Stemming the tide of resistance in TB: development of chemical tools to evaluate mycothiol dependent enzymes in multidrug resistance in mycobacteria

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I, Ewelina Rybak, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I can confirm that this has been indicated in the thesis.

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Abstract

Tuberculosis (TB) is an infectious disease that kills more than a million people a year and poses a significant health threat. Because of the global spread of multi-drug resistant TB and high infection rates, there is a significant unmet clinical need for new drugs. *M. tuberculosis* (Mtb) produces mycothiol (MSH) in place of glutathione as the most abundant low molecular weight thiol. MSH and associated enzymes (e.g. mycothiol-*S*-transferase, MST) are thought to play pivotal roles in cellular protection against various xenobiotics. Successful synthesis of MST inhibitors may lead to the development of a novel strategy for the treatment of TB.

The aim of this project is to validate MST as a novel drug target and understand the role played by MST in mycobacterial physiology *via* the development of MSH analogues as chemical probes. To achieve this a 2-fold approach was adopted. The first approach includes the development of mycothiol analogues. The synthetic routes towards the synthesis of three different mycothiol analogues containing glucosamine and cysteine moieties are described. These simplified analogues provide a potential scaffold for the synthesis of a library of *S*-conjugates that would enable us to probe the hydrophobic pocket of MST and gain some information about MST's structure–activity relationships. The second approach involves the use of kinetic target-guided synthesis (kTGS), a process in which the protein acts as a catalyst or template in the synthesis of its own best inhibitors from the 'choices' provided. Towards this goal, two azido and two azidoacetamido derivatives of the simplified mycothiol analogue were successfully synthesised and can act as a handle within the binding site. Moreover, the above-mentioned azides were used to synthesize several triazoles that can be utilised as standards when kTGS is performed. The significance of these findings to future drug discovery efforts in this area is discussed.

Impact statement

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and is among the top 10 causes of death globally. In 2017, 1.6 million people died because of TB with 10 million people estimated to suffer from it worldwide. TB is extremely difficult to eradicate and has to be treated using a cocktail of antimicrobial drugs that must be taken for a minimum of six months. Over the years, the same anti-TB drugs have been used extensively resulting in the rise of multi-drug resistant and extensively drug-resistant strains of TB. Because of the global spread of multi-drug resistant tuberculosis and high infection rates, there is a great need for new drugs to be brought to the clinic. *M. tuberculosis* produces mycothiol (MSH) as the most abundant low molecular weight thiol. MSH and associated enzymes (e.g. mycothiol-*S*-transferase, MST) are thought to play a major role in combating oxidative stress and to be involved in the detoxification of electrophilic toxins. This is why it is hypothesised that inhibition of MST will lead to the development of a novel strategy for the treatment of TB.

This project resulted in the development of a novel approach towards the synthesis of the cysteine-containing mycothiol scaffolds. Three different mycothiol analogues containing glucosamine and cysteine moieties were successfully obtained. The synthesised scaffolds can be easily derivatized forming a library of *S*-conjugates that can be utilized to explore the role of MST and other MSH-dependent enzymes. The described methodology can be used to generate more mycothiol-based scaffolds if needed. Moreover, relatively short and easy synthetic strategies were designed and applied to successfully form two azido and two azidoacetamido derivatives of the simplified mycothiol analogue. Because of the introduction of the azido group the analogues can be utilized in kinetic target-guided synthesis (kTGS) to produce triazole containing mycothiol analogues. kTGS is a process in which the protein acts as a catalyst or template in the synthesis of its own best inhibitors from the ‘choices’ provided. The use of kTGS to synthesise MST inhibitors will allow further exploration of this technique in lead discovery. Additionally, a diverse chemical library containing azido mycothiol analogue-based triazoles was successfully synthesised using standard ‘click chemistry’ conditions. Thus, providing an easy way to produce a diverse library of potential MST substrates. The biological activity of all of the synthesised

mycothiol analogues will have to be tested. The findings of these tests will allow a better understanding of the role of MSH-dependent enzymes. If a promising candidate is found, it can be further developed into a lead compound and finally novel anti-TB drugs could be identified.

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List of abbreviations

1-L-Ins-1-P	1-L-*myo*-Inositol-1-phosphate
AChE	Acetylcholinesterase
AIBN	2,2'-Azobis(2-methylpropionitrile)
BEMP	2-*t*-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine
BTFFH	Fluoro-*N*,*N*,*N*',*N*'-bis(tetramethylene)formamidinium hexafluorophosphate
CD	Circular dichroism
COMU	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
Cys-GlcN-Ins	1-*O*-[2-[[(2*R*)-2-amino-3-mercapto-1-oxopropyl]amino]-2-deoxy-α-D-glucopyranosyl]-D-*myo*-inositol
DAST	(Diethylamino)sulfur trifluoride
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCS	D-Cycloserine
DCU	Dicyclohexylurea
DEAEA	*N*,*N*-Diethylethylenediamine
DEPC	Diethylphosphoryl cyanide
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
DNP	2,4-Dinitrophenyl group
DSF	Differential scanning fluorimetry
DTNB	5,5'-dithio-bis-(2-nitrobenzoic acid)
EDCI	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
FDA	Food and Drug Administration
FSA	Fluorescamine
GlcNAc-Ins	*N*-Acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-

	glucopyranoside
GlcNAc-Ins-3-P	3-Phospho-1-D-*myo*-inosityl-2-acetamido-2-deoxy-α-D-glucopyranoside
GlcN-Ins	Glucosaminyl-inositol
GSH	Glutathione
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate
HBTU	*N*,*N*,*N*',*N*'-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IC_{50}	Half maximal inhibitory concentration
***i*-Pr_2EtN**	*N*,*N*-Diisopropylethylamine
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITC	Isothermal titration calorimetry
K_d	Dissociation constant
K_i	Inhibitor constant
kTGS	Kinetic target guided synthesis
LAH	Lithium aluminum hydride
LCMS	Liquid chromatography–mass spectrometry
mBCl	Monochlorobimane
Mca	Mycothiol *S*-conjugate amidase
MCPBA	3-Chloroperbenzoic acid
MDR-TB	Multi-drug resistant tuberculosis
MIC	Minimum inhibitory concentration
MscR	Mycothiol-S-nitrosoreductase/-formaldehyde dehydrogenase
MSH	Mycothiol
MSmB	Mycothiol bimane
MSNO	Nitrosomycothiol
MSSM	Mycothiol disulfide
MST	Mycothiol-*S*-transferase

Mtr	Mycothiol disulfide reductase
***N*-AcCysmB**	*N*-acetyl-L-cysteinyl monobimane
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NIS	*N*-Iodosuccinimide
NMM	*N*-Methylmorpholine
NMO	4-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
PAS	*para*-Aminosalicylic acid
PhMe	Toluene
***p*-TsOH**	*p*-Toluenesulfonic acid
R_f	Retention factor
SAR	Structure–activity relationship
STD-NMR	Saturation transfer difference NMR
TB	Tuberculosis
TBAF	Tetra-*n*-butylammonium fluoride
TBTU	*O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate
TCA	Trichloroacetonitrile
TFA	Trifluoroacetic acid
TfOH	Triflic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNTU	*O*-(5-Norbornene-2,3-dicarboximido)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate
Tol-SH	4-Methylbenzenethiol
TPAP	Tetrapropylammonium perruthenate
UDP-GlcNAc	Uridine diphosphate *N*-acetylglucosamine

Part I Introduction

1. Tuberculosis

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and is among the top 10 causes of death globally. In 2017, 1.6 million people died because of TB with 10 million people estimated to suffer from it worldwide.[1] It is primarily an infection of the lungs and is spread through droplet transmission while sneezing or coughing. The symptoms may be mild during first few months and before the disease is diagnosed other people can be infected. It is estimated that one-quarter of the world's population is infected with TB but as the bacteria are dormant in the majority of the cases, infected people neither develop nor transmit the disease to others.[1] Dormant *M. tuberculosis* can reactivate in a patient with a weakened immune system. This is why HIV-positive people are 20 to 30 times more likely to suffer from TB than healthy individuals.[1] Active, drug sensitive TB is treatable using a cocktail of four antimicrobial drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) that must be taken for two months, followed by four months of isoniazid and rifampicin treatment.[2] TB is extremely difficult to eradicate due to the unique composition of the mycobacterial cell wall. The differences between Gram-negative, Gram-positive and mycobacterial cell walls are shown in Fig. 1. The Mtb cell wall is composed of a thin layer of cross-

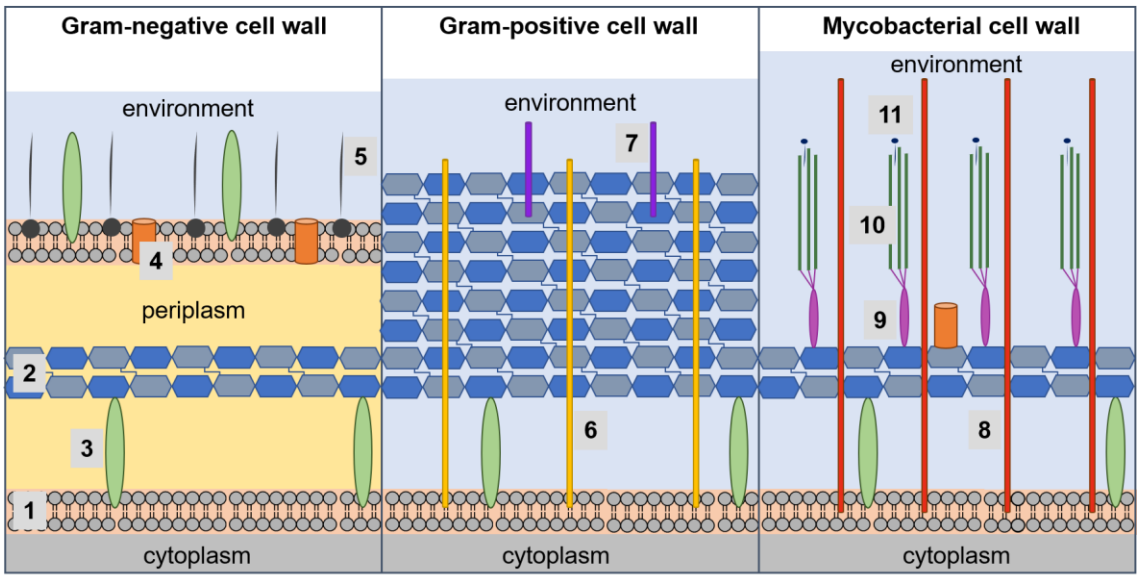
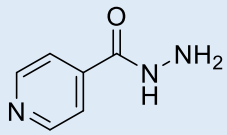
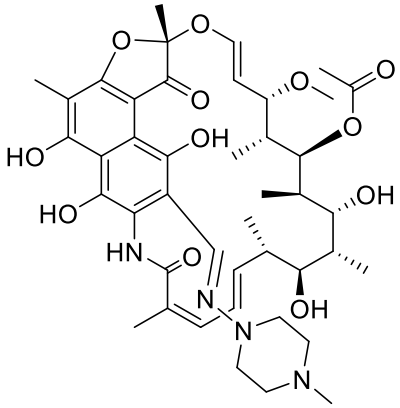
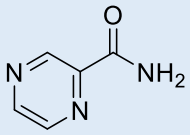
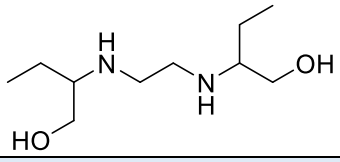
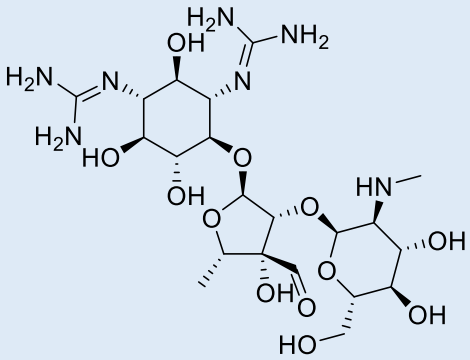
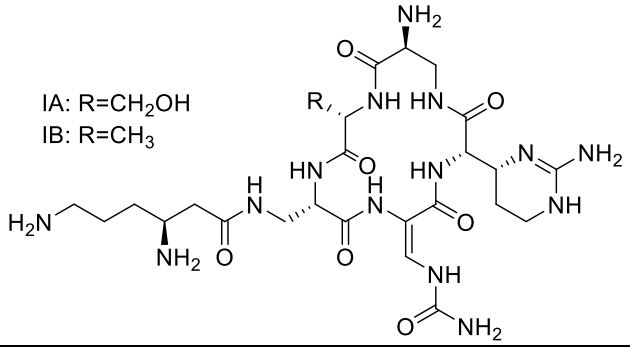
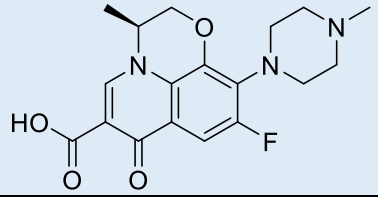
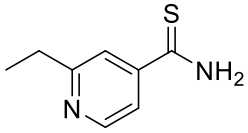
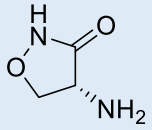
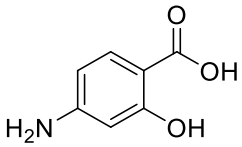
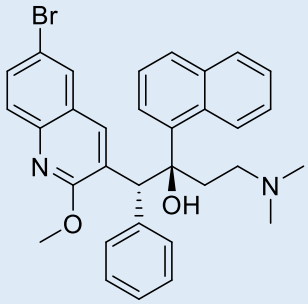
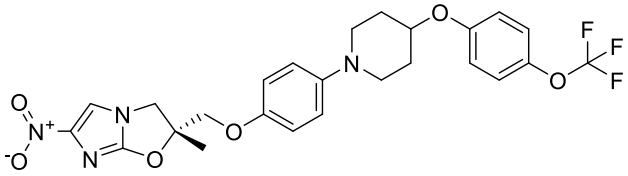
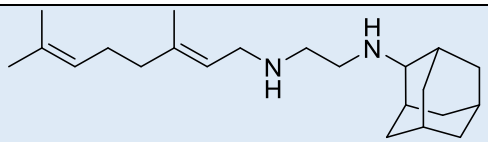
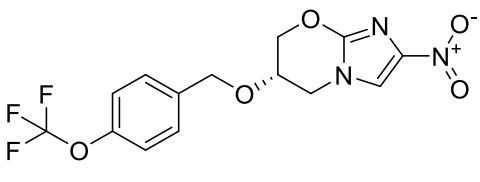


Fig. 1. Scheme depicting bacterial cell walls. 1: cytoplasmic and outer membrane, 2: peptidoglycan, 3: lipoprotein, 4: porin, 5: lipopolysaccharide, 6: lipoteichoic acid, 7: teichoic acid, 8: lipoarabinomannan, 9: arabinogalactan, 10: mycolic acid, 11: glycolipids.[3]

linked peptidoglycan and branched arabinogalactan, to which mycolic acids are attached. Another characteristic is the presence of lipoarabinomannan attached to the cytoplasmic membrane which is not present in Gram-negative and Gram-positive bacteria. The large amount of fatty acids present in the cell wall makes it mostly impermeable. This is why many anti-TB drugs target cell wall biosynthesis.[3] Each of the drugs used have a different mechanism of action. Isoniazid acts *via* inhibition of mycolic acid synthesis, rifampicin blocks the synthesis of RNA, pyrazinamide inhibits translation, while ethambutol inhibits arabinogalactan synthesis (Table 1).[4,5] Because the same treatment has been used for several decades and sometimes the drugs are not used appropriately or correctly, multi-drug resistant strains of TB (MDR-TB) have evolved and spread around the world. In order to be called multi-drug resistant, TB strains must be resistant to isoniazid and rifampicin, which are the most effective first-line anti-TB drugs.[1] MDR-TB is treated using the following drugs: fluoroquinolones (including levofloxacin),[5] aminoglycosides (including streptomycin), capreomycin, ethionamide, D-cycloserine (DCS) and *para*-aminosalicylic acid (PAS) (Table 1). There are also two new drugs available that can be used in combination with previous drugs, namely bedaquiline, which inhibits ATP production and delamanid, which blocks the synthesis of mycolic acid and produces reactive nitrogen species (Table 1). Additionally, there are two novel drugs (SQ109 and pretomanid) currently under development, which act *via* inhibition of mycolic acid synthesis (Table 1).[4] Pretomanid has recently been accepted for review by the FDA,[6] while SQ109 has successfully completed a Phase 2b-3 clinical trial.[7] Because of the spread of multi-drug resistant tuberculosis, there is an urgent need for new drugs that act *via* novel mechanisms to be developed.

Table 1. Antitubercular drugs.[4,5,8]

Drug	Structure	Mechanism of action
Isoniazid		Inhibition of mycolic acid synthesis.
Rifampin		Inhibition of RNA synthesis.
Pyrazinamide		Inhibition of translation. It may also inhibit ATP synthesis.
Ethambutol		Inhibition of arabinogalactan synthesis.
Streptomycin		Inhibition of protein synthesis.
Capreomycin		Inhibition of protein synthesis.

Levofloxacin		Inhibition of DNA synthesis.
Ethionamide		Inhibition of mycolic acid synthesis.
D-Cycloserine		Inhibition of cell wall peptidoglycan synthesis.
***para*-Aminosalicylic acid**		Inhibition of folic acid and thymine nucleotide metabolism.
Bedaquiline		Inhibition of ATP production.
Delamanid		Inhibition of mycolic acid synthesis, production of reactive nitrogen species.
SQ109		Inhibition of mycolic acid synthesis.
Pretomanid		Inhibition of mycolic acid synthesis, production of reactive nitrogen species.

2. Mycothiol

All eukaryotes and most Gram-negative bacteria produce the tripeptide glutathione (GSH) as the major low molecular weight thiol. GSH plays a central role in combating oxidative stress, is involved in the detoxification of electrophilic toxins and serves as a cofactor for various enzymes.[9] It was found, however, that *Actinomycetes*, to which *M. tuberculosis* belongs, instead produce mycothiol (MSH) as the most abundant low molecular weight thiol. It has not been extensively studied, but it is thought to fulfil the same functions as glutathione.[10] The core functional component of GSH and MSH is the cysteine moiety. However, mycothiol, unlike glutathione which is a tripeptide, has two sugar moieties, namely inositol and glucosamine (Fig. 2).[9] The inositol moiety is not found in any other biological thiol.[11]

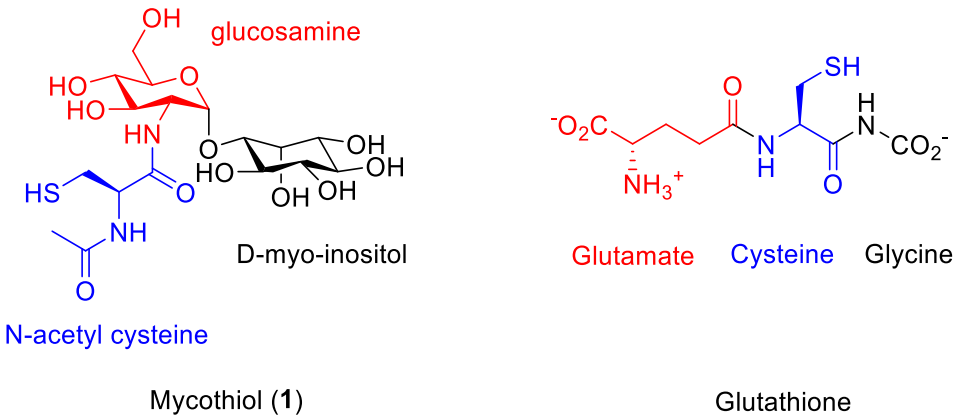


Fig. 2. Structure of mycothiol and glutathione.

Mycothiol, like glutathione, serves as thiol reservoir and is important in maintaining the reducing environment inside the cell as it acts as a redox buffer. Its most important function is protection against various xenobiotics such as oxidizing agents, alkylating agents and antibiotics.[11] It is involved in the detoxification of electrophilic toxins by forming mycothiol-toxin *S*-conjugates, which are subsequently hydrolysed by mycothiol *S*-conjugate amidase (Mca) generating GlcN-Ins and a mercapturic acid-toxin conjugate, which is transported out of the cell.[12] It has also been implicated in resistance to antibiotics such as rifampicin and streptomycin. Moreover, it was found that mycothiol plays an important role in the bioactivation of some antibiotics (e.g. isoniazid and ethionamide).[11] A chemical mutant of *Mycobacterium smegmatis* in which MSH production was disrupted showed a more than 25-fold increase in resistance to

isoniazid compared to the wild type strain.[13] It was also shown that mycothiol lacking mutants: Δ*mshA*, Δ*mshC* and Δ*mshD* of *M. smegmatis* are resistant to ethionamide. High levels of resistance to isoniazid and ethionamide in strains lacking mycothiol confirms that MSH plays an important role in the bioactivation of these pro-drugs. On the other hand, all MSH mutants were shown to be sensitive to streptomycin and the Δ*mshA* mutant of *M. smegmatis* was 10 times more sensitive to rifampicin (determined by *E*-test) as the level of mycothiol in the *M. smegmatis* mutant was considerably decreased.[14] Even though there is conflicting evidence regarding the essentiality of MSH for *M. tuberculosis*, which will be discussed in more detail in the next section, it is generally accepted that it is vital for the survival of Mycobacterium species under stress conditions. Thus, it is considered to be a good drug target.[11]

2.1. Mycothiol biosynthesis

Mycothiol is biosynthesised in five steps that involve the following enzymes: MshA, MshA2, MshB, MshC and MshD, which are found only in *Actinomycetes*. All the enzymes in the mycothiol biosynthetic pathway are currently viewed as potential drug targets, because not only are they not found in humans, but also their inhibition would block the synthesis of mycothiol, which is important in maintaining the viability of *M. tuberculosis*.[12]

The first step is catalysed by MshA, which is a glycotransferase. It transfers *N*-acetylglucosamine from UDP–*N*-acetylglucosamine (UDP–GlcNAc) to 1-L-*myo*-inositol-1-phosphate (1-L-Ins-1-P) generating 3-phospho-1-D-*myo*-inosityl-2-acetamido-2-deoxy-α-D-glucopyranoside (GlcNAc-Ins-3-P) (Fig. 3).[11,12] It has been demonstrated that the Δ*mshA* mutant of *M. smegmatis* is more sensitive to a number of antibiotics including streptomycin and rifampicin and alkylating agents (e.g. iodoacetamide, chlorodinitrobenzene) than the wild type strain.[14] The Δ*mshA* null deletion mutant of *M. smegmatis* was shown to block mycothiol biosynthesis and slow cell growth.[15] There is conflicting evidence regarding the essentiality of the *mshA* gene for *M. tuberculosis*.[11] In 2006, it was found that the *mshA* gene is required for growth of *M. Tuberculosis*,[16] however, two years later it was reported that spontaneous *mshA* mutants of *M. tuberculosis* were viable.[17] MshA is thought to be a good drug target because it is the enzyme that catalyses the first committed step in the mycothiol biosynthesis. To date two inhibitors of

MshA have been reported. The first inhibitor UDP-(5F)-GlcNAc (**2**) (Fig. 4) is a structural analogue of the natural substrate UDP-GlcNAc. It was found to competitively inhibit MshA from *Corynebacterium glutamicum* (K_i ~1.6 μM).[18] The second reported inhibitor was *O*-UDP-GlcNAc (**3**) (Fig. 4) – also a natural substrate analogue. It was shown to inhibit MshA from *M. smegmatis* (IC_{50} 0.2 mM).[19] Solving the crystal structure and developing a method to easily purify active MshA for *M. tuberculosis* would facilitate inhibitor discovery for this enzyme.[11,20]

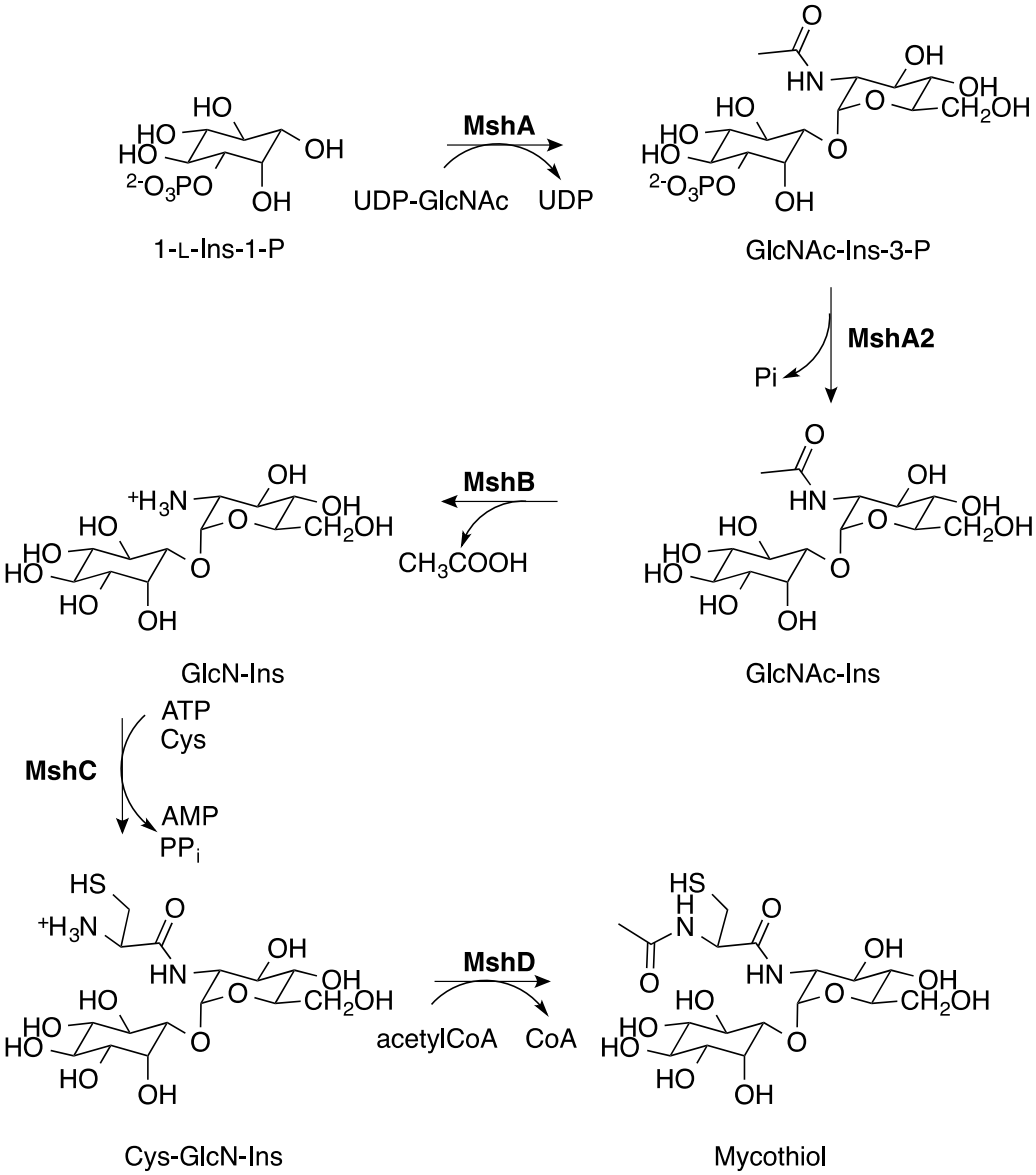
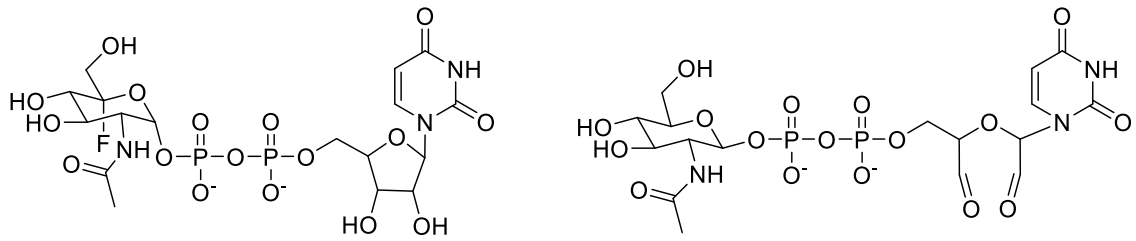


Fig. 3. Biosynthesis of mycothiol.



UDP-(5F)-GlcNAc (**2**) O-UDP-GlcNAc (**3**)

Fig. 4. Structures of MshA inhibitors.

The next step is catalysed by the phosphatase MshA2, which dephosphorylates GlcNAc-Ins-3-P to produce *N*-acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-glucopyranoside (GlcNAc-Ins) (Fig. 3). The gene encoding for MshA2 activity has not been identified and so has yet to be characterized biochemically.[11]

The following step is catalysed by the deacetylase MshB, which deacetylates GlcNAc-Ins to give GlcN-Ins (Fig. 3). MshB isolated from *M. tuberculosis* was found to be a Zn^{2+}-dependent deacetylase. Moreover, it is thought that this is the rate-limiting step in the biosynthesis of mycothiol. MshB is not essential for mycothiol biosynthesis as it was found that the mycothiol *S*-conjugate amidase (Mca) can compensate for *mshB* gene deletion and mycothiol is not produced in a dual Δ*mshB*/Δ*mca* knockout mutant.[11,12] It was also found that *mshB* gene disruption in *M. smegmatis* does not alter its sensitivity to streptomycin, rifampicin, or ethionamide but increases sensitivity to erythromycin and azithromycin.[14]

The first reported inhibitors of MshB were identified by screening a biased chemical library that included compounds with both structural features of the natural substrate (GlcN-Ins) and compounds found to inhibit Mca, which is a closely related homolog of MshB. The greatest advantage of using the latter class of dual inhibitors would be inactivation of both MshB and Mca. Eight analogues (Fig. 5) out of 23 tested showed more than 40% inhibition at 200 μM (fluorescence-detected HPLC assays). The most potent inhibitor (**10**) had an IC_{50} of 7 ±1 μM, so there is scope for further improvements to the activity of this series.[11,21]

Three years later a new set of 21 substrate-mimics of GlcNAc-Ins was tested. The synthesized compounds fall into three categories: cyclohexyl-2-deoxy-2-*C*-alkylglucosides, naphthoquinones and thioglycosides (Fig. 6). All of the

compounds were relatively weak inhibitors. The most promising class of compounds was the naphthoquinones. The four compounds in this series showed inhibition within the range of 57 to 95% at 500 μM substrate and inhibitor concentrations. They also inhibited Mca, but to a lower extent (23 to 44% inhibition at 250 μM).[22] Additionally, two new assays for measuring MshB activity were reported. The first assay is a fluorescence-based assay where fluorescamine (FSA) is used.[23] The second assay is a continuous 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) based assay.[24] The development of those assays will help to screen prospective inhibitors more rapidly.

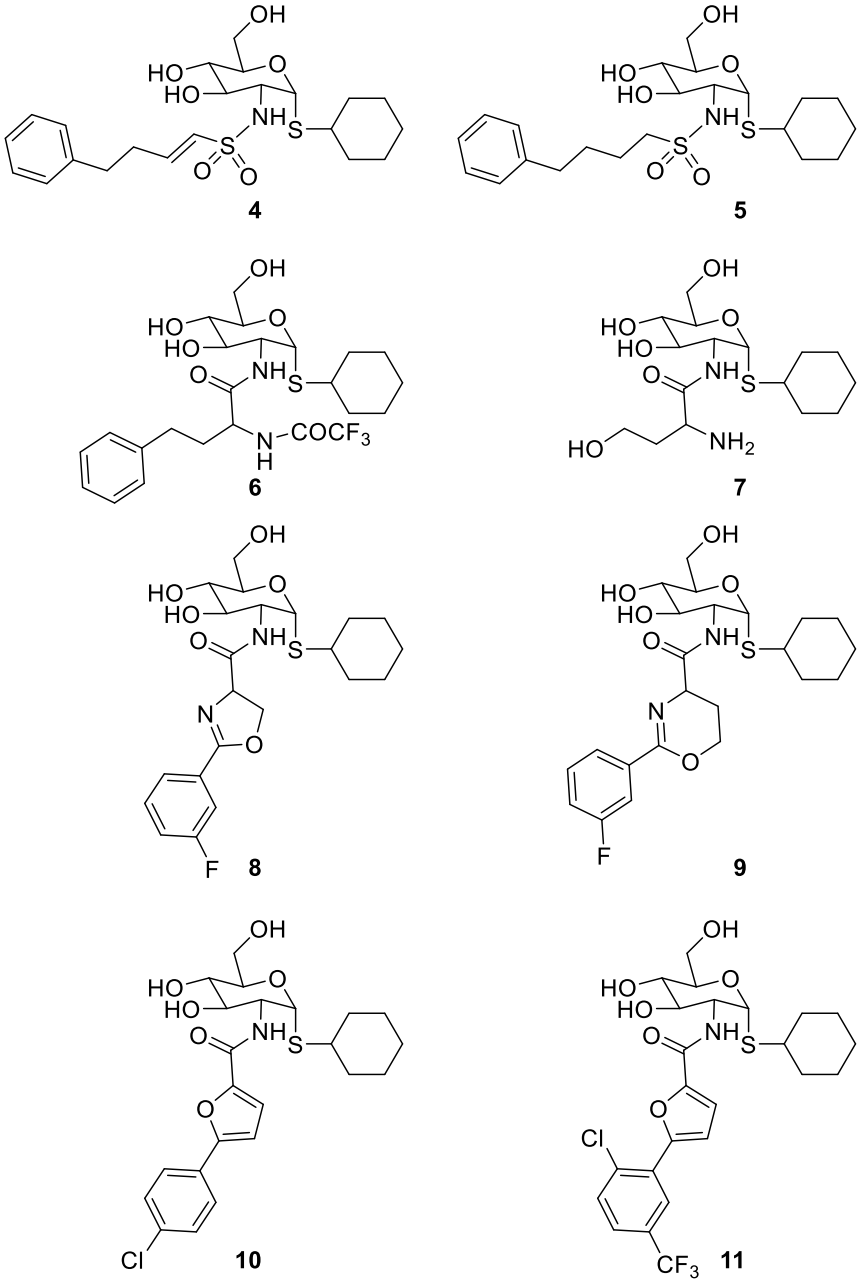


Fig. 5. Structures of MshB inhibitors.

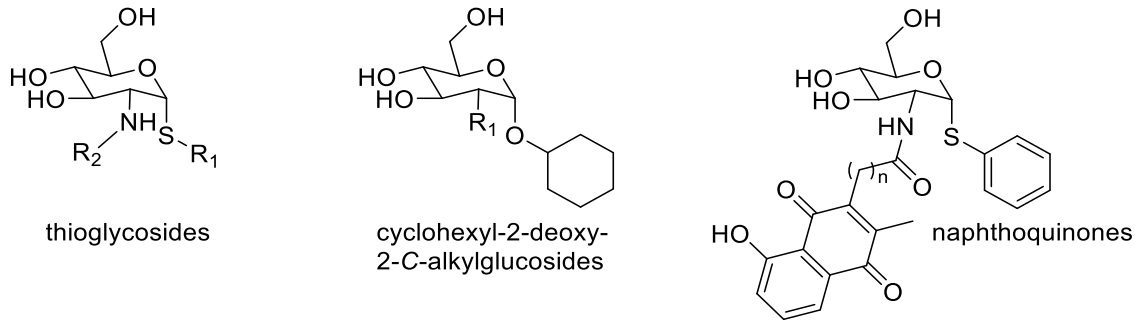


Fig. 6. Structures of MshB inhibitors.

The fourth step in the mycothiol biosynthesis involves the ligase MshC, which catalyses the ATP-dependent conjugation of cysteine with GlcN-Ins to yield Cys-GlcN-Ins (Fig. 3). MshC has been found to be essential for the mycothiol biosynthesis in *M. smegmatis* as well as essential for *in vitro* growth of *M. tuberculosis* suggesting that it is a promising drug target.[11,12] A mutant disrupted in *mshC* gene of *M. smegmatis* has been demonstrated to be more sensitive to erythromycin and azithromycin but not to rifampicin compared with the wild type strain. Moreover, the Δ*mshC* mutant of *M. smegmatis* was more sensitive to oxidative stress and alkylating agents (e.g. iodoacetamide, chlorodinitrobenzene).[14] MshC is thought to be the most attractive drug target amongst the mycothiol biosynthesis enzymes because of its essentiality for *M. tuberculosis* growth. The first identified inhibitor of MshC, NTF1836 (**12**) (IC_{50} ~100 μM) (Fig. 7), was found in a screen of a commercial library containing 2024 compounds. It was demonstrated that **12** inhibits growth of *M. tuberculosis* and kills non-replicating cells.[11,25,26] In a more recent study, a library of 3100 chemically diverse compounds was screened and one compound, namely dequalinium (**13**) (Fig. 7), was identified as a relatively potent, ATP-competitive inhibitor of MshC (IC_{50} 24 μM) that inhibits *M. tuberculosis* growth under aerobic and anaerobic conditions.[11,27]

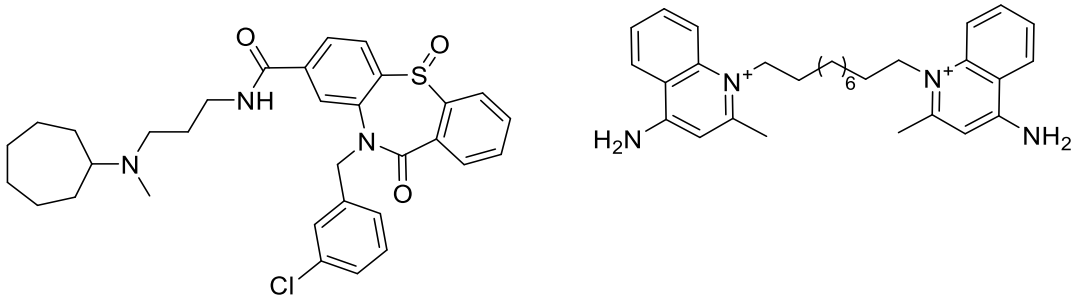


Fig. 7. Structures of MshC inhibitors.

The last step in the biosynthetic pathway of MSH is catalysed by the acetylase MshD, which transfers an acetyl group from acetyl-CoA to Cys-GlcN-Ins to produce mycothiol (Fig. 3). It was shown that deletion of the *mshD* gene in *M. smegmatis* decreases mycothiol production in a cell.[15] It was also demonstrated that disruption in the *mshD* gene of *M. tuberculosis* increases the sensitivity of the strain to oxidative stress (hydrogen peroxide) compared to the wild type strain.[28] Moreover, it was shown that the Δ*mshD* mutant of *M. smegmatis* is more sensitive to a number of antibiotics (e.g. rifampin and vancomycin) and alkylating agents (e.g. iodoacetamide, chlorodinitrobenzene) than the wild type strain.[11,12,14] No inhibitors of MshD have been reported to date.[11]

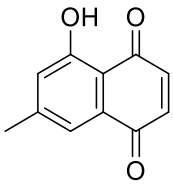
2.2. Mycothiol-dependent enzymes and their inhibitors

Because some anti-tubercular drugs like isoniazid and ethionamide require mycothiol to exert their full effect, blocking its biosynthesis would limit the usefulness of those drugs. This is why mycothiol-dependent enzymes rather than the mycothiol synthesis enzymes may present more appealing drug targets. Inhibitors of mycothiol-*S*-transferase (MST) could work synergistically with current drugs that are detoxified by mycothiol-dependent enzymes like rifampicin and streptomycin and with those that need mycothiol for bioactivation unless MST is involved in the bioactivation.[11]

2.2.1. Mycothiol disulfide reductase (Mtr)

Mycothiol disulfide reductase (Mtr) is an enzyme responsible for the reduction of oxidized mycothiol disulfide (MSSM) to MSH. Maintenance of a high thiol to disulfide ratio is important for the maintenance of redox homeostasis inside the cell. Mtr was characterized as one of the first mycothiol dependent enzymes by sequence homology with GSH reductase. It is an NADPH/NADH dependent flavoprotein. Its crystal structure has not been solved but its catalytic mechanism and overall structure is believed to be analogous to that of the orthologous glutathione reductase (31% sequence identity). It was also found that the inositol moiety of MSSM is not essential for its recognition by Mtr by comparing the steady-state kinetic parameters for MSSM and desmyoinositol mycothione.[9,11,20,29]

Because of the known antimicrobial activity of naphthoquinones, a series of 24 synthetic and natural analogues of naphthoquinone 7-methyljuglone was designed and synthesized as potential Mtr inhibitors. The most potent compound of this series, which was shown to be subversive substrate of Mtr, was 5-hydroxy-7-methyl-1,4-naphthoquinone (**14**) (MIC: 0.5 μg/mL) (Fig. 8). However, the potential Mtr inhibitors were found to be non-specific and they are thought to inhibit other disulfide reductases including those present in mammalian cells.[11,30] Additionally, the development of a continuous DTNB-coupled assay that allows characterisation of slow time-dependent inhibitors of Mtr will help to identify prospective inhibitors more rapidly.[11,31]



5-Hydroxy-7-methyl-1,4-naphthoquinone (**14**)

Fig. 8. Structure of Mtr inhibitor.

2.2.2. Mycothiol *S*-conjugate amidase (Mca)

Mycothiol *S*-conjugate amidase (Mca) is thought to be one of the most important mycobacterial enzymes involved in the detoxification of the electrophilic toxins including some antibiotics. This is believed to be the case because Mca homologues were found within operons for antibiotic biosynthesis in antibiotic-producing *Actinomycetes*. Mca has deacetylase activity and is a zinc metalloenzyme. It hydrolyses the glucosaminyl–amide bond of mycothiol *S*-conjugates to give GlcN-Ins, which is recycled back into the mycothiol biosynthetic pathway, and *S*-conjugated cysteine or mercapturic acid, which is transported out of the cell, probably *via* a yet to be identified efflux transporter (Fig. 9).[9,20,32,33]

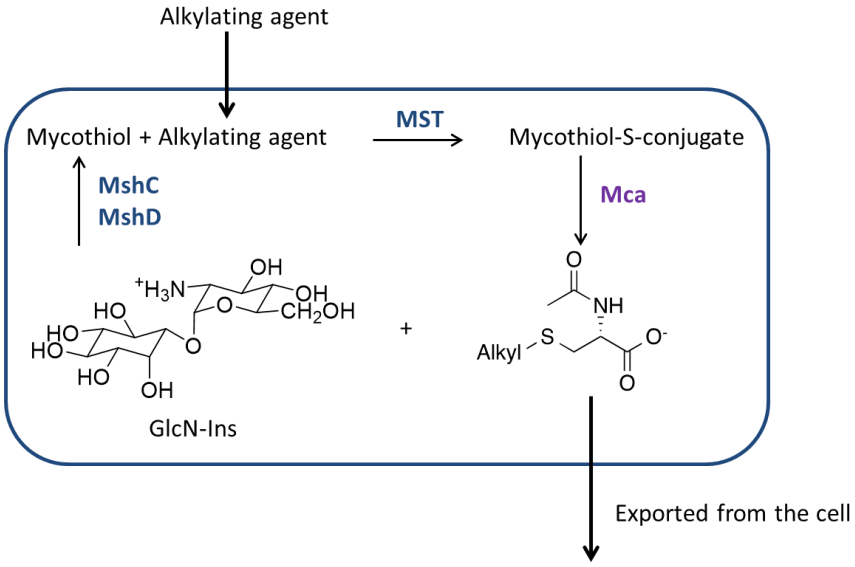
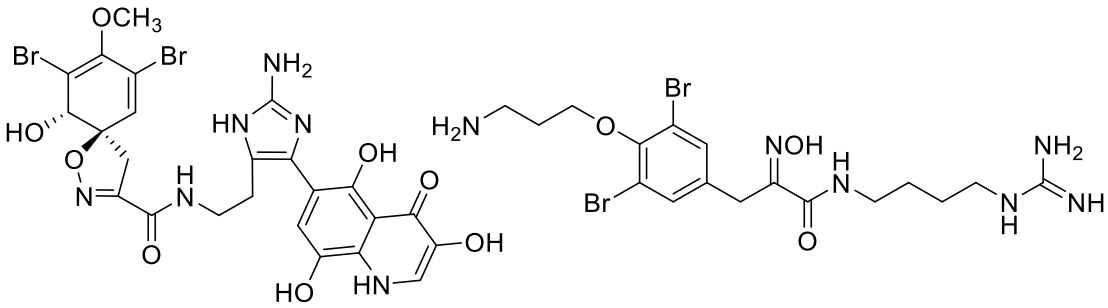


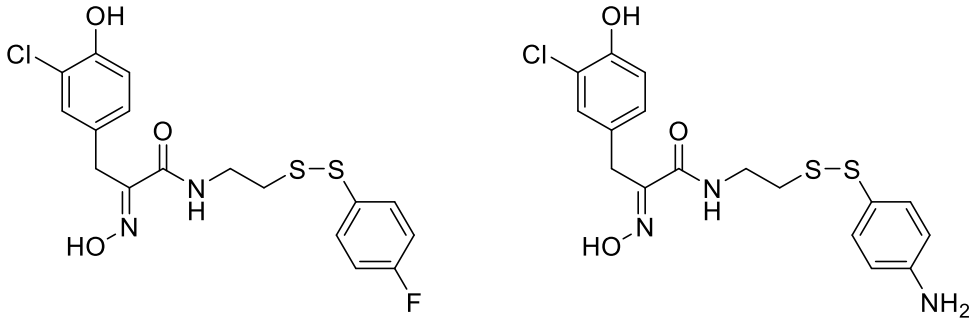
Fig. 9. Scheme depicting the role of Mca in mycothiol dependent detoxification.

An *mca* gene mutant of *M. smegmatis* was found to be more sensitive to streptomycin, electrophilic toxins (e.g. iodoacetamide, chlorodinitrobenzene) and to oxidants (e.g. plumbagin, menadione) than the wild type strain.[34] The crystal structure of Mca has yet to be solved but its overall structure is believed to be analogous to that of MshB (36% sequence identity). Because Mca and MshB are homologs, they have overlapping substrate activity. As mentioned, Mca can compensate for loss of MshB by deacetylating GlcNAc-Ins to give GlcN-Ins in mutants lacking MshB. However, by examining the substrate specificity of Mca with various substrates it was shown that large substrates (e.g. MS–rifamycin) are preferred to small and polar substrates (e.g. MSH) by Mca. Comparison of the kinetic parameters for a wide range of substrates showed that *S*-conjugates can be either small, non-polar molecules or large macrocyclic antibiotics. The acetyl and inositol moieties of mycothiol *S*-conjugates are essential for the substrate recognition, because when substrates lacking those moieties were used no Mca activity was observed.[9,20,32,33] Mycothiol *S*-conjugate amidase is the most studied drug target of all mycothiol-dependent enzymes. The first identified inhibitors of Mca were four bromotyrosine-derived metabolites isolated from *Oceanapia sp.* (a marine sponge). Compounds **15** and **16** showed the strongest inhibition of Mca (IC_{50} 3 μM for both compounds) (Fig. 10).[35,36] In subsequent years, screening of about 1500 marine plants and invertebrates extracts as well as fungal extracts resulted in identification of 13 natural products that inhibited Mca. It was also found that bromotyrosine-derived natural products competitively

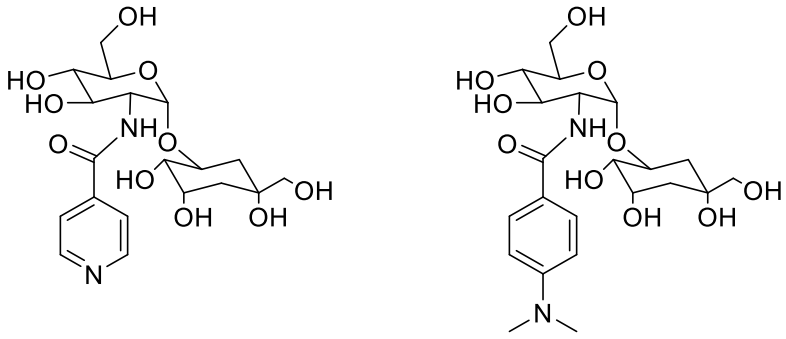
inhibit Mca, while gliotoxin (IC_{50} 50 μM), which is a fungal metabolite, is a non-competitive inhibitor of Mca.[36] In another screen a psammaplin A-inspired combinatorial library[37] was used alongside natural products to identify the first synthetic inhibitors of Mca (e.g. **17** and **18** IC_{50} ~40 μM) (Fig. 10).[38] More recently, 14 substrate-mimic inhibitors of Mca having a quinic acid-derived ring system instead of a *myo*-D-Ins moiety were synthesized and tested as Mca inhibitors (e.g. **19** and **20** ~45% inhibition of Mca at 50 μM) (Fig. 10).[39]



Bromotyrosine-derived natural products **15** and **16**



Psammaplin A synthetic derivatives **17** and **18**



Quinic acid-derived mycothiol analogues **19** and **20**

Fig. 10. Structures of Mca inhibitors.

2.2.3. Mycothiol-*S*-nitrosoreductase/-formaldehyde dehydrogenase (MscR)

MscR is a NAD/mycothiol dependent formaldehyde dehydrogenase. It is involved in the metabolism of formaldehyde, which is naturally produced by the cells as a result of C1 compound metabolism (e.g. methane) and it is toxic even at low concentrations. Mycothiol acts as a cofactor that spontaneously conjugates to formaldehyde in order to form an *S*-hydroxymethyl-mycothiol adduct, which is subsequently oxidized by MscR to give mycothiol formate ester (Fig. 11.a). MscR was also shown to have NADH/mycothiol dependent nitrosomycothiol (MSNO) reductase activity. It catalyses the conversion of nitrosomycothiol to MSSM and nitrate (Fig. 11.b). Moreover, it is believed to be an essential enzyme involved in cell protection against the toxic effects of nitric oxide, as it was demonstrated that a MSH-deficient mutant of *M. smegmatis* was more sensitive to nitric oxide than the wild type strain.[9,20,33] No inhibitors of MscR have been reported to date.

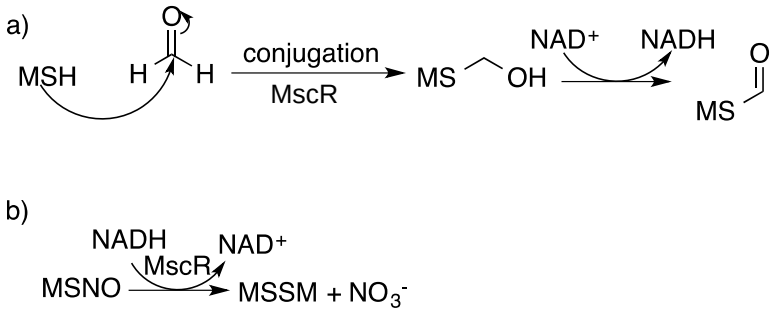


Fig. 11. (a) Formaldehyde dehydrogenase and (b) mycothiol-*S*-nitroso reductase activity of MscR.

2.2.4. Mycothiol-*S*-transferase (MST)

By analogy to the GSH-dependent detoxification pathway it was expected that mycobacteria would produce an enzyme analogous to glutathione-*S*-transferase. It was not until recently that the existence of a mycothiol-*S*-transferase (MST) was identified in *M. tuberculosis*. MST activity of the protein encoded by the Rv0443 gene from *M. tuberculosis* (77% sequence identity to *M. smegmatis* MST) was confirmed using monochlorobimane (mBCl) and MSH as substrates and a mycothiol-bimane conjugate was identified as the product. It was demonstrated that MST like other *S*-transferases catalyses the conjugation of electrophilic toxins to the free thiol group on mycothiol, producing mycothiol *S*-conjugates. Mycothiol *S*-conjugates are subsequently hydrolysed by mycothiol *S*-

conjugate amidase in order to give GlcN-Ins, which is recycled back into the mycothiol biosynthetic pathway, and mercapturic acid, which is transported out of the cell. MST belongs to the DUF664 family which is a part of the DinB superfamily (DNA-damage-induced genes). This superfamily includes proteins known to have thiol-dependent catalytic activity or detoxification activity. The crystal structure of MST has not been solved, but as it is a member of the DinB superfamily of proteins its structure is believed to be similar to other family members (e.g. mycothiol-dependent maleylpyruvate isomerase from *C. glutamicum*). It is likely to contain a four-helix up-down-down-up bundle, form dimers and to be metal-dependent (Fig. 12). An active site consisting of three histidine residues chelating a metal ion is characteristic for DinB proteins and is thought to be a potential binding site for the *N*-acetyl-cysteine moiety of the mycothiol.[11,40] Analysis of the polarity of the surface of a homology model (Fig. 12) revealed a relatively hydrophobic site to the left of the metal-binding site, which may be where electrophilic toxins coupled to mycothiol bind. On the right, a relatively hydrophilic site was revealed, and we propose that it is where the GlcN-Ins moiety of MSH binds.

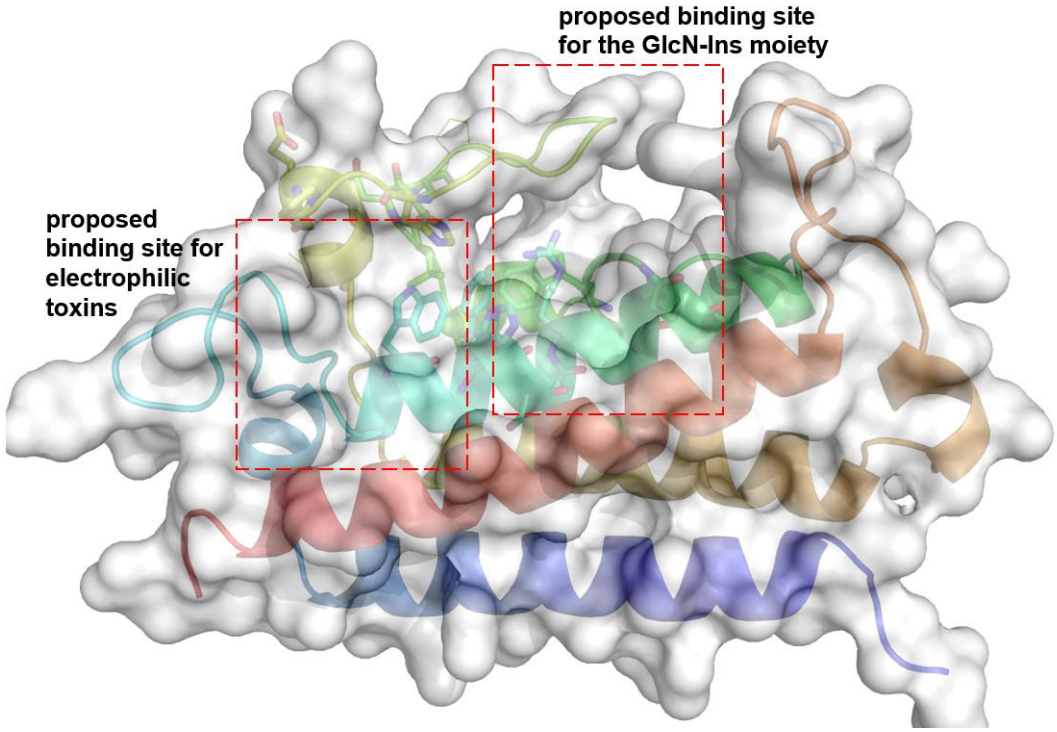


Fig. 12. Homology model of MST (shown as ribbon) based on 3CEX (35.5% identity) made by Dr Shozeb Haider (UCL School of Pharmacy). Image prepared using PyMOL.

It was found that MST was 200-fold more active toward mBCl with MSH than with GSH, showing that MST is substrate selective towards MSH. It was also suggested that inhibition of mycothiol-*S*-transferase could have an important role in impairing detoxification of antibiotics, which could result in increased sensitivity to antibiotics as MST was shown to have good activity when mitomycin C was used as a substrate.[11,40]

2.3. Chemical synthesis of mycothiol

The first attempt to chemically synthesise mycothiol was performed in 2002 by Jardine *et al*.[41] The synthetic plan was to synthesise separately the appropriately protected sugar moieties, link them and finally couple the cysteine moiety to the pseudo-disaccharide. First, the selectively acetylated *myo*-inositol with an unprotected 1'-OH group was synthesised over six steps, finally giving pure D-**25** and L-**25** enantiomers (Fig. 13). Both enantiomers were separately coupled to the protected glucosamine moiety (**26**) and treated with an anion exchange resin to give four pseudo-disaccharides in total: **31** (the desired α-D anomer), **32**, **33**, **34** (Fig. 14) Because synthetic coupling of *N*-acetyl-cysteine to **31** was unsuccessful, it was decided to use a cell-free extract of *M. smegmatis* to produce mycothiol (**1**) (Fig. 15).[41]

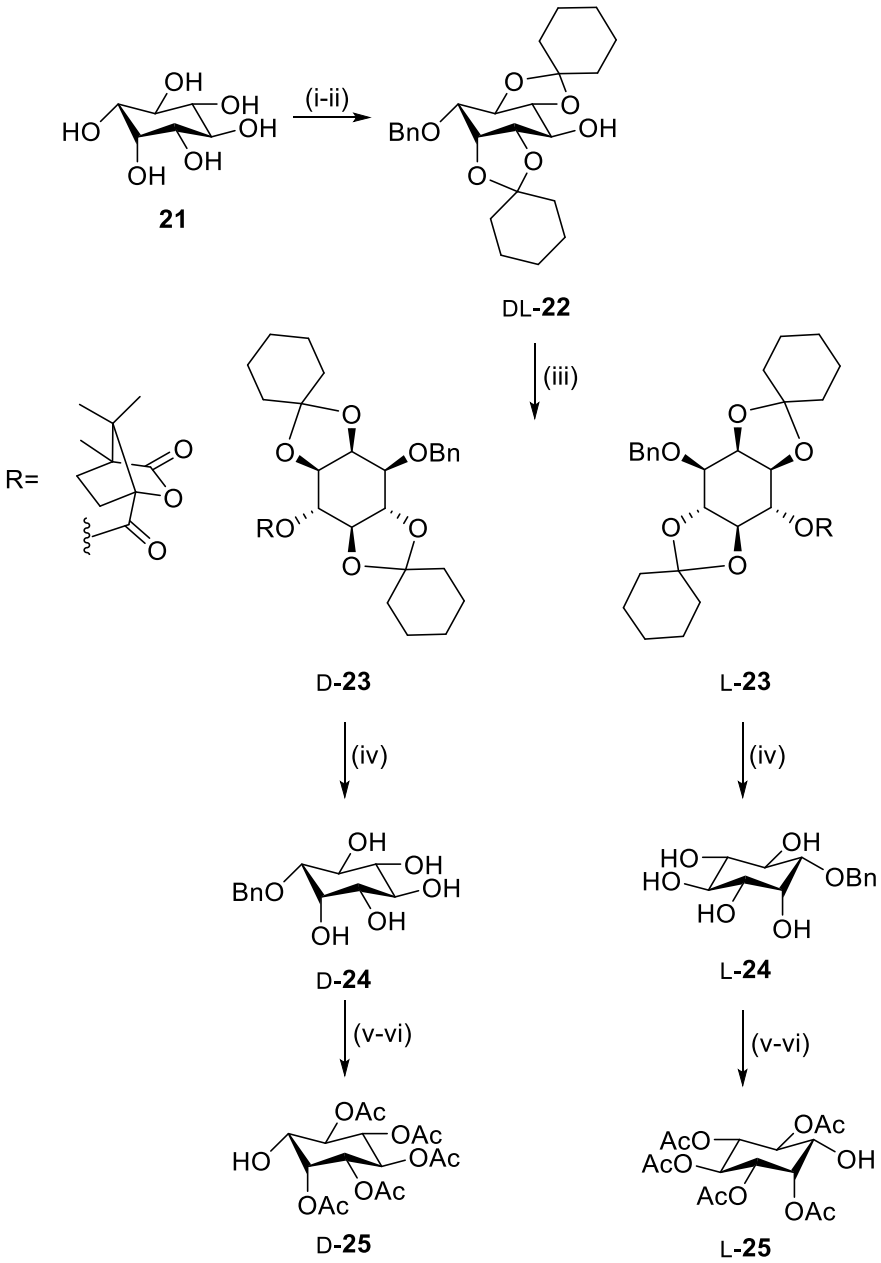


Fig. 13. Towards the synthesis of mycothiol. Reagents: (i) 1-ethoxycyclohexene, *p*-toluenesulphonic acid, DMF; (ii) NaH, BnBr, toluene; (iii) camphanic acid chloride, Et_3N in CH_2Cl_2; (iv) (a) KOH in ethanol; (b) 80% acetic acid (v/v); (v) acetic anhydride, pyridine; (vi) H_2 and Pd/C.[41]

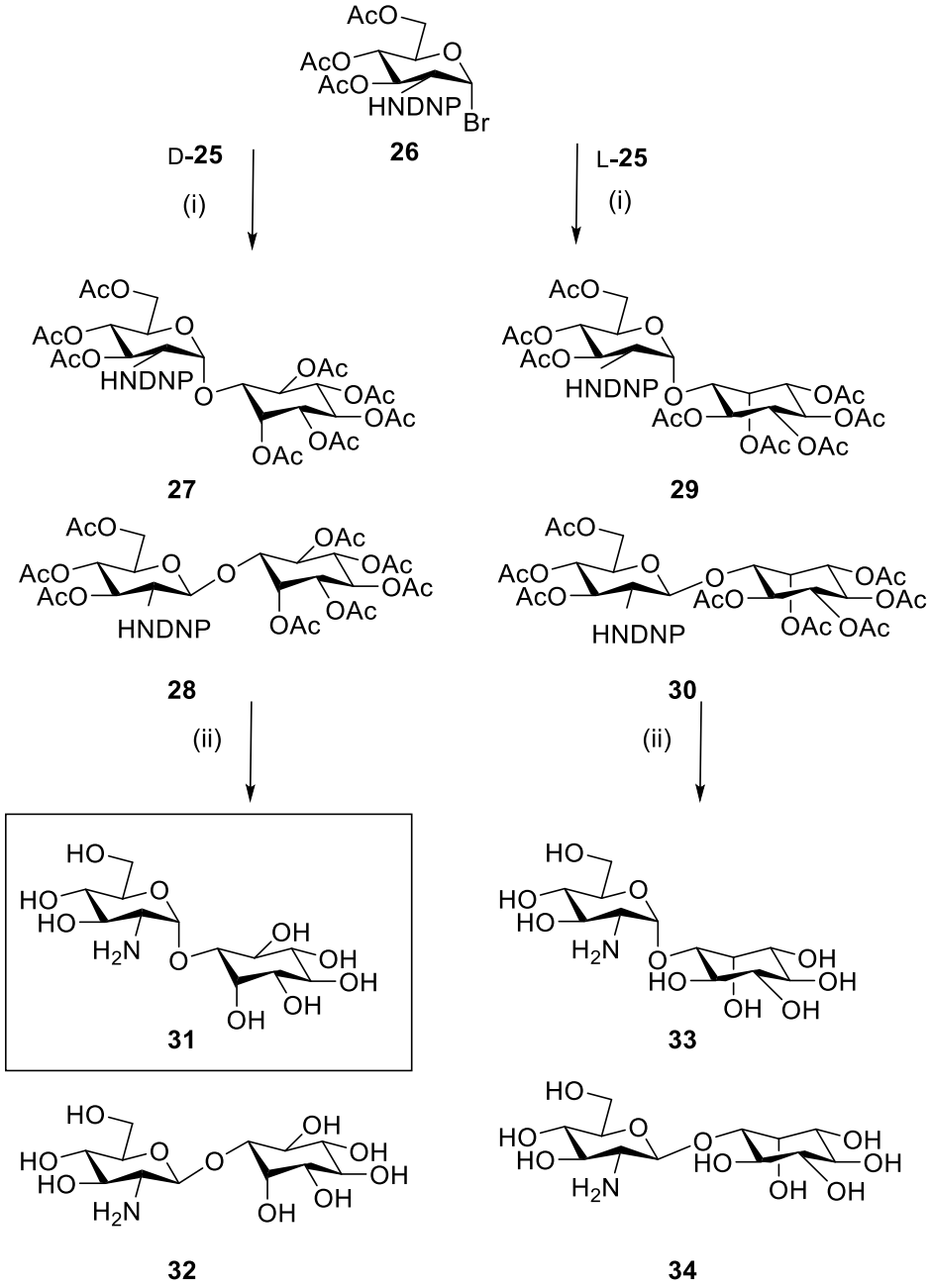


Fig. 14. Towards the synthesis of mycothiol. Reagents: (i) AgOTf, 2,6-tert-dibutylpyridine, CH_2Cl_2; (ii) Amberlite IR400 (OH).[41]

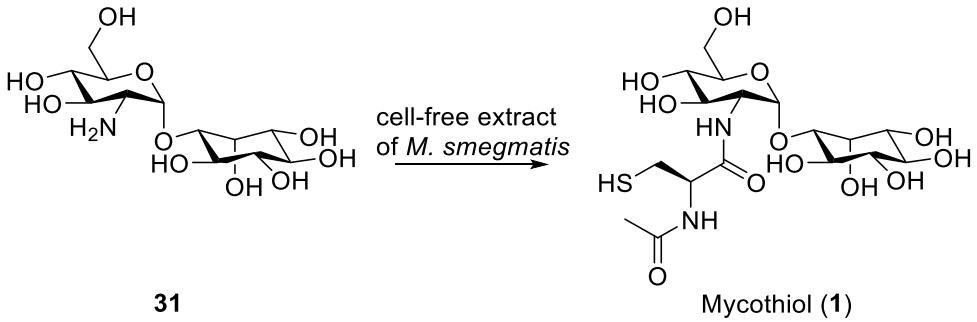


Fig. 15. The synthesis of mycothiol.[41]

The first total chemical synthesis of mycothiol was achieved in 2004 by Lee and Rosazza.[42] A similar synthetic plan of linking three mycothiol building blocks was adopted. As before, a protected D-*myo*-inositol having a free 1'-OH group was synthesised (D-**25**) but using a slightly modified synthetic route (Fig. 16). The second building block, namely *O*-(3,4,6-tri-*O*-acetyl)-2-azido-2-deoxy-α,β-D-glucopyranosyl) trichloroacetimidate (**36**), was then linked to D-**25** using trimethylsilyl trifluoromethanesulfonate (TMSOTf), which gave a 9:1 ratio of α- and β-isomers. The isomers were separated by column chromatography and the desired α-anomer (**37**) was reduced giving the amine (**38**), which was successfully coupled to *N*,*S*-diacetyl-L-cysteine (**39**) using 1-[*bis*(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) as a coupling reagent and after deprotection gave mycothiol (**1**) (Fig. 17).[42]

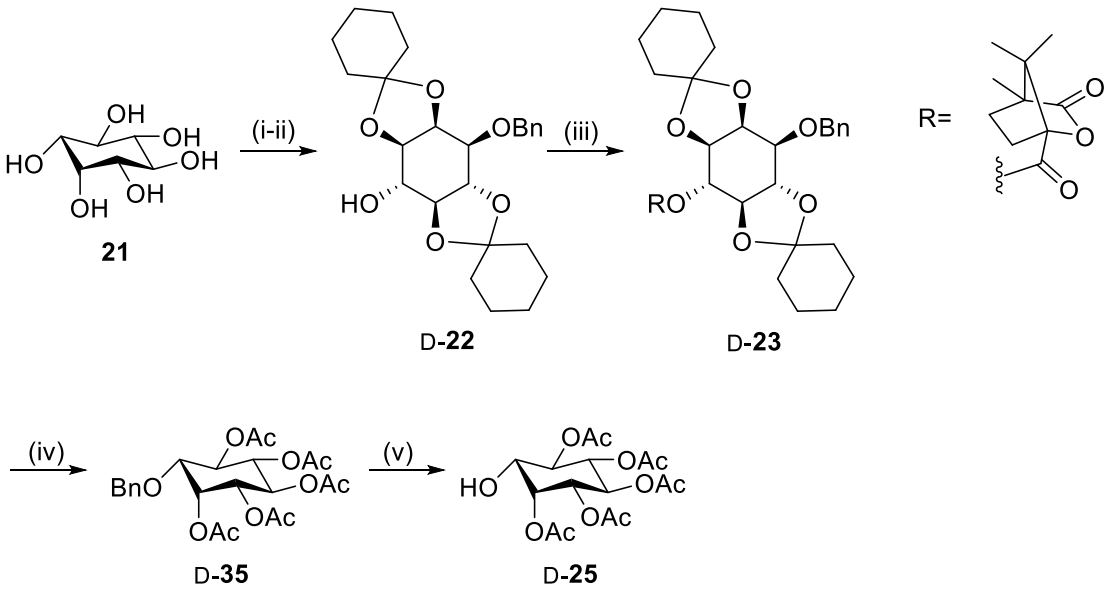


Fig. 16. Towards the synthesis of mycothiol. Reagents and conditions: (i) 1-ethoxycyclohexene, *p*-TsOH, CH_2Cl_2, 100 °C, 2 h, 23%; (ii) BnBr, NaH, PhMe, 130 °C, 6 h to rt, 12 h, 54%; (iii) (1*S*)-(-)-camphanic chloride, DMAP, Et_3N, CH_2Cl_2, rt, 24 h, 49%; (iv) (a) KOH, EtOH, rt, 15 h, 97%, (b) CH_3COOH, H_2O, 100 °C, 2 h, then Ac_2O, pyridine, rt, 17 h, 89%; (v) H_2, Pd-C, EtOAc, rt, 48 h, 97%.[42]

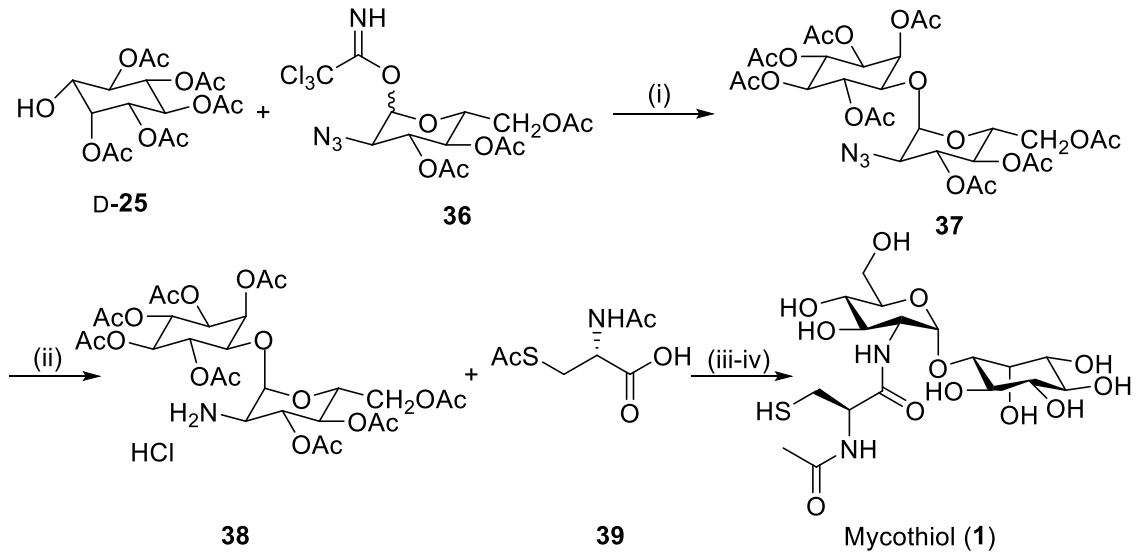


Fig. 17. The synthesis of mycothiol. Reagents and conditions: (i) TMSOTf, molecular sieves, CH_2Cl_2, 0 °C, 1 h, 56%; (ii) H_2, Pd-C, EtOAc, HCl, rt, 6 h, 81%; (iii) HATU, HOAt, collidine, DMF, 0 °C, 2 h to rt, 22 h, 25%; (iv) $Mg(OMe)_2$, MeOH, rt, 2 h, 40%.[42]

Successful synthesis of mycothiol was also accomplished *via* intramolecular *alpha*–glucosaminidation.[43] There are three main components crucial for intramolecular *alpha*–glucosaminidation: a temporary tether (in this case a methylene tether), a removable amine protecting group (*N*-arylsulfonyl group) and a leaving group (*S*-tolyl group). First, the acetyl-protected glucosamine (**40**) was decorated with *S*-tolyl and *N*-arylsulfonyl groups to give the protected glucosamine (**42**) (Fig. 18). Next, the penta-*O*-benzylinositol (**43**) was converted to the chloromethyl derivative (**44**), which was subsequently coupled with the protected glucosamine (**42**) using 2-*t*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) to give the desired glycosylation substrate (**45**) (Fig. 19). The glycosylation substrate (**45**) was treated with PhSeCl and a catalytic amount of AgOTf to furnish the desired glycoside (**46**) (Fig. 19). The advantage of using this method is that only α-anomer of **46** is produced. After general deprotection, the glycoside (**31**) was coupled with *N*-Boc-*S*-acetylcysteine (**47**) using HATU. Finally, after cysteine deprotection using trifluoroacetic acid (TFA) and *S* to *N* acetyl migration, mycothiol (**1**) was obtained (Fig. 19).[43]

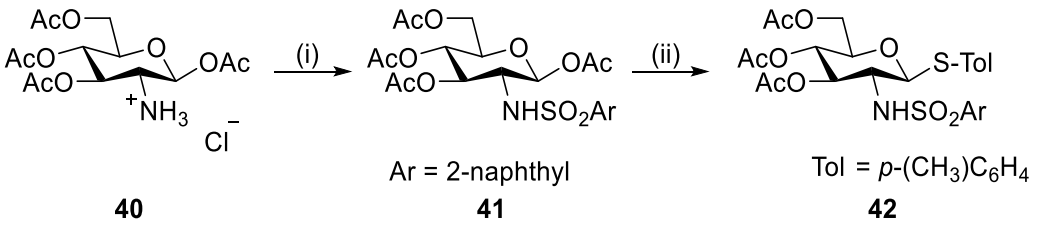


Fig. 18. Towards the synthesis of mycothiol. Reagents: (i) $ArSO_2Cl$, Et_3N, CH_2Cl_2, 95%; (ii) Tol-SH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 93% (9:1 β/α).[43]

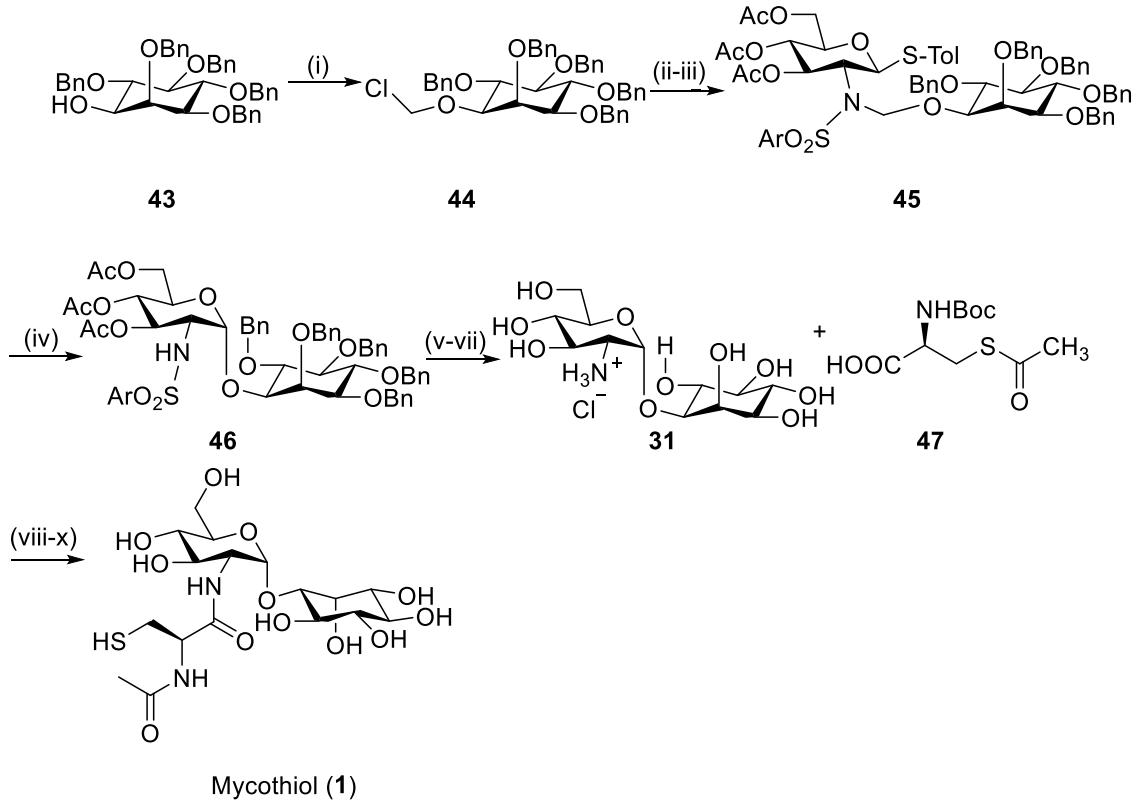


Fig. 19. The synthesis of mycothiol. Reagents and conditions: (i) $MeSCH_2Cl$, NaI, NaH, THF, 8 h, 74%; (ii) SO_2Cl_2, CH_2Cl_2, 0 °C; (iii) **42**, BEMP, THF, 98%; (iv) PhSCl, AgOTf, CH_2Cl_2, CH_3CN, -78 °C to -20 °C, 93%; (v) NaOMe, MeOH; (vi) Na(Hg), Na_2HPO_4, MeOH, 15 min, 74%; (vii) H_2, $Pd(OH)_2$, HCl, aq *t*-BuOH, 6 h, 98%; (viii) HATU, *i*-Pr_2EtN, DMF, 0 °C to 23 °C, 12 h, 77%; (ix) TFA, CH_2Cl_2; (x) pyridine, 100%.[43]

Chung *et al*.[44] has reported a mycothiol synthesis that involved regioselective ketopinyl desymmetrization of benzyl-protected D-*myo*-inositol. 3-*O*-Ketopinyl **49** was linked to the protected D-glucosamine (**48**) using *N*-iodosuccinimide (NIS) and triflic acid (TfOH) to give only the desired α-anomer (**51**) after subsequent cleavage of the ketopinyl group (Fig. 20). Following the global deprotection, the pseudo-disaccharide (**31**) was coupled with protected cysteine (**47**) using HATU as a coupling reagent. Finally, after cysteine deprotection and *S* to *N* acetyl migration, mycothiol (**1**) was obtained (Fig. 20). High overall yield (40%) and α-

stereoselective glucosaminylation are the main benefits of using this method.[44]

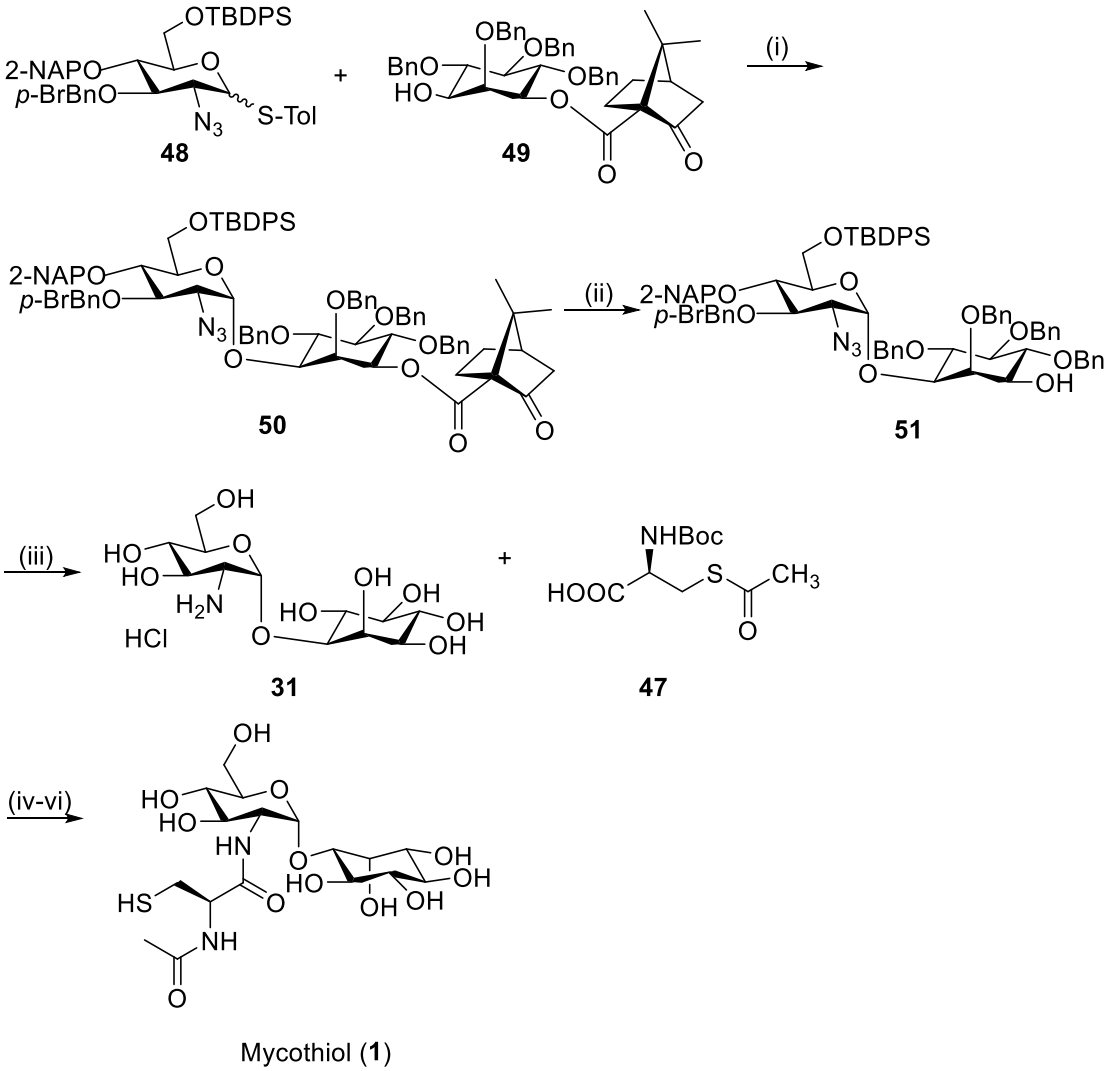


Fig. 20. The synthesis of mycothiol. Reagents and conditions: (i) NIS, TfOH, CH_2Cl_2, -60 °C, 3 h, 84%; (ii) NaOH, EtOH/CH_2Cl_2 (4/1), 60 °C, 30 min, 95%; (iii) TBAF, THF, 95%; (iv) HATU, DIPEA, DMF, 12 h, 78%; (v) TFA; (vi) pyridine 100%.[44]

McConnell, Yu and Nguyen[45] also reported a method for the synthesis of the α-pseudo-disaccharide (**31**), which is an intermediate in the synthesis of mycothiol. In this method, the protected D-*myo*-inositol (**53**) was α-selectively coupled with the *N*-phenyl trifluoroacetimidate derivative of glucosamine (**52**) using a nickel catalyst to give protected α-pseudo-disaccharide (**54**) (Fig. 21). It is worth noting that a mixture of α- and β-anomers of **52** was used and only α-isomer of **54** was produced (yield = 78%). After removal of the 2-trifluoromethyl-benzylidene group, benzyl and acetyl protecting groups the desired α-pseudo-disaccharide (**31**) was furnished (Fig. 21).[45]

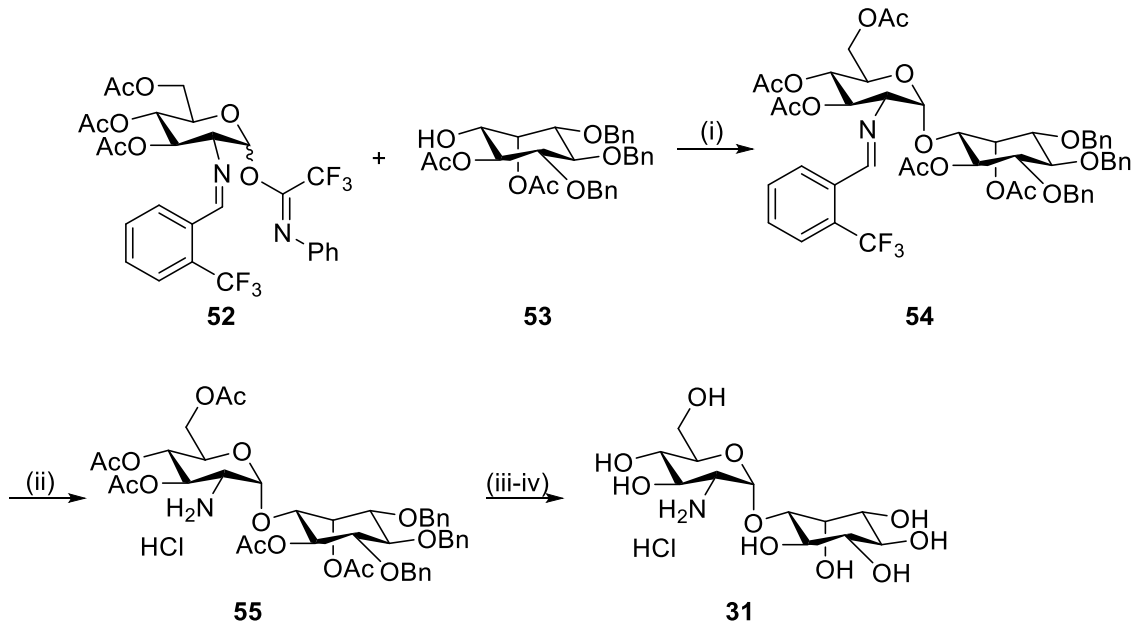


Fig. 21. Towards the synthesis of mycothiol. Reagents and conditions: (i) 10 mol%, $Ni(4\text{-F-PhCN})_4(OTf)_2$, CH_2Cl_2, 35 °C, 78%; (ii) 5 N HCl, acetone, reflux, 5 min, 98%; (iii) $Pd(OH)_2/C$, H_2, *t*-BuOH, pH 4 buffer; (iv) NaOMe, MeOH, 55% (2 steps).[45]

Most recently, Manabe and Ito[46] have reported mycothiol synthesis including anomerization *via* endocyclic cleavage as a key step. First, in order to obtain the β-pseudo-disaccharide (**57**), phthalimido-protected glucosamine thioglycoside (**56**) and protected inositol (**43**) were linked *via* a glycosylation reaction (Fig. 22). Next, protecting groups on the glucosamine ring were removed and a carbamate group was introduced. In the following steps, the glucosamine ring was acetylated and the β-pseudo-disaccharide (**59**) was fully anomerized to the desired α-pseudo-disaccharide (**60**) (Fig. 22). The α-pseudo-disaccharide (**60**) was fully deprotected and coupled to protected cysteine (**47**) using (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) as a coupling reagent. As before[44], after cysteine deprotection and *S* to *N* acetyl migration, mycothiol (**1**) was successfully furnished (Fig. 22).[46]

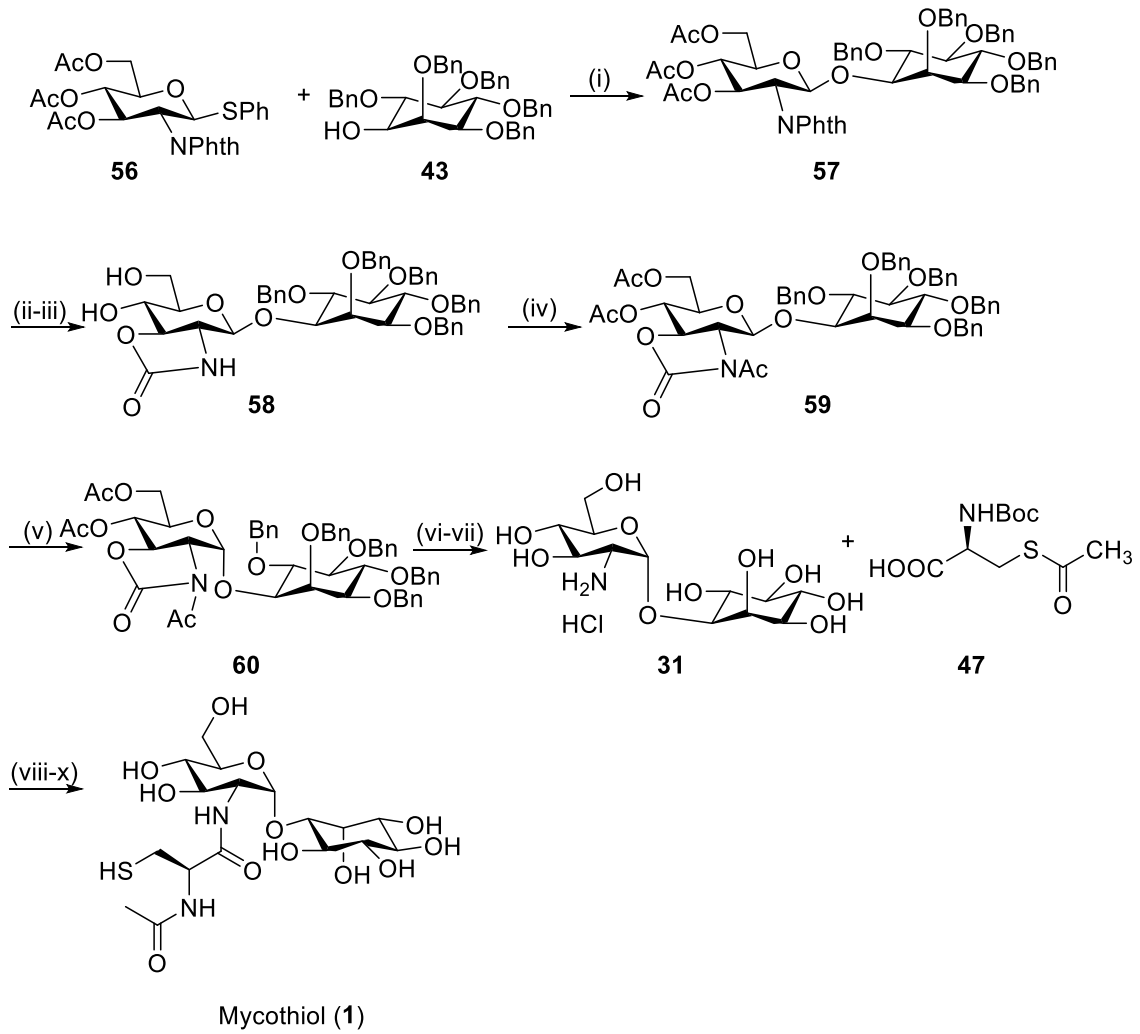


Fig. 22. Towards the synthesis of mycothiol. Reagents: (i) NIS, TMSOTf, CH_2Cl_2, 90%; (ii) ethylenediamine, DMF, 82%; (iii) triphosgene, $NaHCO_3$, CH_3CN-H_2O, 93%; (iv) Ac_2O, pyridine, DMAP, 84%; (v) $BF_3{\cdot}OEt_2$, CH_3CN, quant.; (vi) aq. NaOH, dioxane, 72%; (vii) H_2, $Pd(OH)_2/C$, AcOH, dioxane, H_2O, 85%; (viii) COMU, iPr$_2$NEt, 80%; (ix) TFA; (x) pyridine, quant.[46]

2.4. Chemical synthesis of mycothiol analogues

The first mycothiol analogue was synthesised in 1998 to perform kinetic and mechanistic studies with the mycothione reductase from *M. tuberculosis*. At that time the chemical synthesis of mycothiol was not yet reported, so a truncated analogue, namely oxidised des-*myo*-inositol mycothiol (**65**) (Fig. 23), was synthesised as a mixture of α- and β-isomers. First, glucosamine (**61**) was coupled to *N*-α-Fmoc-*S*-acetamidomethyl-L-cysteine pentafluorophenyl ester (**62**) to produce compound **63**. Then, the Fmoc group was removed and the free amine was selectively *N*-acetylated. Finally, thallium (III) trifluoroacetate was used to produce the target disulphide (**65**). It was shown that both diastereomers

act as substrates for the reductase.[47]

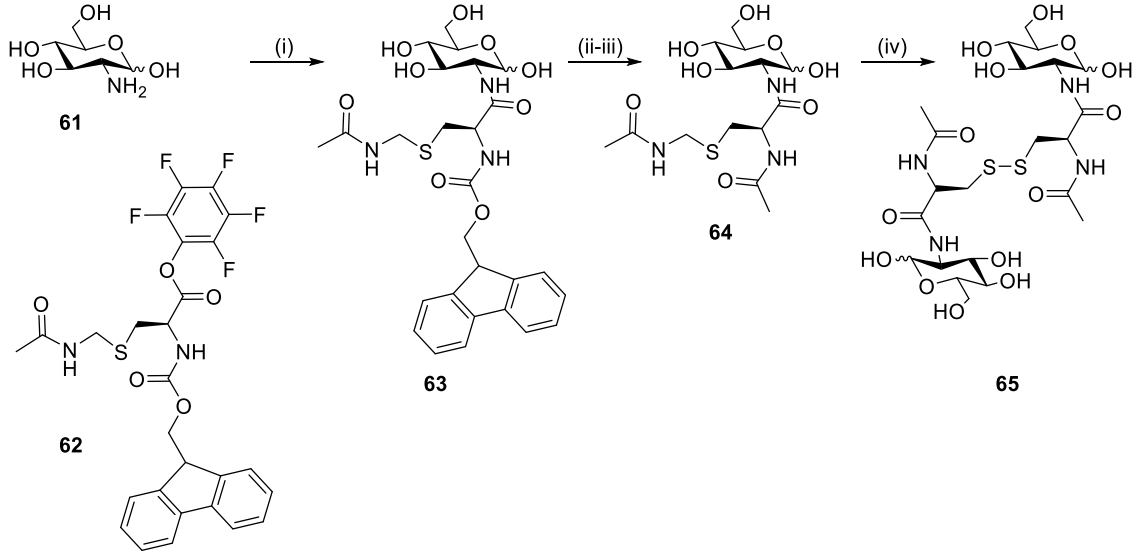


Fig. 23. The synthesis of the truncated mycothiol analogue (**65**). Reagents and conditions: (i) 2 equiv of hydroxybenzotriazole, 5 equiv α-D-glucosamine (freebase), DMF, room temperature, 2 h, 65%; (ii) 5% piperidine in DMF, rt, 0.5 h, 77%; (iii) 1.1 equiv AcO_2 over 0.5 h, 2 equiv K_2CO_3, H_2O, rt, 2 h, 66%; (iv) 1.1 equiv $Tl(CF_3CO_2)_3$, 2 equiv anisole, TFA, 4 °C, 1 h, 28%.[47]

In 2002, in order to confirm the absolute configuration of mycothiol, mycothiol bimane (MSmB) (**70**) was synthesised (Fig. 24). In short, the appropriately protected inositol (**66**) was glycosylated with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride producing a 2:1 mixture of α- and β-anomers. The α-anomer was separated by column chromatography (yield = 44%). After inositol ring deprotection and subsequent acetyl protection and azide reduction, the amine (**69**) was formed. In the next step the amine (**69**) was coupled with *N*-acetyl-L-cysteinyl monobimane and the global deprotection gave mycothiol bimane (**70**). MSmB is produced by *M. smegmatis* and is a substrate for mycothiol *S*-conjugate amidase. Synthetic MSmB was used as a substrate for Mca and the data were compared with the results obtained when MSmB isolated from *M. smegmatis* was used. NMR and CD spectra along with kinetic studies confirmed that mycothiol is composed of D-*myo*-inositol, D-glucosamine and L-cysteine.[48]

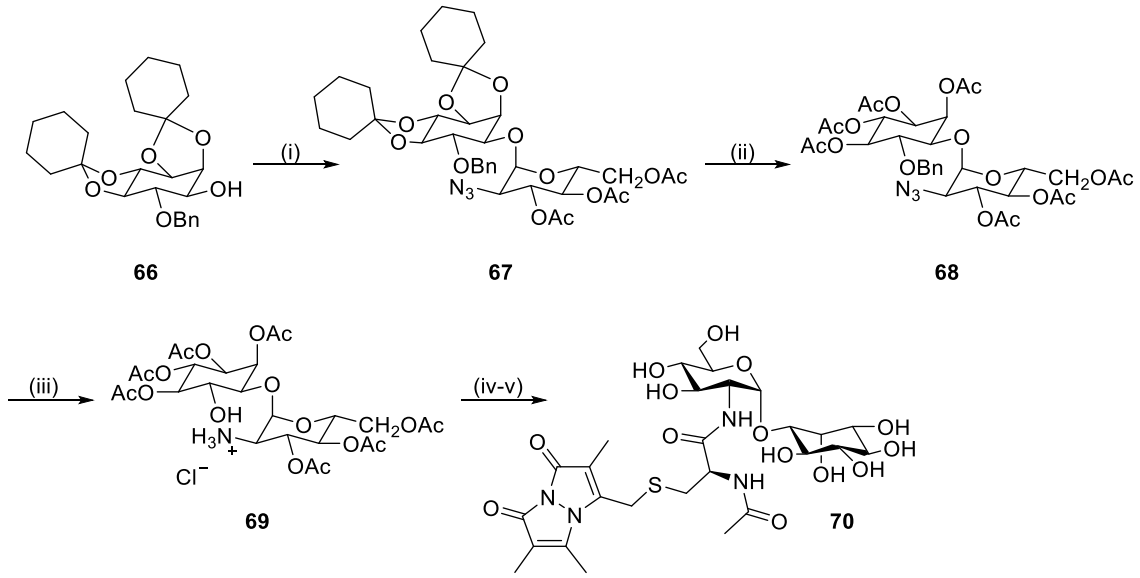


Fig. 24. The synthesis of mycothiol bimane (**70**). Reagents: (i) 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl chloride, AgOTf, 2,6-diisopropyl-4-methyl pyridine, CH_2Cl_2, 44%; (ii) 1. ethylene glycol, (+)-camphor sulfonic acid, CH_3CN 2. Ac_2O, pyridine 80%; (iii) Pd-C, H_2, EtOAc, 86%; (iv) *N*-AcCysmB, DEPC, *i*Pr_2EtN, DMF, 31%; (v) $Mg(OMe)_2$, MeOH (dry), 75%.[48]

In the same year the first mycothiol analogue (**78**) to serve as a scaffold for further development into anti-TB drugs was reported (Fig. 25). In order to simplify the synthesis and isolation, the *myo*-inositol moiety was replaced by *S*-cyclohexyl. A sulphur atom was used in place of the linking oxygen in the natural compound, so that the obtained mycothiol analogue (**78**) would be more chemically and biologically stable and it also sets the stereochemistry at the anomeric centre, so that only the α-isomer is formed. The simplified analogue (**78**) was shown to be a substrate for Mca when *S*-alkylated with bromobimane proving that it is a good scaffold for further development into anti-TB drugs. The simplified mycothiol analogue (**78**) was synthesised as follows (Fig. 25): first, the commercially available acetyl-protected β-glucosamine (**71**) was treated with Lawesson's reagent to furnish the GlcNAc-thiazoline triacetate (**72**). In the second step, **72** was hydrolysed using trifluoroacetic acid to provide the acetamido mercaptan (**73**) (only the desired α-isomer was formed). Next, the cyclohexyl ring was added using a radical thiol-ene coupling to furnish the cyclohexyl thioglycoside (**74**). In the following step, **74** was treated with hydrazine monohydrate to provide the aminotriol (**75**). Then **75** was coupled with *N*-(tert-butoxycarbonyl)-*S*-acetyl-L-cysteine (**47**) in order to furnish the amide (**76**). The penultimate step involves treatment of **76** with trifluoroacetic acid leading to the formation of the ammonium

salt (**77**), and finally **77** was treated with pyridine to furnish the simplified mycothiol analogue (**78**) *via* intramolecular *S*-*N* acetyl transfer.[49]

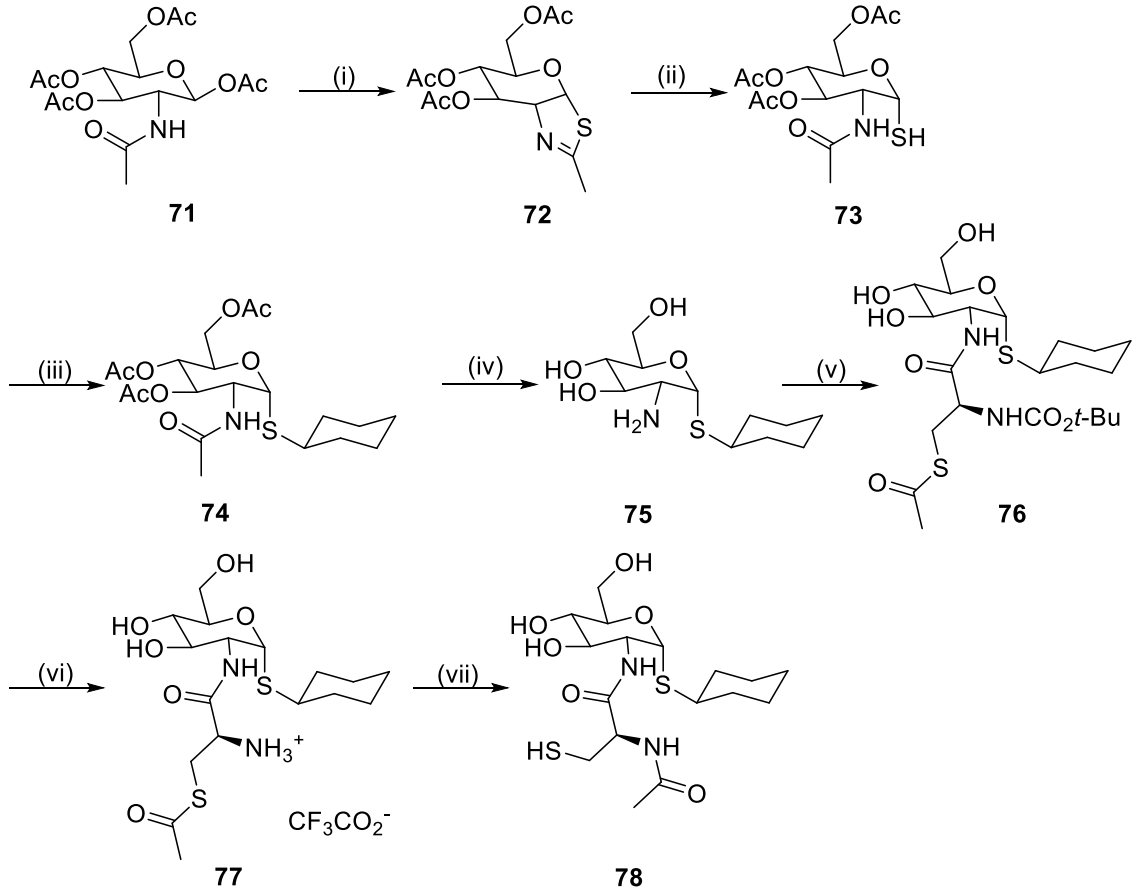


Fig. 25. The synthesis of simplified mycothiol analogue (**78**). Reagents and conditions: (i) Lawesson's reagent, toluene, 80 °C, 1.5 h, 100%; (ii) TFA, wet MeOH, 100%; (iii) C_6H_{10}, AIBN, $CHCl_3$, reflux, 79%; (iv) $NH_2NH_2{\cdot}H_2O$, 120 °C, 24 h, 84%; (v) *N*-Boc-*S*-acetylcysteine (**47**), EDCI, DMF, 82%; (vi) TFA; (vii) 1. pyridine, 2. Sephadex, 100% from **76**.[49]

The aminotriol intermediate (**75**) was then used as a scaffold for the synthesis of a further 40 mycothiol analogues. The aminotriol (**75**) was *N*-substituted with a range of different groups to mimic the transition state of the natural substrates for the mycothiol dependent enzymes like Mca. The analogues were generated using a variety of procedures including amide coupling, alkylation, acylation and sulfonylation. Biological activity against *M. tuberculosis* was evaluated for 27 of the analogues using an Alamar blue growth inhibition assay and four of them **79** – **82** (Fig. 26) showed weak inhibitory activity ranging from 4 to 51% inhibition at 6.25 μg/mL.[50]

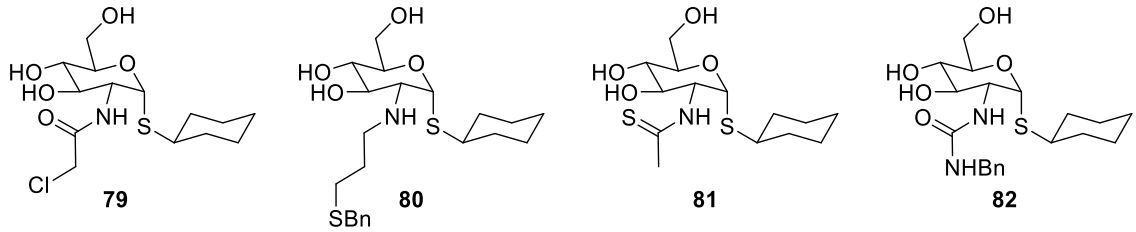


Fig. 26. Structures of mycothiol analogues.[50]

Mycothiol analogues having 2'-hydroxypropyl (**90**) or 2'-oxopropyl (**91**) groups in place of L-cysteine were also synthesised (Fig. 27). The synthesis started with acetyl protected 2-deoxy-2-C-allylglucoside (**83**) that was converted into the glycoside fluoride (**84**). Then **84** was glycosylated with appropriately protected inositol (**43**) to produce **85** as an inseparable 8:1 mixture of α- and β-anomers. After the deacetylation of **85**, the α-anomer (**86**) was separated by column chromatography (yield = 36%). In the following step, the pure α-glycoside (**86**) was benzylated. The desired 2'-hydroxypropyl (**90**) mycothiol analogue was produced *via* epoxidation, subsequent ring opening and global deprotection of **88**. The analogue **91** was obtained *via* oxidation of **88** followed by global deprotection. The analogues were proposed to act as inhibitors of the MshB, thus blocking the biosynthesis of mycothiol. Preliminary biological studies showed that compounds **90** and **91** do not cause growth inhibition of *M. smegmatis in vitro*. However, **90** and **91** inhibited [^{3}H]inositol incorporation by whole cells (*M. smegmatis*) into metabolites containing the inositol moiety proving that **90** and **91** have biological activity.[51]

Because the chemical synthesis of mycothiol is challenging and low-yielding a new mycothiol analogue was designed and synthesised, namely a fused bicyclic thioglycoside (**95**) (Fig. 28). In the proposed synthetic route, the cysteine moiety is introduced in the first step to address the most difficult step at the beginning of the synthetic pathway. Then, the β-thioglycoside (**93**) was *O*-deacetylated and the phthalimido group was removed. The addition of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI) and subsequent deprotection resulted in the formation of the desired bicyclic product (**95**). The inhibitory activity of the analogue (**95**) on enzymes involved in the mycothiol biosynthetic pathway was not reported.[52]

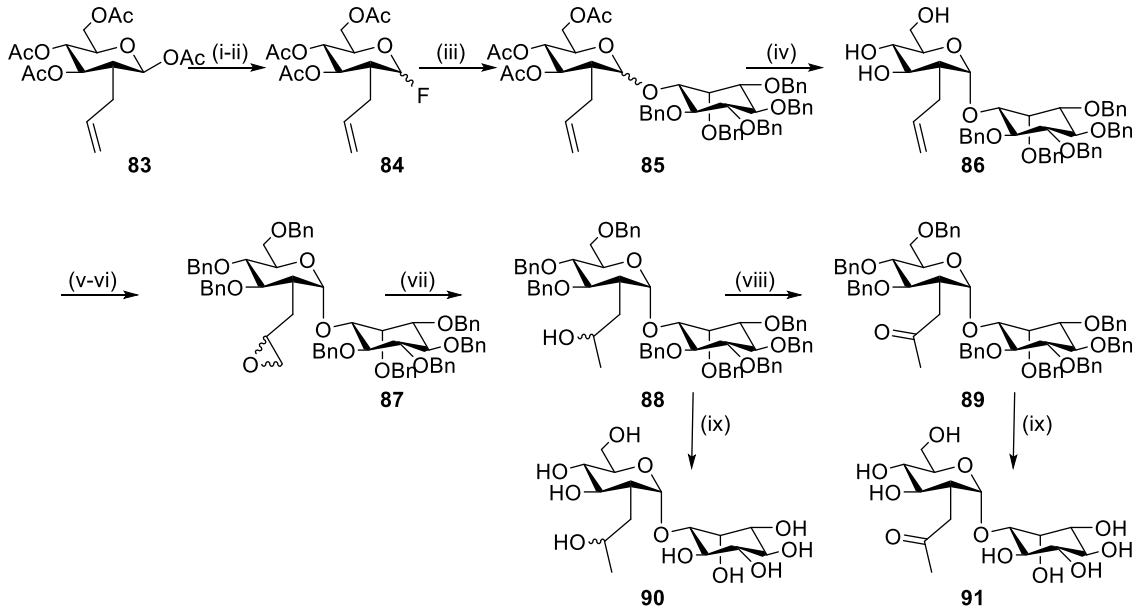


Fig. 27. The synthesis of 2'-hydroxypropyl (**90**) and 2'-oxopropyl (**91**) mycothiol analogues. Reagents and conditions: (i) hydrazine acetate (2 equiv), DMF, 60 °C, 30 min, 92%; (ii) DAST (3 equiv), THF, rt, 30 min, 90% (α:β=2:1); (iii) **43** (1 equiv), **84** (1.6 equiv), $BF_3{\cdot}Et_2O$ (5 equiv), 4 Å ms, rt, 2 h, (α:β =8:1); (iv) NaOMe, MeOH–CH_2Cl_2, rt, 1 h, 36%; (v) NaH, BnBr, THF, reflux, 20 h, 94%; (vi) MCPBA (70%) (6 equiv), CH_2Cl_2, rt, 24 h, 83% (1:1);. (vii) LAH (10 equiv), THF, 0 °C, 1 h, 82% (1:1); (viii) TPAP (cat), NMO (1.5 equiv), CH_2Cl_2, ms, rt, 25 min, 90%; (ix) H_2, 10% Pd/C, EtOAc–MeOH, rt, 3 days, >90%.[51]

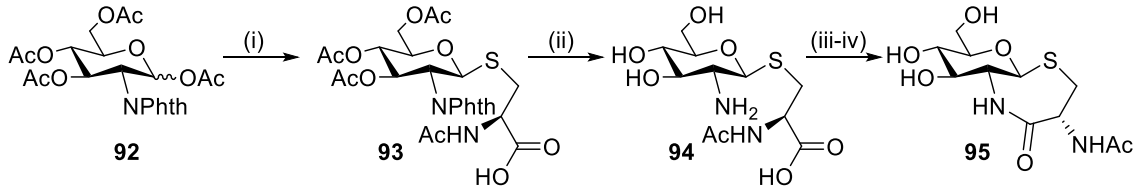


Fig. 28. The synthesis of the fused bicyclic thioglycoside (**95**). Reagents: (i) *N*-acetyl-L-cysteine, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 68%; (ii) $NH_2CH_2CH_2NH_2$, MeOH, 86%; (iii) 1. EDCl, HOBt, DMF, H_2O, 2. Ac_2O, pyridine, 50%; (iv) NaOMe, MeOH, 82%.[52]

More recently, an analogue (**100**) based on a scaffold reported by Knapp *et al.*[49] but having *S*-trichloroethenyl substituted cysteine instead of *N*-acetylcysteine moiety was synthesised (Fig. 29). *S*-chloroalkenyl cysteine is known to be toxic to cells as it undergoes β-lysis that results in production of reactive electrophiles such as thioacyl halides. It was hypothesised that the synthesis of an Mca substrate mimic decorated with *S*-haloalkenyl cysteine would result in cleavage of the toxin selectively in mycobacteria. It is worth noting that the analogue (**100**) also lacks an *N*-acetyl group as a free amino group on the cysteine moiety is required for the binding with the β-lyase enzyme. The synthetic pathway started with α-D-glucosamine pentaacetate (**96**) that was treated with cyclohexanethiol

to give a 2:1 mixture of α- and β-anomers. The anomers **74** (yield = 22%) and **97** were separated by column chromatography and carried separately through the subsequent steps. The anomers **74** and **97** were globally deprotected over 3 steps and coupled with *N*-Boc-*S*-trichloroethenyl-L-cysteine to produce Boc-**100** and Boc-**101**. Finally, the *N*-Boc groups were removed to give the desired analogues **100** and **101**. The analogue **100** and its β-anomer (**101**) (unlike their Boc-protected derivatives) were reported to be taken up by *M. smegmatis* strain and showed comparable growth inhibition (MIC: 250–500 μM for both compounds). However, the analogue **100** and its β-anomer (**101**) were also shown to be cytotoxic to HL-60 (human promyelocytic leukemia) (IC_{50} 76 ±1 and 112 ±18 μM, respectively) and 1A9 (human ovarian carcinoma) cells (IC_{50} 79 ±23 and 121 ±16 μM, respectively), which could suggest that they act *via* different mechanism than β-lysis. Although selectivity should be improved, the *S*-trichloroethenyl substituted mycothiol analogue (**100**) is thought to be promising candidate for further development.[53]

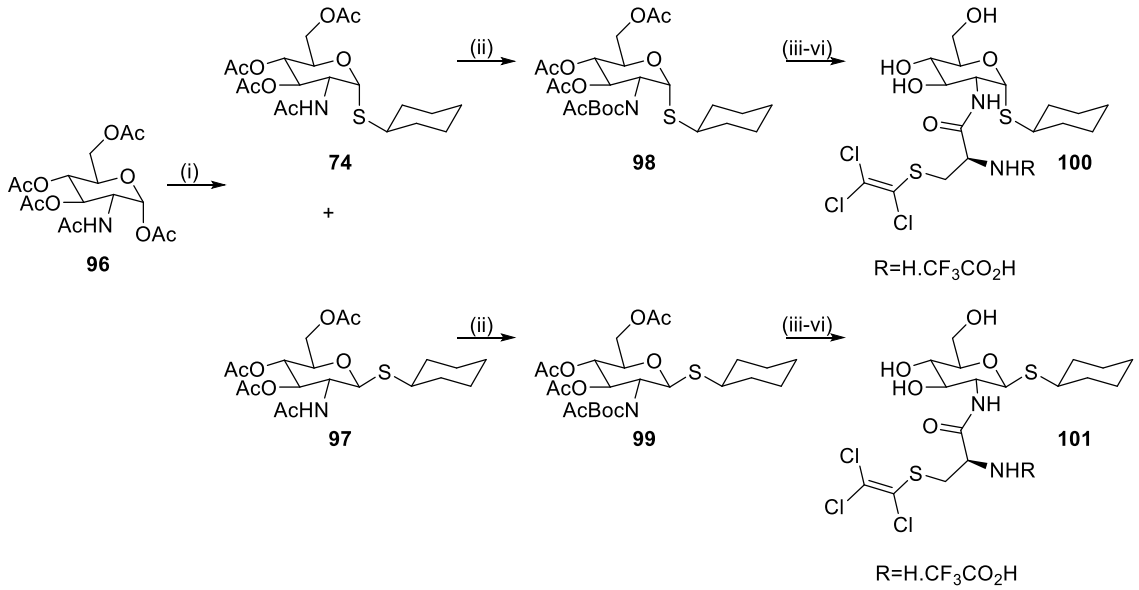


Fig. 29. The synthesis of the *S*-trichloroethenyl substituted mycothiol analogue (**100**). Reagents and conditions: (i) cyclohexanethiol (4 equiv), $BF_3{\cdot}OEt_2$ (2 equiv), 1,2-dichloroethane, 0–55 °C, 3 h (**74** 22%, **97** 11%); (ii) di-*tert*-butyl dicarbonate (5.4 equiv), 4-(*N*,*N*-dimethylamino)pyridine (0.13 equiv), THF, reflux 18 h (**98** quant, **99** 82%); (iii) Na (1 equiv), MeOH, rt, 2 h; (iv) HCl, MeOH/water, rt,18 h; (v) KO^{t}Bu (1 equiv), EDCI (2.5 equiv), *N*-Boc-*S*-trichloroethenyl-L-cysteine (1.5 equiv), DMF, 0 °C – rt, 2 h (Boc-**100** 26%, Boc-**101** 35% over 3 steps); (vi) TFA, 0 °C – rt, 25 min (**100** 35%, **101** quant).[53]

Most recently, two structural analogues (GlcF-Ins **106** and $GlcN_3$-Ins **109**) of GlcN-Ins (**31**) were synthesised as potential inhibitors of MshC. The synthesis of

GlcF-Ins (**106**) (Fig. 30) started with the fluorination of 3,4,6-tri-*O*-acetyl-D-glucal (**102**) that was subsequently acetylated at the anomeric carbon to allow for the separation of the D-glucopyranose from the α-D-mannopyranose by column chromatography. After separation the anomeric carbon of the D-glucopyranose was selectively deprotected to form 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-glucopyranose (**103**). Next, **103** was treated with trichloroacetonitrile (TCA) to produce the TCA donor (**104**) that was glycosylated with the appropriately protected inositol (**66**) to give only the desired α-product (**105**) that was globally deprotected to furnish the GlcF-Ins (**106**). [54]

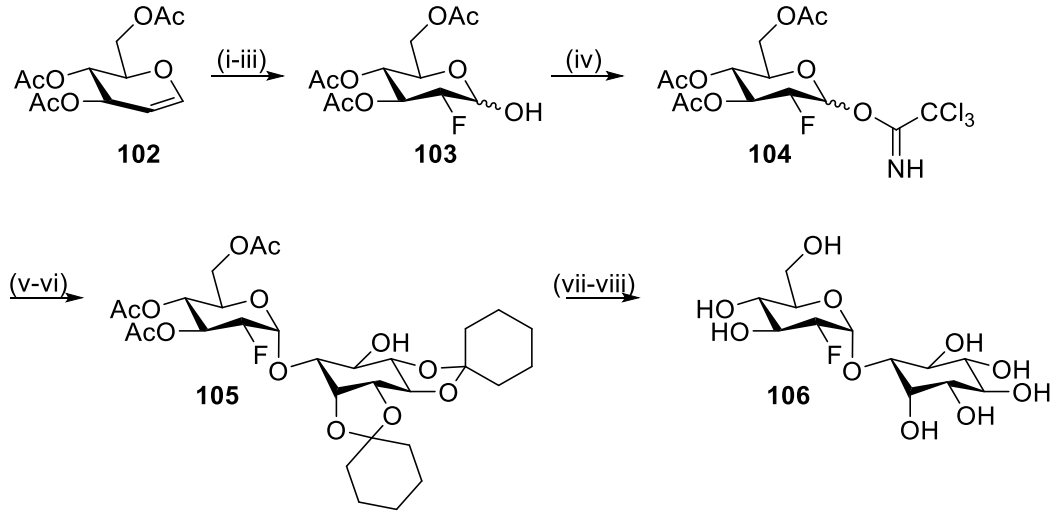


Fig. 30. The synthesis of the GlcF-Ins (**106**). Reagents and conditions: (i) Selectfluor®, DMF : water, RT, 12 h, (ii) Ac_2O, pyridine, DMAP, RT, 12 h, (58%); (iii) ammonium acetate, DMF, RT, 12 h, (96%); (iv) trichloroacetonitrile, K_2CO_3, anhydrous DCM, 0 °C, 16 h, (93%); (v) **66**, TMSOTf, anhydrous DCM, MS-4 Å, -20 °C, 1 h, (78%); (vi) 10% w/w Pd-C, H_2, MeOH, RT, 5 h, (98%); (vii) NaOMe/MeOH, RT, 1 h, (viii) DOWEX 50WX8-100 ion exchange (H^+) resin, MeOH, 35 °C, 1 h, (98%).[54]

In order to synthesise $GlcN_3$-Ins (**109**) (Fig. 31), glucosamine hydrochloride (**107**) underwent a diazo transfer reaction and was subsequently acetylated and selectively deacetylated at the anomeric carbon to give 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-D-glucopyranose (**108**). As before, **108** was treated with TCA to produce the TCA donor (**36**) that was glycosylated with the appropriately protected inositol (**66**) to give only the desired α-product (**67**) that was globally deprotected to furnish the $GlcN_3$-Ins (**109**). The inhibitory activity of the analogues **106** and **109** on MshC was not reported.[54]

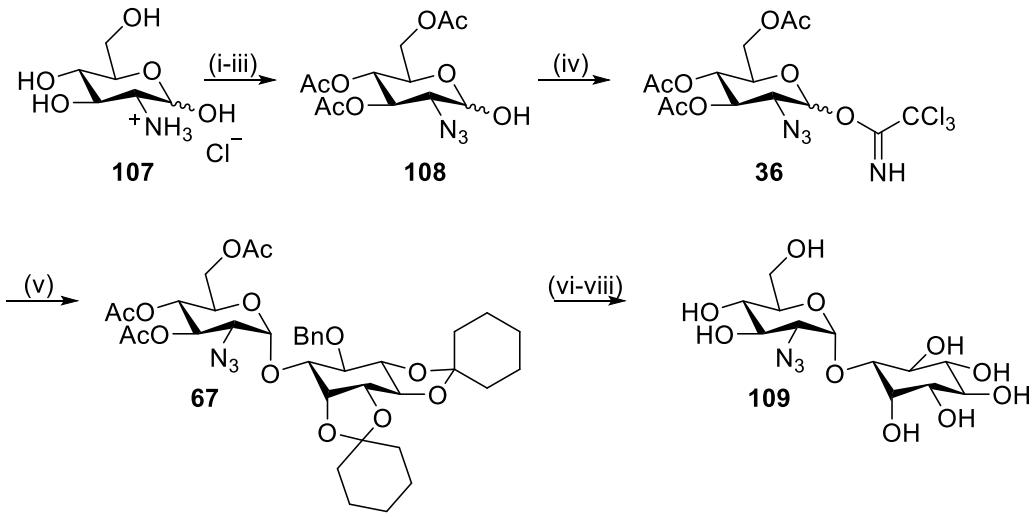


Fig. 31. The synthesis of the $GlcN_3$-Ins (**109**). Reagents and conditions: (i) Stick's diazo transfer reagent, K_2CO_3, $CuSO_4 \cdot 5H_2O$, MeOH, RT, 3 h, (ii) Ac_2O, pyridine, DMAP, RT, 12 h (87%); (iii) ammonium acetate, DMF, RT, 12 h, (98%); (iv) trichloroacetonitrile, K_2CO_3, anhydrous DCM, 0 °C to RT, 16 h, (90%); (v) **66**, TMSOTf, anhydrous DCM, MS-4 Å, -20 °C, 1 h, (81%); (vi) $NaBrO_3$, $Na_2S_2O_4$, EtOAc:H_2O, RT, 6 h, (vii) NaOMe/MeOH, RT, 1 h, (viii) DOWEX 50WX8-100 ion exchange (H^+) resin, MeOH, 35 °C, 1 h, (90%).[54]

In conclusion, there were multiple attempts to obtain a biologically active mycothiol analogues. However, their synthesis proved to be challenging. This is why development of a new synthetic strategy to form a simplified mycothiol scaffold that would be easily derivatised is of great importance. Such a strategy would enable the synthesis of mycothiol-based chemical probes that would help to explore the role of MSH-dependent enzymes including MST.

3. Kinetic target guided synthesis

In kinetic target guided synthesis (kTGS) the protein acts as a catalyst or template in the synthesis of its own best inhibitor from a pool of choices provided. The protein is typically incubated with one fragment that is known to bind to the protein and a pool of fragments that can react with the constant fragment in order to form irreversible bonds (Fig. 32). The archetypal click reaction, namely 1,3 dipolar cycloaddition of alkynes and azides, is considered to be the gold standard in kTGS. The hits can be identified using LCMS and subsequently re-synthesised using standard 'click chemistry' conditions. Binding and inhibition of the hits can be confirmed using a variety of biophysical techniques including saturation transfer difference NMR (STD-NMR), differential scanning fluorimetry (DSF) and isothermal titration calorimetry (ITC). The use of kTGS has many advantages over traditional medicinal chemistry approaches. Most importantly, it enables quick exploration of the protein binding site. Moreover, it reduces synthetic effort at early stages of drug development and produces potent inhibitors from two low-affinity fragments in only one step. However, this approach also has limitations. All of the reactants used as well as products formed should be stable under physiological conditions and cannot cause degradation or denaturation of the protein. Because the inhibitors are generated in very small amounts, they should be easily synthesised using established 'click chemistry' conditions to enable validation of the hits. [55,56]

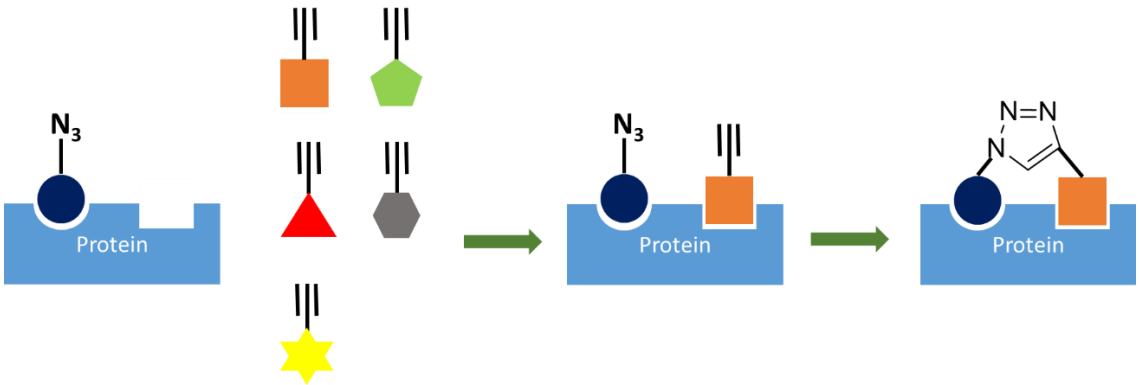


Fig. 32. Principle of the kinetic target guided synthesis.

Kinetic target guides synthesis was first successfully employed to generate inhibitors of acetylcholinesterase (AChE), which is a validated drug target in Alzheimer's disease.[56] AChE has been selected as there are two separate

binding sites located next to each other in its active site. To perform kTGS two known inhibitors of AChE, namely tacrine (K_d =18 nM) and propidium (K_d = 1.1 μM) that bind to different binding sites were chosen. Each inhibitor was equipped with an azido or alkyne linker of a various length. In total 8 analogues of each inhibitor were synthesised. The analogues were incubated in pairs with the enzyme to produce only one compound (K_d = 99 fM) out of 98 possibilities. In another experiment a tacrine azido analogue was used as a handle within the binding site and incubated with 23 alkyne fragments. This less biased approach resulted in an identification of two additional AChE inhibitors. Over the years kTGS was employed to find inhibitors of a wide range of protein targets e.g. carbonic anhydrase, HIV protease and chitinase.[55]

4. Aims

Tuberculosis (TB) is an infectious disease that kills more than a million people a year, thus posing a significant global health threat. TB is extremely difficult to eradicate and has to be treated using a cocktail of antimicrobial drugs that must be taken for a minimum of six months. Over the years, the same anti-TB drugs have been used extensively resulting in the rise of multi-drug resistant and extensively drug-resistant strains of TB.[1] Because of the global spread of multi-drug resistant tuberculosis and high infection rates, there is a great need for new drugs to be brought to the clinic.

M. tuberculosis and related *Actinomycetes* produce mycothiol as the most abundant low molecular weight thiol. Mycothiol (MSH) is thought to fulfil the same roles as glutathione in other species.[10] MSH unlike GSH, which is a tripeptide, contains the functional cysteine moiety decorated with two sugar moieties, namely inositol and glucosamine. It plays a major role in combating oxidative stress, to which bacteria are exposed in host organism due to immune response and antibiotics. It is involved in the detoxification of electrophilic toxins, serves as a cysteine reservoir and as a cofactor for various enzymes (Fig. 33).[9] Even though there is conflicting evidence regarding essentiality of MSH for *M. tuberculosis*, mycothiol and related enzymes are considered to be good drug targets due to its vital role in the survival of *M. tuberculosis* under stressed conditions. Apart from protecting *M. tuberculosis* against some antibiotics (e.g. rifampicin and streptomycin), mycothiol plays an important role in bioactivation of other antibiotics such as isoniazid and ethionamide. Therefore inhibitors targeting mycothiol-dependent enzymes are thought to be more promising drug targets than those involved in mycothiol biosynthesis as they could work synergistically with current drugs that require mycothiol for bioactivation and with those that are detoxified by mycothiol-dependent enzymes.[11]

Mycothiol-*S*-transferase (MST) is a key enzyme involved in the neutralisation of electrophilic toxins in mycobacteria. MST in *M. tuberculosis* has recently been identified and has not been extensively studied. Chemical tools are needed to help to dissect the role played by MST and other MSH-dependent enzymes in *M. tuberculosis*.[11,40] It is hypothesised that inhibition of MST will negatively affect cell viability, enhance sensitivity to existing antibiotics and increase susceptibility to

the human immune system.

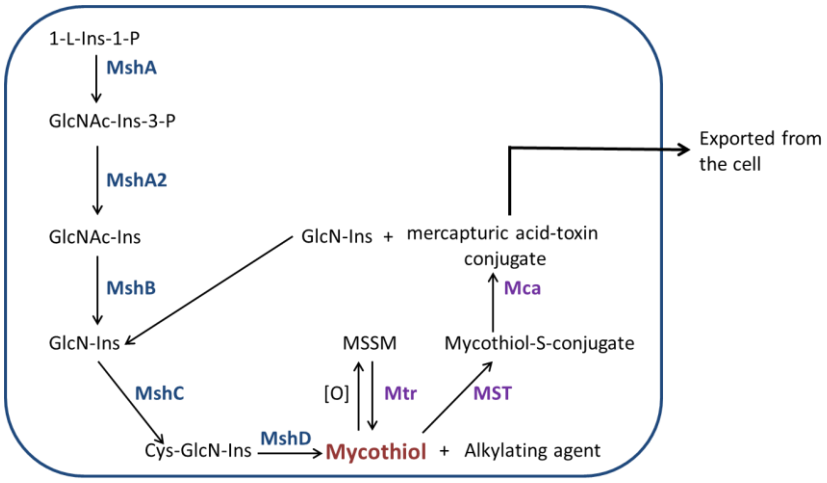


Fig. 33. Scheme depicting the biosynthesis of mycothiol and its role in mycobacterial physiology.

The ultimate aim of this project was to validate MST as a novel drug target. Because chemical synthesis of MSH is complicated and the isolation of mycothiol from cells in sufficient quantities is challenging, potent and selective probes are needed to investigate the role of MST and other MSH-dependent enzymes. Towards the development of chemical probes, a 2-fold approach was taken. The first approach included the development of substrate analogues based on a simplified mycothiol scaffold (**110**) containing cysteine moiety (Fig. 34). The analogues do not have the inositol ring because it was shown that the inositol moiety is not crucial for the substrate recognition by Mtr, so it can be assumed that it will be the case for other MSH dependent proteins.[11] The simplified analogues would provide a scaffold for the synthesis of a varied library of *S*-conjugates that would enable us to probe the hydrophobic pocket of MST and gain some information about the structure–activity relationships of the target protein.

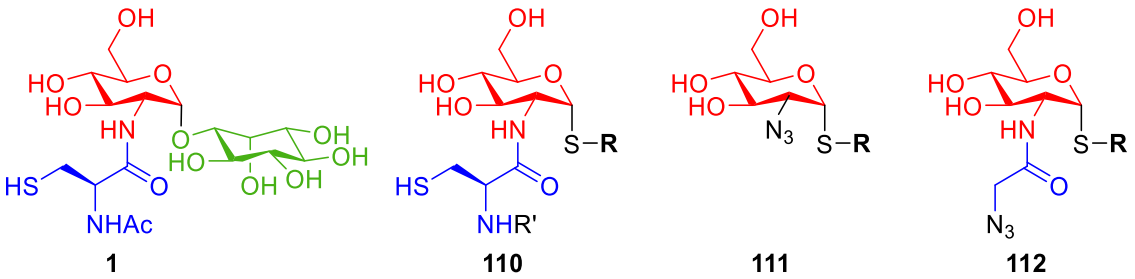


Fig. 34. Structures of mycothiol (**1**) and simplified mycothiol analogues (**110**, **111**, **112**).

The second approach involved the use of kinetic target guided synthesis (kTGS), which is a process whereby the protein acts as a catalyst or template in the synthesis of its own best inhibitors from the 'choices' provided. Towards this goal azido derivatives of the simplified mycothiol analogue (**111** and **112**) (Fig. 34) were designed for synthesis and testing. The analogues would act as a handle within the binding site and would allow us to explore the cysteine-binding site of mycothiol-*S*-transferase. The azido derivatives would be incubated with an alkyne fragment library in the presence of MST in order to find triazole containing hits. The identified hits would be synthesised using standard 'click chemistry' conditions.

The specific objectives are as follows:

(i) to synthesise the cysteine containing mycothiol analogues (**110**);

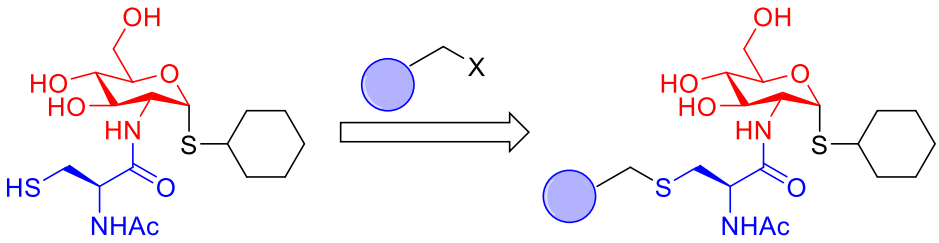
(ii) to synthesise the non-cysteine mycothiol analogues (**111** and **112**);

(iii) to synthesise the triazole containing mycothiol analogues.

Part II Results and discussion

5. Synthesis of the cyclohexyl cysteine mycothiol analogue

In order to explore the role of mycothiol-*S*-transferase it was necessary to synthesise a series of analogues of the natural product. Chemical probes based on a simplified mycothiol scaffold (**78**) that contains a cysteine moiety were designed. It was proposed to follow a published methodology[49] to develop a range of substrate analogue inhibitors of MST. The simplified analogue (**78**) contains a cyclohexyl group in place of the inositol ring present in the natural product, as it was shown that the inositol moiety does not contribute greatly to the binding affinity of mycothiol to Mtr.[11] This simplified analogue (**78**) was planned to be investigated as a substrate for enzyme kinetics studies and would provide a scaffold for the synthesis of a library of *S*-conjugates. A series of novel *S*-conjugates could be synthesised using thiol-ene coupling or *S*-alkylation (Fig. 35). The substituents used would vary in size and polarity to enable us to probe the hydrophobic pocket of MST and gain some information about the structure–activity relationships (SAR) of the target protein.



78

Fig. 35. Scheme outlining the synthesis of substrate analogue inhibitors of mycothiol-*S*-transferase.

5.1. The first synthetic route

It was decided to synthesise the simplified mycothiol analogue (**78**) according to the synthetic route presented by Knapp *et al.*[49] (Fig. 36), which was described in detail in the Introduction. In short, the acetamido mercaptan (**73**) could be obtained using the acetyl-protected β-glucosamine (**71**) as a starting material. Then the cyclohexane ring can be introduced giving the aminotriol (**75**) after global deprotection. Finally, the aminotriol (**75**) would be coupled with *N*-Boc-*S*-acetyl-L-cysteine (**47**) forming the simplified mycothiol analogue (**78**).

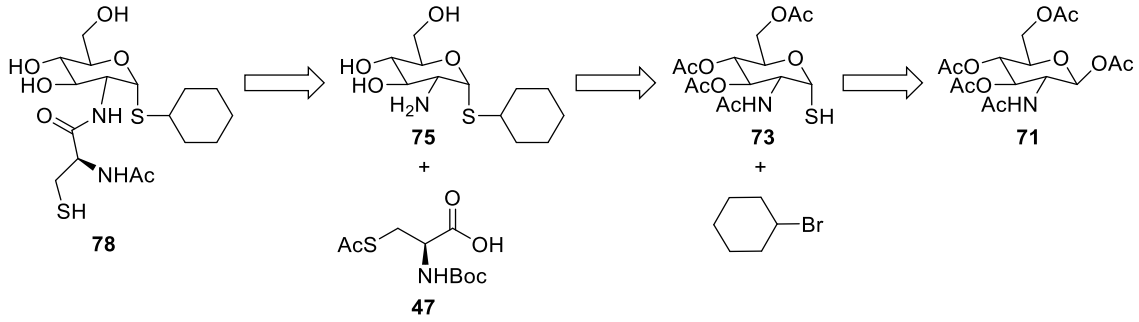


Fig. 36. Retrosynthesis of the simplified mycothiol analogue (**78**).

First, following the published synthetic strategy,[49] the acetyl-protected β-glucosamine (**71**) was treated with Lawesson's reagent and the reaction was heated at 80 °C for 1.5 h (Fig. 37). Lawesson's reagent was chosen, as it is the most widely used reagent for converting carbonyl groups into thiocarbonyls and allows for the preparation of heterocyclic compounds having a sulphur atom.[57] A sulphur atom was used to replace the linking oxygen in the structure of natural mycothiol, so that the mycothiol analogue (**78**) would be more chemically and biologically stable.[58] The product, GlcNAc-thiazoline triacetate (**72**), was successfully obtained in 76% yield.

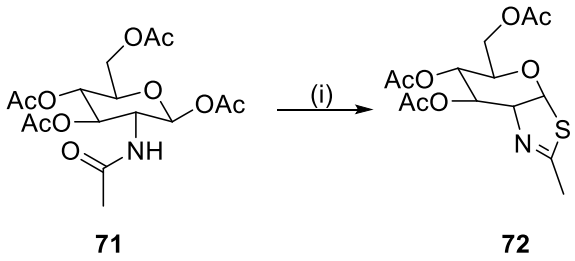
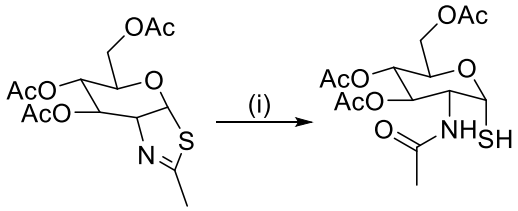


Fig. 37. Synthesis of the GlcNAc-thiazoline triacetate (**72**). Reagents and conditions: (i) Lawesson's reagent, toluene, 80 °C, 1.5 h, 76%.

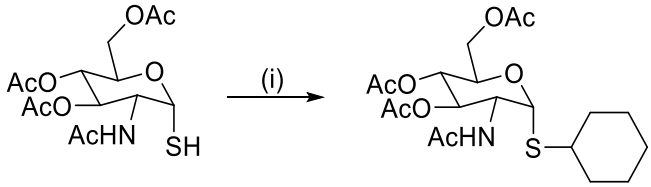
In the second step, the GlcNAc-thiazoline triacetate (**72**) was hydrolysed using trifluoroacetic acid to provide the acetamido mercaptan (**73**) in quantitative yield with no further purification required (Fig. 38). Acidic hydrolysis using TFA was performed, so that the hydrolysis would only occur at the iminium carbon and not at C-1 of **72**.[58] Additionally, use of TFA sets the stereochemistry at the anomeric centre, so that only the α-isomer is formed. The formation of the sulfhydryl group is important as it allows for the addition of the cyclohexyl group in the subsequent step.



72 **73**

Fig. 38. Synthesis of the acetamido mercaptan (**73**). Reagents and conditions: (i) TFA, wet MeOH, 100%.

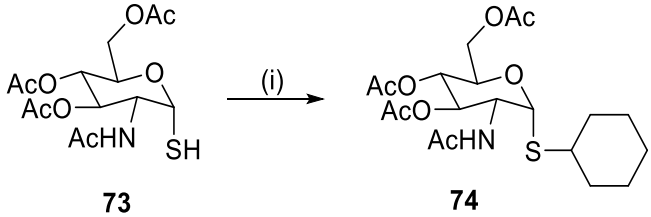
The third step in the synthetic pathway required a free radical addition of the acetamido mercaptan (**73**) to cyclohexene using 2,2'-azobis(2-methylpropionitrile) (AIBN) as a radical initiator (Fig. 39), however, the cyclohexyl thioglycoside (**74**) was obtained in a low yield (8%).



73 **74**

Fig. 39. Synthesis of the cyclohexyl thioglycoside (**74**). Reagents and conditions: (i) C_6H_{10}, AIBN, $CHCl_3$, reflux, 8%.

Because the method reported by Knapp *et al.*[49] proved to be low-yielding in our hands, it was proposed to employ S_N2 nucleophilic substitution to afford the cyclohexyl thioglycoside (**74**). The advantage of this type of reaction is that it does not require the use of potentially explosive reagents such as AIBN and will thus better lend itself to scale up. The acetamido mercaptan (**73**) was dissolved in dry dimethylformamide, which is a polar, aprotic solvent, and treated with anhydrous potassium carbonate to deprotonate the nucleophile **73**, which attacks the cyclohexyl bromide furnishing the cyclohexyl thioglycoside (**74**) (Fig. 40). The reaction was stirred at room temperature for 1 h to give the cyclohexyl thioglycoside (**74**) in 10% yield.



73 **74**

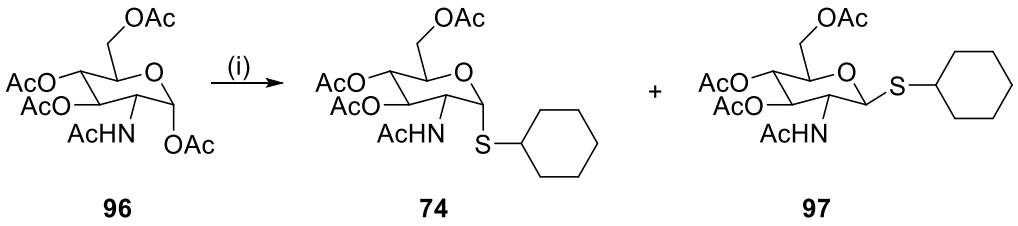
Fig. 40. Synthesis of the cyclohexyl thioglycoside (**74**). Reagents and conditions: (i) $C_6H_{11}Br$, K_2CO_3, DMF, 10%.

In order to increase the yield, it was decided to optimise the reaction conditions (Table 2). Two different solvents were used (DCM or THF) instead of DMF and a range of different bases (Et_3N, NaOH, *N*-methylmorpholine (NMM) and Cs_2CO_3). None of the reaction conditions employed led to significant production of the target compound (**74**).

Table 2. Reagents and conditions for the synthesis of **74**.

Experiment code	Solvent	Base	Temperature	Reaction time	Yield
ERD1	DMF	K_2CO_3	RT	1 h	10%
ERD10	DCM	Et_3N	RT	20 h	-
ERD13	DCM	NaOH	RT	3 h	-
ERD19	THF	NMM	RT	1.5 h	-
ERE27	DMF	Cs_2CO_3	RT	1 h	-

As the reaction yield was not improved an alternative approach to the formation of the cyclohexyl thioglycoside (**74**) was explored. Following the reaction conditions proposed by Riordan *et al*.[53], α-D-glucosamine pentaacetate (**96**) was treated with $BF_3{\cdot}OEt_2$ and cyclohexanethiol to give a mixture of α- and β-anomers (Fig. 41). The anomers **74** and **97** were separated by column chromatography. In our hands the yield of the cyclohexyl thioglycoside (**74**) from this reaction was much lower than that stated by Riordan *et al*.[53] (3% *vs* 22%).

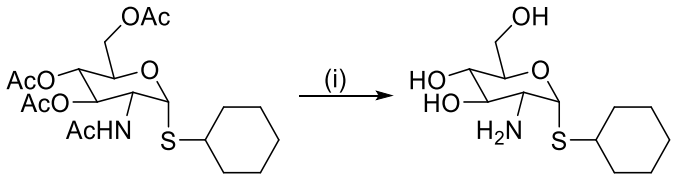


96 **74** **97**

Fig. 41. Synthesis of the cyclohexyl thioglycoside (**74**). Reagents and conditions: (i) cyclohexanethiol, $BF_3{\cdot}OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h, **74** 3%, **97** 10%.

In the next step, the conditions outlined by Knapp *et al.*[49] were slightly changed as the cyclohexyl thioglycoside (**74**) was treated with hydrazine monohydrate and heated at 70 °C *c.f.* 120 °C for 22 h (Fig. 42). Hydrazinolysis is a widely used method for the deprotection of *N*-acetyl and *O*-acetyl groups of aminosugars.[59] In this case the *N*-deacetylation was of the greatest importance, as a free amino

group is needed for the conjugation of the aminotriol (**75**) with *N*-Boc-*S*-acetyl-L-cysteine (**47**) in the next step. Under the stated conditions the desired aminotriol (**75**) was isolated in 84% yield.



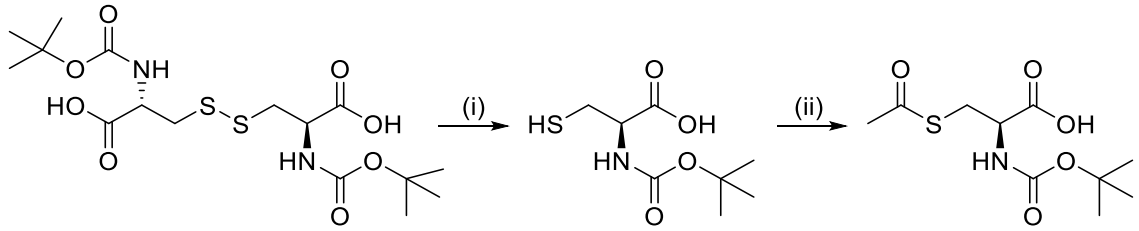
74 **75**

Fig. 42. Synthesis of the aminotriol (**75**). Reagents and conditions: (i) $NH_2NH_2 \cdot H_2O$, 70 °C, 22 h, 84%.

The fifth step involves coupling of the aminotriol (**75**) with *N*-Boc-*S*-acetyl-L-cysteine (**47**) in order to furnish the target amide (**76**). As *N*-Boc-*S*-acetyl-L-cysteine (**47**) is not commercially available it had to be synthesised.

First, following the conditions presented by Knapp *et al.*,[49] the *N*,*N*'-di-Boc-L-cystine (**113**) solution in 5% ethereal acetic acid was treated with zinc dust at 0 °C. After 4 h, acetic anhydride and pyridine were added and the reaction was stirred at room temperature for 2 h to obtain *N*-Boc-*S*-Ac-L-cysteine (**47**) in 35% yield.

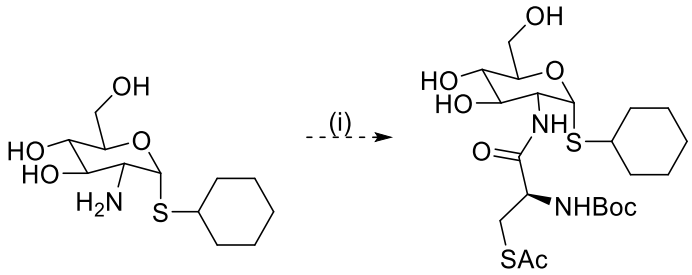
Because a low yield was obtained, it was decided to synthesise *N*-Boc-*S*-acetyl-L-cysteine (**47**) using an alternative approach. *N*-Boc-*S*-acetyl-L-cysteine (**47**) was prepared in a two-step reaction sequence. In the first step, the commercially available *N*,*N*'-di-Boc-L-cystine (**113**) was treated with triphenylphosphine and the reaction was stirred overnight at room temperature to obtain *N*-Boc-L-cysteine (**114**) in a 100% yield (Fig. 43). In this reaction triphenylphosphine acts as a nucleophile and cleaves the S-S bond of the cystine producing triphenylphosphine oxide and *N*-Boc-L-cysteine (**114**).[60] The next step required *S*-acetylation of *N*-Boc-L-cysteine (**114**). In order to acetylate **114** acetic anhydride was added under basic conditions and the reaction was stirred at room temperature for 3 h to give *N*-Boc-*S*-acetyl-L-cysteine (**47**) in a quantitative yield (Fig. 43).



113 114 47

Fig. 43. Synthesis of *N*-Boc-*S*-acetyl-L-cysteine (**47**). Reagents and conditions: (i) PPh_3, THF/H_2O, 100%; (ii) acetic anhydride, $NaHCO_3$, 100%.

With **47** in hand, the coupling reaction to obtain the key intermediate (**76**) could be attempted. Following the reaction conditions outlined by Knapp *et al.*,[49] EDCI was used as a coupling reagent and anhydrous DMF as the solvent (**ERA27**) (Fig. 44.,Table 3). *N*-Boc-*S*-acetyl-L-cysteine (**47**) and the aminotriol (**75**) were azeotroped with toluene to remove any residual water. The residue was dissolved in DMF then EDCI was added at 0 °C and the reaction mixture was allowed to warm to room temperature and stirred for 1 h. The resulting crude material was purified by silica gel column chromatography. However, the target compound (**76**) was not identified.



75 76

Fig. 44. Synthesis of **76**. Reagents and conditions: (i) *N*-Boc-*S*-acetylcysteine (**47**), EDCI, DMF.

In an amide coupling reaction, a base helps in the deprotonation of the carboxyl group and carboxylate ion formed attacks the electron deficient carbon of the coupling reagent. The additive such as 1-hydroxybenzotriazole (HOBt) reacts with the activated carboxyl group to form an activated ester. Finally, the activated ester reacts with the amine to form the desired amide product (Fig. 45).

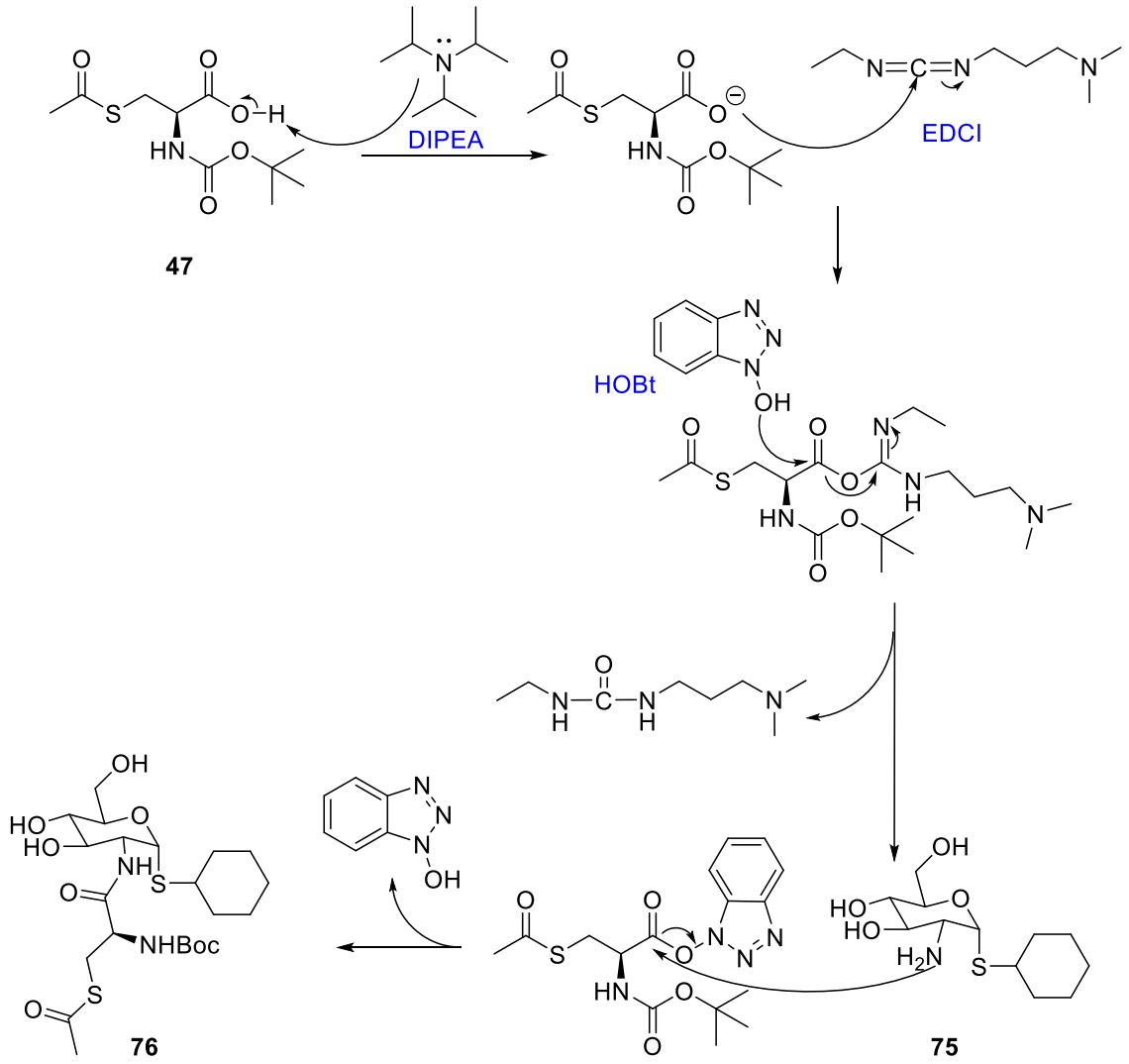


Fig. 45. Amide coupling mechanism. EDCI is used as a coupling reagent, DIPEA as a base and HOBt as an additive.

Because the method presented by Knapp *et al.*,[49] proved to be unsuccessful in our hands alternative coupling conditions were investigated. There are various factors affecting the product formation in an amide coupling reaction. They include: the type of coupling reagent (more or less reactive): EDCI, HATU, COMU, *N*,*N*,*N*',*N*'-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), *N*,*N*'-dicyclohexylcarbodiimide (DCC), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), fluoro-*N*,*N*,*N*',*N*'-bis(tetramethylene)formamidinium hexafluorophosphate (BTFFH), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU) and *O*-(5-norbornene-2,3-dicarboximido)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TNTU); type of a base (stronger or weaker): *N*,*N*-diisopropylethylamine (DIPEA), KO^{t}Bu, Et_3N; solubility of the starting materials

and the product in the solvent chosen for the reaction: DMF, acetonitrile, H_2O, THF, DCM, pyridine, MeOH; use of an additive (HOBt); the equivalents of reagents used in the reaction; the temperature at which the reaction is carried out; the activation and reaction times and finally the purification technique employed. In order to optimise reaction conditions for the synthesis of the desired amide product (**76**) twenty different reaction conditions have been explored (Table 3).

In the first set of test reactions, it was decided to add a base (DIPEA) to the reaction mixture to help in the activation of the cysteine carbonyl group (**ERA32**, **ERA34**, **ERA36**) (Table 3). Additionally, in the reactions **ERA32** and **ERA34** the coupling reagent was changed to HBTU and acetonitrile was used as a solvent in **ERA34**. The crude reaction mixtures were purified by column chromatography (19:1 DCM/MeOH), however, the desired amide (**76**) was not isolated.

In order to find the optimal reaction conditions for the synthesis of **76**, conditions reported by Riordan *et al.*[53] were employed (**ERA38**) (Table 3). The synthetic strategy towards the synthesis of the mycothiol analogue (**100**) was described in detail in the Introduction. Here, the number of equivalents of the coupling reagent was slightly increased (2.5 *c.f.* 2) and KO^{t}Bu was used as a base, nevertheless, the target compound (**76**) was not formed.

Next, it was decided to explore another set of conditions presented by Slättegård, Gammon and Oscarson[52] that were used in the synthesis of the bicyclic mycothiol analogue (**95**). The number of equivalents of the cysteine (**47**) was reduced (1 *c.f.* 2), 3:1 DMF/water mixture was used as a solvent and EDCI as a coupling reagent (**ERA39**) (Table 3). Additionally, an additive (HOBt) was used to help in the formation of an activated ester, however, no product (**76**) formation was observed.

In the next attempt to synthesise **76**, reaction conditions used by Chung *et al.*[44] in the synthesis of mycothiol were explored. An alternative coupling reagent – HATU, 3 equivalents of DIPEA and no additive were employed (**ERA40**) (Table 3). Still the desired amide (**76**) was not isolated.

In effort to optimise the reaction conditions a new set of test reactions was designed (**ERA48** and **ERA49**) (Table 3). The number of equivalents of EDCI was increased (3 *c.f.* 2) and KO^{t}Bu was used as a base. Moreover, the activation time was set to 15 min and the reactions were carried out for 5 h at 0 °C and then

at room temperature for next 22 h (**ERA49**) or 2 days (**ERA48**) to allow the reaction to complete. Once again, the product (**76**) was not obtained in either case.

Inspired by the reaction conditions presented by Jones *et al.*[61] that were used to synthesise various glucosamine-amino acid conjugates (the use of cysteine was not reported), reaction **ERA52** was carried out (Table 3). HOBt was used as an additive, trimethylamine as base and THF as a solvent, however, the aminotriol (**75**) was dissolved in small amount of DMF as it is not soluble in THF. The desired product (**76**) was not identified in any of the fractions collected during column chromatography.

Following the reaction conditions used by Kyas and Feigel[62] to prepare differently glycosidated sugar amino acids based on a glucosamine core, two more reactions were performed (**ERA53** and **ERA57**) (Table 3). The reaction conditions employed in **ERA53** and **ERA57** were similar to the reaction conditions that were used in **ERA52**, but the solvent was changed to DCM. Furthermore, in reaction **ERA57** the coupling reagent used was changed to EEDQ and no base was used. The synthesis of **76** failed in both cases. It was decided to repeat reaction **ERA57** but changing the solvent to DMF, adding an additive (HOBt) and a base (DIPEA) (**ERA62**) (Table 3), nevertheless, the desired amide (**76**) was not isolated.

Inspired by the coupling conditions reported by Yoshimura *et al.*,[63] the reaction was repeated using DCC as the coupling reagent, Et_3N as a base and pyridine as a solvent (the aminotriol (**75**) was dissolved in a small amount of DMF) (**ERA55**) (Table 3). Once again, the reaction did not lead to the formation of the target amide (**76**).

Due-Hansen *et al*.[64] has recently published a novel protocol describing the reaction conditions for the coupling of carboxylic acids with amines that failed when conventional methods were employed. Following the presented method, a fluorouronium reagent BTFFH was used as well as DIPEA and DMF as a solvent (**ERA61**) (Table 3). However, this method proved to be unsuccessful in the synthesis of **76**.

Inspired by the reaction conditions explored by Suttisansanee[65] that led to the synthesis of a truncated mycothiol analogue (**64**) three more reactions were carried out (**ERA74**, **ERA76** and **ERC1**) (Table 3). In each reaction a different

coupling reagent was used (HBTU, TBTU and TNTU respectively). In all reactions DMF was used as a solvent and DIPEA as a base. The activation time was increased to 2 h and the reactions were stirred at room temperature for 24 h. The desired product (**76**) was still not formed.

In further efforts to optimise the reaction conditions COMU was used as a coupling reagent (**ERA80**) (Table 3), because it was reported to be a better choice than HATU in the mycothiol synthesis performed by Manabe and Ito.[46] DMF was used as a solvent, DIPEA as a base and equal equivalents of reagents were used (**ERA80**). There was no activation time and the reaction was carried out at room temperature for 24 h. One more time, no product (**76**) formation was observed.

In the next attempt to synthesise **76**, it was decided to follow the coupling conditions employed by Laughlin and Bertozzi[66] in the synthesis of various azido sugars. Methanol was used as a solvent, EDCI as a coupling reagent, HOBt as an additive and Et_3N as a base (**ERC14**) (Table 3). There was no activation time, but the reaction mixture was stirred at 0 °C for 30 min before EDCI and HOBt were added. Then the reaction was allowed to slowly warm to room temperature and stirred for 17 h. The desired product (**76**) was not isolated.

In summary, after exploring a wide range of different reaction conditions synthesis and purification of the amide product (**76**) was unsuccessful. In most cases the coupling reactions resulted in either no product formation or formation of a complex mixture of products. As the target compound (**76**) is polar it would be advisable to use a reversed-phase chromatography to obtain better separation and to be able to isolate pure product. All reaction mixtures were purified by silica gel column chromatography as other purification methods were unavailable at that time.

Table 3. Reagents and conditions for the synthesis of **76**.

Experiment code	Equivalent					Solvent	Coupling reagent	Base	Additive	Temperature	Activation time	Reaction Time
	Aminotriol (**75**)	*N*-Boc-*S*-Ac-Cys (**47**)	Coupling reagent	Base	Additive							
ERA 27	1	2	2	-	-	DMF	EDCI	-	-	0 °C when adding EDCI then RT	-	1 h
ERA32	1	1	1	3	-	DMF	HBTU	DIPEA	-	RT	-	2 h
ERA34	1	1	1	3	-	MeCN	HBTU	DIPEA	-	RT	-	2 h
ERA36	1	2	2	3	-	DMF	EDCI	DIPEA	-	0 °C when adding EDCI then RT	-	1 h
ERA38	1	1.5	2.5	1	-	DMF	EDCI	KO^tBu	-	0 °C when adding EDCI then RT	10 min	2 h
ERA39	1	1	2	-	2	DMF/H_2O	EDCI	-	HOBt	RT	-	1 h
ERA40	1	2	2	3	-	DMF	HATU	DIPEA	-	0 °C when adding HATU then RT	-	1 h
ERA48	1	2	3	1	-	DMF	EDCI	KO^tBu	-	0 °C for 5 h then RT	15 min	2 days
ERA49	1	2	3	2	-	DMF	EDCI	KO^tBu	-	0 °C for 5 h then RT	15 min	22 h
ERA52	1	1	1.2	1	1.2	THF/DMF	EDCI	Et_3N	HOBt	0 °C for 1 h then RT	1 h	20 h
ERA53	1	1	1.5	1	1.5	DCM	EDCI	Et_3N	HOBt	0 °C for 1 h then RT	1 h	30 h
ERA55	1	1	1.1	1	-	Pyridine/DMF	DCC	Et_3N	-	0 °C for 1 h then RT	1 h	30 h
ERA57	1	1	1.5	-	-	DCM	EEDQ	-	-	0 °C for 1 h then RT	1 h	28 h
ERA61	1	1.3	1.5	4.5	-	DMF	BTFFH	DIPEA	-	0 °C for 1 h then RT	1 h	24 h
ERA62	1	1	1.5	2	1	DMF	EEDQ	DIPEA	HOBt	0 °C for 1 h then RT	1 h	24 h
ERA74	1	2	2	3	-	DMF	HBTU	DIPEA	-	RT	2 h	24 h
ERA76	1	2	2	3	-	DMF	TBTU	DIPEA	-	RT	2 h	24 h
ERA80	1	1	1	1	-	DMF	COMU	DIPEA	-	RT	-	24 h
ERC1	1	2	2	3	-	DMF	TNTU	DIPEA	-	RT	2 h	24 h
ERC14	1	1.5	0.7	2	0.7	MeOH	EDCI	Et_3N	HOBt	0 °C for 0.5 h then RT	-	17 h

5.1.1. Synthesis of the truncated mycothiol analogue

As the synthesis of the amide (**76**) proved to be more challenging than expected, it was decided to synthesise the truncated mycothiol analogue, namely 2-amino-2-*N*-(*N*-tert-butylcarbonyl-*S*-acetyl-L-cysteinyl)-2-deoxy-1-thio-D-glucopyranoside (**115**), which lacks the thioglycoside and cyclohexyl moieties present in **76** (Fig. 46). It was designed to act as a model system to further optimise coupling conditions for the formation of **76** but could also be used to explore SAR of the mycothiol-*S*-transferase. The use of D-glucosamine hydrochloride (**107**), which is commercially available, instead of the aminotriol (**75**) allows test reactions to be performed on a larger scale and the truncated analogue (**115**) could be synthesised in one-step *c.f.* seven steps required for the synthesis of the simplified mycothiol analogue (**78**).

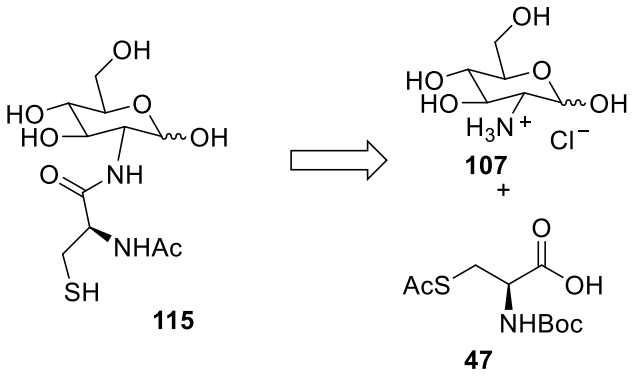


Fig. 46. Retrosynthesis of the truncated mycothiol analogue (**115**).

It was decided to carry out a number of test reactions in parallel. Inspired by the reaction conditions reported by Suttisansanee,[65] in the first set of test reactions HBTU (**ERA81** and **ERA83**) and TNTU (**ERA84** and **ERA85**) were explored as coupling reagents, DMF was used as a solvent and DIPEA as a base (Table 4). Two different activation times were applied when using each coupling reagent, namely 15 min (**ERA81** and **ERA84**) and 2 h (**ERA83** and **ERA85**). All four reactions (**ERA81**, **ERA83**, **ERA84** and **ERA85**) were carried out at room temperature and stirred for 3 h. The crude reaction mixtures were purified by column chromatography using a gradient of 0-20% methanol in dichloromethane (2% methanol increments). However, the desired product (**115**) was not isolated. In the second set of four test reactions (**ERC2**, **ERC3**, **ERC4** and **ERC5**) the reaction conditions were further optimized (Table 4). TNTU was used as a coupling reagent and the activation time was set to 15 min in all test reactions

(**ERC2**, **ERC3**, **ERC4** and **ERC5**). However, the reaction time was extended (3 h *c.f.* 6 h) in order to find out whether it affects the product (**115**) formation. In reactions **ERC2** and **ERC3** the equivalents of TNTU were varied (two and three equivalents were used respectively). In reactions **ERC4** and **ERC5** the number of equivalents of D-glucosamine HCl (**107**) was varied (two and three equivalents were used respectively). The crude reaction mixtures were purified by column chromatography as before, however, the desired truncated analogue (**115**) was not isolated.

Inspired by the work of Ajayi *et al.*,[43] that was described in detail in the Introduction, it was decided to explore an alternative coupling reagent, namely HATU (**ERC7**) (Table 4). The temperature of the reaction mixture was lowered to 0 °C when the coupling reagent was added and then the reaction was allowed to slowly warm to room temperature over 17 h. The crude reaction mixture was chromatographed using 17:3 dichloromethane/methanol as the eluent, nevertheless, the desired amide (**115**) was not obtained.

In summary, after exploring a wide range of different reaction conditions synthesis and purification of the truncated mycothiol analogue (**115**) was unsuccessful.

Table 4. Reagents and conditions for the synthesis of **115**.

Experiment code	Equivalent				Solvent	Base	Coupling reagent	Temperature	Activation time	Reaction time
	GlcN HCl (**107**)	*N*-Boc-*S*-Ac-Cys (**47**)	Coupling reagent	Base						
ERA81	0.6	1	1	2.5	DMF	DIPEA	HBTU	RT	15 min	3 h
ERA83	0.6	1	1	2.5	DMF	DIPEA	HBTU	RT	2 h	3 h
ERA84	1.2	1	1	2.5	DMF	DIPEA	TNTU	RT	15 min	3 h
ERA85	0.6	1	1	2.5	DMF	DIPEA	TNTU	RT	2 h	3 h
ERC2	1	1	2	2.5	DMF	DIPEA	TNTU	RT	15 min	6 h
ERC3	1	1	3	2.5	DMF	DIPEA	TNTU	RT	15 min	6 h
ERC4	2	1	1	2.5	DMF	DIPEA	TNTU	RT	15 min	6 h
ERC5	3	1	1	2.5	DMF	DIPEA	TNTU	RT	15 min	6 h
ERC7	1	1.5	1.5	2.5	DMF	DIPEA	HATU	0 °C when adding HATU then RT	-	17 h

5.2. The second synthetic route

Because the first synthetic strategy after numerous optimisation attempts proved to be unsuccessful another synthetic route towards the synthesis of the simplified mycothiol analogue (**78**) was designed (Fig. 47). Here, it was decided to introduce the cysteine moiety early in the synthetic route to overcome the most difficult step in the synthetic pathway at the beginning. Also, an acetyl-protected glucosamine (**40**) was planned to be used instead of glucosamine HCl (**107**) as it is less polar making the purification and handling of the coupling product (**116**) easier. Then the cyclohexyl group would be introduced and after deprotection the desired simplified mycothiol analogue (**78**) would be obtained.

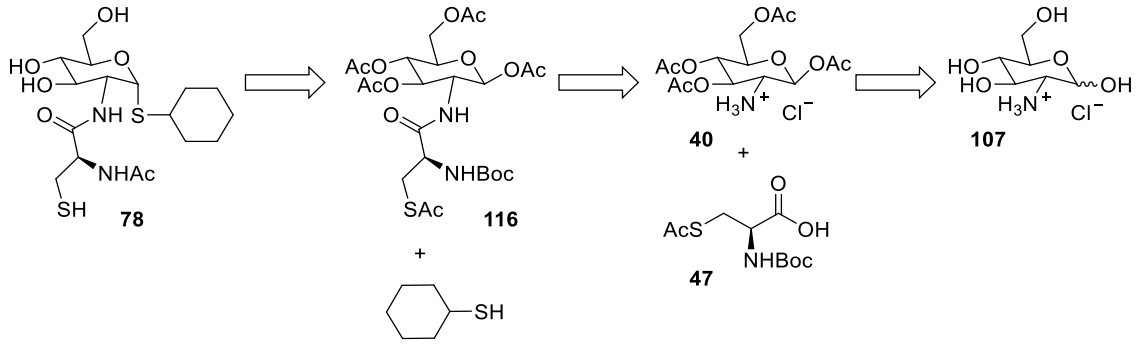


Fig. 47. Retrosynthesis of the simplified mycothiol analogue (**78**).

First, the acetyl-protected glucosamine (**40**) was synthesised. It is commercially available but relatively expensive, so it was decided to synthesise **40** in a three-step reaction sequence according to a published protocol[67] (Fig. 48).

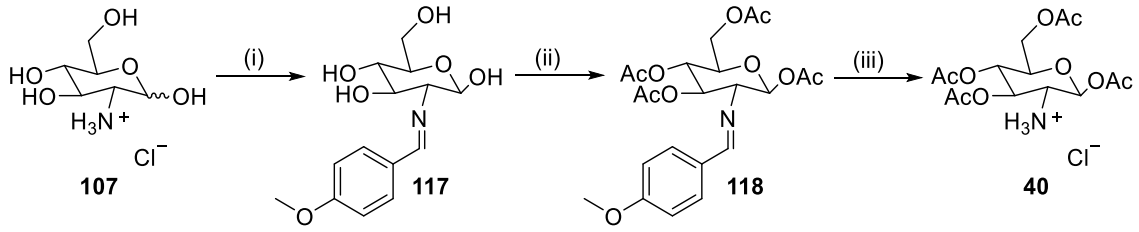
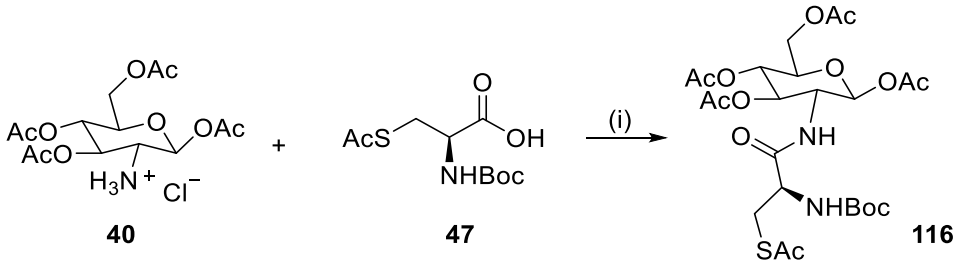


Fig. 48. Synthesis of **40**. Reagents and conditions: (i) *p*-anisaldehyde, 1M sodium hydroxide, 46% (ii) acetic anhydride, DMAP, pyridine, 57% (iii) 5N HCl, acetone, 67%.

In the first step, glucosamine HCl (**107**) was treated with *p*-anisaldehyde in 1 M sodium hydroxide solution in order to obtain 2-*p*-methoxybenzylideneamino-β-D-glucosamine (**117**) in 46% yield. In the next step, **117** was treated with acetic anhydride, 4-(dimethylamino)pyridine (DMAP) and pyridine at 0 °C and the reaction was stirred overnight at room temperature to obtain 2-deoxy-2-*p*-

methoxybenzylideneamino-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**118**) in 57% yield. In the third step, 5 N HCl solution was added dropwise to the solution of **118** in acetone under reflux in order to obtain the acetyl-protected glucosamine (**40**) in 67% yield.

Having the acetyl-protected glucosamine (**40**) and *N*-Boc-*S*-acetyl-L-cysteine (**47**) in hand, the synthesis of the amide (**116**) was attempted. It was decided to employ the reaction conditions used by Zeng *et al*.[68] in the synthesis of glucosamine mimetic peptides where **40** and various amino acids (use of cysteine was not reported) were used as starting materials. DCC was used as a coupling reagent, HOBt as an additive, NMM as a base and THF as a solvent (**ERC38**) (Fig. 49) (Table 5). The activating reaction was carried out at 0 °C for 30 min and then the solution of **40** and *N*-methylmorpholine was added. The reaction mixture was stirred at 0 °C for 1 h followed by 16 h at room temperature. The reaction was filtered to remove dicyclohexylurea (DCU) and worked-up. The residue was chromatographed using 99:1 DCM/MeOH as the eluent to give **116**. Because the yield obtained was very low (5%) it was decided to perform a series of test reactions in order to improve the yield.



40 **47** **116**

Fig. 49. Synthesis of **116**. Reagents and conditions: (i) DCC, HOBt, THF, NMM, 5%.

In the first set of test reactions (**ERC45**, **ERC46**, **ERC47**, **ERC48**) the number of equivalents of the protected glucosamine (**40**) was increased from 1 to 5 (Table 5) In the second set of test reactions (**ERC49**, **ERC50**, **ERC51**, **ERC52**) different bases were used – NMM, no base, DIPEA and 2,4,6-collidine respectively (Table 5). In the third set of test reactions (**ERC53**, **ERC54**, **ERC55**) the number of equivalents of *N*-Boc-*S*-acetyl-L-cysteine (**47**) was increased from 2 to 5 (Table 5). All reactions were monitored by thin layer chromatography (TLC). The best results, when comparing three sets of test reactions, in terms of the product (**116**) formation were obtained when the number of equivalents of the protected glucosamine (**40**) was increased (**ERC46**, **ERC47**, **ERC48**). However, the full

consumption of the protected cysteine (**47**) was observed only when 5 equivalents of **40** were used (**ERC48**). Because traces of DCU were visible in the ^{1}H NMR spectrum of the product (**116**) even after recrystallization, it was decided to use EDCI instead of DCC as a coupling reagent in order to ease purification. The reaction **ERC48** was repeated on a bigger scale (500 mg) and EDCI was used as a coupling reagent (**ERC58**) (Table 5). The reaction was worked-up to obtain the amide (**116**) in 64% yield. Some of the starting material (**40**) was also recovered from the acidic aqueous phase (20%).

In summary, the desired amide (**116**) was synthesised using THF as a solvent, NMM as a base and EDCI as a coupling reagent in a good yield (64%).

Having the two main components (*i.e.* the glucosamine and cysteine moieties) of the simplified analogue (**78**) in place, the introduction of the *S*-cyclohexyl substituent was explored. As before, following the reaction conditions presented by Riordan *et al*.[53], the amide (**116**) was treated with $BF_3{\cdot}OEt_2$ and cyclohexanethiol and heated at 55 °C for 5 h (Fig. 50). Previously when these reaction conditions were employed to synthesise the cyclohexyl thioglycoside (**74**) a mixture of α- and β-anomers was obtained, however, in this case only the β-anomer (**119**) was isolated. Thus, the second synthetic route towards the synthesis of the simplified mycothiol analogue (**78**) proved to be unsuccessful.

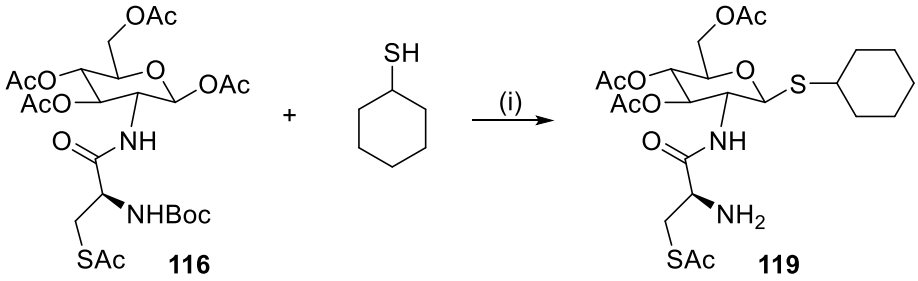


Fig. 50. Synthesis of **119**. Reagents and conditions: (i) $BF_3{\cdot}OEt_2$, DCM/THF, 0–55 °C, 5 h, 18%.

Table 5. Reagents and conditions for the synthesis of **116**.

Experiment code	Equivalent					Solvent	Coupling reagent	Base	Additive	Temperature	Activation time	Reaction Time
	Protected GlcN (**40**)	*N*-Boc-*S*-Ac-L-Cys (**47**)	Coupling reagent	Base	Additive							
ERC38	1	1	1	-	1	THF	DCC	-	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC45	1	1	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC46	2	1	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC47	3	1	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC48	5	1	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC49	1	1	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	-	24 h
ERC50	1	1	1	-	1	THF	DCC	-	HOBt	0 °C for 1h then RT	-	24 h
ERC51	1	1	1	10	1	THF	DCC	DIPEA	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC52	1	1	1	10	1	THF	DCC	2,4,6-collidine	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC53	1	5	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC54	1	3	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC55	1	2	1	10	1	THF	DCC	NMM	HOBt	0 °C for 1h then RT	0.5 h	24 h
ERC58	5	1	1	8	1	THF	EDCI	NMM	HOBt	0 °C for 1h then RT	0.5 h	18 h

5.3. The third synthetic route

As the two synthetic routes that had been explored to obtain the simplified mycothiol analogue (**78**) proved to be unsuccessful a third synthetic strategy was developed (Fig. 51). This approach encompasses the strengths of the two previous strategies. The synthesis begins in the same way as in the first synthetic route. The acetamido mercaptan (**73**) is formed using the acetyl-protected β-glucosamine (**71**) as a starting material. Then the cyclohexane ring is introduced giving only the desired α-anomer (**74**). Next, the cyclohexyl thioglycoside (**74**) would be selectively *N*-deacetylated to give **120**. Finally, the amine (**120**) would be coupled with *N*-Boc-*S*-acetyl-L-cysteine (**47**) and deprotected forming the simplified mycothiol analogue (**78**).

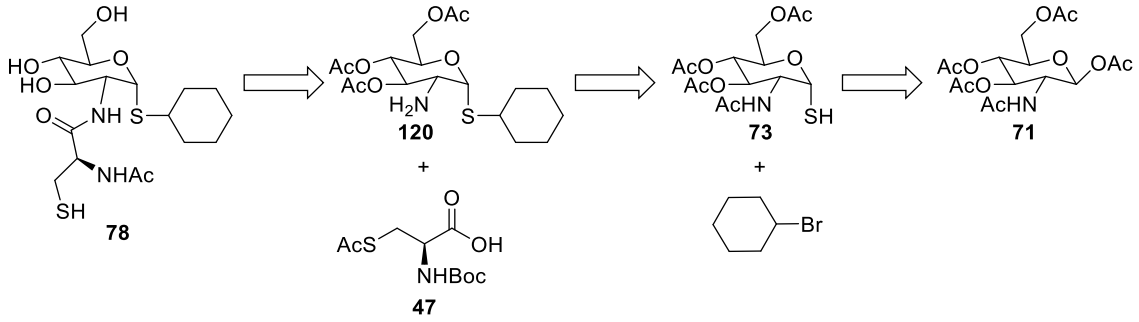


Fig. 51. Retrosynthesis of the simplified mycothiol analogue (**78**).

Having the cyclohexyl thioglycoside (**74**) in hand, the synthesis of the glucopyranoside (**98**) was attempted following the reaction conditions presented by Riordan *et al*.[53] (Fig. 52). The cyclohexyl thioglycoside (**74**) was treated with DMAP and Boc_2O and stirred at 60 °C for 18 h. The resulting crude material was purified by silica gel chromatography to give **98** in 89% yield. The Boc group was introduced to allow the selective *N*-deacetylation in the next step followed by the removal of a Boc group in the subsequent step to produce the free amine (**120**).

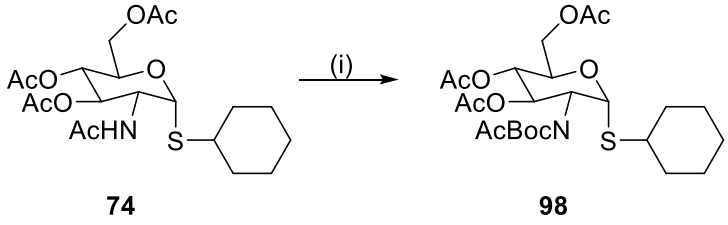


Fig. 52. Synthesis of **98**. Reagents and conditions: (i) DMAP, Boc_2O, THF, 60 °C, 89%.

In the two following steps the reaction conditions employed by Metaferia *et al.*[21] in the synthesis of MshB inhibitors were used. First, the glucopyranoside (**98**) was treated with hydrazine monohydrate and stirred at 0 °C for 1 h to give *N*-deacetylated product (**121**) in 65% yield (Fig. 53). In this step it was important to keep the reaction at 0 °C, otherwise the glucopyranoside (**98**) would be fully deacetylated. In the subsequent step the *N*-Boc protected glucopyranoside (**121**) was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and stirred at 0 °C for 4 h to give the free amine (**120**) in 83% yield (Fig. 53).

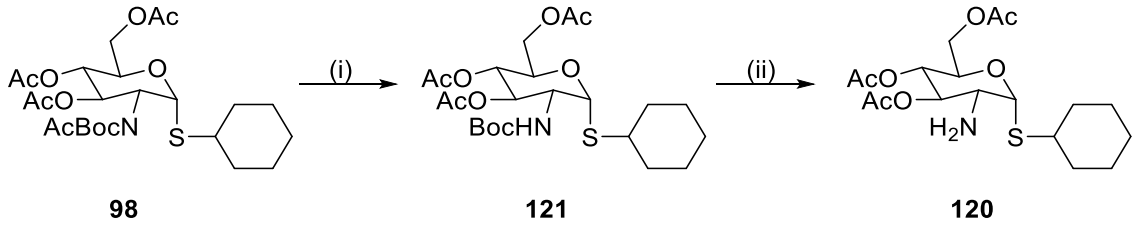


Fig. 53. Synthesis of **120**. Reagents and conditions: (i) $NH_2NH_2 \cdot H_2O$, MeOH, 0 °C, 65%; (ii) TMSOTf, CH_2Cl_2, 0 °C, 83%.

Having the free amine (**120**) in hand, it was decided to synthesise the simplified mycothiol precursor (**122**) following the optimized procedure for the synthesis of the amide (**116**) that was described previously (Fig. 54). In short, EDCI was used as a coupling reagent, HOBt as an additive, NMM as the base and THF as the solvent. The main difference was the decrease in the number of equivalents of the amine (**120**) used (1 *c.f.* 5). After column chromatography the desired amide (**122**) was isolated in 20% yield.

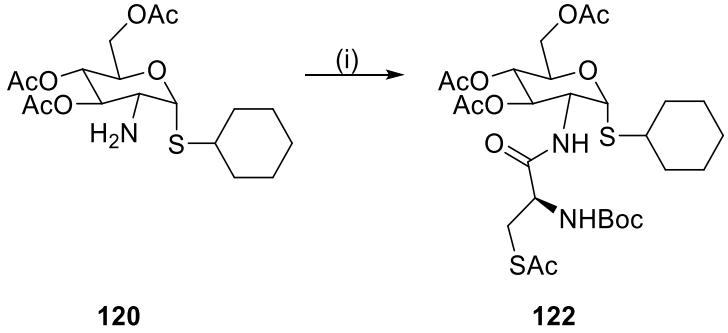
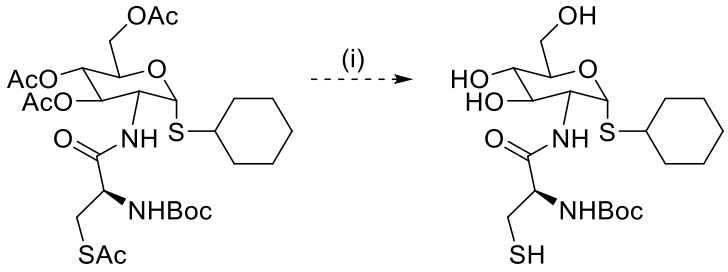


Fig. 54. Synthesis of **122**. Reagents and conditions: (i) *N*-Boc-*S*-Ac-Cys (**47**), EDCI, HOBt, NMM, THF, 20%.

Because it requires 7 steps to obtain the simplified mycothiol precursor (**122**) and the yields are variable only a small amount of the product (**122**) (16 mg) was produced. The deacetylation of the amide (**122**) using sodium methoxide in methanol was attempted (Fig. 55). However, there was not enough material to

fully characterise the compound produced. Therefore, it was not possible to confirm that the reaction was successful.

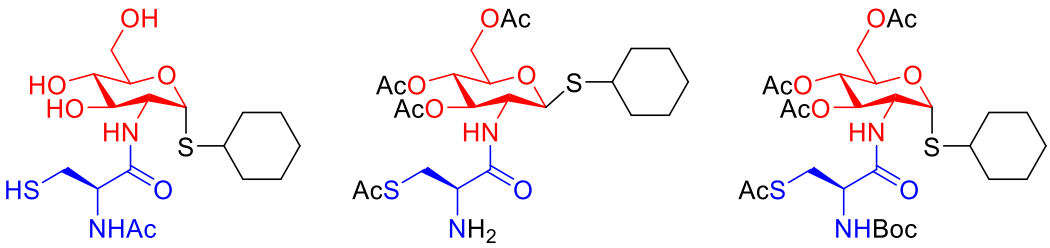


122 **123**

Fig. 55. Synthesis of **123**. Reagents and conditions: (i) CH_3ONa, MeOH.

5.4. Conclusions

The synthesis of the simplified mycothiol scaffold (**78**) proved to be more challenging than expected. The first synthetic route was based on published methodology,[49] but even after extensive optimisation did not lead to the formation of the desired mycothiol analogue (**78**) (Fig. 56). The second synthetic approach that was explored resulted in a formation of the acetyl-protected β-cyclohexyl cysteine mycothiol analogue (**119**) (Fig. 56). Finally, the immediate simplified mycothiol analogue precursor (**122**) (Fig. 56) was successfully synthesised establishing a novel approach towards the synthesis of the cysteine containing mycothiol scaffolds.

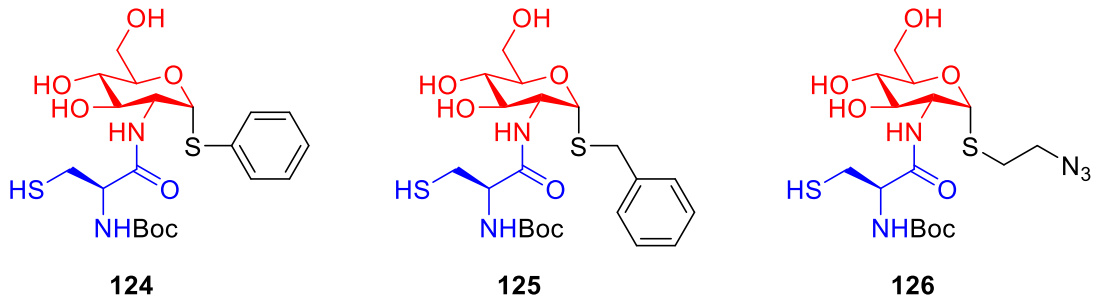


78 **119** **122**

Fig. 56. Structures of **78**, **119** and **122**.

6. Synthesis of other cysteine containing mycothiol analogues

As a novel synthetic strategy towards the synthesis of the cysteine containing mycothiol scaffolds was developed it was decided to synthesise three new mycothiol analogue scaffolds (Fig. 57) to obtain a diverse set of chemical probes to investigate the role of MST. The first two analogues (**124** and **125**) would contain aromatic groups (phenyl and benzyl groups respectively) that are useful chromophores in place of the cyclohexane ring. The phenyl and benzyl α-thioglycosides had been previously shown to act as substrates for MshB,[22,24] so it was expected that the analogues **124** and **125** would bind to MST. The third analogue (**126**) would have a 2-azidoethyl group in place of the cyclohexyl moiety that would enable us to easily modify this side of the compound using 'click chemistry'.



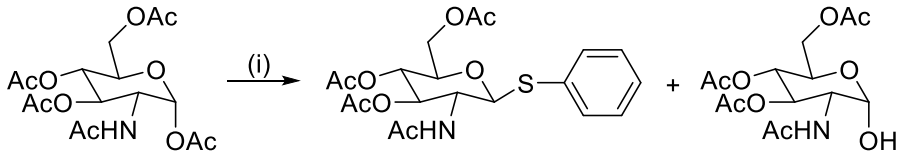
124 **125** **126**

Fig. 57. Structures of the cysteine containing mycothiol analogues (**124**, **125** and **126**).

6.1. The phenyl cysteine mycothiol analogue

In order to synthesise the phenyl cysteine mycothiol analogue (**124**) it was decided to follow the optimized protocol for the synthesis of the cysteine containing mycothiol scaffolds. Previously to obtain the cyclohexyl thioglycoside (**74**) three different synthetic methods were explored. As it is not possible to introduce a phenyl ring *via* S_N2 nucleophilic substitution using the acetamido mercaptan (**73**) and thiophenol as starting materials, it was decided to employ the alternative method where α-D-glucosamine pentaacetate (**96**) was treated with $BF_3{\cdot}OEt_2$ and thiophenol (Fig. 58). When these reaction conditions were used to synthesise the cyclohexyl thioglycoside (**74**) a mixture of α- and β-anomers was obtained, however, in this case only the β-anomer (**127**) was formed. Moreover,

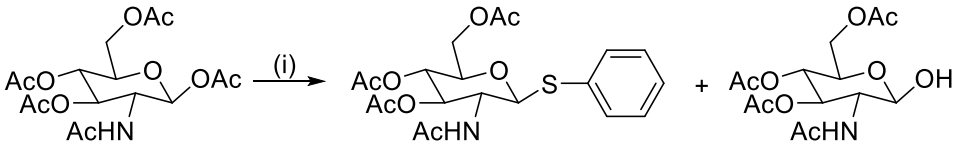
it was not possible to isolate the pure β-product (**127**) using column chromatography (20–100% EtOAc in hexane) as it co-elutes with the side product (**128**).



96 **127** **128**

Fig. 58. Synthesis of the phenyl thioglycoside (**127**). Reagents and conditions: (i) thiophenol, $BF_3 \cdot OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h.

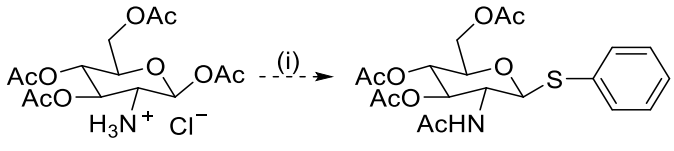
As only the phenyl β-thioglycoside (**127**) was formed when α-D-glucosamine pentaacetate (**96**) was used as a starting material the reaction was repeated using the acetyl-protected β-glucosamine (**71**) instead (Fig. 59). Again, only the β-anomer (**127**) was obtained and as before the product (**127**) co-eluted with the main side product (**129**) even after a second purification by column chromatography (5% MeOH in DCM). Other purification methods (e.g. crystallization) were not explored as the phenyl β-thioglycoside (**127**) was not the target product.



71 **127** **129**

Fig. 59. Synthesis of the phenyl thioglycoside (**127**). Reagents and conditions: (i) thiophenol, $BF_3 \cdot OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h.

In another attempt to synthesise the phenyl α-thioglycoside an acetyl-protected glucosamine (**40**) was used as a starting material (Fig. 60). In this case no product formation was observed and the unreacted starting material was isolated.



40 **127**

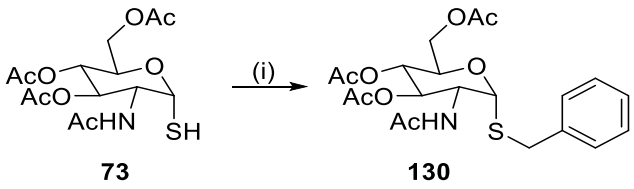
Fig. 60. Synthesis of the phenyl thioglycoside (**127**). Reagents and conditions: (i) thiophenol, $BF_3 \cdot OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h.

Because the synthesis of the phenyl α-thioglycoside proved to be unsuccessful after multiple attempts it was decided to move on to the synthesis of the next

cysteine containing mycothiol scaffold containing the benzyl moiety (**125**).

6.2. The benzyl cysteine mycothiol analogue

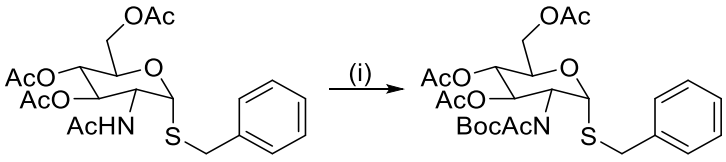
Having the acetamido mercaptan (**73**) in hand, the synthesis of the benzyl thioglycoside (**130**) was attempted. As the benzyl thioglycoside (**130**) is a known compound it was decided to follow the reaction conditions presented by Lamprecht *et al*.[24] to synthesise the target compound (**130**) and not to employ the reaction conditions used to obtain the cyclohexyl thioglycoside (**74**). The acetamido mercaptan (**73**) was dissolved in dichloromethane and treated with benzyl bromide and triethylamine (Fig. 61). The reaction was stirred at room temperature for 20 h to give the phenyl thioglycoside (**130**) in 69% yield.



73 **130**

Fig. 61. Synthesis of **130**. Reagents and conditions: (i) benzyl bromide, Et_3N, CH_2Cl_2, 69%.

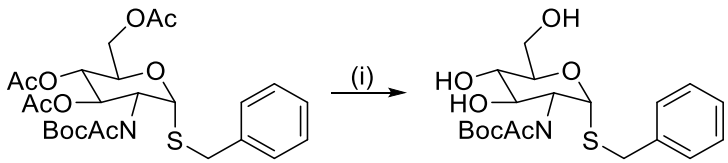
In order to selectively *N*-deacetylate the phenyl thioglycoside (**130**) the Boc group was introduced following the previously optimized reaction conditions. In short, the phenyl thioglycoside (**130**) was treated with DMAP and Boc_2O and stirred at 60 °C for 18 h (Fig. 62). After purification by column chromatography (1% MeOH in DCM) the *N*-Boc benzyl thioglycoside (**131**) was obtained in 88% yield.



130 **131**

Fig. 62. Synthesis of **131**. Reagents and conditions: (i) DMAP, Boc_2O, THF, 60 °C, 88%.

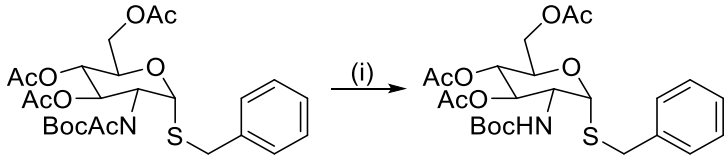
In the next step, hydrazine monohydrate was used at 0 °C to exclusively remove the *N*-acetyl group and give the *N*-Boc protected glucopyranoside (**133**). However, the reaction resulted in a formation of the *O*-deacetylated product instead (**132**) (Fig. 63).



131 132

Fig. 63. Synthesis of **132**. Reagents and conditions: (i) $NH_2NH_2 \cdot H_2O$, MeOH, 0 °C.

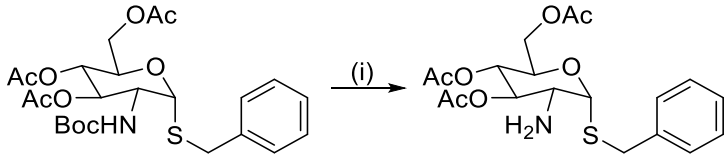
As the use of hydrazine monohydrate did not lead to the isolation of the *N*-Boc protected glucopyranoside (**133**) it was decided to employ milder reaction conditions.[69] The *N*-Boc benzyl thioglycoside (**131**) was dissolved in methanol, treated with *N*,*N*-diethylethylenediamine (DEAEA) and stirred at 0 °C for 1 h to produce the desired *N*-Boc protected glucopyranoside (**133**) in 72% yield (Fig. 64).



131 133

Fig. 64. Synthesis of **133**. Reagents and conditions: (i) DEAEA, MeOH, 0 °C, 72%.

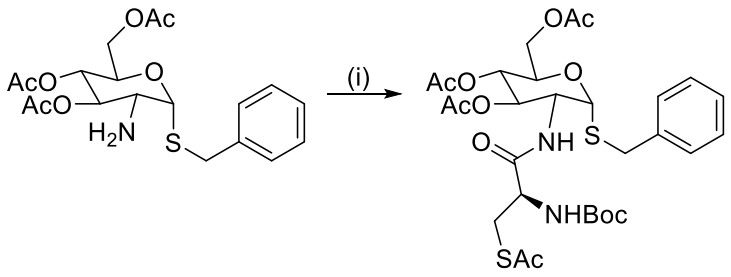
In the following step, the *N*-Boc protected glucopyranoside (**133**) was treated with TMSOTf to remove the Boc group. The reaction was stirred at 0 °C for 2 h to form the free amine (**134**) in 83% yield (Fig. 65).



133 134

Fig. 65. Synthesis of **134**. Reagents and conditions: (i) TMSOTf, CH_2Cl_2, 0 °C, 83%.

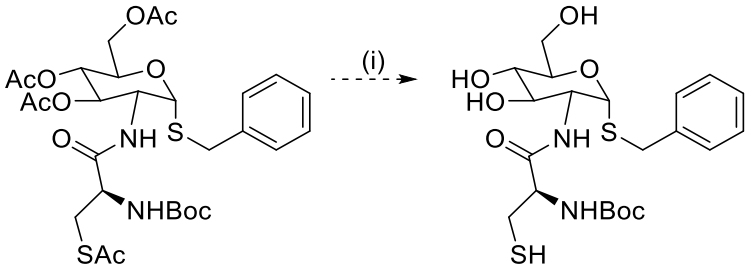
The successful synthesis of the free amine (**134**) made it possible to attempt a coupling reaction leading to the formation of the acetyl-protected benzyl cysteine mycothiol analogue (**135**) (Fig. 66). The same reaction conditions as for the synthesis of the acetyl-protected cyclohexyl cysteine mycothiol analogue (**122**) were employed *i.e.* EDCI was used as a coupling reagent, HOBt as an additive, NMM as a base and THF as a solvent. The benzyl cysteine mycothiol analogue precursor (**135**) was obtained in 9% yield.



134 **135**

Fig. 66. Synthesis of **135**. Reagents and conditions: (i) *N*-Boc-*S*-Ac-Cys (**47**), EDCI, HOBt, NMM, THF, 9%.

As a result of the multi-step synthetic pathway employed only a small amount of the analogue precursor (**135**) was isolated (16 mg). The deacetylation of the amide (**135**) using sodium methoxide in methanol was attempted (Fig. 67). However, there was not enough material to fully characterise the compound produced. Therefore, it was not possible to confirm that the reaction was successful. Time constraints did not allow the repetition of the whole synthetic route in order to produce more product (**135**).

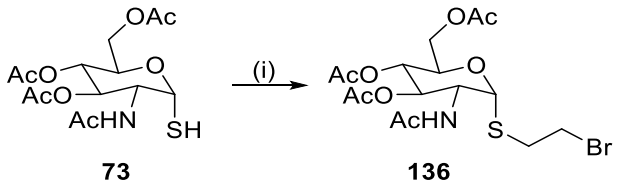


135 **125**

Fig. 67. Synthesis of **125**. Reagents and conditions: (i) CH_3ONa, MeOH.

6.3. The azido cysteine mycothiol analogue

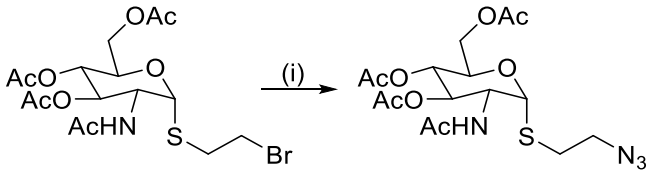
Having successfully synthesised the benzyl cysteine mycothiol analogue precursor (**135**) the synthesis of the azido mycothiol analogue (**126**) was attempted. First, the 2-bromoethyl thioglycoside (**136**) was obtained following the reaction conditions reported by André *et al*.[70] The acetamido mercaptan (**73**) was dissolved in acetone-water mixture and treated with 1,2-dibromoethane and potassium carbonate (Fig. 68). The reaction was stirred at room temperature for 3 h to produce the 2-bromoethyl thioglycoside (**136**) in 62% yield.



73 136

Fig. 68. Synthesis of **136**. Reagents and conditions: (i) 1,2-dibromoethane, K_2CO_3, acetone-water, 62%.

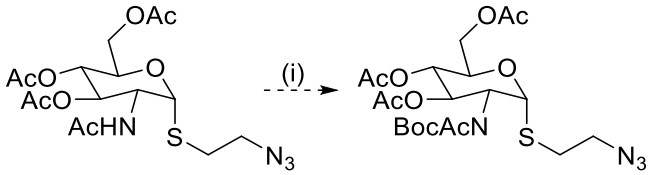
In the next step, the 2-azidoethyl thioglycoside (**137**) was synthesised *via* S_N2 nucleophilic substitution using sodium azide and the 2-bromoethyl thioglycoside (**136**) as starting materials (Fig. 69)[70]. The reaction was heated at 80 °C for 18 h to give the desired product (**137**) in 82% yield.



136 137

Fig. 69. Synthesis of **137**. Reagents and conditions: (i) NaN_3, DMF, tertbutylammonium iodide, 80 °C, 82%.

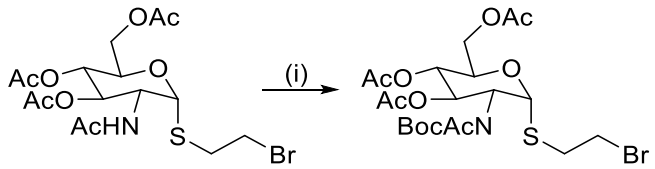
Following the optimized synthetic route, the 2-azidoethyl thioglycoside (**137**) was treated with DMAP and Boc_2O and stirred at 60 °C for 18 h (Fig. 70) in order to introduce the Boc group. However, the formation of the desired compound (**138**) was not observed and the unreacted starting material was isolated.



137 138

Fig. 70. Synthesis of **138**. Reagents and conditions: (i) DMAP, Boc_2O, THF, 60 °C.

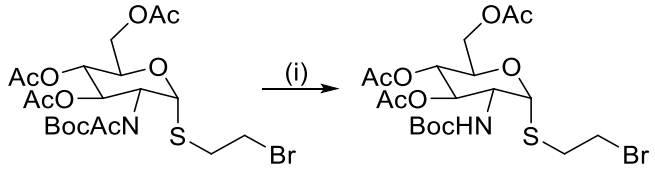
As the Boc group introduction proved to be unsuccessful when the 2-azidoethyl thioglycoside (**137**) was used as a starting material it was decided to repeat the reaction using the 2-bromoethyl thioglycoside (**136**) instead. As previously **136** was treated with DMAP and Boc_2O and stirred at 60 °C for 18 h (Fig. 71). After purification by column chromatography (25-50% EtOAc in hexane) the desired glucopyranoside (**139**) was successfully obtained in 83% yield.



136 **139**

Fig. 71. Synthesis of **139**. Reagents and conditions: (i) DMAP, Boc_2O, THF, 60 °C, 83%.

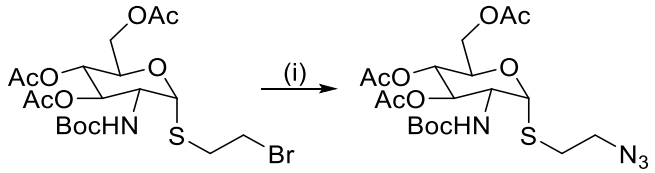
To selectively remove the *N*-acetyl group the intermediate (**139**) was dissolved in methanol, treated with DEAEA and stirred at 0 °C for 1 h (Fig. 72). After column chromatography the desired product (**140**) was isolated in 32% yield.



139 **140**

Fig. 72. Synthesis of **140**. Reagents and conditions: (i) DEAEA, MeOH, 0 °C, 32%.

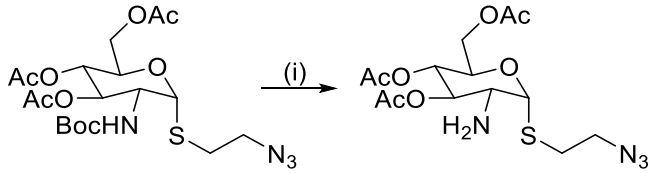
Next, the azido group was introduced using sodium azide[70] (Fig. 73). The reaction was heated at 80 °C for 18 h to give the target compound (**141**) in 65% yield.



140 **141**

Fig. 73. Synthesis of **141**. Reagents and conditions: (i) NaN_3, DMF, tertbutylammonium iodide, 80 °C, 65%.

Having successfully synthesised the *N*-Boc protected 2-azidoethyl glucopyranoside (**141**) the *N*-Boc removal was carried out. **141** was treated with TMSOTf and the reaction was stirred at 0 °C for 2 h to produce the free amine (**142**) in 100% yield (Fig. 74).

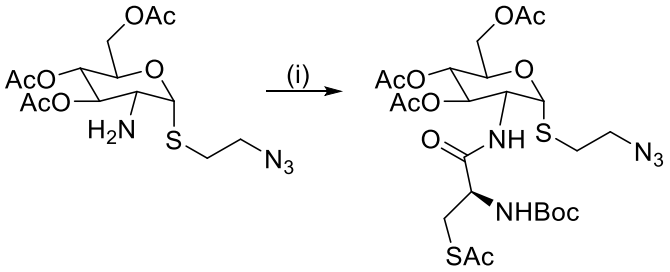


141 **142**

Fig. 74. Synthesis of **142**. Reagents and conditions: (i) TMSOTf, CH_2Cl_2, 0 °C, 100%.

With the free amine (**142**) in hand, it was decided to attempt the synthesis of the

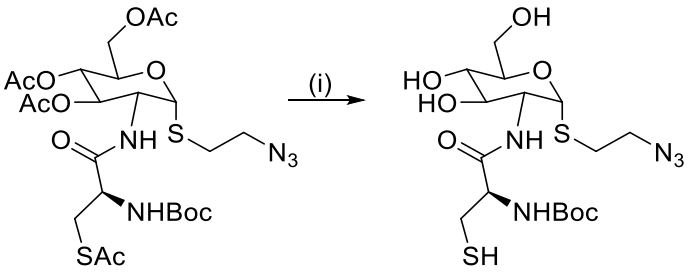
acetyl-protected azido cysteine mycothiol analogue (**143**) following the coupling reaction conditions previously applied to synthesise the cysteine containing mycothiol analogue precursors (**122** and **135**). As before EDCI was used as a coupling reagent and HOBt as an additive, however, in this reaction DIPEA was used in place of NMM and DMF was used as the solvent (Fig. 75). After column chromatography the desired azido cysteine mycothiol analogue precursor (**143**) was isolated in 17% yield.



142 **143**

Fig. 75. Synthesis of **143**. Reagents and conditions: (i) *N*-Boc-*S*-Ac-Cys (**47**), EDCI, HOBt, DIPEA, DMF, 17%.

Finally, the azido cysteine mycothiol analogue precursor (**143**) was deacetylated using sodium methoxide in methanol to give the azido cysteine mycothiol analogue (**126**) in 11% yield (Fig. 76).



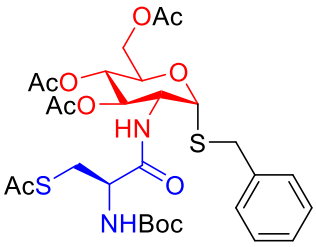
143 **126**

Fig. 76. Synthesis of **126**. Reagents and conditions: (i) CH_3ONa, MeOH, 11%.

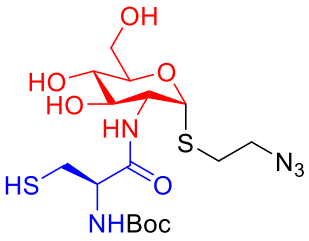
6.4. Conclusions

Three novel cysteine containing mycothiol scaffolds (**124**, **125** and **126**) were designed and their synthesis was attempted following the newly developed synthetic strategy. The synthesis of the phenyl cysteine mycothiol analogue (**124**) was unsuccessful because the phenyl α-thioglycoside, which is the key intermediate in the synthetic pathway, was not obtained after several attempts. The benzyl cysteine mycothiol analogue precursor (**135**) (Fig. 77) was successfully synthesised. The unique benzyl cysteine analogue precursor (**135**)

could be further developed into chemical probes that could be used to investigate the role of MST. Finally, the first azido cysteine mycothiol analogue (**126**) (Fig. 77) was successfully produced and it could be used as a scaffold for the synthesis of various analogues *via* 'click chemistry'.



135



126

Fig. 77. Structures of **135** and **126**.

7. Synthesis of the non-cysteine mycothiol analogues

In order to explore the use of kinetic target guided synthesis (kTGS) in the synthesis of MST inhibitors the azido derivatives of simplified mycothiol analogues were needed. The analogues would act as a handle within the binding site and would allow us to explore the cysteine-binding site of mycothiol-*S*-transferase. During kTGS the azido derivatives would be incubated with an alkyne fragment library in the presence of MST in order to find triazole containing hits. In this way new inhibitors containing a triazole moiety would be synthesised. Two types of derivative were designed: the azido mycothiol analogues and the azidoacetamido mycothiol analogues.

7.1. The azido mycothiol analogues

The first set of the azido derivatives was based on 2-azido-2-deoxy-1-thio-α-D-glucopyranose. Three analogues having the cyclohexyl (**144**), phenyl (**145**) and benzyl groups (**146**) (Fig. 78.) were designed as those moieties had been reported to be good replacements for the inositol ring in the mycothiol structure.[11,22,24]

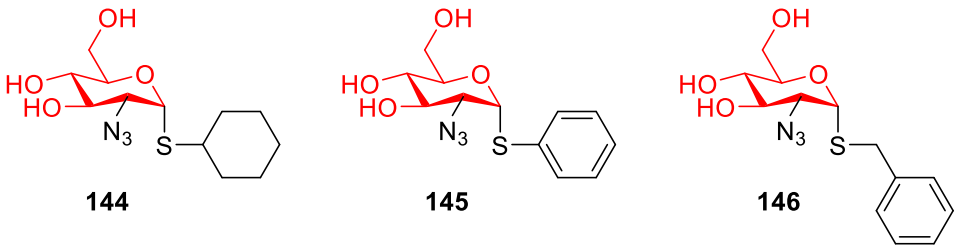
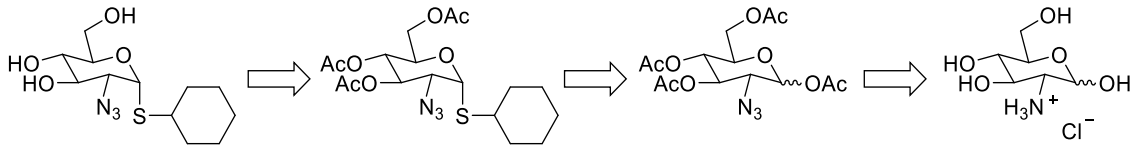


Fig. 78. Structures of the azido mycothiol analogues (**144**, **145** and **146**).

7.1.1. The cyclohexyl azido mycothiol analogue

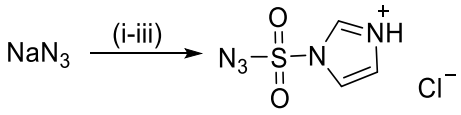
In order to obtain the novel cyclohexyl azido mycothiol analogue (**144**) the following synthetic strategy inspired by the work of Gammon *et al.*[22] was explored (Fig. 79). First, the acetyl-protected 2-azido glucopyranose (**148**) would be generated from glucosamine HCl (**107**). Then **148** would be treated with cyclohexanethiol and $BF_3 \cdot OEt_2$ to give the desired azido analogue (**144**) after global deprotection.



144 147 148 107

Fig. 79. Retrosynthesis of the cyclohexyl azido mycothiol analogue (**144**).

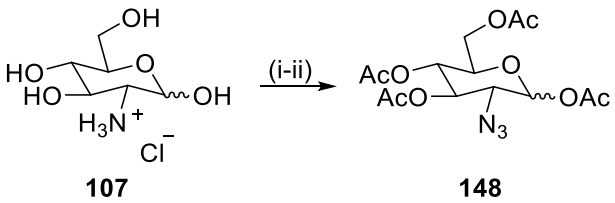
To synthesise the acetyl-protected 2-azido glucopyranose (**148**) using glucosamine HCl (**107**) as a starting material, a diazotransfer reagent (**149**) was required. **149** was synthesised in one pot over three steps according to the methodology published by Goddard-Borger and Stick[71] (Fig. 80). First, sodium azide in acetonitrile was treated with sulfuryl chloride and the reaction mixture was stirred at room temperature overnight. Next, imidazole was added and the reaction was stirred for further 3 h. Finally, a solution of acetyl chloride in ethanol was added and the resulting precipitate was filtered to give imidazole-1-sulfonyl azide HCl (**149**) in 62% yield.



149

Fig. 80. Synthesis of imidazole-1-sulfonyl azide HCl (**149**). Reagents and conditions: (i) SO_2Cl_2, MeCN, (ii) imidazole, (iii) acetyl chloride, EtOH, 62%.

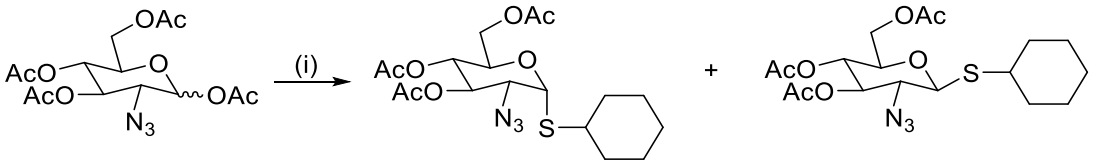
With imidazole-1-sulfonyl azide HCl (**149**) in hand, synthesis of the acetyl-protected 2-azido glucopyranose (**148**) was attempted following the reaction conditions proposed by Goddard-Borger and Stick[71] (Fig. 81). The glucosamine HCl (**107**) was dissolved in methanol and treated with $CuSO_4 \cdot 5H_2O$, potassium carbonate and imidazole-1-sulfonyl azide HCl (**149**). The reaction mixture was stirred at room temperature for 4 h, concentrated and dissolved in pyridine. Then, acetic anhydride was added and the reaction mixture was stirred for another 4 h. After purification by column chromatography (3:1 hexane/EtOAc) the desired azide (**148**) was isolated in 77% yield.



107 148

Fig. 81. Synthesis of **148**. Reagents and conditions: (i) imidazole-1-sulfonyl azide HCl (**149**), K_2CO_3, $CuSO_4 \cdot 5H_2O$, MeOH; (ii) acetic anhydride, pyridine, 77% (over two steps).

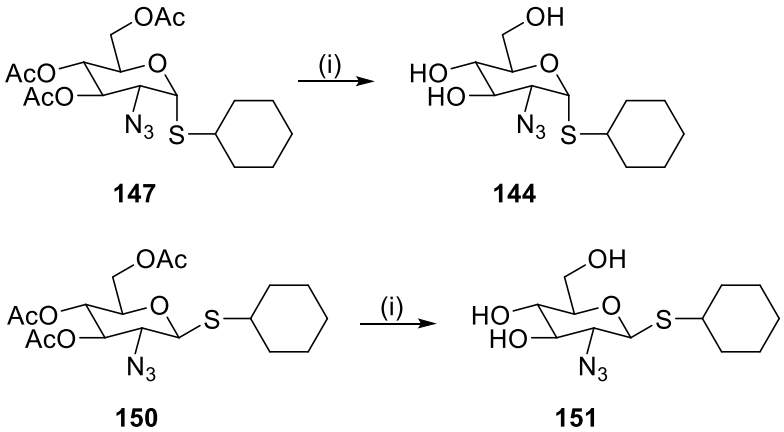
In the next step, the acetyl-protected 2-azido glucopyranose (**148**) was treated with $BF_3 \cdot OEt_2$ and cyclohexanethiol to give a mixture of α- and β-anomers (Fig. 82). The anomers **147** (yield = 5%) and **150** (yield = 10%) were readily separated by column chromatography (3:1 hexane/EtOAc). The yields obtained were modest, however, there was enough material to carry out the final step in the synthetic pathway.



148 147 150

Fig. 82. Synthesis of **147** and **150**. Reagents and conditions: (i) cyclohexanethiol, $BF_3 \cdot OEt_2$, DCM, 0–55 °C, 5 h, **147** 5%, **150** 10%.

In the last step the α- (**147**) and β- (**150**) anomers were separately deacetylated using CH_3ONa in MeOH to successfully produce the novel cyclohexyl azido mycothiol analogue (**144**) (yield = 64%) and its β-anomer (**151**) (yield = 100%) (Fig. 83). **151** could also be tested as a potential MST substrate to see if there is a difference in the binding affinity between the anomers.



147 144

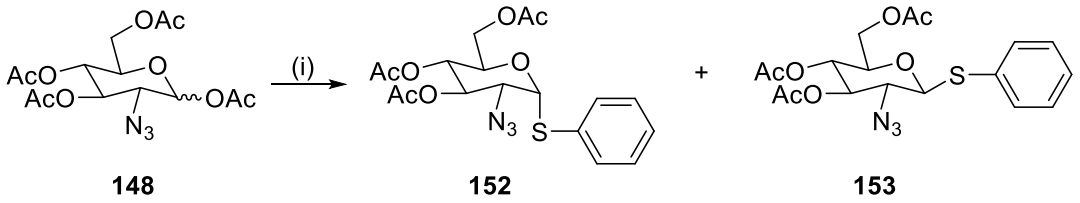
150 151

Fig. 83. Synthesis of **144** and **151**. Reagents and conditions: (i) CH_3ONa, MeOH, **144** 64%, **151** 100%.

7.1.2. The phenyl azido mycothiol analogue

As the cyclohexyl azido mycothiol analogue (**144**) was successfully obtained it was decided to follow the same synthetic strategy to obtain the phenyl azido mycothiol analogue (**145**).

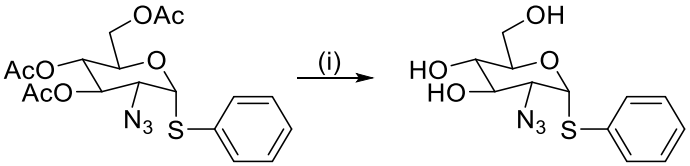
With the acetyl-protected 2-azido glucopyranose (**148**) in hand the synthesis of the acetyl-protected phenyl azido mycothiol analogue (**152**) was carried out. **148** was treated with $BF_3{\cdot}OEt_2$ and thiophenol to give a 3:1 α/β mixture of anomers (Fig. 84). The separation of the anomers by column chromatography was attempted to no avail. Gratifyingly, pure crystals of the acetyl-protected phenyl azido mycothiol analogue (α anomer, **152**) were obtained by crystallization from ethanol (yield = 21%).[22] Unlike previously, the β-anomer (**153**) was not isolated in a pure form from the reaction mixture.



148 **152** **153**

Fig. 84. Synthesis of **152**. Reagents and conditions: (i) thiophenol, $BF_3{\cdot}OEt_2$, DCM, 0–55 °C, 5 h, 21%.

Finally, the acetyl-protected phenyl azido mycothiol analogue (**152**) was globally deprotected using CH_3ONa in MeOH to produce the phenyl azido mycothiol analogue (**145**) in 100% yield (Fig. 85.).



152 **145**

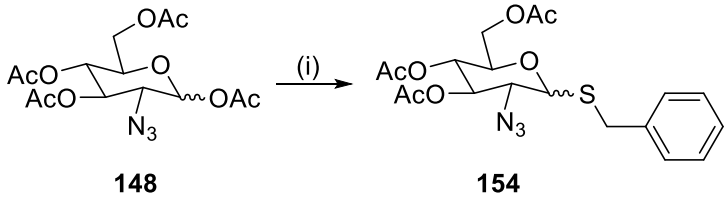
Fig. 85. Synthesis of **145**. Reagents and conditions: (i) CH_3ONa, MeOH, 100%.

7.1.3. The benzyl azido mycothiol analogue

Having successfully synthesised the cyclohexyl (**144**) and the phenyl azido mycothiol analogue (**145**) the synthesis of the benzyl azido mycothiol analogue (**146**) was attempted.

As before, the acetyl-protected 2-azido glucopyranose (**148**) was treated with $BF_3{\cdot}OEt_2$ and benzyl mercaptan to give 1:1 α/β mixture of anomers (**154**) that was inseparable by column chromatography (Fig. 86). After numerous

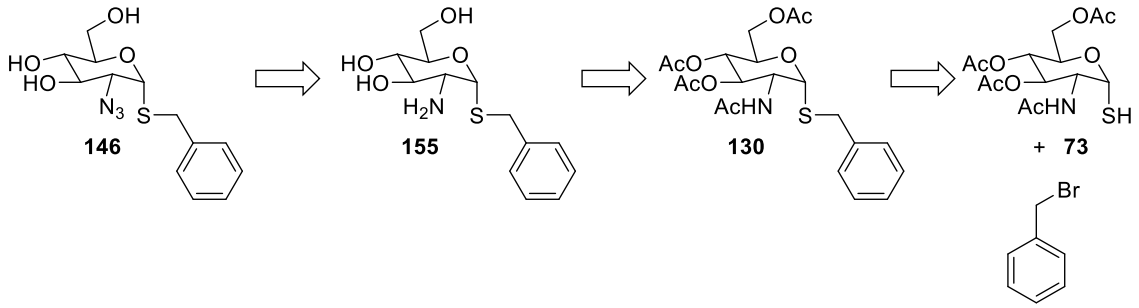
crystallization attempts the pure α-anomer of **154** was not isolated.



148 **154**

Fig. 86. Synthesis of **154**. Reagents and conditions: (i) benzyl mercaptan, $BF_3 \cdot OEt_2$, DCM, 0–55 °C, 5 h, 53%.

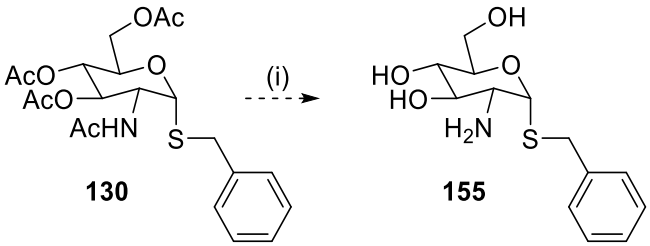
As the previously applied methodology towards the synthesis of the azido mycothiol analogues proved to be unsuccessful in this case, a new synthetic strategy was designed (Fig. 87). The plan involved global deprotection of the benzyl thioglycoside (**130**) producing the benzyl aminotriol (**155**). Then, the amino group could be replaced by an azido group forming the benzyl azido mycothiol analogue (**146**).



146 **155** **130** + **73**

Fig. 87. Retrosynthesis of the benzyl azido mycothiol analogue (**146**).

The benzyl thioglycoside (**130**) was treated with hydrazine monohydrate and heated at 70 °C for 22 h (Fig. 88). However, the desired the benzyl aminotriol (**155**) was not isolated. Most probably, only the *O*-acetyl groups were removed and the *N*-acetyl group remained intact thus not forming the free amine (**155**). As **155** was not obtained it was not possible to proceed to the next step.



130 **155**

Fig. 88. Synthesis of **155**. Reagents and conditions: (i) $NH_2NH_2 \cdot H_2O$, 70 °C, 22 h.

Because the benzyl azido mycothiol analogue (**146**) was not obtained after employing two different synthetic strategies it was decided to move on to the synthesis of the azidoacetamido mycothiol analogues.

7.1.4. Conclusions

To enable the synthesis of the MST inhibitors *via* kinetic target guided synthesis (kTGS) three different azido mycothiol analogues were designed (**144**, **145** and **146**). A short and easy synthetic strategy was used to successfully obtain not only the novel cyclohexyl azido mycothiol analogue (**144**) but also its β-anomer (**151**) (Fig. 89). Following the same synthetic route, the phenyl azido mycothiol analogue (**145**) (Fig. 89) was synthesized from the crystalline **152**. The synthesis of the benzyl azido mycothiol analogue (**146**) proved to be more challenging as an inseparable mixture of anomers of the precursor (**154**) was formed. An alternative synthetic pathway employed did not result in the formation of the benzyl azido mycothiol analogue (**146**) as the synthesis of its precursor (**155**) failed.

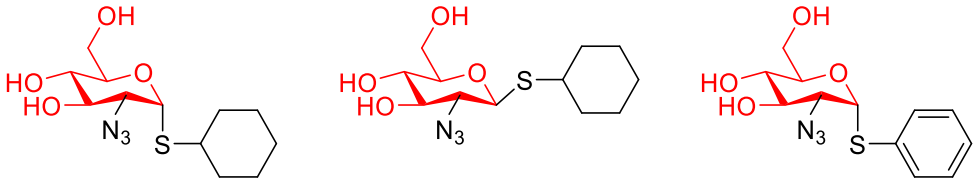
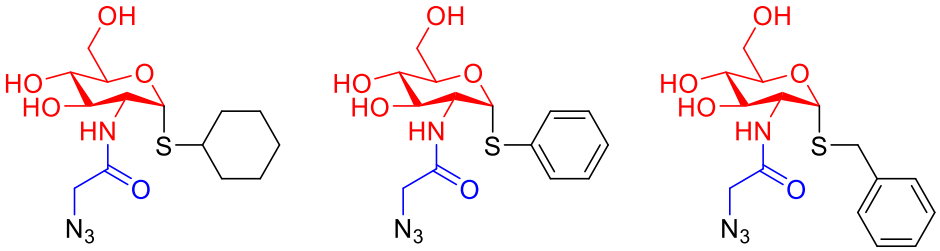


Fig. 89. Structures of **144**, **151** and **145**.

7.2. The azidoacetamido mycothiol analogues

The second set of the azido derivatives was based on 2-azidoacetamido-2-deoxy-1-thio-α-D-glucopyranose. Once again three analogues having the cyclohexyl (**156**), phenyl (**157**) and benzyl groups (**158**) (Fig. 90.) were designed and their synthesis was attempted. The 2-azidoacetamido group was introduced to mimic the cysteine moiety in the mycothiol structure and would also allow the use of the analogues (**156**, **157** and **158**) in the kinetic target guided synthesis.

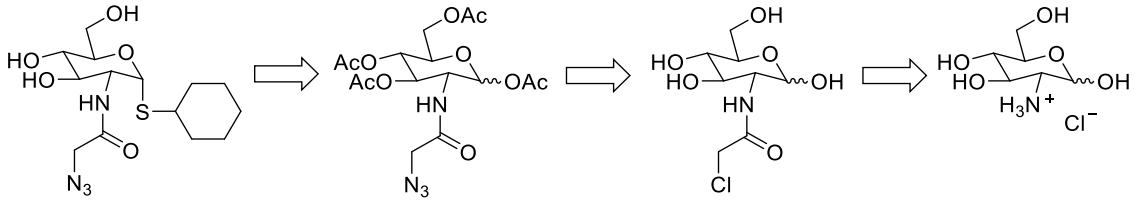


156 **157** **158**

Fig. 90. Structures of the azidoacetamido mycothiol analogues (**156**, **157** and **158**).

7.2.1. The cyclohexyl azidoacetamido mycothiol analogue

In order to form the cyclohexyl azidoacetamido mycothiol analogue (**156**) a synthetic route based on the work presented by Hang *et al.*[72] was designed (Fig. 91). Hang *et al.* described the three-step synthesis of 2-azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-galactopyranose using D-galactosamine hydrochloride as a starting material.[72] It was decided to employ the same reaction conditions to obtain the acetyl-protected 2-azidoacetamido glucopyranose (**159**) starting with glucosamine HCl (**107**). Then **159** could be treated with cyclohexanethiol and $BF_3 \cdot OEt_2$ to produce the desired azidoacetamido analogue (**156**) after global deprotection.

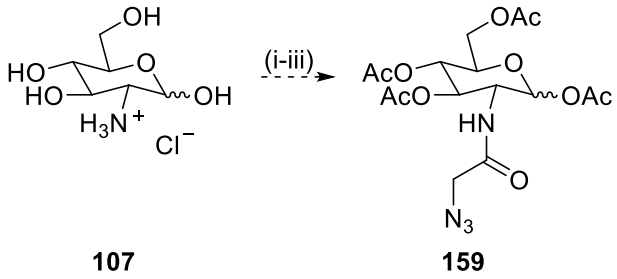


156 **159** **160** **107**

Fig. 91. Retrosynthesis of the cyclohexyl azidoacetamido mycothiol analogue (**156**).

To obtain the key intermediate (**159**), glucosamine HCl (**107**) was treated with sodium methoxide solution in methanol to form a free amine to which chloroacetic anhydride and triethylamine were added and the reaction was stirred at room temperature for 16 h (Fig. 92.). The reaction was passed through a silica plug and the crude material was carried through to the next step. The residue was dissolved in DMF, sodium azide was added and the reaction was stirred at 80 °C for 2 h (Fig. 92.). The reaction mixture was concentrated and dissolved in pyridine. Then acetic anhydride and DMAP were added at 0 °C and the reaction was stirred at room temperature for 2 h (Fig. 92.). As a complex mixture of

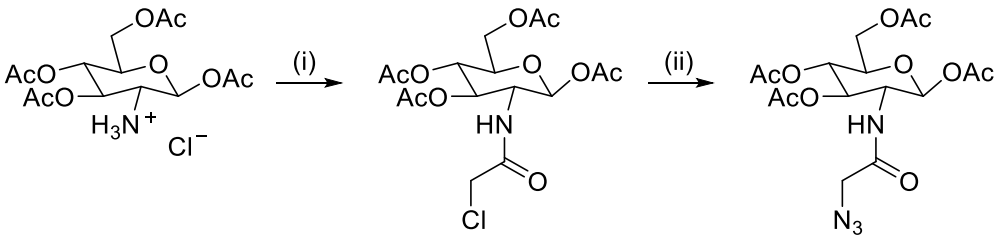
products was formed purification by silica gel column chromatography did not lead to the isolation of pure acetyl-protected 2-azidoacetamido glucopyranose (**159**).



107 **159**

Fig. 92. Synthesis of **159**. Reagents and conditions: (i) chloroacetic anhydride, triethylamine, MeOH; (ii) NaN_3, DMF, 80 °C, 2 h; (iii) acetic anhydride, DMAP, pyridine.

As the previously explored synthetic strategy towards the synthesis of the key intermediate (**159**) proved to be unsuccessful a different approach was taken (Fig. 93). Here, the acetyl-protected glucosamine (**40**) was used as a starting material instead of glucosamine HCl (**107**) to make the purification of the intermediate product (**161**) easier. The previously used reaction conditions were slightly optimized. The acetyl-protected glucosamine (**40**) was dissolved in dry DCM, treated with chloroacetic anhydride and triethylamine and stirred at 0 °C for 1 h. After work up the desired acetyl-protected 2-chloroacetamido glucopyranose (**161**) was isolated in 81% yield. Having successfully formed **161**, the synthesis of the key intermediate (**162**) was attempted (Fig. 93). As before, the acetyl-protected 2-chloroacetamido glucopyranose (**161**) was dissolved in DMF, sodium azide was added and the reaction was stirred at 80 °C for 2 h. The acetyl-protected 2-azidoacetamido glucopyranose (**162**) was successfully obtained in 59% yield.

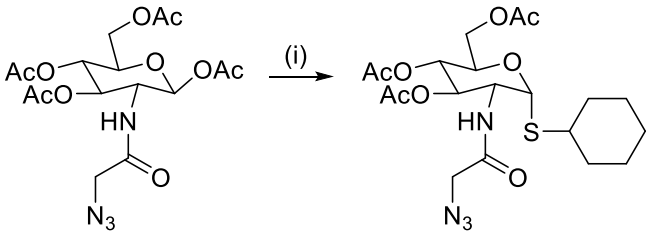


40 **161** **162**

Fig. 93. Synthesis of **162**. Reagents and conditions: (i) chloroacetic anhydride, triethylamine, DCM, 0 °C, 1 h, 81%; (ii) NaN_3, DMF, 80 °C, 2 h, 59%.

Having the key intermediate (**162**) in hand, the synthesis of the cyclohexyl

azidoacetamido mycothiol analogue (**156**) was attempted. First, the acetyl-protected 2-azidoacetamido glucopyranose (**162**) was treated with $BF_3 \cdot OEt_2$ and cyclohexanethiol and the reaction was stirred at 55 °C for 3 h (Fig. 94). Gratifyingly, only the α-anomer (**163**) was formed. The cyclohexyl azidoacetamido mycothiol analogue precursor (**163**) was successfully obtained after purification by column chromatography (20-65% EtOAc in hexane) in 18% yield. As only small amount of the precursor (**163**) was isolated (10 mg) the deacetylation was not attempted but the reaction was repeated on a bigger scale. Surprisingly, when the reaction was scaled up (450 mg *c.f.* 50 mg) a 2:3 α/β mixture of anomers was formed. The mixture was inseparable by silica gel column chromatography.



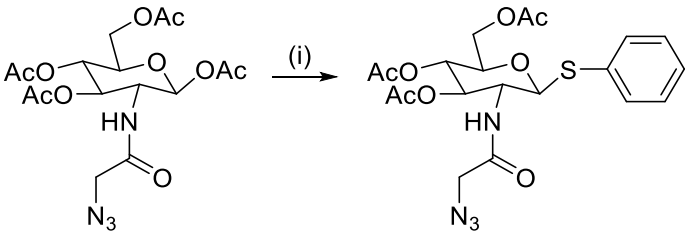
162 **163**

Fig. 94. Synthesis of **163**. Reagents and conditions: (i) cyclohexanethiol, $BF_3 \cdot OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h, 18%.

7.2.2. The phenyl azidoacetamido mycothiol analogue

To obtain the phenyl azidoacetamido mycothiol analogue (**157**) the same synthetic route as for the synthesis of the cyclohexyl azidoacetamido mycothiol analogue (**156**) was employed.

The key intermediate (**162**) was treated with $BF_3 \cdot OEt_2$ and thiophenol to give only the β-anomer (**164**) (Fig. 95). The acetyl-protected phenyl 2-azidoacetamido β-thioglucopyranoside (**164**) was isolated in 45% yield after purification by column chromatography.



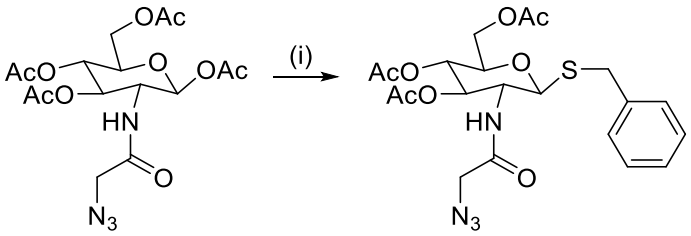
162 **164**

Fig. 95. Synthesis of **164**. Reagents and conditions: (i) thiophenol, $BF_3 \cdot OEt_2$, DCM, 0–55 °C, 3 h, 45%.

As previous attempts to synthesise the phenyl α-thioglycoside using α-D-glucosamine pentaacetate (**96**), β-D-glucosamine pentaacetate (**71**) and the acetyl-protected glucosamine (**40**) as starting materials failed, no other synthetic strategies towards the synthesis of the phenyl azidoacetamido mycothiol analogue (**157**) were explored.

7.2.3. The benzyl azidoacetamido mycothiol analogue

The synthesis of the phenyl azidoacetamido mycothiol analogue (**157**) proved to be unsuccessful, however, it was decided to try to obtain the benzyl azidoacetamido mycothiol analogue (**158**) following the same synthetic pathway. The acetyl-protected 2-azidoacetamido glucopyranose (**162**) was treated with $BF_3{\cdot}OEt_2$ and benzyl mercaptan and the reaction was stirred at 55 °C for 3 h (Fig. 96). As before, only the β-anomer (**165**) was formed. Purification by silica gel column chromatography gave the acetyl-protected benzyl 2-azidoacetamido β-thioglucopyranoside (**165**) in 52% yield.



162 **165**

Fig. 96. Synthesis of **165**. Reagents and conditions: (i) benzyl mercaptan, $BF_3{\cdot}OEt_2$, 1,2-dichloroethane, 0–55 °C, 3 h, 52%.

Because the previously applied synthetic strategy did not lead to the formation of the desired analogue (**158**) a new approach was taken (Fig. 97). Here, it was decided to use the amine (**134**) as a starting material to be sure that only the α-anomer would be formed. Then, the 2-chloroacetamido group was successfully introduced following the previously described reaction conditions to form the acetyl-protected 2-chloroacetamido benzyl thioglucopyranoside (**166**) in 72% yield. Finally, **166** was treated with sodium azide and the reaction was stirred at 80 °C for 2 h. After work up, the benzyl azidoacetamido mycothiol analogue precursor (**167**) was isolated in 82% yield.

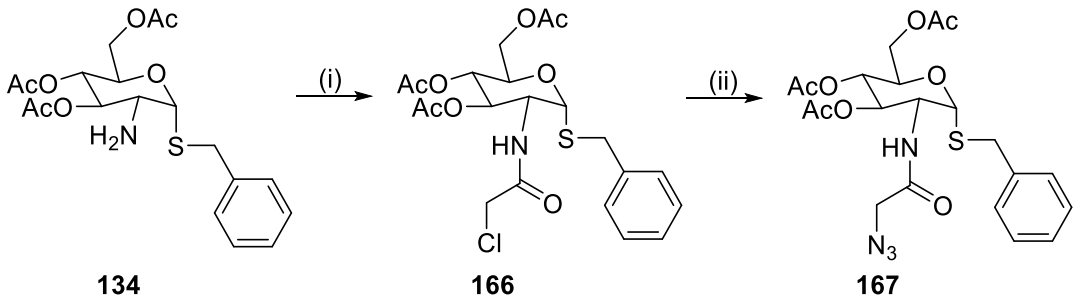


Fig. 97. Synthesis of **167**. Reagents and conditions: (i) chloroacetic anhydride, triethylamine, DCM, 0 °C, 1 h, 72%; (ii) NaN_3, DMF, 80 °C, 2 h, 82%.

The last step in the synthetic pathway towards the formation of the benzyl azidoacetamido mycothiol analogue (**158**) was planned to be the deacetylation of the precursor (**167**) using sodium methoxide in methanol (Fig. 98). However, this reaction was not attempted. As it takes 8 steps to obtain the benzyl azidoacetamido mycothiol analogue precursor (**167**) only a small amount of the compound (**167**) was isolated (90 mg) and it was decided to use it to synthesise a few benzyl azidoacetamido mycothiol analogue-based triazoles.

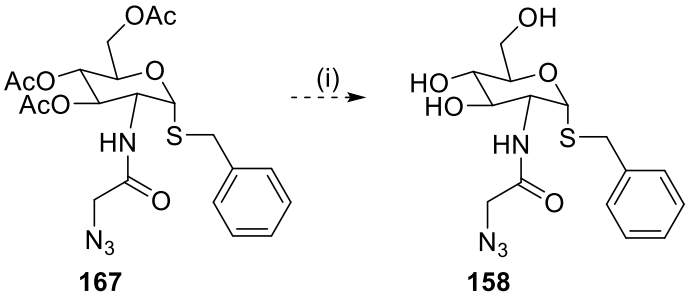
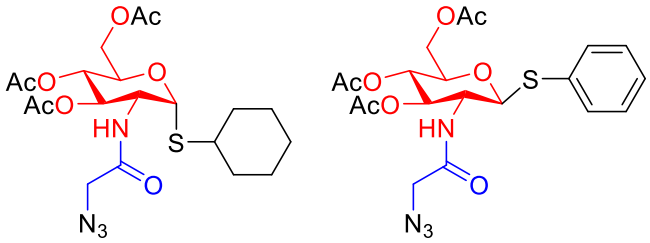


Fig. 98. Synthesis of **158**. Reagents and conditions: (i) CH_3ONa, MeOH.

7.2.4. Conclusions

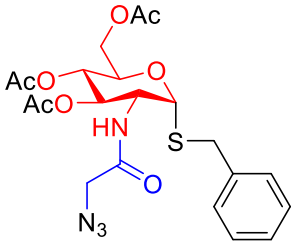
As a result of the synthetic efforts towards the synthesis of the azidoacetamido mycothiol analogues (**156**, **157** and **158**) four novel mycothiol analogue precursors were formed (**163**, **164**, **165** and **167**) (Fig. 99). The cyclohexyl azidoacetamido mycothiol analogue precursor (**163**) was successfully obtained but only when the reaction was performed on a small scale (50 mg), so there is scope for further optimization. The synthesis of the phenyl azidoacetamido mycothiol analogue (**157**) was unsuccessful as only the β-precursor (**164**) was formed, which could be deprotected and tested alongside potential MST substrates as a negative control. In the case of the benzyl azidoacetamido analogue (**158**) synthesis, both pure α- (**167**) and β- (**165**) precursors were isolated when two different synthetic approaches were employed. Both anomers

(**165** and **167**) could be deprotected and their binding affinity to MST could be measured.

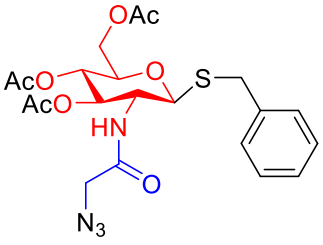


163

164



167



165

Fig. 99. Structures of **163**, **164**, **165** and **167**.

8. Synthesis of the triazole containing mycothiol analogues

Having successfully synthesised two α-azido mycothiol analogues (**144** and **145**) and two α-azidoacetamido mycothiol analogue precursors (**163** and **167**) it was decided to construct a small triazole library. The triazole containing mycothiol analogues (**168** and **169**) (Fig. 100) could be used as standards when kTGS is performed or be directly tested as substrates for mycothiol-*S*-transferase. One analogue of each class was chosen as a model compound for the synthesis of the triazoles (Fig. 100). The phenyl azido mycothiol analogue (**145**) was selected over the cyclohexyl analogue (**144**) as it was produced in higher yields. The same reason stood behind the selection of the benzyl azidoacetamido analogue precursor (**167**).

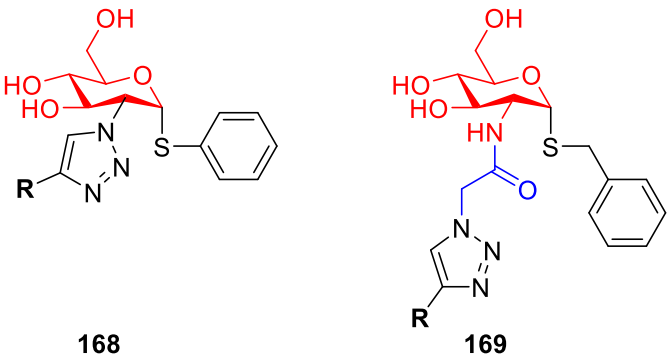
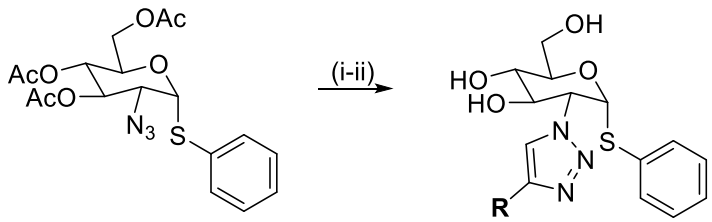


Fig. 100. Structures of the triazole containing mycothiol analogues (**168** and **169**).

8.1. The phenyl azido mycothiol analogue-based triazoles

In order to produce a small library of the phenyl azido mycothiol analogue-based triazoles (**168**) the following synthetic strategy was applied. The acetyl-protected phenyl azido mycothiol analogue (**152**) was used in place of the phenyl azido mycothiol analogue (**145**) to make the purification of the triazole products easier. Following the reaction conditions presented by André *et al*.,[70] the analogue precursor (**152**) was dissolved in a THF/water mixture and treated with an alkyne, copper sulphate pentahydrate and sodium ascorbate (Fig. 101). The reaction was stirred at room temperature for 18 h to give an acetyl-protected triazole that was subsequently deprotected using sodium methoxide in methanol. The phenyl azido mycothiol analogue-based triazole (**168**) was isolated after column chromatography.

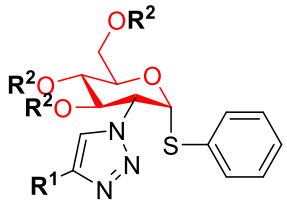


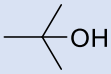
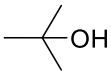
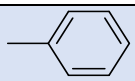
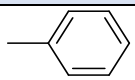
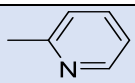
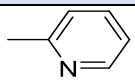
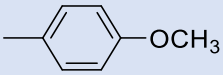
152 **168**

Fig. 101. Synthesis of the phenyl azido mycothiol analogue-based triazoles (**168**). Reagents and conditions: (i) alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/H_2O; (ii) CH_3ONa, MeOH.

To obtain a diverse triazole library alkynes with both aliphatic and aromatic moieties were selected (Table 6). The acetyl-protected triazoles with aliphatic 4-substituents (**170**, **172**, **174**) were easily purified, in most cases the standard work-up was sufficient to isolate the pure compounds (**170**, **172**, **174**) in good yields (57 – 100%) (Table 6). The acetyl-protected triazoles with aromatic 4-substituents (**176**, **178**, **180**) were more difficult to purify and silica gel chromatography was required in each case. The purification of the acetyl-protected 4-methoxyphenyl triazole (**180**) proved to be the most challenging leading to the isolation of the pure product (**180**) in only 13% yield (Table 6). In this case, there was not enough material (**180**) to run the deacetylation reaction. The deprotection of the acetyl-protected triazoles (**170**, **172**, **174**, **176**, **178**) resulted in the formation of the triazoles with aliphatic 4-substituents (**171**, **173**, **175**) in good yields (44 – 67%) and the triazoles with aromatic 4-substituents (**177** and **179**) in low yields (26 and 33% respectively) (Table 6).

Table 6. The phenyl azido mycothiol analogue-based triazoles.

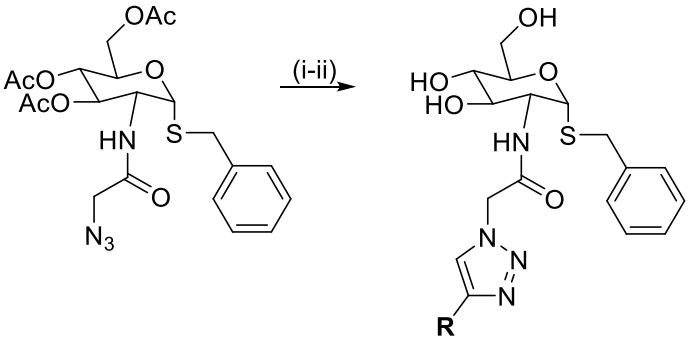


Compound	R^1	R^2	Yield [%]
170		Ac	100
171		H	60
172		Ac	83
173		H	44
174		Ac	57
175		H	67
176		Ac	67
177		H	26
178		Ac	65
179		H	33
180		Ac	13

8.2. The benzyl azidoacetamido mycothiol analogue-based triazoles

As a small library of the phenyl azido mycothiol analogue-based triazoles (**168**) was successfully synthesised it was decided to follow the same synthetic strategy in order to obtain a few benzyl azidoacetamido mycothiol analogue-based triazoles (**169**). In short, the acetyl-protected benzyl azidoacetamido mycothiol analogue (**167**) was treated with an alkyne, copper sulphate pentahydrate and

sodium ascorbate to produce an acetyl-protected triazole that was deacetylated using sodium methoxide in methanol to give a desired triazole (**169**) (Fig. 102).[70]

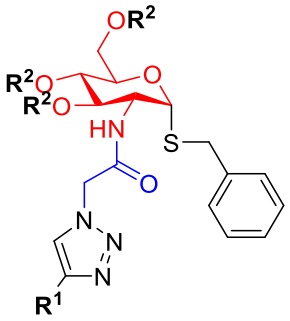


167 **169**

Fig. 102. Synthesis of the benzyl azidoacetamido mycothiol analogue-based triazoles (**169**). Reagents and conditions: (i) alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/H_2O; (ii) CH_3ONa, MeOH.

Because the synthetic pathway towards the formation of the analogue precursor (**167**) is long and the yields are variable there was enough material to carry out only three 'click reactions' (Table 7). One triazole with aliphatic 4-substituent (**182**) and one triazole with aromatic 4-substituent (**184**) were successfully obtained. The synthesis of the acetyl-protected triazole having the benzyl moiety (**185**) was unsuccessful as the purification by silica gel chromatography did not lead to the isolation of the pure product (**185**).

Table 7. The benzyl azidoacetamido mycothiol analogue-based triazoles.



Compound	R^1	R^2	Yield [%]
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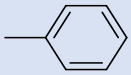
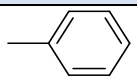
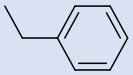
182		H	26
183		Ac	47
184		H	32
185		Ac	-

8.3. Conclusions

A small triazole library based on the phenyl azido mycothiol analogue (**145**) and the benzyl azidoacetamido mycothiol analogue (**158**) was successfully synthesised. Three novel azido triazoles with aliphatic 4-substituents (**171**, **173**, **175**) and one azidoacetamido triazole (**182**) as well as two novel azido triazoles with aromatic 4-substituents (**177** and **179**) and one azidoacetamido triazole (**184**) were obtained using 'click chemistry' conditions (Fig. 103). As both the triazoles with aliphatic 4-substituents and the triazoles with aromatic 4-substituents were formed it could be assumed that many other triazoles could be synthesised using a range of different alkynes following the optimized synthetic pathway if needed. Thus, this provides an easy way to produce a diverse library of potential MST substrates.

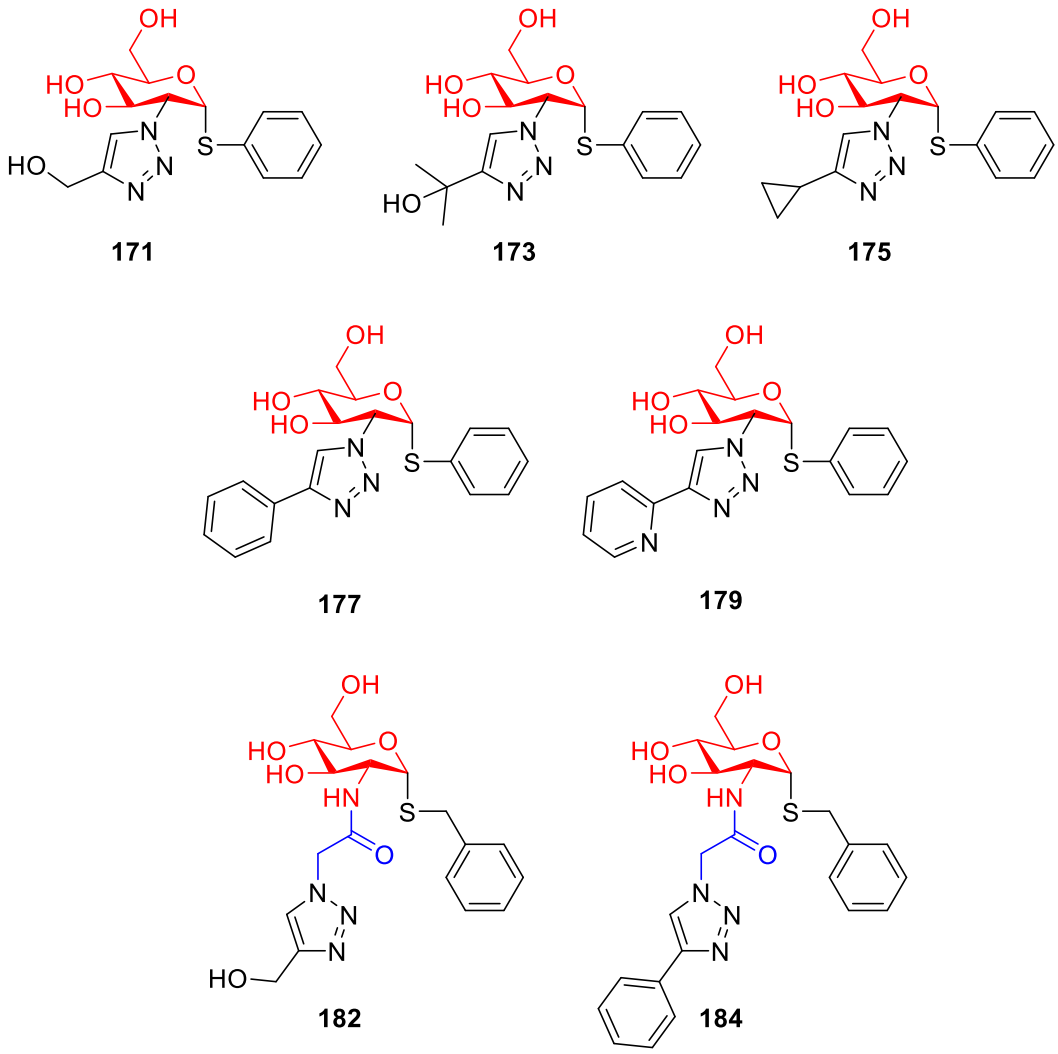


Fig. 103. Structures of the triazole containing mycothiol analogues (**171**, **173**, **175**, **177**, **179**, **182**, **184**).

9. Summary

Two approaches were explored to design and synthesise selective chemical probes to investigate the role of mycothiol-*S*-transferase. The first approach included the development of substrate analogues based on a simplified mycothiol scaffold (**110**) (Fig. 104). Synthesis of a simplified mycothiol analogue (**78**) *via* methodology published by Knapp *et al.*[49] was attempted, however, it was unsuccessful even after extensive optimisation. The second synthetic route was designed that led to the formation of the acetyl-protected β-cyclohexyl cysteine mycothiol analogue (**119**) (Fig. 104). Finally, the third synthetic strategy resulted in the isolation of the immediate simplified mycothiol analogue precursor (**122**) (Fig. 104). Having established a novel approach towards the synthesis of the cysteine containing mycothiol scaffolds another three mycothiol scaffolds (**124**, **125** and **126**) were designed. The phenyl cysteine mycothiol analogue (**124**) was not obtained as the synthesis of the key intermediate, which is the phenyl α-thioglycoside, was unsuccessful. The benzyl cysteine mycothiol analogue precursor (**135**) (Fig. 104) and the azido cysteine mycothiol analogue (**126**) (Fig. 104) were successfully synthesised proving that the synthetic route may lead to the formation of various cysteine containing mycothiol scaffolds.

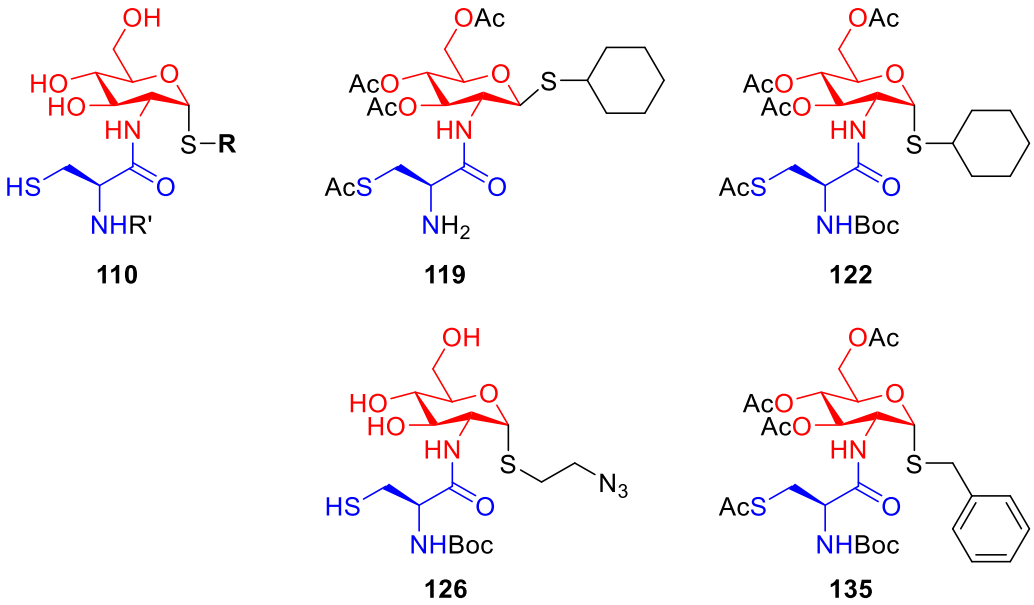


Fig. 104. The cysteine containing mycothiol analogues (**110** and **126**) and the analogue precursors (**119**, **122** and **135**).

The second approach was planned to involve the use of kinetic target guided synthesis (kTGS) in order to find new mycothiol-based chemical probes. Towards

this goal two types of the derivatives were designed: the azido mycothiol analogues (**111**) and the azidoacetamido mycothiol analogues (**112**). First, three different azido mycothiol analogues were designed (**144**, **145** and **146**). Following a short and easy synthetic strategy the novel cyclohexyl azido mycothiol analogue (**144**), its β-anomer (**151**) and the phenyl azido mycothiol analogue (**145**) were successfully obtained (Fig. 105). The synthesis of the benzyl azido mycothiol analogue (**146**) was unsuccessful even when an alternative synthetic route was employed. Then, three novel azidoacetamido mycothiol analogues (**156**, **157** and **158**) were designed and their synthesis was attempted. The cyclohexyl azidoacetamido mycothiol analogue precursor (**163**), the benzyl azidoacetamido analogue precursor (**167**) and its β-anomer (**165**) were successfully isolated (Fig. 105). The phenyl azidoacetamido mycothiol analogue (**157**) was not formed, however, its β-precursor (**164**) was obtained (Fig. 105).

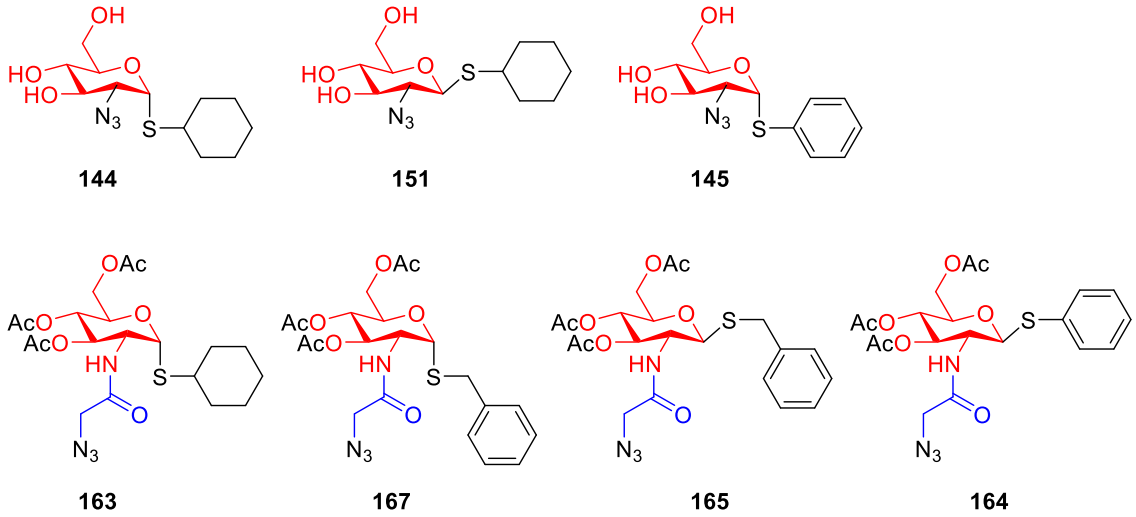


Fig. 105. The azido mycothiol analogues (**144**, **145** and **151**) and the azidoacetamido mycothiol analogue precursors (**163**, **164**, **165** and **167**).

Additionally, a diverse chemical library containing the phenyl azido mycothiol analogue-based triazoles (**168**) and the benzyl azidoacetamido mycothiol analogue-based triazoles (**169**) was successfully synthesised (Fig. 106). Both aliphatic and aromatic alkynes were used to show that the reaction conditions can be employed to synthesise a wide variety of the triazole containing mycothiol analogues.

To conclude, the findings of this research will help to better understand the role played by mycothiol-*S*-transferase in mycobacteria as a diverse set of chemical probes based on mycothiol structure was synthesised. Development of the

mycothiol-based probes into MST inhibitors may lead to a novel strategy for the treatment of tuberculosis. Moreover, the use of kinetic target guided synthesis in lead discovery can be further explored.

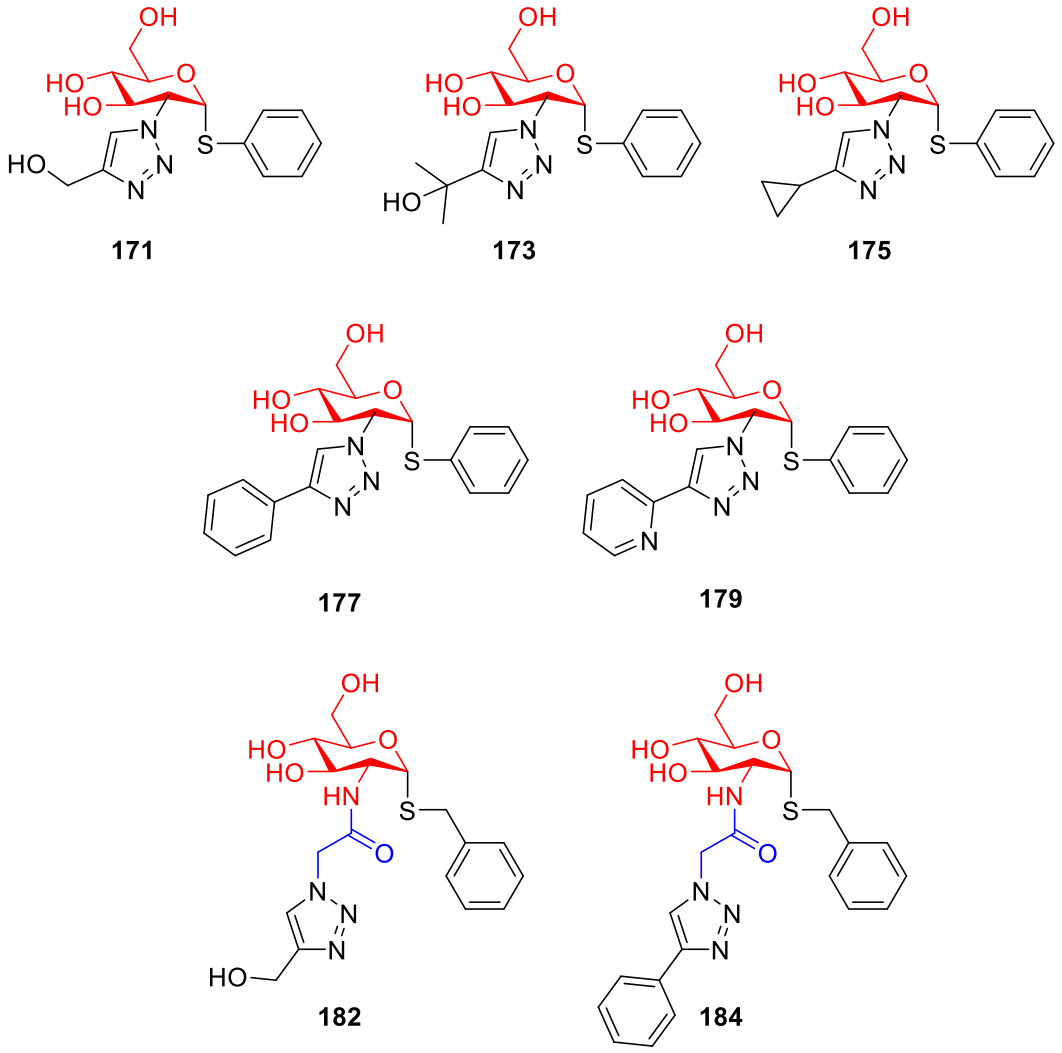
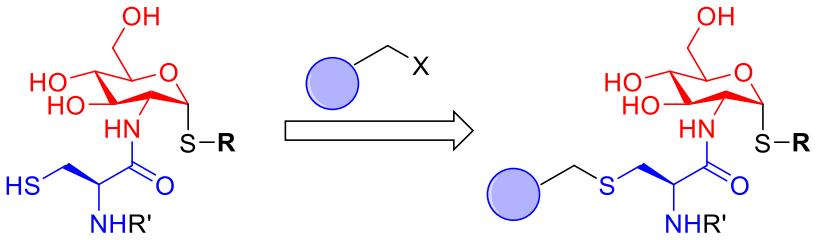


Fig. 106. Structures of the triazole containing mycothiol analogues (**171**, **173**, **175**, **177**, **179**, **182**, **184**).

10. Future work

With the novel mycothiol scaffold (**126**) and scaffold precursors (**122** and **135**) in hand, the synthesis of a small library of novel *S*-conjugates using thiol-ene coupling or *S*-alkylation will be possible (Fig. 107). The substituents used will vary in size and polarity to enable us to probe the hydrophobic pocket of MST and gain some information about the structure–activity relationships (SAR) of the target protein.



110

Fig. 107. Scheme outlining the synthesis of a small library of simplified mycothiol scaffold (**110**) *S*-conjugates.

In the second approach towards the synthesis of the chemical probes to investigate the role of mycothiol-*S*-transferase the use of kinetic target guided synthesis was planned. Because of time constraints and the fact that all of the steps involved required extensive optimisation, only preliminary studies were performed. To the best of our knowledge this would be the first attempt to employ kTGS in the synthesis of MST inhibitors. To attempt kTGS three main components are needed: a protein that would act as a catalyst or a template, an azide and an alkyne that would form a triazole hit. Time constraints limited what could be achieved in this part of the project, but the protein expression and purification steps, and the azide ligand synthesis steps were completed, along with proof of principle studies to show that 1,4-substituted triazoles could be formed using copper-catalysed ‘click’ chemistry.

The first component, which is mycothiol-*S*-transferase, was expressed in *E. coli* using a plasmid provided by Dr Mamta Rawat and purified using standard methods. To confirm protein identity mass spectrometry will be performed. In order to establish that MST is correctly folded and active an enzymatic activity assay is required. To perform the assay[40] mycothiol and bromobimane are needed. As mycothiol is not commercially available it will have to be extracted

and purified from *Mycobacterium* species.

The second component will be a mycothiol analogue containing an azido group. Two azido mycothiol analogues were successfully obtained: the cyclohexyl azido mycothiol analogue (**144**) and the phenyl azido mycothiol analogue (**145**) (Fig. 108). Two azidoacetamido mycothiol analogue precursors were also isolated: the cyclohexyl azidoacetamido mycothiol analogue precursor (**163**) and the benzyl azidoacetamido analogue precursor (**167**) (Fig. 108). The analogue precursors (**163** and **167**) could be easily transformed into respective mycothiol analogues (**156** and **158**) using sodium methoxide in methanol. Before the azido analogues (**144**, **145**, **156** and **158**) could be used in kTGS their binding to mycothiol *S*-transferase will have to be measured, preferably by isothermal titration calorimetry.

The third component of the system is an alkyne. In order to select the most suitable alkynes a library of alkyne fragments will be screened against MST using differential scanning fluorimetry. The alkynes that will show the highest binding affinity to MST will be subsequently used in the kTGS.

Having confirmed MST activity and having checked that the azido analogues (**144**, **145**, **156** and **158**) bind to MST kinetic target guided synthesis will be performed. Alkyne fragments will be grouped into mixtures of 5 based on their molecular masses, so that triazoles of a unique mass can be formed in each group. Then an azide scaffold and purified MST will be added. Two sets of control experiments will also be prepared. One containing bovine serum albumin instead of MST and the other containing no protein. The 96-well plate will be sealed and incubated at 37 °C in a water bath for 3 days. Finally, the triazole hits will be identified using LCMS and subsequently re-synthesised using previously optimized ‘click chemistry’ conditions. As a small triazole library based on the phenyl azido mycothiol analogue (**145**) and the benzyl azidoacetamido mycothiol analogue (**158**) was successfully obtained it can be assumed that the resynthesis method will work for the other two analogues (**144** and **156**). Lastly, the binding and inhibition of the re-synthesised hits will be confirmed using isothermal titration calorimetry and/or differential scanning fluorimetry, together with assays to assess the inhibition of MST enzymatic activity. Finally, the role of inhibitors in cells/bacteria will be tested to establish proof of principle.

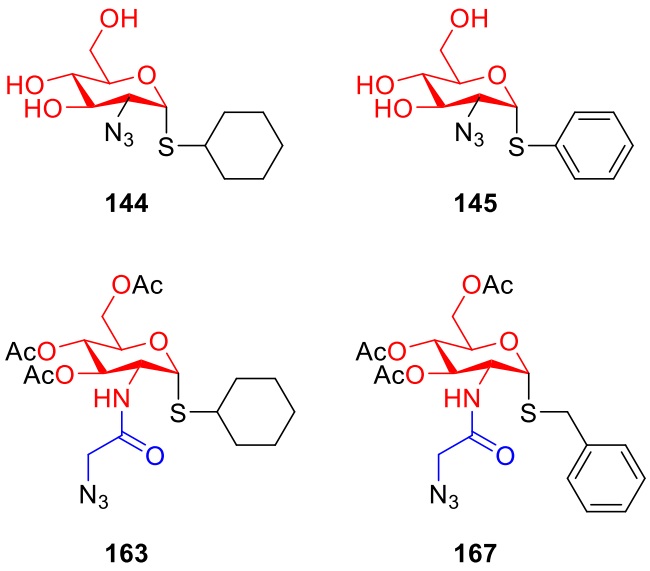


Fig. 108. Structures of the azido mycothiol analogues (**144** and **145**) and the azidoacetamido mycothiol analogues precursors (**163** and **167**).

Part III Experimental section

11. Chemical synthesis

Materials

All materials were purchased from Sigma Aldrich, Fisher, VWR and Alfa Aesar unless stated otherwise. All solvents were analytical or HPLC grade and used as supplied without further purification. Anhydrous solvents were purchased from Sigma Aldrich in a SureSeal^{TM} bottle and used without prior purification.

NMR

^{1}H

Spectra were measured on a Bruker Avance 400 MHz or Bruker Avance 500 MHz spectrometers using the deuterated solvent stated in parentheses. Chemical shifts (δ) are reported in parts per million (ppm) and referenced against tetramethylsilane (TMS). Results are shown as: chemical shift (integration, multiplicity, coupling constant, assignment). The following abbreviations were used to indicate the signal multiplicity: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), ddd (doublet of doublet of doublet), br s (broad singlet). Coupling constants (*J*) are quoted in Hz. Assignments were made using 2D techniques such as COSY and HMQC.

^{13}C

Spectra were measured on a Bruker Avance 400 MHz or Bruker Avance 500 MHz spectrometers with ^{1}H decoupling using the deuterated solvent stated in parentheses. Chemical shifts (δ) are reported in parts per million (ppm).

Mass spectrometry

High resolution mass spectra (HRMS) were obtained from the UCL School of Pharmacy, Structural Chemistry or from the EPSRC National Mass Spectrometry Facility Swansea, College of Medicine, Swansea University, Swansea, operating in positive or negative mode. Low resolution mass spectra were measured on *Shimadzu LCMS-2020,* operating in positive or negative mode, from solutions of methanol. *m/z* values are reported in Daltons (Da).

Liquid chromatography-mass spectrometry

Liquid chromatography-mass spectrometry (LC-MS) spectra were recorded using

Shimadzu LCMS-2020 equipped with an XTerra MS C18 column (4.6 x 50 mm, 2.5 µm) and a flow rate of 0.6 mL/min. The eluent system consisted of eluent A (water with 0.1% formic acid, HPLC grade) and eluent B (acetonitrile with 0.1% formic acid, HPLC grade) with the following conditions: 0.0 – 2.5 min 90% A: 10% B, then a linear gradient from 2.5 min to 5.5 min to a final composition 5% A: 95% B that was maintained for further 2.5 min, then adjusted to 10% A: 90% B over 10.5 min and held for 1.5 min. Total run time = 12 min.

Column chromatography

All column chromatography was performed manually using Merck silica gel 60 (0.040-0.063 mm) in a glass column. Elution was performed with specified analytical or HPLC grade solvents under a positive pressure of compressed air.

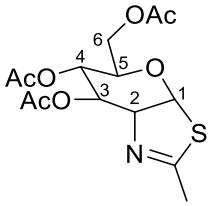
Thin Layer Chromatography

TLC was performed on Merck silica gel 60 F_{254} aluminium-supported plates. Samples were visualised using UV light (254 nm), or by thermal development after staining with $KMnO_4$ or an ethanolic solution of ninhydrin.

Rotary evaporator

All solvents were removed *in vacuo* using either Heidolph or Buchi rotary evaporators.

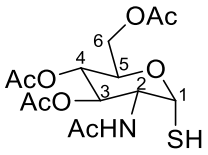
(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(Acetoxymethyl)-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole (72)



To a solution of 2-acetamido-2-deoxy-*β*-D-glucopyranose 1,3,4,6-tetraacetate (**71**) (1.00 g, 2.57 mmol, 1 eq.) in 10 mL of toluene, Lawesson's reagent (0.69 g, 1.70 mmol, 0.66 eq.) was added. The reaction was heated at 80 °C for 1.5 h. The reaction was then cooled to room temperature, neutralized by adding 100 mg of sodium bicarbonate and purified directly by silica gel column chromatography (3:7 ethyl acetate/dichloromethane) furnishing the GlcNAc-thiazoline triacetate (**72**) as a yellow oil (0.68 g, 76%): R_f 0.64 (19:1 dichloromethane/methanol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.17 (1H, d, *J* = 7.1 Hz, C*H*-1), 5.51 (1H, dd, *J* = 3.3, 1.9 Hz, C*H*-3), 4.91-4.87 (1H, m, C*H*-4), 4.45-4.37 (1H, m, C*H*-5), 4.06 (2H, d, *J* = 4.9 Hz, CH_2-6), 3.51-3.46 (1H, m, C*H*-2), 2.25 (3H, d, *J* = 2.3 Hz, N=CCH_3), 2.07 (3H, s, OC(O)CH_3), 2.02 (3H, s, OC(O)CH_3), 2.02 (3H, s, OC(O)CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.6 (S*C*=N), 169.6 (*C*=O), 169.3 (*C*=O), 168.2 (*C*=O), 88.8 (*C*H-1), 76.7 (*C*H-5), 70.7 (*C*H-3), 69.3 (*C*H-4), 68.5 (*C*H-2), 63.3 (*C*H_2-6), 20.9 (N=C*C*H_3), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI^+) *m/z* = 346.45 [$(M+H)^+$, 100%], R_t = 2.90 min; **HRMS** (ESI^+) calculated for $C_{14}H_{20}NO_7S$ $[M+H]^+$ 346.0955 found 346.0956.

The data are in agreement with the reported literature values.[73]

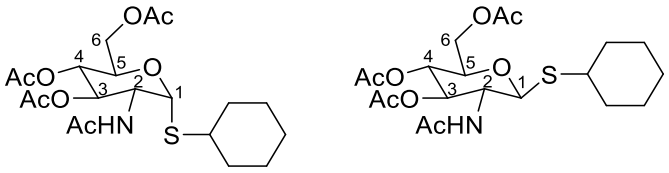
2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranose (73)



The GlcNAc-thiazoline triacetate (**72**) (100 mg, 0.290 mmol) was dissolved in 1 mL of methanol. The solution was cooled to 0 °C and treated with 2 drops of trifluoroacetic acid and 2 drops of water. The reaction was allowed to warm to room temperature over 2 h, after which time it was concentrated *in vacuo* to furnish the acetamido mercaptan (**73**) as a white solid (105 mg, 100%): R_f 0.35 (19:1 dichloromethane/methanol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.82 (1H, d, *J* = 8.4 Hz, N*H*), 5.71 (1H, dd, *J* = 7.1, 5.3 Hz, C*H*-1), 5.10-5.00 (2H, m, C*H*-3 + C*H*-4), 4.45-4.38 (1H, m, C*H*-2), 4.25-4.20 (1H, m, C*H*-5), 4.18 (1H, dd, *J* = 12.8, 5.5 Hz, CH_AH$_B$-6), 4.07 (1H, dd, *J* = 12.8, 5.5 Hz, CH$_A$$H_B$-6), 2.03 (3H, s, OC(O)C$H_3$), 1.99 (3H, s, OC(O)C$H_3$), 1.98 (3H, s, OC(O)C$H_3$), 1.91 (3H, s, NHC(O)C$H_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.8 (NH*C*=O), 170.7 (*C*=O), 170.4 (*C*=O), 169.2 (*C*=O), 78.8 (*C*H-1), 70.7 (*C*H-3), 69.0 (*C*H-5), 67.9 (*C*H-4), 61.8 (*C*H_2-6), 52.7 (*C*H-2), 23.1 (NHC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 361.80 [(M-H)$^-$, 100%], R_t = 3.83 min; **HRMS** (ESI$^+$) calculated for $C_{14}H_{22}NO_8S$ $[M+H]^+$ 364.1061 found 364.1061.

The data are in agreement with the reported literature values.[73]

Cyclohexyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (74) and cyclohexyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (97)



Method A

To a solution of acetamido mercaptan (**73**) (50 mg, 0.138 mmol, 1 eq.) in a 10:1 mixture of cyclohexene/chloroform (2.2 mL), AIBN (5 mg, 0.030 mmol, 0.22 eq) was added and the reaction was heated to 65 °C and stirred for 3 h. Additional portions of AIBN (2 x 5 mg) were added after 1 h and 2 h. After 3 h the reaction was cooled to room temperature and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 DCM/ethyl acetate) furnishing the cyclohexyl thioglycoside (**74**) as a colourless oil (5 mg, 8%).

Method B

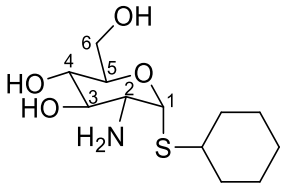
The acetamido mercaptan (**73**) (200 mg, 0.550 mmol, 1 eq.) was dissolved in dry dimethylformamide (2 mL). The solution was treated with anhydrous K_2CO_3 (152 mg, 1.10 mmol, 2 eq.), followed by cyclohexyl bromide (0.14 mL, 1.10 mmol, 2 eq.). The reaction was stirred for 1 h at room temperature, after which time it was diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layer was washed with water (3 x 10 mL) and brine (10 mL) then dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (1:3 ethyl acetate/dichloromethane) furnishing the cyclohexyl thioglycoside (**74**) as a colourless oil (24 mg, 10%).

Method C

To a solution of α-D-glucosamine pentaacetate (**96**) (1.00 g, 2.57 mmol, 1 eq.) in 1,2-dichloroethane (35 mL) cyclohexanethiol (1.26 mL, 10.28 mmol, 4 eq.) was added and the reaction was stirred for 20 min at room temperature under argon. The reaction was then cooled to 0 °C and $BF_3{\cdot}OEt_2$ (0.63 mL, 5.14 mmol, 2 eq.) was added drop-wise. The reaction was subsequently heated to 55 °C and stirred for 3 h. After which time, the reaction was quenched with saturated $NaHCO_3$ (30 mL) and extracted with DCM (3 x 30 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (1:2 hexane/ethyl acetate) furnishing pure α- (**74**) (30 mg, 3%) and β- (**97**) (115 mg, 10%) anomers as a colourless oil and a white solid respectively: $R_{f\alpha}$ 0.56, $R_{f\beta}$ 0.38 (ethyl acetate); **α-anomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.68 (1H, d, *J* = 9.0 Hz, N*H*), 5.47 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.13-5.08 (1H, m, C*H*-4), 5.06-5.01 (1H, m, C*H*-3), 4.53-4.48 (1H, m, C*H*-2), 4.41-4.37 (1H, m, C*H*-5), 4.25 (1H, dd, *J* = 12.3, 4.7 Hz, CH_ACH_B-6), 4.08 (1H, dd, *J* = 12.5, 2.4 Hz, CH$_A$CH_B-6), 2.86-2.80 (1H, m, C*H*'-1), 2.09 (3H, s, OC(O)CH_3), 2.03 (3H, s, OC(O)CH_3), 2.02 (3H, s, OC(O)CH_3), 2.00-1.94 (2H, m, cyclohexyl), 1.95 (3H, s, NHC(O)CH_3), 1.78-1.71 (2H, m, cyclohexyl), 1.62-1.59 (1H, m, cyclohexyl), 1.46-1.28 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.5 (NH*C*=O), 169.9 (*C*=O), 169.8 (*C*=O), 169.3 (*C*=O), 83.7 (*C*H-1), 71.6 (*C*H-3), 68.5 (*C*H-4), 68.2 (*C*H-5), 62.1 (*C*H$_2$-6), 52.3 (*C*H-2), 45.1 (*C*H-1'), 34.2 (cyclohexyl), 33.7 (cyclohexyl), 25.9 (cyclohexyl), 25.8 (cyclohexyl), 25.5 (cyclohexyl), 23.3 (NHC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$); **β-anomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.40 (1H, d, *J* = 9.1 Hz, N*H*), 5.19-5.14 (1H, m, C*H*-3), 5.03-4.97 (1H, m, C*H*-4), 4.70 (1H, d, *J* = 10.4 Hz, C*H*-1), 4.14 (1H, dd, *J* = 12.2, 5.5 Hz, CH_ACH_B-6), 4.06 (1H, dd, *J* = 12.2, 2.3 Hz, CH$_A$CH_B-6), 3.94-3.87 (1H, m, C*H*-2), 3.65-3.60 (1H, m, C*H*-5), 2.88-2.81 (1H, m, C*H*'-1), 2.00 (3H, s, OC(O)CH_3), 1.96 (6H, s, OC(O)CH_3), 1.94-1.89 (2H, m, cyclohexyl), 1.88 (3H, s, NHC(O)CH_3), 1.71-1.65 (2H, m, cyclohexyl), 1.57-1.50 (1H, m, cyclohexyl), 1.35-1.13 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.0 (*C*=O), 170.7 (*C*=O), 170.0 (*C*=O), 83.7 (*C*H-1), 75.7 (*C*H-5), 73.7 (*C*H-3), 68.6 (*C*H-4), 62.5 (*C*H$_2$-6), 52.9 (*C*H-2), 43.6 (*C*H-1'), 34.1 (cyclohexyl), 33.9 (cyclohexyl), 26.1 (cyclohexyl), 26.0 (cyclohexyl), 25.6 (cyclohexyl), 23.4

(NHC(O)*C*H$_3$), 20.8 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 446.05 [(M+H)$^+$, 100%], R_t = 3.87 min (α-anomer); **HRMS** (ESI$^+$) calculated for $C_{20}H_{32}NO_8S$ [M+H]$^+$ 446.1843 found 446.1839 (α-anomer).
The data are in agreement with the reported literature values.[49,53]

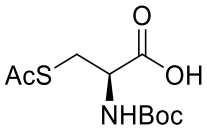
Cyclohexyl 2-amino-2-deoxy-1-thio-α-D-glucopyranoside (75)



A mixture of the cyclohexyl thioglycoside (**74**) (185 mg, 0.416 mmol) and hydrazine monohydrate (5.0 mL) was heated at 70 °C for 22 h. The reaction was then cooled to room temperature and concentrated *in vacuo*. The resulting crude material was dissolved in methanol and purified by silica gel column chromatography (3:1 dichloromethane/methanol) furnishing the aminotriol (**75**) as a colourless oil (97 mg, 84%): R_f 0.39 (3:1 dichloromethane/methanol); **^{1}H NMR** (400 MHz, MeOD) δ 5.54 (1H, d, *J* = 5.3 Hz, C*H*-1), 4.00-3.96 (1H, m, C*H*-5), 3.82 (1H, dd, *J* = 11.9, 2.4 Hz, CH_AH$_B$-6), 3.74 (1H, dd, *J* = 12.0, 5.3 Hz, CH$_A$$H_B$-6), 3.59 – 3.53 (1H, m, C*H*-4), 3.40-3.34 (1H, m, C*H*-3), 3.07 (1H, dd, *J* = 10.4, 5.3 Hz, C*H*-2), 2.87-2.80 (1H, m, C*H*-1'), 1.81-1.73 (2H, m, cyclohexyl), 1.65-1.58 (2H, m, cyclohexyl), 1.44-1.29 (6H, m, cyclohexyl); **^{13}C NMR** (126 MHz, D_2O) δ 84.0 (*C*H-1), 72.8 (*C*H-3), 72.7 (CH-5), 70.0 (*C*H-4), 60.5 (*C*H$_2$-6), 54.7 (*C*H-2), 44.1 (*C*H-1'), 33.8 (cyclohexyl), 33.2 (cyclohexyl), 25.5 (cyclohexyl), 25.4 (cyclohexyl), 25.1 (cyclohexyl); **HRMS** (ESI$^+$) calculated for $C_{12}H_{24}NO_4S$ $[M+H]^+$ 278.1421 found 278.1421.

The data are in agreement with the reported literature values.[49]

N-Boc-*S*-acetyl-L-cysteine (47)



Method A

To a solution of *N*,*N*'-di-Boc-L-cystine (**113**) (1.00 g, 2.27 mmol, 1 eq.) in 5% ethereal acetic acid (20 mL), zinc dust (4.00 g, 61.29 mmol, 27 eq.) was added portion-wise at 0 °C. The reaction was stirred for 4 h, after which time acetic anhydride (5 mL, 52.89 mmol, 23 eq.) was added drop-wise, followed by pyridine (20 mL). The reaction was stirred for 2 h at room temperature, after which time the reaction was filtered through a Celite pad and washed with methanol. The filtrate was concentrated *in vacuo* furnishing a yellow oil, which was dissolved in ethyl acetate. The solution was washed with ice-cold 5% sulphuric acid (10 x 10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (2:98 methanol/dichloromethane) furnishing *N*-Boc-*S*-acetyl-L-cysteine (**47**) as a light yellow oil (0.42 g, 35%).

Method B

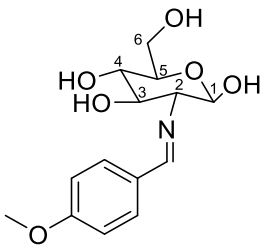
To a solution of *N*,*N*'-di-Boc-L-cystine (**113**) (1.00 g, 2.27 mmol, 1 eq.) in a 10:1 mixture of THF/water (22 mL), triphenylphosphine (656 mg, 2.50 mmol, 1.1 eq.) was added. The reaction was stirred at room temperature for 20 h, after which time the reaction was concentrated *in vacuo* and dissolved in water (10 mL). 6 M NaOH was added until the solution reached pH 10. The aqueous solution was extracted with ethyl acetate (3 x 10 mL). The aqueous layer was collected and acidified with 10% HCl until pH 2 was reached, followed by extraction with ethyl acetate (3 x 10 mL). The organic layer was collected, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing *N*-Boc-L-cysteine (**114**) as a pale yellow oil (1.00 g, 100%).

To a solution of *N*-Boc-L-cysteine (**114**) (1.00 g, 4.54 mmol, 1 eq.) in 5.20 mL of 1 M $NaHCO_3$ at 0 °C, acetic anhydride (7.72 mL, 81.72 mmol, 18 eq.) was added

drop-wise. The reaction was allowed to warm to room temperature over 3 h, after which time it was diluted with water (10 mL). The solution was acidified with 10% HCl until pH 2 was reached and extracted with dichloromethane (3 x 10 mL). The organic layer was collected and concentrated *in vacuo* furnishing *N*-Boc-*S*-acetyl-L-cysteine (**47**) as a light yellow oil (1.19 g, 100%): R_f 0.71 (8:2 dichloromethane/methanol); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.11 (1H, br s, COO*H*), 5.23 (1H, br s, N*H*), 4.47-4.38 (1H, m, αC*H*), 3.37 (1H, dd, J = 14.1, 4.7 Hz, βC*H*$_A$H$_B$), 3.25 (1H, dd, J = 14.1, 6.9 Hz, βCH$_A$*H*$_B$), 2.45 (3H, s, SC(O)C*H*$_3$), 1.39 (9H, s, NHC(O)OC(C*H*$_3$)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 194.7 (S*C*=O), 174.6 (*C*OOH), 155.7 (NH*C*=O), 80.7 (*C*(CH$_3$)$_3$), 53.4 (α*C*H), 30.9 (β*C*H$_2$), 30.5 (SC(O)*C*H$_3$), 28.3 (NHC(O)OC(*C*H$_3$)$_3$); **HRMS** (ESI$^-$) calculated for $C_{10}H_{16}NO_5S$ $[M-H]^-$ 262.0755, found 262.0755.

The data are in agreement with the reported literature values.[49]

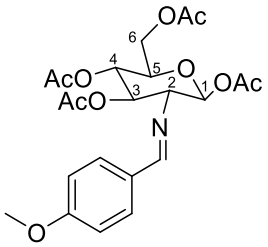
2-(4-Methoxybenzylidene)amino-β-D-glucosamine (117)



To a solution of glucosamine HCl (**107**) (5.00 g, 0.0232 mol, 1 eq.) in 1 M aqueous NaOH (24 mL), anisaldehyde (2.82 mL, 0.0232 mol, 1 eq.) was added *via* syringe under vigorous stirring. The reaction was kept at 0 °C for 1 h. After which time it was filtered and washed with water (2 x 20 mL) and a mixture of methanol and ether (1:1, 2 x 20 mL). The precipitate was dried under vacuum to furnish 2-(4-methoxybenzylidene)amino-β-D-glucosamine (**117**) as a white solid (3.20 g, 46%): R_f 0.80 (1:1 ethyl acetate/hexane); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.18 (1H, s, *H*C=N), 7.75 (2H, d, *J* = 8.8 Hz, Ar*H*), 7.05 (2H, d, *J* = 8.8 Hz, Ar*H*), 6.59 (1H, d, *J* = 6.8 Hz, O*H*-1), 4.98 (1H, d, *J* = 5.3, O*H*-4), 4.87 (1H, d, *J* = 5.6 Hz, O*H*-3), 4.78-4.73 (1H, m, C*H*-1), 4.61 (1H, t, *J* = 5.8 Hz, O*H*-6), 3.86 (3H, s, CH_3), 3.79 (1H, dd, *J* = 12.5, 6.6 Hz, CH_AH$_B$-6), 3.58-3.52 (1H, m, CH$_A$$H_B$-6), 3.52-3.45 (1H, m, C*H*-3), 3.32-3.27 (1H, m, C*H*-5), 3.23-3.17 (1H, m, C*H*-4), 2.79 (1H, dd, *J* = 9.1, 7.8 Hz, C*H*-2); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 161.2 (*C*=N), 161.0 (*C*-4'), 129.6 (Ar), 129.1 (*C*-1'), 113.9 (Ar), 95.6 (*C*H-1), 78.2 (*C*H-2), 76.8 (*C*H-5), 74.6 (*C*H-3), 70.3 (*C*H-4), 61.2 (*C*H_2-6), 55.2 (*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{14}H_{20}NO_6$ $[M+H]^+$ 298.1285, found 298.1287.

The data are in agreement with the reported literature values.[67]

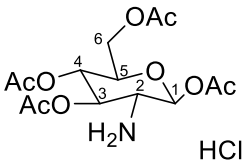
2-(4-Methoxybenzylidene)amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (118)



2-(4-Methoxybenzylidene)amino-β-D-glucosamine (**117**) (3.00 g, 0.0101 mol, 1 eq.) was treated with acetic anhydride (9 mL, 0.0960 mol, 9.5 eq.), pyridine (16 mL, 0.202 mol, 20 eq.) and DMAP (0.025 g, 0.202 mmol, 0.02 eq) in an ice-water bath. The reaction was stirred overnight at room temperature, after which time the solution was poured into 100 mL of ice, forming a white precipitate. The precipitate was isolated by filtration and washed with water (2 x 10 mL) and ether (2 x 10 mL). The solid was dried under vacuum to furnish 2-(4-methoxybenzylidene)amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**118**) as a white solid (2.70 g, 57%): R_f 0.54 (1:1 ethyl acetate/hexane); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.15 (1H, s, *H*C=N), 7.65 (2H, d, *J* = 8.8 Hz, Ar*H*), 6.91 (2H, d, *J* = 8.8 Hz, Ar*H*), 5.94 (1H, d, *J* = 8.3 Hz, C*H*-1), 5.46 – 5.39 (1H, m, C*H*-3), 5.17 – 5.11 (1H, m, C*H*-4), 4.38 (1H, dd, *J* = 12.4, 4.6 Hz, CH_AH_B-6), 4.13 (1H, dd, *J* = 12.4, 2.1 Hz, CH_AH_B-6), 3.97 (1H, ddd, *J* = 10.1, 4.6, 2.1 Hz, C*H*-5), 3.84 (3H, s, CH_3), 3.44 (1H, dd, *J* = 9.7, 8.3 Hz, C*H*-2), 2.10 (3H, s, OC(O)CH_3), 2.03 (3H, s, OC(O)CH_3), 2.02 (3H, s, OC(O)CH_3), 1.88 (3H, s, OC(O)CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.7 (*C*=O), 169.9 (*C*=O), 169.6 (*C*=O), 168.8 (*C*=O), 164.3 (*C*=N), 162.3 (*C*-4'), 130.3 (Ar), 128.3 (*C*-1'), 114.1 (Ar), 93.2 (*C*H-1), 73.3 (*C*H-3), 73.0 (*C*H-2), 72.8 (*C*H-5), 68.0 (*C*H-4), 61.8 (*C*H_2-6), 55.4 (*C*H_3), 20.9 (OC(O)*C*H_3), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3); **HRMS** (ESI^+) calculated for $C_{22}H_{28}NO_{10}$ $[M+H]^+$ 466.1708, found 466.1700.

The data are in agreement with the reported literature values.[67]

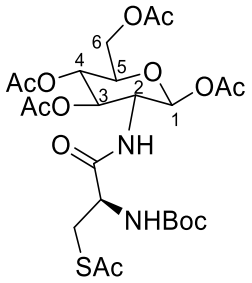
2-Amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose HCl (40)



To a refluxing solution of 2-(4-methoxybenzylidene)amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**118**) (3.00 g, 6.44 mmol) in acetone (15 mL) 5 N HCl was added dropwise (1.5 mL). The reaction was cooled to room temperature. The precipitate was isolated by filtration and washed with acetone (6 mL) and ether (2 x 15 mL). The solid was dried under vacuum to furnish 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose HCl (**40**) as a white solid (1.65 g, 67%): R_f 0.57 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.87 (3H, s, N*H*$_3$), 5.96 (1H, d, *J* = 8.7 Hz, C*H*-1), 5.44-5.38 (1H, m, C*H*-3), 5.01-4.96 (1H, m, C*H*-4), 4.24 (1H, dd, *J* = 12.5, 4.5 Hz, C*H*$_A$H$_B$-6), 4.13-4.08 (1H, m, C*H*-5), 4.05 (1H, dd, *J* = 12.5, 2.1 Hz, CH$_A$*H*$_B$-6), 3.66-3.59 (1H, m, C*H*-2), 2.23 (3H, s, OC(O)C*H*$_3$), 2.08 (3H, s, OC(O)C*H*$_3$), 2.05 (3H, s, OC(O)C*H*$_3$), 2.03 (3H, s, OC(O)C*H*$_3$); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 169.9 (*C*=O), 169.8 (*C*=O), 169.3 (*C*=O), 168.6 (*C*=O), 90.1 (*C*H-1), 71.6 (*C*H-5), 70.3 (*C*H-3), 67.7 (*C*H-4), 61.2 (*C*H$_2$-6), 52.0 (*C*H-2), 20.9 (OC(O)*C*H$_3$), 20.8 (OC(O)*C*H$_3$), 20.5 (OC(O)*C*H$_3$), 20.3 (OC(O)*C*H$_3$); **HRMS** (ESI$^+$) calculated for $C_{14}H_{22}NO_9$ [M-Cl]$^+$ 348.1289, found 348.1293.

The data are in agreement with the reported literature values.[67]

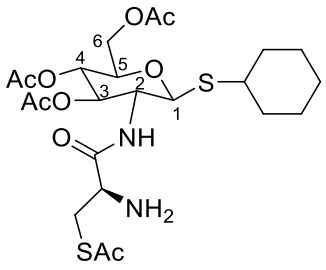
2-Amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (116)



To a solution of *N*-Boc-*S*-acetyl-L-cysteine (**47**) (69 mg, 0.261 mmol, 1 eq.) in anhydrous THF (2 mL) under nitrogen, HOBt (35 mg, 0.261 mmol, 1 eq.) and EDCI (50 mg, 0.261 mmol, 1 eq) were added at 0 °C. The reaction was stirred at 0 °C for 0.5 h under nitrogen, after which time a solution of 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose HCl (**40**) (500 mg, 1.303 mmol, 5 eq.) in anhydrous THF (14 mL) and *N*-methylmorpholine (0.23 mL, 2.088 mmol, 8 eq.) were added. The coupling reaction was stirred at 0 °C for 1 h and then at room temperature overnight, after which time it was concentrated *in vacuo* and dissolved in ethyl acetate (6 mL). The organic solution was washed with saturated $NaHCO_3$ (10 mL), 5% $KHSO_4$ (10 mL) and saturated NaCl (10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* furnishing 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**116**) as a white solid (100 mg, 64%): R_f 0.37 (1:1 hexane/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.49 (1H, d, *J* = 9.3 Hz, N*H*), 5.73 (1H, d, *J* = 8.7 Hz, C*H*-1), 5.23-5.18 (2H, m, C*H*-3 + N*H*Boc), 5.15-5.10 (1H, m, C*H*-4), 4.28 (1H, dd, *J* = 12.5, 4.5 Hz, CH_AH_B-6), 4.26-4.21 (2H, m, C*H*-2 + CysαC*H*), 4.12 (1H, *J* = 12.4, 2.2 Hz, CH_AH_B-6), 3.83-3.79 (1H, m, C*H*-5), 3.31 (1H, dd, *J* = 14.3, 4.2 Hz, CysβCH_AH_B), 3.17 (1H, dd, *J* = 14.3, 7.3 Hz, CysβCH_AH_B), 2.37 (3H, s, SC(O)CH_3), 2.14 (3H, s, C(O)CH_3), 2.09 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 1.45 (9H, s, NHC(O)OC(CH_3)$_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.3 (*C*=O), 171.8 (*C*=O), 171.3 (*C*=O), 170.7 (*C*=O), 93.2 (*C*H-1), 73.8 (*C*H-3), 73.3 (*C*H-5), 69.9 (*C*H-4), 63.0 (*C*H_2-6), 53.0 (Cysα*C*H), 54.1 (*C*H-2), 31.7 (Cysβ*C*H_2), 30.9 (S*C*=O), 28.7 (NC(O)OC(*C*H_3)$_3$), 20.8 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), the N*C*=O and *C*(CH_3)$_3$

signals were not observed in the spectrum; **HRMS** (ESI^+) calculated for $C_{24}H_{37}N_2O_{13}S$ $[M+H]^+$ 593.2011, found 593.2012.

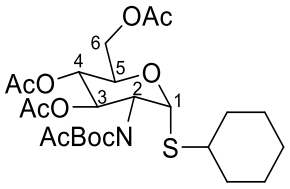
Cyclohexyl 2-amino-2-*N*-(*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (119)



To a solution of 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**116**) (30 mg, 0.051 mmol, 1 eq.) and cyclohexanethiol (12 µL, 0.102 mmol, 2 eq.) in a 1:1 mixture of DCM/THF (2 mL) $BF_3{\cdot}OEt_2$ (28 µL, 0.230 mmol, 4.5 eq.) was added at 0 °C under argon. The reaction was stirred at 55 °C for 5 h. The reaction was then concentrated *in vacuo* and diluted with EtOAc (5 mL), washed with brine (3 x 10 mL) and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% MeOH in DCM) furnishing cyclohexyl 2-amino-2-*N*-(*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**119**) as a white solid (5 mg, 18%): R_f 0.35 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 5.31-5.26 (1H, m, C*H*-3), 5.00-4.95 (1H, m, C*H*-4), 4.86 (1H, d, *J* = 10.5 Hz, C*H*-1), 4.43-4.40 (1H, m, CysαC*H*), 4.26 (1H, dd, *J* = 12.3, 5.3 Hz, CH_ACH_B-6), 4.13 (1H, dd, *J* = 12.3, 2.1 Hz, CH_ACH_B-6), 3.96-2.91 (1H, m, C*H*-2), 3.83 – 3.79 (1H, m, C*H*-5), 2.98-2.91 (1H, m, C*H*-1'), 2.86 – 2.76 (2H, m, CysβCH_2), 2.07 (3H, s, SC(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 2.00-1.97 (1H, m, cyclohexyl), 1.79-1.74 (2H, m, cyclohexyl), 1.66-1.60 (2H, m, cyclohexyl), 1.42-1.28 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, MeOD) δ 170.6 (*C*=O), 169.9 (*C*=O), 169.8 (*C*=O), 84.9 (*C*H-1), 76.8 (*C*H-5), 74.5 (*C*H-3), 70.3 (*C*H-4), 63.6 (*C*H_2-6), 57.2 (Cysα*C*H), 54.6 (*C*H-2) 44.9 (*C*H-1'), 35.3 (cyclohexyl), 35.2 (cyclohexyl), 27.0 (Cysβ*C*H_2), 26.8 (cyclohexyl), 26.8 (cyclohexyl), 26.7 (cyclohexyl), 22.5 (S*C*=O), 20.9 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{23}H_{36}N_2O_9S_2$ $[M+Na]^+$ 571.1760,

found 571.1765.

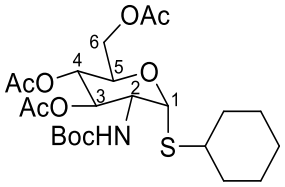
Cyclohexyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (98)



To a solution of the cyclohexyl thioglycoside (**74**) (50 mg, 0.112 mmol, 1 eq.) and DMAP (1.4 mg, 0.0112 mmol, 0.1 eq.) in THF (1.5 mL) Boc_2O (134 mg, 0.616 mmol, 5.5 eq.) was added and the reaction was refluxed at 60 °C for 18 h. After which time, the reaction was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (4:1 hexane/ethyl acetate) furnishing cyclohexyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**98**) as a colourless oil (54 mg, 89%): R_f 0.83 (3:1 DCM/EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.92 (1H, dd, *J* = 11.6, 8.8 Hz, C*H*-3), 5.42 (1H, d, *J* = 5.6 Hz, C*H*-1), 5.05-4.96 (1H, m, C*H*-2), 4.92 (1H, dd, *J* = 10.3, 8.7 Hz, C*H*-4), 4.55 – 4.49 (1H, m, C*H*-5), 4.31 (1H, dd, *J* = 12.2, 4.7 Hz, CH_ACH_B-6), 4.06 (1H, dd, *J* = 12.2, 2.1 Hz, CH$_A$CH_B-6), 2.77-2.66 (1H, m, C*H*-1'), 2.39 (3H, s, NC(O)*C*H_3), 2.06 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.92 (3H, s, C(O)CH_3), 1.91-1.88 (1H, m, cyclohexyl), 1.74-1.65 (2H, m, cyclohexyl), 1.59-1.56 (1H, m, cyclohexyl), 1.54 (9H, s, NC(O)OC(CH_3)$_3$), 1.39-1.22 (6H, m, cyclohexyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.7 (*C*=O), 170.7 (*C*=O), 170.0 (*C*=O), 169.5 (*C*=O), 152.5 (N*C*=O), 85.2 (*C*(CH$_3$)$_3$), 82.9 (*C*H-1), 70.8 (*C*H-4), 70.7 (*C*H-3), 67.8 (*C*H-5), 62.2 (*C*H$_2$-6), 56.3 (*C*H-2), 44.3 (*C*H-1'), 34.2 (cyclohexyl), 33.5 (cyclohexyl), 28.0 (NC(O)OC(*C*H$_3$)$_3$), 26.9 (NC(O)*C*H$_3$), 26.0 (cyclohexyl), 25.8 (cyclohexyl), 25.6 (cyclohexyl), 20.8 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 563.45 [(M+NH$_4$)$^+$, 100%], R_t = 4.92 min; **HRMS** (ESI$^+$) calculated for $C_{25}H_{40}NO_{10}S$ [M+H]$^+$ 546.2367, found 546.2362.

The data are in agreement with the reported literature values.[53]

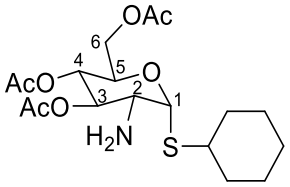
Cyclohexyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (121)



To a solution of cyclohexyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**98**) (50 mg, 0.092 mmol, 1 eq.) in methanol (3 mL) hydrazine monohydrate (9 µL, 0.184 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 1 h. After which time, the reaction was diluted with DCM (5 mL), washed with 0.5 M HCl (5 mL), saturated solution of $CuSO_4$ (5 mL) and $NaHCO_3$ (5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing cyclohexyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**121**) as a colourless oil (30 mg, 65%): R_f 0.89 (3:1 DCM/EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.44 (1H, d, *J* = 5.3 Hz, C*H*-1), 5.11-5.02 (1H, m, C*H*-3), 5.03-4.95 (1H, m, C*H*-4), 4.73 (1H, d, *J* = 9.7 Hz, N*H*), 4.44-4.33 (1H, m, C*H*-5), 4.27-4.22 (1H, m, CH_ACH_B-6), 4.21-4.13 (1H, m, C*H*-2), 4.08 (1H, dd, *J* = 12.3, 1.7 Hz, CH_ACH_B-6), 2.88-2.80 (1H, m, C*H*-1'), 2.08 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.98-1.94 (1H, m, cyclohexyl), 1.79-1.71 (2H, m, cyclohexyl), 1.62-1.58 (1H, m, cyclohexyl), 1.42 (9H, s, NC(O)OC(CH_3)$_3$), 1.36-1.24 (6H, m, cyclohexyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.9 (*C*=O), 170.6 (*C*=O), 169.4 (*C*=O), 154.9 (N*C*=O), 84.2 (*C*H-1), 80.1 (*C*(CH$_3$)$_3$), 71.8 (*C*H-4), 68.5 (*C*H-5), 68.5 (*C*H-3), 62.2 (*C*H$_2$-6), 53.4 (*C*H-2), 45.0 (*C*H-1'), 34.2 (cyclohexyl), 33.6 (cyclohexyl), 28.2 (NC(O)OC(*C*H$_3$)$_3$), 25.9 (cyclohexyl), 25.8 (cyclohexyl), 25.6 (cyclohexyl), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 526.25 [(M+Na)$^+$, 100%], R_t = 4.59 min; **HRMS** (ESI$^+$) calculated for $C_{23}H_{41}N_2O_9S$ [M+NH$_4$]$^+$ 521.2527, found 521.2521.

The data are in agreement with the reported literature values.[21]

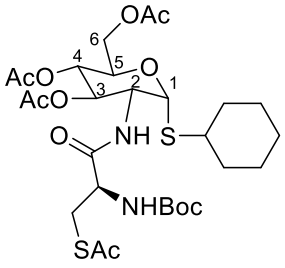
Cyclohexyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (120)



To a solution of cyclohexyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**121**) (30 mg, 0.060 mmol, 1 eq.) in dichloromethane (3 mL) TMSOTf (22 µL, 0.120 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 4 h. After which time, the reaction was quenched with cold saturated $NaHCO_3$ (5 mL) and extracted with dichloromethane (3 x 5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing cyclohexyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**120**) as a colourless oil (20 mg, 83%): R_f 0.53 (3:1 DCM/EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.41 (1H, d, *J* = 5.1 Hz, C*H*-1), 4.97-4.92 (2H, m, C*H*-3 + C*H*-4), 4.49-4.44 (1H, m, C*H*-5), 4.30 (1H, dd, *J* = 12.3, 4.9 Hz, CH_ACH_B-6), 4.06 (dd, *J* = 12.3, 2.3 Hz, CH_ACH_B-6), 3.25-3.18 (1H, m, C*H*-2), 2.89-2.81 (1H, m, C*H*-1'), 2.07 (3H, s, C(O)CH_3), 2.06 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 2.00-1.98 (1H, m, cyclohexyl), 1.80-1.71 (2H, m, cyclohexyl), 1.62-1.57 (1H, m, cyclohexyl), 1.36-1.28 (6H, m, cyclohexyl); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.6 (*C*=O), 169.8 (*C*=O), 86.9 (*C*H-1), 75.1 (*C*H-4), 69.1 (*C*H-3), 68.4 (*C*H-5), 62.4 (*C*H_2-6), 54.8 (*C*H-2), 44.8 (*C*H-1'), 34.4 (cyclohexyl), 33.7 (cyclohexyl), 25.9 (cyclohexyl), 25.8 (cyclohexyl), 25.6 (cyclohexyl), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{18}H_{30}NO_7S$ $[M+H]^+$ 404.1737, found 404.1733.

The data are in agreement with the reported literature values.[21]

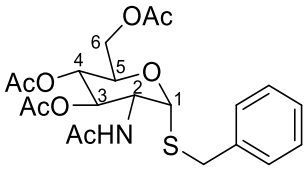
Cyclohexyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (122)



To a solution of *N*-Boc-*S*-acetyl-L-cysteine (**47**) (49 mg, 0.186 mmol, 1.5 eq.) in THF (5 mL), HOBt (17 mg, 0.124 mmol, 1 eq.) and EDCI (36 mg, 0.186 mmol, 1.5 eq) were added at 0 °C. The reaction was stirred at 0 °C for 0.5 h, after which time a solution of cyclohexyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**120**) (50 mg, 0.124 mmol, 1 eq.) in THF (5 mL) and *N*-methylmorpholine (0.11 mL, 0.992 mmol, 8 eq.) were added. The coupling reaction was stirred at 0 °C for 1 h and then at room temperature overnight, after which time it was concentrated *in vacuo* and dissolved in ethyl acetate (5 mL). The organic solution was washed with saturated $NaHCO_3$ (10 mL), 5% $KHSO_4$ (10 mL) and saturated NaCl (10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 DCM/ethyl acetate) furnishing cyclohexyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**122**) as a white solid (16 mg, 20%): R_f 0.71 (3:1 DCM/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.56 (1H, d, J = 7.6 Hz, N*H*), 5.49 (1H, d, J = 5.1 Hz, C*H*-1), 5.18 (1H, d, J = 6.0 Hz, N*H*Boc), 5.11-5.02 (2H, m, C*H*-3 + C*H*-4), 4.40-4.32 (2H, m, C*H*-5 + C*H*-2), 4.23-4.17 (2H, m, CH_ACH_B-6 + CysαC*H*), 4.07 (1H, dd, *J* = 12.3, 2.0 Hz, CH_ACH_B-6), 3.29 (1H, dd, *J* = 14.2, 4.2 Hz, CysβCH_AH$_B$), 3.17 (1H, dd, *J* = 14.2, 7.7 Hz, CysβC$H_A$$H_B$), 2.80-2.73 (1H, m, C*H*-1'), 2.36 (3H, s, SC(O)CH_3), 2.08 (3H, s, C(O)CH_3), 2.01 (6H, s, C(O)CH_3), 2.00-1.94 (1H, m, cyclohexyl), 1.72-1.64 (2H, m, cyclohexyl), 1.62-1.55 (1H, m, cyclohexyl), 1.45 (9H, s, NHC(O)OC(CH_3)$_3$), 1.41-1.26 (6H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.3 (*C*=O), 170.7 (*C*=O), 169.4 (*C*=O), 155.5 (N*C*=O), 84.3 (*C*(CH$_3$)$_3$), 83.7 (*C*H-1), 71.0 (*C*H-3),

68.4 (*C*H-5), 68.3 (*C*H-4), 62.1 (*C*H_2-6), 54.8 (CysαCH), 52.6 (*C*H-2), 45.0 (*C*H-1'), 33.7 (cyclohexyl), 31.2 (CysβCH$_2$), 30.5 (S*C*=O), 29.7 (cyclohexyl), 28.3 (NC(O)OC(*C*H_3)$_3$), 25.9 (cyclohexyl), 25.8 (cyclohexyl), 25.6 (cyclohexyl), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{28}H_{45}N_2O_{11}S_2$ $[M+H]^+$ 649.2459, found 649.2451.

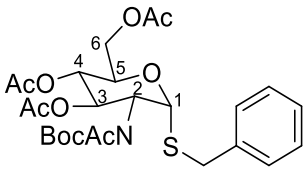
Benzyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (130)



To a solution of the acetamido mercaptan (**73**) (130 mg, 0.358 mmol, 1 eq.) in dichloromethane (1.5 mL) benzyl bromide (0.05 mL, 0.430 mmol, 1.2 eq.) and triethylamine (0.10 mL, 0.716 mmol, 2 eq.) were added. The reaction was stirred at room temperature for 20 h, after which time the reaction was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (2% methanol in DCM) furnishing the benzyl thioglycoside (**130**) as a white foam (112 mg, 69%): R_f 0.32 (2% methanol in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28-7.15 (5H, m, Ar*H*), 5.73 (1H, d, *J* = 8.4 Hz, N*H*), 5.28 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.10-4.94 (2H, m, C*H*-3 + C*H*-4), 4.45-4.37 (1H, m, C*H*-2), 4.26-4.21 (1H, m, C*H*-5), 4.15 (1H, dd, *J* = 12.4, 4.4 Hz, CH_ACH_B-6), 3.89-3.82 (1H, m, CH$_A$CH_B-6), 3.79-3.66 (2H, m, SCH_2), 2.02 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.93 (3H, s, C(O)CH_3), 1.79 (3H, s, NHC(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.4 (*C*=O), 170.7 (*C*=O), 169.9 (*C*=O), 169.3 (*C*=O), 137.2 (Ar), 128.9 (Ar), 128.7 (Ar), 127.5 (Ar), 83.6 (*C*H-1), 71.4 (*C*H-4), 68.6 (*C*H-5), 68.2 (*C*H-3), 61.9 (*C*H$_2$-6), 52.0 (*C*H-2), 35.0 (S*C*H$_2$), 23.1 (NHC(O)*C*H$_3$), 20.8 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 454.25 [(M+H)$^+$, 100%], R_t = 3.46 min; **HRMS** (ESI$^+$) calculated for $C_{21}H_{28}NO_8S$ [M+H]$^+$ 454.1530, found 454.1524.

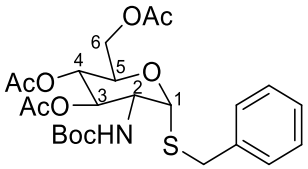
The data are in agreement with the reported literature values.[24]

Benzyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (131)



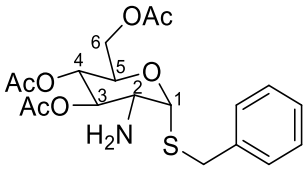
To a solution of the benzyl thioglycoside (**130**) (240 mg, 0.529 mmol, 1 eq.) and DMAP (6.5 mg, 0.0529 mmol, 0.1 eq.) in THF (8 mL) Boc_2O (635 mg, 2.910 mmol, 5.5 eq.) was added and the reaction was refluxed at 60 °C for 18 h, after which time, the reaction was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (1% MeOH in DCM) furnishing benzyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**131**) as a colourless oil (258 mg, 88%): R_f 0.34 (2% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.23-7.20 (4H, m, Ar*H*), 7.18-7.13 (1H, m, Ar*H*), 5.97-5.87 (1H, m, C*H*-3), 5.17 (1H, d, *J* = 5.5 Hz, C*H*-1), 5.02-4.83 (1H, m, C*H*-2 + C*H*-4), 4.40-4.33 (1H, m, C*H*-5), 4.22 (1H, dd, *J* = 12.3, 4.5 Hz, CH_ACH_B-6), 3.79 (1H, dd, *J* = 12.3, 2.0 Hz, CH_ACH_B-6), 3.69-3.56 (2H, m, SCH_2), 2.28 (3H, s, NHC(O)CH_3), 2.01 (3H, s, C(O)CH_3), 1.94 (3H, s, C(O)CH_3), 1.86 (3H, s, C(O)CH_3), 1.45 (9H, s, NHC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.4 (*C*=O), 170.7 (*C*=O), 170.0 (*C*=O), 169.45 (*C*=O), 137.8 (Ar), 128.9 (Ar), 128.5 (Ar), 127.2 (Ar), 82.7 (*C*H-1), 70.7 (*C*H-3), 70.5 (*C*H-4), 68.1 (*C*H-5), 62.0 (*C*H_2-6), 35.1 (S*C*H_2), 27.7 (NHC(O)*C*H_3), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), the N*C*=O and *C*$(CH_3)_3$ signals were not observed in the spectrum; **LCMS** (ESI$^+$) *m/z* = 576.20 [$(M+Na)^+$, 100%], R_t = 4.44 min; **HRMS** (ESI$^+$) calculated for $C_{26}H_{36}NO_{10}S$ $[M+H]^+$ 554.2054, found 554.2048.

Benzyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (133)



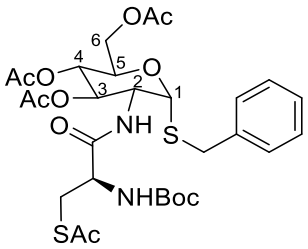
To a solution of benzyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**131**) (90 mg, 0.163 mmol, 1 eq.) in methanol (1 mL) DEAEA (*N*,*N*-diethylethylenediamine) (46 µL, 0.326 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 1 h, after which time the reaction was diluted with DCM (5 mL), washed with 0.5 M HCl (5 mL), saturated solution of $CuSO_4$ (5 mL) and $NaHCO_3$ (5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing benzyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**133**) as a yellow oil (60 mg, 72%): R_f 0.94 (2% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.25-7.22 (4H, m, Ar*H*), 7.20-7.16 (1H, m, Ar*H*), 5.24 (1H, d, *J* = 5.0 Hz, C*H*-1), 5.04-4.95 (2H, m, C*H*-3 + C*H*-4), 4.58 (1H, d, *J* = 9.3 Hz N*H*), 4.28-4.23 (1H, m, C*H*-5), 4.16 (1H, dd, *J* = 12.5, 4.1 Hz, CH_ACH_B-6), 4.11-4.07 (1H, m, C*H*-2), 3.88 (1H, dd, *J* = 12.3, 2.2 Hz, CH$_A$CH_B-6), 3.78-3.67 (2H, m, SCH_2), 2.02 (3H, s, C(O)CH_3), 1.96 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.33 (9H, s, NC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.6 (*C*=O), 171.0 (*C*=O), 170.7 (*C*=O), 154.8 (N*C*=O), 128.9 (Ar), 128.7 (Ar), 127.4 (Ar), 127.3 (Ar), 84.0 (*C*H-1), 80.1 (*C*(CH$_3$)$_3$), 71.7 (*C*H-3), 68.6 (*C*H-5), 68.5 (*C*H-4), 62.0 (*C*H$_2$-6), 53.4 (*C*H-2), 34.9 (S*C*H$_2$), 28.2 (NC(O)OC(*C*H$_3$)$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$), 20.5 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 534.05 [(M+Na)$^+$, 100%], R_t = 4.31 min; **HRMS** (ESI$^+$) calculated for $C_{24}H_{34}NO_9S$ [M+H]$^+$ 512.1949, found 512.1939.

Benzyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (134)



To a solution of benzyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**133**) (60 mg, 0.117 mmol, 1 eq.) in dichloromethane (6 mL) TMSOTf (42 µL, 0.234 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 2 h, after which time the reaction was quenched with cold saturated $NaHCO_3$ (5 mL) and extracted with dichloromethane (3 x 5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing benzyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**134**) as a yellow oil (40 mg, 83%): R_f 0.31 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28-7.23 (4H, m, Ar*H*), 7.21-7.16 (1H, m, Ar*H*), 5.16 (d, *J* = 5.3 Hz, C*H*-1), 4.97-4.92 (1H, m, C*H*-3), 4.92-4.86 (1H, m, C*H*-4), 4.34-4.29 (1H, m, C*H*-5), 4.22 (1H, dd, *J* = 12.3, 4.7 Hz, CH_ACH_B-6), 3.84 (1H, dd, *J* = 12.3, 2.2 Hz, CH_ACH_B-6), 3.78-3.68 (2H, m, SCH_2), 3.18-3.13 (1H, m, C*H*-2), 2.03 (3H, s, C(O)CH_3), 1.99 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.7 (*C*=O), 170.6 (*C*=O), 169.8 (*C*=O), 137.4 (Ar), 129.0 (Ar), 128.6 (Ar), 127.3 (Ar), 86.3 (*C*H-1), 74.8 (*C*H-3), 69.0 (*C*H-4), 68.4 (*C*H-5), 62.2 (*C*H_2-6), 54.6 (*C*H-2), 34.5 (S*C*H_2), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 412.00 [(M+H)$^+$, 100%], R_t = 2.50 min; **HRMS** (ESI$^+$) calculated for $C_{19}H_{26}NO_7S$ [M+H]$^+$ 412.1424, found 412.1418.

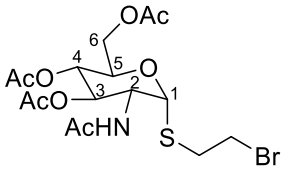
Benzyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (135)



To a solution of *N*-Boc-*S*-acetyl-L-cysteine (**47**) (105 mg, 0.401 mmol, 1.5 eq.) in THF (5 mL), HOBt (36 mg, 0.267 mmol, 1 eq.) and EDCI (77 mg, 0.401 mmol, 1.5 eq) were added at 0 °C. The reaction was stirred at 0 °C for 0.5 h, after which time a solution of benzyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**134**) (110 mg, 0.267 mmol, 1 eq.) in THF (5 mL) and *N*-methylmorpholine (0.23 mL, 2.136 mmol, 8 eq.) were added. The coupling reaction was stirred at 0 °C for 1 h and then at room temperature overnight, after which time it was concentrated *in vacuo* and dissolved in ethyl acetate (5 mL). The organic solution was washed with saturated $NaHCO_3$ (10 mL), 5% $KHSO_4$ (10 mL) and saturated NaCl (10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 DCM/ethyl acetate) furnishing benzyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**135**) as a colourless oil (16 mg, 9%): R_f 0.74 (3:1 DCM/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.25-7.22 (4H, m, Ar*H*), 7.19-7.16 (1H, m, Ar*H*), 6.48 (1H, d, J = 7.4 Hz, N*H*), 5.31 (1H, d, J = 5.4 Hz, C*H*-1), 5.06-5.01 (3H, m, N*H*Boc + C*H*-3 + C*H*-4), 4.35-4.30 (1H, m, C*H*-2), 4.28-4.24 (1H, m, C*H*-5), 4.17 (1H, dd, *J* = 12.4, 4.4 Hz, CH_ACH_B-6), 4.14-4.09 (1H, m, CysαC*H*), 3.86 (1H, dd, *J* = 12.3, 2.2 Hz, CH$_A$CH_B-6), 3.75-3.67 (2H, m, SCH_2), 3.15 (1H, dd, *J* = 14.4, 4.2 Hz, CysβCH_AH$_B$), 3.01 (1H, dd, *J* = 14.3, 7.7 Hz, CysβCH$_A$$H_B$), 2.29 (3H, s, SC(O)C$H_3$), 2.03 (3H, s, C(O)C$H_3$), 1.95 (3H, s, C(O)C$H_3$), 1.94 (3H, s, C(O)C$H_3$), 1.39 (9H, s, NHC(O)OC(CH_3)$_3$); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.4 (*C*=O), 170.7 (*C*=O), 169.4 (*C*=O), 137.2 (Ar), 128.9 (Ar), 128.7 (Ar), 127.5 (Ar), 83.2 (*C*H-1), 70.8 (*C*H-3), 68.5 (*C*H-5), 68.2 (*C*H-4),

61.9 (*C*H_2-6), 54.9 (CysαCH), 52.5 (*C*H-2), 34.9 (S*C*H_2), 31.0 (CysβCH_2), 30.5 (S*C*=O), 28.3 (NC(O)OC(*C*H_3)$_3$), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), the N*C*=O and *C*$(CH_3)_3$ signals were not observed in the spectrum; **LCMS** (ESI^+) *m/z* = 679.05 [$(M+Na)^+$, 100%], R_t = 4.24 min; **HRMS** (ESI^-) calculated for $C_{29}H_{39}N_2O_{11}S_2$ $[M-H]^-$ 655.1995, found 655.1990.

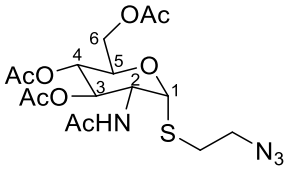
2-Bromoethyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (136)



To a solution of the acetamido mercaptan (**73**) (100 mg, 0.275 mmol, 1 eq.) in an acetone-water mixture (2:1, 1.2 mL) potassium carbonate (46 mg, 0.330 mmol, 1.2 eq.) and 1,2-dibromoethane (0.19 mL, 2.20 mmol, 8 eq.) were added. The reaction was stirred at room temperature for 3 h, after which time it was diluted with DCM (5 mL). The organic layer was collected and the aqueous layer was re-extracted with DCM (5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* furnishing the 2-bromoethyl thioglycoside (**136**) as a yellow solid (80 mg, 62%): R_f 0.37 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, CD_3OD) δ 5.82 (1H, d, *J* = 8.4 Hz, N*H*), 5.50 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.08-5.04 (1H, m, C*H*-4), 5.04-4.98 (1H, m, C*H*-3), 4.49-4.41 (1H, m, C*H*-2), 4.37-4.31 (1H, m, C*H*-5), 4.21 (1H, dd, *J* = 12.3, 5.4 Hz, CH_ACH_B-6), 4.07 (1H, dd, *J* = 12.3, 2.2 Hz, CH_ACH_B-6), 3.59-3.51 (1H, m, CH_ACH_BBr), 3.50-3.43 (1H, m, CH_ACH_BBr), 3.11-3.03 (1H, m, SCH_ACH_B), 3.03-2.95 (1H, m, SCH_ACH_B), 2.07 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.93 (3H, s, NHC(O)CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.6 (*C*=O), 170.6 (*C*=O), 170.1 (*C*=O), 169.3 (*C*=O), 85.1 (*C*H-1), 71.0 (*C*H-3), 68.7 (*C*H-5), 68.2 (*C*H-4), 62.2 (CH_2-6), 52.6 (*C*H-2), 33.9 (SCH_2), 30.3 (CH_2Br), 23.1 (NHC(O)CH_3), 20.8 (OC(O)CH_3), 20.7 (OC(O)CH_3), 20.6 (OC(O)CH_3); **LCMS** (ESI$^+$) *m/z* = 471.85 [(M+H)$^+$, 100%], R_t = 3.94 min; **HRMS** (ESI$^+$) calculated for $C_{16}H_{25}BrNO_8S$ [M+H]$^+$ 470.0479, found 470.0474.

The data are in agreement with the reported literature values.[70]

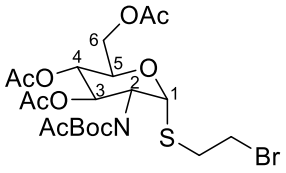
2-Azidoethyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (137)



To a solution of the 2-bromoethyl thioglycoside (**136**) (80 mg, 0.170 mmol, 1 eq.) in DMF (2 mL) tetrabutylammonium iodide (63 mg, 0.170 mmol, 1 eq.) and sodium azide (44 mg, 0.680 mmol, 4 eq.) were added and the reaction was refluxed at 80 °C for 18 h, after which time the reaction was diluted with DCM (5 mL) and washed several times with water. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* furnishing the 2-azidoethyl thioglycoside (**137**) as a yellow oil (60 mg, 82%): R_f 0.82 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.82 (1H, d, *J* = 8.6 Hz, N*H*), 5.48 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.10-5.04 (1H, m, C*H*-4), 5.04-4.99 (1H, m, C*H*-3), 4.48-4.41 (1H, m, C*H*-2), 4.34-4.29 (1H, m, C*H*-5), 4.21 (dd, *J* = 10.0, 7.2 Hz, CH_ACH_B-6), 4.05 (dd, *J* = 12.3, 2.3 Hz, CH_ACH_B-6), 3.51-3.43 (2H, m, CH_2N_3), 2.86-2.79 (1H, m, SCH_ACH_B), 2.78-2.70 (1H, m, SCH_ACH_B), 2.05 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.99 (3H, s, C(O)CH_3), 1.92 (3H, s, NHC(O)CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.6 (*C*=O), 170.5 (*C*=O), 170.1 (*C*=O), 169.3 (*C*=O), 84.8 (*C*H-1), 71.0 (*C*H-3), 68.6 (*C*H-5), 68.1 (*C*H-4), 62.0 (*C*H_2-6), 52.5 (*C*H-2), 50.8 (*C*H_2N_3), 30.9 (S*C*H_2), 23.1 (NHC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3).

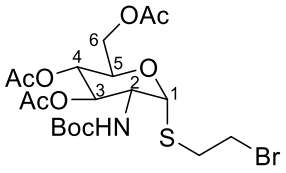
The data are in agreement with the reported literature values.[70]

2-Bromoethyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (139)



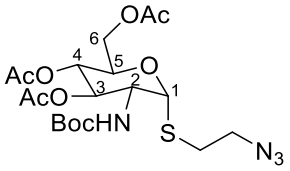
To a solution of the 2-bromoethyl thioglycoside (**136**) (50 mg, 0.106 mmol, 1 eq.) and DMAP (1.3 mg, 0.0106 mmol, 0.1 eq.) in THF (1.5 mL) Boc_2O (127 mg, 0.583 mmol, 5.5 eq.) was added and the reaction was refluxed at 60 °C for 20 h, after which time the reaction was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (25-50% EtOAc in hexane) furnishing 2-bromoethyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**139**) as a yellow oil (50 mg, 83%): R_f 0.80 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.90 (1H, dd, *J* = 11.5, 8.8 Hz, C*H*-3), 5.33 (1H, d, *J* = 5.6 Hz, C*H*-1), 5.05 – 4.95 (1H, m, C*H*-2), 4.87 (1H, dd, *J* = 10.3, 8.7 Hz, C*H*-4), 4.45-4.39 (1H, m, C*H*-5), 4.22 (1H, dd, *J* = 12.3, 5.6 Hz, CH_ACH_B-6), 4.04 (1H, dd, *J* = 12.2, 2.1 Hz, CH$_A$CH_B-6), 3.57-3.49 (1H, m, CH_ACH$_B$Br), 3.43-3.36 (1H, m, CH$_A$CH_BBr), 3.01-2.93 (1H, m, SCH_ACH$_B$), 2.91-2.82 (1H, m, SCH$_A$CH_B), 2.36 (3H, s, NC(O)CH_3), 2.03 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.88 (3H, s, C(O)CH_3), 1.49 (9H, s, NC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 173.6 (*C*=O), 170.6 (*C*=O), 169.9 (*C*=O), 169.4 (*C*=O), 152.2 (N*C*=O), 85.6 (*C*(CH$_3$)$_3$), 84.5 (*C*H-1), 70.7 (*C*H-4), 70.1 (*C*H-3), 68.2 (*C*H-5), 62.3 (*C*H$_2$-6), 55.8 (*C*H-2) 34.2 (S*C*H$_2$), 30.5 (*C*H$_2$Br), 27.7 (NHC(O)*C*H$_3$), 20.8 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 593.80 [(M+Na)$^+$, 100%], R_t = 4.47 min; **HRMS** (ESI$^+$) calculated for $C_{21}H_{33}BrNO_{10}S$ [M+H]$^+$ 570.1008, found 570.1011.

2-Bromoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (140)



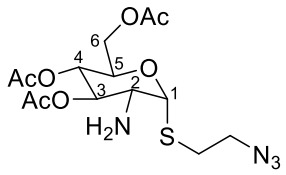
To a solution of 2-bromoethyl 2-acetamido-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**139**) (100 mg, 0.175 mmol, 1 eq.) in methanol (1 mL) DEAEA (*N*,*N*-diethylethylenediamine) (49 µL, 0.350 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 1 h, after which time the reaction was diluted with DCM (5 mL), washed with 0.5 M HCl (5 mL), saturated solution of $CuSO_4$ (5 mL) and $NaHCO_3$ (5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 hexane/EtOAc) furnishing 2-bromoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**140**) as a light yellow oil (30 mg, 32%): R_f 0.50 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.46 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.08-5.02 (1H, m, C*H*-4), 5.02-4.97 (1H, m, C*H*-3) 4.71 (1H, d, *J* = 7.5 Hz, N*H*), 4.38-4.32 (1H, m, C*H*-5), 4.26-4.17 (2H, m, C*H*-2 + CH_ACH_B-6), 4.09 (1H, dd, *J* = 12.3, 2.1 Hz, CH_ACH_B-6), 3.63-3.54 (1H, m, CH_ACH_BBr), 3.52-3.44 (1H, m, CH_ACH_BBr), 3.15-2.98 (2H, m, SCH_2), 2.09 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.42 (9H, s, NC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 171.0 (*C*=O), 170.6 (*C*=O), 169.3 (*C*=O),), 154.9 (N*C*=O), 85.9 (*C*H-1), 80.4 (*C*(CH$_3$)$_3$),71.2 (*C*H-3), 68.8 (*C*H-5), 68.4 (*C*H-4), 62.2 (*C*H$_2$-6), 53.4 (*C*H-2), 34.0 (S*C*H$_2$), 30.1 (*C*H$_2$Br), 28.2 (NC(O)OC(*C*H$_3$)$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$), 20.5 (OC(O)*C*H$_3$); **HRMS** (ESI$^+$) calculated for $C_{19}H_{31}BrNO_9S$ $[M+H]^+$ 528.0897, found 528.0891.

2-Azidoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (141)



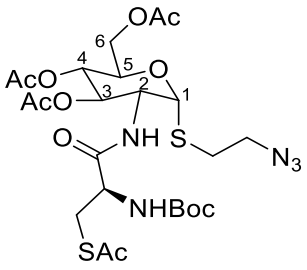
To the solution of 2-bromoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**140**) (100 mg, 0.189 mmol, 1 eq.) in DMF (4 mL) tetrabutylammonium iodide (70 mg, 0.189 mmol, 1 eq.) and sodium azide (49 mg, 0.756 mmol, 4 eq.) were added and the reaction was refluxed at 80 °C for 2 h. The reaction was then diluted with EtOAc (5 mL) and washed several times with water. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* furnishing 2-azidoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**141**) as a yellow oil (60 mg, 65%): R_f 0.90 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.45 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.11-5.05 (1H, m, C*H*-4), 5.05-4.99 (1H, m, C*H*-3), 4.73 (1H, d, *J* = 9.6 Hz, N*H*), 4.37-4.31 (1H, m, C*H*-5), 4.30-4.17 (2H, m, C*H*-2 + CH_ACH_B-6), 4.10 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 3.57-3.45 (2H, m, CH_2N$_3$), 2.92-2.82 (1H, m, SCH_ACH_B), 2.82-2.74 (1H, m, SCH$_A$CH_B), 2.09 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3), 1.42 (9H, s, NC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.3 (*C*=O), 85.8 (*C*H-1), 71.3 (*C*H-3), 68.8 (*C*H-5), 68.3 (*C*H-4), 61.9 (*C*H_2-6), 53.1 (*C*H-2), 50.6 (*C*H_2N$_3$), 30.8 (S*C*H_2), 28.2 (NC(O)OC(*C*H_3)$_3$), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3), the N*C*=O and *C*(CH_3)$_3$ signals were not observed in the spectrum; **HRMS** (ESI$^+$) calculated for $C_{19}H_{34}N_5O_9S$ $[M+NH_4]^+$ 508.2072, found 508.2059.

2-Azidoethyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (142)



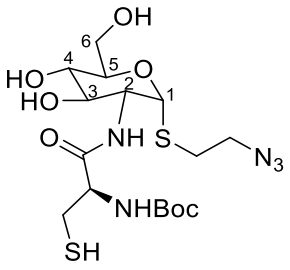
To a solution of 2-azidoethyl 2-amino-*N*-Boc-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**141**) (110 mg, 0.224 mmol, 1 eq.) in dichloromethane (2 mL) TMSOTf (81 µL, 0.488 mmol, 2 eq.) was added at 0 °C and the reaction was stirred at 0 °C for 2 h, after which time the reaction was quenched with cold saturated $NaHCO_3$ (5 mL) and extracted with dichloromethane (3 x 5 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* furnishing 2-azidoethyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**142**) as a yellow solid (87 mg, 100%): R_f 0.43 (5% MeOH in DCM); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.37 (1H, d, *J* = 5.1 Hz, C*H*-1), 4.98-4.95 (2H, m, C*H*-3 + C*H*-4), 4.43-4.38 (1H, m, C*H*-5), 4.30 (1H, dd, *J* = 12.3, 5.0 Hz, CH_ACH_B-6), 4.07 (1H, dd, *J* = 12.3, 2.2 Hz, CH_ACH_B-6), 3.59-3.49 (2H, m, CH_2N$_3$), 3.29-3.24 (1H, m, C*H*-2), 2.96-2.85 (1H, m, SCH_ACH_B), 2.84-2.74 (1H, m, SCH_ACH_B), 2.09 (3H, s, C(O)CH_3), 2.07 (3H, s, C(O)CH_3), 2.03 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ169.2 (*C*=O), 168.7 (*C*=O), 167.7 (*C*=O), 84.3 (*C*H-1), 70.6 (*C*H-3), 67.0 (*C*H-5), 66.6 (*C*H-4), 60.1 (*C*H_2-6), 52.1 (*C*H-2), 49.0 (*C*H_2N$_3$), 29.6 (S*C*H_2), 18.8 (OC(O)*C*H_3), 18.7 (OC(O)*C*H_3), 18.6 (OC(O)*C*H_3); LCMS (ESI$^+$) *m/z* = 413 [(M+Na)$^+$, 50%], R_t = 2.54 min. **HRMS** (ESI$^+$) calculated for $C_{14}H_{23}N_4O_7S$ [M+H]$^+$ 391.1288, found 391.1289.

2-Azidoethyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (143)



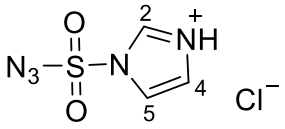
To a solution of *N*-Boc-*S*-acetyl-L-cysteine (**47**) (536 mg, 1.5 eq.) in DMF (15 mL), HOBt (183 mg, 1 eq.) and EDCI (390 mg, 1.5 eq) were added at 0 °C. The reaction was stirred at 0 °C for 0.5 h, after which time a solution of 2-azidoethyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**142**) (530 mg, 1 eq.) in DMF (15 mL) and DIPEA (0.59 mL, 2.5 eq.) were added. The coupling reaction was stirred at 0 °C for 1 h and then at room temperature overnight, after which time it was diluted with ethyl acetate (20 mL). The organic solution was washed with saturated $NaHCO_3$ (30 mL), 5% $KHSO_4$ (30 mL), saturated NaCl (30 mL) and several times with water. The organic layer was collected, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* The resulting crude material was purified by silica gel column chromatography (0-50% EtOAc in DCM) furnishing 2-azidoethyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**143**) as a yellow oil (150 mg, 17%): **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.54 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.12-5.09 (2H, m, C*H*-3 + C*H*-4), 4.45-4.41 (1H, m, C*H*-2), 4.39-4.35 (1H, m, C*H*-5), 4.29-4.25 (2H, m, CH_ACH_B-6 + CysαC*H*), 4.10 (1H, dd, *J* = 12.5, 2.2 Hz, CH_ACH_B-6), 3.57-3.46 (2H, m, CH_2N_3), 3.32-3.28 (1H, m, CysβCH_AH$_B$), 3.06 (1H, dd, *J* = 14.2, 7.6 Hz, CysβCH$_A$$H_B$), 2.90-2.84 (1H, m, SC$H_AH_B$), 2.82-2.75 (1H, m, SCH$_A$$H_B$) 2.37 (3H, s, SC(O)C$H_3$), 2.04 (6H, s, C(O)C$H_3$), 2.03 (3H, s, C(O)C$H_3$), 1.46 (9H, s, NHC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 172.4 (*C*=O), 171.3 (*C*=O), 155.4 (N*C*=O), 84.2 (*C*H-1), 70.4 (*C*H-3), 68.7 (*C*H-4), 68.4 (*C*H-5), 62.0 (*C*H_2-6), 53.5 (CysαCH), 51.5 (*C*H-2), 50.3 (*C*H_2N_3), 30.1 (S*C*H_2), 30.3 (CysβCH$_2$), 28.7 (SC(O)*C*H_3), 26.9 (NHC(O)OC(*C*H_3)$_3$), 18.8 (C(O)*C*H_3), 18.7 (C(O)*C*H_3), the *C*(CH$_3$)$_3$ signal was not observed in the spectrum.

2-Azidoethyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-1-thio-α-D-glucopyranoside (126)



To a solution of 2-azidoethyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**143**) (150 mg, 0.24 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (0.042 mL, 0.20 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 3 h, after which time it was filtered furnishing 2-azidoethyl 2-amino-2-*N*-(*N*-Boc-*S*-acetyl-L-cysteinyl)-2-deoxy-1-thio-α-D-glucopyranoside (**126**) as a white solid (10 mg, 11%): **^{1}H NMR** (500 MHz, DMSO-d_6) δ 7.71 (1H, d, *J* = 7.0 Hz, N*H*), 7.03 (1H, d, *J* = 8.8 Hz, N*H*Boc), 5.44 (1H, d, *J* = 5.1 Hz, C*H*-1), 5.14 (1H, d, *J* = 5.7 Hz, O*H*-4), 4.95 (1H, d, *J* = 5.5 Hz, O*H*-3), 4.61-4.57 (1H, m, O*H*-6), 4.27-4.20 (1H, m, CysαC*H*), 3.82-3.76 (1H, m, C*H*-2), 3.76-3.71 (1H, m, C*H*-5), 3.67 (1H, dd, *J* = 10.4, 5.6 Hz, CH_ACH_B-6), 3.55-3.45 (3H, m, CH_ACH_B-6 + CH_2N$_3$), 3.43-3.37 (1H, m, C*H*-3), 3.19-3.09 (2H, m, C*H*-4 + CysβCH_AH$_B$), 2.83 (1H, dd, *J* = 13.1, 10.0 Hz, CysβCH$_A$*H*$_B$), 2.80-2.68 (2H, m, SCH_2), 1.40 (9H, s, NHC(O)OC(CH_3)$_3$); **^{13}C NMR** (100 MHz, DMSO-d_6) δ 84.3 (*C*H-1), 74.5 (*C*H-5), 71.3 (*C*H-3), 71.2 (*C*H-4), 61.3 (*C*H$_2$-6), 55.0 (*C*H-2), 54.2 (CysαCH), 51.0 (*C*H$_2$N$_3$), 40.8 (CysβCH$_2$), 30.1 (SCH$_2$), 28.8 (NHC(O)OC(*C*H$_3$)$_3$), the N*C*=O and *C*(CH$_3$)$_3$ signals were not observed in the spectrum; **HRMS** (ESI$^-$) calculated for $C_{16}H_{28}N_5O_7S_2$ [M-H]$^-$ 466.1430, found 466.1433.

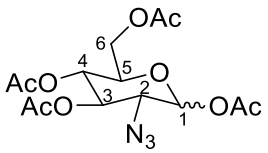
Imidazole-1-sulfonyl azide hydrochloride (149)



Sulfuryl chloride (3.11 mL, 38.46 mmol, 1 eq.) was added dropwise to an ice-cold suspension of sodium azide (2.50 g, 38.46 mmol, 1 eq.) in anhydrous acetonitrile (38 mL). The reaction was stirred at room temperature for 22 h, cooled to 0 °C and imidazole (5.24 g, 76.92 mmol, 2 eq.) was added portion wise. The reaction was stirred at room temperature for a further 3 h, after which time the reaction was diluted with ethyl acetate (100 mL), washed with water (2 x 100 mL) and saturated aqueous sodium hydrogen carbonate (2 x 100 mL). The organic layer was collected, dried over anhydrous $MgSO_4$ and cooled to 0 °C. Acetyl chloride (4.12 mL, 57.69 mmol, 1.5 eq.) was added to ice-cold anhydrous ethanol (17.5 mL) and added dropwise to the ice-cold ethyl acetate solution. The resulting precipitate was filtered and washed with ethyl acetate (2 x 50 mL) to furnish imidazole-1-sulfonyl azide HCl (**149**) as a colourless solid (5.0 g, 62%): R_f 0.42 (50:50 methanol/water, RP C-18 TLC); **^{1}H NMR** (400 MHz, D_2O) δ 9.11 (1H, dd, *J* = 1.6, 1.2 Hz C*H*-2); 7.91 (1 H, dd, *J* = 1.8, 1.6 Hz, C*H*-5), 7.49 (1H, dd, *J* = 1.8, 1.2 Hz, C*H*-4); **^{13}C NMR** (100 MHz, D_2O) δ 137.8 (*C*H-2), 125.1 (*C*H-5), 119.8 (*C*H-4); **LCMS** (ESI^+) *m/z* = 174.05 [$(M+H)^+$, 100%], R_t = 2.40 min; **HRMS** (ESI^+) calculated for $C_3H_4N_5O_2S$ $[M-Cl]^+$ 174.0080, found 174.0076.

The data are in agreement with the reported literature values.[71]

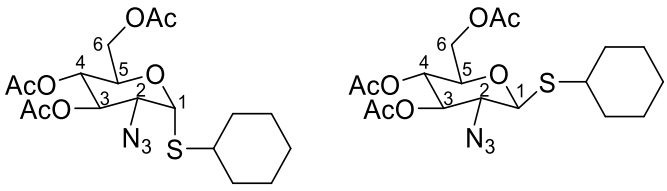
2-Azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranose (148)



To a solution of glucosamine HCl (**107**) (1.00 g, 4.64 mmol, 1 eq.), potassium carbonate (1.28 g, 9.28 mmol, 2 eq.) and copper sulphate pentahydrate (12 mg, 0.05 mmol, 0.01 eq.) in methanol (23 mL) imidazole-1-sulphonyl azide HCl (**149**) (1.17 g, 5.57 mmol, 1.2 eq.) was added and the mixture was stirred at room temperature for 4 h, after which time the reaction was concentrated and co-evaporated with toluene (2 x 20 mL). The residue was dissolved in pyridine (23 mL) and acetic anhydride (3.5 mL) was added. The reaction was stirred at room temperature for 4 h. After which time, it was concentrated, diluted with water (80 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 hexane/ethyl acetate) furnishing the azide (**148**) as a yellow gum (2:1 β/α) (1.34 g, 77%): R_f 0.33 (3:1 hexane/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.21 (1H, d, *J* = 3.6 Hz, C*H*-1α), 5.46 (2H, d, *J* = 8.6 Hz, C*H*-1β), 5.40-5.34 (1H, m, C*H*-3α), 5.05-4.93 (5H, m, C*H*-3β +C*H*-4αβ), 4.24-4.21 (1H, m, CH_ACH_B-6α), 4.21-4.18 (2H, m, CH_ACH_B -6β), 4.05-3.94 (4H, m, CH_ACH_B-6αβ + C*H*-5α), 3.73-3.69 (2H, m, C*H*-5β), 3.60-3.55 (3H, m, C*H*-2αβ), 2.10 (9H, s, C(O)CH_3-αβ), 2.02 (3H, s, C(O)CH_3-α), 2.01 (6H, s, C(O)CH_3-β), 1.99 (9H, s, C(O)CH_3-αβ), 1.96 (3H, s, C(O)CH_3-α), 1.94 (6H, s, C(O)CH_3-β); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.8 (*C*=O), 169.6 (*C*=O), 168.6 (*C*=O), 92.6 (*C*H-1β), 90.0 (*C*H-1α), 72.7 (*C*H-5β), 72.7 (*C*H-3β), 70.7 (*C*H-3α), 69.8 (*C*H-5α), 67.8 (*C*H-4αβ), 62.6 (*C*H-2αβ), 61.4 (*C*H_2-6αβ), 20.9 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{14}H_{23}N_4O_9$ $[M+NH_4]^+$ 391.1460, found 391.1448.

The data are in agreement with the reported literature values.[74]

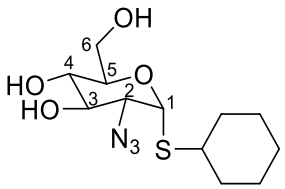
Cyclohexyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (147) and cyclohexyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (150)



To a solution of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**148**) (250 mg, 0.670 mmol, 1 eq.) and cyclohexanethiol (0.16 mL, 1.34 mmol, 2 eq.) in DCM (5 mL) $BF_3{\cdot}OEt_2$ (0.37 mL, 3.02 mmol, 4.5 eq.) was added at 0 °C under argon. The reaction was stirred at 55 °C for 5 h, after which time the reaction was diluted with DCM (5 mL), washed with brine (3 x 10 mL) and the organic layer separated. The aqueous layer was extracted with DCM (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 hexane/ethyl acetate) furnishing pure α- (**147**) (15 mg, 5%) and β- (**150**) (30 mg, 10%) anomers as yellow oils: $R_{f\alpha}$ 0.29, $R_{f\beta}$ 0.21 (3:1 hexane/ethyl acetate); **α-anomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.44 (1H, d, *J* = 5.6 Hz, C*H*-1), 5.20 (1H, dd, *J* = 10.3, 9.4 Hz, C*H*-3), 4.96-4.89 (1H, m, C*H*-4), 4.45-4.40 (1H, m, C*H*-5), 4.23 (1H, dd, *J* = 12.3, 4.9 Hz, CH_ACH_B-6), 4.00 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 3.94-3.89 (1H, m, C*H*-2), 2.80-2.73 (1H, m, C*H*-1'), 2.02 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.97 (3H, s, C(O)CH_3), 1.94-1.86 (2H, m, cyclohexyl), 1.75-1.64 (4H, m, cyclohexyl), 1.57-1.49 (2H, m, cyclohexyl), 1.42-1.31 (2H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.9 (*C*=O), 169.8 (*C*=O), 82.4 (*C*H-1), 71.9 (*C*H-3), 68.4 (*C*H-4), 68.0 (*C*H-5), 62.0 (*C*H_2-6), 61.5 (*C*H-2) 43.5 (*C*H-1'), 34.0 (cyclohexyl), 33.5 (cyclohexyl), 31.6 (cyclohexyl), 25.8 (cyclohexyl), 25.6 (cyclohexyl), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **β-anomer**: **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.01-4.96 (1H, m, C*H*-3), 4.96-4.90 (1H, m, C*H*-4), 4.40 (1H, d, *J* = 10.3 Hz, C*H*-1), 4.16 (1H, dd, *J* = 12.2, 5.5 Hz, CH_ACH_B-6), 4.05-4.01 (1H, m, CH_ACH_B-6), 3.64-3.55 (1H, m, C*H*-5), 3.44-3.39 (1H, m, C*H*-2), 2.94-2.89 (1H, m, C*H*-1'), 2.03 (3H,

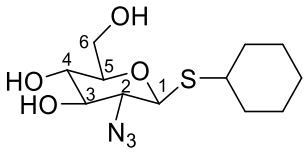
s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.91-1.82 (1H, m, cyclohexyl), 1.75-1.66 (4H, m, cyclohexyl), 1.59-1.51 (2H, m, cyclohexyl), 1.42-1.32 (3H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.0 (*C*=O), 169.6 (*C*=O), 83.9 (*C*H-1), 75.6 (*C*H-5), 74.5 (*C*H-3), 68.4 (*C*H-4), 63.9 (*C*H-2), 62.3 (*C*H_2-6), 44.3 (*C*H-1'), 34.0 (cyclohexyl), 33.8 (cyclohexyl), 31.6 (cyclohexyl), 26.0 (cyclohexyl), 25.6 (cyclohexyl), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **HRMS** (ESI^-) calculated for $C_{18}H_{27}N_3O_7SCl$ $[M+Cl]^-$ 464.1258, found 464.1251 (α-anomer), 464.1262 (β-anomer).

Cyclohexyl 2-azido-2-deoxy-1-thio-α-D-glucopyranoside (144)



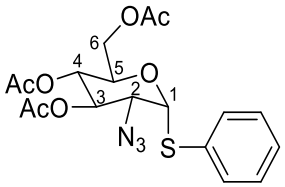
To a solution of cyclohexyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**147**) (15 mg, 0.035 mmol, 1.2 eq.) in methanol (1.5 mL) 25% sodium methoxide solution in methanol (0.006 mL, 0.029 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (8:2 DCM/MeOH) furnishing cyclohexyl 2-azido-2-deoxy-1-thio-α-D-glucopyranoside (**144**) as a yellow oil (7 mg, 64%): R_f 0.20 (8:2 DCM/MeOH); **^{1}H NMR** (500 MHz, MeOD) δ 5.48 (1H, d, *J* = 4.9 Hz, C*H*-1), 4.01-3.97 (1H, m, C*H*-5), 3.80 (1H, dd, *J* = 12.0, 2.4 Hz, CH_ACH_B-6), 3.73 (1H, dd, *J* = 12.0, 5.2 Hz, CH_ACH_B-6), 3.66-3.58 (2H, m, C*H*-2 + CH-3), 3.39-3.34 (1H, m, C*H*-4), 2.93-2.86 (1H, m, C*H*-1'), 2.10-1.98 (2H, m, cyclohexyl), 1.83-1.74 (2H, m, cyclohexyl), 1.66-1.59 (1H, m, cyclohexyl), 1.48-1.29 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, MeOD) δ 84.1 (*C*H-1), 74.5 (*C*H-5), 74.3 (*C*H-3), 72.1 (*C*H-2), 65.2 (*C*H-4), 62.4 (*C*H_2-6), 44.4 (*C*H-1'), 35.3 (cyclohexyl), 34.9 (cyclohexyl), 27.1 (cyclohexyl), 26.9 (cyclohexyl), 26.7 (cyclohexyl); **HRMS** (ESI$^+$) calculated for $C_{12}H_{22}N_3O_4S$ $[M+H]^+$ 304.1326, found 304.1327.

Cyclohexyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (151)



To a solution of 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**150**) (30 mg, 0.070 mmol, 1.2 eq.) in methanol (3 mL) 25% sodium methoxide solution in methanol (0.012 mL, 0.058 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (8:2 DCM/MeOH) furnishing cyclohexyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (**151**) as a yellow oil (21 mg, 100%): R_f 0.45 (8:2 DCM/MeOH); **^{1}H NMR** (500 MHz, MeOD) δ 4.48 (1H, d, *J* = 10.2 Hz, C*H*-1), 3.85 (1H, dd, *J* = 12.1, 2.2 Hz, CH_ACH_B-6) 3.66 (1H, dd, *J* = 12.1, 5.6 Hz, CH_ACH_B-6), 3.39-3.34 (2H, m, C*H*-3 + C*H*-4), 3.29-3.25 (1H, m, C*H*-5), 3.15-3.11 (1H, m, C*H*-2), 3.09-3.02 (1H, m, C*H*-1'), 2.11-1.99 (2H, m, cyclohexyl), 1.83-1.75 (2H, m, cyclohexyl), 1.67-1.60 (1H, m, cyclohexyl), 1.48-1.29 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, MeOD) δ 84.7 (*C*H-1), 82.0 (*C*H-5), 78.4 (*C*H-3), 71.4 (*C*H-4), 68.5 (*C*H-2), 62.7 (*C*H_2-6), 44.5 (*C*H-1'), 35.3 (cyclohexyl), 34.9 (cyclohexyl), 34.8 (cyclohexyl), 27.1 (cyclohexyl), 26.9 (cyclohexyl); **HRMS** (ESI$^+$) calculated for $C_{12}H_{21}N_3O_4SNa$ $[M+Na]^+$ 326.1152, found 326.1152.

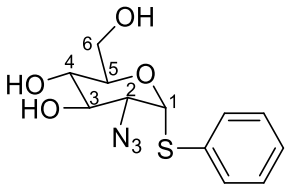
Phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (152)



To a solution of 2-azido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranose (**148**) (250 mg, 0.670 mmol, 1 eq.) and thiophenol (0.14 mL, 1.34 mmol, 2 eq.) in DCM (5 mL) $BF_3 \cdot OEt_2$ (0.37 mL, 3.02 mmol, 4.5 eq.) was added at 0 °C under argon. The reaction was stirred at 55 °C for 5 h, after which time the reaction was diluted with DCM (5 mL), washed with brine (3 x 10 mL) and the organic layer separated. The aqueous layer was extracted with DCM (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (3:1 hexane/ethyl acetate) furnishing 3:1 α/β anomeric mixture as a yellow oil (175 mg, 62%). The α-anomer (**152**) was obtained as white crystals by crystallization from ethanol (60 mg, 21%): R_f 0.31 (3:1 hexane/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46-7.40 (2H, m, Ar*H*), 7.29-7.23 (3H, m, Ar*H*), 5.58 (1H, d, *J* = 5.6 Hz, C*H*-1), 5.30-5.25 (1H, m, C*H*-3), 5.01-4.95 (1H, m, C*H*-4), 4.56-4.50 (1H, m, C*H*-5), 4.23 (1H, dd, *J* = 12.4, 5.1 Hz, CH_ACH_B-6), 4.02 (1H, dd, *J* = 10.6, 5.6 Hz, C*H*-2), 3.97 (1H, dd, *J* = 12.4, 2.1 Hz, CH_ACH_B-6), 2.04 (3H, s, C(O)CH_3), 1.99 (3H, s, C(O)CH_3), 1.97 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.5 (*C*=O), 169.8 (*C*=O), 132.4 (Ar), 132.2 (Ar), 129.3 (Ar), 128.1 (Ar), 86.5 (*C*H-1), 72.1 (*C*H-3), 68.7 (*C*H-4), 68.5 (*C*H-5), 61.9 (*C*H_2-6), 61.6 (*C*H-2), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI^+) *m/z* = 446.10 [$(M+Na)^+$, 100%], R_t = 4.18 min; **HRMS** (ESI^+) calculated for $C_{18}H_{21}N_3O_7SNa$ $[M+Na]^+$ 446.0992, found 446.0981.

The data are in agreement with the reported literature values.[22]

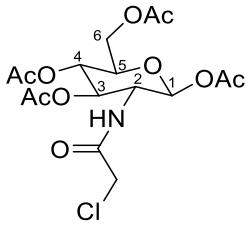
Phenyl 2-azido-2-deoxy-1-thio-α-D-glucopyranoside (145)



To a solution of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (25 mg, 0.059 mmol, 1.2 eq.) in anhydrous methanol (2 mL) sodium methoxide (2.6 mg, 0.049 mmol, 1 eq.) was added under argon. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (19:1 DCM/MeOH) furnishing phenyl-2-azido-2-deoxy-1-thio-α-D-glucopyranose (**145**) as a white solid (18 mg, 100%): R_f 0.17 (19:1 DCM/MeOH); **^{1}H NMR** (500 MHz, MeOD) δ 7.54-7.49 (2H, m, Ar*H*), 7.32-7.23 (3H, m, Ar*H*), 5.55 (1H, d, *J* = 5.2 Hz, C*H*-1), 4.10-4.04 (1H, m, C*H*-5), 3.76-3.71 (2H, m, C*H*-2+CH_2-6), 3.67-3.61 (1H, m, C*H*-3), 3.47-3.37 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 135.5 (Ar), 133.5 (Ar), 130.1 (Ar), 128.7 (Ar), 89.2 (*C*H-1), 75.0 (*C*H-5), 74.7 (*C*H-3), 71.9 (*C*H-4), 65.4 (*C*H-2), 62.2 (*C*H_2-6); **LCMS** (ESI$^+$) *m/z* = 342.20 [(M+FA-H)$^-$, 100%], R_t = 2.79 min; **HRMS** (ESI$^+$) calculated for $C_{12}H_{19}N_4O_4S$ $[M+NH_4]^+$ 315.1122, found 315.1126.

The data are in agreement with the reported literature values.[75]

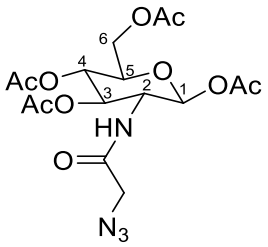
2-Chloroacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (161)



To a solution of 2-amino-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose HCl (**40**) (100 mg, 0.261 mmol) in dichloromethane (5 mL) triethylamine (55 µL, 0.392 mmol, 1.5 eq.) and chloroacetic anhydride (67 mg, 0.392 mmol, 1.5 eq.) were added at 0 °C and the reaction was stirred at room temperature for 1 h, after which time the reaction was quenched with cold saturated Na_2CO_3 (5 mL) and washed with 5% $KHSO_4$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* furnishing 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**161**) as a dark blue solid (90 mg, 81%): R_f 0.10 (4:1 hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.57 (1H, d, *J* = 9.2 Hz, N*H*), 5.81 (1H, d, *J* = 8.6 Hz, C*H*-1), 5.30-5.25 (1H, m, C*H*-3), 5.17-5.12 (1H, m, C*H*-4), 4.29 (1H, dd, *J* = 12.5, 4.6 Hz, CH_ACH_B-6), 4.25-4.18 (1H, m, C*H*-2), 4.13 (1H, dd, *J* = 12.5, 2.2 Hz, CH$_A$CH_B-6), 4.00 (1H, d, *J* = 15.2 Hz, CH_ACH$_B$Cl), 3.97 (1H, d, *J* = 15.2 Hz, CH$_A$CH_BCl), 3.86-3.82 (1H, m, C*H*-5), 2.12 (3H, s, C(O)CH_3), 2.09 (3H, s, C(O)CH_3), 2.05 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.0 (*C*=O), 169.7 (*C*=O), 168.4 (*C*=O), 165.7 (*C*=O), 91.0 (*C*H-1), 71.8 (*C*H-5), 71.0 (*C*H-3), 67.1 (*C*H-4), 60.8 (*C*H$_2$-6), 52.1 (*C*H-2), 41.4 (*C*H$_2$Cl), 19.9 (OC(O)*C*H$_3$), 19.7 (OC(O)*C*H$_3$), 19.6 (OC(O)*C*H$_3$), 19.5 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 445.85 [(M+Na)$^+$, 100%], R_t = 2.94 min; **HRMS** (ESI$^+$) calculated for $C_{16}H_{26}ClN_2O_{10}$ [M+NH$_4$]$^+$ 441.1270, found 441.1269.

The data are in agreement with the reported literature values.[76]

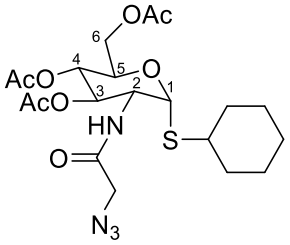
2-Azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (162)



To the solution of 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**161**) (100 mg, 0.236 mmol, 1 eq.) in DMF (3 mL) sodium azide (61 mg, 0.944 mmol, 4 eq.) was added and the reaction was refluxed at 80 °C for 2 h, after which time the reaction was diluted with EtOAc (5 mL) and washed several times with water. The organic layer was collected, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* furnishing 2-azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**162**) as a yellow solid (60 mg, 59%): R_f 0.26 (4:1 hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.38 (1H, d, *J* = 9.2 Hz, N*H*), 5.79 (1H, d, *J* = 8.7 Hz, C*H*-1), 5.26-5.20 (1H, m, C*H*-3), 5.17-5.11 (1H, m, C*H*-4), 4.29 (1H, dd, *J* = 12.5, 4.6 Hz, CH_ACH_B-6), 4.25-4.18 (1H, m, C*H*-2), 4.13 (1H, dd, *J* = 12.5, 2.3 Hz, CH$_A$CH_B-6), 3.92 (2H, s, CH_2N_3), 3.85-3.80 (1H, m, C*H*-5), 2.12 (3H, s, C(O)CH_3), 2.09 (3H, s, C(O)CH_3), 2.05 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.0 (*C*=O), 170.7 (*C*=O), 169.4 (*C*=O), 169.3 (*C*=O), 92.1 (*C*H-1), 72.8 (*C*H-5), 72.2 (*C*H-3), 67.9 (*C*H-4), 61.7 (*C*H$_2$-6), 53.0 (*C*H-2), 52.6 (*C*H$_2$N$_3$), 20.9 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$), 20.5 (OC(O)*C*H$_3$); **LCMS** (ESI$^+$) *m/z* = 509.85 [(M+DMSO+H)$^+$, 100%], R_t = 3.80 min; **HRMS** (ESI$^+$) calculated for $C_{16}H_{22}N_4O_{10}Na$ [M+Na]$^+$ 453.1234, found 453.1231.

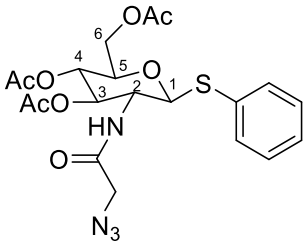
The data are in agreement with the reported literature values.[77]

Cyclohexyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (163)



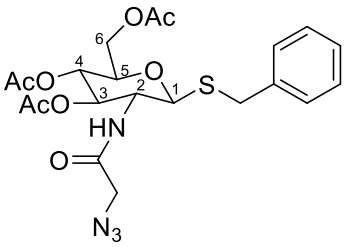
To a solution of 2-azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**162**) (50 mg, 0.116 mmol, 1 eq.) in 1,2-dichloroethane (1 mL) cyclohexanethiol (0.057 mL, 0.464 mmol, 4 eq.) was added and the reaction was stirred at room temperature under argon for 20 min, after which time the reaction was cooled to 0 °C and $BF_3{\cdot}OEt_2$ (0.029 mL, 0.232 mmol, 2 eq.) was added under argon. Then the reaction was stirred at 55 °C for 3 h. After which time, the reaction was quenched with sodium bicarbonate solution and diluted with DCM (5 mL). The organic layer was washed with brine (3 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (20-65% ethyl acetate in hexane) furnishing cyclohexyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**163**) as yellow oil (10 mg, 18%): R_f 0.12 (4:1 hexane/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.58 (1H, d, *J* = 8.9 Hz, N*H*), 5.48 (1H, d, *J* = 5.5 Hz, C*H*-1), 5.14-5.05 (2H, m, C*H*-3 + C*H*-4), 4.52-4.46 (1H, m, C*H*-2), 4.44-4.40 (1H, m, C*H*-5), 4.27 (1H, dd, *J* = 12.4, 4.6 Hz, CH_ACH_B-6), 4.02 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 3.92 (2H, s, CH_2N_3), 2.87-2.80 (1H, m, C*H*-1'), 2.09 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.99-1.93 (2H, m, cyclohexyl), 1.79-1.71 (2H, m, cyclohexyl), 1.64-1.57 (1H, m, cyclohexyl), 1.47-1.24 (5H, m, cyclohexyl); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.3 (*C*=O), 170.7 (*C*=O), 169.4 (*C*=O), 83.4 (*C*H-1), 71.4 (*C*H-3), 68.5 (*C*H-4), 68.1 (*C*H-5), 62.0 (CH_2-6), 52.6 (CH_2N_3), 52.3 (*C*H-2) 45.2 (*C*H-1'), 34.2 (cyclohexyl), 33.7 (cyclohexyl), 25.9 (cyclohexyl), 25.8 (cyclohexyl), 25.5 (cyclohexyl), 20.8 (OC(O)CH_3), 20.7 (OC(O)CH_3), 20.6 (OC(O)CH_3); **HRMS** (ESI$^+$) calculated for $C_{20}H_{31}N_4O_8S$ $[M+H]^+$ 487.1857, found 487.1853.

Phenyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (164)



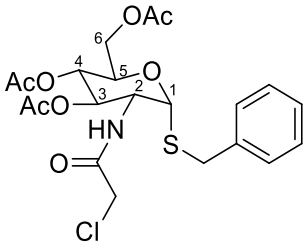
To a solution of 2-azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**162**) (100 mg, 0.232 mmol, 1 eq.) in dry dichloromethane (2 mL) thiophenol (0.10 mL, 0.928 mmol, 4 eq.) was added and the reaction was stirred for 20 min at room temperature under argon. The reaction was then cooled to 0 °C and $BF_3 \cdot OEt_2$ (57 µL, 0.464 mmol, 2 eq.) was added drop-wise. The reaction was subsequently heated to 55 °C and stirred for 3 h, after which time the reaction was quenched with saturated $NaHCO_3$ (5 mL) and extracted with DCM (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (25-65% ethyl acetate in hexane) furnishing phenyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**164**) as a yellow solid (50 mg, 45%): R_f 0.48 (1:2 hexane/ethyl acetate); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45-7.41 (2H, m, Ar*H*), 7.26-7.22 (3H, m, Ar*H*), 6.64-6.58 (1H, m, N*H*), 5.30-5.24 (1H, m, C*H*-3), 5.01-4.95 (1H, m, C*H*-4), 4.91 (1H, d, *J* = 10.4 Hz, C*H*-1), 4.15 (1H, dd, *J* = 12.2, 5.3 Hz, CH_ACH_B-6), 4.12-4.08 (1H, m, CH_ACH_B-6), 3.95-3.83 (3H, m, C*H*-2 + CH_2N_3), 3.72-3.66 (1H, m, C*H*-5), 2.00 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.92 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.9 (*C*=O), 170.6 (*C*=O), 169.4 (*C*=O), 167.0 (*C*=O), 132.8 (Ar), 132.1 (Ar), 129.0 (Ar), 128.3 (Ar), 86.1 (*C*H-1), 75.8 (*C*H-5), 73.3 (*C*H-3), 68.5 (*C*H-4), 62.4 (CH_2-6), 53.5 (*C*H-2), 52.7 (CH_2N_3), 20.8 (OC(O)CH_3), 20.7 (OC(O)CH_3), 20.6 (OC(O)CH_3); **LCMS** (ESI^+) *m/z* = 502.90 [$(M+Na)^+$, 100%], R_t = 3.55 min; **HRMS** (ESI^+) calculated for $C_{20}H_{24}N_4O_8SNa$ $[M+Na]^+$ 503.1212 found, 503.1200.

Benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (165)



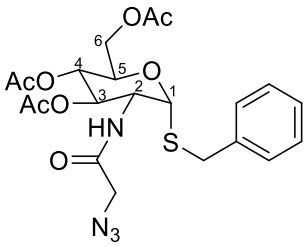
To a solution of 2-azidoacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**162**) (100 mg, 0.232 mmol, 1 eq.) in dry 1,2-dichloroethane (2 mL) benzyl mercaptan (0.11 mL, 0.928 mmol, 4 eq.) was added and the reaction was stirred for 20 min at room temperature under argon. The reaction was then cooled to 0 °C and $BF_3{\cdot}OEt_2$ (57 µL, 0.464 mmol, 2 eq.) was added drop-wise. The reaction was subsequently heated to 55 °C and stirred for 3 h, after which time the reaction was quenched with saturated $NaHCO_3$ (5 mL) and extracted with DCM (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (25-65% ethyl acetate in hexane) furnishing benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**165**) as a white solid (60 mg, 52%): R_f 0.40 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.28-7.18 (5H, m, Ar*H*), 6.25 (1H, d, *J* = 9.4 Hz, N*H*), 5.08-4.99 (2H, m, C*H*-3 + C*H*-4), 4.31 (1H, d, *J* = 10.4 Hz, C*H*-1), 4.17 (1H, dd, *J* = 12.3, 5.2 Hz, CH_ACH_B-6), 4.11-4.04 (2H, m, C*H*-2 + CH_ACH_B-6), 3.87 (1H, d, *J* = 13.0 Hz, SCH_ACH_B), 3.86-3.80 (2H, m, CH_2N$_3$), 3.77 (1H, d, *J* = 13.0 Hz, SCH_ACH_B), 3.55-3.50 (1H, m, C*H*-5), 2.05 (3H, s, C(O)CH_3), 1.95 (3H, s, C(O)CH_3), 1.94 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.9 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 167.0 (*C*=O), 129.1 (Ar), 128.9 (Ar), 128.7 (Ar), 127.5 (Ar), 82.6 (*C*H-1), 75.9 (*C*H-5), 73.4 (*C*H-3), 68.4 (*C*H-4), 62.4 (*C*H_2-6), 53.1 (*C*H-2), 52.5 (*C*H_2N$_3$), 33.9 (S*C*H_2), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 517.10 [(M+Na)$^+$, 100%], R_t = 3.62 min; **HRMS** (ESI$^+$) calculated for $C_{21}H_{26}N_4O_8SNa$ [M+Na]$^+$ 517.1369, found 517.1364.

Benzyl 2-chloroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (166)



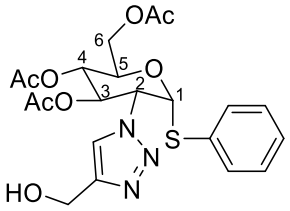
To a solution of benzyl 2-amino-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**134**) (70 mg, 0.170 mmol) in dichloromethane (2.5 mL) triethylamine (35 µL, 0.255 mmol, 1.5 eq.) and chloroacetic anhydride (44 mg, 0.255 mmol, 1.5 eq.) were added at 0 °C and the reaction was stirred at room temperature for 1 h. After which time, the reaction was quenched with cold saturated Na_2CO_3 (1 mL) and washed with 5% $KHSO_4$ and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* furnishing benzyl 2-chloroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**166**) as a violet oil (60 mg, 72%): R_f 0.11 (4:1 hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.34-7.24 (5H, m, Ar*H*), 6.68 (1H, d, *J* = 8.6 Hz, N*H*), 5.32 (1H, d, *J* = 5.3 Hz, C*H*-1), 5.15-5.10 (2H, m, C*H*-3 + C*H*-4), 4.46-4.40 (1H, m, C*H*-2), 4.35-4.31 (1H, m, C*H*-5), 4.26 (1H, dd, *J* = 12.4, 4.3 Hz, CH_ACH_B-6), 3.94 (1H, dd, *J* = 12.4, 2.1 Hz, CH$_A$CH_B-6), 3.90 (1H, d, *J* = 15.2 Hz, CH_ACH$_B$Cl), 3.85 (1H, d, *J* = 15.2 Hz, CH$_A$CH_BCl), 3.82 (1H, d, *J* = 13.6 Hz, SCH_ACH$_B$), 3.78 (1H, d, *J* = 13.6 Hz, SCH$_A$CH_B), 2.10 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.2 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 137.1 (Ar), 132.0 (Ar), 129.5 (Ar), 128.4 (Ar), 83.2 (*C*H-1), 71.1 (*C*H-3), 68.9 (*C*H-5), 68.0 (*C*H-4), 61.8 (*C*H_2-6), 52.6 (*C*H-2), 41.2 (*C*H_2Cl), 35.2 (S*C*H_2), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 510.05 [(M+Na)$^+$, 100%], R_t = 3.81 min; **HRMS** (ESI$^-$) calculated for $C_{21}H_{25}ClNO_8S$ [M-H]$^-$ 486.0989, found 486.1003.

Benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (167)



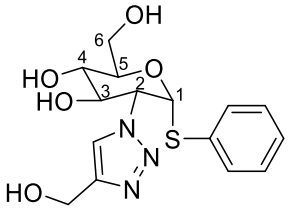
To the solution of benzyl 2-chloroacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**166**) (15 mg, 0.0307 mmol, 1 eq.) in DMF (1 mL) sodium azide (8 mg, 0.123 mmol, 4 eq.) was added and the reaction was refluxed at 80 °C for 2 h, after which time the reaction was diluted with EtOAc (5 mL) and washed several times with water. The organic layer was collected, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* furnishing benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**167**) as a colourless oil (12 mg, 82%): R_f 0.18 (4:1 hexane/EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.37-7.23 (5H, m, Ar*H*), 6.45 (1H, d, *J* = 8.7 Hz, N*H*), 5.33 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.15-5.07 (2H, m, C*H*-3 + C*H*-4), 4.47-4.41 (1H, m, C*H*-5), 4.35-4.30 (1H, m, C*H*-2), 4.25 (1H, dd, *J* = 12.4, 4.3 Hz, CH_ACH_B-6), 3.93 (1H, dd, *J* = 12.3, 1.9 Hz, CH_ACH_B-6), 3.86-3.74 (4H, m, CH_2N_3 + SCH_2), 2.10 (3H, s, C(O)CH_3), 2.04 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.4 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 137.1 (Ar), 129.0 (Ar), 128.7 (Ar), 127.6 (Ar), 83.3 (*C*H-1), 71.2 (*C*H-3), 68.8 (*C*H-5), 68.0 (*C*H-4), 61.8 (*C*H_2-6), 52.4 (*C*H_2N_3), 52.2 (*C*H-2), 35.1 (S*C*H_2), 20.8 (OC(O)*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 516.95 [(M+Na)$^+$, 100%], R_t = 3.82 min; **HRMS** (ESI$^-$) calculated for $C_{21}H_{25}N_4O_8S$ [M-H]$^-$ 493.1393, found 493.1375.

Phenyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (170)



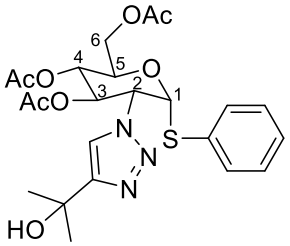
Sodium ascorbate (2.4 mg, 0.012 mmol, 0.25 eq.) and copper sulphate pentahydrate (3.0 mg, 0.012 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (20 mg, 0.047 mmol, 1 eq.) and propargyl alcohol (5.4 µL, 0.094 mmol, 2 eq.) in THF/water (1:1, 1 mL). The reaction was stirred at room temperature for 18 h, after which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* furnishing phenyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**170**) as a white solid (23 mg, 100%): R_f 0.18 (3:7 ethyl acetate/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.66 (1H, s, C*H*, triazole), 7.25-7.20 (5H, m, Ar*H*), 5.85-5.79 (1H, m, C*H*-3), 5.75 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.32 (1H, dd, *J* = 11.7, 5.4 Hz, C*H*-2), 5.18-5.13 (1H, m, C*H*-4), 4.74 (2H, s, CH_2OH), 4.67-4.60 (1H, m, C*H*-5), 4.29 (1H, dd, *J* = 12.4, 5.0 Hz, CH_ACH_B-6), 4.06 (1H, dd, *J* = 12.4, 2.1 Hz, CH$_A$CH_B-6), 2.02 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 1.83 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.1 (*C*=O), 169.5 (*C*=O), 132.2 (Ar), 131.4 (Ar), 129.4 (Ar), 128.5 (Ar), 120.8 (C*C*H, triazole) 87.9 (*C*H-1), 69.1 (*C*H-5), 68.9 (*C*H-4), 68.8 (*C*H-3), 62.1 (*C*H-2), 61.8 (*C*H$_2$-6), 53.5 (*C*H$_2$OH), 20.7 (OC(O)*C*H$_3$), 20.6 (OC(O)*C*H$_3$), 20.5 (OC(O)*C*H$_3$), the *C*CH triazole signal was not observed in the spectrum; **LCMS** (ESI$^+$) *m/z* = 480.05 [(M+H)$^+$, 100%], R_t = 3.31 min.

Phenyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (171)



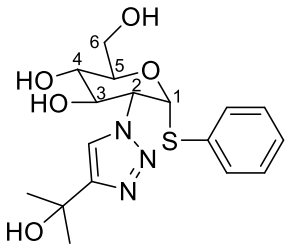
To a solution of phenyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**170**) (20 mg, 0.042 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (8 µL, 0.035 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo* furnishing phenyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**171**) as a yellow solid (9 mg, 60%): **^{1}H NMR** (500 MHz, MeOD) δ 7.99 (1H, s, CC*H*, triazole), 7.24-7.19 (2H, m, Ar*H*), 7.18-7.13 (3H, m, Ar*H*), 5.66 (1H, d, *J* = 5.3 Hz, C*H*-1), 4.88 (1H, dd, *J* = 11.1, 5.3 Hz, C*H*-2), 4.63 (2H, s, C*H*$_2$OH), 4.27-4.19 (1H, m, C*H*-3), 4.15-4.09 (1H, m, C*H*-5), 3.80-3.69 (2H, m, C*H*$_2$-6), 3.55-3.48 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 133.5 (Ar), 130.1 (Ar), 129.0 (Ar), 124.2 (C*C*H, triazole), 90.5 (*C*H-1), 75.4 (*C*H-5), 72.5 (*C*H-4), 71.5 (*C*H-3), 65.8 (*C*H-2), 62.2 (*C*H$_2$-6), 56.6 (*C*H$_2$OH), the *C*CH triazole and quaternary aromatic carbon signals were not observed in the spectrum; **HRMS** (ESI$^+$) calculated for $C_{15}H_{20}N_3O_5S$ $[M+H]^+$ 354.1118, found 354.1121.

Phenyl 2-(4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (172)



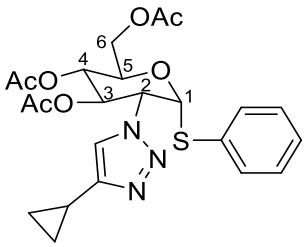
Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (30 mg, 0.071 mmol, 1 eq.) and 2-methyl-3-butyn-2-ol (14 µL, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h. After which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo* furnishing phenyl 2-(4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**172**) as a yellow solid (30 mg, 83%): R_f 0.39 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.59 (1H, s, C*H*, triazole), 7.20 (5H, s, Ar*H*), 5.84-5.78 (1H, m, C*H*-3), 5.73 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.30 (1H, dd, *J* = 11.7, 5.4 Hz, C*H*-2), 5.18-5.13 (1H, m, C*H*-4), 4.64-4.59 (1H, m, C*H*-5), 4.29 (1H, dd, *J* = 12.4, 4.9 Hz, CH_ACH_B-6), 4.06 (1H, dd, *J* = 12.4, 1.9 Hz, CH_ACH_B-6), 2.02 (3H, s, C(O)CH_3), 2.01 (3H, s, C(O)CH_3), 1.80 (3H, s, C(O)CH_3); 1.57 (6H, s, CCH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.0 (*C*=O), 169.5 (*C*=O), 132.2 (Ar), 131.6 (Ar), 129.4 (Ar), 128.4 (Ar), 118.6 (C*C*H, triazole) 87.9 (*C*H-1), 69.1 (*C*H-5), 69.0 (*C*H-4), 68.9 (*C*H-3), 68.5 (*C*$(CH_3)_2$), 62.1 (*C*H-2), 61.8 (*C*H_2-6), 30.6 (C*C*H_3), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.4 (OC(O)*C*H_3), the *C*CH triazole signal was not observed in the spectrum; **LCMS** (ESI^+) *m/z* = 508.20 [$(M+H)^+$, 100%], R_t = 3.46 min; **HRMS** (ESI^+) calculated for $C_{23}H_{29}N_3O_8S$ $[M+H]^+$ 508.1754, found 508.1752.

Phenyl 2-(4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (173)



To a solution of phenyl 2-(4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**172**) (30 mg, 0.059 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (11 µL, 0.049 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing phenyl 2-(4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**173**) as a colourless oil (10 mg, 44%): R_f 0.13 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 7.89 (1H, s, C*H*, triazole), 7.21 – 7.17 (2H, m, Ar*H*), 7.17 – 7.13 (3H, m, Ar*H*), 5.64 (1H, d, *J* = 5.3 Hz, C*H*-1), 4.85 (1H, dd, *J* = 11.2, 5.3 Hz, C*H*-2), 4.25 – 4.20 (1H, m, C*H*-3), 4.12 – 4.08 (1H, m, C*H*-5), 3.78 – 3.71 (2H, m, CH_2-6), 3.53 – 3.48 (1H, m, C*H*-4), 1.51 (6H, s, CCH_3); **^{13}C NMR** (126 MHz, MeOD) δ 133.5 (Ar), 130.2 (Ar), 129.0 (Ar), 121.9 (C*C*H, triazole), 90.6 (CH-1), 75.5 (CH-5), 72.3 (CH-4), 71.4 (CH-3), 65.7 (CH-2), 62.2 (CH_2-6), 30.6 (C*C*H_3), the *C*CH triazole, *C*(CH_3)$_2$ and quaternary aromatic carbon signals were not observed in the spectrum; **LCMS** (ESI$^+$) *m/z* = 382.05 [(M+H)$^+$, 100%], R_t = 2.45 min; **HRMS** (ESI$^+$) calculated for $C_{17}H_{23}N_3O_5S$ [M+H]$^+$ 382.1437, found 382.1443.

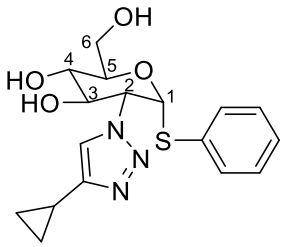
Phenyl 2-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (174)



Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (30 mg, 0.071 mmol, 1 eq.) and cyclopropylacetylene (12 µL, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h. After which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (25-33% EtOAc in hexane) furnishing phenyl 2-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**174**) as a white solid (20 mg, 57%): R_f 0.33 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.36 (1H, s, C*H*, triazole), 7.22 – 7.19 (5H, m, Ar*H*), 5.81 – 5.76 (1H, m, C*H*-3), 5.72 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.28 – 5.22 (1H, m, C*H*-2), 5.16 – 5.11 (1H, m, C*H*-4), 4.63 – 4.59 (1H, m, C*H*-5), 4.28 (1H, dd, *J* = 12.4, 5.0 Hz, CH_ACH_B-6), 4.05 (1H, dd, *J* = 12.6, 2.0 Hz, CH_ACH_B-6), 2.02 (3H, s, C(O)CH_3), 2.00 (3H, s, C(O)CH_3), 1.92 – 1.86 (1H, m, C*H*), 1.82 (3H, s, C(O)CH_3), 0.92 – 0.88 (2H, m, CH_2), 0.78 – 0.76 (2H, m, CH_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.1 (*C*=O), 169.5 (*C*=O), 150.2 (*C*CH, triazole), 132.2 (Ar), 131.6 (Ar), 129.3 (Ar), 128.4 (Ar), 118.9 (*C*H, triazole), 87.9 (*C*H-1), 69.2 (*C*H-5), 68.9 (*C*H-4), 68.8 (*C*H-3), 61.9 (*C*H-2), 61.8 (*C*H_2-6), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3), 8.0 (*C*H_2), 7.9 (*C*H_2), 6.8 (*C*H); **LCMS** (ESI^+) *m/z* = 490.20 [$(M+H)^+$, 100%], R_t = 3.94 min; **HRMS** (ESI^+)

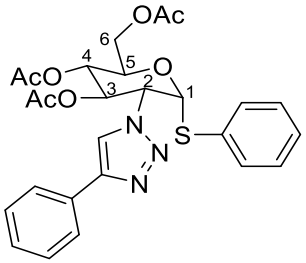
calculated for $C_{23}H_{27}N_3O_7S$ $[M+H]^+$ 490.1648, found 490.1653.

Phenyl 2-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (175)



To a solution of phenyl 2-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**174**) (20 mg, 0.041 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (7 µL, 0.034 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing phenyl 2-(4-cyclopropyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**175**) as a colourless oil (10 mg, 67%): R_f 0.21 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 7.75 (1H, s, C*H,* triazole), 7.21 – 7.18 (2H, m, Ar*H*), 7.17 – 7.14 (3H, m, Ar*H*), 5.61 (1H, d, *J* = 5.3 Hz, C*H*-1), 4.83 – 4.78 (1H, m, C*H*-2), 4.22 – 4.17 (1H, m, C*H*-3), 4.11 – 4.07 (1H, m, C*H*-5), 3.78 – 3.70 (2H, m, CH_2-6), 3.52 – 3.47 (1H, m, C*H*-4), 1.94 – 1.88 (1H, m, C*H*), 0.92 – 0.87 (2H, m, CH_2), 0.73 – 0.69 (2H, m, CH_2); **^{13}C NMR** (126 MHz, MeOD) δ 133.6 (Ar), 130.1 (Ar), 129.0 (Ar), 122.1 (*C*H, triazole), 90.5 (*C*H-1), 75.4 (*C*H-5), 72.3 (*C*H-4), 71.3 (*C*H-3), 65.7 (*C*H-2), 62.2 (*C*H_2-6), 8.2 (*C*H_2), 8.1 (*C*H_2), 7.4 (*C*H), the *C*CH triazole and quaternary aromatic carbon signals were not observed in the spectrum; **LCMS** (ESI$^+$) m/z = 364.00 [(M+H)$^+$, 100%], R_t = 2.81 min; **HRMS** (ESI$^+$) calculated for $C_{17}H_{21}N_3O_4S$ [M+H]$^+$ 364.1331, found 364.1333.

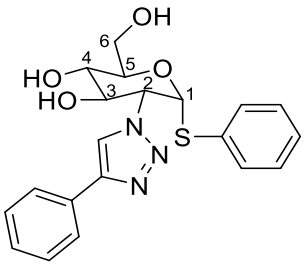
Phenyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (176)



Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (30 mg, 0.071 mmol, 1 eq.) and phenylacetylene (16 µL, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h, after which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (1:3 ethyl acetate/hexane) furnishing phenyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**176**) as a white solid (25 mg, 67%): R_f 0.11 (1:3 ethyl acetate/hexane); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.90 (1H, s, C*H*, triazole), 7.81-7.78 (2H, m, Ar*H*), 7.40-7.36 (2H, m, Ar*H*), 7.31-7.27 (1H, m, Ar*H*), 7.24-7.17 (5H, m, Ar*H*), 5.91-5.85 (1H, m, C*H*-3), 5.78 (1H, d, *J* = 5.5 Hz, C*H*-1), 5.37 (1H, dd, *J* = 11.7, 5.5 Hz, C*H*-2), 5.23-5.16 (1H, m, C*H*-4), 4.69-4.65 (1H, m, C*H*-5), 4.31 (1H, dd, *J* = 12.4, 4.9 Hz, CH_ACH_B-6), 4.09 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 2.04 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.83 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 170.2 (*C*=O), 169.5 (*C*=O), 147.8 (*C*CH, triazole), 132.4 (Ar), 131.5 (Ar), 130.2 (Ar), 129.4 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 125.9 (Ar), 118.8 (C*C*H, triazole) 88.1 (*C*H-1), 69.1 (*C*H-5), 69.0 (*C*H-4), 68.8 (*C*H-3), 62.2 (*C*H-2), 61.9 (*C*H_2-6), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 526.10 [(M+H)$^+$, 100%], R_t = 4.25 min; **HRMS** (ESI$^+$) calculated for $C_{26}H_{27}N_3O_7S$ [M+H]$^+$

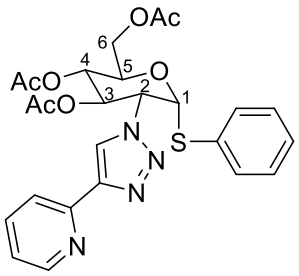
526.1648, found 526.1645.

Phenyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (177)



To a solution of phenyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**176**) (25 mg, 0.048 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (9 µL, 0.040 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing phenyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**177**) as a colourless oil (5 mg, 26%): R_f 0.20 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 8.40 (1H, s, CC*H*, triazole), 7.78-7.75 (1H, m, Ar*H*), 7.38-7.34 (2H, m, Ar*H*), 7.29-7.16 (5H, m, Ar*H*), 7.15-7.12 (2H, m, Ar*H*), 5.70 (1H, d, *J* = 5.3 Hz, C*H*-1), 4.93 (1H, dd, *J* = 11.2, 5.4 Hz, C*H*-2), 4.34-4.28 (1H, m, C*H*-3), 4.17-4.12 (1H, m, C*H*-5), 3.81-3.73 (2H, m, CH_2-6), 3.57-3.53 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 133.7 (Ar), 130.9 (Ar), 130.8 (Ar), 130.1 (Ar), 130.0 (Ar), 129.4 (Ar), 129.0 (Ar), 126.7 (Ar), 122.3 (C*C*H, triazole), 90.5 (*C*H-1), 75.5 (*C*H-5), 72.3 (*C*H-4), 71.4 (*C*H-3), 65.9 (*C*H-2), 60.4 (*C*H_2-6), the *C*CH triazole signal was not observed in the spectrum; **LCMS** (ESI$^+$) *m/z* = 400.05 [(M+H)$^+$, 100%], R_t = 3.19 min; **HRMS** (ESI$^+$) calculated for $C_{20}H_{21}N_3O_4S$ [M+H]$^+$ 400.1331, found 400.1336.

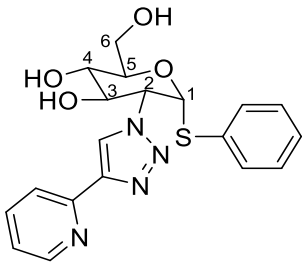
Phenyl 2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (178)



Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (30 mg, 0.071 mmol, 1 eq.) and 2-ethynylpyridine (14 µL, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h, after which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (2% methanol in DCM) furnishing phenyl 2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**178**) as a white solid (24 mg, 65%): R_f 0.47 (2% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.57 – 8.54 (1H, m, Ar*H*), 8.27 (1H, s, C*H*, triazole), 8.11 – 8.08 (1H, m, Ar*H*), 7.73-7.69 (1H, m, Ar*H*), 7.25 – 7.21 (2H, m, Ar*H*), 7.20 – 7.15 (4H, m, Ar*H*), 5.96 – 5.90 (1H, m, C*H*-3), 5.78 (1H, d, *J* = 5.5 Hz, C*H*-1), 5.36 (1H, dd, *J* = 11.7, 5.5 Hz, C*H*-2), 5.21 – 5.16 (1H, m, C*H*-4), 4.71 – 4.67 (1H, m, C*H*-5), 4.32 (1H, dd, *J* = 12.4, 4.9 Hz, CH_ACH_B-6), 4.08 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 2.03 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.82 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.8 (*C*=O), 169.7 (*C*=O), 149.9 (*C*CH, triazole), 149.7 (Ar), 148.5 (Ar), 136.9 (Ar), 132.3 (Ar), 131.6 (Ar), 129.3 (Ar), 128.4 (Ar), 123.1 (Ar), 121.4 (C*C*H, triazole), 120.3 (Ar), 87.9 (*C*H-1), 69.2 (*C*H-5), 68.9 (*C*H-4), 68.8 (*C*H-3), 62.2 (*C*H-2), 61.8 (*C*H_2-6), 20.7 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3); **LCMS** (ESI$^+$) *m/z* = 527.15 [(M+H)$^+$, 100%], R_t = 3.83 min; **HRMS**

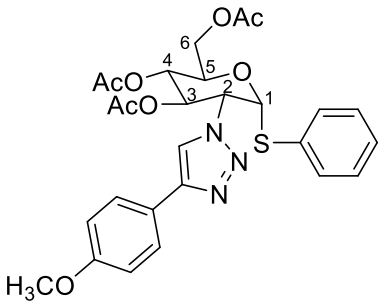
(ESI$^+$) calculated for $C_{25}H_{26}N_4O_7S$ $[M+H]^+$ 527.1600, found 527.1593.

Phenyl 2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (179)



To a solution of phenyl 2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**178**) (20 mg, 0.038 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (7 µL, 0.032 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing phenyl 2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**179**) as a colourless oil (5 mg, 33%): R_f 0.10 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 8.52 (1H, s, C*H*, triazole), 8.51 – 8.49 (1H, m, Ar*H*), 8.04 – 8.01 (1H, m, Ar*H*), 7.86 – 7.82 (1H, m, Ar*H*), 7.31 – 7.28 (1H, m, Ar*H*), 7.24 – 7.21 (2H, m, Ar*H*), 7.15 – 7.12 (3H, m, Ar*H*), 5.73 (1H, d, *J* = 5.4 Hz, CH-1), 4.98 (1H, dd, *J* = 11.3, 5.4 Hz, C*H*-2), 4.32 – 4.26 (1H, m, C*H*-3), 4.17 – 4.13 (1H, m, C*H*-5), 3.81 – 3.74 (2H, m, CH_2-6), 3.57– 3.52 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 150.5 (Ar), 139.0 (Ar), 133.6 (Ar), 130.9 (Ar), 130.2 (Ar), 129.1 (Ar), 124.6 (Ar), 124.2 (C*C*H, triazole), 121.6 (Ar), 90.5 (*C*H-1), 75.5 (*C*H-5), 72.3 (*C*H-4), 71.4 (*C*H-3), 65.9 (*C*H-2), 62.2 (*C*H_2-6), the *C*CH triazole and quaternary aromatic carbon signals were not observed in the spectrum; **LCMS** (ESI^+) *m/z* = 401.10 [$(M+H)^+$, 100%], R_t = 2.66 min; **HRMS** (ESI^+) calculated for $C_{19}H_{20}N_4O_4S$ $[M+H]^+$ 401.1284, found 401.1288.

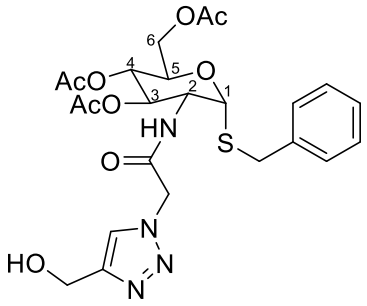
Phenyl 2-(4-(4-methoxyphenyl)-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (180)



Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of phenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**152**) (30 mg, 0.071 mmol, 1 eq.) and ethynylanisole (19 mg, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h, after which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (25-50% EtOAc in hexane) furnishing phenyl 2-(4-(4-methoxyphenyl)-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**180**) as a white solid (5 mg, 13%): R_f 0.39 (2% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.81 (1H, s, C*H*, triazole), 7.73 – 7.70 (2H, m, Ar*H*), 7.24 – 7.21 (2H, m, Ar*H*), 7.20 – 7.18 (3H, m, Ar*H*), 6.93 – 6.89 (2H, m, Ar*H*), 5.90 – 5.85 (1H, m, C*H*-3), 5.77 (1H, d, *J* = 5.5 Hz, C*H*-1), 5.35 (1H, dd, *J* = 11.8, 5.5 Hz, C*H*-2), 5.20 – 5.16 (1H, m, C*H*-4), 4.68 – 4.64 (1H, m, C*H*-5), 4.31 (1H, dd, *J* = 12.4, 4.9 Hz, CH_ACH_B-6), 4.09 (1H, dd, *J* = 12.4, 2.2 Hz, CH_ACH_B-6), 3.79 (3H, s, OCH_3), 2.04 (3H, s, C(O)CH_3), 2.02 (3H, s, C(O)CH_3), 1.83 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 170.6 (*C*=O), 169.9 (*C*=O), 169.5 (*C*=O), 132.4 (Ar), 129.3 (Ar), 128.5 (Ar), 127.2 (Ar), 117.9 (C*C*H, triazole), 114.3 (Ar), 88.1 (*C*H-1), 69.2 (*C*H-5), 69.0 (*C*H-4), 68.8 (*C*H-3), 62.1 (*C*H-2), 61.8 (*C*H_2-6), 55.4 (OCH_3), 20.7 (OC(O)CH_3), 20.6 (OC(O)CH_3), 20.5 (OC(O)CH_3), the *C*CH triazole and quaternary aromatic

carbon signals were not observed in the spectrum; **LCMS** (ESI^+) *m/z* = 556.15 [$(M+H)^+$, 100%], R_t = 4.17 min; **HRMS** (ESI^+) calculated for $C_{27}H_{29}N_3O_8S$ $[M+H]^+$ 556.1754, found 556.1758.

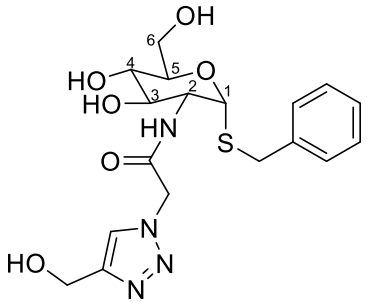
Benzyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-O-acetyl-1-thio-α-D-glucopyranoside (181)



Sodium ascorbate (2.6 mg, 0.013 mmol, 0.25 eq.) and copper sulphate pentahydrate (3.2 mg, 0.013 mmol, 0.25 eq.) were added to a mixture of benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**167**) (25 mg, 0.051 mmol, 1 eq.) and propargyl alcohol (6 µL, 0.102 mmol, 2 eq.) in THF/water (1:1, 1.0 mL). The reaction was stirred at room temperature for 18 h. After which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing benzyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-O-acetyl-1-thio-α-D-glucopyranoside (**181**) as a yellow oil (25 mg, 89%): R_f 0.14 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 (1H, s, C*H*, triazole), 7.28 – 7.18 (5H, m, Ar*H*), 6.01 (1H, d, *J* = 8.2 Hz, N*H*), 5.30 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.04 – 4.98 (1H, m, C*H*-4), 4.94 – 4.88 (1H, m, C*H*-3), 4.87 – 4.77 (2H, m, CH_2N), 4.75 (2H, s, CH_2OH), 4.34 – 4.28 (1H, m, C*H*-2), 4.23 – 4.18 (1H, m, C*H*-5), 4.16 (1H, dd, *J* = 12.3, 4.4 Hz, CH_ACH$_B$-6), 3.83 (1H, dd, *J* = 12.3, 2.0 Hz, CH$_A$CH_B-6), 3.74 – 3.66 (2H, m, SCH_2), 2.02 (3H, s, C(O)CH_3), 1.94 (3H, s, C(O)CH_3), 1.90 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.9 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 137.1 (Ar), 129.0 (Ar), 128.8 (Ar), 127.6 (Ar), 123.2 (*C*H, triazole), 83.0 (*C*H-1), 71.2 (*C*H-3), 68.7 (*C*H-5), 67.8 (*C*H-4), 61.7 (*C*H$_2$-6), 56.6 (CH_2OH), 52.7 (C*H*-2), 52.6 (CH_2N), 35.1 (S*C*H$_2$), 20.8 (OC(O)*C*H$_3$), 20.7 (OC(O)*C*H$_3$), 20.6

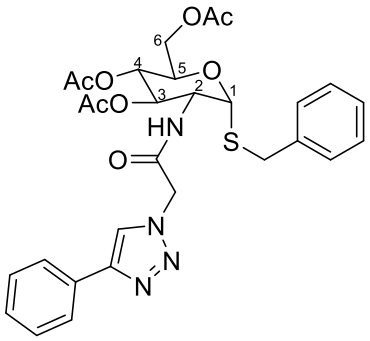
(OC(O)*C*H$_3$), the *C*CH triazole signal was not observed in the spectrum; **LCMS** (ESI$^+$) *m/z* = 551.20 [(M+H)$^+$, 100%], R_t = 3.16 min; **HRMS** (ESI$^+$) calculated for $C_{24}H_{30}N_4O_9S$ [M+H]$^+$ 551.1812, found 551.1819.

Benzyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (182)



To a solution of benzyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-O-acetyl-1-thio-α-D-glucopyranoside (**181**) (25 mg, 0.045 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (8 µL, 0.038 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5-20% methanol in DCM) furnishing benzyl 2-(4-hydroxymethyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**182**) as a yellow oil (5 mg, 26%): R_f 0.09 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 7.76 (1H, s, C*H*, triazole), 7.25 – 7.22 (2H, m, Ar*H*), 7.20 – 7.15 (2H, m, Ar*H*), 7.13 – 7.09 (1H, m, Ar*H*), 5.23 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.05 (1H, d, *J* = 16.2 Hz, CH_ACH_BN), 4.97 (1H, d, *J* = 16.2 Hz, CH$_A$CH_BN), 4.59 (2H, s, CH_2OH), 3.94 (1H, dd, *J* = 11.0, 5.4 Hz, C*H*-2), 3.91 – 3.86 (1H, m, C*H*-5), 3.73 – 3.65 (3H, m, SCH_2 + CH_ACH_B-6), 3.61 (1H, dd, *J* = 12.0, 5.5 Hz, CH$_A$CH_B-6), 3.57 – 3.52 (1H, m, C*H*-3), 3.29 – 3.25 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 149.1 (*C*CH, triazole), 139.5 (Ar), 130.2 (Ar), 129.5 (Ar), 128.1 (Ar), 125.6 (*C*H, triazole), 83.9 (*C*H-1), 74.6 (*C*H-5), 72.9 (*C*H-4), 72.6 (*C*H-3), 62.5 (*C*H$_2$-6), 56.5 (*C*H$_2$OH), 55.8 (*C*H-2), 52.9 (*C*H$_2$N), 35.1 (S*C*H$_2$); **HRMS** (ESI$^+$) calculated for $C_{18}H_{24}N_4O_6S$ $[M+H]^+$ 425.1495, found 425.1499.

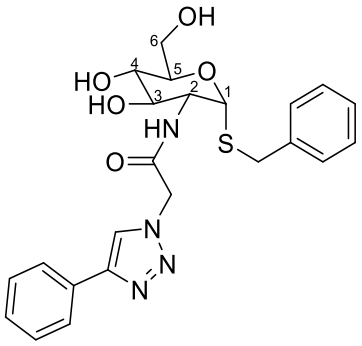
Benzyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (183)



Sodium ascorbate (3.6 mg, 0.018 mmol, 0.25 eq.) and copper sulphate pentahydrate (4.5 mg, 0.018 mmol, 0.25 eq.) were added to a mixture of benzyl 2-azidoacetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**167**) (35 mg, 0.071 mmol, 1 eq.) and phenylacetylene (16 µL, 0.142 mmol, 2 eq.) in THF/water (1:1, 1.5 mL). The reaction was stirred at room temperature for 18 h, after which time the reaction was concentrated *in vacuo* and the residue was dissolved in dichloromethane (5 mL). The mixture was washed with water (3 x 10 mL). The aqueous layer was re-extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (10% methanol in DCM) furnishing benzyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**183**) as a yellow solid (20 mg, 47%): R_f 0.50 (10% MeOH in DCM); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.79 – 7.75 (3H, m, Ar*H* + C*H*, triazole), 7.40 – 7.35 (2H, m, Ar*H*), 7.31 – 7.27 (1H, m, Ar*H*), 7.23 – 7.15 (5H, m, Ar*H*), 6.16 (1H, d, *J* = 8.3 Hz, N*H*), 5.26 (1H, d, *J* = 5.4 Hz, C*H*-1), 5.04 – 4.99 (1H, m, C*H*-4), 4.97 – 4.92 (1H, m, C*H*-3), 4.92 – 4.82 (2H, m, CH_2N), 4.38 – 4.32 (1H, m, C*H*-2), 4.23-4.20 (1H, m, C*H*-5), 4.16 (1H, dd, *J* = 12.3, 4.4 Hz, CH_ACH_B-6), 3.84 (1H, dd, *J* = 12.3, 2.1 Hz, CH_ACH_B-6), 3.72 – 3.64 (2H, m, SCH_2), 2.02 (3H, s, C(O)CH_3), 1.93 (3H, s, C(O)CH_3), 1.87 (3H, s, C(O)CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.5 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 148.5 (*C*CH, triazole), 137.0 (Ar), 130.2 (Ar), 129.0 (Ar), 128.9 (Ar), 128.7 (Ar), 128.5 (Ar), 127.6 (Ar), 125.9 (Ar), 120.90 (C*C*H, triazole), 83.0 (*C*H-1), 71.3 (*C*H-3), 68.7 (*C*H-5), 67.85 (*C*H-4), 61.8 (*C*H_2-6), 52.7

(*C*H_2N), 52.6 (*C*H-2), 35.0 (S*C*H_2), 20.8 (OC(O)*C*H_3), 20.6 (OC(O)*C*H_3), 20.5 (OC(O)*C*H_3); **HRMS** (ESI$^+$) calculated for $C_{29}H_{32}N_4O_8S$ $[M+H]^+$ 597.2019, found 597.2012.

Benzyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (184)



To a solution of benzyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (**183**) (20 mg, 0.034 mmol, 1.2 eq.) in methanol (2 mL) 25% sodium methoxide solution in methanol (6 µL, 0.028 mmol, 1 eq.) was added. The reaction was stirred at room temperature for 2 h, after which time it was concentrated *in vacuo*. The resulting crude material was purified by silica gel column chromatography (5% methanol in DCM) furnishing benzyl 2-(4-phenyl-1,2,3-triazol-1-yl)-2-deoxy-1-thio-α-D-glucopyranoside (**184**) as a white solid (5 mg, 32%): R_f 0.11 (5% MeOH in DCM); **^{1}H NMR** (500 MHz, MeOD) δ 8.16 (1H, s, CC*H*, triazole), 7.75-7.70 (2H, m, Ar*H*), 7.38-7.32 (1H, m, Ar*H*), 7.28-7.05 (7H, m, Ar*H*), 5.25 (1H, d, *J* = 5.1 Hz, C*H*-1), 5.11-5.02 (2H, m, CH_2N), 3.96 (1H, dd, *J* = 11.0, 5.4 Hz, C*H*-2), 3.92-3.87 (1H, m, C*H*-5), 3.74-3.65 (3H, m, SCH_2 + CH_ACH_B-6), 3.62 (1H, dd, *J* = 12.1, 5.5 Hz, CH_ACH_B-6), 3.58-3.53 (1H, m, C*H*-3), 3.30-3.25 (1H, m, C*H*-4); **^{13}C NMR** (126 MHz, MeOD) δ 131.3 (Ar), 130.9 (Ar), 130.1 (Ar), 130.0 (Ar), 129.5 (Ar), 129.4 (Ar), 128.1 (Ar), 126.7 (Ar), 122.2 (C*C*H, triazole), 83.8 (*C*H-1), 74.6 (*C*H-5), 72.9 (*C*H-3), 72.6 (*C*H-4), 62.5 (*C*H_2-6), 55.8 (*C*H-2), 54.8 (*C*H_2N), 35.1 (S*C*H_2), the *C*CH triazole signal was not observed in the spectrum; **HRMS** (ESI$^+$) calculated for $C_{23}H_{26}N_4O_5S$ $[M+H]^+$ 471.1702, found 471.1709.

12. Biological experiments

Chemicals and Buffers

All chemical reagents were purchased from Fisher Scientific, Sigma Aldrich UK, VWR or Life Sciences unless otherwise stated. SDS PAGE gel electrophoresis was carried out using NOVEX™ precast gels and buffers from Invitrogen Ltd. Instant Blue was obtained from Expedeon through Fisher Scientific. All solutions for growth were autoclaved (Priorclave Front Loading Autoclave machine) and stored at room temperature unless otherwise stated. A Beckman Coulter Avanti J-E Centrifuge was used.

Mycothiol-*S*-transferase expression and purification

Mycothiol-*S*-transferase (MST) was overexpressed in *E. coli* BL21(DE3) host cells harbouring the pETMtMST plasmid (from Dr M. Rawat, CSU Fresno). For the expression step, the cells were grown overnight at 37 °C in 5 mL of Luria-Bertani (LB) medium supplemented with ampicillin (100 µg/mL). 1 mL of the overnight culture was transferred to 500 mL of the LB broth containing ampicillin (100 µg/mL) and grown at 37 °C until the measured optical density at 650 nm (OD650) reached 0.5. The cells were induced with 0.5 mM IPTG and incubated overnight at 22 °C. Cells were harvested by centrifugation (15 min, 5000 rpm) and resuspended in 20 mL of LEW buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% glycerol, pH 8.0). Next, the cells were lysed using sonication and the cellular debris was removed by centrifugation (20 min, 13000 rpm). The supernatant was filtered through a 0.45 µm Whatman syringe filter and the protein was purified using a Ni-NTA agarose gel. The column was washed with a Wash Buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% glycerol, pH 8.0, 10 mM imidazole) and then the protein was eluted with the Elution Buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% glycerol, pH 8.0, 250 mM imidazole). SDS-PAGE Gel was used to identify the purity of the protein and the Bradford assay was used to determine the concentration of the protein in each of the fractions collected. The fractions containing the protein were pooled and loaded onto a PD-10 column to undergo buffer exchange with LEW buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10% glycerol, pH 8.0). The protein was stored in 100 µL aliquots at -80 °C.

Bibliography

(1) Tuberculosis Fact sheet, 2018. World Health Organization Web site. http://www.who.int/news-room/fact-sheets/detail/tuberculosis (accessed May 1, 2019).

(2) TB CARE I. *International Standards for Tuberculosis Care*, Edition 3; TB CARE I: The Hague, 2014.

(3) Rohde, M. The Gram-Positive Bacterial Cell Wall. In *Gram-Positive Pathogens*; Fischetti, V. A., Novick, R. P., Ferretti, J. J., Portnoy, D. A., Braunstein, M., Rood, J. I., Eds.; ASM: Washington, DC, USA, 2019; pp 4-10.

(4) Zhang, Y.; Yew, W-W. Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. *Int J Tuberc Lung Dis* **2015**, *19*, 1276–1289.

(5) Arya, N.; Raut, M. K.; Tekale, S. G.; Shishoo, C. J.; Jain K. S. Tuberculosis: New Drug Discovery Pipelines. *Austin J Anal Pharm Chem* **2014**, *1*, 1014–1022.

(6) TB medicine pretomanid enters regulatory process in the United States, 2019. TB Alliance Web site. https://www.tballiance.org/news/pretomanid-enters-FDA-review (accessed May 1, 2019).

(7) Press release from Infectex and Sequella on SQ-109 clinical trial in Russia, 2017. Working Group on New TB Drugs Web site. https://newtbdrugs.org/news/press-release-infectex-and-sequella-sq-109-clinical-trial-russia (accessed May 1, 2019).

(8) Compound summary: Pretomanid, 2020. PubChem Web site. https://pubchem.ncbi.nlm.nih.gov/compound/Pretomanid (accessed March 3, 2020).

(9) Rawat, M.; Av-Gay, Y. Mycothiol-dependent proteins in actinomycetes. *FEMS Microbiol Rev* **2007**, *31*, 278–292.

(10) Nilewar, S. S.; Kathiravan M. K. Mycothiol: A promising antitubercular target. *Bioorg. Chem.* **2014**, *52*, 62–68.

(11) Hernick, M. Mycothiol: a target for potentiation of rifampin and other antibiotics against *Mycobacterium tuberculosis*. *Expert Rev. Anti Infect. Ther.* **2013**, *11*, 49–67.

(12) Fan, F.; Vetting, M. W.; Frantom, P. A.; Blanchard, J. S. Structures and mechanisms of the mycothiol biosynthetic enzymes. *Curr Opin Chem Biol* **2009**,

13, 451–459.

(13) Newton, G. L.; Unson, M. D.; Anderberg, S. J.; Aguilera, J. A.; Oh, N. N.; delCardayre, S. B.; Av-Gay, Y.; Fahey, R. C. Characterization of *Mycobacterium smegmatis* Mutants Defective in 1-D-*myo*-Inosityl-2-amino-2-deoxy-α-D-glucopyranoside and Mycothiol Biosynthesis. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 239–244.

(14) Rawat, M.; Johnson, C.; Cadiz, V.; Av-Gay, Y. Comparative analysis of mutants in the mycothiol biosynthesis pathway in *Mycobacterium smegmatis*. *Biochem. Biophys. Res. Commun.* **2007**, *363*, 71–76.

(15) Xu, X.; Vilchèze, C.; Av-Gay, Y.; Gómez-Velasco, A.; Jacobs Jr., W. R. Precise Null Deletion Mutations of the Mycothiol Synthesis Genes Reveal Their Role in Isoniazid and Ethionamide Resistance in *Mycobacterium smegmatis*. *Antimicrob. Agents Chemother.* **2011**, *55*, 3133–3139.

(16) Buchmeier, N.; Fahey, R. C. The mshA gene encoding the glycosyltransferase of mycothiol biosynthesis is essential in *Mycobacterium tuberculosis* Erdman. *FEMS Microbiol Lett.* **2006**, *264*, 74-9.

(17) Vilchèze, C.; Av-Gay, Y.; Attarian, R.; Liu, Z.; Hazbón, M. H.; Colangeli, R.; Chen, B.; Liu, W.; Alland, D.; Sacchettini, J. C.; Jacobs Jr., W. R. Mycothiol biosynthesis is essential for ethionamide susceptibility in *Mycobacterium tuberculosis*. *Mol Microbiol.* **2008**, *69*, 1316–1329.

(18) Frantom, P. A.; Coward, J. K.; Blanchard, J. S. UDP-(5F)-GlcNAc acts as a slow-binding inhibitor of MshA, a retaining glycosyltransferase. *J. Am. Chem. Soc.* **2010**, *132*, 6626–6627.

(19) Upton, H.; Newton, G. L.; Gushiken, M.; Lo, K.; Holden, D.; Fahey, R. C.; Rawat, M. Characterization of BshA, bacillithiol glycosyltransferase from *Staphylococcus aureus* and *Bacillus subtilis FEBS Lett.* **2012**, *586*,1004–1008.

(20) Newton, G. L.; Buchmeier, N.; Fahey, R. C. Biosynthesis and Functions of Mycothiol, the Unique Protective Thiol of *Actinobacteria*. *Microbiol. Mol. Biol. Rev.* **2008**, *72*, 471–494.

(21) Metaferia, B. B.; Fetterolf, B. J.; Shazad-ul-Hussan, S.; Moravec, M.; Smith, J. A.; Ray, S.; Gutierrez-Lugo, M.-T.; Bewley, C. A. Synthesis of Natural Product-Inspired Inhibitors of *Mycobacterium tuberculosis* Mycothiol-Associated Enzymes: The First Inhibitors of GlcNAc-Ins Deacetylase. *J. Med. Chem.* **2007**,

50, 6326–6336.

(22) Gammon, D. W.; Steenkamp, D. J.; Mavumengwana, V.; Marakalala, M. J.; Mudzunga, T. T.; Hunter, R.; Munyololo, M. Conjugates of plumbagin and phenyl-2-amino-1-thioglucoside inhibit MshB, a deacetylase involved in the biosynthesis of mycothiol. *Bioorg. Med. Chem.* **2010**, *18*, 2501–2514.

(23) Huang, X.; Hernick, M. A fluorescence-based assay for measuring *N*-acetyl-1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-glucopyranoside deacetylase activity. *Anal. Biochem.* **2011**, *414*, 278–281.

(24) Lamprecht, D. A.; Muneri, N. O.; Eastwood, H.; Naidoo, K. J.; Strauss, E.; Jardine, A. An enzyme-initiated Smiles rearrangement enables the development of an assay of MshB, the GlcNAc-Ins deacetylase of mycothiol biosynthesis. *Org. Biomol. Chem.* **2012**, *10*, 5278–5288.

(25) Newton, G. L.; Ta, P.; Sareen, D.; Fahey, R. C. A coupled spectrophotometric assay for L-cysteine:1-D-*myo*-inosityl-2-amino-2-deoxy-α-D-glucopyranoside ligase and its application for inhibitor screening. *Anal. Biochem.* **2006**, *353*, 167–173.

(26) Newton, G. L.; Buchmeier, N.; La Clair, J. J.; Fahey, R. C. Evaluation of NTF1836 as an inhibitor of the mycothiol biosynthetic enzyme MshC in growing and non-replicating *Mycobacterium tuberculosis*. *Bioorg. Med. Chem.* **2011**, *19*, 3956–3964.

(27) Gutierrez-Lugo, M.-T.; Baker, H.; Shiloach, J.; Boshoff, H.; Bewley, C. A. Dequalinium, a New Inhibitor of *Mycobacterium tuberculosis* Mycothiol Ligase Identified by High-Throughput Screening. *J Biomol Screen* **2009**, *14*, 643-652.

(28) Buchmeier, N. A.; Newton, G. L.; Fahey, R. C. A Mycothiol Synthase Mutant of *Mycobacterium tuberculosis* Has an Altered Thiol-Disulfide Content and Limited Tolerance to Stress. *J. Bacteriol.* **2006**, *188*, 6245-6252.

(29) Patel, M. P.; Blanchard, J. S. Expression, Purification, and Characterization of *Mycobacterium tuberculosis* Mycothione Reductase. *Biochemistry* **1999**, *38*, 11827–11833.

(30) Mahapatra, A.; Mativandlela, S. P. N.; Binneman, B.; Fourie, P. B.; Hamilton, C. J.; Meyer, J. J. M.; van der Kooy, F.; Houghton, P.; Lall, N. Activity of 7-methyljuglone derivatives against *Mycobacterium tuberculosis* and as subversive substrates for mycothiol disulfide reductase. *Bioorg. Med. Chem.* **2007**, *15*,

7638–7646.

(31) Hamilton, C. J.; Finlay, R. M. J.; Stewart, M. J. G.; Bonner, A. Mycothiol disulfide reductase: A continuous assay for slow time-dependent inhibitors. *Anal. Biochem.* **2009**, *388*, 91–96.

(32) Newton, G. L; Fahey, R. C. Mycothiol biochemistry. *Arch Microbiol* **2002**, *178*, 388–394.

(33) Jothivasan, V. K.; Hamilton, C. J. Mycothiol: synthesis, biosynthesis and biological functions of the major low molecular weight thiol in actinomycetes. *Nat. Prod. Rep.* **2008**, *25*, 1091–1117.

(34) Rawat, M.; Uppal, M.; Newton, G.; Steffek, M.; Fahey, R. C.; Av-Gay, Y. Targeted Mutagenesis of the *Mycobacterium smegmatis mca* Gene, Encoding a Mycothiol-Dependent Detoxification Protein. *J. Bacteriol.* **2004**, *186*, 6050–6058.

(35) Nicholas, G. M.; Newton, G. L.; Fahey, R. C.; Bewley, C. A. Novel Bromotyrosine Alkaloids: Inhibitors of Mycothiol *S*-Conjugate Amidase. *Org. Lett.* **2001**, *3*, 1543-1545.

(36) Nicholas, G. M.; Eckman, L. L.; Newton, G. L.; Fahey, R. C.; Ray, S.; Bewley, C. A. Inhibition and Kinetics of *mycobacterium tuberculosis* and *Mycobacterium Smegmatis* Mycothiol-*S*-conjugate Amidase by Natural Product Inhibitors. *Bioorg. Med. Chem.* **2003**, *11*, 601–608.

(37) Nicolaou, K. C.; Hughes, R., Pfefferkorn, J. A., Barluenga, S. Optimization and Mechanistic Studies of Psammaplin A Type Antibacterial Agents Active against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Chem. Eur. J.* **2001**, *7*, 4296–4310.

(38) Nicholas, G. M.; Eckman, L. L.; Ray, S.; Hughes, R. O.; Pfefferkorn, J. A.; Barluenga, S.; Nicolaou, K. C.; Bewley, C. A. Bromotyrosine-Derived Natural and Synthetic Products as Inhibitors of Mycothiol-*S*-Conjugate Amidase. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2487–2490.

(39) Metaferia, B. B.; Ray, S.; Smith, J. A.; Bewley, C. A. Design and synthesis of substrate-mimic inhibitors of mycothiol-*S*-conjugate amidase from *Mycobacterium tuberculosis*. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 444–447.

(40) Newton, G. L.; Leung, S. S.; Wakabayashi, J. I.; Rawat, M.; Fahey, R. C. The DinB Superfamily Includes Novel Mycothiol, Bacillithiol, and Glutathione *S*-Transferases. *Biochemistry* **2011**, *50*, 10751−10760.

(41) Jardine, M. A.; Spies, H. S.C.; Nkambule, C. M.; Gammon D. W.; Steenkamp D. J. Synthesis of Mycothiol, 1D-1-*O*-(2-[*N*-Acetyl-L-cysteinyl]amino-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol, Principal Low Molecular Mass Thiol in the Actinomycetes. *Bioorg. Med. Chem.* **2002**, *10*, 875–881.

(42) Lee S.; Rosazza J. P. N. First Total Synthesis of Mycothiol and Mycothiol Disulfide. *Org. Lett.* **2004**, *6*, 365-368.

(43) Ajayi, K.; Thakur, V. V.; Lapo, R. C.; Knapp S. Intramolecular *alpha*–Glucosaminidation: Synthesis of Mycothiol. *Org. Lett.* **2010**, *12*, 2630–2633.

(44) Chung, C.-C.; Zulueta, M. M. L.; Padiyar, L. T.; Hung, S.-C. Desymmetrization of 2,4,5,6-Tetra-Obenzyl-D-myo-inositol for the Synthesis of Mycothiol. *Org. Lett.* **2011**, *13*, 5496–5499.

(45) McConnell, M. S.; Yu, F.; Nguyen, H. M. Nickel-catalyzed α-glycosylation of C(1)-hydroxyl-D-*myo*-inositol: a formal synthesis of mycothiol. *Chem. Commun.* **2013**, *49*, 4313-4315.

(46) Manabe, S.; Ito, Y. Mycothiol synthesis by an anomerization reaction through endocyclic cleavage. *Beilstein J. Org. Chem.* **2016**, *12*, 328–333.

(47) Patel, M. P.; Blanchard, J. S. Synthesis of Des-*myo*-Inositol Mycothiol and Demonstration of a Mycobacterial Specific Reductase Activity. *J. Am. Chem. Soc.* **1998**, *120*, 11538-11539.

(48) Nicholas, G. M.; Kováč, P.; Bewley, C. A. Total Synthesis and Proof of Structure of Mycothiol Bimane. *J. Am. Chem. Soc.* **2002**, *124*, 3492- 3493.

(49) Knapp, S.; Gonzalez, S.; Myers, D. S.; Eckman, L. L.; Bewley, C. A. Shortcut to Mycothiol Analogues. *Org. Lett.* **2002**, *4*, 4337-4339.

(50) Knapp, S.; Amorelli, B.; Darout, E.; Ventocilla, C. C.; Goldman, L. M.; Huhn, R. A.; Minnihan, E. C. A Family of Mycothiol Analogues. *J Carbohyd Chem* **2005**, *24*, 103-130.

(51) Gammon, D. W.; Hunter, R.; Steenkamp, D. J.; Mudzunga, T. T. Synthesis of 2-Deoxy-2-C-Alkylglucosides of *myo*-Inositol as Possible Inhibitors of a *N*-Deacetylase Enzyme in the Biosynthesis of Mycothiol. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2045–2049.

(52) Slättegård, R.; Gammon, D. W.; Oscarson, S. Synthesis of fused bicyclic thioglycosides of N-acylated glucosamine as analogues of mycothiol. *Carbohydr. Res.* **2007**, *342*, 1943–1946.

(53) Riordan, S. W.; Field, J. J.; Corkran, H. M.; Dasyam, N.; Stocker, B. L.; Timmer, M. S. M.; Harvey, J. E.; Teesdale-Spittle, P. H. Synthesis of mycothiol conjugate analogues and evaluation of their antimycobacterial activity. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2152–2155.

(54) Patel, K.; Song, F.; Andreana, P. R. Synthesis of substrate analogues as potential inhibitors for *Mycobacterium tuberculosis* enzyme MshC. *Carbohydr. Res.* **2017**, *453-454*, 10–18.

(55) Mamidyala, S.K.; Finn, M. G. *In situ* click chemistry: probing the binding landscapes of biological molecules. *Chem. Soc. Rev.* **2010**, *39*, 1252–1261.

(56) Oueis, E.; Sabot, C.; Renard, P.-Y. New insights into the kinetic target-guided synthesis of protein ligands. *Chem. Commun.* **2015**, *51*, 12158–12169.

(57) Ozturk, T.; Ertas, E.; Mert, O. Use of Lawesson's Reagent in Organic Syntheses. *Chem. Rev.* **2007**, *107*, 5210-5278.

(58) Knapp, S.; Myers D. S. α-GlcNAc Thioconjugates. *J. Org. Chem.* **2002**, *67*, 2995-2999.

(59) Fujinaga, M.; Matsushima, Y. Studies on Amino-hexoses. IX. The *N*-Deacetylation of Methyl *N*-Acetyl-β-D-glucosaminide, Methyl *N*-Acetyl-3-*O*-methyl-α- and β-D-glucosaminide, and Methyl *N*-Acetyl-4,6-*O*-ethylidene-α-D-glucosaminide. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 185-190.

(60) Overman, L. E.; O'Connor, E. M. Nucleophilic Cleavage of the Sulfur-Sulfur Bond by Phosphorus Nucleophiles. IV. Kinetic Study of the Reduction of Alkyl Disulfides with Triphenylphosphine and Water. *J. Am. Chem. Soc.* **1976**, *98*, 771–775.

(61) Jones, R. A.; Thillier, Y.; Panda, S. S.; Rivera Rosario, N.; Hall, C. D.; Katritzky, A. R. Synthesis and characterisation of glucosamine–NSAID bioconjugates. *Org. Biomol. Chem.* **2014**, *12*, 8325-8335.

(62) Kyas, A.; Feigel, M. Differently Glycosidated 2-Amino-2-deoxy-D-glucopyranosiduronic Acids as Building Blocks in Peptide Synthesis. *Helv. Chim. Acta* **2005**, *88*, 2375–2396.

(63) Yoshimura, J.; Ando, H.; Sato, T.; Tsuchida, S.; Hashimoto, H. Aminosugars. XXVI. Synthesis of Amido-bonded Disaccharides Containing Hexosaminuronic Acids. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2511-2514.

(64) Due-Hansen, M. E.; Pandey, S. K.; Christiansen, E.; Andersen, R.; Hansen,

S. V. F.; Ulven, T. A protocol for amide bond formation with electron deficient amines and sterically hindered substrates. *Org. Biomol. Chem.* **2016**, *14*, 430-433.

(65) Suttisansanee, U. Investigation of the Microbial Glyoxalase System. Ph.D. Dissertation, University of Waterloo, Waterloo, Ontario, Canada, 2011.

(66) Laughlin, S. T.; Bertozzi, C. R. Metabolic labeling of glycans with azido sugars and subsequent glycan-profiling and visualization via Staudinger ligation. *Nat. Protoc.* **2007**, *2*, 2930-2944.

(67) Silva, D. J.; Wang, H.; Allanson, N. M.; Jain, R. K.; Sofia, M. J. Stereospecific Solution- and Solid-Phase Glycosylations. Synthesis of β-Linked Saccharides and Construction of Disaccharide Libraries Using Phenylsulfenyl 2-Deoxy-2-Trifluoroacetamido Glycopyranosides as Glycosyl Donors. *J. Org. Chem.* **1999**, *64*, 5926-5929.

(68) Zeng, L.; Xu, G.; Gao, P.; Zhang, M.; Li, H.; Zhang, J. Design, synthesis and evaluation of a novel class of glucosamine mimetic peptides containing 1,3-dioxane. *Eur. J. Med. Chem.* **2015**, *93*, 109-120.

(69) Grehn, L.; Gunnarsson, K.; Ragnarsson, U. Removal of Formyl, Acetyl, and Benzoyl Groups from Amides with Conversion into the Corresponding *t*-Butyl Carbarnates. *J. Chem. Soc., Chem. Commun.* **1985**, 1317-1318.

(70) André, S.; O'Sullivan, S.; Koller, C.; Murphy, P. V.; Gabius, H.-J. Bi- to tetravalent glycoclusters presenting GlcNAc/GalNAc as inhibitors: from plant agglutinins to human macrophage galactose-type lectin (CD301) and galectins. *Org. Biomol. Chem.* **2015**, *13*, 4190-4203.

(71) Goddard-Borger, E. D.; Stick, R. V. An Efficient, Inexpensive, and Shelf-Stable Diazotransfer Reagent: Imidazole-1-sulfonyl Azide Hydrochloride. *Org. Lett.* **2007**, *9*, 3797-3800.

(72) Hang, H. C.; Yu, C.; Kato, D. L.; Bertozzi C. R. A metabolic labeling approach toward proteomic analysis of mucin-type *O*-linked glycosylation. *PNAS* **2003**, *100*, 14846–14851.

(73) Knapp, S.; Myers D. S. Synthesis of α-GalNAc Thioconjugates from an α-GalNAc Mercaptan. *J. Org. Chem.* **2001**, *66*, 3636-3638.

(74) Vasella, A; Witzig, C.; Chiara, J.-L.; Martin-Lomas, M. Convenient Synthesis of 2-Azido-2-deoxy-aldoses by Diazo Transfer. *Helv. Chim. Acta* **1991**, *74*, 2073-

2077.

(75) Glibstrup E.; Pedersen C. M. Scalable Synthesis of Anomerically Pure Orthogonal-Protected $GlcN_3$ and $GalN_3$ from D-Glucosamine. *Org. Lett.* **2016**, 18, 4424–4427.

(76) Pertel S. S., Seryi, S. A., Kakayan, E. S. A new approach to the synthesis of lactams of muramic, isomuramic and normuramic acids via intramolecular *O*-alkylation: Stereochemical features of the intramolecular nucleophilic substitution. *Tetrahedron* **2018**, *74*, 4857-4867.

(77) Tomaszewska, J.; Kowalska, K.; Koroniak-Szejn, K. Glucosamine- and galactosamine- based monosaccharides with highly fluorinated motifs. *J Fluorine Chem* **2016**, *191*, 1–13.