Chemical synthesis of modified D-mannuronate building blocks: prospects for modified alginates

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Abstract

Alginate is an anionic polysaccharide comprised of β-1,4-linked D-mannuronic acid (M) and L-guluronic acid (G). The polymer is naturally harvested from seaweed and is widely exploited in various biotechnological and biomedical applications, due to the multitude of physicochemical characteristics it possesses, predominantly owed to the presence of charged *C*5-carboxylic acid groups. Modification of the carboxylates would enable the development of alginate systems possessing novel physicochemical properties.

Herein is presented for the first time the synthesis of *C*6-hydroxamate and *C*6-tetrazole D-ManA building blocks. The optimised experimental protocols developed, delivered the building blocks in high yields and multi-gram amounts. *C*6-hydroxamate building blocks were evaluated for the chemical glycosylation of modified alginate oligosaccharides by employing an iterative approach, whereby the non-reducing end was extended. Initial design of the strategy allowed access to the α- and β-linked mixed-system alginate disaccharides. The results derived from their evaluation emphasises the important contribution of both *C*6-hydroxamate donor and acceptor reactivity. An additional objective presented was the chemical synthesis of *C*6-bioisosteric D-ManA 1-phosphate building blocks.

Abbreviations

$(NH_4)_4Ce(SO_4)_4.2H_2O$	Ceric ammonium sulfate dihydrate
3-D	3-Dimensional
5-STs	5-Substituted tetrazoles
Ac_2O	Acetic anhydride
AcCl	Acetyl chloride
AcOH	Acetic acid
AFM	Atomic force microscopy
AgOTf	Silver trifluoromethanesulfonate
aq.	Aqueous solution
BAIB	Bis(acetoxyiodo)benzene
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
Bn	Benzyl
BnBr	Benzyl bromide
BSP	1-Benzenesulfinyl piperidine
Bu_2SnO	Dibutyltin (IV) oxide
BzCl	Benzoyl chloride
$CDCl_3$	Deuterated chloroform
CF	Cystic fibrosis
Ch1	Chitin synthase
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
COSY	COrrelated SpectroscopY
D_2O	Deuterium oxide
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCI	5-Dicyanoimidazole
DIAD	Diisopropyl azodicarboxylate
DIPEA	*N*,*N*-Diisopropylethylamine
DLS	Dynamic light scattering
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dmethyl sulfoxide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl(methylthio)sulfonium trifluoromethanesulfonate

DTT	Dithiothreitol
EDC.HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
EWG	Electron withdrawing group
FA	Formic acid
F_G	Molar fraction of G residues
g	Gas
G	L-Guluronic acid
GDP-ManA	Guanosine-5'-diphosphate D-mannuronic acid
GH	Glycoside hydrolases
GMP	Guanosine-5'-monophosphate
GT	Glycosyltransferase
GTP	Guanosine-5'-triphosphate
H_2NNH_2.HOAc	Hydrazine acetate
H_2NOH.HCl	Hydroxylamine hydrochloride
H_2O	Water
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HAD	Hydrogen-mediated aglycone delivery
hADSCs	Human adipose-derived stem cells
$HBF_4.Et_2O$	Tetrafluoroboric acid diethyl ether complex
HCl	Hydrochloric acid
HFIP	Hexafluoro-*iso*-propanol
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence/Correlation
IAD	Intramolecular aglycone delivery
IL	Interleukin
IM	Inner membrane
K_2CO_3	Potassium carbonate
KI	Potassium iodide
LDA	Lithium diisopropylamide
Lev_2O	Levulinoyl anhydride

LiOH	Lithium hydroxide
M	D-Mannuronic acid
m/*z*	Mass-to-charge ratio
MBOAT	Membrane bound *O*-acetyltransferase
m-CPBA	*meta*-Chloroperoxybenzoic acid
Me_2S_2	Dimethyl disulfide
MeCN	Acetonitrile
MeI	Iodomethane
MeOD	Deuteriated methanol
MeOH	Methanol
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
Min.	Minutes
N_2	Nitrogen
Na	Sodium
Na_2CO_3	Sodium carbonate
$Na_2S_2O_3$	Sodium thiosulfate
Na_2SO_4	Sodium sulfate
$NaBrO_3$	Sodium bromate
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NaOMe	Sodium methoxide
NBS	*N*-Bromosuccinimide
NDP	Nucleotide diphosphate
NGP	Neighbouring group participation
NH_4HCO_3	Ammonium bicarbonate
NH_4OH	Ammonium hydroxide
NHS	*N*-Hydrosuccinimide
NIS	*N*-Iodosuccinimide
N-MIC	*N*-methylimidazole hydrochloride
NMP	Nucleotide monophosphate

NMR	Nuclear magnetic resonance
n*O*e	Nuclear Overhauser effect
N-PTFACl	*N*-phenyltrifluoroacetimidoyl chloride
OM	Outer membrane
ORF	Open reading frame
P. aeruginosa	Pseudomonas aeruginosa
P_2O_5	Phosphorus pentoxide
PBr_3	Phosphorous tribromide
$Pd(OH)_2/C$	Palladium hydroxide (Pearlman's catalyst)
Pd/C	Palladium-on-carbon
PEG	Polyethylene glycol
PGA	Propene glycol esters of alginate
Ph_2SO	Diphenyl sulfoxide
$PhCH(OMe)_2$	Benzaldehyde dimethyl acetal
PhSH	Thiophenol
PL	Polysaccharide lyases
PMBCl	*para*-Methoxy benzyl chloride
PMI	Phosphomannose isomerase
$POCl_3$	Phosphorous (V) oxychloride
PPh_3	Triphenylphosphine
ppm	Parts per million
p-TsOH	*para*-Toluenesulfonic acid
PVA	Polyvinyl alcohol
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
R_f	Retention time
RRV	Relative reactivity values
RT	Room temperature
s	Solid
Sat.	Saturated
SGNH	Serine-glycine *N*-terminal hydrolases
SO_3.pyridine	Sulfur trioxide pyridine complex
spp.	Specie
TBA	Tetrabutylammonium
TBAI	Tetra-butyl ammonium iodide

TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDMSOTf	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
TCA	Trichloroacetimidate
TEM	Transmission electron microscope
TEMPO	2,2,6,6-Tetra-methylpiperidine-1-oxyl
Tf_2O	Trifluoromethanesulfonic anhydride
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TM	Transmembrane
$TMSN_3$	Trimethylsilyl azide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF	Tumour necrosis factor
TPR	Tetratricopeptide
TTBP	2,4,6-Tri-*tert*-butylpyrimidine

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Publications

1) Dimitriou, E., Miller, G. J., “Exploring a glycosylation methodology for the synthesis of hydroxamate-modified alginate building blocks”, *Org. Biomol. Chem.*, **2019**, *17*, 9321-335.

2) Dimitriou, E., Jones, R. H., Ritchard, R. G., Miller, G. J., & O'Brien, M., “Gas-liquid flow hydrogenation of nitroarenes: Efficient access to a pharmaceutically relevant pyrrolobenzo [1,4] diazepine scaffold” *Tetrahedron*, **2018** *74*, 6795-6803.

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1. Alginate sources and chemical structure

Alginate is a non-repeating linear anionic polysaccharide consisting of variable proportions of β-1,4-linked D-mannuronic acid (M) and its *C*5 epimer L-guluronic acid (G) (Figure 1).[1,2,3] Alginate was first extracted from brown algae (*Phaeophyceae*) in the 1880s, and it has been commercially available since the early 20th century. As a naturally occurring anionic and hydrophilic polysaccharide, alginate exhibits different viscosity and gel-forming capacity, as the chain length of the polymer and ratio of M and G residues (block structure) depend on the source from which it is isolated. Alginate is one of the most abundant biosynthesised materials that has been extensively studied for the design and development of new biomaterials that can be used as stabilisers, viscosifiers and gelling agents in the food, beverage, paper and pharmaceutical industries.[4,5,6,7] The wide range of alginate-based biomaterial applications relies on its processability for the development of 3-D scaffolding materials such as hydrogels, sponges, microspheres, microcapsules, fibres and foams.[8] Modifying the chemical structure of alginate results in alternative physicochemical properties useful in the fields of biomedicine and biotechnology.[7,8] This emphasises the potential of modified alginate biopolymers in constructing novel materials for applications such as drug delivery or tissue engineering.[8]

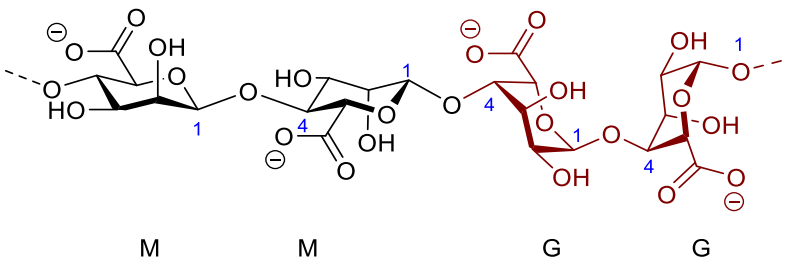


***Figure 1**: Chemical structure of alginate.*

A large part of alginate polysaccharide research has focused on identifying and evaluating the impact of M and G molar fraction on the gelling, permeability, viscosifying and stabilising properties of the polysaccharide.[6,9] The two uronic acids adopt different chair conformations (M: $^4C_1$ and G: $^1C_4$) in order to place the bulky carboxyl group in an energetically favoured equatorial position. Therefore, the glycosidic bonds at positions *C*1 and *C*4 will be equatorial in M and axial in G (Figure 1).[10] This conformational difference makes G-blocks responsible for the specific divalent ion binding which consequently tunes the gelling properties of alginate.[8,9] The resultant interaction between two pairs of G-blocks from a different polymer chain with a divalent cation induces the formation of an 'egg-box'-like structure (Figure 2).[11]

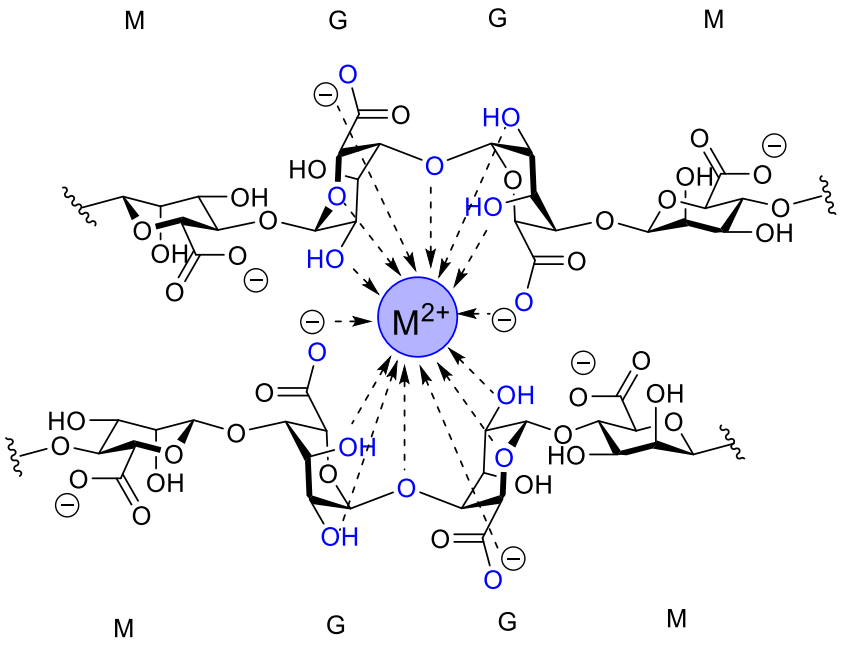


***Figure 2**: An indicative mechanism of ionic interaction between two interchain G-blocks and a divalent cation (e.g. Ca^{2+}, Mg^{2+}, Fe^{2+}, Ba^{2+}, or Sr^{2+}), forming an 'egg-box'-like structure.[1,8]*

It has been proposed that M-blocks and MG-blocks function as elastic 'hinges' between the cross-linked chains that lack the ability of binding divalent cations.[1] The chelating properties of alginate signify its versatility as a biopolymer whereby

chemical modification of it can produce novel biomaterials with the capability to encapsulate and protect cationic drugs or therapeutically useful cells from the biological environment they are subjected to.[8]

Alginate can additionally be produced by two genera of bacteria, *Pseudomonas* and *Azotobacter*.[12] On the one hand, the soil dwelling *A. vinelandii* produces alginate with higher concentrations of G-blocks, which in turn induce the formation of gels in the presence of divalent cations such as Ca^{2+}. Gel formation constitutes an important feature that enhances the structural integrity of alginate produced by *A. vinelandii*.[1,10,13] On the other hand, the opportunistic human pathogen *P. aeruginosa* synthesises acetylated alginate that lacks G-blocks (homopolymeric fragments consisting of G monomers), leading to the formation of thick and highly structured biofilms (Figure 3).[10,14,15] In this instance, alginate is considered a virulence factor, as it participates in the formation of persistent biofilms that protect the bacterium after infection of the lungs of cystic fibrosis (CF) patients.[16]

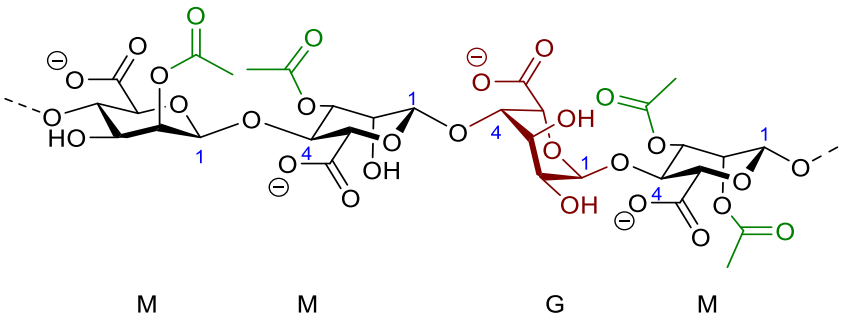


Figure 3: *Chemical structure of P. aeruginosa alginate comprising of β-1,4-linked M and its C5 epimer, G. O-acetylation may occur at the hydroxyl groups at the C2 and/or C3 positions of M.*

The extensive use of alginate in high-value biotechnological and biomedical applications requires a steady supply of material with defined homogeneity in G/M composition and polymer lengths. Specifically, industrial alginate production reaches 30,000 metric tons annually.[17] Due to the abundance of algae in water bodies, a large

proportion of commercially available alginates is obtained from algal sources.[17,18] However, alginate extracted from seaweed suffer from problems with mechanical stability, heterogeneity in G/M composition and wide pore size distribution. Bacterial biosynthesis may provide alginate with more defined chemical structures and physical properties, however, due to the pathogenic nature of *P. aeruginosa*, any form of commercially available alginate is preferably produced by *A. vinelandii* or non-pathogenic Pseudomonas spp.[1]

A considerable amount of literature has been published focusing on alginate biosynthesis in *P. aeruginosa*, nevertheless, further understanding of the biosynthetic pathway is required, especially for the polymerisation, modification and secretion processes.[1,2] The development of structure-function tools to probe the various enzymatic pathways could shed light on the molecular mechanisms, thereby showing important information for the design of potential inhibitors. Considering both the advantageous material properties of alginate and its role as a virulence factor in *P. aeruginosa*, the research presented here divided its focus on the biomaterial aspect of alginate, as well as the biochemical aspect and specifically on the alginate biosynthetic pathway in *P. aeruginosa*. Following the introduction on alginate biosynthesis from the pathogen, the discussion on alginate derivatisation for the production of novel biomaterials, and the chemical synthesis of alginate oligomers, the design of synthetic tools is presented in Section 5.

2. Alginate biosynthesis in *P. aeruginosa*

Pseudomonas aeruginosa and *Azotobacter vinelandii* are the best-characterised organisms that secrete alginate.[19] Despite the fact that the two genera of bacteria utilise similar biosynthetic pathways to produce the heteropolymeric polysaccharide,

they require it for different biological functions.[1,20] In brown algae and *Azotobacter* cysts, alginate serves as a cell wall constituent, whereas in *Pseudomonas* it contributes to the biofilm matrix.[21] A large number of studies explain that the random structure of alginate stems from the fact that the enzymatic modification of M residues takes place at the polymer level. It has also been identified that the gram-negative pathogen secretes at least two other exopolysaccharides, Psl and Pel.[22] However, the biosynthetic pathway of alginate is the best characterised due to its direct involvement in acute and chronic pulmonary infections, especially in CF (Figure 4).[21,23]

Clinical isolates of *P. aeruginosa* obtained from CF patients display biofilms with high levels of alginate secretion (Figure 5).[24,25] The genetic competence of *P. aeruginosa* to self-encapsulate in differentiated three-dimensional biofilm matrices constitutes a key adaptation strategy that augments its defense against hostile external conditions, such as antibiotic treatment, disinfectants, oxidative stresses, nutrient/oxygen restriction, metabolic waste accumulation, interspecies competition, and host immune response (phagocytosis).[15,21,16,25] Particularly, *in vitro* experiments have demonstrated that biofilm properties such as viscoelasticity, bio-volume, cell-to-cell interaction, cell aggregation, cell density, surface attachment, and architecture, are strongly dependent on the composition and chemical structure of alginate.[15]

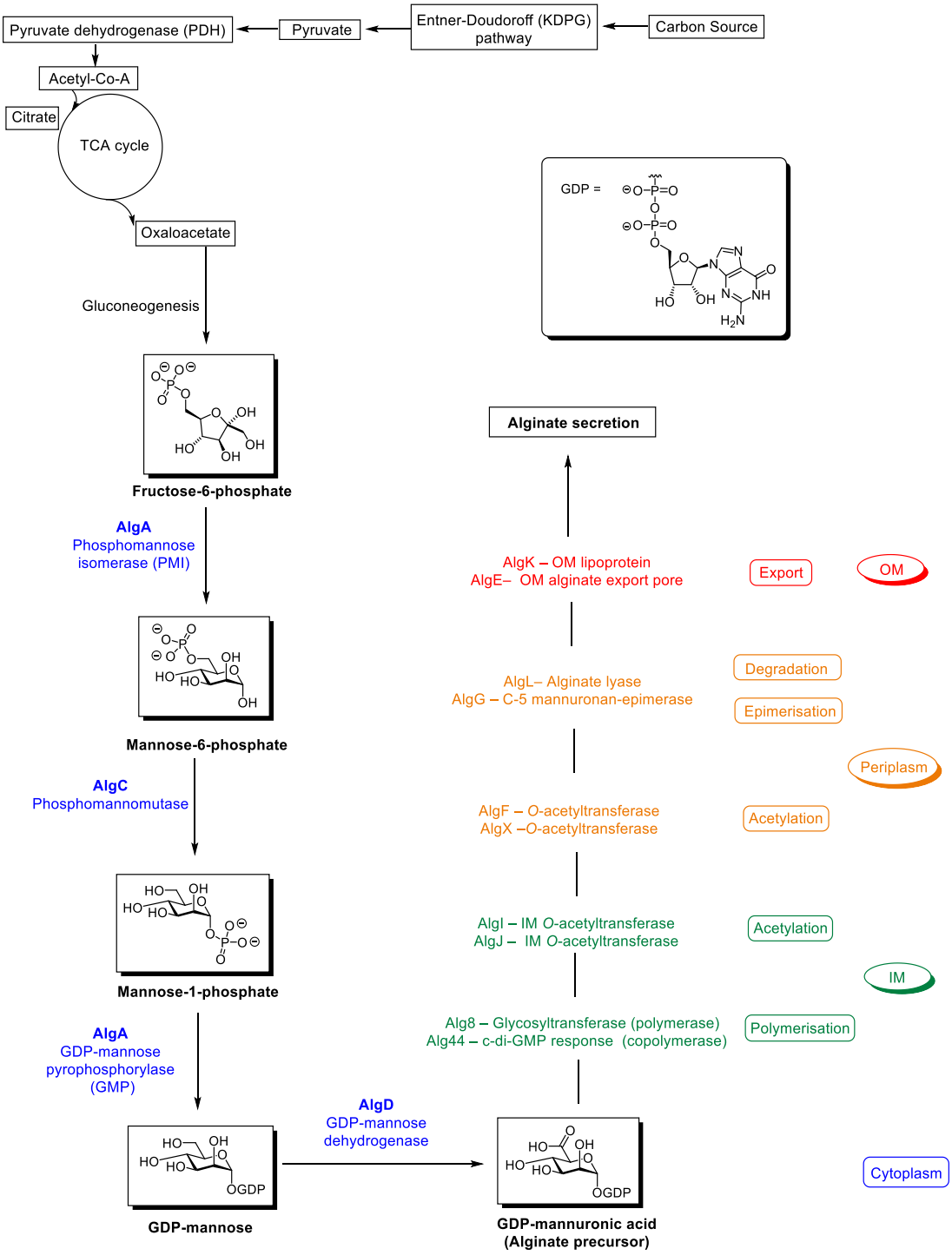


***Figure 4**: Overview of alginate biosynthesis in P. aeruginosa indicating the structures participating in each stage in the cytoplasm (blue). The polymerisation and acetylation processes take place in the inner membrane (IM) (green),acetylation, epimerisation, and degradation take place in the periplasm (orange), and secretion of mature alginate comes out from the outer membrane (OM) (red) [Adapted from Hay et al. (2013)].*[1]

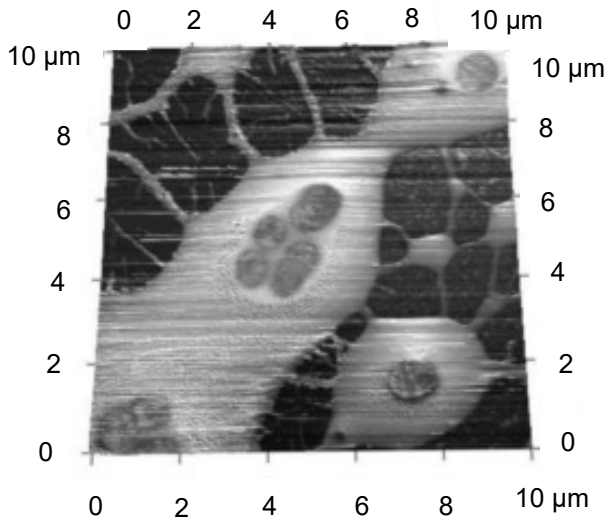


Figure 5*: Atomic Force Microscopy (AFM) image of the mucoid CF P. aeruginosa FRD1 strain, showing alginate as a dense gelatinous structure that surrounds the cells [Adapted from Franklin et al. (2011)].*[21]

Consequently, alginate polysaccharide is essential for the survival of *P. aeruginosa* during pathogenesis and antibiotic treatment, emphasising the need for an in-depth understanding of alginate biosynthesis, as the information extracted from mechanistic pathways could aid in the design and development of inhibitors.[16] In fact, it has been reported that these strains are resistant to almost all categories of widely used antibiotics, including cephalosporins, fluoroquinolones, amino glycosides and carbapenems.[26] The ability of *P. aeruginosa* to switch from non-mucoid strains to the alginate-overproducing mucoid phenotype once it colonises the respiratory epithelium, is linked to the decline of pulmonary function and survival rate of individuals suffering from CF.[19,15,27]

2.1. Alginate precursor synthesis

The formation of the activated sugar nucleotide building block, guanosine diphosphate mannuronic acid (GDP-D-ManA), is an important process for the exopolysaccharide biosynthesis and consequently well-characterised.[1] The

precursor biosynthesis takes place in the cytosol, where a series of metabolic steps mediated by the enzymes AlgA, AlgC and AlgD deliver GDP-ManA to the membrane-bound multiprotein complex composed of the rest of the proteins encoded by the operon.[14,15] To begin with, the bifunctional protein AlgA converts fructose-6-phosphate to mannose-6-phosphate by employing its phosphomannose isomerase (PMI) activity.[28] Mannose-6-phosphate is then converted into mannose-1-phosphate by phosphomannomutase, AlgC.[29] The third step requires the GDP-mannose pyrophosphorylase (GMP) activity of AlgA responsible for the catalysis of activated mannose-1-phosphate to GDP-mannose, using guanosine-5'-triphosphate (GTP).[30] Interestingly, the GMP activity of AlgA favours the reverse reaction; however, the continuous and consecutive conversion of GDP-mannose to GDP-ManA by the activity of AlgD (GDP-mannose-dehydrogenase, GMD) shifts the reaction equilibrium towards the production of the desired sugar-nucleotide.[14,31]

The final step for the conversion of GDP-mannose to GDP-ManA is catalysed by GMD.[32] The oxidation step by AlgD and the high intracellular concentrations of GDP-mannose suggest that this final four-electron transfer is underpinning to the overall alginate biosynthesis, as it supplies the alginate polymerization/secretion multiprotein complex with its precursor.[14,31,33,34] GMD is a nicotinamide adenine dinucleotide (NAD+)-dependent four-electron-transfer dehydrogenase.[35] Snook *et al.* have determined the crystal structure of GMD in complex with its cofactor NAD(H) and GDP-ManA at a resolution of 1.55 Å (Figure 6a).[32]

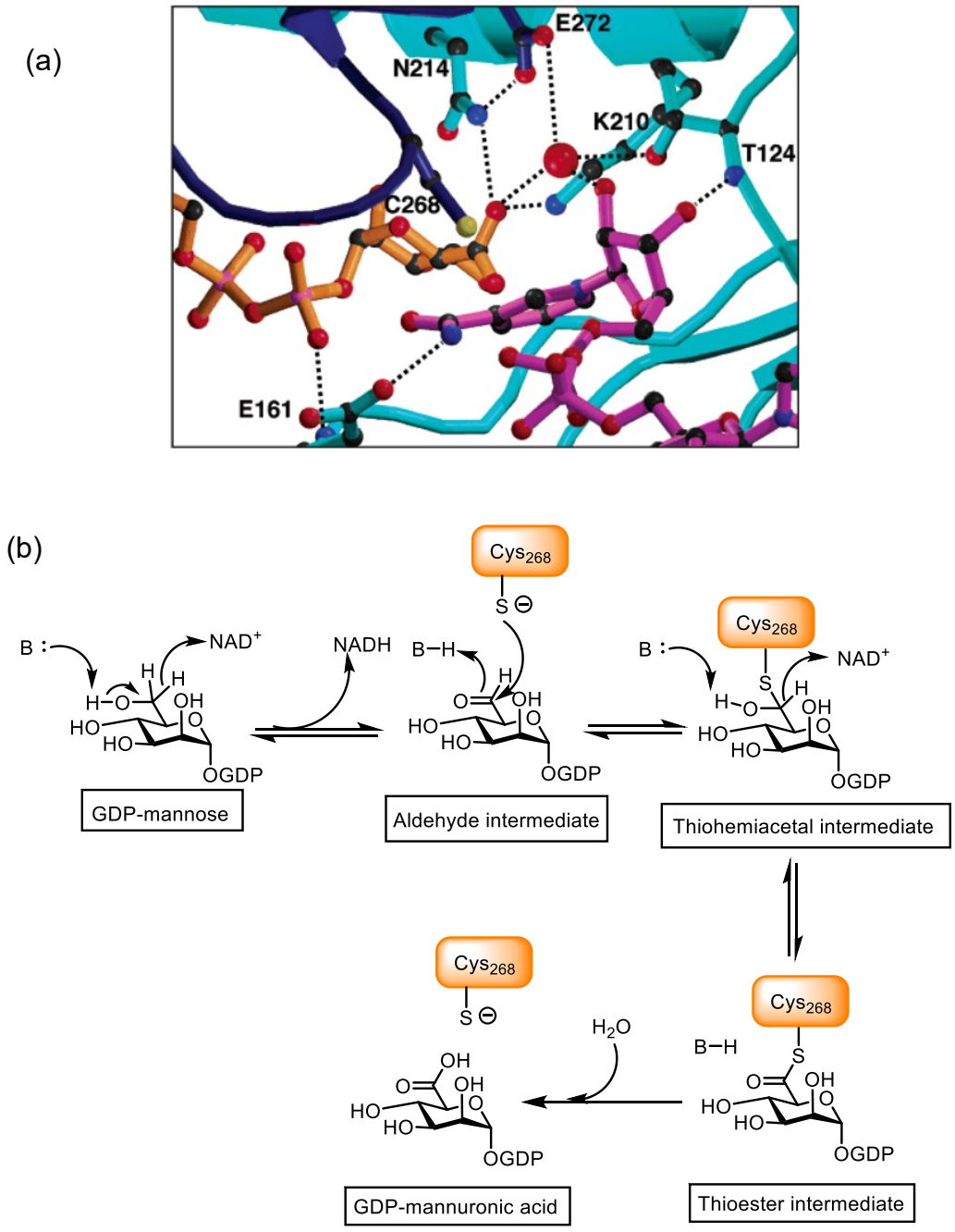


Figure 6: *GMD active site structure and proposed mode of action. (a) Crystal structure of GMD active site with GDP-ManA (gold), a conserved water molecule that contacts the pyrophosphate group. (b) Proposed step-wise mechanism in a bi uni uni bi ping-pong fashion. [Taken by Snook et al. (2003)].*[32]

Important mechanistic information was obtained from the crystal structure, as they categorised the multistep oxidation reaction into four steps: (1) oxidation of the *C*6 hydroxyl to an aldehyde; (2) nucleophilic attack by a thiol group (Cys-268) to form the thiohemiacetal intermediate; (3) oxidation of the intermediate to a thioester; (4)

release of GDP-ManA by hydrolysis (Figure 6b). As a key regulatory enzyme in the alginate biosynthetic pathway, GMD has been conceived as a potential target to inhibit alginate secretion, thus making *P. aeruginosa* susceptible to antibiotic treatment.[1]

2.2. Polymerisation of GDP-D-ManA

To date, the polymerisation and translocation processes of alginate biosynthesis remain unclear. This uncertainty stems from the fact that no *P. aeruginosa* polymerase or polymerase complex has been purified for further detailed studies on function and structure determination.[14] Nevertheless, one of the most significant findings in the alginate polymerisation process is the vital involvement of the inner membrane (IM) proteins, Alg8 and Alg44. In the light of experimental evidence from disruption mutagenesis studies, deletion mutations of *alg8* and *alg44* resulted in the complete termination of alginate biosynthesis.[19,36] The deletion of *algG*, *algK, algX* and *algE* resulted in the secretion of uronic acid oligomers generated by degradation of the alginate polymer caused by the catalytic activity of the periplasmic alginate lyase, AlgL.[37,38,39] This observation suggests that AlgG, AlgK, AlgX and AlgE offer some degree of chemical modification that offers structural protection to alginate from AlgL, as they do not contribute directly to the polymerisation process.[14,40,41] Furthermore, the mucoid phenotype of *P. aeruginosa* is maintained despite the deletion of *algI*, *algJ,* and *algF*. [21,42]

Data from bioinformatic analysis have established that Alg8 is the best candidate for a polymerase.[43] Homology studies classify Alg8 as a member of β-glycosyltransferases of class II (GT-2), a large family of glycosyltransferases that catalyse the transfer of an activated carbohydrate donor to a growing acceptor

carbohydrate chain.[1] Considering that Alg8 shares structural similarity with other members of the transmembrane (TM) GT-2 family, such as cellulose synthase (AcsAB) and chitin synthase (Ch1), it is predicted to possess at least five TM proteins and a large cytoplasmic GT domain.[19,36] Remminghorst *et al.* demonstrated that Asp-188/Asp-190, Asp-295/Asp-296 and Lys-297 of Alg8 were indispensable for *in vivo* polymerase activity, and therefore alginate production.[3] The first pair of aspartic acids, Asp-188/Asp-190, is accommodated in the DXD motif which is believed to be involved in sugar-nucleotide binding.[44] The second pair, Asp-295/Asp-296 is detected in the cytoplasmic GT loop and it is suggested to play an acid-base catalytic role. Site-directed mutagenesis of the proposed conserved catalytic residues indicated loss of alginate production, validating the importance of the catalytic subunit.[3] Further compelling evidence that highlights the significance of Alg8 in alginate polymerisation is the overproduction of alginate caused by the overexpression of Alg8, which finally led to a super mucoid phenotype.[43] In fact, experimental support from *in vitro* polymerisation experiments conducted by Remminghorst and Rehm recommend Alg8 to be indispensable for alginate biosynthesis, as it amplified alginate secretion by a factor of 15 when a plasmid containing only the open reading frame (ORF) of *alg8* (termed pBBR1MCS-5:alg8) was exploited for restoring alginate production in the *alg8* deletion mutant (termed PDO300*Δ*alg8(pBBR1MCS-5), as it subsequently converted it to a super mucoid mutant (termed PDO300*Δ*alg8(pBBR1MCS-5:alg8)) (Figure 7b, 7c, 7e, 7f).[19]

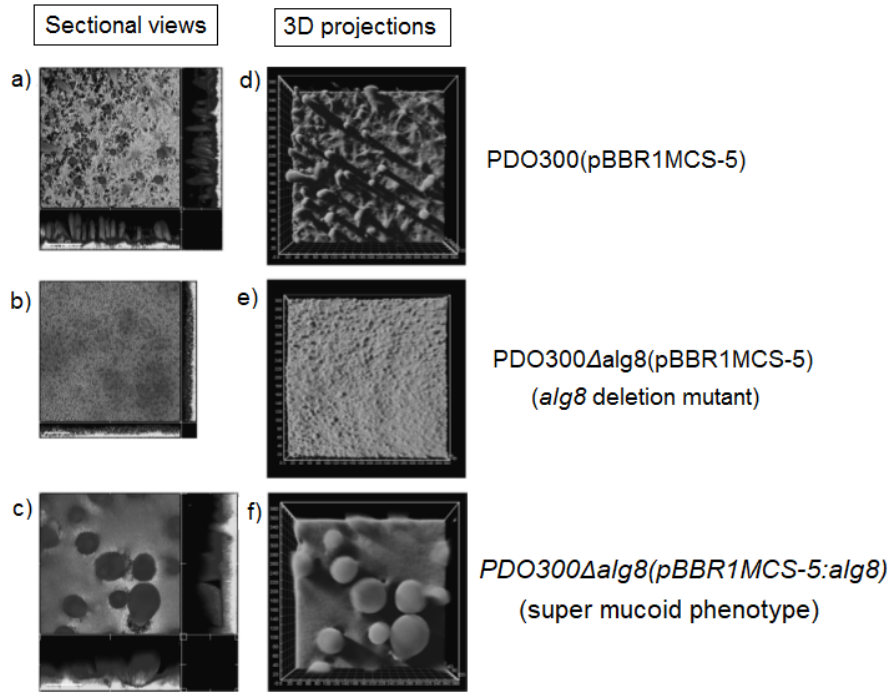


Figure 7*: P. aeruginosa biofilm formation in a continuous-culture flow cell, analysed using a confocal laser scanning microscope(a,d) Control strain showing a mature and fully differentiated biofilm with well-defined finger-like microcolonies (b,e) alg8 deletion strain exhibiting a thinner biofilm with no apparent microcolonies (c,f) supermucoid strain showing the thickest biofilm [Adapted from Hay et al. (2009a)].*[43]

In addition to this, NMR analysis by using ^{14}C labelled-GDP-D-ManA as substrate indicated that additional *alg8* gene copies influence alginate composition and architecture which finally affect properties such as solubility, morphology, and viscosity. The ^{1}H NMR data that derived from the complementary mutant PDO300Δalg8(pBBR1MCS-5:alg8) revealed an increase from 4.7% to 9.3% in the degree of ^{14}C GPD-D-Man acetylation, whereas the same experiment suggested a significant decrease from 38% to 19% in the content of G residues.[19] According to these results, Alg8 overexpression leads to higher level of alginate secretion, a larger degree of *O*-acetylation and lower degree of epimerisation. Their results were consistent with a quantitative analysis provided by Hay *et al*., as their investigation showed that the thickest biofilm (90-145 μm in height) was produced by the super

mucoid strain of *P. aeruginosa* (PDO300*Δ*alg8(pBBR1MCS-5:alg8)), containing high proportions of alginate (Figure 7c). Large quantities of alginate led to the development of exceptionally large microcolonies, verifying its crucial role in microcolony formation.[43] Furthermore, Remminghorst and Rehm have proven using enzymatic *in vitro* alginate synthesis with ^{14}C GPD-D-ManA that alginate polymerase activity is localised in the cell envelope fraction (IM and outer membrane (OM) plus respective proteins), designating that the function of Alg8 strongly relies on the other proteins. This finding, along with the results from the above complementation studies create the following question; If an alginate multi-protein biosynthetic complex is required for alginate production, why does the addition of *alg8* gene copies amplify polymer production? Thus, Remminghorst and Rehm proposed Alg8 to be the bottleneck in alginate biosynthesis.[19]

Prior to the work of Moradali *et al*., the specific role of Alg44 in alginate polymerisation was largely unknown. Alg44 was proposed to play an indirect but significant role in the polymerisation process.[1] This conclusion was based on experimental evidence where deletion of *alg44* led to a complete loss of alginate polymerisation, while its overexpression resulted in alginate overproduction.[45,36] Alg44 was suggested to function as a bridge between the IM protein Alg8 and the OM export protein AlgE, facilitating the transit, modification, and secretion of alginate.[1,45] Moradali *et al*. were the first to elucidate experimentally the direct protein-protein interaction between Alg8 and Alg44, as well as the interaction between Alg44 and AlgK.[15] Previously, Rehman *et al.* identified the interaction between Alg44, AlgX and AlgK, by observing reduced alginate production in *algK* and *algX* deletion mutants.[2] Their findings also confirmed the hypothesis of previous work supporting that Alg44 is important for the structural integrity of the multiprotein alginate biosynthesis

machinery, Alg8-Alg44-AlgX-AlgK-AlgE (Figure 8).[2,15,45]

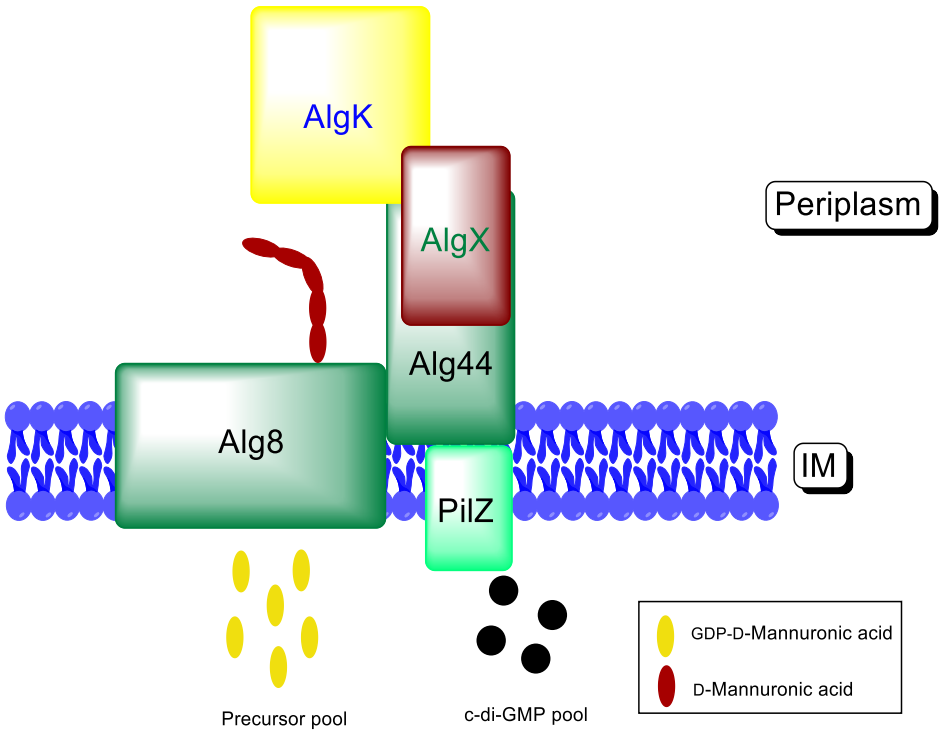


***Figure 8**: Schematic overview of the protein-protein interaction between Alg8-Alg44, Alg44-AlgK, and Alg44-AlgX.*

2.3. Periplasmic translocation and modification of polymannuronate

The post-polymerisation modifications of the linear D-mannuronate homopolymer define the functional properties of mature alginate.[6,42,46] Once the exopolysaccharide is synthesised in the cytoplasm, it is translocated across the IM and undergoes three types of modification in the periplasm. One of the modifications is the *O*-acetylation at the *O*2 and/or *O*3 positions of the M residues by the complex AlgI, AlgJ, AlgF, and AlgX (Figure 9).[47] In general, high degrees of alginate acetylation increase water-holding capacity, viscosity and pseudoplastic rheology of the biofilm and consequently the polymer acts as an impenetrable shield of protection for the bacterium.[1,47,48] The glue-like properties of *O*-acetylated alginate are highly important for mucoid strains of *P. aeruginosa*, as they significantly impact biofilm architecture,

and therefore the cell aggregation at the early stages of surface colonisation, as well as the shape of macrocolonies.[49]

A second modification is the *C*5 epimerisation of selected M residues to G by AlgG (Figure 9).[50,51] Thus far, several studies have examined whether acetylation occurs prior to epimerisation, as studies revealed that acetylation of the M residues prevents the epimerisation to G residues by AlgG.[13,46] In addition, *O*-acetylation is suggested to prevent the third modification that might occur before alginate secretion, which is degradation by AlgL.[52]

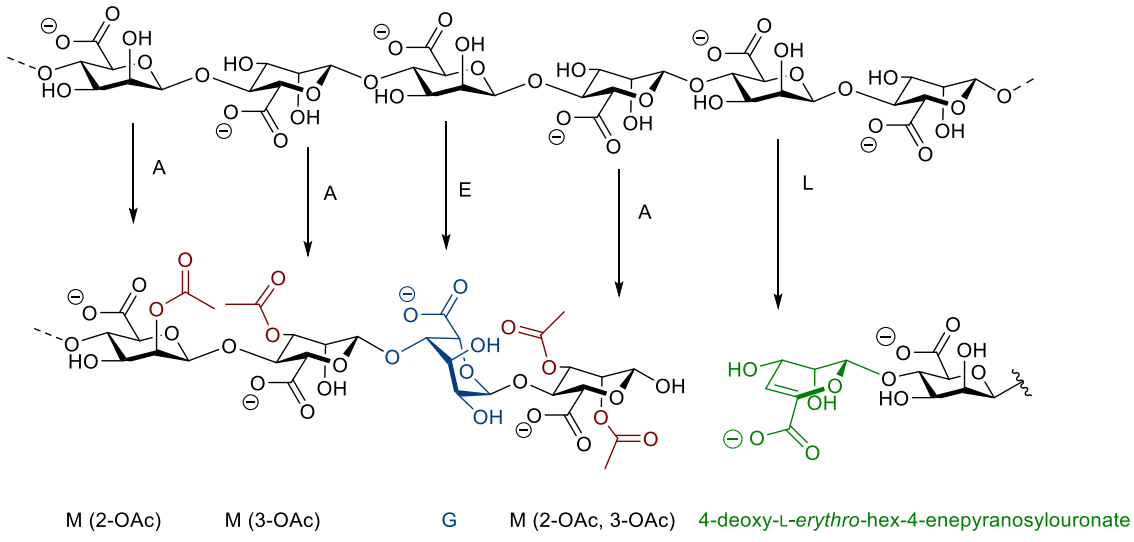


Figure 9: *Enzymatic modifications of alginate in the periplasm. M may be acetylated at C2 and/or C3 positions by AlgX, AlgJ, AlgF, AlgI complex (red), epimerised to G by AlgG (blue), or degraded by AlgL in an unsaturated 4-deoxy-L-erythro-hex-4-enepyranosylouronate residue.*

Therefore, it is speculated that *O*-acetylation occurs first, as it indirectly affects the degree of epimerisation, length of the exopolysaccharide, and therefore its molecular weight.[1,14] In other words, the ability to control the first post-polymerisation modification, which is alginate *O*-acetylation, could allow some level of regulation over the magnitude of the subsequent modifications. Although *P. aeruginosa* does not produce alginate composed of G-blocks, it is worth noting that their existence affects

the mechanical characteristics of the resulting polysaccharide.[1] Specifically, the diaxial *O*-glycosidic bond in G-blocks leads to the formation of linkages with large hindered rotation, which enhance several gelling properties of alginate such as stiffness, swelling and porosity.[1,9]

O-acetylation of alginate is a complicated biological process of great significance for the survival and virulence of *P. aeruginosa*.[25] Data from several sources have identified that this process demands the transportation of an acetyl-donor into the periplasm by a membrane bound *O*-acetyltransferase (MBOAT) and the transfer of the acetate onto the polymannuronate by a periplasmic *O*-acetyltransferase.[50,42,53,54] The four protein complex of *P. aeruginosa* is composed by a MBOAT protein (AlgI), two periplasmic *O*-acetyltransferases (AlgJ, AlgX) and a protein of unknown function (AlgF).[25,42,53,54] AlgF is not expected to possess a catalytic domain and given the current state of knowledge, its function can only be speculated. Moreover, it is currently uncertain why two *O*-acetyltransferases are needed for alginate acetylation.[25,50] It is hypothesised that AlgI catalyses the transfer of an acetate from an unknown cytoplasmic acetyl donor (possibly Coenzyme A or an acyl carrier protein) across the IM to AlgJ and/or AlgF in the periplasm.[54] As opposed to AlgF and AlgX, AlgJ is associated with the IM through a TM domain, setting the enzyme closer to AlgI. In addition, AlgX can bind alginate in a length-dependent manner, and catalyse its direct *O*-acetylation after receiving the acetate or acetyl donor by AlgJ or AlgF.[25] The fact that active site variants of AlgJ and AlgX terminated *O*-acetylation *in vivo*, supports the above model of alginate acetylation process.[50,54]

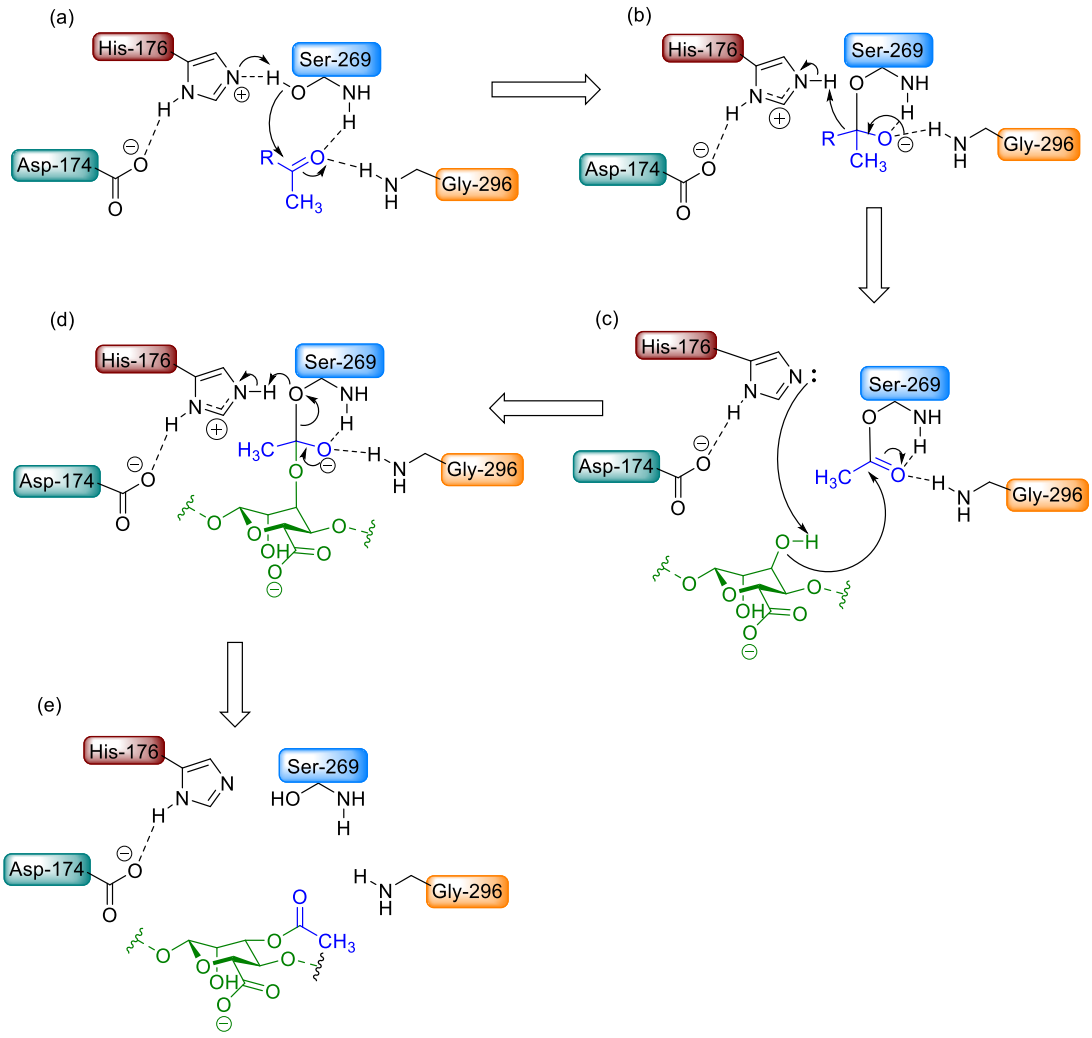


***Figure 10**: Proposed catalytic mechanism of the acetyltransferase activity of AlgX [Adapted from Riley et al. (2013)].*[50]

Riley *et al*. proposed a mechanism of alginate *O*-acetylation, based on their structural and functional analysis of *P. aeruginosa* AlgX crystal structure at 2.15 Å resolution (Figure 10). They suggested Ser-269 to be both a nucleophile and one of the two proton donors in the oxyanion hole, and Gly-296 residue to serve as the second proton donor which stabilises the negative charges of the tetrahedral acetyl oxyanion intermediate. The highly conserved His-176 could act as a catalytic base accountable for the nucleophilic activity of Ser-269, as it could deprotonate its hydroxyl group. The aspartic acid residue Asp-174 present in the catalytic site is important for neutralising the charge developed on His-176 during acylation.[50] As

opposed to the majority of serine-glycine-*N*-terminal hydrolases (SGNH) that possess a conserved asparagine residue responsible for the oxyanion hole stability, PaAlgX$_{27-474}$ has a tyrosine (Tyr-328) residue instead. Specifically, a series of experiments replacing Tyr-328 and Tyr-275 with alanine led to a 40% and a 50% decrease of alginate *O*-acetylation, respectively.[25,50] These results emphasise the importance of these tyrosine residues to situate the polysaccharide chain correctly across the catalytic site.[50] The annotated structures of both AlgJ and AlgX, as well as their phenotypic characterisation, have definitely provided crucial information regarding their role in the *O*-acetylation of the alginate polysaccharide, as both enzymes are part of the SGNH hydrolase family and share the same amino acid residues required for *O*-acetyl-transferase activity.[25,50]

As previously mentioned, *Pseudomonas* does not share the same need for G-blocks in its alginate polymer with Azotobacter and brown algae.[6] As opposed to *Azotobacter vinelandii* that encodes extracellular Ca^{2+}-dependent mannuronan *C*5 epimerases (AlgE1-7), *Pseudomonas spp.* produce only a single periplasmic Ca^{2+}-independent mannuronan *C*5 epimerase, AlgG.[6,55] The periplasmic Ca^{2+}-independent alginate epimerases AlgG of both *Pseudomonas spp.* and *Azotobacter vinelandii* share ~60% sequence identity.[56,57,58] The literature findings discussed above clarify that the correct balance between *O*-acetylation and epimerisation processes plays a pivotal role in the pathogenesis of *P. aeruginosa*, as both modifications affect the material properties of mature alginate and subsequently, the structural integrity of the biofilm.

Wolfram and co-workers proposed an epimerase reaction mechanism based on the information obtained from solving the crystal structure of *P. syringae* AlgG at

2.1 Å resolution (Figure 11).[59]

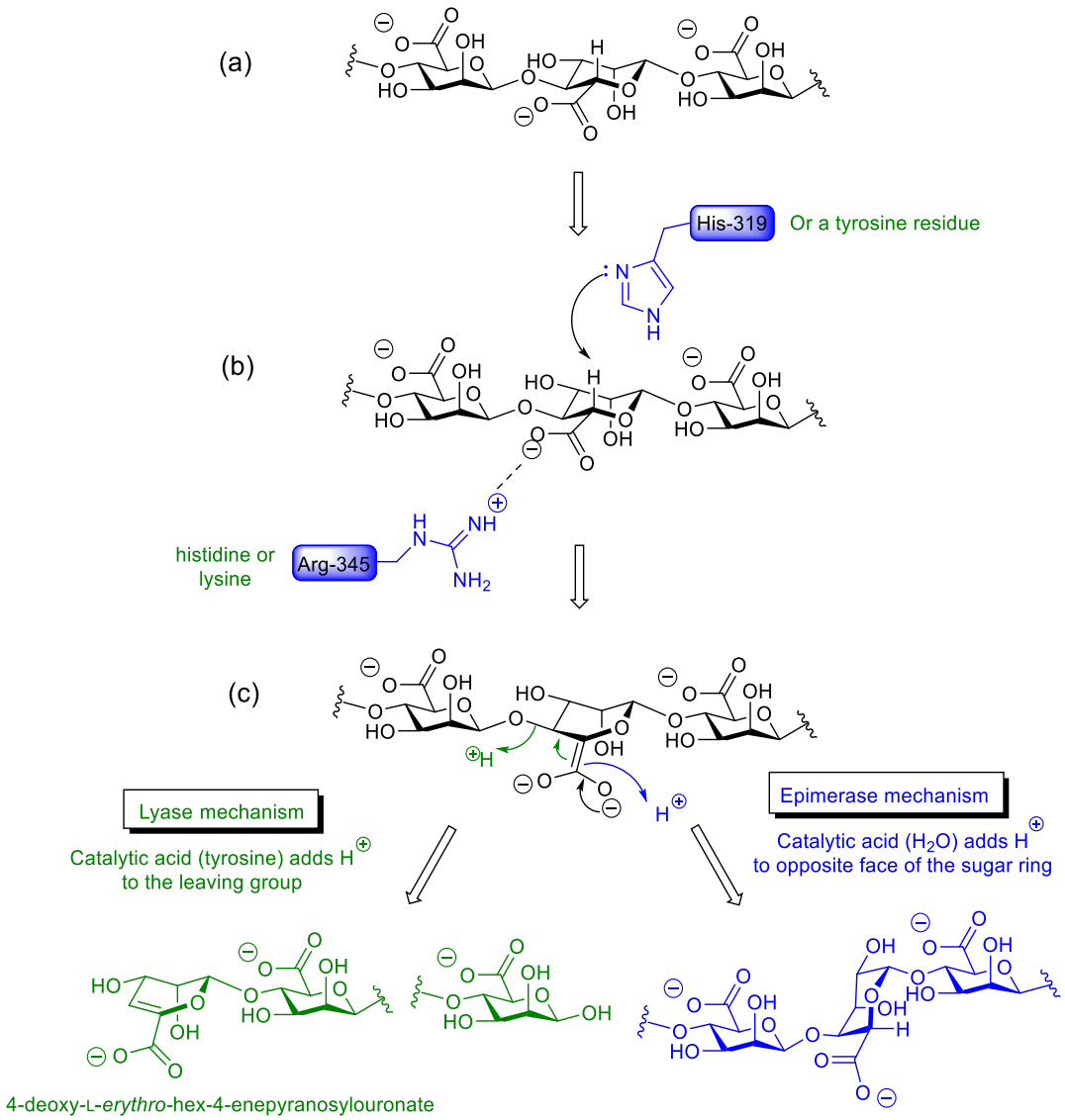


***Figure 11**: Proposed catalytic lyase (green) and P. syringae AlgG epimerase (blue) reaction mechanisms [Adapted from Wolfram et al. (2014)].*[55]

Firstly, they suggested that the negative charge of the carboxylate group on the uronic acid must be neutralised by the positive charge of Arg-345. The proton on *C*5 position is then abstracted by His-319 which acts as the base, forming an enolate group stabilised by resonance. According to the crystal structure of the active site, Wolfram *et al.* demonstrated that the hydrogen bond interaction between His-319 and Asn-320

is responsible for the former to facilitate the abstraction of the proton at *C*5, as their interaction reduces the pK_a value of His-319 leading to its deprotonation at the observed pH range (pH= 6-7.5).[55,56] In the epimerase reaction mechanism, the proton is added to the opposite side of the *C*5 of the pyranose ring by a water molecule, furnishing a G residue. In the case of the lyase reaction, a histidine or a lysine residue might be responsible for neutralising the negative charge of the uronic acid, while a tyrosine residue acts as the base. The lyase reaction mechanism utilises a similar mechanism, and the difference lies in the last step, where β-elimination occurs after turning *O*4 it into a leaving group by protonation. The electron transfer from the carboxyl group leads to a double bond formation between *C*4 and *C*5 creating a new non-reducing end after the cleavage of the *O*-glycosidic bond.[55] The catalytic acid in the lyase mechanism is the same tyrosine residue that acted as a base in the first step of proton abstraction.[6,60]

As expected, point mutations of conserved amino acid residues in the cleft lead to the secretion of the exopolysaccharide lacking G residues.[15,21] In comparison, *algG* deletion mutants result in the production of depolymerised alginate. These results connote that even though AlgG can be enzymatically inactive, its association with the other proteins in the multi-protein machinery contributes to the protection of alginate from AlgL-mediated degradation, as long as the 3-D structure of the protein is maintained.[21,39] This also proposes that the polymer can move through the biosynthetic machinery by interacting with the catalytic site of AlgG.[21] The above conclusions were confirmed by *Moradali et al.* while trying to restore alginate production in AlgG-negative mutants. Specifically, the addition of catalytically inactive variant AlgG(D324A) resulted in the highest alginate molecular mass (4.653 ± 1.1% kDa/ 22.876 uronic acid residues) compared to that of active AlgG, proposing that the

epimerisation event may restrict the acetylation process of alginate. The above experimental evidence also designated that as the molar fraction of G residues (F_G) increases, the molecular mass of the resulting alginate decreases.[15]

AlgG is responsible for converting 22-44% of non-acetylated M residues to G residues in *P. aeruginosa*.[13,14,55] Based on the finding that the composition of alginate reached 75% of G content when D-polymannuronate is incubated with AlgG, Schurks *et al.* and Jerga *et al.* suggested that strict regulation and/or competition between the modification processes might take place *in vivo*.[61,62] This interpretation contrasts with that of Moradali *et al.*, as they proposed that *O*-acetylation and epimerisation are not competitive modification events, but linked.[15] Moradali and his group utilised mutants with catalytically inactive variants of AlgX(S269A) and AlgG(D324A). Interestingly, the highest values of F_G were obtained by the addition of active or inactive copies of AlgX (F_G = 0.36), which supports that AlgX plays a crucial role in alginate epimerisation. Moreover, the addition of active or inactive copies of both AlgG and AlgX resulted in higher degree of alginate epimerisation. It is also of exceptional interest to note that additional copies of both AlgX and AlgG resulted in higher degree of *O*-acetylation, as opposed to additional copies of AlgX alone. All of the above experimental evidence suggest that both AlgX and AlgG are fundamental for the fulfillment of both acetylation and epimerisation processes, indicating some form of auxiliary behaviour.[15] Both modifications convert polymannuronate to mature alginate and as a result, it is protected from degradation by AlgL during transport across the periplasm.[40]

Alginate lyases are produced by organisms that harness alginate as a carbon source. Although alginate-producing bacteria synthesise alginate lyases as well, thus

far, none of them have been reported to utilise alginate as a carbon source.[6] *In P. aeruginosa*, *algL* belongs to the gene cluster that encodes the enzymes required for alginate biosynthesis.[6,63] In general, 7 Polysaccharide lyases (PL) families are currently known, PL-5, -6, -7, -14, -15, -17, -18 and their classification is based on the hydrophobic cluster of their primary structures.[64] Homology modelling studies showed that *P. aeruginosa* AlgL is a member of PL-5 family, and structurally similar to A1-III of *Sphingomonas spp*.[65] In terms of catalytic mode of action, there are endolytic and exolytic alginate lyases, where the former group degrades alginate into unsaturated di-, tri- and tetra-saccharides, and the latter can further cleave alginate polymer into monomers.[66] Tipton *et al.* demonstrated that *P. aeruginosa* AlgL utilises endolytic catalysis, as confirmed by using ^{1}H NMR spectroscopy together with mass spectrometry to characterise the degraded products.[63]

P. aeruginosa AlgL is a periplasmic protein responsible for clearing mislocalised alginate polymer derived from non-functional export complexes.[40] The anionic polysaccharide attracts smaller cations, therefore its accumulation in the periplasm results in increased osmotic pressure which can subsequently cause lysis and cell death.[40,67] Albrecht and Schiller suggested that the main consequence of *algL* deletion is the complete loss of alginate production, which they restored by the addition of the *algL* ORF.[68] In other words, deletion of *algL* is lethal for *P. aeruginosa*, even though the enzyme is not part neither of the biosynthetic cluster or part of the secretion complex.

2.4. Alginate secretion

AlgK and AlgE are the proteins responsible for the translocation and secretion of mature alginate, respectively.[69,70] Keiski *et al.* solved the crystal structure of AlgK

at 2.5 Å resolution and demonstrated that it is a lipoprotein attached to the inner leaflet of the OM *via* its lipid moiety. In addition, Keiski and co-workers proved that AlgK is crucial for the production and secretion of high molecular weight alginate, and that it constitutes a major contributor to the proper localisation of the OM anion-selective channel protein, AlgE.[69] Their former conclusion indicates that AlgK functions as a scaffold that stabilises the alginate biosynthetic complex, while the latter suggests that the interaction between AlgK and AlgE leads to the formation of a new type of secretin, structurally distinct from any other bacterial secretion systems.[21,69,71]

AlgE is an OM β-barrel porin essential for the formation of highly specific anion channel through which mature alginate is secreted.[72,73] The specificity of AlgE for alginate was assessed by electrophysiological analysis.[74] The existence of a more electronegative potential impacted alginate secretion, indicating that AlgE forms an anion-specific channel which facilitates the export of its substrate. Furthermore, *algE* deletion mutant exhibited a non-mucoid phenotype, as it secreted degraded alginate in the form of free uronic acids. These experimental data highlight that AlgE is important for alginate translocation through the periplasm and across the OM.[70] According to all the aforementioned experimental data and conclusions regarding the alginate biosynthesis, chemical modification, translocation and secretion, a model of the biosynthetic machinery was schematically proposed (Figure 12).[1,2]

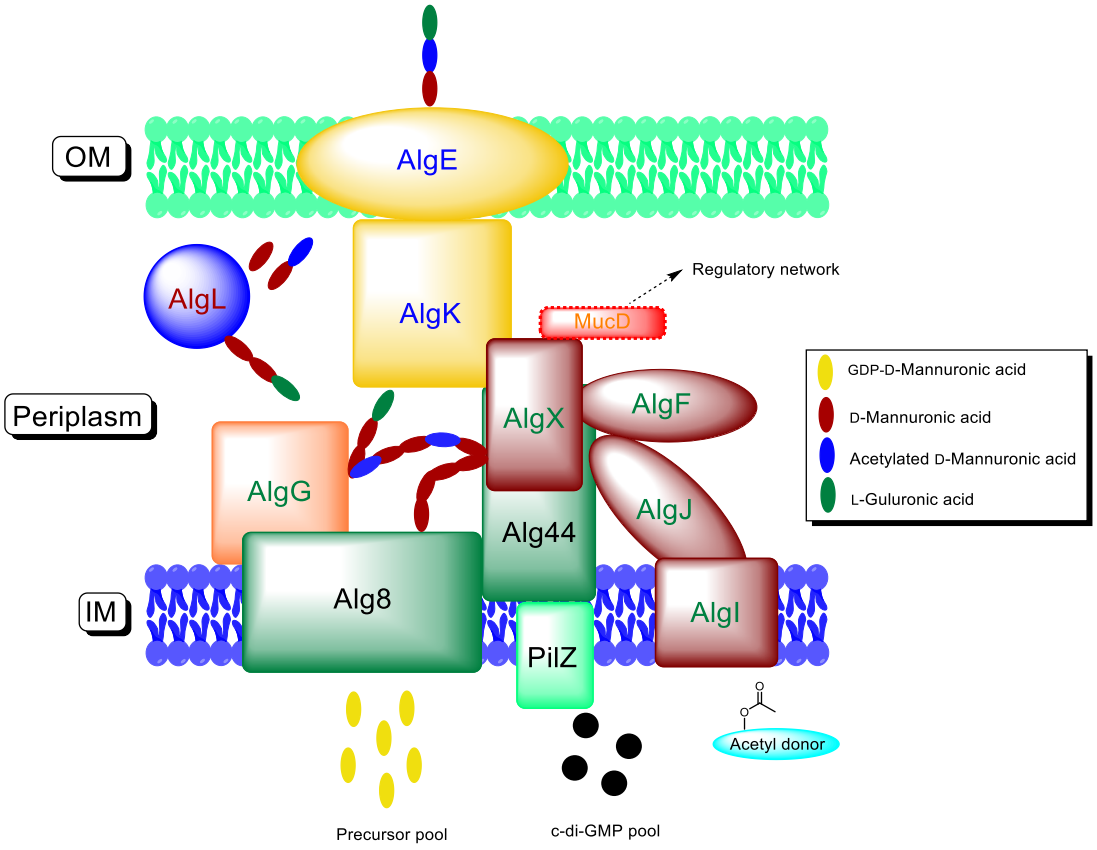


Figure 12: *Proposed model of the alginate biosynthetic multiprotein machinery cluster based on the current state of knowledge established by recent studies. The protein-protein interactions of Alg8-Alg44*[15]*, Alg8-AlgG*[2]*, Alg44-AlgK*[15]*, Alg44-AlgX*[2]*, AlgX-AlgK*[75]*, AlgX-MucD*[75]*, and AlgK-AlgE*[2] *are illustrated. This model denotes the positive regulation of alginate biosynthesis by c-di-GMP binding to Alg44, which in turn increases Alg8 catalytic activity through an unknown mechanism. The polymannuronate is then translocated across the periplasm where O-acetylation and epimerisation occur successively. AlgL is also situated in the periplasm for degrading misguided polysaccharide oligomers. Alginate is finally secreted though AlgE leading to the formation of the bacterial biofilm [Adapted from Hay et al. (2013) and Rehman et al. (2013)].*[1,2]

3. Chemical modification of plant-based alginate

Chemical modification of alginate is applied as a strategy for the construction of materials with enhanced existing properties, such as improvement of ionic gel strength by additional covalent cross-linking.[17] In addition, chemical modification of alginate aims towards the production of materials exhibiting completely novel properties, for example, heparin-like anticoagulant properties by sulfation.[76] In other

words, controlled alginate derivatisation has proven to be advantageous for generating materials that exhibit inherent property enhancement or new property introduction.[17] This highlights the increased potential to design novel alginate biomaterials, as different modifications on the polymer backbone may enable the tailoring of properties such as solubility, hydrophobicity and affinity for specific biomolecules or small molecules.

Alginate modification strategies rely on the solubility, characterisation and reactivity of the polymer.[17] In particular, the degree of alginate derivatisation, and consequently its substitution pattern, is dictated by the solvent system (aqueous, organic or mixed media) in which alginate dissolved. It is important to emphasise the sensitivity of alginates towards acidic, basic and reductive environments during derivatisation reactions, as the biopolymer can degrade rapidly causing drastic reduction of molecular weight and subsequent loss of functional performance.[18] Chemical modification of multiple alginate samples with a range of M/G ratios is often essential for in-depth understanding of the substitution patterns.[17,18] Regarding reactivity, the secondary –OH positions at *C*2/*C*3 or the –COOH at *C*6 can be modified selectively due to the reactivity difference between the two types of functional groups. The main techniques used to date for *C*2/*C*3 –OH modification are oxidation, sulfation, phosphorylation, acetylation, and coopolymerisation (Figure 13).[17,18,77] Chemical modification of the carboxyl groups include esterification, amidation and bis-amidation (Ugi reaction) as part of multicomponent reactions.[77] The literature examples discussed below are focused on the derivatisation of the carboxylate group at the *C*6 position of M and G monomers, as it is relevant to the strategy followed herein.

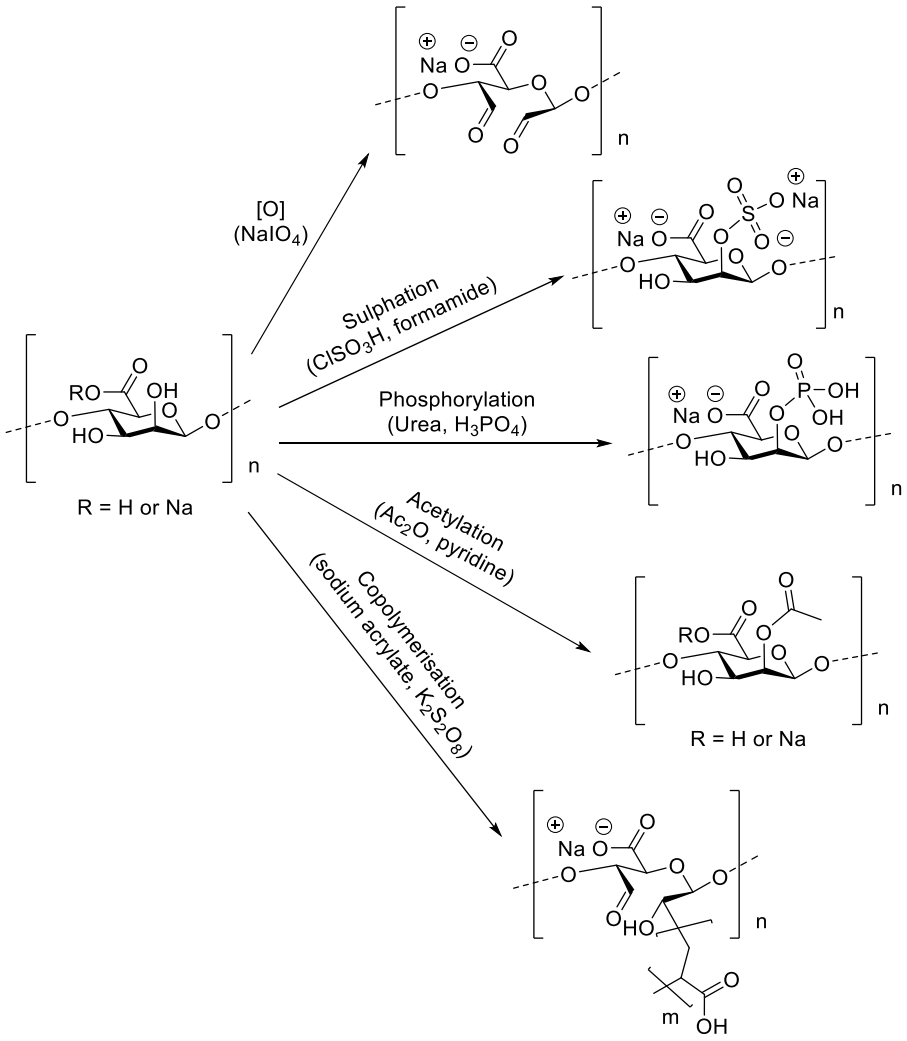


Figure 13: *Examples of alginate hydroxyl group chemical modifications. Note that the degree of any of the depicted modifications is controlled by varying the concentration of the reagents used.*

Native alginate exhibits enhanced hydrophilicity at pH = 5 and above, due to the carboxylate ion on the polymer backbone.[17] The alginate backbone can be readily transformed by esterification whereby alkyl groups can be attached in order to increase the hydrophobic nature of the polymer.[77,78] Propene glycol esters of alginate (PGA) that can be obtained through coupling of alginate with propyleneoxide, were the first commercially valuable derivatives in food industry as a thickener and stabiliser for products of low pH or that contain calcium.[79] Yang, Zhang, and Wen demonstrated that a water soluble amphiphilic cholesteryl ester of alginate obtained though coupling of the carboxylate and cholesterol with DCC and DMAP, can

self-assemble into stable and compact nano-aggregates in aqueous NaCl solution (Figure 14).[80]

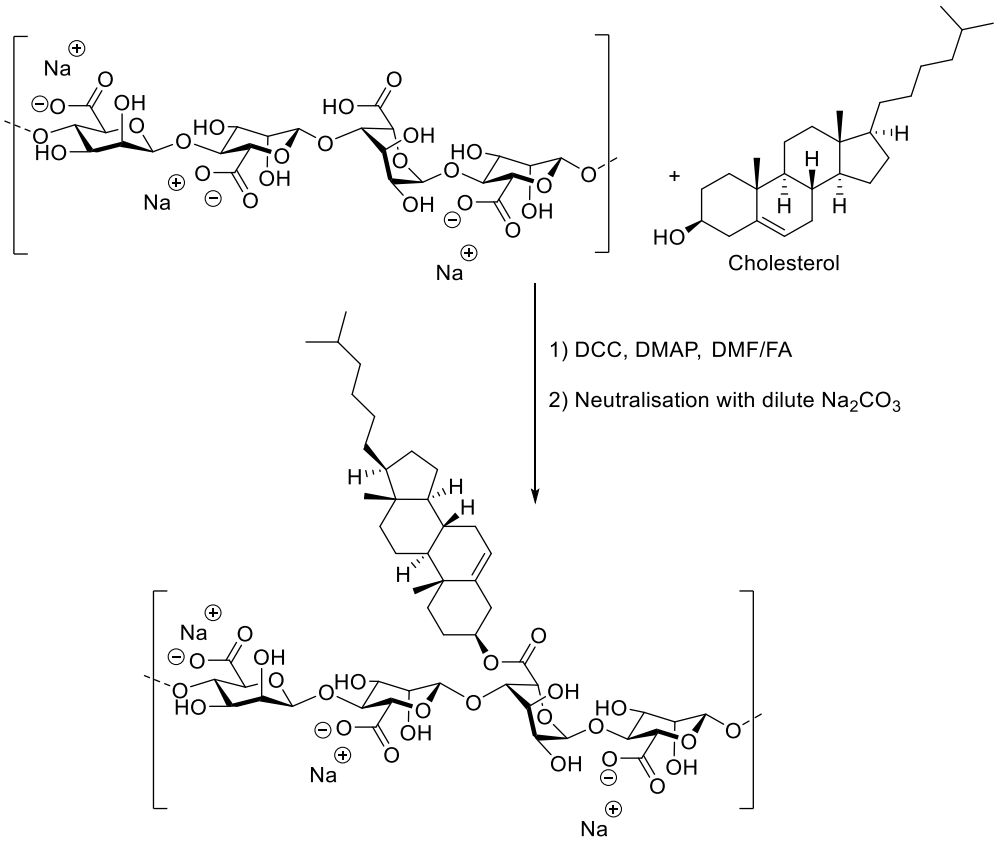


***Figure 14**: Synthesis of alginate derivative grafted with 3 cholesteryl groups per 100 uronic acid residues [Adapted from Yang et al. (2007)].*[80]

A study conducted by Broderick *et al*. showed that a butyl ester of alginate prepared from sodium alginate and BuOH in the presence of catalytic H_2SO_4, is capable of both hydrophilic and hydrophobic molecule encapsulation.[81] An additional method of producing hydrophobically modified alginates is the conversion of the carboxylic acid groups into their TBA salts and subsequent coupling of them with alkyl halides in DMSO.[82]

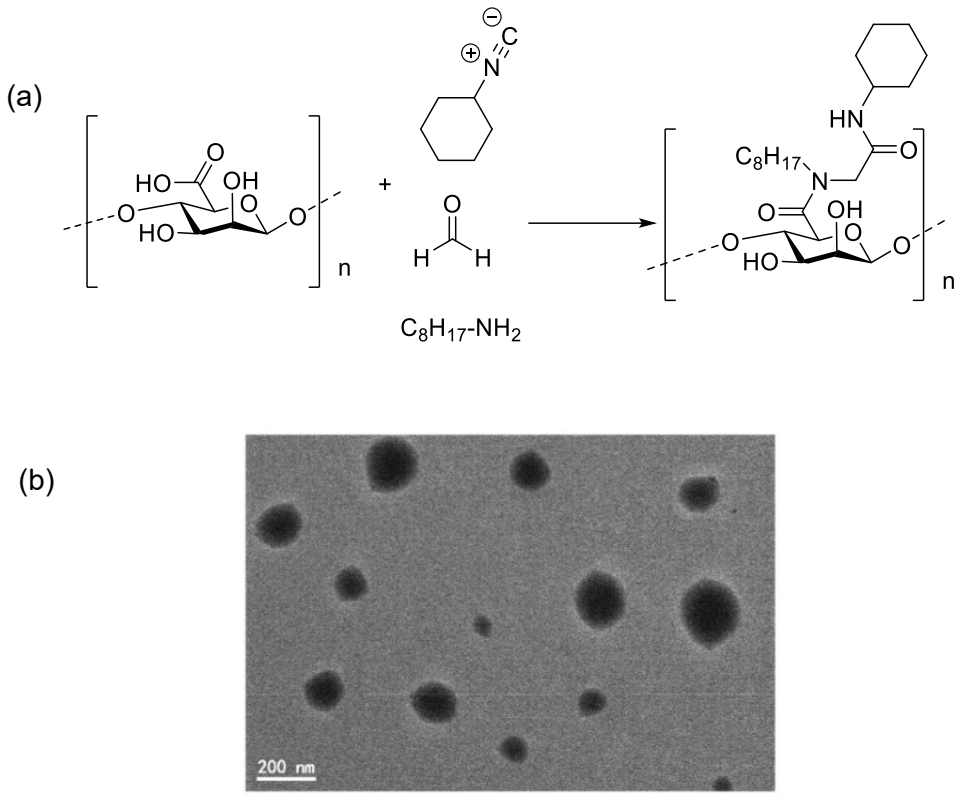


Figure 15: *(a) Four component Ugi reaction (b) Transmission electron microscope (TEM) image and dynamic light scattering (DLS) reveal that stable Ugi-Alg self-aggregated micelle with the average size of 162.3 nm [Adapted from H. Yan et al. (2016)].*[83]

The Ugi reaction is largerly applied in combinatorial chemistry involving the condensation of a ketone or aldehyde, an amine, an isocyanide and a carboxylic acid.[77] A facile procedure was reported as follows: to stirred aqueous solution of acidified native alginate (pH = 3.6) is added consecutively formaldehyde, n-octylamine and cyclohexyl isocyanide (Figure 15a)[77,83]. The linkage formed on the backbone of the polysaccharide was a bis-amide, resulting in a hydrophobically modified alginate that could form stable self-aggregated micelles in aqueous media with high thermal stability (Figure 15b).[83]

An additional strategy followed for the carboxylate modification is amidation. The use of coupling agents such as EDC.HCl and NHS is commonly applied for the coupling of amine-containing molecules and the carboxylate moieties of alginate to

form amide linkages.[18,77] By blending the amphiphilic alginate derivatives with polyvinyl alcohol (PVA) using an electrospinning method, the resultant nanofibres were exploited as hydrophobic drug-carriers.[84]

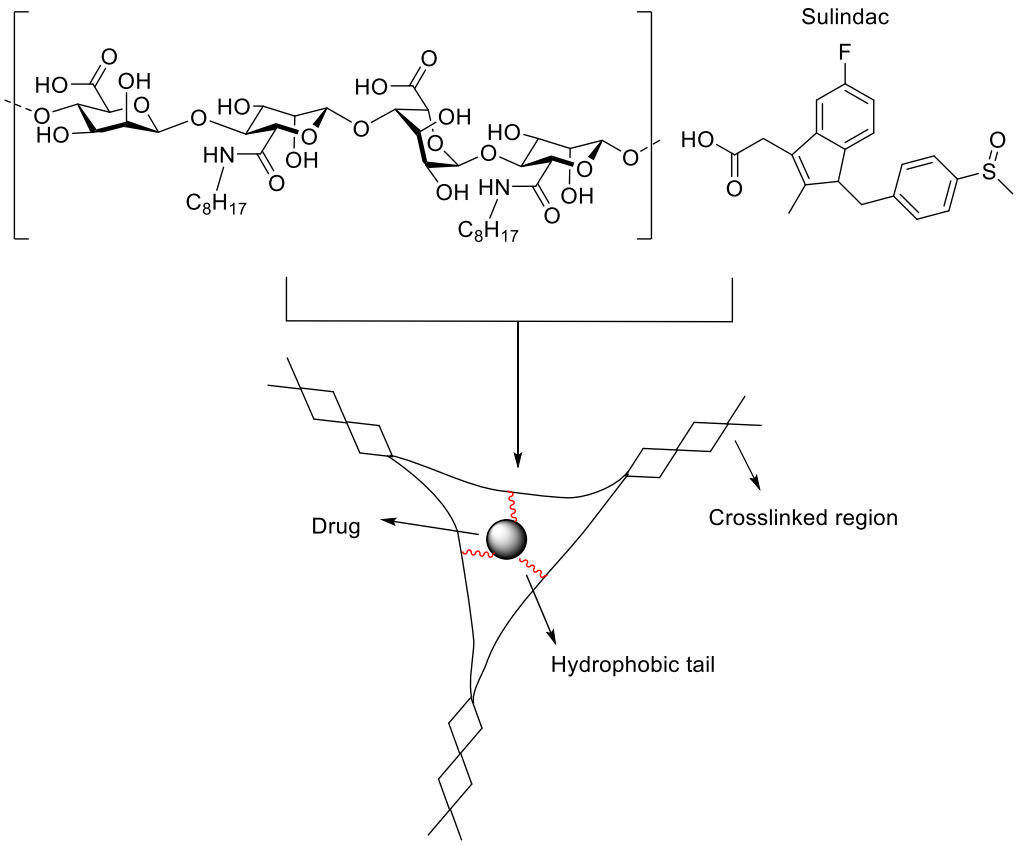


Figure 16: *Schematic representation of hydrophobically modified alginate stabilising sulindac in hydrophobic domain in aqueous media. [Adapted from Choudhary et al. (2018)].*[78]

Recent evidence suggests that hydrophobically modified alginate hydrogels with C_8 alkyl chains are promising candidates for the controlled delivery and release of hydrophobic drugs such as sulindac, as the amphiphilic modified polymer could stabilise the hydrophobic drug molecule in aqueous media (Figure 16).[78] All of the above experimental evidence denotes that chemical transformation of the alginate carboxylic acid groups can impact significantly the overall physicochemical properties of the polysaccharide.

Alginate derivatives with high biomedical significance can be prepared by applying simple chemical modifications, however, the challenge relies on matching the chemical transformations with the required physical properties of the resulted biomaterial. It is noticeable that the main modification of the carboxylate backbone is hydrophobic, as a large proportion of the literature reported coupling with alkyl alcohols or amines.[17,77] As described in the following section, modified alginate has a track record of safe clinical uses. The demonstrated biomedical utility of modified alginate emphasises the need for further chemical manipulation as a means to produce novel alginate-based materials.

3.1. Biomedical and biotechnological applications of modified/native alginate

Alginate-based biomaterials have proven to be of great significance in various human health applications such as drug delivery, wound healing, cartilage repair and bone regeneration.[8,9] They constitute ideal scaffolding materials as they can be exploited as hydrogels, microspheres, porous scaffolds and fibres in a broad range of applications.[8] Properties of alginate such as high mechanical and chemical stability, controllable swelling properties, minimal or negligible cytotoxicity, defined pore size and narrow size distribution, are the key elements that make the polysaccharide a promising tool for regenerative medicine and tissue engineering.[85] In addition, the higher molecular mass of alginate facilitates slower degradation rates, providing a lower number of reactive positions available for hydrolysis.[8] Most importantly, as a naturally-derived material, alginate has been regaining attention owing to its inherent biocompatibility.

3.1.1. CF medicine

The goal of a recent study was the investigation whether G-rich alginate oligomers (OligoG CF-5/20) could detach CF mucus by chelating Ca^{2+}, which is important for normal mucin unfolding.[86,87] The fact that the unfolding process of mucins secreted by the epithelial cells of CF patients is insufficient, they hypothesised that OligoG CF-5/20 would lead to mucus detachment. Indeed, at 1.5%, OligoG CF-5/20 resulted in CF mucus detachment wheres M-rich alginate oligomers failed to produce a similar effect.[86]

3.1.2. Drug delivery vehicles

Alginate-based drug delivery carriers have attracted a lot of interest during the past decades, as they proved to successfully deliver small drugs, as well as biomacromolecules.[88,89] The pH-responsive properties of alginate-based biomaterials profoundly influence degradation, mechanical and swelling properties, long term stability and *in vitro* performance, making them ideal delivery systems for cationic drugs and cells.[4] The construction of alginate-based microcapsules as drug-delivery carriers aims to control the degradation properties of the biomaterial, in terms of calculating the number of encapsulation layers needed for preserving the stability of the drug at different pH values until they reach the targeting site.[8] Formulation of this kind of microcapsule can be achieved by combining different biopolymers or polymers, such as alginate, chitosan or polyethylene glycol (PEG).[90] An additional advantage of using alginate as an oral tablet formulation component is its ability to maintain gel formation at the acidic conditions of the stomach by forming an alginic acid gel. Raft-forming formulations of alginate are also applied for treating heartburn and gastric reflux into the oesophagus, e.g. Gaviscon*TM*.[91]

3.1.3. Wound dressings

Alginate-based sponges, hydrogels and electrospun mats are applied as wound dressings, due to the advantageous characteristics they exhibit including hemostatic capability, good water absorptivity, optimal water vapour transmission rate, conformability, mild antiseptic properties, and gel-forming ability when they absorb wound exudates.[92] The antibacterial properties of alginate-based materials are accompanied by nontoxicity and biodegradability which are important characteristics, considering that wounds constitute a favourable environment for microbial colonisation that often leads to infections that may in turn delay the healing process.

The application of an alginate dressing at the wound surface creates a fibrous gel layer due to the ion exchange that occurs between the Ca^{2+} ions from alginate dressings and the Na^{+} ions that exist in the exudate (Figure 17). Additionally, the gel formation preserves the moisture on the wound which encourages cellular regeneration. The antimicrobial, as well as the moisturising properties of alginate-based wound dressings accelerate the healing process, leading to better cosmetic repair.[8] Interestingly, wound dressings composed by alginate are proposed to amplify wound healing through monocyte stimulation which results in increasing the levels of cytokines, for example interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α), which in turn induce pro-inflammatory factors that lead to wound healing enhancement.[93] Non-woven wound dressings like Kaltostat*TM*, Sorbsan*TM*, Seasorb*TM* exhibit haemostatic properties and have been used for the treatment of dermal and epidermal wounds.[4,93]

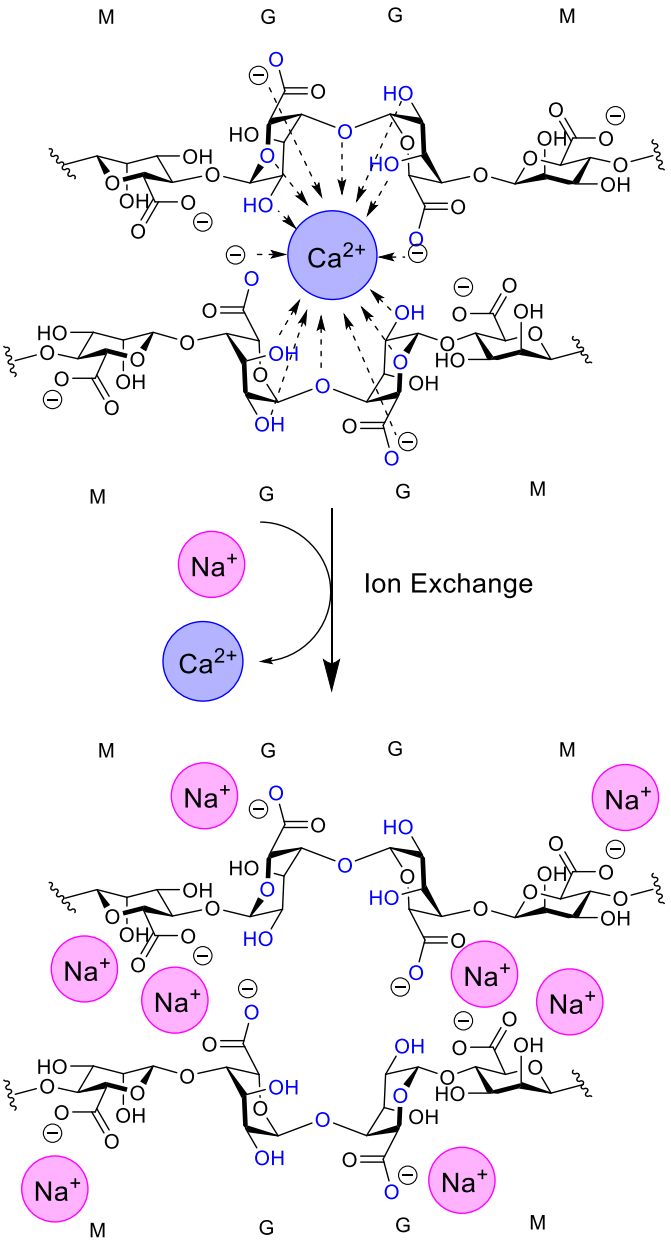


***Figure 17**: Ion exchange triggered by the presence of Na^+ in the wound, releasing Ca^{2+} in the alginate-based wound dressing.*

3.1.4. Regenerative medicine applications

Alginate hydrogels are extensively used for addressing critical challenges encountered in tissue engineering applications. Alginate-based bioinks are used in 3-D bioprinting due to their capability to form cell-compatible hydrogels under physiological conditions.[94,95,96] Viscosity and density constitute key material properties that make alginate suitable as a cell encapsulation material and as a matrix

scaffold to tune a specific 3-D cell growth.[7,95] In order to achieve a homogenous cell suspension throughout the whole printing process, it is essential the density of the biomaterial to be similar to that of the examined cell type, as this defines the desirable homogeneity of cell distribution in the bioink. Jia *et al.* investigated the viscosities of 30 different biodegradable alginates combined with fluorescent-labelled human adipose-derived stem cells (hADSCs), and concluded that as the concentration of alginate in gelatin increased and degree of oxidation decreased, the density and viscosity of the bioink demonstrated an increasing trend (Figure 18).[77] Nevertheless, the metabolic requirements of different cell types may demand oxidised alginate with alternative sets of viscosity and density, in order to permit the optimal diffusion of nutrients, as well as the homogenous distribution of cells.[7] This example highlights the challenge of chemically modifying alginate for tuning its physicochemical properties for specific applications.

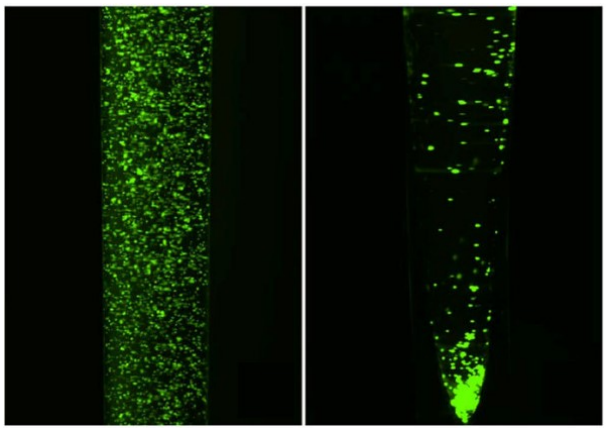


Figure 18*: Calcein-stained hADSCs in the 5% ox.-10% conc. (i.e., alginate with 5% oxidation and 10% w/w in Ca^{2+}-containing gelatin) (left) and 5% ox.-2% conc. (right) material with and without successful cell suspension, respectively (scale bar= 500 μm) [Adapted from Jia et al.(2014)].*[7]

Chemical modification of alginate-based bioinks is of great clinical significance for cartilage, bone and vascular regeneration.[4,8.96] The application of injectable hydrogels, solid- and gel-microspheres composed of alginate proved to be

advantageous for cartilage repair due the stiffness and strength that is formed almost instantly. In addition, the physical properties of an alginate hydrogel at a macroscopic level can be tuned according to the mechanical properties of the native cartilage tissue.[97] As for bone regeneration, *in situ* osteogenesis is a beneficial strategy to pursue.[8] To accomplish this, alginate is utilised as a bioink which is seeded with stem cells to produce an injectable scaffold that leads to bone tissue regeneration.[98,99] To date, a number of studies demonstrated the effectiveness of using injectable alginate-based bioink scaffolds for inducing osteogenesis.[100,101,102] Apart from osteogenic properties, alginate also exhibits angiogenic properties that aid in the design of scaffolds applicable for vascular regeneration.[4,8] Based on the above tissue repair and regeneration examples, it is clear that alginate is a promising substrate material for the successful construction of biopolymers useful for a wide range of biomedical applications.

4. Chemical synthesis of alginate

4.1. Concepts in oligosaccharide synthesis

Synthetic carbohydrate chemistry approaches have been developed for the chemical production of complex carbohydrates of biological, medicinal and biomaterial significance. The plethora of biological functions that carbohydrates and glycoconjugates perform stems from their structural diversity. Polysaccharides owe their complexity to the large variety of monosachharide building blocks from which they can be accessed. Any difference in stereo- and regio-chemical aspects of the glycosidic linkages, may result in alternative polysaccharide structures with a great variety of properties and functions.[103,104] In particular, the stereoselective formation of a glycosidic linkage depends on the α- and β- geometric isomers that exist.

Regioselectivity is based on the possible reaction of the five hydroxyl groups in the glycosyl acceptor with the anomeric position of the glycosyl donor (Figure 19).

Clearly, the aforementioned make the assembly of oligo- and polysaccharides significantly more complicated compared to the synthesis of other natural polymers. The vast majority of polysaccharides derive primarily from bacteria and fungi, and are characterised by well-defined structures with specific types of glycosidic linkages.

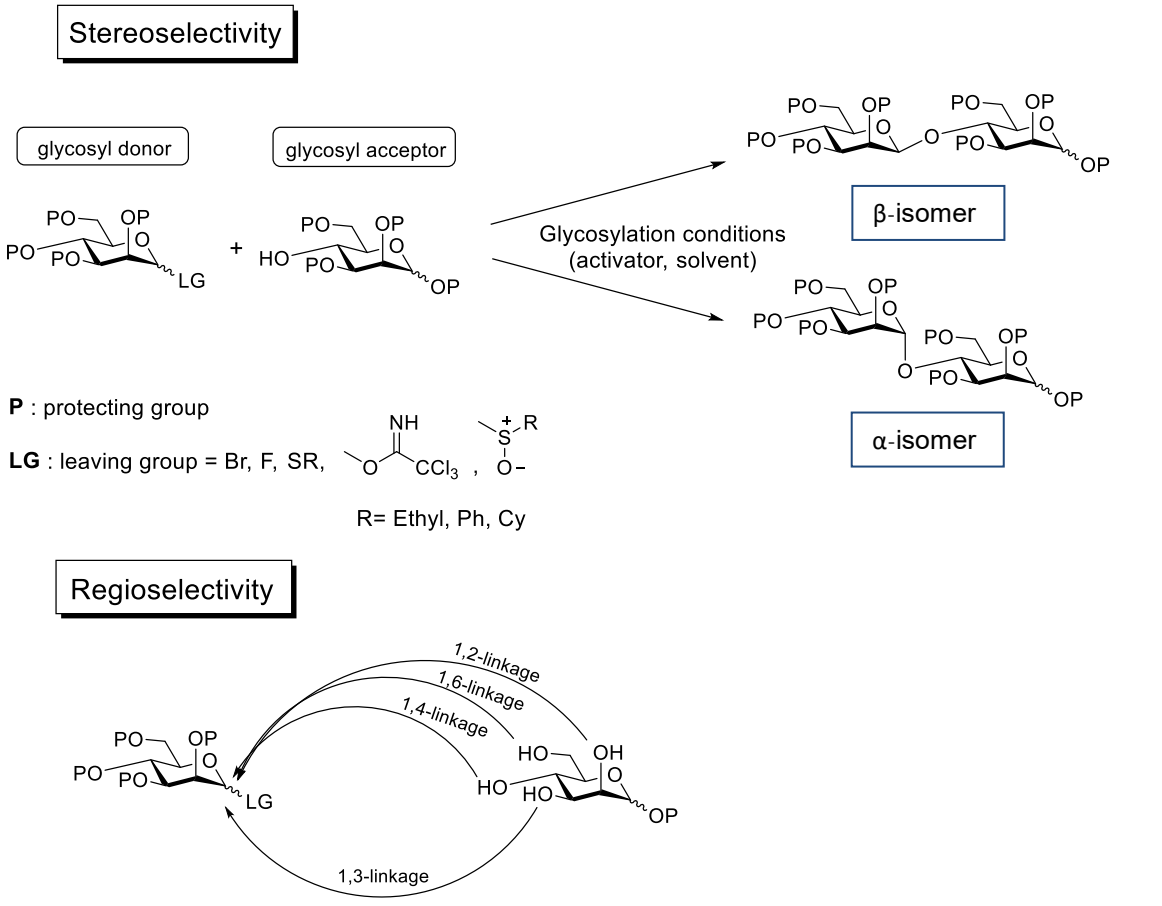


Figure 19*: Concepts in glycosylation. Stereoselectivity refers to the possible formation of two isomers of a mannose dimer. Regioselectivity depends on the position of the hydroxyl group that participates in the formation of the glycosidic linkage.*

To date, a great obstacle in glycochemistry and glycomedicine is the synthesis of structurally well-defined and pure carbohydrates and their glycoconjugates. The chemical synthesis of oligo- or polysaccharides strictly demands perfect control of

both stereo- and regiochemistry, therefore it requires the design of appropriate and efficient glycosylation and protecting group strategies.

Chemical glycosylation may appear as an extremely simple concept, however, the complications in this synthetic process are manifold, often leading to low yields and/or mixture of anomers. The difficulty of controlling the stereochemical outcome of a glycosylation reaction relies on correlating factors, such as the potency of the anomeric leaving group, the activation conditions, the reaction solvent, the temperature, the identity of the glycosyl donor and acceptor, and most importantly, the protecting groups of both glycosylating agents. Stereocontrolled synthesis of both α- and β- or 1,2-*trans* and 1,2-*cis* glycosides is important, as they usually exist in a variety of naturally occurring polysaccharides.

Glycosidic bond formation involves the displacement of a leaving group at the anomeric position of the glycosyl donor by a free hydroxyl group of the glycosyl acceptor, in the presence of a promoter or an activator (Figure 20). Upon activation, the loss of the anomeric leaving group produces a glycosyl cation which is a stabilised oxocarbenium ion. The formed cation at the anomeric carbon is stabilised by electron donation from a lone pair of ring oxygen (*O*5). The hydroxyl group of a suitably protected acceptor acts as the nucleophile in this reaction, attacking either from the top or bottom face of the flattened oxocarbenium intermediate. Thus, the reactions proceed *via* an S_N1 mechanism which may lead to the formation of the 1,2-*cis* or the 1,2-*trans* glycoside, posing a challenge in stereoselectivity. Note that in the *manno*-series glycosylation reactions, 1,2-*trans* linkages are favoured by the anomeric effect (Figure 20a). The employment of an ester protecting group at the *C*2-position of the donor can yield almost exclusively 1,2-*trans* glycosidic linkages,

as in this case, a bicyclic acyloxonium ion is the major intermediate. Considering that the top face of the mannose analogue is blocked during the bicyclic acyloxonium ion formation, the nucleophilic attack by the hydroxyl group of the acceptor would take place at the bottom α-face of the ring (Figure 20b). In some cases, orthoester formation or 1,2-*cis* glycosides may additionally be observed, depending on the reactivity and types of the acceptors used.[105]

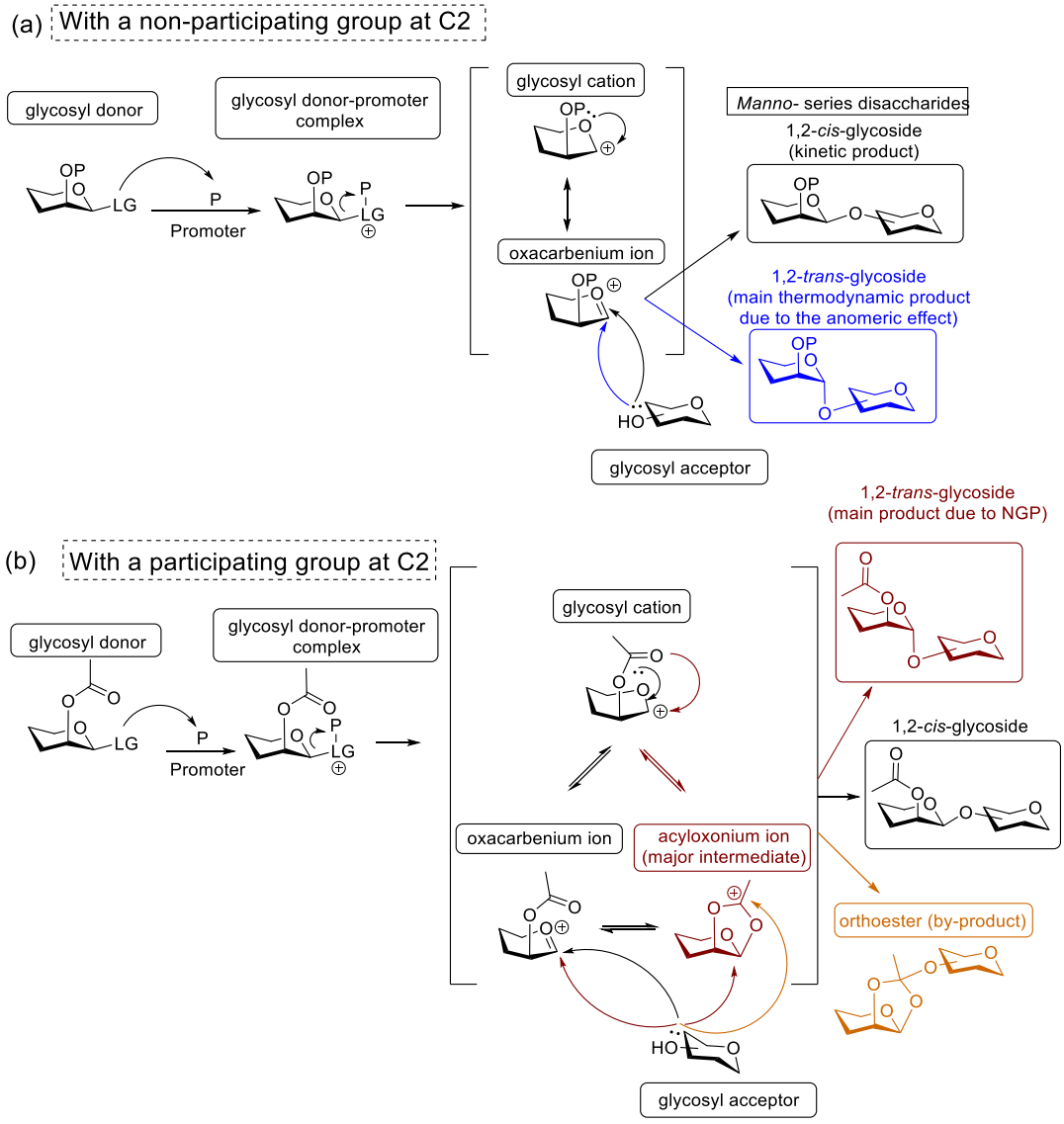


***Figure 20**: General mechanistic outline of chemical glycosylation. (a) Glycosylation mechanism when a non-participating group is placed at C2, where the anomeric effect dictates the formation of a 1,2-trans linkage in mannose derivatives (b) An ester protecting group at C2 promotes the nucleophilic attack of the glycosyl acceptor at the bottom face of the mannopyranoside.*

In general, the four types of glycosidic linkages based on *C*1 and *C*2 geometries are 1,2-*trans*-β, 1,2-*trans*-α, 1,2-*cis*-β and 1,2-*cis*-α (Figure 21). As depicted in Figure 20, the synthesis of 1,2-*trans* saccharides can be succeeded with the use of neighbouring group participation (NGP) on the *C*2-position of the glycosyl donor, while 1,2-*cis* glycosides have proven to be synthetic more challenging for carbohydrate chemists.[106]

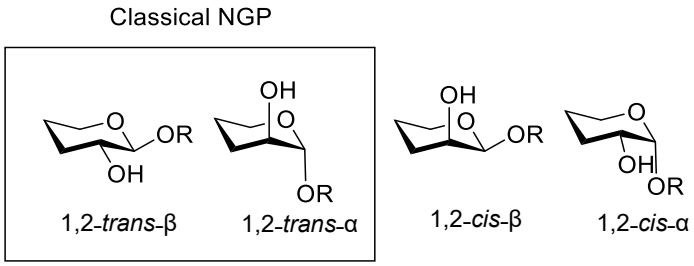


***Figure 21**: Four types of glycosidic linkages based on C1 and C2 geometries.*

Even though a large number of examples in the literature demonstrate excellent 1,2-*cis* stereoselectivity, none of them provides a fully comprehensive method, especially for producing 1,2-*cis*-β-glycosidic linkages.[106,107] In addition to the non-availability of NGP, the reasons behind the synthetic challenge for the construction 1,4-β-linkage are both steric and electronic. The steric factor is based on the repulsive interactions between the nucleophile approaching from the top face of the ring and the axial *C*2 substituent, while the electronic relies on the the anomeric effect, which would favour α-1,4-linkages. The anomeric effect describes the preference of electronegative substituents at the anomeric carbon to adopt an axial orientation (α-anomer) rather than an equatorial (β-anomer) (Figure 22).[108,109] The theory of hyperconjugation is the most widely accepted explanation, whereby the tendency to adopt an axial orientation is a result of electron delocalisation from the ring oxygen (*O*5) lone pair to the anomeric *C*1-X antibonding orbital ($n \rightarrow \sigma^*$), also known as the endo-anomeric effect. Due to orbital misalignment, the β-anomer

cannot be stabilised by the endo-anomeric effect, therefore the equatorial orientation is less favoured.[110] Evidence of a shortened and consequently strengthened *C*1-*O*5 bond accompanied by a longer and weakened *C1*-X bond in compounds supported this theory of hyperconjugation.[111]

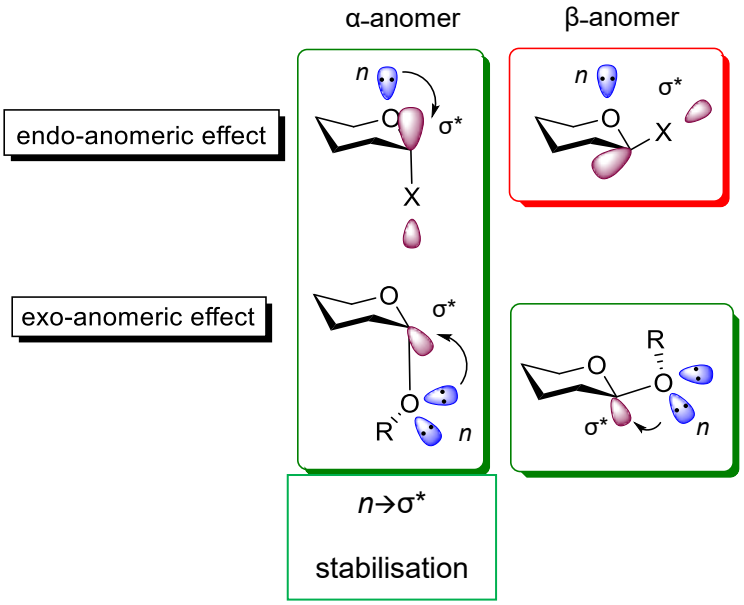


***Figure 22**: Schematic representation of the endo- and exo-anomeric effect based on the hyperconjugation theory. Stabilisation of the α-anomer by the overlap of the n (O5) and σ* (C1-X) syn-periplanar orbitals (top left) and by the overlap of the n (O1) and σ* (C1-O5) syn-periplanar orbitals (bottom left). The endo- anomeric effect is not observed with the β-anomer, therefore no stabilisation can occur (top right). The β-anomer can only be stabilised by the exo-anomeric effect.*[108,109,112]

Furthermore, the existence of an anomeric exocyclic alkoxy –OR gives rise to an additional anomeric effect known as the exo-anomeric effect, whereby the aglycon dictates the conformation.[112] In particular, the anti-periplanar arrangement of a molecular orbital (*n*) on *O*1 and an unoccupied σ* molecular orbital along the *C*1-*O*5 bond stabilises both α- and β-anomeric configuration, as depicted in Figure 22. The α-anomer is stabilised by both endo- and exo-anomeric effects, whereas the β-anomer can only be stabilised by the exo-anomeric effect.

The so-called Δ2 effect constitutes a stereo-electronic factor that also favours the formation of 1,2-*trans*-α-glycosidic linkages in mannosides (Figure 23a).[113,114]

The Δ2 effect was first proposed by Reeves as part of his pioneering work on the shape of the pyranose ring conformation. It describes the occurring destabilisation of the pyranose ring conformation due to the repulsion between the three indicated oxygens in the β-anomer. Notably, this repulsion takes place when the hydroxyl group attached to *C*2 is axial and bisects the torsional angle between the *C*1-*O*5 and *C*1-*O*1 (Figure 23b).[113]

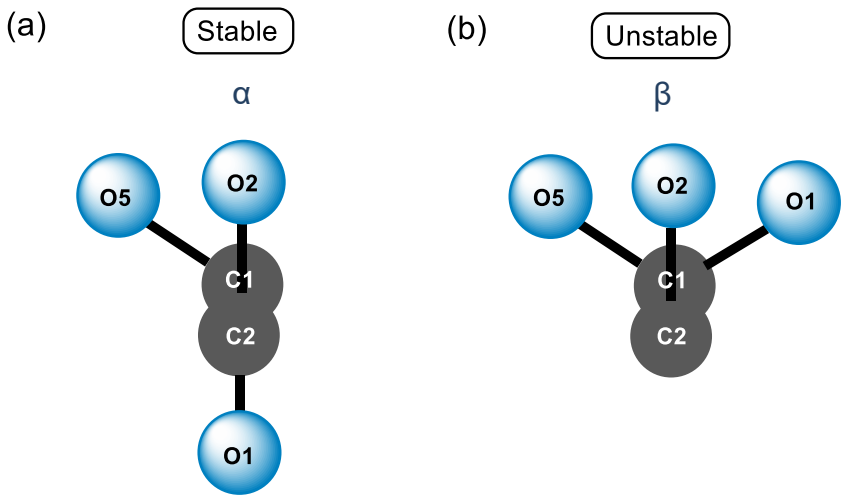


***Figure 23**: The Δ2 effect. Newman projection represents view C2→C1 axis [Adapted from Reeves (1950)].*[113]

In addition, the Woerpel model described that nucleophilic attack of the glycosyl cations formed during an S_N1 mechanism occurs along a pseudoaxial trajectory to maximise the overlap between the nucleophile HOMO and oxoxarbenium ion LUMO.[115] For a $^4H_3$ glycosyl cation, a bottom face attack leads to a more favourable chair-transition state furnishing α-linked products (Figure 24).[115,116] Axial attack from the top face of the $^4H_3$ half-chair conformation results in the higher-energy twist/skew boat conformation, which is responsible for the formation of β-linkages.[116]

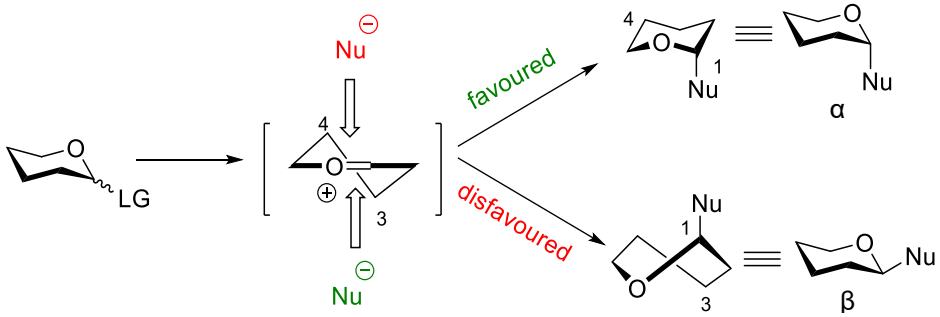


***Figure 24**: Mechanisms of S_N1-type glycosylations leading to α-configurations [Adapted from Woerpel et al. (2003)].*[116]

A large number of efforts have been made for the development of an efficient methodology to overcome these hurdles. These include direct β-mannosylation by employing mannosyl halide glycosyl donors with silver salts,[117–120] inversion of the *C*2 configuration of β-glucosides *via* a sequence of deprotection, oxidation and reduction reactions,[121–126] intramolecular aglycone delivery (IAD),[126–128] anomeric *O*-alkylations,[129–131] application of mannosyl donor protecting groups such as, 4,6-*O*-benzylidene,[132–137] 4,6-*O*-arylboronate[138] or 4,6-*O*-silylene,[139] hydrogen-mediated aglycone delivery (HAD),[140,] application of boronic ester catalyst that derived from the glycosyl acceptor with 1,2-anhydromannosyl donors,[141,142] and use of 2,6-[107] or 3,6-lactones on mannosyl donors[143]. -corrected punctuation Chemical glycosylation reactions often follow an S_N1 mechanistic pathway which involves the loss of the anomeric leaving group as the first step. Therefore, one could suggest that the orientation of this leaving group is irrelevant. However, depending on the activation conditions, chemical glycosylations may proceed *via* a mixture of S_N1 and S_N2 mechanism, where the latter stereospecifically results in the inversion of the anomeric configuration (Figure 25).

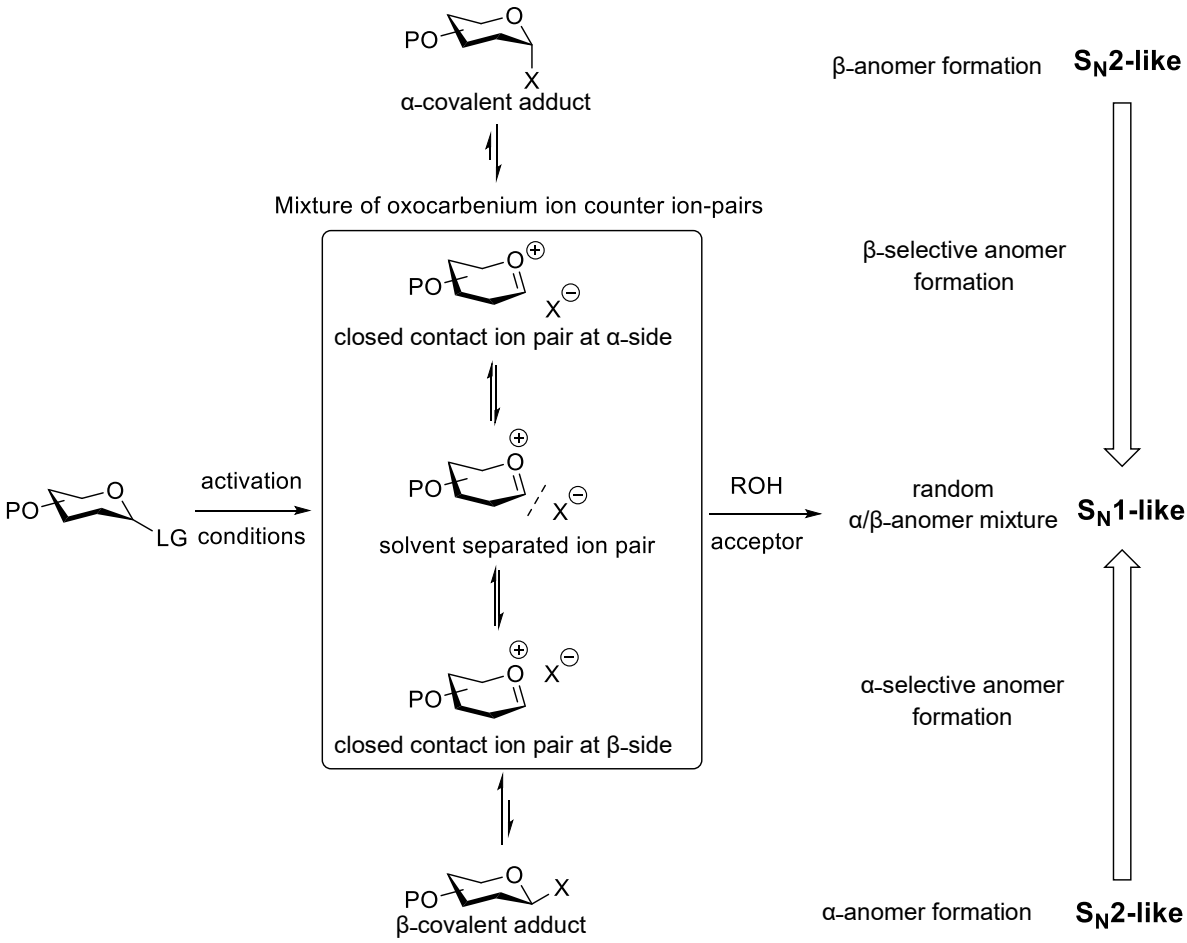


***Figure 25**: Schematic representation of the possible S_N1-like and S_N2-like glycosylation mechanisms deriving from covalent adducts and oxocarbenium ion counter ion-pairs [Adapted from Codée et al. (2017)].*[144]

Notably, S_N2 leaving groups that have been proven to provide mostly 1,2-*cis* stereoselectivity are β-glycosyl halides that derive from their respective α-glycosyl halides with bromonium ions,[145] or from α-thioglycosides in the presence of bromine,[146] glycosyl thiocyanates,[147] and anomeric mannosyl triflates generated *in situ* from thioglycosides or sulfoxides for β-mannosylation.[148,149]

A large and growing body of literature focused on identifying and evaluating the role of protecting groups of the donor in β-mannosylation, as the ring substituents play a decisive role on its reactivity.[150] Paulsen and co-workers were the first to observe that EWG on carbohydrates slow down the production of oxocarbenium ion,

as they destabilise the formation of partial positive charge at the anomeric centre. As a result, the rate of hydrolysis and/or glycosylation is retarded.[151] This observation was formulated as the 'armed-disarmed' concept by Fraser-Reid and co-workers based on their observation where benzylated glycosyl donors (armed) were activated and coupled to acetylated glycosyl donors (disarmed).[152,153] They suggested that this phenomenon is supported by the instability of the positively charged intermediate which stems from the presence of adjacent electron-withdrawing groups on the glycosyl donor.[153] Moreover, the research groups of Wong[154–157] and Ley[158] have examined the reactivity of diversely substituted thioglycosyl donors, and concluded that the nature of the mono- or oligo-saccharide, as well as the location of the substituents on the sugar ring, have a specific influence on the conversion rate of the glycosylation. For example, computational calculations of the relative reactivity values (RRV's) of a specific thiogalactoside with different protection and functional groups at the *C*2-position, were quantified as $-N_3$ > -O(ClAc) > -NPhth > -OBz> -OBn.[155] Most importantly, the 'armed-disarmed' concept is widely applied in chemoselective glycosylation strategies, where the electronic effects of the glycosyl donor and acceptor protecting groups are exploited for enabling orthogonal glycosylation with potential donors as acceptors (Figure 26).[159]

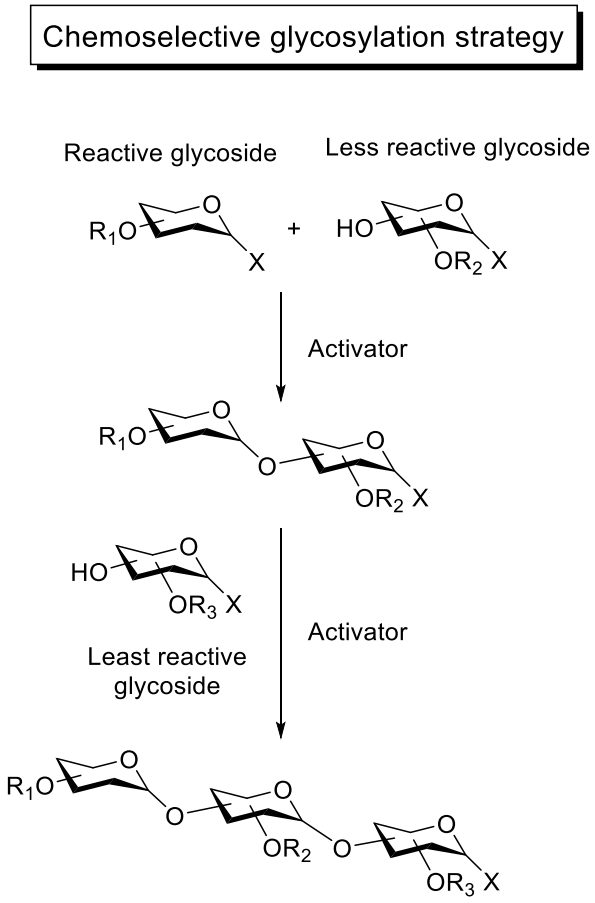


***Figure 26**: Schematic representation of chemoselective glycosylation strategy, employing the 'armed-disarmed' concept [Adapted from Codée et al. (2005)].*[159]

In-depth understanding of the aforementioned fundamental concepts of oligosaccharide synthesis has improved the development of solution-phase synthetic protocols and subsequently provided access to pure and complex oligosaccharides. Considerable effort is still needed for the synthesis of carbohydrate sequences, as stereoselectivity, reactivity and overall structural complexity require the design of a series of methodologies which involve various protecting group manipulations and long synthetic routes.

4.2. Mannuronic acid building blocks in oligosaccharide synthesis

The synthesis of alginate oligosaccharides is highly demanding, not only because of the 1,2-*cis* configuration it possesses, but also because of the carboxylic acid group at the *C*5 position of the carbohydrate core of both M and G monomers.[150] Generally, the construction of mixed-sequence alginate oligosaccharides can be succeeded by the use of M and G as monomeric or GM or MG as dimeric synthons in a pre-glycosylation or post-glycosylation oxidation approach.[160] In order to minimise functional-group alteration at a late stage of the oligosaccharide synthesis, a number of studies focused on investigating the reactivity of various mannuronate donors and acceptors. [150,144,161] Uronic acids are regarded to be among the most unreactive glycosyl pyranoside donors, as the electron withdrawing character of the carboxylate group at *C*5 is considered to have a 'disarming' effect responsible for retarding the formation of positive charge at the anomeric position, and therefore, slowing down the rate of the glycosylation.[150,162]

4.2.1. Effect of glycosyl donor in alginate oligosaccharide synthesis

Codée and co-workers have provided experimental evidence of high β-stereoselectivity by using 2-azido-mannuronate ester as a glycosyl donor.[163] Their choice of this donor relies on its tendency to partake in S_N2-type reactions due to the electron-withdrawing effect of the *C*2 azide that stabilises the anomeric triflate as the reactive intermediate. They explained that the application of Ph_2SO/Tf_2O on α-thiophenol mannuronic acid **1**, leads to the preferential formation of the axial anomeric triflate **3**, which also exists as an oxacarbenium ion intermediate with a $^3H_4$ half-chair conformation **5** (Figure 27).

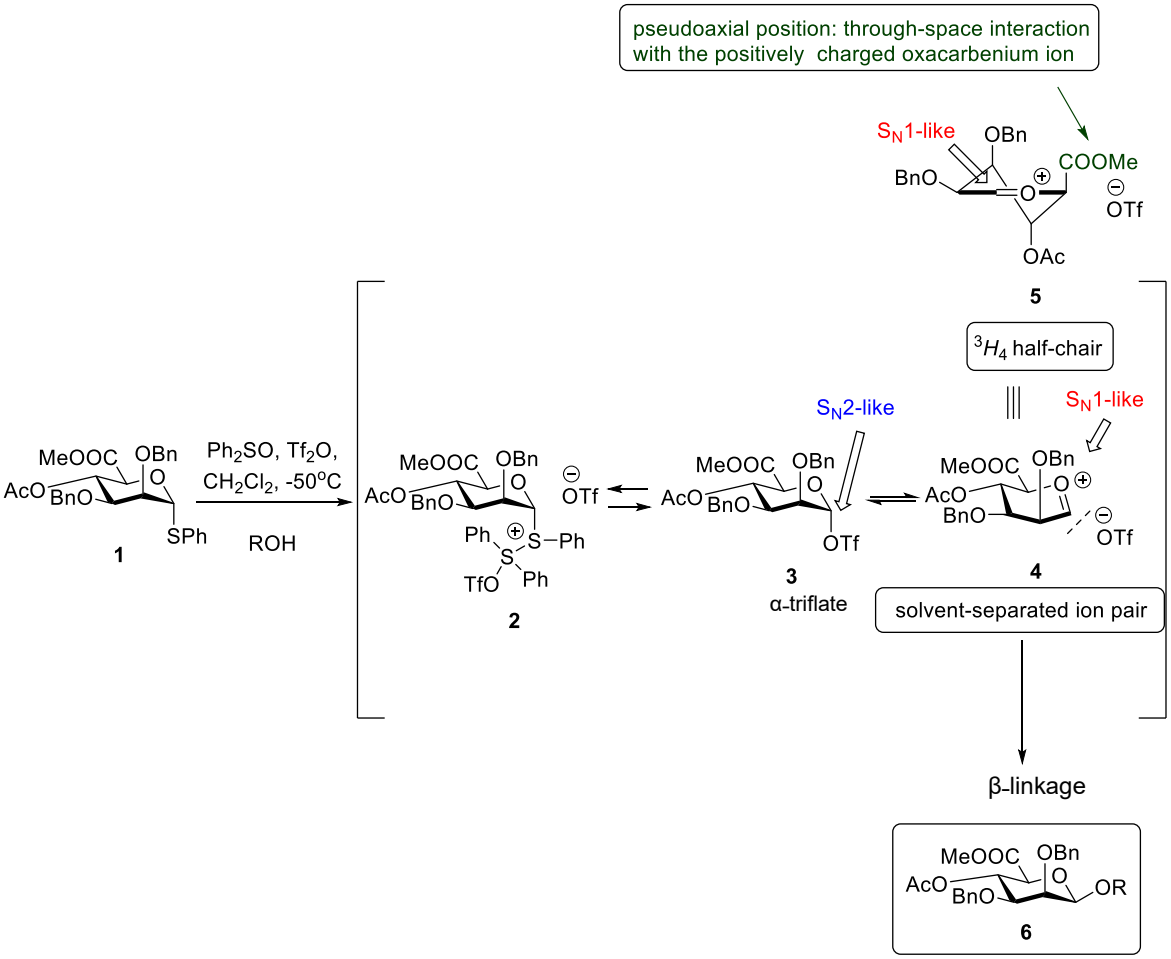


***Figure 27**: β-directing effect of the glycosyl C5-carboxylate in $^3H_4$-oxacarbenium ion intermediate [Adapted from Codée et al. (2009)].*[164]

The α-triflate intermediate **3** follows S_N2-like displacement providing the β-linked product, while the oxocarbenium intermediate proceeds through S_N1-like pathway allowing the nucleophilic attack of the acceptor from the β-face. In addition, they suggested that substituents at *C*3 and *C*4 prefer to occupy pseudoaxial postions, as in this way they exhibit less disarming effect, whereas the *C*2 substituent occupies a pseudoequatorial position on the mannosyl half-chair.[115,165–167] Most importantly, they suggested that the *C*5 carboxylic acid ester group displays strong preference for a pseudoaxial position due to the through-space interaction between its electron-rich carbonyl function and the positively charged oxacarbenium ion (Figure 27).[166–168]

Based on the latter line of recommendation, Codée *et al.* also demonstrated experimentally that the *C*5 carboxylate ester reacts in a highly 1,5-*cis* selective fashion, depending on the steric demands of the nucleophile.[164] Moreover, by employing carboxylbenzyl and *N*-PTFA glycosyl donors that led to the formation of β-mannosidic linkage, showed that the stereoselectivity of these mannosylations does not depend on the type of donor or pre-activation conditions. Their work on the remote stereodirecting effect of the *C*5-carboxylate ester highlighted the importance of the $^{3}H_{4}$ half-chair in the formation of β-mannosidic linkage.[164]

Walvoort *et al.* tested and compared the activation and glycosylation behaviour of α-thio-mannuronic acid donors and their non-*C*6 oxidised counterparts demonstrating that not only α-thio-mannuronic acid donors can provide high β-stereoselectivity in mannosidic linkage formation, but they also exhibited considerably high reactivity.[150] Their experiments showed that α-thio-mannuronic acid **13** is 30 times less reactive than the α-configured 4,6-*O*-acetylated and 4,6-*O*-benzylidene analogues, due to the presence of the *C*5 carboxylic acid ester. Interestingly, the respective β-thio-mannuronic acid donor **7** indicated higher reactivity than the β-configured 4,6-acetylated and 4,6-benzylidene analogues, which again signifies the effect of the formed $^{3}H_{4}$-oxacarbrenium ion intermediate **5** (Figure 28). As opposed to β-thio-mannuronic acid **7**, the α-thio-mannuronic acid donor **13** leads primarily to the formation of the more unstable $^{4}H_{3}$-oxacarbenium ion **12**, where the substituents are positioned in unfavourable orientations. Nevertheless, both α- and β-mannuronates reacted in a stereoselective manner to provide β-mannosidic linkages, which is strongly based on the relatively favourable $^{3}H_{4}$-oxacarbrenium ion intermediate.[150] In other words, the incorporation of a *C*5-carboxylate ester on the

mannopyranosyl building blocks is valuable, making them highly β-selective glycosylating agents for the construction of alginate oligomers.

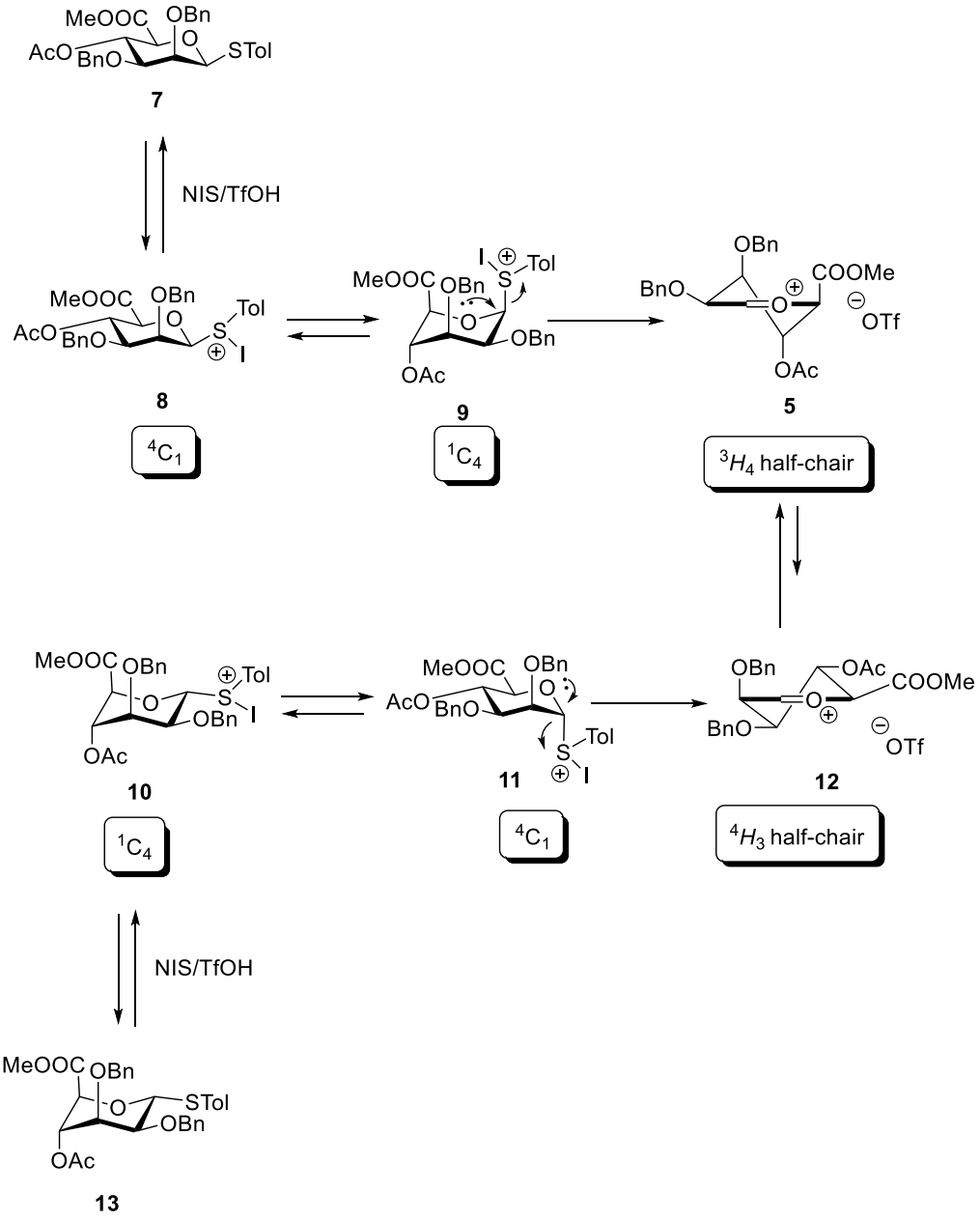


***Figure 28**: Proposed mechanism for the formation of β-mannosidic linkage from α- and β-mannuronic acid donors [Adapted from Walvoort et al. (2011)].*[150]

Codée and co-workers reported the construction of D-ManA alginate oligomers by automated solid phase synthesis incorporating up to twelve 1,2-*cis* mannosidic linkages.[169] The stereoselective formation of β-mannosidic linkages was

carried out successfully by the application of a second-generation automated oligosaccharide synthesiser. Merrifield resin functionalised with a butanediol linker was used, which can be selectively cleaved through cross metathesis with ethylene.[170] They chose to use *N*-PTFA donors prepared from their respective thioglycoside donors. *N*-PTFA donors can be activated by catalytic amounts of Lewis or Brønsted acid, as opposed to thioglycoside donors that prohibit the application of soft electrophiles for activation which was not compatible with the nature of the linker.[171] They demonstrated that solid-phase synthesis offers the capability to construct oligosaccharides of a length initially considered difficult to synthesise in solution. Their methodology produced multi-milligram quantities of the dodecasaccharides that enabled their full characterisation, and their use in biological experiments.[169]

To date, Pan *et al.* reported the first solution-phase synthesis of a β-mannuronic acid alginate hexadecasaccharide introducing the longest synthetic polymannuronate (Figure 29).[172] Their approach was based on the orthogonal and consecutive activation of thioglycosides and glycosyl *N*-phenyl trifluoroacetimidates in a [2+2], [4+4] and [8+8] coupling. The use TfOH was preferred over TMSOTf or TBDMSOTf, due to the higher yields and slight increase in β-selectivity. Their highly convergent strategy exhibited satisfactory coupling efficiency by consuming only 1.5 equiv. of *N*-PTFA donors, as opposed to the 5.0 equiv. of donor used in the solid-phase construction of β-mannuronic acid alginate dodecasaccharide.[169,172]

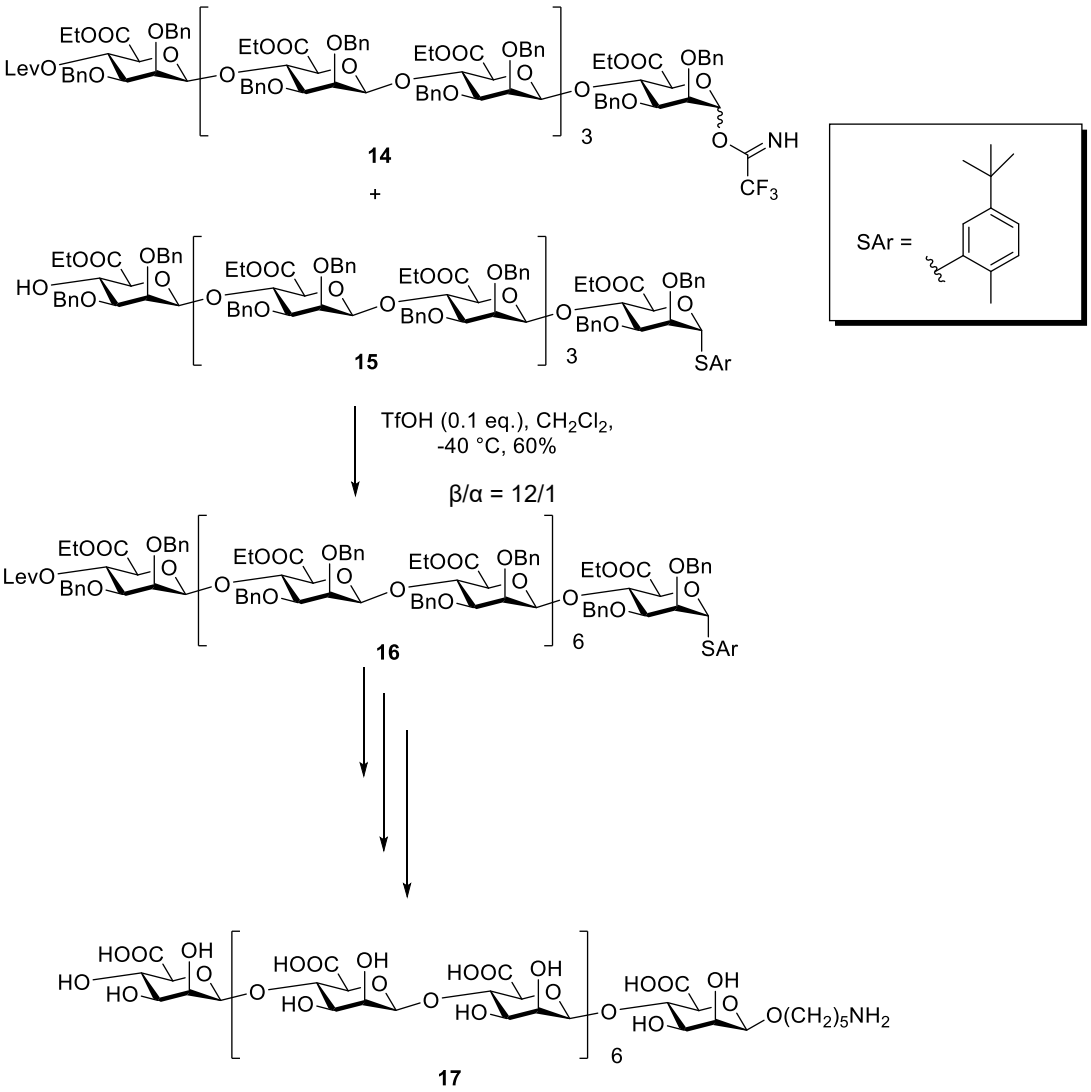


***Figure 29**: Synthesis of the β-mannuronic acid alginate hexadecasaccharide **17** by coupling octamannuronate N-phenyl trifluoroacetimidate donor **14** and the octamannuronate acceptor **15** [Adapted from Pan et al. (2019)].*[172]

4.2.2. Effect of glycosyl acceptor in alginate oligosaccharide synthesis

Previous studies concluded that the reactivity of an acceptor is inversely correlated with the stereoselectivity, as the most reactive alcohols provide the lowest α/β-ratios.[173] It is well established that stronger nucleophiles proceed through faster reactions which are consequently more difficult to direct in terms of the stereochemical outcome.[105] Thus far, several studies have reported that acylated (disarmed) acceptors are less reactive compared to the corresponding benzylated

(armed) acceptors, because of the ester electron-withdrawing effect that reduces the electron density of the adjacent hydroxyl group, and therefore its nucleophilicity.[174–176] Instalment of such electron-withdrawing groups is used for controlling glycosylation reactions, as in this way stereoselectivity may be improved.

An example depicting the reactivity of mannuronate acceptors was showed by Zhang *et al.* in their work where the total synthesis of alginate oligosaccharides containing both M and G residues was performed.[177] In particular, by utilising a G acceptor and a M donor they managed to assemble a set of GM, GMG, GMGM, GMGMG, GMGMGM, GMGMGMG, and GMGGMG alginates in a fully β-stereoselective manner. They explained that the use of GM as a glycosyl acceptor provided low glycosylation yields due to the poor nucleophilicity of G C4-*OH*.[166,178,179] Furthermore, their studies provided further insight in the construction of alginate oligosaccharides, as they confirmed that the nature of the reducing-end anomeric centre, in terms of conformational flexibility, exhibits a tremendous effect on the efficiency and effectiveness with which the disaccharide building blocks were merged. This suggestion derived from a series of glycosylation experiments they conducted for the assembly of four monomers and more, by using glycosyl GM acceptors with different anomeric functionality. NMR analysis established that GM acceptor **20** bearing the β-azidopropanol anomeric group occupies a normal $^4C_1$ conformation, whereas GM acceptor with α-thiocresol anomeric group exists as an equilibrium of a $^4C_1$ (**18**) and $^1C_4$ (**19**) conformation (Figure 30). More importantly, the equilibrium exhibits strong preference towards the $^1C_4$ conformation which is a more reactive conformation that makes C4-*OH* more accessible for the incoming donor. The conformational flexibility of the GM acceptor bearing the α-thiocresol anomeric

group arises from the presence of the *C*5-carboxylate group and the α-anomeric configuration, and both are required for producing the desirable $^{1}C_{4}$ conformation.[177]

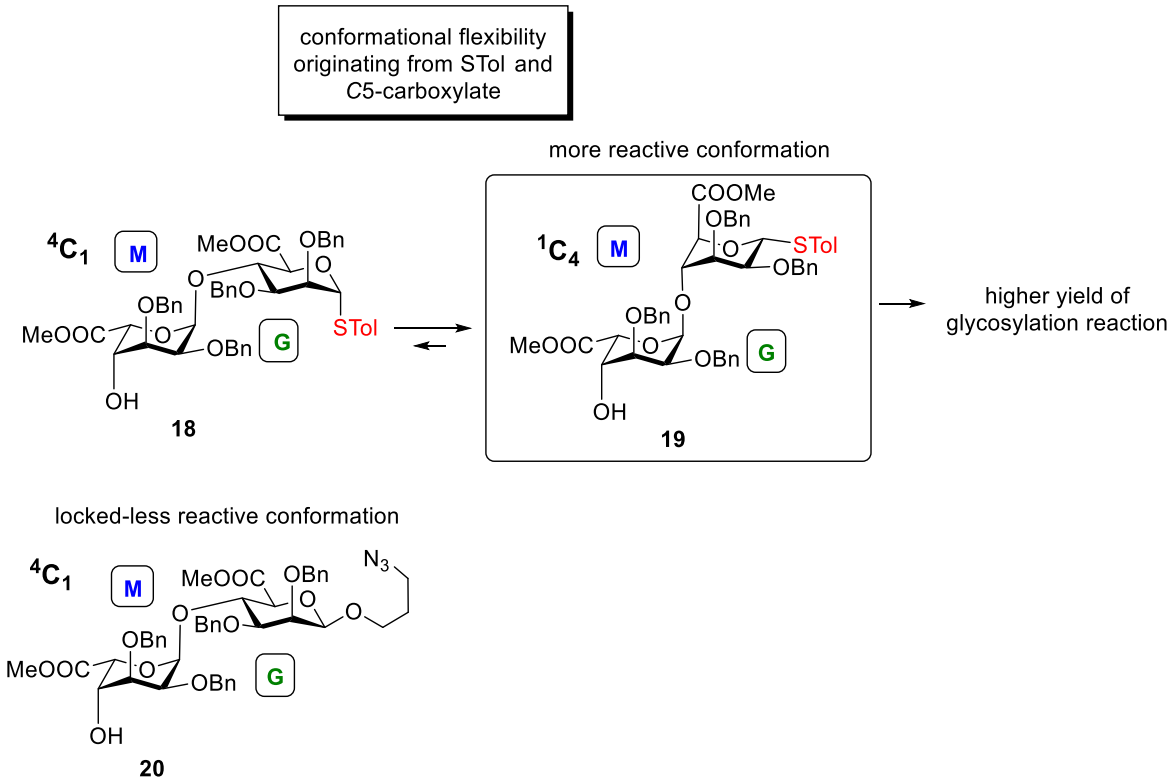


Figure 30: *GM acceptors bearing α-thiocresol and β-azidopropanol in their chair conformation [Adapted from Codée et al. (2015)].*[177]

Preliminary investigation on the relationship between mannuronic acid donors and acceptor reactivity by Codée *et al.* demonstrated that they provide β-selective condensations with all acceptors explored, except with the very unreactive *O*-nucleophile hexafluoro-*iso*-propanol that gave both anomers at a 1:1 ratio.[144] The use of partially fluorinated ethanols as a "toolset" to determine the effect of acceptor nucleophilicity on the stereochemical outcome of a glycosylation showed that S_N1 character is dominant when the acceptor nucleophilicity is decreased (Figure 31). As previously discussed, $^{3}H_{4}$ half-chair oxocarbenium ion **5** is the most stable conformer that tends to be attacked from the top face to give β-linked products **23**. Based on

their results, they concluded that for less reactive *O*-nucleophiles, the higher energy $^{4}H_{3}$ half chair transition state **12** becomes equally favourable with the more stable $^{3}H_{4}$ half chair conformation **5**, leading to the formation of α-*O*-mannuronic acids **24**. Most secondary carbohydrate acceptors are weak nucleophiles, therefore they react with donors that exhibit more carbocation character.

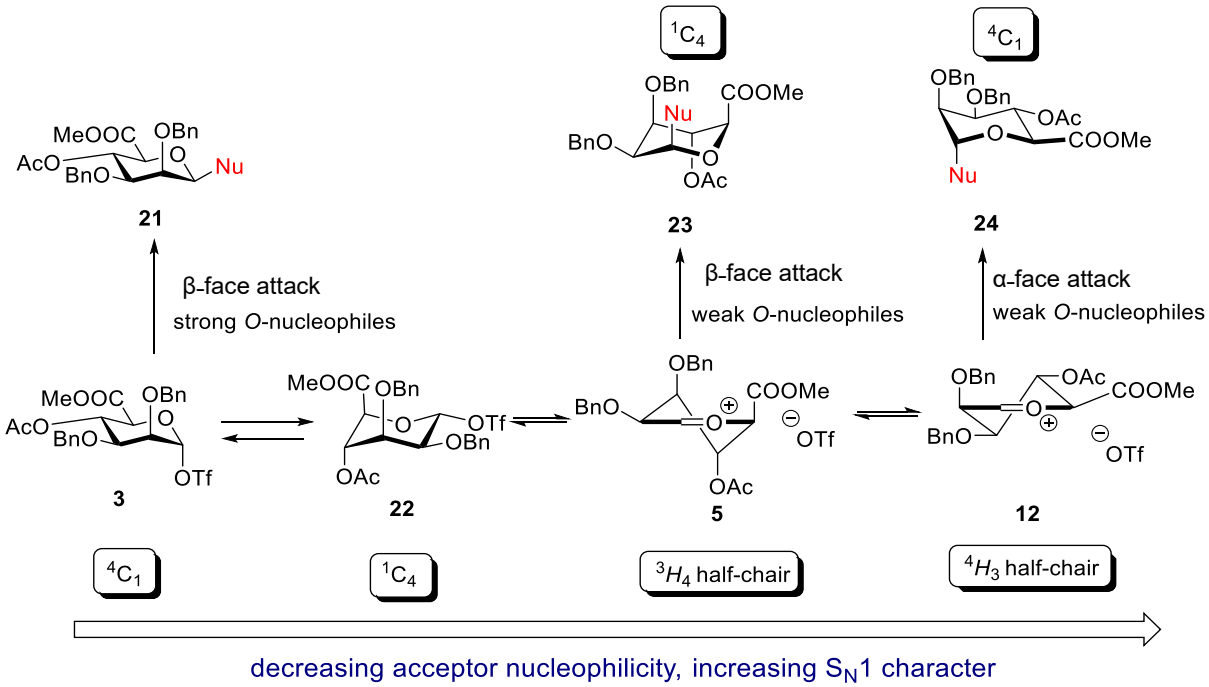


Figure 31: *Conformations and intermediate transitions states to account for the selectivity in glycosylations of mannuronic acid donors [Adapted from Codée et al. (2017)].*[144]

A more recent study by the same group explored the reactivity of uronic acid acceptors and their non-*C*6 oxidised counterparts by employing two conformationally locked thioglycoside donors, 4,6-*O*-benzylidene protected glucose **25** and glucosazide **26** (Figure 32).[180]

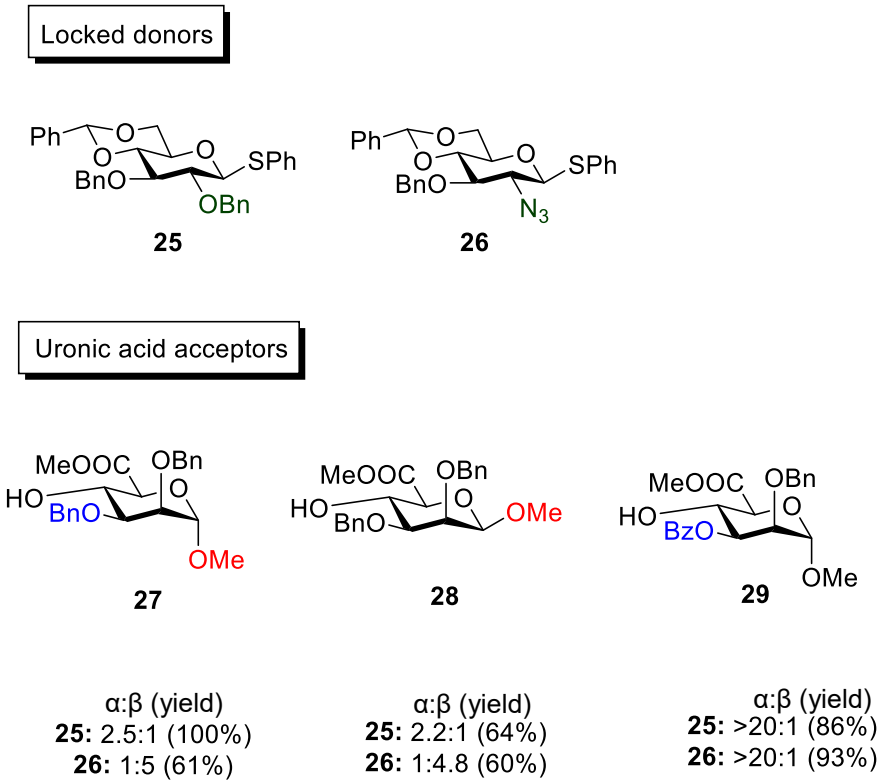


***Figure 32**: Glycosylation stereoselectivities of model donors **25** and **26** with mannuronic acid acceptors **27**-**29** [Adapted from Codée et al. (2018)].*[180]

Again pre-activation conditions were applied for the formation of α-triflate donors, with donor **26** giving a more stable intermediate as a result of the electron-withdrawing effect of *C*2 azide. Consequently, donor **26** exhibited high β-stereoselectivity by participating in S_N2-type reactions. They demonstrated that the anomeric centre configuration does not influence the reactivity of C4-*OH* acceptors, as the employment of α and β-methyl uronic acid acceptors **27** and **28** gave the same α/β ratios with donors **25** and **26**. Although the protecting groups at *C*2 position showed no effect on the glycosylation stereoselectivity, the nature of the protecting group at *C*3 position had an apparent effect. In particular, the exchange of *C*3-Bn with *C*3-Bz functionality in uronic acid acceptor **27**, **29** gave significantly higher α/β ratios with both donors **25** and **26** (>20/1).

Previous research on native alginate synthesis enriched the current state of knowledge with significant information regarding methodology and mechanistic interpretation. This thesis focused mainly on the application of established synthetic methodology for native alginate oligosaccharides in the attempted construction of structurally-defined modified alginate oligosaccharides. As summarised in Section 3.1, alginate is of high significance in various applications, and thus far, hydrophobically-modified alginate is the major biomaterial exploited.

5. Concepts underpinning the work described in this thesis

5.1. *C*6-modified alginate oligosaccharide synthesis

As discussed throughout Section 3, alginate exhibits material properties that can be tailored to become beneficial for high-value medical applications. The examples described on *C*6-carboxylate modification focuse mainly on the production of hydrophobically modified alginate. The work presented here targets the design and synthesis of *C*6-bioisosteric D-ManA building blocks which could then be assembled for the synthesis of structurally defined modified alginate oligosaccharides. The carboxylic acid group bioisosteres selected for this purpose were hydroxamic acid and tetrazole (Figure 33). This novel strategy of alginate derivatisation would generate designer alginates possessing improved or new material properties, such as new ion chelating properties. In addition to the biomaterial aspect, the mimetic alginate oligosaccharides could be exploited as structure-function tools for investigating the epimerase and lyase mode of action.

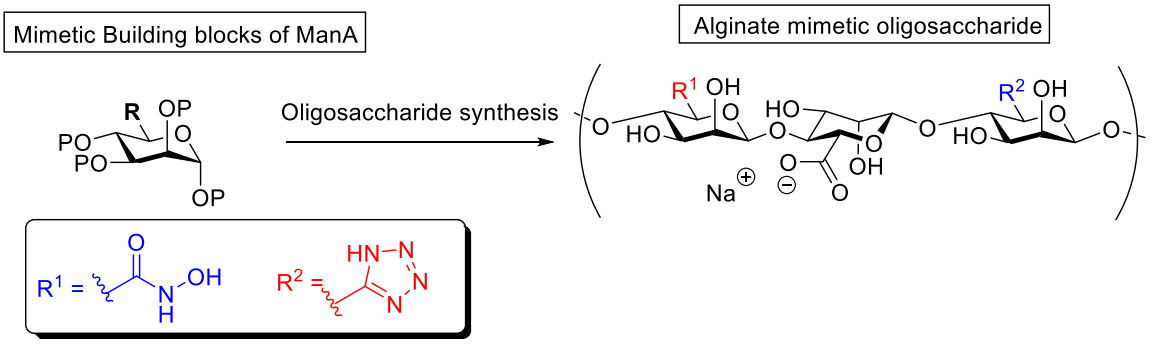


Figure 33: *Generic representation of designer alginate oligosaccharides from mimetic monosaccharide building blocks of D-ManA (P = protecting group).*

5.1.1. Hydroxamic acid

Focusing on the selection of carboxylic acid bioisosteres, hydroxamic acid was chosen based on its coordination properties that have proven advantageous in biological areas including medicine, pharmacology and different agricultural fields.[181] In the context of an anionic oligosaccharide synthesis, the physicochemical parameters to consider are acidity, lipophilicity and permeability.[182] Even though hydroxamic acids are less acidic than carboxylic acids, exhibiting pK_a values in the range of ~8-9, they have been successfully employed in drug design as carboxylic acid bioisosteres.[183,184] In addition, replacement of carboxylic acids with hydroxamic acids results in compounds with increased lipophilicity, leading to favourable activity profiles in cellular assays, in terms of permeability, absorption and distribution.[185]

Hydroxamic acid derivatives are also often utilised for the analysis of trivalent elements such as Fe(III).[186] Extensive research has focused on the ability of hydroxamic acids to inhibit certain metalloenzymes that contain Fe(III) and Zn(II).[187,188] Complexes of hydroxamate species with other metal ions such as Co(II), Co(III), Ni(II), Cu(II) and V(IV) have also been investigated, indicating the variety of coordination complexes that can be formed.[189] The vast majority of the metal

complexation of simple primary hydroxamic acid ligands occurs by deprotonation of the NH–OH which leads to coordination of the metal ion to the carbonylic oxygen and the deprotonated oxygen NH–O^-, giving the hydroamato (1-) species **30** (Figure 34a). A second metal-induced deprotonation can also take place, producing the hydroximato (2-) type of ligand **31** (Figure 34b).[189]

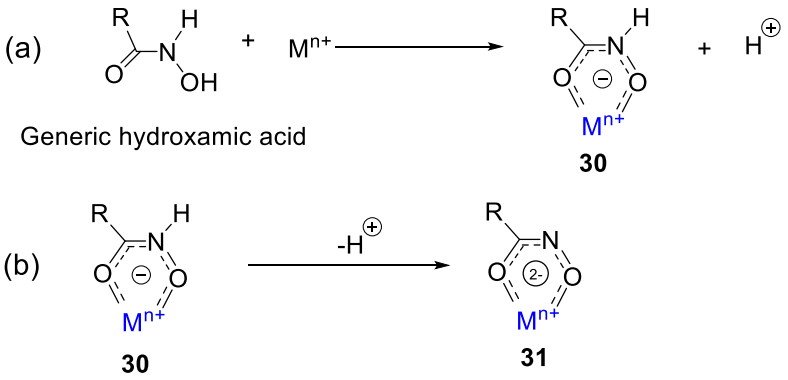


Figure 34: *(a) Metal ion [O,O] coordination by a gereric hydroamato (1-) derivative* ***30*** *(b) metal induced deprotonation leading to metal ion [O,O] coordination by a generic hydroximato (2-) type of species* ***31****.*[189]

To consider an example, hydroxamic acid was employed as a *C*6 functional group in octyl D-glucosides in an attempt to produce biodegradable surfactants with good chelating properties for the removal of contaminant metals in wastewater.[190] By performing flotation experiments using Fe(III) solutions as a model contaminant metal, Ferlin *et al.* demonstrated that hydroxamic acid derivatives exhibit better iron extraction than their respective carboxylic acid derivatives. The ability of compounds containing hydroxamic acid groups to coordinate Fe(III) has categorised them in a highly diverse group of compounds called siderophores.[191,192] The chelating properties of hydroxamate make it an interesting carboxylic acid bioisostere for incorporation in chemical structure-function tools and novel alginate biomaterials.

5.1.2. Tetrazole

Focusing on tetrazole as a bioisostere, 5-Substituted tetrazoles (5-STs) are frequently referred to as tetrazolic acids and exist as a nearly 1/1 ratio of 1*H*- and 2*H*-tautomers. In addition, tetrazolic acids are often described as imidoyl azides (Figure 35a).[183]

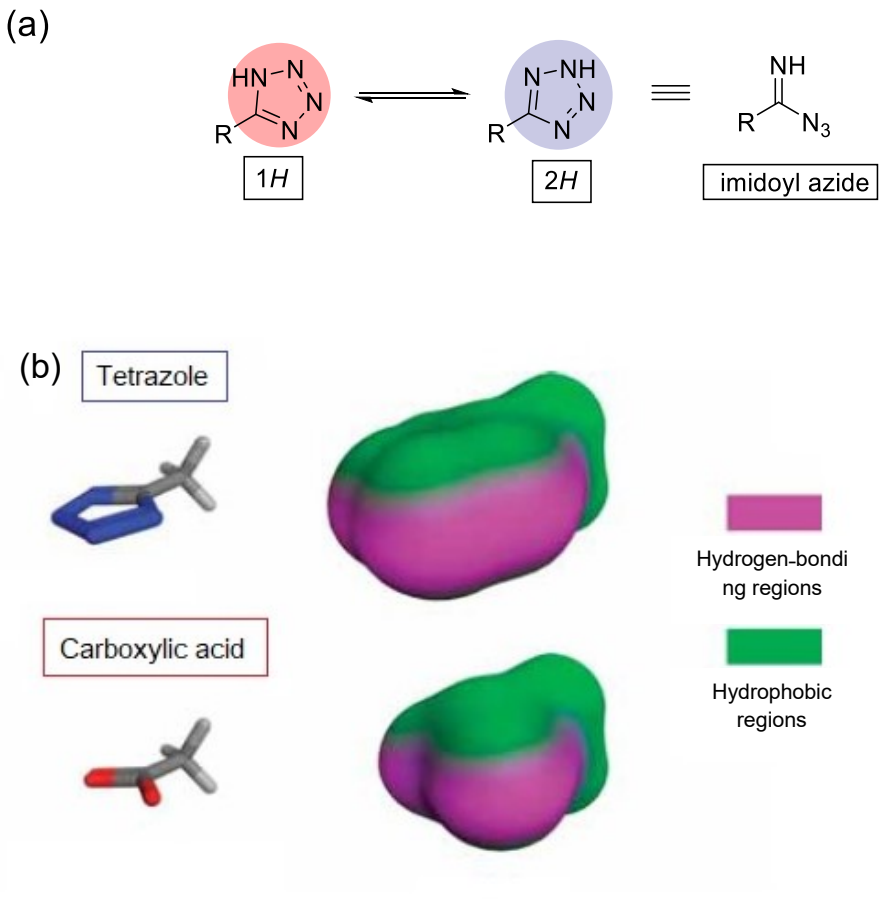


Figure 35: *Tetrazolic acids are carboxylic acid bioisosters (a) Tautomers of 5-substituted tetrazoles.*[193] *(b) Skeleton and surface models of 5-methyltetrazole and acetic acid anions [Adapted from Hamada and Kiso (2012)].*[194]

Lassalas *et al.* showed that a tetrazole surrogate moiety exhibits stronger hydrogen bond interactions than the respective carboxylic acid group, and their findings are consistent with previous studies.[182,195] The enhanced hydrogen bonding capability of tetrazolic anions highlights their increased binding affinity for the biological target site,

as it is apparent that the hydrogen bonding region in tetrazole (magenta on the surface model) is greater than in carboxylate (Figure 35b).[183]

Under physiological conditions (pH = 7.4), carboxylic acids are partially dissociated (pK_a ~ 4). The resulting delocalisation of the negative charge between the two oxygen atoms of the carboxylate anion provide two hydrophobic regions (green on the surface model) that contain a π-orbital above and below the plane of the anion.[194] Therefore, the tetrazole ring can be applied as non-classical carboxylic acid bioisostere, which appears to be more effective as a surrogate moiety than a carboxamide group. The term non-classical isostere refers to the fact that tetrazole, as an aromatic heterocycle, differs in size and number of atoms from the carboxylic acid group.[194,193] According to the surface model in Figure 35b, the hydrophobic region of tetrazoles is larger, therefore they are more lipophilic compared to carboxylate anions and potentially more permeable.[182] The free N-H bond of tetrazoles makes them acidic molecules, hence, they are also ionised at physiological conditions, exhibiting a planar structure.[194] Moreover, the delocalisation of the negative charge around the tetrazole ring is distributed over a larger molecular surface area, which can lead to a more favourable interaction with the targeting site, or it may complicate it. [183,193] Acidic compounds often exhibit low permeability and bioavailability, nevertheless, computational studies calculating the aqueous acidity of tetrazole suggested that it is the most preferred carboxylic acid bioisostere due to its *in vivo* stability.[196] Similarly to the hydroxamic acid group, tetrazole constitutes a compelling candidate for the synthesis of designer alginates possessing diverse physicochemical properties.

5.2. *C*6 and *C*5-modified alginate precursor building blocks

Sugar-nucleotides play a crucial role as precursors or intermediates in carbohydrate metabolism and glycoconjugate biosynthesis.[197] A large and growing body of literature focuses on the development of structural analogues of sugar-nucleotides which can be employed as substrates for enzymatic reactions in carbohydrate synthesis, as enzyme inhibitors, or as innovative tools for assay development and for the study of glycoconjugate biosynthesis.[198,199] The importance for accessing both natural and non-natural sugar-nucleotides is emphasised from the numerous studies focusing on their efficient preparation for subsequent investigation of biological pathways in both mammalian and bacterial cells.[197,198,200,201]

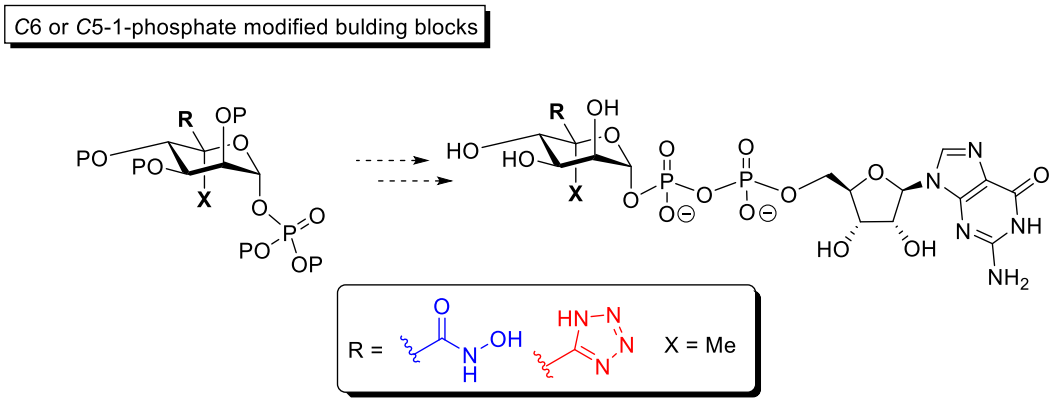


***Figure 36**: Generic representation of C6-modified and C5-substituted GDP-D-ManA analogues from respective 1-phosphates (P = protecting group).*

A secondary aim of this thesis is the synthesis of *C*6-bioisosteric D-ManA 1-phosphates and subsequent production of their sugar-nucleotides (Figure 36). Similar to the strategy discussed in Section 5.1, the targeted structures designed incorporate *C*6-hydroxamic acid and *C*6-tetrazole moieties. *C*6-bioisosteric D-ManA 1-phosphates can be used as structure-function tools to further study of AlgA, while their respective sugar-nucleotides can investigate AlgD, as both enzymes are

crucially involved in alginate precursor (GDP-D-ManA) biosynthesis. In addition, *C*6-modified GDP-D-ManA analogues could be employed to modulate the biosynthesis of alginate in *P. aeruginosa* for biotechnological, as well as therapeutic applications.

An additional research aim presented here focuses on the design and synthesis of *C*5-Me modified alginate building blocks, as a means to probe epimerase AlgG and lyase AlgL (Figure 36). As previously discussed, the mode of action of both enzymes strongly relies on the H_5 abstraction for completing their mechanistic function, therefore the replacement of *C*5-proton with a *C*5-Me group could potentially block this preliminary deprotonation. In other words, *C*5-Me-ManA could be incorporated in the biosynthesised polymannuronate and consequently affect alginate modification and/or production.

6. Synthesis of native and *C*6-modified mannuronate building blocks

The synthetic approach undertaken initially for the synthesis of structurally defined *C*6-modified alginate oligosaccharides was based on a pre-glycosylation oxidation route, as Codée *et al.* clearly demonstrated that the stereodirecting effect of the electron-withdrawing *C*5-carboxylic ester is the major contributor in the formation of β-linkages with mannuronate donors.[166,164] Therefore, the objective was to incorporate the hydroxamate and tetrazole functionality at an early stage, to enable the assembly of modified alginate oligomers in a fashion similar to that seen for the native system (Figure 37).

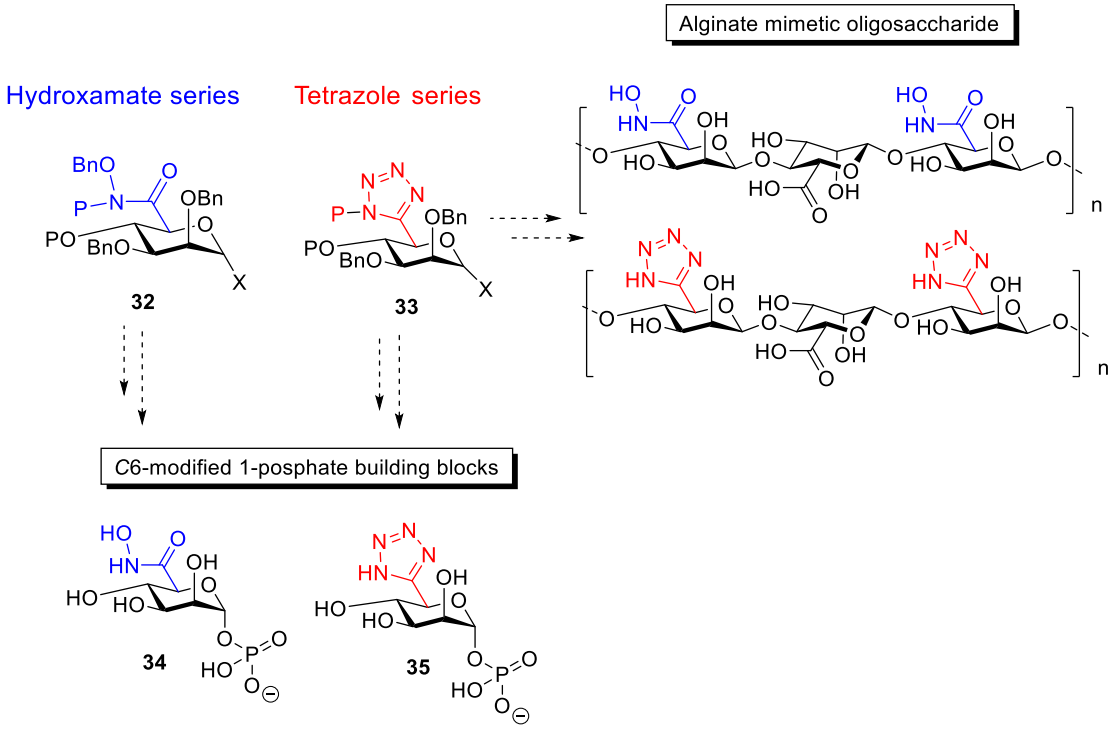


***Figure 37**: C6-modified mannuronate monosaccharide toolset for accessing designer alginate oligosaccharides and sugar-1-phosphates (P = protecting group, e.g. Ac, Lev, TBDMS, PMB, X = SPh, $OCH_2CH_2CH_2N_3$, $OCH_2CH_2CH_2N(H)Cbz$).*

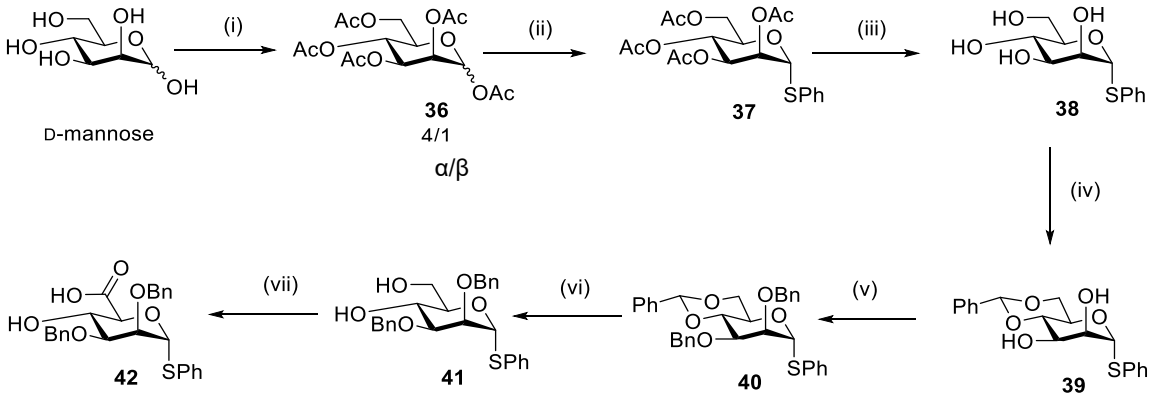
*C*6-modified thioglycosides **32** and **33** were designed as suitable mimetic glycosyl donors for the oligosaccharide assembly. Thioglycosides are extensively used in oligosaccharide synthesis due to the stability of the anomeric thio function under a wide range of conditions applied during protecting group manipulations. Moreover, the thioether anomeric functionality can be activated using varied types of glycosylation conditions, as the soft sulfur atom can selectively react with soft electrophiles. These characteristics emphasise the versatility of thioglycosides, making the thio-functionality (e.g. thiophenol) a suitable anomeric protective group and an efficient leaving group. Considering that alginate is composed of β-1,4-linkages, the protecting group at *C*4 position of **32** and **33** was orthogonal to the rest of the protecting groups, allowing selective deprotection for iterative glycosylation reactions. Access to thioglycosides **32** and **33** was also used as

precursors useful in the synthesis of respective glycosyl acceptors and 1-phosphates **34** and **35** (Figure 37).

The following sections are divided as follows: synthesis of native and *C*6-modified thioglycoside series, conversion to their respective sugar-1-phosphates, alginate disaccharide synthesis using native mannuronate and *C*6-hydroxamate building blocks, synthesis of conjugable *C*6-modified mannuronates and future work and directions.

6.1. Mannuronate donor and acceptor synthesis

The route towards the synthesis of D-ManA building block **42** was completed following established literature procedures (Scheme 1).[202,203,204,205] Uronic acid **42**, reported by Codée and collaborators for automated alginate oligosaccharide synthesis[169], was an important intermediate for the synthesis of both a native and a hydroxamate precursor series.



***Scheme 1**: Synthesis of D-ManA derivative **42** (i) Ac_2O, H_2SO_4, RT, 1 h., 91% (ii) PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, RT, 48 h, 75% (iii) $Na_{(s)}$, MeOH, RT, 2 h, 94% (iv) $PhCH(OMe)_2$, $HBF_4{\cdot}Et_2O$, DMF, RT, 24 h, 67% (v) NaH, BnBr, DMF, RT, 24 h, 74% (vi) p-TsOH·H_2O, MeOH, 80 °C, 2 h, 72% (vii) TEMPO, BAIB, CH_2Cl_2/H_2O (2/1, v/v), RT, 2.5 h, 60%.*

Peracetylation of α/β-D-mannose furnished **36** as α- and β-anomers in a 4/1 ratio, as determined by ^{1}H NMR and NOESY spectra. Equatorial H_1 (6.09 ppm, d, $^{3}J_{H1\text{-}H2}$ = 1.9 Hz) was expected to resonate downfield to the axial H_1 (5.88 ppm, d, $^{3}J_{H1\text{-}H2}$ = 1.1 Hz), and the obtained NOESY spectrum confirmed through space correlation of the axial H_1 (β-anomer) with the axial H_3 (5.15 ppm, dd, $^{3}J_{H3\text{-}H4,\ H3\text{-}H2}$ = 10.0, 3.2 Hz). Thioglycoside **37** was obtained in 75% yield through reaction of **36** with thiophenol and $BF_3{\cdot}Et_2O$. Following thioglycosidation, the acetyl groups were removed by treatment with NaOMe, produced *in situ* by dissolving $Na_{(s)}$ in MeOH to give tetraol **38**. 4,6-*O*-benzylidene protection of **38** produced the *trans*-fused ring system **39** in 67% yield, using benzaldehyde dimethyl acetal under protic catalysis.

Initial attempts towards the synthesis of **40** with NaH and BnBr led to a 74% yield of the desired product. A monobenzylated product was also isolated (15 mg, 4.3%), however, NMR data (COSY, HSQC, HMBC, and NOESY) were inconclusive. H_2 and H_3 were merged in the ^{1}H NMR spectrum, as well as H_1 and the benzylidene-CH. *C*2 and *C*4 were also merged in the HMBC spectrum, making assignment difficult. The ^{1}H NMR spectrum obtained for **43** matched the literature values for a *C*2-benzylated compound, however, X-Ray crystallographic data were produced to further confirm the assignement.[206] As such, 8 mg of **43** was dissolved in 0.5 mL of CH_2Cl_2:hexane (1/1) to successfully produce a crystal sample by slow evaporation at room temperature. Crystal formation was completed after 16 h and the crystallographic data obtained verified that *C*2 position was benzylated, suggesting a relative order of reactivity for the bis-benzylation of **39** (Figure 38). These results can be attributed to the higher acidity of C2-*OH*, as previously demonstrated for diol mono-benzylation using phase-transfer catalysis.[207]

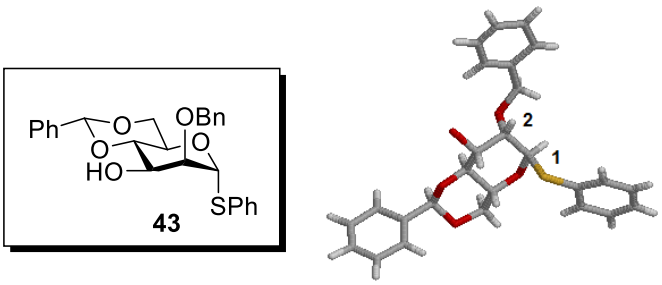
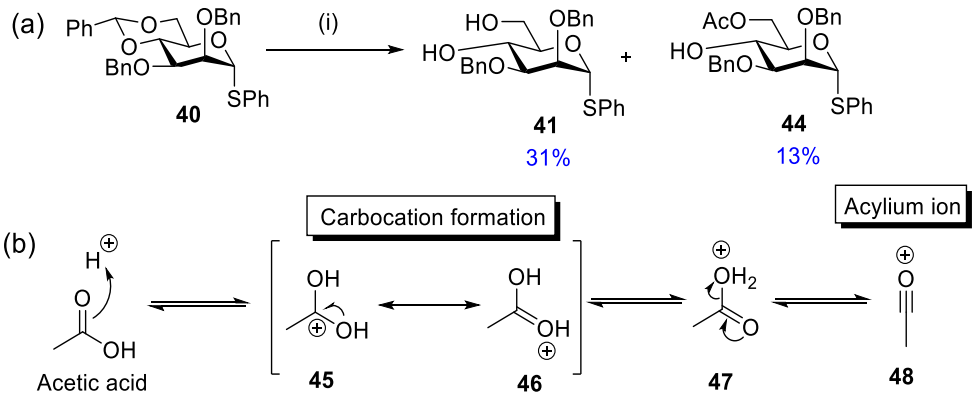


***Figure 38**: Crystal structure of **43** indicating the benzyl group ac C2.*

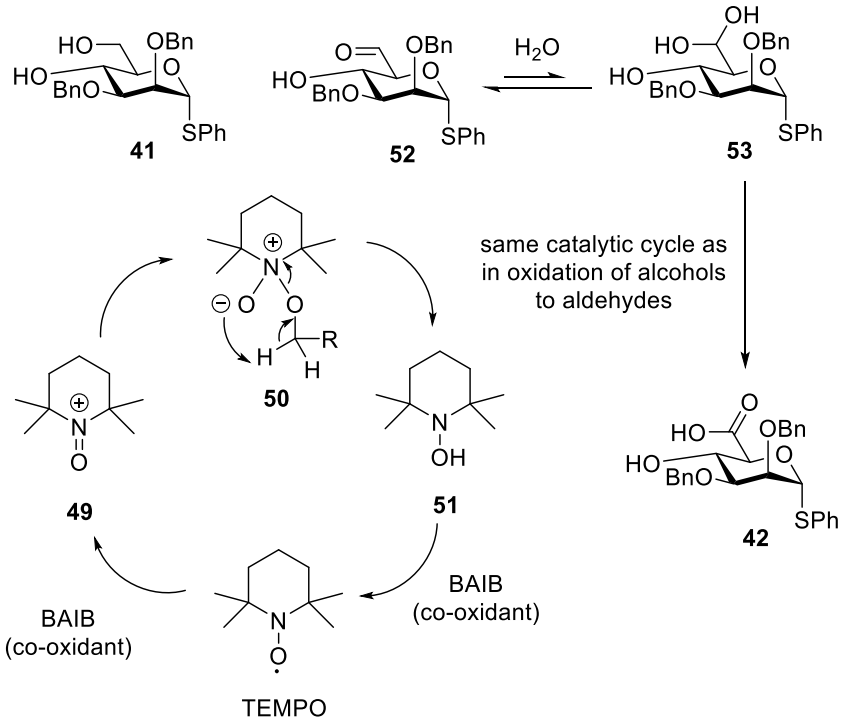
Deprotection of **40** was completed by cleavage of the benzylidene group evaluating two alternative sets of acidic conditions. The first conditions used *p*-TsOH whereas the second used 60% aq. AcOH. The former conditions were more efficient, providing higher yields of **41** (72%). Heating to reflux with 60% aq. AcOH at 120 °C for 6 h gave only 31% of **41** and the starting material recovery was 24%. Moreover, the use of the latter conditions gave additional by-product **44** in 13% yield (Scheme 2a). The ^{1}H NMR data for this by-product were in a good agreement with literature values.[208] *In situ* generation of acylium ion **48** from AcOH through carbocation **45** formation and a series of proton transfers (**46**, **47**) accounts for the formation of **44** (Scheme 2b). The mechanism could also proceed through common ester forming mechanism in acidic conditions.



***Scheme 2**: 4,6-Benzylidene group hydrolysis by using (i) 60% AcOH$_{(aq)}$ yielded 31% **41** and 13% **44**.*

Repeated large scale synthesis of **41** starting from ~ 80 g of D-mannose was achieved for acquiring satisfactory amounts (~ 30 g) of **41**, giving a 15% overall yield.

BAIB and catalytic TEMPO were next employed for a regio- and chemoselective oxidation of **41** to **42**, as these reagents do not readily promote the oxidation of an anomeric thiohemiacetal, or oxidation of a secondary alcohol.[209] The oxidising species generated *in situ* from the reaction of TEMPO and BAIB is the *N*-oxoammonium intermediate **49**, and depending on the reaction medium (anhydrous or aqueous) the oxidation can be tuned by stopping at the aldehyde **52** or carboxylic acid **42** stage (Scheme 3).

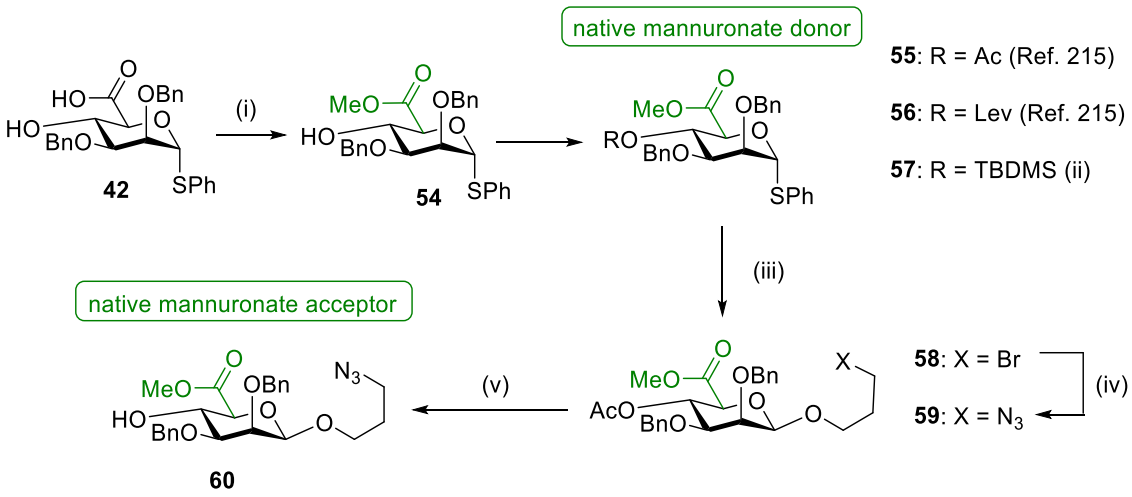


***Scheme 3**: Postulated oxidation mechanism of **42** using TEMPO/BAIB in aqueous medium [Adapted from Parlanti et al. (1997)].*[210]

More specifically, the presence of water hydrates the aldehyde to give **53**, allowing the oxidation towards the carboxylic acid by repeat of the same catalytic steps,

proceeding *via* intermediate **50**.[210] Finally, the formed hydroxylamine **51** is oxidised to TEMPO by BAIB, thus completing the catalytic cycle.

With amount of **42** in hand, literature procedures were followed for the construction of native D-ManA building blocks **54**, **55,** and **56** (Scheme 4).[211] A *C*4-OTBDMS protected donor was accessed by the use of TBDMSOTf, imidazole and DMAP in DMF. The reason behind the synthesis of 3 differentially *C*4-protected mannuronate donors was to examine the deprotection protocols applied in terms of yield and influence on the existing linkages (Section 8.1.). Mannuronate donor **55** was subsequently used with Ph_2SO/Tf_2O and TTBP in CH_2Cl_2, to furnish **58** in 67% yield, which was then refluxed in acetone with NaN_3 for 48 h to give **59** in 97% yield. ^{13}C NMR confirmed the installation of an azide functionality, as the resonance of the α-carbon to N_3 shifted downfield from 30.4 ppm to 48.3 ppm.



Scheme 4*: Synthesis of the native donors and acceptor (i) MeI, K_2CO_3, DMF RT, 24 h, 77% (ii) TBDMSOTf, imidazole, DMAP, DMF, RT, 16 h, 96% (iii) Ph_2SO, TTBP, Tf_2O, 3-bromopropanol, CH_2Cl_2, -60 °C to -90 °C, 1 h, 66% (iv) NaN_3, acetone, 55 °C, 48 h, 97% (v) $Na_{(s)}$, MeOH, RT, 24 h, 87%.*

Finally, basic transesterification of the *C*4-OAc under Zemplén conditions, gave the reducing end mannuronate acceptor **60** in 87% yield. A coupled HSQC spectrum confirmed the expected β-configuration ($^{1}J_{C\text{-}H}$ = 156 Hz) of **60**. It is worth noting here that HSQC experiments obtained without ^{13}C decoupling enable the assignment of the anomeric configuration by providing one-bond CH coupling constants ($^{1}J_{C\text{-}H}$) exhibiting a 10 to 20 Hz difference. An empirical rule is that $^{1}J_{C1\text{-}H1}$ = 170 Hz indicates an equatorial proton at *C*1 (e.g., α-D-glucose), while $^{1}J_{C1\text{-}H1}$ = 160 Hz indicates an axial proton.[212]

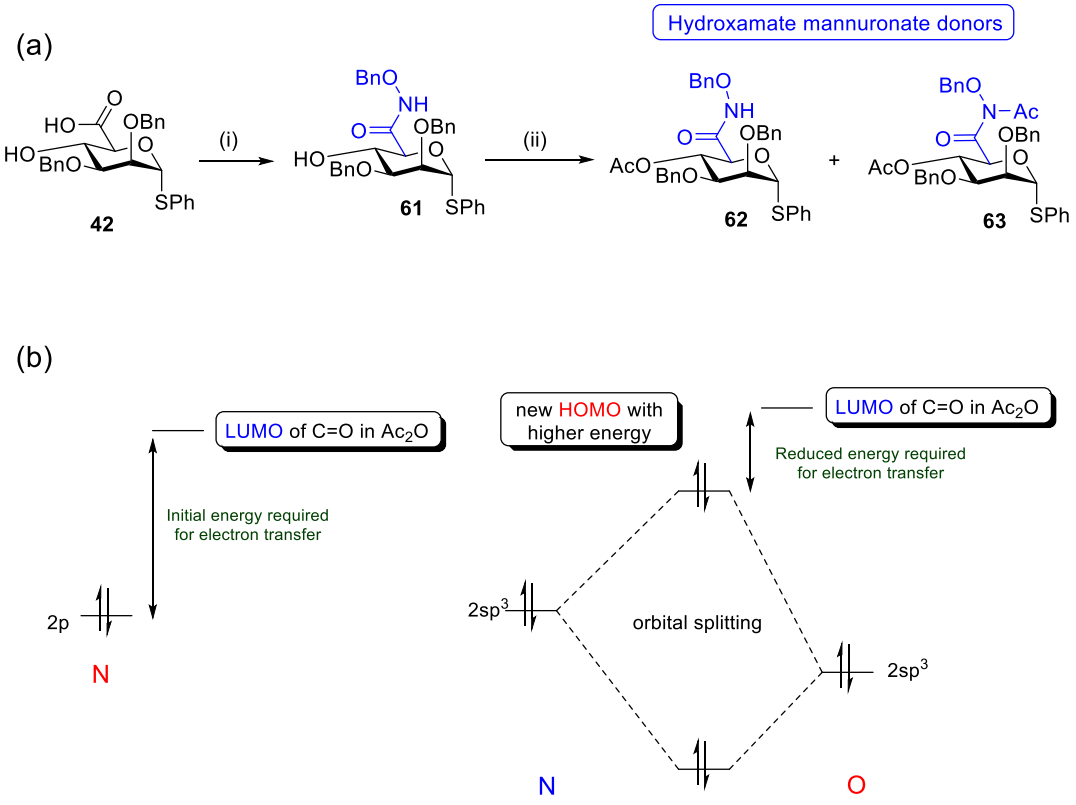
The selection to install the 3-azidopropanol functionality on the native and modified reducing end glycosides was based on its dual advantage of differentiating the anomeric configuration with the thioglycoside donors (Sections 8.1. and 8.2.) and enabling further elaboration of the azide moiety for future conjugation studies, as it has been successfully employed for many applications in carbohydrate chemistry (Section 9.1.).[213]

6.2. 6-*C*-hydroxamate donor and acceptor synthesis

A hydroxamate functionality was installed by coupling *O*-benzylhydroxylamine with **42** using PyBOP in the presence of hindered base DIPEA (Scheme 5a). *C*6-hydroxamate scaffold **61** was delivered in 81% yield and ^{13}C NMR data confirmed the coupling by showing an upfield shift of the carbonyl carbon peak from 172.9 ppm in mannuronic acid **42** to 168.4 ppm in **61**. The next step towards donor **62** was the chemoselective protection of C4-*OH* with an acetate group. Initial attempts using 1.2 and 1.7 equivalents of Ac_2O gave **62**, **63** and a significant amount of starting material. The reaction was completed successfully when 2.2 equivalents of Ac_2O were used leading to **62** in 68% yield, as well as acylation of the hydroxamate nitrogen (**63**,

20%). An increased nucleophilicity of the nitrogen in the hydroxamate group caused by the α-effect could account for the *N*-acylation (Scheme 5b).

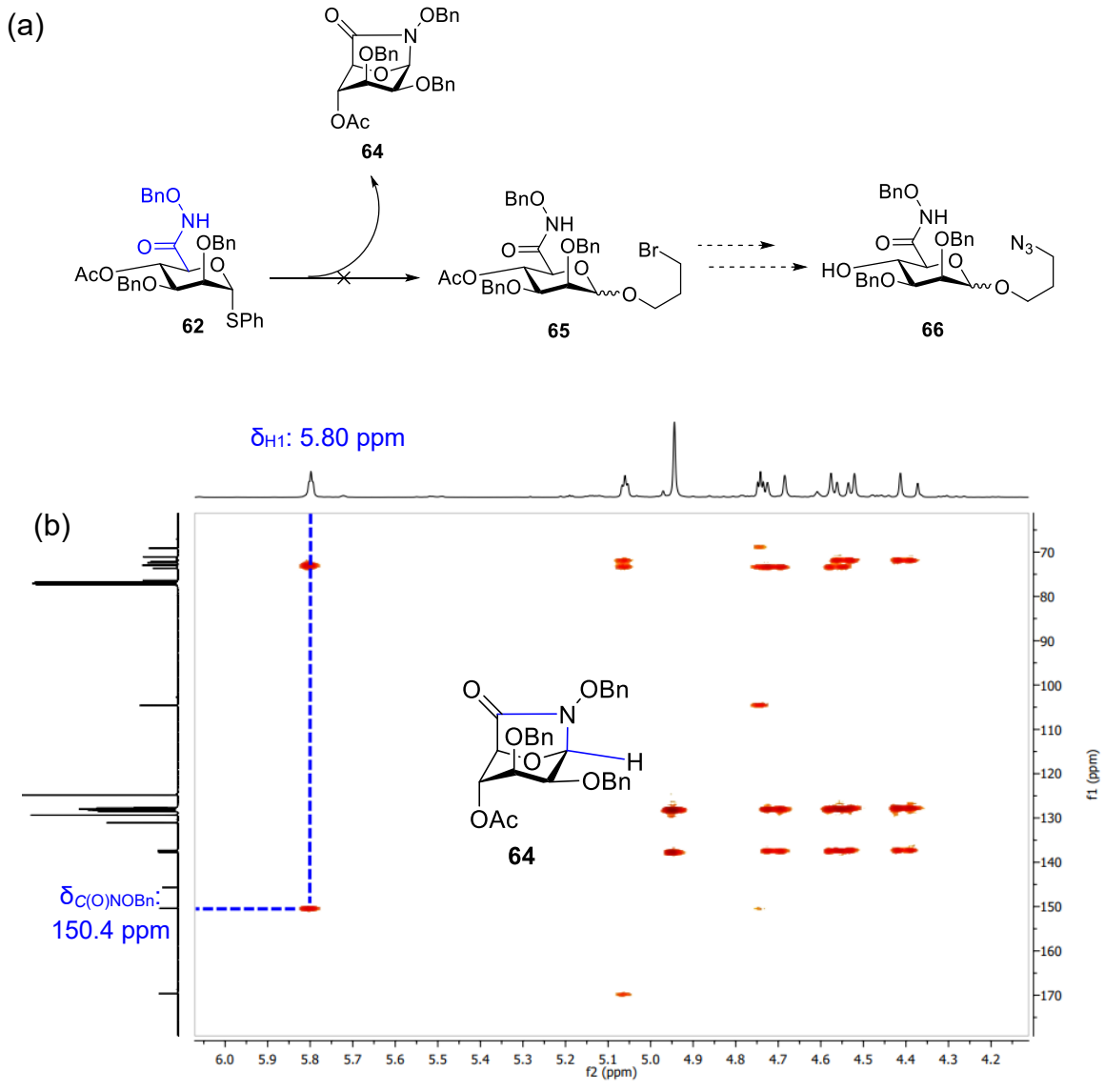
Analogous linker installation conditions with native donor **55** were attempted to access *C*6 hydroxamate glycosyl acceptor **66** from donor **62** (Scheme 6a). Upon activation of the α-thiophenyl group and addition of 3-bromopropanol, TLC analysis suggested conversion to a material with an unexpected higher R_f.



Scheme 5: *(a) Initial synthetic route towards C6-hydroxamate donor* ***62*** *(i) O-benzylhydroxylamine, PyBOP, DIPEA, CH_2Cl_2, RT, 3 h, 90% (ii) Ac_2O, pyridine, CH_2Cl_2, RT, 24 h,* ***62****: 68%,* ***63****: 20%. (b) depiction of α-effect: the resulting π-orbital splitting by the alpha atom (O) increases the energy of the HOMO, requiring less energy for the electron transfer from the 'alpha' nucleophile (N) HOMO to the C=O LUMO of Ac_2O.*

NMR data showed that the 3-bromopropyl unit was not installed and instead the nitrogen of the hydroxamate group had acted as a nucleophile creating a bridge from *C*5 to *C*1, giving **64** in a 62% yield. ^{1}H NMR analysis suggested **64** to be in $^1C_4$

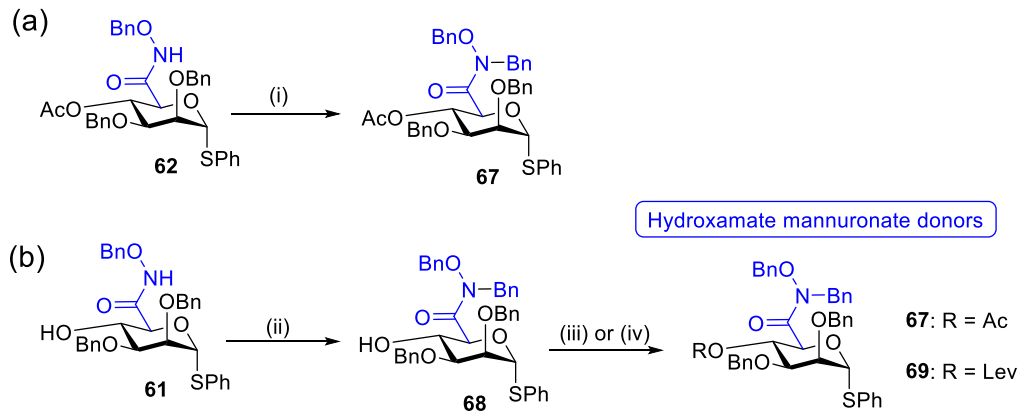
conformation, due to the small ^3J value observed for the apparent triplet peak corresponding to H_4 (5.06 ppm, app. br. t, $^3J_{H4\text{-}H3,\ H4\text{-}H5}$ = 1.9 Hz). The reaction presumably proceeded *via* an intramolecular nucleophilic substitution producing **64** as the sole product. An *N*-C1 bond formation was confirmed by HMBC, where the anomeric proton (H_1: 5.80 ppm, app. t, $^3J_{H1\text{-}H2,\ H1\text{-}H3}$ = 1.2 Hz) showed correlation to the carbonyl carbon of the hydroxamate (*C*(O)NOBn: 150.4 ppm) (Scheme 6b).



Scheme 6: *(a) Attempted synthesis of* ***65*** *using donor* ***62*** *with Ph_2SO, TTBP, Tf_2O, 3-bromopropanol, CH_2Cl_2, -60 °C to -90 °C, 1 h gave* ***64*** *at 62% yield (b) HMBC data confirming the connection of anomeric proton (H_1: 5.80 ppm) and hydroxamate carbonyl carbon (150.4 ppm).*

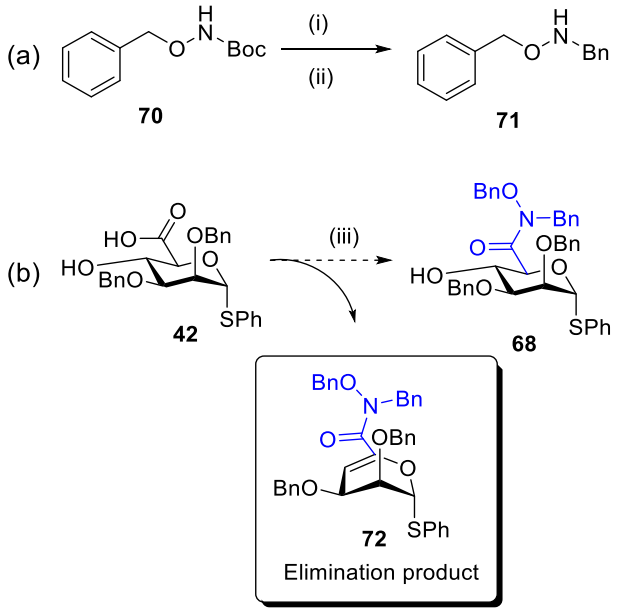
Based on these results, **63** was next employed as the scaffold to attach the linker at the anomeric position using the same glycosylation conditions. Unfortunately, the acetyl protecting group on the hydroxamate nitrogen was hydrolysed, giving **62** as the product of the reaction. Considering the observed instability of an *N*-Ac protecting group during these glycosylations, a benzyl group was introduced instead.

Accordingly, *N*-benzylation of **62** with BnBr and K_2CO_3, afforded **67** in 42% yield (Scheme 7a).[214] The reaction was monitored by TLC every hour (for 6 h), which showed formation of a decomposed material (with a lower R_f) and was responsible for the low yield of **67**.The yield of the reaction remained the same even after 24 h at room temperature. The alternative route designed as a means to improve the overall yield of **67**, started with *N*-benzylation of **61**, giving **68** in varying yields (45% - 64%) (Scheme 7b). The low yield spectrum reported was again due to the formation of the unidentified decomposed material, as determined by TLC and NMR analysis. Acetylation or levulinoyl protection of **68** gave **67** or **69** in 87% and 91% yield, respectively.



***Scheme 7**: Synthetic pathways towards C6-hydroxamate glycosyl donors **67** and **69** (a) (i) K_2CO_3, BnBr, DMF, RT, 15 h, 42% (b) (ii) K_2CO_3, BnBr, DMF, RT, 15 h, 45% - 64% (iii) Ac_2O, pyridine, CH_2Cl_2, RT, 24 h, 87% (iv) Lev_2O, pyridine, CH_2Cl_2, RT, 24 h, 91%.*

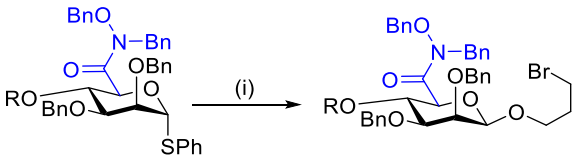
An alternative approach was taken to optimise the yield of the synthetic pathway towards **67** and **69**, evaluating a direct coupling of *N*,*O*-dibenzyl hydroxylamine **71** with uronic acid **42** (Scheme 8). This route required a synthesis of **71** from *tert*-butyl-*N*-(benzyloxy)carbamate **70**, which was then used for coupling with **42**.[215] Reagent **71** was successfully synthesised by *N*-benzylation of **70** followed by treatment with TFA for *N*-Boc cleavage (Scheme 8a).



Scheme 8*: Proposed synthetic route towards **68** via coupling of **42** with **71** (i) NaH, BnBr, DMF, RT, 3 h (ii) 3% TFA in CH_2Cl_2, 24 h, RT, 62% (2 steps) (vi) **71**, PyBOP, DIPEA, CH_2Cl_2, 0 °C, 40 min, gave C4-C5 elimination product **72** in 38% yield.*

In a first attempt to directly synthesise **68** from **42**, two equivalents of **71** were used which delivered an unwanted *C*4-*C*5 elimination product **72**, in 38% yield (Scheme 8b). ^{1}H NMR data of **72** showed a doublet at 5.62 ppm corresponding to vinylic H_4 ($^3J_{H4\text{-}H3}$ = 5.4 Hz), and confirming the absence of H_5. This observation suggested an increased basicity of **71** compared to the *O*-benzylhydroxylamine (used for the synthesis of **61**). In light of this result, the reaction was repeated by stirring **42** with PyBOP and DIPEA at 0 °C, followed by a dropwise addition of **71** (1.3

equiv.). The major product obtained after 40 min. at 0 °C was again **72**, and NMR data showed no evidence of the desired product **68**. These results imply that the basicity of **71** outweighed its nucleophilicity for this reaction and prevented a streamlined access to **68** (and ultimately **67** or **69**) from **42**. Therefore, the route reported in Scheme 7b was applied for the synthesis of building blocks **67** and **69**. *C*6-hydroxamate donors **67** and **69** were synthesised on a multigram scale and were subsequently employed as donors in synthesis of acceptor **78**.



67: R= Ac

69: R= Lev

73: R= Ac

74: R= Lev

Scheme 9*: 3-bromopropyl linker instalment at the anomeric position of* ***67*** *and* ***69*** *(i) Ph_2SO, TTBP, Tf_2O, 3-bromopropanol, CH_2Cl_2, -60 °C to -80 °C, 1 h,* ***73****: 89%,* ***74****: 78%.*

Table 1: Glycosylation conditions attempted for the synthesis of 73 from 67

Entry	Promoter system (equiv.)	Acceptor (equiv.)	Reaction concentration[b] (M)	Duration and temperature (h, °C)	Observation by TLC analysis
Pre-mixing donor and acceptor method[a]					
1	NIS (1.5) TMSOTf (2.0)[c]	1.5	0.05	24 h /RT	Slow conversion (17% isolated yield)
2	NIS (1.5) TMSOTf (2.0)[c]	4.0	0.08	24 h /RT 24 h /35 °C	Slow conversion (not isolated)
3	NIS (1.5) AgOTf (0.5)	1.5	0.06	45 min. /0 °C 15 min. /RT	Slow conversion, inseparable complex mixture
4	NIS (1.5) TfOH (1.0) [c]	2.0	0.07	1 h /0 °C 2 h /RT	Slow conversion (not isolated)
5	NBS (1.5) TMSOTf (0.5)	1.5	0.05	45 min. /0 °C 45 min. /RT	No conversion, inseparable complex mixture
Pre-activation using NIS and equimolar TMSOTf					
6	NIS (1.5) TMSOTf (2.5)	4.0	0.06	1 h /RT 1 h /30°C	No conversion, inseparable complex mixture

[a] **67**, 3-bromopropanol and NIS (1.5 equiv.) were stirred at -10 °C before the addition of Lewis acid.

[b] The solvent used in all attempts was CH_2Cl_2.

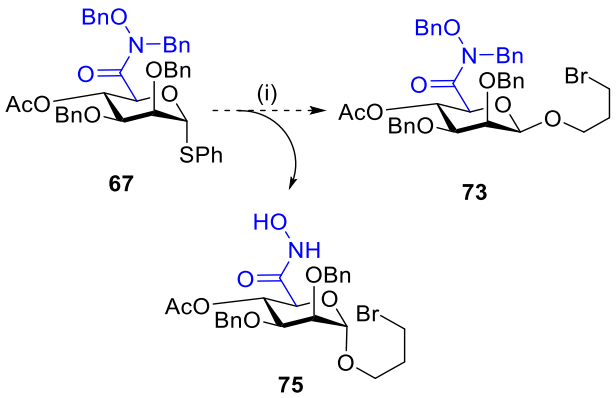
[c] Started from 0.5 equiv. and increased over the given time to the equivalents indicated in the brackets.

Similarly to the synthesis of native acceptor **60**, the route towards *C*6-hydroxamate acceptor **78** required the attachment of a 3-bromopropyl group at the anomeric centre of **67** and **69** (Scheme 9). Multiple reaction conditions were attempted for this transformation and are summarised in Table 1.

All of the glycosylation reactions started with 0.5 equivalents of the indicated Lewis acid, and increased up to 2.0 equivalents within the denoted reaction time. Significant drawbacks of the glycosylation using NIS/TMSOTf (2.0 equiv.) were the slow conversion of **67** and low yield (17%) of **73**, even after stirring for 24 hours at room temperature (Entry 1). Coupled HSQC data obtained for **73** suggested a β-anomeric configuration, with a value similar to the one obtained for the native acceptor ($^{1}J_{C1\text{-}H1}$ = 152 Hz). The same glycosylation conditions were applied with increased amounts of the linker 3-bromopropanol (4.0 equiv.) for 24 hours at room temperature and then 24 h at 35 °C (Entry 2). Again, incomplete conversion was observed by TLC analysis. Changing the Lewis acid to AgOTf (0.5 equiv.) showed the formation of **73** by TLC analysis after 45 min. at 0 °C, alongside a significant amount of starting material **67**. The reaction was left to warm to room temperature and TLC analysis after 15 min. indicated the formation of an inseparable mixture (Entry 3). NMR data showed no evidence of **73**.

NIS and TfOH (1.0 equiv.) were evaluated next as an alternative promoter system, and gave a result similar to that seen with NIS/TMSOTf activation (Entry 4). NBS and TMSOTf (0.5 equiv.) were attempted next, where TLC analysis of the reaction indicated incomplete conversion after 45 min. at 0 °C. The reaction mixture was left stirring for an additional 45 min. at room temperature, which gave a complicated mixture by TLC whereby **73** was not indicated (Entry 5). Interestingly,

TLC analysis suggested the formation a new spot with lower R_f compared to that of **73** and NMR data unexpectedly confirmed the material as **75** (11% yield), a product where the *N*-Bn and *O*-Bn groups were hydrolysed and 3-bromopropanol was glycosylated at the anomeric position (Scheme 10). HMBC data indicated *C*1 (99.2 ppm) correlating through bond with the linker protons OC*H*$_2$CH$_2$CH$_2$Br (3.82 ppm and 3.55 ppm) and also confirmed that the remaining 2 Bn groups (benzylic protons: 4.83 ppm, 4.64 ppm and 4.61 ppm) were attached to *C*2 (74.7 ppm) and *C*3 (76.5 ppm), respectively. Coupled HSQC spectrum showed the formation of an α-linkage ($^1J_{C1\text{-}H1}$ = 172 Hz). Taken together, these observations suggested that glycosylation of 3-bromopropanol occurred prior to hydroxamate debenzylation.

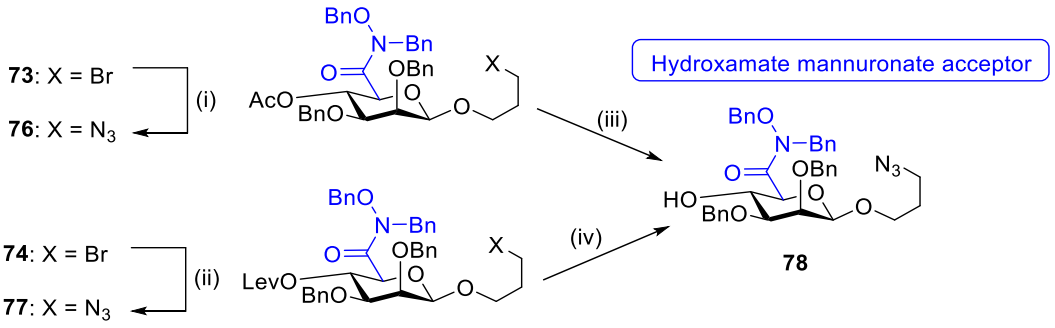


***Scheme 10**: Attempted synthesis of **73** from **67** with NBS/TMSOTf (Entry 5), gave **75** in 11% yield.*

Entries 1-5 in Table 1 are categorised as pre-mixing donor and acceptor methodologies, where the promoter system was added to a stirring mixture containing both the glycosyl donor and acceptor. Pre-activation conditions were also investigated for the synthesis of **73**, whereby an equimolar amount of TMSOTf was added for the activation of **67** with NIS, in the absence of 3-bromopropanol linker (Entry 6). A mixture of **67** and NIS in CH_2Cl_2 was cooled to -40 °C before TMSOTf (2.5 equiv.) was added. The reaction mixture was allowed to warm to -30 °C (15 min.)

whereupon 3-bromopropanol (4.0 equiv.) was added dropwise. The mixture was next allowed to warm to room temperature over 1 h with TLC analysis this time indicating the presence of **67**. The mixture was stirred for an additional 1 h which produced a complicated mixture not containing **73**. Finally, the original pre-activation conditions with Ph_2SO/Tf_2O and TTBP in CH_2Cl_2 that were used for the synthesis of **58**, were applied for a synthesis of **73**. These conditions were successful, delivering the desired product in 89% yield and with a β-configuration ($^1J_{C1\text{-}H1}$ =156 Hz). The same conditions were applied to donor **69**, which led to the formation of β-linked monosaccharide **74** in 78% yield.

Refluxing **73** with NaN_3 in acetone for 48 h gave **76** in 76% yield (Scheme 11). The conditions were optimised by warming a solution of **74**, NaN_3 and TBAI in DMF to give **77** (73%) in a shorter amount of time (24 h). C4-*OH* of **76** was released using NaOMe, giving **78** in 76% yield. C4-*OH* deprotection of **77** with $H_2N\text{-}NH_2$.AcOH in pyridine/AcOH (4/1, *v*/*v*) gave **78** in 92% yield.



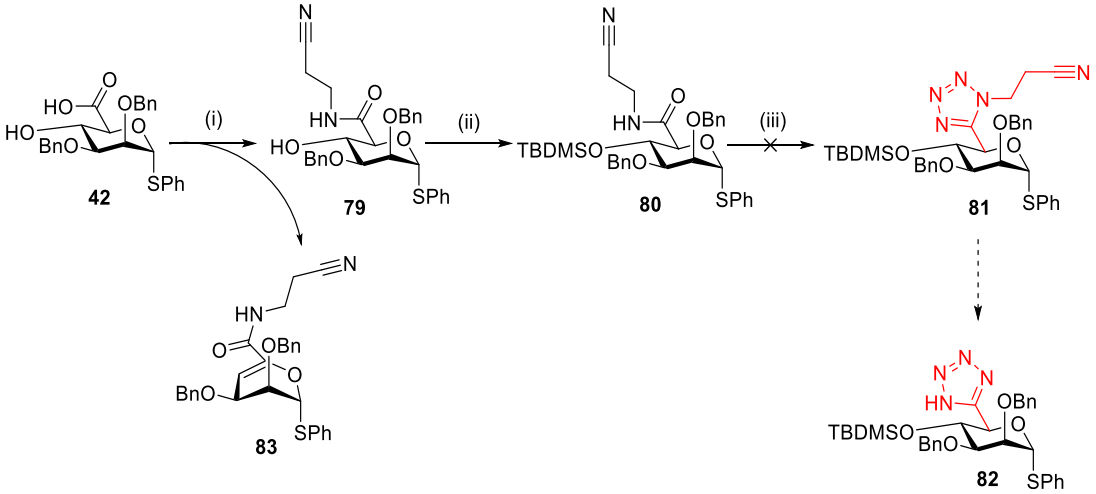
Scheme 11: *Final steps towards the synthesis of hydroxamate mannosyl acceptor* ***78*** (i) *NaN_3, acetone, 55 °C, 48 h,* ***76****: 76% (ii) NaN_3, TBAI, DMF, 65 °C,* ***77****: 73% (iii) $Na_{(s)}$, MeOH, RT, 24 h, 76% (iv) $H_2N\text{-}NH_2$.AcOH, pyridine/AcOH (4/1, v/v), RT, 30 min, 92%.*

The synthetic methodologies developed for the production of *C*6-hydroxamate building blocks **67**, **69** and **78** proved to be efficient, providing them in satisfactory yields for subsequent manipulation (i.e. anomeric phosphorylation (Section 7.1) and

oligosaccharide synthesis (Section 8)). Although multiple glycosylation conditions were attempted for instalment of the 3-bromopropanol linker on thioglycosides **67** and **69**, preactivation protocol using Ph_2SO/Tf_2O and TTBP in CH_2Cl_2 furnished **73** and **74** in 89% and 78% yield respectively. Successful synthesis of acceptor **78** integrated the toolset of hydroxamate building blocks required for oligosaccharide assembly (Section 8).

6.3. 6-*C*-tetrazole precursors synthesis

A number of synthetic methodologies have been developed for the preparation of tetrazole rings. The main synthetic routes towards their synthesis are based on cyclisation reactions using NaN_3.[216] Herein, tetrazole ring synthesis at *C*6 of mannuronic acid derivatives is attempted in two ways; firstly from a *C*6-cyanoalkylamide, followed by a dipolar cycloaddition with NaN_3 and elimination of acrylonitrile, and secondly from a *C*6-nitrile (Schemes 12 and 13).



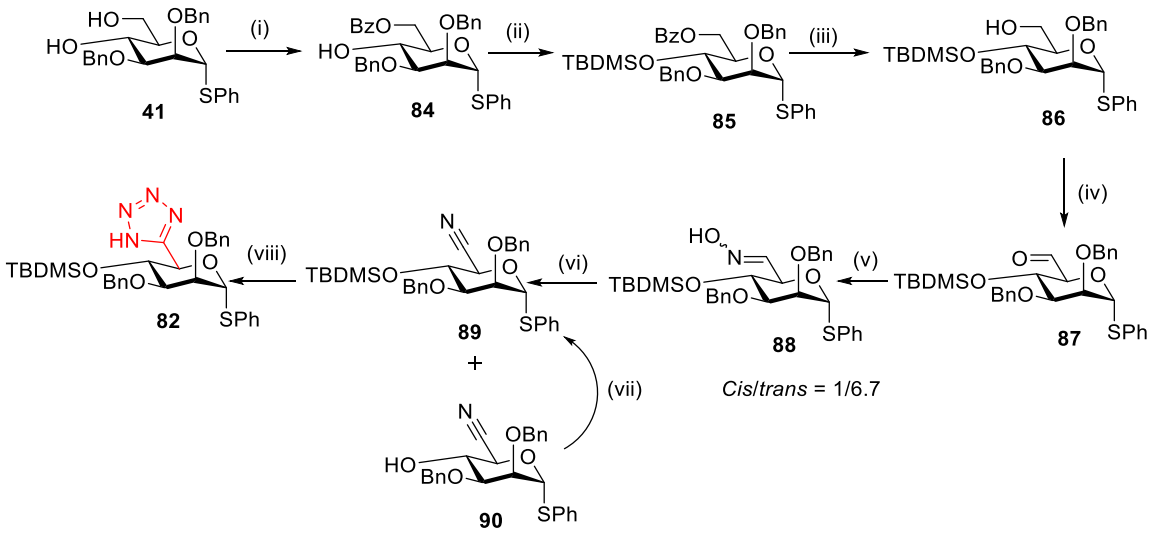
Scheme 12: *Synthetic route from* ***42*** *towards C6-tetrazole derivative* ***82*** *(i) PyBOP, DIPEA, CH_2Cl_2, 3-aminopropionitrile, 0 °C, 40 min,* ***79****: 47%,* ***83****: 44% (ii)TBDMSOTf, imidazole, DMAP, DMF, RT, 24 h, 80% (iii) PPh_3, DIAD, $TMSN_3$, MeCN, 80 °C, 48 h (iv) PPh_3, DIAD, $TMSN_3$, MeCN, 80 °C, 48 h.*

A first route followed towards *C*6-tetrazole derivative **82** involved coupling of 3-aminopropionitrile to **42**, TBDMS-protection of the free C4-*OH*, and Mitsunobu-type reaction conditions, along with $TMSN_3$ to effect tetrazole formation (Scheme 12). The conditions evaluated for the first step included stirring **42** with PyBOP, DIPEA and EDC.HCl for 5 min. in CH_2Cl_2, before 3-aminopropionitrile was added.[182] The reaction was left stirring for 1.5 h at room temperature, resulting in the formation of *C*4-*C*5 elimination compound **83** (35%) as the major product and desired **79** (7%) as the minor. In an attempt to optimise conditions for the formation of **79**, 3-aminopropionitrile was added to a stirred mixture of **79**, PyBOP and DIPEA in CH_2Cl_2 at 0 °C, maintaining this temperature for 40 min. The yield was improved, affording **79** in 47% and **83** in 44% yield, respectively. Silyl protection of C4-*OH* in **79** was successful by applying the same conditions used to convert **54** to **57** (TBDMSOTf, imidazole and DMAP), furnishing **80** in 80% yield. Attempts to convert **80** to a propanenitrile-protected tetrazole by applying Mitsunobu protocol with PPh_3, DIAD and $TMSN_3$ was unsuccessful.[182] TLC analysis and NMR data obtained indicated no conversion of the starting material **80**, even after stirring at 80 °C in MeCN for 41 hours. NMR data obtained also corroborated this.

A second route commenced with selective benzoylation of the primary alcohol in **41**, giving **84** in 90% yield (Scheme 13). The next step was orthogonally protecting C4-*OH* of **84** with a TBDMS group, giving **85** in 78% yield. Treatment of **85** with NaOMe yielded **86** in 90%, which was subsequently subjected to oxidation conditions with DMSO, SO_3.pyridine and Et_3N to deliver aldehyde **87** in 98% crude yield. From aldehyde **87**, oxime **88** was synthesised effectively as a mixture of its *trans* and *cis* (6.7/1) isomers in 70% yield. Subsequent dehydration of **88** using $POCl_3$ gave **89** (22% yield) and **90** (26% yield). The acidic reaction mixture led to the

TBDMS-deprotection of *C*4-*OH* of **89**, which was retrieved by stirring **90** with TBDMSOTf, imidazole and DMAP in DMF (87% yield).

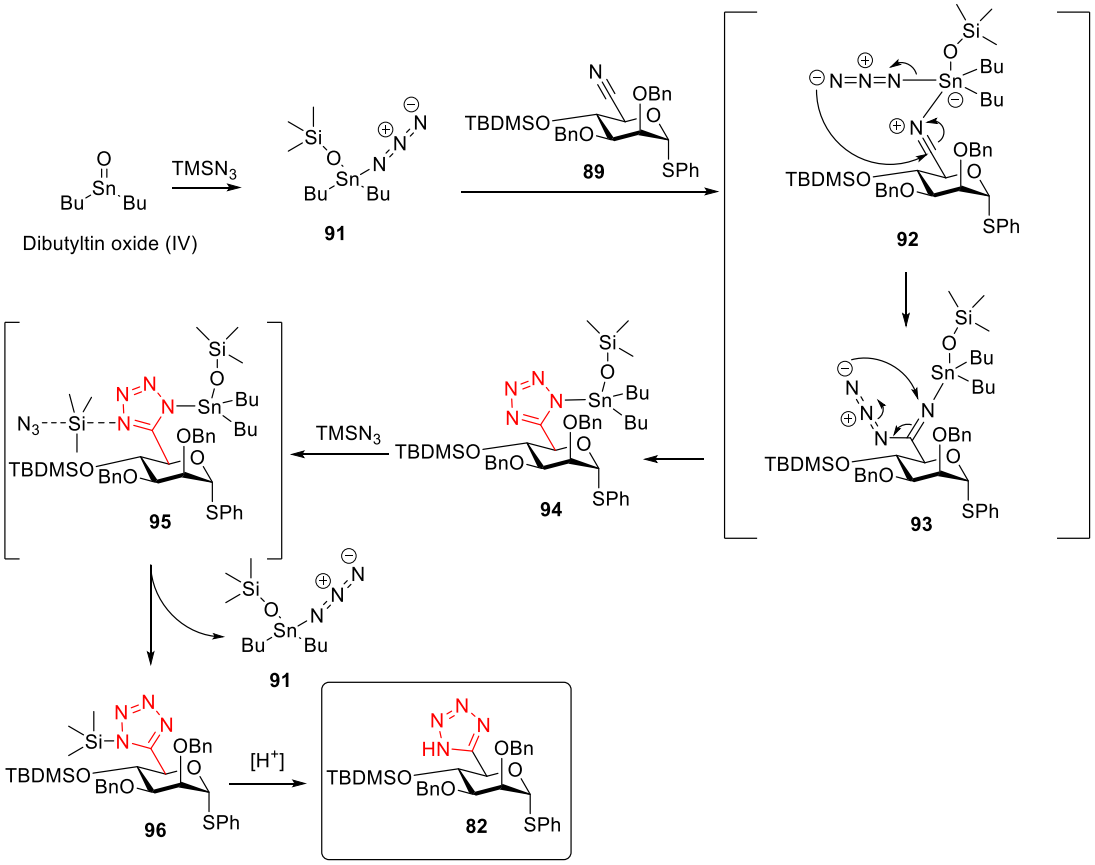
*C*6-nitrile **89** was successfully converted into *C*6-tetrazole **82** in 51% yield using $TMSN_3$ and catalytic amounts of Bu_2SnO.^{13}C NMR data collected from the isolated material showed the presence of a new quaternary carbon (C_q tetrazole) peak at 155.8 ppm and disappearance of the nitrile carbon (*C*≡N) peak at 117.0 ppm, confirming the conversion of *C*6-nitrile to *C*6-tetrazole. HRMS data supported the synthesis of **82**, with the major ion observed at *m*/*z* 605.2628 ($C_{32}H_{41}N_4O_4SSi$ requires $(M+H)^+$,605.2618).



Scheme 13: *Synthetic route from* ***41*** *towards C6-tetrazole derivative* ***82*** *(i) BzCl, DMAP, pyridine, CH_2Cl_2, RT, 24 h, 90% (ii) TBDMSOTf, imidazole, DMAP, DMF, RT, 24 h, 78% (iii) $Na_{(s)}$, MeOH, RT, 16 h, 90% (iv) DMSO, SO_3.pyridine, Et_3N, RT, 1h, 98% (v) $H_2NOH.HCl$, Na_2CO_3, THF, 0 °C to RT, 24 h, 70%, cis/trans = 9%/61% (vi) $POCl_3$, MeCN, RT to 65 °C, 3 h,* ***89****: 22%,* ***90****: 26% (vii) TBDMSOTf, imidazole, DMAP, DMF, RT, 24 h, 87% (viii) $TMSN_3$, Bu_2SnO, toluene, reflux at 120 °C, 16 h, 51%.*

Furthermore, ^1H NMR analysis indicated a doublet peak at 5.64 ppm for H_5 which was further downfield compared to the chemical shifts of H_5 proton peaks (4.54 – 4.56 ppm) observed previously for the mannuronate and hydroxamate series. The

coupling constant calculated for H_5 was $J_{H5\text{-}H4}$ = 8.9 Hz, designating a $^4C_1$ pyranose conformation for **82**.



***Scheme 14**: Proposed mechanism for the synthesis of **82** between nitrile **89** and $TMSN_3$ in the presence of Bu_2SnO.*

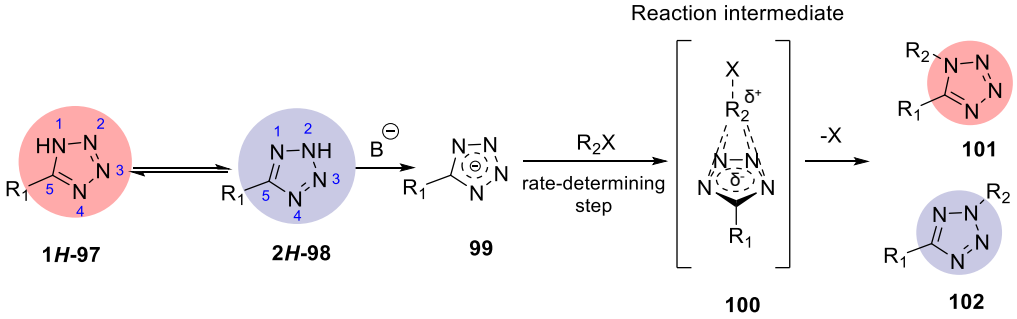
The reaction proceeded *via* intermediates **92** and **93** which occurred from the bond formation between the nitrile nitrogen in **89** and the generated dibutyl(trimethylsilyloxy)tin azide complex **91** (Scheme 14). The generated catalyst **91** activates the nitrile carbon for the attack of the azide group in **92** giving the open-chain intermediate **93**, which subsequently cyclises to the 1-[dibutyl(trimethylsilyloxy)stannyl]-5-substituted tetrazole **94**. The catalytic complex **91** is then regenerated through a S_N2 reaction between **94** and $TMSN_3$ *via* transition state **95**, where the produced *N*-(trimethylsilyl) tetrazole product **96** is hydrolysed into

82 during the acidic workup.[217] The proposed mechanism by Cantillo *et al*. contradicts the one first suggested by Wittenberger *et al*., as the latter recommended the regeneration of Bu_2SnO instead of **91**.[218,219]

In order to reduce reaction times and improve the yields of the three latter synthetic steps towards **82**, a different protocol was performed on *C*6-aldehyde **87**. A one-pot three component strategy of 5-substituted 1*H*-tetrazole has recently been reported, claiming that various aromatic aldehydes in a reaction mixture with $H_2NOH.HCl$, NaN_3 and a catalytic amount of $[(NH_4)_4Ce(SO_4)_4.2H_2O]$ can be converted to 1*H*-tetrazole derivatives in shorter reaction times and in good to moderate yields.[220] Initial attempts showed a considerably slow reaction time (45 °C to 125 °C over 50 h), as TLC analysis indicated *C*6-nitrile formation after 20 h and was completed in 36 h. The reaction was left stirring for additional 14 h at 125 °C; however, the desired *C*6-tetrazole analogue **82** was not isolated due to decomposition of the reaction. A second attempt starting at a higher temperature (100 °C) exhibited again a slow reaction time, giving *C*6-oxime **88** and *C*6-nitrile **89** in 35% and 14% yields respectively over 40 h. The long reaction times as well as the low yields obtained from this set of conditions proved this strategy to be ineffective; therefore the original route depicted in Scheme 13 was followed for the successful synthesis of **82**.

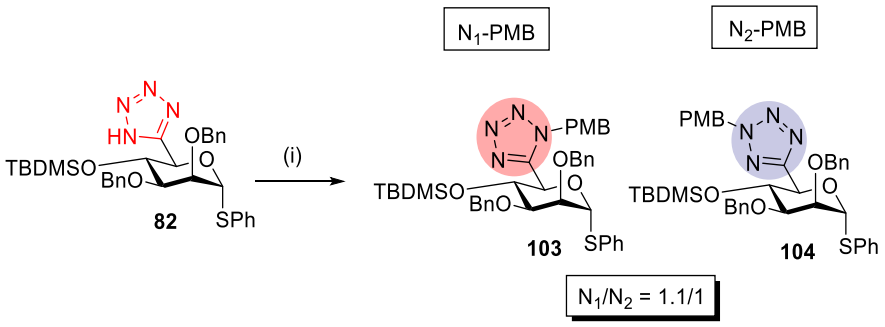
The next step towards the synthesis of a fully protected *C*6-tetrazole glycosyl donor was the nitrogen protection of the tetrazole group. *C*6-tetrazole scaffold **82**, as a 5-ST, exists as two tautomers: 1*H*- (**97**) and 2*H*- (**98**) respectively (Scheme 15). The acidity of such systems relies on their ability to delocalise negative charge after deprotonation, such as in system **99**, making their subsequent substitution poorly

regioselective. As a result, alkylation, arylation and acylation of a tetrazolate anion, leads to a mixture of 1- and 2-substituted tetrazole isomers in various ratios.[217] Ostrovskii *et al.* suggested that the first rate-limiting step of a substitution reaction involves a bimolecular process that aids in the formation of an unstable intermediate **100**, followed by a second step where the isomers **101** and **102** are formed. The properties of the reaction intermediate **100** are influenced by the stereochemical properties of the *C*5-substituent, reactivity of the electrophile used, and the reaction medium.[221]



Scheme 15: *1H- and 2H- tautomers of 5-STs and the bimolecular mechanism that tetrazolate anion undergoes during electrophilic substitution, giving the two isomers [Adapted from Roh et al. (2012)].*[217]

A first attempt to protect the tetrazole group involved the reaction of **82** with PMBCl in DMF, having K_2CO_3 as a base and KI for nucleophilic catalysis. Two isomers **103** and **104** were produced in a ratio of N_1/N_2 = 1.1/1 (Scheme 16).



Scheme 16*: The two isomers **103** and **104**, obtained by paramethoxybenzylation of C6-tetrazole **82** (i) PMBCl, K_2CO_3, DMF, RT, 4 h, 53%.*

HMBC spectra of **103** and **104** were obtained to clarify the position of the PMB group on the tetrazole ring in each case. The HMBC spectrum of **103** showed correlation of tetrazole C_q (152.0 ppm) with the benzylic protons of the PMB group (5.66 ppm, d, ^2J = 15.0 Hz and 5.63 ppm, d, ^2J = 15.0 Hz), H_4 (4.59 ppm, app. t, $^3J_{H4\text{-}H3,\ H4\text{-}H5}$ = 9.4 Hz) and H_5 (5.68 ppm, d, $^3J_{H5\text{-}H4}$ = 9.4 Hz) (Figure 39). The HMBC spectrum of **104** indicated no correlation between tetrazole C_q (164.4 ppm) and the benzylic protons of the PMB group, verifying **104** as the N_2-isomer and **103** the N_1-isomer.

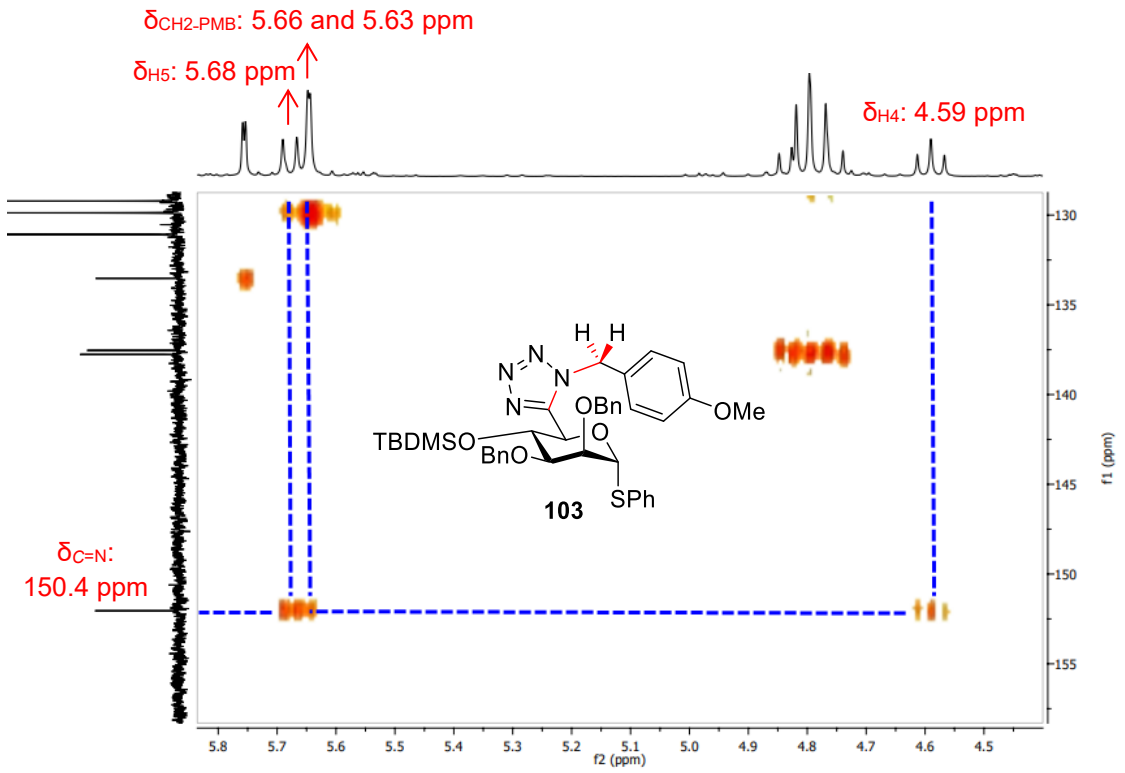
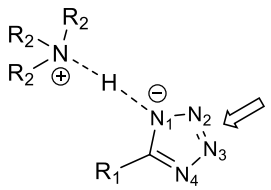


***Figure 39**: HMBC spectrum of **103** confirming its structure as the N_1-isomer.*

It has been suggested that the use of 5-STs as ammonium salts in substitution reactions may lead to a more regioselective outcome.[217] In particular, in aprotic solvents these salts exist as hydrogen-bonded complexes possessing lower aromaticities compared to their tetrazolate anion counterparts such as **99** (Scheme

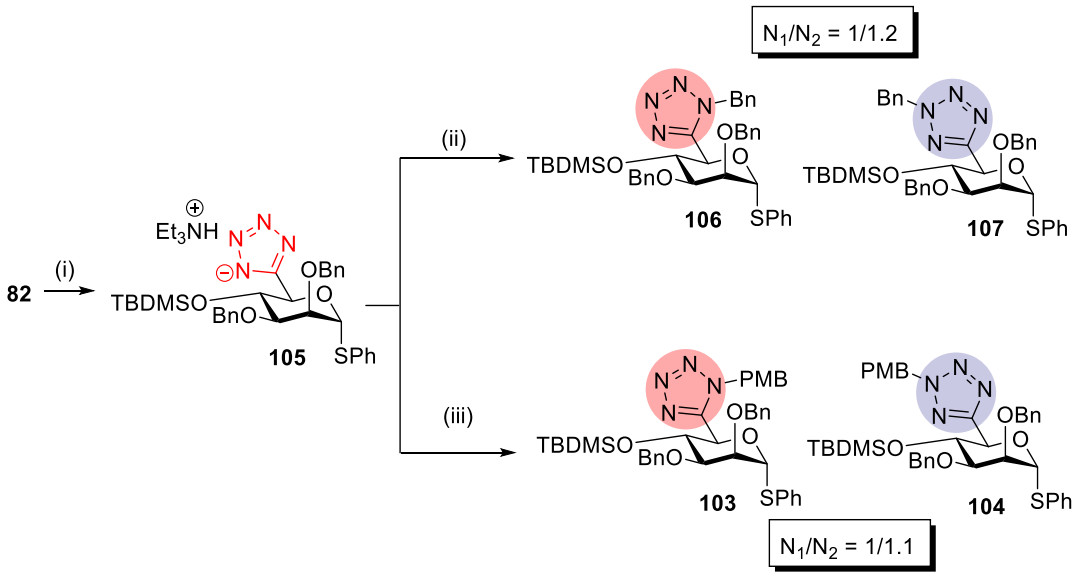
17).[222] It was proposed that the hydrogen-bonded N_1 would direct the electrophilic substitution to the double bond between N_2 and N_3 of the tetrazole ring (not to the plane of the tetrazole as in the case of the tetrazolate anion **99**), giving 2,5-disubstituted tetrazoles. Steric hindrance at the N_1 was an additional argument supporting this hypothesis.[223] Evidence suggest that both triethylammonium and sodium salts of 5-STs gave only 2,5-disubstituted tetrazoles, which proved that the steric aspect is the major driving force of regioselectivity.[224]



***Scheme 17**: Suggested electrophilic attack to an ammonium salt of a 5-ST [Adapted from Roh et al. (2012)].*[217]

In order to try and improve the N_1/N_2 ratio an approach detailed by Ostrovskii *et al.* was adopted here by firstly converting **82** to its respective triethylammonium salt **105**. The reason behind this strategy was to increase or decrease the N_1/N_2 ratio, as this would lead to better separation and consequently higher regioisomeric purity of **103** and **104**. The plan here was to use the N_1-isomer for the sugar 1-phosphate route and the N_2-isomer as a donor for the oligosaccharide synthesis. Subjection of a N_1/N_2 mixture of donors **103** and **104** (or **106** and **107**) to glycosylation conditions for disaccharide synthesis would be problematic considering that each regioisomer would give an anomeric mixture of disaccharides (or/and hydrolysed materials), generating a complex mixture of products. However, reaction of **105** with BnBr in DMF gave the two isomers **106** and **107** in comparable amounts (N_1/N_2 = 1/1.2) in low a low yield (31%) (Scheme 18). TLC analysis showed consumption of **105** and

formation of a complicated mixture that was not separated. Higher yields (98%) were obtained by subjecting **105** to reaction with PMBCl in DMF, producing regioisomers **103** and **104** in similar amounts (N_1/N_2 = 1/1.1). Column chromatography separation of **103** and **104** was challenging, giving the pure regioisomers in low quantities.

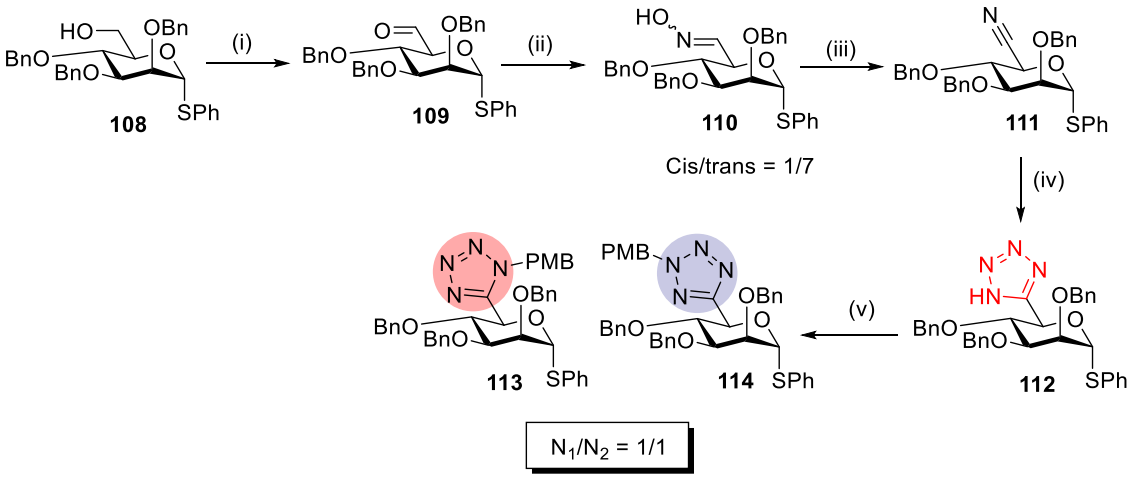


***Scheme 18**: Attempted alkylation of the tetrazole triethylammonium salt **82** (i) Et_3N, CH_2Cl_2, RT, 1 h, 94% (ii) BnBr, DMF, RT, 1 h, 31% (iii) PMBCl, DMF, RT, 24 h, 98%.*

The strategies discussed above (starting from **82** or its triethylammonium salt **105**) led to similar N_1/N_2 ratios (with both Bn and PMB groups), therefore a new synthetic route was revised for the production of *C*6-tetrazole thioglycosides that were exploited as scaffolds for the synthesis of *C*6-tetrazole 1-phosphate building block **35** (Section 7.2.) and 3-aminopropyl *C*6-tetrazole conjugable monosaccharide **174** (Section 9.3.).

The synthetic protocol followed towards **103** and **104** was repeated starting from precursor **108** that was not chemoselectively protected with a TBDMS group, as a means to improve overall yield (Scheme 19). In addition, global deprotection to give the final *C*6-tetrazole 1-phosphate **35** and 3-aminopropyl monosaccharide **174**

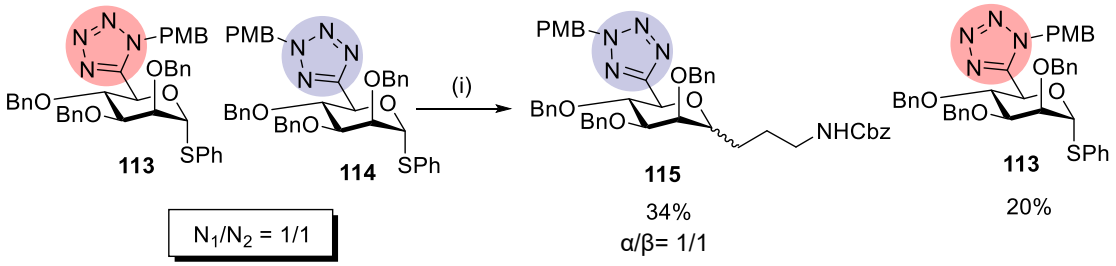
made an orthogonal protection unnecessary. Repeat of the Parikh-Doering oxidation afforded aldehyde **109** in 96% crude yield which was converted to oxime **110** as a mixture of *cis* and *trans* (1/7) isomers in 89% yield. Subsequent dehydration of **110** using $POCl_3$ gave **111** in 59%, which was then converted into *C*6-tetrazole **112** in 55% by using $TMSN_3$ and catalytic Bu_2SnO. Tetrazole **112** was finally stirred with PMBCl, K_2CO_3 and KI in DMF, to give the two isomers **113** and **114** in a ratio of N_1/N_2 = 1/1. Their structure was confirmed by HMBC data analysis, as previously illustrated.



Scheme 19: *(i) DMSO, SO_3.pyridine, Et_3N, RT, 1h, 96% (ii) $H_2NOH.HCl$, Na_2CO_3, THF, 0 °C to RT, 24 h, 89%, cis/trans = 11%/78% (ii) $POCl_3$, MeCN, RT to 65 °C, 3 h, 59% (iv) $TMSN_3$, Bu_2SnO, toluene, RT to 120 °C, 16 h, 55% (v) PMBCl, K_2CO_3, DMF, RT, 16 h, 76%.*

The steps required for completing the route depicted in Scheme 19 were significantly decreased compared to the one in Scheme 13 (9 steps reduced to 5), due to the protecting group manipulation that was required for the latter. Moreover, the overall yield was increased from 9% to 21%, making it a more facile and advantageous route to follow for the synthesis of *C*6-tetrazole thioglycosides precursors.

Thioglycosides **113** and **114** were additionally used as scaffolds for the instalment of an anomeric linker, allowing the synthesis of conjugable *C*6-tetrazole mannuronate **174** which is discussed in further detail in Section 9.3. A 1/1 mixture of **113** and **114** was subjected to glycosylation conditions with NIS/AgOTf and 3-(benzyloxycarbonylamino)-1-propanol for 3 h, giving N_2-PMB tetrazole **115** as an anomeric mixture α/β = 1/1 (Scheme 20). Hydrolysed product (18% recovery) overlapped with **115** on the TLC plate therefore subsequent acetylation of the crude material facilitated purification, furnishing **115** in 34% yield. N_1-PMB tetrazole thioglycoside **113** (20% recovery) demonstrated reduced reactivity during these conditions, contributing further to the low yield of the reaction.

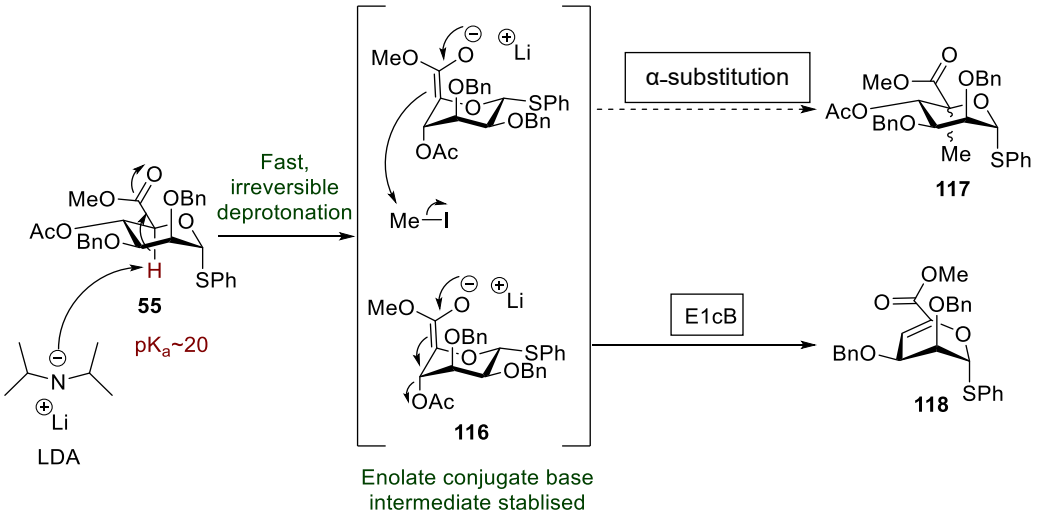


***Scheme 20**: Synthesis of 3-(benzyloxycarbonylamino) propyl (2,3,4-tri-O-benzyl-6-C-(2-para -methoxybenzyl-tetrazol-5-yl)-α/β-D-mannopyranoside **115** (i) NIS, AgOTf, CH_2Cl_2, -30 °C to -10 °C, 3 h, 34%.*

The strategy designed for the synthesis of *C*6-tetrazole thioglycosides was more challenging compared to *C*6-hydroxamate thioglycosides, as production of the 2 regioisomers (**103**, **104**) in equal amounts was unavoidable. Nevertheless, the route for the production of **113** and **114** was more viable, providing sufficient amount of material. The toolset of modified thioglycosides was ready to be utilised in the following sections.

6.4. 5-*C*-methyl-α/β-D-mannopyranoside peracetate

The first route designed for the synthesis of a *C*5-methylated building block was based on the observations of *C*4-*C*5 elimination of mannuronates **55** and **59** under basic conditions (Zemplén deacetylation conditions with pH ≥ 11). In particular, **55** was used as a scaffold in an experimental procedure where LDA was added as base for the generation of enolate **116** *via* an E1cB pathway, and MeI was employed as a suitable electrophile for subsequent *C*5-methylation (Scheme 21).



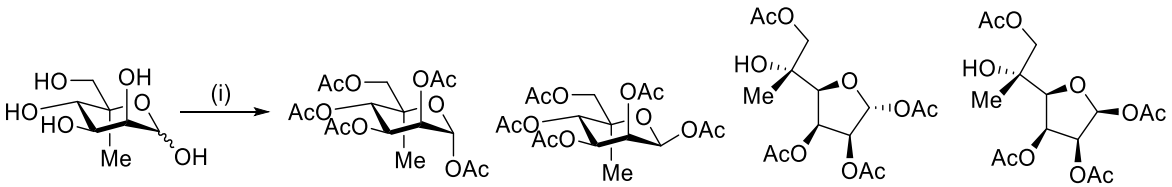
Scheme 21*: Attempted C5-alkylation of* ***55*** *using LDA and MeI for a proof of concept synthesis of C5 methylated analogue* ***117****. The reaction proceeded via an E1cB elimination pathway, giving* ***118*** *in 61% and 67% yield.*

More specifically, the intermediate enolate ion **116** was expected to react through carbon with MeI, as the reaction is dominated by orbital interactions when alkyl halides are used as electrophiles. The designed protocol started with a stirred solution of **55** in THF (0.2 M) being cooled to -70 °C, whereby a 1 M solution of LDA (1.2 equiv.) in THF/hexane was added dropwise. The reaction mixture was kept between -80 °C and -70 °C for 15 min. before MeI (1.5 equiv.) was added dropwise, and warmed to room temperature over 40 min. Unfortunately, desired analogue **117**

was not isolated, and only elimination product **118** was identified (61% yield), suggesting that elimination occurred in preference to α-substitution. A second attempt using an inverse addition, i.e. the addition of LDA (1.2 equiv.) to a stirring solution of **55** and MeI (1.5 equiv.) in THF (0.2 M) at -80 °C to -70 °C gave similar results, as **118** was isolated in 67% yield.

An entirely different approach was thus taken, based on previous work of Davis *et al*.[225] By following their developed methodology, 5-*C*-Me-D-mannose **119** was produced by a colleague in the group and its synthesis is therefore not described here. NMR data of **119** indicated an α/β = 1/2.8 ratio with coupling constants $^{3}J_{H1\text{-}H2}$ = 2.4 Hz for $H_{1\alpha}$ and $^{3}J_{H1\text{-}H2}$ = 1.3 Hz for $H_{1\beta}$. Further information was obtained by coupled HSQC data analysis, where the calculated coupling constants for the α-anomer (*C*1: 94.7 ppm) and the β-anomer (*C*1: 89.3 ppm) were $^{1}J_{C1\text{-}H1}$ = 172 Hz and $^{1}J_{C1\text{-}H1}$ = 164 Hz, respectively. The fact that the β-anomer of **119** dominates at equilibrium in aqueous solution emphasises the steric effect caused by the Me group at the *C*5 position, making a heavily solvated axial hydroxyl group (α-anomer) less favourable.

With **119** in hand, peracetylation was performed with Ac_2O and catalytic H_2SO_4. The reaction gave the expected mixture of products (**120**: 40%, **121**: 43%, **122**: 5%, and **123**: 12%) in 62% overall yield (Scheme 22). Column chromatography provided a mixture of peracetylated products (**120**: 43%, **121**: 45%, **123**: 10%). Coupled HSQC data of the crude material were obtained to verify the reported anomeric ratio, showing **120** (*C*1: 91.1 ppm) with a coupling constant $^{1}J_{C1\text{-}H1}$ = 176 Hz and **121** (*C*1: 86.8 ppm) with a coupling constant $^{1}J_{C1\text{-}H1}$ = 164 Hz.



119 **120** 40% **121** 43% **122** 5% **123** 12%

***Scheme 22**: Peracetylation of 5-C-methyl mannose **119** (i) Ac_2O, H_2SO_4, RT, 45 min, 62%.*

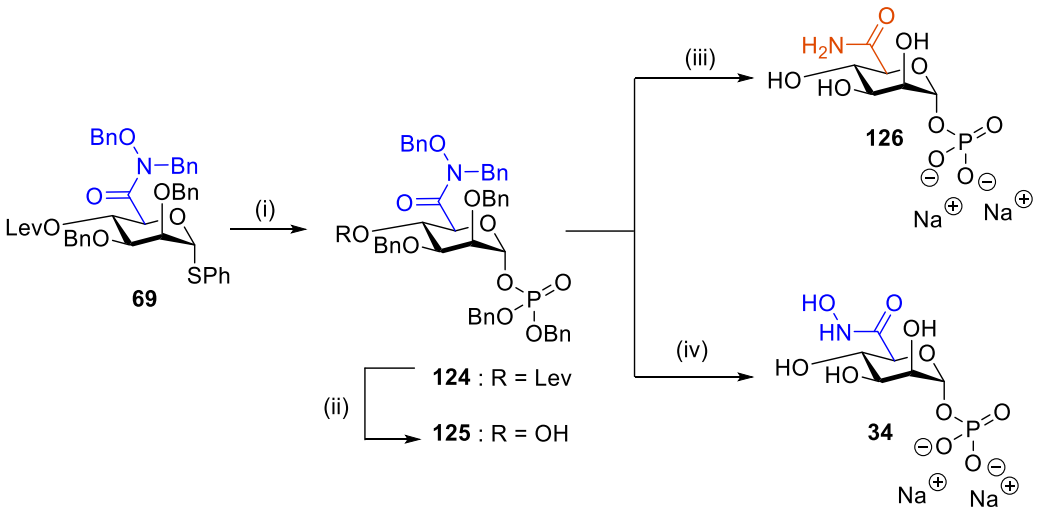
In order to support the above assignment, coupled HSQC data of peracetylated D-mannopyranoside **36** were collected, exhibiting coupling constants $^1J_{C1\text{-}H1}$ = 180 Hz (6.09 ppm) for the α-anomer, and $^1J_{C1\text{-}H1}$ = 164 Hz (5.88 ppm) for the β-anomer, similar to those calculated for **120** and **121**. Considering that Ac_2O/ cat. H_2SO_4 procedure furnished **36** in an α/β = 4/1 and **120**/**121** in an α/β = 1/1.1 ratio, it is clear that the *C*5-Me group in **120** and **121** influences their equilibration under acidic catalysis. More specifically, the acid-catalysed equilibration that occurs under these conditions provided predominantly the α-product of **36**, as dictated by the anomeric effect. However, in the case of 5-*C*-Me pentaacetates, the equilibration gave a ratio with a slight preference for β-product **121** which again highlights the steric effect of the *C*5-Me group on the final anomeric configuration. 5-*C*-Me peracetylated products **120**, **121** and **123** were key intermediates in the synthesis of their respective sugar 1-phosphates, which is further discussed in Section 7.3.

7. Synthesis of *C*6-modified mannuronate 1-phosphate building blocks

7.1. Synthesis of 6-*C*-hydroxamic acid (α-D-mannopyrannoside) 1-phosphate 34

The route designed for the synthesis of *C*6-hydroxamic acid 1-phosphate **34** started by introducing DBP stereoselectively at the anomeric centre of **69** using NIS/AgOTf as a promoter system, giving **124** in 55% yield (Scheme 23).

The expected α-stereochemistry of **124** was confirmed, as coupled HSQC data showed a coupling constant of $^{1}J_{C1\text{-}H1}$ = 180 Hz (C1: 96.0 ppm). Subsequent C4-*OH* deprotection of **124** with $H_2N\text{-}NH_2$.AcOH delivered **125** (72% yield), which was then subjected to hydrogenolysis conditions for global deprotection.



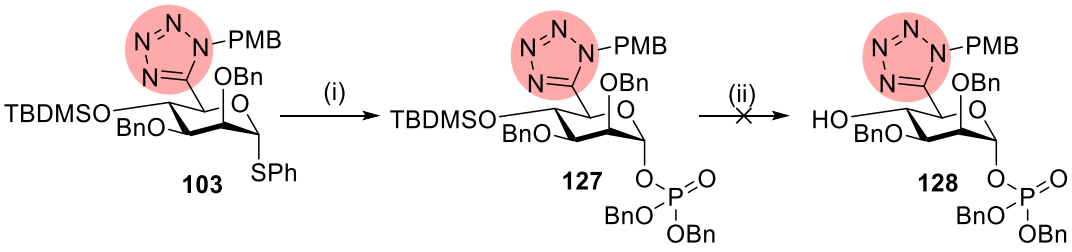
Scheme 23*: Route for the synthesis of C6-hydroxamic acid 1-phosphate* ***34*** *from* ***69****. (i) NIS, AgOTf, DBP, CH_2Cl_2, -30 °C to -10 °C, 2.5 h, 55% (ii) $H_2N\text{-}NH_2$.AcOH, pyridine/AcOH (4/1, v/v), RT, 1 h, 72% (iii) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2$/C (20%), 5% aq. $NaHCO_3$, EtOH/THF (1.5/1, v/v), RT, 32 h, 89% (iv) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2$/C (20%), 5% aq. $NaHCO_3$, EtOH/THF (1.5/1, v/v), RT, 5 h, 67%.*

A first attempt to cleave all Bn groups in **125** under hydrogenolysis conditions with 1.2 equiv. (0.2 equiv. per Bn group) of Pd/C and 1.2 equiv. $Pd(OH)_2$/C in EtOH/THF (1.5/1, *v*/*v*, 0.05 M) and 2.0 equiv. of 5% aq. $NaHCO_3$ gave *C*6-amide 1-phosphate **126** after 32 h. ESI-MS analysis confirmed the structure of **126**, as the major ion was found at *m*/*z* 272.0178 ($C_6H_{11}NO_9P$, **126** requires $(M\text{-}H)^-$ 272.0177). ^{13}C and ^{31}P NMR spectra collected for **126** showed the carbonyl carbon peak of the amide moiety at 174.7 ppm and a phosphorous peak at 1.89 ppm, respectively. To avoid this unwanted reductive cleavage of the OBn group from the hydroxamate, a second attempt was performed using 0.3 equiv. (0.05 equiv. per Bn group) of Pd/C and 0.3 equiv. of $Pd(OH)_2$/C. The reaction was monitored by TLC analysis, and suggested

completion after 5 h. NMR data of the product were different compared to that of **126**. A weak carbonyl carbon peak of the hydroxamic acid appeared at 167.5 ppm while the phosphorous peak indicated a chemical shift of 1.91 ppm. The difference between the ^{31}P NMR spectra of **34** and **126** was not strikingly evident, exhibiting small chemical shift difference. HRMS data gratifyingly confirmed the mass of **34**, with the major ion observed at *m/z* 288.0127 ($C_6H_{11}NO_{10}P$, **34** requires $(M\text{-}H)^-$ 288.0126).

7.2. Synthesis of 6-*C*-tetrazole (α-D-mannopyrannoside) 1-phosphate 35

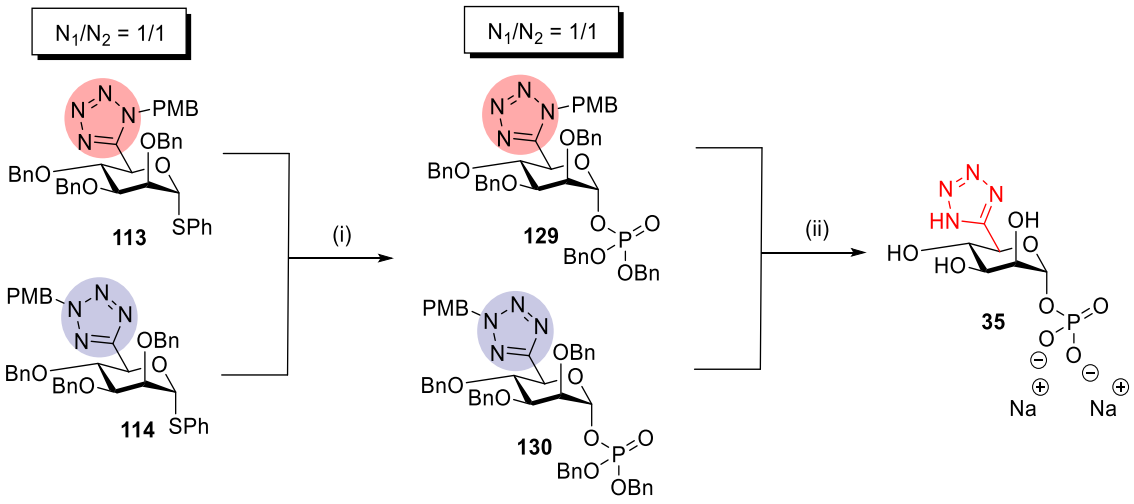
*C*6-tetrazole thioglycoside **103** was used to attempt an anomeric phosphorylation with DBP and NIS/AgOTf conditions, giving **127** in a low yield (16%). The use of AcCl in MeOH for cleavage of the *C*4-OTBDMS led to decomposition of the reaction, thus this method was abandoned (Scheme 24).



Scheme 24: *Towards the synthesis of protected C6-tetrazole 1-phosphate **128** (i) NIS, AgOTf, DBP, CH_2Cl_2, -30 °C to -10 °C, 3.5 h, 16% (ii) AcCl, MeOH, 0 °C to RT, 16 h.*

The next attempt for installing the anomeric phosphate employed both **113** and **114** as starting materials, as cleavage of the PMB group at the final step would result in a single *C*6-tetrazole 1-phosphate compound. Accordingly, a NIS/AgOTf protocol was applied for the glycosylation of DBP, furnishing **129** and **130** in 72% yield (Scheme 25). The N_1/N_2 ratio of thioglycosides **113** and **114** was 1/1, as well as the ratio of 1-phosphates **129** and **130** obtained, indicating that **113** and **114**

exhibited the same reactivity for the anomeric phosphorylation. These results were contradictory to those discussed in Section 6.3., where glycosylation of 3-(benzyloxycarbonylamino)-1-propanol under the same conditions gave only N_2-PMB product **115**. Hydrogenolysis conditions of **129** and **130** with 0.6 equiv. of Pd/C (0.1 equiv. per Bn group) and 0.6 equiv. of $Pd(OH)_2/C$ gave desired compound **35** in 72% yield after 24 h. ^{13}C NMR of **35** showed a weak peak at 160.8 ppm for C_q tetrazole while the phosphorous peak appeared at -2.15 ppm on ^{31}P NMR spectrum. HRMS data confirmed the mass of **35**, as the major ion was identified at *m*/*z* 297.0236 ($C_6H_{10}N_4O_8P$, **35** requires $(M-H)^-$, 297.0233).

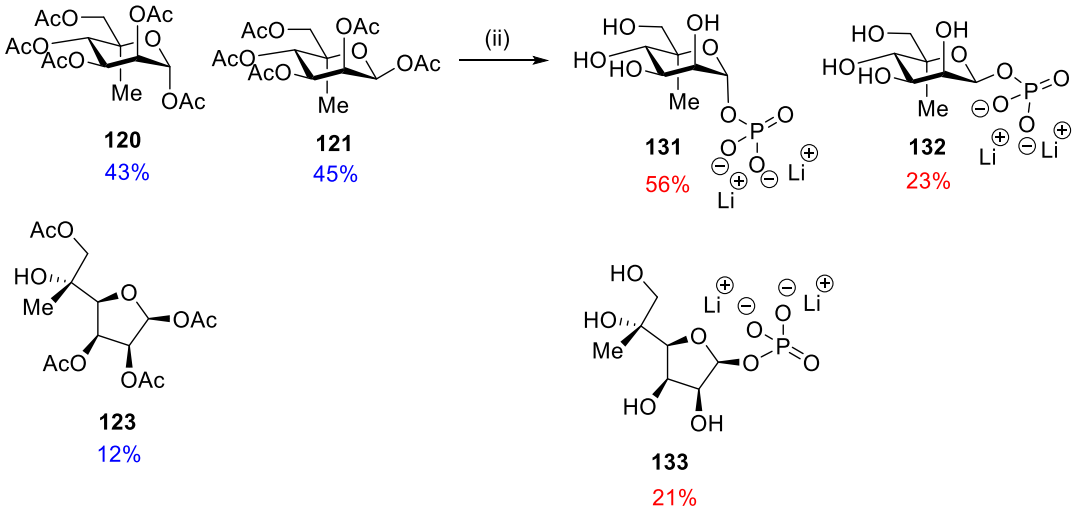


***Scheme 25**: Designed route towards the synthesis of C6-tetrazole 1-phosphate **35** starting from **113** and **114** (i) NIS, AgOTf, DBP, CH_2Cl_2, -30 °C to -10 °C, 3.5 h, 72% (ii) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2/C$ (20%), 5% aq. $NaHCO_3$, EtOH/THF (1.5/1, v/v), RT, 24 h, 72%.*

As part of a program in our group that focuses on the chemoenzymaric synthesis of alginate sugar nucleotide building blocks, *C*6-bioisosteric 1-phosphates **34**, **126** and **35** were used as substrates of GMP to deliver a toolbox of *C*6-modified GDP-ManAs. An additional objective of this program is the design and development of GMD inhibitors and/or structure-function tools, therefore the enzymatically synthesised *C*6-modified sugar nucleotide analogues could be tested.

7.3. Synthesis of 5-*C*-methyl-α/β-D-mannopyranose 1-phosphate 120

A mixture of peracetylated products (**120**: 43%, **121**: 45%, **123**: 10%) was subjected to MacDonald reaction conditions[226]furnishing their respective sugar 1-phosphates **131** (56%), **132** (23%) and **133** (21%) in 19% overall yield (Scheme 26).



***Scheme 26**: Synthesis of 1-phosphates **131**, **132** and **133** (ii) $H_3PO_{4(s)}$, 60 °C, 2 h and then THF, 1 M $LiOH_{(aq.)}$, 0 °C to RT, 72 h, 19%.*

^{1}H NMR data showed chemical shifts at 5.27 ppm (dd, $^3J_{H1\text{-}31P,\ H1\text{-}H2}$ = 9.1, 2.2 Hz) for $H_{1\alpha}$ and 5.19 ppm (dd, $^3J_{H1\text{-}31P,\ H1\text{-}H2}$ = 8.6, 1.1 Hz) for $H_{1\beta}$ with a 2/1 preference for formation of the α-1-phosphate (Figure 40). Coupled HSQC data verified the assignments, as the α-anomer **131** (*C*1: 95.8 ppm) exhibited a coupling constant of $^1J_{C1\text{-}H1}$ = 172 Hz, while the β-anomer **132** (*C*1: 89.3 ppm) showed $^1J_{C1\text{-}H1}$ = 168 Hz. In addition, ^{31}P NMR spectrum showed 2 distinct peaks at 1.85 ppm (α-anomer **131**) and 2.27 ppm (β-anomer **132**).

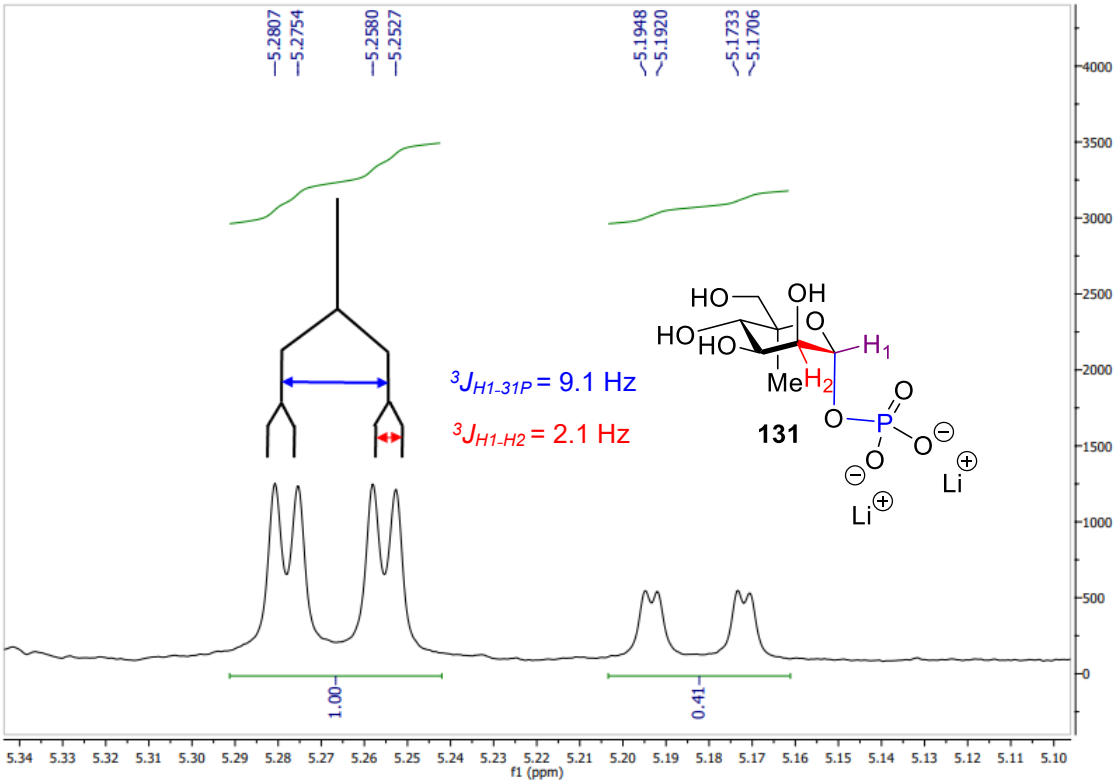


Figure 40: *^{1}H NMR data of **131** and **132**. α-pyranoside **131**: 5.27 ppm with coupling constant of $^3J_{H1\text{-}H2,\ H1\text{-}31P}$ = 2.2, 9.1 Hz (indicated on figure), and β-pyranoside **132**: 5.19 ppm ($^3J_{H1\text{-}H2,\ H1\text{-}31P}$ = 1.1, 8.6 Hz).*

MacDonald phosphorylation of an anomeric mixture of D-mannose pentaacetate **36** conducted by Beswick *et al.* furnished exclusively α-D-mannose-1-phosphate.[227] As previously discussed in Section 6.4., it is clear that the *C*5-Me group sterically affects the outcome of the reaction, introducing a competing pathway for formation of the β-1-phosphate. In addition, MacDonald phosphorylation on substrates **120** and **121** with an α/β = 1/2 (obtained from Ac_2O/pyridine) performed by a colleague in the group, delivered 1-phosphates **131** and **132** in a similar ratio (α/β = 2/1), denoting that the anomeric mixture ratio used as starting material does not influence the anomeric composition of 1-phosphates **131** and **132**. Future work involving **131** will be similar to that described for **34**, **126** and **35**, and is further outlined in Section 10.2.

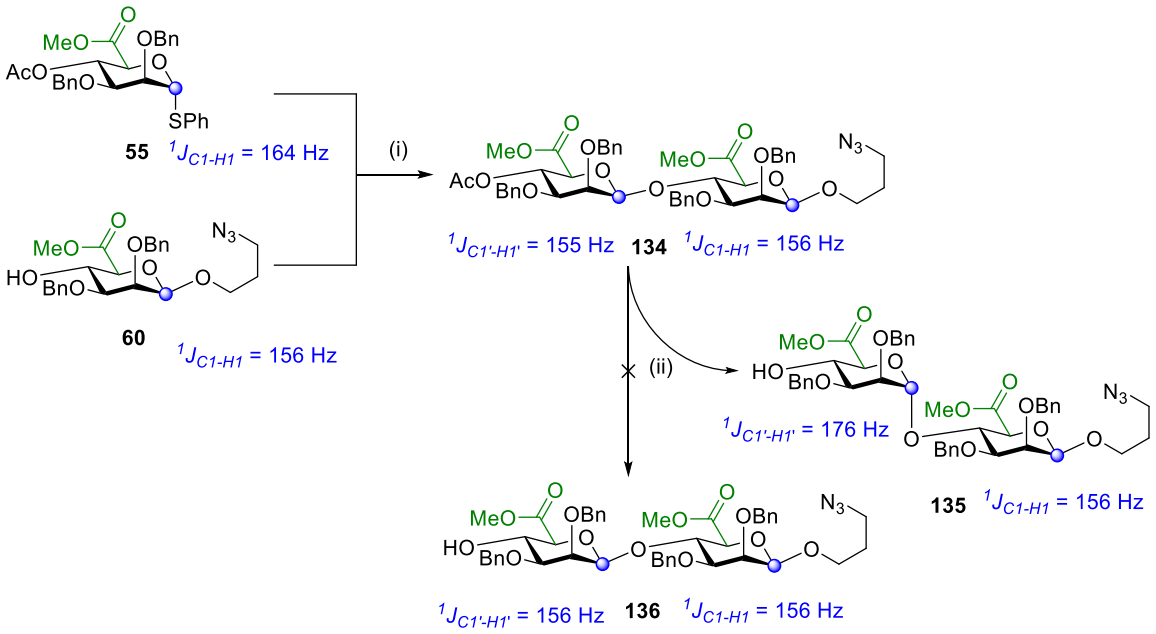
8. Synthesis of *C*6-modified alginate disaccharides

Following their successful preparation, both native and *C*6-modified monosaccharides (donors and acceptors) were utilised for the production of modified alginate disaccharide building blocks. As discussed in Section 6, the initial route designed was based on a pre-glycosylation oxidation approach, as this was considered to promote β-linkage formation through a similar pathway to that demonstrated by *C*5-carboxylate donors.[166,150] Their construction proceeded on an iterative basis, by extending from the non-reducing end of acceptors **60** and **78**. Consequently, after non-reducing end *O*4-position deprotection, the newly formed sachharide would act as the new acceptor in a subsequent glycosylation step with a new donor.

8.1. Synthesis of native D-ManA – D-ManA disaccharide 136

For D-ManA – D-ManA disaccharide synthesis, a mixture of donor **55**, acceptor **60** and NIS in CH_2Cl_2 was cooled to -60 °*C* and a catalytic amount of TMSOTf was added to afford β-linked disaccharide **134** in 56% yield. The β-linkage was determined through coupled HSQC with coupling constant values $^{1}J_{C1'\text{-}H1'}$ = 155 Hz and $^{1}J_{C1\text{-}H1}$ = 156 Hz. (Scheme 26). Codée *et al.* proposed that this NIS/TMSOTf mediated glycosylation event proceeds solely *via* an S_N1 pathway, where the displacement of the initially formed iodosulfonium species takes place, giving the desired β-stereoselectivity. This was demonstrated by pre-activation of donor **55** with NIS and equimolar amount of TMSOTf. They confirmed by TLC analysis that no complete activation of the donor was achieved; however, the addition of the acceptor afforded the same β-disaccharide in high yields. These observations imply that the NIS/TMSOTf glycosylation mechanistic pathway does not proceed through an α-triflate intermediate, but only through the $^{3}H_4$-oxocarbenium ion intermediate.[211]

This highlights the capacity of *C*5 carboxylate esters to form β-mannosidic linkages independently of their anomeric configuration, as this was additionally confirmed by the use of both α- and β-mannuronate thioglycosides as glycosyl donors.[150]



***Scheme 26**: Attempted synthesis of native system **136** (i) NIS, TMSOTf, CH_2Cl_2, -60 °C to RT, 30 min, 56% (ii) $Na_{(s)}$, MeOH, RT, 2 h, 32%.*

Native disaccharide **134** was subsequently subjected to Zemplén deacetylation conditions at room temperature to unexpectedly furnish disaccharide acceptor **135** (Scheme 26). The reaction was left stirring at room temperature for 2 h (pH ~ 9-10) and was quenched with ion exchange Amberlite 120 (H^+) resin (pH ~ 5-6). TLC analysis showed starting material **134** and the slow formation of a lower R_f spot. The NMR data collected for this material were not consistent with the literature,[211] whereby $H_{1'}$ was found at 5.43 ppm instead of 4.73 ppm. Moreover, coupled HSQC showed coupling constants $^1J_{C1'\text{-}H1'}$ = 176 Hz and $^1J_{C1\text{-}H1}$ = 156 Hz (Figure 41a).

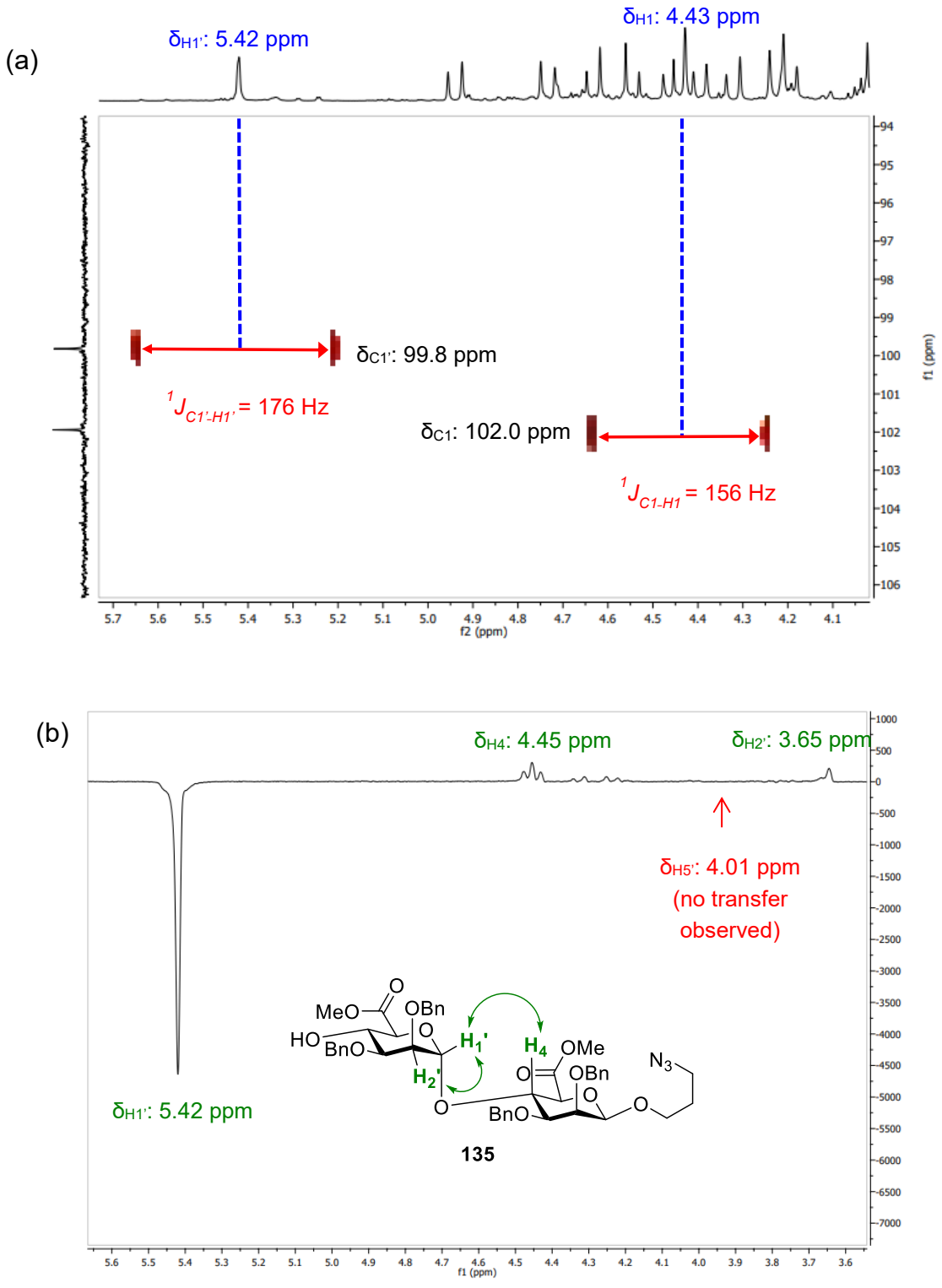


***Figure 41**: NMR data of α-linked disaccharide **135** (a) Coupled HSQC showing C1: 102.0 ppm with $^1J_{C1\text{-}H1}$ = 156 Hz and C1': 99.8 with $^1J_{C1'\text{-}H1'}$ = 176 Hz (b) nOe spectrum of **135**, verifying an α-linked product.*

These data suggested that the applied conditions had liberated C4-*OH* (2.95 ppm, br. s) but also altered the anomeric integrity at the non-reducing glycosidic linkage.

ESI-MS analysis found the sodium adduct of **135** at *m*/*z* 864.3343 which matched the reported literature ($C_{45}H_{51}N_3O_{13}$, **135** requires $(M+Na)^+$ 864.3320).[211] To examine whether the applied NaOMe conditions caused epimerisation at the non-reducing end linkage, the NMR data of **135** were compared to that of the disaccharide containing L-guluronic acid as the reducing end monomer, which was found in the literature ($H_{1'Gul}$: 5.24 ppm).[177] The NMR analysis here did not match, excluding possible epimerisation. The next step taken towards confirming the stereochemistry of the non-reducing end linkage was the implementation of n*O*e experiments, where the anomeric proton of the non-reducing end mannuronate $H_{1'}$ (5.42 ppm, s) was irradiated (Figure 41b). The n*O*e data collected for **135** confirmed the change of the disaccharide linkage from β to α, as $H_{1'}$ showed transfer to H_4 (4.45 ppm, app. t, $^3J_{H4\text{-}H3,\ H4\text{-}H5}$ = 9.3 Hz) and $H_{2'}$ (3.65 ppm-hidden under multiplet with $H_{3'}$) and none to $H_{5'}$ (4.01 ppm, d, $^3J_{H5'\text{-}H4'}$ = 9.7 Hz).

To evaluate whether the anomerisation process had taken place during the basic conditions of the reaction or during stirring with Amberlite 120 (H^+) resin, a repeat was performed, terminating the reaction at 1 h with H^+ resin. ^1H and ^{13}C NMR spectra clearly indicated a mixture of α- and β-linked products with a ratio of 1/4.9 respectively. The same mixture was then stirred overnight with Amberlite 120 (H^+) resin in MeOH and the ^{13}C NMR spectrum obtained showed no further change (Figure 42). The information gathered suggested that the anomerisation process was underway during the basic deacetylation conditions (pH ~ 9-10) and was terminated with the addition of the acidic resin (pH ~ 5-6). It is unclear how this occurred; one explanation could be that an E1cB elimination from the reducing end uronate, mutarotation of the released hemiacetal to the α-anomer which is subsequently re-added to the bottom face of the elimination product, delivering **135** from **134**. The

data obtained also suggest that this happened after *C*4-OAc hydrolysis. Repeated attempt to hydrolyse the acetate group with NH_3 and Et_3N in MeOH at room temperature and 35 °C were unsuccessful, and only **134** was recovered.

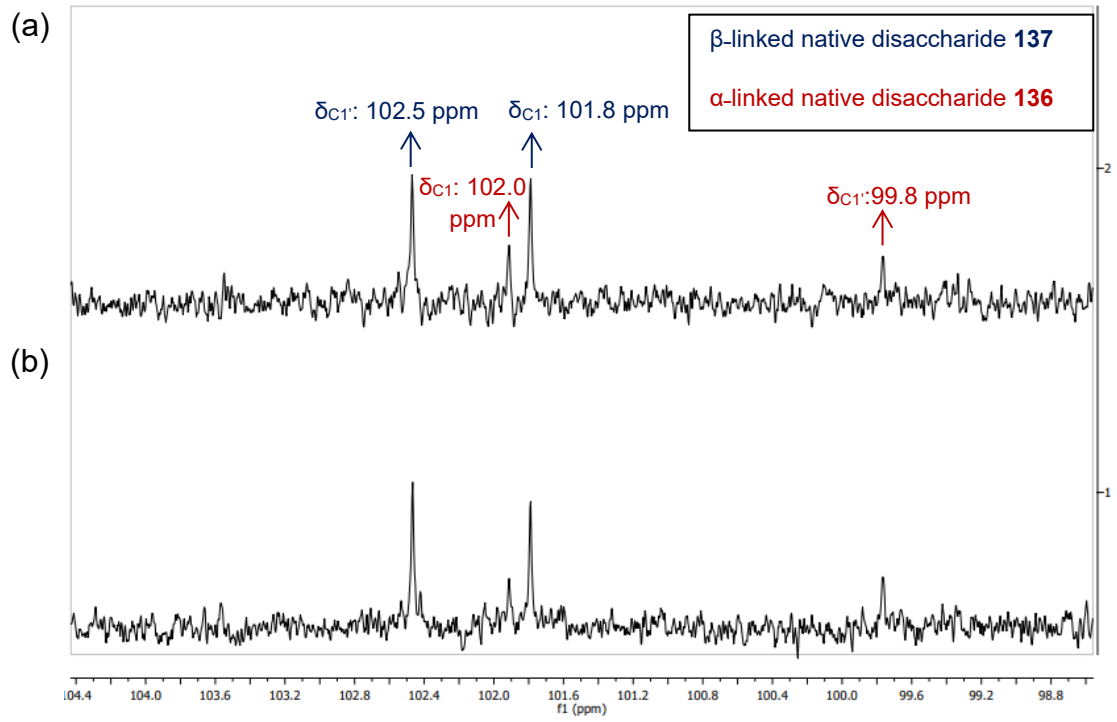
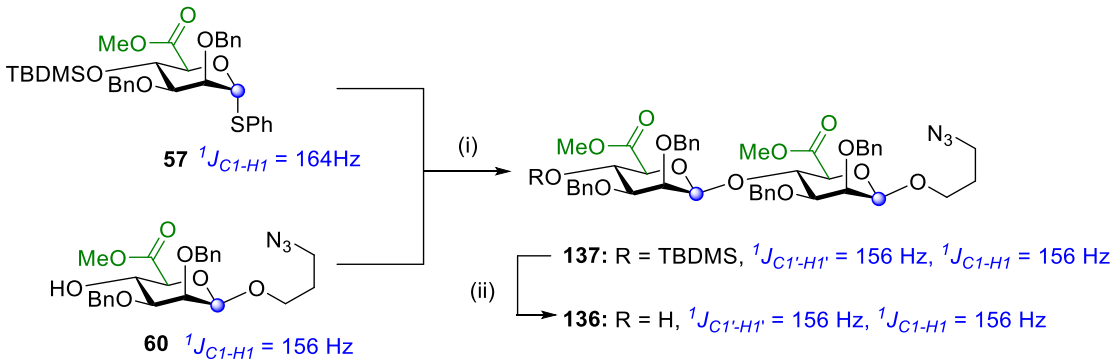


Figure 42: *(a) ^{13}C NMR data taken after 1h of stirring **134** with NaOMe in MeOH (the reaction was quenched with Amberlite 120 (H^+) resin (b) ^{13}C NMR data taken after stirring the mixture with Amberlite 120 (H^+) resin in MeOH O/N. The NMR spectra show no difference, suggesting that the anomerisation is not acid-assisted.*

By employing the same conditions used for the synthesis of **134**, donor **57** and acceptor **60** delivered β-linked disaccharide **137** as the sole product in 61% yield (Scheme 27). Subsequent removal of the TBDMS group with AcCl in MeOH gave **136** in 32% yield, and in this case, the NMR data obtained were in a good agreement with the literature, giving coupling constants values $^1J_{C1\text{-}H1}$ = 156 Hz (*C*1': 102.5 ppm) and $^1J_{C1'\text{-}H1'}$ = 156 Hz (C1: 101.8 ppm).[211]



***Scheme 27**: Synthesis of native β-disaccharide **136** (a) (i) NIS, TMSOTf, CH_2Cl_2, -10 °C to RT, 30 min, 61% (ii) AcCl, MeOH, 0 °C to RT, 16 h, 32%.*

n*O*e data of **136** were obtained to further verify its β-linkage, as well as to compare it with that of **135** (Figure 43). The spectrum clearly showed transfer from $H_{5'}$ (3.59 ppm, d, $^3J_{H5'-H4'}$ = 9.6 Hz) to $H_{1'}$ (4.72 ppm, s) and $H_{3'}$ (3.32 ppm, dd, $^3J_{H3-H4,\ H3-H2}$ = 9.5, 2.8 Hz), and not H_4 (4.45 ppm, app. t, $^3J_{H4-H3,\ H4-H5}$ = 8.6 Hz), suggesting the existence of a β-linkage. The low yield of **136** owed to the formation of a highly polar material which was not isolated.

(b)

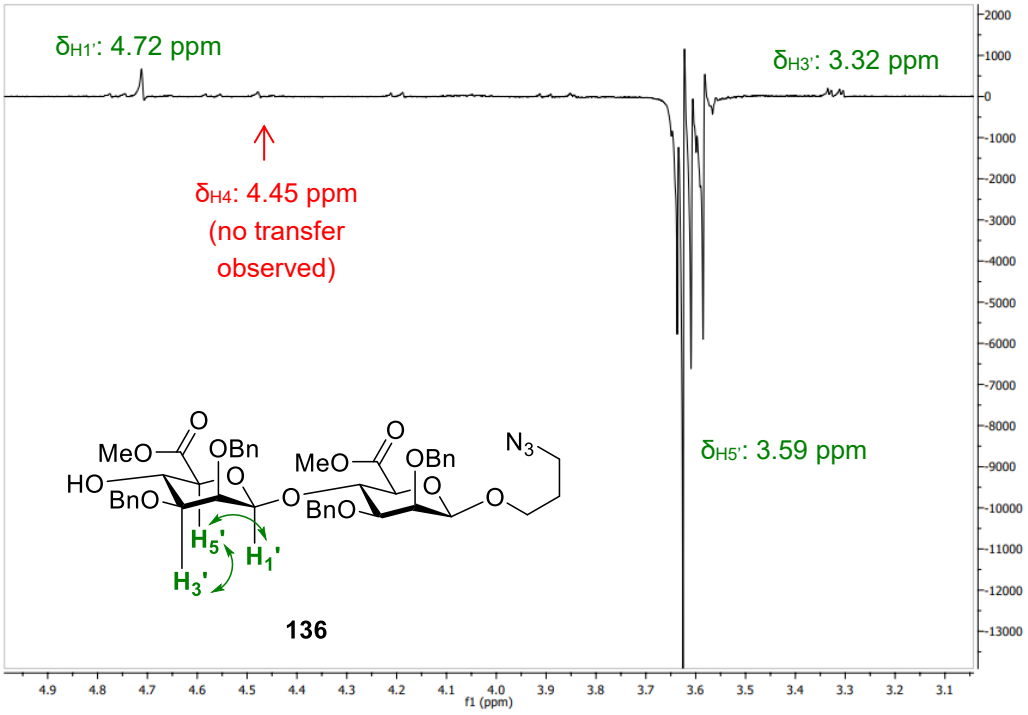
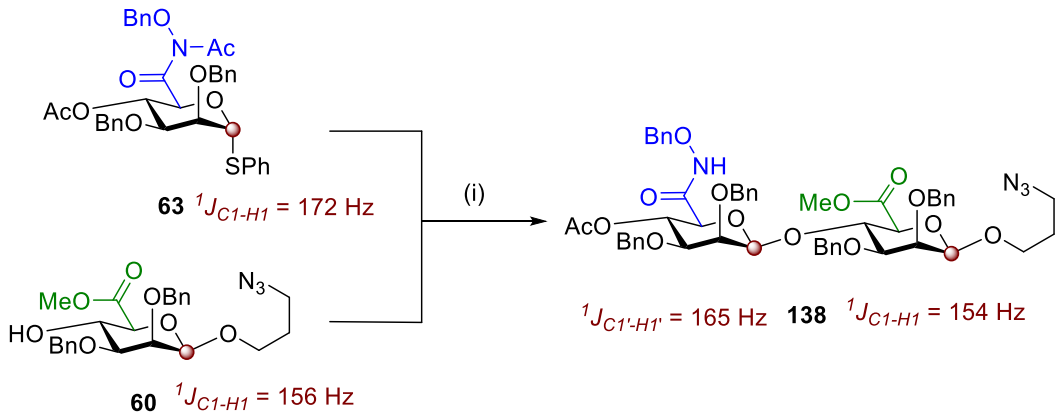


***Figure 43**: nOe spectrum of **136**, verifying a β-linked product.*

8.2. Synthesis of *C*6-hydroxamate D-ManA – D-ManA 140

Synthesis of a mixed disaccharide system was first attempted by coupling *N*-acyl hydroxamate donor **63** and native acceptor **60** with the same promoter system (NIS/TMSOTf) employed for the synthesis of **134**. TLC analysis of the reaction suggested the formation of a complicated mixture after 1 h. Disaccharide **138** was isolated as a single diastereoisomer in 54% yield (Scheme 28). Similar to the glycosidation of **63** to 3-bromopropanol, ^{1}H and ^{13}C NMR data suggested *N*-Ac cleavage of **63** had occurred, which minimised the overall yield. Interestingly, *O*-benzyl (4-*O*-acetyl-2,3-di-*O*-benzyl- bicyclo [3.2.1-*O*]) hydroxamate **64** was not isolated as one of the by-products of the reaction, suggesting that the *N*-Ac hydrolysis might have occurred after the glycosylation between **63** and **60**. Mass spectrum analysis contradicted with the structure extracted from the NMR data, as COSY, HSQC and HMBC data suggest the structure of **138** to be as depicted in Scheme 28.



***Scheme 28**: Synthesis of mixed sequence disaccharide **138** (i) NIS, TMSOTf, CH_2Cl_2, -60 °C, 1 h, 54%.*

The coupled HSQC spectrum showed coupling constant of $^{1}J_{C1'-H1'}$ = 165 Hz (C1': 100.6 ppm), and an expected coupling constant of $^{1}J_{C1-H1}$ = 154 Hz (C1: 102.1 ppm). To confirm the configuration of the newly formed glycosidic bond of **138**, n*O*e

experiments were conducted by irradiating the non-reducing end proton, $H_{5'}$ (3.92 ppm, d, $^{3}J_{H5'\text{-}H4'}$ = 9.9 Hz). The data obtained determined the formation of a β-linkage, as $H_{5'}$ showed transfer to $H_{1'}$ (4.71 ppm, s) and $H_{3'}$ (3.52 ppm, dd, $^{3}J_{H3'\text{-}H4',\ H3'\text{-}H2'}$ = 9.7, 2.8 Hz) indicating an axial position of the $H_{1'}$ and consequently β-stereochemistry of the non-reducing end linkage (Figure 44).

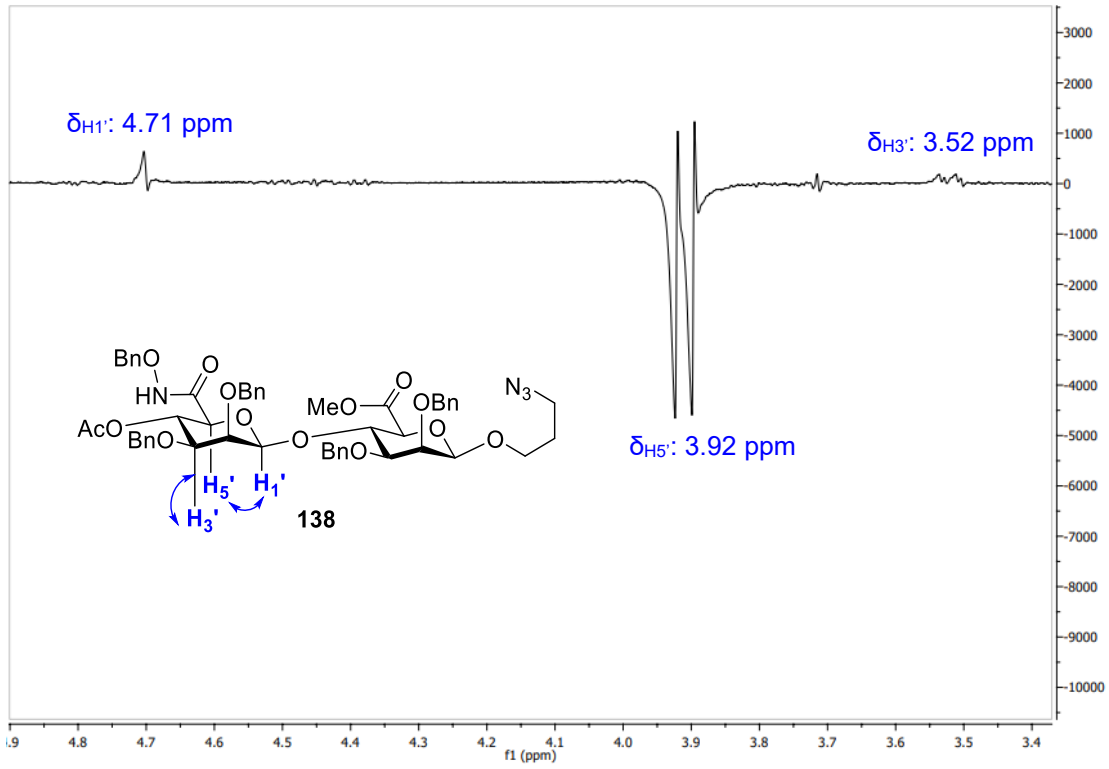
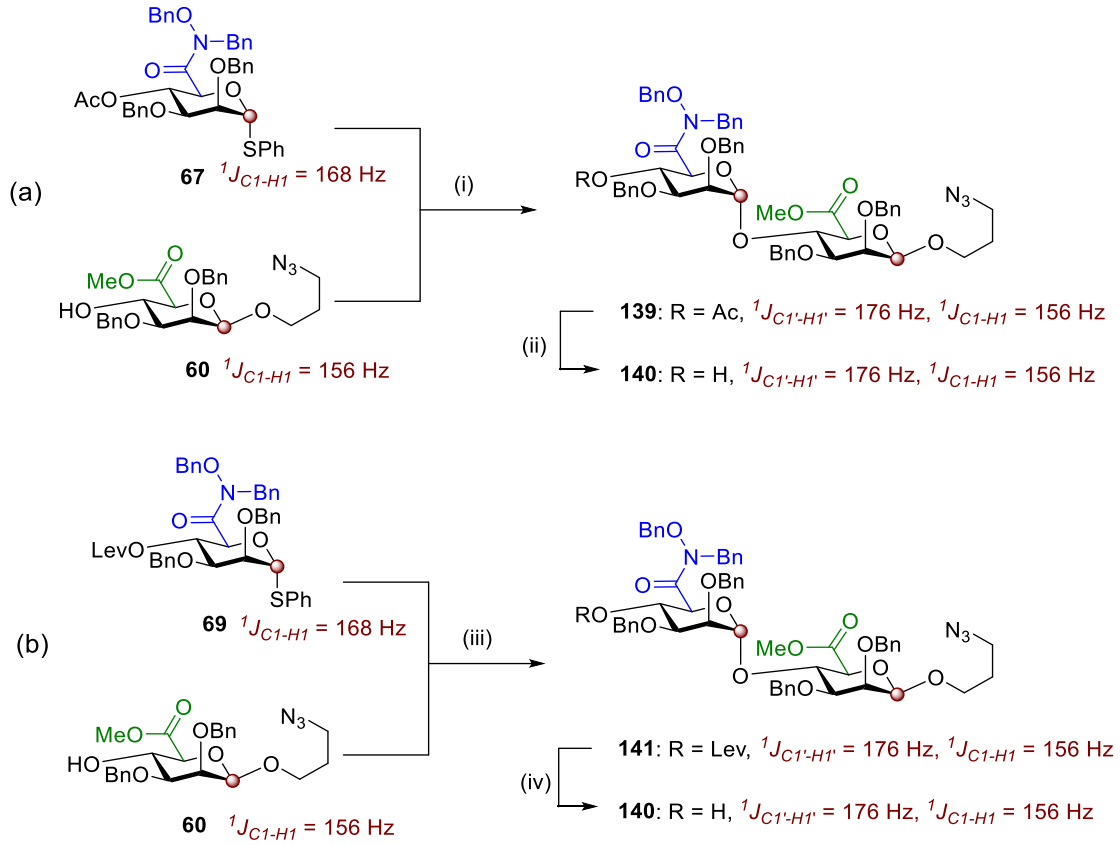


***Figure 44**: nOe spectrum of **138**, verifying a β-linked product.*

A second mixed disaccharide was synthesised using native mannuronate acceptor **60** and *N*-Bn hydroxamate donors **67** and **69** (Scheme 29). Thioglycosides **67** and **69** were considered to be more robust during the glycosylation conditions with NIS and TMSOTf, as opposed to *N*-Ac counterpart **63**. Similar to the synthesis of **138**, TLC analysis indicated the formation of a complicated for **138** mixture over 30 min. at 0 °C. Purification by silica gel flash column chromatography gave **139** in extremely low yield (12%) with an anomeric ratio α/β = 9/1. Coupled HSQC indicated

α-stereochemistry for the non-reducing end linkage, with $^{1}J_{C1'\text{-}H1'}$ = 176 Hz (C1': 99.4 ppm).



***Scheme 29**: Synthesis of mixed disaccharide **140** (i) NIS, TMSOTf, CH_2Cl_2, 0 °C, 30 min, 12% (ii) $Na_{(s)}$, MeOH, RT, 16 h, 54% (iii) NIS, TMSOTf, CH_2Cl_2, -40 °C to -10 °C, 6.5 h, 30% (iv) $H_2N\text{-}NH_2$.AcOH, pyridine/AcOH (4/1, v/v), RT, 30 min, 76%.*

In order to improve the yield, the NIS/TMSOTf promoter system was repeated using substrates **69** and **60** at lower temperatures and for prolonged period of time (1 h at -40 °C, 2 h at -25 °C, 3 h at -20 °C and 30 min. at -10 °C). The yield for this glycosylation was slightly improved but not optimised, as TLC analysis again denoted the formation of a complicated mixture, giving **141** in 30% yield (with 14% recovery of acceptor **60**) and in the same anomeric ratio as before (α/β = 9/1). The α-linkage of **141** was again confirmed *via* coupled HSQC, with $^{1}J_{C1'\text{-}H1'}$ = 176 Hz (C1': 99.4 ppm), the same value observed for **139**. Promoter system Ph_2SO/Tf_2O (30 min. from

-80 to -30 °C) was additionally evaluated for improving the reaction yield, using **69** as the donor. Unfortunately, the yield acquired was similar to that obtained previously (34%), as well as the anomeric configuration (α/β = 9/1).

Mixed disaccharide **140** was successfully delivered by C4-*OH* deprotection of **139** with NaOMe (54% yield) and deprotection of the levulinoyl group in **141** with H_2N-NH_2.AcOH (76% yield). To further examine the stereochemistry of **140**, a coupled HSQC spectrum was obtained showing $^{1}J_{C1'\text{-}H1'}$ = 176 Hz for the non-reducing end (C1': 99.4 ppm), and $^{1}J_{C1\text{-}H1}$ = 156 Hz for the reducing end linkage (C1: 101.9 ppm). The n*O*e spectrum acquired by irradiating $H_{5'}$ (3.98 ppm, d, $^{3}J_{H5'\text{-}H4'}$ = 9.6 Hz) indicated strong transfer only to $H_{3'}$ (3.66 ppm, dd, $^{3}J_{H3'\text{-}H4',\ H3'\text{-}H2'}$ = 9.6, 2.3 Hz) (Figure 45). The absence of a transfer signal from $H_{5'}$ to $H_{1'}$ implied the equatorial configuration of $H_{1'}$.

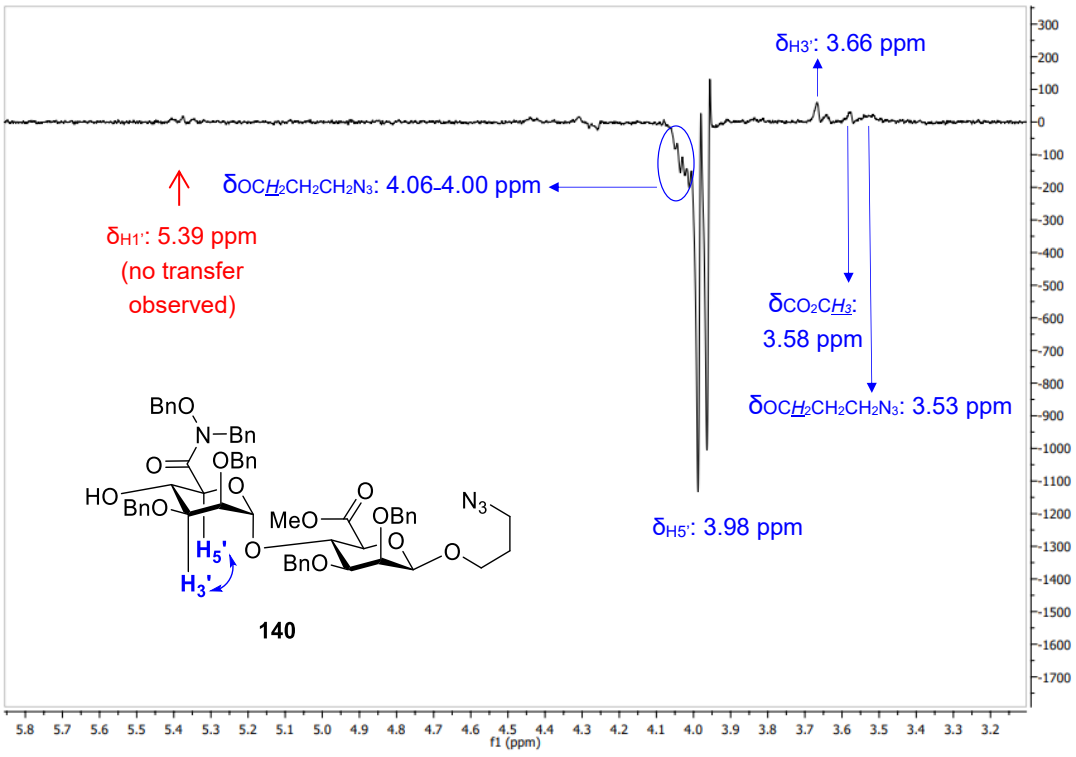
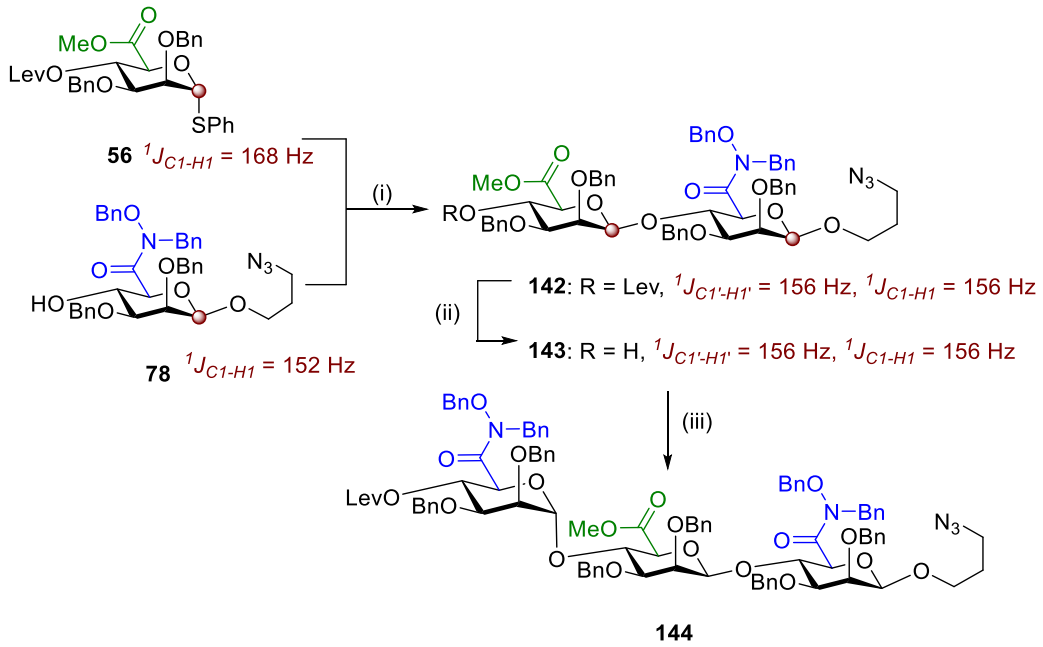


***Figure 45**: nOe spectrum of **140**, verifying an α-linked product.*

8.3. Synthesis of D-ManA – *C*6-hydroxamate D-ManA disaccharide 143 and *C*6-hydroxamate D-ManA – D-ManA – *C*6-hydroxamate D-ManA trisaccharide 144

The inverse sequence disaccharide was next synthesised by employing hydroxamate acceptor **78** and native donor **56** (Scheme 30). The promoter system applied was NIS/TMSOTf, starting from -40 °C. The reaction was monitored and upon complete consumption of the acceptor, was quenched within 45 min. Disaccharide **142** was obtained as a single diastereoisomer ($^1J_{C1'\text{-}H1'}$ = 156 Hz, *C*1': 102.3 ppm, $^1J_{C1\text{-}H1}$ = 156 Hz, *C*1: 101.8 ppm) in 55% yield. The stereochemical outcome was in agreement with the studies conducted by Codée *et al.* regarding the reactivity of mannuronic acid donors.[150,144] Donor **56** was recovered (30%), which explained the reduced yield of the reaction.

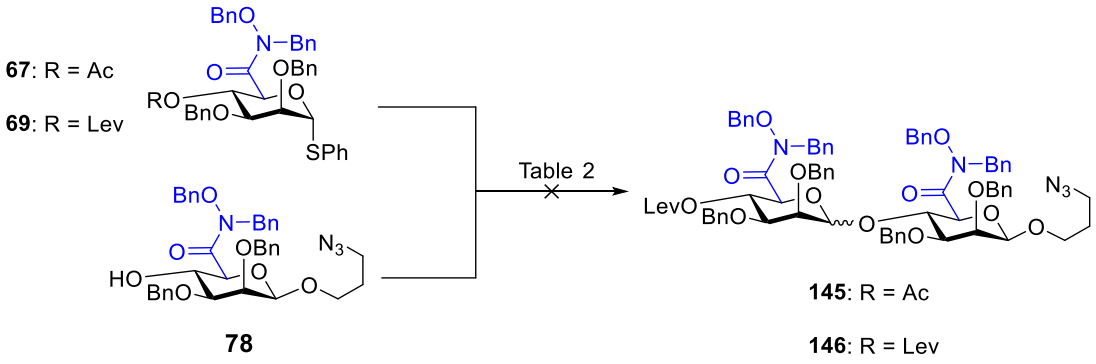


Scheme 30: *Synthesis of mimetic of trisaccharide **144** (i) NIS, TMSOTf, CH_2Cl_2, -40 °C to 0 °C, 45 min, 55% (ii) $H_2N\text{-}NH_2$.AcOH, pyridine/AcOH (4/1, v/v), RT, 30 min, 81% (iii) Ph_2SO, TTBP, Tf_2O, **69**, CH_2Cl_2, -60 °C to -90 °C, 1 h, 14%.*

C4-*OH* liberation using H_2N-NH_2.AcOH furnished disaccharide acceptor **143** in 81% yield, which was successfully glycosylated under Ph_2SO/Tf_2O mediated conditions with hydroxamate donor **69**, giving trisaccharide **144** in 14% yield. (Scheme 30). ESI-MS analysis found the ammonium adduct of **144** at *m/z* 1689.7330 ($C_{97}H_{105}N_6O_{21}$, **144** requires $(M+NH_4)^+$ 1689.7327). Coupled HSQC data obtained for **144** were inconclusive, as the anomeric peaks in the ^{13}C NMR spectrum were in close proximity, making it difficult to calculate the one-bond coupling constant ($^1J_{C1''-H1''}$) for the newly formed linkage. Considering the fact that donor **69** afforded α-linked disaccharide **141** when condensed with native acceptor **60**, the coupling of **69** with disaccharide **143** is proposed to be a *trans*-glycosylation. The poor yield of trisaccharide **144** implies a dismissal for the construction of multiple 1,4-linkages.

8.4. Synthesis of *C*6-hydroxamate D-ManA – *C*6-hydroxamate D-ManA disaccharides 145 and 146

The synthesis of a *C*6-hydroxamate disaccharide system was attempted by using *N*-Bn hydroxamate donors **67** or **69** and *N*-Bn hydroxamate acceptor **78**. Different thioglycoside activation protocols were evaluated for the reaction of **67** or **69** with **78**, as outlined in Scheme 31, and the results are summarised in Table 2.



***Scheme 31**: Attempted synthesis of **145** and **146**.*

Table 2: Glycosylation conditions attempted for the synthesis of 145 and 146				
Pre-activation using Ph_2SO/Tf_2O[a]				
Entry	Acceptor **78** (equiv.)	Reaction concentration (M)	Duration and temperature[b] (°C)	Observations from TLC analysis and NMR data[c]
1	1.0	0.05	30 min. / -10 °C	Unidentified material with low R_f value
2	1.0	0.06	20 min. / -20 °C	Complicated mixture, NMR showed hydrolysed donor (28%), unreacted acceptor (60%) and decomposed material with low R_f value
3	1.1	0.05	15 min. / -40 °C	Similar results with entry 2
Pre-activation using BSP/Tf_2O[d]				
4	1.1	0.05	45 min. / -40 °C	Baseline spot and recovery of donor (77%)
Pre-mixing glycosylation using Me_2S_2/Tf_2O[e]				
5	1.1	0.07	20 min. / -55 °C	Decomposition, recovered donor (23%) and acceptor (12%)
Inverse glycosylation using NIS/TMSOTf				
6	1.1	0.05	3.5 h / -20 °C	Complicated mixture, recovered acceptor (49%) and decomposed baseline material
Standard glycosylation using NIS/TMSOTf				
7	1.1	0.07	3.5 h / -20 °C	Donor hydrolysis (63%) and recovered acceptor (76%)

[a] The equiv. used were 1.3 equiv. of Ph_2SO, 1.3 equiv. of Tf_2O and 2.5 equiv. of TTBP. The results discussed derived from reaction of **69** with **78**. The observations when **67** was used as a donor were similar to that of **69**.

[b] The initial reaction temperature in all entries was -80°C (donor and promoter stirred for 5-10 min), except from entry 6 where the reaction started at -40°C.

[c] HRMS samples of the reaction mixtures were submitted. No trace of the desired products was detected in the reported entries.

[d] The equiv. used were 1.3 equiv. of BSP, 1.3 equiv. of Tf_2O and 2.5 equiv. of TTBP.

[e] Preparation: A 1 M solution of the reagent was made by adding Tf_2O (168 μL, 1 mmol) to a solution of Me_2S_2 (100 μL, 1.1 mmol) in dry CH_2Cl_2 (750 μL) at 0°C and stirring the mixture for 30 min. at the same temperature before use. 1.5 equiv. of the promoter were added to the reaction 0.8 equiv. of DTBMP were used as a base in the glycosylation reaction.

Considering that the pre-activation conditions with Ph_2SO/Tf_2O and TTBP gave high yields for the synthesis of **73** and **74** the same conditions were applied for the synthesis of **145** and **146**. Donor **69** was stirred for 5 min. with Ph_2SO/Tf_2O and TTBP at -60°C before cooling down to -80 °C. At that temperature, acceptor **78**

dissolved in CH_2Cl_2 was added dropwise, and the reaction was stirred for 30 min., allowing the mixture to warm up to -10 °C (Entry 1). TLC analysis showed the presence of a decomposed material close to the baseline which was not isolated. Donor **69** and acceptor **78** were used again under the same glycosylation conditions, but the reaction mixture was only allowed to warm up to -20 °C and stirred for 20 min. TLC analysis showed a complicated mixture, and NMR data revealed that the main components was hydrolysed donor (28%), unreacted acceptor (60%) and the same decomposed material with low R_f value (Entry 2). The same results were obtained when the reaction mixture was allowed to warm to -40 °C and left stirring for 15 min. (Entry 3). The BSP/Tf_2O protocol was next applied, as it was also reported to promote the highly stereoselective formation of β-mannuronate linkages.[228,161] Similarly to the procedure applied with Ph_2SO/Tf_2O, donor **69** was stirred for 5 min. with BSP/Tf_2O and TTBP at -60 °C before cooling down to -80 °C and adding acceptor **78**. The reaction was warmed up to -40 °C and stirred for 45 min, whereby TLC analysis suggested decomposition of the mixture, baseline material formation and unreacted donor thioglycoside was recovered (77%) (Entry 4).

Me_2S_2-Tf_2O was employed as an alternative organosulfur activating promoter system. Fügedi *et al.* demonstrated that by analogy with the preparation of DMTST, Me_2S_2 can react with Tf_2O to give a more electrophilic product, as one of the methyl groups will be replaced by a strongly electron-withdrawing trifluoromethanesulfonyl group.[229] They demonstrated that the use of Me_2S_2-Tf_2O reagent can activate thioglycosides at low temperatures with the glycosylation reaction completed within a short time. TLC analysis for using these conditions suggested decomposition of the mixture and purification by column chromatography recovered donor **69** (23%) and

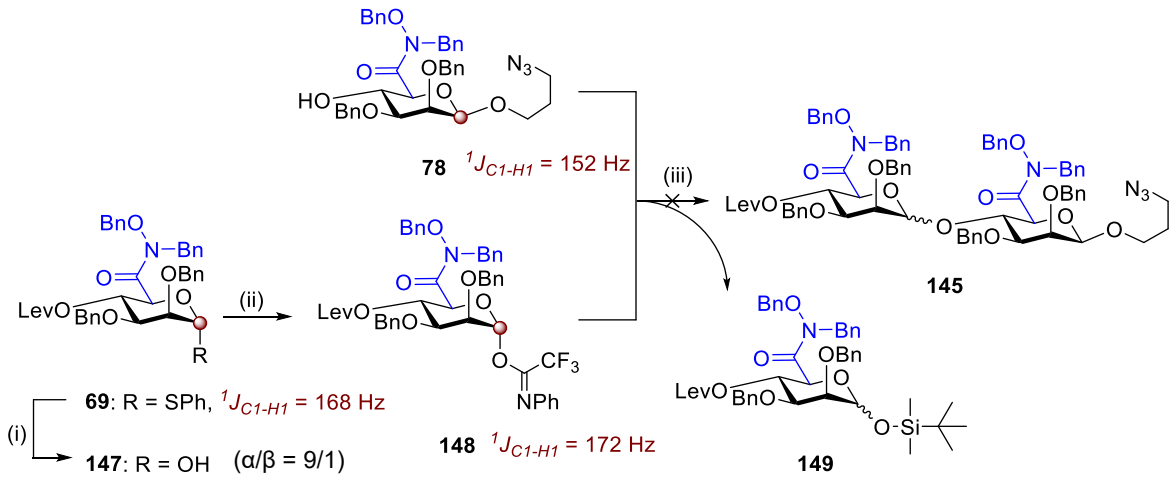
acceptor **78** (12%) (Entry 5). NMR spectra obtained from other isolated materials showed no evidence of **145**, and remain unresolved.

Inverse glycosylation conditions with promoter system NIS/TMSOTf were also applied to substrates **69** and **78**. The reaction started at -40 °C and was monitored while the temperature increased up to -10 °C over 3.5 h. TLC analysis again showed the production of a complicated mixture, indicating consumption of donor **69**. NMR data obtained after purification revealed the presence of the remaining acceptor **78** (49%) and an unidentified material with low R_f that was observed in the previous entries (Entry 6). A final attempt for the synthesis of **146** with standard pre-mixing glycosylation conditions using NIS/TMSOTf was unsuccessful, as TLC analysis showed that the hydroxamate acceptor **78** was not consumed and donor **69** slowly hydrolysed (Entry 7). NMR data confirmed the presence of hydrolysed donor **69** (63%) and recovered acceptor **78** (76%).

The results derived from the aforementioned experiments using *C*6-hydroxamate donors **67**, **69** and acceptor **78** indicate that the application of various thioglycoside activation protocols failed to deliver desired disaccharides **145** and **146**. The main components isolated from the complicated reaction mixtures were hydrolysed donor, unreacted acceptor and a highly polar material. ^{1}H and ^{13}C NMR spectra of this material were complex, however, they showed presence of a Lev group, 4 Bn groups and a carbonyl carbon at 148.8 ppm, suggesting an alteration/ decomposition of donor **69**. This baseline material was not observed when **67** and **69** were glycosylated to native acceptor **60** or when *C*6-hydroxamate acceptor **78** was glycosylated to native donor **56**, implying that there is a delicate balance of reactivity contributed from both *C*6-hydroxamate donors and acceptor. To further

investigate and identify successful conditions for the synthesis of **145** and **146**, new methodologies were examined using different types of *C*6-hydroxamate donors.

Based on the results and observations exhibited in Table 3, a different type of donor was used for the next attempt to synthesise **145** and/or **146**. An *N*-PTFA donor was selected, as it has been reported to be advantageous for the synthesis of β-mannuronates.[164,135] An additional advantage of *N*-PTFA donors derives from their low tendency to undergo side reactions during glycosylations, as opposed to TCA donors where an *N*-glycoside by-product can be formed.[230] Donor **148** was prepared from hemiacetal **147** by treating the latter with PTFA.Cl in the presence of a stoichiometric amount of K_2CO_3 in a water/acetone solvent mixture (Scheme 32).[169] The applied conditions gave **148** which was used directly after for glycosylation with **78**.



***Scheme 32**: Attempted synthesis of disaccharide **145** from N-PTFA donor **148** and acceptor **78** (i) NIS, AgOTf, CH_2Cl_2/H_2O (10/1, v/v), 0 °C, 4 h, 78% (ii) N-PTFA.Cl, K_2CO_3, acetone/H_2O (20/1, v/v), 0 °C to RT, 20 h, 66% (iii) TBDMSOTf, CH_2Cl_2, -78 °C, 24 h, 16%.*

The activator used for the attempted synthesis of **145** was TBDMSOTf, which was previously used for the activation of D-ManA *N*-PTFA donor.[231] Unfortunately, the glycosylation reaction was unsuccessful giving an anomeric mixture (α/β = 1/1)

of by-product **149** (16%), along with the unreacted acceptor **78** (87%). No hydrolysed donor was recovered; however, the results obtained can be attributed to the presence of moisture. The same conditions were tested again with a fresh bottle of promoter TBDMSOTf, giving similar results after stirring the reaction from -78 °C to -30 °C for 24 h. In order to avoid the formation of **149**, TMSOTf was used (from -78 °C to -30 °C, 24 h), whereby TLC and NMR analysis showed the major components of the reaction mixture to be unreacted acceptor **78** (58%) and hydrolysed donor (20%). NMR data obtained after flash column purification of the mixture showed no evidence of desired disaccharide **145**.

A subsequent strategy designed for the condensation of two *C*6-hydroxamate building blocks required the conversion of **147** to the corresponding glycosyl bromide **150** (Scheme 33). Based on the mechanistic study of the Koenigs−Knorr reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, Wulff and Röhle suggested that 1,2-*trans* glycosyl bromides are more favourable for the preparation of 1,2-*cis* glycosides, as a result of a putative associative transition state (Figure 46).[232] Specifically, they proposed that in the presence of non-polar solvents, insoluble silver salts promote an S_N2-type transition state which can yield higher amounts of *cis* glycosides. Their recommendation was also based on the fact that the reaction rate depended on the concentration of both donor and acceptor. The design of this route was based on Westman's approach to condensate iduronic acid bromide with an ethylthio glucosazide, which was successful as it resulted in 60% of the desired β-linked disaccharide.[233]

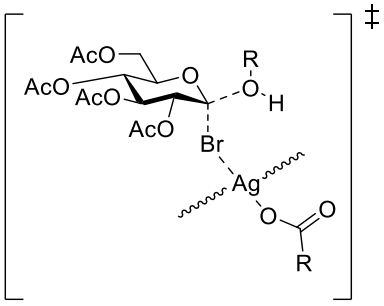
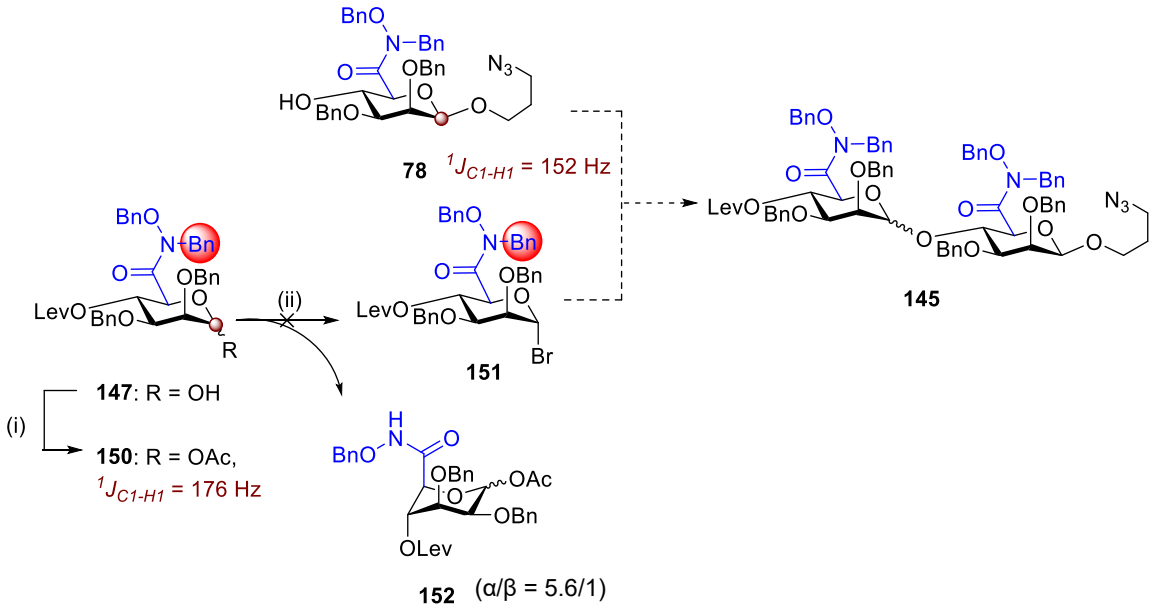


***Figure 46**: Kinetically-derived associative transition state for the reaction of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with alcohols and an insoluble silver salt in diethyl ether [Adapted from Wulff and Röhle (1974)].*[232]

Glycosyl acetate **150** was synthesised in 90% yield from **147** with Ac_2O/pyridine (Scheme 33). NMR analysis of the material isolated indicated only the α-anomer of **150**, with H_1 at 6.18 ppm (d, $^3J_{H1\text{-}H2}$ = 2.2 Hz), while coupled HSQC spectrum showed a coupling constant of $^1J_{C1\text{-}H1}$ = 176 Hz. Following a protocol by Jakeman *et al*.[234] for installing the bromo substituent at the anomeric centre of **150** with PBr_3 and H_2O did not furnish **151**. TLC analysis after 40 min. showed consumption of starting material **150** and the formation of two lower R_f spots. NMR of the reaction suggested a mixture of compounds that did not include the desired glycosyl bromide, as the ^{13}C NMR of the major product showed no significant change at the anomeric centre (**150**: 91.6 ppm, **152**: 91.0 ppm). ^1H and HMBC analysis confirmed the cleavage of a Bn group from the hydroxamate (explaining the difference in R_f), and the existence of the anomeric OAc group. Moreover, **152** was isolated as an anomeric mixture (α/β = 5.6/1), and the ^3J values of both anomers suggested a $^1C_4$ ring conformation. HRMS data confirmed the proposed structure of **152**, as the protonated species was found at *m*/*z* 620.2498 ($C_{34}H_{38}NO_{10}$, **152** requires $(M+H)^+$ 620.2496). *N*-Bn hydrolysis was previously observed during glycosidation of **67** with 3-bromopropanol using NBS/TMSOTf (Table 1, Entry 5),

indicating that *C*6-hydroxamate donors are susceptible to *N*-hydrolysis when exposed to highly acidic conditions.

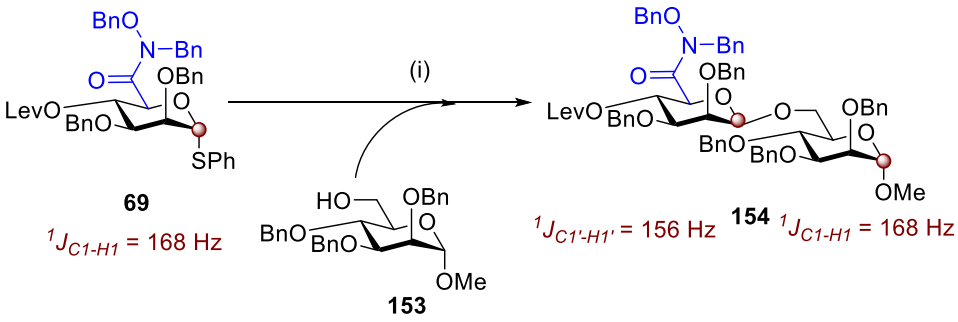


***Scheme 33**: Designed route towards mimetic disaccharide **145** (a) (i) Ac_2O, pyridine, CH_2Cl_2, RT, 24 h, 89% (ii) PBr_3/H_2O, CH_2Cl_2, 0 °C, 40 min, 95%.*

8.5. Discussion of possible mechanistic pathways involving *C*6-hydroxamate donors 67, 69 and acceptor 78

It is important to note that the glycosylation of hydroxamate donors **67**, **69** and acceptor **78** with the respective native acceptor **60** and donor **56** was successful, as desired disaccharides **140** and **143** were obtained (Sections 8.2. and 8.3.). In general, mannuronic acid donors are regarded to be among the most unreactive donors due to their electron-withdrawing *C*6 oxidised moiety.[150,160] However, in a later study Codée *et al.* showed that they owe their high reactivity and β-selectivity to the formation of the relatively favourable $^3H_4$-oxacarbenium ion-like intermediate, and based on their observations, the synthesis of both mixed and fully *C*6-modified disaccharides was designed based on a pre-glycosylation oxidation route.[150] The yield observed from the synthesis of mixed disaccharide **142** (55%) showed that

hydroxamate acceptor **78** displayed similar nucleophilicity with is native counterpart, as the yield obtained was similar with the one for the synthesis of **134** (56%). Taking into consideration the yields for the synthesis of **139** (12%), **141** (30%) and **144** (14%) hydroxamate donors **67** and **69** exhibited reduced reactivity during glycosylation reactions with mannuronate acceptor **60** and disaccharide acceptor **143**. As previously mentioned, the reason behind the failed attempts to synthesise *C*6-hydroxamate disaccharides **145** and **146** could be attributed to the relatively poor reactivity of both hydroxamate donors **67**, **69** and acceptor **78** demonstrated under various glycosylation protocols.



***Scheme 34**: Synthesis of disaccharide **154** for stereochemistry investigation (i) Ph_2SO, TTBP, Tf_2O, **153**, CH_2Cl_2, -78 °C to -20 °C, 2 h, 27%.*

To further investigate the reactivity of the designed hydroxamate donors, **153** bearing a free C6-*OH* was used as a monosaccharide acceptor in a glycosylation reaction with hydroxamate donor **69**. A preactivation protocol with Ph_2SO/Tf_2O and TTBP furnished β-linked disaccharide **154** ($^1J_{C1'\text{-}H1'}$ = 156 Hz, *C*1': 102.2 ppm) in 27% yield (Scheme 34). The yield obtained for **154** was similar to that of **141** (30%), implying that the conversion percentage observed for glycosylation reactions involving donor **69** with other monosaccharides (**60**, **153**) is mainly influenced by the low reactivity of **69**. However, the stereochemical outcome was different when the 2 acceptors (**60**, **153**) were used (α-linkage for **141** and β-linkage for **154**), suggesting

that their reactivity (and steric hindrance) may contribute at a higher degree to the stereochemistry of the glycosidic linkage.

Given the mechanistic explanation provided by Codée *et al.* (Figures 27 and 31), herein a similar mechanistic pathway was proposed based on the results and observations from the glycosylation reactions discussed earlier in this section (Figure 47). Preactivation of donors **67** and **69** could give their respective α-triflate intermediates **156** that undergo a fast β-face (S_N2-like) attack when a strong *O*-nucleophile is present as an acceptor (e.g. primary alcohol, 3-bromopropanol and *O*-glycoside **153**) to give products **159** (Figure 47a). In the presence of weak *O*-nucleophiles (e.g. mannuronate acceptor **60** and disaccharide acceptor **143**), solvent-separated ion pair **157** becomes more favourable, as it exhibits more carbocation character than **156**. Following a S_N1-like pathway, intermediate **157** leads to the formation of α-linked products **158**. A more detailed explanation of the S_N1-like pathway included intermediate transition states **160**, **161** and **162** (Figure 47b). Half-chair oxocarbenium ions **160** and **161** are attacked from the α-bottom face, as in both cases the top face is hindered by *C*6-hydroxamate, leading to α-linked products **158a** and **158b**. $^4H_3$ half-chair oxocarbenium ion **160** was considered to be more favourable compared to $^3H_4$ half-chair oxocarbenium ion **161** because of the steric interactions between *C*3-OBn and *N*,*O*-dibenzyl functionality present in the latter intermediate. An alternative pathway could also exist *via* intermediate **162**, leading to the production of α-glycosides by bottom face attack. Cyclised intermediate **162** was proposed based on the production of by-product **64** that was isolated during attempted glycosidation of **62** with 3-bromopropanol.

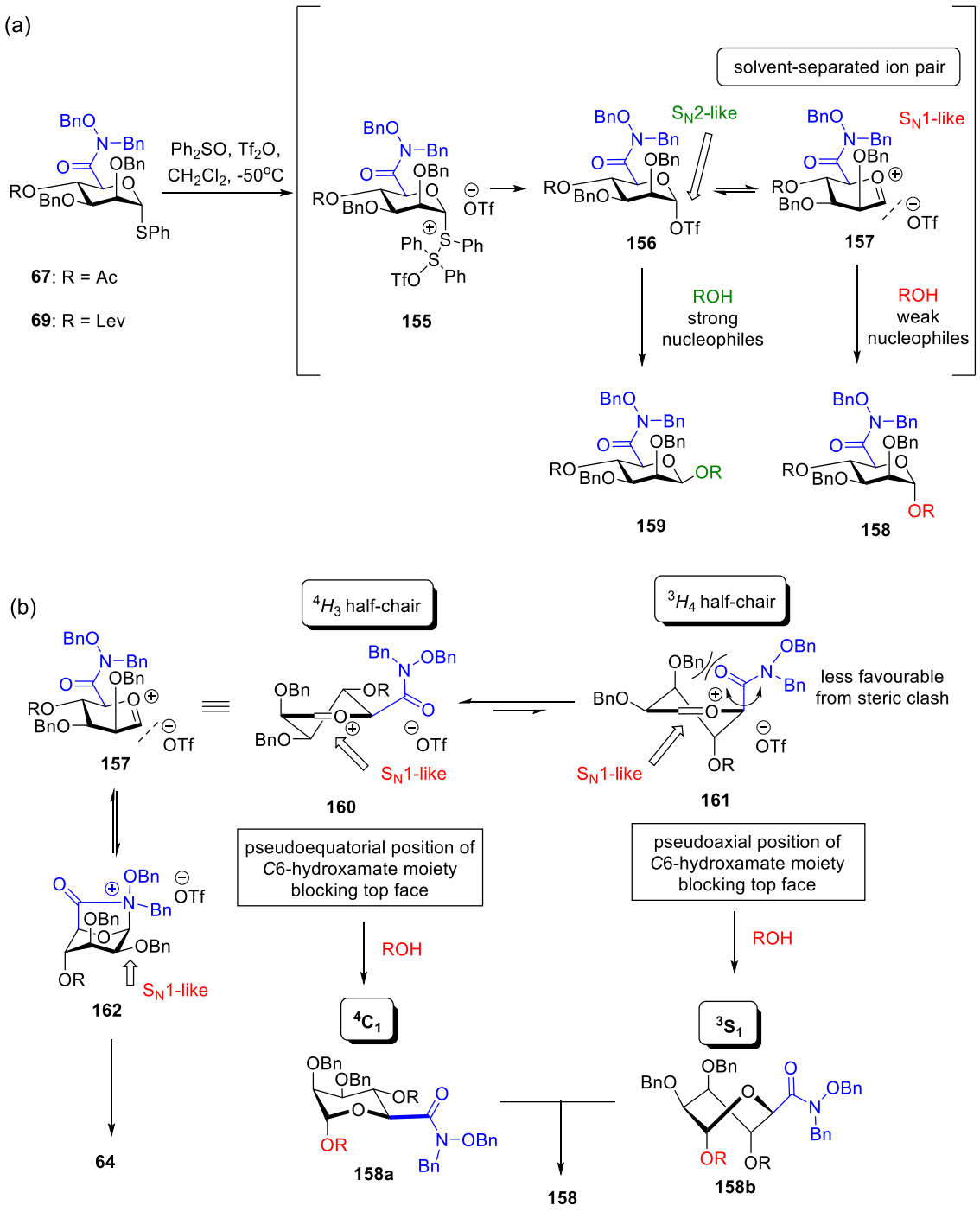


***Figure 47**: Possible mechanistic pathways involving putative transition states and conformations showing dominantly the α-directing effect of hydroxamate donors. S_N1 character increases with decreased acceptor nucleophilicity, leading to α-linked products.*

Given the considerably low yielding glycosylation reactions and the production of α-linked disaccharides (instead of desired linked products) discussed above, a subsequent synthetic methodology was designed based on a late stage oxidation

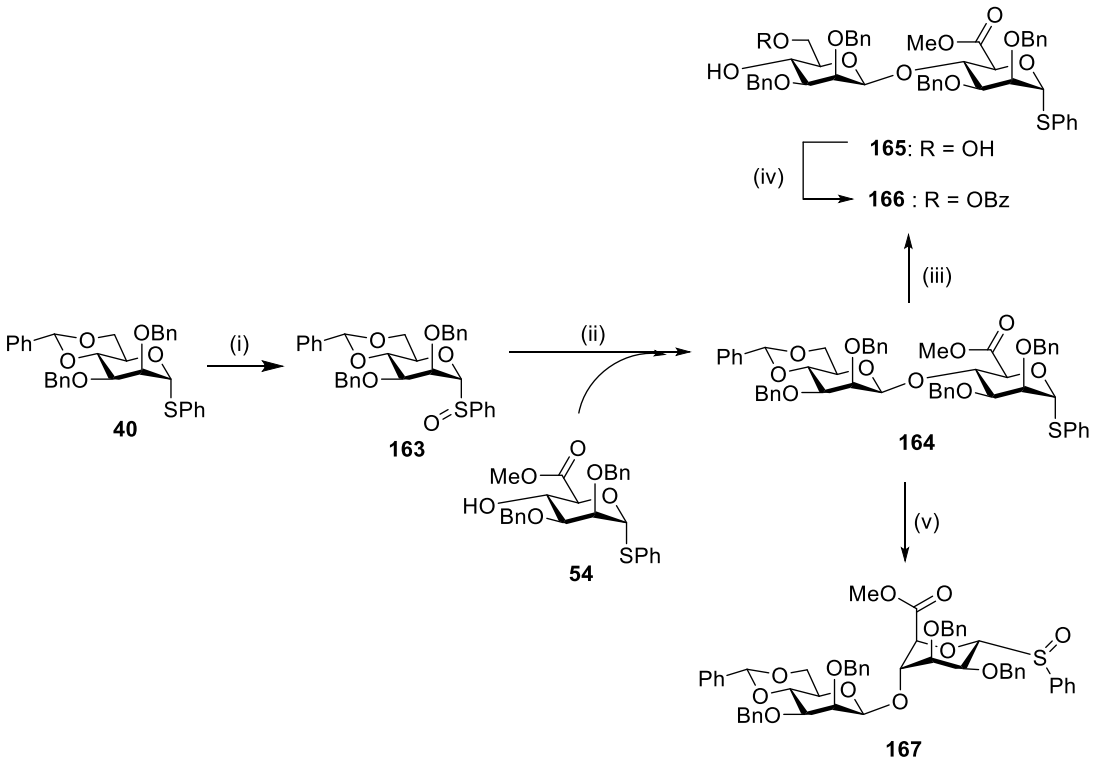
approach. This strategy was considered to be more beneficial in terms of delivering the products in higher yields compared to the use of *C*6-oxidised donors **67**, **69**. The challenge of the following approach laid in developing a methodology that could provide β-linked disaccharide building blocks which could then be elongated with high β-stereoselectivity. An additional challenging point was the ability to easily modify the obtained oligosaccharide at a later stage. Preliminary experimental data are discussed in Section 8.6. and the strategy is further elaborated is Section 10.1.

8.6. Synthesis of modified alginate oligosaccharides involving a late stage *C*6-modification

8.6.1. β-mannosylation approach

The following strategy was constructed based on the advantages offered by Crich's 4,6-benzylidene-mannopyranoside sulfoxide donor **163** and Codée's uronate thio-mannopyranoside building block **54** to provide high β-selectivities (Scheme 35).[148,235,161] In particular, disaccharide building block **164** was synthesised and further manipulated into producing disaccharide thioglycoside acceptor **166** and sulfoxide donor **167**.

Sulfoxide donor **163** was furnished as a single diastereoisomer in 73% yield with the use of oxidant *m*-CPBA in CH_2Cl_2 at -78 °C to -30 °C (Scheme 35).[236] Preactivation of **163** with Tf_2O in the presence of TTBP in CH_2Cl_2 at -78 °C and subsequent dropwise addition of **54** dissolved in CH_2Cl_2, furnished **164** as a β-linked disaccharide in 75% yield ($^1J_{C1'-H1'}$ = 156 Hz, C1': 101.1 ppm). This material was then exploited in producing thioglycoside disaccharide acceptor **166** (with acetal hydrolysis and selective protection of primary alcohol with a benzoyl group), and sulfoxide disaccharide donor **167**.

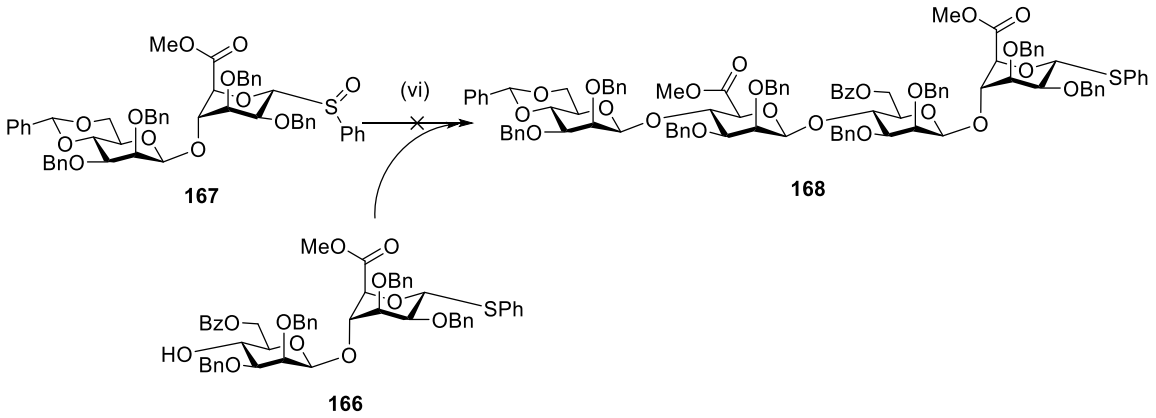


Scheme 35: *Synthesis of disaccharide acceptor **166** and donor **167** (i) m-CPBA, CH_2Cl_2, -78 °C to -30 °C, 3 h, 73% (ii) TTBP, Tf_2O, **54**, CH_2Cl_2, -80 °C, 30 min, 75% (iii) p-TsOH·H_2O, MeOH, 85 °C, 2 h, 69% (iv) BzCl, DMAP, pyridine, CH_2Cl_2, RT, 18 h, 76% (v) m-CPBA, CH_2Cl_2, -78 °C to -40 °C, 2 h, 66%.*

Sulfoxide **167** was isolated as a mixture of two diastereoisomers (3/1), as indicated by NMR data analysis. ^{1}H NMR data of **166** and **167** suggested that the reducing end in both disaccharides adopted a $^1C_4$ conformation. This is based on the coupling constants of the anomeric protons, exhibiting values equal to $^3J_{H1\text{-}H2}$ = 9.1 Hz (H_1: 5.79 ppm) for **166** and $^3J_{H1\text{-}H2}$ = 10.1 Hz (H_1: 5.05 ppm) for **167**, implying an axial relationship with neighbouring H_2.

Unfortunately, the glycosylation between **166** and **167** was fruitless, as tetrasaccharide **168** was not detected by NMR (Scheme 36). TLC analysis 30 min. after the addition of acceptor **167** showed decomposition of **167** and the presence of unreacted **166**. Flash column purification of the mixture did not isolate the hydrolysis

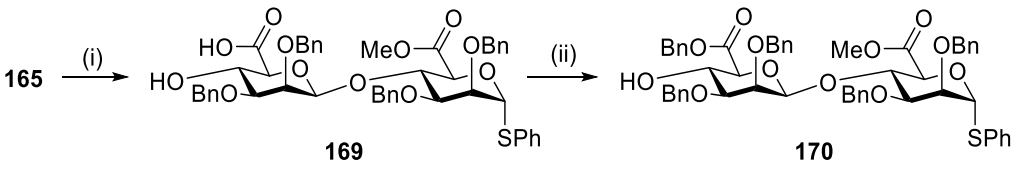
product of **167**, and only acceptor **166** (64%) was recovered. This late-stage modification strategy is further outlined in Section 10.1.1., as it could be a more viable approach for the production of a modified alginate oligosaccharide.



***Scheme 36**: Attempted synthesis of tetrasaccharide **168** for assembly of longer alginate oligosaccharide using a post-glycosylation modification approach (vi) Ph_2SO, TTBP, Tf_2O, **166**, CH_2Cl_2, -80 °C to -30 °C, 1 h.*

8.6.2. Synthesis of disaccharide 170 as a building block for the solid phase synthesis of modified alginate oligosaccharides

The next strategy was designed for the synthesis of a disaccharide building block for the construction of modified alginate oligosaccharides on solid phase. The past decade has seen dramatic developments in the area of solid phase-supported oligosaccharide synthesis.[237] The increasing interest in polymer-supported synthesis relies on the fact that it permits synthesis without the necessity of purifying and characterising the intermediates, as the excess of reagents can be removed by filtration.[105]



Scheme 37*: Synthesis of building block* ***170*** *that could be used for the construction of longer C6-modified alginate sequences in solid-phase synthesiser (i) TEMPO, BAIB, CH_2Cl_2/H_2O (2/1, v/v), RT, 1 h, 70% (ii) BnBr, K_2CO_3, DMF, RT, 24 h, 93%.*

As presented in Section 4.2., Codée and co-workers developed a methodology involving the conversion of thioglycoside donors to *N*-PTFA donors which were efficiently assembled with catalytic amounts of TfOH to construct alginate oligomers.[169] The first step taken towards this approach was the design and synthesis of disaccharide **170** (Scheme 37). *C*6-oxidation of the non-reducing end of **165** under TEMPO/BAIB conditions gave **169** in 70%. ^{13}C NMR data of **169** showed the presence of a new carbonyl carbon peak at 171.0 ppm, corresponding to the new carboxylic acid group. *C*6-esterification using BnBr and K_2CO_3 afforded **170** in 93% yield. NMR and HRMS analysis of **170** confirmed its structure (Found ammonium adduct at *m*/*z* 944.3685, $C_{54}H_{58}NO_{12}S$ **170** requires $(M+NH_4)^+$ 944.3680). The future work envisaged for **170** is further explained is Section 10.1.2.

9. Synthesis of conjugable 3-aminopropyl-D-ManA and 3-aminopropyl *C*6-modified D-ManA monosaccharides

Polymer modification *via* carbohydrate conjugation has attracted tremendous interest over past few decades due to their degradation properties *in vivo*, as well as inherent tunability and chirality. In particular, such biopolymers are ideal for delivery systems, owing to their inherent biocompatibility, biodegradability, and bioadhesive properties.[238,239] The synthetic approaches for sugar-based polymers can be divided into three major categories depending on the role of sugar building blocks involved:

(a) polysaccharide derivatisation, where the polymer backbone is mainly composed of carbohydrates; (b) carbohydrate-functionalised polymers, where sugar molecules decorate the polymer of interest as pendent groups (i.e. glycopolymers), and (c) polymers connected *via* a sugar linkage, where a saccharide or an oligosaccharide is used as a branch site or backbone (Figure 48). Apart from the production of novel polymeric systems, carbohydrates are additionally exploited for functionalisation of nanoparticles (i.e. glyconanoparticles).[240–243] More specifically, glyco-gold nanoparticles are widely applied as nanosensors in studies focused on carbohydrate-protein interactions and *in vivo* cell imaging, or as drug carriers in the development of nano- and personalised medicine.[241]

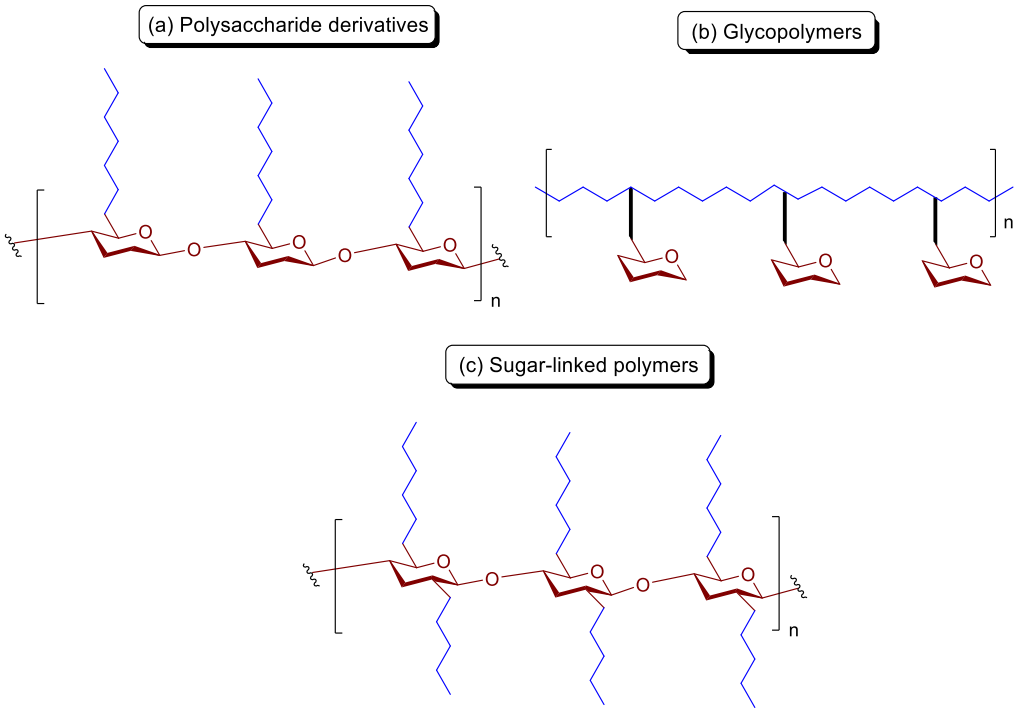


***Figure 48**: Schematic illustration and classification of carbohydrate-modified polymers [Adapted from Zhang et al. (2015)].*[238]

Given the previous discussion on the unsuccessful extension of mixed *C*6-modified alginate disaccharides (Sections 8.2. and 8.3.) and failed synthesis of a non-self oligosaccharide (Section 8.4.), it was considered that native mannuronate

60 and *C*6-bioisosteric mannuronates, **78** and **115** could be used in their deprotected forms to functionalise polymers for generation of novel biomaterials. The azidopropanol linker attached at the anomeric centre of the **60** and **78** offers the opportunity for conjugation to polymers (or nanoparticles) containing alkyne groups *via* click chemistry. In addition, reduction of the azide to the amine is beneficial, as it could be coupled to carboxylic acids for the production of amide-linked materials. In other words, the linker on these monosaccharide building blocks can be used as a handle for their attachment to polymers, producing novel glycopolymers (Figure 48b and 49).

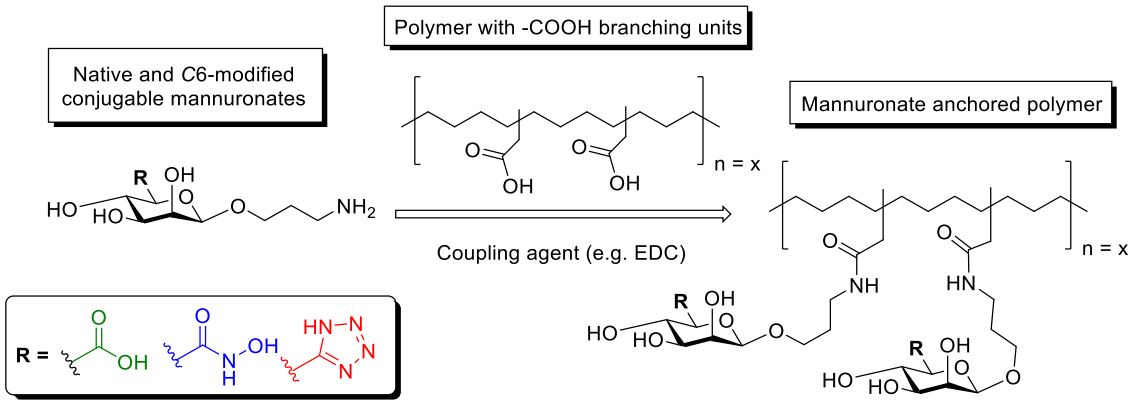


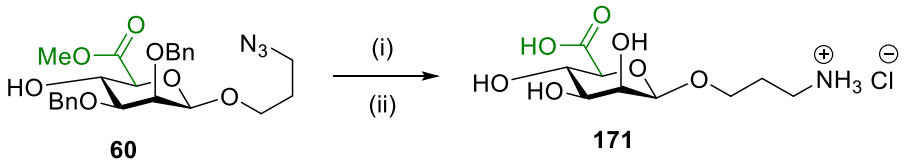
Figure 49: *Schematic representation of native and C6-modified mannuronate conjugation by coupling terminal amine with the carboxylic acid groups of a polymer.*

The approach taken here was towards a global deprotection of **60**, **78** and **115** using hydrogenolysis which would, unavoidably, lead to the production of aminopropanol linkers. The methods evaluated are discussed in further detail below.

9.1. Synthesis of 3-aminopropyl (β-D-mannopyranoside) uronic acid 171

Introductory Section 3 comprises of examples of alginate-based biomaterials that mainly result from hydrophobic derivatisation of alginate, or from crosslinking of it with other polymers. There are currently no examples in the literature where

mannuronic acids are linked to polymers for production of biomaterials exhibiting novel physicochemical properties. For the synthesis of a conjugable mannuronic acid **171**, native acceptor **60** was used (Scheme 38). Hydrolysis of *C*6-methyl ester with 0.45 M aq. KOH, followed by hydrogenolysis with 0.15 equiv. Pd/C, 0.15 equiv. $Pd(OH)_2/C$ and 1.0 equiv. of 0.1 M HCl in EtOH/THF (1.5/1, *v*/*v*, 0.05 M), furnished **171** in 36% yield (over 2 steps). TLC analysis of the hydrogenolysis step showed conversion of the starting material to a baseline product in 32 h. NMR spectra collected confirmed complete deprotection, as no Bn groups were present. HRMS spectrum found major ion at *m*/*z* 250.0928 which corresponds to $(M\text{-}H)^-$ for **171** ($C_9H_{17}NO_7$, **171** requires $(M\text{-}H)^-$, 250.0927).



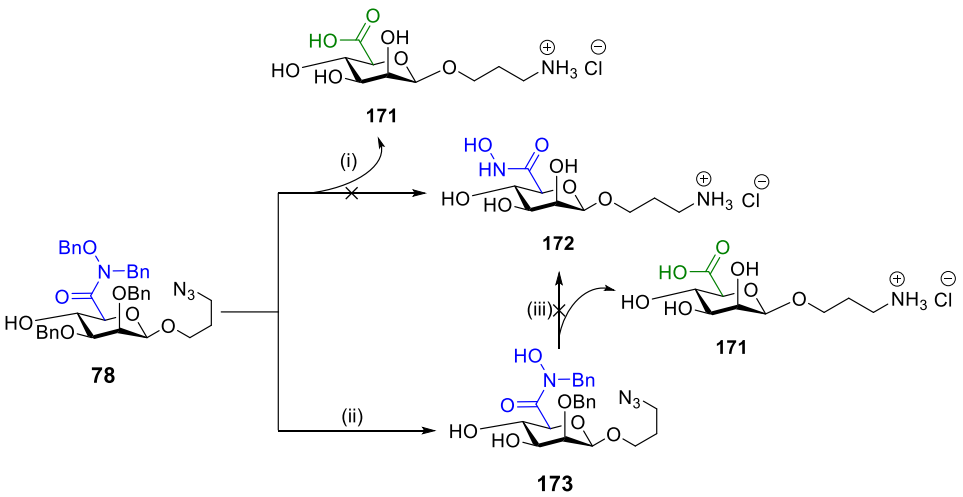
60 **171**

***Scheme 38**: Synthesis of **171** (i) 0.45 M KOH, THF/H_2O (1/1, v/v), RT, 2 h (ii) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2/C$ (20%), 0.1 M aq. HCl, EtOH/THF (1.5/1, v/v), RT, 32 h, 36% (over 2 steps) .*

9.2. Synthesis of 3-aminopropyl (β-D-mannopyranoside) hydroxamic acid 172

Conjugation of *C*6-hydroxamic acid mannuronates to polymers would be compelling to achieve, as these weak acids constitute one of the most important families of organic bioligands, widely used in biomedicine and biotechnology.[244] For example, hydroxamate-containing siderophores are exploited as drug-conjugate complexes for the treatment of iron overload associated with repeated blood transfusions, cancer and malaria.[192] In addition, bacterial cells evolved high-affinity iron uptake mechanisms comprised of siderophores, and are activated under stress conditions caused by restricted access to soluble iron.[245] Considering that one of the

crucial mechanisms of antibiotic resistance in bacteria is decreased outer membrane permeability, siderophore-drug complexes are used to 'smuggle' the antibiotic molecule into the bacterial cell.[246] This strategy is also known as 'Trojan horse' strategy, whereby a Fe(III) siderophore is linked to the drug that is subsequently released in its free active form when the siderophore-drug conjugate reaches the cytoplasm, killing the microorganism.[192,191] The 'Trojan horse' approach may also employ heavy metals (e.g. Ga^{3+}), whose facilitated transport into the bacterial cell can prevent biofilm formation, by bacterial iron metabolism interruption.[192] As a hydroxamate-containing monosaccharide, **172** could be attached on the backbone of polymers, or surface of nanoparticles, producing systems with alternative chelating properties for various applications, such as delivery of metal-based drugs, metals to promote cell apoptosis, or siderophore-drug conjugates.



***Scheme 39**: Attempted synthesis of **172** under hydrogenolysis and oxidative debenzylation conditions (i) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2$/C (20%), 5% aq. $NaHCO_3$, EtOH/THF (1.5/1), RT, 21 h (ii) $NaBrO_3$/$Na_2S_2O_4$, EtOAc, RT, 32 h, 40% (iii) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2$/C (20%), 0.1 M aq. HCl, EtOH/THF (1.5/1), RT, 6 h.*

Table 3: Deprotection conditions used for the conversion of 78 to 172					
Hydrogenolysis conditions [a,b]					
Entry	Pd/C (10 %) total equiv.	$Pd(OH)_2/C$ (20 %) total equiv.	Acid or base used	Duration	Observation by TLC analysis NMR and HRMS data[c]
1	0.35	0.35	0.1 M HCl (1.0 equiv.)	28 h	Unidentified product
2	0.42	0.42	5% aq. $NaHCO_3$ (2.0 equiv.)	21 h	**171**: 84% yield
3	0.5	0.5	5% aq. $NaHCO_3$ (1 equiv.) and 0.1 M HCl (1.0 equiv.)	6 h ($NaHCO_3$) and 3 h (HCl)	Unidentified product
4	0.5	-	5% aq. $NaHCO_3$ (1 equiv.) and 0.1 M HCl (1.0 equiv.)	6 h ($NaHCO_3$) and 3 h (HCl)	Unidentified product
5[e]	0.1	0.1	0.1 M HCl (1.0 equiv.)	6 h	**171**: 94% yield
Oxidative debenzylation conditions with $NaBrO_3$ and $Na_2S_2O_4$					
	$NaBrO_3$ (0.45 M aq.) total equiv.	$Na_2S_2O_4$ (0.2 M aq.) total equiv.	EtOAc	Duration	Observation by TLC analysis NMR and HRMS data
6	9	8	0.08 M	32 h	**173**: 40% yield

[a] The concentration of the reaction during hydrogenolysis was kept 0.05 M and the solvents used initially were EtOH/THF (1.5/1, *v*/*v*) while 2nd and 3rd attempts (same entries) MeOH/H_2O (1/1, *v*/*v*) were used.

[b] The temperature was at RT at all times.

[c] TLC solvent system used was H_2O/MeCN, 1/2.

[d] Theoretical isotope mass $(M+H)^+$ 267.1192, $(M-H)^-$ 265.1041 and $(M+Cl)^-$ 301.0808.

[e] The starting material used was the 3-azidopropyl *N*-benzyl (phenyl 2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate **173** obtained from entry 6.

Global deprotection and reduction of the azido-group in hydroxamate acceptor **78** was not as straightforward as the deprotection of native acceptor **60** discussed above. Scheme 39 and Table 3 summarise the conditions applied to substrate **78** for the synthesis of **172**. Reductive cleavage by hydrogenolysis using Pd/C (0.35 equiv. in total during 28 h), $Pd(OH)_2/C$ (0.35 equiv. in total during 28 h) and 0.1 M HCl (1.0 equiv.) did not furnish desirable material **172** (Entry 1). NMR and HRMS analysis of isolated product was inconclusive. Based on the knowledge obtained from the hydrogenolysis of **125** to *C*6-hydroxamic α-phosphate **34** (Section 7.1.), the same

conditions were applied next using Pd/C (0.42 equiv. in total during 21 h), $Pd(OH)_2/C$ (0.42 equiv. in total during 21 h), and 5% aq. $NaHCO_3$ (2.0 equiv.). TLC analysis showed complete conversion of the starting material **78** to a lower R_f material. NMR and HRMS data showed the production of uronic acid **171** (84% yield), indicating hydrolysis of the hydroxamate functionality under these set of conditions (Entry 2, Scheme 39).

Following these results, a subsequent approach was the application of an oxidative debenzylation protocol, using $NaBrO_3$ and $Na_2S_2O_4$.[247] As opposed to reductive debenzylation, these oxidative debenzylation conditions demonstrated selective removal of benzyl ethers in the presence of azides. Furthermore, $NaBrO_3/Na_2S_2O_4$ were reported to be carried out in high conversion, with ester and amide type of protecting groups remaining intact. TLC analysis of a reaction mixture with **78**, 0.45 M $NaBrO_3$ (4.5 equiv.) and 0.2 M $Na_2S_2O_4$ (4.0 equiv.) displayed a slow conversion after 16 h (Entry 6, Scheme 39). An additional batch of $NaBrO_3/Na_2S_2O_4$ was added to force the reaction to completion, however, after 32 h a mixture of compounds was still present according to TLC analysis. Purification by flash column chromatography isolated *N*-benzylated **173** as the major compound (40% yield). In order to cleave the remaining Bn group, **173** was subjected under hydrogenolysis conditions where the catalysts were added in batches (2 x 0.05 equiv. every 3 h) and the reaction was monitored by TLC analysis (Entry 5, Scheme 39).The addition of 0.1 M HCl was considered to avoid the formation of uronic acid **171** (as observed in entry 2 where 5% aq. $NaHCO_3$ was added), however, these conditions afforded only **171** (94% yield) after 6 h.

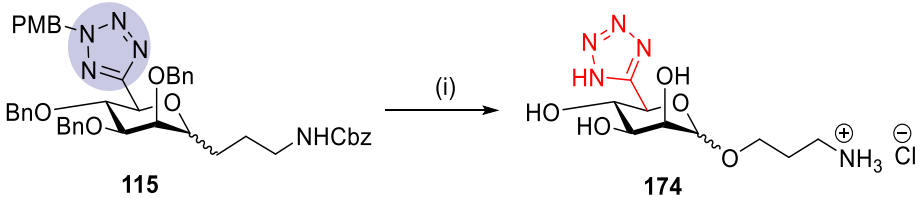
Based on the information gathered from the above observations, $Pd(OH)_2/C$ was regarded to be responsible for the hydrolysis of the hydroxamate functionality to the corresponding carboxylic acid. Therefore, a new bottle of Pearlman's catalyst was used in the next attempt (Entry 3). The reaction was monitored every 3 h and during the initial 6 h (where 1.0 equiv. of 5% aq. $NaHCO_3$ was present in the mixture) TLC analysis suggested that the reaction was quenched presumably by the formed amino-group which poisoned the catalyst. Upon filtration through Celite®, the remaining crude material was re-dissolved in $MeOH/H_2O$ (1/1, *v*/*v*, 0.05 M) and was subjected under the same hydrogenolysis conditions (0.25 equiv. of each catalyst with the exception of adding 1.0 equiv of 0.1 M HCl). TLC analysis after 3 h showed the presence of a deprotected material (R_f = 0.19) which was isolated and characterised. ESI-MS data obtained did not display expected theoretical isotope pattern for **172** ($C_9H_{18}N_2O_7$, **172** requires $(M-H)^-$ 265.1041). ^{13}C NMR spectrum of this material showed carbonyl carbon at 173.5 ppm, and the carbon peak of α-carbon in anomeric linker ($OCH_2CH_2\underline{C}H_2$) appeared to be at 45.6 ppm (instead of 37.5 ppm as observed in the NMR data of **171** and **174**), indicating that a replacement of the amino-group with an oxygen-containing functionality could be responsible for the downfield shift. NMR and HRMS analysis were inconclusive and no plausible structure was proposed, as alkylation of the structure to match the obtained data could not be explained under these conditions. The same results were obtained by repeating the experimental procedure using only Pd/C as a catalyst (Entry 4).

According to the above results, an alternative reaction occurred under hydrogenolysis conditions, which was not observed earlier during deprotection of *C*6-hydroxamate 1-phosphate **125**. Considering that similar conditions (catalyst

equivalents) were used for deprotection of **125** and **78**, the anomeric 3-azidopropanol linker may be accountable for the undesirable reaction.

9.3. Synthesis of 3-aminopropyl (6-*C*-tetrazol-5-yl)-α/β-D-mannopyranoside 174

Similar to Sections 9.1. and 9.2., carbohydrate-functionalisation of polymers (or nanoparticles) using tetrazole-containing monosaccharide **174** could lead to the production of novel biomaterials. As explained in Section 5., tetrazolic acids exhibit stronger hydrogen bond interactions and at the same time are more lipophilic compared to carboxylate anions.[182]



115

α/β= 3/1 (after column chromatography)

174

α/β= 3/1

***Scheme 40**: Synthesis of **174** (ii) $H_{2(g)}$, Pd/C (10%), $Pd(OH)_2$/C (20%), 0.1 M aq. HCl, EtOH/THF (1.5/1, v/v), RT, 56 h, 96%.*

Hydrogenolysis of **115** (α/β = 3/1 after flash column purification) with 0.5 equiv. of Pd/C and $Pd(OH)_2$/C furnished **174** (96% yield) in the same ratio (α/β = 3/1) (Scheme 40). TLC analysis showed conversion of **115** to a lower R_f spot after 24 h, and NMR data collected confirmed its successful deprotection. ^{13}C NMR spectrum indicated 2 peaks corresponding to the quaternary carbons (C_q tetrazole) of the anomeric mixture with a chemical shift of 160.1 ppm, close to that observed for *C*6-tetrazole 1-phosphate **35** (160.8 ppm). ESI-MS spectrum confirmed the mass of **115**, where the major ion was observed at *m*/*z* 276.1309 ($C_9H_{19}N_5O_5$, **115** requires $(M+H)^+$ 276.1308).

10. Conclusions and Future Directions

Herein is presented the first synthesis set of *C*6-hydroxamate and C6-tetrazole mannopyrannoside analogues for the synthesis of modified alginate building blocks. As reported in the sections above, the project faced synthetic obstacles due to the design and methodology development performed at its initial stages. Undoubtedly, considering the information gathered up to this point, new routes could be designed for the construction of designer alginate oligomers.

10.1. Late stage *C*6-modification of alginate oligosaccharides

10.1.1. Future directions for β-mannosylation approach

The synthesis of tetrasaccharide **168** could be attempted again under the same gylcosylation conditions (Section 8.6.1.) or different types of disaccharide donor **167** could be used by replacing sulfoxide anomeric group with other orthogonal groups to thiophenol (e.g. *N*-PTFA, TCA), allowing the application of alternative glycosylation conditions. Following successful synthesis of β-linked tetrasaccharide **168**, conversion of it to donors **175** and thioglycoside acceptor **176** (in the same way **164** was converted) could be achieved to deliver octasaccharide **177** when assembled (Figure 50). Finally, when converted to an *O*-glycoside, **177** could be modified at *C*6 position by hydrolysis (acidic and basic), oxidation and coupling to H_2NOH, to give designer alginate octasaccharide **178**. Hydrolysis of the remaining *C*6-methyl ester groups and global deprotection under hydrogenolysis conditions could provide the modified alginate oligomer.

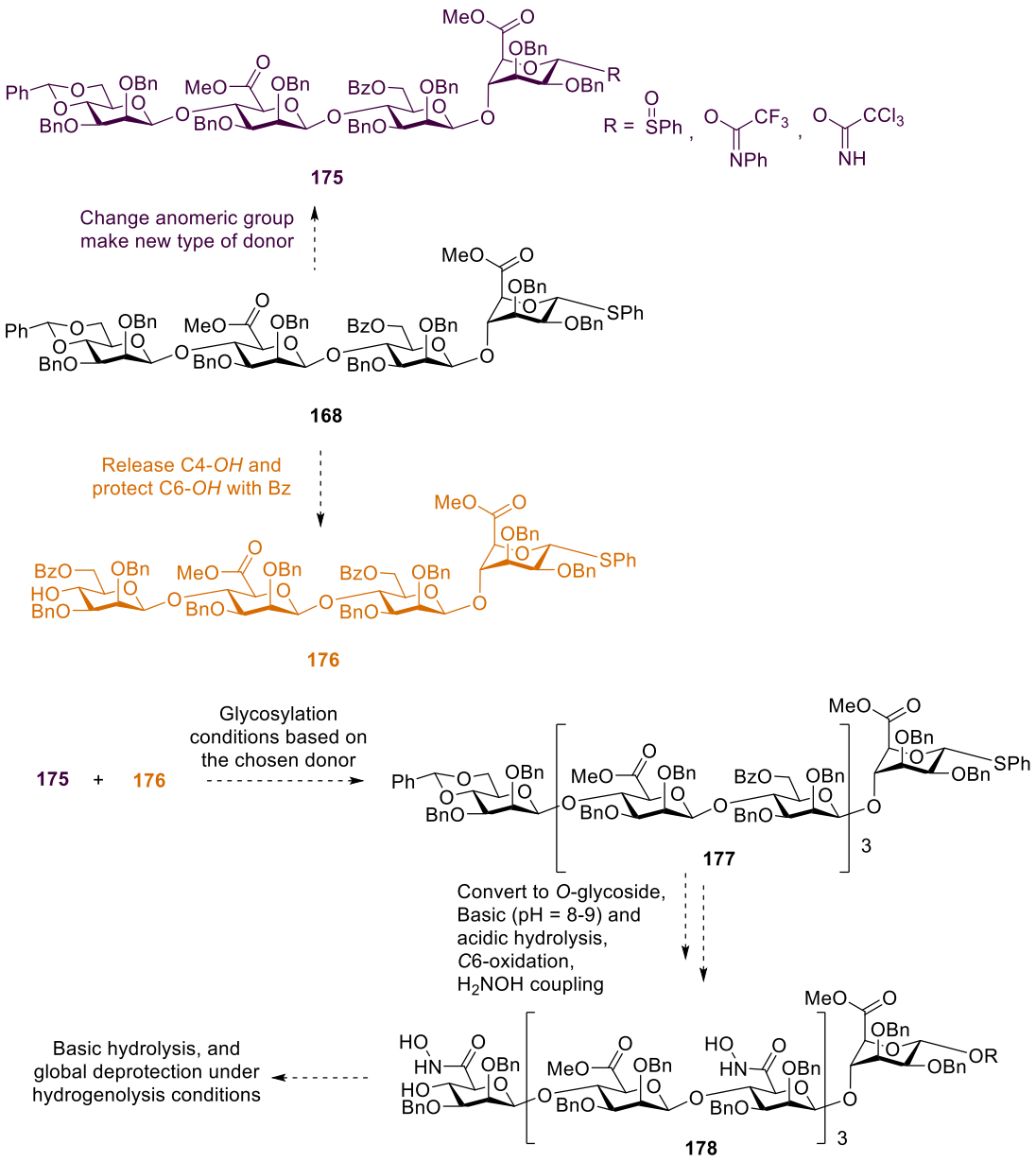


***Figure 50**: Schematic illustration of future work involving the assembly of tetrasaccharides **175** and **176** towards the synthesis of a modified alginate oligosaccharide.*

10.1.2. Future work with disaccharide 170 as a building block for solid phase synthesis

As discussed in Section 8.6.2., disaccharide **170** could be employed as a building block for the solid-phase synthesis of a modified alginate oligosaccharide, as outlined in Figure 51.

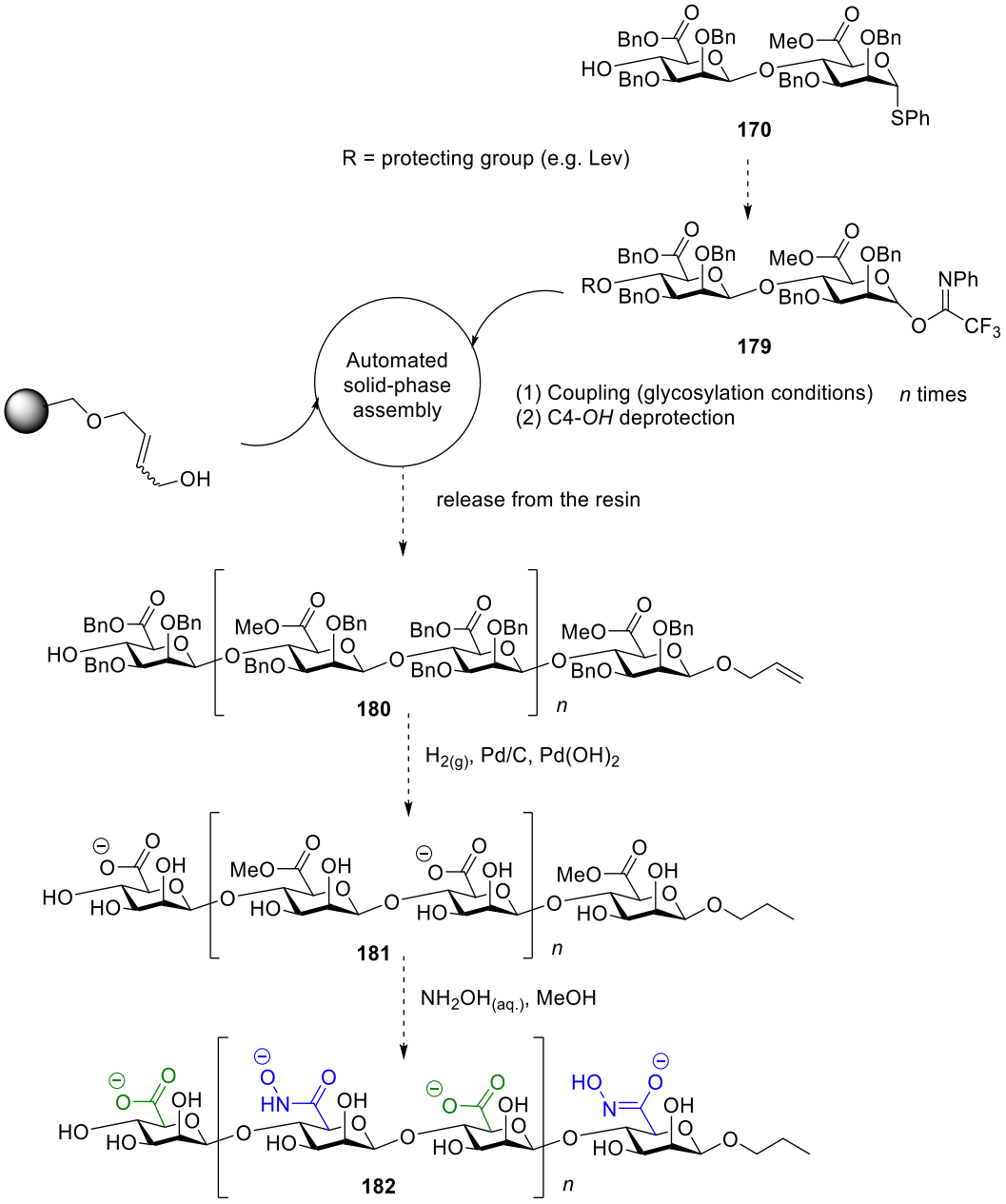


***Figure 51**: Schematic representation of future work for the solid phase assembly of C6-modified alginate oligosaccharide involving building block **170**.*

An acceptor-bound approach is generally considered as more profitable because the attachment of the nucleophile allows the use for excess of the donor which would then drive the reaction to completion.[237] As for the donor-bound approach, side reactions usually occur by decomposition of the reactive species (donor), which results in a direct reduction in overall yield.

Conversion of thioglycoside **170** to *N*-PTFA donor **179** orthogonally protected at *C*4 position, could be first attached on the resin (e.g. Merrifield resin functionalised with a butanediol linker), and C4-*OH* liberation would furnish the acceptor disaccharide that can be then glycosylated with **179** at a specific amount of cycles (Figure 51). Synthesis of an oligosaccharide (e.g. **180**) and subsequent cleavage of it from the resin, could be subjected to a series of reaction steps, leading to *C*6-modification in a chemoselective fashion. For the production of an alginate oligomer bearing *C*6-hydroxamic acid functionalities, the steps that could be taken are hydrogenolysis of the material, and conversion of the remaining methyl esters to hydroxamic acids with aq. H_2NOH in MeOH to give **182**.

10.2. *C*5- and *C*6-modified 1-phosphate building blocks

Sugar 1-phosphates *C*6-hydroxamic acid **34**, *C*6-tetrazole **35** and *C*6-amide **126** could be employed for the chemical synthesis of respective *C*6-modified GDP-D-ManA building blocks *via* a pyrophosphorylation strategy, involving their reaction with activated monophosphate species (e.g. GMP-morpholidate) in the presence of a catalyst (e.g. DCI, *N*-MIC).

Sugar 1-phosphates **126** and **131** can be used as substrates for GMP, as the absence of a charge at the *C*6-position makes them ideal candidates for the enzymatic synthesis of their respective sugar-nucleotide analogues. *C*6-amide GDP-D-ManA could be tested as a GMD inhibitor, or it could be used as 'fuel' for polymerase Alg8, producing a non-anionic alginate polysaccharide in physiological conditions. Moreover, following successful enzymatic synthesis, 5-*C*-Me GDP-D-Man could then be *C*6-oxidised (chemically or enzymatically with GMD if any inhibitory effect is not exhibited) for integrating the production of a 5-*C*-Me GDP-D-ManA

building block **183**. Incorporation of 5-*C*-Me-D-ManA in a polymannuronate chain could consequently probe epimerase AlgG and lyase AlgL (Figure 52).

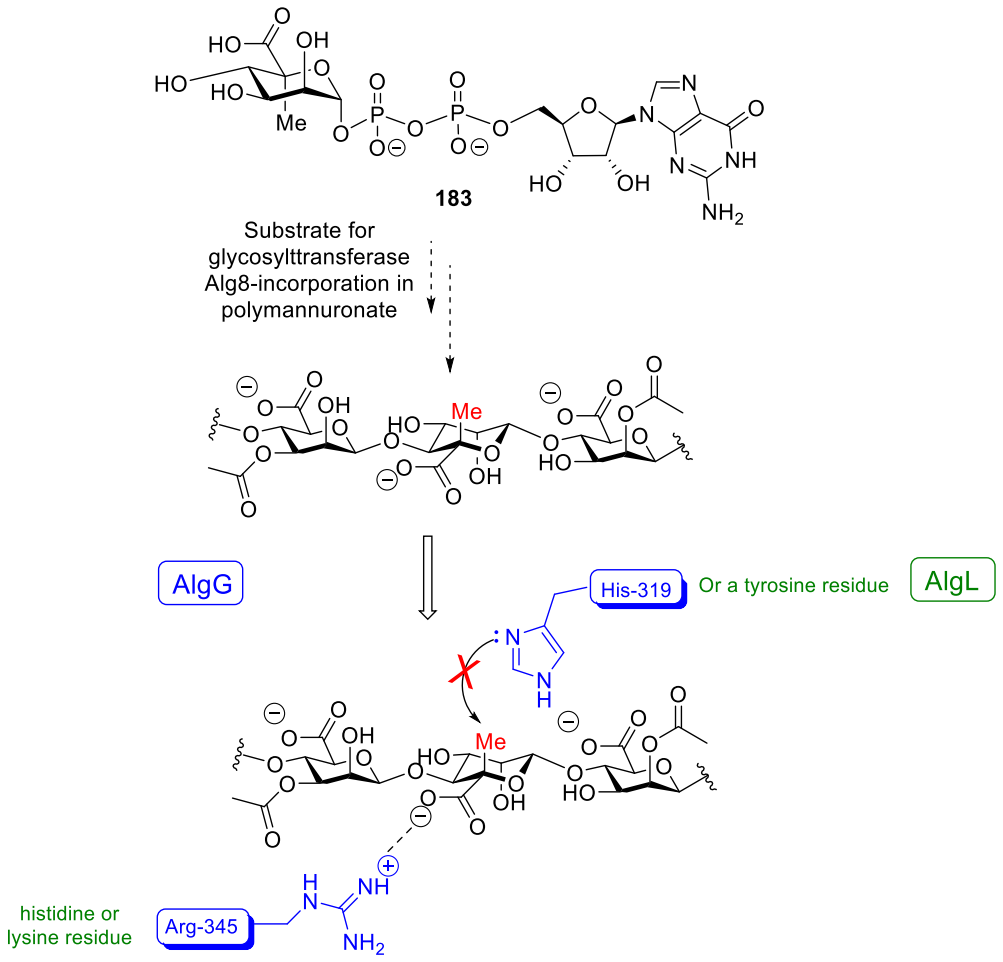


***Figure 52**: 5-C-Me-GDP-D-ManA **183** could be used as an Alg8 substrate to incorporate 5-C-Me-D-ManA in alginate polysaccharide to provide insight on the AlgG (blue) and AlgL (green) biochemical pathway.*

Acidic sugar 1-phosphates **34** and **35** may not be successfully converted to their respective *C*6-modified GDP-D-ManA analogues using GMP (due to their charge in physiological conditions). However, they can be employed as donors for the enzymatic synthesis of a designer alginate, using a phosphorylase. As opposed to sugar nucleotide-dependent glycosyltransferases, phosphorylases can enable efficient and regioselective synthesis of diverse oligosaccharides with sugar

1-phosphates as donors.[248,249] For **34** and **35**, an inverting phosphorylase could promote oligosaccharide assembly *via* axial-to-equatorial substitution on *C*1 through the base catalytic residue (Figure 53). Utilisation of phosphorylases for oligosaccharide synthesis has attracted tremendous interest due to high regioisomeric purity obtained (separation of product isoforms is not required), therefore, the number of reported phosphorylases has gradually increased.[249,250] Recent studies report characterisation and engineering of phosphorylases that could be exploited as tools for this purpose[248], and considering that the phosphorolysis of a glycoside to produce the corresponding sugar 1-phosphate is reversible[249], β-1,4-mannooligosaccharide phosphorylase (GH130) could be structurally modified for oligosaccharide synthesis, instead of hydrolysis.

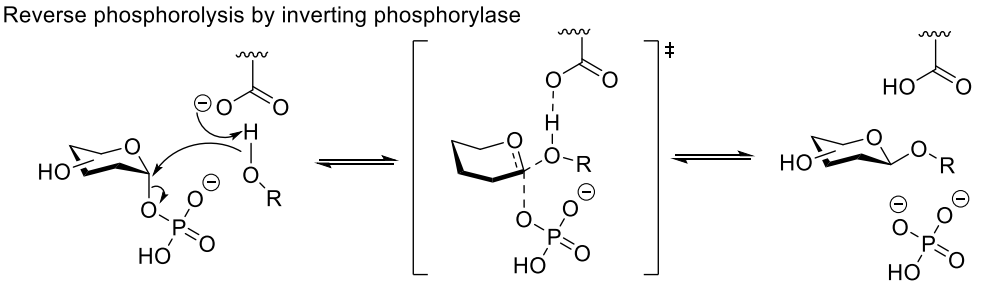
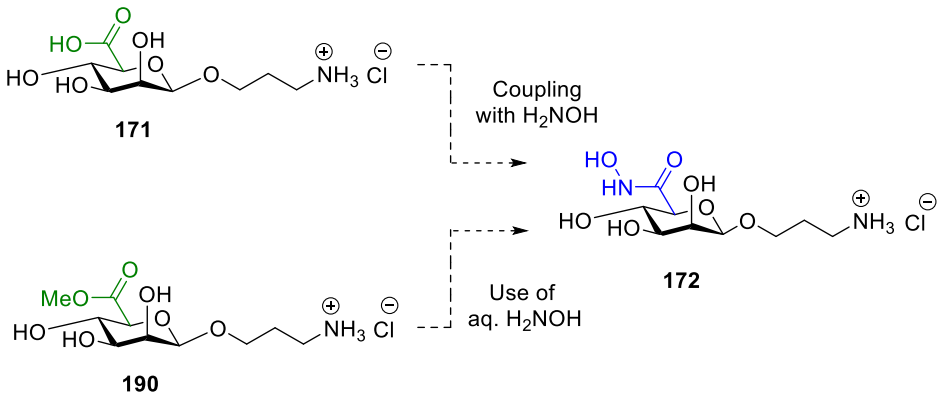


***Figure 53**: Schematic representation of β-stereoselective glycosylation using inverting phosphorylase and α-1-phosphates as donors [Adapted from Benkoulouche et al. (2019)].*[248]

10.3. Conjugable 3-aminopropyl (β-D-mannopyranoside) hydroxamic acid 172

The synthesis of **172** was unsuccessful when **78** was used as the precursor, as unidentified products were produced, or hydrolysis of the hydroxamate group furnished **171** instead. An alternative route to pursue this synthesis could either be the coupling of H_2NOH to the carboxylic acid of **171**, or the conversion of *C*6-methyl ester of **190** to *C*6-hydroxamic acid using aq. H_2NOH in MeOH (Scheme 41).



***Scheme 41**: Alternative routes proposed for the synthesis of **172**.*

10.4. General conclusion on alginate oligosaccharide synthesis

The research presented in this thesis has shown preliminary results obtained from the assembly of novel *C*6-hydroxamate and native mannuronate building blocks towards modified alginate oligosaccharides, by following a pre-glycosylation oxidation strategy which was previously demonstrated to give β-linked products.[150,211] Although synthesis of mixed disaccharide and trisaccharide systems was achieved (i.e. **140**,**143** and **144**), the yields of the glycosylation reactions were low for further extension from the non-reducing end. Moreover, the stereochemistry of the 1,4-linkages constructed using hydroxamate donors **67** and **69** was α instead of the desirable β, as confirmed by coupled HSQC and n*O*e spectra. Consequently, an alternative approach was designed, based on Crich's β-mannosylation methodology and preliminary results are discussed in Section 8.6.1. Synthesis of β-linked disaccharides **166** and **167** for oligosaccharide assembly and late-stage *C*6-modification is considered a more promising approach, compared to the pre-glycosylation strategy followed initially. Further research using this methodology is required for accessing longer modified alginate oligosaccharides, as mentioned in Section 10.1.

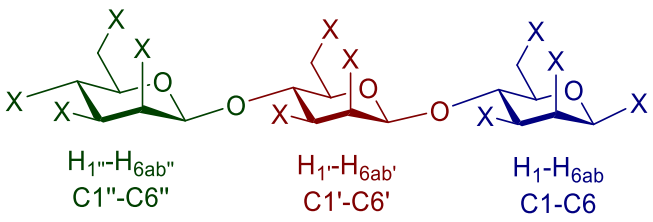
11. Experimental

11.1. General methods and materials

^{1}H NMR spectra were recorded at 400 MHz on a Bruker AVIII400 spectrometer, using deuterochloroform (or other indicated solvent) as reference or internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); app. t (apparent triplet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); ddt (doublet of doublet of triplets); sp (septet) or m (multiplet). The number of protons (n) for a given resonance is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. ^{1}H NMR signals were assigned with the aid of gDQCOSY.

^{13}C NMR spectra were recorded at 100 MHz on a Bruker AVIII400 spectrometer using proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of ppm relative to TMS where δ (TMS) = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. ^{13}C NMR signals were assigned with the aid of gHSQCAD.

NMR data were analysed using Mestrenova software and reported based on the scheme below:



IR spectra were recorded on a Diamond 1000 FTIR spectrometer. Absorption maxima are reported in wavenumbers (cm^{-1}). Intensities of the maxima are quoted as strong (s), medium (m) or weak (w).

Melting points were determined using a Gallenkamp MF-370 or a Electrothermal 9100 melting point apparatuses and are uncorrected.

Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60F254 analytical plates (aluminium support). Visualisation was by absorption of UV radiation (245 nm), or thermal development after dipping in 5 % H_2SO_4 in MeOH.

Manual flash column chromatography was carried out on silica gel 60 (0.043–0.063 mm) under a positive pressure of compressed air.

Automatic Column Flash chromatography was carried out on silica gel (Reveleris® X2 system) under a positive pressure of compressed N_2.

Purification by C18 chromatography was conducted using a Thermoscientific ×30 SPE column (HyperSep C18, 6 mL) eluting with H_2O.

For glycosylation reactions the materials used were co-evaporated with anhydrous toluene (3 x). Oven-dried glassware and magnetic stirrer bars were used.

Reactions requiring low temperatures used the following cooling baths: -80 °C (liquid nitrogen/CH_2Cl_2), -15 °C (NaCl/ ice/water) and 0 °C (ice/water).

Where appropriate and if not stated otherwise, all non-aqueous reactions were performed under an inert atmosphere of nitrogen, using a vacuum manifold with nitrogen passed through 4 Å molecular sieves and self-indicating silica gel.

Dry Solvents (CH_2Cl_2, DMF, Et_2O, toluene, THF) were purified by passing through activated alumina columns and used directly from a Pure Solv-MD solvent purification system and were transferred under nitrogen. Anhydrous MeOH was dried with 4 Å molecular sieves.

Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to *n*-hexane and petroleum ether to the fraction boiling between 40-60 °C.

Chemicals were purchased from Acros UK, Aldrich UK, Avocado UK, Fisher UK or Fluka UK. *In vacuo* refers to the use of a rotary evaporator attached to a diaphragm pump.

Optical rotations were recorded on automatic polarimeter Rudolph autopol I or Bellingham and Stanley ADP430 (concentration in g/100 mL).

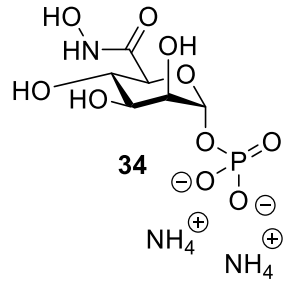
MS and HRMS (ESI) were obtained on Waters (Xevo, G2-XS TOF) or Waters Micromass LCT spectrometers using a methanol mobile phase. High resolution (ESI) spectra were obtained on a Xevo, G2-XS TOF mass spectrometer. HRMS was obtained using a lock-mass to adjust the calibrated mass.

General procedure for strong anion exchange chromatography: BioRad cartridge was pre-washed with minimum 3 column volumes (15 mL) of degassed H_2O. The sample was dissolved in minimal amount of degassed H_2O. The cartridge was eluted with 3 column volumes of degassed H_2O, followed by 3 column volumes of 1.0 M NH_4HCO_3. Upon injection of the sample onto column, the sample was collected in 5 mL fractions. When TLC analysis indicated the frations tha contained the compound, they were collected directly into flask, freezed and lyophilised. The residue was re-dissolved in H_2O and lyophilisation was repeated to ensure all NH_4HCO_3 is

removed. After using the column wash with 3 column volumes of degassed H_2O then 10% MeOH/degassed H_2O to store.

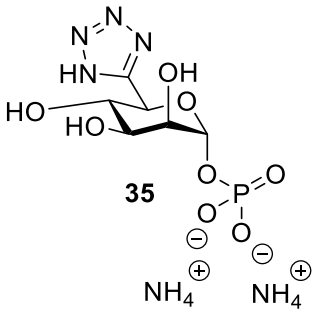
Exchanging salt form: Et_3NH^+: Syringe filled with Dowex H^+ resin was wash with MeOH, acetone, CH_2Cl_2 and H_2O before use. 10% Et_3N solution in H_2O was passed through the resin until pH = ~10/11. The sample was dissolved in minimal amount of H_2O before applied to the resin. The sample was washed through with H_2O directly into a flask, freezed and lyophilised.

11.1.1. (6-*C*-hydroxamic acid) α-D-mannopyranoside 1-phosphate (*bis*-ammonium salt) 34



A suspension of hydroxamate **125** (56 mg, 67 μmol, 1.0 equiv.), 10% Pd/C (21 mg, 20 μmol, 0.3 equiv.), 20% $Pd(OH)_2/C$ (14 mg, 20 μmol, 0.3 equiv.) and 5% aq. $NaHCO_3$ (227 μL, 0.13 mmol, 2.0 equiv.) in a mixture of EtOH/THF (1.3 mL, 1.5/1 *v/v*) was stirred under an atmosphere of hydrogen (1 atm, balloon) at room temperature for 5 h. TLC analysis (hexane/EtOAc, 1/2) showed complete conversion of starting material to a lower R_f spot. The reaction mixture was filtered through Celite®, followed by solvent removal *in vacuo*. Purification *via* strong anion exchange chromatography was conducted manually using a Bio-Scale™ Mini UNOsphere™ Q (strong anion exchange) cartridge (See General methods) and lyophilisation afforded **34** as a white solid (15 mg, 45 μmol, 67%). R_f 0.30 (MeCN/H_2O, 2/1); $[\alpha]_D^{22}$ -6.13 (*c*. 0.66, H_2O); **^{1}H NMR** (400 MHz; D_2O) 5.35 (1 H, dd, *J* = 8.1, 1.2 Hz, H_1), 4.07 (1 H, d, *J* = 8.5 Hz, H_5), 3.95 – 3.78 (3 H, m, H_2, H_3, H_4); **^{13}C NMR** (101 MHz; D_2O) δ 167.5 (*C*(O)N(H)OH), 96.0 (d, *J* = 5.1 Hz, C1), 71.4 (C5), 70.1 (d, *J* = 8.9 Hz, C2), 69.4 (C3), 67.6 (C4); **^{31}P NMR** δ_P (162 MHz, D_2O) 1.91 (s); **HRMS** (ES^-) *m/z* [Found: $(M-H)^-$ 288.0127 $C_6H_{10}NO_{10}P$ requires $(M-H)^-$, 288.0126].

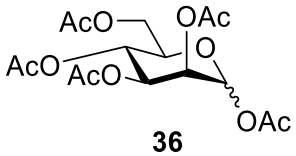
11.1.2. (6-*C*-tetrazol-5-yl)-α-D-mannopyranoside 1-phosphate (*bis*-ammonium salt) 35



35

A suspension of *C*6-tetrazole **129** and **130** (190 mg, 0.22 mmol, 1.0 equiv.), 10% Pd/C (140 mg, 0.13 mmol, 0.6 equiv.), 20% $Pd(OH)_2/C$ (92 mg, 0.13 mmol, 0.6 equiv.) and 5% aq. $NaHCO_3$ (739 µL, 0.44 mmol, 2.0 equiv.) in a mixture of EtOH/THF (4.4 mL, 1.5/1 *v/v*) was stirred under an atmosphere of hydrogen (1 atm, balloon) at room temperature for 24 h. TLC analysis (hexane/EtOAc, 1/2) showed complete conversion of starting material to a lower R_f spot. The reaction mixture was filtered through Celite®, followed by solvent removal *in vacuo*. Purification *via* strong anion exchange chromatography was conducted manually using a Bio-Scale™ Mini UNOsphere™ Q (strong anion exchange) cartridge (See General methods) and lyophilisation afforded **35** as a white powder (53 mg, 0.16 mmol, 72%). R_f 0.42 (H_2O/MeCN, 1/2); $[\alpha]_D^{22}$ -3.0 (*c*. 1.0, H_2O); **^{1}H NMR** (400 MHz; D_2O) δ 5.41 (1 H, dd, *J* = 7.9, 1.7 Hz, H_1), 5.08 (1 H, d, *J* = 9.7 Hz, H_5), 4.05 (1 H, app. t, *J* = 9.6 Hz, H_4), 4.02 – 3.97 (2 H, m, H_2 and H_3); **^{13}C NMR** (101 MHz; D_2O) δ 160.8 (C_q tetrazole), 96.1 (C1), 70.5 (C2), 69.7 (C3), 69.4 (C4), 67.1 (C5); **^{31}P NMR** δ_P (162 MHz, D_2O) -2.15 (s); **HRMS** (ES^-) *m/z* [Found: $(M-H)^-$ 297.0236 $C_6H_{10}N_4O_8P$ requires $(M-H)^-$, 297.0233].

11.1.3. 1,2,3,4,6-Penta-*O*-acetyl-α/β-D-mannopyranoside 36

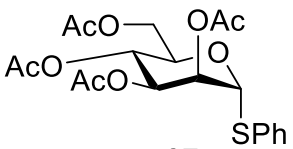


36
4:1

To a stirred mixture of Ac_2O (27.0 mL, d = 1.08, 285.6 mmol, 10.3 equiv.) and α/β-D-mannose (5.0 g, 27.8 mmol, 1.0 equiv.), H_2SO_4 (2 drops) was added at 0 °C, under an atmosphere of N_2. The solution was stirred for 10 min. at 0°C and then allowed to warm to room temperature and stirred for a further 50 min. The mixture was then

diluted with ice–water (100 mL), and the organic phase extracted with EtOAc (100 mL). The extract was washed with H_2O (3 × 100 mL), sat. aq. $NaHCO_3$ solution (100 mL), dried over $MgSO_4$, filtered, and the solvent evaporated to dryness to yield **36** as a pale yellow viscous oil (9.9 g, 25.3 mmol, 91%). R_f 0.25 (EtOAc/hexane, 1/3); α/β = 4/1; **^{1}H NMR** (300 MHz; $CDCl_3$) δ **α-anomer** = 6.09 (1 H, d, *J* = 1.9 Hz, H_1), 5.37 – 5.34 (2 H, m, H_3, H_4), 5.27 (1 H, dd, *J* = 2.4, 1.9 Hz, H_2), 4.29 (1 H, dd, *J* = 12.5, 4.7 Hz, H_{6a}), 4.10 (1 H, dd, *J* = 12.6 Hz, 2.1, H_{6b}), 4.05 (1 H, m, H_5), 2.19 (3 H, s, C(O)C*H*$_3$), 2.18 (3 H, s, C(O)C*H*$_3$), 2.11 (3 H, s, C(O)C*H*$_3$), 2.07 (3 H, s, C(O)C*H*$_3$), 2.02 (3 H, s, C(O)C*H*$_3$); **β-anomer** = 5.88 (1 H, d, *J* = 1.1 Hz, H_1), 5.49 (1 H, dd, *J* = 3.2, 1.1 Hz, H_2), 5.34–5.37 (1 H, m, H_4), 5.15 (1 H, dd, *J* = 10.0, 3.2 Hz, H_3), 4.32 (1 H, dd, *J* = 10.5, 5.0 Hz, H_{6a}), 4.16 (1 H, d, *J* = 2.3 Hz, H_{6b}), 3.82 (1 H, ddd, *J* = 9.9, 5.3, 2.3 Hz, H_5), 2.23 (3 H, s, C(O)C*H*$_3$), 2.11 (3 H, s, C(O)C*H*$_3$), 2.09 (3 H, s, C(O)C*H*$_3$), 2.05 (3 H, s, C(O)C*H*$_3$), 2.01 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (75 MHz; $CDCl_3$) δ **α-anomer** = 170.7 (*C*(O)CH_3), 170.0 (*C*(O)CH_3), 169.8 (*C*(O)CH_3), 169.6 (*C*(O)CH_3), 168.1 (*C*(O)CH_3), 90.5 (C1), 70.5 (C5), 68.7 (C3), 68.3 (C2), 65.4 (C4), 62.0 (C6), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.6 (C(O)*C*H_3); **β-anomer** = 170.2 (*C*(O)CH_3), 169.8 (*C*(O)CH_3), 169.6 (*C*(O)CH_3), 168.4 (*C*(O)CH_3), 166.4 (*C*(O)CH_3), 90.4 (C1), 73.2 (C5), 70.6 (C3), 68.1 (C2), 65.3 (C4), 62.0 (C6), 22.2 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 20.5 (C(O)*C*H_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 90.5 ($^1J_{C1\text{-}H1}$ = 180 Hz, C1 **α-anomer**), 90.4 ($^1J_{C1'\text{-}H1'}$ = 164 Hz, C1 **β-anomer**); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 408.1496 $C_{16}H_{26}NO_{11}$ requires $(M+NH_4)^+$, 408.1500]. These data were consistent with literature values.[202,251]

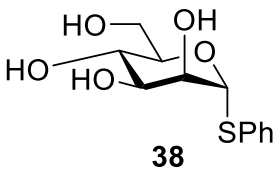
11.1.4. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside 37



37

To a stirred solution of **36** (22.2 g, 56.9 mmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (55 mL) under N_2 at room temperature, were added successively, thiophenol (8.7 mL, d = 1.078, 85.3 mmol, 1.5 equiv.) and $BF_3.Et_2O$ (33.7 mL, d = 1.15, 273.1 mmol, 4.8 equiv.). The yellow-orange solution formed was stirred at room temperature for 2 days, during which it turned deep purple in colour and TLC showed higher R_f spot. The mixture was then washed successively with sat. aq. $NaHCO_3$ (4 x 80 mL), 5% aq. NaOH solution (5 x 70 mL) and brine (3 x 50 mL), dried over $MgSO_4$, filtered and the solvent was removed *in vacuo*. The residue, a pale yellow oil, was crystallised from EtOH (~ 80 mL) to give **37** as a white powder (18.9 g, 43.0 mmol, 75%). R_f 0.73 (EtOAc/hexane, 1/2); mp: 86-88 °C; **1H NMR** (300 MHz; $CDCl_3$) δ 7.50–7.30 (5 H, m, Ar-H), 5.49–5.51 (2 H, m, H_1, H_3), 5.35–5.32 (2 H, m, H_2, H_4), 4.57–4.53 (1 H, m, H_5), 4.31 (1 H, dd, *J* = 12.2, 5.7 Hz, H_{6a}), 4.10 (1 H, dd, *J* = 12.2, 1.5 Hz, H_{6b}), 2.16 (3 H, s, C(O)C*H*$_3$), 2.08 (3 H, s, C(O)C*H*$_3$), 2.06 (3 H, s, C(O)C*H*$_3$), 2.03 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 170.5 (*C*(O)CH_3), 169.9 (*C*(O)CH_3), 169.8 (*C*(O)CH_3), 169.7 (*C*(O)CH_3), 132.7 (C_q Bn), 132.1 (2C), 129.2 (2C), 128.1, 85.7 (C1), 70.9 (C3), 69.6 (C5), 69.4 (C2), 66.4 (C4), 62.5 (C6), 20.9 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.6 (C(O)*C*H_3); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 458.1475 $C_{20}H_{28}NO_9S$ requires $(M+NH_4)^+$, 458.1479]. These data were consistent with literature values.[203]

11.1.5. Phenyl 1-thio-α-D-mannopyranoside 38

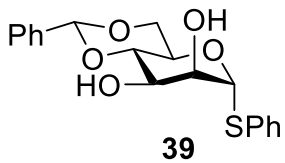


38

To a stirred solution of **37** (7.1 g, 16.1 mmol, 1.0 equiv.) in anhydrous MeOH (50 mL), $Na_{(s)}$ (1.8 mg, 80 µmol, 0.005 equiv.) dissolved in anhydrous MeOH (1 mL) was added

dropwise at room temperature. The mixture was stirred for 2 h, then neutralised with ion exchange Amberlite 120 (H^+) resin (approximately 1.0 g, 5 min), filtered, and concentrated under reduced pressure. The residue was dried over P_2O_5 overnight to give **38** as a white foam (4.1 g, 15.2 mmol, 94%), which was used in the next step without further purification. R_f 0.05 (EtOAc/hexane, 1/2); **^{1}H NMR** (300 MHz; D_6-DMSO) δ 7.53 – 7.26 (5 H, m, Ar-H), 5.33 (1 H, d, *J* = 1.1 Hz, H_1), 5.17 (1 H, br s, C2-O*H*), 4.93 (1 H, d, *J* = 4.4 Hz, C4-O*H*), 4.87 (1 H, d, *J* 3.5 Hz, C3-O*H*), 4.55 (1 H, t, *J* = 5.7 Hz, C6-O*H*), 3.88 (1 H, br s, H_2), 3.77 (1 H, app. t, *J* = 6.4 Hz, H_5), 3.66 (1 H, dd, *J* = 11.8, 3.5 Hz, H_{6a}), 3.55 – 3.41 (3 H, m, H_3, H_4, H_{6b}); **^{13}C NMR** (101 MHz; D_6-DMSO) δ 135.4 (C_q), 131.5 (2C), 129.5 (2C), 127.5, 89.4 (C1), 75.9 (C5), 72.4 (C2), 72.0 (C3), 67.5 (C4), 61.4 (C6); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 290.1061 $C_{12}H_{20}NO_5S$ requires $(M+NH_4)^+$, 290.1062]. These data were consistent with literature values.[203]

11.1.6. Phenyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside 39

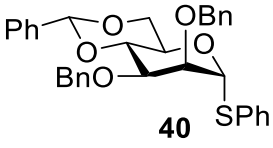


39

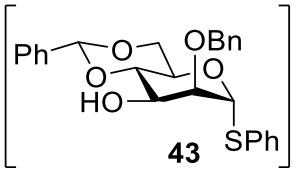
To a solution of **38** (4.6 g, 16.8 mmol, 1.0 equiv.) in anhydrous DMF (60 mL), were added successively, with stirring at 0 °C, benzaldehyde dimethyl acetal (2.52 mL, d = 1.014, 16.8 mmol, 1.0 equiv.) and $HBF_4{\cdot}Et_2O$ (2.31 mL, d = 1.18, 16.8 mmol, 1.0 equiv.). The mixture was stirred at room temperature overnight and then neutralised with Et_3N (9 mL). The residue, a yellow-orange solid was crystallised from absolute EtOH (~ 20 mL) to give **39** (4.1 g, 11.4 mmol, 67%) as a white powder. R_f 0.22 (EtOAc/hexane, 1/2); m.p. 201-203 °C; **^{1}H NMR** (300 MHz; D_6-DMSO) δ 7.51–7.32 (10H, m, Ar-H), 5.64 (1H, s, C*H*Ph), 5.59 (1 H, d, *J* = 4.1 Hz, C2-O*H*), 5.46 (1 H, s, H_1), 5.26 (1H, dd, *J* = 6.1 Hz, C3-O*H*), 4.13–4.03 (2 H, m, H_5, H_{6a}), 4.03 – 3.97 (1 H, m, H_2), 3.94 (1 H, app. t, *J* = 8.9 Hz, H_4), 3.77 (1 H, app. t, *J* = 11.0 Hz, H_{6b}), 3.75 – 3.70 (1 H, m, H_3); **^{13}C**

NMR (101 MHz; D_6-DMSO) δ 138.3 (C_q), 134.2 (C_q), 131.8 (2C), 129.7 (2C), 129.3, 128.5 (2C), 127.9, 126.9 (2C), 101.7 (*C*HPh), 89.8 (C1), 79.0 (C4), 72.9 (C2), 68.6 (C3), 68.1 (C6), 65.8 (C5); **HRMS** *m/z* (ES^+) [Found: $(M+H)^+$ 361.1107 $C_{19}H_{21}O_5S_1$ requires $(M+H)^+$, 361.1104]. These data are consistent with literature values.[203]

11.1.7. Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside 40



40



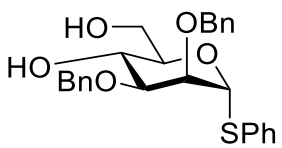
43

A solution of **39** (2.8 g, 7.76 mmol, 1.0 equiv.) in DMF (55 mL) was added to a suspension of NaH (60% in mineral oil, 835 mg, 20.9 mmol, 2.7 equiv.) in DMF (5 mL) at 0 °C and the mixture was stirred for 30 min. BnBr (2.48 mL, d= 1.438, 20.9 mmol, 2.7 equiv.) was then added to the reaction, which was warmed to room temperature and left stirring overnight. The reaction was quenched with MeOH (5 mL) and solvent removed *in vacuo*. The resulting oil was reconstituted between CH_2Cl_2 (50 mL) and H_2O (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 x 70 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a yellow oil. Purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95 and 90/10) afforded a colourless oil. Crystallisation from MeOH yielded **40** (3.1 g, 5.7 mmol, 74%) as white crystals. R_f 0.35 (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.55 – 7.49 (2 H, m, Ar-H), 7.41–7.27 (18 H, m, Ar-H), 5.65 (1 H, s, C*H*Ph), 5.51 (1 H, d, *J* = 0.6 Hz, H_1), 4.82 (1 H, d, *J* = 12.2 Hz, CH_2Ph-attached to C3), 4.73 (2 H, s, CH_2Ph-attached to C2), 4.65 (1 H, d, *J* = 12.2 Hz, CH_2Ph-attached to C3), 4.36–4.19 (3 H, m, H_4, H_5, H_{6a}), 4.05 (1 H, dd, *J* = 2.9, 1.2 Hz, H_2), 3.96 (1 H, dd, *J* = 9.4, 3.1 Hz, H_3), 3.89 (1 H, app. t, *J* = 9.7 Hz, H_{6b}); **^{13}C**

NMR (75 MHz; $CDCl_3$) δ 138.4 (C_q), 137.7 (C_q), 137.6 (C_q), 133.8 (C_q), 131.6, 129.2, 128.9, 128.5, 128.4, 128.25, 128.2, 127.9, 127.7, 126.1, 101.5 (*C*HPh), 87.1 (C1), 79.1 (C4), 78.0 (C2), 76.2 (C3), 73.1 (*C*H_2Ph-attached to C3), 73.0 (*C*H_2Ph-attached to C2), 68.5 (C6), 65.5 (C5); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 558.2304 $C_{33}H_{36}NO_5S$ requires $(M+NH_4)^+$, 558.2309]. These data were consistent with literature values.[204]

43 was isolated as white crystals (15 mg, 33 µmol, 4%). R_f 0.25 (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.55 – 7.26 (15 H, m, Ar-H), 5.58 (2 H, s, H_1, C*H*Ph), 4.75 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph-attached to C2), 4.31 (1 H, app. td, *J* = 9.7, 4.9 Hz, H_5), 4.22 (1 H, dd, *J* = 10.2, 4.9 Hz, H_{6a}), 4.12 – 4.14 (2 H, m, H_2, H_3), 3.99 (1 H, app. t, *J* = 9.5 Hz, H_4), 3.84 (1 H, app. t, *J* = 10.2 Hz, H_{6b}), 2.47 (1 H, d, *J* = 7.8 Hz, C3-*OH*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 137.3 (C_q), 133.7 (C_q), 131.8 (C_q), 129.2, 129.2, 128.7, 128.3, 128.2, 128.1, 127.8, 126.3, 102.2 (*C*HPh), 86.3 (C1), 80.1 (C3), 79.6 (C4), 73.2 (*C*H_2Ph-attached to C2), 69.1 (C2), 68.5 (C6), 64.7 (C5). These data were consistent with literature values.[206]

11.1.8. Phenyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside 41

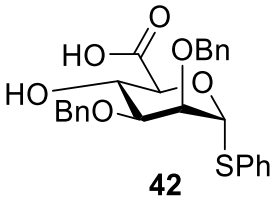


41

A mixture of **40** (2.8 g, 5.18 mmol, 1.0 equiv.) and *p*-TsOH.H_2O (197 mg, 1.03 mmol, 0.2 equiv.) in MeOH (21 mL) was heated at reflux (85 °C) for 2 h. The solution was then cooled to room temperature and quenched with Et_3N (2 mL, pH ~ 7). The solvent was evaporated under reduced pressure and the crude product was dissolved in CH_2Cl_2 (80 mL). The organic layer was washed with H_2O (2 x 50 mL), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a colourless oil. The residue was

purified by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 30/70, 50/50 and 100/0) to afford **41** (1.7 g, 3.76 mmol, 72%) as a white solid. R_f 0.09 (EtOAc/hexane, 1/2); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.43 – 7.20 (15 H, m, Ar-H), 5.51 (1 H, d, *J* = 1.2 Hz, H_1), 4.60 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C2), 4.50 (2 H, s, C*H*$_2$Ph-attached to C3), 4.49 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2), 4.18–4.10 (1 H, m, H_4), 4.09 – 4.02 (1 H, m, H_5), 3.92 (1 H, dd, *J* = 2.9, 1.5 Hz, H_2), 3.80 (2 H, m, $H_{6a,b}$), 3.67 (1 H, dd, *J* = 9.2, 3.0 Hz, H_3), 3.59 (1 H, d, *J* = 3.2 Hz, C4-O*H*), 2.86 (1 H, t, *J* = 5.7 Hz, C6-O*H*); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 138.0 (C_q), 137.8 (C_q), 134.2 (C_q), 131.9, 129.2, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.7, 86.1 (C1), 79.6 (C3), 75.9 (C2), 73.8 (C5), 72.3 (*C*H$_2$Ph-attached to C2), 72.1 (*C*H$_2$Ph-attached to C3), 67.0 (C4), 62.3 (C6); **HRMS** (ES$^+$) *m/z* [Found: $(M+NH_4)^+$ 470.1993 $C_{26}H_{32}NO_5S$ requires $(M+NH_4)^+$, 470.1996]. These data were consistent with literature values.[205]

11.1.9. Phenyl 2,3-di-*O*-benzyl-1-thio-α-D-mannuronic acid 42

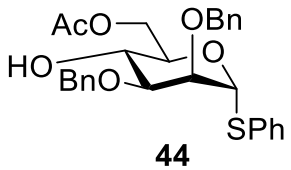


42

To a vigorously stirred solution of **41** (500 mg, 1.10 mmol, 1.0 equiv.) in CH_2Cl_2 (1.3 mL) and H_2O (3.5 mL) was added TEMPO (34 mg, 0.22 mmol, 0.2 equiv.) and BAIB (886 mg, 2.75 mmol, 2.5 equiv.). Stirring was continued for 3h, and the reaction mixture was quenched by the addition of 10% aq. $Na_2S_2O_3$ solution (12 mL) and diluted with CH_2Cl_2 (20 mL). The organic layer was separated and the aqueous phase was acidified with 1.0 M aq. HCl to pH = 4, extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic layers dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude oil was purified by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with MeOH/CH_2Cl_2 (0/100, 5/95, 10/90 + 2% (*v/v*) AcOH) to afford the uronic acid **42** (308 mg, 0.66 mmol,

60%) as a white foam. R_f 0.18 (EtOAc/petroleum ether, 1/1 + 2% (*v/v*) AcOH); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.44 – 7.24 (15 H, m, Ar-H), 5.59 (1 H, d, *J* = 1.7 Hz, H_1), 4.65 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph-attached to C3), 4.57 (1 H, d, *J* = 8.5 Hz, H_5), 4.56 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph-attached to C2), 4.54 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C3), 4.33 (1 H, app. t, *J* = 9.1 Hz, H_4), 3.92 (1 H, d, *J* = 3.5 Hz, H_2),) 3.71 (1 H, dd, *J* = 9.0, 2.8 Hz, H_3); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 172.9 (*C*(O)OH), 137.6 (C_q), 137.4 (C_q), 133.2 (C_q), 131.7, 131.3, 129.3, 128.6, 128.5, 128.1, 128.0, 128.0, 127.9, 85.9 (C1), 78.1 (C3), 75.6 (C2), 72.6 (C5), 72.4 (*C*H_2Ph-attached to C2 or C3), 71.7 (*C*H_2Ph-attached to C2 or C3), 68.3 (C4); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 484.1784 $C_{26}H_{26}O_6SNH_4$ requires $(M+NH_4)^+$, 484.1788]. These data were consistent with literature values.[211]

11.1.10. Phenyl 2,3-di-*O*-benzyl-6-*O*-acetyl-1-thio-α-D-mannopyranoside 44

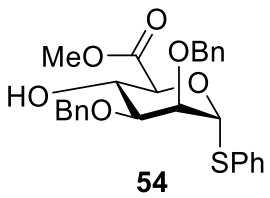


44

To a stirred solution of **40** (2.0 g, 3.70 mmol, 1.0 equiv.) in aq. AcOH (15 mL, 60%, v/v) was heated to reflux (120 °C) for 6h. The solution was then cooled to room temperature and quenched with sat. aq. $NaHCO_3$ solution (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL), and the combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (20/80, 50/50, 90/10) afforded **41** (519 mg, 1.15 mmol, 31%) and **44** (238 mg, 0.48 mmol, 13%) as a colourless oils. R_f 0.46 (EtOAc/petroleum ether, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.47 – 7.24 (15 H, m, Ar-H), 5.61 (1 H, d, *J* = 1.1 Hz, H_1), 4.69 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.53 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.50 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.43 (1 H, dd,

J = 12.0, 5.7 Hz, H_{6a}), 4.34 (1 H, dd, *J* = 12.0, 2.1 Hz, H_{6b}), 4.27 (1 H, ddd, *J* = 9.5, 5.7, 1.9 H_5), 4.01 (1 H, app. t, *J* = 9.6 Hz, H_4), 4.01 (1 H, dd, *J* = 2.9, 1.3 Hz, H_2), 3.68 (1 H, dd, *J* = 9.5, 3.1 Hz, H_3), 2.64 (1 H, br. s,), 2.04 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 171.2 (*C*(O)CH_3), 137.6 (C_q), 134.0 (C_q), 131.6 (C_q), 129.0, 128.6, 128.4, 128.0, 127.9, 127.9, 127.8, 127.6, 85.6 (C1), 79.3 (C3), 75.4 (C2), 71.9 (*C*H_2Ph), 71.8 (*C*H_2Ph), 71.5 (C5), 66.7 (C4), 63.6 (C6), 20.8 (C(O)*C*H_3); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 512.2095 $C_{28}H_{34}NO_6S$ requires $(M+NH_4)^+$, 512.2101]; These data were consistent with literature values.[252]

11.1.11. Methyl (phenyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) uronate 54

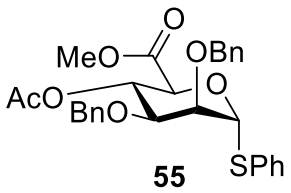


54

To a stirred solution of **42** (2.6 g, 5.57 mmol, 1.0 equiv.) in DMF (8 mL) was added MeI (867 µL, d = 2.28, 13.9 mmol, 2.5 equiv.) and K_2CO_3 (770 mg, 5.57 mmol, 1.0 equiv.). The reaction mixture was stirred overnight and quenched by the addition of MeOH (1 mL).The solvents were removed *in vacuo* and the crude was taken up in EtOAc (100 mL) and washed with H_2O (100 mL). The aqueous layer was extracted with EtOAc (50 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95, 20/80, 70/30, 100/0) afforded **54** as a colourless oil (2.1 g, 4.29 mmol, 77%). R_f 0.38 (EtOAc /hexane, 1/2); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.49–7.24 (15 H, m, Ar-H), 5.62 (1 H, d, *J* = 2.4 Hz, H_1), 4.69 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.65 (1 H, d, *J* = 8.8 Hz, H_5), 4.64 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.59 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.38 (1 H, app. td, *J* = 8.9, 2.3 Hz, H_4), 3.95 (1 H, app. t, *J* = 2.7 Hz, H_2), 3.76 (3 H, s, C(O)OC*H*$_3$), 3.71 (1 H, dd, *J* =

8.9, 2.9 Hz, H_3), 3.09 (1 H, d, *J* = 2.4 Hz, C4-*OH*); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 170.4 (*C*(O)OCH_3), 137.9 (C_q), 137.7 (C_q), 133.7 (C_q), 131.5, 129.2, 128.5, 128.45, 128.04, 127.9, 127.9, 127.7, 86.1 (C1), 78.1 (C3), 75.6 (C2), 72.7 (C5), 72.5 (CH_2Ph), 72.3 (CH_2Ph), 68.5 (C4), 52.7 (C(O)OCH_3); **HRMS** *m/z* (ES^+) [Found: $(M+H)^+$ 481.1684 $C_{27}H_{29}O_6S$ requires $(M+H)^+$, 481.1685]. These data were consistent with literature values.[211]

11.1.12. Methyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) uronate 55

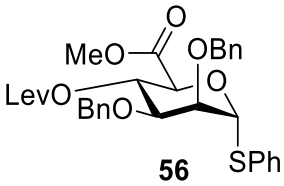


55

Uronate **54** (200 mg, 0.42 mmol, 1.0 equiv.) was dissolved in pyridine/ Ac_2O (3/1) solution (4 mL) and stirred for 3 h, followed by the addition of MeOH (1 mL). The solvents were then removed under reduced pressure and the crude taken up in CH_2Cl_2 (50 mL). The organic layer was washed with 1.0 M aq. HCl (2 x 40 mL) and sat. aq. $NaHCO_3$ solution (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a colourless oil. The crude was then purified by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 30/70, 50/50 and 100/0) to afford **55** as a white solid (158 mg, 0.30 mmol, 72%). R_f 0.09 (EtOAc/hexane, 1/2); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.64 – 7.23 (15 H, m, Ar-H), 5.80 (1 H, d, *J* = 7.1 Hz, H_1), 5.56 (1 H, dd, *J* = 5.9, 4.6 Hz, H_4), 4.62 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.59 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 4.2 Hz, H_5), 4.50 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.55 (1H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 3.80 (1 H, dd, *J* = 6.0, 2.9 Hz, H_3), 3.74 (1 H, bd, *J* = 4.8 Hz, H_2), 3.59 (3H, s, CO_2CH_3), 2.02 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 169.8 (C=O of $C(O)CH_3$ or CO_2CH_3), 168.5 (C=O of $C(O)CH_3$ or CO_2CH_3), 137.5 (C_q), 137.4 (C_q), 133.7 (C_q), 131.0, 128.9, 128.4, 128.2, 128.0, 127.9, 127.2, 82.9 (C1),

73.9 (C2, C3), 72.8 (C5), 72.4 (2 x *C*CH_2Ph), 69.5 (C4), 52.4 (CO_2*C*H_3), 20.9 (C(O)*C*H_3); **^{13}C-GATED** (126 MHz; $CDCl_3$): 82.9 ($^1J_{C1\text{-}H1}$ =164 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 523.1780 $C_{29}H_{31}O_7S$ requires $(M+H)^+$, 523.1785]. These data were consistent with literature values.[211]

11.1.13. Methyl (phenyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) uronate 56

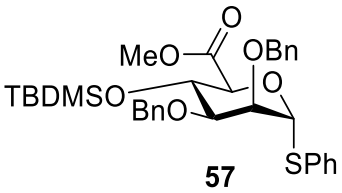


56

To a mixture of **54** (500 mg, 1.04 mmol, 1.0 equiv.) and Lev_2O (250 µL, d = 1.35, 1.56 mmol, 1.5 equiv.) in CH_2Cl_2 (5 mL) was added pyridine (252 µL, d = 0.978, 3.12 mmol, 3.0 equiv.). The reaction mixture was left stirring at room temperature for 18 h. Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed successively with 1.0 M aq. HCl (2 x 30 mL) and sat. aq. $NaHCO_3$ solution (2 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a colourless oil. The crude was purified using silica gel flash column chromatography, eluting with EtOAc/hexane (30/70, 40/60, 50/50, 90/10) to afford **56** as a colourless oil (530 mg, 0.92 mmol, 88%). R_f (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.55 – 7.24 (15 H, m, Ar-H), 5.78 (1 H, d, *J* = 7.2 Hz, H_1), 5.57 (1 H, dd, *J* = 5.9, 4.6 Hz, H_4), 4.64 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.59 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 3.8 Hz, H_5), 4.53 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 3.86 (1 H, dd, *J* = 5.9, 2.8 Hz, H_3), 3.75 (1 H, d, *J* = 6.1 Hz, H_2), 3.58 (3 H, s, CO_2CH_3), 2.74 – 2.69 (2 H, m, C*H*$_2$ Lev), 2.57 – 2.51 (2 H, m, C*H*$_2$ Lev), 2.18 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.1 (C=O Lev ketone), 171.6 (C=O Lev or CO_2CH_3), 168.5 (C=O Lev or CO_2CH_3), 137.7 (C_q), 137.5 (C_q), 133.8 (C_q), 131.1, 128.9, 128.4, 128.1, 127.9, 127.8, 127.8, 127.2, 82.9 (C1), 74.3 (C2), 73.9 (C3), 72.8 (C5), 72.6 (*C*H_2Ph), 72.4

(*C*H_2Ph), 69.8 (C4), 52.3 (CO_2CH_3), 37.8 (*C*H_2 Lev), 29.8 (*C*H_3 Lev), 28.0 (*C*H_2 Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 82.9 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 596.2338 $C_{32}H_{38}NO_8S$ requires $(M+NH_4)^+$, 596.2313]; These data were consistent with literature values.[211]

11.1.14. Methyl (phenyl 4-*O*-*tert*-butyl dimethylsilyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) uronate 57

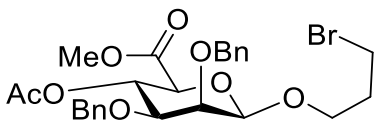


57

To a mixture of **54** (100 mg, 0.21 mmol, 1.0 equiv.), imidazole (mg, 0.62 mmol, 3.0 equiv.) and DMAP (42mg, 0.62 mmol, 0.5 equiv.) in DMF (2 mL) was added TBDMSOTf (144 µL, d = 1.151, 0.62 mmol, 3.0 equiv.). The reaction mixture was left stirring overnight at room temperature and was quenched with H_2O (1 mL). The mixture was concentrated under reduced pressure, and the remaining crude was reconstituted in CH_2Cl_2 (25 mL) and H_2O (20 mL). The organic layer was washed, separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure to furnish a colourless oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 5/95, 10/90) afforded **31**, as a colourless oil (120 mg, 0.20 mmol, 96%). R_f 0.77 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +22.3 (*c*. 4.65, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.62 – 7.20 (15 H, m, Ar-H), 5.68 (1 H, d, *J* = 7.4 Hz, H_1), 4.58 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph), 4.50 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.42 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.40 – 4.35 (2 H, m, H_4, H_5), 3.81 (1 H, dd, *J* = 7.5, 2.6 Hz, H_2), 3.60 (3 H, s, CO_2CH_3), 3.54 (1 H, dd, *J* = 5.6, 2.7 Hz, H_3), 0.80 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.08 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.7 (*C*O_2CH_3), 138.0 (C_q), 137.9 (C_q), 134.0 (C_q), 131.3, 128.8, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.0, 82.9 (C1), 77.3 (C3), 76.4 (C4), 73.7 (C2), 72.5 (*C*H_2Ph), 72.5 (*C*H_2Ph), 69.7 (C5), 52.0 (CO_2CH_3), 25.7

(C(*C*H$_3$)$_3$), 18.0 (*C*(CH$_3$)$_3$), -4.7 (Si(*C*H$_3$)$_2$), -5.2 (Si(*C*H$_3$)$_2$); **^{13}C-GATED** (101 MHz; CDCl$_3$): 82.9 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 612.2832 $C_{33}H_{46}NO_6SSi$ requires (M+NH$_4$)$^+$, 612.2810].

11.1.15. 3-bromopropyl (methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) uronate 58

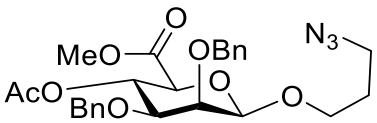


58

A solution of **55** (100 mg, 0.19 mmol, 1.0 equiv.), Ph$_2$SO (50 mg, 0.25 mmol, 1.3 equiv.) and TTBP (125 mg, 0.48 mmol, 2.5 equiv.) in CH$_2$Cl$_2$ (5 mL) was stirred over activated MS4Å for 40 min. The mixture was cooled to -60 °C and Tf$_2$O (41 µL, d = 1.720, 0.25 mmol, 1.3 equiv.) was then added. The mixture was allowed to warm to -40 °C over 10 min. followed by cooling to -90 °C, upon 3-bromopropanol (26 µL, d = 1.537, 0.29 mmol, 1.5 equiv.) in CH$_2$Cl$_2$ (0.7 mL) was added. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. After the addition of Et$_3$N to pH = 7, the organic layer was washed with H$_2$O (10 mL), dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 90/10) afforded **58** as white crystals (72 mg, 0.13 mmol, 67%). R$_f$ 0.37 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -121 (*c*. 0.95, CHCl$_3$); **^{1}H NMR** (400 MHz; CDCl$_3$) δ 7.47–7.21 (10 H, m, Ar-H), 5.52 (1 H, app. t, *J* = 9.4 Hz, H$_4$), 4.92 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph-attached to C2), 4.51 (1 H, d, *J* =12.3 Hz, C*H*$_2$Ph-attached to C3), 4.45 (1 H, d, *J* = 0.8 Hz, H$_1$), 4.38 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.06 (1 H, dt, *J* = 9.9, 5.3 Hz, OC*H*$_2$CH$_2$CH$_2$Br), 3.89 (1 H, d, *J* = 2.4 Hz, H$_2$), 3.86 (1 H, d, *J* = 9.3 Hz, H$_5$), 3.72 (3 H, s, CO$_2$C*H*$_3$), 3.61 (1 H, ddd, *J* = 9.7, 8.2, 4.7 Hz, OC*H*$_2$CH$_2$CH$_2$Br), 3.56–3.42 (3 H, m, H$_3$, OCH$_2$CH$_2$C*H*$_2$Br) 2.22 (1 H, tdt, *J* 8.1, 5.6, 4.1 Hz, OCH$_2$C*H*$_2$CH$_2$Br), 2.09 (1 H, dddd,

J = 14.6, 7.3, 5.1, 2.1 Hz, $OCH_2CH_2CH_2Br$), 2.02 (3 H, s, $C(O)CH_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.6 ($C(O)OCH_3$), 168.0 ($C(O)CH_3$), 138.3 (C_q), 137.8 (C_q), 131.1, 129.3, 128.4, 128.1, 127.8, 127.6, 127.4, 124.8, 101.6 (C1), 78.2 (C3), 73. 9 (CH_2Ph-attached to C2), 73.7 (C5), 73.2 (C2), 71.6 (CH_2Ph-attached to C3), 69.0 (C4), 67.8 ($OCH_2CH_2CH_2Br$), 52.6 (CO_2CH_3), 32.7 ($OCH_2CH_2CH_2Br$), 30.4 ($OCH_2CH_2CH_2Br$), 20.8 ($C(O)CH_3$); **^{13}C-GATED** (126 MHz; $CDCl_3$): 101.6 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 568.1539 $C_{26}H_{35}BrNO_8$ requires $(M+NH_4)^+$, 568.1541]; **IR** ν_{max}/cm^{-1} 1745 (s, C=O), 1045 (s, C-O_{ester}), 1145 (s, C-O_{ether}), 695 (s, C-Br).

11.1.16. 3-azidopropyl (methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) uronate 59

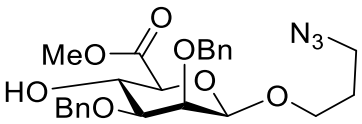


59

Compound **58** (30 mg, 54 µmol, 1.0 equiv.) and NaN_3 (21 mg, 0.33 mmol, 6.0 equiv.) were dissolved in acetone (3 mL) and the reaction mixture was stirred for 2 days at 50 °C. Upon completion, the reaction mixture was cooled to room temperature and petroleum ether (40 mL) was added. The organic layer was washed with H_2O (30 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford **59** as a white solid (27 mg, 52 µmol, 97%). R_f 0.60 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.54 – 7.11 (10 H, m, Ar-H), 5.52 (1 H, app. t, *J* = 9.3 Hz, H_4), 4.92 (1H, d, *J* = 12.5 Hz, CH_2Ph-attached to C2), 4.81 (1 H, d, *J* = 12.6 Hz, CH_2Ph-attached to C2), 4.51 (1 H, d, *J* = 12.3 Hz, CH_2Ph-attached to C3), 4.43 (1 H, s, H_1), 4.37 (1 H, d, *J* = 12.3 Hz, CH_2Ph-attached to C3), 4.09–4.00 (1 H, m, $OCH_2CH_2CH_2N_3$), 3.89 (1 H, d, *J* = 2.6 Hz, H_2), 3.85 (1 H, d, *J* = 9.3 Hz, H_5), 3.72 (3 H, s, CO_2CH_3), 3.56– 3.47 (2 H, m, H_3, $OCH_2CH_2CH_2N_3$), 3.39 (2 H, t, *J* = 6.6 Hz, $OCH_2CH_2CH_2N_3$), 2.02 (3 H, s, $C(O)CH_3$), 1.90 – 1.81 (2 H, m, $OCH_2CH_2CH_2N_3$); **^{13}C**

NMR (101 MHz; $CDCl_3$) δ 169.6 (CO_2CH_3),168.0 ($C(O)CH_3$), 138.3 (C_q), 137.8 (C_q), 128.4, 128.3, 128.1, 127.7, 127.6, 127.5, 101.5 (C1), 78.2 (C3), 73.9 (CH_2Ph-attached to C2), 73.7 (C5), 73.2 (C2), 71.6 (CH_2Ph-attached to C3), 69.0 (C4), 67.0 (1C, $OCH_2CH_2CH_2N_3$), 52.6 (CO_2CH_3), 48.3 ($OCH_2CH_2CH_2N_3$), 29.1 ($OCH_2CH_2CH_2N_3$), 20.8 ($C(O)CH_3$). These data were consistent with literature values.[211]

11.1.17. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-β-D-mannopyranoside) uronate 60

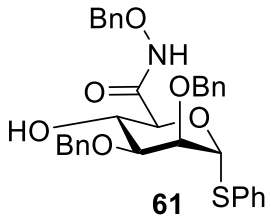


60

To a stirred solution of **59** (100 mg, 0.19 mmol, 1.0 equiv.) in anhydrous MeOH (3 mL), Na (45 µg, 1.9 µmol, 0.01 equiv.) dissolved in anhydrous MeOH (0.5 mL) was added dropwise at room temperature. The mixture was stirred for 24 h, then neutralised with ion exchange Amberlite 120 (H^+) resin (approximately 50 mg, 5 min), filtered, and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 90/10) afforded **60** as a colourless oil (78 mg, 0.16 mmol, 87%). R_f 0.42 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.48 – 7.23 (10 H, m, Ar-H), 4.94 (1 H, d, *J* =12.4 Hz, C*H*$_2$Ph), 4.78 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.51 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.43 (1 H, d, *J* = 0.6 Hz, H_1), 4.30 (1 H, app. t, *J* = 9.5 Hz, H_4), 4.04 (1 H, dt, *J* = 9.7, 5.6 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.88 (1 H, d, *J* = 2.8 Hz, H_2), 3.81 (3 H, s, CO_2CH_3), 3.76 (1 H, d, *J* = 9.6 Hz, H_5), 3.57 (1 H, ddd, *J* = 9.7, 7.8, 5.1 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.40 (2 H, t, *J* = 6.7 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.36 (1 H, dd, *J* = 9.5, 3.0 Hz, H_3), 2.97 (1 H, br. s, C4-O*H*), 2.00 – 1.82 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.8 (CO_2CH_3), 138.4 (C_q), 137.9 (C_q), 128.5, 128.3, 128.2, 127.8, 127.7, 127.6, 102.1 (C1), 80.4 (C3), 75.1 (C5), 74.3 (CH_2Ph), 73.7 (C2), 71.9 (CH_2Ph), 68.3 (C4),

66.9 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.6 (CO$_2$*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (126 MHz; CDCl$_3$): 102.1 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 489.2354 $C_{24}H_{33}N_4O_7N$ requires (M+NH$_4$)$^+$, 489.2344]. These data are consistent with literature values.[211]

11.1.18. *O*-benzyl (phenyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 61

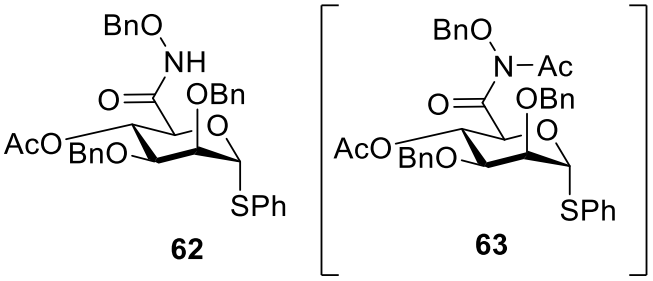


61

To a mixture of **42** (700 mg, 1.50 mmol, 1.0 equiv.) and *O*-benzylhydroxylamine hydrochloride (263 mg, 1.65 mmol, 1.1 equiv.) in CH$_2$Cl$_2$ (5 mL) was added successively PyBOP (858 mg, 1.65 mmol, 1.1 equiv.) and DIPEA (653 µL, d = 0.742, 3.75 mmol, 2.5 equiv.). The reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, the solvent was removed under reduced pressure and the crude material was purified using silica gel flash column chromatography, eluting with EtOAc/hexane (30/70, 40/60, 50/50, 90/10) to afford **61** as a colourless oil (772 mg, 1.35 mmol, 90%). R$_f$ 0.30 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +120 (*c*. 1.0, CHCl$_3$); **^{1}H NMR** (400 MHz; CDCl$_3$) δ 8.84 (1 H, br. s, C(O)N*H*OBn), 7.39 – 7.21 (20H, m, Ar-H), 5.38 (1 H, d, *J* = 1.4 Hz, H$_1$), 4.87 (1 H, d, *J* = 11.1 Hz, C*H*$_2$Ph-attached to C3), 4.84 (1 H, d, *J* = 10.7 Hz, C(O)NHOC*H*$_2$Ph), 4.83 (1 H, d, *J* =11.2 Hz, C*H*$_2$Ph-attached to C3), 4.66 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 12.0 Hz, C(O)NHOC*H*$_2$Ph), 4.58 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C2), 4.54 (1 H, d, *J* = 9.7 Hz, H$_5$), 4.30 (1 H, app. t, *J* = 9.5 Hz, H$_4$), 3.90 (1 H, dd, *J* = 2.8, 1.8 Hz, H$_2$), 3.72 (1 H, dd, *J* = 9.2, 3.0 Hz, H$_3$); **^{13}C NMR** (101 MHz; CDCl$_3$) δ 168.4 (*C*(O)NHOBn), 138.5 (C$_q$), 137.9 (C$_q$), 134. 9 (C$_q$), 133.1 (C$_q$), 132.2, 129.5, 129.4, 128.9, 128.7, 128.5, 128.5, 128.5, 128.2, 128.1, 128.0, 127.95, 127.9, 127.8, 86.7 (C1), 78.4 (C(O)NHO*C*H$_2$Ph), 77.9 (C3), 76.7 (C2), 73.2 (*C*H$_2$Ph-attached to C3), 73.0

(*C*H_2Ph-attached to C2), 71.2 (C5), 69.8 (C4); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 572.2111 $C_{33}H_{34}NO_6S$ requires $(M+H)^+$, 572.2107]; **IR** ν_{max}/cm^{-1} 3313 (m, N-H), 1659 (s, C=O), 1071 (s, C-O_{ether}).

11.1.19. *O*-benzyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 62 and *O*-benzyl *N*-acetyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 63

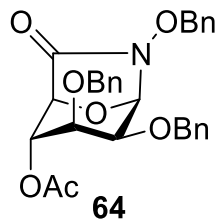


To a stirred solution of **61** (2.2 g, 3.85 mmol, 1.0 equiv.) in CH_2Cl_2 (30 mL) was added Ac_2O (800 µL, d = 1.08, 8.47 mmol, 2.2 equiv.) and pyridine (1.6 mL, d = 0.978, 19.2 mmol, 5.0 equiv.). The reaction mixture was stirred for 16 h. CH_2Cl_2 (15 mL) was added and the organic layer was washed successively with 1.0 M aq. HCl (2 x 15 mL), sat. aq. $NaHCO_3$ solution (2 x 15 mL) and brine (15 mL). The organic layer was then dried over $MgSO_4$ filtered and concentrated under reduced pressure to furnish a yellow oil. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 30/70, 40/60, 90/10) afforded **62** as a white solid (1.6 g, 2.62 mmol, 68%). R_f 0.60 (EtOAc/Toluene, 3/7); m.p. 143-145 °C; $[\alpha]_D^{22}$ +31.4 (*c*. 0.95, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.54 (1 H, br. s, C(O)N*H*OBn), 7.44 – 7.15 (20 H, m, Ar-H), 5.59 (1 H, app. t, *J* = 8.7 Hz, H_4), 5.37 (1 H, d, *J* = 2.8 Hz, H_1), 4.86 (1 H, d, *J* = 11.4 Hz, C(O)NHOC*H*$_2$Ph), 4.76 (1 H, d, *J* = 11.4 Hz, C(O)NHOC*H*$_2$Ph), 4.66 (1 H, d, *J* 12.0 Hz, C*H*$_2$Ph-attached to C2 or C3), 4.64 (1 H, d, *J* =12.0 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* =11.6 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 8.6 Hz, H_5), 4.56 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 3.89 (1 H, app. t, *J* = 2.8 Hz, H_2), 3.80 (1 H, dd, *J* = 8.7, 2.8 Hz,

H_3), 2.11 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.9 (*C*(O)CH_3), 165.0 (*C*(O)NHOBn), 137.6 (C_q), 137.6 (C_q), 135.1 (C_q), 132.6 (C_q), 132.3, 129.7, 129.3, 128.7, 128.5, 128.5, 128.5, 128.2, 128.0, 127.9, 85.8 (C1), 78.2 (C(O)NHO*C*H_2Ph), 75.9 (C2), 75.7 (C3), 72.8 (*C*H_2Ph), 72.6 (*C*H_2Ph), 71.7 (C5), 69.2 (C4), 21.0 (C(O)*C*H_3); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 614.2205 $C_{35}H_{36}NO_7S$ requires $(M+H)^+$, 614.2207]; **IR** ν_{max}/cm^{-1} 3257 (m, N-H), 1749 (s, C=O_{ester}), 1676 (s, C=O_{amide}), 1454, 1494 (C=$C_{aromatic}$), 1110 (s, C-O_{ester}), 1069 (s, C-O_{ether}).

63 was isolated as a colourless oil (505 mg, 0.77 mmol, 20%). R_f 0.78 (EtOAc/Toluene, 3/7); $[\alpha]_D^{22}$ +45.7 (*c*. 4.25, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.45 – 7.20 (20 H, m, Ar-H), 5.63 (1 H, app. t, *J* = 9.6 Hz, H_4), 5.53 (1 H, d, *J* = 1.9 Hz, H_1), 5.05 (2 H, s, C(O)NAcOC*H*$_2$Ph) 4.82 (1 H, d, *J* = 9.7 Hz, H_5), 4.67 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.62 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.54 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.44 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 3.95 (1 H, app. t, *J* = 1.7 Hz, H_2), 3.72 (1 H, dd, *J* = 9.3, 1.8 Hz, H_3), 2.12 (3 H, s, NC(O)C*H*$_3$), 1.83 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.3 (*C*(O)CH_3), 165.0 (N*C*(O)CH_3), 145.6 (*C*(O)NAcOBn), 137.8 (C_q), 137.2 (C_q), 133.5 (C_q), 131.9 (C_q), 128.5, 128.4, 128.4, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 86.2 (C1), 76.8 (2C, C3, C(O)NAcO*C*H_2Ph), 75.7 (C2), 72.3 (*C*H_2Ph-attached to C2), 71.9 (*C*H_2Ph-attached to C3), 70.2 (C5), 67.4 (C4), 20.8 (C(O)*C*H_3), 20.1 (NC(O)*C*H_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.2 ($^1J_{C1\text{-}H1}$ = 172 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 673.2567 $C_{37}H_{41}N_2O_8S$ requires $(M+NH_4)^+$, 673.2578]; **IR** ν_{max}/cm^{-1} 1784 (s, C=O_{ester}), 1753 (s, C=O_{amide}), 1016 (s, C-O_{ester}), 1077 (s, C-O_{ether}).

11.1.20. *O*-benzyl (4-*O*-acetyl-2,3-di-*O*-benzyl- bicyclo [3.2.1-*O*]) hydroxamate 64

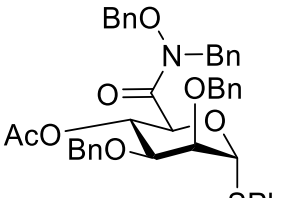


64

A solution of **62** (100 mg, 0.16 mmol, 1.0 equiv.), Ph_2SO (53 mg, 0.26 mmol, 1.6 equiv.) and TTBP (108 mg, 0.41 mmol, 2.5 equiv.) in CH_2Cl_2 (5 mL) was stirred over activated MS4Å for 30 min. The mixture was cooled to -60 °C and Tf_2O (67 µL, d = 1.720, 0.41 mmol, 2.5 equiv.) was then added. The mixture was allowed to warm to -40 °C over 10 min. followed by re-cooling to -90 °C, when 3-bromopropanol (22 µL, d = 1.537, 0.24 mmol, 1.5 equiv.) in CH_2Cl_2 (0.5 mL) was added. The reaction mixture was warmed to room temperature and stirring was continued for further 1 h. The reaction was quenched by the addition of Et_3N until pH = 7 and the crude mixture was filtered through Celite® and concentrated under reduced pressure. The residue was purified by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 30/70, 50/50, 90/10) to afford compound **64** as a colourless oil (50 mg, 0.1 mmol, 62%). R_f 0.28 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +21.0 (*c*. 1.10, $CHCl_3$); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.71 – 7.21 (15 H, m, Ar-H), 5.80 (1 H, app. t, *J* = 1.2 Hz, H_1), 5.06 (1 H, app. br. t, *J* = 1.9 Hz, H_4), 4.94 (2 H, s, C(O)NOC*H*$_2$Ph), 4.74 (1 H, app. t, *J* = 2.0 Hz, H_5), 4.71 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.56 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.54 (1 H, d, *J* = 12.2, Hz, C*H*$_2$Ph-attached to C2), 4.39 (1 H, d, *J* = 12.2, Hz, C*H*$_2$Ph-attached to C2), 3.85 (1 H, ddd, *J* = 4.8, 3.1, 1.6 Hz, H_3), 3.65 (1 H, dd, *J* = 5.0, 1.6 Hz, H_2), 2.09 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.6 (*C*(O)CH_3), 150.4 (*C*(O)NOBn), 145.7, 137.8 (C_q), 137.5 (C_q), 137.3 (C_q), 131.1, 129.3, 128.5, 128.3, 128.2, 128.2, 128.0, 127.8, 127.7, 127.7, 124.8, 104.6 (C1), 76.4 (C(O)N(C1)O*C*H_2Ph), 73.7 (C3), 73.0 (C5), 72.4 (*C*H_2Ph-attached to C3), 72.2 (C2),

71.1 (*C*H_2Ph-attached to C2), 69.1 (C4), 20.9 (C(O)*C*H_3); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 504.2009 $C_{29}H_{30}NO_7$ requires $(M+H)^+$, 504.2017]; **IR** ν_{max}/cm^{-1} 1740 (s, C=O_{ester}), 1701 (m, C=O_{amide}), 1223 (s, C-O_{ester}) 1065 (m, C-O_{ether}).

11.1.21. *O*-benzyl, *N*-benzyl (phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 67



67

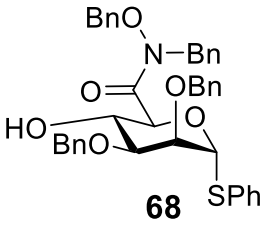
N-benzylation: To a stirred solution of **62** (900 mg, 1.47 mmol, 1.0 equiv.) and K_2CO_3 (244 mg, 1.76 mmol, 1.2 equiv.) in DMF (11 mL) was added BnBr (192 µL, d = 1.438, 1.62 mmol, 1.1 equiv.) at room temperature. The reaction mixture stirred for 15 h, diluted with EtOAc (40 mL), washed with H_2O (40 mL) and brine (40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude product by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95, 20/80 and 90/10) afforded **67** as a colourless oil (435 mg, 0.62 mmol, 42%).

C4-OH Acetylation: To a stirred solution of **68** (1.0 g, 1.51 mmol, 1.0 equiv.) in CH_2Cl_2 (15 mL) was added Ac_2O (214 µL, d= 1.08, 2.27 mmol, 1.5 equiv.) and pyridine (367 µL, d= 0.978, 4.53 mmol, 3.0 equiv.). After 16 h the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1.0 M aq. HCl (30 mL), sat. aq. $NaHCO_3$ solution (30 mL) and brine (30 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (10/90, 20/80, 30/70) afforded **67** as a colourless oil (922 mg, 1.31 mmol, 87%).

R_f 0.80 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +46.7 (*c*. 1.17, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.40 – 7.20 (25 H, m, Ar-H), 5.73 (1 H, app. t, *J* = 9.6 Hz, H_4), 5.55 (1 H, d, *J* = 2.0

Hz, H_1), 5.42 (1 H, d, *J* = 11.9 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.35 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.00 (1 H, d, *J* = 12.5 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.97 (1 H, d, *J* = 12.5 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.65 (2 H, s, C*H*$_2$Ph-attached to C2), 4.63 (1 H, d, *J* = 9.8 Hz, H_5), 4.57 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.49 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 3.98 (1 H, app. t, *J* = 2.5 Hz, H_2), 3.74 (1 H, dd, *J* = 9.4, 2.9 Hz, H_3), 1.80 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.3 (*C*(O)CH_3), 150.7 (*C*(O)N(Bn)OBn), 137.9 (C_q), 137.9 (C_q), 137.8 (C_q), 137.2 (C_q), 133.7 (C_q), 131.7, 129.1, 128.4, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 86.2 (C1), 76.8 (C3), 76.4 (C(O)N(Bn)O*C*H_2Ph), 76.0 (C2), 73.2 (C(O)N(*C*H_2Ph)OBn), 72.4 (*C*H_2Ph-attached to C2), 72.0 (*C*H_2Ph-attached to C3), 71.3 (C5), 68.3 (C4), 20.7 (C(O)*C*H₃); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.2 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 704.2681 $C_{42}H_{42}NO_7S$ requires $(M+H)^+$, 704.2676]; **IR** ν_{max}/cm^{-1} 1751 (m, C=O_{ester}), 1639 (m, C=O_{amide}), 1496, 1454 (C=$C_{aromatic}$), 1223 (s, C-O_{ester}), 1024 (s, C-O_{ether}).

11.1.22. *O*-benzyl, *N*-benzyl (phenyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 68

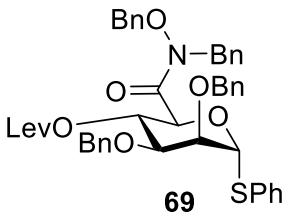


68

To a stirred solution of **61** (180 mg, 0.31 mmol, 1.0 equiv.) and K_2CO_3 (65.3 mg, 0.47 mmol, 1.5 equiv.) in DMF (3.5 mL) was added BnBr (41.2 µL, d = 1.438, 0.35 mmol, 1.1 equiv.) at room temperature. The reaction mixture stirred for 16 h, diluted with EtOAc (60 mL), washed with H_2O (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude product by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95, 20/80 and 90/10) afforded **68** as a colourless oil (133 mg, 0.20 mmol, 64%). R_f 0.75 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +55.7

(*c*. 0.22, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.40 – 7.14 (25 H, m, Ar-H), 5.59 (1 H, d, *J* = 1.3 Hz, H_1), 5.22 (1 H, d, *J* = 12.2 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.16 (1 H, d, *J* = 12.2 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.06 (1 H, d, *J* = 12.3 Hz, C(O)N(Bn)OC*H*$_2$Ph), 5.00 (1 H, d, *J* = 12.3 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.71 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.69 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.62 (2 H, d, *J* = 8.8 Hz, H_5), 4.59 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.43 (1 H, app. td, *J* = 9.6, 2.9 Hz, H_4), 3.96 (1 H, dd, *J* = 2.9, 1.6 Hz, H_2), 3.66 (1 H, dd, *J* = 9.8, 3.0 Hz, H_3), 2.57 (1 H, d, *J* = 2.9 Hz, C4-*OH*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 151.8 (*C*(O)N(Bn)OBn), 138.2 (C_q), 137.9 (C_q), 137.7 (C_q), 136.7 (C_q), 134.0 (C_q), 130.8, 129.2, 128.5, 128.4, 128.4, 128.2, 128.0, 127.94, 127.9, 127.8, 127.7, 127.4, 86.3 (C1), 78.2 (C3), 77.2 (C2), 76.5 (C(O)N(Bn)O*C*H$_2$Ph), 72.8 (*C*H$_2$Ph), 72.6 (*C*H$_2$Ph), 72.3 (C(O)N(*C*H$_2$Ph)OBn), 71.8 (C5), 67.8 (C4); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.3 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 662.2589 $C_{40}H_{40}NO_6S$ requires $(M+H)^+$, 662.2571]; **IR** ν_{max}/cm^{-1} 1640 (m, C=O_{amide}), 1454 (m, C=$C_{aromatic}$), 1223 (s, C-O_{ester}), 1024 (s, C-O_{ether}).

11.1.23. *O*-benzyl, *N*-benzyl (phenyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 69

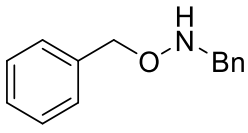


69

To a mixture of **68** (90 mg, 0.13 mmol, 1.0 equiv.) and Lev_2O (43 µL, d = 1.35, 0.27 mmol, 2.0 equiv.) in CH_2Cl_2 (1 mL) was added pyridine (44 µL, 0.54 mmol, 4.0 equiv.). The reaction mixture was left stirring at room temperature for 18 h. Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (15 mL), and the organic layer was washed successively with 1.0 M aq. HCl (2 x 10 mL) and sat. aq. $NaHCO_3$ solution (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a colourless oil. The crude was

purified using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (30/70, 40/60, 50/50, 90/10) to afford **43** as a colourless oil (76 mg, 0.12 mmol, 91%). R_f 0.58 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +51.5 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.22 (25 H, m, Ar-H), 5.74 (1 H, app. t, *J* = 9.6 Hz, H_4), 5.54 (1 H, d, *J* = 2.0 Hz, H_1), 5.39 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.32 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.99 (2 H, s, C(O)N(Bn)OC*H*$_2$Ph), 4.67 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 9.8 Hz, H_5), 4.58 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.53 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 3.98 – 3.94 (1 H, m, H_2), 3.75 (1 H, dd, *J* = 9.4, 2.9 Hz, H_3), 2.66 – 2.38 (3 H, m, C*H*$_2$, C*H*$_2$ Lev), 2.31 – 2.19 (1 H, m, C*H*$_2$ Lev), 2.12 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.1 (C=O Lev), 150.6 (*C*(O)N(Bn)OBn), 137.9 (C_q), 137.9 (C_q), 137.8 (C_q), 137.1 (C_q), 131.6 (C_q), 129.1, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 86.1 (C1), 77.2 (C3), 76.4 (C(O)N(Bn)O*C*H$_2$Ph), 76.1 (C2), 73.1 (C(O)N(*C*H$_2$Ph)OBn), 72.4 (*C*H$_2$Ph-attached to C2), 72.1 (*C*H$_2$Ph-attached to C3), 71.1 (C5), 68.6 (C4), 38.0 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 28.0 (*C*H$_2$ Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.1 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 760.2955 $C_{45}H_{46}NO_8S$ requires $(M+H)^+$, 760.2939].

11.1.24. *N*,*O*-dibenzyl hydroxylamine 71

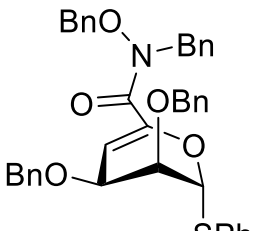


71

A solution of *tert*-butyl-*N*-(benzyloxy)carbamate **70** (1.0 g, 4.48 mmol, 1.0 equiv.) in DMF (30 mL) was added to a suspension of NaH (60% in mineral oil, 270 mg, 6.72 mmol, 1.5 equiv.) in DMF (10 mL) at 0 °C and the mixture was stirred for 30 min. BnBr (800 µL, d= 1.438, 6.72 mmol, 1.5 equiv.) was then added to the reaction, which was warmed to room temperature and left stirring for 3 h. The reaction was quenched with MeOH (3 mL)

and the solvents were removed *in vacuo*. The resulting oil was reconstituted between CH_2Cl_2 (50 mL) and H_2O (40 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were washed with brine (2 x 40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a yellow oil which was directly used for the next step. The crude yellow oil was stirring for 24 h in 3% (*v*/*v*) TFA in CH_2Cl_2 (20 mL). The solvent was then removed under reduced pressure and purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95, 20/80) afforded **71** as a colourless oil (592 mg, 2.78 mmol, 62% in 2 steps). R_f (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.28 (10 H, m, Ar-H), 5.73 (1 H, s, N(*H*)Bn), 4.66 (2 H, s, OC*H*$_2$Ph), 4.05 (2 H, s, N(H)C*H*$_2$Ph); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 137.9 (C_q Bn), 137.6 (C_q Bn), 129.0 (2 C), 128.5 (2 C), 128.4 (2 C), 128.4 (2C), 127.8, 127.4, 76.3 (O*C*H$_2$Ph), 56.6 (N(H)*C*H$_2$Ph); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 214.1226 $C_{14}H_{16}NO$ requires $(M+H)^+$, 214.1243]; These data were consistent with literature values.[215]

11.1.25. *O*-benzyl, *N*-benzyl (phenyl 4,5-ene-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) hydroxamate 72

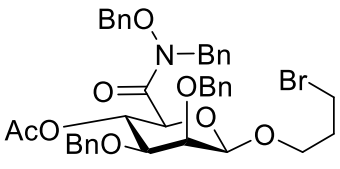


72

To a stirred solution of **42** (100 mg, 0.21 mmol, 1.0 equiv.), PyBOP (278 mg, 0.53 mmol, 2.5 equiv.) and DIPEA (75 µL, d = 0.742, 0.43 mmol, 2.0 equiv.) in CH_2Cl_2 (2 mL) at 0 °C, **71** (49 mg, 0.23 mmol, 1.1 equiv.) in CH_2Cl_2 (0.1 mL) was added. The reaction mixture was left stirring for 40 min. at 0 °C and the solvent was removed under reduced pressure. The remaining crude was purified using silica gel flash column chromatography, eluting with EtOAc/hexane (30/70, 40/60, 50/50, 90/10) to afford **72** as a colourless oil (51 mg, 79 µmol, 38%, 61% based on recovered starting

material-40 mg). R_f 0.59 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +42.8 (*c*. 0.85, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.47 – 7.21 (25 H, m, Ar-H), 5.77 (1 H, dd, *J* = 3.3, 0.8 Hz, H_1), 5.62 (1 H, d, *J* = 5.4 Hz, H_4), 4.74 (1 H, d, *J* = 9.6 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.71 (1 H, d, *J* = 9.7 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.69 (1 H, d, *J* = 12.3 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.66 (2 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 10.8 Hz, C*H*$_2$Ph-attached to C3), 4.61 (1 H, d, *J* = 10.9 Hz, C*H*$_2$Ph-attached to C3), 4.58 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C2), 4.27 (1 H, app. t, *J* = 3.7 Hz, H_2), 3.88 (1 H, ddd, *J* = 5.2, 4.1, 1.0 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 163.7 (*C*(O)N(Bn)OBn), 146.0 (C5), 137.93 (C_q), 137.6 (C_q), 136.0 (C_q), 134.8 (C_q), 132.7 (C_q), 132.6, 129.4 129.1, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 105.3 (C1), 85.0 (C4), 77.2 (C(O)N(Bn)O*C*H$_2$Ph), 72.7 (C3), 72.3 (*C*H$_2$Ph-attached to C3), 71.1 (*C*H$_2$Ph-attached to C2), 68.3 (C2), 52.9 (C(O)N(*C*H$_2$Ph)OBn); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 644.2485 $C_{40}H_{38}NO_5S$ requires $(M+H)^+$, 644.2465]; **IR** ν_{max}/cm^{-1} 1652 (w, $C{=}O_{amide}$), 1454 (m, $C{=}C_{aromatic}$), 1071 (s, $C\text{-}O_{ether}$), 909 (m, $C{=}C_{alkene}$).

11.1.26. 3-bromopropyl (*O*-benzyl, *N*-benzyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate 73

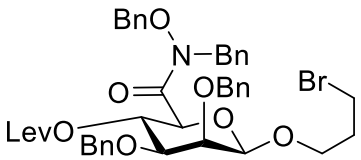


73

A solution of **67** (100 mg, 0.14 mmol, 1.0 equiv.), Ph_2SO (37 mg, 0.18 mmol, 1.3 equiv.) and TTBP (88 mg, 0.35 mmol, 2.5 equiv.) in CH_2Cl_2 (3 mL) was stirred over activated MS4Å for 40 min. The mixture was cooled to -60 °C and Tf_2O (30 µL, d = 1.720, 0.18 mmol, 1.3 equiv.) was then added. The mixture was stirred for 5 min. followed by cooling to -80 °C, upon 3-bromopropanol (19 µL, d = 1.537, 0.21 mmol, 1.5 equiv.) was added. The reaction mixture was allowed to warm up to -20 °C, and stirring was continued for 1 h. At that temperature Et_3N was

added until pH = 7, the organic layer was washed with H_2O (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (10/90, 20/80, 30/170) afforded **73** as a colourless oil (91 mg, 0.12 mmol, 89%). R_f 0.58 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +12.8 (*c*. 0.5, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.42 – 7.23 (25 H, m, Ar-H), 5.63 (1 H, app. t, *J* = 9.9 Hz, H_4), 5.50 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.45 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.99 (1 H, d, *J* = 12.6 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.94 (1 H, d, *J* = 12.6 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.89 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.52 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.39 (1 H, s, H_1) 4.38 (1 H, d, *J* = 11.3 Hz, C*H*$_2$Ph-attached to C3), 3.95 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$Br), 3.89 (1 H, d, *J* = 2.6 Hz, H_2), 3.81 (1 H, d, *J* = 10.0 Hz, H_5), 3.61 – 3.52 (1 H, m, OC*H*$_2$CH$_2$CH$_2$Br), 3.43 (1 H, dd, *J* = 9.7, 3.1 Hz, H_3), 3.46 – 3.39 (2 H, m, OCH$_2$CH$_2$C*H*$_2$Br), 2.09 (2 H, dddd, *J* = 26.8, 21.7, 11.0, 5.6 Hz, OCH$_2$C*H*$_2$CH$_2$Br), 1.77 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.3 (*C*(O)CH$_3$), 150.9 (*C*(O)N(Bn)OBn), 138.5 (C_q), 137.9 (C_q), 137.9 (C_q), 137.3 (C_q), 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.7, 127.7, 127.7, 127.6, 127.4, 127.4, 101.8 (C1), 78.9 (C3), 76.3 (C(O)N(Bn)O*C*H$_2$Ph), 74.1 (2 C, C5 and *C*H$_2$Ph-attached to C2), 73.9 (C2), 73.1 (C(O)N(*C*H$_2$Ph)OBn), 71.4 (*C*H$_2$Ph-attached to C3), 68.1 (C4), 67.4 (O*C*H$_2$CH$_2$CH$_2$Br), 32.8 (OCH$_2$*C*H$_2$CH$_2$Br), 30.2 (OCH$_2$CH$_2$*C*H$_2$Br), 20.7 (C(O)*C*H$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.8 ($^1J_{C1\text{-}H1}$ = 152 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 732.2188 $C_{39}H_{43}BrNO_8$ requires $(M+H)^+$, 732.2167]; **IR** ν_{max}/cm^{-1} 1745 (m, C=O$_{ester}$), 1637 (w, C=O$_{amide}$), 1051 (m, C-O$_{ester}$).

11.1.27. 3-bromopropyl (*O*-benzyl, *N*-benzyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate 74

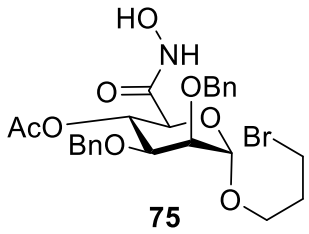


74

A solution of **69** (100 mg, 0.13 mmol, 1.0 equiv.), Ph_2SO (34 mg, 0.19 mmol, 1.3 equiv.) and TTBP (81 mg, 0.33 mmol, 2.5 equiv.) in CH_2Cl_2 (2.5 mL) was stirred over activated MS4Å for 40 min. The mixture was cooled to -60 °C and Tf_2O (28 µL, d = 1.720, 0.17 mmol, 1.3 equiv.) was then added. The mixture was stirred for 5 min. followed by cooling to -90 °C, upon 3-bromopropanol (18 µL, d = 1.537, 0.20 mmol, 1.5 equiv.) was added. The reaction mixture was allowed to warm up to -20 °C, and stirring was continued for 1 h. At that temperature Et_3N was added until pH = 7, the organic layer was washed with H_2O (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (10/90, 20/80, 30/170) afforded **49** as a colourless oil (80 mg, 0.10 mmol, 78%). R_f 0.46 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +39.5 (*c*. 0.84, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.40 – 7.24 (20 H, m, Ar-H), 5.65 (1 H, app. t, *J* = 9.9 Hz, H_4), 5.48 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.42 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.98 (1 H, d, *J* = 12.5 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.95 (1 H, d, *J* = 12.4 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.88 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.52 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.43 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.39 (1 H, s, H_1), 3.94 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$Br), 3.88 (1 H, d, *J* = 2.6 Hz, H_2), 3.83 (1 H, d, *J* = 10.0 Hz, H_5), 3.56 (1 H, ddd, *J* = 9.8, 7.7, 4.8 Hz, OC*H*$_2$CH$_2$CH$_2$Br), 3.44 (1 H, dd, *J* = 9.8, 2.9 Hz, H_3), 3.46 – 3.38 (2 H, m, OCH$_2$CH$_2$C*H*$_2$Br), 2.64 – 2.37 (3 H, m, C*H*$_2$, C*H*$_2$ Lev), 2.27 – 2.15 (1 H, m, C*H*$_2$ Lev), 2.11 (3 H, s, C*H*$_3$ Lev), 2.10 – 1.97 (2 H, m, OCH$_2$C*H*$_2$CH$_2$Br);

^{13}C NMR (101 MHz; $CDCl_3$) δ 206.3 (C=O Lev ketone), 171.2 (C=O Lev), 150.7 (*C*(O)N(Bn)OBn), 138.5 (C_q), 137.9 (2 C, C_q), 137.3 (C_q), 128.4, 128.3, 128.2, 128.1, 128.1, 128.1, 127.7, 127.7, 127.6, 127.6, 127.4, 101.7 (C1), 78.8 (C3), 76.3 (C(O)N(Bn)O*C*H_2Ph), 74.0, 73.9, 73.8 (3C, C2, C5, *C*H_2Ph-attached to C2), 73.1 (C(O)N(*C*H_2Ph)OBn), 71.5 (*C*H_2Ph-attached to C3), 68.3 (C4), 67.4 (O*C*H_2CH$_2$CH$_2$Br), 37.9 (*C*H_2 Lev), 32.7 (OCH$_2$*C*H_2CH$_2$Br), 30.3 (OCH$_2$CH$_2$*C*H_2Br), 29.8 (*C*H_3 Lev), 27.8 (*C*H_2 Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.7 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 788.2465 $C_{42}H_{47}BrNO_9$ requires $(M+H)^+$, 788.2429].

11.1.28. 3-bromopropyl (4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranoside) hydroxamate 75

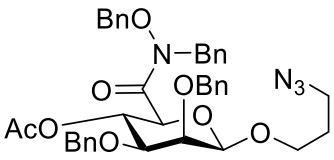


75

To stirred solution of **67** (130 mg, 0.18 mmol, 1.0 equiv.) and 3-bromopropanol (25 µL, d = 1.537, 0.28 mmol, 1.5 equiv.) in CH_2Cl_2 (3.5 mL) over activated MS4Å for 1 h before NBS (49 mg 0.28 mmol, 1.5 equiv.) was added. The reaction mixture was cooled down to -10 °C and TMSOTf (17 µL, d = 1.225, 90 µmol, 0.5 equiv.) was added dropwise. The reaction mixture was stirred for 45 min. at 0 °C and 45 min. at room temperature. The reaction mixture was quenched by the addition of Et_3N to pH = 7 followed by concentration under reduced pressure. Purification using silica gel flash column chromatography, eluting with Et_2O/hexane (5/95, 10/90, 15/85) afforded **39** as a colourless oil (11 mg, 20 µmol, 11 %). R_f 0.40 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -25.2 (*c*. 0.09, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.71 (1 H, s, N(H)O*H*), 7.42 – 7.19 (15 H, m, Ar-H), 5.57 (1 H, t, *J* = 9.6 Hz, H_4), 4.87 (1 H, d, *J* = 2.0 Hz, H_1), 4.83 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C2), 4.61 (2 H, s, C*H*$_2$Ph-attached to C3), 4.24 (1 H,

d, *J* = 9.8 Hz, H_5), 3.87 (1 H, dd, *J* = 9.4, 2.9 Hz, H_3), 3.83 – 3.77 (1 H, m, OC*H*$_2$CH$_2$CH$_2$Br), 3.78 – 3.74 (1 H, m, H_2), 3.56 – 3.51 (1 H, m, OC*H*$_2$CH$_2$CH$_2$Br), 3.42 (2 H, t, *J* = 6.3 Hz, OCH$_2$CH$_2$C*H*$_2$Br), 2.13 – 2.03 (2 H, m, OCH$_2$C*H*$_2$CH$_2$Br), 2.06 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 170.3 (*C*(O)N(H)OH), 169.7 (*C*(O)CH$_3$), 138.1, 137.9 (C_q), 137.9 (C_q), 129.0, 128.5, 128.4, 128.0, 127.9, 127.8, 127.6, 126.8, 125.4, 99.2 (C1), 76.5 (C3), 74.7 (C2), 73.4 (*C*H$_2$Ph-attached to C2), 72.7 (*C*H$_2$Ph-attached to C3), 71.6 (C5), 68.7 (C4), 66.0 (O*C*H$_2$CH$_2$CH$_2$Br), 32.1 (OCH$_2$*C*H$_2$CH$_2$Br), 29.7 (OCH$_2$CH$_2$*C*H$_2$Br), 20.9 (C(O)*C*H$_3$); **^{13}C-GATED** (126 MHz; $CDCl_3$) 99.2 ($^1J_{C1\text{-}H1}$ =172 Hz, C1).

11.1.29. 3-azidopropyl (*O*-benzyl, *N*-benzyl 4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate 76

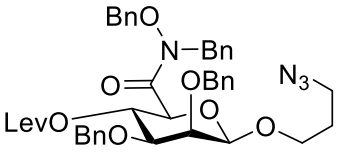


76

Compound **73** (20 mg, 27 µmol, 1.0 equiv.) and NaN_3 (11 mg, 0.18 mmol, 6.5 equiv.) were dissolved in acetone (1.5 mL) and the reaction mixture was stirred for 48 h at 55 °C. Upon completion, the reaction mixture was cooled to room temperature and EtOAc (10 mL) was added. The organic layer was washed with H_2O (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude product. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (10/90, 20/80, 40/60) afforded **76** as a colourless oil (14 mg, 20 µmol, 76%). R_f 0.60 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -50.8 (*c*. 0.93, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.43 – 7.19 (20 H, m, Ar-H), 5.63 (1 H, app. t, *J* = 9.9 Hz, H_4), 5.50 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.45 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.99 (1 H, d, *J* = 12.6 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.94 (1 H, d, *J* = 12.6 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.89 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.52 (1 H, d, *J* = 12.3 Hz,

C*H*$_2$Ph-attached to C3), 4.38 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.37 (1 H, s, H$_1$), 3.94 – 3.85 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.89 (1 H, d, *J* = 2.8 Hz, H$_2$), 3.81 (1 H, d, *J* = 10.0 Hz, H$_5$), 3.48 (1 H, ddd, *J* = 9.8, 7.6, 5.1 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.43 (1 H, dd, *J* = 9.7, 2.8 Hz, H$_3$), 3.38 – 3.25 (2 H, m, OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.85 (2 H, ddd, *J* = 18.1, 11.5, 4.8 Hz, OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.77 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; CDCl$_3$) δ 169.3 (*C*(O)CH$_3$), 150.8 (*C*(O)N(Bn)OBn), 138.5 (C$_q$), 137.9 (C$_q$), 137.8 (C$_q$), 137.3 (C$_q$), 128.4, 128.30, 128.2, 128.1, 128.0, 127.7, 127.7, 127.7, 127.6, 127.5, 127.39, 124.8, 101.7 (C1), 78.8 (C3), 76.3 (C(O)N(Bn)O*C*H$_2$Ph), 74.1 (2 C, C5, *C*H$_2$Ph-attached to C2), 74.0 (C2), 73.1 (C(O)N(*C*H$_2$Ph)OBn), 71.4 (*C*H$_2$Ph-attached to C3), 68.1 (C4), 66.6 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 20.7 (C(O)*C*H$_3$); **^{13}C-GATED** (101 MHz; CDCl$_3$): 101.7 ($^1J_{C1\text{-}H1}$ = 152 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 695.3097 C$_{39}$H$_{43}$N$_4$O$_8$ requires (M+H)$^+$, 695.3075]; **IR** ν_{max}/cm^{-1} 2095 (m, N=N=N), 1746 (m, C=O$_{ester}$), 1637 (w, C=O$_{amide}$), 1050 (s, C-O$_{ester}$).

11.1.30. 3-azidopropyl (*O*-benzyl, *N*-benzyl 4-*O*-levulinoyl-2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate 77

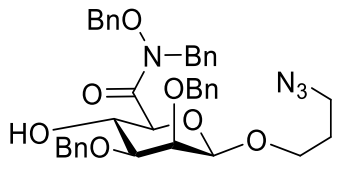


77

Compound **74** (90 mg, 1.14 mmol, 1.0 equiv.) and NaN$_3$ (37 mg, 5.70 mmol, 5.0 equiv.) and TBAI (2.1 g, 5.70 mmol, 5.0 equiv.) were dissolved in DMF (12 mL) and the reaction mixture was stirred for 24 h at 65 °C. Upon completion, the reaction mixture was cooled to room temperature and EtOAc (25 mL) was added. The organic layer was washed with H$_2$O (20 mL), brine (20 mL), dried over MgSO$_4$, filtered and concentrated under reduced pressure to afford the crude product. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (20/80, 40/60, 50/50) afforded **77** as a colourless oil (62 mg, 0.83

mmol, 73%). R_f 0.38 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -41.5 (*c*. 2.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.41 – 7.23 (20 H, m, Ar-H), 5.65 (1 H, app. t, *J* = 9.9 Hz, H_4), 5.48 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.42 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.98 (1 H, d, *J* = 12.4 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.95 (1 H, d, *J* = 12.5 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.88 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.52 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.43 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.37 (1 H, s, H_1), 3.90 – 3.84 (1H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$) 3.88 (1 H, d, *J* = 3.0 Hz, H_2), 3.82 (1 H, d, *J* = 10.0 Hz, H_5), 3.48 (1 H, ddd, *J* = 6.6, 6.0, 3.6 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.44 (1 H, dd, *J* = 9.8, 2.8 Hz, H_3), 3.30 (2 H, ddd, *J* 13.9, 9.9, 4.0 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.63 – 2.37 (3 H, m, C*H*$_2$, C*H*$_2$ Lev), 2.24 (1 H, ddd, *J* = 10.8, 8.8, 4.0 Hz, C*H*$_2$ Lev), 2.10 (3 H, s, C*H*$_3$ Lev), 1.81 (2 H, ddt, *J* = 24.6, 12.3, 6.3 Hz, OCH$_2$CH$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.2 (C=O Lev), 150.7 (*C*(O)N(Bn)OBn), 138.5 (C_q), 137.8 (2 C, C_q), 137.3 (C_q), 128.4, 128.3, 128.1, 128.1, 128.1, 128.0, 127.7, 127.7, 127.6, 127.6, 127.4, 101.7 (C1), 78.7 (C3), 76.3 (C(O)N(Bn)O*C*H$_2$Ph), 74.0, 73.9, 73.9 (3C, C2, C5, *C*H$_2$Ph-attached to C2), 73.1 (C(O)N(*C*H$_2$Ph)OBn), 71.5 (*C*H$_2$Ph-attached to C2), 68.3 (C4), 66.6 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 37.9 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 27.8 (*C*H$_2$ Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.7 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 751.3342 $C_{42}H_{47}N4O_9$ requires $(M+H)^+$, 751.3338].

11.1.31. 3-azidopropyl (*O*-benzyl, *N*-benzyl 2,3-di-*O*-benzyl-1-β-D-mannopyranoside) hydroxamate 78



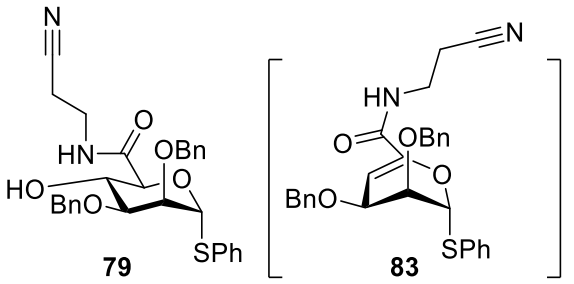
78

To a stirred solution of **76** (280 mg, 0.40 mmol, 1.0 equiv.) in anhydrous MeOH (3.5 mL), Na (93 µg, 4.0 µmol, 0.01 equiv.) dissolved in anhydrous MeOH (0.5 mL) was added dropwise at room temperature. The mixture was stirred for 24 h, then neutralised with ion exchange Amberlite 120 (H^+) resin (approximately 50 mg, 5 min), filtered, and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 90/10) afforded **78** as a colourless oil (198 mg, 0.3 mmol, 76%).

*C*6-hydroxamate **77** (1.9 g, 3.0 mmol, 1.0 equiv.) was dissolved in a mixture of pyridine/AcOH (30 mL, 4/1 *v/v*), after which H_2N-NH_2.AcOH (1.4 g, 14.9 mmol, 5.0 equiv.) was added. The mixture was stirred for 1 h at room temperature and was diluted with EtOAc (100 mL). The organic layer was washed with 1.0 M aq. HCl (2 x 80 mL), sat. aq. $NaHCO_3$ solution (2 x 80 mL) and brine (80 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to furnish a yellow oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 90/10) afforded **78** as a colourless oil (1.8 g, 2.76 mmol, 92%). R_f 0.56 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -31.5 (*c*. 0.65, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.50 – 7.25 (20 H, m, Ar-H), 5.41 (1 H, d, *J* = 12.1 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.32 (1 H, d, *J* = 12.2 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.05 (1 H, d, *J* = 12.1 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.99 (1 H, d, *J* = 12.2 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.93 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.78 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.58 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C3), 4.54 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C3), 4.37 (1 H, s, H_1), 4.32 (1 H,

dd, *J* = 9.5, 2.8 Hz, H_4), 3.97 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.86 (1 H, d, *J* = 2.9 Hz, H_2), 3.71 (1 H, dd, *J* = 9.4, 2.1, H_5), 3.52 – 3.42 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.36 – 3.30 (3 H, m, H_3, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.53 (1 H, d, *J* = 2.9 Hz, C4-O*H*), 1.93 – 1.76 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 151.4 (*C*(O)N(Bn)OBn), 138.6 (C_q), 138.1 (C_q), 137.5 (C_q), 136.9 (C_q), 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.7, 127.5, 127.4, 102.1 (C1), 80.1 (C3), 76.6 (C(O)N(Bn)O*C*H$_2$Ph), 74.8 (C5), 74.5 (C2), 74.3 (*C*H$_2$Ph-attached to C2), 72.8 (C(O)N(*C*H$_2$Ph)OBn), 72.2 (*C*H$_2$Ph-attached to C3), 67.5 (C4), 66.6 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.2 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 102.1 ($^1J_{C1\text{-}H1}$ = 152 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 653.2971 $C_{37}H_{41}N_4O_7$ requires $(M+H)^+$, 653.2970].

11.1.32. 3-propionitrile (phenyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) amide 79 3-propionitrile (phenyl 4,5-ene-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) amide 83



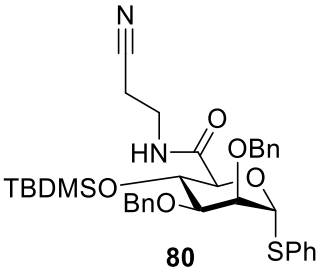
To a stirred solution of **42** (100 mg, 0.21 mmol, 1.0 equiv.), PyBOP (280 mg, 0.53 mmol, 2.5 equiv.) and DIPEA (75 µL, d = 0.742, 0.43 mmol, 2.0 equiv.) in CH_2Cl_2 (2 mL), was added 3-aminopropionitrile (24 µL, d = 0.952, 0.32 mmol, 1.5 equiv.) in CH_2Cl_2 (0.1 mL) at 0 °C. The mixture was left stirring for 40 min. and was diluted with CH_2Cl_2 (10 mL). The organic layer was washed 1.0 M aq. HCl (2 x 10 mL), sat. aq. $NaHCO_3$ solution (2 x 10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with EtOAc/toluene (0/100, 5/95, 10/90, 20/80) afforded **79** as a white solid (51 mg, 0.1 mmol, 47%). R_f

0.29 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ +56.4 (*c*. 7.5, $CHCl_3$); mp: 102-105 °C; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.40 – 7.29 (15 H, m, Ar-H), 6.87 (1 H, t, *J* = 6.1 Hz, C(O)N(*H*)$CH_2CH_2C{\equiv}N$), 5.45 (1 H, d, *J* = 1.5 Hz, H_1), 4.88 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.72 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.68 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.53 (1 H, d, *J* = 9.8 Hz, H_5), 4.38 (1 H, s, C4-*OH*), 4.26 (1 H, app. t, *J* = 9.5 Hz, H_4), 3.94 (1 H, dd, *J* = 2.8, 1.8 Hz, H_2), 3.77 (1 H, dd, *J* = 9.3, 3.0 Hz, H_3), 3.64 (1 H, td, *J* = 12.6, 6.2 Hz, C(O)N(H)C*H*$_2$$CH_2C{\equiv}N$), 3.39 (1 H, ddt, *J* = 13.8, 7.8, 5.9 Hz, C(O)N(H)C*H*$_2$$CH_2CN$), 2.66 (1 H, dd, *J* = 11.7, 5.0 Hz, C(O)N(H)CH_2C*H*$_2$CN), 2.61 – 2.51 (1 H, m, C(O)N(H)CH_2C*H*$_2$C≡N); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 172.1 (*C*(O)N(H)$CH_2CH_2C{\equiv}N$), 138.4 (C_q), 137.8 (C_q), 132.8 (C_q), 132.4, 129.4, 128.5, 128.4, 128.3, 128.0, 127.8, 127.7, 117.4 (C(O)N(H)CH_2CH_2*C*≡N), 86.8 (C1), 78.5 (C3), 77.2 (C2), 73.3 (*C*H_2Ph), 73.1 (*C*H_2Ph), 70.9 (C5), 69.8 (C4), 35.1 (C(O)N(H)*C*$H_2CH_2C{\equiv}N$), 18.3 (C(O)N(H)CH_2*C*$H_2C{\equiv}N$); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 536.2217 $C_{29}H_{34}N_3O_5S$ requires $(M+NH_4)^+$, 536.2219]; **IR** ν_{max}/cm^{-1} 3401 (w, N-H_{amide}), 2249 (w, C≡N), 1655, 1530 (s, C=O_{amide}), 1496, 1454 (m, C=$C_{aromatic}$), 1102 (C-N).

83 was isolated as a colourless oil (46 mg, 90 μmol, 44%). R_f 0.32 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ +72.0 (*c*. 7.5, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.49 – 7.28 (15 H, m, Ar-H), 6.73 (1 H, t, *J* = 6.2 Hz, C(O)N(*H*)$CH_2CH_2C{\equiv}N$), 6.23 (1 H, dd, *J* = 3.5, 0.9 Hz, H_4), 5.59 (1 H, d, *J* = 5.3 Hz, H_1), 4.70 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.69 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 11.9 C*H*$_2$Ph), 4.29 (1 H, app. t, *J* = 3.8 Hz, H_3), 3.85 (1 H, ddd, *J* = 5.1, 4.1, 0.9 Hz, H_2), 3.62 – 3.43 (2 H, m, C(O)N(H)C*H*$_2$$CH_2C{\equiv}N$), 2.69 – 2.52 (2 H, m, C(O)N(H)CH_2C*H*$_2$C≡N); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 161.5 (*C*(O)N(H)$CH_2CH_2C{\equiv}N$), 143.6 (C5), 137.8 (C_q), 137.5 (C_q), 133.4 (C_q), 131.9, 129.4, 128.6, 128.5, 128.5, 128.1, 128.0, 127.9, 127.8,

117.9 (C(O)N(H)CH_2CH_2C≡N), 106.1 (C4), 85.1 (C1), 72.81 (C2), 72.5 (CH_2Ph), 71.2 (CH_2Ph), 68.3 (C3), 35. 6 (C(O)N(H)CH_2CH_2C≡N), 18.2 (C(O)N(H)CH_2CH_2C≡N); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 518.2115 $C_{29}H_{32}N_3O_4S$ requires $(M+NH_4)^+$, 518.2114]; **IR** ν_{max}/cm^{-1} 3354 (w, N-H_{amide}), 2248 (w, C≡N), 1655, 1517 (s, C=O_{amide}), 1454 (m, C=$C_{aromatic}$), 1057 (C-N).

11.1.33. 3-propionitrile (phenyl 4-*O*-*tert*-butyl dimethylsilyl 2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) amide 80

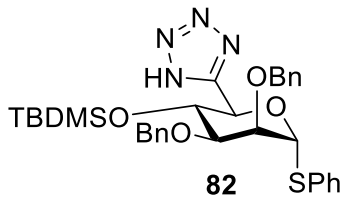


80

To a mixture of **79** (50 mg, 0.1 mmol, 1.0 equiv.), imidazole (20 mg, 0.29 mmol, 3.0 equiv.) and DMAP (5.9 mg, 50 µmol, 0.5 equiv.) in DMF (1 mL) was added TBDMSOTf (66 µL, d = 1.151, 0.29 mmol, 3.0 equiv.) dropwise. The reaction mixture was left stirring overnight at room temperature and was quenched with H_2O (0.1 mL). The mixture was concentrated under reduced pressure, and the remaining crude was reconstituted in CH_2Cl_2 (10 mL) and H_2O (5 mL). The organic layer was washed, separated, dried over Na_2SO_4, filtered and concentrated under reduced pressure to furnish a colourless oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 10/90, 20/80) afforded **80** as a white solid (50 mg, 79 µmol, 80%). R_f 0.46 (EtOAc/hexane, 1/2); $[\alpha]_D^{26}$ +14.0 (*c*. 1.0, $CHCl_3$); mp: 119-122 °C; **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.67 – 7.23 (15 H, m, Ar-H), 6.29 (1 H, t, *J* = 6.1 Hz, C(O)N(*H*)CH_2CH_2C≡N), 5.35 (1 H, d, *J* = 7.4 Hz, H_1), 4.57 (1 H, d, *J* = 12.1 Hz, CH_2Ph), 4.56 (1 H, d, *J* = 11.8 Hz, CH_2Ph), 4.50 (1 H, app. t, *J* = 3.4 Hz, H_4), 4.49 (1 H, d, *J* = 11.8 Hz, CH_2Ph), 4.47 (1 H, d, *J* = 12.0 Hz, CH_2Ph), 4.19 (1 H, d, *J* = 3.9 Hz, H_5), 3.81 (1 H, dd, *J* = 7.3, 2.6 Hz, H_2), 3.56 (1 H, dd, *J* = 5.2, 2.6 Hz, H_3), 3.28 (1 H, dq, *J* = 13.1, 6.6 Hz, C(O)N(H)CH_2CH_2C≡N), 3.19 (1 H, td, *J* = 13.2, 6.5 Hz,

C(O)N(H)CH_2CH$_2$C≡N), 2.31 (1 H, dt, *J* = 16.6, 6.6 Hz, C(O)N(H)CH$_2$CH_2C≡N), 2.25 – 2.12 (1 H, m, C(O)N(H)CH$_2$CH_2C≡N), 0.80 (9 H, s, C(CH_3)$_3$), 0.00 (3 H, s, Si(CH_3)$_2$), -0.08 (3 H, s, Si(CH_3)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.3 (*C*(O)N(H)CH$_2$CH$_2$C≡N), 137.9 (C_q), 137.8 (C_q), 133.5 (C_q), 133.0, 129.2, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 117.6 (C(O)N(H)CH$_2$CH$_2$*C*≡N), 84.1 (C1), 77.5 (C5), 74.2 (C2), 72.4 (*C*H$_2$Ph), 72.4 (*C*H$_2$Ph), 68.7 (C4), 35.3 (C(O)N(H)*C*H$_2$CH$_2$C≡N), 25.8 (C(*C*H$_3$)$_3$), 18.0 (*C*(CH$_3$)$_3$), -4.8 (Si(CH_3)$_2$), -5.0 (Si(CH_3)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 650.3082 $C_{35}H_{48}N_3O_5SSi$ requires (M+NH$_4$)$^+$, 650.3078]; **IR** ν_{max}/cm^{-1} 3217 (w, N-H$_{amide}$), 2255 (w, C≡N), 1678, 1659 (s, C=O$_{amide}$), 1496, 1455 (m, C=C$_{aromatic}$), 1243 (s, Si-C), 1096 (s, C-N), 1068 (s, Si-O).

11.1.34. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(1*H*-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 82

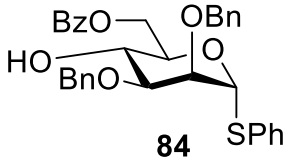


82

Nitrile **89** (60 mg, 0.11 mmol, 1.0 equiv.) was dissolved in toluene (10 mL) and $TMSN_3$ (84 µL, d = 0.872, 0.64 mmol, 6.0 equiv.) and Bu_2SnO (11 mg, 43 µmol, 0.4 equiv.) were added. The mixture was heated to 120 °C and stirred for 16 h. Upon completion, the mixture was cooled down to room temperature, diluted with EtOAc (50 mL) and washed with 0.1 M aq. HCl solution (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of this crude material by silica gel flash column chromatography, eluting with MeOH/CH_2Cl_2 (0/100, 1/99, 2/98) afforded **82** as a brown oil (34 mg, 56 µmol, 51%). R_f 0.71 (MeOH/CH_2Cl_2, 1/2); $[\alpha]_D^{22}$ +88.7 (*c*. 1.75, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.47 – 7.26 (15 H, m, Ar-H), 5.64 (1 H, d, *J* = 8.9 Hz, H_5), 5.54 (1 H, d, *J* = 1.8 Hz, H_1), 4.74 (1 H, d, *J* = 11.7 Hz, CH_2Ph), 4.73 (1 H, d, *J* = 11.8Hz, CH_2Ph), 4.69 (1 H, d, *J* = 11.7 Hz, CH_2Ph), 4.65 (1 H, d, *J* = 11.8

Hz, C*H*$_2$Ph), 4.41 (1 H, app. t, *J* = 8.8 Hz, H$_4$), 4.09 (1 H, app. t, *J* = 2.6 Hz, H$_2$), 3.84 (1 H, dd, *J* = 8.6, 2.8 Hz, H$_3$), 0.78 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.41 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 155.8 (C_q tetrazole), 137.6 (C_q), 137.1 (C_q), 132.8 (C_q), 132.2, 129.2, 128.6, 128.5, 128.3, 128.1, 128.0, 86.8 (C1), 79.7 (C3), 76.5 (C2), 73.3 (*C*H$_2$Ph), 72.7 (*C*H$_2$Ph), 71.0 (C4), 68.5 (C5), 25.6 (C(*C*H$_3$)$_3$), 17.9 (*C*(CH$_3$)$_3$), -4.3 (Si(C*H*$_3$)$_2$), -5.9 (Si(C*H*$_3$)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 605.2628 $C_{32}H_{41}N_4O_4SSi$ requires (M+H)$^+$,605.2618].

11.1.35. Phenyl 2,3-di-*O*-benzyl-6-*O*-benzoyl-1-thio-α-D-mannopyranoside 84

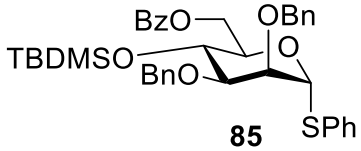


84

To a stirred solution of **41** (1.0 g, 2.21 mmol, 1.0 equiv.), pyridine (357 µL, d = 0.978, 4.42 mmol, 2.0 equiv.), DMAP (81 mg, 0.7 mmol, 0.3 equiv.) in CH_2Cl_2 (10 mL) was added BzCl dropwise (269 µL, d = 1.211, 2.32 mmol, 1.05 equiv.) at 0 °C. The reaction was left stirring overnight at room temperature, and diluted with CH_2Cl_2 (15 mL). The mixture was washed with 1.0 M aq. HCl (10 mL), sat. aq. $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude product. Purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/hexane (0/100, 5/95 and 10/90) afforded **84** as a colourless oil (1.1 g, 2.0 mmol, 90%). R$_f$ 0.37 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +48.2 (*c*. 7.5, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.03 – 7.19 (20 H, m, Ar-H), 5.65 (1 H, d, *J* = 1.3 Hz, H$_1$), 4.69 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2), 4.65 – 4.59 (2 H, m, H$_{6a,b}$), 4.61 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph-attached to C2), 4.55 (1 H, d, *J* = 10.5 Hz, C*H*$_2$Ph-attached to C3), 4.52 (1 H, d, *J* = 10.1 Hz, C*H*$_2$Ph-attached to C3), 4.43 (1 H, dt, *J* = 9.6, 3.9 Hz, H$_5$), 4.15 (1 H, dd, *J* = 9.6 Hz, H$_4$), 4.04 (1 H, dd, *J* = 3.0, 1.6 Hz, H$_2$), 3.73 (1 H, dd, *J* =

9.5, 3.0 Hz, H_3), 2.64 (1 H, br. s, C4-O*H*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 166.7 (*C*(O)Ph), 137.8 (C_q), 137.7 (C_q), 134.1 (C_q), 133.0 (C_q), 131.5, 130.1, 129.8, 129.1, 128.6, 128.4, 128.3, 128.1, 128.0, 127.8, 127.5, 85.7 (C1), 79.6 (C3), 75.7 (C2), 72.1 (*C*H_2Ph), 71.9 (*C*H_2Ph), 71.7 (C5), 66.9 (C4), 64.1 (C6); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 574.2257 $C_{33}H_{32}O_6SNH_4$ requires $(M+NH_4)^+$, 574.2258]; **IR** ν_{max}/cm^{-1} 3477 (br. s, C4-*OH*), 1718 (s, C=O_{ester}), 1273 (s, C-O_{ester}), 1070 (s, C-O_{ether}), 1025 (s, C-OH).

11.1.36. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*O*-benzoyl-1-thio-α-D-mannopyranoside 85

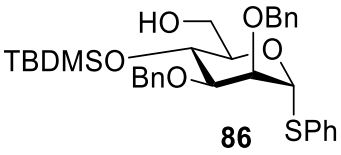


85

To a mixture of **84** (900 mg, 1.62 mmol, 1.0 equiv.), imidazole (330 mg, 4.85 mmol, 3.0 equiv.) and DMAP (99 mg, 0.81 mmol, 0.5 equiv.) in DMF (10 mL) was added TBDMSOTf (1.1 mL, d = 1.151, 4.85 mmol, 3.0 equiv.) dropwise. The reaction mixture was left stirring overnight at room temperature and was quenched with H_2O (2 mL). The mixture was concentrated under reduced pressure, and the remaining crude was reconstituted in CH_2Cl_2 (50 mL) and H_2O (30 mL). The organic layer was washed, separated, dried over $MgSO_4$, filtered and concentrated under reduced pressure to furnish a colourless oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 5/95, 10/90) afforded **85**, as a colourless oil (846 mg, 1.27 mmol, 78%). R_f 0.75 (EtOAc/hexane, 1/2); $[\alpha]_D^{26}$ +57.8 (*c*. 1.37, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.96 – 7.10 (20 H, m, Ar-H), 5.54 (1 H, d, *J* = 1.7, H_1), 4.61 (1 H, dd, *J* = 11.6, 1.8 Hz, H_{6b}), 4.57 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.57 (1 H, s, *J* = 11.9 Hz, C*H*$_2$Ph), 4.52 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.50 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.39 (1 H, dd, *J* = 11.6, 5.8 Hz, H_{6a}), 4.35 – 4.29 (1 H, m, H_5), 4.19 (1 H, t, *J* = 9.1 Hz, H_4), 3.91 (1 H, dd, *J* = 2.7, 2.0 Hz, H_2), 3.61 (1 H, dd,

J = 8.9, 2.9 Hz, H_3), 0.82 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (6 H, d, *J* = 1.5 Hz, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 166.4 (*C*(O)Ph), 138.1 (C_q), 138.1 (C_q), 132.8 (C_q), 131.3 (C_q), 130.1, 129.7, 129.0, 128.3, 128.3, 127.9, 127.6, 127.6, 127.5, 127.3, 85.7 (C1), 80.3 (C3), 76.2 (C2), 72.6 (C5), 72.1 (*C*H$_2$Ph), 71.8 (*C*H$_2$Ph), 68.2 (C4), 64.2 (C6), 26.0 (C(*C*H$_3$)$_3$), 18.2 (*C*(CH$_3$)$_3$), -3.8 (Si(*C*H$_3$)$_2$), -5.0 (Si(*C*H$_3$)$_2$); **HRMS** (ES$^+$) *m/z* [Found: $(M+NH_4)^+$ 688.3127 $C_{39}H_{50}NO_6SSi$ requires $(M+NH_4)^+$, 688.3123]; **IR** ν_{max}/cm^{-1} 1713 (s, C=O$_{ester}$), 1276 (m, C-O$_{ester}$), 1253 (m, Si-C), 1096 (s, Si-O), 1024 (m, C-O$_{ether}$).

11.1.37. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-1-thio-α-D-mannopyranoside 86

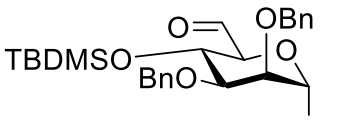


86

To a stirred solution of **85** (800 mg, 1.19 mmol, 1.0 equiv.) in anhydrous MeOH and THF (7 mL, 1/1 *v*/*v*), Na (14 mg, 0.60 mmol, 0.5 equiv.) dissolved in anhydrous MeOH (2 mL) was added dropwise at room temperature. The mixture was stirred overnight, then neutralised with ion exchange Amberlite 120 (H^+) resin (approximately 0.7 g, 10 min), filtered, and concentrated under reduced pressure. Purification by silica gel flash column chromatography, eluting with Et_2O/hexane (0/100, 5/95, 10/90) afforded **86** as a colourless oil (596 mg, 1.07 mmol, 90%). R_f 0.69 (EtOAc/hexane, 1/2); $[\alpha]_D^{26}$ +81.3 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.35 – 7.13 (15 H, m, Ar-H), 5.37 (1 H, d, *J* = 1.8 Hz, H_1), 4.51 (2 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.49 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.44 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.02 – 3.96 (1 H, m, H_4), 3.96 – 3.91 (1 H, m, H_5), 3.82 (1 H, dd, *J* = 2.8, 2.0 Hz, H_2), 3.72 (1 H, ddd, *J* 11.5, 6.6, 2.4 Hz, H_{6b}), 3.64 (1 H, ddd, *J* = 11.6, 6.5, 5.2 Hz, H_{6a}), 3.53 (1 H, dd, *J* = 8.4, 2.9 Hz, H_3), 1.70 (1 H, t, *J* = 6.6 Hz, C6-*OH*), 0.78 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.05 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 138.1

(C_q), 138.0 (C_q), 134.0 (C_q), 132.0, 129.1, 128.4, 128.3, 127.9, 127.7, 127.7, 127.6, 86.2 (C1), 80.4 (C3), 76.4 (C2), 74.8 (C5), 72.5 (*C*H_2Ph), 72.0 (*C*H_2Ph), 67.9 (C4), 62.2 (C6), 26.0 (C(*CH*$_3$)$_3$), 18.2 (*C*(C*H*$_3$)$_3$), -3.8 (Si(*C*H_3)$_2$), -4.9 (Si(*C*H_3)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+NH_4)$^+$ 584.2875 $C_{32}H_{46}NO_5SSi$ requires (M+NH_4)$^+$, 584.2850]; **IR** ν_{max}/cm^{-1} 1454 (w, C=$C_{aromatic}$), 1248 (m, C-Si), 1084 (s, Si-O).

11.1.38. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-aldehyde-1-thio-α-D-mannopyranoside 87

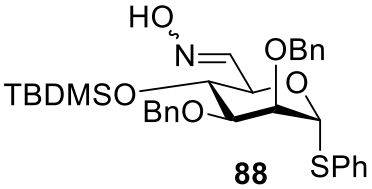


87

To a stirred solution of **86** (60 mg, 0.11 mmol, 1.0 equiv.) in DMSO (1 mL) was added Et_3N (44 µL, d = 0.726, 0.32 mmol, 3.0 equiv.) and sulfur trioxide pyridine complex (51 mg, 0.32 mmol, 3.0 equiv.) at room temperature. The reaction mixture was left stirring for 1 h before it was diluted with EtOAc (30 mL) and H_2O (20 mL). The whole was extracted with EtOAc (3 x 15 mL) and the extracts were washed with H_2O (6 x 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude aldehyde **87** was obtained as a colourless oil (60 mg, 0.11 mmol, 98%) and was carried on the next step without further purification. R_f 0.684 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -14.4 (*c*. 0.33, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 9.77 (1 H, s, C*H*O), 7.56 – 7.25 (15 H, m, Ar-H), 5.56 (1 H, d, *J* = 6.4 Hz, H_1), 4.57 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.52 (2 H, s, C*H*$_2$Ph), 4.43 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.26 – 4.20 (2 H, m, H_4, H_5), 3.83 (1 H, dd, *J* = 6.2, 2.3 Hz, H_2), 3.60 (1 H, dd, *J* = 5.8, 2.5 Hz, H_3), 0.82 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.07 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 198.0 (*C*HO), 137.8 (C_q), 137.6 (C_q), 133.5 (C_q), 132.4, 131.8, 129.0, 128.5, 128.4, 128.0, 127.9, 127.8, 127.5, 83.8 (C1), 81.15 (C5), 77.2 (C3), 73.8 (C2), 72.5 (*C*H_2Ph), 72.3 (*C*H_2Ph), 68.9

(C4), 25.7 (C(*C*H$_3$)$_3$), 18.0 (*C*(CH$_3$)$_3$), -4.6 (Si(*C*H$_3$)$_2$), -5.0 (Si(*C*H$_3$)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 582.2723 $C_{32}H_{44}NO_5SSi$ requires (M+NH$_4$)$^+$, 582.2704].

11.1.39. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-oxime-1-thio-α-D-mannopyranoside 88

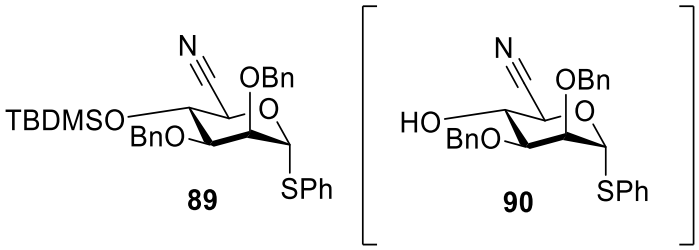


88

Crude aldehyde **87** (4.5 g, 7.97 mmol, 1.0 equiv.) was dissolved in THF (790 mL) and a solution of $H_2NOH.HCl$ (554 mg, 7.97 mmol, 1.0 equiv.) dissolved in H_2O (15 mL) was added dropwise. The mixture was cooled to 0 °C and a solution of Na_2CO_3 (1.0 g, 9.56 mmol, 1.2 equiv.) dissolved in H_2O (9.5 mL) was added dropwise. The solution was slowly warmed to room temperature and stirred for 24 h. The mixture was diluted with H_2O (30 mL) and then extracted with EtOAc (4 x 300 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained crude oil was purified using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (0/100, 5/95, 10/90, 20/80) to furnish **88** as a colourless oil. *Cis* and *trans* (1/6.7) were isolated separately (major isomer: 2.82 g, 4.86 mmol, 61%, minor isomer: 416 mg, 0.72 mmol, 9%) and both were used in the next step; Major isomer R_f 0.78; minor isomer R_f 0.68; (EtOAc/petroleum ether, 1/2); major: $[\alpha]_D^{22}$ +57.7 (*c*. 0.46, $CHCl_3$); minor: $[\alpha]_D^{22}$ +41.3 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) Major isomer δ 7.46 – 7.17 (16 H, m, Ar-H, *H*C=N), 5.40 (1 H, d, *J* = 1.8 Hz, H_1), 4.58 (1 H, d, *J* = 10.5 Hz, C*H*$_2$Ph), 4.57 (1 H, m, H_5), 4.55 (1 H, d, *J* = 10.1 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.50 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.06 (1 H, app. t, *J* = 9.0 Hz, H_4), 3.89 – 3.86 (1 H, m, H_2), 3.59 (1 H, dd, *J* = 8.9, 2.9 Hz, H_3), 0.81 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.01 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 149.3 (H*C*=N), 138.1 (C_q), 137.9 (C_q), 134.0 (C_q), 131.9, 129.1, 128.4, 128.4, 127.9,

127.85, 127.8, 127.7, 127.6, 86.3 (C1), 79.8 (C3), 76.3 (C2), 72.5 (C5), 72.5 (*C*H_2Ph), 72.2 (*C*H_2Ph), 69.8 (C4), 25.8 (C(*CH*$_3$)$_3$), 18.1 (*C*(C*H*$_3$)$_3$), -4.0 (Si(*C*H_3)$_2$), -4.6 (Si(*C*H_3)$_2$); **HRMS** (ES$^+$) *m/z* [Found: $(M+NH_4)^+$ 597.2815 $C_{32}H_{45}N_2O_5SSi$ requires $(M+NH_4)^+$, 597.2813].

11.1.40. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-nitrile-1-thio-α-D-mannopyranoside 89 Phenyl 2,3-di-*O*-benzyl-6-nitrile-1-thio-α-D-mannopyranoside 90

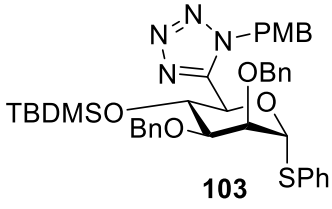


Oxime **88** (120 mg, 0.21 mmol, 1.0 equiv.) was dissolved in dry acetonitrile (21 mL) and $POCl_3$ (19 µL, d = 1.645, 0.21 mmol, 1.0 equiv.) was added at room temperature. The solution was stirred for 5 min. at room temperature, heated up to 65 °C and then stirred for 3 h. The reaction was quenched with sat. aq. $NaHCO_3$ solution (20 mL) and extracted with EtOAc (3 x 60 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (0/100, 5/95, 10/90, 20/80) to furnish **89** as a yellow oil (26 mg, 46 µmol, 22%). R_f 0.90 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +39.4 (*c*. 0.53, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.44 – 7.21 (15 H, m, Ar-H), 5.45 (1 H, d, *J* = 3.0 Hz, H_1), 4.76 (1 H, d, *J* = 8.3 Hz, H_5), 4.60 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.58 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.53 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.21 (1 H, app. t, *J* = 8.2 Hz, H_4), 3.84 (1 H, app. t, *J* = 2.9 Hz, H_2), 3.49 (1 H, dd, *J* = 8.2, 2.9 Hz, H_3), 0.89 (9 H, s, C(C*H*$_3$)$_3$), 0.18 (3 H, s, Si(C*H*$_3$)$_2$), 0.05 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 137.5 (C_q), 137.1 (C_q), 132.9 (C_q), 131.5, 129.4, 129.3, 129.1, 128.5, 128.5,

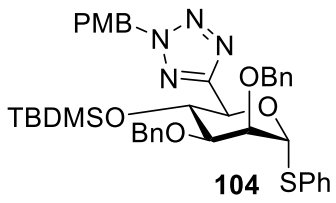
128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 127.6, 127.2, 124.4, 117.0 (*C*≡N), 85.9 (C1), 78.8 (C3), 75.3 (C2), 72.6 (*C*H_2Ph), 72.5 (*C*H_2Ph), 69.4 (C4), 64.7 (C5), 25.8 (C(*C*H_3)$_3$), 18.0 (*C*(C*H*$_3$)$_3$), -4.1 (Si(*C*H$_3$)$_2$), -4.8 (Si(*C*H$_3$)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 579.2732 $C_{32}H_{43}N_2O_4SSi$ requires (M+NH$_4$)$^+$, 579.2707].

90 was isolated as a yellow oil (24 mg, 54 µmol, 26%). R_f 0.82 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +15.4 (*c*. 0.95, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.41 – 7.28 (15 H, m, Ar-H), 5.51 (1 H, d, *J* = 1.7 Hz, H_1), 4.87 (1 H, d, *J* = 9.8 Hz, H_5), 4.66 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.32 (1 H, app. t, *J* = 9.6 Hz, H_4), 3.96 (1 H, dd, *J* = 2.7, 2.0 Hz, H_2), 3.57 (1 H, dd, *J* = 9.3, 2.9 Hz, H_3), 2.92 (1 H, br. s, C4-*OH*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 137.3 (C_q), 137.2 (C_q), 132.7 (C_q), 131.5, 129.4, 128.7, 128.6, 128.3, 128.3, 128.1, 128.0, 128.0, 116.6 (*C*≡N), 86.6 (C1), 78.4 (C3), 75.4 (C2), 72.5 (*C*H_2Ph), 72.4 (*C*H_2Ph), 68.3 (C4), 63.1 (C3); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 465.1857 $C_{26}H_{29}N_2O_4S$ requires (M+NH$_4$)$^+$, 465.1843].

11.1.41. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(1-*para*-methoxybenzyl -tetrazol-5-yl)-1-thio-α-D-mannopyranoside 103 Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(2-*para*-methoxybenzyl-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 104



103



104

To a stirred solution of **58** (130 mg, 0.21 mmol, 1.0 equiv.) in DMF (2 mL) was added successively, KI (53 mg, 0.32

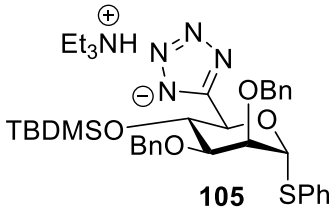
mmol, 1.5 equiv.), K_2CO_3 (44 mg, 0.32 mmol, 1.5 equiv.) and PMBCl (58 µL, d = 1.155, 0.43 mmol, 2.0 equiv.). The reaction was left stirring for 4 h and was diluted with CH_2Cl_2 (10 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with acetone/toluene (1/250, 1/150, 1/100) to furnish isomers **79** and **80** (80 mg, 0.11 mmol, 53%) as colourless oils.

79 was isolated as a yellow oil (42 mg, 58 µmol, 28%). R_f 0.42 (acetone/toluene, 1/50); $[\alpha]_D^{22}$ +25.4 (*c*. 0.53, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.56 – 7.39 (16 H, m, Ar-H), 7.36 (2 H, d, *J* = 8.7 Hz, Ar-H PMB), 6.82 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 5.76 (1 H, d, *J* = 1.8 Hz, H_1), 5.68 (1 H, d, *J* = 9.4 Hz, H_5), 5.66 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 5.63 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 4.83 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph-attached to C2), 4.81 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C3), 4.78 (1 H, d, *J* = 11.1 Hz, C*H*$_2$Ph-attached to C2), 4.75 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph-attached to C3), 4.59 (1 H, app. t, *J* = 9.4 Hz, H_4), 4.22 – 4.19 (1 H, m, H_2), 3.87 (3 H, s, OC*H*$_3$), 3.86 – 3.84 (1 H, m, H_3), 0.79 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (3 H, s, Si(C*H*$_3$)$_2$), -0.53 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 159.7 (C_q PMB), 152.0 (C_q tetrazole), 137.8 (C_q), 137.5 (C_q), 133.5 (C_q), 131.1 (C_q), 129.9, 129.2, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 125.7, 114.1, 86.5 (C1), 80.1 (C3), 76.4 (C2), 72.9 (*C*H$_2$Ph-attached to C2), 72.1 (*C*H$_2$Ph-attached to C3), 70.0 (C4), 67.7 (C5), 55.2 (*C*H$_2$Ph PMB), 50.8 (O*C*H$_3$), 25.6 (C(*C*H$_3$)$_3$), 17.8 (*C*(C*H*$_3$)$_3$), -4.5 (Si(*C*H$_3$)$_2$), -6.1 (Si(*C*H$_3$)$_2$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.5 ($^1J_{C1\text{-}H1}$ =172 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 725.3177 $C_{40}H_{49}N_4O_5SSi$ requires $(M+H)^+$, 725.3187].

80 was isolated as a yellow oil (38 mg, 52 µmol, 25%). R_f 0.48 (acetone/toluene, 1/50); $[\alpha]_D^{22}$ +40.6 (*c*. 0.86, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.43 – 7.21 (18 H,

m, Ar-H), 6.87 (2 H, d, *J* = 8.7 Hz, Ar-H PMB), 5.67 (1 H, d, *J* = 14.1 Hz, C*H*$_2$Ph-PMB), 5.62 (1 H, d, *J* = 14.0 Hz, C*H*$_2$Ph-PMB), 5.56 (1 H, d, *J* = 1.9 Hz, H$_1$), 5.40 (1 H, d, *J* = 9.2 Hz, H$_5$), 4.67 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.60 (1 H, app. t, *J* = 9.1 Hz, H$_4$), 4.57 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.56 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph-attached to C3), 4.52 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C3), 4.01 – 3.99 (1 H, m, H$_2$), 3.79 (3 H, s, OC*H*$_3$), 3.70 (1 H, dd, *J* = 8.9, 2.9 Hz, H$_3$), 0.50 (9H, s, C(C*H*$_3$)$_3$), -0.11 (3 H, s, Si(C*H*$_3$)$_2$), -0.55 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 164.4 (C_q tetrazole), 160.1 (C_q PMB), 138.0 (C_q), 133.9 (C_q), 131.8 (C_q), 130.6 (C_q), 129.0, 128.3, 128.3, 127.8, 127.7, 127.6, 127.5, 125.0, 114.3, 86.5 (C1), 80.2 (C3), 75.7 (C2), 72.1 (*C*H$_2$Ph-attached to C2), 71.6 (*C*H$_2$Ph-attached to C3), 70.4 (C4), 69.1 (C5), 56.5 (*C*H$_2$Ph PMB), 55.3 (O*C*H$_3$), 25.5 (C(*C*H$_3$)$_3$), 17.7 (*C*(CH$_3$)$_3$), -4.1 (Si(*C*H$_3$)$_2$), -5.9 (Si(*C*H$_3$)$_2$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.5 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 725.3192 $C_{40}H_{50}N_4O_5SSi$ requires (M+H)$^+$, 725.3187].

11.1.42. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(1*H*-tetrazol-5-yl)-1-thio-α-D-mannopyranoside triethylammonium salt 105

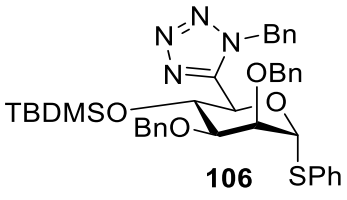


105

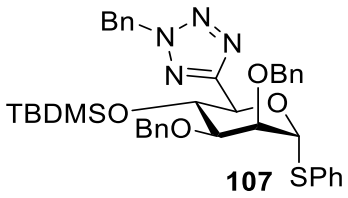
To a stirred solution of **82** (75 mg, 0.12 mmol, 1.0 equiv.) in CH_2Cl_2 (1 mL) was added Et_3N (17 µL, d = 0.726, 0.12 mmol, 1.0 equiv.). The reaction was left stirring for 1 h and then was dried *in vacuo*, giving **105** as a yellow oil (80 mg, 0.11 mmol, 94%). $[\alpha]_D^{22}$ -42.4 (*c*. 0.46, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.51 – 7.26 (15 H, m, Ar-H), 5.62 (1 H, d, *J* = 9.3 Hz, H$_5$), 5.56 (1 H, d, *J* = 1.5 Hz, H$_1$), 4.77 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.74 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.71 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.67 (1 H, d, *J* = 11.3 Hz, C*H*$_2$Ph), 4.53 (1 H, app. t, *J* = 9.1 Hz,

H_4), 4.13 (1 H, app. t, *J* = 2.2 Hz), 3.86 (1 H, dd, *J* = 8.9, 2.9 Hz, H_3), 3.05 (6 H, q, *J* = 7.3 Hz, N(CH_2CH_3)$_3$), 1.23 (9 H, t, *J* = 7.3 Hz, N(CH_2CH_3)$_3$), 0.75 (9 H, s, C(CH_3)$_3$), 0.00 (3 H, s, Si(CH_3)$_2$), -0.43 (3 H, s, Si(CH_3)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 159.6 (C_q tetrazole), 138.2 (C_q), 138.1 (C_q), 134.3 (C_q), 131.9, 128.9, 128.4, 128.3, 128.1, 127.9, 127.8, 127.6, 127.4, 86.9 (C1), 81.0 (C3), 77.2 (C2), 73.1 (CH_2Ph), 72.3 (CH_2Ph), 71.7 (C4), 69.8 (C5), 45.2 (N(CH_2CH_3)$_3$), 25.7 (C(CH_3)$_3$), 17.9 (C(CH_3)$_3$), 8.5 (N(CH_2CH_3)$_3$), -4.4 (Si(CH_3)$_2$), -5.9 (Si(CH_3)$_2$).

11.1.43. Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(1-benzyl -tetrazol-5-yl)-1-thio-α-D-mannopyranoside 106 Phenyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(2-benzyl-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 107



106



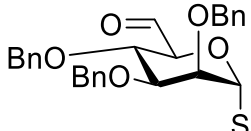
107

To a stirred solution of **105** (80 mg, 0.11 mmol, 1.0 equiv.) in DMF (1.1 mL) was added BnBr (20 µL, d = 1.438, 0.17 mmol, 1.5 equiv.). The reaction was left stirring for 3 h and was diluted with CH_2Cl_2 (10 mL). The organic layer was washed with brine (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with acetone/toluene (1/250, 1/150, 1/100) to furnish the inseparable isomers **106** and **107** as colourless oil in 1/1.2 ratio (24 mg, 34 µmol, 31%). R_f 0.80 (EtOAc/petroleum ether, 1/50); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.41 – 7.18 (40 H, m, Ar-H), 5.73 (1 H, d, *J* = 14.3 Hz, CH_2Ph benzyl tetrazole, N_2-isomer), 5.68 (1 H, d, *J* = 14.2 Hz, CH_2Ph benzyl tetrazole, N_2-isomer), 5.57 (1 H, d, *J* = 2.7 Hz, H_1 N_1-isomer), 5.56 (1 H, d, *J* = 2.4 Hz, H_1 N_2-isomer), 5.54 (2 H, d, *J* = 1.9 Hz, CH_2Ph benzyl tetrazole, N_1-isomer), 5.50 (1 H, d, *J* = 9.5 Hz, H_5

N_1-isomer), 5.40 (1 H, d, *J* = 9.2 Hz, H_5 N_2-isomer), 4.67 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.65 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.61 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.60 (1 H, m, H_4 N_2-isomer) 4.59 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.54 (2 H, s, C*H*$_2$Ph), 4.43 (1 H, app. t, *J* = 9.1 Hz, H_4 N_1-isomer), 4.04 (1 H, app. t, *J* = 2.8 Hz, H_2 N_1-isomer), 4.00 (1 H, app. t, *J* = 2.6 Hz, H_2 N_2-isomer), 3.70 (1 H, dd, *J* = 8.9, 2.9 Hz, H_3 N_1 and N_2-isomers), 0.63 (9 H, s, C(C*H*$_3$)$_3$), 0.51 (9 H, s, C(C*H*$_3$)$_3$), -0.11 (3 H, s, Si(C*H*$_3$)$_2$), -0.17 (3 H, s, Si(C*H*$_3$)$_2$), -0.54 (3 H, s, Si(C*H*$_3$)$_2$), -0.68 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) 164.5 (C_q tetrazole, N_2-isomer), 152.3 (C_q tetrazole, N_1-isomer), 138.0 (C_q), 137.9 (C_q), 137.8 (C_q), 137.5 (C_q), 134.5 (C_q), 133.9 (C_q), 133.6 (C_q), 133.5 (C_q), 132.8, 131.8, 131.0, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.3, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 86.5 (C1, N_1 or N_2-isomer), 86.4 (C1, N_1 or N_2-isomer), 80.2 (C3, N_1 or N_2-isomer), 80.1 (C3, N_1 or N_2-isomer), 76.3 (C2, N_1-isomer), 75.7 (C2, N_2-isomer), 72.9 (*C*H$_2$Ph), 72.1 (2C, *C*H$_2$Ph), 71.6 (*C*H$_2$Ph), 70.4 (C4, N_2-isomer), 69.9 (C4, N_1-isomer), 69.1 (C5, N_2-isomer), 67.8 (C5, N_1-isomer), 56.9 (*C*H$_2$Ph tetrazole, N_2-isomer), 51.2 (*C*H$_2$Ph tetrazole, N_1-isomer), 25.6 (C(*CH*$_3$)$_3$), 25.5 (C(*CH*$_3$)$_3$), 17.8 (*C*(CH$_3$)$_3$), 17.7 (*C*(CH$_3$)$_3$), -4.2 (Si(*C*H$_3$)$_2$), -4.5 (Si(*C*H$_3$)$_2$), -5.9 (Si(*C*H$_3$)$_2$), -6.0 (Si(*C*H$_3$)$_2$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.5 and 86.4 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 695.3084 $C_{39}H_{47}N_4O_4SSi$ requires (M+H)$^+$, 695.3082].

11.1.44. Phenyl

2,3,4-tri-*O*-benzyl-6-aldehyde-1-thio-α-D-mannopyranoside 109

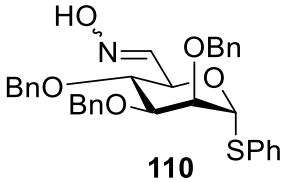


109

To a stirred solution of **108** (420 mg, 0.77 mmol, 1.0 equiv.) in dimethyl sulfoxide (7.7 mL) was added Et_3N (323 µL, d = 0.726, 2.32 mmol, 3.0 equiv.) and sulfur trioxide pyridine

complex (369 mg, 2.32 mmol, 3.0 equiv.) at room temperature. The reaction mixture was left stirring for 1 h before it was diluted with EtOAc (25 mL) and H_2O (20 mL). The whole was extracted with EtOAc (3 x 20 mL) and the extracts were washed with H_2O (5 x 30 mL) and brine (2 x 30 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude aldehyde **109** was obtained as a yellow oil (400 mg, 0.74 mmol, 96%) and was carried on the next step without further purification. R_f 0.83 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +40.5 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 9.73 (1 H, s, C*H*O), 7.49 – 7.27 (20 H, m, Ar-H), 5.59 (1 H, t, *J* = 6.3 Hz, H_1), 4.69 (1 H, d, *J* = 12.9 Hz, CH_2Ph), 4.63 (1 H, d, *J* = 12.8 Hz, CH_2Ph), 4.59 (2 H, d, *J* = 12.0 Hz, CH_2Ph), 4.55 (1 H, d, *J* = 11.9 Hz, CH_2Ph), 4.54 (1 H, d, *J* = 12.5 Hz, CH_2Ph), 4.49 (1 H, d, *J* = 7.7 Hz, H_5), 4.08 (1 H, app. t, *J* = 7.7 Hz, H_4), 3.94 – 3.91 (1 H, m, H_2), 3.87 (1 H, dd, *J* = 7.6, 2.8 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 197.6 (*C*HO), 137.6 (C_q), 137.6 (C_q), 137.6 (C_q), 133.5 (C_q), 131.6, 129.1, 128.5, 128.4, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.6, 84.9 (C1), 77.4 (C3), 77.2 (C5), 75.0 (C2), 74.7 (C4), 74.3 (*C*H_2Ph), 72.3 (*C*H_2Ph), 72.2 (*C*H_2Ph). These data were consistent with literature values.[253]

11.1.45. Phenyl 2,3,4-tri-*O*-benzyl-6-oxime-1-thio-α-D-mannopyranoside 110

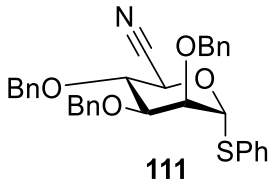


110

Crude aldehyde **109** (4.72 g, 8.73 mmol, 1.0 equiv.) was dissolved in THF (873 mL) and a solution of $H_2NOH.HCl$ (606 mg, 8.73 mmol, 1.0 equiv.) dissolved in H_2O (17.5 mL) was added dropwise. The mixture was cooled to 0 °C and a solution of Na_2CO_3 (1.1 g, 10.5 mmol, 1.2 equiv.) dissolved in H_2O (10.5 mL) was added dropwise. The solution was slowly warmed to room temperature and stirred for 24 h. The mixture was diluted with H_2O (200 mL) and then extracted with EtOAc (4 x 400 mL). The organic layers

were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained crude oil was purified using silica gel flash column chromatography, eluting with Et_2O/petroleum ether (0/100, 5/95, 10/90, 20/80) to furnish **110** as a colourless oil. *Cis* and *trans* (1/7) isomers were isolated separately (major isomer: 3.8 g, 6.54 mmol, 78%, minor isomer: 550 mg, 0.99 mmol, 11%) and both were used for the next step. Major isomer R_f 0.70; minor isomer R_f 0.62 (EtOAc/petroleum ether, 1/2); major: $[\alpha]_D^{22}$ +87.7 (*c*. 3.1, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) major isomer δ 7.46 (1 H, d, *J* = 6.5 Hz, *H*C=N), 7.47 – 7.25 (20 H, m, Ar-H), 5.49 (1 H, d, *J* = 1.5 Hz, H_1), 4.86 (1 H, d, *J* = 10.9 Hz, C*H*$_2$Ph), 4.73 (1 H, dd, *J* = 10.5, 5.6 Hz, H_5), 4.68 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.66 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.65 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.61 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.01 (1 H, app. t, *J* = 9.4 Hz, H_4), 4.01– 3.98 (1 H, m, H_2), 3.87 (1 H, dd, *J* = 9.2, 2.9 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 148.7 (H*C*=N), 138.1 (C_q), 137.7 (C_q), 133.9 (C_q), 131.7 (C_q), 129.1, 128.4, 128.4, 128.3, 128.5, 128.0, 127.8, 127.8, 127.8, 127.6, 86.1 (C1), 79.5 (C3), 76.4 (1 C, C2 or C4), 76.3 (1 C, C2 or C4), 75.1 (*C*H_2Ph), 72.4 (*C*H_2Ph), 72.3 (*C*H_2Ph), 70.6 (C5); **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 578.1993 $C_{33}H_{33}NO_5SNa$ requires $(M+Na)^+$, 578.1977].

11.1.46. Phenyl 2,3,4-tri-*O*-benzyl-6-nitrile-1-thio-α-D-mannopyranoside 111

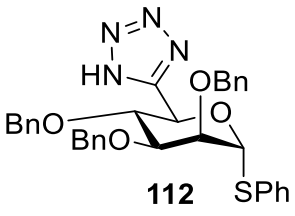


111

Oxime **110** (4.35 g, 7.83 mmol, 1.0 equiv.) was dissolved in dry MeCN (783 mL) and $POCl_3$ (729 µL, d = 1.645, 7.83 mmol, 1.0 equiv.) was added at room temperature. The solution was stirred for 5 min. at room temperature, heated up to 65 °C and then stirred for 3 h. The reaction was quenched with sat. aq. $NaHCO_3$ solution (20 mL) and extracted with EtOAc (3 x 300 mL). The organic layers were combined, dried over Na_2SO_4,

filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (0/100, 5/95, 10/90, 20/80) to furnish **111** as a yellow oil (2.5 g, 4.65 mmol, 59%). R_f 0.76 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +71.0 (*c*. 0.93, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.28 (20 H, m, Ar-H), 5.48 (1 H, d, *J* = 2.2 Hz, H_1), 4.88 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph), 4.88 (1H, d, *J* = 9.7 Hz, H_5), 4.68 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.66 (2 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.19 (1 H, app.t, *J* = 9.2 Hz, H_4), 3.93 (1 H, app. t, *J* = 2.6 Hz, H_2), 3.71 (1 H, dd, *J* = 8.9, 2.9 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 137.5 (C_q), 137.3 (C_q), 137.2 (C_q), 132.7 (C_q), 131.5 (C_q), 129.3, 128.5, 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 128.0, 127.9, 117.1 (*C*≡N), 86.2 (C1), 78.4 (C3), 76.1 (C4), 75.8 (C2 or *C*H_2Ph), 75.7 (C2 or *C*H_2Ph), 72.7 (*C*H_2Ph), 72.6 (*C*H_2Ph), 62.2 (C5); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 538.2068 $C_{33}H_{33}NO_4S$ requires $(M+H)^+$, 358.2052].

11.1.47. Phenyl 2,3,4-tri-*O*-benzyl-6-*C*-(1*H*-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 112

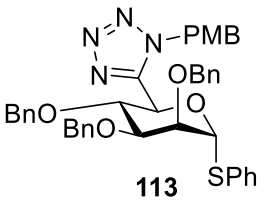


112

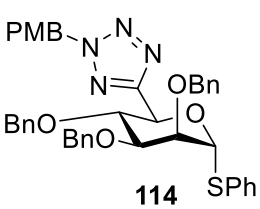
Nitrile **111** (2.5 g, 4.65 mmol, 1.0 equiv.) was dissolved in toluene (465 mL) and $TMSN_3$ (3.7 mL, d = 0.872, 27.9 mmol, 6.0 equiv.) and Bu_2SnO (463 mg, 1.86 mmol, 0.4 equiv.) were added. The mixture was heated to 120 °C and stirred for 16 h. Upon completion, the mixture was cooled down to room temperature, diluted with EtOAc (400 mL) and washed with 0.1 M aq. HCl (250 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude product. Purification of the crude material by silica gel flash column chromatography, eluting with MeOH/CH_2Cl_2 (0/100, 2/98, 5/95) afforded **112** as a brown oil (1.5 g, 2.58 mmol, 55%). R_f 0.65 (MeOH/CH_2Cl_2, 1/9); $[\alpha]_D^{22}$ +95.0 (*c*. 1.96,

$CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.37 – 7.08 (20 H, m, Ar-H), 5.61 (1 H, d, *J* = 9.7 Hz, H_5), 5.54 (1 H, d, *J* = 1.7 Hz, H_1), 4.71 (2 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.70 (1 H, d, *J* = 10.5 Hz, C*H*$_2$Ph), 4.65 (2 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.38 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.19 (1 H, app. t, *J* = 9.4 Hz, H_4), 4.08 (1 H, dd, *J* = 2.7, 2.1 Hz, H_2), 3.99 (1 H, dd, *J* = 9.2, 2.9 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 155.0 (C_q tetrazole), 137.6 (C_q), 137.1 (C_q), 137.0 (C_q), 132.8 (C_q), 132.0, 129.3, 128.7, 128.6, 128.6, 128.4, 128.4, 128.4, 128.1, 128.1, 128.0, 127.9, 86.8 (C1), 79.4 (C3), 76.6 (C2 and C4), 75.0 (*C*H$_2$Ph), 73.3 (*C*H$_2$Ph), 72.8 (*C*H$_2$Ph), 66.5 (C5); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 581.2251 $C_{33}H_{34}N_4O_4S$ requires $(M+H)^+$, 581.2223].

11.1.48. Phenyl 2,3,4-tri-*O*-benzyl-6-*C*-(1-*para*-methoxybenzyl-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 113 Phenyl 2,3,4-tri-*O*-benzyl-6-*C*-(2-*para*-methoxybenzyl-tetrazol-5-yl)-1-thio-α-D-mannopyranoside 114



113



114

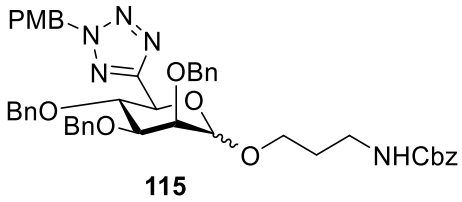
To a stirred solution of **112** (920 mg, 1.37 mmol, 1.0 equiv.) in DMF (10 mL) was added successively, KI (341 mg, 2.06 mmol, 1.5 equiv.), K_2CO_3 (227 mg, 1.65 mmol, 1.2 equiv.) and PMBCl (279 µL, d = 1.155, 2.06 mmol, 1.5 equiv.). The reaction was left stirring for 16 h and was diluted with CH_2Cl_2 (30 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (30 mL) and brine (30 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (5/95, 10/90, 15/85) to furnish isomers **113** and **114** (732 mg, 1.04 mmol, 76%) as colourless oils.

113 was isolated as a yellow oil (374 mg, 0.53 mmol, 39%). R_f 0.72 (EtOAc/petroleum ether, 1/2); $[\alpha]_D^{22}$ +48.5 (*c*. 2.75, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.39 – 6.95 (21 H, m, Ar-H), 7.00 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 6.65 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 5.61 (1 H, d, *J* = 1.6 Hz, H_1), 5.45 (1 H, d, *J* = 9.9 Hz, H_5), 5.28 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 5.19 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 4.72 (2 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.70 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.67 (1 H, d, *J* = 11.0 Hz, C*H*$_2$Ph), 4.65 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.45 (1 H, app. t, *J* = 9.6 Hz, H_4), 4.33 (1 H, d, *J* = 10.6 Hz, C*H*$_2$Ph), 4.08 (1 H, dd, *J* = 2.7, 2.0 Hz, H_2), 3.93 (1 H, dd, *J* = 9.2, 2.8 Hz, H_3), 3.70 (3 H, s, OC*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 159.6 (C_q PMB), 152.2 (C_q tetrazole), 137.8 (C_q), 137.5 (C_q), 137.5 (C_q), 133.3 (C_q), 130.7 (C_q), 129.3, 129.3, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 125.6, 114.1, 86.3 (C1), 79.7 (C3), 76.4 (C2), 76.0 (C4), 75.1 (*C*H$_2$Ph), 72.8 (*C*H$_2$Ph), 72.5 (*C*H$_2$Ph), 65.6 (C5), 55.2 (O*C*H$_3$), 50.5 (*C*H$_2$Ph PMB); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.3 ($^1J_{C1\text{-}H1}$ =172 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 701.2829 $C_{41}H_{42}N_4O_4S$ requires $(M+H)^+$, 701.2798].

114 was isolated as a yellow oil (355 mg, 0.51 mmol, 37%). R_f 0.73 (EtOAc/petroleum ether, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.13 (21 H, m, Ar-H), 6.84 (2 H, d, *J* = 8.0 Hz, Ar-H PMB), 6.76 (2 H, d, *J* = 8.7 Hz, Ar-H PMB), 5.57 (1 H, d, *J* = 1.3 Hz, H_1), 5.52 (1 H, d, *J* = 9.9 Hz, H_5), 5.27 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 5.18 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph-PMB), 4.63 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.59 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.58 (1 H, app. t, *J* = 9.9 Hz, H_4), 4.56 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph), 4.08 – 4.06 (1 H, m, H_2), 3.94 (1 H, dd, *J* = 9.3, 1.2 Hz, H_3), 4.33 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.23 (1 H, d, *J* = 10.8 Hz, C*H*$_2$Ph), 3.67 (1 H, s, OC*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 164.3 (C_q tetrazole), 160.0 (C_q PMB), 138.1 (C_q), 138.1 (C_q), 137.8 (C_q), 133.8 (C_q), 132.0 (C_q), 129.4, 129.3, 128.6,

128.5, 128.3, 128.1, 128.1, 127.9, 127.8, 127.7, 125.3, 114.3, 86.6 (C1), 79.8 (C3), 76.3 (C2), 76.0 (C4), 75.1 (CH$_2$Ph), 72.4 (CH$_2$Ph), 72.2 (CH$_2$Ph), 67.1 (C5), 56.5 (CH$_2$Ph PMB), 55.3 (OCH$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 86.6 ($^1J_{C1\text{-}H1}$ =168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 701.2829 $C_{41}H_{42}N_4O_4S$ requires $(M+H)^+$, 701.2798].

11.1.49. 3-(benzyloxycarbonylamino) propyl (2,3,4-tri-*O*-benzyl-6-*C*-(2-*para*-methoxybenzyl-tetrazol-5-yl)-α/β-D-mannopyranoside 115



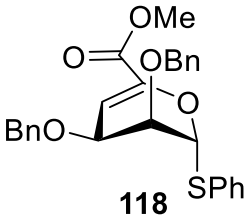
115

A solution of **113** and **114** (290 mg, 0.41 mmol, 1.0 equiv.) and 3-(benzyloxycarbonylamino)-1-propanol (259 mg, 1.24 mmol, 3.0 equiv.) in in CH_2Cl_2 (4.1 mL) was stirred over activated MS4Å for 1 h before NIS (139 mg, 0.62 mmol, 1.5 equiv.) was added. The mixture was cooled to -40 °C before AgOTf (53 mg, 0.21 mmol, 0.5 equiv.) was added. The reaction was warmed up to 0 °C and stirred for 3 h. Upon completion, Et_3N was added until pH = 7, and subsequently diluted with CH_2Cl_2 (20 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (20 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/petroleum (30/70, 40/60 and 50/50) afforded **115** (N_2 isomer) as a colourless oil in an anomeric mixture of α/β = 1/1 ratio (110 mg, 0.14 mmol, 34%). R_f 0.66 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.40 – 7.22 (23 H, m, Ar-H), 7.19 – 7.09 (6 H, m, Ar-H PMB), 6.83 – 6.73 (4 H, m, Ar-H PMB), 5.65 (1 H, d, J = 14.4 Hz, CH_2Ph PMB), 5.60 (1 H, d, J = 14.6 Hz, CH_2Ph PMB), 5.60 (1 H, d, J = 14.6 Hz, CH_2Ph PMB), 5.55 (1 H, d, J = 14.4 Hz, CH_2Ph PMB), 5.09 (1 H, d, J = 12.0 Hz, CH_2Ph), 5.03 (1 H, d, J = 12.4 Hz, CH_2Ph), 4.96 (1

H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.92 (1 H, d, *J* = 9.9 Hz, H$_5$ α-anomer), 4.85 (1 H, d, *J* = 2.2 Hz, H$_1$ α-anomer), 4.83 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.78 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.73 (1 H, d, *J* = 11.2 Hz, C*H*$_2$Ph), 4.69 (2 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.63 (2 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.62 (1 H, d, *J* = 9.6 Hz, H$_5$ β-anomer), 4.59 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 3.0 Hz, H$_1$ β-anomer), 4.53 (1 H, d, *J* = 12.8 Hz, C*H*$_2$Ph), 4.49 (2 H, app. t, *J* = 9.7 Hz, H$_4$ α and β-anomer), 4.47 (1 H, d, *J* = 12.8 Hz, C*H*$_2$Ph), 4.28 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 4.24 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 3.96 (1 H, d, *J* = 2.7 Hz, H$_2$ β-anomer), 3.95 (1 H, dd, *J* = 9.4, 2.9 Hz, H$_3$ α-anomer), 3.93 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$NHCbz α-anomer), 3.90 – 3.79 (2 H, m, H$_2$ α-anomer, OC*H*$_2$CH$_2$CH$_2$NHCbz β-anomer), 3.72 (3 H, s, OC*H*$_3$ α-anomer), 3.70 (3 H, s, OC*H*$_3$ β-anomer), 3.59 (1 H, dd, *J* = 9.4, 2.7 Hz, H$_3$ β-anomer), 3.56 – 3.50 (1 H, m, OC*H*$_2$CH$_2$CH$_2$NHCbz α-anomer), 3.47 – 3.40 (1 H, m, OC*H*$_2$CH$_2$CH$_2$NHCbz β-anomer), 3.28 (4 H, m, OCH$_2$CH$_2$C*H*$_2$NHCbz α and β-anomer), 1.78 (4 H, dt, *J* = 12.9, 6.9 Hz, OCH$_2$C*H*$_2$CH$_2$NHCbz α and β-anomer); **^{13}C NMR** (101 MHz; CDCl$_3$) δ 164.5 (C$_q$ tetrazole α-anomer), 164.0 (C$_q$ tetrazole β-anomer), 160.0 (C$_q$ PMB), 156.4 (C=O CBz), 138.6 (C$_q$), 138.4 (2 C, C$_q$), 138.2 (2 C, C$_q$), 138.1 (C$_q$), 138.1 (C$_q$), 136.6 (C$_q$), 130.0 (C$_q$), 130.0 (C$_q$), 129.1, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 128.0, 127.7, 127.7, 127.7, 127.6, 127.6, 127.4, 114.3, 102.2 (C1 β-anomer), 98.9 (C1 α-anomer), 81.8 (C3 β-anomer), 79.9 (C3 α-anomer), 77.2 (2 C, C4, α and β-anomer), 75.0 (C2 α-anomer), 74.8 (*C*H$_2$Ph), 74.2 (*C*H$_2$Ph), 74.2 (C2 β-anomer), 74.1 (*C*H$_2$Ph), 72.9 (*C*H$_2$Ph), 72.5 (2 C, *C*H$_2$Ph), 71.7 (*C*H$_2$Ph), 69.8 (C5 β-anomer), 67.6 (O*C*H$_2$CH$_2$CH$_2$NHCbz α-anomer), 66.6 (*C*H$_2$Ph), 66.4 (C5 α-anomer), 65.8 (O*C*H$_2$CH$_2$CH$_2$NHCbz β-anomer), 56.4 (2 C, *C*H$_2$Ph PMB), 55.2 (2 C, O*C*H$_3$), 38.4 (OCH$_2$CH$_2$*C*H$_2$NHCbz α-anomer), 38.2 (OCH$_2$CH$_2$*C*H$_2$NHCbz β-anomer), 29.7 (OCH$_2$*C*H$_2$CH$_2$NHCbz β-anomer), 29.5 (OCH$_2$*C*H$_2$CH$_2$NHCbz α-anomer);

^{13}C-GATED (101 MHz; $CDCl_3$): 102.2 ($^1J_{C1\text{-}H1}$ =156 Hz, C1 β-anomer); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 800.3693 $C_{46}H_{51}N_5O_8$ requires $(M+H)^+$, 800.3659].

11.1.50. Methyl (phenyl 4,5-ene-2,3-di-*O*-benzyl-1-thio-α-D-mannopyranoside) uronate 118

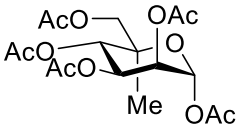


118

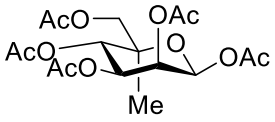
To a stirred solution of **55** (150 mg, 0.29 mmol, 1.0 equiv.) in THF (1.5 mL) being cooled to -70 °C, whereby a 1 M solution of LDA (34 µL, 0.35 mmol, 1.2 equiv.) in THF/hexane was added dropwise. The reaction mixture was kept between -80 °C and -70 °C for 15 min. before MeI (268 µL, d = 2.28, 0.43 mmol, 1.5 equiv.) was added dropwise, and warmed to room temperature over 40 min. The reaction was quenched with sat. aq. NH_4Cl solution (10 mL) and diluted with CH_2Cl_2 15 (mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a yellow oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 20/80, 40/60, 90/10) afforded **118**, as a colourless oil (82 mg, 0.18 mmol, 61%). R_f 0.65 (EtOAc/hexane, 1/2); $[\alpha]_D^{26}$ +68.0 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (300 MHz; $CDCl_3$) δ 7.54–7.25 (15 H, m, Ar-H), 6.25 (1 H, dd, *J* = 2.7, 1.3 Hz, H_4), 5.71 (1 H, d, *J* = 3.8 Hz, H_1), 4.72 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C2), 4.63 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph-attached to C2), 4.61 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C3), 4.56 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C3), 4.34 (1 H, dd, *J* = 4.1, 2.9 Hz, H_3), 3.94 (1 H, td, *J* = 4.0, 1.3 Hz, H_2), 3.79 (3 H, s, CO_2CH_3); **^{13}C NMR** (75 MHz; $CDCl_3$) δ 162.3 (CO_2CH_3), 141.6 (C5), 137.7 (C_q), 137.4 (C_q), 132.8 (C_q), 132.5, 129.2, 128.5, 128.2, 128.2, 128.0, 127.9, 127.7, 110.4 (C4), 85.0 (C1), 72.0 (*C*H$_2$Ph-attached to C2), 71.3 (*C*H$_2$Ph-attached to C3) , 71.1 (C2), 68.8 (C3), 52.5 (CO_2CH_3); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 480.1847

$C_{27}H_{30}NO_5S$ requires $(M+NH_4)^+$, 480.1839]; **IR** ν_{max}/cm^{-1} 1731 (m, C=O_{ester}), 1652 (w, C=C), 1086 (s, C-O_{ether}).

11.1.51. 2,3,4,6-penta-*O*-acetyl-5-*C*-methyl-α/β-D-mannopyranoside 120, 121, 1,2,3,6-tetra-*O*-acetyl-5-*C*-methyl-α/β-D-mannofuranoside 122, 123



120



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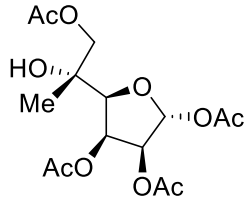
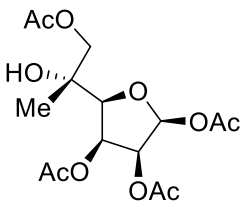


122

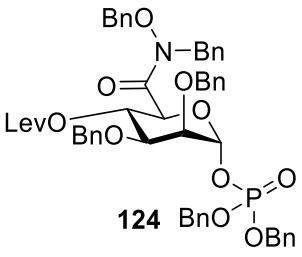


123

To a stirred mixture of Ac_2O (847 µL, d = 1.08, 9.0 mmol, 10.3 equiv.) and 5-*C*-methyl-α/β-D-mannopyranose (170 mg, 0.87 mmol, 1.0 equiv.), H_2SO_4 (47 µL, d = 1.83, 0.87 µmol, 0.001 equiv.) was added at 0 °C, under an atmosphere of N_2. The solution was stirred for 10 min. at 0°C and then allowed to warm to room temperature and stirred for a further 45 min. The mixture was then diluted with ice–water (10 mL), and the organic phase extracted with EtOAc (2 x 10 mL). The extract was washed with H_2O (10 mL), sat. aq. $NaHCO_3$ solution (15 mL), dried over $MgSO_4$, filtered, and the solvent evaporated to dryness to pyranoses **120** (40%), **121** (43%) and furanoses **122** (5%), **123** (12%) as a pale yellow viscous oil (218 mg, 0.54 mmol, 62%); R_f 0.25 (EtOAc/petroleum ether, 1/1); **^{1}H NMR** (400 MHz; $CDCl_3$) δ **α-pyranoside**: 6.11 (1 H, d, *J* = 2.3 Hz, H_1), 5.58 (1 H, d, *J* = 10.5 Hz, H_4), 5.29 – 5.26 (2 H, m, H_2, H_3 α or β), 4.11 (1 H, d, *J* = 12.0 Hz, H_{6a}), 3.96 (1 H, d, *J* = 12.0 Hz, H_{6b}), 2.02 (3 H, s, C(O)CH_3), 2.06 (3 H, s, C(O)CH_3), 2.12 (3 H, s, C(O)CH_3), 2.14 (3 H, s, C(O)CH_3), 2.17 (3 H, s, C(O)CH_3), 1.39 (3 H, s, *C*5-CH_3). **β-pyranoside**: 6.06 (1 H, d, *J* = 1.4 Hz, H_1), 5.48 (1 H, d, *J* = 10.4 Hz, H_4), 5.49 – 5.46 (2 H, m, H_2, H_3 α or β), 4.00 (1 H, d, *J* = 11.9 Hz, H_{6a}), 4.00 (1 H, d, *J* = 12.0 Hz, H_{6b}), 2.01 (3 H, s, C(O)CH_3), 2.05 (3 H, s, C(O)CH_3), 2.10 (3 H, s, C(O)CH_3), 2.12 (3 H, s, C(O)CH_3), 2.21 (3 H, s, C(O)CH_3), 1.40 (3 H, s, *C*5-CH_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 170.6

(*C*(O)CH_3), 170.5 (*C*(O)CH_3), 170.2 (*C*(O)CH_3), 170.0 (*C*(O)CH_3), 169.9 (*C*(O)CH_3), 169.7 (*C*(O)CH_3), 169.5 (*C*(O)CH_3), 169.4 (*C*(O)CH_3), 168.6 (*C*(O)CH_3), 168.4 (*C*(O)CH_3), 99.6 (C1 α-furanoside), 98.3 (β-furanoside) 91.0 (C1 α-pyranoside), 86.8 (C1 β-pyranoside), 78.2 (C5 α-pyranoside), 76.1 (C5 β-pyranoside), 68.9 (C2 α-pyranoside), 68.4 (C3 β-pyranoside), 68.3 (C2 β-pyranoside), 67.3 (C6 α-pyranoside), 67.2 (C6 β-pyranoside), 66.6 (C3 α-pyranoside), 66.5 (C4 α-pyranoside), 66.2 (C4 β-pyranoside), 21.2 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.6 (C(O)*C*H_3), 20.6 (C(O)*C*H_3), 19.3 (C5-*C*H_3 α-pyranoside), 15.0 (C5-*C*H_3 β-pyranoside); **^{13}C-GATED** (101 MHz; $CDCl_3$): 99.6 ($^1J_{C1\text{-}H1}$ = 180 Hz, **143**), 98.3 ($^1J_{C1\text{-}H1}$ = 184 Hz, **144**), 91.1 ($^1J_{C1\text{-}H1}$ = 176 Hz, **141**), 86.8 ($^1J_{C1'\text{-}H1'}$ = 164 Hz, **142**); Pyranosides **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 427.1226 $C_{17}H_{24}O_{11}Na$ requires $(M+Na)^+$, 427.1216]; These data were consistent with literature values.[225]

11.1.52. Di-*O*-benzyl (*O*-benzyl, *N*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-di-*O*-benzyl)-1-phosphate-α-D-mannopyranoside) hydroxamate 124



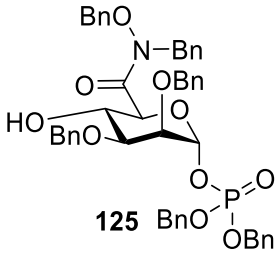
124

Hydroxamate thioglycoside **69** (420 mg, 0.55 mmol, 1.0 equiv.) was stirred with activated MS4Å for 1 h in CH_2Cl_2 (4.5 mL). Dibenzyl phosphate (230 mg, 0.83 mmol, 1.5 equiv.) was added, and the solution was stirred for further 30 min. before being cooled down to -30 °C. NIS (187 mg, 0.83 mmol, 1.5 equiv.) and AgOTf (42 mg, 0.16 mmol, 0.3 equiv.) were added successively and the reaction mixture was stirred for further 2.5 h, allowing the temperature to reach -10 °C. When TLC analysis indicated complete conversion of **69** to a lower R_f value material, the reaction was quenched by the addition of Et_3N (770 µL, d = 0.726, 5.5 mmol, 10.0

equiv.) and diluted with CH_2Cl_2 (20 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (15 mL), brine (15 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/petroleum ether (20/80, 30/70 and 40/60) afforded **124** as a colourless oil (280 mg, 0.30 mmol, 55%). R_f 0.66 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -36.5 (*c*. 0.73, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.34 – 7.22 (30 H, m, Ar-H), 5.70 (1 H, dd, *J* = 6.1, 1.9 Hz, H_1), 5.69 (1 H, app. t, *J* = 9.9 Hz, H_4), 5.40 (1 H, d, *J* = 11.9 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.36 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 4.99 (1 H, d, *J* = 11.7 Hz, OP(O)OC*H*$_2$Ph), 4.97 (1 H, d, *J* = 11.4 Hz, OP(O)OC*H*$_2$Ph), 4.95 (1 H, d, *J* = 11.5 Hz, OP(O)OC*H*$_2$Ph), 4.93 (1 H, d, *J* = 11.7 Hz, OP(O)OC*H*$_2$Ph), 4.92 (1 H, d, *J* = 12.9 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.89 (1 H, d, *J* = 12.7 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.69 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2), 4.57 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2), 4.48 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.45 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C3), 4.28 (1 H, d, *J* = 9.7 Hz, H_5), 3.75 (1 H, dd, *J* = 9.7, 2.9 Hz, H_3), 3.67 (1 H, app. t, *J* = 2.5 Hz, H_2), 2.64 – 2.35 (3 H, m, C*H*$_2$ Lev), 2.29 – 2.14 (1 H, m, C*H*$_2$ Lev), 2.11 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.1 (C=O Lev), 150.3 (*C*(O)N(Bn)OBn), 137.9 (C_q), 137.7 (C_q), 137.6 (C_q), 137.0 (C_q), 135.5 (1 C, d, *J* = 6.8 Hz, C_q), 135.4 (1 C, d, *J* = 6.8 Hz, C_q) 128.7, 128.6, 128.6, 128.6, 128.4, 128.2, 128.2, 128.2, 128.1, 128.1, 127.8, 127.7, 127.7, 127.6, 127.6, 96.0 (1 C, d, *J* = 6.0 Hz, C1), 76.3 (C(O)N(Bn)O*C*H$_2$Ph), 75.5 (C3), 74.2 (d, *J* = 9.2 Hz, C2), 73.5 (C(O)N(*C*H$_2$Ph)OBn), 73.1 (*C*H$_2$Ph-attached to C2), 72.3 (*C*H$_2$Ph-attached to C3), 72.2 (C5), 69.7 (d, *J* = 5.3 Hz, OP(O)O*C*H$_2$Ph), 69.5 (d, *J* = 5.4 Hz, OP(O)O*C*H$_2$Ph), 67.9 (C4), 37.9 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 27.8 (*C*H$_2$ Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 96.0 ($^1J_{C1\text{-}H1}$ = 180 Hz, C1); **^{31}P NMR** δ $_P$ (162

MHz, $CDCl_3$) -2.82 (s); **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 950.3311 $C_{53}H_{54}NO_{12}PNa$ requires $(M+Na)^+$, 950.3276].

11.1.53. Di-*O*-benzyl (*O*-benzyl, *N*-benzyl 2,3-di-*O*-benzyl)-1-phosphate-α-D-mannopyranoside) hydroxamate 125

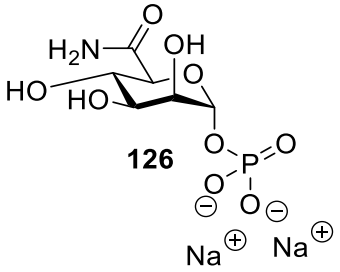


125

*C*6-hydroxamate **124** (270 mg, 0.29 mmol, 1.0 equiv.) was dissolved in a mixture of pyridine/AcOH (3 mL, 4/1 *v/v*), after which H_2N-NH_2.AcOH (53 mg, 0.58 mmol, 2.0 equiv.) was added. The mixture was stirred for 1 h and then was diluted with EtOAc (25 mL), washed with 1 M HCl (20 mL), sat. aq. $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was then dried over $MgSO_4$ filtered and concentrated under reduced pressure to furnish a yellow oil. Purification using silica gel flash column chromatography, eluting with EtOAc/toluene (0/100, 30/70, 40/60, 90/10) afforded **125** as a colourless oil (173 mg, 0.21 mmol, 72%). R_f 0.62 (EtOAc/Toluene, 3/7); $[\alpha]_D^{22}$ +3.34 (*c*. 1.0, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.33 – 7.21 (30 H, m, Ar-H), 5.72 (1 H, dd, *J* = 6.7, 1.6 Hz, H_1), 5.31 (1 H, d, *J* = 12.1 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.27 (1 H, d, *J* = 12.1 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.01 (1 H, d, *J* = 12.1 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.97 (1 H, d, *J* = 11.0 Hz, OP(O)OC*H*$_2$Ph), 4.94 (1 H, d, *J* = 11.6 Hz, OP(O)OC*H*$_2$Ph), 4.93 (1 H, d, *J* = 12.3 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.91 (1 H, d, *J* = 12.0 Hz, OP(O)OC*H*$_2$Ph), 4.89 (1 H, d, *J* = 11.7 Hz, OP(O)OC*H*$_2$Ph), 4.63 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.58 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.49 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.36 (1 H, app. t, *J* = 9.6 Hz, H_4), 4.24 (1 H, d, *J* = 9.6 Hz, H_5), 3.64 (1 H, dd, *J* = 4.7, 2.8 Hz, H_2), 3.62 (1 H, dd, *J* = 9.5, 3.1 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 151.3 (*C*(O)N(Bn)OBn), 138.1 (C_q), 137.6 (C_q), 137.4 (C_q), 136.7 (C_q), 135.5 (d, *J* = 3.2 Hz, C_q), 135.4 (d, *J* = 3.1 Hz, C_q), 128.7, 128.6, 128.6, 128.6, 128.4, 128.4, 128.4, 128.4, 128.1, 128.1, 128.0, 127.9,

127.9, 127.8, 127.7, 127.7, 127.6, 96.2 (d, *J* = 6.0 Hz, C1), 77.2 (C3), 76.5 (C(O)N(Bn)O*C*H$_2$Ph), 74.5 (d, *J* = 8.8 Hz, C2), 73.1 (*C*H$_2$Ph), 73.0 (C5), 72.9 (*C*H$_2$Ph), 72.7 (*C*H$_2$Ph), 69.7 (d, *J* = 5.5 Hz, OP(O)O*C*H$_2$Ph), 69.5 (d, *J* = 5.5 Hz, OP(O)O*C*H$_2$Ph), 67.0 (C4); **^{31}P NMR** δ $_P$ (162 MHz, $CDCl_3$) -2.81 (s); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 830.3121 $C_{48}H_{50}NO_{10}P$ requires $(M+H)^+$, 830.3089].

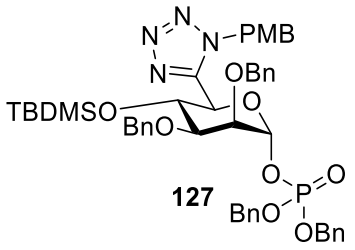
11.1.54. (6-*C*-amide) α-D-mannopyranoside 1-phosphate (disodium salt) 126



126

A suspension of hydroxamate **125** (56 mg, 67 μmol, 1.0 equiv.), 10% Pd/C (86 mg, 81 μmol, 1.2 equiv.), 20% $Pd(OH)_2/C$ (57 mg, 81 μmol, 1.2 equiv.) and 5% aq. $NaHCO_3$ (227 μL, 0.14 mmol, 2.0 equiv.) in a mixture of EtOH/THF (1.4 mL, 1.5/1 *v/v*) was stirred under an atmosphere of hydrogen (1 atm, balloon) at room temperature for 32 h. TLC analysis (hexane/EtOAc, 1/2) showed complete conversion of starting material to a lower R_f spot. The reaction mixture was filtered through Celite®, followed by solvent removal *in vacuo* to afford **126** as a white solid (19 mg, 60 μmol, 89%). R_f (0.14); $[\alpha]_D^{23}$ -8.72 (*c*. 0.46, H_2O); **^{1}H NMR** (400 MHz; D_2O) δ 5.31 (1 H, dd, *J* = 8.4, 1.5 Hz, H_1), 4.20 (1 H, d, *J* = 9.9 Hz, H_5), 3.93 – 3.87 (2 H, m, H_2 and H_3), 3.74 (1 H, app. t, *J* = 9.5 Hz, H_4); **^{13}C NMR** (101 MHz; D_2O) δ 174.7 (*C*(O)NH_2), 95.1 (d, *J* = 4.8 Hz, C1), 71.7 (C5), 70.6 (d, *J* = 7.4 Hz, C2), 69.7 (C3), 68.4 (C4); **^{31}P NMR** δ $_P$ (162 MHz, D_2O) 1.89 (s); **HRMS** (ES^-) *m/z* [Found: $(M-H)^-$ 272.0178 $C_6H_{10}NO_9P$ requires $(M-H)^-$, 272.0177].

11.1.55. Di-*O*-benzyl (2,3,-di-*O*-benzyl-4-*O*-*tert*-butyl dimethylsilyl-6-*C*-(1-*para*-methoxybenzyl -tetrazol-5-yl)-1-phosphate-α-D-mannopyranoside 127

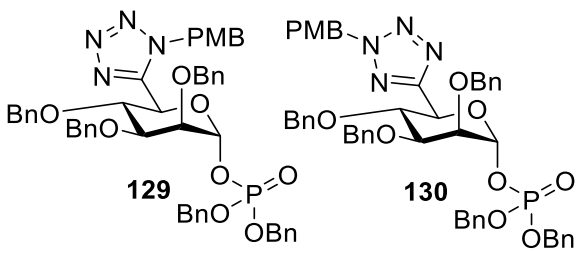


127

*C*6-tetrazole thioglycoside **103** (50 mg, 69 µmol, 1.0 equiv.) was stirred with activated MS4Å for 1h in CH_2Cl_2 (0.7 mL). Dibenzyl phosphate (29 mg, 0.10 mmol, 1.5 equiv.) was added, and the solution was stirred for further 30 min. before being cooled down to -40 °C. NIS (23 mg, 0.10 mmol, 1.5 equiv.) and AgOTf (11 mg, 40 µmol, 0.6 equiv.) were added successively and the reaction mixture was stirred for further 3.5 h, allowing the temperature to reach 0 °C. When TLC analysis indicated complete conversion of **103** to a lower R_f value, the reaction was quenched by the addition of Et_3N (96 µL, d = 0.726, 0.7 mmol, 10.0 equiv.) and diluted with CH_2Cl_2 (10 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (10 mL), brine (5 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/toluene (5/95, 10/90 and 30/70) afforded **127** as colourless oil (10 mg, 10 µmol, 16%). R_f 0.54 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -61.6 (*c*. 0.4, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.33 – 7.27 (21 H, m, Ar-H), 7.22 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 6.67 (1 H, d, *J* = 8.8 Hz, Ar-H PMB), 5.72 (1 H, dd, *J* = 6.4, 2.1 Hz, H_1), 5.48 (1 H, d, *J* = 14.8 Hz, CH_2Ph PMB), 5.39 (1 H, d, *J* = 14.8 Hz, CH_2Ph PMB), 5.17 (1 H, d, *J* = 9.7 Hz, H_5), 4.99 (1 H, d, *J* = 9.2 Hz, OP(O)OCH_2Ph), 4.98 (1 H, d, *J* = 9.3 Hz, OP(O)OCH_2Ph), 4.62 (1 H, d, *J* = 11.4 Hz, CH_2Ph), 4.56 (1 H, d, *J* = 11.5 Hz, CH_2Ph), 4.53 (1 H, d, *J* = 11.9 Hz, CH_2Ph), 4.46 (1 H, d, *J* = 11.7 Hz, CH_2Ph), 4.34 (1 H, app. t, *J* = 9.4 Hz, H_4), 3.72 (3 H, s, OCH_3), 3.73 – 3.70 (1 H, m, H_2), 3.64 (1 H, dd, *J* = 9.0, 2.7 Hz, H_3), 0.59 (9H, s, C(CH_3)$_3$), -0.22 (3 H, s, Si(CH_3)$_2$), -0.79 (3 H, s,

Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 159.8 (C_q PMB), 151.3 (C_q tetrazole), 137.7 (C_q), 137.4 (C_q), 135.3 (d, *J* = 6.1 Hz, C_q OP(O)OBn), 135.2 (d, *J* = 6.1 Hz, C_q OP(O)OBn), 130.1(C_q), 128.9, 128.7, 128.7, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 125.7, 114.2 (Ar-C), 95.9 (d, *J* = 5.7 Hz, C1), 78.7 (C3), 74.5 (C2), 73.8 (*C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 70.0 (d, *J* = 5.7 Hz, OP(O)O*C*H$_2$Ph), 69.9 (d, *J* = 5.5 Hz, OP(O)O*C*H$_2$Ph), 69.1 (C4), 68.8 (C5), 50.7 (*C*H$_2$Ph PMB), 25.6 (C(*CH*$_3$)$_3$), 17.8 (*C*(C*H*$_3$)$_3$), -4.6 (Si(*C*H$_3$)$_2$), -6.1 (Si(*C*H$_3$)$_2$); **^{31}P NMR** δ $_P$ (162 MHz, $CDCl_3$) -2.93 (s); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 893.3719 $C_{48}H_{58}N_4O_9SPSi$ requires (M+H)$^+$, 893.3711].

11.1.56. Di-*O*-benzyl (2,3,4-tri-*O*-benzyl-6-*C*-(1-*para*-methoxybenzyl -tetrazol-5-yl)-1-phosphate)-α-D-mannopyranoside 129 Di-*O*-benzyl 2,3,4-tri-*O*-benzyl-6-*C*-(2-*para*-methoxybenzyl-tetrazol-5-yl)-1-phosphate)-α-D-mannopyranoside 130

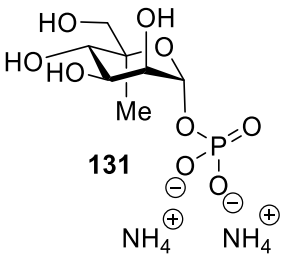


*C*6-tetrazole thioglycoside **113** and **114** (730 mg, 1.04 mmol, 1.0 equiv.) was stirred with activated MS4Å for 1h in CH_2Cl_2 (10 mL). Dibenzyl phosphate (580 mg, 2.08 mmol, 2.0 equiv.) was added, and the solution was stirred for further 30 min. before being cooled down to -30 °C. NIS (350 mg, 1.56 mmol, 1.5 equiv.) and AgOTf (133 mg, 0.52 mmol, 0.5 equiv.) were added successively and the reaction mixture was stirred for further 3.5 h, allowing the temperature to reach 0 °C. When TLC analysis indicated complete conversion of **113** and **114** to a lower R_f value, the reaction was quenched by the addition of Et_3N (1.4 mL, d = 0.726, 10.4 mmol, 10.0 equiv.) and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with 10% aq. $Na_2S_2O_3$ solution (30 mL), brine (30 mL), dried over $MgSO_4$, filtered

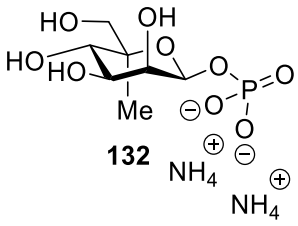
and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/toluene (5/95, 10/90 and 30/70) afforded **129** and **130** as colourless oils in a 50/50 ratio (650 mg, 0.75 mmol, 72%). R_f 0.66 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.27 (35 H, m, Ar-H), 7.14 (2 H, d, *J* = 7.2 Hz, Ar-H PMB), 7.07 (2 H, d, *J* = 8.7 Hz, Ar-H PMB), 6.93 (2 H, d, *J* = 6.7 Hz, Ar-H), 6.82 (2 H, d, *J* = 6.5 Hz, Ar-H), 6.75 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 6.65 (2 H, d, *J* = 8.8 Hz, Ar-H PMB), 5.76 (1 H, dd, *J* = 6.3, 1.8 Hz, H_1 N_2-isomer), 5.69 (1 H, dd, *J* = 6.3, 2.1 Hz, H_1 N_1-isomer), 5.65 (1 H, d, *J* = 14.0 Hz, C*H*$_2$Ph PMB), 5.61 (1 H, d, *J* = 14.2 Hz, C*H*$_2$Ph PMB), 5.33 (1 H, d, *J* = 15.0 Hz, C*H*$_2$Ph PMB), 5.16 (1 H, d, *J* = 13.4 Hz, C*H*$_2$Ph PMB), 5.15 (1 H, d, *J* = 10.0 Hz, H_5 N_1-isomer or N_2-isomer), 5.12 (1 H, d, *J* = 9.7 Hz, H_5 N_1-isomer or N_2-isomer), 5.04 (2 H, d, *J* = 8.6 Hz, OP(O)OC*H*$_2$Ph), 4.96 (2 H, d, *J* = 8.3 Hz, OP(O)OC*H*$_2$Ph), 4.95 (2 H, d, *J* = 8.3 Hz, OP(O)OC*H*$_2$Ph), 4.94 (2 H, d, *J* = 8.3 Hz, OP(O)OC*H*$_2$Ph), 4.72 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.71 (1 H, d, *J* = 11.0 Hz, C*H*$_2$Ph), 4.69 (1 H, d, *J* = 11.2 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.64 (1 H, d, *J* = 10.1 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 10.6 Hz, C*H*$_2$Ph), 4.54 (2 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.53 (1 H, app. t, *J* = 9.6 Hz, H_4 N_2-isomer), 4.49 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.39 (1 H, app. t, *J* = 9.8 Hz, H_4 N_1-isomer), 4.29 (1 H, d, *J* = 10.6 Hz, C*H*$_2$Ph), 4.21 (1 H, d, *J* = 10.7 Hz, C*H*$_2$Ph), 3.89 (1 H, dd, *J* = 9.6, 3.2 Hz, H_3 N_2-isomer), 3.86 (1 H, dd, *J* = 9.6, 3.0 Hz, H_3 N_1-isomer), 3.78 (1 H, dd, *J* = 4.8, 2.2 Hz, H_2 N_2-isomer), 3.74 – 3.72 (1 H, m, H_2 N_1-isomer), 3.71 (3 H, s, OC*H*$_3$), 3.70 (3 H, s, OC*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 163.8 (C_q tetrazole N_2-isomer), 160.0 (C_q PMB), 159.7 (C_q PMB), 151.5 (C_q tetrazole N_1-isomer), 138.1 (C_q), 138.0 (C_q), 137.9 (C_q), 137.7 (C_q), 137.4 (C_q), 137.4 (C_q), 135.6 (d, *J* = 6.7 Hz, C_q OP(O)OBn), 135.5 (d, *J* = 6.7 Hz, C_q OP(O)OBn), 135.2 (d, *J* = 6.2 Hz, C_q OP(O)OBn), 135.1 (d, *J* = 6.4 Hz, C_q OP(O)OBn), 130.0 (C_q), 129.6

(C_q), 128.9, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.4, 125.8, 125.2, 114.3, 114.2, 96.2 (d, *J* = 6.1 Hz, C1 N_2-isomer), 96.1 (d, *J* = 6.1 Hz, C1 N_1-isomer), 78.6 (C3 N_1-isomer or N_2-isomer), 78.4 (C3 N_1-isomer or N_2-isomer), 76.4 (C4 N_2-isomer), 75.1 (2 C, *C*H_2Ph), 76.0 (C4 N_1-isomer), 74.5 (d, *J* = 9.6 Hz, C2 N_1-isomer), 74.3 (d, *J* = 9.3 Hz, C2 N_2-isomer), 73.5 (*C*H_2Ph), 72.9 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.5 (*C*H_2Ph), 70.1 (d, *J* = 5.6 Hz, OP(O)O*C*H_2Ph, 70.0 (d, *J* = 5.7 Hz, OP(O)O*C*H_2Ph), 69.7 (d, *J* = 5.4 Hz, OP(O)O*C*H_2Ph), 69.6 (d, *J* = 5.5 Hz, OP(O)O*C*H_2Ph), 67.9 (C5 N_2-isomer), 66.5 (C5 N_1-isomer), 56.5 (*C*H_2Ph PMB), 55.2 (2 C, O*C*H_3), 50.4 (*C*H_2Ph PMB); **^{31}P NMR** δ_P (162 MHz, $CDCl_3$) -2.88 (s), -2.79 (s); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 869.3370 $C_{49}H_{51}N_4O_9P$ requires $(M+H)^+$, 869.3315].

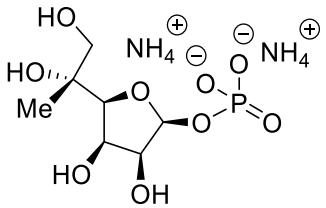
11.1.57. 5-*C*-methyl-α/β-D-mannopyranose 1-phosphate 131, 132, 5-*C*-methyl-α/β-D-mannofuranose 1-phosphate 133



131



132

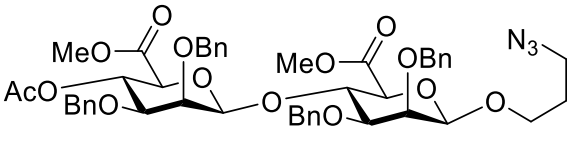


133

H_3PO_4 (240 mg, 2.45 mmol, 10 equiv.) was added to a mixture of **120**, **121**, **123** (100 mg, 0.25 mmol, 1.0 equiv.) in a Schlenk tube under inert atmosphere. The reaction mixture was heated under vacuum for 2 hours at 60 °C. The black solution was cooled and diluted in anhydrous THF (4 mL). TLC (2/1 EtOAc/petroleum ether) showed complete conversion of the starting material (R_f 0.60) to a new baseline product (R_f 0.00). The mixture was stirred for 5 min. before being added to an excess of 1 M LiOH aq. (20 mL) at 0 °C. An off-white precipitate

formed and the solution was stirred for 72 h at room temperature. Upon completion, the reaction mixture was filtered under vacuum through a Whatman GF/A glass microfibre filter paper and ion exchange Amberlite 120 (H^+) resin was added to the filtrate until pH = 7. The solution was filtered again and the filtrate was concentrated *in vacuo*. The residue was treated with MeOH (3 mL) and a cloudy precipitate formed which was centrifuged at 4000 rpm for 10 min. The supernatant was removed and the crude product was dried under vacuum prior to purification *via* strong anion exchange chromatography was conducted manually using a Bio-Scale™ Mini UNOsphere™ Q (strong anion exchange) cartridge (See General methods) and lyophilisation afforded **131** (56%), **132** (23%), and **133** (21%) as a white powder (15 mg, 48 μmol, 19%). R_f 0.36 (MeCN/H_2O, 2/1); **^{1}H NMR** (400 MHz; D_2O) δ **α-anomer** 5.27 (1H, dd, *J* = 9.1, 2.2 Hz, H_1), 4.09 (1H, dd, *J* = 10.2, 3.3 Hz, H_3), 3.93–3.91 (1H, m, H_2), 3.78 (1H, d, *J* = 10.2 Hz, H_4), 3.39 (2H, q, *J* = 11.9 Hz, $H_{6a,b}$); **β-anomer** δ 5.19 (1H, dd, *J* = 8.6, 1.1 Hz, H_1), 3.96 (1H, d, *J* = 2.8 Hz, H_2), 3.84 (1H, dd, *J* = 10.2, 3.4 Hz, H_3), 3.64 (1H, d, *J* = 10.2 Hz, H_4), 3.54–3.45 (2H, m, $H_{6a,b}$); **^{13}C NMR** (101 MHz, D_2O) δ **α-anomer** 95.8 (C1, d, *J* = 3.9 Hz), 79.8 (C5), 71.8 (C2), 68.0 (C4), 67.2 (C6), 66.7 (C3), 18.0 (C5-*C*H_3); **^{13}C-GATED** (101 MHz; D_2O): 95.8 ($^1J_{C1\text{-}H1}$ = 172 Hz, **145**), 89.3 ($^1J_{C1\text{-}H1}$ = 168 Hz, **146**); **^{31}P NMR** δ $_P$ (162 MHz, D_2O) **α-anomer** 1.85 (s). **β-anomer** 2.27 (s); **HRMS** (ES^-) *m/z* [Found: $(M\text{-}H)^-$ 273.0385 $C_7H_{13}O_9P$ requires $(M\text{-}H)^-$, 273.0381].

11.1.58. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl (4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) uronate 134

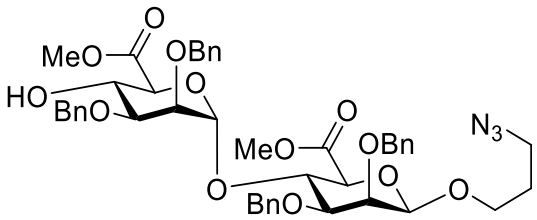


134

A solution of **55** (160 mg, 0.31 mmol, 1.0 equiv.) and **60** (216 mg, 0.46 mmol, 1.5 equiv.) in in CH_2Cl_2 (5 mL) was stirred over activated MS4Å for 30 min. before NIS (90 mg 0.40 mmol, 1.3 equiv.) was added. The mixture was cooled to -60 °C before TMSOTf (5.6 µL, d = 1.225, 31 µmol, 0.1 equiv.) was added. The reaction was left stirring for 30 min. at room temperature, and upon completion, Et_3N was added until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/toluene (0/100, 5/95 and 10/90) afforded **134** as a colourless oil (150 mg, 0.17 mmol, 56%). R_f 0.42 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -35.3 (*c*. 3.65, $CHCl_3$); **^{1}H NMR** (500 MHz; $CDCl_3$) δ 7.50 – 7.21 (20 H, m, Ar-H), 5.44 (1 H, app. t, *J* = 9.8 Hz, $H_{4'}$), 4.88 (1H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.84 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.79 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.78 (2 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.76 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.72 (1 H, d, *J* =11.9 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.52 (1 H, d, *J* = 0.9 Hz, $H_{1'}$), 4.46 (1 H, s, H_1), 4.43 (1 H, app. t, *J* = 8.8 Hz, H_4), 4.03 (1 H, dt, *J* = 9.7, 5.7 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.93 (1 H, d, *J* = 8.8 Hz, H_5), 3.89 – 3.88 (2 H, m, $H_{2'}$, H_2), 3.71 (1 H, d, *J* = 9.8 Hz, $H_{5'}$), 3.67 (3 H, s, CO$_2$C*H*$_3$), 3.66 (1 H, dd, *J* = 8.8, 3.0 Hz, H_3), 3.58 (3 H, s, CO$_2$C*H*$_3$), 3.55 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.49 (1 H, dd, *J* = 9.8, 2.8 Hz, $H_{3'}$), 3.37 (2 H, t, *J* = 6.8 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.02 (3 H, s, C(O)C*H*$_3$), 1.95 – 1.84 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (126 MHz; $CDCl_3$) δ 169.8 (*C*(O)CH**$_3$**), 168.7 (*C*O$_2$CH$_3$),

167.8 (*C*O$_2$CH$_3$), 138.7 (C$_q$), 138.6 (C$_q$), 138.4 (C$_q$), 137.8 (C$_q$), 128.4, 128.3, 128.2, 128.1, 128.1, 127.9, 127.7, 127.5, 127.5, 127.4, 127.3, 127.2, 102.4 (C1'), 101.9 (C1), 79.4 (C3), 78.5 (C3'), 77.6 (C4), 75.0 (C2' or C2), 74.5 (C5), 74.4 (*C*H$_2$Ph), 74.0 (C2' or C2, *C*H$_2$Ph), 73.4 (C5'), 72.2 (*C*H$_2$Ph), 71.7 (*C*H$_2$Ph), 68.7 (C4'), 66.9 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.4 (CO$_2$*C*H$_3$), 52.4 (CO$_2$*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 20.8 (C(O)*C*H$_3$); **^{13}C-GATED** (126 MHz; CDCl$_3$): 102.4 ($^1J_{C1'\text{-}H1'}$ = 155 Hz, C1'), 101.9 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 901.3877 C$_{47}$H$_{57}$N$_4$O$_{14}$ requires (M+NH$_4$)$^+$, 901.3871].

11.1.59. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl (2,3-di-*O*-benzyl-α-D-mannopyranosyl) uronate)-β-D-mannopyranoside) uronate 135

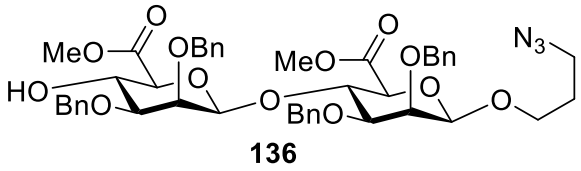


135

To a stirred solution of **134** (50 mg, 56 µmol, 1.0 equiv.) in anhydrous MeOH (1 mL), Na (65 µg, 2.8 µmol, 0.05 equiv.) dissolved in anhydrous MeOH (30 µL) was added at room temperature. The mixture was stirred for 2 h, then neutralised with ion exchange Amberlite 120 (H$^+$) resin (approximately 0.1 g, 5 min), filtered, and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 70/30, 90/10) afforded **85** as a colourless oil (15 mg, 18 µmol, 32%, 46% based on recovered starting material, 16 mg). R$_f$ 0.42 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -17.8 (*c*. 0.74, CHCl$_3$); **^{1}H NMR** (400 MHz; CDCl$_3$) δ 7.65 – 6.80 (20 H, m, Ar-H), 5.42 (1 H, s, H$_{1'}$), 4.94 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.73 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.45 (1 H, app. t, *J* = 9.3 Hz, H$_4$), 4.43 (1 H, s, H$_1$), 4.40 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.32 (1 H, d, *J* = 12.2 Hz,

C*H*$_2$Ph), 4.23 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.22 (1 H, d, *J* = 9.3 Hz, H$_{4'}$), 4.19 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.07 – 3.98 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 4.01 (1 H, d, *J* = 9.7 Hz, H$_{5'}$), 3.91 (1 H, d, *J* = 2.6 Hz, H$_2$), 3.84 (1 H, d, *J* = 9.4 Hz, H$_5$), 3.80 (3 H, s, CO$_2$C*H*$_3$), 3.76 (3 H, s, CO$_2$C*H*$_3$), 3.67 – 3.62 (2 H, m, H$_{2'}$, H$_{3'}$), 3.57 – 3.49 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.43 – 3.36 (2 H, m, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.40 (1 H, dd, *J* = 9.3, 2.7 Hz, H$_3$), 2.95 (1 H, br. s, C4-*OH*), 2.01 – 1.78 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; CDCl$_3$) δ 170.9 (*C*O$_2$CH$_3$), 168.3 (*C*O$_2$CH$_3$), 138.5 (C$_q$), 138.3 (C$_q$), 138.3 (C$_q$), 137.4 (C$_q$), 128.5, 128.4, 128.2, 128.1, 128.0, 127.6, 127.6, 127.5, 127.4, 127.4, 102.0 (C1), 99.8 (C1'), 81.5 (C3), 78.2 (C2'), 75.8 (C5), 75.0 (C3'), 74.7 (C4), 74.1 (*C*H$_2$Ph), 72.9 (C2), 72.4 (2 C, *C*H$_2$Ph), 72.4 (C5'), 71.0 (*C*H$_2$Ph), 68.4 (C4'), 67.0 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.6 (CO$_2$*C*H$_3$), 52.5 (CO$_2$*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (101 MHz; CDCl$_3$): 102.0 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1), 99.8 ($^1J_{C1'\text{-}H1'}$ = 176 Hz, C1'); **HRMS** (ES$^+$) *m/z* [Found: (M+Na)$^+$ 864.3343 C$_{45}$H$_{51}$N$_3$O$_{13}$Na requires (M+Na)$^+$, 864.3320].

11.1.60. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl (2,3-di-*O*-benzyl-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) uronate 136

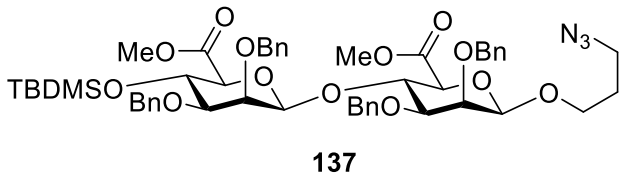


136

To a stirred solution of **137** (70 mg, 73 μmol, 1.0 equiv.) in anhydrous MeOH (0.7 mL) at 0 °C, was added AcCl (1.6 μL, d = 1.104, 20 μmol, 0.3 equiv.) and the reaction was left stirring at room temperature for 24 h. The mixture was then neutralised and diluted with sat. aq. NaHCO$_3$ (20 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 20 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to furnish a colourless oil. Purification by silica gel flash column

chromatography, eluting with Et_2O/toluene (10/90, 20/80, 25/75) afforded **136** as a colourless oil (20 mg, 24 μmol, 32%). R_f 0.30 (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.22 (20 H, m), 4.84 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.79 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.76 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.75 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.72 (1 H, s, $H_{1'}$), 4.67 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.60 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.48 (1 H, d, *J* = 0.7 Hz, H_1), 4.45 (1 H, app. t, *J* = 8.6 Hz, H_4), 4.19 (1 H, app. t, *J* = 9.6 Hz, $H_{4'}$), 4.07 – 3.99 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.90 (1 H, d, *J* = 8.7 Hz, H_5), 3.85 (1 H, d, *J* = 2.3 Hz, H_2), 3.83 (1 H, d, *J* = 2.5 Hz, $H_{2'}$), 3.70 (1 H, dd, *J* = 9.0, 4.4 Hz, H_3), 3.64 (3 H, s, CO_2C*H*$_3$), 3.62 (3 H, s, CO_2C*H*$_3$), 3.59 (1 H, d, *J* = 9.6 Hz, $H_{5'}$), 3.57 – 3.51(1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.36 (2 H, t, *J* = 6.7 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.32 (1 H, dd, *J* = 9.5, 2.8 Hz, $H_{3'}$), 2.93 (1 H, br. s, C4-*OH*), 1.94 – 1.81 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.9 (*C*O_2CH$_3$), 168.6 (*C*O_2CH$_3$), 138.8 (C_q), 138.7 (C_q), 138.4 (C_q), 138.0 (C_q), 128.5, 128.3, 128.2, 128.1, 128.1, 127.8, 127.8, 127.7, 127.5, 127.4, 127.4, 127.1, 102.5 (C1'), 101.8 (C1), 80.4 (C3'), 79.4 (C5'), 77.2 (C4), 75.2 (C2'), 74.8 (C3), 74.6 (*C*H$_2$Ph), 74.5 (C5), 73.9 (C2, *C*H$_2$Ph), 72.1 (*C*H$_2$Ph), 71.9 (*C*H$_2$Ph), 68.2 (C4'), 66.9 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.5 (CO_2*C*H$_3$), 52.4 (CO_2*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 102.5 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1'), 101.8 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: $(M+NH_4)^+$ 859.3781 $C_{45}H_{55}N_4O_{13}$ requires $(M+NH_4)^+$, 859.3766]; These data were consistent with literature values.[211]

11.1.61. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(methyl (4-*O*-*tert*-butyl dimethylsilyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) uronate 137

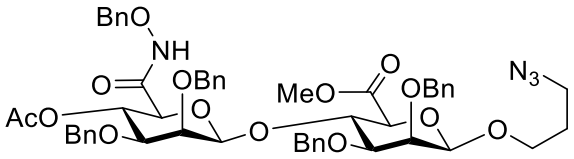


137

A solution of **57** (110 mg, 0.18 mmol, 1.0 equiv.) and **60** (96 mg, 0.20 mmol, 1.1 equiv.) in in CH_2Cl_2 (3.5 mL) was stirred over activated MS4Å for 30 min. before NIS (54 mg, 0.24 mmol, 1.3 equiv.) was added. The mixture was cooled to -10 °C before TMSOTf (6.7 µL, d = 1.225, 40 µmol, 0.2 equiv.) was added. The reaction was left stirring for 30 min. at room temperature, and upon completion, Et_3N was added until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Purification by silica gel flash column chromatography, eluting with EtOAc/toluene (0/100, 5/95, 10/90) afforded **137**, as a colourless oil (105 mg, 0.11 mmol, 61%). R_f 0.76 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ +8.0 (*c*. 0.21, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.41 – 7.15 (20 H, m, Ar-H), 4.82 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.80 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.74 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.71 (1 H, s, $H_{1'}$), 4.70 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph), 4.67 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.55 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.47 (1 H, d, *J* = 11.4 Hz, C*H*$_2$Ph), 4.45 (1 H, s, H_1), 4.41 (1 H, app. t, *J* = 8.6 Hz, H_4), 4.30 (1 H, app. t, *J* = 9.2 Hz, $H_{4'}$), 4.08 – 3.99 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.86 (1 H, d, *J* = 8.6 Hz, H_5), 3.83 (1 H, d, *J* = 2.5 Hz, $H_{2'}$), 3.80 (1 H, d, *J* = 2.2 Hz, H_2), 3.74 (1 H, d, *J* = 9.2 Hz, $H_{5'}$), 3.62 (6 H, s, CO$_2$C*H*$_3$), 3.62 – 3.56 (1 H, m, H_3), 3.52 (1 H, ddd, *J* = 9.6, 7.6, 5.2 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.35 (2 H, t, *J* = 6.7 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.28 (1 H, dd, *J* = 9.2, 2.7 Hz, $H_{3'}$), 1.92 – 1.82 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$), 0.81 (9 H, s, C(C*H*$_3$)$_3$), 0.00 (6 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 168.7 (*C*O$_2$CH$_3$), 168.7 (*C*O$_2$CH$_3$), 139.2 (C_q), 138.8 (C_q), 138.5

(C_q), 137.9 (C_q), 128.4, 128.2, 128.1, 128.1, 128.0, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.2, 102.6 (C1'), 101.7 (C1), 81.8 (C3'), 79.1 (C3), 77.5 (C5'), 77.2 (C4), 75.0 (C2'), 74.6 (C5), 74.5 (CH$_2$Ph), 74.4 (C2), 73.9 (CH$_2$Ph), 72.6 (CH$_2$Ph), 71.4 (CH$_2$Ph), 68.9 (C4'), 66.8 (OCH$_2$CH$_2$CH$_2$N$_3$), 52.3 (CO$_2C$H$_3$), 52.0 (CO$_2C$H$_3$), 48.3 (OCH$_2$CH$_2C$H$_2$N$_3$), 29.1 (OCH$_2C$H$_2$CH$_2$N$_3$), 25.8 (C(CH$_3$)$_3$), 18.0 (C(CH$_3$)$_3$), -3.9 (Si(CH$_3$)$_2$), -5.3 (Si(CH$_3$)$_2$); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 973.4639 $C_{51}H_{69}N_4O_{13}Si$ requires (M+NH$_4$)$^+$, 973.4630].

11.1.62. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(*O*-benzyl-(4-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl) hydroxamate)-β-D-mannopyranoside) uronate 138

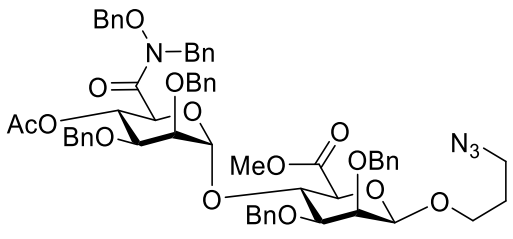


138

A solution of **63** (110 mg, 0.17 mmol, 1.0 equiv.) and **60** (87 mg, 0.18 mmol, 1.1 equiv.) and in CH_2Cl_2 (5 mL) was stirred over activated MS4Å for 1 h before NIS (60 mg 0.27 mmol, 1.6 equiv.) was added. The mixture was cooled to -60 °C before TMSOTf (3.5 µL, d = 1.225, 20 µmol, 0.1 equiv.) was added. The reaction was left stirring for 1 h at room temperature, and upon completion, Et_3N was added until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Purification by Sephadex™ LH-20, eluting with CH_2Cl_2 and purification by Reveleris® automated silica gel flash column chromatography (liquid injection onto column), eluting with EtOAc/toluene (0/100, 5/95 and 10/90) afforded **88** as a colourless oil (90 mg, 92 µmol, 54%). R_f 0.42 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -45.2 (*c*. 0.7, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.37 (1 H, br. s, C(O)N*H*OBn), 7.38 – 7.07 (25 H, m, Ar-H), 5.60 (1 H, app. t, *J* = 9.8 Hz, H$_{4'}$), 4.83 (1 H, d, *J* = 12.3 Hz, CH_2Ph), 4.80 (2 H, d, *J* = 12.2 Hz, CH_2Ph), 4.71 (1 H, s, H$_{1'}$), 4.71 (1 H, d, *J* = 12.5 Hz, CH_2Ph), 4.54 (1 H, d, *J* =

12.2 Hz, C*H*$_2$Ph), 4.48 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.47 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.44 (1 H, app. t, *J* = 9.8 Hz, H$_4$), 4.43 (2 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.40 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.38 (1 H, s, H$_1$), 4.01 (1 H, ddd, *J* = 11.2, 9.4, 5.4 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.92 (1 H, d, *J* = 9.9 Hz, H$_{5'}$), 3.85 (1 H, d, *J* = 2.8 Hz, H$_{2'}$), 3.82 (1 H, d, *J* = 2.8 Hz, H$_2$), 3.79 (1 H, d, *J* = 9.6 Hz, H$_5$), 3.72 (3 H, s, CO$_2$C*H*$_3$), 3.52 (1 H, dd, *J* = 9.7, 2.8 Hz, H$_{3'}$), 3.57 – 3.44 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.39 (1 H, dd, *J* = 6.9, 2.7 Hz, H$_3$), 3.42 – 3.35 (2 H, m, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.07 (3 H, s, C(O)C*H*$_3$), 1.97 – 1.80 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; CDCl$_3$) δ 170.0 (*C*(O)CH$_3$), 169.7 (*C*(O)NHOBn), 168.6 (*C*O$_2$CH$_3$), 138.3 (C$_q$), 138.3 (C$_q$), 138.0 (C$_q$), 137.8 (C$_q$), 137.2 (C$_q$), 128.9, 128.5, 128.5, 128.4, 128.2, 128.2, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 126.3, 124.6, 102.1 (C1), 100.6 (C1'), 79.2 (C3), 79.0 (C3'), 75.2 (C4), 74.7 (C5), 74.7 (2 C, C2' and *C*H$_2$Ph), 73.9 (*C*H$_2$Ph), 73.5 (*C*H$_2$Ph), 73.4 (C2), 73.1 (C5'), 72.4 (*C*H$_2$Ph), 72.2 (*C*H$_2$Ph), 68.1 (C4'), 67.0 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.5 (CO$_2$*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 21.0 (C(O)*C*H$_3$); **^{13}C-GATED** (101 MHz; CDCl$_3$): 102.4 ($^1J_{C1\text{-}H1}$ = 154 Hz, C1), 101.9 ($^1J_{C1'\text{-}H1'}$ = 165 Hz, C1'); **IR** ν_{max}/cm^{-1} 3260 (w, N-H), 2096 (w, N=N=N), 1745 (s, C=O$_{ester}$), 1708 (m, C=O$_{amide}$), 1229 (m, C-O$_{ester}$), 1089 (s, C-O$_{ether}$), 1054 (s, C-O$_{ester}$).

11.1.63. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(*O*-benzyl-*N*-benzyl-(4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl) hydroxamate)-β-D-mannopyranoside) uronate 139



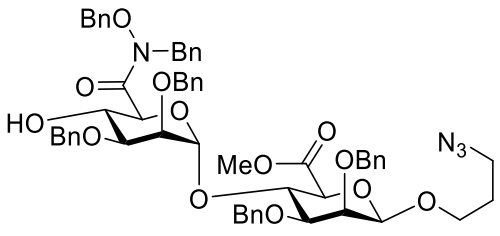
139

A solution of **67** (180 mg, 0.26 mmol, 1.1 equiv.) and **60** (113 mg, 0.23 mmol, 1.0 equiv.) and in CH_2Cl_2 (5 mL) was stirred over activated MS4Å for 1 h before NIS (88 mg 0.39 mmol, 1.5 equiv.) was added. The

mixture was cooled to 0 °C before TMSOTf (4.2 μL, d = 1.225, 20 μmol, 0.1 equiv.) was added. The reaction was left stirring for 30 min. at 0 °C, and upon completion, Et_3N was added until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/toluene (0/100, 5/95 and 10/90) afforded **139** as a colourless oil (32 mg, 28 μmol, 12%, α/β = 9/1). R_f 0.53 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.44 – 7.09 (30 H, m, Ar-H), 5.63 (1 H, app. t, *J* = 9.9 Hz, $H_{4'}$), 5.44 (2 H, s, C(O)N(C*H*$_2$Ph)OBn), 5.39 (1 H, d, *J* = 1.8 Hz, $H_{1'}$), 4.95 (2 H, s, C(O)N(Bn)OC*H*$_2$Ph), 4.92 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph-attached to C2), 4.73 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph-attached to C2), 4.53 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph-attached to C3'), 4.50 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph-attached to C3') 4.42 (1 H, s, H_1), 4.40 (1 H, d, *J* =10.3 Hz, H_4), 4.38 (1 H, d, *J* = 11.2 Hz, C*H*$_2$Ph-attached to C3), 4.34 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2'), 4.23 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph-attached to C2'), 4.18 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph-attached to C3), 4.06 – 3.99 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.95 (1 H, d, *J* = 10.0 Hz, $H_{5'}$), 3.90 (1 H, d, *J* = 2.6 Hz, H_2), 3.81 (1 H, d, *J* = 9.4 Hz, H_5), 3.78 (1 H, dd, *J* = 9.9, 2.7 Hz, $H_{3'}$), 3.65 (1 H, app. t, *J* = 2.3 Hz, $H_{2'}$), 3.59 (3 H, s, CO_2C*H*$_3$), 3.53 (1 H, ddd, *J* = 9.5, 8.0, 5.1 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.38 (2 H, ddd, *J* = 11.2, 8.6, 2.2 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.36 (1 H, dd, *J* = 9.2, 2.3 Hz, H_3) 1.97 – 1.82 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.79 (3 H, s C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.6 (*C*(O)CH$_3$), 168.1 (*C*O$_2$CH$_3$), 151.2 (*C*(O)N(Bn)OBn), 138.4 (C_q), 138.3 (2 C, C_q), 138.2 (C_q), 137.4 (C_q), 137.3 (C_q), 128.5, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.2, 127.2, 102.0 (C1), 99.4 (C1'), 81.5 (C3), 76.1 (C3'), 76.1 (C(O)N(Bn)OC*H*$_2$Ph), 75.8 (C5), 75.4 (C2'), 74.5 (C4), 74.0 (*C*H$_2$Ph-attached to C2), 73.4 (C(O)N(*C*H$_2$Ph)OBn), 72.8 (C2), 72.3 (*C*H$_2$Ph-attached to C2'), 72.1 (*C*H$_2$Ph-attached to C3'), 71.7 (C5'),

71.0 (*C*CH_2Ph-attached to C3), 68.5 (C4'), 67.0 (O*C*$H_2CH_2CH_2N_3$), 52.9 (CO_2*C*H_3), 48.3 (OCH_2CH_2*C*H_2N_3), 29.1 (OCH_2*C*$H_2CH_2N_3$), 20.7 (C(O)*C*H_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 102.0 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1), 99.4 ($^1J_{C1'\text{-}H1'}$ = 176 Hz, C1'); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 1065.4525 $C_{60}H_{65}N_4O_{14}$ requires $(M+H)^+$,1065.4497]; **IR** ν_{max}/cm^{-1} 2095 (m, N=N=N), 1746 (m, C=O_{ester}), 1638 (w, C=O_{amide}), 1229 (m, C-O_{ester}), 1084 (s, C-O_{ether}), 1024 (C-O_{ester}).

11.1.64. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(*O*-benzyl-*N*-benzyl-(4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl) hydroxamate)-β-D-mannopyranoside) uronate 140



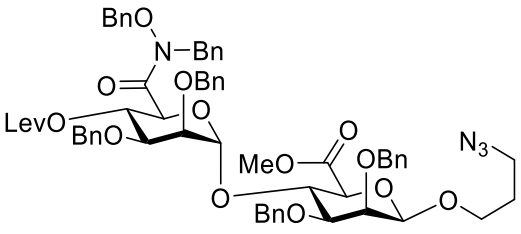
140

To a stirred solution of **139** (10 mg, 9 µmol, 1.0 equiv.) in anhydrous MeOH (0.15 mL), Na (10 µg, 0.4 µmol, 0.05 equiv.) dissolved in anhydrous MeOH (10 µL) was added at room temperature. The mixture was stirred for 16 h, then neutralised with ion exchange Amberlite 120 (H^+) resin (approximately 50 mg, 3 min), filtered, and concentrated under reduced pressure. Flash column chromatography, eluting with Et_2O/petroleum ether (20/80, 50/50, 90/10) afforded **140** as a colourless oil (5 mg, 5 µmol, 54%).

Disaccharide **141** (100 mg, 90 µmol, 1.0 equiv.) was dissolved in a mixture of pyridine/AcOH (4/1 *v/v*, 1.5 mL), after which H_2N-NH_2.AcOH (41 mg, 0.44 mmol, 5.0 equiv.) was added. The mixture was stirred for 1 h at room temperature and was diluted with EtOAc (20 mL). The organic layer was washed with 1 M aq. HCl (2 x 15 mL), sat. aq. $NaHCO_3$ solution (2 x 10 mL) and brine (15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to

furnish a yellow oil. Purification by silica gel flash column chromatography, eluting with EtOAc/hexane (20/80, 50/50, 90/10) afforded **140** as a colourless oil (65 mg, 0.63 mmol, 70%). R_f 0.58 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.17 (30 H, m, Ar-H), 5.39 (1 H, s, $H_{1'}$), 5.39 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 5.33 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 5.05 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.99 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.90 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.71 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.62 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.43 (1 H, s, H_1), 4.42 (1 H, d, *J* = 13.7 Hz, C*H*$_2$Ph), 4.42 (1 H, app. t, *J* = 8.9 Hz, H_4) 4.30 (2 H, s, C*H*$_2$Ph), 4.23 (1 H, app. t, *J* = 9.6 Hz, $H_{4'}$), 4.23 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph), 4.06 – 4.00 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.98 (1 H, d, *J* = 9.6 Hz, $H_{5'}$), 3.90 (1 H, d, *J* = 1.9 Hz, H_2) 3.82 (1 H, d, *J* = 9.1 Hz, H_5), 3.67 (1 H, d, *J* = 2.3 Hz, $H_{2'}$), 3.66 (1 H, dd, *J* = 9.6, 2.3 Hz, $H_{3'}$), 3.58 (3 H, s, CO_2C*H_3*), 3.53 (1 H, dd, *J* = 13.7, 8.6 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.43 – 3.35 (3 H, m, H_3, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.27 (1 H, d, *J* = 2.2 Hz, C4-*OH*), 1.90 (2 H, ddd, *J* = 28.6, 14.0, 7.4 Hz, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 168.2 (*C*O$_2$CH$_3$), 152.4 (*C*(O)N(Bn)OBn), 138.5 (C_q), 138.4 (C_q), 138.3 (C_q), 137.7 (C_q), 137.4 (C_q), 137.1 (C_q), 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 101.9 (C1), 99.4 (C1'), 81.5 (C3), 78.1 (C3'), 76.4 (*C*H$_2$Ph), 75.6 (C5), 75.3 (C2'), 74.0 (*C*H$_2$Ph and C4), 73.3 (*C*H$_2$Ph), 72.9 (C5'), 72.8 (C2), 72.3 (*C*H$_2$Ph), 72.3 (*C*H$_2$Ph), 70.9 (*C*H$_2$Ph), 67.3 (C4'), 66.9 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.9 (CO$_2$*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.9 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1), 99.4 ($^1J_{C1'\text{-}H1'}$ = 176 Hz, C1'); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 1023.4412 $C_{58}H_{63}N_4O_{13}$ requires (M+H)$^+$,1023.4386].

11.1.65. 3-azidopropyl (methyl 2,3-di-*O*-benzyl-4-*O*-(*O*-benzyl-*N*-benzyl-(4-*O*-levulinoyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl) hydroxamate)-β-D-mannopyranoside) uronate 141



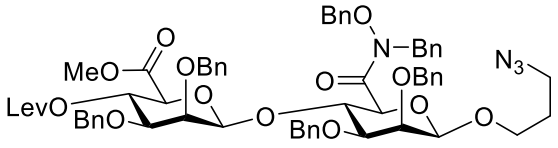
141

A solution of **69** (200 mg, 0.26 mmol, 1.0 equiv.) and **60** (136 mg, 0.29 mmol, 1.1 equiv.) and in CH_2Cl_2 (5 mL) was stirred over activated MS4Å for 1 h before NIS (89 mg 0.39 mmol, 1.5 equiv.) was added. The mixture was cooled to -40 °C before TMSOTf (4.8 µL, d = 1.225, 20 µmol, 0.1 equiv.) was added. The reaction was left stirring for 1 h at -40 °C, 2 h at -25 °C, 3 h at -20 °C and 30 min. at -10 °C, and quenched with Et_3N until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Flash column chromatography, eluting with Et_2O/toluene (0/100, 10/90, 20/80) afforded **141** as a colourless oil (80 mg, 80 µmol, 30%, α/β = 9/1). R_f 0.30 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.39 – 7.10 (30 H, m, Ar-H), 5.64 (1 H, app. t, *J* = 9.9 Hz, $H_{4'}$), 5.43 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 5.40 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 5.38 (1 H, d, *J* = 2.0 Hz, $H_{1'}$), 4.97 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.94 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.92 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.73 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.52 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.52 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.42 (1 H, s, H_1), 4.37 (1 H, d, *J* = 11.3 Hz, C*H*$_2$Ph), 4.36 (1 H, app. t, *J* = 10.5 Hz, $H_{4'}$), 4.34 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.22 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.18 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.03 (1 H, dt, *J* = 9.7, 5.6 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.96 (1 H, d, *J* = 10.0 Hz, $H_{5'}$), 3.90 (1 H, d, *J* = 2.7 Hz, H_2), 3.81 (1 H, d, *J* = 9.4 Hz, H_5), 3.79 (1 H, dd, *J* = 10.4, 2.1 Hz, $H_{3'}$), 3.64 (1 H, app. t, *J* = 2.1

Hz, $H_{2'}$), 3.58 (3 H, s, CO_2CH_3), 3.53 (1 H, ddd, *J* = 9.5, 8.0, 5.1 Hz, $OCH_2CH_2CH_2N_3$), 3.42 – 3.36 (2 H, m, $OCH_2CH_2CH_2N_3$), 3.36 (1 H, dd, *J* = 9.3, 2.7 Hz, H_3), 2.65 – 2.38 (3 H, m, CH_2 Lev), 2.31 – 2.20 (1 H, m, CH_2 Lev), 1.99 – 1.81 (3 H, m, CH_3 Lev), 1.99 – 1.81 (2 H, m, $OCH_2CH_2CH_2N_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.4 (C=O Lev ketone), 171.5 (C=O Lev), 168.1 (C=O CO_2CH_3), 151.1 (*C*(O)N(Bn)OBn), 138.4 (C_q), 138.2 (C_q), 138.2 (C_q), 138.1 (C_q), 137.3 (C_q), 137.3 (C_q), 128.5, 128.3, 128.2, 128.2, 128.1, 128.1, 1280, 127.9, 127.6, 127.5, 127.5, 127.4, 127.2, 127.2, 101.9 (C1), 99.4 (C1'), 81.5 (C3), 76.1, 76.0 (2 C, C3' CH_2Ph), 75.8 (C5), 75.4 (C2'), 74.5 (C4), 74.0 (CH_2Ph), 73.4 (CH_2Ph), 72.7 (C2), 72.3 (CH_2Ph), 72.2 (CH_2Ph), 71.5 (C5'), 70.9 (CH_2Ph), 68.7 (C4'), 67.0 ($OCH_2CH_2CH_2N_3$), 52.9 (CO_2CH_3), 48.3 ($OCH_2CH_2CH_2N_3$), 37.9 (CH_2 Lev), 29.8 (CH_3 Lev), 29.0 ($OCH_2CH_2CH_2N_3$), 27.9 (CH_2 Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.9 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1), 99.4 ($^1J_{C1'\text{-}H1'}$ = 176 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: $(M+H)^+$ 1121.4770 $C_{63}H_{68}N_4O_{15}$ requires $(M+H)^+$, 1121.4754].

11.1.66. 3-azidopropyl (*O*-benzyl-*N*-benzyl-2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) hydroxamate 142

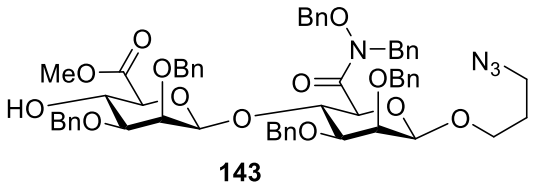
A solution of donor **56** (120 mg, 0.21 mmol, 1.0 equiv.) and acceptor **78** (108 mg, 0.17 mmol, 0.8 equiv.) and in CH_2Cl_2 (4 mL) was stirred over activated MS4Å for 30 min. before NIS (61 mg 0.27 mmol, 1.3 equiv.) was added. The mixture was cooled to -40 °C before TMSOTf (7.5 µL, d = 1.225, 40 µmol, 0.2 equiv.) was added. The reaction was allowed to warm at 0 °C within 45 min, and upon completion, Et_3N was added until pH = 7. The reaction mixture was filtered through Celite® and concentrated under reduced pressure. Flash



142

column chromatography, eluting with Et_2O/toluene (0/100, 5/95 and 10/90) afforded **142** as a colourless oil (129 mg, 0.11 mmol, 55%). R_f 0.38 (EtOAc/toluene, 3/7); $[\alpha]_D^{22}$ -29.6 (*c*. 0.5, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.44 – 7.16 (30 H, m), 5.46 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 5.35 (1 H, app. t, *J* = 9.8 Hz, H_4'), 5.31 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.92 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.88 (1 H, d, *J* = 10.0 Hz, C*H*$_2$Ph), 4.85 (1 H, s, *J* = 13.9 Hz, C*H*$_2$Ph), 4.85 (2 H, s, C*H*$_2$Ph), 4.83 (4 H, d, *J* = 14.2 Hz, C*H*$_2$Ph), 4.67 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.44 (1 H, s, H_1'), 4.39 (1 H, s, H_1), 4.36 (1 H, d, *J* = 11.5 Hz, C*H*$_2$Ph), 4.35 (1 H, d, *J* = 9.4 Hz, H_4), 4.23 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 3.95 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.85 (1 H, d, *J* = 9.7 Hz, H_5), 3.82 (1 H, d, *J* = 2.9 Hz, H_2), 3.76 (1 H, d, *J* = 2.6 Hz, H_2'), 3.53 (3 H, s, CO_2CH_3), 3.50 (1 H, d, *J* = 9.0 Hz, H_5'), 3.46 (1 H, dd, *J* = 9.2, 2.9 Hz, H_3), 3.48 – 3.39 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.31 (2 H, dd, *J* = 9.9, 3.9 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.21 (1 H, dd, *J* = 9.8, 2.8 Hz, H_3'), 2.69 – 2.64 (2 H, m, C*H*$_2$ Lev), 2.55 – 2.46 (2 H, m, C*H*$_2$ Lev), 2.14 (3 H, s, C*H*$_3$ Lev), 1.88 – 1.76 (2 H, m, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.5 (C=O Lev), 167.9 (C=O *C*O_2CH$_3$), 151.8 (*C*(O)N(Bn)OBn), 139.0 (2 C, C_q), 138.8 (C_q), 138.7 (C_q), 138.0 (C_q), 137.1 (C_q), 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 102.3 (C1'), 101.8 (C1), 79.9 (C3), 78.4 (C3'), 77.3 (C4), 76.6 (*C*H$_2$Ph), 75.1 (C2), 74.9 (C2'), 74.6 (C5, *C*H$_2$Ph), 74.3 (*C*H$_2$Ph), 73.3 (C5'), 73.3 (*C*H$_2$Ph), 72.8 (*C*H$_2$Ph), 71.2 (*C*H$_2$Ph), 68.9 (C4'), 66.6 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 52.3 (CO_2CH_3), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 37.9 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 29.2 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 27.9 (*C*H$_2$ Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 102.3 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1'), 101.8 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 1121.4755 $C_{63}H_{69}N_4O_{15}$ requires $(M+H)^+$, 1121.4754].

11.1.67. 3-azidopropyl (*O*-benzyl-*N*-benzyl-2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl (2,3-di-*O*-benzyl-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) hydroxamate 143

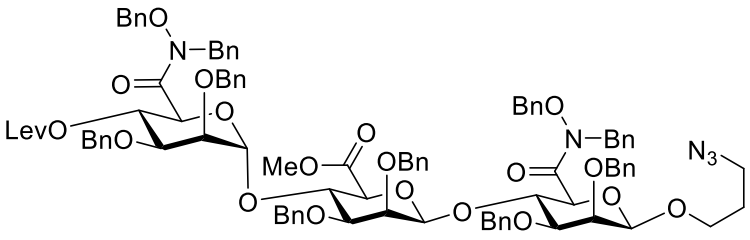


143

Disaccharide **142** (40 mg, 35 µmol, 1.0 equiv.) was dissolved in a mixture of pyridine/AcOH (0.5 mL, 4/1 *v/v*), after which H_2N-NH_2.AcOH (16 mg, 0.18 mmol, 5.0 equiv.) was added. The mixture was stirred for 30 min. and then was diluted with EtOAc (5 mL), washed with 1 M aq. HCl (5 mL), sat. aq. $NaHCO_3$ solution (5 mL) and brine (5 mL). The organic layer was then dried over $MgSO_4$ filtered and concentrated under reduced pressure to furnish a yellow oil. Purification using silica gel flash column chromatography, eluting with Et_2O/toluene (0/100, 30/70, 40/60, 90/10) afforded **143** as a colourless oil (29 mg, 28 µmol, 81%). R_f 0.60 (EtOAc/Toluene, 3/7); $[\alpha]_D^{22}$ -44.5 (*c*. 0.25, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.43 – 7.20 (30 H, m, Ar-H), 5.46 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 5.33 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.93 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.89 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.88 (1 H, d, *J* = 10.6 Hz, C*H*$_2$Ph), 4.86 (1 H, d, *J* = 11.0 Hz, C*H*$_2$Ph), 4.85 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.81 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.52 (1 H, s, $H_{1'}$), 4.43 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.42 (1 H, t, *J* = 9.2 Hz, H_4), 4.41 (1 H, s, H_1), 4.36 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.12 (1 H, td, *J* = 9.6, 1.2 Hz, $H_{4'}$), 3.96 – 3.90 (1 H, m, OC*H*$_2$$CH_2CH_2N_3$), 3.88 (1 H, d, *J* = 9.7 Hz, H_5), 3.84 (1 H, d, *J* = 2.9 Hz, H_2), 3.76 (1 H, d, *J* = 2.2 Hz, $H_{2'}$), 3.59 (3 H, s, CO_2C*H*$_3$), 3.58 – 3.54 (1 H, m, OC*H*$_2$$CH_2CH_2N_3$), 3.50 (1 H, dd, *J* = 9.3, 2.7 Hz, H_3), 3.46 (1 H, d, *J* = 9.6 Hz, $H_{5'}$), 3.32 (2 H, td, *J* = 6.7, 1.5 Hz, OCH_2CH_2C*H*$_2$$N_3$), 3.12 (1 H, dd, *J* = 9.5, 2.8 Hz, $H_{3'}$), 2.85 (1 H, d, *J* = 1.7 Hz, C4-*OH*), 1.90 – 1.77 (2 H, m, OCH_2C*H*$_2$$CH_2N_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 170.0 (C=O

CO_2CH_3), 151.8 (*C*(O)N(Bn)OBn), 139.1 (C_q), 139.0 (C_q), 138.8 (C_q), 138.0 (C_q), 137.1 (C_q), 137.0 (C_q), 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.7, 127.6, 127.5, 127.4, 127.3, 127.3, 102.6 (C1'), 101.7 (C1), 80.3 (C3'), 80.2 (C3), 77.2 (C4), 76.6 (*C*H_2Ph), 75.3 (C2), 75.2 (C2'), 74.9 (C5), 74.8 (*C*H_2Ph), 74.7 (C5'), 74.3 (*C*H_2Ph), 73.3 (*C*H_2Ph), 72.6 (*C*H_2Ph), 71.3 (*C*H_2Ph), 68.0 (C4'), 66.6 (O*C*$H_2CH_2CH_2N_3$), 52.3 (CO_2*C*H_3), 48.3 (OCH_2CH_2*C*H_2N_3), 32.8, 30.3, 29.2 (OCH_2CH_2*C*H_2N_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 102.6 ($^1J_{C1'-H1'}$ = 156 Hz, C1'), 101.7 ($^1J_{C1-H1}$ = 156 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 1023.4412 $C_{58}H_{63}N_4O_{13}$ requires $(M+H)^+$, 1023.4386].

11.1.68. 3-azidopropyl (*O*-benzyl-*N*-benzyl-(2,3-di-*O*-benzyl-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-(*O*-benzyl-*N*-benzyl-(4-*O*-levulinoyl-2,3-di-*O*-benzyl-α-D-mannopyranosyl) hydroxamate)-β-D-mannopyranosyl) uronate)-β-D-mannopyranoside) hydroxamate 144



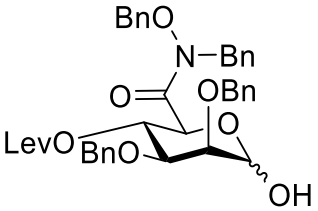
144

A solution of **69** (140 mg, 0.18 mmol, 1.0 equiv.), Ph_2SO (48 mg, 0.24 mmol, 1.3 equiv.) and TTBP (110 mg, 0.46 mmol, 2.5 equiv.) in CH_2Cl_2 (3.5 mL) was stirred over activated MS4Å for 1 h. The mixture was cooled to -60 °C and Tf_2O (39 µL, d = 1.720, 0.24 mmol, 1.3 equiv.) was then added. The mixture was allowed to warm to -40 °C over 10 min. followed by cooling to -90 °C, upon **143** (132 mg, 0.13 mmol, 0.7 equiv.) in CH_2Cl_2 (0.5 mL) was added. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 1 h. After the addition of Et_3N to pH = 7, the organic layer was washed with H_2O (5 mL), dried over $MgSO_4$, filtered and concentrated under reduced

pressure. Purification using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (20/80, 50/50, 90/10) afforded **144** as colourless oil (43 mg, 26 µmol, 14%, α/β = 3.5/1). R_f 0.27 (EtOAc/toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.10 (50 H, m, Ar-H), 5.61 (1 H, app. t, *J* = 9.8 Hz, $H_{4''}$), 5.42 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 5.33 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 5.28 (1 H, d, *J* = 12.8 Hz, C*H*$_2$Ph), 5.25 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.94 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph), 4.91 (1 H, d, *J* = 12.8 Hz, C*H*$_2$Ph), 4.87 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.86 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.84 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.82 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.80 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.79 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.75 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.66 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.51 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.49 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.46 (1 H, s, $H_{1''}$), 4.44 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.43 (1 H, s, $H_{1'}$), 4.41 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.36 (1 H, app. t, *J* = 9.4 Hz, H_4), 4.35 (1 H, s, H_1), 4.33 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 3.87 (1 H, ddd, *J* = 11.4, 10.2, 6.5 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.80 (1 H, d, *J* = 2.8 Hz, H_2), 3.79 (1 H, d, *J* = 3.4 Hz, $H_{2''}$), 3.78 (1 H, d, *J* = 9.7 Hz, H_5), 3.75 (1 H, d, *J* = 9.9 Hz, $H_{5''}$), 3.67 (1 H, d, *J* = 2.7 Hz, H_2), 3.53 (1 H, d, *J* = 9.5 Hz, $H_{5'}$), 3.50 – 3.44 (1 H, m, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.44 – 3.39 (1 H, m, H_3), 3.40 (3 H, s, CO$_2$C*H*$_3$), 3.33 (1 H, dd, *J* = 9.3, 3.1 Hz, $H_{3''}$), 3.31 (2 H, ddd, *J* = 8.9, 7.7, 2.5 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 3.15 (1 H, dd, *J* = 9.2, 3.1 Hz, $H_{3'}$), 2.53 – 2.32 (3 H, m, C*H*$_2$ Lev), 2.18 – 2.10 (1 H, m, C*H*$_2$ Lev), 2.08 (3 H, s, C*H*$_3$ Lev), 1.82 (2 H, dt, *J* = 20.1, 6.8 Hz, OCH$_2$C*H*$_2$CH$_2$N$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.3 (C=O Lev ketone), 171.1 (C=O Lev), 168.9 (C=O *C*O$_2$CH$_3$), 151.6 (*C*(O)N(Bn)OBn), 150.5 (*C*(O)N(Bn)OBn), 139.1 (C_q), 139.0 (C_q), 138.8 (C_q), 138.8 (C_q), 138.7 (C_q), 138.0 (C_q), 137.9 (C_q), 137.3 (C_q), 137.1 (C_q), 137.0 (C_q), 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.7, 127.6, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 127.2,

127.1, 102.4 (C1'), 102.3 (C1''), 101.7 (C1), 80.0 (C3), 79.1 (C3'), 78.6 (C3''), 77.7 (C4'), 77.2 (C4), 76.8 (*C*H_2Ph), 76.5 (2C, C2' and C*H*$_2$Ph), 76.1 (*C*H_2Ph), 75.4 (C2''), 75.1 (2C, C2 and C5), 74.9 (*C*H_2Ph), 74.8 (*C*H_2Ph), 74.6 (C5'), 74.3 (*C*H_2Ph), 74.2 (*C*H_2Ph), 73.6 (C5''), 73.3 (*C*H_2Ph), 73.2 (*C*H_2Ph), 72.8 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.5 (*C*H_2Ph), 71.4 (*C*H_2Ph), 68.2 (C4''), 66.5 (O*C*H_2CH$_2$CH$_2$N$_3$), 52.0 (CO_2*C*H$_3$), 48.3 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 37.9 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 29.1 (OCH$_2$*C*H$_2$CH$_2$N$_3$), 27.8 (*C*H$_2$ Lev); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 1689.7330 $C_{97}H_{105}N_6O_{21}$ requires (M+NH$_4$)$^+$, 1689.7327].

11.1.69. *O*-benzyl, *N*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-hydroxyl-α/β-D-mannopyranoside) hydroxamate 147

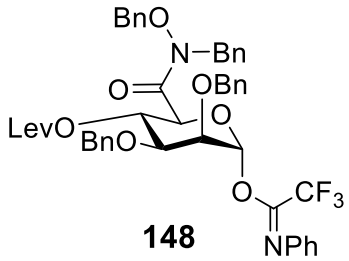


147

A solution of **69** (100 mg, 0.13 mmol, 1.0 equiv.) in CH_2Cl_2/H_2O (1.4 mL, 10/1 *v/v*) was cooled to 0 °C followed by the addition of NIS (30 mg 0.13 mmol, 1.0 equiv.), and catalytic amount of AgOTf (6.8 mg 26 µmol, 0.2 equiv.). The reaction was left stirring at 0 °C for 4 h before it was quenched by the addition of 10% aq. $Na_2S_2O_3$ solution (5 mL) and diluted with CH_2Cl_2 (15 mL). The organic layer was subsequently washed with sat. aq. $NaHCO_3$ solution (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with Et_2O/toluene (5/95, 10/90, 20/80) to furnish **147** as a yellow oil (68 mg, 0.10 mmol, 78%). R_f 0.50 (EtOAc/toluene, 3/7); The NMR data reported refer to the major α-anomer (α/β = 9/1): **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.41 – 7.20 (20 H, m, Ar-H), 5.70 (1 H, app. t, *J* = 9.7 Hz, H$_4$), 5.46 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.42 (1 H, d, *J* = 12.0 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.20 (1 H, dd, *J* = 3.5, 2.2 Hz, H$_1$), 4.97 (1 H,

d, *J* = 12.7 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.93 (2 H, d, *J* = 13.1 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.76 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C2), 4.64 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C2), 4.59 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C3), 4.54 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph-attached to C3), 4.33 (1 H, d, *J* = 9.9 Hz, H$_5$), 3.90 (1 H, dd, *J* = 9.5, 2.9 Hz, H$_3$), 3.77 – 3.74 (1 H, app. t, *J* = 2.6 Hz, H$_2$), 3.38 (1 H, d, *J* = 3.7 Hz, C1-*OH*), 2.63 – 2.35 (4 H, m, C*H*$_2$ Lev), 2.30 – 2.21 (1 H, m, C*H*$_2$ Lev), 2.09 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.4 (C=O Lev ketone), 171.4 (C=O Lev), 151.5 (*C*(O)N(Bn)OBn), 138.2 (C$_q$), 138.2 (C$_q$), 137.7 (C$_q$), 137.2 (C$_q$), 128.3, 128.3, 128.3, 128.2, 128.1, 127.7, 127.7, 127.6, 127.5, 127.5, 93.0 (C1), 76.2 (2C, C3, (C(O)N(Bn)O*C*H$_2$Ph), 74.8 (C2), 73.3 (C(O)N(*C*H$_2$Ph)OBn), 72.9 (*C*H$_2$Ph-attached to C2), 72.2 (*C*H$_2$Ph-attached to C3), 70.5 (C5), 68.7 (C4), 37.9 (*C*H$_2$ Lev), 29.8 (*C*H$_3$ Lev), 27.9 (*C*H$_2$ Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): **α-anomer**: 93.0 ($^1J_{C1\text{-}H1}$ = 176 Hz, C1), **β-anomer**: 93.7 ($^1J_{C1\text{-}H1}$ = 164 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+H)$^+$ 668.2860 $C_{39}H_{42}NO_9$ requires (M+H)$^+$, 668.2854].

11.1.70. *O*-benzyl, *N*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-*O*-Phenyl-*N*-trifluoroacetimidate-α-D-mannopyranoside) hydroxamate 148

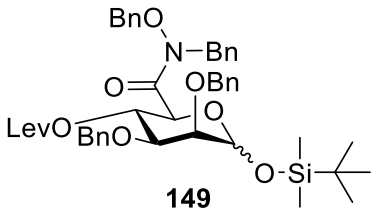


148

Hemiacetal **147** (70 mg, 0.10 mmol, 1.0 equiv.) was dissolved in acetone/H_2O (1.1 mL, 20/1 *v/v*) and the solution was cooled to 0 °C. *N*-PTFA.Cl (25 µL, d = 1.31, 0.16 mmol, 1.5 equiv.) and K_2CO_3 (17 mg, 0.12 mmol, 1.2 equiv.) were added and the resulting suspension was stirred for 20 h at room temperature. The reaction mixture was diluted with EtOAc (10 mL) and H_2O (10 mL), the organic layer was then washed with brine (10 mL), collected dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with Et_2O/toluene (5/95, 10/90,

20/80) to furnish **148** as a colourless oil (58 mg, 69 µmol, 66%). R_f 0.68 (EtOAc/tolene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.38 – 7.22 (25 H, m, Ar-H), 7.11 (1 H, t, *J* = 7.5 Hz, C*H* NPh), 6.69 (2 H, d, *J* = 7.7 Hz, C*H* NPh), 6.26 (1 H, br. s, H_1), 5.76 (1 H, t, *J* = 9.7 Hz, H_4), 5.44 (1 H, d, *J* = 11.9 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.38 (1 H, d, *J* = 11.9 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.02 (1 H, d, *J* = 12.4 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.99 (1 H, d, *J* = 12.5 Hz, C(O)N(Bn)OC*H*$_2$Ph), 4.72 (1 H, d, *J* = 10.2 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 10.3 Hz, C*H*$_2$Ph), 4.61 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.27 (1 H, d, *J* = 9.9 Hz, H_5), 3.82 (1 H, dd, *J* = 9.5, 2.7 Hz, H_3), 3.77 (1 H, s, H_2), 2.68 – 2.41 (3 H, m, C*H*$_2$ Lev), 2.30 – 2.20 (1 H, m, C*H*$_2$ Lev), 2.13 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.2 (C=O Lev), 150.2 (*C*(O)N(Bn)OBn), 143.1 (C_q NPh), 142.3 (q, *J* = 36.0 Hz, C=NPh), 137.7 (C_q), 137.7 (C_q), 137.5 (C_q), 137.0 (C_q), 128.7, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.7, 127.5, 124.5, 119.4 (*C*H NPh), 115.8 (q, *J* = 287.3 Hz, *C*F_3), 94.9 (C1), 75.4, 73.5 (C3), 73.0 (*C*H_2Ph), 72.8 (2C, C2, C5), 72.6 (*C*H_2Ph), 67.7 (C4), 37.9 (*C*H_2 Lev), 29.8 (*C*H_3 Lev), 27.8 (*C*H_2 Lev); **^{13}C-GATED** (101 MHz; $CDCl_3$): 94.9 ($^1J_{C1\text{-}H1}$ = 172 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 861.2972 $C_{47}H_{45}F_3N_2O_9Na$ requires $(M+Na)^+$, 861.2969].

11.1.71. *O*-benzyl, *N*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-*O*-*tert*-butyl dimethylsilyl-α/β-D-mannopyranoside) hydroxamate 149



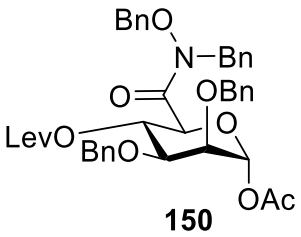
149

A stirred solution of imidate hydroxamate donor **148** (60 mg, 71 µmol, 1.0 equiv.) and hydroxamate acceptor **78** (37 mg, 57 µmol, 0.8 equiv.) in CH_2Cl_2 (0.6 mL) was cooled down to -78 °C before TBDMSOTf (4.9 µL, d = 1.151, 21 µmol, 0.3 equiv.). The reaction was stirred at -78 °C for 24 h before it was quenched by the addition of Et_3N (997 µL, d = 0.726, 0.71 mmol, 10 equiv.). The

reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/toluene (5/95, 10/90 and 15/85) afforded undesirable by-product **149** as a colourless oil in an anomeric ratio of α/β = 1/1 (9 mg, 11 μmol, 16%). R_f 0.84 (Et_2O /toluene, 3/7); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.41 – 7.17 (40 H, m, Ar-H), 5.68 (1 H, app. t, *J* = 9.9 Hz, H_4 α-anomer), 5.62 (1 H, app. t, *J* = 9.9 Hz, H_4 β-anomer), 5.52 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 5.43 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 5.38 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 5.38 (1 H, d, *J* = 10.5 Hz, C*H*$_2$Ph), 5.04 (1 H, d, *J* = 1.9 Hz, H_1 α-anomer), 4.99 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.96 (2 H, d, *J* = 11.3 Hz, C*H*$_2$Ph), 4.95 (2 H, d, *J* = 12.7 Hz, C*H*$_2$Ph), 4.83 (2 H, d, *J* = 13.2 Hz, C*H*$_2$Ph), 4.72 (1 H, s, H_1 β-anomer), 4.60 (1 H, d, *J* = 12.9 Hz, C*H*$_2$Ph), 4.58 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.51 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.42 (1 H, d, *J* = 12.3 Hz, C*H*$_2$Ph), 4.20 (1 H, d, *J* = 9.8 Hz, H_5 α-anomer), 3.86 (1 H, dd, *J* = 9.8, 2.9 Hz, H_3 α-anomer), 3.85 (1 H, d, *J* = 10.0 Hz, H_5 β-anomer), 3.82 (1 H, d, *J* = 2.7 Hz, H_2 β-anomer), 3.53 (1 H, t, *J* = 2.2 Hz, H_2 α-anomer), 3.46 (1 H, dd, *J* = 9.7, 2.8 Hz, H_3 β-anomer), 2.65 – 2.17 (8 H, m, C*H*$_2$ Lev), 2.12 (3 H, s, C*H*$_3$ Lev), 2.10 (3 H, s, C*H*$_3$ Lev), 0.88 (9 H, s, C(C*H*$_3$)$_3$), 0.80 (9 H, s, C(C*H*$_3$)$_3$), 0.07 (3 H, s, Si(C*H*$_3$)$_2$), 0.06 (3 H, s, Si(C*H*$_3$)$_2$), -0.01 (3 H, s, Si(C*H*$_3$)$_2$), -0.06 (3 H, s, Si(C*H*$_3$)$_2$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.4 (C=O Lev ketone), 206.3 (C=O Lev ketone), 171.3 (C=O Lev), 171.2 (C=O Lev), 151.1 (*C*(O)N(Bn)OBn), 151.1 (*C*(O)N(Bn)OBn), 138.8 (C_q), 138.8 (C_q), 138.3 (C_q), 138.1 (C_q), 138.0 (C_q), 138.0 (C_q), 137.3 (C_q), 137.2 (C_q), 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.5, 127.2, 96.8 (C1 β-anomer), 93.4 (C1 α-anomer), 78.7 (C3 β-anomer), 76.3 (C3 α-anomer), 76.2 (*C*H_2Ph), 76.1 (*C*H_2Ph), 75.9 (C2 α-anomer), 75.4 (C2 β-anomer), 74.3

(*C*H$_2$Ph), 73.8 (C5 β-anomer), 73.3 (*C*H$_2$Ph), 73.1 (*C*H$_2$Ph), 72.9 (*C*H$_2$Ph), 72.3 (*C*H$_2$Ph), 71.4 (*C*H$_2$Ph), 70.6 (C5 α-anomer), 68.7 (C4 α or β-anomer), 68.3 (C4 α or β-anomer), 38.0 (*C*H$_2$ Lev), 37.9 (*C*H$_2$ Lev), 29.9 (*C*H$_3$ Lev), 29.8 (*C*H$_3$ Lev), 28.0 (*C*H$_2$ Lev), 27.8 (*C*H$_2$ Lev), 25.7 (C(*CH$_3$*)$_3$), 25.6 (C(*CH$_3$*)$_3$), 17.9 (*C*(CH$_3$)$_3$), 17.8 (*C*(CH$_3$)$_3$), -4.0 (Si(*C*H$_3$)$_2$), -4.7 (Si(*C*H$_3$)$_2$), -5.4 (Si(*C*H$_3$)$_2$), -5.9(Si(*C*H$_3$)$_2$); **^{13}C-GATED** (101 MHz; CDCl$_3$): **α-anomer**: 93.5 (*$^1J_{C1\text{-}H1}$* = 168 Hz, C1), **β-anomer**: 96.8 (*$^1J_{C1\text{-}H1}$* = 156 Hz, C1).

11.1.72. *O*-benzyl, *N*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-*O*-acetyl-α-D-mannopyranoside) hydroxamate 150

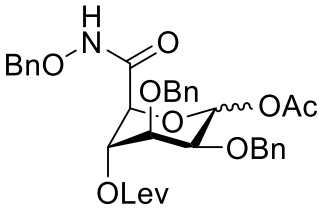


150

To a stirred solution of **147** (150 mg, 0.22 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (2.2 mL) was added Ac$_2$O (42 µL, d = 1.08, 0.44 mmol, 2.0 equiv.) and pyridine (36 µL, d = 0.978, 0.44 mmol, 2.0 equiv.) at room temperature. The reaction mixture was left stirring for 16 h before it was diluted with CH$_2$Cl$_2$ (15 mL). The organic layer was washed successively with 1.0 M aq. HCl (2 x 10 mL), sat. aq. NaHCO$_3$ solution (2 x 10 mL) and brine (15 mL), dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to furnish a yellow oil. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 30/70, 40/60, 90/10) afforded colourless oil **150** as a single anomer (α-anomer) (142 mg, 0.20 mmol, 89%). R$_f$ 0.83 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ +27.5 (*c*. 0.46, CHCl$_3$); **^{1}H NMR** (400 MHz; CDCl$_3$) δ 7.37 – 7.25 (20 H, m, Ar-H), 6.18 (1 H, d, *J* = 2.2 Hz, H$_1$), 5.73 (1 H, app. t, *J* = 9.8 Hz, H$_4$), 5.40 (2 H, s, C(O)N(C*H$_2$*Ph)OBn), 5.00 (1 H, d, *J* = 12.4 Hz, C(O)N(Bn)OC*H$_2$*Ph), 4.96 (1 H, d, *J* = 12.4 Hz, C(O)N(Bn)OC*H$_2$*Ph), 4.75 (1 H, d, *J* = 12.2 Hz, C*H$_2$*Ph-attached to C2), 4.69 (1 H, d, *J* = 12.2 Hz, C*H$_2$*Ph-attached to C2),

4.57 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C3), 4.53 (1 H, d, *J* = 12.7 Hz, C*H*$_2$Ph-attached to C3), 4.19 (1 H, d, *J* = 10.0 Hz, H_5), 3.80 (1 H, dd, *J* = 9.6, 3.0 Hz, H_3), 3.70 (1 H, app. t, *J* = 2.6 Hz, H_2), 2.67 – 2.40 (3 H, m, C*H*$_2$ Lev), 2.29 – 2.20 (1 H, m, C*H*$_2$ Lev), 2.12 (3 H, s, C*H*$_3$ Lev), 2.05 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.2 (C=O Lev ketone), 171.2 (C=O Lev), 168.6 (*C*(O)CH_3), 150.6 (*C*(O)N(Bn)OBn), 137.8 (C_q), 137.7 (C_q), 137.6 (C_q), 137.1 (C_q), 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.5, 91.6 (C1), 76.3 (C(O)N(Bn)O*C*H_2Ph), 75.6 (C3), 73.6 (C(O)N(*C*H_2Ph)OBn), 73.5 (C2), 72.8 (*C*H_2Ph-attached to C2), 72.7 (C5), 72.2 (*C*H_2Ph-attached to C3), 68.1 (C4), 37.9 (*C*H_2 Lev), 29.8 (*C*H_3 Lev), 27.8 (*C*H_2 Lev), 21.0 (C(O)*C*H_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 91.6 ($^1J_{C1\text{-}H1}$ = 176 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 710.2975 $C_{41}H_{44}NO_{10}$ requires $(M+H)^+$, 710.2960].

11.1.73. *O*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-1-*O*-acetyl-α/β-D-mannopyranoside) hydroxamate 152

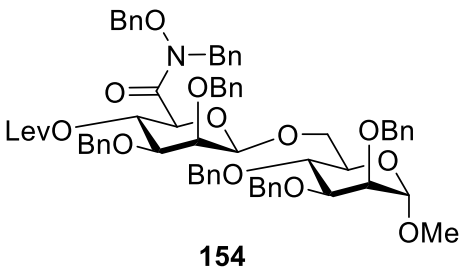


152

Compound **150** (120 mg, 0.17 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (0.6 mL) in a round-bottomed flask, which was stoppered with a septum and cooled in an ice-water bath. PBr_3 (27 µL, d = 2.88, 0.29 mmol, 1.7 equiv.) and H_2O (18 µL, 1.01 mmol, 6.0 equiv.) were added dropwise. After 40 min, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and extracted with H_2O (2 x 20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (2 x 20 mL). The organic layer was dried over Na_2SO_4 filtered and concentrated under reduced pressure to furnish crude colourless oil **152** as a mixture of α and β-anomers (84 mg, 0.13 mmol, 80%, α/β = 5.6/1); R_f 0.83 (EtOAc/toluene, 3/7); data

reported for α-anomer: **^{1}H NMR** (400 MHz; $CDCl_3$) δ 9.75 (1 H, br. s, C(O)N(*H)*OBn), 7.42 – 7.27 (20 H, m, Ar-H), 5.96 (1 H, d, *J* = 6.6 Hz, H_1), 5.78 (1 H, dd, *J* = 5.4, 4.3 Hz, H_4), 4.81 (1 H, d, *J* = 11.0 Hz, C(O)N(H)OC*H*$_2$Ph), 4.76 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph-attached to C3), 4.71 (1 H, d, *J* = 11.0 Hz, C(O)NHOC*H*$_2$Ph), 4.64 (1 H, d, *J* = 12.8 Hz, C*H*$_2$Ph-attached to C2), 4.61 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph-attached to C2), 4.56 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph-attached to C3), 4.36 (1 H, d, *J* = 4.0 Hz, H_5), 3.96 (1 H, dd, *J* = 5.6, 2.9 Hz, H_3), 3.69 (1 H, dd, *J* = 6.6, 2.9 Hz, H_2), 2.86 – 2.60 (4 H, m, C*H*$_2$ Lev), 2.18 (3 H, s, C*H*$_3$ Lev), 2.08 (3 H, s, C(O)C*H*$_3$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.4 (C=O Lev ketone), 171.6 (C=O Lev), 170.3 (*C*(O)CH_3) 163.9 (*C*(O)N(H)OBn), 137.7 (C_q), 137.4 (C_q), 135.1 (C_q), 129.6, 129.3, 129.2, 128.8, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 91.0 (C1), 78.3 (C(O)N(H)O*C*H_2Ph), 74.4 (C5), 73.9 (C3), 73.2 (C2), 72.7 (*C*H_2Ph-attached to C3), 72.6 (*C*H_2Ph-attached to C2), 68.8 (C4), 38.0 (*C*H_2 Lev), 29.8 (*C*H_3 Lev), 28.0 (*C*H_2 Lev), 20.8 (C(O)*C*H_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 91.0 ($^{1}J_{C1\text{-}H1}$ = 180 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 620.2498 $C_{34}H_{38}NO_{10}$ requires $(M+H)^+$, 620.2496].

11.1.74. *O*-methyl (*O*-benzyl-*N*-benzyl- (2,3,4-tri-*O*-benzyl (4-*O*-levulinoyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl)-α-D-mannopyranoside)-hydroxamate 154



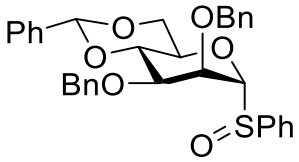
154

A solution of **69** (50 mg, 66 µmol, 1.0 equiv.), Ph_2SO (32 mg, 0.15 mmol, 2.4 equiv.) and TTBP (16 mg, 66 µmol, 1.0 equiv.) in CH_2Cl_2 (1.3 mL) was stirred over activated MS4Å for 45 min. The mixture was cooled to -78 °C and Tf_2O (11 µL, d = 1.720, 69 µmol, 1.05 equiv.) and left stirring for 45 min. until the temperature reached -60 °C. A solution of the *C*6-OH acceptor **153** (30 mg, 66 µmol,

1.0 equiv.) in CH_2Cl_2 (1.3 mL) was slowly added. The reaction mixture was allowed to warm up to -20 °C, and stirring was continued for 3 h. At that temperature Et_3N was added until pH = 7, the organic layer was washed with H_2O (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with acetone/toluene (10/90, 20/80, 30/170) afforded **154** as a colourless oil (20 mg, 18 µmol, 27%). R_f 0.54 (EtOAc/hexane, 1/2); $[\alpha]_D^{26}$ -44.8 (*c*. 0.33, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) 7.40 – 7.18 (35 H, m, Ar-H), 5.62 (1 H, t, *J* = 9.9 Hz, H_4'), 5.47 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 5.39 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.98 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.94 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.91 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.87 (1 H, d, *J* = 11.4 Hz, C*H*$_2$Ph), 4.77 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.69 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.68 (1 H, s, H_1), 4.66 (1 H, d, *J* = 12.6 Hz, C*H*$_2$Ph), 4.61 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.58 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.51 (1 H, d, *J* = 11.4 Hz, C*H*$_2$Ph), 4.46 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.37 (1 H, d, *J* = 12.4 Hz, C*H*$_2$Ph), 4.26 (1 H, s, H_1'), 4.12 (1 H, dd, *J* = 10.6, 1.6 Hz, H_{6a}), 3.88 (1 H, dd, *J* = 8.7, 3.0 Hz, H_3), 3.80 (1 H, d, *J* = 9.8 Hz, H_5') 3.78 (1 H, d, *J* = 10.0 Hz, H_5), 3.77 – 3.71 (3 H, m, H_2, H_2' H_4) 3.49 (1 H, dd, *J* = 10.6, 6.5 Hz, H_{6b}), 3.34 (1 H, dd, *J* = 9.7, 2.8 Hz, H_3'), 3.21 (3 H, s, OC*H*$_3$), 2.64 – 2.37 (3 H, m, C*H*$_2$ Lev), 2.27 – 2.16 (1 H, m, C*H*$_2$ Lev), 2.10 (3 H, s, C*H*$_3$ Lev); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 206.3 (C=O Lev ketone), 171.2 (C=O Lev), 150.8 (*C*(O)N(Bn)OBn), 138.5 (C_q), 138.4 (C_q), 138.3 (C_q), 138.2 (C_q), 137.9 (C_q), 137.9 (C_q), 137.2 (C_q), 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.6, 127.6, 127.2, 102.2 (C1'), 98.8 (C1), 80.2 (C3), 78.4 (C3'), 76.2 (*C*H_2Ph), 74.9 (*C*H_2Ph), 74.7, 74.4, 73.9, 73.7, 73.4 (5 C, C2 or C2', C4, C5, C5', *C*H_2Ph), 73.0 (*C*H_2Ph), 72.7 (*C*H_2Ph), 72.0 (*C*H_2Ph), 71.3 (2 C, C2 or C2', *C*H_2Ph), 68.4 (C4'), 54.6 (O*C*H_3), 37.9 (*C*H_2 Lev), 29.8 (*C*H_3 Lev), 27.8 (*C*H_2 Lev); **^{13}C-GATED**

(101 MHz; $CDCl_3$): 102.2 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1'), 98.8 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 1131.5214 $C_{67}H_{75}N_2O_{14}$ requires $(M+NH_4)^+$, 1131.5213].

11.1.75. Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thio-α-D-mannopyranoside *S*-Oxide 163

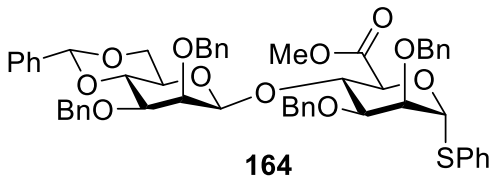


163

To a stirred solution of **40** (3.2 g, 5.92 mmol, 1.0 equiv.) in CH_2Cl_2 (65 mL) at -78 °C was added *m*-CPBA (1.3 g, 5.92 mmol, 77%, 1.0 equiv.) after which the mixture was warmed to -30 °C in 40 min. and stirred for further 3 h before quenching with sat. aq. $NaHCO_3$ solution (50 mL) and washing with brine (60 mL).The organic layer was dried over Na_2SO_4 filtered and concentrated under reduced pressure to furnish a yellow oil. Purification using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (0/100, 30/70, 40/60) afforded **163** as a single diastereoisomer (2.4 g, 4.32 mmol, 73%); R_f 0.63 (EtOAc/petroleum ether, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.54 – 7.21 (20 H, m, Ar-H), 5.63 (1 H, s, C*H*Ph), 4.82 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C3), 4.67 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph-attached to C3), 4.60 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph-attached to C2), 4.56 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph-attached to C2), 4.50 (1 H, d, *J* = 1.0 Hz, H_1), 4.39 (1 H, dd, *J* = 3.1, 1.3 Hz, H_2), 4.33 (1 H, dd, *J* = 10.0, 8.6 Hz, H_4), 4.28 (1 H, dd, *J* = 9.6, 3.2 Hz, H_3), 4.22 (1 H, dd, *J* = 10.3, 4.8 Hz, H_{6a}), 4.15 – 4.06 (1 H, m, H_5), 3.76 (1 H, app. t, *J* = 10.1 Hz, H_{6a}); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 141.6, 138.3 (C_q), 137.3 (C_q), 137.3 (C_q), 131.6 (C_q), 129.4, 129.0, 128.4, 128.3, 128.2, 128.2, 127.9, 127.8, 127.6, 126.1, 124.3, 101.6 (*C*HPh), 97.6 (C1), 78.0 (C4), 76.3 (C3), 73.5 (*C*H$_2$Ph-attached to C3),

73.2 (*C*CH_2Ph-attached to C2), 72.9 (C2), 70.1 (C5), 68.2 (C6); These data were consistent with literature values.[236]

11.1.76. Methyl (phenyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-thio-*α*-D-mannopyranoside*)* uronate 164

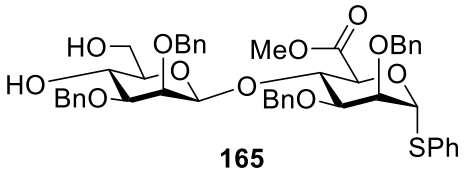


164

To a stirred solution of sulfoxide donor **163** (1.4 g, 2.51 mmol, 1.0 equiv.) and TTBP (1.3 g, 2.03 mmol, 2.0 equiv.) in CH_2Cl_2 (98 mL) cooled at -78 °C was added Tf_2O (453 µL, d = 1.720, 2.76 mmol, 1.1 equiv.) and 5 min. later, a solution of thioglycoside acceptor **54** (1.3 g, 2.63 mmol, 1.05 equiv.) in CH_2Cl_2 (12 mL) was added dropwise. The opaque reaction mixture was stirred at -78 °C for 30 min. and the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution (15 mL) when TLC analysis indicated consumption of the donor. The organic layer was washed with brine (20 mL) dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using silica gel flash column chromatography, eluting with EtOAc/petroleum ether (20/80, 30/70, 50/50) to furnish **164** as a white foam (1.68 g, 1.88 mmol, 75%). R_f 0.71 (EtOAc/petroleum ether, 1/2); $[\alpha]_D^{22}$ -6.2 (*c*. 1.7, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.65 – 7.26 (30 H, m, Ar-H), 5.79 (1 H, d, *J* = 8.4 Hz, H_1), 5.59 (1 H, s, C*H*Ph), 4.80 (1 H, d, *J* = 12.0 Hz, CH_2Ph), 4.75 (1 H, d, *J* = 12.0 Hz, CH_2Ph), 4.70 (1 H, d, *J* = 12.5 Hz, CH_2Ph), 4.62 (1 H, d, *J* = 0.8 Hz, $H_{1'}$), 4.60 (1 H, d, *J* = 12.2 Hz, CH_2Ph), 4.56 (1 H, d, *J* = 12.8 Hz, CH_2Ph), 4.53 (1 H, d, *J* = 11.6 Hz, CH_2Ph), 4.52 (1 H, t, *J* = 10.3 Hz, H_4), 4.51 (1 H, d, *J* = 9.0 Hz, H_5), 4.50 (1 H, d, *J* = 12.3 Hz, CH_2Ph), 4.42 (1 H, d, *J* = 11.6 Hz, CH_2Ph), 4.19 (1 H, dd, *J* = 10.7, 4.5 Hz, $H_{6a'}$), 4.16 (app. t, *J* = 9.6 Hz, $H_{4'}$), 4.04 (1 H, br. dd, *J* 2.4, 10.1 Hz, H_3), 3.90 (1 H, dd, *J* = 0.8, 2.8 Hz, $H_{2'}$), 3.83 (1 H, app. t, *J* = 10.2 Hz, $H_{6b'}$),

3.77 (1 H, dd, *J* = 8.4, 2.7, H_2), 3.57 (3 H, s, CO_2CH_3), 3.57 (1 H, dd, *J* = 9.8, 3.2 Hz, $H_{3'}$), 3.29 (1 H, td, *J* = 9.8, 4.8 Hz, $H_{5'}$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.4 (CO_2CH_3), 138.3 (C_q), 138.2 (C_q), 137.8 (C_q), 137.5 (C_q), 133.9 (C_q), 131.1 (C_q), 128.9, 128.8, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.0, 126.1, 101.5 (*C*HPh), 101.1 (C1'), 82.4 (C1), 78.4 (C4'), 77.6 (C3'), 76.5 (C2'), 75.7 (C4), 75.2 (CH_2Ph), 74.8 (C2), 74.6 (C3), 73.4 (C5), 72.8 (CH_2Ph), 72.4 (CH_2Ph), 72.4 (CH_2Ph), 68.4 (C6'), 67.7 (C5'), 52.2 (CO_2CH_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.1 ($^1J_{C1'-H1'}$ = 156 Hz, C1'), 82.4 ($^1J_{C1-H1}$ = 168 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 928.3766 $C_{54}H_{58}NO_{11}S$ requires $(M+NH_4)^+$, 928.3731].

11.1.77. Methyl (phenyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-β-D-mannopyranosyl)-thio-α-D-mannopyranoside) uronate 165

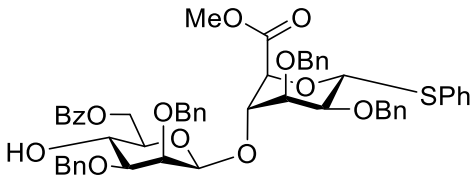


165

A mixture of **164** (1.6 g, 1.76 mmol, 1.0 equiv.) and *p*-TsOH.H_2O (33 mg, 0.17 mmol, 0.1 equiv.) in MeOH (17 mL) was heated at reflux (85 °C) for 2 h. The solution was then cooled to room temperature and quenched with Et_3N (2 mL). The solvent was evaporated under reduced pressure and the crude product was dissolved in CH_2Cl_2 (50 mL). The organic layer was washed with H_2O (2 x 50 mL), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (1 x 50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure furnishing a colourless oil. Purification using silica gel flash column chromatography, eluting with MeOH/CH_2Cl_2 (5/95) afforded **165** as a colourless oil (1.0 g, 1.21 mmol, 69%). R_f 0.53 (MeOH/CH_2Cl_2, 1/9); $[\alpha]_D^{22}$ -32.0 (*c*. 2.45, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.63 – 7.23 (25 H, m, Ar-H), 5.79 (1 H, d, *J* = 7.7 Hz, H_1), 4.78 (1 H, d, *J* = 12.1 Hz, CH_2Ph), 4.64 (1 H, d, *J* = 12.1 Hz, CH_2Ph), 4.61 (2 H, d, *J* = 12.4 Hz, CH_2Ph), 4.60 (1 H, d, *J*

= 2.8 Hz, $H_{1'}$), 4.57 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 10.2 Hz, H_5), 4.54 (1 H, app. t, *J* = 8.1 Hz, H_4), 4.50 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.46 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.31 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 3.97 (1 H, dd, *J* = 10.2, 2.6 Hz, H_3), 3.94 (1 H, app. td, *J* = 9.6, 2.4 Hz, $H_{4'}$), 3.90 (1 H, d, *J* = 2.8 Hz, $H_{2'}$), 3.87 – 3.77 (1 H, m, $H_{6a' \text{ or } b'}$), 3.79 (1 H, dd, *J* = 7.9, 2.4 Hz, H_2), 3.75 – 3.67 (1 H, m, $H_{6a' \text{ or } b'}$), 3.59 (3 H, s, CO_2CH_3), 3.28 (1 H, dd, *J* = 9.5, 3.1 Hz, $H_{3'}$), 3.32 – 3.23 (1 H, m, $H_{5'}$), 2.49 (1 H, d, *J* = 2.3 Hz, C4-O*H*), 2.15 (1 H, t, *J* = 6.5 Hz, C6-O*H*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.4 (CO_2CH_3), 138.3 (C_q), 137.8 (C_q), 137.5 (C_q), 133.9 (C_q), 131.0 (C_q), 128.8, 128.6, 128.4, 128.33, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.0, 100.5 (C1'), 82.7 (C1), 81.2 (C3'), 76.0 (C5'), 75.4 (C4), 74.9 (2C, C2, C3), 74.5 (CH_2Ph), 73.7 (C2'), 73.1 (C5), 72.9 (CH_2Ph), 72.5 (CH_2Ph), 71.1 (CH_2Ph), 67.3 (C4'), 62.9 (C6'), 52.2 (CO_2CH_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 100.5 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1'), 82.7 ($^1J_{C1\text{-}H1}$ = 168 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: $(M+Na)^+$ 845.3032 $C_{47}H_{50}O_{11}SNa$ requires $(M+Na)^+$, 845.2972].

11.1.78. Methyl (phenyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-6-benzoyl-β-D-mannopyranosyl)-thio-α-D-mannopyranoside) uronate 166

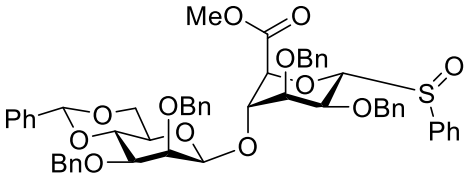


166

To a stirred solution of **165** (470 mg g, 0.57 mmol, 1.0 equiv.), pyridine (46 µL, d = 0.978, 0.57 mmol, 1.0 equiv.), DMAP (7 mg, 57 µmol, 0.1 equiv.) in CH_2Cl_2 (5.7 mL) was added BzCl (69 µL, d = 1.211, 0.60 mmol, 1.05 equiv.) dropwise at 0 °C. The reaction was left stirring overnight at room temperature, and diluted with CH_2Cl_2 (10 mL). The mixture was washed with 1.0 M aq. HCl (10 mL), sat. aq. $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under

reduced pressure to afford the crude product. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (30/70 and 40/60) afforded **166** as a colourless oil (402 mg, 0.43 mmol, 76%). R_f 0.18 (EtOAc/hexane, 1/2); $[\alpha]_D^{22}$ -37.5 (*c*. 1.45, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 8.07 – 7.15 (30 H, m), 5.79 (1 H, d, *J* = 9.1 Hz, H_1), 4.76 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.69 – 4.61 (1 H, m, $H_{6a' \text{ or } b'}$) 4.67 (1 H, d, *J* = 11.4 Hz, C*H*$_2$Ph), 4.66 (1 H, s, $H_{1'}$), 4.60 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph), 4.63 – 4.57 (1 H, m, H_4), 4.58 – 4.52 (1 H, m, $H_{6a' \text{ or } b'}$), 4.58 – 4.52 (1 H, m, H_5, C*H*$_2$Ph), 4.47 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.39 (1 H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.31 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.28 (1 H, d, *J* = 12.1 Hz, C*H*$_2$Ph), 4.13 – 4.08 (1 H, m, H_3), 4.05 (1 H, t, *J* = 9.5 Hz, $H_{4'}$), 3.93 (1 H, d, *J* = 2.8 Hz, $H_{2'}$), 3.66 (1 H, dd, *J* = 9.2, 2.5 Hz, H_2), 3.56 (1 H, ddd, *J* = 10.7, 6.1, 2.6 Hz, $H_{5'}$), 3.45 (3 H, s, $CO_2C\mathit{H}_3$), 3.34 (1 H, dd, *J* = 9.4, 2.9 Hz, $H_{3'}$), 2.68 (1 H, br. s, C4-O*H*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.4 (*C*O_2CH_3), 166.6 (*C*(O)Ph), 138.5 (C_q), 137.8 (C_q), 137.5 (C_q), 133.8 (C_q), 133.0 (C_q), 131.0 (C_q), 129.9, 129.7, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 126.8, 100.6 (C1'), 81.6 (C1), 81.0 (C3'), 75.1 (C4), 74.6 (C5'), 74.2 (3C, C2, C3, *C*H_2Ph), 73.7 (C5), 73.6 (C2'), 72.9 (*C*H_2Ph), 71.8 (*C*H_2Ph), 71.2 (*C*H_2Ph), 66.4 (C4'), 63.8 (C6'), 52.0 ($CO_2C\mathit{H}_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 100.6 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1'), 81.6 ($^1J_{C1\text{-}H1}$ = 164 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 949.3234 $C_{54}H_{54}O_{12}SNa$ requires $(M+Na)^+$, 849.3228].

11.1.79. Methyl (phenyl 2,3-di-*O*-benzyl-4-O-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-thio-α-D-mannopyranoside (R/S) *S*-Oxide) uronate 167

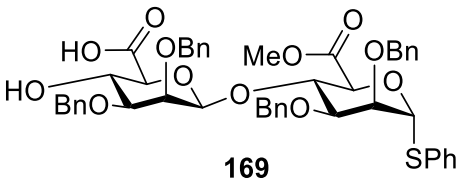


167

To a stirred solurion of disaccharide **164** (120 mg, 0.13 mmol, 1.0 equiv.) in CH_2Cl_2 (3.3 mL) at -78 °C was added *m*-CPBA (32 mg, 0.14 mmol, 77%, 1.1 equiv.) followed by warming to -40 °C over 2 h, whereupon TLC analysis (EtOAc/petroleum ether, 1/2) indicated no starting material remained. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution (25 mL) and the organic layer was separated and washed with brine (2 x 25 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure furnishing crude **125** as a yellow oil. Purification using silica gel flash column chromatography, eluting with EtOAc/hexane (0/100, 20/80, 40/60, 90/10) afforded **124** (79 mg, 86 µmol, 66%) as a mixture of two diastereoisomers (75/25). Analytical data only for major; R_f 0.18 (EtOAc/hexane, 1/2); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.78 – 7.26 (30 H, m, Ar-H), 5.62 (1 H, s, C*H*Ph), 5.05 (1 H, d, *J* = 10.1 Hz, H_1), 4.98 (1 H, d, *J* = 12.0 Hz, CH_2Ph), 4.90 (1 H, d, *J* = 12.0 Hz, CH_2Ph), 4.67 (1 H, d, *J* = 12.5 Hz, CH_2Ph), 4.65 (1 H, d, *J* = 10.1 Hz, CH_2Ph), 4.60 (1 H, d, *J* = 12.5 Hz, CH_2Ph), 4.60 (1 H, d, *J* = 10.4 Hz, CH_2Ph), 4.58 (1 H, s, $H_{1'}$), 4.57 (1 H, d, *J* = 11.9 Hz, CH_2Ph), 4.50 (1 H, m, H_4), 4.48 (1 H, d, *J* = 11.2 Hz, CH_2Ph), 4.39 (1 H, dd, *J* = 10.1, 2.6 Hz, H_2), 4.35 (1 H, d, *J* = 1.4 Hz, H_5), 4.25 – 4.15 (3 H, m, H_3, $H_{4'}$, $H_{6a'}$), 3.99 (1 H, d, *J* = 3.0 Hz, $H_{2'}$), 3.91 – 3.81 (1 H, m, $H_{6b'}$), 3.56 (1 H, dd, *J* = 9.9, 3.1 Hz, $H_{3'}$), 3.41 (3 H, s, CO_2CH_3), 3.30 (1 H, ddd, *J* = 13.8, 9.4, 4.5 Hz, $H_{5'}$); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 168.7 (CO_2CH_3), 140.10 (C_q), 138.4 (C_q), 138.1 (C_q), 137.4 (C_q), 137.4 (C_q), 130.4 (C_q), 129.3, 128.9, 128.7, 128.5, 128.4, 128.4, 128.3,

128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.6, 127.5, 126.1, 126.0, 125.2, 101.5 (*C*HPh), 100.7 (C1'), 87.3 (C1), 78.3 (C3), 77.2 (C3'), 75.8 (C2'), 75.3 (*C*H_2Ph), 74.6 (C4), 74.4 (C5), 73.9 (C4'), 72.7 (*C*H_2Ph), 72.6 (*C*H_2Ph), 72.2 (*C*H_2Ph), 71.3 (C2), 68.3 (C6'), 52.0 (CO_2CH_3); **^{13}C-GATED** (101 MHz; $CDCl_3$): 100.7 ($^1J_{C1'\text{-}H1'}$ = 156 Hz, C1'); **HRMS** (ES^+) *m/z* [Found: $(M+Na)^+$ 949.3283 $C_{54}H_{54}O_{12}SNa$ requires $(M+Na)^+$, 949.3228].

11.1.80. Methyl (phenyl 2,3-di-*O*-benzyl-4-O-(2,3-di-*O*-benzyl-β-D-mannuronic acid)-thio-α-D-mannopyranoside) uronate 169

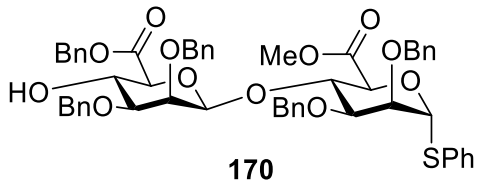


169

To a vigorously stirred solution of **165** (480 mg, 0.58 mmol, 1.0 equiv.) in CH_2Cl_2 (2.6 mL) and H_2O (1.3 mL) was added TEMPO (18 mg, 0.12 mmol, 0.2 equiv.) and BAIB (468 mg, 1.46 mmol, 2.5 equiv.). Stirring was continued for 1 h, and the reaction mixture was quenched by the addition of 10% aq. $Na_2S_2O_3$ solution (10 mL) and diluted with CH_2Cl_2 (15 mL). The organic layer was separated and the aqueous phase was acidified with 1.0 M aq. HCl to pH = 4, extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic layers dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with MeOH/CH_2Cl_2 (0/100, 5/95, 10/90 + 2% (*v/v*) AcOH) to afford the disaccharide **169** (342 mg, 0.41 mmol, 70%) as a white foam. R_f 0.56 (EtOAc/petroleum ether, 2/1 + 2% (*v/v*) AcOH); $[\alpha]_D^{22}$ -10.9 (*c*. 0.86, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.52 – 7.20 (25 H, m, Ar-H), 5.60 (1 H, br. s, H_1), 4.79 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.76 (H, d, *J* = 11.8 Hz, C*H*$_2$Ph), 4.72 (1 H, d, *J* = 12.5 Hz, C*H*$_2$Ph), 4.66 (1 H, s, $H_{1'}$), 4.64 (1 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.62 (1H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.57 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.54 (1 H, d, *J* = 11.7 Hz, C*H*$_2$Ph),

4.54 (1 H, d, *J* = 8.0 Hz, H_5), 4.49 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.44 (1 H, t, *J* = 7.7 Hz, H_4), 4.22 (1 H, t, *J* = 9.5 Hz, $H_{4'}$), 3.87 (1 H, br. s, H_2), 3.82 (1 H, d, *J* = 2.5 Hz, $H_{2'}$), 3.74 (1 H, d, *J* = 9.7 Hz, $H_{5'}$), 3.65 (3 H, s, CO_2CH_3), 3.41 (1 H, dd, *J* = 9.3, 2.9 Hz, $H_{3'}$), 3.38 (1 H, dd, *J* = 7.6, 1.6 Hz, H_3); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 171.0 (*C*(O)OH), 169.3 (CO_2CH_3), 138.2 (C_q), 138.0 (C_q), 137.4 (C_q), 137.0 (C_q), 131.6 (C_q), 129.1, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 125.3, 100.5 (C1'), 85.4 (C1), 80.2 (C3'), 78.1 (C3), 75.7 (2 C, C2, C4), 74.9 (2 C, C2', CH_2Ph), 73.2 (2 C, C5', CH_2Ph), 72.6 (C5 or CH_2Ph), 72.4 (2 C, C5 or CH_2Ph, CH_2Ph), 68.4 (C4'), 52.5 (CO_2CH_3); **HRMS** (ES^+) *m/z* [Found: $(M+NH_4)^+$ 854.3213 $C_{47}H_{52}NO_{12}S$ requires $(M+NH_4)^+$, 854.3210].

11.1.81. Methyl (phenyl 2,3-di-*O*-benzyl-4-O-(benzyl 2,3-di-*O*-benzyl-β-D-mannopyranosyl-benzyluronate)-thio-α-D-mannopyranoside) uronate 170

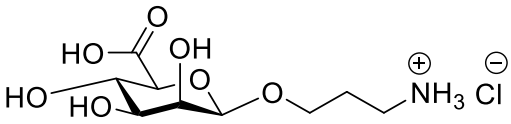


170

To a stirred solution of **169** (320 mg, 0.38 mmol, 1.0 equiv.) in DMF (3.8 mL) was added BnBr (91 µL, d= 1.438, 0.76 mmol, 2.0 equiv.) and K_2CO_3 (79 mg, 0.57 mmol, 1.5 equiv.). The reaction mixture was stirred overnight and the solvents were removed *in vacuo*. The crude was taken up in EtOAc (50 mL) and washed with H_2O (40 mL). The aqueous layer was extracted with EtOAc (50 mL), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification using silica gel flash column chromatography, eluting with with EtOAc/hexane (20/80, 30/70, 40/60) afforded **170** (330 mg, 0.36 mmol, 93%) as a colourless oil. R_f 0.31 (EtOAc/hexane, 2/1); $[\alpha]_D^{22}$ -13.9 (*c*. 3.2, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.64 – 7.14 (30 H, m, Ar-H), 5.77 (1 H, d, *J* = 8.7 Hz, H_1), 5.23 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 5.15 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph),

4.75 (1 H, d, *J* = 12.2 Hz, C*H*$_2$Ph), 4.63 (1 H, d, *J* = 11.3 Hz, C*H*$_2$Ph), 4.56 (1 H, d, *J* = 12.0 Hz, C*H*$_2$Ph), 4.55 – 4.49 (1 H, m, H$_4$), 4.50 (1 H, d, *J* = 9.8 Hz, H$_5$), 4.50 (1 H, d, *J* = 11.9 Hz, C*H*$_2$Ph), 4.43 (2 H, d, *J* = 12.9 Hz, C*H*$_2$Ph), 4.40 (2 H, d, *J* = 11.6 Hz, C*H*$_2$Ph), 4.31 (1 H, td, *J* = 9.5, 2.5 Hz, H$_{4'}$), 4.15 (1 H, br. s, H$_3$), 3.86 (1 H, d, *J* = 2.5 Hz, H$_{2'}$), 3.80 (1 H, d, *J* = 9.6 Hz, H$_{5'}$), 3.72 (1 H, dd, *J* = 8.8, 2.4 Hz, H$_2$), 3.48 (1 H, s, CO$_2$C*H*$_3$), 3.35 (1 H, dd, *J* = 9.5, 2.8 Hz, H$_{3'}$), 2.98 (1 H, d, *J* = 2.5 Hz, C4-*OH*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.4 (*C*O$_2$CH$_3$), 168.9 (*C*O$_2$Bn), 138.4 (C$_q$), 137.9 (C$_q$), 137.8 (C$_q$), 137.8 (C$_q$), 135.0 (C$_q$), 133.9 (C$_q$), 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 127.9, 127.9, 127.8, 127.7, 127.6, 126.9, 101.4 (C1'), 82.2 (C1), 80.0 (C3'), 76.1 (C5), 75.2 (C5'), 74.8 (C3), 74.5 (*C*H$_2$Ph), 74.3 (C2), 74.0 (C2), 73.8 (C4), 73.1 (*C*H$_2$Ph), 72.0 (*C*H$_2$Ph), 71.8 (*C*H$_2$Ph), 68.1(C4'), 67.4 (*C*H$_2$Ph), 52.1 (CO$_2$*C*H$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 101.4 ($^1J_{C1'-H1'}$ = 156 Hz, C1'), 82.2 ($^1J_{C1-H1}$ = 168 Hz, C1); **HRMS** (ES$^+$) *m/z* [Found: (M+NH$_4$)$^+$ 944.3685 $C_{54}H_{58}NO_{12}S$ requires (M+NH$_4$)$^+$, 944.3680].

11.1.82. 3-aminopropyl (α-D-mannopyranoside) uronic acid 171

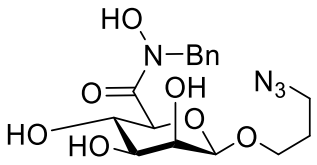


171

Mannuronate **60** (100 mg, 0.21 mmol, 1.0 equiv.) was dissolved in a mixture of THF/H$_2$O (5 mL 1/1 *v/v*) and treated with 0.45 M KOH (0.6 mL) for 2 h (pH = 9-10). The reaction mixture was neutralised with with ion exchange Amberlite 120 (H$^+$) resin (approximately 50 mg, 10 min), until pH = 3, filtered through Celite® and concentrated under reduced pressure. The residual oil (45 mg, 98 µmol, 1.0 equiv.) was then dissolved in EtOH/THF (2.0 mL, 1.5/1 *v/v*), after which Pd/C (10%) (16 mg, 15 µmol, 0.15 equiv.), Pd(OH)$_2$/C (20%) (1 mg, 15 µmol, 0.15 equiv.) and 0.1 M aq. HCl (147 µL, 15 µmol, 1.0 equiv.) were added. The mixture was stirred for 32 h under an atmosphere of hydrogen (1 atm, balloon) at

room temperature. TLC analysis (hexane/EtOAc, 1/2) showed complete conversion of starting material to a lower R_f spot. The reaction mixture was filtered through Celite®, followed by solvent removal *in vacuo* to give **171** as a white powder (22 mg, 76 µmol, 36% over 2 steps). R_f 0.37 (H_2O/MeCN, 1/2); $[\alpha]_D^{22}$ -51.3 (*c*. 0.93, H_2O); **^{1}H NMR** (400 MHz; D_2O) δ 4.63 (1 H, d, *J* = 0.7 Hz, H_1), 3.96 – 3.88 (1 H, m, OC*H*$_2$CH$_2$CH$_2$NH$_3$.Cl), 3.91 (1 H, d, *J* = 2.5 Hz, H_2), 3.83 – 3.73 (1 H, m, OC*H*$_2$CH$_2$CH$_2$NH$_3$.Cl), 3.70 – 3.63 (1 H, m, H_4), 3.59 (1 H, d, *J* = 9.0 Hz, H_5), 3.58 (1 H, dd, *J* = 10.8, 2.1 Hz, H_3), 3.16 – 3.02 (2 H, m, OCH$_2$CH$_2$*C*H$_2$NH$_3$.Cl), 1.96 – 1.88 (2 H, m, OCH$_2$C*H*$_2$CH$_2$NH$_3$.Cl); **^{13}C NMR** (101 MHz; D_2O) δ 176.3 (*C*OOH), 99.8 (C1), 76.1 (C5), 72.7 (C3), 70.3 (C2), 68.7 (C4), 67.3 (O*C*H$_2$CH$_2$CH$_2$NH$_3$.Cl), 37.5 (OCH$_2$CH$_2$*C*H$_2$NH$_3$.Cl), 26.7 (OCH$_2$*C*H$_2$CH$_2$NH$_3$.Cl); **^{13}C-GATED** (101 MHz; D_2O): 99.8 ($^1J_{C1\text{-}H1}$ = 160 Hz, C1); **HRMS** (ES^+) *m/z* [Found: $(M\text{-}H)^-$ 250.0928 $C_9H_{17}NO_7$ requires $(M\text{-}H)^-$, 250.0927].

11.1.83. 3-azidopropyl *N*-benzyl (phenyl 2,3-di-*O*-benzyl-β-D-mannopyranoside) hydroxamate 173

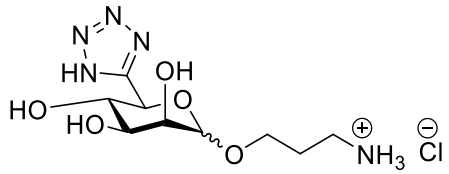


173

To a solution of **78** (85 mg, 0.13 mmol, 1.0 equiv.) in EtOAc (1.6 mL) was added 0.45 M $NaBrO_3$ aq. solution (176 mg, 1.17 mmol, 9.0 equiv.). 0.2 M $Na_2S_2O_4$ aq. solution (181 mg, 1.04 mmol, 8.0 equiv.) was added over 15 min. and the mixture was vigorously stirred for 32 h at room temperature. When TLC analysis indicated conversion of **78** to a lower R_f value, the reaction was quenched by the addition of 10% aq. $Na_2S_2O_3$ solution (5 mL) and extracted with EtOAc (2 x 10 mL).The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Flash column chromatography, eluting with EtOAc/petroleum ether (50/50, 70/30 and 90/10) afforded **140** as a colourless oil (20

mg, 52 µmol, 40%). R_f 0.66 (EtOAc/petroleum ether, 1/2); $[\alpha]_D^{22}$ -67.2 (*c*. 0.31, $CHCl_3$); **^{1}H NMR** (400 MHz; $CDCl_3$) δ 7.39 – 7.33 (5 H, m, Ar-H), 5.26 (1 H, d, *J* = 12.3 Hz, C(O)N(C*H*$_2$Ph)OBn), 5.23 (1 H, d, *J* = 12.3 Hz, C(O)N(C*H*$_2$Ph)OH), 4.52 (1 H, d, *J* = 0.8 Hz, H_1), 4.08 – 4.01 (2 H, m, H_2, H_4), 3.99 (1 H, dt, *J* = 10.0, 5.9 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.81 (1 H, d, *J* = 9.6 Hz, H_5), 3.65 (1 H, ddd, *J* = 10.0, 7.1, 5.6 Hz, OC*H*$_2$CH$_2$CH$_2$N$_3$), 3.60 (2 H, br. s, H_3, -O*H*), 3.46 (1 H, br. s, -*OH*), 3.38 (2 H, td, *J* 6.6, 3.1 Hz, OCH$_2$CH$_2$C*H*$_2$N$_3$), 2.99 (1 H, s, -*OH*), 1.88 (4 H, td, *J* = 12.6, 6.2 Hz, OCH$_2$C*H*$_2$CH$_2$N$_3$), 1.84 (1 H, s, -*OH*); **^{13}C NMR** (101 MHz; $CDCl_3$) δ 169.3 (*C*(O)N(Bn)OH), 135.0 (C_q), 128.6, 128.5, 128.3, 100.4 (C1), 74.6 (C5), 73.4 (C3), 70.1 (C2), 68.9 (C4), 67.5 (C(O)N(*C*H$_2$Ph)OH), 67.0 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 48.2 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 29.0 (OCH$_2$*C*H$_2$CH$_2$N$_3$); **^{13}C-GATED** (101 MHz; $CDCl_3$): 100.4 ($^1J_{C1\text{-}H1}$ = 156 Hz, C1).

11.1.84. 3-aminopropyl (6-*C*-tetrazol-5-yl)-α/β-D-mannopyranoside 174



174

*C*6-tetrazole **115** (30 mg, 38 µmol, 1.0 equiv.) was dissolved in a mixture of EtOH/THF (0.6 mL, 1.5/1 *v/v*), after which Pd/C (10%) (20 mg, 19 µmol, 0.5 equiv.), $Pd(OH)_2$/C (20%) (13 mg, 19 µmol, 0.5 equiv.) and 0.1 M aq. HCl (380 µL, 38 µmol, 1.0 equiv.) were added. The mixture was stirred for 56 h under an atmosphere of hydrogen (1 atm, balloon) at room temperature. TLC analysis (hexane/EtOAc, 1/2) showed complete conversion of starting material to a lower R_f spot. The reaction mixture was filtered through Celite®, followed by solvent removal *in vacuo* to give white powder **174** in an anomeric mixture of α/β = 3/1 (11 mg, 36 µmol, 96%). R_f 0.27 (H_2O/MeCN, 1/2); **^{1}H NMR** (400 MHz; D_2O) δ 4.86 (1 H, s, H_1 α-anomer), 4.82 (1 H, d, *J* = 9.8 Hz, H_5), 4.74 (1 H, s, H_1 β-anomer), 4.61 (1 H, d, *J* = 9.9 Hz, H_5 β-anomer), 4.15 (1 H, app. t, *J* = 9.9 Hz,

H_4 α-anomer), 4.08 (app. t, *J* = 9.9 Hz, H_4 β-anomer), 4.03 (1 H, d, *J* = 3.2 Hz, H_2 β-anomer), 4.01 – 3.98 (1 H, m, H_2 α-anomer), 3.87 (1 H, dd, *J* = 9.8, 3.4 Hz, H_3 α-anomer), 3.84 – 3.76 (2 H, m, $OCH_2CH_2CH_2NH_3.Cl$ α and β-anomer), 3.73 (1 H, dd, *J* = 9.8, 3.2 Hz, H_3 β-anomer), 3.56 (2 H, ddd, *J* = 17.3, 9.7, 4.5 Hz, $OCH_2CH_2CH_2NH_3.Cl$ α and β-anomer), 3.17 – 3.07 (2 H, m, $OCH_2CH_2CH_2NH_3.Cl$ α-anomer), 3.02 (2 H, td, *J* = 12.6, 7.2 Hz, $OCH_2CH_2CH_2NH_3.Cl$ β-anomer), 1.99 (2 H, dq, *J* = 13.6, 6.7 Hz, $OCH_2CH_2CH_2NH_3.Cl$ α-anomer), 1.91 – 1.82 (2 H, m, $OCH_2CH_2CH_2NH_3.Cl$ β-anomer); **^{13}C NMR** (101 MHz; D_2O) δ 160.1 (2 C, C_q tetrazole), 100.6 (C1 β-anomer), 100.5 (C1 α-anomer), 72.7 (C3 β-anomer), 70.5 (C3 α-anomer), 70.5 (C2 β-anomer), 70.2 (C5 β-anomer), 70.0 (C2 α-anomer), 69.6 (C4 β-anomer), 69.5 (C4 α-anomer), 67.6 ($OCH_2CH_2CH_2NH_3.Cl$ β-anomer), 66.6 (C5 α-anomer), 65.3 ($OCH_2CH_2CH_2NH_3.Cl$ α-anomer), 37.6 ($OCH_2CH_2CH_2NH_3.Cl$ β-anomer), 37.4 ($OCH_2CH_2CH_2NH_3.Cl$ α-anomer), 26.7 ($OCH_2CH_2CH_2NH_3.Cl$ α-anomer), 26.6 ($OCH_2CH_2CH_2NH_3.Cl$ β-anomer); **^{13}C-GATED** (101 MHz; D_2O): 100.5 ($^1J_{C1\text{-}H1}$ = 172 Hz, C1 α-anomer); **HRMS** (ES^+) *m/z* [Found: $(M+H)^+$ 276.1309 $C_9H_{19}N_5O_5$ requires $(M+H)^+$, 276.1308].

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