur powder is added to produce disodium disulfide.[27]



Scheme 2.11. Application of disodium disulfide for the synthesis of diallyl disulfide **2**

The use of aqueous disodium disulfide salt has also been patented in the synthesis of diallyl disulfide.[28] Sodium hydroxide was added to supress the formation of hydrogen sulfide formed under acidic conditions.

Diallyl disulfide has also been synthesised in good yields using the minimum quantity of water (Scheme 2.12).[29] This patent claims to perform the reaction in absence of an aqueous phase, only methanol is used as the protic solvent. However concentrated aqueous disodium disulfide was described in the procedure. Although the reaction time is 2 h, compound **45** is fed into the reaction mixture over 2.5 h and produced a mixture of polysulfides that are difficult to separate due to their similar polarity.



Scheme 2.12. Synthesis of **2** using sodium sulfide without a PTC

An alternative to disodium disulfide is dilithium disulfide (Li_2S_2), developed in 1982 and generated from lithium triethylborohydride and sulfur in THF at room temperature.[30] This reagent produced **2** with a 75% yield and required column chromatography.

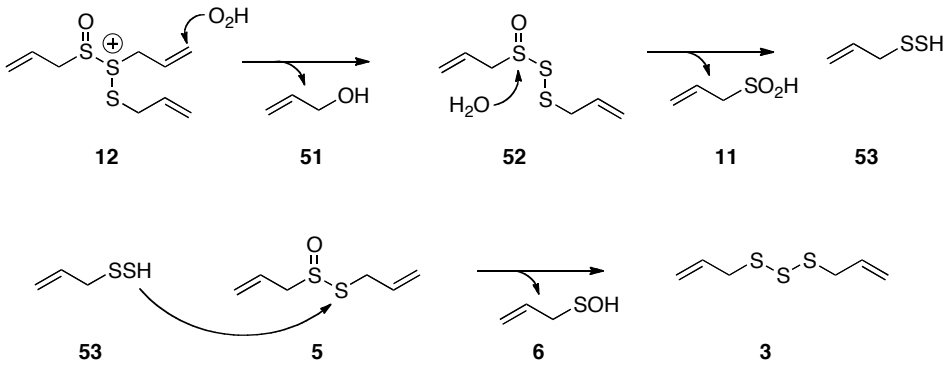
Developing a disulfide synthesis in flow mode will be crucial for the production of **2** for the subsequent synthesis of allicin **5** in chapter 4. The application of PTC will be investigated to improve the reaction.

2.1.3. Synthesis of Symmetrical Trisulfides

Diallyl trisulfide **3** is the second most abundant polysulfide in GOE. This molecule has antibacterial and fungicidal activity comparable to **5** and has several interesting biological properties.[31] Compound **3** is a great source of hydrogen sulfide, which has vital functions within the blood[32] and has also demonstrated suppression of the spread of human colon cancer cells and induces apoptosis.[33]

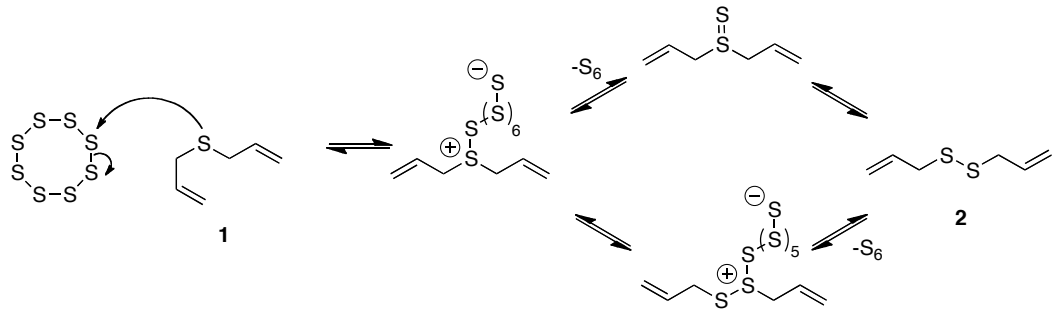
Isolation of **3** and higher diallyl polysulfides from GOE is very difficult. They are formed when **5** is heated in aqueous conditions. The reaction of two molecules of **5** produces the trisulfide cation intermediate **12** (Scheme 1.2, page 7).

Compound **12** reacts with water (Scheme 2.13),[34] which releases another trisulfide intermediate **52** and allyl alcohol **51**. Compound 52 further reacts with water to produce compound **11** and the perthiol, 2-propene-1-sulfenothoic acid **53**. Compound **11** rapidly decomposes to propene and sulfur dioxide.[35] Compound **53** then reacts with **5** to release **3** and **6**.



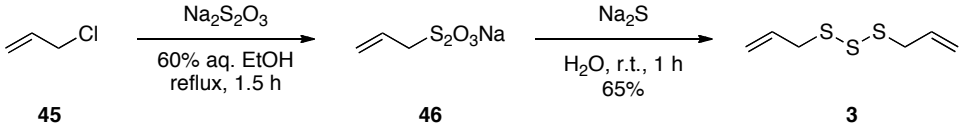
Scheme 2.13. Proposed mechanism for the formation of polysulfides

There are two reported synthetic routes to produce diallyl trisulfide **3**; using Bunte salts or heating diallyl sulfides **1** in the presence of a sulfur source. At temperatures above 120 °C, compound **1** is reported to rearrange to form diallyl thiosulfoxide, which can react with elemental sulfur in a similar mechanism to sodium sulfide.[36,37] A similar approach has been demonstrated by heating **1** in the presence of elemental sulfur (Scheme 2.14).[38] These operating conditions are very high, 90 °C over several days, and the yields are not given.



Scheme 2. 14. Proposed mechanism for diallyl monosulfide **1** reducing elemental sulfur to produce diallyl polysulfides

Other synthetic approaches are more appealing due to the lower temperatures employed compared to Scheme 2.14. The chemical synthesis of **3** has been performed by reacting allyl halides with sodium thiosulfate to produce the Brunte salt **46** and subsequent addition of sodium sulfide (Scheme 2.15).[39]

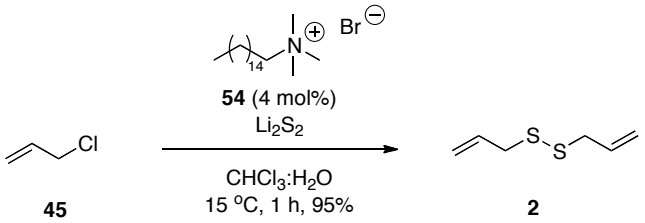


Scheme 2.15. Synthesis of diallyl trisulfide **3** using sodium thiosulfate

Sodium disulfide has also been reacted with the Brunte salt 46. Although diallyl trisulfide **3** was formed as the major product, polysulfide impurities were also formed. Two Chinese publications have also reported the synthesis of **3.**[28,40]

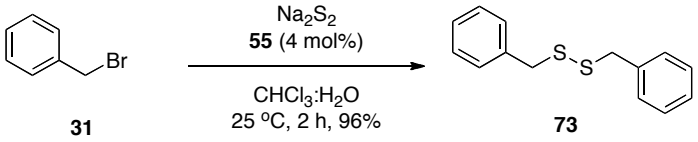
2.1.4. Synthesis of Symmetrical Disulfides using Phase Transfer Catalysis

Phase transfer catalysis facilitates the reaction between two reagents in the presence of a phase boundary and its application in flow chemistry was discussed in the introduction. In the literature, immiscible aqueous-organic solvent systems have been used to improve the synthesis of disulfides. Early work by Hase and Peräkylä used a water-chloroform mixture. Cetyltrimethylammonium bromide **54** was used as a phase transfer catalyst (PTC) to facilitate the reaction in good yields without heating (Scheme 2.16).[41]



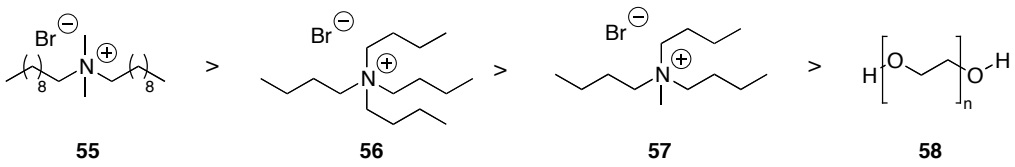
Scheme 2.16. Synthesis of **2** using **54** as a PTC

Sonavane and co-workers developed a reaction in a water-chloroform solvent system using didecyldimethylammonium bromide **55** as a transfer catalyst to produce dibenzyl disulfides **73**.[42] The reaction proceeds at ambient temperature over the period of 2 h.



Scheme 2.17. Synthesis of **73** using **55** as a PTC

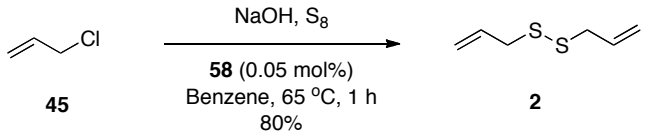
The work also demonstrated the efficiency of several PTC's (Scheme 2.18) and showed that the cheaper tetrabutylammonium bromide **56** performed the reaction with marginally lower yield compared to **55**. Compound **56** has been used to synthesise diallyl disulfide **2** using microwave irradiation that reduced the reaction time to 12 minutes with 82% yield.[43]



Scheme 2.18. Comparison of PTC's reactivity[42]

The application of **58** (0.05 mol%) with aqueous sodium hydroxide and benzene in a biphasic solution has also demonstrated the effectiveness of phase transfer catalysis in the synthesis of disulfides. The synthesis of **2** was completed after an hour with 80 % yield (Scheme 2.19).[44] This reaction was improved in homogenous DMF solution and

microwave irradiation and proceeded in 90% yield after 6 minutes, however silica chromatography was required.[45]



45 **2**

Scheme 2.19. Synthesis of **2** using sulfur and polyethylene glycol-400 **58** as a PTC

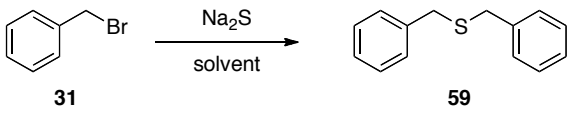
Combining sodium sulfide salts, as a nucleophilic source of sulfur, with phase transfer catalysts should reduce the reaction times in the synthesis of diallyl polysulfides and minimising the formation of by-products.

2.2. Results and Discussion

Garlic oil extract (GOE) contains symmetrical polysulfides. The concentrations of these garlic metabolites are determined using calibrated high performance liquid chromatography (HPLC) and required analytical standards. This chapter aims to produce dialkyl polysulfides using continuous flow technology, specifically mono-, di- and trisulfides. The synthesis was investigated using alkyl halides and nucleophilic dianion sulfur. Phase transfer catalysis was employed to reduce the operating conditions of these reactions. A rapid, clean synthesis of diallyl disulfide **2** is crucial for the subsequent synthesis of ajoene **14**.

2.2.1. Synthesis of Symmetrical Monosulfides

Diallyl monosulfide **1** is the most abundant monosulfide in GOE. Due to the volatility of compound **1**, the model reaction used for investigation was synthesis of dibenzyl monosulfide **59** (Scheme 2.20).



Scheme 2.20. Model reaction for the synthesis of dibenzyl monosulfide

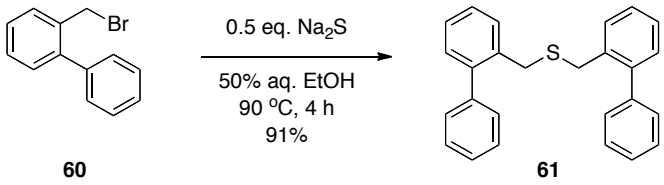
Batch conditions produced **59** in good yields using sodium sulfide (Table 2.1). The reaction shown in Table 2.1, Entry 1 produced the product in a moderate yield of 61%. However, the reaction was biphasic due to the immiscible nature of benzyl bromide in water. To improve this reaction, miscible organic solvents were investigated to produce a homogeneous solution. The reaction yields were improved (Table 2.1, entries 2 and 3), similar to yields reported in the literature.[11]

Table 2.1. Synthesis of dibenzyl monosulfide

Entry	Solvent	Yield (%)	Time (h)
1	Water	61	48
2	50% aq. EtOH	88	18
3	50% aq. MeOH	87	18

Conditions: Sodium sulfide (0.1 M) in water and benzyl chloride (0.2 M) in the specified solvent was heated at 90 °C overnight

The conditions were used to synthesise a novel dialkyl monosulfide. Using the aqueous ethanol solvent, compound **61** was synthesised in batch (Scheme 2.21) and the reaction proceeded with an excellent yield.



Scheme 2.21. Synthesis of **61**

2.2.1.1. Synthesis in Flow Mode

The reaction solvent system (50% aq. EtOH) was then evaluated in flow mode. A syringe was filled with aqueous sodium sulfide solution. Another syringe was filled with benzyl chloride in ethanol. The syringes were placed on a syringe pump. The syringes were pumped through the PTFE reactor and eluted into a collection flask containing brine, shown in Figure 2.3.

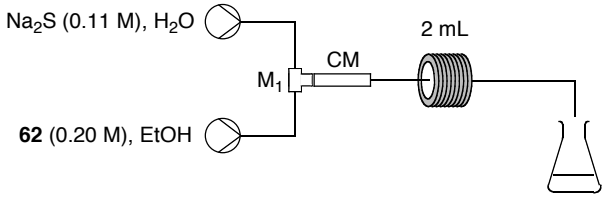


Figure 2.3. Syringe pump flow reactor setup for the synthesis of dibenzyl monosulfide

Table 2.2. Optimisation of dibenzyl monosulfide in flow

Entry	Temp (°C)	Flow rate (mL/h)	Retention time (min)	Yield (%)
1	35	0.5	120	55
2	70	0.5	120	59
3	35	3	20	72
4	35	6	10	41

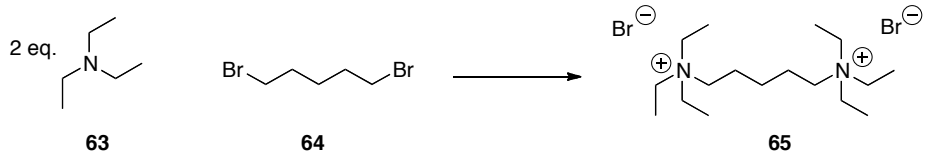
Conditions: Na_2S (0.11 M) in H_2O was pumped at the flow rate indicated. **62** (0.20 M) in EtOH was pumped at the flow rate indicated.

Table 2.2 shows that temperature did not improve the reaction (Table 2.2, entries 1 and 2). The flow rate had the greatest effect on the yield. The ideal flow rate was 3 mL/h (Table 2.2, entry 3), and is likely due to the fluid vectors generated at this flow rate.

2.2.1.2. Synthesis in Batch Mode with a Phase Transfer Catalyst

The introduction of a phase transfer catalyst (PTC) was used to improve the rate of reaction. The PTC investigated, **65**, has been reported for the synthesis of **1** using favourable operating conditions, shown in Scheme 2.1.

The synthesis of **65** was attempted using the reported conditions, however, no product was formed (Table 2.3, entry 1). A modified synthesis by Gmiro *et al*. produced **65** in a good yield.[46] The reaction was attempted with benzene (Table 2.3, entry 2) at room temperature but did not induce product formation. The mixture was refluxed in benzene to produce **65** in a lower yield. Toluene (Table 2.3, entry 5) was utilised as a greener solvent and improved the yield. The reaction did not proceed using cyclohexane as a solvent (Table 2.3, entry 4).

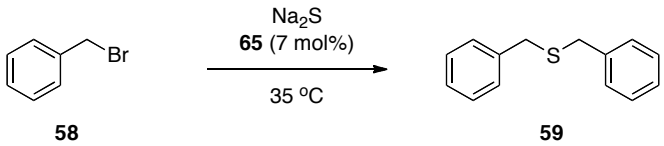


Scheme 2.22. Synthesis of hexaethylpentane-1, 5-diaminium bromide **65**

Table 2.3. Optimisation of hexaethylpentane-1, 5-diaminium bromide **65** synthesis

Entry	Solvent	Temp	Time (h)	Yield (%)
1	EtOH	60 °C	48	n.r.
2	Benzene	r.t.	144	n.r.
3	Benzene	Reflux	18	39
4	Cyclohexane	Reflux	18	n.r.
5	PhMe	Reflux	18	48

The phase transfer-catalysed synthesis of monosulfide **59** was performed using the reported conditions. The reaction proceeded with a much lower yield (Table 2. 4, entry 1). Aqueous miscible solvents were used and ethanol performed the reaction in good yields but a longer reaction time was required (Table 2.4, entry 3).



58 **59**

Scheme 2.23. Synthesis of dibenzyl monosulfide **59** using **65** as a PTC

Table 2.4. Optimisation of dibenzyl monosulfide **59** synthesis using **65**

Entry	Solvent (2:1)	Time (min)	Yield (%)
1	Hexane/water	20	4
2	EtOAc/water	120	20
3	EtOH/water	120	65

Conditions: Sodium sulfide (0.25 M) in water, **58** (0.25 M) and benzyl bromide **65** (7 mol%) in the specified solvent was stirred at 35 °C

In flow mode, results were promising and the experience gained was invaluable to the progress of this thesis. However, due to the commercial availability of the symmetrical monosulfide, the project focused on the synthesis of higher polysulfides.

2.2.2. Synthesis of Symmetrical Disulfides in Flow Mode

In this section, alkyl halides were reacted with sodium disulfide using phase transfer catalysts. The synthesis of diallyl disulfide **2** has been performed with disodium disulfide without a phase transfer catalyst.[29] The reaction time was 2 h and produced an array of polysulfide impurities. Phase transfer catalysts should reduce the reaction time and improve the selectivity. Sonavane and co-workers developed a batch synthesis using tetrabutylammonium bromide **56** as a PTC to produce dialkyl disulfides. Sonavane *et al.*'s batch reagents shown in Scheme 2.17 were assessed in flow mode for the synthesis of **2**.

A syringe was filled with aqueous disodium disulfide solution. Another syringe was filled with allyl bromide in an organic solution. The syringes were placed on a syringe pump. The syringes were pumped through a PTFE reactor and eluted into a collection flask containing brine, shown in Figure 2.4.

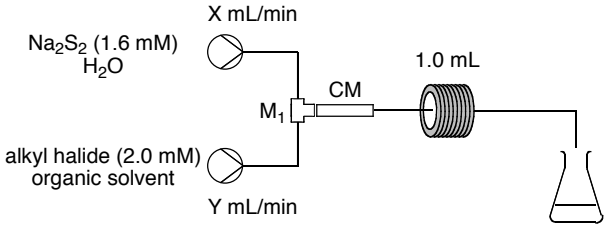
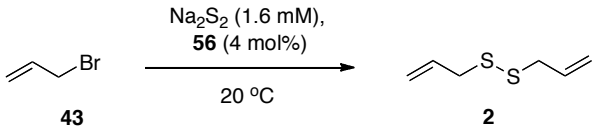


Figure 2.4. Syringe pump flow reactor setup for the synthesis of **2**
[M_1] T-piece, [CM] comet mixer, [R_1] PTFE coil

The concentration of disodium sulfide (1.6 mM) was a limiting factor. The disodium disulfide salt precipitated within the reactor coil and created blockages at the T-piece (M1) that has a reduced diameter.



Scheme 2.24. Synthesis of **2** using **56** as a PTC

Several solvents were examined using this flow reactor setup. The reaction proceeded in a low yield using chloroform as the immiscible organic solvent (Table 2.5, entry 1). Methylene chloride produced **2** in good yields (Table 2.5, entry 2). Aqueous miscible solvents were investigated to determine whether a phase boundary was required. The yield was lower when performed with ethanol and acetonitrile (Table 2.5, entries 3 and 4).

Table 2.5. Solvent screening of **2** synthesis in flow mode

Entry	Organic solvent	Yield **2** (%)
1	$CHCl_3$	10
2	CH_2Cl_2	68
3	EtOH	44
4	MeCN	1

Conditions: Na_2S_2 (0.8 mM) in H_2O was pumped at 0.1 mL/min.
Allyl bromide **43** (2.0 mM) and $^tBu_3NBr$ **56** (4 mol%) in organic solvent was pumped at 0.1 mL/min.

The effect of residence time was investigated using the same flow rate of 0.2 mL/min. Larger reactor coil volumes increased the residence time. A longer residence time resulted in prolonged mixing that reduced the yield (Table 2.6, entry 3). The dominant residence time was 5 minutes using a reactor coil with a volume of 1 mL (Table 2.6, entry 2).

Table 2.6. Residence time optimisation of **2** synthesis in flow mode

Entry	Coil Volume (mL)	Residence Time (min)	Yield **2** (%)
1	0.5	2.5	28
2	1	5	68
3	2	10	48

Conditions: Na_2S_2 (0.8 mM) in H_2O was pumped at 0.1mL/min.
43 (2.0 mM) and **56** (4 mol%) in CH_2Cl_2 was pumped at 0.1mL/min.

The catalyst loading was also examined (Table 2.7). Without the presence of **56** the reaction did not proceed. This is consistent with literature in which high temperatures are required for non-catalysed reaction.[9] However, there is an optimum catalytic concentration for this reaction, entry 2 in Table 2.7 was the best result discovered.

Table 2.7. Catalyst loading for the optimisation of **2** synthesis in flow mode

Entry	**56** (mol %)	Yield **2** (%)
1	0	0
2	4	68
3	8	14

Conditions: Na_2S_2 (0.8 mM) in H_2O was pumped at 0.1 mL/min.
43 (2.0 mM) and **56** (4 mol%) in CH_2Cl_2 was pumped at 0.1 mL/min.

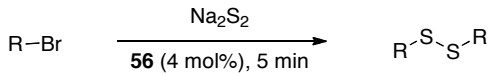
Allyl chloride **45** was evaluated as the substrate. The yield of **2** was improved using the previously optimised conditions (Table 2.8, entry 1). Furthermore, acetone was investigated as a greener solvent to reduce the toxicity and also proceeded in an excellent yield (Table 2.8, entry 2). Tetrabutylammonium iodide **66** was also investigated, but this reduced the yield (Table 2.8, entry 3).

Table 2 8. Optimisation in the **2** synthesis using allyl chloride **45** in flow mode

Entry	PTC	Solvent	Yield **2** (%)
1	**56**	CH_2Cl_2	92
2	**56**	Acetone	95
3	**66**	CH_2Cl_2	38

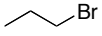
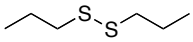
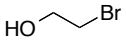
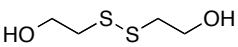
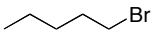
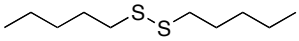
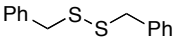
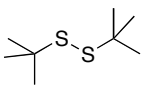
Conditions: Na_2S_2 (0.8 mM) in H_2O was pumped at 0.1 mL/min.
45 (2.0 mM) and PTC (4 mol%) in the solvent indicated was pumped at 0.1 mL/min.

The best conditions were used to examine the synthesis of other symmetrical dialkyl polysulfides (Table 2.9). However, an increase in temperature was required to improve yields. Further optimisation of conditions may be a prerequisite for individual substrate.



Scheme 2.25. General reaction for synthesis of dialkyl disulfides

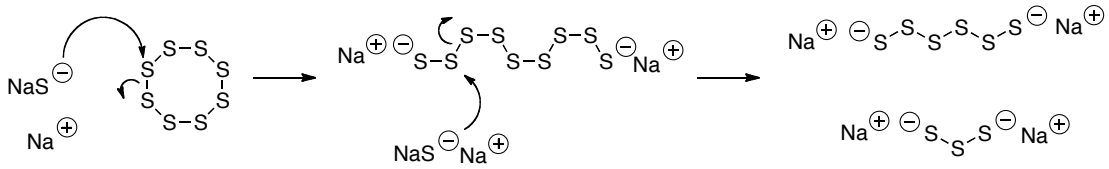
Table 2.9. Synthesis of dialkyl disulfides

Entry	Substrate	Disulfide	Temp (°C)	Yield (%)
1	 **67**	 **68**	40	45
2	 **69**	 **70**	40	70
3	 **71**	 **72**	40	63
4	 **58**	 **73**	30	43
5	 **74**	 **75**	40	n.r.

Conditions: Na_2S_2 (0.8 mM) in H_2O was pumped at 0.1 mL/min.
Alkyl halide (2.0 mM) and **56** (4 mol%) in CH_2Cl_2 was pumped at 0.1 mL/min.

2.2.3. Synthesis of Symmetrical Trisulfide in Flow Mode

The synthesis of diallyl trisulfide was performed using the optimised conditions of the diallyl disulfides **2** synthesis. Disodium trisulfide salt (Na_2S_3) was generated using aqueous disodium sulfide reducing two equivalents of sulfur, as shown in Scheme 2.26.



Scheme 2.26. The reduction of elemental sulfur by disodium sulfide producing sodium trisulfide

The reactivity of sodium trisulfide was investigated using benzyl bromide. The use of benzyl bromide allowed for easier analysis using proton nuclear magnetic resonance (^{1}H NMR). Initial experiments using **45** produced a complicated array of compounds. Several flow rates and intrinsic residence times were investigated (Table 2.10). Reducing the flow rates decreases yields. However, the ratio of polysulfides was not affected.

Table 2.10. Residence time optimisation for the synthesis of dibenzyl polysulfide in flow mode

Entry	Flow Rate (mL/min)	Reaction time (min)	^{1}H NMR Ratio S_2: S_3: S_4: S_5	Combined yield (%)
1	0.025	20	50:26:15:9	65
2	0.05	10	51:25:15:9	83
3	0.1	5	49:25:15:11	94

Conditions: Na_2S_3 (1.0 mM) in H_2O was pumped at desired flow rate. **45** (2.0 mM) and **56** (4 mol%) in acetone was pumped at desired flow rate. The reactor temperature was 20 °C.

These results were reproduced with two different batches of disodium sulfide with minimal variation. The sodium sulfide salt generated was not as selective as claimed in published patents.[27,29] The effect of temperature on the selectivity and yield was negligible (Table 2.11).

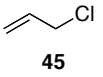
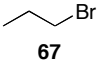
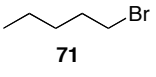
Table 2.11. Temperature optimisation for the optimisation of dibenzyl polysulfide synthesis in flow mode

Entry	Temp (°C)	^{1}H NMR Ratio S_2: S_3: S_4: S_5	Combined yield (%)
1	20	50:26:15:9	94
2	30	49:27:15:9	92
3	40	54:26:13:7	88
4	50	53:25:14:8	92

Conditions: Na_2S_3 (1.0 mM) in H_2O was pumped at 0.1 mL/min.
Benzyl bromide **58** (2.0 mM) and **56** (4 mol%) in acetone was pumped at 0.1 mL/min.
The reactor residence time was 5 min.

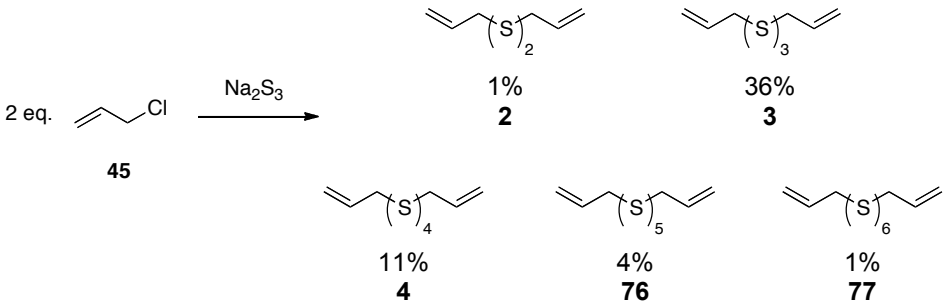
Several substrates were examined using the optimised conditions (Table 2.12). The polysulfides ratio was dependent on the substrate. Allyl bromide **43** produced the trisulfide as the major product. Propyl bromide favors the formation of polysulfides with 4 sulfur atoms. Pentyl bromide favored the formation of the disulfide product. However, gas chromatography mass spectrometry (GC-MS) was used to identify the polysulfides due to overlapping proton NMR signals, in which the ratios could not be determined.

Table 2.12. Synthesis of dialkyl polysulfides

Entry	Substrate	^{1}H NMR Ratio S_2: S_3: S_4: S_5	Combined yield (%)
1	 **45**	22:53:25:0	72
2	 **67**	15:33:52:0	87
3	 **71**	80:20:0:0	80
4	 **74**	n.r.	n.r.

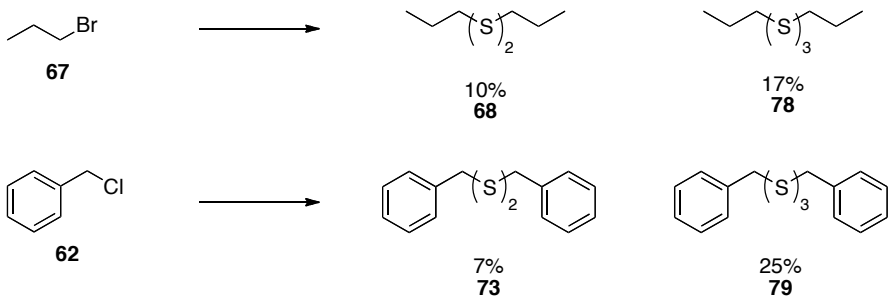
Conditions: Na_2S_3 (1.0 mM) in H_2O was pumped at 0.1 mL/min.
Alkyl halide (2.0 mM) and **56** (4 mol%) in acetone was pumped at 0.1 mL/min.
The reactor residence time was 5 min and the temperature was 20 °C.

The separation and isolation of the diallyl polysulfides was difficult. A patent reported the use of isocratic reverse phase chromatography with 30% aqueous methanol to isolate > 98% pure diallyl trisulfide.[28] Using reverse phase C18 silica chromatography, the diallyl polysulfide mixture (Table 2.12, entry 1) was completely separated (Scheme 2.27). Diallyl trisulfide **3** was still the major product. The isolation of diallyl tetrasulfide, pentasulfide and hexasulfide was achieved. These compounds have identical ^{1}H NMR chemical shifts and assignment was performed using GC-MS.



Scheme 2.27. Isolated diallyl polysulfides

The reverse phase separation of dibenzyl polysulfides (Table 2.11, entry 2) and dipropyl polysulfides (Table 2.12, entry 2) also produced the trisulfide as the major isolated product.



Scheme 2.28. Isolated dialkyl polysulfides

Unfortunately, dipentyl polysulfide (Table 2.12, entry 3) separation was unachievable using this system. However, up to dipentyl hexasulfide was identified by high-resolution mass spectrometry.

2.3. Conclusion

Garlic oil extract (GOE) contains symmetrical polysulfides. The concentrations of these garlic metabolites are determined using calibrated high performance liquid chromatography (HPLC) and required analytical standards. This chapter aims to produce dialkyl polysulfides using continuous flow technology, specifically mono-, di- and trisulfides. The synthesis was investigated using alkyl halides and nucleophilic dianion sulfur. Phase transfer catalysis was employed to reduce the operating conditions of these reactions. Most importantly, the first step of the ajoene synthesis, a rapid method for producing diallyl disulfide, without additional purification, was achieved.

2.4. General Methods

The batch reactions were performed using standard laboratory equipment. Reactions were stirred using magnetic stirring and heated to specified temperatures using hotplates with temperature probe control in dry heating blocks or silicone oil baths. The sonication reactions were performed in Fisherbrand FB15051 Ultrasonic bath. Microwave heated reactions were performed in a CEM SP Discover.

The flow reactions performed using KD Scientific and Fusion 100 syringe pumps with plastic syringes from BD Plastik and glass syringes from SGE. The tubing used was PTFE with P-201X screws and P-200X flangeless ferrules (ETFE, OD = 1/16") purchased from Upchurch Scientific. The static mixer used was a "Comet X-01," available from Techno Applications Co., Ltd., 34-16-204, Hon, Denenchofu, Oota, Tokyo 145–0072, Japan. Flow reactions were also performed using a Vapourtec E-series machine.

Reactions performed at low temperatures were stirred in reaction vessels in an ice/sodium chloride bath (–15 °C), or ice/water (0 °C). Rotary evaporators Büchi B-461, B-481 or B-490 were used for solvent evaporations (reduced pressure to 15 mbar); further drying was performed using high vacuum apparatus. For inert reactions, anhydrous solvents were obtained from the solvent purification system (SPS) MBraun MB SPS-800 or solvents were distilled over a drying agent under inert conditions and stored over molecular sieves. Air and/or moisture sensitive experiments were performed under an inert atmosphere of argon and the glassware was oven dried at 120 °C prior to use.

Other chemicals were purchased from Acros, Aldrich, Alfa Aesar or Fisher and were used without further purification.

2.4.1. Physical Data

^{1}H NMR Spectroscopy

Bruker DPX 500 (500 MHz), Bruker DPX 400 (400 MHz) or Bruker DPX 250 (250 MHz). The chemical shifts δ are given in ppm downfield of tetramethylsilane (δ = 0 ppm). Compounds and crude reaction mixtures are dissolved in either deuterated chloroform ($CDCl_3$), deuterium oxide (D_2O) or deuterated dimethylsulfoxide (DMSO). Coupling constants (*J*) are given in Hertz (Hz). The multiplicity of signals is designated: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dt = doublet of triplets, td = triplet of doublets, m = multiplet. Residual solvent peaks are assigned as follows: 7.26 ppm for chloroform, 4.79 ppm for water, 2.54 ppm for dimethylsulfoxide, 2.05 ppm for acetone.

^{13}C NMR Spectroscopy

Bruker DPX 500 (125 MHz), Bruker DPX 400 (100 MHz), Bruker DPX 250 (62.5 MHz) The chemical shifts δ are reported in ppm downfield of tetramethylsilane (δ = 0 ppm). Compounds and crude reaction mixtures are dissolved in $CDCl_3$, D_2O or DMSO. Residual solvent peaks are assigned as follows: 77.36 ppm for chloroform and 40.45 ppm for dimethylsulfoxide.

Mass Spectrometry

Mass spectrometry was performed by the EPSRC Mass Spectrometry Service Centre at Swansea University using LTQ Orbitrap XL or by R. Jenkins/R. Hick/S. Waller/D. Walker in Analytical Services at Cardiff University using Water LCR Premier XE-tof. Ions were generated by the atmospheric pressure ionisation techniques voltage applied corana discharge pin (APCI), Electrospray (ES) or Electron Ionisation (EI). Mass fragments usually are in atomic mass units per elementary charges (m/z) with relative abundance of ion in percentage (%). The high-resolution mass spectrometry (HRMS) for most of the compounds was carried out at Cardiff University. The molecular ion peak values report the molecular ion (M^+), molecular ion plus hydrogen ($M+H^+$) or molecular ion plus sodium ($M+Na^+$).

IR Spectroscopy

IR spectra were recorded using either the Perkin Elmer FTIR Spectrum RX1 or the Shimadzu IR Affinity 1S. Wavenumbers are reported in cm^{-1}. Crystalline compounds were measured deposited between NaCl disks with CH_2Cl_2, non-crystalline samples were measured as neat film between NaCl disks.

Melting Points

Melting Points (m.p.) were measured using a Stuart SMP 11 with samples in open capillary tubes.

2.4.2. Chromatographic Methods

Thin Layer Chromatography

All reactions were monitored by thin-layer chromatography (TLC) which was performed on precoated aluminum sheets of Merck silica gel 60 F254 (0.20 mm) and visualised by UV radiation (λ= 254 nm) or by staining with potassium permanganate solution prepared with $KMnO_4$ (1.5 g), K_2CO_3 (10 g), NaOH (2.5 M, 1.25 mL) in distilled H_2O (200 mL).

Column Chromatography

Column chromatography was performed with silica gel 60 (Merck, 230-400 mesh) under increased pressure (Flash Chromatography) or as gravitational column chromatography. The mobile phase solvents are indicated in the text.

Biotage Chromatography

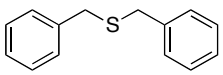
Automated chromatography was performed using a Biotage Isolera 4. The columns and mobile phase solvents are indicated in the text.

High Pressure Liquid Chromatography (HPLC)

HPLC measurements were performed at Cardiff University using an arrangement from Shimadzu. The Shimadzu Class VP consisted of SIL-10ADVP (auto injector), LC-10 ATVP (liquid chromatograph), FCV-10ALVP (pump), DGU-14A (degasser), CTO-10ASVP (column oven), SCL-10AVP (system controller) and a SPD-M10A (diode array detector). The only solvents used were degassed, deionized H_2O and HPLC grade MeCN from Fisher Scientific. Analytical reverse phase column Capital HPLC ODS-NN (4.6 mm × 250 mm).

2.5. Experimental

Dibenzyl disulfide **59** *in batch mode without a phase transfer catalyst*



Sodium sulfide nonahydrate (0.24 g, 1.0 mmol) was dissolved in water (10 mL) at 30 °C. The solvent designated in Table 2. 1 (10 mL) was added. Benzyl bromide (0.24 mL, 2.0 mmol) was added and stirred at 90 °C overnight. The reaction was quenched with brine (10 mL) and the organic product was extracted using Et_2O (3 × 10 mL). The combined organic extracts were dried over $MgSO_4$, and then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum. The product was obtained in an 88% yield (0.188 g, 0.88 mmol) using the above procedure using 50% aqueous ethanol. The spectroscopic data is in agreement with the literature.[47] Colourless solid; m.p. 48-50 °C (Lit m.p. 48 °C)[48]; Proton nuclear magnetic resonance (^{1}H NMR) (250 MHz, $CDCl_3$, 298 K): δ= 7.36-7.11 (m, 10H), 3.59 (s, 4H) ppm.

Dibenzyl disulfide **59** *in batch mode with a phase transfer catalyst*

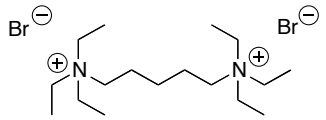
Sodium sulfide nonahydrate (0.12 g, 0.5 mmol) was dissolved in water (2 mL) at 30 °C. An organic solution of benzyl bromide (0.12 mL, 1.0 mmol) and PTC designated in Table 2. 4 (7 mol%) in the solvent designated in Table 2.4 (4 mL) was added. The reaction was stirred at 35 °C for the designated time in Table 2.4. The reaction was quenched with brine (2 mL). The organic layer was extracted then washed with H_2O (3 × 3 mL). The organic extract was dried over $MgSO_4$, then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum. The product was obtained in yields reported in Table 2.4 using the above procedure. The spectroscopic data is in agreement with the literature.[47]

Dibenzyl disulfide **59** *in flow mode with a phase transfer catalyst*

Sodium sulfide nonahydrate (0.26 g, 1.1 mmol) were dissolved in water (10 mL) and stirred at 50 °C for 30 min. Benzyl bromide (0.25 g, 2 mmol) and compound **65** (0.03 g, 7 mol%) were dissolved in EtOH (10 mL). The solutions were loaded into two separate syringes, placed on a syringe pump and pumped 3 mL/h at 35°C, through a Comet mixer which was connected to a PTFE reaction coil (volume: 1 mL, length: 2 m, internal diameter: 0.8 mm). Two reactor volumes (2 RV) were pumped through to reach steady state, and the subsequent 3 RV was collected in a flask containing brine (3 mL). The

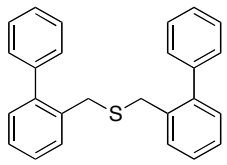
product was extracted using Et_2O (3 mL), Et_2O:EtOAc 1:1 (3 mL) and EtOAc (3 mL). The combined organic extracts were dried with anhydrous $MgSO_4$ and concentrated by vacuum evaporation. The product was obtained in 65% yield (42 mg, 0.20 mmol) using the above procedure. The spectroscopic data is in agreement with the literature.[47]

N,N,N,N',N',N'-Hexaethyl-1,5-pentanediaminium dibromide **65**



Triethylamine (1.2 mL, 8.6 mmol), 1,5-dibromopentane (0.59 mL, 2.5 mmol) and the desired solvent was stirred at the desired temperature for a desired time in Table 2. 3. A brown precipitate formed, then the solvent was decanted. Acetone (4 mL) was added and the mixture was heated to reflux, allowed to cool to produce white solid. The mixture was filtered and the solid was dried under high vacuum. The product was obtained in yields give in Table 2. 3. The spectroscopic data is in agreement with the literature.[49] Colourless solid, m.p. 86-88 °C; ^{1}H NMR (250 MHz, D_2O, 298 K): δ = 3.24-3.11 (m, 16H), 1.71-158 (m, 4H), 1.37-1.28 (m, 2H), 1.15 (t, *J*= 7.25 Hz, 18H) ppm.

bis([1,1'-biphenyl]-2-ylmethyl)sulfide **61**



Sodium sulfide nonahydrate (0.12 g, 0.5 mmol) was dissolved in water (2.5 mL). EtOH (2.5 mL) and 2-phenylbenzyl bromide (0.18 mL, 1.0 mmol) were added. The reaction was stirred at 90 °C for 4 h. The reaction was quenched with brine (10 mL) and the organic product was extracted using Et_2O (3 × 3 mL). The combined organic extracts was dried over $MgSO_4$, and then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum.

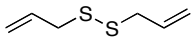
Column chromatography was performed using a Biotage Isolera with the following method. The crude mixture (0.178 g) was loaded onto a Biotage Snap KP-Sil 10 g Flash Column (15 mL column volume). The gradient was performed; 100 % hexane for 10 column volume (CV), then increased to 90:10 hexane: ethyl acetate over 5CV, then to 100% ethyl acetate over 10CV, and held at 100% ethyl acetate for 5CV. The solvent from the appropriate fractions was removed and dried on a high vacuum. The product was

obtained in a 91 % yield (166 mg, 0.49 mmol) using the above procedure. Colourless oil; ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ = 7.30-7.11 (m, 18H), 3.41 (s 4H) ppm; Carbon nuclear magnetic resonance (^{13}C NMR) (125 MHz, $CDCl_3$) δ = 142.0 (2C), 140.9 (2C), 135.3 (2C), 130.2 (2C), 130.0 (2C), 129.2 (4C), 128 (4C), 127.4 (2C), 127.0 (2C), 126.9 (2C), 34.6 (2C) ppm; ν_{max} (NaCl): 3058, 3022, 1479, 1436, 1093, 1073, 1010 cm^{-1}; High resolution mass spectrometry (HRMS) (electrospray ionization [ESI]): calculated for $C_{26}H_{22}S_1$ (M^++Na^+): 389.1346; found 389.1340.

General procedure: Synthesis of symmetrical disulfides in flow

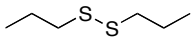
Sulfur (258 mg, 8.0 mmol) and sodium sulfide nonahydrate (2.40 g, 10 mmol) were dissolved in water (5 mL) and stirred at 50 °C for 30 min. The alkyl halide (20 mmol) and tetrabutylammonium bromide (257 mg, 0.8 mmol) were dissolved in EtOH (3.22 mL). The solutions were loaded into two separate 5 mL syringes and placed on a syringe pump with a flow rate of 0.1 mL/min, through a Comet mixer which was connected to a PTFE reaction coil (volume: 1 mL, length: 2 m, internal diameter: 0.8 mm). The reaction mixture was eluted into a flask containing brine to quench the reaction. After the reaction, the mixture was extracted with diethyl ether (3 × 20 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over magnesium sulfate, and the solvents were removed in vacuo.

Diallyl disulfide **2**



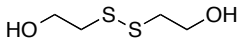
The product was obtained in an 88% yield (769 mg, 7.0 mmol) using the general procedure. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$): δ= 5.81 (ddt, *J*=17.2, 9.9, 7.3 Hz, 2H), 5.21-5.09 (m, 4H), 3.34 (d, *J*= 7.4 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ = 133.4, 118.4, 42.4 ppm; ν_{max} (NaCl): 3082, 3010, 2979, 2906, 1634, 1423, 1398, 1266, 1215, 987, 739 cm^{-1}; High resolution mass spectrometry (HRMS) (electrospray ionization [ESI]): calculated for $C_6H_{10}S_2$ (M^+): 146.0224; found 146.0223.

Dipropyl disulfide **68**



The product was obtained in a 45% (540 mg, 3.6 mmol) yield using the general procedure. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$): δ= 2.60 (t, *J*= 7.4 Hz, 4H), 1.75-1.56 (m, 4H), 0.94 (t, *J*= 7.3 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ = 41.1, 22.5, 13.1 ppm; ν_{max} (NaCl): 2962, 2932, 2873, 1456, 1413, 1290, 1230, 1216, 760 cm^{-1}; HRMS (ESI): calculated for $C_6H_{13}S_2$ (M^+-H): 149.0453; found 149.0450.

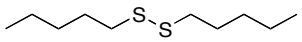
Bis(2-hydroxyethyl) disulfide **70**



70

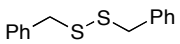
The product was obtained in a 70% yield (539 mg, 3.5 mmol) using the general procedure but on a 62.5 % reduced scale. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ= 2.82 (t, *J*= 5.8 Hz, 4H), 3.84 (t, *J*= 5.8 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ= 60.4, 41.3 ppm; ν_{max} (NaCl): 3390, 3054, 2928, 2877, 1421, 1401, 1266, 1058, 1008, 739, 703 cm^{-1}; HRMS (ESI): calculated for $C_4H_{10}O_2S_2$ ($M+H^+$): 155.0200; found 155.0191.

Dipentyl disulfide **72**



The product was obtained in a 63% yield (1.03 g, 5.0 mmol) using the general procedure. Clear oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ= 2.67 (d, *J*= 7.5, Hz, 4H), 1.81-1.60 (m, 4H) 1.42-1.26 (m, 8H), 0.94-0.86 (t, *J*= 6.94 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ= 39.1, 30.7, 28.9, 22.3, 13.9 ppm; ν_{max} (NaCl): 2957, 2927, 2871, 2858, 1465, 1413, 1378, 1341, 1297, 1271, 1254, 729 cm^{-1}; HRMS (ESI): calculated for $C_{10}H_{22}S_2$ (M^+): 206.1163; found 206.1165.

Dibenzyl disulfide **74**



The product was obtained in a 43% yield (1.06 g, 4.3 mmol) using 50% the equivalents of the general procedure. Colourless solid; m.p. 48-50 °C (Lit m.p. 48-50 °C)[48]; ^{1}H NMR (250 MHz, $CDCl_3$) δ= 7.50–7.37 (m, 10 H), 3.73 (s, 4H) ppm; ^{13}C NMR (125MHz, $CDCl_3$) δ= 137.5, 129.5 (2C), 128.6 (2C), 127.5, 43.4ppm; ν_{max} (NaCl): 3054, 2987, 1265, 739, 705 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{15}S_2$ ($M+H^+$): 247.0610; found 247.0608.

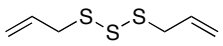
General procedure: synthesis of symmetrical trisulfides in flow

Sulfur (641 mg, 20 mmol) and sodium sulfide nonahydrate (2.40 g, 10 mmol) were dissolved in water (5 mL) and stirred at 50°C for 30 min. The alkyl halide (20 mmol) and tetrabutylammonium bromide (257 mg, 0.8 mmol) were dissolved in EtOH to a total volume of 5 mL. The solutions were loaded into two separate 5 mL syringes and placed on a syringe pump with a flow rate of 0.1 mL/min, through a Comet mixer which was connected to a PTFE reaction coil (volume: 1mL, length: 2 m, internal diameter: 0.8 mm). The reaction mixture was eluted into a flask containing brine to quench the reaction. After the reaction, the mixture was extracted with diethyl ether (3 × 20 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over magnesium sulfate, and the solvents were removed in vacuo.

Diallyl polysulfide using allyl chloride

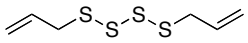
Diallyl polysulfide mixture was obtained as a clear yellow oil (2.64 g) using twice of the amounts for all chemicals as described in the general procedure. From the ^{1}H NMR, the sample contained 22% diallyl disulfide, 53% diallyl trisulfide, and 25% higher diallyl polysulfides. From the reaction mixture, 342 mg was purified on a Biotage Isolera system with a Telos Flash C18 column (12 g) using a solvent gradient (v:v) of water–methanol (50:50) to (20:80) for 25 column volumes (CV), then (20:80) to (0:100) for 15 CV, then (0:100) for 4 CV at a flow rate of 12 mL/min.

Diallyl trisulfide **3**



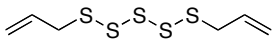
The product was isolated (206 mg, 1.16 mmol) using the above procedure. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ = 6.00-5.81 (m, 2H), 5.32–5.16 (m, 4H), 3.52 (d, *J*= 7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ= 133.0, 119.5, 42.0 ppm; ν_{max} (NaCl): 3082, 3010, 2979, 2906, 1634, 1423, 1398, 1217, 986, 191, 721 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_3$ (M^+): 177.9945; found 177.9940.

Diallyl tetrasulfide **4**



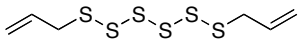
The product was isolated (75 mg, 0.36 mmol) using the above procedure. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.89 (ddt, *J*= 17.1, 9.9, 7.3 Hz, 4H), 5.24-5.11 (m, 2H), 3.51 (d, *J*= 7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=132.4, 119.3, 42.0 ppm; ν_{max} (NaCl): 3084, 3011, 2980, 2908, 1634, 1423, 1398, 1219, 986, 909, 733 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_4$ (M^+): 209.9665; found 209.9661.

Diallyl pentasulfide **73**



The product was isolated (28 mg, 0.12 mmol) using the above procedure. Clear yellow oil; ^{1H} NMR (250 MHz, $CDCl_3$): δ = 5.82 (ddt, *J*= 17.1, 9.9, 7.3 Hz, 4H), 5.25-5.14 (m, 2H), 3.55 (d, *J*= 7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=132.2, 119.9, 42.5 ppm; ν_{max} (NaCl): 3072, 3054, 2982, 2920, 1634, 1423, 1265, 1220, 988, 925, 739 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_5$ (M^+): 241.9386; found 241.9385.

Diallyl hexasulfide **74**

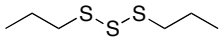


The product was isolated (10 mg, 0.04 mmol) using the above procedure. Clear yellow oil; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 5.89 (ddt, *J*= 17.2, 9.9, 7.3, 4H), 5.34-5.21 (m, 2H), 3.62 (d, *J*= 7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ=132.2, 120, 42.4 ppm; ν_{max} (NaCl): 3081, 2956, 2922, 2849, 1847, 1726, 1634, 1422, 1397, 1261, 1218, 1074, 1020, 945, 921, 859, 801, 720 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_6$ (M^+): 273.9107; found 273.9105.

Dipropyl polysulfide using propyl bromide

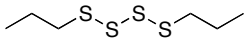
Dipropyl polysulfide mixture was obtained as a yellow oil (1.30 g) using the amounts given in the general procedure. From ^{1}H NMR, the crude reaction product mixture contained 15% dipropyl disulfide, 33% dipropyl trisulfide, and 52% dipropyl polysulfides. From the product mixture, 200 mg was separated on a Biotage Isolera system with a Telos Flash C18 column (12 g) using a solvent gradient (v:v) of water–methanol (50:50) for 3 CV, then (50:50) to (24:86) for 32 CV, held at (24:86) for 13 CV, then (26:84) to (0:100) for 4 CV, then (0:100) for 13 CV at a flow rate of 12 mL/min. The amounts obtained after separation were as follows: dipropyl disulfide (23 mg, 0.15 mmol), dipropyl trisulfide (41 mg, 0.23 mmol), and an inseparable mixture of dipropyl polysulfides (121 mg, 0.56 mmol). Due to overlapping ^{1}H NMR signals, a ratio could not be determined.

Dipropyl trisulfide **78**



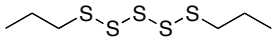
The product was isolated (41 mg, 0.23 mmol) using the above procedure. Clear yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.92 (m, 4H), 1.80 (m, 4H), 1.02 (t, J = 7.33 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CHCl_3$) δ = 41.3, 22.3, 13.1 ppm; ν_{max} (NaCl): 2962, 2929, 2872, 1455, 1411, 1377, 1337, 1290, 1231, 1089, 1051, 897, 781 cm^{-1}; HRMS (ESI): calculated for $C_6H_{14}S_3(M^+)$: 182.0258; found 182.0257.

Dipropyl tetrasulfide



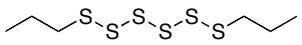
The product was detected by HRMS (ESI): calculated for $C_6H_{14}S_4(M^+)$: 213.9978; found 213.9977.

Dipropyl pentasulfide



The product was detected by HRMS (ESI): calculated for $C_6H_{14}S_5(M^+)$: 245.9699; found 245.9702.

Dipropyl hexasulfide

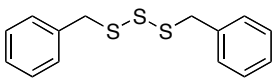


The product was detected by HRMS (ESI): calculated for $C_6H_{14}S_6(M^+)$: 277.9420; found 277.9420.

Dibenzyl polysulfides using benzyl bromide

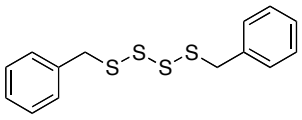
Dibenzyl polysulfides mixture was obtained as a yellow oil (2.4 g) using the amounts given in the general procedure at 33°C. From ^1H NMR, the sample contained 14% dibenzyl disulfide, 71% dibenzyl trisulfide, and 14% dibenzyl polysulfides. A purification of the dibenzyl polysulfides was impossible; how- ever, their existence was detected and verified by mass spectrometry of the mixture. From the product mixture, 261 mg was separated on a Biotage Isolera with a Telos Flash C18 column (12 g) using a solvent gradient (v:v) of water–methanol (30:70) for 3 CV, (30:70) to (20:80) for 25 CV, then (20:80) to (0:100) for 15 CV, then (0:100) for 4 CV at a flow rate of 12 mL/min. The amounts obtained after separation were as follows: dibenzyl disulfides (21 mg, 0.09 mmol), dibenzyl trisulfides (70 mg, 0.25 mmol), and an inseparable mixture of dibenzyl polysulfides (104 mg).

Dibenzyl trisulfide **79**



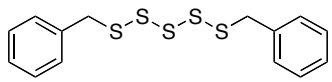
The product was isolated (70 mg, 0.25 mmol) using the above procedure. ^1H NMR (400 MHz, $CHCl_3$) δ =7.65-7.51 (m, 10H), 4.31 (s, 4H) ppm; ^{13}C NMR (100 MHz, $CHCl_3$) δ = 129.4, 128.6, 127.5, 136.4, 43.1 ppm; ν_{max} (NaCl): 3074, 3061, 3028, 2914, 1601, 1494, 1453, 1230, 1199, 1070, 914, 765, 967, 658 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{14}S_3(M^+)$: 278.0258; found 278.0262.

Dibenzyl tetrasulfide



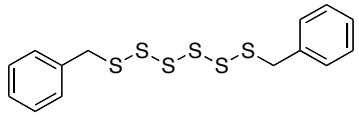
The product was detected by HRMS (ESI): calculated for $C_{14}H_{14}S_4(M^+)$: 309.9978; found 309.9981.

Dibenzyl pentasulfide



The product was detected by HRMS (ESI): calculated for $C_{14}H_{14}S_5(M^+)$: 341.9699; found 341.9695.

Dibenzyl hexasulfide

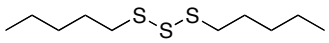


The product was detected by HRMS (ESI): calculated for $C_{14}H_{14}S_6(M^+)$: 373.9420; found 373.9419.

Dipentyl polysulfides using pentyl bromide

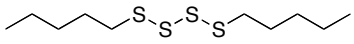
Dipentyl polysulfides mixture was obtained as a yellow oil (165 mg) using 10% of the amounts given in the general procedure. From ^{1}H NMR, the sample contained 80% dipentyl disulfide and 20% dipentyl polysulfides. This mixture proved inseparable using RP chromatography. Their presence was confirmed by high-resolution mass spectrometry of the polysulfide mixture.

Dipentyl trisulfide



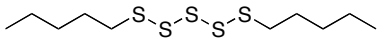
The product was detected by HRMS (ESI): calculated for $C_{10}H_{22}S_3(M^+)$: 238.0884; found 238.0889.

Dipentyl tetrasulfide



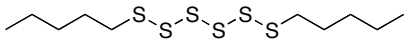
The product was detected by HRMS (ESI): calculated for $C_{10}H_{22}S_4(M^+)$: 270.0604; found 270.0606.

Dipentyl pentasulfide



The product was detected by HRMS (ESI): calculated for $C_{10}H_{22}S_5(M^+)$: 302.0325; found 302.0331.

Dipentyl hexasulfide



The product was detected by HRMS (ESI): calculated for $C_{10}H_{22}S_6(M^+)$: 334.0046; found 334.0039.

2.6. References

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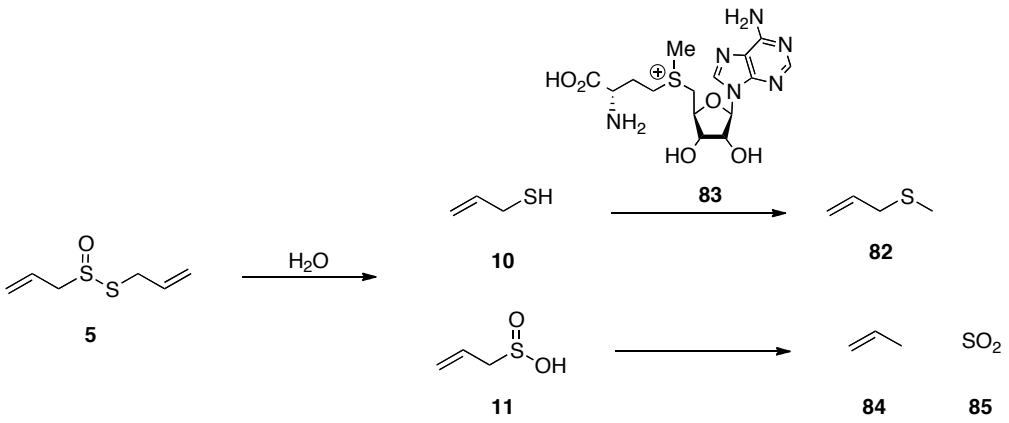
3

Synthesis of Unsymmetrical Monosulfides

3.1. Introduction

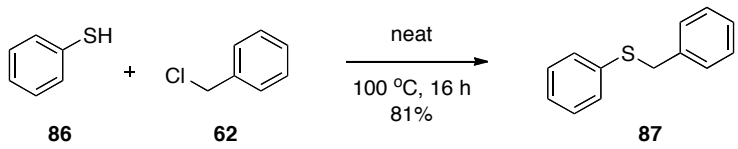
This chapter focuses on the synthesis of unsymmetrical monosulfides found in garlic oil extract (GOE), such as allyl methyl sulfide **82**, (Scheme 3.1). GOE contains a wide array of organic sulfur compounds and the concentrations can be determined by high performance liquid chromatography (HPLC), provided appropriate analytical standards are available. Thus, a flow procedure will be developed to produce unsymmetrical monosulfides for HPLC calibration of GOE.

Compound **82** is a stable garlic metabolite and the main component of garlic breath. It can be detected over 30 hours after consumption of garlic.[1] It is generated when allicin in GOE breaks down in the presence of water, which generates allyl thiol **10**. This reactive allyl thiol is then *S*-alkylated by *S*-adenosylmethionine **83** to produce **82**.[2]



Scheme 3.1. Allyl methyl sulfide formation through the degradation of allicin **5** with water

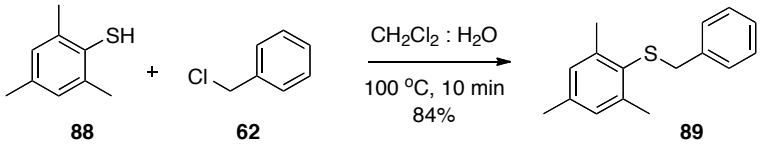
Synthetically, unsymmetrical monosulfides are produced through electrophilic alkylation of thiols. Thiols will react directly with alkyl halides in the absence of solvent and catalysts (Scheme 3.2).[3] In doing so, the overall reaction then poses excellent atom economy. This approach, however, has undesirable operating conditions, high temperatures of 100 °C and long reaction times of 16 h. Improving this reaction can be achieved in several ways; utilising flow chemistry to improve heating, mass transfer and mixing. Catalysts have also been utilised to reduce the reaction time.



86 **62** **87**

Scheme 3.2. Solvent-free *S*-alkylation of thiophenol

A limiting factor of this alkylation was mass transfer as the product formed was a solid and may have eventually led to ineffective mixing. Flow chemistry has been demonstrated to improve mixing and mass transfer.[4] By using flow mode instead of batch, Glasnov *et al*. described that the reaction time of an alkylation of aryl thiol **88** was reduced from hours to minutes, (Scheme 3.3).[5]

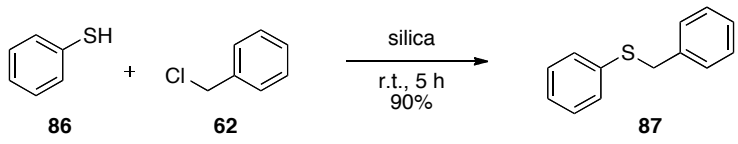


88 **62** **89**

Scheme 3.3. Flow mode *S*-alkylation of thiophenol

Catalysis can be used to improve the rate of reactions. Glasnov *et al*. added tetrabutylammonium bromide **56** to the reaction in Scheme 3. 3 and reduced the reaction time to 2 minutes. However, this reaction still utilised undesirable high temperatures.

Reducing the reaction temperature of *S*-alkylation has been achieved using commercially available silica (Scheme 3.4).[6] The reaction procedure was straightforward; no additional heating was required as the reaction proceeded at ambient temperatures. Furthermore, the solid silica was reusable and easily removed by filtration as the silica is heterogeneous.



86 **62** **87**

Scheme 3.4. Silica-promoted *S*-alkylation of thiophenol

Whilst the lower reaction temperature is advantageous, the reaction time is longer, hours and not minutes. Disulfides were also generated as side products and difficult chromatographic purification was required.[6] This isolation needed additional time that is undesirable in the syntheses described in this chapter.

Heterogeneous solid-supported reagent morpholinomethyl-polystyrene (PS-NMM, £ 2.90 / mmol) has been described for the *S*-alkylation of thiols in the context of the synthesis of thionicotinamide inhibitors.[7] The reaction was performed at 60 °C for 6 h and the work-up only required filtration and centrifugation, however 5 equivalents were used.

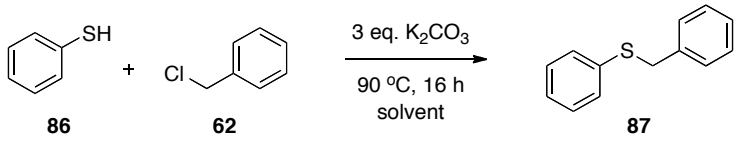
3.2. Results and Discussion

The aim of this chapter it to synthesise unsymmetrical monosulfides, akin to those found in garlic such as allyl methyl sulfide. This investigation evaluates the reactivity of inorganic bases, such as potassium carbonate, as cheap bases to produce unsymmetrical monosulfides from thiols. The inorganic bases were examined in batch mode, then in homogenous flow systems and finally in heterogeneous flow systems. The developed flow system will also investigate the alkylation of other protic substrates.

The synthesis of garlic metabolites substrate would have required the use of allyl thiol. However, due to the volatility of the products, a different model system using thiophenol was investigated.

3.2.1. Synthesis in Batch Mode

The initial batch experiments for the *S*-alkylation used thiophenol **86**, which was alkylated with **62** and an excess of potassium carbonate.



Scheme 3.5. Model reaction of the *S*-alkylation

The reaction proceeded in good yields in organic solvents, such as methanol and ethyl acetate (Table 3.1, entries 1 and 2). The potassium carbonate appeared to remain as a heterogeneous solid. Filtration was not sufficient to remove the potassium carbonate and an aqueous work-up had to be employed. No reaction was observed when hexane was used as a non-protic solvent (Table 3.1, entry 3).

The next approach was to evaluate a homogenous system with the potassium carbonate dissolved in water. The reaction proceeded quantitatively using 50% aqueous methanol (Table 3.1, entry 4). Then, the reaction was performed without an organic solvent (Table 3.1, entry 5) and proceeded with an equally excellent yield.

Table 3.1. The effect of solvent on the *S*-alkylation of model reaction

Entry	Solvent	Yield (%)
1	MeOH	80
2	EtOAc	73
3	anh. hexane	n.r.
4	50% aq MeOH	99
5	H_2O	98

Conditions; Thiophenol, benzyl chloride and K_2CO_3 were stirred in a solvent at reflux overnight.

The results from homogenous batch reaction were promising. However, the reaction temperature may have been excessive. Flow mode was investigated to improve the mixing, heat and mass transfer.

3.2.2. Synthesis in Homogeneous Flow Mode

The reaction solvent system from Table 3.1, entry 4 (50% aq MeOH) was evaluated in flow mode. Two solutions were prepared; an aqueous potassium carbonate solution and an organic reagent solution in methanol, which were attached to a Vapourtec machine using cannula needles. This flow system is not a continuous process. The work-up of this flow process required a discrete phase extraction performed using glassware. The solvents used were methylene chloride and petroleum ether (pet. ether). The product was isolated in average yields (Figure 3.1).

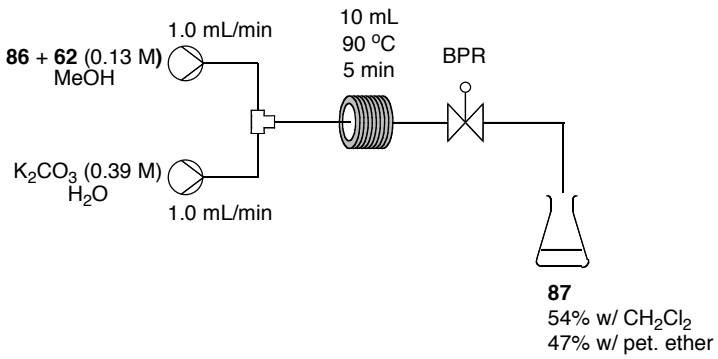


Figure 3.1. Homogenous flow setup for the *S*-alkylation of model reaction
[BPR] backpressure regulator

An inline phase separator (PS), discussed in chapter 1, was developed in the Wirth group for inline organic separations to eliminate a discrete organic extraction.[8] This resulted in a continuous process; the reaction setup is shown in Figure 3.2. In this system, an immiscible organic solvent was pumped into the reactant stream to extract the organic product; that was then pumped through a 2 mL PTFE coil and into the PS to separate the organic phase from the aqueous phase.

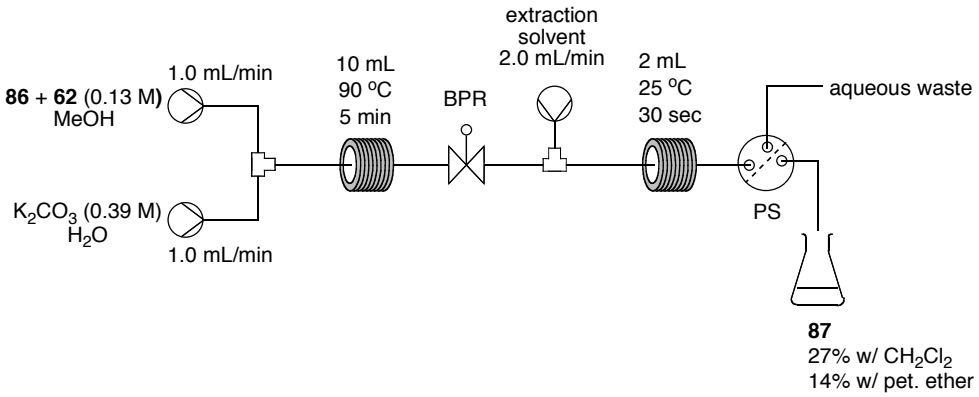


Figure 3.2. Homogenous flow setup with inline PS for the *S*-alkylation of model reaction [BPR] backpressure regulator, [PS] phase separator

The 5 minute reaction time was too short which resulted in a low conversion. Furthermore, the phase separator was not as efficient as the separation performed using glass phase separator. The lower efficiency led to a lower isolated yields comparative to batch.

Multiple passes through the PS with additional extracting solvent may be required to improve the system. However there are now several more advanced PS in the literature,[9] as well as commercially available systems such as Zaiput PS.

3.2.3. Synthesis in Heterogeneous Flow Mode

This *S*-alkylation had the potential to be performed as a heterogeneous reaction in a packed-bed flow reactor system. Solid-supported bases have been used in packed-bed flow reactor systems.[10] The system depended on the solvent remaining anhydrous. The absence of water should prevent potassium carbonate and generated salts from dissolving and eluting. The first principle of green chemistry states that only the solvent has to be removed to isolate the product.[10] This process has the potential to achieve this and would have exceedingly high atom efficiency. A packed-bed flow reactor was used to create a high rate of heat and mass transfer. The narrow diameter allows greater heat transfer. The mass transfer would be improved as the solvent immediately comes into contact with heterogeneous reagent without diffusion.

The system involved a glass column charged with the maximum quantity of inorganic base. A backpressure (BPR) regulator was attached (Figure 3.3) to allow solvents to remain in the liquid phase above their boiling point.

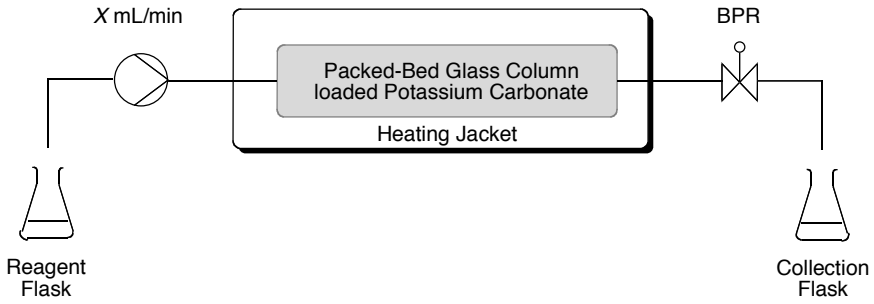


Figure 3.3. Packed-bed reactor setup, [BPR] backpressure regulator

The heterogeneous packed-bed reactor system was developed using a Vapourtec E-series. This system used peristaltic based pumps that are attached to the reagent flasks that are under an inert atmosphere of argon. The reactor consisted of a glass heating jacket and a packed-bed column placed inside. The heating jacket is heated by blowing hot air through with precise temperature control. The packed-bed was an Omnifit glass column (length: 150 mm, internal diameter: 6.6 mm). Full visibility of the contents of the packed-bed reactor was possible as all the components were glass. The packed-bed column was connected to a backpressure regulator (BPR) that eluted the reaction mixture into a collection flask.

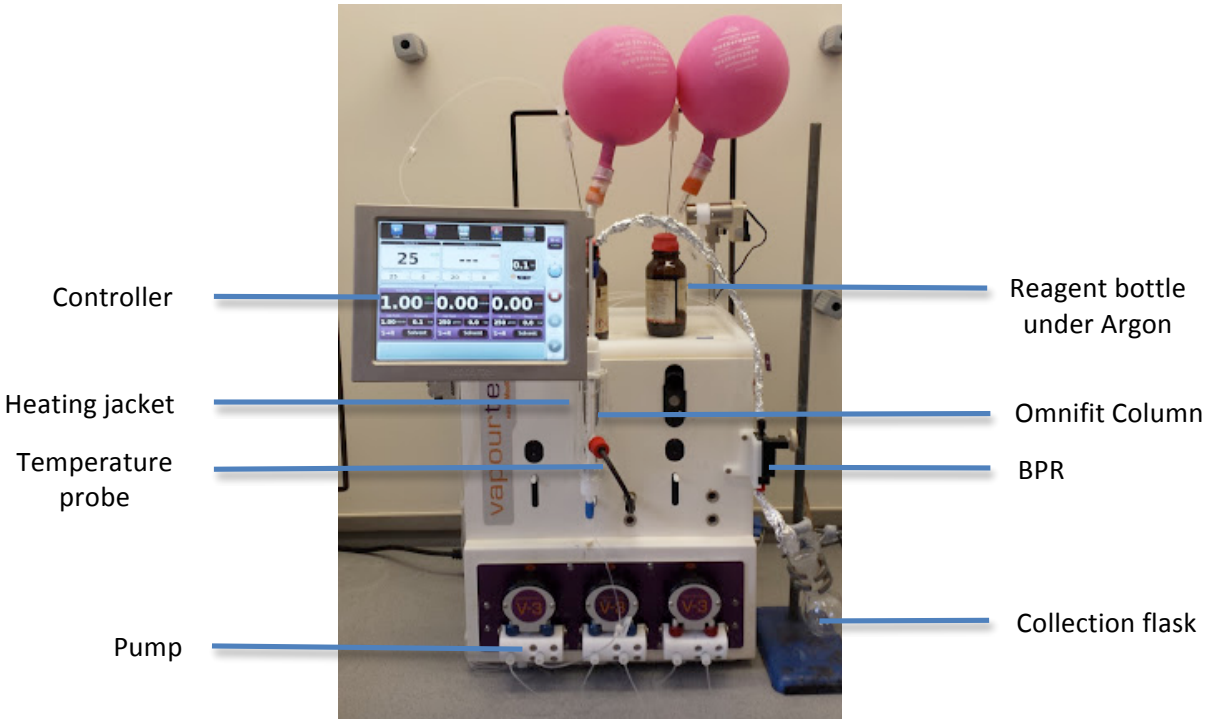
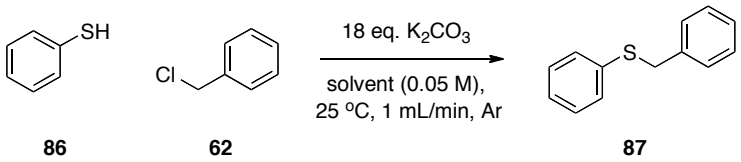


Figure 3.4. Vapourtec setup

3.2.3.1. S-Alkylation

The heterogeneous *S*-alkylation approach was developed to produce unsymmetrical monosulfides found in garlic without an aqueous work-up in a green synthesis.

The *S*-alkylation was investigated using **86** and **62** as the model system (Scheme 3.6). When dry ethanol was used as the solvent, **87** was obtained in good yield (80%).



Scheme 3.6. Model reaction for the *S*-alkylation

Other dry solvents were also investigated, available from solvent purification system (SPS), as shown in Table 3.2 (entries 2-4). Diethyl ether and tetrahydrofuran did not produce any alkylated product. Acetonitrile was the preeminent solvent for this reaction, which has also been demonstrated by Saxena and co-workers in similar work.[11]

Na_2CO_3 was also investigated, however, only a 16% yield of **87** was achieved, as a result of the reduced polarising ability compared to potassium carbonate, which has been previously demonstrated by Iki *et al.*[11] There was a concern that the inorganic base in the packed-bed reactor may have been eluted by water formed in the reaction.

To examine this possibility, the experiment from Table 3.2, entry 5 was repeated and an aqueous work-up was performed to evaluate if any column salt material was in the product. There was no discernible difference in isolated yield indicating no salts were eluted. The solubility of potassium carbonate and potassium chloride is negligible in acetonitrile below 100 °C.[12]

Table 3.2. Solvent screening for the *S*-alkylation of the model reaction

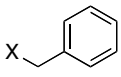
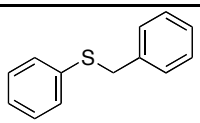
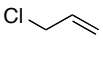
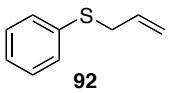
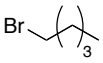
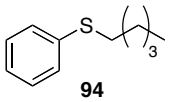
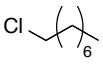
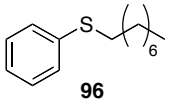
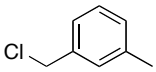
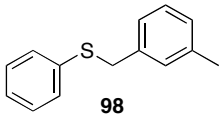
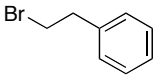
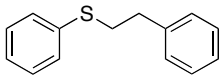
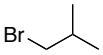
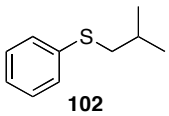
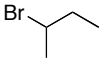
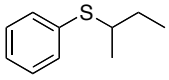
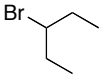
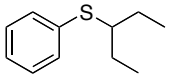
Entry	Solvent	Yield (%)
1	EtOH[a]	80
2	Et_2O	n.r.
3	THF	n.r.
4	MeCN	96
5	MeCN	67[b]

Conditions: Thiophenol and benzyl bromide were dissolved in an anhydrous solvent (0.05M) was pumped with a flow rate of 1 mL/min through an Omnifit column packed with 18 eq.= K_2CO_3 at 25 °C, [a] dried by distillation over Mg/I_2. [b] the concentration is increased to 0.5M.

The reduced yield in Table 3.2, entry 5, was the result of increasing the concentration by a factor of 10. This may indicate that the contact time may not be sufficient or the potassium carbonate is consumed in the reaction, as a reduced yield was isolated. To evaluate the longevity of the potassium carbonate in the packed-bed reactor, the reagent stream was flowed continuously. After 1 hour, unreacted starting material is observed on TLC in 100% hexane and after 5 hours the column is depleted and product formation cannot be observed.

A larger packed-bed reactor that has a greater quantity of potassium carbonate would be required to perform reactions at higher concentrations or for longer periods of time. The concentration of 0.05M remained unaltered during the investigation of the substrate scope of this heterogeneous packed-bed reactor system, which is shown in Table 3.3.

Table 3.3. Results of the *S*-alkylation on thiophenol using several alkyl halides

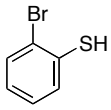
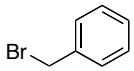
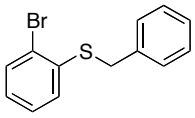
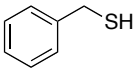
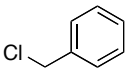
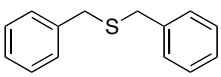
Entry	Alkyl halide	Product	Temperature (°C)	Back Pressure (bar)	Yield (%)
1 2		 **87**	25	-	X = Cl 96 X = Br 99
3	 **45**	 **92**	25	-	99
4	 **71**	 **94**	25	-	84
5	 **95**	 **96**	25	-	93
6	 **97**	 **98**	25	-	99
7	 **99**	 **100**	25	-	75[a,b]
8			25	-	n.r.
9	**101**	**102**	100	4.0	85[a]
10			25	-	20
11	**103**	**104**	100	3.5	85[a]
12			25	-	n.r.
13	**105**	**106**	100	1.7	70[a]

Conditions: anh. MeCN (0.05 M) containing thiophenol and alkyl halide pumped with a flow rate of 1 mL/min through an Omnifit column packed with 18 eq. of potassium carbonate
[a] Purified by column chromatography, [b] performed at 0.10 M concentration

Several alkyl halides (Table 3.3, entries 1-6) reacted with good to excellent yields in clean reactions. The reaction with phenyl ethyl (Table 3. 3, entry 7) bromide proceeded in good yield but required chromatography.

The branched alkyl halides (Table 3.3, entries 8, 10, 12) did not react at 25 °C. However, when the packed-bed reactor was heated to 100 °C the products were obtained in good yields after purification (Table 3.3, entries 9, 11, 13). A backpressure regulator was required as the solvent was heated above the boiling point to keep the acetonitrile in the liquid phase.

Table 3.4. Results of the *S*-alkylation of different thiols and alkyl halides

Entry	Thiol	Alkyl halide	Product	Temp (°C)	Back Pressure (bar)	Yield (%)
1	 **107**	 **31**	 **108**	25	-	98
2	 **109**	 **62**	 **110**	100	1.5	60[a]

Conditions: anh. MeCN (0.05 M) containing the thiol and alkyl halide was pumped with a flow rate of 1 mL/min through an Omnifit column packed with 18 eq. K_2CO_3 at the specified temperature
[a] column performed

A brief evaluation of other thiols was performed. Bromo-substituted thiophenol **107** (Table 3.4, entry 1) reacted in excellent yield; comparable to that obtained using thiophenol (Table 3.3, entry 1). Aliphatic benzyl thiol required both higher temperatures to perform the reaction in good yield and chromatography to isolate the product (Table 3.4, entry 2).

A potential explanation for the reactivity of carbonate packed-bed reactor is related to substrate's aqueous pKa. This reaction proceeds more readily with lower pKa. Comparing the reactivity of thiophenol and benzyl thiol, thiophenol **86** did not require any additional heating to achieve excellent yields of alkylated product. By contrast, benzyl thiol required an elevated temperature of 100 °C to undergo reasonable alkylation. Thiophenol has a greater acidity (pK_a 6.5)[13] than benzyl thiol (pK_a 9.3).[14]

The reaction using allyl thiol **10** to produce garlic metabolites, such as **82**, was promising. However, after column chromatography and solvent removal on high vacuum, the products were lost due the high volatility of the compounds. Isolation using distillation was not investigated due to time constraints.

To summarise, *S*-alkylation of thiols proceeded in good to excellent yields without an aqueous work-up using a packed-bed reactor charged with potassium carbonate. Substrates that required heating (Compounds **102**, **104**, **106**, **110**) did require chromatography.

3.2.3.2. Subsequent oxidation

The eluted reaction mixture has no impurities and only requires solvent removal to isolate the product. A subsequent oxidation was performed on the reaction mixture to test this hypothesis.

A two-step reaction sequence was attempted in continuous-flow mode. Hydrogen peroxide was pumped into the eluting solution from the packed-bed reactor and through a Vapourtec coil (10 mL). The reaction product was analysed by NMR and achieved a 25% conversion to the desired sulfoxide **111**. The maximum residence time possible for the oxidation was 5 minutes, due to the incoming flow rate of 1.0 mL/min of both reaction streams from the packed-bed reactor and the oxidant-containing solution. Longer reaction times were required and a semi-batch approach implemented.

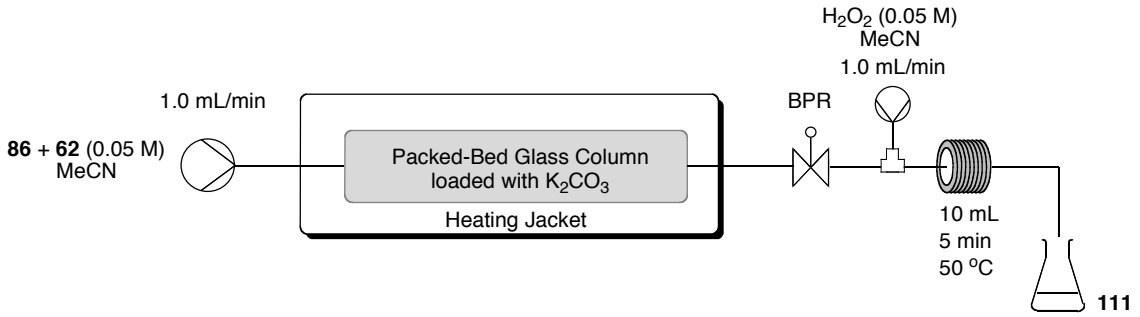


Figure 3.5. Telescoped flow synthesis of **111**

The reaction mixture from the packed-bed flow reactor was eluted into a collection flask containing one equivalent of hydrogen peroxide and the oxidising mixture was stirred overnight (Figure 3.6).

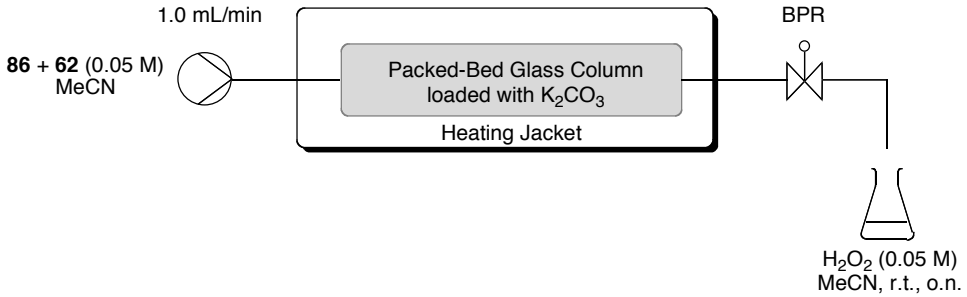
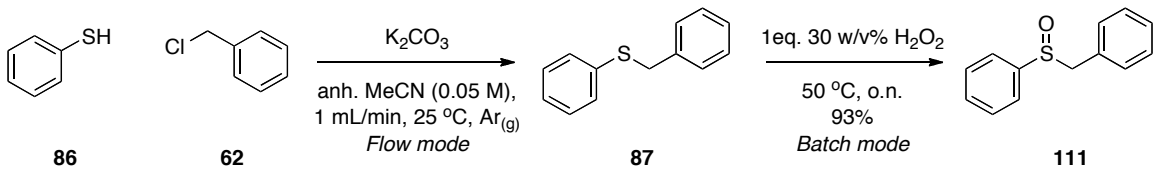


Figure 3.6. Semi-batch setup for the synthesis of **111**

The reaction was successful in semi-batch and the sulfoxide **111** was isolated in 93% yield (Scheme 3.7). Under these reaction conditions, no over-oxidation to the sulfone was observed.



Scheme 3. 7. Semi-batch synthesis of **111**

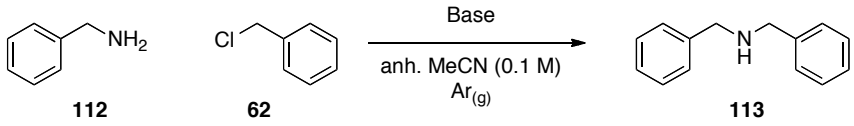
3.2.3.3. N-Alkylations

N-Alkylations of amines were also investigated using the packed-bed reactor system. Selective mono-*N*-alkylation is described in this section

N-Alkylation of Primary Amines

Mono-*N*-alkylation

The mono-alkylation of amines was performed on benzyl amine **112** with **62** (Scheme 3.8). The alkylation was performed at 100 °C with the packed-bed reactor loaded with potassium carbonate, this produced dibenzyl amine as the sole product albeit in a low yield (Table 3.5, entry 1). The yield was improved by increased residence time and temperature (Table 3.5, entries 2 and 3).



Scheme 3.8. Model reaction for *N*-alkylation

Caesium carbonate (Table 3.5, entry 4) demonstrated similar reactivity as potassium carbonate (Table 3.5, entry 1) using the same conditions. Calcium carbonate and magnesium oxide (Table 3.5, entry 5 and 6) required purification by chromatography and only proceeded in low yields. The application of time-consuming chromatography decidedly meant that investigation using calcium carbonate and magnesium oxide was undesirable, as group 1 alkali metal carbonates did not require chromatography.

Table 3.5. Results of the *N*-alkylation of benzyl amine using several inorganic bases

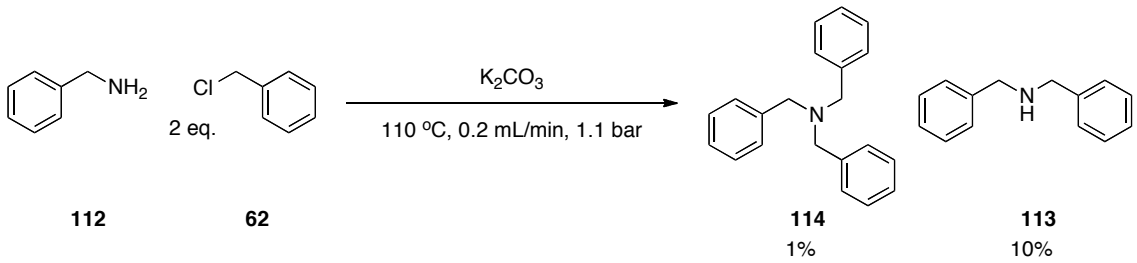
Entry	Temp (°C)	Flow Rate (mL/min)	Back Pressure (bar)	Base	Yield (%)
1	100	1	1.2	K_2CO_3	7
2	100	0.5	1.5	K_2CO_3	15
3	110	0.2	1.1	K_2CO_3	32
4	100	1	3.0	Cs_2CO_3	8
5	100	1	1.8	$CaCO_3$	8[a]
6	20	1	3.5	MgO	8[a]

Conditions: anh. MeCN (0.05M) containing the benzyl amine and benzyl chloride was pumped with a flow rate of 1 mL/min through an Omnifit column packed with an inorganic base,
[a] column chromatography performed

As can be seen in Table 3.5, the monoalkylation of benzyl amine can be performed using heterogeneous inorganic bases, however the yields are low and in some cases purification by chromatography is required. If syringe pumps were used instead of the Vapourtec pumps, lower flow rates could be achieved which would increase the residence time and the yield.

Di-*N*-Alkylation

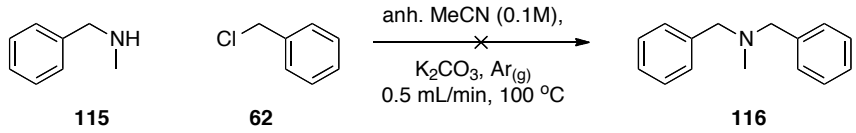
By doubling the equivalents of benzyl chloride, primary amines could be alkylated twice to produce tertiary amines. Experiments with benzyl amine produced a crude product mixture that mainly contained dibenzyl amine, as determined by NMR with respect to unreacted benzyl amine. This method has potential but again is impeded by the residence time provided by the size of the pack-bed reactor.



Scheme 3.9. Attempted di-*N*-alkylation of benzyl amine using 2 equivalents of benzyl chloride (yields determined from ^{1}H NMR)

N-Alkylation of Secondary Amines

N-alkylation of primary amines has already shown some promising results. The *N*-alkylation of secondary amines was hypothesised to proceed faster due to a lower pKa than primary amines. The alkylation of *N*-benzylmethyl amine as a secondary amine was performed. Unfortunately, no formation of tertiary amine using these conditions was detected.

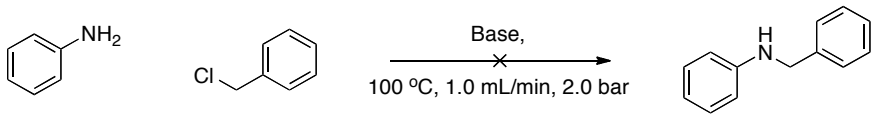


115 **62** **116**

Scheme 3.10. Attempted alkylation of *N*-benzylmethyl amine

N-Alkylation of Aniline

Alkylation of aniline was not achieved using any of the inorganic bases tested; sodium carbonate, potassium carbonate, caesium carbonate, calcium carbonate and magnesium oxide. Although, the mono-alkylation using potassium carbonate has been reported in the literature, additional aluminium oxide and microwave irradiation were required.[15] The addition of aluminium oxide in the pack-bed reactor could improve the reactivity of this packed-bed flow reactor in the future.

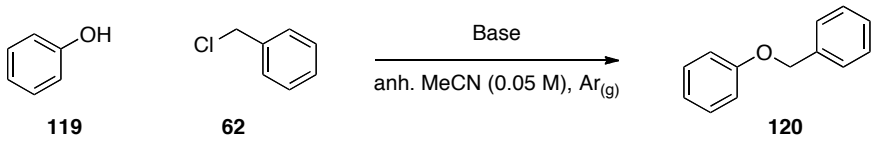


117 **62** **118**

Scheme 3.11. Attempted *N*-alkylation of aniline

3.2.3.4. *O*-Alkylation

Phenol was the initial substrate for the investigation using the packed-bed reactor.



119 **62** **120**

Scheme 3.12. *O*-Alkylation of phenol using benzyl chloride

No reactivity was observed at 25 °C (Table 3.6, entry 1). Higher temperatures increased the yield of **120** (Table 3.6, entry 2). The greatest increase of yield, 82% (Table 3.6, entry 3), was achieved using the lowest flow rate accessible with Vapourtec 3-series pumps (0.1 mL/min). *O*-Alkylation of phenol could be achieved using caesium carbonate in good yield (Table 3.6, entry 5).

Higher temperatures had improved the yield, however at 150 °C the potassium carbonate became coloured. The pressure also reached 10.0 bar, which is the maximum pressure of the system and reaction using these conditions had to be abandoned (Table 3.6, entry 4). Sodium carbonate, calcium carbonate and magnesium oxide showed no reactivity.

Table 3. 6. Results of the *O*-Alkylation of phenol using several inorganic bases

Entry	Temp (°C)	Flow Rate (mL/min)	Back Pressure (bar)	Base	Yield (%)
1	25	1	-	K_2CO_3	n.r.
2	100	1	1.5	K_2CO_3	4
3	100	0.1	1.0	K_2CO_3	20[a]
4	150	0.1	10.0	K_2CO_3	abandoned
6	100	0.1	1.4	Cs_2CO_3	82

Conditions: anh. MeCN (0.05 M) containing the phenol and benzyl chloride was pumped at a flow rate of 1 mL/min through an Omnifit column packed with an inorganic base, [a] NMR yield

4-Methoxy phenol, benzyl alcohol and benzoic acid were also investigated using this packed bed reactor setup and the reaction conditions are included in the appendix. However, these substrates did not produce any *O*-Alkylation products.

3.3. Conclusion

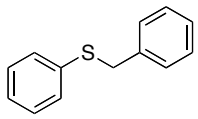
In this chapter, a safe procedure for handling thiols using flow chemistry was developed. The alkylation of thiols was performed using a packed-bed reactor containing an inorganic base that was insoluble in the solvent acetonitrile. Inorganic bases represent a cheaper alternative. Potassium carbonate (£ 0.007 / mmol from Sigma-Aldrich) is less expensive than commercially available heterogeneous solid-supported bases such as morpholinomethyl-polystyrene (PS-NMM, £ 2.90 / mmol from Sigma-Aldrich).

The atom efficiency is excellent using this packed-bed flow reactor method. The inorganic base and waste product salts are not eluted and the resultant solution merely required solvent removal that could be recycled without a work-up. This method was performed on a small scale and the longevity of the heterogeneous base is a factor when using larger quantities of reagents, either in larger concentrations or larger volumes of reactant solutions. The reaction was also clean enough to perform a subsequent oxidation in a semi-batch process producing the sulfoxide in 93%.

This procedure had some success on alkylating benzyl amine and phenol. This procedure could not alkylate aniline, benzoic acid or alcohols. The limiting factor appeared to be the flow rate and reaction vessel size. If both of these parameters could be increased this reaction may proceed with complete conversion. Further investigation with longer residence times and larger reactors may be required to improve the yields of the experiments reported here.

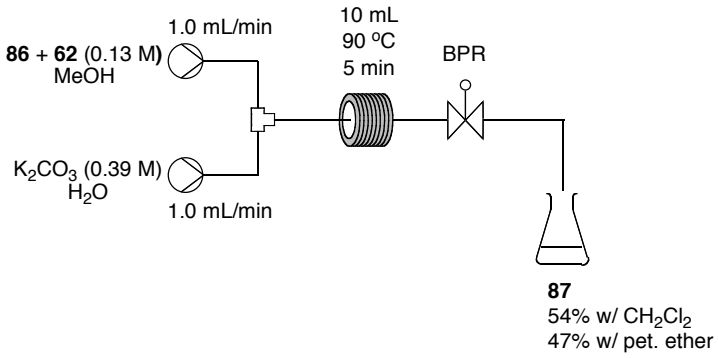
3.4. Experimental

Batch synthesis of benzyl phenyl sulfide **87**



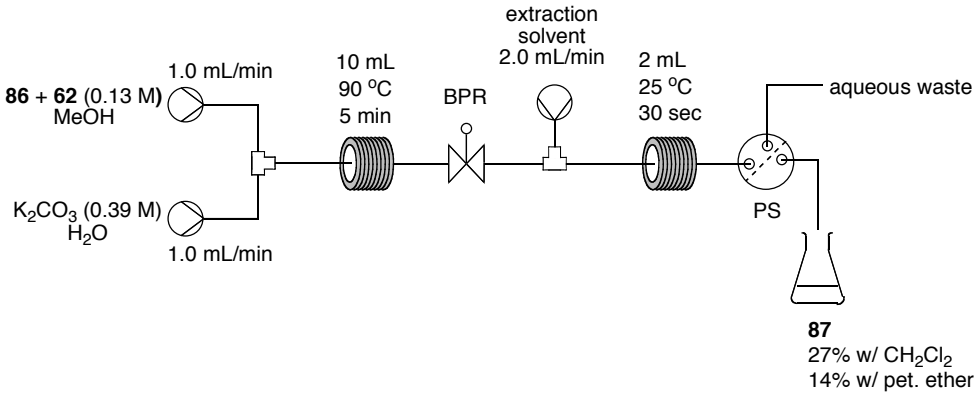
Thiophenol (0.20 mL, 2.0 mmol), benzyl chloride (0.23 mL, 2.4 mmol), K_2CO_3 (0.82 g, 6.0 mmol) and solvent (10 mL) was added to a 50 mL round bottom flask (RBF) with a reflux condenser. The suspension was stirred at reflux overnight. The reaction was quenched with H_2O (10 mL), then extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts was dried over $MgSO_4$, then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum. The product was obtained in the yields given in Table 3. 2. The spectroscopic data is in agreement with the literature.[16] Colourless solid; m.p. 42-44 °C (Lit m.p. 41 °C)[16]; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.27-7.07 (10H, m), 4.02 (2H, s) ppm.

Procedure for the homogeneous flow synthesis of benzyl phenyl sulfide with separating funnel



Potassium carbonate (3.3 g, 24 mmol) was dissolved in H_2O (0.39 M, 60 mL) and attached to pump A with a flow rate of 1 mL/min. Thiophenol (0.81 mL, 8.0 mmol) and benzyl bromide (0.92 mL, 8.0 mmol) were dissolved in MeOH (0.13 M, 60 mL) then sealed and attached to pump B with a flow rate of 1 mL/min. The reaction reached a steady state after 10 minutes, at which point the resulting product was collected for 5 min into a vial containing brine solution (5 mL). The product was extracted with solvent on the diagram (3 × 10 mL), dried over $MgSO_4$ and filtered. The solvent was removed on a rotary evaporator and dried on a high vacuum. The product was obtained in the yield given on the diagram above. The spectroscopic data is in agreement with the literature.[16]

Procedure for the homogeneous flow synthesis of sulfides with inline phase separator



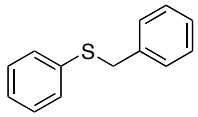
The same reagents and flow rates were used as the above procedure. The extracting solvent was attached to pump C with a flow rate of 2 mL/min and connected to the end of the reagent stream. The reaction was allowed to reach steady state after 10 min, at which point the resulting product was collected for 5 minutes. The solvent was removed on a rotary evaporator and dried on a high vacuum. The product was obtained in the yield given on the diagram above. The spectroscopic data is in agreement with the literature.[16]

General procedure for the heterogeneous flow synthesis of sulfides:

Anhydrous potassium carbonate (2.5 g) was loaded in a dry Omnifit column and attached to a Vapourtec E-series machine. A flask containing 4 Å MS (3.5 g) was charged with dry acetonitrile (100 mL) under argon. Another flask charged with 4 Å MS (3.5 g) under argon and acetonitrile (20 mL, 0.05 M), thiol (1.0 mmol) and alkyl halide (1.0 mmol) were added. The flask was attached to pump A.

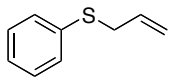
The column was heated to 25 °C and primed with 1 mL/min dry acetonitrile (5 mL) for 5 min. The column was then primed at 1 mL/min from the reagent flask for 5 min to achieve a steady state. The product was subsequently collected for a further 5 min. Removal of the solvent under vacuum gave the desired sulfide.

Benzyl phenylsulfide **87**



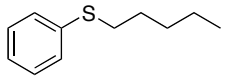
The product was obtained in 96% yield (48 mg, 0.24 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[16]

Allyl phenylsulfide **92**



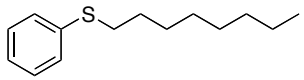
The product was obtained in 99% yield (37 mg, 0.25 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[17] Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.30-7.08 (m, 5H), 5.81 (ddt, *J*= 13.7, 10.0, 6.8 Hz, 1H), 5.07 (ddd, *J*= 17, 1.1 Hz, 1H), 5.00 (ddd, *J*= 10.0, 1.1 Hz, 1H), 3.48 (m, 2H) ppm.

Pentyl phenylsulfide **94**



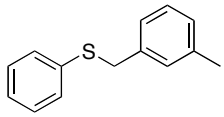
The product was obtained in 83% yield (75 mg, 0.42 mmol) using the general procedure with 0.1 M concentrations. The spectroscopic data is in agreement with the literature.[18] Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.34-7.14 (m, 5H), 2.95 (t, *J*= 7.2 Hz, 2H), 1.69 (m, 2H), 1.40 (m, 4H), 0.93 (t, *J*= 7.1 Hz, 3H) ppm.

Octyl phenylsulfide **96**



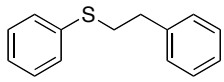
The product was obtained in 93% yield (72 mg, 0.33 mmol) using the general procedure but collected for 7 minutes. The spectroscopic data is in agreement with the literature.[19] Colourless oil; ^{1}H NMR (250 MHz, $CDCl_3$): δ= 7.45-7.13 (m, 5H), 2.99-2.94 (m, 2H), 1.75-1.51 (m, 2H), 1.48-1.45 (m, 2H), 1.40-1.24 (m, 8H), 0.93 (m, 3H) ppm.

3-Methylbenzyl phenylsulfide **98**



The product was obtained in 99% yield (54 mg, 0.25 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[17] Colourless liquid; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.38-7.08 (m, 9H), 4.14 (s, 2H), 2.37 (s, 3H) ppm.

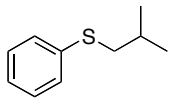
2-Ethyl phenyl (phenyl) sulfide **100**



Column chromatography was performed using a Biotage Isolera 4 according to the following method. The crude product mixture (212 Mg) was loaded onto a Biotage Snap KP-Sil 10g Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 10 column volume (CV), then increased to 90:10 hexane: ethyl acetate over 10CV, then to 100% ethyl acetate over 10CV, and held at 100% ethyl acetate for 5CV. The solvent from the appropriate fractions was removed and dried on a high vacuum.

The product was obtained in 75% yield (81 mg, 0.38 mmol) using the general procedure. Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.30-7.06 (m, 10H), 3.10-3.05 (m, 2H), 2.87-2.79 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 140.1, 136.3, 129.1 (2C), 128.9 (4C), 128.5 (2C), 126.4, 125.9, 35.5, 35.0 ppm; ν_{max} (neat): 3069, 3060, 3027, 3003, 2952, 2924, 2854, 1604, 1584, 1496, 1480, 1453, 1438, 1091, 1071, 1025, 736, 721, 692 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{14}S$ (M^{+}): 214.0816; found 214.0818.

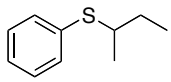
2-Methyl propyl (phenyl) sulfide **102**



The crude product was obtained after 30 minutes of collection using the general procedure at 100 °C and 1.7 bar. After 30 minutes of collection, column chromatography was performed using a Biotage Isolera 4 according to the following method. The crude product mixture (223 mg) was loaded onto a Biotage Snap KP-Sil 10 g Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 10 CV, then to 100 % ethyl acetate over 5 CV, and held at 100% ethyl acetate for 5 CV. The solvent from the appropriate fractions was

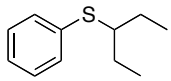
removed and dried on a high vacuum. The product was obtained in 85% yield (212 mg, 13 mmol). The spectroscopic data is in agreement with the literature.[20] Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.37 (m, 4H), 7.22 (m, 1H), 2.89 (d, *J*= 6.9 Hz, 2H), (m, 1H), 1.21(d, *J*= 6.7 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 137.1, 128.7 (2C), 128.6 (2C), 125.4, 42.4, 28.1, 22.0 ppm.

2-Butyl (phenyl) sulfide **104**



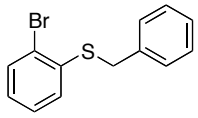
The product was obtained in 85% yield (221 mg, 13 mmol) after 30 minutes of collection using the general procedure at 100 °C and 3.5 bar after 30 minutes of collection. The spectroscopic data is in agreement with the literature.[19] Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.55-7.10 (m, 5H), 3.16 (sext, *J*= 6.7 Hz, 1H), 1.59 (m, 1H), 1.25 (d, *J*= 6.7Hz, 2H), 1.00 (t, *J*= 7.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 135.7, 132.0 (2C), 128.9 (2C), 126.6, 44.9, 29.6, 20.7, 11.6 ppm.

3-Pentyl (phenyl) sulfide **106**



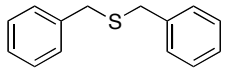
The crude product was obtained after 30 minutes of collection using the general procedure at 100 °C and 1.7 bar after 30 minutes of collection. Column chromatography was performed using a Biotage Isolera with the following method. The crude product mixture (212 mg) was loaded onto a Biotage Snap KP-Sil 10 g Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 10 column volume (CV), then to 100% ethyl acetate over 5CV, and held at 100% ethyl acetate for 5CV. The solvent from the appropriate fractions was removed and dried on a high vacuum. The isolated product was obtained in 70% yield (189 mg, 1.28 mmol). The spectroscopic data is in agreement with the literature.[21] Colourless liquid; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.50-7.45 (m, 2H), 7.41-7.22 (m, 3H), 3.07 (m, 1H), 1.68 (m, 4H), 1.09 (t, *J*= 7.4 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 135.8, 131.6 (2C), 128.6 (2C), 126.9, 52.1, 26.6 (2C), 11.1 (2C) ppm.

Benzyl (2-bromophenyl) sulfide **108**



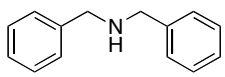
The product was obtained in 98% yield (67.0 mg, 0.25 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[22] Colourless solid; m.p. 42-44 °C (Lit m.p. 44-45 °C)[23]; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.60-7.55 (m, 1H), 7.45-7.23 (m, 7H), 7.10-7.03 (m, 1H), 4.19 (s, 2H) ppm.

Dibenzyl sulfide **110**



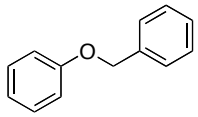
The crude product was obtained after 30 minutes of collection using the general procedure at 100 °C and 1.5 bar after 30 minutes of collection. Column chromatography was performed using a Biotage Isolera 4 according to the following method. The crude product mixture (44 mg) was loaded onto a Biotage Snap KP-Sil 10g Flash Column (15 mL column volume). The gradient was performed; 100 % hexane for 10 CV, then to 100% ethyl acetate over 5 CV, and held at 100% ethyl acetate for 5 CV. The solvent from the appropriate fractions was removed and dried on a high vacuum. The product was obtained in a 62% (33 mg, 0.15 mmol) yield using the general procedure. The spectroscopic data is in agreement with the literature.[24] Colourless solid, m.p. 46-48°C (Lit m.p. 48-49 °C)[25]; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.31-7.16 (m, 10H), 3.55 (s, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 138.1 (2C), 129.0 (4C), 128.3 (4C), 126.9 (2C), 35.5 (2C) ppm.

Dibenzyl amine **113**



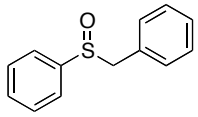
The product was obtained in 32% yield (157 mg, 0.80 mmol) after 50 minutes of collection using the general procedure at 0.1 mL/min, 100 °C and 1.1 bar. The spectroscopic data is in agreement with the literature.[26] Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.33-7.12 (m, 10H), 3.72 (s, 4H), 2.00 (s, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 139.7 (2C), 128.4 (4C), 128.3 (4C), 127.1 (2C), 52.9 (2C) ppm.

(Benzyloxy)benzene **120**



The product was obtained in 82% yield (38 mg, 0.21 mmol) after 50 minutes of collection using the general procedure at 0.1 mL/min, 100 °C and 1.4 bar. The spectroscopic data is in agreement with the literature.[27] Colourless solid, m.p. 38 - 40°C (Lit m.p. 39-40 °C)[28]; ^{1}H NMR (300 MHz, $CDCl_3$): δ= 7.49- 7.26 (m, 7H), 7.01-6.96 (m, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ= 158.8, 137.1, 128.9 (2C), 128.6 (2C), 127.9(2C), 127.5(2C), 120.9, 114.8 , 69.9 ppm.

Procedure for the semi-batch synthesis benzylphenylsulfoxide **111**



The synthesis of benzyl phenyl sulfide was performed but collected into a flask and 30 w/v% H_2O_2 (1 mL, 1 mmol) was added. The mixture was stirred at 50 ºC overnight. The mixture was quenched with saturated $Na_2S_2O_3$ solution (5 mL), then extracted with CH_2Cl_2 (3 × 20 mL) and dried over $MgSO_4$. Column chromatography was performed using a Biotage Isolera 4 according to the following method. The crude product mixture (110 mg) was loaded onto a TELOS 12 g Silica Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 1 CV, then increase to 60:40 hexane: ethyl acetate over 40 CV, then to 100% ethyl acetate over 13 CV, and held at 100% ethyl acetate for 5 CV. The solvent from the appropriate fractions was removed and dried on a high vacuum. The product was obtained in 93% yield (102 mg, 0.93 mmol). The spectroscopic data is in agreement with the literature.[29] Colourless solid, m.p. 120-122 °C (Lit m.p. 123-124 °C)[30]; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.41-7.27 (m, 7H), 7.01-6.91 (m, 3H), 5.06 (s, 2H) ppm.

3.5. References

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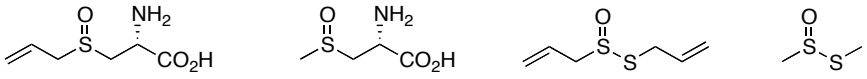
4

Selective Oxidation of Sulfides

4.1. Introduction

4.1.1. Selective Mono-Oxidations of Sulfides

Several organosulfur compounds in garlic contain sulfoxides. The general structural formula is R-S(O)-R[1], a mono-oxidised sulfide. These include the cysteine amino acids, such as alliin **15** and methiin **18**. Thiosulfates **5** and **122**, which are generated after enzymatic cleavage of **15** and **18**, also contains a sulfur atom bonded to one oxygen atom. The lone pair of the sulfoxide moiety creates chiral tetrahedral geometry.



15 **18** **5** **122**

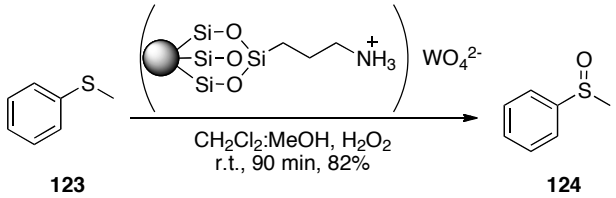
Figure 4.1. Garlic metabolite containing sulfoxides

Further oxidation of sulfides produces sulfone moieties. The sulfur atom is bonded to two oxygen atoms, R-S(O)$_2$-R[1]. The synthesis of sulfones is covered in a recommended[1] review.[2] This 'over-oxidation' producing sulfones is undesirable for the garlic metabolites synthesis in this chapter. There are many oxidising agents that have been utilised for the oxidation of sulfides. These include hydrogen peroxide (H_2O_2),[3] urea-hydrogen peroxide adduct[4] and *tert*-butyl hydroperoxide,[5] Oxone, and peroxy acids; such as peroxybenzoic acid and *meta*-chloroperoxybenzoic acid (*m*-CPBA).

Dimethyldioxirane (DMDO) is a very clean oxidising agent, which can be prepared using Oxone.[6] DMDO is very unstable and formed in low concentrations, however it is synthetically useful and only produces acetone as a waste product and has been reliably used on large scale.[7]

Several oxidising agents have been combined with other reagents that perform the oxidations faster or with greater functional group tolerance. These methods include utilising 1,3,5-triazo-2,4,6-triphosphorine-2,2,4,4,6,6-tetrachloride (10 mol%) with hydrogen peroxide to produce sulfoxides in 5 minutes at room temperature.[8]

Kirihara and co-workers have oxidised sulfides using tantalum carbide in catalytic quantities that could tolerate a functional groups, such as allylic moieties. [9] Solid-supported catalysts have also been developed and perform the oxidation of sulfides at room temperature (Scheme 4.1).[10]

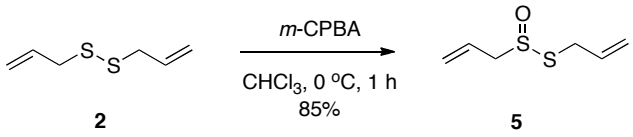


Scheme 4.1. Solid supported catalyst for the oxidation of sulfides

4.1.2. Synthesis of Allicin

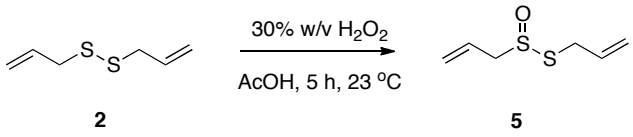
This section of the chapter describes the development of allicin **5** syntheses. Oxidation of diallyl disulfide **2** is the approach cited most frequently to synthesise **5**. This reactive garlic metabolite contains a thiosulfinate moiety, R-S(O)-S-R.

The oxidation of diallyl disulfide **2** to produce allicin was the first synthesis of this thiosulfinate moiety reported in 1947 by Small and co-workers. However, an isolated yield was not reported.[11] *m*-CPBA has been used in chloroform to synthesise allicin. After chromatography pure allicin was isolated in 85% yield (Scheme 4.2).[12,13]



Scheme 4.2. Oxidation of diallyl disulfide **2** using *m*-CPBA

In 1986, Block and co-workers oxidised commercially available **2** using peracetic acid at 0 °C over 30 minutes to produced allicin in 90% yield after chromatography.[14] The oxidation has also been performed using hydrogen peroxide and acetic acid over 5 h to produce allicin with 93% purity (Scheme 4.3).[3]

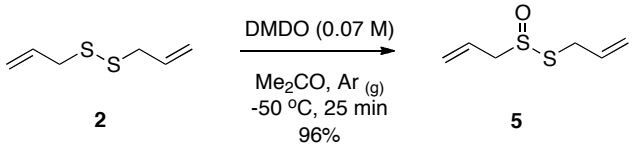


Scheme 4.3. Oxidation of diallyl disulfide using hydrogen peroxide

This method was later performed by using a different group using commercially available kosher diallyl disulfide **2,** which was sold with only 80% purity. Kosher means that the product is a food chemical and meets a strict regulatory criteria during the manufacturing process.

After neutralisation, it was observed that a phase boundary was formed and **5** resided in the lighter fraction.[15] Although the isolation of **5** was simple by extracting the lighter phase, no yield or purity of **5** was reported.

NeemBiotech's patented procedure uses Oxone in 50% aqueous ethanol. The reaction was completed after two hours producing crude **5** in 90% purity.[16] Another patent also uses Oxone in the synthesis of **5**. However, Oxone is utilised to generate a DMDO solution (0.07 M), which was subsequently used to produce **5** in 96% yield after chromatography (Scheme 4.4). This process utilised low temperatures, low concentrations of DMDO, and an inert atmosphere, which creates challenges on large scale.[17] A recent patent has used L-proline and hydrogen peroxide in THF at 40 °C. After chromatography compound **5** was produced in 80% yield with 90% purity.[18]



Scheme 4.4. Oxidation of diallyl disulfide using DMDO

The enzyme-catalysed synthesis of **5** from garlic has also been investigated. NeemBiotech have developed a process of mechanically treating garlic to produce alliinase, to which additional alliin **15** has been added. This increases the quantities of **5** from an extraction of garlic.[19]

An alternative approach uses immobilised alliinase on sodium alginate to produce **5**. The isolation and immobilisation of alliinase from onions was based on Thomas' and Parkin's work.[20] All of the thiosulfinates found in garlic were synthesised using this technique.[21] Alliinase has also been immobilised on other heterogeneous supports and employed using packed-bed flow reactors. The heterogeneous flow system using supported alliinase with a flow rate of 7.0 mL/h of alliin solution produced **5** continuously for 2 weeks with no reported loss of activity.[22]

4.2. Results and Discussion

This chapter's primary objective was to develop a simple synthesis for the production of allicin **5** in large quantities. The oxidation of diallyl disulfide **2** was examined in batch and flow mode. It was hoped that a flow mode would produce a system with a high throughput. In addition, selective oxidations of sulfides in flow mode will also be examined.

4.2.1. Synthesis of Allicin via Oxidation of Diallyl Disulfide

Allicin **5** is a very reactive molecule and decomposes at room temperature.[3] A simple, efficient procedure was developed to produce the quantities of **5** for the investigation of the thermolysis of **5** described in chapter 5.

4.2.1.1. Batch Mode

The oxidation of **2** was performed with oxidants previously used in the literature synthesis of **5**. A low yield was obtained using *m*-CPBA that required purification of the oxidant prior to the reaction (Table 4.1, entry 1). Oxone was investigated and the reaction was maintained at 0 °C using an ice-bath, this produced **5** in an improved 75% yield. (Table 4.1, entry 2).

Table 4.1. Oxidants investigated in the oxidation of diallyl disulfide **2**

Entry	Oxidant	Time (h)	Temp (°C)	Yield (%)
1	mCPBA	1	40	44
2	Oxone	3	0	75
3	H_2O_2	5	0 to 25	95

The preeminent method used hydrogen peroxide as the oxidant, based on the method by Freeman and Kodera (Table 4.1, entry 3).[3] The modified work-up used sodium hydrogen carbonate for the neutralisation instead of potassium hydroxide, as the latter was causing decomposition of **5**. The sodium hydrogen carbonate evolved carbon dioxide gas that helped maintain lower the temperature during the work-up. This method was repeatedly used to produce **5** for the subsequent thermolysis investigation in chapter 5. Commercially available 80% pure kosher **2** has also been used as a substrate in this reaction.

4.2.1.2. Homogenous Flow Mode

The reliable acetic acid/hydrogen peroxide conditions were developed in flow mode. A syringe, filled with a solution of **2** in acetic acid, was placed on a syringe pump. A second syringe, was filled with hydrogen peroxide (30 w/v%) and the placed on another syringe pump. The two syringes were attached to the flow setup shown in Figure 4.2 and pumped through a Comet mixer that was connected to a PTFE reaction coil (volume: 2 mL, length: 4 m, internal diameter: 0.8 mm). The reaction mixture eluted the reactor into a collection flask containing saturated sodium thiosulfate to quench the reaction. With a residence time of 100 minutes, the reaction proceeded in 92% yield.

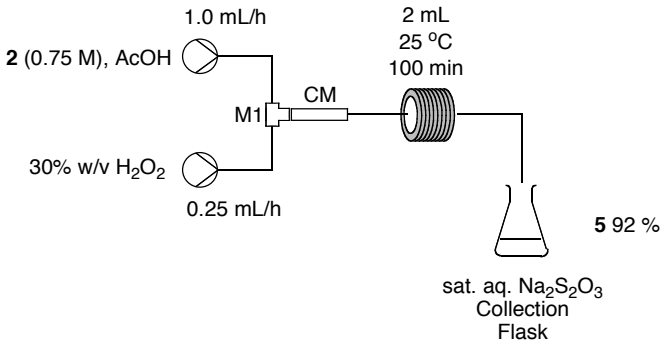


Figure 4.2. Oxidation of diallyl disulfide using hydrogen peroxide in flow mode

However, batch mode was decidedly used for bulk synthesis of **5**. Compared to the batch mode synthesis, the output of this flow setup afforded 351 mg over 6 h. Batch reaction consistently produced **5** in larger quantity with superior conversion in a similar time period. Additionally, a quarter of the starting material was unreacted and required chromatography to recycle the starting material.

4.2.1.3. Heterogeneous Flow Mode

Aqueous Oxone was also investigated in homogenous flow mode. However, precipitation of Oxone in the flow reactor frequently occurred and required low concentrations to be used. The results of the precipitation led to the proposal of using Oxone heterogeneously in a pack-bed reactor setup.

Packed-bed Reactor; Syringe pumps with a Biotage Cartridge

In initial experiments a Biotage column (10 g) was filled with Oxone and sodium hydrogen carbonate. Acetone was pumped through and eluted into a flask containing diallyl disulfide **2** (Figure 4.3).

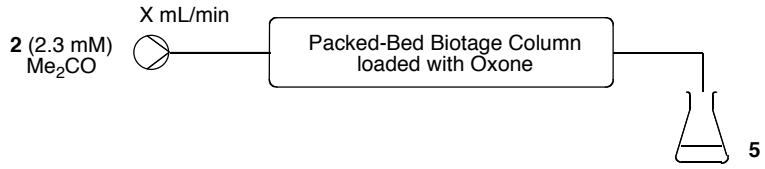


Figure 4.3. Oxidation flow setup using a Biotage column charged with Oxone: $NaHCO_3$ at room temperature

Initially the composition of the column material contained sodium hydrogen carbonate to maintain a basic pH (Table 4.2, entries 1 and 2).[23] Reducing the sodium hydrogen carbonate equivalents increased the quantities of Oxone used and increased the oxidisation of the product (Table 4.2, entry 3). Increasing the residence time, by lowering the flow rate, did not improve the oxidation (Table 4.2, entry 4).

Table 4.2. The effect of column composition and flow rate on the oxidation of diallyl disulfide

Entry	Composition ratio		Flow rate (mL/min)	NMR Conversion (%)
	Oxone	$NaHCO_3$		
1	2	1	1	5
2	3	1	1	15
3	1	0	1	57
4	1	0	0.5	52

Conditions: Acetone was pumped through the Biotage column at the desired flow rate at room temperature

This setup demonstrated that it was possible use Oxone heterogeneously to oxidise diallyl disulfide. These results also suggested that a lower flow rate improves the conversion. This system also did not allow effective temperature control and reproducibility was problematic as the plastic joints warped and began to leak. An alternative system had to be developed.

Packed-bed Reactor; Vapourtec E-series with an Omnifit column

The packed-bed reactor system that was successfully employed in Chapter 3, a Vapourtec E-series with an Omnifit column, was utilised to investigate heterogeneous Oxone in a flow system. A solution of **2** in acetone was pumped by the Vapourtec pump through the glass Omnifit column (length: 150 mm, internal diameter: 6.6 mm), shown in Figure 4.4.

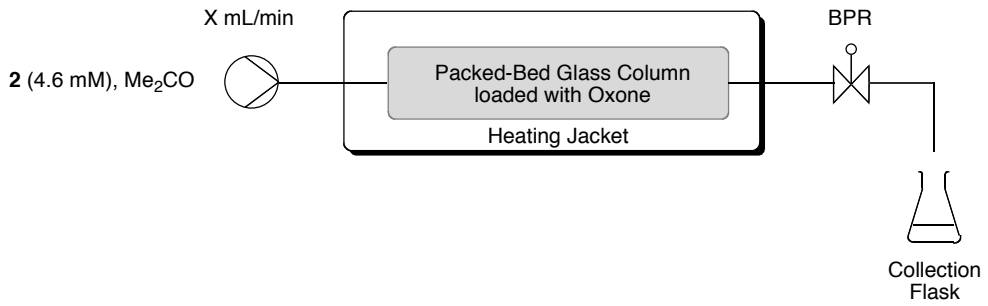


Figure 4.4. Packed-bed reactor setup containing Oxone

The results of the experiments showed higher temperatures appeared to improve the conversion (Table 4.3). Entry 5 was a promising result and achieved 51% conversion. To achieve full conversion, twice as much Oxone would have to be used in the reactor. However, the packed-bed reactor column was already charged with the maximum quantity and an alternative approach had to be developed.

Table 4.3. Oxidation of diallyl disulfide **2** in acetone using Oxone column

Entry	Temp (°C)	Back Pressure (bar)	NMR Conversion (%)
1	25	1.8	35
2	35	1.8	44
3	45	1.8	26
4	65	2.9	31
5	75	3.0	51
6	85	3.3	65

Conditions: Compound **2** (2.3 mM) in acetone was pumped through the Omnifit column at 1 mL/minute at the desired temperature

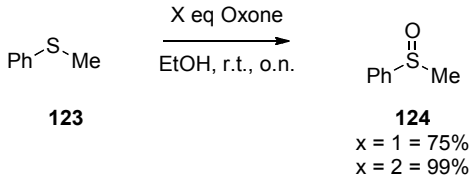
To overcome the limited reactor size and doubling the exposure of the reaction mixture to Oxone should have completely oxidised the product. However, availability of the starting material and reproducibility of the same conversion at 75 °C became issues. Further experimentation was performed using monosulfides that were more readily available in larger quantities.

4.2.2. Oxidation of Monosulfides to Synthesise Sulfoxides

In this section, the efficacy of Oxone for the oxidation of sulfides in flow mode was further investigated using available sulfides.

4.2.2.1. Batch Mode

The investigation used methyl phenyl sulfide as the model substrate and is known chemistry developed by Trost *et al.*[24] The effect of excess Oxone was investigated to evaluate the selectivity of the oxidation. Yu and co-workers had demonstrated that selective oxidation to the sulfoxide was obtained when ethanol was used as the solvent.[25] The results repeating the work by Yu *et al*. confirmed that when ethanol was used as the solvent, only the sulfoxide was produced (Scheme 4.5). The reaction appeared to occur faster with two equivalents of Oxone. Additionally, Oxone appeared to remain solid.



Scheme 4.5. Selective oxidation of methyl phenyl sulfide using excess Oxone

.4.2.2..2 Homogenous Flow mode

Initial experiments began using homogeneous flow in which Oxone was dissolved in an aqueous solution. This was investigated because solubilised Oxone could be prepared on larger scales than the pack-bed reactor. The solvent system developed for this reaction had to overcome the partial solubility of the salts in an aqueous-organic solvent system that were prone to precipitation in the flow reactor.

Aqueous Oxone required a greater flow rate, pumped at 2.5 times the flow rate of the sulfide in acetone, to increase the aqueous concentration of the solvent in the PTFE reactor coil (Figure 4.5). The Vapourtec also enabled temperature control and the reaction was performed at 60 °C to improve the solubility of Oxone.

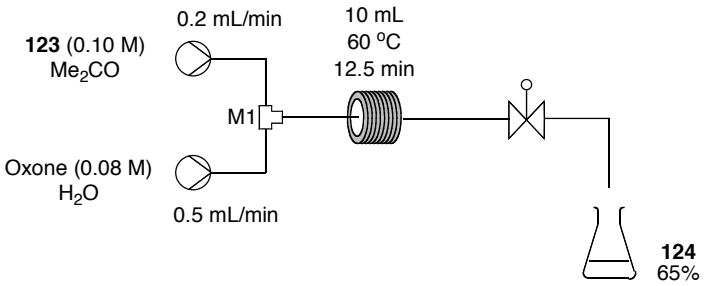


Figure 4.5. Homogenous flow setup for the oxidation of methyl phenyl sulfide

The reaction was successful and produced the sulfoxide as the sole product. However, precipitation still occurred in the reactor and the BPR. The product may still be retained in the reactor causing a lower yield. The oxidations in a homogenous flow system suggested that lower concentrations or larger diameter tubing are required when aqueous Oxone is used as a reagent.

4.2.2.3. Heterogeneous Flow Mode

Heterogeneous Oxone implemented in a packed bed reactor had already shown interesting results for the oxidation of **2**. The oxidation of methyl phenyl sulfide remained the model reaction under investigation, as the oxidation could easily be determined from the crude NMR. The packed-bed reactor setup is shown in Figure 4.6.

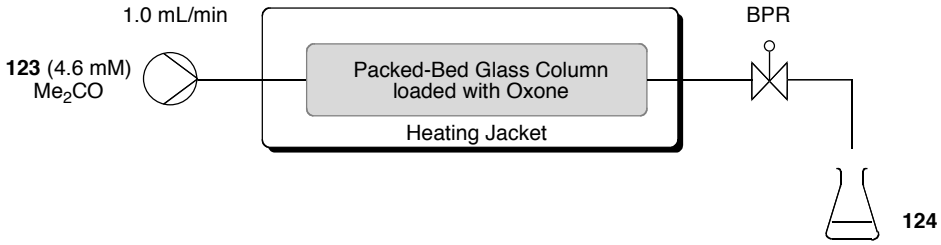


Figure 4.6. Packed-bed reactor setup containing Oxone

Three solvents were investigated; acetone, ethanol and methylene chloride (Table 4.4). Acetone demonstrated good conversion at 45 °C (Table 4.4, entry 1). Reactions performed in ethanol showed that temperature did not increase the yield (Table 4.4, entry 2). Almost no oxidation was observed when using methylene chloride as the solvent (Table 4.4, entry 3). At 55 °C only 7% conversion was observed using methylene chloride.

Table 4.4. ^{1}H NMR Conversion to methyl phenyl sulfoxide using different solvents

Entry	Solvent	^{1}H NMR Conversion		
		25 °C	35 °C	45 °C
1	Me_2CO	44 (1.8)	57 (3.1)	84 (1.9)
2	EtOH	40 (1.7)	-	57 (1.5)
3	CH_2Cl_2	0 (1.9)	>1 (1.9)	>1 (1.8)

Conditions: Compound **123** (4.6 mM) in the specified solvent was pumped at 1.0 mL/min
Values in parentheses is back pressure reported in bar

However, these solvents were not anhydrous with undetermined water content, thus dry solvents were investigated to determine the effect of water in the reaction systems.

Using Anhydrous Conditions

Anhydrous acetone was investigated first, the temperature increased the oxidation of methyl phenyl sulfide to the sulfoxide (Table 4.5) and no over-oxidation to the sulfone was observed. However, the conversion was lower compared to non-anhydrous acetone (Table 4.4).

Table 4. 5. Conversion to methyl phenyl sulfoxide using anhydrous acetone

Entry	Temp (^{o}C)	Back pressure (Bar)	^{1}H NMR Sulfoxide Conversion (%)
1	25	2.3	16
2	45	2.5	30
3	65	2.5	77

Conditions: Compound **123** (4.6 mM) in the specified solvent was pumped at 1.0 mL/min

In anhydrous ethanol the oxidation was achieved at lower temperatures with higher yields. Selective oxidation and complete conversion to the sulfoxide was achieved at the lower temperature using ethanol than acetone (Table 4.6, entry 2 and Table 4.5, entry 2). Furthermore, over oxidation was observed, and at high temperatures the sulfone became the major product (Table 4.6, entry 4).

Table 4.6. Conversion to methyl phenyl sulfoxide using anhydrous ethanol

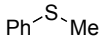
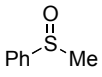
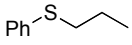
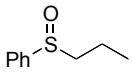
Entry	Temp (^{o}C)	Back pressure (Bar)	NMR Sulfoxide Conversion (%)	^{1}H NMR Sulfone Conversion (%)
1	25	1.2	30	-
2	45	1.4	99	-
3	65	2.0	93	7
4	85	10.0[a]	29	71

Conditions: Compound **123** (4.6 mM) in the specified solvent was pumped at 1.0 mL/min
[a] back pressure increased due to precipitation of Oxone

However, at elevated temperatures, the solubility of Oxone increased. This led to precipitation in the BPR, and was the result of the solution cooling. This was overcome by surrounding the collecting tube with aluminium foil for thermal insulation.

The reaction was performed with two substrates with greater eluted volumes to obtain isolated yields and investigate selectivity. Moderate yields of sulfoxides were obtained without an aqueous work-up (Table 4.7).

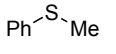
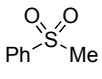
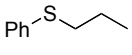
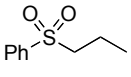
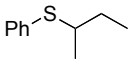
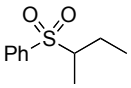
Table 4.7. Oxidation at 45 **°C** to produce sulfoxides

Entry	Sulfide	Sulfoxide	Yield (%)
1	 **123**	 **124**	65
2	 **126**	 **127**	41

Conditions: Sulfide (4.6 mM) in acetone was pumped at 1.0 mL/min at 45 **°C** with 1 bar backpressure

Although the Oxone dissolves at high temperatures, selectivity for sulfone formation has been demonstrated. At 110 °C, only the sulfones were obtained in poor to good yields (Table 4.8), which may be the result of the substrates remaining in the column. No sulfoxides were observed in the product.

Table 4.8. Oxidation at 110 °C to produce sulfones

Entry	Sulfide	Sulfone	Back pressure (Bar)	Yield (%)
1	 **123**	 **128**	4.5	39
2	 **126**	 **129**	4.5	14
3	 **130**	 **131**	4.0	51

Conditions: Sulfide (4.6 mM) in acetone was pumped at 1.0 mL/min at 110 °C

A brief investigation into the functional group tolerance of an aldehyde was also performed using 4-(methylthio)benzaldehyde. The preliminary results showed that the oxidation is very dependant on temperature. Twenty degree increases in temperature were not sufficient to determine the precise temperature for selective oxidation. At low temperature the sulfoxide was formed, and as the temperature increased, so did the percentage of sulfoxide (Table 4.9, entry 1-3). However, between 65 °C and 85 °C the sulfone was formed in larger quantities (Table 4.9, entry 3 and 4). The aldehyde appeared to be unoxidised and was present until 110 °C (Table 4.9, entry 5). A more precise temperature screen needs to be conducted to gain further insight into specific temperature for selective oxidation.

Table 4.9. Oxidation of 4-(methylthio)benzaldehyde

Entry	Temp (°C)	Back pressure (Bar)	^{1}H NMR Conversion (%)		
			Sulfide	Sulfoxide	Sulfone
1	25	1.0	92	8	-
2	45	1.2	82	18	-
3	65	1.2	37	63	-
4	85	4.5	-	60	40
5	110	8.5	-	-	-[a]

Conditions: Sulfide (4.6 mM) in acetone was pumped at 1.0 mL/min
[a] complete elimination of aldehyde peak

An issue with this system is the quantity of oxidant, once it has reacted the column, has to be replenished. Fortunately, the column can be flushed within 10 minutes with water. The system is ideal for small-scale flow reactions with low concentrations.

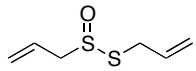
4.3. Conclusion

Selected oxidations of sulfides were successfully performed in flow mode. However, batch mode was the best procedure for the synthesis of **5**, via the oxidation of **2**, in high yields. The oxidation in homogenous flow mode was attempted using hydrogen peroxide in acetic acid but only achieve a 73% yield with a lower throughput. Oxone proved to be unsuitable in biphasic flow as a result of precipitation.

Oxone's insolubility in organic solvents was exploited in a pack-bed flow reactor. The heterogeneous Oxone pack-bed flow reaction worked using acetone or ethanol as solvents. Anhydrous ethanol improved the reactivity and further reduced the operating temperature. Selective mono-oxidation of sulfides to sulfoxides was demonstrated in a rapid flow procedure. The oxidation of sulfides to sulfones requires greater heating; these temperatures also increase the solubility of Oxone and resulted in precipitation in cooler sections of the reactor.

4.4. Experimental

Synthesis of allicin **5** *in batch mode using m-CPBA*



Diallyl disulfide (1.99 g, 13.7 mmol) was dissolved in CH_2Cl_2 (50 mL) and stirred at 0 °C. *m*-CPBA (3.10 g, 14.0 mmol) in CH_2Cl_2 (50 mL) was added slowly. The mixture was then stirred at 40 °C for 1 h. Then the mixture was washed with $NaHCO_3$ (0.6 M, 4 × 50 mL), then H_2O (4 × 50 mL). The organic product was extracted with Et_2O (4 × 100 mL). The organic extracts were combined and dried over $MgSO_4$, then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum. The crude product was purified using silica gel chromatography. The solvent system was 5:1 hexane: EtOAc. The product was obtained in 44% yield (975 mg, 6.02 mmol). The spectroscopic data is in agreement with the literature.[26] Faint yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 6.07-5.87 (m, 2H), 5.53- 5.20 (m, 4H), 3.95-3.70 (m, 4H) ppm.

Synthesis of allicin **5** *in batch mode using Oxone*

Diallyl disulfide (112 mg, 0.8 mmol) was dissolved in EtOH (8 mL) and stirred at 0 °C. Oxone (614 mg, 1.0 mmol) dissolved in H_20 (3 mL) was added and stirred at 0 °C for 3 h. The product was extracted with Et_2O (20 mL), Et_2O:$CHCl_3$ 1:1 (20 mL) and $CHCl_3$ (20 mL). The organic extracts were combined, dried over Na_2SO_4, then filtered. The solvent was removed on a rotary evaporator. The crude product was purified using silica gel chromatography. The solvent system was 5:1 hexane: EtOAc. The product was obtained in 75% yield (110 mg, 0.68 mmol). The spectroscopic data is in agreement with the literature.[26]

Synthesis of allicin **5** *in batch mode using hydrogen peroxide*

Diallyl disulfide (1.00 g, 6.8 mmol) was added to glacial AcOH (7 mL) and stirred at 0 °C in an ice bath. H_2O_2 (30% w/v, 0.77 mL, 6.8 mmol) was added dropwise, then allowed to warm to room temperature over 5 h. The reaction was quenched with water (50 mL), then extracted with CH_2Cl_2 (3 × 35 mL). The organic extracts were combined, dried over $MgSO_4$ and then filtered. The solvent was removed on a rotary evaporator. The reaction was followed by TLC, Hex: EtOAc 4:1. The product was purified using silica gel chromatography. The solvent system was 5:1 hexane: EtOAc. The product was obtained in 95% yield (1.05 g, 6.5 mmol). The spectroscopic data is in agreement with the literature.[26]

Synthesis of allicin **5** *in batch mode using hydrogen peroxide and commercial 80% pure kosher diallyl disulfide*

Commercial kosher 80% pure diallyl disulfide (5 mL) was washed through a silica plug with hexane as the mobile phase. The solvent was removed and the purified diallyl disulfide was dissolved in AcOH (25 mL) and added to a 250 mL round bottom flask at 0 °C. H_2O_2 (1.2 eq, 30% w/v) was added dropwise and allowed to warm to room temperature and stirred overnight. $NaHCO_3$ (38 g, 0.48 mol) and water (50 mL) was added using a dropping funnel with side arm for the gas to escape. Bubbling may persist for 30 minutes, and more water will have to be added. The product was extracted with ether (3 × 50 mL). The organic extractions were combined, dried over $MgSO_4$ and then filtered. The solvent was removed on a rotary evaporator. The product was purified using silica gel chromatography. The solvent system was 5:1 hexane: EtOAc to obtain pure allicin. The spectroscopic data is in agreement with the literature.[26]

Homogenous flow synthesis of allicin **2** *using hydrogen peroxide*

A syringe, filled with diallyl disulfide (654 mg, 4.4 mmol) was dissolved in acetic acid (6 mL) and was placed on a syringe pump with a flow rate of 1.0 mL/h. Another syringe, filled with hydrogen peroxide (2 mL, 20.5 mmol), was loaded onto a syringe pump with a flow rate of 0.25 mL/h.

The solutions were pumped through a Comet mixer that was connected to a PTFE reaction coil (volume: 2 mL, length: 4 m, internal diameter: 0.8 mm) at room temperature. The reaction solution was eluted into a collection flask containing sat. aq. $Na_2S_2O_3$ (2 mL) to quench the reaction. The eluted crude material (4 mL) was extracted with $CHCl_3$ (3 × 6 mL), dried over $MgSO_4$, evaporated under reduced pressure and dried under vacuum.

Column chromatography was performed using a Biotage Isolera with the following method. The crude mixture (480 mg) was loaded onto a Biotage Snap KP-Sil 10 g Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 10 column volume (CV), then increased to 80:20 hexane: ethyl acetate over 20 CV, then to 100% ethyl acetate over 5 CV, and held at 100% ethyl acetate for 5 CV. The solvent from the appropriate fraction was removed and dried on a high vacuum. The product was obtained in 92% yield (351 mg, 2.16 mmol). The spectroscopic data is in agreement with the literature.[26]

Heterogeneous flow reactions of diallyl disulfide using Biotage column/syringe pumps

Oxone and $NaHCO_3$ were prepared in the composition shown in Table 4. 2 and thoroughly mixed. The mixture filled an empty Biotage 10 g column to the maximum capacity. A solution of acetone was placed on a syringe pump at 1 mL/min and attached to the Biotage 10 g column. The solution was collected after 30 min into a beaker containing diallyl disulfide (20 mg). The solvent was removed and dried on a high vacuum. The crude mixture conversion was analysed by NMR.

Heterogeneous flow reactions of diallyl disulfide using Vapourtec

A syringe, filled with diallyl disulfide (120 mg, 0.82 mmol) in acetone (360 mL), was placed on a syringe pump at 1 mL/min. The syringe was attached to the Omnifit column that was charged with Oxone (4.70 g, 76.5 mmol). The solution was pumped through the column and collected after 30 minutes into a beaker. The solvent was removed and dried on a high vacuum. The crude mixture conversion was analysed by NMR.

Oxidation of methyl phenyl sulfide in batch mode

Methyl phenyl sulfide (0.47 mL, 4.0 mmol) in EtOH (10 mL) was stirred at 0 °C. A solution of Oxone (610 mg, 8.0 mmol) in H_2O (10 mL) was added dropwise, allowed to warm to room temperature and left overnight. The organic product was extracted with $CHCl_3$ (3 × 20 mL), dried over Na_2SO_4 and filtered. The solvent removed on rotary evaporator then dried under high vacuum. The product was obtained in yields reported in Scheme 4.5 using the above procedure.

Homogenous oxidation of methyl phenyl sulfide in flow mode

A solution of methyl phenyl sulfide in acetone (0.10 M) was attached to pump A on the Vapourtec with a flow rate of 0.2 mL/min. A solution of aqueous Oxone (0.08 M) was attached to pump B with a flow rate of 0.5 mL/min. They were flowed through the T-piece connected to a PTFE reactor coil (volume: 10 mL, internal diameter: 1.0 mm) at 60 °C and through the backpressure regulator (2.4 bar). Two coil volumes were eluted to reach steady state before the product was collected over 10 minutes. The product was extracted with CH_2Cl_2 (3 × 10 mL), dried over $MgSO_4$ and the solvent was removed and dried on a high vacuum. The crude mixture conversion was analysed by NMR.

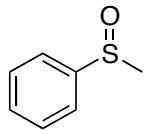
Heterogeneous oxidation of sulfides using Biotage column/syringe pumps

Oxone (28.5 g) was placed into an empty Biotage 10 g column. A solution of acetone was placed on a syringe pump at 1 mL/min and attached to the Biotage 10 g column. The solution was collected after 30 minutes into a beaker containing methyl phenyl sulfide (20 mg). The solvent was removed and dried on a high vacuum. The crude mixture conversion was analysed by NMR.

General oxidation of monosulfide to sulfoxide using Omnifit column/Vapourtec

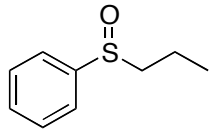
Oxone (4.7 g, 7.6 mmol) was loaded into an Omnifit column (length: 150 mm, internal diameter: 6.6mm). A solution of monosulfide (4.6 mM, 0.46 mmol) in acetone (100 mL) and attached to the Vapourtec pump. The solution was pumped at 1.0 mL/min through the column at 45 °C. The product was collected over 30 min, the solvent was removed and dried on a high vacuum. The crude mixture conversion was analysed by NMR.

Methyl phenyl sulfoxide **124**



The product was obtained in 65% yield (12 mg, 0.086 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[25] Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.65-7.49 (m, 5H), 2.74 (s, 3H) ppm.

Propyl phenyl sulfoxide **127**

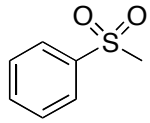


The product was obtained in 41% yield (9 mg, 0.057 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[27] Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.91-7.87 (m, 2H), 7.67-7.51 (m, 3H), 3.09-3.01 (m, 2H), 1.72 (m, 2H), 0.97 (t, *J*= 4.8 Hz, 3H) ppm.

General oxidation of monosulfides to sulfoxides using Omnifit column/Vapourtec

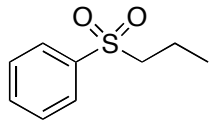
Oxone (4.7 g, 7.6 mmol) was loaded into an Omnifit column (length: 150mm, internal diameter: 6.6mm). A solution of monosulfide (4.6 mM, 0.46 mmol) in acetone (100 mL) was attached to the Vapourtec pump. The solution was pumped at 1.0 mL/min through the column at 110 °C. The steady state was reacted after 15 min and product was collected over 30 min, the solvent was removed and dried on a high vacuum.

Methyl phenyl sulfone **128**



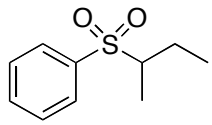
The product was obtained in 39% yield (8 mg, 0.051 mmol) using the general procedure at 45 °C. The spectroscopic data is in agreement with the literature.[25] Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.96-7.93 (m, 2H), 7.76-7.63 (m, 1H), 7.59-7.50 (m, 2H), 3.05 (s, 3H) ppm.

Propyl phenyl sulfone **129**



The product was obtained in 14% yield (7 mg, 0.043 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[28] Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.63-7.59 (m, 2H), 7.54-7.47 (m, 3H), 2.93-2.82 (m, 2H), 1.84-1.64 (m, 2H), 1.30 (t, *J*= 7.4 Hz, 3H)

2-butyl phenyl sulfone **131**



The product was obtained in 51% yield (14 mg, 0.071 mmol) using the general procedure at 110 °C. The spectroscopic data is in agreement with the literature.[29] Faint yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.88-7.79 (m, 2H), 7.66-7.62 (m, 1H), 7.59-7.52 (m, 2H), 2.99-2.89 (m, 1H), 2.05-1.95 (m, 1H), 1.49-1.36 (m, 1H), 1.26-1.23 (d, *J*= 6.9 Hz, 3H), 0.95 (*t*, *J*= 7.5 Hz, 3H).

4.5. References

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5

Thermolysis of Allicin and the Synthesis of Alliin

5.1. Introduction

In this chapter, the syntheses of several garlic metabolites; ajoene **14** and dithiins (**8** and **9**) from the thermolysis of allicin **5,** and the synthesis of the sulfoxide amino acids is described. The thermolysis of **5** is the only known method for the synthesis of **14**,[1] a biologically active molecule that is commercially desirable. The synthesis of dithiins is desirable to produce standards for HPLC calibration of GOE.

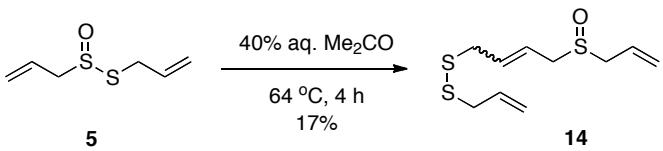
In the first part of this chapter the thermolysis of synthetic **5** was investigated. In the literature, compound **5** is isolated from garlic, which contains other organosulfur compounds. This is currently the process employed by NeemBiotech.[2] To circumvent this process of extraction from garlic, **5** has been synthesised.

The products formed from the thermolysis of **5** are dependant on the conditions. Distillation under aqueous conditions produces diallyl polysulfides, heating of **5** in non-polar solvents produces dithiins, and reflux in aqueous acetone produces ajoene **14**.

5.1.1. Production of ajoene

5.1.1.1. Thermolysis of Allicin to Produce Ajoene

In 1986, the thermolysis of **5** was performed in 40% aqueous acetone at reflux. Compound **14** was isolated in a 17% yield after chromatography.[3] This process was then patented in the same year.[4]



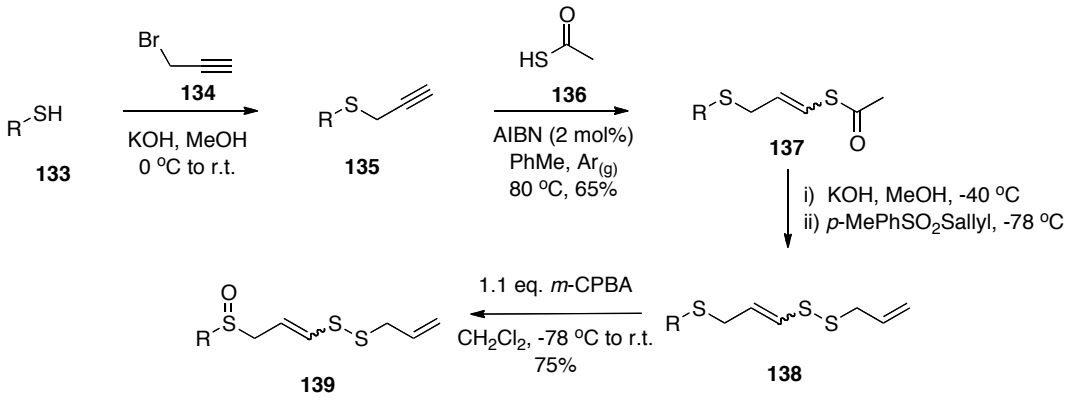
5 **14**

Scheme 5.1. Thermolysis of **5** to produce **14**

Thermolysis of **5** has to be employed widely in the literature, as there are no alternative chemical syntheses reported for the production of **14**.[1]

5.1.1.2. Synthesis of Ajoene

Ajoene **14** has been ingested for thousands of years and has not yet been chemically synthesised. However, the syntheses of ajoene analogues that retain the pharmacophore core have been recently reported. Hunter *et al.* were able to modify the alkyl chain attached to the sulfoxide (Scheme 5.2).[1] Unfortunately the thiol **133** could not contain an allylic moiety, as the radical addition was used to couple the vinyl thioacetate **137** reportedly cyclised.



Scheme 5.2. Synthesis of ajoene analogues

This synthesis of ajoene analogues was expanded (Figure 5.1); by varying the alkyl moiety in the second step, the alkyl chain was modified to produce compound **140**.[5] In addition, the analogues were synthesised with a saturated linker (**142**) and without oxidation to the sulfoxide (**141** and **143**). The analogues synthesised had improved IC_{50} compared to ajoene **14**.

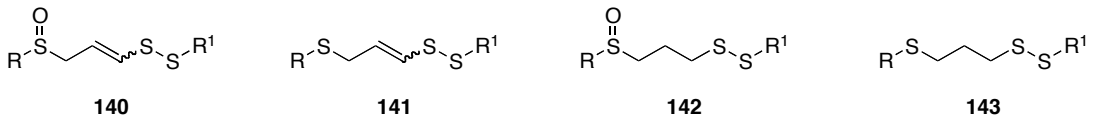
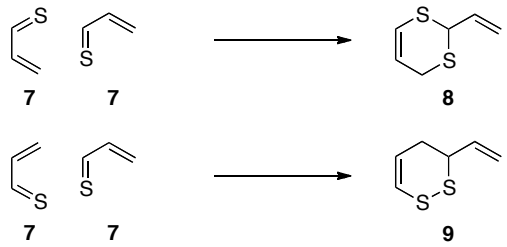


Figure 5.1. Synthesised ajoene analogues

5.1.2. Production of Dithiins

5.1.2.1. Thermolysis of Allicin to Produce Dithiins

A major component of the thermolysis of **5** are the dithiin isomers, **8** and **9**. These are generated through the Diels-Alder dimerization of thioacrolein **7** (Scheme 5. 3).



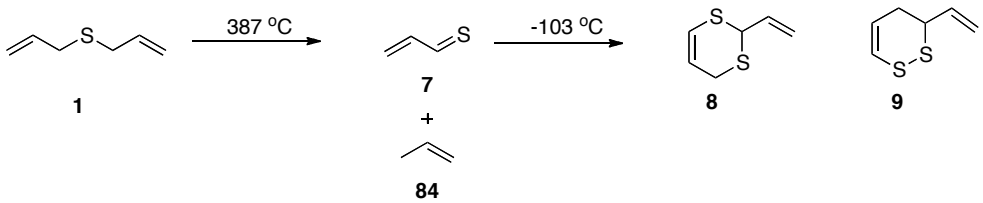
Scheme 5.3. Thioacrolein dimerisation producing dithiin isomers

In early work, dithiins were isolated products from the thermolysis of **5**. However, GOE with a high concentration of **5** were used rather than synthetic **5**. The GOE was stirred in methanol at temperatures of 37 or 25 °C for several days and dithiins (**8** and **9**) were isolated using silica gel chromatography.[6,7]

The extraction technique to isolate GOE containing high concentrations of dithiins.[8,9] Chung and co-workers demonstrated that using petroleum ether for solvent extraction of GOE will lead to **8** and **9** as the major products.[10] However there is another report in the literature that only **8** was the major product using a similar procedure.[8] The thermolysis of synthetic **5** has been performed in acetone: methanol (3:2)[11] or in hexane[3] to produce dithiins.

5.1.2.2. Synthesis of Dithiins

There are several dithiin syntheses other than the thermolysis of **5**. The flash vacuum pyrolysis (Figure 5.2) of diallyl monosulfide (Scheme 5.4) at 387 °C can produce thioacrolein.[12] Upon cooling it was reported that the dimer of thioacrolein was formed. This has apparently been used to synthesise dithiin materials[9,13,14,15] and has been patented for the synthesis of **9**.[16] However, there is no information on the conditions for this method.



Scheme 5.4. Flash vacuum pyrolysis of diallyl disulfide and subsequent cooling producing dithiin dimers

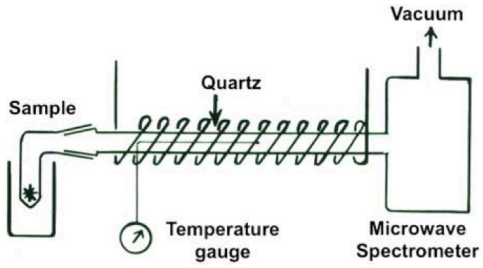
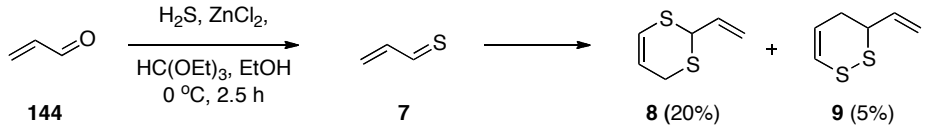


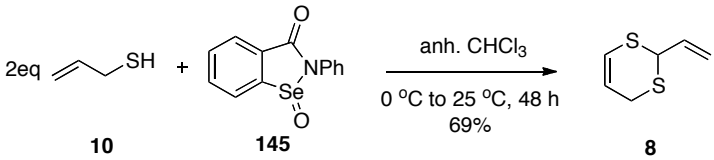
Figure 5.2. Flash vacuum pyrolysis set up[15]

An early synthesis of dithiins was the result of research by Pierre Beslin into flavour compounds in asparagus. Compounds **8** and **9** were detected during the cooking of asparagus.[17] The synthesis in Scheme 5.5 prepared **7** *in situ* from acrolein **144**. The dithiins were then isolated and separated using silica gel chromatography.[18,19] Although yields were reported, the experimental procedure was lacking information for re-evaluation in this thesis.



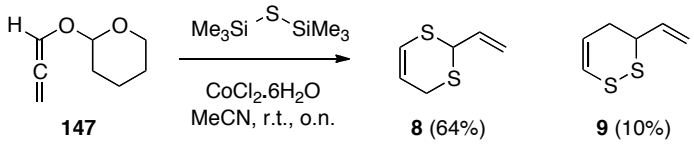
Scheme 5.5. Synthesis of dithiins from acrolein[18]

Ebselen oxide **145** has been reported as a reagent in the synthesis of **8**. When reacted with 2-propene-1-thiol, thioacrolein is generated, which as previously stated is the reactive intermediate that dimerises to produce dithiins. Glass *et al*. successfully purified the reaction products and isolated **8** (Scheme 5.6).



Scheme 5.6. Synthesis of **8** using Ebselen oxide **145**

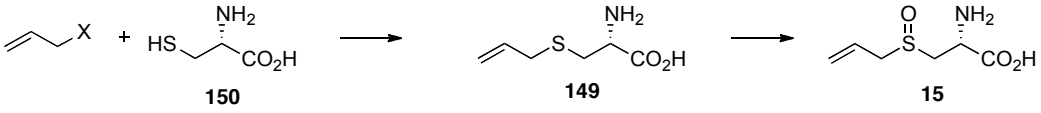
More recently, thioacyl silanes have proven to be a stable reagent to produce both **8** and **9** (Scheme 5.7).[20] Silylated allenes with hexamethyl disilathiane in the presence of cobalt chloride form α, β-unsaturated thioacyl silanes. This intermediate undergoes dimerization producing **8** as the major product. Unfortunately, the experimental section is lacking information and only NMR conversions were reported.



Scheme 5.7. Synthesis of both dithiin isomers using hexamethyl disilathiane and cobalt chloride

5.1.3. Synthesis of Alliin

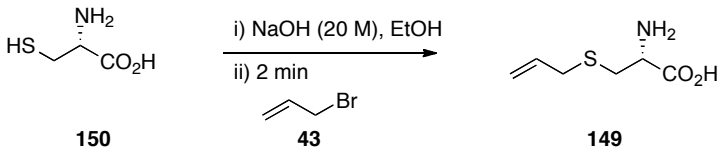
The chiral amino acid precursor of **5** is **15** and only comprises approximately 1% the weight of garlic. In an industrial process, the garlic is mashed to release the enzyme alliinase which reacts with compound **15**; to improve the process extra synthetic alliin **15** is added to the enzymatic solution to increase the yield of **5**.[2] The synthesis of **15** involves the alkylation of L-cysteine **150** and subsequent oxidation, shown in Scheme 5.8.



Scheme 5.8. Synthesis of alliin **15**

The Nobel Laureate du Vigneaud, who's work with sulfur led to the first synthesis of a polypeptide hormone,[21] developed a method to alkylate **150** to produce **149** that is widely used.[22,23,24] The reduction of cysteine with metallic sodium in liquid ammonia was developed to perform alkylation's of L-cysteine with benzyl chloride.[25]

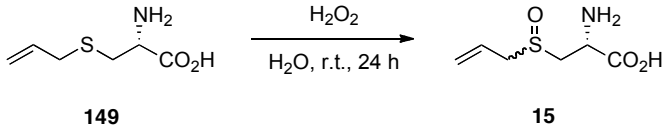
The first synthesis of **149** was performed by Stoll and Seebeck in 1950.[26] Simpler conditions have since been developed. Concentrated sodium hydroxide in EtOH is used to dissolve L-cysteine hydrochloride, which is then reacted with allyl bromide overnight. This procedure has been modified to produce **149**,[24,27,28,29] most notably by Iberl *et al.*,[30] which is cited in Neem Biotech's patent.[2]



Scheme 5.9. Synthesis of **149**

The oxidation was first published by Stoll and Seebeck who first isolated compound **15**,[31] using hydrogen peroxide to oxidise **149**.[26] However, the oxidised product **15** did not have the same specific rotation as the isolated **15** from garlic. It was deduced that the naturally occurring alliin **15** had an additional chiral centre, the *S*-oxide. The following year Stoll and Seebeck

reported the fractional recrystallisation to separate the diastereomers and isolated (+)-**15**.[32] This method has been repeatedly used to synthesise (+)-**15**.[28,29,30,33,34] An accessible and rapid adaptation of the procedure has also been developed by Iberl *et al*. to produce the diastereomers (Scheme 5.10).[30]



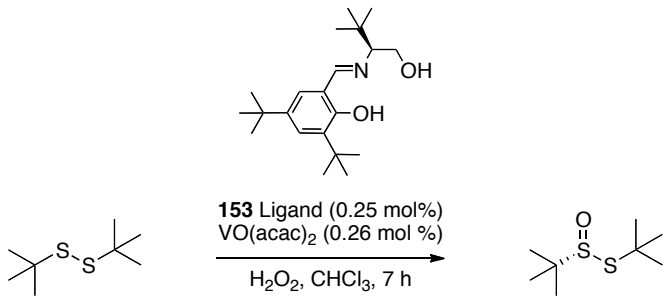
149 **15**

Scheme 5.10. Oxidation of **149**

This method was further modified to be performed without the additional water in NeemBiotech's legacy patent for kilogram production of **15**.[2] This method of oxidation, using H_2O_2, has also been used to synthesise other *Allium* amino acids, such as methiin (+)-**18**.[35]

The chirality of the sulfoxide is known to have an effect on the rate of reaction with alliinase; the (+)-**15** enantiomer reacts four times faster.[36] The asymmetric synthesis of (+)-**15** by Koch and Keusgen had required protection of the amino acid moiety.[37] More recently, the asymmetric oxidation has been performed using an enzyme from *Bacillus thuringiensis*.[38] After isolation using negative ion exchange resin, (+)-**15** was isolated in a 65% yield.

Direct asymmetric oxidation would be the simplest method to produce (+)-**15**. Attempts to improve the selectivity of the oxidation of **149** were performed using (+)-percamphoric acid.[39] Catalytic asymmetric oxidation of sulfides has been reported with chiral metal complexes.[40,41] The catalytic system to be investigated herein is taken from the work of Blum *et al*. using a vanadium catalyst and ligand **153**.[42]



152 **154**

Scheme 5.11. Chiral thiosulfinate synthesis

5.2. Results and Discussion

The initial work done in chapter 2 and chapter 4 were to produce allicin **5**. In this chapter the thermolysis of synthesised allicin **5** is evaluated using an array of conditions to produce high yields of ajoene **14** and dithiins (**8**, **9**). The chapter also evaluates the synthesis of alliin **15** using flow chemistry.

5.2.1. Thermolysis of Allicin to Produce Ajoene

The highest conversion of **5** to ajoene **14** by thermolysis was achieved by Block *et al.* in 1986.[3] Compound **5** (10 wt.%) in 40% aqueous acetone solution was refluxed for 4 h. This section investigates whether this yield can be improved through altering the current aqueous acetone conditions. The analysis of the allicin conversion to ajoene was performed using HPLC, based on Lawson *et al.* conditions, that are reported in the experimental.[43]

5.2.1.1. Effect of Water Concentration

The thermolysis of **5** performed in 40 % aqueous acetone at reflux (64 °C) produces the highest concentration of ajoene. The water concentration is critical to a high conversion of **5** to ajoene (Table 5.1). Above or below 40% aqueous acetone reduces the concentration of ajoene to less than 5%. The conclusion was that the ideal water/acetone ratio was 2:3, and would remain unchanged.

Table 5.1. The effect of water concentration in acetone on the formation of **14**

Entry	Acetone: Water ratio	HPLC Yield (%)
1	1:0	1.5
2	4:1	4.5
3	3:2	17.0
4	1:1	1.3
5	0:1	0.4

Conditions: Compound **5** (10 wt.%) in aqueous acetone heat at 64 °C for 4 h

5.2.1.2. Effect of Temperature

The temperature was the next parameter investigated. Temperatures below the boiling point of the solvent only produce 5% conversion (Table 5.2, entry 1 and 2). The best conversion was obtained at 64 °C (Table 5.2, entry 3). Temperatures above 64 °C also had the same detrimental effect on the conversion of **5** to ajoene (Table 5.2, entries 4 and 5). At these higher temperatures ajoene may have been formed more rapidly, however the prolonged exposure to higher temperatures may have caused the ajoene to decompose. However, shorter reaction

times using higher temperatures were not evaluated. These results showed that the original conditions from the work by Block *et al.* are the best conditions.

Table 5.2. The effect of temperature on the formation of **14**

Entry	Temperature (°C)	HPLC Yield (%)
1	20	2.7
2	40	1.7
3	64	17.0
4	80	1.5
5	100	3.2

Conditions: Compound **5** (10 wt.%) in 40% aqueous acetone heat for 4 h

5.2.1.3. Effect of Acid Additive

In recent literature the addition of an acid (20 mol%), relative to the quantity of allicin **5**, has been shown to improve the geometric ratio in favour of the Z-isomer with an improved 22% yield of ajoene.[44]

Using the optimum conditions, several acids were added to the reaction. Entries 1 and 2 were reported in the patent, however our experiments showed that the concentration of ajoene was reduced. Additional acids available in the laboratory were also used (Table 5.3, entries 4-6) and the same effect was observed. Peracetic acid was used as an acid and as an oxidant. The latter was hypothesised to oxidise diallyl disulfide generated into additional **5**, however this did not improve the conversion to ajoene (Table 5.3, entry 7).

Table 5.3. The effect of an acid additive on the formation of **14**

Entry	Acid	HPLC Yield (%)
1	None	17.0
2	*p*-toluenesulfonic acid	1.5
3	acetic acid	2.1
4	(1S)-(+)-10-camphorsulfonic acid	0.7
5	diphenyl acetic acid	0.2
6	caffeic acid	1.4
7	peracetic acid	0.8

Conditions: Compound **5** (10 wt.%) and acid (20 mol%) in 40% aqueous acetone at 64°C for 4 h

5.2.1.4. Effect of Base Additive

Basic conditions were applied to investigate the effect of a base on the conversion of **5** to ajoene. Deprotonation of the trisulfide cation **12** may increase the elimination of sulfenic acid **6**. The results showed that the yield was reduced to below 7% in all cases.

Table 5.4. The effect of a base additive on the formation of **14**

Entry	Base	HPLC Yield (%)
1	None	17.0
2	Pyridine	1.4
3	Piperidine	1.4
4	Na_2CO_3	5.2
5	sat. Na_2CO_3	4.5

Conditions: Compound **5** (10 wt.%) and base (20 mol%) in 40% aqueous acetone at 64^{o}C for 4 h

5.2.1.5. Effect of Sonication

The thermolysis of **5** has also been performed using sonication as an alternative to reflux conditions. However, no quantitative yields of ajoene were reported.[45] The sonication of **5** (10 wt.%) was performed in several solvents (Table 5.5).

The optimum solvent from the batch thermolysis, 40% aqueous acetone, halved the reaction time (2h) to reach maximum ajoene yield (Table 5.5, entry 1). However the HPLC yield was lower compared to batch reflux thermolysis. After 4 h, prolonged heating decreased the yield of ajoene. All solvents also showed this effect and in most cases the highest conversion was achieved after 2 h.

The best results were obtained with polyethylene glycol-400 (PEG-400) as the solvent with 9.6% ajoene yield, (Table 5.5, entry 4). The PEG-400 extraction procedure was more efficient than the batch reflux thermolysis. In the batch reflux thermolysis the reaction is quenched with saturated ammonium sulfate solution, then extracted with pentane and methylene chloride five times each. With PEG-400 the reaction was diluted with water and extracted three times with ethyl acetate.

Table 5.5. The effect of sonication on the formation of **14** over 4 h

Entry	Solvent	HPLC Yield (%)		
		1 h	2 h	4 h
1	40%aq. Me_2CO	2.9	5.3	4.7
2	H_2O	2.1	2.7	1.1
3	MeOH	-	0.4	-
4	PEG-400	6.7	9.6	2.3
5	PEG-6000	2.5	1.7	2.4
6	PEG-10000	1.5	2.4	1.8

Conditions: Compound **5** (10 wt.%) sonicated in specified solvent for specified time

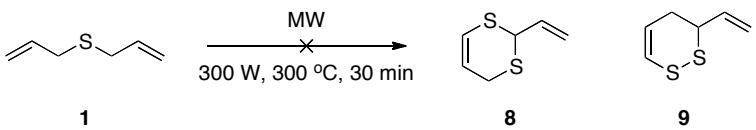
5.2.2. Synthesis of Dithiins

5.2.2.1. Microwaves Thermolysis of Diallyl Monosulfide

In the introduction the synthesis of dithiins was described using flash vacuum pyrolysis of diallyl monosulfide **1** heated to 387 °C.[15,46] This method has been used to obtain dithiin standards for gas chromatography analysis of garlic oil extracts.[13,9] Compared to allicin, the stability and availability of diallyl monosulfide **1** would be a more favourable precursor for the synthesis of dithiins.

Flash vacuum pyrolysis equipment was not available in our laboratory. However, microwave equipment was available and able to reach similar temperatures. Furthermore, continuous flow processes have been combined with microwave synthesis to increase the output.[47]

Diallyl monosulfide **1** was heated in a microwave at maximum power to 300 °C (Scheme 5.12). Product formation was observed by thin layer chromatography, however the results were not reproducible.

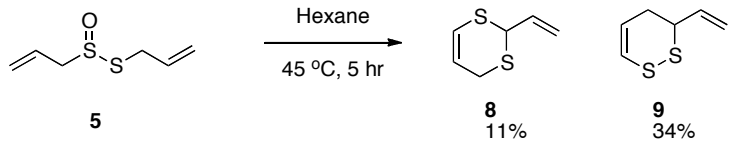


Scheme 5.12. Attempted microwave (MW) thermolysis of **1** to produce dithiins

5.2.2.2. Thermolysis of Allicin

Early work on the thermal degradation of **5** to produce dithiins was performed at room temperature with methanol as a polar solvent.[6] This reaction was attempted, but only on a qualitative scale. Over the course of 6 days, the depletion of allicin was monitored by thin layer chromatography. After 6 days, compound **5** was depleted and dithiins were present, however this reaction was not investigated further due to the long reaction time.

Lawson *et al.* performed the thermolysis of **5** using hexane, a non-polar solvent, to produce dithiins **8** and **9**. However, the yields for the isolated products were not published.[48] This reaction was investigated and followed by thin layer chromatography using hexane as the mobile phase. Compound **5** disappeared after 5 h and the reaction proceeded with good yields of both dithiins. The isomers were isolated using normal phase chromatography. Compound **9** was the major product in a 68% yield and combined yield of both dithiins was 90%. The reaction time was longer than reported in the literature but was an excellent and reliable synthesis of both dithiin isomers.



Scheme 5.13. Thermolysis of **5** to produce dithiins

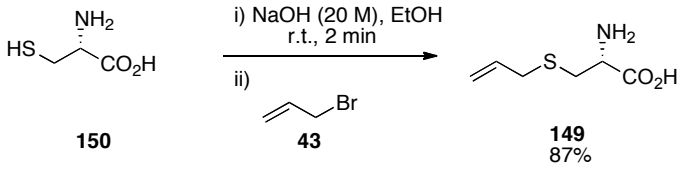
5.2.3. Synthesis of Alliin

5.2.3.1. Synthesis of Deoxyalliin

The synthesis of **149** was performed in a relative straightforward procedure based on the work by du Vigneaud[49] which has seen very minimal variation since the first synthesis in 1951.[32]

Batch Mode

The synthesis proceeds by deprotonation of **150** and the subsequent reaction with **43** to form the desired **149** good yields (Scheme 5.14).[30]

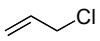
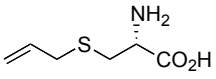
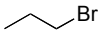
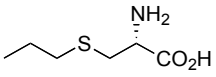
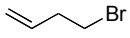
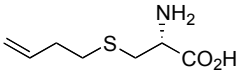
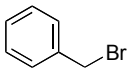
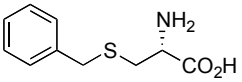
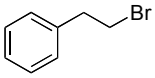
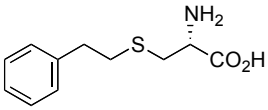


Scheme 5.14. Synthesis of **149**

The conditions were applied to the synthesis of other *S*-alkyl-L-cysteine garlic metabolites and non-natural analogues. The yields were excellent; however the allyl chloride required a longer

reaction time (Table 5.6, entry 1). Compound **160** was produced in a low yield (Table 5.6, entry 5), this maybe the result of an incomplete reaction or low isolation due to the solubility in the recrystallization solvent.

Table 5.6. Synthesis of *S*-alkyl-L-cysteine derivatives

Entry	Alkyl halide	Product	Time (min)	Yield (%)
1	 **45**	 **149**	120	99
2	 **67**	 **156**	20	99
3	 **157**	 **158**	20	89
4	 **31**	 **159**	15	91
5	 **99**	 **160**	20	18

Conditions: L-cysteine hydrochloride monohydrate suspended in EtOH and stirred at room temperature. Aqueous NaOH solution (20 M) was added dropwise. Then alkyl halide was added and stirred.

Homogeneous Flow Mode

The first flow system investigated consisted of two reactor coils, in the primary reaction coil **150** would react with base, followed by the reaction with the alkyl halide in the secondary reactor coil (Figure 5.3).

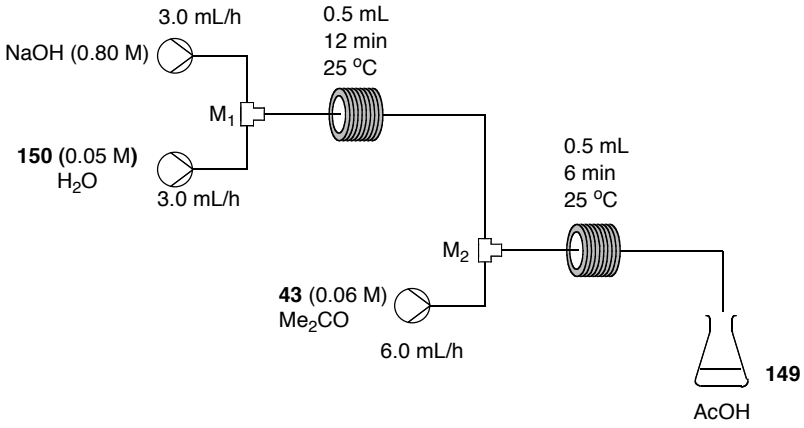


Figure 5.3. Double coil flow synthesis of **149**

This flow system failed to produce the alkylated product. The deprotonating of **150** is likely the limiting step. The flow system was simplified to use an aqueous solution of **150** in sodium hydroxide in a single coil system (Figure 5.4). The reaction was successful and resulted in a 95% yield of **149** after recrystallization.

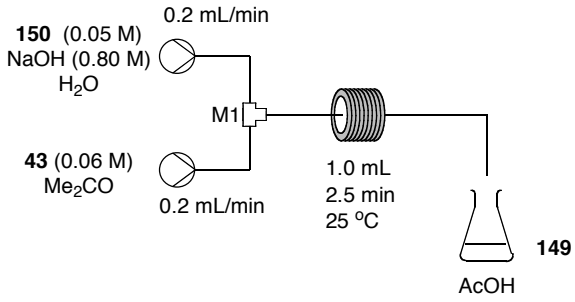


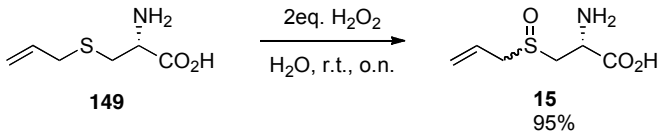
Figure 5.4. Single coil flow synthesis of **149**

5.2.3.2. Synthesis of Alliin via Oxidation of Deoxyalliin

The next reaction evaluated was the oxidation of **149** in batch mode and subsequent homogenous flow mode.

Batch Mode

Iberl *et al.* reported conditions for oxidation of **149** but not a yield.[30] The reaction was performed using these conditions and an excellent yield was obtained. However, oxidation in flow mode, as shown in Chapter 4, have long reaction times. A large reactor coil and low flow rates would have to used to investigate this synthesis.



149 **15** 95%

Scheme 5.15. Oxidation of **149**

Blum *et al.* developed a catalytic oxidative system using hydrogen peroxide.[42] This was applied to the synthesis of **15**. The experiment was evaluated in the absence of a chlorinated solvent. In the initial experiments precipitation of **15** occurred, so the reaction required dilution. The oxidation proceeded much faster in the presence of the catalyst vanadyl acetylacetonate (Table 5.7, entry 2). In the presence of both catalyst and the ligand **153** the reaction was complete in 4 h (Table 5.7, entry 3).

Table 5.7. Oxidation of **149** in water

Entry	Reagents	NMR conversion (%)	
		1 h	4 h
1	No catalyst	18	66
2	Catalyst (1.0 mol%)	38	85
3	Catalyst (1.0 mol%) Ligand (1.5 mol%)	60	100

Conditions: Compound **149** (0.1 M) in D_2O, $VO(acac)_2$ (1 mol%), ligand (1.5 mol%) was added. Then H_2O_2 (0.65 mmol) was added and stirred.

The catalyst and ligand were insoluble in water, and would be difficult to employ in a homogenous flow system. The original system had a biphasic liquid-liquid system using chloroform. Results show that this did not give any improvement in the reaction, but demonstrated that the biphasic liquid-liquid system works (Table 5.8).

Table 5.8. Oxidation of **149** in a biphasic system (1:1 water: chloroform)

Entry	Reagents	NMR conversion (%)	
		1 h	4 h
1	No catalyst	18	66
2	Catalyst (1.0 mol%)	3	78
3	Catalyst (1.0 mol%) Ligand (1.5 mol%)	3	100

Conditions: Compound **149** (0.1 M) in D_2O, $VO(acac)_2$ (1 mol%), ligand (1.5 mol%) in $CDCl_3$ was added. Then H_2O_2 (1.2 eq) was added and stirred.

Homogeneous Flow Mode

The developed catalysed oxidation was attempted in flow mode. The biphasic catalyst solution created segmented flow and the reaction proceeded with a good yield (43%). However, the reaction was performed on a small scale.

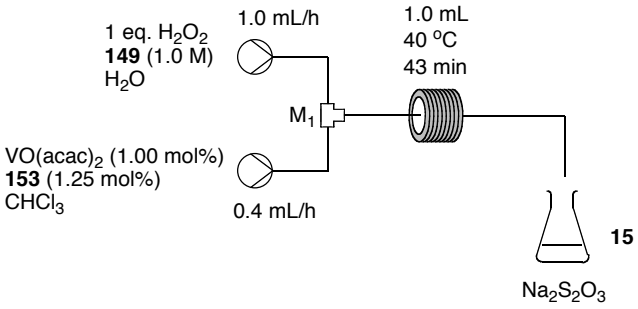


Figure 5.5. Flow synthesis of **15**

5.2.3.2. Telescoped Flow Synthesis

Multistep flow synthesis was attempted by coupling the alkylation flow system and the flow oxidation system. This would have provided a continuous flow synthesis of **15**. However, simply combining the two developed flow systems failed to produce **15**. A likely explanation is that the incoming solvent system (50% aqueous acetone) created a solvent system that was detrimental for the second oxidation flow reaction. Further investigation using this solvent system will need to be investigated.

Semi-batch approach

The system was simplified into a semi-batch process, in the same approach as that in chapter 3. After compound **150** was alkylated in the flow coil, the reaction mixture was eluted into a batch flask. The flask contained hydrogen peroxide as an oxidant and acetic acid to neutralise the sodium hydroxide. The batch reaction was stirred overnight and the reaction proceeded with a 37% yield after recrystallization. The yield was low, however it demonstrates proof of concept. Further investigation into this system is required.

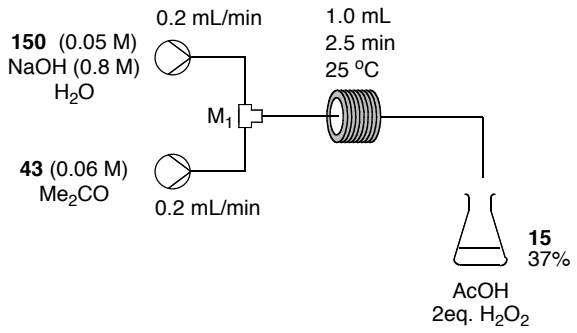


Figure 5.6. Semi-batch synthesis of **15**

5.3. Conclusion

In this chapter several garlic metabolites were successfully synthesised. The thermolysis of allicin **5** was investigated to produced ajoene and dithiins. The traditional, long standing conditions to produce ajoene is the thermolysis of **5** in 40% aq. acetone. This method produces ajoene **14** in 17% yield.[3] Increasing the formation of **14** in this reaction was attempted by varying different conditions. Addition of bases and acids, as well as increasing the temperature and altering the aqueous character of the solvent were investigated. However, the variations failed to improve the reaction yield.

The development of a sonication method had lower HPLC yields. Several solvents were investigated and it was discovered that PEG-400 produce ajoene in a good yield with reduced reaction time and simplified work-up.

The thermolysis of **5** to produce dithiins was very successful. Both isomers were produced in a combined yield of 90%. The thermolysis of more stable monosulfide at high temperatures was unsuccessful using microwave irradiation.

The synthesis of alliin **15** was also successful. The initial alkylation of **150** was achieved rapidly in batch and flow mode in a basic solution. However, a two-step flow system with an initial deprotonation reaction in a primary reactor failed to produce the alkylated product **149**. The oxidation of **149** was also performed in batch mode but with a longer reaction time, unsuitable for flow mode. The addition of catalytic reagents reduced the reaction time to 4 h and was implemented in flow mode with a residence time of 43 min. However, a longer reaction time and high temperatures are required. A telescoped flow synthesis consisting of an alkylation and subsequent oxidation was attempted but failed to produce **15**. However, a semi-batch process successfully produced **15**.

5.4. Experimental

General procedure of the thermolysis of allicin in aqueous acetone

Allicin (162 mg, 1 mmol) was dissolved in the aqueous acetone solvent (1.6 mL) in a 10 mL RBF with a reflux condenser and stirred at the desired temperature for the desired time (Table 5.1-4). The reaction was quenched with 50% aqueous MeOH (6 mL), then extracted with petroleum ether (5 × 10 mL). The aqueous layer was then saturated with ammonium sulfate, and extracted with CH_2Cl_2 (5 × 10 mL). The organic fractions were combined, dried over $MgSO_4$, then solvent was removed on the rotary evaporator and high vacuum. The sample was dissolved in HPLC grade MeOH to make a concentration of 1.0 mg/mL. Neem Biotech provided ajoene standards of 10 mg/mL. Ajoene concentration and yield were determined by HPLC analysis: Capital HPLC ODS-NN (4.6 mm × 250 mm), degassed deionised H_2O: MeCN 55:45 v:v, 1.5 mL/min.

Thermolysis of allicin using sonication

Allicin (162 mg, 1 mmol) was dissolved in the desired solvent (1.6 mL) in a 10 mL RBF with a reflux condenser and placed in a sonicator for the desired time (Table 5.5). The crude mixture was extracted with EtOAc (3 × 10 mL), and washed with water (3 × 10 mL). The organic phases were combined, dried over $MgSO_4$, then filtered. The solvent was removed on a rotary evaporator then dried under high vacuum. The sample was then dissolved in HPLC grade MeOH to make a concentration of 1.0 mg/mL. Neem Biotech provided ajoene standards of 10 mg/mL. Ajoene concentration and yield were determined by HPLC analysis: Capital HPLC ODS-NN (4.6 mm × 250 mm), degassed deionised H_2O: MeCN 55:45 v:v, pumped 1.5 mL/min, the column oven temperature was 20 °C.

Thermolysis of allicin using non-polar solvent

Allicin (67 mg, 0.41 mmol) in hexane (2 mL) was stirred at 45 °C for 5 h with a reflux condenser. Column chromatography was performed using a Biotage Isolera with the following method. The crude mixture (61 mg) was loaded onto a Biotage Snap 10 g Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 30 column volume (CV), then to 80:20 hexane: ethyl acetate over 20CV, then to 100% ethyl acetate over 10CV, and held at 100% ethyl acetate for 10CV.

The solvent from the appropriate fractions was removed and dried under high vacuum. 2-Vinyl-4H-1,3-dithiin **8** was obtained in a 11% yield (7 mg, 0.046 mmol). The spectroscopic

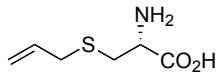
data is in agreement with the literature.[18] Colourless oil; ^{1}H NMR (400 MHz, $CHCl_3$): δ= 6.30 (td, *J*= 10.7, 1.7, 1H), 5.97 (m, 2H), 5.34 (m, 2H), 4.72 (d, *J* = 6.9 Hz, 1H), 3.31 (m, 2H).

3,4-Dihydro-3-vinyl-1,2-dithiin **9** was obtained in a 34% yield (20 mg, 0.14 mmol). The spectroscopic data is in agreement with the literature.[18] Colourless oil; ^{1}H NMR (400 MHz, $CHCl_3$): δ= 6.37 (td, *J* = 10.3, 2.0 Hz, 1H), 6.00 (m, 1H), 5.27 (m, 2H), 3.71 (m, 2H), 2.60 (m, 1H), 2.47 (m, 1H).

Attempted microwave thermolysis of diallyl monosulfide

Diallyl disulfide (50 mg, 0.34 mmol) was placed in a microwave flask in a CEM Discover SP. The reaction was performed at 300 W, 300 °C for 30 mins. The TLC, 9:1 Hex: EtOAc, showed no starting material and the formation of new spots.

(2R)-2-amino-3-[(S)-prop-2-enylthio]propanoic acid **149** *(deoxyalliin or S-allyl-L-cysteine)*

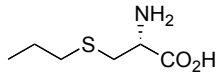


L-cysteine hydrochloride monohydrate (5.00 g, 28.5 mmol) was suspended in EtOH (5 mL) and stirred at room temperature. Aqueous NaOH solution (20 M, 0.1 mL) was added dropwise. Allyl bromide (2.7 mL, 31.4 mmol) was added and stirred for 2 minutes. The solution was acidified to pH 5.0-5.5 with acetic acid at 30 °C using universal indicator paper. The solution was cooled to 0 °C to precipitate crystals. The solid was filtered and washed twice with ice-cold ethanol. The crystals were dried in an oven at 90 °C for 1.5 h. The product was obtained in 87% yield (4.00 g, 24.8 mmol). The spectroscopic data is in agreement with the literature.[14] Colourless solid, m.p. 208-210 °C (dec) [Lit m.p. 208-210 °C (dec)][14]; $[\alpha]_D^{20}$ -12.0 (5 mg/ 1 mL) [Lit $[\alpha]_D^{20}$ -16.0 (10 mg/ 1 mL)][36]; ^{1}H NMR (300 MHz, D_2O): δ= 5.67 (dddd, *J*= 17.2, 14.5, 10.0, 7.3, 1H), 5.05 (m, 2H), 3.75 (dd, *J*= 7.5, 4.3, 1H), 3.06 (d, *J*= 7.3 Hz, 2H), 2.85 (m, 2H) ppm.

General procedure for the synthesis of alkyl-S-cysteine

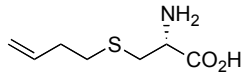
L-cysteine hydrochloride monohydrate (200 mg, 1.14 mmol) was suspended in EtOH (1.5 mL) and stirred at room temperature. Aqueous NaOH solution (20 M, 0.17mL) was added dropwise. Alkyl halide in Table 5.6 (1.25 mmol) was added and stirred for 2 minutes. The solution was acidified to pH 5.0-5.5 with acetic acid (approx. 0.3 mL) at 30 °C on universal indicator paper. The solution was cooled to 0 °C to precipitate crystals. The solid was filtered and washed twice with ice-cold ethanol. The crystals were dried in an oven at 90 °C for 1.5 h.

(2R)-2-Amino-3-[(S)-propylthio]propanoic acid **156**



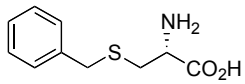
The product was obtained in 99% yield (184 mg, 1.13 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[14] Colourless solid; m.p. 205-206 °C (dec) [Lit m.p. 210-212 °C (dec)][14]; $[\alpha]_D^{20}$ -24.0 (10 mg/ 1 mL) [Lit $[\alpha]_D^{20}$ -11.7 (10 mg/ 1 mL)][51]; ^{1}H NMR (400 MHz, D_2O): δ= 3.77 (dd, *J*= 7.5, 4.3 Hz, 1H), 2.92 (m, 2H), 2.44 (m, 2H), 1.46 (m, 2H), 0.81 (t, *J*= 7.4, 3H) ppm.

(R)-2-Amino-3-(but-3-en-1-ylthio)propanoic acid **158**



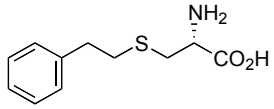
The product was obtained in 89% yield (178 mg, 1.01 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[50] Colourless solid; m.p. 210-212 °C (dec) [Lit m.p. 208-211 °C (dec)][50]; $[\alpha]_D^{20}$ -38.0 (10 mg/ 1 mL); ^{1}H NMR (400 MHz, D_2O): δ= 5.67 (m, 1H), 4.97 (m, 2H), 3.77 (dd, *J*= 7.51, 4.26 Hz, 1H), 2.92 (m, 2H), 2.55 (m, 2H), 2.22 (m, 2H) ppm.

(R)-2-Amino-3-(benzylthio)propanoic acid **159**



The product was obtained in 91% yield (0.220 g, 1.04 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[51] Colourless solid, 212-214 °C (dec) [Lit m.p. 215-216 °C (dec)][51]; $[\alpha]_D^{20}$ +22.0 (10 mg/ 1 mL) [Lit $[\alpha]_D^{20}$ +25.8 (10 mg/ 1 mL)][51]; ^{1}H NMR (250 MHz, D_2O): δ= 7.42-7.27 (m, 5H), 3.78 (m, 3H), 2.94 (m, 2H) ppm.

(R)-2-Amino-3-(phenethylthio)propanoic acid **160**



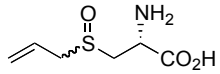
The product was obtained in 18% yield (51.7 mg, 0.21 mmol) using the general procedure. The spectroscopic data is in agreement with the literature.[52] Colourless solid, 178-180 °C (dec)

[Lit m.p. 220-221 °C (dec)][52]; ^{1}H NMR (400 MHz, DMSO): δ= 7.25 (m, 5H), 3.25 (m, 2H), 2.84-2.66 (m, 5H).

Flow synthesis of **149**

L-cysteine (60.5 mg, 0.5 mmol) in D_2O (4 mL) was prepared. NaOH (20 M, 0.2mL) was then filled to 5 mL with extra D_2O. This was loaded into the syringe at a flow rate of 0.2 mL/min. Allyl bromide (52 µL, 0.6 mmol) in acetone (0.12 M, 5 mL) was loaded into another syringe at a flow rate of 0.2 mL/min. The syringes were pumped through a 1 mL coil (length 2 m) at 25 °C. The reaction was quenched in acetic acid (0.1 mL). The solvent was removed by rotary evaporation. The product was recrystallized by refluxing in EtOH (6 mL) then cooled to 0 °C and filtered. The product was obtained in a 95% yield (315 mg, 1.78 mmol). The spectroscopic data is in agreement with the literature.[14]

Batch synthesis of alliin **15**



L-deoxyalliin (0.325 g, 2.02 mmol) was dissolved in H_2O (3.2 mL) and stirred. H_2O_2 (30% w/v, 0.33 mL) was added dropwise and the reaction was stirred overnight. The reaction was complete on TLC using propan-2-ol: AcOH: H_2O (10:5:1). The solvent was removed on rotary evaporator, then recrystallized in MeOH. The product was obtained in a 95 % yield (315 mg, 1.78 mmol). The spectroscopic data is in agreement with the literature.[14] Colourless solid; m.p. 166-168 °C (dec) [Lit m.p. 165 °C (dec)][14]; $[\alpha]_D^{20}$ +14.3 (7 mg/ 1 mL) [Lit $[\alpha]_D^{20}$ +63.2 (10 mg/ 1 mL)][36];[1]H NMR (250 MHz, D_2O): δ= 5.82 (m, 1H), 5.25 (m, 2H), 4.11 (m, 1H), 3.69 (m, 2H), 3.25 (m, 2H).

Catalysed alliin **15** *synthesis using aqueous conditions*

Deoxyalliin (81 mg, 0.5 mmol) in D_2O (5 mL) was added to 10 mL vial. $VO(acac)_2$ (2.5 mg, 1 mol%), ligand (5 mg, 1.5 mol%) were added and stirred. Then H_2O_2 (30 w/v%, 60 µL, 0.65 mmol) was added and stirred. Samples were taken at 1 and 4 h intervals and diluted with D_2O for NMR analysis.

Catalysed alliin **15** *synthesis using biphasic conditions*

Deoxyalliin (81 mg, 0.5 mmol, 0.1 M), in D_2O (5 mL) was added to 10 mL vial. $VO(acac)_2$ (2.5 mg, 1 mol%), ligand (5 mg, 1.5 mol%) in $CDCl_3$ (2 mL) were added and stirred. Then H_2O_2 (30 w/v%, 60 µL, 0.65 mmol) was added and stirred. Samples were taken at 1 and 4 h intervals and diluted with D_2O for NMR analysis.

Flow synthesis of alliin **15**

Deoxyalliin (81 mg, 0.5 mmol, 0.1 M) and H_2O_2 (30 w/v%, 60 µL, 0.65 mmol) in D_2O (5 mL) were loaded into a syringe at a flow rate of 1.0 mL/h. $VO(acac)_2$ (2.5 mg, 1 mol%), ligand (5 mg, 1.5 mol%) in $CDCl_3$ (2 mL) was loaded into a separate syringe at a flow rate of 0.4 mL/h. The syringes were pumped through a PTFE reaction coil (volume: 1 mL, length: 2 m, internal diameter: 0.8 mm) at 40 °C. The reaction solution was eluted into a collection flask containing sat. $Na_2S_2O_3$ (1 mL). The solvent was removed on rotary evaporator. The crude salt product was dissolved in water (0.2 mL), then MeOH (4 mL) was added and cooled to -41 °C using a dry ice/ MeCN cooling bath. The suspension was filtered and washed with cold MeOH (3 × 4 mL) that was chilled on the dry ice/ MeCN cooling bath. The solid was washed with cold Et_2O (2 × 4 mL) that was chilled on the dry ice/ MeCN cooling bath. The crystals were dried in an oven at 120 °C for 1 h. The product was obtained in a 43% yield (0.21 mmol, 38.1 mg). The spectroscopic data is in agreement with the literature.[14]

Semi-batch telescoped flow synthesis of alliin **15**

L-cysteine (60.5 mg, 0.5 mmol) in H_2O (4 mL) was prepared. NaOH (20 M, 0.2 mL) was then diluted to 5 mL with H_2O. This was loaded into syringe at a flow rate of 0.2 mL/min. Allyl bromide (52 µL, 0.6 mmol) in acetone (0.12 M, 5 mL) was loaded into a separate syringe at a flow rate of 0.2 mL/min. The syringes were connected to a PTFE reaction coil (volume: 1 mL, length: 2 m, internal diameter: 0.8 mm) at 25 °C. The reaction was eluted into a collecting flask containing acetic acid (0.5 mL) and H_2O_2 (30 w/v%, 60 µL, 0.65 mmol) and stirred overnight. The reaction was quenched with sat. aq. $Na_2S_2O_3$ (1 mL). The solvent was removed on rotary evaporator. The crude salt product was dissolved in water (0.2 mL), then MeOH (4 mL) was added and cooled to -41 °C using a dry ice/ MeCN cooling bath. Cold Et_2O (20 mL) was added to precipitate the product. This was filtered and washed with cold Et_2O (2 × 10 mL). The crystals were dried in an oven for 1 hour at 120 °C. The product was obtained in a 37% yield (0.18 mmol, 32.9 mg). The spectroscopic data is in agreement with the literature.[14]

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Research Publications

Flow Synthesis of Symmetrical Di- and Trisulfides Using Phase-Transfer Catalysis

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Using mild conditions and short reaction times, symmetrical disulfides have been synthesized in flow chemistry using phase transfer catalysts to facilitate the substitution of organohalides with disodium disulfide. Also, the synthesis of symmetrical trisulfides was possible using this procedure with an additional equivalent of sulfur.

Keywords: flow chemistry, phase transfer catalysts, symmetrical disulfides, symmetrical trisulfides

1. Introduction

Disulfide linkages are important structural features in enzymes and peptidic structures, and also, disulfides themselves are important compounds in biochemistry. There are several established procedures for the chemical synthesis of disulfides. Widely used starting materials are thiols as they can undergo oxidative coupling reactions toward disulfides. Different catalysts can be used, such as anhydrous potassium phosphate [1], reusable ionic liquids [2], aluminium nitrate under heterogeneous reaction conditions [3], as well as metal-free nitrates [4], solid supported basic catalysts [5], the Burgess reagent [6], and others [7].

Using alkyl halides as an alternative precursor for the synthesis of disulfides is a safe and commercially viable alternative. Different methods and reagents as sulfur sources in such syntheses as benzyltriethylammonium tetracosathioheptamolybdate $[(C_6H_5CH_2N(Et)_3)_6Mo_7S_{24}]$ [8] or piperidinium tetrathiomolybdate [9] have been reported.

A promising procedure to develop disulfide synthesis in flow chemistry was reported by Sonavane et al. utilizing didecyldimethylammonium bromide (DDCB) as a transfer catalyst in the reaction between aqueous sodium disulfide and alkyl halides in chloroform to produce symmetrical disulfides under mild reaction conditions [10]. In this work, several other transfer catalysts, such as tetrabutylammonium bromide (TBAB), were screened. This method was investigated in batch and under flow chemistry conditions and extensively screened by reacting allyl bromide **1a** to diallyl disulfide **2a** as the target molecule. This compound is of large importance for the synthesis of garlic metabolites but not commercially available in pure form.

Diallyl trisulfide is another naturally occurring molecule in garlic [11], which has several health benefits through the release hydrogen sulfide [12]. In some cases, diallyl trisulfide has also shown greater effect on cancer cells compared to diallyl disulfide **2a** [13].

Sonavane et al. claimed that the reaction shown in Scheme 1 [10] is also suitable for the synthesis of other polysulfides. In the approach to synthesize the corresponding trisulfide, two equivalents of elemental sulfur were added to the disodium sulfide solution for the generation of disodium trisulfide. In preliminary batch reactions, however, a mixture of different polysulfides was generated. Also, dioxaphosphorinane derivatives have been used to synthesize both symmetrical [14] and unsymmetrical disulfides [15] as well as several novel aromatic and heterocyclic trisulfides in two-step reaction sequences starting from thiols.

2. Results and Discussion

2.1. Synthesis of Symmetrical Disulfides in a Flow Reactor. Disodium disulfide (Na_2S_2) was generated by the reduction of sulfur by sodium sulfide at 50 °C in water. The resulting solution was then loaded onto a syringe pump, with the second syringe containing the alkyl bromide and tetra-*n*-butylammonium bromide (TBAB) in an organic solvent, as shown in Figure 1. After passing through a micromixer [16], the reaction occurs in the reactor coil (Teflon tubing, 0.8 mm diameter, 2 m length, and 1 mL volume) before being quenched with brine in the collection flask.

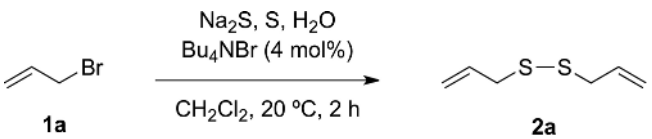
A low concentration (2 mM) of disodium disulfide was required to prevent the compound from precipitating in the flow setup. For optimization studies, a wide range of reaction conditions were investigated including different solvents, flow rates (residence times), and temperatures using allyl bromide **1a** as reactant. All reactions have been performed at 20 °C and are summarized in Table 1.

Initially, the reaction time was modified by increasing the length of the reactor coil, while the (total) flow rate was kept constant at 0.2 mL/min. The optimum reaction time was found to be 5 min (corresponding to a reactor coil length of 2 m, volume 1 mL), as shown in entries 1–3 in Table 1. Dichloromethane and chloroform are leading to a biphasic flow system resulting in segmented flow [17]. Microreactors can offer advantages by intense mixing of immiscible liquids. We have already shown that ester hydrolysis [18], performed under liquid–liquid biphasic reaction conditions or Heck reactions [19], can be accelerated in microreactors. In biphasic flow systems, mass transfer is accelerated as the fluid packets benefit from a continually refreshing interface between adjacent fluid segments and a rapid vortex flow within each fluid packet.

The reaction using ethanol or acetonitrile gave much lower yields compared to dichloromethane. Without TBAB as phase transfer catalyst, the reaction did not proceed and was less efficient when larger amounts of TBAB were used (Table 1, entries 9 and 10). A similar phase transfer catalyst, tetrabutylammonium iodide, was not as efficient (Table 1, entry 6). This protocol was then used to screen different substrates.

Other disulfides can be prepared efficiently, as shown in Table 2, but the reaction temperature had to be increased to 40 °C to ensure high yields within the 5-min reaction time. The

Scheme 1. Synthesis of diallyl disulfide **2a**



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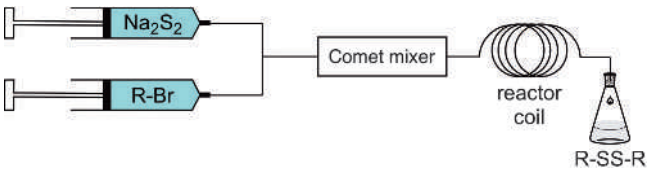


Figure 1. Flow reactor setup for disulfide synthesis

Table 1. Synthesis of diallyl disulfide **2a**

Entry	Solvent	TBAB (mol.%)	Reaction time (min)	2a Yield (%)
1	CH_2Cl_2	4	2.5	28
2	CH_2Cl_2	4	5	68
3	CH_2Cl_2	4	10	48
4	$CHCl_3$	4	5	10
5[a]	CH_2Cl_2	4	5	92
6[a]	CH_2Cl_2	4[b]	5	38
7	EtOH	4	5	44
8	MeCN	4	5	1
9	CH_2Cl_2	0	5	0
10	CH_2Cl_2	8	5	14

[a] Allyl chloride was used instead of allyl bromide.
[b] Tetrabutylammonium iodide was used instead of TBAB.

yields are lower compared to the batch experiments by Sonavane et al. [10]; however, the conditions are optimized for a different substrate.

2.2. Synthesis of Symmetrical Trisulfides in a Flow Reactor. For the synthesis of trisulfides, the corresponding trisulfide bisanion (S_3^{2-}) has to be generated first. The known equilibria between elemental sulfur and monosulfide (S^{2-}) lead to the formation of polysulfide dianions mixtures. For the optimization of the trisulfide synthesis, benzyl bromide **1e** was chosen as the model substrate as the product mixture was easy to analyze by proton nuclear magnetic resonance (^{1}H NMR). In this reaction, different amounts of dibenzyl disulfide **2e**, dibenzyl trisulfide **3e**, dibenzyl tetrasulfide **4e**, and dibenzyl pentasulfide **5e** are formed.

This reaction produced interesting results. Unlike the synthesis of the disulfide derivatives, which was selective, this procedure produces an array of polysulfides, as shown in Table 3. Forming the disulfide as the major product is a likely outcome as the reduction favored the formation of the disodium disulfide ion as a result of insufficient reduction by S^{2-} to form Na_2S_3. These results were reproduced with two different batches of disodium sulfide with minimal variation. The yield is improved with a reduced reaction time, and temperature is shown to have no substantial effect (Table 3, entries 3–6).

Other polysulfide mixtures using allyl bromide **1a**, propyl bromide **1b**, and pentyl bromide **1d** as starting materials have also been synthesized, as shown in Table 3, entries 7–9. The separation of the polysulfide products from these experiments was difficult and only possible using reverse phase C18 silica. Only the diallyl polysulfides were completely separated which lead to the isolation of diallyl tetrasulfide **4a**, pentasulfide **5a**, and some hexasulfide **6a**. These compounds have the identical ^{1}H NMR chemical shifts, and their assignment was only possible using mass spectrometric analysis. The structure of polysulfides **1b**, **1d**, and **1e** was also confirmed by high-resolution mass spectrometry.

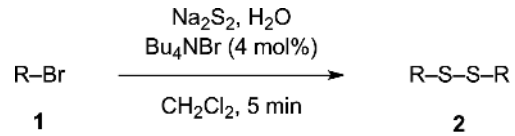
3. Conclusion

Several symmetrical disulfides can be synthesized efficiently in flow with high conversion and high throughput, requiring only an aqueous work-up and no additional purification. Symmetrical trisulfides were also successfully synthesized and isolated using similar reaction conditions; however, higher polysulfides were also produced.

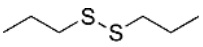
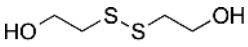
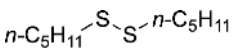
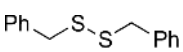
4. Experimental

4.1. General Procedure: Synthesis of Symmetrical Disulfides in Flow. Sulfur (0.258 g, 8 mmol) and anhydrous sodium sulfide (2.40 g, 10 mmol) were dissolved in water (5 mL) and stirred at 50 °C for 30 min. The alkyl halide (20 mmol) and TBAB (0.257 g, 0.8 mmol) were dissolved in EtOH (3.22 mL). The solutions were loaded into two separate 5-mL syringes and placed on a syringe pump with a flow rate of 0.1 mL/min, through a Comet mixer and a 0.8-mm diameter, 2-m-long polytetrafluoroethylene (PTFE) reaction coil. The reaction mixture was quenched by introducing the reactor outlet into brine. After the reaction, the mixture was extracted with diethyl ether

Table 2. Synthesis of dialkyl disulfides **2**

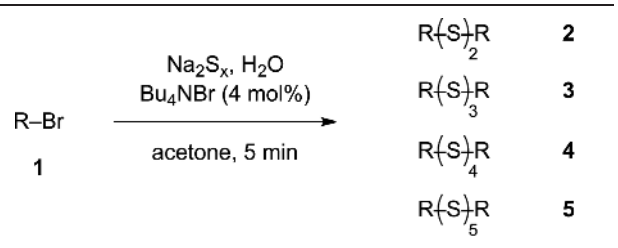


1 **2**

Entry	Substrate **1**[a]	Disulfide **2**	Reaction temperature (°C)	**2** Yield (%)
1	**1b**: R=*n*-C_3H_7	 **2b**	40	45
2	**1c**: R=CH_2CH_2OH	 **2c**	40	70
3	**1d**: R=*n*-C_5H_{11}	 **2d**	40	63
4	**1e**: R=CH_2Ph	 **2e**	30	43

[a] Reaction conditions: 4 mol.% TBAB, CH_2Cl_2, 5-min reaction time.

Table 3. Synthesis of polysulfides **2e**–**5e**



Entry	Substrate	Reaction time (min)	Temperature (°C)	2:3:4:5[a]	Combined yield (%)
1	**1e**	20	20	50:26:15:9	65
2	**1e**	10	20	51:25:15:9	83
3	**1e**	5	20	49:25:15:11	94
4	**1e**	5	30	49:27:15:9	92
5	**1e**	5	40	54:26:13:7	88
6	**1e**	5	50	53:25:14:8	92
7	**1a**	5	20	22:53:35[b]	72
8	**1b**	5	20	15:33:52[c]	87
9	**1d**	5	20	80:20[d]	80

[a] Ratios determined by ^{1}H NMR.
[b] Isolated compounds: **2a**: 1%, **3a**: 50%, **4a**: 15%, **5a**: 5%, **6a** (diallyl hexasulfide): 1%.
[c] 52% polysulfides.
[d] 20% polysulfides.

(3 × 20 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over magnesium sulfate, and the solvents were removed in vacuo.

4.1.1. Diallyl Disulfide (2a). Compound **2a** was obtained as a clear yellow oil (0.769 g, 5.3 mmol) in 88% yield. ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=5.81 (tdd, J=17.2, 9.9, 7.3 Hz, 4H), 5.21–5.09 (m, 2H), 3.34 (d, J=7.4 Hz, 4H) ppm; carbon nuclear magnetic resonance (^{13}C NMR) (125 MHz, $CDCl_3$, 298 K) δ=133.4, 118.4, 42.4 ppm; ν_{max} (NaCl): 3082, 3010, 2979, 2906, 1634, 1423, 1398, 1266, 1215, 987, 739 cm^{-1}; high-resolution mass spectrometry (HRMS) (electrospray ionization [ESI]): calculated for $C_6H_{10}S_2$ (M^+): 146.0224; found 146.0223.

4.1.2. Dipropyl Disulfide (2b). Compound **2b** was obtained as a clear oil (0.54 g, 3.6 mmol) in 45% yield. ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=2.60 (dt, J=7.4, 3.1 Hz, 4H), 1.75–1.56 (m, 4H), 0.94 (dt, J=7.3, 2.6 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ=41.1, 22.5, 13.1 ppm; ν_{max} (NaCl): 2962, 2932, 2873, 1456, 1413, 1290, 1230, 1216, 760 cm^{-1}. HRMS (ESI): calculated for $C_6H_{13}S_2$ (M^+–H): 149.0453; found 149.0450.

4.1.3. 2,2′-Disulfanediyldiethanol (2c). Compound **2c** was obtained as a clear oil (0.517 g, 3.5 mmol) in 70% yield (5 mmol sulfur used in the general procedure). ^{1}H NMR (250 MHz, $CDCl_3$, 298 K) δ=2.82 (t, J=5.8 Hz, 4H), 3.84 (t, J=5.8 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ=60.4, 41.3 ppm; ν_{max} (NaCl): 3390, 3054, 2928, 2877, 1421, 1401, 1266, 1058, 1008, 739, 703 cm^{-1}; HRMS (ESI): calculated for $C_4H_{10}O_2S_2$ ($M+H^+$): 155.0200; found 155.0191.

4.1.4. Dipentyl Disulfide (2d). Compound **2d** was obtained as a clear oil (1.309 g, 5.0 mmol) in 63% yield. ^{1}H NMR (250 MHz, $CDCl_3$, 298 K) δ=2.67 (dd, J=7.5, 7.2 Hz, 4H), 1.81–1.60 (m, 4H) 1.42–1.26 (m, 8H), 0.94–0.86 (t, J=6.94 Hz, 6H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ=39.1, 30.7, 28.9, 22.3, 13.9 ppm; ν_{max} (NaCl): 2957, 2927, 2871, 2858, 1465, 1413, 1378, 1341, 1297, 1271, 1254, 729 cm^{-1}; HRMS (ESI): calculated for $C_{10}H_{22}S_2$: 206.1163; found 206.1165.

4.1.5. Dibenzyl Disulfide (2e). Compound **2e** was obtained as a clear oil (0.834 g, 3.4 mmol) in 43% yield using half the equivalents of the general procedure. ^{1}H NMR (250 MHz, $CDCl_3$, 298 K) δ=7.50–7.37 (m, 10 H), 3.73 (s, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ=137.5, 129.5, 128.6, 127.5, 43.4 ppm; ν_{max} (NaCl): 3054, 2987, 1265, 739, 705 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{15}S_2$ ($M+H^+$): 247.0610; found 247.0608.

4.2. General Procedure: Synthesis of Symmetrical Trisulfides in Flow. Sulfur (0.641 g, 20 mmol) and anhydrous sodium sulfide (2.40 g, 10 mmol) were dissolved in water (5 mL) and stirred at 50 °C for 30 min. The alkyl halide (20 mmol) and tetrabutylammonium bromide (0.257 g, 0.8 mmol) were dissolved in EtOH to a total volume of 5 mL. The solutions were loaded into two separate 5 mL syringes and placed on a syringe pump with a flow rate of 0.1 mL/min and attached to a Comet mixer which is connected to a PTFE reaction coil (length: 2 m, internal diameter: 0.8 mm). The reaction mixture was quenched by introducing the reactor outlet into brine. After the reaction, the mixture was extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over magnesium sulfate, and the solvents were removed in vacuo.

Compound mixtures **2a**, **3a, 4a**, **5a**, and **6a** were obtained as a clear yellow oil (2.639 g) using twice of the amounts for all chemicals as described in the general procedure. From ^{1}H NMR, the sample contained 22% diallyl disulfide, 53% diallyl trisulfide, and 25% higher diallyl polysulfides. From the reaction mixture, 342 mg was purified on a Biotage Isolera system with a Telos Flash C18 column (12 g) using a solvent gradient (*v:v*) of water–methanol (50:50) to (20:80) for 25 column volumes (CV), then (20:80) to (0:100) for 15 CV, then (0:100) for 4 CV at a flow rate of 12 mL/min. This protocol also allowed a separation of **4a**, **5a**, and **6a**, which are indistinguishable by ^{1}H NMR. The amounts obtained after separation were as follows: **2a** (2 mg, 0.01 mmol), **3a** (206 mg, 1.16 mmol), **4a** (75 mg, 0.36 mmol), **5a** (28 mg, 0.12 mmol), and **6a** (10 mg, 0.04 mmol).

4.2.1. Diallyl Trisulfide (3a). ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=6.00–5.81 (m, 2H), 5.32–5.16 (m, 4H), 3.52 (d, J= 7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ= 133.0, 119.5, 42.0 ppm; ν_{max} (NaCl): 3082, 3010, 2979, 2906, 1634, 1423, 1398, 1217, 986, 191, 721 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_3$ (M^+): 177.9945; found 177.9940.

4.2.2. Diallyl Tetrasulfide (4a). ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=5.89 (tdd, J=17.1, 9.9, 7.3 Hz, 4H), 5.24–5.11 (m, 2H), 3.51 (d, J=7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ=132.4, 119.3, 42.0 ppm; ν_{max} (NaCl): 3084, 3011, 2980, 2908, 1634, 1423, 1398, 1219, 986, 909, 733 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_4$ (M^+): 209.9665; found 209.9661.

4.2.3. Diallyl Pentasulfide (5a). ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=5.82 (tdd, J=17.1, 9.9, 7.3 Hz, 4H), 5.25–5.14 (m, 2H), 3.55 (d, J=7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ=132.2, 119.9, 42.5 ppm; ν_{max} (NaCl): 3072, 3054, 2982, 2920, 1634, 1423, 1265, 1220, 988, 925, 739 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_5$ (M^+): 241.9386; found 241.9385.

4.2.4. Diallyl Hexasulfide (6a). ^{1}H NMR (250 MHz, $CDCl_3$, 298 K): δ=5.89 (tdd, J=17.2, 9.9, 7.3, 4H), 5.34–5.21 (m, 2H), 3.62 (d, J=7.3 Hz, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ=132.2, 120, 42.4 ppm; ν_{max} (NaCl): 3081, 2956, 2922, 2849, 1847, 1726, 1634, 1422, 1397, 1261, 1218, 1074, 1020, 945, 921, 859, 801, 720 cm^{-1}; HRMS (ESI): calculated for $C_6H_{10}S_6$ (M^+): 273.9107; found 273.9105.

Compounds **2b**, **3b**, **4b**, and **5b** were obtained as a yellow oil (1.3 g) using the amounts given in the general procedure. From ^{1}H NMR, the crude reaction product mixture contained 15% dipropyl disulfide, 33% dipropyl trisulfide, and 52% dipropyl polysulfides. From the product mixture, 200 mg was separated on a Biotage Isolera system with a Telos Flash C18 column (12 g) using a solvent gradient (*v:v*) of water–methanol (50:50) for 3 CV, then (50:50) to (24:86) for 32 CV, held at (24:86) for 13 CV, then (26:84) to (0:100) for 4 CV, then (0:100) for 13 CV at a flow rate of 12 mL/min. The amounts obtained after separation were as follows: **2b** (23 mg, 0.15 mmol), **3b**

(41 mg, 0.23 mmol), and an inseparable mixture of **3b**, **4b**, **5b**, and **6b** (121 mg, 0.56 mmol). Due to overlapping ^{1}H NMR signals, a ratio could not be determined.

4.2.5. Dipropyl Trisulfide (3b). ^{1}H NMR (400 MHz, $CHCl_3$, 298 K) δ=2.92 (m, 4H), 1.80 (m, 4H), 1.02 (t, J=7.33, 7.33 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CHCl_3$, 298 K) δ=41.3, 22.3, 13.1 ppm; ν_{max} (NaCl): 2962, 2929, 2872, 1455, 1411, 1377, 1337, 1290, 1231, 1089, 1051, 897, 781 cm^{-1}; HRMS (ESI): calculated for $C_6H_{14}S_3$: 182.0258; found 182.0257.

4.2.6. Dipropyl Tetrasulfide (4b). HRMS (ESI): calculated for $C_6H_{14}S_4$: 213.9978; found 213.9977.

4.2.7. Dipropyl Pentasulfide (5b). HRMS (ESI): calculated for $C_6H_{13}S_5$: 245.9699; found 245.9702.

4.2.8. Dipropyl Hexasulfide (6b). HRMS (ESI): calculated for $C_6H_{14}S_6$: 277.9420; found 277.9420.

Compounds **2d**, **3d**, **4d**, and **5d** were obtained as a yellow oil (165 mg) using 10% of the amounts given in the general procedure. From ^{1}H NMR, the sample contained 80% dipentyl disulfide and 20% dipentyl polysulfides. Unfortunately, these could not be separated, and due to overlapping ^{1}H NMR signals, a ratio could not be determined. Their presence was confirmed by high-resolution mass spectrometry of the polysulfide mixture.

4.2.9. Dipentyl Trisulfide (3d). HRMS (ESI): calculated for $C_{10}H_{22}S_3$: 238.0884; found 238.0889.

4.2.10. Dipentyl Tetrasulfide (4d). HRMS (ESI): calculated for $C_{10}H_{22}S_4$: 270.0604; found 270.0606.

4.2.11. Dipentyl Pentasulfide (5d). HRMS (ESI): calculated for $C_{10}H_{22}S_5$: 302.0325; found 302.0331.

4.2.12. Dipentyl Hexasulfide (6d). HRMS (ESI): calculated for $C_{10}H_{22}S_6$: 334.0046; found 334.0039.

Compounds **2e**, **3e**, **4e**, and **5e** were obtained as a yellow oil (2.4 g) using the amounts given in the general procedure at 33 °C. From ^{1}H NMR, the sample contained 14% dibenzyl disulfide **2e**, 71% dibenzyl trisulfide **3e**, and 14% dibenzyl polysulfides. A purification of the dibenzyl polysulfides was impossible; however, their existence was detected and verified by mass spectrometry of the mixture. From the product mixture, 261 mg was separated on a Biotage Isolera with a Telos Flash C18 column (12 g) using a solvent gradient (*v*:*v*) of water–methanol (30:70) for 3 CV, (30:70) to (20:80) for 25 CV, then (20:80) to (0:100) for 15 CV, then (0:100) for 4 CV at a flow rate of 12 mL/min. The amounts obtained after separation were as follows: **2e** (21 mg, 0.09 mmol), **3e** (70 mg, 0.25 mmol), and an inseparable mixture of **3e**, **4e**, **5e**, and **6e** (104 mg).

4.2.13. Dibenzyl Trisulfide (3e). ^{1}H NMR (400 MHz, $CHCl_3$, 298 K) δ=7.65–7.51 (m, 10H), 4.31 (s, 4H) ppm; ^{13}C NMR (100 MHz, $CHCl_3$, 298 K) δ=129.4, 128.6, 127.5, 136.4, 43.1 ppm; ν_{max} (NaCl): 3074, 3061, 3028, 2914, 1601, 1494, 1453, 1230, 1199, 1070, 914, 765, 967, 658 cm^{-1}; HRMS (ESI): calculated for $C_{14}H_{14}S_3$: 278.0258; found 278.0262.

4.2.14. Dibenzyl Tetrasulfide (4e). HRMS (ESI): calculated for $C_{14}H_{14}S_4$: 309.9978; found 309.9981.

4.2.15. Dibenzyl Pentasulfide (5e). HRMS (ESI): calculated for $C_{14}H_{14}S_5$: 341.9699; found 341.9695.

4.2.16. Dibenzyl Hexasulfide (6e). HRMS (ESI): calculated for $C_{14}H_{14}S_6$: 373.9420; found 373.9419.

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Flow Alkylation of Thiols, Phenols, and Amines Using a Heterogenous Base in a Packed-Bed Reactor

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Rapid alkylations of thiols are performed in a packed-bed flow reactor where potassium carbonate acts as a heterogeneous base in anhydrous solvents at ambient temperature. The reaction also has a high efficiency as the removal of the solvent is the only work up required to isolate the product. The products can be used in a subsequent oxidation which was performed sequentially in semibatch mode. The alkylations of phenol and benzyl amine have been demonstrated on an array of bases, but higher temperatures and longer reaction times are required than with thiols.

1. Introduction

Electrophilic alkylation of thiols is usually performed using alkyl halides with a base. It has been demonstrated that thiols will react directly with alkyl halides in the absence of solvent and catalysts; however, high temperatures and long reaction times were necessary [1] (Figure 1).

The reaction rates can be improved with catalysts, especially in combination with flow chemistry. Very efficient protocols use phase transfer catalysts such as tetrabutylammonium bromide to shorten reaction times [2]. The use of a copper catalyst with microwaves provides a rapid route at elevated temperatures for 120 °C using aqueous potassium *tert*-butoxide as a base [3]. These processes still require high temperatures even when performed in microreactors with superior thermal transfer properties than batch reactors. Saxena and coworkers utilized nickel nanoparticles for the catalytic oxidative coupling of a thiol with an alcohol in a batch reaction performed at ambient temperature [4]. Further optimization revealed acetonitrile to be an optimal solvent for that reaction. Alkylation of thiophenol has also been demonstrated with readily available and cheap bases such as potassium carbonate. However, additional triethylamine was required, and the resultant mixture required chromatography [5].

Silica gel has also been used as a heterogeneous catalyst for the alkylation of thiols. The surface facilitated the reaction to proceed at room temperature but generated a disulfide side product that still required chromatographic purification [6]. The application of a solid supported base, such as morpholinomethyl-polystyrene (PS-NMM, £2.90/mmol) has been described for the alkylation of thiols in the context of the synthesis of thionicotinamides inhibitors [7]. A heterogeneous inorganic base in a packed-bed flow reactor would be a cheaper and more accessible alternative to solid supported bases that have been used in flow mode [8].

2. Results and Discussion

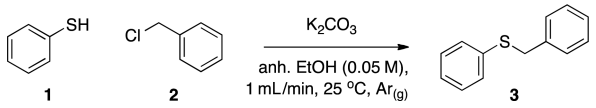
Our approach was to use readily available materials in flow mode. The use of a microreactor would also create a high rate of heat and mass transfer. We investigated the use of a dry organic solvent flown through a packed-bed reactor of an inexpensive inorganic base.

A glass Omnifit column [9] was loaded with the maximum quantity of potassium carbonate (2.5 g) and used as the packed-bed reactor that can withstand higher pressures generated by solvents heated above their boiling points (Figure 1).

Potassium carbonate (£0.007/mmol) does not dissolve in organic solvents and allows an application as a heterogeneous base in flow using anhydrous reaction conditions. This process has an exceedingly high atom efficiency, which follows the first of the principle of green chemistry, as only the solvent has to be removed to isolate the product [10].

In initial investigations, thiophenol **1** and benzylchloride **2** were used as a model reaction. When dry ethanol was used as the solvent, thioether **3** was obtained in good yield (80%) (Scheme 1).

Scheme 1. Benzylation of thiophenol as model reaction



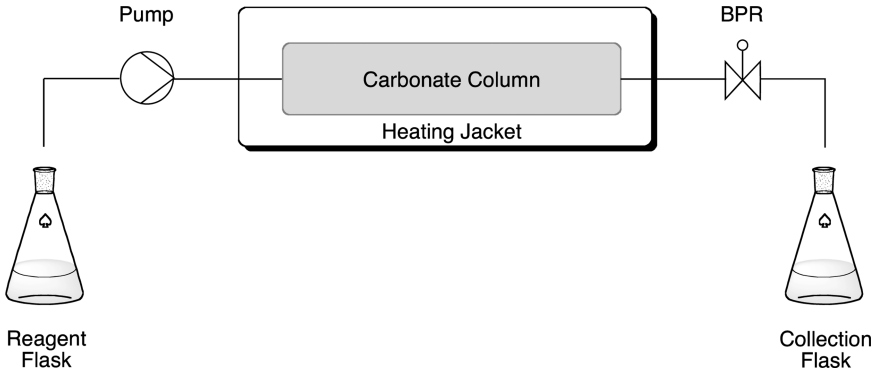


Figure 1. Reaction set up. BPR: back pressure regulator

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Other dry solvents were also investigated as shown in Table 1 (entries 2–4). We discovered that acetonitrile is an ideal solvent for this reaction, which is in accordance with results from Saxena and coworkers [4]. The solubility of potassium carbonate and potassium chloride is negligible in acetonitrile below 100 °C [11]. An aqueous workup was performed to evaluate if any salt was in the product, but there was no discernible difference in isolated yields indicating no salts were eluted (Table 1).

Table 1. Solvents investigated in the model reaction. Thiophenol and benzyl bromide were dissolved in dry acetonitrile (0.05 M) and flowed at 1 mL/min through an Omnifit column packed with 18.1 equivalents K_2CO_3 at 25 °C

Entry	Solvent	**3** Yield (%)
1	EtOH	80
2	Et_2O	–
3	THF	–
4	MeCN	96
5	MeCN	67[a]

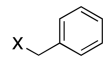
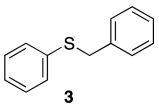
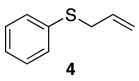
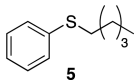
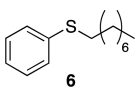
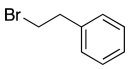
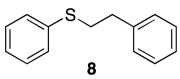
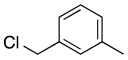
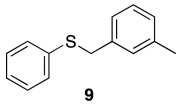
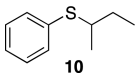
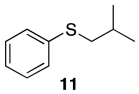
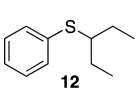
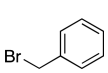
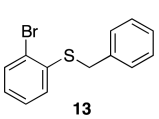
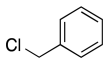
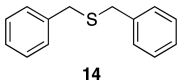
[a] Concentration of **1** and **2**: 0.5 M.

Increasing the concentration led to a reduced yield of **3** (Table 1, entry 5) indicating that the K_2CO_3 is consumed in the reaction. The reagent stream was flowed continuously; after 1 h, unreacted starting material is observed on TLC in 100% hexane. The column is depleted after 5 h, and product formation cannot be observed.

The amount of potassium carbonate is then the limiting factor; for higher concentrations, a larger packed-bed reactor would be required. Na_2CO_3 as a base achieved only 16% yield, resulting from the polarising ability compared to K_2CO_3, which has been reported in literature [12].

Most substrates reacted with good yields at room temperature. Short chain alkyl halides (Table 2, entries 8–10) required

Table 2. Dry acetonitrile (0.05 M) containing thiophenol and alkyl halide was flowed at 1 mL/min through an Omnifit column packed with 18.1 equivalents K_2CO_3 at 25 °C without back pressure regulator

Entry	Alkyl halide	Product	Temperature (°C)	Back pressure (bar)	Yield[a] (%)
1		 **3**	25	–	X = Cl 96 X = Br 99
2		 **4**	25	–	99
3		 **5**	25	–	84
4		 **6**	25	–	93
5	MeI	 **7**	25	–	83
6		 **8**	25	–	75[a]
7		 **9**	25	–	99
8		 **10**	100 (25)	3.5 (−)	89 (20)
9		 **11**	100 (25)	4.0 (−)	86 (n.r.)
10		 **12**	100 (25)	1.7 (−)	85 (n.r.)
11		 **13**	25	–	98
12		 **14**	100 (25)	1.5 (−)	60[b] (30)

[a] Values in parenthesis are yields at 25 °C.
[b] Column chromatography performed.

heating to 100 °C which was accompanied by attaching the Vapourtec back pressure regulator.

Some other thiols substrates were investigated as well (Table 2, entries 11 and 12). 2-Bromothiophenol reacted at room temperature to form **13** in almost quantitative yield, whereas benzyl thiol required heating and also further purification to remove impurities. The use of allyl thiol to produce garlic metabolites such as allyl methyl sulfide was successful and the product identified by NMR, but could not be purified due to its volatility.

We investigated also the alkylation of other nucleophiles under basic reaction conditions. *N*-Alkylations of amines were attempted in the flow system, and it was found that higher temperatures were required. Increasing the residence time also improved the reaction. Prolonged exposure to potassium carbonate explains the increase in yield when the flow rate is reduced. The reaction shown in Scheme 2 was also performed using several other bases; sodium carbonate, caesium carbonate, magnesium oxide, and calcium carbonate. The reaction did not proceed as readily, and the results are reported in the supporting information. Attempts to improve the yield using DMF and acetone were unsuccessful (Scheme 2).

Secondary amines should proceed more readily because the pK_a is lowered with each successive alkylation of the amine. We evaluated the effect of performing the alkylation using two equivalents of **2**, which produced a crude mixture that contained mainly **16**, but the NMR revealed only traces of tribenzyl amine. We also attempted to produce the tertiary amine **17** by reacting **2** with *N*-benzylmethyl amine. Although the secondary amine has a lower pK_a, the reaction did not proceed. This method could be very useful for selectively mono-alkylating primary amines.

The alkylation of phenol has only shown low levels of reactivity and maybe impeded by the size of the reactor and other limits of the equipment. Increasing the temperature and reaction time improves the yield. The lowest flow rate that can be achieved on the Vapourtec E-series is 0.1 mL/min. At a temperature of 150 °C, the reaction had to be abandoned as the caesium carbonate begins to turn brown and backpressure spikes to the machine limit (10 bar). Compared to thiols, phenol did not react as readily using potassium carbonate. We have shown that this reaction does proceed when using sodium carbonate, caesium carbonate, magnesium oxide, and calcium carbonate. The largest rate of reaction was achieved with caesium carbonate leading to ether **19** in 82% yield as shown in Scheme 3.

The reaction with aniline and benzyl alcohol was also investigated using the packed-bed reactor loaded with different bases. Neither showed any reactivity on any of the bases and was eluted from the system unreacted.

To demonstrate that the reaction mixture from the primary reaction is clean enough to perform a second reaction without work up, a subsequent oxidation was performed. The approach taken was to have a semibatch reaction, in which the reaction mixture from the flow reactor was added to one equivalent of hydrogen peroxide. The oxidizing mixture was stirred overnight, and the sulfoxide **20** was isolated in 93% yield (Scheme 4). Under these reaction conditions, no over-oxidation to the sulfone was observed.

This two-step reaction sequence was also attempted in a continuous-flow synthesis. With the available reactor coil (10 mL), we achieved a 25% conversion to the desired sulfoxide **20**. The maximum residence time possible for the oxidation was 5 minutes, due to the incoming flow rate of 1.0 mL/min of both reaction streams from the packed-bed reactor and the oxidant-containing solution. Longer reaction times were required, hence why we utilized a semibatch approach to obtain a high yield (93%).

3. Conclusions

The use of heterogeneous potassium carbonate packed-bed flow reactor has been demonstrated in flow mode for the alkylation of thiols using primary alkyl halides at ambient temperatures. This procedure only needs evaporation to isolate the product and has eliminated the aqueous work up required in previous literature procedures. The reaction is clean enough so that a subsequent reaction could be performed. Amines and phenol have shown some reactivity in this system with an array of bases. However, higher temperatures and longer residence times are required when using amines and phenols.

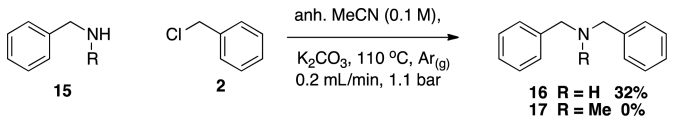
4. Method

4.1. General Method for the Flow Synthesis of Sulfides. All the glassware was oven dried at 120 °C prior to use. Anhydrous solvents were obtained from a MBRAUN SPS-800 solvent purification system.

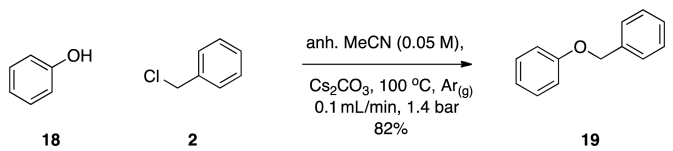
Anhydrous potassium carbonate (2.5 g, 18.1 mmol) is loaded in a dry Omnifit column and attached to a Vapourtec E-series machine.

A solvent flask was prepared, charged with 4 Å MS (3.5 g) under argon, into which acetonitrile (100 mL) was added. The flask was

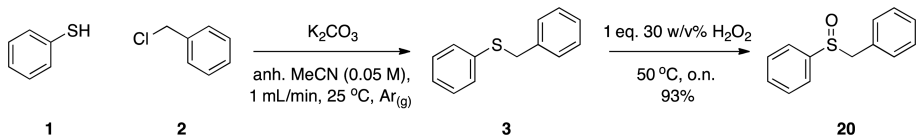
Scheme 2. *N*-Alkylation of benzyl amines



Scheme 3. *O*-Alkylation of phenol



Scheme 4. Subsequent oxidation in batch directly after the flow alkylation



attached to Vapourtec V3 pump. The column was heated to 25 °C and primed with 1 mL/min dry acetonitrile (5 mL) for 5 min.

A reagent flask was prepared, charged with 4 Å MS (3.5 g) under argon, and into which, acetonitrile (20 mL, 0.05 M), thiol (1.0 mmol), and alkyl halide (1.0 mmol) were added. The flask was attached to Vapourtec V3 pump. Then the column was additionally primed with 1 mL/min from the reagent flask for 5 min to achieve a steady state, and the eluted product was discarded. Then the product was collected over a further 5 min. The solvent was removed under vacuum to produce the desired sulfide.

4.2. Semibatch Synthesis of Benzylphenylsulfoxide. The above process was performed but collected into a flask, and 30 w/v% H_2O_2 (1 mL, 1 mmol) was added. The mixture was stirred at 50 °C overnight. The mixture was quenched with saturated $Na_2S_2O_3$ solution (5 mL) and then extracted with dichloromethane (3×20 mL) and dried over $MgSO_4$. Column chromatography was performed using a Biotage Isolera with the following method. The crude mixture (110 mg) was loaded onto a TELOS 12 g Silica Flash Column (15 mL column volume). The gradient was performed; 100% hexane for 1 column volume (CV), then increased to 60:40 hexane–ethyl acetate over 40 CV, then to 100% ethyl acetate over 13 CV, and held at 100% ethyl acetate for 5 CV. The solvent from the appropriate fractions was removed and dried on a high vacuum to produce the title compound as a colorless solid (102 mg, 93%).

Supporting Information

Electronic Supplementary Material (ESM) is available in the online version at doi: 10.1556/1846.2015.00009.

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