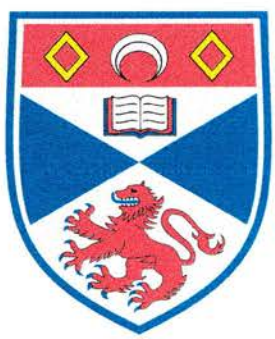
Development of Methods for the Isotopic Labelling of Glucosinolates

A thesis presented for the degree of
Doctor of Philosophy
to the
University of St Andrews
in September 2004



By

David N. Milne

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Acknowledgments

During my studies, I have been helped by a great many people. My thanks must firstly go to Dr. Nigel Botting who has been an excellent supervisor. His door has always been open to me and I thank him for all his help, enthusiasm and optimism. I must also thank all the members of the group, past and present, as well as my other colleagues and members of staff. They have provided me with an excellent environment in which to work, and have always had time to help me whenever I have needed it.

There are many technical staff who have helped me a great deal: Mrs Melanja Smith and Dr Tomas Lebl (NMR), Mrs Caroline Horsburgh, Dr Catherine Botting and Dr Robin Antrobus (MS), Mrs Sylvia Williamson (CHN), Mr Jim Bews (Computing), Mrs Marjory Parker and Mr Andrew Watson (Purchasing and Store) and all those in the workshops who have mended the things I've broken.

I would like to thank Kate for all her help, during my time in the lab, but especially when writing my thesis. Her support, both in and out of work, has helped me more than she will know.

Finally, I would like to thank my parents and sister. They have helped me in so many ways and always given me the support and encouragement I have needed. Without their help I would never have come so far.

Abbreviations

Ac	Acetyl
APCI	Atmospheric pressure chemical ionisation
CI	Chemical ionisation
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMF.DMA	*N,N*-Dimethylformamide dimethyl acetal
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EI	Electron impact
GC	Gas chromatography
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionisation time-of-flight
MS	Mass spectrometry
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NMR	Nuclear magnetic resonance
PAPS	3-Phosphoadenosine 5'-phosphosulfate
QR	Quinone reductase
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UDPG	Uridine-5'-diphosphate glucose

Contents

Abstract

Synthetic routes suitable for the preparation of isotopically labelled glucosinolates have been investigated. Firstly, a synthetic route was devised for glucobrassicin starting from indole-3-acetic acid, which can be purchased with ^{13}C and ^{14}C isotopic labels. The synthesis was taken though to the acetylated desulfoglucosinolate in 5 steps and 10% yield overall.

A formal synthesis was also developed for ^{13}C-labelled glucoraphanin, whereby the side chain was constructed using diethyl malonate and cyanide ion as precursors, both of which are readily available in isotopically labelled form. The starting material was β-bromostyrene which was first converted to the nitrile using a nickel catalysed procedure. Further steps gave cinnamyl bromide which was then coupled with diethyl malonate to give the desired 5-carbon chain. Functional group transformation allowed the incorporation of the terminal thiomethyl unit. The key step was then oxidative cleavage of the alkene to provide 5-thiomethylpentanal, required for the glucoraphanin side-chain, via a two step procedure.

Several thiomethyl alkyl aldoximes have been prepared as biosynthetic precursors to glucosinolates. These were synthesised for Prof. Clint Chapple, Purdue University, USA, to be used in biological testing to probe the biosynthesis of glucosinolates. 5-Thiomethylpentaldoxime was prepared from ethyl 5-bromovalerate, 8-thiomethyloctaldoxime from 8-bromooctanoic acid and 9-thiomethylnonaldoxime from 9-bromononanol.

Introduction

1.1 History

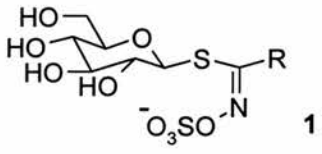
Although interest in glucosinolates has increased greatly in the past couple of decades, in actual fact they have been investigated for many years. In attempting to understand the sharp taste of mustard seeds, the first observations on glucosinolates (sometimes termed the mustard oils) were recorded as far back as the seventeenth century.[1] In 1831, the first crystalline glucosinolate, sinalbin, was isolated from white mustard seeds,[2] while sinigrin was also isolated in the 1830s, from black mustard seeds.[1]

The first general structure of glucosinolates was proposed by Gadamer[3] in 1897 and was assumed to be correct until 1956 when Ettlinger and Lundeen proposed the correct structure.[4] A review of the discovery and early history of glucosinolates and myrosinase was published by Challenger in 1959.[5] Since then, a great number of papers have been published on many different aspects of glucosinolates. Over one hundred and twenty glucosinolates have been characterised,[6,7] and this number continues to rise with the recent discovery of novel glucosinolates such as L-prolinium 4-(methylsulfinyl)butyl glucosinolate, isolated from *Cardaria draba*,[8] and others isolated from woad (*Isatis tinctoria* L.). New sources of glucosinolates are also being discovered, such as Maca (*Lepidium meyenii*), a tuber high in minerals.[9]

1.2 Background

Glucosinolates are an important class of sulfur containing glycoside. They are a structurally homogenous family, but a very diverse class of compound. Their structure consists of a β-D-glucose unit linked through sulfur to a sulfated oxime moiety, with a side-chain R **1**. The main areas of research have focussed on their presence in *Brassica*

vegetables, such as broccoli and Brussels sprouts. *Brassica* vegetables are a member of the *Brassicaceae* family, which has three hundred and fifty genera and three thousand species and is only one of sixteen families of higher plants that contain glucosinolates.



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Individual plants, however, don't often contain a great variety of glucosinolates, normally around four,[10] although *Arabidopsis Thaliana* L. contains over thirty.[11] It is also interesting to note that only a few structural moieties predominate, with straight and branched carbon chain glucosinolates being the most prevalent.[1] They can be divided into three principal groups - the aliphatic group **2**, the indolic group **3** and the aromatic group **4**. (Figure 1) Glucosinolates are biosynthesised from amino acids and this becomes evident when considering the side chain structure.

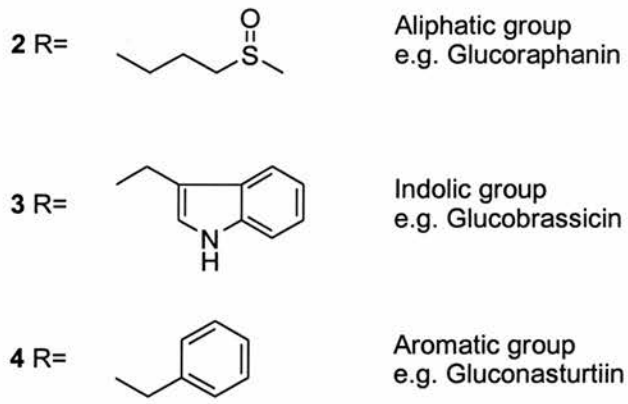
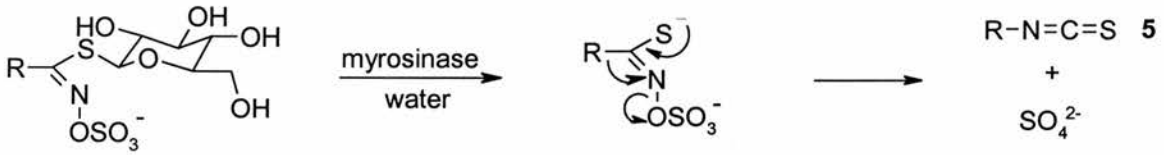


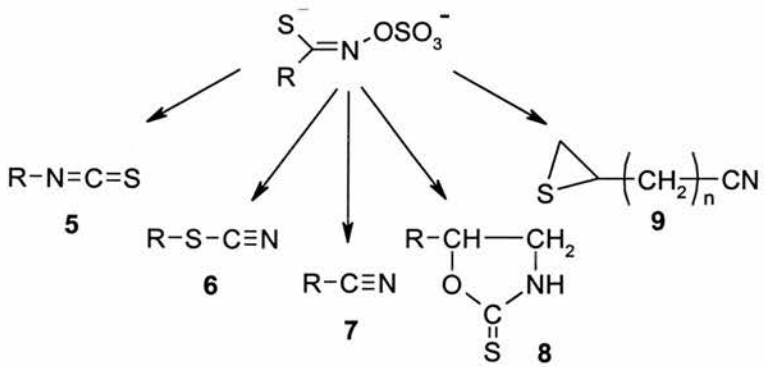
Figure 1: Glucosinolate side-chains can be divided into three principle groups

Most interest in glucosinolates has centred on the crucifers, since they have such a major contribution to the human and also animal diet. No cruciferous plant has been found that

cannot synthesise a glucosinolate.[1] Research has not just focussed on the glucosinolates themselves. The breakdown products are also of interest. Members of the *Brassicaceae* contain an enzyme called myrosinase, a β-thioglucoside glucohydrolase, which is the only enzyme class recognised as being capable of catalysing the hydrolysis of glucosinolates.[12] When this occurs, myrosinase catalyses the hydrolytic cleavage of the glucosyl group at the carbon-sulfur bond to give a thiohydroxamic acid. This molecule is unstable and undergoes a Lössen type rearrangement to yield equimolar quantities of glucose, sulfate and an isothiocyanate. (Scheme 1) Although breakdown gives predominantly isothiocyanates **5**, depending on conditions, thiocyanates **6**, nitriles **7**, oxazolidine-2-thiones **8** and epithionitriles **9** can also be formed. (Scheme 2)



Scheme 1: Glucosinolate hydrolysis by myrosinase

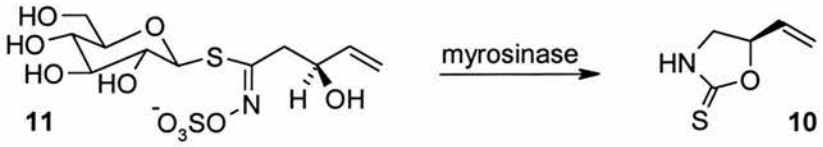


Scheme 2: Thiohydroxamic acid breakdown to give a variety of products

Isothiocyanates, often the major product of hydrolysis, are usually produced at neutral pH, while nitriles are often produced at more acidic pH and/or where a reducing agent, such as

cysteine or ferrous ions, is present.[13] Isothiocyanates with 2-hydroxy functionality spontaneously cyclise to give oxazolidine-2-thiones **8**.[14] Thiocyanates **6** seem to be produced by the hydrolysis of only three glucosinolates:[14] allyl-, benzyl- and 4-(methylthio)-butyl glucosinolates. The mechanism of this transformation in unknown, but a theory put forward by Hasapis and MacLeod[15] suggests that an isomerase enzyme causes the stereochemistry of the aglycone to change from *Z* to *E* and that only glucosinolates with side chains that give stable cations are able to undergo rearrangement to give the thiocyanate. Epithionitriles **9** can also be formed from alkenyl glucosinolates when a protein, known as epithiospecifier protein, is present during hydrolysis. Epithiospecifier protein, a small protein of 30-40 kDa, does not have myrosinase activity, but interacts with myrosinase to assist the sulfur transfer from the S-glucose moiety to the terminal carbon of the double bond of an alkene group.[14] The effects of epithiospecifier protein are apparent during the degradation of progoitrin. In the presence of epithiospecifier protein, an epithionitrile is the main product, whereas the reaction in its absence results in the formation of oxazolidine-2-thiones.[14] It has also been shown that ferrous ions are vital for the formation of epithionitriles. In *B. napus*, epithiospecifier protein was shown to be inactive in the absence of ferrous ions.[16]

The properties of myrosinase can be exploited to artificially produce some glucosinolate breakdown products, as it can often be difficult to obtain these by normal synthetic methods. One such case is the production of *epi*-goitrin **10** from *epi*-progoitrin **11**, which can be extracted from *Crambe abyssinica*. By using immobilised myrosinase, a high yielding chemo- and stereo-controlled reaction gave the enantiomerically pure *epi*-goitrin, which may be useful as a synthetic starting material.[13] (Scheme 3)



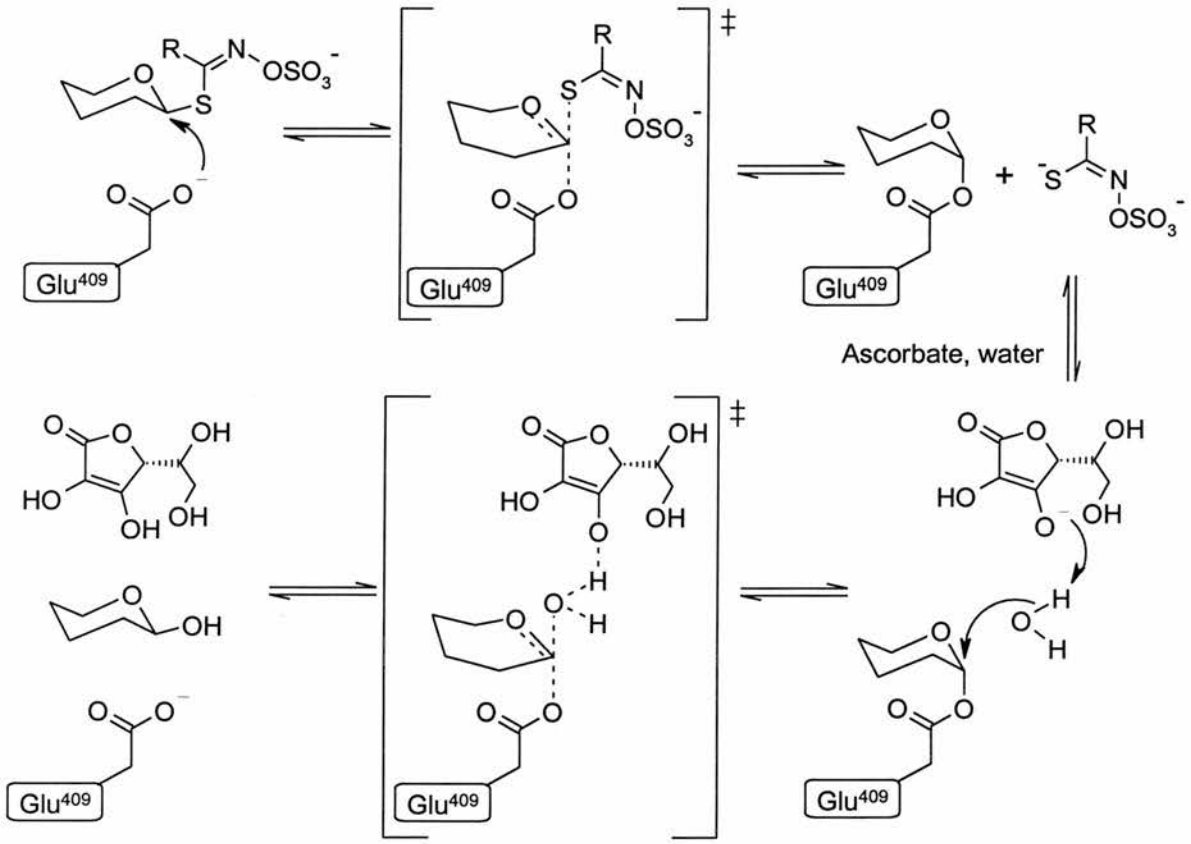
11 **10**

Scheme 3: Hydrolysis of epi-*progoitrin to give* epi-*goitrin*

Although the term myrosinase has been used to indicate the enzyme able to catalyse the hydrolysis of glucosinolates, it actually covers a whole family of enzymes. Myrosinases occur in plant families, fungi, intestinal bacteria and cruciferous aphids and always appear to be associated with at least one glucosinolate.[14] In many plants, more than one myrosinase enzyme has been isolated and purified, such as in *Brassica juncea*[17] which showed two myrosinase isoenzymes and in *Sinapis alba*, where two myrosinases were isolated,[18] although crude extracts have since been shown to contain at least 14 myrosinase isoenzymes.[14] The glucosinolate-myrosinase system is therefore considerably more complicated than it first appears and the function of the system may be wide-ranging. Other than a role in the defence of the plant, glucosinolates may act as a sink for nutrients such as nitrogen, sulfur and glucose.[14]

Ascorbic acid has been shown to alter myrosinase activity in some plant species.[14,19] It was found that the addition of 1 mM ascorbic acid increased the enzymatic activity of *Brassica juncea* myrosinase for sinigrin hydrolysis by 25-fold.[19,20] Until recently, it was thought that ascorbic acid activated the enzyme by introducing a change in conformation of the protein structure. However, with the publication of the crystal structure of a myrosinase isolated from *Sinapis alba*[21] and subsequent research, this was proved to be incorrect. Ascorbic acid was found to be a cofactor for myrosinase and to function as a catalytic base.[22] It has been shown that the glucosinolate binds to the active site, but that the enzyme complex is unstable. The bound glucosinolate is cleaved by the nucleophile $Glu^{\underline{409}}$ to form the

glucosyl enzyme and the aglycone. When the glucosyl enzyme is formed, a water molecule is positioned above the C-1 position by hydrogen bonding to Gln^{187}, promoting the final step of the reaction to give glucose. The hydrogen bonding effect of the Gln^{187} is not optimal, however. The binding of an ascorbate molecule alters the position of the water such that it interacts with the ascorbate O-3 and not Gln^{187}. Ascorbate binds to the active site and, acting as a base, partially abstracts a proton from a water molecule, enhancing the nucleophilicity of the water for attack at the anomeric centre. Glucose and ascorbate are then released from the active site.[22] (Scheme 4)



Scheme 4: Effect of ascorbic acid on the hydrolysis of glucosinolates

Although it is understood how myrosinase and ascorbic acid act upon glucosinolates, it is unclear exactly how these different components are located within the plant. If myrosinase and glucosinolates were located together, instant hydrolysis would occur, so some special

system is required to prevent the contact of the components until the hydrolysis becomes necessary. Matile[23] suggested that the stability of glucosinolates was due to the location of glucosinolates and myrosinase in distinct subcellular compartments of the same cell. Evidence suggested that myrosinase was present in extracellular compartments and associated with the cytoplasmic side of internal membranes while the glucosinolates were located in vacuoles. Further work has also suggested that both glucosinolates and ascorbic acid are located in the vacuoles of cells,[23,24,25] with 99.5% of the ascorbic acid shown to be located in the vacuoles.[26] This compartmentalised system was termed the 'mustard-oil bomb'.[27] (Figure 2)

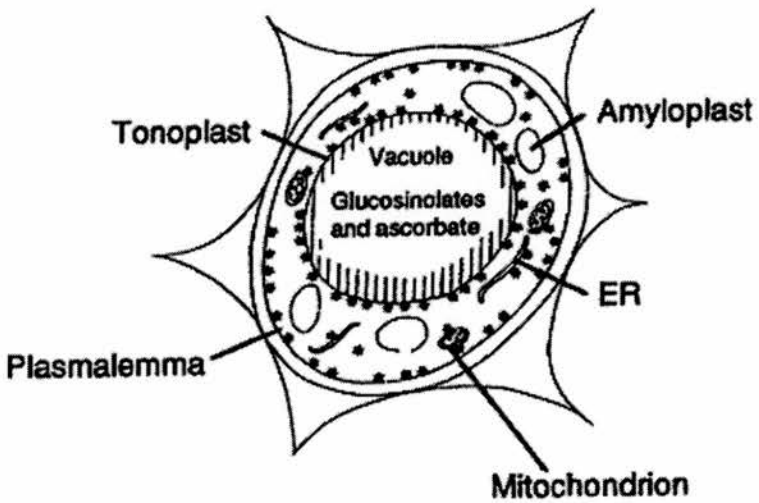


Figure 2: The 'Mustard-oil Bomb'
* indicate suggested location of myrosinase,
but myrosinase has since been shown to be localised in myrosin cells
Taken from Bones and Rossiter 1996[14]

Since then, however, myrosinase has been shown to be located in the vacuoles of myrosin cells,[28,29] a special type of cell first discovered by Heinricher[30] in 1884. The name 'myrosin cell' was ascribed by Guignard[31] in 1890, but it has taken over a century to prove this theory correct. The locations of other proteins associated with myrosinase are still

unknown, so the cellular organisation in the glucosinolate-myrosinase system is still unclear.

1.3 Biosynthesis

Elucidating the biochemical mechanism for the production of glucosinolates has proved to be, in some areas, both challenging and contentious. Of the 120 or so glucosinolates identified,[32] only seven have side chains that correspond directly to a protein amino acid. These are the glucosinolates derived directly from alanine **12**, valine **13**, leucine **14**, isoleucine **15**, phenylalanine **16**, tyrosine **17** and tryptophan **18**. (Figure 3)

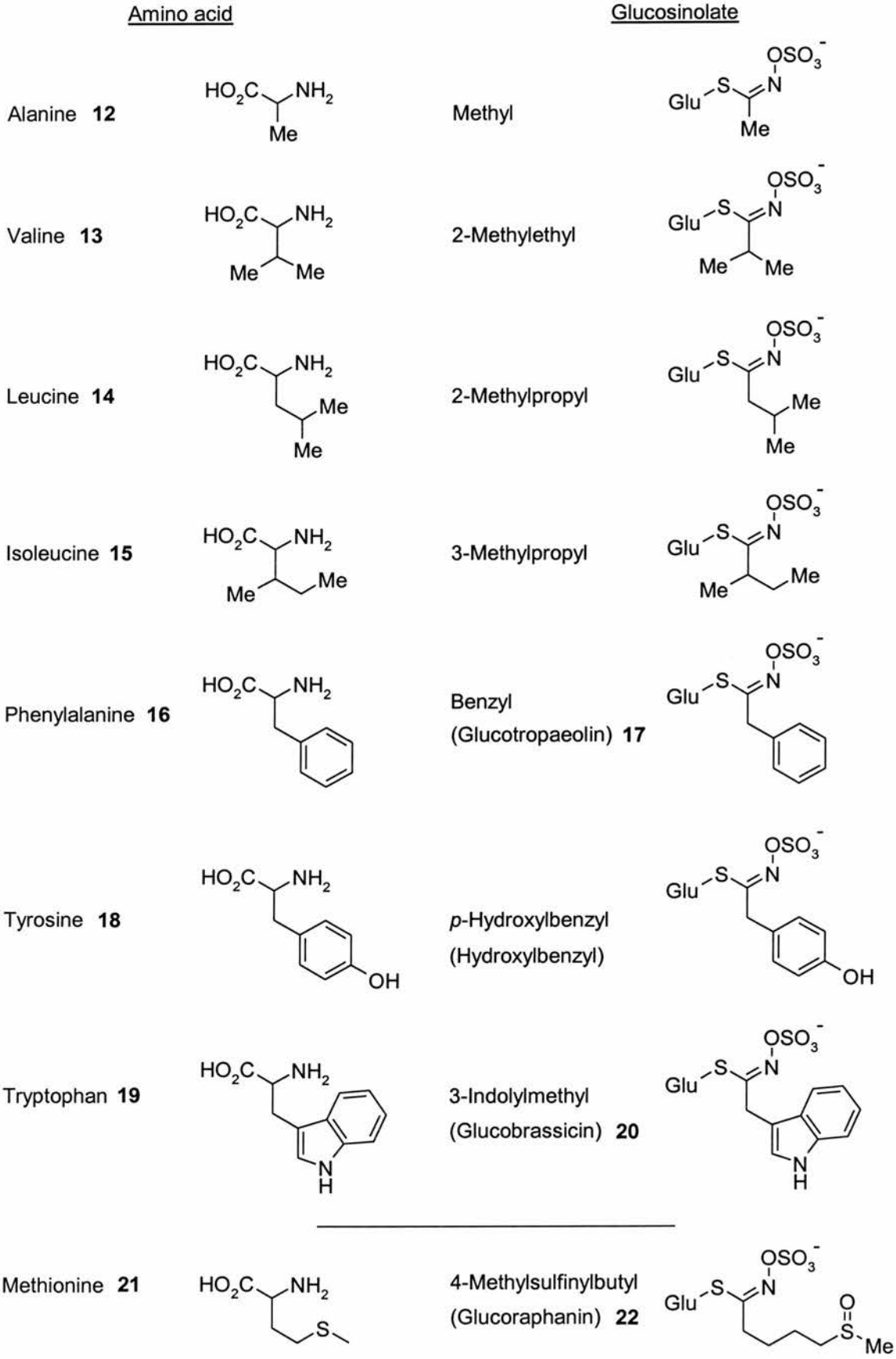
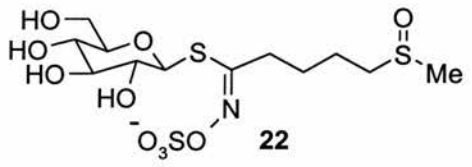


Figure 3: Amino acids with their directly corresponding glucosinolates and methionine, from which a great many glucosinolates are derived

The remaining glucosinolates can be split into three families:

1. A large number of glucosinolates are derived from chain extended forms of amino acids, particularly methionine **21**, but also phenylalanine **16** and branched-chain amino acids.[33]
2. The side chain of the amino acid may also be modified by a variety of methods, such as oxidation, hydroxylation, methoxylation, desaturation or sulfation,[34] e.g. glucoraphanin **22** and progoitrin **23**.
3. Some glucosinolates also have complicated side-chains that have been glycosylated such as *o*-(α-L-rhanmnopyranosyloxy)benzyl glucosinolate **24**.[33]



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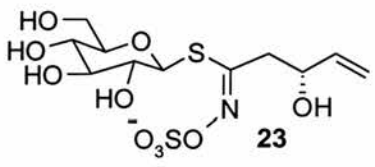

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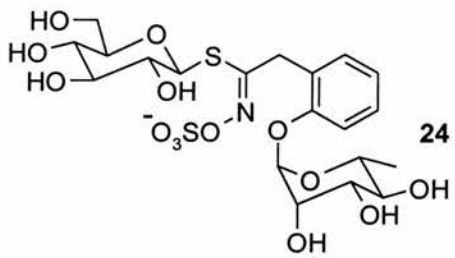

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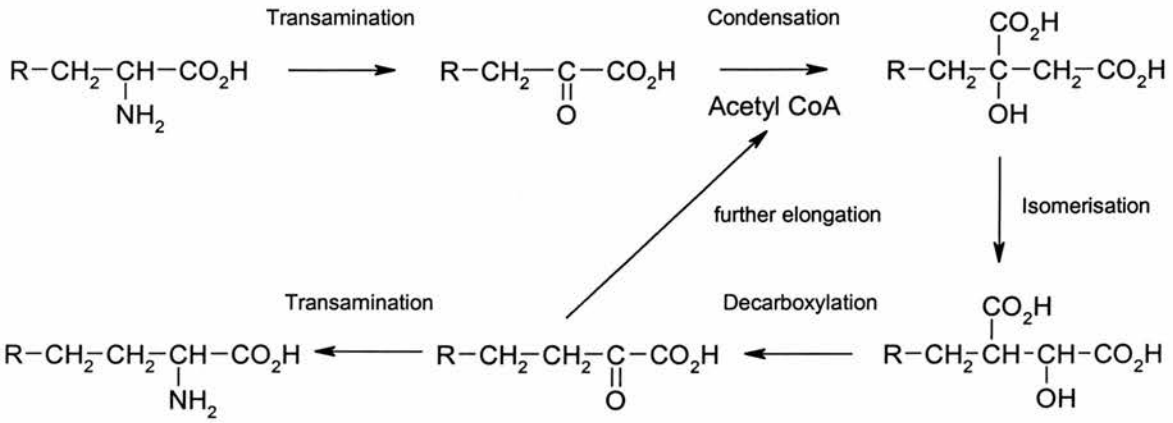
Of all the different glucosinolates, approximately 50% are derived from chain extended methionine, 10% from tryptophan (the indolyl glucosinolates), 10% from phenylalanine and/or tyrosine (the aromatic glucosinolates) and 10% from elongated phenylalanine. The rest are probably derived from branch-chain amino acids, alanine or methionine.[33] Repeated cycles of elongation and modification can result in homologous series of glucosinolates, such as when the R-group is $CH_3SO(CH_2)_3$ through to $CH_3SO(CH_2)_{11}$.

The evolution of the biosynthetic pathway is one of the contentious areas of research. It has been speculated by a large number of workers that glucosinolate biosynthesis has an evolutionary relationship with that of the cyanogenic glucosides.[33] Both these classes of secondary metabolites can be derived from phenylalanine and tyrosine and have oximes as biosynthetic intermediates. Cyanogenic glucosides occur widely in flowering and non-flowering plants, whereas glucosinolates are far less prevalent, occurring in only a small number of plant families. Glucosinolates and cyanogenic glucosides were actually thought to be mutually exclusive until benzylglucosinolate and cyanogenic glucosides were both found in *Carica Papaya*.[35,36] It has been suggested that if an oxime had been diverted from cyanogenic glucoside biosynthesis by conjugation with cysteine, the highly reactive thiohydroximate product could be conjugated with sulfate and then glucose to detoxify the compound, resulting in a glucosinolate.[33] This, however, has only been speculated and no direct biochemical evidence is currently available to support this theory.[34]

The biosynthesis of glucosinolates can be divided into three distinct parts. These are chain elongation of the precursor amino acids, synthesis of the glucone moiety and side-chain modification.[37]

The chain elongation of the amino acids is thought to proceed by a mechanism similar to the biosynthesis of leucine from acetate and valine.[32,38] Although some glucosinolates are formed from chain-extended versions of phenylalanine and valine, as previously stated, the majority of glucosinolates are synthesised from extended forms of methionine **21**, which can have between one and nine additional methylene units.[33] Most species, however, only have certain ranges of chain length and *Brassica* crops usually only have short chain lengths, with between one and four extra methylene units.

In the proposed pathway, a 2-oxo-acid is produced by deamination of the precursor amino acid and this condenses with acetyl CoA to form a malate derivative.[39] Isomerisation then takes place, shifting a hydroxyl group one carbon along the chain, followed by oxidative decarboxylation to give an elongated keto acid which is finally transaminated to form the extended amino acid. (Scheme 5)



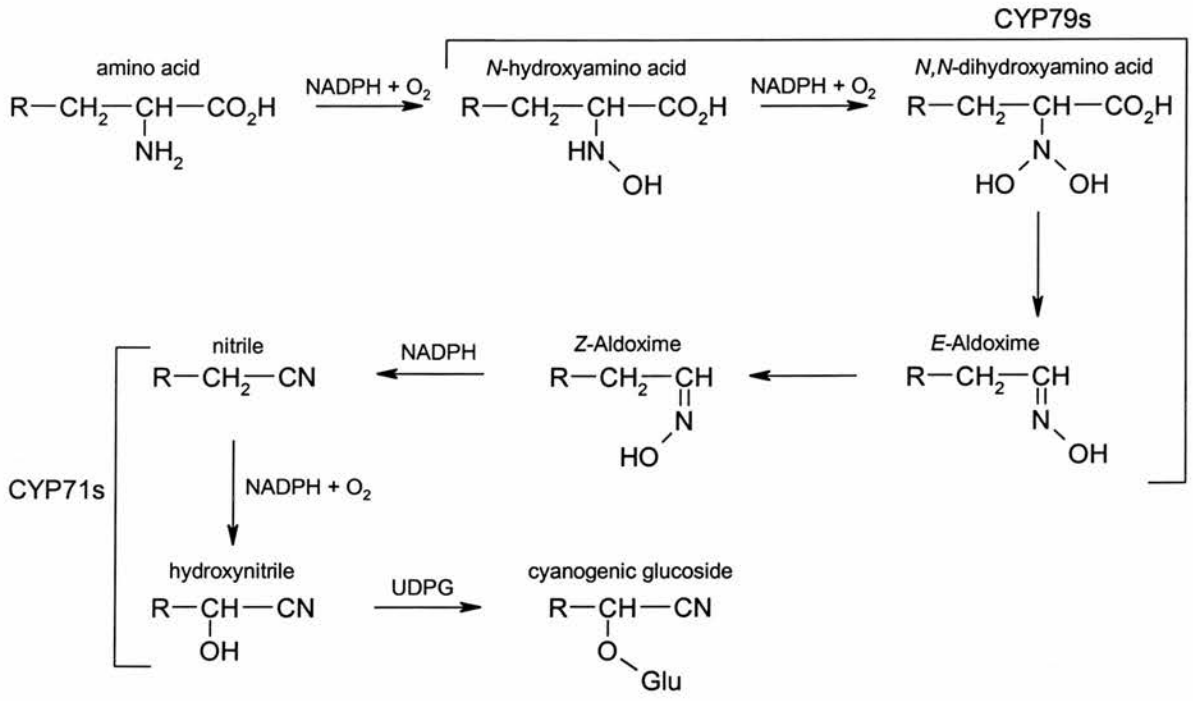
Scheme 5: Proposed biosynthetic pathway for amino acid chain extension

It has been postulated that the elongated keto acid can undergo further condensations with acetyl CoA to give the multiple chain extended derivatives observed.[33] Morse *et al.*[40] have shown that chain elongation can have a considerable effect on the insect resistance and anti-cancer activity of glucosinolates. It has also been shown by Graser *et al.*[41] that the transamination and keto acid elongation reactions are likely to occur in the same subcellular compartment, since in a study using [^{15}N]methionine, they found that two thirds of the labelled amino group removed in the deamination step was reincorporated into the elongated amino acid.

Following any chain extension, the first common step in the biosynthetic pathway is the conversion of the amino acid to an aldoxime.[32] The mechanism by which molecules are

carried into the biosynthesis of the core glucosinolate structure, rather than undergoing a further chain elongation cycle is not known.[32] Another unknown is the process by which the amino acids are converted to their corresponding aldoximes and this has been the source of much controversy.

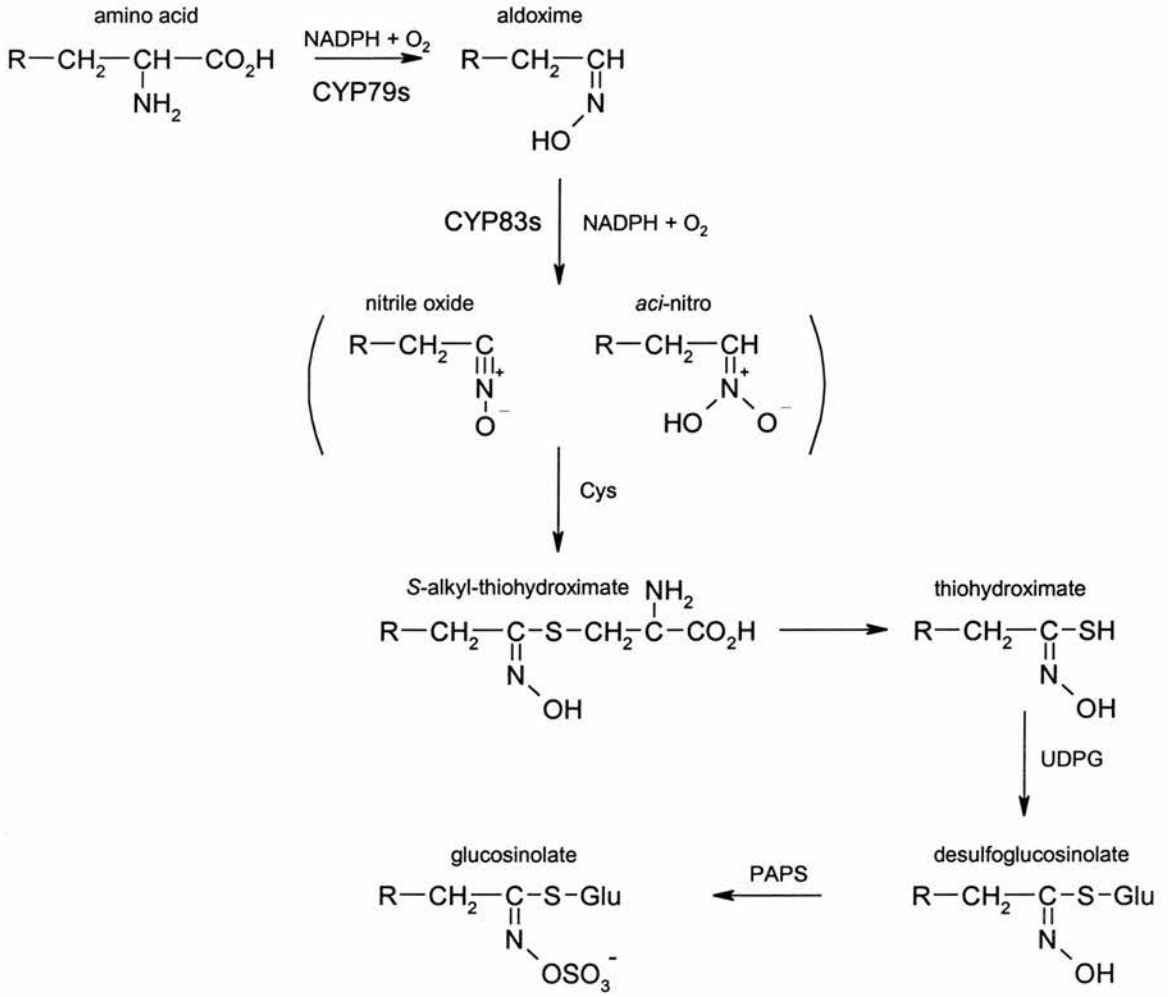
As mentioned earlier, it has been speculated that glucosinolates have an evolutionary relationship with the cyanogenic glucosides.[33] The biosynthetic pathway for the cyanogenic glycosides has been elucidated and also involves the conversion of an amino acid to an aldoxime. (Scheme 6) In this case, the conversion is catalysed by a cytochrome P450, of the CYP79 family,[32] which catalyses two consecutive NADPH and O_2 dependent *N*-hydroxylations. In the biosynthesis of the cyanogenic glucoside dhurrin, found in sorghum, the cytochrome P450 enzyme CYP79A1 was shown to catalyse the conversion of tyrosine to *p*-hydroxyphenylacetaldoxime.[42] The *N,N*-dihydroxyamino acid resulting from the two *N*-hydroxylations then dehydrates and decarboxylates to give the corresponding aldoxime.[34]



Scheme 6: Biosynthesis of cyanogenic glucosides

It is generally believed that cytochrome P450 enzymes also catalyse the conversion of amino acids to aldoximes in glucosinolate biosynthesis[32,33,38] although this has not yet been proven and conflicting data have also suggested other possible means of oxidation. Chen and Andreasson[34] state that three enzyme systems have been shown to catalyse the conversion of amino acids to oximes, namely flavin-containing monooxygenases and plasma membrane peroxidases as well as cytochrome P450. When Bennett *et al.*[43] investigated the conversion of amino acids to aldoximes in oilseed rape, they found that the enzymes had no characteristics of cytochrome P450s, and later suggested that it was flavoprotein monooxygenases that catalyse the conversion,[44] although this paper has not been followed up with further work. It is possible that all three suggested enzyme systems convert amino acids to oximes in glucosinolate biosynthesis, but that some are more utilised than others depending on the glucosinolate structure and the plant involved.

The conversion of the aldoxime to the thiohydroximate is not well understood, as the intermediates between the two species have not been identified.[33] In 1968, Ettlinger and Kjaer[45] proposed that the oxime was oxidised to give an *aci*-nitro compound, though it has also been suggested that oxidation may give a nitrile oxide.[32] In *Arabidopsis Thaliana,* CPY83A1 and CYP83B1 enzymes have been shown to be able to catalyse the oxidation of various oximes,[32] so it is possible that these enzymes are responsible for this step in the biosynthesis. Subsequent reaction of the oxidised species with a thiol donor occurs to form an *S*-alkylthiohydroximate.[32] The most likely thiol donor has been shown to be cysteine,[46] but in some species, methionine has been reported to be as effective.[32] The reaction may be catalysed by glutathione-*S*-transferase enzymes, although these have not yet been characterised.[34] The *S*-alkylthiohydroximate is then hydrolysed by what is thought to be a C-S lyase[34] to give the thiohydroximate, although again no enzymes have been isolated that catalyse this step. Subsequent *S*-glucosylation that is catalysed by a soluble (uridine diphosphoglucose) UDPG: thiohydroximate glucosyl transferase will produce a desulfoglucosinolate.[33] The last step is the sulfation of the desulfoglucosinolate to give the complete glucosinolate framework, catalysed by 3'-phosphoadenosine-5'-phosphosulfate: desulfoglucosinolate sulfotransferase.[34] (Scheme 7) Evidence has shown that post-aldoxime enzymes generally have high specificity for the functional group and low specificity for the side chain, although a sulfotransferase purified from *Lepidium sativa* has been shown to have limited substrate specificity for the side-chain.[32] The efficiency of *L. sativa* was three fold higher when using desulfobenzyl glucosinolate than when using desulfoallylglucosinolate. [47] This has led to the conclusion that there may be more than one sulfotransferase enzyme present to encompass the diverse range of glucosinolates found in certain plants.[32] The same may also be true of the C-S lyase enzymes mentioned above.



Scheme 7: Proposed biosynthetic pathway of glucosinolates

It has been stated that the diversity of glucosinolate structures may be explained as an interaction of the amino acid elongation enzymes and those governing chain modifications.[33] These modifications must be made on either the desulfoglucosinolate or the complete glucosinolate, since it has been found that the presence of an unoxidised sulfur in the substrate during the formation of the core glucosinolate structure is important.[43] This implies that the substrate binds to the active site of the enzymes through the sulfur atom. If the sulfur atom had been oxidised prior to the generation of the core structure, binding to the enzyme would be ineffective and therefore the glucosinolate could not be fully synthesised. Consequently, any modification must occur at the final stage of

glucosinolate biosynthesis. The biochemistry of these modifications has not been extensively studied, with the exception of the final hydroxylation of alkenyl glucosinolates, which may be catalysed by a cytochrome P450 hydroxylase.[33]

The location of glucosinolate biosynthesis at the cellular level has not yet been established, but some indications have come from cloned genes in *Arabidopsis Thaliana*. The enzymes that are responsible for the chain elongation in the amino acid precursors (MAM synthases) have plastid targeting sequences, suggesting that elongation takes place in plastids. Also, as the CYP79 gene family sequences suggest that they are targeted to the endoplasmic reticulum, the conversion of the amino acids to the aldoxime may take place there.[33]

1.4 The role of glucosinolates in plants

Plants have to cope with a great deal of environmental stress. Weather conditions, insects and animals often make a plant's life difficult. Secondary metabolites are believed to play an important part in helping to cope with these problems. Counted amongst these compounds are glucosinolates.

The type and levels of glucosinolate within the plant can vary. The glucosinolate content is usually around 1% of the dry weight of a Brassica vegetable, but this can approach 10% in some seeds.[1] The plants themselves have an ability to regulate glucosinolate levels in response to stress. Under stress, plants often build up a store of basic metabolites, which can be converted to secondary metabolites. The changes in type and concentration also affect the degree of protection given to the plant by the breakdown products, such that

some stages of plant growth are more susceptible to attack by insects. Age can also have a very pronounced effect on the level of glucosinolates and it was found that levels of sulforaphane, a breakdown product of glucoraphanin, were one order of magnitude higher in young leaves compared to older leaves.[48] It has also been found that 3-day old sprouts of broccoli and cauliflower can contain 10 to 100 times the levels of glucoraphanin than mature plants.[49] The particular stage in the plant cycle also has a significant effect. In *B. juncea* and *B. nigra*, the concentration of sinigrin decreased from seedling to early flowering stage, increased during late flowering and then decreased again during seed maturation.[50] Temperature also has an effect on the levels of glucosinolates found in a plant. In a study evaluating the effects of temperature on the glucosinolate content of broccoli sprouts, it was found that seedlings cultivated at 30/15 °C day/night temperatures had considerably higher levels of glucosinolates than those cultivated at lower temperatures. However, higher and lower constant temperatures stimulated higher glucosinolate levels in the sprouts.[51]

Glucosinolates can act as a form of storage for nitrogen, carbon and sulfur and high sulfur levels can increase glucosinolate levels. In some seeds, glucosinolates can represent up to half the sulfur content.[1] In a study evaluating the effect of sulfur and nitrogen application on the glucosinolate content of vegetable turnip rape, Kim *et al.*[52] found that glucosinolate content is strongly affected by nitrogen and sulfur application. The effect of sulfur application was to increase the total glucosinolate content of the plant, although Mailer[53] has found that an excess of sulfur can reduce glucosinolate concentration. In addition, it was shown that the balance between nitrogen and sulfur is important in the regulation of the synthesis and/or build up of glucosinolates. Since sulfur uptake is suppressed by high levels of nitrogen, glucosinolate production is reduced by nutrient imbalance. The type of

glucosinolate is also altered by the application of sulfur and nitrogen. The proportion of indole glucosinolates was larger with an increase in nitrogen application.[52] It has also been suggested that indole glucosinolates may act as a potential source of indole acetic acid, a plant hormone, by the combination of myrosinase and nitrilase enzymes.[54]

Glucosinolates can also act as plant defence agents. The breakdown products following myrosinase catalysed hydrolysis of glucosinolates can be lethal to insects. This explains why they are present at higher levels in younger more vulnerable plants. It is ironic, however, that this defence system can be used against the plant. Certain insects use glucosinolates and their breakdown products as markers for identifying a host for feeding or egg laying. The knowledge of this property is useful in its own way and can be used to study the oviposition of insects. It has been discovered that glucosinolates with an indolic side-chain are the most potent oviposition kairomones.[55] Some insects even make use of the properties of glucosinolates in their own defence. Larvae of the sawfly *Athalia rosae ruficornis* fed on glucosinolate-containing plants, have been shown to sequester certain glucosinolates. When the larvae are subsequently attacked, the glucosinolates are released, providing an intriguing method of defence.[56]

1.4.1 Isothiocyanates as herbicides, pesticides and the effects of intercropping

Isothiocyanates have phytotoxic properties. This was shown when some species of *Brassica* were used as a natural manure crop, temporarily preventing the growth of weeds.[57] This property can be useful in several ways: it can help reduce the use of herbicides and can aid the control of herbicide-resistant weeds. If the crop is planted in winter, there are also other beneficial properties. Soil erosion and nutrient leaching is reduced and some soil-borne diseases are suppressed.[57] The effects are not just restricted to

weeds. Nematodes, a type of worm, can do considerable damage to potatoes. They are normally controlled by synthetic compounds, but a study showed that a 100% mortality was achieved by treatment with gluconasturtiin and added myrosinase. No effect was observed with the pure glucosinolate,[58] suggesting that the active compounds are isothiocyanates. These results show that potatoes and other crops susceptible to nematode attack may be easily protected by the use of glucosinolates and myrosinase, removing the need for environmentally harmful synthetic treatments.

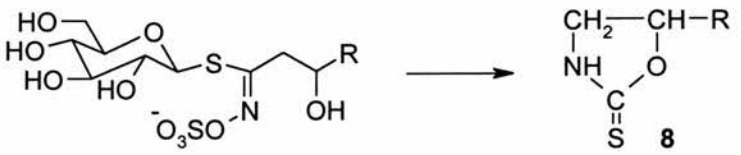
Glucosinolate-containing plants may also be able to repel insects. A study investigating the effects of intercropping kale with cabbage and cauliflower found that insect attack on cabbage was eliminated and that although cauliflower was attacked, this was very late in the growing season.[59] It was suggested that phenolic compounds deposited on the surface of the kale leaves and volatile compounds released from the plant repelled insects, thus suggesting that the use of insecticide could be avoided by planting kale with cabbage and possibly a variety of other crops.

Glucosinolate degradation products are known to suppress a wide variety of plant pathogens[60] and this may have implications in the disease resistance of certain Brassica species. Broccoli is susceptible to a disease known as bacterial soft rot, which can lead to 100% crop loss. It has been shown, however, that broccoli cultivars with high glucosinolate levels have the lowest disease incidence.[60]

1.5 The Biological Activity of Glucosinolates and Isothiocyanates

1.5.1 Undesirable effects of glucosinolates and breakdown products

Early reports on glucosinolates often discussed the undesirable effects that these compounds can have. There have been reports of toxic effects due to glucosinolates and their breakdown products. Glucosinolates found in rapeseed have been implicated in liver damage in poultry,[61] and contact dermatitis has developed amongst humans handling *Brassicas* and their leaves.[62] Another unfavourable effect of glucosinolate breakdown products is that they can have goitrogenic properties. This can manifest itself in two ways. The first is where the thiocyanate ion competes with iodine for uptake into the thyroid gland. An iodine deficiency in the diet allows goitre to develop and over 96% of human goitre is due to an iodine deficiency.[63] In this case, increasing the amount of iodine in the diet will treat the condition. Oxazolidine-2-thiones **8**, compounds formed by the cyclisation of glucosinolate breakdown products, can also cause goitre by the inhibition of thyroxine synthesis.[10] (Scheme 8) In this case, increasing the amount of dietary iodine has a minimal effect. The link between human goitre and glucosinolate breakdown products is lacking in evidence, but the anti-nutritive properties of glucosinolates are far more prominent and problematic in cattle. With this sort of evidence, it is easy to see why glucosinolates have been given a bad name, but as with so many things, this was not the whole story. Only a few glucosinolates display goitrogenic properties, mainly those from rape seed crops,[1] and at subtoxic levels, the effects of glucosinolates can be very different.



Scheme 8: Glucosinolate breakdown to give oxazolidine-2-thione

1.5.2 Antifungal properties of isothiocyanates

The antifungal properties of glucosinolates have been known for many years. Isothiocyanate breakdown products are particularly active against fungi and bacteria. Those with more lypophilic character are more active, possibly due to their greater ability to pass through cell membranes. It was found that aromatic isothiocyanates are more toxic than their aliphatic counterparts and that fungal toxicity of aliphatic glucosinolates decreases with increasing length of the side chain.[48]

1.5.3 Anti-carcinogenic effects of isothiocyanates

For centuries, *Brassica* vegetables such as cabbage and mustard have been used as wound poultices and anti-tumour agents.[1] It is quite likely that these exploit the biological properties of isothiocyanates. In Thailand and other parts of south-east Asia, the leaves, leaf juice and seeds of *Cleome viscosa* are used in a range of medicines due to the presence of glucosinolates.[64] Watercress, a native glucosinolate-containing plant of south-east Europe has been described as a medicinal plant since the 1st century AD.[65] Medicinally, it has been considered a diuretic, expectorant, purgative, stimulant, stomachic and tonic and has also been used to treat anaemia, eczema, kidney and liver disorders, tuberculosis, boils warts and tumours.[66] In more recent times, isothiocyanates are again being investigated for

medicinal purposes. It is widely believed that isothiocyanates can be used to protect against cancer, and this will shortly be discussed.

As is widely accepted, the inclusion of fruit and vegetables into the human diet has beneficial effects on health. It has been found that an intake of 400-600 grams per day of fruit and vegetables is associated with a reduced risk of cancer.[67] In certain cancers of the aerodigestive tract, this can be a reduction of 50%. In the 1960s, a great deal of interest was shown in the possibility that certain aromatic and indolyl glucosinolate hydrolysis products might influence carcinogenesis,[68] while the consumption of cruciferous vegetables has been known to induce detoxification enzymes in experimental systems for more than twenty years.[69] Therefore it is highly likely that *Brassicas* will have a protective effect against cancer. Indeed, a review by van Poppel *et al.*[70] provides compelling evidence that *Brassica* vegetables have cancer chemoprotective effects, especially against cancers of the lung and intestinal tract.

It has been estimated that around 35% of all cancers in industrialised countries are due to diet,[71] while cigarette smoking is the leading cause of bladder cancer and is thought to be responsible for 50% of bladder cancers in men and 25% in women.[72] In the UK, 90% of cancers arise from epithelial cells and 80% of these are associated with the bladder, lungs and alimentary tract.[73] The prevention of these diseases is obviously very important and glucosinolates, along with their isothiocyanate breakdown products, may provide a method for doing this. More than twenty isothiocyanates are known to protect cells against a wide variety of chemical carcinogens and inhibit carcinogen-induced cancers in many rodent organs, including the bladder, colon, oesophagus, liver, lung, mammary gland and small intestine.[72]

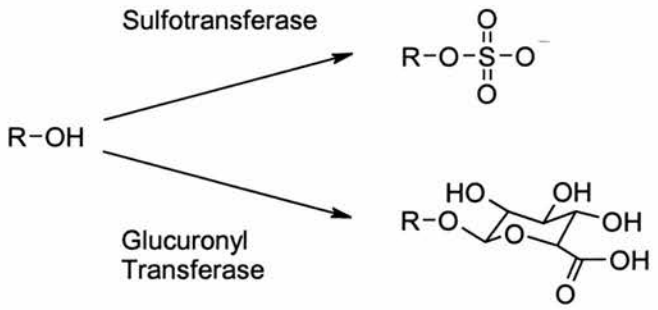
1.5.3.1 Cancer Prevention

Certain hydrolysis products of glucosinolates have shown cancer preventative properties and naturally occurring and synthetic isothiocyanates are amongst the most effective chemopreventative agents known.[74] The results of 6 cohort studies showed inverse associations between the consumption of brassicas and the risk of lung cancer, stomach cancer and all cancers taken together.[70] Of 74 case control studies, 64% showed an inverse relationship between the consumption of one or more brassica and the risk of cancer. It was shown that the association was most consistent for cancers of the lung, stomach and colon. In tests with rodents treated with carcinogens, it was found that isothiocyanates blocked the production of tumours and were effective inhibitors of cancer induction.[10,74,75,76] Sulforaphane has even been counted as one of forty of the most potent cancer inhibitors.[77] From these studies, the potential of isothiocyanates in the fight against cancer is quite clear.

1.5.3.2 Mechanism of anti-cancer effects

Any foreign compound entering the body will be metabolised with the ultimate goal of producing a compound that can be excreted. This process usually begins with the reaction of a phase I enzyme that catalyses the addition of oxygen to the xenobiotic. Subsequent coupling of this compound with a water soluble moeity, such as sulfate or glucuronic acid, leads to a compound that can be easily excreted. This process is catalysed by phase II enzymes. (Scheme 9) The most important phase I enzymes are a class of enzymes known as cytochrome P450 enzymes, of which over 100 forms are known in mammalian

species.[78] Phase II enzymes include glutathione *S*-transferases, uridine diphospho-glucuronyl transferases, sulfotransferases and quinone reductase.



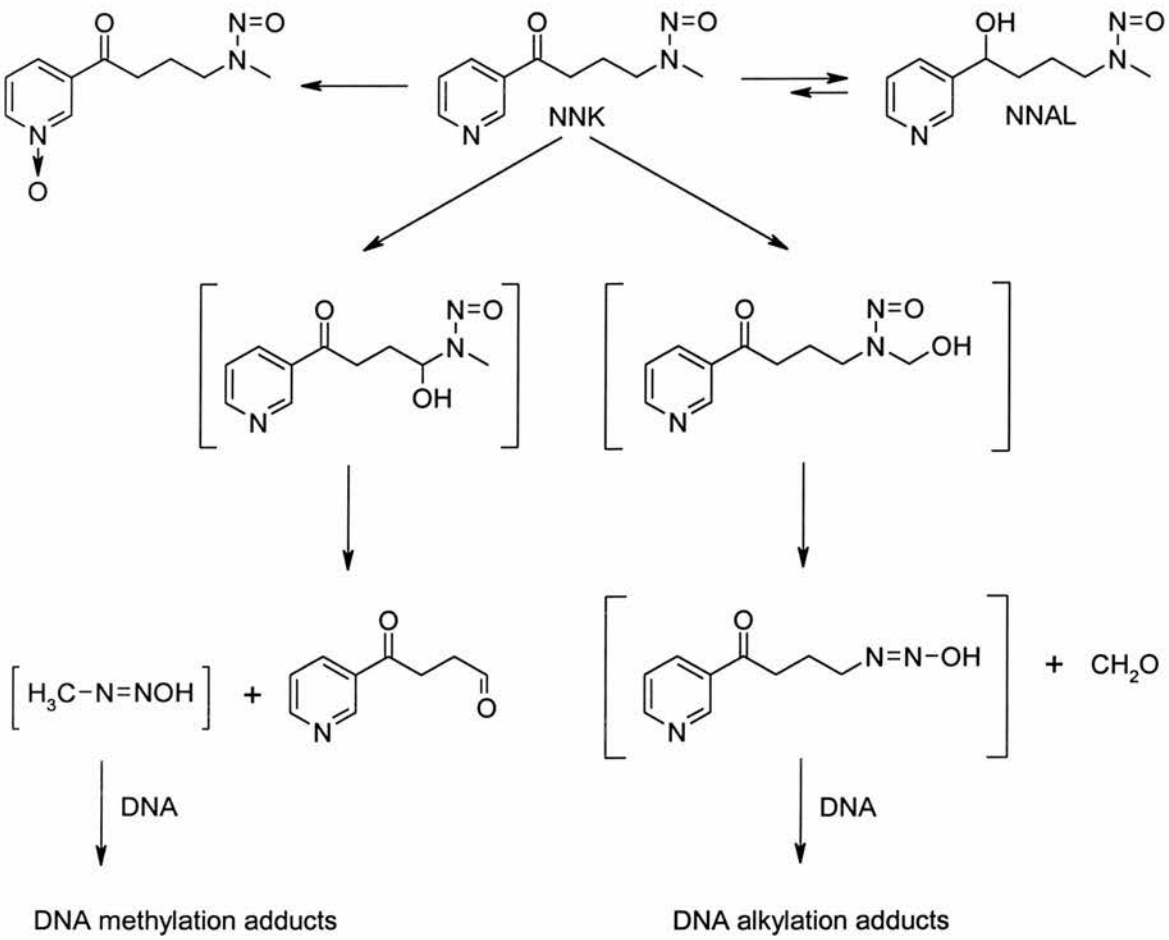
Scheme 9: Phase II enzymes add water soluble moiety to compound

Nearly all dietary or environmental carcinogens to which humans are exposed need an enzymatic transformation before they can exert their carcinogenic effects.[74] In fact, the majority of chemical carcinogens are known to be non DNA-damaging until they are metabolised to reactive electrophiles.[72] Reaction of the procarcinogen with a phase I enzyme is one of the most common methods of activation and many cytochrome P450 enzymes have been shown to be able to catalyse such a reaction.[72] This can produce electrophiles that can react at nucleophilic sites in critical molecules, such as DNA, RNA and proteins. The products of these reactions are adducts. DNA adducts that are not repaired can cause miscoding and therefore mutations in critical genes.

Carcinogenesis is a multi-stage process in which at least three distinct phases can be recognised.[2] The first of these is initiation, a genetic event that itself occurs in two stages: molecular lesions are induced into DNA and then fixed by DNA replication. The second phase is promotion, which is followed by progression. Charged electrophiles are the ultimate carcinogen in the process. Initiation can be blocked either by detoxification of a

procarcinogen or removal of the carcinogen. Once genetic damage has occurred and clonal expansion can arise, progression can be suppressed by either reduced cell proliferation or apoptic removal of the cell.[73]

An example of a carcinogen that requires metabolic activation is NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), a compound found in tobacco smoke. A variety of pathways can be undertaken, some of which can cause DNA damage. In humans and animals, NNK is converted to 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanal (NNAL) by carbonyl reductase enzymes. NNAL is a potent pulmonary carcinogen, but is partially converted to a glucuronide (NNAL-Gluc), a compound believed to be a detoxification product of NNK. *N*-Oxidation of NNK or NNAL also gives detoxification products. However, hydroxylation at either of the α-carbons gives intermediates which can spontaneously decompose to give aldehydes and electrophilic diazohydroxides. The diazohydroxides can then methylate or alkylate DNA producing permanent mutations.[79] (Scheme 10)



Scheme 10: Reactions of NNK. Some products can form DNA adducts

The way in which isothiocyanates work in cancer prevention is not fully understood, though the consumption of foods rich in glucosinolates has been recognised to modify the enzyme pathways associated with carcinogen and drug metabolism for over a decade.[73] The ability of a carcinogen to fulfil its potential is based on the balance between activating enzymes.[80]

1.5.3.3 Theory of Cancer Prevention by Isothiocyanates

A comprehensive and convincing theory of cancer prevention has been put forward by Das *et al.*,[2,80] who suggest that isothiocyanates work in six ways:

- Induction of Phase I enzymes
- Inhibition of enzyme activation
- Induction of Phase II enzymes
- Modification of hormone binding
- Scavenging of electrophiles
- Protection against oxidative damage

Phase I enzymes, as previously mentioned, are the first step in the body's route for disposal of xenobiotics. The reaction adds a hydroxyl group to the toxicant to make it more polar. Induction of phase I enzymes will increase their concentration and help with this conversion.

The inhibition of enzyme activation is also thought to be a key step in preventing cancer. As mentioned earlier, most chemical carcinogens require some kind of activation before they can take effect. By preventing this process, many carcinogens will never be activated and the threat of cancer greatly reduced.

Phase II enzymes then add the water soluble moeity to the products of the phase I reaction. This is usually sulfate, glucose or glucuronic acid. This process is particularly important in

the prevention of cancer and induction of phase II enzymes upregulates the body's natural defences, allowing this to be undertaken more effectively.

Isozymes of cytochrome P450 can metabolise oestrogen in the body.[2] The formation of various oestrogen metabolites is linked to breast and uterine cancers. Indole-3-carbinol, a glucosinolate breakdown product, induces CYP1A2, which mediates the effect of oestrogen-2-hydroxylase. By modifying the steroid hormone metabolism in this way, glucosinolate breakdown products can be useful in preventing hormone related cancers.

Glucosinolate breakdown products induce endogenous anti-oxidant defences in cells. This process will also protect against cancer through prevention of oxidative damage.

There is a large amount of evidence supporting this theory, but it is by no means conclusive. It seems somewhat ambiguous to suggest the induction of phase I enzymes, would prevent metabolism of a carcinogen since phase I enzymes themselves have the potential to activate carcinogens. It would seem more logical that the inhibition of phase I enzymes would prevent the activation of carcinogens, as suggested by Thornalley.[81] This area is therefore rather contentious and recent work has suggested that chemopreventive activity is due to an overall favourable modification of phase I and phase II carcinogen metabolism.[73,79,82,83]

1.5.3.4 Role of Phase I enzymes

One of the reasons that the role of phase I enzymes is so controversial is likely due to their sheer number. As mentioned, over 100 cytochrome P450 enzymes have been identified and each isoform will have a different activity and specificity. It has been shown that isothiocyanates can inhibit rat and human cytochrome P450 1A1, 1A2, 2A6, 2B1, 2B6, 2C9, 2D6, 2E1 and 3A4 isoforms.[72,81] These enzymes had been shown to be involved in the activation of many environmental carcinogens and that isothiocyanates inhibited them by competitive, non-competitive or mechanism-based inhibition, depending on the isoform.[81] Covalent binding of isothiocyanates to the cytochrome P450 apoprotein or the heme moiety can result in a modification of the structure and loss of activity and covalent modification by atomic sulfur, produced through oxidative desulfuration, may also inactivate the P450 enzymes.[84]

Obviously, the above-mentioned enzymes are only a small proportion of the total number of cytochrome P450 enzymes and although other isoforms may be found to be inhibited by isothiocyanates, it is likely that others will not. This may explain the confusing role of phase I enzymes, such that certain isoforms may be induced and others inhibited by the presence of isothiocyanates. This indeed appears to be the case, with literature evidence showing that tumour inhibitory activity of isothiocyanates is due to inhibition of specific cytochrome P450 enzymes that activate carcinogens.[72,78,84] For example, the inhibition of NNK-induced lung tumours in mice was shown to be closely correlated to the inhibition of NNK-activating enzymes, especially CYP 2B1.[72]

1.5.3.5 Role of Phase II enzymes

Although the role of phase I enzymes is complex, that of phase II enzymes is much clearer and glutathione *S*-transferase, quinone reductase (QR) and UDP-glucuronyl transferases (UGT) have been shown to play a critical role in the detoxification of reactive carcinogens.[72] Of all the phase II enzymes studied, the glutathione *S*-transferase (GST) family has received the most attention.[73] Phase II enzymes can either catalyse the conjugation of endogenous ligands with the carcinogen to form an easily excretable product, or catalyse reactions to destroy the reactive centres of carcinogens,[72] so an induction of phase II enzymes will lead to increased conjugation and a faster excretion of carcinogens.[84] It has been shown that many phase II enzymes are controlled by the same regulatory DNA element, known as the anti-oxidant response element (ARE),[72] or electrophile response element.[84] Isothiocyanates have been shown to activate ARE and to therefore induce a variety of phase II enzymes, in a variety of cells and animal organs.[72] The selective induction of phase II enzymes has recently been demonstrated in the urinary bladder of rats by allyl isothiocyanate, where even minimal doses of 10 μmol $kg^{-1}day^{-1}$ caused a significant increase in the activity of quinone reductase and/or glutathione *S*-transferase.[85] It was suggested that such a level could be attained in humans through the consumption of *Brassica* vegetables and that this could contribute to the lower incidence of bladder cancer among individuals who regularly consume *Brassicas*.

Approximately 50% of the Caucasian population have no GST-M1 activity, due to a homozygous deletion of the GST-M1 gene.[81] Individuals lacking this gene, GST-T1 or QR1 were found to be at a much greater risk of developing uroepithelial cancers and that in the majority of cases of bladder cancer, UGT expression was either absent or at a very low

level.[72] The lack of these genes may therefore put the individual at a greater risk of developing cancer, since they may not be able to dispose of carcinogens adequately. Since many isothiocyanates are known to upregulate the expression of GST, QR and UGT,[72] this may contribute to their cancer chemoprotective effects. In a study of lung cancer in Chinese men, London *et al.*[86] found a correlation between the concentration of isothiocyanate metabolites in the urine and the risk of lung cancer. The study, which was carried out over 10 years, found that individuals with detectable levels of isothiocyanate metabolites were at a reduced risk of cancer, but that the effect was only observed in individuals with missing GST-M1 and/or GST-T1 genes. It was concluded that the reduced metabolic capacity of those lacking the GST genes was compensated for by their slower excretion of isothiocyanates in those consuming a diet high in Brassica vegetables.

1.5.3.6 Role of Isothiocyanates and Apoptosis

Recent evidence suggests that glucosinolate breakdown products may also have a role in preventing cancer where DNA damage has already occurred. This may be by the inhibition of cell proliferation and induction of apoptosis, or programmed cell death.[10,73,78] Failure of apoptosis allows mutated cells to enter the cell cycle and can result in the build up of active oncogenes and defective tumour suppressor genes. An imbalance between cell proliferation and apoptosis has been linked with the development of bladder cancer.[72] *In vitro* studies showed that the cytotoxicity of isothiocyanates was selective for tumour cells, but the reason for the selectivity was unknown.[81] The mechanism of induction of apoptosis by isothiocyanates is not fully understood, but the tumour suppressor protein p53 is known to be involved, although this is not essential since apoptosis has been demonstrated in cells absent of p53.[81] It is suggested that low doses of isothiocyanates induce ARE-coordinated

changes in gene expression i.e. alteration of the balance of phase I and II enzymes and that higher or more extended exposure induced apoptosis.[72,81] In a recent study, it was found that allyl isothiocyanate inhibited the proliferation of human prostate cancer cells by causing arrest in the G_2/M phase (Figure 4) and inducing apoptosis.[87] It was also found that normal prostate cells were minimally affected at concentrations of the isothiocyanate that were highly cytotoxic to the cancerous cells.

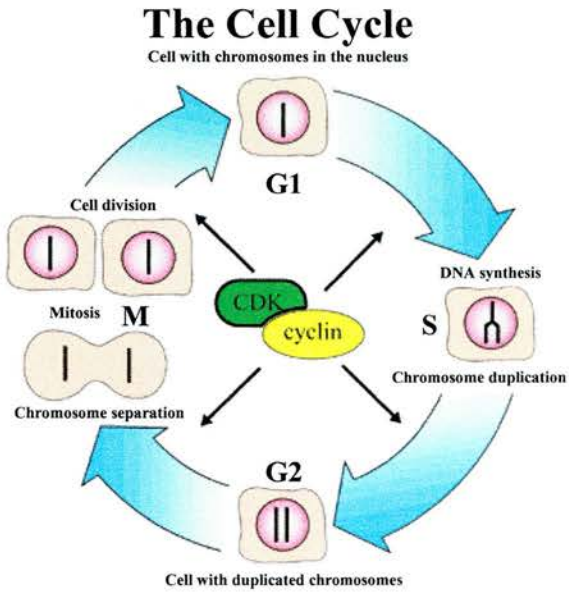


Figure 4: The cell cycle. Allyl isothiocyanate can cause arrest in the G_2/M phase Taken from Nobelprize.org[88]

1.5.3.7 Isothiocyanate Structure and Anti-cancer effects

The structure of the isothiocyanate also has a considerable effect on its anti-cancer effect. Jiao *et al.*[89] found that the isothiocyanate functional group was essential for the inhibitory effects in NNK-induced lung tumourigenesis. Structure-activity studies have also shown that the efficacy of arylalkyl isothiocyanates increases with chain length up to C-6 and then declines with further increases in chain length,[78] leading to the suggestion that the binding

of isothiocyanates is governed by lipophilicity.[68] The target tissue is also greatly affected by isothiocyanate structure. In rats treated with NNK, phenethyl isothiocyanate inhibited lung cancer, but no effect was observed in the liver or nasal cavities.[90] Similarly, in mice, several isothiocyanates effectively inhibit lung and stomach tumours but none have been shown to be effective against skin carcinogenesis.[91]

1.5.3.8 Studies on Isothiocyanates and Cancer

In a search for phase II inducer activity in broccoli, sulforaphane, the hydrolysis product of glucoraphanin, was identified.[92] It proved to be a very potent inducer of phase II activity and has been one of the most extensively studied isothiocyanates over recent years. It has been found to mediate growth arrest and apoptosis in human prostate cancer cells,[93,94] leukaemia cells[95,96] and melanoma cells.[95] Other compounds, such as 4-(methylthio)butyl isothiocyanate obtained from glucoerucin present in rocket, are less well studied, but also have implications for human health. Like sulforaphane, 4-(methylthio)butyl isothiocyanate has also been found to affect the cell cycle progression and apoptosis induction in human leukaemia and to do this selectively, since non-cancerous cells were unaffected.[97]

Other studies have concentrated not on single isothiocyanates, but on the effects of *Brassica* vegetables themselves. In a recent study, the effects of *Brassica* vegetables on breast cancer in Chinese women were investigated by measuring urinary isothiocyanate levels. It was found that greater *Brassica* consumption was associated with a significantly reduced risk of breast cancer.[98] A study on the effects of Brussels sprouts and red cabbage on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced lesions in rats found that heterocyclic-amine-induced lesions were prevented in the liver and colon,[99,100] while

Brussels sprouts have also been shown to protect against benzo[*a*]pyrene induced DNA damage in human cells.[101]

It must be mentioned that some studies have also found the potential for cancer-causing effects of glucosinolates, such as the finding that glucoraphanin can increase susceptibility to cancer by activation of phase I enzymes.[102] Other studies have found an increase in tumour formation due to isothiocyanates,[84] but it has been suggested that the doses required for this are relatively high and are therefore irrelevant to human dietary consumption of isothiocyanate-containing plants.[78]

The results of the studies on cruciferous vegetables and isothiocyanates have led to the suggestion that *Brassicas* could be developed that have enhanced levels of glucosinolates to aid prevention of cancer. This has proved to be possible with the development of isothiocyanate enriched broccoli, which proved to have 80 times the ability to reduce quinone reductase, a standard assay of phase II enzyme induction potential, than standard broccoli.[103] Also of note is that phenethyl isothiocyanate is currently being investigated in Phase 1 clinical trials as a chemopreventive agent in lung cancer.[78,84]

Further developments from isothiocyanate research includes the development of a compound called oxomate, a sulforaphane derivative. It has been suggested that the compound, which has been shown to be seven times lower in toxicity than sulforaphane,[104] could be developed into a once-a-day pill or a vitamin component for cancer prevention and that such a tablet could be on the market in seven to ten years.[105]

1.5.4 Bioavailability of Isothiocyanates

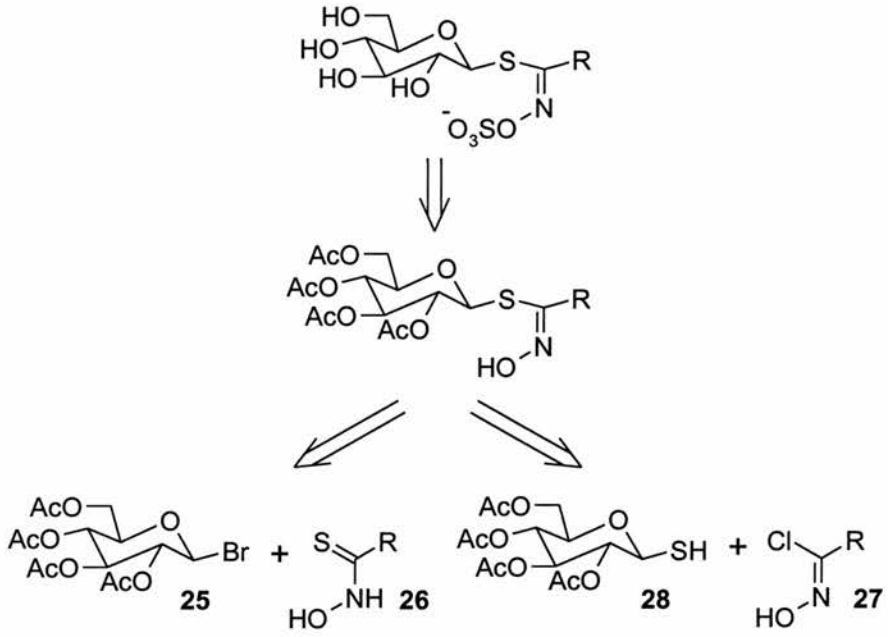
The widespread use of glucosinolate-containing plants, mainly Brassica vegetables, in the human diet is obviously very important and the methods of storage of vegetables can have a significant effect on the quantity of glucosinolates present in the vegetables consumed. It has been found that the methods of storing broccoli after harvest have a significant effect on the levels of glucosinolates within the plant. A study found that storage at 20 °C in open boxes resulted in a 55% loss of glucosinolate concentration and that storage in plastic bags gave approximately the same result. It was found that storage in modified atmosphere packaging at 4 °C maintained glucosinolate concentration and also visual quality for at least 10 days.[106]

The method of preparation of broccoli also has a significant effect on glucosinolate and isothiocyanate levels. A study into the isothiocyanate content of daikon, Japanese white radish, found that the total isothiocyanate content of grated daikon was seven times higher after 30 minutes of cooking than that of cut daikon.[107] The method of cooking can also have a great impact on the quantity of glucosinolates present. In a study into a variety of standard cooking methods, microwave cooking reduced total glucosinolate content by 74%, while high and low pressure boiling had loss values of 33 and 55%. Steaming proved to be the best method of cooking, with regard to glucosinolate content, showing only a slight loss.[108] The bioavailability of isothiocyanates from cooked broccoli is, however, considerably lower than that of the fresh vegetable. Research has found that the bioavailability is three times higher from fresh broccoli compared to cooked broccoli.[109] This is caused by the inactivation of myrosinase due to the heat of cooking, suggesting that cooking broccoli and other vegetables, may reduce the benefits to health. Intact

glucosinolates reaching the large intestine can be degraded by gut microflora, however, resulting in a release of isothiocyanates, possibly explaining the protective effects of cruciferous vegetables on the colon epithelium.[110]

1.6 Chemical Synthesis of Glucosinolates

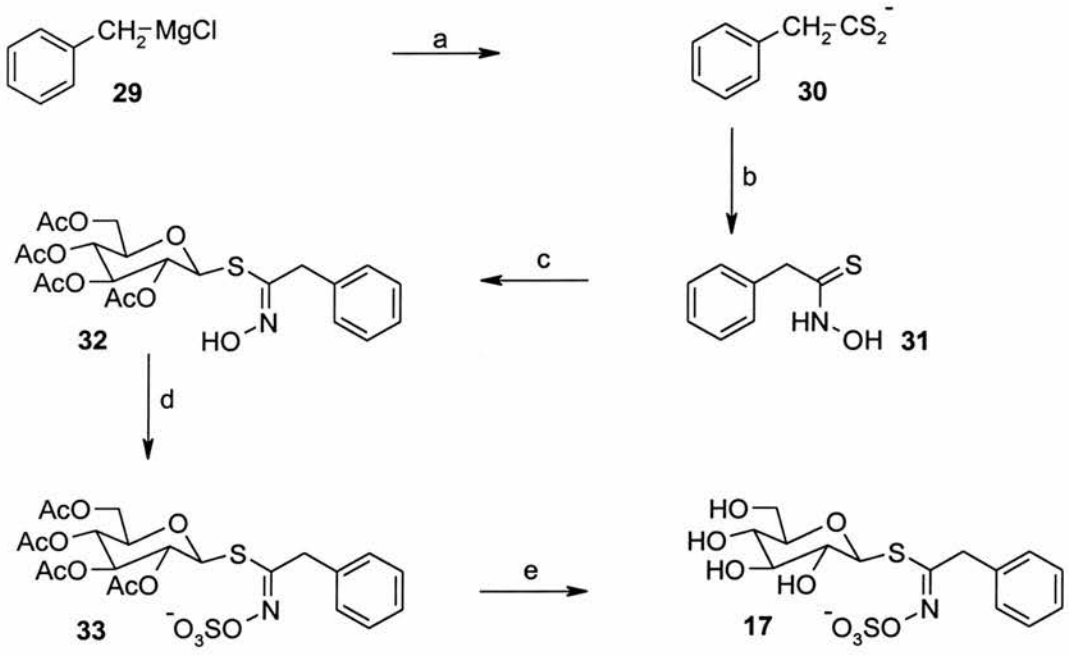
The first reported chemical synthesis of a glucosinolate was the synthesis of glucotropaeolin, then termed the glucotropaeolate ion, by Ettlinger and Lundeen in 1957.[111] Not only was this the first chemical synthesis of a glucosinolate, but it also provided definitive proof of the structure, which had previously been proposed incorrectly.[3] There are two likely approaches to the synthesis of a glucosinolate, as illustrated by the retrosynthetic scheme. (Scheme 11) The first is the coupling of a glucosyl donor **25** with a thiohydroxamic acid **26** and the second is where the sulfur has been previously added to the glucose, such that the coupling takes place between an oximyl chloride **27** and a thioglucose derivative **28**. In both routes, a protected sugar would need to be used so that sulfation of the oxime moiety could be achieved without sulfation of free glucose hydroxyl groups. Generally, this has been achieved by using an acetylated sugar, which can be deprotected after the sulfation has taken place.



Scheme 11: Retrosynthesis of a glucosinolate

1.6.1 Synthesis of Natural Glucosinolates

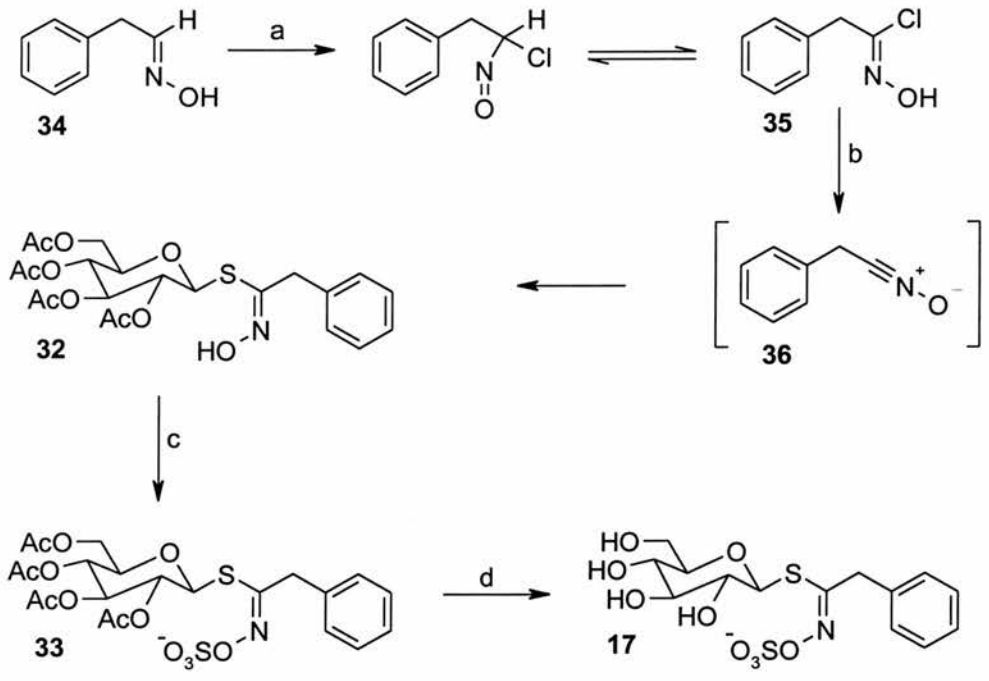
In Ettlinger and Lundeen's synthesis,[111] benzylmagnesium chloride **29** was treated with carbon disulfide to furnish the dithiophenylacetate **30**, which was treated with hydroxylamine hydrochloride to give phenylacetothiohydroxamic acid **31**. Treatment with potassium hydroxide and acetobromoglucose gave the acetylated desulfoglucosinolate **32** which was further reacted with pyridine-sulfur trioxide to give the acetylated glucosinolate **33**. The acetyl groups were removed by treatment with methanolic ammonia to give the final glucosinolate, glucotropaeolin **17**. (Scheme 12) The synthetic glucosinolate was hydrolysed with myrosinase to give benzyl isothiocyanate, which was determined and isolated as benzylthiourea, therefore confirming the synthesis of the glucosinolate and the correct structure.



Scheme 12: Ettlinger and Lundeen's Synthesis of Glucotropaeolin

a) CS_2, Et_2O b) $NH_2OH.HCl$, H_2O (33%) c) Acetobromoglucose, KOH, $MeOH/AcCH_3$ (47%)
d) Pyridine-SO_3 (53%) e) NH_3/MeOH (94%)
Overall yield to glucosinolate 8%

Six years after the first synthesis, Benn proposed a new synthesis of glucotropaeolin **17**,[112] based on the synthesis of thiohydroximates by addition of thiols to nitrile oxides,[113] which would provide a more facile method of preparing the basic framework of the glucosinolate. This method used an oxime **34** as the precursor, which was chlorinated with chlorine to give the corresponding oximyl chloride **35**. Treatment of the oximyl chloride with base produced the nitrile oxide **36**, which was reacted with acetylated thioglucose to produce the thiohydroximate **32**, in this case an acetylated desulfoglucosinolate. The final steps used the procedure of Ettlinger and Lundeen to sulfate and deprotect to give the final glucosinolate **17**. (Scheme 13)

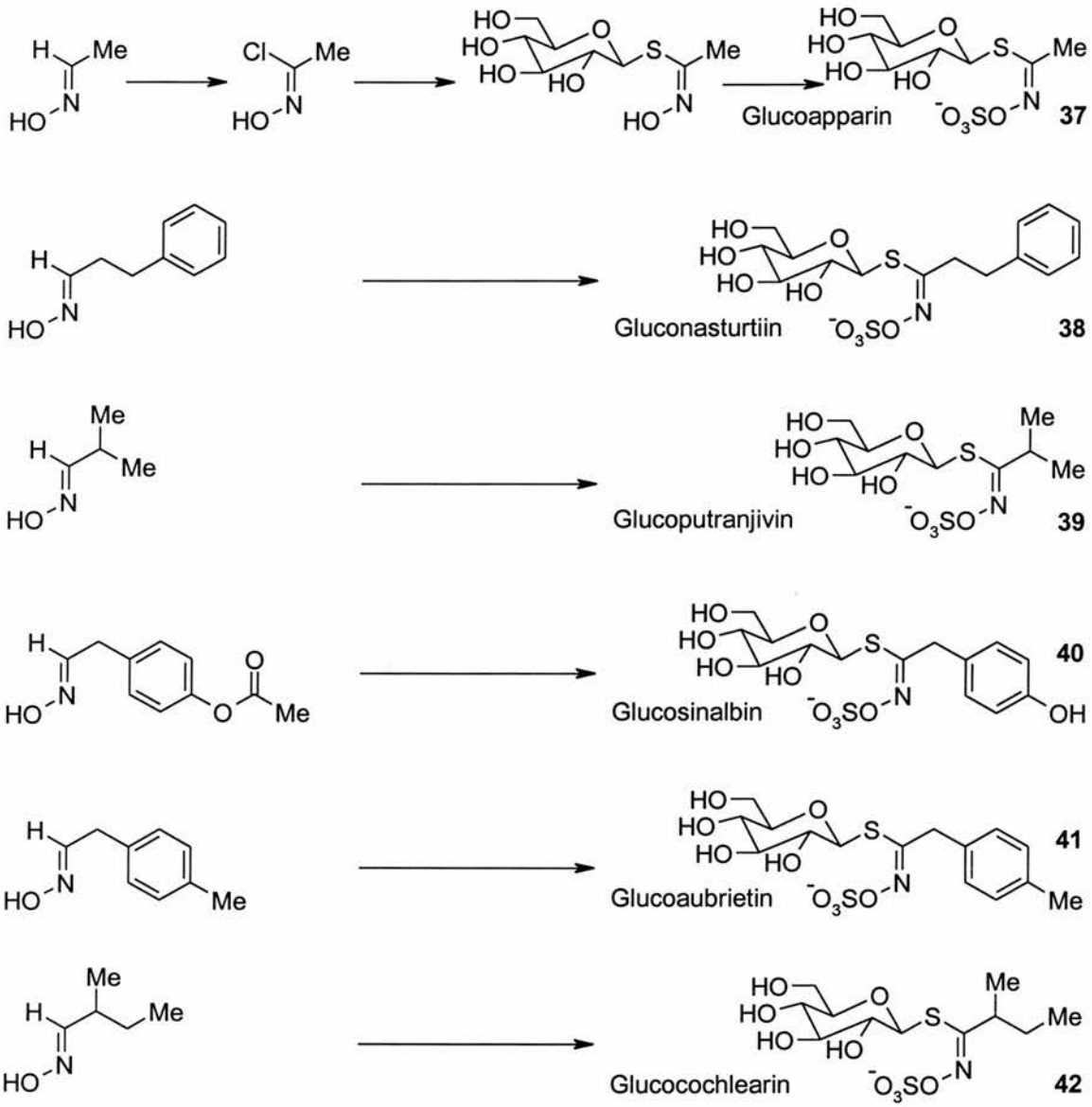


Scheme 13: Benn's Synthesis of Glucotropaeolin

a) Cl_2, $CHCl_3$ (48%) b) Acetylated thioglucose, NEt_3, Et_2O (81%)
c) Pyridine-SO_3, then $KHCO_3$ (17%) d) NH_3/MeOH (100%)
Overall yield to glucosinolate 7%

In both the above schemes, an acetylated sugar was used in the synthesis. This was to avoid problems during the sulfation reaction, where free hydroxyl groups would also be susceptible to sulfation and would be likely to give a mixture of unwanted products. This practice seems to have been universally adopted, since the final synthetic steps seem to have changed little in the years since the first glucosinolate synthesis. Also of note is that glucosinolates are only ever produced in the *Z* configuration. The is due to the nature of the coupling step. When the oximyl chloride is treated with base, a nitrile oxide is formed. Attack on the nitrile oxide by the nucleophilic thioglucose results in a *trans* configuration of the oxime with the lone pair on the nitrogen, forcing the hydroxyl group *cis* to the thioglucose.[114]

A great deal of the early synthetic work was carried out by Benn and co-workers, who synthesised many glucosinolates by this method.[112,115-120]



Scheme 14: Synthesis of glucosinolates from oximes

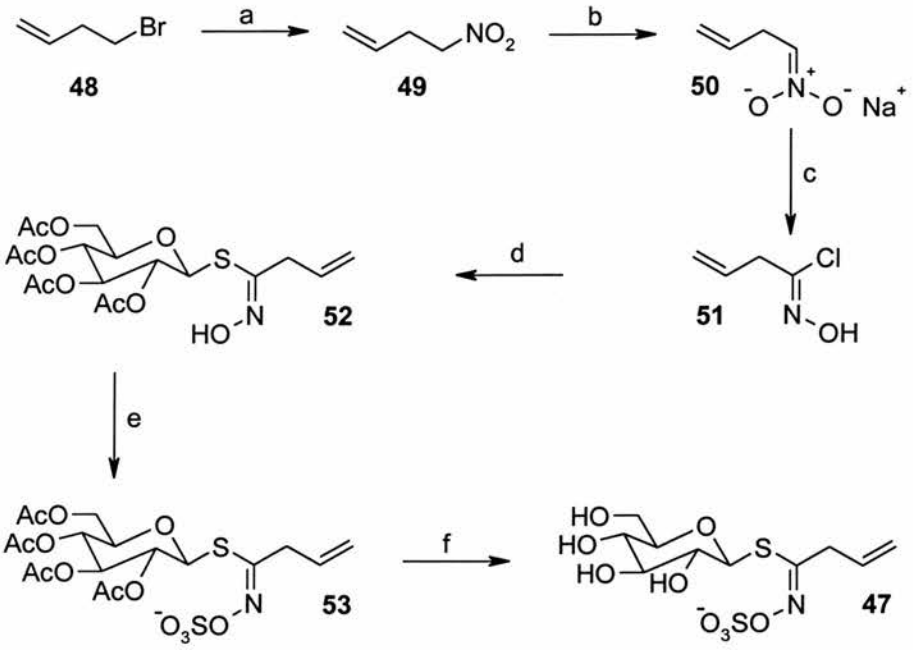
This synthetic route generally proved to be successful, though on occasions the formation of the oxime could prove difficult, resulting in low yields.[119] An alternative method was put forward in 1965, by Benn and Ettlinger,[119] which did not require the formation of an oxime. Instead, a nitro-compound could be used in a reaction noted by Kornblum and

Brown.[121,122] The nitro-compound was converted into its sodium salt, which was treated with a lithium chloride-hydrochloric acid mixture to give the hydroximyl chloride directly. The formation of nitro compounds **43** can, however, be troublesome, since nitrite esters **44** are also formed in the reaction. Further to this, nitrite esters and nitro compounds can give a side reaction to produce a nitrosated nitro compound **45** and an alcohol **46**.[123] (Scheme 15)

43 **44** **45** **46**

Scheme 15: Side reaction of nitro compound and nitrite ester

A procedure developed by Kornblum *et al.* used urea to increase the solubility of sodium nitrite in DMF, one of the only solvents in which sodium nitrite will dissolve, resulting in a faster reaction thus minimising the side reaction.[124] These procedures were used in the synthesis of sinigrin **47**, which was prepared from but-3-enyl bromide **48**. The bromide was treated with sodium nitrite and urea in DMF and the resulting nitro-compound **49** was converted to the sodium salt **50** with sodium ethoxide. Treatment with lithium chloride-hydrochloric acid gave the but-3-enohydroximoyl chloride,[121] which was treated with triethylamine and acetylated thioglucose to give the thiohydroxamic acid **52**. Sulfation **53** and deacylation in the usual manner furnished the glucosinolate, sinigrin **47**. (Scheme 16)

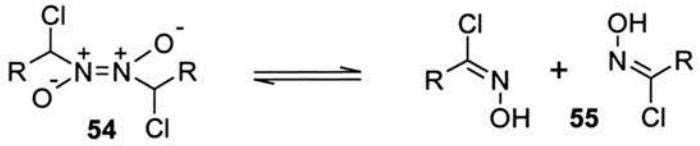


Scheme 16: Synthesis of Sinigrin

a) $NaNO_2$, urea, DMSO b) NaOEt c) LiCl-HCl d) Acetylated thioglucose, NEt_3
e) Pyridine-SO_3 then $KHCO_3$ f) NH_3/MeOH
Overall yield to glucosinolate 7%

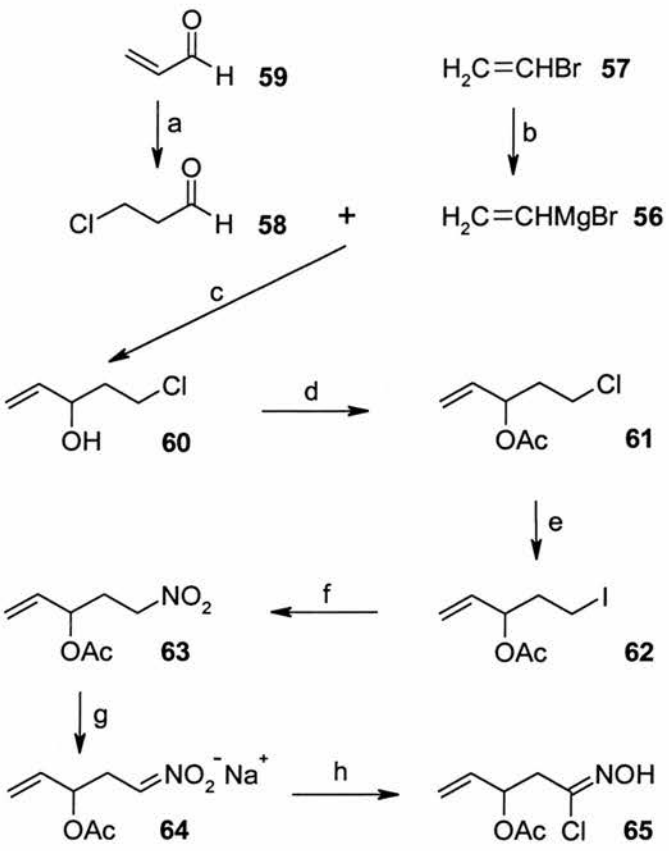
Since then, little change has been made to the general synthetic route towards glucosinolates. Both oxime and nitro-compound routes have been widely used, with only minor adjustments made to certain steps. This applies to Gil and MacLeod's synthesis of gluconasturtiin, which was to be prepared on the gram scale.[125] Initially the oxime to oximyl chloride route was used, but this proved to be low yielding. The nitro-compound route was then attempted and a modification of a method of Casnati and Ricca[126] was used to prepare the oximyl chloride compound in a far greater yield, by employing hydrochloric acid in place of chlorine. The temperature used for crystallisation proved to be the key to the success, with a low temperature of -60 °C giving particularly good results. Analysis showed that the crystals were in fact dimers **54** of the compound, which may form prior to crystallisation. It was suggested that this process only occurs at low temperatures and could explain why previous methods were inconsistent. The dimer formation does not prove

problematic during subsequent reactions, since it dissociates in solution to give the free monomer **55**. (Scheme 17)



Scheme 17: Dimer dissociates to give oximyl chloride in solution

This slight modification allowed 2.4 g of gluconasturtiin **38** to be synthesised successfully and in an acceptable overall yield (7% over six steps). MacLeod and Rossiter then synthesised 2-hydroxybut-3-enylglucosinolate (progoitrin) **23** by a similar method, this time using thionyl chloride in chloroform at -30 °C to produce the hydroximyl chloride from the nitro compound.[127] Reaction of the Grignard compound **56** of vinyl bromide **57** with 3-chloropropanal **58**, which had been prepared by reaction of hydrochloric acid with propionaldehyde **59**, gave the chlorohydroxy alkene **60**. Acetyl protection **61** and conversion of the chloride to iodide **62** allowed the nitro compound **63** to be produced by treatment with sodium nitrite. The nitronate salt **64** was then produced by treatment with sodium in butan-2-ol and reaction with thionyl chloride furnished the oximyl chloride **65**. The coupling to the sugar was carried out in the usual manner. (Scheme 18) In this case, the oximyl chloride could not be isolated, again indicating the difficulty of its successful synthesis and underlining it as one of the key steps in glucosinolate synthesis.

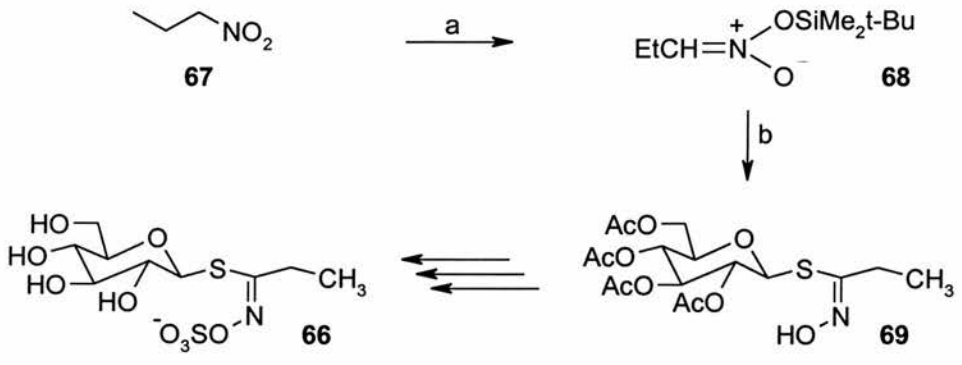


Scheme 18: Synthesis of Progoitrin Oximyl Chloride

a) HCl, Et_2O, -42 °C b) Mg, I_2, THF c) -10 °C, Et_2O/THF (61%)
d) Ac_2O, 0 °C (85%) e) NaI, $AcCH_3$ (78%) f) $NaNO_2$, DMF 0 °C to RT (39%)
g) Na, butan-2-ol (69%) h) $SOCl_2$, $CHCl_3$, -60 °C
Overall yield to glucosinolate 1%

The requirement of the formation of an oximyl chloride was removed in a synthetic route developed by Benn *et al.* in 1984 for the formation of glucolepidiin **66**.[128] Thiohydroximates were prepared by base-induced condensation of nitro-alkanes with thiols by Copenhaver.[129] These reactions, however, required particularly harsh conditions and resulted in only very low yields of the desired products, but a modification of the route i.e. replacement of the nitroalkane with a nitronate, proved to be successful. 1-Nitropropane **67** was treated with lithium di-isopropylamide and *tert*-butyldimethylsilyl chloride was added to quench the reaction, giving the trialkylsilyl derivative of the nitronate **68**. Addition of this nitronate to a solution of acetylated thioglucose and

triethylamine gave two thiohydroximates **69**, which were found to be the *E* and *Z* isomers. Only the *Z* isomer is used to generate a glucosinolate, but the *E* isomer was found to undergo thermal and photochemical isomerisation in solution to give the *Z* isomer. Treatment of the *Z*-thiohydroximate with pyridine-SO_3 and subsequent deprotection of the sugar with methanolic ammonia generated the glucosinolate in the usual manner. (Scheme 19) Although the yields of this route were good, it does not seem to have been adopted, this being the only example of its use in glucosinolate synthesis.

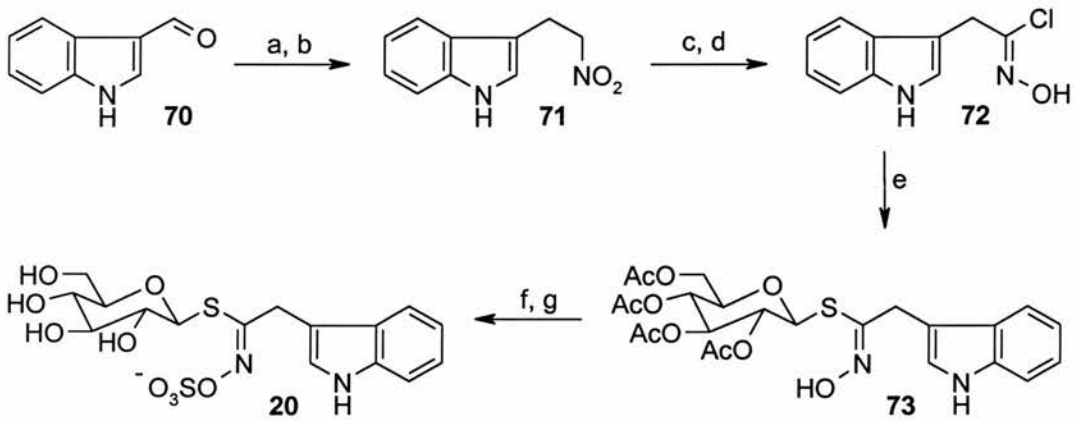


Scheme 19: Synthesis of Glucolepidiin

a) LDA, TBDMS-Cl, THF (70%) b) NEt_3, Acetylated thioglucose, THF (84%)
Overall yield to glucosinolate 50%

In the more recent past, the work of Rollin and co-workers has dominated glucosinolate synthesis. In 1990, Viaud and Rollin proposed the first synthesis of an indole glucosinolate,[129] compounds which have proved to be the most difficult glucosinolates to synthesise. 3-Formylindole **70** was used as the starting material and was treated with nitromethane and ammonium acetate in a Knoevenagel-type condensation. Subsequent reduction with sodium borohydride on silica gave 3-(2'-nitroethyl)-indole **71**, which was converted to the oximyl chloride **72** by treatment with sodium methoxide and thionyl chloride as per the above procedure and coupled with acetylated thioglucose in the usual

manner **73**. Again, pyridine-SO_3 was used for the *O*-sulfation and deacetylation was undertaken by treatment with triethylamine to give glucobrassicin **20**. (Scheme 20)

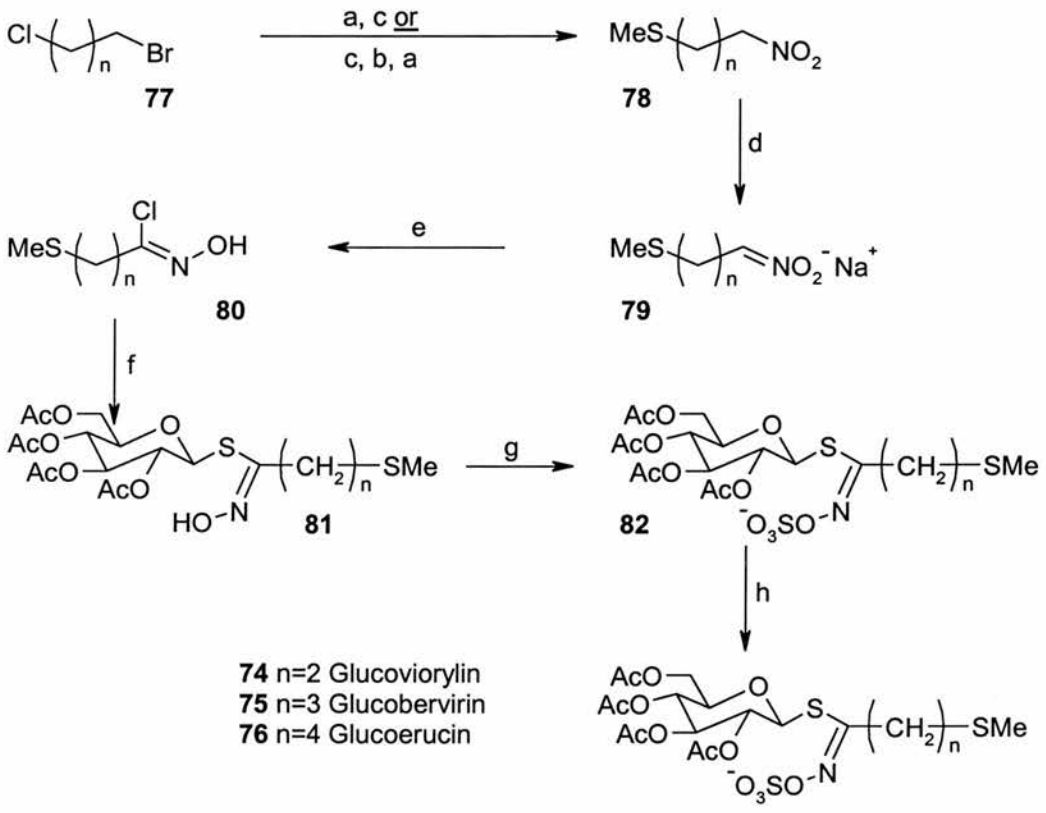


Scheme 20: Synthesis of Glucobrassicin

a) $MeNO_2$, $AcONH_4$, 100 °C b) $NaBH_4$, SiO_2, $CHCl_3$, *i*-PrOH (56% over two steps)
c) NaOMe, MeOH, Et_2O d) $SOCl_2$, DME, -78 °C
e) Acetylated thioglucose, NEt_3, Et_2O (50% over three steps)
f) Pyridine-SO_3, CH_2Cl_2, then $KHCO_3$ (85%) g) NEt_3, MeOH, H_2O (96%)
Overall yield to glucosinolate 23%

In 1996 Rollin *et al.* reported the first synthesis of glucosinolates with an external thio-funcationality.[130] Glucoviorylin **74**, glucoibervirin **75** and glucoerucin **76** were all synthesised by the same protocol (Scheme 21). A 1-bromo-ω-chloroalkane **77** of appropriate chain length was treated to give the corresponding 1-nitro-ω-thiomethyl compound **78**. The bromo-chloroalkane was treated with either sodium nitrite, then sodium thiomethoxide, or alternatively sodium thiomethoxide, sodium iodide, then sodium nitrite. It was necessary to use reaction conditions specific for each chain length since 3-nitro-2-isoxazoline could be formed when n=2 and cyclic sulfonium salts could be formed when n=3 or 4 if a reaction was attempted with the reactive methylthio functionality already present in the compound. The nitronate salt **79** was prepared by treatment with sodium methoxide and was subsequently reacted with thionyl chloride to give the oximyl chloride

80. Reaction with acetylated thioglucose in the presence of triethylamine furnished the acetylated desulfoglucosinolate **81**, which was sulfated with chlorosulfonic acid in pyridine and deprotected with potassium methoxide to give the final glucosinolate **74-76**.

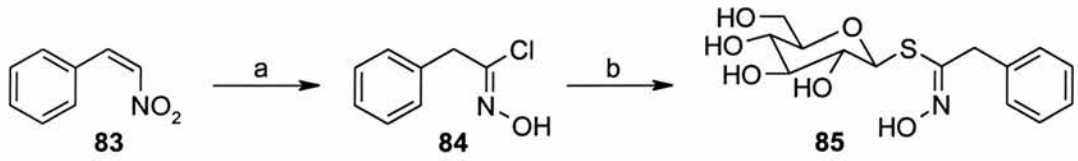


Scheme 21: Synthesis of Glucosinolates with External Thiofunctionality

a) $NaNO_2$, DMSO b) NaI, $AcCH_3$, reflux
c) NaSMe, MeOH, reflux (40-65% from bromochloroalkane) d) NaOMe, MeOH/Et_2O
e) $SOCl_2$, $CHCl_3$ f) Acetylated thioglucose, NEt_3, CH_2Cl_2/Et_2O (32-40% over three steps)
g) HSO_3Cl, pyridine, CH_2Cl_2, 0 °C (61-86%) h) KOMe, MeOH (100%)
Overall yields to glucosinolates min 8%, max 22%

In 1998, another alternative method of producing the oximyl chloride was proposed by Rollin *et al.*[132] A method devised by Kumaran and Kulkarni[132] used $TiCl_4$-Et_3SiH to chlorinate ω-nitrostyrenes **83** to produce phenylacetohydroximyl chlorides **84** and this

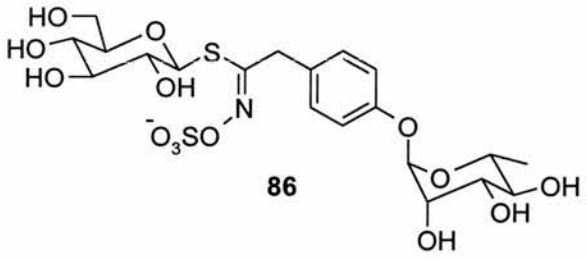
method was adopted to produce arylalkyl **85** and indolylmethyl glucosinolates directly from allylic nitro compounds. (Scheme 22)



Scheme 22: Synthesis of Arylalkyl Glucosinolates

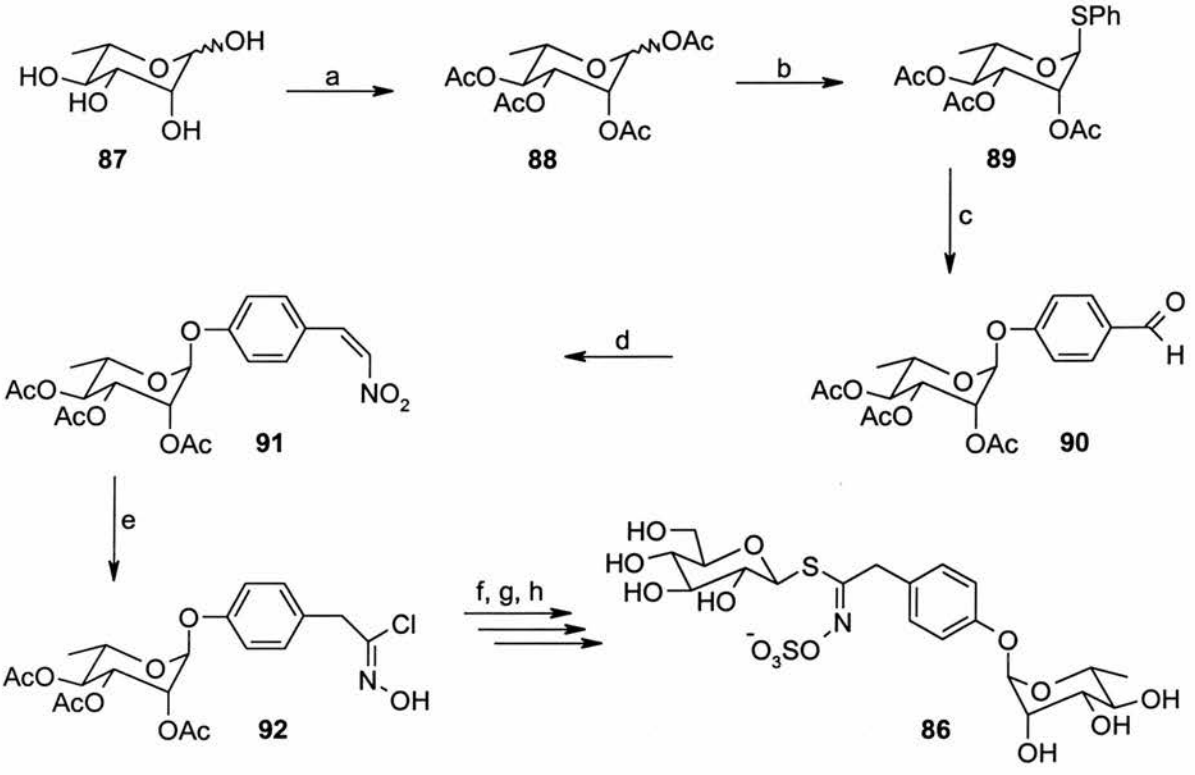
a) TiCl4, Et_3SiH, CH_2Cl_2 b) Acetylated thioglucose, NEt_3, CH_2Cl_2/Et_2O
Yield over two steps 50-84%

The first synthesis of an *O*-glycosylated glucosinolate was reported in 2000, again by Rollin and co-workers.[133] The glucosinolate formed was of the variety with a complicated side-chain structure, the biosynthesis of which could not be easily rationalised. It is, however, a natural glucosinolate and is found in *Moringa oleifera* (Moringaceae), a tree found extensively in the tropics. It would seem that no name has yet been ascribed to this glucosinolate, but it is a *O*-rhamnosylated form of glucosinalbin **86**.



L-Rhamnose **87** was acetylated by the method of Fischer *et al.*,[134] and reacted to give the α-phenyl thioglycoside **89**. Activation using *N*-iodosuccinimide allowed reaction with *p*-hydroxybenzaldehyde, through a modification of a reaction demonstrated by Leuck and Kunz,[135] to give the aryl α-L-rhamnoside **90**. This was subsequently reacted with

nitromethane and ammonium acetate to give the allylic nitro compound **91**, which was reacted under Kumaran and Kulkarni's conditions[132] with acetylated thioglucose to give the acetylated desulfoglucosinolate. *O*-Sulfation using SO_3-pyridine and standard deprotection generated the final glucosinolate **86**. (Scheme 23)



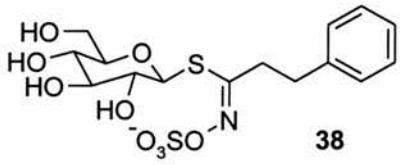
Scheme 23: Synthesis of an O-Rhamnosylated Glucosinolate

a) Ac_2O, pyridine b) PhSH, $BF_3.Et_2O$, CH_2Cl_2
c) *N*IS, TfOH, *p*-hydroxybenzaldehyde, CH_2Cl_2 (59%) d) CH_3NO_2, $AcONH_4$ (83%)
e) $TiCl_4$, Et_3SiH, CH_2Cl_2 f) Acetylated thioglucose, NEt_3, CH_2Cl_2 (65% over two steps)
g) Pyridine-SO_3, then $KHCO_3$ h) KOH, MeOH (58% over two steps)
Overall yield to glucosinolate from phenyl thioglycoside 18%

1.6.2 Isolation of glucosinolates

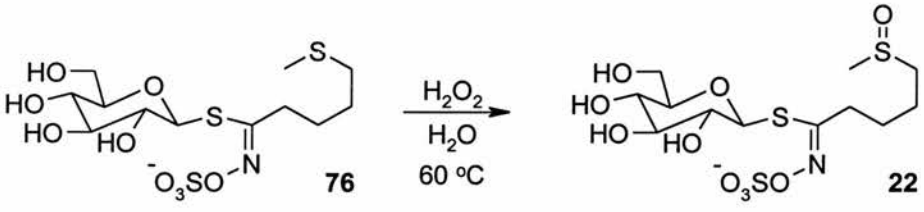
Another method of obtaining glucosinolates is by their isolation from plants. This method can prove to be difficult if more than one glucosinolate is present in the plant, since

separation can prove to be challenging. Gluconasturtiin **38** has been isolated from *Barbarea verna* where it is the only glucosinolate compound present in the seeds.[136] By grinding the seeds in boiling water and subsequent purification of the extracted mixture, gluconasturtiin was isolated in a yield of nearly 3%, by weight, from starting seeds.



38

Glucoraphanin **22** has also been produced by a semi-synthetic method, by treatment of glucoerucin, which was extracted from the seeds of rocket (*Eruca sativa*).[137] Glucoerucin **76** was found to be present in nearly pure form and in good amount, approximately 3% by weight of rocket leaves. It was converted to glucoraphanin **22** by treatment with hydrogen peroxide in quantitative yield, providing a semi-synthetic route to this important glucosinolate. (Scheme 24)



76 **22**

Scheme 24: Treatment of glucoerucin with hydrogen peroxide yields glucoraphanin

1.6.3 Synthesis of Glucosinolate Analogues

Other compounds recently synthesised include a variety of glucosinolate analogues, often for studies with myrosinase to provide a greater understanding of the enzyme mechanism.

A series of glucobrassicin analogues was synthesised by Rollin *et al.*,[138] this time from a variety of different sugars, namely D-galactose **93**, D-mannose **94**, 2-*N*-acetyl-D-glucose **95**, L-rhamnose **96**, D-xylose **97**, D-arabinose **98**, maltose **99** and cellobiose **100**. These compounds were used as model substrates in the study of the active site of myrosinase. (Figure 5)

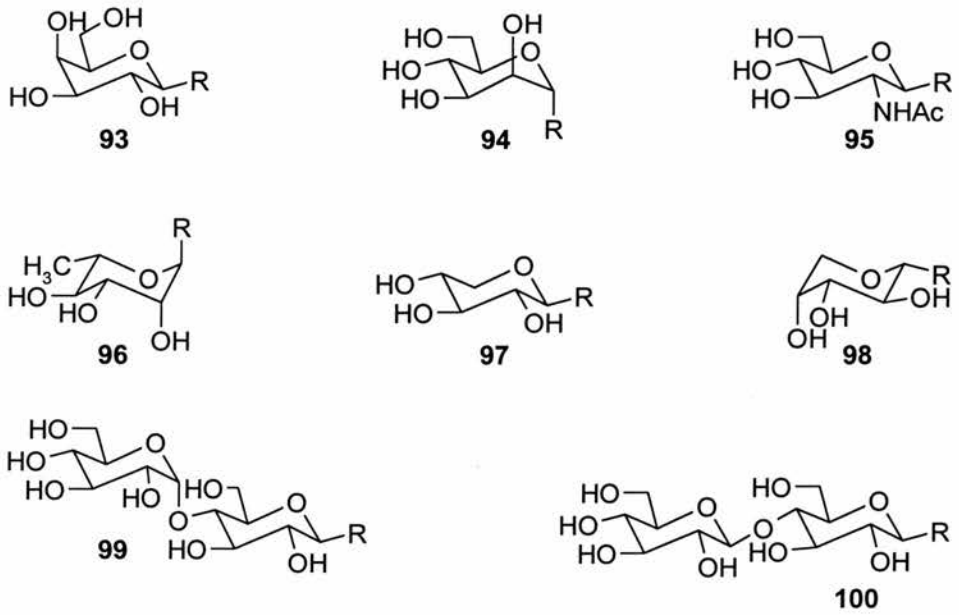
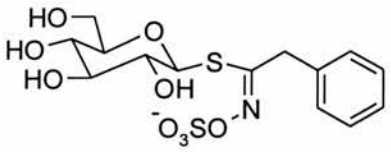
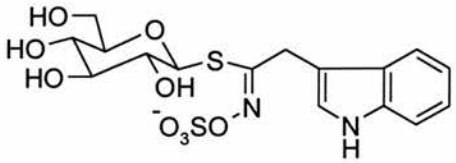


Figure 5: Sugars used to make glucobrassicin analogues

A series of deoxy derivatives of glucotropaeolin and glucobrassicin were also synthesised.[139] Again this involved the modification of the sugar moiety, this time by removal of a specific hydroxyl group. Since there are four free OH groups in the sugar of a glucosinolate, this allowed the formation of four different deoxy derivatives each of glucotropaelin and glucobrassicin. Another paper illustrated the synthesis of 2-deoxy-2-fluoroglucotropaeolin **101** as a potential mechanism-based inhibitor of myrosinase.[140] (Figure 6)

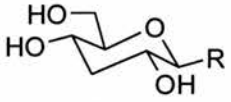


Glucotropaeolin **17**



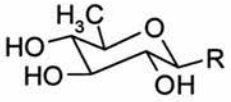
Glucobrassicin **20**

2-deoxy

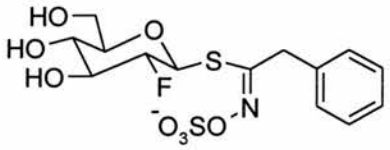


3-deoxy

4-deoxy



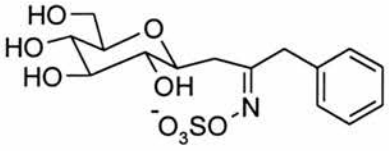
6-deoxy



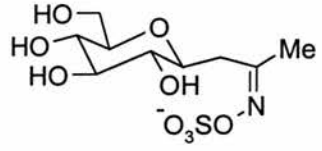
2-deoxy-2-fluoroglucotropaeolin **101**

Figure 6: Glucosinolates and analogues

The first ever synthesis of a *C*-analogue of a glucosinolate, *C*-glucotropaeolin **102**, was published in 1999. This compound was also a potential myrosinase inhibitor.[141] The sulfur atom attached to the sugar was replaced with a CH_2 group and it was hoped that myrosinase would be unable to cleave the bond and therefore inhibit the enzyme. Further work also included the synthesis of *C*-glucoapparin[142] **103**, but unfortunately, neither of the analogues bound to the enzyme.

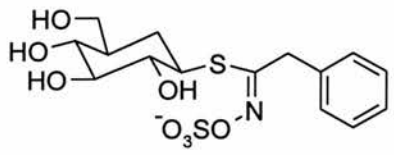


C-Glucotropaeolin **102**



C-Clucocapparin **103**

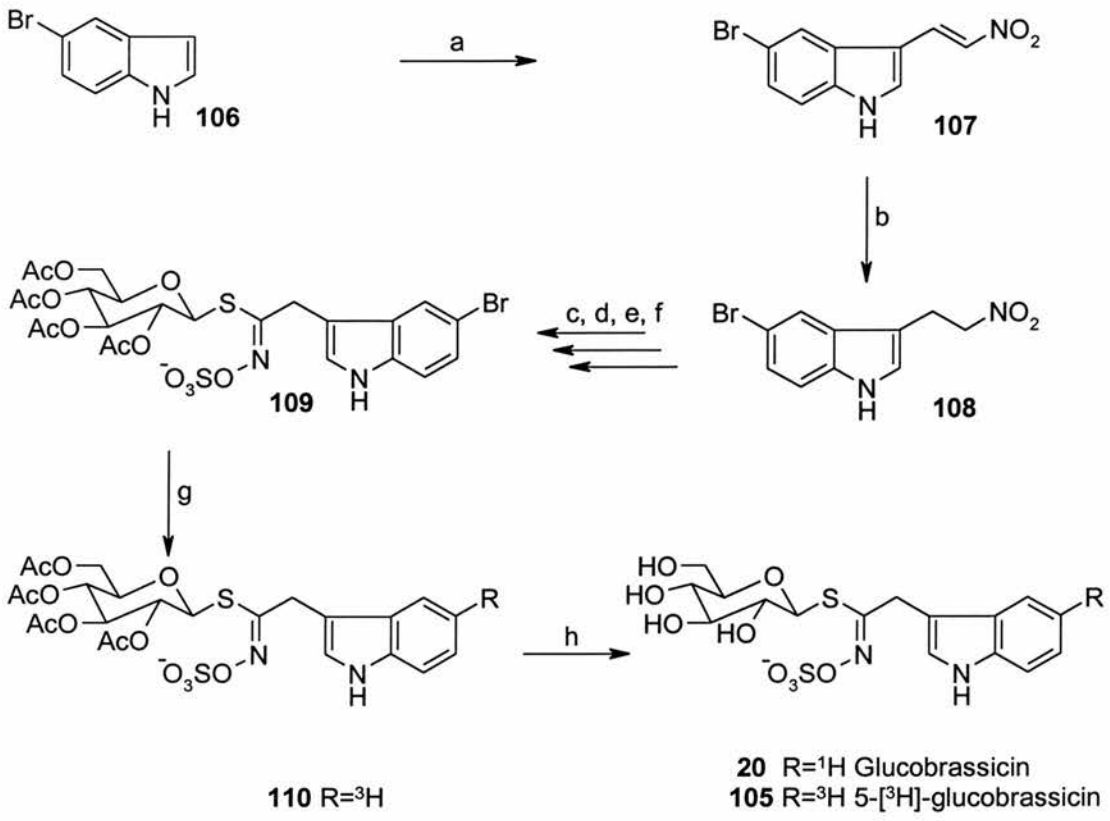
Recently, a new carba-analogue of glucotropaeolin **104** was synthesised, resulting in a substituted cyclohexane ring, rather than the oxygen-containing ring of glucose.[143] This compound performed as desired and became the first non-hydrolysable glucosinolate analogue to inhibit myrosinase.[143]



104

1.6.4 Synthesis of Isotopically Labelled Glucosinolates

In order to carry out specific biological tests, it is often useful to have an isotopically labelled analogue of the compound under investigation to easily distinguish it from the natural compound. Radiolabelling is one method of doing this and Rollin *et al.*[144] synthesised a tritium-labelled glucobrassicin **105**. This was to be used to study the *in vivo* metabolism of glucobrassicin. The synthesis of glucobrassicin **20** has been described above (Section 1.6.1) and a modification of this route was used to provide the labelled analogue. 5-Bromoindole **106** was reacted with *N,N*-dimethyl-2-nitrovinylamine and subsequently reduced to give 5-bromo-3-(2'-nitroethyl)-indole **108**. The oximyl chloride, prepared via the nitronate salt followed by reaction with thionyl chloride, was treated with acetylated thioglucose in the usual manner. Following sulfation, a hydrogenation step was employed to convert the bromide **109** to tritium **110**, before the standard deprotection was used to afford the labelled glucosinolate **105**. (Scheme 25)



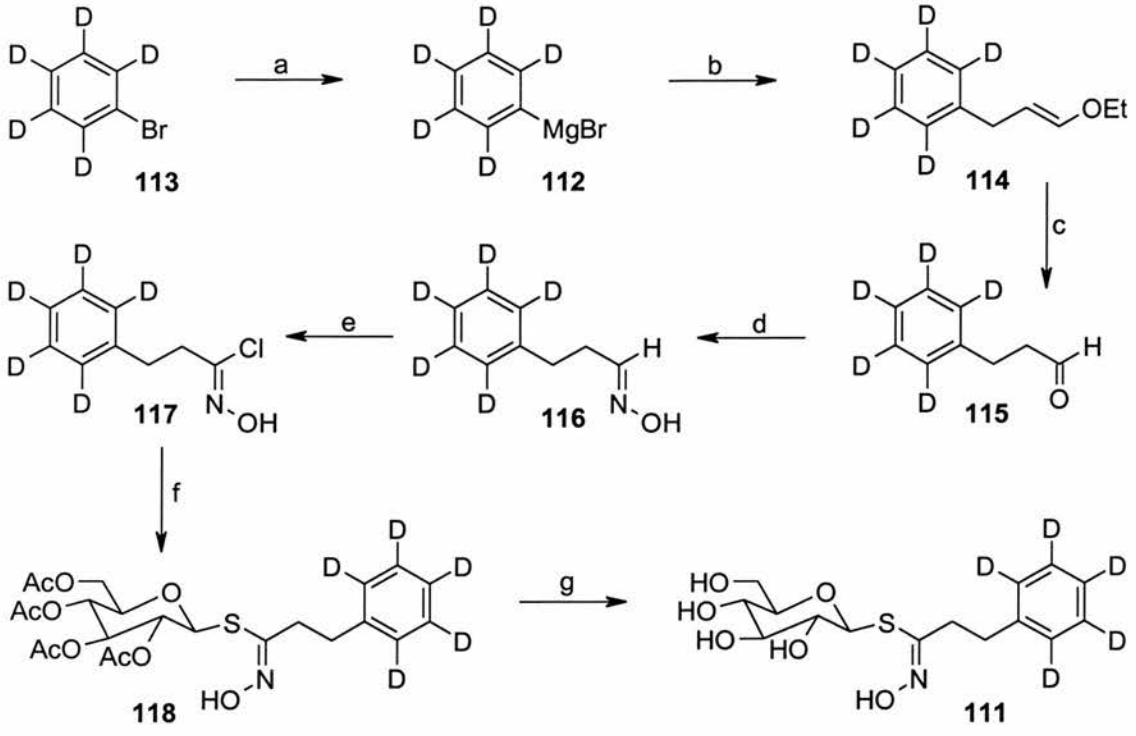
Scheme 25: Synthesis of Tritium Labelled Glucobrassicin

a) *N,N*-Dimethyl-2-nitrovinylamine, CF_3COOH, 0 °C (80%) b) $NaBH_4$, MeOH
c) NaOMe, MeOH d) $SOCl_2$, DME e) Acetylated thioglucose, NEt_3, Et_2O/CH_2Cl_2 (72% over 3 steps)
f) Pyridine, HSO_3Cl, CH_2Cl_2, then $KHCO_3$ (87%) g) H_2 or T_2, Pd/C, NEt_3 h) MeOK, MeOH

The preparation of deuterium labelled desulfoglucosinolates for use as internal standards in LC-MS analysis was reported by Botting and co-workers.[145] Techniques to identify glucosinolates by LC-MS have been developed,[146] but in order to quantify the compounds reproducibly, a suitable internal standard had to be employed. Such a standard would require a structure similar to the analyte and per-deuterated analogues were deemed to be the most appropriate. Since a great deal of analysis is carried out by HPLC, gluosinolates are normally enzymatically degraded to desulfoglucosinolates to give a compound more suitable to the analytical method. Thus, deuterated desulfoglucosinolates were chosen as

very suitable targets and deuterated analogues of desulfogluconasturtiin and methoxy-desulfoglucobrassicins were synthesised.

Deuterated desulfogluconasturtiin **111** was prepared by preparation of the Grignard reagent **112** from per-deuterated bromobenzene **113** and reaction with acrolein diethyl acetal. Hydrolysis of the enol ether **114** gave the aldehyde **115** which was converted to the oxime **116** by reaction with hydroxylamine hydrochloride. Preparation of the oximyl chloride **117** and subsequent reaction with acetylated thioglucose gave the acetylated desulfoglucsinolate **118** which was deprotected using sodium methoxide to give the final desulfoglucosinolate **111**. (Scheme 26)

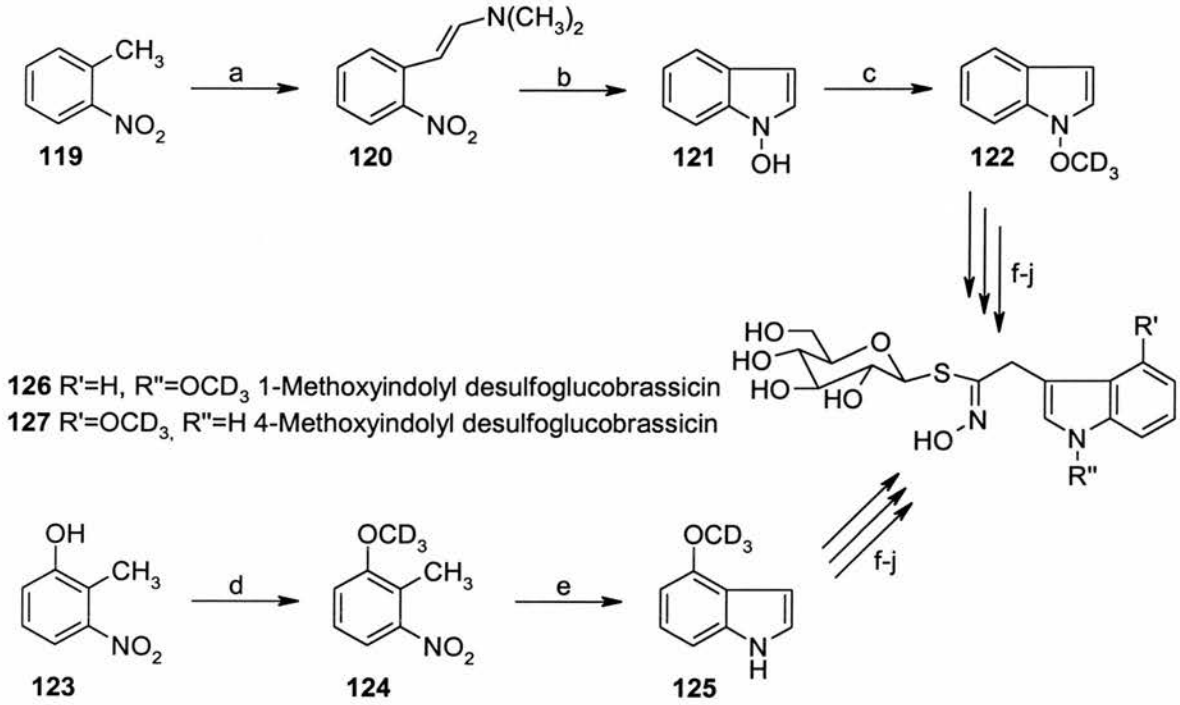


Scheme 26: Synthesis of Deuterium Labelled Desulfogluconasturtiin

a) Mg, I_2, Et_2O b) Acrolein diethyl acetal, 5% CuBr, THF (100% over two steps)
c) Conc. HCl, $AcCH_3/H_2O$ (87%) d) $NH_2OH.HCl$, $NaOAc.3H_2O$, $EtOH/H_2O$ (49%)
e) *N*CS, pyridine, $CHCl_3$ (49%) f) Acetylated thioglucose, NEt_3, THF (37%) g) NaOMe, MeOH (94%)
Overall yield to desulfoglucosinolate 7%

In the synthesis of a labelled desulfoglucobrassicin, methoxy derivatives were deemed to be the most suitable compounds to prepare, by addition of deuterated methoxy groups to either the benzene ring, or the nitrogen. In latter case, 2-nitrotoluene **119** was reacted with *N,N*-dimethylformamide dimethylacetal (DMF.DMA) and the nitro group was reduced using zinc under acidic conditions. Cyclisation then gave the 1-hydroxyindole **121** which was trapped with deuteromethyl iodide to give the 1-methoxy indole **122**. In the case of the 4-methoxy derivative, deuteromethylation of 2-methyl-3-nitrotoluene **123** allowed the incorporation of the labels into the compound. The nitro group was reduced with titanium (III) chloride and subsequent cyclisation gave the 4-methoxyindole **125**.

Both indole compounds were then treated with phosphorus oxychloride and dimethyl formamide, nitromethane, then sodium borohydride to give the corresponding nitroethyl indoles. These were converted to the oximyl chlorides by treatment with sodium in methanol followed by reaction with thionyl chloride. Subsequent coupling to the acetylated thioglucose followed by deprotection gave the deutero desulfoglucobrassicins **126, 127**. (Scheme 27)

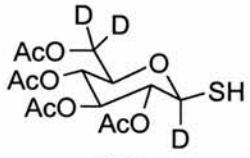


Scheme 27: Synthesis of Deuterium-labelled Glucobrassicins

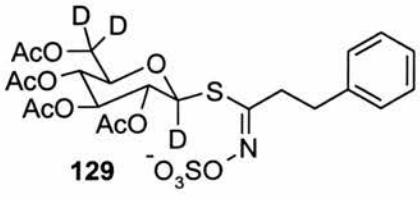
a) DMF.DMA, DMF b) Zn, NH_4Cl, Et_2O, H_2O c) CD_3I, pyridine (40% over three steps)
d) DMSO, KOH, CD_3I (87%) e) Pyrollidine, DMF.DMA, $TiCl_3$ (71%)
f) $POCl_3$, DMF (73%) g) CH_3NO_2, NH_4OAc (89%), h) $NaBH_4$, $CHCl_3$, *i*-PrOH, SiO_2 (37%)
i) Na, MeOH j) $SOCl_2$, DME, -40 °C (100% over two steps)
Overall yields: 1-Methoxyindolyl desulfoglucobrassicin 10%
4-Methoxyindolyl desulfoglucobrassicin 15%

An alternative method of incorporating isotopic labels into glucosinolates to produce internal standards for LC-MS analysis is by preparation of a labelled sugar fragment. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-[1-$^2H_1$,6-$^2H_2$]glucopyranose **128** was synthesised by

Robertson[55] and a deuterated analogue of desulfogluconasturtiin **129** was produced. The advantage of this method of labelling is that the same deuterated sugar can be used to synthesise each glucosinolate. However, this strategy is of no use when studying the metabolites of glucosinolates.



128



129

1.6.5 Summary of Chemical Syntheses

There have been a significant number of chemical syntheses developed for a variety of glucosinolate structures, but there are many features that are recurrent. As discussed earlier, there are two possible methods of coupling the sugar moiety with the aglycone. The sulfur linker could be incorporated either into the aglycone unit or the glucose unit. The second method seems to have been almost universally adopted, with coupling taking place between an oximyl chloride and the thioglucose compound. Another key feature is that the protection used to prevent sulfation of the glucose hydroxyl groups was, almost without exception, acetyl protection. The method of preparation of the oximyl chloride has proved to be a point at which some variation is found. The two routes, either via the oxime or the nitronate salt, have both been shown to successfully furnish an oximyl chloride, but with varying degrees of success. The success of a particular route seems to be a consequence of the individual side chain rather than the method, since neither route has been distinguished as being superior.

Although there have been several syntheses of glucosinolate analogues and isotopically labelled glucosinolates, no syntheses of ^{13}C-labelled glucosinolates have been reported. Although the precursors of the established pathways may not be useful initially, they can provide the basis for the synthesis of ^{13}C-labelled compounds after initial construction of the labelled side-chain.

1.7 Aims and Objectives

Glucosinolates and their breakdown products are currently of considerable interest due to their biological effects. Of most importance is their cancer preventative activity which has the potential to be beneficial to human health. However, before the cancer preventative effects of these compounds can be exploited, questions still have to be answered regarding the chemical mechanism of the effects and also, possibly more importantly, the bioavailability of the glucosinolates and isothiocyanates.

One way to investigate the bioavailability of chemicals is to employ isotopically labelled compounds. This allows the compounds and metabolites to be more readily traced. Radioactive isotopes (e.g. ^{14}C, ^{3}H) offer some advantages in terms of detection at very low levels but are unsuitable for use in humans. Therefore, stable isotopes (e.g. ^{13}C, ^{2}H, ^{15}N) are often more useful.

The aim of this project is to develop synthetic routes that are suitable for the incorporation of stable isotopic labels into glucosinolates. The isotopes must be incorporated into the side-chain, such that breakdown products will also be labelled. The synthetic procedures will be dominated by the commercial availability of isotopically labelled starting materials,

at reasonably low cost. The nature of the starting materials will, by necessity, determine the synthetic route employed. Initial work was aimed at the development of synthetic routes for isotopically labelled versions of glucobrassicin and glucoraphanin.

Results and Discussion

2.0 Results and Discussion

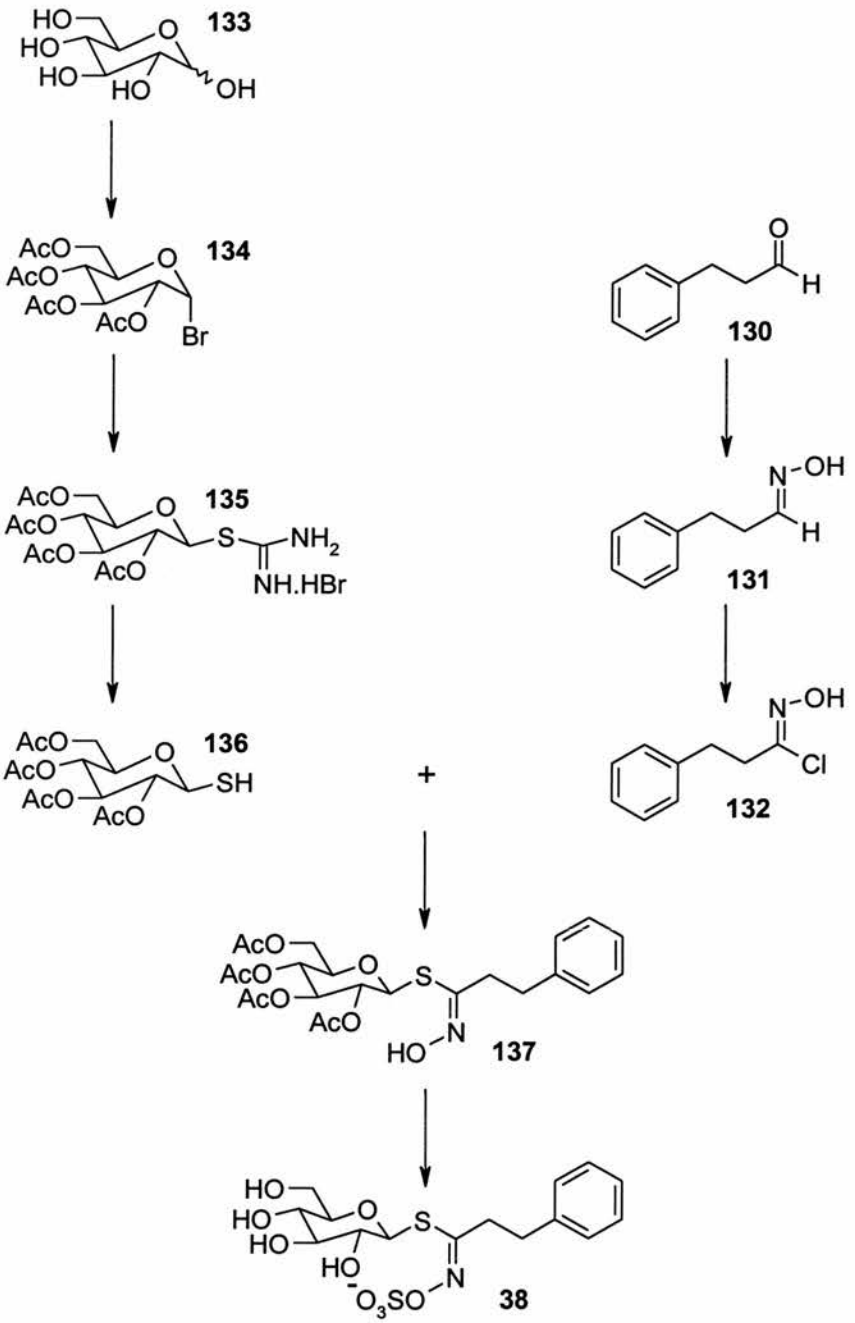
The ability to synthesise glucosinolates is important as it allows studies on the properties of compounds that may otherwise be difficult to obtain in sufficient quantities via isolation procedures. Methods which can be modified to allow the synthesis of isotopically labelled compounds open up further possibilities. Isotopically labelled glucosinolates can be used to distinguish between natural and synthetic compounds and their metabolites in studies on biological activity and metabolism. Isotopic labelling allows easy identification of the compounds, primarily by mass spectrometry, since the labelled compound would have a higher mass than its corresponding unlabelled analogue.

2.1 Synthesis of Gluconasturtiin

Early work was mostly based upon the procedures of Robertson[55] for the synthesis of gluconasturtiin to provide experience of glucosinolate synthesis, and to synthesise a compound to be analysed by MALDI-TOF mass spectrometry. Gluconasturtiin has previously been synthesised, but no mass spectrum of the intact glucosinolate has been possible due to the ionic nature of the sulfate group. MALDI-TOF mass spectrometry allows the identification of ionic compounds, and negative ion mode would provide an ideal method of identifying a glucosinolate.

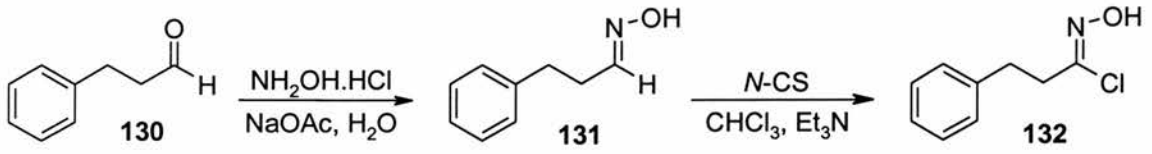
The overall synthetic route to gluconasturtiin **38** is depicted in Scheme 28 The synthesis can be initially split into two halves. Firstly 3-phenylpropionaldehyde (hydrocinnamaldehyde) **130** is converted to its oxime **131**, and then to the oximyl chloride **132**. The other half of the molecule is prepared from D-glucose **133**, which is acylated and

brominated **134**, converted to an isothiouronium bromide **135**, then finally to thioglucopyranose **136**. The two halves are then coupled together **137**. The product is then sulfated and deprotected to yield gluconasturtiin **38**.



Scheme 28: Synthetic route for gluconasturtiin

2.1.1 Preparation of oximyl chloride

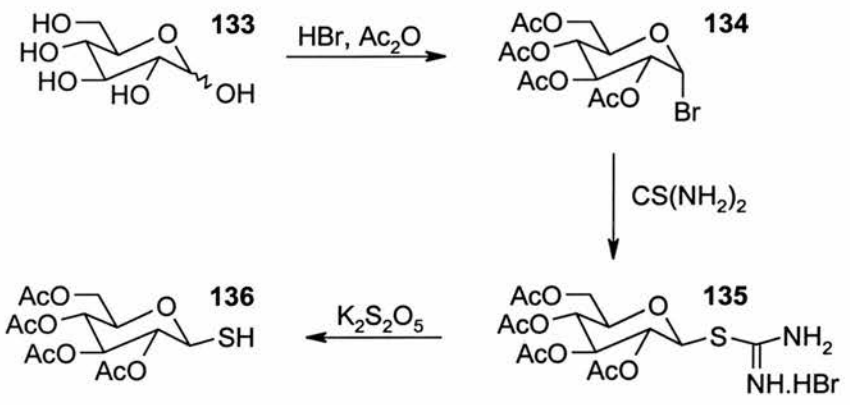


Scheme 29: Synthesis of 3-phenylpropyl oximyl chloride

3-Phenylpropionaldehyde **130** and hydroxylamine hydrochloride were heated together in water. Sodium acetate was added to yield the free hydroxylamine from the hydrochloride salt. After recrystallisation from ethanol, a yield of 79% was obtained offering a reasonable comparison with that obtained by Robertson[55] (93%). The IR spectrum showed peaks at 3200 and 1660 cm^{-1} consistent with the formation of an OH group, and a C=N group respectively. No peak was detected for C=O, indicating that reaction had taken place. In the ^{1}H NMR spectrum, the terminal CH resonance moved significantly upfield from 9.80 to 6.75 and 7.50 ppm, as a result of the change from CHO to CH=N. The two peaks showed the *E* and *Z* isomers of the product **131**. In the ^{13}C NMR spectrum, the peak of the former carbonyl carbon had moved 50 ppm upfield with the change to the oxime.

The oximyl chloride **132** was then prepared using *N*-chlorosuccinimide. The reaction was carried out in chloroform, with triethylamine present as a base. Previously, a solid product had been obtained,[55] but a golden oil was formed in this case. Due to the instability of the product, it could not be purified, but analysis showed that the oximyl chloride **132** had indeed been formed. The two CH=N peaks in the ^{1}H NMR spectrum of the oxime were not present, indicating that the proton had been replaced. In the ^{13}C NMR spectrum, the C=N peak had also moved significantly upfield, from 152 to 146 ppm, indicating a reaction had taken place.

2.1.2 Preparation of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose



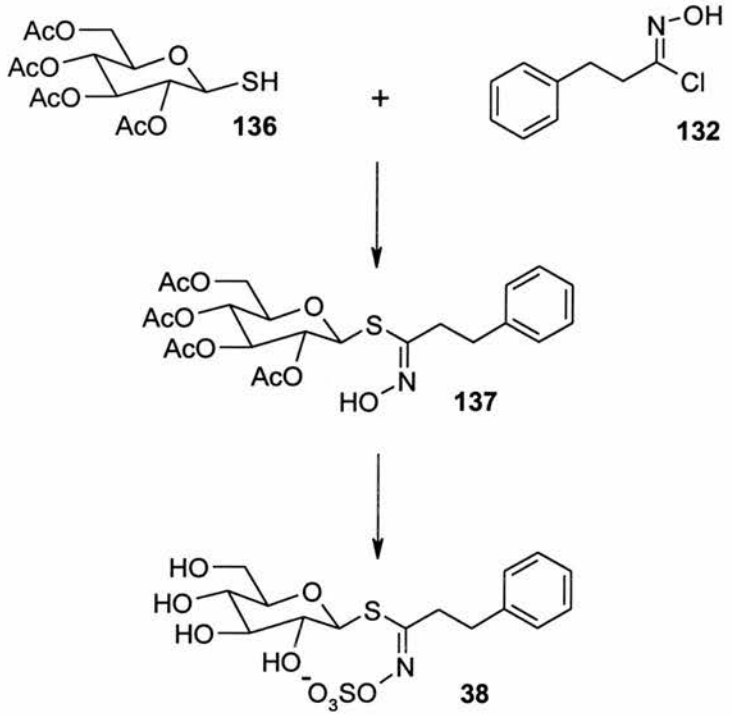
Scheme 30: Synthesis of 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranose (acetylated thioglucose)

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) **134** was prepared by reaction of D-glucose **133** with acetic anhydride and hydrobromic acid. In this reaction, acetic anhydride was also used as the solvent. Upon recrystallisation, the product was obtained in a respectable 70% yield. In the IR spectrum, the absence of an OH peak suggested that all the hydroxyl groups had been acylated, and the appearance of a carbonyl peak helped to confirm this. In the ^{1}H NMR spectrum, four singlets at around 2 ppm corresponded to the presence of acetyl CH_3 groups, the ratios indicating twelve protons, i.e. four acetyl groups. Additional peaks were observed in the ^{13}C NMR spectrum in two groups at around 21 and 170 ppm, corresponding to the presence of the acetyl groups, with two carbon atoms per group. The presence of the bromine atom was shown in the mass spectrum by the characteristic pattern of two molecular ion peaks in a 1:1 ratio, at 428 and 430 mass units. Acetobromoglucose can be obtained commercially, but its instability means that the compound is better prepared fresh in-house.

The isothiouronium bromide **135** was prepared by heating a solution of acetobromoglucose **134** in acetone to reflux in the presence of thiourea. This results in displacement of the bromine. Following recrystallisation, a 50% yield was obtained. Whilst this is not an impressive yield, it compares very well with that obtained by Robertson.[55] The IR spectrum of the product showed a peak corresponding to the presence of an NH group. In the ^{1}H NMR spectrum, the *H*-1 peak had moved significantly upfield from 6.60 to 5.38 ppm. The *H*-2 peak had also moved upfield, though not to the same extent. In the ^{13}C NMR spectrum, the *C*-1 and *C*-2 peaks had also moved upfield, and the presence of a new peak at 170.3 ppm, due to a new C=N group, confirmed that reaction had taken place.

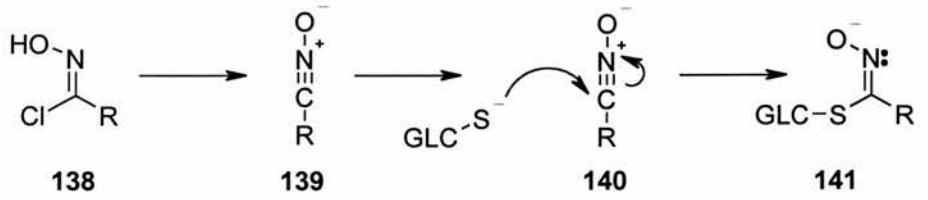
The isothiouronium bromide **135** was then converted to the glucopyranose **136** by heating to reflux in a biphasic solution of water and DCM with potassium metabisulfite. After recrystallisation, a 92% yield of the product was obtained, comparing favourably with the 100% yield obtained by Robertson.[55] A peak at 3460 cm^{-1} due to SH was observed in the IR spectrum, indicating that the isothiouronium group had been cleaved. In the ^{1}H spectrum, the H-1 peak moved significantly upfield to 4.55 ppm, and the *C*-2 peak in the ^{13}C NMR spectrum moved downfield from 67.2 to 74.0 ppm. The *C*-1 peak, however, remained in the same place. The C=N peak of the starting material was also absent.

2.1.3 Construction of gluconasturtiin



Scheme 31: Construction of gluconasturtiin

The synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phenethyl thiohydroximate **137** involved the coupling of the two previously prepared halves of the molecule. The coupling reaction involves conversion of the oximyl chloride **138** to the more reactive nitrile oxide **139**. Nitrile oxides can be regarded as stabilised nitrilium ions, and kinetic studies have found that all of these reactions are stereospecific, giving a single *Z* oxime as the product.[115] It was shown that the lone pair of the nitrogen and the incoming thioglucose are situated in a *trans* relationship **140**, such that the hydroxyl group is pushed into a position *cis* to the thioglucose, leading to a *Z* product **141**. (Scheme 32) *Ab initio* molecular orbital calculations have suggested that the stereospecificity of the reaction is due to a stereoelectronic effect, and that the addition is concerted leading to a *Z* configuration of the product.[114]



Scheme 32: Nucleophilic addition to nitrile oxide always gives Z-configured product

The oximyl chloride **132** and glucopyranose **136** were dissolved in THF in the presence of triethylamine. Following purification by column chromatography, the product was obtained in a 72% yield. It is difficult to ascertain by NMR spectroscopy that the two reactants had coupled. However, in the ^{13}C NMR spectrum, the C=N peak had returned to a position similar to that in the oxime. In the IR spectrum, no SH peak was observed, but this could have been masked by the broad OH peak. The mass spectrum, however, confirmed that the two reactants had indeed coupled, showing the expected M^+ peak at 511 mass units.

The sulfation step of this reaction was performed by adding a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phenethyl thiohydroximate **137** to chlorosulfonic acid in dry pyridine and dry dichloromethane. Part of the work up involved the use of sodium bicarbonate to neutralise the solution. At this point it was discovered that acyl deprotection had taken place making further purification difficult. The compound was, however, suitable for analysis by MALDI-TOF mass spectrometry, which confirmed the correct mass of the gluconasturtiin anion while other spectral data confirmed that gluconasturtiin **38** had been produced.

The broad peak at around 3000 cm^{-1} in the IR spectrum indicated the presence of an OH group, and the carbonyl peak of the starting material was absent. In the ^1H NMR spectrum,

the peaks due to the methyl protons of the acyl groups were missing, and the peaks of hydrogens 6a and 6b could be observed separately, having moved upfield from their positions in the starting material. In the ^{13}C NMR spectrum, the methyl and carbonyl carbon peaks of the acyl groups in the starting material were also absent. The MALDI-TOF mass spectrum showed the mass of the anion to be 422, as expected for the product.

MALDI-TOF mass spectrometry is particularly useful for analysing glucosinolates because it is very sensitive. It was found that detection could be achieved with as little as 0.5 ng on the plate.[147] The negative ion mode is also very useful as it allows the intact glucosinolate to be analysed. Other methods give poor resolution and require the glucosinolate to be desulfated to provide a sample suitable for analysis, although recently LC-MS methods for intact glucosinolates have been developed.[148] Other advantages of MALDI-TOF MS include its high speed of analysis and its ability to analyse complex mixtures, such that several glucosinolates can be identified in one sample. It was found that very clear spectra were obtained from crude plant extracts prepared from cauliflower florets, rutabaga peel and turnip, and strong peaks corresponding to specific glucosinolates were observed.[147]

2.2 Proposed Synthesis of Glucobrassicin

The aim of this project was to develop novel synthetic routes for glucosinolates with a view to isotopic labelling. One target compound was glucobrassicin, whose breakdown products have been shown to prevent various forms of cancer.[149,150] In the case of glucobrassicin **20**, a logical compound from which to start the synthesis was indole-3-acetic acid **142**, since it is commercially available in various isotopically labelled forms (^{13}C **143**, ^{14}C **144a, 144b**). (Figure 7)

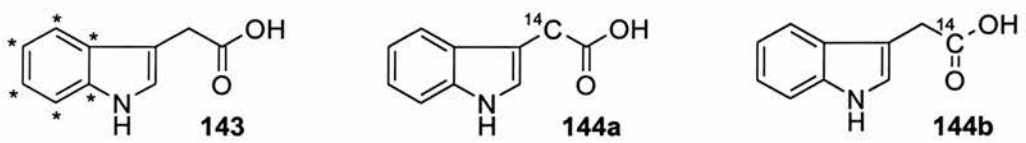
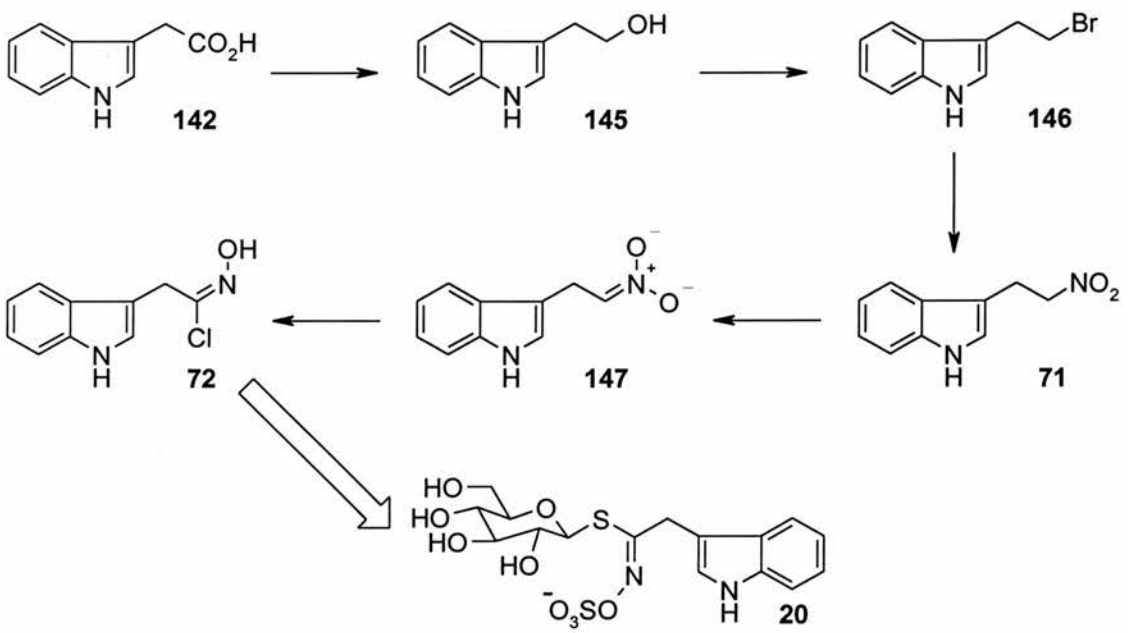


Figure 7: Various commecially available isotopically labelled indole-3-acetic acid compounds

The usual synthetic route for glucobrassicin, however, does not start from indole acetic acid. The route devised by Rollin *et al.*[130] used 3-formylindole as the precursor, which was reacted with nitromethane to produce the vinylnitro derivative. Reduction of this compound then gave the 3-(2'-nitroethyl)-indole **71**, which was taken through the remainder of the synthetic route outlined in Scheme 20 (Section 1.6.1).

The first step in the proposed new synthetic route involved the reduction of indole-3-acetic acid to give 3-(2'-hydroxyethyl)indole **145**, commercially known as tryptophol. The hydroxyl group would then be replaced by a bromide **146** to provide a good leaving group for the nitration step. Nucleophilic substitution would then give the nitro compound **71** and from this, the nitronate salt **147** could be formed. Following this step, the oximyl chloride **72** would be produced, and then coupled to the same glucopyranose **136** moiety as used in

the synthesis of gluconasturtiin. The same deacylation and sulfation steps as for gluconasturtiin would also be used to yield glucobrassicin **20**. (Scheme 33)



Scheme 33: Proposed synthesis of glucobrassicin

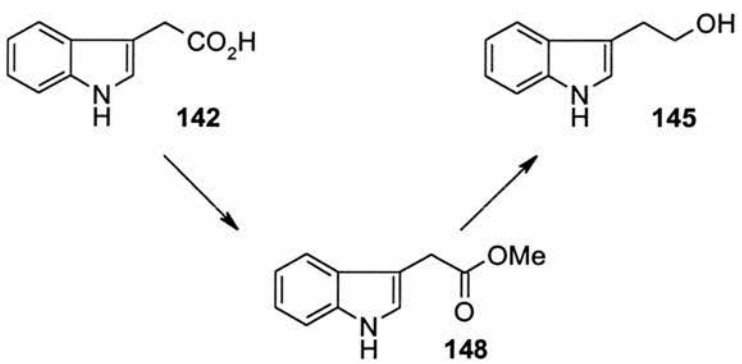
2.2.1 Reduction of indole-3-acetic acid

Scheme 34: Reduction of indole-3-acetic acid

Initially, indole-3-acetic acid **142** was reduced to 3-(2'-hydroxyethyl)-indole **145** by stirring with an excess of lithium aluminium hydride in dry diethyl ether. The yield, however, was only 38%, with a large proportion of the starting material left unreacted. The melting point obtained was only slightly lower than the literature value,[151] 52-54 °C compared to 59 °C. In the IR spectrum, the peak corresponding to a carbonyl group,

observed in the starting material, had disappeared, but the OH and NH peaks remained at 3300 and 3387 cm^{-1}. This is consistent with the change from carboxyl to hydroxyl functionality. The ^{1}H NMR spectrum showed two CH_2 groups as triplets at 3.16 and 4.04 ppm, as expected. In the ^{13}C NMR spectrum, the carbon of the former carboxyl group had moved significantly upfield from 172.7 to 63.0 ppm, with the change to hydroxyl functionality. The mass spectrum showed the expected molecular ion peak at 161 mass units, and a peak at 130, consistent with the loss of the CH_2OH group.

One of the reasons suspected for the low yield of the reduction may be that one of the hydride ions removes the proton of the carboxylic acid, rather than attacking at carbon atom. Adding excess lithium aluminium hydride would solve this problem, but the salt produced when the proton is removed is less reactive to attack by hydride. A solution to this problem was to esterify the acid, and reduce the ester instead. (Scheme 35)



Scheme 35: Modification of reduction of indole acetic acid. The acid is first convered to its methyl ester

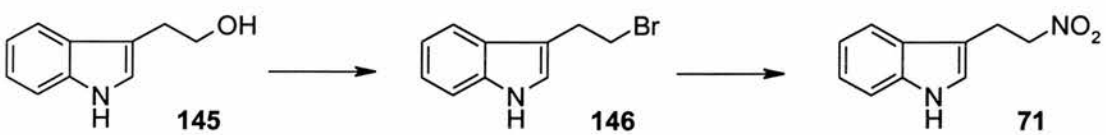
Indole acetic acid **142** was, therefore, converted to the corresponding methyl ester **148** by heating it with methanol in the presence of thionyl chloride. Following purification by column chromatography on silica, the product was obtained as an off-white solid in a yield

of 74%. In the ^{1}H NMR spectrum, a new singlet peak with an integral ratio of 3 was observed, corresponding to the newly formed methyl group of the ester. The singlet peak of *H*-2 had also moved significantly upfield, likely due to the change of the electron withdrawing effect.

The reduction of methyl indole-3-acetate **148** was then accomplished by treatment with di-isobutylaluminium hydride in dry diethyl ether. After purification by column chromatography on silica, the product was obtained as an off-white solid in a yield of 63%.

Converting indole acetic acid to the methyl ester and reducing it was a worthwhile exercise as the overall yield (60%) of both esterification and reduction steps was higher than the 38% achieved for the direct reduction of the acid.

2.2.2 Synthesis of 3-(2'-nitroethyl)indole



Scheme 36: Conversion of 3-(2'-hydroxyethyl)-indole to the nitro compound

It was then necessary to convert the alcohol to the bromide in order to carry out the subsequent nitration step. The first method attempted for this synthesis involved adding phosphorus tribromide to 3-(2'-hydroxyethyl)-indole **145** in dry diethyl ether. A poor yield of 23% was obtained from this reaction, possibly due to the phosphorus tribromide having decomposed, and a different method was thus sought.

An alternative reaction employed carbon tetrabromide and triphenylphosphine, in dry dichloromethane as solvent, and gave a yield of 85% following purification. The melting point was consistent with the literature value and in the IR spectrum only the peak due to the NH, at 3395 cm^{-1}, was observed, indicating that the hydroxyl functionality had been replaced. In the ^{1}H NMR spectrum, it was observed that the peak due to the CH_2 group attached directly to the heterocyclic ring had moved slightly downfield from 3.16 to 3.35 ppm. There was a much larger difference for the terminal CH_2 group, the peak for which had moved upfield from 4.04 to 3.65 ppm, indicating a change in the functionality on the terminal group. The ^{13}C NMR spectrum also confirmed this with a significant shift from 63.0 to 32.8 ppm for the terminal carbon. In the mass spectrum, two MH^+ peaks, in a 1:1 ratio, at 224 and 226 mass units were observed, characteristic of the presence of a bromine atom in the molecule. Peaks at 144 and 130 mass units were also observed signifying the loss of the bromine and CH_2Br groups respectively. This method proved to be far more successful, and was consistently so. This may be because the reagents are much more stable.

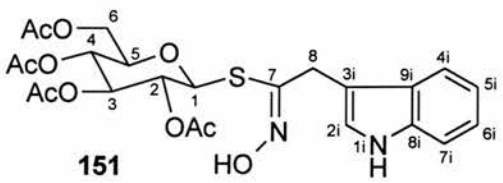
Several methods were attempted to convert 3-(2'-bromoethyl)-indole **146** to the corresponding nitro compound **71**. Initial attempts followed the procedure of Rollin *et al.*[131] using sodium nitrite in dimethyl formamide under reflux, but purification of the product proved troublesome. The reaction temperature produced many decomposition products that were difficult to remove, so a different synthesis was sought. The ion exchange method[152] used to convert 1-bromo-5-chloropentane **149** to its nitro equivalent **150** (Section 2.3) was employed. This method used Amberlite IRA 900 resin in the NO_2^- form to exchange the bromide functionality for NO_2 functionality. Unfortunately, no reaction was observed. Many methods involving silver nitrite were found in the

literature,[153-156] but several attempts did not produce any of the desired product. In most cases, the bromide starting material **146**, or 3-(2'-hydroxyethyl)indole **145**, was recovered from the reactions. Tetraethylammonium nitrite was synthesised to react with the bromo indole in dry acetonitrile, but again, no reaction was observed.

It was found that treatment of 3-(2'-bromoethyl)indole **146** with sodium nitrite and potassium iodide in acetone, using 18-crown-6 to help solubilise the inorganic salts, was the best method. The potassium iodide acts as a catalyst, initially exchanging the bromide group of the substrate for iodide, which is a better leaving group allowing a faster reaction with sodium nitrite. Following purification by column chromatography on silica, the product was obtained as an off-white solid in 52% yield.

The melting point of the product was only slightly lower than the literature value. The ^{1}H NMR spectrum showed that the peak for the CH_2 group attached to the heterocyclic ring had only shifted slightly downfield, but the terminal CH_2 group had shifted quite considerably from 3.65 to 4.66 ppm. The changes in the ^{13}C NMR spectrum were even more pronounced, with the terminal carbon moving downfield by over 45 ppm, from 29.3 to 75.8 ppm. In the mass spectrum, the MH^+ peak at 191 mass units was consistent with the product, and the same peaks at 144 and 130 mass units were observed as in the spectrum of 3-(2'-bromoethyl)-indole, indicating the loss of the NO_2 and CH_2NO_2 groups respectively.

2.2.3 Synthesis of acetylated desulfoglucobrassicin



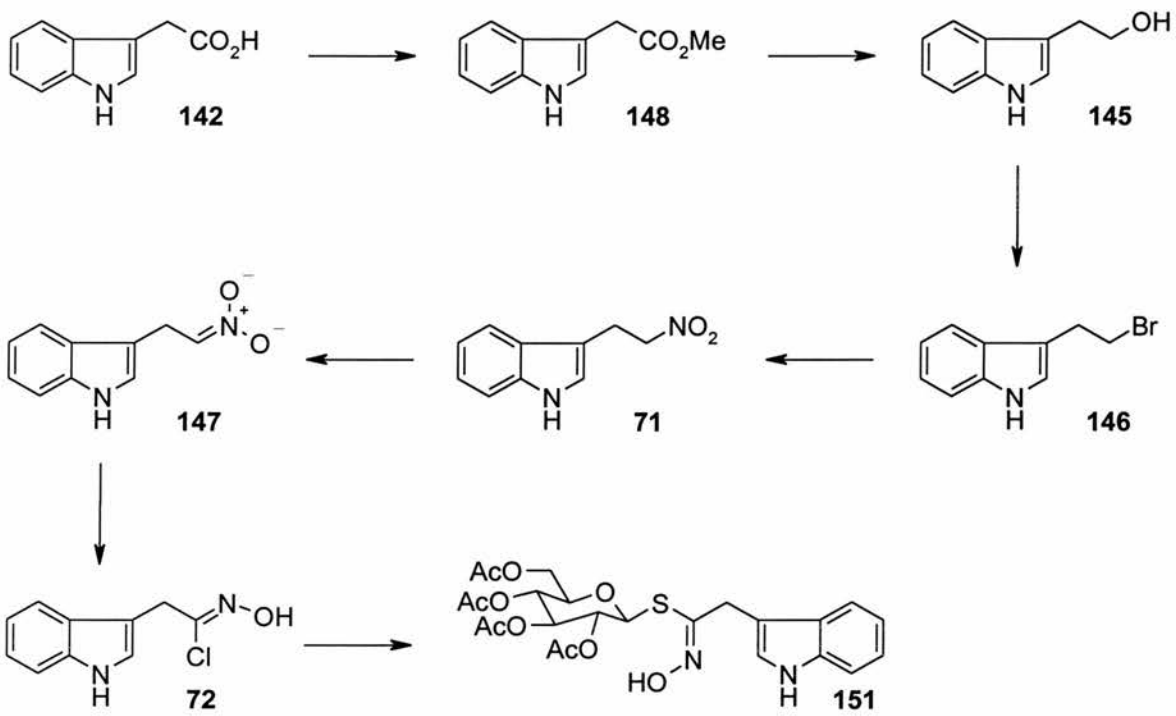
151

The oximyl chloride **72** of the 3-(2'-nitroethyl)-indole was firstly prepared by treatment of the nitro compound **71** with sodium in methanol to yield the corresponding nitronate **147**, which was reacted with thionyl chloride in 1,2-dimethoxyethane at -40 °C. The hydroximyl chloride **72** was then coupled to the sugar unit **136** using dry triethylamine in a mixture of dichloromethane and diethyl ether. Purification by column chromatography on silica gave the product **151** as a light brown solid in a yield of 39%.

The yield is a reasonable one considering the three steps involved in the coupling reaction, and is the same as that obtained by Robertson.[55] The IR spectrum showed the presence of a hydroxyl group, consistent with the oxime functionality of the product. In the ^{1}H NMR spectrum, the triplet for the protons on *C*-7 had disappeared, showing the change in the functionality at the carbon. The peak representing the protons of the CH_2 group adjacent to the indole group had moved from 3.48 to 3.4 ppm with the change in functionality. The oximyl carbon peak had moved considerably downfield from 75.8 to 151.9 ppm in the ^{13}C NMR spectrum. The mass spectrum showed the expected peak at 556, the product bound to sodium.

2.2.4 Conclusion

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl glucobrassicin thiohydroximate **151** was thus prepared in 7 steps from indole-3-acetic acid **142**. The proposed synthetic route only required slight modification, the incorporation of the initial esterification step, to make it a viable one. (Scheme 37)

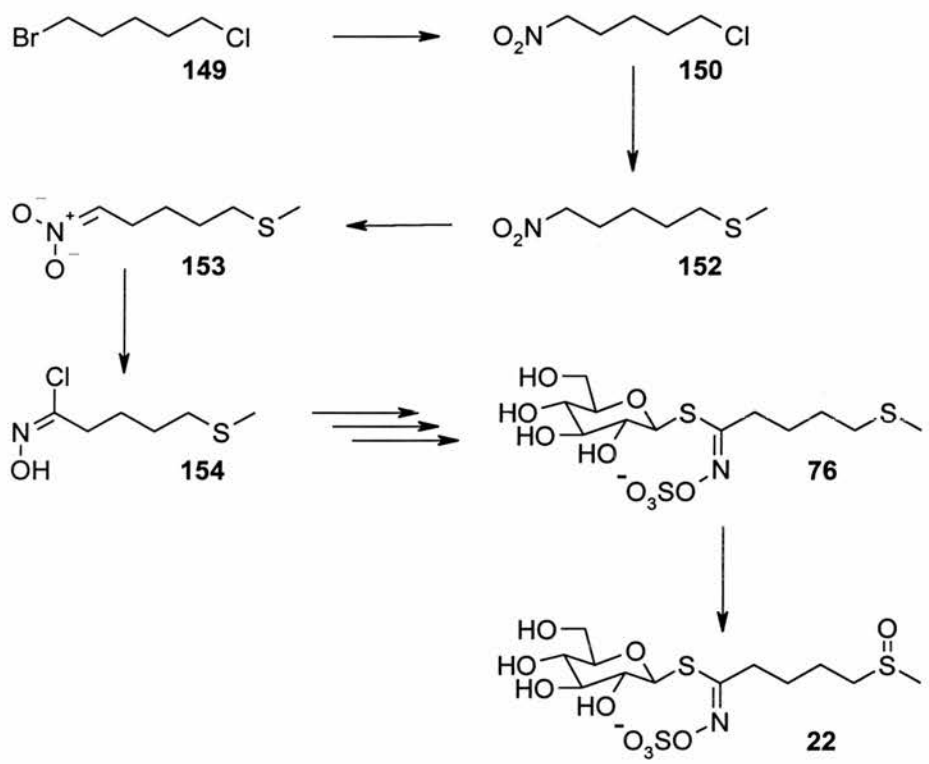


Scheme 37: Overall synthetic route from indole-3-acetic acid to acetylated desulfoglucobrassicin

Further reaction to produce glucobrassicin was not attempted since these reactions have been successfully carried out in the past, and it was therefore deemed unnecessary to repeat them. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl glucobrassicin thiohydroximate **151** was successfully prepared in a respectable overall yield of 10% over five steps from indole-3-acetic acid **142**.

2.3 Studies on Methods for the Isotopic Labelling of Glucoraphanin

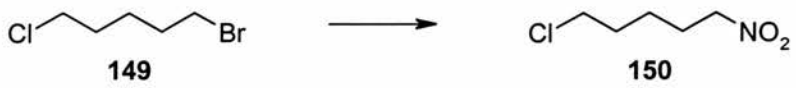
Glucoraphanin **22** is another glucosinolate in which there is a great deal of interest, due to its potential cancer-preventative properties, but very few synthetic routes have been employed for this compound. Rollin *et al.*[131] developed a route (Scheme 38), using 1-bromo-5-chloropentane **149** as starting material. This was converted to chloronitropentane **150** then thiomethylated **152**, converted to the nitronate salt **153**, and the oximyl chloride **154**. Coupling to the sugar unit **136** proceeded as in the synthesis of glucobrassicin. Sulfation and deprotection give glucoerucin **76**, which is oxidised using hydrogen peroxide to give glucoraphanin **22**.



Scheme 38: Synthetic route for unlabelled glucoraphanin

2.3.1 Synthesis of 1-Chloro-5-nitropentane

It was suggested that the synthesis of the unlabelled glucosinolate would be useful to investigate reactions that may be used in a labelled synthesis of glucoraphanin. The synthesis of Rollin *et al.*[130] was used as a basis for this synthesis.



149 **150**

Scheme 39: Conversion of 1-bromo-5-chloropentane to the nitro-compound

1-Chloro-5-nitropentane **150** was first prepared by reaction of 1-bromo-5-chloropentane **149** with sodium nitrite in acetone, using 18-crown-6 to help solubilise the sodium nitrite. Although this method did produce the desired product, the yield, estimated at 40%, was low. This may be due to the nature of the nitrite salt, which may react through the oxygen as well as the nitrogen. The nitrite ester form of the product would not be stable, and would likely hydrolyse to leave the hydroxyl functionality after work up.

The second method involved the use of ion-exchange resin.[152] The resin has bound anions, initially Cl^- ions, but washing the resin with sodium nitrite solution exchanged the Cl^- ions with NO_2^- ions, converting it to the nitrite form. The resin can then exchange a suitable functional group of a substrate with its bound anion. In this case, the resin, in nitrite form, was stirred with 1-bromo-5-chloropentane in toluene and gave 1-chloro-5-nitropentane in a yield of 85% following purification.

^{1}H NMR spectroscopy showed that the peak at 3.41 ppm due to the CH_2Br in the starting material had moved to 4.42 ppm, and the multiplet at 1.86 ppm, due to the convergence of

the triplets of the 2- and 4-CH_2 quintets, had separated to give two distinct quintets at 1.80 and 2.05 ppm respectively. The ^{13}C NMR spectrum showed that the *C*-5 carbon had moved significantly from 33.7 to 75.7 ppm, and the *C*-4 had moved slightly upfield. The mass spectrum showed the expected molecular ion peaks in the ratio consistent with the presence of a chlorine atom, and other peaks in the spectrum corresponded to the loss of the chlorine, at 116 mass units, and the loss of the NO_2 group, at 105 and 107 mass units.

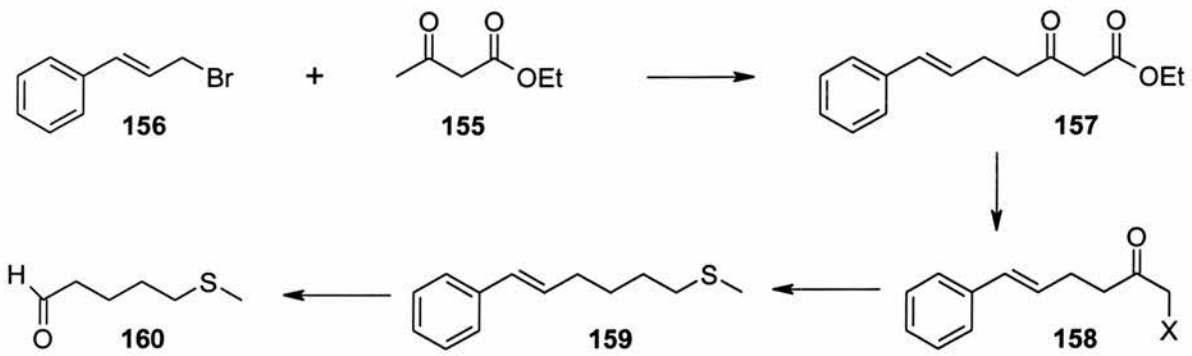
This method did not prove to be reliable, however, and the purification of the product was also very difficult. The route was abandoned to be returned to later on. Meanwhile, the development of an alternative route for the synthesis of labelled glucoraphanin was the next target.

2.3.2 Synthesis of Labelled Glucoraphanin

The greatest difficulty with the synthesis of isotopically labelled glucoraphanin was the identification of suitable commercially available starting materials. Certainly, none of the precursors employed by Rollin's route[131] are available and so a different strategy had to be developed to introduce the labelled atoms.

Ethyl acetoacetate **155**, can be purchased with either one or two ^{13}C atoms. Being a four carbon unit, it would therefore require some form of chain extension to give the five carbon chain required for the target. It was decided to use cinnamyl bromide **156** as the compound to couple with ethyl acetoacetate for a variety of reasons. Firstly, it can provide the extension to the chain that is required, with only a simple decarboxylation required to give the desired five carbon moiety. Secondly, the use of an aromatic unit is useful for

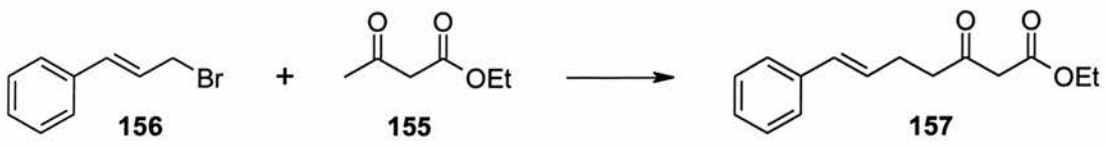
TLC analysis to allow visualisation by UV, and thirdly, it will add some mass to the intermediates through the synthesis, decreasing their volatility, and preventing loss of material. The removal of the cinnamyl group could be achieved at the end by oxidative cleavage of the alkene to give the aldehyde **160**, possibly via ozonolysis. (Scheme 40)



Scheme 40: Proposed synthetic route for ^{13}C-labelled glucoraphanin

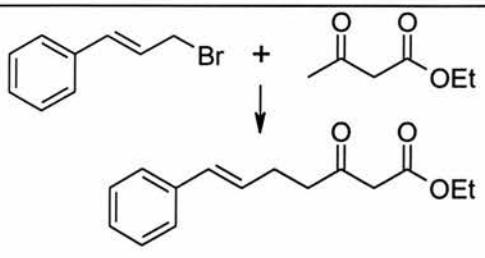
Thus the two starting materials are first coupled to give the ester **157**, which has some form of leaving group added to the carbon between the two carbonyl groups. Hydrolysis and decarboxylation would leave only a single carbonyl group in the chain **158**, as well as having functionality that could easily be substituted with a thiomethyl group. Reduction would remove the second carbonyl group to leave a compound **159** which, following ozonolysis, would give **160**, a suitable pentane derivative.

2.3.2.1 Studies on coupling reactions



Scheme 41: Coupling of cinnamyl bromide and ethyl acetoacetate

It was initially difficult to find a suitable method to couple cinnamyl bromide **156** and ethyl acetoacetate **155**. NMR spectra of the products of various different reactions were very messy and did not look promising. The difficulty in the reaction was that the di-anion of ethyl acetoacetate had to be created, so that reaction would occur at the methyl carbon rather than at the central CH_2. An initial treatment with base will remove a proton from the central CH_2 group since these protons are the most acidic. A second treatment with base, however, will remove the next most acidic proton, a proton from the methyl group, to create the di-anion. Since the second proton to be removed is less acidic, the nucleophilicity of the terminal anion will be substantially higher, and this will therefore react preferentially. The choice of bases is therefore crucial to create an anion that will be stable in solution. A successful reaction was found, however, and the desired product was prepared by treating ethyl acetoacetate **155** with sodium hydride and *n*-butyllithium in dry THF at 0 °C. Addition of cinnamyl bromide **156** to the solution furnished the desired product. Purification by column chromatography on silica gave the product **157** as a pale yellow oil in a yield of 48%.



Bases used	*Result*
Na, NH_3, Fe(III)NO_3	Unsuccessful
LDA, *n*-BuLi	Unsuccessful
NaH, *n*-BuLi	**Successful**
NaH, KHMDS	Unsuccessful

Table 1: Different methods attempted in coupling cinnamyl bromide and ethyl acetoacetate

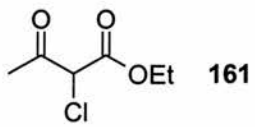
In the ^{1}H NMR spectrum, the former CH_2Br peak had moved upfield with the change in functionality. The peak of the ketone methyl group of ethyl acetoacetate had also moved, in this case, slightly downfield, and showed the change in integral ratio from three to two protons. In the ^{13}C spectrum, the peak for the same carbon had also shifted significantly from 30 ppm to 43 ppm. There was a slight change for the $CHCH_2$ peak, but it was much less pronounced. The mass spectrum showed the expected molecular ion peak at 247, and also a fragment that would represent the molecule with the loss of CO_2Et.

The purification of this compound proved to be difficult, however, due to ethyl acetoacetate having a very similar retention time on silica. It was decided that the compound would be carried through to the decarboxylation step without purification, since the ethyl acetoacetate decarboxylation product would be acetone, which could be easily removed in the work up.

Hydrolysis and decarboxylation were carried out in a single step by heating the ester **157** in aqueous hydrochloric acid to reflux. The product **158** (X=H) was purified by column chromatography. The yield for the decarboxylation step is most likely quantitative, since the yield of 48% over two steps is the same as that achieved for the coupling reaction.

The ^{1}H NMR spectrum peaks corresponding to the ethyl group were not present, but a peak at 2.3 ppm with an integral ratio of 3 was present, confirming the loss of the ester group and formation of the terminal methyl group. In the ^{13}C NMR spectrum, the peaks for the ethyl group, and the ester carbonyl carbon were not present, also showing the loss of the ester.

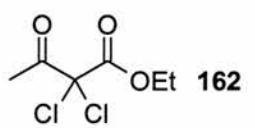
Since the coupling and decarboxylation had proceeded smoothly, a leaving group had to be introduced to facilitate the addition of the thiomethyl functionality. It was anticipated that chlorination between the two carbonyls could be achieved by employing sulfuryl chloride. This reaction was tested on ethyl acetoacetate **155**, which was treated with sulfuryl chloride in dichloromethane at room temperature to furnish the monochlorinated product **161** in quantitative yield. Purification was not required.



161

The ^{1}H NMR spectrum showed that chlorination had taken place, with the shifting of the peak for the former CH_2 protons downfield from 3.4 ppm in the starting material to 4.8 ppm in the product. The integral ratio had also dropped from 2 to 1 proton. In the ^{13}C NMR spectrum, the change was also observed for the CHCl carbon peak, with a slight shift upfield from the starting material, and also the upfield shifting of the ketone carbon peak.

It was also discovered that leaving the reaction longer allowed the production of the dichlorinated species **162**. The reaction took place in almost quantitative yield, and with very high purity.



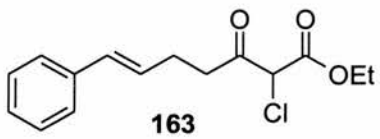
162

In the ^{1}H spectrum, the change to the dichlorinated compound **162** was observed with the disappearance of the peak for the CH_2 group that was present in the starting material. The ^{13}C spectrum showed that the peak for the CCl_2 carbon had shifted significantly from 50

ppm in the starting material to 82 ppm in the product. As with the monochlorinated compound, the ketone carbonyl peak had shifted slightly upfield.

With this in hand, reactions were attempted to chlorinate between the carbonyls of the coupled product **163**. This, however, did not go to plan, and resulted in a complex mixture of products. Analysis of the crude product by ^{1}H NMR showed that the peaks corresponding to the double bond were barely visible, suggesting that the sulfuryl chloride was reacting at the double bond rather that at the desired point. Reactions employing a mild base and *N*-chlorosuccinimide were attempted, but these also proved fruitless.

The next logical step was the coupling of cinnamyl bromide and ethyl 2-chloroacetoacetate.



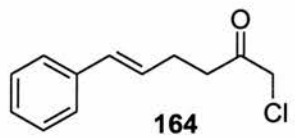
163

The chlorinated β-diketone was synthesised by creating the di-anion of ethyl 2-chloroacetoacetate **161** and reacting it with cinnamyl bromide, as per the synthesis of ethyl 3-oxo-7-phenyl-hept-6-enoate **157**. Purification on column chromatography yielded the product in a very poor 1% yield.

The ^{1}H NMR spectrum showed that the peak due to the C*H*Cl proton had moved significantly downfield in comparison with the protons in the same position of the non-chlorinated compound. The integral ratio had dropped from 2 protons in the non-chlorinated compound **157** to 1 in the chlorinated **163**. No significant differences were

observed in the ^{13}C spectrum. The mass spectrum showed the two expected molecular ion peaks, in the correct ratio, for the presence of a chlorine atom in the molecule.

The poor yield could be attributed to lithium halogen exchange between the *n*-butyllithium and the chlorine of ethyl chloroacetoacetate. If this occurs in preference to deprotonation then the previously synthesised non-chlorinated derivative will be produced. Potassium bis(trimethylsilyl)amide was tried as a substitute for the butyllithium, but no reaction was observed.



164

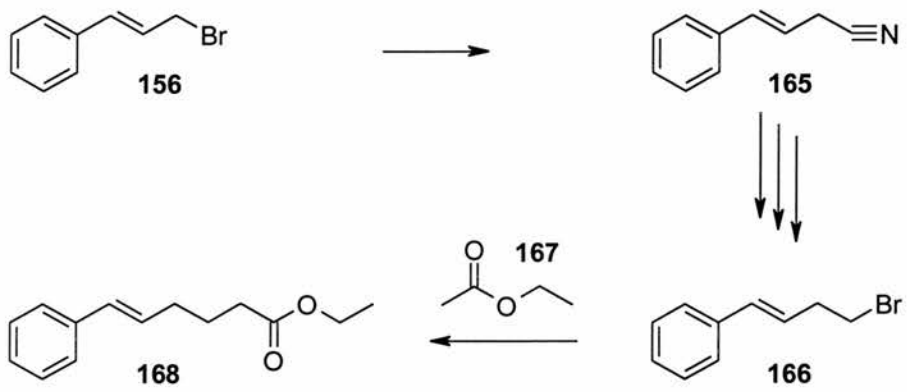
2-Chloro-6-phenylhex-5-en-2-one **164** was prepared in the same way as that of the non-chlorinated compound, by heating in aqueous hydrochloric acid. The product was purified by column chromatography on silica to give the desired product in 2% yield, based upon the quantities of the compounds used in the previous step.

The ^{1}H NMR spectrum showed that the peaks due to the ester functionality were no longer present, and that the peak of the protons on the now terminal carbon had moved upfield due to the change. In comparison with the non-chlorinated compound, the peak of the terminal protons was significantly upfield in the chlorinated compound. The ^{13}C spectrum also showed the peaks for the ester had disappeared, and in comparison with the non-chlorinated compound, the peak for the terminal carbon was significantly downfield. The mass spectrum showed the two molecular ion peaks in the correct ratio for the presence of a chlorine atom in the molecule.

Although it was possible to produce a small amount of the chlorinated hexenone, the yield of 2% over the two steps is obviously far too low to attempt any further synthesis through this route. No other bases were found that would allow cinnamyl bromide and ethyl acetoacetate to be coupled together, without the risk of removing a halogen in the reaction of the chlorinated derivative. A different approach to the problem therefore had to be developed.

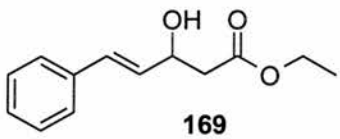
2.3.2.2 Attempted synthesis with Ethyl Acetate

The investigation of the commercial availability of alternative ^{13}C labelled compounds found ethyl [$^{13}C_2$]acetate to be an interesting option. Although its coupling with cinnamyl bromide would mean that the chain was one carbon too short, it would incorporate two ^{13}C atoms into the molecule. The extra carbon unit could easily be incorporated by the nucleophilic substitution of cyanide on cinnamyl bromide. Since isotopically labelled cyanide is a good cheap source of ^{13}C label, it would provide a simple method for adding a further carbon label to the compound. Previous work has often used ^{13}C-labelled cyanide for this purpose.[157,158] Ideally, the extra carbon unit would be added to the cinnamyl unit prior to the addition of ethyl acetate, so that the expensive labelled ethyl acetate would be taken through as few synthetic steps as possible. (Scheme 42)



Scheme 42: Proposed extension of cinnamyl bromide and reaction with ethyl acetate would give a compound able to furnish the desired 5-carbon unit

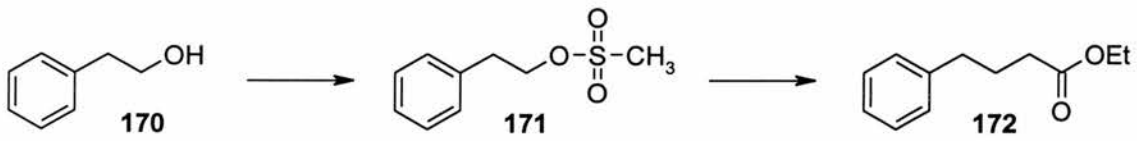
A literature procedure for the addition of ethyl acetate to cinnamaldehyde was found.[159] This reaction was tested as the basis for the addition of ethyl acetate to the cinnamyl unit.



The lithium stabilised enolate of ethyl acetate was created by treatment of ethyl acetate **167** with lithium bis(trimethylsilyl)amide at –78 °C. Subsequent reaction with cinnamaldehyde generated the product **169** in a yield of 30%. The ^{1}H NMR spectrum of the product showed a multiplet peak at 2.75 ppm, consistent with the CH_2 protons situated between the carbonyl and hydroxyl carbons, the move downfield from 2.04 ppm of ethyl acetate. A peak at 4.80 ppm for the proton on the hydroxyl carbon showed the change from aldehyde to alcohol functionality. The ^{13}C spectrum also showed some major changes due to the changes in functionality. The *C*-2 carbon peak had moved downfield from 21 to 42 ppm, the *C*-4 peak had also shifted downfield, while the greatest change was of the *C*-3 carbon, the peak of which moved upfield from 194 to 69 ppm.

Unfortunately, the yield of 30% did not compare favourably with the literature yield of 94%, but the reaction was not optimised, so it is unsurprising that the yield was poor. The reaction did yield the correct product, however, and it was decided to attempt the reaction on some other suitable substrates. Since the reaction would have to be carried out on a non-vinylic substrate, due to the need to extend the cinnamyl chain, cinnamyl bromide was initially ruled out. A suitable alternative was found in phenethyl bromide. Unfortunately, this reaction failed to give any of the desired product. It was thought that the bromide was not sufficiently labile, so a better leaving group was investigated.

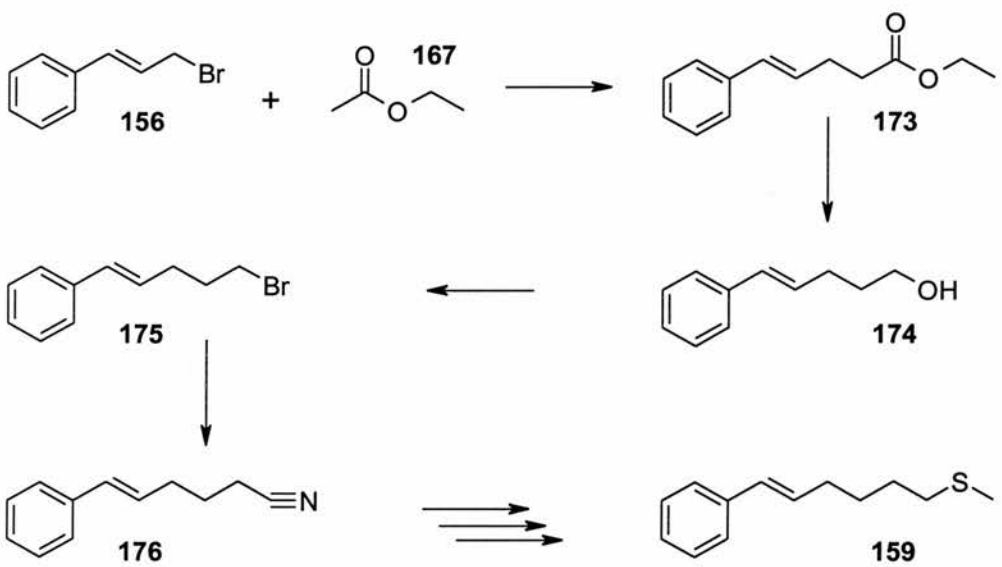
Phenethyl alcohol **170** was treated with methanesulfonyl chloride at –23 °C in the presence of triethylamine. The mesylate solution was added to a solution of ethyl acetate that had been treated with lithium bis(trimethylsilyl)amide.



Scheme 43: Proposed mesylation of phenethyl alcohol and reaction with ethyl acetate

Analysis of the product showed that the mesylate **171** had indeed been formed, but that no subsequent reaction had taken place. The ^{1}H NMR spectrum showed a new peak due to the methyl group of the mesylate compound, and this reflected in the ^{13}C spectrum where a new peak for the carbon of the methyl group was present. The peak for the protons of the former hydroxyl carbon had also shifted significantly downfield in the proton NMR spectrum, and again the change, though less pronounced, was observed in the ^{13}C spectrum.

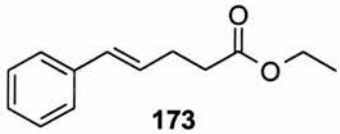
The mesylate leaving group had proved to be unreactive, and even survived purification by column chromatography, so a reaction with cinnamyl bromide **156** and ethyl acetate **167** was undertaken. If the ethyl acetate was added to cinnamyl bromide, the chain would be one carbon short, thus necessitating a change to the route to incorporate another carbon unit. (Scheme 44) This change would be detrimental to the route, since the incorporation of ethyl acetate would occur earlier, and any losses in yield would therefore include losses of the expensive labelled compound.



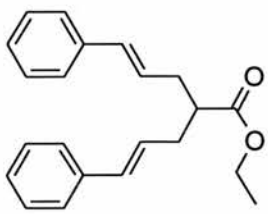
Scheme 44: Proposed reaction of ethyl acetate with cinnamyl bromide and cyanide extension to give 5-carbon moiety

Ethyl acetate **167** was treated with lithium bis(trimethylsilyl)amide to generate its enolate. Reaction of the enolate with cinnamyl bromide **156** and subsequent purification by column chromatography gave a clear oil. Initial analysis suggested that the compound had been formed correctly, but with some impurity. Closer inspection, however, revealed that a mixture of the correct product **173**, as well as a product of two cinnamyl bromide molecules and one ethyl acetate molecule had formed **177**. This was most evident in the

mass spectrum where separate analyses found molecular ion peaks for both compounds. Re-evaluation of the ^{1}H NMR spectrum allowed for full interpretation with the integral ratios matching a mixture of the products in an approximate ratio of monomer to dimer 5:1. The ^{13}C NMR spectrum was also able to be fully assigned, helped by later work which successfully created the monocinnamyl compound.



173



177

Several attempts were made to try to form only the desired product, or at least minimise the dicinnamyl compound, but these were in vain. This route therefore appeared to be unsuitable.

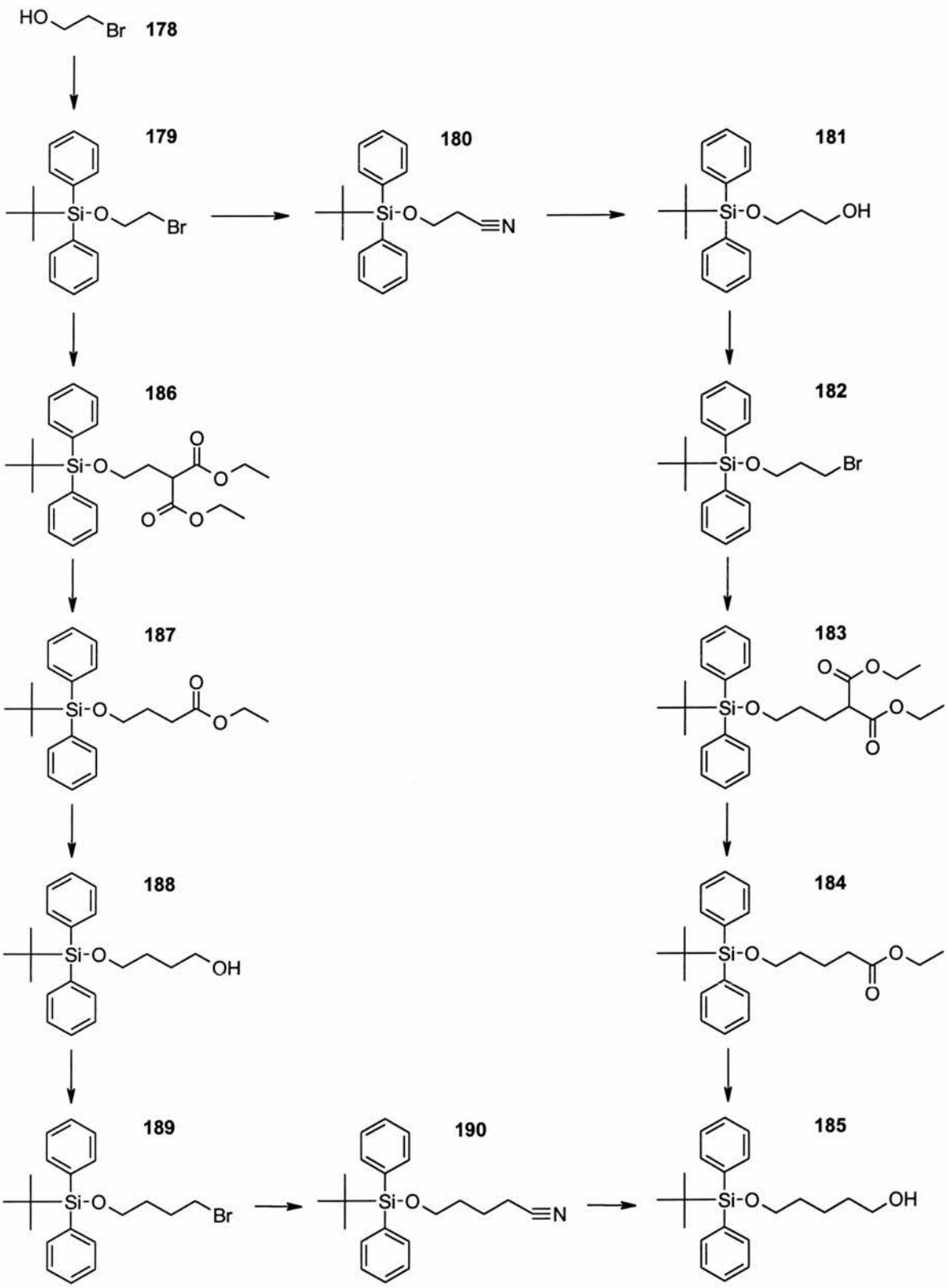
2.3.2.3 Attempted Synthesis using 2-Bromoethanol

Another compound which provoked interest was 2-bromoethanol **178**. Again, this compound was available with two ^{13}C atoms, and it had functionality well suited to the proposed synthetic route. The bromide would allow the addition of a carbon unit to increase the chain length, and the alcohol could be oxidised and converted to an oxime in the final steps of the synthesis.

It was decided to protect the alcohol with a *t*-butyldiphenylsilyl (TBDPS) group. The extra mass would prevent loss of the compound due to its high volatility, and protecting the alcohol would remove the possibility of side reactions occurring. A TBDPS group was

chosen for the purpose because of its higher stability under acidic and basic conditions than TBDMS (*t*-butyldimethylsilyl) group, whilst allowing UV visualisation of the compound by TLC.

Diethyl malonate was chosen to provide extra carbon units because of its ability to perform S_N2 reactions on an alkyl bromide, and because it can be purchased with a ^{13}C label between the two carbonyls. Again, this would necessitate the extension of the chain, which could easily be carried out using cyanide. If ^{13}C labelled malonate was to be used, the cyanide chain extension would ideally be carried out prior to the addition of the malonate unit. If this was not possible, unlabelled malonate could be used in the reaction, and a further label added through the cyanide. Decarboxylation would remove the unwanted side chain, then reduction and bromination would provide a site suitable for thiomethylation. (Scheme 45)



Scheme 45: Proposed synthetic strategies for developing 5-carbon chain from 2-bromoethanol

Bromoethanol **178** was treated with *t*-butyl diphenylsilyl chloride in dichloromethane at room temperature. The product was purified by suction column chromatography to give a colourless semisolid **179** in 80% yield.

The infra-red spectrum gave the best evidence for the reaction. No OH peak was present in the spectrum, but there were signals for the *t*-butyl group and an Si-O-C chain. Little change was observed in either the ^{1}H or ^{13}C NMR spectra of the product compared to the starting materials. The mass spectrum, however, gave peaks at 385 and 387 for the molecular ions plus sodium, and at 386 and 388 for the molecular ions plus hydrogen and sodium. The pattern observed was also consistent with a compound containing one bromine atom.

Since the reaction of diethyl malonate with the protected bromide was likely to be more problematic than the cyanation, it was decided to attempt this reaction prior to chain extending the compound.

Sodium was dissolved in ethanol to give sodium ethoxide, and diethyl malonate was added to the basic solution. The protected bromide **179** was added to the solution which was heated under reflux. Following purification by column chromatography, the product **186** was obtained as a colourless oil in 27% yield.

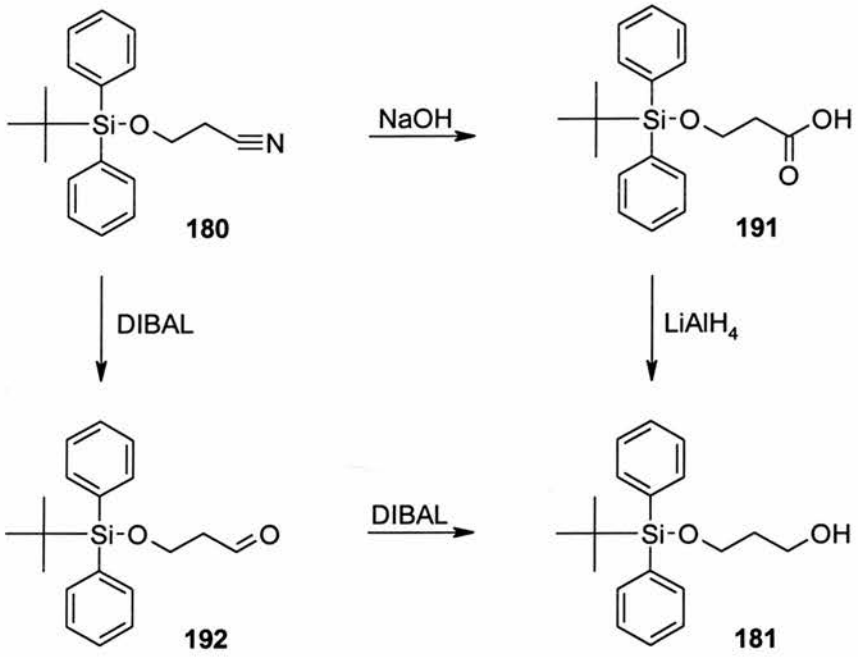
The most significant difference was observed in the ^{1}H NMR spectrum. The peak for the proton between the two carbonyl groups had shifted downfield from 3.33 ppm to 3.69 ppm. The integral ratios of the peaks in the proton spectrum were also consistent with the coupled product. In the ^{13}C NMR spectrum, the former CH_2 peak between the carbonyl

groups had also shifted downfield indicating the change. This was confirmed by the mass spectrum which showed peaks corresponding to $[M+Na]^+$ and $[MH]^+$ at 301 and 279 mass units respectively.

Only the poor yield of the reaction was of slight concern at this point, but since the reaction had proceeded, it was decided to carry on since the yield was likely to improve with optimisation. The next logical step seemed to be to attempt the cyanation of the protected bromide. This was undertaken by treating the bromide in acetonitrile with potassium cyanide at room temperature. Following purification by column chromatography, the protected propionitrile **180** was obtained in a reasonable 77% yield.

The infra-red spectrum showed the presence of a CN group in the product, and the mass spectrum was consistent with the product, with peaks for $[M+K]^+$ and $[M+Na]^+$ at 348 and 332 mass units respectively. The main difference in the proton NMR spectrum was the upfield movement of the former CH_2Br peak, which had shifted from 3.43 to 2.54 ppm with the change of functionality to CH_2CN. An extra peak was also present in the carbon NMR spectrum, at 118 ppm, consistent with a nitrile carbon.

To continue through the synthetic route required the conversion of the nitrile **180** to an alcohol **181**, which could then be brominated to allow the thiomethyl unit to be added to the compound. A standard procedure would be to hydrolyse the nitrile, then reduce the acid **191** to give the alcohol **181**. (Scheme 46) This would, however, require the use of strong acid or base, neither of which was likely to be compatible with the TBDPS group. A literature procedure was found which used DIBAL to reduce the nitrile **180** to the aldehyde[160] **192**. This could then be further reduced to give the alcohol **181**. (Scheme 46)



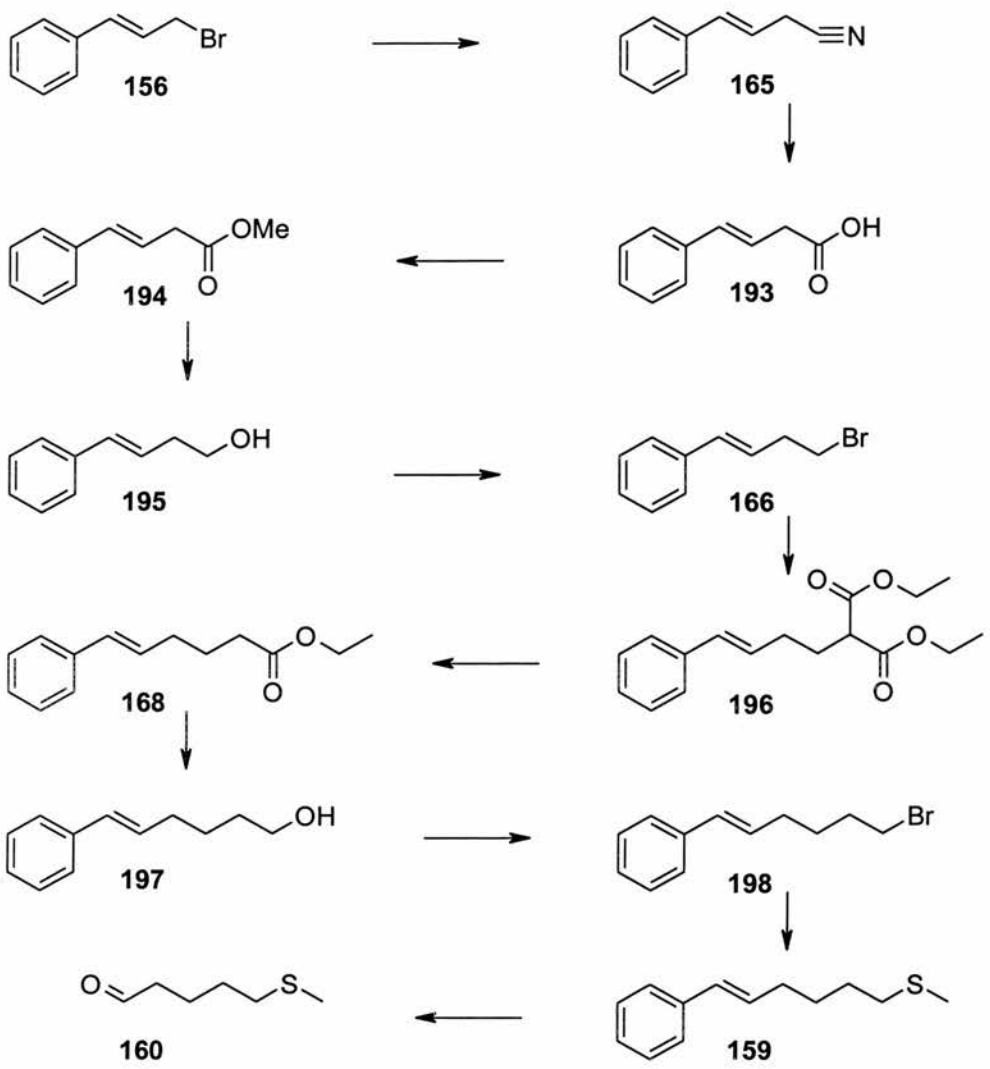
Scheme 46: Cyanide extension of TBDPS-protected bromoethanol

The DIBAL reduction was attempted, but analysis showed that the compound had decomposed. It is likely that one of the decomposition products was TBDPS-OH, though it can only be speculated as to how it formed. A work up method developed by GSK from a procedure of Wadsworth *et al.*[161] was also attempted since it had proved useful as a very mild acidic workup for a DIBAL reduction. This used a slurry of silica gel, tartaric acid, water and diethyl ether as a mildly acidic medium, but this still failed to stop the compound decomposing. The reaction was attempted several times, but little or no product was ever recovered. For the sake of completeness, the hydrolysis reaction was also attempted using sodium hydroxide, but, as expected, the TBDPS group was cleaved from the molecule.

It seemed unlikely that either route from the protected bromide **179** to the protected pentanol **185** was likely to succeed, since they both required some form of hydrolysis or reduction along the way. This route was therefore abandoned.

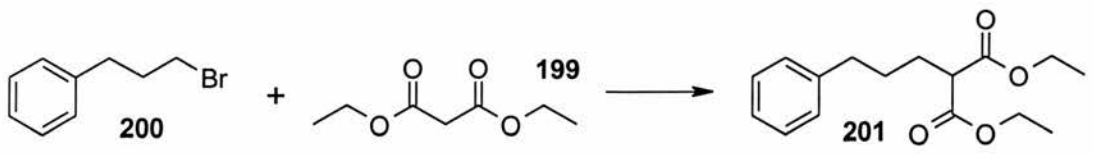
2.3.2.4 Attempted Synthesis using Diethyl Malonate

The ability of diethyl malonate to be added easily to a non-vinylic substrate, demonstrated in the previous route, was of great encouragement. A re-evaluation of all the previous methods showed that using cinnamyl bromide **156**, instead of the protected bromoethanol **179**, would be a practical solution. Cinnamyl bromide **156** could be cyanated **165**, to add the extra carbon unit, and the product converted to the alcohol **195**. This would then be brominated **166**, and reacted with the malonate to provide the correct number of carbon atoms **196**. Decarboxylation **168** and reduction would furnish the alcohol **197**, which, following bromination **198**, could be thiomethylated **159**. Ozonolysis could then be used to furnish the aldehyde **160**. (Scheme 47)



Scheme 47: Proposed extension of cinnamyl bromide, reaction with diethyl malonate and cleavage to give thiomethylpentanal

As a test reaction, the anion of diethyl malonate **199** was created by reaction with sodium in ethanol, and 1-bromo-3-phenylpropane **200** was added to this to give the coupled product **201** in an excellent 98% yield. (Scheme 48). No purification was undertaken.



Scheme 48: Reaction of diethyl malonate with 1-bromo-3-phenylpropane

The ^{1}H NMR spectrum revealed that the peaks of the propane unit had shifted quite dramatically. The CH_2CH peak had shifted from 2.76 ppm in the starting material to 1.95 ppm in the product, and the central CH_2 of the former propane unit had shifted from 2.14 ppm to 1.68 ppm. Although the peak for the proton between the two carbonyl groups had not shifted significantly, the peak had changed from a singlet to a triplet, and the integral ratio had dropped to one, all indicating that the two compounds had reacted successfully. The only significant change in the ^{13}C NMR spectrum was the change in the CH peak of the carbon between the two carbonyl groups. It had shift downfield from 41.8 ppm to 52.2 ppm, illustrating a significant change in the functionality surround the atom. The successful reaction was confirmed by the mass spectrum, which showed peak for $[M+Na]^+$ and $[MH]^+$ at 301 and 279 mass units respectively.

The reaction seemed ideal to allow the addition of the malonate unit to an extended cinnamyl chain, so it was decided to synthesise the extended bromide **166**. Cinnamyl bromide **156** was thus treated with potassium cyanide in ethanol to give the phenyl substituted butenonitrile **165** in an excellent yield of 94%.

The infra-red spectrum showed the presence of nitrile functionality with a peak at 2230 cm^{-1}. In the proton NMR spectrum, the CH_2 peak had shifted upfield 0.89 ppm to 3.29 ppm, and in the carbon NMR spectrum, the change was reflected with an upfield shift from 33.9 ppm to 20.8 ppm. There was also a new peak present at 117.4 ppm, indicating the new carbon of the compound. The mass spectrum confirmed the change, showing a peak at 166 mass units, for $[M+Na]^+$.

It was decided to carry through the product without purification, since it was of high purity, and to avoid losing any compound, therefore maximising the yield through the route. The nitrile **165** was therefore treated with sodium hydroxide solution under reflux to give the acid **193** as a yellow oil in 81% yield. Again, no purification was required. The infra-red spectrum showed no peak for a nitrile, but peaks were present for both hydroxyl and carbonyl functionality. Little change was observed in the proton NMR spectrum, but the ^{13}C NMR spectrum showed considerable change. The CH_2 peak had moved downfield 17.4 ppm to 38.2 ppm, whilst the former nitrile carbon had shifted 60.5 ppm downfield to 177.9 ppm in the acid. The mass spectrum showed peaks at 163, 162 and 145 mass units, for $[MH]^+$, $[M]^+$ and $[M\text{-}OH]^+$ respectively, as well as a peak at 117 corresponding to $[PhenylCHCHCH_2]^+$.

Previous work had shown that reduction of acids did not proceed in consistently good yield, and in many cases, conversion to the ester first gave a better yield, even over two steps. The acid **193** was therefore converted to its methyl ester **194** by treatment with thionyl chloride in methanol under reflux. This gave the product as a yellow oil in a good yield of 93%.

The IR spectrum showed that the OH of the starting material had disappeared, but that the carbonyl functionality was still present. The ^1H NMR spectrum showed a new singlet peak at 3.73 ppm, corresponding to a methoxy group whilst a new peak was also present in the carbon NMR spectrum, at 52.0 ppm. No other differences were observed in either NMR spectrum. The mass spectrum confirmed the product, showing peaks consistent with $[MH]^+$, $[M]^+$, and $[M\text{-}OCH_3]^+$ at 177, 176 and 145 mass units, as well as the same cinnamyl fragment as seen in the starting material at 117 mass units.

The ester **194** was then reduced to the alcohol **195** by treatment with DIBAL at room temperature which gave the product as a yellow oil in an excellent 97% yield. The IR spectrum showed the presence of hydroxyl functionality, with a broad peak at 3600-3000 cm^{-1}, but that the carbonyl functionality had been removed. The proton NMR spectrum showed that the singlet due to the CH_3 group had disappeared, while the original CH_2 peak had moved upfield to 2.48 ppm from 3.26 ppm. A new peak at 3.76 ppm had the correct integral ratio for two protons, and both CH_2 peaks had the correct splitting patterns for the $CHCH_2CH_2OH$ chain.

Following this, the alcohol **195** was converted to its corresponding bromide **166**, to allow the addition of the malonate unit. This was done by treating the alcohol with carbon tetrabromide and triphenylphosphine in dichloromethane at room temperature. The product was purified by column chromatography to remove the triphenylphosphine oxide that was generated in the reaction. This gave the bromide **166** as a yellow oil in 93% yield.

The ^1H NMR spectrum showed that the terminal CH_2 peak had moved upfield to 3.47 ppm from 3.76 ppm, and the second CH_2 had moved downfield to 2.76 ppm from 2.48 ppm with the change from hydroxyl to bromide functionality. In the ^{13}C NMR spectrum, the $CHCH_2$ peak had not moved, but the terminal carbon had shifted greatly from 62.3 ppm in the alcohol to 32.4 ppm in the bromide. No other data were collected because the product was deemed to be unstable, and was used straight away.

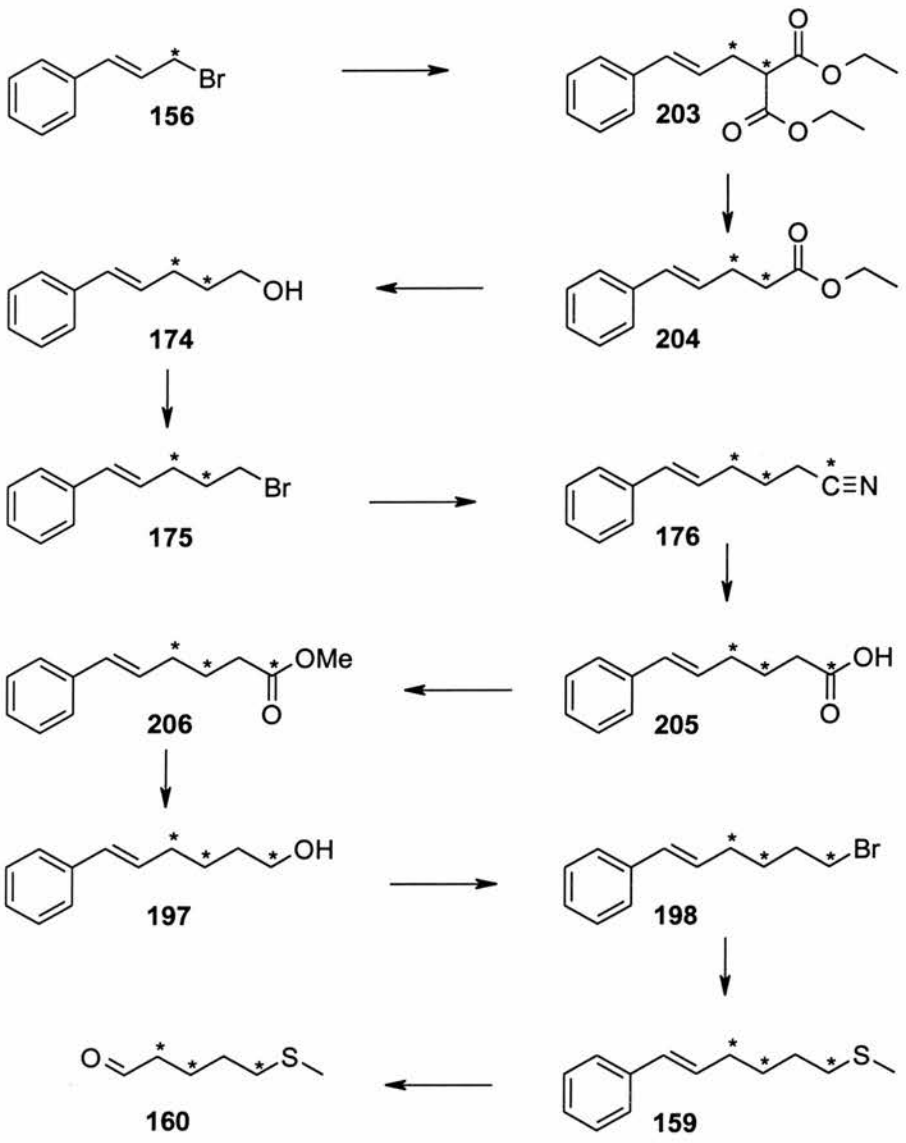
Unfortunately, the coupling reaction did not proceed as expected. Under the basic conditions of the reaction with the diethyl malonate anion it was suggested that the

elimination of HBr took place to give the conjugated alkene **202**, (Scheme 49), though no evidence for this was found.

Scheme 49: Possible base-catalysed elimination of HBr from 1-bromo-4-phenylbut-3-ene

2.3.2.5 Re-evaluation of Diethyl Malonate Synthesis

A re-evaluation of the route showed that altering the order of the steps could possibly alleviate this problem. If the diethyl malonate **199** was reacted with cinnamyl bromide **156**, and the cyanation then took place after that, there should not be a problem with elimination. This route was initially avoided because the expensive labelled diethyl malonate would undergo a greater number of steps than it would if it was added later in the synthesis. (Scheme 50).

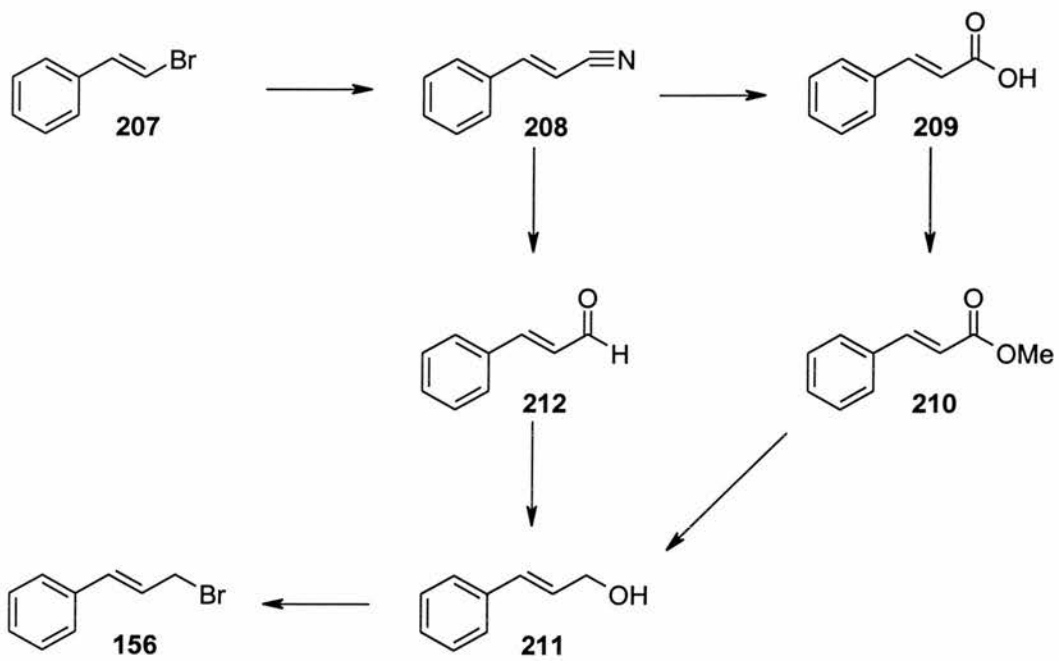


Scheme 50: Modification of route using diethyl malonate.
Initial reaction of cinnamyl bromide and dicthyl malonate, followed by extension with cyanide

Another issue regarding this, and the previously attempted route, was that only two ^{13}C could be easily incorporated into the chain. It is possible to purchase diethyl malonate with 3 × ^{13}C atoms in the chain, but decarboxylation of the malonate unit would remove one label, and this was deemed unnecessarily wasteful of what was a particularly expensive compound. It was therefore decided to devise a route to synthesise cinnamyl bromide with a ^{13}C label in the terminal position, which would then be incorporated into the product.

2.3.2.6 Synthetic Route for ^{13}C-Labelled Cinnamyl Bromide

The easiest, and cheapest, way to synthesis labelled cinnamyl bromide **156** would be to use cyanide to add an extra carbon unit to β-bromostyrene **207**. Hydrolysis of the nitrile **208** would give the acid **209**, and reduction of this, or its corresponding methyl ester **210**, would give the alcohol **211**. Subsequent bromination would then furnish the bromide **156**. The reduction of the nitrile to give the aldehyde **212**, using DIBAL, was also considered since this would reduce the number of steps in the synthesis by one. (Scheme 51)



Scheme 51: Proposed strategy to synthesise labelled cinnamyl bromide from β bromostyrene

The cyanation step was inevitably going to be the most difficult, due to the bromine being attached directly to the double bond, and therefore preventing simple S_N2 reactions from taking place. A literature search revealed that transition metal catalysts were often used in this type of reaction,[162,163] and palladium tetrakis(triphenylphosphine) seemed to be a popular choice. Initial reactions were attempted using the procedure of Yamamura,[164]

using commercially available palladium tetrakis(triphenylphosphine), but the catalyst proved to be air-sensitive, so fresh catalyst was prepared from triphenylphosphine and palladium acetate. This, however, did nothing to improve the progress of the reaction, which, despite showing some product by ^{1}H NMR spectroscopy, never looked like it would proceed in a yield sufficient for labelling. Interestingly, the quoted literature yield was 94%.

Sakakibara *et al.*[163] used a nickel catalyst to couple cyanides to various β-bromostyrenes. The catalyst used was nickel tetrakis(triphenylphosphine), but this was generated *in situ* from bis(triphenylphosphine)nickel(II) dibromide, zinc and triphenylphosphine. This proved to be a far more reliable method for performing the cyanation, and β-bromostyrene **207** was treated with cyanide in DMF using the catalyst, to give the product **208** as a pale yellow oil in a very reasonable 73% yield.

The infra-red spectrum showed the presence of nitrile functionality with a peak at 2185 cm^{-1}, while the ^{13}C NMR spectrum showed a new peak at 118.2 ppm, consistent with a nitrile carbon. The *C*-2 peak had shifted upfield from 106.7 ppm to 96.4 ppm, and the *C*-3 peak had shifted in the opposite direction, from 137.3 ppm to 150.6 ppm. In the ^{1}H NMR spectrum, the PhC*H* peak had shifted downfield into the peaks for the aryl protons, while the former C*H*Br peak had moved upfield from 6.75 ppm to 5.88 ppm. The mass spectrum confirmed the structure of the product, with an $[M]^{+}$ of 129 mass units.

It was decided to attempt the shorter reduction method to give the aldehyde **212**. Cinnamonitrile **208** was therefore treated with DIBAL in dichloromethane, initially at 0 °C, then at room temperature. The GSK workup method, discussed earlier (Section 2.3.2.3),

was employed since it proved to give higher yields to this particular reduction. In this case cinnamaldehyde **212** was obtained in an excellent 94% yield.

The IR spectrum showed the change in functionality with the loss of the CN peak, and the appearance of a carbonyl peak. The change was reflected in the ^{13}C NMR spectrum, which showed that the peak of the terminal carbon had shifted downfield from 118.2 ppm to 193.8 ppm with the change in functionality, while in the ^{1}H spectrum, the peak for the C*H*-2 proton had shifted downfield from 5.88 ppm to 6.73 ppm. A new peak was also present at 9.72 ppm for the aldehydic proton. The mass spectrum confirmed the compound, with a peak at 132 mass units for $[M]^+$. Cinnamyl alcohol **211** was then prepared by further reduction of cinnamaldehyde **212** with DIBAL in THF at room temperature. A standard acid workup was used, and gave the product **211** as a white solid in an excellent yield of 93%.

The ^{1}H NMR spectrum showed a new multiplet at 4.33 ppm for the CH_2OH protons, indicating a coupling to the OH proton, as well as the adjacent CH proton. The aldehydic proton peak had also disappeared, and this was reflected in the ^{13}C NMR spectrum which showed no carbonyl peak. The IR spectrum also confirmed the reduction, with the expected broad peak at 3600-3000 cm^{-1} while the mass spectrum showed an M^+ of 134 mass units.

Cinnamyl bromide **156** was prepared by treatment of cinnamyl alcohol **211** with phosphorus tribromide in diethyl ether. This route was chosen over the previously used carbon tetrabromide method to remove the need for purification by column chromatography. Previous experience had shown that cinnamyl bromide would hydrolyse

on silica, and would therefore defeat the purpose of the reaction. The reaction with phosphorus tribromide allowed the phosphorus compound to be removed in the work up, and gave the product **156** as a brown solid in 84% yield.

Few peaks were identified in the IR spectrum, but the disappearance of the OH peak indicated that reaction had taken place. Little change was observed in ^{1}H NMR spectrum either, though the OH peak had disappeared. The greatest difference was seen in the ^{13}C NMR spectrum, which showed a large upfield shift for the CH_2 peak from 63.7 ppm to 33.6 ppm, the position consistent with an alkyl bromide. The mass spectrum confirmed the structure, with the expected pattern of $[MH]^+$ peaks at 197 and 199 mass units. It also showed the cinnamyl fragment at 117 mass units.

A route had thus been successfully devised that would allow the synthesis of labelled cinnamyl bromide **156** from β-bromostyrene **207**, in an overall yield of 53% over 4 steps. It was then necessary to couple the cinnamyl bromide **156** to diethyl malonate **199**.

2.3.2.7 Reaction of Diethyl Malonate with Cinnamyl Bromide

Initial attempts at the coupling of cinnamyl bromide **156** and diethyl malonate **199** followed the procedure describe earlier (Section 2.3.2.4) by dissolving sodium in ethanol, and allowing the sodium ethoxide formed to deprotonate the diethyl malonate. The anion would then perform a S_N2 reaction, displacing the bromine from cinnamyl bromide. Initially it was thought that the reaction was proceeding well. However, the ^{1}H NMR spectrum showed that the integral ratios of the ethyl peaks were incorrect, and their splitting patterns were overly complicated. It was deduced that, like the reaction of ethyl

acetate with cinnamyl bromide (Section 2.3.2.2), the product was being deprotonated and reacting with another molecule of cinnamyl bromide. This was confirmed by mass spectrometry, which showed peaks for $[M+Na]^+$ of the monocinnamyl compound, and for $[M+Na]^+$ of the dicinnamyl compound. Obviously, this was of no use, so an alternative means of coupling the two components together was sought.

A procedure employed by Barbry *et al.*[165] used a phase transfer catalyst to perform a similar reaction with diethyl malonate. The reaction was attempted using diethyl malonate **199** and cinnamyl bromide **156** in dichloromethane treated with potassium carbonate in water. Tetrabutylammonium chloride was used as the phase transfer catalyst, and the reaction proceeded very well, giving the desired product **203** as a yellow oil in an excellent 96% yield.

The ^1H NMR spectrum showed that the former CH_2Br peak had shifted significantly upfield, to 2.92 ppm from 4.16 ppm, while the former malonate CH_2 had shifted downfield slightly to 3.61 ppm from 3.31 ppm. The multiplicity of the peak had also changed to a triplet, while the integral ratio had dropped to one proton, confirming that the compound had reacted. In the ^{13}C NMR spectrum, little change was observed in the cinnamyl portion of the compound, but the former CH_2 peak of the malonate had shifted significantly downfield from 41.8 ppm to 52.4 ppm. The mass spectrum confirmed the product with peaks for $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$ at 315, 299 and 277 mass units respectively.

This procedure proved particularly useful, since there was no evidence of the formation of the dicinnamyl compound. It also eliminated the requirement for the use of dry diethyl malonate, which was necessary to undertake the sodium/ethanol reaction. Drying the

diethyl malonate could have proved a difficult and wasteful procedure on small amounts of ^{13}C-labelled material.

The next step was the decarboxylation of the malonate derivative **203**. Standard decarboxylation techniques use acid or base to initially hydrolyse the esters, which subsequently lose carbon dioxide. This would give the product as the acid, but this was not desirable, since the step after was to reduce the compound to an alcohol. A literature procedure by Krapcho[166] was found, however, which described a dealkoxycarbonylation procedure that would leave the compound as a monoester **204**. The reaction was attempted, and the pentenoate diester **203** was treated with sodium chloride and wet dimethyl sulfoxide at 170 °C. The product **204** was obtained as a yellow oil in an excellent 96% yield.

The infra-red spectrum showed the presence of carbonyl functionality with a peak at 1731 cm^{-1}, but did not show the presence of hydroxyl functionality, indicating that the ester had not been hydrolysed. The ^{1}H NMR spectrum showed that the CHC*H*$_2$ peak had shifted slightly upfield, as had the peak for the for the former C*H*CO protons, giving a multiplet with an integral ratio of four protons between 2.45 and 2.59 ppm. The ^{13}C NMR spectrum showed that the *C*-2 peak had shifted significantly from 52.4 ppm to 34.1 ppm, indicating the change in functionality at the position. The mass spectrum showed the expected $[MH]^+$ peak at 205 mass units, and a peak corresponding to $[M\text{-}OEt]^+$ at 159 mass units. Accurate mass found an $[MH]^+$ weight of 205.1229, and the compound required 205.1229.

The dealkoxycarbonylation had proceeded particularly well, and the monoester **204** was treated with DIBAL in THF at room temperature to furnish the alcohol **174** as a yellow oil in an almost quantitative 99% yield.

The IR spectrum showed that the carbonyl functionality had been removed, while a broad peak at 3700-3000 cm^{-1} showed the presence of an OH group. In the proton NMR spectrum, the peaks of the ethyl ester group had disappeared, and three peaks with an integral ratio of two protons were present. These had multiplicities of quartet (2.51 ppm), quintet (1.76 ppm) and triplet (3.72 ppm) indicating the expected splitting patterns of the $CHCH_2CH_2CH_2OH$ chain. In the ^{13}C spectrum, again the ethyl signals were absent. The carbonyl signal was also missing, replaced by a signal at 62.5 ppm, consistent with the carbon of an alcohol. The mass spectrum showed peaks for $[M]^+$, $[M\text{-}OH]^+$ and the cinnamyl fragment respectively at 162, 145 and 117 mass units, while the accurate mass was consistent with the expected formula, giving a mass of 162.1046 which compared well to the expected 162.1045 mass units.

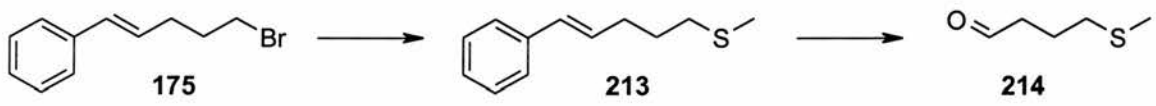
The alcohol **174** was then treated with carbon tetrabromide and triphenylphosphine in dichloromethane, and following purification by column chromatography on silica, yielded the bromide **175** as a yellow oil in 71% yield.

The ^1H NMR spectrum showed that the peak due to the CH_2OH had moved slightly upfield with the change to CH_2Br, from 3.72 ppm to 3.48 ppm. The peak due to the adjacent CH_2 moved more significantly from 1.76 ppm in the starting material to 2.05 ppm in the product. The ^{13}C NMR spectrum showed a prominent change in the peak for the terminal carbon. It shifted downfield from 62.5 ppm to 32.4 ppm, consistent with a change from

hydroxyl to bromide functionality. The mass spectrum showed the expected peak pattern for a bromo compound, with peaks at 225 and 227 mass units for the $[MH]^+$ peaks, and at 224 and 226 mass units for the $[M]^+$ peaks. An $[M\text{-}Br]^+$ fragment, and the ubiquitous cinnamyl fragment were also present, at 145 and 117 mass units.

2.3.2.8 Oxidative Cleavage via Ozonolysis

With the bromide **175** in hand, it was decided that a trial run of the ozonolysis step would be prudent before the synthesis was taken any further. To replicate the conditions as accurately as possible, the bromide **175** was to be thiomethylated, and then used to test the ozonolysis reaction. (Scheme 52)



Scheme 52: Conversion of 1-bromo-5-phenylpent-4-ene to the thiomethyl compound which would be used to test the ozonolysis reaction

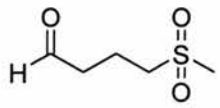
The bromide **175** was treated with sodium thiomethoxide in methanol to give the product **213** as a brown oil in an excellent yield of 96%. The ^1H NMR spectrum showed that the terminal CH_2 peak had shifted significantly upfield from 3.48 ppm to 2.67 ppm. The adjacent CH_2 had also shifted upfield from 2.05 ppm to 1.78 ppm, while a new singlet peak with an integral ratio of three protons was present at 2.23 ppm, indicating the thiomethyl protons. In the ^{13}C NMR spectrum, little change was observed to the peaks for the CH_2 carbons, the greatest shift being for the *C*-2 peak which shifted to 28.9 ppm from 33.3 ppm, though a new peak at 15.7 ppm was present, consistent with the new thiomethyl

carbon. The mass spectrum confirmed the product, with peaks at 193, 192 and 145 mass units, corresponding to $[MH]^+$, $[M]^+$ and $[M\text{-}SCH_3]^+$ ions.

The substituted thiomethylpentene **213** was then treated with ozone in dry dichloromethane, using triphenylphosphine to break down the ozonide. Purification was attempted, but even after column chromatography, a mixture of products was obtained. The mixture showed aromatic functionality in the ^{1}H NMR spectrum, which was attributed to triphenylphosphine oxide, but in lower range of the spectrum, there was sufficient clarity to identify the desired alkyl chain from the ozonolysis product.

The high polarity solvents required to remove the product from the column indicated that the compound was considerably more polar than would have been expected for a thiomethyl substituted aldehyde, and the ^{1}H NMR spectrum showed that the thiomethyl CH_3 peak had shifted downfield to 2.86 ppm. This shift is consistent with a change of oxidation state of the sulfur. All three CH_2 peaks were able to be identified, at 2.68, 2.09 and 3.01 ppm, with the expected triplet, quintet, triplet splitting pattern for the $C(O)CH_2CH_2CH_2S$ chain, while the aldehyde peak was identified at 9.73 ppm. In the low range of the ^{13}C NMR spectrum, four peaks were identified, corresponding to the three CH_2 and one CH_3 carbons. The CH_3 peak had shifted significantly downfield to 41.0 ppm, also indicating a change in the oxidation state of the sulfur. The carbonyl peak was identified at 200.7 ppm, confirming that the compound had undergone cleavage. Infra red spectroscopy helped identify the compound, with a peak at 1694 cm^{-1} confirming the carbonyl functionality, but more interestingly, peaks were observed at 1305 and 1120 cm^{-1}, consistent with SO_2 functionality. This was confirmed by mass spectrometry, which found

a peak at 173 mass units, consistent with an $[M+Na]^+$ ion for the sulfone product. No peak was observed for a sulfoxide compound.



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It should be noted that only one aldehyde peak was present in the ^1H NMR spectrum of the purified product mixture, compared to the two found in the crude product. This indicated that the initial identification of the aromatic region as triphenylphosphine oxide was likely to be correct. Had the compound been benzaldehyde, from the other half of the starting material, a second aldehyde peak would have been present in the spectrum of the purified product. Indeed, comparison of the ^1H NMR spectrum with that of authentic triphenylphosphine oxide showed near perfect consistency. This indicated that the benzaldehyde had been removed by the purification procedure.

The ozonolysis had therefore generated the sulfone product **215** rather than the desired sulfide **214**. It was a result that was not totally unexpected, since there is literature precedence for such a reaction,[167] but could nevertheless be of some use. The sulfone product would still give an alternative natural glucosinolate, glucoerysolin. Reduction of the sulfone to the sulfoxide was considered, but very few procedures for this transformation exist. Only a procedure of Still and Ablenas[168] was found that has been shown to perform this transformation. Since this was the only procedure likely to reduce the sulfone, it seemed that this procedure would not be straightforward. It was thought that an alternative method of oxidative cleavage could furnish the desired product, and therefore be more profitable.

2.3.2.9 Alternative Methods of Oxidative Cleavage

A literature search was performed, and the most suitable candidates for reaction appeared to be osmium tetroxide and sodium periodate, which would perform the cleavage in two steps. This combination would be useful, since it would allow a much greater control over the oxidation. References were found that showed the reaction of osmium tetroxide with an alkene in the presence of a sulfide without any oxidation of the sulfur,[169,170] while sodium periodate is known to cleave the 1,2-diol created by such an osmium tetroxide reaction.[171,172] It has been shown that periodate can oxidise sulfur compounds, but that reaction proceeds only to the sulfoxide.[173] It was hoped therefore, that periodate would react preferentially with the diol rather than with the sulfur.

2.3.2.10 Chain Extension

Though the ozonolysis step had failed to produce the desired product, it was decided to continue the synthesis of the full-length chain and attempt the osmium tetroxide/periodate cleavage steps with the full length chain. To extend the chain, the bromophenyl pentene **175** was treated with potassium cyanide in acetonitrile to furnish the nitrile **176** as a pale yellow oil in 84% yield.

The infra-red spectrum showed the presence of nitrile functionality with a peak at 2247 cm^{-1}. In the proton NMR spectrum, the peak belonging to the former CH_2Br had shifted a significant 1.05 ppm upfield to 2.43 ppm with the addition of the nitrile unit. In the ^{13}C NMR spectrum, an extra peak was present at 120.0 ppm, consistent with a nitrile carbon, while the peak due to *C*-2 had shifted from 32.4 ppm to 16.9 ppm. The *C*-3 peak had also

shifted upfield, though the change was less considerable. The mass spectrum confirmed the compound with the expected molecular ion peak at 171 mass units. Also observed were peaks at 130 and 117 mass units respectively corresponding to the $[M\text{-}CH_3CN]^+$ fragment and the usual cinnamyl fragment.

It was decided to convert the nitrile **176** to the alcohol **197** by hydrolysis and reduction, via the ester, rather than by reducing the nitrile, and reducing the aldehyde. This was mainly due to the inconsistency of the nitrile reduction method which, although it often produced good results, was not sufficiently reliable to be of use to a labelled synthesis. The nitrile **176** was thus hydrolysed by treatment with aqueous sodium hydroxide solution in methanol under reflux. This afforded the acid **205** as a yellow oil in 85% yield.

The infra-red spectrum showed that there was no longer any nitrile functionality in the compound, but that hydroxyl and carbonyl groups were present due to peaks at 3600-2800 cm^{-1} and 1709 cm^{-1} respectively. In the ^{13}C NMR spectrum, the change was illustrated with the movement of the former nitrile peak from 120.0 ppm to 181.0 ppm, while the *C*-2 peak shifted downfield to 34.4 ppm from 16.9 ppm. The mass spectrum confirmed the product, with the molecular ion peak at 190 mass units.

The acid **205** was once again converted to the methyl ester **206** prior to reduction, by treatment with thionyl chloride in methanol under reflux. The product was given as a yellow oil in an almost quantitative 99% yield.

In the NMR spectra, new peaks were present indicating the presence of the methyl group of the ester at 3.69 ppm in the ^1H spectrum and 51.5 ppm in the ^{13}C spectrum. The change

was confirmed by the mass spectrum, which showed the molecular ion peak at 204 mass units, and peaks for $[M\text{-}OMe]^+$, $[M\text{-}Acetic\ acid]^+$ and the cinnamyl fragment at 173, 130 and 117 mass units.

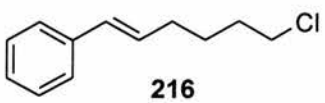
The ester **206** was then treated with DIBAL in THF to furnish the alcohol **197** in 95% yield. The IR spectrum showed no carbonyl functionality, but that a hydroxyl group was present in the compound due to a broad peak at 3600-3100 cm^{-1}. In the ^1H NMR spectrum, the singlet peak of the CH_3 ester group had disappeared while a new triplet peak, integral ratio two, was present, signalling a change in the oxidation state of the carbon. The peak of the adjacent CH_2 group had shifted upfield from 2.40 ppm to between 1.54 and 1.75 ppm, sitting on top of the peak for it's other adjacent CH_2 group. In the carbon NMR spectrum, the peak for the ester CH_3 group had disappeared, as had the carbonyl peak, but a new peak was present at 62.8 ppm, consistent with a hydroxyl group on the carbon. The mass spectrum showed the expected molecular ion peak at 176 mass units, while fragments indicating the loss water and loss of ethanol were present at 158 and 130 mass units.

The subsequent step was the bromination of the alcohol, which was carried out in the usual manner by treatment of the alcohol **197** with carbon tetrabromide and triphenylphosphine in dichloromethane. Purification by column chromatography gave a colourless oil which appeared to be only one spot by TLC, but analysis revealed that more than one compound was present.

The ^1H NMR spectrum showed a triplet peak at 3.48 ppm, consistent with the protons of a CH_2Br group, but another triplet peak was also observed at 3.62 ppm while the peak of the CH_2-2 protons had become a multiplet. The ^{13}C spectrum showed the expected peaks for

C-2 at 27.9 ppm, and CH_2Br at 33.8 ppm, but also extra peaks at 26.6 ppm and 45.0 ppm. This indicated that a second compound was present in the product, and that it had to be very similar to the bromide **198**, since the chemical shifts were not radically different.

Since none of the starting material remained, the number of options was limited. Had triphenylphosphine attached to the alcohol and not been displaced by bromide, the aromatic region of the proton NMR would have been substantially different, whilst TLC analysis would more than likely have shown a spot for a second compound. Another possibility was that the reaction had somehow managed to generate a significant quantity of chloride ion which had reacted in place of the bromide. This indeed proved to be the case, when the mass spectrum showed peaks for both the molecular ions of the bromide compound **198**, at 238 and 240 mass units, as well as the chloride compound **216** at 194 and 196 mass units, with their distinctive isotope patterns.



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It is unclear exactly how the reaction came to generate such a quantity of chloride. The obvious source was the dichloromethane that was used as the solvent, but since DCM had been used as the solvent in many other bromination reactions, it is perplexing that any such effects occurred in only this reaction. The reaction was repeated, and similar results were obtained. It was conceivable that a batch of carbon tetrabromide was contaminated, but since two different batches were used, and the compound was checked for consistency by ^{13}C NMR spectroscopy, this was ruled out. This left dichloromethane as the only possible source of the chloride.

It was decided to carry both compounds through the thiomethylation step, and hope that both compounds would react. A mixture of the compounds **198**, **216** was treated with sodium thiomethoxide in methanol, with a catalytic amount of potassium iodide. The reaction was stirred at room temperature, but TLC analysis showed that starting material still remained. This was likely to be the chloride since it persisted even after the addition of more sodium thiomethoxide. The reaction was then heated at 40 °C, which allowed it to proceed to completion, and gave the product **159**, after purification, as a clear oil. The yield over the two steps was a very reasonable 73%.

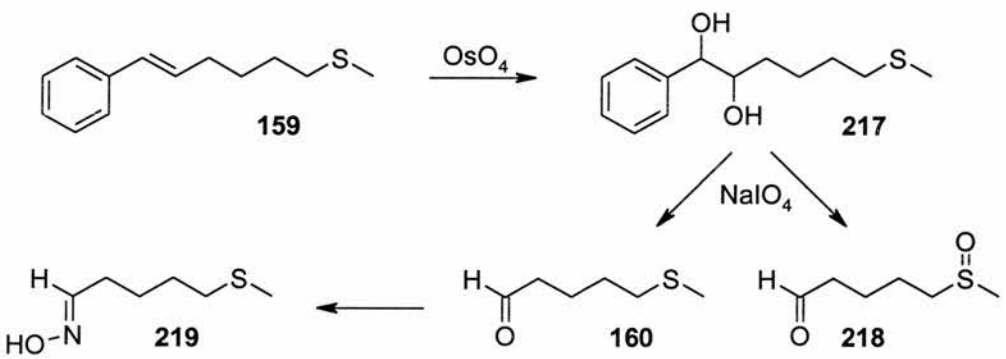
The ^{1}H NMR spectrum showed that the two triplet peaks of the starting materials had disappeared, and were replaced with a single triplet peak at 2.46 ppm, an upfield shift from both starting materials. A new singlet peak was also present at 2.03 ppm, with an integral ratio of three protons, consistent with an S*C*H_3 group, while the carbon of the group was represented by a new peak at 15.6 ppm in the ^{13}C NMR spectrum. The two *C*-1 peaks of the starting material were not present, replaced by a single peak at 34.2 ppm. The mass spectrum confirmed the product with $[MH]^+$ and $[M\text{-}SCH_3]^+$ peaks present at 207 and 159 mass units.

2.3.2.11 Oxidative Cleavage

As was discussed earlier, treatment of the alkene with osmium tetroxide, then sodium periodate appeared to be the best means of cleaving the double bond. It was found that in cases where oxidation of another functional group was to be avoided, osmium tetroxide was used quantitatively, rather than in a catalytic amount with a co-oxidant such as NMO.[169,170] Normally a co-oxidant is employed to avoid using large quantities of the

highly toxic osmium compound. As the reaction proceeds, the reduced osmium compound is re-oxidised by the co-oxidant until all the co-oxidant is itself reduced. Avoiding the use of a co-oxidant would presumably allow greater control over the oxidation, since the exact quantity of oxidant was known. It is also conceivable that the co-oxidant could potentially oxidise the sulfur of the substrate in these reactions.

As mentioned, sodium periodate can be used to oxidise sulfur compounds.[173] It was hoped that the diol would be more susceptible to oxidation that the sulfur, such that the diol could be cleaved without a change of oxidation state of the sulfur. The oxidation of the sulfur could prove to be beneficial, however, since the synthesis of glucoraphanin would require the oxidation of the sulfide to sulfoxide, thus if the oxidation took place with the cleavage step, a separate step would not be needed later in the synthesis. It was therefore hoped that if sodium periodate was used to cleave the diol created by osmium tetroxide, the oxidation of the sulfur could be controlled to give either the sulfide or the sulfoxide. (Scheme 53)



Scheme 53: Proposed oxidative cleavage of the alkene bond by treatment with osmium tetroxide and sodium periodate to give either the sulfide or sulfoxide

The alkene **159** was thus treated with osmium tetroxide in pyridine at room temperature, which furnished the diol **217**, after purification, as a colourless oil in 63% yield.

The infra-red spectrum showed the presence of hydroxyl functionality due to the presence of a broad peak at 3700-3000 cm^{-1}. The distinctive cinnamyl double bond peaks present in the starting material at 6.13 and 6.31 ppm were absent in the ^1H NMR spectrum of the product, but new peaks at 3.63 and 4.38 ppm each had an integral ratio of one proton. A broad singlet peak was observed at 2.54 ppm, consistent with an O*H* group. The second was deduced to be lying under the CH_2S peak at 2.36 ppm, with the integral ratio for the multiplet observed as three protons. In the carbon NMR spectrum, the *C*-6 and *C*-5 peaks, previously at 130.5 and 130.2 ppm, had shifted upfield to 78.3 and 76.2 ppm respectively. The mass spectrum confirmed the product, showing an $[M+Na]^+$ peak at 263 mass units.

As discussed earlier, sodium periodate was to be used to cleave the diol **217**. There was the possibility that the sulfoxide could be created at this stage, since the synthesis of glucoraphanin would require the oxidation of the sulfur to the sulfoxide. It was decided, however, to initially attempt to only cleave the diol, and leave the sulfur unoxidised, since attempting both reactions in one may have caused more problems than it would have solved.

The diol **217** was treated with sodium metaperiodate in a THF and water solution. Due to the product's volatility **160**, it was not isolated, but instead, was treated with hydroxylamine hydrochloride in water, in the presence of sodium acetate. This yielded the expected mixture of products comprising benzaldehyde oxime and thiomethylpentaldoxime **219**. The amount of product isolated was particularly low, so purification was achieved by preparative TLC to give the desired oxime **219** as a mixture of isomers.

The ^{1}H NMR spectrum showed no aromatic functionality indicating that the cleavage had indeed occurred. Both *E* and *Z* oximyl protons were identified at 6.73 and 7.43 ppm respectively, as were their corresponding hydroxyl protons, at 7.67 and 7.31 ppm. The ratio of *E* to *Z* was estimated at 1:1, as defined by the integral ratios of the ^{1}H NMR spectrum. At the opposite end of the molecule, the peaks for *E* and *Z* isomers often overlapped, so that the CH_2S peak was observed as a quartet at 2.52 ppm.

The carbon spectrum proved more difficult to assign because peaks were observed for each individual carbon in both isomers of the product, with the exception of the thiomethyl carbons. A TOCSY HSQC experiment allowed the spectrum to be deconvoluted, by separation of the peaks into two different spin systems, and correlating with the proton spectrum that had been fully assigned. (Figure 8) The methyl group was observed at 15.5 ppm, little changed from the dihydroxy precursor. Two peaks were observed at 152.0 and 152.7 ppm, corresponding to the *Z* and *E* oxime carbons respectively. The mass spectrum identified the compound, with $[MH]^+$ and $[M]^+$ peaks at 148 and 147 mass units respectively.

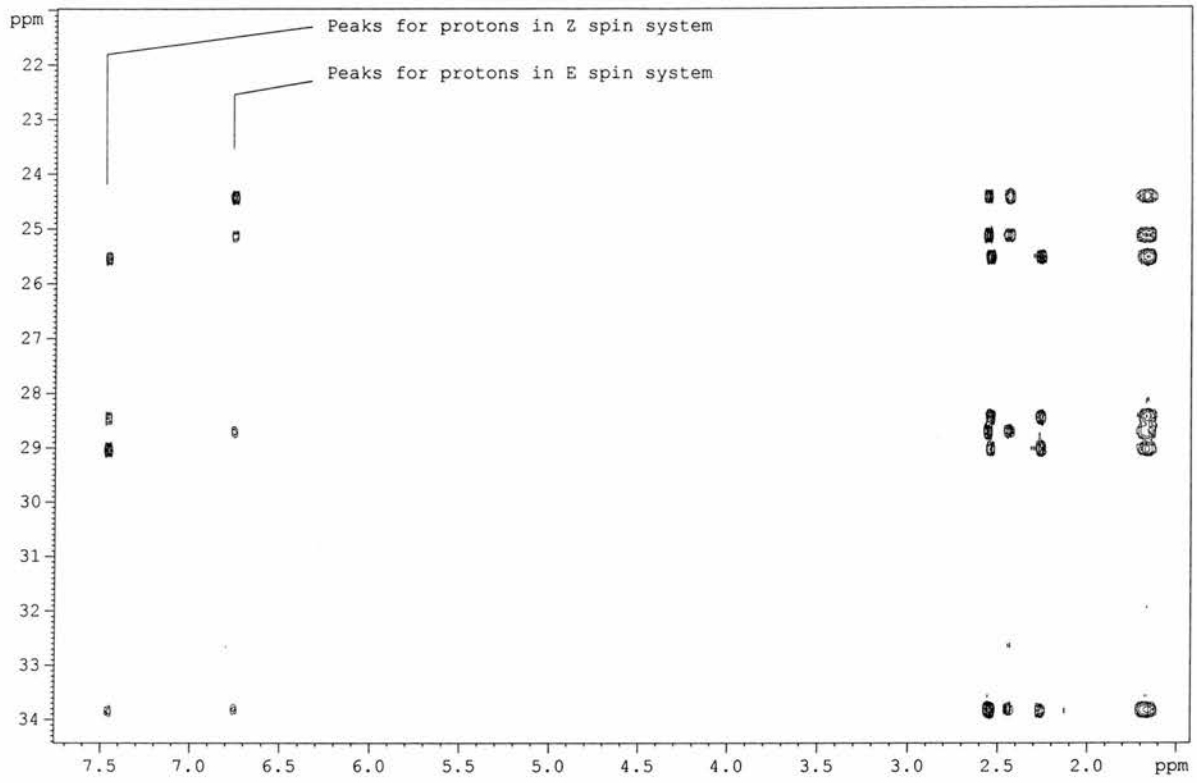


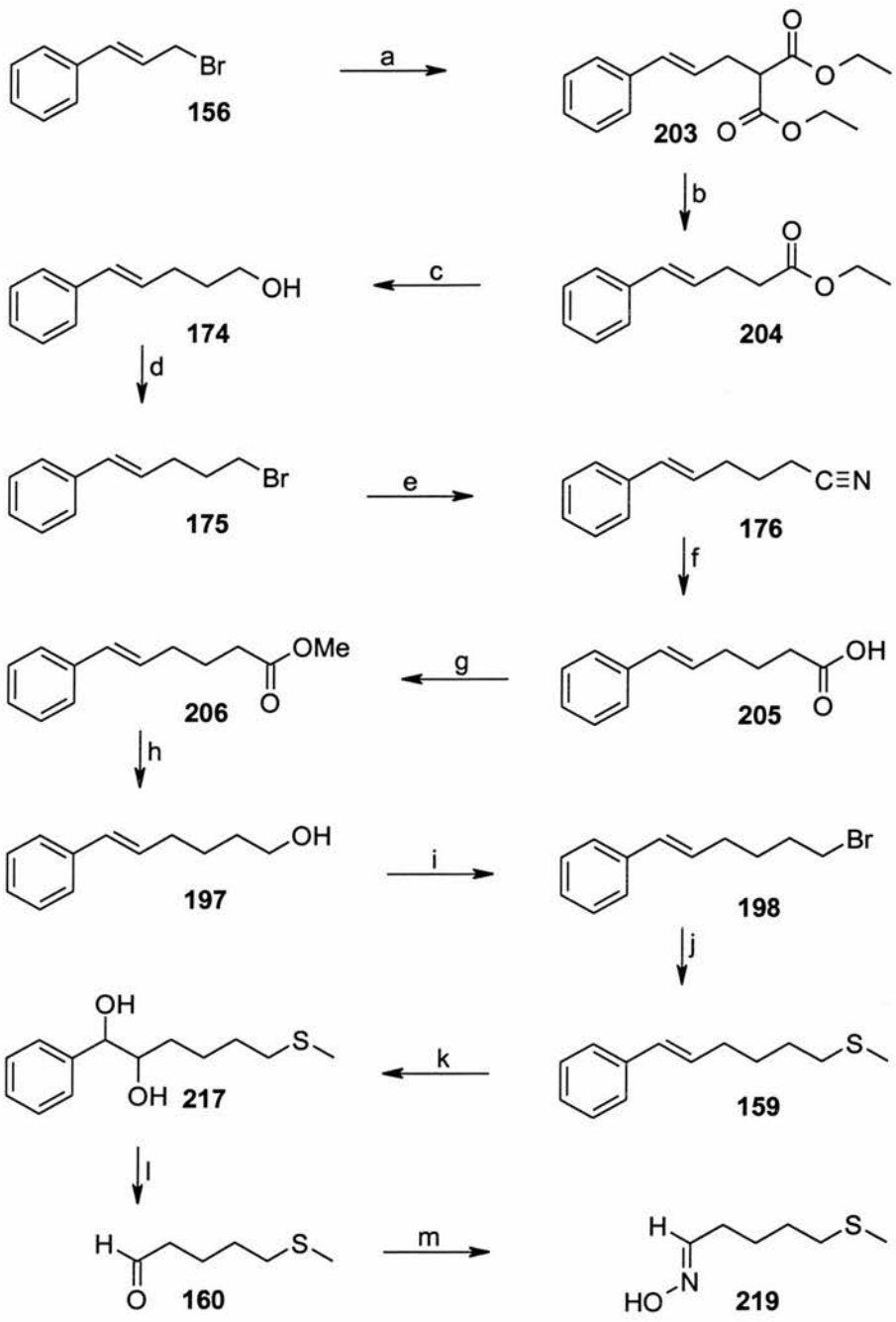
Figure 8: TOCSY HSQC NMR spectrum of 5-thiomethylpentaldoxime

The poor yield over the last two steps is most likely due to the diol cleavage step, since the oxime reaction normally proceeds in good yield. It is possible that the reaction had not proceeded to completion, since one of the compounds identified in the purification of the oxime was the diol precursor. Therefore, it must be ensured that this reaction has indeed gone to completion before the workup is commenced. The most obvious place that compound could be lost was in the concentration of the aldehyde, where the volatility of the compound may have resulted in some of it being removed along with the solvent. The use of a lower boiling solvent such as diethyl ether may have prevented this, and would be a worthwhile change if the reaction was to be repeated.

2.3.2.12 Summary

A route has successfully been devised that would allow the production of a labelled glucoraphanin compound. (Scheme 54) Many of the steps are high yielding, as was desired for a labelled synthesis. Unfortunately, the oxidative cleavage steps proved to be the lowest yielding of the synthesis, but it is anticipated that these could be optimised to furnish the oxime in a better yield. Indeed, it may be a consequence of the volatility of the compound at this stage that prevents a greater yield being attained. The possibility of producing the sulfoxide compound during the periodate cleavage step may help reduce any loss of yield in this manner, since the sulfoxide would be a far less volatile compound, and would prove much simpler to handle. If this route was employed, the possibility to optimise the two steps to the oxime individually would arise, and may also be helpful in providing a higher overall yield.

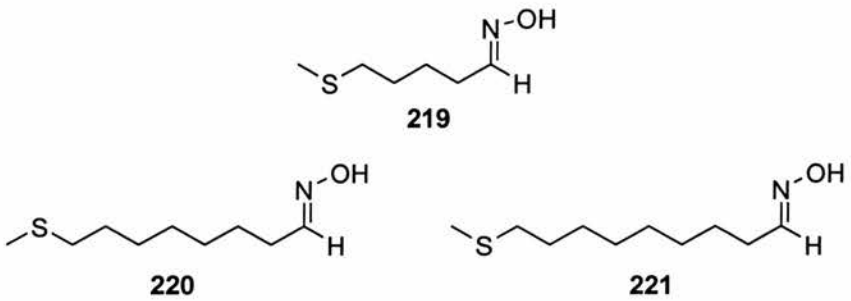
Although the route was not completed, the final steps in the synthesis have been carried out previously on unlabelled material. Conversion of the oxime to the oximyl chloride would be followed by coupling to the thioglucose unit. Sulfation followed by deprotection would give a glucosinolate compound, glucoerucin, which could be oxidised to give glucoraphanin, as previously described.



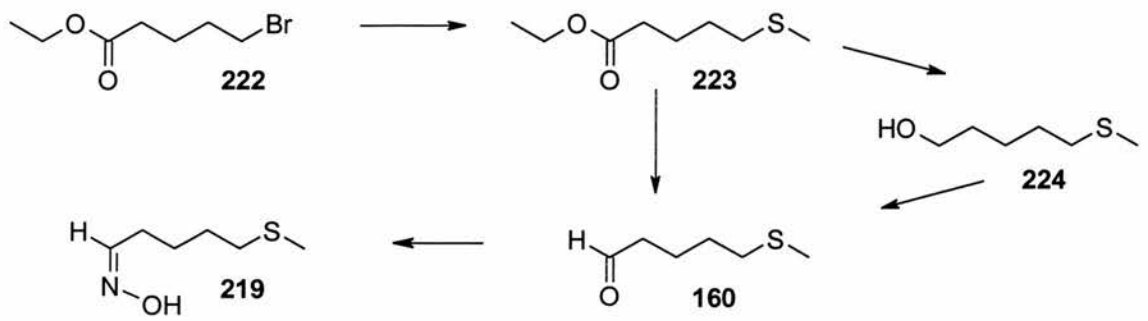
Scheme 54: Overall synthesis of 5-thiomethylpentaldoxime
a) Diethyl malonate, K_2CO_3, NBu_4Cl, DCM, H_2O (96%) b) NaCl, DMSO, H_2O (96%)
c) DIBAL, THF (99%) d) CBr_4, PPh_3, DCM (71%) e) KCN, 18-crown-6, MeCN (84%)
f) NaOH, H_2O, MeOH (85%) g) $SOCl_2$, MeOH (99%) h) DIBAL, THF (95%)
i) CBr_4, PPh_3, DCM j) NaSMe, MeOH (73% over 2 steps) k)OsO_4, py (63%)
l) $NaIO_4$, H_2O, THF m) $NH_2OH.HCl$, NaOAc, H_2O, EtOH (13% over 2 steps)
Overall yield 3%

2.4 Synthesis of Glucosinolate Biosynthetic Precursors

In collaboration with Professor Clint Chapple of Purdue University, USA, a number of compounds were synthesised for biological testing. These compounds were 5-, 8- and 9-thiomethylalkyloximes **219-221**, precursors of natural methionine-derived glucosinolates, and were to be used to probe the biosynthesis of glucosinolates.



Since these compounds did not require any isotopic labelling, no restrictions were placed upon the starting materials. The first compound to be synthesised was 5-thiomethylpentaldoxime **219**, and ethyl 5-bromovalerate **222** was judged to be a suitable starting material. It would provide a suitable leaving group that could be easily displaced with thiomethoxide **223**, and ester functionality that could be reduced to an aldehyde **160**, the desired oxime precursor. If necessary, the ester reduction could proceed to the alcohol **224**, and a controlled oxidation would be used to furnish the aldehyde **160**. (Scheme 55)



Scheme 55: Proposed synthetic route for 5-thiomethylpentaldoxime

As a precursor of glucoraphanin **22**, the formation of the oxime would also allow the synthesis of the glucosinolate by conversion of the oxime **219** to the oximyl chloride, with subsequent coupling to the protected thioglucose. Sulfation and deprotection would furnish glucoerucin **76.** (Scheme 55) The synthesis of the glucosinolate, however, was not undertaken.

2.4.1 Synthesis of 5-thiomethylpentaldoxime

The first step in the synthesis was a nucleophilic substitution of the bromide of the starting material with thiomethoxide anion. Ethyl 5-bromovalerate **222** was thus treated with sodium thiomethoxide in dry ethanol to furnish the desired product **223** in 70% yield. No purification was required.

A new singlet due to the thiomethyl group was observed in the ^{1}H NMR spectrum at 2.18 ppm. The protons of the CH_2 group adjacent to the sulfur had also shifted from 3.4 ppm in the starting material to 2.6 ppm in the product, further illustrating the change in functionality. In the ^{13}C NMR spectrum, few changes were observed, bar the change of the

former CH_2Br peak which shifted upfield to 16 ppm from 24 ppm. In the mass spectrum, the MH^+ peak was observed at 177 ppm, consistent with the structure.

2.4.1.1 Conversion of ethyl 5-thiomethylvalerate to aldehyde

The key step in the synthesis was the reduction of the ester **223** to an aldehyde **160**. DIBAL should accomplish this in a single step, since it is a reasonably mild reducing agent, and is known to reduce esters to aldehydes. Ethyl 5-thiomethylvalerate **223** was thus treated with di-isobutyl aluminium hydride in dry THF at –78 °C to give the desired aldehyde **160**. Purification by column chromatography on silica gave the desired aldehyde in a reasonable yield of 51%.

The ^1H NMR spectrum showed the change from the ester to the aldehyde, with the disappearance of the peaks due to the ethyl group. A new peak at 9.80 ppm was observed, due to the aldehyde proton. The changes were also reflected in the ^{13}C NMR spectrum, where the peaks of the ethyl group had also disappeared, and the carbonyl peak had moved significantly from 173 to 202 ppm.

However, the yield of only 51% was not ideal and attempts to optimise the yield were unsuccessful. It reflects the fact that the aldehyde is more susceptible to reduction than the ester and the reaction was worked up when the starting material and what was thought to be the alcohol were both present by TLC. The reaction used two equivalents of DIBAL, rather than the expected one. This was because using one equivalent gave a particularly slow reaction, and allowed a greater quantity of the alcohol to form. Using two equivalents

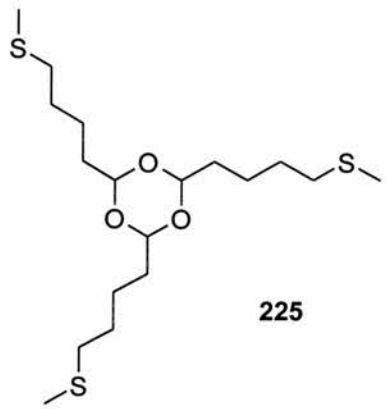
gave a faster reaction, which permitted the isolation of a larger amount of the aldehyde before it was further reduced to the alcohol.

Since the yield could not be improved any further by this method, it was decided to reduce the ester **223** to the alcohol **224**, then selectively oxidise the alcohol up to the aldehyde **160**. Ethyl 5-thiomethylvalerate **223** was treated with di-isobutyl aluminium hydride at room temperature to furnish the desired alcohol **224** in a yield of 88%. No purification was required.

The ^{1}H NMR spectrum showed that the ethyl group of the ester had been removed due to the disappearance of the relevant peaks. The integral ratio of the multiplet at 1.4 to 1.7 ppm had also grown to 6 protons, consistent with the incorporation of the new CH_2 protons. The ^{13}C spectrum also reflected the changes with the disappearance of the ester carbon peaks, and the peak due to the former carbonyl carbon had moved greatly upfield with the change to hydroxyl functionality.

The oxidation of the alcohol **224** to the aldehyde **160** was then attempted by means of a Swern oxidation,[174] which is used to oxidise alcohols to aldehydes and ketones. In the case of aldehydes, it avoids problems of the reaction proceeding to the carboxylic acid. Initial analysis of the product looked promising, but surprisingly, no aldehyde peak was present in the ^{1}H NMR spectrum. An unexpected resonance was observed in the spectrum at 4.83 ppm and the mass spectrum gave m/z of 396 mass units, three times the expected mass. These data suggested that the compound was in fact a trimer of the aldehyde. Taking this into consideration, the ^{1}H NMR spectrum was reviewed, and the unexpected peak had a resonance consistent with the aldehyde-equivalent peak of the trimer **225**. It is unclear

exactly how this compound formed. One possibility is that under the reaction conditions, the sulfur atom in the initial product acts as a nucleophile and attacks the aldehyde in an intramolecular fashion. The oxygen anion thus produced can then attack a second molecule at the aldehyde and then a further reaction produces a trimeric species. Finally cyclisation will give the observed product. Indeed, several examples of this type of structure have been found in the literature,[175,176] but these are usually formed in the presence of a catalyst. It is not clear why this reaction takes place during the Swern oxidation reaction. The 5-thiomethylpentanal produced subsequently seemed to be stable in solution and no trimer was observed during any other of its reactions.



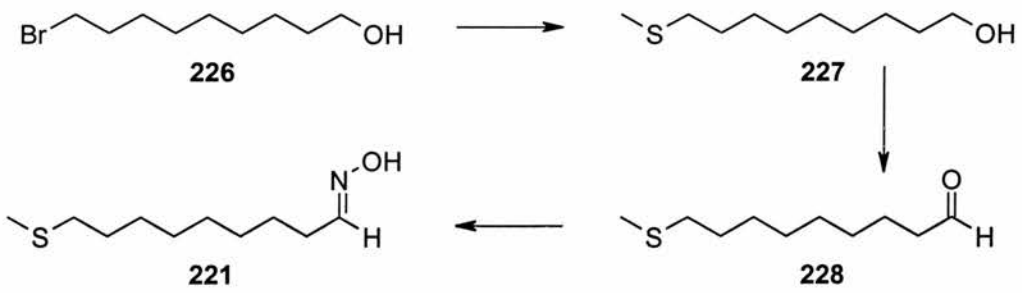
225

Since the oxidation of the alcohol to the aldehyde was so problematic, this synthetic step was abandoned, and it was decided to proceed with the reduction of the ester to the aldehyde. The reduction proved less problematic than the oxidation, and although the yield was not ideal, it was sufficient to produce the necessary quantity of the aldehyde to carry through to the oxime. 5-Thiomethylpentanal **160** was thus treated with hydroxylamine hydrochloride in the presence of sodium acetate to yield 5-thiomethylpentaldoxime. After recrystallisation, the product **219** was obtained as a white flaky solid in a yield of 30%, and in a ratio *E*:*Z* of 1:1.

In the ^1H NMR spectrum, the peak for the aldehyde proton had disappeared, and new peaks at 6.73, 7.31, 7.43 and 7.67 ppm showed the presence of both isomers of the oxime, with both the CHN peaks and the hydroxyl peaks all visible. The change was also reflected in the carbon NMR spectrum, with the peak for the former aldehyde moving upfield to give the two CN peaks at 152.0 and 152.7 ppm. The mass spectrum confirmed the structure with peaks for MH^+ and the molecular ion, as well as fragments consistent with the product.

2.4.2 Synthesis of 9-Thiomethylnonaldoxime

The second compound to be prepared was 9-thiomethylnonaldoxime **221**. It was anticipated that formation of the cyclic trimer would not occur in this synthesis due to the extended length of the chain, thus 9-bromononan-1-ol **226** was chosen as the starting material since it was deemed that oxidation to the aldehyde would be simpler than reduction of an ester. In any case, 9-bromononanoate esters do not appear to be commercially available. The synthesis would therefore require the bromide to be replaced with thiomethyl functionality **227**, oxidation of the alcohol to the aldehyde **228** and lastly conversion to the oxime **221**. (Scheme 56)



Scheme 56: Proposed synthetic route for 9-thiomethylnonaldoxime

9-Bromononan-1-ol **226** was treated with sodium thiomethoxide in ethanol at room temperature which furnished the product **227** as a colourless oil in 95% yield. The ^{1}H NMR spectrum showed a new peak at 2.12 ppm with an integral ratio of 3 protons, consistent with the addition of the thiomethyl group, and the former CH_2Br peak had shifted upfield from 3.41 ppm to 2.48 ppm. The changes were reflected in the ^{13}C NMR spectrum which also showed a new peak at 15.4 ppm for the thiomethyl group.

Subsequently, the alcohol **227** was oxidised under Swern conditions. Following purification by column chromatography, the product **228** was obtained as a colourless oil in 54% yield. The infra-red spectrum showed no presence of OH functionality, but that carbonyl functionality was present due to a peak at 1720 cm^{-1}. The ^{1}H NMR spectrum showed that the former CH_2OH peak had shifted from 3.64 ppm in the starting material to sit on top of the CH_2S peak at 2.43 ppm. The aldehyde proton peak was also observed at 9.77 ppm. The change was confirmed by the mass spectrum which showed peaks at 227 and 189 mass units for $[M+K]^+$ and $[MH]^+$ ions.

The oxidation did not produce the cyclic trimer that was observed when 5-thiomethylpentan-1-ol **224** was oxidised under the same conditions. This would suggest that the proximity of the sulfur to the alcohol/aldehyde was an important factor in the formation of the trimer, and adds credibility to the theory that the sulfur caused the cyclisation. The fact that the sulfur and oxygen atoms were six bonds apart in the five-carbon analogue, and therefore be able to form a six-membered ring, certainly adds some weight to the theory.

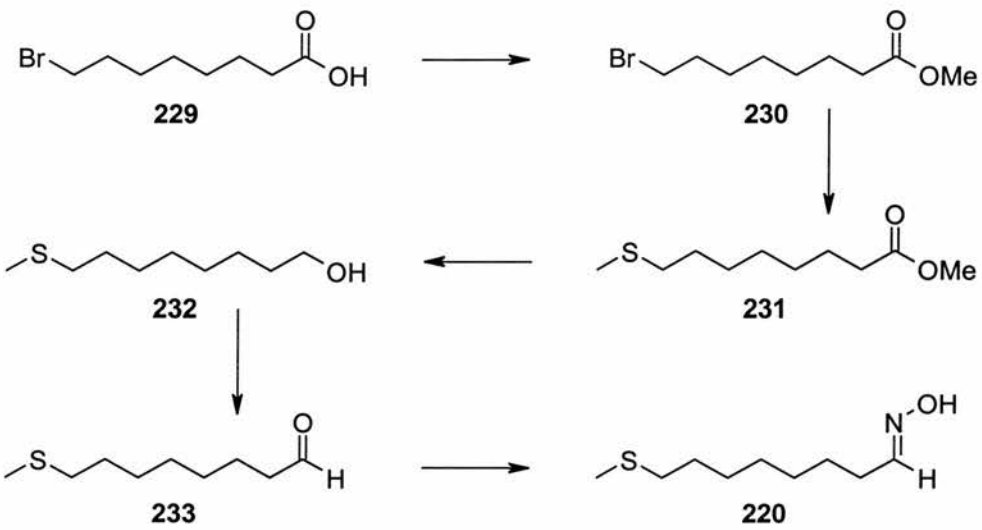
The aldehyde **228** was converted to the oxime **221** in the usual manner, by treatment with hydroxylamine hydrochloride in the presence of sodium acetate. Purification by column chromatography yielded the compound as a colourless solid in 61% yield.

The proton NMR spectrum showed the presence of hydroxyl functionality with a broad peak at 3500-3000 cm^{-1}, as well as a peak at 1663 cm^{-1} indicating the C=N group. No carbonyl peak was observed. The proton NMR spectrum was slightly complicated due to the presence of both isomers of the oxime, but showed that the former $CH_2C(O)$ peak had shifted downfield, to 2.18 ppm for the *E* isomer and 2.37 ppm for the *Z* isomer. Peaks for both oxime protons were present at 7.41 and 6.71 ppm for the *E* and *Z* isomers respectively, and had integral ratios showing a ratio of *Z*:*E* (3:2). The carbon NMR spectrum was also complicated, showing different peaks for carbons in both isomers. Peaks at 152.4 and 153.1 ppm were consistent with oxime functionality. The mass spectrum showed peaks for the $[M+K+Na]^+$ at 265, $[M+K]^+$ at 242, $[M-2H+Na]^+$ at 224 and $[M-H]^+$ at 202 mass units, confirming the mass, and showing a pattern consistent with the compound. CHN microanalysis found percentages of 58.73 for C, 10.73 for H and 6.59 for N, consistent with the respective calculated percentages of 59.07, 10.41 and 6.89.

The desired 9-thiomethylnonaldoxime **221** had successfully been synthesised. Some steps would require optimisation to produce this compound on a large scale, but the yields were adequate to produce a sufficient quantity of compound for Prof Chapple's research.

2.4.3 Synthesis of 8-thiomethyloctaldoxime

8-Bromooctanoic acid **230** was found to be the closest commercially available compound to 8-thiomethyloctaldoxime **220**. This required two extra steps over the synthetic route used for the nonaldoxime **221**, since the compound could only be purchased as the acid rather than the alcohol. The most obvious synthetic route was to convert the acid **229** to its corresponding ester **230**, which would be reduced to the alcohol **232**. Oxidation would again furnish the aldehyde **233**, which would be converted to the oxime **220**. (Scheme 57)



Scheme 57: Proposed synthetic route for 8-thiomethyloctaldoxime

8-Bromooctanoic acid **229** was treated with thionyl bromide in methanol under reflux. No purification was required and the product **230** was obtained as a yellow oil in 85% yield.

The infra-red spectrum showed the expected carbonyl peak at 1740 cm^{-1}, but the OH peak of the starting material had disappeared. Little change was observed in the proton NMR spectrum, but the presence of a new peak at 3.78 ppm with an integral ratio of three protons was in the expected position for the methyl group of the ester. In the carbon NMR

spectrum, a new peak was observed at 51.6 ppm, consistent with the methyl ester group, while the carbonyl peak had shifted slightly upfield, from 181 ppm to 174.2 ppm. The mass spectrum confirmed the compound with the expected pattern of peaks for a bromide compound at 259 and 261 mass units for $[M+Na]^+$ ions.

The bromoester **230** was then treated with sodium thiomethoxide in methanol at room temperature to furnish the product **231** as a colourless oil in 81% yield.

The proton NMR spectrum showed that the former CH_2Br peak had shifted significantly upfield from 3.51 ppm to 2.48 ppm, while a new peak at 2.09 ppm, with an integral ratio of three protons, showed the presence of the thiomethyl group. A new peak was also present in the ^{13}C NMR spectrum, at 15.7 ppm, consistent with the thiomethyl carbon.

DIBAL treatment of the thiomethyl ester **231** in THF at room temperature yielded the alcohol **232** as a colourless oil in 92% yield. The infra-red spectrum showed no presence of carbonyl functionality, but hydroxyl functionality was present due to a broad peak at 3600-3000 cm^{-1}. The ^1H NMR spectrum showed that the integral ratio of the multiplet at 1.30-1.46 ppm had increased by two protons due to the shifting of the former $CH_2C(O)$ peak into the multiplet. A new triplet peak was present at 3.65 ppm, with an integral ratio of two protons, indicating the reduction of the ester to alcohol functionality. In the ^{13}C NMR spectrum, one less carbon peak was observed with the loss of the methyl group of the ester, and the carbonyl peak had been replaced with a peak at 63.0 ppm.

The alcohol **232** was treated under Swern conditions[174] to furnish the aldehyde **233** in an excellent 99% yield. The infra-red spectrum showed that the alcohol functionality had been

removed, and a peak at 1686 cm^{-1} confirmed the presence of carbonyl functionality. The integral ratio of the low-range multiplet at 1.26-1.45 ppm had dropped by two protons, returning to the pattern that it had shown in the methyl ester of the compound, with the peak of the CH_2 group adjacent to the former CH_2OH group moving downfield to 2.47 ppm. The former CH_2OH peak had also disappeared, but a new peak at 9.78 ppm confirmed the aldehyde functionality. The ^{13}C NMR spectrum showed the presence of carbonyl functionality with a peak at 202.8 ppm, while the peak of its adjacent carbon had shifted to 44.0 ppm.

The oxime **220** was once again prepared from the aldehyde **233** by treatment with hydroxylamine hydrochloride in the presence of sodium acetate. Following purification by column chromatography, the product was obtained as a colourless solid in 53% yield.

The IR spectrum showed the presence of hydroxyl functionality with a broad peak at 3600-3000 cm^{-1} and a peak at 1675 cm^{-1} was consistent with C=N functionality. The ^1H NMR spectrum again showed both isomers of the oxime, with peaks at 6.72 and 7.43 ppm respectively for the C*H*N protons of the *Z* and *E* compounds. Both oximyl carbon peaks were also observed, at 152.7 ppm for the *E* isomer and 153.4 ppm for the *Z* isomer. Accurate mass spectrometry confirmed the compound with an $[MH]^+$ of 190.1271.

2.5 Summary

Using the previously developed synthesis, a sample of gluconasturtiin **38** has been successfully prepared for MALDI-TOF analysis.[147] Work has then moved on to the investigation of novel routes for glucosinolate synthesis that are suitable for the incorporation of ^{13}C isotopic labels.

Indole-3-acetic acid **142**, which is available in labelled form, was identified as a suitable precursor for glucobrassicin **20**. A synthetic strategy was developed for the synthesis of labelled glucobrassicin, and followed through to the acetylated desulfoglucosinolate stage. It was deemed unnecessary to pursue the route any further since this route has already been developed and tested. Further work on this compound would be to develop a synthesis of indole acetic acid to incorporate several ^{13}C labels, since a limited range of compounds is commercially available. Any compounds that are available are particularly expensive.

The development of a synthetic route to produce labelled glucoraphanin proved to be challenging. Initial routes using ethyl acetoacetate, 2-bromoethanol and diethyl malonate proved to be unsuitable. However, after considerable investigation, an appropriate method was developed. For incorporation of three ^{13}C atoms, diethyl malonate **199** and cyanide anion were the two small building blocks. Initially, cinnamyl bromide **156** was prepared from β-bromostyrene **207** and cyanide using a nickel catalysed procedure, allowing one ^{13}C atom to be incorporated. The rest of the 5-carbon side chain of glucoraphanin was then added using diethyl malonate **199**, employing a phase transfer catalyst method in high yield. Through a series of functional group transformations, the terminal thiomethyl group was then added. The next key step was the oxidative cleavage of the double bond. The

aromatic group had been initially included to produce less volatile intermediates and species more easily visualised on TLC, but had to be removed near the end of the synthesis. Ozonolysis was originally the method of choice, but this also oxidised the thiomethyl group to the sulfone. Conversion of the sulfone back to the sulfoxide is possible but not straightforward, however this does give a synthesis of another glucosinolate, namely glucoerysolin. The problem was overcome using a 2-step procedure involving dihydroxylation of the alkene using osmium tetroxide then oxidative cleavage of the diol using sodium periodate. The required aldehyde **160** was then converted to the oxime **219** precursor for the glucosinolate synthesis. Oxidation of the thiomethyl group to the sulfoxide can be carried out selectively at a later stage. This therefore represents a new formal synthesis for glucoraphanin suitable for incorporation of three ^{13}C-atoms into the side chain.

Three aldoximes were successfully synthesised for biological testing by Prof. Chapple's group. For the synthesis of 5-thiomethylpentaldoxime, ethyl 5-bromovalerate **222** was used as the starting material. Reduction of the ester gave the required aldehyde precursor but in only reasonable yield. Interestingly, the alternative of reduction to the alcohol and selective oxidation to the aldehyde was unsuccessful. This gave a cyclic trimeric species **225** presumably due to some involvement of the thiomethyl group. Conversion of the aldehyde, produced directly by reduction of the ester, to the oxime **219** was achieved by the usual method of treatment with hydroxylamine hydrochloride. 9-Thiomethylnonaldoxime **221** was simply prepared from 9-bromononan-1-ol **226** and 8-thiomethyloctaldoxime **220** was prepared in a similar manner from 8-bromooctanoic acid **229** without any major problems.

2.6 Future Work

A great deal of work has been carried out to develop routes suitable for ^{13}C labelled synthesis of glucosinolates, but inevitably, there is still a great deal of work to be done. Firstly, glucobrassicin could be synthesised by the method developed using the labelled indole acetic acid compound, while more advanced work could involve the in-house synthesis of a labelled indole.

In the case of glucoraphanin, a synthesis has been developed that would allow the synthesis of a labelled compound, but the final steps would require some optimisation. Further investigation of the osmium tetroxide and sodium periodate reactions should allow for higher yielding steps which would improve the feasibility of the route. It would also be worth investigating the possibility of producing the sulfoxide compound during the periodate cleavage step, since this could remove a step later in the synthesis.

Another route to consider would be the formation of a cinnamyl compound by means of a Wittig reaction with benzaldehyde. This would allow the possibility of shortening the synthesis if the ylide had sufficient chain length to require the addition of only one species to provide the necessary 5-carbon chain. A three-carbon ylide would require only a two carbon attachment, which could easily be achieved by addition of malonate as previously detailed. Obviously, this route would be dependent on the availability of suitable labelled starting materials, but is one worthy of investigation.

Finally, there is a vast array of glucosinolates, and very few have yet been synthesised with a view to isotopic labelling. Many are likely to possess interesting biological properties, so the identification and synthesis of other glucosinolates should be pursued.

Experimental

3.0 Experimental details

Melting points were determined using an Electrothermal melting point apparatus and are uncorrected.

NMR spectra were recorded on a Varian Gemini 2000 FT spectrometer (^{1}H, 300 MHz; ^{13}C, 74.76 MHz), a Varian Gemini FT spectrometer (^{1}H, 200 MHz; ^{13}C, 50.31 MHz), a Bruker AM 300 FT spectrometer (^{1}H, 300 MHz, ^{13}C, 75.4 MHz), a Bruker Avance 300 FT spectrometer (^{1}H, 300 MHz, ^{13}C, 75.4 MHz), a Bruker Avance 500 FT spectrometer (^{1}H, 500 MHz, ^{13}C, 125.7 MHz) and a Varian UnityPlus FT spectrometer (^{1}H, 500 MHz, ^{13}C 125.7 MHz). ^{1}H NMR spectra were referenced on chloroform or TMS. NMR spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quadruplet, p-pentet, m-multiplet, dd-doublet of doublets, dt-doublet of triplets and br-broad), coupling constant ($J_{x,y}$ Hz if applicable) and assignment.

IR spectra were taken on a Perkin–Elmer Paragon 1000 FT-IR spectrometer. The samples were prepared as nujol mulls, thin films between sodium chloride discs, or added in solution to PTFE substrate IR cards. Absorption maxima are given in wavenumbers (cm^{-1}).

Solvents were dried according to the methods of Perrin and Armarego.[177] Flash chromatography was performed according to the procedure of Still[178] using Sorbisil C60 (40-60 mm) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV_{254}) and compounds were visualized by UV fluorescence or potassium permanganate solution.

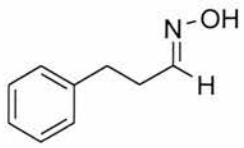
Electron Impact (EI) and Chemical Ionisation (CI) mass spectra were acquired using a VG Autospec spectrometer and a Micromass GCT (Time of Flight) spectrometer with Agilent 6890 GC unit. Electrospray (ES) and APCI mass spectra and were acquired using a Micromass LCT (Time of Flight) spectrometer, coupled with a Waters 2975 HPLC and MALDI-TOF mass spectra were acquired using a Micromass TofSpec 2E spectrometer.

CHN Microanalyses were recorded on a CE Instruments CHNS elemental analyser.

Optical rotations were measured at room temperature using an Optical Activity Ltd. AA 1000 polarimeter with 20 cm path-length cells.

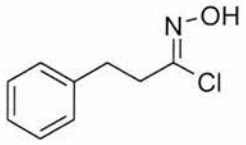
3.1 Synthesis of Gluconasturtiin

3.1.1 3-Phenylpropionaldehyde oxime **131**



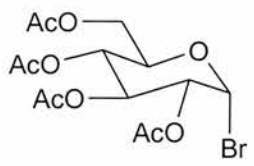
Sodium acetate trihydrate (5 g, 37 mmoles) and hydroxylamine hydrochloride (3 g, 43 mmoles) were dissolved in water (30 cm^3). 3-Phenylpropionaldehyde (3.27 cm^3, 25 mmoles) was dissolved in a small amount of ethanol and added to the aqueous solution. The reaction was heated to 70 °C for 30 minutes, then cooled to 0 °C. The oxime crystallised out of solution, was filtered off and washed with ice-cold water. The product was recrystallised from ethanol to yield a colourless flaky solid that was found to be a mixture of isomers (2.93 g, 79%); m.p. 78-82 °C (lit.,[179] 93-94.5 °C); (Found: C, 72.23; H, 7.94; N, 9.35. Calc for $C_9H_{11}NO$: C, 72.46; H, 7.43; N, 9.39%); ν_{max} (nujol)/cm^{-1} 3200-3110 (OH) and 1660 (C=N); δ_H (300 MHz; $CDCl_3$) 2.43-2.56 (0.5 × 2H, m, CH_2CHN, *E*), 2.64-2.77 (0.5 × 2H, m, CH_2CHN, *Z*), 2.77-2.88 (2H, m, PhCH_2, *E* and *Z*) 6.75 (0.5H, t, *J* 3.5, C*H*=N, *Z*), 7.15-7.37 (5H, m, C_6H_5), 7.50 (0.5H, t, *J* 3.5, C*H*=N, *E*); δ_C (75.45 MHz; $CDCl_3$) 27.2 (*C*H_2CHN, *Z*), 31.8 (*C*H_2CHN, *E*), 32.3 (PhCH_2, *Z*), 33.2 (PhCH_2, *E*), 126.0 (*C*-4), 129.0 (*C*-3 and 5), 129.2 (*C*-2 and 6), 141.2 (*C*-1, *E*), 141.4 (*C*-1, *Z*), 151.8 (*C*H=N, *E*), 152.1 (*C*H=N, *Z*); *m/z* (EI) 149 ($[M]^+$, 10%), 117 (23, $[M\text{-}NOH_2]^+$), 104 (51, $[M\text{-}CH_2NOH]^+$) and 91 (100, $[PhCH_2]^+$).

3.1.2 3-Phenylpropionaldehyde oximyl chloride **132**[180]



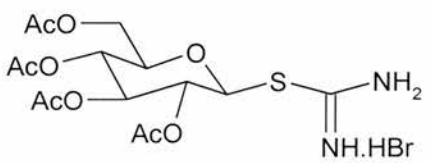
3-Phenylpropionaldehyde oxime (2 g, 13 mmoles) was dissolved in chloroform (30 cm^3) and dry triethylamine (0.54 cm^3). *N*-Chlorosuccinimide (2 g, 15 mmoles) was added slowly to the solution, which had been cooled to 0 °C. The reaction was stirred for 45 minutes, then poured onto ice-water (40 cm^3). The solution was extracted with diethyl ether (2 × 80 cm^3), then the organic layer washed with water (2 × 40 cm^3). The organic layer was dried ($MgSO_4$) and the solvent removed under reduced pressure. The product was obtained as a golden oil (3.43 g, 93%). The product was used without further purification due to its instability; ν_{max} (nujol)/cm^{-1} 3200 (OH); δ_H (300 MHz; $CDCl_3$) 2.69-2.86 (2H, m, PhCH_2), 2.86-2.98 (2H, m, CH_2CClN), 7.00-7.35 (5H, m, C_6H_5), 8.57 (1H, s, O*H*); δ_C (50.3 MHz; $CDCl_3$) 33.0 (Ph*C*H_2), 38.9 (*C*H_2CClN), 126.8 (*C*-4), 128.9 (*C*-3 and 5), 129.0 (*C*-2 and 6), 144.0 (*C*-1), 146.0 (*C*=N); *m/z* (CI) 184, 186 ($[MH]^+$, 31, 10%), 130 (100, $[M\text{-}ClOH_2]^+$), 91 (30, $[PhCH_2]^+$) and 80 (94, $[CHClNOH_2]^+$).

3.1.3 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Acetobromoglucose) **134**



D-Glucose (10.01 g, 55 mmoles) was dissolved in acetic anhydride (36 cm^3) at 0 °C under a nitrogen atmosphere. Hydrogen bromide in acetic acid (45% w/v, 15 cm^3, 84 mmoles) was added dropwise to the solution with stirring. The solution was stirred for 3½ hours, then further hydrogen bromide in acetic acid (45 cm^3, 253 mmoles) was added and the solution was stirred overnight. Dichloromethane (75 cm^3) was added and the reaction mixture poured into ice-water (125 cm^3). The organic phase was washed with saturated sodium bicarbonate solution (150 cm^3), then dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a golden oil that solidified on cooling. The product was recrystallised from diethyl ether to yield a colourless crystalline solid (16.06 g, 70%); m.p. 86-87 °C (lit.,[181] 88-89 °C); $[\alpha]_D$ +195.9 (c 2.42 in $CHCl_3$) (lit.,[182] +197.84 (c 2.42 in $CHCl_3$)); ν_{max} (nujol)/cm^{-1} 1730 (CO); δ_H (300 MHz; $CDCl_3$) 2.00-2.10 (12H, 4s, 4 × OC(O)CH_3), 4.09-4.16 (1H, m, *H*-5), 4.25-4.36 (2H, m, *H*-6a and 6b), 4.85 (1H, dd, $J_{1,2}$ 5, $J_{2,3}$ 10, *H*-2), 5.15 (1H, t, $J_{3,4}$ 10, $J_{4,5}$ 10, *H*-4), 5.55 (1H, t, $J_{2,3}$ 10, $J_{3,4}$ 10, *H*-3), 6.60 (1H, d, $J_{1,2}$ 5, *H*-1); δ_C (50.3 MHz; $CDCl_3$) 21.0 & 21.1 (4 × OC(O)*C*H_3), 61.4 (*C*-6), 67.6 (*C*-4), 70.6 (*C*-2), 71.0 (*C*-3), 72.6 (*C*-5), 86.6 (*C*-1), 169.9, 170.2, 170.3, 170.9 (4 × O*C*(O)CH_3); *m/z* (CI) 428, 430 ($[M+NH_4]^+$, 18%), 331 (8, $[M-Br]^+$) and 213 (29, $[M-Br-2OAc]^+$).

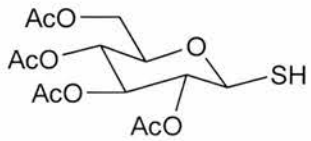
3.1.4 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosylisothiouronium bromide **135**



Acetobromoglucose (10 g, 25 mmoles) was dissolved in dry acetone (23 cm^3). Thiourea (1.9 g, 25 mmoles) was added and the solution heated to reflux for 15 minutes. A white

precipitate appeared and the mixture was cooled to 0 °C. The precipitate was removed by filtration and recrystallised from acetone. The product was obtained at a colourless solid (5.94 g, 50%); m.p. 200 °C decomposes (lit.,[183] 205 °C); $[\alpha]_D$ -17.1 (c 1.0 in MeOH) (lit.,[184] -17.3 (1.0 in MeOH)); ν_{max} (nujol)/cm^{-1} 3310-3160 (NH), 1750 (CO) and 1655 (NH); δ_H (300 MHz; D_2O) 1.94-2.05 (12H, 4s, 4 × OC(O)C*H*$_3$), 4.08-4.17 (1H, m, *H*-5), 4.18 (1H, dd, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12.5, *H*-6a), 4.29 (1H, dd, $J_{5,6b}$ 2.5, $J_{6a,6b}$ 12.5, *H*-6b), 5.12 (1H, t, *J* 10, *H*-2), 5.23 (1H, t, *J* 10, *H*-4), 5.34 (1H, t, *J* 10, *H*-3), 5.38 (1H, d, *J* 10, *H*-1); δ_C (50.3 MHz; D_2O) 14.9, 18.0, 18.1, 18.2 (4 × OC(O)*C*H$_3$), 59.9 (*C*-6), 65.7 (*C*-4), 67.2 (*C*-2), 71.4 (*C*-3), 73.9 (*C*-5), 79.2 (*C*-1), 170.3 (*C*=N), 170.5, 170.7, 171.0, 171.7 (4 × O*C*(O)CH$_3$); *m/z* (EI) 331 ([M-CH_4BrN_2S]$^+$, 11%), 169 (35, [$C_8H_9O_4$]$^+$ and 127 (18, [$C_6H_7O_3$]$^+$).

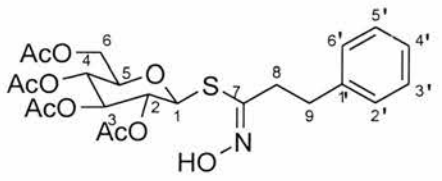
3.1.5 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose **136**



Potassium metabisulfite (2.3 g, 10 mmoles) was dissolved in water (30 cm^3). The solution was heated to 75 °C. Dichloromethane (60 cm^3) was carefully added, followed by 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylisothiouronium bromide (6 g, 25 mmoles). The biphasic solution was then heated under reflux for 30 minutes before being cooled to room temperature. The organic phase was washed with water (3 × 30 cm^3) and the aqueous layer with dichloromethane (40 cm^3). The combined organic layers were dried ($MgSO_4$) and the solvent removed at reduced pressure to yield a colourless solid. The product was

recrystallised from methanol to yield a colourless crystalline solid (4.15 g, 92%); m.p. 74-75 °C (lit.,[185] 75 °C); $[\alpha]_D$ -9.8 (c 1.5 in EtOH) (lit.,[186] -8.3 (c 1.5 in EtOH)); ν_{max} (nujol)/cm^{-1} 3460 (SH) and 1735 (CO); δ_H (300 MHz; $CDCl_3$) 2.00-2.10 (12H, 4s, 4 × OC(O)C*H*$_3$), 2.15 (1H, s, S*H*), 3.70 (1H, t, $J_{3,4}$ 10, $J_{4,5}$ 10, *H*-4), 4.13 (1H, dd, $J_{5,6a}$ 2.5, $J_{6a,6b}$ 12.5, *H*-6a), 4.25 (1H, dd, $J_{5,6b}$ 5, $J_{6a,6b}$ 12.5, *H*-6b), 4.55 (1H, d, $J_{1,2}$ 10, *H*-1), 4.98 (1H, t, $J_{1,2}$ 10, $J_{2,3}$ 10, *H*-2), 5.11 (1H, t, $J_{3,4}$ 10, $J_{4,5}$ 10, *H*-4), 5.20 (1H, t, $J_{2,3}$ 10, $J_{3,4}$ 10, *H*-3); δ_C (50.3 MHz; $CDCl_3$) 21.0, 21.2 (4 × OC(O)*C*H$_3$), 62.4 (*C*-6), 68.5 (*C*-4), 74.0 (*C*-2), 76.8 (*C*-3), 77.5 (*C*-5), 79.1 (*C*-1), 169.8, 170.1, 170.6, 171.1 (4 × O*C*(O)CH$_3$); *m/z* (CI) 382 ($[M+NH_4]^+$, 100%), 331 (7, $[M-SH]^+$) and 322 (22, $[M-OAc]^+$).

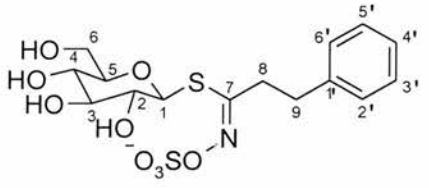
3.1.6 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl phenethyl thiohydroximate **137**



3-Phenylpropionaldehyde oximyl chloride (0.8 g, 4 mmoles) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (0.68 g, 2 mmoles) were dissolved in dry tetrahydrofuran (150 cm^3) under nitrogen. Dry triethylamine (5.73 cm^3) was then added and the reaction stirred for 18 hours. The solvent was removed under reduced pressure and the residue was taken up in diethyl ether (50 cm^3) and the solution was washed with 1 M sulphuric acid (50 cm^3). The acid layer was extracted with ethyl acetate (20 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed at reduced pressure. The product was obtained as a yellow-brown solid (1.02 g). It was purified by column chromatography on silica, using ethyl acetate/hexane (1:1) as eluent, to yield an off-white powdery solid (0.70

g, 72%); m.p. >150 °C decomposes (lit.,[187] 198 °C); $[\alpha]_D$ +16.4 (c 1.0 in $CHCl_3$) (lit.,[187] +11.1 (c 0.2 in CCl_4)); (Found: C, 54.30; H, 5.81; N, 2.86. Calc for $C_{23}H_{29}NO_{10}S$: C, 54.00; H, 5.71; N, 2.74%); ν_{max} (nujol)/cm^{-1} 3300 (OH) and 1750 (C=O); δ_H (300 MHz; $CDCl_3$) 1.90-2.05 (12H, 4s, 4 × OC(O)CH_3), 2.75-2.90 (2H, m, *H*-8), 2.90-3.00 (2H, m, *H*-9), 3.64-3.72 (1H, m, *H*-5), 4.07-4.14 (2H, m, *H*-6a and 6b), 4.95-5.10 (3H, m, *H*-1, 2, 4), 5.20 (1H, t, *J* 10, *H*-3), 7.20-7.35 (5H, m, C_6H_5); δ_C (50.3 MHz; $CDCl_3$) 21.0-21.3 (4 × OC(O)CH_3), 33.7 (CH_2), 34.7 (CH_2), 62.8 (*C*-6), 68.4 (*C*-4), 70.5 (*C*-2), 74.1 (*C*-3), 76.5 (*C*-5), 80.2 (*C*-1), 127.1 (*C*-4'), 128.7 (*C*-3' and 5'), 129.1 (*C*-2' and 6'), 140.9 (*C*-1'), 152.2 (*C*=N), 169.8, 169.9, 170.8, 171.2 (4 × O*C*(O)CH_3); *m/z* (EI) 511 ($[M]^+$, 3%), 494 (8, $[M\text{-}OH]^+$), 331(79, $[M\text{-Aglycone}]^+$), 271 (27, $[M\text{-Aglycone-AcOH}]^+$), 169 (96, $[C_8H_9O_4]^+$) and 109 (73, $[PhCH_2{+}OH_2]^+$).

3.1.7 Gluconasturtiin **38**[125]

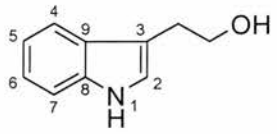


Dry pyridine (50 cm^3) and dry dichloromethane (50 cm^3) were cooled to 0 °C under nitrogen. Chlorosulfonic acid (2.3 cm^3) in dry dichloromethane (50 cm^3) was added to the solvent mixture over thirty minutes. A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phenethyl thiohydroximate (1.071 g, 2.1 mmoles) in dry dichloromethane (25 cm^3) was then added and the reaction was stirred for 24 hours at room temperature. Saturated aqueous sodium bicarbonate solution (50 cm^3) was then added and the biphasic solution was stirred for thirty minutes. The solvent was removed under reduced pressure.

The concentrate was then dissolved in the minimum volume of dichloromethane and heated under reflux. Upon cooling, no crystallisation took place, so the solvent was removed under reduced pressure. This yielded gluconasturtiin within a mixture of salts: ν_{max} (nujol)/cm^{-1} 3600-3000 (OH); δ_H (300 MHz; D_2O) 2.80-3.00 (4H, m, CH_2CH_2), 3.20-3.40 (4H, m, *H*-2, 3, 4, 5), 3.50 (1H, dd, $J_{5,6b}$ 5, J_{gem} 12.5, *H*-6b), 3.70 (1H, dd, $J_{5,6a}$ 2.5, J_{gem} 12.5, *H*-6a), 4.85 (1H, d, $J_{1,2}$ 10, *H*-1), 7.18-7.32 (5H, m, C_6H_5); δ_C (75.45 MHz; D_2O) 32.5, 33.8 (2 × CH_2), 60.6 (*C*-6), 69.1 (*C*-4), 71.9 (*C*-2), 77.1 (*C*-3), 80.1 (*C*-5), 81.7 (*C*-1), 126.7 (*C*-4'), 128.8 (*C*-3' and 5'), 128.9 (*C*-2' and 6'), 140.7 (*C*-1'), 163.5 (*C*=N); *m/z* (MALDI-TOF) 422 $[M]^-$.

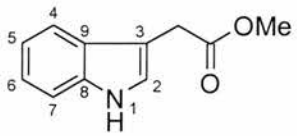
3.2 Synthesis towards Glucobrassicin

3.2.1 3-(2'-Hydroxyethyl)indole **145**



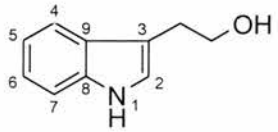
Indole-3-acetic acid (5 g, 28.5 mmoles) was dissolved in dry diethyl ether (200 cm^3). Lithium aluminium hydride (1.036 g, 27 mmoles) was added to the solution, which was stirred overnight. Water was then carefully added to the solution until all the lithium aluminium hydride had reacted. The mixture was extracted with diethyl ether (2 × 200 cm^3) and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a brownish solid (1.70 g, 38%); mp 52-54 °C (lit.,[151] 59 °C); ν_{max} (nujol)/cm^{-1} 3300 (OH), 3386.9 (NH); δ_H (300 MHz; $CDCl_3$) 1.80 (1H, t, *J* 7, O*H*), 3.16 (2H, t, *J* 7, C$H_2$$CH_2OH$), 4.04 (2H, q, *J* 7, CH_2OH), 7.15 (1H, s, *H*-2), 7.27 (1H, t, *J* 7, *H*-5), 7.35 (1H, t, *J* 7, *H*-6), 7.48 (1H, d, *J* 7, *H*-7), 7.76 (1H, d, *J* 7, *H*-4), 8.23 (1H, br s, N*H*); δ_C (75.4 MHz; $CDCl_3$) 29.1 (CH_2CH_2OH), 63.0 (CH_2OH), 111.6 (*C*-7), 112.6 (*C*-3), 119.2 (*C*-4), 119.8 (*C*-5), 122.5 (*C*-6), 122.9 (*C*-2), 127.8 (*C*-9), 136.8 (*C*-8); *m/z* (EI) 161 ($[M]^+$, 23%), 130 (100, $[M-CH_2OH]^+$), 103 (7, $[C_8H_7]^+$) and 77 (11, $[C_6H_5]^+$).

3.2.2 Methyl indole-3-acetate **148**



Indole-3-acetic acid (5 g, 28.5 mmoles) was dissolved in dry methanol (65 cm^3). Thionyl chloride (3.62 g, 2.21 cm^3, 30.4 mmoles) was added to the solution, which was then heated under reflux for 2 hours. The solution was allowed to cool, then the methanol was removed under reduced pressure yielding a dark purple oil. The product was washed with saturated aqueous sodium bicarbonate solution (50 cm^3), extracted into diethyl ether (2 × 50 cm^3) and dried ($MgSO_4$). The solvent was removed under reduced pressure, giving a brownish oil which was purified by column chromatography on silica, using petroleum ether (40-60 °C)/diethyl ether (3:2) to yield an off-white solid (3.98 g, 74%); mp 48-49 °C (lit.,[188] 47-48 °C); ν_{max} (nujol)/cm^{-1} 1730 (C=O); δ_H (300 MHz; $CDCl_3$) 3.85 (3H, s, CH_3), 3.92 (2H, s, CH_2), 7.2 (1H, s, *H*-2), 7.22-7.35 (2H, m, *H*-5 and 6), 7.45 (1H, d, *J* 7, *H*-7), 7.75 (1H, d, *J* 7, *H*-4), 8.23 (1H, br s, N*H*); δ_C (75.4 MHz; $CDCl_3$) 31.6 (CH_2), 52.4 (CH_3), 108.4 (*C*-3), 111.8 (*C*-7), 119.1 (*C*-4), 120.0 (*C*-5), 122.5 (*C*-6), 123.8 (*C*-2), 127.5 (*C*-9), 136.5 (*C*-8), 173.3 (*C*=O); *m/z* (ES) 212 ($[M+Na]^+$, 59%), 190 (100, $[MH]^+$) and 130 (40, $[M-CO_2Me]^+$).

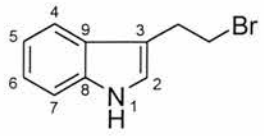
3.2.3 3-(2'-Hydroxyethyl)indole **145**



Methyl indole-3-acetate (1.040 g, 5.5 mmoles) was dissolved in dry diethyl ether (100 cm^3). Lithium aluminium hydride (0.5 g, 13 mmoles) was added to the stirred solution. The reaction was monitored by TLC (diethyl ether/petroleum ether (40-60 °C) (1:1)) and water (50 cm^3) was added when no starting material could be observed. The product was extracted into diethyl ether (2 × 100 cm^3) and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a green oil (0.72 g, 81%). The product was purified by

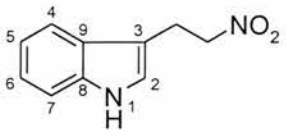
column chromatography on silica to yield an off white solid (0.56 g, 63%); data as per 3.2.1

3.2.4 3-(2'-Bromoethyl)indole **146**



3-(2'-Hydroxyethyl)indole (2.3 g, 14.3 mmoles) was dissolved in dry dichloromethane (100 cm^3). Triphenylphosphine (7.63 g, 29 mmoles) and carbon tetrabromide (9.448 g, 28.5 mmoles) were added to the solution, which was stirred overnight. The solution was washed with saturated aqueous potassium bicarbonate solution (100 cm^3), then extracted with ethyl acetate (2 × 100 cm^3). The combined organic layers were then dried ($MgSO_4$) and the solvent was removed at reduced pressure. A viscous brown liquid was obtained. This was purified by column chromatography on silica using diethyl ether/petroleum ether (40-60 °C) 1:1 to yield an air sensitive colourless crystalline solid (2.7 g, 85%); m.p. 85-87 °C (lit.,[189] 90-95 °C); ν_{max} (nujol)/cm^{-1} 3394 (NH); δ_H (200 MHz; $CDCl_3$) 3.35 (2H, t, *J* 8, CH_2CH_2Br), 3.65 (2H, t, *J* 8, CH_2Br), 7.10 (1H, s, *H*-2), 7.17 (1H, t, *J* 8, *H*-5), 7.25 (1H, t, *J* 8, *H*-6), 7.40 (1H, d, *J* 8, *H*-7), 7.62 (1H, d, *J* 8, *H*-4), 8.05 (1H, br s, N*H*); δ_C (75.4 MHz; $CDCl_3$) 29.3 (CH_2CH_2Br), 32.9 (CH_2Br), 111.3 (*C*-7), 113.5 (*C*-3), 118.5 (*C*-4), 119.6 (*C*-5), 122.2 (*C*-2 and 6), 126.9 (*C*-9), 136.1 (*C*-8); *m/z* (CI) 224, 226 ($[MH]^+$, 71, 70 %), 144 (100, $[M-Br]^+$) and 130 (11, $[M-CH_2Br]^+$).

3.2.5 3-(2'-Nitroethyl)indole **71**



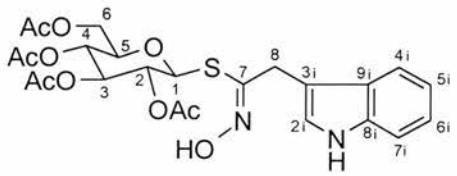
Method 1

3-(2'-Bromoethyl)indole (0.1 g, 0.4 mmoles) and sodium nitrite (0.04 g, 0.58 mmoles) were dissolved in dry dimethylformamide (50 cm^3). The solution was stirred at room temperature for 70 hours, but TLC analysis showed that no reaction had taken place. The solution was then heated under reflux for 90 minutes. After cooling to room temperature, the reaction mixture was washed with saturated aqueous potassium bicarbonate solution (50 cm^3) and extracted with ethyl acetate (2 × 50 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The product was purified by column chromatography on silica using diethyl ether/petroleum ether (1:2) then (1:1) to give the desired product as a brownish solid (0.05 g, 53%); mp 49-51 °C (lit.,[190] 56.5-57 °C); ν_{max} (nujol)/cm^{-1} 3385 (NH); δ_H (500 MHz; $CDCl_3$) 3.48 (2H, t, *J* 7, $CH_2CH_2NO_2$), 4.65 (2H, t, *J* 7, CH_2NO_2), 7.04 (1H, s, *H*-2), 7.16 (1H, t, *J* 4.4, *H*-5), 7.22 (1H, t, *J* 7, *H*-6), 7.37 (1H, d, *J* 7, *H*-7), 7.58 (1H, d, *J* 7, *H*-4), 8.06 (1H, br s, N*H*); δ_C (75.4 MHz; $CDCl_3$), 23.5 ($CH_2CH_2NO_2$), 75.8 (CH_2NO_2), 110.1 (*C*-3), 111.6 (*C*-7), 118.2 (*C*-4), 120.0 (*C*-5), 122.7 (*C*-2 and 6), 126.8 (*C*-9), 136.4 (*C*-8); *m/z* (CI) 191 ($[MH]^+$, 5), 144 (5, $[M\text{-}NO_2]^+$) and 130 (9, $[M\text{-}CH_2NO_2]^+$).

Method 2

3-(2'-Bromoethyl)indole (4.9 g, 21.9 mmoles) was dissolved in acetone (300 cm^3). Sodium nitrite (5 g, 72 mmoles), potassium iodide (3.8 g, 22.9 mmoles) and 18-crown-6 (17.5 g, 66.2 mmoles) were added to the solution which was stirred for 3 days at room temperature. The acetone was removed under reduced pressure and the product was washed with water (100 cm^3). The product was extracted with diethyl ether (2 × 150 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure. Purification on a silica column using petroleum ether (40-60 °C)/diethyl ether (4:1) yielded the product as an off-white solid (2.19 g, 52%); data as per Method 1

3.2.6 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl glucobrassicin thiohydroximate **151**



Sodium (0.18 g, 7.8 mmoles) was dissolved in dry methanol (15 cm^3) under nitrogen. 3-(2'-Nitroethyl)indole (1.5 g, 7.9 mmoles) in dry diethyl ether (10 cm^3) was added and the solution stirred at room temperature for 15 minutes. The solvent was removed under reduced pressure, then the product was put under high vacuum for 15 minutes.

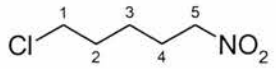
The nitronate was dissolved in dry 1,2-dimethoxyethane (40 cm^3) and the solution was cooled to -40 °C. Thionyl chloride (3.03 g, 1.86 cm^3, 25 mmoles) in 1,2-dimethoxyethane (12.5 cm^3) was added dropwise and the solution stirred for 30 minutes. Water (60 cm^3) was

then added and the product was extracted with dichloromethane (2 × 100 cm^3). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The product was placed under high vacuum for a further 15 minutes.

The oximyl chloride was dissolved in dry diethyl ether (40 cm^3) and dry dichloromethane (20 cm^3). 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranose (2.51 g, 6.9 mmoles) and dry triethylamine (1.815g, 2.5 cm^3, 17.9 mmoles) in dry dichloromethane (20 cm^3) were added to the solution, which was stirred for 1 hour. The reaction was then acidified with sulfuric acid (0.5 M, 100 cm^3) and extracted with dichloromethane (2 × 100 cm^3). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield the product as a dark oil. The product was purified twice by column chromatography on silica gel, firstly using petroleum ether (40-60 °C)/ethyl acetate (6:4) as the eluent and secondly using dichloromethane/methanol (97:3) as the eluent. This yielded the product as a light brown solid (1.45 g, 39%); m.p. 78-81 °C (lit.,[55] 83-86 °C); ν_{max} (nujol)/cm^{-1} 3350 (NH, OH), 1750 ($C(O)CH_3$); δ_H (300 MHz; $CDCl_3$) 2.02-2.18 (4 × 3H, 4 s, $OC(O)CH_3$), 3.34-3.42 (1H, m, *H*-5), 4.05 (1H, dt, J_{gem} 12, $J_{6a,5}$ 2.5, *H*-6a), 4.15-4.30 (3H, m, *H*-6b, 8a and 8b), 5.05-5.15 (4H, m, *H*-1, 2, 3 and 4), 7.20 (1H, s, *H*-2i), 7.25 (1H, t, *J* 8, *H*-5i), 7.35 (1H, t, *J* 8, *H*-6i), 7.50 (1H, d, $J_{7i,6i}$ 8, *H*-7i), 7.70 (1H, d, $J_{4i,5i}$ 8, *H*-4i), 8.20 (1H, br s, O*H*), 8.30 (1H, br s, N*H*); δ_C (125.8 MHz; $CDCl_3$) 18.5, 18.6, 21.0, 21.2 (4 × $OC(O)CH_3$), 29.0 (*C*-8), 58.6 (*C*-6), 68.3 (*C*-4), 70.5 (*C*-2), 74.2 (*C*-3), 75.9 (*C*-5), 79.9 (*C*-1), 110.3 (*C*-3i), 112.2 (*C*-7i), 118.6 (*C*-4i), 120.1 (*C*-5i), 122.8 (*C*-6i), 123.5 (*C*-2i), 127.0 (*C*-9i), 136.8 (*C*-8i), 151.9 (*C*-7), 170.0, 170.1, 170.9, 171.5 (4 × $OC(O)CH_3$); *m/z* (ES) 556 ($[M+Na]^+$, 100%).

3.3 Synthesis towards Glucoraphanin

3.3.1 1-Chloro-5-nitropentane **150**



Sodium Nitrite method[131]

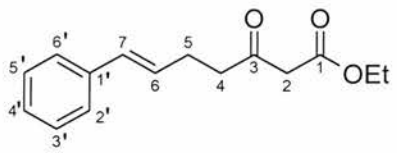
1-Bromo-5-chloropentane (0.6 cm^3, 4.6 mmoles), sodium nitrite (0.65 g, 9.4 mmoles) and 18-crown-6 (2.45 g, 9.3 mmoles) were stirred together in acetone (20 cm^3) at room temperature for one hour. Diethyl ether (50 cm^3) was then added to the solution, followed by water (30 cm^3). The biphasic solution was separated and the aqueous layer extracted with diethyl ether (50 cm^3). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the product was obtained as a yellow oil. This was purified by column chromatography on silica using petroleum ether (40-60 °C)/diethyl ether 5:1 as eluent to give the product as a yellow oil. As only a small amount of purified product was obtained for characterisation, no yield was calculated: ν_{max} (film)/cm^{-1} 1551 and 1383 (NO_2); δ_H (300 MHz; $CDCl_3$) 1.58 (2H, p, *J* 7, 3-CH_2), 1.80 (2H, p, *J* 7, CH_2-2), 2.05 (2H, p, *J* 7, CH_2-4), 3.55 (2H, t, *J* 7, CH_2Cl), 4.40 (2H, t, *J* 7, CH_2NO_2); δ_C (75.4 MHz; $CDCl_3$) 24.0 (*C*-3), 27.0 (*C*-4), 32.1 (*C*-2), 44.6 (*C*H_2Cl), 75.7 (*C*H_2NO_2); *m/z* (CI) 152, 154 ($[MH]^+$, 100, 32%), 116 (9, $[M\text{-}Cl]^+$), 105,107 (81, 25, $[M\text{-}NO_2]^+$) and 69 (50, $[C_5H_9]^+$).

Ion Exchange method[153]

Amberlite IRA 900 ion exchange resin was converted to the NO_2^- form by stirring with aqueous sodium nitrite solution (1 M) overnight. The resin was then washed with water, ethanol and toluene and dried under reduced pressure at 40 °C for four hours.

1-Bromo-5-chloropentane (1 cm^3, 7.6 mmoles) was stirred with the dried resin (9 g) in toluene (30 cm^3) overnight. The resin was then removed by filtration and washed with diethyl ether. The solvent was removed under reduced pressure. The product was purified by Kugelrohr distillation at 80 °C, 1.2mm Hg to yield a yellow oil (1.00 g, 87%); Data as per sodium nitrite method.

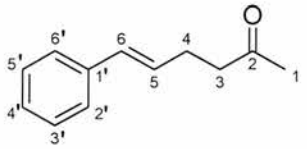
3.3.2 Ethyl 3-oxo-7-phenylhept-6-enoate **157**[191]



A solution of sodium hydride (60%, 0.45 g, 11.3 mmoles) in dry THF (25 cm^3) was cooled to 0 °C. Ethyl acetoacetate (1.3 g, 10 mmoles) was added dropwise and the solution was stirred for 10 minutes at 0 °C. *n*-Butyl lithium (2.5 M, 6 cm^3, 15 mmoles) was added dropwise and the solution was again stirred for 10 minutes at 0 °C. Cinnamyl bromide (2.17 g, 11 mmoles) in dry THF (2 cm^3) was added to the reaction solution, which was stirred for a further 15 minutes. The reaction was quenched with aqueous hydrochloric acid (2 cm^3 HCl, 5 cm^3 water) and the reaction mixture was diluted with diethyl ether (50 cm^3).

The organic layer was washed with water until neutral, then dried over $MgSO_4$. The product was obtained as a brown oil. The product was purified by column chromatography on silica using hexane/diethyl ether (19:1). A pale yellow oil was obtained (1.17 g, 48%); ν_{max} (film)/cm^{-1} 1718 (C=O) and 966 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.40 (3H, t, *J* 8, CH_3), 2.60 (2H, q, *J* 8, $CHCH_2$), 2.85 (2H, t, *J* 8, CH_2CH_2CO), 3.55 (2H, s, $C(O)CH_2C(O)$), 4.30 (2H, q, *J* 8, OCH_2CH_3), 6.30 (1H, dt, *J* 8, *J* 16, $CHCH_2$), 6.55 (1H, d, *J* 16, PhC*H*), 7.27-7.48 (5H, m, C_6H_5); δ_C (75.4MHz, $CDCl_3$) 14.5 (CH_3), 27.2 (*C*-5), 42.9 (*C*-4), 50.5 (*C*-2), 61.8 (CH_2CH_3), 126.4 (*C*-2' and 6'), 127.6 (*C*-6), 128.7 (*C*-4'), 128.9 (*C*-3' and 5'), 131.4 (*C*-7), 137.7 (*C*-1'), 167.5 (*C*-1), 202.4 (*C*-3); *m/z* (CI) 247 (MH^+, 100%), 175 (50, $[C_{12}H_{15}O]^+$), 143 (32) and 117 (50, $[C_9H_9]^+$).

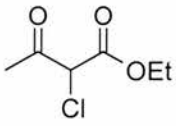
3.3.3 6-Phenylhex-5-en-2-one **158**[192]



Ethyl 3-oxo-7-phenylhept-6-enoate (~15 g, 73 mmoles) was heated under reflux with aqueous hydrochloric acid (100 cm^3 HCl, 100 cm^3 water) for 2 hours. The product was extracted with diethyl ether (2 × 100 cm^3) and the combined organic layers were washed with aqueous sodium bicarbonate solution (100 cm^3). The ethereal solution was dried ($MgSO_4$) and concentrated under reduced pressure to give the product as a dark brown oil which was purified by column chromatography on silica using petroleum ether (40-60 °C)/diethyl ether (19:1). This gave the product as a pale yellow oil (9.90 g, 48% over 2 steps): ν_{max} (film)/cm^{-1} 1717 (C=O) and 967 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 2.30 (3H,

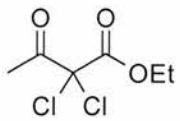
s, CH_3), 2.60 (2H, q, *J* 8, CH_2-4), 2.75 (2H, t, *J* 8, CH_2-3), 6.30 (1H, dt, *J* 8, *J* 16, C*H*-5), 6.55 (1H, d, *J* 16, C*H*-6), 7.30-7.50 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 27.5 (CH_3), 30.4 (*C*-4), 43.5 (*C*-3), 126.4 (*C*-2' and 6'), 127.5 (*C*-5), 128.9 (*C*-3' and 5'), 129.2 (*C*-4'), 131.1 (*C*-6), 137.8 (*C*-1'), 208.4 (*C*=O); *m/z* (CI) 175 (MH^+, 100%), 117 (16, $[M-C_3H_5O]^+$) and 58 (9, $[C_3H_6O]^+$).

3.3.4 Ethyl 2-chloroacetoacetate **161**[193]



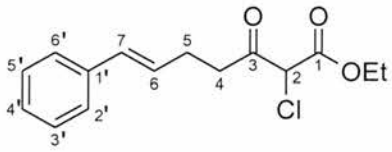
Ethyl acetoacetate (6.5 g, 50 mmoles) was dissolved in dry dichloromethane (40 cm^3). Sulfuryl chloride (7.87 g, 4.7 cm^3, 58 mmoles) was added to the solution which was stirred at room temperature for 1 hour. The reaction was quenched by pouring onto water (200 cm^3). The organic layer was washed with brine (20 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to yield a colourless oil (8.20 g, 100%); ν_{max} (film)/cm^{-1} 1757 (C=O); δ_H (300 MHz; $CDCl_3$) 1.40 (3H, t, *J* 8, CH_2CH_3), 2.60 (3H, s, $COCH_3$), 4.50 (2H, q, *J* 8, CH_2), 4.80 (1H, s, C*H*Cl); δ_C (75.4 MHz; $CDCl_3$) 14.1 (CH_2CH_3), 23.8 ($COCH_3$), 44.2 (*C*HCl), 65.0 (CH_2), 163.6 (O*C*(O)), 191.6 (CH_3CO); *m/z* (CI) 165, 176 (MH^+, 10, 3%) and 58 (62, $[C_3H_6O]^+$).

3.3.5 Ethyl 2,2-dichloroacetoacetate **162**[194]



Ethyl acetoacetate (5.3 g, 40.7 mmoles) was dissolved in dry dichloromethane (125 cm^3). Sulfuryl chloride (13.4 g, 8 cm^3, 99 mmoles) was added to the solution which was stirred overnight at room temperature. The reaction was added to water (400 cm^3). The organic layer was shaken with brine (100 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to yield a colourless oil (7.79 g, 96%); ν_{max} (film)/cm^{-1} 1757 (C=O); δ_H (300 MHz, $CDCl_3$) 1.40 (3H, t, *J* 8, CH_2CH_3), 2.60 (3H, s, $C(O)CH_3$), 4.45 (2H, q, *J* 8, CH_2); δ_C (75.4 MHz, $CDCl_3$) 14.2 (CH_2CH_3), 23.8 ($C(O)CH_3$), 65.0 (CH_2), 82.2 (CCl_2), 163.6 (OC(O)), 191.6 ($CH_3C(O)$); *m/z* (CI) 199, 201, 203 (MH^+, 20, 13, 2%), 57 (8, $[C_3H_5O]^+$) and 43 (100, $[C_2H_3O]^+$).

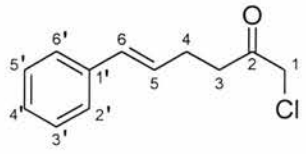
3.3.6 Ethyl 2-chloro-3-oxo-7-phenylhept-6-enoate **163**



Sodium hydride (60%, 0.535 g, 13.4 mmoles) was dissolved in dry THF (25 cm^3) and the solution was cooled to 0 °C. Ethyl 2-chloroacetoacetate (0.859 g, 5.2 mmoles) was added to the solution dropwise at 0 °C and the solution was stirred for 15 minutes. *n*-Butyl lithium (2.5 M, 4 cm^3, 10 mmoles) was then added dropwise to the solution, which was stirred for a further 15 minutes. Cinnamyl bromide (1.1 g, 5.6 mmoles) in THF (2 cm^3) was added to the solution and the solution was stirred for 20 minutes, allowing to warm to room temperature. Aqueous hydrochloric acid (2 cm^3 HCl, 5 cm^3 water) was added to quench the reaction, which was subsequently diluted with ether (15 cm^3). The organic layer was washed with water (2 × 30 cm^3) and brine (20 cm^3), then dried ($MgSO_4$). Upon

concentration at reduced pressure, the product was obtained as a brown oil, which was purified by column chromatography on silica, using initially petroleum ether (40-60 °C), then petroleum ether (40-60 °C)/diethyl ether (9:1) as the eluent. The product was obtained as a pale yellow oil (0.02g, 1%); δ_H (300 MHz; $CDCl_3$) 1.40 (3H, t, *J* 8, C*H*$_3$), 2.65 (2H, q, *J* 8, C*H*$_2$-5), 3.05 (2H, t, *J*, 8, C*H*$_2$-4), 4.40 (2H, q, *J* 8, OC*H*$_2$CH$_3$), 4.90 (1H, s, C*H*Cl), 6.30 (1H, dt, *J* 8, *J* 16, C*H*-6), 6.55 (1H, d, *J* 16, C*H*-7), 7.30-7.50 (5H, m, C$_6$*H*$_5$); δ_C (75.4 MHz; $CDCl_3$) 14.3 (*C*H$_3$), 27.6 (*C*-5), 39.2 (*C*-4), 49.2 (*C*HCl), 63.6 (*C*H$_2$CH$_3$), 126.4 (*C*-2' and 6'), 127.6 (*C*-6), 128.1 (*C*-4'), 128.9 (*C*-3' and 5'), 131.7 (*C*-7); *m/z* (CI) 281, 283 (MH^+, 81%, 28%), 247 (100, $[C_{15}H_{19}O_3]^+$) and 155 (28).

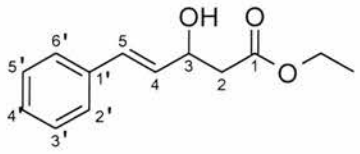
3.3.7 1-Chloro-6-phenylhex-5-en-2-one **164**[195]



Crude ethyl 2-chloro-3-oxo-7-phenylhept-6-enoate (~3 g) was heated to reflux with aqueous hydrochloric acid (50 cm^3 HCl, 50 cm^3 water) for 3 hours. The solution was allowed to cool, then extracted with diethyl ether (2 × 100 cm^3). The organic layers were washed with aqueous sodium bicarbonate solution (50 cm^3), dried ($MgSO_4$) and concentrated under reduced pressure to yield the product as a brown oil. The product was purified by column chromatography on silica using hexane/diethyl ether (19:1) as eluent to yield the product as a pale yellow oil (0.04 g, 2%); ν_{max} (film)/cm^{-1} 1734 (C=O) and 966 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 2.65 (2H, q, *J* 8, C*H*$_2$-4), 2.90 (2H, t, *J* 8, C*H*$_2$-3), 4.20 (2H, s, C*H*$_2$Cl), 6.30 (1H, dt, *J* 8, *J* 16, C*H*-5), 6.55 (1H, d, *J* 16, C*H*-6) 7.28-7.51 (5H, m,

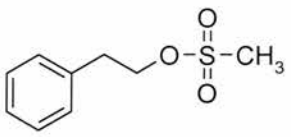
C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 27.3 (*C*-4), 39.7 (*C*-3), 48.6 (CH_2Cl), 126.4 (*C*-2' and 6'), 127.6 ($CHCH_2$), 128.3 (*C*-4'), 128.9 (*C*-3' and 5'), 131.7 (PhCH), 137.5 (*C*-1'), 202.2 (*C*=O); *m/z* (CI) 209, 211 (MH^+, 100, 33%), 175 (26, $[C_{13}H_{15}O]^+$), 117 (25, $[C_9H_9]^+$), 58 (66) and 56 (63).

3.3.8 Ethyl 3-hydroxy-5-phenyl pent-4-enoate **169**[159]



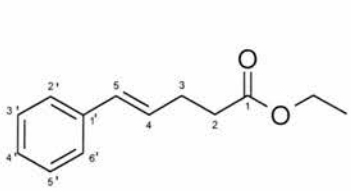
Lithium bis(trimethylsilyl)amide (1M, 16 cm^3, 16 mmoles) in THF was cooled to -78 °C. Dry ethyl acetate (1.4 g, 1.55 cm^3, 15.9 mmoles) was added dropwise and the solution was stirred for 15 minutes. Cinnamaldehyde (2.10 g, 2 cm^3, 15.9 mmoles) was added to the solution, which was stirred for a further 5 minutes. Aqueous hydrochloric acid (20%, 5 cm^3) was then added to the solution and it was left to warm to room temperature. The solution was diluted with diethyl ether (50 cm^3) and the organic layer was washed with water (50 cm^3) and brine (50 cm^3). It was then dried ($MgSO_4$) and the solvent removed under reduced pressure. This yielded the product as a dark orange oil (1.05 g, 30%); ν_{max} (film)/cm^{-1} 3600-3000 (OH), 1734 (C=O) and 968 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.40 (3H, t, *J* 8, CH_3), 2.70-2.76 (2H, m, $CH_2C(O)$), 4.30 (2H, q, *J* 8, OCH_2CH_3), 4.80 (1H, m, CHOH), 6.35 (1H, dd, *J* 8, *J* 16, $CHCH_2$), 6.80 (1H, d, *J* 16, PhCH), 7.30-7.50 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.6 (CH_3), 42.0 ($CH_2C(O)$), 61.2 (OCH_2), 69.2 (CH(OH)), 126.9 (CH), 128.1 (*C*-4'), 128.9 (CH), 130.4 (CHCH(OH)), 131.1 (*C*-5), 136.9 (*C*-1'), 172.5 (C(O)); *m/z* (CI) 220 (M^+, 9%), 203 (100, $[M\text{-}OH]^+$) and 157 (24, $[C_{11}H_9O]^+$).

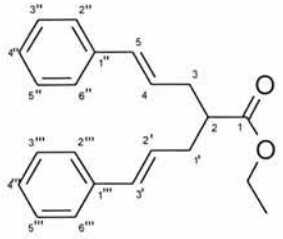
3.3.9 Formation of 1-methanesulfonyl-2-phenyl ethane **171** and attempted reaction with lithio-ethyl acetate[196]



Phenethyl alcohol (1.02 g, 1 cm^3, 8.3 mmoles) and triethylamine (1.27 g, 1.75 cm^3, 12.6 mmoles) were dissolved in THF (20 cm^3). The solution was cooled to -23 °C and methane sulfonyl chloride (0.96 g, 0.65 cm^3) was added. The solution was then stirred at room temperature for 30 minutes. The mesylate solution was then added to a cooled (-78 °C) solution of ethyl acetate in THF, that had been treated with lithium bis(trimethylsilyl)amide. The solution was stirred and allowed to warm to room temperature overnight. Aqueous hydrochloric acid (20%, 5 cm^3) was added to the solution, which had turned green overnight and the solution turned yellow. The solution was diluted with diethyl ether (50 cm^3) and the organic layer was washed with water and brine until it appeared that the trimethylsilylamide salts had been removed. It was then dried ($MgSO_4$) and the solvent removed under reduced pressure. A pale yellow oil was obtained (0.73 g, 44%) that was found to be the mesylate compound: ν_{max} (film)/cm^{-1} 1354 and 1174 (OSO_2); δ_H (300 MHz, $CDCl_3$) 2.90 (3H, s, CH_3), 3.15 (2H, t, *J* 8, $PhCH_2$), 4.55 (2H, t, *J* 8, OCH_2), 7.30-7.50 (5H, m, C_6H_5); δ_C (75.4 MHz, $CDCl_3$) 36.0 (SCH_3), 37.6 ($PhCH_2$), 70.8 (CH_2O), 127.4 (*C*-4), 129.1 (*C*H), 129.4 (*C*H), 136.7 (*C*-1); *m/z* (CI) 201 (MH^+, 11%) and 105 (100, $[C_8H_8]^+$).

3.3.10 Ethyl 5-phenylpent-4-enoate **173**[197] and Ethyl 2-(3-phenylprop-2-enyl)-5-phenylpent-4-enoate **177**[198]

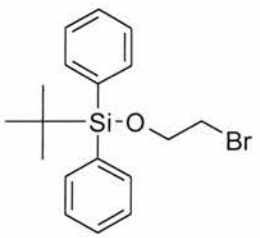




Lithium bis(trimethylsilylamide) (0.8M, 18 cm^3, 14.4 mmoles) was added to dry THF (30 cm^3) and the solution was cooled to -78 °C. Dry ethyl acetate (1.37 cm^3, 1.24 g, 14 mmoles) was added to the solution which was stirred for 25 minutes at -78 °C. Cinnamyl bromide (2.76 g, 14 mmoles) was dissolved in dry THF (5 cm^3) and added to the reaction solution. The solution was allowed to warm to -50 °C and stirred at -45 to -50 °C for 2 hours. The reaction was allowed to warm to room temperature and hydrochloric acid (20%, 5 cm^3) was added. The solution was extracted with diethyl ether (2 × 100 cm^3) and washed with water (100 cm^3) and brine (100 cm^3). The combined organic extracts were dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. This was purified by suction column chromatography on silica to give a mixture of the named products as a colourless oil (1.75 g); ν_{max} (film)/cm^{-1} 1730 (C=O) and 966 (CH=CH, *E*); monomer: δ_H (300 MHz; $CDCl_3$) 1.18-1.30 (3H, m, CH_3), 2.40-2.72 (4H, m, 2 × CH_2), 4.08-4.18 (2H, m, OCH_2), 6.08-6.26 (1H, m, C*H*CH_2), 6.43 (1H, d, *J* 16, PhC*H*), 7.15-7.38 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.5 (*C*H_3), 28.5 (*C*-3), 34.2 (*C*-2), 60.5 (O*C*H_2), 126.3 (*C*-2'), 128.6 (*C*-3' and 5'), 131.1 (*C*-4), 132.4 (*C*-5), 137.5 (*C*-1'), 173.8 (*C*=O); *m/z* (CI) 205 ($[MH]^+$, 100%), 204 (32, $[M]^+$), 159 (55, $[M\text{-}OEt]^+$), 117 (23, $[PhCHCHCH_2]^+$); dimer: δ_H (300 MHz; $CDCl_3$) 1.18-1.30 (3H, m, CH_3), 2.40-2.72 (5H, m, 2 × CH_2 and C*H*), 4.08-4.18 (2H, m, OCH_2), 6.08-6.26 (2H, m, C*H*CH_2), 6.43 (2H, d, *J* 16, PhC*H*), 7.15-7.38

(10H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.5 (CH_3), 35.3 (*C*-3 and 1'), 45.8 (*C*-6), 60.5 (OCH_2), 126.3 (*C*-2", 6", 2''' and 6'''), 127.1 (*C*-4 and 2'), 127.3 (*C*-4" and 4'''), 128.6 (*C*-3", 5", 3''' and 5'''), 132.4 (*C*-5 and 3'), 137.5 (*C*-1" and 1'''), 174.9 (*C*=O); *m/z* (MALDI-TOF) 343 ($[M+Na]^+$, 35%), 342 (100, $[M-H+Na]^+$).

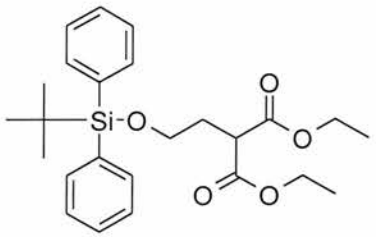
3.3.11 1-Bromo-2-(*tert*-butyldiphenylsilyloxy)ethane **179**[199]



Bromoethanol (5.67 cm^3, 10 g, 80 mmoles) was dissolved in dry dichloromethane (250 cm^3). *tert*-Butyl(chloro)diphenylsilane (21.8 cm^3, 23 g, 84 mmoles) was added to the solution, followed by 4,4-dimethylaminopyridine (1.96 g, 16 mmoles) in dichloromethane (10 cm^3), then triethylamine (14.7 cm^3, 10.67 g, 105 mmoles). The reaction was stirred for 4 hours at room temperature, then water (200 cm^3) was added. The aqueous layer was extracted with dichloromethane (2 × 200 cm^3) and the combined extracts were dried over magnesium sulfate. The solvent was removed under reduced pressure to give a colourless semi-solid. This was purified by suction column chromatography on silica, eluting with 2.5% ethyl acetate in petroleum ether (40-60 °C) to give a colourless semi-solid (23.29 g, 80%); ν_{max} (nujol)/cm^{-1} 1375 ($C(CH_3)_3$) and 1112 (Si-O-C); δ_H (300 MHz; $CDCl_3$) 1.06 (9H, s, 3 × CH_3), 3.43 (2H, t, *J* 8, CH_2Br), 3.92 (2H, t, *J* 8, CH_2O), 7.34-7.48 (6H, m, aryl), 7.64-7.72 (4H, m, aryl); δ_C (75.4 MHz; $CDCl_3$) 19.5 ($C(CH_3)_3$), 27.0 (CH_3), 33.3 (CH_2Br),

64.2 (CH_2O), 128.0 (*C*-3' and 5'), 130.0 (*C*-4'), 133.4 (*C*-1'), 135.8 (*C*-2' and 6'); *m/z* (ES) 386, 388 ($[M+H+Na]^+$, 8, 9%) and 385, 387 (98, 100, $[M+Na]^+$).

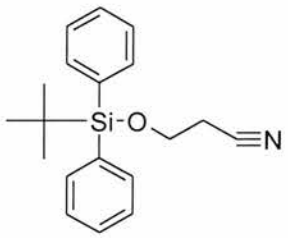
3.3.12 Diethyl 2-(*tert*-butyldiphenylsilyloxy)malonate **186**[200]



Sodium (0.063 g, 2.75 mmoles) was dissolved in dry ethanol (20 cm^3) and diethyl malonate (0.42 cm^3, 0.44 g, 2.75 mmoles) was added dropwise to the solution. 1-Bromo-2-(*tert*-butyldiphenylsilyloxy)ethane (1 g, 2.75 mmoles) was dissolved in ethanol (10 cm^3) and added to the solution which was heated for 3 hours, stirred overnight at room temperature, then heated under reflux for a further 8 hours. The solvent was removed under reduced pressure and water (50 cm^3) was added to the concentrate. The mixture was extracted with ethyl acetate (3 × 50 cm^3) and the combined organic fractions were washed with brine (50 cm^3). The solution was concentrated under reduced pressure to give a yellowish oil. This was purified by suction column chromatography on silica, eluting with 2.5% ethyl acetate in petroleum ether (40-60 °C), to give the product as a colourless oil (0.32 g, 27%); ν_{max} (film)/cm^{-1} 1734 (C=O), 1375 ($C(CH_3)_3$) and 1110 (Si-O-C); δ_H (300 MHz; $CDCl_3$) 1.05 (9H, s, 3 × CCH_3), 1.26 (6H, t, *J* 8, 2 × CH_2CH_3), 2.18 (2H, q, *J* 8, CH_2CH), 3.65-3.76 (3H, m, CH_2OSi, *CH*), 4.09-4.24 (4H, m, 2 × CH_2O), 7.33-7.47 (6H, m, aryl), 7.61-7.72 (4H, m, aryl); δ_C (75.4 MHz, $CDCl_3$) 14.3 (2 × CH_3 (ethyl)), 19.4 ($C(CH_3)_3$), 27.0 ($C(CH_3)_3$), 31.7 ($CHCH_2$), 48.8 (*C*H), 61.4 (CH_2OSi), 61.6 (2 × CH_2CH_3),

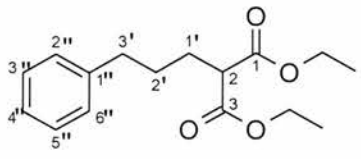
127.9 (*C*-3' and 5'), 129.8 (*C*-4'), 133.7 (*C*-1'), 135.7 (*C*-2' and 6'), 169.7 (2 × *C*=O); *m/z* (ES) 465 ($[M+Na]^+$, 100%).

3.3.13 3-(*tert*-Butyldiphenylsilyloxy)propionitrile **180**[201]



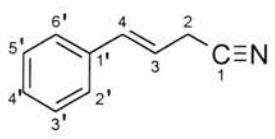
1-Bromo-2-(*tert*-butyldiphenylsilyloxy)ethane (10 g, 27.5 mmoles) and 18-crown-6 (7 g, 26.5 mmoles) were dissolved in dry acetonitrile (100 cm^3). Potassium cyanide (2 g, 30.7 mmoles) was added to the solution, which was stirred for 2 days at room temperature. The solvent was removed under reduced pressure and water (100 cm^3) was added to the concentrate. The mixture was extracted with dichloromethane (3 × 100 cm^3) and the combined organic layers were washed with brine (100 cm^3). The organic solution was dried over magnesium sulfate and concentrated under reduced pressure to give an off-white solid. This was purified by suction column chromatography on silica, eluting with 2.5% ethyl acetate in petroleum ether (40-60 °C) to give the product as a colourless crystalline solid (6.58 g, 77%); m.p. 48-51 °C (lit.,[201] 50-51 °C); ν_{max} (nujol)/cm^{-1} 2350 (CN), 1390 ($C(CH_3)_3$) and 1112 (Si-O-C); δ_H (300 MHz; $CDCl_3$) 1.08 (9H, s, 3 × CH_3), 2.54 (2H, t, *J* 8, CH_2CN), 3.86 (2H, t, *J* 8, CH_2O), 7.36-7.51 (6H, m, aryl), 7.62-7.71 (4H, m, aryl); δ_C (75.4 MHz; $CDCl_3$) 19.2 ($C(CH_3)_3$), 21.5 (CH_2CN), 26.7 (CH_3), 59.1 (CH_2O), 118.0 (*C*N), 127.9 (*C*-3' and 5'), 130.0 (*C*-4'), 132.7 (*C*-1'), 135.6 (*C*-2' and 6'); *m/z* (ES) 348 ($[M+K]^+$, 16%) and 332 (28, $[M+Na]^+$).

3.3.14 Diethyl 3-phenyl 2-(3-phenylpropyl)malonate **201**[202]



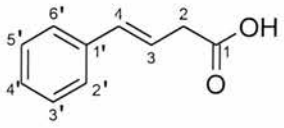
Under a nitrogen atmosphere, sodium (0.72 g, 31 mmoles) was dissolved in ethanol (120 cm^3). Diethyl malonate (4.74 cm^3, 5 g, 31 mmoles) was added dropwise to the solution, which was then stirred for 10 minutes. 1-Bromo-3-phenylpropane (6 g, 30 mmoles) was then added and the solution was heated under reflux for 3 hours. The solvent was removed under reduced pressure and water (75 cm^3) was added to the concentrate. The mixture was extracted with ethyl acetate (3 × 80 cm^3) and the combined organic layers were dried over magnesium sulfate. The solution was concentrated under reduced pressure to give the product as a pale yellow oil (8.23 g, 98%). The compound was used without purification; ν_{max} (film)/cm^{-1} 1733 (C=O); δ_H (300 MHz; $CDCl_3$) 1.27 (6H, t, *J* 8, 2 × CH_3), 1.68 (2H, p, *J* 8, CH_2-4), 1.88-2.00 (2H, m, CH_2CH), 2.67 (2H, t, *J* 8, $PhCH_2$), 3.36 (1H, t, *J* 8, *CH*), 4.20 (4H, q, *J* 8, 2 × OCH_2CH_3), 7.12-7.33 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.3 (CH_3), 28.6 (*C*-1'), 29.3 (*C*-2'), 35.7 (*C*-3'), 52.2 (*C*-2), 61.5 (OCH_2), 126.1 (*C*-4''), 128.5 (*C*-2'', 3'', 5'' and 6''), 141.9 (*C*-1''), 169.6 (*C*=O); *m/z* (ES) 301 ($[M+Na]^+$, 100%) and 279 (16, $[MH]^+$).

3.3.15 4-Phenylbut-3-enonitrile **165**



Cinnamyl bromide (0.5 g, 2.54 mmoles) and 18-crown-6 (0.67 g, 2.53 mmoles) were dissolved in dry ethanol (20 cm^3) and added to potassium cyanide (0.17 g, 2.61 mmoles). The reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure and water (40 cm^3) was added to the concentrate. The mixture was extracted with dichloromethane (3 × 50 cm^3) and the combined organic extracts were washed with brine (50 cm^3). The organic solution was dried over magnesium sulfate and concentrated under reduced pressure to give a yellow solid (0.34 g, 94%). The product was used without further purification; m.p. 53-57 °C (lit.,[203] 59.5-60 °C) ν_{max} (nujol)/cm^{-1} 2230 (CN); δ_H (300 MHz; $CDCl_3$) 3.29 (2H, d, *J* 7, CH_2), 6.05 (1H, dt, *J* 7, *J* 16, $CHCH_2$), 6.74 (1H, d, *J* 16, PhC*H*), 7.20-7.42 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 20.8 (CH_2), 116.9 (*C*-3), 117.4 (*C*N), 126.5 (*C*-2' and 6'), 128.3 (*C*-4'), 128.7 (*C*-3' and 5'), 134.4 (*C*-4), 135.7 (*C*-1'); *m/z* (MALDI-TOF) 166 ($[M+Na]^+$, 60%).

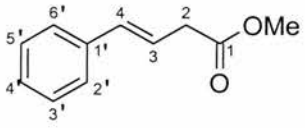
3.3.16 4-Phenylbut-3-enoic acid **193**[204]



4-Phenylbut-3-enonitrile (3.23 g, 22.6 mmoles) was dissolved in methanol (80 cm^3). Sodium hydroxide solution (3M, 240 cm^3) was added to the solution, which was heated under reflux for 24 hours. The solution was allowed to cool and was acidified to pH 5 with hydrochloric acid. The mixture was extracted with ethyl acetate (3 × 100 cm^3) and the combined organic layers were washed with brine (100 cm^3). The organic solution was dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil

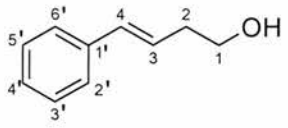
(2.96 g, 81%). The product was used without purification; ν_{max} (nujol)/cm^{-1} 3200-2500 (OH), 1707 (C=O) and 979 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 3.31 (2H, d, *J* 7, C*H*$_2$), 6.28 (1H, dt, *J* 7, *J* 16, C*H*CH$_2$), 6.52 (1H, d, *J* 16, PhC*H*), 7.14-7.42 (5H, m, C$_6$*H*$_5$); δ_C (75.4 MHz; $CDCl_3$) 38.2 (*C*H$_2$), 121.0 (*C*-3), 126.5 (*C*-2' and 6'), 127.8 (*C*-4'), 128.7 (*C*-3' and 5'), 134.1 (*C*-4), 136.8 (*C*-1'), 177.9 (*C*=O); *m/z* (CI) 163 ([MH]$^+$, 100%), 162 (53, [M]$^+$), 145 (46, [M-OH]$^+$) and 117 (71, [PhCHCHCH$_2$]$^+$).

3.3.17 Methyl 4-phenylbut-3-enoate **194**[205]



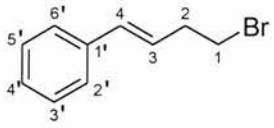
4-Phenylbut-3-enoic acid (1 g, 6.17 mmoles) was dissolved in dry methanol (30 cm^3). Thionyl chloride was added to the solution, which was heated under reflux for 2 hours. The solution was allowed to cool and the solvent was removed under reduced pressure. The concentrate was washed with sodium bicarbonate solution (30 cm^3) and extracted with ethyl acetate (3 × 50 cm^3). The combined organic layers were washed with brine (50 cm^3), dried over magnesium sulfate and concentrated under reduced pressure to give the product as a pale yellow oil (1.01 g, 93%); ν_{max} (film)/cm^{-1} 1739 (C=O) and 970 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 3.26 (2H, d, *J* 7, C*H*$_2$), 3.73 (3H, s, C*H*$_3$), 6.29 (1H, dt, *J* 7, *J* 16, C*H*CH$_2$), 6.49 (1H, d, *J* 16, PhC*H*), 7.22-7.40 (5H, m, C$_6$*H*$_5$); δ_C (75.4 MHz; $CDCl_3$) 38.3 (*C*H$_2$), 52.0 (*C*H$_3$), 121.7 (*C*-3), 126.4 (*C*-2' and 6'), 127.7 (*C*-4'), 128.6 (*C*-3' and 5'), 133.6 (*C*-4), 136.9 (*C*-1'), 172.0 (*C*=O); *m/z* (CI) 177 ([MH]$^+$, 100%), 176 (42, [M]$^+$), 145 (48, [M-OCH$_3$]$^+$) and 117 (51, [PhCHCHCH$_2$]$^+$).

3.3.18 4-Phenylbut-3-en-1-ol **195**[206]



Methyl 4-phenylbut-3-enoate (0.8 g, 4.54 mmoles) was dissolved in dry THF (20 cm^3). DIBAL (1M, 10 cm^3, 10 mmoles) was added to the solution which was stirred for 2 hours at room temperature. Brine (50 cm^3) was added to the solution, which was then diluted with diethyl ether (200 cm^3). The organic phase was washed with brine until all the aluminium salts had been removed. The solution was dried over magnesium sulfate and concentrated under reduced pressure to give the product as a yellow oil (0.65 g, 97%). The compound was used without purification; ν_{max} (film)/cm^{-1} 3600-3000 (OH) and 970 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 2.48 (2H, q, *J* 7, CHCH_2), 3.76 (2H, q, *J* 7, CH_2OH), 6.21 (1H, m, C*H*CH_2), 6.45 (1H, dt, *J* 7, *J* 16, PhC*H*), 7.16-7.42 (5H, m, C_6H_5); δ_C (125.7 MHz; $CDCl_3$) 36.6 (CH*C*H_2), 62.3 (*C*H_2OH), 126.3 (*C*-2' and 6'), 126.5 (*C*-3), 127.5 (*C*-4'), 128.8 (*C*-3' and 5'), 133.1 (*C*-4), 137.5 (*C*-1'); *m/z* (EI) 148 ($[M]^+$, 31%), 117 (31, $[PhCHCHCH_2]^+$), 115 (91, $[C_9H_7]^+$) and 91 (36, $[PhCH_2]^+$).

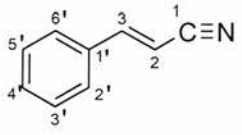
3.3.19 1-Bromo-4-phenylpent-3-ene **166**[207]



5-Phenylbut-3-en-1-ol (0.68 g, 4.6 mmoles) was dissolved in dry dichloromethane (50 cm^3). Carbon tetrabromide (3.05 g, 9.2 mmoles) and triphenylphosphine (2.41 g,

9.2 mmoles) were added to the solution, which was stirred at room temperature for 3 hours. The solution was washed with aqueous potassium bicarbonate solution (70 cm^3), then extracted with ethyl acetate (3 × 70 cm^3). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. This was purified by column chromatography on silica, eluting with petroleum ether (40-60 °C)/ethyl acetate (7:1), to give the product as a yellow oil (0.90 g, 93%); δ_H (300 MHz; $CDCl_3$) 2.76 (2H, q, *J* 7, CHC*H*$_2$), 3.47 (2H, t, *J* 7, C*H*$_2$Br), 6.18 (1H, dt, *J* 7, *J* 16, C*H*CH$_2$), 6.49 (1H, d, *J* 16, PhC*H*), 7.13-7.42 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 32.4 (*C*H$_2$Br), 36.5 (CH*C*H$_2$), 126.3 (*C*-2' and 6'), 126.8 (*C*-3), 127.6 (*C*-4'), 128.7 (*C*-3' and 5'), 132.9 (*C*-4), 137.2 (*C*-1').

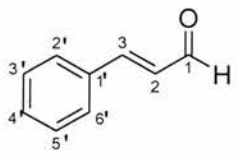
3.3.20 Cinnamonitrile **208**[208]



Zinc dust (0.196 g, 3 mmoles), triphenylphosphine (0.520 g, 2 mmoles) and Bis(triphenylphosphine)nickel(II) dibromide (0.74 g, 1 mmole) were stirred in a flask under argon. Potassium cyanide (2.54 g, 39 mmoles) was added to the mixture, which was left to stir under argon for 10 minutes. β-Bromostyrene (5.49 g, 30 mmoles) was dissolved in dry DMF (8 cm^3) and the solution was added to the reaction mixture. The reaction was stirred at room temperature for 45 minutes, then heated at 50 °C for 40 hours. The solvent was removed under reduced pressure and water (40 cm^3) was added to the concentrate. The mixture was then extracted with dichloromethane (3 × 50 cm^3) and the combined organic layers washed with water (50 cm^3). Following a wash with dilute hydrochloric acid (30

cm^3), the organic layer was dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. This was purified by suction column on silica using petroleum ether (40-60 °C), then petroleum ether/ethyl acetate (24:1) to give the product as a pale yellow oil (2.81 g, 73%); ν_{max} (film)/cm^{-1} 2185 (CN) and 942 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 5.88 (1H, d, *J* 16, C*H*CN), 7.35-7.53 (6H, m, C_6H_5 and PhC*H*); δ_C (75.4 MHz; $CDCl_3$) 96.4 (*C*-2), 118.2 (*C*N), 127.4 (*C*-2' and 6'), 129.2 (*C*-3' and 5'), 131.3 (*C*-4'), 133.6 (*C*-1'), 150.6 (*C*-3); *m/z* (CI) 131 ($[MH_2]^+$, 10%), 130 (100, $[MH]^+$), 129 (15, $[M]^+$).

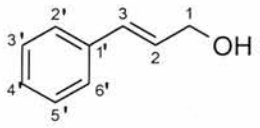
3.3.21Cinnamaldehyde **212**[209]



Cinnamonitrile (1 g, 7.74 mmoles) was dissolved in dry dichloromethane (20 cm^3). The solution was cooled on ice and DIBAL (1M, 10 cm^3, 10 mmoles) was added slowly. The reaction was stirred for 3 days at room temperature. Silica gel (4.63 g, 77 mmoles), tartaric acid (11.62 g, 77 mmoles) and water (1.39 cm^3) were stirred in a large beaker. Diethyl ether (25 cm^3) was added to the mixture to produce a slurry. The reaction mixture was then poured onto the slurry and left to stir for 5 hours at room temperature. The mixture was filtered under vacuum and washed with diethyl ether. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure to give the product as a yellow oil (0.96 g, 94%). The compound was used without purification; ν_{max} (film)/cm^{-1} 1678 (C=O) and 974 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 6.73 (1H, dd, *J* 8, *J* 16, C*H*C(O)), 7.42-7.61 (6H, m, C_6H_5 and PhC*H*), 9.72 (1H, d, *J* 8, C*H*O); δ_C (75.4 MHz; $CDCl_3$), 128.7 (*C*-2' and 6'), 128.8 (*C*-2), 129.3 (*C*-3' and 5'), 131.4 (*C*-4'), 134.2 (*C*-1'), 152.9 (*C*-3),

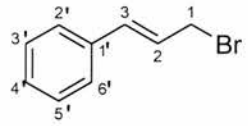
193.8 (*C*=O); *m/z* (EI) 132 ($[M]^+$, 42%), 131 (100, $[M-H]^+$), 103 (45, $[M-CHO]^+$) and 77 (37, $[Ph]^+$).

3.3.22 Cinnamyl alcohol **211**[210]



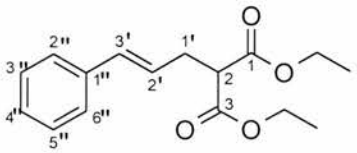
Cinnamaldehyde (1 g, 7.57 mmoles) was dissolved in THF (20 cm^3). DIBAL (1M, 15 cm^3), 15 mmoles) was added to the solution which was stirred at room temperature for 4 hours. Water (50 cm^3) and diethyl ether (100 cm^3) were added to the solution and sufficient dilute hydrochloric acid was added to dissolve the aluminium salts. The aqueous layer was further extracted with diethyl ether (2 × 80 cm^3) and the combined organic layers were dried over magnesium sulfate. The solution was concentrated under reduced pressure to yield the product as a colourless solid (0.94 g, 93%). The compound was used without purification; m.p. 33-35 °C (lit.,[210] 30-32 °C); ν_{max} (film)/cm^{-1} 3600-3000 (OH) and 969 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.35-1.44 (1H, m, O*H*), 4.28-4.37 (2H, m, CH_2OH), 6.37 (1H, dt, *J* 8, *J* 16, C*H*CH_2), 6.62 (1H, d, *J* 16, PhC*H*), 7.18-7.43 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 63.7 (*C*-1), 126.6 (*C*-2' and 6'), 127.8 (*C*-4'), 128.6 (*C*-2), 128.7 (*C*-3' and 5'), 131.2 (*C*-3), 136.7 (*C*-1'); *m/z* (CI) 135 ($[MH]^+$, 8%), 134 (24, $[M]^+$), 133 (38, $[M-H]^+$) and 117 (100, $[PhCHCHCH_2]^+$).

3.3.23 Cinnamyl bromide **156**[211]



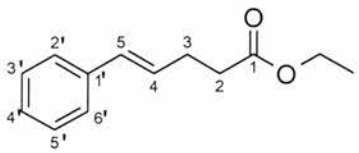
Cinnamyl alcohol (1 g, 7.4 mmoles) and pyridine (0.12 g, 1.5 mmoles) were dissolved in dry diethyl ether (30 cm^3) under nitrogen. The solution was cooled to 0 °C then phosphorus tribromide (2 g, 7.4 mmoles) was added dropwise. The reaction was stirred for 30 minutes at 0 °C then allowed to warm to room temperature. The solution was poured into an ice/brine mixture and stirred until all the ice had melted. The aqueous layer was extracted with diethyl ether (3 × 50 cm^3) and the combined organic layers were washed with brine (50 cm^3). The organic solution was dried over magnesium sulfate and concentrated under reduced pressure to give a brown semi-solid (1.23 g, 84%). No purification was required; ν_{max} (film)/cm^{-1} 962 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 4.16 (2H, d, *J* 8, CH_2), 6.38 (1H, dt, *J* 8, *J* 16, C*H*CH_2), 6.64 (1H, d, *J* 16, PhC*H*), 7.23-7.42 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 33.6 (*C*-1), 125.4 (*C*-2), 126.9 (*C*-2' and 6'), 128.5 (*C*-4'), 128.8 (*C*-3' and 5'), 134.7 (*C*-3), 136.0 (*C*-1'); *m/z* (CI) 197, 199 ($[MH]^+$, 17%) and 117 (100, $[PhCHCHCH_2]^+$).

3.3.24 Diethyl 3-phenyl 2-(3-phenylprop-2-enyl)malonate **203**[212]



Cinnamyl bromide (5.678 g, 29 mmoles) and diethyl malonate (4.5 g, 28 mmoles) were dissolved in dichloromethane (40 cm^3). Potassium carbonate (13 g, 94 mmoles) and tetra-butyl ammonium chloride (8 g, 29 mmoles) were dissolved in water (40 cm^3) and added to the organic solution. The biphasic solution was heated at 80 °C for 24 hours, then allowed to cool to room temperature. The aqueous layer was extracted with dichloromethane (2 × 50 cm^3) and the combined organic layers were washed with water (50 cm^3) and brine (50 cm^3). The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure to yield a yellow oil (7.61 g, 96%). The compound was used without purification; ν_{max} (film)/cm^{-1} 1733 (C=O) and 968 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.39 (6H, t, *J* 8, 2 × CH_3), 2.92 (2H, q, *J* 8, CH_2), 3.61 (1H, t, *J* 8, *CH*C(O)) 4.31 (4H, m, *J* 8, 2 × OCH_2), 6.14-6.33 (1H, m, *CH*CH_2), 6.58 (1H, d, *J* 16, PhC*H*), 7.28-7.46 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.5 (*C*H_3), 32.6 (*C*-1'), 52.4 (*C*-2), 61.9 (OCH_2), 126.0 (*C*-2'), 126.6 (*C*-2" and 6"), 127.8 (*C*-4"), 128.9 (*C*-3" and 5"), 133.2 (*C*-3'), 137.4 (*C*-1") 168.9 (*C*=O); *m/z* (ES) 315 ($[M+K]^+$, 16%), 299 (100, $[M+Na]^+$) and 277 (40, $[MH]^+$).

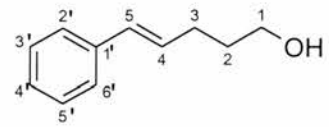
3.3.25 Ethyl 5-phenylpent-4-enoate **204**[212]



Ethyl 5-phenyl 2-(ethyl acetyl) pent-4-enoate (13.068 g, 47 mmoles) was dissolved in dimethyl sulfoxide (100 cm^3). Sodium chloride (3.45 g, 59 mmoles) and water (4 cm^3) were then added to the solution which was heated at 170 °C overnight. The mixture was allowed to cool, then poured onto water (100 cm^3). The solution was extracted with ether/hexane (1:1) (3 × 100 cm^3) and the combined organic layers were washed with brine

(2 × 50 cm^3). The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure to yield a yellow oil (9.23 g, 96%). The product was used without further purification; *m/z* (Found: $[M+H]^+$ 205.1229. $C_{13}H_{17}O_2$ requires 205.1229); ν_{max} (film)/cm^{-1} 1731 (C=O) and 966 (CH=CH, *E*); δ_H (300 MHz, $CDCl_3$) 1.26 (3H, t, *J* 8, CH_3), 2.45-2.59 (4H, m, 2 × CH_2), 4.14 (2H, q, *J* 8, OCH_2), 6.20 (1H, dt, *J* 8, *J* 16, $CHCH_2$), 6.43 (1H, d, *J* 16, PhC*H*), 7.16-7.36 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 14.3 (CH_3), 28.3 (*C*-3), 34.1 (*C*-2), 60.4 (OCH_2), 126.1 (*C*-2' and 6'), 127.3 (*C*-4), 128.5 (*C*-3', 4' and 5'), 131.0 (*C*-5), 137.4 (*C*-1'), 173.0 (*C*=O); *m/z* (CI) 205 ($[MH]^+$, 100%) and 159 (7, $[M-OCH_2CH_3]^+$).

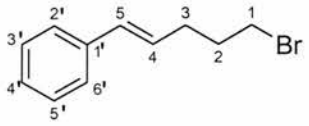
3.3.26 5-Phenylpent-4-en-1-ol **174**[213]



DIBAL (1M in hexanes, 25 cm^3, 25 mmoles) was carefully added to a solution of ethyl 5-phenylpent-4-enoate (2.2 g, 10.8 mmoles) in dry THF (25 cm^3). The solution was stirred at room temperature for 4 hours, before water (20 cm^3) was carefully added to quench the reaction. Dilute hydrochloric acid was then added to dissolve the aluminium salts that had precipitated. The mixture was extracted with ethyl acetate (3 × 50 cm^3) and the combined extracts were washed with brine (50 cm^3). The organic phase was dried over magnesium sulfate and concentrated under reduced pressure to yield a yellow oil (1.73 g, 99%); *m/z* (Found: M^+ 162.1046. $C_{11}H_{14}O$ requires 162.1045); ν_{max} (film)/cm^{-1} 3700-3000 (OH) 965 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.53 (1H, br s, O*H*), 1.76 (2H, p, *J* 8, CH_2-2), 2.51 (2H, q, *J* 8, $CHCH_2$), 3.72 (2H, t, *J* 8, CH_2OH), 6.23 (1H, dt, *J* 8, *J* 16, $CHCH_2$), 6.42 (1H,

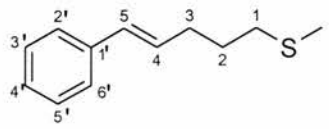
d, *J* 16, PhC*H*), 7.14-7.40 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 29.5 (*C*-3), 32.4 (*C*-2), 62.5 (*C*-1), 126.1 (*C*-2' and 6'), 127.1 (*C*-4'), 128.7 (*C*-3' and 5'), 130.2 (*C*-4), 130.5 (*C*-5), 137.8 (*C*-1'); *m/z* (CI) 162 ($[M]^+$, 7%), 145 (100, $[M\text{-}OH]^+$), 117 (52, $[PhCHCHCH_2]^+$) and 91 (98, $[PhCH_2]^+$).

3.3.27 1-Bromo-5-phenylpent-4-ene **175**[214]



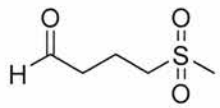
5-Phenylpent-4-en-1-ol (1.73 g, 10.7 mmoles) was dissolved in dry dichloromethane (50 cm^3). Carbon tetrabromide (7.566 g, 22.8 mmoles) and then triphenylphosphine (5.98 g, 22.8 mmoles) were added to the solution which was stirred overnight at room temperature. The solvent was then removed under reduced pressure and the product dissolved in diethyl ether (100 cm^3). The insoluble triphenylphosphine oxide was removed by filtration. The ethereal layer was then dried over magnesium sulfate and concentrated under reduced pressure to yield a brown oil. This was purified by column chromatography on silica using petroleum ether (40-60 °C), then petroleum ether with 1% ethyl acetate to yield the product as a yellow oil (1.70 g, 71%); δ_H (300 MHz; $CDCl_3$) 2.05 (2H, p, *J* 7.5, CH_2-2), 2.40 (2H, q, *J* 7.5, CHCH_2), 3.48 (2H, t, *J* 7.5, CH_2Br), 6.18 (1H, dt, *J* 7.5, *J* 16, C*H*CH_2), 6.47 (1H, d, *J* 16, PhC*H*), 7.20-7.38 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 31.5 (*C*-3), 32.4 (*C*-1), 33.3 (*C*-2), 126.2 (*C*-2' and 6'), 127.3 (*C*-4'), 128.6 (*C*-4), 128.7 (*C*-3' and 5'), 131.5 (*C*-5), 137.6 (*C*-1'); *m/z* 225, 227 ($[MH]^+$, 9, 8%), 224, 226 (75, 72, $[M]^+$), 145 (39, $[M\text{-}Br]^+$), 117 (100, $[PhCHCHCH_2]^+$), 91 (75, $[PhCH_2^+]$) and 77 (17, $[Ph]^+$).

3.3.28 5-Phenyl-1-thiomethylpent-4-ene **213**[215]



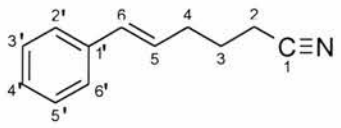
5-Phenyl-1-bromo-pent-4-ene (0.5 g, 2.22 mmoles) was dissolved in dry methanol (30 cm^3). Sodium thiomethoxide (0.21 g, 3 mmoles) was added to the solution, which was stirred overnight at room temperature. The reaction was monitored by TLC and further sodium thiomethoxide (0.05 g, 0.71 mmoles) was added. The reaction was stirred for a further 4 hours, then more sodium thiomethoxide (0.1 g, 1.42 mmoles) was added. The reaction was stirred for a further 2 hours, then water (40 cm^3) was added to the solution. The solution was extracted with ethyl acetate (3 × 50 cm^3) and the combined organic fractions were washed with brine (50 cm^3) and dried over magnesium sulfate. The solution was concentrated under reduced pressure to give the product as a brown oil (0.41 g, 96%). The compound was used without purification; ν_{max} (film)/cm^{-1} 966 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.78 (2H, p, *J* 7.5, C*H*$_2$-2), 2.23 (3H, s, C*H*$_3$), 2.44 (2H, q, *J* 7.5, CHC*H*$_2$), 2.67 (2H, t, *J* 7.5, C*H*$_2$S), 6.32 (1H, dt, *J* 7.5, *J* 16, C*H*CH$_2$), 6.53 (1H, d, *J* 16, PhC*H*), 7.36-7.52 (5H, m, C$_6$*H*$_5$); δ_C (75.4 MHz; $CDCl_3$), 15.7 (*C*H$_3$), 28.9 (*C*-2), 32.2 (*C*-3), 33.9 (*C*-1), 126.1 (*C*-2' and 6'), 127.1 (*C*-4'), 128.6 (*C*-3' and 5'), 129.8 (*C*-4), 130.8 (*C*-5), 137.8 (*C*-1'); *m/z* (CI) 193 ([MH]$^+$, 86%), 192 (32, [M]$^+$) and 145 (57, [M-SCH$_3$]$^+$).

3.3.29 4-(Methylsulfonyl)butanal **215**



5-Phenyl-1-thiomethylpent-4-ene (0.105 g, 0.55 mmoles) was dissolved in dry dichloromethane (10 cm^3). Oxygen was bubbled through the solution as it was cooled to -78 °C. Ozone was then bubbled through the solution until it turned blue and the indicator (potassium iodide in aqueous acetic acid) turned black. Oxygen was then bubbled through the solution to dissipate any remaining ozone. Triphenylphosphine (0.285 g, 1.1 mmoles) was then added to the solution to breakdown the ozonide. The solvent was then removed under reduced pressure to give a pale yellow oil. The oil was purified by column chromatography on silica, eluting with hexane, then ethanol and methanol, but a mixture of products was obtained. Data is given for the sulfone **215**; *m/z* (Found: $[MH]^+$ 151.0429. $C_5H_{11}O_3S$ requires 151.0429); ν_{max} (film)/cm^{-1} 1694 (C=O), 1305 and 1120 (SO_2); δ_H (300 MHz; $CDCl_3$) 2.09 (2H, p, *J* 7, CH_2-3), 2.68 (2H, t, *J* 7, CH_2CO), 2.86 (3H, s, CH_3), 3.01 (2H, t, *J* 7, CH_2S), 9.73 (1H, s, CHO); δ_C (75.4 MHz, $CDCl_3$) 15.6 (*C*-3), 41.0 (CH_3), 42.1 (*C*-2), 53.7 (*C*-4), 200.7 (*C*=O); *m/z* (MALDI-TOF) 173 ($[M+Na]^+$, 100%).

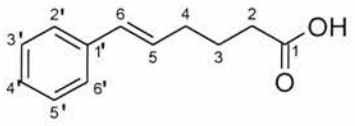
3.3.30 6-Phenylhex-5-enonitrile **176**[216]



A mixture of 18-crown-6 (2.38 g, 9 mmoles), potassium cyanide (0.6 g, 9.2 mmoles) and 1-bromo-5-phenylpent-4-ene (1.7 g, 7.55 mmoles) in dry acetonitrile (40 cm^3) was heated under reflux for 5 hours, then stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue dissolved in dichloromethane (50 cm^3) and water (50 cm^3). The aqueous layer was extracted with dichloromethane (2 × 40 cm^3) and the combined organic layers were washed with brine (50 cm^3) and water (50 cm^3). The

solution was dried over magnesium sulfate and concentrated under reduced pressure to yield a brown oil. The product was purified by suction column chromatography on silica, eluting with petroleum ether (40-60 °C)/ethyl acetate (10:1 to 4:1), to yield the compound as a pale yellow oil (4.23 g, 84%); ν_{max} (film)/cm^{-1} 2247 (CN) and 969 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.87 (2H, p, *J* 7.5, C*H*$_2$-3), 2.37-2.46 (4H, m, 2 × C*H*$_2$), 6.15 (1H, dt, *J* 7.5, *J* 16, C*H*CH$_2$), 6.47 (1H, d, *J* 16, PhC*H*), 7.20-7.38 (5H, m, C$_6$*H*$_5$); δ_C (75.4 MHz; $CDCl_3$) 16.9 (*C*-2), 25.4 (*C*-3), 32.1 (*C*-4), 120.0 (*C*N), 126.5 (*C*-2' and 6'), 127.8 (*C*-4'), 128.0 (*C*-5), 129.0 (*C*-3' and 5'), 132.4 (*C*-6), 137.5 (*C*-1'); *m/z* (EI) 171 ([M]$^+$, 84%), 130 (80, [M-CH$_3$CN]$^+$), 117 (100, [PhCHCHCH$_2$]$^+$) and 91 (66, [PhCH$_2$]$^+$).

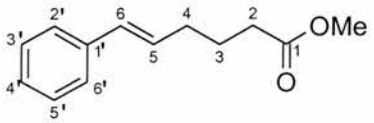
3.3.31 6-Phenylhex-5-enoic acid **205**[217]



6-Phenylhex-5-enonitrile (1.154 g, 6.74 mmoles) was dissolved in methanol (40 cm^3). Sodium hydroxide solution (3M, 60 cm^3) was added and the biphasic solution heated under reflux overnight. After cooling, the methanol was removed under reduced pressure, then ethyl acetate (50 cm^3) was added. The mixture was acidified to pH 5 using hydrochloric acid and the mixture extracted with ethyl acetate (2 × 80 cm^3). The combined organic extracts were washed with brine (80 cm^3), then dried over magnesium sulfate. Concentration of the solution under reduced pressure yielded the product as a yellow oil (1.09 g, 85%). The product was used without purification; ν_{max} (film)/cm^{-1} 3600-2800 (OH), 1709 (C=O) and 966 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.85 (2H, p, *J* 7.5, C*H*$_2$-3), 2.30 (2H, q, *J* 7.5, CHC*H*$_2$), 2.42 (2H, t, *J* 7.5, C*H*$_2$CO$_2$H), 6.23 (1H, dt, *J* 7.5, *J* 16, C*H*CH$_2$),

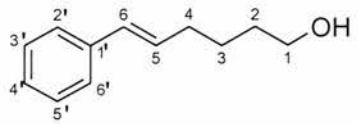
6.44 (1H, d, *J* 16, PhC*H*), 7.19-7.42 (5H, m, C_6H_5), 12.06 (1H, br s, O*H*); δ_C (75.4 MHz; $CDCl_3$) 24.8 (*C*-3), 32.7 (*C*-4), 34.4 (*C*-2), 126.4 (*C*-2' and 6'), 127.4 (*C*-4'), 128.9 (*C*-3' and 5'), 129.9 (*C*-5), 131.2 (*C*-6), 138.0 (*C*-1'), 181.0 (*C*=O); *m/z* (EI) 190 ($[M]^+$, 68%), 130 (100, $[M\text{-}CH_3CO_2H]^+$), 117 (88, $[PhCHCHCH_2]^+$), 115 (91, $[C_9H_7]^+$) and 91 (65, $[PhCH_2]^+$).

3.3.32 Methyl 6-phenylhex-5-enoate **206**[218]



6-Phenylhex-5-enoic acid (1.091 g, 5.63 mmoles) was dissolved in dry methanol (20 cm^3). Thionyl chloride (1.03 g, 0.63 cm^3, 8.66 mmoles) was carefully added to the solution which was subsequently heated under reflux for 2 hours. The solution was allowed to cool and was stirred at room temperature for 2 hours. The solution was washed with sodium bicarbonate solution (20 cm^3), then extracted with ethyl acetate (3 × 30 cm^3). The combined organic phases were dried over magnesium sulfate and concentrated under reduced pressure to yield the product as a yellow oil (1.16 g, 99%). No purification was necessary; ν_{max} (film)/cm^{-1} 1738 (C=O) and 967 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.76 (2H, p, *J* 7.5, CH_2-3), 2.28 (2H, q, *J* 7.5, $CHCH_2$), 2.40 (2H, t, *J* 7.5, $CH_2C(O)$), 3.69 (3H, s, CH_3), 6.21 (1H, dt, *J* 7.5, *J* 16, $CHCH_2$), 6.43 (1H, d, *J* 16, PhC*H*), 7.18-7.42 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 24.5 (*C*-3), 32.4 (*C*-4), 33.4 (*C*-2), 51.5 (CH_3), 126.0 (*C*-2' and 6'), 127.0 (*C*-4'), 128.5 (*C*-3' and 5'), 129.5 (*C*-5), 130.1 (*C*-6), 137.6 (*C*-1'), 174.0 (*C*=O); *m/z* (EI) 204 ($[M]^+$, 74%), 173 (20, $[M\text{-}OMe]^+$), 130 (100, $[M\text{-}CH_3CO_2H]^+$), 117 (66, $[PhCHCHCH_2]^+$), 115 (85, $[C_9H_7]^+$) and 91 (59, $[PhCH_2]^+$).

3.3.33 6-Phenylhex-5-en-1-ol **197**[218]

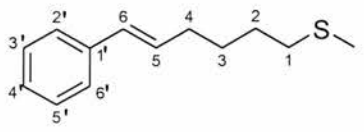


Methyl 6-phenylhex-5-enoate (1.092 g, 5.36 mmoles) was dissolved in dry THF (30 cm^3). DIBAL (1M, 12 cm^3, 12 mmoles) was added carefully to the solution which was subsequently stirred for 2 hours at room temperature. The reaction was quenched carefully with water (30 cm^3), then the aluminium salts were dissolved by the addition of hydrochloric acid. The mixture was extracted with ethyl acetate (3 × 50 cm^3) and the combined organic extracts were washed with brine (50 cm^3). The organic solution was dried over magnesium sulfate, then concentrated under reduced pressure to yield the product as a yellow oil (0.89 g, 95%). The compound was used without purification; ν_{max} (film)/cm^{-1} 3600-3100 (OH) and 965 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.54-1.75 (5H, m, 2 × CH_2, O*H*), 2.27 (2H, q, *J* 7.5, CHCH_2), 3.68 (2H, t, *J* 7.5, CH_2OH), 6.24 (1H, dt, *J* 7.5, *J* 16, C*H*CH_2), 6.43 (1H, d, *J* 16, PhC*H*), 7.16-7.41 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 25.5 (*C*-3), 32.3 (*C*-2), 32.8 (*C*-4), 62.8 (*C*-1), 126.0 (*C*-2' and 6'), 126.9 (*C*-4'), 128.5 (*C*-3' and 5'), 130.2 (*C*-5), 130.6 (*C*-6), 137.8 (*C*-1'); *m/z* (EI) 176 ($[M]^+$, 73%), 158 (32, $[M-H_2O]^+$), 130 (92, $[M-EtOH]^+$), 117 (98, $[PhCHCHCH_2]^+$), 115 (100, $[C_9H_7]^+$), 104 (59, $[PhCHCH_2]^+$) and 91 (86, $[PhCH_2]^+$).

3.3.34 1-Bromo-6-phenylhex-5-ene **198**[219] and 1-Chloro-6-phenylhex-5-ene **216**[220]

6-Phenylhex-5-en-1-ol (0.893 g, 5.07 mmoles) was dissolved in dry dichloromethane (20 cm^3). Carbon tetrabromide (3.39 g, 10.7 mmoles) then triphenylphosphine (2.69 g, 10.7 mmoles) were added to the solution, which was stirred at room temperature for 90 minutes. Water (50 cm^3) was added to the reaction mixture, which was then washed with sodium bicarbonate solution (50 cm^3). The aqueous layer was extracted with dichloromethane (3 × 50 cm^3) and the combined extracts were washed with brine (50 cm^3). The solution was dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. This was purified by column chromatography on silica using petroleum ether (40-60 °C) to yield the products (ratio bromide:chloride 9:1) as a colourless oil (1.01 g); δ_H (300 MHz; $CDCl_3$) 1.67 (2H, p, *J* 7.5, C*H*$_2$-3), 1.82-2.03 (2H, m, C*H*$_2$-2 bromide and chloride), 2.30 (2H, q, *J* 7.5, C*H*$_2$-4), 3.48 (2H, t, *J* 7.5, C*H*$_2$Br), 3.62 (2H, t, *J* 7.5, C*H*$_2$Cl), 6.25 (1H, dt, *J* 7.5, *J* 16, C*H*CH_2), 6.45 (1H, d, *J* 16, PhC*H*), 7.22-7.43 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 26.6 (*C*-2 chloride), 27.9 (*C*-2 bromide), 32.1 (*C*H_2), 32.3 (*C*H_2), 33.8 (CH_2Br), 45.0 (CH_2Cl), 126.0 (*C*-2' and 6'), 127.0 (*C*-4'), 128.6 (*C*-3' and 5'), 130.0 (*C*-5), 130.5 (*C*-6), 137.7 (*C*-1'); *m/z* (EI) 238, 240 ($[M]^+$(bromide), 56, 59%), 194, 196 ($[M]^+$(chloride), 16, 6%), 159 (17, $[M\text{-}Hal]^+$), 117 (100, $[PhCHCHCH_2]^+$), 115 (86, $[C_9H_7]^+$) and 91 (79, $[PhCH_2]^+$).

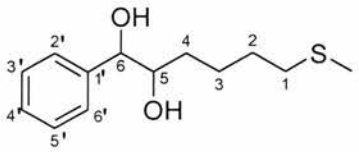
3.3.35 6-Phenyl-1-thiomethyl hex-5-ene **159**



A mixture of 1-bromo-6-phenylhex-5-ene and 1-chloro-6-phenylhex-5-ene (1.010 g) was dissolved in dry methanol (20 cm^3). Potassium iodide (0.07g, 0.42 mmoles) was added

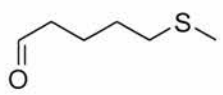
followed by sodium thiomethoxide (0.44 g, 6.28 mmoles). The reaction was stirred overnight at room temperature. TLC analysis showed that some starting material remained. Further sodium thiomethoxide (0.2 g, 2.85 mmoles) was added and the solution stirred overnight at room temperature. TLC analysis showed that the reaction had not gone to completion, so the solution was heated at 40 °C for 6 hours. Water (30 cm^3) and ethyl acetate (30 cm^3) were then added to the solution. The aqueous layer was twice extracted with ethyl acetate (2 × 50 cm^3) and the combined organic layers were washed with brine (50 cm^3). The solution was dried over magnesium sulfate and concentrated under reduced pressure to yield the product as a colourless oil. The crude oil was purified by column chromatography on silica, using petroleum ether (40-60 °C) then petroleum ether/ethyl acetate (10:1), to yield the product as a colourless oil (0.79 g, 73% over 2 steps); *m/z* (Found: $[MH]^+$ 207.1208. $C_{13}H_{19}S$ requires 207.1207); ν_{max} (film)/cm^{-1} 965 (CH=CH, *E*); δ_H (300 MHz; $CDCl_3$) 1.46-1.66 (4H, m, 2 × CH_2), 2.03 (3H, s, CH_3), 2.17 (2H, q, *J* 7.5, $CHCH_2$), 2.46 (2H, t, *J* 7.5, CH_2S), 6.13 (1H, dt, *J* 7.5, *J* 16, $CHCH_2$), 6.31 (1H, d, *J* 16, PhC*H*), 7.08-7.39 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$), 15.6 (CH_3), 28.5 (*C*-3), 28.7 (*C*-2), 32.6 (*C*-4), 34.2 (*C*-1), 126.0 (*C*-2' and 6'), 126.9 (*C*-4'), 128.5 (*C*-3' and 5'), 130.2 (*C*-5), 130.5 (*C*-6), 137.8 (*C*-1'); *m/z* (CI) 207 ($[MH]^+$, 100%), 159 (37, $[M\text{-}SCH_3]^+$), 117 (16, $[PhCHCHCH_2]^+$) and 91 (12, $[PhCH_2]^+$).

3.3.36 6-Phenyl-5,6-dihydroxy-1-thiomethylhexane **217**



6-Phenyl-1-thiomethylhex-5-ene (0.068 g, 0.33 mmoles) was dissolved in dry pyridine (4 cm^3) and added to osmium tetroxide (0.125 g, 0.49 mmoles). The solution was stirred at room temperature for 2 hours, then the solvent was removed under reduced pressure. Sodium metabisulfite (3 g, 15.8 mmoles), water (1 cm^3) and THF (30 cm^3) were added to the reaction which was then heated at 65 °C overnight. After cooling, the mixture was filtered through celite. The filtrate was dried over sodium sulfate and concentrated under reduced pressure to give a brown oil. The oil was purified by suction column chromatography on silica, eluting with petroleum ether (40-60 °C)/ethyl acetate (3:1 to 1:1), to yield the product as a colourless oil (0.05 g, 63%); *m/z* (Found: $[M+Na]^+$ 263.1078. $C_{13}H_{20}O_2SNa$ requires 263.1082); ν_{max} (film)/cm^{-1} 3700-3000 (OH); δ_H (300 MHz; $CDCl_3$) 1.22-1.58 (6H, m, 3 × CH_2), 1.98 (3H, s, CH_3), 2.30-2.40 (3H, m, CH_2S, O*H*) 2.54 (1H, br s, O*H*), 3.57-3.68 (1H, m, $CH_2CH(OH)$), 4.38 (1H, d, *J* 8, PhC*H*(OH)), 7.21-7.34 (5H, m, C_6H_5); δ_C (75.4 MHz; $CDCl_3$) 15.9 (CH_3), 25.2 (*C*-3), 29.3 (*C*-4), 32.6 (*C*-2), 34.5 (*C*-1), 76.2 (*C*-5), 78.3 (*C*-6), 127.2 (*C*-2' and 6'), 128.5 (*C*-4'), 129.0 (*C*-3' and 5'), 141.5 (*C*-1'); *m/z* (MALDI-TOF) 285 ($[M+2Na-H]^+$, 13%) and 263 (100, $[M+Na]^+$).

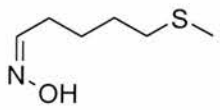
3.3.37 5-Thiomethylpentanal **160**[221]



6-Phenyl-5,6-dihydroxy-1-thiomethylhexane (0.05 g, 0.21 mmoles) was dissolved in THF (5 cm^3). Sodium metaperiodate (0.053 g, 0.25 mmoles) was dissolved in water (8 cm^3) and added to the organic solution. The reaction was stirred for 90 minutes at room temperature, then the solvent was removed under reduced pressure. The mixture was saturated with sodium chloride and extracted with ethyl acetate (3 × 30 cm^3). The combined organic

layers were dried over magnesium sulfate and concentrated under reduced pressure to give a pale yellow oil. Due to the product's volatility, it was carried straight into the next reaction. For data, see 3.4.2.

3.3.38 5-Thiomethylpentaldoxime **219**[222]

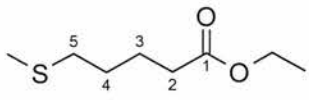


Sodium acetate trihydrate (0.204 g, 1.5 mmoles) and hydroxylamine hydrochloride (0.118 g, 1.7 mmoles) were dissolved in water (10 cm^3). A mixture of 5-thiomethylpentanal and benzaldehyde (0.12 g) were dissolved in three drops of ethanol and added to the aqueous solution. The solution was heated at 70 °C for 2.5 hours, then cooled to room temperature. The solution was extracted with ethyl acetate (3 × 30 cm^3) and the combined organic layers were dried over magnesium sulfate. The solution was concentrated under reduced pressure to yield a pale yellow oil. The product was purified by preparative TLC on silica, using petroleum ether (40-60 °C)/ethyl acetate (4:1) as the mobile phase, to yield the desired product, as a mixture of isomers (ratio *E*:*Z* 1:1), as a colourless crystalline solid (0.01 g, 13% over 2 steps); m.p. 35-38 °C; *m/z* (Found: $[MH]^+$ 148.0791. $C_6H_{14}NOS$ requires 148.0796); ν_{max} (PTFE)/cm^{-1} 3700-3000 (OH) and 1660 (C=N); δ_H (500 MHz; $CDCl_3$) 1.48-1.66 (4H, m, 2 × CH_2, *E* and *Z*), 2.10 (3H, s, CH_3, *E* and *Z*), 2.31 (1H, q, *J* 7, CH_2CN, *Z*), 2.41 (1H, q, *J* 7, CH_2CN, *E*), 2.51 (1H, t, *J* 7, CH_2S, *Z*), 2.52 (1H, t, *J* 7, CH_2S, *E*), 6.73 (0.5H, t, *J* 7, C*H*N, *E*), 7.31 (0.5H, br s, O*H*, *Z*), 7.43 (0.5H, t, *J* 7, C*H*N, *Z*), 7.67 (0.5H, br s, O*H*, *E*); δ_C (125.7 MHz; $CDCl_3$) 15.5 (CH_3, *E* and *Z*), 24.5 (*C*-2, *E*), 25.1 (*C*-3, *E*), 25.5 (*C*-3, *Z*), 28.5 (*C*-4, *Z*), 28.7 (*C*-4, *E*), 29.1 (*C*-2, *Z*), 33.81 (*C*-5, *E*), 33.84 (*C*-5, *Z*), 152.0

(*C*N, *Z*), 152.7 (*C*N, *E*); *m/z* (EI) 148 ($[MH]^+$, 73%), 147 (100, $[M]^+$), 131 (53, $[MH^+-OH]^+$), 130 (48, $[M-OH]^+$) and 103 (63, $[M-CHNOH]^+$).

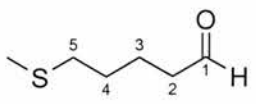
3.4 Synthesis of Glucosinolate precursor oximes

3.4.1 Ethyl 5-thiomethylvalerate **223**[223]



Ethyl 5-bromovalerate (1.321 g, 1 cm^3, 6.3 mmoles) was dissolved in dry ethanol (20 cm^3). Sodium thiomethoxide (0.6 g, 8.6 mmoles) was added to the solution, which was stirred for 6 hours at room temperature. Water (30 cm^3) and diethyl ether (30 cm^3) were then added to the solution. The organic layer was washed with brine (2 × 30 cm^3), dried ($MgSO_4$) and concentrated at reduced pressure. The product was obtained as a colourless oil (0.77 g, 70%); ν_{max} (film)/cm^{-1} 1734 (C=O); δ_H (300 MHz; $CDCl_3$) 1.35 (3H, t, *J* 8, CH_2CH_3), 1.68-1.88 (4H, m, CH_2-3, CH_2-4), 2.18 (3H, s, SCH_3), 2.40 (2H, t, *J* 8, CH_2CO), 2.60 (2H, t, *J* 8, CH_2S), 4.20 (2H, q, *J* 8, OCH_2CH_3); δ_C (75.4 MHz; $CDCl_3$) 14.6 (CH_2CH_3), 15.8 (SCH_3), 24.4 (*C*-4), 28.9 (*C*-3), 34.1, 34.2 (*C*-2 and 5), 60.6 (CH_2CH_3), 173.6 (*C*=O); *m/z* (CI) 177 ($[MH]^+$, 100%) and 131 (61, $[M-OCH_2CH_3]^+$).

3.4.2 5-Thiomethylpentanal **160**[221]



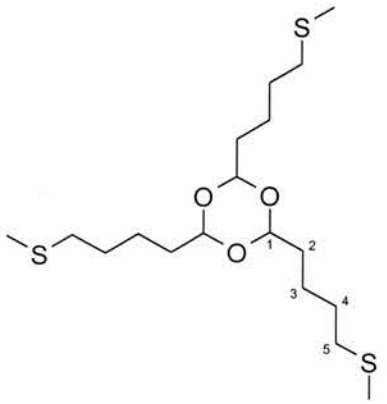
Ethyl 5-thiomethylvalerate (0.5 g, 2.8 mmoles) was dissolved in THF (20 cm^3) and the solution was cooled to -78 °C. Di-isobutyl aluminium hydride (1 M in hexanes, 7 cm^3, 7 mmoles) was added to the solution, which was stirred at -78 °C for 20 minutes. Aqueous ammonium chloride (20 cm^3) was added to the solution, which was then allowed to warm to room temperature. The reaction solution was diluted with diethyl ether (50 cm^3) and the organic layer was washed with water and brine until all the aluminium salts had been removed. The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure to yield the product as a colourless liquid. The product was purified by column chromatography, using petroleum ether (40-60 °C)/diethyl ether (4:1) as the eluent. This yielded the product as a colourless oil (0.19 g, 51%); ν_{max} (film)/cm^{-1} 1734 (C=O); δ_H (300 MHz; $CDCl_3$) 1.60-1.80 (4H, m, CH_2CH_2), 2.15 (3H, s, CH_3), 2.55 (4H, m, CH_2CO, CH_2S), 9.80 (1H, s, CHO); δ_C (75.4 MHz; $CDCl_3$) 15.8 (CH_3), 21.4 (CH_2), 28.78 (CH_2), 34.1 (CH_2S), 43.7 (CH_2CHO), 202.4 (CHO); *m/z* (CI) 133 ($[MH]^+$, 100%), 117 (20, $[M-CH_3]^+$) and 85 (23, $[M-SCH_3]^+$).

3.4.3 5-Thiomethylpentan-1-ol **224**[224]

Ethyl 5-thiomethylvalerate (0.5 g, 2.8 mmoles) was dissolved in THF (20 cm^3). Di-isobutyl aluminium hydride (1 M in hexanes, 6 cm^3, 6 mmoles) was added to the solution, which was stirred at room temperature for 2 hours. Aqueous ammonium chloride (20 cm^3) was added to the solution, which was then diluted with diethyl ether (50 cm^3). The organic layer was washed with water and brine until all the aluminium salts had been removed, dried ($MgSO_4$) and then concentrated under reduced pressure to yield the product as a

colourless liquid (0.33 g, 88%); ν_{max} (film)/cm^{-1} 3600-3000 (OH); δ_H (300 MHz; $CDCl_3$) 1.40-1.70 (6H, m, 3 × CH_2), 2.10 (3H, s, CH_3), 2.50 (2H, t, *J* 8, CH_2S), 3.65 (2H, t, *J* 8, CH_2OH); δ_C (75.4 MHz; $CDCl_3$) 15.81 (CH_3), 25.29 (CH_2), 29.22 (CH_2), 32.55 (CH_2), 34.49 (CH_2S), 62.75 (CH_2OH); *m/z* (CI) 135 ($[MH]^+$, 73) and 117 (24, $[M-OH]^+$).

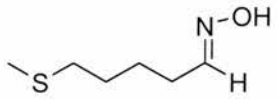
3.4.4 Attempted synthesis of 5-Thiomethylpentanal



A solution of oxalyl chloride (3.8 g, 30 mmoles) in dichloromethane (10 cm^3) was cooled to -78 °C. Dimethyl sulfoxide (2.4 g, 30 mmoles) was dissolved in dichloromethane (10 cm^3) and added to the solution, which was stirred for one hour at -78 °C. 5-Thiomethylpentan-1-ol (2 g, 15 mmoles) in dichloromethane (20 cm^3) was then added to the solution which was stirred for a further hour at -78 °C. Triethylamine (7.6 g, 75 mmoles) was then added to the solution, which was allowed to warm to room temperature. Ethyl acetate (50 cm^3) was added to the reaction and the organic solution was washed with water (50 cm^3), dilute hydrochloric acid (50 cm^3), sodium bicarbonate solution (50 cm^3) and brine (50 cm^3). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure to give a brown oil. This was purified by column chromatography on silica, eluting with petroleum ether (40-60 °C)/diethyl ether (4:1), to give the product as a

brown oil (0.61 g, 15%); *m/z* (Found: $[M]^+$ 396.1826. $C_{18}H_{36}O_3S_3$ requires 396.1827) ν_{max} (film)/cm^{-1} 1128, 1064 (C-O-C); δ_H (300Mhz; $CDCl_3$) 1.43-1.75 (6H, m, 3 × CH_2), 2.06 (3H, s, CH_3), 2.48 (2H, t, *J* 7, CH_2S), 4.83 (1H, t, *J* 7, *CH*); δ_C (75.4 MHz; $CDCl_3$) 15.7 (CH_3), 23.0 (*C*-3), 29.1 (*C*-4), 34.1 (*C*-2), 34.2 (*C*-5), 101.5 (*C*-1); *m/z* (EI) 396 ($[M]^+$, 25%), 133 (100, $[C_6H_{13}OS]^+$), 132 (67, $[C_6H_{12}OS]^+$), 104 (23, $[C_5H_{12}S]^+$), 85 (49, $[C_5H_9O]^+$) and 61 (38, $[C_2H_5S]^+$).

3.4.5 5-Thiomethylpentaldoxime **219**[222]



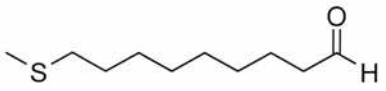
Sodium acetate trihydrate (0.23 g, 1.7 mmoles) and hydroxylamine hydrochloride (0.14 g, 2 mmoles) were dissolved in water (10 cm^3). To the solution was added 5-thiomethylpentanal (0.15 g, 1.1 mmoles) in ethanol (1 cm^3). The solution was heated at 70 °C for 30 minutes, then cooled on ice, to yield a yellow precipitate. The product was filtered off and washed with cooled ethanol. The product was recrystallised from aqueous ethanol to yield a colourless flaky solid (0.05 g, 30%); Data as per 3.3.38.

3.4.6 9-Thiomethylnonan-1-ol **227**[225]

9-Bromononan-1-ol (1 g, 4.5 mmoles) was dissolved in dry ethanol (20 cm^3). Sodium thiomethoxide (0.43 g, 6.1 mmoles) was added to the solution, which was stirred at room

temperature for 2 hours. The solvent was removed under reduced pressure and water (40 cm^3) was added to the concentrate. The mixture was extracted with ethyl acetate (3 × 40 cm^3) and the combined organic fractions were dried over magnesium sulfate. Concentration of the solution under reduced pressure gave the product as a colourless oil (0.81 g, 95%). The product was used without purification; ν_{max} (film)/cm^{-1} 3700-3100 (OH); δ_H (300 MHz; $CDCl_3$) 1.25-1.45 (10H, m, 5 × CH_2), 1.52-1.65 (4H, m, 2 × CH_2), 2.12 (3H, s, CH_3), 2.48 (2H, t, *J* 7, CH_2S), 3.64 (2H, t, *J* 7, CH_2OH); δ_C (75.4 MHz; $CDCl_3$), 15.4 (CH_3), 25.8 (CH_2), 28.8 (CH_2), 29.1 (2 × CH_2), 29.4 (CH_2), 29.5 (CH_2), 32.8 (CH_2), 34.2 (CH_2S), 62.7 (CH_2OH); *m/z* (CI) 191 ($[MH]^+$, 17%), 189 (21, $[M-H]^+$), 173 (100, $[M-OH]^+$).

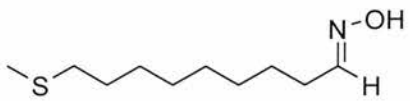
3.4.7 9-Thiomethylnonanal **228**



A solution of oxalyl chloride (0.26 g, 2 mmoles) in dichloromethane (2 cm^3) was cooled to -78 °C. Dimethyl sulfoxide (0.16 g, 2 mmoles) in dichloromethane (2 cm^3) was then added to the solution, which was stirred for 1 hour at -78 °C. 9-Thiomethylnonan-1-ol (0.2 g, 1 mmole) in dichloromethane (5 cm^3) was added and the solution was stirred for a further hour at -78 °C. Triethylamine (0.53 g, 0.73 cm^3, 5 mmoles) was then added to the solution, which was allowed to warm to room temperature. The solution was extracted with ethyl acetate (3 × 20 cm^3) and the combined organic layers were washed with brine (30 cm^3). The solution was dried over magnesium sulfate and concentrated under reduced pressure to give a brown oil. This was purified by column chromatography on silica, using petroleum ether (40-60 °C)/ethyl acetate (12:1) as the elutant, to give the title compound as a

colourless oil (0.11 g, 54%); *m/z* (Found: $[MH]^+$ 189.1310. $C_{10}H_{21}OS$ requires 189.1313); ν_{max} (film)/cm^{-1} 1720 (C=O); δ_H (300 MHz; $CDCl_3$), 1.25-1.45 (8H, m, 4 × CH_2), 1.52-1.69 (4H, m, 2 × CH_2), 2.08 (3H, s, CH_3), 2.37-2.52 (4H, m, CH_2S and CH_2CO), 9.77 (1H, s, CHO); δ_C (75.4 MHz; $CDCl_3$) 15.7 (CH_3), 23.7 (CH_2), 28.9 (CH_2), 29.28 (CH_2), 29.33 (CH_2), 29.46 (CH_2), 29.53 (CH_2), 34.5 (CH_2S), 44.0 (CH_2CO), 203.0 (C=O); *m/z* (ES) 227 ($[M+K]^+$, 100%) and 189 (13, $[M+H]^+$).

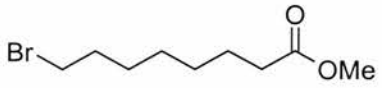
3.4.8 9-Thiomethylnonaldoxime **221**



Sodium acetate trihydrate (0.33 g, 2.4 mmoles) and hydroxylamine hydrochloride (0.19 g, 2.7 mmoles) were dissolved in water (20 cm^3). 9-Thiomethylnonanal (0.3 g, 1.6 mmoles) was dissolved in a minimum volume of ethanol and was added to the aqueous solution. The solution was heated at 70 °C for 2.5 hours, then cooled to 0 °C. The solution was extracted with ethyl acetate (3 × 30 cm^3) and the combined organic layers were dried over magnesium sulfate. The solution was concentrated under reduced pressure to give a yellow oil which was purified by column chromatography on silica, eluting with petroleum ether (40-60 °C)/ethyl acetate (15:1 to 4:1). This gave the title compound as a colourless solid with ratio *Z*:*E* 3:2 (0.20 g, 61%); m.p. 54-55 °C; (Found: C, 58.73; H, 10.73; N, 6.59. Calc for $C_{10}H_{21}ONS$: C, 59.07; H, 10.41; N, 6.89%); *m/z* (Found: $[MH]^+$ 204.1425. $C_{10}H_{22}NOS$ requires 204.1422); ν_{max} (nujol)/cm^{-1} 3500-3000 (OH) and 1663 (C=N); δ_H (300 MHz; $CDCl_3$) 1.25-1.65 (12H, m, 6 × CH_2, *E* and *Z*), 2.09 (3H, s, CH_3, *E* and *Z*), 2.18 (0.5 × 2H, q, *J* 8, CH_2CN, *E*), 2.37 (0.5 × 2H, q, *J* 8, CH_2CN, *Z*), 2.48 (2H, t, *J* 8, CH_2S, *E* and *Z*),

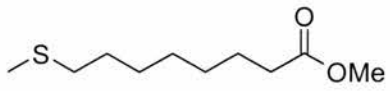
6.71 (0.5H, t, *J* 8, C*H*N, *Z*), 7.41 (0.5H, t, *J* 8, C*H*N, *E*); δ_C (75.4 MHz; $CDCl_3$), 15.7 (*C*H_3), 21.1 (*C*H_2), 26.2 (*C*H_2CN, *Z*), 26.7 (*C*H_2), 28.9 (*C*H_2), 29.2 (*C*H_2), 29.30 (*C*H_2), 29.35 (*C*H_2), 29.38 (*C*H_2), 29.5 (*C*H_2), 29.7 (*C*H_2CN, *E*), 34.5 (*C*H_2S, *E* and *Z*), 152.4 (*C*=N, *E*), 153.1 (*C*=N, *Z*); *m/z* (CI) 204 ($[MH]^+$, 22%), 188 (29, $[M\text{-}Me]^+$), 186 (100, $[M\text{-}OH]^+$), 138 (66, $[M\text{-}MeS\text{-}H_2O]^+$.

3.4.9 Methyl 8-bromooctanoate **230**[226]



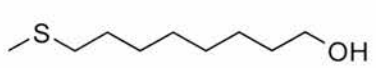
8-Bromooctanoic acid (4 g, 18 mmoles) was dissolved in dry methanol (100 cm^3). Thionyl bromide (11.27 g, 4.2 cm^3, 54 mmoles) was added to the solution, which was heated under reflux for 2 hours. The reaction was cooled and the solvent was removed under reduced pressure. The concentrate was extracted with diethyl ether (3 × 80 cm^3) and washed with sodium bicarbonate solution (100 cm^3). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give a yellow liquid (3.65 g, 85%). No purification was required; ν_{max} (film)/cm^{-1} 1740 (C=O); δ_H (300 MHz; $CDCl_3$) 1.41-1.61 (6H, m, 3 × C*H*$_2$), 1.64-1.80 (2H, m, C*H*$_2$), 1.97 (2H, p, *J* 8, C*H*$_2$), 2.42 (2H, t, *J* 8, C*H*$_2$CO), 3.51 (2H, t, *J* 8, C*H*$_2$Br), 3.78 (3H, s, C*H*$_3$); δ_C (75.4 MHz; $CDCl_3$) 25.0 (*C*H_2), 28.1 (*C*H_2), 28.6 (*C*H_2), 29.1 (*C*H_2), 32.9 (*C*H_2), 34.0 (*C*H_2), 34.1 (*C*H_2), 51.6 (O*C*H_3), 174.2 (*C*=O); *m/z* (ES) 259, 261 ($[M+Na]^+$, 100, 92%).

3.4.10 Methyl 8-thiomethyloctanoate **231**[227]



Methyl 8-bromooctanoate (3 g, 14.8 mmoles) was dissolved in dry methanol (80 cm^3). Sodium thiomethoxide (1.5 g, 21 mmoles) was added to the solution which was stirred at room temperature overnight. The solvent was removed under reduced pressure and the concentrate was extracted with ethyl acetate (3 × 80 cm^3). The combined organic layers were washed with water (50 cm^3) and brine (50 cm^3), then dried over magnesium sulfate. The solution was concentrated under reduced pressure to give the product as a colourless oil (2.10 g, 81%); ν_{max} (film)/cm^{-1} 1740 (C=O); δ_H (300 MHz; $CDCl_3$) 1.28-1.46 (6H, m, 3 × CH_2), 1.53-1.68 (4H, m, 2 × CH_2), 2.09 (3H, s, SCH_3), 2.30 (2H, t, *J* 8, CH_2CO), 2.48 (2H, t, *J* 8, CH_2S), 3.66 (3H, s, OCH_3); δ_C (75.4 MHz; $CDCl_3$) 15.7 (SCH_3), 25.0 (CH_2), 28.7 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 34.2 (CH_2S), 34.4 (CH_2CO), 51.5 (OCH_3), 174.2 (C=O); *m/z* (CI) 205 ($[MH]^+$, 8%), 173 (100, $[M\text{-}OMe]^+$), 157 (9, $[M\text{-}SMe]^+$), 155 (18, $[CHCH(CH_2)_5CO_2Me]^+$), 125 (8, $[M\text{-}OMe\text{-}MeSH]^+$).

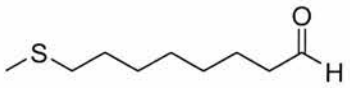
3.4.11 8-Thiomethyloctan-1-ol **232**[225]



Methyl 8-thiomethyloctanoate (1.99 g, 9.7 mmoles) was dissolved in dry THF (50 cm^3). DIBAL (1M in hexanes, 20 cm^3, 20 mmoles) was added to the solution, which was stirred at room temperature. The reaction was monitored by TLC (petroleum ether (40-60

°C)/ethyl acetate 5:1) and water was added to the reaction when it had reached completion. Dilute hydrochloric acid was added to dissolve the DIBAL salts and the mixture was extracted with ethyl acetate (3 × 80 cm^3). The combined organic layers were washed with brine (100 cm^3) and dried over magnesium sulfate. The solution was concentrated under reduced pressure to give the product as a colourless oil (1.59 g, 92%); ν_{max} (film)/cm^{-1} 3600-3000 (OH); δ_H (300 MHz; $CDCl_3$) 1.30-1.46 (8H, m, 4 × CH_2), 1.52-1.65 (4H, m, 2 × CH_2), 2.11 (3H, s, CH_3), 2.51 (2H, t, *J* 8, SCH_2), 3.65 (2H, t, *J* 8, CH_2OH); δ_C (75.4 MHz; $CDCl_3$) 15.7 (CH_3), 25.8 (CH_2), 28.9 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 32.9 (CH_2), 34.4 (CH_2S), 63.0 (CH_2OH); *m/z* (CI) 177 ($[MH]^+$, 13%), 175 (11, $[M-H]^+$), 159 (100, $[M-OH]^+$).

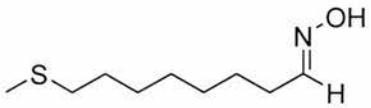
3.4.12 8-Thiomethyloctanal **233**



Dimethyl sulfoxide (1.25 g, 16 mmoles) in dry dichloromethane (15 cm^3) was added to a solution of oxalyl chloride (2 g, 16 mmoles) in dichloromethane (15 cm^3) at -78 °C. The solution was stirred at -78 °C for 1 hour then 8-thiomethyloctan-1-ol (1.4 g, 7.4 mmoles) in dichloromethane (20 cm^3) was added and the solution was stirred for a further hour. Triethylamine (4 g, 5.5 cm^3, 40 mmoles) was added and the solution was allowed to warm to room temperature. Ethyl acetate (50 cm^3) was added to the solution, which was washed with water (80 cm^3) and brine (80 cm^3). The solvent was removed under reduced pressure to give an oil, but salts were observed so water (30 cm^3) was added and the mixture was re-extracted with ethyl acetate (2 × 50 cm^3). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give the title compound as

a yellow oil (1.37 g, 99%); *m/z* (Found: $[MH]^+$ 175.1153. $C_9H_{19}OS$ requires 175.1157); ν_{max} (film)/cm^{-1} 1686 (C=O); δ_H (300 MHz; $CDCl_3$) 1.26-1.45 (6H, m, 3 × CH_2), 1.51-1.68 (4H, m, 2 × CH_2), 2.09 (3H, s, CH_3), 2.38-2.53 (4H, m, CH_2S and CH_2CO), 9.78 (1H, s, CHO); δ_C (75.4 MHz; $CDCl_3$) 15.7 (CH_3), 22.1 (CH_2), 28.7 (CH_2), 28.8 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 34.4 (CH_2S), 44.0 (CH_2CO), 202.8 (C=O); *m/z* (CI) 175 ($[MH]^+$, 100%), 159 (50, $[M\text{-}Me]^+$), 109 (25, $[M\text{-}SMe\text{-}H_2O]^+$).

3.4.13 8-Thiomethyloctaldoxime **220**



Sodium acetate trihydrate (1.16 g, 8.5 mmoles) and hydroxylamine hydrochloride (0.68 g, 9.8 mmoles) were dissolved in water (60 cm^3). 8-Thiomethyloctanal (1 g, 5.7 mmoles) was dissolved in a minimum volume of ethanol and added to the aqueous solution. The reaction was heated at 70 °C for 2.5 hours, then cooled to 0 °C. The solution was extracted with ethyl acetate (3 × 80 cm^3) and the combined organic layers were washed with brine (100 cm^3). The solution was dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil. This was purified by suction column on silica, eluting with petroleum ether (40-60 °C)/ethyl acetate (10:1), to give the title compound as a colourless solid (ratio *Z*:*E* 11:1) (0.581 g, 53%); m.p. 42-43 °C; *m/z* (Found: $[MH]^+$ 190.1271. $C_9H_{20}NOS$ requires 190.1266); ν_{max} (nujol)/cm^{-1} 3600-3000 (OH) and 1675 (C=N); δ_H (300 MHz; $CDCl_3$) 1.24-1.66 (10H, m, 5 × CH_2, *E* and *Z*), 2.09 (3H, s, CH_3, *E* and *Z*), 2.19 (0.5 × 2H, q, *J* 8, CH_2CN, *E*), 2.38 (0.5 × 2H, q, *J* 8, CH_2CN, *Z*), 2.49 (2H, t, *J* 8, CH_2S, *E* and *Z*), 6.72 (0.5H, t, *J* 8, CHN, *Z*), 6.84 (0.5H, br s, OH, *E*), 7.12 (0.5H, br s, OH, *Z*), 7.43

(0.5H, t, *J* 8, C*H*N, *E*); δ_C (75.4 MHz; $CDCl_3$) 15.7 (*C*H_3, *E* and *Z*), 25.1 (*C*H_2CN, *Z*), 26.2 (*C*H_2), 28.8 (*C*H_2), 29.1 (*C*H_2), 29.3 (*C*H_2), 29.4 (*C*H_2CN, *E*), 34.4 (*C*H_2S, *E* and *Z*), 152.7 (*C*=N, *E*), 153.4 (*C*=N, *Z*); *m/z* 190 ($[MH]^+$, 13%), 174 (41, $[M\text{-}Me]^+$), 172 (100, $[M\text{-}OH]^+$), 124 (51, $[M\text{-}MeS\text{-}H_2O]^+$).

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