THE SYNTHESIS OF NATURAL AND NOVEL GLUCOSINOLATES

Susan Elizabeth Cobb

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

2012

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The Synthesis of Natural and Novel Glucosinolates

Susan Elizabeth Cobb

May 2012

A thesis presented for the degree of Doctor of Philosophy to the School of Chemistry, University of St Andrews

Supervisor Dr Nigel P. Botting
This thesis is dedicated to the memory of Dr Nigel Botting
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I, Susan Elizabeth Cobb, hereby certify that this thesis, which is approximately 39,200 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

This thesis describes the synthesis of natural and novel glucosinolates \( 1 \).

Chapter 1 gives an overview of glucosinolates, from isolation and biosynthesis to previous chemical syntheses. Chapter 2 describes an improved method for the formation of thiohydroximate bonds and its application over a variety of substrates. Chapter 3 focuses on exploring new chemistry on intact glucosinolates and details oxidative chemistry on an alkene containing side chain glucosinolate. Furthermore, the total synthesis of novel glucosinolates 150, 156 and 161 is illustrated.

Chapter 4 describes a model study for the solid phase synthesis of glucosinolates. Finally experimental procedures for the compounds synthesised in the thesis are described in Chapter 5.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>amu</td>
<td>Atomic Mass units</td>
</tr>
<tr>
<td>BAIB</td>
<td>Bis(acetoxy)iodobenzene</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>Cl</td>
<td>Chemical ionisation</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess Martin periodinane</td>
</tr>
<tr>
<td>DMS</td>
<td>Dimethyl sulfide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>ESP</td>
<td>Epithiospecifier protein</td>
</tr>
<tr>
<td>et al.</td>
<td>Et alia (Latin), and others</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione S-transferase</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>IBX</td>
<td>2-iodoxybenzoic acid</td>
</tr>
<tr>
<td>ITC</td>
<td>isothiocyanate</td>
</tr>
<tr>
<td>Min</td>
<td>minutes</td>
</tr>
<tr>
<td>MNDO</td>
<td>Modified neglect of differential overlap</td>
</tr>
<tr>
<td>NCS</td>
<td>N-Chlorosuccinimide</td>
</tr>
<tr>
<td>NFKb</td>
<td>Nuclear factor kappa b</td>
</tr>
<tr>
<td>NIS</td>
<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>nm</td>
<td>nanometres</td>
</tr>
<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NPG</td>
<td>Nitrophenyl-β-D-glucoside</td>
</tr>
<tr>
<td>NSP</td>
<td>Nitrile specifier protein</td>
</tr>
<tr>
<td>NQO1</td>
<td>NADH quinone oxidoreductase</td>
</tr>
<tr>
<td>PAPs</td>
<td>3-Phosphoadenosine 5’-phosphosulfate</td>
</tr>
<tr>
<td>PTAS</td>
<td>p-Toluenesulfonic acid</td>
</tr>
<tr>
<td>TBAF</td>
<td>Tetra-n-butylammonium fluoride</td>
</tr>
<tr>
<td>TBS</td>
<td>tert-Butyldimethylsilyl chloride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TS</td>
<td>Transition state</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-Tetramethylpiperidine N-oxide radical</td>
</tr>
<tr>
<td>UDPG</td>
<td>Uridine-5’-diphosphate glucose</td>
</tr>
</tbody>
</table>
Acknowledgments

First and foremost I would like thank Dr Nigel Botting for providing me with the privilege to do my PhD under his supervision. He gave me the inspiration, enthusiasm and freedom to explore my project whilst always having an open door and time to puzzle through a problem together whenever they came up. I dedicate my thesis to him.

I would like to thank Prof. David O’Hagan for his ongoing encouragement and unwavering support over the last 12 months. I would also like to thank Iain Smellie for help, advice and technical expertise in organic synthesis over the course of my PhD.

Thank you to Prof. Derek Woollins and Prof. Alex Slawin for their support over the last year and also Alex for X-ray crystallography data.

I would like to thank Dr. Catherine Botting for her sound words of advice and help in particular during writing up.

Thank you to the Botting Group for making my time in the lab enjoyable; Nikos (a great friend), Kate (the number 1 project student), Gavin, Andy, Aga, Qing and Kirsten. I would also like to thank our lab neighbours in the Florence group, in particular, Ross, Katy, Jo & Jo. I would also like to thank my friend, postdoc, and on call mac-support, Neil Keddie.

I am grateful for all the assistance from the analytical and technical staff of the School of Chemistry at the University of St Andrews. Thanks to Melanja Smith (NMR), Tomas Lebl (NMR) and Bobby Cathcart (workshop). In particular, thank you to Caroline Horsborough for HRMS and for making me laugh.

On a personal note I would like to thank my parents, Catherine and George, who have never questioned my endeavours and have always supported me in whatever I have decided to do. I would like to thank my brothers, George and Steven and their wives Kelly and Victoria for their support. My warmest thanks to my wonderful troop of nieces and nephews, who have all been born along the way of the PhD adventure; Freddie, Henry, George, Gretchen and Charlotte.
Last, but by no means least, I would like to thank my friends, Becks, Claire, Kirsty, Anna, Emma and Jess, without them the St Andrews experience would not have been the same. In particular I’d like to thank Lisa and Katy – not only for endless proof reading of my thesis (you both have the eye of the tiger!) but for all Team Beaver adventures of the last 4 years. It’s been a journey and a half but I’m glad to have the T-shirt to prove it!
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Introduction

1.1 Glucosinolates

Glucosinolates are a family of natural products found in all Cruciferae, including Brassica vegetables (Brassica Oleracea) such as Brussels sprouts, broccoli and oilseed rape, as well as mustard, horseradish and rocket. These secondary metabolites have the general structure of Type 1 (Figure 1) containing a thioglucose unit attached to a β-thiohydroximate bond that is O-sulfated. Additionally, attached to the carbon of the thiohydroximate moiety lies a side chain varying as an aliphatic, aromatic or indole group.

![Figure 1: General glucosinolate structure of Type 1 with examples of side chains.](image)

To date, there have been approximately 150 naturally occurring glucosinolates isolated\(^1\) with the first reported in 1831 by Robiquet and Boutron.\(^2\) It was Gadamer\(^3\) in 1897 who proposed the first, but erroneous, isomeric structure, whereby the side chain would be linked to nitrogen and the sulfate directly attached to the carbon of the thiohydroximate. However, this would be later revised by Ettlinger and Lundeen\(^4\) in 1957 to give the currently accepted structure (Figure 2) whereby the β-thioglucose unit is connected to a thiohydroximate moiety and the sulfate is attached to the nitrogen with the Z-stereochemistry.
The nature of the variable side chain, R, is dependant upon the amino acid used in the early stages of the biosynthesis.

### 1.2 Biosynthesis of glucosinolates

Glucosinolates are biosynthesised in three distinct stages; side chain elongation, formation of the glycone moiety and then secondary side chain modification.\(^5\)

In the initial side chain elongation, the appropriate \(\alpha\)-amino acid undergoes a transamination reaction to generate the corresponding \(\alpha\)-keto acid (Scheme 1). This is followed by an aldol reaction with acetyl CoA. A dehydration, rehydration sequence, followed by oxidation and then decarboxylation occurs. Finally a second transamination takes place to recover the elongated amino acid functionality.\(^5\)

![Scheme 1: Initial side chain elongation.](image)

The substrate can be subjected to this cycle a number of times, adding another carbon atom with each iteration. For example methionine \(2\), which is used as a precursor for aliphatic glucosinolates, can be converted to homomethionine \(3\) and then to dihomomethionine \(4\) and so forth using this biosynthetic cycle, (Scheme 2).
Labelling experiments have been used to probe this biosynthetic pathway. The most recent study by Graser\textsuperscript{6} in 2000 used isotopically enriched $[1,2-{\textsuperscript{13}}C_2]$-acetate and universally labelled $[U-{\textsuperscript{13}}C]$-methionine that were incorporated into \textit{Eruca sativa} (more commonly known as ‘rocket’) in a feeding study. In turn this led to the isolation of desulfoglucosinolates 5 and 6 from the leaves, where $^{13}$C-NMR analysis was used to establish isotope enrichments, which were found at the corresponding positions as summarised in Scheme 3.

Scheme 2: An example of chain extension.

\begin{center}
\begin{tikzpicture}

\node (a) at (0,0) {2};
\node (b) at (1.5,0) {3};
\node (c) at (3,0) {4};

\node (d) at (0,-1) {OH};
\node (e) at (0,-2) {NH$_2$};

\node (f) at (1.5,-1) {OH};
\node (g) at (1.5,-2) {NH$_2$};

\node (h) at (3,-1) {OH};
\node (i) at (3,-2) {NH$_2$};

\node (j) at (0,-3) {$\text{Scheme 2}$: An example of chain extension.}
\end{tikzpicture}
\end{center}

The second stage of the biosynthesis involves the formation of the aglycone moiety and is initiated by cytochrome P$_{450}$ oxidation of the amino acid precursor to give an N-hydroxyl amino acid. Little data is available regarding these steps due to the instability of the intermediates involved, although it is proposed that the oxime is initially converted to an aci-nitro compound, which is an acceptor for a thiol donor. The identity of the sulfur donor has recently been shown to be GSH, the reduced form of the tripeptide of glutamic acid, cysteine and glycine.\textsuperscript{7} A C-S lyase enzyme then cleaves the cysteine adduct to give the thiohydroximate, which undergoes S-thioglucosylation by a soluble UDPG:thiohydroximate glucosyltransferase to yield the desulfoglucosinolate. The second stage of the biosynthesis concludes with sulfation by a 3'-$
phosphoadenosine 5'-phosphosulphate (PAPS) dependent enzyme to yield the complete glucosinolate $^5$ (Scheme 4).

Scheme 4: Second stage of glucosinolate biosynthesis.

In individual cases the biosynthesis continues with secondary side-chain modifications such as methylation, oxidation and hydrolysis. It is by these modifications that such a diverse range of glucosinolates is formed $^8$ (Scheme 5). For example the thiomethyl glucosinolate 7, can be oxidised to give the S-oxygenated glucosinolate, which in turn is converted to the hydroxylalkyl glucosinolate, 8. Alternatively thiomethyl glucosinolate undergoes methylsulfide elimination to give the alkenyl glucosinolate, 10, which can be derivatised further to a hydroxyalkenyl glucosinolate, 11. In further biosynthetic modifications the hydroxylalkyl side chain is esterified by the benzoate hydrolysing enzyme (BZO) to afford benzoyloxy glucosinolate, 9.
1.3 Enzymatic hydrolysis of glucosinolates

Myrosinase catalyses the hydrolysis of glucosinolates when plants are damaged, for example by harvesting, freezing, thawing or chewing. By this process the glucosinolate 1 is cleaved at the thioglycoside linkage 9 (Scheme 6). This releases the aglycone 12, a thiohydroximate-O-sulfonate, which readily undergoes Lossen rearrangement, in a non-enzymatic step, to form the corresponding isothiocyanate.

The mode by which myrosinase hydrolyses glucosinolates is very similar to the hydrolysis of O-glycosides by β-glycosidase enzymes. However unlike β-glycosidase, myrosinase does not require a glutamate residue in order to protonate the departing aglycone as the glucosinolate aglycone is a good leaving group. Myrosinase has been shown only to hydrolyse those O-glycosides with good leaving groups such as p-NPG (p-nitrophenyl-β-D-glucoside) and o-NPG. In myrosinase the catalytic glutamate is replaced by...
glutamine and it is believed that this leads to a more favourable interaction with the sulfate of the glucosinolate.\textsuperscript{11} The glutamine is positioned in order to deliver a water molecule for the hydrolysis of the glycosyl-enzyme intermediate. Although the myrosinase binding site has a preference for the sugar and sulfate group a wide variety of side chains are clearly tolerated (Figure 3).

**Figure 3:** Proposed mechanism for glucosinolate hydrolysis by myrosinase.\textsuperscript{10}

The myrosinase catalysed glucosinolate hydrolysis can be modified by the presence of other proteins. For example, if specifier proteins are present then the formation of the isothiocyanate is impeded. Two such proteins have been identified to date; the epithiospecifer protein (ESP) from *Arabidopsis thaliana* and the nitrile-specifier protein from *Pieris rapae*.\textsuperscript{12} They do not share any sequence similarity, however both have been shown to encourage the formation of nitriles. The defining reason for this change in product profile is not yet known, however the outcome of the rearrangement is also largely dependent on pH, temperature and the presence of ferrous ions.\textsuperscript{13}
Scheme 7: The outcome of glucosinolate breakdown is dependant on conditions as well as side chain functionality.

In selected cases, such as indole or hydroxylalkenyl side chains, the initially produced isothiocyanate can undergo further rearrangements to give cyclic (Scheme 7) or dimeric by-products (Scheme 8), due to the inherent reactivity of the isothiocyanate intermediate. There do not appear to be any enzymes involved in these reactions and the products emerge as a result of the inherent instability of the initially formed isothiocyanate.

Scheme 8: Indole isothiocyanates can undergo breakdown by rearrangements to give dimeric compounds.
1.4 Biological activity of glucosinolates

Glucosinolates and their breakdown components have many important roles within nature. The earliest report of glucosinolates, or more precisely their isothiocyanate breakdown products, acting as a natural pesticide emerged in 1967 from Drobnica et al.\textsuperscript{14} Following this account there has been a lack of research in the area, properly establishing good structure-activity relationships.\textsuperscript{15} Despite the limited examples, phenethyl isothiocyanate \textsuperscript{13} is known to be a feeding deterrent for snails (\textit{Phsella spp.}), caddisflies (\textit{Hesperophylax designatus} and \textit{Limnephilus spp.}) and amphipods (\textit{Gammanus pseudolimnaeus}).\textsuperscript{16}

\begin{center}
\begin{tikzpicture}
\draw[thick] (-0.5,0) -- (0.5,0) -- (0.5,1) -- (-0.5,1) -- cycle;
\draw[thick] (0,0) -- (0,1);
\draw[thick] (0,0.5) -- (0.5,0.5);
\draw[thick] (0,1) -- (0.5,1.5);
\draw[thick] (0,1.5) -- (0.5,1.5);
\node at (0,0.5) {N};\node at (0.5,0.5) {C};\node at (0.5,1.5) {S};\end{tikzpicture}
\end{center}

13

In some specialised cases pests, such as the diamond-back moth (\textit{Plutella xylostella}),\textsuperscript{17} have evolved to use glucosinolates found on the leaf surface as a stimulus for oviposition.\textsuperscript{18} This is a process whereby pests use glucosinolates as a signalling agent and then burrow down into the root via the stem of the plants to lay eggs near the root. Upon hatching the larvae will eat the root of the plant at a large cost to crop yields and therefore impact significantly on commercial return.

The antifungal and antimicrobial activity of glucosinolates and isothiocyanates are also widely known. For example phenethyl isothiocyanate and 4-methylsulfonylbutyl isothiocyanate have been reported to inhibit the growth of \textit{Staphylococcus aureus} and \textit{Penicillium glaucum}.\textsuperscript{19} Studies have also shown that glucosinolates induce a significant suppression of soil-borne pathogens and inhibit weed seed germination\textsuperscript{20} which suggests a role in agriculture as a green manure.

By far the most widely recognisable aspect of glucosinolates are their breakdown products, which give the sharp, bitter and distinctive taste to foods such as broccoli or mustard. However a regular intake of brassica vegetables, for example as little as 10 g of broccoli per day, has been reported to lower the incidence of lung, prostate and colon cancer.\textsuperscript{21} This appears to be due to the
presence of the glucosinolate, glucoraphanin 14, which is abundant in broccoli, and breaks down to give the isothiocyanate sulforaphane 15 (Scheme 9).

![Chemical structure](image)

Scheme 9: Glucoraphanin 14 hydrolysis to generate sulforaphane 15, a putative chemoprotective 22,23

Greater health benefits have been shown to come from the consumption of raw as opposed to cooked vegetables.24 Cooking has a detrimental effect on the levels of myrosinase present and by consequence the level of glucosinolate breakdown products.

The mode by which the glucosinolate isothiocyanates exert their chemoprotective properties appears to be via a signalling pathway, involving nuclear factor kappa b (NFKb) whereby isothiocyanates act as external stimuli. This leads to increased expression of antioxidant and detoxification processes induced by the phase II enzymes glutathione-S-transferase (GST) and NAD(P)H: quinone oxidoreductase 1 (NQO1).24 Increased levels of phase II enzymes then lead to increased protection against other carcinogenic species.

As discussed above, specifer proteins can impede the formation of isothiocyanates in favour of nitriles. Studies have been conducted in order to assess the ecological functions of plant specifer proteins, such as ESP or NSP. Due to the complexity of the glucosinolate-myrosinase system and the difference in volatility and reactivity, it has proven difficult to assess the function of isolated glucosinolate hydrolysis products.25 However, more recent artificial diet studies of the larvae of the generalist pest Spodoptera littoralis have suggested that nitriles do not act as a deterrent to feeding,25 highlighting that there are differences in activity between the breakdown products produced.
1.5 Epithiospecifier protein (ESP)

In the presence of ESP, glucosinolates have been reported to favour nitrile formation upon breakdown. However, upon metabolism of alkenyl glucosinolates the sulfur of the thioglycosidic bond undergoes an intramolecular reaction, to give an unusual thiirane motif 16 which has been verified, by labelling experiments.\textsuperscript{26} The exact mechanism for this thiirane formation is unknown, although it has been hypothesised that its formation progresses via a radical mechanism,\textsuperscript{27} due to the 1:1 ratio of enantiomers formed (Scheme 10),\textsuperscript{28,29} and that it is comparable to the reaction catalysed by isopenicillin-N synthase.\textsuperscript{12} However another study involving the exclusion of oxygen and addition of radical scavengers did not affect the absolute or relative amounts of epithionitrile formed suggests that radicals may not actually be involved.\textsuperscript{12}

\[\text{Scheme 10: Proposed mechanism for ESP-catalysed reaction.}\textsuperscript{27}\]

The mode by which ESP promotes nitrile formation is still unclear and a debate as to whether these proteins are co-factors or true enzymes is ongoing. As yet no other natural or synthetic compound has been reported to serve as a substrate for ESP and the instability of the aglycone intermediate does not allow for rigorous kinetic studies.\textsuperscript{12} Nonetheless, studies performed on an optimal assay of myrosinase combined with ESP by Burow et al.,\textsuperscript{12} failed to observe any ESP activity when the two proteins were spatially separated by dialysis membranes, implying that the two proteins need to be in close proximity. In addition, ESP attached to a resin bound antibody was used to investigate a stable interaction between ESP and myrosinase. However it was found that when ESP was eluted from the resin, myrosinase activity was not detected. The study investigated whether ESP was a co-factor of myrosinase or a separate enzyme, by monitoring the ratio of epithionitrile to isothiocyanate formed from an alkyl glucosinolate. It was found that myrosinase activity was increased by the addition of L-ascorbate, and consequently there was a
decrease in the epithionitrile to isothiocyanate ratio. This contradicts a proposal that ESP is acting as an allosteric co-factor,\textsuperscript{30} therefore it seems likely that ESP is an enzyme but one that rapidly intercepts the unstable intermediate as its substrate (Figure 4).

**Figure 4:** a) Model of the putative allosteric interaction b) without ESP c) and in close proximity for the intervention of the reactive intermediate.

### 1.6 Previous syntheses of glucosinolates

The first glucosinolate synthesis by Ettlinger and Lundeen in 1957 used an ‘electrophilic’ sugar approach.\textsuperscript{4} By this approach, glucosinolate 1 is synthesised via the intermediate thiohydroximate 17 following sulfation and deprotection of the acetyl protecting groups. In turn, 17 is generated by the coupling of 18 with thiohydroxamic acid 19 (Scheme 11).

![Scheme 11: Retrosynthetic analysis of ‘electrophilic’ sugar approach to glucosinolate synthesis.](image)

Until recently, this was the only example of this method which suffered from poor coupling yields of 18 and 19.\textsuperscript{4} In 2011, Rollin \textit{et al.},\textsuperscript{31} revisited this approach and developed a milder technique which improved the low yields by employing a ‘nucleophilic’ sugar. The authors found that it was possible to synthesise aliphatic side chains by lactone ring opening, which can be converted efficiently to bis-O-silylated hydroxamic acids, 20. Upon treatment with triflic anhydride this can progress, \textit{via} a nitrile oxide, to an efficient coupling with thioglucose to obtain a thiohydroximate – the precursor to glucosinolates.
Introduction

(Scheme 12b). This method was successful for a range of substrates as well as for aromatic side chains via the per-silylation of preformed hydroxamic acids.

\[
\begin{array}{c}
\text{A.} & \text{MgCl} & \text{S} & \text{N} & \text{H} & \text{OH} \\
\text{Ph} & \text{NH} & \text{Ph} & \text{S-} & \text{Glucose} \\
\end{array}
\]

\[
\begin{array}{c}
\text{B.} & \text{O} & \text{TBSO} & \text{N} & \text{OTBS} & \text{OH} \\
\text{NH} & \text{K} & \text{TBSO} & \text{N} & \text{S-} & \text{Glucose} \\
\end{array}
\]

**Scheme 12:** A. Ettlinger & Ludeen method. *Reagents and conditions* a) i. CS\(_2\), ii. NH\(_2\)OH \cdot HCl, 0°C, 33%  
b) 18, KOH, 6 h, rt, 47%; B. Rollin method. *Reagents and conditions* a) i. HON\(_3\)Cl, KOH, MeOH ii. TBSCI, imidazole, DMF b) i. T\(_2\)O, Et\(_3\)N, DCM, 0°C ii. 21, Et\(_3\)N, DCM.

The most common method used for the synthesis of glucosinolates is that of Benn\(^{32}\) (Scheme 13) reported over forty years ago. It has been the most widely utilised.\(^{33}\) This robust method constructs the intact glucosinolate 1 from a thiohydroximate 17, which in turn derives from the coupling of tetra-O-acetyl thioglucopyranose 21, a nucleophilic sugar, and an oximyl chloride 22.

\[
\begin{array}{c}
\text{R = aromatic, indole or aliphatic side chains} \\
\end{array}
\]

**Scheme 13:** Retrosynthetic analysis of Benn.\(^{32}\)

The sugar unit 21 can be readily accessed from glucose, an inexpensive and readily available starting material, by a route devised by Cerny *et al.*\(^{34}\) (Scheme 14).

**Scheme 14:** Retrosynthetic analysis of 2,3,4,6-tetra-O-acetyl-1-thio-\(\delta\)-glucopyranose 21.
For fragment 22, there are thee main synthetic approaches reported for the synthesis of glucosinolates (Scheme 15). These are the aldoxime, nitronate or nitroalkene pathways, which are all detailed in the following sections.

**Scheme 15:** Three retrosynthetic routes toward the intermediate oximyl chloride 22.33

### 1.7 The aldoxime pathway for glucosinolate synthesis

Benn’s pioneering work on the synthesis of many natural glucosinolates including; glucoapparin, gluconasturtin, glucoputranjivin, glucosinalbin, glucaubrietin, glucocochlearin32,33,35-41 used the aldoxime pathway. The key step for this method requires the chlorination of an oxime, synthesised from the corresponding aldehyde, using electrophilic chlorinating agents (Scheme 16). Chlorine gas42 and NCS43 are often employed. One advantage of this route is that the starting alcohols, aldehydes or even oximes are commercially available. However there have been reports of low yields40 of thiohydroximate formation and difficulty in purifying the oximyl chlorides, which has led to the development of complementary routes.
1.8 The nitronate pathway towards glucosinolate synthesis

Methodology emerged in 1954 by Copenhaveur whereby a thiohydroximate is formed by using base induced condensation of primary nitroalkanes with thiols (Scheme 17).44

Benn later developed these conditions to explore their application towards glucosinolates.45 The original synthetic studies proved unsuccessful as they required harsh conditions, which were likely to deprotect the thioglucose moiety, and thus proved low yielding. However they observed that there was an intermediate nitronate anion, which could be formed under a range of milder conditions (Scheme 18). This nitronate has proven widely applicable in the area of glucosinolate synthesis.
During the coupling of the two fragments, 24 and 21, formation of the unnatural $E$ isomer, 26, was observed, however this was found to be labile and it could be converted to the natural $Z$ isomer, 25, by the addition of acid (Scheme 18). In more recent studies,\textsuperscript{48} as detailed in Scheme 19, the key stage of the reaction requires nucleophilic chlorination of the nitronate intermediate. Various techniques have been reported to achieve this for glucosinolates including dry HCl\textsuperscript{46} and thionyl or lithium chloride.\textsuperscript{40,47} This pathway has been particularly favoured in the synthesis of glucosinolates, such as sinigrin 27,\textsuperscript{48} which contain alkene functionality in order to circumvent the possibility of alkene halogenation (Scheme 19).

Scheme 18: Benn’s milder conditions.\textsuperscript{45} Reagents and conditions a) NEt$_3$, THF, 56% and 28%.

Scheme 19: An example of the nitronate pathway in the synthesis of sinigrin.\textsuperscript{48} Reagents and conditions a) NaNO$_2$, urea, DMSO; b) NaOEt; c) LiCl-HCl; d) acetylated thioglucose, NEt$_3$; e) py·SO$_3$; f) NH$_3$/MeOH.
1.9 Nitroalkene pathway towards glucosinolate synthesis

In 1994 Kulkarni developed an alternative method for the formation of hydroximyl chlorides \( 29 \) via nitroalkene precursors \( 28 \) (Scheme 20).\(^{49}\)

\[
\begin{align*}
\text{Ar}-\overset{\text{NO}_2}{\longrightarrow} & \quad \overset{\text{a}}{\longrightarrow} \quad \text{Ar}-\overset{\text{NOH}}{\longrightarrow} \\
28 & \quad & 29
\end{align*}
\]

Scheme 20: Kulkarni methodology. Reagents and conditions a) TiCl\(_4\), Et\(_3\)SiH, CH\(_2\)Cl\(_2\), rt, 1 h, 76%.

Realising the potential in this methodology Rollin et al., applied it to the preparation of a range of glucosinolates bearing aryl alkyl and indolymethyl functionalities.\(^{50}\) They found that the approach had the advantage of a shorter reaction pathway than the nitronate method and that it was applicable to a wider range of substrates including indoles, such as \( 30 \), which had previously proven troublesome in glucosinolate syntheses. An example of this method is detailed in Scheme 21.\(^{51}\)

\[
\begin{align*}
\text{O} & \quad \overset{\text{a}}{\longrightarrow} \quad \overset{\text{O}_2\text{N}}{\longrightarrow} & \quad \overset{\text{b}}{\longrightarrow} \quad \overset{\text{HO-}}{\longrightarrow} \\
\text{N} & \quad \overset{\text{c}}{\longrightarrow} \quad \overset{\text{AcO}}{\longrightarrow} & \quad \overset{\text{d}}{\longrightarrow} \quad \overset{\text{AcO}}{\longrightarrow} & \quad \overset{\text{e}}{\longrightarrow} \quad \overset{\text{SO}_3\text{NaAc}}{\longrightarrow} \\
\text{H} & \quad \overset{\text{N}}{\longrightarrow} & \quad \overset{\text{N}}{\longrightarrow} & \quad \overset{\text{N}}{\longrightarrow}
\end{align*}
\]

Scheme 21: An example of the nitroalkene route in the synthesis of glucobrassicin.\(^{51}\) Reagents and conditions a) i. CH\(_3\)NO\(_2\), AcONH\(_4\); ii) Ac\(_2\)O, DMAP; b) TiCl\(_4\), Et\(_3\)SiH, CH\(_2\)Cl\(_2\); c) 21, NEt\(_3\); d) py·SO\(_3\), pyridine; e) MeOK, MeOH.

1.10 The synthesis of novel and unnatural glucosinolates

The synthesis of novel and unnatural glucosinolates play a vital role in understanding the many biological aspects of these natural products. For example Rollin et al., synthesised sugar motifs, deoxygenated at various
positions (31-34, Figure 5), and screened them against natural glucotropaeolin 35 and glucobrassicin 36 in order to probe the activity of myrosinase. During this study it was found the C-2 deoxyglucose (31, Figure 5) showed a significant decrease in inhibition. This was suggested to be due to two factors; i) the absence of a C-2 hydroxyl in 31 disturbs favourable alignment of the sugar moiety in the binding site. This effect was also apparent for the C-6 deoxygenated glucose analogue 34; and ii) C-2 being close to the glycoside bond will impact on the polarisation required for inducing nucleophilic attack at the anomeric carbon.

2-Deoxy-2-fluoroglucotropaeolin 37 (Figure 6) was synthesised and proved successful in inhibiting myrosinase, however this substrate was found to undergo rapid Lossen rearrangement. Therefore the sulfide functionality was removed in order to prepare a non-hydrolysable substrate, C-glucotropaeolin, 38 (Figure 6). It was anticipated that in doing so, information regarding substrate positioning, conformation and binding in the active site might be obtained with the use of X-ray data. Unfortunately this substrate did not bind to the enzyme, suggesting that the sulfide is essential for binding to myrosinase. This led to further developments by Rollin and co-workers in the synthesis of the first carbo-glucosinolate, 39 (Figure 6), whereby the endo-oxygen was replaced by a methylene group. This analogue was found to be an inhibitor of myrosinase.

Figure 5: Deoxygenated sugar analogues synthesised by Rollin et al. 52
Figure 6: Modified structures of glucopaeolin; 2-deoxy-2-fluoroglucotropaeolin 37, C-glucotropaeolin 38, carbo-glucotropaeolin 39.53-56

To date the only structural change tolerated at the anomeric centre and to undergo enzymatic hydrolysis by myrosinase has been that of selenoglucosinolate 40 in Figure 7.57 Breakdown products were analysed by ‘trapping’ the volatile breakdown products in reactions with piperidine where adducts such as 41 were observed.

Figure 7: Selenoglucosinolate 40 and trapped adduct 41.

Finally, a ‘pseudo-glucosinolate’ 42 has also been synthesised by Rollin and co-workers58 that possesses an α-thioglycosidic linkage instead of the naturally occurring β-thioglycoside bond.
1.11 The synthesis of isotopically labelled glucosinolates

Isotopically labelled glucosinolates have provided vital information regarding the biosynthesis (Section 1.2) and metabolism of glucosinolates.\textsuperscript{59} For example, plant metabolism has been probed in feeding experiments with isotopically labelled precursors. In this way the labelled compounds are introduced into the living plant and their metabolites can then be traced, either by following the radioactivity by scintillation counting of purified extracts or with stable isotopes, or by analytical methods such as LC-MS or NMR spectroscopy. For example in 1977 Luthy and Benn used 2-[1-\textsuperscript{14}C]-propenyl glucosinolate to demonstrate the conversion to 3-thiocyanato [3-\textsuperscript{14}C]-propenyl glucosinolate where the aglycone \textbf{43} is believed to undergo a rearrangement as illustrated in Scheme 22.\textsuperscript{60}

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\includegraphics[width=0.4\textwidth]{scheme22.png}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 22:} S\textsubscript{N}2\textsuperscript{'} rearrangement of sinigrin aglycone \textbf{43}.

More recently Rossiter \textit{et al.},\textsuperscript{61} used (E)-2-[2,3-\textsuperscript{2}H\textsubscript{2}]-propenyl glucosinolate to study the enzymatic hydrolysis of sinigrin. Sinigrin \textbf{27}, is a widely documented\textsuperscript{62} alkene glucosinolate isolated from broccoli and Brussel sprouts. During this study Rossiter \textit{et al.},\textsuperscript{61} were able to confirm the rearrangement of the thiohydroximate moiety (Scheme 22) and demonstrated by \textsuperscript{2}H-NMR that the deuterium atoms were retained and emerged at the double bond terminus of \textbf{44} in an S\textsubscript{N}2\textsuperscript{'} type rearrangement.

Isotopically labelled glucosinolates have also been used to answer questions regarding metabolism of glucosinolates in humans. For example, the chemoprotective properties of glucosinolate breakdown products such as sulforaphane, are well documented\textsuperscript{63} and the issue of human exposure is complex. As previously discussed (Section 1.3) myrosinase levels, affected by processes such as cooking or chewing, will in turn affect the level of isothiocyanates produced and therefore have an effect on the level of chemoprotective activity. In order to gain an insight into human exposure levels
to isothiocyanates, stable isotope enriched glucosinolates have been synthesised to search for new biomarkers and to be used as internal standards for LC-MS.\(^{64}\)

Previous work within the Botting group has reported the synthesis of [phenyl-\(^2\)H\(_5\)]-gluconasturtiin 45 and its metabolites (Figure 8).\(^{65}\) In order to search for new biomarkers a study was performed whereby labelled and unlabelled compounds were fed to rats and the isotopically enriched products were used to identify metabolites by a difference of 5 amu using LC-MS. Additionally these isotopically labelled compounds have also been employed as internal LC-MS references compounds in various studies, including a study on the analysis of blood plasma following dietary studies in order to quantify glucosinolate exposure.\(^{66}\)

1.12 Aims of research

This programme of research set out to develop new methods for the synthesis of novel and natural glucosinolates by developing improved methodologies. A key area to be addressed was the formation of the thiohydroximate bond, a current synthetic hurdle, which is time consuming and generally gives products in low yield (Scheme 23).\(^{40}\)

\[\text{Thiohydroximate 17 assembly by coupling of thioglucose 21 and oximyl chloride 22.}\]
Development of new methodology such as side chain functional group conversions was of particular interest, as this would introduce improved techniques in the area of introducing isotope labels into glucosinolates. For example, the establishment of chemical transformations that could be successfully performed at a late stage in glucosinolate assembly, using commercially available isotopically labelled starting materials, would allow access to desirable labelled glucosinolates via less expensive and more efficient methods (Scheme 24).

Scheme 24: Schematic of novel approach to introduce labelled side chains from a common intermediate.

A successful programme would also allow the synthesis of novel glucosinolates, which could then be used to explore biological questions, such as the ESP mechanism (Section 1.5). Therefore at the outset it was important to establish a method to allow the synthesis of glucosinolates, containing alkene side chains. In addition synthetic challenges, such as glucosinolate-thiohydroximate protections, were explored to allow for intact-glucosinolate chemistry to be fully investigated.

Strategies regarding purification and handling of glucosinolates need to be considered, e.g. during the sulfation step. Thus another objective explored alternative counter ions for sulfation, particularly cyclohexylammonium, to improve stability and processability (Figure 9).

Figure 9: Cyclohexylammonium is an example of an organic counter ion for glucosinolate salts.
An alternative approach for simplifying glucosinolate preparation and purification, involved exploring a solid phase synthesis of glucosinolates (Scheme 25). This offers particular benefit at the sulfation and final deprotection stages where the products are often difficult to handle. In this programme a solution phase model study with a resin mimetic was explored as a prelude to attempting an on resin synthesis. Solid phase synthesis of glucosinolates has never previously been reported.

Scheme 25: Proposed method for the solid phase synthesis of glucosinolates.
Optimising glucosinolate synthesis

2.1 Introduction and background

In order to improve on previous total syntheses of glucosinolates, it was necessary to establish a synthesis of a representative example of the glucosinolate family in the laboratory. To this end a total synthesis of but-3-enyl glucosinolate 46 was planned.

A number of factors make but-3-enyl glucosinolate an interesting target. It had previously been prepared in low yields and the efficiency of the synthesis remained unsatisfactory.\textsuperscript{67} Secondly it is known to be a substrate for ESP breakdown. Access to the natural product would allow the role of ESP to be addressed in more depth (Section 1.5). Finally, the alkene functionality should enable further chemistry to be developed on intact glucosinolates in order to prepare novel entities (Section 1.12) a strategy that has been relatively unexplored to date.
2.2 Results and Discussion

The approach taken explored retrosynthetic disconnections as previously described by Benn\textsuperscript{32} (Scheme 26). This involved the coupling of β-thioglucose \textsuperscript{21} and the oximyl chloride \textsuperscript{48}. Following this it was envisaged that thiohydroximate \textsuperscript{47} would be converted to the glucosinolate \textsuperscript{46} by O-sulfation and removal of the acetate protecting groups.

![Scheme 26: Retrosynthesis of 46 via the Benn approach.\textsuperscript{32}](image)

2.3 Synthesis of β-thioglucose 21

The synthesis of β-thioglucose followed the route developed by Cerny \textit{et al.}\textsuperscript{34} Thus starting from D-glucose a one-pot acetylation using HBr (45\% in acetic acid) and acetic anhydride gave rise to the peracetylated glucose \textsuperscript{49}. This intermediate was not isolated, instead addition of a further 3.3 equivalents of HBr (45\% in acetic acid) promoted C-1 bromination. The product was secured by extraction into ether and then recrystallisation from diethyl ether. This provided \textsuperscript{18} in an excellent yield (85\%) and the reaction was carried out on a 50 g scale.

![Scheme 27: Reagents and conditions a) HBr (45\% in acetic acid), acetic anhydride, 24 h, 85\%.](image)

Although the C-2 acetate group is likely to be involved in neighbouring group participation giving rise to the β-anomer, it has been observed that the β-
bromide will equilibrate under the reaction conditions to give the more thermodynamically stable $\alpha$-anomer 18 (Scheme 28).

![Scheme 28: The proposed mechanism for bromination.](image)

The $\alpha$-anomer is thermodynamically more favoured than the $\beta$-anomer due to anomeric stabilisation. This stereoelectronic effect is a result of the lone pair of the pyranose oxygen being able to donate into the antibonding orbital ($\sigma^*$) of the C-Br bond. This is only possible due to the overlap of the two orbitals and is not achievable in the $\beta$-anomer where the orbitals are essentially at right angles to each other (Figure 10a). Also with the bromide in the equatorial position an additional electrostatic repulsion occurs between the bromine and the endo oxygen, which is relaxed with the bromide in the axial position (Figure 10b) thus disfavouring the $\beta$-anomer.

![Figure 10: a) Anomeric effect comparing the $\alpha$-anomer and $\beta$-anomer. b) Electrostatic repulsion comparing the $\alpha$-anomer and $\beta$-anomer.](image)

Introduction of the sulfur was then required. Displacement of the bromide of 18 was accomplished with thiourea, in an S$_{\text{N}2}$-type reaction to give 50. The resulting salt (50) was purified via recrystallisation from acetone and was recovered in good yield (71%). In contrast to 18, the anomeric stereochemistry of intermediate 50 favoured the $\beta$-anomer, although coupling constants could
not be measured at this point to assign stereochemistry, as the H-1 signal overlapped with those from H-2 and H-4.

![Chemical structure](image)

**Scheme 29**: Reagents and conditions a) Thiourea, acetone, reflux, 15 mins, 71%; b) sodium metabisulfite, DCM: water, reflux, 20 mins, 76%.

Finally, in order to obtain β-thioglucose 21, the hydrolysis of thiourea 50 was performed (Scheme 29). Treatment with sodium metabisulfite in a biphasic system at reflux provided the product in an excellent yield (76%). The product 21 was crystallised from methanol and the β-stereochemistry was confirmed by a distinctive doublet at 4.49 ppm and with a $^3J$ coupling constant of 9.1 Hz consistent with H-1 being in the axial position.

### 2.4 Synthesis of the oximyl chloride fragment

The discussion in Chapter 1 outlined that three independent routes have been explored for the formation of oximyl chlorides; a nitroalkane, nitroalkene or an aldoxime approach. The nitroalkene route used for the synthesis of sinigrin 27 was first explored.

![Chemical structure](image)
2.4.1 Synthesis of oximyl chloride via Nitroalkene route

The desired nitroalkene 54 was approached in two different ways (Scheme 30). In each case the integrity of the material was confirmed by $^1$H and $^{13}$C NMR and was consistent for both methods.

The first used penten-1-ol 51 that was brominated, used carbon tetrabromide and triphenylphosphine to generate intermediate 53, a reaction which proceeds in a moderate yield (48%). The second method developed by Meyer et al.,$^{69}$ started from 1,5-dibromopentane 52 to give 5-bromopentene 53 in an E2 elimination reaction. In situ distillation allowed recovery of the desired product in good yield (73%) and high purity. This method proved the more practical for greater accessibility to the desired bromide on a gram scale.

Nitration using sodium nitrite was favoured over the use of silver nitrite due to its lower cost and lower toxicity. The resultant nitro compound 54 was generated in a moderate yield (37%). Perhaps the low yield is due to the ambident nature of the nitrite anion nucleophile, which can either react via nitrogen or oxygen, although none of the O-alkylated product 55 was observed (Scheme 31).

It was not possible to improve the yield of the nitration step and thus access to significant quantities for further investigation was particularly limited. Therefore the alternative aldoxime pathway was explored.
2.4.2 Synthesis of oximyl chloride via aldoxime pathway

The availability of straight chain alkenes with an aldehyde functionality is limited and the few compounds that are of this class, available from commercial suppliers, are expensive. Therefore it was envisaged that oxidation of the more readily available alcohols would prove to be more viable (Scheme 32).

![Scheme 32: Reagents and conditions a) IBX, DCM, 50 °C, 48 h, quant; b) NH₂OH·HCl, NaOAc, MeCN : H₂O, 16 h, 73%.]

There are of course numerous methods available for the oxidation of an alcohol to an aldehyde. In this case methods involving TEMPO were first explored. However these methods proved largely unsuccessful. IBX, the precursor to the well-known Dess-Martin periodinane (DMP) was then explored. This gave a quantitative conversion of the alcohol to the aldehyde, showing the distinctive formyl proton signal at 9.72 ppm in the ¹H NMR spectrum. An added advantage of this method is the low solubility of IBX and its reduced by-product (Figure 11) in organic solvents, allowing the reagent residue to be filtered off once the reaction was complete. Further purification of the aldehyde was not required after work up.

![Figure 11: Structures of TEMPO and IBX.]

Although IBX is relatively easy to synthesise, large amounts were required for scale up. Batch syntheses of 50 g were undertaken for IBX but this proved time consuming and costly for the amount of reagent required to secure the aldehyde on a desirable scale. This was a bottle-neck particular as an early stage step and thus an alternative method was sought.

It was found that a more cost-effective Claisen rearrangement, could be used to convert commercially available allyl vinyl ether 58 to the desired aldehyde 56 in
quantitative yields by heating in a sealed tube (Scheme 33). This also had the benefit that the product 56 did not require further purification after the reaction.

\[
\text{Scheme 33: Claisen rearrangement of allyl vinyl ether 58. Reagents and conditions a) Sealed tube, 150 °C, 16 h, quant.}
\]

With aldehyde 56 in hand, conversion to the desired oxime intermediate 57 was then required. This was achieved using hydroxylamine hydrochloride in a biphasic solution of acetonitrile and water, and generated the desired product in high yield (73%). The oxime was isolated as a 1:1 mixture of E:Z isomers, readily identifiable by the two signals of the oxime protons, in the \(^1\)H-NMR spectrum at 7.43 and 6.72 ppm, as well as two oxime resonances in the \(^{13}\)C-NMR spectrum at 149.8 and 149.4 ppm. A separation of the geometric isomers was not attempted as the E and Z oximes both proceed via the common intermediate 59 during the coupling reaction (Scheme 34).

\[
\text{Scheme 34: E and Z oximes progress via the nitrile oxide 59 during oximyl chloride formation.}
\]

For the conversion of oxime 57 to the oximyl chloride 48 a variety of methods have been used. These have included the use of chlorine gas\(^{42}\) or an alternative ‘Cl’ source such as NCS.\(^{43}\) A previous synthesis of isotopically labelled 4-butenyl glucosinolate by Rossiter\(^{74}\) used NCS and achieved a yield of 19% for the thiohydroximate product. As this was a key stage in our synthesis of glucosinolates, we sought to develop an alternative and more reliable protocol to effect this transformation.

After repeating Rossiter’s\(^{74}\) method (Scheme 35) it was observed that the intermediate oximyl chloride was low yielding and proved difficult to purify and then to characterise. It became apparent that, due to the instability of the oximyl

---

*Note: The diagrams and chemical structures are not transcribed in the plain text representation.*
chloride, a ‘one-pot’ coupled synthesis from oxime 57 to thiohydroximate 47 would be advantageous, to avoid handling of the sensitive oximyl chloride 48.

Scheme 35: Chlorination was attempted using NCS.

2.5 An improved one-pot coupling protocol

There is evidence that the coupling of the oximyl chloride and β-thioglucose occurs via a nitrile oxide intermediate 59 (Scheme 34). It was noted that a mild method for the formation of nitrile oxides had been reported by Engberts et al., in 1998, although its use in glucosinolate synthesis had not been explored. This method simply used sodium hypochlorite (NaOCl) treatment to efficiently oxidise the oxime to a nitrile oxide for cycloaddition to the alkene. The reaction with styrene is illustrated in Scheme 36.

Scheme 36: Engberts formation of nitrile oxides using sodium hypochlorite.

The aldoxime route is not generally used in the synthesis of glucosinolates bearing alkene side chains due to the possibility of alkene chlorination. Although NaOCl is an electrophilic source of chlorine it was however found in a preliminary study with oxime 57, that chlorination of the terminal alkene did not occur (Scheme 37) and the process might be suitable in the development of a 'one-pot' procedure.

Scheme 37: Preliminary analytical study: Oxime 57 treated with a NaOCl : DCM biphasic solution. Mass spectra of product. A m/z (ES⁺) of 134 was observed consistent with production of 48.
An attempt was made to carry out a one-pot process however a strongly exothermic reaction occurred and only the dimeric sugar 60 was isolated as evidenced by mass spectrometry. Such a structure is consistent with a disulfide bond formation although this was never securely identified.

In order to investigate and overcome oxidative dimerisation, it was necessary to prepare the oximyl chloride 48 independently and then add the resulting solution directly to the $\beta$-thioglucose 21. This was achieved by simply shaking a solution of oxime in DCM with commercially available laboratory NaOCl in a separating funnel. The use of DCM as the solvent is advantageous as a range of oximes are readily soluble and conveniently, it is denser than the NaOCl solution and the lower layer can be added directly to the $\beta$-thioglucose 21 coupling reaction. Addition of the NaOCl to a solution with oxime and DCM results in a strong blue colour indicating the formation of a nitroso intermediate, most probably 61 (Scheme 38) as well as transient dimeric intermediate 62. The strong blue absorbance at 660 nm is most probably due to a N=O, $\pi^*\pi^*$,electron transition.46

Scheme 38: Proposed mechanism for thiohydroximate formation.76
After the blue colour had dissipated the DCM fraction containing the oximyl chloride was then added directly to a stirred solution of $\beta$-thioglucose 21. It was not necessary to dry or purify the oximyl chloride in any way before addition. Also during optimisation it was noted that the order of addition was key to a successful outcome for the reaction. It was observed that addition of triethylamine to the $\beta$-thioglucose component before the oximyl chloride led exclusively to the formation of the disulfide dimeric sugar 60. Also it was presumed that early addition of base to the oximyl chloride would generated the nitrile oxide 59 which in turn could lead to unwanted by-products. Such by-products could be 1,3-dipolar cycloaddition adducts such as furoxans 63, (Scheme 39a) or tricyclic adducts such as 64, (Scheme 39b) although, none of these products were isolated.

For the most efficient outcome the sugar 21 and oximyl chloride 48 are combined before the addition of triethylamine. Also during optimisation it was observed that both the desired adduct and $\beta$-thioglucose co-eluted on flash chromatography making it difficult to isolate the product in high purity. This issue was addressed by lowering the amount of $\beta$-thioglucose to 0.6 equivalents, which improved the conversion with no unreacted starting material being observed by $^1$H-NMR spectrum. Finally, increased yields were obtained when an excess of both base (3 eq) and sodium hypochlorite (5 eq) were used in the respective steps.

Scheme 39: Origin of putative dimeric products.
Following the formation of the intermediate nitrile oxide, the nucleophile, in this case β-thioglucose, attacks the nitrile oxide to generate the desired β-thiohydroximate bond (Scheme 40). This gave rise to the Z-isomer exclusively, consistent with the previous literature of glucosinolate coupling.\(^{32}\) The formation of the Z-thiohydroximate is stereoelectronically favoured as the incoming nucleophile and the nitrogen lone pair will result in an antiperiplanar arrangement analogous to the atomic rearrangement that takes place during 1,3-dipolar cycloadditions of nitrile oxides. A debate regarding the mechanism of nucleophillic addition to nitrile oxides has led to conflicting computational studies regarding whether the reaction proceeds via a stepwise or concerted mechanism. The first report was by Sharma and Aggarwal,\(^ {76}\) which showed the progression via a stepwise addition based on semiempirical MNDO calculations. By this mechanism the reaction is reported to progress via two discrete transition states, TS1 and TS2, as shown in Scheme 41.\(^ {77}\)

\[
\begin{align*}
R-\overset{\text{N}^+}{\overset{\text{O}}{\text{C}}} & \rightarrow [ \overset{\text{R}}{\overset{\text{C}}{\overset{\text{N}^+}{\overset{\text{O}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\text{H}}}}}}}}} ] \rightarrow \overset{\text{R}}{\overset{\text{C}}{\overset{\text{N}^+}{\overset{\text{O}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\text{H}}}}}}}}} \\
\text{TS1} & \\
\overset{\text{R}}{\overset{\text{C}}{\overset{\text{N}^+}{\overset{\text{O}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\text{H}}}}}}}}} & \rightarrow \overset{\text{R}}{\overset{\text{C}}{\overset{\text{N}^+}{\overset{\text{O}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\text{H}}}}}}}}} \rightarrow \overset{\text{R}}{\overset{\text{C}}{\overset{\text{N}^+}{\overset{\text{O}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{O}}{\text{H}}}}}}}}}
\end{align*}
\]

**Scheme 41:** Proposed non-concerted mechanism of Sharma and Aggarwal\(^ {77}\) deduced by semiempirical MNDO calculations.
By contrast, Hegarty et al. using \textit{ab initio} calculations\textsuperscript{78} suggest an asynchronous concerted process, whereby proton transfer to the oxygen begins at the transition state and progresses without any further energy barrier, (Scheme 42). Despite the dispute in the status of the transitions state both methods are in agreement that $Z$-selectivity will be favoured as the stereochemical outcome of thiohydroximate formation.

\[
\begin{align*}
R\text{-C}&&\text{N}&&\text{O}^+ \quad \text{Nu}&&\text{H} \\
+ \\
\text{Nu}&&\text{H} \\
\rightarrow \\
\left[ \begin{array}{c} \\
R \\
\text{Nu} \\
\rightarrow \text{H} \\
\text{O} \\
\end{array} \right] \\
\rightarrow \\
R&&\text{N}&&\text{OH} \\
\text{Nu} \\
\end{align*}
\]

\textbf{Scheme 42: Proposed concerted mechanism \textit{via} ab initio calculations\textsuperscript{78}}

2D NMR spectroscopy was used to confirm the formation of a new C-S bond, showing a HMBC correlations between H-1 (5.01 ppm) in the $^1$H-NMR spectrum and thiohydroximate carbon (156.9 ppm) in the $^{13}$C-NMR spectrum.

In addition it was also found that the product crystallised and a suitable crystal was submitted for X-ray crystallographic analysis (Figure 12). The resulting structure confirmed the $Z$-stereochemistry of the thiohydroximate moiety with a S-C=N-O torsion angle of -1.0°.
2.6 Viability of coupling on other analogues

The applicability of this new thiohydroximate bond formation was explored with a variety of substrates in order to assess its generality in glucosinolate synthesis. This new procedure proved to be highly efficient with all of the oximes that were studied, giving improved yields over previous two-step methods as detailed in Table 1. Entry 1, this gave a 76% yield and is the precursor to gluconasturtiin, which had been made before in the Botting laboratory in a 61% yield, over two steps via the standard method involving isolation of the oximyl chloride. Similarly it was also prepared by McLeod in 69% yield. The oxime of octanal (Entry 2) was coupled to give heptyl glucosinolate, previously preformed in 61% yield over two steps. This could be improved to a 78% isolated yield. Good yields were also obtained with both alkenyl and alkynyl side chains (Entries 3 and 4). In previous syntheses of glucosinolates, with unsaturated side chains, the oximyl chlorides were prepared via nucleophilic chlorination due as the presence of the alkene in the side chain which was anticipated to be problematic for electrophillic methods. However, it is striking that but-4-enyl oxime could be obtained in 63% yield (Entry 3), compared to only 13% with the nitroalkane protocol over two steps.
Entries 5 and 6 are aromatic oxime components for novel glucosinolate structures. The final oxime (Entry 7) gives a $\beta$-thiohydroximate with a TBS-protected alcohol in the side chain. This was found to be stable under the reaction conditions, and coupled in an excellent yield.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxime</th>
<th>Product</th>
<th>Product number</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Oxime" /></td>
<td><img src="image2" alt="Product" /></td>
<td>65</td>
<td>76%</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3" alt="Oxime" /></td>
<td><img src="image4" alt="Product" /></td>
<td>66</td>
<td>78%</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Oxime" /></td>
<td><img src="image6" alt="Product" /></td>
<td>47</td>
<td>63%</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7" alt="Oxime" /></td>
<td><img src="image8" alt="Product" /></td>
<td>67</td>
<td>77%</td>
</tr>
<tr>
<td>5</td>
<td><img src="image9" alt="Oxime" /></td>
<td><img src="image10" alt="Product" /></td>
<td>68</td>
<td>92%</td>
</tr>
<tr>
<td>6</td>
<td><img src="image11" alt="Oxime" /></td>
<td><img src="image12" alt="Product" /></td>
<td>69</td>
<td>65%</td>
</tr>
<tr>
<td>7</td>
<td><img src="image13" alt="Oxime" /></td>
<td><img src="image14" alt="Product" /></td>
<td>70</td>
<td>83%</td>
</tr>
</tbody>
</table>

*Table 1:* Range of oximes coupled to β thioglucose by NaOCl method. Yields are of purified products.
2.7 Attempted synthesis of indole glucosinolate

One of the most challenging side chains to prepare in the glucosinolate family is that of the indole moiety. In order to test the viability of such substrates in this one-pot oxidative method, a synthesis of 73 using indole-oxime 72 was explored. The oxime 72 was readily prepared from commercially available indole-aldehyde 71, as illustrated in Scheme 43.

![Scheme 43: Reagents and conditions a) NH₂OH·HCl, NaOAc, MeCN : H₂O, 16 h. 52% b) NaOCl, NEt³, DCM.](image)

The coupling reaction failed to yield the desired indole thiohydroximate 73 under our conditions and only a dimeric sugar product was generated as well as unreacted oxime 72. It was thought, due to the recovery of the oxime that poor solubility of 72 may be the cause of the unsuccessful outcome. The reaction was therefore explored using a range of solvents. Although a solvent change from DCM to ethyl acetate increased the solubility of oxime 72 there was no evidence for the formation of the desired indole hydroximate, 73.

2.8.1 Attempted synthesis of N-glycoside glucosinolates

Only a few examples are known of glucosinolates that have alternative heteroatoms to sulfur at the anomeric centre, Section 1.10. It was decided to explore the synthesis of this linkage with nitrogen to generate the aminoxide moiety 74 using the new methodology. In addition to probing the scope of the reaction, these products would clearly be of biological interest as potential substrate analogues to test their ability to undergo enzymatic hydrolysis with myrosinase.
In order to generate the amidoxime analogues of glucosinolate 77 a suitable amino sugar 75 was required which could be coupled with 3-phenylpropiopeoxime 76 (Scheme 44). The amino sugar 75 has been prepared previously from bromide 18, an intermediate used for the synthesis of β-thioglucose 21.

**Scheme 44:** Proposed synthesis of amidoxime analogue of glucosinolates.

The requisite amino sugar 75 was prepared by heating 18 with sodium azide in a sealed tube in acetone and water (Scheme 45). The desired azide 79 was generated in good (69%) yield. The β-anomer was formed exclusively as indicated by a coupling constant of 9.0 Hz at 4.68 ppm in the ¹H spectrum. This suggests a process which progresses via neighbouring group participation involving the C-2 acetate moiety. The next step in the process involved reducing the azide via a Staudinger reaction. Such a reduction on 79 was however inconclusive. Although the mass spectrum indicated an ion of m/z 348 (ES⁺), consistent with the desired product, it was not possible to purify.
Therefore a hydrogenation, using the H-Cube apparatus was selected for direct reduction of the azide using a 10% palladium on carbon cartridge (10% Pd/C).

\[
\begin{align*}
\text{O} & \quad \text{Ac} \quad \text{O} \\
\text{Ac} & \quad \text{O} \\
\text{Br} & \quad \text{N}_3 \\
\end{align*}
\]

18

\[
\begin{align*}
\text{O} & \quad \text{Ac} \quad \text{O} \\
\text{Ac} & \quad \text{O} \\
\text{N}_3 & \quad \text{O} \\
\end{align*}
\]

79

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

75

**Scheme 45:** *Reagents and conditions* a) NaN₃, sealed tube, 16 h, 69%; b) H-Cube, 10% Pd/C 30 min, quant.

The H-Cube has the benefit of performing the hydrogenation under flow conditions and the active palladium catalyst is contained in a pre-packed cartridge. The equipment eliminates the requirement for handling flammable powdered palladium on carbon (Figure 13).

\[
\begin{align*}
\text{A} & \quad \text{A solution of the reactants are pumped into H-Cube with a continuous flow.} \\
\text{B} & \quad \text{Hydrogen gas is produced } \textit{in situ} \text{ from the electrolysis of water.} \\
\text{C} & \quad \text{The catalyst is contained within a pre-packed cartridge within the reaction chamber. The pressure and temperature of the reaction chamber can be altered as required.} \\
\text{D} & \quad \text{Hydrogenated product is pumped through the system to an awaiting collection vessel.}
\end{align*}
\]

**Figure 13:** Schematic of H-Cube equipment.

The method proved successful and allowed reduction of the azide to the amine to take place in a quantitative yield in a 30 minute run with no need for purification.
With this amino sugar 75 in hand attempts were made to couple with 3-phenylpropional oxime 76 using the method described in Section 2.5. However the reaction did not however go to completion as determined by the $^1$H NMR spectra. The $^1$H NMR of the product (Figure 14a) showed the starting amino sugar as well as a second sugar derivative (<10%). These products co-elute by flash chromatography and it was not possible to separate them for characterisation. In order to confirm the presence of the coupled amine, a D$_2$O shake was performed. This resulted in the disappearance of the NH signal (6.20 ppm) in the $^1$H-NMR spectrum as well as a change from a doublet of doublets to a doublet at 4.62 ppm with a $^3$J coupling of 9.0 Hz, for the H-1 proton (Figure 14).
A sample of the reaction mixture was submitted to electrospray mass spectrometry and a \( m/z \) (ES\(^+\)) 517 [M+Na]\(^+\), consistent with the desired product, as well as \( m/z \) (ES\(^+\)) 348 [M+H]\(^+\) for unreacted \( 75 \) were observed.

In an attempt to improve upon the preliminary result and to drive the reaction to completion a screen of different conditions was undertaken. This involved increasing the equivalents of oxime, NaOCl and base. Also a variety of alternative oximes were screened. In addition to this the reaction was performed at various temperatures. Despite this the reaction could not be pushed towards completion.
2.9 Conclusions

In summary a new coupling method for β-thiohydroximate bond formation has been developed for the glucosinolate family of natural products using inexpensive and readily available NaOCl as the chlorinating agent. The method exploited a known oxidation of oximes but used this activation protocol for glucosinolate coupling. It proved very effective over a range of substrates. This simplified one-step procedure is straightforward to carry out, rapid and it gives good to excellent yields over a range of substrates. The method is undoubtedly an improvement on existing protocols, and should be the choice for future synthetic routes to glucosinolates.
Exploring Glucosinolate chemistry

3.1 Introduction and background

Our interest in a combinatorial approach to the synthesis of new glucosinolates is two fold. The first is that little is known in the literature regarding the chemistry of intact glucosinolates. The second is that access to a variety of substrates would be highly desirable in order to evaluate glucosinolates in the presence of the epithiospecifier protein (ESP). As discussed in Section 1.5, ESP acts in concert with the enzyme myrosinase to convert glucosinolates, containing terminal alkenes, to thiirane breakdown products (Scheme 47).

Scheme 47: Breakdown pathways of glucosinolates.

It is proposed that a Wittig reaction could be used to introduce a range of alternative alkene side chains from aldehydes (Scheme 48). However in order to achieve this, a novel glucosinolate, one that contains an aldehyde side chain, such as 81, would be required.
Scheme 48: Proposed synthetic of novel alkene containing glucosinolates 80 via Wittig olefination of aldehyde 81.

In addition to Wittig chemistry, an aldehyde would also be a useful synthetic intermediate to allow access to a variety of novel glucosinolates analogues, for example, amines of type 82 (Scheme 49). As previously outlined in Section 1.12 advancing the area of intact glucosinolate chemistry would open up the possibility of preparing libraries of novel glucosinolates from a common intermediate, such as 81. In particular it is proposed that this would offer an alternative more cost effective method for achieving isotopically labelled glucosinolates.

Scheme 49: Proposed synthetic pathway towards amine side chain glucosinolate 82 from 81.

Two complementary routes were envisaged that offer potential access to the desired aldehyde 81, as illustrated in Scheme 50. The first route involved the oxidation of the terminal alcohol side chain in 83 and the second would utilise oxidative cleavage of a terminal alkene 84. Common to both routes is the requirement for oxime protection, a previously unexplored area in glucosinolate chemistry.
Scheme 50: Two retrosynthesis approaches to the aldehyde side chain 81.

With respect to a study of ESP a range of substrates would be required in order to probe the stereochemical outcome of the generated thiiranes (section 1.5) and to explore structure activity relationships of the enzymes. To do this constrained ring systems and elongated substrates, such as those illustrated in Figure 15, were envisaged as potential synthetic targets.

Figure 15: Target compounds for mechanism and enzyme probes of ESP.

The reaction catalysed by ESP is reported to follow a radical mechanism (Scheme 51).\(^{29,83,84}\) Therefore, it was rationalised that the incorporation of side chains containing cyclopropane rings could act as mechanistic probes since these are known to ring open on contact with radicals.\(^85\) It was anticipated that rearrangement products could inform upon the ESP breakdown mechanism of glucosinolate, as represented in Scheme 51.

Scheme 51: The proposed breakdown of a cyclopropane side chains of radical intermediates.
3.2 Results and discussion

3.2.1 Alternative synthesis of β-thioglucose 21

β-Thioglucose 21, is required for both approaches as it represents the sugar unit for each of the desired glucosinolates. Previously β-thioglucose 21 has been synthesised using a one-pot method (Scheme 52). However large scale (>50 g) preparation is limited due to the expense and safety issues concerning larger quantities of hydrobromic acid. A relatively large quantity of hydrobromic acid would be required to complete both synthetic pathways, therefore an alternative more practical protocol was sought.

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{HO} & \quad \text{AcO} \\
\text{OH} & \quad \text{AcO} \\
\text{HO} & \quad \text{OAc} \\
\end{align*}
\]

\[\text{D-Glucose} \rightarrow 18\]

Scheme 52: Reagents and conditions a) HBr (45% in acetic acid), 24 h, 85%.

The process was separated into two steps as detailed in Scheme 53. In doing so peracetylated glucose 49 is formed independent of the hydrobromic acid step. The key advantage being that the overall volume of hydrobromic acid required is significantly reduced.

\[
\begin{align*}
\text{HO} & \quad \text{AcO} \\
\text{HO} & \quad \text{OAc} \\
\text{HO} & \quad \text{OAc} \\
\text{OH} & \quad \text{OAc} \\
\end{align*}
\]

\[\text{D-Glucose} \rightarrow 49 \rightarrow 18\]

Scheme 53: Reagents and conditions a) Acetic anhydride, sodium acetate, 4 h 87%; b) 45% HBr in acetic acid, DCM, 3 h, 63%.

Acetylation of D-glucose was explored on a small scale in a number of ways, most simply by treatment with acetic anhydride and pyridine\textsuperscript{86} or acetic anhydride and iodine.\textsuperscript{87} For scale up (>50 g), the former would require the use of large quantities of anhydrous pyridine. Also iodine, which is used as a catalyst, proved to be difficult to remove on a small scale and required column chromatography therefore on a larger scale this would become a more
significant problem. Therefore, a protocol using acetic anhydride and sodium acetate was selected for scale up.\textsuperscript{88}

In the event, a slurry of D-glucose and sodium acetate in acetic anhydride was heated to reflux until the solution was clear, before pouring into an ice-water slurry. It was found that after 3 h stirring, a white solid had precipitated, and was isolated by filtration. The product was then recrystallised from ethanol to generate a white crystalline solid of the peracylated product, 49. \textsuperscript{1}H NMR data indicated this to be a 1:9 ratio of \(\alpha\) and \(\beta\) anomers of 49. It was not necessary to separate the diastereomers at this stage. By this method, 49 could be obtained reliably in batches of 200 g.

Peracetylated 49, was then progressed to 18 via anomic bromination. This was achieved using HBr (45\% in acetic acid). It was found that using an aqueous work up, washing with sodium hydrogen carbonate solution, could purify the crude product 18. Upon concentration of the combined organic fractions a golden oil was generated, which was then recrystallised from diethyl ether to furnish 18 in good yield (63\%). The \(\alpha\)-anomer was formed exclusively. \textsuperscript{1}H-NMR spectroscopy indicated a coupling constant of 4.0 Hz at 4.83 ppm for H-1 indicating a vicinal \textit{gauche} relationship between the C-1 and C-2 hydrogens. This stereochemical preference is due to the instability of the \(\beta\)-anomer that equilibrates to the \(\alpha\)-anomer during the reaction. It was found that the two steps could be performed sequentially allowing the process to be completed in 8 h rather than the 24 h required for the previous one-pot reaction.

![Scheme 54: Reagents and conditions](image)

The glycosyl bromide 18, was then converted to thiourea 50 in high yield (71\% as already discussed in Section 2.3. Finally, \(\beta\)-thioglucose 21 was generated by the hydrolysis of 50 with sodium metabisulfite in a biphasic system to give 21 in excellent yield (76\%) (Scheme 54).
3.3 Routes towards an aldehyde terminated side chain

It was envisaged that β-thioglucose 21 would be coupled to the two respective oximes, via Method 1 and Method 2 (Scheme 55) in order to obtain target aldehyde 81.

Scheme 55: Two proposed pathways to aldehyde 81.

3.4 Method 1: Via a terminal alcohol oxidation

It was envisaged that oximyl chloride 87 could be generated from butane-1,4-diol 86, as illustrated in Scheme 56. This would require a number of functional group transformations. The oxime requires protection of its hydroxyl group and it is necessary that the selected protecting group can be removed orthogonally with respect to the acetate esters of the sugar unit. Another obvious consideration is that following coupling with 21 to generate the thiohydroximate intermediate 88 a further protecting group will be required to enable a Wittig olefination to take place. Thiohydroximate protection had not been attempted in the glucosinolate literature and would need to be explored. In the final stages, this approach would require deprotection of the side chain alcohol and selection of a suitable mild oxidation method to generate the desired aldehyde 81.
3.4.1 Synthesis of oxime fragment 92

Butane-1,4-diol 86 was mono-protected using the classical strategy of tert-butyldimethylsilyl chloride and imidazole (Scheme 57). The silyl protecting group was selected as it can be removed under mild conditions in the presence of a variety of functional groups including acetate esters.

Scheme 56: Proposed synthetic pathway towards intermediate aldehyde 81.

Scheme 57: Reagents and conditions a) TBS-Cl, imidazole, 16 h, 75%.

The inexpensive diol is kept in excess of the tert-butyldimethylsilyl chloride and gave rise to predominately the mono-protected silyl ether. The product could be readily purified by column chromatography.

Two methods were explored for the oxidation of alcohol 89. The first involved a modified Parikh-Doering oxidation using pyridine·sulfur trioxide complex, DMSO and Hünig's base. This furnished the desired aldehyde 83 in excellent yield (83%) (Scheme 58).

Scheme 58: a) DIPEA, py·SO₃, 2 h, DMSO : DCM, 83%.

This reaction progresses firstly by DMSO reacting with pyridine.sulfur trioxide complex to form the reactive intermediate 91, which then undergoes nucleophilic attack from the alcohol oxygen (Scheme 59). Deprotonation of N-
N-diisopropylethylamine generates a sulfur ylide, which in turn triggers an intramolecular 1,4-hydrogen transfer and fragmentation to give the desired aldehyde with dimethyl sulfide as a by-product.

Scheme 59: Proposed mechanism for Parikh-Doering oxidation.

As large quantities (< 10 g) of aldehyde 90 were required an alternative method avoiding the use of toxic pyridine·sulfur trioxide complex was explored; an Anelli oxidation.\(^90\) It was found in practise that this method, which utilised TEMPO and NaOCl, could readily and rapidly generate 90, in high yield (74%).

Scheme 60: Reagents and conditions: a) TEMPO, NaOCl, DCM, NaBr, 20 min, 74%.

With aldehyde 90 in hand, the next step required the conversion to oxime 91. In the event this involved the use of hydroxylamine hydrochloride and sodium acetate. The oxime was isolated as a 1:1 mixture of E:Z isomers that were clearly identifiable by \(^1\)H NMR, with two diagnostic triplets at 7.47 ppm and 6.72 ppm.

Scheme 61: Reagents and conditions: a) NH\(_2\)OH·HCl, NaOAc, MeCN : H\(_2\)O, 8 h, 77%.

This stereochemical mixture was not separated, as the geometric isomers are each converted to a common intermediate during the coupling process, as discussed in Section 2.4.2.
3.4.2 Thiohydroximate formation for Method 1

The two fragments, β-thioglucose 21 and oxime 92, were coupled using the NaOCl methodology described in Chapter 2. This gave the novel thiohydroximate 70 in excellent yield (83%), as illustrated in Scheme 62.

\[ \begin{align*}
\text{Scheme 62: Reagents and conditions a) NaOCl, NEt}_3, \text{DCM, 3 h, 83%}. \\
\end{align*} \]

To allow for a greater range of chemistry to be performed on the intact glucosinolate, the thiohydroximate hydroxyl required protection. To date, very little is known in the literature regarding protections of this type. The glucosinolate systems are known to undergo the Lossen rearrangement upon enzymatic hydrolysis, thus care had to be taken in the selection of a protecting group not to promote such a rearrangement (Scheme 63).

\[ \begin{align*}
\text{Scheme 63: Proposed mechanism for Lossen type rearrangement breakdown of glucosinolates.} \\
\end{align*} \]

The use of 2,2-dimethoxypropane has previously been reported for oximes however it had not been applied to the thiohydroximate functionality found in glucosinolates. In the event, the protection of the thiohydroximate, 70, was achieved in a high yield (91%) with the use of 2,2-dimethoxypropane and a catalytic amount of \( p \)-toluenesulfonylic acid (PTSA).
In order to progress with the synthesis the reaction was scaled up, from 200 mg to 5.0 g. It was observed by $^1$H NMR that breakdown of 70 occurred if a non-linear scale up of solvents were used for the increased quantity of substrate. Following 2D NMR spectroscopy the major breakdown product was identified to be 94. This reaction on a larger scale had to be carried out on a larger volume (16g/litre).

With protected thiohydroximate 92 in hand the silyl protecting group was now removed using tetra-n-butylammonium fluoride (TBAF). It was found that if this reaction was performed at room temperature the exclusive product was 95 but it was generated in low yield (36%). This is consistent with an intermolecular acetate migration promoted by generation of the intermediate alkoxylate anion (Scheme 65).

At lower temperature (0 °C) silyl group deprotection using TBAF generated alcohol 96 in an excellent yield (80%).
Scheme 66: Reagents and conditions a) TBAF, THF, 0 °C, 2 h, 80%.

3.4.3 Oxidation of the alcohol side chain

Oxidation of alcohol 96 to aldehyde 97 was required. As the substrate is decorated with several sensitive functional groups, mild oxidation conditions were explored.

Scheme 67: Oxidation of terminal alcohol 96 to aldehyde 97. See Table 2 for reagents.

A range of mild oxidation methods are available in the literature for such a transformation although on this system many of these proved to be either unsuccessful or low yielding, as summarised in Table 2.

<table>
<thead>
<tr>
<th>Entry</th>
<th>General conditions</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMP</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>DMP/pH 7 buffer</td>
<td>Starting material 96 recovered</td>
</tr>
<tr>
<td>3</td>
<td>DMP/NaHCO₃</td>
<td>Starting material 96 recovered</td>
</tr>
<tr>
<td>4</td>
<td>IBX</td>
<td>Starting material 96 recovered</td>
</tr>
<tr>
<td>5</td>
<td>BAIB/TEMPO/DCM</td>
<td>Aldehyde 97 (22%)</td>
</tr>
<tr>
<td>6</td>
<td>IBX/Tempo</td>
<td>Aldehyde 97 (99%)</td>
</tr>
</tbody>
</table>

Table 2: Summary of results for conditions of 96 to 97 oxidation.
For example the Dess–Martin periodinane, DMP,\textsuperscript{95} (Entry 1) proved to be too acidic and resulted in deprotection of the thiohydroximate moiety to afford \textit{98}.

\[
\begin{align*}
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{S} & \quad \text{OH} \\
\text{OH} & 
\end{align*}
\]

\textit{98}

Attempts were made to generate a neutral system with the use of a buffer at pH 7 (Entry 2) and also a more basic set of conditions (Entry 3). However in both cases this led only to the recovery of starting material \textit{96}.

IBX, the precursor to DMP and a mild oxidising agent, is also known to convert alcohols to aldehydes.\textsuperscript{96} In addition IBX has the advantage of being sparingly soluble in a range of solvents and is readily removed by filtration on work up. Unfortunately treatment of alcohol \textit{96} with IBX (Entry 4) also failed to give aldehyde \textit{97} after 24 h at reflux and only starting material was recovered.

2,2,6,6-Tetramethyl piperidin-1-ylloxy (TEMPO) is known to oxidise alcohols with the use of co-oxidants such as BAIB,\textsuperscript{97} Entry 5. These conditions proved successful on this system and the desired aldehyde was isolated but only in a modest yield (22%).

Encouraged by this reaction (Entry 5), a range of conditions involving TEMPO and hypervalent iodine compounds were screened. It was found that alcohol \textit{96} could be reacted with IBX and a catalytic amount of TEMPO (Entry 6) after 3 h at reflux to access aldehyde \textit{97} in excellent yield (99%) (Scheme 68).

\begin{align*}
\begin{array}{c}
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{S} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{OH} & \\
\end{array} & \xrightarrow{a} & \\
\begin{array}{c}
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{AcO} & \quad \text{AcO} \\
\text{S} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\end{array}
\end{align*}

\textit{96} \quad \textit{97}

\textbf{Scheme 68: Reagents and conditions} \textsuperscript{a} IBX, cat. TEMPO, DCM, 3 h, 99%.
The reaction most probably progresses with the alcohol moiety of the glucosinolate side chain reacting with TEMPO. The resultant adduct 101 can then undergo a 1,5-hydrogen transfer to give the aldehyde and the reduced TEMPO, 100. The active TEMPO 99 can then be regenerated by oxidation with IBX.

To our knowledge the use of IBX as a co-oxidant for TEMPO is a novel oxidation technique, that has not previously been reported in the literature. It is presented as a mild, novel technique for oxidation of alcohols in complex molecular systems.

In summary this synthesis gave the desired aldehyde 97 in eight linear steps. The synthesis required column chromatography in six of the seven steps and was found to be time consuming in generating material. A more expedient route to the aldehyde was explored alongside this method.

### 3.5 Side chain modifications after sulfations on Method 1

Forming the aldehyde 103 at a later stage in the synthesis would reduce the number of independent modifications required to form each glucosinolate in a collection, as summarised in Scheme 70.
With intermediate thiohydroximate 70 in hand, 104 was generated by a sulfation using pyridine•sulfur trioxide complex in good yield (68%) (Scheme 71).

Deprotection of 104 was attempted using TBAF, however this proved to be unsuccessful and the desired primary alcohol could not be isolated. It was proposed that this may be due to the polar nature of 102 that renders it water soluble during work up. In order to prevent this, Dowex-50W resin that can be filtered directly was explored\(^6\) as a deprotection method, however in practise, this also failed to generate the desired alcohol 102.
Attempts to cleave silyl ether were unsuccessful with TBAF and Dowex-50W resin.

The polar nature of this compound as its potassium salt was clearly making the product difficult to isolate and at this stage the approach was abandoned.

### 3.6 Formation of aldehyde via alkene side chain oxidation

Alkene oxidation offered an alternative strategy to 81. Such an approach required the formation of the thiohydroximate intermediate 47 carrying a terminal alkene side chain. This approach would arise from the coupling of 21 and 48 (Scheme 73). It was anticipated that protection of thiohydroximate 47 would enable a wider range of transformations to be performed for structural diversification. The final steps in the synthesis would require the oxidation of the terminal alkene 47 to aldehyde 81. Such an alkene cleavage is a novel strategy on an intact glucosinolate and required detailed investigation.

![Scheme 73: Proposed synthesis of aldehyde 81 via terminal alkene oxidation.](image-url)
3.6.1 Formation of thiohydroximate 47

In order to explore terminal alkene oxidation the coupling of pent-4-enal oxime 57 and β-thioglucose 21 was required (Scheme 74). This synthesis has been described, in detail, in Section 2.5. In practise the reaction was performed in 10 g batches of 52 and proved a reliable method for access to terminal alkene 47.

![Scheme 74: Reagents and conditions](image)

**Scheme 74: Reagents and conditions** a) Sealed tube, 160 °C, 16 h, quant; b) NH₂OH·HCl, NaOAc, MeCN : H₂O, 73%; c) NaOCl, 21, NEt₃, DCM, 63%.

3.6.2 Investigating novel thiohydroximate protections

Following the success of the protection strategy for thiohydroximate 70, the acetal protecting group was selected for thiohydroximate 47 (Scheme 75). It was found that treatment of 47 with dimethoxypropane and PTSA generated 105 in good yields (77%).

![Scheme 75: Reagents and conditions](image)

**Scheme 75: Reagents and conditions** a) Dimethylmethoxy propane, pTSA, DCM, 16 h, 77%.

Other protecting groups were investigated at this stage. Silyl groups were selected to protect the free hydroxyl of 47 and thus 47 was treated with TBSCI and TBDPSCI. The two silyl protections of 47 proved straightforward, as illustrated in Scheme 76 and gave the corresponding protected thiohydroximates, 106 and 107, in excellent yields (92% and 87% respectively).
3.7 Novel oxidation chemistry on intact glucosinolates

With the thiohydroximate protection strategies in place a number of oxidation methods were now explored. General methods for the oxidation of an alkene to an aldehyde are illustrated in Scheme 77. These include functional group interconversions such as hydroboration and then oxidation, ozonolysis or dihydroxylation followed by oxidative fragmentation. To date, these reaction conditions have not been explored on glucosinolate systems and thus they merited further investigation.

Scheme 77: General strategies for the conversion of the terminal alkene to an aldehyde.
3.7.1 Hydroboration oxidation of the terminal alkene side chain.

Hydroboration of 105 will clearly retain all carbons of the side chain and generated the homologated alcohol 108 (Scheme 78). However, this transformation was screened in order to probe the viability of such a transformation on an intact glucosinolate.

![Scheme 78: Proposed hydroboration of alkene side chain glucosinolate.](image)

The method was of particular interest as it had potential to be adapted, via the use of hydroboration-amination, to generate novel amines, such as 109 (Scheme 79).

![Scheme 79: Proposed hydroboration-amination of glucosinolate 105 to amine 109.](image)

In the event the alkene was treated with borane-tetrahydrofuran complex followed by a hydrogen peroxide/sodium hydroxide oxidative work up. Upon work up, the crude \(^1\)H NMR spectroscopy did not indicate any residual alkene functionality. However the acetate protecting groups had been removed as evidenced by a characteristic shift of the sugar protons from 5.50-4.04 ppm, up field, to the region of 4.20-3.50 ppm. This is likely to be due to the sodium hydroxide employed during the oxidative work up. It was concluded that these reaction conditions are too harsh to be performed on intact glucosinolates and other methods were explored.
3.7.2 Studies towards aldehyde via epoxide

Scheme 80: Proposed pathway towards aldehyde via epoxide.

Epoxides can offer an indirect precursor to an aldehyde. This would require three steps. Epoxide could also serve as a versatile intermediate for further functionalisation (Scheme 81).

Scheme 81: Examples of proposed transformations of the epoxide functionality of 110.

The Prilezhaev reaction, utilises m-chloroperoxybenzoic acid (mCPBA), (Figure 16) to form epoxides from electron-rich alkenes.

Figure 16: Structure of mCPBA and by-product m-chlorobenzoic acid.

Reaction of mCPBA with alkene resulted in the loss of the thiohydroximate protecting group. This could be due to the formation of m-chlorobenzoic acid as a by-product, which could clearly promote thiohydroximate deprotection (Scheme 82).
Scheme 82: The proposed mechanism of deprotection of 105.

Dimethyldioxirane (DMDO) 113, has found use as an alternative reagent for epoxidation and it gives rise to acetone as a by-product, Scheme 83.\textsuperscript{102} Thus a suitable preparation of DMDO was explored. It was found that formation of 113, using sodium bicarbonate and Oxone, was time consuming as it rapidly degraded over a 24 h time period even upon storage at -20 °C.

\[ \text{Scheme 83: DMDO synthesis. Reagents and conditions a) NaHCO}_3, \text{H}_2\text{O, 'oxone' 4 h.} \]

Therefore an alternative procedure whereby the DMDO was formed \textit{in situ}\textsuperscript{103} proved more practical. However, epoxide 110 was only formed in low yield (31\%) and attempts to improve the yield were unsuccessful (Scheme 84).

\[ \text{Scheme 84: Reagents and conditions a) Oxone, acetone : water, NaHCO}_3, 16 h, 31\%.} \]

With a small quantity of epoxide 110 available an attempt was made to open the epoxide under acidic conditions to form diol 114 (Scheme 85). Although a range of acids and concentrations were explored, it was found that the glucosinolate decomposed into a multitude of unidentifiable by-products.
Therefore, although diol 114 was not formed, a novel epoxide containing glucosinolate 110 was prepared in this study. This may prove to be a useful intermediate for further investigations in intact glucosinolate chemistry.

### 3.7.3 Studies towards aldehyde 97 via a dihydroxylation pathway

Aldehyde 97 could clearly be formed by the oxidative fragmentation of diol 114. In order to form the desired diol, oxidation of alkene 105 with potassium permanganate, was explored (Scheme 86).

This proved successful and diol 114, could be isolated in a modest yield (45%). The remainder of the material was recovered as a mixture of the starting alkene 105 and the unprotected thiohydroximate alkene 47. Further optimisation of the reaction was explored by varying the equivalents of the reagents used, as well as altering the ratio of solvents. However this did not lead to any further improvement in the yield obtained.

Magnesium sulfate was added to the reaction to precipitate magnesium hydroxide. However this reagent system was still too acidic and resulted in the
loss of the thiohydoximate protecting group. In order to prevent the loss of the protecting group a phosphate buffer (pH 7) was explored. However the buffered reaction failed to yield any of the desired diol 114 and only starting material 105 was recovered.

In order to improve the oxidation an alternative method was sought. Sharpless et al.,\(^{104}\) have reported dihydroxylation of alkenes using ruthenium (III) chloride and sodium metaperiodate. Ruthenium tetroxide 115 the active oxidant, is generated \textit{in situ}, and a co-oxidant is added to recycle the ruthenium and regenerate the active species (Scheme 87).

\[
8 \text{Ru}^{3+} (aq) + 5 \text{IO}_4^- (aq) + 12 \text{H}_2\text{O} \rightarrow 8 \text{RuO}_4 + 5 \text{I}^- (aq) + 24 \text{H}^+ (aq)
\]

\textbf{Scheme 87}: Oxidation of Ru\(^{3+}\) with periodate to form active species 115.

This system was explored and was promising with no alkene remaining as judged by \(^1\)H NMR, although deprotection of the oxime had occurred. This system was then buffered at pH 7 using a phosphate buffer, however this again only led to the recovery of starting material 105.

At this stage it was clear that a change in protecting group strategy was required due to the propensity of 105 to undergo acetyl cleavage. Therefore, the TBS protected thiohydroximate 106 was selected, because the silyl ether (described in Section 3.6.2) offers a less labile protection under the acidic reaction conditions.

Oxidation with RuO\(_4\) did not remove the protecting group but there was an apparent intermolecular migration of an acetate group from the sugar motif to give 116 in low yields (13\%) (Scheme 88). Once again, an attempt was made to buffer the reaction with a phosphate buffer and also to run the reaction at a lower temperature to overcome this migration but in both cases only starting materials were recovered.
Potassium permanganate proved to be the best reagent as diol 117 could be isolated in modest yields (48%) (Scheme 89). Upon optimisation it was found that by altering the order of addition and the ratio of solvents from 2:1 to 3.5:0.5 ethanol:water, the yield could be significantly increased from 48 to 99% (Scheme 89).

Having established access to the vicinal diol 117, oxidative cleavage to the required aldehyde 118 was required (Scheme 90). A range of conditions was explored (Scheme 90) to identify suitable conditions for this oxidative cleavage. These are illustrated in Table 3.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent system</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaIO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>no aldehyde 118</td>
</tr>
<tr>
<td>2</td>
<td>Pb(OAc)&lt;sub&gt;4&lt;/sub&gt;</td>
<td>aldehyde 118 (6 %)</td>
</tr>
<tr>
<td>3</td>
<td>NaOCl</td>
<td>Decomposition of 117</td>
</tr>
<tr>
<td>4</td>
<td>NIS</td>
<td>117 recovered</td>
</tr>
<tr>
<td>5</td>
<td>IBX/DMSO</td>
<td>Aldehyde 118 (4%)</td>
</tr>
<tr>
<td>6</td>
<td>DMP</td>
<td>Aldehyde 118 (trace)</td>
</tr>
</tbody>
</table>

Table 3: Reaction conditions screened for the oxidative cleavage of 117.

The use of sodium metaperiodate is generally the reagent of choice<sup>105</sup> for the oxidative cleavage of 1,2-diols. However on treatment of 117 (Entry 1) with sodium metaperiodate the diol was consumed but did not yield the desired aldehyde 118. Instead the reaction gave a polar product consistent with over-oxidation to carboxylic acid 119 (Scheme 91).

\[ \text{Scheme 91: Proposed over-oxidation of diol 117 to acid 119.} \]

Aldehyde 118 could be isolated in a low yield (6%) with the use of lead tetraacetate<sup>106</sup> (Entry 2), however it was found to degrade quickly even upon storage at -20 °C. The majority of the material from this reaction had decomposed into a range of unidentifiable by-products. Similarly the use of sodium hypochlorite<sup>107</sup> (Entry 3) also led to decomposition of the starting material. N-Iodosuccinimide (NIS)<sup>108</sup> has been reported for glycol bond cleavage by McDonald et al., however in our hands (Entry 4) only starting material was recovered.
Scheme 92: *Reagents and conditions* a) IBX, DCM, 4 h, 4%.

An IBX and DMSO system (Entry 5) had previously been reported by Frigerio et al., for the cleavage of 1,2 diols. This reaction progresses with the IBX reacting with DMSO *in situ* and is detailed in Scheme 93.

Scheme 93: Proposed mechanism for IBX oxidative diol fragmentation.

With the use of IBX and DMSO (Entry 5), it was found that diol 117 was converted to aldehyde 118 in a low yield (4%). Analysis revealed that the majority of the recovered product was unreacted starting material.

Reports by Dess and Martin have shown that the Dess-Martin periodionane DMP, more commonly used for oxidation, can also cleave glycol bonds. The use of DMP on an intact glucosinolate previously led to decomposition (detailed in Section 3.4.3). This was also observed when attempting to cleave diol 111 with DMP. The breakdown of the glucosinolate may be occurring due to the release of acetic acid as the DMP degrades prior to the reaction. Therefore an attempt was made to circumvent this by forming DMP *in situ*. To do this, acetic anhydride was reacted with excess IBX in order to generate DMP before the addition of diol 111 (Scheme 94).
Upon work up the desired aldehyde 118 was isolated, but in a trace amount only, with significant recovery of the starting diol 117.

To conclude, dihydroxylation is shown to occur in good yields to generate novel glucosinolate diol 117, an intermediate that could be used for further research. Limited success however was made in the oxidative cleavage reaction to the aldehyde. It was thought a more direct approach would be necessary, therefore ozonolysis was explored.

### 3.7.4 Synthesis of aldehyde 97 via ozonolysis

Ozonolysis would offer a single step transformation of alkene 105 to furnish aldehyde 97 (Scheme 95).

This is expected to progress first with 1,3 dipolar cycloaddition of the alkene and ozone to obtain the molozonide intermediate 120 (Scheme 96). This is an unstable intermediate that will undergo a retro 1,3 cycloaddition to furnish a aldehyde and a carbonyl oxide. The aldehyde will then adopt the orientation required to allow a further 1,3 cycloaddition to give trioxolane 121 which, on reductive work up with triphenylphosphine or dimethyl sulfide (DMS), should produce the desired aldehyde.
First attempts at ozonolysis proved to be successful but low yielding (10%) when triphenyphosphine was used for the reductive work up. The difficulty was the removal of the triphenylphosphine oxide by-product 122. Also it was not clear how stable aldehyde 97 was and repeated chromatography may have led to degradation and loss of material.

Two alternative reductive work up procedures were employed in order to circumvent the purification issues described. The first explored the use of solid-supported triphenylphosphine, which would allow the by-products to be filtered from the crude reaction mixture. The second used DMS in the work up procedure, which would give rise to dimethyl sulfoxide (DMSO) as an alternative by-product to triphenylphosphine.

The solid phase resin did improve the recovery of the desired aldehyde, however, although the isolated yields were still low (22%). The low level of recovery could not justify the significant cost involved in using this solid phase reagent. Similarly, a change to DMS on work up did make purification easier however the aldehyde was recovered in low conversion (22-26%). Therefore further optimisation of this approach was required.

The low isolated yields may be due to poor side chain stability leading to product breakdown. A large excess of base, typically potassium carbonate, is used in ozonolysis as ozone reacts with DCM to give HCl.\textsuperscript{113} There are concerns too regarding the stability of the glucosinolates (Section 3.4.3) and thus the use of base in ozonolysis maybe contributing towards decomposition.

\textbf{Scheme 96:} Criegees ozonolysis mechanism.\textsuperscript{112}
This led to an investigation of a new ozonolysis system in which no base would be required. An acetone/water system reported by Schiaffo et al.,¹¹⁴ suggested that the reaction would proceed via ozonolysis intermediate 123 reacting with water to form 124, which in turn will break down to generate the desired aldehyde with loss of hydrogen peroxide (Scheme 97). Such a hypothesis suggests that the reaction does not progress via the trioxolane 114, as in the Criegee mechanism, and that there is no requirement for a reductive work up.

\[
\begin{align*}
R\ &= \overset{\text{O}}{R}O\overset{\text{O}}{O}\overset{\text{O}}{O} + R\overset{\text{O}}{O}^{-}H_{2}O \\
&\rightarrow R\overset{\text{O}}{O}^{-}O\overset{\text{O}}{O}H_{2}O \\
&\rightarrow R\overset{\text{O}}{O}^{-}O\overset{\text{O}}{O}H_{2}O_{2}
\end{align*}
\]

Scheme 97: Proposed mechanism for acetone/water ozonolysis.

The reaction was performed at 0 °C and gave the desired aldehyde in low conversion (13%). The major product was β-thioglucose 21, arising from hydrolytic cleavage of the thiohydroximate bond during the reaction (Scheme 98).

\[
\begin{align*}
\text{OAc} &\text{OAc} \\
\text{OAc} &\text{OAc}
\end{align*}
\]

Scheme 98: Oxidative fragmentation occurring across thiohydroximate bond of 105 resulted in formation of 21.

An attempt was made to circumvent this breakdown by lowering the reaction temperature to -40 °C, however no improvement was noted and once again cleavage across the thiohydroximate bond occurred.

An alternative base-free ozonolysis system reported by Dussault et al.¹¹⁵ used N-oxides such as N-methylmorpholine N-oxide (NMO), as illustrated in Scheme 99. Upon break down of the molozonide 120, the intermediate 123 reacts with the NMO to give 125. This intermediate undergoes loss of oxygen and reduction of the N-oxide to give the aldehyde and NMM (Scheme 99).
It was clear that anhydrous conditions were required and the reaction benefited from the use of 4 Å molecular sieves. Optimisation, as illustrated in Table 4, also found that this method required ten equivalents of NMO (Entry 3 and 4) as three equivalents (Entry 1) gave only a 10% yield of the desired aldehyde 97.

<table>
<thead>
<tr>
<th>Entry</th>
<th>NMO equivalents</th>
<th>Time (h)</th>
<th>Conversion to 97 by ¹H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
<td>&gt;10%</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>1</td>
<td>32%</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>1</td>
<td>56%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>5</td>
<td>30%</td>
</tr>
</tbody>
</table>

Table 4: Optimisation results for NMO assisted ozonolysis on alkene 97.

Increasing the reaction time from 1 h to 5 h (Entry 3 and 4) led to a lower yield of aldehyde 97. This was partially due to the breakdown of the aldehyde in solution as well as its previously observed instability on both silica or alumina. Attempts to use the crude aldehyde 97 directly in subsequent reactions were unsuccessful however it was found that an aqueous work up gave reasonable recovery. This could now be explored in Wittig reactions.
3.8 Ozonolysis of potassium salt 126

Another strategy explored ozonolysis on the potassium salt 126. This had the advantage that it would shorten the synthetic sequence to access a range of novel glucosinolates (Scheme 100).

Ozonolysis of the potassium salt 126 in DCM did not give a reaction (Scheme 101). This was most probably due to the insolubility of 126 in DCM. Additionally if methanol was used as a co-solvent with DCM (1:9, MeOH:DCM) for the ozonolysis of 126 then $^1$H NMR analysis of the reaction product showed the loss of the alkene protons, but no aldehyde proton.

A hemiacetal, of type 128 (Scheme 102), may be forming therefore a $^1$H NMR study was undertaken. As salt 126 is only sparingly soluble in both chloroform and methanol, analogue 97 was used in the $^1$H NMR study. A distinctive
aldehyde peak at 9.85 ppm was observed when only deuterated chloroform was used. However the $^1$H NMR of 97 recorded in methanol (Figure 17), indicated a disappearance of the aldehyde peak, and the emergence of the distinctive methyl hemiacetal at 3.31 ppm, Scheme 102 as well as an additional proton signal in the region of 5.40-5.37 ppm corresponding to the hemiacetal methine proton.

![Scheme 102](image)

**Scheme 102**: Hemiacetate 128 formation on the addition of MeOH to aldehyde 97.

![Figure 17](image)

**Figure 17**: Overlays of $^1$H NMR a) aldehyde 97 in chloroform b) aldehyde 97 in methanol

Although it appears that the desired aldehyde is forming on reaction of ozone with potassium salt 126, it appears then to be reacting with methanol to form a hemiacetal of 127. Further work in this area is required.
In order to increase the solubility of 126 in organic solvents, a change of counterion was envisaged. In the event, sulfation was achieved using pyridine·sulfur trioxide complex and the reaction was quenched using an aqueous cyclohexylamine solution to furnish 129 in high yield (72%).

Scheme 103: Reagents and conditions: a) Py·SO₃, pyridine, 16 h, 72%.

It was found that 129 generated good crystals and this allowed a suitable crystal to be submitted for X-ray structure analysis. The resultant structure is shown in Figure 18. To our knowledge this is the only reported example of an X-ray crystal structure for a sulfated glucosinolate.

Figure 18: The resultant X-ray crystal structure of glucosinolate 129 as a cyclohexylammonium salt.
Preliminary ozonolysis studies on 129 to 130 indicated that only starting material was recovered. Further investigations are clearly warranted as this would shorten the synthetic pathway towards a collection of novel alkenes.

Scheme 104: Preliminary ozonolysis studies on 129 were unsuccessful.

3.9 Conclusions on the oxidation of the alkene side chain.

In order to access aldehyde 97 from alkene 105 a variety of methods were utilised. It was found that hydroboration was not suitable on glucosinolates due to harsh work up conditions. Epoxidation of 105 generated novel intermediate 110, which was explored as a point of divergence, however this failed to ring open to give the desired diol. Dihydroxylation was achieved, to obtain novel diol 117, however oxidative cleavage to generate aldehyde 118 was only achieved in low yields. Ozonolysis was found to mediate the desired transformation to aldehyde 97, in a good yield (56%).
3.10 Using aldehyde 97 to form alkene side chains

3.10.1 Wittig reaction

It was envisaged that a Wittig reaction\textsuperscript{116} could be performed on aldehyde 97, in order to install the desired alkene functionality. It is clear that a range of alkene-containing glucosinolate analogues could be synthesised \textit{via} this method if it proved to be successful. In order to probe the viability of the Wittig strategy, benzyltriphenylphosphonium bromide was selected in the first instance (Scheme 105).

![Chemical structure]

\textit{Scheme 105: Reagents and conditions} a) nBuLi, Ph$_3$P$^+$CH$_2$PhBr$^-$, THF, 4 h, 30%; b) Dowex-50W, MeOH, 5 h, 98%.

Aldehyde 97 was reacted accordingly with benzyltriphenylphosphonium bromide and n-butyllithium in THF to generate the target alkene 131. This reaction proved to be successful and alkene 131 was isolated in a 30% yield. The alkene was obtained as a mixture of \textit{E:Z} isomers. It was not possible to separate the diastereomers at this stage by column chromatography.

To investigate the synthetic strategy further the diastereomeric mixture of 131 was progressed through to the next stage. Removal of the acetal group of 131 would require acidic conditions. It was found that Dowex-50W resin, which was readily removed by filtration, could be used to generate the thiohydroximate 132 in excellent yields (98%). Previous studies had found sulfation of the thiohydroximate to be troublesome on a small scale therefore sulfation of 132 was not attempted.

A second example, one that would furnish the aliphatic side chain 133, was now explored. For this butyltriphenylphosphonium bromide was selected, and alkene 133 was recovered in a 28% yield (Scheme 106).
To conclude, Wittig chemistry was shown to successfully install alkene functionality from aldehyde 97. However due to the relatively modest yields, further optimisation is required before a compound collection can be generated.

3.10.2 A one-pot ozonolysis-Wittig reaction

A one-pot ozonolysis-Wittig reaction that would furnish 134 from 106 was explored. If successful this would allow the direct conversion from 106 to 134, without the requirement to isolate the unstable aldehyde (Scheme 107). This procedure had previously been reported by Montgomery and co-workers\textsuperscript{117} and utilised stabilised ylides.

In the event the reaction proved to be low yielding (35%) and \textsuperscript{1}H NMR suggested that deprotection of the thiohydroximate had partially occurred. Therefore the product was subjected to deprotection to convert 134 to 135 with the use of Dowex-50W resin.
Attempts were made to optimise the yield achieved by the one-pot procedure. This involved investigating the reductive work up to use DMS. In addition a variety of reaction times were explored however no improvement was noted.

In summary the one-pot method generated the thiohydroximate alkenes in a 35% yield in one step compared to 17% over two steps from Section 3.10.1.

### 3.11 Additional mechanism probes for ESP

#### 3.11.1 Cycloaddition reactions on alkene glucosinolates

Cycloadditions to alkenes are well documented for the production of ring systems, such as 136 and 137. By exploring cycloaddition reactions on an alkene containing glucosinolate such as 105, it was envisaged that a variety of novel side chains could be synthesised.

![Scheme 108: Examples of cycloaddition products on an alkene.](image)

**Scheme 108:** Examples of cycloaddition products on an alkene.

#### 3.11.1.1 The use of Diels-Alder chemistry on alkene 106

As a ring containing glucosinolate such as 138 would be of particular interest to an ESP study (Section 1.5), a Diels-Alder reaction between alkene 105 and a variety of dienes was explored (Scheme 109).

![Scheme 109: Diels-Alder chemistry on glucosinolate side chains.](image)

**Scheme 109:** Diels-Alder chemistry on glucosinolate side chains.
Although there is a lack of commercially available dienes, 2,3-dimethylbutadiene and dimethoxybutadiene are readily available and they were reacted with the alkenyl glucosinolate 105 (Figure 19).

![dimethylbutadiene and dimethoxybutadiene](image)

**Figure 19:** Dimethylbutadiene and dimethoxybutadiene.

Both of these dienes failed to yield Diels-Alder products even after screening a range of solvents, extended reaction times and temperature. Cyclopentadiene was also explored as it is generally a good diene in Diels-Alder reactions. In order to use cyclopentadiene it must be cracked from the commercially available dimeric form, and this was carried out following a literature method.\(^{120}\) After cracking the diene, it was reacted immediately with 105 but again there was no conversion to the desired cycloaddition product.

### 3.11.1.2 The use of 1,3 dipolar cycloadditions on alkene 105

As discussed in Section 2.5, nitronates can be formed from the corresponding oxime with the use of NaOCl. Engberts and co-workers\(^{75}\) originally used this methodology for generating nitrile oxides for 1,3-dipolar cycloadditions. Clearly a 1,3-dipolar cycloaddition of alkene 106 would generate a dihydroisoxazole core side chain and give access to a novel class of glucosinolate side chains. In the event oxime 76 was converted to the corresponding nitrile \(N\)-oxide via the oximyl chloride and this was immediately reacted with 106 and triethylamine in DCM. This produced 139 in an excellent yield (89%) and as a 1:1 mixture of diastereomers (Scheme 110).
Scheme 110: Reagents and conditions a) Oxime 76, NaOCl, NEt₃, DCM, 3 h, 89%.

The regiochemistry predicted for this reaction was first confirmed via COSY couplings showing Hₓ coupling to the diastereotopic Hᵧ protons (Figure 20a). Further to this an HMBC coupling from the carbon of the imine bond to the carbon with a pair of diastereotopic protons was also observed (Figure 20b).

Figure 20: a) COSY correlations; b) HMBC correlations.

This is the first example of this sort of reaction on an intact glucosinolate. Clearly intermediate 139 could be used to expand into a range of novel glucosinolates (Scheme 111). For example, work by Curran¹²¹,¹²²,¹²³ demonstrated the high yielding reduction of dihydroisoxazole rings with Raney nickel give to keto-alcohol 140. Following olefination to 142 this would lead to a novel glucosinolate.

Scheme 111: Proposed conversion of 139 to 142.
3.12 Synthesis of glucosinolates containing ring side chains

The original approach of Benn\(^{32}\) (as discussed in Section 1.6) was now explored to construct novel ring systems. The commercially available aldehyde 143 was converted to the corresponding oxime 144 as summarised in Scheme 112. This oxime was then successfully coupled to \(\beta\)-thioglucose to obtain thiohydroximate 145 in a good yield (61\%) over the two steps (Scheme 112).

\[ \text{Scheme 112: Reagents and conditions} \text{ a) NH}_2\text{OH-HCl, NaOAc, MeCN, H}_2\text{O, 16 h; b) NaOCl, NEt}_3, \text{ DCM, 3 h, 61\% (over 2 steps)} \]

The thiohydroximate 145 was isolated as an inseparable mixture of the diastereoisomers of 145a and 145b (Figure 21).

\[ \text{Figure 21: Diastereomers of 145.} \]

Sulfation of 145 was explored using pyridine·sulfur trioxide complex and quenching with potassium hydrogen carbonate however this proved unsuccessful. The reaction was repeated using cyclohexylammonium as an alternative counter ion, however this also failed to yield sulfated 146 (Scheme 113).
Scheme 113: Sulfation failed to yield desired product 146 as a potassium or cyclohexylammonium salt.

Oxime 148 was prepared as shown in Scheme 114. This was obtained by coupling the oxime generated from aldehyde 147 with β-thioglucose 21 to obtain thiohydroximate 148 in good yield (69%). The $^1$H NMR was relatively complex consistent with a mixture of diastereomers. Sulfation of 148 to 149 was successfully achieved as a potassium salt (Scheme 114).

Scheme 114: Reagents and conditions a) i. NH$_2$OH·HCl, NaOAc, MeCN, H$_2$O, 16 h; ii. 21, NaOCl, NEt$_3$, DCM, 69% (over 2 steps); b) Py·SO$_3$, pyridine, 16 h, 48%.

This also led to a mixture of diastereomers as indicated by $^{13}$C NMR spectra with multiple signals for the thiohydroximate. There are four possible diastereomers for 149 (Figure 22). The structural integrity of 149 was however confirmed with the use of 2D NMR and mass spectrometry.
The use of potassium carbonate in methanol facilitated acetate cleavage and provided 150 in excellent yield (Scheme 115).

Scheme 115: Reagents and conditions a) K$_2$CO$_3$, MeOH, 16 h, 88%.

3.13 The total synthesis of novel glucosinolates

It was anticipated that novel elongated side chains with a terminal alkene functionality could be obtained using the Benn synthesis$^{32}$ (Scheme 116).

Scheme 116: Proposed pathway for the formation of elongated side chains.

Aldehyde 152 was accessed by the oxidative fragmentation of commercially available diol 151 in an excellent yield (92%) (Scheme 117). The aldehyde 152 was then converted to the desired oxime 153 using hydroxylamine.
hydrochloride. The reaction was conducted in a biphasic system, and this generated **153** as a 1:1 mixture of \(E:Z\) isomers.

![Scheme 117: Reagents and conditions](image)

**Scheme 117**: Reagents and conditions a) NaIO₄, 3 h, 92%; b) NH₂OH·HCl, NaOAc, MeCN, H₂O, 16 h, 78%.

Coupling of the oxime to the protected thioglucose **21** was achieved using the NaOCl method outlined in Section 2.5 to generate **154**. This reaction was straightforward and gave **154** in an excellent yield (71%) (Scheme 118).

![Scheme 118: Reagents and conditions](image)

**Scheme 118**: Reagents and conditions a) NaOCl, NEt₃, DCM, 3 h, 71%.

The glucosinolate was a crystalline solid and it was subject to X-ray structure analysis. The structure of **154** showed two distinct conformers in the solid state with different orientations of the alkene moiety (Figure 23). A similar disorder was apparent in the structure of **129** (Figure 23).
Figure 23: X-ray crystal structure of 154 showing two distinct conformers around the terminal alkene.

With alkene 154 in hand, sulfation with pyridine sulfur trioxide complex using a potassium counter ion was achieved to give 155 in good yield (59%) (Scheme 119). Deacetylation was successfully achieved using potassium carbonate in methanol, to furnish the desired glucosinolate in high yield (73%) (Scheme 119).

Scheme 119: Reagents and conditions a) Py·SO₃, pyridine, DCM, 16 h, 59%; b) K₂CO₃, methanol, 24 h, 73%.
The synthesis of 157 was undertaken in an effort to prepare a glucosinolate analogue with a mid chain double bond.

Oxime 159 was prepared in an excellent yield (83%) by reacting commercially available, Z-alkeneic aldehyde 158 with hydroxylamine hydrochloride. Using the one-pot coupling technique now developed in this thesis, oxime 159 was coupled to thioglucose, generating 157, in an excellent yield (85%) (Scheme 120).

With thiohydroximate 157 in hand sulfation was carried out to generate the cyclohexylammonium salt 160. A variety of methods were attempted to purify the sulfate 160 at this stage, however these were found to be time consuming and gave low isolation yields. Sulfation of thiohydroximate 157 was repeated and a cyclohexylammonium salt used as a counter ion. This gave 160 as a pale yellow solid. Deprotection was undertaken using potassium carbonate in methanol and this furnished the glucosinolate 161 as a cyclohexylammonium salt in excellent yield (81%) (Scheme 121).
Scheme 121: Reagents and conditions  a) Py·SO₃, pyridine, 5% cyclohexylamine in water, 16 h, 75%; b) K₂CO₃, MeOH, 24 h, 81%. 
3.14 Conclusions

Three novel glucosinolates, 150, 156, 161, have been prepared in good yields (Figure 24).

![Chemical structures of glucosinolates 150, 156, and 161]

Figure 24: Total synthesis of 150, 156, and 161 has been completed

This involved using our NaOCl coupling strategy and also sulfation and then mild conditions for per-acetate ester hydrolysis. This route allows for practical access to a range of novel glucosinolates and should find utility in the future.
Towards the solid phase synthesis of a glucosinolate

4.1 Background and introduction

Merrifield\textsuperscript{124} introduced the concept of solid phase synthesis in 1963 when he described a technique for reacting amines and carboxylic acids together with one covalently linked to an insoluble solid matrix.\textsuperscript{125} By physically separating the reactants from the unwanted reagents and by-products the need to use flash chromatography is removed and replaced with a simple filtration and washing procedure. Initially solid phase synthesis was developed primarily for use in peptide synthesis, and indeed, the first use by Merrifield\textsuperscript{124} involved the synthesis of the tetrapeptide Leu-Ala-Gly-Val\textsuperscript{162}.

\begin{center}
\includegraphics[width=0.5\textwidth]{peptide.png}
\end{center}

4.2 Components of a solid phase reagent

Solid phase reagents can be divided into two key components. There is the insoluble matrix which forms the backbone of the resin and then there is the linker to which the substrate is covalently attached (Figure 25). Finally the substrate is likely to have other functionality attached to allow further modifications to be performed. The properties of the resin, for example solubility, can be tuned via alterations to each of these components.
4.2.1 The matrix backbone

Classically the matrix backbone is insoluble in the reaction medium and is required to be sufficiently stable to allow for agitation and filtration.\textsuperscript{124,125} In addition it must also have appropriate functionality to allow attachment directly to the substrate or, more commonly, \textit{via} a linker. Merrifield investigated many polymers in his initial studies of matrices but found that modified polystyrene (PS) (Figure 26) was most successful in achieving the desired porous gel structure required for reagent diffusion.

More recently controlled pore glass (CPG) was introduced an alternative matrix.\textsuperscript{125} Unlike PS it has wide solvent compatibility, as it does not require swelling prior to use. However, CPG systems based on a silica network, have the disadvantage of not being compatible with silyl protecting groups as deprotection, typically with a fluoride ion source, leads to breakdown of the matrix. Therefore the use of this matrix is not favoured in carbohydrate synthesis.\textsuperscript{125}
4.2.2 The linker component

The linker component can be used to tune the reactivity and physical properties of the backbone of the resin. For example, the incorporation of poly(ethylene glycol) (PEG), 163, linkers improves the compatibility of the PS-based systems in polar solvents due to the improved swelling characteristics of the PEG side chain.\textsuperscript{129}

\[
\text{HO-}\begin{array}{c}
\text{O} \\
\text{O} \\
\text{O}
\end{array}\text{H}
\]

163

The linker also plays a vital role in the cleavage conditions required for removal of the substrate from the resin. Typically, linkers can be cleaved via acid,\textsuperscript{128} base,\textsuperscript{129} oxidation,\textsuperscript{130} reduction,\textsuperscript{131} photolysis\textsuperscript{132} or alkene metathesis\textsuperscript{133} depending on their design (Figure 27). Therefore, it is possible to select a linker/resin system whereby the cleavage conditions are orthogonal to those of the protecting groups or the functionality contained within the substrate.

\[ \text{Ozonolysis} \quad \text{Base (NEt}_3\text{)} \quad \text{Acid (TFA)} \]

\[ \text{Reduction (LiAlH}_4\text{)} \quad \text{Hydrogenation (Raney-Nickel)} \quad \text{Photolysis (320 nm)} \]

\textbf{Figure 27:} Various solid phase synthesis linkers that have been developed with their cleavage conditions.\textsuperscript{129-133}
4.3 Alternatives to classical solid phase reagents

Research focused on finding supported reagents is an emerging field in the area of solid phase research. Recently the use of highly fluorinated tags, Figure 28, for the development of fluorous solid phase extraction (FSPE) has become fashionable.\textsuperscript{134}

Figure 28: Examples of fluorous reagents.

This technique has been successful in many recently reported syntheses of complex motifs. These include library synthesis of bioactive heterocycles\textsuperscript{137} such as hydantoins and thiohydantoins \textbf{164} (Scheme 122a), as well as natural product-like scaffolds such as spiropiperidinones\textsuperscript{136} \textbf{165} (Scheme 122b).

Scheme 122: Examples of FSPE in synthesis.

The advantage of FSPE is in the ease of purification. This is due to the specialist cartridge systems, on which the tagged FSPE molecules are retained. A change in solvent gradient can then be used to elute the tagged substrate, as illustrated in Figure 35. Currently the major limitation for this methodology is the high cost of both the reagent tags and the specialist purification scavenger
systems, but they have found increasing utility in the synthesis of high value products.

Figure 29: Schematic of fluorous solid phase extraction.

4.4 Carbohydrates on solid phase

The first example of solid phase carbohydrate synthesis was reported in 1971 by Frechet et al. Frechet described the synthesis of disaccharide, and used a novel poly [p-(1-propen-3-ol-1yl) styrene] resin, which allowed cleavage by ozonolysis.

Since this pioneering publication various synthetic strategies have been reported for attaching carbohydrate substrates to resins. In early work, Gagnaire explored the use of an ester linkage to the C-6 hydroxyl group for the formation of disaccharide, as shown in Scheme 123a. Subsequently the synthesis of gentiotetraose utilised a benzoyl propionate linker that could be cleaved under mild conditions with hydrazine acetate, Scheme 123b.
Frechet and co-workers\textsuperscript{138} developed an alternative pathway using the resin bound boronic acid (Scheme 124). This method allowed selective preparation for the $\beta$-anomer as it had previously been reported that glycosylation preferred formation of $\alpha$-anomer linkages.\textsuperscript{139}

An alternative and notable strategy was introduced by Guthrie and co-workers\textsuperscript{140} involving a novel concept by performing a co-polymerisation of 169 with styrene in order to gain covalent resin attachment while also crucially forming the polymeric scaffold (Scheme 125).
Automation has revolutionised the synthesis of bioactive carbohydrates enabling their exploration as novel candidates in the drug discovery process. Current market leaders in this class include acarbose 170 (Figure 36) a Type II diabetes drug first synthesised by Shibata et al. A further example is heparin 171 (Figure 30), an injectable anticoagulant, which was first prepared by Sinaÿ et al.

Glucosinolates are thioglycosides but they have an additional sulfate moiety, a structural feature that presents a difficulty in their purification and isolation during synthesis. It was envisaged that it may be possible to ease purification, in particular through the latter stages of their synthesis, by developing a solid phase synthesis of a glucosinolate. Such an approach has never been reported.
4.6 Model study

4.6.1 Resin selection

Before using solid phase reagents a solution phase model study was required to investigate the potential suitability of the chemical transformations involved. Merrifield resin with its high loading capacity, compatibility and broad range of possible reaction conditions, durability and low price\textsuperscript{143} made it the ideal resin for the synthesis. In order to mimic this resin a solution phase study used the benzyl group as it shares the same chemical reactivity characteristics of the resin (Figure 31).

![Figure 31: Benzyl group and Merrifield resin.](image)

4.6.2 Selecting a position for resin attachment

It was attractive to consider attaching the resin to the sugar moiety instead of through the side chain of the glucosinolate (Figure 32).

![Figure 32: Schematic of potential resin attachment points.](image)

This has the potential for the synthesis of a range of glucosinolates on the solid phase using the sugar unit as a common motif. The C-2 hydroxyl group was not considered as the attachment point as its location may interfere with neighbouring group participation, a feature that is required to control the stereochemical outcome during manipulations at the anomeric centre (Section 2.3). It would also be challenging to obtain selectivity at the C-6 hydroxyl without the use of elaborate protecting group manipulations prior to resin attachment, therefore this position was not selected. The C-3 and C-4 hydroxyls emerged as candidates, however the C-3 hydroxyl looked most promising as
this could be selectively attached via the use of diacetone glucose, an inexpensive and readily available starting material (Scheme 126).

![Scheme 126](image)

**Scheme 126**: Proposed benzylolation/resin attachment on the C-3 hydroxyl.

### 4.6.3 Solution phase model study.

The synthesis began with the protection of the C-3-hydroxyl group of diacetone glucose as a benzyl ether (Scheme 127). Removal of the acetal groups should result in spontaneous cyclisation to give the pyranose ring form of 173. Protection of 173 would then generate the key intermediate, 174. There are many routes available for the formation of β-thioglucose 175 and these approaches have been discussed previously in Section 2.3 and will be revisited in the context of this study. The route progresses with the coupling of the oximyl chloride and the resin bound thioglucose 175, via the NaOCl method developed in this thesis (Section 2.5). The intermediate thiohydroximate requires to be sulfated to intermediate 176 and then the acetyl esters hydrolysed in the presence of the benzyl group in an orthogonal strategy to obtain the resin bound glucosinolate. Finally, benzyl deprotection strategies will be screened to identify appropriate conditions for the release the final glucosinolate 1.
Scheme 127: Proposed glucosinolate synthesis on the solid phase.
4.7 Results and discussion

4.7.1 Synthesis of 3-O-benzyl-1,2,4,6-tetra-O-acetyl glucose, 177

Kojima et al.\textsuperscript{145} have recently reported that the C-3 hydroxyl group of diacetone glucose can be selectively functionalised. They used this for the attachment point for a FSPE reagent 178 for the total synthesis of cucurbitoside A, 179 (Figure 33).

In the event successful benzylation was achieved with the use of sodium hydride and benzyl bromide in DMF\textsuperscript{146} and the ether was isolated in a good yield (78\%) after further purification by column chromatography (Scheme 129).
for this deprotection\textsuperscript{147} (Scheme 129). The resin has the advantage that it allows a straightforward filtration on work up. In our hands this gave an excellent yield (95\%) of 3-benzylglucose 173 in a 2:3 ratio of \(\alpha:\beta\) anomers and a purity of greater than 95\% (by \(^1\text{H} \text{NMR, Figure 34}) was achieved. No further purification was required following concentration of the filtrate.

![Figure 34: \(^1\text{H} \text{NMR of the 173 after Dowex-50W treatment of 177. Characteristic protons at 5.10 ppm (1H, d, J 3.7 Hz, CH-1\(\alpha\)) and 4.53 ppm (1H, d, J 8.6 Hz, CH-1\(\beta\)).}]

Although this reagent system might not be suitable for a solid phase approach, it was necessary for this model study to obtain a reference sample of the desired product in good quantity. On the solid phase the reagent roles will be reversed, therefore the use of aqueous acid would be compatible with the glucose motif attached to the solid support.

Upon deprotection, rearrangement of the furanose, form of the sugar to the pyrano glucose form occurs (Scheme 130).\textsuperscript{148} Although both furanose and glucopyranose are possible, the 6-membered ring is favoured thermodynamically due to lower torsional strain.\textsuperscript{149}
Scheme 130: Proposed mechanism for ring opening of 177.

The final step towards key intermediate 174 required acetylation of the four remaining free hydroxyl groups of 173 (Scheme 131). The acetate protecting group was selected as it could be orthogonally deprotected relative to the resin, and its use has been previously documented in a related system (Section 2.3). A variety of acetylation conditions have been reported, for example acetic anhydride and iodine or acetic anhydride and pyridine. Previous work within the group on related substrates has however shown these methods to be unsuccessful, low yielding or difficult to purify. Instead the desired reaction has been achieved in moderate yield (54%) with the use of acetic anhydride and sodium acetate. The main advantage of this method was that the by-products are water soluble, therefore 174 could be purified readily by pouring into an ice/water slurry. The desired peracetylated product 174, precipitated as a pale yellow solid. It was purified by recrystallisation from ethanol.

Scheme 131: Reagents and conditions a) Acetic anhydride, sodium acetate, 110 °C, 54%.
4.7.2 The synthesis of thioglucose, 175

The acetylated sugar 174 occupies a branch point in the synthesis as illustrated in Scheme 132.

Scheme 132: Proposed pathways for 175 synthesis.

Three different methods were explored for the conversion of 174 to the β-thioglucose 175. Method 1 explored a progression via the trichloroacetamide intermediate 181 and Method 2 via bromination at C-1 to give 182. Finally Method 3 investigated direct formation of β-thioglucose 175 from tetra-acetyl glucose 174.

4.7.2.1 Method 1 - via a trichloroacetamide.

Selective deprotection of the anomeric acetyl group was required prior to the formation of the trichloroacetamide intermediate, 181. A range of conditions for such a selective hydrolysis have previously been reported including amide formation with hydrazine acetate\textsuperscript{150} or benzylamine.\textsuperscript{151}

Scheme 133: Schmidt donor synthesis. Reagents and conditions a) Benzyamine, THF, 16 h, rt; b) trichloroacetonitrile, DBU, DCM, 16 h.
This can be difficult to achieve when the anomeric centre is not orthogonally protected with respect to the other positions. However this method exploits the slightly higher reactivity at the anomeric position, as the leaving group is a hemiacetal rather than an alcohol (Scheme 134).

![Scheme 134: Anomeric deprotection of 174.](image)

Selective deacetylation used benzylamine in THF was explored because it had been shown previously within the research group to be efficient. However, in the event, the hemiacetal proved difficult to purify as the amide co-product, co-eluted during purification by flash chromatography. After failed attempts to purify the product by recrystallisation it was decided to progress the crude material though to the next step and purify after reaction with trichloroacetonitrile. Clearly on the solid phase this purification would not be necessary, as the sugar will remain attached to the solid support. The trichloroacetonitrile donor, also known as a Schmidt donor, was then explored. Althought crude product analysis suggested that this reaction had been successful but with a low conversion, it appeared that a diastereomeric mixture had been produced. As it would not be possible separate these diastereomers on solid phase, a concern was that this would introduce diastereomers of the thioglucose product (Scheme 135). This method was not developed any further.

![Scheme 135: Diastereomeric mixture of 181 may lead to diastereomeric mixture of 175.](image)
4.7.2.2 Method 2 – via bromination of intermediate 181

Method 2 envisaged a route toward thioglucose 175 via the brominated intermediate 182 (Scheme 136).

Scheme 136: One-pot bromination of 174.

The major advantage of this approach is that α-bromination of carbohydrates is a well-documented method. The desired C-1 β-anomer of 175 can be generated with good stereochemical control from reaction of 182 with thiourea. The stereochemical control arises from neighbouring group participation of the C-2 acetate (Section 2.3).

At the outset bromination was explored with the use of hydrogen bromide (45% v/v in acetic acid) using the one-pot method, as described Section 2.3. It was found, upon analysis of the 1H-NMR spectrum, that the benzyl group of the C-3 hydroxyl had been cleaved during the reaction. This was most likely due to the acidic conditions of the reaction (Scheme 137) and an alternative method was sought.

Scheme 137: Proposed mechanism for debenzylation of 174.

The Appel reaction has previously been reported for bromination and offered a method of bromination under milder conditions, Scheme 138. Initial deacetylation of the anomeric centre to generate 183 was required before bromination could be attempted. In the event, the deacetylation was achieved by the use of benzylamine however the isolation of hemiacetal 183 proved
difficult and therefore it was used as a crude product in order to progress to the bromination.

![Chemical structure](image1)

Scheme 138: Reagents and conditions a) CBr₄, PPh₃, 5 h, 22%.

The reaction is reported to progress by formation of the bromotriphenylphosphonium tribromomethanide 185 upon reaction of triphenylphosphine and carbon tetrabromide (Scheme 139). The anomeric hydroxyl of 183 acts as a nucleophile and attacks the reactive intermediate 185, displacing bromide and forming phosphonium oxide 186. Anomeric displacement results in the loss of triphenylphosphine oxide to generate 187, which can then undergo nucleophilic attack by bromide to obtain the β-anomer of 182. This will then equilibrate to the more thermodynamically stable α-anomer.

![Chemical structure](image2)

Scheme 139: Proposed mechanism for Appel bromination of 183 to give 182.

The yield of 182α was disappointingly low (22%) and it was also found to rapidly decompose. Due to this a full analysis could not be obtained however the ¹H NMR (Figure 35) was consistent with desired structure. An attempt was made to optimise this method purifying 183 by column chromatography. Additionally alterations were made to reagent equivalents and alternative
purifications of 182 were attempted without success. The poor recovery created a bottle-neck in available material at an early stage of the synthesis therefore it was decided to explore an alternative method toward \( \beta \)-thioglucose 175.

![Image](image.png)

Figure 35: \(^1\)H NMR of 182. Anomeric proton at 6.57 ppm (1H, d, J 3.5 Hz, CH-1).

4.7.2.3 Method 3 – a one-pot thioglycosylation

Method 3 involved using the fully protected pyranose 174 and converting it to 175 via an intermediate thiourea 188.\(^{157}\)

![Image](image.png)

**Scheme 140:** Reagents and conditions a) Thiourea, BF\(_3\)·OEt\(_2\), MeCN, 2 h; b) sodium metabisulfite, water, 62% (over 2 steps).

This direct route was preferred as it would eliminate three steps from the synthesis compared to Method 1 and Method 2.
A recent report by Chiara et al.,\textsuperscript{158} on a similar substrate showed that the sulfur could be installed into the anomeric position without prior deacetylation (Scheme 141). This would be an advantage as deprotection to generate 183 proved to be problematic during purification in the previous approaches (Section 4.7.2.1 and 4.7.2.2).

**Scheme 141:** Reagents and conditions a) Thiourea, BF$_3$•OEt$_2$, MeCN, 80 °C, 94%.

The method required isolating the intermediate 188 before an additional hydrolysis step to generate 175. In our hands it was found that it was possible to react 174 with thiourea at reflux for 15 min before performing a hydrolysis with sodium metabisulfite. Only the β-anomer was obtained which is indicative of a process involving neighbouring group participation in the formation of intermediate 189, followed by an $S_N 2$ reaction with thiourea to generate 188, Scheme 142. Finally hydrolysis, with sodium metabisulfite, gave β-thioglucose 175 in a 62% yield (Scheme 140).

**Scheme 142:** Mechanistic rational for the stereochemical outcome for β-thioglucose 175.

The stereochemistry was assigned via $^1$H NMR spectroscopy. The product had a $^3J_{HH}$ coupling of 9.4 Hz for the H-1 at the anomeric centre, which is consistent with β-thioglucose 175. Recrystallisation of this material was not straightforward and it was found that purification required column chromatography.
Figure 36: $^1$H NMR of 3Bn-thioglucose, 175. Anomeric proton at 5.13 ppm (1H, dd, $J$ 10.1, 9.4 Hz, CH-1).

To our knowledge this is the first model system which has been used to achieve orthogonally protected thioglucose and clearly this substrate may have wider applications than glucosinolate synthesis. For example, Fiore et al.\textsuperscript{159} have recently reported the synthesis of S-glycosyl amino acids. It is anticipated that the use of resin bound 175 could lead to a rapid synthesis of highly polar structures, such as 190, without the need for time consuming purification.
4.7.3 Formation of glucosinolates

With β-thioglucose 175 in hand the next stage in the formation of a glucosinolate was to couple with an oxime of choice (Scheme 143). Thus the intermediate thiohydroximate 191 would then be required to undergo sulfation to obtain 192 as a potassium salt. It is at this stage that a solid phase synthesis would be most beneficial as previous syntheses of glucosinolates have found this product difficult to purify as they are often isolated with an excess of potassium salts. Salt 193 would then be generated after deacetylation. Finally in order to realise the desired glucosinolate 194 a variety of debenzylation methods will need to be explored.

\[
\begin{align*}
\text{175} & \quad \text{191} & \quad \text{192} \\
\end{align*}
\]

Scheme 143: Propose synthesis for glucosinolate 194.

4.7.4 Formation of thiohydroximate bond

In the event oxime 57 was selected as it was readily prepared in good yield as discussed in Section 2.4.2. Thiohydroximate 195 was generated by the novel coupling methodology developed in Section 2.5 and this was achieved in an excellent yield (86%) (Scheme 144).

\[
\begin{align*}
\text{175} & \quad \text{57} & \quad \text{195} \\
\end{align*}
\]

Scheme 144: Reagents and conditions a) NaOCl, DCM, NEt₃, 5 h, 86%.
Sulfation was performed using the pyridine·sulfur trioxide complex by the method already described in Section 3.12. Electrospray mass spectrometry was used to confirm product formation and this method showing the desired mass peak at \( m/z \) \( (ES^-) \) 588.

![Scheme 145](image)

Scheme 145 Reagents and conditions a) Py·SO₃, pyridine, 2M KHCO₃, DCM, 16 h.

The \(^1\)H NMR of 196 was however difficult to interpret due to an excess of potassium salts and attempts to further purify this material were not successful. Therefore it was felt that a change in the counter ion may be advantageous in order to help purify the glucosinolate at this stage. Therefore a sulfation was performed, but this time using a cyclohexylammonium as the counterion, 197 (Scheme 146). Sulfate 197 was obtained in a moderate but workable yield (53%) and allowed investigations into the final deprotections stages of the model synthesis.

![Scheme 146](image)

Scheme 146: Reagents and conditions a) Py·SO₃, pyridine, 5 % aq cyclohexylamine solution, DCM, 16 h, 53%.
4.7.5 Deprotection of 197

A deacetylation using potassium carbonate in methanol was attempted. It was observed by $^1$H NMR and mass spectrometry analysis that a mixture of partially deprotected material had formed, whereby one acetate moiety remained.

\[
\text{Scheme 147: Reagents and conditions a) } K_2CO_3, \text{ MeOH.}
\]

2D NMR spectroscopy techniques were used to investigate at which position the acetate group was located however the results were not definitive. Extending the reaction time to drive the hydrolysis to completion was explored, however it was found that even after 6 days a mixture remained. Attempts were made to separate the desired compound 198 from the mixture using reverse phase chromatography however without success.

Zemplen conditions, of sodium metal in methanol,$^{160}$ proved too harsh and gave a range of decomposed by-products. Therefore in order to progress further, investigations in the deprotection of the acetate esters would need to be explored.
4.8 Conclusion

Diacetone glucose was successfully protected as its a benzyl ether 177 at C-3, as a mimic for Merrifield’s resin. It was found that 177 be could readily converted to 173 in excellent yield with the use of Dowex resin. Acetylation was achieved with acetic anhydride and sodium acetate to obtain workable quantities of 174.

Various methods were explored at this stage in order to generate 2-Bn-thioglucose 175. Successful methods used a one-pot thioglycosylation-hydrolysis system to achieve 175 in good yield (62%) over the two steps.

Coupling between 175 and 57 was achieved in good yields using the NaOCl method previously developed in this thesis, as detailed in Section 2.5. In the end deprotections proved to be problematic. The use of potassium carbonate suggested that one acetate remained intact. It was not possible to determine at exactly which position the acetate remained.

It is clear that there are now prospects for a solid phase synthesis of glucosinolates after optimisation of the late stage deprotection methodology.
Applications of synthesised glucosinolates

In summary a new coupling method for β-thiohydroximate bond formation has been developed for the glucosinolate family of natural products using inexpensive and readily available NaOCl as the chlorinating agent. The method proved very effective over a range of substrates exploiting the oxidative activation of oximes for glucosinolate coupling. This simplified one-step procedure is straightforward to carry out, rapid and it gives good to excellent yields over a range of substrates. It is envisaged that this methodology could prove highly useful in the formation of isotopically labeled natural and novel glucosinolates, as detailed in Scheme 148. The method is undoubtedly an improvement over existing protocols, and should be the first choice for the synthesis of glucosinolates to gain efficient access to materials for further biological, metabolism and enzymatic studies.

![Diagram](attachment:image.png)

Scheme 148: Alternative route towards [Phenyl\(^2\text{H}_5\)]-gluconaturtiin

This thesis has also developed a novel concept of using the side chain functionality of glucosinolates to synthesis novel and natural glucosinolates. Further developments of this methodology could include the synthesis of labelled glucosinolates by side chain modifications. For example the synthesis of a terminal alkene labelled sidechain glucosinolate, as detailed in Scheme 149, could be obtained via the Wittig reaction of aldehyde 97 and an isotopically
labelled Wittig salt. In turn, it is envisaged that such an analogue could be used in biological studies to gain further insight into the ESP mechanism by monitoring the breakdown products produce by analytical techniques such as LCMS and NMR spectroscopy.

\[ \text{Scheme 149: Wittig chemistry would allow isotopically label to be installed at terminal alkene.} \]

Additionally aldehyde 97 could also allow access to cyclopropane side chain glucosinolate (Scheme 150). As discussed in Section 3.1 this would allow the proposed radical mechanism of ESP to be explored by monitoring the metabolites produced.

\[ \text{Scheme 150: Wittig reaction on 97 could be used to obtain ESP mechanism probe.} \]

The intermediate aldehyde 97 could be used to synthesis novel amine glucosinolates via the reductive amination of aldehyde 97 and a desired amine.
Scheme 151: Reductive amination on intermediate 97 would allow access to amine containing glucosinolates.

The three novel glucosinolates, 150, 156, 161, synthesed in Chapter 3 would offer the opportunity for insight into the ESP mechanism. Little is known about the structural activity relationship of ESP therefore bulky substrates, such as 150, and elongated sidechains, such as 156 and 161 would allow a substrate profile of the ESP-myrosinase system to be developed.

Novel glucosinolate such as the 1,3-dipolar intermediate 139 would not only allow further chemical transformations to be explored but also insight into the tolerance of the myrosinase hydrolysis.

Finally, the transfer of the chemistry developed in Chapter 4 to solid support would allow for rapid access to glucosinolates which are immobilized thereby overcoming the difficulties associated with their high polarity. A further
implementation of the solid supported synthesis would be their use on the solid support as biological probe upon identification of a suitable biocompatible linker.
Experimental

General considerations:

Materials were obtained from commercial suppliers and were used without further purification unless otherwise noted. All moisture and air-sensitive reactions were carried out under an atmosphere of nitrogen, which had been dried via passage through a Drierite column, using oven or flame dried glassware.

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

Microanalysis was carried out by the University of St Andrews microanalytical laboratory on a Carlo Erba EA1110 CHNS analyser.

IR spectra were recorded as either: nujol mulls or liquid films between sodium chloride discs; as potassium bromide pellets unless otherwise stated on a Perkin Elmer GX FT-IR instrument. Absorption maxima are given in wavenumbers (cm$^{-1}$) relative to a polystyrene standard.

$^1$H NMR spectra were recorded using a Bruker Avance 300 at 300 MHz, Bruker Avance II 400 (400 MHz) or Bruker Avance 500 (500 MHz) spectrometers. $^{13}$C NMR spectra were recorded using a Bruker Avance 300 (75 MHz), Bruker Avance 400 (100 MHz) or Bruker Avance 500 (125 MHz) spectrometers using a DEPT-Q pulse sequence. $^1$H and $^{13}$C NMR spectra were referenced to deuterochloroform (CDCl$_3$), d$_4$-methanol or D$_2$O. NMR spectra are described in parts per million (ppm) downfield shift from TMS and are reported consecutively as position ($\delta_H$ or $\delta_C$), relative intergral, multiplicity (s=singlet, br s=broad singlet, d = doublet, br d = broad doublet, dd = doublet of doublets and assignment. Coupling constants ($J$) are recorded to 0.1 Hz and identical coupling constants averaged.
Low resolution and high resolution chemical isonisation (CI) mass spectra were recorded on a Micromass GCT spectrometer with methane as the ionisation gas. Electrospray (ES) spectra were recorded using a Micromass LCT operating in positive or negative mode from solutions of acetonitrile, methanol or water.

Preparative flash column chromatography was performed with Apollo Scientific silica gel 60 (40-63 micron grade) under a positive pressure of air. Thin layer chromatography was performed on Merck silica gel 60 F$_{254}$ glass plates backed, and visualised using UV light, 10% H$_2$SO$_4$ in ethanol stain or alkaline potassium permanganate stain.

NaOCl refers to sodium hypochlorite solution. (wt/mL at 20 °C 1.24 -1.26g)

Ozone experiments were performed using a Fisher Technology OZ/500/5 ozone generator.

Specific optical rotations were measure using a Perkin Elmer Model 341 polarimeter, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (c) is expressed in g/100 mL. Specific rotations are denoted as $[\alpha]_T^\circ$ and are given in implied units of $10^{-1}$ deg cm$^2$ g$^{-1}$ (T = ambient temperature in °C).
6.2 Experimental procedures

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide,\textsuperscript{161} 18

Method 1: Hydrogen bromide (45\% w/w in acetic acid, 60 mL, 237.6 mmol, 1.1 eq) was added dropwise to a solution of d-glucose (39.0 g, 216.0 mmol, 1.0 eq) in acetic anhydride (150 mL) at 0 °C. After 4 h further hydrogen bromide (45\% w/w in acetic acid, 180 mL, 712.8 mmol, 3.3 eq) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was taken up in DCM (300 mL) and poured into ice/water (500 mL). The layers were separated and the organic layer was washed with an ice/saturated sodium hydrogen carbonate solution (600 mL). The organic phase was separated and washed with saturated sodium hydrogen carbonate solution (600 mL). After effervescence had ceased the organic layer was separated, dried over magnesium sulfate and solvent removed under reduced pressure. The resulting oil was crystallised from diethyl ether to give 18 as a crystalline solid (75.4 g, 85\%):

Method 2: Hydrogen bromide (45\% w/w in acetic acid, 36.3 mL 140.1 mmol, 1.1 eq) was added dropwise to a solution of (S)-1,2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl (50.0 g, 128.0 mmol, 1.0 eq) in DCM (20 mL) under a nitrogen atmosphere and at 0 °C. Following addition and 1 h of stirring at 0 °C the solution was allowed to warm to room temperature and was stirred for a further 2 h. The solution was poured into water (ca. 300 mL) and the organic layer separated before being washed repeatedly (5 × 200 mL) with saturated sodium hydrogen carbonate solution until the organic layer was neutral at pH 7. The organic fraction was dried over magnesium sulfate before the solvent was removed under reduced pressure to obtain a golden oil which was crystallised from diethyl ether to give 18 (33.2 g, 63\%) as a colourless crystalline solid.

Data was consistent for both methods:
Experimental

mp 86-87 °C. [lit 88-89 °C]¹⁶³; \( \nu_{\text{max}} \) (nujol)/cm\(^{-1}\) 2960 (CH), 1740 (CO), 609 (C-Br); \( \delta_{\text{H}} \) (300 MHz, CDCl\(_3\)) 6.59 (1H, d, \( J \) 4.0 Hz, CH-1), 5.55 (1H, dd, \( J \) 10.4, 10.4 Hz, CH-3), 5.19-5.10 (1H, m, CH-4), 4.83 (1H, dd, \( J \) 10.4, 4.0 Hz, CH-2), 4.34-4.25 (2H, m, CH\(_2\)-6), 4.14-4.08 (1H, m, CH-5), 2.09, 2.08, 2.04, 2.02 (12H, 4 x s, CH\(_3\)C(O)O); \( \delta_{\text{C}} \) (75 MHz, CDCl\(_3\)) 170.9, 170.3, 170.2, 169.9, (4 x CH\(_3\)C(O)O), 86.9 (C-1), 72.5 (C-5), 70.9 (C-3), 70.6 (C-2), 67.5 (C-4) 61.4 (C-6), 21.1 21.0 (2C), 20.9 (4 x CH\(_3\)C(O)O); \( m/z \) (ES\(^{+}\)) 435 (98%, [M+Na]\(^{+}\), \(^{81}\)Br), 433 (100%, [M+Na]\(^{+}\), \(^{79}\)Br).

S (2,3,4,6-Tetra-O-acetyl-\( \beta \)-d-glucopyranosyl) isothiouronium bromide,¹⁶² 50

Thiourea (6.86 g, 90.1 mmol, 1.0 eq) was added in one portion to a solution of 2,3,4,6-tetra-O-acetyl-\( \alpha \)-d-glucopyranosyl bromide 18 (37.0 g, 90.1 mmol, 1.0 eq) in dry acetone (100 mL), and the solution heated under reflux. After 15 min the reaction was first cooled to room temperature, then to 0 °C. The resulting precipitate was filtered and recrystallised from acetone to give 50 as a crystalline solid (31.2 g, 71%): mp 200-204 °C dec [lit¹⁶⁴ 205 °C]; \( \nu_{\text{max}} \) (nujol)/cm\(^{-1}\) 3320 (NH), 1750 (CO), 1655 (NH); \( \delta_{\text{H}} \) (300 MHz, D\(_2\)O) 5.40-5.09 (4H, m, CH-1,2,3,4), 4.34-4.26 (2H, m, CH\(_2\)-6), 4.18-4.07 (1H, m, CH-5), 2.01, 1.99, 1.97, 1.94 (12H, 4 x s, CH\(_3\)C(O)O); \( \delta_{\text{C}} \) (75 MHz, D\(_2\)O) 171.7, 171.0, 170.5, 170.3 (4 x CH\(_3\)C(O)O), 79.2 (C-1), 73.9 (C-5), 71.4 (C-3), 67.2 (C-2), 65.7 (C-4), 59.9 (C-6), 18.2, 18.1 (2C), 18.0 (4 x CH\(_3\)C(O)O).
Potassium metabisulfite (1.15 g, 6.0 mmol, 1.0 eq) was added to water (75 mL) and the resulting solution was heated, with stirring, to 75 °C. DCM (100 mL) was added and then 50 (3.0 g, 6.0 mmol, 1.0 eq), and the biphasic mixture heated under reflux for 20 min. Upon cooling, the organic layer was separated, washed with water (3 × 100 mL), dried over magnesium sulfate and the solvent removed under reduced pressure to yield a colourless oil that crystallised upon cooling to -20 °C. The solid was recrystallised from methanol yielding 21 (1.65 g, 76%) as a colourless solid: mp 74-76 °C [lit.\textsuperscript{165} 75 °C]; [\alpha]_D\textsuperscript{20} +5.0 (c 5.0, CHCl\textsubscript{3}); \nu_{\text{max}} (nujol)/cm\textsuperscript{-1} 2920 (CH), 2580 (SH), 1740 (C=O); \delta_H (300 MHz, CDCl\textsubscript{3}) 5.16-5.01 (2H, m, CH\textsubscript{3}3,4), 4.90 (1H, dd, J 9.3, 9.1 Hz, CH-2), 4.49 (1H, dd, J 9.1, 9.1 Hz, CH-1), 4.18 (1H, dd, J 12.6, 4.9 Hz, CH\textsubscript{2}6a), 4.05 (1H, dd, J 12.6, 2.4 Hz, CH\textsubscript{2}6b), 3.70-3.67 (1H, m, CH-5), 2.03, 2.02, 1.96 1.94 (12H, 4 × s, CH\textsubscript{3}C(O)O); \delta_C (75 MHz, CDCl\textsubscript{3}) 171.0, 170.5, 170.0, 169.7 (4 × CH\textsubscript{3}C(O)O), 79.1 (C-1), 77.5 (C-5), 76.7, (C-3), 73.9, (C-2), 68.5 (C-4), 62.4 (C-6), 21.2, 21.1 (2C), 20.1 (4 × CH\textsubscript{3}C(O)O); m/z (ES)+ 387 (100%, [M+Na]+).
**Experimental**

**1-Bromopent-4-ene**,\(^{69,164}\) 53

\[ \text{Br} \]

Method 1.\(^{164}\) Carbon tetrabromide (8.70 g, 26.6 mmol, 1.1 eq) was added to a solution of 4-pentene-1-ol 51 (2.00 g, 23.8 mmol, 1.0 eq) in DCM (10 mL) and the solution was cooled to 0 °C before the portionwise addition of triphenylphosphine (6.82 g, 26.0 mmol, 1.1 eq). After 4 h stirring at room temperature, the solution was reduced in volume and the residue was poured into petroleum ether (30 mL). After 5 min the resulting precipitate was filtered and washed with further petroleum ether. The resulting brown oil was fractionally distilled to give the 53 (1.64 g, 48%) as a colourless oil.

Method 2.\(^{69}\) 1,5-Dibromopentene (20.0 g, 87.8 mmol, 1.0 eq) was added to a distillation apparatus and heated to 195 °C. HMPA (18.9 g, 105.3 mmol, 1.2 eq) was added dropwise over 1 h via a syringe pump and 53 (9.51 g, 73%) was collected in the receiver flask as a colourless oil: bp 122-125 °C [Lit.\(^{69}\) bp 122-124 °C]; \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3100 (HC), 1640 (C=C), 630 (C-Br); \(\delta_H\) (300 MHz, CDCl\(_3\)) 5.82 -5.69 (1H, ddt, \(J\) 16.7, 10.6, 6.4 Hz, CH-4), 5.03-4.96 (2H, m, CH\(_2\)-5), 3.34 (2H, t, \(J\) 6.4 Hz, CH\(_2\)-1), 2.18-2.09 (2H, m, CH\(_2\)-3), 1.87 (2H, m, CH\(_2\)-2); \(\delta_C\) (75 MHz, CDCl\(_3\)) 137.1 (C-4), 116.3, (C-5), 33.5, 32.4, 32.2.
4-Bromopent-1-ene 53 (4.0 g, 27.0 mmol, 1.0 eq) was added to a stirred solution of sodium nitrite (2.23 g, 32.4 mmol, 1.2 eq) in dry DMF (50 mL) at 0 °C, and reaction mixture was stirred for 3 h at ambient temperature. The reaction mixture was then poured into an ice-water slurry and once most of ice had melted the product was extracted into diethyl ether (3 × 150 mL). The organic extracts were then combined and washed with water (100 mL) before drying over magnesium sulfate and the solvent removed under reduced pressure. The crude yellow oil was purified by flash column chromatography (5 : 95, diethyl ether : petroleum ether) to give 54 (1.15 g, 37%) as a colourless oil: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3080 (HC), 2920 (CH), 1640 (C=C), 1580 (NO$_2$), 1350 (NO$_2$); $\delta_H$ (300 MHz, CDCl$_3$) 5.74 (1H, ddt, $J_{16.7, 10.4, 6.7}$ Hz, CH-2), 5.10-5.01 (2H, m, CH$_2$-1), 4.36 (2H, t, $J_{6.8}$ Hz, CH-5), 2.18-2.03 (4H, m, CH$_2$-3,4); $\delta_C$ (75 MHz, CDCl$_3$) 136.2 (C-2), 117.1 (C-1), 75.1 (C-5), 30.6 (C-4), 26.7 (C-3).
**2-Iodoxybenzoic Acid (IBX)**

2-Iodobenzoic acid (50 g, 0.20 mol, 1.0 eq) was added to a solution of oxone (181.0 g, 0.29 mol, 1.5 eq) in distilled water (650 mL) and the resulting slurry was mechanically stirred at 73 °C for 5 h. After this time the suspension was cooled to 5 °C for 1.5 h. The resulting precipitate was isolated by filtration and washed with water (6 × 100 mL), acetone (2 × 100 mL) and allowed to dry at room temperature for 16 h, yielding IBX (45.9 g, 81%) as a colourless solid; δ\textsubscript{H} (300 MHz, CDCl\textsubscript{3}) 8.13 (1H, d, J 8.0 Hz, CH-2), 8.05-7.94 (2H, m, CH-3, 5), 7.86-7.79 (1H, m, CH-4) and 2.49 (1H, br s, OH).

**Pent-4-enal**, 56

Method 1: IBX (39.6 g, 141.4 mmol, 2.0 eq) was added in a single portion to a solution of 4-penten-1-ol (6.1 g, 7.3 mL, 70.7 mmol, 1.0 eq) in DCM (200 mL) and the resulting slurry was heated under reflux for 48 h. Upon cooling the slurry was filtered and the residue washed with DCM (2 × 150 mL). The filtrate was concentrated under reduced pressure to yield 56 (4.8 g, 99%) as a colourless oil.

Method 2: Allyl vinyl ether 58 (10.0 g, 118.9 mmol, 1.0 eq) was heated in a sealed tube for 15 h at 150 °C to obtain 56 (9.9 g, 99%) as a colourless oil; δ\textsubscript{H} (300 MHz, CDCl\textsubscript{3}) 9.72 (1H, t, J 1.5 Hz, CH-1), 5.78 (1H, ddt, J 16.8, 10.3, 6.7 Hz, CH-4), 5.05-4.93 (2H, m, CH\textsubscript{2}-5), 2.62-2.58 (2H, m, CH\textsubscript{2}-2), 2.38-2.30 (2H, m, CH\textsubscript{2}-3); δ\textsubscript{C} (75 MHz, CDCl\textsubscript{3}) 201.5 (C-1), 136.5 (C-4), 116.9, (C-5), 43.3 (C-2), 27.1 (C-3).
General procedure A: Oxime formation

Sodium acetate (1.0 eq) and hydroxylamine hydrochloride (1.0 eq) were added to a solution of aldehyde (1.0 eq) in acetonitrile and water (2:1). The resulting biphasic solution was allowed to stir at room temperature for 16 h before the organic layer was separated. The aqueous layer was extracted with DCM (3 ×) before the organic fractions were combined and washed with saturated sodium hydrogen bicarbonate and brine. The organic layer was then dried over magnesium sulfate and the solvent removed under reduced pressure to yield the oxime.

Pent-4-enal oxime, 57

General procedure A was employed using 56 (8.00 g, 95.2 mmol, 1.0 eq), sodium acetate (9.40 g, 114.1 mmol, 1.2 eq) and hydroxylamine hydrochloride (6.6 g, 95.3 mmol, 1.0 eq) in water (48 mL) and acetonitrile (96 mL) to obtain 57 (6.88 g, 73%) as a pale yellow oil and as a 1 : 1 mixture of E : Z isomers: ν\text{max} (film)/cm\(^{-1}\) 3280 (N-OH), 2980 (CH), 2920 (CH), 1640 (CN); δ\text{H} (300 MHz, CDCl\(_3\)) 7.43 (1H, t, J 5.8 Hz, CH-1 \text{anti}), 6.72 (1H, t, J 5.2 Hz, CH-1 \text{syn}) 5.88-5.72 (1H, m, CH-4), 5.10-4.95 (2H, m, CH\(_2\)-5), 2.52-2.33 (2H, m, CH\(_2\)-3), 2.32-2.20 (2H, m, CH\(_2\)-2); δ\text{C} (75 MHz, CDCl\(_3\)) 149.8 (C-1 \text{anti}), 149.4 (C-1 \text{syn}), 135.0 (C-4), 134.7(C-4), 113.8 (C-5), 113.7 (C-5), 31.9 (C-3), 30.9 (C-3), 28.8 (C-2), 28.1 (C-2).
General procedure B: Thiohydroximate formation

NaOCl (5.0 eq) was added to a solution of oxime (1.0 eq) in DCM in a separating funnel. The mixture was shaken and a colour change from colourless to vibrant blue to pale yellow was observed. The DCM layer was then added directly to a stirred solution of 21 (0.6 eq) in DCM before the addition of triethylamine (3.0 eq). The reaction was allowed to stir at room temperature until completion. The solution was washed with H$_2$SO$_4$ (0.5 M, 3 ×), water (3 ×) and brine and dried over magnesium sulfate. The solvent was removed under reduced pressure to yield the crude thiohydroximate. Purification was carried out using flash chromatography (with a gradient of ethyl acetate: petroleum ether), unless otherwise stated.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-pen-4-ene thiohydroximate, 47

General procedure B was employed using 57 (1.0 g, 10.1 mmol, 1.0 eq) in DCM (40 mL), NaOCl (31.0 mL, 50.5 mol, 1.6 M, 5.0 eq), 21 (2.2 g, 6.0 mmol, 0.6 eq) and triethylamine (3.0 g, 4.2 mL, 30.3 mmol, 3.0 eq). The reaction was allowed to stir for 3 h and was purified using flash chromatography (40:60, ethyl acetate: petroleum ether) to yield 47 (2.9 g, 63%) as a colourless solid: mp 148-152 °C; $[\alpha]_D^{30}$ -17.4 (c 1.0, CHCl₃); νmax (KBr)/cm⁻¹ 3320 (NOH), 3080 (CH), 2920 (CH), 2850 (CH), 1750 (CO), 1640 (C=C), 1620 (CN); δH (300 MHz, CDCl₃) 5.79 (1H, ddt, J 16.7, 10.6, 6.4 Hz, CH-10), 5.23-5.17 (1H, m, CH-3), 5.06-4.93 (5H, m, CH₂-11 and CH-1,2,4), 4.15-4.03 (2H, m, CH₂-6), 3.71-3.65 (1H, m, CH-5), 2.65-2.46 (2H, m, CH₂-8), 2.39-2.32 (2H, m, CH₂-9), 2.02, 1.98, 1.97, 1.94 (12H, 4 × s, CH₃C(O)O); δC (75 MHz, CDCl₃) 170.2, 169.4, 169.2 (4 × CH₃C(O)O), 156.9 (C-7), 136.6 (C-10), 115.9 (C-11), 79.9 (C-1), 76.0 (C-5), 73.8 (C-3), 70.1 (C-2), 68.1 (C-4), 62.3 (C-6), 31.9 (C-8), 30.8 (C-9), 20.7, 20.6 (2C), 20.5 (4 × CH₃C(O)O); m/z (ES⁺) 484 (100%, [M+Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ C₁₆H₂₅NO₁₀NaS 484.1243, found 484.1253.
General procedure A was employed using 3-phenylpropanal (1.0 g, 7.5 mmol, 1.0 eq), hydroxylamine hydrochloride (0.52 g, 7.5 mmol, 1.0 eq), sodium acetate (0.73 g, 8.9 mmol, 1.2 eq), acetonitrile (20 mL) and water (10 mL) to obtain 76 (0.80 g, 72%) as a pale yellow solid and 1 : 1 mixture of E : Z isomers: mp 86-88°C [lit.\textsuperscript{170} 85.5-88°C]; $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3200 (NO), 3060 (Ar-CH), 2860 (CH), 1660 (C=N); $\delta$\textsubscript{H} (300 MHz, CDCl\textsubscript{3}) 7.46 (1H, t, $J$ 7.0 Hz, CH-1), 7.35-7.27 (2H, m, 2 × CH-5), 7.25-7.18 (3H, m, 2 × CH-6, CH-7), 6.77 (1H, t, $J$ 3.5 Hz, CH-1 syn), 2.88-2.79 (2H, m, CH\textsubscript{2}-3), 2.77-2.68 (1H, m, CH\textsubscript{2}-2a), 2.58-2.48 (1H, m, CH\textsubscript{2}-2b); $\delta$\textsubscript{C} (75 MHz, CDCl\textsubscript{3}) 152.2 (C-1), 151.8 (C-1), 141.0 (C-4), 140.9 (C-4), 128.9 (C-5), 128.8 (C-6), 126.7 (C-7), 33.2 (C-3), 32.4 (C-3), 31.6(C-2), 26.8 (C-2); $m/z$ (ES\textsuperscript{+}) 172 (100%, [M+Na]\textsuperscript{+}).
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-3-phenylpropane thiohydroximate,\textsuperscript{169} \textsuperscript{65}

General procedure B was employed using using \textbf{21} (0.21 g, 0.6 mmol, 0.6 eq), 3-phenylpropanal oxime (0.14 g, 1.0 mmol, 1.0 eq), NaOCl (3.1 mL, 1.6 M, 5.0 mmol, 5.0 eq), triethylamine (0.42 mL, 3.0 mmol, 3.0 eq) and DCM (15 mL) to obtain \textbf{65} (0.23 g, 76\%) as a colourless solid; mp 196-198\°C [lit.\textsuperscript{172} 198 \°C]; \([\alpha]_D^20\) -17.0 (c 0.5, CHCl\textsubscript{3}); \(\nu_{\max}\) (KBr)/cm\textsuperscript{-1} 3310 (NOH), 3030 (C=C), 1710 (C=O), 1600 (CN); \(\delta\textsubscript{H}\) (300 MHz, CDCl\textsubscript{3}), 7.28-7.10 (5H, m, CH-11,12,13), 5.20-5.13 (1H, m, CH-3), 5.03-4.89 (3H, m, CH-1,2,4), 4.06 (1H, dd, J 12.1, 5.7 Hz, CH\textsubscript{2}-6a), 4.02 (1H, dd, J 12.1, 2.1 Hz, CH\textsubscript{2}-6b), 3.66-3.58 (1H, m, CH-5), 2.96-2.64 (4H, m, CH\textsubscript{2}-8,9). 1.99, 1.96, 1.94, 1.85 (12H, s, 4 \times CH\textsubscript{3}C(O)O); \(\delta\textsubscript{C}\) (100 MHz, CDCl\textsubscript{3}) 171.2, 170.8, 169.9, 169.8 (4 \times CH\textsubscript{3}C(O)O), 152.2 (C-7), 140.9 (C-10), 129.1 (2C, C-12), 128.7 (2C, C-11), 126.9 (C-13), 80.3 (C-1), 76.5 (C-5), 74.1 (C-3), 70.5 (C-2), 68.4 (C-4), 62.6 (C-6), 34.8 (C-8), 33.5 (C-9), 21.0, 20.9 (4 \times CH\textsubscript{3}C(O)O); \(m/z\) (ES\textsuperscript{+}) 534 (100\%, [M+Na]\textsuperscript{+}).
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-octane thiohydroximate, 66

General procedure B was employed using octanal oxime (1.44 g, 10.1 mmol, 1.0 eq), DCM (10 mL), NaOCl (31.0 mL, 50.5 mmol, 1.6 M, 5.0 eq), 21 (2.20 g, 6.0 mmol, 0.6 eq) and triethylamine (3.00 g, 4.2 mL, 30.0 mmol, 3.0 eq) in DCM (40 mL). Purification by flash chromatography (40 : 60, ethyl acetate : petroleum ether) gave 66 (2.3 g, 78%) as a colourless solid; mp 122-124°C [lit.172 127°C]; νmax (KBr)/cm⁻¹ 3300 (OH), 2930 (CH) 1730 (CO), 1600 (C=N); δH (300 MHz, CDCl₃) 5.40 (1H, dd, J 9.0 Hz, CH-3), 5.24 (1H, d, J 10.0 Hz, CH-1), 5.05 (2H, m, CH-2,4), 4.25 (1H, dd, J 12.4, 4.6 Hz, CH₂-6a), 4.13 (1H, m, dd, J 2.3, 12.4 Hz, CH₂-6b), 3.98 (1H, m, CH-5), 2.55 (2H, t, J 6.1 Hz, CH₂-8), 2.07, 2.04, 2.05, 2.07 (12H, 4 × s, CH₃C(O)O), 1.70-1.52 (2H, m, CH-9), 1.45-1.30 (8H, m, CH-10,11,12,13), 0.96 (3H, m, CH₃-14). m/z (ES⁺) 528 (100%, [M+Na⁺])
**S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-pen-4-yne thiohydroximate, 67**

General procedure A was employed using pen-4- nal (1.02 g, 12.4 mmol, 1.0 eq) in acetonitrile: water (2 : 1, 20 : 10 mL), sodium acetate (1.22g, 14.9 mmols, 1.2 eq) and hydroxylamine hydrochloride (0.86 g, 12.4 mmol, 1.0 eq) to yield pen-4-ynal oxime (1.06 g, 88%) as a mixture of E : Z isomers and as a yellow oil which was used without further purification.

General procedure B was employed using pent-4-ynal oxime (1.0 g, 10.3 mmol, 1.0 eq), NaOCl (32.0 mL, 51.4 mmol, 5.0 eq), 21 (2.2 g, 6.2 mmol, 0.6 eq) and triethylamine (3.1 g, 4.27 mL, 30.9 mmol, 3.0 eq) in DCM (15 mL) to obtain the product as an off-white solid which was purified by flash chromatography (30 : 70, ethyl acetate : petroleum ether) to obtain 67 (2.12 g, 77%) as white crystalline solid: mp 262-264 °C; [α]_D^20 -22.6 (c 1.0, CHCl_3); ν_{max} (nujol)/cm\(^{-1}\)

3290 (NOH), 2920 (CH), 1640 (CN), 1460; δ_\(H\) (300 MHz, CDCl_3) 5.35-5.24 (1H, m, CH-3), 5.17-5.06 (3H, m, CH-1,2,4), 4.26-4.18 (2H, m, CH-2-6), 3.83 (1H, ddd, J 9.3, 5.4, 2.3 Hz, CH-5), 2.93-2.73 (2H, m, CH-2-8), 2.65-2.55 (2H, m, CH-2-9), 2.14, 2.08 (6H, 2 × s, 2 × CH_3C(O)O), 2.06-2.02 (7H, m, 2 × CH_3C(O)O and CH-11); δ_\(C\) (75 MHz, CDCl_3) 170.7, 170.2, 169.4, 169.2 (4 × CH_3C(O)O), 150.7 (C-7), 82.7 (C-10), 80.0 (C-1), 76.2 (C-11), 75.9 (C-5), 73.7 (C-3), 70.1 (C-2), 68.1 (C-4), 62.3 (C-6), 31.7 (C-9), 29.8 (C-8), 20.8 (2C), 20.5 (2C) (4 × CH_3C(O)O); m/z (ES\(^{+}\)) 482 (100%, [M+Na\(^+\)); HRMS (ES\(^{+}\)) calculated [M+Na\(^+\] C_{19}H_{25}NO_{10}NaS 482.1097, found 482.1101.
4-Bromobenzaldehyde oxime

General procedure A was employed using 4-bromobenzaldehyde (5.0 g, 27.0 mmol, 1.0 eq), sodium acetate (2.7 g, 32.4 mmol, 1.2 eq) and hydroxylamine hydrochloride (1.9 g, 27.0 mmol, 1.0 eq) in water and acetonitrile (1 : 2, 40 : 80 mL). This gave 4-bromobenzaldehyde oxime (4.32 g, 79%) as a colourless solid and exclusively as the Z isomer: mp 110-112 °C [lit. 171 111-113 °C]; ν_{max} (nujol)/cm^{-1} 3660 (NO-H), 3050 (Ar-CH), 1650 (C=N), 930 (N-O); δ_{H} (300 MHz, CDCl₃) 7.98 (1H, s, CH₁); 7.44 (2H, d, J 8.3 Hz, CH₃,5), 7.52 (2H, d, J 8.3 Hz, CH₂,4); δ_{C} (75 MHz, CDCl₃) 149.8 (C-5), 132.5 (C-2), 131.5 (C-3), 128.9 (C-1), 124.7 (C-4); m/z (ES⁺) 200 (98%, [M+H]⁺, ⁷⁹Br), 202 (100%, [M+H]⁺, ⁸¹Br).
**Experimental**

\[ S-(2,3,4,6-Tetra-O-acetyl-\beta-D-glucopyranosyl) \quad Z-4-bromophenyl methane thiohydroximate, 68 \]

General procedure B was employed using 4-bromobenzaldehyde oxime (0.20 g, 1.00 mmol, 1.0 eq), NaOCl (3.10 mL, 1.6 M, 5.0 mmol, 5.0 eq). 21 (0.21 g, 0.6 mmol, 0.6 eq), triethylamine (0.3 g, 0.42 mL, 3.0 mmol, 3.0 eq) and DCM (15 mL) to obtain 68 (0.30 g, 92%) as a colourless solid: mp 156-160 °C; \([\alpha]_D^{20}\) +7.0 (c 1.0 in CHCl\(_3\)); \(v_{\text{max}}\) (KBr)/cm\(^{-1}\) 3320 (NOH), 2940 (C=O), 1620 (CN); \(\delta_H\) (300 MHz, CDCl\(_3\)) 7.52 (2H, d, \(J\ 8.0\) Hz, 2 × CH-9), 7.39 (2H, d, \(J\ 8.0\) Hz, 2 × CH-10), 5.04-4.90 (3H, m, CH-1,2,3), 4.50-4.41 (1H, m, CH-4), 4.06 (1H, dd, \(J\ 12.0\), 5.7 Hz, CH\(_2\)-6a), 3.92 (1H, dd, \(J\ 12.5\), 2.3 Hz, CH\(_2\)-6b), 3.16-3.10 (1H, m, CH-5), 2.04-1.92 (12H, 3 × s, 4 × CH\(_3\)C(O)); \(\delta_C\) (100 MHz, CDCl\(_3\)) 171.2, 170.8, 169.9, 169.8 (4 × CH\(_3\)C(O)), 151.0 (C-7), 132.4 (C-8), 132.1 (2C, C-9,10), 130.9 (C-11), 81.9 (C-1), 76.2 (C-5), 74.0 (C-3), 70.4 (C-2), 68.2 (C-4), 62.3 (C-6), 21.2, 21.0 (2C), 20.9 (4 × CH\(_3\)C(O)). m/z (ES\(^+\)) 584 (98%, [M+Na]\(^+\), \(^{79}\)Br), 586 (100%, [M+Na]\(^+\), \(^{81}\)Br); HRMS (ES\(^+\)) calculated [M+Na]\(^+\) \(\text{C}_{21}\text{H}_{24}\text{NO}_{16}\text{NaS}^{79}\text{Br}\) 584.0200, found 584.0168.
**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-4-pyridylmethane thiohydroximate, 69**

General procedure B was employed using 4-pyridinecarboxaldehyde oxime (0.12 g, 1.00 mmol, 1.0 eq), NaOCl (3.1 mL, 5.0 mmol, 5.0 eq), 21 (0.21 g, 0.60 mmol, 0.6 eq) and triethylamine (3.0 g, 0.42 mL, 3.0 mmol, 3.0 eq) in DCM (15 mL) to obtain the product as an off-white solid. Purification by flash chromatography (30:70, ethyl acetate : petroleum ether) gave 69 (0.18 g, 65%) a white crystalline solid: mp 138-142 °C; $\left[\alpha\right]_{D}^{20} +12.8$ (c 1.0, CHCl₃); $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3330 (NOH), 2940(C=C), 1610 (CN); $\delta$H (500 MHz, CDCl₃), 9.34 (1H, s, OH), 8.64 (2H, d, J 4.2 Hz, 2 × CH-10), 7.50 (2H, d, J 4.2, Hz, 2 × CH-9), 5.06-4.92 (3H, m, CH-1,3,2), 4.63-4.57 (1H, m, CH-4), 4.10-4.00 (1H, m, CH₂-6a), 3.92 (1H, dd, J 12.6, 2.1, Hz, CH₂-6b), 3.23-3.14 (1H, m, CH-5), 2.00 (6H, s, 2 × CH CH₃C(O)O), 1.97 (6H, s, 2 × CH₃C(O)O); $\delta$C (100 MHz, CDCl₃) 170.1, 170.0, 169.9, 169.8 (4 × CH₂C(O)O), 150.5 (C-7), 148.9 (2 × C-10), 135.7 (C-8), 123.4 (2 × C-9), 81.8 (C-1), 76.4 (C-5), 74.0 (C-3), 70.6 (C-2), 68.3 (C-4), 62.5 (C-6), 21.2 (4 × OC(O)CH₃); m/z (ES$^+$) 507 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{20}$H$_{24}$N$_{2}$O$_{10}$NaS 507.1049, found 507.1051
1H-Indole-3-carbaldehyde oxime, 171 72

General procedure A was employed using 1H-indole-3-carbaldehyde (1.00 g, 6.9 mmol, 1.0 eq), hydroxylamine hydrochloride (0.48 g, 6.9 mmol, 1.0 eq), sodium acetate (0.68 g, 8.3 mmol, 1.2 eq), acetonitrile (20 mL) and water (10 mL) to obtain 72 (0.58 g, 52%) as a pale pink solid and as a mixture of E : Z isomers: ν\text{max} (nujol)/cm\(^{-1}\) 3390 (OH), 3160 (NO), 2920 (CH), 640 (CN); δ\text{H} (300 MHz, CDCl\(_3\)) 10.71 (1H, br s, NH), 10.42 (1H, br s, NOH), 8.42, 8.39 (1H, 2 × s, CH-9), 7.83 (1H, dd, J 6.9, 1.6 Hz, CH-5), 7.73 (1H, s, CH-8), 7.51 (1H, d, J 7.6 Hz, CH-2), 7.43-7.14 (2H, m, CH-3,4); δ\text{C} (75 MHz, acetone) 145.7 (C-9), 139.5 (C-9), 131.6 (C-9), 127.6 (C-6), 123.4 (C-6), 123.0 (C-5), 121.1 (C-4), 118.9 (C-4), 112.5 (C-2), 107.8 (C-7); m/z (ES\(^{+}\)) 161 (100%, [M+H]\(^{+}\)).
2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl azide, 79

Sodium azide (0.23 g, 3.65 mmol, 1.0 eq) was added to a sealed tube containing a solution of 18 (1.50 g, 3.65 mmol, 1.0 eq) in acetone (9 mL) and water (1 mL). The resulting solution was heated to 70 °C for 16 h before allowing to cool to room temperature. The brown solution was poured into water (10 mL) and extracted with DCM (3 × 15 mL) before being dried over sodium sulfate and removing the solvent under reduced pressure. The pale yellow solid was triturated from diethyl ether before being isolated by filtration to obtain 79 (0.95 g, 69%) as a colourless solid. The product was stored at -20 °C to avoid decomposition: $\left[\alpha\right]_{D}^{20} -25.0$ (c 1.0, CDCl$_3$); $\delta$$_H$ (300 MHz, CDCl$_3$) 5.23 (1H, dd, $J$ 9.5, 9.5 Hz, CH-3), 5.17 (1H, dd, J 9.3, 9.3 Hz, CH-4), 4.99 (1H, dd, J 9.3, 9.3 Hz, CH-2), 4.68 (1H, dd J 9.0 Hz, H-1) 4.30 (1H, dd J 12.6, 4.9 Hz, CH$_2$-6a), 4.20 (1H, dd, J 12.6, 2.3 Hz, H-6b), 3.87 (1H, ddd, J 9.3, 4.9, 2.3 Hz, CH-5), 2.13, 2.11, 2.06, 2.04 (12H, s, CH$_3$C(O)O); $\delta$$_C$ (75 MHz, CDCl$_3$) 170.6, 170.1, 169.3, 169.2 (4 × CH$_3$C(O)O), 88.0 (C-1), 74.0 (C-5), 72.6 (C-3), 70.7 (C-2), 67.9 (C-4), 61.7 (C-6), 20.7 (2C), 20.6 (2C) (4 × CH$_3$C(O)O); m/z (ES$^+$) 396 (100%, [M+Na]$^+$).
2,3,4,6-Tetra-\(O\)-acetyl-\(\beta\)-d-glucopyranosyl amine,\(^{173}\) 75

A solution of 2,3,4,6-tetra-\(O\)-acetyl-\(\beta\)-d-glucopyranosyl azide 79 (100 mg, 0.268 mmol, 1 eq) in acetone (5.3 mL), was passed though a 10% Pd/C cartridge using the H-Cube. The solvent was removed under reduced pressure to obtain 75 (90 mg, 96%) as a colourless solid which did not require further purification; mp 110-114 °C [lit.\(^{176}\) 113-116 °C]; \(\nu\max(KBr)/\text{cm}^{-1}\) 3480 (NH), 2970 (CH), 2980, 2120, 1750 (CO), 1370, 1050; \(\delta_H\) (300 MHz, CDCl\(_3\)) 5.27 (1H, t, \(J_{9.5}\) Hz CH-3) 5.02 (1H, dd, \(J_{9.9}\), 9.9 Hz, CH-2), 4.85 (1H, dd, \(J_{9.3}\), 9.3 Hz, CH-4), 4.25-4.20 (2H, m, CH\(_2\)-6a and CH-1), 4.12 (1H, dd, \(J_{12.6}\), 2.4 Hz, CH\(_2\)-6b), 3.71 (1H, ddd, \(J_{9.4}\), 4.9, 2.4 Hz, CH-5) 2.11, 2.09, 2.04, 2.03 (4 \(\times\) 3H, s, CH\(_3\)C(O)O); \(\delta_C\) (75 MHz, CDCl\(_3\)) 170.7, 170.3, 170.2, 169.6 (4 \(\times\) CH\(_3\)C(O)O), 84.9 (C-1), 72.3 (C-3), 72.0 (C-5), 71.1 (C-2), 67.2 (C-4), 61.5 (C-6) 20.7 (2C), 20.6 (2C) (4 \(\times\) CH\(_3\)C(O)O); \(m/z\) (ES\(^+\)) 348 (100%, [M+H]\(^+\)).
**Experimental**

[S-1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose,\(^{174}\) 49](#)

![Chemical Structure](image)

A slurry of D-glucose (200 g, 1.1 mmol, 1.0 eq), sodium acetate (41 g, 0.5 mmol, 0.5) and acetic anhydride (733 mL, 7.8 mmol, 7.0 eq) was heated to 120 °C with vigorous stirring. Upon dissolution the yellow reaction mixture was carefully poured onto an ice-water slurry. After 2 h of stirring 49 precipitated from the solution as a chalky colourless solid, which was recrystallised from ethanol to obtain 49 (373 g, 87%) as a colourless crystalline solid and as a 1 : 9 mixture of α : β: \(\nu_{\text{max}}\) (KBr)/cm\(^{-1}\) 2920 (CH), 2850, 1750 (CO); \(\delta_H\) (300 MHz, CDCl\(_3\)) 6.33 (1H, d, \(J\) 3.7 Hz, CH-1), 5.72 (1H, d, \(J\) 8.2 Hz, CH-1), 5.29-5.10 (3H, m, CH-2,3,4), 4.21 (2H, m, CH-6), 3.85 (1H, ddd, \(J\) 9.9, 4.5, 2.2 Hz, CH-5), 2.19-2.02 (15H, m, 5 \(\times\) CH\(_3\)C(O)O).

[4-(tert-Butyldimethylsilyloxy)butan-1-ol,\(^{175}\) 89](#)

![Chemical Structure](image)

Butane-1,4-diol 86 (20.0 g, 222.0 mmol, 3.2 eq) was added to a solution of DMAP (0.90 g, 7.4 mmol, 0.1 eq) and triethylamine (9.1 g, 12.6 mL, 90.0 mmol, 1.3 eq) in DCM (70 mL) before the dropwise addition of TBS chloride (11.2 g, 70.0 mmol, 1.0 eq) in DCM (30 mL). The resulting solution was allowed to stir at room temperature for 16 h before washing with a saturated NaHCO\(_3\) solution (3 \(\times\) 50 mL), brine (2 \(\times\) 50 mL) and removing the solvent under reduced pressure. The resulting yellow oil was purified by flash chromatography (90 : 10, petroleum ether : ethyl acetate) to yield 89 (10.7 g, 75%) as a colourless oil: \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3350 (OH), 2930 (CH) 2860 (CH), 1250 (Si-CH\(_3\)), 1090 (Si-OR); \(\delta_H\) (300 MHz, CDCl\(_3\)) 3.60-3.52 (4H, m, CH\(_2\)-1,4), 3.15 (1H, br s, OH), 1.59-1.46 (4H, m, CH\(_2\)-2,3) 0.90 (9H, s, 3 \(\times\) CH\(_3\)-6), 0.00 (6H, s, 2 \(\times\) CH\(_3\)-5); \(\delta_C\) (75 MHz, CDCl\(_3\)) 63.7 (C-1), 63.2 (C-4), 30.7 (C-2), 30.3 (C-3), 26.4 (C-8),18.7 (C-7) -4.9 (C-5); m/z (ES\(^{+}\)) 227 (100%, [M+Na]\(^{+}\)).
4-( tert-Butyldimethylsilyloxy)butyraldehyde, 175 90

Method 1: A solution of 89 (1.0 g, 4.89 mmol, 1.0 eq) and DIPEA (1.89 g, 14.69 mmol, 3.0 eq) in DMSO:DCM (1:3, 5:15 mL) was cooled to 0 °C before the addition of pyridine sulfur trioxide (2.33 g, 14.69 mmol, 3.0 eq). The reaction mixture was allowed to stir at room temperature for two hours before diluting with diethyl ether (100 mL) and washing with 0.5 M HCl (3 × 50 mL), brine (2 × 50 mL) and drying over magnesium sulfate. The solvent was removed under reduced pressure to yield a colourless oil (0.83 g, 83%) that was used without further purification.

Method 2: To a solution of 89 (840 mg, 4.11 mmol, 1.0 eq) was added TEMPO (27 mg, 0.17 mmol, 0.04 eq) and the solution cooled to 0 °C. To this NaBr (68.4 mg, 0.67 mmol, 0.16 eq), NaOCl (1.6 M, 36 mL), water (11 mL) and saturated sodium hydrogen carbonate solution (11 mL) were added. The biphasic solution was allowed to stir for 10 min before the addition of methanol (10 mL). The organic layer was separated and the aqueous phase extracted with DCM (3 × 25 mL) and ethyl acetate (3 × 25 mL). The combined organics were dried over magnesium sulfate and the solvent removed under reduced pressure to give 90 (650 mg, 74%) as a colourless oil. ν_{max} (film)/cm^{-1} 2950, 2890, 1720 (C=O) 1260 (Si-CH₃), 1100 (Si-OR); δ_{H} (300 MHz, CDCl₃), 9.74 (1H, t, J 1.7 Hz, CH-1), 3.60 (2H, t, J 6.0 Hz, CH₂-4), 2.46 (2H, dt, J 7.1, 1.7 Hz, CH₂-2), 1.84 (2H, tt, J 6.0, 7.1 Hz, CH₂-3), 0.87 (9H, s, 3 × CH₃-6), 0.04 (6H, s, 2 × CH₃-5); δ_{C} (75 MHz, CDCl₃) 202.8 (C-1), 62.3 (C-4), 40.8 (C-2), 25.8, (C-3), 25.6, (C-6), 19.2 (C-7), -5.2 (C-5).
4-(tert-Butyldimethylsilyloxy)butyraldehyde oxime, 92

General procedure A was employed using 4-(tert-butyldimethylsilyloxy)butyraldehyde (2.00 g, 9.9 mmol, 1.0 eq), sodium acetate (0.97 g, 11.9 mmol, 1.2 eq), hydroxylamine hydrochloride (0.68 g, 9.9 mmol, 1.0 eq), acetonitrile (60 mL) and water (30 mL) to yield 92 (1.65 g, 77%) as a 1:1 mixture of E:Z isomers and as a yellow oil; \( \nu_{\text{max}} \text{(film)}/\text{cm}^{-1} \) 3270 (N-OH), 2930 (CH) 2880 (CH), 2850 (CH), 1660 (CN), 1250 (Si-CH\(_3\)), 1100 (Si-OR); \( \delta_H \) (300 MHz, CDCl\(_3\)) 7.47 (1H, t, J 6.0 Hz, CH-1\text{anti}), 6.72 (1H, t, J 5.4 Hz, CH-1\text{syn}), 3.64-3.58 (2H, m, CH-4), 2.52-2.47 (1H, m, CH\(_2\)-2a), 2.29-2.21 (1H, m, CH\(_2\)-2b), 1.75 (2H, m, CH\(_2\)-3) 0.08 (9H, s, CH\(_3\)-6) and 0.00 (6H, s, CH\(_3\)-5); \( \delta_C \) (75 MHz, CDCl\(_3\)) 152.3 (C-1\text{anti}), 151.9 (C-1\text{syn}), 62.8 (C-4), 62.4 (C-4), 29.8 (C-2), 29.3 (C-2), 26.5 (C-6), 26.2 (C-7), 25.9 (C-7), 22.3 (C-3), -3.4 (C-5), -5.1 (C-5); \( m/z \) (ES\(^+\)) 218 (100%, [M+H]\(^+\)); HRMS (ES\(^+\)) calculated [M+H]\(^+\) C\(_{10}\)H\(_{24}\)NO\(_2\)Si 218.1567, found 218.1576.
Experimental

S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-4-(tert-butyltrimethylsilyloxy)butane thiohydroximate, 70

General procedure B was employed using 92 (1.00 g, 4.62 mmol, 1.0 eq), NaOCl (14.3 mL, 23.1 mmol, 5.0 eq), 21 (1.01 g, 2.78 mmol, 0.6 eq) and triethylamine (1.93 g, 13.9 mmol, 3.0 eq) in DCM (80 mL). 70 (1.33 g, 83%) was obtained as a colourless oil: $\nu_{\text{max}}$ (film)/cm$^{-1}$ 3410 (N-OH), 2950 (CH) 2880 (CH), 2850 (CH), 1750 (C=O), 1600 (CN), 1230 (Si-CH$_3$), 1090 (Si-OR); $\delta$H (300 MHz, CDCl$_3$) 5.15-5.13 (1H, m, CH-1) 5.04-4.96 (2H, m, CH-2,3), 4.36-4.20 (1H, m, CH-4), 4.12 (1H, dd, $J$ 12.3, 5.2 Hz, CH$_2$-6a), 4.00 (1H, dd, $J$ 12.3, 2.3 Hz, CH$_2$-6b), 3.70-3.64 (1H, m, CH-5), 3.62-3.54 (2H, m, CH$_2$-10), 2.55-2.47 (2H, dd, $J$ 9.5, 6.1 Hz, CH$_2$-8), 1.97, 1.95, 1.93, 1.91 (12H, 4 x s, CH$_3$C(O)O), 1.83-1.71 (2H, m, CH$_2$-9), 0.81 (9H, s, CH$_3$-13), 0.00 (6H, s, CH$_3$-11); $\delta$C (75 MHz, CDCl$_3$) 170.6, 169.8 (2C), 169.6 (4 x CH$_3$C(O)O), 152.9 (C-7), 80.3 (C-1), 76.5 (C-5), 74.3 (C-3), 70.4 (C-2), 68.5 (C-4), 62.5 (C-6), 62.4 (C-10), 30.7 (C-8), 29.4 (C-12), 26.4 (C-9), 21.4, 21.1, 21.0, 20.9 (4 x CH$_3$C(O)O), 20.1 (C-13), -4.8 (C-11); $m/z$ (ES$^+$) 602 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{24}$H$_{41}$NNaO$_{11}$Si 602.2067, found 602.2064.
General procedure C: 2,2-Dimethoxypropane protection of thiohydroximates

p-Toluenesulfonic acid monohydrate (0.1 eq) and 2,2-dimethoxypropane (4.0-5.0 eq) were added to a solution of thiohydroximate (1.0 eq) in dry DCM (30 mL) at 0 °C. The resulting solution was allowed to warm to room temperature and stirred for 16 h. Sodium hydrogen carbonate solution (1 M, 50 mL) was used to wash the reaction mixture before the organic layer was dried and the solvent was removed under reduced pressure. Purification was undertaken using flash chromatography.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(2-methoxypropan-2-yl)-4-(tert-butyltrimethylsilyloxy)butane thiohydroximate, 93

General procedure C was employed using 70 (0.50 g, 0.86 mmol, 1.0 eq), DCM (30 mL), p-toluenesulfonic acid monohydrate (0.02 g, 0.10 mmol, 0.1 eq) and 2,2-dimethoxypropane (0.51 mL, 4.20 mmols, 4.8 eq). The solvent was reduced and the resulting brown syrup was purified via flash chromatography (30% Ethyl acetate: petroleum ether) to furnish 93 (0.51 g, 91%) as a colourless solid: mp 65-67 °C; $\left[\alpha\right]_D^{20}$ -21.0 (c 1.0, CHCl$_3$); $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3470 (NO), 2950 (CH), 2860, 1750 (CO), 1580, 1440, 1370, 1090 (Si-OR); $\delta_{\text{H}}$ (300 MHz, CDCl$_3$) 5.22–5.14 (1H, m, CH$_{-3}$), 5.05–4.96 (3H, m, CH-1,2,4) 4.15 (1H, dd J 12.6, 4.9 Hz, CH$_2$-6a), 4.06 (1H, dd, J 12.6, 2.3 Hz, CH$_2$-6b) 3.73-3.67 (1H, m, CH-5), 3.63-3.58 (2H, m, CH$_2$-10), 3.14 (3H, s, CH$_3$-13), 2.50-2.56 (2H, m, CH-8), 1.99, 1.97, 1.95, 1.93 (12H, 4 × s, CH$_3$C(O)O), 1.84-1.72 (2H, m, CH$_2$-9), 1.37 (6H, s, 2 × CH$_3$-1), 0.85 (9H, s, CH$_3$-16), 0.00 (6H, s, CH$_3$-14); $\delta_{\text{C}}$ (75 MHz, CDCl$_3$) 170.8, 170.4, 169.6, 169.4 (4 × CH$_3$C(O)O) 151.9 (C-7), 104.6 (C-11), 80.4 (C-1), 76.1 (C-5), 74.3 (C-3), 70.4 (C-2), 68.3 (C-4), 62.5 (C-6), 60.7 (C-10), 49.6 (C-13), 30.9 (C-8), 29.3 (C-9) 26.4 (C-16), 24.2 (C-12), 21.4 (C-15), 21.0, 20.9, 20.8, 20.7 (4 × CH$_3$C(O)O), -4.9 (C-14); $m/z$ (ES$^+$) 674 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{28}$H$_{46}$NO$_{12}$SSiNa 674.2642, found 674.2634.
**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-NO-4-acetyloxybutane thiohydroximate, 95**

TBAF (1.0 M in THF, 9.5 ml, 9.5 mmol, 2.5 eq) was added to a solution of 93 (2.50 g, 3.83 mmol, 1 eq) in THF (40 mL) at 0 °C, and resulting solution allowed to stir at room temperature for 3 h. The reaction was then quenched using saturated NH₄Cl solution (30 mL) and allowed to warm to room temperature. The resulting yellow solution was extracted into ethyl acetate (3 × 50 mL) and the combined organics were washed using brine (50 mL) before the solvent was removed under reduced pressure and the resulting oil was purified by column chromatography (30 : 70, ethyl acetate : petroleum ether) to yield 95 (0.69 g, 36%) as a colourless solid: mp 118-122°C; $[\alpha]_{D}^{20} -30.0$ (c 1.0, CHCl₃); $\nu_{\text{max}}$ (KBr)/cm⁻¹ 2920 (CH), 2850, 2730, 1750 (CO); $\delta_{H}$ (300 MHz, CDCl₃) 5.34-5.09 (4H, m, CH-1,2,3,4), 4.39-4.12 (4H, m, CH₂-6,10), 3.80 (1H, ddd, $J$ 10.3, 5.6, 2.3 Hz CH-5), 2.73-2.53 (2H, m, CH₂-8), 2.17-2.02 (17H, m, 5 × CH₃C(O)O, CH₂-9); $\delta_{C}$ (75 MHz, CDCl₃) 171.1, 170.6, 170.2, 169.4, 169.2 (5 × CH₃C(O)O), 151.1 (C-7), 80.9 (C-1), 76.2 (C-5), 73.9 (C-3), 70.1 (C-2), 68.0 (C-4), 62.1 (C-6), 61.6 (C-10), 29.3 (C-8), 26.04 (C-9), 20.9, 20.8 (5 × CH₃C(O)O); $m/z$ (ES⁺) 530 (100%, [M+Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ C₂₀H₂₉O₁₂NNaS 530.1306, found 530.1308.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(2-methoxypropan-2-yl)-4-hydroxybutane thiohydroximate, 96

TBAF (1.0 M in THF, 9.5 ml, 9.5 mmol, 2.5 eq) was added to a solution of 93 (2.50 g, 3.8 mmol, 1.0 eq) in THF (40 mL) at 0 °C under an inert atmosphere, and the resulting solution allowed to stir at 0 °C for 2 h. The reaction was then quenched at 0 °C using saturated NH₄Cl solution (30 mL) and the resulting solution was allowed to warm to room temperature with stirring. The solution was extracted using ethyl acetate (3 × 50 mL). The combined organics were further washed using brine (50 mL) and dried over magnesium sulfate. The solvent was removed under reduced pressure and the resulting oil was purified via column chromatography (40 : 60, ethyl acetate : petroleum ether) to yield 96 (1.6 g, 80%) as a colourless solid: mp 92-94 °C; [α]D^30^-9.7 (c 1.2, CHCl₃); νmax (nujol)/cm⁻¹ 3430 (OH), 2920 (CH), 1750 (CO); δH (300 MHz, CDCl₃) 5.20 (1H, dd J 9.0, 9.3, CH-3), 5.07-4.93 (3H, m, CH-1,2,4), 4.17-4.06 (2H, m, CH₂-6), 3.74 (1H, ddd, J 10.2, 4.9, 2.3 Hz, CH-5), 3.70-3.58 (2H, m, CH₂-10), 3.14 (3H, s, CH₃-OMe), 2.65 (2H, m, CH-8), 2.01, 1.98, 1.96, 1.95 (12H, s, CH₃C(O)O), 1.94-1.90 (2H, m, CH-9) and 1.38 (6H, s, 2 × CH₃); δC (75 MHz, CDCl₃) 171.0, 170.6, 169.8, 169.6 (4 × CH₃C(O)O), 152.3 (C-7), 104.9 (C-11), 170.6, 169.8, 169.6 (4 × CH₃C(O)O), 152.3 (C-7), 104.9 (C-11), 80.2 (C-1), 76.2 (C-5), 74.3 (C-3), 70.5 (C-2), 68.6 (C-4), 62.7 (C-6), 61.9 (C-10), 49.8 (C-13), 30.4 (C-8), 29.5 (C-9), 24.2 (C-12a), 24.1 (C-12b), 21.1 (2C), 20.9 (2C) (4 × CH₃C(O)O); m/z (ES⁺) 560 (100%, [M+Na⁺]); HRMS (ES⁺) calculated [M+Na⁺] C_{22}H_{35}NO_{12}NaS 560.1778, found 560.1783.
Experimental

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-NO-(2-methoxypropan-2-yl) 4-oxobutane thiohydroximate, 97

Method 1: IBX (156 mg, 0.55 mmol, 3.0 eq) and TEMPO (5.8 mg, 0.04 mmol, 0.2 eq) were added to a solution of 96 (100 mg, 0.18 mmol, 1.0 eq) in DCM (4 mL), and the resulting slurry heated under reflux for 3 h. After cooling to room temperature the slurry was passed through a plug of celite and washed repeatedly with DCM (100 mL). Solvent was reduced by 75% under a reduced pressure and washed with saturated sodium hydrogen carbonate (20 mL), dried over magnesium sulfate and then the solvent was removed under reduced pressure to yield 97 (98.6 mg, 99%) as a colourless solid. No further purification was undertaken. The material was stored at -20 °C to prevent decomposition.

Method 2: NMO (0.11 g, 0.94 mmol, 4.9 eq) and potassium carbonate (anhydrous, 1.0 g) were added to a solution of 105 (100 mg, 0.19 mmol, 1.0 eq) in DCM (50 mL). The slurry was cooled to -78 °C and a stream of oxygen was bubbled through. After 5 min the oxygen was replaced by a oxygen/ozone flow (50 mL, 30 W) until a strong blue colour remained. Ozone was flushed from the system using a stream of oxygen before flushing with a stream of nitrogen. The slurry was then allowed to warm to room temperature and stirred for a further 30 min before pouring the slurry into water (20 mL) and extracting with DCM (3 × 50 mL). The combined organic fractions were dried over magnesium sulfate and solvent removed under reduced pressure. 97 (35 mg, 35%) was isolated following chromatography (50:50, ethyl acetate: petroleum ether) as a colourless solid. The product was stored at -20 °C to prevent decomposition.
Experimental

mp 72-78 °C; \([\alpha]^{20}_D\) -23.0 (c 1.0, CHCl₃); \(\delta_H\) (300 MHz, CDCl₃) 9.82 (1H, s, CH-10), 5.29-5.21 (1H, m, CH-3), 5.10-5.01 (3H, m, CH-1,2,4), 4.15 (2H, m, CH-6), 3.77 (1H, ddd \(J\) 10.0, 5.4, 2.5 CH-5), 3.17 (3H, s, CH₃-13), 2.92-2.89 (4H, m, CH₂-8,9) 2.05, 2.03, 2.02, 2.01, (4 × 3H, s, CH₃C(O)O), 1.43 (6H, s, CH₃-11); \(\delta_C\) (75 MHz, CDCl₃) 200.7 (C-10), 170.9, 170.5, 169.8, 169.6, (4 × CH₃C(O)O) 149.9 (C-7), 105.0 (C-11), 80.3 (C-1), 76.5 (C-5), 74.2 (C-3), 70.4 (C-2), 68.5 (C-4), 62.6 (C-6), 49.7 (C-12), 40.3 (C-9), 29.7 (C-8), 24.1 (C-12a), 24.2 (C-12b), 21.1 (2C), 20.9 (2C) (4 × CH₃C(O)O); \(m/z\) (ES⁺) 558 (100%, \([M+Na]^+\)); HRMS (ES⁺) calculated \([M+Na]^+\) C₂₂H₃₃NO₁₂NaS 558.1621, found 558.1611.

General procedure D: Sulfation of a desulfoglucosinolate with a potassium counterion

A solution of desulfoglucosinolate (1.0 eq) in dry DCM was added dropwise to a slurry of pyridine.sulfur trioxide (5.0 eq) in anhydrous pyridine (10.0 eq). The resulting pink slurry was allowed to stir for 16 h. After this time the solvent was removed under reduced pressure before the residue was diluted with KHCO₃ (2M) allowed to stir for 30 min before the addition of diethyl ether followed by a further 30 mins stirring. The resulting precipitate was isolated by filtration and washed with KHCO₃ (2 M), water, ethanol and diethyl ether. The tetra-O-acetyl glucosinolate was isolated as its potassium salt.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-4-(tert-butyldimethylsilyloxy)butane thiohydroximate NO-sulfate, potassium salt, 104

General procedure D was employed using 2,3,4,6-tetra-O-acetyl-β-D-4-(tert-butyldimethylsilyloxy) butyl thiohydroximate (1.00 g, 1.72 mmol, 1.0 eq), pyridine sulfur trioxide complex (1.4 g, 8.6 mmol, 5.0 eq), pyridine (1.4 g, 17.3 mmol, 10.0 eq), DCM (8 mL) to yield 104 (0.49 g, 68%) as a colourless solid following purification by flash chromatography (10:90, ethanol: DCM); mp 180 °C decomposed; νmax (nujol)/cm⁻¹ 3440 (NO), 2920 (CH), 2850 (CH), 1740 (CO), 1650 (CN), 1220 (Si-CH₃), 1060 (Si-OR); δH (300 MHz, CD3CN) 5.24 (1H, t J 9.6 Hz, CH-3), 5.11 (1H, d, J 9.8 Hz, CH-1), 4.99-4.88 (2H, m, CH-2,4) 4.11-3.94 (2H, m, CH₂-6), 3.86-3.77 (1H, m, CH-5), 3.63 (2H, t, J 6.3 Hz, CH-10) 2.61-2.53 (2H, m, CH₂-8), 1.93-1.87 (12H, m, CH₃C(O)O), 1.81-1.72 (2H, m, CH₂-9), 0.80 (9H, s, CH₃-tBu), 0.00 (6H, s, CH₃-Si); δC (75 MHz, CDCl₃) 169.9, 169.5, 169.2, 169.0, (4 × CH₃C(O)O), 153.3 (C-7), 79.5 (C-1), 74.3 (C-5), 72.7 (C-3), 69.9 (C-2), 67.9 (C-4), 62.5 (C-10), 61.9 (C-6), 30.2 (C-8), 27.9 (C-9), 25.8 (C-13), 20.4(2C), 20.3 (2C), (4 × CH₃C(O)O), 17.9 (C-12), -5.3 (C-11) : m/z (ES⁺) 658 (100%, [M-K]⁺); Anal. Calc. (Found C, 40.9; H, 5.7; N, 2.0. C₂₄H₄₀KO₄S₂Si requires C, 41.3 H, 5.8, N, 2.0%)
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(2-methoxypropan-2-yl) pent-4-ene thiohydroximate, 105

General procedure C was employed using 47 (200 mg, 4.30 mmol, 1.0 eq), DCM (20 mL), p-toluenesulfonic acid monohydrate (8 mg, 0.43 mmol, 0.1 eq) and 2,2-dimethoxypropane (0.26 mL, 21.5 mmol, 5.0 eq). The resulting solution was allowed to stir for 3 h before the solvent was removed under reduced pressure. The resulting brown syrup was purified by column chromatography (30:70, ethyl acetate in petroleum ether) to yield 105 (0.17g, 77%) as a colourless solid: mp 102-106 °C; [α]D20-11.6 (c 1.0, CHCl3); δH (300 MHz, CDCl3) 5.86 (1H, ddt J 16.7, 10.3, 6.4 Hz, CH-10), 5.26 (1H, dd, J 9.3, 9.1 Hz, CH-3), 5.13-4.97 (5H, m, CH2-11 and CH-1,2,4), 4.23-4.07 (2H, m, CH2-6), 3.74 (1H, J 10.3, 4.9, 2.3 Hz, CH-5), 3.16 (3H, s, CH3-14) 2.76-2.53 (2H, m, CH2-8), 2.50-2.39 (2H, m, CH2-9), 2.06, 2.05, 2.04, 2.01 (4 × 3H, s, CH3C(O)O) and 1.46 (6H, s, 2 × CH3-13); δC (75 MHz, CDCl3) 170.6, 170.2, 169.4, 169.2 (4 × CH3C(O)O), 151.6 (C-7), 136.7 (C-10), 115.9 (C-11), 104.8 (C-12), 80.3 (C-1), 76.4 (C-5), 74.2 (C-3), 70.5 (C-2), 68.5 (C-4), 62.7 (C-6), 49.3 (C-14), 32.9 (C-9), 31.3 (C-8), 24.8 (C-13), 21.1, 21.0 (2C), 20.9 (4 × CH3C(O)O); m/z (ES+) 556 (100%, [M+Na]+); HRMS (ES+) calculated [M+Na]+ C23H35NO11NaS 556.1841, found 556.1829
A solution of tert-butyldimethylsilyl chloride (0.59 g, 3.93 mmol, 1.0 eq) in dry DCM (20 mL) was added to a solution of 47 (2.00 g, 4.33 mmol, 1.1 eq) and imidazole (0.59 g, 8.66 mmol, 2.2 eq) in dry DCM (30 mL) at 0 °C and under a nitrogen atmosphere. The reaction was allowed to stir for 3 h at 0 °C, under an inert atmosphere, before being quenched using saturated sodium bicarbonate (mL). After allowing to warm to room temperature. The aqueous layer was then extracted using DCM (3 × 20 mL) and the combined organic fractions were dried over magnesium sulfate. The solvent was removed under reduced pressure to yield a yellow oil. 106 (2.29 g, 92%) was isolated as colourless solid following flash chromatography (30:70, ethyl acetate : petroleum ether); mp 62-68 °C; $[\alpha]_{D}^{20} -10.2$ (c 1.0, CH$_3$Cl); $\nu$$_{\text{max}}$ (KBr)/cm$^{-1}$ 3340 (NO), 2910 (CH), 1730 (C=O), 1240 (Si-R); $\delta$$_{H}$ (300 MHz, CDCl$_3$) 5.72 (1H, ddt, $J$ 16.9, 10.6, 6.4 Hz CH-10), 5.10 (1H, dd, $J$ 9.3, 9.1 Hz, CH-3), 4.98-4.77 (5H, m, CH-1,2,4), 4.06-3.98 (2H, m, CH$_2$-6), 3.58 (1H, ddd, $J$ 10.1, 5.7, 2.3 Hz, CH-5), 2.59-2.35 (4H, m, CH$_2$-8,9), 1.90-1.85 (12H, m, CH$_3$C(O)O), 0.76 (9H, s, CH$_3$-14) and 0.00 (6H, s, CH$_3$-12); $\delta$$_{C}$ (75 MHz, CDCl$_3$) 170.9, 170.6, 169.7, 169.5 (4 × CH$_3$C(O)O), 155.6 (C-7), 137.5 (C-10), 116.0 (C-11), 80.3 (C-1), 77.0 (C-5), 76.4 (C-3), 74.3 (C-2), 68.5 (C-4), 62.8 (C-6) 31.8 (C-9), 31.1 (C-8), 26.4 (C-12), 21.5, 21.1, 21.0, 20.9 (4 × CH$_3$C(O)O), 18.6 (C-13), -3.18 (C-12a); m/z (ES$^+$) 598 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{25}$H$_{41}$NO$_{10}$NaSSi 598.2118, found 598.2122.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(tert-butyldiphenylsilyloxy) pent-4-ene thiohydroximate, 107

A solution of tert-butyldiphenylsilyl chloride (1.30 g, 3.89 mmol, 1.0 eq) in dry DCM (20 mL) was added to a solution of 47 (2.00 g, 4.30 mmol, 1.1 eq) and imidazole (0.59 g, 8.60 mmol, 2.2 eq) in dry DCM (30 mL) at 0 °C. The reaction was allowed to stir for 4 h at 0 °C before being quenched using saturated sodium hydrogen carbonate solution (20 mL). After allowing to warm to room temperature, the aqueous layer was then extracted using DCM (3 × 20 mL) and the combined organic fractions were dried over magnesium sulfate and the solvent removed under reduced pressure to yield a yellow oil. Following flash chromatography (20:80, ethyl acetate : petroleum ether) 97 (2.63 g, 87%) was obtained as colourless crystalline solid; mp 68-72 °C: $[\alpha]_D^{20}$ -12.0 (c 1.0, CHCl$_3$); $\nu_{\text{max}}$ (nujol)/cm$^{-1}$ 3390 (NO), 2920 (CH), 1760 (CO), 1730, 1590, 1460, 1380, 1250 (Si-R), 1210, 1040 (Si-OR), 960, 810; $\delta_H$ (300 MHz, CDCl$_3$) 7.71-7.66 (4H, m, CH$_2$-Ar), 7.44-7.31 (6H, m, CH$_2$-Ar), 5.76 (1H, ddt, $J_{16.9, 10.6, 6.4}$ Hz, CH$_2$-10), 5.28 (1H, dd, J 9.3, 9.1 Hz, CH-3), 5.15-4.90 (5H, m, CH-1,2,4, CH$_2$-11), 4.20 (1H, dd, J 12.4, 5.8 Hz, CH$_2$-6a), 4.16-4.09 (1H, m, CH$_2$-6b), 3.74 (1H, ddd, J 10.3, 5.8, 2.3 Hz, CH-5), 2.75-2.53 (2H, m, CH$_2$-8), 2.42-2.38 (2H, m, CH$_2$-9), 2.06, 2.04, 2.03, 2.02 (12H, s, CH$_3$C(O)O), 1.08 (9H, s, CH$_3$-13); $\delta_C$ (75 MHz, CDCl$_3$) 170.6, 170.2, 169.4, 169.1 (4 × CH$_3$C(O)O), 156.7 (C-7), 137.0 (C-10), 135.5 (C-Ar), 133.3 (C-Ar, 133.2 (C-Ar), 129.7 (C-Ar), 127.6 (C-Ar), 115.6 (C-11), 80.1 (C-1), 76.1 (C-5), 73.8 (C-3), 70.2 (C-2), 68.2 (C-4), 62.3 (C-6), 31.5 (C-9), 30.8 (C-8), 26.9 (C-13), 20.7, 20.6 (4 × CH$_3$C(O)O), 19.5 (C-12); m/z (ES$^+$) 722 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{35}$H$_{45}$NO$_{10}$NaSSi 722.2431, found 722.2437.
S-(2,3,4,6-Tetra-O-acetyl-\(\beta\)-D-glucopyranosyl) \(Z\)-NO-(2-methoxypropan-2-yl) 4,5-epoxypentane thiohydroximate, 110

Sodium hydrogen carbonate (0.38g, 4.4 mmol, 12.0 eq) was added to a solution of 105 (0.20 g, 0.37 mmol, 1.0 eq) in acetone: water (2 : 1, 5.0 : 2.5 mL) and cooled to 0 °C before the addition of oxone (0.69 g, 4.5 mmol, 12 eq). The resulting slurry was allowed to stir at 2 h at 0 °C before warming to room temperature and stirring for 16 h. The slurry was filtered before the filtrate was added to DCM (20 mL) and water (10 mL). The organic layer was separated and washed further with brine (10 mL), dried using magnesium sulfate and the solvent removed at reduced pressure. 110 (0.15 g, 37%) was isolated as a colourless oil after column purification (50 : 50, ethyl acetate : petroleum ether) and as a mixture of diastereoisomers; \(\delta\)H (300 MHz, CDCl\(_3\)) 5.62 (1H, dd, \(J\) 9.3 Hz, CH-3), 5.28 (1H, dd \(J\) 9.3, 9.5 Hz, CH-4), 5.02 (1H, dd, \(J\) 9.0, 10.6 Hz, CH-2), 4.87 (1H, d, \(J\) 10.6 Hz, CH-1), 4.14 (1H, dd, \(J\) 12.6, 4.8 Hz, CH\(_2\)-6a), 4.02 (1H, dd, \(J\) 12.6, 2.3 Hz, CH\(_2\)-6b), 3.68-3.59 (1H, m, CH-5), 3.34 (3H, s, CH\(_3\)-14), 3.02-2.95 (3H, m, CH\(_2\)-10), 3.72-2.64 (3H, m, CH\(_2\)-8, CH-11a) 2.43 (1H, dd, CH-11b), 2.00, 1.98, 1.96 (CH\(_3\)C(O)O) 1.90-1.69 (2H, m, CH\(_2\)-9), 1.56 (3H, s, CH\(_3\)-13a) 1.48 (3H, s, CH\(_3\)-13b); \(\delta\)C (75 MHz, CDCl\(_3\)) 170.30, 169.1, 168.9, (4 \(\times\) CH\(_3\)C(O)O), 151.6 (C-7), 107.1 (C-12), 86.1, 84.5 (2 \(\times\) C-1), 81.0 (C-5), 73.2 (C-3), 67.5 (C-2), 66.5 (C-4), 61.5 (C-6), 50.9, 50.8, (2 \(\times\) C-10) 51.2 (C-11), 47.0, 46.9 (2 \(\times\) C-14), 31.9, 29.9, (2 \(\times\) C-8), 27.1, 27.0 (2 \(\times\) C-9), 24.2, 23.5 (2 \(\times\) C-13), 20.6, 20.5 (4 \(\times\) CH\(_3\)C(O)O); \(m/z\) (ES\(^+\)) 603 [M+MeOH+Na]\(^+\)
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-NO-(tert-butyldimethylsilyloxy) 4-hydroxy-5-acetoxypentane thiohydroximate, 116

To a solution of 106 (61 mg, 0.10 mmol, 1.0 eq) in MeCN : H₂O (8 : 2 mL) at 0°C was added RuCl₃ (1 mg) and NaIO₄ (33 mg, 0.15 mmol, 1.5 eq). The resulting solution was allowed to stir for 1 hr before being filtered through a plug of celite. The solvent was removed under vacuum and the resulting oil was purified by flash chromatography (40: 60, ethyl acetate : petroleum ether) to obtain a clear oil (9 mg, 13%) that was tentatively assigned as 116; δH (300 MHz, CDCl₃) 5.14-5.09 (1H, m, CH-1), 4.98-4.85 (3H, m, CH-2,3,4) 4.04-4.03 (2H, m, CH₂-6), 3.71-3.61 (3H, m, CH-5, CH₂-11), 3.38-3.31 (1H, m, CH-10), 2.61-2.32 (4H, m, CH₂-8,9), 1.93-1.85 (15H, m, 5 × CH₃C(O)O); m/z (ES⁺) 674 (100%, [M+Na]⁺).
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-NO-(tert-butylidimethylsilyloxy) 4,5-dihyroxypentane thiohydroximate, 117

A solution of potassium permanganate (142 mg, 0.89 mmol, 2.4 eq) in water (0.5 mL) was added, over 10 min, to a solution of 106 (200 mg, 0.37 mmol, 1.0 eq) and magnesium sulfate (162 mg, 1.34 mmol, 3.6 eq) at 0 °C in ethanol (3.5 mL), was added. The resulting brown slurry was allowed to stir overnight before being filtered though a plug of celite and washed repeatedly with ethanol. The solvent was removed under reduced pressure and 117 (223 mg, 99%) was achieved as a colourless oil. No further purification was undertaken.

\[ \alpha_d^{30} \] -13.8 (c 1.0, CHCl3); \( \nu_{\text{max}} \) (KBr)/cm\(^{-1} \) 3300 (OH); \( \delta_H \) (300 MHz, CDCl3) 5.14-5.09 (1H, m, CH-3), 4.98-4.85 (3H, m, CH-1,2,4), 4.10-3.98 (2H, m, CH2-6), 3.71-3.61 (3H, m, CH-5, CH2-11), 3.38-3.31 (1H, m, CH-10), 2.71-2.53 (2H, m, CH-8), 1.93-1.87 (13H, m, 4 \( \times \) CH3C(O)O, CH2-9a), 1.73-1.63 (1H, m, CH-9b), 0.88 (9H, s, CH3-14), 0.00 (6H, s, CH3-12); \( \delta_C \) (75 MHz, CDCl3) 170.5, 169.9, 169.2, 169.0, (4 \( \times \) CH3C(O)O), 155.1, (C-7), 79.7 (C-1), 75.9 (C-5), 73.6 (C-3), 71.7 (C-2), 70.1 (C-10), 69.8 (C-4), 66.4 (C-11), 61.9 (C-6), 30.2 (C-8), 28.4 (C-9), 26.4 (C-14), 21.0, 20.6, 20.3, 19.9 (4 \( \times \) CH3C(O)O), 18.6 (C-13), -4.5 (C-12); \( m/z \) (ES\(^+\)) (100%, 632 [M+Na]\(^+\)); HRMS calculate [M+Na]\(^+\) C\(_{26}\)H\(_{43}\)NO\(_{12}\)NaSi 632.2173, found 632.2173.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(tert-butyldimethylsilyloxy) 3-carboxypropane thiohydroximate, 119

To a solution of 117 (30 mg, 0.05 mmol, 1.0 eq) in MeOH : H2O (1 : 0.5 mL), NaIO4 (27 mg, 0.01 mmol, 2.4 eq) was added and allowed at room temperature for 2 hr. The resultant solution was diluted with ethyl acetate (5 mL) and extracted with water (2 × 5 mL). The solvent was removed from the organic layer under vacuum to obtain a white solid (18 mg, 61%) that was tentatively assigned as 119; δH (300 MHz, CDCl3) 5.10-5.07 (1H, m, CH-1), 5.01-4.98 (3H, m, CH-2,3,4), 4.04-4.00 (2H, m, CH2-6), 3.69-3.72 (1H, m, CH-5), 2.97-2.78 (3H, m, CH2-8,9a), 2.56-2.34 (1H, m, CH2-9b), 1.92-1.81 (12H, m, CH3COO), 0.80 (9H, s, CH3-14), 0.00 (6H, s, CH3-12); m/z (ES⁺) 593 (100%, [M+Na]⁺);
**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-pent-4-ene thiohydroximate NO-sulfate, potassium salt, 126**

General procedure D was employed using pyridine · sulfur trioxide (0.52 g, 3.3 mmol, 5.0 eq), pyridine (0.51 g, 0.52 mL, 6.50 mmol, 10.0 eq), 47 (0.30 g, 0.65 mmol, 1.0 eq) and DCM (3 mL). The resulting precipitate was isolated by filtration and washed with KHCO$_3$ solution (2M, 3 mL), water (3 mL), ethanol (2 mL) and diethyl ether (3 mL) and then purified by flash chromatography (10 : 90, ethanol : DCM) gave 126 (0.31 g, 82%) as a colourless solid: mp 200-206 °C dec; $[\alpha]_D^{20}$ -8.0 (c 0.5, H$_2$O); $\nu_{\text{max}}$(nujol)/cm$^{-1}$ 3420 (N-O), 2950 (CH), 1750 (C=O), 1630 (CN), 1210 (C-O); $\delta_H$ (300 MHz, CD$_3$CN) 5.93 (1H, ddt, J 17.0, 10.4, 6.4 Hz, CH-10), 5.37-5.32 (1H, m, CH-1), 5.24 (1H, d, J 10.0 Hz, CH-3), 5.19-5.11 (1H, m, CH-2), 5.07-4.98 (3H, m, CH$_2$-11, CH-4), 4.17-4.05 (2H, m, CH$_2$-6), 3.98-3.91 (1H, m, CH-5), 2.75-2.69 (2H, m, CH$_2$-8), 2.47-2.39 (2H, m, CH$_2$-9), 2.06, 2.05 2.04, 2.01 (12H, 4 × s, CH$_3$C(O)O); (75 MHz, CDCl$_3$) 170.1, 169.3 (2C), 169.1 (4 × CH$_3$C(O)O), 163.0 (C-7), 135.4 (C-10), 114.9 (C-11), 80.1 (C-1), 75.9 (C-5), 72.5 (C-3), 70.1 (C-2), 68.1 (C-4), 61.9 (C-6), 31.0 (C-9), 29.8 (C-8), 20.7, 20.6 (2C), 20.5 (4 × CH$_3$C(O)O); $m/z$ (ES$^-$) 540 (100%, [M-K]$^-$); HRMS (ES-) calculated [M]$^-$ C$_{19}$H$_{28}$NO$_{13}$S$_2$ 540.0852, found 540.0846.
General procedure E: Sulfation of a thiohydroximate with a cyclohexylammonium counterion

A solution of desulfoglucosinolate (1.0 eq) in dry DCM was added dropwise to a slurry of pyridine·sulfur trioxide (5.0 eq) in anhydrous pyridine (10.0 eq). The resulting pink slurry was allowed to stir for 16 h. After this time the solvent was removed under reduced pressure before the residue was diluted with aqueous cyclohexylamine solution (5% w/w cyclohexylamine/water). The solution was allowed to stir for 30 min before the addition of diethyl ether, followed by a further 30 min stirring. The resulting precipitate was isolated by filtration and washed with aqueous cyclohexylamine solution (5% w/w cyclohexylamine/water) water, and diethyl ether. The resulting tetra-O-acetyl glucosinolate, as a cyclohexylammonium salt, was isolated as a solid.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-pent-4-ene thiohydroximate NO-sulfate, cyclohexylammonium salt, 129

General procedure E was employed using 47 (0.52 g, 1.12 mmol, 1.0 eq), DCM (5 mL) pyridine · sulfur trioxide complex (0.81 g, 5.60 mmol, 5.0 eq) and pyridine (0.90 mL, 11.2 mmol, 10.0 eq) to obtain 129 (0.46 g, 72%) as a pale yellow solid: mp 150-154 °C; $[\alpha]_D^{20} = -11.0$ (c 0.5, D$_2$O); $\nu_{\text{max}}$(KBr)/cm$^{-1}$ 2920 (CH), 2850 2730, 1740 (CO), 1580, 1510, 1460, 1380, 1220, 1150, 1060, 920; $\delta_H$ (300 MHz, CDCl$_3$) 5.80 (1H, ddt, J 17.0, 10.4, 6.4 Hz, CH-10), 5.20 (1H, dd, J 9.3, 9.1 Hz, CH-3), 5.07-4.90 (5H, m, CH-1,2,4, CH$_2$-11) 4.13-4.05 (2H, m, CH$_2$-6), 3.71 (1H, ddd, J 10.3, 5.4, 2.5 Hz, CH-5), 3.12-3.09 (1H, m, CH-1"), 2.74-2.52 (4H, m, CH$_2$-8,9), 2.45-2.33 (4H, m, CH$_2$-2"), 2.00, 1.99, 1.97, 1.94 (4 × 3H, s, 4 × CH$_3$(O)O), 1.76-1.69 (4H, m, CH$_2$-3"), 1.56-1.53 (2H, m, CH$_2$-4"a), 1.41-1.28 (1H, m, CH$_2$-4"a); $\delta_C$ (75 MHz, CDCl$_3$) 157.9 (C-7), 136.6 (C-10), 116.0 (C-11), 80.2 (C-1), 76.1 (C-5), 73.7 (C-3), 69.9 (C-2), 67.8 (C-4), 62.2 (C-6), 51.2 (C-1"), 32.0 (C-2C, C-2"), 31.2 (C-9), 30.7 (C-8), 24.6 (2C, C-3"), 24.3 (C-4"), 20.7, 20.7, 20.6, 20.5 (4 × CH$_3$(O)O); m/z (ES$^+$) 540 (100%, [M$^+$]); HRMS (ES$^+$) calculated C$_{19}$H$_{26}$NO$_{13}$S$_2$ 540.0852, found 540.0842.
General procedure F: Wittig reaction on intact glucosinolate

$n$BuLi (1.0 eq) was added to a stirred solution of Wittig salt (1.0 eq) in THF and allowed to stir at -78 °C for 1 h. following this a solution of aldehyde (1.0 eq) was added and the allowed to warm to 0°C and stirred until no aldehyde remained. Ammonia chloride was added to the solution and resulting biphasic solution allowed to warm to room temperature. Ethyl acetate was used to extract and upon drying over magnesium sulfate and concentration under reduced pressure. The resulting material was purified by column chromatography.
Experimental

S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-NO-(2-methoxypropan-2-yl) 5-phenylpent-4-ene thiohydroximate, 131

General procedure F was employed using 97 (600 mg, 1.12 mmol, 1.0 eq), nBuLi (1.6M in THF, 0.738 mL, 1.18 mmol, 1.0 eq) and benzyltriphenylphosphonium bromide (474 mg, 1.09 mmol, 0.97 eq). Following purification by flash column chromatography (30:70, ethyl acetate : petroleum ether) 131 (199 mg, 30%) was obtained as a colorless oil and as a mixture of diastereomers; [α]D20 -25.6 (c 0.3, CHCl3); νmax (KBr)/cm⁻¹ 3420 (N-O), 3120 (CH-C=C), 2970 (CH), 1730 (CO), 1640 (C=C), 1610 (CN); δH (300 MHz, CDCl3) 7.39-7.27 (3H, m, CH-14,15) 7.27-7.16 (2H, m, CH-13), 6.53-6.40 (1H, m, CH-10), 6.30-6.19 (1H, m, CH-11), 5.33-5.23 (1H, m, CH-3), 5.17-4.90 (3H, m, CH-1,2,4) 4.19-4.13(1H, m, CH2-6a), 4.09-4.00 (1H, m, CH2-6b), 3.78 (1H, m, CH-5), 3.21 (3H, s, CH3-19), 2.84-2.56 (4H, m, CH2-8,9), 2.09, 2.06, 2.04 (12H, s, 4 × CH3C(O)O), 1.47 (6H, s, 2 × CH3-18); δC (75 MHz, CDCl3) 170.6, 170.5, 169.5, 169.4, 169.2 (4 × CH3C(O)O), 150.8, 150.4 (2 × C-7), 137.3 (C-12), 131.2, 130.4 (2 × C-10), 128.7 (C-13), 128.4 (C-14),127.2, 127.1 (2 × C-11), 126.0 (C-15), 104.5 (C-17) 81.2, 80.8 (2 × C-1), 76.3, 75.8 (2 × C-5), 73.8, 73.6 (2 × C-3), 70.4, 70.1 (2 × C-2), 70.0, 69.9 (2 × C-2), 69.8, 68.6 (2 × C-4), 62.2, 61.9 (2 × C-6), 49.4, 45.3 (2 × C-19), 32.5, 32.3 (2 × C-8), 30.3, 29.7 (2 × C-9), 26.2, 25.4, 23.9, 23.8 (2 × C-18), 20.8, 20.7, 20.6 (4 × CH3C(O)O); m/z (ES⁺) 632 [M+Na]+; HRMS (ES⁺) calculated [M+Na]+ C29H39NO11NaS 632.2142, found 632.2140.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-5-phenylpent-4-ene thiohydroximate, \(132\)

131 (100 mg, 0.16 mmols, 1.0 eq) was added to a solution of Dowex-50W resin (20 mg) in methanol (2 mL) and stirred for 5 h. 132 (84 mg, 98\%) was isolated following filtration and removal of the solvent as a colourless oil and as inseparable mixture of diastereoisomers; \(\nu_{\text{max}}\) (KBr)/cm\(^{-1}\) 2960 (CH), 1760 (CO), 1620 (CN); \([\alpha]_D^{20}\) -11.5 (c 0.2 in CHCl\(_3\)); \(\delta_H\) (300 MHz, CDCl\(_3\)) 7.39-7.27 (3H, m, 2 × CH-14, CH-15), 7.27-7.16 (2H, m, 2 × CH-13), 6.51-6.39 (1H, m, CH-10), 6.30-6.19 (1H, m, CH-11), 5.32-5.21 (1H, m, CH-3), 5.17-4.90 (3H, m, CH-1,2,4) 4.19-4.08 (2H, m, CH-6), 3.78-3.70 (1H, m, CH-5), 2.79-2.50 (4H, m, CH\(_2\)-8,9), 2.07, 2.04, 2.02 (12H, s, 4 × CH\(_3\)C(O)O); \(\delta_C\) (75 MHz, CDCl\(_3\)) 169.5, 169.5, 169.4, 169.2 (4 × CH\(_3\)C(O)O), 151.8, 151.4 (2 × C-7), 137.3 (C-12), 131.3, 130.4 (2 × C-10), 129.0, 128.7 (2 × C-14), 128.4, 128.3 (2 × C-13), 127.4, 127.1 (2 × C-11), 126.0 (C-15) 81.2, 80.8 (2 × C-1), 76.2, 75.9 (2 × C-5), 73.7, 73.6 (2 × C-3), 70.5, 70.1, (2 × C-2), 69.9, 69.8 (2 × C-4), 68.6, 62.2 (2 × C-6), 32.5, 32.3 (2 × C-8), 30.3, 29.7 (2 × C-9), 20.8 (2C), 20.6 (2C) (4 × CH\(_3\)C(O)O); \(m/z\) (ES\(^+\)) 560 [M+Na]\(^+\); HRMS (ES\(^+\)) calculated [M+Na]\(^+\) C\(_{25}\)H\(_{31}\)NO\(_{10}\)NaS 560.1566, found 560.1567.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-NO(2-methoxypropan-2-yl) oct-4-ene thiohydroximate, 133

General procedure G was employed using 97 (600 mg, 1.12 mmol, 1.0 eq), n-BuLi (1.6M in THF, 0.738 mL, 1.18 mmol, 1.0 eq) and butyltriphenyl phosphonium bromide (447 mg, 1.18 mmol, 1.0 eq). Following purification by flash column chromatography (30:70, ethyl acetate, petroleum ether) 133 (180 mg, 28%) was obtained as a colorless oil and as a mixture of diastereomers; 

\[ \alpha \]$_D$$^{20}$ -8.2 (c 1.0, CHCl$_3$); δ$_H$ (300 MHz, CDCl$_3$) 5.48-5.35 (2H, m, CH$_3$-10), 5.26 (1H, dd, J 9.0, 8.7 Hz, CH-4), 5.10-4.98 (3H, m, CH-1, 2,11), 4.20 (1H, dd, J 12.1, 5.6 Hz, CH$_2$-6a) 4.12 (1H, dd, J 12.1, 2.3 Hz, CH$_2$-6b), 3.74 (1H, ddd, J 10.3, 5.6, 2.3 Hz, CH-5), 3.22 (3H, s, CH$_3$-17), 2.69-2.60 (1H, m, CH$_2$-12a), 2.53-2.48 (1H, m, CH$_2$-12b), 2.46-2.41 (2H, m, CH$_2$-9), 2.06-2.01 (14H, m, 4 × CH$_3$C(O)O, CH$_2$-8), 1.49 (6H, s, 2 × CH$_3$-16), 1.41-1.35 (2H, m, CH$_2$-13), 0.91 (3H, t, J 7.0 Hz, CH$_3$-14); δ$_C$ (75 MHz, CDCl$_3$) 170.6, 170.2, 169.4, 169.2 (4 × CH$_3$C(O)O), 150.8 (C-7), 131.5 (C-10), 127.6 (C-11), 104.4 (C-15), 80.0 (C-1), 76.0 (C-5), 73.9 (C-3), 70.2 (C-2), 68.0 (C-4), 62.2 (C-6), 49.4, 41.6 (2 × C-17), 32.5, 29.4 (2 ×C-8), 26.2, 25.2 (2 × C-9), 23.9 (C-12), 23.7 (2 × C-16), 22.8 (C-13) 20.7, 20.5, 20.4 (4 × CH$_3$C(O)O), 14.1, 13.8 (2 × C-14); m/z (ES$^+$) 598 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{28}$H$_{41}$NO$_{11}$NaS 598.2298, found 598.2286
Experimental

S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z- (5-ethoxycarbonylpent-4-ene) thiohydroximate, 135

A stream of oxygen was passed through a stirred slurry of 106 (100 mg, 0.17 mmol, 1.0 eq) in DCM (50 mL) and potassium carbonate (1.0 g) whilst cooling to -78 °C. After 5 min a stream of oxygen/ozone was passed though the solution until the blue colour remained constant. A stream of oxygen was used to flush excess ozone from the solution before the addition of triphenylphosphine (4.46 g, 1.70 mmol, 10.0 eq). After 2 min of vigorous stirring ethyl triphenylphosphoranylidene acetate (0.12 g, 0.34 mmol, 2.0 eq) was added and solution was allowed to warm up to room temperature. The resulting slurry was stirred overnight before being concentrated under reduced pressure and filtered though a plug of celite. After washing the residue with DCM, the filtrate was removed under reduced pressure and the resulting brown oil was purified via column chromatography (30 : 70, ethyl acetate : petroleum ether) to obtain 134 (39.1 mg, 35%) as a colourless oil:

To a solution of 134 (35 mg, 0.054 mmol, 1.0 eq) in methanol (2 mL) was added Dowex-50W 200 mesh resin (cat.) that had been pre washed with HCl (1M, 20 mL) and stirred for 4 h at room temperature. The resin was removed by filtration and the solvent removed under reduced pressure. The yellow residue was purified by chromatography (50 : 50, ethyl acetate : petroleuem ether.) to yield 135 (27 mg, 96%) as a colourless oil; $[\alpha]_{D}^{20} - 4.0 \; (c \; 1.0, \; CHCl_3)$; $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 2950 (CH), 1730 (CO); $\delta_{H}$ (300 MHz, CDCl$_3$) 8.45 (1H, br s , NOH), 6.96-6.84 (1H, m, CH-10), 5.82 (1H, d, J 15.0 Hz, CH-11), 5.25-5.16 (1H, m, CH-3), 5.06-4.96 (3H, m, CH-1,2,4), 4.17-4.03 (4H, m, CH$_2$-6, 13), 3.74-3.66 (1H, m, CH-5),
2.74-2.48 (4H, m, CH$_2$-8,9), 2.05-1.96 (12H, m, 4 × CH$_3$C(O)O), 1.22 (3H, t, J 7.5 Hz, CH$_3$-14); δ$_C$ (75 MHz, CDCl$_3$) 170.5, 170.1, 169.4, 169.08 (4 × CH$_3$C(O)O), 166 (C-12), 154.1 (C-7), 146.9 (C-10), 122.3 (C-11), 79.8 (C-1), 76.1 (C-5), 73.8 (C-3), 70.2 (C-2), 68.2 (C-4), 62.4 (C-6), 60.4 (C-13), 30.5 (C-8), 28.89 (C-9), 20.6 (2C), 20.7 (2C) (4 × CH$_3$C(O)O), 14.2 (C-14); m/z (ES$^+$) 556 [M+Na]$^+$: HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{22}$H$_{31}$NO$_2$NaS 556.1467, found 556.1465.
**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-N-(tert-butyldimethylsilyloxy) (3-phenethyl-4,5-dihydroisoxazole-5-yl)propane thiohydroximate, 139**

A solution of 3-phenylpropanal oxime (36 mg, 0.24 mmol, 1.0 eq) was added to a separating funnel containing NaOCl (0.7 mL, 121.5 mmol, 5.0 eq) and the biphasic solution was shaken and a vivid blue to yellow colour change was observed. The organic phase was added to a stirred solution of 106 (100 mg, 0.17 mmol, 0.6 eq) in DCM (3mL) before the addition of triethylamine (0.1 mL, 0.73 mmol, 3.0 eq) and the reaction mixture was stirred for 3 h. The solution was washed using H$_2$SO$_4$ (0.5 M, 2 mL) and brine before drying over magnesium sulfate. The solvent was removed to obtain a pale yellow oil that was purified using chromatography (40% ethyl acetate: petroleum ether) to yield 139 (154 mg, 89%) as a colourless oil and as mixture of diastereomers; $[\alpha]_D^{30}$-17.0 (c 1.0, CHCl$_3$); (300 MHz; CDCl$_3$) 7.16-7.05 (5H, m, CH$_3$-16,17,18), 5.12 (1H, m, CH-3), 5.00-4.88 (3H, m, CH-1,2,4), 4.51-4.39 (1H, m, CH-10), 4.08-3.96 (2H, m, CH$_2$-6), 3.79-3.69 (1H, m, CH-5), 2.89-2.74 (3H, m, CH$_2$-8, 11a), 2.58-2.40 (5H, m, CH$_2$-9, 11b, 13), 1.93-1.86 (12H, m, 4 × CH$_3$C(O)O), 1.82-1.71 (2H, m, CH$_2$-14), 0.80 (9H, s,CH$_3$-tBu), 0.00 (6H, s, CH$_3$-Si);(75 MHz; CDCl$_3$): 170.6, 170.2, 170.1, 169.5, 169.4, 169.2, 169.1 (4 × CH$_3$C(O)O), 158.6, 158.5 (2 × C-12), 156.1, 155.9 (2 × C-7), 140.5, 140.4 (2 × C-15), 128.6 (2C, 2 × C-18), 128.3 (2C, 2 × C-17), 126.4 (2C, 2 × C-16), 79.8, 79.5 (2 × C-1), 78.81, 78.75 (2 × C-5), 75.7, 75.6 (2 × C-3), 74.0, 74.1 (2 × C-2), 70.3, 70.1 (2 × C-4), 67.9, 67.9 (2 × C-5), 62.1 (2 × C-6), 42.6, 42.3 (2 × C-13), 32.9, 32.8 (2 × C-8), 32.7, 32.6 (2 × C-14), 29.6, 29.5 (2 × C-10), 27.9, 27.8 (2 × C-11), 26.3 (C-21), 26.0 (2 × C-9), 20.7, 20.6, 20.6 (4 × CH$_3$C(O)O), 18.2 (C-20), -5.21, -5.24 (C-
19); \( m/z \) (ES\(^+\)) 745 (100%, [M+Na]\(^+\)); HRMS (ES\(^+\)) calculated [M+Na]\(^+\) \( C_{34}H_{50}N_2O_{11}NaSSi \) 745.2802, found 745.2807.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-cyclohex-3-enyl methane thiohydroximate, 145

General procedure A was employed using cyclohex-3-enecarbaldehyde (2.0 g, 18.2 mmol, 1.0 eq), sodium acetate (1.79 g, 21.8 mmol, 1.2 eq), hydroxylamine hydrochloride (1.49 g, 18.2 mmol, 1.0 eq), acetonitrile (20 mL) and water (10 mL) to yield the cyclohex-3-enecarbaldehyde oxime (1.68 g, 74%) as a yellow oil and as a mixture of diastereomers. This product was used without further purification.

General procedure B was employed using cyclohex-3-enecarbaldehyde oxime (1.00 g, 8.00 mmol, 1.0 eq), NaOCl (25 mL, 1.6 M, 40.0 mmol, 5.0 eq), 21 (1.73 g, 4.80 mmol, 0.6 eq), triethyl amine (3.35 ml, 24.00 mmol, 3.0 eq) and DCM (80 mL). The product was purified by chromatography (30 : 70, ethyl acetate : petroleum ether) to obtain 145 (1.42 g, 61%) as a colourless solid and mixture of diasteriomers: mp 150-154 °C; ν_max(nujol)/cm⁻¹ 2910 (CH), (CO); δ_H (300 MHz, CDCl₃) 8.33 (1H, br s, NOH), 5.76–5.70 (2H, m, CH₁₀, 11), 5.33-5.26 (1H, m, CH-1), 5.20-5.05 (3H, m, CH-2,3,4), 4.27-4.11 (2H, m, CH₂-6) 3.79-3.71 (1H, m, CH-5), 2.74-2.62 (1H, m, CH-8), 2.38-2.23 (2H, m, CH₂-9) 2.22-2.14 (2H, m, CH₂-12), 2.12-2.05 (13H, m, 4 × CH₃C(O)O, CH₂-13a), 1.79-1.69 (1H, m, CH₂-13b); δ_C (75 MHz, CDCl₃) 170.7, 170.3, 170.2, 169.4, 169.3 (4 × CH₃C(O)O), 154.6, 154.6 (2 × C-7), 126.8, 126.5 (2 × C-10), 126.0, 125.5 (2 × C-11), 80.6, 80.3 (2 × C-1), 76.2, 76.0 (2 × C-5), 74.5 (C-3), 70.4, 70.3 (C-2), 68.2, 68.2 (2 × C-4), 62.2 (C-6), 39.2, 38.9 (2 × C-8), 30.7, 29.6 (2 × C-9), 28.2, 27.2 (2 × C-12), 25.5, 25.4 (C-13), 20.7, 20.6 (4 × CH₃C(O)O); m/z (ES⁺) 510 (100%, [M+Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ C₂₁H₂₉NO₁₀NaS 510.1410, found 510.1407.
Experimental

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) S(-2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-2,4-dimethylcyclohex-3-en-1-ylmethane thiohydroximate, 148

General procedure A was employed using 2,4-dimethylcyclohex-3-ene carbaldehyde aldehyde (2.0 g, 14.5 mmol, 1.0 eq), sodium acetate (1.76 g, 21.5 mmol, 1.2 eq), hydroxylamine hydrochloride (1.00 g, 14.5 mmols, 1.0 eq), acetonitrile (20 mL) and water (10 mL) to yield 2,4-dimethylcyclohex-3-ene carbaldehyde oxime (1.97 g, 89%) as a yellow oil and as a 1:1 mixture of E:Z isomers and was used without further purification.

General procedure B was employed using 2,4-dimethylcyclohex-3-ene carbaldehyde oxime (1.0 g, 6.52 mmol, 1.0 eq), NaOCl (20 mL, 1.6 M, 32.63 mmol, 5.0 eq), 21 (1.42 g, 3.91 mmol, 0.6 eq) and triethylamine (3.35 mL, 24.00 mmol) in DCM (80 mL). The material was purified via chromatography (30 : 70, ethyl acetate: petroleum ether) to 148 (1.38 g, 69%) as a colourless oil and as an inseperable mixture of diastereomers: ν_{max}(KBr)/cm⁻¹ 3050, 2950, 1740; δ_{H} (300 MHz, CDCl₃) 8.35 (1 H, br s, N-OH), 5.23-5.15 (3H, m, CH-1,3,10) 5.13-4.98 (2H, m, CH-2,4), 4.21 (1H, dd J 12.5, 5.4 Hz, CH₂-6a), 4.14 (1H, dd, J 12.5, 2.3 Hz, CH₂-6b), 3.75–3.64 (1H, m, CH-5), 2.17-2.12 (1H, m, CH-8), 2.10-1.90 (17H, m, 4 × CH₃C(O)O, CH-9, CH-12, CH₂-13), 1.66 (3H, s, CH₃-15), 0.96 (3H, d J 7.0 Hz, CH₃-14); δ_{C} (75 MHz, CDCl₃) 170.7, 170.6, 170.3, 169.4, 169.3 (4 × CH₃C(O)O), 153.9, 153.6 (2 × C-7), 132.9, 132.5 (2 × C-11), 127.2, 126.8 (2 × C-10), 80.9, 80.3 (2 × C-1), 76.1, 75.9 (2 × C-5), 73.9 (C-3), 70.5, 70.4 (2 × C-2), 68.2 (C-4), 62.3, 62.2 (2 × C-6), 47.3, 46.9 (2 × C-8), 34.3, 33.6 (2 × C-9), 30.1, 30.0 (2 × C-12), 28.8, 28.5 (2 × C-14), 23.4 (C-15), 20.7 (C-13), 20.6, 20.2 (2C), 20.1 (4 × CH₃C(O)O); m/z (ES⁺) 538 (100%, [M +Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ C₂₃H₃₃NO₁₀SNa 538.1723, found 538.1725.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl)  Z-2,4-dimethylcyclohex-3-enylmethane thiohydroximate  NO-sulfate, potassium salt, 149

General procedure E was employed using 148 (300 mg, 0.581 mmol, 1.0 eq), pyridine·sulfur trioxide complex (460 mg, 2.90 mmol, 5.0 eq), pyridine (0.48 mL, 5.81 mmol, 10.0 eq) and DCM (5 mL) to yield the potassium salt of 149 (193 mg, 48%) as a pale yellow solid and mixture of diastereomers; δH (300 MHz, D2O); 5.32 (1H, dd, J 9.7, 9.7, Hz, CH-3), 5.24-5.19 (1H, m, CH-1), 5.10-5.03 (2H, m, CH-2,10), 4.93 (1H, dd, J 9.9, 9.3 Hz, CH-4), 4.28-4.10 (2H, m, CH2-6), 3.83-3.78 (1H, m, CH-5), 2.25-2.17 (1H, m, CH-8), 2.01-1.93 (17H, m, 4 × CH3C(O)O, CH-9, CH-12, CH2-13), 1.57 (3H, s, CH3-15), 0.87-0.84 (3H, m, CH3-14) δC (75 MHz, D2O) 171.2, 170.6 (2C), 170.1 (CH3C(O)O), 161.3, 161.1 (C-7), 133.4, 132.9 (C-11), 126.2, 125.9, (C-10), 81.4 (C-1), 75.2 (C-5), 73.6 (C-3), 71.0 (C-2), 70.8, 67.9 (2 × C-4), 61.9 (C-6), 48.3, 48.0 (2 × C-8), 33.8, 33.5 (2 × C-9), 29.8, 29.5 (2 × C-12), 23.5 (C-15), 21.2 (C-14), 20.8 (C-13), 19.9 (4 × CH3C(O)O); m/z (ES⁻) (100%, 594 [M]); HRMS (ES⁻) calculated [M] C23H32NO13S2 594.1315, found 594.1320.

General procedure G: Acetate deprotection of glucosinolates

To a stirred solution of potassium carbonate (anhydrous) in methanol was added 2,3,4,6-tetra-O glucosinolate and the mixture was stirred for 16 h. The slurry was then filtered though a plug of cotton wool and the solvent removed under reduced pressure to obtain the glucosinolate.
S-(β-D-Glucopyranosyl) Z-2,4-dimethylcyclohex-3-enylmethane thiohydroximate NO-Sulfate, Potassium salt, 150

General procedure D was employed using 149 (200 mg, 0.31 mmol, 1.0 eq), potassium carbonate (anhydrous, 40 mg) and methanol (2 mL) gave 150 (128 mg, 88%) as a clear oil and as a mixture of diastereoisomers; δH (300 MHz, D2O) 6.47 (1H, d, J 9.8 Hz, CH-10a), 6.36 (1H, d J 9.8 Hz, CH-10b), 5.32-5.29 (1H, m, CH-1a), 5.02-4.88 (1H, m, CH-1b), 5.02-4.88 (3H, m, CH-2,3,4), 3.90-3.87 (1H, m, CH-8), 3.62-3.42 (4H, m, CH2-12, 13), 3.15 (3H, CH3-15), 2.48 (3H, d, J 6.9 Hz, CH3-14a) 2.45 (3H, d, J 6.9 Hz, CH3-14b) ; δC (75 MHz, D2O) 167.3, 166.3, 164.2 (C-7), 137.2, 136.8 (C-11), 129.2, 129.1 (C-10), 84.9, 84.3 (C-1), 82.6, 82.5 (C-5), 79.6, 79.6 (C-3), 74.6, 74.5 (C-2), 71.4, 71.3 (C-4), 62.8 (C-6), 51.5 (C-8), 26.5, 25.9 (C-9), 25.1 (C-15), 25.0 (C-12), 21.9 (C-14), 21.8 (C-13); m/z (ES-) (100%, 426 [M-K]-); HRMS (ES-) calculated [M]- C15H24NO9S2 426.0893 found 426.0883.
Hept-6-enal,\textsuperscript{176} 152

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An aqueous solution of sodium metaperiodate (8.14 g, 38.17 mmol, 1.1 eq in 47.5 mL water), was added over 45 min to a stirred emulsion of 7-octen-1,2-diol \textbf{157} (5.00 g, 34.7 mmol, 1.0 eq) in water (20 mL) at room temperature. The resulting mixture was stirred for 2 h at room temperature before separating the organic phase. The organic phase was dried over sodium sulfate and the solvent removed under reduced pressure to yield hept-6-enal (2.84 g). The aqueous fraction was then saturated with sodium chloride and extracted into DCM (3 \times 40 mL), dried over magnesium sulfate and concentrated under reduced pressure to yield give the second batch of \textbf{152} (0.73 g). The two fractions were combined to give hept-6-enal (3.57 g, 92%) as a colourless oil; δ\textsubscript{H} (300 MHz, CDCl\textsubscript{3}) 9.67 (1H, t, J 1.8 Hz, CH-1), 5.70 (1H, ddt, J 17.2, 10.6, 6.4 Hz, CH-6), 4.96-4.84 (2H, m, CH\textsubscript{2}-7), 2.35 (2H, dt, J 7.3, 1.5 Hz, CH\textsubscript{2}-2), 1.94 (2H, dt, J 7.3, 1.5 Hz, CH\textsubscript{2}-5), 1.62-1.50 (2H, m, CH\textsubscript{2}-3), 1.40-1.29 (2H, m, CH\textsubscript{2}-4); δ\textsubscript{C} (75 MHz, CDCl\textsubscript{3}) 202.4 (C-1), 138.2 (C-6) 114.7 (C-7), 43.6 (C-2), 33.4 (C-5), 28.2 (C-3), 21.4(C-4); m/z (ES)\textsuperscript{+} 121 (100%, [M+Na]\textsuperscript{+}).
Hept-6-enal oxime, 153

General procedure A was employed using hept-6-enal (3.80 g, 34.3 mmol, 1.0 eq), sodium acetate (3.39 g, 41.1 mmol, 1.2 eq), hydroxylamine hydrochloride (2.38 g, 34.2 mmol, 1.0 eq) in water (48 mL) and acetonitrile (96 mL). The procedure afforded 153 (3.39 g, 78%) as a colorless oil and a 1:1 mixture of E:Z isomers: \( \nu_{max} \) (film)/cm\(^{-1}\) 3280, 2930 (CH), 2620, 1640 (C\(=\)C), 1620 (CN); \( \delta_H \) (300 MHz, CDCl\(_3\)) 9.98 (1H, br s, N-OH), 7.41 (1H, t, \( J \) 6.0 Hz, CH-1 \textit{anti}), 6.70 (1H, t, \( J \) 5.4 Hz, CH-1 \textit{syn}), 5.84-5.69 (1H, m, CH-2-6), 5.02-4.90 (2H, m, CH-2-7), 2.42-2.34 (1H, m, CH\(_2\)-2a), 2.21-2.15 (1H, m, CH\(_2\)-2b), 2.09-2.01 (2H, m, CH\(_2\)-5), 1.55-1.35 (4H, m, CH\(_2\)-3,4); \( \delta_C \) (75 MHz, CDCl\(_3\)) 152.6 (C-1 \textit{anti}), 152.1 (C-1 \textit{syn}), 138.5 (C-6), 114.7 (C-7), 33.4 (C-5), 29.3 (C-4), 28.5 (C-2), 28.2 (C-2), 25.9 (C3) and 25.4 (C-3); \( m/z \) (Cl\(^+\)) 128 (100\%, [M+H]\(^+\)); HRMS (Cl\(^+\)) calculated [M+H]\(^+\) \( C_7H_{14}NO \) 128.1075, found 128.1078.
S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-hept-6-ene thiohydroximate, 154

General procedure B was employed using hept-6-enal oxime 153 (1.28 g, 10.1 mmol, 1.0 eq), DCM (10 mL), NaOCl (31.0 mL, 50.5 mol, 1.6 M, 5.0 eq), 21 (2.20 g, 6.0 mmol, 0.6 eq) and triethylamine (3.00 g, 4.2 mL, 30.3 mmol, 3.0 eq) in DCM (100 mL). Purification by flash chromatography (40:60, ethyl acetate: petroleum ether) gave 154 (2.08 g, 71%) as a colourless solid: mp 122-126 °C; [α]D30 -14.6 (c 0.5, CHCl3); v max (KBr)/cm⁻¹ 3320 (NOH), 3080 (CH), 2920 (CH), 2850 (CH), 1750 (CO), 1640 (C=C), 1610 (CN); δH (300 MHz, CDCl₃) 5.72 (1H, ddt, J 16.7, 10.6, 6.4 Hz, CH-12), 5.19 (1H, m, CH-3), 5.05-4.86 (5H, m, CH₂-13 and CH-1,2,4), 4.16-4.02 (2H, m, CH₂-6), 3.65-3.60 (1H, m, CH-5), 2.45-2.39 (2H, m, CH₂-11), 2.05-1.93 (14H, m, 4 × CH₃ and CH₂-8), 1.65-1.53 (2 H, m, CH₂-9), 1.44-1.34 (2H, m, CH-10); δC (75 MHz, CDCl₃) 171.0, 170.6, 169.8, 169.6, (4 × CH₃C(O)O), 152.7 (C-7), 138.6 (C-12), 115.4 (C-13), 80.4 (C-1), 76.4 (C-5), 74.2 (C-3), 70.5 (C-2), 68.5 (C-4), 62.5 (C-6), 33.8 (C-11), 32.8 (C-8), 28.8 (C-9), 26.9 (C-10), 21.1, 21.0 (2C), 20.9 (4 × CH₃C(O)O); m/z (ES⁺) 512 (100%, [M+Na]⁺); Anal. Calc. (Found C, 51.4; H, 6.1; N, 2.7. C₂₁H₃₁NO₁₀S requires C, 51.5; H, 6.3; N, 2.9%).
**S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-hept-6-ene thiohydroximate NO-sulfate, potassium salt, 155**

General procedure D was employed using **154** (0.78 g, 1.6 mmol, 1.0 eq), pyridine (1.3 g, 1.2 mL, 16.0 mmol 10.0 eq), pyridine - sulfur trioxide complex (1.3 g, 8.0 mmol, 5 eq) and DCM (80 mL) to obtain **155** (0.54 g, 59%) as a colourless solid: mp >150 °C decomposed; $[\alpha]_{D}^{20}$ -10.0 (c 1.0, H$_2$O); $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3500 (N=O), 2930 (CH), 2850, 2720, 1750 (C=O), 1630 (CN), 1250 (CO), 1050; $\delta$H (300 MHz, D$_2$O) 5.72 (1H, ddt, J 16.7, 10.6, 6.4 Hz, CH-12), 5.34-5.29 (1H, m, CH-3), 5.05-4.86 (5H, m, CH$_2$-13 and CH-1,2,4), 4.28 (1H, m, CH$_2$-6a), 4.12-4.00 (2H, m, CH-5, CH$_2$-6b), 3.98-3.92 (1H, m, CH-5), 2.61-2.56 (2H, m, CH$_2$-11), 2.05-1.93 (14H, m, 4 × CH$_3$ and CH$_2$-8), 1.65-1.55 (2H, m, CH$_2$-9), 1.44-1.34 (2H, m, CH-10); $\delta$C (75 MHz, CDCl$_3$) 173.5, 172.9, 172.6, 172.3, (4 × CH$_3$C(O)O), 162.6 (C-7), 139.3 (C-12), 114.0 (C-13), 79.5 (C-1), 75.1 (C-5), 73.8 (C-3), 69.9 (C-2), 67.7 (C-4), 61.9 (C-6), 32.6 (C-11), 32.0 (C-8), 30.2 (C-11) 27.2 (C-9), 26.2 (C-10), 20.0 (2C), 19.9, 19.8 (4 × CH$_3$C(O)O); $m/z$ (ES$^-$) 568 (100%, [M-K]$^-$); HRMS (ES$^-$) calculated [M]$^-$ C$_{21}$H$_{30}$NO$_{13}$S$_2$ 568.1159, found 568.1152.
S-(β-d-Glucopyranosyl) Z-hept-6-ene thiohydroximate NO-Sulfate, potassium salt, 156

General procedure G was employed using 155 (200 mg, 0.33 mmol, 1.0 eq), potassium carbonate (anhydrous, 40 mg) and methanol (2 mL) to obtain 156 (105 mg, 73%) as a colourless amorphous solid; [α]D 20 -4.6 (c 0.5, H2O); νmax (nujol)/cm⁻¹ 3400 (NO), 2850 (CH), 1560 (C=C), 1250; δH (500 MHz, D2O) 5.84-5.69 (1H, m, CH-12), 4.92-4.89 (2H, m, CH-13), 3.83 (1H, d, J 12.0 Hz, CH-1), 3.70-3.57 (3H, m, CH-2,3,4), 3.39-3.28 (4H, m, CH-1,5, CH2-6), 2.59 (2H, t, J 7.5 Hz, CH2-8), 2.04-1.94 (2H, m, CH2-11), 1.63-1.42 (2H, m, CH2-9), 1.43-1.37 (2H, m, CH2-10); δC (125 MHz, D2O) 164.7 (C-7), 139.6 (C-12), 114.6 (C-13), 81.8 (C-1), 79.8 (C-5), 77.0 (C-3), 72.3 (C-2), 69.4 (C-4), 60.8 (C-6), 32.9 (C-11), 29.8 (C-8), 27.7 (C-9), 25.4 (C-10); m/z (ES⁻) 400 (100%, [M-K]⁻); HRMS (ES⁻) calculated [M⁻] C13H22NO9S2 400.0736, found 400.0730.
S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-hept-4-ene thiohydroximate, 157

General procedure A was employed for using cis-heptenal aldehyde (5.0 g, 44.6 mmol, 1.0 eq), sodium acetate (3.36 g, 44.57 mmol, 1.0 eq), hydroxylamine hydrochloride (4.48 g, 53.5 mmol, 1.0 eq), acetonitrile (20 mL) and water (10 mL) to yield the cis-hept-enal oxime 158 (4.70 g, 83%) as a yellow oil and a 1:1 mixture of E:Z isomers.

General procedure B was employed using cis-hept-enal oxime 158 (1.0 g, 7.9 mmol, 1.0 eq) NaOCl (24.5 mL, 1.6 M, 39.3 mmol, 5.0 eq), 21 (1.72 g, 4.7 mmol, 0.6 eq) and triethylamine (1.89 mL, 23.5 mmol, 3.0 eq) in DCM (60 mL). The product was purified via chromatography (30:70, ethyl acetate : petroleum ether) to obtain 157 (1.96 g, 85%) as a colourless solid: mp 130-132 °C; $\left[\alpha\right]_{D}^{20}$ - 12.4 (c 1.0, CHCl$_3$); $\nu_{max}$ (KBr)/cm$^{-1}$ 3430 (N=O), 3100 (CH), 2970 (CH), 1730 (CO), 1650 (C=C), 1610 (CN); $\delta$H (300 MHz, CDCl$_3$) 8.97 (1H, br s, N=O-H), 5.50-5.46 (1H, m, CH$_{10}$), 5.38-5.32 (2H, m, CH$_{11}$, CH$_{3}$), 5.10-5.04 (3H, m, CH$_{1}$,2,4) and 4.20 (1H, dd, J 12.0, 5.4 Hz, CH$_{2}$-6a), 4.13 (1H, dd J 12.3, 2.0 Hz, CH$_{2}$-6b), 3.76-3.65 (1H, m, CH-5), 2.68-2.50 (2H, m, CH$_{2}$-8), 2.48-2.34 (2H, m, CH$_{2}$-9), 2.13-1.97 (14H, m, CH$_{2}$-12, 4 $\times$ CH$_{3}$C(O)O), 0.97 (3H, t, J 7.5 Hz, CH$_{3}$-13); $\delta$C (75 MHz, CDCl$_3$); 170.7, 170.3 , 169.4, 169.2 (4 $\times$ CH$_{3}$C(O)O), 151.8 (C-7), 133.6 (C-10) 126.5 (C-11), 79.9 (C-1), 76.0 (C-5), 73.8 (C-3), 70.1 (C-2), 68.1 (C-4), 62.2 (C-6), 32.5 (C-8), 24.7 (C-9), 20.7 (C-12), 20.6, 20.5 (4 $\times$ CH$_{3}$C(O)O), 14.3 (C-13); m/z (ES$^+$) 512 (100%, [M+Na]$^+$); HRMS (ES$^+$) calculated [M+Na]$^+$ C$_{21}$H$_{31}$NO$_{10}$NaS 512.1566, found 512.1570.
**S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-hept-4-ene thiohydroximate NO-sulfate, Cyclohexylammonium salt, 160**

General procedure E was employed using S-(2,3,4,6-tetra-O-acetyl-β-d-glucopyranosyl) Z-(cis-hept-enal) thiohydroximate (1.0 g, 2.04 mmol, 1.0 eq), pyridine sulfur trioxide complex (1.63 g, 10.2 mmol, 5.0 eq), pyridine (1.60 g, 1.64 mL, 20.4 mmol, 10.0 eq) and DCM (5mL) to yield 160 (1.03 g, 75%) as a pale yellow solid: mp 152-156 °C; $[\alpha]_D^{20}$ -18.3 (c 0.5, CHCl$_3$); $\nu_{\text{max}}$ (KBr)/cm$^{-1}$ 3330 (N-O), 3000 (HC), 2970 (CH), 1760 (CO), 1650 (C=C), 1610 (CN); $\delta$H (300 MHz, CDCl$_3$) 5.52-5.40 (1H, m, CH-10), 5.38 (1H, m, CH-11), 5.26 (1H, dd $J$ 9.3, 9.3 Hz, CH-3), 5.12-4.96 (3H, m, CH-1,2,4), 4.22-4.09 (2H, m ,CH$_2$-6), 3.81-3.76 (1H, m CH-5), 2.76 (1H, m, CH-8a), 2.60-2.56 (1H, m, CH-8b), 2.50-2.38 (2H, m, CH$_2$-9) 2.01- 1.89 (16H, m, 4 × CH$_3$C(O)O, CH$_2$-2'a, CH$_2$-12), 1.65-1.53 (2H, m, CH$_2$-2'b), 1.40-1.16 (6H, m, 2×CH$_2$-3', CH$_2$-4'), 0.97 (3H, t, $J$ 7.5 Hz, CH-7); $\delta$C (75 MHz, CDCl$_3$) 170.7, 170.3, 170.0, 169.8 (4 × CH$_3$C(O)O), 155.9 (C-7), 133.8 (C-10), 128.5 (C-11), 80.2 (C-1), 76.4 (C-5), 74.2 (C-3), 71.0 (C-2), 70.8 (C-2), 69.1 (C-4), 60.6 (C-6) 51.9 (C-1'), 32.9 (2C, C-2'), 32.5 (C-8), 30.7 (2C, C-9, 12), 25.0 (2C, C-3'), 24.6 (C-4'), 20.7, 20.6 (2C), 20.5 (4 × CH$_3$C(O)O) and 14.3 (C-13); $m/z$ (ES') 568 [M-C$_6$H$_{14}$N]; HRMS (ES') calculated [M]$^-$ C$_{24}$H$_{30}$NO$_{15}$S$_2$ 568.1159, found 568.1155.
S-(β-d-Glucopyranosyl) Z-hept-4-ene thiohydroximate NO-sulfate, Cyclohexylammonium salt, 161

General procedure D was employed using 160 as a cyclohexylammonium salt (100 mg, 0.15 mmol, 1.0 eq), potassium carbonate (anhydrous, 40 mg) and methanol (6 mL) to obtain 161 (60 mg, 81%) as a colourless amorphous solid: $[\alpha]_{D}^{20}$ -13.0 (c 1.0, CDCl$_3$); $\nu_{\text{max}}$(KBr)/cm$^{-1}$ 3400 (OH); $\delta_{H}$(300 MHz, CDCl$_3$) 5.49-5.38 (1H, m, CH$_2$-10), 5.34-5.24 (1H, m, CH-11), 4.90 (1H, d, J 9.3 Hz, CH-1), 3.76-3.62 (1H, m, CH-5), 3.58 (1H, dd, J 12.7, 5.6 Hz, CH-6a), 3.48-3.28 (4H, m, CH-2,3,4,6b), 3.04-2.92 (1H, m, CH-1”), 2.61 (3H, m, CH$_2$-8a, 9), 2.41-2.31 (1H, m, CH$_2$-8b), 1.97-1.90 (2H, m, CH$_2$-12), 1.87-1.80 (2H, m, 2 × CH$_2$-2”a), 1.73-1.62 (2H, m, 2 × CH$_2$-2”b), 1.56-1.43 (2H, m, CH$_2$-4”), 1.19-1.15 (4H, m, 2 × CH$_2$-3”), 0.81 (3H, t, J 7.6 Hz, CH$_3$-13); $\delta_C$ (75 MHz, CDCl$_3$) 164.3 (C-7), 134.5 (C-10), 126.4 (C-11), 81.8 (C-1), 80.0 (C-5), 77.1 (C-3), 71.9 (C-2), 69.1 (C-4), 60.6 (C-6), 50.5 (C-1’), 33.3 (2C, C-2’), 32.0 (2C, C-9, C-12), 25.0 (2C, C-3’), 24.8 (2C, C-4’), 13.0 (C-13); m/z (ES$^-$) 400 (100%, [M$^-$]); HRMS (ES$^-$) calculated [M$^-$] $C_{13}H_{22}NO_9S_2$ 400.0736, found 400.0746
Experimental

3-Benzylidacetoneglucose,\textsuperscript{177} 177

Sodium hydride (60% wt in mineral oil, 1.6 g, 42.2 mmol, 1.1 eq) was added to a solution of diacetone-\(\delta\)-glucose (10.0 g, 38.4 mmol, 1.0 eq), in DMF (100 mL) at 0 °C. The resulting suspension was allowed to stir for 20 min before the addition of benzyl bromide (5.02 mL, 4.22 mmol, 1.1 eq) and further stirring for 3 hr. The reaction mixture was poured into water (200 mL) and extracted with ethyl acetate (3 \(\times\) 100 mL). The organic layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting product was purified via column chromatography (5:95, ethyl acetate:petroleum ether) to yield \textbf{177} (10.4 g, 78%) as a colourless oil: \([\alpha]_D^{20}\) \(-20.5\) (c 1.1, CHCl\(_3\)) \[Lit.\textsuperscript{180} -25.2\ (c 2.87, CHCl\(_3\))]; \(\delta_H\) (300 MHz, CDCl\(_3\)) 7.32-7.27 (3H, m, 2 \(\times\) CH-13 CH-15), 7.24-7.16 (2H, m, 2 \(\times\) CH-14), 5.82 (1H, d, \(J\) 3.8 Hz, CH-1), 4.70-4.64 (2H, m, CH\(_2\)-11), 4.60-4.57 (1H, m, CH-2), 4.29 (1H, m, CH-4), 4.05 (2H, m, CH\(_2\)-3,5), 3.96-3.91 (2H, m, CH-6), 1.41, 1.35, 1.29, 1.22 (12H, 4 \(\times\) s, 4 \(\times\) CH\(_3\)); \(\delta_C\) (75 MHz, CDCl\(_3\)) 138.1 (C-12), 128.8 (C-13), 128.2 (C-14), 128.0 (C-15), 112.1 (C-7), 109.9 (C-9), 105.7 (C-1), 83.0 (C-2), 82.1 (C-3), 81.7 (C-4), 73.0 (C-11), 67.8 (C-6), 27.2 (CH\(_3\)), 27.1 (CH\(_3\)), 26.7 (CH\(_3\)), 25.8 (CH\(_3\)); \(m/z\) (ES\(^+\)) 373 (100%, [M+Na]\(^+\))
3-Benzylxyglucose\textsuperscript{178} 173

Pre-activated Dowex-50W 200 resin (2.00 g) was added to a solution of 177 (5.00 g, 14.2 mmol, eq) in water (40 mL) and the resulting slurry heated under reflux for 16 h. Upon cooling, the resin was filtered and the solvent removed under reduced pressure to yield 173 (3.66 g, 95\%) as a colourless solid and a 2:3 mixture of $\alpha$:$\beta$ anomers: $\text{mp} 118$-$122 ^\circ\text{C}$ [Lit.\textsuperscript{181} 137-140 \]; $[\alpha]_D^{20} + 24.0$ (c 1.0, H$_2$O); $\delta_H$ (300 MHz, D$_2$O) 7.23-7.46 (5H, m, CH-9,10,11), 5.10 (1H, d, J 3.7 Hz, CH-1$\alpha$), 4.77 (1H, d, J 8.6 Hz, CH$_2$-7a), 4.75 (1H, d, J 8.6 Hz, CH$_2$-7b), 4.53 (1H, d, J 8.6 Hz, CH-1$\beta$), 3.81 (3H, m, CH-2,3,4), 3.52-3.18 (3H, m, CH-5, CH$_2$-6); $m/z$ (ES$^+$) 271 (100\%, [M+H]$^+$).
3-O-Benzyl-1,2,4,6-tetra-O-acetyl glucose,\textsuperscript{179} \textsuperscript{174}

To a solution of 3-Bn-Glucose (0.30 g, 1.11 mmol, 1.0 eq) in acetic anhydride (0.6 mL, 0.63 mmol, 5.7 eq) was added sodium acetate (0.18 g, 2.22 mmol, 2.0 eq). The resulting slurry was heated to 100 °C with stirring until the solution had become yellow and transparent. Heating was removed and the solution poured on to an ice water slurry (ca. 20 mL). After 2 h of stirring the resulting precipitate was isolated by filtration and the recrystallised from ethanol to yield 3-O-benzyl-1,2,4,6-tetra-O-acetyl glucose (0.26 g, 54%) as a pale yellow solid: m.p 100-106 °C [Lit.\textsuperscript{182} 107 °C ]; $[\alpha]_D^{20}$ + 6.3 (c 1.0, CHCl\textsubscript{3}); $\nu$\textsubscript{max} (KBr)/cm\textsuperscript{-1} 3030, 2940 (CH), 1720 (C=O); $\delta_H$ (300 MHz, CDCl\textsubscript{3}) 7.34-7.27 (3H, m, 2 × CH-10, CH-11), 7.21-7.19 (2H, d, J 7.9 Hz, 2 × CH-9), 6.29 (1H, d, J 3.5 Hz, CH-1\textalpha{)}, 5.62 (1H, d, J 8.2 Hz, CH-1\textbeta{)}, 5.17-5.09 (2H, m, CH-2,4), 4.58 (2H, s, CH\textsubscript{2}-7), 4.18 (1H, dd , J 12.5, 5.5 Hz, CH\textsubscript{2}-6a) 4.06 (1H, dd, J 12.5, 2.3 Hz, CH\textsubscript{2}-6b), 3.75-3.67 (2H, m, CH-3,5), 2.07-1.94 (12H, 3 × s, 3 × CH\textsubscript{3}C(O)O); m/z (ES\textsuperscript{+}) 461 (100%, [M+Na]\textsuperscript{+}); HRMS (ES\textsuperscript{+}) calculated [M+Na]\textsuperscript{+} C\textsubscript{21}H\textsubscript{26}O\textsubscript{10}Na 461.1424, found 461.1414.
2,4,6-Tetra-O-acetyl-3-benzyl-1-thio-β-d-glucopyranose, 175

A solution of 3-O-benzyl-1,2,4,6-tetra-O-acetyl glucose (456 mg, 1.04 mmol, 1.0 eq) and thiourea (98 mg, 1.2 mmol, 1.2 eq) and BF₃·OEt₂ (0.324 g, 2.6 mmol, 2.5 eq) in acetonitrile was heated under reflux for 3 h. After this time a solution of sodium metabisulfite (197 mg, 1.04 mmol, 1.0 eq) in water (10 mL) was added and the biphasic solution was heated for a further 3 h at reflux. Upon cooling the organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic fractions were combined and washed with saturated sodium hydrogen carbonate (2 × 50 mL), brine (2 × 50 mL) and dried over magnesium sulfate. Following flash column chromatography (40:60, ethyl acetate: petroleum ether) 175 (296 mg, 62%) was obtained as a pale yellow solid; $[\alpha]_D^{20} +17.3$ (c 1.0, CHCl₃); $\nu_{\text{max}}$ (KBr)/cm⁻¹ 2920 (CH), 2570 (SH), 1730 (C=O); $\delta_H$ (300 MHz, CDCl₃) 7.36-7.28 (3H, m, 2 × CH-9, CH-11), 7.24-7.21 (2H, m, 2 × CH-10), 5.13 (1H, dd, $J$ 10.1, 9.4 Hz, CH-1), 5.01 (1H, dd, $J$ 9.5, 9.5 Hz, CH-4), 4.44 (1H, dd, $J$ 9.3, 9.4, CH-2), 4.16 (1H, dd, $J$ 12.5, 5.4 Hz, CH₂-6a), 4.10 (1H, dd, $J$ 12.5, 2.3 Hz, CH₂-6b), 3.67 (1H, t, $J$ 9.3Hz, CH-3), 4.62 (2H, m, CH₂-7), 3.60 (1H, ddd, $J$ 9.5, 5.4, 2.3, Hz, CH-5), 2.31 (1H, d, $J$ 10.1 Hz, SH), 2.08, 2.03, 1.95 (9H, 3 × s, 3 × CH₃C(O)O); $\delta_C$ (75 MHz, CDCl₃) 170.9, 169.7, 169.4 (3 × CH₃C(O)O), 138.2 (C-8), 128.5 (C-10), 127.7 (C-9), 127.4 (C-11), 81.3 (C-3), 79.5 (C-1), 76.8 (C-5), 75.2 (C-2), 74.3 (C-7), 69.5 (C-4), 62.5 (C-6), 20.9, 20.8, 20.7 (3 × CH₃C(O)O); $m/z$ 435 (100%, [M+Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ calculated C₁₉H₂₄O₆NaS 435.1090, found 435.1094.
S-(2,4,6-Tetra-O-acetyl-3-O-benzyl-β-D-glucopyranosyl) pent-4-ene thiohydroximate, 195

General procedure B was employed using 2,4,6-tetra-3-benzyl-O-acetyl-1-thio-β-D-glucopyranose 21 (2.0 g, 4.07 mmol, 0.6 eq), pent-4-enal oxime (0.69 g, 6.79 mmol, 1.0 eq), NaOCl (1.6 M, 63.8 mL, 102 mmol, 5.0 eq), DCM (40 mL), and NEt₃ (2.78 mL, 20.4 mmol, 3.0 eq). Purification was undertaken using flash chromatography (40 : 60, ethyl acetate : petroleum ether) to obtain 195 (1.79 g, 86%) as a colourless solid; $[\alpha]_D^{20}$-15.6 (c 1.1 in CHCl₃); $\nu_{\text{max}}$ (nujol)/cm⁻¹ 3230 (NOH), 2920 (CH), 1750 (CO); $\delta$H (300 MHz; CDCl₃) 8.46 (1H, br s, N-OH), 7.40-7.31 (3H, m, 2 × CH-15, CH-16), 7.25-7.20 (2H, m, 2 × CH-14), 5.88 (1H, ddt, J 17.0, 10.4, 6.5 Hz, CH-10), 5.18-5.04 (4H, m, CH-2,4,11), 4.97 (1H, d, J 10.1 Hz, CH-1), 4.66-4.59 (2H, m, CH₂-12), 4.19-4.12 (2H, m, CH₂-6), 3.80, (1H, t, J 9.3 Hz, CH-3), 3.68 (1H, m, CH-5), 2.76-2.55 (2H, m, CH₂-8), 2.48-2.40 (2H, m, CH₂-9), 2.08-2.0 (9H, 3 × s, CH₃C(O)O). $\delta$C (75 MHz, CDCl₃) 170.7, 169.4, 169.1 (3 × CH₃C(O)O), 152.0 (C-7), 137.0 (C-13), 128.5, 128.0, 127.8 (3 × CH-Ar), 115.8 (C-11), 81.4 (C-3), 80.2 (C-1), 76.4 (C-5), 74.3 (C-4), 71.3 (C-12), 69.5 (C-6), 62.7 (C-5), 31.8 (C-9), 30.8 (C-8), 20.8 (CH₃C(O)O); m/z (ES⁺) 532 (100%, [M+Na]⁺); HRMS (ES⁺) calculated [M+Na]⁺ C₄₅H₃₁NO₉NaS 532.1617, found 532.1627.
S-(2,4,6-Tetra-O-acetyl-3-O-benzyl-β-D-glucopyranosyl) pent-4-ene thiohydroximate NO-sulfate, cyclohexylammonium salt, 197

General procedure E was employed using 195 (1.00 g, 1.96 mmol, 1.0 eq) pyridine-sulfoxide complex (1.55 g, 9.8 mmol, 5.0 eq), pyridine (1.55 mL, 19.6 mmol, 10.0 eq), DCM (10 mL) gave the cyclohexylammonium salt of 197 (0.71 g, 53%) as a colourless solid: [α]_D^20 = -22.3 (c 0.5, CHCl₃); δ_H (500 MHz, CDCl₃) 7.35-7.27 (3H, m, CH-Ar), 7.24-7.21 (2H, m, CH-Ar), 5.87-5.77 (1H, m, CH-10), 5.13-4.93 (5H, m, CH₂-11, CH-1,2,4), 4.63-4.57 (2H, m, CH₂-12), 4.13-4.09 (2H, m, CH₂-6), 3.76 (1H, dd, J 9.3, 9.3 Hz, CH-3), 3.70-3.64 (1H, m, CH-5), 3.17-3.04 (1H, m, CH-1'), 2.75-2.51 (2H, m, CH₂-8), 2.49-2.29 (4H, m, CH₂-9, 2'a), 2.04, 1.99, 1.96 (3 × 3H, s, 3 × CH₃C(O)O), 1.75-1.66 (2H, m, CH₂-2'b), 1.60-1.33 (2H, m, CH₂-3'a), 1.28-1.06 (4H, m, CH₂-3'b, 4'); δ_C (MHz, CDCl₃) 170.7, 169.4, 169.2 (3 × CH₃C(O)O), 152.0 (C-7), 137.5 (C-Ar), 136.8 (C-10), 128.5, 128.0, 127.8 (C-16), 115.8 (C-11), 81.4 (C-3), 80.2 (C-1), 76.4 (C-5), 74.3 (C-12), 71.3 (C-4), 69.5 (C-2), 62.7 (C-6), 50.6 (C-1'), 32.0 (C-9), 31.8 (C-8), 30.5 (2C, C-2'), 24.2 (2C, C-3'), 23.8 (C-4'), 20.9, 20.8, 20.8 (3 × CH₃C(O)O); m/z (ES⁺) (100%, 588 [M⁺]); HRMS calculated [M⁺] C₂₄H₃₀NO₁₂S₂ 588.1209, found 588.120.
References


63. For recent studies please refer to: C.C. Conaway, C. X. Wang, B. Pittman, Y-M Yang, J. E. Schwartz, D. Tian, E. J. McIntee, S. S. Hecht


References


<table>
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<th>Journal</th>
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<th>Year</th>
<th>Pages</th>
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<td>161</td>
<td>S. Karjala and K. P. Link</td>
<td>J. Am. Chem. Soc.</td>
<td>62</td>
<td>1940</td>
<td>917-920</td>
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<td>162</td>
<td>B. H. Brauns</td>
<td>J. Am. Chem. Soc.</td>
<td>47</td>
<td>1925</td>
<td>1280-1284</td>
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<td>164</td>
<td>T. W. Baughman, J.C. Sworen and K.B. Wagener</td>
<td>Tetrahedron</td>
<td>60</td>
<td>2004</td>
<td>10943-10948</td>
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<td>166</td>
<td>M. Frigerio, M. Santagostino and S. Sputore</td>
<td>J. Org. Chem.</td>
<td>64</td>
<td>1999</td>
<td>4537-4538</td>
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<td>169</td>
<td>M. H Benn</td>
<td>J. Chem. Soc.</td>
<td>1964</td>
<td>4072-4074</td>
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Appendices

Crystal data and structure refinement

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-pent-4-ene thiohydroximate, 47

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-hept-6-ene thiohydroximate, 154

S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) Z-pent-4-ene thiohydroximate
NO-Sulfate, cyclohexylammonium salt, 129

Publication

S-(2,3,4,6-Tetra-O-acetyl-β-d-glucopyranosyl) Z-pent-4-ene thiohydroximate, 47

Table 1. Crystal data and structure refinement for scnb2.

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Independent reflections 5625 [R(int) = 0.0784]
Completeness to theta = 25.29° 96.8 %
Absorption correction Multiscan
Max. and min. transmission 1.0000 and 0.8355
Refinement method Full-matrix least-squares on F²
Data / restraints / parameters 5625 / 15 / 560
Goodness-of-fit on F² 1.068
Final R indices [I>2sigma(I)] R1 = 0.1019, wR2 = 0.2684
R indices (all data) R1 = 0.1157, wR2 = 0.2974
Absolute structure parameter -0.12(17)
Extinction coefficient 0.042(8)
Largest diff. peak and hole 0.779 and -0.373 eÅ⁻³

Table 2. Atomic coordinates (x 10⁴) and equivalent isotropic displacement parameters (Å² x 10⁻³) for scnb2. U(eq) is defined as one third of the trace of the orthogonalized Uᵢⱼ tensor.
Table 1. Crystal data and structure refinement for scnb1

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**Table 1. Crystal data and structure refinement for scnb3.**

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An improved procedure for the preparation of β-thiohydroximates for glucosinolate synthesis

Susan E. Cobb, Kate F. Morgan, Nigel P. Botting

Tetrahedron Letters 52 (2011) 1605-1607

doi: 10.1016/j.tetlet.2011.01.117

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